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# Development of a liquid chromatography–tandem mass spectrometric method for the determination of methamphetamine and amphetamine using small volumes of rat serum

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**Abstract**

The aim of this paper was to develop LC/MS/MS methodology for the determination of methamphetamine (METH) and amphetamine (AMP) using low microliter volumes (20–150 μl) of rat serum and demonstrate the use of this method for the study of serum pharmacokinetics in the rat. The analytes were extracted from rat serum using solid-phase extraction followed by an isocratic separation on a narrow-bore Hypersil $C_{18}$ column. Lower limits of quantitation for METH and AMP were 0.3 ng/ml using positive ion electrospray tandem mass spectrometry. The accuracy of the method was within 20% of the actual values over a wide range of serum concentrations. The within-day and between-day precision was better than 20% (R.S.D.). Ion-suppression matrix effects on electrospray ionization were evaluated for extracted rat serum. The LC/MS/MS method was further validated by comparing serum concentrations of METH and AMP to serum concentrations previously determined using an LC/[$^3$H]-METH assay with radiochemical detection. Finally, the LC/MS/MS method was used to study the pharmacokinetics of METH and AMP after a 1 mg/kg intravenous bolus dose of METH to female Sprague–Dawley rats.


*Keywords:* Methamphetamine; Amphetamine

## 1. Introduction

(+)-Methamphetamine ((+)-METH) is an addictive and toxic drug that can adversely affect the cardiovascular and central nervous systems [1]. The underlying mechanism(s) leading to long-term (+)-METH abuse in humans are not adequately understood. Thus, appropriate animal models are needed to aid in our understanding of the adverse effects and abuse of the drug [2], and for the pre-clinical testing of medications for the treatment of (+)-METH abuse in humans.

A rugged and validated analytical method for determining METH and its pharmacologically active metabolite, (+)-amphetamine (AMP), is essential for investigating the relationship between tissue concentrations and effects. Nevertheless, quantitation of METH and AMP serum concentrations in pharmacokinetic studies using small animal models like the rat is particularly challenging. First, the analytical sensitivity must be high and have the ability to accurately quantitate low serum concentrations found at later time points following METH dosing. Second, the most accurate serum pharmacokinetic parameters are obtained by collecting a complete serum concentration–time profile from an individual animal, instead of pooling samples from different animals at individual time points. This experimental design places constraints on the sample size (e.g., ≤50–300 μl of whole blood per sample) so that the health of the animal and the experimental outcome are not compromised due to repeated blood sampling. Third, extensive extravascular distribution of METH in rats and humans results in extremely low concentrations of METH and AMP in the serum after dosing [3,4].

Liquid chromatographic procedures with various detection strategies have been described for the separation and detection of amphetamine-like compounds. Takayama and co-workers demonstrated an on-column lower limit of

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quantitation (LLOQ) of 2 pg for METH and AMP using pre-column derivatization and chemiluminescence detection [5–7]. Kitaichi et al. have used chemiluminescence for the determination of METH and AMP plasma concentrations in rats following a 5 mg/kg intravenous (i.v.) dose of METH [8]. They reported a limit of quantitation of 5 ng/ml using 50 μl of rat plasma.

Liquid chromatography coupled to atmospheric pressure ionization mass spectrometry has become the method of choice for the determination of small molecules in biological matrices, including amphetamine-like compounds [9–12]. A recent review by Marquet lists several LC/MS methods for the quantitation of AMP and METH for clinical and forensic toxicology studies [13]. For example, liquid chromatography coupled to time of flight mass spectrometry has been used to quantitate amphetamine-like compound in 200 μl of saliva with an LLOQ of 0.4 ng/ml [12].

