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Determination of the Oxime, 1-[1-(3-Butynyloxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium Chloride, in Plasma by HPLC

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APPLIED TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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Determination of the Oxime, 1-[1-(3-Butynyloxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium Chloride, in Plasma by HPLC (Toxicology Series 251)--FERRARIS and KORTE

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An assay for the quantitation of the oxime, 1-[1-(3-METHYL-3-BUTYNYLOXYMETHYL)]-2-HYDROXYIMINOMETHYL-3-METHYLIMIDAZOLIUM CHLORIDE, [ICD# [illegible]; WR254,416] in plasma using high performance liquid chromatography is described. The above oxime (WR 254,416) 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, 2-hydroxyiminomethyl-[illegible]-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium chloride (WR255737), 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.[illegible] 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 i[illegible]

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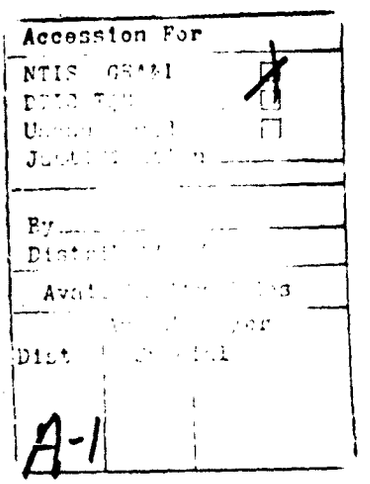
ABSTRACT(cont.).

both ranges. Accuracy was evaluated from the analysis of blind, spiked samples which gave a coefficient of variation of ≤5.1%. The recovery was greater than 86%; interday variability was less than 5%; intraday variability was less than 3%. Stability was determined for WR 254,416 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, 1-(1-(3-butynyloxymethyl))-2-hydroxyiminomethyl-3-methyl imidazolium chloride, [WR 254,416; ICD #301] in plasma using high performance liquid chromatography is described. The above oxime (WR 254,416) 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, 2-hydroxyiminomethyl-3-methyl-1-[2-(3-methyl-3-nitrobutyloxymethyl)]imidazolium chloride (WR 255,737), 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 $\leq$ 5.1%. The recovery was greater than 86%; interday variability was less than 5%; intraday variability was less than 3%. Stability was determined for WR 254,416 in plasma at -15°C and -80°C. There was no degradation at either temperature after twenty-three weeks.

KEY WORDS: Oxime, 1-[1-(3-butynyloxymethyl)]-2-hydroxyiminomethyl-3-methyl imidazolium chloride, WR 254,416, Ion-pair Extraction, HPLC, Analytical Method, Plasma.



PREFACE

TYPE REPORT: Analytical Method Development Study Report

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

PRINCIPAL INVESTIGATOR: Suellen Ferraris, PhD

TEST SUBSTANCE: 1-[1-(3-Butynyloxymethyl)]-2-hydroxyimino-methyl-3-methyl imidazolium chloride

OBJECTIVE: The object of this study was to develop a sensitive, reproducible HPLC method for the quantitation in plasma of 1-[1-(3-butynyloxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium chloride (WR 254,416; ICD #301).

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

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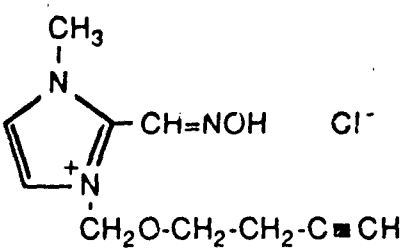
Determination of the Oxime, 1-[1-(3-Butynyloxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium Chloride in Plasma by HPLC--FERRARIS and KORTE

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).

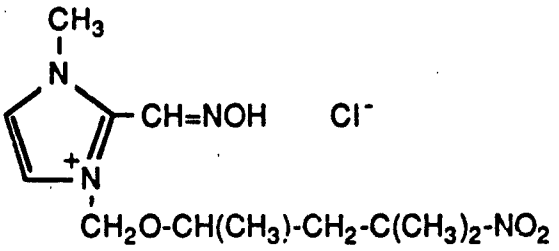
Various methods have been developed to measure oximes, including colorimetric (2), spectrophotometric (3,4) and liquid chromatographic (5-7) methods. The HPLC methods for measuring oximes in biological fluids are the most sensitive and least time-consuming. Thus, more work has been done recently using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

This report describes an assay for the quantitation of an oxime [WR 254,416; ICD #301] 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 254,416 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 255,737; ICD #467) was used as the internal standard.



