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Method Development and Routine Assay

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CONTRACTING ORGANIZATION: University of California, San Francisco

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INTRODUCTION

This report describes technical work accomplished and information gained in performance of contract number DAMD17-92-C-2028, entitled "Analysis of Investigational Drugs in Biological Fluids - Method Development and Routine Assay," for the US Army Medical Research and Development Command (USAMRDC).

For many years our research group has been actively involved in the development of analytical methods to assay for drug substances in biological fluids for pharmacokinetic, bioavailability, drug metabolism and drug monitoring studies. This report describes the approach we took to develop sensitive (nanograms per milliliter of biological matrix), specific and quantitative analytical methods to support pharmacokinetic and bioavailability studies of candidate chemical warfare antidotes, antiparasitic drugs, radioprotectants and anti-infectious disease drugs.

In addition, routine analyses of biological specimens to support pharmacokinetic and bioavailability studies as part of preclinical and clinical investigations undertaken for the purpose of new drug development were performed as a significant adjunct to method development objectives. Within our routine analysis laboratory, we developed the capability to assay up to 10,000 samples per year.

There are many reasons for the U.S. military to develop various new drugs to protect or to treat soldiers confronted with the hazards of the modern battlefield. Like any pharmaceutical company, however, the military has to provide documentation in support of Investigational New Drug (IND) submissions to the Food and Drug Administration (FDA). Therefore, a great deal of work involving animal studies, preclinical and clinical trials, toxicity, metabolism and formulations must be carried out before a drug can be tried in the field. All of these studies depend on the adequacy of the analytical method for the particular compound. The route of administration and the dosage form are not necessarily the same in the field as in the clinic. For example, pyridostigmine is given prophylactically in the field, but the dose and route of administration are different for the treatment of myasthenia gravis or in anesthesiology.¹ Since military personnel are constantly involved in areas where they can be infected by parasites, including tropical or subtropical zones with drug-resistant forms, the U.S. Army needs to organize programs so that highly active and more effective new drugs can be discovered. These types of programs are generally ignored by private industry due to limited markets and profits.

This contract has offered us an interesting and stimulating challenge to utilize and extend our considerable capabilities to conduct method development and routine analysis in support of pharmacokinetic and

bioavailability studies. Our participation in this contract was possible by virtue of the experience and expertise of our staff in the area of pharmacokinetics, which requires assurance of extensive and rigorous internal and external analytical quality. As a result of our extensive involvement in these analytical programs, the staff members working on this project are the best in the field and have acquired a broad range of experience in the analysis of organic compounds in diverse media.

NATURE OF PROBLEM

Using the experimental procedures described in this report, we maintain the capability to complete projects on up to one new compound per year in terms of method development, validation, and characterization. We demonstrate sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describe the extent of recovery for the method, and report on the stability of compounds of interest in specimens during storage and drug analysis. Validation of sensitive and specific analytical methods follow procedures described in the Analytical Section Procedural Manual, Procedure 2D-3.1 and earlier versions "Procedure for Validation." Methods developed are such that a single technician can complete at least 15 clinical samples in one day. These methods are robust and portable enough to be transported to other laboratories. Within our routine analysis laboratory, we maintain the capability to assay up to 5,000 samples per year. Routine sample analysis will be performed in accordance with applicable procedures described in the Analytical Section Procedural Manual, Procedure 2D-4.2 and earlier versions. We have sufficient equipment and personnel to develop several candidate agents simultaneously and to be able to respond to changing priorities. We prepare and submit required reports in accordance with the contracted schedule.

BACKGROUND OF PREVIOUS WORK

Studies conducted over the 8 years prior to contract DAMD17-92-C-2028 under previous contracts including DAMD17-86-C-6150, DAMD17-85-D-0008, and DAMD17-83-C-3004 are listed in Tables 1 (study reports) and 2 (routine analyses reports).

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Minimum Quantitation Limit
01	8/26/83	Analytical Procedure for the determination of WR 6026 in Plasma	WR 6026 WR211,789•2HCl WR 6026 WR211,789•2HCl	Plasma Plasma Blood Blood	6.44 ng/ml 8.00 ng/ml 6.44 ng/ml 8.00 ng/ml
03	1/22/85	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Plasma	Pyridostigmine	Plasma	1.4 ng/ml
04	8/23/85	Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite, WR 178,460, in Blood and Plasma	halofantrine WR 178,460 halofantrine WR 178,460	Plasma Plasma Blood Blood	0.900 ng/ml 1.40 ng/ml 0.900 ng/ml 1.40 ng/ml
05	7/21/86	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Plasma Using Silica Gel Column and an Aqueous Mobile Phase	Pyridostigmine	Plasma	1.39 ng/ml
06	1/8/88	High Pressure Liquid Chromatography (HPLC) of Mefloquine in Plasma	Mefloquine	Plasma	10.0 ng/ml
07	1/12/88	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Urine	Pyridostigmine	Urine	13.7 ng/ml
08	9/23/88	High Pressure Liquid Chromatography (HPLC) of Physostigmine in Plasma with Ultraviolet Detection	Physostigmine	Plasma	1 ng/ml
09	9/12/88	Quantitation of Physostigmine & Eseroline in Plasma by HPLC with Fluorescence Detection	Physostigmine eseroline	Plasma Plasma	0.1 ng/ml 0.1 ng/ml
10	9/14/89	Quantitation of WR 6026 (Free Base) in Plasma & Blood by HPLC	WR 6026 WR 6026	Plasma Blood	0.980 ng/ml
11	9/28/89	Quantitation of WR 2721 in Plasma by HPLC with Electrochemical Detection	WR 2721	Plasma	0.100 µg/ml

**TABLE 1: PREVIOUS STUDY REPORTS
(Continued)**

Report No.	Report Date	Report Title	Test Article	Test System	Minimum Quantitation Limit
12	11/14/89	Quantitation of WR 3689 in Plasma by HPLC with Electrochemical Detection	WR 3689	Plasma	0.0990 µg/ml
13	11/17/89	Quantitation of WR 238605 by HPLC	WR 238,605 WR 238,605	Plasma Blood	0.815 ng/ml 1.91 ng/ml
14	8/29/89	Quantitation of Mefloquine (Free Base) in Plasma by HPLC, Extraction Method	Mefloquine	Plasma	8.00 ng/ml
15	12/19/90	Quantitation of Ribavirin and WR 249,992 (free base) in Plasma by HPLC with C18 Bonded Silica Gel Columns and Acidic Aqueous Mobile Phases	Ribavirin WR 249,992	Plasma Plasma	20 ng/ml 10 ng/ml
14	8/29/89	Quantitation of Mefloquine (Free Base) in Plasma by HPLC, Extraction Method	Mefloquine	Plasma	8.00 ng/ml
15	12/19/90	Quantitation of Ribavirin and WR 249,992 (f. b.) in Plasma by HPLC with C18 Bonded Silica Gel Columns and Acidic Aqueous Mobile Phases	Ribavirin WR 249,992	Plasma Plasma	20 ng/ml 10 ng/ml
16	Cancelled	β-arteether project cancelled	WR 255663		
17	4/25/90	Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	halofantrine WR 178,460 halofantrine WR 178,460	Plasma Plasma Blood Blood	0.960 ng/ml 0.928 ng/ml 0.960 ng/ml 0.928 ng/ml
18	Status report: 7/31/91	Quantitation of WR 6026 and WR 211,789 (WR 6026 Meta-bolite) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 6026 WR 211789	Plasma Blood	0.980 ng/ml 1.21 ng/ml
19	Status report: 1/14/92	Tentative title: Quantitation of Mefloquine and its Metabolite, WR 160972 in Biological Fluids	mefloquine WR 160972	plasma blood	7.36 ng/ml -
20	Status: 10/16/ 91	Tentative title: Quantitation of Artelinic acid in Plasma	Artelinic acid	plasma	20.2 ng/ml

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis of Halofantrine Plasma Samples Obtained from Protocol Titled "The Relative Bioavailability of Three Oral Formulations of Halofantrine Hydrochloride"	10/23/87	Halofantrine WR 178,460	plasma plasma	971 971	AY 86-1D
Phase III Comparative Clinical Trial of 4 Regimens of Halofantrine and Chloroquine in Treatment of <i>P. falciparum</i> Malaria	6/27/90	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood	470 470 468 468	Hal/BP 89-7
Routine Analysis for Protocol Titled "Pharmacokinetics of Intravenous Halofantrine HCl"	12/18/90	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood sol'ns	434 434 429 429 20	Hal/PB 90-5
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained under the Protocol Titled "52-Week Chronic Oral Toxicity Study of WR 171,669 HCl (Halofantrine Hydrochloride) in Dogs" and "Analysis of Blood and Plasma to Verify in vitro Metabolism of Halofantrine and Partition of Halofantrine and WR 178,460"	7/16/91	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood	83 83 48 48	Hal/P 91-1&2
Routine Analysis of Plasma and Blood Samples for the Protocol Titled "Disposition Kinetics of IV Desbutyl Halofantrine and the Effects of Gastric pH on the Bioavailability of Halofantrine-HCl"	2/4/92	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood dosing sol'ns	756 756 754 754 18	Hal/BP 91-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained under the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR 171,669•HCl (Halofantrine Hydrochloride) in Rats"	9/23/91	Halofantrine WR 178,460	plasma plasma	118 118	Hal/P 91-4
Routine Analysis for Halofantrine and WR 178,460 (free bases) in Blood Samples Obtained for the Protocol Titled "Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria"	1/21/92	Halofantrine WR 178,460	blood blood	107 107	Hal/B 91-5
Routine Analysis of Mefloquine Plasma Samples obtained from Six Clinical Protocols from Thailand	2/25/88	Mefloquine	plasma	781	Mef/P 87-1B
Routine Analysis of Plasma Samples from Thailand for Mefloquine Concentrations	12/7/88	Mefloquine	plasma	388	Mef/P 88-11
Routine Analysis of Blood Samples for Mefloquine (Free Base) Concentrations	2/12/91	Mefloquine	blood	18	Mef/B 90-3
Routine Analysis of Physostigmine Plasma Samples from the Protocol Titled "Bioavailability and Pharmacokinetic Study of Physostigmine (WR 006570) in Beagle Dogs"	8/26/88	Physostigmine Eseroline	plasma plasma	198 198	Phy/P 88-5
Routine Analysis of Physostigmine Plasma Samples from the Protocol Titled "Bioavailability and Pharmacokinetic Study of Physostigmine (WR 006570 AM) in Rhesus Macaques"	9/15/88	Physostigmine Eseroline	plasma plasma	196 196	Phy/P 88-6

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Pilot Study - Analysis of Rat Plasma	9/14/88	Physostigmine Eseroline	plasma plasma	45 45	Phy/rP 88-8
Pilot Study - Analysis of Rat Perfusate	9/14/88	Physostigmine	perfus	37	Phy/rP 88-9 r, pilot
Pilot Study - Analysis of Monkey Plasma	5/5/88	Physostigmine Eseroline	plasma plasma	8 8	"Phy/m 88-10 P, pilot"
Routine Analysis of Physostigmine (free base) and Eseroline (free base) Rat Plasma Bile, and Tube Binding Samples for Samples Obtained from WRAIR	1/18/90	Physostigmine Eseroline Physostigmine Eseroline	plasma plasma bile etc bile etc	92 92 20 20	Phy/rP, 89-6 pilot
Pyridostigmine in plasma (Israel)	5/14/86	Pyridostigmine	plasma	427	PY 85-4
"Pyridostigmine in plasma" (PY85-6-2 and PY85-6-3 combined) (Johns Hopkins, Millers)	7/3/86	Pyridostigmine	plasma	32	PY 85-6-4
Routine Analysis of Pyridostigmine Plasma Samples from Battelle Laboratories-MREF Protocol 27 (Battelle)	7/9/86	Pyridostigmine Pyridostigmine	plasma plasma, blind	648 22	PY 85-2-3
Routine Analysis of Pyridostigmine Plasma Samples Obtained from Protocol Titled "Pharmacokinetics of Orally Administered Pyridostigmine and Comparative Bioavailability of Liquid and Tablet Formulations" (Subjects 1-30)	12/3/86	Pyridostigmine Pyridostigmine	plasma dose sol	1698 12	PY 85-1
"Pyridostigmine in plasma (Johns Hopkins, Sub.1-24)"	1/12/87	Pyridostigmine pyridostigmine	plasma infusate	969 23	PY 85-6-5
"Pyridostigmine in plasma (Johns Hopkins, Sub.1-24)"	3/12/87	Pyridostigmine Pyridostigmine	plasma dose sol	1102 27	PY 85-6- 5B

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples		Report No.
Routine Analysis of Pyridostigmine Plasma Samples obtained from Protocol Titled "Development of a Primate Model for Evaluating Efficacy of Treatment Regimens Against Nerve Agent Poisoning: Part I: Pharmacokinetics of Pralidoxime Chloride, Atropine Sulfate, and Pyridostigmine Bromide" (PY85-3-1 through PY85-3-5 combined)	5/29/87	Pyridostigmine	plasma, monkey	439	PY	85-3-6B
Battelle Rat Study Pyridostigmine in plasma (revised letter report)	7/28/87	Pyridostigmine	plasma, rat	102	none	none
Battelle Dosing Sol's Pyridostigmine in plasma (revised letter report)	7/28/87	Pyridostigmine	dose sol	92	none	none
Routine Analysis of Pyridostigmine Plasma Samples Obtained from Protocol Titled "14 day pilot dose range oral toxicity study in dogs" (Battelle)	7/30/87	Pyridostigmine Pyridostigmine	plasma dose sol	152 2	PY	85-2-2B
Pyridostigmine in plasma (Huntingdon dog)	9/30/87	Pyridostigmine	plasma dog	336	PY	85-5-3C
Routine Analysis of Pyridostigmine Plasma Samples obtained from Protocol titled "Comparative Bioavailability Studies of Pyridostigmine Bromide in Male Beagle Dogs" (31 July 1985) (Huntingdon dog)	10/7/87	Pyridostigmine	plasma, dog	324	PY	85-5C

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis of Pyridostigmine Urine Samples from Protocol Titled "Bioavailability of Oral Pyridostigmine and Inhibition of Red Blood Cell Acetylcholinesterase by Oral and Intravenous Pyridostigmine"	2/3/88	Pyridostigmine	urine	110	Pyr/U 86-3B (rename d from AY86- 3)
Routine Analysis of Pyridostigmine Plasma and Urine Samples from Protocol Titled "Pharmacokinetics and Pharmacodynamics of Sustained, Low-dose, Intravenous Infusions of Pyridostigmine"	2/24/88	Pyridostigmine Pyridostigmine Pyridostigmine	plasma urine infusate	498 72 24	Pyr/PU 87-2B
Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled "Comparative Oral Bioavailability Studies of Two Wax Matrix Formulations of Pyridostigmine Bromide in Male Beagle Dogs"	3/29/88	Pyridostigmine	plasma	341	Pyr/P 88-1
Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Sustained Release Pyridostigmine in Healthy Men," dated 9/18/87	8/3/88	Pyridostigmine	plasma	558	Pyr/P 88-2
"Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Sustained Release Pyridostigmine in Healthy Men," dated Sept. 30, 1987"	8/2/88	Pyridostigmine	plasma	476	Pyr/P 88-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Protocol Titled "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of Pyridostigmine Administered by an Osmotic-Delivery Module (osmetr) compared to Pyridostigmine Syrup in Healthy Men"	5/12/89	Pyridostigmine	plasma	374	Pyr/P 89-2
Routine Analysis for protocol titled "Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Single Oral Doses of a Commercial Formulation of Sustained-Release Pyridostigmine in Healthy Men."	5/16/89	Pyridostigmine	plasma	120	Pyr/P 89-3
Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-Release Pyridostigmine in Healthy Men & the Influence of Food on Oral Pyridostigmine Pharmacokinetics	11/13/90	Pyridostigmine	plasma	1250	Pyr/P 89-8
Routine Analysis for Protocol Titled "Effect of chronic pyridostigmine administration on heavy exercise in hot environments"	9/11/90	Pyridostigmine	plasma	37	Pyr/P 90-2
Routine Analysis for Protocol Titled "Effects of Pyridostigmine Pretreatment on Physiological Responses to Heat & Moderate-to Intense Exercise"	2/20/91	Pyridostigmine	plasma	142	Pyr/P 90-4
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	4/13/89	WR 238605 WR 238605	plasma blood	62 62	WR5/B 89-1 P, pilot

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED
(Continued)

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	6/1/89	WR 238605 WR 238605	plasma blood	88 88	WR5/B 89-4 P, pilot
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	8/25/89	WR 238605 WR 238605	plasma blood	240 240	WR5/B 89-5 P
Routine Analysis of WR 6026 Plasma Samples Obtained from Clinical Protocol Titled "Single-Dose Absorption and Pharma- cokinetics of WR 6026 Hydrochloride in Healthy Subjects"	6/24/87	WR 6026	plasma	192	AY 86-2D
Routine Analysis of Blood Samples from the Protocol Titled "Multiple-Dose Pharmacokinetics, Safety and Tolerance of WR 6026 Hydrochloride in Healthy Subjects"	4/21/89	WR 6026	blood	571	Wr6/B 88-7
Routine Analysis for WR 6026 and WR 211,789 (as Free Bases) of Plasma Samples Obtained from WRAIR - Preliminary Report	2/13/91	WR 6026 WR 211789	plasma plasma	13 13	Wr6/PB 90-6

PURPOSE OF THE PRESENT WORK

Work on development and/or validation of analytical methodologies during the first half of the contract focused on assays for WR 238,605, halofantrine (and its metabolite), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), and primaquine (and its metabolite). Work on routine analyses of biological specimens during this period was performed for studies that

required determination of concentrations of WR 238,605, halofantrine (and its metabolite), WR 6026 (and its metabolites), mefloquine (and its metabolite), *p*-aminoheptanophenone (and related compounds), and primaquine (and its metabolite).

METHODS OF APPROACH

The general development plan is described with emphasis on the laboratory procedures used. Using the procedures described in this report, we were able to work simultaneously on seven projects (WR 238,605, halofantrine (and its metabolite), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), and primaquine (and its metabolite)) in terms of method development, validation, and characterization. We worked on demonstrating sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describing the extent of recovery for the method, and reporting on the stability of compounds of interest in specimens during storage and drug analysis. Validation of sensitive and specific analytical methods follow procedures described in the Analytical Section Procedural Manual, Procedure 2D-3.1 and earlier versions "Procedure for Validation of an Assay Methodology." Methods are developed such that a single technician could complete at least 15 clinical samples in one day. These methods are to be robust and portable enough to be transported to other laboratories.

All drug standards received from the USAMRDC were logged into our record book and stored at -20°C. If necessary, they were checked for chemical purity or radiopurity by high pressure liquid chromatography or thin layer chromatography, purified through recrystallization or chromatography, and hygroscopic samples were dried according to USP methods.

Sample Preparation for Assay Development

Spiked samples of biological media are prepared by spiking different amounts of drug from known stock solutions into the biological media. Samples are mixed, then equilibrated for one hour at room temperature, unless the compound of interest is unstable, in procedures in which it is especially important for measuring drug concentrations in blood, since drugs may take some time to reach equilibrium with erythrocytes.

Sample Preparation Procedures

Suitable preparation of the biological specimens is essential for the successful application of an analytical technique. The preparation procedure should be as simple as possible, yet allow for the specific measurement of the drug in the presence of numerous biological components. The extent of sample work-up is therefore largely determined by the selectivity and

sensitivity of the analytical technique. Interfering endogenous substances must be removed before analysis. A second objective in devising preparation steps for a biological specimen is to protect the analytical apparatus from contamination by proteins and undissolved particles. Biological sample preparation thus varies according to the technical demands of the various analytical instruments utilized. Since the advent of highly selective analytical methods that combine chromatographic separation and detection in one unit [e.g., HPLC], the importance of the second objective has become more critical.

Protein Precipitation

Protein precipitation methods are rapid; they involve mixing the sample with water-miscible organic solvents. Acetonitrile yields a protein precipitate that can be readily centrifuged into a small pellet. Use of protein precipitation alone, without further work-up, is a popular application in HPLC analysis. It is possible, using appropriate measurement devices such as electrochemical or fluorescence detectors, to obtain adequate sensitivity so that measurements in the nanogram per milliliter range can be made for drugs using small aliquots of the biological sample. We have used the protein precipitation method of sample preparation extensively in the development of analytical assays, including for antibiotics that are zwitterionic in nature, generally possess very low water-to-oil partition coefficients and, thus, are extremely difficult to extract efficiently. Also, protein precipitation is one method of choice for sample preparation, since a simpler sample preparation procedure reduces the risk of degradation. We use the direct protein precipitation method for our studies whenever possible (as demonstrated in Study Report 6 for mefloquine, Study Report 11 for WR 2721 and Study Report 12 for WR 3689).

Quantitation limits with ultraviolet (UV) detectors are usually at about 50 ng/ml concentrations when the protein precipitation method is used. If UV detection is required, organic solvent extraction and solid phase extraction are more useful methods for preparation of biological samples for subsequent analysis. Extraction also limits column overloading and removes assay interferences.

Solvent Extraction

Three major variables were considered in the design of suitable organic solvent extraction procedures: the polarity of the organic solvent, the pH of the aqueous phase, and the volumes of the organic and aqueous phases (as demonstrated in Study Reports 8 and 9 for physostigmine and its metabolite eseroline in plasma and Study Report 10 for WR 6026). A higher pH is often desirable since many endogenous substances are acidic and will not be extracted at alkaline pH. Consideration of pH is therefore important even when assays are developed for neutral drugs. Lipophilic bases are quite

uncommon in body fluids, so it should be relatively easy to analyze many of the lipophilic basic drugs by extracting at high pH (as shown in Study Report 13 for WR 238,605 in plasma and blood and Study Report 14 for mefloquine). However, one solvent partitioning step alone is not always capable of separating bases from acids and neutral compounds. In such cases, multiple extraction steps must be employed.

A sample preparation method combining protein precipitation with acetonitrile and extraction with organic solvent is also a viable option. This method has been successfully used in our halofantrine assay (² and Study Report 17).

Commercial prepacked solid phase columns [e.g. Bond Elut™] with different types of packing materials, such as silica, C2, C8, C18 and ion exchange were employed. These columns are very useful for sample purification. Two approaches can be utilized: 1] the column separates desired compound(s) from interferences, or 2] the column retains desired compound(s), undesired endogenous substances are washed away, and the desired compound(s) are eluted with a suitable solvent. For low nanogram or picogram per milliliter concentrations, the method of retaining the desired compound on the column is preferred. This method has been successfully used in our laboratory for charged, water soluble compounds (pyridostigmine (see Study Report 5 for plasma and Study Report 7 for urine)), or highly nonpolar lipophilic, weakly basic and nonvolatile compounds (WR 6026³ and halofantrine²) in biological fluids. For example WR 6026 and halofantrine are non-polar lipophilic compounds which are retained on C8 columns. Pyridostigmine, a quaternary amine, will not elute with CH₃CN alone. A 2 ml CH₃CN wash after loading the biological sample onto the C8 column eliminates undesired substances. The drug is subsequently eluted with CH₃CN containing SDS and tetramethylammonium chloride (TMA+Cl-) or 1% HCl culminating in a quantitation limit of 2 ng/ml with UV detection.

Specific functional groups in molecules of interest can also be advantageously used to purify biological samples by solid phase extraction. Diol functional groups can adsorb on a boronate column and subsequently be eluted with an acidic solution. This turned out to be our method of choice in the ribavirin and WR 249,992 assay development project (see Study Report 15).

Adsorption losses to glass or other apparatus for the low level lipophilic antimalarial drugs probably explains the inconsistent results reported by many investigators. The significance of this adsorption should be considered, especially when several extraction steps are to be employed. This was demonstrated during our development of the assay for halofantrine (WR 171,669) and its active metabolite, WR 178,460, in which WR 194,965 was used as the internal standard (² and Study Report 17). The compounds were

adsorbed by the glassware after reconstitution of the extract with organic solvent. In our experience, a true measurement of drug was obtained with the addition of a small amount of surface active agent to the solvent system before delivery onto the HPLC column. Adsorption loss can also occur in the port of delivery.

Detector Selection

The detector is a device that supplies an output in response to the presence of the compound(s) of interest. It is connected to the outlet of the column to monitor the column effluent in real time. The detector can be the most sophisticated and one of the most expensive components of a chromatographic system. Optical detectors, which currently dominate the field for biological samples in HPLC, include UV-visible absorbance detectors and fluorescence detectors. Depending on the measured difference between incidental and transmitted light intensity, these instruments can detect down to 9 to 10 ng of sample if the direct precipitation method is used. Electrochemical (EC) detectors are also used for routine work due to their specificity and/or sensitivity.

UV-Visible Absorbance Detector

Since the analytical methods for this contract required the quantitation of nanogram per milliliter concentrations of drug in biological samples, samples assayed with the UV detector required an extensive extraction work-up. For example, the pyridostigmine plasma assay was capable of quantitating 2 ng/ml concentrations of pyridostigmine (free base) (see Study Report 5) with UV detection only because of the extensive extraction procedure.

Fluorescence Detector

Fluorescence detection is more selective than UV spectroscopy. However, more structural requirements must be met to produce a high fluorescence yield ($\emptyset$) and to allow measurement above a negligible background (i.e., better quantitation limits). Minimum detection limits for the fluorescence detector can extend below the nanogram per milliliter level for favorable samples. (See Study Reports 9, 13, and 17).

Fluorescence intensity can be manipulated both by changes in solvent components and the pH of the solvent system. For example, quinoline is non-fluorescent in hexane but fluoresces in ethanol, while indomethacin shows fluorescence at a pH above 12. Most of the synthetic antimalarial drugs are asymmetrically conjugated, not strongly ionic and, hence, would be expected to fluoresce. Fluorescence detection might therefore be expected to be the method of choice for measuring antimalarial drugs due to the sensitivity, selectivity and lower dependence on instrumental stability (from pressure and temperature changes) of the detector.

Two different light sources at various wavelengths are used in commercial fluorescence detectors. They are the deuterium and the xenon arc lamps. The xenon arc lamp has high intensity and the energy is more evenly distributed at different wavelengths, whereas the deuterium lamp emits at lower energy than the xenon arc and the intensity is drastically diminished at wavelengths above 280 nm.

Since the intensity of emitted fluorescence is dependent upon the intensity of the excitation source, it would appear that the sensitivity of a fluorescence assay can be increased without limit by using the most intense source. Many researchers do not realize that marked differences can be found with different lamp sources in commercial detectors.

8-Amino-quinoline antimalarial drugs, such as WR 6026, WR 238,605 and mefloquine (Study Reports 6, 10, 13, 14, 18, and 19) are highly conjugated and the excitation wavelengths were expected to be high. The xenon arc source equipped with monochromometers to collect both the excitation and emitted energy wavelengths provided us with maximum flexibility in fluorescence detection. With these devices, specific wavelengths for optimum sensitivity and/or selectivity were conveniently selected.

Electrochemical Detector

Electrochemical detectors (EC) are also used in methods of choice for applying liquid chromatography to trace (sub-nanogram) analysis. EC detection can provide the sensitivity and selectivity necessary for practical analytical procedures in a variety of situations. Material eluted from the chromatographic column acts at an electrode surface under controlled potential conditions and the current which results from the net exchange of electrons is monitored as a function of time. Since the amount of material converted by the electrochemical reaction is proportional to the instantaneous concentration, the current will be directly related to the amount of compound eluted as a function of time. The flow through a thin layer electrochemical cell is ideally suited for LC analysis since it can be easily constructed with a very small dead volume (1 μ l) and maintain extreme sensitivity toward electroactive compounds. Several configurations using glassy carbon, carbon paste, or mercury-gold electrodes have been developed. If chromatographic conditions are carefully controlled, EC detection is quite precise and quantitative data can be obtained at the picomole level (total injected amount) for many compounds. In addition to being extremely sensitive, the electrochemical detector is quite specific in that only compounds electroactive at a given potential are detected. A large number of extremely important endogenous compounds, drugs, drug metabolites, food additives and organic pollutants are electroactive and therefore can be studied by EC. It is the method of choice for the detection of catecholamines and their analogs; numerous assay methods using EC detection have been published in

the recent literature. We have been successful in using this detector for measuring the morphine analog, nalbuphine in urine. When determining whether or not a particular compound can be successfully analyzed by EC, it is not sufficient to know that the compound can react electrochemically. The type of electrode surface, the nature of the solvent system and relative ease of oxidation or reduction must be carefully considered before one can ascertain whether such an analysis is feasible (see Study Reports 11 and 12 for phosphorothioate assays). Many important compounds have been studied in detail and conditions for analysis have been optimized. In order to assess fully the possibility of developing a new assay, it is desirable to carry out voltametric studies. This is equivalent to measuring an adsorption spectrum prior to using a UV detector.

With detection in the reductive mode, analysis of blood for artesunic acid and dihydroquinhaosu had been successfully carried out in Walter Reed Army Institute of Research.⁴

Phosphorothioates ($R-SPO_3H_2$) are potential radioprotective drugs investigated by the US Army. Neither UV nor fluorescence detection is suitable for this type of compound unless some other functional group in these molecules can be derivatized. To make matters worse, phosphorothioates are readily hydrolyzed to free sulfhydryl compounds in vivo (metabolism) and in vitro (degradation) and possibly further oxidized to disulfides. However, phosphorothioates can be detected by EC with dual mercury/gold electrode detectors connected in series. These can be very useful for the simultaneous determination of thiols and disulfides. Two Hg/Au electrodes are utilized in a series arrangement with reduction of disulfide to thiol at the upstream electrode, followed by conventional thiol detection downstream. The upstream electrode behaves as a novel on-line post column reactor of negligible dead volume. Phosphorothioates, thiols and disulfides are all readily quantitated in this detector and suitable separation is achieved by the HPLC system. It is interesting to recall that disulfide is actually being detected as the corresponding free thiol. No confusion occurs in measurements, however, because thiols are chromatographically resolved from the disulfide and thus separately detected.

Solvent System and Column

One of the most important steps in the development of an HPLC assay is selection of a suitable solvent system (mobile phase) and stationary phase. They are both closely related for maximum separation. Practical approaches are discussed in this section.

Reverse-Phase and Bonded Phase Columns

We intended to use reverse-phase systems for the majority of the analytical methods developed for HPLC assay described in this contract, since

such bonded phase columns have several advantages for applications involving biological fluids. Reverse phase columns are stable since the stationary phase is chemically bonded to the support and cannot easily be removed or lost during use. Therefore, a pre-column and/or presaturation of the two phases is/are not required. Reverse-phase columns have minimal irreversible retention which is compatible with a large variety of solvents; it is often possible to inject an aqueous sample without further treatment. As a result, bonded phase columns (BPC) are especially suited for samples containing components with widely varying K' (column capacity factor). The availability of a wide variety of functional groups on BPC packing allows reverse phase and ion paired chromatography to be carried out in a relatively simple, straight-forward manner.

In reverse-phase liquid chromatography, water is the polar solvent and any less polar, water-miscible solvent can be used in conjunction. Common examples of the second solvent include methanol, acetonitrile and tetrahydrofuran. The design of a successful LC separation depends on matching the right mobile phase to a given column and sample ion pairing mode.

Aqueous Mobile Phase and Silica Columns

The recent use of an unbonded silica stationary phase and an aqueous mobile phase has been successfully used in our laboratory for the liquid chromatographic separation of lipophilic amines. When C18 bonded phase columns are used, it is often necessary to employ amine mobile phase modifiers to ensure good retention times and peak shapes in the ion-suppression mode. Recent work suggests that unbonded silica gel, with the maximum concentration of surface silanol groups, is a preferable stationary phase for these compounds. Use of unbonded silica as the stationary phase permits the separation of a wide variety of amine compounds with a simple mobile phase containing an organic solvent and an aqueous phosphate buffer at neutral to alkaline pH. The retention volumes are lower and the peaks are more symmetrical when silica, rather than a C18 bonded support, is used as the stationary phase. The method is especially suitable for assays of biological fluids, since endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column while cationic aliphatic amine drugs will be retained. The interfering substances in biological fluids are eluted at the solvent front, leaving a very clean base line about the drug's retention time. Using a silica column and an aqueous solvent system, we obtained a quantitation limit of 2 ng/ml for plasma samples for pyridostigmine (free base) (see Study Report 05).

Selectivity and Resolution Modification

As a general approach to increasing (column selectivity) and improving resolution, several options are available and can be ranked in order of decreasing promise.

Modification of Mobile Phase

Many different properties of the solvent must be considered, including solvent strength and selectivity. Polar compounds are best separated by a polar solvent system, while non-polar compounds should be separated with a less polar system. Separation may be defined as the ability of the solvent system and column material to retain the compound of interest on the column for a longer period of time than the undesired components. We found that a change from methanol to acetonitrile can sometimes enhance the selectivity of the column. We tend to use acetonitrile as the solvent modifier since it has a lower viscosity and tends to increase the efficiency of the column; it is also characterized by increased miscibility with non-polar samples.

TABLE 3: DRUGS IN PLASMA ASSAYED WITH A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE

Drug	Detection Limit (ng/ml)	Detection Mode	Sample Preparation	Mobile Phase	Retention Time (min.)
WR 238605	0.8	Fluorescence	Liquid extraction	50% CH ₃ CN 5 mM (NH ₄) ₂ HPO ₄ pH = 7.0	6.50
WR 6026	1.0	UV	Liquid extraction	60% CH ₃ CN 5 mM (NH ₄) ₂ HPO ₄ pH = 7.0	7.00
Halofantrine	1.0	Fluorescence	Liquid extraction	80% CH ₃ OH 5 mM (NH ₄) ₂ HPO ₄ pH = 8.2	7.00
Pyridostigmine	1.4	UV	Solid Phase extraction	50% CH ₃ CN, 0.05% TMAC 5 mM (NH ₄) ₂ HPO ₄ pH = 7.2	16.4
Mefloquine	8.0	UV	Liquid extraction	80% CH ₃ OH 5 mM (NH ₄) ₂ HPO ₄ pH = 7.5	10.1

Change of pH and Ionic Strength

Aqueous buffers are commonly employed to suppress ionization of the ionizable sample components in reverse-phase analyses. The pH of the

mobile phase is varied and the resulting changes in K' (column capacity) and α (column selectivity) are examined.

Strength of buffers or ion-pairing agents can also influence the retention times of many drugs. Most of the antimalarial drugs are highly hydrophobic in nature, hence a high ratio of organic solvent modifier should be required and ion-pair techniques will be involved.

For silica stationary and aqueous mobile phase systems, the interaction between silica and amine is electrostatic and the separation mechanism is similar to an ion-exchange mechanism. Here, the pH of the mobile phase (pH 7-9.5) and pKa of the amine are very important in determining retention time, while the pH of the mobile phase in bonded phase systems (pH 2-5) is not as critical. Ionic strength is also critical. Thus for silica gel-aqueous mobile phase systems, mobile phase pH and ionic strength are more important to retention time determination than the organic modifier (e.g. CH_3CN , CH_3OH), which is the critical determinant in bonded gel - reverse-phase systems.

Change of Stationary Phase

A change of stationary phase is less convenient than a change in mobile phase composition and is less commonly used. Further adjustment of the mobile phase composition is usually required when a new column packing or stationary phase is used to optimize of solvent strength and K' values.

Most aromatic antimalarial drugs are very non-polar. It is reasonable to expect that the retention time will be shorter on a more polar C8 column (tend not to retain) than a non-polar C18 column. This was found to be true during the development of our first WR 171,669 (halofantrine) assay. The retention time for this compound is reduced by 1/3 by changing from a C18 column to a C8 column when the same mobile phase is used.

Temperature Change

The fourth technique for varying K' values is to increase (or decrease) the temperature. Since an increase in temperature normally reduces all sample K' values, it is usually necessary to decrease solvent strength to compensate for this effect. A change in temperature usually has little effect on sample K' values in liquid-liquid chromatography, but it is important in ion exchange and ion-pair chromatography. For this reason, a change in temperature for improvement of K' in ion-exchange and ion-pair chromatography is generally more promising than a change in stationary phase.

Complexation

A final means of changing K' values, sometimes dramatically, is through chemical complexation. A well known example is the use of metal ions (e.g.

Ag^+NO_3^-) in the solvent system to separate various olefinic compounds. The complexation of olefin and metal ion causes dramatic changes in retention time and selectivity.⁵ This technique is probably applicable for some antimalarial drugs.

For the most part, we intended to use C8 columns and/or ion-pair techniques to develop assay methodologies. However, silica gel column - aqueous mobile phase systems are our general method of choice for amines. Since measurement concentrations of 5 to 20 ng/ml are required, we expected to use 5 μm particle size columns for separation of drugs.

Derivatization

Derivatization is an important adjunct to HPLC assays. The choice of derivatization procedure is dependant upon the type of detector that is used. We are actively involved in pre-column and post-column derivatization as well as in structure modification studies to increase detection sensitivity. A wealth of information on potentially useful derivatives is available from the disciplines of qualitative organic analysis⁶ and protective group synthesis.⁷ In choosing a derivative for HPLC, ideally the reaction should be specific, quantitative, free from side reactions, complete in a relatively short time, and done under mild conditions. This kind of information is not readily available in the literature, and therefore, derivatization studies can be a rather time consuming venture. The design or choice of a derivatizing agent is critical.

Post column derivatization or degradation is also an excellent way to increase sensitivity of an assay. The technique of post column hydrolysis at alkaline pH and post column oxidation reactions with potassium permanganate or potassium periodate can be applied to assays that employ fluorescence detection. Post-column photo-irradiation is another way to increase sensitivity. First, the drug of interest is separated from other components of the sample by HPLC. Then, the sensitivity is enhanced by photo-irradiation which may rearrange the chromophore or otherwise break bonds to form a fluorescent species

Assay Validation

Validation of the methods were performed using biological fluids obtained from same species, when possible. This process indicates sample stability, method precision, accuracy and selectivity, and the feasible sample concentration range for use in pharmacokinetic or bioavailability studies. Validation procedures are part of our standard operating procedures (SOP) which are written in accordance with our program to meet Good Laboratory Practice (GLP) regulations. The procedures are described in the Analytical Section Procedural Manual, Procedure 2D-3.1 "Procedure for Validation of an Assay Methodology." This section summarizes Procedure 2D-3.1.

Specificity

The specificity should be evidenced by showing with chromatograms that: Test compounds are separated from major metabolites (if metabolite standard is available); Test compounds are separated from co-administered drugs (if any); At least three different sources of biological fluid should be free of possible interference of endogenous peaks at the retention times of test compounds.

All assay methods developed required use of an internal standard. Analogs of the compounds under study or chemicals with similar functional groups were preferred as internal standards. The internal standard must elute at a different time than the drug of interest, yet separate from endogenous substances in the biological sample. In addition, it should have similar extraction or partition properties as the drug of interest during the sample preparation process.

Linearity

Linearity is demonstrated by acceptable spiked vs. calculated (or vs. peak response ratios), y-intercept, and coefficient of determination (r^2) values for the standard curve (concentration vs. response).

Calibration curves were constructed by using the peak height (or peak area) ratio of drug to internal standard versus spiked concentration of drug. The calibration curve was used for quantitation of the chemical sample concentration. Linear regression (unweighted or weighted method) to obtain calculated concentrations, correlation coefficient (r or r^2), slope of regression line, and y-intercept were used to evaluate sample concentrations.

In the weighted least squares linear regression method, weights (w) = $1/y_i$, the intercept, b , is defined by:

$$b = \frac{((\sum w_i x_i^2)(\sum w_i y_i) - (\sum w_i x_i)(\sum w_i x_i y_i))}{((\sum w_i)(\sum w_i x_i^2) - (\sum w_i x_i)^2)}$$

and the slope, m , is defined by:

$$m = \frac{((\sum w_i)(\sum w_i x_i y_i) - (\sum w_i x_i)(\sum w_i y_i))}{((\sum w_i)(\sum w_i x_i^2) - (\sum w_i x_i)^2)}$$

Two standard curves may be calculated from the same set of standard curve calibrators (unless the weighted linear regression method is used). The low range curve is calculated from low concentration standard curve points

and is used to derive concentrations from samples with peak response ratios at or below the calculated peak response ratio of the highest standard curve point used in the low range curve. The high range curve is calculated from all standard curve points and is used to derive concentrations from samples with peak response ratios above the calculated peak response ratio of the highest standard curve point used in the low range curve.

The results are reported as a table containing spiked concentrations, peak response ratios, calculated concentrations, slope(s), intercept(s) and r^2 value(s) of a typical standard curve that was used in the method validation and as a table containing all slopes, intercepts and r^2 values of standard curves run in "intraday" and "interday" studies.

Limit of Quantitation

The quantitation limit is defined as the lowest standard curve concentration which can be reasonably, accurately, and precisely quantitated. Six samples spiked to the lowest standard curve concentration and a standard curve are prepared. The samples are run together within one day (or one run). The 6 lowest point of the standard curve sample concentrations, and their mean, S.D., C.V. (percent) and deviation (percent) are calculated. These data are used as the quantitation limit intraday result.

The 6 calculated lowest point of the standard curve concentrations that were obtained in the interday precision study and their means, S.D.s, C.V. percents and deviation percents are used as the quantitation limit interday result.

Recovery

It is important to check the recovery of compounds of interest during the assay in order to assess the uniformity of recovery during the assay or whether or not a better recovery can be obtained. Radio-labelled drugs, when necessary, were added to the sample and either the direct precipitation, solid phase purification or the extraction procedure was utilized to evaluate recovery. If labelled compounds were not available, a recovery study similar to those for WR 6026,³ halofantrine and its metabolite, WR 178,460,² and pyridostigmine⁸ were carried out. In brief, the recoveries of these drugs from plasma or whole blood were determined by comparison of the drug-to-internal standard peak height ratios of blood or plasma versus water samples spiked with the drug. In each case, the internal standard was added after sample was eluted from the solid phase column, extraction from organic solvent, or direct precipitation with CH_3CN to insure that the internal standard did not bind to the blood or plasma or to the cartridge during the preparation.

Precision

Precision is expressed as the standard deviation (S) of the assayed concentration where X_i are the repeated concentration measurements of an individual sample and $\bar{x}$ is the mean concentration.

$$S = \left(\frac{\sum_{i=1}^N (X_i - \bar{x})^2}{(N-1)} \right)^{\frac{1}{2}}$$

The coefficient of variation (C.V.) was used for determination of the precision. The sample number was 6. The bias of an assay method is determined by comparing biological sample results with spiked values. The significance of the bias is established by setting a confidence limit.

$$\text{Percent C.V.} = \frac{S \cdot 100}{\bar{x}}, \quad (N \geq 6)$$

If needed, the assay results were compared to those obtained with an assay of proven reliability and specificity. For example, the Pearson correlation coefficient (r) can be used. Maximum r value indicates exact correlation between the two variables and $r \approx 0$ indicates complete independence.

$$r = \frac{(\sum (X_i - \bar{x})(Y_i - \bar{y}))}{\sqrt{\sum (X_i - \bar{x})^2 \sum (Y_i - \bar{y})^2}}$$

The within-run precision was determined by measuring the amount of drug in a number of biological samples, in duplicate. The duplicate mean results are used to calculate the standard deviation. The between-run precision is measured on separate days with replicate samples at low, intermediate and high drug concentrations. From these three sets of replicate samples, the between-run standard deviation is calculated for each drug concentration.

Accuracy

Accuracy was determined by assaying a series of blind samples prepared according to the project director of DAMD.

Stability in Biological Specimens

Stability studies of a drug in biological media serve to establish the procedure for proper storage of the samples and furnishes information to clinical researchers on how best to handle these occasionally labile samples. We have a great deal of experience in planning and executing the required

stability studies. In methods developed for analytical and clinical studies, drug stability may play a particularly important role.

Known amounts of sample in different biological media are measured at various times after preparation (up to 6 months) and assayed for the drug, in duplicate. Variables, including light exposure, storage conditions (container type) and pH of the biological samples, are evaluated if necessary.

Since clinical samples are often repeatedly assayed and samples are thawed and refrozen, it is necessary to check for any instability of samples during these processes. Practically, this study can be done by using two concentration samples (High and Low). The same volume of biological fluid used to prepare standard curve samples is aliquoted to the appropriate number of tubes. Samples (in duplicate) are thawed and refrozen (a cycle) for 5 cycles. Samples are repeatedly thawed and refrozen according to the following table. Samples are thawed as if for sample preparation to room temperature and are left to stand at room temperature for 1 hour.

Cycle	Keep these samples in freezer
1	a
2	a, b
3	a, b, c
4	a, b, c, d
5	a, b, c, d, e

Following Cycle 5, all of the samples are thawed to room temperature and assayed with a standard curve. Test sample concentrations are calculated and reported in a table for each concentration (n=2) of mean concentrations (n=2) at each test point (n=5).

Stability studies are performed according to the methodology developed in this laboratory.

Purity of Standard Chemicals

The standard chemicals used in a study are USP™ reference standards (if available) or pure chemicals provided by the sponsor, unless otherwise instructed.

A chemical purchased from a general chemical company is not used as a standard, except in unusual circumstances and when its purity can be verified against a USP™ reference standard or the sponsor's standard verified by a certificate of analysis. The internal standard is not under this restriction.

USP™ reference standards can be regarded as 100% pure (unless specified), and no purity correction factor for concentration calculations is necessary. However, a sponsor's standard chemical must be regarded as possibly impure and a correction factor should be considered.

When verifying non USP™ reference standard chemical purity, the working standard solution is run under the method used for assaying biological samples. Each solution is injected 3 times, and two standard solutions are prepared for each standard chemical.

A copy of the supplier's certification sheet is saved with the method validation files.

Routine Assay Procedure

The following are the steps carried out when samples arrive for routine analysis. Sample arrival is recorded in the sample log-in book and on the log-in sheet, which includes the name of the shipper, arrival date, number of samples, sample storage location, and sample condition. An analytical standard operating procedure (SOP) is normally completed prior to routine analysis. In this SOP, the method description is condensed to about 3-5 pages that contain information regarding instrumentation, assay conditions, source of chemicals, preparation of stock solutions, sample preparation and a representative chromatogram.

For routine sample analysis, standard curve, blank and control samples are also analyzed. Sets of equipment consisting of a pump, detector, column, integrator and autosampler were set up for routine assay. Each system is tested by assigning personnel to run a series of controls; the performance of equipment and technical personnel are validated before routine sample assay.

Carry over testing is performed for each run by assay of a blank sample (not spiked with internal standard or with drug standard) immediately following the assay of a high concentration control.

To monitor variation during the course of an assay, a series of controls are prepared beforehand and stored in the freezer. Control samples are run together with standards and routine assay samples. Each set of controls normally includes four different concentrations within the range of the standard curve. For every treatment or up to 20 routine assay samples, a set of controls (extra low, low, medium and high) is included to validate the results. Since the concentrations of the controls are known, it is possible to judge whether the routine assay samples must be repeated on the basis of the results obtained for the controls.

Assay samples are prepared by spiking known volumes of biological sample with a known amount (constant over all samples) of internal standard (IS). Standard curve samples are generated by spiking interference free biological samples with known amounts of standard compound and IS. These standard curve and assay samples are prepared according to the analytical SOP, then injected onto an HPLC column for separation and subsequent detection. The peak height ratio of standard compound to IS is calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and standard compound to IS peak height ratios of the standard curve samples are fit by weighted or non weighted least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = standard compound concentration), and standard compound concentrations in assay samples are calculated by this equation from the standard compound to IS peak height ratios obtained by HPLC.

Assay findings are then sent in a report with a complete assay methodology including detailed methods, statistical evaluation of methods, routine assay sample results, results from the control samples, and one representative set of calibration chromatograms. Results can be sent by disc or through a modem for pharmacokinetic evaluation.

EXPERIMENTAL METHODS

The goals of the research under contract DAMD17-92-C-2028 are 1) to develop and validate methods to assay for drug substances in biological fluids for pharmacokinetic, bioavailability, drug metabolism and drug monitoring studies, and 2) to use these methods to perform routine analyses of biological specimens to support pharmacokinetic and bioavailability studies as part of preclinical and clinical investigations undertaken for the purpose of new drug development.

METHOD DEVELOPMENT AND/OR VALIDATION RESULTS

The following section describes the status of specific methods developed and validated or currently being developed and validated during the first half of the contract.

TABLE 4: STUDY REPORTS

Report No.	Report Date Status	Report Title	Test Article	Test System	Minimum Quantitation Limit
13, Supp I	5/2/94 in review	Quantitation of WR 238605 as Free Base in Rat Plasma by HPLC and Fluorescence Detection	WR 238,605	Rat Plasma	1.00 ng/ml
13, Supp II	4/11/94 in revision	Quantitation of WR 238605 as Free Base in Dog Plasma by HPLC and Fluorescence Detection	WR 238,605	Dog Plasma	1.00 ng/ml
17	4/25/90 in revision	Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	halofantrine WR 178,460 halofantrine WR 178,460	Plasma Plasma Blood Blood	0.960 ng/ml 0.928 ng/ml 0.960 ng/ml 0.928 ng/ml
18	Status report: 7/31/91	Quantitation of WR 6026 and WR 211,789 (WR 6026 Metabolite) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 6026 WR 211789	Plasma Blood	0.980 ng/ml 1.21 ng/ml
19	Status report: 1/14/92	Quantitation of Mefloquine in Blood By HPLC	mefloquine	blood	7.36 ng/ml
20	7/27/94 final	Quantitation of Artelinic acid in Plasma by HPLC with a C18 Bonded Column	Artelinic acid	human plasma	4.96 ng/ml
21	in prep	Tentative title: Quantitation of <i>p</i> -Aminoheptanophenone in Dog Plasma by HPLC	<i>p</i> -Aminoheptanophenone		
22	7/18/94 in review	Quantitation of WR 6026, WR 211,789, and WR 254,421 (as Free Bases) in Human Urine By HPLC	WR 6026 WR 211,789 WR 254,421	human urine	5.17 ng/ml 5.09 ng/ml 45.4 ng/ml
23	in prep	Tentative title: Quantitation of Primaquine and Metabolite in Dog Plasma by HPLC	primaquine metabolite	human plasma	

Quantitation of WR 238,605 in Human Plasma and Blood and Rat and Dog Plasma

Study Characteristics: Study Report 13B

Test Article:	WR 238,605
Test System:	human plasma and blood
Internal Standard:	WR 6026
Sample Assay Volume	0.2 ml
Sample Cleanup:	extract with methyl <i>t</i> -butyl ether

Analytical System

Detector:	Fluorescence with excitation - 375 nm; emission - 480 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	acetonitrile/water (1:1, v/v) final concentration of 5 mM (NH ₄) ₂ HPO ₄ at pH 7.0.

Validation Results: human plasma

Quantitation Limit:	0.815 ng/ml
Standard curve range:	0.815-408 ng/ml
Interday Precision	
Concentration Range:	1.63-163 ng/ml
CV Range:	6.64-8.70%
Intraday Precision	
Concentration Range:	1.63-163 ng/ml
CV Range:	5.44-9.07%
Blind Sample Assay	
Concentration Range:	1.20-179.4 ng/ml
Bias Range:	-4.68 to +31.7%
Mean Recovery:	82.5%
Stable Plasma Storage:	-20°C for 4 months

Validation Results (continued): human blood

Quantitation Limit:	1.91 ng/ml
Standard curve range:	1.91-383 ng/ml
Interday Precision	
Concentration Range:	3.82-143 ng/ml
CV Range:	2.94-7.75%
Intraday Precision	
Concentration Range:	3.82-143 ng/ml
CV Range:	7.69-9.15%
Blind Sample Assay	
Concentration Range:	2.39-239.16 ng/ml
Bias Range:	-22.7 to -5.86%
Mean Recovery:	97.3%
Stable Blood Storage:	-20°C for 30 days

The original validation Study Report (No. 13B) for WR 238,605 described validation for human plasma and blood. To satisfy requirements of our current laboratory SOP on validation, a short validation study was performed, Study Report 13B, Supplement I titled "Quantitation of WR 238,605 as Free Base in Rat Plasma by High-Performance Liquid Chromatography and Fluorescence Detection," consisting of precision (a shortened 3 run interday analysis and the normal intraday analysis) and recovery tests with rat plasma.

Study Characteristics: Short Validation Supplement I to SR13

Test System:	Rat Plasma
Validation Results:	
Quantitation Limit:	1.00 ng/ml
Precision CV:	6.72%
Precision Error:	21.8%
Standard curve range:	1.00-400 ng/ml
Interday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	0.54-6.46%
Intraday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	4.62-10.1%
Mean Recovery:	66.5%

An additional short validation study, Study Report 13B, Supplement II titled "Quantitation of WR 238,605 as Free Base in Dog Plasma by High-Performance Liquid Chromatography and Fluorescence Detection," with dog plasma was performed.

Study Characteristics: Short Validation Supplement II to SR13

Test System:	Dog Plasma
Validation Results:	
Quantitation Limit:	1.00 ng/ml
Interday CV:	15.0%
Interday Error:	25.3%
Standard curve range:	1.00-400 ng/ml
Interday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	4.60-10.2%
Intraday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	1.20-10.8%
Mean Recovery:	66.4%

Study Description

WR 238,605 succinate (N4-[2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy]-8-quinoliny]-1,4, pentanediamine succinate), an 8-aminoquinoline derivative, has been chosen by the U.S. Army Drug Development Program to be developed as an anti-malarial drug to replace primaquine.⁹ Although primaquine is the best available drug for the curative treatment of *Plasmodium vivax* malaria¹⁰, problems associated with its toxicity¹¹ and the development of resistant strains,¹² led to the development of WR 238,605 succinate, which was found to be 12.8 times more potent on a molar basis than primaquine in curing the rhesus monkey of *P. cynomolgi*, the simian equivalent of *P. vivax*.⁹

Plasma samples were analyzed for WR 238,605 (free base) (N4-[2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy]-8-quinoliny]-1,4, pentanediamine base), an 8-aminoquinoline derivative, with an HPLC procedure that uses a silica gel column, an (acetonitrile/water) aqueous mobile phase, a fluorescence detector, and 0.2 ml plasma samples. Sample cleanup consisted of extraction into methyl *t*-butyl ether, evaporation of the organic phase and reconstitution of plasma sample extracts in acetonitrile/water prior to separation by HPLC. The attached method SOPs (Appendix A) contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known volumes of plasma with a known amount (constant over all samples) of WR 6026 internal standard (IS). Standard curve samples were generated by spiking interference free plasma samples with known amounts of WR 238,605 (free base) and IS. These standard curve and assay samples were extracted, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratio of WR 238,605 (free base) to IS was calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and WR 238,605 (free base) to IS peak height ratios of the standard curve samples were fit by weighted or non weighted least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = WR 238,605 (free base) concentration), and drug concentrations in assay samples were calculated by this equation from the WR 238,605 (free base) to IS peak height ratios obtained by HPLC.

Plasma samples (0.2 ml aliquots unless otherwise specified) to be assayed for WR 238,605 (free base) were pipetted into glass culture tubes. Next, 20 μ l of internal standard (WR 6026, 6-methoxy-8-(6-diethylamino-hexylamino) lepidine dihydrochloride) solution (27.9 μ g/ml) and 0.1 ml of 0.1 N NaOH buffer were added and vortexed. Then, 3 ml of methyl *t*-butyl ether extracting solvent was added, and the samples were vortexed for 1 min, twice, and centrifuged for 10 min at 3000 g. The organic layer of each sample was

pipetted to a clean tube and evaporated to dryness under nitrogen. The residue was reconstituted with 200 μ l of acetonitrile/water (1:1, v/v), transferred to a WISP insert, and injected onto the HPLC column.

This project will be complete upon review, revision (if required), and approval as final by the COR of supplemental reports I and II to Study Report 13.

