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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF EPERISONE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Eperisone hydrochloride in bulk and pharmaceutical dosage form. The stationary phase used was silica gel precoated aluminum plate $60F_{254}$ plates. The mobile phase used was a mixture of ethyl acetate: methanol: toluene (4:3:3, v/v/v). The detection of spots was carried out at 272 nm. The method was validated in terms of specificity, accuracy, linearity, precision and accuracy. The calibration curve was found to be linear between 100-700 ng/band. The proposed method can be successfully used to determine the drug Eperisone hydrochloride in bulk and pharmaceutical formulation.

KEYWORDS

Eperisone, HPTLC, Validation and Precision.

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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[1]. Chemically it is 4-ethyl-2-methyl-piperdino prophenone hydro-chloride. The chemical structure of Eperisone hydrochloride was shown in Figure No.1. Eperisone hydrochloride also facilitates voluntary movement of the upper and lower extremities without reducing muscle power, it is therefore useful during the initial stage of rehabilitation and as a supporting drug during subsequent rehabilitative therapy[2-4].

High performance thin-layer chromatography (HPTLC) is an easy, cheap, rapid and widely used method for the analysis of drugs and pharmaceuticals[5,6]. Literature survey revealed that few HPTLC methods[7-10] were reported for the estimation of Eperisone hydrochloride alone or in combination with other drugs in pharmaceutical formulations. Hence a new, sensitive, precise and accurate HPTLC method was developed and validated as per ICH guidelines[11,12] for the estimation of Eperisone hydrochloride in bulk and in pharmaceutical formulation.

MATERIAL AND METHODS

Instrumentation

Computerized Camag HPTLC system (Camag, Muttenz, Switzerland) consisting of a Camag 100 microlitre sample syringe (Hamilton, Bonded, Switzerland) on silica gel precoated aluminum plate $60F_{254}$ plates, [20 cm × 20 cm width 200µm thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance absorbance mode at 272 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 nm and 400 nm. A Camag glass twin-trough development chamber, different size pipettes, volumetric flasks, measuring cylinders, micro syringes and ruler were used.

Chemicals and solvents

The reference sample of Eperisone hydrochloride was obtained from Spectrum Pharma Research Solutions, Hyderabad, India. Commercially available Eperisone hydrochloride tablets claimed to contain 50 mg of Eperisone hydrochloride was purchased from local market. Ethyl acetate, methanol and toluene were purchased from Merck Chemicals, Mumbai, India.

Preparation of standard solution

Accurately weighed and transferd 100 mg of Eperisone hydrochloride into a 100mL clean dry volumetric flask, about 10mL of diluent was added and made volume upto the mark by using diluent. Further 1mL from above stock solution was transferred into100mL volumetric flask and diluted upto the mark by adding diluent. From this 1mL of the solution was pipetted into a 100mL volumetric flask and made upto the mark with diluent. From this serial dilutions were prepared for Eperisone hydrochloride to construct the calibration curve.

Preparation of sample solution

Twenty tablets were accurately weighed and crushed in to a fine powder in a mortar. An amount of powder equivalent to 50 mg of Eperisone hydrochloride into 100mL volumetric flask and 50mL of diluent was added to it. The mixture was sonicated for 20 min to dissolve and made volume upto the mark with diluent, then solution was filtered through 0.45 µm filter paper. Further 8mL of solution was pipetted and transferred to 100mL volumetric flask, made volume upto the mark by using diluent. From the above stock solution, 1mL of the solution pipetted into a 100mL volumetric flask and volume was made upto the mark with mobile phase to yield concentration 400 ng/band of Eperisone hydrochloride.

HPTLC method and chromatographic conditions

TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50^0C for 5-10 min. The chromatographic conditions maintained were precoated silica gel $60F_{254}$ aluminum sheets (10x10 cm) as stationary phase, ethyl acetate: methanol: toluene (4:3:3, v/v/v) as mobile phase, chamber and plate saturation time of 35 min, migration distance allowed was 72 mm, keeping the slit dimension at 5x0.45 mm. A deuterium lamp provided the source of radiation. Ten micro liters of standard solution of Eperisone hydrochloride was spotted and developed at constant temperature. Wavelength was selected by scanning standard solution over 200-400 nm wavelength. Eperisone hydrochloride show maximum absorbance at 272 nm in reflectance mode with Camag TLC Scanner 3 using Win CATS software.

Calibration curve

Aliquot of 100, 200, 300, 400, 500, 600 and 700 ng/band of standard solution of Eperisone hydrochloride was applied on the TLC plate. TLC

plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was plotted using regression analysis with Microsoft excel.

Validation of the method

The developed method was validated in terms of specificity, linearity, system precision, method precision and intermediate precision, accuracy, limit of detection and limit of quantitation.

RESULTS AND DISCUSSION

For HPTLC, silica gel precoated aluminum plates $60F_{254}$ was chosen as the stationary phase for the separation and determination of Eperisone hydrochloride. Mixture of commonly used solvents like ethyl acetate, toluene and methanol with or without formic acid in different combinations were tested as mobile phase. The choice of the optimum composition was based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of ethyl acetate: methanol: toluene (4:3:3, v/v/v) was proved to be the most suitable of all the combinations since the densitogram peak obtained was well defined peaks are measured at 272 nm, better resolved. The R_f values of the Eperisone hydrochloride was found to be 0.45.

