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Development and Validation of Stability Indicating RP-HPLC Assay Method of Eperisone HCl In Pure and Pharmaceutical Dosage Form

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ABSTRACT

Simple, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed and validated for the determination of Eperisone HCl using a Phenomenex Luna C18 (250 x 4.6 mm) 5µm column and a mobile phase of buffer and methanol in the proportion of (30:70) pH 3.0 adjusted with o-phosphoric acid. The retention time of Eperisone HCl were found to be 9.050min respectively. Linearity was established for Eperisone HCl in the range of 50.25 to 150.75µg/ml, respectively. The percentage recovery of Eperisone HCl was found to be in the range of 98.96-99.68% respectively. The selected drug were subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, photolytic and UV degradation and the degradation studies revealed, Eperisone HCl is well resolved from the pure form with significant differences in their retention time values. This method can be successfully employed for the quantitative analysis of Eperisone HCl in pure and formulation.

Keywords: Eperisone HCl, stability indicating method, RP-HPLC.

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INTRODUCTION

EperisoneHCl[1,2][Figure 1],4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride is used in the symptomatic treatment of muscle spasm, It exists as a white crystalline powder, almost odorless, slightly soluble in water but freely soluble in alcohol, chloroform and solvent ether.

Literature survey revealed three liquid chromatography-ESI-tandem mass spectrometry in human plasma[3-5] and two high-performance liquid chromatographic (HPLC)[6,7] and one spectrophotometric method[8] for its determination either in single or in combined dosage forms. However, yet no stability indicating analytical method was reported for its estimation by reverse phase HPLC. Consequently, the implementation of an analytical methodology to determine Eperisone HCl, in presence of its degradation products is rather a challenge for pharmaceutical analyst. Therefore, it made necessary to study the stability of Eperisone HCl under acidic, alkaline, oxidative, UV and photolytic conditions. This paper reported an validated stability-indicating RP-HPLC method for the determination of Eperisone HCl in presence of their degradation products. The proposed RP-HPLC method is simple, accurate, reproducible, stability-indicating and suitable for routine analysis of Eperisone HCl either in single or in combined dosage form. The method was validated in compliance with ICH guidelines.

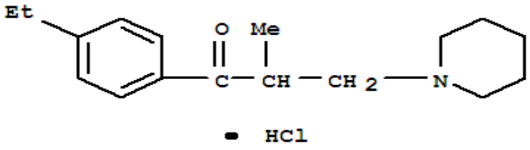

Figure.1. Molecular structure of Eperisone Hydrochloride

MATERIALS AND METHODS:

Eperisone HCl (99.65% pure) of pharmaceutical grade was supplied as gifted sample by Sun Pharmaceuticals, India. Ammonium acetate(AR grade),Methanol and water used were of HPLC grade and were purchased from Spectrochem Pvt. Ltd. Mumbai, India.

The tablet formulation of Eperisone HCl was procured from local market and used for analysis of marketed formulation. The liquid chromatographic system was of Waters (USA), series consisting of Waters 2695 Separation module, Waters 2996 Photo diode array Detector equipped with Waters Empower2 software.

The chromatographic analysis was carried on a Phenomenex Luna C_{18} (250 x 4.6 mm), 5μm column. In addition, an electronic balance (Shimadzu AX200), a pH meter (Systronics model EQMK VI), a sonicator (Spectra Lab, model UCB 40), a hot air oven (Labhosp), UV chamber (Labhosp) were used in this study.

Preparation of Mobile Phase and Stock solutions:

Mobile phase preparation:

Mix buffer and methanol in the proportion of (30:70). This mixture was degassed and sonicated for 10 min and filtered through 0.22µm membrane filter and used as mobile phase. [Buffer: Weigh accurately 0.77 g of ammonium acetate in 1000 ml water].

