Forensic Science International 217 (2012) 87–92

Contents lists available at SciVerse ScienceDirect

Forensic Science International

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

Determination of the active principles of *Catha Edulis*: Quali–quantitative analysis of cathinone, cathine, and phenylpropanolamine

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ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 28 September 2011
Accepted 29 September 2011
Available online 22 October 2011

Keywords:
Catha Edulis
Cathinone
Cathine
Phenylpropanolamine
GC/MS
GC/FID

ABSTRACT

In the last years, all the vegetable material supposed to belong to the *Catha Edulis* species, seized at the Malpensa and Orio al Serio airports, were analyzed in our laboratory on behalf of the Tribunals of Busto Arsizio and Bergamo, respectively. After a preliminary botanic examination, the quali–quantitative determination of the active principles cathinone, cathine and phenylpropanolamine (PPA) was carried out by means of GC/MS and GC/FID techniques, which meet the requirements of the forensic analyses. We developed a fast, effective and reliable derivatization procedure which allowed to simultaneously detect cathine and PPA, whose discrimination is mandatory since PPA is not a psychoactive agent. Cathine was distributed in the various parts of the plant (leaves and stems) and its quantity ranged from 0.03% to 0.17% of the weight of the vegetable material; PPA was not detected in the twigs and its quantity in the leaves ranged from 0.07% to 0.16%. The quantitative determination of cathinone was carried out directly on the methanol solution after maceration of the vegetable material, its quantity ranging from 0.02% to 0.10%. No significant difference in the content of the two active principles was found between the fresh and the dried material.

1. Introduction

Catha Edulis (khat) is a flowering evergreen shrub or small tree belonging to the Celastraceae family. It reaches a height of 4 m and is characterized by oval opposite finely toothed leaves. The plant is native and mainly cultivated in East Africa and Arabian Peninsula [1–3], where the chewing of fresh leaves and shoots is a common social and traditional habit, especially in Yemen and Ethiopia [4]. Dried plant material can also be smoked or drunk as an infusion (Abyssinian tea). The main effect of khat assumption is a psychoactive stimulation similar to that produced by amphetamines, that is mild excitation and euphoria leading to increased energy and communicativeness, diminished concentration and, sometimes, nervousness and agitation [5]. The substances responsible for the central nervous system (CNS) stimulatory effect are alkaloids with an amphetamine-like structure: (*S*)-(−)-α-aminopropiophenone (cathinone, **1**), (*S*,*S*)-(+)-norpseudoephedrine (cathine, **2**) and (*R*,*S*)-(−)-norephedrine (phenylpropanolamine, PPA, **3**), whose structures are shown in Fig. 1 [6,7]. Cathinone is considered the most active agent, it can be assumed that khat-induced psychostimulation is predominately, or even exclusively due to the cathinone content of the leaves; its stimulatory effect is believed to be mediated by the dopaminergic system. Cathine is less active as a stimulant, and PPA has no psychotropic effect [8]. Both cathinone and cathine are regulated as controlled substances in many countries even if the criminal penalty is higher for offenses involving cathinone. This alkaloid, after harvesting, is converted into cathine and norephedrine by an enzymatic reduction [6]; in fact, to achieve the maximum stimulating effects, khat should be picked up in the morning and chewed the same afternoon. Until the 1990s, the limited shelf life of the most active component in the fresh vegetable material, has confined the use of khat in the areas of its primary cultivation, but recently air transport made possible a rapid global distribution of this perishable material. The storage of the plant material is crucial for the identification and quantification of the psychoactive components, in fact it was demonstrated that cathinone is stable for years in the dried khat, but it undergoes a rapid decomposition in the fresh or frozen vegetable material [9].

