



Extraction of amphetamines and methylenedioxyamphetamines from urine using a monolithic silica disk-packed spin column and high-performance liquid chromatography–diode array detection

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ABSTRACT

To overcome the limitations of solid-phase extraction, we developed a device comprising a spin column packed with octadecyl silane-bonded monolithic silica for extracting amphetamines and methylenedioxyamphetamines from urine. Urine (0.5 mL), buffer (0.4 mL), and methoxyphenamine (internal standard) were directly put into the preactivated column. The column was centrifuged (3000 rpm, 5 min) for sample loading and washed. The adsorbed analytes were eluted and analyzed by high-performance liquid chromatography, without evaporation. The results were as follows: linear curves (drug concentrations of 0.2–20 µg/mL); correlation coefficients >0.99; detection limit, 0.1 µg/mL. The proposed method is not only useful for drugs from biological materials but also highly reproducible for the analysis of these drugs in urine.

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1. Introduction

The separation of drugs or medicines from biological matrices is one of the most important objects in investigations on the toxicological and pharmaceutical properties of drugs. Liquid–liquid or solid-phase extraction (SPE) methods are widely used for extracting, purifying, and enriching drugs and medicines from biological materials. However, these methods involve laborious, intensive, and expensive preparatory procedures. In addition, the organic solvents used are toxic to both the humans and the environment. Therefore, recent investigations have focused on methods to reduce the sample volume required, the analytical time, and the cost, and to eliminate the use of chlorinated solvents. Solid- and liquid-phase microextraction have been reported as novel extraction techniques that offer many advantages in that they require a small sample volume, are easy to perform, enable rapid detection, do not require the use of a solvent [1–3], and can be applied for biological analyses [4–7]. However, these technologies require waiting for at least half an hour for the extraction because the extraction mode of the

analytes is the partition equilibrium between the sample and the extraction phase. A new technique that can be used widely for the extraction of various analytes from different matrices is required. Recently, microextraction by packed sorbent [8] or sorbent packed tip [9–11] has been developed and used for extraction of drugs and medicines. The amount of the adsorbed analytes was limited because the amount of sorbent of the above devices was small; therefore, it was difficult to use analysis of the abuser's samples. The risk of contamination is also caused because the sample and the solution flow in both directions.

Monolithic silica has been investigated as a new type of separation material for high-performance liquid chromatography (HPLC) [12–14]. Although the conventional materials used for SPE were similar to those used for HPLC, monolithic silica has the potentials for drug extraction and purification from biological materials. In our previous study, we packed monolithic silica into a capillary glass tube [internal diameter (ID) 0.2 mm], and the extraction device was created by connecting a microsyringe to the capillary column. We demonstrated that amphetamines in urine could be extracted using this device [15,16]. However, sample filtration was required to avoid blockage within the tube, and thus only one sample could be obtained through a batch processing cycle. In order to overcome these problems, monolithic silica disk was packed into a spin

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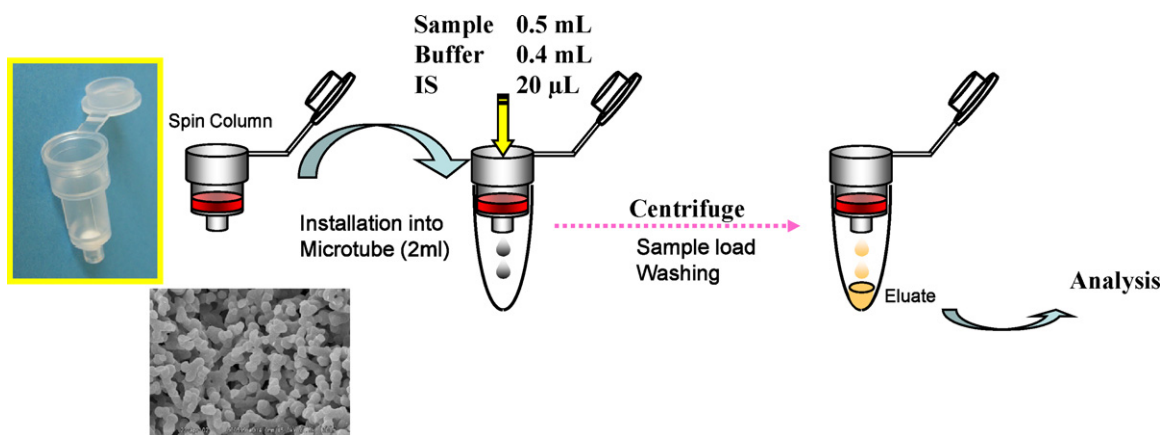


Fig. 1. Summary of the handling procedures for the extraction of amphetamines from urine.

column (Fig. 1), wherein the structure of monolithic silica combined the support body and the surface area for each unit volume is wide by comparing with particle-type silica. The handling procedures such as sample loading, washing, and elution of the target drugs were only exhibited by a centrifugation of the column. In addition, many samples can be processed simultaneously. This method has many advantages: its operation procedure is simple, requires a low eluate volume and does not involve solvent evaporation. When the manifold was used for the extraction of analytes, the scientists need taken care not to dry the column. In this column, the scientists wait for the centrifugation time, and the next step was prepared.

