



## Improved GC method for the determination of the active principles of *Catha edulis*



Lucia Dell'Acqua, Gabriella Roda\*, Sebastiano Arnoldi, Chiara Rusconi, Lorenzo Turati, Veniero Gambaro

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Luigi Mangiagalli 25, 20133 Milano, Italy

### ARTICLE INFO

#### Article history:

Received 23 October 2012

Accepted 14 April 2013

Available online 18 April 2013

#### Keywords:

*Catha edulis*

Cathinone

Cathine

Phenylpropanolamine

GC/MS

GC/FID

### ABSTRACT

The GC method previously reported by our research group for the analysis of the active principles of *Catha edulis*, i.e. cathine, cathinone and phenylpropanolamine, was considerably improved. *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) as derivatizing agent was employed, thus allowing an accurate determination of the analytes and a suitable internal standard for quantitative analyses (nicotinamide) was introduced. Moreover the chromatographic conditions were carefully studied to improve the separation of the alkaloids and sensitivity. To this end different chromatographic capillary columns and temperature gradients were investigated. The optimized GC method was validated and resulted adequate for the application in forensic analysis. Finally on behalf of the Tribunal, *C. edulis* vegetable material seized by the police in northern Italy was analyzed, the quantity of cathine ranging from 0.095 to 0.29%, the quantity of PPA from 0.010 to 0.21% and the quantity of cathinone from 0.025 to 0.374% of the weight of the vegetable material.

© 2013 Elsevier B.V. All rights reserved.

### 1. Introduction

*Catha edulis* (khat) is native and mainly cultivated in East Africa and Arabian Peninsula [1–4], where the chewing of fresh leaves and shoots is a common social and traditional habit [5]. Dried plant material can also be smoked or drunk as an infusion (Abyssinian tea). The plant is a flowering evergreen shrub or small tree belonging to the Celastraceae family, which reaches a height of 4 m and is characterized by oval opposite finely toothed leaves. The substances responsible for the main effect of khat assumption, i.e. 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 [6], are alkaloids with an amphetamine-like structure: (*S*)-(–)- $\alpha$ -aminopropiophenone (cathinone, **1**), (*S,S*)-(+)-norpseudoephedrine (cathine, **2**) and (*R,S*)-(–)-norephedrine (phenylpropanolamine, PPA, **3**), whose structures are shown in Fig. 1 [7,8].

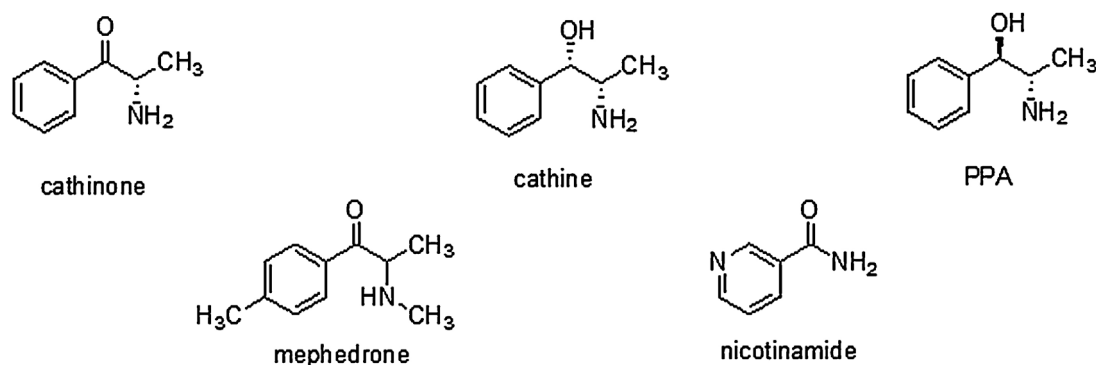
Khat-induced psychostimulation is predominately, or even exclusively due to cathinone, whose stimulatory effect is believed to be mediated by the dopaminergic system. Cathine is less active and PPA, the diastereomer of cathine has no psychostimulant effect

[9]. Both cathinone and cathine are included in the list of the illegal substances in many countries, offenses involving cathinone being considered more serious from a legal point of view. This alkaloid, after harvesting and in the older parts of the plant is converted into cathine and norephedrine by an enzymatic reduction [7]; in fact, young leaves are the most prized vegetable material and to achieve the maximum stimulating effects, khat should be picked up in the morning and chewed the same afternoon. For this reason, the storage of the plant material is crucial for the identification and quantification of the most active component, 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 [10].

