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LC determination of atropine sulfate and scopolamine hydrobromide in pharmaceuticals

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Abstract

An accurate, simple, reproducible and sensitive method for the determination of atropine sulfate and scopolamine hydrobromide has been developed and validated. Atropine sulfate and scopolamine hydrobromide were separated using a μBondapack C_{18} column by isocratic elution with flow rate 1.0 ml/min. The mobile phase composition was methanol, water, formic acid (165:35:1; v/v/v) and pH adjusted 8.3 with triethylamine. The samples were detected at 230 nm using photo-diode array detector. The linear range of detection for atropine sulfate (**I**) and scopolamine hydrobromide (**II**) were between 10.38 and 1038 μg/ml with a limit of quantification (LOQ) of 10.38, 10.00 and 1034 μg/ml with an LOQ of 10.00 μg/ml respectively. The linearity, range, peak purity, selectivity, system performance parameters, precision, accuracy, robustness and ruggedness for (**I**) and (**II**) were also shown acceptable values.

Keywords: Atropine sulfate; Scopolamine hydrobromide; High-performance liquid chromatography; Validation

1. Introduction

The tropane alkaloids; atropine and scopolamine are widely used as parasympatolytic, anticholinergic and antiemetic drugs that are produced by solanaceous plants. Thus rapid and simple analytical methods are often needed to enable the analysis of numerous samples in a short period of time.

The tropane alkaloids have been analyzed by many methods including aqueous and non-aqueous titrimetry, UV, visible spectrophotometry and TLC [1–3]. Tropane alkaloids are also analyzed by several methods including gas chromatography (GC) [3,4], high performance liquid chromatography (HPLC) [4–11], capillary zone electrophoresis (CZE) [12,13] and immunological methods [14].

Most of these methods are not suitable for the determination of atropine sulfate and scopolamine hydrobromide in pharmaceutical formulations. HPLC can successfully used for the separation and quantitative determination of

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closely related tropane alkaloids. Thus rapid and simple analytical methods are needed to enable the analysis of samples in a short analytical time.

We used improved HPLC procedure for the quality control analysis of atropine sulfate and scopolamine hydrobromide as a drug substance, atropine sulfate injections and binary mixture containing hyoscine-*N*-butyl bromide-acetaminophen; hyoscine-*N*-butyl bromide-analgin in pharmaceutical dosage forms. It is applicable to a wide variety of drug formulations for individual tablet assays, as a rather simple analytical method, as well as composite assays. This method can not be developed and used as a stability indicating assay method.

2. Experimental

2.1. Chromatographic systems

The assays were performed with a LC system consisting of a Waters model 515 solvent-delivery system and a Waters model 996 Photodiode-array detector (Milford, MA, USA). Samples were injected with a Waters 717plus autosampler using a 20-μl sample loop. The system was controlled and data analyses were performed with the Millennium 2010 software. The column, a μBondapak C_{18} (10 μm, 300 × 3.9-mm I.D.; Waters, Milford, MA, USA) was thermostatted at 25°C by waters temperature control module and waters column heater. A guard column (10-μm Bondapak C_{18} in disposable plastic inserts and Waters Guard-Pak holder) was used to safeguard the analytical column. Spectrophotometric analysis was carried out on a Unicam UV-500 visible spectrometer with a fixed (2 nm) slit width.

2.2. Reagent and chemicals

Atropine sulfate and scopolamine hydrobromide, analytical grade formic acid and triethylamine, HPLC-grade methanol and water were obtained from Merck Chemicals, Inc. and used as is.

2.3. Standard working solution

Standard working solutions were prepared individually in mobile phase for (**I**) and (**II**). Aliquots from each working solution were combined and diluted with mobile phase to yield a solution with final concentrations of 253 (**I**) and 236.5 μg/ml (**II**). Studies on the stability of analytes in standard working solution showed that there were no decomposition products in the chromatogram and difference in area ratios during analytical procedure.

