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Stability indicating RP-HPLC method for determination of eperisone hydrochloride and diclofenac sodium in tablet dosage form

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Abstract

A simple HPLC method has been developed and subsequently validated for the simultaneous determination of Eperisone hydrochloride and Diclofenac sodium. Optimum chromatographic separations among the eperisone, diclofenac sodium and stress-induced degradation products were achieved by using Waters -ODS 5 µ C18 column (250 X 4.6mm) as stationary phase with Acetonitrile and 0.05% TEA pH 3.5 (75:25% v/v) as mobile phase at a flow rate of 1.0 mL min with detection at 273 nm. ICH guidelines were used to validate the developed method. Linearity was established for Eperisone hydrochloride and Diclofenac sodium in the range of 15-75 µg/ml and 10-60 µg/ml, respectively. Eperisone hydrochloride and Diclofenac sodium were exposed to acid, base and neutral hydrolysis, oxidation and photolytic stress conditions and the stressed samples were analyzed by the proposed method. Also the kinetics of degraded sample was evaluated for all the hydrolytic conditions. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in combined dosage form.

Keywords: Eperisone hydrochloride, Diclofenac sodium, Kinetics, Validation.

Introduction

Eperisone is (2RS)-1-(4-ethylphenyl)-2-methyl-3-(1-piperidyl)propan-1-one. It is an antispasmodic drug. Eperisone acts by relaxing both skeletal muscles and vascular smooth muscles, and demonstrates a variety of effects such as reduction of myotonia, improvement of circulation, and suppression of the pain reflex. The drug inhibits the vicious cycle of myotonia by decreasing pain, ischaemia, and hypertonia in skeletal muscles, thus alleviating stiffness and spasticity, and facilitating muscle movement.

Diclofenac sodium is 2-(2-(2, 6-dichlorophenylamino) phenyl)acetic acid. A non-steroidal anti-inflammatory analgesic with potent cycloxygenase inhibition activity. This drug is commonly used for pain control and treatment of rheumatic diseases.

There are several papers available for Development and validation of RP-HPLC method for simultaneous estimation of Eperisone hydrochloride and Diclofenac sodium in capsule dosage form. The present paper has focused on stability testing of Eperisone and Diclofenac sodium using solid state and solution state stress testing and the quantitative determination of drug in presence of its degradation product.

Materials and Method

1. Eperisone and Diclofenac (EPERI and DICLO)

- **Mix stock solution (ED):** An accurately weighed quantity about 30.0 mg of EPERI and 20.0 mg of DICLO was transferred in 100.0 mL volumetric flask, dissolved in mobile phase and volume made up to the mark with mobile phase (concentration: 300 µg/mL of EPERI and 200 µg/mL of DICLO).
- **Mix working standard Solution (ED1):** A 1.0 mL portion of the above solution was further diluted up to 10.0 mL with mobile phase (concentration is 30.0 µg/mL of EPERI and 20.0 µg/mL of DICLO).
- **Preparation of mobile phase:** Approximately 0.5 mL of Triethyl amine was dissolved in 1000.0 mL of double distilled water and pH was adjusted to 3.5 with ortho-phosphoric acid. The mobile phase was prepared by mixing 0.05% Triethyl amine buffer (pH-3.5) and Acetonitrile in the ratio 25:75% v/v. Mobile phase was sonicated and filtered through 0.45 µm membrane filter paper.

A 20.0µL of solution ED1 was injected through manual injector and chromatogram was recorded. The chromatogram of blank and standard is shown in Fig. 1a and 1.b respectively.

2. Study of Linearity (Calibration Curve)

- **Eperisone and Diclofenac Sodium (EPERI and DICLO):** Aliquots of solution**(ED)**prepared earlier were further diluted with mobile phase so as to get concentration in the range 15 to 75 µg/mL of EPERI and 10- 60 µg/mL of DICLO. The plot of curve area vs concentration was constructed and is shown in Fig. 2.a and 2.b for EPERI and DICLO respectively.

3. **Stability Testing Studies:** To evaluate the stability indicating ability of the proposed methods, drugs were exposed to the various environmental conditions (stress testing) like acidic, alkaline, oxide, photolytic, thermal exposure were applied and the drugs were estimated in presence of their degradation products (if any).
   - **General procedure for preparation of Exposed formulation /Standards (DICLO and EPERI):** Twenty tablets were weighed, powdered and thoroughly mixed. Accurately weighed quantity of tablet powder equivalent was transferred to a series of different 25 mL volumetric flasks. To it 10 mL of reagent (acid, alkali, 3% hydrogen peroxide and distilled water) were added. Each flask was placed in oven at 50°C. Exposed marketed formulation was withdrawn at an interval of 30, 60, 90, 120, 150, 180 min and standard at 180 min. Each flask was cooled to room temperature and mobile phase was added to adjust the volume up to the mark. The contents were sonicated for 20 min. The solution was filtered separately through whatmann filter paper (no. 41). It was again further diluted and all the samples were chromatographed separately using optimized chromatographic conditions.

   The amount of un-degraded drug in sample and standard was calculated using the formula (1) and (2).

