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Reversed Phase High Performance Liquid Chromatographic Method for Simultaneous Estimation of Eperisone Hydrochloride and Paracetamol in Tablet Dosage Form

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ABSTRACT

Eperisone Hydrochloride is a skeletal muscle relaxant and Paracetamol is cyclooxygenase inhibitor. These drugs in combination are used as antispasmodics. A simple, specific, precise and accurate method was developed, namely reverse phase high performance liquid chromatography for simultaneous estimation of Eperisone Hydrochloride and Paracetamol in tablet dosage form. In RP-HPLC method separation was achieved by HiQ silC-18HS column having 250 mm× 4.6 mm, with mobile phase containing Methanol: 0.05 mM Ammonium acetate buffer: Acetonitrile (60:30:10) and adjusted to pH 5.8 using Glacial acetic acid for RP-HPLC system. The flow rate was 1.0 ml/min and effluent was monitored at 264 nm. The retention time of EPE and PAR were 6.45 min and 3.05 min respectively. The linearity for EPE and PAR were in the range of 5-25 µg/mL. The recoveries of EPE and PAR were found in the range of 99.96-100.52% and 99.87-100.11%, respectively. The proposed method was validated as per ICH guidelines and successfully applied to the estimation of EPE and PAR in tablet dosage form.

KEYWORDS: Eperisone Hydrochloride; Paracetamol; RP-HPLC.

1. INTRODUCTION

Eperisone Hydrochloride is a chemically (2RS)-1-(4-Ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one monohydrochloride (1:1) (Fig. 1). EPE is a new generation antispasmodic drug [1]. It exhibits both skeletal muscle relaxant and vasodilator properties because of its actions within the central nervous system and on vascular smooth muscles and demonstrates a variety of pharmacological effects such as cervical spondylosis, headache and low back pain [2]. EPE is official in Japanese Pharmacopeia and described potentiometric method for its estimation [3]. Literature survey divulge that ESI-MS method for estimation of EPE in human plasma [4], HPLC/MS, GC/MS, NMR, UV and IR analytical techniques to identify a degradation product of EPE in the tablets dosage form [5] are available. More recently spectrophotometric method for simultaneous estimation of EPE and Diclofenac sodium in synthetic mixture has been reported [6].

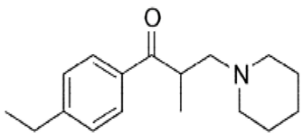

Fig. 1. Structure of Eperisone HCl.

PAR is a chemically N-(4-Hydroxyphenyl) acetamide (Fig. 2). PAR is a non-opioid, non-salicylate analgesic with an unclear mechanism of action. PAR is official in IP [7], BP [8] and USP [9]. Literature survey reveals U.V. and chromatographic methods are available for estimation of PAR in single and combined dosage forms [10-17]. Literature survey also reveals LC-MS, GC-MS, IR [18] and HPTLC [19] methods are reported for estimation of PAR with other drugs in combination.

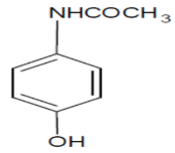

Fig. 2. Structure of Paracetamol.

EPE is a potent new generation antispasmodic drug which is used in the treatment of moderate to severe pain in combination with PAR. Literature survey reveals that no RP-HPLC method has been reported for estimation of EPE and PAR in combination. The objective of the present work is to develop new RP-HPLC method for estimation of EPE and PAR in tablet formulation with good accuracy, simplicity, precision

and economy over other chromatographic methods and which can be used for routine analysis.

2. EXPERIMENTAL

2.1. Standard and chemical reagents

The standard drug Eperisone Hydrochloride was obtained from Abbott Healthcare Pvt., Ltd., Mumbai, India. Paracetamol was obtained from Wockhardt Ltd., Aurangabad, India. Deionised distilled water (DIW) used was obtained from Loba Chemie Mumbai, India. HPLC grade methanol Merck Ltd., India, HPLC-grade acetonitrile, Merck Ltd., India. Buffering agent's ortho phosphate, tri ethylamine was procured from Fisher scientific, Mumbai. India. Ortho phosphoric acid was obtained from SD fine, Mumbai. India.

