Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [5] April 2021 : 108-115

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

ICH Guideline in Practice: Validated Inherent Stability-Indicating HPLC-Dad Method for Simultaneous Determination of Eperisone Hydrochloride and Diclofenac Sodium in Marketed Formulation

Minal T. Harde,*[1]Sameer H. Lakade,[2,3] Neha K. Kakade,[1] Laxmi R. Chaudhary,[1] Snehal V. Gosavi,[1] Rohan R. Patekar,[2] Heena B. Choudhary[2]

[1] Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Sector no. 21, Yamunanagar, Nigdi, Pune, (M.S) India.

[2] Department of Pharmaceutics, RMD Institute of Pharmaceutical Education & Research, Pune,(M.S) India.

[3] E-mail: sameer_patil97@rediffmail.com)

ABSTRACT

A simple, rapid, precise and accurate isocratic reversed-phase stability-indicating HPLC with diode array detection method was developed and validated for the simultaneous determination of Eperisone hydrochloride (EPS) and Diclofenac sodium (DLF) in commercial capsules. EPS and DLF were degraded together under different stress test conditions prescribed by International Conference on Harmonization. The samples generated were used to develop a stability-indicating high performance liquid chromatographic (HPLC) method for both the drugs. The drugs were well separated from degradation products using a reversed-phase C 18 (4.6 x 250mm, 5µm particle size) column with isocratic elution of the mobile phase comprising of acetonitrile : water, in the ratio of 90:10 v/v (pH 3.5 adjusted with OPA). The mobile phase flow rate was maintained at 0.9 ml/min with the detection wavelength used for quantification of EPS and DLF was 276 nm. The drugs were subjected to different stress conditions like neutral, acidic and alkaline hydrolysis, oxidation, photolysis and thermal degradation. Degradation products produced was a result of stress studies did not interfered with the detection of EPS and DLF, thus the assay can thus be considered stability-indicating. Analytical performance of the proposed HPLC procedure was thoroughly validated with respect to system suitability, linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. The developed procedure is also applicable to the determination of instability of the drugs in commercial formulations.

Keywords*: Eperisone hydrochloride, Diclofenac sodium, Stability indicating assay, forced degradation, HPLC, Diode array detection.*

Received 11.01.2021 Revised 01.03.2021 Accepted 29.03.2021

INTRODUCTION

Eperisone hydrochloride (EPS) is chemically, 4-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride. (Fig.1). [1]. It is official in Japanese pharmacopoeia (JP). It is estimated by potentiometric method as per JP.[2] EPS, an antispasmodic agent and a centrally acting muscle relaxant acts by relaxing both skeletal muscle and vascular smooth muscle, and demonstrates a variety of effect such as reduction of myotonia, improvement of circulation, and suppression of the pain reflex.

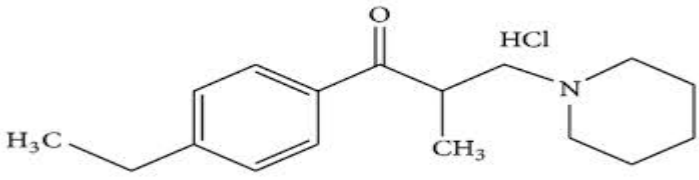


Fig. 1. Chemical Structure of Eperisone Hydrochloride (EPS)

The drug also improves dizziness and tinnitus associated with cerebrovascular disorders or cervical spondylosis. Diclofenac sodium (DLF) chemically it is 2-[2, 6 dichlorophenylamino] benzene acetic acid sodium salt (Fig. 2). DLF is official in IP, BP and USP. IP [3] & BP [4] describes liquid chromatography method & USP [5] describes the potentiometric method for estimation of DLF. It is classified as a non-steroidal anti-inflammatory agent. It is used to treat pain or inflammation caused by arthritis or

ankylosing sypondylitis.

