Journal of Chromatography A, 1356 (2014) 258–265

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Utilization of micellar electrokinetic chromatography–tandem mass spectrometry employed volatile micellar phase in the analysis of cathinone designer drugs

Martin Švidrnoch[a], Ludmila Lněníčková[a], Ivo Válka[b], Peter Ondra[b], Vítězslav Maier[a,*]

[a] Regional Centre of Advanced Technologies and Materials, Department of Analytical Chemistry, Faculty of Science, Palacký University in Olomouc, 17. Listopadu 12, Olomouc CZ-77146, Czech Republic
[b] Department of Forensic Medicine and Medical Law, Faculty Hospital, Hněvotínská 3, Olomouc CZ-77515, Czech Republic

ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form 13 June 2014
Accepted 17 June 2014
Available online 25 June 2014

Keywords:
Cathinone
Designer drug
Micellar electrokinetic chromatography
Mass spectrometry
Perfluorocarboxylic acid
Toxicological analysis

ABSTRACT

A micellar electrokinetic chromatography method with tandem mass spectrometry has been developed for the selective separation, identification and determination of twelve new designer drugs from the group of synthetic cathinones. Ammonium salt of perfluorooctanoic acid at various concentrations as a volatile background electrolyte (BGE) to create micellar phase was studied for separation of selected synthetic cathinones with direct tandem mass spectrometry without significant loss of detection sensitivity. The optimized BGE contained 100 mM perfluorooctanoic acid with 200 mM ammonium hydroxide providing acceptable resolution of studied drugs in the MEKC step. In order to minimize interferences with matrix components and to preconcentrate target analytes, solid phase extraction was introduced as a clean-up step. The method was linear in the concentration range of 10–5000 ng mL^{-1} and the limits of detection were in the range of 10–78 ng mL^{-1}. The method was demonstrated to be specific, sensitive, and reliable for the systematic toxicological analysis of these derivatives in urine samples.

1. Introduction

Along with the rapid development of new pharmaceuticals for humans, the clandestine synthesis of new illegal drugs is increasing as well. There are many derivatives of prohibited substances [1,2] collectively referred to as "New Designer Drugs (NDDs)" which appear on the illegal market much faster than either the governments or the law enforcement agencies are able to respond and include them to the list of prohibited substances. Because of the fact that most of the NDDs may have unknown physiological effects or can cause serious health consequences [3], it is desirable to develop new analytical methods for identification and determination of such compounds in various samples of forensic and toxicological interests.

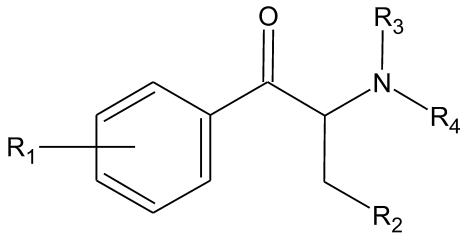
Moreover, most of the NDDs often could not be detected by the standard screening methods (e.g. immunochemical or colour preliminary tests) [3]. Among the wide group of NDDs the synthetic cathinones are one of the class, whose consumption and spread have increased lately. Synthetic cathinones are derivatives of a naturally occurring β-keto phenylethylamine molecule based on the structure of cathinone. Cathinones are abused for their cocaine and amphetamine-like physiological effects. Overview of the chemistry, pharmacology and toxicology was published by Kelly [4]. Synthetic cathinones undergoes phase I. metabolism in human body. The main metabolic pathway consists of reduction of the β-keto group to an alcohol which is catalysed by the liver microsomal enzymes. Studies on the metabolism of methcathinone derivatives in rats and humans have shown that they are *N*-demethylated, the keto group is reduced to hydroxyl, and the ring alkyl groups are oxidized. Generally, cathinones are metabolized after administration, but the unmetabilized cathinones are also presented in urine after administration [5]. Otherwise, few formal studies have been made on the pharmacokinetics or pharmacodynamics of the ring-substituted cathinones [5,6].

However, pharmacokinetics and also pharmacodynamics is rather unpredictable because of the variable concentration and purity of cathinone products abused [7].

Separation methods combined with a mass spectrometry detection such as LC–MS and GC–MS have been the most frequently used techniques for identification and determination of synthetic cathinones for analytical toxicology purposes in

* Corresponding author. Tel.: +420 585634542; fax: +420 585634433.
E-mail address: vitezslav.maier@upol.cz (V. Maier).

http://dx.doi.org/10.1016/j.chroma.2014.06.058
0021-9673/



Compound	R_1	R_2	R_3	R_4
Cathinone	H	H	H	H
Buphedrone	H	Me	H	Me
Mephedrone	4-Me	H	H	Me
4-methylethcathinone	-Me	H	H	Et
Methedrone	4-MeO	H	H	Me
3-fluoromethcathinone	3-F	H	H	Me
Flephedrone	4-F	H	H	Me
Ethylone	3,4-methylendioxy	H	H	Et
Methylone	3,4-methylendioxy	H	H	Me
Ephedrone	H	H	H	Me
Methylendioxypyrovalerone	3,4-methylendioxy	Et	Pyrrolidinyl	
Naphyrone	β-naphthyl (instead of Ph)	Et	Pyrrolidinyl	

Fig. 1. The scheme of chemical structures of studied synthetic cathinon derivatives.

