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Fatal *Datura* poisoning: identification of atropine and scopolamine by high performance liquid chromatography/photodiode array/mass spectrometry

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Abstract

A forensic method comprising solid phase extraction and HPLC analysis was developed for the detection and confirmation of atropine and scopolamine, the main toxic alkaloids of *Datura stramonium* and *Datura ferox*. This method allowed the direct coupling of an electrospray (ZMD) mass selective detector to the HPLC system. Under these conditions, atropine and scopolamine were well separated from other components and detected on the PDA (LOD = 1 μg/ml) and ZMD ($LOD_{atropine}$ = 10 pg/ml; $LOD_{scopolamine}$ = 100 pg/ml) detectors. Four geographically isolated populations of each of *D. stramonium* and *D. ferox* were analysed for seed alkaloids and it was found that the two species were diagnostically different in their atropine–scopolamine ratios. The optimised HPLC method was used to analyse three viscera samples of an adult Caucasian male whose death was ascribed to a fatal heart attack. Atropine and scopolamine were detected in the stomach and its contents, which contained *Datura* seeds. The chemical profile of the seeds found in the stomach contents was similar to those from four geographically different *D. ferox* plants.

Keywords: *Datura stramonium*; *Datura ferox*; Atropine; Scopolamine; HPLC; Mass spectrometry; Poisoning; Forensic

1. Introduction

Datura stramonium L. (Family Solanaceae), also called Apple of Peru, Devil's Apple, Devil's Trumpet, Jamestown Weed, Mad-apple, Stinkweed or Thorn apple, is an erect, subherbaceous annual up to 1.5 m high with dark green or purplish leaves which are usually paler below. Flowers are white, mauve or purplish with a narrow funnel shape. The erect fruit capsules are brown in color, 50 mm long and covered with slender spikes up to 10 mm long [1]. These capsules are filled with numerous brown to black, kidney-shaped seeds of approximately 3 mm in length [2]. *Datura ferox* is very similar to *Datura stramonium* and is often mistaken for it, but the capsules have fewer, larger and much thicker spines [3]. The flowers of *D. ferox* are also narrowly funnel-shaped but are only white in color.

The toxicity of *Datura* species is well known and has been linked to deaths and poisonings for centuries; some authors consider it responsible for the losses suffered by the army of Mark Anthony in 36 BC [4]. The main toxic principles are tropane alkaloids: hyoscyamine, which forms a diastereomeric (epimeric) mixture known as atropine (upon isolation) and scopolamine [5]. Atropine (Fig. 1) (1) has a molecular mass of 289.37. Atropine has anticholinergic activity and causes blurred vision, suppressed salivation, vasodilation, increased heart rate and delirium [2,3,6–8]. It also reduces rigidity in parkinsonism and is used as an antidote to poisoning with parasympathomimetic agents, e.g. nerve gases and organophosphorus insecticides [8]. Scopolamine (also called hyoscine, Fig. 1) (2) has a molecular mass of 303.36. Scopolamine is an antimuscarinic agent (used as an

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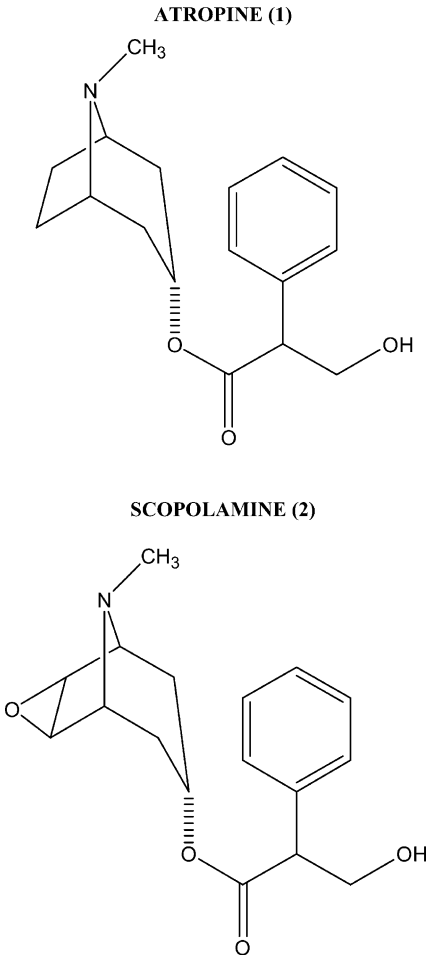

Fig. 1. Structures of scopolamine and atropine.

analgesic) and a smooth muscle relaxant. It is also an antispasmodic agent with antinauseant properties, and is extensively used in the treatment of motion sickness and in pre-operative medication [2,3,8].

