

2



AD-A210 938

Institute Report No. 376

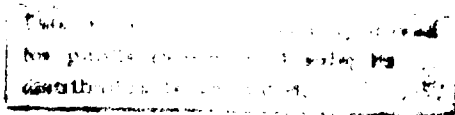
Determination of the Oxime, 2-Hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium Chloride, in Plasma by HPLC

*Suellen Ferraris, PhD
and
Don W. Korte, Jr., PhD, LTC, MSC*

**APPLIED TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY**

HTIC
9-27-89
(Signature)

June 1989



Toxicology Series: 252

**LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129**

89 7 27 06 4

Determination of the Oxime, 2-Hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium Chloride, in Plasma by HPLC (Toxicology Series 252)--
FERRARIS and KORTE

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

 22 June 1989
Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE			4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 376		
6a. NAME OF PERFORMING ORGANIZATION Applied Toxicology Branch Division of Toxicology			6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T		7a. NAME OF MONITORING ORGANIZATION Walter Reed Army Institute of Research
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800			7b. ADDRESS (City, State, and ZIP Code) Washington, DC, 20307-5100		
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO. 62734	PROJECT NO. A875	TASK NO. BC WORK UNIT ACCESSION NO. DA0H0360
11. TITLE (Include Security Classification) (U) Determination of the Oxime, 2-Hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium Chloride, in Plasma by HPLC					
12. PERSONAL AUTHOR(S) S Ferraris and DW Korte, Jr.					
13a. TYPE OF REPORT Institute		13b. TIME COVERED FROM 30JAN87 to 16JUL88		14. DATE OF REPORT (Year, Month, Day) June 1989	
15. PAGE COUNT 26					
16. SUPPLEMENTARY NOTATION Toxicology Series No. 252					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Ion-pair Extraction, Analytical Method, WR255,737, Plasma, HPLC, 2-Hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium Chloride,		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) An assay for the quantitation of the oxime, 2-HYDROXYIMINOMETHYL-3-METHYL-1-[2-(3-METHYL-3-NITROBUTYLOXYMETHYL)]IMIDAZOLIUM CHLORIDE, WR#255,737: ICD# 467, in plasma using high performance liquid chromatography is described. The above oxime (WR 255,737) was extracted from plasma by an ion-pairing liquid/liquid method, and analyzed by normal phase HPLC and UV detection. After the addition of the internal standard, 1-[1-(3-butynyloxymethyl)]-2-hydroxyiminomethyl-3-methyl imidazolium chloride (WR 254,416), the sample was extracted into methylene chloride and subsequently back-extracted into a solution of 0.001M tetrabutylammonium hydrogen sulfate. A portion of this solution was removed with a syringe and placed in a HPLC sample vial for analysis. Flow rate was 1.2 ml/min and the retention times were 2.1 min for WR 254,416 and 2.9 min for WR 255,737. The assay was developed over two concentration ranges, 10-500 ng/ml and 100-1000 ng/ml, and was linear in both					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED		
22a. NAME OF RESPONSIBLE INDIVIDUAL EDWIN S. BEATRICE, COL, MC			22b. TELEPHONE (Include Area Code) (415) 561-3600		22c. OFFICE SYMBOL SGRD-ULZ

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

18 SUBJECT TERMS(cont.). Oxime.

19 ABSTRACT(cont.).

ranges. Accuracy was evaluated from the analysis of blind, spiked samples which gave a coefficient of variation of 2.6%. The recovery was greater than 84%; interday variability was less than 7.8%; intraday variability was less than 3.3%. Stability was determined for WR 255,737 in plasma at -15°C and -80°C. There was no degradation at either temperature for 23 weeks.

ABSTRACT

An assay for the quantitation of the oxime, 2-hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium chloride, [WR 255,737; ICD #467] in plasma using high performance liquid chromatography is described. The above oxime (WR 255,737) was extracted from plasma by an ion-pairing liquid/liquid method, and analyzed by normal phase HPLC and UV detection. After the addition of the internal standard, 1-[1-(3-butynyl-oxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium chloride (WR 254,416), the sample was extracted into methylene chloride and subsequently back-extracted into a solution of 0.001M tetrabutylammonium hydrogen sulfate. A portion of this solution was removed with a syringe and placed in a HPLC sample vial for analysis. Flow rate was 1.2 ml/min and the retention times were 2.1 min for WR 254,416 and 2.9 min for WR 255,737. The assay was developed over two concentration ranges, 10-500 ng/ml and 100-1000 ng/ml, and was linear in both ranges. Accuracy was evaluated from the analysis of blind, spiked samples which gave a coefficient of variation of $\leq 2.6\%$. The recovery was greater than 84%; interday variability was less than 7.8%; intraday variability was less than 3.3%. Stability was determined for WR 255,737 in plasma at -15°C and -80°C. There was no degradation at either temperature for twenty-three weeks.

KEY WORDS: Oxime, 2-hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium chloride, WR 255,737, Ion-pair Extraction, HPLC, Analytical Method, Plasma.



Accession For	
NTIS CRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

PREFACE

TYPE REPORT: Analytical Method Development Study Report

TESTING FACILITY:

U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

U.S. Army Medical Research and Development Command
Walter Reed Army Institute of Research
Washington D.C., 20307-5100
Project Officer: Alan C. Schroeder, PhD, CPT, MSC

PROJECT/WORK UNIT/APC: 3M463764D995/176/TLHO

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC

PRINCIPAL INVESTIGATOR: Suellen Ferraris, PhD

TEST SUBSTANCE: 2-Hydroxyiminomethyl-3-methyl-1-
[2-(3-methyl-3-nitrobutyloxymethyl)]
imidazolium chloride

OBJECTIVE: The object of this study was to develop
a sensitive, reproducible HPLC method
for the quantitation in plasma of 2-
hydroxyiminomethyl-3-methyl-1-[2-(3-
methyl-3-nitrobutyloxymethyl)]imidazolium
chloride (WR 255,737; ICD #467).

