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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Eperisone Hydrochloride and Diclofenac Sodium in Bulk and Pharmaceutical Dosage form

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ABSTRACT

A simple, rapid and precise Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of Eperisone hydrochloride and Diclofenac sodium in pharmaceutical dosage form by reverse phase Pinnacle DB C-18 column (250 mm, 4.6 mm, and 5 µm). The sample was analyzed using 50mM ammonium acetate buffer containing 0.2% triethylamine (pH-4.0 adjusted with glacial acetic acid): Acetonitrile (40:60, v/v), as a mobile phase at a flow rate of 1.0 ml/min. and detection at 273 nm. The retention time for Eperisone hydrochloride and Diclofenac sodium was found to be 3.07 min and 5.56 min, respectively. The linearity of developed method was achieved in the range of 10-100 µg/ml for Eperisone hydrochloride and 10-100 µg/ml for Diclofenac sodium. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines.

Keywords: RP-HPLC, Eperisone hydrochloride (EPE), Diclofenac sodium (DIC), validation.

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INTRODUCTION

Eperisone hydrochloride is chemically 1-(4-ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one (figure 1) and is the well known antispasmodic drug.[1, 2, 3, 4] Mechanism of action of Eperisone includes inhibition of angiotensin II-induced relaxations, mediated possibly by endogenous PGI_2.[5] Eperisone also possesses the property of a Ca^{2+} antagonist on smooth muscle tissues, in addition to the action of antispastic agent, i.e., this agent blocks the voltage-dependent influx of Ca^{2+} at the smooth muscle membrane.[6,7] Eperisone hydrochloride is official in Japanese Pharmacopoeia only[8] and non-aqueous titrimetric method is reported for the estimation. Many methods like HPLC, [9] HPLC-MS [10,11,12] and GC-MS [13] methods were reported for estimation of Eperisone hydrochloride.

Diclofenac sodium is an anti-inflammatory agent and phenylacetic acid derivative compound (figure 2). Mechanism of action of Diclofenac sodium includes inhibition of arachidonic acid cyclo-oxygenase enzyme (COX) and thereby inhibits production of prostaglandins. [14,15] Diclofenac sodium is official drug in Indian Pharmacopoeia,[16] British Pharmacopoeia[17] and United state Pharmacopoeia.[18] Many methods like UV-Visible spectroscopy, HPLC, HPTLC AAS and capillary electrophoresis methods are reported for estimation Diclofenac sodium in single as well as combined dosage form.[19]

Diclofenac sodium and Eperisone hydrochloride available in sustained release capsule dosage form is used in the treatment of acute musculoskeletal spasm associated with low back pain.[20,21] Combination is approved by CDSCO in year 2012 and very few analytical methods were reported for estimation of combination of Diclofenac sodium and Eperisone hydrochloride in pharmaceutical dosage form. [22, 23]

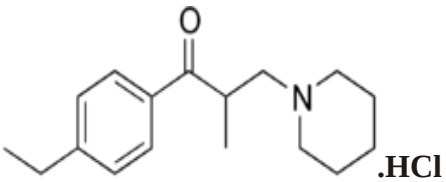

Figure 1: Structure of Eperisone HCl

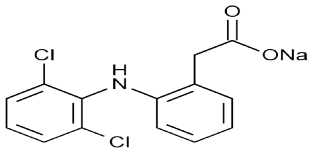

Figure 2: Structure of Diclofenac Na

MATERIALS AND METHODS:

Chemicals and solvents:

Ammonium acetate (GR grade), Triethylamine (GR grade) and glacial acetic acid (GR grade) were used for preparing the buffer. HPLC grade Acetonitrile was used. All reagents were provided from Astral pharmaceuticals Pvt. Ltd. Pure sample of Eperisone hydrochloride was a gift sample from Unin laboratories. Diclofenac sodium was provided by Babaria institute of

pharmacy.

Chromatographic Conditions:

A High pressure liquid chromatography (Shimadzu LC- 10AT - vp) with variable wavelength programmable UV Visible detector and Pinnacle DB C-18 column [250mm, 4.6m, 5µm] was used. A freshly prepared mixture of 50 mM ammonium acetate buffer containing 0.2% triethylamine (pH-4.0 adjusted with glacial acetic acid): Acetonitrile (40:60 v/v) was used as the mobile phase. Buffer solution was prepared by dissolving 3.85 gms of ammonium acetate in 900ml of water. 2 ml of triethylamine was added, adjust ph 4.0 using glacial acetic acid and volume was then adjusted upto 1000ml with water. Mobile phase was filtered through a 0.45 µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.0 ml/min.