Certain authors reported the origin of *D. stramonium* as uncertain [1], but most agreed that this plant originated from the tropical areas of Central and South America [1–3] and it is now a cosmopolitan weed in temperate regions. It is commonly found along riverbanks, roadsides and disturbed sites and is widely distributed through South Africa [1–3] with its first documented appearance in 1714 [4]. Although *D. stramonium* and *D. ferox* are listed in South Africa as declared weeds [1], they are widely used as medicinal plants for the treatment of asthma and to reduce pain. Weak infusions are used as hypnotics by the elderly and as aphrodisiacs by adults. Other medicinal uses include the treatment of gout, boils, abscesses and wounds [2]. *Datura* species have also been used as medicinal plants in Argentina and by the American Indians. The native populations of the western Amazon, Andes, Colombia, Ecuador, Bolivia and Chile have used various *Datura* species as hallucinogens [4,9]. *Datura* species have also been used in criminal activities [6,10]. In Europe, the seeds and plant extracts of *D. stramonium* were used in the treatment of mania, epilepsy, melancholy, rheumatism and convulsions [9].

Accidental *Datura* poisoning is quite common and various cases have been reported: *Datura* poisoning from eating a hamburger [11], scopolamine poisoning from homemade ‘‘moon flower’’ wine [12], atropine poisoning after drinking contaminated Indian tonic water [13], atropine poisoning after eating porridge contaminated with *D. stramonium* [14], atropine poisoning after drinking tainted comfrey tea [15], drinking of a tea blend containing *D. stramonium* leaves [16] and atropine poisoning after eating contaminated honey [17]. In Botswana, a large number of people were affected after consuming sorghum flour contaminated with *Datura* seeds [18]. Many fatalities have occurred, especially among children who are attracted to the capsules and seeds [9]. Intentional poisoning with *D. stramonium* has also been reported in several cases, namely a fatal poisoning with *D. stramonium* [19], the smoking and ingestion of *D. stramonium* for its mind-altering properties [20] and the eating and chewing of *Datura* seeds in a suicide attempt [21].

An actual case study will be presented here where a middle-aged Caucasian male was admitted to hospital after a suspected heart attack. He was stabilized and returned home only to be rushed back to hospital the next day after another heart attack. The medical staff was unable to stabilize the patient and he died shortly thereafter. As the patient had a history of heart problems, no foul play was suspected, but after an anonymous telephone call to the local police station, an autopsy was ordered. The autopsy was performed that same evening as the body was due for cremation the next morning, and viscera samples (stomach and its contents, liver and kidney) were submitted to the Forensic Chemistry Laboratory of Johannesburg (FCL JHB) for chemical analysis.

2. Material studied, methods and techniques

2.1. Standards and reagents

(±)-Atropine (99%), (−)-scopolamine hydrochloride (98%), potassium chloride, sodium chloride, potassium dihydrogen phosphate and sodium monohydrogen phosphate (>99.5%) were obtained from Sigma Chemical Company, USA. Ammonium acetate (98%) was obtained from BDH Laboratory Supplies, England. Sulfuric acid (Suprapur 96%), hydrochloric acid (Suprapur 96%) and ammonia (GR 25%) Pro Analisi was obtained from E-Merck, Darmstadt, Germany. Acetonitrile (gradient quality 200 nm UV cutoff) and methanol (gradient quality 205 nm UV cutoff) were obtained from Romil, England. HPLC grade water (20 MΩ) was obtained from a Milli-Q/reversed osmosis system (Millipore, USA). Oasis HLB solid phase extraction (SPE) cartridges (60 mg) were obtained from Waters Corporation, Milford, USA. The phosphate-buffered saline

solution (PBS) was prepared according to the prescribed method supplied by Waters, Milford, USA [22]. According to this method, PBS is prepared by adding the anhydrous salts of KCl (200 mg), NaCl (8000 mg), KH_2PO_4 (200 mg) and Na_2HPO_4 (1150 mg) to a one liter flask. One liter of deionized water is added and stirred to dissolve. Finally the pH is adjusted to 7.0 with 10% phosphoric acid. HCL-PBS was prepared as described above, but the pH was lowered to 5.0 with hydrochloric acid.

2.2. Instruments and conditions

A Waters 2690 HPLC system equipped with a 996 PDA detector and a Z spray mass selective detector (ZMD) (Micromass, UK) (electrospray mode (ESI); maximum mass 2000 *m/z*) was used in the ESI^+ mode. Various Waters Xterra HPLC columns were evaluated (RP-C18, MS-C18 and Phenyl), but the Waters Xterra Phenyl HPLC column (150 mm × 2.1 mm, 5 μm) performed the best and was used for all experiments. A Harvard syringe pump (Model 11) was obtained from Harvard Apparatus, Holliston, USA and was used for all direct injection experiments on the ZMD detector. Sample mixing was done on a Heidolph rotating sample mixer, and sample cleanup was done by centrifuging all samples at 4000 rpm using a Centronic S-577 centrifuge.