Standard preparation:

Weigh accurately about 50mg of eperisone HCl into 50ml volumetric flask. Add 30 ml of diluent and swirl gently to dissolve and make-up the volume with diluent [Methanol: Water (70:30)]. Take 5.0ml of this solution to 50 ml volumetric flask, dilute and make up with diluent up to the mark. The linearity of response for eperisone HCl was determined by preparing and injecting solutions with concentrations of about 50.25 to 150.75 µg/ml for assay level concentration of eperisone HCl using eperisone HCl working standard respectively.

Formulation preparation:

10 tablets of brand Skelat (manufactured by Sun Pharmaceuticals Limited) containing 50mg of Eperisone HCl were weighed, average weight determined and finely crushed to powder. Weigh accurately about 50mg of Eperisone HCl into 50 ml volumetric flask. Add 30 ml of diluent and swirl gently to dissolve and make-up to the volume with diluent, use this solution for degradation.Transfer 5.0ml of this solution to 50ml with diluent. Use this solution for assay

The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of buffer and methanol in the proportion of (30:70) pH 3.0 adjusted with o-phosphoric acid on Phenomenex Luna C_{18} (250 x 4.6 mm), 5µm with 1.0ml/min flow rate at 254nm using UV detector.

Calibration Curves of Eperisone HCl and Dosage Formulations:

For standard:

The linearity of response for eperisone HCl standard was determined by preparing and injecting solutions with concentrations of about 50.25 to 150.75 µg/ml for assay level concentration of eperisone HCl using eperisone HCl working standard respectively. The solutions were injected using a 10µl fixed loop system and chromatograms were recorded Calibration curves were constructed by plotting average peak areas versus concentrations and regression equations were computed for eperisone HCl (Table.1).

For Dosage formulations:

10 Tablets containing 50mg of Eperisone HCl of was accurately weighed and transferred into a into 50ml volumetric flask. Add 30 ml of diluent and swirl gently to dissolve and make-up to the

volume with diluents.

The above solution was filtered using Whatman filter paper No 1. Appropriate volume of the aliquots was transferred to a 10ml volumetric flask and the volume was made upto the mark with mobile phase to obtain a solution containing 50.25 to 150.75 µg/ml of Eperisone HCl. A 10µl volume of above sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions.

RESULTS AND DISCUSSION:

Method validation:

The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, detection limit, quantitation limit, accuracy, precision and robustness.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formulae: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve.

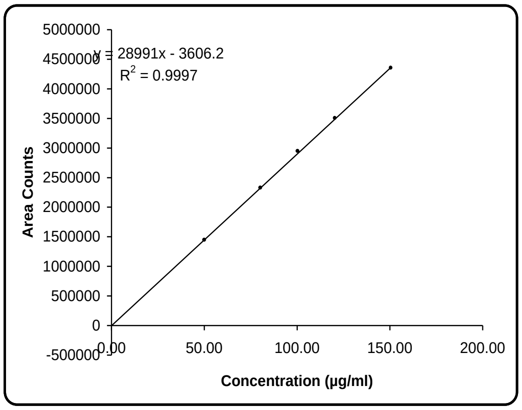

Figure.2.Calibration graph of Eperisone HCl

Precision studies of Eperisone HCl was carried out by injecting six replicate injections of the standard preparation of Eperisone HCl into HPLC system and mean and RSD of area counts were calculated of Eperisone HCl, respectively.

The accuracy of the method was determined by calculating percentage recovery of Eperisone HCl. For Eperisone HCl, recovery studies were carried out by applying the method to drug sample to which known amount of Eperisone HCl corresponding to 50, 100 and 150% of label claim had been added (standard addition method). At each level of the amount six determinations were performed and the results obtained were compared.

System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 50µg/ml for Eperisone HCl, respectively to check the reproducibility of the system and the results are shown in Table.2 & Figure.3 respectively.