The specificity of method was performed by comparing the densitograms of blank, standard and sample. The absence of additional peaks in the densitogram indicates non-interference of the commonly used excipients in the tablets and hence the method was specific. The linearity was found 100-700 ng/band of Eperisone hydrochloride. The regression equation of the linearity curve between concentrations of Eperisone hydrochloride over its peak areas were found to be Y=13.08X+253.7 (where Y is the peak area and X is the concentration of Eperisone hydrochloride in ng/band). The linearity curve of Eperisone hydrochloride was shown in Figure No.2. The linearity results were furnished in Table No.1.

To study the system precision, six replicate standard solutions of Eperisone hydrochloride was injected. The %RSD was calculated and it was found to be 0.38 of Eperisone hydrochloride, which are well within the acceptable criteria of not more than 2.0. The method precision study was carried out on six preparations from the same tablet samples of Eperisone hydrochloride percent amount of both were calculated. The %RSD of the assay result of six preparations in method precision study was found to be 0.61. The intermediate precision study was carried out by different analysts; from the same tablet of Eperisone hydrochloride in the percent amount of Eperisone hydrochloride was calculated. The %RSD of the assay result of six preparations in intermediate precision study was 0.65. The lower %RSD values reveals that the method quite precise.

The limit of detection and limit of quantitation was found to be 7 ng/ band and 21 ng/ band. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method was sensitive. The method validation parameters were shown in Table No.2.

The accuracy of the method was determined by recovery studies. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent recovery and %RSD at each level was calculated. Satisfactory recoveries ranging from 99.28 to 99.89% of Eperisone hydrochloride was obtained by the proposed method. The percent individual recovery and the %mean recovery at each level were within the acceptable limits. This indicates that the method was accurate. The recovery studies results are furnished in Table No.3. The densitogram of Eperisone hydrochloride was shown in Figure No.3.

Table No.1: Linearity study of Eperisone hydrochloride

S.No	Concentration of Eperisone hydrochloride (ng/band)	Mean peak area
1	100	1556
2	200	2867
3	300	4169
4	400	5541
5	500	6762
6	600	8101.2
7	700	9413.4
8	Slope	13.08
9	Intercept	253.7
10	Correlation Coefficient	0.999

Table No.2: Validation parameters of Eperisone hydrochloride

S.No	Parameters	Values
1	Linearity range (ng/band)	100-700
2	Limit of detection (LOD)	7 ng/band
3	Limit of quantitation (LOQ)	21 ng/band
4	Precision (% RSD System precision (n=6) Method precision (n=6) Intermediate precision (n=6)	 0.38 0.61 0.65

Table No.3: Recovery results of Eperisone hydrochloride

S.No	Level	Concentration added (ng/band)	Concentration found (ng/band)	% Recovery	Mean recovery
1	50%	200	198.56	99.28%	99.64%
2	100%	400	399.11	99.77%	
3	150%	600	599.35	99.89%	

