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Short communication

Gas chromatographic determination of methamphetamine and its metabolite amphetamine in human plasma and urine following conversion to N-propyl derivatives

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Abstract

A gas chromatographic method for the simultaneous determination of methamphetamine and its metabolite amphetamine in human plasma and urine is described. The method utilizes reductive alkylation with propionaldehyde and sodium borohydride to produce N-propyl derivatives, which have excellent chromatographic properties. Structural analogs of the analytes, *p*-methylmethamphetamine and *p*-methylamphetamine, are used as internal standards. The method has good precision and accuracy for concentrations ranging from less than 10 ng/ml to 5000 ng/ml and has been used to measure plasma concentrations as part of a pharmacokinetic/pharmacodynamic study of methamphetamine in humans.

1. Introduction

Methamphetamine is a significant drug of abuse worldwide, as well as a drug used for treatment of obesity and attention deficit disorders [1]. It is a sympathomimetic amine producing central nervous system stimulation and mood elevation. Methamphetamine has predominantly α-adrenergic agonist effects which result in increased blood pressure and cardiac work. Some of the adverse health effects of methamphetamine use are believed to be mediated by its effects on the cardiovascular system.

To carry out pharmacokinetic/pharmacodynamic studies of methamphetamine in humans, we required a sensitive and accurate method for the determination of methamphetamine and its metabolite amphetamine in biological fluids. Previously reported methods for the determination of methamphetamine and amphetamine in biological fluids include immunoassay techniques [2], high-performance liquid chromatography (HPLC) [3,4], gas chromatography (GC) with flame-ionization detection (FID) [5], nitrogen–phosphorus detection

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(NPD) [6] or electron-capture detection (ECD) [7–11], or combined gas chromatography–mass spectrometry (GC–MS) [12–14]. Of the various methods, the GC methodology appears to be most suitable for pharmacokinetic studies in terms of providing adequate sensitivity for measuring low levels in plasma.

Amphetamine and methamphetamine generally require derivatization of the amino group prior to GC analysis in order to improve their chromatographic properties and/or to produce a derivative that gives high sensitivity in ECD. Derivatives used in previous methods include acetyl [5], trifluoroacetyl [6,14], trichloroacetyl [8,10], heptafluorobutyryl [7], pentafluorobenzoyl [9,12], and pentafluorobenzenesulfonyl [11]. We have previously found that various secondary amines are readily converted to N-alkyl derivatives by reductive alkylation with sodium borohydride and an aldehyde [15,16]. The resulting tertiary amine derivatives have excellent chromatographic properties, and can be carried through acid–base partitioning steps to clean up and concentrate the extracts. This is a useful alternative to the more commonly used acylations, in some cases giving cleaner extracts from biological samples.

In this paper we describe a sensitive gas chromatographic method for the determination of methamphetamine and its metabolite amphetamine in biological fluids. The method involves reductive alkylation to N-propyl derivatives followed by capillary gas chromatography with nitrogen–phosphorous detection. Application of the method to a pharmacokinetic study in humans is also described.

2. Experimental

2.1. Reagents and standards

(±)-Methamphetamine hydrochloride and (±)-amphetamine sulfate were obtained from Sigma (St. Louis, MO, USA). Ethyl ether (HPLC grade), *n*-butyl acetate (HPLC grade), propionaldehyde (97%), sodium borohydride (99%), and tri-*n*-butylamine (99%) were from Aldrich (Milwaukee, WI, USA). Glacial acetic acid (reagent grade), isopropyl alcohol (HPLC grade), toluene (HPLC grade), *tert*-amyl alcohol (reagent grade), anhydrous potassium carbonate (ACS certified reagent), and ammonium hydroxide (reagent grade) were obtained from Fisher (Pittsburgh, PA, USA). Sodium hydroxide (reagent) and sulfuric acid (reagent) were from Mallinckrodt (St. Louis, MO, USA). The internal standards, 1-(4-methylphenyl)-2-aminopropane hydrochloride (*p*-methylamphetamine) and 1-(4-methylphenyl)-2-(methylamino)propane hydrochloride (*p*-methylmethamphetamine) were synthesized as described below.