- **Formula for sample ----------(1)**

$$\% \text{ Drug un-degraded} = \frac{Au_{(expose)}}{As_{(unexposed)}} \times \text{Conc of std}_{(unexposed)} \times \text{Dilution factor} \times \frac{Avg.wt}{Wtab_{(exposed)}} \times \frac{100}{L.C.}$$

- **Formula for standard---------- (2)**

$$\% \text{ Drug un-degraded} = \frac{As_{(exposed)} \times \text{Conc of std}_{(unexposed)}}{As_{(unexposed)} \times W\ std_{(exposed)}} \times \text{Dilution factor} \times 100$$

Where,

Au	=	peak area of sample (exposed)
As	=	peak area of Standard (unexposed)
W std	=	wt of std drug
W tab	=	wt of tablet powder
Avg.wt	=	Average weight of tablet
L.C.	=	Label Claim in mg of tablet

DICLO and EPERI (100 and150)

4. **Hydrolysis studies DICLO and EPERI:** Accurately weighed quantities of standard and sample EPERI (~15 mg) and DICLO(~10 mg) were transferred to a series of 6 different 25.0 mL dry volumetric flasks. To it 10.0 mL of reagent (0.5M HCl, 0.5M NaOH, 3% H_2O_2 and distilled water) was added and placed in oven at 50˚C for a period of 180 min. After 180 min, a 0.5 mL portion of solution was further diluted to get Concentration of 30 µg/mL for EPERI and 20µg/mL for DICLO. The chromatograms of Marketed Formulation and standard are shown in Fig 3.a to 3.h for acidic, alkaline, neutral and oxidative hydrolysis of exposed standard and marketed formulation respectively.

5. **Solid State Analysis**
   - **Preparation of Standard and Marketed Formulation Preparation of Standard Solution (Exposed):** An accurately weighed quantity exposed standard was withdrawn and transferred to 25.0 mL volumetric flask. To it 10.0 mL of mobile phase was added, shaken and volume was made up to the mark with mobile phase. Further dilution was made with mobile phase so as to get the final concentration. A 20 µL volume was injected and chromatographed.
   - **Preparation of Sample Solution (Stressed condition):** An accurately weighed quantity of powdered marketed formulation equivalent to desired weight was transferred to25.0 mL volumetric flask. To it 10.0 mL of mobile phase was added, shaken and volume was made up to the mark with mobile phase. The content was sonicated for 20 min and filtered through whatmann filter paper (no.41). Further dilution was made so as to get the final concentration (on label claim basis). A 20 µL volume was injected and chromatographed.

6. **Humidity studies (40˚C/75% RH):** It was performed by placing standard drug and tablet powder (Marketed formulation) in humidity chamber separately. The drugs were spread in separate petridish and exposed to 40˚C/75% RH. The standard and Marketed formulation(as per

label claim) and marketed formulation were withdrawn the 1st, 5th, 10th and 15th day. A 20µL volume of standard and Marketed sample solution were chromatographed separately.

- **Photo stability studies**

a. **UV Light:** According to ICH Guidelines sample should be exposed to light to providing an overall illumination of not less than 200 watt hours/ square meter. The stability chamber was calibrated using appropriate UV meter for UV Light study and the study was carried out for 24h and 48 h.

Standard drug and tablet powder was exposed by spreading in two separate petridishes and kept in stability chamber under UV light exposure. The standard drug (as per label claim) and marketed formulation were withdrawn after 24h and 48 h.

b. **Thermal studies:** It was performed by keeping standard drug and marketed formulation in oven at 50°C for 3 h. Standard drug and tablet powder was spread in separate petridishes and kept in oven at 50°C. The standard drug (as per label claim) and marketed formulation were withdrawn after3 h.

c. **Humidity studies /UV Light / Thermal studies for DICLO and EPERI:** Accurately weighed quantity of standard and sample EPERI (~15 mg) and DICLO(~10 mg)was transferred to 25.0 mL volumetric flask. The standard and sample of marketed formulation were prepared by following the general procedure as described earlier to get concentration of EPERI 10 µg/mL and PARA 65 µg/mL. The Overlain chromatogram for standard and marketed formulations humidity studies, UV light and thermal studies are shown in Fig. 4a to 4e respectively.

d. **Kinetics of Solution State Degradation Studies:** The kinetics of degraded sample was evaluated for all the hydrolytic conditions. The plot of regression coefficient (r) obtained and the best fit observed indicates the order of degradation reaction.

- Values of concentration against time (zero- order kinetics)
- Log of concentration verses time (first-order kinetics)
- Reciprocal of concentration verses time (second-order kinetics)

Application of proposed method for assay of drugs in marketed formulations (DICLO and EPERI)

- **Standard solution:** The standard solution was prepared in similar manner as described under study of system suitability parameters (Concentration-30 µg/mL EPERI and 20 µg/mL DICLO).
- **Sample solution:** Twenty capsules were weighed removing capsule shell and average weight was calculated. The contents were triturated thoroughly and mixed. An accurately weighed quantity of capsule powder ~ 150 mg EPERI (~100 mg DICLO) was transferred to 100.0 mL volumetric flask. The mobile phase was added to the flask, shaken and volume was made up to the mark with mobile phase. The content was sonicated for 20 minutes and was filtered through whatmann filter paper (no.41). A 1.0 mL of the filtrate was diluted to 25.0 mL with mobile phase. A 5.0 mL of the filtrate was diluted to 10.0 mL with mobile phase. The sample solution was then prepared and Injected the Concentration 30 µg / mL of EPERI and 20 µg / mL of DICLO. The chromatogram is shown in Fig. 5. The estimation of DICLO and EPERI in marketed formulation is shown in Table 4.

Results and Discussion

1. **Selection of Chromatographic parameters:** Selection of chromatographic parameters like mobile phase (composition), flow rate, detection wavelength for the analysis of the drug was done on trial and error basis that will give well defined peak, symmetrical and resolved peaks. Wavelength was such selected that drug shows sufficient absorption units in terms of mili-volts.
2. **Hydrolysis studies DICLO and EPERI:** From the chromatogram results were calculated using the formula 1 and 2. DICLO and EPERI was found to degrade around 19-25% and 18-31% in alkaline, acid, neutral as well as oxidative hydrolysis. The study of chromatograms 3.a to 3.h shows the presence of two additional peaks at Rt 7.053 and 8.14. The observation and results are shown in Table 1.1 - 1.4 for acidic, alkaline, neutral and oxidative hydrolysis of exposed standard and marketed formulation respectively.
3. **Humidity studies /UV Light / Thermal studies for DICLO and EPERI:** The amount of drug undegraded for each standard and sample and calculated using formula 1 and 2. In humidity studies, standard DICLO and EPERI were found to degrade around 44% and 34% while in exposed formulation DICLO and EPERI degraded to 45% and 60% respectively. Also it was observed that for standard drug DICLO and EPERI degraded to 4% and 6% and in formulation EPERI degraded to 9% under UV light while DICLO was stable towards UV light. In thermal exposure for DICLO and EPERI, degrade 4% and 7% degradation in standard drug and 20% and 28% in exposed formulation. The observation and results of humidity /light/thermal studies are shown in Table 2.1, 2.2 and 2.3 respectively.
4. **Kinetics of Solution State Degradation Studies:** The kinetics of solution state degradation was studied using acid, alkali, oxidative, and neutral

hydrolysis. The observation and results of kinetics of degradation is shown in Table 3.

