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Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF EPERISONE HYDROCHLORIDE IN BULK DRUG

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ABSTRACT

Objective: A new, economical, precise and accurate stability indicating HPTLC method was developed and validated for the determination of Eperisone hydrochloride in bulk drug.

Methods: Sample and standard solutions of Eperisone hydrochloride was applied on precoated silica gel G 60 F_{254} HPTLC plate and the plate was developed using Toluene: Chloroform: Ethanol (4:4:1v/v/v) as mobile phase. The detection was performed at 266 nm

Results: The calibration curve was found to be linear between 100 to 700 ng/spot for Eperisone hydrochloride with correlation coefficients 0.999. The R_f value for Eperisone hydrochloride was found to be 0.26±0.03. The LOD and LOQ were found to be 100 ng/spot and 300 ng/spot for Eperisone hydrochloride. The selected drug was subjected to acid, alkali and oxidative degradation and study revealed, Eperisone hydrochloride is well resolved from pure form with significant differences in their R_f this method can be successfully employed for quantitative analysis of Eperisone hydrochloride in bulk drug.

Conclusion: The proposed method has been validated as per ICH Q2 (R1) guidelines. This method can be used for routine quality control analysis of Eperisone hydrochloride in bulk drug.

Keywords: Validation, Eperisone hydrochloride, HPTLC, Chromatography.

INTRODUCTION

Eperisone hydrochloride is used as an antispasmodic and centrally acting muscle relaxant in treatment of acute and chronic muscle spasm [1]. Chemically it is 4'-ethyl-2-methyl-3-piperidinopropiophenone hydro chloride. It exists as a white crystalline powder, odorless, slightly soluble in water and freely soluble in alcohol, chloroform and methanol [1]. Literature survey revealed few spectrophotometric [2,3], RP-HPLC [4,5,6,7], HPTLC [8] and LC-ESI-MS in human plasma [9,10] methods for the determination of Eperisone hydrochloride either as single or in combination with other drug. But, to the best of our knowledge, a simple, specific and economical method for estimation of Eperisone hydrochloride by HPTLC for routine laboratory analysis is not yet reported. So the aim of present work was to develop and validate stability indicating HPTLC method for determination and quantitative estimation of Eperisone hydrochloride.

Fig. 1: Chemical structure of Eperisone hydrochloride

MATERIALS AND METHODS

Instrumentation

Pre-coated silica gel 60F_{254} aluminium plates (10 x 10 cm, 250 µm thickness; Merck, Germany), Automatic TLC sampler 4 (Camag, Switzerland), twin trough chamber (10 x 10 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner 4 (Camag, Switzerland), winCATS version 1.4.6 software (Camag, Switzerland) were used in the study. Ultrasonic bath (PowerSonic405, Hwashin technology, Korea) and Electronic Balance Shimadzu AX200, (Shimadzu Corporation, Japan) were used in the study.

Chemicals and reagents

Methanol, Toluene, Chloroform are used were of analytical grade.

Eperisone hydrochloride was obtained as gift sample from Sharon Biomedicine, India Ltd Mumbai. Tablet Eprisan® (Eisai Pharmaceuticals India Pvt. Ltd) was purchased from local market, containing Eperisone hydrochloride 50 mg per tablet.

Experimental

Preparation of standard solutions

Standard stock solution of Eperisone hydrochloride was prepared by dissolving 10 mg of accurately weighed Eperisone hydrochloride in 10 ml of methanol. This standard solution was further diluted with the same solvent to obtain 10 µg/ml of Eperisone hydrochloride.

Selection of mobile phase

A trial and error method was used to optimize the mobile phase on which different mobile phases were tried. The solvent system of Toluene: Chloroform: Ethanol in the ratio 4:4:1v/v/v was the most appropriate mobile phase for the HPTLC analysis of Eperisone hydrochloride.