Halofantrine and Metabolite in Human Plasma and Blood, Rat Liver, Rat Bile - and Rat Perfusate

Study Characteristics: Study Report 17

Test Article:	halofantrine, WR 178,460
Test System:	human plasma and blood
Internal Standard:	procainamide hydrochloride
Sample Assay Volume	0.5 ml
Sample Cleanup:	Precipite with acetonitrile extract with methyl <i>t</i> -butyl ether

Analytical System

Detector:	fluorescence
Column Type:	silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	CH ₃ OH/water (80:20, v/v) 5 mM (NH ₄) ₂ HPO ₄

Validation Results: halofantrine in human plasma

Quantitation Limit:	0.960 ng/ml
Standard curve range:	0.960-115 ng/ml
Interday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	4.24-10.6%
Intraday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	3.82-13.6%
Mean Recovery:	73.0%
Stable Plasma Storage:	-80°C for 4 months

Validation Results: WR 178,460 in human plasma

Quantitation Limit:	0.928 ng/ml
Standard curve range:	0.928-111 ng/ml
Interday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	4.90-6.65%
Intraday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	4.21-7.94%
Mean Recovery:	95.5%
Stable Plasma Storage:	-80°C for 4 months

Validation Results: halofantrine in human blood

Quantitation Limit:	0.960 ng/ml
Standard curve range:	0.960-115 ng/ml
Interday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	3.42-10.5%
Intraday Precision	
Concentration Range:	1.88-75.4 ng/ml
CV Range:	4.60-10.0%
Mean Recovery:	68.6%
Stable Blood Storage:	-80°C for 4 months

Validation Results: WR 178,460 in human blood

Quantitation Limit:	0.928 ng/ml
Standard curve range:	0.928-111 ng/ml
Interday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	6.49-7.88%
Intraday Precision	
Concentration Range:	1.85-73.9 ng/ml
CV Range:	5.70-9.40%
Mean Recovery:	95.9%
Stable Blood Storage:	-80°C for 4 months

Study Description

Study Report 17 presents a second approach to the HPLC analysis of blood and plasma samples for determination of the free base concentrations of halofantrine (WR 171,669) and of its metabolite (WR 178,460). Study Report No. 4 (dated August 23, 1985 under contract DAMD 17-83-C-3004) describes an ion-paired liquid chromatographic assay for halofantrine and its metabolite as free bases in blood & plasma. The assay involves protein precipitation and a column elution step prior to HPLC separation. However, due to successes in this laboratory in assays for several amines in which a silica gel stationary phase was used^{13,14} the same approach was tried in an assay for halofantrine and its metabolite (as free bases). The second method involves the use of a silica gel column run with an aqueous mobile phase which results in cleaner baselines and a higher signal to noise ratio at concentrations similar to the ion-paired method.¹⁵ All glassware used in this assay must be silanized to limit error due to absorption.

Plasma samples for analysis are pipetted (0.5 ml) into silanized tubes. Approximately 1.5 ng of the internal standard, procainamide hydrochloride,

and 1 ml of CH_3CN to precipitate the proteins were added. The samples are vortexed and centrifuged and the resulting supernatant is transferred to a silanized tube and evaporated under N_2 to about 0.5 ml. Water (0.5 ml) and 0.1 N NaOH buffer (0.5 ml) were added to make the solution alkaline. Methyl *t*-butyl ether (5 ml), the extracting solvent, is added and the sample is vortexed, centrifuged and frozen in a dry ice/MeOH bath. The organic layer is poured into a 13x100 mm silanized tube. This extraction step is repeated with another 5 ml of extracting solvent which is poured into the same 13x100 mm silanized tube. The solvent is evaporated to dryness under N_2 , the residue is reconstituted with 200 μl of methanol/water (80:20) containing 0.001% HCl, and 25 to 150 μl is injected onto the column.

Blood samples are treated similarly except 0.5 ml water to lyse the cells and 2 ml of CH_3CN to precipitate the proteins were added. For standard curve samples, appropriate amounts of drug and metabolite are added, and samples are left to equilibrate at room temperature for one hour.

A silica gel column and a mobile phase composition of methanol/water (80:20, v/v) with a final concentration 5 mM $(\text{NH}_4)_2\text{HPO}_4$ were used to separate halofantrine and WR 178,460 (as free bases) from the internal standard and interfering endogenous substances in an isocratic elution. In typical chromatograms, halofantrine and WR 178,460 (as free bases) for both plasma and blood are sufficiently separated from each other and from endogenous compounds to permit successful sample assay. Halofantrine (free base) eluted at 8 minutes, WR 178,460 (free base) eluted at 11 minutes, and the internal standard eluted at 26 minutes.

A linear relationship was demonstrated between the halofantrine and WR 178,460 (as free bases) concentrations in plasma or blood and the peak height ratios of the halofantrine or WR 178,460 peak to the internal standard peak. The minimum detection limits, 0.928 and 0.960 ng/ml, were determined as the halofantrine and WR 178,460 (as free bases) concentrations, respectively, at which the signal to noise ratio was 3 to 1. Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level drug or metabolite (as free bases) concentrations, two standard curves were constructed from the same set of standard curve data points.

Data points from 0 to 14.4 ng/ml were used to construct a low halofantrine (free base) concentration standard curve with which to calculate the low concentration samples. All data points (0 to 115 ng/ml) were used to construct a high halofantrine (free base) concentration standard curve for the high concentration samples. Concentrations of samples with peak height ratios above that calculated at 14.4 ng/ml from the low concentration standard curve were calculated with the high concentration standard curve.

Data points from 0 to 13.9 ng/ml were used to construct a low WR 178,460 (free base) concentration standard curve with which to calculate the low

concentration samples. All data points (0 to 111 ng/ml) were used to construct a high WR 178,460 (free base) concentration standard curve for the high concentration samples. Concentrations of samples with peak height ratios above that calculated at 13.9 ng/ml from the low concentration standard curve were calculated with the high concentration standard curve.

For representative standard curves, the coefficients of determination for halofantrine (free base) in plasma were 0.9995 and 0.9950 and for halofantrine (free base) in blood were 0.9985 and 0.9985 for low and high concentration ranges, respectively. The coefficients for WR 178,460 (free base) in plasma were 0.9998 and 0.9983 and for WR 178,460 (free base) in blood were 0.9956 and 0.9993 for low and high concentration ranges, respectively.

Blind blood samples, prepared April 1, 1993 were assayed by the method described in Study Report 17, modified by incorporation of an analog to halofantrine (WR 122,455) as an alternate internal standard. The results, and changes induced by using a different internal standard, will be incorporated in Study Report No. 17.

Study Characteristics: Study Report 17

Internal Standard: WR 122,455

Validation Results: Halofantrine in human blood

Blind Sample Assay

Concentration Range: 2.04-183.6 ng/ml

Bias Range: -8.21 to +0.368%

Validation Results: WR 178,460 in human blood

Blind Sample Assay

Concentration Range: 1.97-177.5 ng/ml

Bias Range: -2.47 to +33.8%

Short validations are underway for halofantrine assays of the following rat tissues and fluids

Sample Type	Study Method	Status
liver	precipitation	assays done, data in preparation
bile	precipitation	assays done, data in preparation
bile	extraction	assays in progress
perfusate	precipitation	assays done, data in preparation
perfusate	extraction	assays in progress

WR 6026 and Metabolite in Human Plasma

Study Characteristics: Study Report 18

Test Article:	WR 6026, WR 211,789
Test System:	human plasma and blood
Internal Standard:	chlorpheniramine
Sample Assay Volume	0.5 ml
Sample Cleanup:	methyl <i>t</i> -butyl ether extraction

Analytical System

Detector:	UV at 263 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	acetonitrile/water (3:2, v/v) final concentration of 5 mM (NH ₄) ₂ HPO ₄ at pH 8.8

Validation Results: WR 6026 in human plasma

Quantitation Limit:	0.980 ng/ml
Standard curve range:	0.980-98.0 ng/ml
Interday Precision	
Concentration Range:	2.06-77.3 ng/ml
CV Range:	3.05-6.82%
Intraday Precision	
Concentration Range:	2.06-77.3 ng/ml
CV Range:	3.22-9.39%
Blind Sample Assay	see Appendix A
Mean Recovery:	74.5%
Stable Plasma Storage:	-20°C for 3 months

Validation Results: WR 211,789 in human plasma

Quantitation Limit:	1.21 ng/ml
Standard curve range:	1.21-121 ng/ml
Interday Precision	
Concentration Range:	2.14-80.1 ng/ml
CV Range:	5.19-8.98%
Intraday Precision	
Concentration Range:	2.14-80.1 ng/ml
CV Range:	4.42-7.86%
Blind Sample Assay	see Appendix A
Mean Recovery:	93.8%

Validation Results: WR 6026 in human blood

Quantitation Limit:	0.980 ng/ml
Standard curve range:	0.980-98.0 ng/ml
Interday Precision	
Concentration Range:	1.96-78.4 ng/ml
CV Range:	1.56-6.38%
Intraday Precision	
Concentration Range:	1.96-78.4 ng/ml
CV Range:	2.31-5.36%
Stable Plasma Storage:	-20°C for 1 month -70°C for 3 months

Validation Results: WR 211,789 in human blood

Quantitation Limit:	1.21 ng/ml
Standard curve range:	1.21-121 ng/ml
Interday Precision	
Concentration Range:	2.40-96.0 ng/ml
CV Range:	1.74-5.12%
Intraday Precision	
Concentration Range:	2.40-96.0 ng/ml
CV Range:	1.76-4.85%

Study Report 18

Sets of blind plasma and blood samples, prepared April 1, 1993, were received. Blind plasma sample results were enclosed with Quarterly Report 8. Upon analysis of blood samples, results will be forwarded to the COR. Acceptable results will be incorporated into Study Report 18, "Quantitation of WR 6026 and WR 211,789 (as Free Bases) in Plasma and Blood by High-Performance Liquid Chromatography." The test of stability is in progress. Procedures Required to Complete Validation

The following list details changes that were instituted for plasma sample analysis, but that have not been tested for validation of the blood sample analytical method.

1. Following addition of 5 ml of methyl-*t*-butyl ether, vortex [not rotate] samples for 1 [not 15] min.
2. Adjust the mobile phase pH to 8.8 [not 7.0].
3. Stock and working solutions were stored at -20°C [not 4°C].

The following list details validation tests that have not been done.

1. Stability of WR 211,789 (free base) at -80°C and -20°C in blood and plasma.
2. Recovery of WR 6026 and WR 211,789 from blood.
3. Precision of WR 6026 and WR 211,789 (as free bases) in blood with mobile phase pH = 8.8, storage of stock and working solutions at -20°C, and vortexing extraction samples for 1 min.
4. Accuracy for WR 6026 and WR 211,789 (as free bases) in plasma and blood on blind spiked samples prepared by the Walter Reed Army Institute of Research.
5. Interference: To determine whether known compounds would interfere with detection of WR 6026 or WR 211,789 (as free bases), the retention times relative to CPA in mobile phase of several WR 6026 (free base) analogues could include WR 225,742 and WR 254,421 (free base).

Study Description

WR 6026 (dihydrochloride) (6-methoxy-8-(6-diethyl amino hexyl amino) lepidine dihydrochloride) (see figure below), is a very effective antileishmanial drug in hamsters infected with *Leishmania donovani*.¹⁶

Because antimony compounds are not always effective and the other drugs in use have toxic effects,^{17,18} alternative therapies are needed. Since WR 6026 (dihydrochloride) is a likely candidate and since WR 6026 (dihydrochloride) is scheduled for clinical testing in the near future, it is extremely important to develop an analytical method capable of measuring concentrations of WR 6026 (free base) at nanogram per milliliter concentrations in biological samples.

This report describes an assay developed to determine the concentrations of WR 6026 and of its mono dealkylated metabolite, WR 211,789, (as free bases) in blood and plasma. This new assay provides significant improvements over capabilities of earlier assays with increased sensitivity for the detection of WR 6026 (free base)¹⁹ and inclusion of WR 211,789 (free base) in the methodology (Study Report 10).

Plasma samples (0.5 ml transferred with a plastic tipped pipetter to silanized culture tubes (see SOP #3-11 for silanization procedure)) were vortexed with 100 µl of a 1.00 µg/ml chlorpheniramine maleate internal standard working solution and 100 µl of a 1 N NaOH solution for 10 s. Next, 5 ml of methyl-*t*-butyl ether was added and samples were vortexed for 1 min, then centrifuged for 10 min at 3000 g. Then, for each sample, the aqueous layer was frozen in a dry ice/methanol bath and the organic layer were decanted into a new silanized culture tube. Finally, the sample's organic layer was evaporated to dryness under prepurified nitrogen, reconstituted in 200 µl of mobile phase, vortexed for 1 min, transferred to silanized WISP inserts, and injected onto the HPLC column.

Blood samples (0.5 ml transferred with a plastic tipped pipetter to silanized culture tubes) were vortexed for 1 min with 0.5 ml of nanopure water, and the mixtures were sonicated for 10 min. Then, these samples were prepared like plasma samples beginning with addition of 100 μ l of the internal standard working solution.

No degradation of WR 6026 (free base) in plasma frozen at -20°C or blood frozen at -80°C was seen for the duration of the stability study. However, noticeable degradation of WR 6026 (free base) in blood frozen at -20°C was observed by the third month at all concentrations.

Two standard curves for each assay were constructed from the chromatographic data; a low range curve from the 0 to 14.7 ng/ml for WR 6026 and 0 to 18.1 ng/ml for WR 211,789 standard curve samples and a high range curve from the 0 to 98.0 ng/ml for WR 6026 and 0 to 121 ng/ml (i.e. all) standard curve samples in order to obtain more accurate determinations of low level WR 6026 and WR 211,789 (free base) concentrations. The low range standard curve was used to calculate drug or metabolite concentrations for assayed samples when the peak height ratio of the sample was less than or equal to the calculated peak height ratio at the highest concentration of the low range curve (as calculated from the low range curve). The high range curve was used to calculate results for samples with peak height ratios greater than the calculated peak height ratio at the highest concentration of the low range curve (as calculated from the low range curve).

Typical plasma and blood chromatograms show WR 6026 (free base), WR 211,789 (free base) and internal standard, chlorpheniramine, peaks that are baseline separated and separated from other components of the sample.

A linear relationships was demonstrated between the WR 6026 and WR 211,789 (free base) spiked concentrations to the WR 6026 and WR 211,789 (free base) to internal standard peak height ratios for the plasma and blood assays. Linear regression analysis of concentration versus the peak height ratio gave coefficients of determination (r^2) of 0.989 or better for these typical standard curves. The linear range of the standard curves covered WR 6026 (free base) concentrations in plasma and blood in the range 0.980 to 98.0 ng/ml and WR 211,789 (free base) concentrations in plasma and blood in the range 1.21 to 121 ng/ml. The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange²⁰ and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid

compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. The interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug.

Validation trials in our laboratory for an earlier study (Study Report 10) were undertaken to include in the WR 6026 (free base) assay the capability to measure WR 211,789 (free base), a mono dealkylated metabolite of WR 6026 (free base), concentrations in biological samples. Large variations between spiked and recovered concentrations were observed in that study. Although WR 211,789 has been detected in a rat microsomal preparation,²¹ it has not been detected in plasma in human studies, perhaps because the detection limit of the assay used was only 10 ng/ml.¹⁹ WR 211,789 plasma standard curves in the trials were of higher quality than blood standard curves. The current report describes an adaptation of the WR 6026 (free base) methodology or a modification of the methodology presented in the earlier report (Study Report 10), in which a 5 to 10 fold increase in sensitivity has been gained that makes detection of WR 211,789 (free base) in human plasma possible at higher WR 6026 (dihydrochloride) doses.

In addition, compared to an even earlier methodology,¹⁹ the WR 6026 (free base) HPLC method presented here offers increased sensitivity and extends the range of biological fluids that can be assayed. The earlier method measured WR 6026 (free base) in plasma cleaned by protein precipitation (with acetonitrile) and column elution (from a C2 extraction column), had a 6.44 ng/ml WR 6026 (free base) detection limit, used WR 223,658 as an internal standard, required a C8 bonded silica gel HPLC column, used a 60:40 (v/v) acetonitrile/water mobile phase at pH 5.5 with 0.2% final concentrations of SDS and glacial acetic acid, and measured WR 211,789 (free base) with a minimum detection limit of 8 ng/ml. The newer method measures WR 6026 (free base) in plasma and blood cleaned by extraction with 99:1 (v/v) pentane/acetonitrile, has a 0.980 ng/ml WR 6026 (free base) detection limit, uses chlorpheniramine maleate as an internal standard, requires an unbonded silica gel HPLC column, uses a 70:30 (v/v) acetonitrile/water mobile phase at pH 7.0 with 5 mM final concentration of dibasic ammonium phosphate, but could not measure WR 211,789 (free base) with a minimum detection limit much better than 8 ng/ml. The current modified method measures WR 6026 and WR 211,789 (free base) in plasma and blood cleaned by extraction with methyl-*t*-butyl ether, has 0.980 ng/ml WR 6026 and 1.21 ng/ml WR 211,789 (as free bases) detection limits, uses chlorpheniramine maleate as an internal standard, requires an unbonded silica gel HPLC column, uses a 60:40 (v/v) acetonitrile/water mobile phase at pH 8.8 with 5 mM final concentration of dibasic ammonium phosphate.

HPLC assays for basic amine drugs in biological samples that make use of a silica gel column and an aqueous mobile phase have been operated in this

laboratory for over 5 years.^{68,69,70} In the WR 6026 (free base) HPLC method presented here, the use of an unbonded silica gel column, an aqueous mobile phase, and UV detection at 263 nm yields satisfactory results for the determination of WR 6026 and WR 211,789 (as free bases) in (0.5 ml) plasma and blood samples. The method is simple in that a single extraction step and evaporation of solvent prior to injection are required. Recovery of WR 6026 (free base) averaged 74.5%, while recovery of WR 211,789 (free base) averaged 93.8% from plasma. The minimum quantitation limits of the assay were 0.980 ng/ml for WR 6026 (free base) and 1.21 ng/ml for WR 211,789 (free base) for blood and plasma. The coefficients of variation of the inter- and intraday assay precision analyses were less than 10% at all concentrations. The method is simple, precise, more sensitive, and includes the capability of quantitating WR 211,789 (free base) as well as the parent drug compared to earlier methods.

Mefloquine in Human Blood

Study Characteristics: Study Report 19

Test Article:	Mefloquine
Test System:	human blood
Internal Standard:	chlorpheniramine
Sample Assay Volume	0.5 ml
Sample Cleanup:	pentane/methylene chloride (7:3, v/v) extraction

Analytical System

Detector:	UV at 280 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	methanol/water (4:1, v/v) final concentration of 5 mM (NH ₄) ₂ HPO ₄ at pH 7.5

Validation Results: Mefloquine in blood

Quantitation Limit:	7.36 ng/ml
Standard curve range:	7.36-2210 ng/ml
Interday Precision	
Concentration Range:	14.7-1472 ng/ml
CV Range:	3.94-8.41%
Intraday Precision	
Concentration Range:	14.7-1472 ng/ml
CV Range:	2.74-10.9%
Blind Sample Assay	
Concentration Range:	11.52-1536 ng/ml
Bias Range:	-12.6 to +7.20%
Mean Recovery:	91.5%
Stable Blood Storage:	-20°C for 4 months

Study Description

Mefloquine (hydrochloride), (WR 142,490: erythro-a-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride), is an alternative curative agent for the treatment of falciparum malaria.^{25,26} Mefloquine (hydrochloride) has also been shown to prophylactically suppress mosquito induced infections by *Plasmodium vivax* and *P. falciparum* in human volunteers.^{27,28} Published plasma and/or blood analytical methods employ gas-liquid chromatography (GLC)^{29,30,31} thin layer chromatography (TLC),³² gas chromatography-mass spectrometry (GC-MS)³³ or high performance

liquid chromatography (HPLC) (Study Reports 6 and 14).^{34,35,36,37,38,39,40} The GLC methods require derivitization, and sample volume in the method described by Nakagawa, *et al.* uses 5 ml samples. The TLC method has no internal standard and is insufficiently sensitive. The GC-MS method requires derivitization and the increased expense of mass spectrometry.

Many HPLC have been reported. The method reported by Grindel, *et al.*, required three times extraction from 5 ml plasma samples and, upon solvent evaporation, the residues required overnight storage in a vacuum desiccator. Kapetanovic, *et al.* used a 3 step extraction of 1 ml samples. Our earlier study (Study Report 6) described a protein precipitation method for 0.2 ml plasma samples. Franssen, *et al.*, described a method for plasma and blood analysis for mefloquine and its carboxylic acid metabolite with 50 ng/ml mefloquine and 100 ng/ml metabolite detection limits. Karbwang, *et al.*, described a 50 ng/ml detection limit, 100 ng/ml quantitation limit method for mefloquine in blood and plasma. Coleman, *et al.*, measured mefloquine at 10 ng/ml in liver perfusate. Riviere *et al.*, presented a method with a 20 ng/ml detection limit and 100 ng/ml quantitation limit in plasma. Bergqvist, *et al.*, describe two HPLC methods for determination of mefloquine and its principal metabolite in plasma and blood, the first with 30 ng/ml plasma and 150 ng/ml blood quantitation limits and the second with 75 ng/ml quantitation limits for both compounds.

We reported (Study Report 14) the development of a simple and rapid HPLC assay for mefloquine (free base) that requires 0.5 ml plasma samples and a one step extraction, has an 7.36 ng/ml quantitation limit and produces chromatograms with a cleaner baseline than our previous method. Study Report 19 describes the extension of our plasma method to include analysis of blood samples. Study Report 19 also describes status of steps taken toward extension of the method for determination of the main mefloquine metabolite, WR 160,972 (2,8-bis-(trifluoromethyl)-4-quinoline carboxylic acid).⁴¹

The blood method was modified from the plasma method described in Study Report No. 14, "Quantitation of Mefloquine (Free Base) in Plasma by High-Performance Liquid Chromatography, Extraction Method." The blood method primarily differs from the plasma method in sample preparation by:

1. Allowing blood standard curve calibrator samples to equilibrate for 1 hour following spiking with mefloquine working solutions;
2. Addition of 0.5 ml water; and
3. Sonication for 10 min prior to addition of internal standard.

Blood samples for analysis are pipetted (0.5 ml) into screw top tubes. Add 100 μ l of a saturated solution of sodium carbonate and vortex the mixture for 1 min. Then, add 100 μ l of the internal standard working solution (CPA, 12

µg/ml) and vortex the mixture for 1 min. The sample is extracted with 5 ml of pentane/methylene chloride (7:3, v/v), evaporated to dryness under nitrogen, resuspended in 200 µl of mobile phase and injected (40-80 µl) onto the HPLC column.

An addendum with blind sample results enclosed with Quarterly Report 6 completed Study Report 19 (Status Report, dated January 14, 1992 and titled "Quantitation of Mefloquine (Free Base) in Blood by High-Performance Liquid Chromatography, Extraction Method." Further work on this assay is scheduled to include WR 160,972 method development, but work on this aspect of the project has been assigned a low priority by the COR.

Artelinic Acid in Human Plasma

Study Characteristics: Study Report 20

Test Article:	artelinic acid (WR 255,663)
Test System:	human plasma
Internal Standard:	meclofenamic acid
Sample Assay Volume	1 ml
Sample Cleanup:	Precipitation with acetonitrile SAX ion exchange solid phase extraction

Analytical System

Detector:	UV at 236 nm
Column Type:	C18 bonded silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	acetonitrile/water (1:1, v/v) 50 mM NH ₄ H ₂ PO ₄ at pH 5.00.

Validation Results: artelinic acid

Quantitation Limit:	4.96 ng/ml
Interday CV:	19.8%
Interday Error:	8.47%
Standard curve range:	4.96-1270 ng/ml
Interday Precision	
Concentration Range:	9.96-797 ng/ml
CV Range:	2.62-8.97%
Intraday Precision	
Concentration Range:	9.96-797 ng/ml
CV Range:	1.76-7.81%
Blind Sample Assay	
Concentration Range:	23.54-401.4 ng/ml
Bias Range:	-13.1 to +0.52%
Mean Recovery:	85.3%
Stable Plasma Storage:	-20°C for 6 months

Study Description

Malaria has continued to be a major health problem in many regions of the world for two reasons. One is that the *Anopheles* mosquito has developed resistance towards a number of insecticides widely used for vector control. But, the more alarming reason is that the malarial parasite has developed resistance to chloroquine and mefloquine. This phenomenon is

rapidly increasing in both degree and prevalence throughout the world.^{42,43} As a result, the search for more effective antimalarial agents with differing mechanisms of action is more important than ever before. There is considerable interest in developing natural products as antimalarials, and one of the prime candidates to be examined is qinghaosu (QHS), also known as artemisinin (I, Fig. 1). This compound was isolated from the plant *Artemisia annua* L. and characterized by the Chinese in 1972.^{44,45,46} The herb itself has been known to Chinese medicine since 160 BC.⁴⁶ Qinghaosu, which has been used effectively for the treatment of malaria, is a sesquiterpene lactone with an endo-peroxide bridge. A number of derivatives of the parent compound have been prepared, among which the methyl ether of reduced QHS, called artemether (II, Fig. 1), dihydroqinghaosu (DQHS) (III, Fig. 1) and the succinate hemi-ester of DQHS, artesunic acid (IV, Fig. 1) and its sodium salt are currently used in China for the treatment of multi-drug resistant malaria patients with excellent results.⁴⁷ The Steering Committee of the Scientific Working Group on Malaria Chemotherapy of the World Health Organization in Geneva, Switzerland (SWG-CHEMAL), selected the ethyl ether of DQHS, β -arteether (V, Fig. 1), for clinical investigation.⁴⁸

Qinghaosu and its derivatives were found to be thermally labile^{49,50,51} and lack UV chromophores or functional groups for derivative formation. Sensitive and selective analytical methods are essential for studying metabolic and pharmacological aspects of drugs and development of such methods for these new drugs has been challenging chemists for some time. Among the several methods developed for the estimation of qinghaosu and its derivatives mentioned previously are included a radioactivity measure of tritium labelled drug following TLC separation, a GC-MS method, HPLC with UV detection after derivatization or degradation and HPLC separation with direct reductive electrochemical detection. Two different modes of HPLC determination of these drugs in biological fluids have been attempted; UV detection of a derivative or pre-column acid or base-catalyzed decomposition product which is UV absorbing after HPLC separation,^{52,53,54,55} and reductive electrochemical detection using gold/mercury amalgam or glassy carbon electrodes following HPLC separation.^{48,56} The first mode lacks specificity in that metabolites of the drugs are also liable to be converted to identical UV absorbing products. Furthermore, the derivative formation can take place only with compounds having a suitable functional group. For example, DQHS with an OH group at position 12 can react with diacetyl dihydro fluorescein to form an intensely UV absorbing derivative.⁵⁷ This method can detect DQHS at concentrations as low as 18 ng/ml, with good reproducibility, and the limit of detection is given as 0.1 ng of DQHS at 235 nm UV wavelength. The chemical structure of β -arteether contains no such functional group, thus eliminating derivatization as a method for increasing UV detectability. It is reported that base catalyzed degradation is effective only for QHS and artesunic acid but not for β -arteether. The acid catalyzed

decomposition of β -arteether followed by UV detection has been reported and validation of the assay down to 60 ng/ml of plasma was provided. Bearing in mind the previously mentioned shortcomings of pre-column degradative methods, the reported assay method for β -arteether cannot be considered completely satisfactory. The reductive electrochemical detection following HPLC separation (LC-EC) is the only highly sensitive (5 ng/ml) method available presently for the determination of β -arteether in plasma. Since the detector specifically reduces the peroxide bridge in the compound, interference from many other compounds extracted from plasma is eliminated. Thus, the technique is both sensitive and selective for these drugs. However, the reductive electrochemical detector has several practical difficulties inherent to it which makes its use as a routine analytical method difficult. Free oxygen is easily reduced at the detector electrode and this gives rise to a large signal. Accordingly, the following precautions have to be rigorously adhered to in order to obtain the expected results: 1. the complete HPLC and detector system need purging with inert gas to eliminate aerial oxygen; 2. the mobile phase has to be continuously purged to keep the system oxygen free; and 3. the samples have to be injected through an oxygen free system, such as a specially designed autosampler which automatically deoxygenates and injects samples.

It was felt that a method for the estimation of β -arteether in plasma, which was less demanding in instrumental facilities than reductive electrochemical detection following HPLC would be of value for routine laboratory analysis of large numbers of samples. Attempts to develop such an assay here are described in the discussion. Studies on three types of possible detection systems following HPLC separation are reported: i. oxidative electrochemical detection; ii. UV detection both following post column photochemical activation; and iii. a chemical derivatization followed by UV detection, since the two previous methods were not successful. An analytical procedure using the third detection system was not capable of quantitating β -arteether with a minimum quantitation limit greater than currently available methods.

In view of the outcome of these studies on the detection of β -arteether in plasma, project emphasis for this laboratory was shifted towards development of a methodology for quantitation of artelinic acid (VI, Fig. 1), which is synthetically derived from quinghaosu, has a water soluble sodium salt, is stable in potassium carbonate solution, has demonstrated antimalarial activity against *Plasmodium berghei* in mice, and is considered a candidate drug for cerebral malarial treatment.⁵⁸ An HPLC assay for artelinic acid in plasma with an estimated 50 ng/ml detection limit was reported by Idowu, et al.⁵⁹ We present an approach to the HPLC analysis of a plasma sample for determination of the concentration of artelinic acid that employs a solid phase extraction step with a trimethyl aminopropyl or an aminopropyl

bonded elution cartridge. Chromatographic separation was accomplished with an ODS column run with an acetonitrile/50 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (1:1, v/v), pH adjusted to 5.00 with H_3PO_4 mobile phase. UV absorbance was measured at 236 nm. The system produced chromatograms with clean baselines and high signal to noise ratios at low concentrations. The minimum quantitation limit of the method for plasma samples is approximately 5 ng/ml.

The assay requires that all plasma samples must be stored at a temperature no higher than -20°C .

Plasma samples for analysis are pipetted (1 ml) into culture tubes and vortexed. A constant amount, approximately 160 ng, of the internal standard, meclofenamic acid, was added. The samples are vortexed, precipitated with 2 ml acetonitrile, then evaporated to 200 μl and combined with 1 ml water. The aqueous solution is loaded onto a 500 mg SAX (or NH_2) elution cartridge (prepared by sequential washes with 3 ml methanol, 2 ml (6 ml for NH_2 cartridge) 0.1 M hydrochloric acid, 3 ml water and 6 ml 0.1 M monobasic ammonium phosphate). The cartridges are then washed with 3 ml water, 3 ml acetonitrile (completely vacuum dry NH_2 cartridge), and 0.5 ml 0.5 M formic acid (wash NH_2 cartridge twice with formic acid and vacuum dry) in acetonitrile. The compounds are eluted from the cartridges with 2 ml 0.5 M formic acid in acetonitrile (use three 0.5 ml aliquots of formic acid for the NH_2 cartridge). The eluents are evaporated to dryness under N_2 and reconstituted in 200 μl of 50% acetonitrile. Then, 60 μl (30 μl for the NH_2 cartridge) is injected onto the HPLC column.

All validation tests were performed with the NH_2 cartridge elution sample clean up procedure. For comparison, the SAX cartridge elution sample clean up procedure was used to repeat the precision and recovery tests.

An ODS column and a mobile phase composition of acetonitrile/50 mM monobasic ammonium phosphate (1:1, v/v) with pH adjusted to 5.00 with phosphoric acid were used to separate artelinic acid from the internal standard and interfering endogenous substances in an isocratic elution.

The minimum quantitation limit (MQL), approximately 5 ng/ml, was determined as the artelinic acid concentration at which the signal to noise ratio was at least 3 to 1. Table 2 compares the NH_2 and SAX method MQL's, at the lowest and second to the lowest points of the standard curve.

Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level drug concentrations, two standard curves were constructed from the same set of standard curve data points. Data points from 0 to 79.3 ng/ml were used to construct a low artelinic acid concentration standard curve with which to calculate the low concentration samples. All data points (0 to 1270 ng/ml) were used to construct a high

artelinic acid concentration standard curve for the high concentration samples. Concentrations of samples with peak height ratios above that calculated at 79.3 ng/ml from the low concentration standard curve were calculated with the high concentration standard curve.

Typical SAX method chromatograms for blank plasma samples and plasma samples spiked with artelinic acid and the internal standard, meclofenamic acid show early elution of most endogenous interferences and baseline separation of the two compounds, with retention times of artelinic acid at approximately 19 minutes and the internal standard at approximately 16 minutes.

For the representative SAX method standard curve, the coefficients of determination for artelinic acid in plasma were 0.9989 and 0.9994 for low and high concentration ranges, respectively. Percent errors for the interday mean calculated concentration of replicate analyses of samples spiked at the lowest point of the standard curve (the minimum quantitation limit) were 7.45% for the NH2 method (n=5) and 8.47% for the SAX method (n=4).

Meclofenamic acid was found to be an acceptable internal standard since amounts used were below quenching levels, the retention time was sufficiently later than early eluting interferences, and the retention time was sufficiently close to that of artelinic acid to limit error caused by changes in peak shape that arise as columns age with use.

We tested coumarin derivatization with an earlier sample preparation procedure⁶⁰ to enable fluorescence detection. Since many peaks were detected, coumarin is postulated to react with hydroxy, amino, and/or carboxylic moieties. A gradient elution program was required to separate late peaks from artelinic acid (Figure 3). Ibuprofen was also derivatized and used as the internal standard, but produced a double peak (not shown). The detection limit was estimated to be comparable with that of the UV method.

WRAIR has GS-MS and HPLC-EC reductive mode methods available to perform routine analysis of plasma samples for determination of the β -arteether concentrations. Our initial investigations did not reveal promising avenues for an improved β -arteether method. Thus, our efforts towards developing improved methods for quantitation of qinghaosu derivatives were shifted by WRAIR from continued study of a β -arteether method to a search for an improved artelinic acid method.

Since the fluorescence method required derivatization and gradient elution to achieve about the same sensitivity as the UV method, it was judged a currently unacceptable method to proceed with validation. A dicarboxylic acid derivative is being considered as an internal standard for future fluorescence detection experiments.

We observed and evaluated a lot dependent inconsistency with NH_2 , 500 μg ion-exchange cartridges during our validation of stability for this methodology. A quaternary aminopropyl elution cartridge (SAX ion exchange) was tested that eliminated the lot dependent inconsistency and that was shown to have comparable precision and improved recovery relative to the NH_2 ion-exchange cartridge. The recovery, precision and blind sample assays were, and any impending studies should be, performed with the SAX cartridge sample clean up procedure.

By use of a solid phase extraction step for sample clean-up, an ODS column combined with an acetonitrile/ 50 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (1:1, v/v), pH adjusted to 5.00 with H_3PO_4 mobile phase for separation, and the superior capability of ultraviolet detection, artelinic acid can be quantitatively and reliably measured in biological samples. The method is improved over our earlier method by a 4 fold increase in sensitivity and elimination of the use of silanized glassware.⁶⁰ The assay described in this report requires 1 ml plasma samples to determine the concentrations of artelinic acid. The method involves sample cleanup with quaternary amine solid phase extraction cartridge columns, separation on an ODS column run with an aqueous mobile phase, and ultraviolet detection. The minimum artelinic acid quantitation limit for the assay of plasma is approximately 5 ng/ml with a signal to noise ratio of 3 to 1. Metabolites of artelinic acid should derive from liberation of benzoic acid and should retain chromophores which may induce chromatographic interferences, but would probably be sufficiently polar to elute sufficiently early to be non-interfering in the assay. Mean recoveries over the working range of the methodology ranged from 80.1% to 88.5% for SAX cartridges. The SAX method coefficients of variation (CVs) of the inter- and intraday assay precision analysis for the concentrations of artelinic acid ranged from 1.76 to 7.81% for intraday precision and 2.62 to 8.97% for interday precision, respectively, in plasma. Blind sample assay percent bias ranged -13.1 to +0.523% when SAX cartridge elution is used. Drug stability in human plasma was demonstrated for storage up to 6 months at -20°C and for repeated sample analysis.

Validation Study Report 20C, dated July 27, 1994 and titled "Quantitation of Artelinic Acid in Human Plasma by High Performance Liquid Chromatography with a C18 Bonded Column," was submitted as final with the COR's approval, dated May 13, 1994, and appended to quarterly report 10. Complete validation results are presented in Appendix A

p-Aminoheptanophenone and Metabolites in Dog Plasma and Rat Plasma

Study Characteristics: Study Report 21

Test Article:	WR 269,410 (<i>p</i> -aminoheptanophenone)
Test System:	dog plasma
Internal Standard:	WR 258,948 (<i>p</i> -aminooctanophenone)
Sample Assay Volume	0.5 ml
Sample Cleanup:	methyl <i>t</i> -butyl ether extraction

Analytical System

Detector:	UV at 316 nm
Column Type:	C18 bonded silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	acetonitrile/water (1:1, v/v) and 0.15% H ₃ PO ₄

Validation Results:

Quantitation Limit:	4.08ng/ml
Standard curve range:	4.08-816 ng/ml
Blind Sample Assay	see Appendix A

Method validation is underway and when complete, will be reported in Study Report No. 21 (preparation in progress covering *p*-aminoheptanophenone (PAHP, WR 269,410) *p*-aminooctanophenone (PAOP, WR 258,948) *p*-aminopropiophenone (PAPP, WR 302)). Results from the analysis of blind spiked (by WRAIR) dog plasma samples are presented in Appendix A.

WR 6026 and Metabolites in Human Urine

Study Characteristics: Study Report 22

Test Article:	WR 6026 WR 211,789 WR 254,421
Test System:	human urine
Internal Standard:	verapamil
Sample Assay Volume	0.5 ml
Sample Cleanup:	methyl <i>t</i> -butyl ether extraction

Analytical System

Detector:	UV at 350 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5 μ particle size
Mobile Phase:	acetonitrile/0.0075% phosphoric acid (80:20, v/v) at pH 6.9.

Validation Results WR 6026 free base

Quantitation Limit:	5.17 ng/ml
Interday CV:	14.8%
Interday Error:	2.44%
Standard curve range:	2.17-414 ng/ml
Interday Precision	
Concentration Range:	10.4-259 ng/ml
CV Range:	3.90-7.42%
Intraday Precision	
Concentration Range:	10.4-259 ng/ml
CV Range:	3.83-28.4%
Blind Sample Assay	
Concentration Range:	5.20-101.2 ng/ml
Bias Range:	-10.6 to +33.9%
Mean Recovery:	97.2%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Validation Results WR 211,789 free base

Quantitation Limit:	509 ng/ml
Interday CV:	14.7%
Interday Error:	4.55%
Standard curve range:	5.09-407 ng/ml
Interday Precision	
Concentration Range:	10.2-255 ng/ml
CV Range:	4.07-10.0%
Intraday Precision	
Concentration Range:	10.2-255 ng/ml
CV Range:	5.12-23.3%
Blind Sample Assay	
Concentration Range:	5.20-102.6 ng/ml
Bias Range:	-11.8 to +33.9%
Mean Recovery:	92.8%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Validation Results WR 254,421 free base

Quantitation Limit:	45.4 ng/ml
Interday CV:	7.51%
Interday Error:	1.60%
Standard curve range:	45.4-3630 ng/ml
Interday Precision	
Concentration Range:	90.8-2270 ng/ml
CV Range:	3.09-5.86%
Intraday Precision	
Concentration Range:	90.8-2270 ng/ml
CV Range:	3.55-10.0%
Blind Sample Assay	
Concentration Range:	50.1-979.4 ng/ml
Bias Range:	-10.7 to +9.63%
Mean Recovery:	94.2%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Study Description

Study Report No. 22 "Quantitation of WR 6026, WR 211,789 and WR 254,421 (as Free Bases) in Human Urine by High Performance Liquid

Chromatography," was submitted for review July 18, 1994. This method is a modified version of the plasma method.

WR 6026 (dihydrochloride) (6-methoxy-8-(6-diethyl amino hexyl amino) lepidine dihydrochloride) (Figure 1), is a very effective antileishmanial drug in hamsters infected with *Leishmania donovani*.¹⁶ Because antimony compounds are not always effective and the other drugs in use have toxic effects,^{17,18} alternative therapies are needed. Since WR 6026 (dihydrochloride) is a likely candidate and since WR 6026 (dihydro-chloride) is scheduled for clinical testing, it is extremely important to develop an analytical method capable of measuring concentrations of WR 6026 (free base) at nanogram per milliliter concentrations in biological samples. This report describes an assay developed to determine the concentrations (as free bases) of WR 6026 and of its metabolites, WR 211,789 (6-methoxy-8-(6-ethyl-aminohexylamino) lepidine dihydrochloride, hemihydrate) and WR 254,421 (8-(6'-N,N-diethylaminohexyl)amino-4-hydroxymethyl-6-methoxyquinoline, dihydrochloride) in urine. WR 211,789 has been detected in a rat microsomal preparation.²¹ This assay adds the capability of detection of WR 6026, WR 211,789 and WR 254,421 (as free bases) in urine to earlier assays for WR 6026 and WR 211,789 in plasma and blood.

Assay samples were prepared by spiking known volumes of human urine with a known amount (constant over all samples) of the verapamil internal standard (IS). Standard curve samples were generated by spiking interference free human urine samples with known amounts of WR 6026, WR 211,789 and WR 254,421 (as free bases) and IS. These standard curve and assay samples were extracted, then injected onto an HPLC column for separation and subsequent ultraviolet detection. The peak height ratios of WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, standard curve concentrations and WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS peak height ratios of the standard curve samples were fit by least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = WR 6026, WR 211,789 or WR 254,421 (as free bases) concentrations), and drug concentrations in assay samples were calculated by this equation from the WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS peak height ratios obtained by HPLC.

Sample volume taken for analysis was 0.5 ml of urine. A constant amount, approximately 5 μ g, of the internal standard, verapamil, was added to and mixed with each sample. Next, 100 μ l of 1 N NaOH was added to and mixed with each sample. Then, samples were extracted with 5 ml of methyl *t*-butyl ether. The extraction solution was transferred to a second culture tube, evaporated to dryness under nitrogen, and reconstituted in 200 μ l of mobile phase. Finally 20-160 μ l of the sample was injected onto the HPLC column.

In typical chromatograms for blank urine and urine samples spiked with WR 6026, WR 211,789 or WR 254,421, WR 6026, WR 211,789 or WR 254,421 eluted at 15.3, 14.3, and 18.2 minutes, respectively, and the internal standard eluted at 12.4 minutes. The coefficients of determination for WR 6026, WR 211,789 or WR 254,421 interday and intraday precision standard curves were 0.9825 or higher.

The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. The interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug.

HPLC assays for basic amine drugs in biological samples that make use of a silica gel column and an aqueous mobile phase have been operated in this laboratory for over 5 years. By use of an organic solvent extraction step for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of ultraviolet detection, the free base concentrations of WR 6026, WR 211,789 and WR 254,421 can be quantitatively and reliably measured in human urine samples. The assay described in this report requires 0.5 ml urine samples to determine the free base concentrations of WR 6026, WR 211,789 or WR 254,421. The method involves sample cleanup with a methyl *t*-butyl ether extraction, separation on an unbonded silica gel column (5 μ m particle size) run with an aqueous mobile phase, and ultraviolet detection. The minimum quantitation limits of the assay are 5.17, 5.09, and 45.4 ng/ml for WR 6026, WR 211,789 and WR 254,421 free base, respectively, with a signal to noise ratio of 3 to 1. Average mean recoveries over the working range of the standard curve were 97.2, 92.8, and 94.2 percent for WR 6026, WR 211,789 and WR 254,421 free base, respectively. The respective percent coefficients of variation (CVs) of the inter- and intraday assay precision analysis for the free base concentrations of WR 6026 ranged from 3.90% to 7.42% and 3.83% to 28.4%; of WR 211,789 ranged from 4.07% to 10.0% and 5.12% to 23.3%; and of WR 254,421 ranged from 3.09% to 5.86% and 3.55% to 10.0%. No discernable pattern of degradation was observed in long term or autosampler stability tests.

Primaquine and Metabolite in Human Plasma

Study Characteristics: Study Report 23

Test Article: primaquine phosphate (WR 002,975),
carboxy-primaquine (WR 249,725)

Test System: human plasma

Internal Standard: mebendazole

Sample Assay Volume 0.5 ml

Sample Cleanup: ether acetate extraction

Analytical System

Detector: UV at 280 nm

Column Type: C18 bonded silica

Column Size: 4.6x250 mm, 5 μ particle size

Mobile Phase: methanol/acetonitrile/water/phos-
phoric acid/TMACl (33%, 18.7%, 48%,
0.13%, 0.2%) with a final pH of 5.05.

Study Description

Method validation is underway and when complete, will be reported in Study Report No. 23 (preparation in progress covering primaquine (free base) and carboxy-primaquine).

Assay samples were prepared by spiking known volumes of human plasma or serum with a known amount (constant over all samples) of mebendazole internal standard (IS). Standard curve samples were generated by spiking interference free human plasma samples with known amounts of primaquine (free base), carboxy-primaquine, and IS. These standard curve and assay samples were extracted, then injected onto an HPLC column for separation and subsequent UV absorbance detection. The peak height ratios of primaquine (free base) and carboxy-primaquine to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and primaquine (free base) or carboxy-primaquine to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = primaquine (free base) or carboxy-primaquine concentration), and drug and metabolite concentrations in assay samples were calculated by these equations from the primaquine (free base) and carboxy-primaquine to IS peak height ratios obtained by HPLC.

Sample Preparation

Plasma or serum samples to be assayed for primaquine (free base) and carboxy-primaquine were thawed and vortexed for 1 min, if frozen. For each sample, 0.5 ml was transferred into a culture tube. Next, 100 μ l of internal standard (mebendazole) solution (1.50 μ g/ml) were added and the sample vortexed. Then, 3 ml of ether acetate extracting solvent was added, the samples were vortexed twice for 30 s and centrifuged for 10 min at 3000 g. Each sample was frozen in dry ice/methanol, then the organic layer was transferred to a clean tube and evaporated to dryness under nitrogen. A second identical extraction was performed with 2 ml of ether acetate. The residue was reconstituted in 100 μ l of acetonitrile/water (2:3, v/v) by vortexing samples for 1 min. Reconstituted samples were centrifuged for 10 min at 3000 g, transferred to a WISP insert, and injected onto the HPLC column.

ROUTINE ASSAY RESULTS

The following section presents short descriptions of specific routine sample assays completed or currently in progress during the first half of the contract. Complete data findings are presented in Appendix B.

TABLE 5: ROUTINE ANALYSES PERFORMED

Report Title	Report Status	Test Article	Test System	No. of Results	Report No.
Routine Analysis for Halofantrine and WR 178,460 (as free bases of Plasma Samples Obtained for the Initial Year of the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats"	3/31/93 in review	halofantrine WR 178,460	rat plasma	118 118	Hal/P 91-4
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Blood Samples Obtained under the Protocol Titled "Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria"	6/23/92 in review	mefloquine	human blood	107	Mef/B 91-5
Results assoc. with Hal/P 91-1	4/28/92 final data report	halofantrine WR 178,460	dog plasma	29 29	Hal/P 91-6
Routine Analysis for Mefloquine (as Free Base) in Plasma Samples Obtained under the Protocol Titled "Evaluation of the Tolerance of Prophylactic Mefloquine Regimens"	3/1/93 final report	mefloquine	human plasma	660	Mef/P 91-7
Study continued as WR6/PU 93-1	8/3/92 data	WR 6026 WR 211,789	plasma	194 194	WR6/P 92-1
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained for the Second Year of the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats, HWA Study No. 193-558"	3/31/93 report in review	halofantrine WR 178,460	rat plasma	154 154	Hal/P 92-2

**TABLE 5: ROUTINE ANALYSES PERFORMED
(Continued)**

Report Title	Report Status	Test Article	Test System	No. of Results	Report No.
Routine Analysis for WR 238,605 (as free base) of Blood and Plasma Samples Obtained for the Protocol Titled "Rising Single Oral Dose Safety and Tolerance Study of WR 238,605 Succinate"	7/7/94 report in review	WR 238,605	human plasma, blood	893 74	WR5/PB 92-3
Routine Analysis for WR 6026, WR 211,789 and WR 254,421 (as free bases) in Plasma and Urine Samples Obtained under the Protocol Titled "Phase II Clinical Trial of Oral WR 6026 2HCl in Patients with Visceral Leishmaniasis - Initial Dose Ranging for Efficacy, Safety and Tolerance"	3/12/93 data	WR 6026 WR 6026 WR 211,789 WR 254,421	human plasma, urine	117 68 68 68	WR6/PU 93-1
Routine analysis for Halo-fantrine and WR 178,460 (as free bases) of Plasma Samples Obtained for the Protocol Titled "Pharmacokinetics of a New Multiple Dose Halofantrine Regimen"	7/7/93 final data report in prepara tion	halofantrine WR 178,460		642 642	Hal/P 93-2
No protocol	2/25/94 data	p-aminohep- tanophenone	dog plasma	876	Pah/P 93-3
Routine Analysis for WR 238,605 (as free base) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Dogs"	4/25/94 report in review	WR 238,605	dog plasma	330	WR5/P 93-4
Routine Analysis for WR 238,605 (as free base) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Rats"	1/20/94 revised report in prepara tion	WR 238,605	rat plasma	154	WR5/P 93-5

**TABLE 5: ROUTINE ANALYSES PERFORMED
(Continued)**

Report Title	Report Status	Test Article	Test System	No. of Results	Report No.
Routine Analysis for Primaquine and Carboxyprimaquine of Serum Samples Obtained for the Protocol Titled "Primaquine and Several Recommended Prophylactic Drugs against Falciparum Malaria: Field Trial II"	7/6/94 preliminary results	primaquine carboxy metab	human serum	60	Pri/P 93-6
Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Rat Liver, Bile and Perfusate Samples	data in preparation	halofantrine	rat liver perfusate bile		Hal/lpb 93-7
Routine Analysis for WR 238,605 (as free base) Human Plasma and Blood Samples Obtained for the Protocol Titled "Pharmacokinetics, Pharmacodynamics, Safety and Tolerance of a Single Oral Dose of WR 238605 Succinate"	data in preparation	WR 238,605	human plasma blood	120 120	WR5/PB 93-8
Routine Analysis for <i>p</i> -Aminoheptanophenone of Dog Plasma Samples Obtained for the Protocol Titled " <i>p</i> -Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs"	assay in progress	<i>p</i> -aminoheptanophenone	dog plasma	189	Pah/P 93-9
Routine Analysis for WR 238,605 (as free base) Monkey Plasma Samples	samples to arrive	WR 238,605	monkey plasma	12	WR5/P 94-1
Routine Analysis for <i>p</i> -Aminoheptanophenone Rat Plasma Samples Obtained for the Protocol Titled " <i>p</i> -Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats"	assay in progress	<i>p</i> -aminoheptanophenone	rat plasma	152	Pah/P 94-2

p-Aminoheptanophenone (PAHP, WR 269,410) *p*-Aminooctanophenone (PAOP, WR 258,948) *p*-Aminopropiophenone (PAPP, WR 302)

Pah/P 93-3

Results will be reported in Analysis Report No. 93-3. Status of samples received is described in the table below. Report completion requires completion of method validation.

No. of Samples	Description	Date Received	Status
106	dog plasma	3/3/93	Results Faxed to COR 9/23/93
52	dog blood	3/3/93	Not to be assayed
645	dog plasma	9/21/93	Results Faxed to COR 2/25/94
36	blind spiked dog plasma	9/30/93 11/2/93	Results Faxed to COR 1/25/94
125	dog plasma	10/21/93	Results Faxed to COR 2/25/94

Pah/P 93-9

Samples (189 dog plasma) were received July 12, 1994 to be analyzed in accordance with the protocol titled "*p*-Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs." Report completion requires completion of method validation.

Pah/P 94-2

Samples (152 rat plasma) were received July 12, 1994 to be analyzed in accordance with the protocol titled "*p*-Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats." Report completion requires completion of method validation.

HALOFANTRINE

Hal/P 91-4

Analysis Report Nos. Hal/P 92-2 initially titled "Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Plasma Samples Obtained under the Protocol Titled 'Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats, HWA Study No. 193-558'" and Hal/P 91-4 from the same protocol were completed and revised Analysis Report Nos. Hal/P 92-2 and Hal/P 91-4 were submitted to the

COR for review March 31, 1993, and with approval will be appended to the then current quarterly report as final reports.

Analysis of 118 rat plasma samples for determination of the free base concentrations of halofantrine (WR 171,669) and of its metabolite (WR 178,460) was accomplished by use of an HPLC method described in Study Report No. 17, dated April 25, 1990 and developed under contract DAMD17-86-C-6150. The samples were obtained from Hazleton Laboratories America, Inc., in accordance with the protocol titled "Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats." Analytical results are presented in this report for plasma samples from the initial year of a 2 year study of rats given daily oral gavage doses of halofantrine hydrochloride and were obtained from analyses performed from May 29 through June 20, 1991.

Hal/P 91-6

Results associated with Hal/P 91-1. No further action planned for this routine assay.

Hal/P 92-2

Analysis Report Nos. Hal/P 92-2 initially titled "Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Plasma Samples Obtained under the Protocol Titled 'Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats, HWA Study No. 193-558'" and Hal/P 91-4 from the same protocol were completed and revised Analysis Report Nos. Hal/P 92-2 and Hal/P 91-4 were submitted to the COR for review March 31, 1993, and with approval will be appended to the then current quarterly report as final reports.

Hal/P 93-2

Final results of the routine analysis of 339 human plasma samples received March 3, 1993 and 303 human plasma samples received April 14, 1993 for halofantrine and WR 178,460 (free base) concentrations for samples obtained under the protocol "Pharmacokinetics of a New Multiple Dose Halofantrine Regimen" faxed to the COR July 7, 1993 and enclosed in Quarterly Report No. 6 will be reported. Analysis Report Hal/P 93-2 (now in preparation). Report completion requires completion of method validation.

Analysis of 642 human plasma samples for determination of the free base concentrations of halofantrine (WR 171,669) and of its metabolite (WR 178,460) was accomplished by use of an HPLC method described in Study Report No. 17, developed under contract DAMD17-86-C-6150. The samples were obtained from the South Florida Drug Research Corporation, Inc., in accordance with the protocol titled "Pharmacokinetics of a New Multiple

Dose Halofantrine Regimen." Analytical results are presented in this report for plasma samples from human male subjects from analyses performed from April 30 through June 8, 1993.

Hal/lpb 93-7

Sample Receipt

No. of Samples	Description	Date Received
115	15 liver; 23 bile; 77 perfusate	1/26/94
305	14 liver; 91 bile; 200 perfusate	1/27/94
285	15 liver; 90 bile; 180 perfusate	2/16/94

Hal/lpb 93-7 Assay Status

Study Method	Sample Type	No. of Samples	Status
precipitation	liver	44	assay done, data in preparation
precipitation	bile	204	final results faxed 5/25/94
extraction	bile	204	assay in progress
precipitation	perfusate	457	preliminary results faxed 4/29/94
extraction	perfusate	457	assay done, data in preparation

Report completion requires completion of method validation.

MEFLOQUINE AND WR 160,972 (MEFLOQUINE METABOLITE) IN BLOOD AND PLASMA

Mef/91-5

Analysis Report Mef/91-5, titled "Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Blood Samples Obtained under the Protocol Titled 'Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria,'" described the routine analysis of 107 human blood samples for the determination of the mefloquine free base concentrations, and was submitted to the COR on June 23, 1992 for review, and upon her approval will be appended to the then current quarterly report.

Analysis of 107 human blood samples for determination of the free base concentration of mefloquine (WR 171,669) was accomplished by use of an HPLC method described in Study Report No. 19, dated Jan 14, 1992 and developed under contract DAMD17-86-C-6150. The samples were obtained from the Armed Forces Research Institute of Medical Sciences and the Royal Thai Army, in accordance with the protocol titled "Efficacy of Halofantrine

and Mefloquine in the Treatment of Falciparum Malaria." Analytical results are presented in this report for blood samples from humans given halofantrine (1500 mg) and/or mefloquine (750 mg) and were obtained from analyses performed April 17-21, 1992.

