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Sensitive and simple gas chromatographic–mass spectrometric determination for amphetamine in microdialysate and ultrafiltrate samples

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Abstract

A gas chromatographic–mass spectrometric (GC–MS) method is described for the measurement of amphetamine (AMP) using negative chemical ionization (NCI) mode. Without prior extraction AMP was derivatized with 2,3,4,5,6-pentafluorobenzoyl chloride (PFBC) and simultaneously extracted into toluene. The toluene extract was injected directly into GC–MS equipped with a HP-1 capillary column. The method is simple and more sensitive than most of the previously published methods. The limit of quantification of amphetamine is 25 pg (1.4 pg on column) with a very limited sample volume (25 μl). The within-day precision was from 1.7 to 5.1% and between-day precision was from 2.2 to 7.3%. The method has been used for the measurement of several thousand microdialysate and ultrafiltrate samples and proven reliable.

Keyword: Amphetamine

1. Introduction

Amphetamine is a sympathomimetic amine and a major central nervous system (CNS) stimulant. In addition, it has anorectic, hyperthermic and cardiovascular effects. For these reasons, amphetamine has attracted extensive attention in basic science and toxicology studies for over 20 years.

It has been reported that schizophrenic patients showed enhanced amphetamine-induced dopamine release in the striatum compared to control subjects [1,2]. A rodent model of the disease has been developed in our labs to mimic these changes. Amphetamine has dopamine–nor-epinephrine releasing properties, by blocking D2 auto receptors and displacing catecholamines from storage vehicles. The vesicular uptake process has broad substrate specificity and can transport amphetamine with a higher affinity than dopamine. Moreover, chronic treatment of this drug irreversibly blocks the dopamine transporter. Following subcutaneous injection of amphetamine we need to analyze microdialysate and ultrafiltrate samples collected via a dual probe microdialysis system in the frontal cortex and striatum and from the peripheral using ultra-filtration techniques. A simple and sensitive method for amphetamine levels in these fluids is required because of the extremely small volume of sample, usually ∼30 μl.

The quantitative measurement of amphetamine in human plasma, urine and hair has been described in numerous gas chromatographic (GC) [3–5], high-performance liquid chromatographic (HPLC) [6–8], gas chromatographic–mass spectrometric (GC-MS) [9–11], and radioimmunoassay (RIA) [12,13] methods. The method described here was developed for the analysis of amphetamine in 25 μl of microdialysate or ultrafiltrate samples. It has the advantage of a single simultaneous derivatization and extraction procedure without the requirement of further sample cleanup before injection into the GC–MS. The sensitivity of the method is extremely high, resulting in a quantitation limit of 25 pg/25 μl (1.4 pg on column).

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2. Experimental

2.1. Reagents and materials

Amphetamine and the internal standard, amphetamine-d_6 were purchased from Cerilliant (Austin, TX, USA) with 100 and 99% purities, respectively. 2,3,4,5,6-Pentafluorobenzoyl chloride (PFBC) was from Sigma (St. Louis, MO, USA). All other chemicals used were reagent grade and obtained from Fisher Chemical (Springfield, NJ, USA). Standard solutions of amphetamine and the internal standard were prepared in 0.01 N HCl and were kept at 4 °C for 6 months. Carbonate buffer of 0.6 M (pH 9.0) was prepared by dissolving 32 g of sodium carbonate and 30 g of potassium bicarbonate into 1 l of distilled water. Saline was prepared by dissolving 9 g sodium chloride into 1 l of distilled water.

2.2. Collection of microdialysis samples

Animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Male Sprague–Dawley rats (280–320 g) bred in our animal colony were used. The microdialysis and ultra-filtration surgical experimental procedures are described in detail elsewhere [14]. After allowing recovery from surgery a 30 min collection of microdialysate or ultrafiltrate was collected at a rate of 1 μl/min. The rats were then challenged with a subcutaneous amphetamine injection (1 mg/kg dose) and samples collected in 30 min aliquots for an additional 210 min. The aliquots were kept at −80 °C until analyzed.

2.3. Sample processing

To 25 μl of a microdialysate or ultrafiltrate sample in a tapered tip borosilicate glass tube containing 0.5 ml of 0.01 N HCl, 5 ng of deuterated (±)-amphetamine (internal standard) was added followed by the addition of 0.5 ml of 0.6 M carbonate buffer (pH 9.0) and 35 μl of 2,3,4,5,6-pentafluorobenzoyl chloride in toluene (30 μl/10 ml). The sample was mixed on a tilt table mixer at 30 oscillations/min for 30 min at room temperature. After centrifugation, the lower aqueous layer was removed, the vial re-centrifuged and the upper organic layer transferred to an automatic injection vial and 2 μl subsequently injected into the GC–MS.