Table.2: System suitability parameters of the proposed RP-HPLC method

System suitability parameters	Results
Ret Time	9.050
Area	2836255
% Area	100.0
USP Tailing	1.72
Purity1 Angle	0.089
Purity Threshold	0.240
USP Plate Count	-4072.77

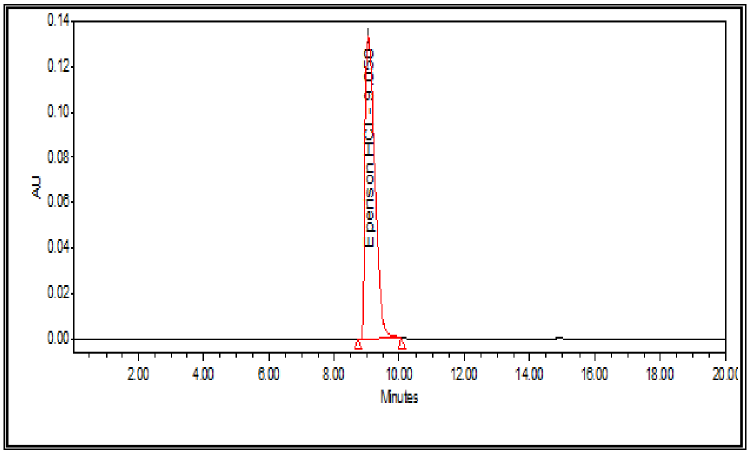

Figure.3.Validative chromatogram of system suitability

For robustness evaluation of HPLC method a few parameters like flow rate, percentage of methanol in the mobile phase and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 50µg/ml for Eperisone HCl respectively.

Forced degradation studies:

Forced degradation studies of Eperisone HCl were carried out under conditions of acid and base hydrolysis, dry heat, oxidation, UV light and photolysis.

Forced degradation in acidic media was performed by transferring the 50 mg of eperisone HCl API to 50 ml volumetric flask. To it, added 10 ml of diluent. Further, added 2 ml of 1N HCl and mixed the content and heated in water bath at 80°C for 30 mins, cooled and neutralised with 2 ml of 1N NaOH. Then cooled and diluted to volume with diluent and mixed. This solution was injected into the HPLC column for analysis.

Forced degradation in basic media was performed by transferring 50 mg of eperisone HCl(pure) to 50 ml volumetric flask. To it, added 10 ml of diluent. Further, added 5 ml of 0.1 N NaOH and mixed the content and flask kept at room temperature for 30 mins, then neutralized with 5 ml of 0.1N HCl. Then cooled and diluted to volume with diluent and mixed.

Degradation with hydrogen peroxide was carried by transferring, accurately weighed, 50 mg of eperisone HCl API to 50 ml volumetric flask. To it, added 10 ml of diluent. Further, added 2 ml of 3% H_2O_2, mixed this content and heated in water bath at 80°C for 30 mins, cooled and diluted to volume with diluent and mixed.

Thermal degradation was done by transferring, the accurately weighed, 50 mg of eperisone HCl API to 50 ml volumetric flask. To it, added 10 ml of diluent. This content was heated in water bath at 80°C for 30 mins, cooled and diluted to volume with diluent and mixed. This solution was injected into the HPLC system.

The mobile phase consisting of methanol: water (70:30, v/v) pH 3.0 adjusted with *o*phosphoric acid, at 1ml/min flow rate was optimized which gave two sharp, well-resolved peaks with minimum tailing factor for Eperisone HCl. The retention times for Eperisone HCl were 9.050min respectively. UV overlain spectra of Eperisone HCl showed that the selected drugs absorbed appreciably at 254nm, so this wavelength was selected as the detection wavelength.The absorbance for the different concentrations (50.25 to 150.75µg/ml) was recorded at 254nm. The regression equation of the calibration curve was found to be y=28991x-3606.6 with a correlation coefficient of 0.9997. The calibration curve is shown in Figure. II and represented in Table.1.

Table.1: Linearity results of the proposed RP-HPLC method

Concentration (µg/ml)	**Mean Area Counts**
50.25	1439833
80.40	2320398
100.50	2942969
120.60	3498133
150.75	4348478
Slope	28990.73
Intercept	-3606.23
Correlation Coefficient (r)	0.9997

The LOD for Eperisone HCl were found to be 3.39µg/ml, while LOQ were 11.31µg/ml µg/ml, respectively.