2.2. Chromatographic conditions

Liquid chromatography was performed on JASCO Isocratic HPLC system model LC-NET II/ADC (JASCO Corporation, Japan). The system built with UV-2070 as UV-VIS detector and HiQ sil C18HS (4.6 × 250 mm, 5µm) column with a 20 µL manual sample injector. The HPLC system was equipped with Chrom-NAV software for data processing. All compounds were eluted off the column with a mobile phase consisting of Methanol: 0.05 mM Ammonium acetate buffer: Acetonitrile (60:30:10) at a flow rate of 1.0 mL/min in isocratic mode. The mobile phase was filtered through a 0.45 µm nylon filter and then ultrasonicated for 30 min. The injection volume was 20 µL and the eluent was detected at 264.0 nm, which was selected as wavelength for further analysis. The retention time of EPE and PAR was around 6.28 and 3.05 min, respectively and the total run was 10 min (Table 1). The method was validated in accordance with the International Conference on Harmonization guidelines for validation of analytical procedures.

Table 1. Optimal chromatographic conditions of tablet formulation.

Aspect	Description
Mobile phase	Methanol: 0.05 mM Ammonium acetate buffer: Acetonitrile (60:30:10), pH 5.8
HPLC Column	HiQ sil C18HS (4.6 × 250 mm, 5µm)
Flow rate	1.0 mL/min
Injection volume	20 µL
Retention time	for EPE 6.28 min and for PAR 3.05 min Runtime 10 min

2.3. Specificity and selectivity

These parameters were determined by comparing the chromatograms of the EPE and PAR standard, tablet drug Myosone Plus and mobile phase as a solvent.

2.4. Linearity

The linearity of an analytical procedure is its ability within a given range to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample [21-22]. The linearity was tested for EPE and PAR in the concentration range value of 5-25 µg/mL.

2.5. Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150%. Known amounts of standard EPE and PAR were added to the pre-analyzed samples and were subjected to the proposed HPLC method.

2.6. Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The repeatability was calculated by the relative standard deviation with three replications and three different concentrations during the same day. Intermediate precision was studied by comparing the assays on two different days.

2.7. Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of detection can be calculated using the following equation as per ICH guidelines [21-22].

$$LOD = 3.3 \times N/S$$

where, N is the standard deviation of the peak area of the drug and S is the slope of thecorresponding calibration curve.

2.8. Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Limit of quantification can be calculated using the following equation as per ICH guidelines [21-22].

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the peak area of the drug and S is the slope of the corresponding calibration curve.

2.9. Sample preparation

A sample solution was prepared by taking accurately weighed twenty tablets (Myosone Plus) and finely powdered. A precisely weighed portion of the powder equivalent to 5 mg of EPE and 32.5 mg of PAR were extracted with the mobile phase. The extract was transferred to a 100 mL volumetric flask and volume was made up to the mark with the mobile phase. The solution was filtered through 0.45 µm nylon filter to remove particulate matter, if any. Then sample solution was ultrasonicated for 15 min. The tablet extract was appropriately diluted with mobile phase to obtain a concentrations 5-25 µg/mL. The amount of drug present in the sample solution was calculated by using the calibration curve. The chromatogram was hold up

Table 2. Analysis of Myosone Plus tablet formulation.

Formulation	Label claim (mg)		Amount fount (mg) ±SD, n=5		% Amount found ±SD, n=5	
	EPE	PAR	EPE	PAR	EPE	PAR
Myosone Plus	50	325	49.96±0.142	324.86±0.241	99.96±0.041	99.99±0.015

to 10 min. The chromatogram obtained is shown in Fig. 3D and the area obtained in each chromatogram of five replicates was correlated with regression equation and the amount of EPE and PAR was calculated, which was within the limit of label claim as mentioned in Table 2.

2.10. Method optimization

Four parameters were optimized to get better separation. These parameters were mobile Phase, flow rate, wavelength and injection volume.

