Quantification of Atropine and Scopolamine in Different Plant Organs of *Datura Metel*: Development and Validation of High-Performance Liquid Chromatography Method

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Abstract

Datura metel (Solanaceae) from south Lebanon. The different parts of this plant contain the tropane alkaloids atropine (AT) and scopolamine (SC), which are naturally muscarinic receptor antagonists. A method has been developed for the extraction and HPLC-UV analysis of the AT and SC in different parts of *D. metel*, namely seeds, capsule, leaf, and stem. This analytical method was validated and gave a good detection response with linearity over a dynamic range of 0.03-0.17 mg mL⁻¹ and recovery in the range of 93.9–108.76%. Limit of detection (LOD) and limit of quantification (LOQ) values were 32 and 98 µg.mL⁻¹ for atropine and 31 and 93 µg.mL⁻¹ for scopolamine, allowing a reliable quantitation of the target alkaloids. The solvent system Methanol/acetonitrile was the better choice for extracting tropane alkaloids from different *Datura* parts. Capsule parts of the plant accumulate the highest amount of scopolamine, while seeds accumulate the higher amount of atropine. Briefly, the order of scopolamine concentrations in *Datura metel* parts, from Lebanon, was in capsules > seeds > leaves > stems and for atropine, the concentrations were in seeds > capsules > stems > leaves.

Keywords: tropane alkaloids; high-performance liquid chromatography (hplc); *datura metel*; atropine; scopolamine

Introduction

Datura metel (*D. metel*) is a wild-growing plant of the Solanaceae family [1]. The name *Datura* is assumed to originate from Sanskrit *Dustura* or *Dahatura* [2], but is mostly known as Angel's and Devil's trumpet, Locoweed, Jimson weed, or *Datura* [3]. In Lebanon however, where it is widely distributed in several regions [4]; it is known as "Jawz-meaael". It comprises different types of phytochemicals such as flavonoids and glycosides. Its leaves and seeds are rich in alkaloids, such as atropine, scopolamine, and hyoscyamine [2]. Due to its alkaloid content, this plant was primarily used as an intoxicant and hallucinogen [3, 5].

Alkaloids, another class of nitrogen-containing secondary metabolites, are known to have wide pharmacological activity and have great potential for the development of new drugs to treat a wide array of diseases. Recently, *in silico* studies showed an affinity of tested alkaloids for binding to the receptor-binding domain of the SARS-CoV-2 spike

protein, putatively preventing it from binding to the host cell [6]. Results show that the alkaloids are interesting compounds with potential use as bioactive agents against SARS-CoV-2 [7].

Furthermore, tropane alkaloids represent a group of over 200 compounds occurring primarily as metabolites produced by members of the Solanaceae family. They have in common a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbons atoms [8]. Many alkaloids have been isolated from *D. metel* including hyoscyamine, hyoscyamine, meteloidine, scopolamine, ticlopidine, tropine, withametelline, and datumetine [9].

Atropine and scopolamine (also known as hyoscyamine) are the predominant tropane alkaloids naturally occurring in many members of the Solanaceae family, including *Datura* genera, which are widely spread throughout the world [10]. Scopolamine is an antimuscarinic agent and a smooth muscle relaxant. It is used in the treatment of motion sickness and preoperative

medication [11, 12]. It must be taken before the onset of motion sickness to be effective. Scopolamine is used to prevent nausea and vomiting caused by motion sickness or from anesthesia given during surgery.

Many healthy people have died from ingesting *Datura*, usually as a result of respiratory paralysis or heart failure [13]. For this reason, the assessment of tropane alkaloids concentration in plants of the Solanaceae family is highly important for toxicological and forensic purposes [14].

In our previous works, different part extracts of *D. metel* were tested for phytochemical screening, total phenols, flavonoids, alkaloids contents, and antioxidant activities [15, 16]. The study revealed that the methanolic extracts exhibit a richness in secondary metabolites. The free radical scavenging methods DPPH and reducing power assay results prove a marked antioxidant activity of the extract [15]. On the other hand, there are few reports about the volatile components of *D. metel* EO's. Consequently, the present study was carried out to develop a validated method to quantify the atropine and scopolamine in extracts from different plant parts (leaves, stem, capsules, and seeds).

Materials and Methods

Chemicals and Reagents

All of the chemicals used were of analytical grade. Methanol (MeOH), ethanol (EtOH), and acetonitrile (ACN) were purchased from BDH (England). Atropine and scopolamine hydrobromide used as standards was bought from Alfa Aesar and Fluka Chemika respectively. The water used in all procedures was an ultrapure one, obtained from a water purification system (TKA MICROMED, Germany). Samples were weighed using a RADWAG XA 82/220/2X laboratory balance. The dried leaves and the dry bark were grinded using a POLYMIX grind mill. The absorbance values of the solutions were measured using a VWR UV-6300PC double beam.

