



## Development and Validation of a Rapid Derivative Spectrophotometric Method for Determination of Tropicamide in Eye Drops

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### Abstract

Tropicamide is an antimuscarinic agent used as eye drops for refractive examinations. The aim of this study was to develop a simple and suitable analytical method for determination of tropicamide in eye drops in the presence of excipients. A zero-crossing third and fourth derivative spectrophotometric method was described for determination of tropicamide in eye drops. The measurements were carried out at wavelengths of 263.8 and 255.4 nm for third- and fourth-derivative, respectively. The method was found to be linear ( $r^2 > 0.999$ ) in the range of 10-100  $\mu\text{g/ml}$  for tropicamide in the presence of excipients. The limit of determination was 10  $\text{ng/ml}$  for tropicamide. The method was successfully applied for determination of tropicamide in eye drops without any interference from excipients or need to prior separation before analysis.

*Keywords:* Derivative spectrophotometry; Eye drop; Tropicamide, Validation; Zero-crossing.

*Received:* January 10, 2010; *Accepted:* April 7, 2010

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### 1. Introduction

Tropicamide, (R, S)-N-ethyl-3-hydroxy-2-phenyl-N-(pyrid-4-yl-methyl) propionamide, is an antimuscarinic agent with short duration of mydriatic and cycloplegic effect. Tropicamide is used for refractive examinations and is available as 0.5 or 1% (w/v) ophthalmic solution containing excipients and preservative for optimal eye tolerance and activity [1].

Since tropicamide use is increasing, it is

very much essential to develop simple and suitable analytical method with sufficient sensitivity and selectivity for its determination in dosage forms for routine quality control analysis. Non-aqueous titration method is reported in EP, USP and BP for determination of tropicamide in raw material [2-4]. Spectrophotometric method for determination of tropicamide in eye drops is also reported in USP and BP after extraction of the active compound into chloroform and back extraction into dilute sulfuric acid [3, 4]. Literature survey showed spectrophotometric [5], and HPLC methods for determination of tropicamide [6-8]. All of these methods are time-consuming and relatively complicated

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for routine analysis. Direct UV-visible spectrophotometric method is not suitable for determination of tropicamide in eye drops due to the spectral overlapping of tropicamide and other ingredients in dosage form. Derivative spectrophotometry provides a greater selectivity than common spectrophotometry and offers a powerful approach for resolution of band overlapping in quantitative analysis of multi-component mixtures without prior chemical or physical separation. In the last 20 years, this technique has been rapidly gained its application in the field of pharmaceutical analysis to overcome the problem of interferences due to substances other than analytes, commonly present in pharmaceutical formulations, or for combination of two or more drug substances [9-22]. The purpose of the present study was to investigate the utility of derivative spectrophotometry for determination of tropicamide in the presence of excipients in eye drops without any pre-treatment.

## 2. Materials and methods

### 2.1. Chemicals

Tropicamide was from Iwaki Pharmaceutical Co. Ltd, Tokyo, Japan and kindly donated by Sina-Darou Pharmaceutical Company (Tehran, Iran). Benzalkonium chloride (>99.5% pure) sodium chloride and EDTA (Titriplex III) were from Merck (Darmstadt, Germany).

### 2.2. Instrumentation

Absorption and derivative spectra were recorded in 1 cm quartz cells using a Shimadzu UV-160 double beam UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) with a fixed bandwidth (2 nm) and data processing capacity. The zero-order absorption spectra were recorded over the wavelength range 200-400 nm against a solvent blank. The derivative spectra were obtained over the same range at different slit width ( $\Delta\lambda$ ). The ordinate, maximum and minimum, was adjusted to the magnitude of derivative values.

### 2.3. Standard stock solutions

Standard stock solution of tropicamide was prepared by dissolving 100 mg of drug in 100 ml distilled water to give a final

