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Research Article

METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPY FOR THE ESTIMATION OF EPHEDRINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Development and validation of simple, rapid, accurate, precise and sensitive UV-Spectrophotometric method for the estimation of Ephedrine hydrochloride in bulk drug and Injection dosage form was performed in the current research. Quantitative determination of Ephedrine hydrochloride was done using distilled water as a solvent. λmax of Ephedrine hydrochloride in distilled water was measured at 270 nm. Linearity range for Ephedrine hydrochloride was 2-10 µg/mL and coefficient of correlation for Ephedrine hydrochloride was 0.999. Accuracy was performed and the percentage recovery of Ephedrine hydrochloride was found to be in the range of 98.6-99.17. The % relative standard deviation (RSD) for precision was less than 2%, LOD & LOQ was 0.079 µg/mL and 0.24 µg/mL respectively. The results suggest that method can be employed for routine analysis of Ephedrine hydrochloride in bulk drug and in pharmaceutical Dosage form.

Keywords: Ephedrine hydrochloride, Distilled water, UV spectrophotometry, Validation.

1. INTRODUCTION

Ephedrine hydrochloride (2-(methylamino)-1- phenyl-propane-1-ol hydrochloride) is a sympathomimetic drug used for the treatment of cold, asthma, and allergies due to decongestion of the nasal mucosa in allergic states and stimulating central nervous system. Due to easy operation and high accuracy, UV spectrophotometry is used to estimate the Ephedrine hydrochloride [1, 2]. The literature survey reveals that Ephedrine was analyzed by UV-visible spectroscopy at the wavelength between 190 and 900 nm and it was found that Eph has an absorption peak at the wavelength 257 nm, while the adsorption of $CoCl_2$ was observed on the wavelength of 315 nm. The UV–visible of the complex mixture of Eph and cobalt (II) shows an adsorption peak at 389 nm using the chloroform and CS_2 as a blank solution. These results confirm that there are no interferences [3, 4]. Other methods based on capillary electrophoresis [5], HPLC method [6] and Flow Injection analysis [7] were also reported. So far estimation of this drug as a single has not been reported in injection dosage form. Thus, the present study was undertaken to develop and validate a simple, accurate, precise and sensitive UV-Spectrophotometric method for Ephedrine hydrochloride.

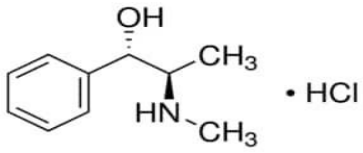

Fig. 1: Chemical structure of Ephedrine hydrochloride

2. MATERIAL AND METHODS

2.1. Apparatus

Instruments used were UV-Visible double beam spectrophotometer (PG Instruments) with 1cm matched quartz cells, Micropipette and Digital balance.

2.2. Reagents and Materials

Ephedrine hydrochloride pure drug was obtained from local pure drug supplier and marketed formulation from Local pharmacy. Methanol was obtained from Finar chemicals. All the chemicals and reagents used were of analytical grade.

2.3. Methodology

2.3.1. Preparation of Stock Solution

Ten mg of Ephedrine hydrochloride was accurately weighed and transferred to a 10mL volumetric flask, 1mL of methanol was used for solubilising the drug and volume was made up to the mark with distilled water to give 1mg/mL. It was further diluted to give 10µg/mL and the solution was scanned from 200-400 nm and λmax was 270 nm.

2.4. Method Validation

Developed method was validated according to ICH Q2B guidelines[8-10].

2.4.1. Linearity

Aliquots of 2 mL, 4 mL, 6 mL, 8 mL and 10 mL were pipetted from standard solution. 10µg/mL was transferred into a 10 mL volumetric flask and volume was made up with distilled water to give concentration ranging 2-10 µg/mL and the absorbance of all the solutions were measured at 270 nm. The linearity was calculated by the least square regression method. A calibration curve was plotted against absorbance and concentration.