WR 254,416

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



WR 255,737 (internal standard)

2-hydroxyiminomethyl-3-methyl-
1-[2-(3-methyl-3-nitrobutyl-oxymethyl)]
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 (Internation 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 Banks 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:	2.10 min

Preparation of Stock Solutions

WR 255,737 was used as the internal standard for the quantitation of WR 254,416. 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 254,416 and internal standard WR 255,737 were thawed and diluted to 10 µg/ml (Solution A) and 1 µg/ml (Solution B). These diluted solutions were used to prepare the samples for the standard curves. The six concentrations of WR 254,416 in plasma used for Standard Curve I were prepared by adding various amounts of the diluted solutions A (10 µg/ml) and B (1 µg/ml) as shown in Table 1. The five concentrations of WR 254,416 in plasma used 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 WR 254,416/ml of plasma, respectively, and 100 ng of WR 255,737 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 WR 254,416, respectively, and 800 ng of WR 255,737 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 254,416 (ng/ml) in plasma versus the peak height ratio of the two oxime peaks (416/737). 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 WR 254,416/ml of plasma (Assay I and Assay II).

Precision

Interday variability was calculated for all eleven concentrations of WR 254,416 in plasma (Table 3). The complete standard curves were run on six different days. The coefficient of variation (CV) ranged from 1.1 to 5.0%. 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% for all concentrations.

Accuracy

The accuracy of the method was verified by the analysis of blind spiked samples. The concentrations used were 16.5, 45.0, and 305 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 254,416 from plasma was determined by comparing the peak height of WR 254,416 obtained from extracted plasma samples with the peak height of WR 254,416 from non-extracted spiked water samples. Recoveries were evaluated for all concentrations of WR 254,416 in plasma and were 80% or above. The mean recoveries were slightly higher (Table 6).

Stability

No appreciable degradation was observed for WR 254,416 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, 1-[1-(3-butynyloxymethyl)]-2-hydroxyiminomethyl-3-methylimidazolium chloride (WR 254,416), in plasma and was demonstrated to be linear over two concentration ranges: 10-500 and 100-1000 ng of WR 254,416/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 254,416 was greater than 86% at all concentration levels and the range of recoveries was from 80-108% for all samples. The intraday assay variability was less than 3%; the interday assay variability was less than 5%. The compound was stable in plasma for 23 weeks when stored at -15°C. or -80°C.

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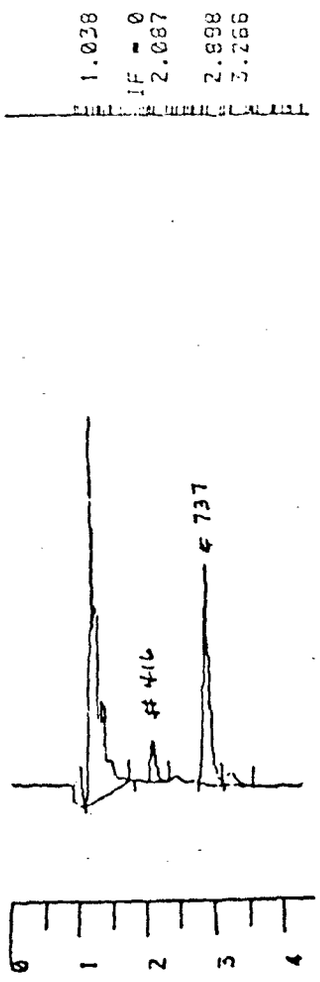


Fig. 1. Chromatogram of extracted plasma, #416 concentration (10 ng/ml), 2.5 ng #416 on column