Application of standard solutions

HPTLC pre-coated plate of silica gel G 60 F_{250} (10×10) was employed for the spotting of standard solutions. 10 µg/ml standard solution of 100, 200, 300, 400, 500, 600 and 700 ng/band of Eperisone hydrochloride were applied in the seven tracks respectively.

Calibration curve for Eperisone hydrochloride

Adequate dilutions were made from standard stock solution to obtained concentration of 100, 200, 300, 400, 500, 600 and 700 ng/band respectively. The relationship between peak area and concentration was established by the simple regression equation method. The regression equation was obtained and this relationship is presented in the calibration curve (Figure 4).

Development of spot

Twin Trough chamber containing 10 ml of mobile phase system was used for developing the spotted plate and saturated for 15 minutes. The plate were dried after development and viewed under UV lamp

to evaluate the spot obtained. The spot were uniform and there was no tailing.

Scanning by HPTLC scanner

After setting up the instrument parameters, the spot was scanned from 200 – 400 nm and the spot showed maximum absorption at 266 nm using the Camag TLC scanner 4. The R_f values were found to be 0.26 for Eperisone hydrochloride. Typical chromatogram obtained for Eperisone hydrochloride is shown in (Figure 2).

Assay of tablet formulation

Ten tablets of Eprisan® containing 50 mg of Eperisone hydrochloride were weighed, average weight was determine and triturate to fine powder. Tablet powder equivalent to 10 mg of Eperisone hydrochloride was transferred in 100 ml volumetric flask and dissolved in methanol. The solution was sonicated for 20 min and filtered through Whatmman filter paper No.41. The filtrate was appropriately diluted with methanol to obtained 50 µg/ml of Eperisone hydrochloride. The plate was developed under previously described chromatographic conditions. The peak area was measured at 266 nm and concentration in the sample were determine using multilevel calibration developed on the same plate under the same condition using the linear regression equation (Table 1).

Method validation

The method was validated for linearity, accuracy and intra-day and inter-day precision and specificity, in accordance with ICH guidelines Q2 (R_1).

Linearity and range

Response to Eperisone hydrochloride was linear in the concentration range of 100 - 700 ng/spot. The regression equations were y = 12.69 x + 131 respectively, where y is response and x the concentration of drug. The correlation coefficient were 0.999

Method precision

Repeatability

The precision of the method was checked by repeated analysis of (n = 6) standard solutions of Eperisone Hydrochloride (400 ng/band). Percentage relative standard deviation (% RSD) value was found to be below 2% which indicated that proposed method is precise (Table 2).

Intermediate precision

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 6 times on the same day and on 3 different days over a period of 1 week for standard solutions of 400 ng/band Eperisone Hydrochloride. The results were reported in terms of relative standard deviation (RSD) (Table 2).

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Eperisone hydrochloride by the standard addition method. Known amount of standard of Eperisone hydrochloride (80%, 100%, and 120%) was added to sample solutions prepared from tablet dosage form. The amounts of Eperisone hydrochloride was estimated by substituting values in the regression equations (y = 12.69 x + 131). The values prove that the method is accurate (Table 3).

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

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability. Sven sets of known concentrations (100-700 ng/band) were prepared and scanned. By using these spectra, regression equation was obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are given.

Specificity

Specificity study was performed by analyzing standard solution of drug and sample. The spot for Eperisone hydrochloride in sample was confirmed by comparing its R_f of sample spot with the spot obtained from standard solution

Forced degradation studies

Acid degradation

20 mg of pure drug was transferred to the round bottom flask. To this, 40 ml of 0.1M HCL was added and this reaction mixture was refluxed at 80^0C for about 3 hrs. After every 30 min, 5 ml of refluxed sample was removed. Further, 1 ml of the above solution was neutralized using 1 ml of 0.1M NaOH solution and then diluted to 10 ml with diluents to obtain concentration of 50 µg/ml of Eperisone hydrochloride.

