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Research Article

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, selective, precise and accurate Reverse Phase High Pressure Liquid Chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of eperisone hydrochloride and diclofenac sodium in marketed sustained release (SR) capsules. The method was validated for accuracy, specificity, linearity and robustness as per the ICH guidelines. The results of the developed method were found to be reproducible and within official limits of USP. Isocratic elution at a flow rate of 1.0 mL per min was employed on symmetry C_{18} column at temperature of 10^{o} C. The method was successfully applied to estimate eperisone hydrochloride and diclofenac sodium in marketed SR capsules since there was no interference from the excipients.

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

INTRODUCTION

The combination of eperisone hydrochloride (EPE) and diclofenac sodium (DIC), available in sustained release capsule dosage form, is used in the treatment of acute musculoskeletal spasm associated with low back pain[1]. The combination of these two drugs in the ratio of (EPE) 150: (DIC) 100 is approved by Central Drugs Standard Control Organization (CDSCO) in India in the year 2012.

Eperisone hydrochloride is chemically 1-(4-ethylphenyl)-2-methyl-3-piperidin-1-yl-propan-1-one (Fig. 1) and is a well known antispasmodic drug[2]. It has a relatively low incidence of central depression when compared with other antispasmodic drugs, which makes it widely useful for the therapeutic treatment of spastic patients to relieve skeletal muscle stiffness and back pain[3]. It is useful in diabetical angiopathy and Raynaud's syndrome[4]. Eperisone hydrochloride is official in Japanese Pharmacopoeia only[5] in which non-aqueous titrimetry is reported for its estimation. Many methods like HPLC[6], HPLC - MS[7], GC - MS[8] and electrospray ionization method[9] are reported for the estimation of eperisone hydrochloride.

Diclofenac sodium is phenylacetic acid derivative and chemically it is 2-(2, 6 dichlorophenylamino)

benzeneacetic acid sodium salt[10] (Fig. 2). It is a non-steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic and antipyretic activities in both animals and human beings[11]. Diclofenac sodium is used for the relief of signs and symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. It is often used to treat chronic pain associated with cancer[12]. Diclofenac sodium is official in Indian Pharmacopoeia[13], British Pharmacopoeia[14], and United States Pharmacopoeia[15]. Many methods like UV-Visible spectroscopy[16], HPLC[17], HPTLC[18], atomic absorption spectroscopy (AAS)[19] and capillary electrophoresis[19] are reported for the estimation of diclofenac sodium in single as well as in combined dosage forms.

The present article deals with the development of RP-HPLC method for the estimation of eperisone hydrochloride and diclofenac sodium from the marketed sustained release (SR) capsules.

MATERIALS AND METHODS

Reagents and chemicals

Ammonium acetate (GR grade), glacial acetic acid (GR grade) and distilled water (HPLC grade) were used for preparing the buffer solution. Acetonitrile, used as a mobile phase along with the buffer, was of HPLC grade. Methanol was also of HPLC grade. All the reagents were provided by Wanbury R & D Centre Pvt. Ltd., Turbhe, Navi Mumbai. Pure sample of eperisone hydrochloride was obtained as a gift sample from Sharon Bio-Medicine Ltd., Vashi, Navi Mumbai, and pure diclofenac sodium was provided by Pharmaceutical Products of India Ltd., Turbhe, Navi Mumbai.

Preparation of mobile phase

A freshly prepared mixture of ammonium acetate buffer (pH=6.0 adjusted with glacial acetic acid) and acetonitrile (50:50 v/v) was used as the mobile phase. Buffer solution was prepared by dissolving 3.9 g of ammonium acetate in 900 mL of water and by adjusting its pH to 6.0 ± 0.05 using glacial acetic acid. The volume was then adjusted to 1000 mL with water. Mobile phase was filtered through a 0.45 µ membrane filter and sonicated before use.

HPLC operating conditions

All chromatographic experiments were performed in the isocratic mode. The high pressure liquid chromatograph from Waters - 2487, equipped with dual wavelength absorbance detector, was used for the estimation of diclofenac sodium and eperisone hydrochloride from the marketed sustained release capsules. One more high pressure liquid chromatograph from Prominence, equipped with UV/VIS detector, was also used for the optimization studies. For both the systems, the reverse phase C_{18} column (150 x 4.6 mm, 5 µ) from Waters was used. The flow rate was maintained at 1.0 mL/min and the eluates were monitored at 256 nm. The sample was injected using a 20 µL fixed loop and the total run time was 10 min.

