Research Article

HPLC Method Development and Validation for Estimation of Eperisone Hydrochloride from Bulk and Marketed Formulation

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ABSTRACT

Rapid, accurate and precise method for identification and quantitation of Eperisone Hydrochloride (EPE) was developed by reversed-phase high performance liquid chromatographic (RP-HPLC) and validated. The solvent system and wavelength were optimized in order to maximize the sensitivity of the proposed method, Eperisone Hydrochloride shows the maximum absorbance at 255 nm. The Agilent HPLC 1200 series system, employed with software EZ Chrom Elite was used for proposed analytical work. Column chromatographic development was carried out with the help of reversed-phase mode using HiQSil C18 (4.6 mm X 250 mm, 5 mm i.d., 5 µm particle size). The optimized mobile phase consisted of Methanol: Double distilled water (pH maintain 3) (90:10 %v/v) as the mobile phase and detection wavelength of 255 nm.Flow rate was kept at 0.8 mL min-1. Drug- Eperisone Hydrochloride was well resolved and retained at 2.9 min. Performance characteristics of HPLC method for estimation of EPE in bulk and its marketed dosage form were statistically validated as per recommendations of ICH guidelines of analytical method validation. This method was validated for accuracy, precision, linearity, LOD & LOQ of sample solution.The HPLC method was found to be linear with R2 value of 0.9942 and across the range 10 µg/ml- 90 µg/ml. The LOD 0.645 µg/ml and LQD values were found to be 0.645 µg/ml and 1.957µg/ml respectively. The method was found to be accurate, precise, robust and economical for the analysis of EPE from bulk and its formulation. Thus this method can be safely and successful employed for analysis of routine samples and quality control of drugs in pharmaceutical formulations.

Keywords: Reversed-phase high performance liquid chromatographic, Eperisone Hydrochloride, Method Development, Validation, ICH guidelines.

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].Literature survey showed that a number of spectrophotometric methods for simultaneous estimation [5-7], reversed phase high pressure liquid chromatographic (RP-HPLC) for simultaneous[8-14] as well as stability indicating assay methods[15] and high pressure thin layer chromatographic methods for simultaneous estimation [16] have been developed for quantitative estimation of EPE. Also 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 [12]. However, in the scientific literature that was referred, no HPLC method has been found for the estimation of EPE as

single chemical entity. RP-HPLC involves the separation of molecules on the basis of hydrophobicity. Excellent resolution that can be achieved under a wide range of chromatographic conditions. Chromatographic selectivity can be manipulated through changes in mobile phase characteristics.The objective of present research work was to develop accurate, precise, specific and economic analytical method over other chromatographic methods and which can be used for routine analysis of EPE in bulk and in marketed formulation. Considering the predefined objective of the research work, for estimation of EPE in bulk and its marketed formulation, an attempt was made to HPLC method was develop and then duly validate as per the recommendations of ICH guidelines of analytical method validation.

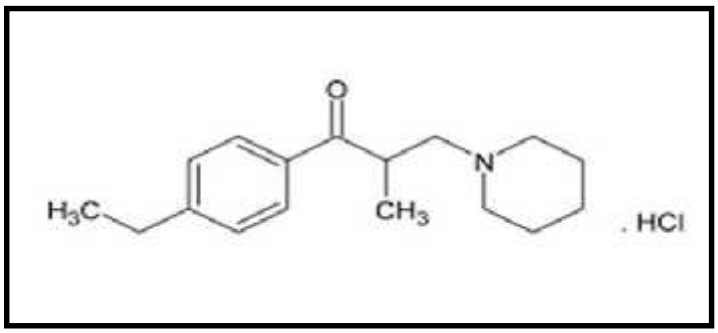

Figure 1: Structure of EPE

Materials And Methods

Materials and marketed formulation

Pure drug of Eperisone Hydrochloride was procured along with thecertificate of analysis as generous gift sample for the purpose of academic research from Sharon Bio-Medicine Pvt.Ltd., Vashi, Navi Mumbai. Commercial tablets containing Eperisone Hydrochloride (150 mg) were used for the study.

Reagents

Chemicals of A.R. and HPLC grade were purchased from S.D. Fine Chemicals, Mumbai, Maharashtra, India.

