Iranian Journal of Pharmaceutical Sciences
Winter 2007: 3(1): 43-50
www.ijps.ir

Original Article

Spectrophotometric Determination of Tropicamide in Bulk and Pharmaceutical Formulations

Massoud Amanlou*, Effat Souri, Shiva Izady, Hassan Farsam

Department of Medicinal Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

Abstract

A simple and sensitive extractive spectrophotometric method is described for determination of tropicamide. The method is based on the reaction of tropicamide and bromocresol green. The ion-paired colored complex was extracted with chloroform at pH 3. The extracted complex showed maximum absorbance at 423 nm. The complex was stable up to 2 days and obeyed Beer's law over the concentration ranges of 1.32-100.81 µg/ml. No significant interference was observed from the excipients, coloring and flavoring agents commonly used in the tropicamide pharmaceutical preparations. The proposed method was applied successfully for determination of tropicamide in commercial eye drop dosage forms.

Keywords: Bromocresol green; Ion-pair complex; Spectrophotometry; Tropicamide.
Received: September 8, 2006; *Accepted*: November 12, 2006.

1. Introduction

Tropicamide (TPC), (*R,S*)-*N*-ethyl-3-hydroxy-2-phenyl-*N*-(pyrid-4-ylmethyl) propionamide, is a tropic acid derivative endowed with short duration of anti-muscarinic activity and available in 0.5 and 1% ophthalmic solutions. 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 most other mydriatics. Its uses are generally much the same as those described for other mydriatics [1, 2]. Since tropicamide use is increasing, it is very much essential to develop simple and suitable analytical method for its quantification in bulks and formulations. Such method should provide proper sensitivity and selectivity and could be easily adapted for routine quality control analysis, pre-formulation or similar studies.

There is little information in the literature for quantification of tropicamide in pharmaceutical raw materials and dosage forms [3, 4]. The reported analytical methods for determination of TPC are TLC [5], spectrophotometry [6, 7] and HPLC [8]. The United States Pharmacopoeia (USP) and British Pharmacopoeia (BP) [9, 10] have described a non-aqueous titration for determination of tropicamide in raw material and an extractive spectrophotometric method for its pharmaceutical preparations. These methods are time consuming and costly for

*Corresponding author: Dr. Massoud Amanlou, Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran,14155-6451, Iran.
Tel (+98)21-66959067, Fax (+98)21-66461178
E-mail: amanlou@tums.ac.ir

routine analysis. Therefore, having a simple, fast and accurate method for determination of TPC in raw material and pharmaceutical dosage forms, which can be used in quality control laboratories is a necessity.

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs. Therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [11-16]. So far, no ion-pair extractive spectrophotometry method has been reported for an estimation of TPC. The present study concerns the reaction of TPC with BCG followed by extraction of the ion-paired complex into chloroform. This method is simple, fast and successfully applied for determination of TPC in pharmaceutical formulations.

2. Materials and methods

2.1. Apparatus

A Shimadzu UV-160A, UV-VIS spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements. The pH value of all buffers was adjusted using a Metrohm 692 pH meter.

2.2. Chemicals and reagents

Tropicamide (TPC) was obtained from Sina Daru Pharmaceutical Company (Tehran, Iran). All chemicals were of analytical reagent grade of Merck (Germany) unless otherwise specified. Double distilled water was used to prepare all solutions. Freshly prepared solutions were always employed. USP standard buffer solution (pH = 3) was prepared by diluting 50 ml of 0.2 M potassium hydrogen phthalate and 22.3 ml of 0.2 M HCl to 200 ml with distilled water. Bromocresol green solution (BCG, 1×10^{-4} M) was prepared in distilled water. Eye drops containing 0.5 and 1% of active material were supplied from local stores.

2.3. Standard solution of the drug

A stock standard solution of TPC (1×10^{-3} M) was prepared by dissolving adequate quantity of TPC in double distilled water. Working standard solutions were prepared by suitable dilution of stock standard solution with distilled water.

