Simultaneous Identification of Amphetamine and Methamphetamine Using Solid-Phase Extraction and Gas Chromatography/Nitrogen Phosphorous Detection or Gas Chromatography/Mass Spectrometry

Robert W. Taylor* and Sam D. Le
Los Angeles County Sheriff’s Department, Scientific Services Bureau, Toxicology Section, 2020 West Beverly Blvd., Los Angeles, California 90057-2494
Susan Philip and Naresh C. Jain
Toxicology Department, Rancho Los Amigos Medical Center, Downey, California 90242 and Department of Pharmacology and Nutrition, USC School of Medicine, Los Angeles, California 90033.

Abstract

A method for the simultaneous detection and quantitation of amphetamine and methamphetamine in urine is described. Using solid-phase extraction, amphetamine, methamphetamine, and n-propylamphetamine (internal standard) are extracted from urine samples. Drugs in their free form are identified using gas chromatography/nitrogen-phosphorus detection (GC/NPD), whereas their heptfluorobutyric anhydride derivatives are detected by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring (SIM) mode. Limits of detection for both amphetamine and methamphetamine are approximately 35 ng/mL. The procedure is simple, rapid, and suitable for a large number of specimens (25 or more).

Introduction

As a result of the increase in requests for methamphetamine testing, both clinical and forensic laboratories are developing an intense interest in analytical procedures that can provide simple, rapid, and sensitive methods for analyzing amphetamine and methamphetamine in biological materials. Immunoassays, which can screen a large number of samples for both amphetamine and methamphetamine, are not entirely specific, cross-reacting with other amphetamine-like substances (1). A confirmatory method must be used to verify the presence of amphetamine and methamphetamine. Confirmatory methods for the determination of amphetamine and methamphetamine by gas chromatography with flame ionization detection have been used by many laboratories (2-3). Recently, there have been papers on specific and highly sensitive methods for determination of amphetamine and methamphetamine by gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) (4-6). However, these and other methods describe the use of liquid–liquid extraction procedures as a means of removing amphetamine and methamphetamine from biological materials. These extractions are more lengthy and often involve back extraction techniques to arrive at a cleaner extract.

Consequently, this paper focuses on the solid-phase extraction procedure using Detectabase GC/MS grade extraction columns. The sample extracts are analyzed using a gas chromatograph equipped with either a nitrogen–phosphorus detector or a mass selective detector. This study also investigates the recovery, linearity, and reproducibility of amphetamine and methamphetamine extracted from urine samples using solid-phase extraction.

Experimental

Equipment

Hewlett-Packard 5890A gas chromatographs with either NPD or MSD were used for the analyses. Unimetric’s Chromaton CB-5 methylphenyl polysiloxane fused-silica capillary columns, 15 m × 0.25 mm i.d. with a 0.25-μm film thickness were installed in both GC systems. The injection ports were capillary split injectors with split silanized glass inserts.

For the GC/MS, the carrier gas, helium, was at a flow rate of 1.0 mL/min at 150°C oven temperature with a split ratio of 20:1. The septum purge was 2.0 mL/min. The injector and interface temperatures were 250 and 280°C, respectively. The temperature program was 110 to 140°C at 10°C/min, then 30°C/min to 220°C, and held at 220°C for 3 min. The MSD was used in the electron impact SIM mode programmed to detect the following ions for the heptfluorobutyric anhydride (HFBA) derivatives of amphetamine: 91, 118, and 240; for methamphetamine: 118, 210, and 254; and for n-propylamphetamine: 91, 240, and 282. A dwell time of 100 ms was used for each ion.

Similar conditions were used for GC/NPD with the exception that the detector temperature was 300°C. A Varian 4270 integrator was used to monitor the detector signal. Solid-phase Detectabase extraction columns, used in conjunction with a Multi-Prep 28-place work station, were obtained from Biochemical Diagnostics.

Reagents

All reagents and solvents were of analytical grade. The d-amphetamine HCl, d-methamphetamine HCl, and n-propylamphetamine HCl were purchased from Aldrich Applied Science. Heptfluorobutyric anhydride was purchased from Supelco. The 1M phosphate buffer was prepared by dissolving 176 g of potassium dibasic phosphate in 1 L of deionized water and adjusting the pH to 6.0 with concentrated HCl.

* Author to whom correspondence should be addressed.
Preparation of standards

Two separate stock solutions of \( d \)-amphetamine and \( d \)-methylamphetamine were prepared in methanol at a concentration of 1.0 ng/mL for each drug and stored at 4°C. One stock solution and negative urine sample were used to prepare a standard calibrator at a concentration of 500 ng/mL each of \( d \)-amphetamine and \( d \)-methamphetamine. The other stock solution and negative urine sample were used to prepare a positive control at a concentration of 1000 ng/mL of each drug. A stock solution of \( n \)-propylamphetamine was prepared in methanol at a concentration of 0.75 mg/mL. A working internal standard was prepared by further dilution with methanol to give a concentration of 75 \( \mu \)g/mL.