5. **Validation of Proposed Method**

- **Accuracy studies:** Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The %RSD for EPIRE and DICLO was found to be 0.334 and 1.2604 respectively.
- **Precision:** Precision of proposed method was ascertained by replicate analysis of homogeneous samples. Precision of any analytical method is expressed as S.D and % RSD of series of measurements.
- **Ruggedness:** The ruggedness of the proposed method was ascertained by carrying out the analysis of marketed formulation under three different conditions i.e. Intraday (same day), Interday(different day) and different analyst.

The sample was prepared and analyzed at intervals of 0h, 1h, 2h, and 3h for intraday study and on 1st, 3rd, and 7thday for interday study. % RSD for interday precision was found to be 0.5466 and 0.5602 for EPERI and DICLO. The % RSD for intraday precision was found to be 0.4786 and 0.7779 for both drugs. The results are shown in Table 5.1 and 5.2 for different times, for different days and for different analyst. All parameters of % RSD are less than 2. This clearly indicates that proposed method is precise.

- **Linearity and Range:** Eperisone and Diclofenac (EPERI and DICLO). The procedure for the determination of linearity and range is as same as that for assay of marketed formulation. The plot of Concentration vs AUC was obtained and the data for linearity clearly indicates that proposed method is Linear. The observations are shown in Table 6.
- **Robustness:** Deliberate change was made in the optimized chromatographic parameters and robustness of the method was studied by evaluating system suitability parameter data after varying the flow rate, detection wavelength and change in mobile phase pH. The observation so recorded are shown in Table 7.
- **Limit of Detection and Limit of Quantification:** The standard deviation of Y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ for all the three drugs using the following formulae.

$$LOD = 3.3\ (\sigma)/SLOQ = 10\ (\sigma)/S$$

Where, σ = Standard deviation, S= slope

Table 1.1: Observation and Results under Acid Hydrolysis and alkaline hydrolysis

Sr. No.	Time (min)	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
1	30	----	----	102.70	99.38
2	60	----	----	100.33	97.62
3	90	----	----	98.75	93.95
4	120	----	----	95.93	91.90
5	150	----	----	94.16	89.84
6	180	80.64	74.07	87.81	86.87

Table 1.2: Observation and Results under Alkali Hydrolysis

Sr. No.	Time (min)	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
1	30	----	----	100.94	88.84
2	60	----	----	98.42	85.08
3	90	----	----	98.17	83.31
4	120	----	----	97.20	79.74
5	150	----	----	95.27	78.42
6	180	81.13	72.55	92.13	71.28

Table 1.3: Observation and Result under Neutral Hydrolysis

Sr. No.	Time (min)	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
1	30	----	----	94.69	101.81
2	60	----	----	94.03	97.95
3	90	----	----	90.14	95.04
4	120	----	----	81.54	89.51
5	150	----	----	82.91	81.19
6	180	96.83	82.32	74.62	79.70

Table 1.4: Observation and Result under Oxidative Hydrolysis

Sr. No.	Time (min)	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
1	30	----	----	98.19	96.33
2	60	----	----	93.65	91.32
3	90	----	----	90.91	90.87
4	120	----	----	89.12	89.60
5	150	----	----	85.14	83.86
6	180	76.23	69.34	79.64	76.30

Table 2.1: Observation and Results under Humidity studies

Stressed condition	Interval time	% Drug Un-degraded			
		STD		MF	
		EPERI	DICLO	EPERI	DICLO
Humidity studies	Day-1	98.26	90.20	70.37	88.13
	Day-5	76.71	78.17	56.52	64.33
	Day-10	66.53	54.80	41.21	56.19

Table 2.2: Observation and Results under UV light studies

Stressed condition	Interval time	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
UV Light	24h	98.14	100.06	101.39	89.56
	48h	99.12	103.72	101.03	91.17

Table 2.3: Observation and Results under Thermal studies

Stressed condition	Interval time	% Drug Un-degraded			
		STD		MF	
		DICLO	EPERI	DICLO	EPERI
Thermal Studies	3 h	96.85	92.98	79.80	78.66

Table 3: Observation and Results of kinetics of degradation studies

Sr. No.	Degradation study	Condition	Value of 'r'		Order of reaction	
			DICLO	EPERI	DICLO	EPERI
1	Acid Hydrolysis	0.5M HCl, 50˚C	0.940	0.992	First–order	First–order
2	Alkali Hydrolysis	0.5M NaOH, 50˚C	0.933	0.951	First–order	Zero-order
3	Oxidative Hydrolysis	3% H_2O_2, 50˚C	0.975	0.892	Zero-order	Zero-order
4	Neutral Hydrolysis	Distilled Water, 50˚C	0.917	0.970	Zero–order	Zero–order

