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Analysis of enloplatin by liquid chromatography and of platinum by atomic absorption spectrometry in various biological fluids

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Abstract: Analytical methods were developed and validated for the determination of enloplatin (an anticancer agent) in plasma by reversed-phase LC and for platinum (an elemental component of enloplatin) in plasma, plasma ultrafiltrate (PUF) and whole blood by flameless atomic absorption spectrometry (FAAS). The LC procedure involved protein precipitation with dilute perchloric acid. The supernatant was mixed with sodium phosphate buffer and injected into the LC system. A C_{18} or a cyano column was used, depending on sample matrix, with UV detection at 230 nm. The LC method was linear from 0.50 to 50.0 μg ml^{-1}. Inter-day and intra-day precision (RSD%) and accuracy (relative error%) were $<\pm 14\%$. The FAAS procedure utilized a graphite furnace, a hollow cathode platinum (Pt) lamp, and Zeeman background correction. An aliquot of plasma, PUF, or whole blood was mixed with a solution of Triton X-100 and Antifoam-B and injected into the FAAS system. The FAAS method showed goodness of fit from 0.05 to 10.0 μg Pt/ml. Inter-day and intra-day precision and accuracy were $<\pm 15\%$. The methods were developed to support pharmacokinetic studies in humans, dogs and rats.

Keywords: *Enloplatin; platinum; reversed-phase LC; flameless atomic absorption spectrometry (FAAS); assay validation.*

Introduction

Enloplatin is a water soluble platinum (Pt) compound ([1,1-cyclobutanedicarboxylato (2-)-O,O′] (tetrahydro-4H-pyran-4,4-dimethamine-N,N′) platinum) exhibiting anticancer properties (Fig. 1). It is generally believed that platinum coordination complexes direct their cytotoxic effects by targeting DNA [1–4]. Liquid chromatography has been used to quantitate unchanged platinum complexes such as cisplatin and carboplatin [5–7], whereas atomic absorption has been used to determine total elemental platinum from parent drug and metabolites in various biological fluids [8, 9]. To support preclinical enloplatin studies in rat and dog, and clinical trials for refractory lymphoma, chronic lymphocytic leukaemia, and refractory advanced ovarian carcinoma, specific methods were developed to quantitate unchanged enloplatin in plasma and total elemental platinum in plasma, plasma ultrafiltrate (PUF) and whole blood. This paper describes the reversed-phase LC method that was developed and validated for the analysis of enloplatin in rat, dog and human plasma and the flameless atomic absorption spectrophotometric (FAAS) method that was developed and validated to determine the total Pt concentration in the plasma and PUF from human, rat and dog, and in human whole blood. The detailed analytical methods and the validation results are presented.

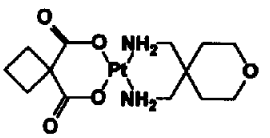

Figure 1
Molecular structure of enloplatin.

Experimental

Materials

Enloplatin was obtained from American Cyanamid Co. (Pearl River, NY). Ammonium

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dihydrogen phosphate monobasic ($NH_4H_2PO_4$), sodium dihydrogen orthophosphate monobasic (NaH_2PO_4), perchloric acid (all ACS grade), Triton X-100, Antifoam-B and ammonia solution (28.0–30.0%) were purchased from BDH Inc. (Toronto, Ontario, Canada). Sodium hydroxide (5 N, certified) and 2-propanol (optima) were obtained from Fisher Scientific (Nepean, Ontario, Canada). Platinum reference solution (certified 1000 μg ml^{-1}) and ammonium hexachloroplatinate (IV) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Centrifree micropartition cartridges were obtained from Amicon (part No. 4104, Beverly, MA). Heparinized rat and dog plasma were purchased from Rockland (Gilbertsville, PA) and Buckshire Corp. (Perkasie, PA), respectively. Human plasma and blood were purchased from Biological Speciality (Lansdale, PA). Water was deionized through the Millipore Type I Filtering System, Millipore Corp. (Bedford, MA).

Reagents

Plasma protein precipitation solution: 6 ml of perchloric acid (commercially available as 70% solution) was added to a 100-ml volumetric flask and diluted to volume with water.

Ammonium dihydrogen phosphate monobasic (0.01 M) was prepared in deionized water.

Sodium dihydrogen orthophosphate monobasic (1 M, pH 7.5) was prepared in deionized water and the pH was adjusted with the dropwise addition of sodium hydroxide (5 N).

Ammonium hydroxide (0.84–0.90%) was prepared by adding 3.0 ml of the concentrated ammonia solution to a 100-ml volumetric flask and diluted to volume with deionized water.

Triton X-100 and Antifoam-B solution (0.4%) was prepared by adding 2 ml of Triton X-100 and 2 ml of Antifoam-B to a 500-ml volumetric flask and diluted to volume with deionized water.

Triton X-100 and Antifoam-B (0.2%) solution was prepared as above using 1 ml of each. The Triton X-100 and Antifoam-B solutions were passed through a 1-μm microfiber filter prior to use.

Preparation of PUF from plasma

An aliquot (400 μl) of plasma was pipetted into each Centrifree micropartition cartridge and centrifuged at 3000 rpm for 1 h at 20°C. Alternatively, if a 35° fixed angle rotor is available, the preferred method would be to centrifuge at 3500 rpm (1500*g*) for 15 min at 20°C. The PUF yield is about one-half the volume of plasma added to the Centrifree micropartition cartridge.