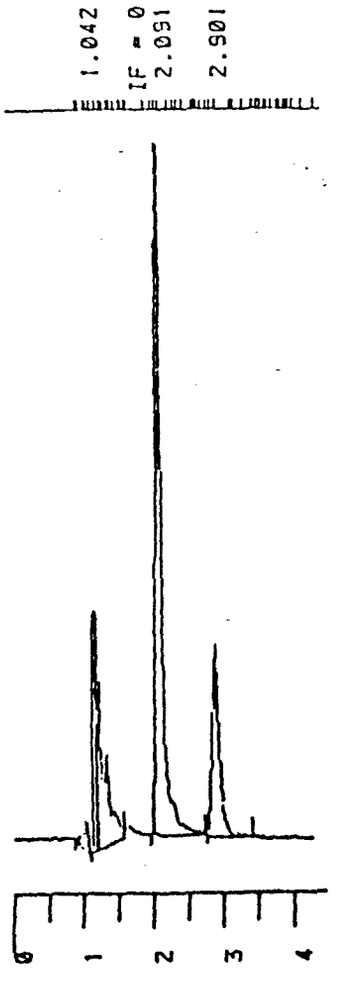


Fig. 2. Chromatogram of extracted plasma, #416 concentration (200 ng/ml), 50 ng #416 on column

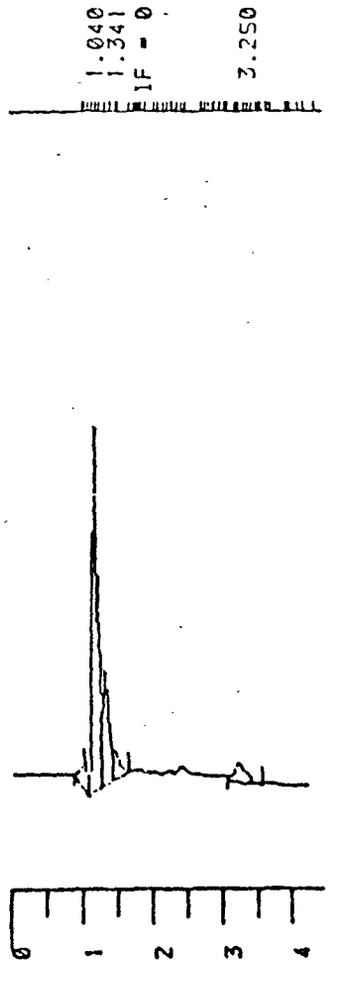


Fig. 3. Chromatogram of extracted blank plasma

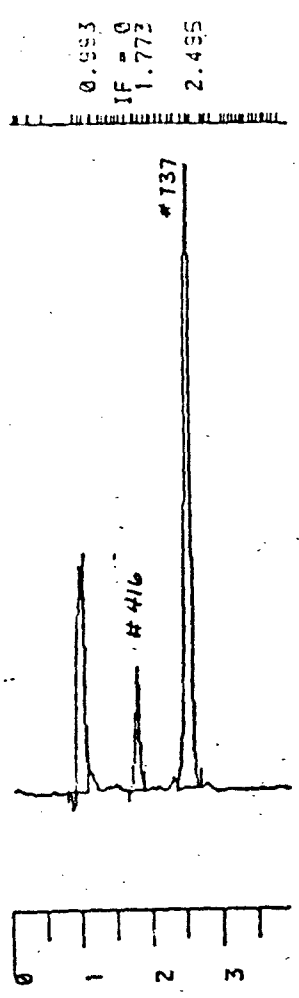


Fig. 4. Chromatogram of extracted plasma, #416 concentration (100 ng/ml), 12.5 ng #416 on column