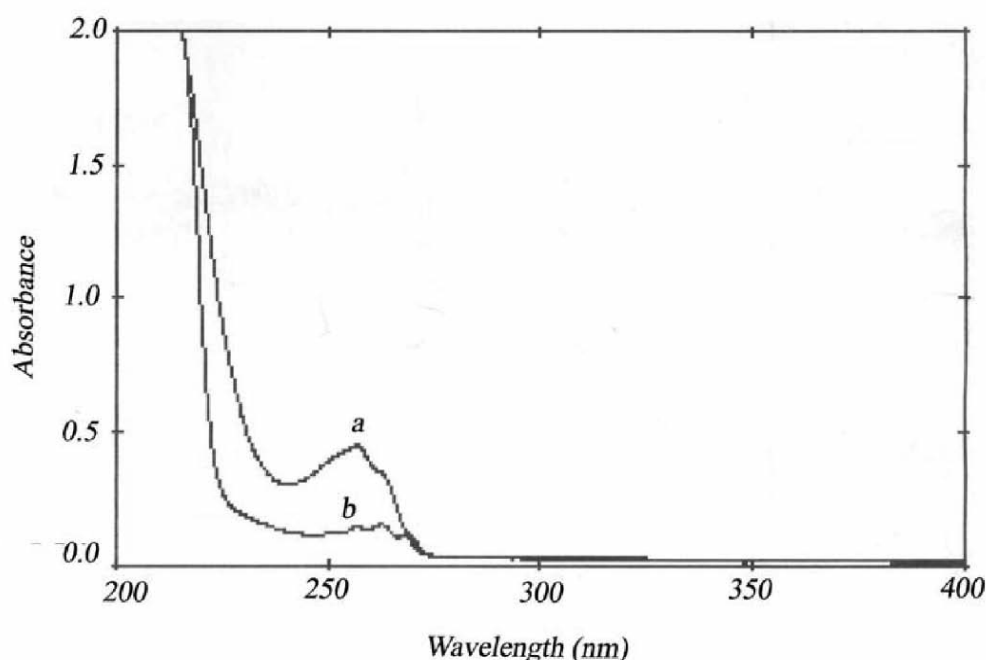


Figure 1. Zero-order spectra of (a) tropicamide (50 µg/ml) and (b) eye drop excipients solution.

**Table 1.** Statistical data of calibration curves of tropicamide in mixtures with different concentrations using third derivative and fourth derivative spectra.

Parameters	Third-derivative	Fourth-derivative
Linearity	10-100 µg/ml	10-100 µg/ml
Regression equation	$y=0.016x+0.018^a$	$y=0.012x+0.017^b$
SD of slope	$1.20 \times 10^{-4}$	$8.90 \times 10^{-5}$
RSD of slope (%)	0.75	0.74
SD of intercept	0.0023	0.0019
Correlation coefficient	0.9996	0.9998

<sup>a</sup> $y=bx+a$ , where x is the concentration of tropicamide in µg/ml and y is the amplitude  $d^3A/d\lambda^3$  value at 263.8 nm.

<sup>b</sup> $y=bx+a$ , where x is the concentration of tropicamide in µg/ml and y is the amplitude  $d^4A/d\lambda^4$  value at 255.4 nm.

concentration of 1000 µg/ml. Standard solutions of tropicamide (10, 20, 30, 40, 50, 60, 80 and 100 µg/ml) was prepared by subsequent dilution. A stock solution of excipients was prepared by dissolving 0.85 g sodium chloride, 0.1 g benalkonium chloride and 0.1 g EDTA in 100 ml distilled water and reached to pH=6.0. This solution was diluted and used for spectrophotometric measurements. Standard solutions of tropicamide in the presence of excipients were prepared at the same concentration range containing 1 ml of prepared stock solution of excipients in 100 ml. All these solutions were stored at 4°C.

#### 2.4. Pharmaceutical preparation

A commercial pharmaceutical preparation, Mydrax® 1% (Sina-Darou Pharm. Co., Tehran, Iran, Lot No: 709 016), containing 1% tropicamide, sodium chloride, EDTA and benzalkonium chloride was assayed.

#### 2.5. Spectrophotometric measurements

Zero-order spectra of standard solutions of tropicamide (50 µg/ml) and excipients versus its solvent blank were recorded in the range of 200-400 nm. The third and fourth derivative spectra of tropicamide solution and excipients solution were obtained in the same range of wavelength against their blanks. The calibration curves for derivative spectrophotometry were constructed by plotting the  $d^3A/d\lambda^3$  and  $d^4A/d\lambda^4$  values at 263.8 and 255.4 nm (zero-crossing of excipients) versus the drug concentration.

#### 2.6. Linearity

Calibration curves were constructed using six series of tropicamide solutions between 10-100 µg/ml in the presence of excipients. The calibration curves were constructed and statistical analysis was performed.

#### 2.7. Precision

To establish the repeatability and reproducibility of the proposed method three replicate of standard solutions at three different concentrations (10, 50 and 100 µg/ml) were assayed on one day and three separate days and the CV values were calculated.

#### 2.8. Accuracy

For accuracy assays the same synthetic mixtures mentioned above were analyzed by the proposed method and the accuracy values were calculated.