Instrumentation

The HPLC system used was HPLC binary gradient system equipped with HPLC Agilent technology 1200 series and variable wavelength UV detector. A PGB100 analytical balance was used and WUC-41L sonicator used. Specifications of HPLC instrument are given in Table 1.

Table 1: HPLC instrument and specifications.

Sr. No.	Instruments	Specification
1.	Make and model	HPLC Agilent technology 1200 series
2.	Column	Hemochrom Intsil C18 (4.6 mm X 250 mm, 5 mm i.d., 5 μm particle size)
3.	Software	EZChrome elite software (version 3.2.1)
4.	Sampling mode	Autosampler
5.	Detection	Ultraviolet (UV) detector

Experimental Work

Analytical Method Development

Preparation of standard stock solution: Standard solution of EPE was prepared by dissolving 100 mg of drug in 100 ml methanol to obtain concentration 1000μg/ml (1000 ppm). This solution was filtered using Whatmann filter paper to remove the undissolved matter. For calibration curve, a series of solutions (10, 20, 30, 40, 50, 60, 70, 80, and 90 ppm) were prepared by diluting stock standard solution in 10 ml standard volumetric flasks and volume was made upto the mark with mobile phase.

Preparation of sample solution

Exact ten tablets, each comprising 150 mg of EPE, were weighed and finely powdered. An amount of powder that is equivalent to 10 mg of EPE was added in a 10 ml volumetric flask. It was dissolved completely in methanol and the volume of the solution was made up to 10 ml with methanol. It was sonicated for 30 minutes in ultra-sonication bath for complete dissolution of drug. The solution was double filtered, first through 0.45μm Whatman filter paper and after that through 0.45μm syringe filter in order to get clear solution. Further, necessary dilutions were prepared to get the desired dilutions.

Selection of stationary phase

The column used was Hemochrom Intsil C18 (4.6 mm X 250 mm, 5 mm i.d., 5μm particle size).

Selection of detection wavelength

UV absorption spectrum for 10μg/ml (10 ppm) solution of EPE was generated by scanning over the range of 200-400 nm and the spectrums were recorded to get λmax of analyte in mobile Phase.

Selection of mobile phase composition

A trial and error method was used to select the optimized mobile phase composition.

Analytical Method Validation (AMV)

The HPTLC method, so developed, was appropriately validated as per suggestions given by "ICH guidelines Q2 (R1) for validation of analytical procedures: text and methodology" [17] Refer Table 2 for parameters and procedure followed for AMV.

Table 2: Analytical method validation: Parameters and procedures followed [18].

Sr. No.	Parameters	Procedure Followed	
1.	Linearity	As per ICH guidelines, for determination of linearity, a minimum of 5 concentrations are suggested. By plotting peak area against standard concentration and calculating regression coefficient (R2).	
2.	Specificity	As per ICH, specificity should be carried out to make sure the identity of an analyte. The specificity of the method was determined by comparing the Retention time (R_t) value of standard EPE with sample (tablet extract).	
3.	Precision	Precision was carried out at two levels, as follows ,	
		Repeatability Repeatability was estimated by using minimum of 9 determinations covering the described range for the procedure (e.g., 3 concentrations/ 3 replicates each)	Intermediate Precision Intermediate Precision was established to study the consequences of random events i.e. days, on the precision of the analytical procedure. Intraday and inter-day precision studies were conducted by taking 9 determinations of 3 concentrations/3 replicates each, at 3 different times in a same day and on 3 different days, respectively.
		Precision is reported as a standard deviation and coefficient of variation (relative standard deviation) for each type of precision investigated.	
4.	Limit of Detection (LOD) and Limit of Quantification (LOQ)	The values of Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated from the standard deviation of the response and the slope of calibration graph. The quantitation was done with the help of equations. For LOD = 3.3 σ/ s, LOQ = 10 σ/ s, where σ = Standard Deviation of replication, s = Slope of calibration curve.	
5.	Accuracy	Accuracy should be reported as a percent recovery by the assay of known added amount of analyte in the sample. Accuracy should be evaluated by carrying out at Least 9 determinations over at least of 3 concentrations levels that cover a definite range (e.g., 3 concentrations / 3 replicates each of entire analytical process). In the existing the percent recovery was calculated by performing recovery studies in triplicates of 3 concentration level viz. 80%, 100%, 120% by putting known amount of standard solution of EPE. These samples were then analysed and the results, thus obtained, were compared against expected results.	
6.	Robustness	The robustness of an analytical method is a degree of its likelihood to remain unaffected by small, but deliberate variations in process parameters and offers a mark of its dependability throughout normal usage. For inspecting the robustness of the developed analytical method following parameters were purposely changed, 1. Composition of Mobile Phase 2. Flow rate (ml/min)	