Tandem mass spectrometric methods generally provide superior LLOQ, sensitivity, and improved selectivity [14]. For example, Wood et al. have described an LC/MS/MS method for the quantitation of six amphetamine-like compounds, including AMP and METH, in human plasma and oral fluid [15]. The LLOQ was 0.5 ng/ml for AMP and METH using only 50 μl of sample (i.e., plasma or saliva), and the on-column LLOQ was 1 pg. Neither solid-phase nor liquid-phase extraction was necessary for reliable quantitation, due in part to the excellent selectivity of the MS/MS technique and the lack of coeluting peaks from the human plasma matrix. The selectivity of the method relied heavily upon the mass selectivity of the instrument. Attempts in our laboratory to apply this methodology for the quantitation of METH and AMP in rat serum were not successful. To our knowledge, analytical methodology with the sensitivity and selectivity necessary to obtain accurate pharmacokinetic parameters for METH and AMP in rat serum is not available.

The aim of these studies was to develop a sensitive and rugged LC/MS/MS method for the determination of METH and AMP concentrations in serum samples derived from pharmacokinetic studies. To accomplish this goal, we used a solid-phase extraction (SPE) procedure to optimize the signal-to-noise ratio (S/N), and an isocratic LC separation combined with tandem mass spectrometric detection to reduce biological matrix effects [16]. The performance of the methodology was characterized for ion-suppression matrix effects, precision, accuracy, dynamic range, and LLOQ for both METH and AMP. We also compared the result obtained from the LC/MS/MS technique with a combined liquid chromatography and radiochemical detection technique that is currently used in our laboratory [17]. Finally, the real-world utility of the method was demonstrated by measuring METH and AMP (a pharmacologically active metabolite of METH) concentrations in rat serum samples of 25–150 μl. These serum samples were obtained from female Sprague–Dawley rats after i.v. doses of METH.

## 2. Experimental

### 2.1. Chemicals

(+)-Methamphetamine chloride and (+)-amphetamine sulfate were obtained from the National Institute on Drug Abuse (Bethesda, MD). (±)-Amphetamine-$d_{11}$ and zinc sulfate were purchased from Sigma (Sigma–Aldrich, St. Louis, MO). The tritiated (+)-METH ((+)-[2′,6′-$^3$H(*n*)]methamphetamine, 23.5 Ci/mmol) was synthesized with the radiolabel at the 2- and 6-positions of the aromatic ring, which are metabolically stable sites. The synthesis was performed by the Research Triangle Institute (Research Triangle Park, NC) for the National Institute on Drug Abuse. HPLC grade methanol, acetonitrile, glacial acetic acid and ammonium hydroxide were purchased from Fisher Scientific (Houston, TX). Water was purified through a Milli-Q Synthesis A10 system (Millipore Corporation, Bedford, MA) prior to use.

### 2.2. Sample preparation

Two stock solutions of 1 mg/ml METH and AMP (calculated as the free base) were prepared in drug-free normal rat serum (Pel Freez, Rogers, AR) and stored at −20 °C. We have determined previously in our laboratory that METH is freely soluble in this matrix at 1 mg/ml. Fresh stock solutions were prepared every 4 weeks. These standard solutions were serially diluted in rat serum to prepare working standards for calibration and quality control (QC). The calibration standards were 0.3, 1.0, 3.0, 10, 20, 30, 100, 300, and 1000 ng/ml. QC standards of 0.5, 10, and 800 ng/ml were prepared from a separate stock solution (1 mg/ml) by serial dilution. These standards were prepared fresh every 4 weeks. All standards and samples were allowed to come to room temperature and vortex-mixed prior to analysis. Each day, six replicates of a different QC standard level were assayed to assess within-day precision of the method. Analysis of each QC standard on four different days established the between-day precision.

Attempts to develop a rapid sample preparation (i.e., organic solvent protein precipitation, evaporation of supernatant, and LC/MS/MS analysis of reconstituted residue) were not compatible with the matrix or the resolution and sensitivity of the mass spectrometer (Quattro LC). Therefore, we developed a simple solid-phase extraction method, derived from previous methodology developed in our laboratory [17]. Briefly, Oasis HLB (1 ml/30 mg) SPE cartridges (Waters Corp., Milford, MA) were conditioned with 1 ml of acetonitrile, 1 ml of water containing 10% methanol, followed by 1 ml of water. Amphetamine-$d_{11}$ (10 μl of a 100 ng/ml solution) was added to each standard and sample as an internal standard. All serum samples were treated with 200 μl of 10% (w/v) zinc sulfate to precipitate serum proteins and improve flow through the extraction cartridge. Samples were centrifuged for 5 min at 6600 rpm and the supernatant was quantitatively transferred to the

