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Gas Chromatographic–Mass Spectrometric Determination of British Anti-Lewisite in Plasma

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Abstract

British anti-Lewisite (BAL) (2,3-dimercapto-1-propanol) is a potential therapeutic compound when used against the effects of cutaneous sulfur mustard, and a method for its determination in plasma has been developed. BAL and the internal standard (IS) ethane dithiol were isolated from plasma samples through solid-phase extraction and then reacted with 1-pentafluoropropionylimidazole, forming stable pentafluoropropionyl derivates that are sensitive to gas chromatographic–mass spectrometric analysis. Examination of concentration versus peak-area ratios of the BAL and IS derivatives demonstrated the method to be linear over a concentration range of 0.48 to 124 ng/mL in plasma when fit to a weighted ($1/y^2$) least-squares regression. Correlation coefficients were 0.9943 to 0.9995 for six runs, and coefficients of variation (CV) were 2.5 to 8.7% over the eight concentrations tested. The intra- and interday accuracy and precision of this method was measured by examining six groups of eight unknown test samples ($n = 6$). Intraday accuracy, as expressed by percent error, was found to range from –15.4 to 0.21%, whereas the precision, expressed as %CV, was less than 9.8% over all sample concentrations. Interday test unknown sample results were similar in that the accuracy was shown to be –7.1 to 0.4%, and precision was 4.7 to 9.5%. BAL levels in frozen plasma (–70°C) remained constant for more than 14 days with a CV of less than 10% for the eight concentrations tested. The data indicate that the method will provide accurate and precise determination of BAL at concentrations down to approximately 1 ng/mL in plasma. This procedure has been applied to determine preliminary time-concentration profile studies of BAL in the hairless guinea pig.

Introduction

British anti-Lewisite (BAL), or 2,3-dimercapto-1-propanol, was developed prior to the end of World War II for treatment against Lewisite gas exposure and is still used clinically as a chelating agent against acute heavy metal poisoning (1). The mechanism of this chelation is based on the formation of a stable water-soluble cyclic complex with heavy metals (arsenite, As^{+3}; in the case of lewisite poisoning), allowing excretion from the body. For the most part, disposition studies following the treatment of heavy metal intoxication focus on examining the elimination of stable BAL-heavy metal complexes. However, little is known about the concentration-time course of the free drug in plasma. Recent studies have indicated that BAL administration has the potential to reduce dermal inflammation subsequent to sulfur mustard exposure (2). To better understand the relationship between the efficacy of BAL and plasma concentrations, the pharmacokinetics of the compound need to be examined and characterized.

Dithiols such as BAL are difficult to analyze in plasma because of extensive binding to proteins resulting from the rapid formation of disulfide bridges with thiol groups of cysteine residues (3). Research involving two water-soluble analogues of BAL, 2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercaptopropane-1-sulfonic acid (DMPS), have utilized dithiothreitol (DTT) to cleave the disulfide bridges these dithiols form with plasma proteins. These reduced and unbound BAL-analogues can then be extracted from plasma and subsequently derivatized, utilizing the reactive dithiol moieties (4). Additional research involving detection of BAL in biological matrices is retrospective biomonitoring for Lewisite exposure. These studies exploit the formation of the Lewisite hydrolysis product 2-chlorovinylarsonous acid (CVAA). British anti-Lewisite quickly forms a stable CVAA-BAL complex that can be isolated from biological samples with solid-phase extraction (SPE) and subsequently derivatized with heptafluorobutyryl imidazole (HFBI) (5). This volatile HFBI derivative of the CVAA-BAL complex was then analyzed with gas chromatography–mass spectrometry (GC–MS) separation and detection.