Plant Material and Extracts Preparation

The *D. metel* plant was collected in February of 2020, from the south of Lebanon in Blida (33°08'0"N 35°31'0"E) region; was first, placed to dry, for several weeks in the laboratory. The samples were kept at room temperature until processing. The harvested plant materials initially underwent natural drying (in the shade, at room temperature) for four weeks, followed by segregating them into different parts namely leaves, capsules, stems, and seeds (Figure 1). Then they were grinded to fine powder, using a manual grinder. Finally, the grinded materials were stored in a well-sealed container for the extraction step.

Figure 1: *Datura's leaves, stems, and capsules*

15 g of *Datura's* grinded seeds/leaves/stems/capsules, were separately macerated for 24 hr in either MeOH/ACN (80/20) or EtOH/ACN (80/20). The experimental steps were performed in triplicates. The extraction

protocol was illustrated in Figure 2. Finally, the weighted *Datura* extracts (DEs) were placed in Eppendorf in the fridge for analyses later on.

Figure 2: A schematic representation of the extraction protocol used for the preparation of DE samples.

Thin Layer chromatography

Thin-layer chromatography (TLC) was used for the preliminary screening of alkaloids in DEs. To analyze DEs samples, samples were spotted as a single spot with a capillary tube onto the TLC plate. 20×20 cm TLC plates coated with silica gel 60G F254 (Merk) were used. The eluent is a mixture of ammonia, MeOH, and chloroform (0.1:8:1.9, v/v/v). A methanolic solution of scopolamine hydrobromide (5 mg.mL⁻¹) and another one of atropine sulfate (5 mg.mL⁻¹) were used as standards. After development, the plate was dried by a hairdryer. It was then sprayed with diluted Dragendorff's reagent to visualize the alkaloids. A red-orange spot indicated the presence of the alkaloid in the extract.

HPLC-DAD system

All measurements were accomplished using an HP 1100 Series LC system (Hewlett Packard, Palo Alto, CA, USA) equipped with a quaternary pump, a vacuum degasser, a column compartment, an auto sample, and a diode-array detector, and controlled by the HP Chemstation chromatography software. The analytical column was LiChrospher 60 RP-select B 5 µm (250 ×4 mm). Other equipments such as pH meter CG 820 (SCHOTT GERATE, made in West Germany), Ultrasonic cleaner (BRANSON 200, made in Taiwan), and vortex made by Daihan Scientific Co. (Korea) are also used in this study.

HPLC-DAD method: development and validation

For the separations, the mobile phase used, in isocratic mode, was a mixture of MeOH/ACN/ammonium acetate 25:60:15 (pH adjusted with acetic acid to 4.6) respectively. The detection was carried at 210 nm by pumping the mobile phases at a flow rate of 1 mL.min⁻¹ while the temperature of the column was maintained at 25 °C. The injection volume was 20 µL for DE and standard mixtures. Before injection, all the standards, sample solution as well as mobile phase were filtered through membrane filter 0.45 µm, then sonicated. All the samples were analyzed using the same conditions according to the analytical method optimized. After running, the peak areas corresponding to AT and SC were used to validate the method and quantify the target compounds in the plant parts.

The characteristics and the procedures used for validation were performed following the recommendations from the International Conference of

Harmonization (ICH) guidelines [17]. The performance criteria; linearity, selectivity, intra-assay and inter-assay precision, accuracy, the limit of detection (LOD), and limit of quantitation (LOQ) were determined.

Results and Discussion

Thin layer Chromatography is used to separate the constituents of secondary metabolites of plant extracts [18]. To ensure the presence of those major alkaloids, TLC was performed on the different extracts and the retention factors (R_f) were calculated and compared against standards for identification. The outcomes of the TLC experiment of *D. metel* extract after visualization is shown in Figure 3 which reveals the presence of two orange spots in the extract solution, one at the same R_f (0.75) as standard scopolamine and the other at the same R_f (0.5) as standard atropine, assuring their identities.

Figure 3: TLC plate of total leaves extract visualized by Dragendorff reagent.

The high-performance TLC was employed by Sharma et al. [19] to determine alkaloid “fingerprints” for morphotypes of *D. metel* and quantify atropine and scopolamine. Although TLC can be performed exceptionally rapidly, it regularly has destitute affectability: stain colors can blur, imtemperate foundation recoloring frequently happens, and numerous components are not UV-active (or stain-reactive), making correct quantitative estimations exceptionally troublesome [20]. Several analytical methods have been employed for the analysis of tropane alkaloids including enzyme-linked immunosorbent assay (ELISA), paper chromatography, gas chromatography (GC), gas chromatography-mass spectrometry (GCMS), capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC) [14].