**SIGNATURES OF PRINCIPAL SCIENTISTS
INVOLVED IN THE STUDY**

We, the undersigned, declare that this study was performed under our supervision, according to the procedures described herein, and that the report is an accurate record of the results obtained.

Don W. Korte Jr. 19 June 89

Don W. Korte Jr., PhD/Date
LTC, MSC
Study Director

Suellen Ferraris 22 May 89

Suellen Ferraris, PhD/Date
Principal Investigator

TABLE OF CONTENTS

Abstract.....	i
Preface.....	iii
Signature page.....	iv
Table of contents	v
INTRODUCTION.....	1
MATERIALS AND METHODS.....	2
Equipment.....	2
Reagents.....	2
HPLC Parameters.....	3
Preparation of stock solutions.....	3
Preparation of samples for standard curve.....	3
Extraction.....	4
RESULTS.....	4
Separation.....	4
Linearity.....	5
Precision.....	5
Accuracy.....	5
Recovery.....	5
Stability.....	5
SUMMARY.....	6
REFERENCES.....	7
APPENDICES	22
Official Distribution List.....	26

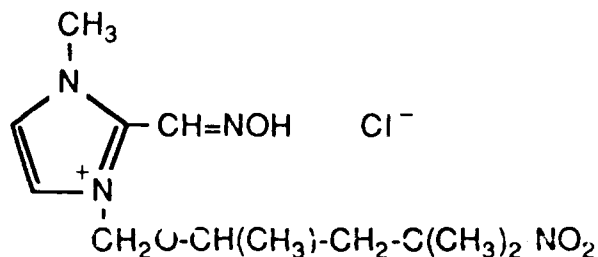
Determination of the Oxime, 2-Hydroximinomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium Chloride in Plasma by HPLC--FERRARIS and KORTE

INTRODUCTION

Oximes have been used for many years in the treatment of poisoning with organophosphates. The organophosphorylation of the enzyme, acetylcholinesterase, can be lethal *in vivo*, because the enzyme does not regenerate. Oximes are capable of reactivating the organophosphorylated enzyme by forming an oxime-phosphonate, leaving the regenerated enzyme (1).

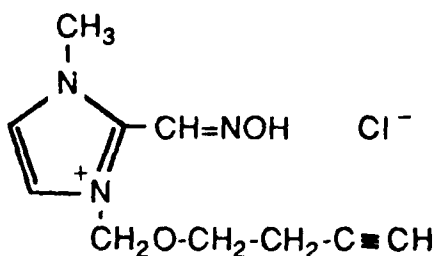
Various methods have been developed to measure oximes, including colorimetric (2), spectrophotometric (3,4) and liquid chromatographic (5-7) methods. The HPLC methods for measuring oximes in biological fluids are the most sensitive and least time-consuming. Thus, more work has been done recently using HPLC methods than any other (8-11).

This report describes an assay for the quantitation of an oxime (WR 255,737; ICD #467) in plasma, which is more sensitive than any other HPLC method for oximes reported in the literature to date. A liquid/liquid ion-pair extraction method was used, followed by liquid chromatographic analysis with UV-detection at 270 nm. Quantitation of WR 255,737 was linear for the concentration range 10-1000 ng/ml of plasma. The limit of detection was 1 ng on column. Another imidazolium oxime (WR 254,416; ICD #301) was used as the internal standard.



WR 255,737

2-hydroxyiminomethyl-3-methyl-
1-[2-(3-methyl-3-nitrobutyl-oxymethyl)]
imidazolium chloride



WR 254,416
(Internal Standard)

1-[1-(3-Butynyloxymethyl)]-2-
hydroxyiminomethyl-3-methyl-
imidazolium chloride

MATERIALS AND METHODS

Equipment

The chromatography was performed on a Hewlett-Packard 1090 high pressure liquid chromatograph with an 85B Personal Computer and DPU Multichannel Integrator (Hewlett-Packard, Santa Clara, CA). An IEC PR 6000 Centrifuge (International Equipment Company, Needham Heights, MA) was used in the extraction procedure.

Reagents

Solvents were HPLC grade and chemicals were reagent grade. Acetonitrile and methylene chloride were obtained from American Burdick and Jackson (Muskegon, MI). The water used in preparation of all HPLC solutions was deionized, distilled, and purified of organics, with an Organicpure[®] water purifier by Barnstead (Boston, MA). Tetramethylammonium chloride (TMA) and tetrabutylammonium hydrogen sulfate (TBA) were obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium dihydrogen phosphate and picric acid were obtained from Baker Chemical Company (Phillipsburg, NJ). WR 254,416 and WR 255,737 were supplied by CPT Alan Schroeder, Division of Experimental Therapeutics, WRAIR. Both swine plasma and human plasma were used. Preliminary studies were done with swine plasma, but human

plasma was used for the bulk of the study. Results from both types of plasma were in agreement. Human plasma was obtained from LAIR Division of Blood Research. This plasma had been collected in accordance with the American Association of Blood Bank Standards under an approved type protocol for blood collection.

