

Development and Validation of RP-HPLC method for simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, precise and accurate Reverse-phase High Performance Liquid Chromatographic method has been developed for the simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in combined pharmaceutical dosage form. The chromatographic separation was carried out on Phenomenex C18 column (250 × 4.6mm) by isocratic elution using 30mM phosphate buffer pH 2.5: methanol (20:80 v/v), as mobile phase at a flow rate of 1ml/min. Detection and quantification of all the analytes was carried out at 268 nm by using photo diode array detector. The retention time of Eperisone and Diclofenac was found to be 2.803 and 6.793 min respectively. The calibration curves of Eperisone and Diclofenac were linear over the concentration range of 15 - 90 µg/ml and 10 - 60 µg/ml with correlation of 0.9992 and 0.9994 respectively. The % amount of Eperisone and Diclofenac in capsule dosage form was found to be 99.64% and 99.27% respectively. The developed method was validated as per ICH guidelines. The limit of detection and limit of quantification were 0.45 and 1.36 µg/ml for Eperisone and 0.14 and 0.43 µg/ml for Diclofenac respectively. As there was no interference of excipients, the proposed method can be applied for the simultaneous estimation of the two drugs in routine quality control analysis of formulation.

Keywords: Eperisone Hydrochloride, Diclofenac Sodium, RP-HPLC, Validation, ICH guidelines

INTRODUCTION

Eperisone Hydrochloride (EPE) is chemically (2RS)-1-(4-Ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one monohydrochloride (1:1) (Fig.1). It is an antispasmodic drug exhibiting both skeletal muscle relaxant and vasodilator properties. It is official in Japanese Pharmacopoeia [1] and potentiometric method was described for its estimation. EPE acts on CNS, primarily at the level of the spinal cord, to relax hypertonic skeletal muscles by inhibiting spinal reflexes and by reducing muscle spindle sensitivity via reduction of γ -motor neuron efferent discharge. [2] The vasodilatory action of EPE enhances circulation and inhibits the pain reflex pathway. [3]

Diclofenac Sodium (DICLO) is chemically 2-(2-(2,6-

dichlorophenylamino)phenyl)acetic acid (Fig.1). It is official in IP, [4] BP, [5] and USP. [6] It is having anti-inflammatory and analgesic properties. It works by inhibiting prostaglandin synthesis by inhibition of cyclooxygenase (COX), inhibiting DNA synthesis. [7]

Literature survey reveals Liquid chromatography-ESI-tandem mass spectrometry and GC-MS methods for estimation of EPE in human plasma; [8] HPLC/MS, GC/MS, NMR, UV and IR analytical techniques to identify a degradation product of EPE in the tablets; [9] UV and HPLC methods were available for estimation of DICLO individually. [10&11] Spectrophotometric, HPLC and HPTLC methods were reported for simultaneous estimation of DICLO with other drugs. [12-15] Spectrophotometric method was available for simultaneous estimation of EPE and DICLO. [16-17] The combination of these two drugs is not official in any Pharmacopoeia, hence no official analytical method has reported for their simultaneous estimation. The aim of the present study is to develop a simple, specific, rapid, precise and accurate RP-HPLC method for simultaneous estimation of Eperisone

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Hydrochloride and Diclofenac Sodium in Pharmaceutical Dosage Form. The proposed method has been validated as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

AGILENT HPLC 1200 series with EZchrom Elite software containing C₁₈ Phenomenex column (250 × 4.6mm, 5μ) with PDA detector was employed for chromatographic separation. Digital balance (Essae) and pH Meter (ELICO LI 615) were used in the study.

Chemicals and Reagents

Eperisone Hydrochloride (EPE) was kindly gifted from Triveni Chemicals, Gujarat and Diclofenac Sodium was obtained from Nicholas Piramal Industries Ltd., Mumbai. The capsule dosage form EPRY-D SR containing EPE 150 mg and DICLO 100 mg was procured from local market. Methanol (HPLC Grade, Merck, Mumbai) was used as diluent. Potassium di hydrogen phosphate and Ortho Phosphoric acid (Fisher Scientific, Mumbai) were used in the study.

