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A Review on Determination of Atropine and Scopolamine by RP-HPLC

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Determination Of Atropine And Scopolamine By Rp-Hplc

Abstract: A reverse phase high- performance liquid chromatography (HPLC) equipped with a UV-PDA detector was used for the analysis of the main tropane alkaloids, which were found in the roots, aerial parts and seeds of the Pojark plants. Scopolamine was the main alkaloid of the two populations. The populations from Ashtian mountain and Zangane village had the highest and lowest tropane alkaloids content. The alkaloid content of seeds was higher than other parts. The linearity of the method was between 4 and 400 g/ml for atropine and between 0. 8 and 80 g/ml for scopolamine. Limit of detection was 5. 15 and 17. 4 g/ml, and limit of quantification was 1. 92 and 6. 4 g/ml.

I. INTRODUCTION

The 8-aza-bicyclo [3. 2. 1] octane structure is the same as that of tropane alkaloids. They are usually found in the Solanaceae, Erythroxylaceae, and Convolvulaceae families. More than 250 natural tropane alkaloids have been isolated from different plant taxa and their biological properties have been the subject of many studies.

The more stable enantiomer of atropine, as well as tropane alkaloids, are some of the natural products that are used in drugs. The muscarinic acetylcholine receptor is classified as an anticholinergic agent byMateus et al.Due to the high cost of the industrial synthesis of tropane alkaloids, the investigation for new sources is still going on. There are a number of analytical methods, including gas chromatography (GC) (Majlat, 1982), gas chromatography-mass spectrometry (GC-MS) (Hashimoto and Yamada, 1983), and LCMS (Vepoorte).A review of tropane alkaloids and related compounds has been done recently. There are 19 herbaceous species in the flora of Iran and seven of which are endemic. The biennial herb is 1. 5 to 0. 8 m in height and has dense tomentose trichomes. The plant is distributed in Iraq and some parts of Iran. We don't know if there is a report on the alkaloid composition. The aim of the present study was to determine the main tropane alkaloids in the roots, aerial parts and seeds of the plants.

Table 1. Sources nd geographical locations of H.arachnoideus populations tested

Sample code	locality	altitude
1.	Malaier-Tore road, 5 Km before Zangane Village	2040m
2.	Malaier-Tore road, 5 Km before Zangane Village	2096m
3.	Ashtian road, 9 km before Khalajestan Village	2050m
4.	10th km Hamadan to Malaier road	1700m
5.	Ashtian Mountain, Markazi province	1800m

II. MATERIALS AND METHODS

A. Plant Materials

Different populations of H. arachnoideus were gathered after seed ripening in some sections of Markazi and Hamadan provinces based on the geographical data reported in Table 1. The voucher specimens were deposited at the Herbarium of Natural Resources Faculty, University of Arak, Arak, Iran. Plant materials were collected and divided into roots, aerial parts, and seeds before being dried at room temperature to extract tropane alkaloids.

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B. Chemicals

Chloroform and methanol were obtained from Panreac (Spain), hyoscyamine and scopolamine standards from Sigma (USA), ammonium solution 25% from Fluka (Switzerland), sulfuric acid 85 to 88%, anhydrous sodium sulfate 99%, and potassium dihydrogen orthophosphoric acid 99% from Merck (Germany), and HPLC grade acetonitrile from Caledon (Canada) chemical.

C. Instrumentation

Extractions were performed in a power sonic 405 (Hwashin Technologies, Korea) ultrasonic chamber. A pH-meter, model CG840 (Schott Gerate Gmbh, Germany), was used to regulate pH at various phases. HPLC analysis was performed on a C18 Lichrospher 100 column (5 µm, 250 x 4.6 mm) with a K1001 pump, K-2800 UV-PDA detector, and a 20 µl injection loop from Knauer (Germany). A 10 mm C8 pre-column was connected to the analytical column.

