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Research

Development and Validation of Novel Stability Indicating RP-HPLC Method for the Estimation of Eperisone hydrochloride in Bulk Drug and Pharmaceutical Dosage Form

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Abstract

A simple, novel, precise and accurate stability indicating RP-HPLC method was developed and validated for the estimation of Eperisone hydrochloride in bulk drug and pharmaceutical dosage form. A Fortis H2O (150 mm × 4.6 mm I.D., 5 µm particle size) column was used as stationary phase with mobile phase consisting of 10 mM ammonium formate: acetonitrile in the ratio of 65:35 v/v (pH was adjusted to 3.8 with 0.1% formic acid). The flow rate was maintained at 1.0 mL/min and effluents were monitored at 259 nm. The retention time was 8.150 min. The linearity of the method was observed in the concentration range of 1-100 µg/mL with correlation coefficient of 0.999. The method developed was validated for linearity, precision, accuracy, system suitability and forced degradation studies like acidic, alkaline, oxidative and thermal stress conditions were performed as per ICH guidelines. The results obtained in the study were within the acceptable limits and hence this method can be used for the estimation of Eperisone hydrochloride in pharmaceutical dosage form.

Key words: Eperisone; HPLC; Validation; Dosage Form.

Introduction

Eperisone hydrochloride (Figure 1) 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 hydrochloride. 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].

Literature survey revealed that few HPLC methods [5-8] were reported for the estimation of Eperisone hydrochloride in pharmaceutical formulations. Hence a new, sensitive and efficient HPLC method was developed and validated as per ICH guidelines [9, 10] for the estimation of Eperisone hydrochloride in bulk and pharmaceutical dosage form.

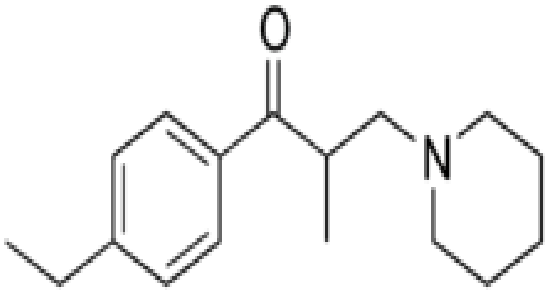

Figure 1: Chemical Structure of Eperisone

Materials and Methods

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Eperisone hydrochloride using isocratic Waters HPLC instrument on a Fortis H20 (150 mm × 4.6 mm I.D , 5 µm particle size) analytical column was used. The instrument is equipped with a pump-515, auto sampler-2707 and PDA detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower2 software.

Chemicals and solvents

The reference sample of Eperisone hydrochloride was obtained from Eisai Co. Ltd, Vishakhapatnam, India. Commercially available Eperisone hydrochloride tablets claimed to contain 50 mg of Eperisone hydrochloride was purchased from local market. Ammonium formate, acetonitrile and formic acid were purchased from S.D. Fine Chemicals, Mumbai, India.

Chromatographic conditions

A mixture of 10 mM ammonium formate: acetonitrile (65:35, v/v, pH 3.8 was adjusted with 0.1% formic acid) was found to be the most suitable mobile phase for ideal chromatographic separation of Eperisone hydrochloride. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 259 nm. The run time was set at 10 min.

Preparation of mobile phase and diluents

650 mL of the 10 mM ammonium formate buffer was mixed with 350 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution

Accurately weighed and transferd 10 mg of Eperisone hydrochloride working standard into a 100 mL clean dry volumetric flask, about 10 mL of diluent was added and volume was made volume upto the mark with the diluent. Further 1 mL of the solution was pipetted from above stock solution into 100 mL volumetric flask and diluted upto the mark with diluent.

Preparation of sample solution

Twenty tablets were weighed and finely powdered. An accurately weighed the portion of powder equivalent to 50 mg of Eperisone hydrochloride was transferred into a 100 mL volumetric flask, to this 50 mL of diluent was added. The contents of flask were sonicated for 20 min, then solution was filtered through a 0.45 µm membrane filter and then final volume of the solution was made upto 100 mL with diluent. Further 2 mL of the solution was pipetted from the above stock solution into 100 mL volumetric flask to get the concentration containing 10 µg/mL of Eperisone hydrochloride.

