Determination of tropane alkaloids atropine and scopolamine by liquid chromatography–mass spectrometry in plant organs of *Datura* species

Silvia Jakabová [a,b], Lajos Vincze [c], Ágnes Farkas [d], Ferenc Kilár [a,e], Borbála Boros [f], Attila Felinger [a,*]

[a] *Department of Analytical and Environmental Chemistry, Faculty of Science, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary*
[b] *Department of Chemistry, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Tr.A. Hlinku 1, 94974 Nitra, Slovakia*
[c] *Simkon Ltd. Színjátszó utca 30, H-1163 Budapest, Hungary*
[d] *Institute of Pharmacognosy, Medical School, University of Pécs, Rókus utca 2, H-7624 Pécs, Hungary*
[e] *Institute of Bioanalysis, Medical School, University of Pécs, Szigeti út 12, H-7624 Pécs, Hungary*
[f] *Institute of Viticulture and Oenology, University of Pécs, Pázmány Péter u. 4., H-7634 Pécs, Hungary*

ARTICLE INFO

ABSTRACT

Hyoscyamine (atropine) and scopolamine are the predominant tropane alkaloids in the *Datura* genus, occurring in all plant organs. The assessment of the alkaloid content of various plant parts is essential from the viewpoint of medical use, but also as a potential risk of toxicity for humans and animals. Therefore, a reliable method for the determination of tropane alkaloid content is of high importance. The present work aimed at the elaboration of a rapid method for determination of the most abundant *Datura* alkaloids by LC–MS technique using a new generation of core–shell particle packed column. Tropane alkaloid content was investigated in various plant organs of four *Datura* taxa (*D. innoxia*, *D. metel*, *D. stramonium*, and *D. stramonium* var. *tatula*), grown under the same conditions, in two developmental stages. We have developed a rapid LC–MS method for the quantitative determination of atropine and scopolamine, which was successfully applied to quantify the alkaloids in different plant organs (leaves, flowers, stems, seeds) of thorn apples after a simple sample preparation step. Elaboration and validation of the method and analysis of plant extracts were done by UFLC–MS technique, employing an Ascentis Express C18 column. Detection was done in positive ionization mode (ESI+) and the method suitability was evaluated by several validation characteristics. Quantitation limits are 333 and 167 pg mL^{-1} for scopolamine and atropine, respectively, and the method shows very good repeatability. The analysis of *Datura* extracts revealed significant differences depending on the species, the organ and the sampling period. Atropine was found to be dominant over scopolamine in three out of the four taxa investigated. *D. innoxia* showed the highest concentrations of scopolamine in all organs examined, whereas *D. metel* accumulated the lowest scopolamine levels. Hyoscyamine, measured as atropine, was the highest in *D. stramonium* var. *tatula*, and the lowest in *D. innoxia*. Samples collected in summer had higher scopolamine levels than autumn samples, concerning both stems and leaves.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The genus *Datura* belongs to the *Solanaceae* family, which is well known for synthesizing a number of tropane alkaloids. Thorn apple species, indigenous to America and Asia, are widespread with higher abundance in tropical and sub-tropical regions [1,2], but they are also planted in other regions, including Europe, as ornamentals and medicinal plants.

Traditionally *Datura* plants have been used for mystic and religious purposes [3] and as natural drugs with narcotic effects or to treat asthma [4]. Well known psychoactive effects make *Datura*

a tempting choice for sensation-seeking young people. Plants are consumed or smoked to achieve hallucinogenic experiences [5–9]. On the other hand, several accidental intoxications of humans and animals coming from food sources contaminated with *Datura* plants have also been reported [10,11].

According to a large-scale survey conducted in the US between 1983 and 2009, *Datura*-related intoxications are the most frequent among plant exposures with fatal outcomes, being responsible for 20% of the fatalities [12]

Phytochemical composition of *Datura* species makes them attractive for conventional medicine. Especially *D. metel* and *D. innoxia* are cultivated for pharmaceutical purposes, since they have been recognized as rich sources of hyoscyamine and scopolamine, however, the tropane alkaloids mentioned occur in the plant organs of all *Datura* species [8,13,14].

* Corresponding author. Tel.: +36 72 501 500x24582; fax: +36 72 501 518.
E-mail address: felinger@ttk.pte.hu (A. Felinger).

Atropine (a racemic mixture of optical isomers, also referred to as $(\pm)$-hyoscyamine), and scopolamine (also called hyoscine) are among the principal natural alkaloids of medicinal interest [15]. The application of the anticholinergic agents is very wide. They are used in ophthalmology diagnostics, as antispasmodics, preoperative and postoperative medications, as analgesics, narcotics, sedatives, as well as in treatment of asthma or sialorrhea, Parkinson's disease and motion sickness [16–20].

In the field of medical defense, atropine was shown to be effective against nerve gases Sarin, VX, VR, Tabun [18]. Antidote efficiency was proved also against organophosphate poisoning, where *D. stramonium* extract significantly decreased the mortality of experimental rats [21].

Besides medicinal usage, *Datura* extracts were recommended as cheap and efficient insecticides with almost no side effects on the environment [2].