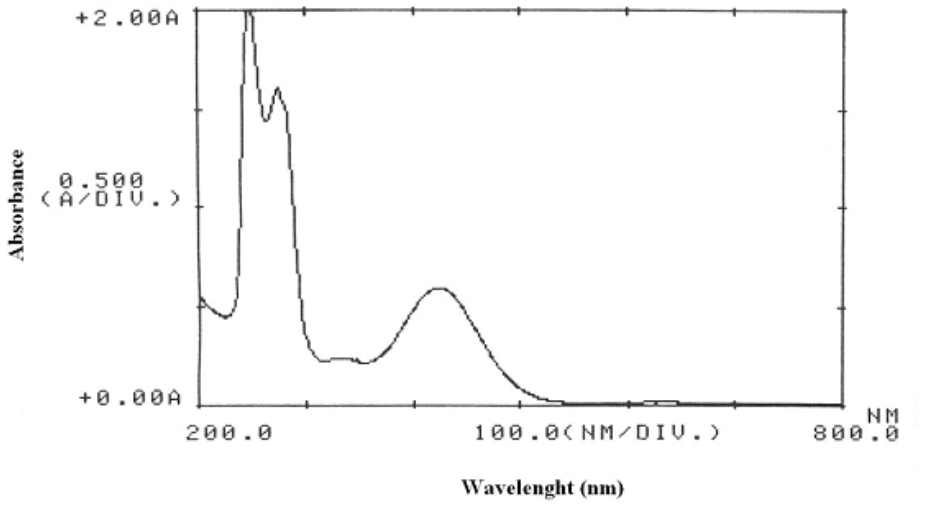


Figure 1. Absorption spectra of tropicamide (100 µg/ml)-bromocresol green (20×10^{-4} M) ion-paired complex in chloroform obtained through scanning at various wavelengths. The maximum wavelength (max) was 423 nm.

2.4. Measurement procedure

Into a series of 100 ml separating funnel flasks, 10 ml of buffer solution of pH 3.0 and 20 ml of BCG (1×10^{-4} M) were placed. An appropriate volume of 10^{-4} M standard drug solution (0.25-20 ml) was added to each funnel and mixed well for few seconds. The funnels were shaken vigorously with 2×5 ml chloroform for 2 min. and then allowed to stand for clear separation of the two phases. Each separated organic phase was transferred to a 25 ml beaker, dried over anhydrous sodium chloride, and transferred to a 10 ml volumetric flask and were made up to the mark with chloroform and mixed well. The absorbance of the organic phase was measured at 423 nm against chloroform as a blank. The standard calibration curve was prepared to calculate the amount of the analyte drug in unknown samples.

2.5. Procedure for measuring TPC in dosage form

The content of five eye drop container were mixed in a beaker and a portion of the solution were diluted to 100 µg/ml of TPC and transferred into a 100 ml volumetric flask. The volume was adjusted to volume with distilled water.

3. Results and discussion

3.1. Spectral characteristics

Absorption spectra of the yellow color TPC-BCG ion-pair complex is shown in Figure 1 with a maximum absorbance (λ_{max}) at 423 nm. The TPC-BCG ion-pair complex formation was completed immediately after all reagents were added, no heating or standing time was needed. The color complex was stable for at least 24 h at the room temperature (25 °C) as determined by proposed method.

3.2. Optimization of variables

A number of preliminary experiments were performed to optimize the necessary conditions for rapid and quantitative formation of colored ion-paired complex. Optimum condition was fixed by varying one parameter at a time while keeping other parameter constant and observing its effect on the absorbance at 423 nm.

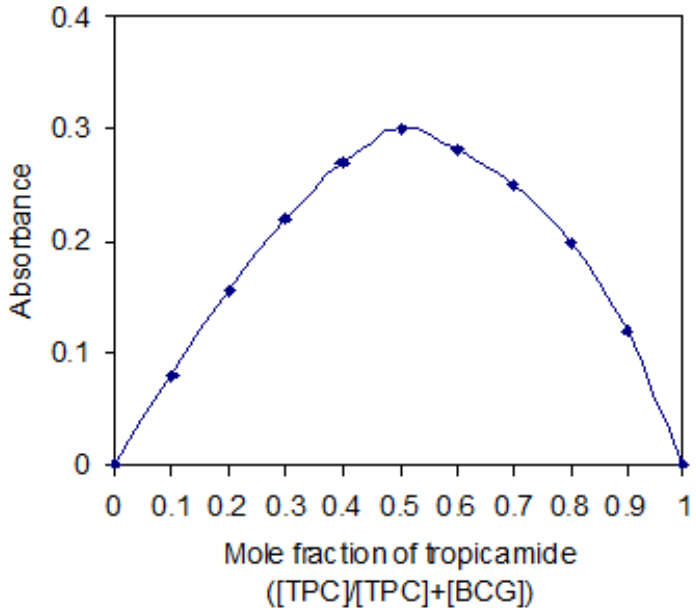


Figure 2. Job's method of continuous variation plot for ion-pair complexes of tropicamide (TPC, 1×10^{-4} M) with bromocresol green (BCG, 1×10^{-4} M) in chloroform. Extrapolation of linear portion can be used to locate the position of break point.

