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HPLC Method Development And Validation for Estimation of Eperisone Hydrochloride from Bulk And Dosage Form

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ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the estimation of Eperisone Hydrochloride in pure and tablet formulation. The proposed method is based on the estimation of drug and tablet formulation by reversed-phase mode using BDS HYPERSIL C18 (4.6mmø×250mm) analytical column. The optimized mobile phase consisted of Acetonitrile: phosphate buffer (pH 4.5) in the ratio of 70:30 v/v. Flow rate was kept at 0.8 mL min^{-1}. The estimation was carried out at detection wavelength of 255 nm. Drug- Eperisone Hydrochloride was well resolved and retained at 4.5 min. The method was statistically validated as per ICH guideline for analytical method validation. The validated method was used for estimation of Eperisone Hydrochloride from their marketed tablet formulation.

Keywords: Eperisone Hydrochloride, RP-HPLC, Validation.

INTRODUCTION

Eperisone hydrochloride is 4'-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride, a centrally acting muscle relaxant with a low incidence of central depression, is widely used for the therapeutic treatment of spastic patients to relieve muscle stiffness and back pain [1]. Its molecular formula is $C_{17}H_{25}NO.HCL$, molecular weight is 295.83 gm/mole, IUPAC name is 1-propanone, 1-(4-ethylphenyl)-2-methyl-3-(1-piperidinyl)-, hydrochloride, Structure shown in figure.1. Mechanism of action Eperisone Hydrochloride is skeletal muscle relaxant as well as vasodilator because of its actions within the Central Nervous System and on vascular smooth muscles so it is used in different conditions as cervical spondilysis, headache and low-back pain. Eperisone represents a valuable and safer alternative to other muscle relaxant agents for the treatment of low back pain. Eperisone does not seem to have its anti-spastic activity by simply inhibiting cycloxygenase. It is hypothesized that it may act by exerting its blocking activity on post junctional α1 and α2-adrenergic, muscarinic, serotonergic receptors and pre-junctional α2 –adrenoreceptors. So it has properties of both neuromuscular blockers as well as spasmolytics [2]. It is official in Japanese Pharmacopoeia (JP) [3]. Drug sold in Japan, India, Philippines, and Bangladesh [4].

Japanese Pharmacopoeia and described potentiometric method for its estimation. Literature survey reveals ESI-MS method for estimation of Eperisone Hydrochloride in human plasma, HPLC/MS, GC-MS, NMR,

UV and IR analytical technique to identify a degradation product for Eperisone Hydrochloride in the tablet dosage form are available [5]. The objective of the present work is to develop and validate new analytical method for estimation of Eperisone hydrochloride in tablet formulation with good accuracy, simplicity, precision and economy over other chromatographic methods and which can be used for routine analysis.

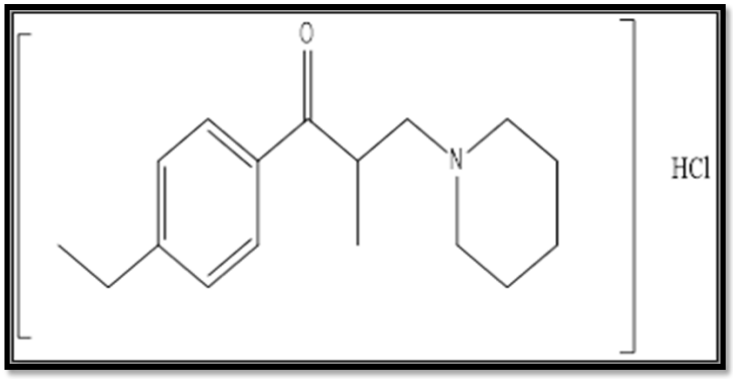

Figure1: Chemical Structure of eperisone hydrochloride

MATERIALS AND METHODS

Materials and Reagents: Pure drug of Eperisone Hydrochloride was kindly gifted by Sharon Bio-Medicine Pvt.Ltd., Vashi, Navi Mumabi. The tablet formulation containing Eperisone hydrochloride (150mg tablet, marketed by Macleods Pharmaceutical Ltd) purchased by local market. HPLC grade Methanol and Acetonitrile were purchased from SD Fine Chemicals, Mumbai.

