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RP-HPLC method for the determination of zaltoprofen in bulk and pharmaceutical dosage form

Purnima Gandi, Y. Srinivasa Rao*, K. Vara Prasada Rao and T. Hemant Kumar

Department of Pharmaceutical Analysis and Quality Assurance, Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam, India

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ABSTRACT

A new RP-HPLC method has been developed and validated for determination of Zaltoprofen in bulk and tablet dosage form. The estimation was carried out on Enable C18G (250 mm ×4.6 mm, 5 µm) column using Acetonitrile and 0.1% v/v Acetic acid in the ratio of 95:5 % v/v as mobile phase. The flow rate was 1.0 ml/min and the effluent was monitored by UV detector at 331 nm. The retention time was 3.688 min and linearity was observed in the concentration range of 10-60 µg/ml. The percentage recovery was in good agreement with the labelled amount in the pharmaceutical formulations and the method was simple, precise and accurate for the determination of Zaltoprofen in bulk and pharmaceutical formulation.

Key words: Zaltoprofen, RP-HPLC, Validation and Tablet dosage form

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INTRODUCTION

Zaltoprofen, 2 - (10, 11 – dihydro – 10 – oxodibenzo [b, f] thiepin – 2 - yl) propionic acid is a potent nonsteroidal anti-inflammatory drug (NSAID)[1]. It is a preferential COX-2 inhibitor, exhibited a potent inhibitory action on the nociceptive responses induced by a retrograde infusion of bradykinin into the right common carotid artery in rats[2] . It is used in the treatment of rheumatoid arthritis, osteoarthritis, and other chronic inflammatory pain conditions.

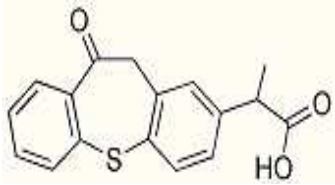

Figure 1. Structure of Zaltoprofen

Literature review revealed the drug estimation by HPLC in plasma[3-7] .There is a chiral HPLC method for enantioselective analysis[8-10] , Stability-Indicating LC method in bulk drug and formulations[11] and UV spectrophotometric method[12,13] and RP-HPLC method[14]. The present work aims to develop a novel, simple, specific, sensitive, precise and accurate RP-HPLC method for the determination of Zaltoprofen in pure form and in tablet dosage form.

MATERIALS AND METHODS

Instrumentation

The HPLC system consists of Enable ODS reverse phase (250 mm x 4.6 mm, 5µm particle size) C_{18} column, a Rheodyne valve injector equipped with a 20 µl sample loop, variable wavelength programmable UV Detector SPD-20A VP with manual mode of injection. The HPLC system equipped with LC solutions software.

Materials and Reagents

Tablet formulation Zaltokin–80 (Zaltoprofen Tablets) containing Zaltoprofen 80 mg that was purchased from local market was used in present study. All reagents and chemicals used were of HPLC Grade.

Preparation of 0.1% v/v acetic acid

Glacial acetic acid (0.1 ml) was taken in 100 ml volumetric flask and dissolved in 10 ml of HPLC water and the volume was made up to the mark with HPLC water to produce 0.1% v/v acetic acid solution.

Preparation of mobile phase

The mobile phase was prepared by using acetonitrile and 0.1% v/v acetic acid in the ratio of 95:5 v/v i.e. 95 volumes of acetonitrile and 5 volumes of 0.1% v/v acetic acid. It was then filtered through 0.45 µm membrane filter to remove any particles if present and kept for sonification for 15 minutes and was then used.

Preparation of Zaltoprofen standard stock solution (100 µg/ml)

Standard solution of Zaltoprofen was prepared by accurately weighing and dissolving 100 mg of the drug in 100 ml of mobile phase (Acetonitrile and 0.1% v/v acetic acid, 95:5 v/v) and sonicated for 5 minutes. 10 ml of this solution was taken and further diluted to 100 ml with the same to get a standard concentration of 100 µg/ml.

Chromatographic conditions

The mobile phase consists of Acetonitrile and 0.1% v/v acetic acid in the ratio of 95:5 (v/v) and was pumped at a flow rate of 1.0 ml/min in isocratic mode while the detection was monitored at a wave length of 331 nm on Enable ODS reverse phase (250 mm x 4.6 mm, 5 µm particle size) C_{18} column. The mobile phase was degassed and vacuum filtered prior to use.