Table 4: Observation and Results of estimation in marketed

EPERY DSR – 150 mg EPERI and 100mg DICLO Average wt. = 453.8 mg							
Sr. No.	Wt. of tablet powder taken (mg)	AUC OF MF((µV)		Amt. estimated in Avg. wt. of tablet (mg)		% Labeled claim	
		DICLO	EPERI	DICLO	EPERI	DICLO	EPERI
1	444.2	1877459	1781685	99.95	149.38	99.959	99.59
2	455.2	2004429	1855250	102.28	151.80	102.28	101.20
3	441.3	1865835	1793380	98.20	151.39	98.20	100.93
4	453.2	1956908	1875885	99.72	153.28	99.72	102.19
5	457.2	1997618	1892143	101.48	154.14	101.48	102.76
6	439.6	1882004	1760966	99.87	148.32	99.87	98.88
				Mean		100.25	100.92
				± S.D.		1.4388	1.483
				% R.S.D		1.435	1.424

Table 5.1: Observation and Results of Intraday and Interday Estimation

Sr. No.	Time	Wt of tablet powder taken (mg)	A.U.C of Sample (µV)		% Labeled Claim		Time	A.U.C of Sample (µV)		% Labeled Claim	
			EPERI	DICLO	EPERI	DICLO		EPERI	DICLO	EPERI	DICLO
1	**0h**	455.2	1855250	2004429	101.20	102.28	**DAY-1**	1831413	1974251	99.90	100.75
2	**1h**		1845169	1996008	100.65	101.85	**DAY-3**	1824084	1961704	99.50	100.10
3	**2h**		1836009	1987180	100.15	101.40	**DAY-7**	1814006	1944068	98.95	99.20
4	**3h**		1832880	1978560	99.98	100.96	--	--	--	--	--
Mean					100.49	101.62	--	--	--	99.45	100.01
± SD					0.5493	0.5693	--	--	--	0.476	0.778
% RSD					0.5466	0.5602	--	--	--	0.4786	0.7779

Table 5.2: Observation and Results for Analyst to Analyst variation

Sr. No.	Time	Wt of tablet powder taken (mg)	A.U.C of Sample (µV)		% Labeled Claim	
			EPERI	DICLO	EPERI	DICLO
1	**Analyst-1**	439.6	1760966	1882004	99.88	99.87
2	**Analyst-2**	452.1	1852643	1968610	101.75	101.14
3	**Analyst-3**	445.2	1791850	1899790	99.93	99.12
	Mean				100.52	100.04
	± SD				1.065	1.021
	% RSD				1.059	1.020

Table 6: Observation of Linearity and Range

Sr. No.	Wt. of tablet powder taken equivalent to % Label claim	A.U.C. (µV)	
		EPERI	DICLO
1	80	1378923	1537325
2	90	1582431	1726260
3	100	1768966	1955241
4	110	1958636	2167525
5	120	2160896	2405406

Table 7: Observation and Result of Robustness study

Sr. No	Deliberate Changes	R.T.		Asymmetry		Theoretical plates	
		DICLO	EPERI	DICLO	EPERI	DICLO	EPERI
1	Standard Condition	2.444	4.484	1.319	1.212	9926	7840
2	Change in flow rate (1.1mL)	2.221	4.045	1.360	1.294	9725	7650
3	Change in flow rate (0.9mL)	3.018	5.243	1.314	1.304	10065	8010
4	Change in Wavelength (278nm)	2.412	4.420	1.382	1.293	10045	7995
5	Change in Wavelength (268nm)	2.410	4.450	1.360	1.291	10245	7660
6	Change in pH (3.3)	2.450	4.521	1.401	1.267	9810	7565
7	Change in pH (3.7)	2.429	4.312	1.421	1.335	9895	7790
8	Change organic phase (+10%)	2.010	3.910	1.415	1.370	9745	7535
9	Change organic phase (-10%)	2.965	5.250	1.394	1.345	9985	7890
	± SD	0.321	0.462	0.040	0.046	0.918	0.961