D. Extraction of Alkaloids and Chromatographic Conditions

Plant materials were pulverized and sonicated for 10 minutes with 10 ml of chloroform-methanol-ammonium hydroxide (25%) (15:15:1) per 100 mg sample. The extraction container was left at room temperature for an hour before being filtered and washed twice with 1 mL of chloroform. Following solvent evaporation, 5 ml of chloroform and 2 ml of sulfuric acid (1 N) were added to the dried sample and thoroughly combined. The chloroform fraction was then extracted, and the pH was adjusted to 10 using NH4OH. The alkaloids were extracted three times with chloroform (1 to 2 ml). After adding anhydrous Na2SO4, the extract was filtered and the residue was washed with 1 to 2 mL of chloroform. Finally, the extracted solvent was evaporated and the samples were dissolved in 0.5 ml methanol and kept at -8°c until analysis.

The samples were analyzed in a buffer containing 50 mM potassium dihydrogen orthophosphoric acid adjusted to pH 3.0 with an 80:20 v/v ratio of orthophosphoric acid to acetonitrile. The mobile phase was pumped at a constant flow rate of 1.4 mL min-1, and detection was performed at a wavelength of 215nm.

E. Preparation of Calibration Curves

Calibration curves were constructed by plotting peak areas versus concentration of atropine and scopolamine, and regression equations were calculated.

F. Method Validation

The method was verified in terms of sensitivity, linearity, accuracy, and recovery using the International Committee of Harmonization's (ICH) criteria (1996).

III. RESULTS AND DISCUSSION

Table 2 shows the results of HPLC analysis, which compares the levels of atropine and scopolamine in various organs of H. arachnoideus plants obtained from five different regions of the country. A satisfactory separation was achieved at a resolution of 3.42. The retention times for atropine and scopolamine were 6.66 and 4.56, respectively (Figure 1). The method's linearity was tested with standard solutions of atropine and scopolamine. The calibration curve for atropine was linear from 4 to 400 µg/ml, with a correlation coefficient of 0.9997.

Table 2. Summary of validation parameters.

Parameters	HPLC method	
	Atropine	Scopolamine
Linearity range (µg/ml)	4.0-400.0	0.8-80.0
Correlation efficient	0.9997	0.9991
LOD (µg/ml)	5.2	1.9
LOQ (µg/ml)	17.4	6.4
Recovery %	82.3	78.5
Intra-day precision (RSD %)	2.8-6.2	4.1-10.7
Inter-day precision (RSD %)	1.9-5.5	6.8-9.3

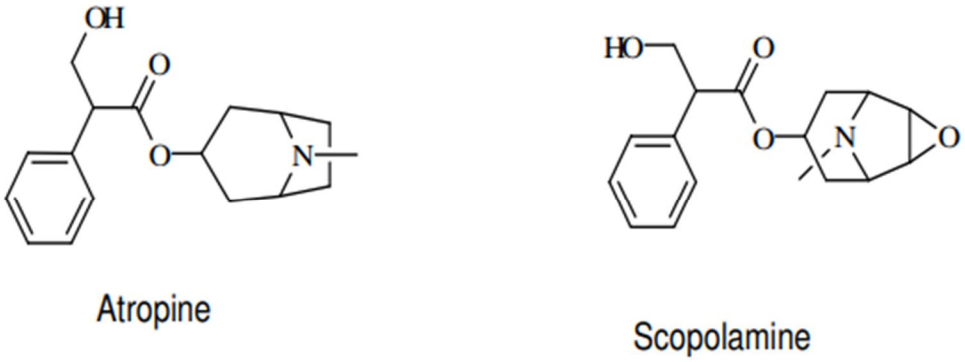
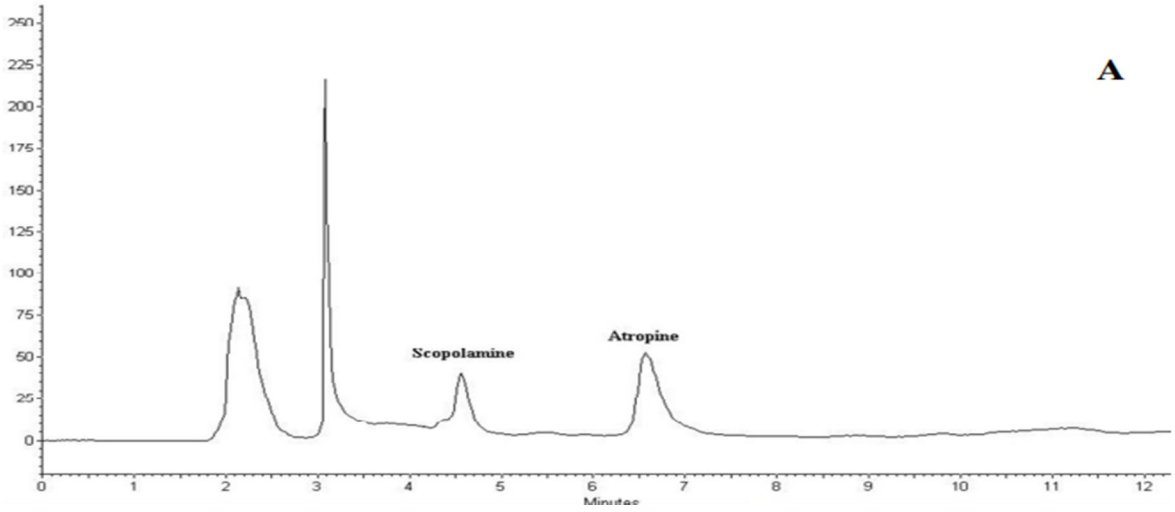


