Journal of Chromatography A, 1447 (2016) 47–56

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of amphetamines in hair by integrating sample disruption, clean-up and solid phase derivatization

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ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form 14 April 2016
Accepted 14 April 2016
Available online 20 April 2016

Keywords:
Amphetamines
Matrix-solid phase dispersion (MSPD)
Hair
Drug analysis
Solid phase derivatization
Liquid chromatography

ABSTRACT

The utility of matrix solid phase dispersion (MSPD) for the direct analysis of amphetamines in hair samples has been evaluated, using liquid chromatography (LC) with fluorescence detection and precolumn derivatization. The proposed approach is based on the employment of MSPD for matrix disruption and clean-up, followed by the derivatization of the analytes onto the dispersant-sample blend. The fluorogenic reagent 9-fluorenylmethyl chloroformate (FMOC) has been used for derivatization. Different conditions for MSPD, analyte purification and solid phase derivatization have been tested, using amphetamine (AMP), methamphetamine (MET), ephedrine (EPE) and 3,4-methylenedioxymethamphetamine (MDMA) as model compounds. The results have been compared with those achieved by using ultrasound-assisted alkaline digestion and by MSPD combined with conventional solution derivatization. On the basis of the results obtained, a methodology is proposed for the analysis of amphetamines in hair which integrates sample disruption, clean-up and derivatization using a C_{18} phase. Improved sensitivity is achieved with respect to that obtained by the alkaline digestion or by the MSPD followed by solution derivatization methods. The method can be used for the quantification of the tested amphetamines within the 2.0–20.0 ng/mg concentration interval, with limits of detection (LODs) of 0.25–0.75 ng/mg. The methodology is very simple and rapid (the preparation of the sample takes less than 15 min).

1. Introduction

In recent years hair has gained popularity as an alternative to urine and blood in the investigation of illicit amphetamines consumption because sample collection is non-invasive and can be used to establish long intake history of abusers. In addition, the risk of sample adulteration and/or deterioration during storage and transportation is minimized. The analysis of amphetamines in hair has also become important in the clinical and forensic fields [1]. Consequently, an increasing number of methods are being developed to identify and quantify amphetamines as well as many other drugs in hair samples.

The analysis of amphetamines in hair typically entails a prior washing step for sample decontamination, followed by the extraction of the analytes from the hair matrix and finally, the processing of the obtained extracts by LC or by gas chromatography (GC). Many procedures also include a derivatization step to improve the chromatographic separation and/or detection [2,3]. Whereas the sensitivity attainable by modern analytical techniques is suitable for most applications, the isolation of amphetamines from hair continues to be a critical step of the analysis. An alkaline digestion is typically used to release the target compounds from the sample matrix [4–6], but other strategies such as the extraction with an acid solution, an organic solvent or with mixtures of solvents have also been proposed [7–15]. Most of the described methods require an additional treatment of the extracts in order to remove co-extracted substances, especially if mass spectrometry (MS) with electrospray ionization is going to be used for detection [2]. For analyte purification a reextraction into an organic solvent and/or clean-up of the extracts by solid-phase extraction (SPE) is the most commonly used options [3]. As a result, sample preparation is generally time-consuming and prone to contamination and loss of the analytes.

Today, many research efforts are oriented towards the development of simplified sample treatments which reduce the time of analysis, the consumption of chemicals and the generation of

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http://dx.doi.org/10.1016/j.chroma.2016.04.036
0021-9673/

wastes. In this regard, different techniques have been developed to simplify the analysis of solid samples, and also to overcome the problems typically encountered with traditional treatments such as Soxhlet extraction and digestion-based methods. Successful examples for the extraction of amphetamines from hair are microwave-assisted extraction [16] and ultrasound-assisted extraction [17,18]. An alternative method termed micropulverized extraction has been described by Miyaguchi et al. [19,20] which involves the pulverization of the samples in extraction tubes and the simultaneous extraction into a buffer using a specially designed extraction device.

Matrix solid phase dispersion (MSPD) has emerged as an attractive approach for the analysis of solid and semi-solid samples. This technique involves the grinding of a portion of the sample with an abrasive solid material, typically a SPE sorbent. The grinding process disrupts the sample structure and disperses it over the surface of the extractive material. The blend is then packed into a pre-fritted SPE tube, and then the analytes are selectively desorbed with an appropriate solvent. In most procedures, the purity of the final MSPD extracts is improved by subsequent extraction, many often by SPE, or simply by packing an extra sorbent layer at the bottom of the SPE tube. Today MSPD is well implemented in areas such as food or environmental analysis, but its application in the clinical and forensic fields is still scarce [21,22]. MSPD was first applied for the isolation of abuse drugs from hair by Míguez-Framil et al. [23–25]. The authors used alumina as dispersant, and the extracts collected after MSPD were further purified by SPE with Oasis HLB cartridges. Then, the purified extracts were treated by a conventional approach which involved evaporation to dryness, redisolution, derivatization and GC analysis. This approach was applied to the analysis of cocaine and opiates.