Results And Discussion

Analytical Method Development

Selection of Detection wavelength

UV absorption spectrum for 10 ppm solution of EPE (Figure 2) was generated using Shimadzu 1800 Series UV – visible spectrophotometer and software, 255 nm wavelength was selected as a detection wavelength for chromatographic determination of EPE because at the wavelength of 255 nm EPE showed maximum absorbance

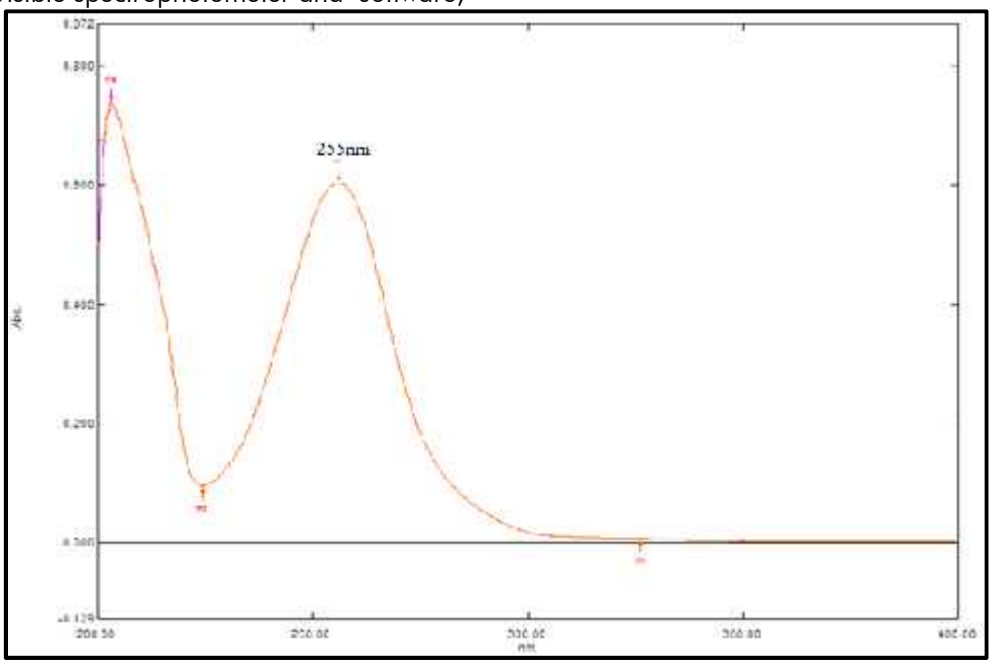

Figure 2: UV absorption spectra of EPE

Selection of mobile phase

Different mobile phase and its proportions were tried (Table no. 3) in order to achieve HPLC analysis of EPE with good R_f value and within sufficient period of time keeping in mind accuracy, precision, specificity and economical point.

Table 3: Trials of Mobile Phase

Sr. No.	Mobile phase	pH of Double distilled water (DDW)	Ratio (% V/V)	Flow rate (ml/min)	Observation
1.	ACN:DDW	-	(60:40, 70:30, 80:20, 90:10)	1	Peak was not observed
2.	ACN: Phosphate Buffer (pH 6.5)	6.5	(60:40, 70:30)	1	Very long retention time, broad peak
3.	ACN:Phosphate Buffer (pH 4.5)	4.5	(60:40, 70:30)	1	Long retention time, slight broad peak along with tailing
4.	DDW: MeOH	-	(90:10, 80:20, 70:30, 60:40)	1	Very long retention time, negligible peak observed
5.	MeOH:DDW	9	90:10	1	Very long retention time, small peak observed
6.	MeOH:DDW	6	90: 10	1	Long retention time, slight broad peak
7.	MeOH:DDW	3	90: 10	1	Sharp peak, desired retention time

Ultimately Methanol: double distilled water pH maintained by acetic acid (90:10 % v/v) was finalized as the mobile phase. Along with composition of mobile phase, other conditions such as flow rate, pH of distilled water etc. were also optimized by laboratory studies. All of the optimized chromatographic conditions are given in Table 4.

