WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 6, 883-891 Research Article ISSN 2278 – 4357

HPTLC METHOD DEVELOPMENT AND VALIDATION OF EPERISON HYDROCHORIDE IN API AND PHARMACEUTICAL DOSAGE FORM

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Article Received on
27 March 2017,

Revised on 16 April 2017,
Accepted on 06 May 2017

DOI:10.20959/wjpps20176-9234

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ABSTRACT

High-performance thin layer chromatography (HPTLC) is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of drug. A suitable HPTLC method was developed for the identification of API - Eperisone hydrochloride in formulation – Myosone using the stationary phase: TLC Silica gel 60 F_{254} and mobile phase: Toluene-Methanol (8:2) *v/v*. After the application of bands using CAMAG Automatic TLC Sampler 4, the plate was developed in the solvent system up to 70mm in CAMAG Twin Trough Chamber. Band was detected at 267nm. The method was validated in terms of linearity, accuracy, precision, LOD-LOQ, reproducibility and specificity according to ICH guidelines.

KEYWORDS: HPTLC, Eperison HCl, Validation, CAMAG, Methanol ,Toluene

INTRODUCTION

Eperisone Hydrochloride acts by relaxing both skeletal muscles and vascular smooth muscles, and demonstrates a variety of effects such as reduction of myotonia, improvement of circulation, and suppression of the pain reflex. Eperisone is contraindicated in patients with known hypersensitivity to the drug. In literature survey very few methods have been reported for the analysis of Eperison Hydrochride in dosage forms which include RP-HPLC, UV spectroscopy , LC-MS/MS. IUPAC name of Eperison Hydrochride1-(4-ethylphenyl)-2-methyl-3-(piperidin-1-yl)propan-1-one. Solubility of this drug is soluble in methanol and has melting point 169-171^0 c

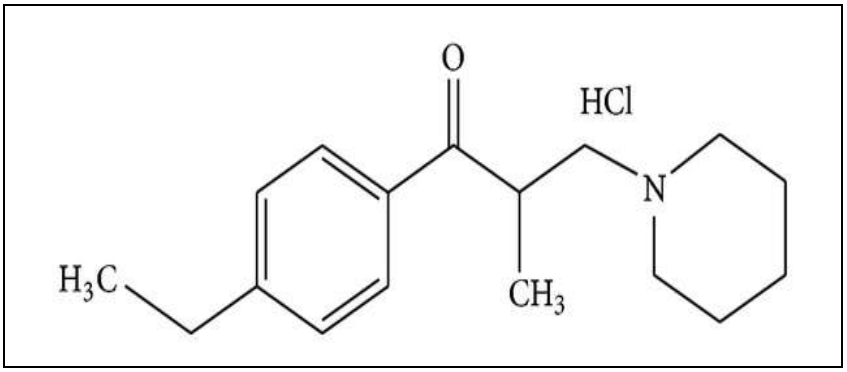


Fig 1: Chemical Structure of Eperison hydrochloride.

MATERIALS AND METHODS

.Eperison Hydrochride gift sample was kindly provided by Macleods Pharmaceuticals Ltd., (Mumbai, Maharashtra.) "Myosone 50mg" Tablet were procured from local market. AR grade of solvents used for this study were purchased from Merck Pvt. Ltd, Mumbai.

PREPARATION OF SOLUTIONS

Preparation of standard (100µg/mL or 0.1 mg/mL)

Accurately weighed and transferred 10 mg of Eperison hydrochride working standard into a 10 ml cleaned and dried volumetric flask and dissolved in 5 ml of diluent with sonication and volume made up to the mark with the diluent. Pipette out 1mL from the above prepared stock solution was, pipetted out transfered into 10mL of volumetric flask and volume was adjusted 100µg/mL**.**

Sample preparation (100µg/mL or 0.1mg/mL)

Eperison HCl tablets (n=3) were accurately weighed and triturated to a fine powder. From the crushed tablets, amount equivalent to 50 mg of Eperison hydrochloride was accurately weighed and transferred in a 50 ml of cleaned and dried volumetric flask. Initially about 15 mL of diluent was added and sonicated to dissolve. The solution was Allowed it to settle for 5 minutes, and adjusted the volume with diluent. Further dilute 1mL was diluted to 10 mL with diluent and mixed thoroughly.