Instrument: JASCO double beam UV/Visible Spectrophotometer (Model V-630) with spectral bandwidth of 1 nm and 10 mm a matched quartz cell was used for scanning drug spectrogram.
The HPLC system used was Agilent 1200 series equipped with variable wavelength detector. The chromatogram was recorded using EZChrom software. All weighing were done on electronic balance (Model Shimadzu), Ultrasonicator model were used.

Chromatographic conditions: Acetonitrile: Phosphate buffer (pH 4.5) in the ratio 70:30 v/v was used as mobile phase and was filtered before use through 0.45 μm membrane filter. A constant flow of 1.0ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 255nm.

Analytical Method Development
Preparation of Standard Stock and Working Solution: 100 mg Eperisone Hydrochloride was accurately weighed and transferred into 100 mL volumetric flask separately and volume was made upto 100 mL with methanol.
Working solution was prepared from standard solution. 1mL from each of stock solutions were pipetted out and transferred to 10mL volumetric flask and volume was made upto the mark with mobile phase.

Preparation of Sample Solution for Estimation from Marketed Tablet Formulation: Twenty tablets each containing 150mg of Eperisone Hydrochloride were weighed. Their average weight was determined and finely powdered using glass mortar and pestle. The weight of powder equivalent to 150 mg of Eperisone Hydrochloride was transferred into 100mL volumetric flask and dissolved in methanol. The mixture was sonicated to dissolve drugs and then volume was made up to the mark with methanol. The solution was filtered through 0.45 μm filter paper. 1mL was pipetted out from this resulting solution and transferred into 100mL volumetric flask. Volume was made upto the mark with mobile phase to yield concentration of Eperisone Hydrochloride (150 μg mL^{-1}) [6, 7].

Selection of Detection Wavelength: UV absorption spectrum for 10 ppm solution of Eperisone Hydrochloride was generated by scanning over the range of 200-400 nm and the spectrums were recorded to get λmax of analyte in Mobile Phase [8].

Analytical Method Validation: Performance characteristics of analytical HPLC method were statistically validated as per ICH guideline for analytical method [9, 10], by means of the following parameters.

Table 1: Analytical Method Validation: Parameters and their determination

<table>
<tr><th>Parameter</th><th colspan="2">Method / Procedure followed</th></tr>
<tr><td>Specificity</td><td colspan="2">As per ICH, Specificity should be carried out to ensure identity of an analyte. To determine specificity chromatograms were obtained for blank and Eperisone Hydrochloride individually.</td></tr>
<tr><td>Accuracy</td><td colspan="2">Accuracy was established across the specified range of analytical procedure by adding known added quantities of analyte to the synthetic mixture of drug product components and to the dosage form. As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentrations levels in triplicate. (e.g., 3 concentrations/ 3 replicates each)
Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of 10ppm solution of synthetic mixture of Eperisone Hydrochloride and Recovery studies were also performed on tablet containing Eperisone Hydrochloride.</td></tr>
<tr><td rowspan="3">Precision</td><td colspan="2">Precision was carried out at two levels.</td></tr>
<tr><td>Repeatability</td><td>Intermediate Precision</td></tr>
<tr><td>Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/ 3 replicates each)</td><td>Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure. Intraday and interday precision studies were performed by taking 9 determinations of 3 concentrations/3 replicates each, at 3 times in a same day and on 3 different days, respectively.</td></tr>
<tr><td colspan="2">Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.</td></tr>
<tr><td>Detection Limit and Quantification Limit</td><td colspan="2">Detection limit and quantification limit is determined based on the standard deviation of the response and the slope.
<table><tr><th>DL (LOD)</th><th>QL (LOQ)</th></tr><tr><td>$LOD = \frac{3.3\,\sigma}{S}$</td><td>$LOQ = \frac{10\,\sigma}{S}$</td></tr></table>σ = Standard deviation of response estimated based on the calibration curve.
S = Slope of the calibration curve.</td></tr>
<tr><td>Linearity</td><td colspan="2">A linear relationship was evaluated across the range of 2 to 30 mg for drug namely Eperisone Hydrochloride.
As per ICH, for the establishment of linearity, a minimum of 5 concentrations are recommended.
Linearity is reported by the value of the correlation coefficient, y-intercept, and slope of the regression line along with a plot of the data.
Robustness
Robustness was evaluated for proving the reliability of an analytical method</td></tr>
</table>