2.4. Calibration solutions

To establish the linear detection range for each compound individual standard stock solutions were prepared in mobile phase in 10-ml volumetric flasks. Aliquots of these solutions were diluted and analyzed to determine method linearity. Limit of quantification (LOQ) values were estimated from serial dilution and analyzed for each compound. A sample was made up at that estimated concentration. This sample was then incorporated as the minimum concentration into the calibration plot. Calibration ranges for (**I**) and (**II**) were from 10.38 to 1038 and 10.00 to 1034 μg/ml respectively. The mean of the peak areas at each concentration was plotted versus concentration (μg/ml) and the calibration curves were constructed. The least-square equation of regression line and correlation coefficient were calculated.

2.5. Pharmaceutical preparation

Atropine sulfate 1 ml injection (Military Pharmaceutical Industry, Turkey) labeled to contain 0.5 mg atropine sulfate and 9 mg NaCl per injection.

3. Procedure

LC analysis was performed using isocratic elution. The mobile phase composition was methanol:water:formic acid (165:35:1: v/v/v) adjusted to pH 8.3 by using triethylamine then filtered through 0.45 μm nylon membrane filter and degassed in sonicator for 10 min.

Three-dimensional chromatograms (wavelength; time; absorbance) were obtained to select the optimum detector wavelength for (**I**) and (**II**). From the result of three-dimensional chromatograms, it was determined that quantitation could be best achieved at 230 nm. A total of 10-μl volume of the each prepared solution was injected into the chromatograph.

Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k'), selectivity (α), resolution (R) and peak asymmetry (T). The chromatographic run time was 10.00 min and the column void volume was 1.0 min.

3.1. Analysis of atropine sulfate injection

A total of 20 injections (atropine sulfate injection) accurately poured in a beaker. The 1 ml volume (0.5 mg atropine sulfate and 9 mg NaCl in per ml) were accurately taken and dissolved in mobile phase in 10-ml calibrated flasks. The solution was filtered through 0.45 μm milipore filter and a 10.0 μl volume of the final solution was injected to chromatographic system.

For comparison of HPLC method, the UV-absorption spectra of the atropine sulfate solutions, prepared at different concentrations were recorded at 230 nm and the calibration curve was constructed. The UV-absorption spectra of the final solutions were recorded and calculated against 0.9 mg/ml NaCl in mobile phase for the determination of atropine sulfate in selected pharmaceutical formulation.

4. Results and discussion

4.1. Method development

Several chromatographic parameters including; methanol–water ratio in mobile phase composition (155:45–175:25; v/v), flow rate (0.8–1.2 ml/min), pH of mobile phase (6.7–8.3), and column temperature (20–40°C) were changed to develop a new method for the determination and quantitation of (**I**) and (**II**).

A mobile phase consisting methanol, water, formic acid (165:35:1 v/v/v) and pH adjusted 8.3 with triethylamine was selected to achieve maximum separation and sensitivity.

A flow rate of 1.0 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using reversed-phase C_{18} column, the retention times for atropine sulfate (**I**) and scopolamine hydrobromide (**II**) were observed to be 5.462 and 3.545 min respectively. Total time of analysis was less than 6 min.

The maximum absorption of (**I**) and (**II**) were found to be at 230 nm and this wavelength was chosen for the analysis.

4.2. Development and validation of proposed method

4.2.1. Linearity and range

Each of the five different concentration standards for each analyte was injected three times. The peak areas obtained for the three analyses were averaged at each concentration. The average peak areas were plotted versus concentration. A linear response between peak area and concentration for all compounds was observed. Table 1 presents the equation of the regression line, determination coefficient, RSD% values of the slope and intercept for each compound. Excellent linearity was obtained for compounds between peak-area and concentrations of 10.38 and 1038 μg/ml with $r^2 = 0.9999$ and 10.00 and 1034 μg/ml with $r^2 = 0.9997$ for (**I**) and (**II**), respectively.