Table 4: Optimized chromatographic conditions

Sr. No.	Parameters	Optimised conditions
1.	Stationary phase	Hemochrom Intsil C18 (4.6 mm X 250 mm, 5 mm i.d., 5 μm particle size)
2.	Mobile phase composition	Methanol: Distilled water (pH maintain 3 by acetic acid)
3.	Ratio of mobile phase	90:10
4.	Injection Volume	20 μl
5.	Detection Wavelength	255nm
6.	Flow Rate	0.8 ml/min
7.	ColumnTemperature (°C)	25±2

Chromatogram obtained using these optimized chromatographic conditions showed that EPE was well resolved and retained at 2.9 min. Chromatogram of EPE is shown in Figure 3.

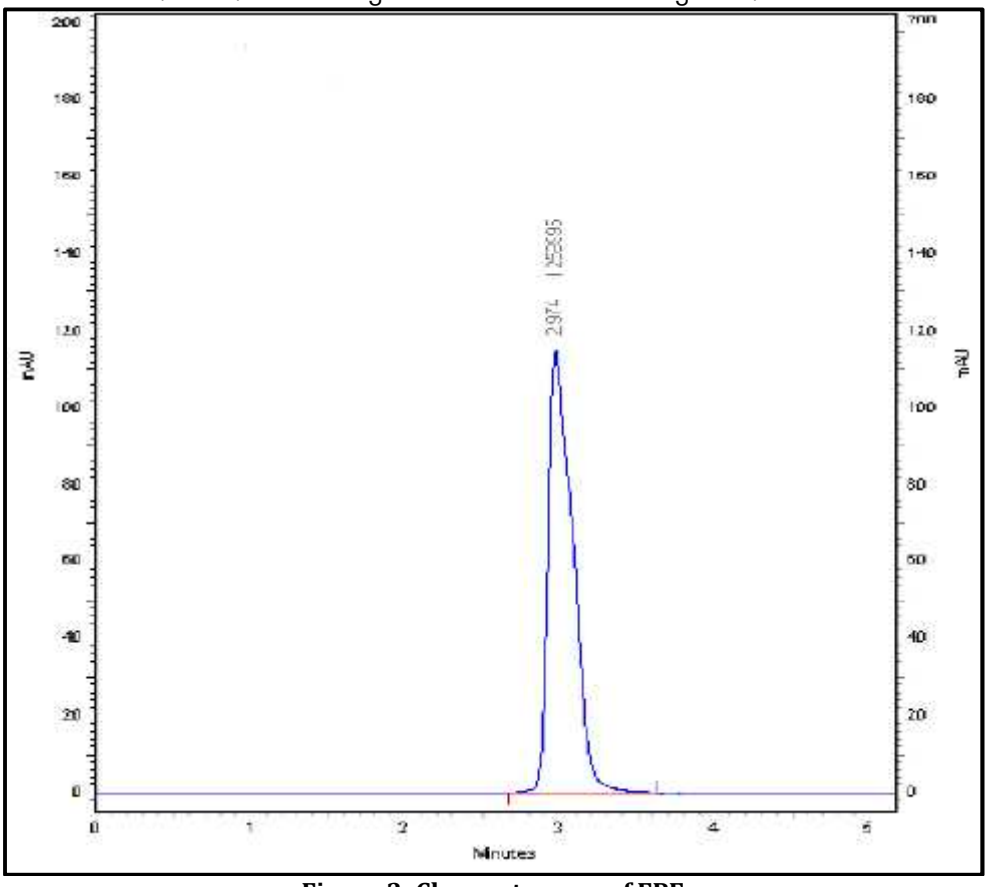

Figure 3: Chromatogram of EPE

Analytical Method Validation

Linearity

Linear relationship was observed by plotting peak area against different concentrations of standard solution. The calibration graph indicated that EPE produced a linear response across the range of 10 - 90 μg/ml (Figure 4). The linear regression data of calibration plot for EPE is given in Table 5.