Preparation of solutions for assay

Standard solution: A mixture of 150 mg of eperisone hydrochloride (AR Grade) and 100 mg of diclofenac sodium (AR Grade) was transferred into 100 mL volumetric flask. The mixture was dissolved in 60 mL of methanol by sonication and the volume was made up to the mark with the same (concentration of EPE: 1500 ppm and DIC: 1000 ppm). Five mL of this standard stock solution was diluted to 50 mL with mobile phase (concentration of EPE 150 ppm and DIC 100 ppm).

Sample solution (Marketed SR capsules)

The capsule claim of each marketed SR capsule was 340 mg. The content of twenty capsules was transferred to a mortar, crushed and mixed intimately. The powder equivalent to the average weight (340 mg) of contents of one capsule was transferred to a 100 mL volumetric flask. To this powder 60 mL of methanol was added and the mixture was sonicated for 45 min in ice cold water and dissolved in methanol to make up the volume to the mark. This solution was centrifuged at 3500 rpm for 5 min. Five mL of this supernatant solution was diluted to 50 mL with mobile phase.

Optimization of HPLC method

Various mobile phases were used in order to find the optimum conditions for the separation of eperisone hydrochloride and diclofenac sodium from each other. It was found that mobile phase containing ammonium acetate buffer (pH = 6.0, adjusted with glacial acetic acid) : acetonitrile (50 : 50 v/v), at a flow rate of 1.0 mL/min with detection at 256 nm gave satisfactory results with sharp, well defined and resolved peaks with minimum tailing, as compared to the other mobile phases. Under these conditions the retention time was typically 5.1 min for eperisone hydrochloride and 2.7 min

for diclofenac sodium (Fig. 3).

VALIDATION OF THE METHOD[20-24]

The ICH guidelines were followed for validation of developed analytical method. The method was validated for the following parameters.

Linearity

Linearity was demonstrated from 50 % to 150 % of standard concentration (EPE 150 µg/mL and DIC 100 µg/mL) using minimum five calibration levels (50, 80, 100, 120 and 150 %) for eperisone hydrochloride and diclofenac sodium, which gave an assurance of analytical method with respect to linearity.

Precision

The precision of the method was studied and determined by repeatability study and by determining, interday and intraday precision. Repeatability studies were performed by analysis of three different concentrations like 50 %, 100 % and 150 % of the drug for six times on the same day. Intraday precision studies were carried out by analyzing sample solutions at different time intervals on the same day. The interday precision studies were determined by injecting the sample solutions on different days.

Accuracy

To confirm the accuracy of the proposed method, recovery studies were carried out by adding a known amount of the standard solution of a pure drug (eperisone hydrochloride and diclofenac sodium) to a pre-analyzed sample solution. These studies were carried out at 50 %, 100 % and 150 % levels. The per cent recoveries of EPE and DIC at each level and each replicate were determined. The mean per cent recovery (n=3) of both the drugs and the relative standard deviation (RSD) were calculated.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, minute variations in the optimized method parameters were done. For this purpose, the effect of change in pH of the mobile phase, change in wavelength and flow rate on the retention time, theoretical plates, area under the curve and per cent content of eperisone hydrochloride and diclofenac sodium was studied. The solution having mixture of eperisone hydrochloride (100 %) and diclofenac sodium (100 %) was injected into the sample injector of RP-HPLC system thrice under the varied conditions.

Ruggedness of method

Solution containing mixture of eperisone hydrochloride (100 %) and diclofenac sodium (100 %) was prepared and analyzed by two different analysts using the same operational and environmental conditions. From the area under the curve, the amount of both the drugs was calculated.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for eperisone hydrochloride and diclofenac sodium by RP - HPLC are summarized in Table 1.

By using the mobile phase of acetonitrile and buffer (50: 50), two drugs, eperisone hydrochloride and diclofenac sodium, could be resolved clearly from each other. The retention time for eperisone hydrochloride and diclofenac sodium was 5.1 and 2.7 min, respectively, (Fig. 3).

Linearity

The drug response was linear for eperisone hydrochloride (r^2 = 0.9999) and for diclofenac sodium (r^2 = 0.9999) over the concentration range of 50-150 µg/mL (Figures 4 and 5, respectively).

Precision

The developed method was found to be precise as the RSD value for intra-day and interday precision studies was < 2 %, which is in the limit as per the recommendations of ICH guidelines.

