

Analytical method development & validation of Eperisone Hydrochloride and Diclofenac Sodium in Rapisone D SR Capsules by RP-HPLC

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ABSTRACT

A simple, sensitive, rapid, accurate, precise and reproducible Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed for the simultaneous quantitative determination of Diclofenac Sodium (DIC) & Eperisone Hydrochloride (EPE) in capsule dosage form and statistically validated as per ICH guidelines. The Chromatographic separation was carried out on a Thermo Hypersil C-8 column (250×4.6mm, 5µm) by gradient elution mode. The mobile phase consists of Methanol, Phosphate Buffer(0.1M, pH 6) and Acetonitrile in the ratio of 30:40:30 (v/v/v) pumped at a flow-rate of 1.0 mL/min. The PDA detector was employed at a wavelength of 261nm. The retention times of Diclofenac Sodium & Eperisone Hydrochloride were found to be 2.21 min and 3.6 min respectively. The calibration curves were linear in the range of 20-80µg/mL & 30-120 µg/mL for DIC & EPE. The method is accurate with %w/w recoveries of 99.03% & 100.9 % for Diclofenac Sodium and Eperisone Hydrochloride respectively & the method is precise with % RSD < 2. The proposed method is rapid, simple and does not require any separation process and it has been successfully applied to the assay of pharmaceutical dosage form (RAPISONE D SR capsules).

Keywords: Diclofenac Sodium, Eperisone Hydrochloride, RP-HPLC, Phosphate buffer, RAPISONE D SR capsules, Validation, ICH.

INTRODUCTION

Eperisone (EPE) is chemically 4-ethyl-2-methyl-3-piperidinopropiopenone (Figure 1 [2]) is a well known antispasmodic drug & is official in Japanese Pharmacopoeia (JP). JP [1] describes potentiometric method for its estimation. Literature survey reveals use of HPLC/MS, GC/MS, NMR, UV and IR to Identify Degredation product of eperisone hydrochloride in the tablets[11]. Literature survey also reveals UV spectrophotometry [12] and HPLC [13] method for the determination of EPE with other drugs in combination. Diclofenac sodium (DIC) is chemically 2-[2,6dichlorophenylamino] benzene acetic acid sodium salt (Figure 2 [3]). Diclofenac sodium (DIC) is official in IP and BP. IP [3] and BP [4] describes liquid chromatography method for its estimation. Literature survey reveals UV spectrophotometric[14], HPLC [15]

& HPTLC [16] method for the determination of DIC with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of EPE and DIC in their combined dosage forms. Literature survey reveal spectrophotometric method for simultaneous estimation of EPE and DIC in synthetic mixture [17] but no RP-HPLC method for dosage form. The present communication describes simple, sensitive, rapid, accurate & precise RP-HPLC[5-8] method for simultaneous estimation of both drugs in their combined dosage form.

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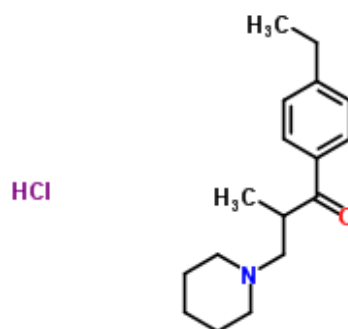


Figure 1: Structure of Eperisone Hydrochloride

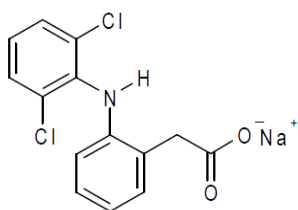


Figure 2: Structure of Diclofenac sodium

MATERIAL AND METHODS

2.1. Apparatus

A WATERS 2690 HPLC with empower software & waters 996 PDA detector, a SYSTRONICS 119 Spectrophotometer double beam UV-Visible spectrophotometer, digital pH meter DPH 500(Global), Vacuum filtration Unit EPCOS, a Wensar PGB 100 analytical balance & an ultrasonic bath (Frontline FS 4) was used in the study.

