

## Estimation Of Tropicamide In Bulk And Eye Drop Dosage Form Using Area Under Curve Method

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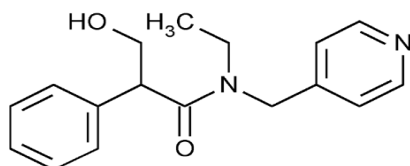
### Abstract:

A simple, rapid, accurate and economical UV-Spectrophotometric method has been developed for estimation of tropicamide from bulk and eye drop formulation. The  $\lambda_{\text{max}}$  of tropicamide in water was found to be 257 nm. The drug follows linearity in the concentration range 10-60  $\mu\text{g/ml}$  with correlation coefficient value 0.999. The proposed method was applied to Tropicacyl eye drop and percent amount of drug estimated 99.24% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels 80%, 100% and 120%. The percent recovery was found to be in the range 99.28% – 100.87%. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The %R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of tropicamide in bulk and in pharmaceutical dosage form.

**Keywords:** Tropicamide, UV-Spectrophotometry, Area Under Curve, Validation

### INTRODUCTION

Tropicamide is N-Ethyl-2-phenyl-N-(4-pyridylmethyl)hydracrylamide (Figure 1).



**Figure 1. Chemical structure of Tropicamide**

It is a tropic acid derivative act as an anticholinergic having short duration of antimuscarinic activity. It produces short acting mydriasis (dilation of the pupil) and cycloplegia. It is used to allow better examination of the lens, vitreous humor, and retina. Due to its relatively short duration of effect (6 hours), it is typically used during eye examinations and also be used before or after eye surgery. Anticholinergic preparation blocks the responses of the sphincter muscle of the iris and the ciliary muscle to produce mydriasis[1]. It is occasionally administered in combination with p-hydroxyamphetamine or phenylephrine hydrochloride.

A detailed literature survey for tropicamide revealed that several analytical techniques have been described for routine determination. Shoaibi and Gouda have described extractive spectrophotometric method for determination of tropicamide, based on formation of drug with bromocresol purple and methyl orange in acidic buffer solution.[2] Also derivative spectroscopic method[3], HPLC[4,5], HPLC-FT-IR[6], were developed for estimation of tropicamide in bulk, combination formulation and pharmaceutical dosage form.

However area under curve method have not been developed and validated for pharmaceutical dosage form. Hence, our study reports a simple, precise and economical UV- Spectrophotometric method for estimation of tropicamide in eye drop formulation. The method was validated according to ICH guidelines[7].

### MATERIAL AND METHOD

#### Reagents and Materials

Tropicamide working standard was obtained as gift sample from Sigma Aldrich. The drug was used without further purification. An eye drop Tropicacyl 1% tropicamide (B.NO.- TR-1307, Expiry date- 12/2015) was

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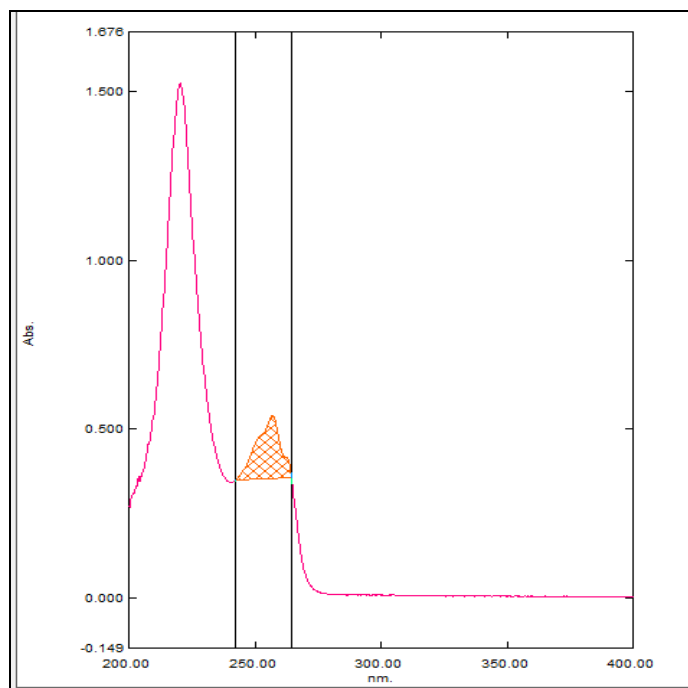
purchased from local market. Analytical grade solvents were used for the experiment.

#### *Preparation of standard stock and working standard solution*

The standard stock solution of tropicamide was prepared by dissolving accurately weighed 10 mg of the drug in 100 mL volumetric flask with water to obtain a final concentration of 100 µg/mL.

#### *Selection of Wavelength for analysis*

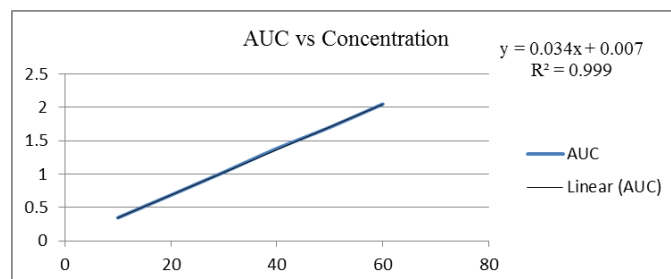
Appropriately 1.0 mL of standard stock solution of tropicamide was transferred into 10 mL volumetric flask and diluted up to the mark with water to give concentration of 10 µg/mL. The resulting solution was scanned in UV range (200 nm–400 nm). In spectrum tropicamide showed absorbance maximum at 257 nm (Figure 2).



