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Research Article

STABILITY INDICATING CHIRAL HPLC METHOD FOR THE ESTIMATION OF ZALTOPROFEN ENANTIOMERS IN PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: A stability indicating chiral high performance liquid chromatographic (HPLC) method was developed and validated for the separated (S) and (R) Zaltoprofen in raw material and its determination in the presence of degradation products formed during forced degradation studies

Methods: In the present study an isocratic NP-HPLC method was developed with stationary phase as ACI Cellu 1 (150 x 4.6 mm i.d., 5 µ) column and acetonitrile: 25 mM sodium perchlorate (80:20, v/v) as mobile phase. The entire study was performed using 1.0 mL/min as flow rate and the detection wavelength at 254 nm. The zaltoprofen (R and S) was exposed to various stress condition such as hydrolytic (acid and base), neutral, oxidative and photolytic. The stressed samples were analyzed by the proposed method.

Results: The described method was linear over the range of 2 - 4 µg/mL for S-Zaltoprofen and 3 - 5 µg/mL for R-Zaltoprofen. The Limit of detection and limit of quantification of S-Zaltoprofen and R-Zaltoprofen were found to be 4.16 µg/mL and 12.61 µg/mL respectively. The recovery study of S and R-Zaltoprofen from tablets formulation ranged from 98.04 %.

Conclusion: The method provides good sensitivity and excellent precision and reproducibility. The developed method can be applied in the quality control of drug products.

Keywords: Stability-indicating method**,** Validation**,** Chiral, Zaltoprofen.

INTRODUCTION

Zaltoprofen (ZLT) is a non-steroidal anti-inflammatory drug, and has excellent effects even on post-surgery or post-trauma chronic inflammation. The chemical name of ZLT is (±)-2-(10, 11-dihydro-10-oxodibenzo [b,f] thiepin-2-yl) propionic acid and its structure[1] is shown in Fig 1. ZLT selectively inhibits cyclooxygenase-2 activity and prostaglandin E2 production; it is used in the treatment of rheumatoid arthritis, osteoarthritis, and other chronic inflammatory Pain conditions. ZLT is a unique compound that also has anti-bradykinin activity. It is not only of cyclooxygenases but also of bradykinin-induced 12-lipoxygenase inhibitors. Earlier publications have described a high-performance liquid chromatography (HPLC) methodology useful for the quantification of ZLT in human plasma samples was reported [1-8].

The 2-arylpropionic acids are an important group of chiral NSAIDs, most of which are marketed as racemates, even though they are known to be stereo selective in both action and disposition. Although the prostaglandin synthetase inhibiting effect of zaltoprofen is attributable to the S antipode, in therapeutics it continues to be used in the racemic form.

The stability-indicating assay methods (SIAMS) are employed for the analysis of stability samples in pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, requirement of establishing stability-indicating assay method (SIAM) has become mandated [9]. The guidelines explicitly require conduct of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc. and separation of drug from degradation products.

The developed method is expected to allow analysis of individual degradation products. The ICH guideline Q1A (R2) [10] emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy of the drug, must be examined by validated stability indicating testing method. As per Q1 (R2) information on the stability of the drug substance is an integral part of the systematic approach to stability evaluation. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways, the intrinsic stability of the molecule, and validate the stability indicating power of the developed analytical procedures. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved.

From the literature review it is observed that no stability indicating assay method for the determination of zaltoprofen enantiomers was available keeping in the view of susceptibility of zaltoprofen enantiomers under variety of conditions. It was felt that an (SIAMS) that separates the drug enantiomers from their degradation products formed need to be developed.

MATERIALS AND METHODS

Solvents and chemicals

Acetonitrile (HPLC grade), sodium perchlorate were supplied by Qualigens fine chemicals and S.D. Fine chemicals. Reference standards of Zaltoprofen enantiomers were procured from Sigma Aldrich limited, Mumbai, India. Working standard of Zaltoprofen RS (99.20%) was obtained as gift sample from Shanghai Titanchem Co Ltd., China. Commercially available tablets Zaltoprofen were purchased commercially from the local market, Ooty, Tamilnadu, India.

Apparatus and instrument conditions

Chromatographic separation and quantitative determination were performed using a high pressure liquid chromatography (HPLC) containing a Shimadzu gradient pump (LC-20 AD solvent delivery system), a UV detector (Shimadzu).

