Journal of Chromatography, 573 (1992) 313–317
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6130

Short Communication

Electron-capture gas chromatographic procedure for simultaneous determination of amphetamine and N-methylamphetamine

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(First received July 2nd, 1991; revised manuscript received August 21st, 1991)

ABSTRACT

An electron-capture gas chromatographic procedure for the simultaneous determination of amphetamine and N-methylamphetamine in biological samples is described. The method employs extraction with the ion-pairing reagent bis(2-ethylhexyl)phosphoric acid, and back-extraction with 0.5 *M* hydrochloric acid. The hydrochloric acid phase is basified, and the amphetamines and the internal standard benzylamine are derivatized with pentafluorobenzenesulfonyl chloride prior to analysis on a gas chromatograph equipped with a capillary column. Levels of amphetamine and N-methylamphetamine have been determined in the urine and liver of rats treated chronically with (−)-deprenyl.

INTRODUCTION

Amphetamine and N-methylamphetamine (methamphetamine) are drugs of abuse. In addition these drugs are metabolites of other drugs such as the anti-parkinsonian agent (−)-deprenyl [1–3]. In the present report we describe a rapid and simple procedure for the simultaneous determination of amphetamine and N-methylamphetamine using pentafluorobenzenesulfonyl chloride (PFBSCl) for extractive derivatization of these drugs for subsequent analysis by gas chromatography with electron-capture detection (GC–ECD). PFBSCl has proved to be a useful reagent for the derivatization of tyrosyl peptides [4], nucleic acid pyrimidine bases [5], tranylcypromine [6], *p*-chloroamphetamine [7] and 2-phenylethylamine [8] for GC–ECD analysis. In the past, several procedures have been developed for quantitating amphetamine and its analogues, including immunoassay techniques (for a review see ref. 9), GC with nitrogen–phosphorus detection [10], GC with mass spectrometric (MS) detection [11], high-performance liquid chromatography [12], as well as a variety of derivatizing procedures for GC–ECD (*e.g.* refs. 13 and 14). Many of the methods previously employed for amphetamine analysis involve time-consuming and tedious extraction procedures or have insufficient selectivity and/or sensitivity for analysis in tissues and body fluids. MS procedures, although highly sensitive and specific, are often outside the financial limits of small laboratories and require highly trained personnel. The present procedure

0378-4347/92/$05.00

involving derivatization with PFBSCl provides a relatively less expensive yet rapid and highly sensitive method for the simultaneous quantitation of amphetamine and N-methylamphetamine. Its application to urine and liver samples obtained from rats treated with (−)-deprenyl is described here.

EXPERIMENTAL

Animals

Male Sprague–Dawley rats weighing 225–275 g at the time of surgery were obtained from Bio-Science Animal Services (Ellerlsie, Alberta, Canada). The animals were housed in pairs on a 12-h light–dark cycle at a temperature of 21 ± 1°C. Food and water were freely available. The animals were anesthetized with methoxyflurane and an osmotic minipump (Alzet 2002, Alza, Palo Alto, CA, USA) was implanted subcutaneously in the dorsal thoracic region of each rat. Pumps were replaced after fourteen days to continue the same drug regimen. Experimental groups consisted of nine animals. Filling concentrations for the minipumps were determined using the program described by Greenshaw [15]. Following 24 days of chronic administration of (−)-deprenyl (1 mg/kg per day of the HCl salt) or distilled water, each rat was placed in a metabolic cage and a 24-h urine sample was collected. Rats were then returned to their home cage. After 28 days of drug treatment the rats were killed by guillotine decapitation and the liver was dissected out and immediately frozen on solid carbon dioxide. Urine and tissue samples were stored at −80°C until the time of analysis.