Figure 1. Chemical structures of atropine and scopolamine.

The calibration curves for atropine and scopolamine were represented by the linear equations Y=15292X+46705 and Y=9088.3X+6902.4, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were estimated using the equations LOD=3.3×N/B and LOQ=10×N/B, respectively. N represents the standard deviation of peak area (n=3) as a measure of noise, and B represents the slope of the calibration curve. Scopolamine's LOD and LOQ values were 1.92 and 6.40 ppm, while atropine's were 5.15 and 17.40 ppm. The sample analysis' intra-day and inter-day precision were expressed as a percentage of the relative standard deviation (RSD) with regard to peak area.

The former ranged from 2.84 to 6.2% for atropine and 4.11 to 10.7% for scopolamine. Scopolamine has smaller quantities in the examined samples, as indicated by relatively larger RSD% readings. The interday precision for atropine and scopolamine was determined three times per week (Table 3). The accuracy of the procedure was determined by estimating the recovery of atropine and scopolamine using the usual addition method. The examined samples were spiked with extra concentrations of 100 ppm atropine and scopolamine, and the mixes were reanalyzed using the same procedure. According to the findings of this investigation, scopolamine was the sole major alkaloid found in the seeds of two H. arachnoideus populations. In contrast, atropine was found to be the primary alkaloid in practically all portions, particularly the roots. The highest and lowest tropane alkaloids content were found in plants from Zangane hamlet and Ashtian Mountain, respectively. However, seeds had a higher alkaloid concentration than the other tissues studied (Table 2). Tropane alkaloids are partitioned differently in plants that produce them, as has been consistently reported. In line with our findings, it has been reported that the total amount of vanished alkaloids in Datura stramonium developing parts is equal to that detected in the seeds (Demeyer and Dejaegere, 1997).

Miraldi et al. (2001) discovered that atropine is the primary alkaloid in various plant parts at different phases of development. These authors identified stems as the plant portion with the highest concentration of tropane alkaloids (atropine and scopolamine). Chalabian and Majd (2004) discovered that hyoscyamine was produced at a higher rate than scopolamine in all samples obtained from various phenological stages of Hyoscyamus reticulatus.



