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Determination of tropicamide in pharmaceutical formulations using high-performance liquid chromatography

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Abstract

An isocratic, reversed-phase liquid chromatographic method was developed for determination of tropicamide using atropine as an internal standard in a pharmaceutical dosage form. Tropicamide and atropine sulfate were separated using a μBondapak ODS (C_{18}) column by isocratic elution of mobile phase with flow rate of 2.0 ml/min. The mobile phase composition was methanol–50 mM phosphate buffer (pH 4; 30:70, v/v). The eluate was monitored at 257 nm with detector range setting fixed at 0.01 AUFS. Under these conditions, the retention times were 4.81 min for atropine and 11.89 min for tropicamide. The standard calibration curve was linear over a sample concentration range from 2 to 300 μg/ml, with limit of detection of 0.15 μg/ml. The assay linearity was good (typically $r^2 = 0.9992$) and the standard curves were linear in the detection range. The precision of the method (expressed by relative standard deviation) and the accuracy (mean error in percent) were <5% for both intra- and inter-day assays. Recovery at 80–120% of labeled claim ranged from 98.4 to 100.7% for tropicamide. The proposed method was satisfactorily applied to the determination of tropicamide in pharmaceutical preparation and stability indicating studies.

Keywords: HPLC; Tropicamide; Determination

1. Introduction

Tropicamide, (*R*,*S*)-*N*-ethyl-3-hydroxy-2-phenyl-*N*-(pyrid-4-ylmethyl)propionamide, is a tropic acid derivative endowed with antimuscarinic activity of short duration and available in 0.5 and 1% ophthalmic solutions for use where mydriasis is produced by relaxation of the sphincter of muscle of the iris, allowing the adrenergic innervation of the radial muscle to dilate the pupil and commonly used for refractive examinations. Its maximum effect is achieved in about 20–25 min and lasts about 20 min, with complete recovery being noted in about 6 h. Its action is more rapid in onset and wears off more rapidly than of most other mydriatics. To achieve mydriasis either 0.5 or 1% concentration should be used, although cycloplegia is achieved only with the stronger solution. Its uses are much the same as those described in general for other mydriatics [1,2].

Since tropicamide use is increasing, it is very much essential to develop simple and suitable analytical method for its estimation in bulk and in formulations. Such method should provide sensitivity and selectivity and could be easily adapted for routine quality control analysis, pre-formulation or similar studies.

There is very little information in the literature for quantification of tropicamide in pharmaceutical raw material and dosage forms [3]. Few analytical procedures have been described for tropicamide [4]. Among the quantification methods described for the determination of tropicamide in pharmaceutical preparation are those based on UV spectrophotometry [5].

A survey of literature has not revealed any HPLC method specifically developed for determination of tropicamide; however, three liquid chromatographic methods have been

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reported [6–8]. The first one is a HPLC method developed for separation of tropicamide enantiomers which is not simple and only suitable for separation of enantiomers. The second one is a reversed-phase HPLC method, which has been used for determination of phenylephrine and tropicamide in human aqueous humor.

In the last one, cyclodextrin bonded stationary phase have been used for analysis of pyridine derivates and not mainly focused on tropicamide. Moreover, limit of detection and quantification was not reported.

The USP and BP [9,10] described a non-aqueous titration for determination of tropicamide in raw material and extractive spectrophotometric method for pharmaceutical preparations. These methods mostly lack specifity and selectivity for routine analysis.

In the present study, a simple, economical, accurate and reproducible analytical method, based on the method reported for determination of atropine in USP, is reported for determination of tropicamide in raw material and its pharmaceutical dosage forms.

2. Experimental

2.1. Materials

Tropicamide and atropine sulfate, both USP reference standard were obtained commercially. Methanol was HPLC grade, phosphoric acid and potassium dihydrogenphosphate were of analytical reagent grade all from Merck (Darmstadt, Germany). Highly pure water, as prepared by a Millipore purification system, was used for preparation of all aqueous standard and buffer solutions. Tropicamide eye drops were obtained from local market. These eye drops normally contain common additives like preservative (benzalkonium chloride), chelating agent (EDTA) and polyvinyl alcohol.