the last several years [8–10]. An HPLC–QqQ–MS method for separation and determination of 16 cathinones and ephedrines (namely cathinone, methcathinone, ethcathinone, amfepramone, mephedrone, flephedrone, methedrone, methylone, butylone, cathine, norephedrine, ephedrine, pseudoephedrine, methylephedrine and methylpseudoephedrine) in a human liver and post-mortem materials was published by Sorensen et al. [11]. Deproteination by methanol was applied on real samples and the observed LODs varied from 0.5 to 3 $\mu g\,L^{-1}$. Another screening method for designer amphetamines, tryptamines and piperazines in the serum employed LC–MS/MS was published by Wohlfarth et al. with LODs varied between 1.0 and 5.0 $ng\,mL^{-1}$ [12]. A review dealing with some aspects of characteristic fragmentations based on the GC–EI/MS and LC–ESI/QTOF–MS spectra of cathinones was published by Zuba [13]. An HPLC–QqQ–MS method for an analysis of thirty new designer drugs (phenylethylamines, tryptamines, cathinones and piperazines) after the SPE extraction in post-mortem materials with the LODs about 10 $pg\,mL^{-1}$ was reported by Swortwood et al. [14]. Shima et al. [15] published an article concerning the cathinones methabolites.

High resolution mass spectrometry (LTQ/Orbitrap) was used for the direct identification of six cathinone homologues in forensic samples [16]. For further reading, Meyer et al. [17] published a review which summarizes the procedures for the analysis of β-keto-amphetamines, pyrrolidinophenones, tryptamines, and synthetic cannabinoids in the last four years.

A GC–MS screening method without any derivatization procedure for separation and identification of 16 cathinone derivatives for forensic purposes was published by Nic Daeid et al. [18], although the quantification and validation were not reported. A metabolic study of the β-naphyrone derivatives by GC–MS and LC–HR–MS/MS was published by Meyer et al. [19]. Martin et al. published a method for determination of mephedrone after chronic abuse in hair using CG–MS [20]. The observed concentrations in hair were in the range from 0.2 to 313.2 $ng\,mg^{-1}$. A CE–MS method for separation and identification of the cathinone-based new designer drugs has not been published yet. Several articles [21–23] deal with an enantioseparation by CE of chiral cathinones only.

The CE coupled with tandem mass spectrometry detection (CE–MS/MS) represents another alternative which allows both the separation and the identification or quantification of the NDDs in a large scale of samples including biological material such as blood, serum or urine as the most frequent samples for toxicological purposes. The CE has been claimed to offer advantages over chromatography for the separation of amphetamines, cathinones, phenylethylamines which are difficult to be analysed by the GC without derivatizations and by LC where the sorption of the basic amines occurs in common types of stationary phases (reversed phase) [10]. Since the structures of the cathinones are similar and also the pK_a values are very close, it is claimed to be very difficult to separate them by the CE with a sufficient resolution. Generally, hyphenation of the CE with MS has some experimental limitations due to the necessity of using volatile buffers and additives only, thus the manipulation of selectivity to reach sufficient resolution between the analytes in a reasonable time is complicated.

In this work we focused on development of the micellar electrokinetic chromatography with tandem mass spectrometry (MEKC–ESI–MS/MS) method for the separation and detection of selected most abused cathinones. Chemical structures of the studied designer drugs are schematically shown in Fig. 1. At the CE side it consisted of the selection of suitable volatile electrolyte in terms of achieving the best selectivity and resolution, respectively. At the MS/MS side parameters such as the electrospray voltage, sheath gas flow, pressure and temperature, sheath liquid composition, flow rate and collision energies were all evaluated to obtain the highest detection sensitivity for the studied cathinones. The fragmentation of studied cathinones and appropriate collision energies for a selected reaction monitoring (SRM) were also determined. Furthermore, a partial validation was done evaluating mainly the linearity, limits of detection, quantification, repeatability and recovery. The developed method was applied on the urine analysis after the SPE clean-up and extraction.

2. Materials and methods

2.1. Chemicals

Drug standards of buphedrone, cathinone, ephedrone, ethylone, flephedrone, 3-fluormethcathinone, 4-methylethcathinone, 3,4-methylendioxypyrovalerone, methylone, methedrone, mephedrone and naphyrone were obtained from Lipomed (Arleshaim, Switzerland) as solid standards. Methanol, water and propane-1-ol were from Merck (Darmstadt, Germandy), acetonitrile (all solvents were in LC–MS grade), formic acid, acetic acid, ammonium hydroxide solution (25%), propane-2-ol (LC–MS grade), perfluorooctanoic acid and 2.0 M ammonium solution in methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The deionized water was prepared by a water purification system (18 MΩ, Millipore, Molsheim, France). All chemicals were of analytical grade purity.

2.2. Standard solutions and BGE preparation

The stock solutions of studied cathinones were prepared by dissolving the appropriate amount of each standard in methanol to obtain the concentration of 1.0 $mg\,mL^{-1}$ and stored at −18 °C in a refrigerator. Sample mixtures were then prepared by mixing the standards and subsequent dilution with water. Ammonium formate and ammonium acetate based BGEs were prepared by dissolving an appropriate amount of formic or acetic acid in the deionized water and pH values were adjusted by ammonium hydroxide to the desired values. BGE consisted of perfluorooctanoic acid (PFOA, studied concentrations were from 40 to 120 mM), ammonium hydroxide (studied concentrations were from 100 to 250 mM) and an organic modifier (acetonitrile, methanol, propane-1-ol or propane-2-ol, 0–5%, v/v) were prepared according to the