Table 1. Optical characteristics and quantitative parameters of the proposed method.

Parameters	Results
λ_{max} (nm)	423
Beer's law limit (µg/ml)	1.32-100.80
Molar absorptivity (1/mol.cm)	1.65×10^4
Sandell's sensitivity (µg.cm^{-2} per 0.001 absorbance unit)	0.039
Linear regression equation[a] $y= mC + b$	
Slope (m)	0.007
Intercept (b)	0.012
Correlation coefficient (R^2)	0.9994

[a]Where y is absorbance and C is the concentration (µg/ml).

3.3. Effect of pH

The influence of pH of buffer solution on the development and stability of color were tested using different buffer systems as phthalate, potassium hydrogen phthalate, phosphate and acetate buffers. Potassium hydrogen phthalate-HCl buffer solution was the buffer of choice which did not interfere and gave the highest sensitivity to complex formation and extraction. The absorbance of TPC-BCG ion-pair was examined at different pH ranges (1-6). The maximum color intensity was observed at pH ranges of 2.5-3.5 and maximum absorbance were achieved with 10 ml of pH 3 buffer solution.

3.4. Selecting the extracting solvent

The color intensity of the ion-pair complexes and extraction efficiency of solvents were examined using chloroform, dichloromethane, dichloroethane, toluene and carbon tetrachloride. Chloroform was preferred to other solvents for extraction of TPC-BCG complexes yielding maximum absorbance intensity and greatest stability of the extracted product. Consequently, a single extraction with 10 ml of chloroform was adequate to achieve a quantitative recovery of the complex in shortest time to reach the equilibrium between both phases.

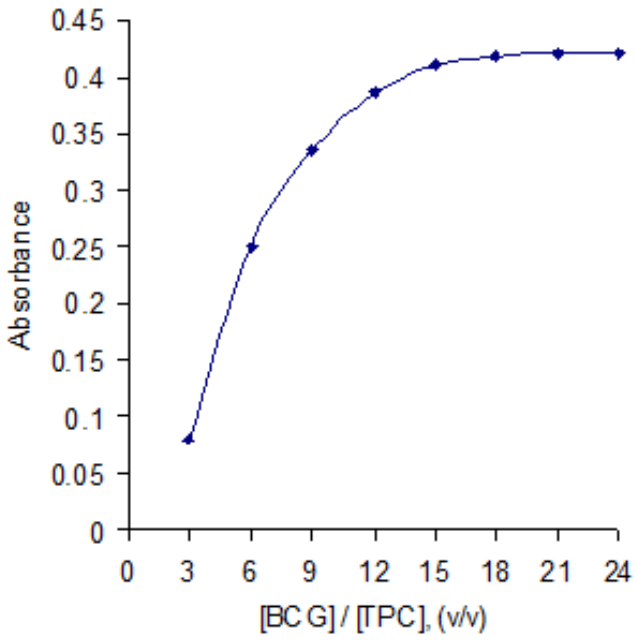
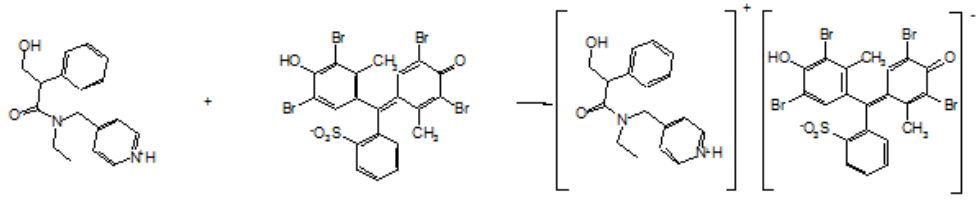


Figure 3. Mole-ratio plot for tropicamide-bromocresol green (TPC-BCG) ion-pair complex in chloroform (BCG 1×10^{-4} M and TPC = 50 µg/ml). The absorbance reaches a plateau with 20 ml of bromocresol green.



Scheme 1. Structure of analyte and formed ion-paired complex, positively charged nitrogen of tropicamide and negatively charged sulfonate of BCG forms an ion-pair complex soluble in organic solvents.

3.5. Composition of ion-pair complexes

Anionic dyes such as BCG form ion-pair complexes with the positively charged nitrogen-containing molecule. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic binding. The suggested mechanism of TPC-BCG ion-pair complex formation is displayed in Scheme 1.