LC calibration standards and quality control (QC) samples in rat, dog and human plasma

Stock solutions of enloplatin were prepared in water at concentrations of 1.0, 0.1, and 0.01 mg ml^{-1}. To make the desired concentrations of calibration standards and QCs, volumes of the appropriate stock solution were added to control plasma. The volume of stock solution added to control plasma did not exceed 10% of the total volume. Calibration standards at 7 or 8 concentrations (range 0.50–50.0 μg ml^{-1}), depending on the sample matrix, were used to generate calibration curves. The QC concentrations were 1.00 or 2.00, 7.50, 15.0 and 40.00 μg ml^{-1} in rat and dog plasma and 0.50, 1.50, 30.0 and 40.0 μg ml^{-1} in human plasma.

FAAS calibration standards and QC samples

Platinum stock solution (1000 μg ml^{-1}) was used as supplied.

Enloplatin stock solution (1000 μg Pt/ml) was prepared by dissolving 63.08 mg of enloplatin (97.8% purity) in 25.0 ml of deionized water.

Ammonium hexachloroplatinate (IV) stock solution (1000 μg Pt/ml) was prepared by dissolving 22.75 mg in 10.0 ml of deionized water.

Calibration standards were prepared by adding appropriate volumes of platinum stock solution to control human biological fluid (plasma, PUF, or whole blood). QC samples were prepared by adding appropriate volumes of enloplatin stock solution to biological fluids from human, rat, and dog. As with the LC calibration standards and QCs, the volume of stock solution added to the control biological fluid did not exceed 10% of the total volume. Plasma and PUF standards were prepared with platinum stock solutions whereas whole blood standards were prepared with ammonium hexachloroplatinate (IV) stock solutions to prevent blood precipitation and agglutination.

For analysis of total Pt in plasma and PUF, two separate calibration curves were used. The low range calibration set spanned the concentration range of 0.05–1.00 μg ml^{-1}. The high

range calibration set spanned the concentration range of 0.50–10.0 μg ml^{-1} for human, dog and rat PUF or 1.00–10.0 μg ml^{-1} for human, dog and rat plasma. For human whole blood, the concentration range was 0.10–10.0 μg ml^{-1}. For human, dog and rat PUF, the low range QC concentrations were 0.08, 0.50 and 0.80 μg ml^{-1} and the high range concentrations were 0.80, 5.00 and 8.00 μg ml^{-1}. For human, dog and rat plasma, the low range QC concentrations were 0.15, 0.31, 0.51 and 0.92 μg ml^{-1} and the high range concentrations were 3.05, 5.08, 7.12 and 9.15 μg ml^{-1}. For human whole blood, the QC concentrations were 0.10, 0.30, 3.00 and 8.00 μg ml^{-1}.

LC procedures

Sample preparation. Plasma protein precipitation solution (300 μl) was added to each 300 μl aliquot of the calibration standards and QCs. The samples were mixed and centrifuged for about 6 min at approximately 3000 rpm. An aliquot (300 μl) of the supernatant was transferred to an autosampler vial followed by the addition of 300 μl of monobasic sodium phosphate (pH 7.5, 1 M). The samples were mixed and 50 μl was injected for rat samples or 100 μl for dog and human samples.

LC system and conditions. An isocratic LC system was used for analysis of rat plasma. The LC system consisted of an ISO Chrom LC pump (Spectra–Physics Analytical, (San Jose, CA), a SP 8875 autosampler (Spectra–Physics), a SP 8760 autosampler cooler maintained at 4°C (Spectra–Physics), a Waters 484 UV detector operated at 230 nm (Waters Assoc., Inc., Milford, MA), a ChromJet integrator (Spectra–Physics) and a LABNET data acquisition system (Spectra–Physics). Chromatographic separation was achieved on a Hypersil-ODS column (250 mm × 4.6 mm i.d., 5 μm particle size) supplied by CSC Scientific (Fairfax, VA). The mobile phase was a mixture of ammonium phosphate (0.01 M) and 2-propanol (99:1, v/v) pumped at a flow rate of 1.5 ml min^{-1}. The retention time of enloplatin was approximately 15 min and the chromatographic run time was 17 min.

Due to interferences, a column switching method was used for the determination of enloplatin in dog and human plasma. This LC setup consisted of the same equipment as above except for an additional pump (model A-60-S, Eldex Laboratories, San Carlos, CA), and a VICI switching valve (Vici Valco Instruments, Houston, TX). Chromatographic separation for dog plasma was achieved on a nucleosil C_{18} precolumn (150 × 4.6 mm i.d., 3 μm particle size) and a cyano analytical column (150 × 4.6 mm i.d., 5 μm particle size) both supplied by CSC. The mobile phase for pump A was monobasic ammonium phosphate (0.01 M) and 2-propanol (97:3, v/v). The mobile phase for pump B was monobasic ammonium phosphate (0.01 M) and 2-propanol (98:2, v/v). The flow rate was 0.75 ml min^{-1} for pump A and 1.2 ml min^{-1} for pump B. The retention time of enloplatin was approximately 8.7 min and the chromatographic run time was 15 min. Chromatographic separation for human plasma was achieved on an ODS-2 precolumn (250 × 4.6 mm i.d., 5 μm particle size) and an Exsil 80 phenyl analytical column (100 × 4.6 mm i.d., 5 μm particle size) both supplied by CSC. The mobile phase for pumps A and B was monobasic ammonium phosphate (0.01 M) and 2-propanol (96:4, v/v). The flow rate was 1.20 ml min^{-1} for pump A and 1.25 ml min^{-1} for pump B. All mobile phase solutions were filtered through a 0.45-μm membrane filter and degassed under vacuum prior to use. The retention time of enloplatin was approximately 10.5 min and the chromatographic run time was 12 min.