**Figure 2. Area under curve spectrum of Tropicamide in water Linearity study**

Different aliquots of tropicamide in range 1-6 mL were transferred into series of 10 mL volumetric flasks and the volume was made up to the mark with water to get concentrations 10, 20, 30, 40, 50 and 60 µg/mL, respectively. The solutions were scanned on

spectrophotometer Shimadzu 2450 in the UV range 200–400 nm. The two wavelengths 242.40 and 264.60 nm were selected for the determination of Area Under Curve (AUC). The calibration plot was constructed as Area Under Curve vs Concentration (Figure 3).



**Figure 3. Calibration curve for tropicamide**

#### *Validation of the method*

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

#### *Accuracy*

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method (Table 1).

**Table 1: Recovery studies**

Drug	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL, n=3]	%Recovered	%RSD
Tropica mide	20	12	32.02	100.08	0.43 14
	20	20	40.35	100.87	0.02 52
	20	28	47.65	99.28	0.25 37

#### *Precision*

Precision of the method was carried out using repeatability, intra-day and inter-day variations. Repeatability was carried out for 40 µg/mL in six replicates (Table 2).

**Table 2: Repeatability**

Drug	Amount taken [µg/mL, n=6]	Amount found [µg/mL, n=6] ± SD	% Amount found ± SD
Tropicami	40	40.63 ± 0.1084	101.58 ± 0.27

de	% RSD	0.2668	0.2668
Intra-day precision was determined by analyzing the 20, 30 and 40µg/ml of tropicamide solutions for three times in the same day. Inter-day precision was determined by analyzing the 20, 30 and 40µg/ml of tropicamide solutions for three days (Table 3).			

**Table 3: Precision studies**

Drug	Concentration [µg/mL]	Intra-day [n=3]	%RSD	Inter-day[n=3]	%RSD
Tropicamide	20	99.85± 0.86	0.866 1	99.73 ± 1.07	1.079 5
	30	100.62 ± 0.60	0.596 6	101.21 ± 0.52	0.516 8
	40	100.40 ± 1.82	1.822 0	101.38 ± 0.16	0.158 0

#### Sensitivity

The sensitivity of measurements of tropicamide by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation  $LOD = 3.3 \times N/B$  and  $LOQ = 10 \times N/B$ , where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

**Table 4: Ruggedness studies**

Drug	Analyst – 1		Analyst – 2	
	% Amount found ± SD [n=3]	% RSD	% Amount found ± SD [n=3]	% RSD
Tropicamide	99.15 ± 0.9763	0.9846	99.31 ± 1.2999	1.3088

#### Ruggedness

Ruggedness of the method was determined for 40µg/ml concentration of tropicamide by two analysts using same operational and environmental conditions (Table 4).

#### Determination of Tropicamide in bulk

Accurately weighed 10 mg of tropicamide was transferred to a 100 mL volumetric, 50 mL water was added and sonicated for 10 min. Volume was made up to the mark with water. An appropriate aliquot was taken to get concentration 40µg/mL. The concentration of the drug was calculated from linear regression equation (Table 5).

**Table 5: Analysis of Bulk material**

Drug	Amount taken [µg/mL, n=6]	Amount found [µg/mL, n=6]± SD	(%) Amount found± SD
Tropicamide	40	40.63 ± 0.1084	101.58 ± 0.2710
	% RSD	0.2668	0.2668

**Table 6: Analysis of Tropicamide in Eye drop Formulation**

Drug	Label - claim [mg]	Amount taken [µg/mL]	Amount found [µg/mL]± SD	% Amount found
Tropicamide	50	40	39.69 ± 0.3767	99.24 ± 1.0317
		% RSD	0.9491	1.0397

#### Application of proposed method to Pharmaceutical formulation

For analysis of commercial formulation Tropicacyl eye drop (50mg/5mL), 1mL equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask, 50 mL water was added and sonicated for 10 min. Volume was made up to the mark with water. From this solution an appropriate aliquot was taken to get concentration 40µg/mL. The concentration of the drug was calculated from linear regression equation (Table 6).

#### RESULTS AND DISCUSSION

The linear regression data for the calibration curves showed good linear relationship over the concentration range 10-60µg/ml for tropicamide. Linear regression equation was found to be  $y = 0.034x + 0.007$  and correlation coefficient  $r^2 = 0.999$ . Percent recovery was found between 99.28% - 100.87%. Which means method

is accurate. The precision of the method was expressed in terms of repeatability and intermediate precision. The repeatability and intermediate precision was found to be less than 2 %RSD. So this method is precise for the estimation of tropicamide. The sensitivity of method expressed in terms of LOD and LOQ was found to be 1.24 $\mu$ g/mL and 3.77 $\mu$ g/mL respectively. Method found to be rugged, when two analysts carried out the analysis with less than 2 %RSD. The proposed method was applied to estimation of tropicamide in eye drop Tropicacyl. Percent label claim was found to be 99.24%.

### CONCLUSION

The method was validated and found to be simple, sensitive, accurate, and precise. Hence, the method can be used successfully for routine analysis of pharmaceutical dosage form of tropicamide. The proposed spectrophotometric method will not replace the presently known methods available for the analysis of tropicamide. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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