Among the techniques used, HPLC is the most widely developed method for the quantification of tropane alkaloids. Thus we aimed to develop an HPLC-DAD method to quantify tropane alkaloids in the Lebanese *D. metel*. To ensure the similarity or the unique quality of plant materials from different locations, it is necessary to apply validated analytical methods and ensure that the developed methods meet their design goals. Evaluation of the developed method for selectivity for AT and SC was carried out by injecting 10 µL of blank, standard, and sample solutions

separately. The resulting chromatograms revealed no interfering signal appears at the retention times of interest (Figure 4, A). The peaks were identified by comparing the retention times of the analyzed compounds with authentic standards. SC (Figure 4, B) comes out with a retention time of around 3.3 min, while AT (Figure 4, C) will be eluted around 4.8 min. The resolution observed, between the AT and SC peaks, was greater than 1.5, which is acceptable when UV detection is applied [21].

The linearity and range of the method were tested using six different standard working solutions for each compound. Working solutions, of 0.03, 0.05, 0.07, 0.11, 0.13, and 0.17 mg.mL⁻¹, were prepared by diluting a standard stock methanolic solution 10 mg.mL⁻¹ with methanol. Each concentration was analyzed in triplicate under the same conditions. Finally, linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

The results of the linearity study (Figure 5 & Figure 6) gave a linear relationship over the studied range. From the regression analysis, a linear equation was obtained: $y = 15133x - 2.1728$ and $y = 27092x + 263.52$ for SC and AT respectively. The correlation coefficient (r^2) was found to exceed 0.99 for in the two cases, indicating a linear relationship between the concentration of the analyte and the area under the peak.

Figure 5: Calibration curve of SC.

Figure 6: Calibration curve of AT.

The precisions were determined by the evaluation of the repeatability of the proposed methods. It was established by repeated measurements of standard solutions, 14 replicates (intraday) over three days (interday). The data obtained expressed in % RSD, are listed in Table 1.

		% RSD	
		Atropine (20 µg.mL ⁻¹)	Scopolamine (10 µg.mL ⁻¹)
Intraday	Day 1	0.94 ^a	0.86 ^a
	Day 2	0.6 ^a	1.4 ^a
	Day 3	0.47 ^a	0.6 ^a
Interday		0.27 ^b	0.28 ^b

^a Data are mean values from fourteen determinations
^b Data are mean values from 42 determinations

Table 1: Intra- and interday precision of the peak area of SC and AT in standard solutions

The results of repeatability showed that the method was repeatable within the acceptable limits. The RSD for both scopolamine and atropine

solutions were within limits. Low variation was observed in the peak areas intraday variation, ranged from 0.47 to 1.4. The % RSD decrease to about

0.28 for both standards in interday variations. The low values of standard deviation (better than $\pm 2\%$) showed the precision and the repeatability of the retention time to be very good [22].

The limits of detection (LOD) and limits of quantification (LOQ) were calculated from the data obtained in the linearity study through the following equations (eq. 1) [17].

$$\text{LOD} = 3.3 \frac{\sigma}{m} \quad (\text{eq. 1})$$

$$\text{LOQ} = 10 \frac{\sigma}{m} \quad (\text{eq. 2})$$

where, (σ) is the standard deviation of y-intercepts of regression analysis and m the calibration curve slope (m).

The results showed that LOD and LOQ (Table 2) for scopolamine were 31 and 93 $\mu\text{g.mL}^{-1}$ respectively and that for atropine were 32 and 98 $\mu\text{g.mL}^{-1}$ respectively. Although our numbers are still higher than obtained via LC-MS [21], the method can be used and applied. Further optimization is needed to increase the sensitivity of the method.

Compounds	LOD in $\mu\text{g.mL}^{-1}$	LOQ in $\mu\text{g.mL}^{-1}$
Scopolamine	31	93
Atropine	32	98

Table 2: LOD and LOQ results for AT and SC standards

The accuracy of the assay method was determined by recovery studies at six concentration levels over three days.

The percent recovery of the method was determined from the founded and added concentrations. The percent recovery was found to range between 93 - 106 % for SC, and between 95 - 110 % for scopolamine. The average % RSD was 1.1 for SC and 1.5 for AT. % Recovery and % RSD were within the accepted limits from 80 % to 120 % [23], and not more than 5 % [13], respectively, which indicates the applicability of the method for scopolamine and atropine analysis.

Concentrations of Scopolamine and Atropine in Different DEs:

The developed method was applied to determine the concentration of AT and SC in the different DEs. DEs were diluted and the concentration of scopolamine and atropine in these extracts were calculated according to the calibration curve obtained before.