HPLC Parameters

Column: Brownlee Silica 5 μm (100 x 4.6 mm)
(Brownlee Labs, Inc. Santa Clara, CA)
Guard column: Brownlee New Guard Silica 7 μm
Buffer: 0.01 M sodium dihydrogen phosphate
0.0020 M TMA in HPLC water, pH 3.0 with
sulfuric acid, filtered through a 0.22 μm
filter.
Mobile Phase: 18% acetonitrile, 82% buffer
Flow: 1.2 ml/min
Injection volume: 25-50 μl
Wavelength: 270 nm; 400 nm (reference)
Run time: 4.0 min
Peakwidth: 0.18 min
Retention time: 2.90 min

Preparation of Stock Solutions

WR 254,416 was used as the internal standard for the quantitation of WR 255,737. Stock solutions of both oximes (1 mg/ml) were prepared in water and aliquots of each solution were placed in microcentrifuge tubes and stored in the freezer (-14°C) for subsequent use. These solutions were used to spike plasma and water samples for the standard curve.

Preparation of Plasma Samples for the Standard Curve

The concentration range studied was 10-1000 ng/ml. This range was too broad to maintain accuracy at the very low and very high ends of the standard curve, so the assay was divided into two ranges which overlapped. Assay I was 10-500 ng/ml and assay II was 100-1000 ng/ml. The standard solutions used were prepared as follows. The stock solutions (1 mg/ml) of WR 255,737 and internal standard WR 254,416 were thawed and diluted to 10 $\mu\text{g/ml}$ (Solution A) and 1 $\mu\text{g/ml}$ (Solution B). These diluted solutions were used to prepare the samples for the standard curves. The six concentrations of WR 255,737 in plasma used for Standard Curve I were prepared by adding various amounts of the diluted solutions A (10 $\mu\text{g/ml}$) and B (1 $\mu\text{g/ml}$) as shown in Table 1. The five concentrations of WR 255,737 in plasma

For Standard Curve II were prepared by adding various amounts of the diluted solution A (10 µg/ml) to plasma as shown in Table 1. These samples were extracted and analyzed by HPLC.

Extraction

Plasma samples were extracted into methylene chloride and then back-extracted into TBA using a modification of the method of de Ruyter, et al. (12). To a PTFE-lined screw-cap culture tube (150 x 16 mm) were added 1.0 ml of plasma, 20 µl of internal standard (10 µg/ml), 0.5 ml of 0.1 M picric acid (pH adjusted to 7 with 2 M sodium hydroxide), and 0.5 ml of 0.1 M sodium dihydrogen phosphate. The picric acid was added to form an ion-pair with the quaternary amine, thus facilitating its dispersion into the methylene chloride layer. This mixture was vortexed (15 seconds) and extracted with 10 ml of water-saturated methylene chloride by vigorously shaking, immediately vortexing (10 sec), shaking by hand again, and vortexing (10 sec). Following centrifugation (1000 g, 10 min) the aqueous phase (top layer) was removed with a Pasteur pipette using vacuum suction, leaving behind the organic phase and an emulsified interface. To break up any additional emulsion, another 2 ml of water-saturated methylene chloride was added to the tube and the contents were shaken and vortexed (15 sec). The mixture was centrifuged (1000 g, 7 min) and the organic phase decanted into another PTFE-lined screw-cap culture tube (150 x 16 mm); 200 µl of 0.001 M TBA was then added. This final mixture was vigorously shaken, vortexed (10 sec), shaken by hand, vortexed (10 sec) and then centrifuged (1000 g, 7 min). The majority of the aqueous phase was then removed using a microliter syringe and placed in a sample vial for HPLC analysis.

RESULTS

Separation

Under the chromatographic conditions described previously, WR 254,416 (#416) elutes at approximately 2.1 min and WR 255,737 (#737) elutes at approximately 2.9 min. Typical chromatograms from Assay I for extracted plasma samples are shown in Figures 1, 2 and 3. Figures 1 and 2 are chromatograms of extracted plasma containing 10 and 200 ng of #737/ml of plasma, respectively, and 50 ng of #416 as internal standard. Chromatograms of Assay II for extracted plasma samples are shown in Figures 4, 5 and 6. Figures 4 and 5 contain 100 and 800 ng of #737, respectively and 200 ng

of #416 as the internal standard. Chromatograms of extracted plasma containing no oximes (blank plasma) show no interfering peaks (Figures 3 and 6).

Linearity

The standard curve was determined by performing a linear regression analysis of the concentration of WR 255,737 (ng/ml) in plasma versus the peak height ratio of the two oxime peaks (737/416). Figures 7 and 8 show the linearity of the calibration plot.

Values from typical standard curves (Table 2) show that the assay is linear ($r = 0.9999$) over the ranges 10-500 and 100-1000 ng of #737/ml of plasma (Assay I and Assay II).

Precision

Interday variability was calculated for all eleven concentrations of WR 255,737 in plasma (Table 3). The complete standard curves were run on six different days. The coefficient of variation (CV) ranged from 2.0 to 7.1. Intraday variation was run on 6 replicate samples of a low and a high concentration in each standard curve (Table 4). The CV was less than 3.2 for all concentrations.

Accuracy

The accuracy of the method was verified by the analysis of blind spiked samples. The concentrations used were 18.5, 135.0, and 335 ng/ml. Results are shown in Table 5. The bias is a measure of the deviation of the mean value from that of the spiked value.

$$\text{Bias} = \frac{(\text{Measured concentration} - \text{Spiked concentration})}{\text{Spiked concentration}} \times 100$$

Recovery

The recovery of WR 255,737 from plasma was determined by comparing the peak height of WR 255,737 obtained from extracted plasma samples with the peak height of WR 255,737 from non-extracted spiked water samples. Recoveries were evaluated for all concentrations of WR 255,737 in plasma and were 80% or above. The mean recoveries were slightly higher (Table 6).

Stability

No appreciable degradation was observed for WR 255,737 in plasma over 23 weeks when stored at -15°C or -80°C, as shown in Tables 7 and 8. The stability of the oxime allows samples to be collected and stored frozen for subsequent analysis.