Mef/91-7

Analysis of 660 human plasma samples for determination of the free base concentration of mefloquine hydrochloride (WR 142,490), was accomplished by use of the HPLC method described in Study Report No. 14B dated August 29, 1989 under contract DAMD17-86-C-6150. The samples were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, in accordance with the protocol titled "Evaluation of the Tolerance of Prophylactic Mefloquine Regimens." Analytical results are presented in this report for plasma samples from humans given mefloquine hydrochloride and were obtained from analyses performed January 27-April 13, 1992.

Primaquine and its carboxy metabolite

Pri/P 93-6

Samples (60 human serum) were received August 18, 1993 for analysis under the protocol (received July 29, 1993) titled "Primaquine and Several Recommended Prophylactic Drugs against Falciparum Malaria: Field Trial II." Preliminary results were faxed to the COR on June 6, 1994.

Analysis of 60 human serum samples for determination of the concentrations of primaquine (WR 002,975AW) free base and carboxy-primaquine (WR 249,725) was accomplished by use of an HPLC method described in Study Report No. 23, in preparation and being developed under contract DAMD17-92-C-2028. The samples were obtained from the Walter Reed Army Institute of Research in accordance with the protocol titled "Primaquine and Several Recommended Prophylactic Drugs against Falciparum Malaria: Field Trial II." Analytical results are to be presented for serum samples from male and female children from analyses performed from April 4-15, 1994.

WR 238,605

WR5/P 92-3

Analysis Report WR5/BP 92-3 was submitted for review on July 7, 1994. Upon acceptance by the COR, the report will be appended to the then current quarterly report as a final report.

Analysis of 893 human plasma and 74 human blood samples for determination of the WR 238,605 (free base) (the salt is 8-[(4-amino-1-

methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy)quinoline succinate) concentration was accomplished by use of an HPLC method described in Study Report No. 13, dated March 29, 1989 and developed under contract DAMD17-86-C-6150. The samples were obtained from the South Florida Drug Research Corporation according to the protocol titled "Rising, Single Oral Dose Safety and Tolerance Study of WR 238605 Succinate." The analytical results for plasma and blood samples from human subjects given oral doses of WR 238,605 (succinate) or placebo were obtained from analyses performed between 12/12/92 and 4/28/94 and are presented in this report.

WR5/P 93-4

Results of the analysis of 330 dog plasma samples received on 7/14/93 were reported in Routine Analysis Report WR5/P 93-4, which was submitted for review on April 25, 1994. The protocol for this study is titled "Thirteen week oral toxicity study of WR 238605 with a thirteen week recovery period in dogs." Upon acceptance by the COR, these reports will be appended to the then current quarterly report as final reports.

Analysis of 320 dog plasma samples for determination of the free base concentration of WR 238,605 was accomplished by use of an HPLC method described in Study Report No. 13 Dog Plasma Assay Supplement, dated April 11, 1994 and developed under contract DAMD17-92-C-2028. The samples were obtained from the University of Illinois at Chicago, in accordance with the protocol titled "Thirteen Week Oral Toxicity Study of WR 238605 with a Thirteen Week Recovery Period in Dogs." Analytical results are presented in this report for plasma samples from male and female dogs from analyses performed from September 30, 1993 through January 11, 1994.

WR5/P 93-5

Routine analysis of 154 rat plasma samples received on 7/14/93 was completed (final data was faxed to COR on December 29, 1993 and enclosed with Quarterly Report 8). Analysis Report WR5/P 93-5 titled "Routine Analysis for WR 238,605 (as free base) of Plasma Samples Obtained for the Protocol Titled 'Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Rats'" was completed January 20, 1994, and submitted to the COR for review. Upon approval by the COR, Analysis Report WR5/P 93-5 will be appended to the then current quarterly report. In response to review comments for this report described in a letter dated April 1, 1994 from the COR, a revised study report was submitted for review on May 2, 1994. Upon acceptance by the COR, these reports will be appended to the then current quarterly report as final reports.

Analysis of 154 rat plasma samples for determination of the free base concentration of WR 238,605 was accomplished by use of an HPLC method

described in Study Report No. 13 Rat Plasma Assay Supplement, dated January 20, 1994 and developed under contract DAMD17-92-C-2028. The samples were obtained from the University of Illinois at Chicago, in accordance with the protocol titled "Thirteen Week Oral Toxicity Study of WR 238605 with a Thirteen Week Recovery Period in Rats." Analytical results are presented in this report for plasma samples from male and female rats from analyses performed from October 12 through 21, 1993.

WR5/P 93-8

Routine analysis of 359 human plasma and 359 human blood samples was completed for Analysis Report WR5/BP 93-8 for samples received in accordance with the protocol titled "Pharmacokinetics Pharmacodynamics, Safety and Tolerance of a Single Oral Dose of WR 238605 Succinate." Current status of recent samples received are summarized below.

No. of Samples	Description	Date Received	Status
120 plasma 120 blood	subjects 1-6	3/15/94	data in preparation
119 plasma 119 blood	subjects 7-12	3/22/94	data in preparation
120 plasma 120 blood	subjects 13-18	4/19/94	data in preparation

WR5/P 94-1

Receipt of samples for study WR5/P 94-1 (12 monkey plasma) is anticipated. The analysis is set to proceed with use of blank human plasma for standard curve and control samples and blank monkey plasma as duplicate controls.

WR 6026, WR 211,789 and WR 254,421 in Plasma, Blood and Urine

WR6/PU 93-1

Routine Analysis Report WR6/PU 93-01, titled "Routine Analysis for WR 6026 and WR 211,789 in Plasma and WR 6026, WR 211,789 and WR 254,421 in Urine of Samples Obtained under the Protocol Titled 'Phase II Clinical Trial of Oral WR 6026 2 HCl in Patients with Visceral Leishmaniasis - Initial Dose Ranging for Efficacy, Safety and Tolerance,'" will cover results from WR6/P 92-01 and WR6/PU 93-01.

No. of Samples	Description	Date Received	Status
64	plasma (WR6/P 92-01)	3/31/92	Faxed to COR 6/3/92
130	plasma (WR6/P 92-01)	6/3/92	Faxed to COR 6/24/92
117	plasma (WR6/P 93-01)	2/11/93	Data in preparation
68	urine (WR6/P 93-01)	2/11/93	Faxed to COR 3/12/93

CONCLUSIONS

Work on development and/or validation of analytical methodologies during the first half of the contract focused on assays for WR 238,605, halofantrine (and its metabolite), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), and primaquine (and its metabolite). Work on routine analyses of biological specimens during this period was performed for studies that required determination of concentrations of WR 238,605, halofantrine (and its metabolite), WR 6026 (and its metabolites), mefloquine (and its metabolite), *p*-aminoheptanophenone (and related compounds), and primaquine (and its metabolite). Planning for future work follows.

Planning for analysis of blood and plasma for WR 238,605 (free base): Planned work includes release of final data and a draft report for study WR5/BP 93-8. Receipt of samples for study WR5/P 94-1 (12 monkey plasma) is anticipated.

Results of assay of blood samples spiked by WRAIR (for determination of halofantrine and WR 178,460 (as free bases) in blood) as well as validation results with internal standard WR 122,455 will be incorporated into Study Report 17.

Completion of Analysis Report No. Hal/P 93-2 on routine assay of samples for determination of halofantrine and WR 178,460 (as free bases) in plasma is planned.

Planning for mefloquine and WR 160,972 method development: Work on including an assay for WR 160,972 has been assigned a low priority by the COR. Planned work involves testing extraction solvent combinations and other sample clean-up methods for Study Report 19.

Planning for WR 6026 and WR 211,789 method development and routine assay: Work on development of the plasma and blood assay methodology on this project has been assigned a low priority by the COR. Planned work has been postponed on completion of validation procedures, as described in Study Report 18A (Status Report) and this quarterly report except for completion of analysis of blind plasma and blood samples. Planned work includes completion of stability studies. Completion of a draft report on routine analysis study WR6/PU 93-1 is planned.

Planning for PAHP, PAOP, and PAPP method development and routine assay: Method development requires validation for rat plasma and completion of the study report. Planned work includes completion of Analysis Report 93-3 and analysis of samples for studies 93-9 (189 dog plasmas) and 94-2 (152 rat plasmas).

Planning for primaquine and des-amino-carboxy-primaquine: Planned work involves completion of the validation report, release of final data and a draft of Analysis Report 93-6.

REFERENCES

- ¹ Anticholinesterase Agents. Palmer Taylor in *"The Pharmacological Basis of Therapeutics"* (Editors) A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad. Seventh Edition, MacMillan Publishing Co. Chapter 6, pp. 118-129 (1985).
- ² Ion-Paired Liquid Chromatographic Method for the Analysis of Blood and Plasma for the Antimalarial Drug Halofantrine and its Putative Mono-debutylated Metabolite. M. Gawienowski, L.Z. Benet, L. Fleckenstein and E.T. Lin. *J. Liq. Chromatogr.* **30**, 412-419 (1988).
- ³ Analysis of the Antileishmanial Compound WR 6026 by High Performance Liquid Chromatography. C.M. Chen, W.L. Gee, L.Z. Benet, J. von Bredow and L. Fleckenstein. *APhA Academy of Pharmaceutical Science* **14**, 216 (1984).
- ⁴ Analysis of Artesunic Acid and Dihydroqinghaosu in Blood by High-Performance Liquid Chromatography with Reductive Electrochemical Detection. Z.M. Zhou, J.C. Anders, H. Chung and A.D. Theoharides. *J. Chromatogr.* **414**, 77-90 (1987).
- ⁵ Separation of Isomeric Insect Pheromonal Compounds Using Reversed Phase HPLC with AgNO₃ in the Mobil Phase. P.L. Phelan and J.R. Miller. *J. Chromatogr. Sci.* **19**, 13-17 (1981).
- ⁶ *Organic Functional Group Analysis by Micro and Semimicro Methods*. N.D. Cheronis and T.S. Ma. Interscience, New York (1964).
- ⁷ *Protective Groups in Organic Chemistry*. J.F.W. McOmie. Plenum, New York (1973).
- ⁸ Ion-Paired Liquid Chromatographic Method for the Analysis of Pyridostigmine in Plasma. O. Yturralde, R-Y. Lee, L.Z. Benet, L. Fleckenstein, and E.T. Lin. *J. Liq. Chromatogr.* **10**, 2231-2246 (1987).
- ⁹ J.M. Karle and R. Olmeda, "Rapid and Sensitive Quantitative Analysis of the New Antimalarial N⁴-[2,6-Dimethoxy-4-Methyl-5-[(3-Trifluoromethyl)phenoxy]-8-Quinoliny]-1,4-Pentanediamine in Plasma by Liquid Chromatography and Electrochemical Detection" *J. Chromatogr.*, 424(1988) 347.
- ¹⁰ I.M. Rollo in *The Pharmacological Basis of Therapeutics*, Goodman and Gilman (Editors), MacMillan Publishing, New York, 6th Edition, 1980, pp. 1038-1060.
- ¹¹ L.T. Webster in *The Pharmacological Basis of Therapeutics*, Goodman, Goodman and Gilman (Editors), MacMillan Publishing, New York, 7th Edition, 1985, pp. 1029-1048.
- ¹² J. Arnold, A.S. Alving, and C.B. Clayman, "Induced Primaquine Resistance in Vivax Malaria," *Trans. R. Soc. Trop. Med. Hyg.*, 55(1961)345.
- ¹³ Shi RJY, Benet LZ, and Lin ET, High Performance Liquid Chromatographic Assay of Basic Amine Drugs in Plasma and Urine Using a

Silica Gel Column and an Aqueous Mobile Phase. 1. Amiloride. *J. Chromatogr.* 377, 399 (1986).

¹⁴ Shi RJY, Gee WL, Williams RL, and Lin ET, High Performance Liquid Chromatographic Assay of Metoclopramide in Plasma and Urine Using a Silica Gel Column and an Aqueous Mobile Phase. *Anal. Lett.*, 20, 131 (1987).

¹⁵ Shi RJY, Gee WL, Williams RL, and Lin ET, HPLC Assay for Basic Amine Drugs in Plasma and Urine Using a Silica Gel Column and an Aqueous Mobile Phase, II. Chlorpheniramine. *J. Liq. Chromatogr.* 10, 3101 (1987).

¹⁶ Kinnamon, K.E.; Steck, E.A.; Loizeaux, P.S.; Hanson, W.L.; Chapman, W.L.; and Waits, V.B. *Amer. J. Trop. Med. Hyg.*, 27(4):751 (1978).

¹⁷ Locksley, R.M. and Plorde, J.J.: "Leishmaniasis," in Harrison's Principles of Internal Medicine, Tenth edition. Edited by Petersdorf, R.G.; Adams, R.P.; Braunwald, E.; Isselbacher K.J.; Martin, J.B.; and Wilson, J.D. McGraw-Hill Book Co., New York, pp. 1193-6, 1983.

¹⁸ Physicians' Desk Reference, 40th Edition. Publisher; Edward R. Barnhart, pp. 1073 and 1744-5.

¹⁹ Lin, E.T.; Benet, L.Z.; Upton, R.A.; and Gee, W.L. Annual Report "Development of Analytical Methods for the Determination of Drug Substances in Biological Fluids," US Army Medical Research and Development Command Contract No. DAMD17-83-C-3004, August 26, 1983.

²⁰ Bidlingmeyer, B.A.; Del Rios, J.K.; and Korpi, J. Separation of Organic Amine Compounds on Silica Gel with Reversed-Phase Eluents. *Anal. Chem.* 54:442 (1982).

²¹ Theoharides, A.D.; Chung, H.; and Velazquez, H. Metabolism of a Potential 8-Aminoquinoline Antileishmanial Drug in Rat Liver Microsomes," *Biochem. Pharmacol.* 34:181 (1985).

²² Shi, R.J.Y.; Benet, L.Z.; and Lin, E.T. High Performance Liquid Chromatographic Assay of Basic Amine Drugs in Plasma and Urine Using a Silica Gel Column and an Aqueous Mobile Phase. *J. Chromatogr.* 377:399-404 (1986).

²³ Shi, J.Y.R.; Gee, W.L.; Williams, R.L.; and Lin, E.T. High Performance Liquid Chromatographic Assay of Metoclopramide in Plasma Using a Silica Gel Column and an Aqueous Mobile Phase. *Anal. Lett.* 20:131 (1987).

²⁴ Shi, J.Y.R.; Gee, W.L.; Williams, R.L.; and Lin, E.T. High Performance Liquid Chromatographic Assay for Basic Amine Drugs in Plasma and Urine Using a Silica Gel Column and an Aqueous Mobile Phase. II. Chlorpheniramine. *J. Liq. Chromatogr.* 10(4), 3101-3112 (1987).

²⁵ C.J. Canfield and R. S. Rozman, *Bull. WHO*, 50 (1974) 203.

²⁶ G.M. Trenholme, R. L. Williams, R. Z. Desjardins, H. Frischer, P. E. Carson, K.H. Rieckman, and C. J. Canfield, *Science*, 190 (1975) 792.

²⁷ K.H. Rieckman, G.M. Trenholme, R.L. Williams, R.E. Carson, H. Frischer, and R.Z. Desjardins, *Bull. WHO*, 51 (1974) 375.

²⁸ D.F. Clyde, V.C. McCarthy, R.M. Miller, and R.B. Hornick, *Antimicrob. Agents. Chemother.*, 9 (1976) 384.

- 29 T. Nakagawa, T. Higuchi, J.L. Haslam, R.D. Shaffer, and W.D. Mendenhall, *J. Pharm. Sci.*, 68 (1979) 718.
- 30 S.M. Mansor, V. Navaratnam, M. Mohamad, S. Hussein, A. Kumar, A. Jamaludin, W.H. Wernsdorfer, Single dose kinetic study of the triple combination mefloquine/sulphadoxine/pyrimethamine (Fansimef®) in healthy male volunteers. *Br. J. Clin. Pharmacol.*, 27(1989)381-6.
- 31 D. Dadgar, J. Climax, R. Lambe, A Darragh. Gas chromatographic determination of mefloquine in human and dog plasma using electron-capture detection. *J. Chromatogr.*, 337(1985)47.
- 32 D.E. Schwartz, in A. Frigerio and M. McCamish (Editors), *Recent Developments in Chromatography and Electrophoresis*, Vol. 10, Elsevier, Amsterdam, 1980, p. 69.
- 33 D.E. Schwartz and U.B. Ranalder, *Biomed. Mass Spectrom.*, 8 (1981) 589.
- 34 J.M. Grindel, P.F. Tilton, and R.D. Shaffer. Quantitation of the antimalarial agent, mefloquine, in blood, plasma, and urine using high-pressure liquid chromatography. *J. Pharm. Sci.*, 66:6 (1977) 834.
- 35 I.M. Kapetanovic, J.D. DiGiovanni, J. Bartosevich, V. Melendez, J. von Bredow and M. Heiffer. Analysis of the antimalarial, mefloquine, in blood and plasma using high-performance liquid chromatography. *J. Chromatogr.* 277 (1983) 209.
- 36 G. Franssen, B. Rouveix, J. Lebras, J. Bauchet, F. Verdier, C. Michon, F. Bricaire. Divided-dose kinetics of mefloquine in man. *Br. J. Clin. Pharmacol.*, 28(1989)179-184.
- 37 J. Karbwang, P. Molunto, K.N. Bangchang, D. Bunnag. Determination of mefloquine in biological fluids using high performance liquid chromatography. *S.E. Asian J. Trop. Med. Pub. Hlth.*, 20:1(1989)55-60.
- 38 M.C. Coleman, L. Fleckenstein, L.A. Shipley, M.H. Heiffer. Disposition of the antimalarial, mefloquine, in the isolated perfused rat liver. *Biochem. Pharmacol.*, 37:2(1988)235-239.
- 39 J.H. Riviere, D.J. Back, A.M. Breckenridge, R.E. Howells. The pharmacokinetics of mefloquine in man: lack of effect of mefloquine on antipyrine metabolism. *Br. J. Clin. Pharmacol.*, 20(1985)469-474.
- 40 Y. Bergqvist, U. Hellgren, F.C. Churchill. High-performance liquid chromatographic assay for the simultaneous monitoring of Mefloquine and its acid metabolite in biological samples using protein precipitation and ion-pair extraction. *J. Chromatogr.*, 432(1988)253-263.
- 41 R. Jauch, E. Griesser, G. Oesterhelt. Metabolismus von Ro 21-5998 (Mefloquin) bei der Ratte. *Arzneim. Forsch./Drug Res.*, 30(1980)60-67.
- 42 Payne D, *Parasitology Today* 1987, 2, 241-246.
- 43 Oduola AM; Milhous WK; Salako LA; Walker O; Desjardins RE. Reduced in-vitro susceptibility to mefloquine in West African isolates of *Plasmodium falciparum*. *Lancet*, 1987 Dec 5, 2(8571):1304-5.
- 44 K'o Hsueh T'ung Pao, 1977, 22, 142; *Chem. Abstr.* 1977, 87, 98788g.
- 45 Klaymen, DL. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* 1985, 228, 1049-1056.

- 46 Acton N, Klayman DL, and Rollman IJ. Reductive electrochemical HPLC assay for artemisinin (qinghaosu). *Planta Med.* 1985, 5, 445-6.
- 47 Brossi A; Venugopalan B; Dominguez Gerpe L; Yeh HJ; Flippen-Anderson JL; Buchs P; Luo XD; Milhous W; Peters W. Arteether, a new antimalarial drug: synthesis and antimalarial properties. *J. Med. Chem.*, 1988, 31(3), 645-50.
- 48 Melendez V, Peggin JO, Brewer TG, Theoharides AD. Determination of the antimalarial arteether and its deethylated metabolite, dihydroartemisinin, in plasma by high-performance liquid chromatography with reductive electrochemical detection. *J. Pharm. Sci.*, 1991, 80(2), 132-8.
- 49 Lin AJ, Klayman DL, Hoch JM, Silvertin JV, and George CF. Thermal rearrangement and decomposition products of artemisinin (qinghaosu). *J. Org. Chem.* 1985, 50, 4504-4508.
- 50 Theoharides AD; Smyth MH; Ashmore RW; Halverson JM; Zhou ZM; Ridder WE; Lin AJ. Determination of dihydroqinghaosu in blood by pyrolysis gas chromatography/mass spectrometry. *Anal. Chem.*, 1988, 60(2), 115-120.
- 51 Lin AJ, Theoharides AD, and Klayman DL. Thermal decomposition products of dihydroartemisinin (dihydroqinghaosu). *Tetrahedron.* 1986, 42, 2181-2184.
- 52 Zhao SS. High-performance liquid chromatographic determination of artemisinin (qinghaosu) in human plasma and saliva. *Analyst*, 1987, 112(5), 661-4.
- 53 Zhao S, Zeng M-Y. Application of Precolumn Reaction to High-Performance Liquid Chromatography of Qinghaosu in Animal Plasma. *Anal. Chem.* 1986, 58, 289-292.
- 54 Idowu OR; Edwards G; Ward SA; Orme ML; Breckenridge AM. Determination of arteether in blood plasma by high-performance liquid chromatography with ultraviolet detection after hydrolysis acid. *J. Chromatogr.*, 1989, 493(1), 125-36.
- 55 Edlund P.O., Westerlund D., Carlqvist J., Bo-Liang W. and Yunhua J. Determination of artesunate and dihydroartemisinin in plasma by liquid chromatography with post-column derivatization and UV-detection. *Acta Pharm. Suec.* 1984, 21, 223-234.
- 56 Analysis of Artesunic Acid and Dihydroqinghaosu in Blood by High-Performance Liquid Chromatography with Reductive Electrochemical Detection. Z.M. Zhou, J.C. Anders, H. Chung and A.D. Theoharides. *J. Chromatogr.* 414, 77-90 (1987).
- 57 Luo X, Xie M, and Zou A. Sub-nanogram detection of dihydroartemisinin after chemical derivatization with diacetyldihydrofluorescein followed by high-performance liquid chromatography and UV absorption. *Chromatographia*, 1987, 23(1), 112-114.
- 58 Lin, AJ, Klayman, DL, Milhous, WK. Antimalarial activity of new water-soluble dihydroartemisinin derivatives. *J. Med. Chem.* 1987, 30, 2147-50.

⁵⁹ Idowu OR, Ward SA, Edwards G. Determination of artelinic acid in blood plasma by high-performance liquid chromatography. *J. Chromatogr.*, 1989, 495, 167-77.

⁶⁰Lin ET, Benet LZ, Upton RA, Levai F, and Gee WL. Quantitation of Artelinic Acid in Plasma by High-Performance Liquid Chromatography with a C8 Bonded Column and an Acidic Aqueous Mobile Phase. Status Report 20B, 6/24/1992, Contract No. DAMD17-92-C-2028.

Appendix A: Method Validation Data

Study 13, Supplement I: WR 238,605 Rat Plasma Assay

Study 13, Supplement II: WR 238,605 Dog Plasma Assay

Study 17: Halofantrine and WR 178,460 Human Plasma and Blood Assay

Study 18: WR 6026 and WR 211,789 Human Plasma and Blood Assay

Study 19: Mefloquine Human Blood Assay

Study 20: Artelinic Acid Human Plasma Assay

Study 21: *p*-Aminoheptanophenone Dog Plasma Assay

Study 22: WR 6026, WR 211,789, and WR 254,421 Human Urine Assay

**LABORATORY METHODOLOGY FOR WR 238,605 AS FREE BASE RAT
PLASMA ASSAY,* STUDY REPORT 13 SUPPLEMENT I**

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA) or equivalent.
2. Altex Model 100A Solvent Delivery Module (Beckman Instruments Inc., Berkeley, CA) or equivalent.
3. Shimadzu RF 535 Fluorescence Detector (ISI Instruspec Inc., Walnut Creek, CA) or equivalent.
4. Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA) or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. WR 238,605 succinate (Walter Reed Army Institute of Research, Washington D.C.), Bottle No. BK 73252, expiration date not available.
4. WR 6026 dihydrochloride (Walter Reed Army Institute of Research, Washington D.C.), Bottle No. BK01845, expiration date not available.
5. Phosphoric acid (85%) (Fisher Scientific, Fair Lawn, NJ).
6. Acetonitrile and methanol (Fisher Scientific, Fair Lawn, NJ).
7. Sodium hydroxide (Fisher Scientific, Fair Lawn, NJ).
8. Dibasic ammonium phosphate (Fisher Scientific, Fair Lawn, NJ).
9. Methyl celvol ether (Fisher Scientific, Fair Lawn, NJ).
10. Type I reagent grade water (deionized with a Nanopure II system, Barnstead, Boston, MA).

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C. ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength: excitation - 375 nm, emission - 480 nm

Sensitivity: high

Range: 4

Response: medium

Lamp

Ushio xenon, type UXL-155-LCA(S-LC)

2. COLUMN

Phenomenex Silica, 5 μ m particle size, 4.6 x 250 mm
(Phenomenex Inc., Rancho Palos Verdes, CA).

3. SOLVENT SYSTEM

Combine and mix H₂O (2 L) + (NH₄)₂HPO₄ (20 mL of 1 M
(NH₄)₂HPO₄) + CH₃CN (2 L).

Adjust pH to 7.0 with 85% H₃PO₄.

4. FLOW RATE

1.2 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a freezer and were checked for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds (solutions are discarded when a more than 10% change in the ratio is observed or 6 months after the preparation date). Solution storage bottles were amber or covered with aluminum foil.

a. WR 238,605 (free base)

Preparation date: 8/18/93					
Solution Type	Weight of Standard (mg)	Purity Factor*	QS Volume (ml)	Solvent	Conc. (μ g/ml)
Standard Curve	12.56	0.79695	50	methanol	200
Preparation date: 6/21/93					
Precision	12.79	0.79695	50	methanol	204

* = Molecular weights of WR 238605 free base/WR 238605 succinate

b. WR 6026 internal standard.

Preparation date: 6/21/93					
Solution Type	Weight of Standard (mg)	Purity Factor	QS Volume (ml)	Solvent	Conc. (µg/ml)
Internal Std	11.16	1	100	methanol	112

2. WORKING SOLUTIONS - Store solution in a freezer and discard within 6 months.

a. WR 238605.

Preparation date: 8/18/93				
Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
Standard Curve	200	1:100	methanol	2.00
Standard Curve	2.00	1:10	methanol	0.200

Preparation date: 7/8/93				
Precision	204	1:100	methanol	2.04
Precision	2.04	1:10	methanol	0.204

b. WR 6026 (Internal Standard).

Preparation date: 6/21/93				
Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
Internal Std.	112	1:4	methanol	27.9

7. RETENTION TIMES (subject to change depending on temperature and column performance). HPLC system is at room temperature.

a. WR 238,605 as free base - 5.1 min

b. WR 6026 as free base (Internal Standard) - 7.2 min

8. BLANK RAT PLASMA

Rat plasma (3.8% sodium citrate) Pel-Freez Biologicals, Rogers, AK.

9. INJECTION VOLUME

50-100 µl

10. QUANTITATION

By peak height ratio of drug peak relative to internal standard peak. Standard curves calculated by weighted linear regression with a weight of $1/y_i$.

11. MINIMUM QUANTITATION LIMIT OF METHOD (The minimum quantitation limit was determined as the WR 238,605 (free base) standard curve concentration at which the signal to noise ratio was at least 3 to 1.)

1.00 ng/ml WR 238,605 (free base) in plasma.

12. SAMPLE VOLUME MEASUREMENT

Plasma sample volumes were measured with a variable volume Eppendorf pipetter.

13. WISP OPERATING TEMPERATURE

Room temperature.

D. SAMPLE STORAGE

All samples are to be kept frozen at -20°C before analysis and thawed at room temperature for preparation (within 30 min) and analysis.

E. SAMPLE PREPARATION

1. If frozen, thaw rat plasma sample at room temperature and vortex for 1 min. Pipet 0.2 ml of rat plasma into a 13 x 100 glass culture tube.
2. Spike standard curve samples with 00,* 0,** 1, 2, 4, 8, or 15 μl of 0.200 $\mu\text{g}/\text{ml}$ WR 238605 working solution or 3, 5, 10, 20, or 40 μl of 2.00 $\mu\text{g}/\text{ml}$ WR 238605 working solution to make a standard curve. Since 0.2 ml plasma samples are assayed, this procedure is equivalent to making standard curve samples with WR 238605 concentrations corresponding to 00, 0, 1.00, 2.00, 4.00, 8.00, 15.0, 30.0, 50.0, 100, 200 and 400 ng/ml. Vortex for 20 s.
3. Add 20 μl of internal standard working solution (27.9 $\mu\text{g}/\text{ml}$ WR 6026), except to 00 standard curve sample. Vortex 30 s.

* 00 = Sample with no drug and no internal standard

** 0 = Sample with no drug but with internal standard.

4. Add add 0.1 ml of 0.1 M NaOH. Vortex 30 s.
5. Add 3 ml of methyl *t*-butyl ether. Vortex for 1 min, twice, and centrifuge for 10 min at 3000 *g*.
6. Transfer the organic layer to a second 13 x 100 tube with a pasteur pipette and evaporate to dryness under nitrogen.
7. Reconstitute with 200 μ l of 50% CH₃CN, transfer to WISP insert and inject onto column.

F. QUALITY CONTROL

1. Content and frequency of blanks

No special blank was used except for the standard curve blank.

2. Pipette Calibration

See ASOP 2C-1.1.

3. Balance Calibration

See ASOP 2C-2.1.

G. RECOVERY

Assay recovery was assessed at four different concentrations by comparing the WR 238,605 (as free base) to internal standard peak height ratios in a reference sample to the peak height ratios in plasma. Plasma (0.2 ml) and reference samples were spiked with corresponding amounts of WR 238,605 (as free base). Each plasma sample was prepared as described in "Sample Preparation" (Section E), except samples were vortexed for 10 minutes (step 7), 2.5 ml of sample was taken for evaporation (step 8), and the internal standard was added after the evaporation (step 8). The reference samples were spiked prior to extraction (step 5) with drug and after evaporation (step 8) with internal standard.

H. GENERATION OF PRECISION SAMPLES

Samples for precision analysis were generated by spiking 0.2 ml plasma specimens with WR 238,608 (as free base) working solutions as shown below. These samples were then prepared as described in Sample Preparation (Section E) above.

Generation of Precision Samples

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (μ l)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	0.204	200	2.04
Low	15	0.204	200	15.3
Med.	5	2.04	200	51.0
Hi	20	2.04	200	204

I. RESULTS

1. STANDARD CURVE

Chromatograms for each point in a representative standard curve for WR 238,605 as free base appear in Figure 1. Peak height ratios for these calibrators appear in Table 1.

2. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 2 and 3.

3. STUDY STANDARD CURVE CALIBRATOR STATISTICAL PARAMETERS

Results for this evaluation appears in Table 4

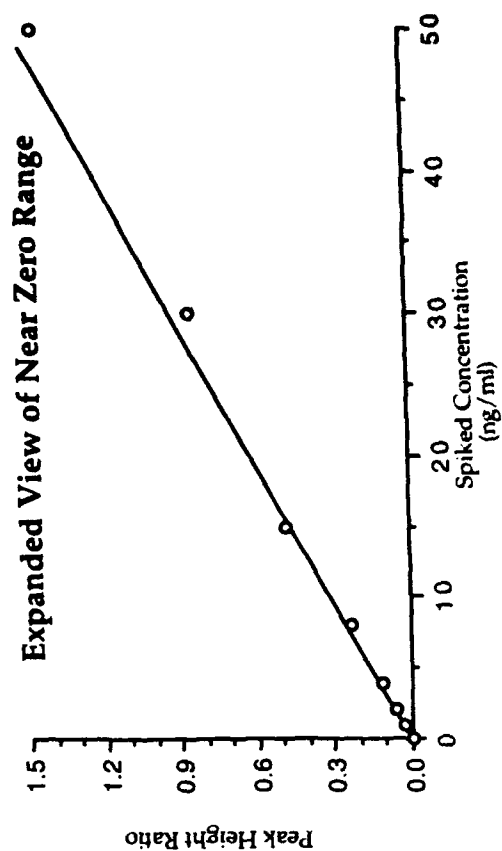
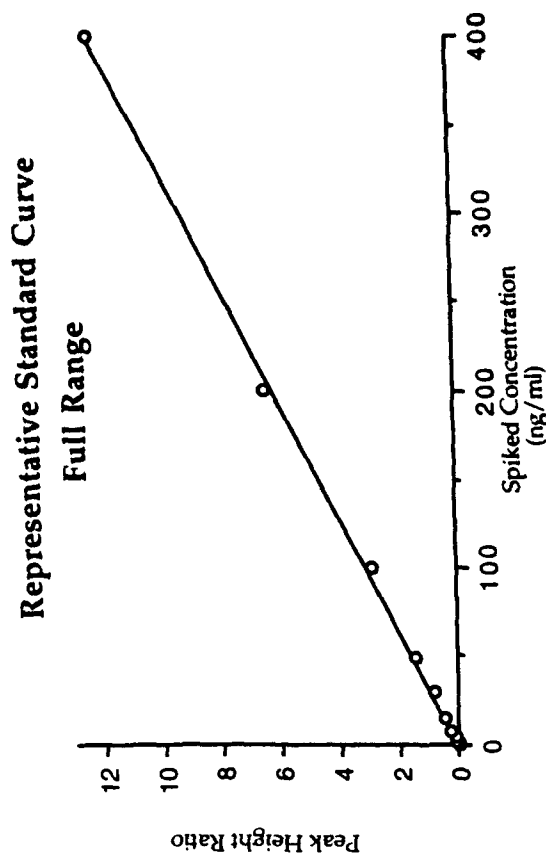
4. RECOVERY

Results for this evaluation appear in Table 5.

TABLE 1: REPRESENTATIVE STANDARD CURVE FOR
WR 238,605 (FREE BASE) RAT PLASMA ASSAY,
STUDY REPORT 13, SUPPLEMENT NO. 1

SPIKED AMOUNT (ng)*	STANDARD CURVE CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
0	0	0	-
0.200	1.00	0.034	1.13
0.400	2.00	0.065	2.15
0.801	4.00	0.116	3.81
1.60	8.00	0.230	7.53
3.00	15.0	0.483	15.8
6.01	30.0	0.849	27.7
10.0	50.0	1.457	47.6
20.0	100	2.944	96.1
40.0	200	6.413	209
80.1	400	12.242	400

Regression equation:
 $y = 0.03060x - 0.00074$, $r^2 = 0.9987$



* Into 0.2 ml of biological sample.

** Ratio of drug peak height to internal standard peak height.

*** Standard curve calculated by weighted linear regression where
weight = $1/y_i$.

TABLE 2: INTERDAY PRECISION OF WR 238,605 FREE BASE RAT PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER			MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3 Measured Concentrations* (ng/ml)				
2.04	2.13	2.09	1.94	2.05	0.100	4.88	0.654
15.3	14.8	14.9	16.4	15.4	0.896	5.83	0.436
51.0	54.6	45.9	55.5	52.0	5.30	10.2	1.96
204	230	218	239	229	10.5	4.60	12.3

TABLE 3: INTRADAY PRECISION OF WR 238,605 FREE BASE RAT PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6 Measured Concentrations (ng/ml)				
2.04	2.35	2.07	1.86	2.21	1.75	2.03	2.05	0.220	10.8	0.245
15.3	15.0	14.9	14.6	14.3	14.8	15.1	14.8	0.293	1.98	-3.38
51.0	43.4	44.2	46.8	46.6	42.3	46.8	45.0	1.98	4.39	-11.7
204	215	210	212	216	216	216	214	2.56	1.20	4.98

* Measured concentrations are averages of two analyses.

**TABLE 4: STUDY STANDARD CURVE CALIBRATOR STATISTICAL
PARAMETERS FOR WR 238,605 RAT PLASMA ASSAY,
STUDY REPORT 13 SUPPLEMENT I**

Spiked Concentration (ng/ml)	n	Mean (ng/ml)	Standard Deviation (ng/ml)	Percent C.V.	Percent Deviation
1.00	3	1.25	0.188	15.0	25.3
2.00	3	2.05	0.111	5.40	2.33
4.00	3	3.89	0.067	1.71	-2.83
8.00	3	7.75	0.276	3.56	-3.13
15.0	3	15.4	0.551	3.57	2.89
30.0	3	27.5	0.473	1.72	-8.22
50.0	3	46.0	2.86	6.21	-8.00
100	3	93.2	2.51	2.70	-6.80
200	3	210	1.73	0.82	5.00
400	3	405	6.81	1.679	1.33

TABLE 5: RECOVERY OF WR 238,605 FROM RAT PLASMA

SAMPLE ID	SPIKED CONCENTRATION Range (ng/ml)		PEAK HEIGHT RATIO		MEAN PERCENT RECOVERY
			REFERENCE	PLASMA	
<u>WR 238.605</u>					
1	X Low	5.00	0.087	0.052	63.9
2			0.075	0.048	
3			0.071	0.049	
Mean (± SD)			0.078 ± 0.008	0.050 ± 0.002	
1	Low	15.0	0.535	0.379	69.5
2			0.469	0.356	
3			0.507	0.335	
Mean (± SD)			0.504 ± 0.033	0.350 ± 0.025	
1	Medium	50.0	1.774	1.183	68.7
2			1.772	1.123	
3			1.698	1.299	
Mean (± SD)			1.748 ± 0.043	1.202 ± 0.089	
1	High	200	7.513	5.135	63.5
2			7.421	4.799	
3			7.932	4.577	
Mean (± SD)			7.622 ± 0.272	4.837 ± 0.281	
AVERAGE MEAN RECOVERY = 66.4					

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**LABORATORY METHODOLOGY FOR WR 238,605 AS FREE BASE
DOG PLASMA ASSAY,* STUDY REPORT 13 SUPPLEMENT**

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA) or equivalent.
2. Altex Model 100A Solvent Delivery Module (Beckman Instruments Inc., Berkeley, CA) or equivalent.
3. Shimadzu RF 535 Fluorescence Detector (ISI Instruspec Inc , Walnut Creek, CA) or equivalent.
4. Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA) or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. WR 238,605 succinate (Walter Reed Army Institute of Research, Washington D.C.), Bottle No. BK 73252, expiration date not available.
4. WR 6026 dihydrochloride (Walter Reed Army Institute of Research, Washington D.C.), Bottle No. BK01845, expiration date not available.
5. Phosphoric acid (85%) (Fisher Scientific, Fair Lawn, NJ).
6. Acetonitrile and methanol (Fisher Scientific, Fair Lawn, NJ).
7. Sodium hydroxide (Fisher Scientific, Fair Lawn, NJ).
8. Dibasic ammonium phosphate (Fisher Scientific, Fair Lawn, NJ).
9. Methyl *t*-butyl ether (Fisher Scientific, Fair Lawn, NJ).
10. Type I reagent grade water (deionized with a Nanopure II system, Barnstead Co., Boston, MA).

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C. ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength: excitation - 375 nm, emission - 480 nm

Sensitivity: high

Range: 4

Response: medium

Lamp

Ushio xenon, type UXL-155-LCA(S-LC)

2. COLUMN

Phenomenex Silica, 5 μ m particle size, 4.6 x 250 mm
(Phenomenex Inc., Rancho Palos Verdes, CA).

3. SOLVENT SYSTEM

CH₃CN/H₂O (50:50, v/v) and 5 mM (NH₄)₂HPO₄, pH = 7.0
(adjusted with 85% H₃PO₄).

4. FLOW RATE

1.2 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a 4°C refrigerator and were checked for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds (solutions are discarded when a more than 10% change in the ratio is observed or 6 months after the preparation date). Solution storage bottles were amber or covered with aluminum foil.

a. WR 238,605 (free base)

Preparation date: 1/6/94					
Solution Type	Weight of Standard (mg)	Purity Factor*	QS Volume (ml)	Solvent	Conc. (μ g/ml)
Standard Curve	12.56	0.79695	50	methanol	200

Preparation date: 6/21/93					
Precision	12.79	0.79695	50	methanol	204

* = Molecular weights of WR 238605 free base / WR 238605 succinate

b. WR 6026 internal standard.

Preparation date: 1/6/94					
Solution Type	Weight of Standard (mg)	Purity Factor	QS Volume (ml)	Solvent	Conc. (µg/ml)
Internal Std	11.16	1	100	methanol	112

2. WORKING SOLUTIONS - Store solution at 4°C and discard within 6 months.

a. WR 238605.

Preparation date: 1/6/94				
Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
Standard Curve	200	1:100	methanol	2.00
Precision	204	1:100	methanol	2.04

Preparation date: day of use				
Standard Curve	2.00	1:10	methanol	0.200
Precision	2.04	1:10	methanol	0.204

b. WR 6026 (Internal Standard).

Preparation date: 6/21/93				
Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
Internal Std.	112	1:4	methanol	27.9

7. RETENTION TIMES (subject to change depending on temperature and column performance).

a. WR 238,605 as free base - 5.1 min

b. WR 6026 as free base (Internal Standard) - 7.2 min

8. BLANK DOG PLASMA

Dog plasma (3.8% sodium citrate, heparin) Pel-Freez Biologicals, Rogers, AK.

9. INJECTION VOLUME

50-100 µl

10. QUANTITATION

By peak height ratio of drug peak relative to internal standard peak. Standard curves calculated by weighted linear regression.

11. MINIMUM QUANTITATION LIMIT OF METHOD (The minimum quantitation limit was determined as the WR 238,605 (free base) standard curve concentration at which the signal to noise ratio was at least 3 to 1.)

1.00 ng/ml WR 238,605 (free base) in plasma.

12. SAMPLE VOLUME MEASUREMENT

Plasma sample volumes were measured with a variable volume Eppendorf pipetter.

13. WISP OPERATING TEMPERATURE

Room temperature.

D. SAMPLE STORAGE

All samples are to be kept frozen at -20°C before analysis and thawed at room temperature for preparation (within 30 min) and analysis.

E. SAMPLE PREPARATION

1. Pipet 0.2 ml of dog plasma into a 13 x 100 culture tube.
2. Spike standard curve samples with 00, 0, 1, 2, 4, 8, or 15 µl of 0.200 µg/ml WR 238605 working solution or 3, 5, 10, 20, or 40 µl of 2.00 µg/ml WR 238605 working solution to make a standard curve. Since 0.2 ml plasma samples are assayed, this procedure is equivalent to making standard curve samples with WR 238605 concentrations corresponding to 00, 0, 1.00, 2.00, 4.00, 8.00, 15.0, 30.0, 50.0, 100, 200 and 400 ng/ml. Vortex for 20 s.
3. Thaw clinical samples at room temperature.
4. Vortex for 1 min.
5. Add 20 µl of internal standard working solution (27.9 µg/ml WR 6026). Vortex 30 s.
6. Add add 0.1 ml of 0.1 M NaOH. Vortex 30 s.

7. Add 3 ml of methyl *t*-butyl ether. Vortex for 2 min and centrifuge for 10 min at 3000 g.
8. Transfer the organic layer to a second 13 x 100 tube and evaporate to dryness under nitrogen.
9. Reconstitute with 200 µl of 50% CH₃CN and inject onto column.

F. QUALITY CONTROL

1. Content and frequency of blanks

No special blank was used except for the standard curve blank.

2. Pipette Calibration

See ASOP 2C-1.1.

3. Balance Calibration

See ASOP 2C-2.1.

G. RECOVERY

Assay recovery was assessed at four different concentrations by comparing the WR 238,605 (as free base) to internal standard peak height ratios in a reference sample to the peak height ratios in plasma. Plasma (0.2 ml) and reference samples were spiked with corresponding amounts of WR 238,605 (as free base). Each plasma sample was prepared as described in "Sample Preparation" (Section E), except samples were centrifuged for 10 minutes (step 7), 2.5 ml of sample was taken for evaporation (step 8), and the internal standard was added after the evaporation (step 8). The reference samples were spiked prior to extraction (step 5) with drug and after evaporation (step 8) with internal standard.

H. GENERATION OF PRECISION SAMPLES

Samples for precision analysis were generated by spiking 0.2 ml plasma specimens with WR 238,608 (as free base) working solutions as shown below. These samples were then prepared as described in Sample Preparation (Section E) above.

Generation of Precision Samples

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (μ l)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	0.204	200	2.04
Low	15	0.204	200	15.3
Med.	5	2.04	200	51.0
Hi	20	2.04	200	204

I. RESULTS

1. STANDARD CURVE

Chromatograms for each point in a representative standard curve for WR 238,605 as free base appear in Figure 1. Peak height ratios for these calibrators appear in Table 1.

2. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 2 and 3.

3. STUDY STANDARD CURVE CALIBRATOR STATISTICAL PARAMETERS

Results for this evaluation appears in Table 4

4. RECOVERY

Results for this evaluation appear in Table 5.

TABLE 1: REPRESENTATIVE STANDARD CURVE FOR
WR 238,605 (FREE BASE) RAT PLASMA ASSAY,
STUDY REPORT 13 SUPPLEMENT

SPIKED AMOUNT (ng)*	STANDARD CURVE CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
0	0	0	-
0.200	1.00	0.031	1.13
0.400	2.00	0.061	2.15
0.800	4.00	0.107	3.72
1.60	8.00	0.219	7.55
3.00	15.0	0.466	16.0
6.01	30.0	0.826	28.3
10.0	50.0	1.527	52.2
20.0	100	2.501	85.5
40.0	200	6.253	214
80.0	400	11.817	404

Regression equation:

$$y = 0.0293x - 0.00198, \quad r^2 = 0.9951$$

* Into 0.2 ml of biological sample.

** Ratio of drug peak height to internal standard peak height.

*** Standard curve calculated by weighted linear regression where

$$w = 1/v$$

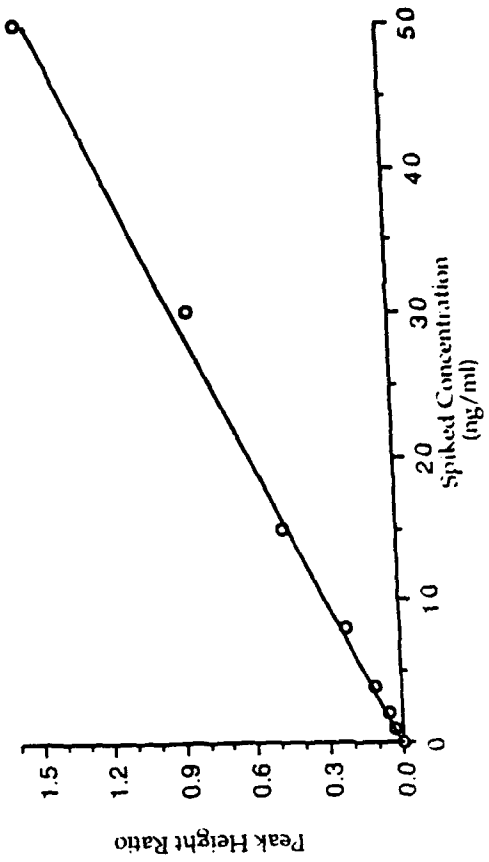
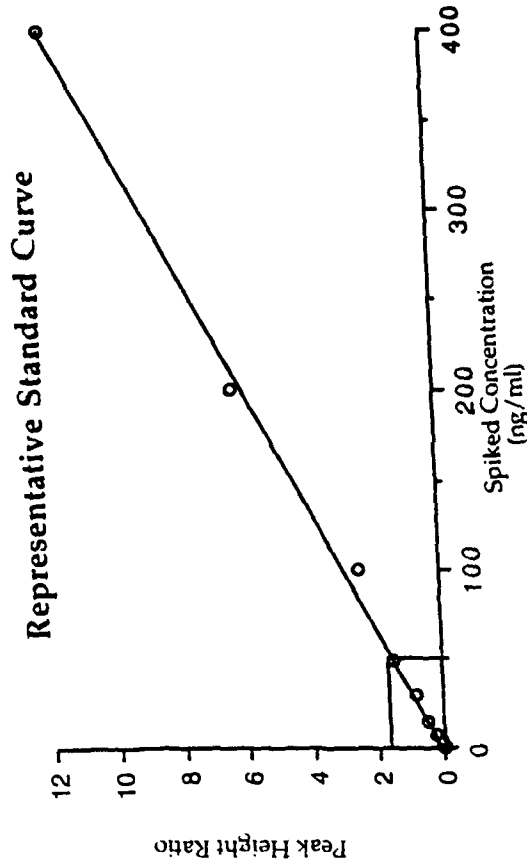


TABLE 2: INTERDAY PRECISION OF WR 238,605 FREE BASE DOG PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER			MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3 Measured Concentrations* (ng/ml)				
2.04	2.44	2.43	2.38	2.41	0.033	1.38	18.3
15.3	14.3	14.1	14.4	14.3	0.076	0.54	-6.75
51.0	54.4	51.5	59.0	55.1	3.56	6.46	8.07
204	222	208	221	218	6.54	3.01	6.62

TABLE 3: INTRADAY PRECISION OF WR 238,605 FREE BASE DOG PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6 Measured Concentrations (ng/ml)				
2.04	2.34	2.04	2.21	2.01	1.91	2.14	2.11	0.154	7.31	3.35
15.3	14.9	14.4	13.7	16	13.1	14.6	14.5	1.00	6.93	-5.56
51.0	50.4	60.7	46.7	51.9	47.1	49.1	51.0	5.15	10.1	-0.033
204	217	200	213	201	192	199	204	9.42	4.62	-0.163

* Measured concentrations are averages of two analyses.

**TABLE 4: STUDY STANDARD CURVE CALIBRATOR STATISTICAL
PARAMETERS FOR WR 238,605 DOG PLASMA ASSAY,
STUDY REPORT 13 SUPPLEMENT**

Spiked Concentration (ng/ml)	n	Mean (ng/ml)	Standard Deviation (ng/ml)	Percent C.V.	Percent Deviation
1.00	4	1.22	0.0818	6.72	21.8
2.00	4	2.09	0.0920	4.40	4.50
4.00	4	3.73	0.135	3.62	-6.81
8.00	4	7.47	0.199	2.66	-6.59
15.0	4	14.4	1.11	7.75	-4.33
30.0	4	27.5	1.35	4.93	-8.50
50.0	4	52.1	0.926	1.78	4.10
100	4	97.8	8.39	8.58	-2.18
200	4	211	6.95	3.30	5.38
400	4	396	9.26	2.34	-1.13

TABLE 5: RECOVERY OF WR 238,605 FREE BASE FROM DOG PLASMA

SAMPLE ID	SPIKED CONCENTRATION Range (ng/ml)		PEAK HEIGHT RATIO		MEAN PERCENT RECOVERY
			REFERENCE	PLASMA	
<u>WR 238,605 free base</u>					
1	X Low	5.00	0.069	0.048	70.4
2			0.066	0.045	
3			0.068	0.050	
Mean (± SD)			0.068 ± 0.002	0.048 ± 0.003	
1	Low	15.0	0.511	0.371	72.7
2			0.505	0.366	
3			0.484	0.356	
Mean (± SD)			0.501 ± 0.016	0.364 ± 0.008	
1	Medium	50.0	1.614	1.105	63.3
2			1.721	1.008	
3			1.566	0.989	
Mean (± SD)			1.634 ± 0.079	1.034 ± 0.062	
1	High	200	7.368	4.342	59.7
2			8.223	4.882	
3			BC	4.749	
Mean (± SD)			7.796 ± 0.605	4.658 ± 0.281	
AVERAGE MEAN PERCENT RECOVERY = 66.5					

**LABORATORY METHODOLOGY FOR HALOFANTRINE (FREE BASE) AND
WR 178,460 (FREE BASE) HUMAN PLASMA AND BLOOD ASSAY* ,
STUDY REPORT 17**

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA) or equivalent.
2. Beckman Model 100A Solvent Delivery Module (Beckman Instruments Inc., Berkeley, CA) or equivalent.
3. Shimadzu RF 535 Fluorescence Detector (ISI Instruspec Inc., Walnut Creek, CA) or equivalent.
4. Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA) or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. Halofantrine hydrochloride, WR 171,669 (Walter Reed Army Institute of Research, Washington D.C.), bottle number BB 43807, expiration date not available.
3. WR 178,460 hydrochloride (Walter Reed Army Institute of Research, Washington D.C.), bottle number BK 21070, expiration date not available.
4. Procainamide hydrochloride (Internal Standard), (Pfaltz & Bauer, Inc., Flushing, NY) lot number not available, expiration date not available.
5. Methanol (Fisher Scientific, Fair Lawn, NJ), lot number not available, expiration date not available.
6. Acetonitrile (Fisher Scientific, Fair Lawn, NJ), lot number not available, expiration date not available.

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

7. Methyl *t* butyl ether (Baxter, Burdick & Jackson, Muskegon, MI), lot number not available, expiration date not available.
8. Sodium hydroxide (Mallinckrodt Co., Paris, KY), lot number KTMA, expiration date not available.
9. Water (deionized by Nanopure II, Barnstead Co., Boston, MA).
10. Dibasic ammonium phosphate (Fisher Scientific, Fair Lawn, NJ), lot number 892838, expiration date not available.

C. ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength; excitation - 300 nm, emission - 375 nm

Sensitivity; 8

Lamp

Ushio xenon, type UXL-155-LCA(S-LC), No. 882936

2. COLUMN

Altex Ultrasphere Silica, 5 μ m particle size, 4.6 x 250 mm
(Beckman Instruments Inc., Berkeley, CA).

3. SOLVENT SYSTEM

CH₃OH/H₂O (80:20, v/v) + 5 mM (NH₄)₂HPO₄ (final concentration)

4. FLOW RATE

1.0 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a -20°C freezer, covered in aluminum foil, and checked for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds and internal standard (solutions are discarded when a more than 10% change in the ratio is observed or by 6 months after the preparation date).

- a. Halofantrine (free base) - Solution preparation date 5/3/89, expiration date 11/3/89.

Dissolve 5.00 mg of halofantrine hydrochloride in and qs to 100 ml with methanol.

Conc. of halofantrine hydrochloride = 50.0 μ g/ml

Conc. of halofantrine (free base) = 47.1 μ g/ml

Halofantrine (free base) - Solution preparation date 9/25/89, expiration date 3/25/90.

Dissolve 5.10 mg of halofantrine hydrochloride in and qs to 100 ml with methanol.

Conc. of halofantrine hydrochloride = 51.0 $\mu\text{g/ml}$

Conc. of halofantrine (free base) = 48.0 $\mu\text{g/ml}$

- b. WR 178,460 (free base) (Halofantrine metabolite) - Solution preparation date 5/3/89, expiration date 11/3/89.

Dissolve 5.00 mg of WR 178,460 hydrochloride in 100 ml of methanol.

Conc. of WR 178,460 hydrochloride = 50.0 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 46.2 $\mu\text{g/ml}$

- WR 178,460 (free base) (Halofantrine metabolite) - Solution preparation date 9/25/89, expiration date 3/25/90.

Dissolve 5.02 mg of WR 178,460 hydrochloride in 100 ml of methanol.

Conc. of WR 178,460 hydrochloride = 50.2 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 46.4 $\mu\text{g/ml}$

- c. Procainamide hydrochloride- Internal standard stock solution
Preparation date 5/3/89, expiration date 11/3/89.

Dissolve 2.95 mg of procainamide in 100 ml of methanol.

Conc. = 29.5 $\mu\text{g/ml}$

Preparation date 9/25/89, expiration date 3/25/90.

Dissolve 2.99 mg of procainamide in 100 ml of methanol.

Conc. = 29.9 $\mu\text{g/ml}$

6. WORKING SOLUTIONS - Solutions were stored in a -20°C freezer, covered in aluminum foil, and discarded when stock solutions were discarded or by 6 months after the preparation date).

- a. Mixed halofantrine and WR 178,460 (as free bases) solutions.

Combine 1 ml of halofantrine (free base) stock solution and 1 ml of WR 178,460 (free base) stock solution and q.s. to 50 ml with methanol.

High concentration solution preparation date 5/3/89,
expiration date 11/3/89.