Method Development

Preparation of Mobile Phase

30mM potassium di hydrogen phosphate buffer (pH 2.5): methanol (20:80 v/v) was used as mobile phase. The buffer solution was prepared by dissolving 4.68 gm of potassium di hydrogen phosphate in 1000 mL water and pH was adjusted to 2.5 with 10% (v/v) OPA. The solution was filtered through 0.45 μm membrane filter.

Preparation of Standard Stock Solution

Accurately weighed 15 mg of EPE and 10 mg of DICLO were dissolved in methanol in 100 ml volumetric flask and the volume was made up to the mark with methanol to get a concentration of 150μg/ml EPE and 100μg/ml DICLO.

Optimization of RP-HPLC Method

Chromatographic conditions were optimized by injecting standard solution (45μg/ml EPE and 30μg/ml DICLO) into HPLC system and allowed to run in different mobile phases like Acetonitrile: water, phosphate buffer (pH4):methanol, phosphate buffer

(pH2.5):methanol and phosphate buffer (pH2.5):Acetonitrile in order to find the optimum conditions for separation of EPE and DICLO. It was found that a satisfactory separation of EPE and DICLO with less tailing factor and good resolution was achieved with mobile phase of phosphate buffer (pH-2.5): methanol (20:80 v/v), maintained at 1.0 ml/min flow rate. Detection was carried out at 268 nm. Optimized chromatographic conditions were given in Table 1. Under these conditions retention times were 2.80 min and 6.79 min for EPE and DICLO respectively.

System Suitability Studies

Freshly prepared sample solution containing 45 μg/ml of EPE and 30 μg/ml of DICLO was injected six times under optimized chromatographic conditions and the parameters were studied to evaluate the suitability of the system.

Assay of EPE and DICLO in Capsule Dosage Form

A quantity of capsule powder equivalent to 15mg EPE and 10mg DICLO was accurately weighed, dissolved in 50 ml methanol and sonicated for 10 min. The volume was made up to 100 ml with methanol to get a concentration of 150 μg/ml EPE and 100 μg/ml DICLO and the solution was filtered through Whatmann filter. 3 ml of above clear filtrate was diluted to 10 ml with mobile phase to obtain a solution containing EPE 45 μg/ml and DICLO 30 μg/ml. 10μl of sample solution was injected under the optimized chromatographic conditions with the help of auto sampler and response was recorded. The amounts of EPE and DICLO were estimated by applying obtained values to the respective regression line equation.

Validation of the Method

Validation of the proposed RP-HPLC method was carried out as per ICH guidelines by means of following parameters. [18]

Linearity (Calibration Curve)

Suitable aliquots of mixed standard stock solutions were diluted to 10 ml with mobile phase to get concentration of 15-90 μg/ml for EPE and 10-60 μg/ml for DICLO. 10 μl of each solution was injected in

to the HPLC system under the optimized chromatographic conditions and the responses were recorded. Calibration curves for EPE and DICLO were plotted using peak area versus concentration and regression equations were determined.

Precision

The precision of the proposed method was ascertained by repeatability, interday and intraday precision. Repeatability (Method Precision) of proposed method was performed by preparing and injecting the sample solution containing 45 µg/ml EPE and 30 µg/ml DICLO six times without changing the parameters of the proposed method. Intraday and Interday precision was determined by analyzing the sample solutions at different time intervals on the same day and on different days over a period of one week respectively for three different concentrations of EPE (15, 45 & 75 µg/ml) DICLO (10, 30 & 50 µg/ml).

Accuracy (Recovery Studies)

The accuracy of the proposed method was determined by recovery studies using standard addition method. The % recovery studies of EPE and DICLO were carried out in triplicate at 50, 100 and 150% levels by spiking previously analyzed samples of the tablet (30µg/ml for EPE and 20µg/ml for DICLO) with known amounts of standard drug solutions of EPE and DICLO.

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

As per ICH guidelines LOD and LOQ of two drugs were calculated using following equations.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, 'σ' is the standard deviation of response and 'S' is the slope of the calibration curve.