Preparation of standard stock solutions:

Reference standard of Eperisone hydrochloride 50 mg and Diclofenac sodium 50 mg was transferred to 50 ml volumetric flask separately and dissolved in mobile phase. The flask was shaken for 30 min and the volume was made up to the mark with mobile phase to obtain standard stock solution of Eperisone hydrochloride (1000 µg/ml) and Diclofenac sodium (1000 µg/ml). Stock solution was filtered through a 0.2 µm membrane filter. The working standard solution of Eperisone hydrochloride and Diclofenac sodium was prepared from suitable aliquots of stock solution.

Preparation of Sample Solution:

Take equivalent weight 150 mg of EPE and 100 mg of DIC and transferred in to a 100 ml volumetric flask and sonicated for 20 min. The solution was filtered through whatman filter paper and the volume was adjusted up to the mark with mobile phase. This solution is expected to contain 1500 µg/ml EPE and 1000 µg/ml DIC. This solution (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with mobile phase to get a mixture of EPE (150µg/ml) and DIC (100 µg/ml). From this solution(8 ml) was taken in to a 20 ml volumetric flask and the volume was adjusted up to mark with mobile phase to get a final concentration of EPE (60 µg/ml) and DIC (40 µg/ml).

Optimization of the HPLC method:

The pure drug solution of Eperisone hydrochloride and Diclofenac sodium were injected individually into HPLC system and allow run in different mobile phases like ACN: phosphate buffer, ACN: acetate buffer, methanol: Acetonitrile: water, ACN: water and methanol: water were tried in order to find the optimum conditions for the separation of Eperisone hydrochloride

and Diclofenac sodium. It was found that mobile phase containing 50 mM ammonium acetate buffer containing 0.2% triethylamine (pH - 4.0 adjusted with glacial acetic acid): Acetonitrile (40:60 v/v), at a flow rate of 1.0 ml/min with detection at 273 nm gave satisfactory results with sharp well defined and resolved peaks with minimum tailing as compared to other mobile phases. Under these conditions the retention times were typically 3.07 min for Eperisone hydrochloride and 5.56 min for Diclofenac sodium (Fig. 3).

VALIDATION OF THE METHOD: [24]

Validation of the optimized HPLC method was carried out with respect to the following parameters.

Linearity and range:

From standard stock solution, aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain concentration of 10-100 µg/ml for Eperisone hydrochloride and 10-100 µg/ml for Diclofenac sodium. The solution of 10 µl was injected into column with the help of autosampler. All measurements were repeated three times for each concentration. The calibration curves of the area under curve Vs concentration were recorded for both drugs.

Precision:

The precision of the method was verified by repeatability, interday and intraday precision. Repeatability studies were performed by analysis of three different concentrations of the drug in six times on the same day. Intraday precision was determined by analyzing sample solutions at different time intervals on the same day and on different day for interday precision.

Accuracy:

Recovery studies were carried out by adding a known amount of standard solution of pure drug (Eperisone hydrochloride and Diclofenac sodium) to a pre analysed sample solution. These studies were carried out at 50%, 100% and 150% level.

Limit of detection and limit of quantitation:

The LOD and LOQ were separately determined based on the calibration curves. The Standard Deviation of the y- intercept and slope of the regression line were used.

The LOD and LOQ were calculated using the formulas,

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

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

Where, S = Slope of regression line

D = Standard deviation of y- intercept on the regression line

Robustness of method:

To evaluate the robustness of the developed RP-HPLC method, minute variations in the optimized method parameters were done. The parameters such as, effect of change in pH of mobile phase, flow rate, effect of mobile phase ratio and effect of temperature on the retention time, theoretical plates, area under curve and percentage content of Eperisone hydrochloride and Diclofenac sodium were studied. The solution containing, Eperisone hydrochloride (50µg/ml) and Diclofenac sodium (50µg/ml) was injected into sample injector of RP- HPLC three times under the varied conditions.