Selection of wavelength for Detection

The working standard of Eperison hydrochride in methanol was scanned by Camag TLC scanner 4 with UV visible detector over wavelength range 200 to 400 nm. Wavelength 267 nm was selected for further studied (Figure 2)

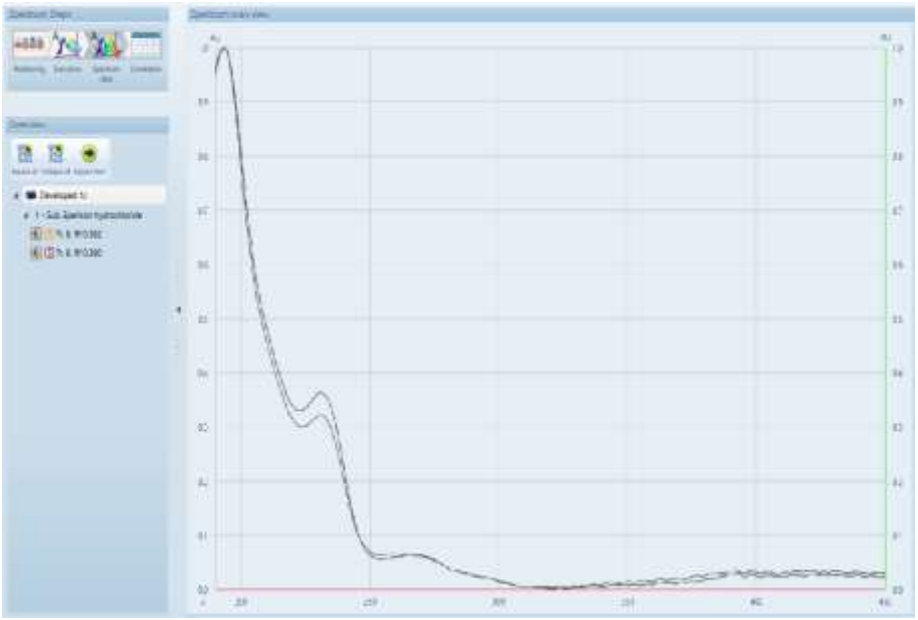


Fig. 2: The overlain UV spectra of 100 ng/ml Eperison Hydrochloride (API and sample) between 200 and 400 nm.

CHROMATOGRAPHIC CONDITIONS

This analysis was performed on Camag HPTLC system (Switzerland). It is equipped with a Linomat-5 applicator, 100 µl sample syringe (Hamilton, Switzerland) and Camag TLC scanner-4. On the basis of trial and error method using different solvent system, following chromatographic conditions were choosen for analysis. Pre-coated silica gel 60 F254 TLC (E-Merck, Germany) plates (10x10 cm) was used as stationary phase. TLC plates were pre-washed with methanol and activated at 110°C for 10 min prior to application. The standard samples of Eperison Hydrochloride_were spotted on pre-coated TLC plates In the form of bands of length 4 mm using 100 µl sample syringe with a Linomat-5 applicator. The chromatographic development was carried using Toluene:Methanol (8:2 *v/v*) as mobile phase with chamber saturation time of 20 minutes and the migration distance of 70 mm. Densitometric scanning was performed using Camag TLC scanners at 267 nm, operated by win CATS Software (Version 1.4.3, Camag).

Preparation of Calibration Curve

Different concentration of working standand solution are made and applied on TLC Plate, Replicate analysis of solution containing 100-400 µg/ml for Eperison Hydrochloride was recorded. Calibration curves were constructed by plotting by taking concentrations on X axis and mean ratio of absorbance of standards on Y-axis and regression equation were obtained in this range (Figure 3).