4.2.2. Limits of detection and limits of quantification

The lower limit of detection (LOD) was estimated and confirmed by analysis of a sample at that concentration based on a signal-to-noise (S/N) ratio of 3, also from the detectable chromatographic peak. The limit of quantification was estimated by two criteria: first one is the S/N ratio of not less than 10, and the second is the RSD% not more than 5% for six replicate injection of the LOQ solution. The limit of detection was calculated to be 5.19 and 5.17 μg/ml and the limit of quantification was calculated to be 10.38 and 10.00 μg/ml for (**I**) and (**II**), respectively (Table1).

4.2.3. Peak purity

Chromatographic peaks of the standard mixture were UV-scanned at the peak apex. The UV spectrum for each component in the mixture was compared to that obtained for the individual components under the same chromatographic conditions to estimate the similarity index.

4.2.4. Selectivity and system performance parameters

The selectivity and system performance parameters of the proposed chromatographic method were tested by injecting a standard working solution containing 253 (**I**) and 236.5 μg/ml (**II**). The chromatograms at 230 nm (Fig. 1), the contour plot (Fig. 2), as well as the three-dimensional (Fig. 3) of the chromatograms showed a complete resolution of all peaks. The system performance parameters of six repetitive injections were calculated and recorded in Table 2. The calculated resolution values between each peak-pair were not less than 4.00 and the selectivity were not less than 1.80. Capacity factors (k') were found to be 4.65 and 2.57 for (**I**) and (**II**), respectively.

4.2.5. Precision

The precision of the method was checked by the

Table 1
Linearity results, limit of detection (LOD) and limit of quantification (LOQ)[a]

Compound	λ	Equation	R^2	Slope RSD%	Intercept RSD%	LOD (μg/ml)	LOQ (μg/ml)
Atropine sulfate	230	$Y = 1\,663\,258.99X - 4477.47$	0.9999	0.445	5.083	5.19	10.38
Scopolamine hydrobromide	230	$Y = 1\,383\,330X + 7977.87$	0.9997	0.449	0.636	5.17	10.00

[a] X, concentration (μg/ml); Y, peak area.

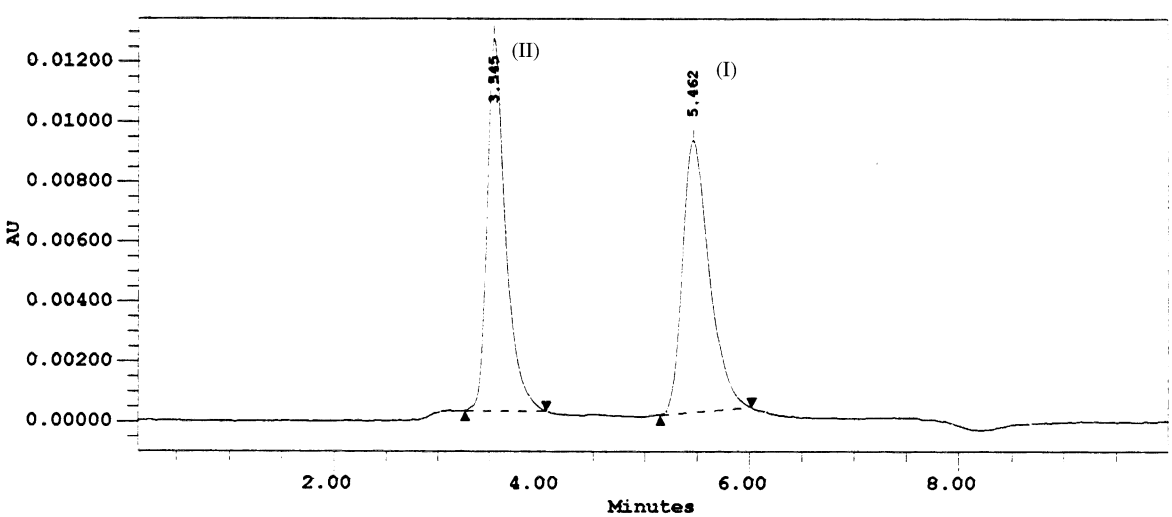

Fig. 1. Chromatogram of the mixture of atropine sulfate (**I**) and scopolamine hydrobromide (**II**) at 230 nm by developed LC method.

analysis of six replicate injections of (**I**) and (**II**) at the LOQ level. The precision of the method, expressed as the RSD at the LOQ level were 3.54 and 4.44% for (**I**) and (**II**), respectively (Table 3).