Drugs and chemicals

Amphetamine and methamphetamine were obtained from Smith, Kline & French (Indianapolis, IN, USA). Methoxyflurane was obtained from MTC Pharmaceuticals (Mississauga, Canada). Benzylamine HCl, di(2-ethylhexyl)phosphate (DEHPA) and PFBSCl were obtained from Sigma (St. Louis, MO, USA). The HCl salt of (−)-deprenyl was obtained from Research Biochemicals (Natick, MA, USA). All solvents employed were of highest purity commercially available. Water was double-distilled in a Corning AG-3 all-glass distillation apparatus.

Extraction and derivatization

Partially thawed rat livers were cut into small pieces, weighed and homogenized in five volumes of ice-cold 0.1 *M* perchloric acid (containing 10 mg/100 ml disodium EDTA and 0.05 m*M* ascorbic acid) using a Tri-R Stir-R (Model S 63C) homogenizer and a Teflon pestle and glass mortar (clearance 0.1–0.15 mm). The homogenates were centrifuged (IEC, B-20 centrifuge) for 10 min at 12 000 *g*. Aliquots (3 ml) of supernatant were transferred to a set of tubes and 125 ng of benzylamine were added as an internal standard to all tubes. A set of authentic standards was also included in each assay run and carried through the entire procedure. For urine analysis, frozen samples were allowed to thaw out completely and an aliquot (250 μl to 1 ml) was poured into tubes to which 1 ml of water and 250 ng of benzylamine (internal standard) had been added.

The perchloric acid supernatants of the liver and the diluted urine samples were basified by the addition of solid potassium bicarbonate and the precipitate was removed by a brief centrifugation (1000 *g* for 5 min). The resultant supernatants were transferred to another set of tubes, one tenth the volume of sodium phosphate buffer (pH 7.8) was added, and the supernatants were extracted by shaking with 5 ml of chloroform containing the liquid ion-pairing agent DEHPA (2.5%, v/v) [16]. Following a brief centrifugation (1000 *g* for 5 min), the top aqueous layers were aspirated off and the chloroform layers were each shaken vigorously with 4.0 ml of 0.5 *M* hydrochloric acid for 5 min. After centrifuging the tubes (1000 *g* for 5 min), the acidic layers were transferred to another set of tubes and basified with solid sodium bicarbonate. A solution (4 ml) of ethyl acetate–acetonitrile–PFBSCl (9:1:0.01) was added to each tube. The tubes were shaken vigorously for 15 min and centrifuged (1000 *g* for 5 min); the top organic layers were transferred to a set of clean tubes and taken to dryness under a stream of nitrogen. Each residue was taken up in 300 μl of toluene and an aliquot (1 μl) was used for GC analysis.

Gas chromatography

A Hewlett-Packard (HP) 5890 gas chromatograph fitted with a 15-mCi ^{63}Ni linear electron-

capture detector and a narrow-bore fused-silica capillary column (15 m × 0.255 mm, 0.25-μm film of 5% phenylmethylsilicone as stationary phase) obtained from J & W Scientific (Rancho Cordova, CA, USA) was employed. The carrier gas was helium at a flow-rate of 2 ml/min, and the make-up gas was methane–argon (5:95) at a flow-rate of 35 ml/min. The injection port and detector temperatures were 200 and 325°C, respectively. A splitless injection system was used. The oven temperature was initially set at 105°C, maintained at that level for 0.5 min, and increased at a rate of 15°C/min to a final temperature of 270°C which was maintained for 10 min. A HP 3392A integrator was used to measure the peak areas.

Gas chromatography–mass spectrometry

Coupled GC–MS was used to confirm the structures of the derivatives using both derivatized authentic standards and derivatized urine and liver extracts. The mass spectrometer was a HP 5985A. The system also consisted of a HP 5840A gas chromatograph as the inlet, a HP 2648A graphics terminal, a HP 9876A printer, a HP 7920 disc drive and a HP 21 MX Series E computer. Operating conditions were as follows: ion source temperature, 200°C; interface temperature, 275°C; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 100 a.m.u./s; dwell time, 200 ms. The capillary column and oven temperature program were the same as described above for the GC analysis.