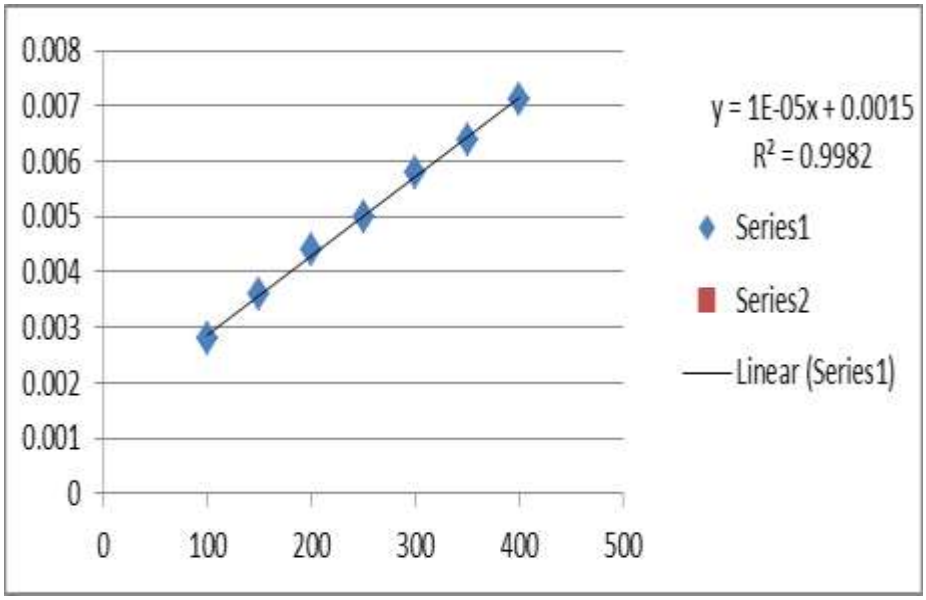


Fig 3: Calibration Curve of Eperison Hydrochloride.

METHOD VALIDATION

Validation is to ensure varied inputs lead to consistent and high quality outputs and The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities.

1. Linearity and Range

A linear relationship should be evaluated across the range of the analytical procedure**.** The linearity was determined by using working standard solutions between 100-400 ng/spot. The spectrums of these solutions were recorded at wavelength 267 nm. Calibration curves was constructed by plotting by taking concentrations on X axis and mean ratio of absorbance of standards on Y-axis and regression equation was obtained.

Table 2: Linearity and Range of Eperison hydrochloride.

Concentration ng/spot	Absorbance
100	0.0028
150	0.0036
200	0.0044
250	0.0050
300	0.0058
350	0.0064
400	0.0071

2. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample.

2.1 Repeatability

Repeatability study was performed by preparing a minimum 6 determinations of standard solution of Eperison Hydrochloride and analyzed by Camag scanner and absorbance were recorded at 267 nm. Relative standard deviation (%RSD) was calculated (Table 3)

Concentration (ng/spot)	Absorbance	Mean absorbance	%RSD
250	0.0043	0.004431	1.72
250	0.0044		
250	0.0045		
250	0.0044		
250	0.0045		
250	0.0045		

***n=6, % RSD = % Relative Standard Deviation.**

2.2 Reproducibility

In The intra-day and inter-day precision made 6 concentration of Eperison Hydrochloride (250ng/spot) of working standard were made it can be analyzed 3 on the same day and 3 on next day . The results were reported in terms of percentage relative standard deviation (%RSD). The results were tabulated in (Table 4).

Drug	Concentration (ng/spot)	% RSD	
		Interday	Interday
Eperison hydrochloride	250	0.95	1.17
	250	0.97	1.19
	250	0.92	1.15

***n=3**

3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Nine sets of known concentrations (0.001-0.004 µg/spot) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation (Table 5) and following formulae as;

LOD = 3.3 SD/S

LOQ = 10 SD/S

Where,

SD =is standard deviation of y-intercept of the calibration curves

S= is mean slope of five calibration curves

Table 5: LOD and LOQ of Eperison Hydrochloride.