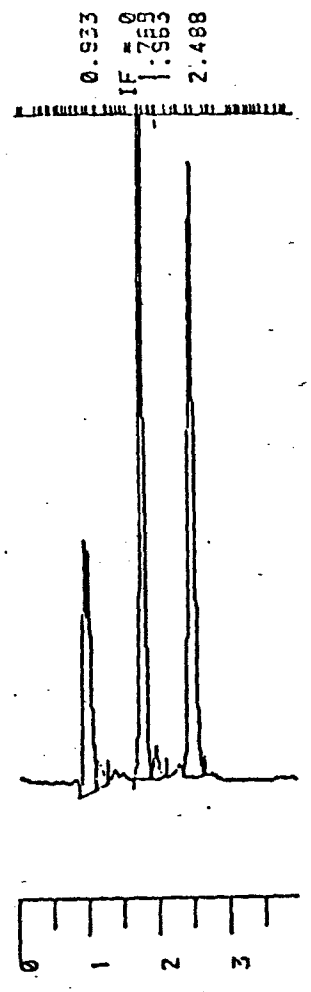


Fig. 5. Chromatogram of extracted plasma, #416 concentration (800 ng/ml), 100 ng #416 on column

Fig. 6. Chromatogram of extracted blank plasma

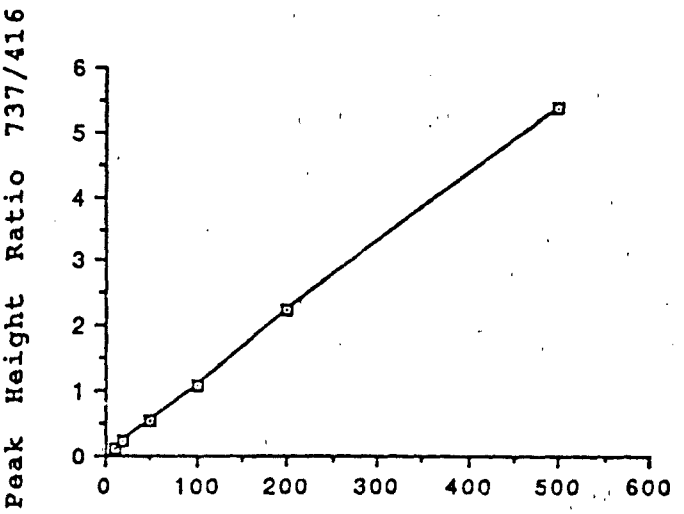


Fig. 7. Standard Curve I

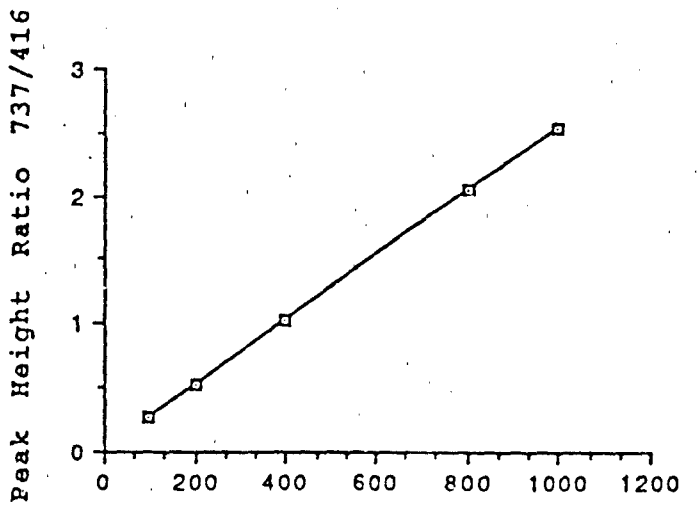


Fig. 8. Standard Curve II

TABLE 1: PREPARATION OF SAMPLES FOR STANDARD CURVES

Conc. of WR 254,416 ng/ml plasma	ng on column	µl of WR 254,416	µl of WR 255,737	plasma (ml)
		Standard Curve I[a]		
10	2.5	10(B)	100(B)	1
20	5.0	20(B)	100(B)	1
50	12.5	50(B)	100(B)	1
100	25.0	100(B)	100(B)	1
200	50.0	20(A)	100(B)	1
500	125.0	50(A)	100(B)	1
		Standard Curve II[b]		
100	12.5	10(A)	80(A)	1
200	25.0	20(A)	80(A)	1
400	50.0	40(A)	80(A)	1
800	100.0	80(A)	80(A)	1
1000	125.0	100(A)	80(A)	1

[a]50-µl injection
[b]25-µl injection

TABLE 2: LINEARITY OF WR 254,416 IN PLASMA

Standard Curve I

Concentration (ng/ml)	Peak Ht Ratio 416/737	SD	CV
10	0.180	±0.0059	3.3
20	0.3665	0.0135	3.7
50	0.8938	0.0243	2.7
100	1.782	0.0572	3.2
200	3.604	0.0391	1.1
500	8.704	0.3131	3.6

n = 6; Slope = 0.017; Intercept = 0.036;
Correlation coefficient = 0.9999.

