Forensic Science International 228 (2013) 154–159

Contents lists available at SciVerse ScienceDirect

Forensic Science International

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

Capillary electrophoretic and extraction conditions for the analysis of *Catha edulis* FORKS active principles

Gabriella Roda [a,*], Vincenzo Liberti [a], Sebastiano Arnoldi [a], Antonella Argo [b], Chiara Rusconi [a], Sonia Suardi [a], Veniero Gambaro [a]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] Istituto di Medicina Legale e delle Assicurazioni Policlinico Universitario, Via del Vespro n. 129, 90127 Palermo, Italy

ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 12 February 2013
Accepted 22 February 2013
Available online 28 March 2013

Keywords:
Catha edulis
Cathinone
Cathine
Phenylpropanolamine
CE

ABSTRACT

A capillary electrophoretic method, which allowed the detection and separation of the active principles of *Catha edulis*, i.e. cathinone, cathine and phenylpropanolamine, was developed. A suitable internal standard (nicotinamide), which permitted the quantification of the analytes reducing the variability of the migration times due to EOF changes, was identified. The analytical method was validated, assessing linearity, sensitivity and repeatability, showing optimal features for the analysis of the vegetable material. Moreover extraction conditions were investigated to achieve the exhaustion of the plant material in the fastest and most efficient way to meet the requirements of the Court.

1. Introduction

Catha edulis (khat), a flowering evergreen shrub or small tree belonging to the Celastraceae family, characterized by oval opposite finely toothed leaves, is native and mainly cultivated in East Africa and Arabian Peninsula [1–3]. The chewing of fresh leaves or the smoking of dried plant material is a common social and traditional habit in these countries [4]. The effect of khat assumption is mild excitation and euphoria, that is a psychoactive stimulation similar to that produced by amphetamines [5]. Khat contains three alkaloids with an amphetamine-like structure (Fig. 1): cathinone [(*S*)-(−)-α-aminopropiophenone, **1**], cathine [(*S*,*S*)-(+)-norpseudoephedrine, **2**] and phenylpropanolamine [(*R*,*S*)-(−)-norephedrine, PPA, **3**] [6,7], but the psychostimulation is predominately, or even exclusively due to cathinone, whose effect is believed to be mediated by the dopaminergic system.

Cathine is less active as a stimulant, and PPA has no psychotropic effect [8]. After harvesting, cathinone is enzimatically converted into cathine and norephedrine [6] and due to the limited shelf life of the most active component in the fresh vegetable material, the storage of the plant is crucial for the identification and quantification of this psychoactive component, which is, together with cathine, regulated as a controlled substance in many countries. In fact it was demonstrated that cathinone is stable for years in the dried khat, but it undergoes rapid decomposition in the fresh or frozen vegetable material [9]. Several analytical methods were developed for the determination of khat alkaloids either involving GC and HPLC techniques [10–14]; in the last years, the Tribunal of Busto Arsizio and Bergamo gave us the task to analyze all the materials suspected to contain khat active principles, coming from the seizures at the Malpensa and Orio al Serio airports in northern Italy. Thus, due to the high number of samples to be analyzed, we developed a fast, effective and reliable GC analytical procedure able to simultaneously detect, cathine and PPA, whose discrimination was mandatory because PPA is not a controlled psychoactive agent, exploiting a derivatization protocol which allowed an effective separation of the two components without dramatically increasing the time of analyses [15].

In this frame we were interested in the application of capillary electrophoresis, a technique, complementary to GC, endowed with similar characteristics of rapidity, selectivity and sensitivity, which is gaining importance in the forensic analysis [16] and which was applied to the separation of several amphetamine derivatives in the presence of chiral additives [17]. In this work, being not interested in chiral separations because the active principles contained in the vegetable material are enatiomerically pure, we tried to simplify the analytical method and to optimize the

* Corresponding author. Tel.: +39 02 50319328; fax: +39 02 50319359.
E-mail address: gabriella.roda@unimi.it (G. Roda).

0379-0738/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2013.02.034

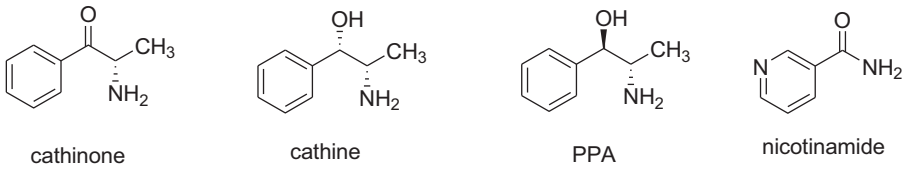

Fig. 1. Chemical structures of *S*-(−)-α-aminopropiophenone (cathinone), (*S*,*S*)-(+)-norpseudoephedrine (cathine) and (*R*,*S*)-(−)-norephedrine (phenylpropanolamine, PPA), pyridine-3-carboxamide (nicotinamide).

extraction procedure to meet the requirements of the Court. Several parameters of the extraction protocol were considered in order to find the most suitable conditions for the treatment of this perishable vegetable material; moreover the electrophoretic method was validated following the guidance on validation published by the European Medicines Agency [18].