Herein is presented a novel GC–MS method developed for the detection of unbound BAL in plasma. We used DTT reduction and subsequent SPE to remove the free drug from plasma. This was followed by reaction with pentafluoropropionylimidazole (PFPI) to form a pentafluoropropionyl derivative of BAL. Analysis of this stable BAL derivative was conducted with

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GC–MS separation and detection and was shown to be reproducible, selective, and sensitive. This method was also utilized in preliminary time-concentration studies of BAL in hairless guinea pigs.

Experimental

All chemicals and solvents were of HPLC grade or higher. The internal standard, ethane dithiol (EDT), BAL, and DTT were purchased from Sigma-Aldrich Co. (St. Louis, MO). Plasma was separated from swine (*Sus scrofa*) whole blood obtained from Archer Farms Inc. (Darlington, MD). Pentafluoropropionylimidazole was obtained from Sigma Chemical Co. and Pierce Chemical Co (Rockford, IL). Oasis (HLB; Hydrophilic-Lipophilic Balance) SPE Cartridges (30 mg HLB sorbent) were purchased from Waters Corporation (Milford, MA).

Apparatus

GC separations were performed on an Agilent 6890 series II GC equipped with a DB-5MS bonded phase column (30 m × 0.25-mm i.d., 0.25-µm film thickness, J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a column head pressure of 12 psi. The initial oven temperature of 50•C was held for 2 min, then ramped to 150°C at 10°C/min, and finally increased to 280°C at 40°C/min. An Agilent 7673 autosampler introduced 1 µL into a 265°C injection port with splitless injection, and an inlet purge of 50 mL/min was begun 1 min post-injection. The inlet liner was an Agilent deactivated borosilicate single-taper with glass wool packing. The GC transfer line was held at 265°C.

The GC was interfaced with an Agilent 5973N mass selective detector for conducting electron impact MS analysis. The MS analysis was conducted using selected ion monitoring (SIM) mode for both pentafluoropropionyl derivatives of EDT (*m/z* 193, 206, 207) and BAL (*m/z* 203, 219, 382, 383) with a dwell time of 50 ms each, resulting in a total scan time of 6.94 cycles/min. The MS conditions were as follows: ion source pressure approximately 1.5×10^{-5} torr, source temperature 230?C, electron energy 70 eV, electron emission current 50 µA, and the electron multiplier voltage +400 V relative to the autotune setting. The electron multiplier (EM) was increased 400 V above the standard autotune setting to produce an increase in the abundances of the ions monitored. This increase in EM voltage and subsequent greater sensitivity was particularly successful because of the high signal-to-noise ratio evident with this method.

Methods

Sample preparation

Plasma was separated from swine whole blood by centrifugation at 2000 rpm for 30 min and frozen until needed for each experiment. Stock solutions of BAL (12.4 µg/mL) for use in standard curves and the internal standard (IS) EDT (0.725 µg/mL) were prepared in acetonitrile, whereas DTT (100mM) was prepared in deionized water (18 mega ohm). All solutions were stored at 4°C. Dithiothreitol (30 µL) was added to each 255 µL plasma sample with gentle shaking for 1 min. Ethane dithiol (5 µL) was then added with an additional 30 s of shaking, resulting in an IS concentration of 12.5 ng/mL. SPE cartridges were conditioned with 1 mL of methanol followed by 1 mL of water. The resulting 290-µL sample was then split into duplicate 145-µL aliquots that were passed through two separate SPE cartridges at a 1 mL/min flow rate. The SPE cartridges were washed with 1 mL of water and dried by applying vacuum for 20 min (15 in-Hg or 7.4 psi). The samples were eluted from the cartridge with the addition of 750 µL of methylene chloride, further dried with the addition of anhydrous sodium sulfate (approximately 150 mg), and spun at 2000 rpm for 5 min at 5°C. The methylene chloride was decanted into a fresh vial where 10 µL of PFPI acylation reagent was added and shaken for 5 min. The reaction was quenched with the addition of 1 mL of water with 45 s of vigorous shaking. The samples were spun at 2000 rpm for 5 min at 5°C after which the organic layer was removed for GC–MS analysis.

