Journal of Chromatography B, 1038 (2016) 19–25

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Amphetamine and derivatives in natural weight loss pills and dietary supplements by capillary electrophoresis-tandem mass spectrometry

Vagner Bezerra dos Santos[a], Daniela Daniel[b], Melina Singh[c], Prof. Dr. Claudimir Lucio do Lago[a,*]

[a] Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, CEP 05508-000 São Paulo, SP, Brazil
[b] Agilent Technologies, Alameda Araguaia, 1142, CEP 06455-000 Barueri, SP, Brazil
[c] Bishop's University, Sherbrooke, Canada

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 14 October 2016
Accepted 16 October 2016
Available online 17 October 2016

Chemical compounds studied in this article:
Amphetamine (PubChem CID 3007)
β-methylphenylethylamine (PubChem CID 11398)
Phentermine (PubChem CID 4771)
Methamphetamine (PubChem CID 10836)
Methylenedioxyamphetamine (PubChem CID 297310)
Methylenedioxymethamphetamine (PubChem CID 1615)
Methylenedioxyethylamphetamine (PubChem CID 3057998)

Keywords:
Capillary electrophoresis
Tandem mass spectrometry
Amphetamines
Amphetamine derivatives
Natural weight loss pills
Illegal drugs

ABSTRACT

A capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method for amphetamine (AM), phentermine (PTM), methamphetamine (MAM), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) in commercial samples of homeopathic and phytotherapic medicines and dietary supplements is presented. The samples were submitted to a modified QuEChERS extraction procedure (at apparent pH 13) followed by electrophoretic separation in 0.1 mol L^{-1} formic acid electrolyte (pH 2.4) and detection by ESI–MS/MS. A polyvinyl alcohol coated capillary was employed to prevent the adsorption of the analytes to the capillary wall. The limits of detection and quantitation were from 0.02 to 0.06 μg L^{-1} and from 0.06 to 0.21 μg L^{-1}, respectively, with recovery ranging from 85 to 123% and the standard deviations were not greater than 6.1%. In addition, the separation occurs in less than six minutes.

1. Introduction

The characterization and quantification of chemical substances in a wide variety of matrices is a challenge faced by forensic scientists. Many forensic samples are complex mixtures, such as biological extracts or debris associated with the crime scene, and analysis generally requires separation prior to identification of chemical species [1,2]. This task has traditionally been carried out using gas chromatography (GC) [3–7] and high-performance liquid chromatography (HPLC) [8–19]. Capillary electrophoresis has proven to be a good alternative to classical techniques for forensic analysis. CE has the potential to provide more rapid separations and higher number of plates than are generally achieved by HPLC, and it can be applied to separation of thermally unstable analytes, in contrast to GC [2,20]. In this sense, the use of capillary electrophoresis (CE) [1,21–23], as analytical separation technique, coupled to mass spectrometry (MS), as the detection method, can provide significant advantages by combining the high separation efficiency of CE with the identification power of MS [20,24–29].

Amphetamine and its derivatives are powerful stimulants of the central nervous system, and the abuse of amphetamine (AM), methamphetamine (MAM), phentermine (PTM), and

* Corresponding author at: Instituto de Química – Universidade de São Paulo, Av. Prof. Lineu Prestes 748, CEP 05508-000, São Paulo, SP, Brazil.
E-mail addresses: vagnerbs@ufpa.br (V.B. dos Santos), daniel@agilent.com (D. Daniel), MSINGH13@ubishops.ca (M. Singh), claudemi@iq.usp.br (C.L. do Lago).

http://dx.doi.org/10.1016/j.jchromb.2016.10.019
1570-0232/

methylenedioxy-derivatives, such as methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDEA), has increased enormously during the last few years. The escalation of the abuse of these drugs has caused serious health and social problems: chronic use of amphetamines often leads to hallucinations and psychosis, as well as dysphoria and depression upon withdrawal [30,31]. Despite the prohibition of the use at sporting events by national and international anti-doping agencies [22,32,33], amphetamines has been used by some athletes, because of their psychological and performance-enhancing effects. In addition, amphetamine has been used to treat obesity.