Investigation of alkaloid production and accumulation in plant organs during different stages of development provides important information regarding the optimal time of collecting plant material. Iranbakhsh et al. [22] monitored atropine and scopolamine levels in *D. stramonium* from the point of germination. According to their analysis, alkaloid production starts at the second week after germination. Alkaloid biosynthesis increases in different organs up to the tenth week of growth, and then decreases. In the first stage (emerging plants) the total alkaloid content was shown to be higher in roots than in aerial parts. Rapid vegetative growth of young plants till flower formation may lead to a decrease in the total alkaloid content of the roots and leaves. Further ontogenetic stages promote mainly fruit and seed development and are characterized by increasing alkaloid biosynthesis. The stage of immature fruits in summer period was reported to have the highest alkaloid concentration in the vegetative organs (stems, petioles, leaves). Generally the younger parts of plants contain more alkaloids than the older ones. The last stage is characterized by a decrease of alkaloid content in senescent plants and alkaloids are retained in seeds [22,23].

Hyoscyamine (atropine) and scopolamine are considered to be the end products of the biosynthetic pathway, and their ratio and content have been used for monitoring of ontogenetic variations [14,23]. Concentrations of hyoscyamine and atropine in different plant organs and various *Datura* species have been reported in several studies, e.g. Al-Humaid and co-workers [24].

Tropane alkaloids are useful also for the chemotaxonomical analysis of the *Solanaceae* family. Chemotaxonomic studies based on alkaloid profiles were published in several papers [8,13,23,25]. Doncheva et al. [13] reported sixty-six alkaloids in the tribe *Datureae*. The variable alkaloid pattern of *Datura* samples (even from the same species) collected from different geographic regions suggested that environmental conditions (e.g. climate, soil factors, nutritional status) significantly affected the production of secondary metabolites. Chemotaxonomical studies, based on tropane alkaloids, require excluding such environmental factors, which can be achieved by cultivating different *Datura* species at identical environmental circumstances. Under these conditions, only the genotype will determine the alkaloid spectra and accumulation [23,25].

Several analytical techniques have been employed for the analysis of hyoscyamine (atropine) and scopolamine. Besides the enzyme-linked immunosorbent assay (ELISA) [26], and the spectrometric methods (based on indirect determination of alkaloid drugs by formation of ion-associate metal complexes of the drugs) like atomic emission spectrometry (AES) [27], atomic absorption spectrometry (AAS) [28], UV spectroscopy [26], and various separation techniques are frequently applied. Capillary electrophoresis (CE) has been recommended as an inexpensive method for the study of pharmacological and plant samples. Several approaches have been published for capillary zone electrophoresis (CZE) [16,29–33] and micellar electrokinetic chromatography–mass spectrometry (MEKC–MS) [34]. Recently, CZE with MS interface [15] was shown to provide fast separation and high resolution. The disadvantage of common CE techniques is their relatively higher detection limit (LOD is around $5 \,\mu g \, mL^{-1}$) [16], however, coupling an MS detector and using SIM mode can improve the detection limit by three to four orders of magnitude [33].

Chromatographic methods have been represented by thin-layer chromatography (TLC) [26,30,35,36], high performance thin-layer chromatography–densitometry (HPTLC-densitometry) [37], gas chromatography (GC) [30], and gas chromatography–mass spectrometry (GC–MS) [8,13,23,25,26,38–41], which were used for studying alkaloid patterns and/or quantities in various biological samples. Namera et al. [39] validated the GC–MS method and they achieved LOD at $5 \, ng \, mL^{-1}$.

Quantitative analysis of atropine and scopolamine was also performed by reversed-phase high-performance liquid chromatography with UV detection (RP-HPLC) [26,30,37,38,42–47]. Coupling the LC with electrospray mass spectrometry (LC–MS) allows the development of efficient methods for the simultaneous determination and quantification of atropine and scopolamine in biological fluids [16,17,48–53]. To our knowledge, the lowest limits of quantitation (LOQ) for LC–MS were reported in our previous work, at levels below $1 \, ng \, mL^{-1}$ both for atropine and scopolamine [17].

Modern demands on development of the new LC methods shift the interest on a use of the newly developed sub 3-μm particle packed columns, which are linked with the modern UFLC or UHPLC systems. A new generation of reverse-phased columns has been designed for rapid and efficient separations. A significant contribution to the acceleration of LC analysis is ascribed to the superficially porous particles. The core–shell structure allows the migration of mobile phase and analyte only through the porous layer, which results in decrease of the diffusion time and path compared with totally porous packing materials of the same size, thus higher efficiency may be expected [54].

Sample preparation reported for tropane alkaloids is usually done by liquid–liquid extraction (LLE) [50,51] and liquid-solid extraction with applying sonication [17,37,44] or by applying solid-phase extraction (SPE) using conventional sorbents (on C18 cartridges) [30,37,44,49,50,52,53].

Therapeutic values, as well as intoxication risks by tropane alkaloids necessitate the development of a reliable, efficient, and fast method for their determination in pharmaceutical products, plant extracts, as well as human and animal samples.

The present study aimed at the development and evaluation of a new LC–MS method for determination of key alkaloids – atropine and scopolamine – in three *Datura* species.

A novel method was developed for the rapid determination of the most abundant tropane alkaloids from their herbal extracts, which were prepared by a simple extraction procedure.

The efficiency of the method was characterized by a rapid evaluation of a number of validation parameters. The current analytical method has better limits of detection and quantitation than other published methods.

Since the plants were grown under the same environmental conditions, this experimental setting allowed us to study atropine and scopolamine levels as a function of the botanical origin of the samples. Besides the role of the genetic background, the influence of the developmental stage on the alkaloid levels was also investigated in different plant organs, which is important from the viewpoint of harvesting for medical purposes.