To ensure the maximum retention of basic alkaloids, the initial chromatographic conditions were 10% acetonitrile and 90% water containing 10 mM ammonium acetate, the pH adjusted to 10.5 with ammonia (25%). After injection, the chromatographic conditions were gradually changed through a linear gradient profile to 80% acetonitrile and 20% of the original aqueous mobile phase in 20 min. These conditions were kept stable for four minutes whereafter the HPLC system re-equilibrated the analytical column to the initial conditions. The flow rate was kept constant at 0.2 ml/min. The electrospray ionization (ESI) probe of the ZMD detector was optimized by direct injection via a syringe pump using an atropine test solution (10 μg/ml). The desolvation temperature was set to 400 °C and the source block temperature to 120 °C. The capillary voltage was set to 0.4 V, the extractor to 2.0 V, the RF lens to 0.5 V and the cone voltage to 25 V.

2.3. Determination of LOD, LOQ and calibration range

The limit of detection (LOD) and the limit of quantitation (LOQ) for the two detectors used are summarized in Table 1. The LOD was determined experimentally, and was taken as the concentration that produced a detector signal that could be clearly distinguished from the baseline noise (±3 times baseline noise). The LOQ was taken as the concentration that produced a detector signal ± 10 times greater than the LOD signal. The poor LOD and LOQ values of the PDA detector can be ascribed to the poor chromophoric properties of atropine and scopolamine (Fig. 2). A calibration curve was prepared covering the 100–10 000 ng/ml concentration range. The coefficient of determination was 0.9987 and was obtained using a second order curve fitting algorithm ($r = 0.9987$; $r^2 = 0.9982$).

2.4. Chemical fingerprinting of D. stramonium and D. ferox plants

To evaluate the alkaloid profile of seeds from the two *Datura* species, four plants of each species were collected from geographically isolated areas. For *D. stramonium*, four plants from each color variety (white flowers and purple flowers) were collected. Table 2 gives sample details of the 12 *Datura* plants evaluated. Five seeds from the dry capsules of each plant were dried at 40 °C for 48 h. The seeds were ground up with a pestle and mortar and the pulp was extracted with three 5 ml aliquots of HCl-PBS buffer for 15 min. The HCl-PBS buffer extracts of each plant were combined and were eluted through an Oasis HLB (60 mg) solid phase extraction cartridge and washed with 5 ml deionized water. The SPE cartridges were dried using vacuum and the alkaloids eluted with 5 ml methanol. The methanol fractions were evaporated under reduced pressure and reconstituted in 1 ml of methanol.

2.5. Chemical extraction and analysis of viscera samples

As the fatality under investigation was submitted to the Forensic Chemistry Laboratory as a suspected heart attack case, a general extraction procedure was initially followed. An aliquot of liquefied stomach (including contents), liver and kidney contents (3 g each) were mixed with 20 ml of PBS solution and shaken for thirty minutes on the rotating sample mixer. After centrifugation for 15 min the aqueous phase was passed through an Oasis HLB cartridge and the absorbed compounds stripped from the solid phase material with 5 ml methanol. The methanol was evaporated under reduced pressure and reconstituted in 0.5 ml of methanol. The sample (in methanol) was injected (10 μl) directly into the HPLC system and analyzed using the optimized HPLC method described.

Table 1
LOD and LOQ results for the PDA and ZMD detectors

Detector	LOD scopolamine	LOD atropine	LOQ scopolamine	LOQ atropine
PDA (200–600 nm)	1 μg/ml	1 μg/ml	10 μg/ml	10 μg/ml
ZMD (100–400 amu)	100 pg/ml	10 pg/ml	1 ng/ml	100 ng/ml