2.2. Synthesis of internal standards

1-(4-Methylphenyl)-2-nitropropene

A solution of 4-methylbenzaldehyde (Aldrich) (20 g, 167 mmol), nitroethane (25 ml, 26.1 g, 348 mmol), and ammonium acetate catalyst (1 g, 13 mmol) was heated under reflex using a Dean–Stark trap for water removal. After 8 h, about 2 ml (111 mmol) of the solution were collected. The solvent was removed using a rotary evaporator to give a dark red oil. This was triturated with 30 ml of ice-cold methanol to give a solid which was filtered, washed with 10 ml of cold methanol, and air dried to give 6 g (20% yield) of a yellow crystalline solid, which was used directly in the next step. Recrystallization of a portion from methanol provided yellow needles, melting point (m.p.) 51.5–52.5°C (Ref. [17]: m.p. 46–47°C).

1-(4-Methylphenyl)-2-propanone

Iron powder (reduced, electrolytic, 10 g, 180 mmol) in 40 ml of 90% aqueous acetic acid was heated on a steam bath until there was gentle gas evolution. The above nitropropene (2.7 g, 15 mmol) was added portionwise with stirring, which led to a mildly exothermic reaction. After heating for 1 h on the steam bath, the dark, viscous, somewhat gummy mixture was poured into 200 ml of water. Methylene chloride (100 ml) was added, the mixture was stirred for 30 min, and filtered through Celite. The filter cake was washed with methylene chloride, and the

phases of the filtrate were separated. The organic phase was washed with aqueous potassium bicarbonate until there was no more gas evolution, and then evaporated using a rotary evaporator. Bulb-to-bulb distillation (Kugelrohr, 100 mmHg) gave 1.9 g (13 mmol, 87% yield) of a colorless liquid distilling at 140–150°C (Ref. [18]: b.p. 70°C at 0.3 mmHg (0.0399 kPa)). GC–MS analysis revealed one peak with a mass spectrum consistent with the structure: *m/z* (relative intensity) 148 ($M^{+\cdot}$, 25), 106 (25), 105 ($M - CH_3CO$, 100), 77 (23), 43 (CH_3CO^+, 35).

1-(4-Methylphenyl)-2-(methylamino)propane hydrochloride (p-methylmethamphetamine)

To a solution of 0.4 g (2.7 mmol) of the above ketone in 50 ml of methanol containing 7 g of ammonium acetate was added 0.5 g (8 mmol) of sodium cyanoborohydride, with stirring. The solution was allowed to stand overnight, then poured into 200 ml of dilute aqueous sodium hydroxide (pH > 12) and extracted with 150 ml of methylene chloride. The extract was shaken with 50 ml of dilute sulfuric acid, and the acid layer (pH < 2) was separated. This was made basic with sodium hydroxide and extracted with 100 ml of methylene chloride. The extract was dried over anhydrous potassium carbonate, the solvent was removed with a rotary evaporator, and the residue was distilled (Kugelrohr, oven 130–140°C, 100 mmHg) to give 0.3 g (75% yield) of the amine as a colorless liquid. This was dissolved in 2 ml of isopropyl alcohol, acidified with concentrated aqueous HCl (3 drops, to pH < 2), and then diluted with 50 ml of anhydrous diethyl ether, with stirring, which led to the formation of a white solid. Filtration, washing with anhydrous ether, and air drying provided 0.14 g, m.p. 148–149°C (Ref. [19]: 151–152°C). The mass spectrum was consistent with the structure: *m/z* (relative intensity) 149 ($M^{+\cdot}$, 0.1), 134 (0.5), 105 (3), 91 (3), 77 (4), 44 ($M - ArCH_2^{\cdot}$, 100).

1-(4-Methylphenyl)-2-(methylamino)propane hydrochloride (p-methyl-methamphetamine)

A 40% aqueous solution of methylamine (20 ml) was mixed with 50 ml of methanol, and enough glacial acetic acid was added to bring the pH to 6. To this was added 0.4 g of 1-(4-methylphenyl)-2-propanone and 0.5 g of sodium cyanoborohydride, and the mixture was stirred overnight. The product was extracted, distilled, and converted to the hydrochloride salt as described above for *p*-methylamphetamine. A 0.17-g amount of white solid was obtained m.p. 144–145°C. The mass spectrum was consistent with the structure: *m/z* (relative intensity) 163 ($M^{+\cdot}$, 0.1), 148 (0.6), 105 (3), 91 (2), 77 (4), 58 ($M - ArCH_2^{\cdot}$, 100) [20].