Robustness

Robustness of the method was evaluated by deliberate variations in the method parameters such as, flow rate variation by ± 0.1 ml/min, wavelength by ± 2 nm and composition of mobile phase by ± 2 ml organic solvent. The solution containing 45µg/ml EPE and 30µg/ml DICLO was injected under the varied

conditions and change in the responses of EPE and DICLO were noted.

Ruggedness

Sample solution containing 45 µg/ml of EPE and 30 µg/ml of DICLO was prepared and analyzed by two different analysts under same experimental conditions. The amount of two drugs was calculated and %RSD was reported.

Results and discussion

The RP-HPLC method developed for simultaneous estimation of EPE and DICLO using 30mM potassium di hydrogen phosphate buffer (pH-2.5): Methanol (40: 60 v/v) as mobile phase was validated as per ICH guidelines and results were reported below.

System Suitability Studies

The column efficiency, peak asymmetry and resolution were calculated for the standard drug solution of EPE and DICLO and the values obtained demonstrated the suitability of the system for analysis of this two drugs combination. The results were reported in Table 2.

Analysis of Marketed Formulation

Capsule dosage form was analyzed and the results of assay showed that the amount of drugs were in good agreement with the label claim of formulation as indicated by % assay, 99.64% for EPE and 99.27% for DICLO. The retention times were found to be 2.803min and 6.793min respectively (Fig.2). All the results were found to be within the limits and therefore the proposed method was found to be free from interferences from excipients. The results of analysis were reported in Table 3.

Linearity

EPE obeyed Beer-Lambert's law in the concentration range of 15-90 µg/ml with a correlation coefficient of 0.9992 (Fig.3), whereas DICLO was observed linear over the concentration of 10-60 µg/ml with correlation of 0.9994 (Fig.4). The results were reported in Table 4.

Precision

The proposed method was found to be highly precise as the % RSD values of Repeatability, intraday and interday studies were < 2% which is under the limit as per recommendations of ICH guidelines. The results were reported in Table 4.

Accuracy

The % recovery of EPE and DICLO were found to be 98.5-100.09% and 98.01-99.91% respectively which is in the limit of 98-102%. Hence the method was said to be accurate. The results were shown in Table 5.

LOD and LOQ

LOD and LOQ values of EPE and DICLO were determined from their respective calibration curves. LOD and LOQ were 0.45µg/ml and 1.36µg/ml for EPE and 0.14µg/ml and 0.43µg/ml for DICLO. The results were shown in Table 4.

Robustness and Ruggedness

Robustness studies revealed that the method was remained unaffected by the deliberate changes in wavelength, flow rate and organic phase ratio in mobile phase. The % RSD value for ruggedness studies was found to be < 2%, which shows the ruggedness of the proposed method.

CONCLUSION

A simple and economic RP-HPLC method has been developed for simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in combined dosage form and was found to be specific, robust, precise and accurate as well as having good reproducibility. It followed all the parameters for validation as per ICH guidelines. The excipients present in the formulation do not interfere in the analysis of EPE and DICLO in the method, hence the developed method can be applied successfully for routine quality control analysis of EPE and PCM in bulk and marketed formulation.

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Table 1: Conditions of Optimized RP-HPLC method

Method Parameters	Optimized value
Column	Phenomenex C ₁₈ (250×4.6mm, 5µ)
Analytical Wavelength	268 nm
Mobile phase	30mM phosphate buffer (pH-2.5): methanol (20: 80 v/v)
Pump mode	Isocratic
Flow rate	1.0 ml/min
Volume of Injection	10 µL
Run Time	10 min

Table 2: System Suitability Parameters of Epe and Diclo

Parameter	Eperisone HCl	Diclofenac Na
Retention time	2.803min	6.793min
Theoretical plates	4839	8941
Tailing factor	1.293	1.029
Resolution	-	22.72
Peak area	1582649	1020963
% RSD	0.328	0.264

Table 3: Analysis of Capsule Dosage Form

Drug	Label claim (mg)	Amount found (mg)	% Labeled amount
EPE	150	149.47	99.64
DICLO	100	99.27	99.27