Monitoring of amphetamines and its derivatives in drug seizures and natural weight loss pills is a timely topic not only in forensic science, but also in consumer protective law [29,32]. These compounds should be absent in pills and phytotherapic medicine formulations that does not specify amphetamines as the active principle[9]. However, to accelerate weight loss, many of these formulations rely on the indiscriminate and illegal addition of these compounds in commercial products. Another problem associated with these so-called "natural products" is the complexity of the matrix. An effective sample treatment is necessary to obtain reliable analytical results. Solid phase extraction (SPE) [17] or liquid-liquid extraction (LLE) [3] can be used, but they are time-consuming. A simple and efficient modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) has been proposed as treatment of samples to determine amphetamines and derivatives [34].

The QuEChERS method was developed by Lehotay and Anastassiades in 2003 as a rapid and inexpensive method for the analysis of pesticides in foods[35]. The procedure involves initial single-phase extraction followed by liquid-liquid partitioning. The main advantages of the QuEChERS with respect to liquid/liquid extraction are a cleaners extract and easy isolation of the organic layer, due to the absence of emulsions. QuEChERS is simple and can be easily modified. There is a great number of versions of the modified QuEChERS technique, which were developed to extend the method for multiresidue analysis in complex matrices [34,36,37].

In the present work, a capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method was developed for the simultaneous determination of amphetamines and derivatives in samples of commercial phytotherapic medicine, dietary supplements, and weight loss pills according to the AOAC guideline for validation of chemical methods for dietary supplements [38]. A simple modified QuEChERS extraction was used as the only sample treatment step.

2. Experimental

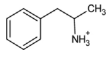
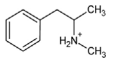
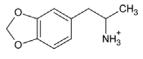
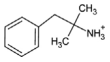
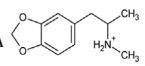
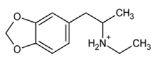
2.1. Materials and methods

A Certified Reference Material (CRM) of LC/MS Toxicology Test Mixture (ULTRA) composed by AM, PTM, MAM, MDA, MDMA, and MDEA at $1.00 \pm 0.01\ \mu g\ mL^{-1}$ in methanol were purchased from Agilent technologies (part number 5190-0470) and kept at 5 °C. β-Methylphenylethylamine (β-AM) was acquired from Sigma-Aldrich (part number 180076-5G, 99%). Formic acid (98–100%) and the methanol (Chromasolv LC–MS grade, ≥99.9%) were acquired from MERCK (Darmstadt, Germany) and Fluka (St. Louis, MO, USA), respectively. Deionized water (18 MΩ cm) was obtained from a Millipore Milli-Q (Molsheim, France) water purification system.

Samples of natural weight loss pills, tablets and tea were obtained from local drugstores, and they were classified as homeopathic medicine (sample 1), phytotherapic medicine (sample 2 and 3), or dietary supplements (sample 4–6). Fresh artichokes (sample 7) were acquired in a street market.

The background electrolyte (BGE) was 0.1 mol L^{-1} formic acid, pH 2.4, and was prepared on a weekly basis. Standard solutions were prepared daily by appropriate dilution of different aliquots of the stock solutions in BGE. The sheath liquid for the ESI source was 0.02 mol L^{-1} formic acid, pH 2.7, prepared on methanol/water 50:50 (v/v), and its flow rate was 6.0 $\mu L\ min^{-1}$.

Table 1
Migration time (t_M) and MS/MS acquisition parameters used for the identification and quantification of amphetamine and its derivatives.

Analyte[a]	t_M (min)	Q_1[b] (*m/z*)	Q_3[c] (*m/z*)	CE[d] (V)	FE[e] (V)
AM 	5.02	136.1	91.1[f]	20	70
			119.1	10	
MAM 	5.11	150.1	91.1[f]	20	75
			65.0	44	
MDA 	5.24	180.1	163.1[f]	4	80
			105.1	24	
PTM 	5.33	150.1	91.1[f]	10	75
			133.1	10	30
MDMA 	5.33	194.1	163.1[f]	8	80
			105.1	24	
MDEA 	5.65	208.1	163.1[f]	8	98
			105.1	24	

[a] Main form at pH 2.4.
[b] Precursor ion (Q_1).
[c] Fragment ions (Q_3).
[d] Collision energy.
[e] Fragmentor energy.
[f] More intensity MRM transition used for quantification purposes.