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. After seizing, the vegetable material underwent a preliminary botanical characterization, but

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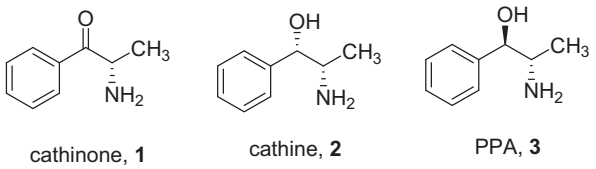
doi:10.1016/j.forsciint.2011.09.028



cathinone, **1** cathine, **2** PPA, **3**

Fig. 1. Chemical structures of *S*-(−)-α-aminopropiophenone (cathinone, **1**), (*S*,*S*)-(+)-norpseudoephedrine (cathine, **2**) and (*R*,*S*)-(−)-norephedrine (phenylpropanolamine, PPA, **3**).

the unequivocal dosage of the active principles was indispensable to establish the criminal penalty. Thus, due to the high number of samples to be analyzed, we developed a fast, effective and reliable analytical procedure able to detect cathinone directly after maceration and cathine and PPA, after derivatization. To this end, HPLC and cation-exchange liquid chromatography methods have been reported in the literature [10,11], but we adopted a GC/MS method for qualitative determinations and a GC/FID combined system for quantitative analysis. These choices are related to our expertise in applying the above cited chromatographic instrumental techniques, which are endowed with high specificity and sensitivity, to the characterization of drugs of abuse. Moreover, most of the police and carabineer laboratories routinely use GC-based analytical procedures, so their application to khat analysis should allow direct comparison of quantitative data with those elaborated by law enforcement.

Furthermore, in the case of khat active principle determination, the chosen analytical method should be undoubtedly fast. As a matter of fact, given the fast decomposition of the most active component, the vegetable material should be frozen immediately after confiscation and the analyses performed in a time as short as possible. All GC techniques well meet these requirements.

A problem which was encountered when developing the GC procedures, was the coelution of cathine and PPA, whose discrimination is mandatory because PPA is not a psychoactive drug. To overcome this limitation, we studied an appropriate derivatization protocol to quantitatively separate the two components without dramatically increasing the overall analysis time.

2. Experimental

2.1. Reagents and standards

Methanol (99.8% purity) and 1 M solution of sodium hydroxide were purchased from J.T. Baker B.V. (The Netherlands); cyclohexanone, pyridine (>99.8% purity), sulfuric acid, toluene, triethylamine and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) were obtained from Fluka (Switzerland); 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. (Italy); phenylpropanolamine hydrochloride and ethyl acetate (>99.5% purity) were obtained from Sigma–Aldrich (Italy); diethylamine was purchased from Riedel de Haën (Germany); trifluoroacetic anhydride was obtained from SUPELCO (Italy). Oxalic acid dihydrate was purchased form Carlo Erba (Italy).

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

2.2. Sample preparation

Five different police seizures were taken into account containing 216 (seizure A), 217 (seizure B), 223 (seizure C), 50 (seizure D) and 100 (seizure E) bundles of vegetable material supposed to belong to the *Catha Edulis* species. All the seizures were conserved by freezing before being delivered to our laboratory for the analysis.

The vegetable material was allowed to reach room temperature, the leaves were separated from the stems and 1.0000 g of leaves and stems for each seizure were separately drawn and submitted to the extraction and derivatization protocols.

2.2.1. Extraction conditions

Maceration with methanol: Methanol (10 mL) was added to the vegetable material (1.0000 g); the mixture was shaken and macerated overnight; the organic solvent was then filtered with 0.45 μm White Nylon filters (Millipore).

Liquid–liquid extraction: 1 N oxalic acid (10 mL) was added to the vegetable material (1.0000 g); the mixture was shaken, macerated overnight and then centrifuged (centrifuge 4263 A, A.L.C. s.r.l., Italy; 2500 rpm) for 15 min. The supernatant (1 mL) was adjusted to pH 12 with 1 M NaOH and extracted with ethyl acetate (1 mL) with a rotary extractor (F205, FALC Instruments s.r.l., Italy) for 2 h. The mixture was centrifuged (2500 rpm) for 15 min and the organic phase was submitted to the following analyses.

