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DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR THE ESTIMATION OF ZALTOPROFEN ENANTIOMERS IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, fast, specific and precise LC-MS/MS method was developed and validated for the determination of zaltoprofen enantiomers in pharmaceutical formulation. The interface used was Atmospheric Pressure Chemical Ionisation Technique. Analysis was performed using a ACI cellu 1 column (150 x 4.6 mm I.D., particle size 5 µ) by isocratic elution with 0.1% Ammonia solution: Acetonitrile (10: 90 v/v) and flow rate was 0.5 ml/min. The calibration plot was linear over the range of 18-54 ng/ml of S-zaltoprofen and 22-66 ng/ml of R-zaltoprofen with a correlation coefficient of 0.999 for S and R zaltoprofen. This optimized mobile phase separated S-zaltoprofen at 2.4 min and R-zaltoprofen at 3.9 min respectively. The proposed LC-MS/MS method is suitable for analysis of zaltoprofen enantiomers in pharmaceutical formulations and quality control analysis.

KEY WORDS: Zaltoprofen, LC-MS/MS, Atmospheric Pressure Chemical Ionisation, Enantiomers, Pharmaceutical formulation

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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. However, there are no reports concerning the analysis of zaltoprofen enantiomer in pharmaceutical formulations by LC/MS-MS. So it is felt necessary to develop a liquid chromatography mass spectroscopy (LC-MS/MS) procedure which would serve as a rapid and reliable method for the determination of zaltoprofen enantiomer in pharmaceutical formulations.

MATERIALS AND METHODS

Solvents and chemicals

Acetonitrile (HPLC grade), ammonia were supplied by Qualigens fine chemicals and S.D. Fine chemicals. Water (HPLC grade) was obtained from Milli Q RO system. All the reagents and chemicals used were of HPLC and analytical grade. Reference standard of enantiomers of zaltoprofen 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.

Apparatus and instrument conditions

The LC-MS/MS was performed using a Shimadzu API 3000 LC-MS/MS with auto injector and Analyst 1.31 data solution. ACI cellu 1 column (150 x 4.6 mm I.D., particle size 5 µ) was used. Sample volume of 10 µl was injected. LC separation was carried out using mobile phase of 0.1% Ammonia solution: Acetonitrile (10: 90 v/v) and flow rate was 0.5 ml/min. The working conditions for APCI MS/MS were as follows: The probe temperature was set at 400 ºC and the polarity was maintained at positive ion mode, ion at m/z 299 was assigned to (M+H) of Zaltoprofen. This ion was monitored and quantified.

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 light resistant containers. Aliquots of S-zaltoprofen (18-54 ng/ml) and R-Zaltoprofen (22-66 ng/ml) were prepared in the mobile phase for analysis.

Preparation of sample solution

Twenty tablets were weighed; the average weight was determined and finely powdered. The powder equivalent to 5 mg of S and R form of zaltoprofen (equivalent to 10 mg of racemic 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 S and R enantiomers of zaltoprofen from one formulation (zaltokin tablet containing 80 mg of zaltoprofen) were extracted in mobile phase. The standard and sample solutions were

analysed by the optimized chromatographic conditions, the chromatograms were recorded.

METHOD VALIDATION

Linearity
Standard solutions of 18-54 ng/ml of S-zaltoprofen and 22-66 ng/ml of R-zaltoprofen were analyzed to check the linearity of response (Fig 2).

Specificity
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 M+H peak.

Precision
Six injections at three different concentrations of S-zaltoprofen (18, 36, 54 ng/ml) and R-zaltoprofen (22, 44, 66 ng/ml) enantiomers were made and analyzed to examine the precision of the method. The mean peak area, standard deviation and % RSD were calculated.

Accuracy (Recovery)
Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at a single level by adding a known quantity of zaltoprofen S and R enantiomers to the drug products of pre analyzed samples and the mixtures were reanalyzed. The average recoveries obtained from each sample were calculated.

Ruggedness and Robustness
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 as stated in ICH guidelines.

Limit of detection and Limit of quantification
The limit of detection (LOD) and the limit of quantification (LOQ) of the developed method were performed as stated in ICH guidelines[9-10].