In the present study, we compared the characteristics of a spin column packed with octadecyl silane (C_{18})-bonded monolithic silica disk with those of an SPE cartridge for the extraction of amphetamines and methylenedioxyamphetamines. The preconcentration efficiency of the spin column was excellent as compared to that of the conventional SPE cartridge.

2. Experimental

2.1. Materials

Methamphetamine (MA) hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan). Amphetamine (AP) hydrosulfate was synthesized in our laboratory, and its purity was verified at the National Institute of Technology and Evaluation (Tokyo, Japan). 3,4-Methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) were purchased from Sigma (St. Louis, MO, USA). Methoxyphenamine [internal standard (IS)] was obtained from Wako (Osaka, Japan). To obtain the stock standard solutions (1.0 mg/mL), the drugs were dissolved in 0.01 M HCl and stored at 4 °C in a refrigerator. The spin column was supplied by GL Sciences (Tokyo, Japan). Drug-free urine was collected from a healthy adult male and was maintained at –20 °C until the time of analysis.

2.2. Spin column

Monolithic silica was manufactured by combining the sol–gel reaction with phase separation and a subsequent solvent exchange treatment [17]. Tetraethoxysilane (Shin-Etsu Chemical, Japan) was added to 1 M aqueous solution of nitric acid [in the presence of polyethylene oxide (PEO; Aldrich, USA) with an average molecular mass of 100,000]. The mixture was stirred for 15 min at room temperature. The solution was kept at 40 °C for gelation and aging over 15 h. The aged gel was immersed in 1.5 M aqueous urea solu-

tion at 110 °C for 20 h. After drying at 40 °C for 24 h, the gel was heat-treated at 600 °C for 5 h. The surface of monolithic silica gel was chemically modified to octadecyl groups (C_{18}) for applying to the reversed solid phase [18]. A monolithic silica rod (C_{18} -bonded; ID, 4.3 mm, silica skeleton size, 10 µm), with a through-pore size of 15 µm and a meso-pore size of 10 nm, was prepared for phase separation by polymerizing tetraethoxysilane in the presence of PEO. The monolithic silica rod was then cut to a length of 1.5 mm and fixed into the spin column via supersonic adhesion.

2.3. Extraction

When sample loading, washing, and elution of the analytes, the spin column was installed into a siliconized microtube (2 mL). Prior to extraction, the column was preactivated with 0.5 mL of acetonitrile and 0.5 mL of deionized water by centrifugation at 3000 rpm for 5 min. The urine sample (0.5 mL), buffer (0.4 mL), and methoxyphenamine (IS; concentration, 0.1 mg/mL; volume, 20 µL) were directly put into the preactivated spin column, and the column was centrifuged at 3000 rpm for 5 min. The column was then washed with 0.5 mL of a suitable solvent by centrifugation. Finally, the column was installed into a new microtube, and the analytes that were adsorbed onto the column were eluted with the mobile phase (0.2 mL). Further, 10 µL of the eluate was introduced into the HPLC system.

If the amphetamine and methylenedioxyamphetamine concentrations in the urine samples were expected to exceed the upper limit of the calibration range, the samples were diluted 1:10 prior to extraction.

2.4. HPLC conditions

The chromatographic analysis was performed on a Shimadzu (Kyoto, Japan) LC-10ADVP system consisting of a SIL-10AD auto-injector, an LC-10AD model pump, a CTO-10A column oven, an M-10A diode array detector, and a DGU-4A degasser. The data were analyzed on a Fujitsu FMV computer with an LC workstation (Class VP). The analysis conditions have been described in a previous report [19]. Separation was achieved using a reversed-phase column (Discovery C_{18} ; 150 mm × 4.6 mm ID; Supelco, Tokyo, Japan). The mobile phase was composed of acetonitrile–phosphate buffer (pH 3.0, containing 20 mM sodium octanesulfate) (25:75, v/v). The analysis was conducted at 40 °C, and the flow rate was 1.0 mL/min. The absorbance of the analytes was monitored at 200–350 nm for qualitative analysis and at 215 nm for quantitative analysis.

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