The stationary phase, ACI Cellu 1 (150 x 4.6 mm i.d., 5 µ particle size) was used. The mobile phase consisted of 80 volumes of acetonitrile and 20 volumes of sodium perchlorate. The flow rate of the mobile phase was 1.0 mL min, with the detection wavelength at 254nm.

Preparation of Standard solution

The stock solutions containing 1 mg/mL of S and R form of Zaltoprofen were prepared in methanol. These stock solutions were

stored in air tight containers. Aliquots of S-Zaltoprofen (2 - 4 μg/mL) and R-Zaltoprofen (3 - 5 μg/mL) were prepared in the mobile phase for analysis.

Preparation of sample solution

Twenty tablets were weighed, to determine the average weight and finely powdered. The powder equivalent to 5 mg of S and R form of Zaltoprofen was accurately weighed and transferred into a 10 mL volumetric flask. To this 5 mL of mobile phase was added and sonicated for 10 min. The resulting solution was made up to 10 mL with mobile phase and filtered using whatmann filter paper No. 42. The components R and S enantiomers of Zaltoprofen from one formulation (Zaltokin-80 tablet containing 80 mg of Zaltoprofen RS of Ipca Laboratories Limited) were extracted in mobile phase. The standard and sample solutions were analyzed by the optimized chromatographic conditions, the chromatograms were recorded.

Forced degradation study of Zaltoprofen

In order to establish whether the analytical method and the assay were indicating stability, pure active pharmaceutical ingredient of both zaltoprofen enantiomers was forced under various stress conditions to conduct degradation studies. As the two enantiomers are freely soluble and stable in methanol, it was used as a co-solvent in all the forced degradation studies. All solution used in forced degradation studies, were prepared by dissolving API in small volume of methanol and later diluted with aqueous hydrogen peroxide, distilled water, aqueous hydrochloric acid or aqueous sodium hydroxide to achieve a concentration of 100 mcg/mL of zaltoprofen. Photo degradation studies were performed in methanol. The solutions were exposed to sunlight during the day time for 4 days. The resultant solutions were analyzed every day, control samples which were protected from light with aluminum foil were also placed in the day light concurrently, and studied for any degradation with the optimized chromatographic condition.

RESULTS AND DISCUSSION

Optimized of chromatographic conditions

A solvent combination of acetonitrile: sodium perchlorate (80:20, % v/v) on an ACI Cellu 1 (150 x 4.6 mm i.d., 5 μ) as stationary phase gave satisfactory separation of S and R Zaltoprofen and their degradation products formed under various stress conditions. The detection was carried out at 254 nm with a flow rate of 1.0 mL/min. The retention times of Zaltoprofen R and S were observed to be 3.1 min and 4.5 min, respectively (Fig. 1 and 2).

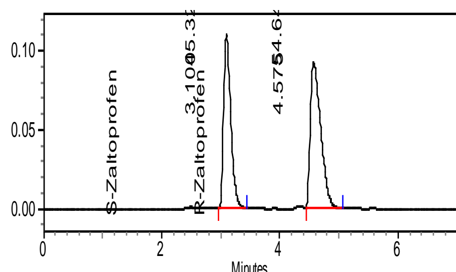

Fig. 1: Typical HPLC chromatogram of Zaltoprofen standard solution

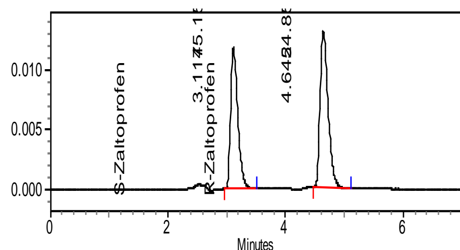

Fig. 2: Typical HPLC chromatogram of Zaltoprofen sample I solution

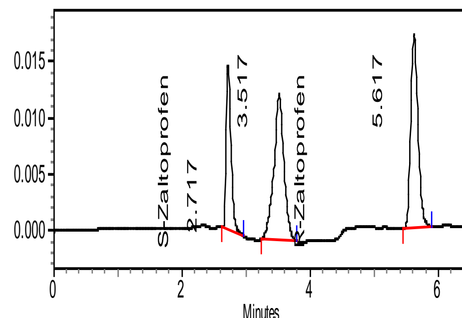

Fig. 3: Typical HPLC chromatogram of acid degradation sample of Zaltoprofen (R&S) with 0.1N HCl at 24 hrs