Analysis of marketed SR capsule

Experimental results for the amount of eperisone hydrochloride and diclofenac sodium in marketed SR capsules, were in good agreement with their label claims. This suggested that there was no interference from any of the excipients, which are normally present in marketed SR capsules. The replicate analysis (n=6) of eperisone hydrochloride and diclofenac sodium by the proposed method showed that the content of eperisone hydrochloride and diclofenac sodium in the SR capsules was 101.60 % and 102.22 %, respectively the results are summarized in table 2. The retention time of diclofenac sodium and eperisone hydrochloride was found to be 2.7 min and 5.1 min, respectively, and hence, the analysis was very fast (Fig. 3).

CONCLUSION

The proposed RP-HPLC method for the

simultaneous estimation of eperisone hydrochloride and diclofenac sodium from their mixture in the marketed sustained release capsules is simple, rapid, selective, accurate, precise, linear, rugged and robust. Hence, it can be adopted efficiently and easily for routine quality control (QC) analysis with accuracy and reproducibility of the results.

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Table 1: Summary of validation parameters

Parameter	Eperisone Hydrochloride	Diclofenac Sodium
Linearity range (µg/ mL)	50-150	50-150
Correlation Coefficient (%)	0.9999	0.9999
Precision (% RSD)	0.15	0.21
Repeatability (at 50, 100, and 150 %)	0.17, 0.11, 0.15	0.23, 0.14, 0.17
Accuracy (%)	101.60	102.22
Recovery (150 %)	98.24	100.74
Recovery (50 %)	104.27	103.71
Retention Time (min)	5.7	2.1
Robustness	Robust	Robust

Table 2: Summary of analysis of marketed SR capsules

Content	Amount Present (mg) / Capsule	Amount Found in (mg)	% RSD	Theoretical plates	Tailing Factor
Eperisone Hydrochloride	150	101.60	0.15 %	4489.88	1.90
Diclofenac Sodium	100	102.22	0.21 %	5895.89	1.25

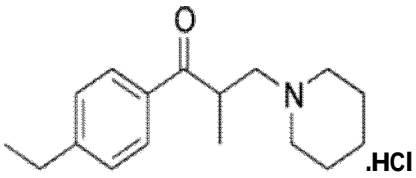

Fig. 1: Structure of eperisone hydrochloride

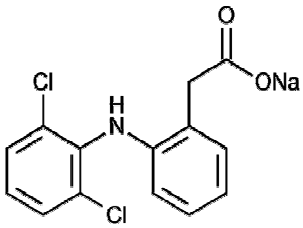

Fig. 2: Structure of diclofenac sodium

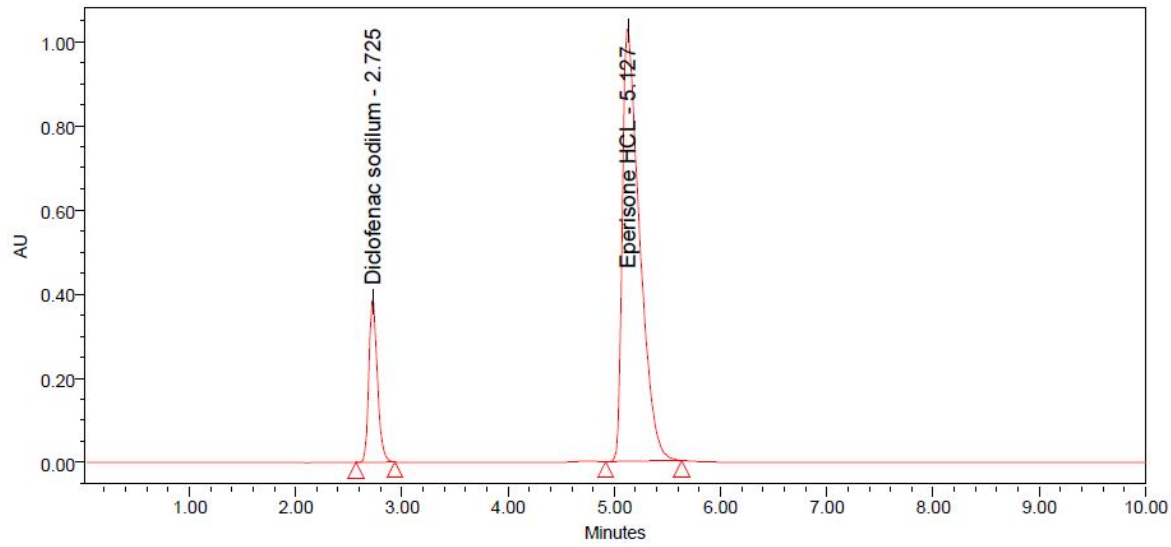

Fig. 3: Chromatogram of a mixture of diclofenac sodium and eperisone hydrochloride