4.2.6. Accuracy

Analysis of solution spiked with known amounts of atropine sulfate and scopolamine hydrobromide showed the accuracy of the method. A standard working solution mixture containing; 253 (**I**) and 236.5 μg/ml (**II**) was injected six times. The accuracy was expressed in term of% deviation of the measured concentration from the spiked concentration. Since the results obtained are within the acceptable range of $\pm 6\%$, the method is deemed to be accurate (Table 4).

4.2.7. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using standard working solution, same chromatographic system and the same column on different days. Small differences in areas and good constancy in retention times were observed for repetitive 2-day time periods (Table 5). The RSD of less than 2.68% for areas and 0.19% for retention times were obtained. The comparable detector responses obtained on different days are indicated that the method is capable of producing results with high precision on different days.

4.2.8. Method robustness

The robustness of the method was evaluated by changing the pH of mobile phase (6.7–8.3),

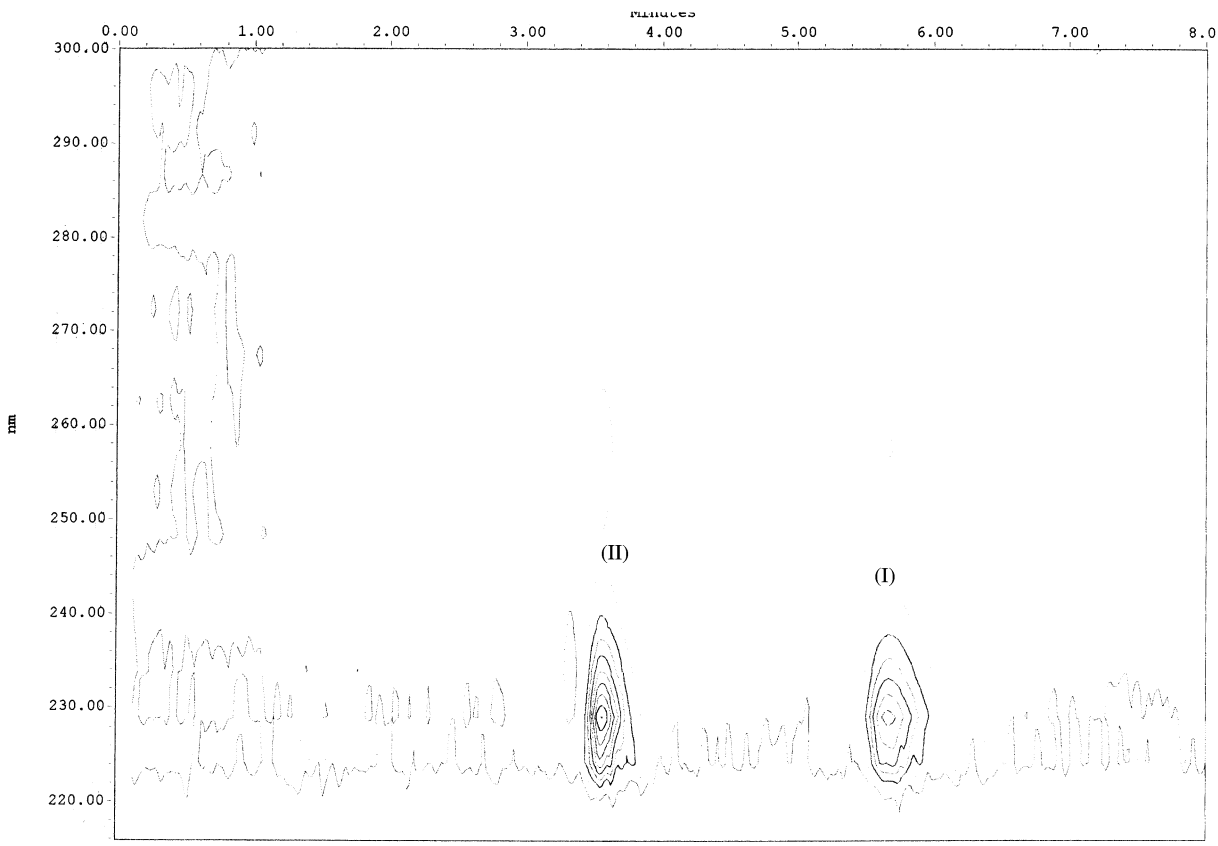

Fig. 2. Contour plot chromatogram of the mixture of atropine sulfate (**I**) and scopolamine hydrobromide (**II**) by developed LC method.

column temperature (20–40°C), methanol–water ratio in mobile phase composition (155:45–175:25) and flow rate (0.8–1.2 ml/min.). To determine the effects of change on resolution, a standard working solution was injected three times after each change. Preliminary inspection of chromatograms obtained under these various conditions suggested that the method is fairly robust, but pH of mobile phase should be more than 7.5 for good separation and sensitivity.

4.2.9. Analysis of pharmaceutical formulations

The validity of the proposed method for pharmaceutical preparations were studied by assaying