SUMMARY

An assay was developed for the extraction and quantitation of the oxime, 2-hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium chloride (WR 255,737) in plasma and was demonstrated to be linear over two concentration ranges: 10-500 and 100-1000 ng of WR 255,737/ml of plasma. The method is sensitive, rapid, and accurate. The limit of detection is 1 ng on column. The extraction is fast; it does not include an evaporation step used by other methods. The extraction is also clean; interfering substances are removed, resulting in sharp, reproducible peaks of the oxime on the HPLC. The mean extraction recovery of WR 255,737 was greater than 84% at all concentration levels and the range of recoveries was from 80-108% for all samples. The intraday assay variability was less than 3.3%; the interday assay variability was less than 7.2%. The compound was stable in plasma for 23 weeks when stored at -15°C. or -80°C.

REFERENCES

1. Gilman AG, Goodman LS, Gilman A. The Pharmacological Basis of Therapeutics. Macmillan Pub. Co. New York. 1980, p 110.
2. Askew BM, Davies DR, Green AL, Holmes R. The nature of the toxicity of 2-oxo-oximes. Brit J Pharmacol 1956; 11: 424.
3. Johnson DP. Spectrophotometric determination of oximes and unsubstituted hydroxylamine. Anal Chem 1968;40:646.
4. Groff WA, Ellin RI. A new and rapid determination of pyridinium aldoximes in blood and urine. Clin Chem 1969;15:72.
5. Brown ND, Sleeman HK. Determination of N,N'-trimethylene-bis(pyridinium-4-aldoxime) dibromide by ion-pair high-performance liquid chromatography. J Chromatogr 1977;138:449-52.
6. Bovenkamp JW, LaCroix BV, Henshaw PF. The retention times of oximes in reversed-phase high-performance liquid chromatography. J Chromatogr 1984;301:492-6.
7. Utley D. Analysis of formulation containing pralodoxime mesylate by liquid chromatography. J Chromatogr 1987;396:237-50.
8. Benschop HP, Konings KAG, Kossen SP, Ligtenstein DA. Determination of some pyridinium aldoxime compounds by means of ion-pair reversed-phase high-performance liquid chromatography: application in biological material. J Chromatogr 1981;225:1107-14.
9. Unger PD, McMahon FJ. High-performance liquid chromatography of cyclohexanone oxime in urine and plasma. J Chromatogr 1981;210:360-4.
10. LeBel M, Ericson JF, Pitkin DH. Improved high-performance liquid chromatographic (HPLC) assay method for ceftizoxime. J Liq Chromatogr 1984;7:961-8.
11. Duff R, Murrill E. Determination of L-buthionine-(S,R)-sulfoximine in plasma by high-performance liquid chromatography with o-phthalaldehyde derivatization and fluorometric detection. J Chromatogr 1987;385:275-82.

REFERENCES (cont.)

12. de Ruyter MGM, Cronnelly R, Castagnoli N Jr. Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds; determination of pyridostigmine, neostigmine, and edrophonium in biological fluids. *J Chromatogr* 1980;183:193-201.



Fig. 3. Chromatogram of extracted blank plasma

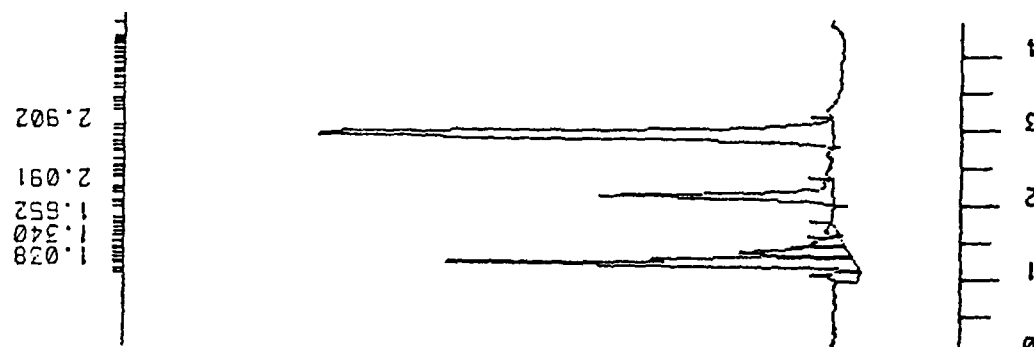


Fig. 2. Chromatogram of extracted plasma, #737 concentration (200 ng/ml), 50 ng #737 on column

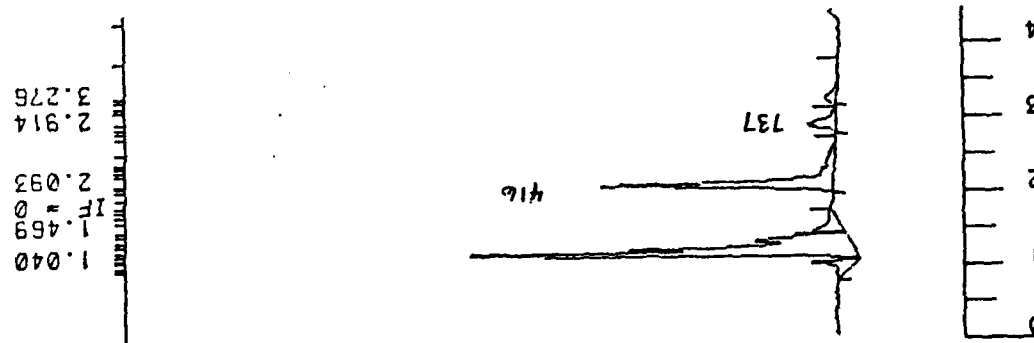


Fig. 1. Chromatogram of extracted plasma, #737 concentration (10 ng/ml), 2.5 ng #737 on column