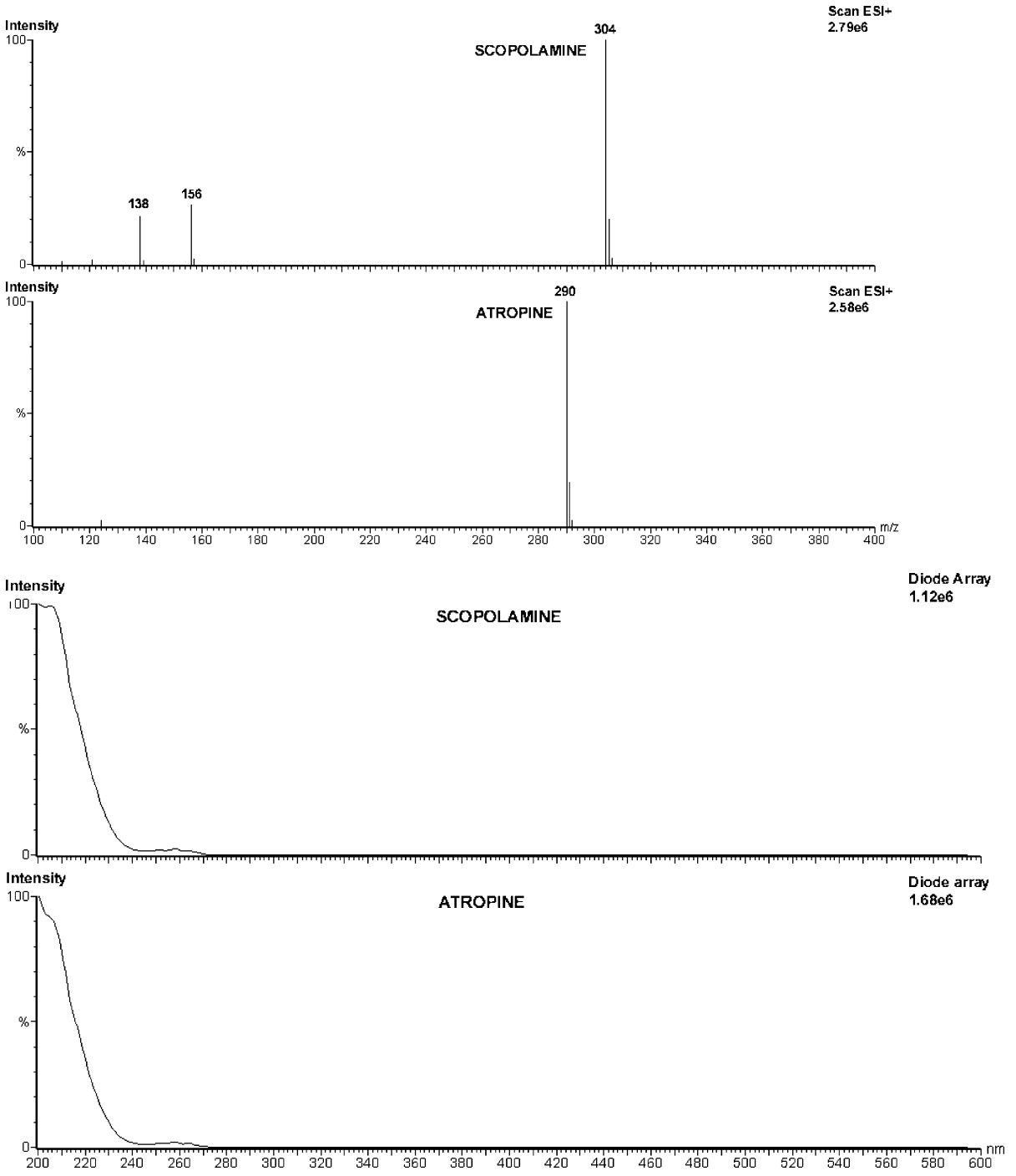

Fig. 2. UV and ESI + spectra of scopolamine and atropine.

3. Results and discussion

Standard toxicological screening of body fluids and tissues for poisonous plant components varies from one Laboratory to another, but the development of a highly sensitive screening method for alkaloids would be of great value to any forensic laboratory, especially if the method is robust, able to handle plant and viscera samples and includes a secondary confirmation without the need for a MS^n mass spectrometer.

Table 2
Datura species used, their geographical locations and the alkaloid contents of the seeds

Species	Collection site	Reference in text	Atropine:scopolamine ratio in seeds
Datura stramonium (White flowers)			
	Witfield, Boksburg, Gauteng Province	WFDSW	6:1
	Melville Koppies, Johannesburg, GP	MVDSW	4.6:1
	East Rand Mall, Boksburg, GP	ERMDSW	4.8:1
	Zeerust, North-West Province	ZRDSW	6.9:1
Datura stramonium (Purple flowers)			
	Elsburg, Germiston, GP	EBDSP	8.4:1
	Witfield, Boksburg, GP	WFDSP	8.6:1
	Edenvale, Kempton Park, GP	EDVDSP	6.9:1
	Zeerust, North-West Province	ZRDSP	7.6:1
Datura ferox (White flowers)			
	Witfield, Boksburg, GP	WFDFW	1:8.4
	Zeerust, North-West Province	ZRDFW	1:6.9
	Elsburg, Germiston, GP	EBDFW	1:5.6
	Edenvale, Kempton Park, GP	EDVDFW	1:7.2