Conc. of halofantrine (free base) = 0.942 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 0.924 $\mu\text{g/ml}$

High concentration solution preparation date 9/25/89,
expiration date 3/25/90.

Conc. of halofantrine (free base) = 0.960 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 0.928 $\mu\text{g/ml}$

Combine 1 ml of halofantrine (free base) stock solution and 1
ml of WR 178,460 (free base) stock solution and q.s. to 100 ml
with methanol.

Low concentration solution preparation date 5/3/89,
expiration date 11/3/89.

Conc. of halofantrine (free base) = 0.471 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 0.462 $\mu\text{g/ml}$

Low concentration solution preparation date 9/25/89,
expiration date 3/25/90.

Conc. of halofantrine (free base) = 0.480 $\mu\text{g/ml}$

Conc. of WR 178,460 (free base) = 0.464 $\mu\text{g/ml}$

b. Procainamide - Internal standard working solution

Preparation date 5/3/89, expiration date 11/3/89.

Make a 1:100 dilution of procainamide stock solution (29.5
 $\mu\text{g/ml}$) with methanol.

Conc. = 0.295 $\mu\text{g/ml}$

Make a 1:10 dilution of 0.295 $\mu\text{g/ml}$ procainamide working
solution with methanol.

Conc. = 0.0295 $\mu\text{g/ml}$

Preparation date 9/25/89, expiration date 3/25/90.

Make a 1:100 dilution of procainamide stock solution (29.9
 $\mu\text{g/ml}$) with methanol.

Conc. = 0.299 $\mu\text{g/ml}$

Make a 1:10 dilution of 0.299 $\mu\text{g/ml}$ procainamide working
solution with methanol.

Conc. = 0.0299 $\mu\text{g/ml}$

7. RETENTION TIMES (subject to change depending on temperature and column performance).

- a. Halofantrine (free base) - 8 min
- b. WR 178,460 (free base) - 11 min
- c. Procainamide (Internal Standard) - 26 min

8. BLANK PLASMA AND BLOOD

Human plasma and blood (CPD or CPDA-1 as anticoagulant) is obtained from San Francisco Irwin Memorial Blood Bank.

9. INJECTION VOLUME

25-150 μ l

10. QUANTITATION

By peak height ratio of drug peak and metabolite peak relative to internal standard peak.

11. MINIMUM QUANTITATION LIMITS OF METHOD

The minimum quantitation limits, 0.960 ng/ml for halofantrine and 0.928 ng/ml for WR 178,460 (as free bases), was determined as the halofantrine and WR 178,460 (as free bases) concentrations at which the signal to noise ratios were at least 3 to 1.

12. SAMPLE VOLUME MEASUREMENT

Plasma sample volumes were measured with a 200 μ l or a 1000 μ l Gilson Pipetman. Blood sample volumes were measured with Eppendorf pipettes. See SOP 3-4 for calibration procedure.

13. WISP OPERATING TEMPERATURE

Room temperature.

D. SAMPLE STORAGE

All samples were kept frozen at -80°C before analysis and thawed at room temperature for preparation and analysis.

E. SAMPLE PREPARATION

PLASMA SAMPLES

- 1. Pipet 0.5 ml of plasma into a 13x100 mm silanized tube.

2. Spike standard curve samples with 00* , 0** , 1, 2, 4, 8, or 15 µl of low concentration (0.480 µg/ml halofantrine (free base) and 0.464 µg/ml WR 178,460 (free base)) standard curve working solution of halofantrine and WR 178,460 (as free bases), or 15, 30, or 60 µl of high concentration (0.960 µg/ml halofantrine (free base) and 0.928 µg/ml WR 178,460 (free base)) standard curve working solution of halofantrine and WR 178,460 (as free bases) to make final halofantrine (free base) concentrations of 00, 0, 0.960, 1.92, 3.84, 7.68, 14.4, 28.8, 57.6, and 115 ng/ml and WR 178,460 (free base) concentrations of 00, 0, 0.928, 1.86, 3.71, 7.42, 13.9, 27.8, 55.7, and 111 ng/ml. Vortex for 20 seconds.
3. Add 50 µl of internal standard working solution (procainamide hydrochloride, 0.0295 or 0.0299 µg/ml). Vortex for 20 s.
4. Add 1 ml acetonitrile. Vortex for 2 min. Centrifuge for 10 min at 3000 g.
5. Transfer supernatant to 16x125 mm silanized tube and evaporate to 0.5 ml.
6. Add 0.5 ml water and 50 µl of 0.1 N NaOH. Vortex for 20 s.
7. Add 5 ml methyl-*t*-butyl ether. Vortex for 2.5 min. Centrifuge for 10 min at 3000 g.
8. Freeze mixture in dry ice/methanol bath. Transfer organic phase to silanized 13x100 mm tube and begin evaporation.
9. Repeat steps 7 and 8, transferring organic phase to same 13x100 mm tube and evaporate to dryness.
10. Reconstitute residue with 200 µl of 80% methanol containing 0.001% HCl. Vortex for 2 min.
11. Transfer to silanized glass WISP inserts and inject onto column.

BLOOD SAMPLES

1. Pipet 0.5 ml of blood into a 13x100 mm silanized tube.
2. Follow step 2 as in plasma sample preparation. Vortex for 20 s, and let stand for 1 h.

* 00 = Sample with no drug and no internal standard.

** 0 = Sample with no drug but with internal standard.

3. Add 0.5 ml water. Vortex for 10 s.
4. Sonicate for 10 min in water bath.
5. Add 50 μ l of internal standard working solution (procainamide, 0.0598 μ g/ml). Vortex for 20 s.
6. Add 2 ml acetonitrile. Vortex for 2.5 min. Centrifuge for 15 min at 3000 g.
7. Follow steps 5-11 as in plasma sample preparation.

F. QUALITY CONTROL

1. Content and frequency of blanks

No special blank was used except for the standard curve blank.

G. PREPARATION OF VALIDATION SAMPLES

Samples for precision analysis and recovery were prepared by spiking 0.5 ml plasma or blood specimens with 2 or 10 μ l of 0.480 μ g/ml halofantrine and 0.464 μ g/ml WR 178,460 (as free bases) low concentration working solution or with 20 or 40 μ l of 0.960 μ g/ml halofantrine and 0.928 μ g/ml WR 178,460 (as free bases) high concentration working solution to make final halofantrine (free base) concentrations of 1.92, 9.60, 38.4, and 76.8 ng/ml and final WR 178,460 (free base) concentrations of 1.86, 9.28, 37.1, and 74.2 ng/ml. For intraday blood samples, 2 or 10 μ l of 0.471 μ g/ml halofantrine and 0.462 μ g/ml WR 178,460 (as free bases) low concentration working solution or with 20 or 40 μ l of 0.942 μ g/ml halofantrine and 0.924 μ g/ml WR 178,460 (as free bases) high concentration working solution were used to make final halofantrine (free base) concentrations of 1.88, 9.42, 37.7, and 75.4 ng/ml and final WR 178,460 (free base) concentrations of 1.85, 9.24, 37.0, and 73.9 ng/ml.

H. RECOVERY

Recovery was assessed by comparing the halofantrine or WR 178,460 (as free bases) to internal standard peak height ratios in reconstitution solvent (0.2 ml) to the peak height ratios in plasma or blood. Plasma or blood samples (0.5 ml) were spiked with 2 or 10 μ l of the low concentration working solution or with 20 or 40 μ l of the high concentration working solution. Each sample was prepared as described in "Sample Preparation" (Section E), except the internal standard was added after the reconstitution step (step 11) and the

solvent samples (80% methanol containing 0.001% HCl) were neither extracted nor evaporated.

I. STABILITY

Pooled plasma and blood samples spiked with halofantrine and WR 178,460 (as free bases) at four different concentrations were mixed on a rotator for one hour. The resulting samples were divided into 0.5 ml fractions, placed in culture tubes and stored in the freezer at -80°C until assayed for stability. Samples were assayed according to the method described in Study Report No. 4 dated Aug. 23, 1985 and titled "Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite (WR 178,460) in Blood and Plasma."

J. RESULTS

1. STANDARD CURVE

Chromatograms for each point in representative standard curves for halofantrine and WR 178,460 (as free bases) appear in Figures 4 and 5. Peak height ratios for these calibrators appear in Tables 1A-D.

2. RECOVERY

Results for this evaluation appear in Tables 2A-D.

3. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 3A-D and 4A-D.

5. STABILITY

Results appear in Table 6A-B.

TABLE 1A: REPRESENTATIVE STANDARD CURVE FOR HALOFANTRINE (FREE BASE) HUMAN PLASMA ASSAY, STUDY REPORT 17

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	0
0.960	0.028	1.03 ^a
1.92	0.050	1.88 ^a
3.84	0.098	3.74 ^a
7.68	0.205	7.89 ^a
14.4	0.371	14.3 ^a
28.8	0.771	25.7 ^b
57.6	1.625	52.3 ^b
115	3.747	118 ^b

Regression equations:**

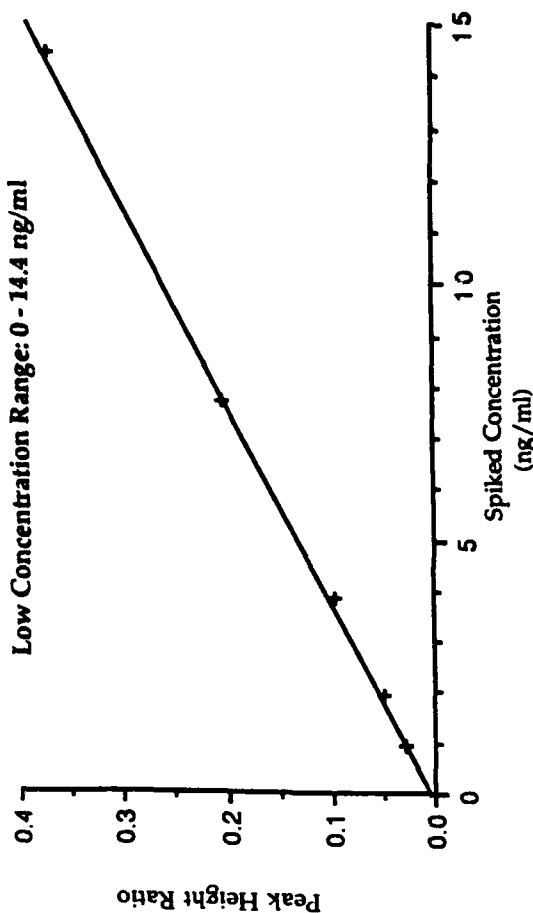
$$^a y = 0.02581x + 0.0014, \quad r^2 = 0.9995 \text{ (Low Range: 0 - 14.4 ng/ml)}$$

$$^b y = 0.03210x - 0.0548, \quad r^2 = 0.9950 \text{ (High Range: 0 - 115 ng/ml)}$$

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Standard Curve
Low Concentration Range: 0 - 14.4 ng/ml



High Concentration Range: 0 - 115 ng/ml

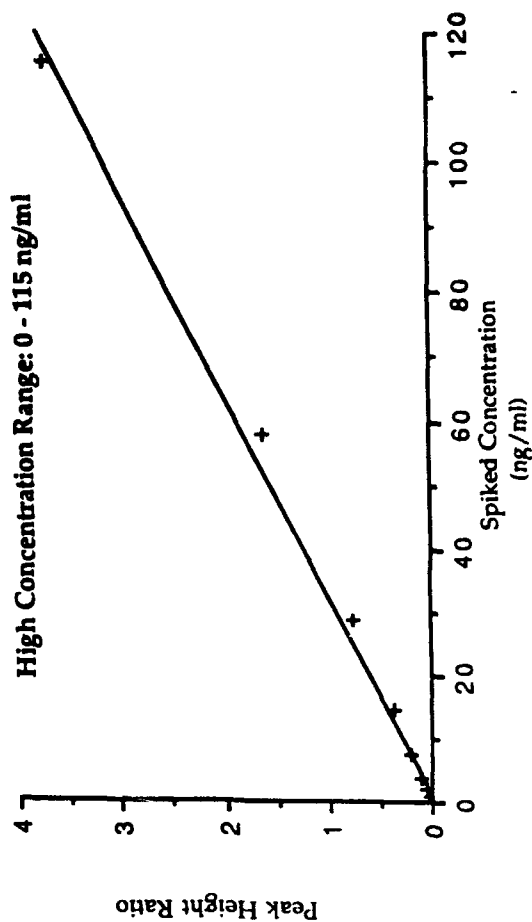


**TABLE 1B: REPRESENTATIVE STANDARD CURVE FOR WR
178,460 (FREE BASE) HUMAN PLASMA ASSAY,
STUDY REPORT 17**

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	0
0.928	0.039	0.959 ^a
1.86	0.072	1.85 ^a
3.71	0.143	3.75 ^a
7.42	0.283	7.51 ^a
13.9	0.519	13.8 ^a
27.8	1.041	26.2 ^b
55.7	2.120	52.5 ^b
111	4.597	113 ^b

Regression equations:

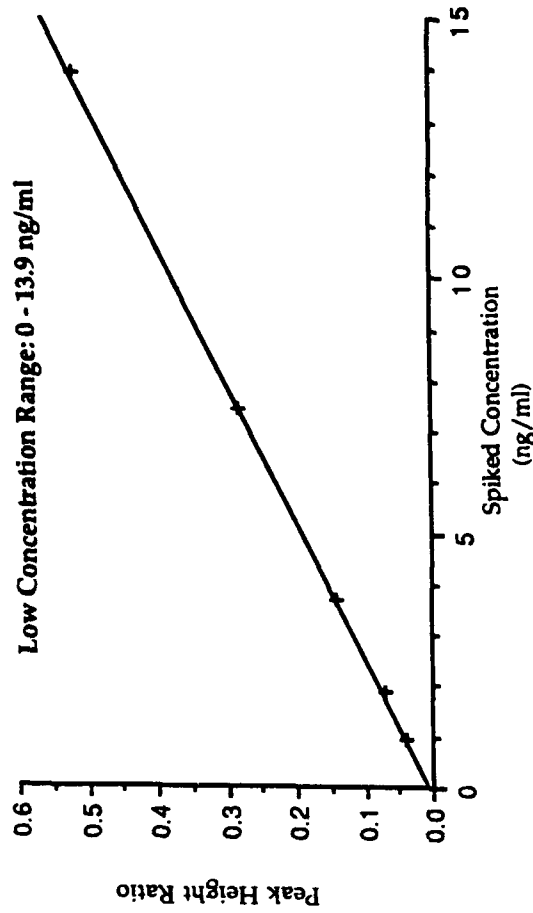
$$a)y = 0.03726x + 0.0033, \quad r^2 = 0.9998 \text{ (Low Range: 0 - 13.9 ng/ml)}$$

$$b)y = 0.04098x - 0.0329, \quad r^2 = 0.9983 \text{ (High Range: 0 - 111 ng/ml)}$$

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Standard Curve
Low Concentration Range: 0 - 13.9 ng/ml



High Concentration Range: 0 - 111 ng/ml

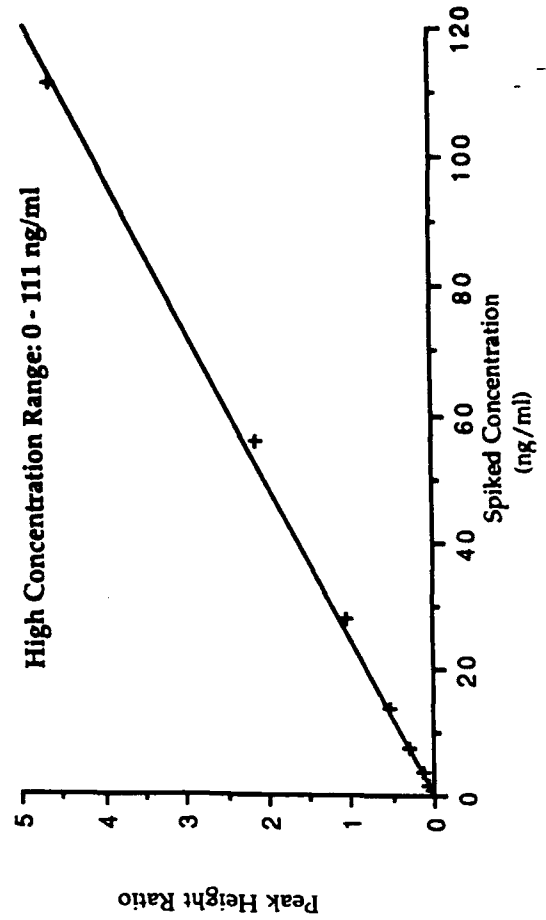


**TABLE 1C: REPRESENTATIVE STANDARD CURVE FOR
HALOFANTRINE (FREE BASE) HUMAN BLOOD
ASSAY, STUDY REPORT 17**

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	0
0.960	0.023	0.76 ^a
1.92	0.065	1.71 ^a
3.84	0.160	3.84 ^a
7.68	0.342	7.93 ^a
14.4	0.626	14.3 ^a
28.8	1.542	32.5 ^b
57.6	2.662	55.9 ^b
115	5.487	115 ^b

Regression equations:

^a $y = 0.04452x - 0.01105$, $r^2 = 0.9985$ (Low Range: 0 - 14.4 ng/ml)

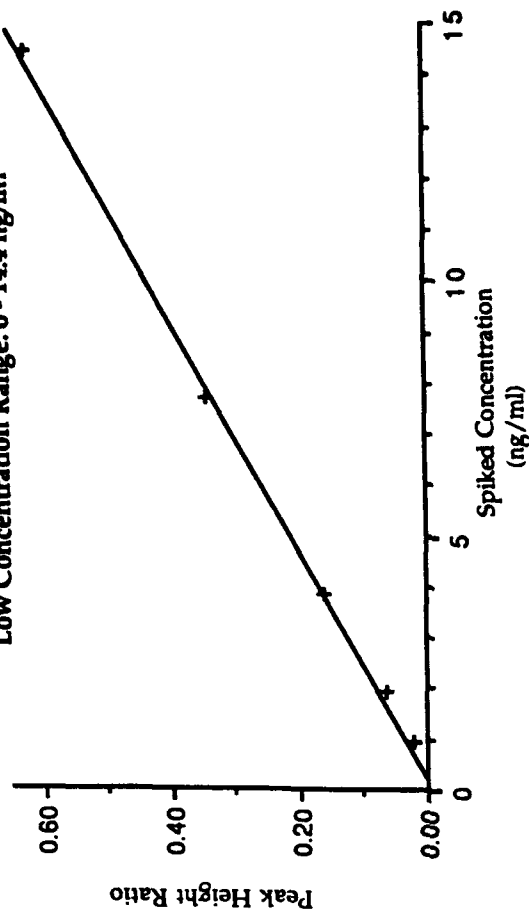
^b $y = 0.04776x - 0.0098$, $r^2 = 0.9985$ (High Range: 0 - 115 ng/ml)

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Standard Curve

Low Concentration Range: 0 - 14.4 ng/ml



High Concentration Range: 0 - 115 ng/ml

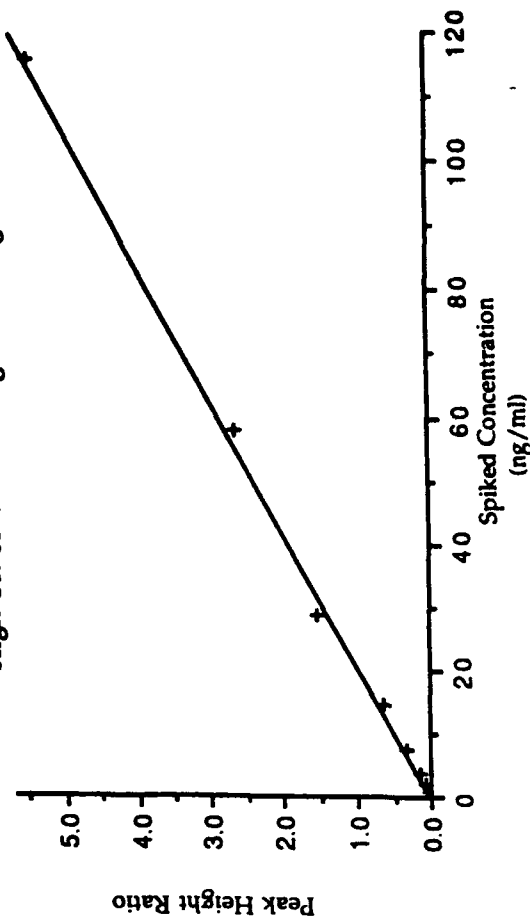


TABLE 1D: REPRESENTATIVE STANDARD CURVE FOR WR
178,460 (FREE BASE) HUMAN BLOOD ASSAY,
STUDY REPORT 17

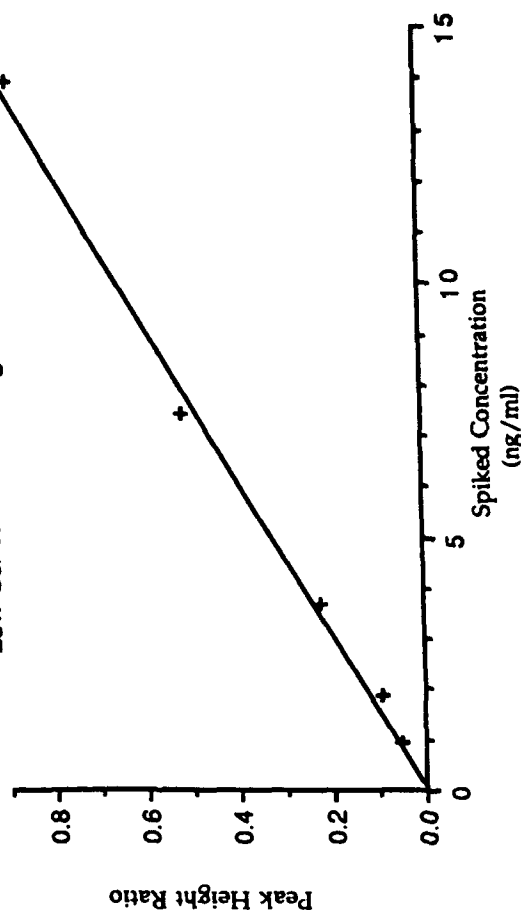
SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	0
0.928	0.054	0.931 ^a
1.86	0.095	1.56 ^a
3.71	0.223	3.52 ^a
7.42	0.520	8.06 ^a
13.9	0.886	13.7 ^a
27.8	2.055	30.2 ^b
55.7	3.769	55.5 ^b
111	7.508	111 ^b

Regression equations:

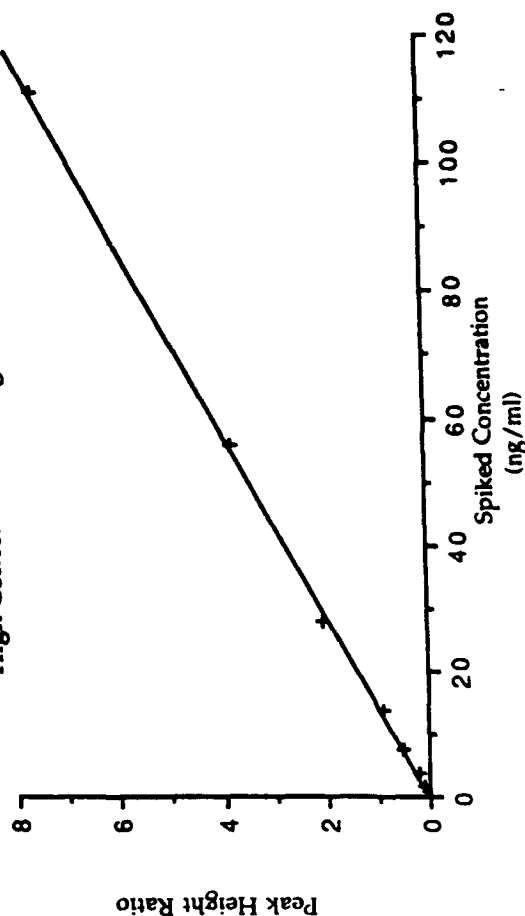
$$a_y = 0.06540x - 0.0069, \quad r^2 = 0.9956 \text{ (Low Range: 0 - 13.9 ng/ml)}$$

$$b_y = 0.06786x + 0.0027, \quad r^2 = 0.9993 \text{ (High Range: 0 - 111 ng/ml)}$$

Representative Standard Curve
Low Concentration Range: 0 - 13.9 ng/ml



High Concentration Range: 0 - 111 ng/ml



* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

TABLE 2A: RECOVERY OF HALOFANTRINE (FREE BASE) FROM HUMAN PLASMA

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		SOLVENT	PLASMA
1	1.92	0.056	0.043
2		0.069	0.045
3		0.071	bc
MEAN $\pm$ SD		0.065	0.044
MEAN RECOVERY (0.044/0.065)100 = 67.7%			
1	9.60	0.282	0.203
2		0.256	0.232
3		0.243	0.201
MEAN $\pm$ SD		0.260	0.212
MEAN RECOVERY (0.212/0.260)100 = 81.5%			
1	38.4	1.079	0.890
2		1.168	0.805
3		1.087	0.872
MEAN $\pm$ SD		1.111	0.856
MEAN RECOVERY (0.856/1.111)100 = 77.1%			
1	76.8	2.308	1.594
2		2.444	1.394
3		2.002	1.443
MEAN $\pm$ SD		2.251	1.477
MEAN RECOVERY (1.477/2.251)100 = 65.6%			

OVERALL AVERAGE RECOVERY = 73.0%

bc = bad chromatograph

TABLE 2B: RECOVERY OF WR 178,460 (FREE BASE) FROM HUMAN PLASMA

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		SOLVENT	PLASMA
1	1.86	0.064	0.062
2		bc	0.077
3		0.069	0.059
MEAN $\pm$ SD		0.067	0.076
MEAN RECOVERY (0.076/0.067)100 = 98.5%			
1	9.28	0.299	0.240
2		0.320	0.294
3		0.239	0.270
MEAN $\pm$ SD		0.286	0.268
MEAN RECOVERY (0.268/0.286)100 = 93.7%			
1	37.1	0.989	1.062
2		1.140	1.155
3		1.034	1.044
MEAN $\pm$ SD		1.054	1.087
MEAN RECOVERY (1.087/1.054)100 = 103%			
1	74.2	2.359	2.137
2		2.511	1.952
3		2.071	1.945
MEAN $\pm$ SD		2.314	2.011
MEAN RECOVERY (2.011/2.314)100 = 86.9%			
OVERALL AVERAGE RECOVERY = 95.5%			

bc = bad chromatograph

TABLE 2C: RECOVERY OF HALOFANTRINE (FREE BASE) FROM HUMAN BLOOD

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		SOLVENT	BLOOD
1	1.92	0.067	0.038
2		0.073	0.049
3		bc	0.053
MEAN $\pm$ SD		0.070	0.047
MEAN RECOVERY (0.047/0.070)100 = 67.1%			
1	9.60	0.278	0.208
2		0.274	0.222
3		0.303	0.177
MEAN $\pm$ SD		0.285	0.202
MEAN RECOVERY (0.202/0.285)100 = 70.9 %			
1	38.4	1.272	0.819
2		1.194	0.687
3		1.214 tube broken	
MEAN $\pm$ SD		1.227	0.753
MEAN RECOVERY (0.753/1.227)100 = 61.4 %			
1	76.8	2.150	1.864
2		2.322	1.488
3		2.197	1.647
MEAN $\pm$ SD		2.223	1.666
MEAN RECOVERY (1.666/2.223)100 = 74.9%			
OVERALL AVERAGE RECOVERY = 68.6%			

bc = bad chromatograph

TABLE 2D: RECOVERY OF WR 178,460 (FREE BASE) FROM HUMAN BLOOD

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		SOLVENT	BLOOD
1	1.86	0.061	0.054
2		0.076	0.061
3		bc	0.057
MEAN $\pm$ SD		0.069	0.057
MEAN RECOVERY (0.057/0.069)100 = 82.6%			
1	9.28	0.239	0.301
2		0.254	0.283
3		0.271	0.269
MEAN $\pm$ SD		0.255	0.284
MEAN RECOVERY (0.284/0.255)100 = 111%			
1	37.1	0.974	0.997
2		0.959	1.056
3		1.184	tb
MEAN $\pm$ SD		1.039	1.027
MEAN RECOVERY (1.027/1.039)100 = 98.9%			
1	74.2	2.276	2.227
2		2.264	2.103
3		2.360	1.964
MEAN $\pm$ SD		2.300	2.098
MEAN RECOVERY (2.098/2.300)100 = 91.2 %			

OVERALL AVERAGE RECOVERY = 95.9%

bc = bad chromatograph
tb = test tube broken

**TABLE 3A: INTRADAY PRECISION OF HALOFANTRINE (FREE BASE)
HUMAN PLASMA ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.92	1.80	1.80	2.49	2.36	2.09	2.26	2.13	0.289	13.6
9.60	8.77	8.14	8.96	8.87	8.98	8.46	8.70	0.332	3.82
38.4	33.0	33.7	37.1	34.7	32.0	35.8	34.4	1.87	5.44
76.8	72.5	75.2	81.6	75.9	70.4	80.6	76.0	4.40	5.79

**TABLE 3B: INTRADAY PRECISION OF WR 178,460 (FREE BASE) HUMAN
PLASMA ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.86	1.81	1.79	2.09	2.02	1.81	2.02	1.92	0.134	6.98
9.28	8.71	9.49	9.15	8.74	9.52	8.75	9.06	0.381	4.21
37.1	33.5	35.4	34.8	36.0	38.4	35.5	35.6	1.62	4.55
74.2	71.7	70.8	76.1	72.9	65.1	82.5	73.2	5.81	7.94

* Measured concentrations are averages of two analyses.

**TABLE 3C: INTERDAY PRECISION OF HALOFANTRINE (FREE BASE)
HUMAN PLASMA ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.92	2.04	1.92	1.79	1.94	1.80	2.10	1.93	0.125	6.48
9.60	9.61	9.44	8.52	9.02	10.9	10.1	9.60	0.833	8.68
38.4	40.6	38.0	41.7	30.9	39.4	42.0	38.8	4.13	10.6
76.8	73.2	74.8	78.9	75.5	73.3	81.2	76.2	3.23	4.24

**TABLE 3D: INTERDAY PRECISION OF WR 178,460 (FREE BASE) HUMAN
PLASMA ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.86	1.91	1.96	1.74	2.09	1.79	1.96	1.91	0.127	6.65
9.28	9.81	8.31	8.90	9.09	9.91	9.52	9.26	0.609	6.58
37.1	39.7	37.0	38.1	36.4	40.5	41.0	38.8	1.90	4.90
74.2	69.34	73.5	71.7	81.4	78.0	80.6	75.8	4.95	6.53

* Measured concentrations are averages of two analyses.

**TABLE 4A INTRADAY PRECISION OF HALOFANTRINE (FREE BASE)
HUMAN BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.88	2.16	1.92	2.20	1.80	1.70	1.96	1.96	0.196	10.0
0.942	8.65	9.74	9.70	10.1	9.56	10.1	9.64	0.534	5.54
37.7	34.2	35.2	42.3	36.6	35.3	40.4	37.3	3.26	8.74
75.4	69.2	67.0	68.2	64.6	74.1	67.9	68.5	3.15	4.60

**TABLE 4B: INTRADAY PRECISION OF WR 178,460 (FREE BASE) HUMAN
BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.85	2.14	1.90	1.66	1.75	1.72	1.82	1.83	0.172	9.40
9.24	8.49	9.74	9.70	9.99	9.88	9.54	9.56	0.545	5.70
37.0	34.3	34.2	41.8	35.7	34.7	40.2	36.8	3.32	9.02
73.9	69.9	65.9	66.8	62.2	76.0	65.4	67.7	4.76	7.03

* Measured concentrations are averages of two analyses.

**TABLE 4C: INTERDAY PRECISION OF HALOFANTRINE (FREE BASE)
HUMAN BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.92	2.03	1.78	2.04	2.06	2.01	1.94	1.98	0.105	5.30
9.60	9.12	9.73	8.87	10.1	8.92	9.60	9.39	0.496	5.28
38.4	33.9	36.3	36.9	35.6	37.4	35.6	36.0	1.23	3.42
76.8	81.5	87.9	71.1	69.5	79.0	67.4	76.1	8.01	10.5

**TABLE 4D: INTERDAY PRECISION OF WR 178,460 (FREE BASE) HUMAN
BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
1.86	1.75	1.58	2.07	1.88	2.04	1.70	1.89	0.149	7.88
9.28	9.04	9.52	8.38	9.22	8.79	10.1	9.18	0.596	6.49
37.1	35.4	36.6	34.8	34.3	41.4	39.1	36.9	2.78	7.11
74.2	78.4	80.5	77.2	68.5	76.0	69.3	75.0	4.95	6.60%

* Measured concentrations are averages of two analyses.

**TABLE A: ACCURACY OF HALOFANTRINE (FREE BASE) HUMAN
BLOOD ASSAY (BLIND STUDY RESULTS) May 93**

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
1	0	*	Mean =
12		*	SD =
13		*	Percent CV =
24		*	Percent Bias =
2	2.04	1.82	Mean = 2.05
11		2.05	SD = 0.170
15		2.09	Percent CV = 8.31
22		2.23	Percent Bias = 0.368
3	20.4	20.0	Mean = 19.8
10		20.0	SD = 0.300
14		19.6	Percent CV = 1.52
23		19.4	Percent Bias = -3.19
4	40.8	40.8	Mean = 39.3
8		36.5	SD = 2.19
17		41.3	Percent CV = 5.58
21		38.7	Percent Bias = -3.62
5	102	90.9	Mean = 93.6
9		92.9	SD = 2.24
16		96.1	Percent CV = 2.39
20		94.6	Percent Bias = -8.21
6	183.6	171	Mean = 173
7		172	SD = 2.63
18		173	Percent CV = 1.52
19		177	Percent Bias = -5.64

[#] n = 3, unless a chromatogram is determined to be unacceptable.

* = Below assay sensitivity.

TABLE B: ACCURACY OF WR 178,460 (FREE BASE) HUMAN BLOOD ASSAY (BLIND STUDY RESULTS) May 93

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
6	0	0.893	Mean = 1.03
7		1.05	SD = 0.121
18		0.982	Percent CV = 11.8
19		1.18	Percent Bias =
5	1.97	2.52	Mean = 2.635
9		2.50	SD = 0.150
16		2.71	Percent CV = 5.70
20		2.81	Percent Bias = 33.8
4	19.7	20.2	Mean = 19.8
8		19.3	SD = 0.45
17		19.6	Percent CV = 2.27
21		20.2	Percent Bias = 0.635
3	39.4	38.2	Mean = 38.4
10		37.6	SD = 0.665
14		38.8	Percent CV = 1.73
23		39.1	Percent Bias = -2.47
2	98.6	95.2	Mean = 98.3
11		96.3	SD = 3.12
15		102	Percent CV = 3.18
22		99.7	Percent Bias = -0.304
1	177.5	178	Mean = 181
12		178	SD = 3.79
13		186	Percent CV = 2.10
24		180	Percent Bias = 1.69

[#] n = 3, unless a chromatogram is determined to be unacceptable.

TABLE 6A: STABILITY OF HALOFANTRINE AND WR 178,460 (AS FREE BASES) IN PLASMA*

HALOFANTRINE (FREE BASE) CONCENTRATION IN PLASMA STORED AT -80°C				
CONCENTRATION (ng/ml)				
Spiked Concentration:	4.50	10.8	28.8	63.0
DAYS STORED				
0	4.68	9.96	26.9	59.0
1	4.76	11.2	26.2	56.9
2	4.20	9.76	25.8	57.6
29	4.85	10.8	24.5	56.2
60	3.99	10.3	30.1	63.5
92	3.68	9.41	21.4	45.6
112	3.81	8.91	23.4	55.2
126	4.02	10.2	27.3	55.4
MEAN	4.25	10.1	25.7	56.2

WR 178,460 (FREE BASE) CONCENTRATION IN PLASMA STORED AT -80°C				
CONCENTRATION (ng/ml)				
Spiked Concentration:	7.00	16.8	44.8	98.0
DAYS STORED				
0	7.78	17.4	50.7	101
1	6.30	17.3	47.8	104
2	7.73	21.6	51.2	108
29	8.42	19.7	46.7	104
60	6.91	16.6	48.4	100
92	5.34	15.3	45.0	95.4
112	7.16	16.6	44.6	106
126	7.40	18.1	45.7	86.4
MEAN	7.13	17.8	47.5	101

Concentrations are means of multiple (usu. 3) analyses.

* Table taken from Project Status Report No. 7, dated Dec. 23, 1987 from data obtained according to the method described in Study Report No. 4, dated Aug. 23, 1985 and titled "Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite (WR 178,460) in Blood and Plasma."

TABLE 6B: STABILITY OF HALOFANTRINE AND WR 178,460 (AS FREE BASES) IN BLOOD*

HALOFANTRINE (FREE BASE) CONCENTRATION IN BLOOD STORED AT -80°C				
		CONCENTRATION (ng/ml)		
Spiked Concentration:	4.50	10.8	28.8	63.0
DAYS STORED				
0	5.57	12.0	31.2	65.0
1	5.29	11.2	28.9	61.4
4	4.62	9.98	28.5	63.4
29	3.72	9.25	24.4	51.7
61	4.47	10.7	27.9	60.3
90	4.86	11.5	28.2	45.9
110	5.30	13.9	30.5	55.5
128	3.99	11.6	27.3	60.1
MEAN	4.73	11.3	28.4	57.9

WR 178,460 (FREE BASE) CONCENTRATION IN BLOOD STORED AT -80°C				
		CONCENTRATION (ng/ml)		
Spiked Concentration:	7.00	16.8	44.8	98.0
DAYS STORED				
0	8.62	19.1	50.6	102
1	10.1	17.6	39.7	107
4	6.73	17.7	47.4	101
29	7.84	21.2	49.5	108
61	6.66	17.1	44.2	94.2
90	8.84	20.0	46.1	69.2
110	7.77	16.5	44.9	85.6
128	5.80	18.2	45.1	90.5
MEAN	7.80	18.4	45.9	94.7

Concentrations are means of multiple (usu. 3) analyses.

* Table taken from Project Status Report No. 7, dated Dec. 23, 1987 from data obtained according to the method described in Study Report No. 4, dated Aug. 23, 1985 and titled "Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite (WR 178,460) in Blood and Plasma."

I. LABORATORY METHODOLOGY FOR WR 6026 AND WR 211,789 (AS FREE BASES) PLASMA ASSAY,* STUDY REPORT 18

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA), or equivalent.
2. Altex Model 100A Solvent Delivery Module (Beckman Instruments, Inc., Berkeley, CA), or equivalent.
3. Kratos Spectroflow 783 UV Detector (Kratos Analytical Instruments, Ramsey, NJ), or equivalent.
4. Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA), or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. WR 6026•2HCl - (WRAIR, Washington, D.C.).
4. WR 211,789•2HCl•1/2H₂O - (WRAIR, Washington, D.C.).
5. Chlorpheniramine maleate (Internal Standard) - (Sigma Chemical Co., St. Louis, MO).
6. NaOH (Mallinckrodt, Paris, KY).
7. Water prepared by Nanopure II system (Barnstead Co., Boston, MA).
8. Methyl-*t*-butyl ether (Baxter, Burdick & Jackson, Muskegan, MI).
9. Dibasic ammonium phosphate (Fisher Scientific, Fairlawn, NJ).
10. Methanol (Fisher Scientific, Fairlawn, NJ).
11. Acetonitrile (Fisher Scientific, Fairlawn, NJ).
12. Phosphoric acid (Fisher Scientific, Fair Lawn, NJ).

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C. ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength; 263 nm

Sensitivity; 0.004 aufs

Rise Time; 1 s

Lamp

ABI Analytical, Inc. (Ramsey, NJ).

2. COLUMN

Altex Ultrasphere Si gel, 5 μ m silica, 4.6 x 250 mm - (Beckman Instruments Inc., Berkeley, CA).

3. MOBILE PHASE

CH₃CN/water (60:40, v/v) with a final concentration of 5 mM (NH₄)₂HPO₄ and solution adjusted to pH 8.8.

4. FLOW RATE

1.0 ml/min

5. STOCK SOLUTIONS - Store at -20°C, protect from light, and check for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds (discard solutions if a more than 10% change in the ratio is observed. In any case, discard solutions within 6 months.

a. WR 6026 and WR 211,789 (as free base bases).

Standard curve stock solution. Dissolve 5.95 mg of WR 6026•2HCl and 7.60 mg of WR 211,789•2HCl•1/2H₂O in and bring to 100 ml with methanol/water (1:1, v/v).

WR 6026

Conc. = 59.5 μ g/ml (dihydrochloride)

Conc. = 49.0 μ g/ml (free base)

WR 211,789

Conc. = 76.0 μ g/ml (dihydrochloride, hemihydrate)

Conc. = 60.3 μ g/ml (free base)

7. RETENTION TIMES (subject to change depending on temperature and column performance).

- a. WR 6026 (free base) - 7.6 min
- b. WR 211,789 (free base) - 6.6 min
- c. Chlorpheniramine (Internal Standard) - 11.4 min

8. QUANTITATION

By peak height ratio of drug or metabolite peak relative to internal standard peak.

9. MINIMUM DETECTION LIMIT OF METHOD

The minimum detection limits, 0.980 ng/ml WR 6026 and 1.21 ng/ml WR 211,789 (as free bases) in plasma, was determined as the WR 6026 (free base) and WR 211,789 (free base) concentrations at which the signal to noise ratio was at least 3 to 1.

10. INJECTION VOLUME

50 to 190 μ l

11. SAMPLE VOLUME MEASUREMENT

Pipetters used for sample volume measurement were Rainin, Gilson Pipettman and/or Eppendorf. See SOP 3-4 for calibration procedure.

12. PLASMA

Plasma (CPD or CPDA-1 as anticoagulant) was obtained from Irwin Memorial Blood Bank, San Francisco, CA.

13. WISP OPERATING TEMPERATURE

Room Temperature

D. SAMPLE STORAGE

All plasma samples were kept frozen at -80°C before analysis and thawed at room temperature for preparation and analysis.

E. SAMPLE PREPARATION

PLASMA

- 1. Pipette 0.5 ml plasma into 16x125 mm silanized screw-top tube.

Control stock solution. Dissolve 6.09 mg of WR 6026•2HCl and 7.19 mg of WR 211,789•2HCl•1/2H₂O in and bring to 100 ml with methanol/water (1:1, v/v).

WR 6026

Conc. = 60.9 µg/ml (dihydrochloride)

Conc. = 50.2 µg/ml (free base)

WR 211,789

Conc. = 71.9 µg/ml (dihydrochloride, hemihydrate)

Conc. = 57.1 µg/ml (free base)

b. Chlorpheniramine maleate (Internal Standard)

Dissolve 10.0 mg of chlorpheniramine maleate in and bring to 100 ml with acetonitrile/water (1:1, v/v).

Conc. = 100 µg/ml

6. WORKING SOLUTIONS (Store solutions at -20°C, protect from light, and discard if deterioration is observed in the stock solutions). In any case, solutions are discarded within 6 months.

a. WR 6026 and WR 211,789 (as free base bases).

Standard curve working solution. Dilute 1.0 ml of 49.0 µg/ml WR 6026 (free base) and 60.3 µg/ml WR 211,789 (free base) stock solution to 100 ml with methanol/water (1:1, v/v).

Conc. = 0.490 µg/ml WR 6026 (free base)

Conc. = 0.603 µg/ml WR 211,789 (free base)

Control working solution. Dilute 1.0 ml of 50.2 µg/ml WR 6026 (free base) and 57.1 µg/ml WR 211,789 (free base) stock solution to 100 ml with methanol/water (1:1, v/v).

Conc. = 0.502 µg/ml WR 6026 (free base)

Conc. = 0.571 µg/ml WR 211,789 (free base)

b. Chlorpheniramine maleate (Internal Standard).

Internal standard working solution. Dilute 1.00 ml of 100 µg/ml chlorpheniramine maleate stock solution to 100 ml with acetonitrile/water (1:1, v/v).

Conc. = 1.00 µg/ml

2. Spike calibration samples with 00,* 0,** 1, 2, 3, 5, 8, 15, 25, 40, 75, or 100 μl of WR6026 (0.490 $\mu\text{g}/\text{ml}$ as free base) and WR 211,789 (0.600 $\mu\text{g}/\text{ml}$ as free base) working solution to make final free base concentrations of 00, 0, 0.980, 1.96, 2.94, 4.90, 7.84, 14.7, 24.5, 39.2, 73.5, and 98.0 ng/ml of WR6026 (free base) and 00, 0, 1.21, 2.41, 3.62, 6.03, 9.65, 18.1, 30.2, 48.2, 90.5, and 121 ng/ml of WR 211,789 (free base).
3. Add 100 μl of internal standard working solution (chlorpheniramine maleate, 1 $\mu\text{g}/\text{ml}$).
4. Add 100 μl of 1 M NaOH. Vortex for 10 s.
5. Add 5 ml methyl-*t*-butyl ether. Vortex for 1 min.
6. Centrifuge for 10 min at full speed.
7. Freeze aqueous layer in dry ice/methanol bath.
8. Pour organic layer (ether) into a 13 x 100 mm silanized culture tube and evaporate to dryness.
9. Reconstitute in 200 μl mobile phase.
10. Transfer to silanized WISP inserts and inject onto column.

BLOOD

1. Pipette 0.5 ml blood into 16x125 mm silanized screw-top tube.
2. Spike calibration samples with 00,* 0,** 1, 2, 3, 5, 8, 15, 25, 40, 75, or 100 μl of WR6026 (0.490 $\mu\text{g}/\text{ml}$ as free base) and WR 211,789 (0.600 $\mu\text{g}/\text{ml}$ as free base) working solution to make final free base concentrations of 00, 0, 0.980, 1.96, 2.94, 4.90, 7.84, 14.7, 24.5, 39.2, 73.5, and 98.0 ng/ml of WR6026 (free base) and 00, 0, 1.21, 2.41, 3.62, 6.03, 9.65, 18.1, 30.2, 48.2, 90.5, and 121 ng/ml of WR 211,789 (free base). Vortex samples for 20 s, and let stand 1 h.
3. Add 0.5 ml nanopure water, vortex 1 min, and sonicate for 10 min.
4. Follow Steps 3-11 of Plasma Sample Preparation (above).

* 00 = Sample with no drug and no internal standard.

** 0 = Sample with no drug, but with internal standard.

F. QUALITY CONTROL

1. CONTENT AND FREQUENCY OF BLANKS

No special blank was used except for the standard curve and patient blanks.

2. PIPETTE CALIBRATION

See SOP 3-4.1.

3. BALANCE CALIBRATION

See SOP 3-19.1

G. PREPARATION OF PRECISION VALIDATION SAMPLES

Samples for precision analysis were prepared by spiking 0.5 ml plasma or blood specimens with 2, 8, 50, or 75 μ l of 0.515 μ g/ml WR 6026 and 0.534 μ g/ml WR 211,789 (as free bases) working solution to make final WR 6026 concentrations of 2.06, 8.24, 51.5, and 77.3 ng/ml and final WR 211,789 (free base) concentrations of 2.14, 8.54, 53.4, and 80.1 ng/ml. Samples were prepared and run with standard curve samples.

H. RECOVERY

Recovery was assessed by comparing the peak height ratios of WR 6026 and WR 211,789 (as free bases) to the internal standard in plasma and in blood to the peak height ratios of WR 6026 (free base) and WR 211,789 (as free bases) to the internal standard in mobile phase. Plasma or blood samples (0.5 ml) were spiked with 2, 8, 50, or 75 μ l of 0.515 μ g/ml WR 6026 and 0.534 μ g/ml WR 211,789 (as free bases) working solution to make final WR 6026 concentrations of 2.06, 8.24, 51.5, and 77.3 ng/ml and final WR 211,789 (free base) concentrations of 2.14, 8.54, 53.4, and 80.1 ng/ml. Each sample was prepared as described in Sample Preparation (Section E), except that the internal standard was not added to the blood or plasma samples until after the nitrogen evaporation step. Reference samples were made by adding the proper amounts of WR 6026 and WR 211,789 (as free bases) and internal standard solutions to 200 μ l of mobile phase.

I. STABILITY

Pooled plasma samples (20 ml) were spiked with 9, 40, 140, or 320 μ l of 4.9 μ g/ml WR 6026 (free base) working solution to make 2.20, 9.78, 34.1, and 77.2 ng/ml WR 6026 (free base) concentrations, mixed on a

rotator for one hour at room temperature, then pipetted in 0.5 ml fractions into silanized culture tubes and kept frozen at -20°C until assayed for stability. Pooled blood samples (25 ml) were spiked with 10, 50, 175, or 400 µl of 4.9 µg/ml WR 6026 (free base) working solution to make 1.96, 9.78, 34.1, and 77.2 ng/ml WR 6026 (free base) concentrations, or pooled blood samples (25 ml) were spiked with 11, 48, 155, or 375 µl of 4.9 µg/ml WR 6026 (free base) working solution to make 2.16, 9.39, 30.2, and 72.4 ng/ml WR 6026 (free base) concentrations, mixed on a rotator for one hour at room temperature, then pipetted in 0.5 ml fractions into silanized culture tubes and kept frozen at -20°C or -80°C until assayed for stability.

J. RESULTS

1. STANDARD CURVE

Chromatograms for each point in a representative standard curve for WR 6026 (free base) appear in Figure 5 for plasma samples and Figure 6 for blood samples. Peak height ratios for these calibrators appear in Tables 1A and B.

2. RECOVERY

Results for this evaluation appear in Tables 2A-B.

3. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 3 and 4.

4. STABILITY

Results appear in Table 5.