RESULTS AND DISCUSSION

The procedure is rapid and the derivatives produced were stable and exhibited excellent chromatographic properties (see Fig. 1 for typical GC traces). The retention times of the derivatives of benzylamine (the internal standard), amphetamine and N-methylamphetamine were 11.7, 12.4 and 12.9 min, respectively. The standard curves were linear from 1 to 50 ng (liver studies) and from 10 to 1000 ng (urine studies) for both amphetamine and N-methylamphetamine ($r^2 >$ 0.99). The limits of detection were < 1 and 2 ng/g (liver) and 10 ng/ml (urine), respectively. The mean absolute recoveries as determined by using

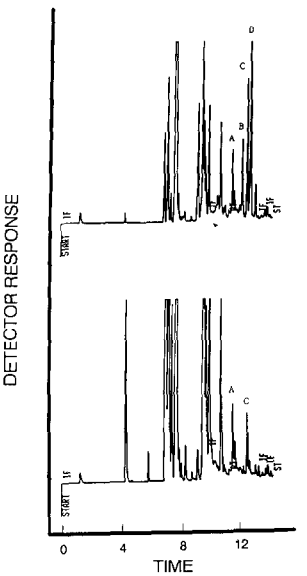

Fig. 1. Gas chromatograms, using a fused-silica capillary column, of derivatized extracts of a urine sample from (top) a rat treated with (−)-deprenyl (1.0 mg/kg per day, subcutaneously, continuous 28-day administration) and (bottom) a vehicle-treated rat. Levels of amphetamine and N-methylamphetamine in the top urine sample were 4.2 and 38.3 μg per 24 h, respectively. Peaks: PFBS derivatives of benzylamine (A) (the internal standard), amphetamine (B), 2-phenylethylamine (C) and N-methylamphetamine (D).

100 ng amphetamine and N-methylamphetamine standards were 94.5 and 78.5%, respectively. Intra-assay coefficients of variation [determined at a variety of concentrations of the drugs ranging from 1 to 1000 ng (n = 6)] ranged from 1.9 to 7.6 and 2.0 to 8.0% for amphetamine and N-methylamphetamine, respectively. The inter-assay coefficients of variation [determined at drug concentrations of 10 and 250 ng per sample (n = 6)] were 8.1 and 2.4%, respectively, for amphetamine and 7.1 and 9.5%, respectively, for N-methylamphetamine. The values obtained for control rat urines spiked with 100 ng each of amphetamine and N-methylamphetamine were 105.9 ± 5.4 and 107.2 ± 13.6 ng, respectively (mean ± S.D., n = 6). The proposed electron-impact MS fragmentations of the PFBS derivatives of benzylamine, amphetamine and N-methylamphetamine are shown in Figs. 2–4.

The mean amphetamine levels in rats treated with (−)-deprenyl were 5.8 ± 1.1 μg per 24-h urine sample and 10.7 ± 1.7 ng/g for liver tissue.

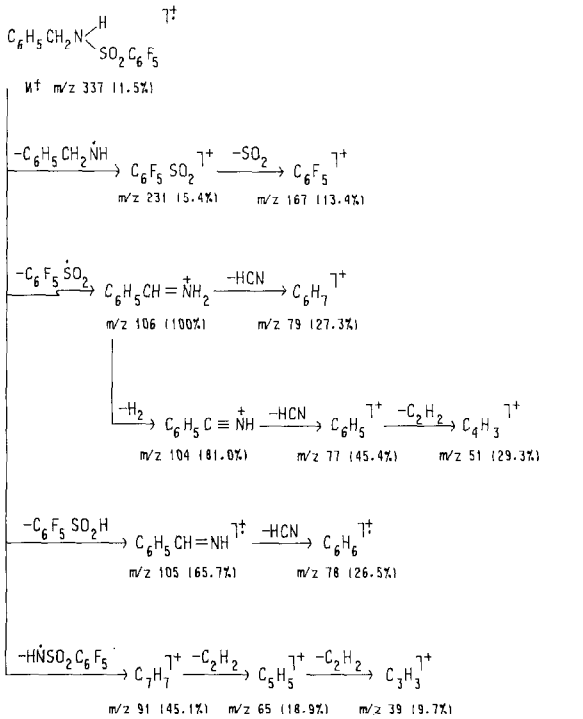

Fig. 2. Proposed electron-impact mass fragmentations of the PFBS derivative of benzylamine, the internal standard. Numbers in parentheses are relative abundances of the individual fragments.