2.2.2. Derivatization conditions

Derivatization with cyclohexanone: (a) 100 μL of sample or standard methanol solution were withdrawn, 100 μL cyclohexanone were added and after vigorous shaking the mixture was transferred into a GC vial, capped and heated for 30 min at 70 °C.

(b) 100 μL of ethyl acetate solution were withdrawn and the solvent evaporated with a gentle stream of nitrogen. To the residue 100 μL methanol and 100 μL cyclohexanone were added and after vigorous shaking the mixture was transferred into a GC vial, capped and heated for 30 min at 70 °C.

Derivatization with triluoroacetic anhydride: 100 μL of sample or standard solution were withdrawn and the solvent evaporated with a gentle stream of nitrogen. To the residue 100 μL trifluoroacetic anhydride were added and after vigorous shaking the mixture was transferred into a GC vial, capped and heated for 15 min at 60 °C. The reagent was evaporated and 50 μL pyridine were added to the residue. After vigorous shaking, the solvent was evaporated again and the residue dissolved in 100 μL methanol.

Derivatization with N-methyl-N-trimethylsilyl-trifluoroacetamide: 100 μL of sample or standard solution were withdrawn and the solvent evaporated with a gentle stream of nitrogen. A 1:4 solution of MSTFA in toluene was added (100 μL) and after vigorous shaking the mixture was transferred into a GC vial, capped and heated for 30 min at 70 °C.

2.2.3. Controlled drying

The leaves and the stems of the vegetable material were separated and put in heater at 35 °C. The weight was checked every 15 min until it reached a constant value (6 h).

2.3. Instrumentation and gas chromatographic–mass spectrometric conditions

Qualitative determinations: The analyses were performed on a Varian 3900 GC system, with a Varian CP 8400 autosampler, a split–splitless injection system and a Saturn 2100T ionic trap MS detector operated in electron impact mode (70 eV) and chemical ionization. The GC was equipped with a HP5-MS capillary column (15 m × 0.25 mm i.d., film thickness 0.25 μm) from Agilent (USA).

The GC–MS system was operated under following conditions: injector temperature 280 °C; interface transfer line 280 °C; ion source 230 °C; oven temperature program initial 70 °C (0 min), then 40 °C/min to 110 °C and 15 °C/min to 300 °C (3 min). Helium was used as the carrier gas at a flow rate of 1.2 mL/min.

Quantitative determinations: The analyses were performed on a Agilent 6890 GC system, with a Agilent 6890 Series Injector autosampler, a split–splitless injection system and a flame ionization detector (FID). The GC was equipped with a DB-5 phenyl methyl-polisiloxane fused silica gel capillary column (15 m × 0.32 mm i.d., film thickness 0.25 μm) from Agilent (USA).

The GC-FID system was operated under following conditions: injector temperature 280 °C; oven temperature program initial 70 °C (0 min), then 40 °C/min to 110 °C and 15 °C/min to 300 °C (3 min). Helium was used as the carrier gas at a flow rate of 1.2 mL/min.

3. Results and discussion

3.1. Botanical characterization

When smuggled, khat is generally packaged in bundles and hidden in passenger luggage or sent via express mail in boxes. A bundle, composed of 4 or 5 smaller sub-bundles secured with a husk-like twine each, typically contains about 40 leafed twigs 30–40 cm in length. To maintain moisture and freshness, each bundle is then rolled in wet paper, wrapped in a large banana leave secured with a husk-like twine, and packed in plastic bags or newspapers; if possible, during transportation plants are frequently sprinkled with water.

A preliminary botanic characterization of the seized vegetable material was necessary because the whole plant *Catha Edulis* is included in the schedules of controlled substances in many countries. All the bundles were therefore examined by a botanist, which identified the material as belonging to the *Catha Edulis* species.