LC column switching setup for analysis of human and dog plasma. Figure 2 illustrates the column switching technique used for LC analysis of human and dog plasma. With the valve set to the load position at the time of injection, mobile phase delivered through pump A was used to carry the sample from the injector to the precolumn. At 3.8 min for dog plasma or 5.05 min for human plasma, the valve was switched from the load to the inject position to allow the flow of mobile phase through pump B to carry enloplatin from the precolumn to the analytical column. At 5.4 min for dog plasma or 6.6 min for human plasma, the valve was switched to its initial load position to wash the precolumn and to equilibrate it prior to the next injection.

LC data analysis. Enloplatin concentrations were based on the external standard method of quantitation using peak height. A calibration curve was plotted by fitting a weighted (1/

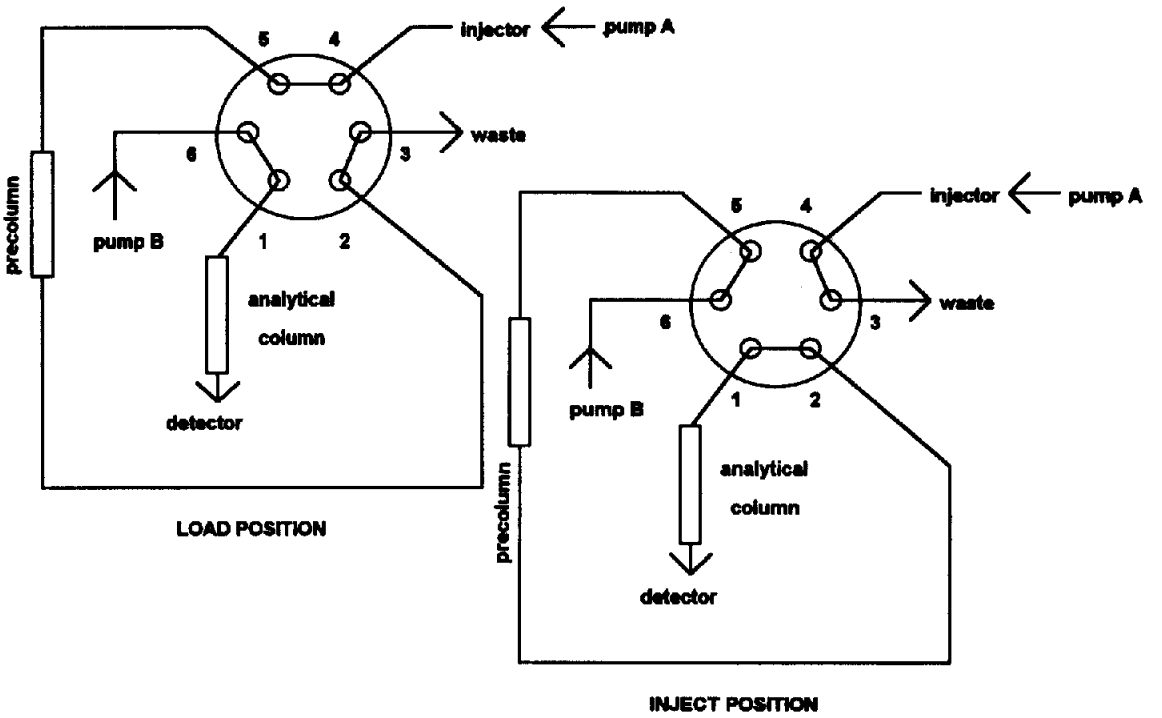

Figure 2
Column switching diagram for the LC analysis of enloplatin in dog plasma.

conc.) least squares regression line ($y = mx + b$) through points defined by the standard concentration versus the standard peak height response.

FAAS procedures

Sample preparation. A 100 μl aliquot of plasma or PUF, or 125 μl of whole blood was transferred to a 5-ml polypropylene tube. For the high concentration range analysis of plasma and PUF samples, 2 ml of Triton X-100 and Antifoam-B solution (0.2%) was added to each sample. For the low concentration range analysis, 400 μl of the 0.4% solution was added. For the whole blood samples, 1 ml of the 0.4% solution was added. The samples were capped and mixed immediately on a vortex mixer. The whole blood samples were shaken at high speed for 15 min. The resulting samples were placed in the autosampler and 20 μl was injected for FAAS analysis.

FAAS system and conditions. A SpectrAA-400 Zeeman atomic absorption spectrometer (Varian Analytical, Sunnyvale, CA) with Zeeman background correction, a graphite furnace, and Report Management software for data reduction were used. The FAAS conditions are shown in Table 1 and the graphite furnace parameters are shown in Tables 2 and 3.

FAAS data analysis. A natural log (ln) polynomial regression was performed to determine the concentration of Pt using the equation [10]; $\ln(y) = a[\ln(x)]^2 + b[\ln(x)] + c$, where y = peak height of Pt absorbance, x = Pt concentration and a,b,c = curve coefficients.

Results

Liquid chromatography

Assay validation. The LC method for the analysis of enloplatin in rat, dog and human plasma was validated and the following assay validation results were obtained.

Selectivity. Control rat, dog and human heparinized plasma were analysed and the chromatograms exhibited no interfering peaks due to endogenous plasma components or reagents around the retention time of enloplatin (representative chromatograms of rat and dog plasma are shown in Figs 3–6).