3.1. Chromatography and detection of atropine and scopolamine

Tropane alkaloids are easily separated and detected by TLC [23,24] but this technique is not specific enough for forensic purposes. Gas chromatography (GC) still remains as one of the most commonly used techniques to analyze for these alkaloids in high concentrations [5,25], but is prone to many problems like decomposition or on-column dehydration, which can only be eliminated by preliminary silylation [23,24]. Gas chromatography coupled to a mass spectrometer is commonly used [26–30], but is also hampered by the problems experienced with GC analysis and must be derivatized prior to analysis [5]. These problems may also result in failing to detect the active component(s) of an intoxication caused by ingestion of plant material, or may result in very low recoveries of the suspected toxic compound(s) [31]. Another useful technique is HPLC that gives good resolution using reversed phase chromatography or ion pairing chromatography [23,30,32–34], and can even differentiate between S-(−) and R-(+)-hyoscyamine as well as S-(−) and R-(+)-scopolamine using a chiral stationary phase [35]. The typical chromatographic conditions of these techniques are unfortunately not conducive to coupling of the HPLC system to a mass selective detector. This is mainly due to the presence of involatile buffers, ionpairing reagents [32], complexometric reagents (chemiluminescence detection) [36] or derivatization reagents (fluorescence detection) [37]. In a previous publication [38], the chromatography of anabasine was highlighted, and many of the problems experienced with anabasine are also applicable to atropine and scopolamine, and relates well to those problems highlighted by other authors [32,39]. In a comprehensive review of poisoning by plant material [40], the authors commented on the remarkable absence of general screening methods in forensic toxicology. It was also mentioned that few methods exist for the analysis of tropane alkaloids and various other compounds. A method based on HPLC-MS-MS was developed and successfully applied to three forensic cases. However, this method could not be duplicated in the FCL JHB due to the absence of a MS-MS detector and the use of halogenated solvents in the extraction process.

Due to the unstable nature of scopolamine [7,8], atropine was used in the initial chromatographic studies. The high pK_a value (10.2) for atropine posed some problems as most analytical columns can not function at pH values higher than nine due to degradation of the silica support structure of the reversed phase analytical columns. Lowering the pH will reduce column bleed, but results in poor peak shape and markedly reduced sensitivity. To enable direct coupling of the HPLC to the ZMD detector, a mobile phase containing ammonium acetate in the aqueous portion was evaluated. The Xterra range of columns from Waters, USA (RP C18, MS C18 and Phenyl), which can be used at a pH > 9, were evaluated and found to be suitable for the chromatography of atropine. The introduction of scopolamine to the test mixture resulted in increased tailing of the atropine peak. This hampered the baseline resolution between atropine and scopolamine as scopolamine eluted just after the atropine peak. The Xterra Phenyl stationary phase did not suffer from this problem as the elution order reversed—scopolamine eluted first followed by atropine with a much improved peak shape.

A mobile phase containing 10 mM ammonium acetate, adjusted to a pH of 10.5 with ammonia, was found to result in reproducible chromatography without reduction in sensitivity. A typical chromatogram of an atropine/scopolamine test mixture (50 ng/ml of each analyte) (Fig. 3) shows