A nine-point calibration standard curve at the concentration levels of 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, 5.0 and 10.0 ng in 25 μl of saline solution was prepared, and processed similarly with each batch of samples. Three levels of quality control samples (run in duplicate) were 0.075, 0.75 and 7.5 ng in 25 μl of saline solution and included with each day's analysis.

2.4. Instrumentation and data acquisition

A HP ChemStation data system was used to control a HP 5988B GC–MS system and to collect and quantitate the data. The GC–MS fitted with an HP-1 capillary column (12 m × 0.2 mm i.d., 0.33 μm) was operated in a negative chemical ionization (NCI) mode using methane:ammonia (95:5) as the reagent gas. The column was programmed from 80 °C (holding for 1 min) to 280 °C at a rate of 30 °C/min. The ion-source temperature was 200 °C, and the temperatures of the injector and the interface between the chromatograph and the spectrometer were set at 280 °C.

Peak area of the target compound and its internal standard were measured using HP ChemStation data acquisition system with RTE integration. The chromatographic data were automatically processed for peak area ratios followed by least square regression of these data. All data were calculated from curves fitted using a second-degree equation (quadratic) with intercepts.

3. Results and discussion

Pentafluorobenzoyl chloride (PFBC) was selected as a derivatizing agent because of its superior ability to combine with many primary or some secondary amine groups of small compounds in an aqueous solution under specific pH conditions. When combined with PFBC, amphetamine was converted to a PFB derivative, which is soluble in organic solvent and thus selectively extracted at the time the derivatization occurred. The simultaneous derivatization and extraction process produced the PFB derivative which has high specificity and sensitivity. Fig. 1 shows the negative chemical ionization (NCI) mass spectra of the derivatives of amphetamine and its internal standard, amphetamine-d_6. The molecular ion peaks, $[M]^+$ at m/z 523 from amphetamine and at m/z 529 from amphetamine-d_6 were used for quantitation, and the fragment peaks at m/z 505 and 511 as confirming ions, respectively.

The method required high sensitivity for the target compound since the amount of the sample was limited (∼ 30 μl). The lower limit for the quantitation of amphetamine in this method was 25 pg/25 μl when 1.4 pg was injected on column, with a signal-to-noise ratio circa. 10:1 (Fig. 2) and R.S.D.% of 5.1 ($n = 5$). The high sensitivity permits the assay of amphetamine with a sample of only 10 μl before processing. The method also showed high specificity using the classical isotope dilution technique with confirmatory ions. Fig. 3 shows the single ion chromatograms (SIM) of PFB-derivatives extracted from 0.1 ng of amphetamine in 25 μl of saline, a blank (25 μl of saline) and 25 μl of sample microdialysate solution, in which 81 pg of amphetamine was measured, indicating that there were no interfering peaks observed at or near the peaks of interest.

Quantitation was achieved by the peak area of amphetamine to the calibration standards at the concentration