Sensitivity. The lower limit of quantitation

Table 1
FAAS conditions for analysis of plasma and PUF of rats, dogs and humans, and human whole blood

FAAS conditions	Parameters
Argon pressure	30 psi
Wavelength	265.9 nm
Slit width	0.2 nm
Lamp	Pt, hollow cathode
Lamp current	10 mA
Blank	Same Triton X-100/Antifoam-B solution added to samples
Modifier	NH_4OH solution (0.84–0.90%)
Modifier volume	5 μl (added to all samples and blanks)
Volume injected	20 μl

Table 2
Graphite furnace parameters (temperature gradient) for high and low concentration range analysis of plasma and PUF

Step no.	Temperature (°C)	Time (s)	Gas flow (l min^{-1})	Gas type	Read command
Low concentration range					
1	85	5	3	norm	no
2	85	40	3	norm	no
3	120	20	3	norm	no
4	500	10	3	norm	no
5	500	5	3	norm	no
6	1350	5	3	norm	no
7	1350	2	0	norm	no
8	2750	0.7	0	norm	yes
9	2750	1.7	0	norm	yes
High concentration range					
1	85	5	3	norm	no
2	85	40	3	norm	no
3	120	20	3	norm	no
4	500	10	3	norm	no
5	500	5	3	norm	no
6	1200*	5	3	norm	no
7	1200*	2	0	norm	no
8	2750	0.7	0	norm	yes
9	2750	2	0	norm	yes
10	3000	1	3	norm	no

*Use 1200°C for plasma or 1350°C for PUF.

Table 3
Graphite furnace parameters (temperature gradient) for analysis of whole blood

Step no.	Temperature (°C)	Time (s)	Gas flow (l min^{-1})	Gas type	Read command
1	85	5	3	norm	no
2	95	60	3	norm	no
3	120	20	3	norm	no
4	500	10	3	norm	no
5	500	10	3	norm	no
6	1175	10	3	norm	no
7	1175	10	0	norm	no
8	2750	0.8	0	norm	yes
9	2750	1.5	0	norm	yes
10	2950	1.3	3	norm	no

was 0.50 μg ml^{-1} for rat, dog and human plasma. The precision, measured by the coefficient of variation (RSD) [standard deviation/mean × 100], at this concentration was 6.5% (n = 8) for rat plasma, 9.2% (n = 8) for dog plasma, and 7.5% (n = 10) for human plasma. The accuracy, measured by the relative error (RE%) [(mean observed conc. − nominal conc.) × 100/nominal conc.], was 0.8, 6.8 and 2.5%, respectively.

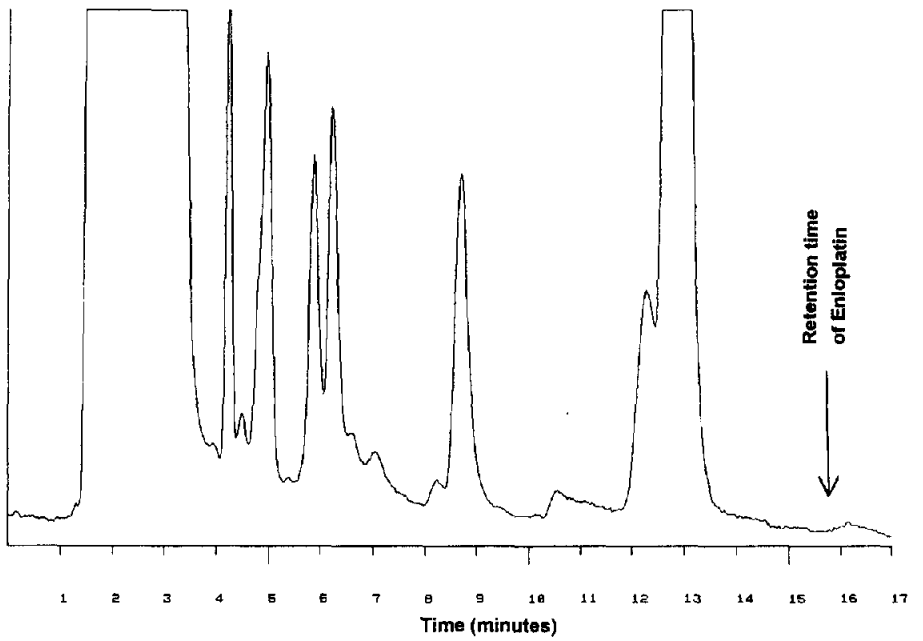

Figure 3
LC chromatogram of control rat plasma.

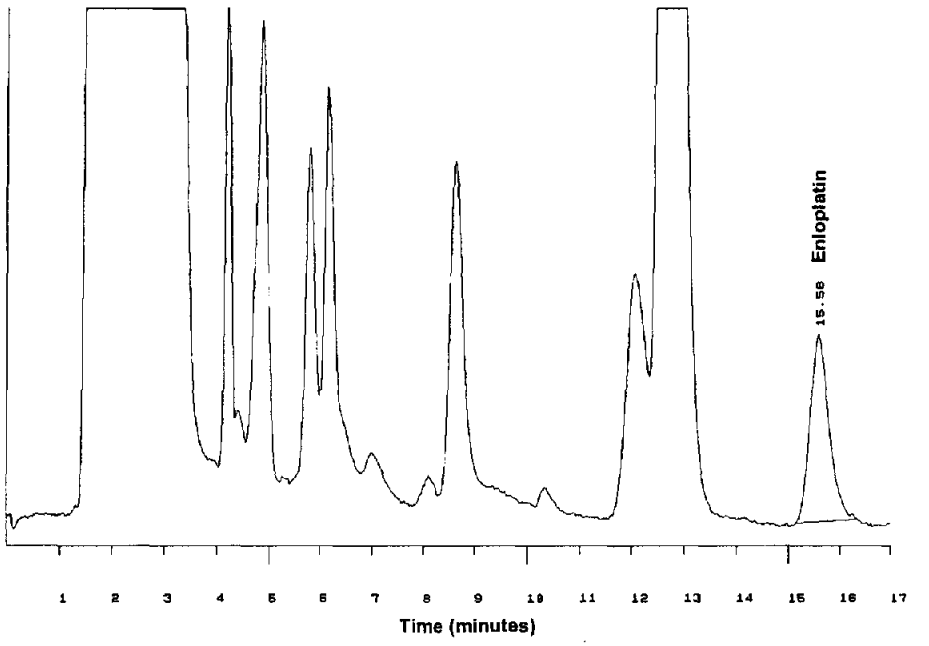

Figure 4
LC chromatogram of rat plasma QC sample with 7.5 μg enloplatin/ml.

