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Determination of MDMA, MDA, MDEA and MBDB in oral fluid using high performance liquid chromatography with native fluorescence detection

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Abstract

This paper describes the analytical methodology for the determination of MDMA, MDA, MDEA and MBDB in oral fluid. After a liquid–liquid extraction, the analysis was carried out by high performance liquid chromatography (HPLC), with fluorescence detection. The detector wavelength was fixed at 285 nm for excitation and 320 nm for emission. The mobile phase, a mixture of phosphate buffer (pH = 5) and acetonitrile (75:25), and the column, Kromasil 100 C8 5 μm 250 mm × 4.6 mm, allowed good separation of the compounds in an isocratic mode in only 10 min. The method was validated and showed good limits of detection (2 ng/mL) and quantitation (10 ng/mL) for all the amphetamine derivatives. No interfering substances were detected. A stability study of these compounds in oral fluid stored at three different temperatures (−18, 4 and 20 °C) over 10 weeks was conducted, showing a time-dependent degradation of the four compounds.

Keywords: MDMA; MDA; MDEA; MBDB; Oral fluid; HPLC; Fluorescence

1. Introduction

Drug dependency is a very serious health problem in the world. Many substances are used as recreational drugs including the amphetamine derivatives, MDMA (3,4-methylenedioxymethamphetamine), MDA (3,4-methylenedioxyamphet amine), MDEA (3,4-methylenedioxyethylamphetamine) and MBDB (*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butamine), as stimulants or hallucinogens. Their use is increasing sharply in the last few years. In Spain the number of tablets confiscated increased 62% between 2001 and 2002, whereas the number of abuse and dependent ecstasy treatments increased from 226 in 1996 to 335 in 2001 [1].

For these reasons it is very useful to have a very quick, sensitive and specific procedure for detecting these drugs in biological samples in clinical and forensic settings.

Oral fluid (OF) is considered as the main alternative matrix to blood to document recent use of medicines or drugs of abuse [2]. OF contains saliva and other fluids and substances which are present in oral cavity. Substances tend to be detectable in OF for shorter periods than in urine, typically for the 12–24 h after consumption [3]. The greatest advantages of OF are non-invasive sample collection and staff can directly observe patients when they produce the samples, usually by placing a collection tube or device in

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their mouth; this assures staff that the sample comes from the patient and the procedure respects the patients' dignity. The disadvantage of OF is that people are sometimes unable to produce sufficient amounts of fluid for analysis.

Only a few studies have published the detection of amphetamine derivatives in OF. The technologies applied were GC–MS [2,4] and LC–MS/MS [5–7]. GC–MS is a very sensitive and specific method, but its main disadvantage is that amphetamines need derivatization, which is time consuming and expensive. LC–MS/MS is one of the best technologies but there are a lot of toxicology laboratories that cannot afford such instrumentation.

HPLC with fluorescence detection is among the most used techniques for the analysis of MDMA and analogue compounds. In the literature Sadeghipour and Veuthey developed a method in serum and tablets [8], Herráez-Hernández et al. in plasma and urine [9], Clauwaert et al. in whole blood, serum, vitreous humor and urine [10], da Costa and da Matta Chasin in urine [11], Tagliaro et al. [12] and Kaddoumi et al. [13] in hair. Mancinelli et al. [14] applied a fluorimetric procedure for the determination of amphetamines in biological matrices and street samples, and gave a brief reference to OF.

Stability must be included in the method validation. Knowledge of the stability of a drug is of importance for toxicologists in several situations, limitations of a logistic nature often introduce variable time intervals between sampling of the matrices and analysis. Even in these kinds of situations, the toxicologist should be able to determine if interpretation of the obtained quantitative data can be performed reliably [15]. Only two stability studies were found in the literature. Garret et al. [16] investigated the stability of MDA and MDMA during 47 days in frozen plasma, and Clauwaert et al. [15] studied the stability of MDA, MDMA, MDEA and MBDB over a period of 21 weeks in serum, whole blood, water and urine. No references were found on the stability of MDMA, MDA, MDEA and MBDB in OF.

We present a rapid, simple and specific validated method that allows the simultaneous determination of MDMA, MDA, MDEA and MBDB in OF using HPLC with fluorescence detection, including a preliminary stability study of amphetamine derivatives over a period of 10 weeks in OF.

2. Experimental

2.1. Chemicals

All the reagents were of analytical-reagent grade. Acetonitrile, methanol, potassium dihydrogen phosphate, sodium hydroxide were purchased from Merck (Darmstadt, Germany), and sodium sulphate anhydride from Panreac (Barcelona, Spain). Standards of MDMA, MDA, MDEA and MBDB were obtained from Lipomed (Arlesheim, Switzerland). Standard solutions of each compound were prepared at a concentration of 1 mg/mL in methanol. Toxitubes A® from Varian (Middelburg, The Netherlands) were used for the liquid–liquid extraction.

2.2. Equipment and chromatography

The chromatographic system used was a model 616 Pump, model 717 plus Autosampler and model 474 Scanning Fluorescence Detector from Waters (Milford, Massachusetts). The detector wavelength was fixed at 285 nm for excitation and 320 nm for emission, Gain 100. A Kromasil 100 C8 5 μm 250 mm × 4.6 mm column from Teknokroma (Barcelona, Spain) was used for the separation of analytes and, as guard cartridge, a Spherisorb® S5 C8 4.6 mm × 10 mm from Waters (Milford, Massachusetts). The mobile phase used was a mixture of potassium dihydrogen phosphate (KH_2PO_4) 0.03 M pH = 5 and acetonitrile (75:25) in isocratic mode, pumped at 1 mL/min flow-rate.

2.3. Sample preparation

2.3.1. Spiked samples

Different volumes of the working standard solutions, that had been prepared in water, were added to 1 mL of drug-free OF (obtained from laboratory staff) to obtain spiked OF samples at 10, 25, 50, 125 and 250 ng/mL.

2.3.2. Liquid–liquid extraction procedure

One milliliters of OF and 1 mL of distilled water were introduced in a Toxitube A®. After shaking during 15 min and centrifuging during 10 min at 4800 rpm, the organic layer (1.4 mL) was collected and transferred to a clean tube. Five milliliters of NaOH 0.05 M was added. After shaking for 15 min, and before centrifuging 10 min at 4800 rpm, 8.5 g of anhydrous sodium sulphate was added.

One milliliters of the organic layer was transferred to a conical tube containing 50 μL of methanolic HCl (5 M hydrochloric acid in methanol) and evaporated to dryness under a fine stream of nitrogen at 30 °C. The residue was reconstituted with 200 μL of mobile phase and a 20 μL aliquot was injected into the HPLC system.

2.4. Validation of the method

The method was validated for linearity, intra- and inter-assay precision, recovery, limits of detection and quantitation, selectivity and stability. The analytical validation was performed according to the guidelines of the FDA [17].

2.4.1. Linearity

Calibration curves were carried out for MDA, MDMA, MDEA and MBDB at concentrations between 10 and 250 ng/mL (10, 25, 50, 125, 250 ng/mL). The simplest model that adequately described the concentration–response relationship was linear regression.