On behalf of the Court in the last years a lot of vegetable material suspected to belong to the *C. edulis* species, seized at the airports in northern Italy, was brought to our laboratory to be analysed.

Thus, due to the high number of samples, in the past a fast, effective and reliable analytical procedure able to detect cathinone directly after maceration and cathine and PPA, after derivatization was developed [11]. To this end, HPLC and cation-exchange liquid chromatography methods have been reported in the literature [12,13], but because of our expertise in applying GC techniques, we adopted a GC/MS method for qualitative determinations and a GC/FID combined system for quantitative analysis, which are endowed with high specificity and sensitivity in the characterization of drugs of abuse. On the other hand, this method suffered from some limitations: the derivatizing agent, i.e. cyclohexanone [14,15]

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



**Fig. 1.** Chemical structures of *S*-(-)- $\alpha$ -aminopropiophenone (cathinone), (*S,S*)-(+)-norpseudoephedrine (cathine) and (*R,S*)-(-)-norephedrine (phenylpropanolamine, PPA), (*RS*)-2-methylamino-1-(4-methylphenyl)propan-1-one (mephedrone), pyridine-3-carboxamide (nicotinamide).

was effective only for the determination of cathine and PPA, while cathinone was directly determined on the methanol extraction solution. In this way the preparation of two samples was necessary to analyse the vegetable material, thus leading to increased time of analysis and higher variability. The separation of cathine and PPA, even though sufficient for a quantitative determination, was not optimal; this point is crucial because only cathine is a controlled substance. Moreover, no suitable internal standard (IS) could be identified. In this work all these problems have been overcome, studying a different derivatizing agent, an adequate IS, and applying different chromatographic columns and different chromatographic conditions to improve the separation of the two analytes. Furthermore the analytical method was validated [16] and applied to the analyses of different *C. edulis* vegetable materials seized by the police.

## 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, nicotinamide and ethyl acetate (>99.5% purity) were obtained from Sigma–Aldrich (Italy); Stock solutions of 100  $\mu$ g/mL of cathinone, cathine, phenylpropanolamine and nicotinamide (IS) were prepared in methanol.

### 2.2. Vegetable material

The studied vegetable materials were seized in February 2012 and July 2012 in northern Italy and were delivered to our laboratory by the Tribunal of Busto Arsizio. The first seizure was composed of 38 bunches (total weight: 2.961 kg) of *C. edulis*, the second, the third and the fourth respectively of 15 (total weight: 8.060 kg), 24 (total weight: 12.565 kg) and 12 (total weight: 12.545 kg) envelopes of fresh vegetable material (mainly leaves) contained in a travelling bag. The vegetable material was completely frozen after the seizure and it was kept refrigerated until the analyses. Six representative bundles were withdrawn from the first seized material to be analyzed. Every bundle was composed of a variable number of 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.

Every bundle was weighted, before and after the elimination of the envelope, dried at 35 °C for 24 h and homogenized. Three representative withdrawals were carried out from each of the other seizures and the vegetable material was dried and homogenized as described before. The weights of the finds analyzed are reported in Table 1S (see supplementary material).

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.

### 2.3. Instrumentation and gas chromatographic–mass spectrometric conditions

GC/MS: 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 VF-xMS (Varian FactourFour™) capillary column (30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu$ m).

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 10 °C/min to 300 °C (3 min). Helium was used as the carrier gas at a flow rate of 1.2 mL/min.

GC/FID: 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 VF-5MS (Varian FactourFour™, 30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu$ m) or VF-xMS (Varian FactourFour™, 30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu$ m) capillary column.

Alternatively a Thermo Electron “Trace GC” with a AS2000 (TE) autosampler, a split-splitless injection system and FID detector equipped with a ZB Multiresidue 2 (Zebtron™ Phenomenex) capillary column (30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu$ m) was used. The retention times of the analytes with the three different columns (method 1) are reported in Table 1.

**Table 1**  
Retention times of the analytes with the different columns.

	Retention times (min)		
	VF-5ms	VF-Xms	ZB-Multiresidue-2
Cathine	7.675	7.265	6.747
PPA	7.712	7.408	6.800
Cathinone	7.747	7.658	7.737
IS (nicotinamide)	7.952	8.156	8.572

Download English Version:

<https://daneshyari.com/en/article/1212982>

Download Persian Version:

<https://daneshyari.com/article/1212982>

[Daneshyari.com](https://daneshyari.com)