Linear range. Linearity was observed over the concentration range of 0.50–50.0 μg ml^{-1}. The RSD (n = 8) of the slopes from standard curves in duplicate over four independent days of analysis in dog and rat plasma were 5.6 and 13.2%, respectively, with correlation coefficients of 0.994 or better. The RSD (n = 4) of the slopes from standard curves over four independent days of analysis in human plasma was 3.8% with correlation coefficients of 0.999 or better. Linearity was further confirmed by the precision and accuracy as described below.

Precision and accuracy. Inter-day precision and accuracy was assessed by analysing QC

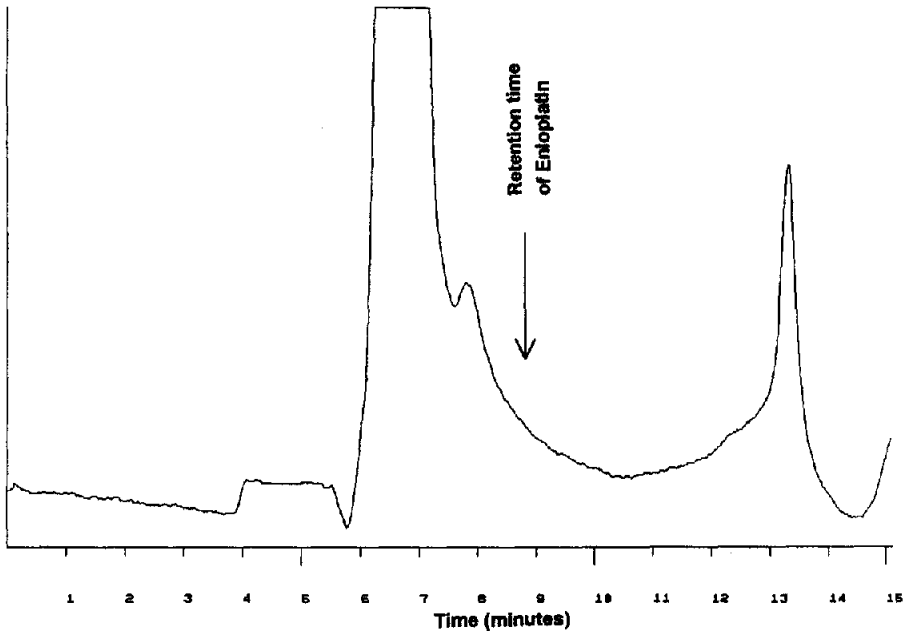

Figure 5
LC chromatogram of control dog plasma.

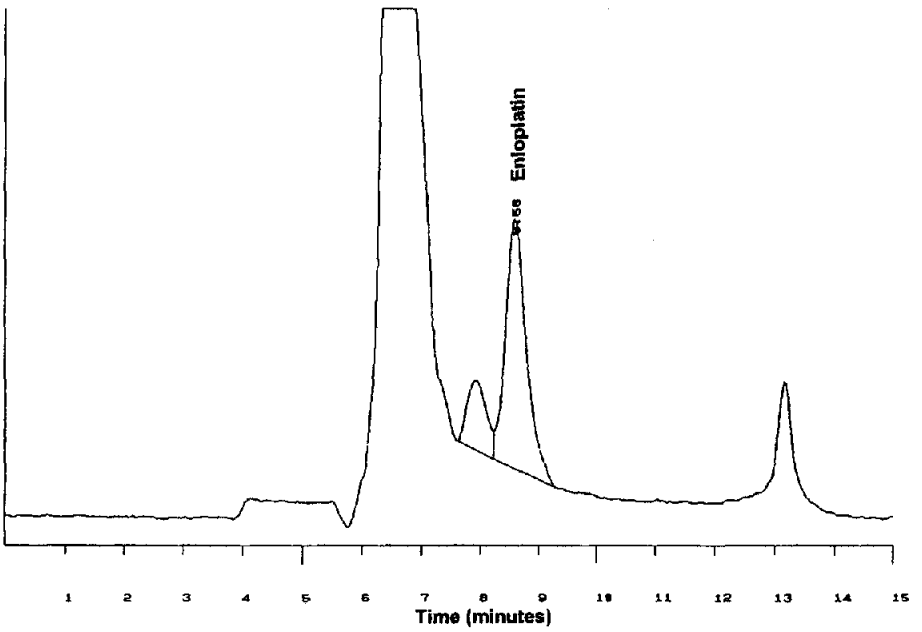

Figure 6
LC chromatogram of dog plasma QC sample with 7.5 μg enloplatin/ml.

samples at four concentrations within the calibration curve range on different days. The assay's precision was determined by calculating the RSD at each concentration. The RSD was generally found to be higher at the lower QC concentration. Similarly, the assay's accuracy was determined by calculating the RE% (from nominal values) at each concentration. Table 4 summarizes the range of values obtained for RSD and RE% for the four QC concentrations in rat, dog and human plasma. On one day of analysis, replicate sets of QCs were analysed to determine the intra-day precision and accuracy. These results are also summarized in Table 4.