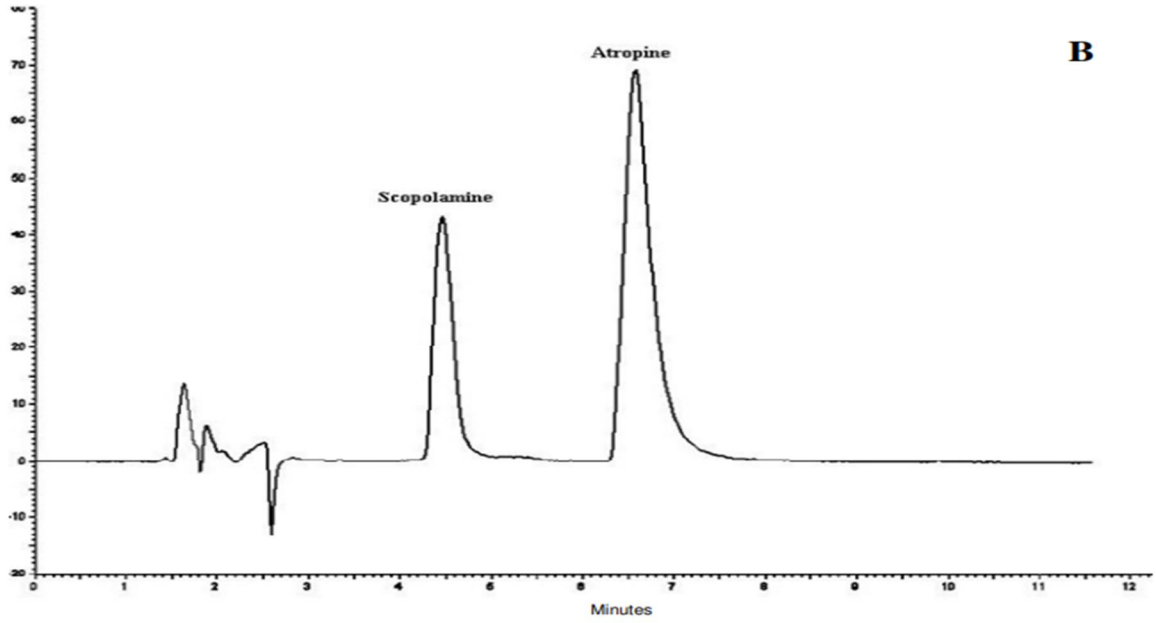


Figure 2. HPLC chromatogram of a sample (A) and standard (B).

Table 3. Atropine and scopolamine composition of different populations of *H. arachnoideus*.

Sample code		Amount (mg/Kg) DW		Atropine/Scopolamine
		Scopolamine	Atropine	
Roots	1	44.6 ± 3.0	180.0 ± 1.5	4.0
	2	4.8 ± 0.3	195.0 ± 1.5	40.0
	3	15.7 ± 0.9	255.1 ± 1.1	16.0
	4	4.6 ± 0.4	395.1 ± 1.8	85.8
	5	12.6 ± 0.3	275.6 ± 1.2	21.0
Aerial parts	1	86.3 ± 1.0	120.0 ± 1.2	1.4
	2	21.2 ± 0.6	190.0 ± 3.0	9.0
	3	34.0 ± 0.4	310.1 ± 3.0	9.0
	4	42.6 ± 1.2	50.1 ± 1.1	1.1
	5	48.8 ± 0.5	125.1 ± 1.2	2.6
Seeds	1	431.0 ± 3.0	448.0 ± 4.0	1.0
	2	829.0 ± 4.3	477.0 ± 4.1	0.6
	3	52.3 ± 0.8	90.0 ± 0.9	1.7
	4	81.2 ± 1.0	29.2 ± 0.4	0.4
	5	50.8 ± 0.6	65.0 ± 0.7	1.3

In this study, the atropine to scopolamine ratio was high in roots, while it decreases in aerial portions and seeds close, or even below, 1. This phenomena can be linked to the conversion of atropine to scopolamine via transfer to aerial portions. The present investigation also revealed differences in the ability of the groups studied to produce tropane alkaloids. The residents of Zangane village were recognized as the most capable of accumulating these medicinally significant chemicals. The observed variability could be due to genetic factors, climatic conditions, or a combination of the two (Hadian et al., 2010; Loziene and Venskutonis, 2005).