2.2. Reagents and Materials

DIC bulk powder was kindly gifted by Dr.Reddys laboratories, Hyderabad, Andhra Pradesh, India. EPE

bulk powder was kindly gifted by Sun Pharmaceuticals Ltd., Mumbai. HPLC grade solvents Methanol & Water obtained from Mercks & Acetonitrile obtained from Molychem, Disodium hydrogen phosphate obtained from Finer chemicals Ltd., Orthophosphoric acid obtained from Mercks and Whatman filter paper no. 45 (Millipore, USA) were used in the study. RAPISONE D SR capsules containing DIC (100mg) & EPE (150mg) were purchased from local pharmacy.

2.3. Chromatographic conditions

Chromatographic separation was performed with gradient elution. Initially mobile phase was tried with Methanol: Water, Acetonitrile: Water, Acetonitrile: Water: Methanol & then finally with Phosphate Buffer: Acetonitrile: Methanol at various pH conditions. The following optimised parameters were used as a final method for the simultaneous estimation of Diclofenac Sodium and Eperisone Hydrochloride.

Table 1: Optimized Chromatographic Conditions

Column	Hypersil C8 (4.6 x 250mm, 5µm, waters)
Buffer	7.5 grams of Disodium Hydrogen Phosphate in 1000 mL water; pH 6 was adjusted with Orthophosphoric acid.
Mobile phase	40% Buffer, 30% Methanol, 30% Acetonitrile.
Flow rate	1 mL/ min
Wavelength	261 nm
Injection volume	10 µL
Run time	7 min

2.4. Preparation of standard stock solutions

Accurately weighed amount of 100mg of Diclofenac Sodium & 150 mg of Eperisone Hydrochloride were taken to a 100 mL cleaned and dried volumetric flask. This was then diluted with 70mL of diluent and was sonicated. The volume was made to 100 mL with the same solvent. This was marked and labeled as Stock solution.

2.5. Preparation of sample solution (dosage form):

Take 20 RAPISONE D SR capsules & weigh them (w_1). Now remove the shells of capsules & weigh empty shells (w_2). Calculate the weight of the capsule contents by subtracting the empty shells weight from

capsules weight $w_3 = (w_1 - w_2)$. Crush the contents into powder form. Calculate a weight equivalent to 100mg of Diclofenac Sodium & 150 mg of Eperisone Hydrochloride & take into a 100mL volumetric flask. Add 50mL of Mobile Phase & sonicate for 10min. Filter under vacuum filtration unit & make up the volume. From this 0.4mL was pipetted into a 10 mL volumetric flask & make up with mobile phase such that the final concentrations are 40µg/mL of Diclofenac Sodium & 60 µg/mL of Eperisone Hydrochloride.

2.6. Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [5-10].

2.6.1. Linearity & range:

The calibration curves were plotted over a concentration range of 20-80 µg/mL for DIC and 30-120 µg/mL for EPE. Accurately measured stock solution of 0.2,0.3,0.4,0.5,0.6,0.7 & 0.8mL were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase such that the final concentrations are 20, 30, 40, 50, 60, 70 & 80 µg/mL for DIC & 30, 45, 60, 75, 80, 105 & 120 µg/mL for EPE. Inject each into the chromatographic system and measure the peak area. Plot a calibration graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

2.6.2. Intra & Inter-Day Precision:

From the stock solution a concentration of 40 µg/mL of DIC & 60 µg/mL of EPE prepared by dissolving 0.4mL in 10mL mobile phase in a 10mL volumetric flask. Both intra-day & inter-day precision was performed by injecting five injections at regular intervals on same day & on five alternate days over a period of 10 days. The results are reported in the form of %RSD.

2.6.3. Accuracy

The accuracy of the method was determined by calculating % recovery of EPE and DIC by analyzing 50, 100 and 150 % level sample solutions of EPE and DIC. Three injections were done for each sample & results are obtained in % mean recovery.

2.6.4. Robustness:

From stock solution 0.4 mL was diluted to 10mL with the mobile phase to get 40 µg/mL of DIC & 60 µg/mL of EPE. Robustness performed by both flow rate variation (0.8, 1 & 1.2mL/min) & temperature variation (20°, 25° & 30 °C).Two injections for each parameter variation were done & the acceptance criteria for tailing factor & USP plate count were determined.