#### 2.9. Analysis of eye drop

The proposed procedure was applied for the analysis of tropicamide in eye drop. 0.5 ml of commercial eye drop was transferred into a 100 ml volumetric flask and diluted with distilled water. The general procedure was followed and the concentration of tropicamide was calculated by comparing with an appropriate standard solution of tropicamide in the same concentration and pH value.

### 3. Results

#### 3.1. Derivative spectrophotometric method

Zero-order absorption spectra of

**Table 2.** Accuracy and precision data of determination of tropicamide (10-100 µg/ml) in the presence of excipients by third derivative spectrophotometry.

Added tropicamide (µg/ml)	Within-day (n=3)			Between-day (n=9)		
	Found (µg/ml)	CV (%)	Accuracy (%)	Found (µg/ml)	CV (%)	Accuracy (%)
10.00	9.86±0.07	0.71	98.60	9.82±0.11	1.12	98.20
50.00	50.61±0.68	1.34	101.22	50.34±0.93	0.93	100.68
100.00	100.02±0.43	0.43	100.02	99.44±0.49	0.40	99.44

tropicamide and eye drop excipients solution is shown in Figure 1. Also the third derivative and fourth derivative spectra traced with  $\Delta\lambda=9.8$  (n=7) and  $\Delta\lambda=9.6$  (n=8) are shown in Figures 2 and 3. Zero-crossing points of eye drop excipients solution (263.8 and 255.4 nm at third derivative and fourth derivative) were used for determination of tropicamide in the presence of excipients.

### 3.2. Calibration curves and statistical analysis

Under the optimized conditions, the absorbance of the standard solutions of tropicamide in the presence of excipients were measured using third derivative and fourth derivative spectra at the specified wavelengths. The calibration curves were constructed by plotting the  $d^3A/d\lambda^3$  and  $d^4A/d\lambda^4$  values versus the tropicamide concentration. Separate determinations (six repetitions) at same concentration levels were

performed. The statistical data are summarized in Table 1.

### 3.3. Limit of quantification

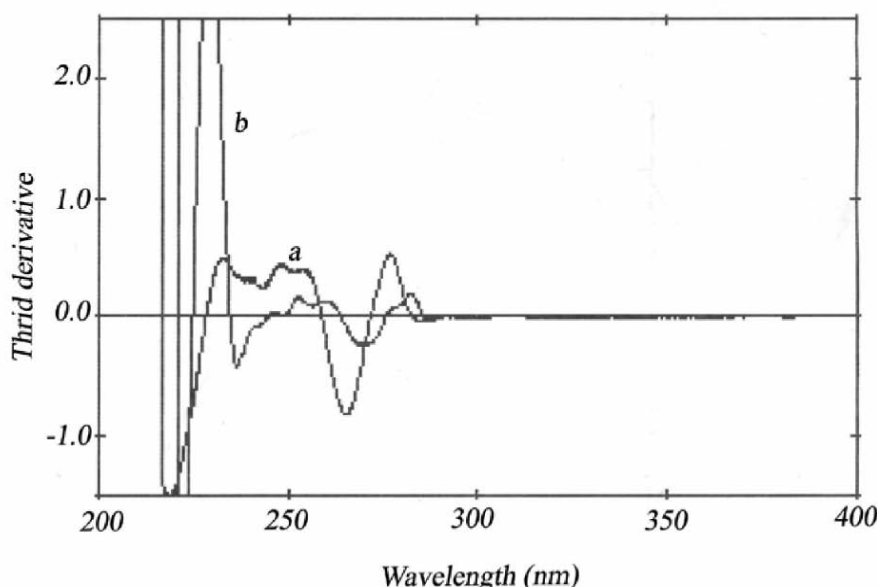
The limit of quantification was found to be 10 µg/ml for tropicamide in the presence of excipients (CV<1.4%). The limit of detection that can be reliably detected with a S/N ratio of 3 was found to be 2 µg/ml.

### 3.4. Accuracy and precision

In order to determine the accuracy and precision of the method, synthetic mixtures of tropicamide and eye drop excipients were prepared and analyzed in three replicates in three days. The mean accuracy and CV values are illustrated in Tables 2 and 3.

### 3.5. Specificity

No interference was observed from the presence of benzalkonium chloride, EDTA

**Figure 2.** Third derivative spectra of (a) tropicamide (50 µg/ml) and (b) eye drop excipients solution.

**Table 3.** Accuracy and precision data of determination of tropicamide (10-100 µg/ml) in the presence of excipients by fourth derivative spectrophotometry.