2. Materials and methods

2.1. 2.1. Chemicals

(±)-Atropine (99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). (−)-Scopolamine hydrochloride (Reag. Ph. Eur.) was purchased from Merck (Darmstadt, Germany). Formic acid (eluent additive for LC–MS) and water (LC–MS Chromasolv) were obtained from Fluka (Buchs, Switzerland). Methanol (LC–MS Chromasolv) was purchased from Riedel-de Haën GmbH & Co. (Seelze, Germany).

2.2. Plant samples

Plant samples were collected in the Melius Medicinal Plant Garden of the University of Pécs, Hungary in 2009. Plant material was collected at two stages of development: fully developed plants in bloom (summer samples) and plants with fruits (autumn samples). Harvested *Datura* species and organs, together with sampling periods, are listed in Table 1.

2.3. LC–MS system

The LC–MS system consisted of a liquid chromatograph (Prominence Liquid Chromatograph LC-20 AD, Shimadzu), a micro vacuum degasser (Prominence Degasser LC-20 A3, Shimadzu), an auto sampler (Prominence Auto Sampler SIL-20 ACHT, Shimadzu), a diode array detector (Prominence Diode Array Detector system SPD-M20A, Shimadzu), a column oven (Prominence Column Oven CTO-20 AC, Shimadzu), a controller (Prominence Controller CBM-20 A, Shimadzu), and an MS detector with electrospray ion source and quadrupole analyzer (Liquid Chromatograph Mass Spectrometer LCMS-2020, Shimadzu). The LabSolutions (Shimadzu) software was used to control the LC–MS system and for data processing. The column effluent passed through a diode array detector before arriving in the MS interface.

2.4. LC–MS method

Chromatographic separations were performed on an Ascentis Express C18 column (50 mm × 2.1 mm, 2.7 μm, Supelco, USA). For the separations, a gradient of mobile phase A (1% (v/v) formic acid in water) and mobile phase B (1% (v/v) formic acid in methanol) was used. The gradient profile was set as follows: 0.00 min 10% B eluent, 10.00 min 90% B eluent, 17.00 min 90% B eluent, 17.10 min 10% B eluent, 23.00 min 10% B eluent. The flow rate was 0.2 mL min^{-1}, the column temperature was 50 °C. The injection volume was 2 μL for *Datura* extracts and standard mixtures. The ions of the compounds and their retention times are referred in Sections 3.1.1 and 3.1.2.

The electrospray source was operated in positive mode and the interface conditions were as follows: capillary voltage of 2.1 kV, curved desolvation line (CDL) voltage of 0.0 V, CDL temperature of 200 °C and deflector voltage of 0.0 V. The nebulizing gas flow rate was 1.5 L min^{-1}, the drying gas flow rate was 1.5 L min^{-1} and was obtained from a nitrogen generator. The detector voltage was 0.95 kV.

2.5. Procedures

2.5.1. Standard solutions

From the methanolic stock solution of atropine and scopolamine (each 10 mg mL^{-1}), standard solutions were prepared for the calibration in the concentration range from 10^{-6} to 10^{-3} mg mL^{-1} by dilution of stock solution with solvent mixture (1% (v/v) formic acid in methanol:water = 7:93). The solutions were stored at 4 °C and used for a week.

2.5.2. Sample preparation

Plant part samples (stems, leaves, flowers, fruits and seeds) from four *Datura* taxa (*D. innoxia*, *D. metel*, *D. stramonium* and *D. stramonium* var. *tatula*) were air dried at room temperature and stored in paper bags until the laboratory analysis. Plant material was pulverized, and 100 mg of the herbal powder was weighed to volumetric flasks. Extraction was ensured with 30 min. ultrasonication after addition of 10 mL solvent mixture ($CH_3OH:H_2O = 3:2$ (v/v)) to each sample. Purification of extracts was done by centrifugation (15 min at 10,000 min^{-1}) and filtration through a 0.45 μm pore size Syringeless filter (Mini-Uniprep, Whatman). Samples were stored in the dark at 4 °C until the LC–MS analysis was carried out. Appropriate dilution of samples was performed according to need before the injection to LC–MS system.

3. Results and discussion

3.1. Rapid method validation

Verification of the reliability of the developed method was carried out by a rapid validation. Several validation parameters were evaluated to assert that the LC–MS method had performances compatible with those required for routine analysis of tropane alkaloids in *Datura* samples.

3.1.1. Selectivity

Resolution, observed between the peaks of scopolamine and atropine was higher than 1.5, which is acceptable even when applying UV detection. Nevertheless, in the field of tropane alkaloid analysis, MS detection is generally preferred because of its inherent high selectivity, especially in single ion monitoring mode (SIM). Fig. 1 shows typical MS-SIM chromatograms of the dominant alkaloids in the extracts of the species examined. The chromatograms correspond to the pseudo molecular ions [M+H]$^+$, where m/z 304 presents scopolamine and m/z 290 atropine.

3.1.2. Repeatability and intermediate precision

A high-speed reversed-phase column – Ascentis Express C18, packed with core–shell particles, was used for the separations. The packing particle contains solid silica core (1.7 μm diameter) coated with thin porous shell (0.5 μm) of high-purity chemically modified silica. This structure allowed the decrease of retention time, resulting in separation in 4.5 min. Compared to the previous study [17], the decrease of retention time of more than 1 min was reached for both compounds. The total time of each run, including washing and equilibration of the column, was less than 23 min.