2.4.2. Precision

The precision was determined by repeatability (intraday Precision) and intermediate precision by comparing the results of two different analysts and the results were reported as % RSD for a statistically significant number of replicate measurements.

2.4.3. Accuracy

Accuracy is done by recovery studies and was carried out by spiking the samples solution with standard solution at 50%, 100%, and 150% at 3 replicates and % recovery was calculated.

2.4.4. LOD and LOQ

The detection limit of an individual's analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

$$LOD = 3.3\ Sa/b$$

$$LOQ = 10\ Sa/b$$

Where, Sa = the standard deviation of the intercept, b = Slope of the calibration curve

2.4.5. Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the parameters. The robustness was checked by changing the λ_{max} of the drug.

2.4.6. Assay

The marketed formulation of Ephedrine hydrochloride injection dosage form was procured from local pharmacy. 1mL containing 30mg of it was dissolved in 100 mL of distilled water to give 0.3 mg/mL. From above solution 1mL was taken and made up with 10 mL of distilled water. Finally, from second stock solution, pipetted out 2 mL and made the volume upto 10 mL with distilled water to get 6µg/mL.

3. RESULTS AND DISCUSSION

3.1. Absorption spectrum

Absorption spectrum or λmax Ephedrine hydrochloride was found to be 270 nm and shown in Fig.2.

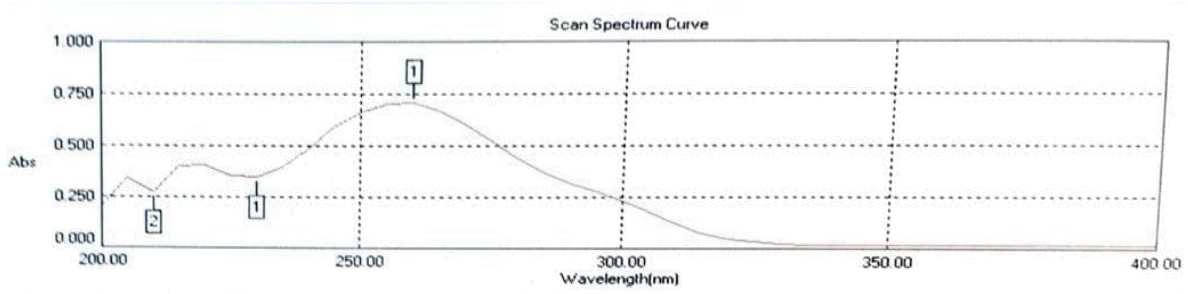

Fig. 2: Absorption spectrum of Ephedrine hydrochloride

3.2. Linearity

From the graph it was found that Ephedrine hydrochloride obeys Beers law and the linearity concentration lies between 2-10 µg/mL. The linearity data and calibration curve were shown in Table 1 and Fig.3.

Calibration curve was plotted and correlation coefficient was found to be 0.999. So, there was a good relation between absorbance and concentration.

Table 1: Linearity data of Ephedrine hydrochloride

S. No	Concentration (µg/mL)	Absorbance
1	2	0.151
2	4	0.306
3	6	0.457
4	8	0.608
5	10	0.751
	Intercept	0.001
	Slope	0.075
	R^2	0.999

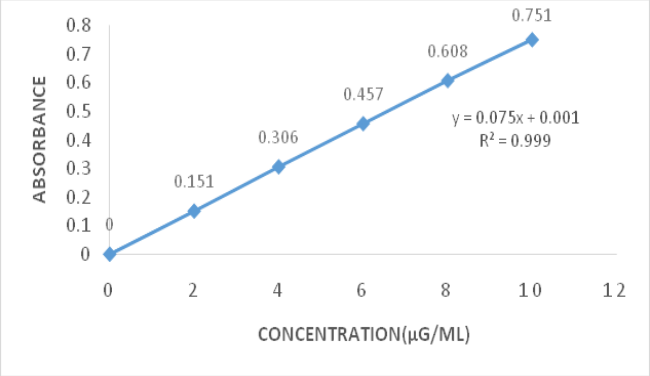

Fig. 3: Calibration curve of Ephedrine hydrochloride

3.3. Precision

Inter day and Intermediate precision data was shown in Tables 2 and 3 respectively.