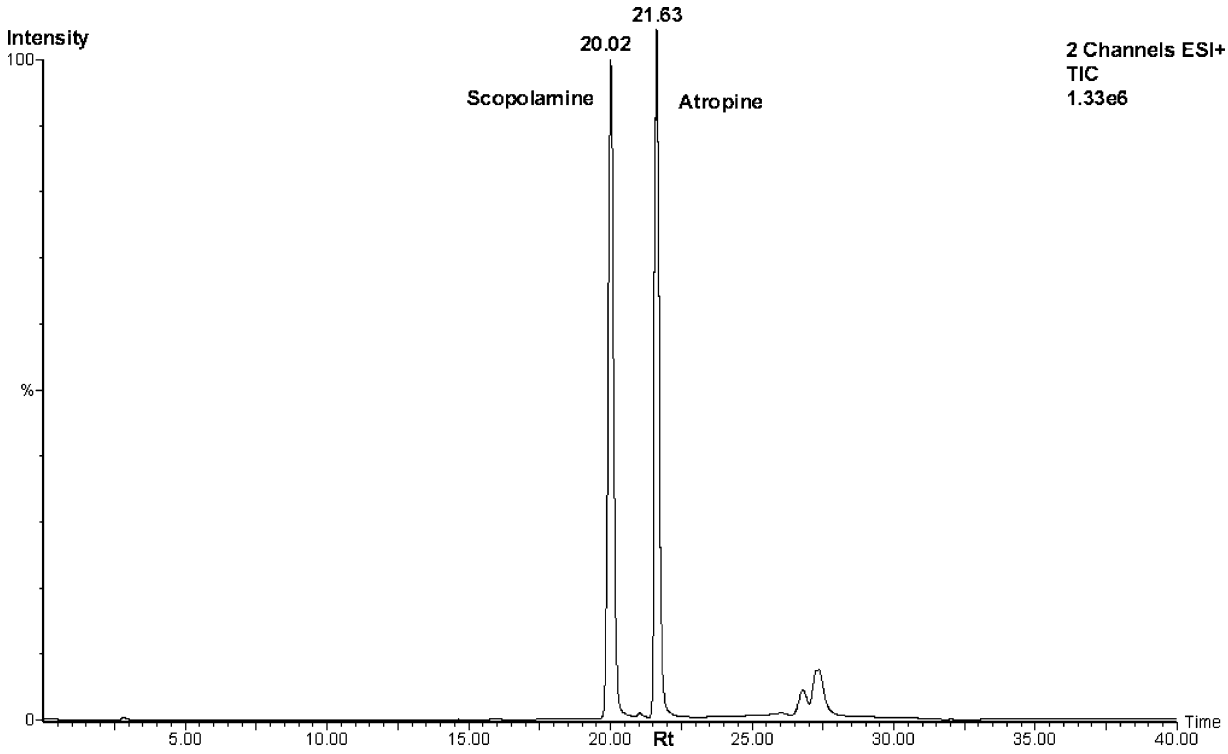

Fig. 3. Typical ESI + chromatogram of a 50 ng/ml mixture of scopolamine and atropine.

scopolamine with a retention time of 20.02 min and atropine with a retention time of 21.63 min. Under these chromatographic and mass spectrometer conditions, atropine produced an ESI + mass spectrum with a $(M+H)^+ =$ 290.4 amu (which is also the base peak), while scopolamine produced an ESI + massspectrum with a $(M+H)^+ =$ 304.4 amu (also the base peak). Atropine displayed very little dissociation under these conditions, while scopolamine displayed minor in-source collision-induced dissociation (mass fragments at 138.3 and 156.4 amu) (Fig. 2). In-source collision-induced dissociation (ISCID) can be induced by increasing the cone voltage from 25 to 50 V. This resulted in a characteristic fragmentation pattern for each alkaloid that can serve as a second confirmation of the analyte.

3.2. Chemical fingerprinting of D. stramonium and D. ferox populations

The extracts of the seeds from the eight *D. stramonium* plants (Table 2) produced similar chromatograms with atropine as main component and scopolamine as minor component. The ratio between scopolamine and atropine was on average 1:7 for both color varieties, but it must be noted that the ratio varied between populations and between color varieties (1:8 average for purple and 1:6 average for white) (Table 1). The results differ from published results that stated that the scopolamine-atropine ratio is 1:2 [5]. The published results were obtained after alkalizing the plant material with NaOH prior to extraction with dichloromethane.

The extracts of the seeds from the four *D. ferox* plants (WFDFW, ZRDFW, EBDFW and EVDFW) produced similar chromatograms with scopolamine as the main component and atropine as minor component. The ratio between scopolamine and atropine was on average 7:1, but it must be noted that the ratio varied between populations (Table 2).

Visual differentiation between the seeds of *D. ferox* and *D. stramonium* was hampered by the large variation in size and color between the species as well as within species. The chemical profile, however, clearly distinguished between the two species and was sufficiently uniform (both qualitatively and semi-quantitatively) in their alkaloid contents to allow a reliable characterization of the species.

3.3. Evaluation of SPE extraction and analysis of viscera samples

Due to the fact that the case was submitted to the FCL as an unknown case, a general extraction of the viscera was performed. Traces of atropine was detected on the LC-MS system in the liquefied stomach sample, but could not be confirmed on the GC-MS system. A closer inspection of the minced stomach revealed several brown to black