Repeatability (evaluated as intra- and interday precision) of the method was tested by six replicate injections of both the standard solution (50 ng mL^{-1}), and a *D. metel* (flower + fruit) sample extract. The responses measured on each chromatogram were the retention time of atropine and scopolamine peaks and the corresponding peak area of the [M−H]$^+$ ions. Intra- and interday variations of retention times and concentrations, expressed in RSD %, are listed in Tables 2 and 3. Very low variation was observed in the retention times, with RSD values not exceeding 0.9%. RSD values for the concentration of alkaloids ranged from 0.03 to 3.53. The low values of standard deviation showed the precision and the repeatability of the retention time to be very good.

3.1.3. Determination of LOD, LOQ, and calibration range

The limit of detection (LOD) was determined experimentally, and was taken as the concentration, which produced a detector signal clearly distinguishable from the baseline noise (3 times the baseline noise). The limit of quantitation (LOQ) was taken as the concentration, which produced a detector signal 10 times higher than the baseline noise. A calibration curve was calculated based

Table 1
Datura samples: species, collected organs, sampling period.

Species	Stem		Leaf		Flower summer	Seed autumn
	Summer	Autumn	Summer	Autumn		
D. innoxia Mill.	−	+	+	+	+	-
D. metel L.	+	+	+	+	+	+
D. stramonium L.	+	+	+	+	+	−
D. stramonium L. var. *tatula*	+	−	+	+	+	−
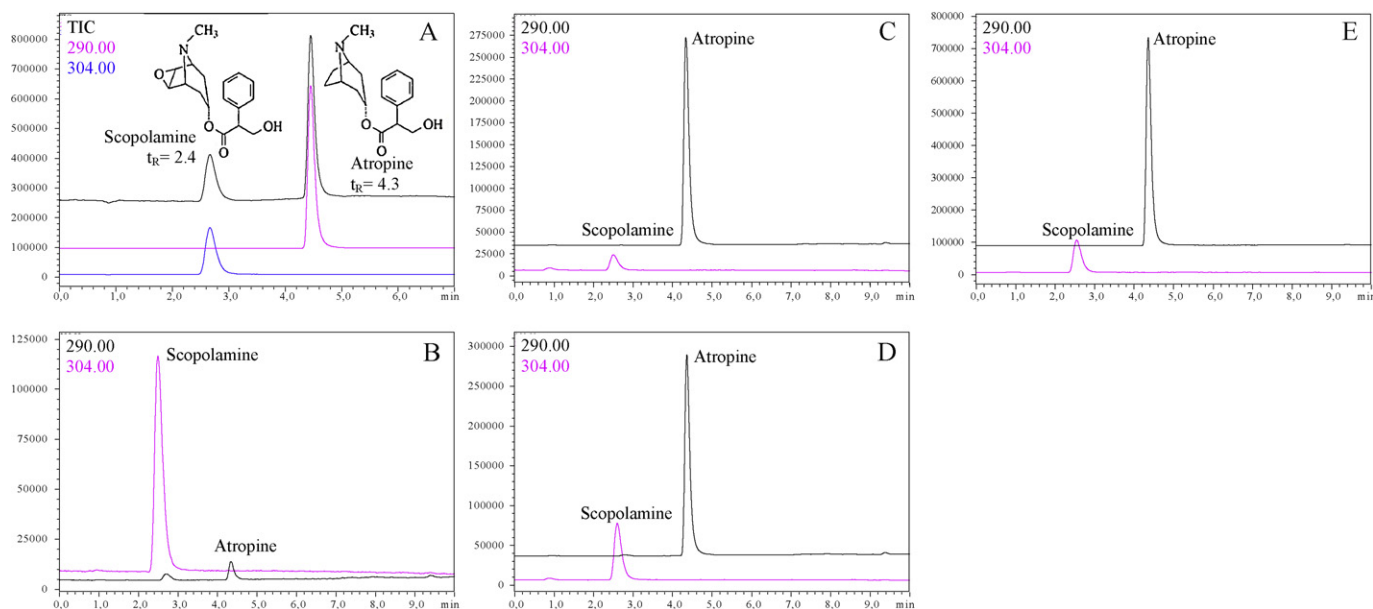
Fig. 1. LC–MS chromatograms of atropine and scopolamine obtained in SIM mode. TIC: total ion chromatogram. A: LC–MS chromatogram of standard solution ($c = 5 \times 10^4$ mg mL^{-1}); B: LC–MS chromatogram of *Datura innoxia* leaf; C: LC–MS chromatogram of *Datura metel* leaf; D: LC–MS chromatogram of *Datura stramonium* leaf; E: LC–MS chromatogram of *Datura stramonium* var. *tatula* stem.

on the peak areas, obtained from the chromatograms of seven different concentrations of the standard solution ranging from 1 ng to 1 μg mL^{-1}. Each concentration level was injected three times ($n = 3$). The calibration ranges adequately covered the variations in the amounts of atropine and scopolamine in the samples. The correlation coefficients (r^2) were 0.9999 and 1.0000 for scopolamine and atropine, respectively.

The LOD and LOQ values are summarized in Table 4. The LOQ limits are around 33% lower than those reported in our previous work [17].