Method optimization

The method was optimized for the recovery of BAL, as measured by area under the curve (AUC) of PFPI-derivatized product subsequent to GC–MS analysis. The two variables examined to accomplish this optimization were the concentration and reaction times of DTT and PFPI. The cleavage of BAL from plasma proteins was addressed by varying both the volume (5, 15, 25, 35 µL) and concentration of DTT added to the plasma samples, and by examining different reaction times (1, 5, 15, 30, 60, 120 min). For PFPI, the volume (5, 10, 15, 20 µL) of PFPI added to the extracted and dried methylene chloride and the optimal reaction times (5, 15, 30, 60, 120 min) were examined.

Standard curves

Standard curves were prepared and analyzed on seven separate days as follows. Plasma (255 µL) spiked with 124 ng/mL BAL was serially diluted with equal volumes of fresh plasma. Final standard curve concentrations of 0.48, 0.97, 1.94, 3.88, 7.75, 15.5, 31.0, 62.0, and 124.0 ng/mL were processed as described in Sample preparation. For each mass chromatogram, the AUC was determined for both BAL and IS peaks. A weighted regression line ($1/y^2$) was generated from the mean AUC ratios (BAL/IS) as a function of actual concentration, and was used to quantify samples with unknown BAL concentrations.

Accuracy and precision

The accuracy and precision of this method was assessed by examining the intra- and interday variability of eight unknown test samples (0.727, 1.45, 2.91, 5.81, 11.63, 23.25, 46.50, and 93.0 ng/mL). For interday studies, plasma test samples were prepared and analyzed each day over a period of six days. Intraday studies used the same concentrations as those for interday experiments; however, all concentrations were prepared and analyzed the same day ($n = 6$). Precision was measured by

calculating the coefficient of variation (CV) for each group of test sample concentrations. Accuracy was expressed as percent error by examining differences between the calculated and the expected concentrations [(calculated – expected)/expected × 100].

Stability

Stability was examined utilizing the same concentrations as those for the standard curve studies. Sample stability was assessed at three stages of sample preparation and under storage conditions of 4°C or frozen at –70°C. The first stage examined the stability of BAL in plasma. Second, the stability of BAL subsequent to SPE in dry methylene chloride was observed. These samples were derivatized as stated in Sample preparation every other day for 14 days. Lastly, the stability of the pentafluoropropionyl derivative of BAL was assessed every other day for 14 days after derivatization.

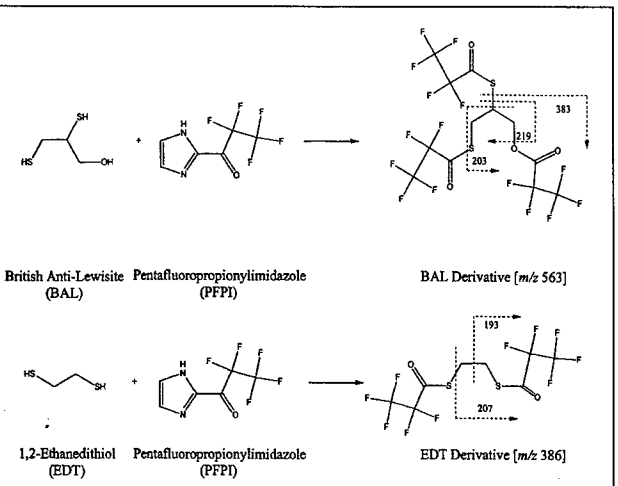

Figure 1. The chemical structures of BAL, EDT, PFPI, and the resulting pentafluoropropionyl derivative products are depicted. The proposed ion fragmentation patterns are also shown.