Absolute recovery. The mean absolute recovery of enloplatin from rat and dog plasma

Table 4
Inter-day and intra-day precision and accuracy for the determination of enloplatin by LC

Biological matrix	Inter-run results*	Intra-run results†
Precision range (RSD%)		
Rat plasma	3.4–7.7% (n = 6)	4.7–7.5% (n = 8)
Dog plasma	5.6–8.0% (n = 8)	3.4–9.2% (n = 6)
Human plasma	1.5–7.5% (n = 8)	0.8–7.8% (n = 10)
Accuracy range (RE%)		
Rat plasma	−2.5 to −1.4% (n = 6)	−3.8–1.1% (n = 8)
Dog plasma	−13.9–1.5% (n = 8)	−8.9–1.6% (n = 6)
Human plasma	−6.0–2.5% (n = 8)	−8.8–5.6% (n = 10)

* Determined by analysing duplicate sets of QCs at four concentrations on three independent days of analysis for rat and dog plasma and on four independent days of analysis for human plasma.
† Determined by analysing replicate setes of QCs for four concentrations on one day of analysis.

was determined for seven calibration standard concentrations in duplicate by comparing the mean peak height of the extracted standard to that of an unextracted standard spiked in water which represented 100% recovery. The mean recovery of enloplatin from rat and dog plasma was 96.1% (RSD = 10.3%) and 86.1% (RSD = 5.6%), respectively. For human plasma, the % recovery was determined at three QC concentrations (n = 6 for each concentration). The mean recovery of enloplatin from human plasma was 85.3% (RSD = 6.3%).

Stability. Enloplatin stability was assessed by subjecting QC samples prepared in rat, dog and human plasma to a variety of conditions. The QC samples in rat plasma (2.00, 7.52, 15.0 and 40.1 μg ml^{-1}) and dog plasma (1.00, 7.52, 15.0 and 40.1 μg ml^{-1}) were analysed as single determinations at each concentration except for long-term freezer storage stability. For the determination of room temperature (benchtop) stability and freeze/thaw stability in human plasma, the QC sample concentrations were 0.50 and 39.94 μg ml^{-1}. For processed (autosampler) sample stability the QCs were 0.50, 1.50, 29.96 and 39.94 μg ml^{-1}. Stability was determined by comparing the observed concentrations of the stability samples with reference samples at the same concentrations. Stability results were calculated by using the formula; (observed conc. of the stability sample/observed conc. of the reference sample) × 100 except for the processed human plasma samples. The latter was determined by calculating the % deviation from the mean of replicates (n = 10 at each concentration) spanning a 19-h period in the autosampler.

The stability of enloplatin was determined under the following conditions:

Room temperature (benchtop) stability in rat, dog, and human plasma. The mean stability of enloplatin kept at room temperature for 6 h in rat plasma or in dog plasma was 91.6 and 90.6%, respectively. For human plasma, a comparison of stability samples kept at room temperature for 4.75 h with reference samples gave a mean stability of 92.1% ($n \geq 6$).

Processed (autosampler) sample stability. The stability of enloplatin after precipitation with perchloric acid solution was assessed after 48 h at room temperature, 4°C and −80°C. For processed rat samples the mean stability at room temperature, 4°C and −80°C was 93.5, 100.9 and 97.6%, respectively. For processed dog samples, the mean stability was 99.9, 101.4 and 102%, respectively. Processed human plasma samples kept at room temperature showed a deviation from the mean of <7.5% except for two values which were −11 and 17.4%.

Freeze/thaw stability. The mean stability of enloplatin in rat, dog, or human plasma after three freeze (−80°C) and thaw cycles was 101.0, 95.1 and 103.2%, respectively.

Long-term freezer storage stability. Stability samples at three concentrations were analysed after being stored in a freezer for approximately 3 months. The mean stability of enloplatin in rat plasma stored at −80°C for 85 days was 91.9% (RSD = 2.4%, n = 4 for each conc.). The mean stability of enloplatin in dog plasma stored at −80°C for 91 days was

101.5% (RSD = 26.3%, $n = 6$ for each conc.). Long-term human plasma stability is in progress.

FAAS

Assay validation. The FAAS method for the analysis of Pt in the plasma and PUF of humans, dogs and rats, and human whole blood was validated and the following assay validation results were obtained.

Selectivity. The control biological fluids used for FAAS analysis were found to be free of endogenous plasma components that would interfere with the quantitation of Pt.

Sensitivity. The lower limit of quantitation was 0.050 μg Pt/ml in human plasma and PUF, and 0.100 μg Pt/ml in human whole blood. The RSD at this concentration was ≤12% ($n \geq 8$) and the absolute RE was ≤1.5% for all of the human biological fluids.

Goodness of fit. Goodness of fit was observed over the concentration range of 0.050–1.00 μg ml^{-1} (low concentration range) and 1.00–10.0 μg ml^{-1} (high concentration range) in human plasma. For human PUF, the concentration range was 0.05–1.00 μg ml^{-1} (low range) and 0.50–10.0 μg ml^{-1} (high range). For human whole blood, the concentration range was 0.10–10.0 μg ml^{-1}. The correlation coefficients of the calibration curves were ≥0.99 ($n \geq 4$). Goodness of fit was further confirmed by the precision and accuracy as described below.

Precision and accuracy. Inter-day (4 days or more) precision and accuracy was assessed by analysing QC samples at three or four concentrations within the calibration curve range. As with LC, the RSD% was, in general, higher at the lower QC concentration. Table 5 summarizes the range of values obtained for RSD% and RE% for the four QC concentrations in various biological fluids. On one day of analysis, replicate sets of QCs were analysed to determine the intra-day precision and accuracy. These results are also summarized in Table 5.