Ruggedness of method:

Standard solution containing mixture of Eperisone hydrochloride (50µg/ml) and Diclofenac sodium (50µg/ml) was prepared from stock solution and analyzed by two different analyst using same operational and environmental conditions. From the area, the amounts of both the drugs were calculated.

System Suitability Parameters:

As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of Eperisone hydrochloride and Diclofenac sodium of both drugs. 10µl solution was injected under optimized chromatographic condition and following parameters were studied to evaluate the suitability of the system.

Analysis of a Synthetic Mixture:

The tablet powder equivalent to Eperisone hydrochloride (150mg) and Diclofenac sodium (100mg) was weighed, transferred to a 100 ml volumetric flask and dissolved in mobile phase, shake for 30 min and the volume was made up to the mark with mobile phase. The content was ultra sonicated for 20 min. The solution was filtered through a 0.2 µm membrane filter paper. This solution was further diluted with mobile phase to obtain mixed sample solutions in the Beer's and Lamberts range containing 60 µg/ml of Eperisone HCl and 40 µg/ml of Diclofenac Na.

RESULTS AND DISCUSSION:

The results of validation studies on simultaneous estimation method developed for Eperisone hydrochloride and Diclofenac sodium in the current study involving 50 mM ammonium acetate buffer containing 0.2% triethylamine (pH-4.0): Acetonitrile (40:60 v/v), as the mobile phase for HPLC are given below.

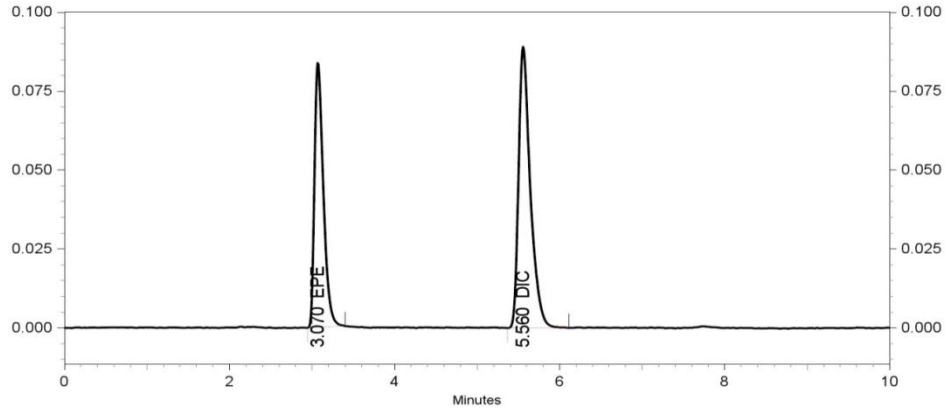

Figure 3: Chromatogram of Eperisone hydrochloride (t_R 3.07 min) and Diclofenac sodium (t_R 5.56 min).

Linearity:

The drug response was linear (r^2 = 0.9994 for Eperisone hydrochloride and 0.9997 for Diclofenac sodium) over the concentration range between 10-100 µg/ml for Eperisone hydrochloride and 10-100 µg/ml for Diclofenac sodium. (Table-1 & 2)