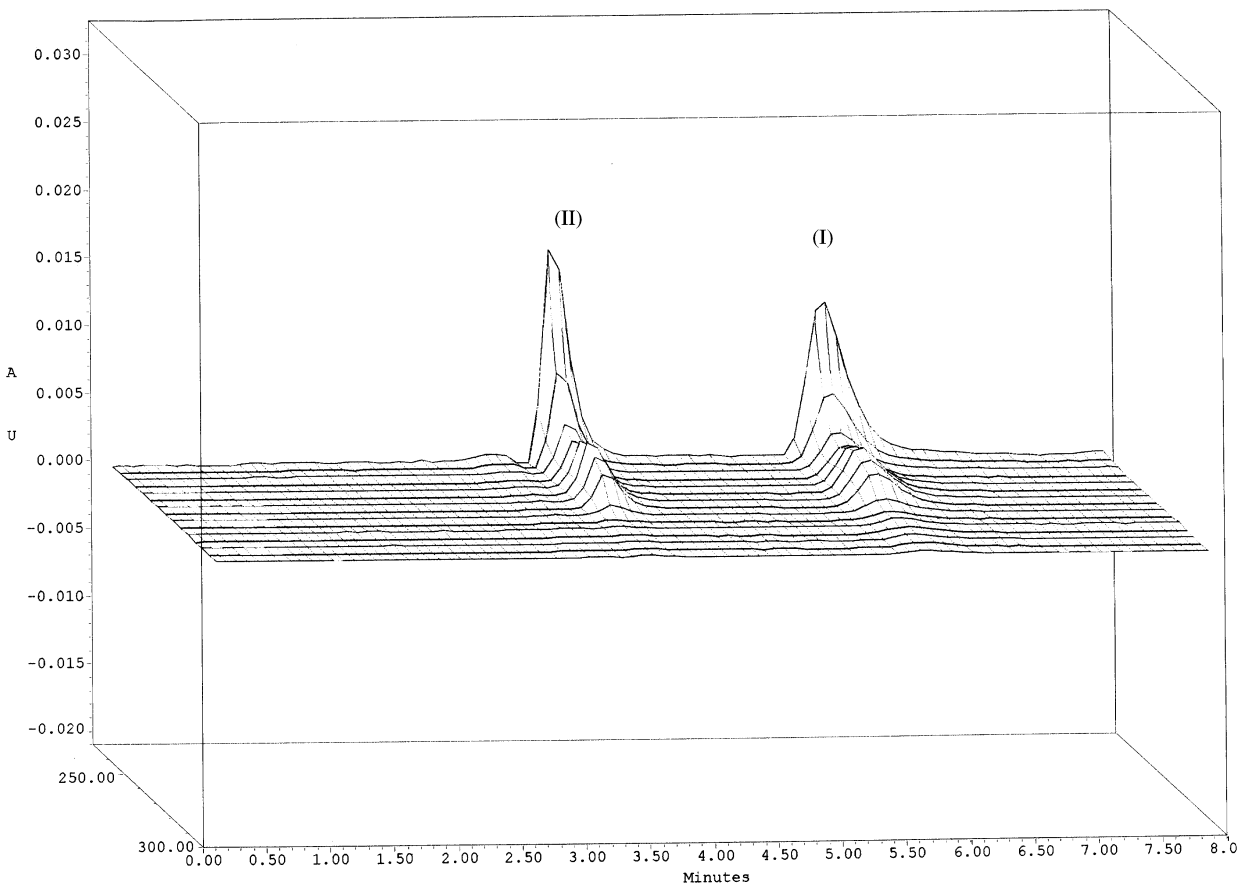

Fig. 3. Three-dimensional chromatogram of the mixture atropine sulfate (**I**) and scopolamine hydrobromide (**II**) by developed LC method.

Table 2
System performance parameters of atropine sulfate and scopolamine hydrobromide[a]

Compound	t_r ($n = 6$, mean)	Area ($n = 6$, mean)	k'	T	R	α
Atropine sulfate	5.662 (0.09)	393 618.66 (1.04)	4.65	1.62	4.251 (3.05)	1.812 (0.29)
Scopolamine hydrobromide	3.573 (0.19)	339 100.17 (1.19)	2.57	1.63		

[a] RSD% values are given in the parenthesis.

Table 3
Precision of the developed method at the LOQ level ($n = 6$)

Compound	λ	Peak area ($n = 6$, mean)	RSD%[a]
Atropine sulfate	230	14 907.33	3.54
Scopolamine hydrobromide	230	40 702.33	4.44

[a] RSD% = (SD/mean) × 100.

atropine sulfate injection (labeled to contain 0.5 mg atropine sulfate and 9 mg NaCl) and the results were shown in Table 6. The RSD% values of the quantitative analysis of atropine sulfate in formulation were found to be 2.32 by HPLC and 0.74 by UV as a reference method.

5. Conclusion

The method described is suitable for the identification and quantification of atropine sulfate and scopolamine hydrobromide as a drug substance and in atropine sulfate injections. The developed method allows the identification of the peaks at the level of the detection limits and the

Table 4
Accuracy of the developed method

Compound	Spiked concentration (μg/ml)	Measured concentration (μg/ml, mean ± SD)	RSD%	% deviation[a]