conditioned SPE cartridges. The cartridges were washed with 1 ml of water followed by 1 ml of water containing 10% (v/v) methanol. Analytes were eluted in 1 ml of acetonitrile containing 0.1% (v/v) acetic acid. Flow through the SPE cartridges was accomplished by centrifugation at 1000 rpm for 1 min. The eluent was quantitatively transferred to a 1.5 ml silicon-treated polypropylene tube (Fisher Scientific) and evaporated under ultra high purity nitrogen at 36 ± 1 °C. The residue was reconstituted in 100 μl of 10 mM ammonium acetate, pH 3.7 containing 5% (v/v) methanol.

### 2.3. LC/MS/MS conditions

Optimum chromatographic separation was achieved on a 100 mm × 2.1 mm i.d., 3 μm Hypersil BDS $C_{18}$ column (Thermo Hypersil-Keystone, Bellefonte, PA) at a column temperature of 55 °C. A 10 mm × 2.1 mm, 3 μm Hypersil BDS $C_{18}$ guard column (Thermo Hypersil-Keystone) was used to extend the life of the analytical column. The mobile phase consisted of 10 mM ammonium acetate buffer, pH 3.7 with 25% (v/v) acetonitrile and 2.5% (v/v) methanol, operated at a flow rate of 0.2 ml/min. The injection volume was 25 μl. The mobile phase was filtered using 0.2 μm Durapore membrane filters (Millipore) and then degassed using an in-line degassing system on the chromatographic system. The chromatographic system consisted of a Waters Alliance 2690 liquid chromatography system and autoinjector controlled by Masslynx 3.4 software (Waters Corp.).

A Quattro LC triple quadrupole mass spectrometer (Waters Corp.) fitted with a Z-Spray ion interface was used for all analyses. Ionization was achieved using electrospray in the positive ionization mode. The following parameters were optimized for METH and AMP analysis: capillary voltage, 2.0 kV; source block temperature, 110 °C; and desolvation gas (nitrogen) heated to 325 °C and delivered at a flow rate of 600 l/h.

Multiple-reaction monitoring (MRM) conditions were established for each analyte and the internal standard by mixing 10 μg/ml of each compound (10 μl/min) with mobile phase (200 μl/min) and infusing the mixture via a tee-union into the mass spectrometer. Each compound was run separately. The cone voltage was adjusted to maximize the intensity of the protonated molecular ion (precursor). Collision-induced dissociation (CID) of each precursor ion was facilitated using argon at a pressure of $2.0 \times 10^{-3}$ mbar. The collision energy was adjusted to optimize the signal of the product ions that were subsequently used for quantitation. Specifically, METH, AMP, and the internal standard (AMP-$d_{11}$), were monitored at *m/z* transitions of 150 → 119, 136 → 119, and 147 → 98, respectively. All data were acquired using a dwell time of 0.25 s and inter-channel delay of 0.08 s. Additional transitions were explored for METH (*m/z* 150 → 91) and AMP (*m/z* 136 → 91) quantitation but were not used since a significant coeluting peak was observed in extracted blank serum samples.

### 2.4. Assay validation

#### 2.4.1. Extraction recovery and absolute recovery

Matrix effects, recovery, and process efficiency were assessed at 0.5, 10, and 800 ng/ml by comparing peak areas of standards prepared in injection solvent (set A), standards spiked after serum extraction (set B), and standards prepared in serum (set C). This approach has recently been described by Matuszewski et al. [18]. The following peak area ratios were used to evaluate, matrix effect (ME), recovery from solid-phase extraction (RE), and recovery from the complete sample process (process efficiency (PE)):

$$\text{ME (\%)} = \frac{B}{A} \times 100\%$$

$$\text{RE (\%)} = \frac{B}{C} \times 100\%$$

$$\text{PE (\%)} = \frac{C}{A} \times 100\%$$

where RE expresses the recovery from solid-phase extraction and PE expresses the total recovery from all sample handling.