Robustness	Robustness was evaluated for proving the reliability of an analytical method with respect to deliberate variations in method parameters. To establish robustness of analytical method following factors were studied 1. Influence of variations of pH in a mobile phase 2. Influence of variations in mobile phase composition 3. Temperature 4. Flow rate

RESULTS AND DISCUSSION

Selection of Wavelength: UV absorption spectra for 10 ppm solution of Eperisone Hydrochloride individually given maximum absorption (Figure 2) at 255 nm was selected as a detection wavelength for chromatographic determination of Eperisone Hydrochloride.

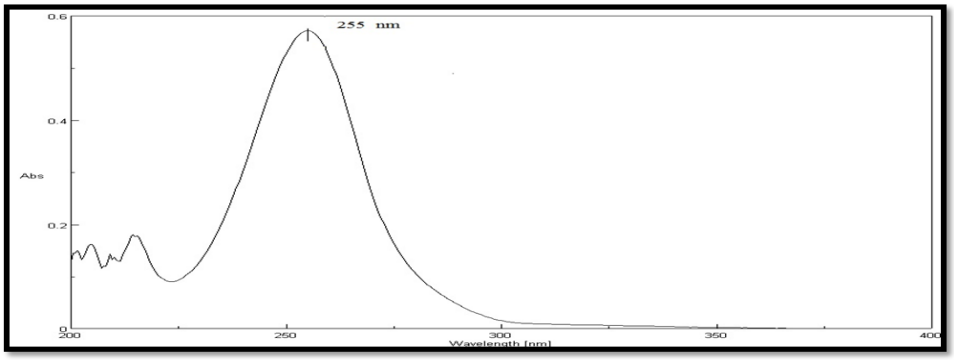

Figure 2: UV absorption spectra of eperisone hydrochloride

Optimization of Chromatographic Conditions: According to the literature survey, it was observed that the drug Eperisone Hydrochloride was well retained on C18 column respectively. Thus, in order to get optimum resolution simultaneously C18 column was selected. Many preliminary trials were carried out for selection of mobile phase; some are tabulated in table 2.

Table 2: Optimization trials for mobile phase composition

Mobile phase components	Compositions
Water :Methanol	(90:10)
ACN: Phosphate Buffer (pH 6.5)	(60:40), (70:30)
ACN : Phosphate Buffer (pH 4.5)	(60:40), (70:30)

Different flow rate in the range of 0.6 to 1.0 mL min^{-1} and different injection volumes in the range of 20 μL to 100 μL were tried. Optimized mobile phase selected was composed of Acetonitrile: 30 mM Phosphate Buffer (pH 4.5) (70:30). Optimized chromatographic conditions are tabulated in table 3.

Table 3: Optimized chromatographic conditions

Mobile Phase	Acetonitrile: Phosphate Buffer (30mM)
Ratio	70:30
pH of mobile phase	4.5
Stationary Phase	HiQSil C-18 (4.6 mm ϕ X 250 mm)

Flow Rate	0.8 ml/min
Detection Wavelength	255nm
Injection Volume	20 μl

Chromatogram obtained using these optimized chromatographic conditions showed that drug namely Eperisone Hydrochloride was well resolved and retained at 3.5 min. Chromatogram of Eperisone Hydrochloride is shown in Figure 3.