Finally, our findings validated and expanded upon prior research on the various patterns of tropane alkaloids accumulation in producing plant organs (Chalabian and Majd, 2004; Demeyer and Dejaegere, 1997; Miraldi et al., 2001). With a few exceptions, atropine was the main alkaloid in all plant components, and the ratio of atropine to scopolamine dropped as the plant moved from roots to seeds.

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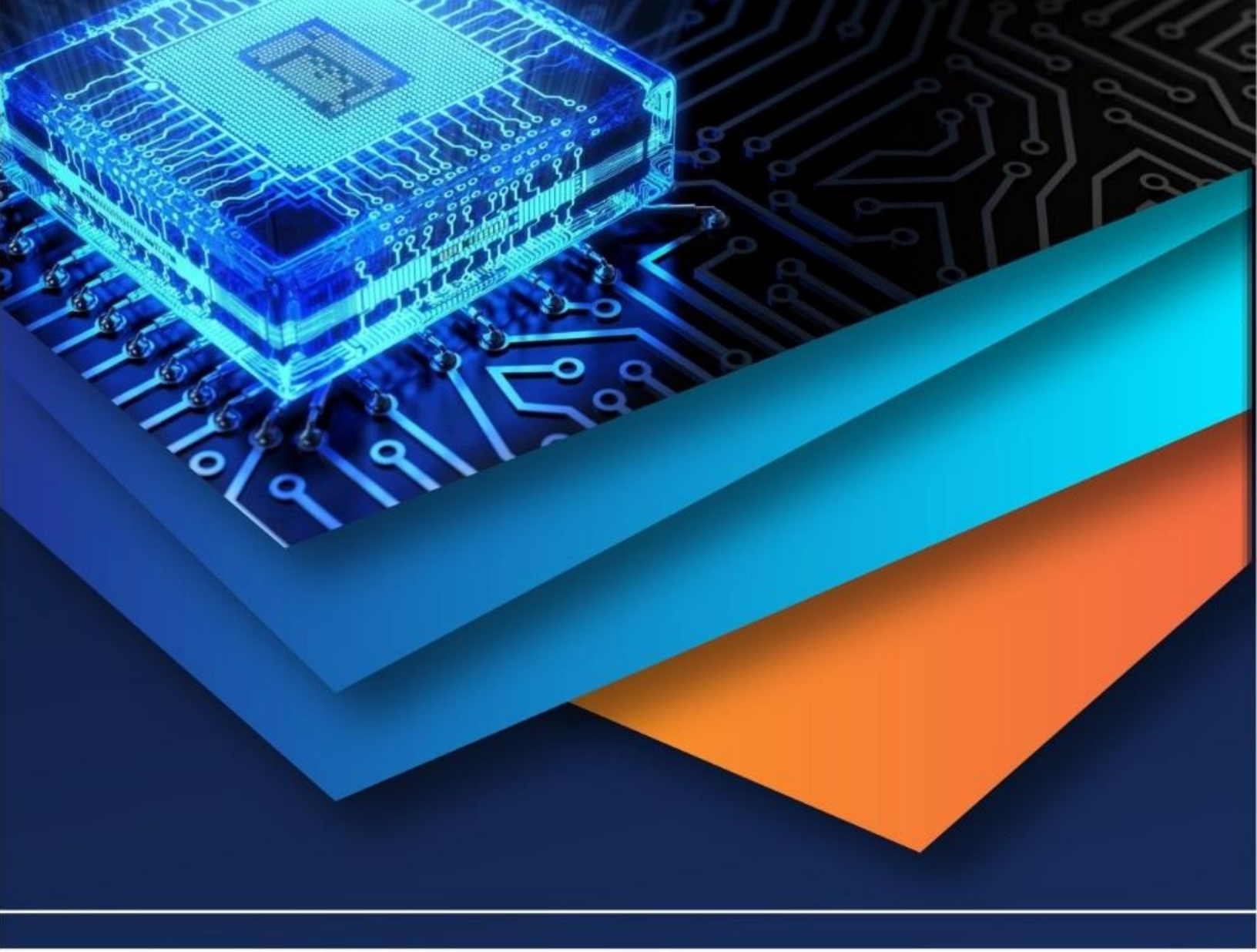

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