TABLE 1A: REPRESENTATIVE STANDARD CURVE FOR
WR 6026 (FREE BASE) PLASMA ASSAY,
STUDY REPORT 18

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	-
0.980	0.021	1.25 ^a
1.96	0.041	2.16 ^a
2.94	0.053	2.70 ^a
4.90	0.096	4.66 ^a
7.84	0.149	7.06 ^a
14.7	0.328	15.2 ^a
24.5	0.514	19.5 ^b
39.2	0.950	34.0 ^b
73.5	2.183	75.1 ^b
98.0	2.939	100 ^b

Regression equations:

$$a_y = 0.0220x - 0.0065, \quad r^2 = 0.9926 \text{ (Low Range: 0 - 14.7 ng/ml)}$$

$$b_y = 0.0300x - 0.0719, \quad r^2 = 0.9930 \text{ (High Range: 0 - 98.0 ng/ml)}$$

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Plasma Standard Curve

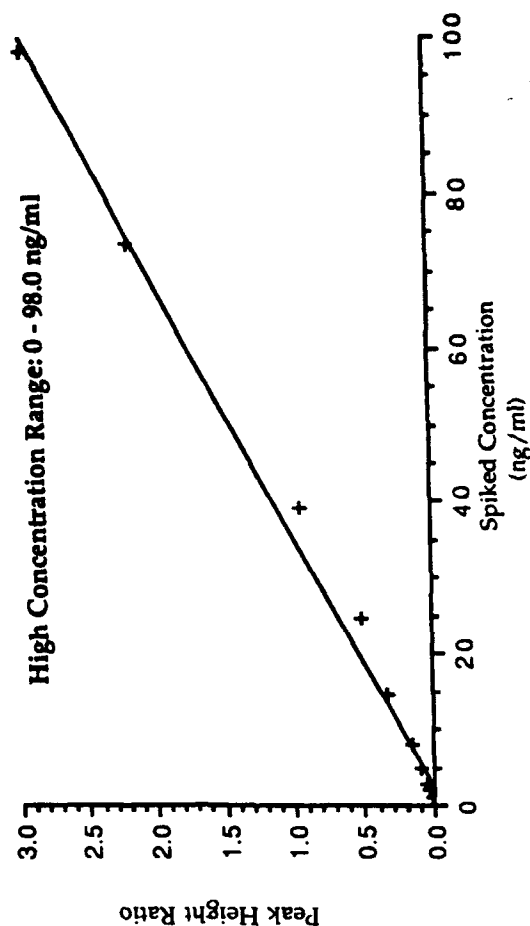
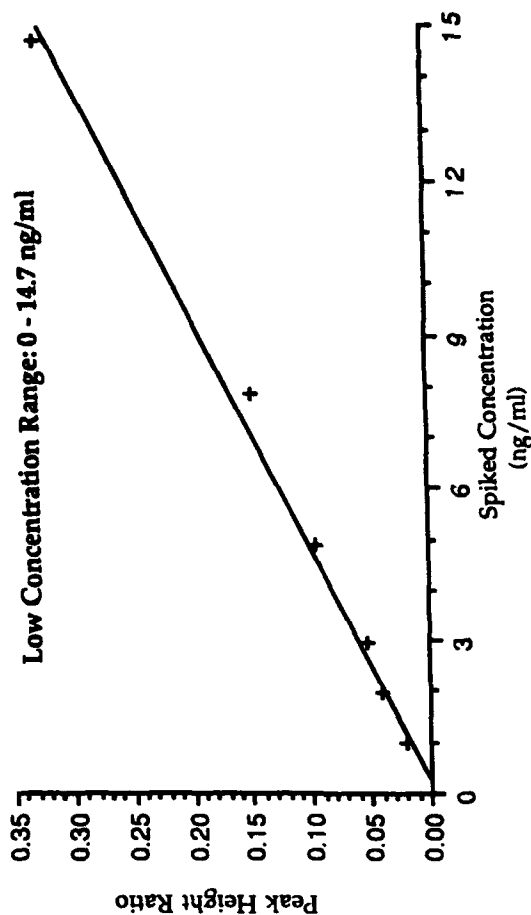


TABLE 1B: REPRESENTATIVE STANDARD CURVE FOR
WR 211,789 (FREE BASE) PLASMA ASSAY,
STUDY REPORT 18

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	-
1.21	0.028	1.38 ^a
2.41	0.066	2.56 ^a
3.62	0.102	3.68 ^a
6.03	0.166	5.67 ^a
9.65	0.253	8.38 ^a
18.1	0.590	18.9 ^a
30.2	0.931	29.2 ^b
48.2	1.725	54.2 ^b
90.5	2.991	94.0 ^b
121	3.703	116 ^b

Regression equations:

$$a_y = 0.0321x - 0.0163, \quad r^2 = 0.9890 \text{ (Low Range: 0 - 18.1 ng/ml)}$$

$$b_y = 0.0318x + 0.0035, \quad r^2 = 0.9955 \text{ (High Range: 0 - 121 ng/ml)}$$

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Plasma Standard Curve

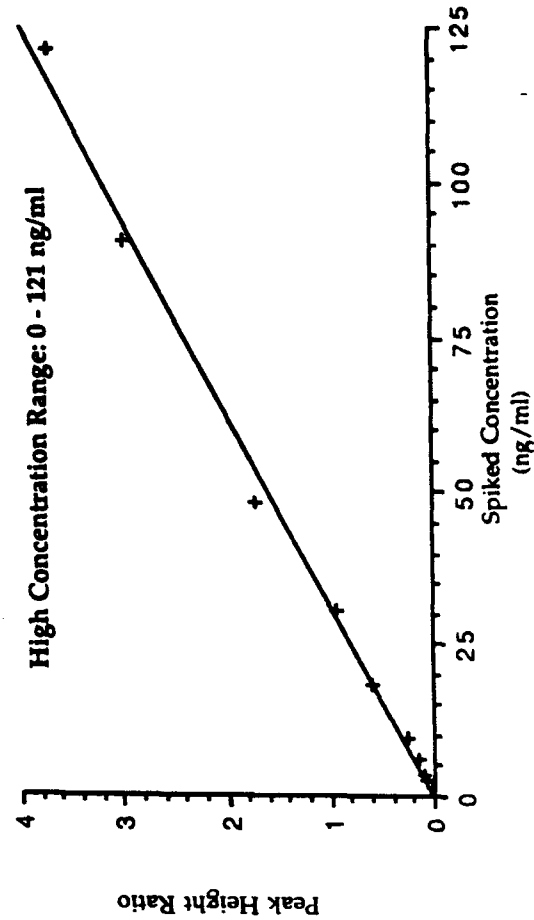
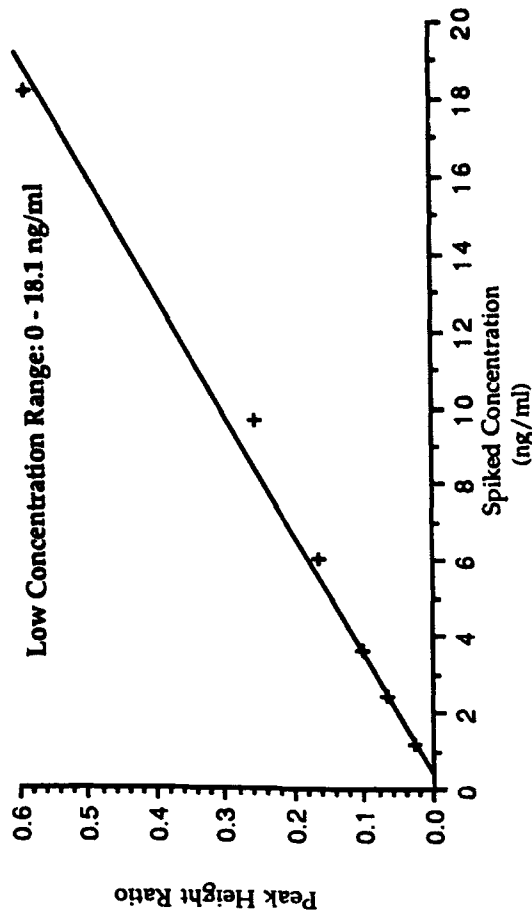


**TABLE 1C: REPRESENTATIVE STANDARD CURVE FOR
WR 6026 (FREE BASE) BLOOD ASSAY,
STUDY REPORT 18**

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	-
0.980	0.026	1.00 ^a
1.96	0.051	1.94 ^a
2.94	0.082	3.10 ^a
4.90	0.125	4.72 ^a
7.84	0.206	7.76 ^a
14.7	0.393	14.8 ^a
24.5	0.654	24.1 ^b
39.2	1.070	39.2 ^b
73.5	1.978	72.2 ^b
98.0	2.717	99.0 ^b

Regression equations:^{**}

$a y = 0.0266x - 0.0007$, $r^2 = 0.9995$ (Low Range: 0 - 14.7 ng/ml)

$b y = 0.0275x - 0.0073$, $r^2 = 0.9997$ (High Range: 0 - 98.0 ng/ml)

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Blood Standard Curve

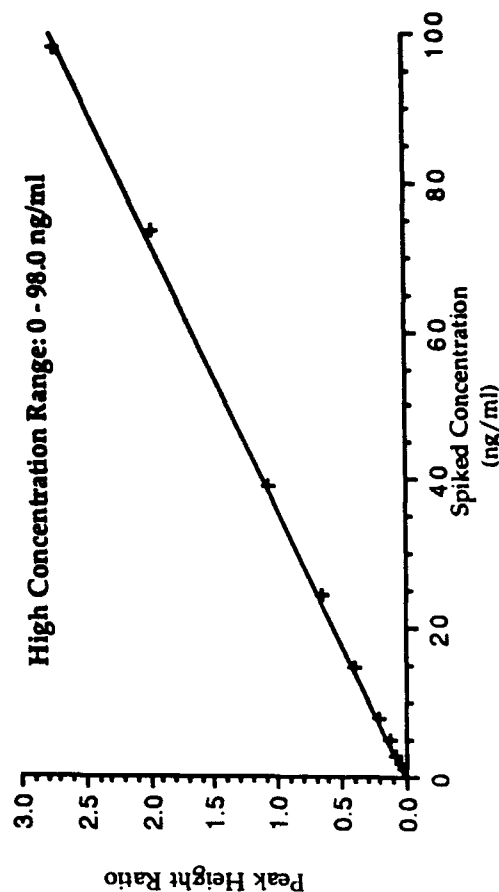
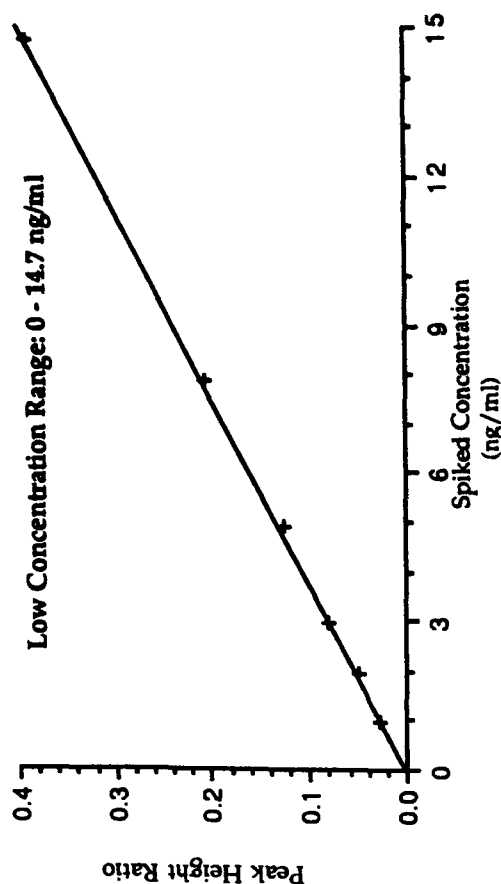


TABLE 1D: REPRESENTATIVE STANDARD CURVE FOR
WR 211,789 (FREE BASE) BLOOD ASSAY,
STUDY REPORT 18

SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO*	CALCULATED CONCENTRATION (ng/ml)
0	0	-
1.21	0.027	1.23 ^a
2.41	0.059	2.36 ^a
3.62	0.094	3.60 ^a
6.03	0.162	6.00 ^a
9.65	0.252	9.17 ^a
18.1	0.513	18.4 ^a
30.2	0.860	30.2 ^b
48.2	1.371	47.9 ^b
90.5	2.540	88.4 ^b
121	3.532	123 ^b

Regression equations:

$a_y = 0.0284x - 0.0080$, $r^2 = 0.9982$ (Low Range: 0 - 18.1 ng/ml)

$b_y = 0.0289x - 0.0140$, $r^2 = 0.9994$ (High Range: 0 - 121 ng/ml)

* Ratio of drug peak height to internal standard peak height.

** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Blood Standard Curve

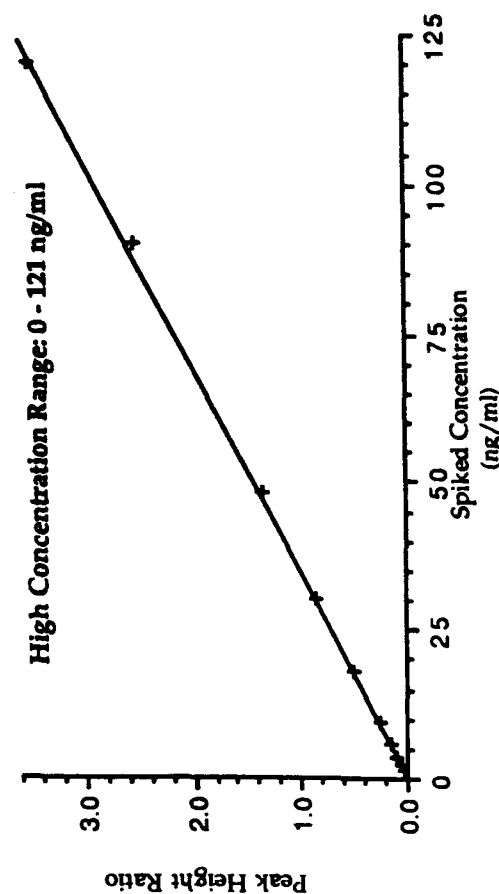
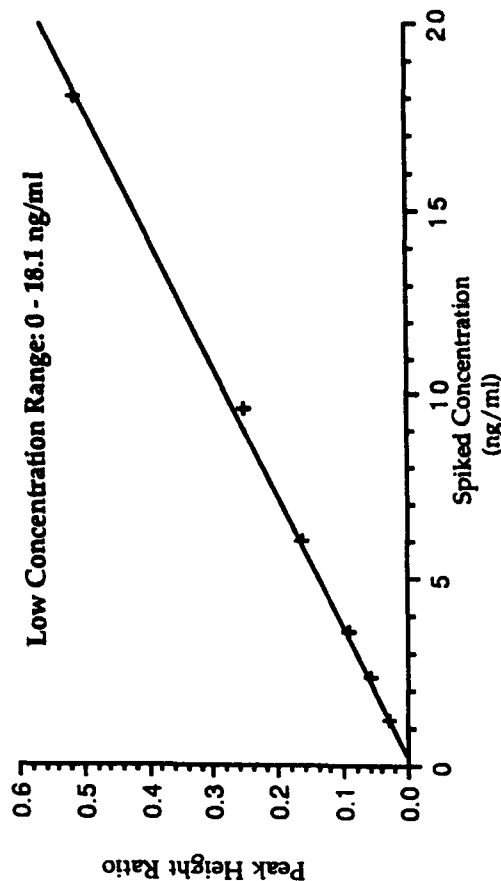


TABLE 2A: RECOVERY OF WR 6026 (FREE BASE) FROM HUMAN PLASMA

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		REFERENCE	PLASMA
1	77.3	2.354	1.883
2		2.248	1.890
3		2.417	2.406
MEAN $\pm$ SD		2.340 $\pm$ 0.085	2.060 $\pm$ 0.300
MEAN RECOVERY (2.060/2.340)100 = 88.0%			
1	51.5	1.490	1.083
2		1.667	1.056
3		1.737	1.049
MEAN $\pm$ SD		1.631 $\pm$ 0.127	1.063 $\pm$ 0.018
MEAN RECOVERY (1.063/1.631)100 = 65.1%			
1	8.24	0.228	0.165
2		0.222	0.147
3		0.238	0.164
MEAN $\pm$ SD		0.229 $\pm$ 0.008	0.159 $\pm$ 0.010
MEAN RECOVERY (0.159/0.229)100 = 69.2%			
1	2.06	0.049	0.037
2		0.056	0.041
3		0.050	0.039
MEAN $\pm$ SD		0.052 $\pm$ 0.004	0.039 $\pm$ 0.002
MEAN RECOVERY (0.039/0.052)100 = 75.4%			
OVERALL AVERAGE RECOVERY = 74.5%			

TABLE 2B: RECOVERY OF WR 211,789 (FREE BASE) FROM HUMAN PLASMA

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		REFERENCE	PLASMA
1	80.1	3.387	2.960
2		3.147	2.952
3		3.316	3.380
MEAN ± SD		3.283 ± 0.123	3.097 ± 0.245
MEAN RECOVERY (3.097/3.283)100 = 93.7%			
1	53.4	2.092	1.921
2		2.120	1.984
3		2.210	1.902
MEAN ± SD		2.141 ± 0.062	1.936 ± 0.043
MEAN RECOVERY (1.936/2.141)100 = 90.4%			
1	8.54	0.287	0.276
2		0.292	0.261
3		0.305	0.289
MEAN ± SD		0.295 ± 0.009	0.275 ± 0.014
MEAN RECOVERY (0.275/0.295)100 = 93.4%			
1	2.14	0.064	0.066
2		0.072	0.067
3		0.070	0.067
MEAN ± SD		0.069 ± 0.004	0.067 ± 0.001
MEAN RECOVERY (0.067/0.069)100 = 97.0%			
OVERALL AVERAGE RECOVERY = 93.8%			

TABLE 3A: INTERDAY PRECISION OF WR 6026 (FREE BASE) HUMAN PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations [*] (ng/ml)								
77.3	75.1	79.1	75.8	75.0	78.7	80.5	77.4	2.36	3.05
51.5	52.3	53.3	53.7	44.7	53.4	52.2	51.6	3.44	6.67
8.24	7.76	8.85	8.32	7.59	8.60	7.56	8.11	0.553	6.82
2.06	2.03	2.02	2.28	1.90	2.10	2.01	2.06	0.127	6.17

TABLE 3B: INTRADAY PRECISION OF WR 6026 (FREE BASE) HUMAN PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations (ng/ml)								
77.3	80.2	79.3	79.8	82.0	85.7	84.4	81.9	2.64	3.22
51.5	55.2	54.6	54.5	60.5	56.8	58.6	56.7	2.43	4.29
8.24	9.30	9.17	8.95	10.0	9.12	9.00	9.26	0.385	4.16
2.06	2.59	2.06	2.46	2.33	2.50	2.72	2.44	0.229	9.39

* n = 2

TABLE 3C: INTERDAY PRECISION OF WR 211,789 (FREE BASE) HUMAN PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations [*] (ng/ml)								
80.1	76.8	85.4	82.9	80.1	85.2	89.0	83.2	4.32	5.19
53.4	54.3	57.5	57.1	51.2	59.0	59.3	56.4	3.11	5.51
8.54	8.37	9.95	8.49	8.44	7.72	8.07	8.51	0.764	8.98
2.14	1.94	2.24	2.31	2.01	2.29	2.20	2.17	0.154	7.10

TABLE 3D: INTRADAY PRECISION OF WR 211,789 (FREE BASE) HUMAN PLASMA ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations (ng/ml)								
80.1	81.3	80.5	81.7	84.6	89.2	88.2	84.3	3.73	4.42
53.4	55.3	56.0	55.2	62.3	58.4	60.7	58.0	3.00	5.17
8.54	9.16	9.46	8.67	9.69	9.47	10.6	9.51	0.641	6.74
2.14	2.34	2.02	2.49	2.37	2.32	2.55	2.34	0.184	7.86

^{*} n = 2

TABLE 4A: INTERDAY PRECISION OF WR 6026 (FREE BASE) HUMAN BLOOD ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations [*] (ng/ml)								
78.4	77.2	79.2	79.8	79.7	79.7	77.3	78.8	1.23	1.56
34.3	33.8	35.5	34.2	32.2	35.4	35.0	34.4	1.25	3.63
9.80	9.31	9.35	9.35	10.0	9.33	9.39	9.46	0.27	2.84
1.96	1.88	1.98	2.08	2.15	1.85	2.13	2.01	0.13	6.38

TABLE 4B: INTRADAY PRECISION OF WR 6026 (FREE BASE) HUMAN BLOOD ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations (ng/ml)								
78.4	78.0	76.9	75.2	79.7	80.0	78.6	78.1	1.80	2.31
34.3	34.0	33.9	32.0	32.4	31.7	35.2	33.2	1.38	4.14
9.80	10.1	9.83	9.57	9.98	9.49	9.57	9.76	0.25	2.57
1.96	1.78	2.04	1.96	2.00	2.08	1.93	1.97	0.11	5.36

^{*} n = 2

TABLE 4C: INTERDAY PRECISION OF WR 211,789 (FREE BASE) HUMAN BLOOD ASSAY

SPIKED CONC. (ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations* (ng/ml)								
96.0	93.9	96.7	96.5	95.6	97.4	93.2	95.6	1.67	1.74
42.0	42.9	41.8	40.2	41.7	43.5	41.3	41.9	1.17	2.80
12.0	12.2	11.6	11.4	12.7	11.3	11.3	11.75	0.58	4.90
2.40	2.59	2.25	2.29	2.37	2.39	2.46	2.39	0.12	5.12

TABLE 4D: INTRADAY PRECISION OF WR 211,789 (FREE BASE) HUMAN BLOOD ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations (ng/ml)								
96.0	99.5	96.9	99.6	101	99.3	96.5	98.8	1.74	1.76
42.0	43.0	42.0	41.7	41.8	41.7	44.0	42.4	0.94	2.22
12.0	12.0	11.9	11.8	12.1	12.3	11.7	11.97	0.22	1.81
2.40	2.34	2.31	2.38	2.49	2.31	2.60	2.41	0.12	4.85

* n=2

TABLE 5A: STABILITY OF WR 6026 (FREE BASE) IN HUMAN PLASMA***WR 6026 (Free Base) Concentration of Samples Stored at -20°C**

Spiked Concentration	CONCENTRATION** (ng/ml)			
	77.2	34.1	9.78	2.20
DAYS STORED				
0	74.7	31.8	9.65	2.09
1	76.1	33.2	9.93	2.17
2	76.7	32.1	9.66	2.37
4	75.8	32.1	9.70	2.27
7	74.2	32.2	9.56	2.32
14	74.9	32.3	10.0	1.96
22	75.7	32.2	9.89	2.29
29	72.5	30.6	9.28	1.98
59	71.6	31.3	9.69	2.17
90	75.3	32.3	9.44	2.10

* Table obtained from Study Report 10C, "Quantitation of WR 6026 (Free Base) in Plasma and Blood by High-Performance Liquid Chromatography," dated September 14, 1989.

** n = 3

TABLE 5B: STABILITY OF WR 6026 (FREE BASE) IN HUMAN BLOOD***WR 6026 (Free Base) Concentration of Samples Stored at -20°C**

		CONCENTRATION** (ng/ml)			
Spiked Concentration		77.2	34.1	9.78	1.96
DAYS STORED					
0		79.3	34.5	9.32	1.98
1		75.0	32.8	9.28	2.01
3		75.5	34.4	8.31	2.09
7		75.9	30.3	8.15	1.83
14		78.5	31.7	8.19	1.63
21		70.9	29.1	7.61	1.84
28		72.2	31.6	8.19	1.99
60		74.4	31.2	8.33	1.64
90		59.5	27.4	6.83	1.12

WR 6026 (Free Base) Concentration of Samples Stored at -80°C

		CONCENTRATION** (ng/ml)			
Spiked Concentration		72.4	30.2	9.39	2.16
DAYS STORED					
0		73.4	29.8	9.02	2.14
1		71.7	28.3	9.69	2.20
2		74.3	30.2	10.2	2.26
30		75.3	31.9	8.60	2.44
60		72.6	29.8	9.19	2.09
90		75.1	30.2	9.33	1.86

* Table obtained from Study Report 10C, "Quantitation of WR 6026 (Free Base) in Plasma and Blood by High-Performance Liquid Chromatography," dated September 14, 1989.

** n = 3

	WR 6026 (ng/ml)	WR 211789 (ng/ml)
1	10.1	50.7
2	*	103
3	2.61	25.2
4	28.6	11
5	50.7	2.89
6	107	*
7	*	102
8	27.2	10.4
9	50.5	2.47
10	105	*
11	2.53	26.4
12	10.3	50.2
13	30.6	10.5
14	114	*
15	52.4	3.22
16	2.02	28.2
17	*	104
18	9.6	54.6
19	48.6	3.25
20	94.3	*
21	*	106
22	2.66	24.4
23	9.52	50.6
24	32.4	11.3

* = below assay sensitivity

LABORATORY METHODOLOGY FOR MEFLOROQUINE (FREE BASE) HUMAN BLOOD ASSAY*

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA) or equivalent.
2. Beckman Model 100A Solvent Delivery Module (Beckman Instruments Inc., Berkeley, CA) or equivalent.
3. Kratos Spectroflow 783 Absorbance Detector (Kratos Analytical Instruments, Ramsey, NJ) or equivalent.
4. Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA) or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. Mefloquine hydrochloride (Walter Reed Army Institute of Research, Washington D.C.), bottle number BK 11592, expiration date not available.
4. Chlorpheniramine (Internal Standard) maleate, (Sigma Chemical Co., St. Louis, MO), lot number 122F 0067, expiration date not available.
5. Methanol (Fisher Scientific, Fair Lawn, NJ), lot number not available, expiration date not available.
6. Acetonitrile (Fisher Scientific, Fair Lawn, NJ), lot number not available, expiration date not available.
7. Methylene chloride (Fisher Scientific, Fair Lawn, NJ), lot number not available, expiration date not available.
8. Dibasic ammonium phosphate (Fisher Scientific, Fair Lawn, NJ), lot number 892838, expiration date not available.

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

9. Sodium carbonate (J.T. Baker Chemical Co., Phillipsburg, NJ), lot number not available, expiration date not available.
10. Pentane (American Burdick and Jackson, Muskegon, MI), lot number not available, expiration date not available.
11. Water (deionized by Nanopure II, Barnstead Co., Boston, MA).

C. ASSAY CONDITIONS

1. DETECTOR SETTINGS

Settings

Wavelength; 280 nm

Sensitivity; 0.005

Rise Time; 1 s

Lamp

ABI Analytical, Inc. (Ramsey, NJ) Model 420-LA 6VDC.

2. COLUMN

Altex Ultrasphere Silica, 5 μ m particle size, 4.6 x 250 mm
(Beckman Instruments Inc., Berkeley, CA).

3. SOLVENT SYSTEM

CH₃OH/H₂O (80:20, v/v) + 5 mM (NH₄)₂HPO₄ (final concentration), pH = 7.5.

4. FLOW RATE

1.0 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a 4°C refrigerator and were checked for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds (solutions are discarded when a more than 10% change in the ratio is observed).

a. Mefloquine (free base)

Standard curve solution preparation date 7/16/90, expiration date 1/16/91.

Dissolve 10.08 mg of mefloquine hydrochloride in 50 ml of methanol

Conc. of mefloquine hydrochloride = 201.6 μ g/ml

Conc. of mefloquine (free base) = 184 μ g/ml

Control solution preparation date 7/16/90, expiration date 1/16/91.

Dissolve 10.05 mg of mefloquine hydrochloride in 50 ml of methanol

Conc. of mefloquine hydrochloride = 201 $\mu\text{g/ml}$

Conc. of mefloquine (free base) = 183 $\mu\text{g/ml}$

b. Chlorpheniramine Maleate

Internal standard solution preparation date 7/12/90, expiration date 1/12/91.

Dissolve 4.55 mg in 50 ml of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v).

Conc. = 91.0 $\mu\text{g/ml}$

Internal standard solution preparation date 9/13/90, expiration date 3/13/91.

Dissolve 8.81 mg in 100 ml of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v).

Conc. = 88.1 $\mu\text{g/ml}$

Internal standard solution preparation date 1/17/91, expiration date 7/17/91.

Dissolve 10.1 mg in 100 ml of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v).

Conc. = 101 $\mu\text{g/ml}$

6. WORKING SOLUTIONS - Solutions were stored in a 4°C refrigerator.

a. Mefloquine (free base) -

Standard curve working solutions preparation date 7/16/90, expiration date 1/16/91.

Dilute stock solution 10:1 with methanol.

Conc. = 18.4 $\mu\text{g/ml}$

Dilute stock solution 100:1 with methanol.

Conc. = 1.84 $\mu\text{g/ml}$

Control working solutions preparation date 7/16/90, expiration date 1/16/91.

Dilute stock solution 10:1 with methanol.

Conc. = 18.3 $\mu\text{g/ml}$

Dilute stock solution 100:1 with methanol.

Conc. = 1.83 $\mu\text{g/ml}$

b. Chlorpheniramine Maleate (Internal Standard).

Dilute stock solution (91.0 $\mu\text{g/ml}$) 100:1 with $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v). Preparation date 7/12/90.

Conc. = 0.910 $\mu\text{g/ml}$

Dilute stock solution (88.1 $\mu\text{g/ml}$) 100:1 with $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v). Preparation date 9/13/90.

Conc. = 0.881 $\mu\text{g/ml}$

Dilute stock solution (101 $\mu\text{g/ml}$) 3:1 with $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1, v/v). Preparation date 1/17/91.

Conc. = 33.7 $\mu\text{g/ml}$

7. RETENTION TIMES (subject to change depending on temperature and column performance).

a. Mefloquine (free base) - 7.6 min

b. Chlorpheniramine (Internal Standard) - 14 min

8. BLANK BLOOD

Human blood (CPD or CPDA-1 as anticoagulant) from San Francisco Irwin Memorial Blood Bank.

9. INJECTION VOLUME

50-150 μl

10. QUANTITATION

By peak height ratio of drug relative to internal standard peak.

11. MINIMUM DETECTION LIMIT OF METHOD

The minimum detection limit, 7.36 ng/ml mefloquine (free base), was determined as the mefloquine (free base) concentration at which the signal to noise ratio was at least 3 to 1.

12. SAMPLE VOLUME MEASUREMENT

Blood sample volumes were measured with a 200 μ l or a 1000 μ l Gilson Pipetman. See SOP 3-4 for calibration procedure.

13. WISP OPERATING TEMPERATURE

Room temperature.

D. SAMPLE STORAGE

All samples were kept frozen at -20°C before analysis and thawed at room temperature for preparation and analysis.

E. SAMPLE PREPARATION

1. Pipet 0.5 ml of blood into a screw top tube.
2. Spike standard curve samples with 00*, 0**, 2, 4, 8, 15 or 30 μ l of 1.84 μ g/ml mefloquine (free base) working solution or 8, 15, 30 or 60 μ l of 18.4 μ g/ml mefloquine (free base) working solution to make final a standard curve. Spiked mefloquine (free base) amounts are 00, 0, 3.68, 7.36, 14.7, 27.6, 55.2, 147, 276, 552, and 1100 ng. Since these amounts are spiked into 0.5 ml blood samples, they correspond to sample concentrations of 00, 0, 7.36, 14.7, 29.4, 55.2, 110, 294, 552, 1100 or 2210 ng/ml. Vortex for 20 seconds and let stand for 1 hour.
3. Add 0.5 ml water and sonicate for 10 min.
4. Add 50 μ l of the internal standard working solution (CPA, 0.881 μ g/ml). Vortex for 1 min.
5. Add 100 μ l of a saturated Na₂CO₃ solution and vortex for 1 min.
6. Add 5 ml pentane/methylene chloride (7:3, v/v).
7. Rotate for 15 min and centrifuge for 15 min at 3000 g.
8. Freeze aqueous layer on dry ice/methanol.
9. Pour organic layer into a 13x100 culture tube.
10. Evaporate to dryness under purified nitrogen.

* 00 = Sample with no drug and no internal standard.

** 0 = Sample with no drug but with internal standard.

11. Add 200 μ l of mobile phase. Vortex for 1 min.
12. Transfer to WISP inserts and inject onto column.

F. QUALITY CONTROL

1. CONTENT AND FREQUENCY OF BLANKS

No special blank was used except for the standard curve blanks.

2. PIPETTE CALIBRATION

See SOP 3-4.1

3. BALANCE CALIBRATION

See SOP 3-19.1.

G. PREPARATION OF PRECISION VALIDATION SAMPLES

Samples for precision analysis were prepared by spiking 0.5 ml blood specimens with 4 or 20 μ l of 1.84 μ g/ml mefloquine (free base) working solution or with 20 or 40 μ l of 18.4 μ g/ml mefloquine (free base) working solution to make final mefloquine (free base) concentrations of 14.7, 73.6, 736, and 1470 ng/ml.

H. RECOVERY

Samples for analysis of recovery were prepared by spiking 0.5 ml blood specimens with 4 or 20 μ l of 1.84 μ g/ml mefloquine (free base) working solution or with 20 or 40 μ l of 18.4 μ g/ml mefloquine (free base) working solution to make final mefloquine (free base) concentrations of 14.7, 73.6, 736, and 1470 ng/ml. Recovery was assessed by comparing the mefloquine (free base) to internal standard peak height ratios in mobile phase (200 μ l) to the peak height ratios in blood. Each 0.5 ml blood sample was prepared as described in "Sample Preparation" (Section E), except the internal standard was added after the nitrogen evaporation step. The mobile phase samples were neither extracted nor evaporated.

I. STABILITY

Pooled blood samples (40 ml) spiked with 0.08 or 0.20 ml of 18.3 μ g/ml mefloquine (free base) or 0.20 or 0.30 ml of 183 μ g/ml mefloquine (free base) solution and were mixed on a rotator for one hour to make final concentrations of 36.5, 91.0, 910, and 1360 ng/ml mefloquine (free base). The resulting samples were divided into 0.5

ml fractions, placed in culture tubes and stored in the freezer at -20°C or -80°C until assayed for stability.

J. RESULTS

1. STANDARD CURVE

Chromatograms for each point in a representative standard curve for mefloquine (free base) appear in Figure 1. Peak height ratios for these calibrators appear in Table 1.

2. RECOVERY

Results for this evaluation appear in Table 2.

3. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 3 and 4.

4.. STABILITY

Results appear in Table 5.

TABLE 1: REPRESENTATIVE STANDARD CURVE FOR
MEFLOQUINE HUMAN BLOOD ASSAY,
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SPIKED AMOUNT (ng)*	STANDARD CURVE		PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
	CONCENTRATION (ng/ml)	CONCENTRATION (ng/ml)		
0	0	0	0	-
3.68	7.36	0.025	0.025	5.88 ^a
7.36	14.7	0.069	0.069	15.0 ^a
14.7	29.4	0.133	0.133	28.2 ^a
27.6	55.2	0.277	0.277	57.9 ^a
55.2	110	0.525	0.525	109 ^a
147	294	1.414	1.414	289 ^b
276	552	2.717	2.717	552 ^b
552	1100	5.432	5.432	1100 ^b
1100	2210	10.899	10.899	2210 ^b

Regression equations:

$$a_y = 0.004846x - 0.00351, \quad r^2 = 0.9985 \text{ (Low Range: 0 - 110 ng/ml)}$$

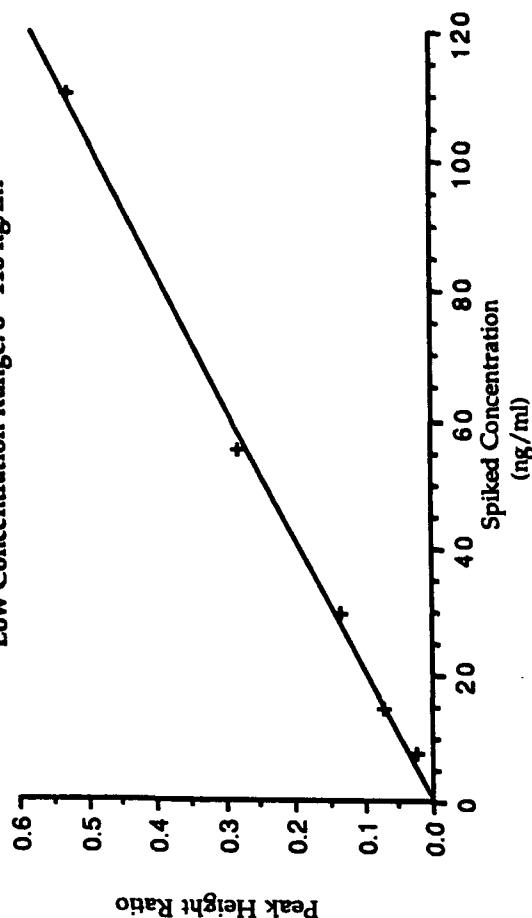
$$b_y = 0.004937x - 0.00968, \quad r^2 = 1.0000 \text{ (High Range: 0 - 2208 ng/ml)}$$

* Into 0.5 ml of biological sample.

** Ratio of drug peak height to internal standard peak height.

*** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level (free base) concentrations, two standard curves were constructed from the same set of standard curve data points.

Representative Standard Curve
Low Concentration Range: 0 - 110 ng/ml



High Concentration Range: 0 - 2210 ng/ml

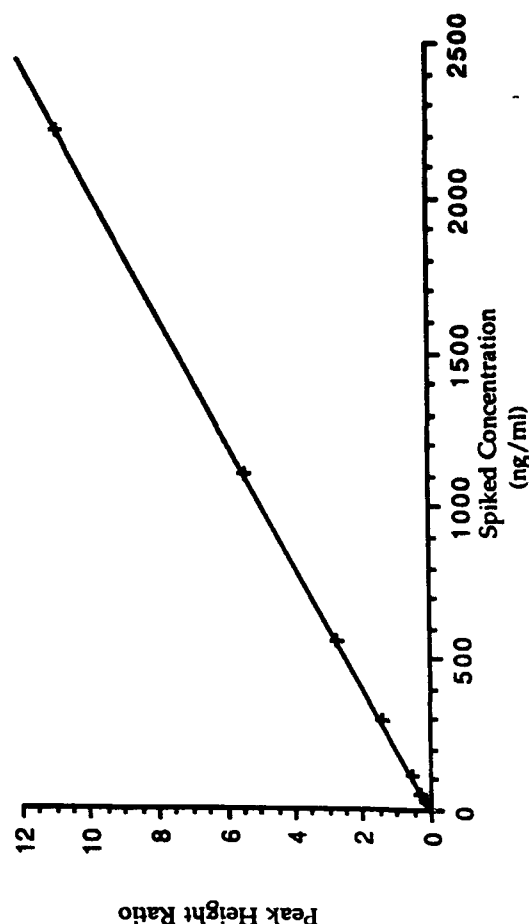


**TABLE 2: RECOVERY OF MEFLOQUINE (FREE BASE)
FROM HUMAN BLOOD**

SAMPLE	SPIKED CONCENTRATION (ng/ml)	PEAK HEIGHT RATIO	
		SOLVENT	BLOOD
1	14.7	0.070	0.056
2		0.073	0.058
3		0.072	0.069
MEAN		0.072	0.061
MEAN RECOVERY $(0.061/0.072)100 = 84.7\%$			
1	73.6	0.362	0.332
2		0.390	0.343
3		0.377	0.342
MEAN		0.376	0.339
MEAN RECOVERY $(0.339/0.376)100 = 90.2\%$			
1	736	3.698	3.443
2		3.880	3.568
3		3.667	3.409
MEAN		3.748	3.473
MEAN RECOVERY $(3.473/3.748)100 = 92.7\%$			
1	1472	7.837	7.634
2		7.507	7.207
3		7.328	7.453
MEAN		7.557	7.431
MEAN RECOVERY $(7.431/7.557)100 = 98.3\%$			

OVERALL AVERAGE RECOVERY = 91.5%

**TABLE 3: INTERDAY PRECISION OF MEFLOQUINE (FREE BASE)
HUMAN BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations*								
	(ng/ml)								
14.7	15.1	14.7	15.7	15.3	15.9	16.4	15.5	0.61	3.94
73.6	62.2	74.0	73.7	71.7	80.9	75.5	73.0	6.14	8.41
736	690	649	709	750	693	730	704	35.1	4.99
1472	1455	1387	1405	1563	1455	1519	1464	66.9	4.57

**TABLE 4: INTRADAY PRECISION OF MEFLOQUINE (FREE BASE)
HUMAN BLOOD ASSAY**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN	S.D.	Percent C.V.
	1	2	3	4	5	6			
	Measured Concentrations								
	(ng/ml)								
14.7	14.8	14.8	14.8	12.6	17.0	17.0	15.2	1.66	10.9
73.6	72.1	80.9	78.7	78.7	80.9	76.5	78.0	3.31	4.24
736	706	724	724	760	758	762	739	24.0	3.25
1472	1462	1460	1501	1556	1520	1548	1508	41.3	2.74

* Measured concentrations are averages of the analysis of two samples.

TABLE A: ACCURACY OF MEFLOQUINE (FREE BASE) HUMAN BLOOD ASSAY (BLIND STUDY RESULTS) JULY 93

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
1	0	*	Mean = *
12		*	SD =
14		*	Percent CV =
24		*	Percent Bias = 0
2	11.52	12.6	Mean = 12.4
11		11.8	SD = 1.75
13		10.4	Percent CV = 14.2
22		14.6	Percent Bias = 7.20
3	30.72	29.9	Mean = 29.4
10		30.2	SD = 0.975
16		28.0	Percent CV = 3.32
23		29.3	Percent Bias = -4.46
4	153.6	133	Mean = 136
9		143	SD = 8.72
15		125	Percent CV = 6.41
21		143	Percent Bias = -11.5
5	345.6	308	Mean = 304
8		324	SD = 23.0
18		271	Percent CV = 7.56
19		313	Percent Bias = -12.0
6	1536	1380	Mean = 1343
7		1390	SD = 59.1
17		1340	Percent CV = 4.40
20		1260	Percent Bias = -12.6

[#] n = 3, unless a chromatogram is determined to be unacceptable.

TABLE 5: STABILITY OF MEFLOQUINE IN HUMAN BLOOD**Mefloquine (Free Base) Concentration of Samples Stored at -20°C**

		CONCENTRATION (ng/ml)			
Spiked Concentration:	36.5	91.0	910	1362	
TIME STORED					
0	36.3	84.7	807	1247	
1	32.9	86.2	864	1320	
2	32.3	80.2	773	1166	
3	37.0	92.7	860	1364	
1st week	33.6	90.2	895	1367	
2nd week	33.9	85.1	857	1280	
3rd week	37.2	95.1	958	1409	
1st mo.	37.8	91.7	905	1340	
2nd mo.	36.8	94.5	894	1311	
3rd mo.	38.8	101	957	1403	
4th mo.	39.6	93.8	917	1292	

Mefloquine (Free Base) Concentration of Samples Stored at -80°C

		CONCENTRATION (ng/ml)			
Spiked Concentration:	36.5	91.0	910	1362	
TIME STORED					
0	35.9	93.1	807	1250	
1	36.0	85.2	878	1298	
2	32.0	78.2	780	1167	
3	38.4	92.4	893	1319	
1st week	32.4	92.5	926	1385	
2nd week	34.3	81.1	847	1289	
3rd week	37.9	94.4	931	1429	
1st mo.	38.6	95.1	908	1388	
2nd mo.	40.4	92.0	915	1367	
3rd mo.	40.5	98.4	984	1462	
4th mo.	39.9	94.8	934	1421	

LABORATORY METHODOLOGY FOR ARTELINIC ACID HUMAN PLASMA ASSAY*

A. INSTRUMENTS

1. Waters Associates WISP 710B (Waters Associates, Milford, MA), or equivalent.
2. Shimadzu LC 6A (Shimadzu Scientific Instruments, Inc., Columbia, MD), or equivalent.
3. Kratos Spectroflow 783 UV Absorbance Detector (Kratos Analytical Instruments, Ramsey, NJ), or equivalent.
4. Hewlett-Packard Integrator 3390A (Hewlett-Packard Co., Santa Clara, CA), or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. Artelinic acid, WR 255663AK (Walter Reed Army Institute of Research, Washington D.C.), bottle number BM04131, expiration date not available.
3. Meclofenamic acid (Internal Standard), (Mylan Pharmaceuticals, and WV), purified by ET Lin.
4. Methanol (Fisher Scientific, Fair Lawn, NJ).
5. Acetonitrile (Fisher Scientific, Fair Lawn, NJ).
6. 85% Phosphoric acid (Fisher Scientific, Fair Lawn, NJ).
7. Type 1 reagent grade water: prepared with a Nanopure II system, Barnstead Co., Boston, MA).

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength: 236 nm

Sensitivity: 0.008 aufs

Lamp: Applied Biosystems deuterium, model 120-LC

2. COLUMN

Axxiom ODS, 5 μ m particle size, 4.6 x 250 mm (Richard Scientific, Novato, CA).

3. SOLVENT SYSTEM

CH₃CN/50 mM NH₄H₂PO₄ (1:1, v/v), pH adjusted to 5.00 with H₃PO₄

4. FLOW RATE

1.0 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a -20°C freezer and checked for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds and internal standard (solutions are discarded when a more than 10% change in the ratio is observed or by 6 months after the preparation date).

a. ARTELINIC ACID (Standard Curve Stock Solution)

10.035 mg of artelinic acid dissolved in and q.s. to 50.0 ml with methanol.

Purity factor = 0.9487*

Conc. of artelinic acid stock = 0.190 mg/ml

b. ARTELINIC ACID (Control Stock Solution)

12.594 mg of artelinic acid dissolved in and q.s. to 50.00 ml with methanol.

Purity factor = 0.9487

Conc. of artelinic acid stock = 0.239 mg/ml

* Report No. 703 titled "Assay of 10-O-(4'-Carboxybenzyl) dihydroartemisinin Hemihydrate, 4-(10'-Dihydroartemisininoxymethyl) benzoic Acid Hemihydrate (Artelinic Acid), WR-255663AK, BM04131," p. 9.

c. INTERNAL STANDARD: Meclofenamic acid

2.945 mg of meclofenamic acid dissolved in 50.0 ml of methanol.

Conc. of meclofenamic acid stock = 58.9 $\mu\text{g/ml}$

6. WORKING SOLUTIONS - Solutions were stored in a 4°C refrigerator and discarded when stock solutions were discarded or by 6 months after the preparation date.

a. ARTELINIC ACID (Standard Curve Working Solution)

Dilute 10.0 ml of 0.190 mg/ml artelinic acid stock solution to 24.0 ml with methanol.

Conc. of artelinic acid working solution = 79.3 $\mu\text{g/ml}$

Dilute 1.0 ml of 79.3 $\mu\text{g/ml}$ artelinic acid working solution to 16 ml with methanol.

Conc. of artelinic acid working solution = 4.96 $\mu\text{g/ml}$

b. ARTELINIC ACID (Control Working Solution)

Dilute 5.00 ml of 0.239 mg/ml artelinic acid stock solution to 15.0 ml with methanol.

Conc. of artelinic acid working solution = 79.7 $\mu\text{g/ml}$

Dilute 1.0 ml of 79.7 $\mu\text{g/ml}$ artelinic acid working solution to 16 ml with methanol.

Conc. of artelinic acid working solution = 4.98 $\mu\text{g/ml}$

c. MECLOFENAMIC ACID (Internal standard).

Dilute 1.0 ml of 58.9 $\mu\text{g/ml}$ meclofenamic acid stock solution to 18 ml with methanol.

Conc. of meclofenamic acid working solution = 3.27 $\mu\text{g/ml}$

7. RETENTION TIMES (subject to change depending on temperature and column performance).

a. Artelinic acid - 20 min

b. Meclofenamic acid (Internal Standard) - 17 min

8. BLANK PLASMA

Human plasma (CPD or CPDA-1 as anticoagulant) was obtained from San Francisco Irwin Memorial Blood Bank.

9. INJECTION VOLUME

60 μ l

10. QUANTITATION

By peak height ratio of drug relative to internal standard peak.

11. MINIMUM QUANTITATION LIMIT OF METHOD

The minimum quantitation limit, 5.23 ng/ml for artelinic acid, was determined as the artelinic acid concentration at which the signal to noise ratio was at least 3 to 1.

12. SAMPLE VOLUME MEASUREMENT

Plasma sample volumes were measured with a 1000 μ l Gilson Pipetman. See SOP 3-4 for calibration procedure.

13. WISP OPERATING TEMPERATURE

Room temperature.

14. CARTRIDGE CONDITIONING

QUATERNARY AMINOPROPYL (SAX) ION EXCHANGE
CARTRIDGE (500 mg)

- a. Wash cartridge with 3 ml methanol.
- b. Wash with 2 ml 0.1 M HCl.
- c. Wash with 3 ml H₂O.
- d. Wash with 6 ml 0.1 M NaH₂PO₄, pH 7.0.

NH₂ ION EXCHANGE CARTRIDGE (500 mg)

Same as SAX cartridge conditioning, except:

- b. Wash with 6 ml 0.1 M HCl.
- d. Wash with 6 ml 0.1 M NaH₂PO₄, pH 7.01.

D. SAMPLE STORAGE

All samples must be kept frozen at -80°C before analysis and thawed at room temperature for preparation and analysis.

E. SAMPLE PREPARATION

SAX CARTRIDGE ELUTION

1. Pipette 1 ml plasma into culture tube.

2. Spike standard curve samples with 00,* 0,** 1, 2, or 8 μl of 4.96 $\mu\text{g}/\text{ml}$ artelinic acid working solution or 1, 2, 4, 8, or 16 μl of 79.3 $\mu\text{g}/\text{ml}$ artelinic acid working solution to make a standard curve. This procedure is equivalent to addition of 4.96, 9.92, 19.8, 39.7, 79.3, 159, 317, 635, or 1270 ng of artelinic acid to each tube. Since 1 ml of biological sample is assayed, these amounts correspond to artelinic acid concentrations of 00, 0, 4.96, 9.92, 19.8, 39.7, 79.3, 159, 317, 635, or 1270 ng/ml.
3. Add 50 μl of internal standard solution (3.27 $\mu\text{g}/\text{ml}$ meclofenamic acid). Vortex 10 s.
4. Add 2 ml acetonitrile. Vortex for 1 min. Centrifuge for 10 min at 3000g.
5. Transfer supernatant into clean 12x75 mm tubes.
6. Evaporate to 200 μl under nitrogen.
7. Add 1 ml water. Vortex for 1 min.
8. Pour sample onto pre-conditioned 500 mg SAX cation-exchange cartridge.
9. Wash cartridge with 3 ml of water followed by 3 ml of acetonitrile by gravity elution.
10. Wash cartridge with 0.5 ml of 0.5 M formic acid in acetonitrile by gravity elution.
11. Elute sample with 2 ml of 0.5 M formic acid in acetonitrile by gravity elution.
12. Evaporate eluent to dryness under nitrogen.
13. Reconstitute sample in 200 μl of 50% acetonitrile, transfer to WISP vial and inject 60 μl onto the HPLC column.

NH_2 CARTRIDGE ELUTION: SAME AS ABOVE EXCEPT:

8. Pour sample onto pre-conditioned 500 mg NH_2 cation-exchange cartridge.
9. Wash cartridge with 3 ml of water followed by 3 ml of acetonitrile by gravity elution. Completely dry cartridge on Vacut.

* 00 = Sample with no drug and no internal standard.

** 0 = Sample with no drug but with internal standard.

10. Wash cartridge twice with 0.5 ml of 0.5 M formic acid in acetonitrile by gravity elution. Completely dry cartridge on Vacelut.
11. Elute sample with three 0.5 ml aliquots of 0.5 M formic acid in acetonitrile by gravity elution.
13. Reconstitute sample in 200 μ l of 50% acetonitrile, transfer to WISP vial and inject 30 μ l onto the HPLC column.

F. QUALITY CONTROL

1. CONTENT AND FREQUENCY OF BLANKS

No special blank sample was assayed, except for the standard curve and blanks.

2. PIPETTE CALIBRATION

See SOP 3-4.1.

3. BALANCE CALIBRATION

See SOP 3-19.1

G. GENERATION OF PRECISION SAMPLES

Precision samples were made and assayed with calibration standards. Samples for precision analysis were prepared by spiking blank 1 ml plasma specimens with artelinic acid control working solution to make final artelinic acid concentrations corresponding to 9.96, 39.8, 398, or 797 ng/ml. See table.

Generation of Precision Samples (NH₂)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	1	9.50	1	9.50
Low	4	9.50	1	38.0
Med.	4	95.0	1	380
Hi	8	95.0	1	760

Generation of Precision Samples (SAX)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	4.98	1	9.96
Low	8	4.98	1	39.8
Med.	5	79.7	1	398
Hi	10	79.7	1	797

H. GENERATION OF RECOVERY SAMPLES

Assay recovery was assessed at three different concentrations by comparing the artelinic acid to internal standard peak height ratios in solvent to the peak height ratios in plasma. Plasma (1 ml) and solvent (methanol) samples were spiked with corresponding amounts of artelinic acid. Each plasma sample was prepared as described in "Sample Preparation" (Section E), except the internal standard was added after the eluents were collected (step 11) and the solvent samples were not extracted.

I. GENERATION OF FREEZE THAW SAMPLES

The effect of repeated freeze and thaw cycles on stability of artelinic acid in human plasma samples was determined as follows: Spiked (40 and 800 ng/ml artelinic acid concentrations) pooled biological sample were aliquoted (1 ml) to screw top culture tubes and subjected to five thaw/freeze cycles. Each cycle, a duplicate set of thaw/freeze samples was generated.

Run the study with the following procedure:

- Prepare high and low concentration samples labelled H-1, H-2 ... H-5, and L-1, L-2 ... L-5, in duplicate.
- Store all samples until frozen at the specified temperature.
- Repeatedly thaw and refreeze samples according to the following table. Thaw as if for sample preparation to room temperature. Let thawed samples stand at room temperature for 1 hour.

Cycle	Keep these samples in freezer	Thaw these samples
1	1	2, 3, 4, 5
2	1, 2	3, 4, 5
3	1, 2, 3	4, 5
4	1, 2, 3, 4	5
5	1, 2, 3, 4, 5	none

- d. Following Cycle 5, take out all of the samples, thaw to room temperature, and assay the samples with a standard curve.

J. RESULTS

1. STANDARD CURVE

Chromatograms for each point in representative standard curves for artelinic acid appear in Figure 1. Peak height ratios for these calibrators appear in Table 1.

2. RECOVERY

Results for this evaluation appear in Table 2.

3. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 3 and 4.

4. STANDARD CURVE STUDY STATISTICS

Mean, standard deviation, percent coefficient of variation and percent deviation from corresponding calibrator concentrations of the standard curve calibrators for this study appear in Table 5.

5. LOW POINT VALIDATION

Take the 6 back calculated lowest standard calibrator concentrations that were obtained in the interday precision-accuracy study as the quantitation limit interday result. Results appear in Table 6.

6. STABILITY

a. Freeze and Thaw

Results appear in Table 7.

b. Freezer Storage Stability

Freezer storage stability data is presented in Table 8.

**TABLE 1: REPRESENTATIVE STANDARD CURVE FOR
ARTELINIC ACID HUMAN PLASMA ASSAY
STUDY REPORT 20**

SPIKED AMOUNT (ng)	STANDARD CURVE		PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
	CONCENTRATION* (ng/ml)	CONCENTRATION		
0	0	0	0	-
4.96	4.96	0.031	0.031	5.40 ^a
9.92	9.92	0.059	0.059	10.5 ^a
19.8	19.8	0.112	0.112	20.2 ^a
39.7	39.7	0.209	0.209	37.8 ^a
79.3	79.3	0.441	0.441	80.1 ^a
159	159	0.910	0.910	158 ^b
317	317	1.804	1.804	315 ^b
635	635	3.779	3.779	661 ^b
1270	1270	7.180	7.180	1260 ^b

Regression equations:***

a $y = 0.00549x + 0.0013$, $r^2 = 0.9989$; (Low Range: 0 - 79.3 ng/ml)

b $y = 0.00570x + 0.0069$, $r^2 = 0.9994$; (High Range: 0 - 1270 ng/ml)

* When 1 ml of biological sample is used.

** Ratio of drug peak height to internal standard peak height.

*** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level concentrations, two standard curves were constructed from the same set of standard curve data points.

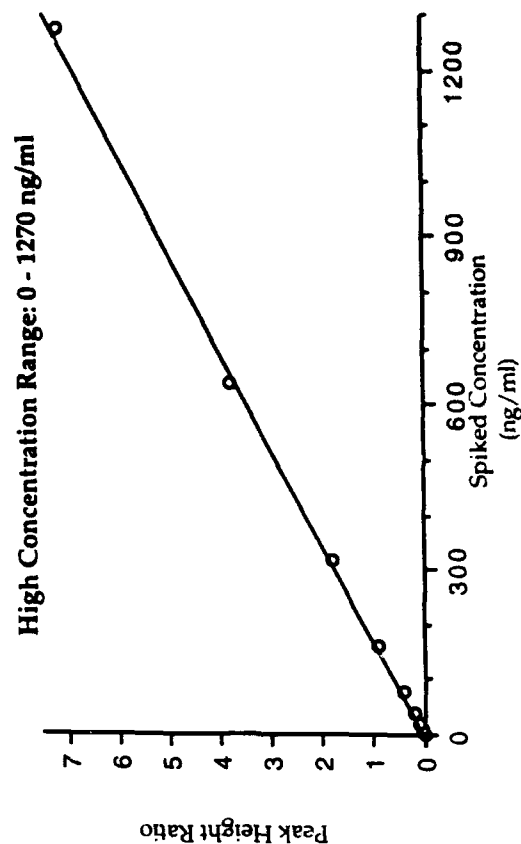
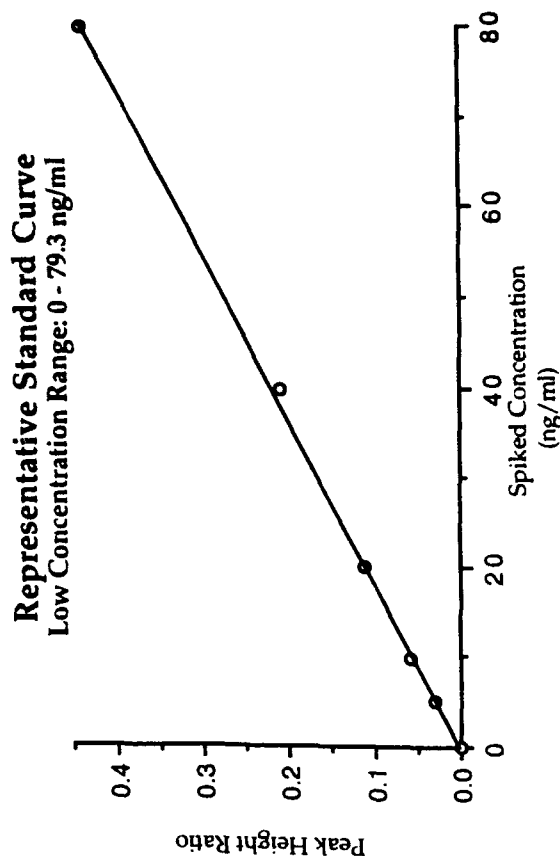


TABLE 2: ARTELINIC ACID MINIMUM QUANTITATION LIMIT

NH2 Method

Spiked Concentration	(9.49 ng/ml)	(9.5 ng/ml)	(4.75 ng/ml)
	Measured Concentrations (ng/ml)		
	Interday	Intraday	Interday
	9.03	12.1	5.20
	9.81	8.35	4.55
	9.03	9.72	5.20
	9.29	9.03	bc
	9.77	9.72	6.38
	9.82	11.8	4.19
Mean	9.46	10.1	5.10
Standard Deviation	0.386	1.51	0.835
Percent CV	4.09	14.9	16.4
Percent Error	-0.334	6.53	7.45

SAX Method

Spiked Concentration	(9.92 ng/ml)	(9.96 ng/ml)	(4.96 ng/ml)
	Measured Concentrations (ng/ml)		
	Interday	Intraday	Interday
	9.97	9.95	6.13
	bc	10.5	6.30
	8.75	9.77	5.09
	8.92	8.32	bc
	9.98	10.1	bc
	9.51	9.41	4.00
Mean	9.43	9.68	5.38
Standard Deviation	0.575	0.755	1.06
Percent CV	6.10	7.81	19.8
Percent Error	-4.98	-2.86	8.47

TABLE 3: RECOVERY OF ARTELINIC ACID FROM HUMAN PLASMA

SAMPLE ID	SPIKED CONCENTRATION (ng/ml)	PERCENT RECOVERY	PEAK HEIGHT RATIO	
			SOLVENT	PLASMA
SAX CARTRIDGE				
Low Concentration Range				
1	19.9		0.122	0.113
2			0.123	0.107
3			0.124	0.076
Mean (± SD)		80.7	0.123 ± 0.001	0.098 ± 0.020
Medium Concentration Range				
1	159		1.194	1.028
2			1.147	1.013
3			1.126	0.980
Mean (± SD)		87.1	1.156 ± 0.035	1.007 ± 0.025
High Concentration Range				
1	319		2.192	2.012
2			2.222	1.898
3			2.311	2.041
Mean (± SD)		88.5	2.242 ± 0.062	1.984 ± 0.076
AVERAGE RECOVERY =		85.3		
NH2 CARTRIDGE				
Low Concentration Range				
1			0.052	0.039
2			0.052	0.032
3			0.052	0.033
Mean (± SD)		67.4	0.052 ± 0.000	0.035 ± 0.004
Medium Concentration Range				
1			0.427	0.332
2			0.431	0.318
3			0.441	0.340
Mean (± SD)		76.2	0.433 ± 0.007	0.330 ± 0.011
High Concentration Range				
1			1.786	1.348
2			1.797	1.340
3			1.803	1.274
Mean (± SD)		73.6	1.795 ± 0.009	1.321 ± 0.041
AVERAGE RECOVERY =		72.4		

**TABLE 4A: INTERDAY PRECISION OF ARTELINIC ACID HUMAN
PLASMA ASSAY (NH2 METHOD)**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
9.50	9.79	9.62	9.79	8.85	10.1	9.59	9.62	0.42	4.36	1.30
38.0	37.9	39.5	37.9	33.9	35.9	38.2	37.2	1.99	5.35	-2.06
380	376	340	396	365	338	320	356	28.1	7.91	-6.36
760	757	842	793	756	741	736	771	40.2	5.21	1.43

**TABLE 4B: INTRADAY PRECISION OF ARTELINIC ACID HUMAN
PLASMA ASSAY (NH2 METHOD)**

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
9.50	12.1	8.35	9.72	9.03	9.72	11.8	10.1	1.51	14.9	6.53
38.0	34.3	33.6	37.4	35.3	38.4	42.2	36.9	3.19	8.65	-2.98
380	369	344	393	359	349	382	366	19.1	5.21	-3.68
760	758	671	733	707	758	773	733	38.39	5.24	-3.51

* Measured concentrations are averages of two analyses.