The %RSD for precision was found to be 0.20 respectively(Table.3). The % recovery(accuracy) was found to be in the range of 98.96% to 99.68 with the %RSD of 0.22(Table.4)

Table.3: Method Precision results of the proposed RP-HPLC method

Preparation	% Assay(on dried basis)
1	99.29
2	98.80
3	98.77
4	98.99
5	99.03
6	99.11
Mean	99.00
SD	0.20
% RSD	0.20

Table.4:Accuracy results (Recovery) of the proposed RP-HPLC method

Simulated Dosage Nominal	Preparation	Amount Recovered %	% Recovery
50	1	49.72	99.44
	2	49.84	99.68
	3	49.70	99.40
100	1	99.23	99.23
	2	99.17	99.17
	3	98.96	98.96
150	1	149.03	99.35
	2	148.80	99.20
	3	148.59	99.06
Mean			99.28
± Standard Deviation			0.22
% Relative Standard Deviation			0.22

The degradation studies indicated that Eperisone HCl was stable to stable to acid, base, H_2O_2, dry heat and UV radiation under experimental conditions without giving any additional degradation peaks (Figure.4-8). The chromatogram of the base degraded sample of Eperisone HCl showed two additional peaks (Figure.5) respectively.

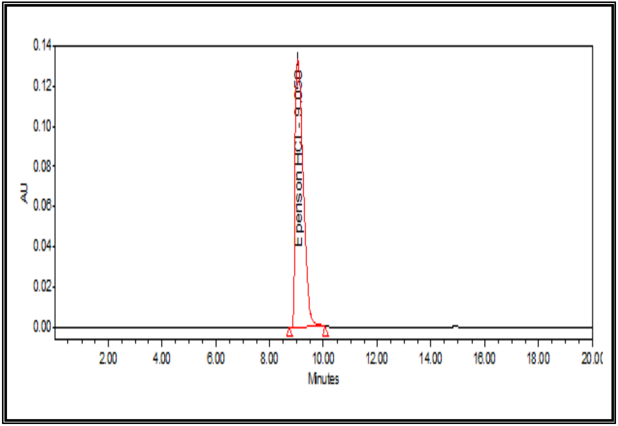

Figure.4.Validation chromatogram for specificity in 0.1M HCl

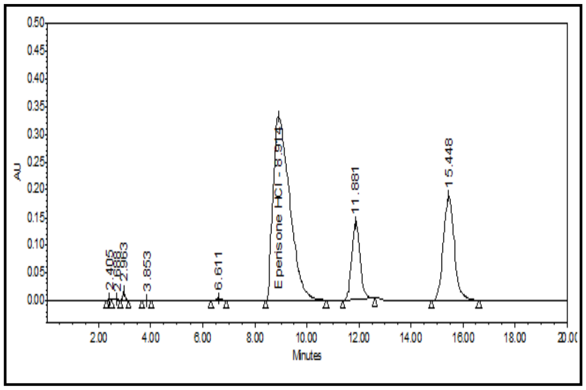

Figure.5.Validation chromatogram for specificity in 0.1M NaOH

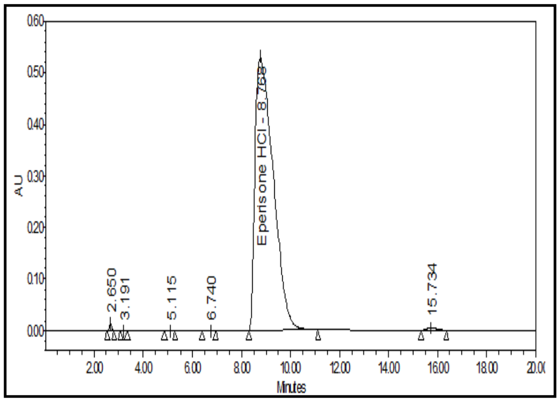

Figure.6.Validation chromatogram for specificity in 3%H_2O_2

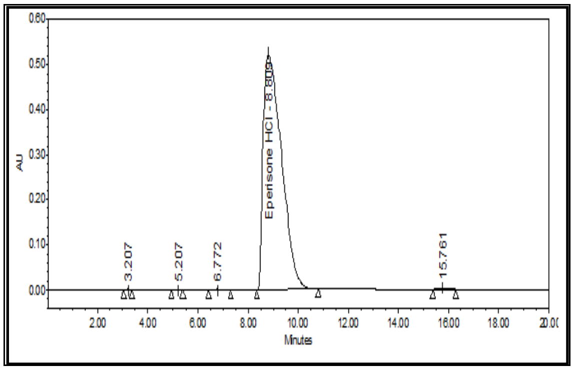

Figure.7.Validation chromatogram for specificity in Thermal degradation (80^{o}C)

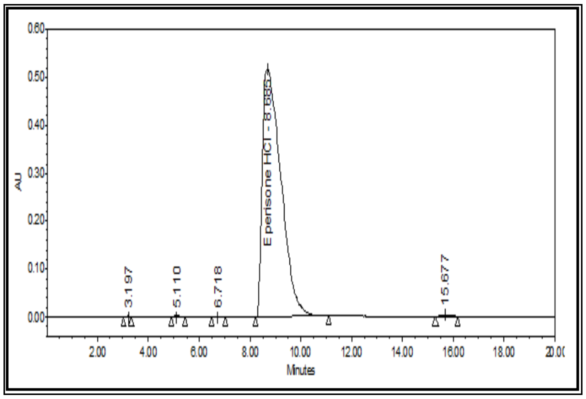

Figure.8.Validation chromatogram for specificity in UV- degradation

Results for robustness evaluation for both the drugs are presented in Table.5. Insignificant differences in peak areas and less variability in retention times were observed.

Table.5:Robustness of the proposed RP-HPLC method

Parameter	Original	Changed conditions
Column	Luna C_{18} (250 x 4.0 mm), 5µm (Phenomenex)	Luna C_{18} (250 x 4.0 mm), 5µm (Phenomenex)