Standard Curve II

Concentration (ng/ml)	Peak Ht Ratio 416/737	SD	CV
100	0.216	±0.003	1.6
200	0.405	0.018	4.4
400	0.817	0.029	3.5
800	1.628	0.048	2.9
1000	2.043	0.049	2.4

n = 6; Slope = 0.016; Intercept = 0.004;
Correlation coefficient = 0.9999.

SD = Standard deviation
CV = (SD/Mean) x 100

TABLE 3 INTERDAY PRECISION

Sample Number 1	2	3	4	5	6	Mean conc.	SD	CV
Assay I								
10 ng/ml[a]								
8.8	9.2	9.0	8.3	8.6	8.6	8.8	±0.32	3.6
20 ng/ml								
20.0	20.3	20.2	18.5	18.9	18.8	19.5	0.80	4.1
50 ng/ml								
51.0	51.0	50.9	48.6	48.0	48.7	49.7	1.4	2.8
100 ng/ml								
105.6	102.1	102.5	99.0	97.5	97.3	100.7	3.3	3.3
200 ng/ml								
207.8	203.6	203.3	207.7	202.9	205.9	205.2	2.23	1.1
500 ng/ml								
526.7	500.4	474.9	498.6	483.1	503.5	497.9	18.0	3.6
Assay II								
100 ng/ml								
105.4	103.9	105.9	101.9	100.9	103.9	103.6	±1.9	1.9
200 ng/ml								
207.2	204.7	199.3	180.1	191.9	198.8	197.0	9.8	5.0
400 ng/ml								
413.1	409.7	403.3	383.6	376.8	410.7	399.5	15.5	3.9
800 ng/ml								
824.6	807.4	808.4	800.5	748.4	798.1	797.9	25.9	3.3
1000 ng/ml								
1033	1018	1000	1007	956	997	1002	26.2	2.6

[a]Spiked concentration

TABLE 4: INTRADAY PRECISION

Sample Number						mean		
1	2	3	4	5	6	conc.	SD	CV
Assay I								
20 ng/ml[a]								
20.5	20.4	20.9	20.0	19.6	19.9	20.2	±0.47	2.3
400 ng/ml								
415.5	408.7	419.1	417.5	416.2	422.0	416.5	4.5	1.1
Assay II								
200 ng/ml								
206.7	200.7	194.4	195.4	204.2	200.3	200.3	4.8	2.4
800 ng/ml								
802.1	781.8	792.8	774.6	802.1	773.5	787.8	13.0	1.7

[a]Spiked concentration

TABLE 5: ACCURACY OF WR 254,416 DETERMINATION IN PLASMA

Sample Number	Prep'd Conc.	Meas Conc.	Mean	SD	CV	Bias[a]
4	16.5	16.9				
5		16.2	15.9	0.8	5.1	3.6
9		15.1				
12		15.4				
1	45.0	45.1				
3		46.3	44.7	1.5	3.4	0.7
8		43.8				
10		43.1				
2	305.0	313.8				
6		300.6	307.35	5.6	1.8	0.8
7		309.2				
11		305.8				