The composition of the ion pairs associates was established by Job's method of continuous variation and mole-ratio method [17, 18]. In these methods, TPC and BCG were prepared in the same concentration (1×10^{-4} M). Different amounts of TPC and BCG were added to each flask and extracted in the same manner as recommended procedure. The absorbance of formed ion-pair complex between TPC-BCG was measured at 423 nm. The absorbance was plotted against [TPC]/[TPC]+[BCG] for Job's method (Figure 2) and [BCG]/[TPC] for mole-ratio method (Figure 3). In Job's plot (Figure 2), the plot reached a maximum value at a mole fraction of 0.5, which indicated the formation of 1:1 (TPC-BCG) complex.

The influence of the volume of BCG (1×10^{-4} M) in the ranges of 3-24 ml on the absorbance of complex was examined (Figure 3) using the mole-ratio method. The absorbance of complex increased with the change of BCG volume up to 20 ml. Above this value, absorbance remained nearly constant. Therefore, 20 ml of BCG (1×10^{-4} M) was selected as optimal and used for complete ion-pair formations throughout the experiment.

The extraction equilibrium can be represented as follows:

$TPC^{+}_{(aq)} + D^{-}_{(aq)} \leftrightarrow TPC{+}D^{-}_{(aq)} \leftrightarrow TPC{+}D^{-}_{(org)}$

where TPC+ and D- represent the protonated TPC and the anion of the BCG, respectively. The subscript (aq) and (org) refer to the aqueous and organic phases. These findings supports that the interaction of TPC and BCG takes place at only one site, which is the nitrogen atom of pyridine ring.

3.6. Linearity and range

Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, and correlation coefficient were determined for the proposed method (Table 1). A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range of 1.32-100.81 μg/ml for TPC in the final measured volume of 10 ml. Regression analysis of the Beer's law plots at λ_{max} revealed a good correlation (R^2=0.9994). The graph showed negligible intercept and

Table 2. Evaluation of accuracy and precision for the proposed method.

Amount taken (μg/ml)	Recovery(%)[a]	RSD(%)[b]	RE(%)[b]
3	101.83	1.25	+1.83
10	97.1	3.6	-2.9
50	99.5	0.97	-0.5

[a]Average of five determinations.
[b]RSD = relative standard deviation, RE = relative error.

Table 3. Determination of tropicamide in the presence of excipients.

Material	Amount added (mg)	Recovered TPC[a]±SD[b]
Lactose	20	99.31 ± 1.33
Dextrose	20	98.92 ±1.24
Ethanol	20	99.11 ± 1.01
Starch	20	101.08 ± 0.59
Propylene glycol	20	99.01 ± 0.97
Hydroxyproyl methylcellulose	10	99.44 ± 1.43
Sodium alginate	10	99.10 ± 1.05
Cellulose	10	99.33 ± 0.48

[a]50 µg/ml of tropicamide (TPC) taken.

[b]Average of five determinations.

were described by the regression equation, $y = 0.007C + 0.012$ (where y is the absorbance of a 1 cm layer, 0.007 is the slope, 0.012 is the intercept and C is the concentration of the measured TPC in µg/ml). The high molar absorptivity of the resulting colored complex indicates the high sensitivity of the method.

3.7. Validation of the method

Samples of pure TPC at three different concentrations were prepared and tested in five replicates using the proposed procedure. The complete set of validation assays was performed. The results are given in Table 2. The accuracy of the method is indicated by the good recovery (97.10-101.83 %), and the precision is supported by the low relative standard deviation <3.6 %.

3.8. Interference studies

Various amounts of commonly used excipients and other additives were added to a known amount of TPC (50 µg/ml) and were examined according to the procedure. Results of the recovery analysis are presented in Table 3. Excipients up to the concentrations shown in Table 3 do not interfere with the assay.

3.9. Application to dosage forms

The proposed method was successfully applied for determination of TPC in commercial eye drops. The applicability of the proposed methods for assay of TPC in formulations was examined by analyzing various formulations and the results are tabulated in Table 4. Determinations were made in five replicates. Results were in a good agreement with the labeled claims (Table 4) for different batches. The results were reproducible with low RSD values.

3.10. Comparison with the official method

To evaluate the validity of the developed method, a comparison was performed between the results of the presented method with non-aqueous titration method procedure described in USP and EP [9, 10]. The results were tested by Wilcoxon test and no significant difference was seen between these two methods (p<0.05). The results were reproducible and accurate with low RSD values as indicated in Table 2. The reliability of the method was established by parallel determination against non-aqueous titration method [9, 10].

The results of analysis of the commercial

Table 4. Determination of TPC in ophthalmic pharmaceutical preparations.