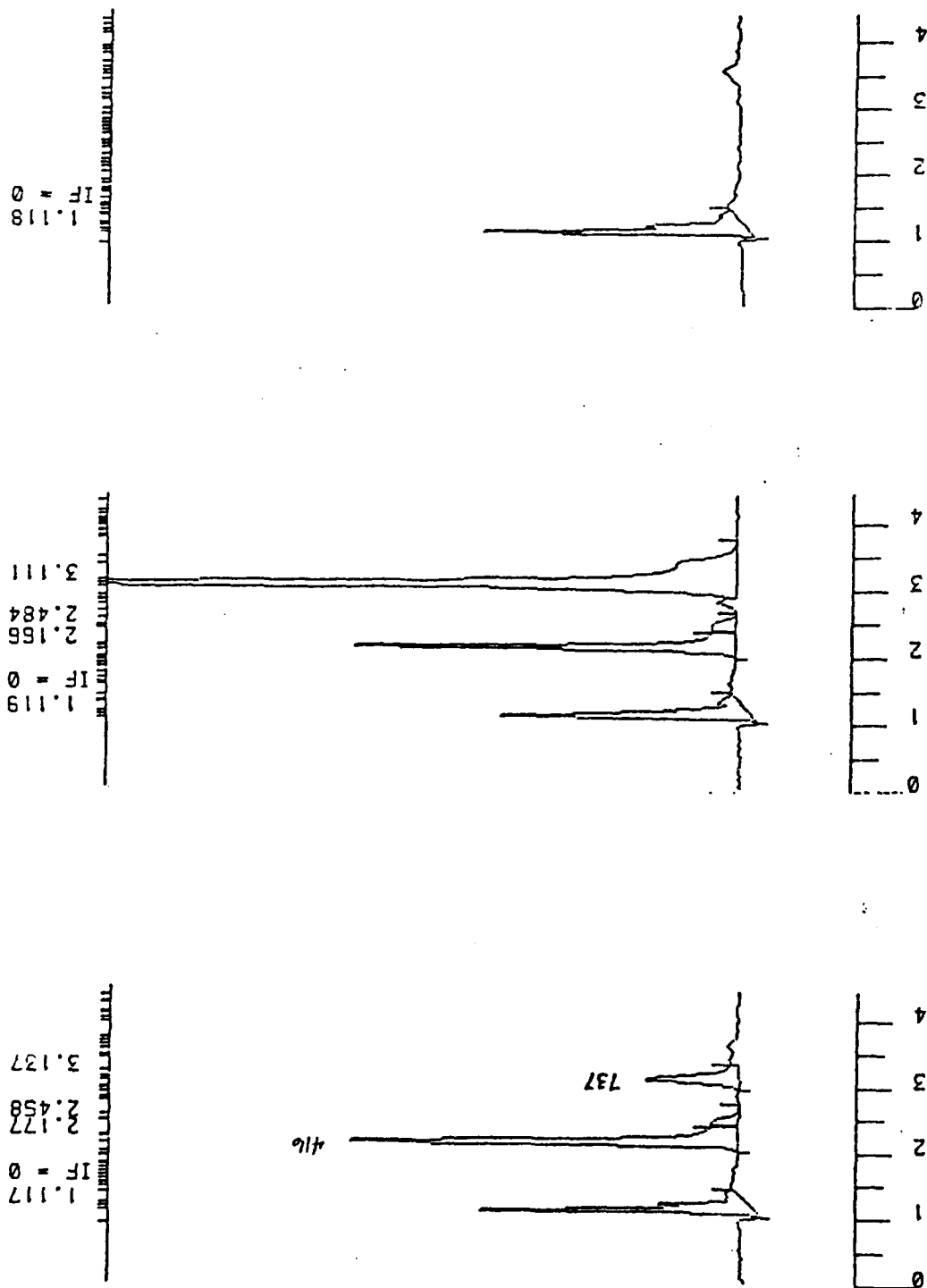


Fig. 4 . Chromatogram of extracted plasma, #737 concentration (12.5ng/ml), 100ng #737 on column

Fig. 5 . Chromatogram of extracted plasma, #737 concentration (800ng/ml), 100ng #737 on column