#### 2.4.2. Linearity, accuracy, and precision

The internal standard approach was used for assessing the accuracy and precision of the method. To conduct these experiments, a low range serum calibration curve (0.3 – 10 ng/ml) and a high range curve (10–1000 ng/ml) were constructed by plotting (([AMP-$d_{11}$] × peak area AMP)/peak area AMP-$d_{11}$) versus the analyte concentration in the calibration standard. A linear least-squares (1/*x* weight) equation was fit to these data points to determine the concentration of each QC standard and each pharmacokinetic sample from the rat studies. The two standard curves were used to quantitate values that fell within the low and high range standard curves. Linearity of the method was evaluated by serum calibration curves on different days ($N = 4$). Each QC standard was run to establish the within-day ($N = 6$) and between-day precision ($N = 4$) and accuracy of the assay. Between-day precision and accuracy was evaluated for each QC standard while the QC standards were stored at −20 °C between each run. Calibration standards were prepared separately and not subjected to the same freeze–thaw cycle. The precision was expressed as the relative or percent standard deviation (R.S.D.). Accuracy was expressed as: ((observed value/nominal value) × 100%). The LLOQ was defined as the lowest concentration of analyte that could be measured with a R.S.D. value of >20% and an analytical accuracy of <20%.

### 2.5. METH pharmacokinetic studies

Female Sprague–Dawley rats ($N = 3$) with indwelling jugular and femoral vein catheters (Silastic medical-grade tubing, 0.020 in. inner diameter and 0.037 in. outer diameter;

Dow Corning Corporation, Midland, MI) were purchased from Hilltop Laboratories (Scottsdale, PA). The patency of the catheter was maintained by flushing every morning with 0.2 ml saline, followed by 0.05 ml of saline containing 25 units/ml of heparin. The rats were housed separately and fed each day with three food pellets to maintain their body weight between 240 and 270 g throughout the experiment.

For the pharmacokinetic studies, each animal was placed in a Rodent Experimental Conditioning Unit (Braintree Scientific, Braintree, MA) during i.v. administration of METH and for the initial 5 min of blood sampling. Animals were transferred to metabolic cages and the remaining blood samples were collected. Serial blood samples were collected from the jugular catheter before dosing and at 1, 2, 5, 20, 60, 120, 210, 300, and 390 min after METH dosing. In all cases, the blood volume collected during the experiment was kept to <10% of the rats total blood volume. The total blood volume collected during the experiment was optimized to allow adequate assay sensitivity, while maintaining the hematocrit at a healthy level for the animal. Blood samples (50–300 μl) were allowed to clot at room temperature for 1 h and then centrifuged for 10 min at 10,000 rpm. The serum was then removed and stored at −80 °C until analyzed. QC standards were stored with authentic rat serum samples an then run at the same time the authentic samples were assayed. All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences and were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

### 2.6. Comparison method

To aid in the validation of our new LC/MS/MS method, a separate pharmacokinetic study was performed in another group ($N = 3$) in which the animals were administered an i.v. bolus dose of METH (1 mg/kg) along with a tracer dose of [$^3$H]-METH, which allowed quantitation of METH and AMP after liquid chromatography separation followed by liquid scintillation spectrophotometric quantitation of the METH and AMP containing fractions [19]. This is the method previously used in our laboratory for quantitation of METH and AMP in pharmacokinetic studies. Briefly, rats were dosed with 1 mg/kg i.v. METH containing a tracer dose of 120 μCi of $^3$H-METH. Blood samples were collected before dosing and at 2, 5, 10, 20, 40, 60, 90, 240, 330, and 420 min. Pharmacokinetic parameters obtained from the two analytical methods (i.e., LC/MS/MS and radiochemical) were compared using a two-tailed non-parametric test (Graphpad Software Inc., San Diego, CA).