2.3. Extraction and derivatization procedure

To 0.5 ml of sample in a 13 × 100 mm screw-top culture tube were added 100 μl of 2 μg/ml combined internal standard (ISTD) [*p*-methylamphetamine (amphetamine ISTD) and *p*-methylmethamphetamine (methamphetamine ISTD)], 1 ml of ethyl ether and 0.5 ml of 2 *M* aqueous K_2CO_3. The tube was then capped, vortex-mixed for ~2 min, centrifuged for ca. 15–20 min at 1500 *g* and subsequently immersed in a dry ice–acetone bath to freeze the bottom aqueous layer. The top, organic layer was decanted into a clean 13 × 100 mm culture tube containing 100 μl of methanol–conc. aqueous HCl (10:1, v/v) and evaporated to dryness in a warm water bath at ~60°C with a current of air.

To the residue was added 0.5 ml of an isopropyl alcohol–acetic acid–propionaldehyde mixture (40:11:5, v/v). The tube was vortex-mixed briefly by hand and allowed to react for at least 5 min at room temperature. A 2 *M* aqueous solution of sodium borohydride in 0.5 *M* aqueous sodium hydroxide (200 μl, in two 100-μl portions) was then added to the tube while it was vortex-mixing in a Kraft (Mineola, NY, USA) vortex-mixer, to convert the analytes and internal standards to the N-propyl derivatives. The tube was removed from the mixer and allowed to stand at room temperature for at least 5 min.

To the derivatization mixture was added 2 ml of toluene-*tert.*-amyl alcohol (9:1, v/v) and 1 ml of 2 *M* K_2CO_3. The tube was vortex-mixed for ca. 2 min, centrifuged for ca. 5 min, then immersed in the dry ice–acetone bath to freeze

the aqueous layer. The organic layer was decanted into a clean 13 × 100 mm culture tube containing 0.5 ml of 0.25 *M* H_2SO_4. The tube was vortex-mixed for 2 min, centrifuged for 5 min, and placed in the dry ice–acetone bath to freeze the aqueous layer. The organic layer was poured off and discarded. After the acid layer had thawed, 175 μl of *n*-butyl acetate containing 0.1% (by volume) tributylamine and 2 ml of 0.4 *M* K_2CO_3 in 0.2% aqueous ammonium hydroxide were added to the tube. The tube was vortex-mixed for 2 min, centrifuged for 5 min, frozen in the acetone bath, and the organic layer was transferred to a 200-μl microvial for injection into the GC system.

2.4. Gas chromatography

Gas chromatographic (GC) analyses were performed using a Hewlett-Packard (HP) 5890 GC equipped with an HP 7673A autosampler, a nitrogen–phosphorous detector (NPD), and an HP 59970C GC Chemstation data collection and processing system (Hewlett-Packard, Palo Alto, CA, USA). An HP Ultra-I fused-silica capillary column (25 m × 0.2 mm I.D.) was used, with a stationary phase of crosslinked methyl-silicone gum (0.33 μm film thickness). A 5-m Restek deactivated, uncoated fused-silica guard column (0.25 mm I.D.) was connected to the analytical column to prolong column life and improve performance. Helium was utilized as the carrier gas with a head pressure of 138 kPa, which resulted in a flow-rate of approximately 2 ml/min (25 cm/s) at 110°C.

The sample (3 μl) was injected in the splitless mode via the autosampler, with a septum purge on-time of 1 min and an injection port temperature of 250°C. The column oven temperature was programmed from 110°C (after a 0.5-min hold) to 150°C at a rate of 5°C/min and held for 7.25 min, then ramped to 300°C at 50°C/min and held for another 3 min. Typical retention times for the N-propyl derivatives were as follows: 9.76 min (amphetamine), 11.72 min (methamphetamine), 12.51 min (*p*-methylamphetamine) and 15.39 min (*p*-methylmethamphetamine).

2.5. Mass spectroscopy

Mass spectroscopy (MS) data was obtained using a Hewlett-Packard (HP) 5890 Series II GC with an HP 7673 autosampler, an HP 5971 mass-selective detector (MSD), and an HP G1034C MS chemstation data collection and processing system. Analyses were performed with the same type of column (HP Ultra-I) under the same conditions (flow-rate, oven program, head pressure) as the GC-NPD analyses. The mass spectrometer was operated in the electron-ionization (EI) mode. Data were collected in the scan mode (30–300 amu), or SIM mode for derivatized methamphetamine and derivatized amphetamine in biological fluids. The mass spectra of the propyl derivatives were consistent with the structures. Methamphetamine derivative: *m/z* (relative intensity) 191 ($M^{+\cdot}$, 0.001), 100 (M − $ArCH_2^{\cdot}$, 100), 91 (18), 58 (20). Amphetamine derivative: 171 ($M^{+\cdot}$, 0.002), 91 (19), 86 (M − $ArCH_2^{\cdot}$, 100), 44 (16).