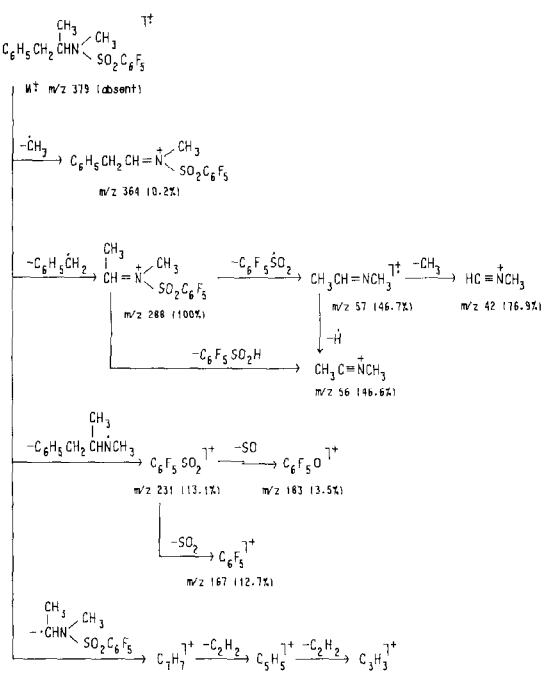

Fig. 4. Proposed electron-impact mass fragmentations of the PFBS derivative of N-methylamphetamine. Numbers in parentheses are relative abundances of the individual fragments.

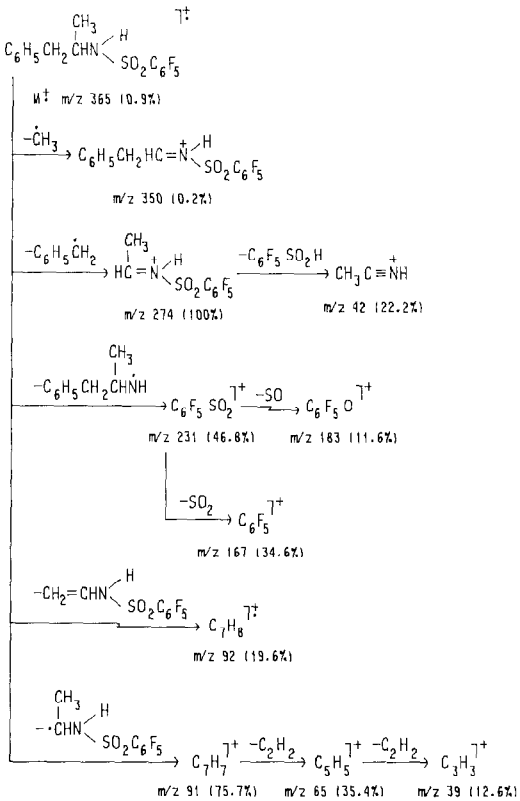

Fig. 3. Proposed electron-impact mass fragmentations of the PFBS derivative of amphetamine. Numbers in parentheses are relative abundances of the individual fragments.

The mean N-methylamphetamine levels in the same rats were 41.3 ± 4.7 μg per 24-h urine sample and 11.9 ± 1.5 ng/g for liver tissue. Peaks corresponding to derivatized amphetamine and N-methylamphetamine were absent in vehicle-treated animals.

In summary, a rapid, convenient procedure has been developed for simultaneous extraction and quantitation of amphetamine and N-methylamphetamine. The method should be readily applicable to other neurochemistry laboratories and particularly useful to those researchers who do not have ready access to mass spectrometry.

ACKNOWLEDGEMENTS

Funding was provided by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

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