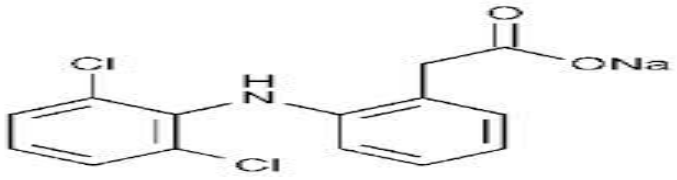


Fig. 2. Chemical Structure of Diclofenac Sodium (DLF)

In recent times, there is an increased tendency towards the development of stability-indicating assays, using the approach of stress testing as incorporated in the International Conference on Harmonization (ICH) guideline Q1AR(2). Even this approach is being extended to drug combinations, to allow accurate and precise quantitation of multiple drugs, their degradation products, and interaction products, if any. The combination capsule formulation containing EPS and DLF having antispasmodic and NSAID activity respectively and extensively used for the treatment of patients with acute musculoskeletal spasm associated with lower back pain. There are several UV [6] and HPLC [7] procedures known for the analysis of EPS and DLF individually, and some methods even exist for simultaneous analysis of the two drugs, either in a combination with other drug [8] in biological fluids or in combination [9]. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for EPS and DLF in their combined dosage forms. To our knowledge, there is no RP-HPLC-DAD method reported for the combination available of an HPLC method with high sensitivity and selectivity will be very useful for the estimation of EPS and DLF in combined pharmaceutical dosage form in presence of their degradation product. Therefore the aim of study was to develop and validate sensitive, precise, accurate and specific RP-HPLC-DAD method for determination of EPS and DLF in formulation developed using the ICH approach of stress testing. The method was also extended to marketed products.

MATERIAL AND METHODS

Materials

Working standards of pharmaceutical grade EPS, was received as gift sample from Sharon Bio-medicine Pharmaceuticals Ltd, Mumbai, India and DLF was obtained as generous gift from Emcure Pharmaceuticals Ltd, Pune. Combination products containing the two drugs were purchased from local pharmacy shop. HPLC grade acetonitrile, water and methanol were purchased from Merck Chemicals, India.

Equipment and chromatographic condition

The modular HPLC system used was equipped with Waters 510 HPLC pump with a Rheodyne injector (20 µl) and PDA 6000 LP detector. A DataAce Chromatography Data system was used to record and evaluate the data collected during and following chromatographic analysis.[10] The chromatographic separation was achieved on a Kromasil C 18, (250 mm × 4.6 mm i. d., and 5µm particle size). The eluent was monitored using photo diode array (PDA) detection at a wavelength of 279 nm. The mobile phase water: methanol: acetonitrile was used and column was maintained at ambient temperature at a flow rate of 1 ml/min. The mobile phase was filtered through a 0.45 µm nylon filter prior to use and degassed in an ultrasonic bath (Biomedica, India) [11].A precision water bath equipped with MV controller (i-therm, Biomedica, India) was used to carry out selected reactions in solution. Thermal stability study was carried out in dry air oven (Biotechnics BTI–20D, Mumbai, India). Other equipments used were sonicator (Biomedica, India), analytical balance (Schimadzu AUX 220, Japan) and autopipettes (Eppendorf, Hamburg, Germany).

Degradation studies

The study was intended to ensure the effective separation of EPS, DLF and its degradation peaks in the bulk drug and marketed formulation. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. In general, degradation studies were carried out at a concentration of 1 mg/ml of each drug and capsule formulation in the solution. All samples were then diluted in mobile phase to give a final concentration of 60 & 40µg/mL for EPS and DLF respectively and filtered before injection in the chromatographic system. For hydrolysis in water, the solution was refluxed for 3h at 70°C. Acid decomposition was carried out in 0.5 M HCl and refluxed for 2h at 70°C and alkaline degradation was conducted using 1M NaOH and refluxed for 1h at 70°C. After cooling the solutions were neutralized and diluted with mobile phase. Degradation was also carried out in solid state by exposing pure drugs and formulation to dry heat at 70°C for 6 h, and in dark. The photochemical stability of the drug was studied by exposing the stock solution to intense UV radiation for 24h. Oxidation study was carried out using 6% oxidizing agent hydrogen peroxide for 5h at room temperature. Samples were withdrawn periodically and subjected to analysis after suitable dilution. The same stress conditions were applied to placebo and blank solution [12-14].

Development of method

HPLC studies were carried out on all the reaction solutions containing API individually, and on a marketed formulation. The separations were achieved by isocratic elution using acetonitrile: water (90:10 v/v, pH adjusted to 3.5 with OPA) as a mobile phase. It was filtered through 0.45 µm nylon filter and degassed before use. The injection volume was 20µl and mobile phase flow rate was 0.9 ml/min. The detection was carried out at 276 nm [15-16].