Fig. 6 . Chromatogram of extracted non-spiked plasma

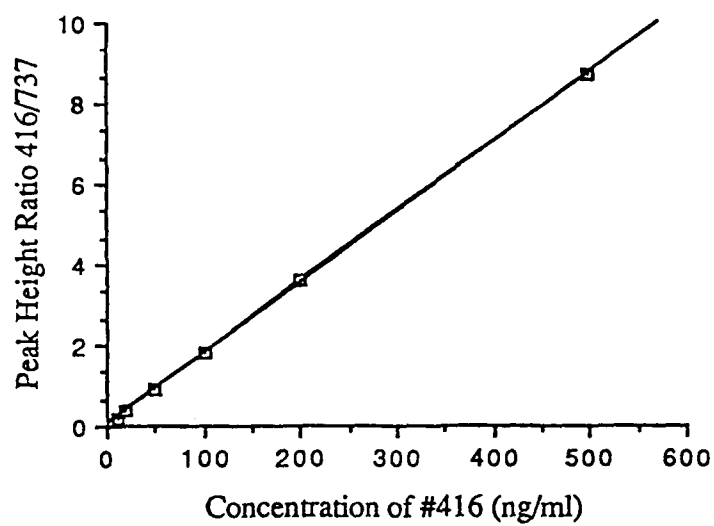


Fig. 7. Standard Curve I

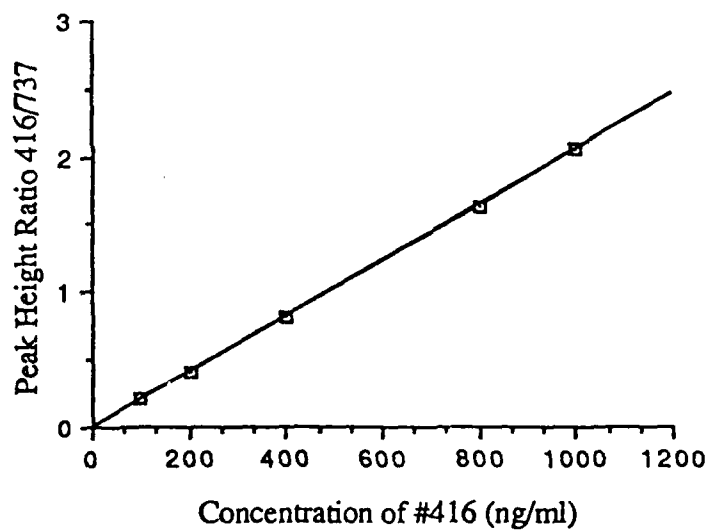


Fig. 8. Standard Curve II

TABLE 1: PREPARATION OF SAMPLES FOR STANDARD CURVES

Concentration of WR	255,737 μ l of WR	μ l of WR	plasma
ng/ml plasma	ng on column	255,737	254,416 (ml)

Standard Curve I^a

10	2.5	10 (B)	50 (B)	1
20	5.0	20 (B)	50 (B)	1
50	12.5	50 (B)	50 (B)	1
100	25.0	100 (B)	50 (B)	1
200	50.0	20 (A)	50 (B)	1
500	125.0	50 (A)	50 (B)	1

Standard Curve II^b

100	12.5	10 (A)	20 (A)	1
200	25.0	20 (A)	20 (A)	1
400	50.0	40 (A)	20 (A)	1
800	100.0	80 (A)	20 (A)	1
1000	125.0	100 (A)	20 (A)	1

^a50- μ l injection^b25- μ l injection

TABLE 2: LINEARITY OF WR 255,737 IN PLASMA

Standard Curve I

Concentration (ng/ml)	Peak Ht Ratio 737/416	SD	CV
10 ^a	0.116	±0.0069	5.9
20 ^a	0.238	0.0105	4.4
50 ^b	0.558	0.0210	3.8
100 ^b	1.096	0.0340	3.1
200 ^b	2.251	0.0440	2.0
500 ^b	5.397	0.2270	4.2

$a_n = 7$; $b_n = 5$; Slope = 0.0108; Intercept = 0.021;
Correlation coefficient = 0.9999.

Standard Curve II

Concentration (ng/ml)	Peak Ht Ratio 737/416	SD	CV
100	0.262	±0.016	6.1
200	0.513	0.018	3.5
400	1.029	0.042	4.1
800	2.042	0.055	2.7
1000	2.543	0.078	3.1

$n = 6$; Slope = 0.0203; Intercept = 0.0108; Correlation
coefficient = 0.9999.

SD = Standard deviation

CV = (SD/Mean) x 100

TABLE 3: INTERDAY PRECISION

Sample Number						Mean	SD	CV	
1	2	3	4	5	6	conc.			
Assay I									
10 ng/ml ^a									
7.7	8.1	9.1	9.7	9.1	9.0	8.6	8.8	±0.63	7.1
20 ng/ml									
18.1	19.5	20.3	20.9	21.3	19.6	20.5	20.0	0.99	4.9
50 ng/ml									
48.3	49.9	50.8	52.2	47.2			49.7	1.98	4.0
100 ng/ml									
97.2	100.0	104.8	98.4	97.1			99.5	3.2	3.2
200 ng/ml									
209.9	208.4	208.8	205.1	199.8			206.4	4.1	2.0
500 ng/ml									
498.2	467.3	511.9	521.8	492.6			496.6	21.2	4.3
Assay II									
100 ng/ml									
92.6	91.8	105.2	97.3	108.0	104.4		99.9	±6.9	6.9
200 ng/ml									
207.7	190.7	200.6	192.7	204.5	206.1		200.4	7.2	3.6
400 ng/ml									
406.7	388.9	394.1	427.6	411.4	382.6		401.9	16.6	4.1
800 ng/ml									
805.9	774.8	814.2	799.9	835.4	807.9		803.0	22.5	2.8
1000 ng/ml									
1040	981	1012	957	980	1020		998.4	30.7	3.1

^aSpiked concentration

TABLE 4: INTRADAY PRECISION

Sample Number						mean	SD	CV
1	2	3	4	5	6	conc.		
Assay I								
20 ng/ml ^a								
21.6	22.5	22.9	23.8	23.2	22.6	22.8	±0.74	3.2
400 ng/ml								
434.7	434.9	430.2	431.5	446.2	418.9	432.7	8.8	2.0
Assay II								
100 ng/ml								
101.7	102.0	106.8	104.4	102.0	104.4	103.6	2.0	1.9
850 ng/ml								
870.9	898.5	835.4	857.9	858.3		864.2	23.1	2.7

^aSpiked concentration

TABLE 5: ACCURACY OF WR 255,737 DETERMINATION IN PLASMA

Sample Number	Prep'd Conc.	Meas Conc.	Mean	SD	CV	Bias ^a
1	18.5	17.9	19.0	1.1	5.8	2.6
3		18.2				
7		19.8				
11		20.1				
2	135.0	137.0	137.8	2.9	2.1	2.0
4		135.1				
9		137.2				
12		142.0				
5	335.0	331.3	339.4	10.3	3.0	1.3
6		329.8				
8		346.9				
10		349.7				