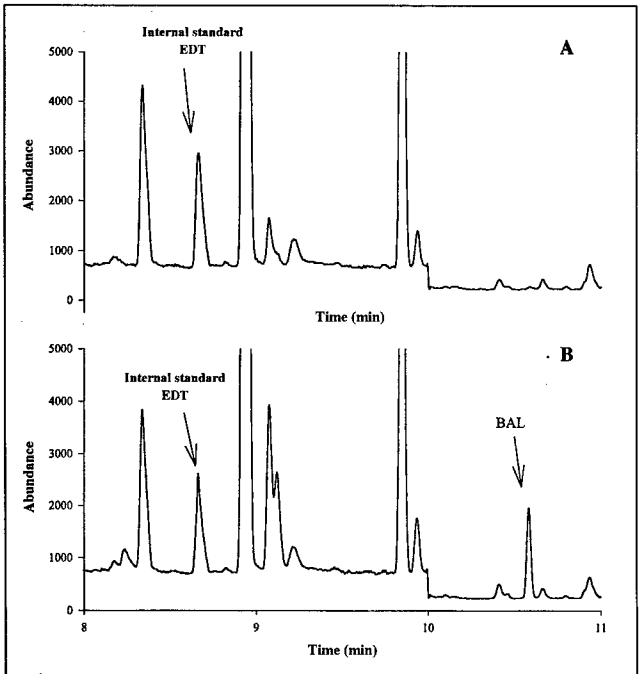

Figure 2. Representative mass chromatograms of BAL and EDT extracted from hairless guinea pig plasma: samples were derivatized with PFPI as described in Sample preparation. (A) Represents initial control blood sample with IS (time 0), (B) represents 120 min after im administration of BAL (0.05 mg/kg) and a resulting concentration of 10.1 ng/mL with IS.

Hairless guinea pig studies

To demonstrate the utility of the assay, preliminary studies examining the concentration-time course of BAL in hairless guinea pigs were performed. In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (NRC 1996). Plasma concentrations of BAL were determined using the described assay following intramuscular (im) administration of the compound. In preliminary experiments, a time-course profile was produced by examining blood samples at 0, 2, 10, 15, 30, 60, and 120 min post-BAL administration for doses of 0.25, 0.5, 2.5, 5.0, 25, and 50 mg/kg (n = 1). Subsequent studies at doses 0.025 and 0.05 mg/kg (n = 3) included additional blood samples at 1, 240, and 360 min. Immediately following the collection of the terminal blood sample, plasma was separated and examined as described in Sample preparation.

Results

The chemical structures for BAL and EDT and the proposed pentafluoropropionyl derivatives of these compounds are presented in Figure 1. A tri-substituted derivate is illustrated in the case of BAL and di-substituted for EDT, along with the proposed fragmentation patterns for each. Figure 2 illustrates a representative mass-chromatogram of BAL and EDT extracted from hairless guinea pig plasma and derivatized with PFPI as described in Sample preparation. The top trace (Figure 2A) represents the initial control blood sample (time 0), and the bottom trace (Figure 2B) represents 120 min after im administration of BAL (0.05 mg/kg) and resulting concentration of 10.1 ng/ml. The chromatogram demonstrates that BAL and EDT are adequately separated and are free from confounding peaks.

Method optimization

Figures 3 and 4 display the relationship between several parameters that were optimized relative to the amount of derivatized BAL recovered from plasma as measured by MS abundance. Figure 3A demonstrates the optimal amount of PFPI added (10 µL) to the extracted and dried methylene chloride. The 10-µL volume was chosen because it resulted in an 8% increase in BAL abundance over the next highest abundance found with 5 µL. The PFPI-BAL derivatization reaction was shown to occur within a relatively short period at room temperature (Figure 3B). The amount of product formed was relatively similar between the 5 and 15 min reaction time. The 30 min time and beyond exhibited either lower levels of derivatized BAL ($p < 0.05$; 30 min), or an increased variability compared to

earlier time points. The 5 min reaction time was chosen for the method because it provided one of the better yields in terms of amount of product formed, along with a short reaction time to expedite sample processing.