Linearity

Several aliquots of standard solution of Eperisone hydrochloride was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Eperisone hydrochloride were in the range of 1 to 100 µg/mL. Evaluation of the drug was performed with PDA detector at 259 nm, peak area was recorded for all the peaks. The correlation coefficient value was 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Precision

The precision was determined for Eperisone hydrochloride in terms of system precision, method precision and intermediate precision. To study the system precision, six replicate standard solutions of Eperisone hydrochloride was injected. The %RSD was 0.18%. The method precision study was carried out on six preparations from the same tablet samples of Eperisone hydrochloride. The %RSD was 0.38%. The intermediate precision study was carried out by different analysts, from the same tablet formulation of Eperisone hydrochloride and the percent amount of Eperisone hydrochloride was calculated. The %RSD was 0.34% (limit %RSD < 2.0%).

Accuracy

The accuracy of the method was assessed by recovery study of Eperisone hydrochloride in the dosage form at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The %mean recovery was 98.93% that shows there is no interference from excipients and the lower values of %RSD indicate the method is accurate.

System suitability

System suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Eperisone hydrochloride was found to be 0.08 µg/mL and the LOQ for Eperisone hydrochloride was found to be 0.30 µg/mL.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions. Stability studies

Control sample

Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 50 mg of Eperisone hydrochloride was transferred into a 100 mL volumetric flask containing 50 mL of the diluent. The contents of the flask were sonicated for about 30 min for complete solubility of the drug with intermittent shaking at controlled temperature and then cooled the solution to room temperature and volume was made up with further quantity of diluent. Then this mixture was filtered through 0.45 µm membrane filter. 2 mL of the above filtered sample solution was pipetted into a 100 mL volumetric flask and diluted to volume with diluent. 10 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation sample

To the stock solution, 5 mL of 0.1N hydrochloric acid was added, refluxed for 8 hours at 60°C, then cooled to room temperature, neutralized with 0.1N sodium hydroxide and diluted to volume with diluent. 2 mL of the above solution was pipetted into a 100 mL volumetric flask and diluted to volume with diluent.

Alkali degradation sample

To the stock solution, 5 mL of 0.1N sodium hydroxide was added, refluxed for 8 hours at 60°C, then cooled to room temperature, neutralized with 0.1N hydrochloric acid and diluted to volume with diluent. 2.0 mL of the above solution

was pipetted into a 100 mL volumetric flask and diluted to volume with diluent.

Oxidative degradation sample

To the stock solution, 2 mL of 3% hydrogen peroxide was added, refluxed for 8 hours at 60°C, then cooled to room temperature and diluted to volume with diluent. 2.0 mL of the above solution was pipetted into a 100 mL volumetric flask and diluted to volume with diluent.

Thermal degradation sample

The drug powder was exposed to heat at 105°C for about 8 hours and then the stock solution was prepared. 2.0 mL of the above solution was pipetted into a 100 mL volumetric flask and diluted to volume with diluent.

Results and Discussion

In the present work, a simple, novel, precise and accurate stability indicating HPLC method has been optimized, developed and validated for the determination of Eperisone hydrochloride in pharmaceutical formulations with PDA detector by using Fortis H2O (150 mm × 4.6 mm I.D., 5 µm particle size) in isocratic mode with mobile phase composition of 10 mM ammonium formate: acetonitrile in the ratio of 65:35 v/v (pH was adjusted to 3.8 with 0.1% formic acid) resulted the chromatographic peak obtained was in good shape, better resolved and almost free from tailing. The flow rate was 1.0 mL/min and the drug component was measured with PDA detector at 259 nm. The results of optimized HPLC conditions were shown in Table 1.

The method was linear in the range of 1 to 100 µg/mL for Eperisone hydrochloride with correlation coefficient of 0.999. The linearity results were shown in Table 2 and the linearity curve was shown in Figure 2.