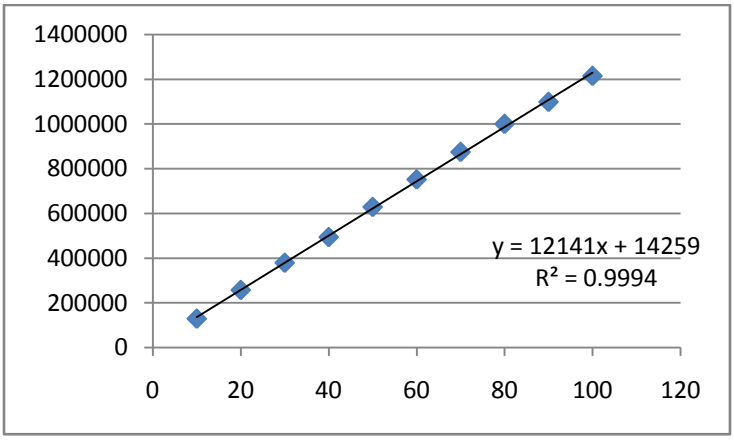

Figure 4: Calibration curve of Eperisone HCl

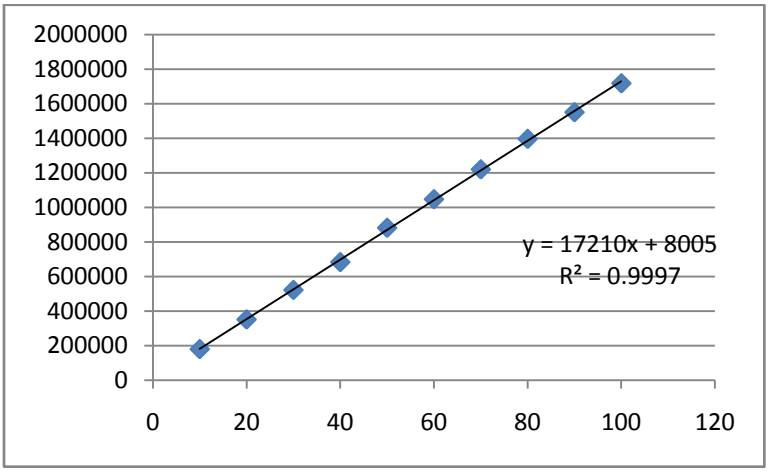

Figure 5: Calibration curve of Diclofenac Na

Table-1: Linearity Data of Eperisone hydrochloride and Diclofenac sodium by Propose method

Sr No.	Concentration of Drug (ppm)		Peak Area ±SD	
	Eperisone HCl	Diclofenac Na	Eperisone HCl	Diclofenac Na
1	10	10	128259 ± 323.4	178948 ± 234.8
2	20	20	256478 ± 845.7	350649 ± 356.6
3	30	30	378183 ± 651.9	521437 ± 219.4
4	40	40	493233 ± 183.7	682747 ± 910.8
5	50	50	627839 ± 983.7	881423 ± 807.3
6	60	60	750990 ± 287.3	1046557 ± 731.4
7	70	70	874018 ± 345.9	1219553 ± 932.9
8	80	80	999321 ± 239.7	1396119 ± 482.7
9	90	90	1097783 ± 945.7	1550354 ± 942.7
10	100	100	1214092 ± 820.3	1717803 ± 184.3

Table-2: Characteristics of HPLC method

Drug	Parameters Determined	Obtained Value
Eperisone hydrochloride	Linearity range (µg/ml)	10-100
	Slope	12141
	Intercept	14259
	Regression Coefficient(r2)	0.9994
	LOD(µg/ml)	0.24
	LOQ(µg/ml)	0.72
Diclofenac sodium	Linearity range (µg/ml)	10-100
	Slope	17210
	Intercept	8005
	Regression Coefficient(r2)	0.9997
	LOD(µg/ml)	0.36
	LOQ(µg/ml)	1.08

Table-3: Precision study (Repeatability)

Drug	Injection	Peak area	Conc.(µg/ml)	%R.S.D.
Eperisone hydrochloride	1	620060	49.89	0.397
	2	618478	49.77	
	3	621892	50.05	
	4	618103	49.74	
	5	614667	49.45	
	6	618474	49.77	
Diclofenac sodium	1	890787	51.29	0.632
	2	890994	51.31	
	3	880400	50.69	
	4	887145	51.08	
	5	878266	50.56	
	6	881377	50.75	

Precision:

The results of the repeatability, intra-day and inter-day precision experiments are shown in Table -3 and Table -4. The developed method was found to be precise as the RSD values for

repeatability of intra-day and interday precision studies were < 2 %, respectively which is under limit as per recommendations of ICH guidelines.

Table-4: Inter-day and Intra-day precision

Drug	Concentration (µg/ml)	Inter-day precision		Intra-day precision	
		Mean* ± S.D	%R.S.D	Mean* ± S.D	%R.S.D
Eperisone hydrochloride	20	99.90±0.199	0.999	98.55±0.347	1.762
	50	98.74±0.684	1.385	100.01±0.425	0.853
	80	98.65±0.536	0.679	98.44±0.532	0.676
Diclofenac sodium	20	99.65±0.262	1.315	99.40±0.071	0.357
	50	100.94±0.521	1.032	100.88±0.467	0.926
	80	98.71±0.324	0.411	99.29±0.478	0.602

*Average of three determinations

LOD and LOQ:

The LOD and LOQ were separately determined based on the calibration curves for Eperisone hydrochloride and Diclofenac sodium. The LOD and LOQ were found to be 0.24µg/ml and 0.72µg/ml for Eperisone hydrochloride and 0.36µg/ml and 1.08µg/ml for Diclofenac sodium respectively. (Table-2)

Robustness and Ruggedness:

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. Results shows low values of % RSD, as shown in Table -7 and Table -8 signify the robustness and ruggedness of the method.