During the past years, we have developed different methods for the analysis of amphetamines and other amines based on their isolation from the matrix with different solid sorbents, followed by a chemical derivatization of the retained analytes onto the sorbents. This methodology offers clear advantages over derivatizations carried out in liquid phase such as the improvement of the reaction rates, the simplification of the overall derivatization procedure and versatility, as it can be used with a variety of analytes, derivatization reagents and extraction formats, including SPE cartridges [26] and disks [27], packed precolumns [28,29] and open capillary columns connected to the chromatographic column [30], and SPME fibers [31]. The reliability of the solid phase derivatization approach was demonstrated for the analysis of amphetamines in liquid samples such as plasma and urine. In principle, the solid phase derivatization approach could be combined with MSPD if a retentive supporting material is used as dispersant. However, to the best of our knowledge, no methods which integrate MSPD and solid phase derivatization have been reported for the analysis of solid samples.

In the present work, we have evaluated for the first time the possibility of combining MSPD and the solid phase derivatization technique as an alternative to simplify sample preparation for the determination of amphetamines in hair. Based on previous studies, FMOC has been selected for the precolumn derivatization of the analytes as it reacts with a variety of amines under mild conditions, providing excellent sensibility with either UV or fluorescence detection [28,32]. The reliability of the proposed approach has been tested by comparing the results with those obtained by using ultrasound-assisted alkaline digestion and by MSPD followed by a conventional liquid-phase derivatization. On the basis of the results obtained, a new approach is proposed for the analysis of AMP, MET, EPE and MDMA in hair which integrates sample disruption, analyte isolation and derivatization.

2. Experimental

2.1. Reagents and solutions

All the reagents were of analytical grade. AMP sulphate, MDMA hydrochloride, EPE hydrochloride and MET hydrochloride were obtained from Sigma (St. Louis, MO, USA). FMOC was purchased from Aldrich (Stenheim, Germany). Acetonitrile and methanol were of HPLC grade (Scharlau, Barcelona, Spain). Sodium hydrogen carbonate, sodium hydroxide and hydrochloric acid were obtained from Panreac (Barcelona, Spain).

Stock standard solutions of the amphetamines (1000 μg/mL) were prepared in water. Working solutions of the analytes were prepared by dilution of the stock solutions with water. Water was deionized and filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain). All solutions were stored in the dark at 4 °C.

The FMOC solutions, at concentrations ranging from 0.01 to 1 mM, were prepared daily by dissolving the compound (97% purity) in acetonitrile. The 0.05 M carbonate buffer used throughout the study was prepared by dissolving the appropriate amount of sodium hydrogen carbonate in water, and then by adjusting the pH to the required value by adding 10% NaOH (w/v).

Bondesil C_{18} phase (40 μm) was obtained from Agilent Technologies (Waldbronn, Germany). Florisil (60–100 mesh) and silica gel (70–230 mesh) were obtained from Aldrich.

2.2. Apparatus and chromatographic conditions

The chromatographic system consisted of a quaternary pump (Hewlett-Packard 1050 Series, Palo Alto, CA, USA), a high-pressure six-port injection valve (Rheodyne Model 7000) with a 20 μL injection loop, and a fluorimetric detector (Hewlett-Packard, 1046 Series), which operated at excitation and emission wavelengths of 264 nm and 313 nm, respectively. The detector was linked to a data system (Hewlett-Packard, HPLC ChemStation) for data acquisition and calculation.

A LiChrospher 100 RP18, 5 μm, 125 mm × 4 mm i.d. column (Merck, Darmstadt, Germany) was used for separation under gradient elution conditions. In preliminary experimets the gradient elution program was optimized to achieve suitable resolution of the FMOC-amphetamine derivatives in the minimum time of analysis. The initial composition of the mobile phase was water-acetonitrile 60:40 (v/v). The acetonitrile content was increased to reach 80% at 12 min, and then kept constant until the end of the chromatographic run (16 min). At the end of the run the mobile-phase composition was linearly returned to the original values in 2 min. These conditions were maintained for 3 min before injecting the next sample. The mobile-phase flow rate was 1 mL/min. The solvents were filtered through 0.45 μm nylon membranes (Teknokroma) and degassed with helium before use. In all instances, the volume of sample injected into the chromatograph was 20 μL and samples were filtered using 0.22 μm nylon syringe filters (Teknokroma).

An ultrasonic bath (300 W, 40 kHz, Sonitech, Guarnizo, Spain) was employed for the alkaline digestion method.

Microscopic images were taken with a Nikon microscope ECLIPSE E200LED MV Series (Nikon Corporation, Tokyo, Japan) under bright-field illumination and using a 10× objective. A NisElements 4.20.02 software (Nikon Corporation) was used for acquiring the images.

2.3. Analysis of hair samples

2.3.1. Sample pretreatment

Hair samples were collected from different volunteers. Portions of 1 g of hair were washed three times with 100 mL of water, and