*Each value is a mean ± standard deviation of three determinations

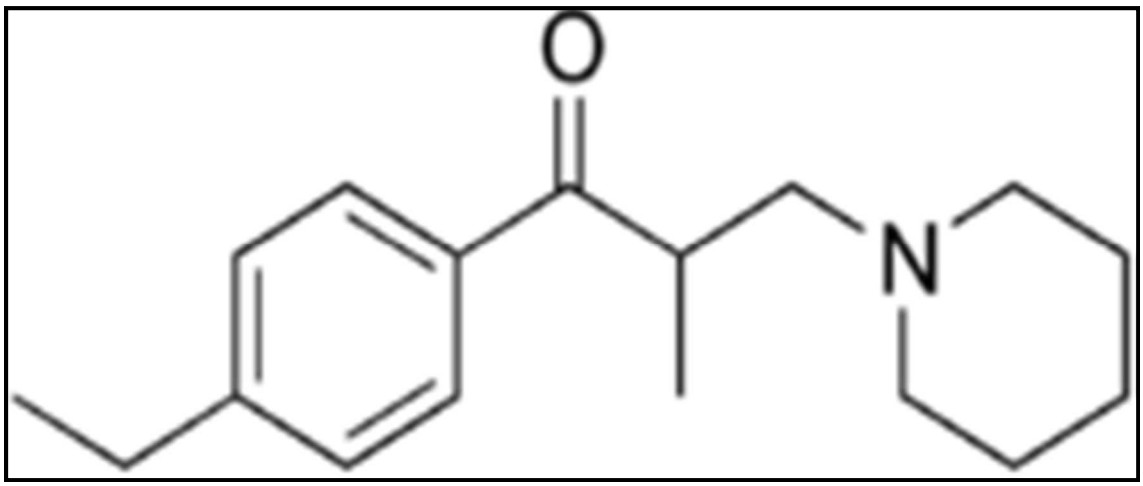

Figure No.1: Chemical structure of Eperisone

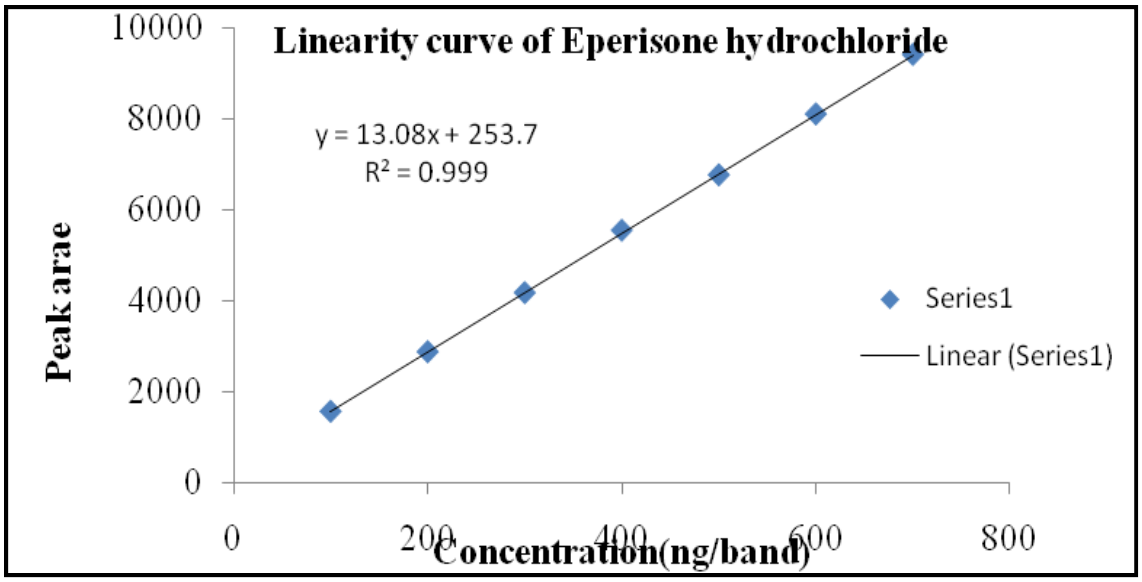

Figure No.2: Linearity plot of Eperisone hydrochloride

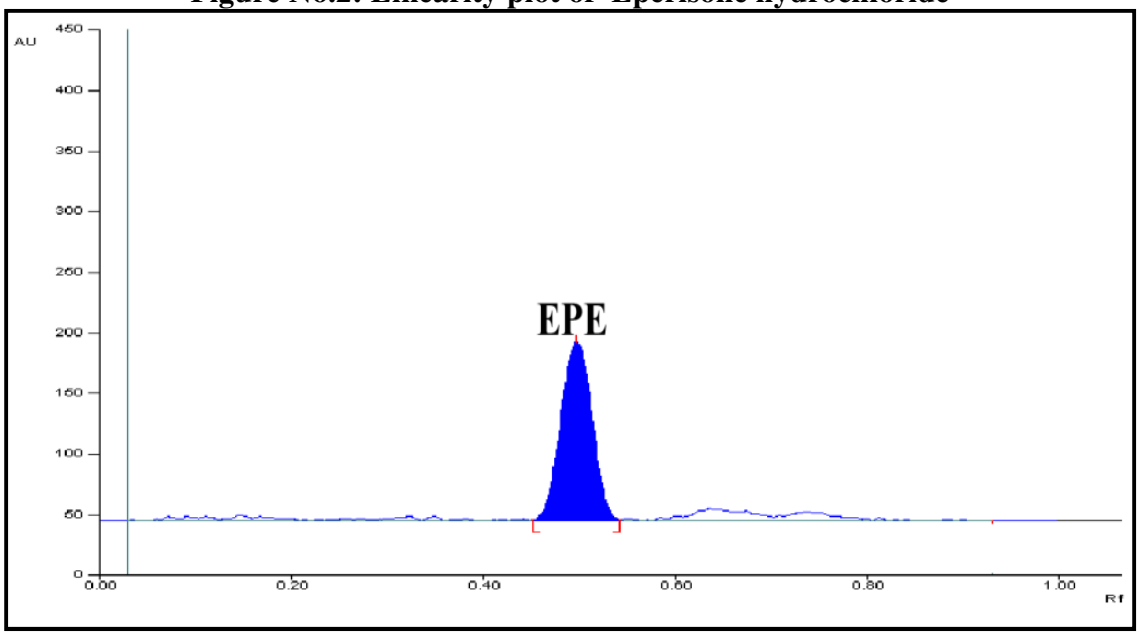

Figure No.3: Typical densitogram of Eperisone hydrochloride

CONCLUSION

The developed HPTLC method for the estimation of Eperisone hydrochloride is simple, specific, precise, accurate and reproducible. The amounts found in formulations are well agreed with label claim. The proposed method can be applied to routine analysis in quality control laboratories for the estimation of Eperisone hydrochloride in bulk and in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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