Alkali degradation

20 mg of pure drug was transferred to the round bottom flask. To this, 40 ml of 0.1M NaOH was added and this reaction mixture was refluxed at 80^0c for about 3 hrs. After every 30 min, 5 ml of refluxed sample was removed. Further, 1 ml of the above solution was neutralized using 1 ml of 0.1M HCL solution and then diluted to 10 ml with diluents to obtain concentration of 50 µg/ml of Eperisone hydrochloride.

Hydrogen peroxide degradation

20 mg of pure drug was transferred to round bottom flask. To this 40 ml of 3% H_2O_2 was added and this reaction mixture was kept at room temperature for about 4 hrs. After every 1 hr, 5 ml of sample was removed. Further 1 ml of the above solution was diluted to 10 ml with diluent to obtain concentration of 50µg/ml.

RESULTS AND DISCUSSION

Linearity were found to be linear in the concentration range of 100-700 ng/spot for Eperisone hydrochloride withr^2= 0.999 respectively in mobile phase Toluene: Chloroform: Ethanol in the ratio 4:4:1v/v/v. The detection was done at 266 nm R_f value was found to be 0.26 for Eperisone hydrochloride. The proposed method was also evaluated by the assay of commercially available tablet and % assay was found to be 101% for Eperisone hydrochloride. The accuracy of the proposed method was studied by recovery studies at three levels (80%, 100% and 120%).

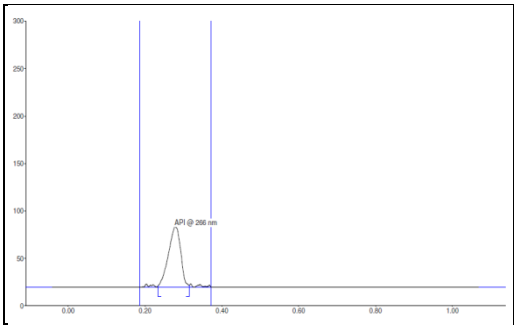

Fig. 2: Typical chromatogram obtained for Standard

The % recovery was found to be in the range of 99.61 to 101.87 for Eperisone hydrochloride shows the accuracy of method. The precision of the proposed method was studied by interday and intraday precision. The method was found to be accurate and

precise, as indicated by recovery studies and % RSD not more than 2 reflects the method is precise. The summary of validation parameters of proposed HPTLC method is given in (Table 4).

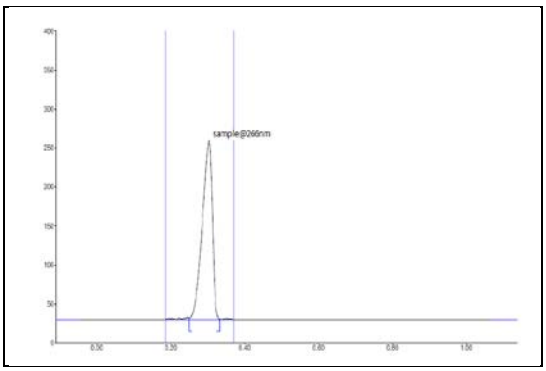

Fig. 3: Typical chromatogram obtained for sample

The degradation studies indicated that Eperisone hydrochloride was stable to acid under experimental condition without giving any additional degradation peak (Figure 5). The chromatogram of base and oxidative degraded sample of Eperisone hydrochloride showed one degradation peak in each condition (Figure 6, 7).

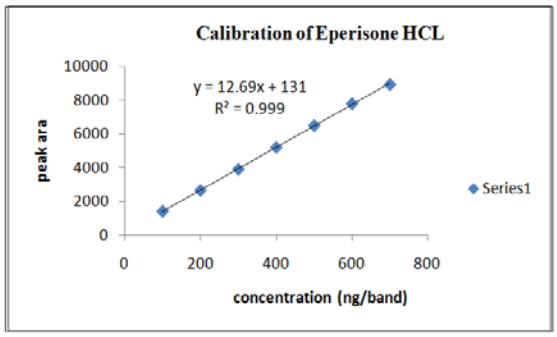

Fig. 4: Calibration curve for Eperisone hydrochloride

Table 1: Assay of Tablet Dosage Form (Eprisan®)