Drug	LOD	LOQ
Eperison Hydrochloride	1.18	3.5

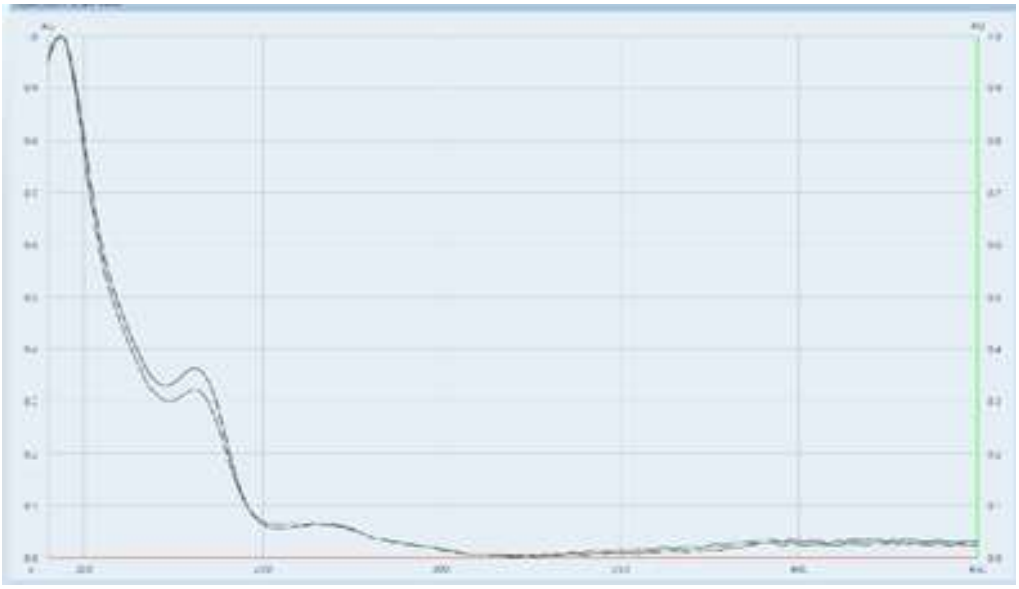
4. Accuracy

The accuracy of the method was determined by calculating recoveries of Eperison hydrochloride HCl by the standard addition method. Known amount of Eperison hydrochloride HCl at 80 %, 100 %, 120 % were added to a pre quantified sample solution. Each level of accuracy was carried out in a triplicate in the presence of placebo. The Mean recoveries for Eperison hydrochloride at 80% to 120% levels were estimated by applying the obtained values to the regression equation of the calibration curve.

Concentration taken in ng/spot (A)	Standard addition in ng/spot (B)	Total drug Concentrati0on (ng/spot) (A+B)	Area	Average	% Recovery
100	80	180	4402	4334	89.14
			4330		
			4270		
100	100	200	4607	4621	86.54
			4644		
			4612		
100	120	220	4946	4948	85.26
			4920		
			4978		

5 Specificity

Specificity is the ability of the method to measure the analyte in the presence of other relevant components those are expected to be present in a sample. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. These might include impurities, degradants, matrix. The spot for drug in sample was confirmed by comparing the R_f and spectra of the spot with that of standard drug spot.



RESULTS AND DISCUSSION

An HPTLC method was preferred for estimation of Eperison Hcl. Preliminary experiments were carried out to achieve the best chromatographic conditions for the determination of the drug substance. Several mobile phase and injection volume trial were carried out. The detection Wavelength was selected as 267 nm.The Calibration curve was plotted for Eperison hydrochloride absorbance *v/s* Concentration. The generated regression equation was y=0.0014x+0.0015 = (R^2= 0.998). The R^2 value as 0.998 indicates that developed method was linear. The calibration curve was obtained in the range of 100-400 ng/spot. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80%, 100% and 120% levels 89.14%, 86.54%, 85.26% showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 0.50 ng/spot and 2.9 ng/spot respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Eperison Hydrochloride in formulation.

CONCLUSION

The developed HPLC method was found to be fast, simple, sensitive and economic. The method was validated and found to be specific, linear, accurate, precise . A new, simple, and sensitive HPTLC method has been successfully developed and validated for determination of Eperison Hydrochloride in bulk and pharmaceutical dosage form.

ACKNOWLEDGEMENT

Authors are thankful to M/S Macleods Pharmaceuticals Ltd.,(Mumbai, Maharashtra). for providing the gift sample of Eperison Hydrochloride. Authors also thank to Anchrom Enterprises Pvt. Ltd. Mumbai, India and Sinhgad College of Pharmacy, Pune, (Savitribai phule pune university) India for providing necessary facilities to complete this project.

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