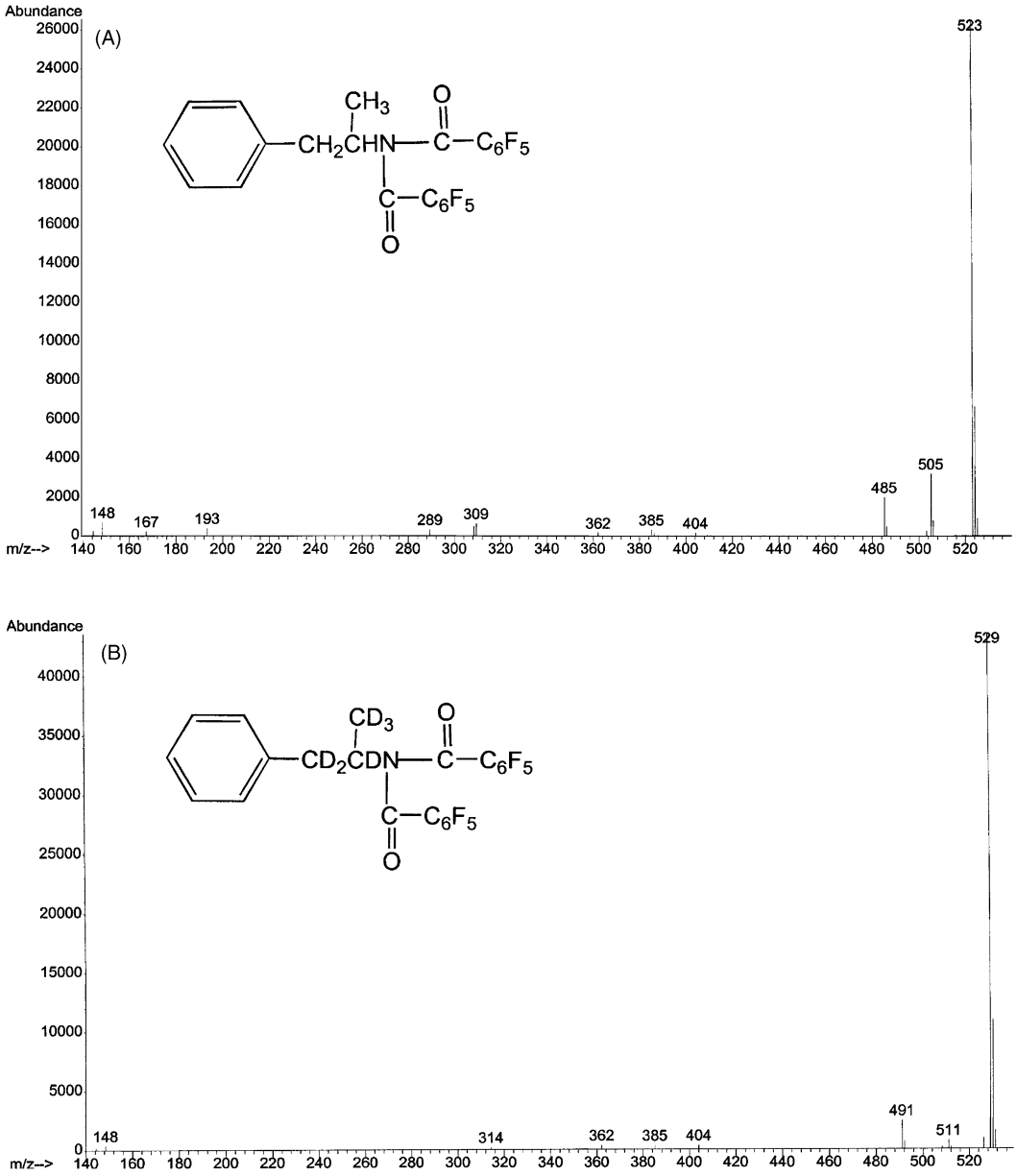

Fig. 1. Mass spectra (NCI) for: (A) PFB-amphetamine and (B) the internal standard, PFB-amphetamine-d_6.

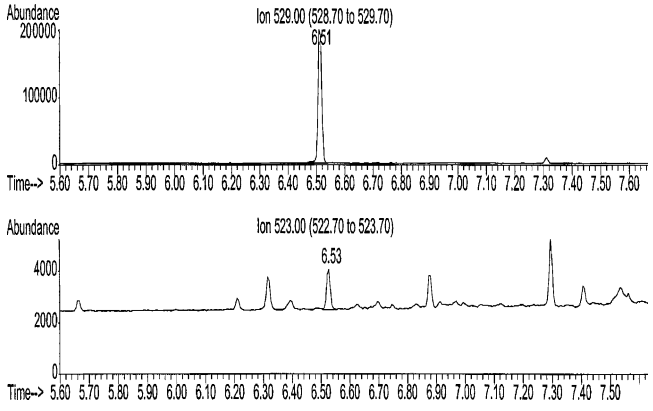

Fig. 2. Detection of 25 pg of amphetamine (1.4 pg on column).

levels in the range of 0.025–10.0 ng in 25 μl of saline solution. The within-day precision was determined by using five replicates of each level of the standard curve and 10 quality control samples at three concentration levels of amphetamine. The relative standard deviation (R.S.D.) for the quality control samples ranged from 1.7 to 5.1% (Table 1). The between-day precision of the method was determined by analyzing quality control samples with each batch of samples, giving the R.S.D. of 2.2, 3.0, and 7.3%, respectively. The accuracy of the method, measured as the percentage difference between the mean concentrations found and the amounts added, ranged from 98 to 104 (Table 1).