Sample analysis

Five mL each of standard calibrator, positive control, negative control, or unknown urine samples and 50 \( \mu \)L of the working internal standard were transferred into separate 16-\( \times \)125-mm disposable glass tubes. Two mL of 1M phosphate buffer (pH 6.0) was added to each tube and vortexed for 15 s. The extraction columns were assembled on the Multi-Prep extraction module. The columns were conditioned with 5 mL methanol, followed by 5 mL of 1M phosphate buffer (pH 6.0). The urine samples were poured into the appropriately labeled extraction columns with the vacuum at 3 in. of Hg. After the samples passed through the columns, the top cotton plugs were removed and discarded. The columns were washed with 2 mL of deionized water followed by 2 mL of isopropanol/water (25:75). The extraction beds were dried under vacuum (20 in. of Hg for 5 min), and then 2 mL of hexane was added to each column and again dried under vacuum at 20 in. of Hg for 5 min. The analytes were eluted with 2 mL of 1% HCl in methanol into 13-\( \times \)100-mm screw capped glass tubes. All tubes were dried under a fine stream of dry nitrogen at 30°C. Contents were dissolved in 50 \( \mu \)L of ethyl acetate. This extract was ready for GC/NPD analysis.

Derivatization for GC/MS analysis was accomplished by adding 50 \( \mu \)L of HFBA to each tube. The tubes were capped, vortexed, and incubated at 75°C for 20 min. After 20 min the tubes were uncapped and dried under nitrogen at 30°C. The contents were dissolved in 50 \( \mu \)L of ethyl acetate and 1 \( \mu \)L was injected into the GC/MS. The ions used for quantitation were \( m/z \) 240 for amphetamine, \( m/z \) 254 for methamphetamine, and \( m/z \) 282 for \( n \)-propylamphetamine.

Results and Discussion

Figure 1 is a gas chromatogram of the extracted urine standard using NPD. Figure 2 is the GC/MS report of the same extracted urine standard after derivatization.

Extraction recovery was determined by comparing the peak areas of amphetamine and methamphetamine of extracted urine samples with the peak areas of methanolic standards at the concentration of 500 ng/mL. Recovery was approximately 78% for amphetamine and about 87% for methamphetamine. The assay was linear for both amphetamine and methamphetamine from 50 ng/mL to 7,000 ng/mL.

The within-run and between-run precision was determined for each analyte by analyzing six samples from a prepared urine pool containing 500 ng/mL of amphetamine and methamphetamine each day for five days. The within-run and between-run precision for amphetamine was 6.7% and 8.9%, respectively, and for methamphetamine was 4.9% and 5.6%, respectively.

Deuterated internal standards are normally the chemicals of choice for GC/MS analysis. In this case, a common internal standard was used.
standard was needed that could be used for both GC/MS and GC/NPD analyses. Therefore, \( n \)-propylamphetamine was chosen because it separated well from amphetamine and methamphetamine by GC/NPD and gave suitable ion fragments for GC/MS. The lower cost of \( n \)-propylamphetamine was also a consideration.

HFBA proved to be an acceptable derivatizing reagent (5). Its stability at room temperature was investigated by reinjection of the capped samples for one week with quantitation based on the original calibration data. After seven days, there was no significant difference in quantitation levels or in the chromatography data.

Phenyethylamines, such as phenylpropanolamine, ephedrine, and phentermine, were analyzed by this method to determine if there were any interferences with amphetamine and methamphetamine detection. No interferences were found using this method.

Positive results for methamphetamine may be possible due to the use of Vicks Inhalers which contain the \( R^- \) optical isomer of methamphetamine. Although this method does not differentiate between the \( R^- \) and the \( S^- \) enantiomers, it would be possible to use this extraction procedure and then perform on-column chiral derivatization with \( n \)-trifluoroacetyl-L-prolyl chloride to differentiate the stereoisomer of methamphetamine present in the urine sample (4).

The method is simple, rapid and detects both amphetamine and methamphetamine in urine with a single extraction and analytical procedure. Either GC/NPD or GC/MS can be used to detect either drug. With both detection systems, the peak symmetry and quantitation have been very good and reproducible. The method has proven to be reliable, specific, and sensitive for the determination of amphetamine and methamphetamine in both clinical and forensic toxicology.

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**References**


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