2.6. Quantitation

Standards and controls were prepared by diluting a solution containing 10 μg/ml or 1 μg/ml of methamphetamine and amphetamine in 0.01 *M* H_2SO_4 with blank plasma (containing 0.13% NaF) or blank urine (pH 2.33). Quantitation was achieved by integration of detector responses and constructing standard curves of the response (peak-height) ratios of analyte/internal standard versus concentration of amount ratios of analyte/internal standard by linear regression. For this assay, the linear range was shown to be from at least 0 to 10 μg/ml.

Plasma

Ten plasma standards containing both analytes spanning the range of 2 to 500 ng/ml were used to construct low level and high level standard curves; one from 2 to 50 ng/ml and the other from 2 to 500 ng/ml. Equations for typical standard curves are given in Table 1. Five combined plasma controls of methamphetamine/amphetamine (10/10 ng/ml, 50/50 ng/ml, 75/75 ng/ml, 200/200 ng/ml, and 125/0 ng/ml) were

Table 1
Equations for standard curves. N-propyl derivatives

Analyte	Biofluid	Concentration range (ng/ml)	Slope	Intercept	Coefficient of determination (r^2)
Methamphetamine	Plasma	2–50	1.50	0.00031	0.998
		2–500	1.53	−0.0061	0.998
	Urine	10–200	2.13	−0.0123	0.999
		10–10 000	2.16	0.274	0.998
Amphetamine	Plasma	2–50	0.527	−0.00995	0.998
		2–500	0.561	−0.00939	0.997
	Urine	10–200	0.713	−0.00775	0.999
		10–10 000	0.709	0.0649	0.998

Equations were determined by linear regression: response ratio = slope × amount ratio + intercept.

used in duplicate for all sample runs to assess run quality.

Urine

The urine assay utilized 11 standards containing both analytes spanning the range 10 ng/ml–10 μg/ml. These were used to construct low level (10–200 ng/ml) and high level (10 ng/ml–10 μg/ml) standard curves. Equations for typical standard curves are given in Table 1. Five combined urine controls (50/50 ng/ml, 100/100 ng/ml, 500/500 ng/ml, 1500/1500 ng/ml, and 0/3000 ng/ml of amphetamine/methamphetamine) were analyzed with each run.

3. Results and discussion

Conversion of methamphetamine to the N-propyl derivative was readily achieved by reductive alkylation with sodium borohydride and propionaldehyde (Fig. 1), as previously described for nornicotine [15] and other tobacco alkaloids [16]. The metabolite amphetamine likewise underwent reductive alkylation to a propyl derivative. The alkylation stopped at the monopropyl stage, although it would be theoretically possible to produce the dipropyl derivative. The derivatives of both amphetamine and methamphetamine have good chromatographic properties, giving symmetrical peaks on fused-silica capillary columns with injection of subnanogram quantities. Without derivatization, sensitivity was poor and, even at concentrations as high as 10 μg/ml, peaks were broad and tailing.

The advantages of using internal standards that are close structural analogs of the analytes are well documented. Consequently, we used *p*-methylmethamphetamine [1-(4-methylphenyl)-2-(methylamino)propane] and *p*-methylamphetamine [1-(4-methylphenyl)-2-aminopropane] as internal standards (Fig. 1). These compounds are extracted and derivatized analogously to the analytes, and effectively correct for losses occurring during sample processing.

An extraction procedure was developed that is convenient for processing large batches of plasma or urine samples generated in pharmacokinetic studies. Solvents that remain liquid at −78°C were chosen so that phase separations

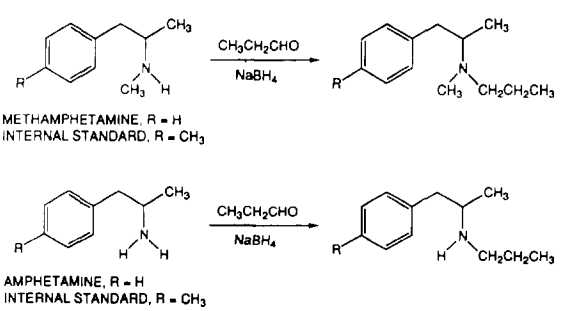

Fig. 1. Conversion of methamphetamine, amphetamine, and internal standards to N-propyl derivatives.

could be made by freezing the aqueous layers with a dry ice–acetone bath, and pouring the organic layers into a new tube. An advantage of the N-alkyl derivatives over the acyl derivatives that are more frequently used to derivatize primary and secondary amines is that the alkyl derivatives are basic (instead of neutral), and they can be carried through acid-base partitioning steps to clean up and concentrate the extracts.