RESULTS AND DISCUSSION

In the spectral investigation by LC/MS/MS in the SCAN mode, standard solution of Zaltoprofen showed major peak at m/z of 299, which was assigned to the [M+H] ion of zaltoprofen (Fig 3). Optimization of the method was carried out using various concentrations of acetonitrile while keeping the aqueous phase constant. A solvent combination of 0.1% ammonia: acetonitrile (10:90 % v/v) gave a satisfactory separation of the enantiomers of interest. This optimized mobile phase separated S-zaltoprofen and R-zaltoprofen at 2.4 and 3.9 min respectively. The typical chromatograms of the standard and the sample solutions are shown in Fig 4-5. The calibration curves of S-zaltoprofen and R-zaltoprofen were linear in the range of 18-54 ng/ml and 22–66 ng/ml respectively (Table 1). Linear regression equation and correlation coefficient are shown in Table 4. The precision of the method was demonstrated by reproducibility studies. The mean, standard deviation and % RSD were calculated and are presented in Table 2. The % RSD values of less than 2% revealed that the methods were precise. The accuracy of the optimized method was determined by absolute recovery experiments. The percentage recovery values for S and R zaltoprofen was found to be 45.63 and 56.04 % respectively. 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 3). Detection limits and quantification limits of S-zaltoprofen and R-zaltoprofen were found to be 0.016 ng/ml and 0.051 ng/ml respectively (Table 4). No marked changes in the chromatogram occurred on changing the operator and chromatographic conditions indicating that the developed method was rugged and robust. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and are presented in Table 4. The values obtained demonstrated the suitability of the system for the analysis of S-zaltoprofen and R-zaltoprofen in combined form in pharmaceutical formulation.