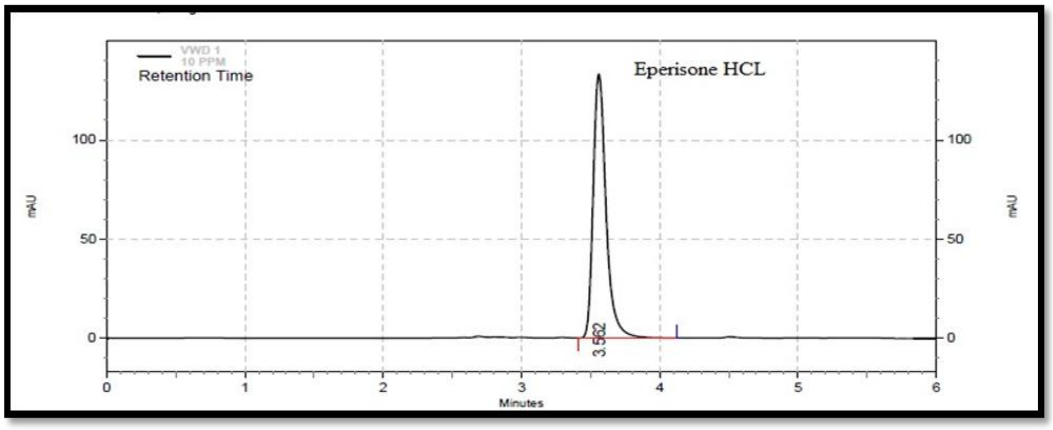

Figure 3: Chromatogram of Eperisone Hydrochloride

Specificity: Separate chromatograms were obtained for blank, Eperisone hydrochloride individually to ensure the identity of analyte under study namely, the chromatograms of blank, Eperisone hydrochloride individually shown in figure 4.

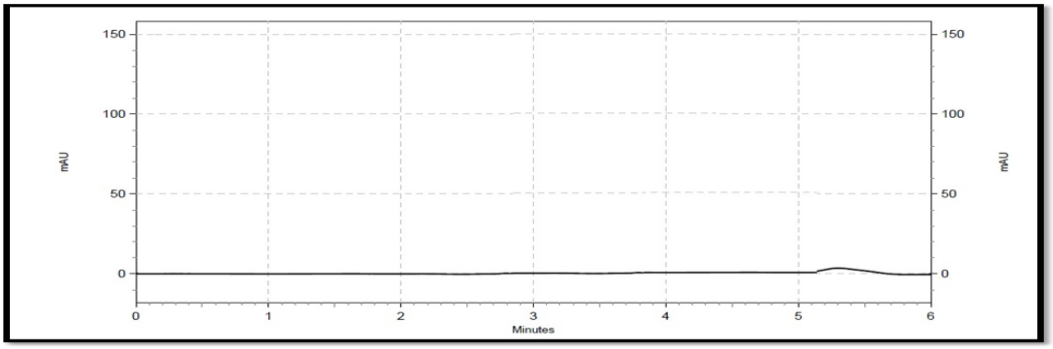

Figure 4: Blank chromatogram

Linearity: Seven serial dilutions of Eperisone hydrochloride were prepared using a standard stock solution and dilutionwere made with mobile phase. [Acetonitrile : Phosphate Buffer (pH 4.5) (70:30)]. Responses were recorded as peak area. The peak areas were plotted against concentrations to obtain the calibration curve. Eperisone hydrochloride was found linear in the range of 2-30 ppm. The linearity plot of eperisone hydrochloride is given in figure 5. The values of correlation coefficient, y intercept and slope of regression line are shown in table 4.