### 2.7. Statistics and pharmacokinetic data analysis

To determine the pharmacokinetic profile of METH in serum, the METH concentration versus time curves from individual animals were analyzed by model-dependent methods using a non-linear least-squares fitting routine. A curve was fit to the data points using two- and three-compartment i.v. bolus models, with no weighting, $1/y$, or $1/y^2$ weighting. The best-fit line and appropriate pharmacokinetic model were then chosen from these analyses after visual inspection of the best-fit line, assessing statistical results, and analysis of the residuals. To determine the terminal elimination half-life ($t_{1/2\lambda Z}$) for AMP (a pharmacologically active metabolite of METH), a straight line was fit to the terminal elimination phase of the log concentration versus time data for AMP using non-compartmental analysis. All pharmacokinetic analyses were performed using WinNonlin V3.0 (Pharsight Corporation, Mountain View, CA).

## 3. Results and discussion

### 3.1. Sample preparation, matrix effects, recovery, and selectivity

We have developed a rugged SPE methodology that allows accurate quantitation of METH and AMP in low volumes of rat serum using LC/MS/MS. High throughput SPE was possible since the flow through of each extraction cartridge was facilitated by low speed centrifugation at 1000 rpm for 1 min. The number of samples that could be prepared in a given time was only limited to the available space on the evaporator (27 spaces). Incorporation of this simple SPE method increased the overall selectivity of the method, and it overcame the limits of mass selectivity and sensitivity of the mass analyzer. While Wood et al. report better LLOQ values for METH and AMP in human saliva, this is a simpler biological matrix than the serum used in our studies.

Several investigators have proposed methods for evaluating matrix effects associated with LC/MS. Müller et al. evaluated the effects of different extraction strategies on the ion suppression of codeine and glafenine by post-column addition of these analytes to drug-free extracted biological samples. Matuszewski et al. have reported a different approach [16,18], where matrix effects, extraction recovery, and overall recovery were assessed simply without the need for post-column infusion of analyte(s). Ion suppression and/or ion enhancement of analytes were evaluated by spiking extracted serum prior to chromatographic separation. The term process efficiency was defined by Matuszewski et al. to distinguish the overall recovery of analyte, or PE, from the extraction recovery, and matrix effects [18]. This approach was used in the current study to determine if ion-suppression matrix effects led to the poorer LLOQ values obtained in our current study in rat serum relative to those reported by Wood et al. [15] which were conducted for human saliva.

Table 1 shows the ion-suppression matrix effects, recovery and process efficiency for METH and AMP at 0.5, 10,

Table 1
Matrix effect (ME), recovery (RE), and process efficiency (PE) for METH and AMP in rat serum

| Nominal concentration (ng/ml) | ME (%) | | RE (%) | | PE (%) | |
|---|---|---|---|---|---|---|
| | METH | AMP | METH | AMP | METH | AMP |
| 0.5 | 63 | 52 | 87 | 67 | 55 | 34 |
| 10 | 59 | 42 | 103 | 89 | 61 | 37 |
| 800 | 44 | 39 | 108 | 110 | 74 | 43 |

ME = peak area set B/peak area set A; RE = peak area set C/peak area set B; PE = peak area set C/peak area set A. Set A QCs were prepared in injection solvent. Set B QCs were prepared by spiking QC into extracted serum. Set C QCs were prepared in serum and extracted. Values are the means ($N = 6$).

and 800 ng/ml. The ME, RE, and PE values for AMP-$d_{11}$, were 43, 84, and 36%, respectively. There was no significant difference of ME, RE, or PE values when comparing AMP and the internal standard AMP-$d_{11}$. It is clear from these data that ion-suppression matrix effects are a major contributor to loss in analyte signal for METH and AMP.

To demonstrate the selectivity of the method, Fig. 1 shows representative chromatograms for blank serum (A) and spiked serum at a METH and AMP concentration of 1 ng/ml (B). These chromatograms were generated in the MRM mode using the *m/z* transitions of 150 → 119, 136 → 119, and 147 → 98. Blank serum samples treated using the organic precipitation method did not yield a satisfactory blank chromatogram, because significant peaks were observed in the blank serum that coeluted with METH and AMP (results not shown). These interferences were observed in both product ion chromatograms (*m/z* 119 and 91). Though the results are not shown, other MS detection modes were investigated in an attempt to increase selectivity. Neither the selective ion monitoring (SIM) mode nor the scanning mode was satisfactory in terms of selectivity and sensitivity when compared with the MRM mode.

