Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 814 (2005) 93–98
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of amphetamine-derived designer drugs in human urine by SPE extraction and capillary electrophoresis with mass spectrometry detection

Gianpiero Boatto*, Maria Nieddu, Antonio Carta, Amedeo Pau, Michele Palomba, Battistina Asproni, Riccardo Cerri

Dipartimento Farmaco Chimico Tossicologico, Università di Sassari, Italy

Received 25 June 2004; accepted 4 October 2004
Available online 11 November 2004

Abstract

In recent years, a number of newer designer drugs have entered the illicit drug market. The methylenedioxy-derivates of amphetamine represent the largest group of designer drugs. This paper describes a method for screening for and quantification of ten 2,5-methylenedioxy-derivates of amphetamine and phenylethylamine in human urine, using capillary electrophoresis coupled to electrospray ionisation–mass spectrometry (CE–ESI–MS). Prior to CE–MS analysis, a simple solid-phase extraction (SPE) was used for sample cleanup. The method was validates according to international guidelines.

Keywords: Methylenedioxy-derivates; Amphetamine; Phenylethylamine; Designer drug; Urine; Capillary electrophoresis; Mass spectrometry; Solid-phase extraction (SPE)

1. Introduction

In the last few decades, the amphetamine designer drugs have gained popularity as recreational drugs and are used mainly for their agreeable stimulating effects, especially in gatherings known as “raves” [1]. The popularity of the methylenedioxyamphetamine derivates can be attributed to their psychotropic effects and the so-called entactogenic effects [2].

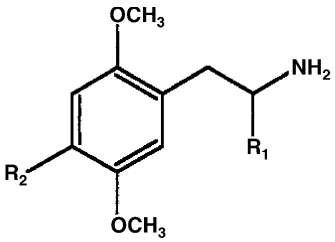
The methylenedioxy-derivates of amphetamine represent the largest group of designer drugs. The term “designer drug” includes compounds that have been chemically altered from federally controlled substances in order to bypass the legal regulations and to produce more potent substances. Examples are derivates with one or two methoxy groups over the phenyl-ring, with halogens, sulphur and methyl group attached against each other [3].

Up until now, nearly 200 different derivates have been synthesised and described by Shulgin and Shulgin [4]. Only a limited number of these derivates are known from Europe, although both halogen and sulphur-derivates have been detected in confiscated tablets or biological samples [5–7].

Monitoring of amphetamines and designer drugs in human urine is successfully used for clinical and forensic application and in surveillance of drug substitution. To date, the determination of amphetamines in urine samples has been mainly on GC–MS [8–12] and HPLC–DAD [7,13–14]. In the last few years, the liquid chromatography coupled mass spectrometry (LC–MS) has developed rapidly in forensic and clinical applications as well as in analysis of amphetamines in biological samples [15–17]. On the other hand, the on-line combination of capillary electrophoresis (CE) and mass spectrometry (MS) has been established as a powerful method for forensic urine samples screened [18–21].

* Corresponding author. Tel.: +39 079228725; fax: +39 079228727.
E-mail address: gboatto@uniss.it (G. Boatto).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.10.010



	Compounds	R_1	R_2
1	2,5-dimethoxy-amphetamine HCl	CH_3	H
2	2,5-dimethoxy-4-methyl-phenethylamine HCl	H	CH_3
3	2,5-dimethoxy-4-methyl-amphetamine HCl	CH_3	CH_3
4	2,5-dimethoxy-4-chloro-amphetamine HCl	CH_3	Cl
5	2,5-dimethoxy-4-nitro-phenethylamine HCl	H	NO_2
6	2,5-dimethoxy-4-nitro-amphetamine HCl	CH_3	NO_2
7	2,5-dimethoxy-4-bromo-phenethylamine HCl	H	Br
8	2,5-dimethoxy-4-bromo-amphetamine HCl	CH_3	Br
9	2,5-dimethoxy-4-iodo-phenethylamine HCl	H	I
10	2,5-dimethoxy-4-iodo-amphetamine HCl	CH_3	I

Fig. 1. Chemical structures of amphetamine derivates studied in this work.

This paper describes a method for screening for and quantification of ten 2,5-methylenedioxy-derivates of amphetamine and phenylethylamine (Fig. 1) in human urine, by capillary electrophoresis coupled to electrospray ionisation–mass spectrometry (CE–ESI–MS). Using an aqueous pH 4.5 buffer composed of ammonium acetate/acetic acid, the CE–MS analysis provided data that permitted the unambiguous confirmation of these drugs in human urine.

This procedure is simple, clean and should be easily applied to epidemiological and clinical studies. In addition, the mass spectrum of these amphetamine derivates can be useful for future their identification with CE–MS in biological matrices as well as in confiscated tablets.

2. Experimental

2.1. Materials

The 2,5-dimethoxy-derivates of amphetamine and phenylethylamine (Fig. 1) was synthesised in our laboratory at their maximum level of purity using a slight modification of a method described in literature [4]. The product characterisation by ^1H and ^{13}C NMR spectrometry was carried out using a Bruker AMX 500. Melting points (mp) were determined with a Kofler hot stage microscope. IR spectra were carried out using a Perkin-Elmer 1760-X IFT.

Deionised and distilled water was purified through a Milli Q water system (Millipore). Other reagents and solvents used were purchased at the highest commercial quality. Bond Elut C_{18} solid-phase extraction (SPE) columns (100 mg/ml) were purchased from Alltech (Italy) and mounted on a VacElut vacuum manifold (Supelco, USA).

Aqueous stock solutions (1.0 mg/ml) of amphetamine derivates were prepared, stored at −20 °C, and diluted with Milli Q water to appropriate concentrations before use.

Drug-free urine collected from a healthy adult male was used to make blank and spiked samples containing amphetamine derivates. The urine samples were kept frozen at −20 °C until analysed.

2.2. CE–electrospray ionisation (ESI)–MS set up

Separations in capillary electrophoresis were performed using a model Hewlett-Packard^{3D} CE system coupled with at Agilent 1100 series LC/MSD (Agilent Technologies) via an electrospray ionisation interface.