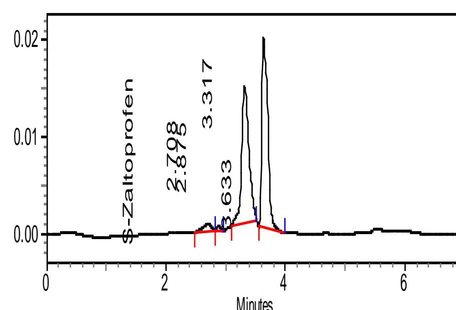

Fig. 4: Typical HPLC chromatogram of basic degradation sample of Zaltoprofen (R&S) with 0.1N NaOH at 24 hrs

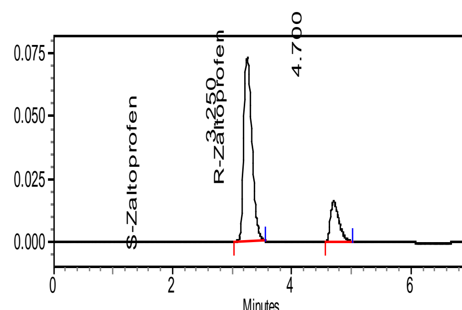

Fig. 5: Typical HPLC chromatogram of oxidative degradation sample of Zaltoprofen (R&S) with 3% H_2O_2 at 24 hrs

The HPLC studies of samples obtained on stress testing of zaltoprofen enantiomers under different conditions suggested the following degradation behaviors (Table 3). Complete degradation of R and S zaltoprofen was found with either 1N HCl or 1N NaOH hence 0.1N HCl or 0.1N NaOH was used for the degradation studies after 1, 2, 4, 8, 12 and 24 h (Fig. 3,4 and 5).

Zaltoprofen enantiomers were found to be stable under acidic condition up to 6 h. Only around 4% of the zaltoprofen enantiomers were degraded through 24 h and the main analyte were eluted at 3.1 and 4.5 min during HPLC analysis. In alkaline stress conditions only 25% decomposition of the zaltoprofen enantiomers was degraded as compared to the standard solution of the drug. The drug enantiomers were completely degraded when utilized 30% H_2O_2 but falling by 8% by using 3% H_2O_2 through 24 h (Fig.3). Under neutral conditions R and S zaltoprofen enantiomers were found to be stable up to 24 h, moreover very slight degradation was observed on exposure of solution and solid drug powder to sunlight. Temperature stress studies R and S zaltoprofen enantiomers were found to be stable at 24 h.

The calibration curves of S- Zaltoprofen and R- Zaltoprofen were linear in the range of 2-4 μg/mL and 3-5 μg/mL respectively. Linear

regression equation and correlation coefficient are shown in (Table 1). The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % RSD were calculated. The % RSD values were found to be less than 2% revealed that, the method were precise. The accuracy of the optimized methods was determined by absolute recovery experiments. An analysis of the results showed that the percentage recovery values were close to 100 % thus establishing that the developed method is accurate and reliable (Table 2). Detection limits and quantification limits of S-Zaltoprofen and R-Zaltoprofen were found to be 4.16 µg/mL and 12.61 µg/mL respectively (Table 3). No marked changes in the chromatogram occurred on changing the instrument; operator and chromatographic conditions indicated that the developed method was rugged and robust. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. Signal to noise ratio of 3 and 10 are generally considered as limit of detection (LOD) and limit of quantification (LOQ), respectively. The LOD and LOQ of S and R zaltoprofen obtained by the method were 4.16 µg/mL and 12.61 µg/mL (Table 4). The values obtained demonstrated the suitability of the system for the analysis of Zaltoprofen enantiomers in pharmaceutical formulation. Additionally the developed HPLC method was also applied for the estimation of Zaltoprofen in pharmaceutical formulations.