[a]Bias = [(Measured - Prepared) / Prepared] x 100

TABLE 6: RECOVERY OF WR 254,416 FROM PLASMA

Sample Number	Recovery[a] of WR 254,416	Recovery[b] of WR 255,737
Assay I Concentration: 10 ng/ml		
1	87.4	95.4
2	108.5	103.1
3	84.0	93.4
4	97.2	98.9
5	81.1	86.2
6	95.8	99.5
7	88.8	93.1
8	80.7	82.7
9	91.2	92.4
10	83.6	92.3
11	81.0	91.8
Mean±SD	89.0±8.7	93.5±5.8
CV[c]	9.7	6.2
Assay I Concentration: 20 ng/ml		
1	102.8	100.0
2	103.9	98.9
3	103.3	98.3
4	86.5	94.0
5	88.2	94.8
6	99.1	96.6
7	93.4	91.9
8	94.2	92.9
9	96.6	93.2
10	90.5	91.2
11	93.7	96.0
12	96.3	97.1
Mean±SD	95.7±5.8	95.4±2.8
CV	6.0	3.0
Assay I Concentration: 50 ng/ml		
1	90.8	92.5
2	97.1	102.9
3	96.1	103.0
4	90.5	94.9
5	88.2	93.2
6	94.7	99.8
Mean±SD	92.9±3.5	97.7±4.8
CV	3.8	4.9

TABLE 6 (cont.): RECOVERY OF WR 254,416 FROM PLASMA

Sample Number	Recovery[a] of WR 254,416	Recovery[b] of WR 255,737
Assay I Concentration: 100 ng/ml		
1	100.5	97.1
2	94.9	104.8
3	94.9	101.8
4	88.2	93.7
5	84.3	91.5
6	92.0	99.0
Mean±SD	92.5±5.7	98.0±5.0
CV	6.1	5.1
Assay I Concentration: 200 ng/ml		
1	93.7	96.9
2	96.3	102.7
3	88.1	100.9
4	88.0	93.5
5	85.4	92.6
6	95.1	100.2
Mean±SD	91.1±4.5	97.8±4.1
CV	4.9	4.2
Assay I Concentration: 500 ng/ml		
1	94.4	93.4
2	87.2	98.4
3	89.4	103.8
4	97.2	102.8
5	80.0	88.1
Mean±SD	89.6±6.7	97.3±6.6
CV	7.5	6.7

TABLE 6 (cont.): RECOVERY OF WR 254,416 FROM PLASMA

Sample Number	Recovery[a] of WR 254,416	Recovery[d] of WR 255,737
Assay II Concentration: 400 ng/ml		
1	87.0	93.1
2	87.5	96.3
3	88.4	94.8
4	86.1	92.7
5	87.4	94.4
6	90.6	96.6
Mean±SD	87.8±1.5	94.7±1.6
CV	1.7	1.7
Assay II Concentration: 800 ng/ml		
1	84.2	89.5
2	90.5	87.7
3	87.1	85.8
4	89.7	88.6
5	88.2	91.7
6	89.6	89.2
Mean±SD	88.4±2.1	88.8±2.0
CV	2.4	2.2
Assay II Concentration: 1000 ng/ml		
1	82.2	85.8
2	89.3	83.6
3	86.6	91.6
4	89.7	91.1
5	87.5	89.0
Mean±SD	87.1±3.0	88.2±3.4
CV	3.4	3.0

[a] Recovery = (Peak ht. in plasma/Peak ht. in water) x 100
[b] Internal Standard #737 concentration: 100 ng/ml
[c] CV = (SD/Mean) x 100
[d] Internal Standard #737 concentration: 800 ng/ml

TABLE 7: STABILITY OF WR 254,416 IN PLASMA AT -15°C.

	Plasma Concentration (ng/ml)					
Day	Prep'd	Meas'd	Prep'd	Meas'd	Prep'd	Meas'd
	13.0		100.0		400.0	
0		12.9		99.5		392.5
1		11.4		106.9		423.5
2		12.4		93.5		373.2
7		13.3		92.8		357.8
11		13.3		95.8		374.1
21		12.1		94.8		370.1
29		11.8		94.0		369.1
42		12.1		91.1		370.7
56		13.2		94.8		379.0
84		9.9		94.2		371.5
132		12.8		96.4		380.2
161		11.1		92.2		361.4
Mean±SD		12.2±0.98		95.5±4.01		376.9±16.41
CV[a]		8.1		4.2		4.3
Bias[b]		6.1		4.5		5.8

[a]CV = (SD/Mean) x 100
[b]Bias = [(Measured - Prepared)/ Prepared] x 100

TABLE 8: STABILITY OF WR 254,416 IN PLASMA AT -80°C.