Table 4: Parameters of the performance of proposed method

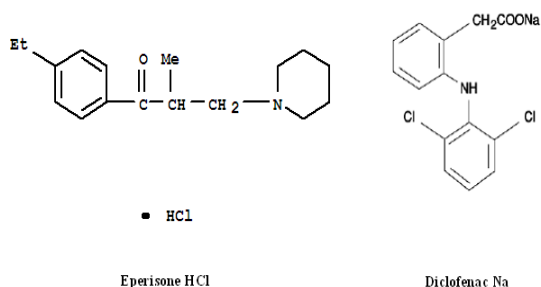
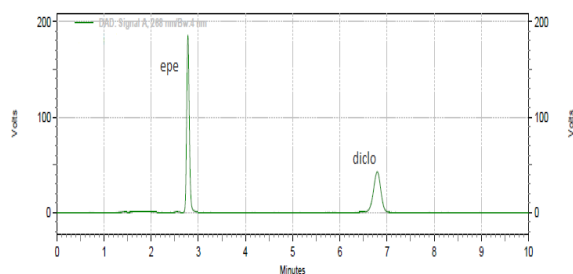
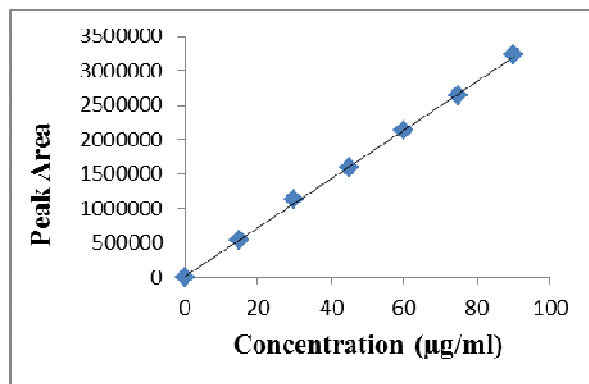
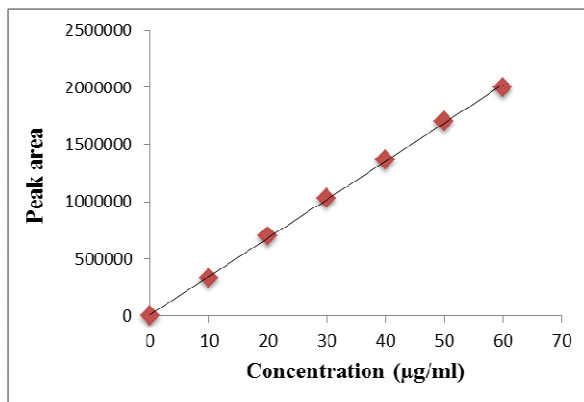
Parameter	Eperisone Hydrochloride	Diclofenac Sodium
Linearity range	15 - 90 µg/ml	10 - 60 µg/ml
Regression Equation	Y = 35416x + 14618	Y = 33557x + 12554
Correlation Coefficient	0.9992	0.9994
LOD (µg/ml)	0.45	0.14
LOQ (µg/ml)	1.36	0.43
Repeatability (%RSD, n=6)	0.32	1.03
Intra-day precision (%RSD, n=3)	0.15-0.71	0.31-0.86
Inter-day precision (%RSD, n=3)	0.24-0.87	0.47-1.56

n= number of determinations

Table 5: Recovery studies of Epe and Diclo

Drug	Level	Amount of Drug Taken ($\mu\text{g/ml}$)	Amount of Drug Spiked ($\mu\text{g/ml}$)	% Recovery* \pm SD	% RSD
EPE	50 %	30	15	98.55 \pm 0.305	0.31
	100 %	30	30	99.65 \pm 0.417	0.42
	150 %	30	45	100.09 \pm 0.832	0.83
DICLO	50 %	20	10	98.01 \pm 0.560	0.57
	100 %	20	20	98.97 \pm 1.187	1.18
	150 %	20	30	99.91 \pm 0.514	0.51