Preparation of tablets for assay

Twenty tablets were weighed, crushed and mixed in a mortar and pestle for 20 min. A portion of powder equivalent to 37.5mg EPS and 25mg DLF was accurately weighed and transferred into each of six 25 ml A-grade volumetric flasks, 15 ml mobile phase was added and sonicated for 20 min for complete dissolution of the EPS and DLF and the solutions were then diluted up to volume with mobile phase. Aliquots of the solution were filtered through a 0.45µm nylon filter. From the filtrate, 1ml of the filtered solution was transferred to a 25ml A-grade volumetric flask and made up to volume with mobile phase, to yield final concentrations of drugs in the range of linearity previously described. [17, 18].

Validation of the method

The method was validated for linearity, precision (inter-day, intra-day), accuracy, specificity, selectivity, LOD, LOQ and robustness. Standard plots were constructed for both EPS and DLF in the range of 100-500 and 20- 100 µg/ml, respectively. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively. The method precision and system precision was determined by repeating the experiment six times. Accuracy was determined by fortifying the mixture of degraded solutions with three known concentrations of the drugs. Further, specificity of the method was assessed by study of the resolution factor of the drug peaks from nearest resolving peaks. The selectivity was determined by checking peak purity of all the peaks, including those of degradation products, using a PDA detector [19-23].

RESULTS AND DISCUSSION

Development of the stability-indicating HPLC method and its Degradation behavior

An isocratic method was found necessary to optimize the separation of major degradation products formed under various stress conditions. The best resolution was achieved with initial run of acetonitrile: water in the ratio of (90:10 v/v, pH 3.5 adjusted with OPA). The obtained chromatogram is represented in fig. 3 shows the Rt of EPS and DLF at 3.59 and 9.95 respectively. The method worked well with the mixture of degradation solutions and was even applicable to degraded formulations. Fig. 4(a–f) shows the obtained chromatographic resolution of EPS and DLF from its degradation product generated during various stress conditions.

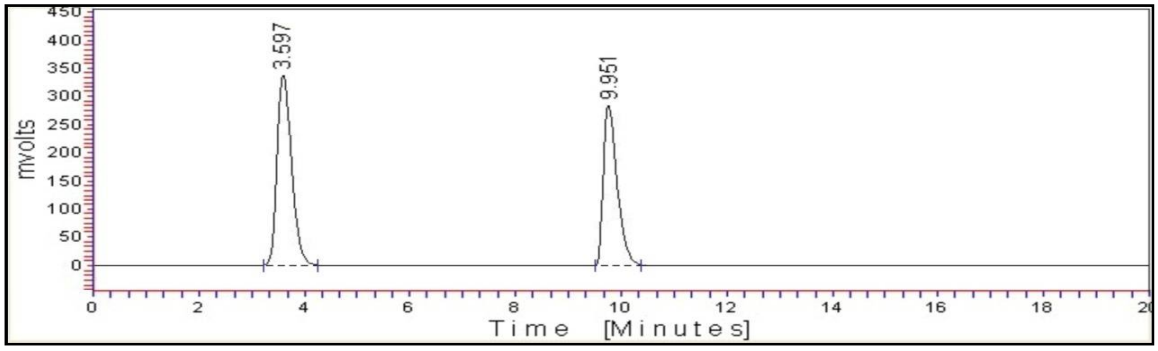


Fig. 3. Typical Chromatogram of EPS (Rt = 3.59 min) and DLF (Rt= 9.95 min) respectively.

Degradation behavior

HPLC studies on the combination under different stress conditions indicated the following degradation behavior.

Acidic condition

Both the drugs were found to be highly labile showed degradation within 2h at 70oC in 0.5M HCl. EPS showed little higher degradation as compared to DLF. The major degradation products formed were at retention times (RTs) 1.3, 2.2 and 7.2 min.

Degradation in alkali

The combination was refluxed in alkaline condition. EPS showed degradation within 1h at 70^{o}C in 1M NaOH, while DLF was found stable in alkaline condition. The major products appeared at RTs 2.4, and 6.3

min.

Neutral (water) degradation

Sufficient degradation was observed upon refluxing the combination for 3h at 70ºC. DLF showed little higher degradation as compared to EPS. The major degradation products appeared at RTs 0.6, 5.1 and 5.6 min.