	Plasma Concentration (ng/ml)					
Days	Prep'd	Meas'd	Prep'd	Meas'd	Prep'd	Meas'd
	13.0		100.0		400.0	
0		12.9		99.5		392.5
1		11.8		97.0		411.8
2		12.7		95.0		376.8
7		13.9		96.7		378.8
11		13.4		98.6		394.7
21		12.4		97.9		382.7
29		11.8		98.4		375.3
42		12.4		96.3		372.7
56		13.4		97.7		381.9
84		11.0		94.1		387.7
132		12.8		99.2		399.5
161		11.1		93.0		375.2
Mean±SD		12.5±0.87		97.0±1.95		385.8±11.33
CV[a]		7.0		2.0		2.9
Bias[b]		4.1		3.0		3.6

[a]CV = (SD/Mean) x 100
[b]Bias = [(Measured-Prepared)/Prepared] x 100

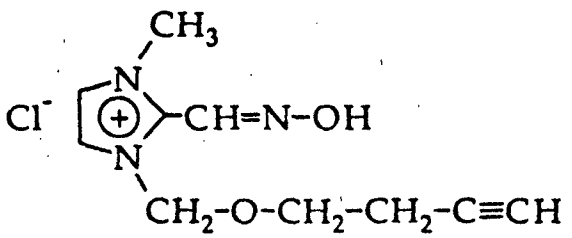
Appendix I: CHEMICAL DATA

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

LAIR code number: TP74

Walter Reed code number: WR 254,416

Chemical structure:



Molecular formula: $C_{10}H_{14}N_3O_2Cl$

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}.[1]

NMR: (300 MHZ, D_2O) α 2.39 (S,1H $-C\equiv CH$), 2.48 (M,J=3.8,2H, $OCH_2CH_2C\equiv$) 3.52 (t,J=6.0,2H,$OCH_2CH_2C\equiv$), 3.98 (S,2H,NCH_3), 5.77 (S,2H,NCH_2O-), 7.92 (d,J=2.1,1H, aromatic proton meta to NCH_2OCH_2-); 8.03 (d,J=2.1,1H, aromatic proton ortho α;NCH_2OCH_2-) 8.53 (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 NaH_2PO_4, 0.0025 M tetramethylammonium hydrogen sulphate, pH adjusted to 3 with H_2SO_4), 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

[1]Wheeler 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.
[2]Wheeler CR. Toxicity testing and antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.5, pp 15-16. 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 72. Letterman Army Institute of Research, Presidio of San Francisco, CA.

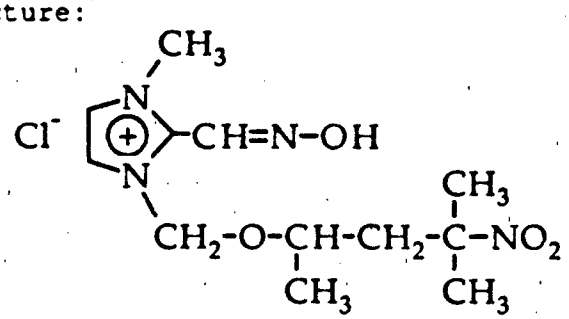
Appendix II: CHEMICAL DATA

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 $OCH(CH_3)C(CH_3)_2NO_2$), 1.41 (S,6H, $OCH(CH_3)C(CH_3)_2NO_2$) 3.97 (S,3H,NCH_3), 4.21 (m,J=6.3, 1H,$OCH(CH_3)C(CH_3)_2NO_2$), 5.76 (m,J=1.1,2H,NCH_2O-), 7.91 (S, 1H, aromatic proton meta to NCH_2O), 8.02(d,J=1.8,1H, aromatic proton ortho to NCH_2O) 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 NaH_2PO_4, 0.0025 M tetramethylammonium hydrogen sulfate, pH adjusted to 3 with H_2SO_4), 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 #85-12-024.5, 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.

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