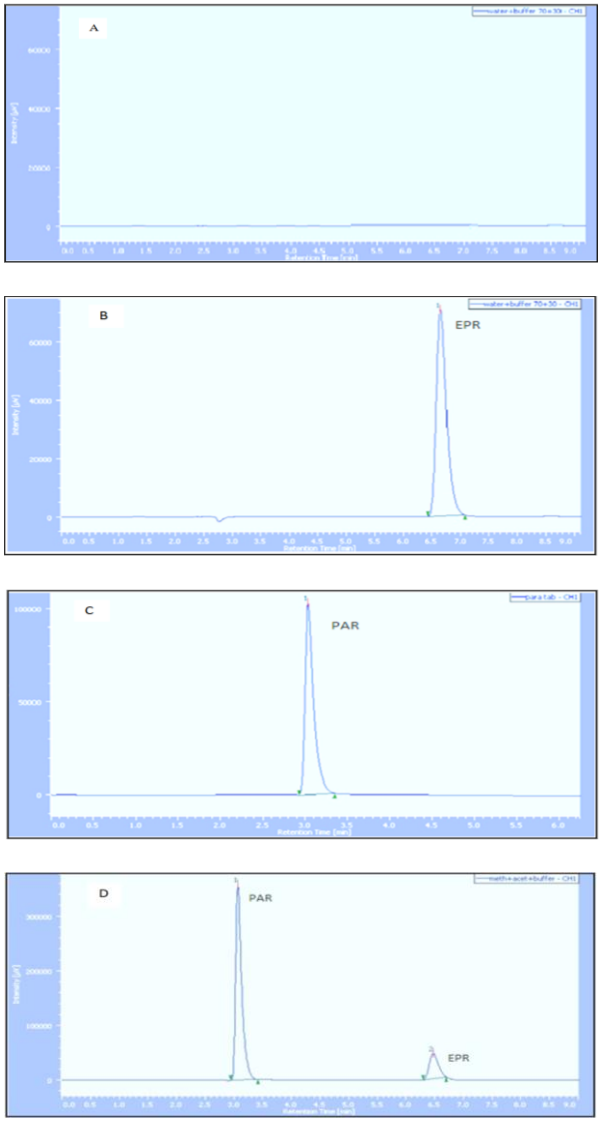

Fig. 3. (A) Blank chromatogram for selected mobile phase, (B) Chromatogram of 10 µg/mL of Eperisone Hydrochloride, (C) Chromatogram of 10 µg/mL of Paracetamol and (D) Chromatogram of 10 µg/mL of tablet.

3. RESULTS AND DISCUSSIONS

3.1 Analytical method development

The optimization of mobile phase, flow rate, wavelength and injection volume is considered very important to achieve good separation and peak area. In proposed method, the estimation these four parameters were optimized individually for EPE and PAR then optimized for in combination. In this study, we observed no significant difference in the results obtained with the mobile phase Methanol: 0.05 mM Ammonium acetate buffer: Acetonitrile (60:30:10, pH-5.8). The mobile phase made up of 100% methanol produced too late peak with an area lower than last mobile phase, may be this is attributed buffer effect. In case of these three mobile phases (methanol /acetonitrile, 50:50; methanol/acetonitrile, 60:40; methanol/acetonitrile/phosphate buffer, 70:30:10) less resolution and late elution peak was obtained. Different trials (methanol: phosphate buffer; 70:30 v/v) were conducted at varying of PH range (2-6) of phosphate buffer with satisfactory results, but non-symmetrical peak and smaller number of theoretical plates were observed. The mobile phase chosen for analytical method validation was Methanol: 0.05 mM Ammonium acetate buffer: Acetonitrile (60:30:10) at pH 5.8 , presented a mobile phase holdup time of 6.45 min for EPE and 3.05 min for PAR and giving good separation, well defined peak with more number of theoretical plates. The flow rate was optimized with (0.8, 1.0, 1.5 and 2 mL/min). At 0.8 mL/min, there is no peak appeared in the chromatogram with 3 replications. This is attributed to the insufficient flow rate to elute EPE and PAR through the column. However, a significant difference was observed among all the rest flow rates. Based on the results obtained, 1 mL/min showed the best results in terms of peak area and retention time. An optimization on the flow rates EPE and PAR analysis shown in Table 3.

3.2 Analytical method validation

3.2.1. Linearity

The linearity of the method was determined by constructing calibration curves. Tablet solution of the EPE and PAR of different concentrations at the range of (5-25 µg/mL) were used for this purpose. Each measurement was carried out in five replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients which are presented in Table 4.