Product ion spectra for METH and AMP are shown in Fig. 2A and B, respectively. These product ion spectra are similar to those recently presented by Slawson et al. for the collision-induced dissociation of METH and AMP [10]. Slawson et al. successfully quantitated METH and AMP concentrations from extracted human plasma in the MRM mode using *m/z* 150 → 91 and 136 → 91, respectively. We also found these transitions produced more intense product ions relative to *m/z* 119. But we observed the highest selectivity using *m/z* 119 product ions for METH and AMP. But as discussed earlier, significant coeluting peaks were observed in extracted blank rat serum samples at the *m/z* 91 product ion. The intra-day and between-day precision CV for METH was 7–10 and 6–14%, respectively, while the predicted METH concentration ranged from 95 to 120% of the nominal value. The intra-day and between-day precision CV for AMP was 5–10 and 6–17%, respectively, while the predicted AMP concentration ranged from 99 to 118% of the nominal value. We think the precision for METH

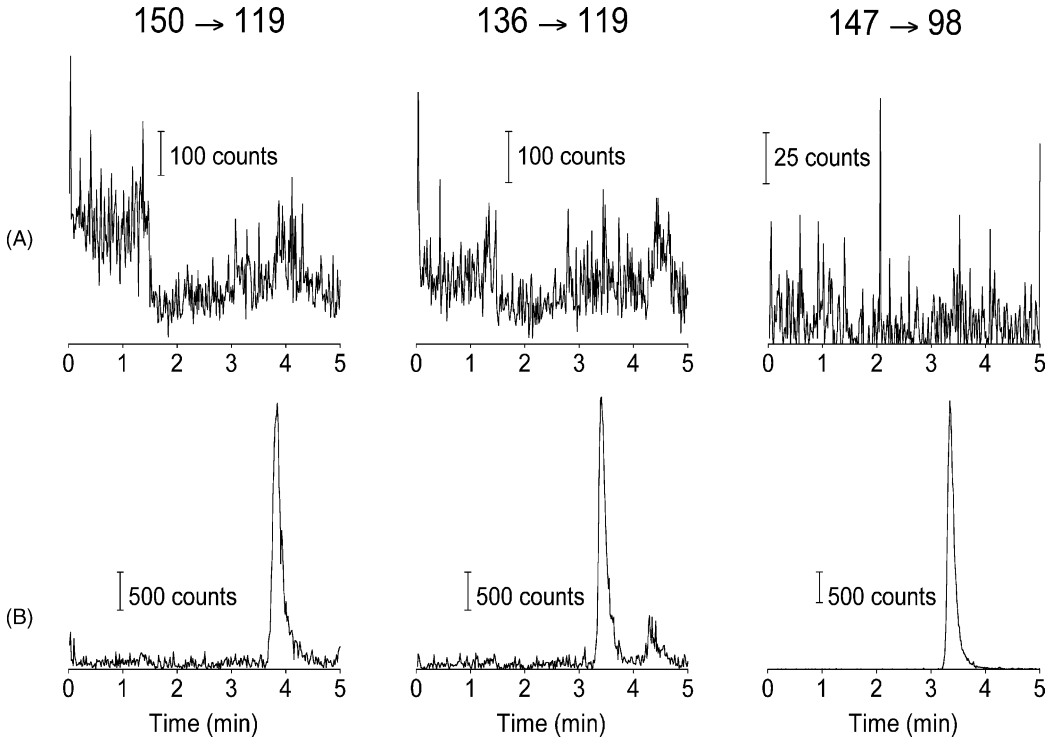


Fig. 1. Representative LC/MS/MS chromatograms showing: (A) 200 μl of extracted blank serum; (B) 100 μl of extracted serum containing 1 ng/ml METH and AMP with 10 ng/ml (±)-AMP-$d_{11}$ as the internal standard. The injection volume was 25 μl. METH, AMP, and (±)-AMP-$d_{11}$, were detected in the multiple-reaction monitoring mode at *m/z* 150 → 119, 136 → 119, and 147 → 98, respectively.