3.1.4. Recovery

The recovery of the method was determined by the standard addition method on selected *Datura* extract sample (*Datura metel* L., small leaf, summer 2009). In spiked samples ($n = 3$) the concentrations of the tropane alkaloids were increased by 50%, 100%, and 150%. These spiked *Datura* extract samples were analyzed ($n = 3$) and the amount of analyte recovered was calculated. The

recoveries for the tropane alkaloids are between 94.8% and 100.6% (mainly between 96% and 98%).

3.2. Scopolamine and atropine levels in Datura plants

The alkaloid levels and scopolamine to atropine ratios (S/A) in different organs of *Datura* plants at two different stages of maturation are summarized in Table 5. Concentrations are expressed in mg g^{-1} of dry material.

3.2.1. Datura innoxia

Scopolamine was found to be predominant in all plant organs of this species. Maximum concentration was found in leaves of medium size and in flower samples, collected in summer period. The ageing process resulted in decrease of scopolamine content to a half in autumn period. Hyoscyamine content, measured as atropine, was found to be highest in stems; however, the stems were collected only from fruit producing plants in the case of this species.

Table 2
Intraday precision of the retention time of scopolamine and atropine in standard solution (concentration of each compound was 5×10^{-5} mg mL^{-1}) and intraday precision of concentration of scopolamine and atropine in *Datura metel* sample extract (flower + fruit).

Compounds	Standard solution ($c = 5 \times 10^{-5}$ mg mL^{-1})				*D. metel* extract (flower + fruit)			
	Retention time		Concentration		Retention time		Concentration	
	min[a]	RSD%	mg mL^{-1} [a]	RSD%	min[a]	RSD%	mg mL^{-1} [a]	RSD%
Scopolamine	2.22	0.66	4.89×10^{-5}	1.20	2.48	0.90	4.47×10^{-5}	1.56
Atropine	4.28	0.22	5.19×10^{-5}	0.65	4.34	0.36	2.45×10^{-4}	0.79

[a] Data are mean values from six determinations.

Table 3

Interday precision of the retention time of atropine and scopolamine in standard solution (concentration of each compound was 5×10^{-5} mg mL^{-1}) and interday precision concentration of scopolamine and atropine in *Datura metel* sample extract (flower + fruit).

Retention time

Tropane alkaloid	Standard solution ($c = 5 \times 10^{-5}$ mg mL^{-1}) Retention time					D. metel extract (flower + fruit) Retention time				
	Day 1[a], min	Day 2[a], min	Day 3[a], min	Mean, min	RSD%	Day 1[a], min	Day 2[a], min	Day 3[a], min	Mean, min	RSD%
Scopolamine	2.22	2.20	2.21	2.21	0.48	2.47	2.49	2.50	2.48	0.50
Atropine	4.29	4.26	4.27	4.27	0.34	4.34	4.33	4.33	4.34	0.12

Concentration

Tropane alkaloid	Standard solution ($c = 5 \times 10^{-5}$ mg mL^{-1}) Concentration					D. metel extract (flower + fruit) Concentration				
	Day 1[a], mg mL^{-1}	Day 2[a], mg mL^{-1}	Day 3[a], mg mL^{-1}	Mean, mg mL^{-1}	RSD%	Day 1[a], mg mL^{-1}	Day 2[a], mg mL^{-1}	Day 3[a], mg mL^{-1}	Mean, mg mL^{-1}	RSD%
Scopolamine	4.83×10^{-5}	4.58×10^{-5}	4.52×10^{-5}	4.64×10^{-5}	3.53	4.43×10^{-5}	4.47×10^{-5}	4.57×10^{-5}	4.49×10^{-5}	1.23
Atropine	5.75×10^{-5}	6.14×10^{-5}	6.07×10^{-5}	5.98×10^{-5}	3.49	2.45×10^{-4}	2.45×10^{-4}	2.45×10^{-4}	2.45×10^{-4}	0.03

[a] Data are mean values from six determinations.

Table 4

LOD and LOQ results for alkaloid standards.

Compounds	LOD, pg mL^{-1}	LOQ, pg mL^{-1}
Scopolamine	100	333
Atropine	50	167

Atropine content in big leaves declined in autumn approximately to one-third level of the concentration measured in summer. From the four investigated taxa, S/A ratios were found to be the highest in *D. innoxia*, considering all plant parts and sampling periods, confirming the extremely high ratio of scopolamine to atropine, measured also in our previous work, which focused on potential toxicity of *Datura* nectar [17].

Our observations are in agreement with the studies of Doncheva et al. [13] and Gadzikowska et al. [35], who reported approximately 4 times and 1.5 times higher scopolamine levels compared to atropine levels, respectively, expressed as percentage of total ion current in *D. innoxia* roots and as the result of a 2D-TLC separation of *D. innoxia* plant extract. In contrast, *D. innoxia* plants, grown on sandy soils in Saudi Arabia, were reported to contain hyoscyamine as their dominant alkaloid, whose content decreased in the order of leaves and fruits, followed by stems, roots and crowns [24]. Berkov and Zayed [25] confirmed that alkaloid ratios did not only depend on the plant organ, but also on the geographical origin of samples: scopolamine was found in the seeds of *D. innoxia* from Bulgary and Egypt, hyoscyamine in the roots of Bulgarian samples, and tropine in the roots of Egyptian samples to be dominant.