The present GC–MS method demonstrates the feasibility of measuring rapidly the level of amphetamine in large numbers of microdialysate and ultrafiltrate samples.

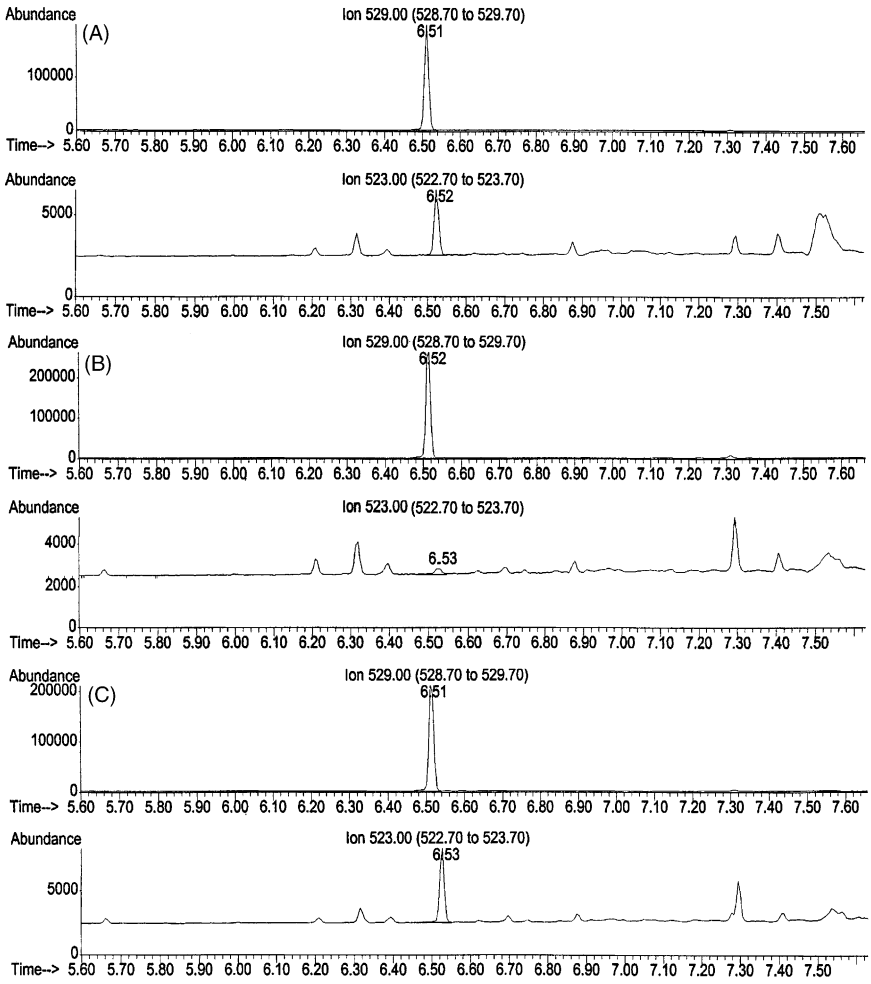

Fig. 3. Chromatogram of PFB-derivatives extracted from: (A) 0.1 ng of amphetamine in 25 μl of saline; (B) a blank (25 μl of saline); and (C) 25 μl of sample microdialysate solution, in which 81 pg of amphetamine was measured. All of them contain amphetamine-d_6.

Table 1
Intra- and inter-assay precision and accuracy of the assay

Added (ng/25 μl)	Mean (ng/25 μl)	S.D.	R.S.D.%	Accuracy
Intra-assay precision				
$n = 5$ at each concentration				
10.000	9.988	0.055	0.55	99.9
5.000	4.985	0.067	1.34	99.7
2.500	2.551	0.061	2.41	102.0
1.000	0.976	0.032	3.30	97.6
0.500	0.500	0.016	3.24	100.0
0.250	0.256	0.010	4.02	102.4
0.100	0.101	0.006	6.14	101.0
0.050	0.051	0.005	9.74	101.2
0.025	0.025	0.004	14.88	101.6
$n = 10$ at each concentration				
7.5	7.797	0.135	1.73	104.0
0.75	0.733	0.013	1.83	97.7
0.075	0.074	0.004	5.07	98.0
Inter-assay precision				
$n = 12$ consecutive days with duplicate run at each concentration				
7.5	7.575	0.170	2.24	101.00
0.75	0.739	0.218	2.95	98.50
0.075	0.076	0.006	7.29	101.47