Atropine sulfate	253	239.35 ± 2.46	1.03	5.40
Scopolamine hydrobromide	236.5	239.43 ± 2.91	1.22	1.24

[a] % Deviation = (spiked concentration-mean measured concentration × 100)/spiked concentration.

Table 5
Day to day variability according to retention time and area

	March 8, 2000		March 9, 2000	
	Atropine sulfate	Scopolamine hydrobromide	Atropine sulfate	Scopolamine hydrobromide
Retention time				
Average	5.662	3.573	5.676	3.576
SD	0.0053	0.0066	0.0033	0.0033
RSD%	0.093	0.19	0.058	0.091
Area				
Average	393 618.67	339 100.17	397 483.5	352 681.5
SD	4095.24	4025.53	8907.42	9461.33
RSD%	1.040	1.19	2.24	2.68

Table 6
Assay results of atropine sulfate injection[a]

	HPLC method atropine sulfate injection (labeled 500 μg)	UV method as reference atropine sulfate injection (labeled 500 μg)
Amount found ± SD	501.58 ± 11.64	518.63 ± 3.82
RSD%	2.32	0.74

[a] Mean values of three determinations.

purity of the peaks at the level of the quantitation limits. Minor changes in pH of mobile phase, flow rate, solvent composition and column temperature did not have any effect on separation and chromatographic parameters. In our last studies, improved HPLC procedure was used for the quality control analysis of binary mixture containing hyoscine-*N*-butyl bromide-acetaminophen; hyoscine-*N*-butyl bromide-analgin in pharmaceutical dosage forms and it is going to be published very soon.

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