Development And Analytical Validation Of Eperisone Hydrochloride By Using RP-HPLC Method In Pharmaceutical Dosage Form

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ABSTRACT:
A simple, specific and accurate reverse phase HPLC method was developed for the simultaneous determination of Eperisone hydrochloride in table dosage forms. Hypersil BDS $C_{18,}$ 250mm X 4.6 mm, 5µ Column with mobile phase 0.01M Ammonium Acetate Salt: Acetonitrile (80:20) was used. The flow rate was 1 ml/min and effluent was monitored at 257 nm. The retention times of Eperisone hydrochloride was 5.5 min, respectively. The method was validated for specificity, linearity, accuracy, and precision, robustness limit of detection and limit of quantitation. Linearity, accuracy, and precision were acceptable in the ranges. The linearity range for Eperisone hydrochloride was in the range of 50-150%, respectively. The proposed method was also validated and successfully applied to the estimation of Eperisone hydrochloride tablet formulations. High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.
Keywords: Eperisone hydrochloride, validation, RP-HPLC

I.INTRODUCTION

Eperisone hydrochloride: 1-(4-Ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one hydrochloride

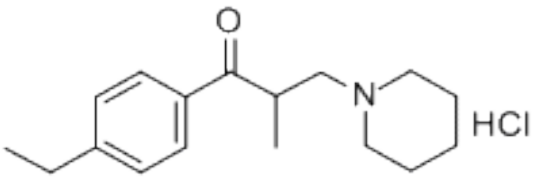

Figure:1 Structure of Eperisone hydrochloride

Molecular Formula : $C_{17}H_{26}ClNO$
Molecular Weight : 295.85 g/mol
Category : Antispasmodic drug.
Solubility : Freely soluble in chloroform, glacial acetic acid, ethanol and water.

Mechanism of Action:

Eperisone 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. The drug inhibits the vicious cycle of myotonia by decreasing pain, ischaemia, and hypertonia in skeletal muscles, thus alleviating stiffness and spasticity, and facilitating muscle movement. Eperisone also improves dizziness and tinnitus associated with cerebrovascular disorders or cervical spondylosis.

Chromatographic Method:

A simple and sensitive reverse phase HPLC method has been developed for the analysis of Eperisone hydrochloride Tablets. The method utilizes sample preparation followed by separation on a Column Hypersil BDS $C_{18,}$ 250mm X 4.6 mm, 5µ or

equivalent. Analytes were monitored by UV detection at 257nm using an isocratic mode with Mixture of 0.01M Ammonium Acetate Salt: Acetonitrile (80:20) as mobile phase. The flow rate was set at 1ml/min and effluent was monitored at 257nm. The retention time was 5.5min. Calibration curves for Eperisone hydrochloride was found respectively.

Equipment and Apparatus used:

- Electronic balance
- Schimadzu HPLC Separation Module
- UV Detector
- Chromatographic data Software : LC solutions
- Hypersil BDS $C_{18,}$ 250mm X 4.6 mm, 5µ.
- Vacuum filter pump
- Mobile phase reservoir
- Ultra Sonicator , Membrane filter(0.45 and 0.2microns)

Reagents:

- Acetonitrile HPLC grade
- Water (HPLC)
- Ammonium acetate salt
-

II.METHOD DEVELOPMENT

Preparation of the Eperisone hydrochloride Standard & Sample Solution:

Standard Solution Preparation: Weighed accurately and transferred about 50mg of Eperisone hydrochloride working standard into a 100ml volumetric flask and dissolved in diluent and was made up to the volume with diluent. Further diluted 5ml of the above solution to 50ml with diluent and mixed. Filtered the solution through 0.45µm nylon filter or 0.45µm PVDF membrane filter.

Sample Solution Preparation: 50mg of Eperisone hydrochloride sample weighed accurately a quantity equivalent to 50mg of Eperisone hydrochloride working standard and transferred into a 100ml volumetric flask and diluted to the volume with diluent.

Further diluted 5ml of the above solution to 50ml with diluent and mixed. The above solution was filtered through 0.45µm nylon filter/0.45µm PVDF membrane filter.