The routine analysis of scopolamine and atropine was achieved under the conditions of the method developed by HPLC. Figure 4 D shows typical

HPLC chromatograms for standard materials of scopolamine and atropine. Based on the obtained concentrations, the first observation was that using methanol is by far better than ethanol one which results the highest concentration of alkaloids. Secondly, the results showed that AT and SC concentrations vary according to the investigated tissue of *D. metel*. The maximum amount of scopolamine in methanol extract was in the flower and seeds. SC concentration in methanol extract was 6.43 mg.g^{-1} DW in flowers and 6.29 mg.g^{-1} DW in seeds. Lesser concentrations of SC were in leaves and stems. The maximum amount of AT in methanol extract was in roots with a concentration of 0.894 mg.g^{-1} DW then in flowers, while the lowest concentration was in stems and leaves (Figure 7). In terms of ratio, the investigated plant part showed extremely high scopolamine to atropine ratios, this ratio is normally characterizing another species the *D. innoxia* [21]. On the other hand, it's known that varieties of the same species can possess characteristic differences in their alkaloid content [21]. Furthermore, the absence of pubescent leaves that distinguish *D. innoxia* from most other weedy *Datura* species [24], supports our results. Issaravanich et al. [25] find the lower amount of scopolamine in the leaves of *Datura metel* from Thailand.

Figure 7: Comparison between the amounts of scopolamine in different studies. Data are mean of triplicates $\pm$ SD.

Figure 4: HPLC chromatograms of different solutions. A: chromatogram of blank solution; B: chromatogram of standard solution SC; C: chromatogram of standard solution AT; D: chromatogram of *Datura metel* leaf extract.

By comparing the concentrations of SC and AT measured in the *D. metel* from Lebanon with those reported for the same species in other countries (Table 3), we find big variations. AT and SC concentrations in leaves of *D. metel* growing wild in Russia ranged between 0.33 mg.g⁻¹ DW for SC and 0.90 mg.g⁻¹ DW for AT which was the maximum amount between different parts of *D. metel*. Those concentrations were 0.02 mg.g⁻¹ DW for SC and 0.01 mg.g⁻¹ DW for AT in stems, a 0.63 mg.g⁻¹ DW of SC and

0.11 mg.g⁻¹ DW of AT in flowers. The maximum amount of SC has recorded in seeds with a concentration of 1.7 mg.g⁻¹ DW, while AT concentration was 0.23 mg.g⁻¹ DW. Another study was carried out on *Datura stramonium*, concentrations of SC and AT were determined in three parts of the plant: leaves, stems, and roots. The maximum amount of SC was in the leaves with a concentration of 1.08 mg.g⁻¹ DW and that of AT was in roots with a concentration of 1.39 mg.g⁻¹ DW.

	Compounds	Leaves	Stems	Seeds	Capsules	Roots
Our results	SC (mg.g ⁻¹)	1.14 ± 0.15	0.61 ± 0.01	6.29 ± 0.08	6.43 ± 0.09	—

	AT (mg.g ⁻¹)	0.040 ± 0.001	0.043 ± 0.001	0.89 ± 0.01	0.27 ± 0.01	—
Russia [26]	SC (mg.g ⁻¹)	0.33 ± 0.03	0.02 ± 0.02	1.7 ± 0.10	0.63 ± 0.05	—
	AT (mg.g ⁻¹)	0.90 ± 0.07	0.01 ± 0.001	0.23 ± 0.02	0.11 ± 0.01	—
Poland [27]	SC (mg.g ⁻¹)	1.08 ± 0.09	1.01 ± 0.06	—	—	0.19 ± 0.01
	AT (mg.g ⁻¹)	0.12 ± 0.01	1.28 ± 0.05	—	—	1.39 ± 0.11

Table 3: Comparison of the amounts of AT and SC in between different locations.

Though the observation of the studies carried out on *Datura* gives importance to the study of the extent of these compounds despite the different content depending on plant part studied and place of growing. Environmental factors such as climatic conditions likely play an important role in the differences seen in plant contents of those alkaloids.

Conclusion

In the *Datura* plant, the main alkaloids were recorded to be scopolamine and atropine. In this research, we were able with both TLC and HPLC to identify qualitatively and quantitatively the presence of scopolamine and atropine compared to their standards. An HPLC method has been developed, and several validation parameters were evaluated to affirm that the method is compatible with the analysis of tropane alkaloids in *Datura* samples. The outcomes showed that the technique was suitable for quantification. The analytical method conditions and the mobile phase solvents provided good resolution for AT and SC. MeOH/ACN solvent system was the better choice for extracting tropane alkaloids from different *Datura* parts. The alkaloid content of *D. metel* varies to a great extent depending on the plant part concerned. The capsule was the tissue that accumulates the higher amount of SC, while seeds accumulate the higher amount of AT. SC concentrations in *D. metel* parts decrease as follows capsules > seeds > leaves > stems and for atropine the highest concentrations were in seeds and the lowest in leaves.

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