2.2. Apparatus

The HPLC system consisted of Waters (Milford, MA, USA) analytical liquid chromatograph equipped with reversed-phase 300 mm × 3.9 mm i.d. 10-μm particles, μBondapak ODS (C_{18}) column, a 510 HPLC pump, a 717 plus Autosampler, variable-wavelength 480 UV detector and 746 data module all from Waters. The column and the HPLC system were kept in ambient conditions. The mobile phase was methanol–phosphate buffer (50 mM) with pH 4 (30:70, v/v) and filtered through a 0.22 μm membrane filter using Millipore HPLC solvent filtration assembly, and were delivered at a flow rate of 2 ml/min. The injection volume was 20 μl. The eluate was analyzed at a wavelength of 257 nm with detector range setting fixed at 0.01 AUFS.

2.3. Standard and sample preparation

Stock solutions of tropicamide (20 mg/ml) and atropine sulfate (internal standard, I.S., 10 mg/ml) were prepared in deioinized water. Appropriate dilutions of these solutions were made with water to produce working solutions containing 0–300 μg/ml for tropicamide and 100 μg/ml for internal standard. Calibration standards containing 0–300 μg/ml tropicamide and 100 μg/ml of internal standard were prepared by diluting the working solution with deionized water. The solution of other concentration was prepared as needed.

The prepared dilutions were injected serially. The obtained peaks were integrated and the area under the curve (AUC) were calculated. The stability of the solution of tropicamide during analysis was determined by repeated analysis of samples during the course of the experiment on the same day and also on different days after storing at laboratory bench conditions and in the refrigerator.

2.4. Assay procedure

Two commercially available eye drops of tropicamide (Brands A and B), taken randomly from Iranian market were estimated by the proposed method. For each brand, three samples were thoroughly mixed and an accurately measured aliquot amount (equivalent to 5 mg of tropicamide) was transferred to a series of 25 ml volumetric flasks (five in each case), volume was adjusted by deionized water and analyzed. From the AUC, the drug content was calculated.

2.5. Validation

(a) Accuracy and precision: Five separate samples of tropicamide standard and test solutions (200 μg/ml of each) were prepared in duplicate from freshly prepared stock solution and analyzed as given procedure.
(b) Linearity: Five solutions of the tropicamide ranging from 100 to 300 μg/ml were prepared from the stock solution and analyzed as mentioned.
(c) Limit of detection (LOD) and limit of quantification (LOQ): LOD and LOQ were calculated on the basis of response and slope of the regression equation and signal-to-noise ratio. Experiments were performed to as described.
(d) To double check the accuracy of the proposed method, recovery experiments were performed by adding the known amount of pure drug to pre-analyzed samples of commercial dosage forms. The percent analytical recovery calculated by comparing the concentration obtained from spiked samples with actual added concentrations. The effect of excipients on the UV absorbance of the drug was studied by adding common excipients to the known concentration of the pure drug and estimated as before.

3. Results and discussion

A typical chromatogram for tropicamide and I.S. is shown in Fig. 1. The two peaks are well separated with retention times of 4.10 and 11.89 for is and tropicamide, respectively (Fig. 1). The selected wavelength (257 nm) showed less