Drug trade name	Label claim (%)	Recovery of TPC ± SD[a]	
		Proposed method	Official method[b]
Tropicamide	0.5	0.49 ± 0.05	0.49 ± 0.09
	1	1.01 ± 0.07	1.08 ± 0.11
Tropicamide	0.5	0.48 ± 0.12	0.50 ± 0.08
	1	1.02 ± 0.09	1.06 ± 0.10

[a]Average of five determination.

[b]Ref. 9,10

formulation and the recovery study of drug suggested that commonly used additives and excipients do not interfere with the assay procedure. The proposed method can be used for determination of TPC in eye drops. The method is rapid, simple and has a great sensitivity and accuracy. The proposed method is sufficiently sensitive to permit determination of low concentration of TPC (1.32 μg/ml).

The advantages of the presented method is its simplicity, sensitivity, and rapidity. Also, it is economic and does not need expensive instruments in comparison to reported techniques and can be used for determination of TPC in pure form, as well as in pharmaceutical preparations, and is recommended for use in routine quality control laboratories.

Acknowledgment

This study was supported by grant No. 230/121435 GSD from the research council of Tehran University of Medical Sciences, Tehran, Iran.

References

[1] Hardman JG, Goodman Gilman A, Limbird LE. *The pharmacological pasis of therapeutics*, 10th ed. New York: McGraw-Hill, 2001; pp. 1673-85.

[2] Delgabo JN, Remers WA. (editors.), *Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry*. 10th ed. New York: Lippincott-Raven Publ., 2004; p. 585.

[3] Timm U, Gober B, Dohnert H, Pfeifer S. Analysis and the stability of tropicamide. *Pharmazie* 1977; 32: 331-5.

[4] Blessel KW, Rudy BC, Senkowski BZ. Tropicamide. *Anal Profile Drug Subst* 1974; 3: 565-89.

[5] Alani SD. The ophthalmic rod: A new ophthalmic drug delivery system I. *Graefes Arch Clin Exp Ophthalmol* 1990; 228: 297-301.

[6] Pohloudek-Fabini R, Martin E, Gallasch V. Contribution to the stability of tropicamide solutions. *Pharmazie* 1982; 37: 184-7.

[7] Dubey BK, Upadhyayr R, Tiwari AK, Shukla IC. Assay of some ophthalmic drugs with ammonium metavanadate reagent. *J Indian Chem Soc* 2004; 81: 511-4.

[8] Amanlou M, Asmardi G, Andalibi PC, Javadi N, Khodadady F, Omarny ZB. Determination of tropicamide in pharmaceutical formulations using high-performance liquid chromatography. *J Chromatogr* A 2005; 1088:136-9.

[9] *United States Pharmacoepia 29*. Rockville: United States Pharmacoepial Convention, 2005; pp. 295, 2223.

[10] *British Pharmacopoeia.* London: Her Majesty's, Stationery Office, 2005; p. 2028.

[11] Kepekci SE, Oztunc A. Determination of maprotiline hydrochloride by ion-pair extraction using bromophenol blue and bromocresol purple. *Acta Pharmaceutica Turcica* 2005; 47: 65-71.

[12] Sudhir Kumar R, Manjunatha DH, Shaikh SMT, Seetharamappa J, Harikrishna K. Sensitive extractive spectrophotometric methods for determination of trazodone hydrochloride in pharmaceutical formulations. *Chem Pharm Bull* 2006; 54: 968-71.

[13] Goyal A, Singhvi I. Visible spectrophotometric methods for estimation of ambroxol hydrochloride from tablet formulation. *Asian J Chem* 2005; 17: 2836-8.

[14] Ashour S, Al-Khalil R. Simple extractive colorimetric determination of levofloxacin by acid-dye complexation methods in pharmaceutical preparations. *IL Farmaco* 2005; 60: 771-5.

[15] Amanlou M, Khosravian P, Souri E, Dadrass OG, Dinarvand R, Alimorad MM, Akbari H. Determination of buprenorphine in raw material and pharmaceutical products using ion-pair formation. *Bull Korean Chem Soc* 2006; 28: 183-7.

[16] Nour El-Dien FAF, Mohamed GG, Mohamed NA. Spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides through ion-pair formation using methyl orange and bromocresol green reagents. *Spectrochimica Acta Part A* 2006; 65: 20-6.

[17] Job P. *Spectrochemical methods of analysis*. New York: Wiley Intersience, 1971; p. 346.

[18] Inczedy J. *Analytical applications of complex equilibria*. Chichester: Ellis Horwood, 1976; p. 137.