Parameter	Condition
Mobile phase	10 mM ammonium formate:acetonitrile (65:35, v/v)
pH	3.8
Diluent	Mobile phase
Column	Fortis H_2O (150 mm × 4.6 mm, 5 µm)
Column temperature	Ambient
Wave length	259 nm
Injection volume	20 µL
Flow rate	1.0 mL/min
Run time	10 min
Retention time	8.150 min

Table 1: Optimized chromatographic conditions of Eperisone hydrochloride

Concentration (µg/mL)	Peak area
1	43503
2	82751
5	204118
10	479801
20	1040398
50	2689376
100	5430271

Table 2: Linearity results of Eperisone hydrochloride

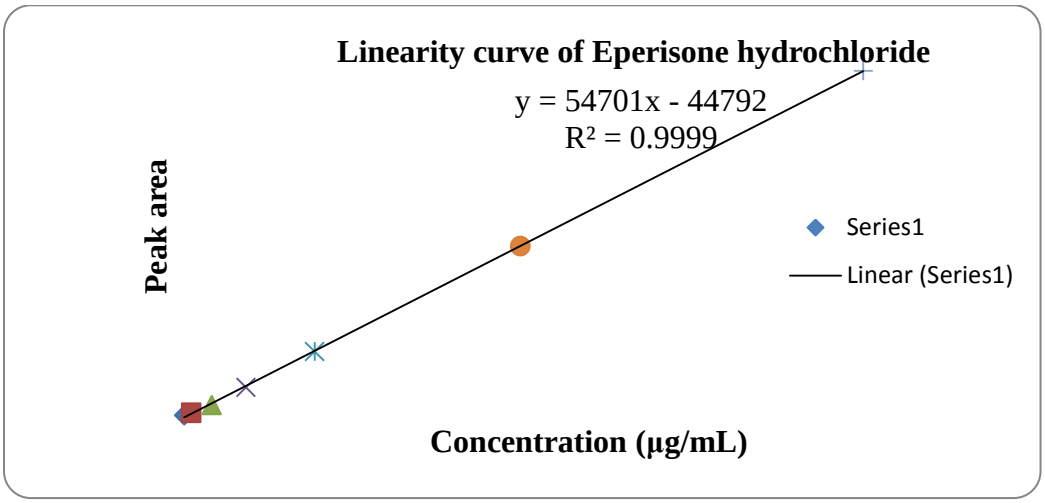

Figure 2: Linearity Curve of Eperisone Hydrochloride

The %RSD for system precision, method precision and intermediate precision for Eperisone hydrochloride was found to be 0.18, 0.38 and 0.34, which indicates the method is precise. The results of precision studies were shown in Table 3. The %recoveries of Eperisone Hydrochloride were found in the range of 98.50-99.60% and the %mean recovery was found to be 98.93%, which indicates the method is accurate. The results of recovery studies were shown in Table 4.

Precision	%RSD
System precision	0.18
Method precision	0.38

Intermediate precision	0.34

Table 3: Precision results of Eperisone hydrochloride

Level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	%RSD
50%	5	4.93	98.60%	0.98
100%	10	9.85	98.50%	1.7
150%	15	14.94	99.60%	0.4

Table 4: Recovery results of Eperisone hydrochloride

The retention time of Eperisone hydrochloride was 8.150 min, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 9354 and tailing factor was 1.1 for Eperisone hydrochloride, which indicates efficient performance of the column. Typical chromatogram of drug Eperisone hydrochloride was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug Eperisone hydrochloride by the proposed HPLC method.