Table 2: Interday Precision Values for Ephedrine hydrochloride

Precision		
	Morning	Afternoon
S. No	Abs	Abs
1	0.456	0.455
2	0.457	0.454
3	0.457	0.451
4	0.456	0.452
5	0.455	0.451
6	0.456	0.454
Mean	0.456167	0.452833
SD	0.000753	0.001722
%RSD	0.165021	0.380361

Table 3: Intermediate precision values for Ephedrine hydrochloride

Ephedrine	Analyst 1	Analyst 2
1	0.452	0.456
2	0.454	0.455
3	0.455	0.458
4	0.454	0.457
5	0.454	0.458
6	0.456	0.457
Mean	0.4542	0.4568
SD	0.0013	0.0012
%RSD	0.2927	0.2559

The % RSD for Intraday and Intermediate precision was found to be < 2%. It indicates that the method was precise.

3.4. Accuracy

Recovery studies were carried out by spiking the samples solution with standard solution at 50%, 100%, and 150% at 3 replicates and data was shown in Table 4.

Table 4: Accuracy data of Ephedrine hydrochloride

Accuracy	Ephedrine			
%Level (N=3)	Target Sample Solution	Amt. Of Std. Spiked	% Recovery	Mean
50%	6	3	98.88	
50%	6	3	99.77	99.17
50%	6	3	98.88	
100%	6	6	99.16	
100%	6	6	99.16	98.94
100%	6	6	98.5	
150%	6	9	98.6	
150%	6	9	98.7	98.60
150%	6	9	98.66	

The average % recovery of Ephedrine hydrochloride was found to be in the range of 98.6-99.17.

3.5. Limit of Detection and Limit of Quantification

LOD and LOQ value for Ephedrine hydrochloride was shown in Table 5.

LOD and LOQ values for Ephedrine hydrochloride were found to be 0.079 µg/mL and 0.240µg/mL. It indicates that the method was sensitive.

Table 5: Limit of detection and limit of quantification

Parameter	Ephedrine Hydrochloride (µg/mL)
LOD	0.079
LOQ	0.240

3.6. Robustness

Robustness data was as shown in Table 6.

Table 6: Robustness values for Ephedrine hydrochloride

S. No	Conc (µg/mL)	268(nm) Abs	270(nm) Abs	272(nm) Abs
1	6	0.436	0.457	0.421
2	6	0.436	0.457	0.421
3	6	0.435	0.456	0.424

There was no much variation in the absorbance with change in wavelength.

All the analytical validation parameters are summarized in Table 7

Table 7: Analytical validation parameters

PARAMETER	RESULT
Absorption maxima(nm)	270
Linearity Range (µg/mL)	2-10
Standard Regression Equation	0.0754x + 0.0019
Correlation Coefficient (r2)	0.9999
Accuracy (% recovery ±SD)	0.254526
Precision (%)	0.165021 and 0.380361
LOD (µg/mL)	0.079
LOQ (µg/mL)	0.240
% Drug found in formulation	100.10

Table 8: Assay of Ephedrine hydrochloride

Label claim(mg)	Amount found(mg)	Assay%±SD
30	30.03	100.10±0.15

Ephedrine hydrochloride % assay was 100.1±0.15 which was comparable with the label claim amount. It shows that UV Visible method developed was successful in determining Ephedrine hydrochloride from injection formulation.

4. CONCLUSION

The developed UV spectrophotometric method was simple, rapid, accurate, precise and sensitive for the estimation of Ephedrine hydrochloride in bulk drug and pharmaceutical dosage form. Hence this method can be used in the routine work of quality control laboratories.

5. ACKNOWLEDGEMENT

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Conflict of interest

None declared

Source of funding

None declared

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