TABLE 4C: INTERDAY PRECISION OF ARTELINIC ACID HUMAN PLASMA ASSAY (SAX METHOD)

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations [*] (ng/ml)									
9.96	9.15	9.64	9.33	9.10	11.4	9.43	9.68	0.867	8.97	-2.86
39.8	42.1	38.8	41.6	36.2	36.9	41.0	39.4	2.51	6.37	-0.921
398	401	398	397	420	414	419	408	10.7	2.62	2.55
797	813	790	754	839	804	810	802	28.3	3.53	0.586

TABLE 4D: INTRADAY PRECISION OF ARTELINIC ACID HUMAN PLASMA ASSAY (SAX METHOD)

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
9.96	9.95	10.5	9.77	8.32	10.1	9.41	9.68	0.755	7.81	-2.86
39.8	39.5	40.0	41.5	40.7	39.5	41.3	40.4	0.882	2.18	1.55
398	386	397	383	399	400	398	394	7.36	1.87	-1.05
797	789	780	783	767	772	806	783	13.8	1.76	-1.78

^{*} Measured concentrations are averages of two analyses.

**TABLE 5: PRECISION STANDARD CURVE CALIBRATOR STATISTICAL
PARAMETERS FOR ARTELINIC ACID HUMAN PLASMA ASSAY**

Standard Curve Concentration (ng/ml)	n	Mean (ng/ml)	Standard Deviation (ng/ml)	Percent Coefficient of Variation	Percent Deviation
NH2 METHOD					
4.75	6	5.08	0.750	14.8	6.84
9.49	7	9.40	0.388	4.13	-0.978
19.0	7	19.1	1.32	6.93	0.376
38.0	7	36.5	2.74	7.51	-3.95
75.9	7	76.6	1.48	1.93	0.960
152	7	151	6.23	4.13	-0.752
304	7	316	17.3	5.48	3.85
608	6	600	61.5	10.3	-1.29
1220	7	1217	26.9	2.21	-0.234
SAX METHOD					
4.96	5	5.38	0.922	17.1	8.55
9.92	6	9.61	0.676	7.04	-3.18
19.8	7	19.8	1.30	6.58	0.144
39.7	7	38.3	1.55	4.06	-3.63
79.3	7	80.0	0.796	0.994	0.937
159	7	162	6.23	3.85	1.80
317	6	318	8.48	2.66	0.421
635	7	646	20.0	3.09	1.80
1270	7	1264	12.7	1.01	-0.450

**TABLE 6: ACCURACY OF ARTELINIC ACID HUMAN PLASMA ASSAY
(BLIND STUDY RESULTS) May 93**

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
1	0	*	Mean =
12		*	SD =
18		*	Percent CV =
20		*	Percent Bias =
2	23.54	21.6	Mean = 20.5
7		21.4	SD = 1.23
17		19.6	Percent CV = 6.00
19		19.2	Percent Bias = -13.1
3	41.3	36.3	Mean = 40.4
10		37.6	SD = 4.48
15		41.3	Percent CV = 11.1
21		46.3	Percent Bias = -2.24
4	124.0	111	Mean = 119
11		123	SD = 5.26
14		119	Percent CV = 4.44
24		121	Percent Bias = -4.44
5	200.7	196	Mean = 196
8		200	SD = 3.69
13		191	Percent CV = 1.88
22		196	Percent Bias = -2.47
6	401.4	406	Mean = 404
9		393	SD = 8.35
16		413	Percent CV = 2.07
23		402	Percent Bias = 0.523

[#] n = 3, unless a chromatogram is determined to be unacceptable.

TABLE 7: STABILITY OF ARTELINIC ACID IN HUMAN PLASMA**Artelinic Acid Concentration of Samples Stored at -20°C**

		CONCENTRATION** (ng/ml)			
Spiked Concentration:		9.50	38.0	380	760
TIME STORED					
0 days	bc	36.9	407	811	
1 day	bc	38.8	388	737	
2 days	bc	21.0	180	640	
3 days	8.07	40.6	310	688	
4 days	7.96	39.5	390	813	
1 week	7.92	30.6	509	820	
11 days	bc	46.1	225	814	
2 week	13.8	40.5	383	813	
22 days	11.4	50.5	414	868	
1 month	11.1	38.7	371	765	
2 months	10.5	39.4	379	776	
3 months	9.68	32.9	295	682	
6 months	10.4	42.4	392	717	

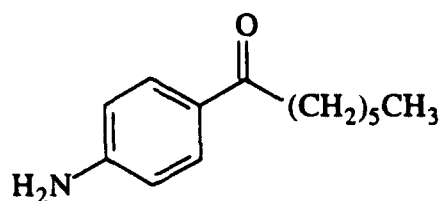
TABLE 8: EFFECT OF REPEATED FREEZE AND THAW CYCLES ON ARTELINIC ACID SPIKED HUMAN PLASMA SAMPLES

Spiked Concentration Cycle	ARTELINIC ACID	
	Low	High
	Concentration#	Concentration
	(40.0 ng/ml)	(800 ng/ml)
1	35.4	770
2	40.4	731
3	33.9	676
4	32.0	728
5	38.2	735

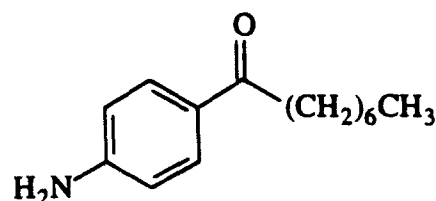
** Measured concentrations are averages of two analyses.

Concentrations are mean (n = 2) results, except when chromatograms are unacceptable.

**ANALYTICAL STANDARD OPERATING PROCEDURE (SOP) FOR AUTOMATED HPLC ASSAY
FOR *p*-AMINOHEPTANOPHENONE IN DOG PLASMA**



p-Aminoheptanophenone
(WR 269,410)



Internal Standard
p-Aminooctanophenone
(WR 258,948)

APPROVALS:

This Analytical Standard Operating Procedure is approved for use in

Study Number: _____

INITIALS DATE

Study Leader: _____

QA Officer: _____

INSTRUMENTS:

PUMP: LC-600 Shimadzu Pump, or equivalent.
 INJECTOR: Waters Intelligent Sample Processor Model 710 B (WISP), or equivalent.
 COLUMN: Beckman ODS 5 μ m, 25 cm X 4.6 mm, or equivalent.
 DETECTOR: Kratos Spectorflow 773, or equivalent.
 INTEGRATOR: Hewlett Packard Integrator 3392A, or equivalent.

CONDITIONS:

FLOW: 1.3 ml/min
 INJECTION VOLUME: 50 - 80 μ l
 RUN TIME: 22 min (PAOP (Internal Standard): 16.5 min; PAHP: 10.2 min)
 DETECTOR SETTINGS: Wavelength: 316 nm
 Absorption Range: 0.006 aufs
 Rise Time 1.0 s
 MOBILE PHASE: Acetonitrile/Water (50:50, v/v) and 0.15% H₃PO₄

STANDARDS:

1. STOCK SOLUTIONS

p-Aminoheptanophenone (WR 269,410)-(WRAIR, Washington, D.C.), bottle number BM 11565.

10.22 mg / 100 ml in methanol
Conc. = 102 µg/ml

p-Aminooctanophenone Internal Standard (WR 258,948) - (WRAIR, Washington, D.C.), bottle number BM 11207.

10.5 mg / 100 ml in methanol
Conc. = 105 µg/ml

2. WORKING SOLUTIONS

p-Aminoheptanophenone - Take 2.0 ml of 102 µg/ml stock and q.s. to 20 ml with methanol.

Conc. = 10.2 µg/ml

p-Aminoheptanophenone - Take 2.0 ml of 10.2 µg/ml working and q.s. to 20 ml with methanol.

Conc. = 1.02 µg/ml

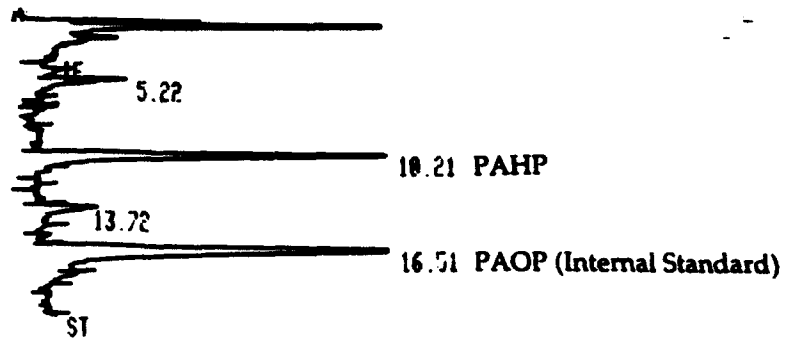
p-Aminooctanophenone Internal Standard - Take 1.0 ml of 105 µg/ml stock and q.s. to 100 ml with methanol.

Conc. = 1.05 µg/ml

SAMPLE PREPARATION:

1. Pipet 0.5 ml of dog plasma samples into 16 X 125 culture tubes.
2. Spike standard curve samples with 0, 0, 2, 4, 8, or 15 µl of 1.02 µg/ml *p*-aminoheptanophenone working solution or 3, 6, 10, 20, or 40 µl of 10.2 µg/ml *p*-aminoheptanophenone working solution to make a standard curve. This procedure is equivalent to addition of 0, 0, 2.04, 4.08, 8.16, 15.3, 30.6, 61.2, 102, 204, and 408 ng of *p*-aminoheptanophenone, respectively, to each sample. Since 0.5 ml plasma clinical samples are assayed, these amounts correspond to 0, 0, 4.08, 8.16, 16.3, 30.6, 61.2, 122, 204, 408, and 816 ng/ml *p*-aminoheptanophenone concentrations. Vortex for 30 s.
3. Add 50 µl of 1.05 µg/ml *p*-aminooctanophenone internal standard solution. Vortex for 1 min.
4. Add 50 µl of 1N NaOH. Vortex 30 s.
5. Add 5 ml methyl *t*-butyl ether and cap tubes. Vortex 1 min. Centrifuge 10 min at 3000 g.
6. Freeze in dry ice/methanol bath. Transfer organic layer to silanized 13 X 100 culture tubes and evaporate to dryness.
7. Immediately reconstitute residue with 200 µl of mobile phase. Vortex for 1 min.
8. Transfer to WISP inserts and inject 50 - 80 µl onto column.

COMMENTS: Use reverse side if necessary.



RUN # 29

JUN/11/93 03:22:27

ISTD

RT	HEIGHT	TYPE	CAL#	AMOUNT
5.22	2398	BB		0.000
10.21	11677	BB	1R	0.983
13.72	1578	BB		0.000
16.51	11882	BB	2L	1.000

LIST: METH @

RUN PRMTRS

ZERO = 30

ATT 2↑ = 1

CHT SP = 0.2

PK WD = 0.16

THRSH = 2

AR REJ = 0

TOTAL HGHT= 27535

ISTD AMT= 1.0000E+00

MUL FACTOR= 1.0000E+00

RPRT OPTNS

2. RF UNC PKS= 0.0000E+00

3. MUL FACTOR= 1.0000E+00

4. PK HEIGHT MODE YES

5. EXTEND RT NO

6. RPRT UNC PKS YES

TIME TBL

0.00 INTG # = 9

4.00 INTG # = -9

4.00 INTG # = 2

13.00 STOP

CALIB TBL

ISTD

CALIB RUNS 1

REF % RTW= 10.00

% RTW= 10.00

CAL #	RT	AMT	AMT/HEIGHT
1R	11.15	1.0000E+00	1.0000E+00
2L	18.10	1.0000E+00	1.0000E+00

WRITTEN BY:

DATE:

NEW SOP_____

REVISION_____

		concentrations in ng/ml	
Sample ID	PAPP	PAHP	PAOP
1	845	438	*
2	346	99.5	20.7
3	*	*	692
4	5.11	4.79	690
5	878	23.9	4.80
6	825	694	111
7	16.8	674	356
8	83.1	4.79	20.2
9	86.4	*	709
10	*	*	*
11	*	24.8	4.99
12	16.3	438	113
13	331	24.9	113
14	351	694	691
15	3.96¥#	701	*
16	87.8	99.5	355
17	*	24.3	21.0
18	15.2	453	20.4
19	7.17	5.80	6.46
20	321	101	21.8
21	2.74\$#	*	354
22	15.9	462	116
23	843	457	*
24	*	664	*
25	75.1	6.86	337
26	15.4	629	361
27	16.7	*	115
28	317	5.24	5.07
29	339	5.70	5.48
30	4.01£	96.8	716
31	814	97.9	21.4
32	86.4	26.5	721
33	91.8	456	*
34	807	*	112
35	*	22.2	5.71
36	5.71	99.4	361
MQL	4.04	4.08	4.16
* = below assay sensitivity			
¥ = below assay sensitivity but one chromatogram had a peak that calculated to 3.96 ng/ml.			
\$ = below assay sensitivity but one chromatogram had a peak that calculated to 2.74 ng/ml.			
£ = below assay sensitivity but one chromatogram had a peak that calculated to 4.01 ng/ml.			
# = controls were unacceptably low and may indicate these values should be higher.			

I. LABORATORY METHODOLOGY FOR WR 6026, WR 211,789 AND WR 254,421 (AS FREE BASES) URINE ASSAY,* STUDY REPORT 22

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 712 (Waters Associates, Milford, MA), or equivalent.
2. Altex Model 100A Solvent Delivery Module (Beckman Instruments, Inc., Berkeley, CA), or equivalent.
3. Kratos Spectroflow 783 UV Detector (Kratos Analytical Instruments, Ramsey, NJ), or equivalent.
4. Hewlett-Packard Reporting Integrator #3390A (Hewlett-Packard Co., Santa Clara, CA), or equivalent.

B. REAGENTS

1. All solvents are HPLC grade.
2. All chemicals are reagent grade.
3. WR 6026•2HCl - bottle no. BK 01845 (WRAIR, Washington, DC).
4. WR 211,789•2HCl•1/2H₂O - bottle no. BK 50713 (WRAIR, Washington, DC).
5. WR 254,421•2HCl - bottle no. BK 18756 (WRAIR, Washington, DC).
6. Verapamil (Internal Standard) - (USP Reference Lot # F-1)
7. NaOH (Mallinckrodt, Paris, KY).
8. Type I reagent grade water (deionized with a Nanopure II system, Barnstead Co., Boston, MA).
9. Methyl-*t*-butyl ether (Baxter, Burdick & Jackson, Muskegan, MI).
10. Methanol (Fisher Scientific, Fairlawn, NJ).
11. Acetonitrile (Fisher Scientific, Fairlawn, NJ).
12. Phosphoric acid (Fisher Scientific, Fair Lawn, NJ).

* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C. ASSAY CONDITIONS

1. DETECTOR

Settings

Wavelength; 350 nm

Sensitivity; 0.005 aufs

Rise Time; 1 s

Lamp

ABI Analytical, Inc. (Ramsey, NJ).

2. COLUMN

Axxiom Si, 5 μ m particle size, 4.6 x 250 mm - (Richard Scientific, Novato, CA).

3. MOBILE PHASE

CH₃CN/0.0075% H₃PO₄ (80:20, v/v) with final pH of 6.9.

4. FLOW RATE

1.0 ml/min

5. STOCK SOLUTIONS - Store at 4°C, protect from light, and check for deterioration by following the ratio of drug to internal standard peak heights for a diluted solution containing both compounds (discard solutions if a more than 10% change in the ratio is observed). In any case, discard solutions within 6 months.

a. Precision solutions.

Preparation date: 2/9/93					
Solution Type	Weight of Standard (mg)	Purity Factor*	QS Volume (ml)	Solvent	Free Base Conc. (mg/ml)
WR 6026	3.140	0.8249*	10	50% meOH	0.259
WR 211,789	2.138	0.7938**	10	50% meOH	0.170
WR 254,421	1.821	0.8314***	10	50% meOH	0.151
Verapamil Internal Std.	6.873	1	6.873	50% meOH	1

*= Molecular weights of WR 6026 free base/WR 6026 • 2HCl

**= Molecular weights of WR 211,789 free base/WR 211,789 • 2HCl • 1/2H₂O

***= Molecular weights of WR 254,421 free base/WR 254,421 • 2HCl

- b. Stability and blind sample analysis solutions. (WR 6026 and WR 211,789 solutions same as precision solutions.)

Preparation date: 3/26/93

Solution Type	Weight of Standard (mg)	Purity Factor*	QS Volume (ml)	Solvent	Free Base Conc. (µg/ml)
WR 254,421	14.346	0.8314		50% meOH	543

6. WORKING SOLUTIONS - (Store solutions at 4°C, protect from light, and discard if deterioration is observed in the stock solutions). In any case, solutions are discarded within 6 months.

- a. Combine and dilute stock precision solutions.

Preparation date: 2/9/93

Solution Type	Conc. Diluted (mg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
WR 6026	0.259	1:100	50% meOH	2.59
WR 211,789	0.170	1.5:100	50% meOH	2.55
WR 254,421	0.151	15:100	50% meOH	22.7

- b. Combine and dilute stability and blind sample analysis solutions.

Preparation date: 3/26/93

Solution Type	Conc. Diluted (mg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
WR 6026	0.259	4:18.4	50% meOH	56.3
WR 211,789	0.170	6:18.4	50% meOH	55.4
WR 254,421	1.19	8.4:18.4	50% meOH	543

- c. Stability and blind sample analysis solutions.

Preparation date: 3/26/93

Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio	Solvent	Conc. (µg/ml)
WR 6026	56.3	4.6:10	50% meOH	25.9
	25.9	1:10	50% meOH	2.59
WR 211,789	55.4	4.6:10	50% meOH	25.5
	25.5	1:10	50% meOH	2.55
WR 254,421	543	4.6:10	50% meOH	250
	250	1:10	50% meOH	25.0

d. Verapamil (Internal Standard).

Preparation date: 2/9/93

Solution Type	Conc. Diluted ($\mu\text{g}/\text{ml}$)	Dilution Ratio	Solvent	Conc. ($\mu\text{g}/\text{ml}$)
Internal Std	1000	1:10	50% meOH	100
Working IS	100	1:2	50% meOH	50

7. RETENTION TIMES (subject to change depending on temperature and column performance). Approximate run time: 23 min.

- a. Verapamil (Internal Standard) - 12.4 min
- b. WR 211,789 (free base) - 14.3 min
- c. WR 6026 (free base) - 15.3 min
- d. WR 254,421 (free base) - 18.2 min

8. QUANTITATION

By peak height ratio of drug peak relative to internal standard peak. Standard curves calculated by nonweighted linear regression.

9. MINIMUM DETECTION LIMIT OF METHOD FOR PRECISION ANALYSIS

The minimum detection limits, 5.17 ng/ml WR 6026, 5.09 ng/ml WR 211,789, and 45.4 ng/ml WR 254,421 (as free bases) in urine, was determined as the WR 6026, WR 211,789, and WR 254,421 (as free bases) concentrations at which the signal to noise ratio was at least 3 to 1.

10. INJECTION VOLUME

20 to 160 μl

11. SAMPLE VOLUME MEASUREMENT

Pipetters used for sample volume measurement were Rainin, Gilson Pipettman and/or Eppendorf. See SOP 3-4.1 for calibration procedure.

12. BLANK URINE

Spiked urine samples were made with interference-free urine obtained from UCSF Department of Pharmacy staff volunteers.

13. WISP OPERATING TEMPERATURE

Room Temperature

D. SAMPLE STORAGE

Urine samples were kept frozen, if required at -70°C before analysis and thawed at room temperature for preparation and analysis.

E. SAMPLE PREPARATION

1. Pipette 0.5 ml urine into sample culture tube.
2. Spike precision standard curve samples with 00,* 0,** 1, 2, 3, 5, 10, 20, 40, or 80 µl of WR 6026, WR 211,789, and WR 254,421 standard curve working solution mixture (conc. 2.59 µg/ml of WR 6026, 2.55 µg/ml of WR 211,789 and 22.7 µg/ml of WR 254,421 as free bases) to make standard curves. This procedure is equivalent to addition of 00, 0, 2.59, 5.17, 7.76, 12.9, 25.9, 51.7, 103, or 207 ng of WR 6026, and 00, 0, 2.55, 5.09, 7.64, 12.7, 25.5, 50.9, 102, or 204 ng of WR 211,789 and 00, 0, 22.7, 45.4, 68.1, 114, 227, 454, 908, or 1820 ng of WR 254,421 to each sample. Since 0.5 ml urine clinical samples are assayed, these amounts correspond to 00, 0, 5.17, 10.3, 15.5, 25.9, 51.7, 103, 207, and 414 ng/ml WR 6026, 00, 0, 5.09, 10.2, 15.3, 25.5, 50.9, 102, 204, and 407 ng/ml WR 211,789 and 00, 0, 45.4, 90.8, 136, 227, 454, 908, 1820, and 3630 ng/ml WR 254,421 concentrations. Vortex 10 s.
3. Add 100 µl of internal standard (50 µg/ml verapamil) working solution. Vortex 10 s.
4. Add 100 µl of 1 N NaOH. Vortex 10 s.
5. Add 5 ml methyl *t*-butyl ether, cap and vortex 1 min.
6. Centrifuge 10 min at 3000 g.
7. Freeze in dry ice/methanol and transfer organic layer to a 13x100 mm culture tube.
8. Evaporate to dryness under nitrogen.
9. Reconstitute in 200 µl of mobile phase, vortex 1 min and inject 20-160 µl onto HPLC column.

F. QUALITY CONTROL

1. Content and frequency of blanks

* 00 = Sample without drug and without internal standard.

** 0 = Sample without drug and with internal standard.

No special blank was used except for the standard curve blank.

2. Pipette Calibration

See SOP 3-4.1.

3. Balance Calibration

See SOP 3-19.2.

G. RECOVERY

Assay recovery was assessed at three different concentrations by comparing the WR 6026, WR 211,789, and WR 254,421 (as free bases) to internal standard peak height ratios in reference samples to the peak height ratios in urine. Urine and reference samples were spiked with corresponding amounts of WR 6026, WR 211,789, and WR 254,421 (as free bases). Each urine sample was prepared as described in "Sample Preparation" (Section E), except internal standard was added after the evaporation (step 8). The reference samples were spiked with WR 6026, WR 211,789, and WR 254,421 (as free bases) and with internal standard, but were not extracted and not evaporated.

H. GENERATION OF PRECISION SAMPLES

Samples for precision analysis were generated by spiking 0.5 ml urine specimens with WR 6026, WR 211,789, and WR 254,421 (as free bases) working solution as shown below. These samples were then prepared as described in Sample Preparation (Section E) above.

Generation of Precision Samples

WR 6026 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	2.59	0.5	10.4
Low	5	2.59	0.5	25.9
Med.	30	2.59	0.5	155
Hi	50	2.59	0.5	259

WR 211,789 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	2.55	0.5	10.2
Low	5	2.55	0.5	25.5
Med.	30	2.55	0.5	153
Hi	50	2.55	0.5	255

WR 254,421 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	22.7	0.5	90.8
Low	5	22.7	0.5	227
Med.	30	22.7	0.5	1360
Hi	50	22.7	0.5	2270

I. GENERATION OF STABILITY SAMPLES

Long term stability samples were generated by spiking rat plasma samples as shown below.

WR 6026 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	2.59	0.5	10.4
Low	5	2.59	0.5	25.9
Med.	3	25.9	0.5	155
Hi	5	25.9	0.5	259

WR 211,789 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	2.55	0.5	10.2
Low	5	2.55	0.5	25.5
Med.	3	25.5	0.5	153
Hi	5	25.5	0.5	255

WR 254,421 (as free base)

	Volume Spiked (μ l)	Spiking Solution Concentration (μ g/ml)	Control Volume (ml)	Precision Sample Nominal Concentration (ng/ml)
X-Lo	2	25.0	0.5	100
Low	5	25.0	0.5	250
Med.	3	250	0.5	1500
Hi	5	250	0.5	2500

Autosampler stability samples were generated by spiking 0.5 ml human urine specimens with WR 6026, WR 211,789, and WR 254,421 working solution as shown above for long term stability sample.

J. RESULTS

1. STANDARD CURVE

Chromatograms for each point in a representative standard curve for WR 6026, WR 211,789, and WR 254,421 (as free bases) appear

in Figure 3. Peak height ratios for these calibrators appear in Tables 1A-C.

2. LOW POINT VALIDATION

Take the 6 back calculated lowest standard calibrator concentrations that were obtained in the interday precision-accuracy study as the quantitation limit interday result. Results appear in Table 2.

3. RECOVERY

Results for this evaluation appear in Table 3.

4. INTRA- AND INTERDAY PRECISION

Results for these evaluations appear in Tables 4-6.

5. BLIND SAMPLE ANALYSIS

Results for this evaluation appear in Tables 7A-C.

6. STABILITY

Results for long term stability appear in Table 8. Results for autosampler stability appear in Table 9.

**TABLE 1A: REPRESENTATIVE STANDARD CURVE FOR
WR 6026 HUMAN URINE ASSAY
STUDY REPORT 22**

SPIKED AMOUNT (ng)	STANDARD CURVE		PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
	CONCENTRATION* (ng/ml)	CONCENTRATION		
0	0	0	0	-
2.59	5.17	0.050	0.050	3.99 ^a
5.17	10.3	0.148	0.148	12.2 ^a
7.76	15.5	0.190	0.190	15.7 ^a
12.9	25.9	0.301	0.301	25.0 ^a
25.9	51.7	0.620	0.620	51.8 ^a
51.7	103	1.358	1.358	126 ^b
103	207	2.060	2.060	194 ^b
207	414	4.357	4.357	414 ^b

Regression equations:

a $y = 0.01192x + 0.0024$, $r^2 = 0.9967$; (Low Range: 0 - 51.7 ng/ml)

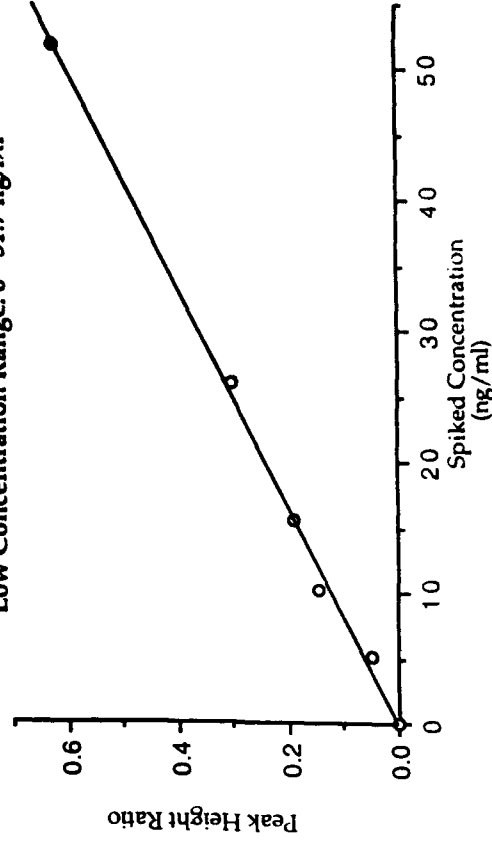
b $y = 0.01040x + 0.0476$, $r^2 = 0.9951$; (High Range: 0 - 414 ng/ml)

* When 0.5 ml of biological sample is used.

** Ratio of drug peak height to internal standard peak height.

*** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level concentrations, two standard curves were constructed from the same set of standard curve data points.

**Representative Standard Curve
Low Concentration Range: 0 - 51.7 ng/ml**



High Concentration Range: 0 - 414 ng/ml

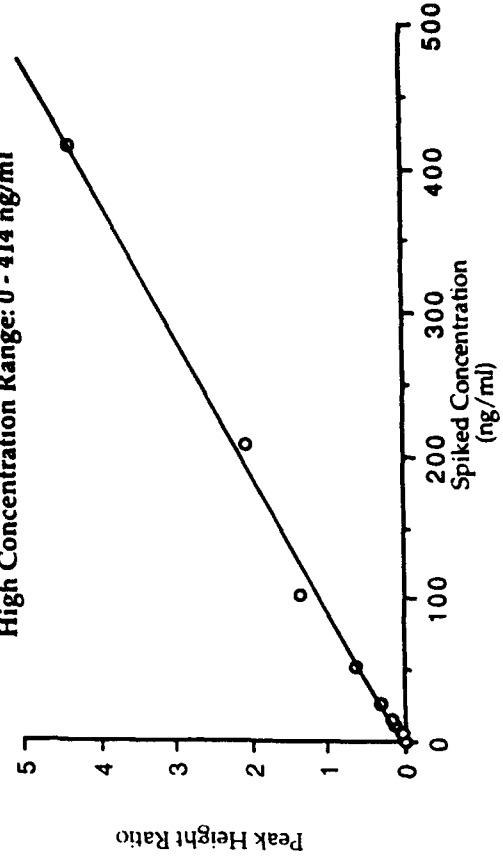


TABLE 1B: REPRESENTATIVE STANDARD CURVE FOR
WR 211,789 HUMAN URINE ASSAY
STUDY REPORT 22

SPIKED AMOUNT (ng)	STANDARD CURVE CONCENTRATION* (ng/ml)	PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
0	0	0	-
2.55	5.09	0.056	4.48 ^a
5.09	10.2	0.124	11.3 ^a
7.64	15.3	0.185	17.4 ^a
12.7	25.5	0.253	24.2 ^a
25.5	50.9	0.519	50.8 ^a
50.9	102	1.185	117 ^b
102	204	1.753	173 ^b
204	407	4.224	418 ^b

Regression equations:

a $y = 0.01000x + 0.0112$, $r^2 = 0.9948$; (Low Range: 0 - 50.9 ng/ml)

b $y = 0.01009x + 0.0028$, $r^2 = 0.9912$; (High Range: 0 - 407 ng/ml)

* When 0.5 ml of biological sample is used.

** Ratio of drug peak height to internal standard peak height.

*** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level concentrations, two standard curves were constructed from the same set of standard curve data points.

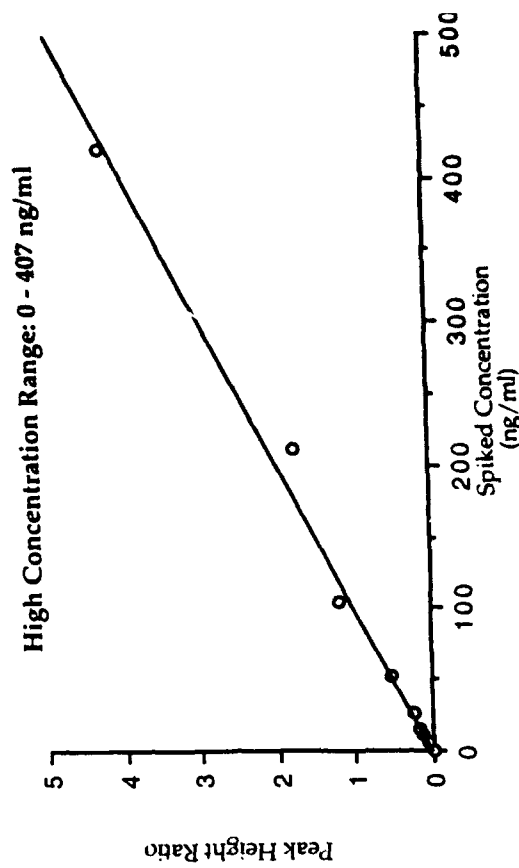
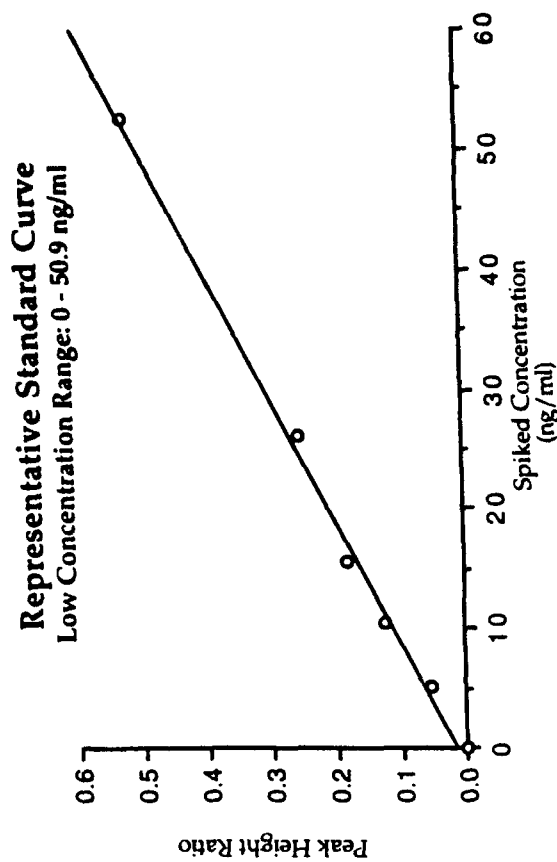


**TABLE 1C: REPRESENTATIVE STANDARD CURVE FOR
WR 254,421 HUMAN URINE ASSAY
STUDY REPORT 22**

SPIKED AMOUNT (ng)	STANDARD CURVE		PEAK HEIGHT RATIO**	CALCULATED CONCENTRATION (ng/ml)
	CONCENTRATION* (ng/ml)	CONCENTRATION		
0	0	0	0	-
22.7	45.4	0.497	0.497	46.1 ^a
45.4	90.8	1.022	1.022	98.0 ^a
68.1	136	1.458	1.458	141 ^a
114	227	2.189	2.189	213 ^a
227	454	4.664	4.664	458 ^a
454	908	9.868	9.868	1070 ^b
908	1820	15.346	15.346	1680 ^b
1820	3630	32.938	32.938	3650 ^b

Regression equations:

^a $y = 0.01012x + 0.0306$, $r^2 = 0.9979$; (Low Range: 0 - 454 ng/ml)

^b $y = 0.00893x + 0.2975$, $r^2 = 0.9958$; (High Range: 0 - 3630 ng/ml)

* When 0.5 ml of biological sample is used.

** Ratio of drug peak height to internal standard peak height.

*** Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level concentrations, two standard curves were constructed from the same set of standard curve data points.

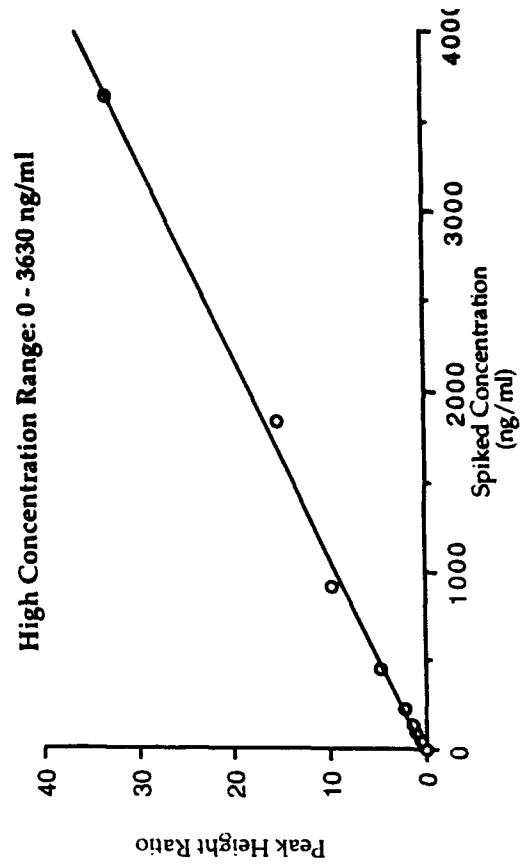
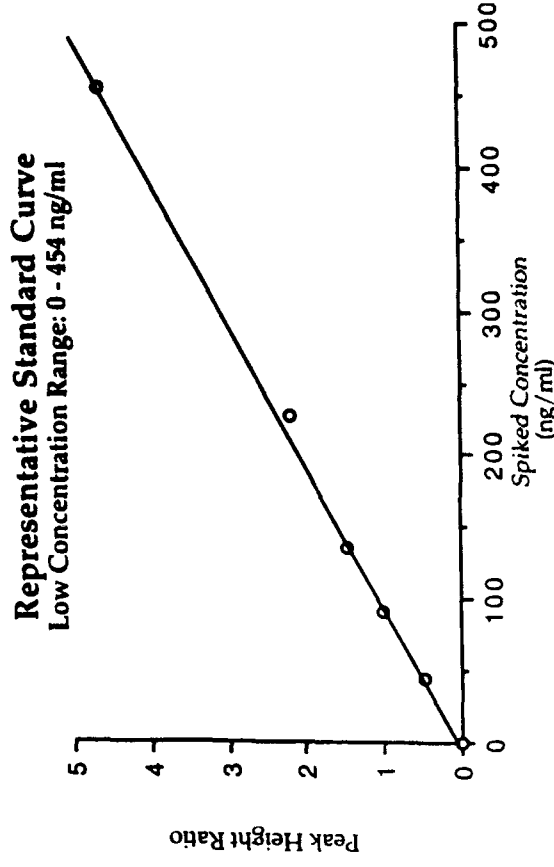


**TABLE 2: WR 6026, WR 211,789, AND WR 254,421 (AS FREE BASES)
MINIMUM QUANTITATION LIMITS**

INTERDAY

Spiked Concentration	WR 6026 (5.17 ng/ml)	WR 211,789 (5.09 ng/ml)	WR 254,421 (45.4 ng/ml)
	Measured Concentrations (ng/ml)		
	bc	6.51	53.3
	6.10	4.76	46.8
	5.60	5.88	44.9
	5.39	5.50	45.3
	3.99	4.48	46.1
	5.40	4.80	42.4
Mean	5.30	5.32	46.1
Standard Deviation	0.785	0.78	3.47
Percent CV	14.8	14.7	7.51
Percent Error	2.44	4.55	1.60

bc = bad chromatogram.

**TABLE 3: RECOVERIES OF WR 6026, WR 211,789 AND WR 254,421
(AS FREE BASES) FROM HUMAN URINE**

SAMPLE ID	SPIKED CONCENTRATION Range (ng/ml)	PEAK HEIGHT RATIO		MEAN PERCENT RECOVERY
		REFERENCE	URINE	
<u>WR 6026</u>				
1	Low	0.299	0.291	91.4
2		0.353	0.284	
3		0.340	0.335	
4		0.339	0.306	
Mean (± SD)		0.333 ± 0.023	0.304 ± 0.023	
1	Medium	1.254	1.380	100
2		1.519	1.393	
3		1.427	1.416	
4		1.381	1.394	
Mean (± SD)		1.395 ± 0.110	1.396 ± 0.015	
1	High	2.153	2.201	100
2		2.205	2.248	
3		2.204	2.261	
4		2.346	2.215	
Mean (± SD)		2.227 ± 0.083	2.231 ± 0.028	
AVERAGE (MEAN PERCENT RECOVERY) =				97.2

<u>WR 211,789</u>				
1	Low	0.316	0.290	86.5
2		0.334	0.255	
3		0.333	0.320	
4		0.344	0.283	
Mean (± SD)		0.332 ± 0.012	0.287 ± 0.027	
1	Medium	1.226	1.315	96.0
2		1.519	1.320	
3		1.421	1.319	
4		1.339	1.330	
Mean (± SD)		1.376 ± 0.124	1.321 ± 0.006	
1	High	2.129	2.011	96.0
2		2.158	2.115	
3		2.156	2.174	
4		2.310	2.100	
Mean (± SD)		2.188 ± 0.082	2.100 ± 0.067	
AVERAGE (MEAN PERCENT RECOVERY) =				92.8

**TABLE 3: RECOVERIES OF WR 6026, WR 211,789 AND WR 254,421
(AS FREE BASES) FROM HUMAN URINE (CONTINUED)**

SAMPLE ID	SPIKED CONCENTRATION Range (ng/ml)	PEAK HEIGHT RATIO		MEAN PERCENT RECOVERY
		REFERENCE	URINE	
<u>WR 254.421</u>				
1	Low	2.422	2.366	91.8
2		2.561	2.212	
3		2.711	2.512	
4		2.668	2.421	
Mean (± SD)		2.591 ± 0.129	2.378 ± 0.126	
1	Medium	10.126	9.939	94.6
2		10.942	10.282	
3		11.223	10.259	
4		10.896	10.388	
Mean (± SD)		10.797 ± 0.470	10.217 ± 0.194	
1	High	16.446	16.116	96.2
2		17.711	17.015	
3		17.265	16.684	
4		17.428	16.43	
Mean (± SD)		17.213 ± 0.543	16.561 ± 0.381	
AVERAGE (MEAN PERCENT RECOVERY) =				94.2

TABLE 4A: INTERDAY PRECISION OF WR 6026 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations ** (ng/ml)									
10.4	11.2	11.7	12.1	11.5	9.74	10.8	11.1	0.83	7.42	7.44
25.9	23.0	25.8	23.2	24.3	25.3	22.8	24.1	1.27	5.28	-7.08
155	143	151	138	138	144	150	144	5.62	3.90	-7.10
259	255	275	220	257	248	245	250	18.0	7.22	-3.47

TABLE 4B: INTRADAY PRECISION OF WR 6026 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
10.4	11.9	10.9	7.82	13.2	bc	6.37	10.0	2.85	28.4	-3.48
25.9	24.5	23.5	bc	24.2	25.3	26.2	24.7	1.04	4.21	-4.48
155	152	145	142	145	158	151	149	5.91	3.97	-3.98
259	237	232	239	236	237	258	240	9.20	3.83	-7.40

** Measured concentrations are averages of two analyses.
bc = bad chromatogram.

TABLE 5A: INTERDAY PRECISION OF WR 211789 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations** (ng/ml)									
10.2	9.20	11.2	11.1	11.9	10.2	11.4	10.8	0.97	8.98	6.21
25.5	21.5	25.9	22.7	21.8	27.2	22.5	23.6	2.36	10.0	-7.45
153	141	145	134	135	145	148	141	5.75	4.07	-7.63
255	255	270	215	253	253	240	248	18.6	7.52	-2.88

TABLE 5B: INTRADAY PRECISION OF WR 211789 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
10.2	12.0	12.4	10.9	7.00	8.89	7.66	9.81	2.28	23.3	-3.84
25.5	22.3	25.5	20.8	24.7	27.1	19.1	23.3	3.04	13.1	-8.82
153	150	146	143	155	136	137	145	7.40	5.12	-5.56
255	241	233	227	228	218	255	234	12.9	5.52	-8.37

** Measured concentrations are averages of two analyses.

TABLE 6A: INTERDAY PRECISION OF WR 254421 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations** (ng/ml)									
90.8	88.0	98.9	101	97.7	89.5	96.4	95.3	5.28	5.54	4.90
227	206	238	217	208	215	212	216	11.5	5.34	-4.85
1360	1270	1290	1240	1230	1270	1340	1273	39.3	3.09	-6.37
2270	2230	2370	1990	2260	2180	2130	2193	128.5	5.86	-3.38

TABLE 6B: INTRADAY PRECISION OF WR 254421 HUMAN URINE ASSAY

SPIKED CONC. (ng/ml)	SAMPLE NUMBER						MEAN (ng/ml)	S.D. (ng/ml)	Percent C.V.	Percent Error
	1	2	3	4	5	6				
	Measured Concentrations (ng/ml)									
90.8	117	99.3	99.2	102	85.4	103	101	10.1	10.0	11.2
227	216	212	215	215	206	230	216	7.92	3.67	-4.99
1360	1310	1270	1290	1260	1380	1260	1295	45.9	3.55	-4.78
2270	2060	2000	2100	2040	2080	2260	2090	90.1	4.31	-7.93

** Measured concentrations are averages of two analyses.

**TABLE 7A: ACCURACY OF WR 6026 (FREE BASE) HUMAN URINE ASSAY
(BLIND STUDY RESULTS)**

Sample Number	Spiked Level (ng/ml)	Measured Level# (ng/ml)	Statistics (ng/ml)
6	2.60	*	Mean =
7		*	SD =
16		*	Percent CV =
22		*	Percent Bias =
1	5.2	7.12	Mean = 6.96
12		7.87	SD = 0.732
17		6.74	Percent CV = 10.5
23		6.12	Percent Bias = 33.9
2	15.5	12.5	Mean = 13.9
11		15.3	SD = 1.32
13		14.6	Percent CV = 9.51
24		13.0	Percent Bias = -10.6
3	56.2	52.2	Mean = 53.9
8		52.9	SD = 1.63
14		54.6	Percent CV = 3.03
21		55.8	Percent Bias = -4.14
4	78.7	72.3	Mean = 75.6
9		76.3	SD = 2.32
18		77.7	Percent CV = 3.07
20		76.2	Percent Bias = -3.91
5	101.2	97.1	Mean = 97.0
10		95.7	SD = 0.991
15		98.1	Percent CV = 1.02
19		97.2	Percent Bias = -4.13

n = 3, unless a chromatogram is determined to be unacceptable.

**TABLE 7B: ACCURACY OF WR 211,789 (FREE BASE) HUMAN URINE
ASSAY (BLIND STUDY RESULTS)**

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
6	2.60	*	Mean =
7		*	SD =
16		*	Percent CV =
22		*	Percent Bias =
1	5.20	6.68	Mean = 6.97
12		7.92	SD = 0.720
17		7.04	Percent CV = 10.3
23		6.22	Percent Bias = 33.9
2	15.7	12.7	Mean = 13.9
11		13.8	SD = 1.03
13		15.2	Percent CV = 7.42
24		13.7	Percent Bias = -11.8
3	57.0	52.8	Mean = 54.8
8		54.2	SD = 1.60
14		55.7	Percent CV = 2.93
21		56.4	Percent Bias = -3.90
4	79.8	76.9	Mean = 77.2
9		76.8	SD = 0.655
18		78.2	Percent CV = 0.848
20		77.0	Percent Bias = -3.23
5	102.6	97.1	Mean = 98.1
10		97.4	SD = 1.94
15		101	Percent CV = 1.98
19		96.9	Percent Bias = -4.39

[#] n = 3, unless a chromatogram is determined to be unacceptable.

**TABLE 7C: ACCURACY OF WR 254,421 (FREE BASE) HUMAN URINE
ASSAY (BLIND STUDY RESULTS)**

Sample Number	Spiked Level (ng/ml)	Measured Level [#] (ng/ml)	Statistics (ng/ml)
6	5.0	*	Mean =
7		*	SD =
16		*	Percent CV =
22		*	Percent Bias =
1	50.1	56.2	Mean = 54.9
12		61.9	SD = 5.34
17		51.6	Percent CV = 9.72
23		50.0	Percent Bias = 9.63
2	150.3	124	Mean = 134
11		135	SD = 7.80
13		135	Percent CV = 5.81
24		143	Percent Bias = -10.7
3	544.1	513	Mean = 522
8		521	SD = 7.85
14		520	Percent CV = 1.51
21		532	Percent Bias = -4.15
4	761.6	745	Mean = 742
9		753	SD = 9.00
18		735	Percent CV = 1.21
20		734	Percent Bias = -2.61
5	979.4	935	Mean = 951
10		948	SD = 13.6
15		953	Percent CV = 1.43
19		968	Percent Bias = -2.90

[#] n = 3, unless a chromatogram is determined to be unacceptable.

TABLE 8: STABILITIES OF WR 6026, WR 211,789 AND WR 254,421 IN HUMAN URINE

WR 6026

Spiked Concentration (ng/ml):	10.4	25.9	155	259
TIME STORED	WR 6026 Free Base Concentration of Samples Stored at -70°C** (ng/ml)			
0 days	9.53	19.8	144	187
1 day	12.2	25.7	132	186
2 days	11.5	25.1	151	203
4 days	9.81	20.9	137	185
1 week	10.9	24.0	139	197
2 weeks	9.82	22.0	131	218
3 weeks	7.22	24.2	145	241
1 month	8.40	20.5	160	239
4 months	10.7	23.6	153	229

WR 211,789

Spiked Concentration (ng/ml):	10.2	25.5	153	255
TIME STORED	WR 211,789 Free Base Concentration of Samples Stored at -70°C** (ng/ml)			
0 days	11.0	20.0	147	201
1 day	12.5	25.5	130	194
2 days	12.6	23.7	146	195
4 days	9.78	20.2	135	187
1 week	9.29	23.5	137	191
2 weeks	9.47	22.8	126	215
3 weeks	7.38	22.9	141	236
1 month	9.77	19.7	156	240
4 months	9.25	23.3	144	221

WR 254,421

Spiked Concentration (ng/ml):	100	250	1500	2500
TIME STORED	WR 254,421 Free Base Concentration of Samples Stored at -70°C** (ng/ml)			
0 days	96.0	205	1500	2110
1 day	121	261	1470	2095
2 days	102	252	1560	2070
4 days	96.7	223	1440	1940
1 week	87.3	226	1390	1940
2 weeks	98.4	221	1310	2190
3 weeks	96.6	233	1480	2400
1 month	97.9	219	1570	2370
4 months	96.2	232	1450	2110

** Measured concentrations are averages of two analyses.

TABLE 9: AUTOSAMPLER STABILITY OF WR 6026, WR 211,789 AND WR 254,421 IN HUMAN URINE

WR 6026

Spiked Concentration (ng/ml):	10.4	25.9	155	259
TIME STORED	WR 6026 Free Base Concentration of Samples Stored at Room Temperature* (ng/ml)			
0 hours	11.1	26.4	159	238
24 hours	12.3	25.7	146	193
48 hours	14.5	26.6	149	224

WR 211,789

Spiked Concentration (ng/ml):	10.2	25.5	153	255
TIME STORED	WR 211,789 Free Base Concentration of Samples Stored at Room Temperature* (ng/ml)			
0 hours	10.5	25.7	142	229
24 hours	11.5	24.4	138	183
48 hours	11.1	25.9	139	205

WR 254,421

Spiked Concentration (ng/ml):	100	250	1500	2500
TIME STORED	WR 254,421 Free Base Concentration of Samples Stored at Room Temperature* (ng/ml)			
0 hours	114	250	1480	2280
24 hours	112	253	1300	1730
48 hours	115	260	1300	1900

* Measured concentrations are averages of two analyses.

Appendix B: Routine Assay Data

Routine Analysis Study: Hal/P 91-4
Routine Analysis Study: Mef/B 91-5
Routine Analysis Study: Hal/P 91-6
Routine Analysis Study: Mef/P 91-7
Routine Analysis Study: WR6/P 92-1
Routine Analysis Study: Hal/P 92-2
Routine Analysis Study: WR5/PB 92-3
Routine Analysis Study: WR6/PU 93-1
Routine Analysis Study: Hal/P 93-2
Routine Analysis Study: Pah/P 93-3
Routine Analysis Study: WR5/P 93-4
Routine Analysis Study: WR5/P 93-5
Routine Analysis Study: Pri/P 93-6
Routine Analysis Study: Hal/lpb 93-7

ANALYTICAL DATA

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
9990	1	male	*	*
9991	1	male	*	*
9992	1	male	*	*
9993	1	male	*	*
9994	1	male	*	*
9995	1	male	*	*
9996	1	male	*	*
9997	1	male	*	*
9998	1	male	*	*
9999	1	male	*	*
10050	1	female	*	*
10051	1	female	*	*
10052	1	female	*	*
10053	1	female	*	*
10054	1	female	*	*
10055	1	female	*	*
10056	1	female	*	*
10057	1	female	*	*
10058	1	female	*	*
10059	1	female	*	*

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
10230	3	male	178	414
10231	3	male	261	433
10232	3	male	194	395
10233	3	male	378	324
10234	3	male	609	448
10235	3	male	178	296
10236	3	male	180	440
10238	3	male	510	115
10239	3	male	290	229
10240	3	male	197	445
10290	3	female	779	261
10291	3	female	1500	249
10292	3	female	293	146
10293	3	female	462	150
10294	3	female	765	153
10296	3	female	496	145
10297	3	female	394	118
10298	3	female	389	143
10299	3	female	2360	94.8
10300	3	female	437	130

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
10110	2	male	36.8	34.5
10111	2	male	38.6	97.3
10112	2	male	46.0	41.0
10113	2	male	37.8	56.6
10114	2	male	90.1	34.1
10115	2	male	34.9	62.6
10116	2	male	55.5	24.1
10117	2	male	34.5	73.9
10118	2	male	30.8	31.1
10119	2	male	40.4	69.5
10170	2	female	44.6	11.9
10171	2	female	53.3	10.5
10172	2	female	55.2	13.5
10173	2	female	58.1	16.1
10174	2	female	57.5	8.65
10175	2	female	51.6	12.2
10176	2	female	44.1	11.3
10177	2	female	71.3	7.71
10178	2	female	86.9	12.9
10179	2	female	77.8	14.8

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
10350	4	male	459	936
10351	4	male	665	1140
10352	4	male	644	1070
10353	4	male	452	1110
10354	4	male	598	654
10355	4	male	650	795
10356	4	male	819	1180
10357	4	male	810	1260
10358	4	male	514	940
10359	4	male	415	1130
10410	4	female	703	486
10411	4	female	1210	503
10413	4	female	925	488
10414	4	female	1000	357
10415	4	female	1250	439
10416	4	female	1450	466
10417	4	female	1370	344
10418	4	female	934	325
10419	4	female	1020	351
10420	4	female	1270	577

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
10470	5	male	962	1840
10471	5	male	3270	1740
10472	5	male	779	2500
10473	5	male	1170	2060
10474	5	male	793	1630
10475	5	male	811	2010
10476	5	male	1450	3700
10477	5	male	593	2180
10478	5	male	919	1110
10479	5	male	495	1800
10480	5	male	919	1920
10481	5	male	1360	4580
10482	5	male	692	3710
10483	5	male	1310	4370
10484	5	male	824	2060
10485	5	male	694	1880
10486	5	male	1130	6280
10487	5	male	1420	2790
10488	5	male	1720	3940
10489	5	male	1020	2100

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-4
PLASMA RESULTS

Animal Number	Dose Group	Sex	WR 171,669 Free Base Concentration (ng/ml)	WR 178,460 Free Base Concentration (ng/ml)
10490	5	female	2800	562
10491	5	female	1650	735
10492	5	female	7120	1790
10493	5	female	1670	899
10494	5	female	2340	2060
10495	5	female	4540	955
10496	5	female	2620	771
10497	5	female	2500	881
10498	5	female	2090	1890
10500	5	female	4680	709
10501	5	female	2210	1050
10502	5	female	2940	1120
10503	5	female	3020	3620
10505	5	female	1950	719
10506	5	female	3240	738
10507	5	female	2090	760
10508	5	female	2570	647
10509	5	female	1440	695

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

Analytical Data
Mef/P 91-5

Subject	4	5	6	7	8	9	10	11	12	13
Scheduled Sampling Time (hrs.)	Mefloquine Free Base Concentration (ng/ml)									
0.00	766.	578.	368.	638.	280.	674.	247.	408.	779.	551.
12.00	730.	659.	518.	680.	306.	672.	244.	339.	526.	585.
18.00	791.	1700.	441.	630.	345.	757.	219.	342.	576.	426.
24.00	816.	660.	689.	682.	366.	814.	271.	351.	674.	478.
48.00	NR	609.	NR	730.	389.	895.	318.	380.	568.	332.
72.00	NR	509.	NR	720.	337.	724.	216.	169.	452.	423.
168.00	310.	328.	345.	397.	198.	373.	170.	198.	275.	247.
240.00	NR	304.	287.	370.	150.	364.	133.	NR	238.	209.
336.00	NR	223.	228.	237.	131.	233.	89.0	NR	197.	156.
504.00	106.	148.	196.	293.	90.0	230.	64.3	87.5	212.	90.5
672.00	NS	105.	127.	NS	63.2	NS	65.7	NR	167.	75.6

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat,
NR = Not Run, insufficient Sample to Run, NA = Not Applicable

Halofantrine Study
Hal/P 91-6
PLASMA RESULTS

Sample ID	Dose (mg/kg)	Halofantrine Conc. (ng/ml)	WR 178,460 Conc. (ng/ml)
28146	0	*	++
28147	0	*	++
28148	0	*	++
28149	0	*	++
28150	0	*	++
28151	0	*	++
28152	0	*	++
28153	0	*	++
28154	2	496	77.8
28155	2	239	74.3
28156	2	123	32.5
28157	2	119	39.9
28158	2	292	22.1
28159	2	304	29.4
28160	2	285	17.0
28161	2	153	76.1
28162	5	531	113
28163	5	1850	283
28164	5	1230	127
28165	5	970	137
28166	5	1210	141
28167	5	492	68.7
28168	5	1520	168
28169	5	1920	277
28172	20	3720	331
28173	20	3180	227
28175	20	2440	BC
28176	20	3330	483
28177	20	2760	146

* = Below Assay Sensitivity

+ = Controls Not Acceptable and Insufficient Sample to Repeat

BC = Bad Chromatogram, Insufficient Sample to Repeat

NS = No Sample, NR = Not Run, NA = Not Applicable

ANALYTICAL RESULTS FOR STUDY MEE/P 91-7

Subject 1006

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	404
48	330
72	318
168	NS
504	296
840	NS
1176	923
1512	641
1848	835

Subject 1026

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	218
48	216
72	166
168	154
504	368
840	709
1176	632
1512	844
1848	1080

Subject 1017

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	183
48	460
72	793
168	665
504	756
840	816
1176	486
1512	912
1848	923

Subject 1038

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	88.4
48	80.2
72	80.7
168	84.1
504	221
840	305
1176	306
1512	375
1848	398

Subject 1055

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	196
48	199
72	167
168	152
504	395
840	377
1176	857
1512	508
1848	390

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 1069

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	224
48	235
72	241
168	215
504	462
840	608
1176	NS
1512	742
1848	1060

Subject 1088

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	177
48	193
72	138
168	183
504	285
840	403
1176	703
1512	512
1848	546

Subject 1104

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	229
48	498
72	640
168	520
504	676
840	619#
1176	632
1512	566
1848	451

Subject 1073

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	258
48	505
72	764
168	595
504	431
840	500
1176	420
1512	321
1848	505

Subject 1100

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	316
48	230
72	276
168	232
504	454
840	969
1176	783
1512	407
1848	618

Subject 1115

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	
48	
72	80.7
168	81.2
504	357
840	479
1176	473
1512	NS
1848	142@

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable

#Missed mefloquine dose for 504 hr.
@No mefloquine dose for 7 weeks preceding final sample.