Stability. Although there was no basis to question the stability of elemental platinum, stability evaluations were conducted to determine if any of the experimental conditions would prevent the accurate quantification of platinum. QC samples prepared at different enloplatin concentrations in various biological fluids were subjected to a variety of conditions. Stability was determined by comparing the observed Pt concentration or Pt response (peak height) of the stability sample with a reference sample at the same concentration that was injected immediately after preparation. Alternatively, stability was assessed by comparing the observed QC concentration to

Table 5
Inter-day and intra-day precision and accuracy for the determination of Pt by FAAS for the low and high concentration ranges as listed in text

	Inter-run results*		Intra-run results†	
Biological matrix	Low conc. range	High conc. range	Low conc. range	High conc. range
Precision range (RSD%)				
Human plasma	5.5–7.4% ($n \geq 61$)	4.9–6.6% ($n \geq 28$)	2.2–4.9% ($n \geq 8$)	1.4–2.8% ($n = 4$)
Dog plasma	4.5–11.7% ($n \geq 15$)	5.1–9.9% ($n \geq 12$)	1.8–6.1% ($n = 4$)	4.0–7.9% ($n = 4$)
Rat plasma	5.2–8.6% ($n \geq 13$)	3.1–11.1% ($n \geq 12$)	3.5–12.2% ($n = 4$)	2.4–6.7% ($n = 4$)
Human PUF	5.1–10.6% ($n = 27$)	5.5–11.8% ($n = 38$)	3.9–7.7% ($n = 5$)	1.4–4.0% ($n = 6$)
Dog PUF	5.7–10.3% ($n = 27$)	4.4–8.5% ($n = 21$)	3.4–7.2% ($n = 5$)	1.9–8.8% ($n = 5$)
Rat PUF	2.6–8.2% ($n = 27$)	3.4–11.2% ($n = 26$)	1.1–10.2% ($n = 5$)	1.7–12.3% ($n = 5$)
Human whole blood	3.2–15.4% ($n = 8$)		5.2–14.1% ($n = 7$)	
Accuracy range (RE%)				
Human plasma	−5.1–0.9% ($n \geq 61$)	−0.2–5.1% ($n \geq 28$)	−9.6–0.7% ($n \geq 8$)	−3.3–1.3% ($n = 4$)
Dog plasma	−1.0–1.7% ($n \geq 15$)	0.5–8.2% ($n \geq 12$)	−2.4–11.8% ($n = 4$)	−6.8–5.0% ($n = 4$)
Rat plasma	0.8–3.6% ($n \geq 13$)	−0.8–6.7% ($n \geq 12$)	−9.1 to −2.5% ($n = 4$)	−0.1–12.8% ($n = 4$)
Human PUF	−9.6–10.5% ($n = 27$)	−2.1–1.3% ($n = 38$)	−9.1–3.6% ($n = 5$)	−14.7 to −1.4% ($n = 6$)
Dog PUF	−7.2–0.4% ($n = 27$)	−5.2–1.5% ($n = 21$)	−4.2–7.9% ($n = 5$)	−8.0–0.4% ($n = 5$)
Rat PUF	−0.8–1.8% ($n = 27$)	2.9–6.5% ($n = 26$)	0.1–1.8% ($n = 5$)	−1.7–1.7% ($n = 5$)
Human whole blood	−5.1–4.0% ($n = 8$)		−8.3–4.8% ($n = 7$)	

* Determined by analysing replicate sets of QCs at three or four concentrations on ≥ four independent days of analysis.
† Determined by analysing replicate sets of QCs at three or four concentrations on one day of analysis.

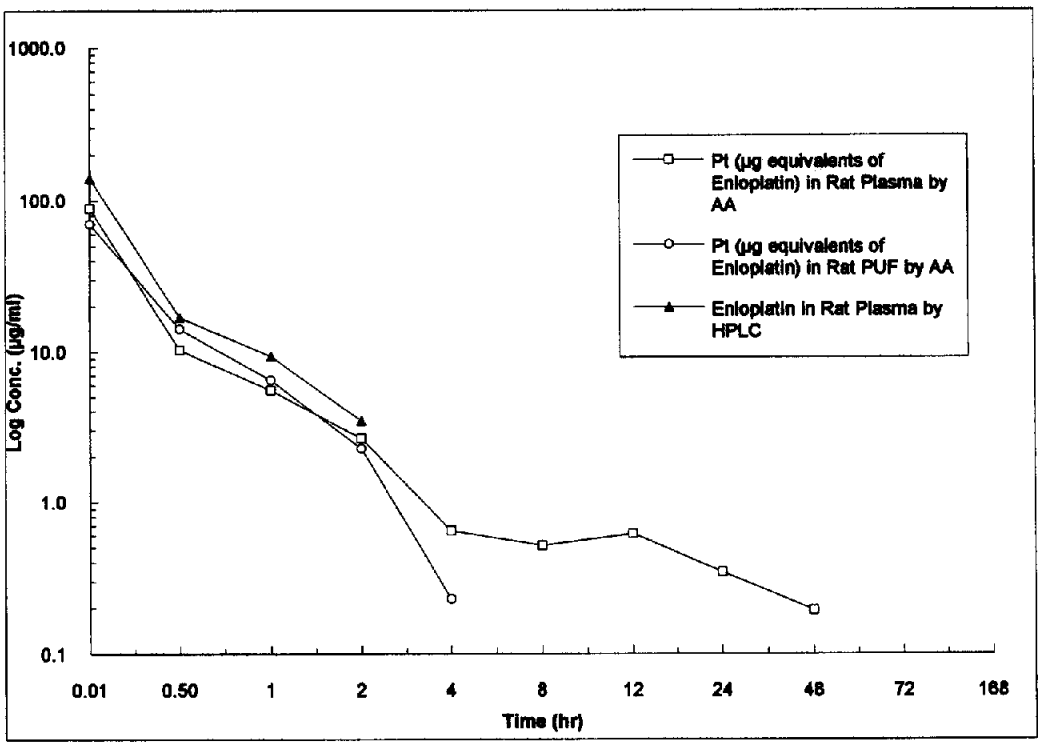

Figure 7
Mean concentrations ($\mu g\ ml^{-1}$) of enloplatin in rat plasma by LC and of platinum in rat plasma and PUF by AA following IV administration (25 mg kg^{-1} given once every 3 weeks, samples collected on day 189).