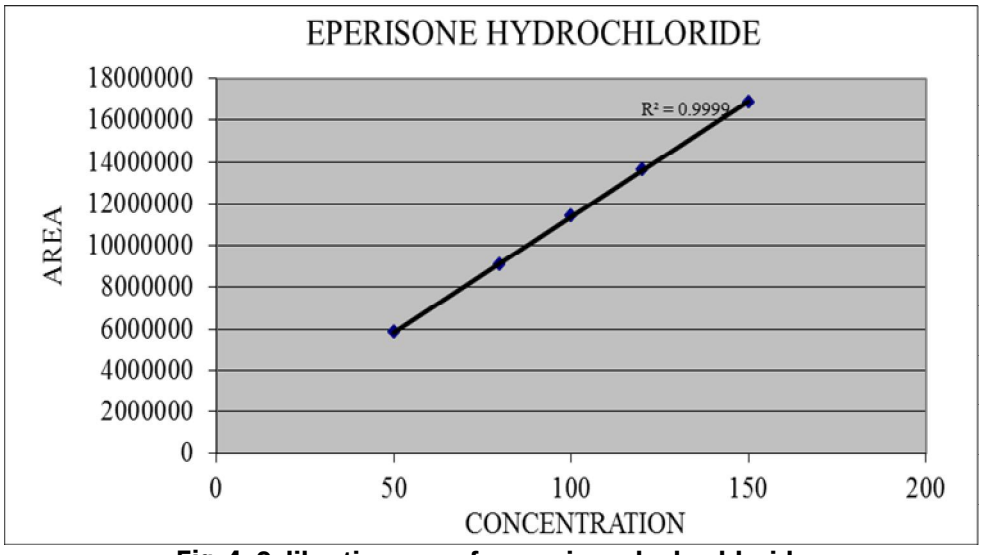

Fig. 4: Calibration curve for eperisone hydrochloride

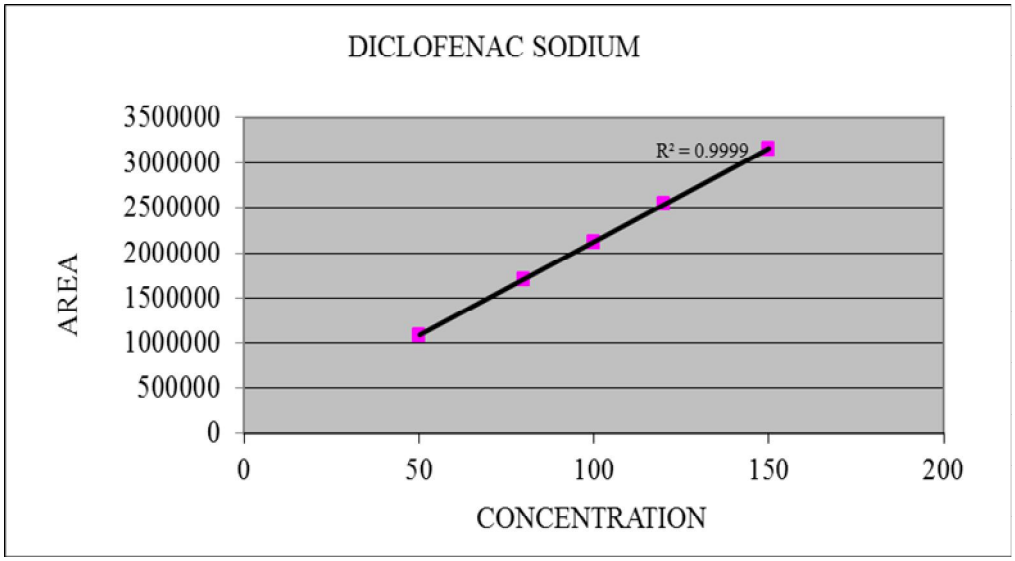

Fig. 5: Calibration curve for diclofenac sodium

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