Oxidative degradation

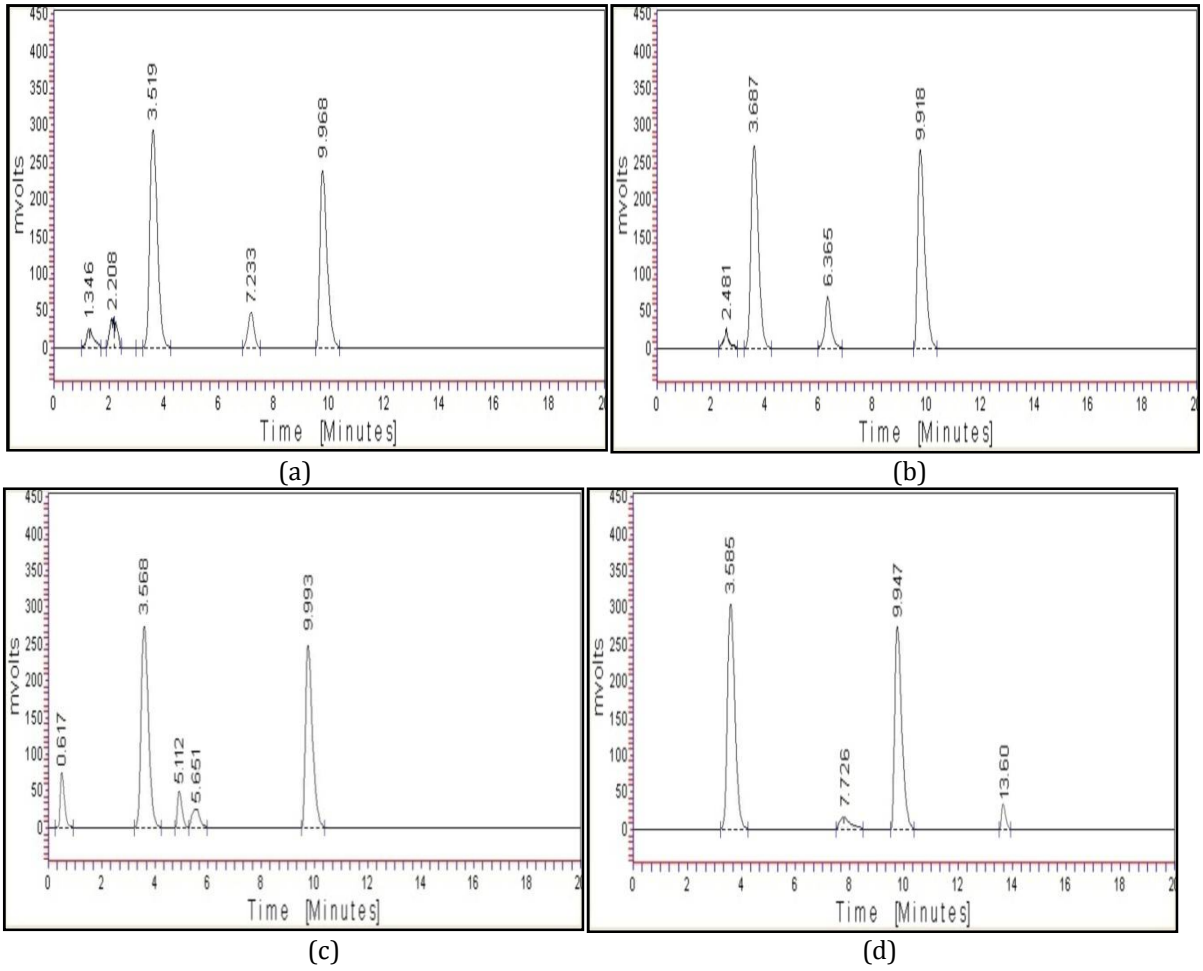
The drugs showed sufficient degradation when the combination was degraded in 6% H_2O_2 for 5h at room temperature. DLF found to be more prone to oxidation than EPS. The two major degradation products appeared at 7.7and 13.6 min.

Photolytic degradation

Drugs were exposed to UV radiation for 24 h, similar to oxidative degradation, DLF was found to be more susceptible to photolytic degradation than EPS. The major degradation products found at 10.7, 11.1 and 12.0 min.