Preparation of sample drug solution from Pharmaceutical formulation

Accurately 20 tablets of zaltoprofen were weighed, average weight of twenty tablets were calculated and triturated to fine powder. Tablet powder equivalent to 100 mg of Zaltoprofen was weighed and dissolved in 10 ml of mobile phase with shaking, sonicated for 15 min and centrifuged. The supernatant liquid was transferred to 100 ml volumetric flask and final volume was made up to 100 ml with the mobile phase. This was then filtered through 0.2 µm membrane filter to get concentration of 1 mg/ml solution. This was then diluted to prepare the working concentration of 100 µg/ml with mobile phase. From the above solution 35 µg/ml was prepared, filtered through 0.2 µm membrane filter, sonicated and then the sample was injected.

RESULTS AND DISCUSSION

Method Validation[15]

The developed method was validated as per ICH guidelines, and accordingly the parameters were evaluated for Linearity, Specificity, Accuracy, Precision and Robustness.

System Suitability

System suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The parameters like retention time, number of theoretical plates, tailing factor, HETP were investigated by injecting standard solutions of the drugs six times and the results are given in Table 1. From the results it was observed that all the values are present within the limits indicating good performance of the system.

Table 1. System Suitability Parameters

Parameter	Result
Retention time	3.688 (mins)
Theoretical plates	4590
Tailing factor	1.1
HETP	32.67

Specificity

The optimized solvent system yielded a symmetric peak for the drug with retention time 3.688 min. The peak for the bulk drug was identified by comparing the retention time and also comparing its peak area with that obtained from standard drug. Peak purity values were good for the drug, which shows that the analyte peaks were pure and there were no interferences from excipients in the analyte peak. Therefore, it could be said that developed method was highly specific.

Linearity and Range

Various concentrations from standard solution of Zaltoprofen were prepared and the calibration graph was plotted by the values of the peak area versus concentration (µg/ml) which were found to be linear over the concentration ranges of 10-60 µg/ml and the linearity data was shown in the figure 2. From the data obtained, co-relation coefficient, slope and y-intercept were calculated and the results were shown in Table 2.

Table 2. Data of linearity

Concentration(µg/ml)	Peak area	Statistical Analysis
10	271758	**Regression equation**
20	540173	Y=26941X+743.07
30	811263	**Correlation coefficient**
40	1071456	0.998
50	1348659	**Slope,m**
60	1619435	26941
		Intercept,
		743.07

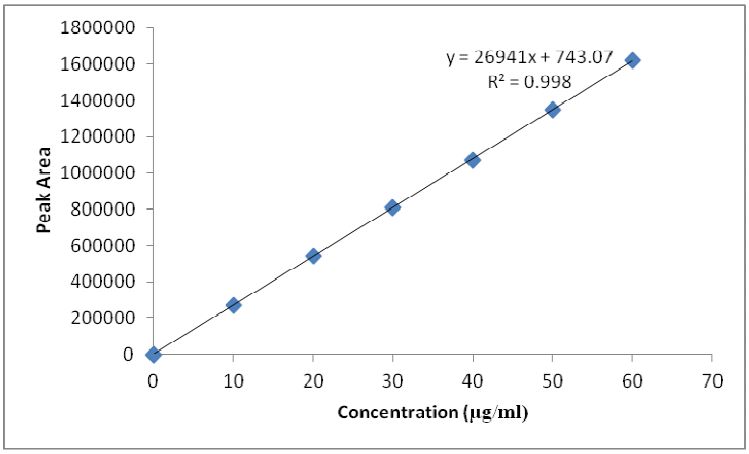

Figure 2. Linearity Plot of Zaltoprofen

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. It was analysed by 6 different solutions of same concentration and peak areas were noted. The result was indicated by % RSD. The results for system, intraday and inter-day precision were shown in Table – 3, 4 &5.

Table 3. System Precision results for Zaltoprofen

Injection	Concentration (µg/ml)	Peak area	%Assay
1	35	938971	99.29
2	35	939542	99.35
3	35	940634	99.46
4	35	944278	99.85
5	35	942957	99.71
6	35	941126	99.51
	%RSD	0.2155	

Table 4. Intraday Precision results for Zaltoprofen

Injection	Concentration (µg/ml)	Peak area	%Assay
1	35	939273	99.32
2	35	943632	99.78
3	35	942991	99.71
4	35	942457	99.66
5	35	943856	99.8
6	35	944574	99.88
	%RSD		0.2054

Table 5. Inter-day Precision results for Zaltoprofen

Day	Concentration (µg/ml)	Peak area	%Assay
1	35	940142	99.41
2	35	942739	99.68
3	35	941358	99.54
4	35	941471	99.55
5	35	943135	99.73
6	35	942614	99.67
7	35	939176	99.31
	%RSD		0.1537

Accuracy

To determine the accuracy of the proposed method, different amounts of drug samples (80%, 100%, and 120%) of Zaltoprofen within the linearity range were taken. Solutions were prepared in triplicates and accuracy was indicated by % recovery. The results were recorded in Table 6.