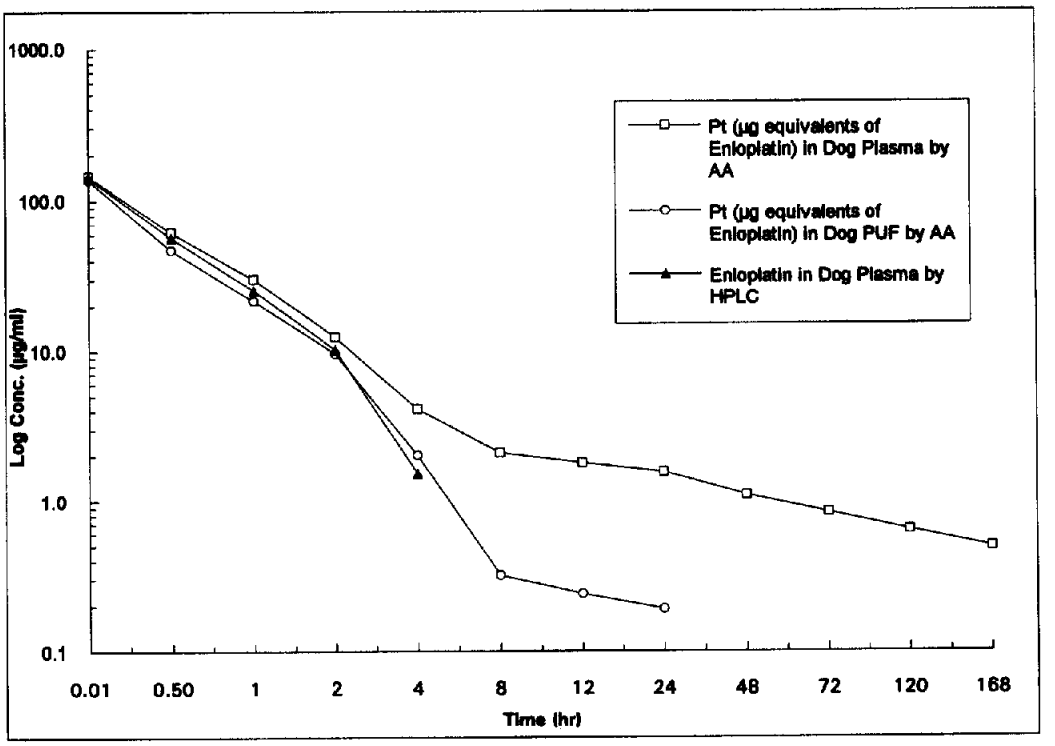

Figure 8
Mean concentrations ($\mu g\ ml^{-1}$) of enloplatin in dog plasma by LC and of platinum in dog plasma and PUF by AA following IV administration (25 mg kg^{-1} given once every 3 weeks, samples collected on day 189).

its nominal concentration. Platinum stability was determined for the following conditions.

Freeze/thaw stability. The mean stability of Pt in the PUF of humans, dogs and rats, human whole blood and human plasma after three freeze (−80°C) and thaw cycles ranged from 93.2 to 109.2% (RSD ≤ 12.4%, $n \geq 3$ for each biological matrix).

Benchtop (room temperature) stability. The stability of Pt in the PUF of humans, dogs and rats, human whole blood and human plasma kept at room temperature for a minimum of 7.5 h ranged from 86.7 to 105.8% (RSD ≤ 10.7%, $n \geq 3$ for each biological matrix).

Processed (autosampler) stability. Replicate QC samples at different concentrations were injected by the FAAS autosampler at various times during a run which typically lasted about 2 h. The stability results of these QC samples ranged from 85.6 to 109.2% in human plasma, 75.5–118.3% in human whole blood, 91.3–123.1% in rat PUF, 85.7–117.7% in dog PUF and 81.9–116.1% in human PUF.

Long-term freezer storage stability. The mean stability of Pt in human, dog, and rat plasma stored at −80°C for 109, 170 and 258 days, respectively, ranged from 96.4 to 106.2% (RSD ≤ 9.4%, $n \geq 3$ for each biological matrix).

Stability of enloplatin stock solution based on its Pt concentration. A value of 95.3% (RSD ≤ 5.4%, $n = 8$) was obtained by comparing the peak height absorbance of enloplatin stock solution stored at 4°C for 176 days versus a freshly prepared solution of enloplatin at the same concentration.

Discussion

This paper presented precise, accurate, and selective LC and FAAS methods that were developed and validated for the determination of enloplatin and its platinum component in various biological fluids. LC analysis was used for measuring the amounts of unchanged (unmetabolized) enloplatin in plasma. FAAS was used to determine the total platinum concentrations in plasma, PUF, and whole blood. FAAS analysis did not distinguish between the platinum contained in unchanged drug or in any possible metabolites. The information obtained from both methodologies was useful for making pharmacokinetic comparisons. Figures 7 and 8 show representative profiles of enloplatin and platinum concentrations vs time after 189 days following an IV dose (25 mg kg^{-1}) given once every 3 weeks for 10 cycles in rat and dog toxicity studies. Analysis of enloplatin by LC showed the drug to be stable under typical laboratory handling, processing, and storage conditions. The stability of Pt in biological matrices prepared with enloplatin was also demonstrated under similar conditions by FAAS analysis. The methods were found to be rugged and suitable for the analysis of various biological fluids from different species in preclinical and clinical pharmacokinetic studies.

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