The relationship between amount of DTT (microliters of 100mM solution) added to BAL spiked plasma as a function of recovered derivatized BAL is presented in Figure 4A. The addition of increasing volumes of DTT (100mM) produced greater amounts of recovered BAL. The 30-µL volume was chosen because it was the smallest volume that also provided maximum BAL recovery. Similar experiments utilizing higher concentration of BAL indicated no additional increase in recovery but produced interfering peaks in the mass chromatogram in the area of interest. The incubation time for DTT in BAL-spiked plasma as a function of recovered derivatized BAL is presented in Figure 4B. The DTT reaction occurred at 1 min, with abundances at 1, 5, and 15 min being statistically similar. Times beyond 30 min (60 and 120 min) were statistically less than at 1 min ($p < 0.05$). Based on these observations, the shortest reaction time (1 min) was chosen for incubation of the DTT with plasma.

Standard curves

The standard curve ($n = 7$) that demonstrates the mean ratio (BAL/EDT) versus concentration is shown in Figure 5. Data were fit to a weighted ($1/y^2$) least-squares regression analysis ($r^2 = 0.9981$). The method showed linearity over a concentration range of 0.48 to 124.0 ng/mL in plasma with correlation coefficients ranging from 0.9995 to 0.9943 for seven runs and CVs of 2.5 to 8.7% over the eight concentrations tested.

Accuracy and precision

Accuracy and precision data for intra- and interday plasma samples are presented in Table 1. For intraday samples the accuracy, as expressed by percent error, ranged from 0.21 to –15.4%, and the precision, as expressed by %CV, was less than 9.8% over all sample concentrations ($n = 6$). The results of the interday tests of unknown samples were similar in that the accuracy was shown to be 0.4 to –7.1%, whereas precision was 4.7 to 9.5% ($n = 6$).

Stability

The BAL-spiked plasma remained unchanged in each of the eight concentrations tested up to 14 days at either +4°C or –70°C, with CVs less than 10%. Solutions of derivatized BAL and EDT in methylene chloride were also stable for 14 days, with CVs of less than 10% when stored at either +4°C or –70°C. Non-derivatized solutions of BAL and EDT in methylene chloride were stable for up to one week with CVs less than 10% at both +4°C and –70°C, but variation began to increase beyond 1 week. This variation was seen as an increase in the BAL/IS ratio. (Data not shown.)

Hairless guinea pig studies

The mean plasma concentration-time profile for BAL at 0.025 and 0.05 mg/kg in hairless guinea pigs following im administration is presented in Figure 6 ($n = 3$). This plot represents mean concentrations plus or minus the standard error of the mean and illustrates a concentration maximum of 14.7 and

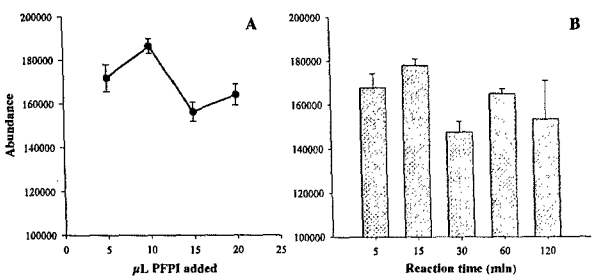

Figure 3. (A) Method optimization examining the volume of pentafluoropropionylimidazole (PFPI) added to the extracted and dried methylene chloride ($n = 6$) and (B) the reaction time of PFPI at room temperature for the derivatization process to be completed ($n = 6$). Both factors are measured as a function of derivatized BAL abundance with associated standard error of mean.