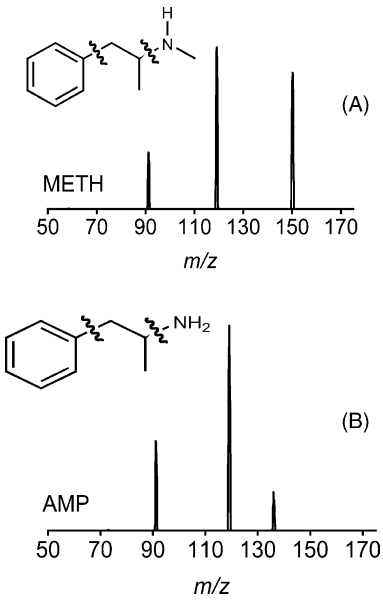


Fig. 2. Product ion spectra for: (A) METH; (B) AMP. Pure analyte in methanol at 10 μg/ml was mixed at a ratio of 1:20 with mobile phase through a mixing tee. Analyte flow rate was 10 μl/min and the mobile phase flow rate was 200 μl/min.

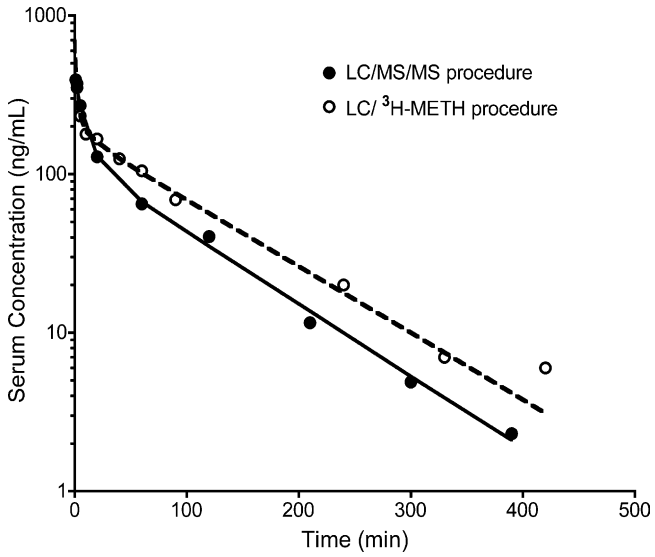


Fig. 3. A representative serum METH concentration–time profile, determined by the LC/MS/MS method (closed circles) and the LC/radiochemical method (open circles) in female Sprague–Dawley rats following a 1 mg/kg i.v. bolus dose of METH. The solid line and dashed line on the concentration–time data represent the best-fit lines to the data points for LC/MS/MS and radiochemical determined concentrations.

measurements could improve if a stable isotope of METH, as a second internal standard, was used.

### 3.2. Calibration curves

By using two separate calibration curves (0.3–10 and 10–1000 ng/ml), we more accurately predicted the serum concentrations and extended the dynamic range of measurement (0.3–1000 ng/ml). Linear least-squares equations (with $1/x$ weighting) for METH and AMP from the low range standard curves were $y = 1.2(\pm0.2)x + 0.004$ and $y = 1.04(\pm0.03)x - 0.01$, respectively. For the high range standard curves, the equations were $y = 1.7(\pm0.4)x - 0.5$ and $y = 1.4(\pm0.2)x - 0.4$. The $r^2$ values for METH and AMP from all standard curves were ≥0.998. The within-day precision of the calibration curve slopes was better ±5%.

The LLOQ for this LC/MS/MS method was 0.3 ng/ml (in 100 μl of serum) for both METH and AMP. This is equivalent to an on-column LLOQ of 10 pg, which is one order of magnitude higher than the 1 pg value recently reported by Wood et al. [15]. We think that the higher-end instrument used by Wood et al. provided better LLOQ values for METH and AMP. However, the use of SPE methodology allowed us added selectivity and increased sensitivity when combined with the liquid chromatographic tandem mass spectrometric method.