* mean of three determinations

Figure 1: Chemical Structures of Eperisone HCl and Diclofenac Na**Figure 2:** Chromatogram of Eperisone Hydrochloride & Diclofenac Sodium in Formulation**Figure 3:** Calibration Curve of Eperisone Hydrochloride**Figure 4:** Calibration Curve of Diclofenac Sodium**REFERENCES**

- Japanese Society of Pharmacopoeia. Japanese Pharmacopoeia. 15th ed. Tokyo; 2006. p. 618.
- Inoue S, Bian K, Okamura T, Okunishi H, Toda N. Mechanisms of action of Eperisone on isolated dog saphenous arteries and veins. *Jpn J Pharmacol* 1989; 50(3): 271-82.
- Fujioka M, Kuriyama H. Eperisone an antispastic agent, possesses vasodilating actions on the guinea-pig basilar artery. *J Pharmacol Exp Ther* 1985; 235(3): 757-63.
- Ministry of health and family welfare. Indian pharmacopoeia. 6th ed. Vol II. New Delhi: The Controller of Publications; 2010. p.1710.
- Medicinal and healthcare products regulatory agency. British Pharmacopoeia. Vol II. London; 2009. p. 2606.
- United States Pharmacopoeia Convention, Food and drug administration. United States pharmacopoeia National Formulary. 34th ed. Vol II. Rockville; 2011. p. 2546.
- Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2007.
- Ding L, Wei X, Zhang S, Sheng J, Zhang Y. Rapid and sensitive liquid chromatography electrospray ionization-mass spectrometry method for the determination of Eperisone in human plasma: Method and clinical applications. *Journal of Chromatographic Science* 2004; 42: 254-8.
- Ding L, Wang X, Yang Z, Chen Y. The use of HPLC/MS, GC/MS, NMR, UV and IR to identify a degradation product of Eperisone hydrochloride in the tablets. *J Pharm Biomed Anal* 2008; 46(2): 282-7.

10. Khaskheli AR, Abro K, Sherazi ST, Afridi HI, Mahesar SA, Saeed M. Simpler and faster spectrophotometric determination of Diclofenac Sodium in Tablets, Serum and Urine samples. *Pak J Anal Environ Chem* 2009; 10: 53-58.
11. Atto RA. New method for determination of Diclofenac sodium by High Performance Liquid Chromatography. *Tikrit J Pharma Sci* 2012; 8(1): 60-67.
12. Patel SA, Hariyani KP. Spectrophotometric method for simultaneous estimation of Tolperisone hydrochloride and Diclofenac sodium in synthetic mixture. *Int Res J pharma* 2012; 3(9): 162-165.
13. Marolia BP, Vanparia DJ, Satani BH, Prajapati PB, Shah SA, Shah DR. Application of RP-HPLC method for simultaneous estimation of Thiocolchicoside and Diclofenac in commercially available capsules. *American J PharmTech Res* 2012; 2(3): 806-818.
14. Nayak D, Vankar K, Patnaik A. Simultaneous Estimation of Rabeprazole Sodium and Diclofenac Sodium by RP-HPLC method in combined tablet dosage form. *Int J PharmTech Res* 2010; 2(2): 1488-92.
15. Sunil R, vidhya K. Validated HPTLC method for simultaneous estimation of Diclofenac Sodium and Misoprostol in bulk Drug and Formulation. *Asian journal of Pharmaceutical and Biological Research* 2011; 1(1): 15-21.
16. Patel PU, Patel SK, Patel UJ. Spectrophotometric estimation of Eperisone Hydrochloride and Diclofenac Sodium in synthetic mixture. *Int Res J Pharm* 2012; 3(9): 203-6.
17. Suthar J, Patel P, Shah N. Development and validation of derivative spectrophotometric method for simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in combined capsule dosage form. *Novus Int J Chem* 2012; 1(1): 24-32.
18. International Conference on Harmonization (ICH), Draft guidelines on validation of Analytical Procedure, Definition and Terminology, Federal Register. 1995; 60: 11260.

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