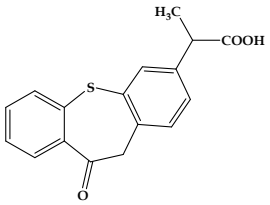

Figure 1
Structure of Zaltoprofen

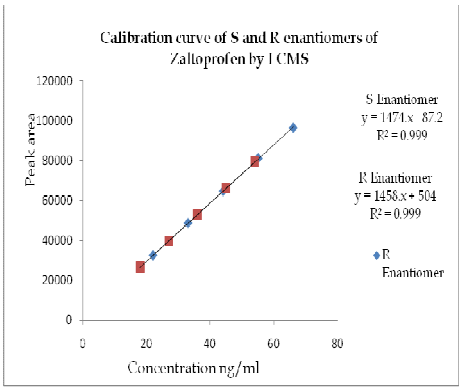

Figure 2
LC-MS/MS Calibration curve of S and R Zaltoprofen

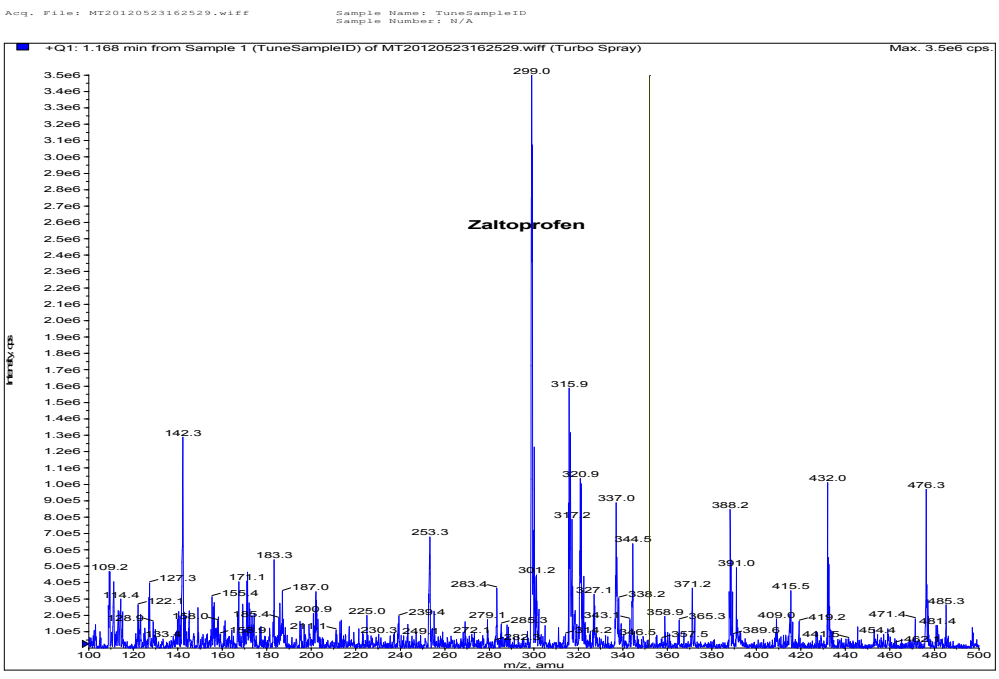

Figure 3
Typical MS chromatogram of Zaltoprofen

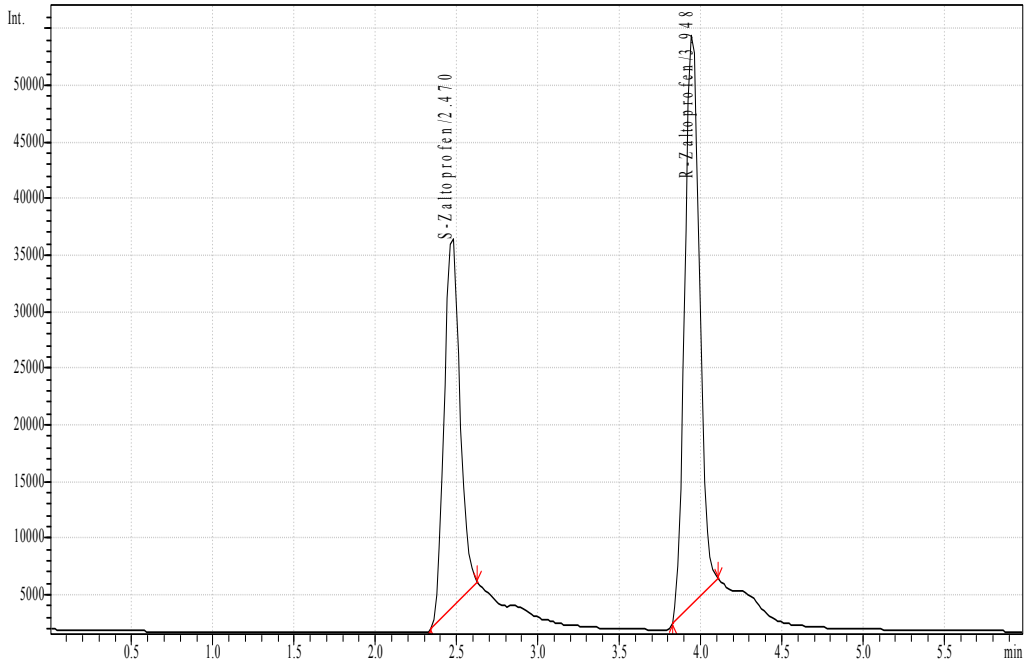

Figure 4
Typical LC-MS/MS chromatogram of S and R Zaltoprofen standard solution

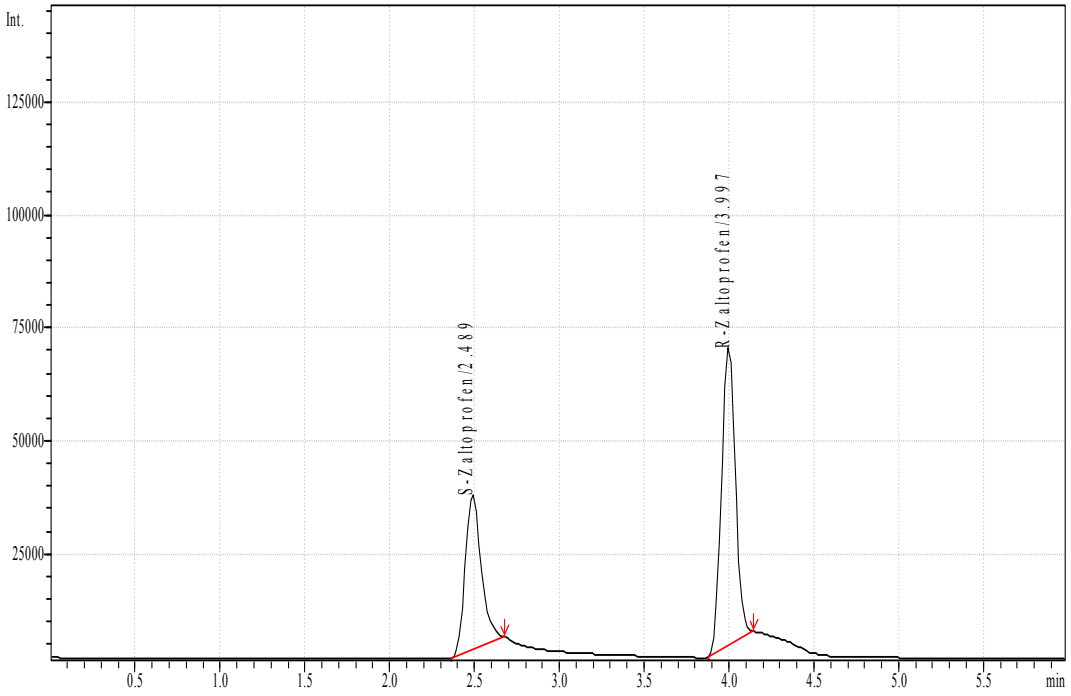

Figure 5
Typical LC-MS/MS chromatogram of sample I containing S and R Zaltoprofen

Table 1
Linearity and range for S and R zaltoprofen enantiomers by LC-MS/MS

S. No	S – Zaltoprofen		R – Zaltoprofen	
	Concentration ng/ml	Peak area	Concentration ng/ml	Peak area
1	18	26531	22	32425
2	27	39784	33	48673
3	36	52674	44	64775
4	45	66401	55	81124
5	54	79572	66	96425

Table 2
Precision studies for S and R zaltoprofen enantiomers by LC-MS/MS

S. No	S – Zaltoprofen (ng/ml)			R – Zaltoprofen (ng/ml)		
	18	36	54	22	44	66
1	26531	52647	69572	32425	64775	96425
2	26489	52603	69401	32586	64989	95987
3	26499	52535	69856	32585	64995	95892
4	26488	52457	69494	32591	63943	95908
5	26432	52413	69889	32590	63974	95985
6	26410	52478	69834	32587	64908	95887
Mean	26474.83	52522.16	69674.33	32560.66	64597.33	96014