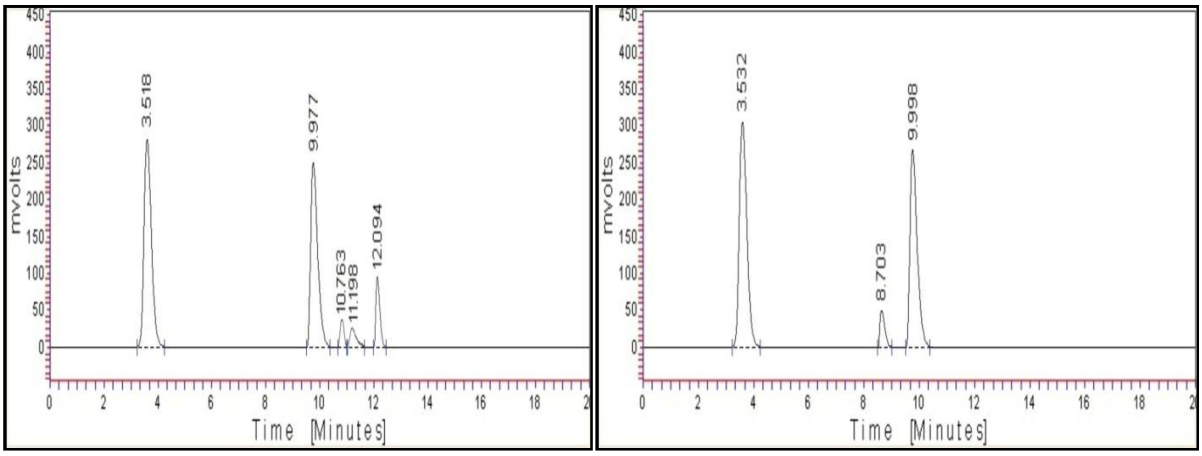
Thermal degradation

Dry heat degradation studies showed that the combination was unstable in dark. Enough degradation was observed when the combination was exposed to dry heat at 70 ˚C for 6h. DLF showed appreciable stability and did not influenced for thermal degradation while EPS undergone degradation. The major degradation products observed at 8.7 min.



(a) (b)

(c) (d)



(e) (f)

Fig. 4. Indicates the obtained chromatograms of stress studies of EPS and DLF as: a) Acid degradation b) Alkali degradation c) Neutral degradation d) Oxidative degradation e) Photolytic degradation f) Thermal degradation

Validation of the developed stability-indicating method

The analytical method was validated with respect to parameters such as linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity, recovery and robustness/ruggedness.

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve The constructed calibration curves were linear over the concentration range of 100–500µg/ml and 20–100µg/ml for EPS (n = 3) and DLF (n = 3), respectively. Peak areas of EPS and DLF were plotted versus their respective concentrations and linear regression analysis performed on the resultant curves. Correlation coefficients (n=3) were found to be 0.999 for both the drugs with %RSD was found to be > 2. Typically, the regression equations were: $y = 1709x + 1422$ ($r^2 = 0.999$) for EPS and $y = 710.5\ xs + 0.047$ ($r^2 = 0.999$) for DLF, respectively.

LOQ and LOD

The LOQ was determined as the lowest amount of analyte that was reproducibly quantified above the baseline noise following triplicate injections. The LOQ that produced the requisite precision and accuracy was found to be 3.36µg/ml for EPS and 2.45µg/ml for DLF, respectively. The LOD was determined based on signal-to-noise ratios and was determine using an analytical response of three times the background noise. The LOD for both EPS and DLF were found to be 0.96µg/ml and 0.82µg/ml, respectively.

Precision

The intra- and inter-day variability or precision data were summarized in table 1 and 2 respectively and were assessed by preparation of standard solutions to produce solutions of three different concentrations of EPS and DLF. Repeatability or intra-day precision was investigated by injecting six replicate samples of each of the samples of three different concentrations. Inter-day precision were assessed by injecting the sample of three different concentrations over three consecutive days.

Table.No.01: Intra-day Precision Data

Parameters	EPS			DLF		
	Concentration (µg/ml)			Concentration (µg/ml)		
	48	60	72	32	40	48
% Estimated	99.57	99.40	99.45	99.55	99.22	99.26
S. D.	± 0.5139	± 0.8328	± 0.4952	± 0.3734	± 0.7355	± 0.3437
C. V	0.5161	0.8378	0.4979	0.3751	0.7413	0.3462

* Mean of six determinations, S.D: Standard Deviation, C.V: Coefficient of variance

Table.No.02: Inter-Day Precision Data

Parameters	EPS			DLF		
	Concentration (µg/ml)			Concentration (µg/ml)		
	48	60	72	32	40	48
% Estimated	99.14	99.40	99.13	99.45	99.92	99.78
S. D.	± 0.4457	± 0.6833	± 0.5663	± 0.5888	± 0.6420	± 0.6445
C. V	0.4495	0.6874	0.5712	0.5920	0.6425	0.6459

* Mean of six determinations, S.D: Standard Deviation, C.V: Coefficient of variance

Accuracy

Accuracy study was performed by standard addition method by adding pure drug in a powder of marketed formulation at three different levels 80%, 100% and 120%. In each case, the percent relevant error and %RSD was calculated and found to be less than 0.59 for EPS and 1.71 DLF. The data obtained from recovery study for the determination of each compounds of interest are summarized in table 3.