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 1126

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	236
48	459
72	597
168	522
504	405
840	337
1176	573
1512	419
1848	499

Subject 1137

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	207
48	134
72	165
168	132#
504	316
840	407
1176	622
1512	591
1848	649

Subject 1153

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	217
48	207
72	157
168	132
504	325
840	715
1176	540
1512	NS
1848	NS

Subject 1127

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	136
48	157
72	153
168	107
504	316
840	402
1176	431
1512	469
1848	538

Subject 1150

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	178
48	349
72	NS
168	407
504	458
840	477
1176	450
1512	540
1848	537

Subject 1169

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	224
48	177
72	195
168	183
504	398
840	484
1176	727
1512	594
1848	717

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
#168 hr tube was dated 9/10, while a second tube dated 9/24 was entered into the 504 hr sampling time.

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 1177

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	180
48	397
72	544
168	537
504	363#
840	815
1176	794
1512	538
1848	417

Subject 1199

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	219
48	205
72	193
168	172
504	287
840	494
1176	348
1512	605
1848	907

Subject 2007

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	182
48	305
72	574
168	342
504	422
840	NS
1176	385
1512	487
1848	510

Subject 1185

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	221
48	213
72	187
168	159
504	292@
840	293
1176	NS
1512	NS
1848	64.1

Subject 2004

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	170
48	181
72	205
168	136
504	418
840	330
1176	517
1512	568
1848	826

Subject 2022

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	249
48	454
72	648
168	775
504	736
840	701
1176	692
1512	761
1848	689

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
 #This 504 hr tube was dated 9/23, while a second tube dated 9/6 was entered at the 72 hr sampling time.
 @No further mefloquine after this sampling time.

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 2025

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	281
48	223
72	225
168	98.4
504	382
840	456
1176	510
1512	416
1848	605

Subject 2037

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	255
48	589
72	850
168	572
504	921
840	729
1176	743
1512	508
1848	730

Subject 2047

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	262
48	354
72	663
168	627
504	916
840	1130
1176	980
1512	785
1848	857

Subject 2029

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	171
48	310
72	364
168	NS
504	NS
840	NS
1176	NS
1512	NS
1848	NS

Subject 2039

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	430
48	303
72	341
168	364
504	363
840	454
1176	642
1512	787
1848	710

Subject 2062

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	325
48	639
72	765
168	651
504	517#
840	NS
1176	856
1512	851
1848	1170

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
#Missed mefloquine dose on preceding week.

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 2064

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	296
48	273
72	243
168	161
504	332
840	700
1176	661
1512	428
1848	603

Subject 2075

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	138
48	140
72	130
168	58.9#
504	138
840	211
1176	511
1512	399
1848	367

Subject 3004

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	57.6
48	253
72	316
168	270
504	390
840	537
1176	NS
1512	NS
1848	NS

Subject 2069

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	303
48	616
72	732
168	675
504	887
840	913
1176	1060
1512	1030
1848	865

Subject 2076

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	274
48	486
72	556
168	239
504	289
840	233
1176	407@
1512	305
1848	343

Subject 3005

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	170
48	134
72	148
168	109
504	228
840	385
1176	532
1512	557
1848	502

* = Below Assay Sensitivity; NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
 # Missed mefloquine dose at 168 hr.
 @ Mefloquine labeled week 9 (1512 h).

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 3009

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	256
48	500
72	704
168	527
504	421
840	423
1176	504
1512	617
1848	570

Subject 3017

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	203
48	410
72	696
168	553
504	518
840	595
1176	668
1512	710
1848	635

Subject 3030

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	193
48	174
72	152
168	411
504	364
840	363
1176	523
1512	701
1848	394

Subject 3015

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	352
48	NS
72	186
168	90.1
504	149#
840	281
1176	337
1512	361
1848	366

Subject 3023

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	178
48	354
72	505
168	355
504	NS
840	467
1176	426
1512	545
1848	543@

Subject 3032

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	203
48	NS
72	NS
168	345
504	255
840	331
1176	312
1512	321
1848	431

* = Below Assay Sensitivity; NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
 #This 504 hr tube had no date, while a second tube dated 9/26 was assayed at 257 ng/ml.

@Missed mefloquine dose on preceding week.

ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject 3039

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	232
48	496
72	750
168	478
504	532
840	547
1176	951
1512	1040
1848	743

Subject 3048

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	413
48	948
72	1180
168	719
504	918
840	1010
1176	1410
1512	1460
1848	1120

Subject 3058

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	191
48	439
72	635
168	509
504	575
840	748
1176	818#
1512	NS
1848	NS

Subject 3042

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	89
48	219
72	164
168	67.2
504	294
840	400
1176	616
1512	356
1848	709

Subject 3054

Sample Time (hrs.)	Mefloquine Free Base Conc. (ng/ml)
24	62.4
48	164
72	133
168	107
504	360
840	329
1176	455
1512	266
1848	433@

* = Below Assay Sensitivity; NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
#Data for preceding week. @Data for 1680 hr instead of 1848 hr.

END STUDY LEVELS
ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject ID	Mefloquine Free Base Conc. (ng./ml)
1001	573
1007	*
1008	*
1010	*
1011	605
1012	*
1013	627
1018	*
1019	521
1021	*
1022	1050
1023	*
1024	990
1025	*
1027	682
1028	471
1030	1120
1031	*
1033	*
1035	493
1036	*
1037	702
1039	*
1040	536

Subject ID	Mefloquine Free Base Conc. (ng./ml)
1041	438
1042	*
1043	704
1045	*
1047	255
1048	*
1049	*
1050	765
1051	752
1052	761
1053	*
1054	*
1056	435
1057	683
1058	1070
1059	774
1062	*
1063	*
1064	*
1065	759
1066	459
1067	*
1068	*
1070	387

Subject ID	Mefloquine Free Base Conc. (ng./ml)
1071	484
1072	*
1074	11.1
1076	*
1077	336#
1079	320
1080	709
1081	*
1082	*
1084	*
1085	*
1087	449
1089	616
1090	445
1091	*
1093	*
1094	*
1095	910
1096	424
1097	502
1098	581
1099	373
1101	*

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
 #This result is the average between two analysis' (322 ng/ml and 349 ng/ml) per phone discussion between COR and QA officer.

END STUDY LEVELS
ANALYTICAL RESULTS FOR STUDY MEF/P 91-7

Subject ID	Mefloquine Free Base Conc. (ng/ml)
1102	*
1106	762
1107	*
1108	1110
1109	243
1110	377
1111	983
1113	*
1114	*
1116	*
1117	755
1118	544
1119	*
1120	450
1121#	498
1123	*
1124	932
1128@	421
1129	*
1130	*
1131	653
1132	602
1133	754
1134	1560

Subject ID	Mefloquine Free Base Conc. (ng/ml)
1135	*
1136	576
1138	615
1139	615
1140	569
1141	*
1143	*
1145	363
1147	*
1148	*
1149	1370
1154	*
1155	564
1156	874
1158	*
1159	*
1161&	645
1163	677
1164	515
1165	*
1167	432
1168(9/16)	587
1168(9/18)	739
1168(10/7)	342

Subject ID	Mefloquine Free Base Conc. (ng/ml)
1170	*
1171#	612
1172	751
1173	690
1174	*
1176	*
1178	253
1180\$	24.7
1181	560
1182	*
1183	*
1184	808
1186	729
1187	830
1188	447
1189	*
1191	*
1192	642
1194	*
1195	473
1196	712
1198	*
1200	708
1201	*

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
 # After 10 doses. @ After 5 doses. & After 7 doses. \$ Patient treated with Chloroquine and also with Mefloquine on 9/15/91.

**END STUDY LEVELS
ANALYTICAL RESULTS FOR STUDY MEF/P 91-7**

Subject ID	Mefloquine Free Base Conc. (ng/ml)	Subject ID	Mefloquine Free Base Conc. (ng/ml)	Subject ID	Mefloquine Free Base Conc. (ng/ml)
1202	*	1229	*	2035	762
1203	*	2001	576	2038	933
1205	*	2002	574	2041@	460
1206	302	2003	*	2042	946
1210	*	2006	*	2043	825
1211	*	2008	*	2044	*
1212	673	2009	441	2045	*
1213	746	2011	*	2046	*
1214#	402	2013	11.5	2048	*
1215	609	2014	798	2049	567
1216	*	2015	*	2050	*
1217	875	2016	*	2051	497
1218	*	2017	584	2052	*
1220	*	2018	625	2054	673
1221	*	2020	*	2055	*
1222	*	2021	*	2056	551
1223	926	2026	629	2057&	524
1224	648	2027	*	2058	*
1225(9/13)	*	2028	455	2059	*
1225(9/16)	*	2030	*	2060^	464
1225(9/18)	*	2031	477	2066	488
1226	1140	2032	525	2067	245
1227	270	2033	*	2068	568
1228	*	2034	650	2072	768

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
After 9 doses. @ After 6 doses. & After 12 doses in loading dose patient. ^ After 5 doses.

**END STUDY LEVELS
ANALYTICAL RESULTS FOR STUDY MEF/P 91-7**

Subject ID	Mefloquine Free Base Conc. (ng/ml)
3069	*
3070	451

Subject ID	Mefloquine Free Base Conc. (ng/ml)
3035(9/04)	251
3035(9/12)	118
3035(9/19)	135
3037	582
3038	321
3040	*
3041	*
3043	*
3044	317
3045	542
3046	*
3047	665
3050	602
3051	*
3052	943
3053	803
3055	*
3056	*
3057	*
3061	865
3064	460
3065	673
3067	*
3068	*

Subject ID	Mefloquine Free Base Conc. (ng/ml)
2073	756
2077	539
3001	788
3002	*
3003	342
3007	963
3008	333
3010	644
3012	600
3013#	595
3014	*
3016	*
3018	*
3019	755
3020	442
3022	423
3024	320
3026	*
3027	*
3028	786
3029	*
3031	645
3033	*
3034	638

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable
= Patient only completed 7 doses at time blood sample was taken.

**WR 6026 Study WR6/P 92-1
PLASMA RESULTS**

Subject 040K

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. # (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
8-Jun	17.1	*
9-Jul	42.7	*
10-Jul	83.7	*
11-Jul	38.9	*
12-Jul	20.0	*
15-Jul	17.0	*
19-Jul	132. ASC	*
22-Jul	52.3	*

Subject 1242

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
8-Jan	112. ASC	4.90
14-Jan	191. ASC	44.7
21-Jan	450. ASC	96.7
28-Jan	18.7	5.12
4-Feb	4.88	3.81
11-Feb	*	*
18-Feb	*	5.72
25-Feb	*	20.5
4-Mar	*	*

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range

BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range

CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

= Relative retention times of chromatographic peaks used to quantitate WR 6026 (free base) concentrations for dog subject 040K corresponded to a non WR 6026 peak eluting slightly earlier than WR 6026 in some human samples assayed for this study.

PRELIMINARY DATA

**WR 6026 Study WR6/P 92-1
PLASMA RESULTS**

Subject 1241

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
8-Jan	CO 113. ASC	4.13
14-Jan	CO 389. ASC	98.8
21-Jan	CO 96.8	56.2
4-Feb	5.53	3.20
11-Feb	CO *	*
18-Feb	CO *	8.64
25-Feb	CO *	3.49
28-Feb	CO 8.04	5.50
4-Mar	CO *	1.33

Subject 1208

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
6-Nov	CO 218. ASC	5.73
12-Nov	CO 392. ASC	65.4
19-Nov	CO 108.	47.7
3-Dec	CO *	*
10-Dec	CO *	*
2-Jan	CO *	*
20-Mar	CO *	*

PRELIMINARY DATA

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

ASC =Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range

BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range

CO =Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

**WR 6026 Study WR6/P 92-1
PLASMA RESULTS**

Subject 1234

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
11-Dec	*	12.4
18-Dec	375. ASC	52.9
24-Dec	52.0	32.8
2-Jan	23.4	*
7-Jan	*	*
11-Feb	*	*

Subject 1185

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
22-Oct	*	*
29-Oct	*	*
4-Dec	*	*

Subject 1209

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
26-Oct	476. ASC	80.6
2-Nov	324. ASC	120
15-Nov	6.28	2.76
16-Nov	7.27	3.10
7-Dec	*	*
17-Dec	19.5	*

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range

BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range

CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

PRELIMINARY DATA

**WR 6026 Study WR6/P 92-1
PLASMA RESULTS**

Subject 1251

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
29-Jan	*	1.70
4-Feb	143. ASC	43.8
18-Feb	3.34	4.55
25-Feb	5.01	CO 2.63
4-Mar	*	2.41

Subject 1203

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
29-Oct	*	14.8
8-Nov	20.4	CO 5.20
10-Nov	*	*
17-Dec	*	*

PRELIMINARY DATA

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

ASC =Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range

BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range

CO =Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

**WR 6026 Study WR6/P 92-1
PLASMA RESULTS**

Subject 1225

Scheduled Sampling Time (Date)	WR6026 (free base) Conc. (ng/ml)	WR 211,789 (free base) Conc. (ng/ml)
20-Nov	198. ASC	9.02
26-Nov	104. ASC	52.6
3-Dec	259. ASC	64.3
4-Dec	90.7	46.6
17-Dec	*	CO 2.76
2-Jan	*	CO 1.22 BSC
26-Feb	*	12.9

PRELIMINARY DATA

* = Below Assay Sensitivity

BC = Bad Chromatogram, Insufficient Sample to Repeat

ASC =Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range

BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range

CO =Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

WR 6026 Study WR6/P 92-1
PLASMA RESULTS

Subject 1234

Sample ID	WR 6026 (free base) Conc. (ng/ml)	WR 211789 (free base) Conc. (ng/ml)
11-Dec 10:30	*	*
11-Dec 12:30	322. ASC	5.60
18-Dec 9:50	33.1	18.7
18-Dec 13:20	156. ASC	22.3
24-Dec 11:15	29.4	17.3
?	4.75	4.69

Subject 1251

29-Jan 10:00	*	*
29-Jan 12:00	22.4	*
4-Feb 10:20	123.	42.2
5-Feb 11:40	212. ASC	37.1
11-Feb 10:00	80.9	44.7
11-Feb 12:45	192.	40.2

Subject 1209

26-Oct 9:00	408. ASC	61.1
26-Oct 12:15	428. ASC	56.8
2-Nov 9:30	264. ASC	85.4
2-Nov 12:30	428. ASC	72.7
8-Nov 9:30	14.0	*
16-Nov 9:30	3.50	*

Subject 1225

20-Nov 3:20	*	*
20-Nov 5:30	218. ASC	6.57
26-Nov 9:30	82.3	41.4
4-Dec 9:00	63.8	32.8
3-Dec 10:05	144.	37.6
3-Dec 12:10	225. ASC	50.1

Subject 1208

6-Nov 9:00	*	*
6-Nov 11:30	192. ASC	4.96
12-Nov 10:20	164. ASC	64.9
12-Nov 12:30	341. ASC	70.3
19-Nov 9:30	94.5	45.7
19-Nov 11:30	119. ASC	42.7
3-Dec 9:00	1.89	*

* = Below Assay Sensitivity; BC = Bad Chromatogram, Insufficient Sample to Repeat
 ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range
 BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range
 CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

WR 6026 Study WR6/P 92-1
PLASMA RESULTS

Subject 1241

8-Jan 9:10	*	*
8-Jan 11:15	77.7	3.10
14-Jan 9:30	83.4	42.9
14-Jan 11:30	100.	24.3
21-Jan 9:30	91.3	54.6
21-Jan 11:45	235. ASC	64.8
21-Jan 11:00	102. ASC	27.3

Subject 1531

24-Feb :?	*	*
26-Feb 9:35	12.6	2.12
2-Mar 9:30	25.8	10.9
3-Mar 12:00	53.5	5.93
9-Mar 10:09	20.6	10.5
9-Mar 12:17	118. ASC	15.0
16-Mar 10:00	18.9	10.8
16-Mar 12:00	97.2	15.7
23-Mar 9:15	30.8	15.0
23-Mar 12:00	161. ASC	17.4
30-Mar 9:00	BSC 2.16	*
7-Mar 9:45	*	*

Subject 1185

29-Oct 10:20	2.21	*

Subject 1242

8-Jan 9:30	*	*
8-Jan 11:55	107.	3.94
14-Jan 9:55	196.	46.0
21-Jan 9:35	178. ASC	66.9
21-Jan 11:45	434. ASC	78.9
28-Jan 11:15	12.5	*
4-Feb 9:20	7.82	*

Subject 1521

20-Jan :?	*	*
28-Jan 2:10	47.3	*
29-Jan :trough	96.3	3.00
3-Feb 9:25	204. ASC	52.0
3-Feb 12:05	520. ASC	55.6
10-Feb 9:50	157. ASC	45.2
10-Feb 12:20	257. ASC	45.1
17-Feb 9:35	48.9	19.8
17-Feb 12:20	52.8	21.0
24-Feb :peak	77.7	19.0
24-Feb :trough	85.5	26.1
2-Mar 9 :55	4.70	*
9-Mar 10 :10	BSC 1.86	*

* = Below Assay Sensitivity; BC = Bad Chromatogram, Insufficient Sample to Repeat
 ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range
 BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range
 CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

WR 6026 Study WR6/P 92-1
PLASMA RESULTS

Subject 1518

20-Jan :?	*	*
21-Jan 12:05	135. ASC	2.79
23-Jan 10:00	48.5	8.05
27-Jan 9:05	58.9	20.5
27-Jan 12:00	208. ASC	31.7
3-Feb 9:10	78.5	27.7
3-Feb 12:05	238. ASC	40.6
10-Feb 9:45	85.1	33.5
10-Feb 12:00	179. ASC	34.5
17-Feb 10:00	102. ASC	39.6
17-Feb 12:25	107. ASC	20.9
25-Feb 9:00	*	*
25-Feb 12:00	140. ASC	3.74
2-Mar 9:15	*	*

Subject 1540

9-Apr :?	*	*
14-Apr 2:00	228. ASC	20.2
22-Apr :?	136. ASC	43.4
27-Apr 10:00	44.0	20.6
27-Apr 12:35	106.	26.3
4-May 8:50	53.6	27.4
4-May 12:00	106. ASC	32.2
11-May 12:05	52.5	17.7

Subject 1532

10-Feb 10:45	*	*
18-Feb 7:00	90.9	2.18
19-Feb 10:00	13.9	*
24-Feb 9:45	11.3	11.5
24-Feb 12:00	85.8	14.1
2-Mar 10:30	16.9	8.80
3-Mar 12:10	6.84	3.09
9-Mar 10:10	10.1	5.83
9-Mar 12:10	34.4	18.2
16-Mar 10:35	1.95	*
16-Mar 12:07	5.38	3.35
23-Mar 10:04	*	*
25-Mar 12:08	*	*
30-Mar 10:00	*	*
13-Apr 9:35	*	7.20

Subject 1546

13-Apr :?	*	*
22-Apr 9:20	*	*
22-Apr 12:00	88.0	1.92
27-Apr 9:15	76.8	43.3
27-Apr 12:30	109. ASC	38.4
4-May 8:50	66.4	52.7
4-May 12:00	170. ASC	57.1
11-May 12:00	141. ASC	40.0

* = Below Assay Sensitivity; BC = Bad Chromatogram, Insufficient Sample to Repeat
ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range
BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range
CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

WR 6026 Study WR6/P 92-1
PLASMA RESULTS

Subject 1535

24-Feb :?	1.71	*
25-Feb 12:00	246. ASC	3.04
26-Feb 9:15	50.2	3.94
2-Mar 9:15	54.6	25.5
3-Mar 12:05	187. ASC	27.0
9-Mar 10:00	24.1	10.2
9-Mar 12:40	108.	16.5
16-Mar 10:20	18.9	8.48
16-Mar 12:05	64.6	10.5
23-Mar 9:45	38.8	17.1
23-Mar 12:00	94.7	14.3
30-Mar 9:45	1.33	*
7-Mar 9:45	*	*
13-Apr 10:00	*	*

Subject 040K

8-Jun :	*	*
9-Jul :	*	*
10-Jul :	*	*
11-Jul :	*	*
12-Jul :	*	*
15-Jul :	*	*
19-Jul :	*	*
22-Jul :	*	*

* = Below Assay Sensitivity; BC = Bad Chromatogram, Insufficient Sample to Repeat
 ASC = Bad Chromatogram, Insufficient Sample to Repeat - Result Above Standard Curve Range
 BSC = Bad Chromatogram, Insufficient Sample to Repeat - Result Below Standard Curve Range
 CO = Bad Chromatogram, Insufficient Sample to Repeat - Controls Out, Result Not Acceptable

Analytical Data Hal/P 92-02

Subject ID	Halofantrine (free base) Concentration (ng/ml)	WR 178,460 (free base) Concentration (ng/ml)	Subject ID	Halofantrine (free base) Concentration (ng/ml)	WR 178,460 (free base) Concentration (ng/ml)
10001	*	*	10141	254	73.2
10002	*	*	10144	87.8	32.7
10004	*	*	10148	238	51.4
10007	*	*	10150	403	179
10010	*	*	10158	110	20.5
10011	*	*	10161	102	36.8
10014	*	*	10163	215	22.8
10019	*	*	10165	108	37.6
10022	1.56	*	10166	113	52.1
10027	*	*	10167	127	108
10028	*	*	10184	152	40.1
10029	*	*	10186	521	72.0
10031	*	*	10187	99.9	19.6
10033	*	*	10188	135	22.5
10039	*	*	10191	99.2	10.4
10041	*	*	10192	370	211
10042	*	*	10194	234	87.0
10044	*	*	10196	183	48.7
10060	*	*	10197	160	17.7
10061	*	*	10200	140	15.6
10063	*	*	10201	193	58.7
10069	*	*	10202	BC	BC
10073	*	*	10203	BC	BC
10074	*	*	10206	124	212
10080	*	*	10209	171	35.3
10083	*	*	10210	204	17.1
10086	*	*	10213	138	16.8
10088	1.40	*	10216	169	30.5
10091	*	*	10218	118	231
10092	*	*	10219	80.2	13.8
10094	*	*	10224	174	9.34
10095	*	*	10225	94.5	9.62
10098	*	*	10227	135	19.0
10099	*	*	10228	164	28.7
10100	*	1.63	10229	144	15.7
10103	*	*	10244	1680	698
10106	*	*	10249	961	423
10108	*	*	10252	896	350
10109	*	*	10260	1170	316
10120	177	43.1	10261	2840	399
10121	478	233	10265	671	410
10122	98.5	40.1	10268	2890	602
10123	117	84.3	10275	1920	790
10128	177	58.9	10279	618	401
10132	83.8	48.3	10280	452	255
10138	78.0	20.3	10283	347	372

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat,
NR = Not Run, NA = Not Applicable

Analytical Data Hal/P 92-02

Subject ID	Halofantrine (free base) Concentration (ng/ml)	WR 178,460 (free base) Concentration (ng/ml)	Subject ID	Halofantrine (free base) Concentration (ng/ml)	WR 178,460 (free base) Concentration (ng/ml)
10289	1310	286	10435	3650	771
10301	2270	231	10436	3140	3070
10302	1230	639	10437	2210	652
10303	1220	209	10439	2020	614
10307	3110	2420	10440	4180	1600
10310	590	511	10443	6740	6870
10311	1190	164	10445	2220	527
10313	758	246	10447	4590	2620
10314	692	232	10448	2990	1930
10315	2030	1340	10453	2000	305
10316	835	267	10456	3770	3760
10319	664	222	10457	4100	921
10323	455	141	10460	5180	711
10324	1190	401	10462	2120	625
10325	2310	599	10467	2110	1790
10326	1000	175	10468	1890	2970
10327	1090	325			
10333	1140	319			
10334	902	162			
10335	1590	1330			
10343	1830	1750			
10344	447	145			
10345	1210	254			
10361	6260	2050			
10365	18300	1930			
10369	1080	1360			
10380	3990	823			
10381	1040	931			
10383	1350	4320			
10384	3170	1260			
10386	2340	1190			
10388	5640	1100			
10395	2270	1910			
10398	12000	1460			
10400	2700	1380			
10403	1730	1300			
10407	2050	1020			
10408	1900	954			
10409	2350	1320			
10421	7440	757			
10424	1430	550			
10425	11600	2080			
10426	1610	637			
10429	1140	634			
10431	2260	644			
10432	2730	3930			

Notes:

Halofantrine data for Subject ID 10289 and 10323 through 10343 are presented despite a high control over 15%, since the diluted control error was less than 10% and insufficient sample remained for repeat analysis.

Chromatograms for Subject ID 10202 and 10203 were unacceptable because of unexplained large solvent fronts that appeared to adversely affect compound to internal standard peak height ratios. Concentrations obtained for these chromatograms were:

Subject ID	Halofantrine (free base) Concentration (ng/ml)	WR 178,460 (free base) Concentration (ng/ml)
10202	37.8	9.62
10203	17.8	8.71

* = Below Assay Sensitivity, NS = No Sample, BC = Bad Chromatogram, Insufficient Sample to Repeat, NR = Not Run, NA = Not Applicable

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group II

Subject 6 WR 238605

Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	3.17
	8.00	6.60
	12.00	8.04
	24.00	4.21
	48.00	4.45
	72.00	4.45
	96.00	4.13
	168.00	2.69
	216.00	*
	264.00	*

Subject 8 WR 238605

Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 10 WR 238605

Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 7 WR 238605

Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	4.11
	8.00	1.80
	12.00	4.38
	24.00	2.48
	48.00	2.62
	72.00	4.11
	96.00	4.11
	168.00	3.57
	216.00	1.94
	264.00	3.02

Subject 9 WR 238605

Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	5.73
	48.00	3.65
	72.00	4.21
	96.00	4.37
	168.00	4.69
	216.00	*
	264.00	*

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group III

Subject 11 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	8.52
	8.00	12.8
	12.00	12.4
	24.00	9.64
	48.00	11.3
	72.00	9.12
	96.00	9.00
	168.00	NS
	216.00	NS
	264.00	NS

Subject 13 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	7.30
	8.00	11.3
	12.00	14.8
	24.00	10.3
	48.00	14.9
	72.00	12.7
	96.00	11.6
	168.00	NS
	216.00	NS
	264.00	NS

Subject 15 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	NS
	216.00	NS
	264.00	NS

Subject 12 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	NS
	216.00	NS
	264.00	NS

Subject 14 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	3.09
	8.00	5.97
	12.00	9.80
	24.00	9.56
	48.00	7.40
	72.00	8.04
	96.00	5.81
	168.00	NS
	216.00	NS
	264.00	NS

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group III Repeat

Subject 112 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	6.68
	8.00	9.29
	12.00	15.0
	24.00	8.58
	48.00	10.7
	72.00	11.3
	96.00	6.04
	168.00	5.33
	216.00	6.12
	264.00	6.12

Subject 114 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	8.72
	8.00	11.6
	12.00	13.2
	24.00	10.9
	48.00	9.38
	72.00	8.26
	96.00	8.00
	168.00	5.90
	216.00	5.30
	264.00	5.40

Subject 113 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	8.10
	8.00	14.8
	12.00	18.7
	24.00	15.6
	48.00	12.1
	72.00	19.6
	96.00	12.5
	168.00	17.5
	216.00	12.3
	264.00	11.1

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group IV

Subject 16 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	*
8.00	8.00	*
12.00	12.00	*
24.00	24.00	*
48.00	48.00	*
72.00	72.00	*
96.00	96.00	*
168.00	168.00	*
216.00	216.00	*
264.00	264.00	*

Subject 20 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	24.1
8.00	8.00	22.1
12.00	12.00	30.1
24.00	24.00	24.6
48.00	48.00	21.4
72.00	72.00	21.8
96.00	96.00	23.5
168.00	168.00	21.7
216.00	216.00	19.8
264.00	264.00	17.2

Subject 18 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	*
8.00	8.00	*
12.00	12.00	*
24.00	24.00	*
48.00	48.00	*
72.00	72.00	*
96.00	96.00	*
168.00	168.00	*
216.00	216.00	*
264.00	264.00	*

Subject 17 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	11.2
8.00	8.00	12.3
12.00	12.00	23.8
24.00	24.00	11.2
48.00	48.00	11.0
72.00	72.00	11.5
96.00	96.00	18.7
168.00	168.00	14.5
216.00	216.00	14.5
264.00	264.00	13.5

Subject 19 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	8.93
8.00	8.00	15.4
12.00	12.00	20.4
24.00	24.00	17.5
48.00	48.00	29.5
72.00	72.00	17.4
96.00	96.00	18.5
168.00	168.00	13.4
216.00	216.00	15.4
264.00	264.00	10.8

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group V

Subject 21 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	*
8.00	8.00	*
12.00	12.00	*
24.00	24.00	*
48.00	48.00	*
72.00	72.00	*
96.00	96.00	*
168.00	168.00	*
216.00	216.00	*
264.00	264.00	*

Subject 23 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	91.2
8.00	8.00	107.
12.00	12.00	111.
24.00	24.00	83.8
48.00	48.00	68.9
72.00	72.00	96.2
96.00	96.00	68.8
168.00	168.00	111.
216.00	216.00	75.7
264.00	264.00	52.3

Subject 25 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	*
8.00	8.00	*
12.00	12.00	*
24.00	24.00	*
48.00	48.00	*
72.00	72.00	*
96.00	96.00	*
168.00	168.00	*
216.00	216.00	*
264.00	264.00	*

Subject 22 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	51.8
8.00	8.00	82.2
12.00	12.00	96.5
24.00	24.00	86.4
48.00	48.00	51.7
72.00	72.00	77.9
96.00	96.00	62.4
168.00	168.00	43.0
216.00	216.00	43.4
264.00	264.00	28.3

Subject 24 WR 238605		
Plasma	Time	Conc.
(hrs.)	(hrs.)	(ng/ml)
0.00	0.00	*
4.00	4.00	53.4
8.00	8.00	55.0
12.00	12.00	54.8
24.00	24.00	42.4
48.00	48.00	47.8
72.00	72.00	42.4
96.00	96.00	40.9
168.00	168.00	39.0
216.00	216.00	29.5
264.00	264.00	24.0

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group VI

Subject 26 WR 238605			Subject 28 WR 238605			Subject 30 WR 238605		
Plasma	Time	Conc.	Plasma	Time	Conc.	Plasma	Time	Conc.
	(hrs.)	(ng/ml)		(hrs.)	(ng/ml)		(hrs.)	(ng/ml)
	0.00	*		0.00	*		0.00	*
	4.00	*		4.00	107.		4.00	64.8
	8.00	*		8.00	108.		8.00	94.2
	12.00	*		12.00	72.5		12.00	84.0
	24.00	*		24.00	65.8		24.00	97.3
	48.00	*		48.00	80.0		48.00	89.8
	72.00	*		72.00	96.5		72.00	102.
	96.00	*		96.00	86.9		96.00	87.3
	168.00	*		168.00	81.3		168.00	70.2
	216.00	*		216.00	88.7		216.00	65.8
	264.00	*		264.00	72.2		264.00	68.0

Subject 27 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	64.1
	8.00	62.0
	12.00	81.1
	24.00	72.6
	48.00	50.6
	72.00	57.9
	96.00	59.9
	168.00	33.7
	216.00	31.9
	264.00	8.49

Subject 29 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group VII

Subject 31 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml) *
	0.00	64.2
	4.00	132.
	8.00	74.5
	12.00	72.5
	24.00	61.2
	48.00	55.6
	72.00	51.7
	96.00	53.8
	168.00	38.8
	216.00	29.0
	264.00	

Subject 33 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml) *
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 35 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml) *
	0.00	90.5
	4.00	83.3
	8.00	83.3
	12.00	70.0
	24.00	71.1
	48.00	93.7
	72.00	79.5
	96.00	67.0
	168.00	56.7
	216.00	49.3
	264.00	

Subject 32 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml) *
	0.00	45.3
	4.00	64.6
	8.00	96.4
	12.00	46.4
	24.00	73.5
	48.00	53.8
	72.00	70.3
	96.00	71.0
	168.00	50.7
	216.00	32.9
	264.00	

Subject 34 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml) *
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group VIII

Subject 36 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	105.
	8.00	108.
	12.00	129.
	24.00	109.
	48.00	92.3
	72.00	87.9
	96.00	88.5
	168.00	84.6
	216.00	63.5
	264.00	72.7

Subject 38 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 40 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 37 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	134.
	8.00	110.
	12.00	58.4.
	24.00	89.4
	48.00	71.5
	72.00	135.
	96.00	111.
	168.00	103.
	216.00	69.3
	264.00	68.4

Subject 39 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	181.
	8.00	189.
	12.00	158.
	24.00	134.
	48.00	122.
	72.00	90.2
	96.00	105.
	168.00	95.1
	216.00	73.2
	264.00	75.3

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group IX

Subject 41 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	26.3
	8.00	30.3
	12.00	43.0
	24.00	52.3
	48.00	39.1
	72.00	35.0
	96.00	28.7
	168.00	32.3
	216.00	NS
	264.00	NS

Subject 45 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	42.5
	8.00	49.4
	12.00	56.5
	24.00	51.0
	48.00	46.7
	72.00	44.3
	96.00	42.6
	168.00	36.7
	216.00	34.5
	264.00	31.2

Subject 43 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 42 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	*
	8.00	*
	12.00	*
	24.00	*
	48.00	*
	72.00	*
	96.00	*
	168.00	*
	216.00	*
	264.00	*

Subject 44 WR 238605		
Plasma	Time	Conc.
	(hrs.)	(ng/ml)
	0.00	*
	4.00	38.4
	8.00	36.1
	12.00	44.3
	24.00	53.4
	48.00	54.8
	72.00	44.0
	96.00	41.4
	168.00	40.0
	216.00	39.4
	264.00	28.6

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group X			Subject 46 WR 238605		
	Time	Plasma Conc.		Time	Plasma Conc.
	(hrs.)	(ng/ml)		(hrs.)	(ng/ml)
	0.00	*		0.00	81.0
	4.00	*		4.00	72.2
	8.00	*		8.00	126.
	12.00	*		12.00	78.6
	24.00	*		24.00	79.8
	48.00	*		48.00	106.
	72.00	*		72.00	106.
	96.00	*		96.00	64.1
	168.00	*		168.00	56.4
	216.00	*		216.00	47.9
	264.00	*		264.00	33.2
	384.00	NS		384.00	16.2
	552.00	NS		552.00	13.3
	720.00	NS		720.00	8.97
	888.00	NS		888.00	

Subject 47 WR 238605			Subject 48 WR 238605		
	Time	Plasma Conc.		Time	Plasma Conc.
	(hrs.)	(ng/ml)		(hrs.)	(ng/ml)
	0.00	*		0.00	*
	4.00	105.		4.00	*
	8.00	111.		8.00	*
	12.00	131.		12.00	*
	24.00	102.		24.00	*
	48.00	102.		48.00	*
	72.00	104.		72.00	*
	96.00	103.		96.00	*
	168.00	84.4		168.00	*
	216.00	69.8		216.00	*
	264.00	53.3		264.00	*
	384.00	39.2		384.00	33.7
	552.00	22.0		552.00	24.9
	720.00	18.6		720.00	17.4
	888.00	16.5		888.00	12.4

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XI			Subject 53 WR 238605			Subject 55 WR 238605		
Subject 51 WR 238605			Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00	*		0.00	*		0.00	*	
4.00	110.		4.00	*		4.00	99.5	
8.00	165.		8.00	*		8.00	116.	
12.00	150.		12.00	*		12.00	110.	
24.00	162.		24.00	*		24.00	112.	
48.00	143.		48.00	*		48.00	110.	
72.00	139.		72.00	*		72.00	103.	
96.00	137.		96.00	*		96.00	107.	
168.00	107.		168.00	*		168.00	99.9	
216.00	102.		216.00	*		216.00	96.5	
264.00	79.7		264.00	*		264.00	72.3	
384.00	65.4		384.00	*		384.00	65.4	
552.00	55.8		552.00	*		552.00	55.5	
720.00	41.6		720.00	*		720.00	41.7	
888.00	32.4		888.00	*		888.00	48.0	
Subject 52 WR 238605			Subject 54 WR 238605					
Time	Plasma Conc.		Time	Plasma Conc.				
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*			
0.00	*		0.00	*				
4.00	*		4.00	84.7				
8.00	*		8.00	117.				
12.00	*		12.00	100.				
24.00	*		24.00	87.4				
48.00	*		48.00	97.8				
72.00	*		72.00	73.2				
96.00	*		96.00	86.4				
168.00	*		168.00	73.7				
216.00	*		216.00	74.2				
264.00	*		264.00	54.6				
384.00	*		384.00	51.8				
552.00	*		552.00	41.2				
720.00	*		720.00	28.2				
888.00	*		888.00	19.5				

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XII			Subject 56 WR 238605			Subject 58 WR 238605			Subject 60 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00	153.		0.00	124.		0.00	124.		0.00		
4.00	181.		4.00	149.		4.00	149.		4.00		
8.00	212.		8.00	162.		8.00	162.		8.00		
12.00	188.		12.00	146.		12.00	146.		12.00		
24.00	188.		24.00	143.		24.00	143.		24.00		
48.00	196.		48.00	129.		48.00	129.		48.00		
72.00	159.		72.00	130.		72.00	130.		72.00		
96.00	120.		96.00	129.		96.00	129.		96.00		
168.00	109.		168.00	88.3		168.00	88.3		168.00		
216.00	129.		216.00	84.1		216.00	84.1		216.00		
264.00	95.6		264.00	76.9		264.00	76.9		264.00		
384.00	77.4		384.00	56.4		384.00	56.4		384.00		
552.00	56.9		552.00	40.3		552.00	40.3		552.00		
720.00	42.8		720.00	30.3		720.00	30.3		720.00		
888.00			888.00			888.00			888.00		

Subject 57 WR 238605			Subject 59 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00	126.		0.00		
4.00	146.		4.00		
8.00	138.		8.00		
12.00	126.		12.00		
24.00	117.		24.00		
48.00	123.		48.00		
72.00	97.4		72.00		
96.00	97.8		96.00		
168.00	92.7		168.00		
216.00	78.4		216.00		
264.00	77.6		264.00		
384.00	59.5		384.00		
552.00	49.7		552.00		
720.00	29.7		720.00		
888.00			888.00		

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XIII			Subject 61 WR 238605			Subject 62 WR 238605			Subject 63 WR 238605			Subject 64 WR 238605			Subject 65 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00		*	0.00		*	0.00		*	0.00		*	0.00		*	0.00		*
4.00		*	4.00		*	4.00		*	4.00		*	4.00		*	4.00		*
8.00		*	8.00		*	8.00		*	8.00		*	8.00		*	8.00		*
12.00		*	12.00		*	12.00		*	12.00		*	12.00		*	12.00		*
24.00		*	24.00		*	24.00		*	24.00		*	24.00		*	24.00		*
48.00		*	48.00		*	48.00		*	48.00		*	48.00		*	48.00		*
72.00		*	72.00		*	72.00		*	72.00		*	72.00		*	72.00		*
96.00		*	96.00		*	96.00		*	96.00		*	96.00		*	96.00		*
168.00		*	168.00		*	168.00		*	168.00		*	168.00		*	168.00		*
216.00		*	216.00		*	216.00		*	216.00		*	216.00		*	216.00		*
264.00		*	264.00		*	264.00		*	264.00		*	264.00		*	264.00		*
384.00		*	384.00		*	384.00		*	384.00		*	384.00		*	384.00		*
552.00		*	552.00		*	552.00		*	552.00		*	552.00		*	552.00		*
720.00		NS	720.00		NS	720.00		NS	720.00		NS	720.00		NS	720.00		NS
888.00		NS	888.00		NS	888.00		NS	888.00		NS	888.00		NS	888.00		NS
Subject 61 WR 238605			Subject 62 WR 238605			Subject 63 WR 238605			Subject 64 WR 238605			Subject 65 WR 238605			Subject 66 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00		*	0.00		*	0.00		*	0.00		*	0.00		*	0.00		*
4.00		*	4.00		*	4.00		*	4.00		*	4.00		*	4.00		*
8.00		*	8.00		*	8.00		*	8.00		*	8.00		*	8.00		*
12.00		*	12.00		*	12.00		*	12.00		*	12.00		*	12.00		*
24.00		*	24.00		*	24.00		*	24.00		*	24.00		*	24.00		*
48.00		*	48.00		*	48.00		*	48.00		*	48.00		*	48.00		*
72.00		*	72.00		*	72.00		*	72.00		*	72.00		*	72.00		*
96.00		*	96.00		*	96.00		*	96.00		*	96.00		*	96.00		*
168.00		*	168.00		*	168.00		*	168.00		*	168.00		*	168.00		*
216.00		*	216.00		*	216.00		*	216.00		*	216.00		*	216.00		*
264.00		*	264.00		*	264.00		*	264.00		*	264.00		*	264.00		*
384.00		*	384.00		*	384.00		*	384.00		*	384.00		*	384.00		*
552.00		*	552.00		*	552.00		*	552.00		*	552.00		*	552.00		*
720.00		NS	720.00		NS	720.00		NS	720.00		NS	720.00		NS	720.00		NS
888.00		NS	888.00		NS	888.00		NS	888.00		NS	888.00		NS	888.00		NS

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XIV			Subject 66 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00			0.00		
4.00	73.4		4.00	*	154.
8.00	140.		8.00	*	228.
12.00	151.		12.00	*	281.
24.00	129.		24.00	*	191.
48.00	144.		48.00	*	231.
72.00	116.		72.00	*	188.
96.00	158.		96.00	*	212.
168.00	98.6		168.00	*	NS
216.00	102.		216.00	*	NS
264.00	93.0		264.00	*	NS
384.00	65.3		384.00	*	116.
552.00	51.5		552.00	*	67.3
720.00	30.4		720.00	NS	43.3
888.00	31.7		888.00	*	49.1

Subject 67 WR 238605			Subject 68 WR 238605		
Time	Plasma Conc.		Time	Plasma Conc.	
(hrs.)	(ng/ml)	*	(hrs.)	(ng/ml)	*
0.00			0.00		
4.00	*		4.00	*	
8.00	*		8.00	*	
12.00	*		12.00	*	
24.00	*		24.00	*	
48.00	*		48.00	*	
72.00	*		72.00	*	
96.00	*		96.00	*	
168.00	*		168.00	*	
216.00	*		216.00	*	
264.00	*		264.00	*	
384.00	*		384.00	*	
552.00	*		552.00	*	
720.00	*		720.00	*	
888.00	*		888.00	*	

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XV		Subject 71 WR 238605		Subject 73 WR 238605		Subject 75 WR 238605	
Time	Plasma Conc.	Time	Plasma Conc.	Time	Plasma Conc.	Time	Plasma Conc.
(hrs.)	(ng/ml)	(hrs.)	(ng/ml)	(hrs.)	(ng/ml)	(hrs.)	(ng/ml)
0.00	*	0.00	*	0.00	*	0.00	*
4.00	69.8	4.00	*	4.00	*	4.00	332.
8.00	186.	8.00	*	8.00	*	8.00	375.
12.00	196.	12.00	*	12.00	*	12.00	287.
24.00	193.	24.00	*	24.00	*	24.00	291.
48.00	187.	48.00	*	48.00	*	48.00	327.
72.00	143.	72.00	*	72.00	*	72.00	298.
96.00	138.	96.00	*	96.00	*	96.00	253.
168.00	129.	168.00	NS	168.00	*	168.00	239.
216.00	109.	216.00	NS	216.00	*	216.00	185.
264.00	136.	264.00	*	264.00	*	264.00	164.
384.00	80.3	384.00	*	384.00	*	384.00	145.
552.00	48.0	552.00	*	552.00	*	552.00	87.3
720.00	38.6	720.00	*	720.00	*	720.00	66.0
888.00	33.5	888.00	*	888.00	*	888.00	45.7
Subject 72 WR 238605		Subject 74 WR 238605					
Time	Plasma Conc.	Time	Plasma Conc.				
(hrs.)	(ng/ml)	(hrs.)	(ng/ml)				
0.00	*	0.00	*				
4.00	*	4.00	248.				
8.00	*	8.00	217.				
12.00	*	12.00	173.				
24.00	*	24.00	216.				
48.00	*	48.00	226.				
72.00	*	72.00	205.				
96.00	*	96.00	134.				
168.00	*	168.00	204.				
216.00	*	216.00	115.				
264.00	*	264.00	195.				
384.00	*	384.00	121.				
552.00	*	552.00	59.5				
720.00	*	720.00	49.9				
888.00	*	888.00	39.3				

NS = No sample; * = Below assay sensitivity

WR5/BP 92-3 Analytical Results
WR 238,605 (as Free Base) in Human Plasma and Blood

Dose Group XV			Subject 73 WR 238605			Subject 75 WR 238605		
Subject 71 WR 238605			Time			Time		
	Blood Conc.		(hrs.)	(ng/ml)		(hrs.)	(ng/ml)	
	(ng/ml)							
	*		0.00	*		0.00	*	
	157.		4.00	*		4.00	607.	
	248.		8.00	*		8.00	842.	
	298.		12.00	*		12.00	653.	
	298.		24.00	*		24.00	573.	
	279.		48.00	*		48.00	548.	
	248.		72.00	*		72.00	530.	
	227.		96.00	*		96.00	474.	
	201.		168.00	*		168.00	389.	
	191.		216.00	NS		216.00	348.	
	156.		264.00	*		264.00	389.	
	147.		384.00	*		384.00	235.	
	104.		552.00	*		552.00	153.	
	85.3		720.00	*		720.00	94.4	
	59.5		888.00	*		888.00	71.6	
Subject 72 WR 238605			Subject 74 WR 238605					
Time			Time					
	Blood Conc.		(hrs.)	(ng/ml)				
	(ng/ml)							
	*		0.00	*				
	*		4.00	386.				
	*		8.00	383.				
	*		12.00	457.				
	*		24.00	385.				
	*		48.00	380.				
	*		72.00	340.				
	*		96.00	304.				
	*		168.00	290.				
	*		216.00	287.				
	*		264.00	215.				
	*		384.00	146.				
	*		552.00	100.				
	*		720.00	66.6				
	*		888.00	48.7				

NS = No sample; * = Below assay sensitivity

Routine Analysis Study: WR6/PU 93-1

DATA
PRELIMINARY

Result of WR6026, 211789, 254421 in Urine on Mar 93

Result of WR6026,WR211789(Met I) and WR254421(Met II) In Urine mail 1518 Feb 92											
Name	CRC#	Date	Urine Volume ml	Conc. ng/ml		6026	211789	254421	Amount mcg		
				WR	WR				WR	WR	
Benjamin Kirpotich	1518	26-27-Jan-92	900	280.00	83.20	624.00	251.69	74.86	561.87		
Benjamin Kirpotich	1518	27-28-Jan-92	400	99.30	33.70	190.00	39.71	13.49	75.90		
Benjamin Kirpotich	1518	2-3-Feb-92	1000	201.00	70.30	666.00	201.20	70.34	666.23		
Benjamin Kirpotich	1518	3-4-Feb-92	1000	261.00	91.60	592.00	260.76	91.60	592.00		
Benjamin Kirpotich	1518	9-10-feb-92	700	184.00	72.70	696.00	128.99	50.86	487.20		
Benjamin Kirpotich	1518	10-11-Feb-92	650	209.00	73.80	971.00	135.99	47.98	632.20		
Benjamin Kirpotich	1518	16-17-Feb-92	unkn Miss	NS	NS	NS	NS	NS	NS		
Benjamin Kirpotich	1518	17-18-Feb-92	unkn	344.00	115.00	*	NA	NA	NA		
Benjamin Kirpotich	1518	25-26-Feb-92	1200	*	*	*	*	*	*		
Benjamin Kirpotich	1518	26-27-Feb-92	unkn Extra	7.94	*	*	*	*	*		
Benjamin Kirpotich	1518	1-2-Mar-92	1800	*	*	*	*	*	*		
James Kipsaset	1521	28-29-Jan-92	1200	580.00	11.20	876.00	696.34	13.48	1051.17		
James Kipsaset	1521	2-3-Feb-92	950	352.00	73.50	1180.00	334.70	69.85	1119.14		
James Kipsaset	1521	3-4-Feb-92	860	358.00	80.50	1690.00	308.21	69.00	1451.70		
James Kipsaset	1521	9-10-Feb-92	1000	388.00	112.00	1170.00	388.42	111.93	1170.90		
James Kipsaset	1521	10-11-Feb-92	1500	444.00	111.00	1040.00	666.18	166.58	1564.94		
James Kipsaset	1521	16-17-Feb-92	1250	738.00	234.00	1300.00	922.34	291.98	1630.94		
James Kipsaset	1521	23-24-Feb-92	1700	97.00	15.10	62.10	164.82	25.69	105.64		
James Kipsaset	1521	24-25-Feb-92	1700	262.00	61.30	362.00	445.59	104.29	616.11		
James Kipsaset	1521	2-3-Mar-92	1300	44.20	*	55.30	57.40	*	71.92		
James Kipsaset	1521	8-9-Mar-92	1100	19.70	*	*	21.72	*	*		
Kipchumba Chepkong	1531	24-Feb-92	50	*	*	*	*	*	*		
Kipchumba Chepkong	1531	1-2-Mar-92	900	175.00	1860.00	157.09	157.09	20.04	1674.66		

Result of WR6026, 211789, 254421 in Urine on Mar 93

Kipchumba Chepkong	1531	2-3-Mar-92	950	122.00	1640.00	116.01	116.01	17.54	1553.31
Kipchumba Chepkong	1531	8-9-Mar-92	700	88.80	1360.00	62.17	62.17	19.33	955.09
Kipchumba Chepkong	1531	9-10-Mar-92	850	106.00	1700.00	90.38	90.38	24.09	1441.63
Kipchumba Chepkong	1531	15-16-Mar-92	700	.	.	.	.	.	.
Kipchumba Chepkong	1531	16-17-Mar-92	200	126.00	219.00	25.23	25.23	4.62	43.75
Kipchumba Chepkong	1531	22-23-Mar-92	650	66.50	107.00	43.20	43.20	8.32	69.67
Kipchumba Chepkong	1531	23-24-Mar-92	500	140.00	209.00	69.87	69.87	11.80	104.87
Kipchumba Chepkong	1531	29-30-Mar-92	500	.	.	.	.	.	.
Kipchumba Chepkong	1531	6-7-Apr-92	1200	.	.	.	.	.	.
Kipchumba Chepkong	1531	7-8-Apr-92	1000	.	.	.	.	.	.
Kipchumba Chepkong	1531	8-9-Apr-92	1100	.	.	.	.	.	.
PRELIMINARY DATA									
Kibet Kamuren	1532	23-24-Feb-92	700Miss	NS	NS	NS	NS	NS	NS
Kibet Kamuren	1532	24-25-Feb-92	1850	32.00	13.40	729.00	59.23	24.79	1394.32
Kibet Kamuren	1532	1-2-Mar-92	300	163.00	39.00	1090.00	49.01	11.69	326.56
Kibet Kamuren	1532	2-3-Mar-92	400	87.40	18.60	1560.00	34.97	7.43	625.31
Kibet Kamuren	1532	8-9-Mar-92	1000	262.00	78.90	1590.00	262.28	78.88	1594.51
Kibet Kamuren	1532	15-16-Mar-92	500	176.00	56.40	1010.00	87.94	28.18	506.82
Kibet Kamuren	1532	16-17-Mar-92	900	178.00	58.70	1540.00	159.87	52.83	1388.22
Kibet Kamuren	1532	23-24-Mar-92	450	142.00	27.70	1050.00	63.86	12.48	473.53
Kibet Kamuren	1532	30-31-Mar-92	700	.	.	.	.	.	.
Kibet Kamuren	1532	5-6-Apr-92	unkn	.	.	.	.	.	.
Thomas Lekwava	1535	24-Feb-92	50	.	14.90	1030.00	.	0.08	51.50
Thomas Lekwava	1535	25-26-Feb-92	500	385.00	.	2790.00	192.46	.	1395.00
Thomas Lekwava	1535	1-2-Mar-92	800	336.00	52.10	1980.00	269.19	41.70	1584.00
Thomas Lekwava	1535	2-3-Mar-92	600	627.00	177.00	3590.00	376.07	106.20	2154.00
Thomas Lekwava	1535	8-9-Mar-92	900	308.00	45.60	1880.00	276.97	41.00	1692.00
Thomas Lekwava	1535	9-10-Mar-92	600	262.00	87.70	1360.00	157.34	52.60	816.00
Thomas Lekwava	1535	16-17-Mar-92	600	261.00	35.60	1420.00	156.90	21.40	852.00
Thomas Lekwava	1535	23-24-Mar-92	500	111.00	30.00	1090.00	55.44	15.00	545.00
Thomas Lekwava	1535	30-31-Mar-92	unkn	.	.	.	.	.	.
Thomas Lekwava	1535	5-6-Apr-92	unkn	.	.	.	.	.	.