This method can be used as an adjunct to other HPLC methods used in microdialysis techniques to evaluate the effect of amphetamine administration on changes in biogenic amines release, for example, the concentration of amphetamine can be monitored in addition to changes in dopamine and other biogenic amines from small aliquots of microdialysate samples. Fig. 4 shows the amphetamine concentration change in 30 min for the microdialysate and ultrafiltrate samples collected from prefrontal cortex, striatum and peripheral respectively. Amphetamine was given subcutaneously at 1 mg/kg dose at starting the second sample. Values are the mean ± S.E.M. (n = 3–6) and the amphetamine concentrations were shown as an actual value in 30 min samples. More detailed discussion on the effect of amphetamine on dopamine release will be shown in another paper.

In summary, a one-step simultaneous derivatization–extraction GC–MS method for amphetamine in microdialysate and ultrafiltrate samples has been developed and validated. The method has been utilized in the measurement of several thousand microdialysate and ultrafiltrate samples.

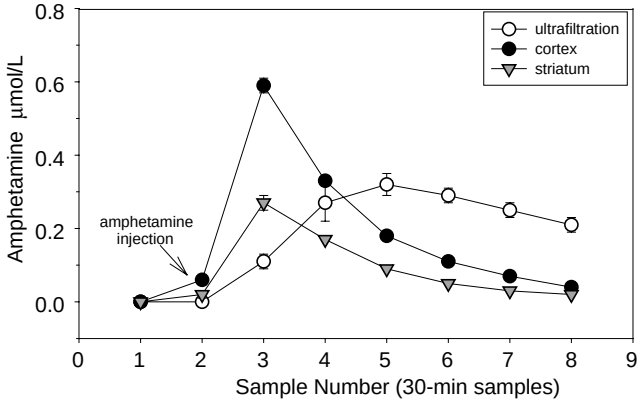

Fig. 4. Extracellular levels of amphetamine in brain and periphery.

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References

[1] A. Breier, C.M. Adler, N. Weisenfeld, T.P. Su, I. Elman, L. Picken, A.K. Malhotra, D. Pickar, Synapse 29 (1998) 142.
[2] M. Laruelle, A. Abi-Dargham, C.H. Van Dyck, R. Gil, C.D. D'Souza, J. Erdos, E. McCance, W. Rosenblatt, C. Fingado, S.S. Zoghbi, R.M. Baldwin, J.P. Seibyl, J.H. Krystal, D.S. Charney, R.B. Innis, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 9235.
[3] N. Raikos, K. Christopoulou, G. Theodoridis, H. Tsoukali, D. Psaroulis, J. Chromatogr. B 789 (2003) 59.
[4] S.J. Asghar, G.B. Baker, G.A. Rauw, P.H. Silverstone, J. Pharmacol. Toxicol. Method 46 (2001) 111.
[5] S. Cheung, H. Nolte, S.V. Otton, R.F. Tyndale, P.H. Wu, E.M. Sellers, J. Chromatogr. B 690 (1997) 77.
[6] N.A. Santagati, G. Ferrara, A. Marrazzo, G. Ronsisvalle, J. Pharm. Biomed. Anal. 30 (2002) 247.
[7] M.E. Soares, F. Carvalho, M.L. Bastos, Biomed. Chromatogr. 15 (2001) 452.
[8] T.K. Wang, M.S. Fuh, J. Chromatogr. B 686 (1996) 285.
[9] M. Sato, T. Mitsui, J. Pharm. Biomed. Anal. 16 (1997) 139.
[10] M. Nishida, A. Namera, M. Yashiki, T. Kojima, J. Chromatogr. B 789 (2003) 65.
[11] M. Yonamine, N. Tawil, R.L. Moreau, O.A. Silva, J. Chromatogr. B 789 (2003) 73.
[12] C. Ward, A.J. Mcnally, D. Rusyniak, S.J. Salamone, J. Forensic Sci. 39 (1994) 1486.
[13] O. Quintela, A.M. Bermejo, M.J. Tabernero, S. Strano-Rossi, M. Chiarotti, A.C. Lucas, forensic Sci. Int. 107 (2000) 273.
[14] A. Balla, A. Hashim, S. Burch, D.C. Javitt, A. Lajtha, H. Sershen, Neurochem. Res. 26 (2001) 1001.