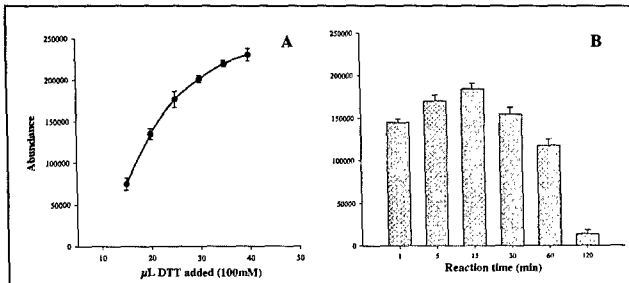

Figure 4. (A) Method optimization detailing the amount of DTT (100mM) added to 255-µL plasma samples that resulted in the greatest BAL recovery and least interference ($n = 6$). (B) Reaction times were studied by adding 30 µL DTT to plasma containing BAL as a function of derivatized BAL abundance with associated standard error of mean ($n = 6$).

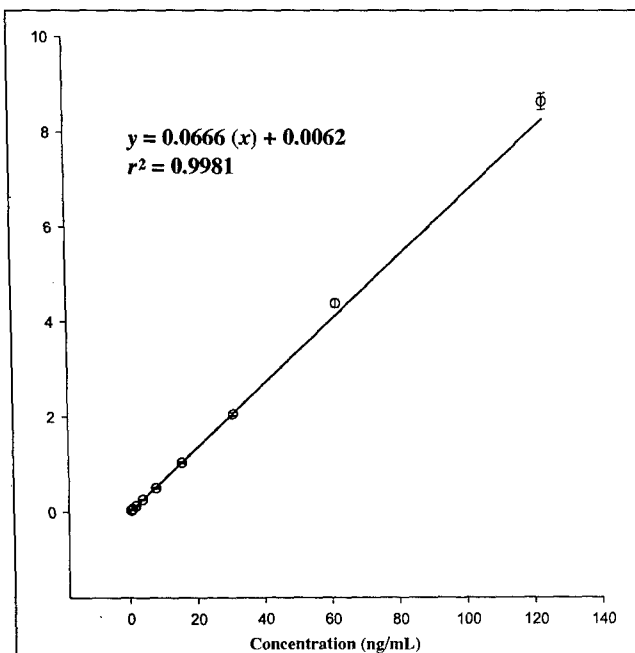

Figure 5. Standard curve with weighted ($1/y^2$) least-squares regression analysis, the resulting equation of the line and correlation coefficient of 0.9981. Data represent mean values of each standard curve ($n = 7$) and the corresponding standard error of the mean.

52.5 ng/mL at a time of 5 min for 0.025 and 0.05 mg/mL, respectively. A dose response was also observed in additional preliminary studies examining 0.25, 0.5, 2.5, 5.0, 25, and 50 mg/kg administered im (n = 1). (Data not shown.)

Discussion

British scientists first synthesized BAL in 1940, yet there is little published research on the pharmacokinetics of the free drug in plasma. Investigative efforts have historically focused on the elimination of BAL-heavy metal complexes that are formed due to the chelating properties of the drug (5,6). Recently, BAL has demonstrated potential in reducing sulfur mustard-induced dermal inflammation (2). Knowledge of the time-course of the free drug would help elucidate doses and subsequent plasma levels needed for efficacy. The determination of BAL in plasma presents a unique series of challenges that must be overcome in developing a sensitive, selective, and reproducible GC–MS assay. The method must extract the previously protein-bound drug from plasma, isolate it, and then create a volatile derivative amenable to GC–MS analysis.

BAL appears to be completely protein bound in plasma. This was supported by the observation that when DTT was not added to the samples, no BAL was recovered. Dithiothreitol has been used previously to overcome the characteristic binding of thiols to the cysteine residues of plasma proteins (3,4,7). These earlier reported assays targeted two water-soluble analogues of BAL, DMPS and DMSA, reducing the dithiol bonds resulting in free drug in plasma. The GC–MS method described herein applies this concept to the reduction of the BAL-protein complex allowing straightforward SPE of the analyte along with the added IS, EDT. With the analyte bound to the HLB sorbent material, it can then be eluted and reacted under anhydrous conditions with PFPI. Although PFPI undergoes rapid hydrolysis in aqueous conditions, the resulting PFPI-BAL derivative is stable. Previous assays employed a liquid–liquid extraction from urine coupled with derivatization of DMPS with the fluorophore bromobimane to render a fluorescence product for separation via HPLC (4). The sensitivity of the fluorescence detection of this bromobimane derivative has been reported as 1µM in urine. In contrast, the BAL-PFPI derivative reported herein is detectable to less than 1nM in plasma with selectivity unique to MS detection.