Added tropicamide (µg/ml)	Within-day (n=3)			Between-day (n=9)		
	Found (µg/ml)	CV (%)	Accuracy (%)	Found (µg/ml)	CV (%)	Accuracy (%)
10.00	9.87±0.07	0.71	98.70	9.88±0.14	1.42	98.80
50.00	50.57±0.29	0.57	101.14	50.41±0.46	0.91	100.82
100.00	99.24±0.30	0.30	99.24	99.61±0.43	0.43	99.61

and sodium chloride in the amounts commonly present in eye drops.

### 3.6. Stability

Study of stability of tropicamide in solutions during the analytical method showed that the analyte were stable for at least one week in solutions when protected from light.

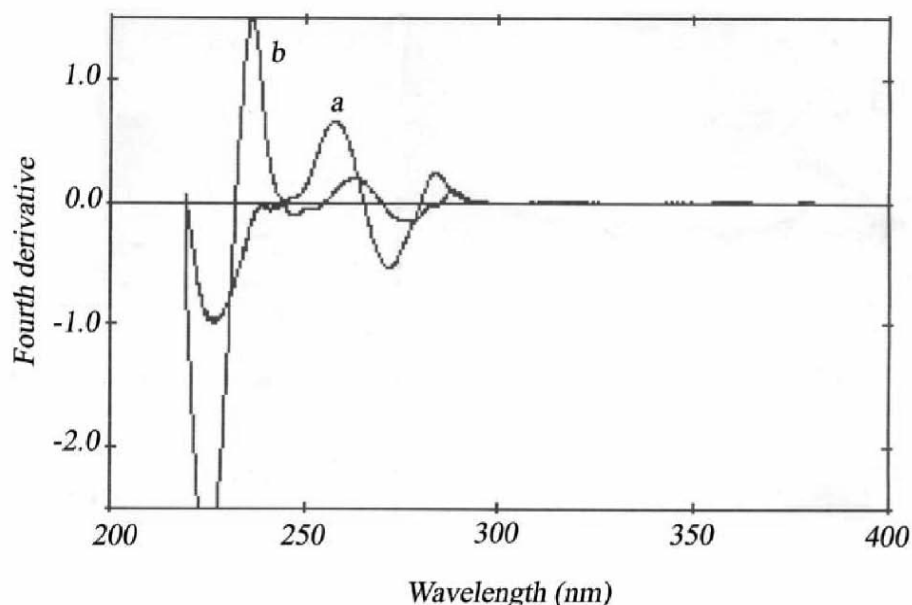
### 3.7. Application

The proposed method was successfully applied to the analysis of a commercial formulation (Mydrax® 1%). No interference from the sample matrix was observed. The results were in good agreement with the labeled content and the error of the determination was lower than 2.0%.

## 4. Discussion

The zero-order absorption spectra of tropicamide and eye drop excipients solution

showed overlapping bands from 200 to 300 nm which prevents the direct determination of tropicamide in the presence of excipients. Derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem. In this study the spectrophotometric parameters were optimized through derivative spectra of tropicamide and eye drop excipients solution at different orders (one to fourth) and  $\Delta\lambda$  values to select a suitable spectrum to be used for determination of tropicamide in the presence of excipients. Several specific signals were single out in the various spectra but the third derivative and fourth derivative spectra traced with  $\Delta\lambda = 9.8$  (n=7) and  $\Delta\lambda = 9.6$  (n=8) respectively showed zero-crossing points with evidently useful characteristics from the analytical view point. The zero-crossing points (263.8 and 255.4 nm at third derivative and fourth derivative)

**Figure 3.** Fourth derivative spectra of (a) tropicamide (50 µg/ml) and (b) eye drop excipients solution.

which showed the best linear response to analyte concentration, least interference of other components, and also lower RSD values were used for determination of tropicamide in the presence of excipients.

Constructing the calibration curves at the range of 10-100 µg/ml using the mentioned wavelengths for third and fourth derivative spectra showed that the proposed method obeys Beer's law with the high values of correlation coefficients ( $r^2 > 0.999$ ) of the regression equations.

The within-day and between-day CV and the accuracy values (%) were considered very satisfactory in all three selected concentrations. The data indicate that the proposed derivative spectrophotometric method is highly reproducible during one run and between different runs.

From the results of this study it can be concluded that the proposed third derivative and fourth derivative spectrophotometric method can be used directly for determination of tropicamide in the presence of eye drop excipients. This method is simple, rapid, practical, reliable and economic and can be used for routine analysis of tropicamide eye drops without any prior separation in quality control laboratories.

### Acknowledgement

The authors would like to thank the Pharmaceutical Sciences Research Center, Medical Sciences/ University of Tehran for the financial support of this research project.

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