3.2.2. Datura metel

It has been reported that the scopolamine-to-atropine ratio (S/A) is higher than 1 in the whole plant of *D. metel*, including its roots [55–57]. In our study, however, higher S/A ratio was not universal for each aerial part analyzed. Scopolamine was found to be dominant to atropine only in the case of the summer stems (S/A ratio was 6.67) and autumn seeds (S/A ratio was 6.54). Pramod et al. [56] reported even higher S/A ratios (10 and 55 in stems and leaves, respectively) in young *D. metel* plants. In contrast with the above mentioned studies, we found the atropine levels to be higher than those of the scopolamine in the case of leaf samples during both sampling periods. The S/A ratios in the leaves changed from 0.133 in summer to 0.189 in autumn. The scopolamine content in autumn stems decreased significantly compared to the summer ones.

Results, which are in agreement with ours have been reported by Doncheva et al. [13], who found nearly double atropine percentage compared to scopolamine in *D. metel* roots, grown in greenhouse conditions.

Growth conditions (including environmental, soil-climate conditions, artificial cultivation media, additions of mineral or organic nutrients, etc.) have been confirmed as the most determining factor for development of the secondary metabolites in *Datura* species. Differences in the above conditions may be in the background of the differences measured in our study and that of Doncheva et al. [13], compared with other previous studies for *D. metel*.

3.2.3. Datura stramonium

Different alkaloid levels were measured in two *D. stramonium* varieties grown under the same conditions: all organs except the stem were found to contain significantly higher alkaloid concentrations in purple flowering var. *tatula* than in white flowering var. *stramonium*. In var. *tatula*, the scopolamine levels were 1.5 and 2 times higher in the leaves and flowers, respectively, than in the white-flowering var. *stramonium*. The difference was even more conspicuous in the atropine concentrations, where var. *tatula* contained approximately 2.5 times higher levels than var. *stramonium*, both in the leaves and in the flowers.

Table 5
Content of scopolamine and atropine in dry plant material of *Datura* species.

Datura species	Sample	Sampling period	Scopolamine (S)		Atropine (A)		S/A ratio
			mg g^{-1}	RSD%	mg g^{-1}	RSD%	
Datura innoxia Mill.							
D. innoxia Mill.	Small and big leaf	Summer 2009	2.00	0.88	0.06	1.25	33.83
D. innoxia Mill.	Medium leaf	Summer 2009	4.53	1.88	0.02	0.70	283.13
D. innoxia Mill.	Flower	Summer 2009	3.94	0.36	0.03	1.65	131.17
D. innoxia Mill.	Big leaf	Autumn 2009	0.94	1.72	0.02	2.40	41.00
D. innoxia Mill.	Stem	Autumn 2009	1.95	2.15	0.40	0.38	4.90
Datura metel L.							
D. metel L.	Small leaf	Summer 2009	0.28	1.52	1.43	0.13	0.20
D. metel L.	Big leaf	Summer 2009	0.13	0.97	0.95	1.21	0.13
D. metel L.	Stem	Summer 2009	3.42	3.35	0.51	1.33	6.67
D. metel L.	Flower + fruit	Summer 2009	0.45	0.92	2.45	0.71	0.18
D. metel L.	Leaf	Autumn 2009	0.01	0.78	0.07	0.77	0.19
D. metel L.	Fruit + seeds	Autumn 2009	3.44	2.26	0.53	1.37	6.54
D. metel L.	Stem	Autumn 2009	0.01	1.06	0.19	0.83	0.03
Datura stramonium L.							
D. stramonium L.	Small leaf	Summer 2009	1.16	1.56	1.45	0.54	0.80
D. stramonium L.	Big leaf	Summer 2009	1.02	1.32	1.91	1.03	0.54
D. stramonium L.	Stem	Summer 2009	3.32	1.45	5.51	1.42	0.60
D. stramonium L.	Flower	Summer 2009	1.36	0.16	1.69	0.71	0.81
D. stramonium L.	Leaf	Autumn 2009	0.13	2.23	0.43	0.56	0.30
D. stramonium L.	Stem	Autumn 2009	0.02	1.36	0.36	0.47	0.05
Datura stramonium var. *tatula* L.							
D. stramonium var. *tatula* L.	Small leaf	Summer 2009	1.79	2.22	3.68	2.07	0.49
D. stramonium var. *tatula* L.	Big leaf	Summer 2009	1.72	1.72	4.71	2.58	0.37
D. stramonium var. *tatula* L.	Stem	Summer 2009	2.51	0.92	5.91	0.67	0.42
D. stramonium var. *tatula* L.	Flower	Summer 2009	2.74	1.01	3.97	0.67	0.69
D. stramonium var. *tatula* L.	Leaf	Autumn 2009	0.23	1.92	1.07	3.85	0.22
D. stramonium var. *tatula* L.	Stem	Autumn 2009	2.13	4.92	4.72	3.32	0.45

Each plant organ of both var. *stramonium* and var. *tatula* contained more atropine than scopolamine, with S/A ratios ranging from as low as 0.05 to as high as 0.80. Our findings are in agreement with the previous studies of Miraldi et al. [14], Iranbakhsh et al. [22], Friedman [26], Gaillard and Pepin [51] and Steenkamp et al. [53], who also reported higher atropine levels relative to scopolamine levels in *D. stramonium*.