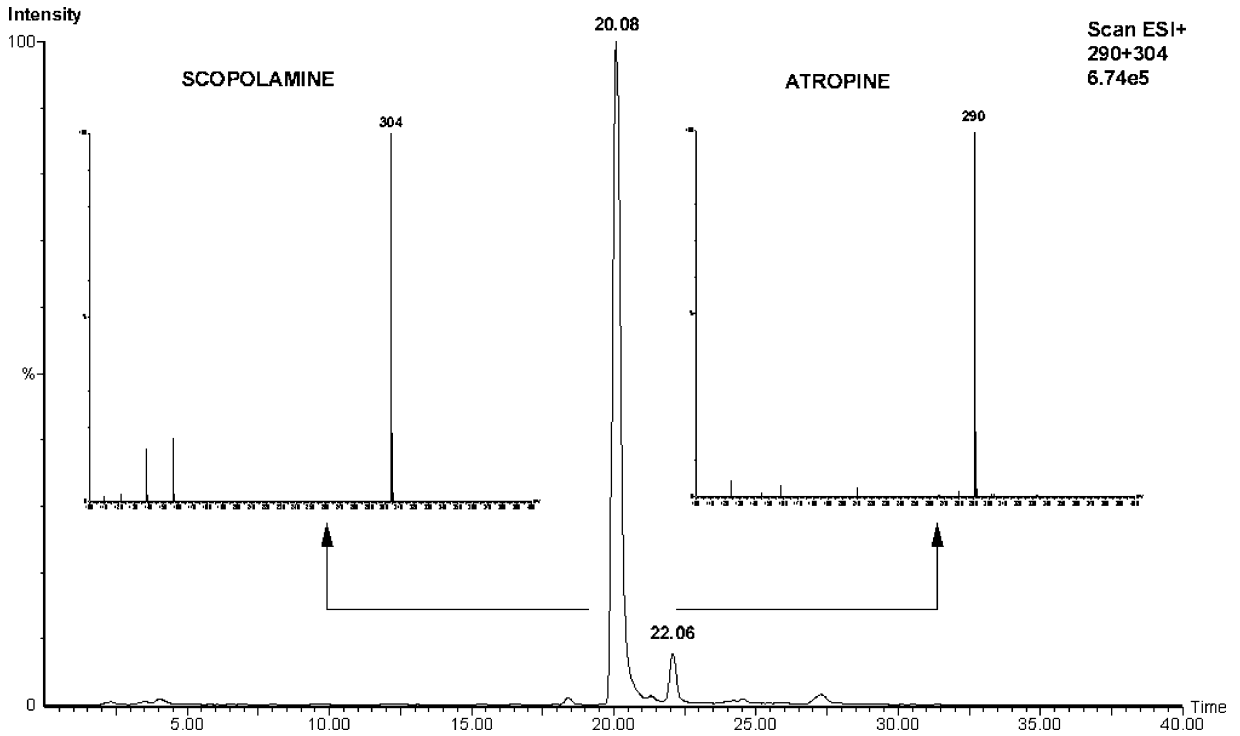

Fig. 4. Analysis results of the intact seeds found in the stomach.

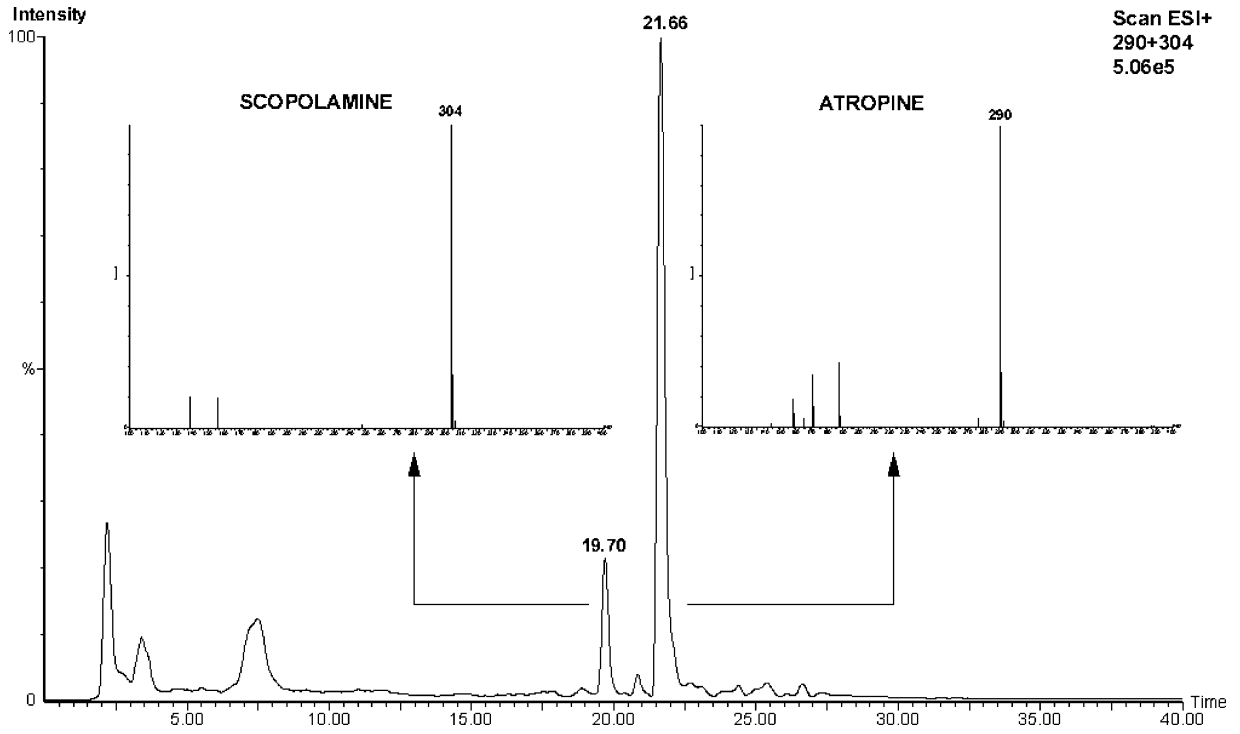

Fig. 5. Analysis results of the minced stomach sample.