^aBias = [(Measured - Prepared) / Prepared] x 100

TABLE 6: RECOVERY OF WR 255,737 FROM PLASMA

Sample Number	Recovery ^{a,b} of WR 254,416	Recovery of WR 255,737
------------------	--	---------------------------

Assay I Concentration: 10 ng/ml

1	91.8	88.3
2	105.3	107.8
3	88.1	106.0
4	88.2	108.1
5	87.4	104.0
6	82.9	90.7
7	82.3	89.2
8	80.3	82.8
9	83.5	87.8
Mean±SD	87.8±7.1	96.1±9.6
CVC	8.0	10.0

Assay I Concentration: 20 ng/ml

1	94.7	104.7
2	91.6	100.5
3	88.9	100.4
4	89.4	98.1
5	92.1	95.0
6	89.6	89.9
7	82.8	89.7
8	82.1	93.3
9	83.8	93.9
10	84.7	96.1
Mean±SD	88.0±4.1	96.2±4.6
CV	4.7	4.7

Assay I Concentration: 50 ng/ml

1	93.5	94.7
2	83.3	93.5
3	89.8	96.4
4	87.1	95.7
5	90.7	96.5
6	84.3	91.4
Mean±SD	88.1±3.9	94.7±2.0
CV	4.4	2.1

TABLE 6 (cont.): RECOVERY OF WR 255,737 FROM PLASMA

Sample Number	Recovery ^{a,b} of WR 254,416	Recovery of WR 255,737
------------------	--	---------------------------

Assay I Concentration: 100 ng/ml

1	95.6	101.5
2	95.9	103.4
3	86.0	93.5
4	83.9	86.1
5	93.4	96.5
6	85.7	91.0
Mean±SD	90.1±5.5	95.2±6.5
CV	6.1	6.8

Assay I Concentration: 200 ng/ml

1	93.1	102.5
2	92.3	97.9
3	91.8	95.6
4	84.1	87.7
5	89.1	91.4
6	95.5	96.6
Mean±SD	91.0±3.9	95.3±5.2
CV	4.3	5.4

Assay I Concentration: 500 ng/ml

1	86.8	89.7
2	99.1	92.5
3	87.3	90.4
4	82.0	86.8
5	91.3	92.8
6	93.7	99.0
Mean±SD	90.0±6.0	91.9±4.1
CV	6.7	4.5

TABLE 6 (cont.): RECOVERY OF WR 255,737 FROM PLASMA

Sample Number	Recovery ^{a,d} of WR 254,416	Recovery of WR 255,737
------------------	--	---------------------------

Assay II Concentration: 400 ng/ml

1	87.5	88.0
2	80.7	90.6
3	89.8	91.0
4	95.1	89.2
Mean $\pm$ SD	88.3 $\pm$ 6.0	89.7 $\pm$ 1.4
CV	6.7	1.5

Assay II Concentration: 800 ng/ml

1	80.1	80.0
2	91.1	89.3
3	87.5	93.7
4	86.8	85.2
5	88.9	89.3
6	89.6	91.5
Mean $\pm$ SD	88.1 $\pm$ 4.7	88.2 $\pm$ 4.9
CV	5.3	5.5

Assay II Concentration: 1000 ng/ml

1	82.5	80.4
2	82.1	82.3
3	88.3	89.9
4	81.1	82.0
5	93.3	89.7
6	93.0	93.1
Mean $\pm$ SD	86.7 $\pm$ 5.6	86.2 $\pm$ 5.3
CV	6.4	6.1

^a Recovery = (Peak ht. in plasma/Peak ht. in water) x 100^b Internal Standard #416 concentration: 50 ng/ml^c CV = (SD/Mean) x 100^d Internal Standard #416 concentration: 200 ng/ml

TABLE 7: STABILITY OF WR 255,737 IN PLASMA AT -15°C.

Days	Plasma Concentration (ng/ml)					
	Prep'd	Meas'd	Prep'd	Meas'd	Prep'd	Meas'd
	17.0		100.0		400.0	
0		16.7		99.2		397.8
1		15.7		101.2		409.0
7		14.6		101.3		414.8
11		15.6		100.0		399.2
21		17.2		99.9		396.5
29		19.1		99.1		388.2
42		17.6		100.4		406.4
56		17.1		97.6		385.8
84		16.6		100.4		420.6
132		16.6		97.6		380.9
161		16.9		101.6		409.0
Mean±SD		16.7±1.1		99.8±1.3		400.7±11.9
CVA ^a		6.7		1.3		3.0
Bias ^b		2.3		0.2		0.18

^aCV = (SD/Mean) x 100^bBias = [(Measured - Prepared) / Prepared] x 100

TABLE 8: STABILITY OF WR 255,737 IN PLASMA AT -80°C.

Days	Plasma Concentration (ng/ml)					
	Prep'd	Meas'd	Prep'd	Meas'd	Prep'd	Meas'd
	17.0		100.0		400.0	
0		16.7		95.2		397.8
1		16.5		97.6		408.3
7		16.9		105.2		421.1
11		16.0		100.1		402.2
21		17.9		103.8		405.2
29		-a		101.1		398.1
42		18.0		102.5		396.6
56		16.1		98.8		398.2
84		16.9		100.5		402.1
132		16.2		97.3		388.0
161		16.0		102.4		410.3
Mean±SD		16.7±0.7		100.4±2.9		402.5±8.3
CV ^b		4.2		2.9		2.1
Bias ^c		1.8		0.4		0.63

^asample contaminated during pipetting.