3.2.4. Chemotaxonomic implications of atropine and scopolamine content

Secondary metabolites are considered as important characteristics in distinction of plant species. These compounds are mostly involved in the relationship of the organism with its environment, e.g. in plant defense mechanisms or as signal compounds; their synthesis is often induced by different kinds of biotic or abiotic stresses [40].

Since the *Datura* taxa investigated were grown under the same environmental conditions, the differences in atropine and scopolamine accumulation trends are supposed to come mainly from genetic base. Atropine was found to be dominant over scopolamine in three out of the four investigated taxa, i.e. *D. metel* and the two *D. stramonium* varieties, while *D. innoxia* was unique with its inverse S/A ratio (Table 5). *D. innoxia* showed the highest concentrations of scopolamine in all examined organs, whereas *D. metel* accumulated the lowest scopolamine levels. Atropine levels were the highest in *D. stramonium* var. *tatula* and the lowest in *D. innoxia*.

Our studies proved that only the alkaloid content of samples collected at the same developmental stage can be compared. The summer samples (at the stage of bloom) had higher alkaloid levels than the autumn samples (at the fruit-bearing stage). Another important aspect of comparative chemotaxonomic evaluations is that the alkaloid content highly varies from organ to organ within the same species (Table 5). Comparisons between different species can be made only for the same organ, from the same developmental stage.

4. Conclusions

A high performance liquid chromatography method coupled with electrospray mass spectrometric detection has been developed for the determination of atropine and scopolamine in *Datura* plant extracts. The validation characteristics tested showed low quantitation limits, allowing the determination of the alkaloids in solutions and in extracts at the concentration levels of sub-ng mL^{-1}. The detection limits for both alkaloids are rather good: 100 pg mL^{-1} for scopolamine and 50 pg mL^{-1} for atropine. The method requires a rather simple sample preparation, and it could be also applied to the analysis of nearly any type of plant material containing atropine and scopolamine alkaloids. The results showed that the technique is repeatable and accurate, the limit of detection is rather low. The separation performed on core–shell RP column and the employment of UFLC instrumentation coupled with mass spectrometer result in a rapid analysis.

Our analysis confirmed that alkaloid levels of *Datura* species vary depending on the taxon, the investigated plant part and the ontogenetic stage. *D. innoxia*, characterized by extremely high scopolamine to atropine ratios can be easily distinguished from other *Datura* species which feature an inverse ratio of the two main alkaloids. Even the varieties of the same species can possess characteristic differences in their alkaloid content, as illustrated by the example of *D. stramonium*. When comparing summer and autumn samples, a strong decreasing trend was found in the concentration of the dominant alkaloids in the vegetative organs. The variability of *Datura* alkaloid levels should be taken into account in several fields like chemotaxonomy, phytomedicine and toxicology.

is acknowledged for the loan of the Ascentis Express C18 column and the LC–MS system.