S. No.	Sample solution concentration (µg/ml)	Amount found (%)	Mean ± SD	% RSD*
1	50	101.2	101.04±0.088	0.307
2	50	101.4		
3	50	101.1		

*n=3, SD=Standard Deviation, % RSD = % Relative Standard Deviation

Table 2: Precision results for Eperisone hydrochloride

Drug	Concentration of drug (ng/band)	Absorbance (Mean ± S. D)*	% RSD*
Repeatability	400	5213.1+2.13	0.0409
Intraday	400	5224+1.9	0.0376
Interday	400	5225+0.8164	0.0156

*n=6

Table 3: Accuracy Results for Eperisone hydrochloride

Spike level	Amount added(ng/band)	Amount recovered (ng/band)	% Recovery	Mean% recovery	%RSD
80%	720	723	100.42	100.06	0.430
		721.1	100.1		
		717	99.50		
100%	800	810	101.2	101.11	0.181
		807.8	100.9		
		808.9	101.1		
120%	880	874	99.32	99.46	0.161
		874.9	99.42		
		876.8	99.64		

*n=3, SD=Standard Deviation, % RSD = % Relative Standard Deviation

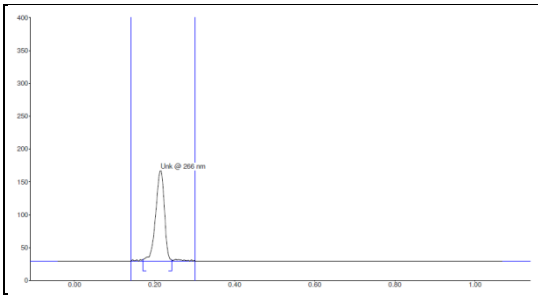

Fig. 5: Chromatogram of Acid degradation condition

Table 4: Summary of Validation Parameters

Parameter	Eperisone hydrochloride
Linearity range (ng/ spot)	100-700
Correlation co-efficient	0.999
Slope (m)	12.69
Intercept (c)	131
Precision (intraday) %RSD	0.0376
Precision (interday) %RSD	0.0156
Accuracy (Mean % recovery)	101.11%
LOD (ng /spot)	100
LOQ (ng /spot)	300

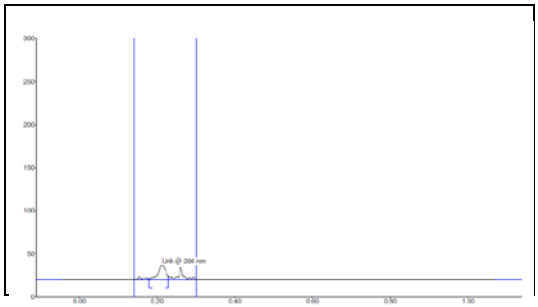

Fig. 6: Chromatogram of alkaline degradation condition

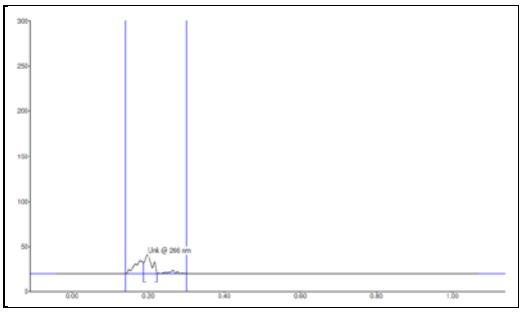

Fig. 7: Chromatogram of Oxidative degradation condition

CONCLUSION

A stability-indicating RP-HPLC method of Eperisone hydrochloride was developed and validated as per ICH guidelines. The degradation behavior of Eperisone hydrochloride was studied under various stress conditions of acid, alkali and oxidation. Drug was found to be unstable in basic and oxidative condition. The developed method is rapid, economic, accurate and precise for quantitative analysis of Eperisone hydrochloride in bulk drug.

CONFLICT OF INTERESTS

Declared None

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