Flow rate	1.0 ml/min	0.8 ml/min
Mobile phase	Buffer: MeOH (300:700)	Buffer: MeOH(290:730)
Pump	Waters 2695	Waters 2695
Detector	Waters 2996 PDA	Waters 2996 PDA
Analyst	I	II
Assay	99.00%	98.81 %
Single degradation	0.28	0.33
Total degradation	0.29	0.32
% deviation from mean assay value obtained in precision studies	-0.19 %	Limit ± 2.0 %

The proposed method was successfully applied to the determination of Eperisone HCl in tablet dosage form. The results obtained with the develop RP-HPLC method were compared with the already reported methods revealing that the developed method was found to be specific, since it was able to separate other excipients present in tablet dosage forms (Table.6).

Table.6: Assay of Eperisone Hydrochloride in Tablet dosage formulation

Pharmaceutical Formulation	Labeled Amount (mg)	Amount found** (mg) ±S.D by the Proposed Method	Found by reference method[8]±S.D	% recovery by proposed methods
SKELAT	50	49.98 ± 0.12	49.99 ± 0.15	99.97

** Average of six determinations

CONCLUSIONS:

In the present paper a stability-indicating RP-HPLC method was developed for the determination of Eperisone HCl and validated as per ICH guidelines. Statistical analysis proved that developed RP-HPLC method was accurate, precise, repeatable, sensitive and selective for analysis of Eperisone HCl without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. Assay results of dosage form using the proposed method showed 99.97 % for Eperisone HCl indicating the suitability of the developed method to study stability of Eperisone HCl under various forced degradation conditions *viz.*acid, base, dry heat, neutral, photolytic and UV degradation and therefore can be concluded that the developed method may be employed for analysis of Eperisone HCl in dosage formulations successfully..

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