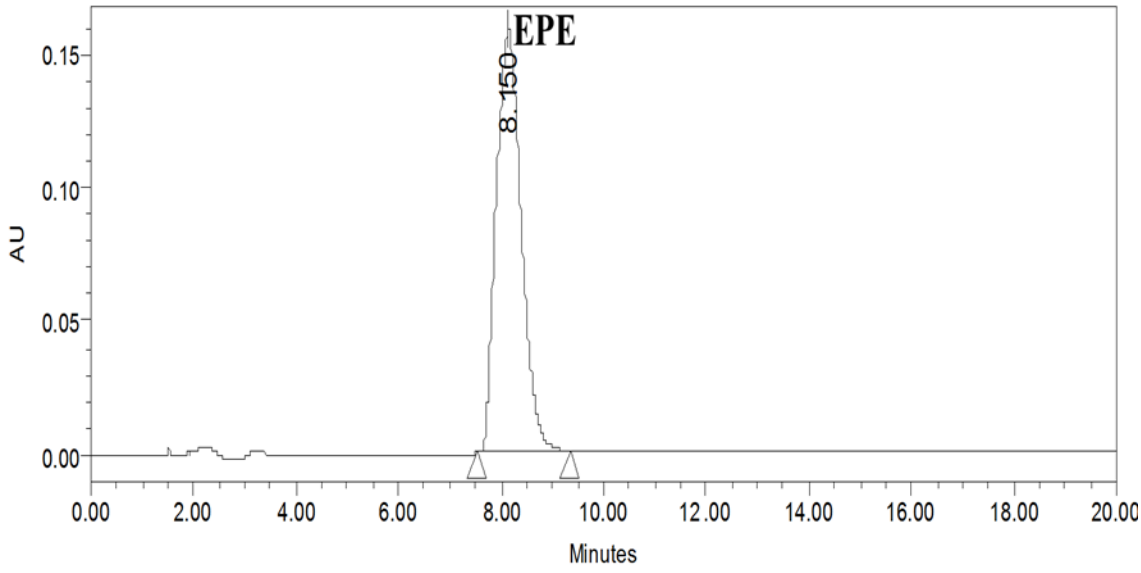

Figure 3: Typical chromatogram of Eperisone hydrochloride

Selectivity of the method was demonstrated by the absence of any interfering peaks at the retention time of the drug. The limit of detection and limit of quantitation for Eperisone hydrochloride were found to be 0.08 µg/mL and 0.30 µg/mL, which indicate the sensitivity of the method. A system suitability test was performed to evaluate the chromatographic parameters and the summary of system suitability parameters were shown in Table 5.

Parameter	Results

Linearity range (µg/mL)	1-100
Correlation coefficient	0.999
Theoretical plates (N)	9354
Tailing factor	1.1
LOD (µg/mL)	0.08
LOQ (µg/mL)	0.30

Table 5: System suitability parameters results of Eperisone hydrochloride

Robustness was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study were showed in Table 6.

Condition	**%Assay**	**%Difference**
Unaltered	100	-
Flow rate at 0.8 mL/min	99.1	0.9
Flow rate at 1.2 mL/min	98.96	1.04
Mobile phase:		
Ammonium formate(67):acetonitrile (33) v/v	99.36	0.64
Ammonium formate (63):acetonitrile (37) v/v	99.14	0.86
pH of buffer at 3.6	98.2	1.8
pH of buffer at 4.0	98.14	1.8

Table 6: Robustness study of Eperisone hydrochloride

Validated method was applied for the determination of Eperisone hydrochloride in commercial formulations.

Stability studies of Eperisone hydrochloride under different stress conditions indicated the following degradation behavior. In acidic degradation, the degradation product was appeared at retention time of 8.157 min and the %degradation is 1.4%. In alkali degradation, the degradation product was appeared at retention time of 8.404 min and the %degradation is 4.4%. In oxidative degradation, the degradation product was appeared at retention time of 8.410 min and the %degradation is 2.68%. In thermal degradation, the degradation product was appeared at retention time of 8.157 min and the %degradation is 1.68%. The results of stability studies were shown in Table 7. The typical chromatograms of degradation behavior of Eperisone hydrochloride in different stress conditions are shown in Figure 4 to Figure7.

Stress conditions	**%Assay**	**%Degradation**
Control	100	-
Acid	98.6	1.4
Alkali	95.6	4.4