Preparation of Buffer solution:

0.7708 gm of Ammonium Acetate Salt was dissolved in 1000ml of milli Q water. Filtered through 0.45µm or a finer porosity membrane filter and degassed.

Preparation of mobile phase:

Mixed Buffer and Acetonitrile in the ratio of 800:200v/v respectively and degassed

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector

Column : Hypersil BDS $C_{18,}$ 250mm X 4.6 mm, 5µ

Flow rate : 1ml per min

Wavelength : 257nm

Injection volume : 10 µl

Column oven : Ambient

Run time : 15min

Assay:

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and percentage purity was found out by following formulae.

Procedure

Preparation of Buffer solution:

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Standard preparation: Weighed accurately and transferred about 50mg of Eperisone hydrochloride working standard into a 100ml volumetric flask and dissolved in diluent and was made up to the volume with diluent. Further diluted 5ml of the above solution to 50ml with diluent and mixed. Filtered the solution through 0.45µm nylon filter or 0.45µm PVDF

membrane filter.

Test preparation: 50mg of Eperisone hydrochloride sample weighed accurately a quantity equivalent to 50mg of Eperisone hydrochloride working standard and transferred into a 100ml volumetric flask and diluted to the volume with diluent. Further diluted 5ml of the above solution to 50ml with diluent and mixed. The above solution was filtered through 0.45µm nylon filter/0.45µm PVDF membrane filter.

III.RESULTS AND DISCUSSION

Method development

Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples. Correspondingly, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminary optimized to develop a HPLC method for validation report for assay of Eperisone hydrochloride with short analyses time and acceptable resolution (Rs> 2).

In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested. Acetonitrile produced a high retention time for Eperisone hydrochloride highcolumn pressures due to the high viscosity. Acetonitrile was found to display advantageous separations. Change of percentage of acetonitrile in the mobile phase brought about a great influence on retention time.

The system suitability parameters prove that the proposed method is equally suitable for validation of, the Eperisone hydrochloride chromatogram were found to be satisfactory Hypersil BDS $C_{18,}$ 250mm X 4.6 mm, 5µ or equivalent, using mobilephase composition of 0.01M Ammonium Acetate Salt: Acetonitrile (80:20)with flow rate of 1ml/min.

The above method is suitable routine pharmaceutical applications involving the validation of Eperisone hydrochloride.

Method Validation:

Validation of analytical method for determination of assay of Eperisone hydrochloride 10 mg tablets was performed for the parameters including – Specificity, Linearity and Range, Precision (System precision, Method precision), Intermediate precision (Ruggedness), Accuracy and Robustness values are in given below tables.

Table: 1 System Suitability Parameters

Parameters	EP.Hcl
Area under curve	713657
	692478
	721594
	690465
	708509
Mean	705341
%RSD	1.92
Retention time (min)	6.750
Theoretical plates	6268
Tailing factor	1.29

Accuracy:

Table: 2 Accuracy results of EP. Hcl sample

Conc	Sample ID	Amount added(ppm)	Amount found(ppm)	% Recovery	Statistical analysis	
50%	Sample-1	50.50	50.89	101.8	Mean	101.6
	Sample-2	50.62	50.93	101.4		
	Sample-3	50.68	51.06	101.7		
100%	Sample-1	100.57	100.81	100.5	Mean	100.8
	Sample-2	100.51	100.88	100.8		
	Sample-3	100.46	100.96	101.1		
150%	Sample-1	150.83	150.24	99.1	Mean	99.5
	Sample-2	150.75	150.29	99.3		
	Sample-3	150.79	150.83	100.1		

Table: 3 Linearity results of EP.Hcl

Level	Concentration	Average Area	Statistical Analysis	
1	50%	396488	Slope	16564.8775
2	75%	564298	Y-intercept	16474.767
3	100%	753961	% of Y-intercept	1.76
4	125%	937571	Correlation coefficient	0.9996
5	150%	1136449	Residual sum of squares	9374.2632