Table 4: Linear regression data for calibration curve

Drug	R^2	y-intercept	Slope
Eperisone hydrochloride	0.9981	10583	83676

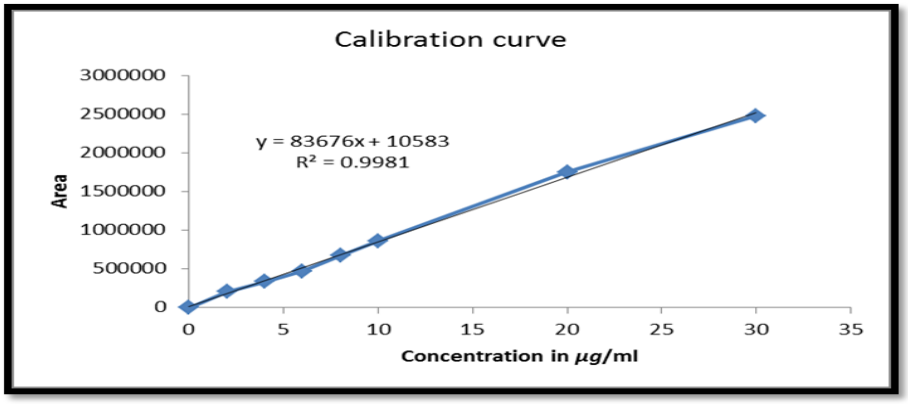

Figure 5: Calibration plot for Eperisone Hydrochloride

Limit of Detection and limit of Quantization: Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slope of regression line. The calculated values of limit of detection and limit of quantitation for Eperisone hydrochloride are shown in table 5.

Table 5: LOD and LOQ of Eperisone hydrochloride

Parameters	Result
LOD	0.235297 μg mL^{-1}
LOQ	0.713022 μg mL^{-1}

Accuracy: Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of 10 ppm solution of synthetic mixture of Eperisone hydrochloride. Results are tabulated in table 6.

Table 6: Accuracy: Recovery studies on Eperisone hydrochloride

Drug	Level of percentage recovery (%)	Amount present in extract (μg/mL)	Amount added (μg/mL)	Total amount (μg/mL	% recovery	% RSD	Inference
Eperisone HCL	80	15	12	27	99.29	0.40	Acceptable recovery hence accurate
	100	15	15	30	101.94	0.19	
	120	15	18	33	99.22	0.39	

Precision: The results of intraday and interday precision studies are tabulated in table 7 and 8 respectively. Percent RSD values for both intraday and interday precision were found within acceptable limit.

Table 7: Intraday precision studies

		Eperisone hydrochloride			Inference
Concentration levels		**Low**	**Mid**	**High**	Acceptable % RSD, hence precise
Concentration (µg/mL)		**4**	**8**	**20**	
Peak area	**1**	335583	648322.3	1780911.3	
	2	345451	659210	1778883.3	
	3	336045.6	654702	1779761.3	
Average peak area		339026.5	654078.1	1779852	
Standard Deviation		5568.5	5470.5	1017.0	
% RSD		1.64	0.83	0.05	

Table 8: Interday precision studies

		Eperisone hydrochloride			Inference
Concentration levels		**Low**	**Mid**	**High**	Acceptable % RSD, hence precise
Concentration (µg/mL)		**4**	**8**	**20**	
Peak area	**Day 1**	358888.3	660324.6	1788578	
	Day 2	354281.3	657698	1731436.3	
	Day 3	347575.7	656492.6	1759601.6	
Average peak area		353581.7	658171.7	1759872	
Standard Deviation		5688.6	1959.4	28571.7	
% RSD		1.60	0.29	1.62	

Robustness: To determine robustness of analytical HPLC method changes observed in retention time and response were recorded. Method was found to be reliable and robust as method performance (retention time and response) is not much affected by deliberate variations in mobile phase composition and its pH, column temperature and flow rate. The results obtained are tabulated in table 9.

Table 9: Robustness: Effect on retention time and response by variation in mobile phase composition and its pH, column temperature and flow rate

Method Parameters and variations	**Level of variations**	**%RSD**	**Retention time (Min.)**
Proportion of organic phase in mobile phase 70:30 (±2)	+2	0.38	0.032
	-2	0.44	0.02
Flow Rate (0.8± 0.2)	+2	0.55	0.69
	-2	0.50	0.54

CONCLUSIONS

The developed RP-HPLC method has been statistically validated by ICH guidelines and it is found to be specific, accurate, precise and robust. Validation studies indicated that the proposed method is suitable for the estimation of Eperisone hydrochloride in bulk and in pharmaceutical formulation. The method can be conveniently adopted for routine analysis of the formulations containing Eperisone hydrochloride.

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