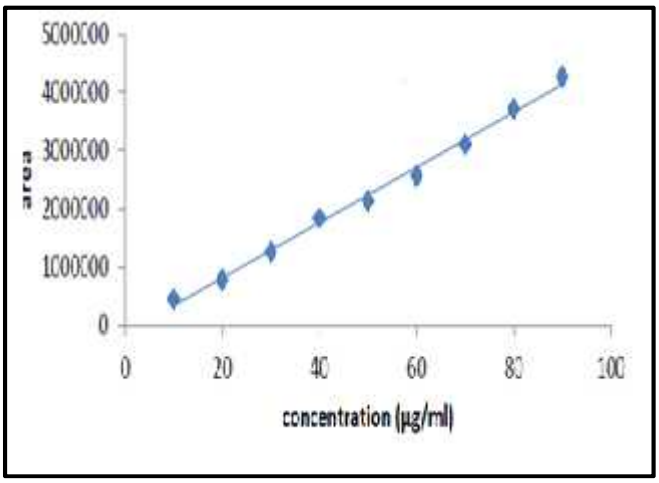

Figure 4: Calibration curve of EPE

Table 5: Linear regression data of calibration plot

Sr. No.	Parameter	Results
1.	Range	10 - 90 µg/ml
2.	R2	0.942
3.	y- intercept	131018
4.	Slope	47412

Specificity

Separate chromatograms were obtained for blank, EPE individually to ensure the identity of analyte under study namely, the chromatograms of blank, EPE individually shown in figure 5. Therefore the method developed can be deemed specific.

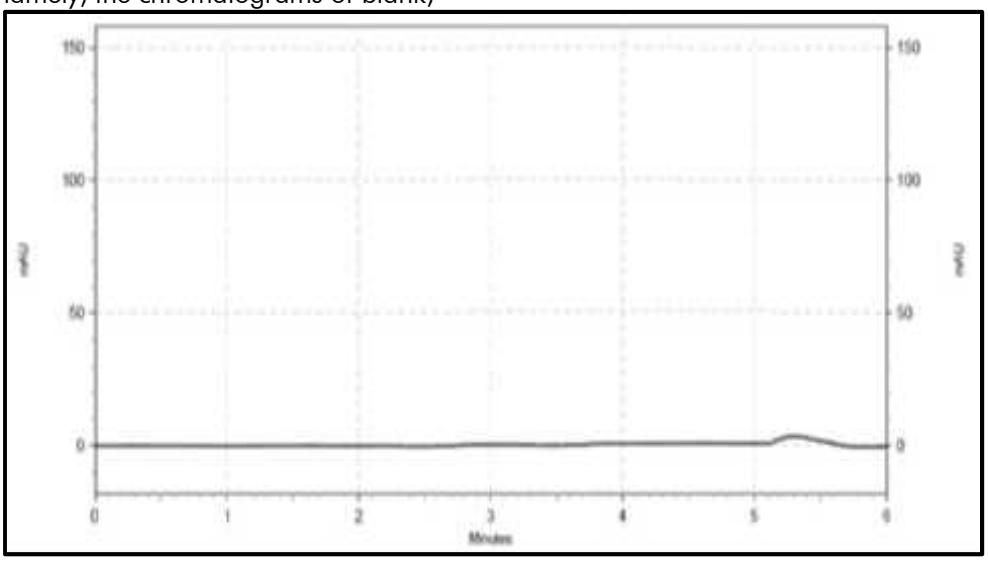

Figure 5: Blank chromatogram

Precision

a) Intra-day precision-

It was performed at three different concentration levels low (20 µg/ml), mid (50 µg/ml) and high (80 µg/ml) respectively within the same day at three different times (session 1, 2, 3).

b) Inter-day precision-

It was carried out at same concentration levels on three consecutive days, using same homogeneous sample.