3.2.2. Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplicate by the standard addition method at 50%, 100% and 150%. Known amounts of standard EPE and PAR were added

Table 3. The optimization of flow rate on EPE and PAR analysis

Flow rate ml/min	RT (min) ± SD,		Peak area (µV/S) ± SD*	
	EPE	PAR	EPE	PAR
0.8	No Peak	No Peak	No Peak	No Peak
1	6.45	3.05	104582	587865
1.5	4.45	3.15	85451	42154
2.0	5.12	4.18	88545	45514

Table 4. Linearity data for EPE and PAR

Conc. In µg/mL	Peak Area (µV/S)*	
	ESE	PAR
5	587814	104582
10	1206101	241634
15	1843320	2402488
20	2402488	467840
25	2852092	558474
Slope	23480	11449
Intercept	3242	60880
Correlation coefficient	0.998	0.995
% RSD	0.018703	0.039349

Table 5. Results of recovery study by standard addition procedure

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	Percent recovery ± SD*	RSD%
EPE	5	8	13.112	100.92±0.57	0.571
	5	10	14.895	99.30±0.87	0.898
	5	15	20.026	99.87±0.15	0.164
PAR	5	8	12.916	98.84±0.42	0.312
	5	10	15.114	100.76±0.27	0.275
	5	12	19.956	99.10±0.69	0.614

Table 6. Precision of method development on EPE and PAR analysis

Repeatability					Intermediate Precision		
Drug	conc. µg/mL	Rt±SD	Peak area ±SD*	RSD %	Rt ±SD	Peak area ±SD*	RSD %
EPE	5	6.45	104565.3±48.333	0.04623	6.45	104656±14.933	0.03934
	15	6.45	351358.3±103.982	0.02958	6.45	351335± 34.176	0.00972
	25	6.45	558455.3±111.634	0.01999	6.45	558410.7±47.542	0.00851
PAR	5	3.05	587849±115.831	0.01970	3.05	587838.3±30.550	0.00519
	15	3.05	1843404±352.719	0.01913	3.05	1843637±623.799	0.03383
	25	3.05	2851935±232.419	0.00815	3.05	2852249±358.714	0.01257

to the pre-analyzed samples and were subjected to the proposed HPLC method. Tablet solution of Myosone Plus presented good recoveries and agreement with the standards of method validation [21-22] as shown in Table 5.

3.2.3 Precision

The precision of the method was evaluated based on the results of the analysis of three samples with three replications for each one at day 1 and the results from intermediate precision from other three samples at day 2. The values were compared with the standards [21-22], thus all values demonstrated good results as shown in Table 6.

3.2.4. Limits of quantification (LOQ) and detection (LOD)

The LOD and LOQ were calculated using signal to noise ratio method according to the guidance of ICH guidelines of method validation [21-22]. LOD was taken as the concentration of the analyte where the signal to-noise ratio was 3, and for EPE and PAR it was found to be 0.270 µg/mL and 0.038 µg/mL respectively. LOQ defined as the analyte concentration at a signal-to-noise ratio of 10 and it was 0.818 µg/mL and 0.121 µg/mL, respectively for EPE and PAR.

3.2.5. Selectivity

Comparison of the chromatograms obtained from the mobile phase (blank), EPE, PAR standard and the tablet revealed no significant interference, using same chromatographic conditions for all samples. Fig. 3A-3D refers to the selective method for the analyte concerned.

4. CONCLUSIONS

The results show that the HPLC method presented here can be considered suitable for the analytical determination of EPE and PAR in tablet dosage form. The proposed method is advantageous over the other developed methods because of low concentration range used for linearity, high selectivity and specificity, high

precision and adequate accuracy at the concentrations studied. The proposed method uses a simple mobile phase which can be available easily as compared to other multi-component mobile phases in many reported methods. Also the separation and determination were achieved at an ambient temperature. Thus, it offers the advantages of low column back pressure, good peak shape, improved column efficiency, higher theoretical plates and consistent retention time, better resolution and use of simple mobile phase over analytical methods. The developed method suggested no interference of formulation excipients in the estimation. Hence it can be easily and conveniently adopted for routine analysis.

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CONFLICT OF INTEREST

The authors certify that no actual or potential conflict of interest in relation to this article exists.

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