[illegible]

**PRELIMINARY
DATA**

ANALYTICAL RESULTS

HAL/P 93-2

Halofantrine in Plasma

Subject 1 WR 171,669 Halofantrine			Subject 2 WR 171,669 Halofantrine		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	32.3	*	0.00	44.0	*
0.50	33.8	5.40	0.50	46.7	*
1.00	33.4	72.5	1.00	87.8	2.47
2.00	34.3	497.	2.00	262.	519.
3.00	55.1	801.	3.00	288.	1760.
4.00	61.0	1160.	4.00	261.	3560.
5.00	61.9	419.	5.00	260.	2780.
6.00	75.9	671.	6.00	240.	2070.
6.50	78.3	1150.	6.50	220.	2060.
7.00	106.	1270.	7.00	241.	1650.
8.00	146.	2020.	8.00	291.	2330.
9.00	208.	1960.	9.00	370.	2900.
10.00	245.	1470.	10.00	419.	2980.
11.00	250.	1480.	11.00	404.	2840.
12.00	229.	1490.	12.00	406.	3070.
12.50	234.	1660.	12.50	348.	2670.
13.00	241.	1530.	13.00	354.	2350.
14.00	347.	1720.	14.00	407.	2320.
15.00	369.	1720.	15.00	389.	3050.
16.00	374.	1560.	16.00	384.	2990.
20.00	497.	854.	20.00	479.	1820.
24.00	260.	637.	24.00	358.	1580.
48.00	164.	248.	48.00	251.	689.
72.00	160.	537.	72.00	200.	602.
120.00	182.	259.	120.00	188.	578.
144.00	174.	257.	144.00	199.	644.
148.00	386.	733.	148.00	405.	2440.
152.00	386.	644.	152.00	351.	1630.
156.00	266.	456.	156.00	321.	1320.
168.00	168.	258.	168.00	187.	612.
172.00	145.	251.	172.00	220.	616.
176.00	157.	255.	176.00	248.	651.
192.00	118.	230.	192.00	149.	411.
219.00	83.1	98.8	219.00	112.	301.
267.00	65.2	86.2	267.00	81.3	203.
363.00	50.8	64.3	363.00	30.8	88.4.
435.00	49.0	67.3	435.00	25.8	63.7
507.00	44.3	61.0	507.00	24.5	70.9

* = Below assay sensitivity; NS = No sample;

BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

Subject 3 WR 171,669 Halofantrine			Subject 4 WR 171,669 Halofantrine		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	6.72	0.00	*	NS
0.50	8.40	8.47	0.50	*	NS
1.00	42.8	48.5	1.00	7.32	NS
2.00	84.4	543.	2.00	66.2	NS
3.00	103.	1920.	3.00	118.	NS
4.00	107.	2350.	4.00	133.	NS
5.00	82.9	2700.	5.00	106.	NS
6.00	81.5	2060.	6.00	101.	NS
6.50	83.4	3040.	6.50	92.6	NS
7.00	83.7	3330.	7.00	103.	NS
8.00	92.1	4750.	8.00	101.	NS
9.00	117.	4670.	9.00	131.	NS
10.00	130.	4680.	10.00	225.	NS
11.00	130.	3120.	11.00	332.	NS
12.00	117.	2210.	12.00	355.	NS
12.50	132.	2620.	12.50	396.	NS
13.00	128.	2230.	13.00	549.	NS
14.00	139.	2610.	14.00	807.	NS
15.00	126.	3310.	15.00	700.	NS
16.00	285.	3550.	16.00	668.	NS
20.00	177.	2780.	20.00	686.	NS
24.00	134.	1580.	24.00	423.	NS
48.00	187.	525.	48.00	322.	NS
72.00	143.	459.	72.00	157.	NS
120.00	92.4	444.	120.00	139.	NS
144.00	71.1	435.	144.00	151.	NS
148.00	188.	2290.	148.00	385.	NS
152.00	208.	1300.	152.00	398.	NS
156.00	211.	1210.	156.00	359.	NS
168.00	94.9	430.	168.00	141.	NS
172.00	110.	419.	172.00	144.	NS
176.00	BC	752.	176.00	161.	NS
192.00	52.4	240.	192.00	86.7	NS
219.00	38.9	164.	219.00	61.3	NS
267.00	18.5	107.	267.00	33.2	NS
363.00	12.8	73.0	363.00	NS	NS
435.00	11.5	60.2	435.00	NS	NS
507.00	14.4	48.2	507.00	NS	NS

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

Subject 5 WR 171,669 Halofantrine			Subject 6 WR 171,669 Halofantrine		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	29.1	0.00	40.8	*
0.50	*	28.2	0.50	48.7	*
1.00	8.48	58.6	1.00	73.4	20.9
2.00	102.	721.	2.00	643.	1230.
3.00	164.	1020.	3.00	858.	2290.
4.00	242.	1670.	4.00	1080.	2280.
5.00	236.	1050.	5.00	833.	1740.
6.00	194.	829.	6.00	506.	1570.
6.50	241.	590.	6.50	831.	2090.
7.00	210.	508.	7.00	866.	2400.
8.00	246.	797.	8.00	962.	3950.
9.00	268.	1370.	9.00	759.	2860.
10.00	285.	1710.	10.00	877.	2340.
11.00	289.	2110.	11.00	977.	2180.
12.00	313.	1800.	12.00	778.	2140.
12.50	303.	1660.	12.50	759.	2030.
13.00	292.	1850.	13.00	907.	2150.
14.00	348.	1220.	14.00	833.	3680.
15.00	667.	1380.	15.00	1200.	2920.
16.00	1190.	1490.	16.00	1740.	2590.
20.00	1610.	2260.	20.00	1910.	1520.
24.00	759.	1160.	24.00	1180.	985.
48.00	452.	516.	48.00	387.	393.
72.00	329.	444.	72.00	241.	417.
120.00	301.	456.	120.00	251.	410.
144.00	247.	558.	144.00	211.	467.
148.00	577.	1700.	148.00	257.	2470.
152.00	935.	397.	152.00	332.	1410.
156.00	638.	808.	156.00	320.	1340.
168.00	325.	462.	168.00	163.	473.
172.00	308.	506.	172.00	273.	470.
176.00	296.	436.	176.00	253.	541.
192.00	211.	344.	192.00	147.	315.
219.00	161.	261.	219.00	126.	253.
267.00	94.1	114.	267.00	110.	163.
363.00	56.3	84.6	363.00	46.4	106.
435.00	42.6	89.5	435.00	36.2	91.9
507.00	58.9	92.2	507.00	37.2	103.

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

Subject 7 WR 171,669 Halofantrine			Subject 8 WR 171,669 Halofantrine		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	15.0	0.00	*	8.82
0.50	3.90	44.9	0.50	*	9.76
1.00	16.7	400.	1.00	1.65	19.5
2.00	58.9	1220.	2.00	9.91	414.
3.00	70.9	1420.	3.00	25.9	1510.
4.00	105.	1340.	4.00	53.0	2090.
5.00	91.8	962.	5.00	57.1	2740.
6.00	98.4	847.	6.00	70.0	1390.
6.50	81.5	836.	6.50	80.7	1840.
7.00	116.	1510.	7.00	206.	1860.
8.00	139.	2570.	8.00	78.1	1280.
9.00	146.	1860.	9.00	84.2	1790.
10.00	169.	1830.	10.00	98.9	2370.
11.00	170.	1270.	11.00	104.	2150.
12.00	168.	1230.	12.00	80.3	1550.
12.50	167.	1100.	12.50	127.	2150.
13.00	141.	1200.	13.00	121.	2350.
14.00	153.	1570.	14.00	89.6	1530.
15.00	178.	2050.	15.00	116.	1730.
16.00	193.	2340.	16.00	85.8	1970.
20.00	197.	1360.	20.00	730.	1510.
24.00	176.	823.	24.00	605.	887.
48.00	169.	393.	48.00	224.	416.
72.00	120.	314.	72.00	68.6	341.
120.00	192.	316.	120.00	90.8	339.
144.00	169.	387.	144.00	88.9	337.
148.00	269.	1110.	148.00	152.	1630.
152.00	394.	1440.	152.00	216.	791.
156.00	361.	1030.	156.00	190.	726.
168.00	139.	324.	168.00	89.4	316.
172.00	167.	445.	172.00	91.9	353.
176.00	154.	371.	176.00	89.7	321.
192.00	79.5	236.	192.00	65.4	215.
219.00	44.6	120.	219.00	47.6	162.
267.00	31.5	121.	267.00	27.7	89.9
363.00	19.1	47.0	363.00	16.2	62.4
435.00	19.5	40.9	435.00	19.0	63.7
507.00	15.5	NS	507.00	15.9	58.5

* = Below assay sensitivity; NS = No sample;
BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

<u>Subject 9 WR 171,669 Halofantrine</u>		
<u>Scheduled Time (hrs.)</u>	<u>Treat A Conc. (ng/ml)</u>	<u>Treat B Conc. (ng/ml)</u>
0.00	30.6	*
0.50	41.1	12.8
1.00	96.4	205.
2.00	134.	1580.
3.00	181.	1240.
4.00	156.	858.
5.00	155.	637.
6.00	167.	832.
6.50	143.	1000.
7.00	157.	813.
8.00	194.	1180.
9.00	243.	1430.
10.00	276.	1080.
11.00	203.	1500.
12.00	191.	1520.
12.50	201.	1300.
13.00	203.	1250.
14.00	193.	1140.
15.00	194.	2490.
16.00	170.	2460.
20.00	265.	880.
24.00	270.	564.
48.00	370.	360.
72.00	358.	360.
120.00	235.	395.
144.00	340.	953.
148.00	350.	2630.
152.00	502.	1520.
156.00	492.	1380.
168.00	236.	382.
172.00	188.	357.
176.00	219.	408.
192.00	141.	307.
219.00	94.2	250.
267.00	99.4	171.
363.00	41.8	52.8
435.00	39.6	38.5
507.00	38.8	29.0

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

Subject 1 WR 178,460			Subject 2 WR 178,460		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	26.9	*	0.00	17.8	*
0.50	24.6	*	0.50	17.1	*
1.00	23.3	2.00	1.00	21.3	*
2.00	22.6	13.7	2.00	43.4	6.59
3.00	26.8	27.2	3.00	50.5	27.4
4.00	31.5	34.4	4.00	65.1	50.1
5.00	33.2	21.1	5.00	75.4	57.6
6.00	40.7	25.4	6.00	82.8	71.3
6.50	36.9	76.3	6.50	73.1	94.5
7.00	48.0	82.8	7.00	95.3	117.
8.00	54.9	84.3	8.00	96.6	139.
9.00	63.4	122.	9.00	129.	193.
10.00	69.9	121.	10.00	141.	212.
11.00	82.2	138.	11.00	133.	225.
12.00	85.9	429.	12.00	147.	233.
12.50	85.2	149.	12.50	129.	214.
13.00	86.4	158.	13.00	136.	221.
14.00	97.1	196.	14.00	136.	283.
15.00	98.0	208.	15.00	147.	319.
16.00	108.	227.	16.00	153.	326.
20.00	158.	212.	20.00	204.	397.
24.00	166.	181.	24.00	203.	491.
48.00	180.	238.	48.00	286.	521.
72.00	169.	216.	72.00	314.	596.
120.00	198.	287.	120.00	359.	676.
144.00	226.	310.	144.00	390.	679.
148.00	262.	311.	148.00	451.	544.
152.00	264.	372.	152.00	493.	751.
156.00	252.	386.	156.00	466.	716.
168.00	268.	295.	168.00	413.	721.
172.00	250.	324.	172.00	459.	715.
176.00	266.	297.	176.00	411.	642.
192.00	242.	311.	192.00	376.	614.
219.00	162.	259.	219.00	342.	225.
267.00	113.	216.	267.00	227.	175.
363.00	106.	152.	363.00	82.3	57.3
435.00	71.9	113.	435.00	52.4	44.1
507.00	65.4	88.4	507.00	40.8	34.6

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS
HAL/P 93-2
Halofantrine in Plasma

Subject 3 WR 178,460			Subject 4 WR 178,460		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	6.68	0.00	*	NS
0.50	*	5.55	0.50	*	NS
1.00	3.55	7.05	1.00	*	NS
2.00	13.7	16.8	2.00	*	NS
3.00	25.2	36.1	3.00	25.3	NS
4.00	34.4	62.1	4.00	35.2	NS
5.00	35.8	80.3	5.00	36.5	NS
6.00	43.9	97.1	6.00	43.6	NS
6.50	60.3	119.	6.50	46.4	NS
7.00	37.0	144.	7.00	40.3	NS
8.00	51.7	174.	8.00	47.2	NS
9.00	56.9	215.	9.00	58.5	NS
10.00	71.4	244.	10.00	76.3	NS
11.00	55.0	273.	11.00	104.	NS
12.00	71.0	274.	12.00	122.	NS
12.50	80.8	290.	12.50	215.	NS
13.00	81.6	319.	13.00	122.	NS
14.00	86.3	336.	14.00	151.	NS
15.00	85.6	356.	15.00	150.	NS
16.00	87.0	378.	16.00	154.	NS
20.00	119.	430.	20.00	178.	NS
24.00	118.	500.	24.00	183.	NS
48.00	224.	557.	48.00	313.	NS
72.00	296.	654.	72.00	313.	NS
120.00	262.	674.	120.00	290.	NS
144.00	269.	796.	144.00	328.	NS
148.00	288.	900.	148.00	416.	NS
152.00	381.	986.	152.00	401.	NS
156.00	328.	896.	156.00	366.	NS
168.00	286.	862.	168.00	367.	NS
172.00	368.	854.	172.00	402.	NS
176.00	308.	936.	176.00	358.	NS
192.00	253.	725.	192.00	298.	NS
219.00	230.	621.	219.00	268.	NS
267.00	160.	357.	267.00	156.	NS
363.00	73.0	163.	363.00	NS	NS
435.00	51.9	117.	435.00	NS	NS
507.00	33.3	101.	507.00	NS	NS

* = Below assay sensitivity; NS = No sample;
BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS

HAL/P 93-2

Halofantrine in Plasma

Subject 5 WR 178,460			Subject 6 WR 178,460		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	16.9	0.00	19.5	*
0.50	*	16.6	0.50	22.1	*
1.00	*	18.3	1.00	22.7	*
2.00	*	33.2	2.00	49.8	17.0
3.00	11.5	52.9	3.00	73.3	56.5
4.00	21.1	79.3	4.00	88.3	80.9
5.00	31.3	84.7	5.00	87.1	92.9
6.00	38.6	121.	6.00	82.2	112.
6.50	55.7	123.	6.50	137.	129.
7.00	55.5	117.	7.00	178.	158.
8.00	58.3	139.	8.00	195.	217.
9.00	58.3	182.	9.00	197.	234.
10.00	101.	171.	10.00	246.	253.
11.00	117.	233.	11.00	229.	263.
12.00	135.	222.	12.00	216.	283.
12.50	140.	246.	12.50	208.	283.
13.00	135.	258.	13.00	216.	290.
14.00	144.	229.	14.00	134.	285.
15.00	182.	277.	15.00	128.	359.
16.00	172.	297.	16.00	250.	363.
20.00	299.	350.	20.00	381.	387.
24.00	792.	535.	24.00	436.	389.
48.00	402.	589.	48.00	521.	350.
72.00	488.	733.	72.00	469.	452.
120.00	486.	739.	120.00	566.	441.
144.00	475.	769.	144.00	474.	452.
148.00	494.	922.	148.00	464.	525.
152.00	608.	368.	152.00	480.	533.
156.00	556.	851.	156.00	505.	626.
168.00	534.	749.	168.00	354.	568.
172.00	548.	883.	172.00	449.	537.
176.00	541.	792.	176.00	428.	496.
192.00	462.	791.	192.00	327.	457.
219.00	440.	635.	219.00	315.	368.
267.00	350.	538.	267.00	263.	311.
363.00	116.	137.	363.00	65.4	151.
435.00	88.8	96.4	435.00	53.9	106.
507.00	72.2	78.4	507.00	36.1	73.0

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS

HAL/P 93-2

Halofantrine in Plasma

Subject 7 WR 178,460			Subject 8 WR 178,460		
Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)	Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	*	10.5	0.00	*	5.65
0.50	*	10.2	0.50	*	6.03
1.00	*	23.8	1.00	*	9.38
2.00	6.57	59.6	2.00	*	14.9
3.00	17.2	105.	3.00	*	39.9
4.00	25.1	114.	4.00	12.6	65.4
5.00	29.0	120.	5.00	18.8	98.4
6.00	34.8	151.	6.00	22.4	98.3
6.50	38.3	162.	6.50	29.6	110.
7.00	43.5	182.	7.00	280.	118.
8.00	52.4	239.	8.00	38.6	153.
9.00	62.6	252.	9.00	46.8	180.
10.00	72.3	252.	10.00	52.3	224.
11.00	77.9	235.	11.00	70.4	220.
12.00	87.8	226.	12.00	66.3	246.
12.50	92.8	250.	12.50	61.1	228.
13.00	93.1	253.	13.00	71.9	255.
14.00	112.	259.	14.00	71.4	292.
15.00	107.	299.	15.00	79.9	341.
16.00	130.	301.	16.00	72.3	320.
20.00	161.	282.	20.00	126.	347.
24.00	146.	344.	24.00	132.	430.
48.00	175.	446.	48.00	225.	598.
72.00	217.	465.	72.00	234.	549.
120.00	283.	506.	120.00	322.	596.
144.00	318.	635.	144.00	307.	620.
148.00	365.	572.	148.00	342.	737.
152.00	472.	705.	152.00	415.	700.
156.00	480.	668.	156.00	359.	673.
168.00	390.	202.	168.00	378.	620.
172.00	459.	280.	172.00	380.	677.
176.00	432.	230.	176.00	349.	638.
192.00	317.	149.	192.00	286.	539.
219.00	306.	80.4	219.00	259.	562.
267.00	217.	81.0	267.00	193.	424.
363.00	106.	29.6	363.00	113.	167.
435.00	66.5	25.7	435.00	75.5	143.
507.00	50.8	NS	507.00	60.1	138.

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

ANALYTICAL RESULTS

HAL/P 93-2

Halofantrine in Plasma

Subject 9 WR 178,460

Scheduled Time (hrs.)	Treat A Conc. (ng/ml)	Treat B Conc. (ng/ml)
0.00	44.5	*
0.50	44.0	*
1.00	52.8	5.52
2.00	59.1	31.0
3.00	58.1	46.8
4.00	70.6	66.0
5.00	93.7	70.8
6.00	105.	73.3
6.50	98.5	97.7
7.00	92.7	84.0
8.00	BC	108.
9.00	117.	139.
10.00	131.	148.
11.00	140.	183.
12.00	144.	202.
12.50	108.	202.
13.00	138.	224.
14.00	146.	239.
15.00	142.	330.
16.00	146.	324.
20.00	196.	308.
24.00	187.	366.
48.00	281.	460.
72.00	413.	500.
120.00	353.	605.
144.00	492.	607.
148.00	527.	739.
152.00	544.	705.
156.00	456.	807.
168.00	411.	807.
172.00	415.	674.
176.00	457.	630.
192.00	369.	572.
219.00	350.	521.
267.00	464.	456.
363.00	233.	189.
435.00	142.	121.
507.00	94.9	107.

* = Below assay sensitivity; NS = No sample;
 BC = Bad chromatogram, insufficient sample to repeat.

Routine Analysis Study: Pah/P 93-3

SUBJ # CL117 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
0	.
2	28.4
3	73.7
4	74.5
5	51.2
6	29.7
7	20.6
8	13.5
9	10.7
10	6.98
11	6.13
12	.
13	.
14	.

SUBJ # EBWAH 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	74.2
3	88.1
4	75.9
5	55.4
6	33.9
7	22.8
8	17.1
9	13.1
10	10.0
11	8.71
12	7.96
13	.
14	.

SUBJ # EDWAA 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	126
3	111
4	61.9
5	68.1
6	35.0
7	29.3
8	15.7
9	10.0
10	9.38
11	5.85
12	5.85
13	4.13
14	.

SUBJ # 1AO6 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	.
2	54.7
3	88.1
4	86.3
5	55.0
6	30.0
7	20.0
8	12.7
9	8.44
10	7.11
11	6.05
12	.
13	.

SUBJ # EDWAA 0.4 mg/kg POG suspension

Sample Number	(ng/ml) CONC
1	.
2	95.6
3	79.9
4	65.3
5	49.3
6	28.2
7	18.8
8	15.9
9	11.1
10	7.11
11	4.78
12	5.84

SUBJ # EBWAA 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	110
3	100
4	81.5
5	72.9
6	31.1
7	22.0
8	18.2
9	12.6
10	7.84
11	5.39
12	4.13
13	5.85
14	5.49
15	.
16	.

FINAL PAPP RESULTS

SUBJ # EFWAA 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	.
2	39.2
3	85.3
4	54.0
5	34.8
6	23.0
7	14.9
8	11.0
9	7.90
10	5.44
11	4.06
12	.

SUBJ # ECWAG 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	81.6
3	88.0
4	82.9
5	64.6
6	38.5
7	25.8
8	18.4
9	14.3
10	9.80
11	6.83
12	5.27
13	5.13
14	.

SUBJ # EBWAH 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	111
3	123
4	115
5	85.8
6	53.1
7	30.1
8	22.0
9	16.4
10	13.2
11	10.1
12	7.57
13	6.57
14	6.30
15	5.39

SUBJ # EBWAH 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	.
2	78.5
3	96.2
4	60.0
5	34.5
6	25.3
7	13.4
8	9.06
9	7.39
10	6.40
11	5.14
12	.

SUBJ # EFWAA 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	63.0
3	66.0
4	52.8
5	44.1
6	30.4
7	19.8
8	11.6
9	6.75
10	7.89
11	6.75
12	6.94
13	.
14	.

SUBJ # 4612 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	109
3	100
4	82.9
5	58.7
6	33.0
7	34.8
8	17.5
9	10.1
10	6.90
11	8.82
12	4.67
13	.

FINAL PAPP RESULTS

SUBJ # EDWAA 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	.
2	119
3	91.4
4	50.0
5	27.7
6	18.4
7	13.4
8	8.67
9	7.75
10	4.29
11	4.91

SUBJ # ECWAI 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	58.2
3	72.0
4	64.4
5	52.5
6	27.4
7	21.0
8	14.6
9	5.18
10	6.20
11	6.26
12	4.99
13	5.35
14	4.29

SUBJ # DJWAI 0.4 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	.
2	27.2
3	75.8
4	100
5	73.7
6	51.3
7	32.3
8	22.8
9	16.6
10	9.20
11	8.48
12	6.75
13	7.57
14	.

SUBJ # ECWAG 0.4 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	.
2	89.9
3	73.8
4	56.0
5	38.8
6	23.7
7	19.1
8	12.7
9	8.59
10	9.21
11	5.83

SUBJ # EDWAA 0.4 mg/kg IV-Anes

SUBJ # EDWAA 0.4 mg/kg IV

Sample Number	(ng/ml) CONC
1	.
2	584
3	453
4	334
5	188
6	130
7	111
8	98.9
9	62.5
10	46.7
11	32.4
12	24.3
13	24.8
14	20.4
15	15.0
16	16.0
17	11.9
18	13.1
19	9.58
20	9.17

SUBJ # EFWAA 0.4 mg/kg IV-Anes

SUBJ # EFWAA 0.4 mg/kg IV

Sample Number	(ng/ml) CONC
1	.
2	475
3	302
4	172
5	173
6	105
7	86.9
8	59.4
9	46.5
10	33.6
11	25.0
12	18.6
13	20.1
14	14.0
15	9.91
16	12.6
17	9.09
18	8.01
19	4.47

Sample Number	(ng/ml) CONC
1	.
2	369
3	254
4	194
5	190
6	140
7	99.5
8	63.4
9	40.1
10	27.3
11	17.3
12	13.2
13	9.43
14	9.50
15	9.69
16	9.57
17	7.22

FINAL PAPP RESULTS

SUBJ # EBWAH 0.4 mg/kg IV

Sample Number	(ng/ml) CONC
1	.
2	336
3	254
4	218
5	205
6	169
7	142
8	40.5
9	60.9
10	41.6
11	31.1
12	23.8
13	14.7
14	13.4
15	10.8
16	10.8
17	9.85
18	6.67

SUBJ # EDWAA 0.4 mg/kg IV-Anes

Sample Number	(ng/ml) CONC
1	.
2	381
3	287
4	203
5	211
6	142
7	94.9
8	58.5
9	46.6
10	32.4
11	26.7
12	22.9
13	16.3
14	11.6
15	11.0
16	11.0
17	9.88
18	8.67
19	6.45

SUBJ # ECWAG 0.4 mg/kg IV

Sample Number	(ng/ml) CONC
1	.
2	384
3	312
4	298
5	257
6	185
7	171
8	104
9	63.1
10	42.8
11	34.5
12	28.9
13	24.4
14	16.0
15	14.0
16	11.4
17	9.01
18	7.42
19	5.91

SUBJ # EBWAH 0.4 mg/kg IV-Anes

Sample Number	(ng/ml) CONC
1	.
2	494
3	266
4	204
5	163
6	100
7	91.2
8	86.2
9	54.4
10	49.4
11	32.8
12	25.9
13	25.0
14	17.3
15	13.7
16	14.7
17	10.1
18	9.58
19	8.26

SUBJ # **EBWAA** 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	145
3	289
4	370
5	331
6	305
7	232
8	167
9	150
10	126
11	118
12	91.4
13	74.5
14	66.7
15	NS
16	61.0

SUBJ # **ECWAI** 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	215
3	470
4	495
5	594
6	343
7	318
8	244
9	180
10	163
11	148
12	134
13	102
14	83.0
15	77.5
16	66.4
17	56.4
18	52.1

SUBJ # **4612** 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	244
3	319
4	396
5	335
6	335
7	306
8	268
9	233
11	181
12	183
13	124
13.5	113
14	102
15	101
16	84.5
17	101
18	67.5

SUBJ # **DJWAU** 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	194
3	228
4	303
5	294
6	219
7	180
8	128
9	127
10	94.8
11	78.6
12	71.0
13	65.1
14	53.2
15	44.8
16	56.0

* = below assay sensitivity
NS = No sample

SUBJ # EDWAA 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	200
3	352
4	371
5	376
6	251
7	210
8	174
9	153
10	127
11	122
12	99.3
13	82.7
14	90.1
15	65.8
16	59.0
17	64.6
18	95.9

SUBJ # EFWAA 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	80.1
3	403
4	799
5	582
6	447
7	329
8	274
9	222
10	181
11	170
12	135
13	103
14	94.1
15	72.8
16	64.3
17	85.4
18	70.3

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FINAL PAHP RESULTS
FINAL PAHP RESULTS
2/25/94

SUBJ # EBWAH 6 mg/kg POG Solution

Sample Number	(ng/ml) CONC
1	*
2	354
3	390
4	271
5	214
6	176
7	155
8	141
9	103
10	87.2
11	94.5
12	78.0
13	74.7
14	56.1
15	62.1
16	43.9
17	46.0

SUBJ # DJWAS 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	274
3	302
4	315
5	312
6	254
7	219
8	176
9	160
10	128
11	131
12	120
13	83.9
14	76.8
15	65.8
16	64.1
17	74.4
18	96.8

* = below assay sensitivity

NS = No sample

SUBJ # ECWAG 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	407
3	481
4	596
5	653
6	426
7	358
8	265
9	232
10	183
11	160
12	148
13	114
14	102
15	82.2
16	79.7
17	98.6
18	69.6

SUBJ # EBWAH 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	39.1
3	102
4	336
5	175
6	54.3
7	115
8	97.6
9	90.8
10	78.0
11	67.8
12	64.4
13	50.1
14	42.7
15	40.8
16	38.1
17	34.2
18	31.3

SUBJ # 4632 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	49.4
3	407
4	592
5	494
6	336
7	249
8	216
9	189
10	170
11	129
12	120
13	86.8
14	94.6
15	88.9
16	75.4
17	77.0
18	54.9

SUBJ # DJWAU 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	83.3
3	133
4	136
5	56.5
6	113
7	40.5
8	73.1
9	64.1
10	54.5
11	53.7
12	56.6
13	47.8
14	35.1
15	37.1
16	34.9

* = below assay sensitivity
NS = No sample

SUBJ # EDWAA 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	297
3	499
4	562
5	475
6	326
7	247
8	265
9	146
10	134
11	119
12	104
13	87.3
14	70.8
15	63.9
16	58.0
17	48.8
18	45.9
19	42.1

SUBJ # EBWAA 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	273
3	471
4	431
5	359
6	305
7	219
8	142
9	159
10	138
11	132
12	116
13	91.5
14	68.6
15	63.5
16	57.2
17	54.3
18	39.8
19	40.8

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FINAL PAHP RESULTS
FINAL PAHP RESULTS
2/25/94

SUBJ # ECWAI 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	314
3	802
4	593
5	433
6	285
7	224
8	164
9	164
10	147
11	130
12	126
13	93.3
14	77.9
15	63.2
16	53.9
17	50.6
18	46.1

SUBJ # 4612 6 mg/kg POG Suspension

Sample Number	(ng/ml) CONC
1	*
2	172
3	215
4	202
5	212
6	168
7	159
8	144
9	126
10	106
11	112
12	106
13	91.5
14	75.0
15	62.8
16	57.4
17	57.0
18	49.2
19	46.6

* = below assay sensitivity
NS = No sample

SUBJ # **EDWAA** 6 mg/kg IV

Sample Number	(ng/ml) CONC
1	*
2	3220 **
3	2620 **
4	2840 **
5	1810 **
6	1340 **
7	1560 **
8	1290 **
9	602
10	694
11	455
12	308
13	280
14	251
15	271
16	224
17	188
18	175
19	145
20	129
21	109
22	98.1
23	104
24	114
25	87.7

SUBJ # **ECWAI** 6 mg/kg IV

Sample Number	(ng/ml) CONC
1	*
2	1960 **
3	1500 **
4	1200 **
5	1270 **
6	1070 **
7	1110 **
8	657
9	596
10	292
11	311
12	313
13	250
14	338
15	233
16	210
17	188
18	146
19	135
20	112
21	109
22	102
23	92.2
24	93.8
25	81.1
26	63.5

SUBJ # **EBWAA** 6 mg/kg IV

Sample Number	(ng/ml) CONC
1	*
2	2260 **
3	2530 **
4	2190 **
5	2310 **
6	1390 **
7	1620 **
8	1020 **
9	779
10	682
11	452
12	355
13	302
14	237
15	212
16	227
17	182
18	165
19	139
20	127
21	104
22	100
23	89.3
24	76.3
25	65.5

SUBJ # **EBWAH** 6 mg/kg IV

Sample Number	(ng/ml) CONC
1	*
2	NS
3	1720 **
4	1870 **
5	948 **
6	966 **
7	923 **
8	674
9	463
10	465
11	296
12	283
13	283
14	256
15	223
16	243
17	210
18	232
19	237
20	165
21	217
22	164
23	144
24	135
25	133
26	103

* = below assay sensitivity
NS = No sample

Sample Number	(ng/ml) CONC
1	*
2	1930 **
3	1750 **
4	1330 **
5	1350 **
6	1710 **
7	953 **
8	828 **
9	668
10	603
11	544
12	450
13	425
14	363
15	353
16	282
17	247
18	282
19	197
20	136
21	114
22	97.0
23	92.9
24	70.7

* = below assay sensitivity
NS = No sample

[illegible]

Pah/P 93-3.final data

PAPP		PAHP		PAOP	
SUBJ #		SUBJ #		SUBJ #	
1A07		4612		DJWAS	
PD53		PD76		PD37	
TIME	CONC ng/ml	TIME	CONC ng/ml	TIME	CONC ng/ml
0	*	0	*	0	*
5	243	1	2760	30	29.6
10	165	3	2760	60	337
20	162	5	2220	120	555
30	146	10	2100	150	595
40	111	15	1560	180	549
50	110	20	1410	210	481
60	91.1	30	984	240	368
75	63.9	45	764	270	306
90	71.5	60	627	300	305
120	49.2	90	514	360	205
150	36.0	120	415	420	166
180	27.1	150	386	480	138
210	24.6	180	312	540	119
240	BC	210	281	600	104
270	#	240	282	720	NS
		270	216		
		300	224		
		360	194		
		420	178		
		480	140		
		540	106		
		600	101		
		660	62.0		
# The original chromatogram was a BC, and the repeat result was 41.0 ng/ml.					
This sample should be repeated for pharmacokinetic reasons, but insufficient sample remains for repeat analysis.				NS = no sample * = below assay sensitivity	

Analytical Data
WR 238,605 (as free base) in Dog Plasma
WR5/P 93-4

Male 0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7505:-1	*
7505:4	1.48
7505:8	3.20
7505:13	1.44
7512:-1	*
7512:4	4.69
7512:8	1.93
7512:13	3.68
7512:18	*
7512:26	*
7515:-1	*
7515:4	1.64
7515:8	3.23
7515:13	1.57
7515:18	*
7515:26	*
7520:-1	*
7520:4	2.45
7520:8	4.36
7520:13	39.6
7521:-1	*
7521:4	5.56
7521:8	5.27
7521:13	4.33
7531:-1	*
7531:4	2.03
7531:8	2.61
7531:13	2.03
7531:18	*
7531:26	*
7532:-1	*
7532:4	*
7532:8	1.51
7532:13	57.9
7532:18	*
7532:26	*
7533:-1	*
7533:4	2.71
7533:8	2.90
7533:13	40.6

Female 0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7541:-1	*
7541:4	2.45
7541:8	1.06
7541:13	80.0
7541:18	*
7541:26	*
7542:-1	2.56
7542:4	1.22
7542:8	3.62
7542:13	389
7549:-1	*
7549:4	*
7549:8	1.02
7549:13	4.40
7549:18	*
7549:26	*
7555:-1	*
7555:4	2.74
7555:8	2.06
7555:13	2.42
7557:-1	*
7557:4	*
7557:8	4.62
7557:13	16.4
7557:26	*
7558:-1	*
7558:4	1.74
7558:8	1.96
7558:13	843
7566:-1	*
7566:4	1.51
7566:8	1.44
7566:13	2.32
7566:18	*
7566:26	*
7573:-1	*
7573:4	2.90
7573:8	1.48
7573:13	50.6

* = Below assay sensitivity

Analytical Data
WR 238,605 (as free base) in Dog Plasma
WR5/P 93-4

Male 0.1 mg/kg/day

Sample Identification	Concentration (ng/ml)
7503:-1	*
7503:4	88.0
7503:8	86.2
7503:13	100
7517:-1	*
7517:4	75.9
7517:8	62.9
7517:13	58.8
7519:-1	4.09
7519:4	48.1
7519:8	37.2
7519:13	84.9
7519:18	1.86
7519:26	1.68
7523:-1	*
7523:4	49.2
7523:8	28.7
7523:13	38.7
7527:-1	*
7527:4	28.6
7527:8	19.4
7527:13	111
7527:18	*
7527:26	*
7528:-1	*
7528:4	73.7
7528:8	79.2
7528:13	391
7529:-1	*
7529:4	65.9
7529:8	52.7
7529:13	78.5
7529:18	*
7529:26	*
7536:-1	*
7536:4	44.2
7536:8	32.9
7536:13	62.2
7536:18	*
7536:26	*

Female 0.1 mg/kg/day

Sample Identification	Concentration (ng/ml)
7543:-1	*
7543:4	67.2
7543:8	76.5
7543:13	220
7543:18	1.86
7543:26	1.68
7545:-1	*
7545:4	48.3
7545:8	57.7
7545:13	46.7
7545:18	*
7545:26	*
7550:-1	*
7550:4	41.6
7550:8	29.9
7550:13	78.3
7552:-1	*
7552:4	85.7
7552:8	73.4
7552:13	59.1
7552:18	4.99
7552:26	*
7553:-1	*
7553:4	60.9
7553:8	52.5
7553:13	142
7553:18	*
7553:26	*
7560:-1	*
7560:4	59.0
7560:8	40.3
7560:13	195
7567:-1	*
7567:4	74.2
7567:8	68.7
7567:13	2.95
7569:-1	*
7569:4	26.8
7569:8	26.4
7569:13	126

* = Below assay sensitivity

Analytical Data
WR 238,605 (as free base) in Dog Plasma
WR5/P 93-4

Male 2.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7502:-1	*
7502:4	432
7502:8	459
7502:13	441
7506:-1	*
7506:4	590
7506:8	788
7506:13	747
7510:-1	*
7510:4	712
7510:8	704
7510:13	676
7510:18	32.2
7510:26	*
7514:-1	*
7514:4	544
7514:8	378
7514:13	446
7516:-1	*
7516:4	530
7516:8	422
7516:13	559
7516:18	20.0
7516:26	*
7522:-1	*
7522:4	508
7522:8	470
7522:13	543
7522:18	2.25
7522:26	*
7538:-1	*
7538:4	906
7538:8	762
7538:13	639
7538:18	26.4
7538:26	*
7576:-1	*
7576:4	475
7576:8	427
7576:13	579

Female 2.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7548:-1	*
7548:4	614
7548:8	729
7548:13	897
7548:18	32.1
7548:26	*
7556:-1	*
7556:4	745
7556:8	743
7556:13	871
7561:-1	*
7561:4	573
7561:8	449
7561:13	1030
7561:18	8.15
7561:26	*
7562:-1	*
7562:4	661
7562:8	644
7562:13	696
7562:18	27.4
7562:26	*
7564:-1	*
7564:4	1000
7564:8	885
7564:13	816
7571:-1	*
7571:4	415
7571:8	379
7571:13	964
7571:18	*
7571:26	*
7572:-1	*
7572:4	510
7572:8	390
7572:13	528
7574:-1	*
7574:4	459
7574:8	468
7574:13	518

* = Below assay sensitivity

Analytical Data
WR 238,605 (as free base) in Dog Plasma
WR5/P 93-4

Male 6.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7507:-1	12.5
7507:4	830
7507:8	1120
7507:13	1310
7507:18	87.0
7507:26	2.78
7508:-1	*
7508:4	1010
7508:8	1180
7508:13	1310
7509:-1	*
7509:4	556
7509:8	578
7509:13	832
7511:-1	*
7511:4	1290
7511:8	1290
7511:13	1150
7511:18	171
7511:26	4.54
7518:-1	*
7518:4	1490
7518:8	1420
7518:13	2100
7524:-1	*
7524:4	1230
7524:8	1160
7524:13	1320
7530:-1	*
7530:4	994
7530:8	1190
7530:13	1160
7530:18	239
7530:26	3.63
7535:-1	*
7535:4	1590
7535:8	1830
7535:13	1930
7535:18	472
7535:26	10

Female 6.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
7539:-1	*
7539:4	1070
7539:8	1180
7539:13	1750
7539:18	346
7539:26	6.24
7540:-1	*
7540:4	949
7540:8	951
7540:13	1470
7540:18	73.5
7540:26	3.10
7544:-1	*
7544:4	1430
7544:8	1000
7544:13	1140
7546:-1	*
7546:4	1690
7546:8	1520
7546:13	1620
7551:-1	*
7551:4	773
7551:8	628
7551:13	975
7551:18	*
7554:-1	*
7554:4	1220
7554:8	1090
7554:13	2500
7554:18	303
7554:26	6.40
7563:-1	*
7563:4	1020
7563:8	836
7563:13	4000
7563:18	9.37
7563:26	*
7568:-1	*
7568:4	1080
7568:8	1370
7568:13	1810

* = Below assay sensitivity

ANALYTICAL DATA
Analysis Report WR5/P 93-5
WR 238,605 Free Base in Rat Plasma

0 mg/kg/day

Sample Identification	Concentration (ng/ml)
803-13	*
804-13	*
805-13	*
808-13	*
812-13	*
813-13	*
817-13	*
818-13	*
819-13	*
820-13	*
821-13	*
822-13	2.25
825-13	1.68
826-13	*
828-13	*
829-13	*
832-13	*
833-13	*
838-13	*
839-13	2.33

0.5 mg/kg/day

Sample Identification	Concentration (ng/ml)
841-13	17.2
842-13	28.2
843-13	25.4
845-13	20.4
847-13	30.6
849-13	23.9
854-13	22.3
855-13	34.3
856-13	32.7
857-13	26.4
862-13	25.6
863-13	27.1
865-13	25.9
866-13	45.1
869-13	45.3
870-13	26.3
872-13	42.7
875-13	37.6
876-13	28.3
878-13	56.1

0 mg/kg/day

Sample Identification	Concentration (ng/ml)
801-27	*
802-27	*
806-27	*
807-27	*
809-27	*
810-27	*
811-27	*
814-27	*
815-27	*
816-27	*
823-27	*
824-27	*
827-27	*
830-27	*
831-27	*
834-27	*
835-27	*
836-27	*
837-27	*
840-27	*

* = Below Assay Sensitivity

ANALYTICAL DATA
Analysis Report WR5/P 93-5
WR 238,605 Free Base in Rat Plasma

0.5 mg/kg/day

Sample Identification	Concentration (ng/ml)
844-27	*
846-27	*
848-27	*
850-27	*
851-27	*
852-27	*
853-27	*
858-27	*
859-27	*
860-27	*
861-27	*
864-27	*
867-27	*
868-27	*
871-27	*
873-27	*
874-27	*
877-27	*
879-27	*
880-27	*

6.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
884-27	*
887-27	*
889-27	*
890-27	*
893-27	*
895-27	*
896-27	*
897-27	*
899-27	*
900-27	*
901-27	*
902-27	*
904-27	*
908-27	*
909-27	*
912-27	*
915-27	*
916-27	*
918-27	*
919-27	*

6.0 mg/kg/day

Sample Identification	Concentration (ng/ml)
881-13	303
882-13	624
883-13	439
885-13	466
886-13	328
888-13	268
891-13	326
892-13	609
894-13	460
898-13	237
903-13	557
905-13	518
906-13	459
907-13	639
910-13	697
911-13	661
913-13	555
914-13	422
917-13	537
920-13	662

* = Below Assay Sensitivity

ANALYTICAL DATA
Analysis Report WR5/P 93-5
WR 238,605 Free Base in Rat Plasma
18 mg/kg/day

18 mg/kg/day

Sample Identification	Concentration (ng/ml)
924-13	1700
925-13	1940
928-13	1260
931-13	1470
938-13	1910
943-13	1470
945-13	1850
946-13	1950
947-13	1600
948-13	2580
950-13	1140
951-13	1330
952-13	1550
955-13	2140
958-13	1660
922-27	*
923-27	*
927-27	*
929-27	*
930-27	*

Sample Identification	Concentration (ng/ml)
932-27	*
933-27	*
935-27	*
939-27	*
940-27	*
941-27	*
942-27	2.06
944-27	1.18
949-27	*
953-27	*
954-27	1.15
957-27	*
959-27	1.67
960-27	*

* = Below Assay Sensitivity

Routine Analysis Study: Pri/P 93-6

Primaquine Clinical Sample List

VOLUNTEERS TAKING PRIMAQUINE

Clinical Sample Date: No label Date (Week 6)

Study #	Name	Primaquine(002975AW) ng/ml	WR249725(Met.) ng/ml
93A-003	Manas Oyuga	Below Sensitivity	315
93A-010	Jackline Akoth	B. S.	420
93A-017	Kennedy Omondi	B.S.	946
93A-023	Maurice Otieno	B. S.	571
93A-024	Josiah Nyakwaka	B. S.	241
93A-032	Tabitha Odera	B. S.	431
93A-041	Clement Seje	B. S.	377
93A-043	Rose Adhiambo	B. S.	327
93A-051	Tobias Okach	B. S.	543
93A-057	Roseline Achieng	B. S.	587
93A-064	Reuben Onyango	B.S.	51.2
~~93A-069~~	~~Silvia Atieno~~		
93A-074	Millicent Akinyi	B. S.	490
93A-083	Jael Tabu	B. S.	1100
93A-092	Josinter Anyango	47.9	224
93A-102	Kennedy Ochieng	B. S.	62.4
93A-117	Elizabeth Akinyi	B. S.	638
93A-123	Rahel Atieno	B. S.	535
93A-126	Lawi Okoko	B. S.	492
93A-132	Denis Juma	B. S.	388
93A-145	Mormc Tabitha	40.4	1180
93A-156	Evans Odhiambo	53.4	691
93A-162	Damaris Atieno	B. S.	652
93A-168	John Eric	B. S.	215
93A-177	Antony Oduor	B. S.	1180
93A-189	Elizabeth Akinyi	B. S.	446
93A-196	Samuel Ourma	B. S.	307
93A-205	Grace Akinyi	B. S.	494
93A-208	Rosemary Atieno	B. S.	580
93A-213	Isaya Okoth	46.6	756
93A-218	Joyce Obama	B. S.	501
93A-221	Pamela Atieno	B. S.	323

PRELIMINARY DATA

VOLUNTEERS TAKING PRIMAQUINE

Clinical Sample Date: 7/7/93 or 8/7/93

(Week 11)

Study #	Name	Primaquine(002975AW) ng/ml	WR249725(Met.) ng/ml
93A-003	Manas Oyuga	Below Sensitivity	356
93A-010	Jackline Akoth	B. S.	B. S.
93A-017	Kennedy Omondi	B. S.	693
93A-023	Maurice Otieno	B. S.	570
93A-024	Josiah Nyakwaka	B. S.	138
93A-032	Tabitha Odera	B. S.	452
93A-041	Clement Seje	B. S.	373
93A-043	Rose Adhiambo	B. S.	239
93A-051	Tobias Okach	B. S.	434
93A-057	Roseline Achieng	B. S.	312
93A-064	Reuben Onyango	42.5	B. S.
~~93A-069~~	~~Silvia Atieno~~		
93A-074	Millicent Akinyi	B. S.	555
93A-083	Jael Tabu	B. S.	59.5
93A-092	Josinter Anyango	B. S.	254
93A-102	Kennedy Ochieng	B. S.	296
93A-117	Elizabeth Akinyi	B. S.	481
93A-123	Rahel Atieno	B. S.	54.6
93A-126	Lawi Okoko	37.3	1130
93A-132	Denis Juma	B. S.	402
93A-145	Morme Tabitha	45.5	804
93A-156	Evans Odhiambo	40.2	752
93A-162	Damaris Atieno	29.5	93
93A-168	John Eric	B. S.	377
93A-177	Antony Oduor	B. S.	793
93A-189	Elizabeth Akinyi		
93A-196	Samuel Ourma	B. S.	235
93A-205	Grace Akinyi	B. S.	561
93A-208	Rosemary Atieno		
93A-213	Isaya Okoth	B. S.	B. S.
93A-218	Joyce Obama	B. S.	501
93A-221	Pamela Atieno	170	328

PRELIMINARY DATA

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

1 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	20.3
P2	NS
P3	6.94
P4	4.00
P5	2.00
P6	1.07
P7	0.735
P8	0.595
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

2 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	39.9
P2	39.0
P3	37.7
P4	35.9
P5	36.1
P6	34.9
P7	35.3
P8	31.7
P9	32.8
P10	31.9
P11	27.9
P12	
P13	
P14	
P15	

3 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	23.0
P2	13.7
P3	7.67
P4	5.44
P5	4.14
P6	1.82
P7	1.19
P8	0.947
P9	0.862
P10	0.740
P11	0.601
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

4 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	27.6
P2	15.3
P3	8.60
P4	5.07
P5	2.54
P6	1.40
P7	1.06
P8	0.841
P9	0.802
P10	0.807
P11	0.757
P12	
P13	
P14	
P15	

5 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	28.3
P2	21.9
P3	11.7
P4	6.92
P5	5.45
P6	1.85
P7	1.18
P8	0.975
P9	0.757
P10	0.785
P11	0.729
P12	
P13	
P14	
P15	

6 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	18.7
P2	11.8
P3	6.59
P4	3.72
P5	2.20
P6	1.09
P7	0.696
P8	0.550
P9	0.545
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

7 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	17.7
P2	9.88
P3	5.15
P4	2.17
P5	1.10
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

8 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	22.3
P2	14.4
P3	8.06
P4	4.35
P5	3.01
P6	1.76
P7	0.870
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

9 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	20.6
P2	12.0
P3	4.15
P4	1.68
P5	0.688
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

10 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	23.6
P2	16.5
P3	9.85
P4	5.43
P5	2.45
P6	0.728
P7	0.522
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

11 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	25.9
P2	15.9
P3	7.24
P4	3.64
P5	1.92
P6	0.898
P7	0.528
P8	*
P9	0.579
P10	0.545
P11	*
P12	0.545
P13	0.562
P14	*
P15	0.561

12 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	20.6
P2	12.5
P3	6.09
P4	3.23
P5	1.82
P6	0.928
P7	0.754
P8	0.621
P9	0.650
P10	0.574
P11	0.534
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

13 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	28.6
P2	15.7
P3	8.03
P4	4.54
P5	2.87
P6	1.41
P7	1.13
P8	0.853
P9	0.706
P10	0.908
P11	0.847
P12	
P13	
P14	
P15	

14 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	23.1
P2	12.3
P3	5.88
P4	3.57
P5	2.03
P6	1.13
P7	1.43
P8	0.632
P9	0.528
P10	*
P11	*
P12	0.545
P13	*
P14	*
P15	0.651

15 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	24.2
P2	14.9
P3	8.58
P4	5.17
P5	3.02
P6	1.24
P7	0.783
P8	0.534
P9	0.557
P10	*
P11	0.594
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

16 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	15.1
P2	6.57
P3	3.85
P4	2.40
P5	1.50
P6	0.893
P7	0.598
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

17 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	12.6
P2	4.94
P3	1.90
P4	1.21
P5	0.802
P6	0.581
P7	*
P8	*
P9	*
P10	*
P11	*
P12	0.564
P13	0.581
P14	0.564
P15	0.547

18 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	28.6
P2	18.5
P3	10.2
P4	6.43
P5	3.91
P6	2.18
P7	1.91
P8	1.05
P9	0.913
P10	0.814
P11	0.808
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

19 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	12.1
P2	6.20
P3	3.32
P4	1.90
P5	1.36
P6	0.958
P7	0.630
P8	0.625
P9	0.619
P10	0.575
P11	0.675
P12	0.553
P13	0.647
P14	0.614
P15	0.608

20 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	16.1
P2	11.2
P3	6.78
P4	4.05
P5	2.38
P6	1.09
P7	0.575
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

1 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	17.1
P2	10.3
P3	4.96
P4	2.53
P5	1.60
P6	0.791
P7	0.619
P8	0.547
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

2 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	33.6
P2	24.4
P3	13.7
P4	9.04
P5	5.92
P6	2.85
P7	1.58
P8	1.08
P9	0.776
P10	0.641
P11	0.635
P12	
P13	
P14	
P15	

3 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	18.7
P2	11.6
P3	6.04
P4	3.04
P5	1.82
P6	0.946
P7	0.658
P8	*
P9	0.558
P10	*
P11	0.588
P12	
P13	
P14	
P15	

4 CL-HM

(hrs) Time	(µg) CONC
P0	*
P1	30.2
P2	19.2
P3	13.1
P4	6.83
P5	4.74
P6	3.13
P7	1.67
P8	1.40
P9	0.792
P10	0.789
P11	0.808
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

5 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	29.8
P2	17.4
P3	9.67
P4	6.27
P5	3.87
P6	1.89
P7	1.35
P8	0.976
P9	0.746
P10	0.670
P11	0.670
P12	
P13	
P14	
P15	

6 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	32.6
P2	21.7
P3	12.8
P4	7.52
P5	4.47
P6	2.10
P7	1.44
P8	1.09
P9	0.899
P10	0.887
P11	0.729
P12	
P13	
P14	
P15	

7 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	30.2
P2	21.3
P3	14.7
P4	7.92
P5	6.10
P6	2.67
P7	1.34
P8	1.12
P9	1.05
P10	0.823
P11	0.776
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

8 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	49.4
P2	28.4
P3	15.1
P4	9.05
P5	6.35
P6	3.37
P7	2.46
P8	2.03
P9	1.91
P10	1.74
P11	1.70
P12	
P13	
P14	
P15	

9 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	14.7
P2	10.1
P3	5.74
P4	3.41
P5	2.26
P6	1.01
P7	0.680
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

10 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	36.5
P2	25.3
P3	16.3
P4	11.5
P5	8.26
P6	4.43
P7	2.75
P8	1.81
P9	1.23
P10	1.02
P11	0.881
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

11 CL₋-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	19.8
P2	14.6
P3	8.73
P4	5.55
P5	3.68
P6	1.69
P7	1.08
P8	0.737
P9	0.630
P10	0.598
P11	0.577
P12	
P13	
P14	
P15	

12 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	41.4
P2	28.6
P3	15.8
P4	9.28
P5	6.10
P6	2.88
P7	1.66
P8	1.09
P9	0.921
P10	0.891
P11	0.978
P12	
P13	
P14	
P15	

13 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	18.6
P2	9.03
P3	4.06
P4	2.30
P5	1.62
P6	1.45
P7	1.12
P8	1.06
P9	1.18
P10	1.15
P11	1.21
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
Halofantrine Free Base Concentrations
Preliminary Analytical Results (4/29/93)

14 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	39.7
P2	17.9
P3	9.74
P4	6.39
P5	4.62
P6	2.53
P7	1.97
P8	1.61
P9	1.38
P10	1.35
P11	1.46
P12	
P13	
P14	
P15	

15 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	23.6
P2	11.7
P3	4.90
P4	2.82
P5	1.87
P6	1.08
P7	0.951
P8	0.637
P9	0.599
P10	0.623
P11	0.744
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

1 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	NS
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

2 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

3 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

4 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

5 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

6 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity; NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

7 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	0.842
P2	0.737
P3	0.631
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

8 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

9 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	0.581
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

10 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

11 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

12 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

13 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

14 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

15 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

16 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	0.703
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

17 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

18 CL-HAL

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

19 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

20 CL-HAL

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	*
P13	*
P14	*
P15	*

1 CL-HM

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity; NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

2 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

3 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

4 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

5 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

6 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	0.532
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

7 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

8 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	0.573
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

9 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

10 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	0.666
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity; NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

11 CL-HM

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

12 CL-HM

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

13 CL-HM

(hrs) Time	($\mu\text{g/ml}$) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.

Hal 93-7
Rat Perfusate
WR 178,460 Free Base Concentrations
Preliminary Analytical Results (4/29/93)

14 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

15 CL-HM

(hrs) Time	(µg/ml) CONC
P0	*
P1	*
P2	*
P3	*
P4	*
P5	*
P6	*
P7	*
P8	*
P9	*
P10	*
P11	*
P12	
P13	
P14	
P15	

* = Below assay sensitivity: NS = No sample.



SCHOOL OF PHARMACY
DEPARTMENT OF PHARMACY

SAN FRANCISCO, CALIFORNIA 94143

May 25, 1994

Jean M. Karle, Ph.D.
Walter Reed Army Institute of Research
Forest Glen Annex, Bldg. 500
9100 Brookville Road
Silver Spring, MD 20910

Dear Dr. Karle,

As you requested by fax on May 12, 1994, we rechecked rat bile halofantrine concentrations for 2 samples in the data sent to you on May 6, 1994. We found unacceptable chromatograms for these two samples and, as a consequence, rechecked all rat bile chromatograms. A total of 5 chromatograms were found to be unacceptable. The five samples were reassayed with results as shown below.

Sample Identification	Free Base Halofantrine Concentration from Unacceptable Chromatogram ($\mu\text{g/ml}$)	Free Base Halofantrine Concentration from Reanalysis ($\mu\text{g/ml}$)
HAL 5-B3	3.72	5.53
HAL 7-B4	0.495	2.84
HAL 9-B1	1.57	5.51
HAL 9-B5	3.13	4.56
HAL 10-B1	0.667	5.53

This data makes the rat bile results for this study final.

Please let me know if I can be of any assistance.

Thank you,

A handwritten signature in cursive script that reads "Richard Bonderud".

Richard Bonderud, Staff Research Associate for
Emil T. Lin, Professor