2.6.5. Ruggedness:

performed by analyst variation. A concentration of 40 µg/mL of DIC & 60 µg/mL of EPE was prepared from stock solution & analysed by using two different analyst. Five injections were done by each analyst & the results are reported in the form of % recovery.

2.6.6. Limit of detection and Limit of quantification

LOD and LOQ values decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte, while LOQ is the lowest quantifiable concentration. The LOD & LOQ are calculated from the calibration curve by using the formulas (Eq..1 & Eq..2)

$$\text{LOD} = 3.3 \times \sigma / S \dots\dots\dots \text{Eq.1}$$

$$\text{LOQ} = 10 \times \sigma / S \dots\dots\dots \text{Eq.2}$$

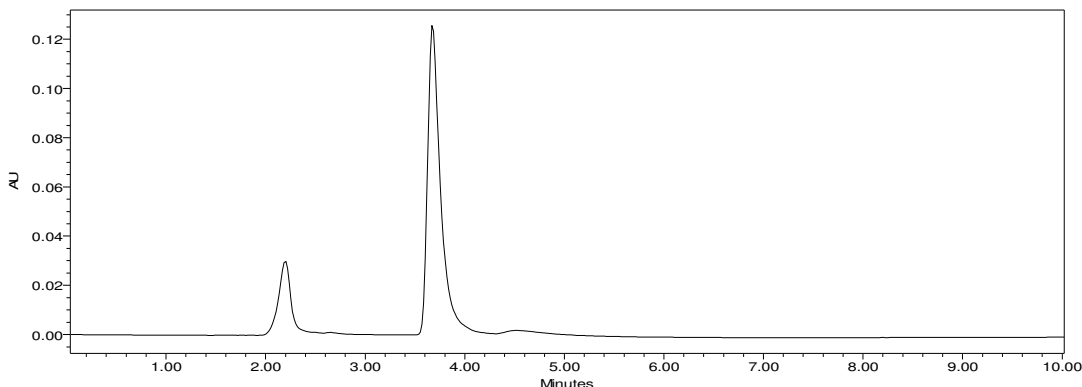
Where, σ = the standard deviation and S = slope of the calibration curve

RESULTS AND DISCUSSION

The standard solutions of EPE and DIC were scanned separately in the UV range and the maximum separation of both drugs was obtained at 261 nm with good resolution. The optimized chromatogram was shown in figure 3 & system suitability parameters are given in table 2. Linear correlation was obtained between peak areas and concentrations of EPE and DIC in the concentration ranges of 30-120 µg/mL & 20-80 µg/mL, respectively. The % RSD values of EPE & DIC was found to be 0.92 & 0.45% for inter day precision and 0.26 & 0.38 % for intra-day precision respectively. Relative standard deviation was less than 2 %, which indicates that proposed method is precise. The mean % recoveries were 99.03 and 100.9% for DIC and EPE, respectively, which indicates that the proposed method is highly accurate. The LOD & LOQ results indicate that the method is sensitive. The proposed method was analysed by different analysts & the results in % recovery indicates the method can be rugged. All the above results are given in table 3. The method is unaffected by small deliberate changes like change in flow rate & change in temperature & the results obtained are given in table 4 indicates the method is robust. The proposed

validated method was successfully applied to determine EPE and DIC in their capsule dosage form. The results obtained for EPE and DIC were comparable with the corresponding labeled amounts

(Table 5). No interference of the excipients with the peaks of interest appeared; hence the proposed method is applicable for the routine analysis of EPE and DIC in pharmaceutical dosage forms.