The derivatization of BAL with PFPI produces a tri-substituted compound with a corresponding molecular weight of *m/z* 563. Single ion monitoring focused on four fragments at *m/z* 219, 203, 382, and 383 (7.7:6.7:1.3:1) providing the highest abundance with little or no interference at a retention time of 10.6 min. The pentafluoropropionyl derivatization at both thiol groups of EDT yielded a product with a parent ion at *m/z* 386. This had a characteristic fragmentation pattern with predominant ions of *m/z* 207, 206, and 193 (9.5:6:1). The derivatization reaction occurs at room temperature within 5 min, whereas earlier investigations with HFBI derivatization required heating to 50°C for 60 min (5). In this earlier research, the CVAA-BAL-HFBI complex led to the formation of two isomers due to the chiral arsenic center in CVAA. The tri-substituted BAL derivative however, absent of any stereoisomers, resulted in a single detectable conformation. The limit of detection for the CVAA-BAL-HFBI derivative was also previously reported at 20 pg on column, whereas the PFPI-BAL derivative limit of detection was 0.12 pg on-column (0.12 ng/mL; 1 µL injected).

The 10 µL addition of PFPI represents the amount added to the 255-µL sample returning the greatest abundance of BAL and equals an excess greater than 100 times the molar equivalence of PFPI when compared with BAL at the 124.0 ng/mL concentration. In addition to BAL, PFPI also reacts with the two thiol positions of EDT, as well as with the DTT that is added in large excess to the plasma to reduce the dithiol bonds. The PFPI reaction times of 5–60 min provided equal BAL abundance and similarly consistent results; however, the time of 5 min was chosen due to time constraints in preparing the quantity of samples within experiments. The 120 min time was found to have a significant ($p < 0.05$) drop in BAL abundance, when compared with 5 min, with a concomitant increase in variability. The concentration of DTT is similar to previously published methods utilizing the reducing agent (8), and the volume represents the smallest amount that yields consistent and maximum BAL abundance but alleviates any confounding peaks that begin to occur at higher levels. The time required for DTT to reduce the dithiol bonds in the BAL-exposed plasma seemed to occur at less than 1 min, and beyond 60 min resulted in lower BAL recovery and greater variability.

The standard curves generated utilizing this method were linear across three orders of magnitude (0.48–124 ng/mL). The data were fit to a weighted regression due to the proportionally small SEM evident at lower concentrations of the standard curve, in contrast to the relatively

Table I. The Precision and Accuracy of the Method*

	Intraday			Interday		
Expected Concentration	Mean Calculated Concentration (ng/mL)	Precision CV (%)	Accuracy Error (%)	Mean Calculated Concentration (ng/mL)	Precision CV (%)	Accuracy Error (%)
0.727	0.728	7.0	0.21	0.68	7.4	–6.1
1.45	1.24	9.8	–14.6	1.26	8.9	–5.9
2.91	2.55	5.3	–12.1	2.43	8.6	–6.2
5.81	5.21	8.6	–10.3	5.28	4.7	–5.3
11.63	10.38	8.9	–10.7	10.07	9.5	–6.1
23.25	19.60	2.9	–15.4	20.52	6.2	–7.1
46.50	40.77	6.4	–12.3	41.82	8.1	–3.8
93.0	85.62	7.9	–7.9	87.89	7.2	0.4

* For interday studies, plasma test samples were prepared and analyzed each day over a period of six days. Intraday studies used the same concentrations as those for interday experiments; however, all concentrations were prepared and analyzed the same day. Precision was measured by calculating the coefficient of variation (CV) expressed as a percentage for each group of test sample concentrations. Accuracy was expressed as percent error by examining differences between the calculated and the expected concentrations [(calculated – expected)/expected x 100].