The methamphetamine, amphetamine, and internal standard derivatives extracted from plasma and urine were readily separated on a methylsilicone fused-silica capillary column (Figs. 2 and 3) and measured at low concentrations with a nitrogen–phosphorous detector. The identity of the methamphetamine and amphetamine propyl derivatives in plasma and urine extracts was confirmed by GC–MS. Ion chromatograms of m/z 58, 86, and 100, the major ions produced by electron ionization of methamphetamine propyl derivative, and ion chromatograms of m/z 44, 86, and 91, the major ions produced by electron ionization of amphetamine propyl derivative, displayed peaks with retention times identical to those of the derivatized standards.

There was relatively little interference from endogenous compounds, allowing accurate determination of concentrations ranging from less than 10 ng/ml to 5 μg/ml (Tables 2 and 3). In urine, precision (relative standard deviation) ranged from 1.0 to 13.2%, and accuracy (percent

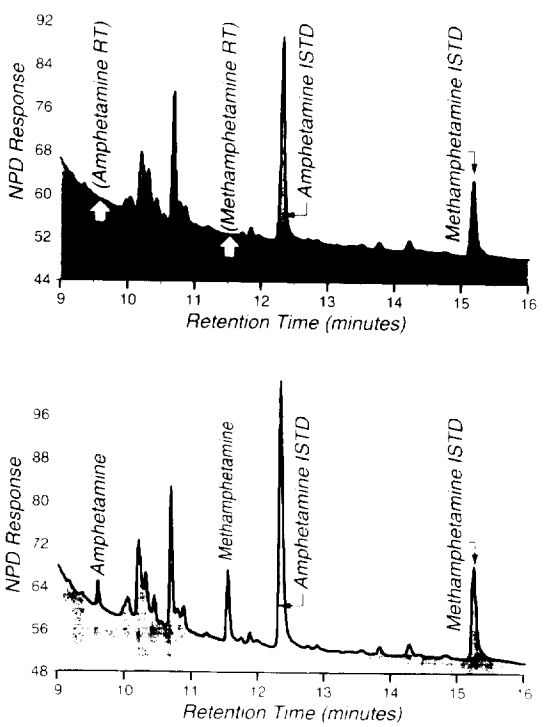

Fig. 2. Chromatograms of plasma extracts derivatized with propionaldehyde and sodium borohydride. (Upper panel) Blank plasma; (lower panel) plasma of subject following methamphetamine administration (30 mg dose, 5 h after dose) containing 82.7 ng/ml methamphetamine and 26.4 ng/ml amphetamine.

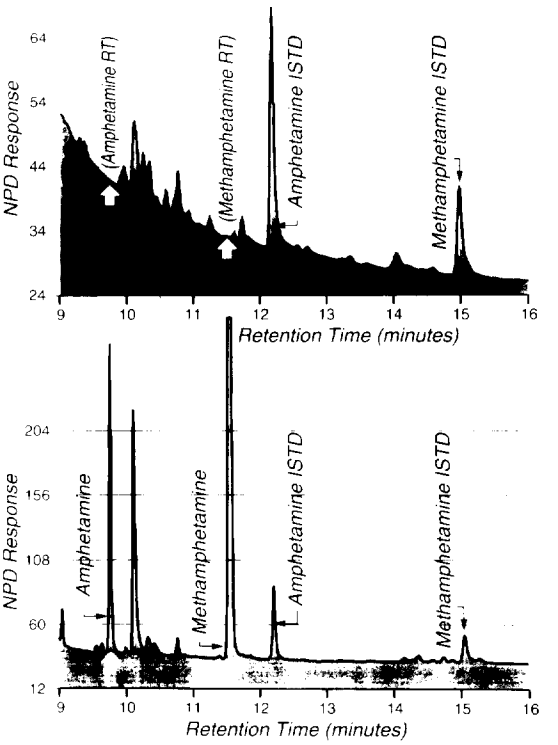

Fig. 3. Chromatograms of urine extracts derivatized with propionaldehyde and sodium borohydride. (Upper panel) Blank urine; (lower panel) urine of subject following methamphetamine administration (30 mg dose, 48 h after dose) containing 2963 ng/ml methamphetamine and 1072.8 ng/ml amphetamine.