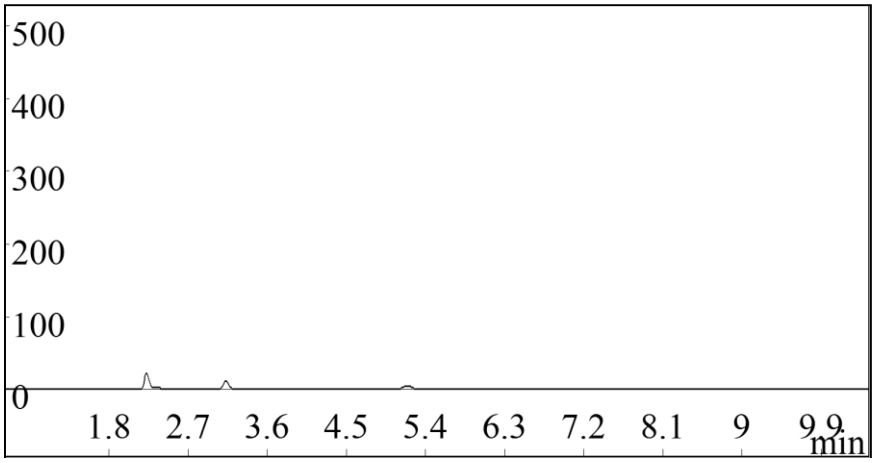

Fig 1.a: Chromatogram of Blank

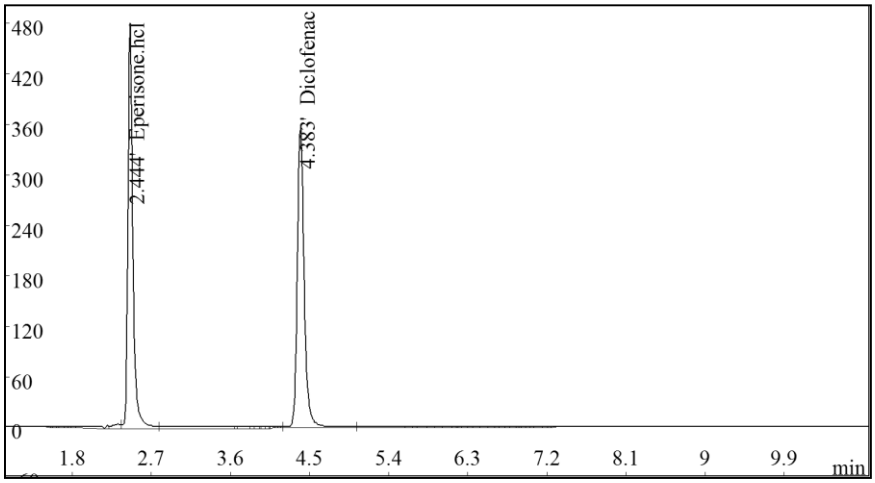

Fig 1.b: Chromatogram of Standard EPERI and DICLO

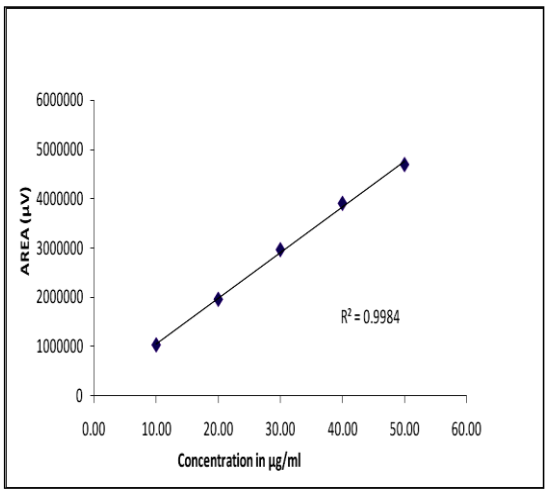

Fig. 2.a: Linearity curve for EPERI

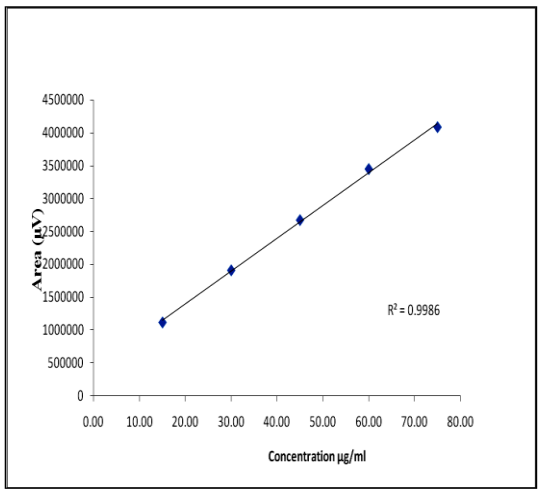

Fig. 2.b: Linearity curve for DICLO

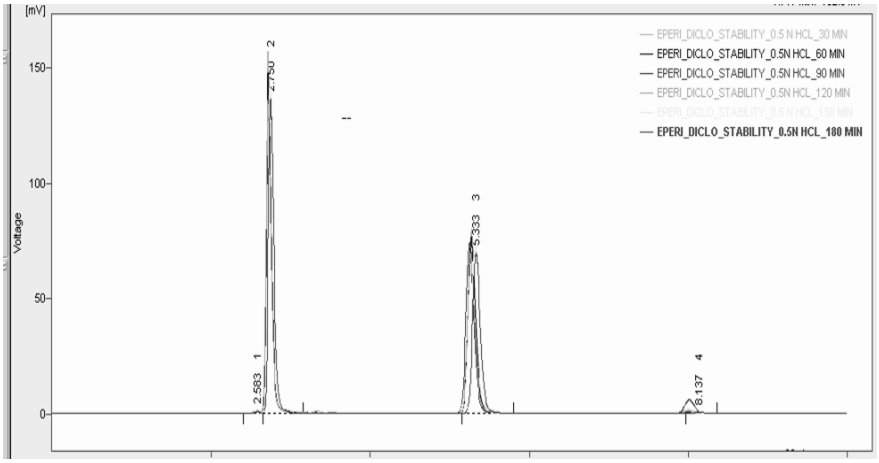

Fig 3.a: Overlain Chromatogram of MF under Acid hydrolysis

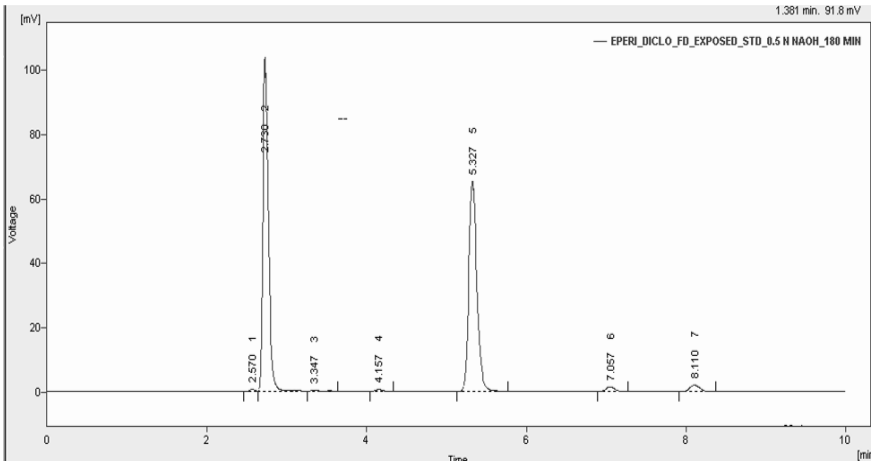

Fig 3.b: Chromatogram of mix Standard under Acid hydrolysis

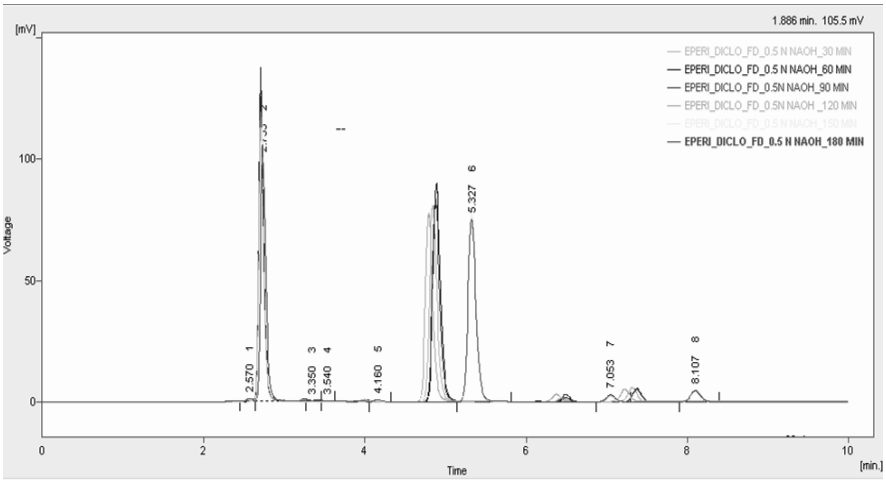

Fig 3.c: Overlain Chromatogram of MF under Alkali hydrolysis