### 3.3. Pharmacokinetics of METH and AMP in rats

We chose to demonstrate the utility of the method using the *S*-form of the drug since this is the isomer abused by humans [3]. Clearly, the analytical method in the current study is equally applicable to both enantiomeric forms of METH and AMP, but it would not be able to distinguish the isomers in a racemic mixture. Fig. 3 shows the serum METH concentration–time profiles following a 1 mg/kg i.v. bolus dose of METH in a rat. We collected blood volumes of about 50–300 μl. After the blood clotted we had about 25–150 μl of serum for the actual analysis. The larger volumes of serum (e.g., 150 μl) were needed to have sufficient concentrations for measurement at the later time points. The use of anticoagulants during serum sample collection was avoided to prevent changes in animal hemodynamics and potential effect on the distribution of the drug. The selectivity and sensitivity of the assay was sufficient to accurately quantitate both METH and AMP (results not shown) for about five to seven elimination half-lives (390 min) of the drugs.

Table 2
Comparison of pharmacokinetic parameters for METH and AMP in separate groups of rats determined by two different analytical methods[a]

| Parameter | LC/[$^3$H]-METH ($N = 3$) | LC/MS/MS ($N = 3$) |
|---|---|---|
| $Cl_s$ (ml/(min kg)) | 51 ± 4 | 66 ± 8 |
| $Vd_{ss}$ (l/kg) | 5.0 ± 0.8 | 5.8 ± 0.8 |
| AUC (ng min/ml) | 19826 ± 1462 | 14557 ± 1938 |
| $t_{1/2\lambda z}$ METH (min) | 72 ± 17 | 72 ± 10 |
| $t_{1/2\lambda z}$ AMP (min) | ND[b] | 100 ± 9 |

[a] No values were found to be significantly different ($P < 0.05$).
[b] Not determined because the LC/[$^3$H]-METH analytical method lacked sufficient analytical sensitivity to quantitate AMP at later blood collection time points following a 1 mg/kg i.v. dose of METH.

The two curves for METH show concentrations derived from the LC/MS/MS method (closed circles) and LC/radiochemical method (open circles). Pharmacokinetic parameters derived from each of these serum concentration–time profiles for METH determined from each method are shown in Table 2. Statistical analysis of the data (non-parametric test) indicated that the pharmacokinetic values were not significantly different at the 95% confidence interval.

## 4. Conclusions

Other investigators have reported METH and AMP pharmacokinetic parameters in rats similar to those we report here (Table 2) [8,17]. Parameters reported by Kitaichi et al. were obtained after rats received a 5 mg/kg i.v. dose, which was a five-fold higher dose than we report here [8]. The analytical method used by Kitaichi et al. required a lengthy pre-column derivatization step to facilitate chemiluminescent detection. Melega et al. have determined the plasma pharmacokinetics of METH and AMP in rats using a GC/MS method [20]. In this study, 200 μl of plasma was required to obtain an LLOQ of 17 ng/ml and 170 ng/ml for AMP and METH, respectively. While the analytical method used by Riviere et al. to quantitate METH and AMP serum concentrations was accurate and reproducible, it did not have the high sensitivity of the current method and it required the use of high levels of tritium-labeled METH [17].

In summary, we found that a simple SPE extraction procedure was an essential component of the method and led to additional selectivity that was not possible with tandem mass spectrometric detection alone. Ion-suppression matrix effects were significant and alternative ionization modes (e.g., atmospheric pressure chemical ionization) were not investigated here in an attempt to lessen these matrix effects. The analytical liquid chromatographic separation of METH and AMP with tandem mass spectrometric detection afforded an accurate and reproducible quantitation of these compounds in low microliter volumes of rat serum. Considered together, this analytical system allows for a complete serum pharmacokinetic profile of METH and AMP in an individual small animal, without significantly stressing the animal by altering its homeostatic blood volume.

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