Table 2
Intra-day precision and accuracy for determination of amphetamine and methamphetamine in plasma

Actual[a]		Measured mean		Accuracy (%)		Relative standard deviation (%)		Replicate analyses	
Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine
2	2	2.90	2.44	145	122	14.5	18.8	11	10
5	5	5.20	5.45	104	109	6.20	8.10	10	10
10	10	9.81	10.8	98.1	108	9.60	13.1	10	10
20	20	20.8	21.4	104	107	9.60	13.7	11	9
50	50	50.9	52.3	102	105	6.80	5.80	10	9
100	100	95.2	100.62	95.2	101	8.40	7.20	11	9
200	200	193	208	96.6	104	5.30	5.90	9	9
500	500	511	517	102	104	4.30	6.80	9	9

[a] Spiked blank plasma; target concentration.

of target concentration) ranged from 93.3 to 112% over the concentration range of 10–5000 ng/ml. In plasma, precision and accuracy ranged from 4.3 to 13.7% and 92.3 to 108%, respectively, over the concentration range of 5–500 ng/ml (Tables 2 and 3).

The method has been applied to a study of pharmacokinetic and pharmacodynamic interactions of methamphetamine and ethanol in humans [21]. Concentrations of methamphetamine were reliably determined in plasma for 48 h following a 30-mg intravenous dose of methamphetamine peaking at about 350 ng/ml, and dropping to less than 10 ng/ml at about 48 h. Levels of the metabolite amphetamine in plasma were low, peaking at 11.1 ng/ml, approximately 24 h following administration (Fig. 4).

In summary, a sensitive gas chromatographic method has been developed for determination of methamphetamine and its metabolite amphetamine in biological fluids. The method has good precision and accuracy in the concentration ranges typically found in plasma and urine following modest doses of methamphetamine, and

Table 3
Inter-day precision and accuracy for determination of amphetamine and methamphetamine in plasma

Actual[a]		Measured mean		Accuracy (%)		Relative standard deviation (%)		Replicate analyses	
Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine	Amphetamine	Methamphetamine
10	10	9.23	9.49	92.3	94.9	7.3	7.80	26	28
50	50	48.9	48.9	97.8	97.7	6.3	6.70	30	30
75	75	70.8	72.3	94.4	96.4	6.4	6.60	29	29
200	200	199	199	99.5	99.5	6.0	6.00	30	30
0	125	–	124	–	99.0	–	4.90	27	25

[a] Spiked blank plasma; target concentration.

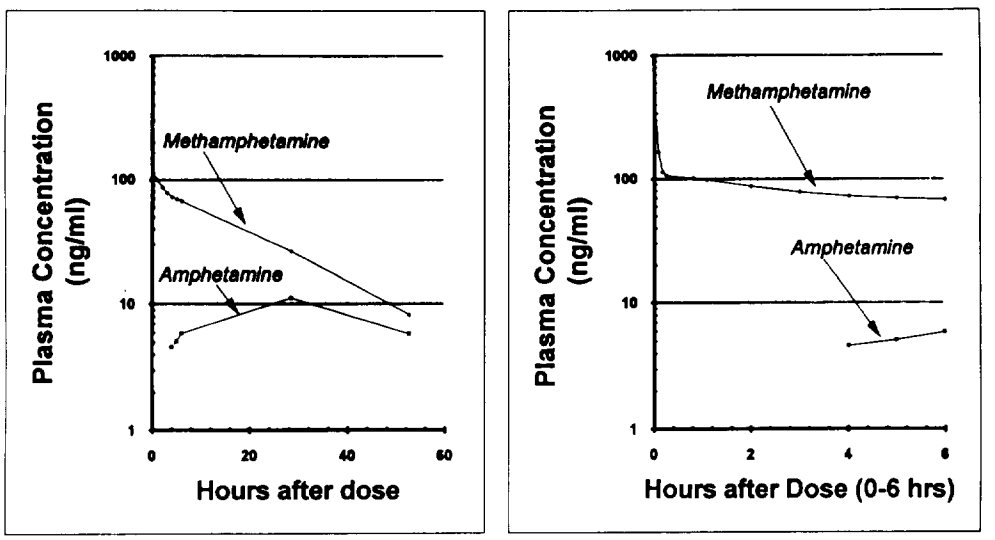

Fig. 4. Concentrations of methamphetamine and amphetamine in plasma of a subject following intravenous administration of 30 mg of methamphetamine and 1 g/kg ethanol.

is well suited for pharmacokinetic studies in humans.

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