The % RSD values for both intra-day and inter-day precision were found within acceptable limit as shown in Table 6 and 7 respectively.

Table 6: Intra-day precision studies

		Eperisone Hydrochloride			Inference
Concentration levels		Low	Mid	High	Acceptable % RSD, hence Precise
Concentration(μg/ml)		20	50	80	
Peak area	Session 1	801730	2149872	3719370	
	Session 2	803486	2144838	3720833	
	Session 3	806185	2139900	3700213	
Average Peak area		803800.33	2144870	3713472	
Standard Deviation		2244.0723	4986.077	11505.91	
% RSD		0.2791	0.23246	0.30984	

Table 7: Inter-day precision studies

		Eperisone Hydrochloride			Inference
Concentration levels		Low	Mid	High	Acceptable % RSD, hence Precise
Concentration (μg/ml)		20	50	80	
Peak area	Session 1	801730	2137044	3723454	
	Session 2	803849	2131441	3708764	
	Session 3	796432	2147626	3720099	
Average Peak area		800670.3	2138704	3717439	
Standard Deviation		3820.359	8219.15	7697.774	
% RSD		0.4771	0.3843	0.2070	

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

Values of LOD and LOQ calculated using slope of calibration plot for OLM is tabulated in Table 7.

Table 7: LOD and LOQ

Sr. No.	Parameters	Results obtained
1.	LOD	0.645 μg/ml
2.	LOQ	1.957μg/ml

Accuracy

Accuracy of the method is reported as percent recovery of known added amount of analyte in the sample. The accuracy of the method was established by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% by adding known amount of EPE. Results obtained are given in Table 8.

Table 8: Accuracy- recovery studies

Drug	Level of % recovery (%)	Amount present in extract (μg/ml)	Amount added (μg/ml)	Total amount (μg/ml)	% recovery	Average % recovery	% RSD	Inference
EPE	80	40	32	72	100.03	100.006	0.087	Acceptable recovery Hence accurate
	100	40	40	80	99.968		0.117	
	120	40	48	88	100.142		0.05	

The inference drawn here is that the % RSD is acceptable and thus the method is accurate.

Robustness

To determine robustness of analytical HPLC method deliberate changes were made in the mobile phase composition and flow rate. Effect of these changes on both the Rt values and peak areas were evaluated by calculating the relative standard deviations (% RSD). The results obtained are tabulated in Table 9.

Table 9: Robustness results

Robustness parameters	Parameter changed	Change in Retention time (min)	%RSD of area
Composition of organic phase in mobile phase	+2 % -2 %	0.14 0.21	0.168 0.153
Flow rate (ml/min)	+0.2 -0.2	0.26 0.29	0.286 0.253

Conclusion

The method developed by HPLC was found to be fast, simple and economic. The method so developed was validated and was found to be specific, linear, accurate, precise and robust. Hence the HPLC method can be conveniently adopted for routine analysis of the formulations containing EPE. Validation studies indicated that the proposed method is suitable for the estimation of EPE in bulk and in pharmaceutical formulation. Hence the HPLC method can be conveniently adopted for routine analysis of the formulations containing EPE.