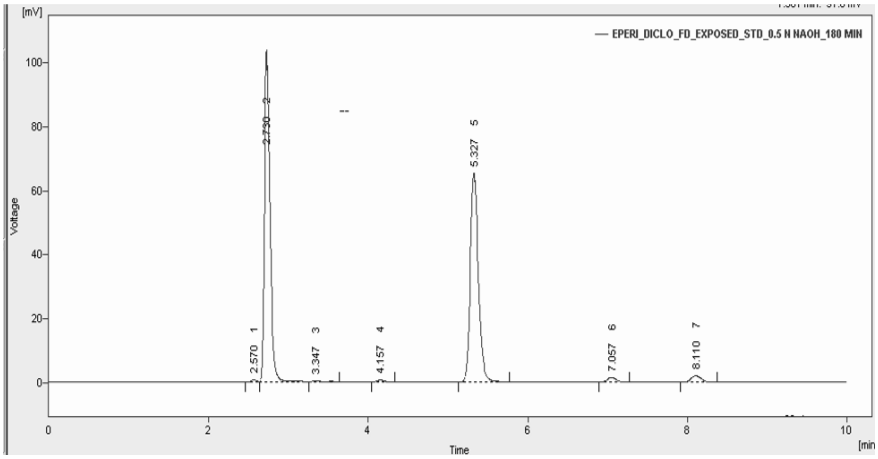

Fig 3.d: Chromatogram of Standard under Alkali hydrolysis

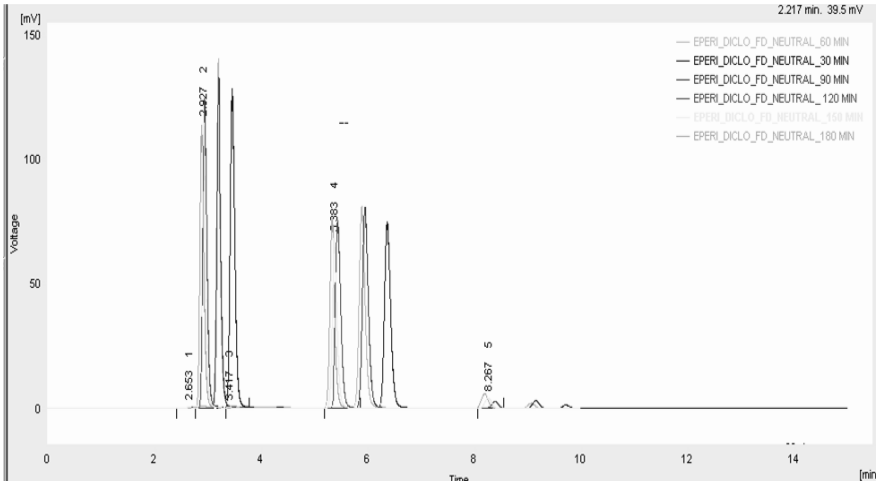

Fig 3.e: Overlain Chromatogram of MF under Neutral hydrolysis

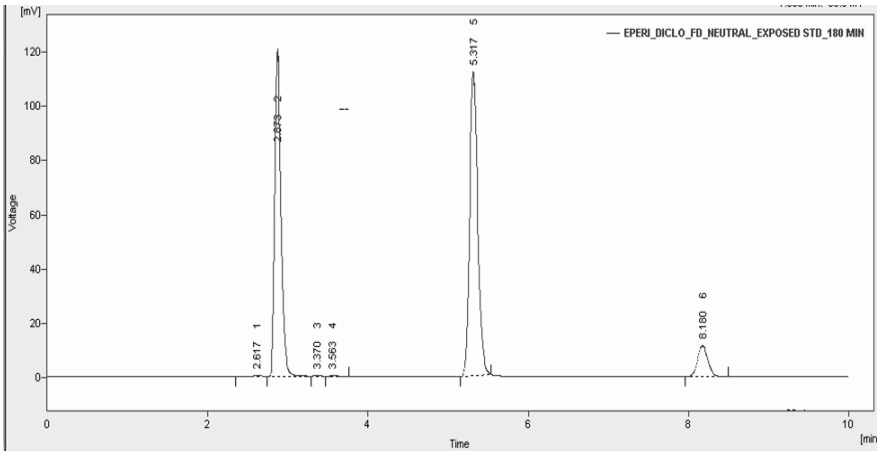

Fig 3.f: Chromatogram of Standard under Neutral hydrolysis

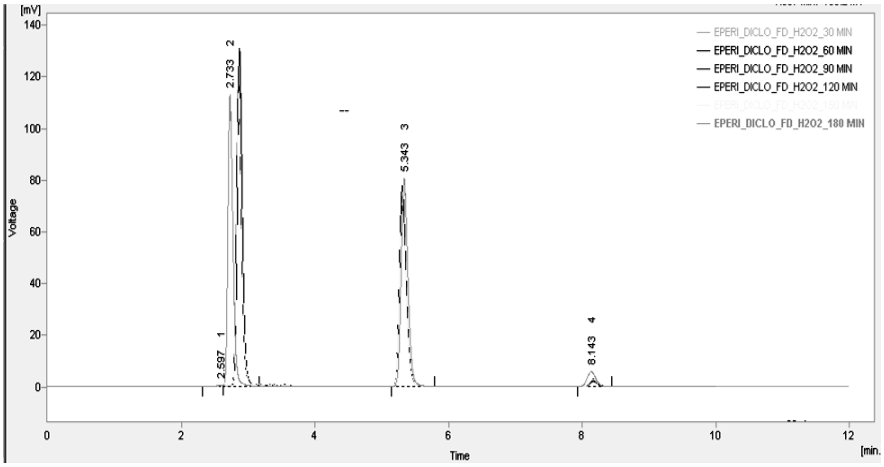

Fig 3.g: Overlain Chromatogram of MF under Oxidative hydrolysis

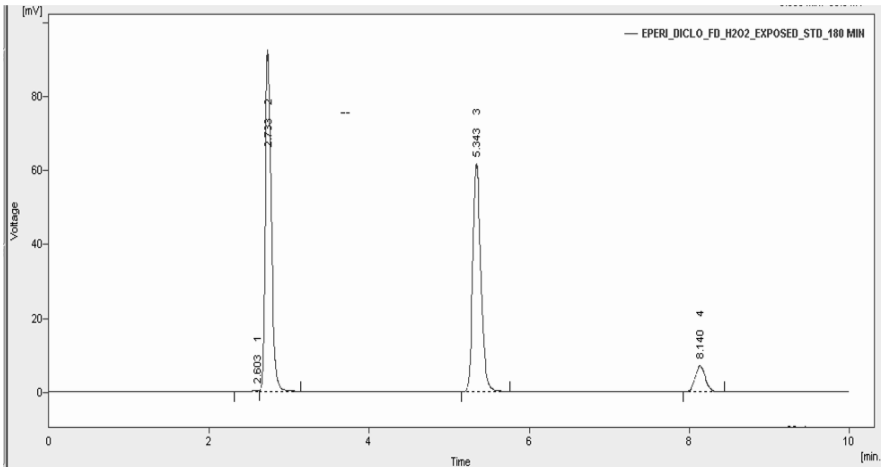

Fig 3.h: Chromatogram of Standard under Oxidative hydrolysis

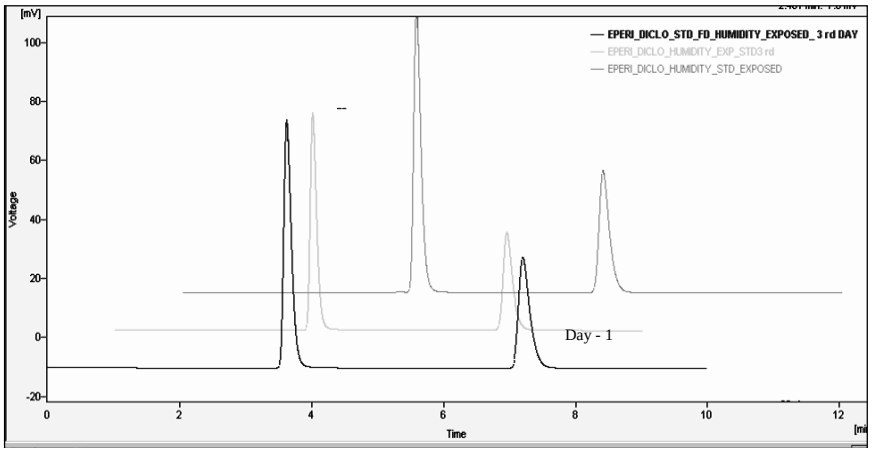

Fig 4.a: Overlain Chromatogram of mix Standard under Humidity studies