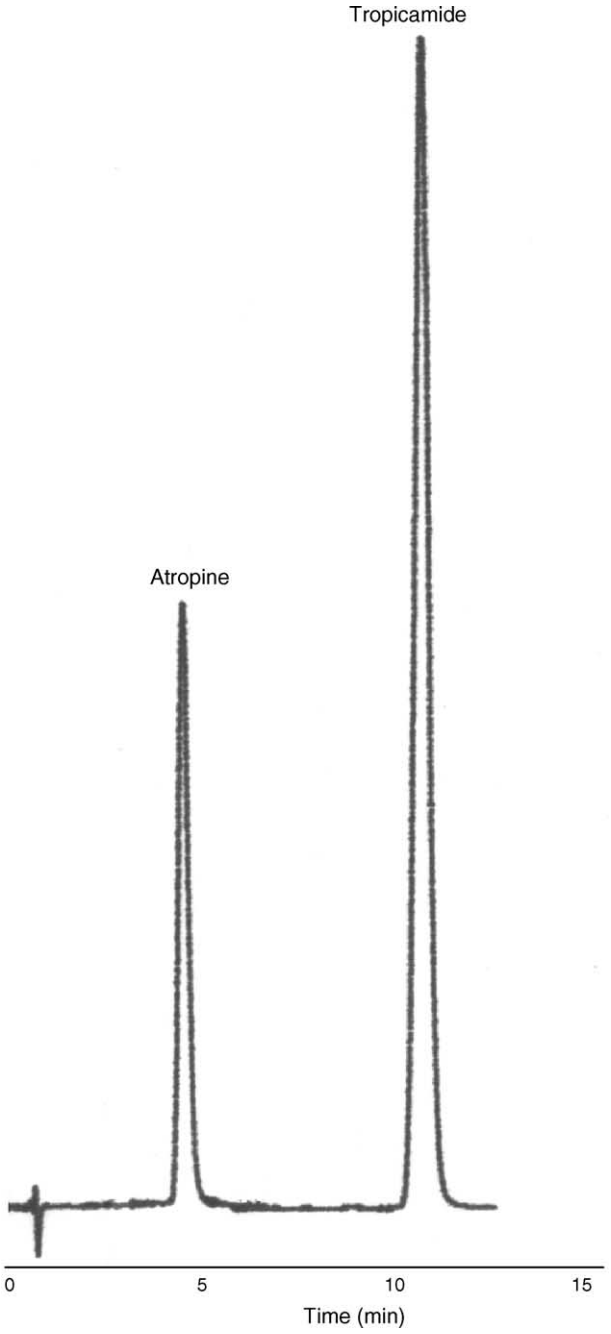

Fig. 1. Typical RP-HPLC chromatogram of atropine and tropicamide.

interference of mobile phase with highest sensitivity according to UV analysis. The common excipients of the formulation did not affect the absorbance or interfering with peak separation of the drug.

Analysis of a series of tropicamide with known concentrations in water, ranging from 2 to 300 μg/ml, yielded a straight-line calibration curve which passed through the origin when peak area ratio (tropicamide: internal standard) was plotted against the concentration of tropicamide ($r^2 = 0.9992$).

LODs were established at a signal-to-noise ratio (S/N) of 3 while LOQs were established at a S/N of 9. The LOD and LOQ were experimentally verified by five injections of tropicamide at the LOD and LOQ concentrations. The calculated LOD and LOQ were 0.1 and 1 μg/ml, respectively.

Table 1
Recovery analysis of tropicamide

Concentration (μg/ml)	Amount found (μg/ml)	Recovery (%)	SD	RSD
50	49.58	99.16	1.12	0.56
200	199.69	99.84	1.43	2.86

Table 2
Intra- and inter-day precision and accuracy for tropicamide assay

Concentration (μg/ml)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
	Conc. found (μg/ml)	SD	RSD	Conc. found (μg/ml)	SD	RSD
50	49.14	1.09	0.53	50.88	0.19	0.10
200	198.71	1.68	3.33	00.37	2.64	5.30

Table 3
Assay results of tropicamide in powder form and two brands of eye drops

Product	Claimed (mg/ml)	Found (mg/ml)	SD
Powder	10	9.897	0.061
Tropicamide eye drops A	10	9.919	0.19
Tropicamide eye drops B	10	9.758	0.11

The accuracy of the method was >99% (Table 1). The AUC versus concentration (μg/ml) was found to be linear ($y = ax + b$, $r^2 = 0.xxx$). The low values of standard deviation established the precision of the proposed method (Table 1). The results of the precision of the method is displayed in Table 2. As seen from Table 2 the RSDs of drug for 50 and 200 μg/ml were 0.53 and 3.33% for intra-day and 0.1 and 5.30 for inter-day, respectively.

The decomposition products, tropic acid and ethyl(gama-picolyl)amine, of tropicamide solution in diluted sulfuric acid did not interfere in the assay procedure then this factor can probably be efficient in the studies regarding stability of the formulation of this drug.

The proposed method was used to estimate the total drug content in two commercially available eye drops of tropicamide (Table 3). As seen for this table the data obtained is in a very good agreement with the claimed amounts of the assayed eye drops and the amount of the raw material.

The aim of this study was to develop a rapid and sensitive method for determination of tropicamide. The presented method is simple, rapid and sensitive and more easier and more practical than those methods reported in other papers and official sources [8–10].

4. Conclusions

The proposed HPLC method is simple and rapid and precision with satisfactory accuracy with no pretreatment step and can used in determination of tropicamide and its pharmaceutical preparations in routine analysis and quality

control laboratories, as well as stability determination of dosage forms of this drug.

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