^bCV = (SD/Mean) x 100

^cBias = [(Measured-Prepared)/Prepared] x 100

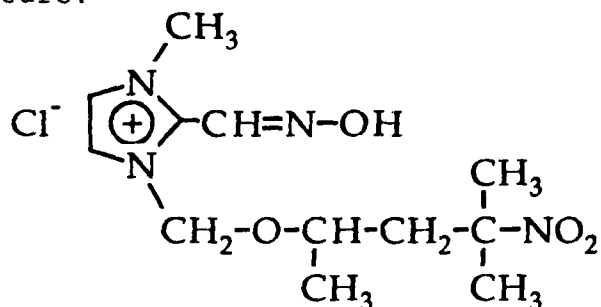
Appendix I: CHEMICAL DATA

Chemical name: 2-Hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)] imidazolium chloride

LAIR code number: TP76

Walter Reed code number: WR 255,737

Chemical structure:



Molecular formula: $C_{11}H_{19}N_4O_4Cl$

Molecular weight: 306.75

Physical state: white crystalline solid

Analytical data:

IR (KBr): The major peaks in the infrared spectrum of the compound were observed at: 3496, 3136, 3066, 2993, 1617, 1535, 1514, 1404, 1352, 1099, 1000, 764 cm^{-1} .¹

NMR: (300 MHz, D_2O) α 1.16 (d, J=6.6, 3H OCH(CH₃)C(CH₃)₂NO₂), 1.41 (s, 6H, OCH(CH₃)C(CH₃)₂NO₂) 3.97 (s, 3H, NCH₃), 4.21 (m, J=6.3, 1H, OCH(CH₃)C(CH₃)₂NO₂), 5.76 (m, J=1.1, 2H, NCH₂O-), 7.91 (s, 1H, aromatic proton meta to NCH₂O), 8.02 (d, J=1.8, 1H, aromatic proton ortho to NCH₂O) 8.44 (s, 1H, CHNOH).²

HPLC: The compound was analyzed by HPLC under the following conditions: column, 5 μm silica (Brownlee, 100 x 4.6 mm): mobile phase, 82% A (0.01 M NaH₂PO₄, 0.0025 M tetramethylammonium hydrogen sulfate, pH adjusted to 3 with H₂SO₄), 18% B (acetonitrile); flow rate, 1.0 ml/min; wavelength monitored, 275 nm. The compound eluted at 4.37 min. No other peaks were observed to 15 min.³

Source: SRI International

Lot number: BHH-0113

¹ Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 45. Letterman Army Institute of Research, Presidio of San Francisco, CA.

² Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.6, pp 21-22. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³ Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 74. Letterman Army Institute of Research, Presidio of San Francisco, CA.

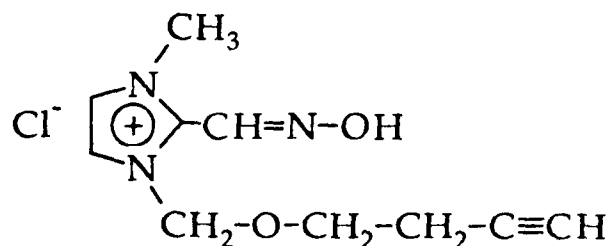
Appendix II: CHEMICAL DATA

Chemical name: 1-(1-(3-butyloxymethyl))-2-hydroxyiminomethyl-3-methylimidazolium chloride

LAIR code number: TP74

Walter Reed code number: WR 254,416

Chemical structure:



Molecular formula: C₁₀H₁₄N₃O₂Cl

Molecular weight: 243.7

Physical state: slightly off-white crystalline solid

Analytical data:

IR (KBr): The major peaks in the infrared spectrum of the compound were observed at: 3199, 3087, 3003, 2831, 1621, 1517, 1236, 1067, 1003, 782, 746, 741 cm⁻¹.

NMR: (300 MHz, D₂O) α 2.39 (S,1H -C≡CH), 2.48 (M, J=3.8, 2H, OCH₂CH₂C≡) 3.52 (t, J=6.0, 2H, OCH₂CH₂C≡), 3.98 (S, 2H, NCH₃), 5.77 (S, 2H, NCH₂O-), 7.92 (d, J=2.1, 1H, aromatic proton meta to NCH₂OCH₂-), 8.03 (d, J=2.1, 1H, aromatic proton ortho α ; NCH₂OCH₂-) 8.53 (S, 1H, CHNOH)²

HPLC: The compound was analyzed by HPLC under the following conditions: column, 5 μ m silica (Brownlee, 100 x 4.6 mm): mobile phase, 82% A (0.01 M NaH₂PO₄, 0.0025 M tetramethylammonium hydrogen sulphate, pH adjusted to 3 with H₂SO₄), 18% B (acetonitrile); flow rate, 1.0 ml/min; wavelength monitored, 275 nm. The compound eluted at 3.37 min. No other peaks were observed to 15 min.³

Source: SRI International

Lot number: BHH-0063

¹Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 39. Letterman Army Institute of Research, Presidio of San Francisco, CA.

²Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.6, pp 15-16. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.4, p 72. Letterman Army Institute of Research, Presidio of San Francisco, CA.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
& Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

DASG-AAFJML
Army/Air Force Joint Medical Library
Offices of the Surgeons General
5109 Leesburg Pike, Room 670
Falls Church, VA 22041-3258

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University of
Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
Walter Reed Army Institute of Research
ATTN: SGRD-UWZ-L
Washington, DC. 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Materiel Development
Activity
Fort Detrick, Bldg T622
Frederick, MD 21701-5009

Commander
US Army Biomedical Research and
Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute of
Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Institute of Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Medical Research Institute of
Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research
Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

USAF School of Aerospace Medicine
Document Section
USAFSAM/TSKD
Brooks Air Force Base, TX 78235-5301

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000