Table 6. Accuracy results for Zaltoprofen

S.No.	(%) level	Actual conc. (µg/ml)	Conc. Added (µg/ml)	Conc.found (µg/ml)	%Recovery ±%RSD	%Mean recovery ±%RSD
1.	80%	35	28	27.99	99.97±0.4851	
2.	100%	35	35	34.83	99.53±0.6053	99.75±0.2175
3.	120%	35	42	41.89	99.75±0.476	

Application of method to formulation

The assay of the method was performed to determine the % recovery of formulation. A 35 µg/ml of sample solution was prepared and injected. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The results were shown in Table 7.The chromatogram was shown in Figure 3.

Table 7. Estimation of Zaltoprofen in Tablets

Formulation	Amount of drug taken from tablet (mg)	Mean amount of drug found from tablet* (mg)	% Mean Assay*±%RSD
Zaltokin 80 (Tablets)	100	99.60	99.60±0.3112

**Average of three determinations*

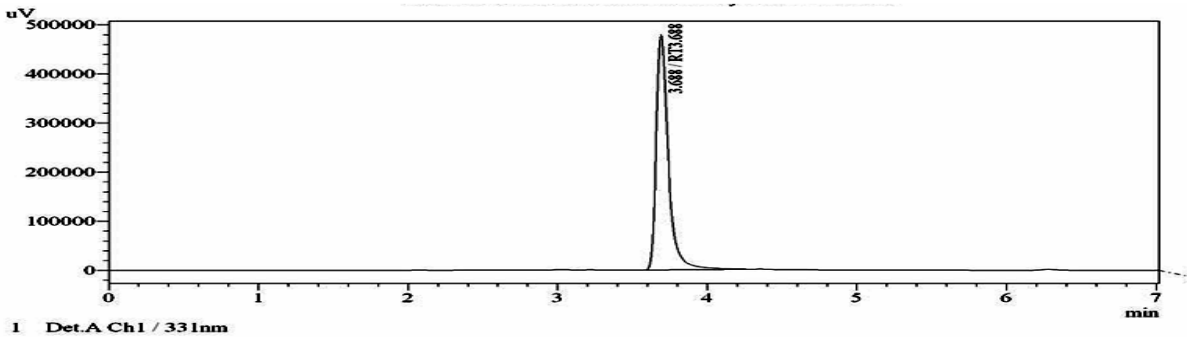

Figure 3. Chromatogram of the drug Zaltoprofen

Robustness

To evaluate the robustness of the developed method, small variations in the optimised method parameters were done. The effect of change in flow rate and wavelength were studied. The method was found to be unaffected by small changes in the mobile phase flow rate (± 10%) & changing the wavelength (± 5 nm) (Table 8).

Table 8. Results from robustness study

Parameter	Condition	Peak area	%Assay±%RSD
Flow rate±10% of optimum flow rate	0.9 ml	941918	99.3±0.434
	1.1 ml	559829	99.98±0.522
Wavelength±5nm of optimum wavelength	326 nm	560082	99.94±0.417
	336 nm	559612	99.86±0.379

Limit of Quantification (LOQ) and Limit of Detection (LOD)

LOD and LOQ were determined based on statistical calculation from the calibration curves, where LOD = (3.3 ×σ)/m; LOQ= (10.0×σ)/m (σ is the standard deviation of the y-intercepts of the three regression lines and m is mean of the slopes of the three calibration curves) .The LOD and LOQ values of the developed method were found to be 1.2 µg/ml and 3.88 µg/ml respectively indicating that the method was sensitive.

CONCLUSION

The RP – HPLC method proposed for the determination of Zaltoprofen is simple, sensitive and economical with reasonable precision and accuracy. Parameters and statistical comparison justify this method for application in estimation of Zaltoprofen in bulk and tablet dosage form. Commercial formulation of Zaltoprofen was successfully analysed and results were calculated. There was no interference of additives or excipients for the assay and evaluation of Zaltoprofen in pharmaceutical tablet dosage form. Hence it can be applied routine analysis of Zaltoprofen in formulation.

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