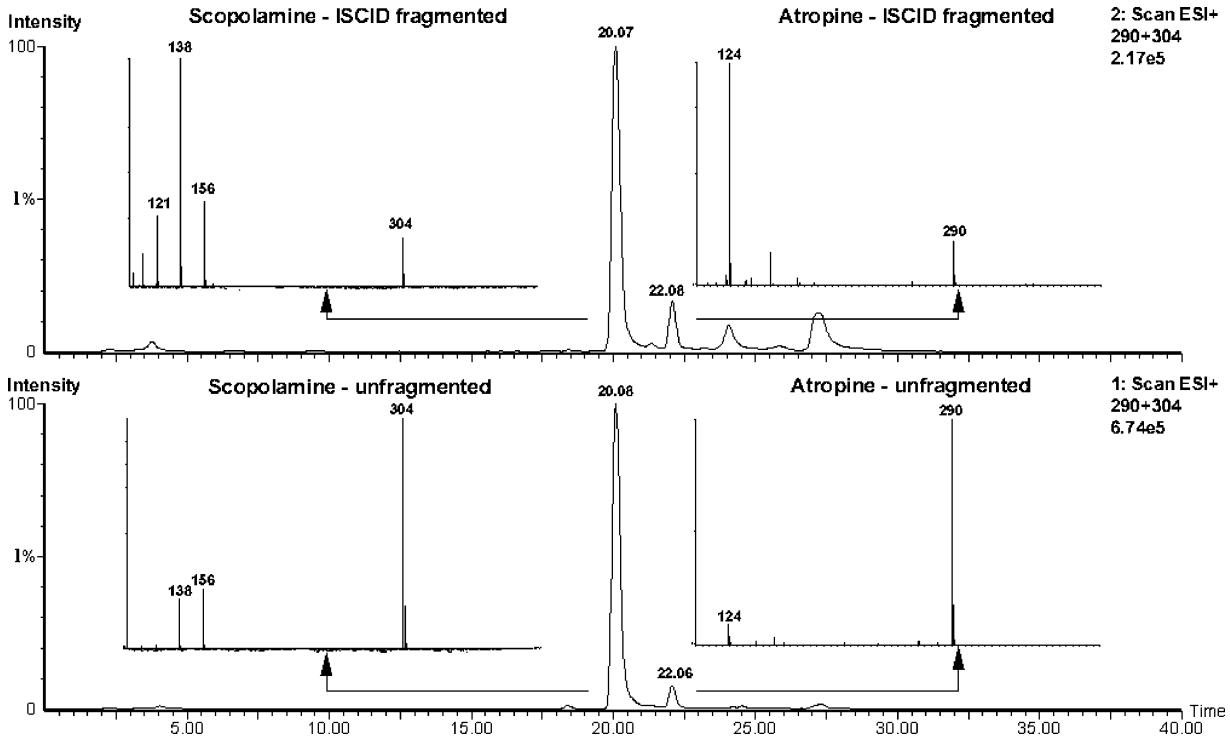

Fig. 6. Confirmation of atropine and scopolamine by ISCID ESI + mass spectrometry.

kidney-shaped seeds, which was removed from the sample for separate analysis. A methanol extract of the intact seeds produced no significant results, but an extract of the ground seeds tested positive for atropine and scopolamine as the major and minor components, respectively (Fig. 4). The chromatographic and mass spectral profiles obtained were identical to that of the *D. ferox* seed extracts.

The liquefied stomach sample, without any visible seeds present, as well as the liquefied liver and kidney samples were extracted utilizing the HCl-PBS buffer method. Atropine (major component) and scopolamine (minor component) were detected in the stomach sample but not in the liver or kidney sample (Fig. 5). These results were initially confusing as the same alkaloid profile as the intact seeds from the stomach (typical *D. ferox* alkaloid profile) was expected (scopolamine:atropine = 7 : 1). The obtained profile was more comparable with a *D. stramonium* alkaloid profile (scopolamine:atropine = 1 : 7). In an attempt to mimic the conditions in the stomach, a few *D. ferox* seeds were ground as before, but the HCl-PBS buffer was acidified to pH 1.5 with hydrochloric acid. The extract obtained from this experiment was analyzed with the optimized HPLC-ZMD method and a very good match was obtained with the liquefied stomach sample that contained no seeds. This confirmed the hypothesis that *D. ferox* seeds were administered to the patient, and that the scopolamine released from the macerated seeds was hydrolyzed at strong acidic pH, but that the more stable atropine remained intact.

As a final confirmation of atropine and scopolamine in the seeds and liquefied stomach sample, the samples were reanalyzed by utilizing a dual function MS method: Function 1 used a cone voltage of 25 V while Function 2 used a cone voltage of 50 V to induce ISCID. The results clearly confirmed the presence of atropine and scopolamine in the samples (Fig. 6).

It is clear that HPLC coupled to an ESI mass selective detector offers a reliable and very sensitive method for the identification and confirmation of atropine and scopolamine in biological samples. By using the optimized technique described above, the seeds of *D. stramonium* can be distinguished from those of *D. ferox* by comparing their atropine-scopolamine ratios. We have demonstrated that the method is suitable for application in forensic toxicology.

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