Oxidative	97.32	2.68
Thermal	98.32	1.68

Table 7: Forced degradation study results of Eperisone hydrochloride

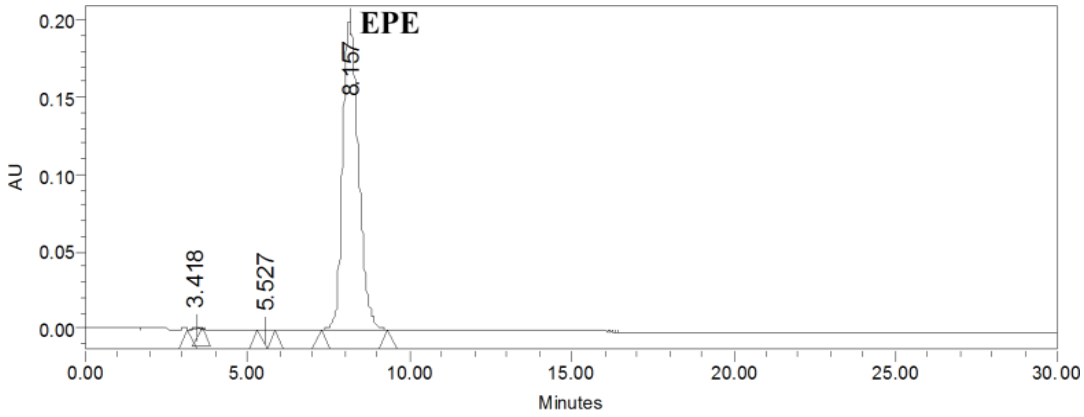

Figure 4: Chromatogram of Eperisone hydrochloride in acid degradation

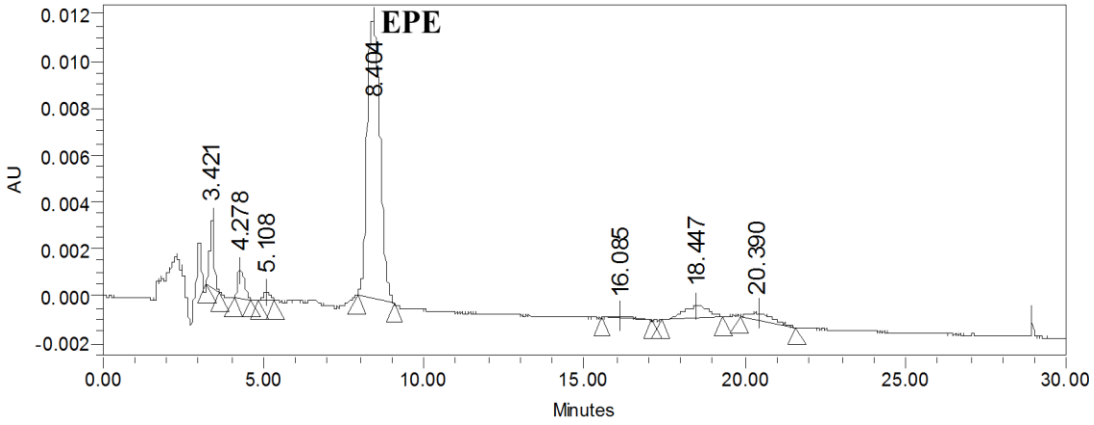

Figure 5: Chromatogram of Eperisone hydrochloride in alkali degradation

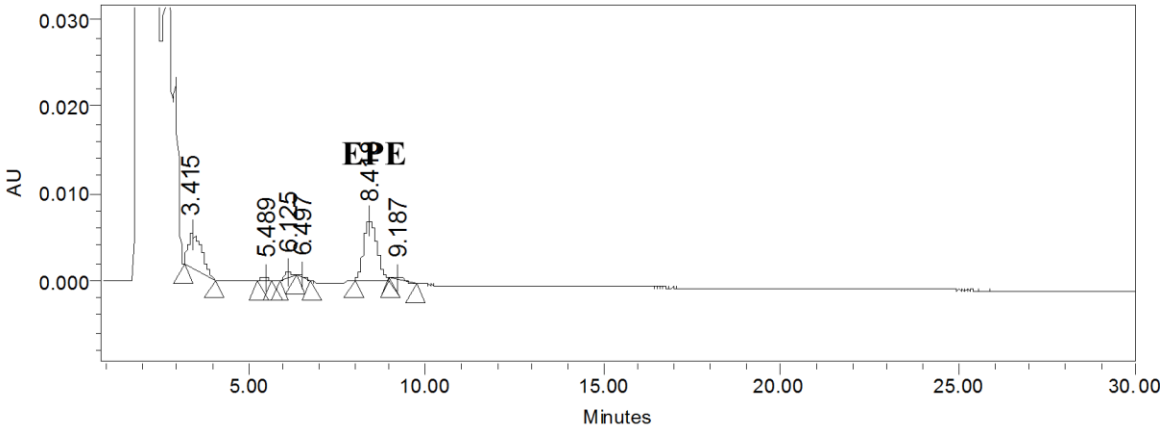

Figure 6: Chromatogram of Eperisone hydrochloride in oxidative degradation

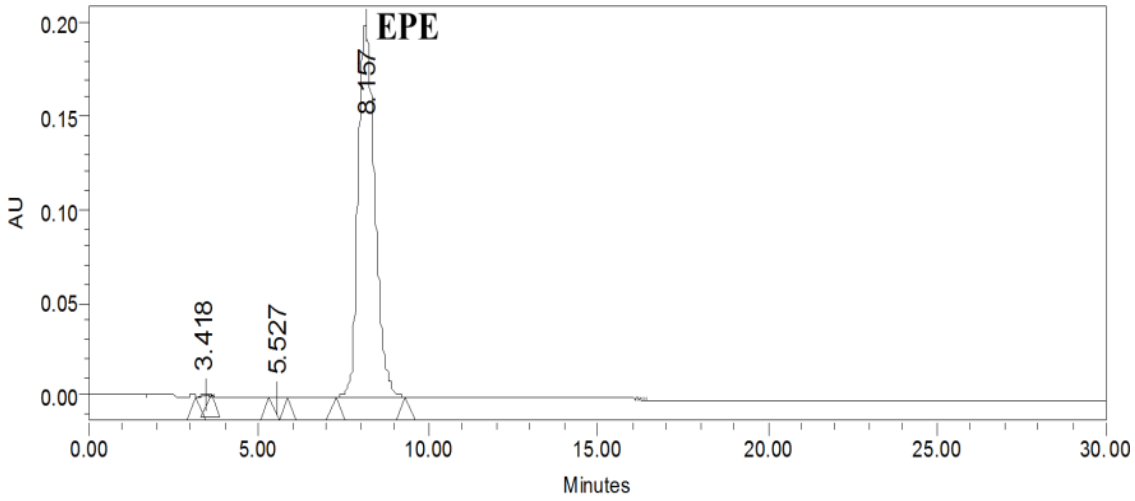

Figure 7: Chromatogram of Eperisone hydrochloride in thermal degradation

Conclusion

The present study represents the development of a stability indicating RP-HPLC method for determination of Eperisone hydrochloride by following the recommendations of ICH guidelines. The proposed method showed acceptable accuracy, precision, selectivity and wide linear concentration range. The results of analysis proved that the method is suitable for the determination of Eperisone hydrochloride in bulk and tablet dosage form without any interference from the degradation products and it is recommended for routine quality control analysis of the Eperisone hydrochloride in pharmaceutical formulation.

References

1. Matunga M, Umera Y, Yonemoto Y, Tanaka S, Nishiwaza Y et.al (1997) Long lasting muscel relaxant activity of Eperisone hydrochloride after percutaneous administration in rats. The Japanese Journal of Pharmacology 73: 215-220.
2. Cabitza P , Randelli P (2008) Efficacy and safety of Eperisone in patients with low back pain: a double blind randomized study. European Review of Medical and Pharmacological Sciences 12: 229-235.