2. Experimental

2.1. Reagents

Phosphoric acid, sodium hydroxide, trizma base, acetone, methanol, phenylpropanolamine hydrochloride, nicotinamide and ethyl acetate were obtained from Sigma–Aldrich. All reagents used were of analytical grade and all reagents, standard solutions and buffers were prepared with water obtained from a Milli-Q water purification system (Millipore).

2.2. Standards

Cathinone was purchased from LGC PROMOCHEM s.r.l., while a 1 mg/mL solution of cathine in methanol from S.A.L.A.R.S. s.p.a.

Stock solutions of 100 μg/mL of cathinone, cathine and phenylpropanolamine were prepared in methanol.

2.3. Vegetable material

The vegetable material studied (bundle 1) was seized in January 2011 and was delivered to our laboratory by the Tribunal of Busto Arsizio. It was composed by 120 bunches contained in a traveling bag. The vegetable material was completely frozen after the seizure and it was kept refrigerated until the analyses. 10 representative bundles were withdrawn from the seized material to be analyzed. Every bundle was composed of a variable number of *C. edulis* twigs divided into small groups and tied with strands of raffia. The leaves and stems of the vegetable material were wrapped with blotting paper and covered with a banana leaf.

The vegetable material from which bundle 2 was taken was seized in February 2012 and was composed of 38 bunches of *C. edulis*, the vegetable material from which bundle 3 was taken was composed of 15 bunches, seized in July 2012 in northern Italy and were delivered to our laboratory by the Tribunal of Busto Arsizio.

The plant material underwent a preliminary botanical examination in order to establish the species. From a legal point of view, therefore it was necessary to unequivocally determine and quantify the active principles.

2.4. Method development

Several parameters were taken into account in the development of the extraction protocol: solvent, method of extraction and time of contact. All the proofs were carried out starting from 1 g of dry vegetable material, which was suspended in 10 mL of extracting solvent.

2.4.1. Preparation of the vegetable material

Frozen leaves and stems were manually separated and dried at room temperature on blotting paper for 48 h. The dry vegetable material was stored up in plastic bags until use.

2.4.2. Solvent of extraction

Three different solvents were evaluated: methanol, ethyl acetate and chloroform. Leaves and stems were chopped and mixed in order to obtain a homogeneous vegetable material (1 g), which was added with the extraction solvent (10 mL). The mixture was vigorously stirred for 1 min and then macerated at room temperature. The solvent (100 μL) was withdrawn every 24 h to control the progress of the extraction. In the case of ethyl acetate and chloroform the solvent was evaporated and the residue recovered with water.

2.4.3. Method of extraction

Three different extraction methods were compared:

Maceration: 1 g of leaves and stems manually chopped in pieces of about 0.5 cm were suspended in 10 mL of solvent in a round flask, vigorously mixed for 1 min and macerated for 24 h. Every hour the solvent (100 μL) was withdrawn to control the progress of the extraction.

Extraction with a rotary extractor: the vegetable material suspended in the organic solvent was put on a rotary extractor and rotated for 7 h. Every hour the solvent (100 μL) was withdrawn to control the progress of the extraction.

Ultrasound extraction: three samples (1 g of leaves and 1 g of stems) were suspended in 10 mL of solvent. The first sample was sonicated for 20 min and then allowed to stand at room temperature for 40 min, The second was sonicated for 40 min and allowed to stand for 20 min. The third was sonicated for 60 min. After 60 min the solvent (100 μL) was withdrawn to control the progress of the extraction.

2.4.4. Time of contact

The progress of the extraction (1 g of leaves and 1 g of stems suspended in 10 mL of solvent) was monitored during the first 7 h withdrawing 100 μL of solvent, then the solvent was analyzed after 24 h and once a day for 5 days, either changing the solvent every day or without changing the solvent, in order to evaluate the time necessary to exhaust the vegetable drug. To shorten the time of extraction other two proofs were carried out (with or without changing the solvent) with 100 mg of vegetable material.

2.4.5. Evaluation of the distribution of the active principles

The vegetable material was separated into leaves, tender stems and woody stems. 1 g of the different parts of the plant were exactly weighted and suspended in 10 mL of solvent.

2.4.6. Drying of the vegetable material

The stability of the active principles was evaluated: 3 samples of frozen leaves (1 g) and 3 samples of frozen stems (1 g) were directly extracted; 3 samples of frozen leaves (1 g) and 3 samples of frozen stems (1 g) were dried at 35 °C and then extracted. In order to simplify the preparation of the vegetable material a whole