SD	45.05	89.88	210.84	66.50	501.26	206.27
% RSD	0.170	0.171	0.303	0.204	0.775	0.218

Table 3
Results of analysis of drug products and recovery studies for S and R zaltoprofen enantiomers by LC-MS/MS

Label* claim (mg)	Amount present (mg/tablet) ± % RSD*			% Label Claim			% Recovery ± % RSD**
	S&R form	S-form	R-form	S&R form	S-form	R-form	
2	80	80.20 ±0.42 53	35.99 ±0.8783	44.20 ±0.9212	101.67 ±0.2735	45.63 ±0.7758	56.04 ±0.9591

Zaltokin Tablets containing 80mg of Zaltoprofen (RS)
***** RSD of three determination***

Table 4
System suitability studies for estimation of S and R zaltoprofen enantiomers by LC-MS/MS

S. No	Parameters	S - Zaltoprofen	R– Zaltoprofen
1	Linearity range	18 - 54 ng/ml	22 - 66 ng/ml
2	Regression equation Y = mx + c	Y= 1474X – 87.2	Y = 1458X + 504
3	Correlation coefficient	0.999	0.999
4	Resolution factor	1.8	
5	Asymmetric factor	1.02	1.01
6	LOD (ng/ml)	0.016	0.016
7	LOQ (ng/ml)	0.051	0.051

CONCLUSION

The developed LC-MS/MS method in the present study for the estimation was found to be simple, rapid, accurate, precise, specific, linear and rugged. It is thus suitable for the estimation of Zaltoprofen enantiomers in raw materials and formulations.

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