References

[1] C. Rätsch, Enzyklopädie der psychoaktiven Pflanzen, AT Verlag, 2007.
[2] N. Kuganathan, S. Ganeshalingam, E-J. Chem. 8 (2011) 107.
[3] L. Ajungla, P.P. Patil, R.B. Barmukh, T.D. Nikam, Indian J. Biotechnol. 8 (2009) 317.
[4] J.F. Dessanges, J. Aerosol Med. 14 (2001) 65.
[5] V.A. Boumba, A. Mitselou, T. Vougiouklakis, Vet. Hum. Toxicol. 46 (2004) 81.
[6] V. de Germond-Burquier, F. Narring, B. Broers, Presse Med. 37 (2008) 982.
[7] D. Diker, D. Markovitz, M. Rothman, U. Sendovski, Eur. J. Intern. Med. 18 (2007) 336.
[8] A. El Bazaoui, M.A. Bellimam, A. Soulaymani, Fitoterapia 82 (2011) 193.
[9] B. Marc, A. Martis, C. Moreau, G. Arlie, P. Kintz, J. Leclerc, Presse Med. 36 (2007) 1399.
[10] T.W. Naude, R. Gerber, R.J. Smith, C.J. Botha, J. S. Afr. Vet. Assoc. 76 (2005) 107.
[11] N. Oshiro, K. Kuniyoshi, A. Nakamura, Y. Araki, K. Tamanaha, Y. Inafuku, J. Food Hyg. Soc. Jpn. 49 (2008) 376.
[12] E.P. Krenzelok, R. Mrvos, Clin. Toxicol. 49 (2011) 142.
[13] T. Doncheva, S. Berkov, S. Philipov, Biochem. Syst. Ecol. 34 (2006) 478.
[14] E. Miraldi, A. Masti, S. Ferri, I.B. Comparini, Fitoterapia 72 (2001) 644.
[15] L. Mateus, S. Cherkaoui, P. Christen, J.L. Veuthey, J. Pharm. Biomed. Anal. 18 (1998) 815.
[16] T. Bo, K.A. Li, H.W. Liu, J. Pharm. Biomed. Anal. 31 (2003) 885.
[17] B. Boros, A. Farkas, S. Jakabova, I. Bacskay, F. Kilar, A. Felinger, Chromatographia 71 (2015) S43.
[18] M.G. Hamilton, P.M. Lundy, Toxicology 233 (2007) 8.
[19] S. Lukasewycz, M. Holman, P. Kozlowski, C.R. Porter, E. Odom, C. Bernards, N. Neil, J.M. Corman, Can. J. Urol. 17 (2010) 5377.
[20] A. Rapoport, J. Pain Symptom Manage. 40 (2010) 783.
[21] T.C. Bania, J. Chu, D. Bailes, M. O'Neill, Acad. Emerg. Med. 11 (2004) 335.
[22] A. Iranbakhsh, M.A. Oshaghi, A. Majd, Acta Biol. Cracov. Ser. Bot. 48 (2006) 13.
[23] S. Berkov, T. Doncheva, S. Philipov, K. Alexandrov, Biochem. Syst. Ecol. 33 (2005) 1017.
[24] A.I. Al-Humaid, J. Agric. Rural Dev. Trop. Subtrop. 104 (2003) 151.
[25] S. Berkov, R. Zayed, Z. Naturforsch. C: J. Biosci. 59 (2004) 184.
[26] M. Friedman, J. Chromatogr. A 1054 (2004) 143.
[27] S. Khalil, J. Pharm. Biomed. Anal. 21 (1999) 697.
[28] M.A. El Ries, S. Khalil, J. Pharm. Biomed. Anal. 25 (2001) 3.
[29] S. Cherkaoui, L. Mateus, P. Christen, J.L. Veuthey, J. Pharm. Biomed. Anal. 21 (1999) 165.
[30] B. Drager, J. Chromatogr. A 978 (2002) 1.
[31] M. Eeva, J.P. Salo, K.M. Oksman-Caldentey, J. Pharm. Biomed. Anal. 16 (1998) 717.
[32] S. Fleury-Souverain, L. Vernez, C. Weber, P. Bonnabry, Eur. J. Hosp. Pharm. Sci. 15 (2009) 53.
[33] L. Mateus, S. Cherkaoui, P. Christen, J.L. Veuthey, Electrophoresis 20 (1999) 3402.
[34] L. Mateus, S. Cherkaoui, P. Christen, K.M. Oksman-Caldentey, Phytochemistry 54 (2000) 517.
[35] M. Gadzikowska, A. Petruczynik, M. Waksmundzka-Hajnos, M. Hawryl, G. Jozwiak, JPC-J. Planar Chromatogr.-Modern TLC 18 (2005) 127.
[36] S. Kagale, T. Marimuthu, B. Thayumanavan, R. Nandakumar, R. Samiyappan, Physiological, Mol. Plant Pathol. 65 (2004) 91.
[37] T. Mroczek, K. Glowniak, J. Kowalska, J. Chromatogr. A 1107 (2006) 9.
[38] A.A. Vitale, A. Acher, A.B. Pomilio, J. Ethnopharmacol. 49 (1995) 81.
[39] A. Namera, M. Yashiki, Y. Hirose, S. Yamaji, T. Tani, T. Kojima, Forensic Sci. Int. 130 (2002) 34.
[40] R. Amdoun, L. Khelifi, M. Khelifi-Slaoui, S. Amroune, E.H. Benyoussef, D.V. Thi, C. Assaf-Ducrocq, E. Gontier, Plant Sci. 177 (2009) 81.
[41] S. Berkov, R. Zayed, T. Doncheva, Fitoterapia 77 (2006) 179.
[42] T. Ceyhan, M. Kartal, M.L. Altun, F. Tulemis, S. Cevheroglu, J. Pharm. Biomed. Anal. 25 (2001) 399.
[43] A. Djilani, B. Legseir, R. Soulimani, A. Dicko, C. Younos, J. Braz. Chem. Soc. 17 (2006) 518.
[44] L. Kursinszki, H. Hank, I. Laszlo, T. Szoke, J. Chromatogr. A 1091 (2005) 32.
[45] J. Nino, C.M. Gallego, Y.M. Correa, O.M. Mosquera, Plant Cell Tissue Organ Cult. 74 (2003) 289.
[46] O. Rbeida, B. Christiaens, P. Hubert, D. Lubda, K.S. Boos, J. Crommen, P. Chiap, J. Pharm. Biomed. Anal. 36 (2005) 947.
[47] R. Zarate, B. Hermosin, M. Cantos, A. Troncoso, J. Chem. Ecol. 23 (1997) 2059.
[48] C. Abbara, I. Bardot, A. Cailleux, G. Lallement, A. Le Bouil, A. Turcant, P. Clair, B. Diquet, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 874 (2008) 42.
[49] H.X. Chen, Y. Chen, P. Du, F.M. Han, H. Wang, H.S. Zhang, J. Pharm. Biomed. Anal. 40 (2006) 142.
[50] H.X. Chen, Y. Chen, P. Du, F.M. Han, Chromatographia 65 (2007) 413.
[51] Y. Gaillard, G. Pepin, J. Chromatogr. B 733 (1999) 181.
[52] R. Oertel, K. Richter, U. Ebert, W. Kirch, J. Chromatogr. B 750 (2001) 121.
[53] P.A. Steenkamp, N.M. Harding, F.R. van Heerden, B.E. van Wyk, Forensic Sci. Int. 145 (2004) 31.
[54] F. Gritti, G. Guiochon, J. Chromatogr. A 1166 (2007) 30.
[55] S. Afsharypuor, A. Mostajeran, R. Mokhtary, Planta Med. 61 (1995) 383.
[56] K.K. Pramod, S. Singh, C. Jayabaskaran, Plant Sci. 178 (2010) 202.
[57] G. Petri, T.P.S. Bajaj, Biotechnol. Agric. Forest. 7 (1989) 145.