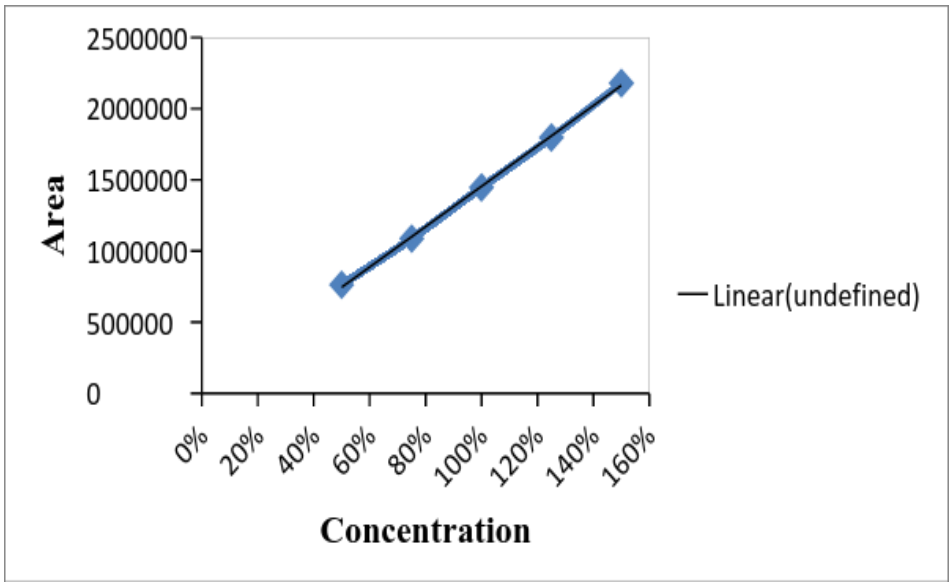

Fig.2.Linearity curve for Eperisone hydrochloride

Precision:

Sample	EP.Hcl	
	RT	**Area**
1	5.549	794394
2	5.548	800023
3	5.549	798873
4	5.547	798665
5	5.548	798644
Mean	5.548	798120
%RSD	**0.06**	**0.27**

Table: 4 Observations of Trails in Method Development

S.No	Mobile phase	Observation
1	0.1 M Ammonium Acetate Buffer: ACN (60:40)	Eperisone hydrochloride getting eluted with the split peaks
2	0.2M Ammonium Acetate (p^H3.5): Acetonitrile (80:20)	Splitting of peak at the tip was observed
3	0.1% Triflouroacetic acid (pH 3.0): Acetonitrile (80:20)	Multiple Peaks splitting of was observed
4	0.02M Ammonium Acetate (pH4.0): Acetonitrile (80:20)	There is scope of tailing at both ends
5	0.01M% Ammonium Acetate (pH 2.8): Acetonitrile (75:25)	There is scope of tailing in Eperisone.Hcl. Peak
6	0.01M Ammonium Acetate Salt: Acetonitrile (80:20)	Eperisone.Hcl peak was eluted with good resolution and with less run time

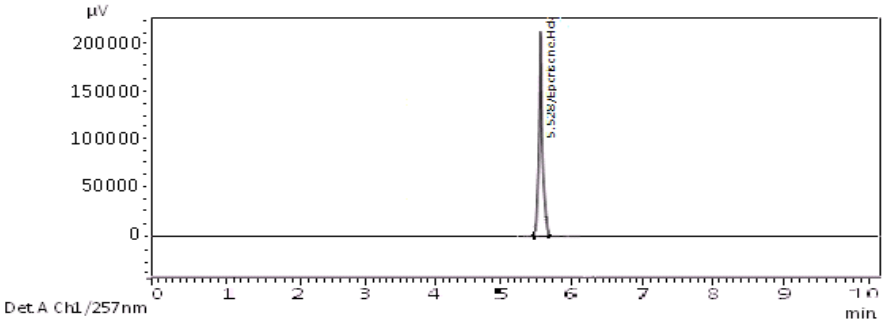

Figure:3 Chromatography of eperisone hcl

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