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References

1. Matsunaga, Manabu, Y. Uemura, Y. Yonemoto, K. Kanai, H. Etoh, S. Tanaka, Y. Atsuta, Y. Nishizawa, and Y. Yamanishi, "Long-lasting muscle relaxant activity of eperisone hydrochloride after percutaneous administration in rats," Jpn. J. Pharmacol. 73(3), 215-220 (1997).
2. H. Batool, K. Asad , M. Hanif, M. Ahmad, "Eperisone Hydrochloride: A Centrally Acting Muscle Relaxant," Int. J. Pharm. Integrated Life Sci. 2(2), 8-13 (2013).
3. Japanese Pharmacopoeia, (Tokyo: Japnease society of pharmacopeia, MHLW Ministerial Notification No. 285, 15th ed., 2007), pp. 618.
4. M. Sittig, Pharmaceutical Manufacturing Encyclopaedia, (William Andrew Publishing; New York, NY, US, vol. 2, 3rd ed., 2007).
5. S. G. Khanage, P. B. Mohite, S. Jadhav, "Development and validation of UV-visible spectrophotometric method for simultaneous determination of eperisone and paracetamol in solid dosage form," Adv. Pharm. Bull. 3(2), 447 (2013).
6. K. K. Dhokale, D. D. Deore, M. A. Nagras, "UV spectrophotometric method for simultaneous estimation of diclofenac salt and eperisone hydrochloride in bulk and capsule dosage form," Int. J. Pharm. Sci. & Res. 7(9), 3810-4 (2016).
7. A. Jawed, J. Prajapati, M. Mujahid, and G. O. Elhassan, "Development and validation of derivative spectrophotometric method for simultaneous estimation of lornoxicam and eperisone in their synthetic mixture," As. J. Res.Chem. 8(7), 465-470 (2015).
8. Locatelli, Marcello, R. Cifelli, C. D. Legge, R. C. Barbacane, N. Costa, M. Fresta, C. Celia, C. Capolupo, and L. D. Marzio, "Simultaneous determination of eperisone hydrochloride and paracetamol in mouse plasma by high performance liquid chromatography-photodiode array detector" J. Chromat. 1388, 79-86 (2015).
9. P. B. Patel, Z. N. Patel, J. D. Modi, N. N. Parikh, B. N. Lad, P. K. Pradhan, and U. M. Upadhyay, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Diclofenac Sodium and Eperisone HCl in Tablet Dosage Form," Pharm. Sci. Monitor. 5(2), 133-41 (2014).
10. Form, Eperisone Hydrochloride in Pharmaceutical Dosage. "Development and Validation of RP-HPLC Method for Eperisone Hydrochloride in Pharmaceutical Dosage Form," J. Pharma. Search. 9(1), 3 (2014).
11. Divya A, Vishwanadham Y. Mounika, "Development and Validation of RP-HPLC Method for Simultaneous Determination of Diclofenac Sodium and Eperisone Hydrochloride in Pharmaceutical Dosage Form," Pharm. Anal. Acta. 8(552), 2 (2017).
12. K.R. Gupta, K. Keche, A. V. Ganorkar, "Stability indicating RP-HPLC method for determination of eperisone hydrochloride and diclofenac sodium in tablet dosage form," Int. J. Pharma. Chem. & Anal. 3(4), 205-18 (2016).
13. B. P. Mohite, G. S. Khanage, S. S. Jadhav, "Reversed Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Eperisone Hydrochloride and Paracetamol in Tablet Dosage Form," Biquart. Ira. J. Anal. Chem. 2(1): 57-62 (2015).
14. Maniteja, K., PurvilChovatia, Jaya Naimisha, ChPavani, and PrathimaPatil, "Simultaneous Estimation of Eperisone Hydrochloride and Diclofenac Sodium in Pharmaceutical Dosage Form by RP-HPLC." SYSTEM. 1379(13.54), 0-98 (2014).
15. R. S. Sakhare, S. S. Pekamwar, K. A. Dannak, "Stability Indicating HPTLC Method for Simultaneous Estimation of Eperisone Hydrochloride and Diclofenac Sodium in Bulk and Solid Dosage Form," Eura. J. Anal. Chem.12(3), 245-56 (2017).

16. N. Uchadadiya, F. Mehta, P. Sanchaniya, "HPTLC-densitometric analysis of eperisone hydrochloride and paracetamol in their combined tablet dosage form," Chromat. Res. Int. (2013).
17. ICH Harmonised Tripartite Guideline Validation of analytical procedures: text and methodology Q2 (R1) (*International Conference on Harmonization, Geneva, Switzerland* Nov. 2005), available at https://www.academia.edu/34386868/ICH_HARMONISED_TRIPARTITE_GUIDELINE_VALIDATION_OF_ANALYTICAL_PROCEDURES_TEXT_AND_METHODOLOGY_Q2_R1,accessed Sept. 2017.
18. T. Raja, A. L. Rao, "Validated RP-HPLC method for simultaneous estimation of Metformin hydrochloride and Sitagliptin phosphate in bulk drug and pharmaceutical formulation. International Journal of Pharmaceutical," Chem. & Biol. Sci. 2(4) 696-702 (2012).