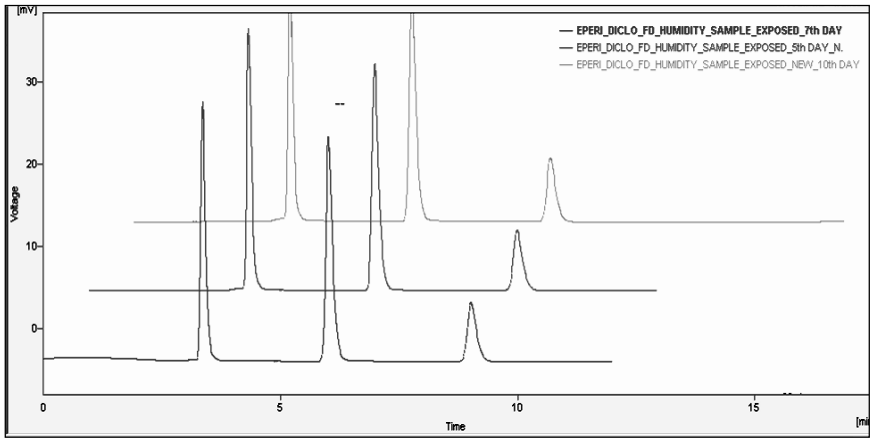

Fig 4.b: Overlain Chromatogram of MF under Humidity studies

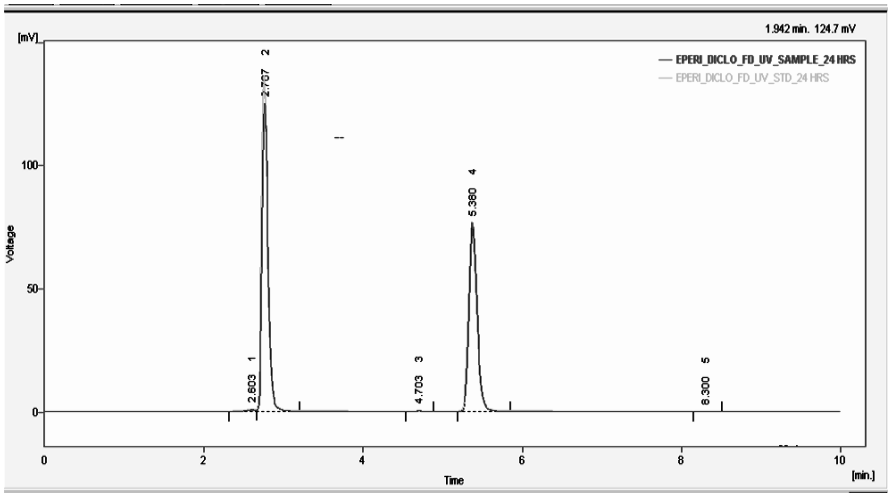

Fig 4.c: Overlain Chromatogram of MF and mix standard under UV Light after 24h

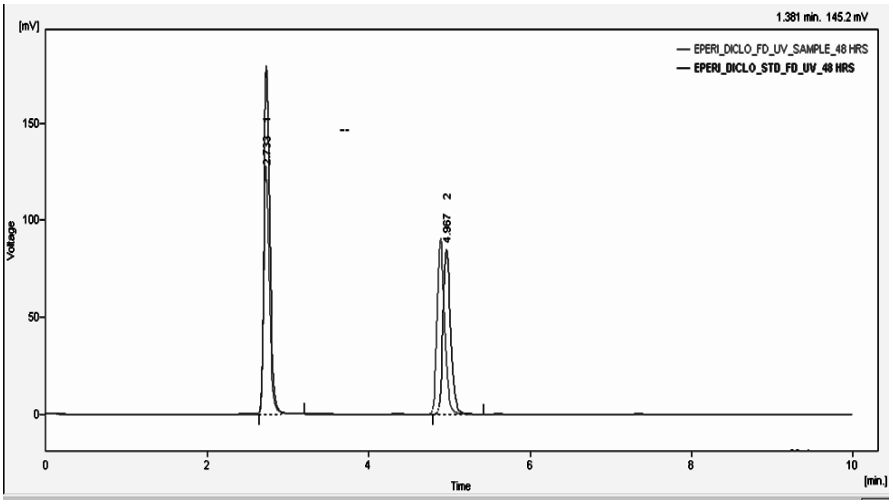

Fig 4.d: Overlain Chromatogram of MF and mix standard under UV Light after 48h

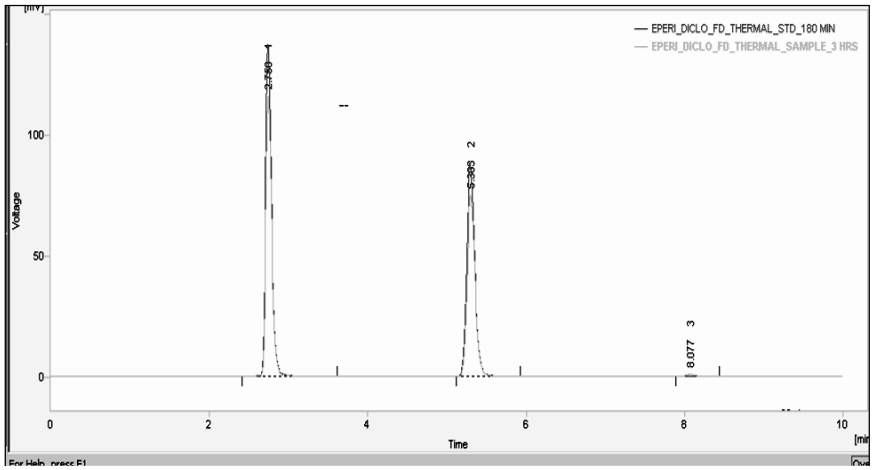

Fig 4.e: Overlain Chromatogram of MF and mix standard under Thermal Studies

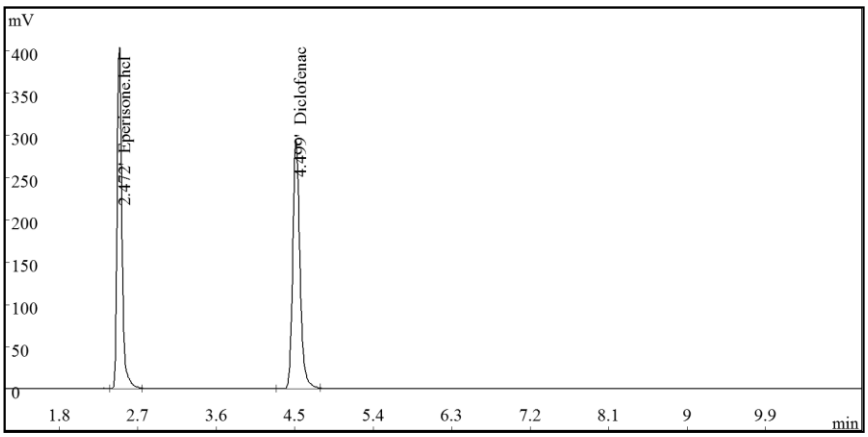

Fig 5: Chromatogram of Marketed formulation of DICLO and EPERI

Conclusion

Review of literature reveals that no stability indicating HPLC method was reported for estimation of Eperisone and Dcilofenac sodium in combined solid dosage form. So, the HPLC method has been developed for estimation of Eperisone and Dcilofenac sodium in combined tablet dosage form. Simple, accurate, precise and reproducible method has been developed. The proposed method gives good resolution of Eperisone, Diclofenac Sodium and degradant product in degradation study as no interference was found with the degradants formed under various stress conditions.

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