Table-7: Robustness testing

Factor	Level	Retention time		Theoretical plate		Peak area	
		EPE	DIC	EPE	DIC	EPE	DIC
Flow rate (ml/min)	0.9	3.40	6.07	3749	7564	630210	872475
	1.0	3.06	5.45	3662	7416	622570	873552
	1.1	2.81	5.01	3433	6928	626443	874717
pH	3.9	3.08	5.47	3368	6798	620899	882430
	4.0	3.06	5.45	3662	7416	622570	873552
	4.1	3.03	5.38	3486	7089	632014	877678
Mobile phase ratio	42:58	3.21	5.70	3667	7540	626001	873073
	40:60	3.06	5.45	3662	7416	622570	873552
	38:62	2.91	5.18	3379	7132	634537	878269
Temperature (˚C)	22	3.06	5.54	3614	7378	631036	908863
	25	3.06	5.45	3662	7416	622570	873552
	28	3.06	5.34	3664	7426	622511	886191

Table -8: Ruggedness studies

Drug	Label Claim(mg)	Amount Found (%)	
		Analyst-1	Analyst-2
Eperisone hydrochloride	150	148.45	149.29
Diclofenac sodium	100	98.28	99.56

Recovery studies:

As shown from the data in Table 6, good recoveries of the Eperisone hydrochloride and Diclofenac sodium in the range from 98 to 102 % were obtained at various added concentrations.

Table-6: Recovery study

Drug	Amount Present(µg/ml)	Amount added(µg/ml)	Total amount(µg/ml)	Amount recovered(µg/ml)	Recovery (%)* ± S.D.
Eperisone HCl	30	15(50%)	45	29.85	99.52±1.00
	30	30(100%)	60	29.65	98.85±1.21
	30	45(150%)	75	30.56	101.88±0.60
Diclofenac Na	20	10(50%)	30	20.07	100.36±0.74
	20	20(100%)	40	20.05	100.27±1.16
	20	30(150%)	50	20.20	101.0±1.51

* Average of three determinations

Analysis of pharmaceutical formulation:

Experimental results of the amount of Eperisone hydrochloride and Diclofenac sodium in pharmaceutical formulation, expressed as a percentage of label claims were in good agreement, thereby suggesting that there is no interference from any of the excipients which are normally present in formulation. In the replicate analysis (n=6) of Eperisone hydrochloride and Diclofenac sodium by proposed method showed that the content of Eperisone hydrochloride and Diclofenac sodium was 97.82% and 101.78% respectively. The retention times of Eperisone hydrochloride and Diclofenac sodium was found to be 3.07 min. and 5.56 min respectively and the result of the analysis of capsule are given in Table 5.

Table -5: Analysis of mixture by proposed method

Drug	Amount present(µg/ml)	Observed amount(µg/ml)	%Amount found* ±SD	% R.S.D.
Eperisone hydrochloride	60	58.91	97.82±0.611	0.625
Diclofenac sodium	40	40.71	101.79±0.937	0.921

*average of six determinations

Table-9: System Suitability Studies

System Suitability Parameters	Proposed Method	
	Eperisone hydrochloride	Diclofenac sodium
Retention Time (tR)	3.07	5.50
Capacity Factor (k)	2.07	4.50
Theoretical Plate Number (N)	3621	7427
Tailing Factor (T)	1.43	1.45
Resolution Factor (R)	--	10.58

System suitability studies:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions

and the results are expressed in Table 9. The values obtained demonstrated the suitability of the system for analysis of this drug combination.

CONCLUSION:

The proposed RP-HPLC method for the simultaneous estimation of Eperisone hydrochloride and Diclofenac sodium in combined capsule dosage forms is accurate, precise, linear, rugged, robust, simple, rapid, and selective. It can be adopted efficiently and easily for routine quality control (QC) analysis of raw materials, formulations and dissolution testing with accuracy and repeatability of results.

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