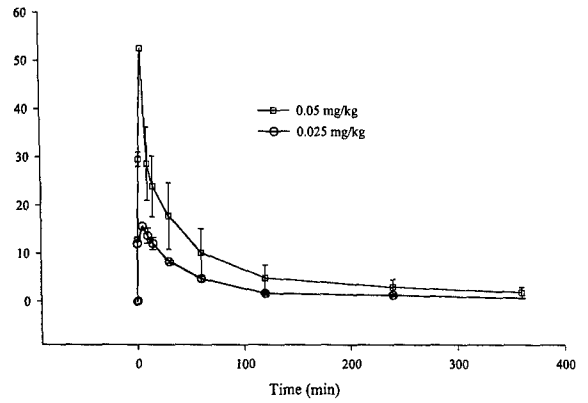

Figure 6. Plot represents studies investigating the concentration-time profile of BAL after im administration in hairless guinea pigs ($n = 3$). Hairless guinea pigs received 0.05 or 0.025 mg/kg at time 0; afterwards serial blood samples were obtained and treated as described in Sample preparation.

elevated associated SEM of data at higher concentrations. The weighting factor of $1/y^2$ results in the equation of the line closely approximating data points with the smallest associated error, allowing more accurate results for predicted concentrations when compared with unweighted least-squares regression analysis (9).

The precision of this method was demonstrated with the CVs calculated for both intra- and interday samples. Both data sets show that CVs are below 10% across all concentrations tested. The percent error was lowest among the interday group at less than 7.1%, and the intraday showed a percent error of almost double these figures. This is surprising given the precision shown of these same data. This can be attributed to, in part, a dilution error that was transferred down the concentrations prepared within that day. These data indicate that the assay will provide reproducible and accurate results as reflected by the respective %CV and percent error determined in these studies.

It has been reported that BAL is unstable when stored in distilled H_2O and retains no titratable mercapto groups after seven days (10). Data from these studies indicate that BAL standards remained stable when stored in acetonitrile diluent for at least 21 days. Levels of BAL also remain steady in frozen plasma for greater than 14 days with an associated CV of less than 10%, perhaps due in part to stabilizing dithiol bonds with the plasma proteins. When derivatized BAL and EDT were stored in methylene chloride they also remained stable for 14 days. When BAL and EDT were left underivatized, the CV increased to greater than 10% beyond five days because of decreasing abundance for BAL, when compared with EDT, causing increased changes in the BAL/EDT ratio.

The utility of this assay has been demonstrated by examining the concentration-time profile of BAL following im administration to hairless guinea pigs. In these preliminary BAL administration studies, plasma levels indicated a dose-response among the single experiments conducted at each dose of 50, 25, 5.0, 2.5, 0.5, and 0.25 mg/kg ($n = 1$), and also at 0.05 and 0.025 mg/kg levels ($n = 3$). British anti-Lewisite was rapidly absorbed and produced maximal plasma levels at 5 min following im administration. These hairless guinea pig maximal plasma concentrations ranged from 43.3 and 14.7 µg/mL for doses of 50 and 25 mg/kg, to 52.5 and 15.6 ng/mL for doses of 0.05 and 0.025 mg/kg, respectively. The drug levels were still within detectable limits 360 min following BAL administration.

Conclusions

A novel GC–MS method for the detection of BAL in plasma has been developed. The method has been shown to be reproducible, selective, and sensitive for the PFPI derivative of BAL. The utility of the method has been demonstrated by examining the concentration-time profile of BAL using serial blood sampling after im administration to hairless guinea pigs.

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

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