Table No.03: Statistical Validation for Recovery Study

Level of recovery	% Mean Recovery		Standard Deviation		% R.S.D.		S.E	
	EPS	**DLF**	**EPS**	**DLF**	**EPS**	**DLF**	**EPS**	**DLF**
80 %	98.94	99.33	±0.229	±0.590	0.231	0.593	0.132	0.341
100 %	99.29	99.38	±0.588	±0.671	0.592	0.675	0.339	0.387
120 %	99.26	98.41	±0.547	±1.689	0.551	1.716	0.316	0.975

*Average of three determinations, R.S.D: Relative Standard deviation, S.E.M: Standard error of mean

Specificity

The results of stress testing studies in addition to that of monitoring standard solutions of each drug in the presence of their degradants indicated a high degree of specificity of this method for both EPS and DLF. The degradation product(s) of each of the parent compounds was found to be similar for both the tablets and API powders assessed. The method has sufficient specificity and selectivity as the two drugs and even degradation products were well separated from each other, with the resolution factor of >2 in all cases. All the peaks were pure, which was proved through PDA purity studies. Data of peak purity index and purity threshold values indicates the degradants peaks are well separated from the drug peak. The established mass balance study ensured that all degradants were adequately detected. The above study is shown in table 4.

Table No. 04: Mass balance and Peak Purity study

Stress Condition	% degradation		Purity Angle		Purity Threshold		% Assay		Mass Balance	
	EPS	DLF	EPS	DLF	EPS	DLF	EPS	DLF	EPS	DLF
Acid 0.5M HCl for 2h at 70°C	20.0	17.23	0.092	0.428	0.586	1.028	78.43	82.7	98.43	99.93
Alkaline 1 M NaOH for 1h at 70°C	23.12	-	0.141	0.257	0.791	0.988	76.26	98.53	99.38	98.53
Neutral H_2O for 3 h at 70°C	18.14	23.05	0.268	0.192	0.886	0.863	81.66	76.06	99.8	99.11
Oxidative 6 % H_2O_2 for 5h at 70°C	7.24	18.73	0.376	0.326	0.751	0.927	92.81	80.28	100.0	99.01
Photolytic 24 h in UV-Vis radiation	6.97	26.01	0.405	0.387	0.911	0.723	91.91	73.99	98.88	100
Thermal 6h at 70°C	12.84	0.0	0.229	0.492	0.837	1.101	86.87	98.97	99.71	98.97

*Average of three determinations

Robustness

The robustness of the method was investigated under a variety of conditions including changes of detection wavelength, flow rate and of organic phase composition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operators has proven that the method is robust as R.S.D was found to be <1%.

CONCLUSION

This study presents a simple, rapid, accurate, precise, economic and validated stability-indicating HPLC method for simultaneous estimation of EPS and DLF in the presence of degradation products. The result of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveals that the method is selective and stability-indicating. The method could be applied with success even to the analysis of marketed products, as no interference was observed due to excipients or other components present.

CONFLICT OF INTEREST

We have no conflict of interest to declare.

ACKNOWLEDGMENTS

The authors are thankful to Emcure Pharmaceuticals Ltd, Pune, India and Sharon Bio-medicine Pharmaceuticals Ltd, Mumbai, India for providing gift sample Diclofenac sodium and Eperisone hydrochloride, respectively as a pure drug. Authors are also thankful to Principal, Modern college of pharmacy for providing necessary instrumental and infrastructure facility.

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CITATION OF THIS ARTICLE

Minal T. Harde, Sameer H. Lakade, Neha K. Kakade, Laxmi R. Chaudhary, Snehal V. Gosavi, Rohan R. Patekar, Heena B. Choudhary. ICH Guideline In Practice: Validated Inherent Stability-Indicating Hplc-Dad Method For Simultaneous Determination Of Eperisone Hydrochloride And Diclofenac Sodium In Marketed Formulation. Bull. Env.Pharmacol. Life Sci., Vol10[5] April 2021 :108-115