3. Chromatogram of Diclofenac Sodium & Eperisone Hydrochloride

Table 2: System suitability parameters

Parameters	Diclofenac sodium	Eperisone hydrochloride
Resolution	6.755598	
Retention time (min)	2.216	3.682
No. of Theoretical plates	9440	10954
Tailing factor	1.02	1.6

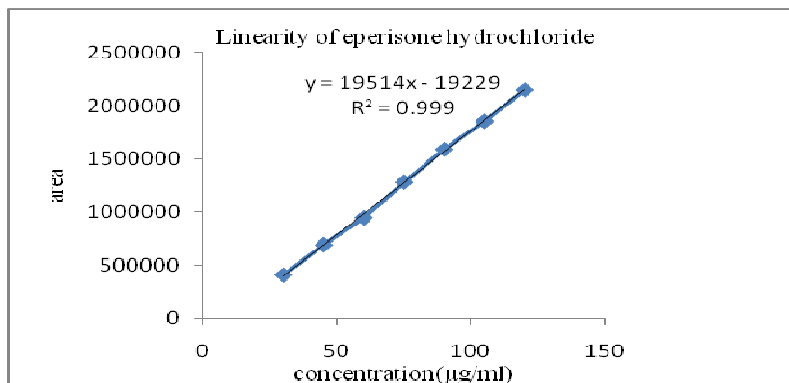


Fig.4 : Calibration graph of Eperisone hydrochloride

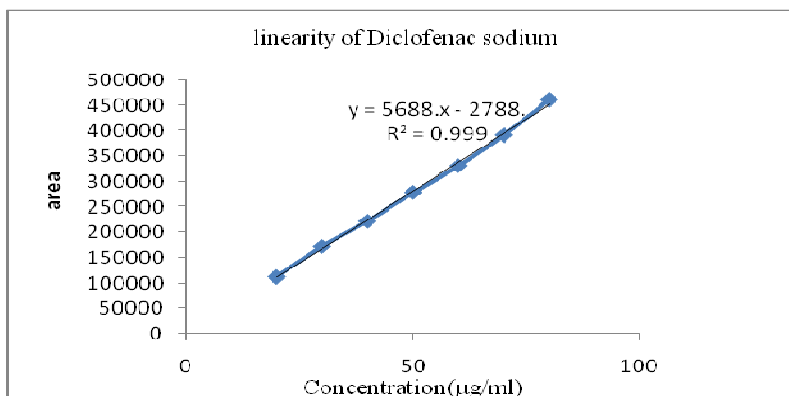


Fig.5. calibration graph of Diclofenac sodium

Table 3: Representation of Results of Various Parameters of EPE & DIC

Parameter	Eperisone Hydrochloride	Diclofenac Sodium
Linearity & Range	30-120ppm	20-80ppm
Regression equation	$y = 19514x - 19229$	$y = 5688.x - 2788.$
Correlation coefficient	0.999	0.999
Precision (%RSD)	Interday	0.92
	Intraday	0.26
Accuracy(%Recovery)	100.9%	99.03%
Ruggedness (%Recovery)	Analyst 1	0.57
	Analyst 2	0.36
LOD	0.0006	0.0012
LOQ	0.0018	0.0038

Table 4: Results of Robustness for Diclofenac Sodium & Eperisone Hydrochloride

Robustness Parameters	Diclofenac sodium		Eperisone hydrochloride	
	USP plate count	USP tailing	USP plate count	USP tailing
Temperature (°C)	20	9504.88	1.057	10962.19
	25	9831.36	1.171	10974.48
	30	9786.33	1.155	10942.93
Flow Rate (ml/min)	0.8	9504.88	1.057	10962.19
	1.0	9831.36	1.171	10974.48
	1.2	9786.33	1.155	10942.93

Table 5: Application of method for the analysis of commercial formulations

Formulation	Drug	Mean (% w/w recovery)
RAPISONE D SR capsules	EPE	100.1
	DIC	99.02

CONCLUSION

A RP-HPLC method was developed for the simultaneous estimation of Diclofenac Sodium & Eperisone Hydrochloride in RAPISONE D SR capsules & validated as per ICH guidelines. The developed method utilized a set of conditions which are not reported previously including a high pH mobile phase with gradient elution & does not required any special separation techniques. The method exhibited good selectivity & sensitivity. The developed method is successfully applied to the analysis of dosage form.

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