3. Hasegwa Y, Kaku S, Arakian H, Otomo S (1992) Sigma receptor modulation of the muscler action of Eperisone. European Neuropsychopharmacology 2: 157-159.
4. Chandan WA, Choprta A, Goregonkar A, Medhi B, Gaikwad S et.al (2011) Evaluation of Eperisone hydrochloride in the treatment of acute musculoskeletal spasm associated with low back pain. Postgraduate Medical Journal 57: 278-285.
5. Amol AD, Satish KM , Kishore NG (2015) Development and validation of RP-HPLC method for determination of Eperisone hydrochloride in bulk and dosage form. Inventi Impact Pharm Analysis & Quality Assurance.
6. Prasanth V, Abhinav K, Swamy SS, Jyothsna B (2016) Development and analytical validation of Eperisone hydrochloride by using RP-HPLC method in pharmaceutical dosage form. International Journal of Advanced Research in Medical & Pharmaceutical Sciences 1: 6-10.
7. Manisha K, Priyanka M, Vilasrao K (2015) HPLC method development and validation for estimation of Eperisone hydrochloride from bulk and dosage form. Journal of Applicable Chemistry 4:1766-1773.
8. Sanjay P, Suvarna V, Rajendra P, Tushar D (2018) Development and validation of stability indicating HPLC method for determination of Eperisone HCl in bulk and in formulation. International Journal of Research in Applied Science & Engineering Technology 6: 1937-1942.
9. ICH Harmonised Tripartite Guideline Q2 (R1) (2005) Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, Geneva 1-13.
10. ICH Harmonised Tripartite Guideline Q1A (R2) (2003) Stability testing of new drug substances and products. International Conference on Harmonization Geneva 1-18.