Table 1: Linearity and range for S and R Zaltoprofen enantiomers by HPLC

S. No.	S Zaltoprofen		R Zaltoprofen	
	Concentration µg/mL	Peak area	Concentration µg/mL	Peak area
1	2	51663	3	63143
2	2.5	61995	3.5	75772
3	3	73475	4	87253
4	3.5	80364	4.5	103325
5	4	91844	5	114806

Table 2: Results of analysis of drug products and recovery studies for S and R Zaltoprofen enantiomers by HPLC

Sample	Label claim (mg)	Amount present (mg/Tablet) ± % RSD*			% Label Claim			% Recovery ± % RSD*
		S&R	S	R	S&R	S	R	
I	80	79.24±0.5397	35.56±0.3452	43.67±0.7145	100.46±0.6747	45.08±0.2127	55.37±0.5342	98.04±0.4478

*n=3

Sample I: Zaltokin tablets containing 80 mg of Zaltoprofen (SR)

Table 3: Stress degradation studies of Zaltoprofen SR

S. No	Time (hrs)	Acid Hydrolysis (% degradation)		Basic Hydrolysis (% degradation)		Neutral Degradation (% degradation)		Oxidative Degradation (% degradation)		Photo Degradation (% degradation)	
		S	R	S	R	S	R	S	R	S	R
		1N HCl		1N NaOH				30 % H_2O_2		Powder form	
1	0	0	0	0	0			0	0		
2	2	55	45	41.49	50.94			41.28	53.65	24 hrs	
		0.1 N HCl		0.1N NaOH				3 % H_2O_2		0.21	0.42
1	0	0	0	0	0	0	0	0	0		
2	2	0.31	0.025	2.46	0.03	0.295	3.20	3.74	49.12		
3	4	1.74	0.35	7.74	4.33	0.325	3.26	5.22	49.23	Solution form	
4	6	4.20	2.95	8.047	4.62	0.455	3.70	28.23	49.29	24 hrs	
5	8	6.16	3.19	8.43	9.76	0.651	3.78	31.99	49.47	0.98	1.61
6	12	7.08	7.90	13.29	11.19	1.27	1.54	33.058	49.79		
7	24	7.75	8.45	14.53	13.69	1.49	6.14	36.11	49.93		

Table 4: System suitability studies for estimation of S and R Zaltoprofen enantiomers by HPLC

S. No.	Parameters	S - Zaltoprofen	R – Zaltoprofen
1	Linearity range	2 - 4 µg/ml	3- 5 µg/ml
2	Regression equation Y = mx + c	y = 19746x + 12630	Y = 26176X – 15843
3	Correlation coefficient	0.995	0.997
4	Theoretical plate/meter	56411	54089
5	Resolution factor	1.85	
6	Asymmetric factor	1.02	1.01
7	LOD (µg/mL)	4.16	4.16
8	LOQ (µg/mL)	12.61	12.61

Method validation

The RP HPLC method was validated according to ICH guidelines. The linearity of S and R Zaltoprofen were studied by preparing and assaying a series calibration standard at six different concentrations within the range from 2-4 µg/mL of S-Zaltoprofen and 3-5 µg/mL of R-Zaltoprofen. A good linear relationship was observed between the peak area ratios of S and R Zaltoprofen and the concentrations in the investigated concentration range.

The specificity of the method was ascertained by analyzing the standards and the samples. The peaks of S and R Zaltoprofen in samples were confirmed by comparing the retention time and spectra of the standards. Six injections at three different concentrations of S-Zaltoprofen (2, 3, 4 µg/mL) and R-Zaltoprofen (3, 4, 5 µg/mL) enantiomers were made and analyzed to examine the precision of the method. The mean peak area, standard deviation and % RSD were calculated.

The accuracy of the method was determined by recovery of Zaltoprofen from its pharmaceutical formulation. The recovery test was performed at three levels, namely, 80%, 100%, and 120% of the nominal concentration of Zaltoprofen, during degradation studies. Triplicate samples were prepared for each recovery level. The solutions were analyzed and the percentage of recoveries was calculated from the calibration curves. The ruggedness of the proposed method was determined by carrying out the experiment on different operators. Robustness of the method was determined by making small changes in the chromatographic conditions like flow rate and mobile phase composition were deliberately varied and resolution between the two peaks is found greater than 2.0 and % RSD of all the compounds were within the limit and this illustrates the robustness of the method as stated in ICH guidelines.

CONCLUSION

A highly specific stability–indicating chiral HPLC assay method was developed for the quantification of zaltoprofen enantiomers in presence of their degradation products. The enantioseparation was carried out by the use of cellulose based chiral column. The total run time for the developed method is 10 min. The method provides good sensitivity and excellent precision and reproducibility.

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Conflict of interest

The authors declare no conflict of interest.

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