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R E S E A R C H A R T I C L E

Development and Validation of Analytical Method for the Estimation of Zaltoprofen Using RP- HPLC Method

Kumarraja Jayavarapu*, Dr. S. Parimalakrishnan, Dr. V. D Sundar

Research Scholar, Department of Pharmacy, Annamalai University, Tamilnadu – 608002.

A B S T R A C T

A simple, sensitive, linear, precise and accurate RP – HPLC method for the estimation of Zaltoprofen was developed and validated. Zaltoprofen [(2RS)-2-(10-oxo-10,11-dihydrodibenzo[b,f]thiepin-2-yl)propionic acid] is a potent NSAID with powerful anti-inflammatory and analgesic effects on inflammatory pain. It selectively inhibits PGE_2 production at inflammatory sites. Zaltoprofen has anti-bradykinin activity also. It has been used clinically for treatment of post-operative pain and low back pain for more than ten years. It has fewer side effects on gastro intestinal tract and induces apoptosis in variety of cell lines. In the present study utilizes Inertsil ODS-2 (150 x 4.6 mm, 5µm) column with mobile phase consisting of Buffer : Acetonitrile (45:55 v/v) used. The detection wavelength is 240nm and the flow rate is 0.8 mL/min. the developed method was statistically validated for linearity, specificity. The method was linear over the range of 0.05-0.15 mg/ml. the linearity of Zaltoprofen shows a correlation coefficient of 0.999. The percentage recovery ranges from 99.56 – 100.29 %. The proposed method can be useful in the quality control of Zaltoprofen.

Keywords: Zaltoprofen, RP-HPLC, Specificity.

A R T I C L E I N F O

Corresponding Author
Kumarraja Jayavarapu
Research Scholar, Department of Pharmacy,
Annamalai University, Tamilnadu – 608002.
MS-ID: JPBMAL3892

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C O N T E N T S

1. Introduction

Zaltoprofen is a nonsteroidal anti-inflammatory drug (NSAID) used as an analgesic, antipyretic, and anti-inflammatory agent. It is a selective COX-2 inhibitor and also inhibits bradykinin-induced pain responses without blocking bradykinin receptors. It has been used clinically for treatment of post-operative pain and low back pain for more than ten years. It has fewer side effects on gastro intestinal tract and induces apoptosis in variety of cell

lines. ZPF is biotransformed by cytochrome P450 (CYP) and UDP-glucuronosyl transferase (UGT) in human liver microsomes to S-oxide-Zaltoprofen (M-2), 10-hydroxy Zaltoprofen (M-3), and S-oxide-10-hydroxy Zaltoprofen (M-5). It is freely soluble in acetone, soluble in methanol, ethanol and practically insoluble in water.

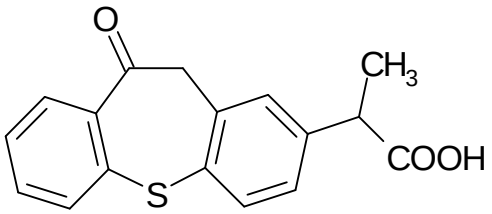

Fig 1: Chemical Structure

2. Materials and Methods

Instrumentation:

A water-alliance HPLC system (Alliance 2695) equipped with Photo Diode Array Detector (2996), UV-Visible detector (2489) and Empower2 software was used**.**

Reagents and chemicals:

ZPF and its three process related impurities (imp-1, imp-2, imp-3) were supplied by Hetero (R&D), Hyderabad. All the chemicals and solvents used were of analytical grade. Milli Q water was used throughout the experiment**.**

SOLUTIONS:

a) Preparation of Sample Solution:

10 mg of ZPF was accurately weighed and transferred into 20 ml of volumetric flask. The material is dissolved and diluted to mark with diluent viz. acetonitrile.

b) Preparation of Impurities Solutions:

10 mg of impurity-1, 2 or 3 were accurately weighed and transferred into 20 ml of volumetric flask. The material is dissolved and diluted to mark with diluent.

c) Assay Solutions:

i. Standard Preparation-1:

10.0 mg of ZPF reference standard was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

ii. Standard Preparation-2:

10.0 mg of ZPF reference standard was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

iii. Assay Preparation-1:

10.0 mg of ZPF test sample was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

iv. Assay Preparation-2:

10.0 mg of ZPF test sample was transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent to volume and mixed.

d) Preparation of Buffer:

1.36 g of potassium dihydrogen orthophosphate mono hydrate is dissolved in 1000 ml of Milli Q water. pH was adjusted to 3.0 with orthophosphoric acid.

e) Chromatographic Conditions:

Column : Inertsil ODS-2, 150 x 4.6 mm, 5µm
Elution : Isocratic
Mobile Phase : Buffer: Acetonitrile (45:55 v/v)
Flow rate : 0.8 ml/min
Detector wavelength : 240 nm
Column oven temperature : 27 ºC
Injection volume : 20 µL
Diluent : Acetonitrile
Runtime : 30 min

3. Results and Discussion

I have made many trails using different solvent systems with different mobile phase concentration ratios and finally concluded the following results.

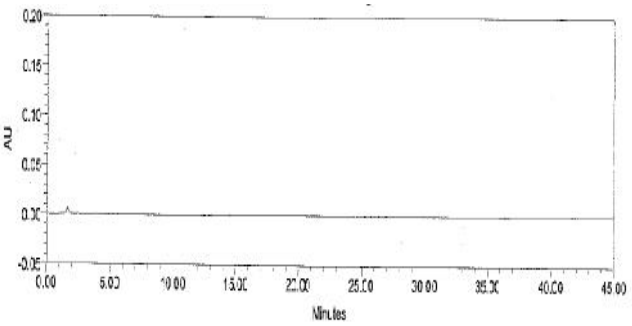

Fig. 2: HPLC Blank Chromatogram

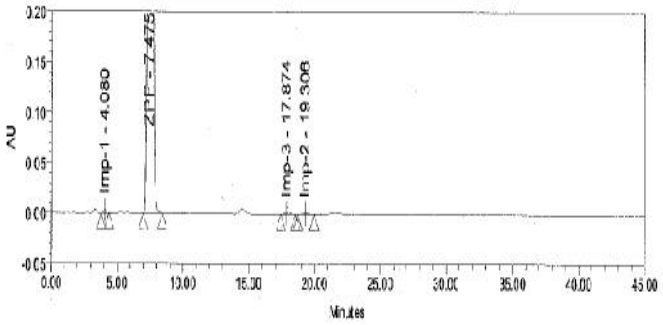

Fig 3: HPLC Chromatogram Representing ZPF Spiked with 0.15 % of Impurities

ii. Forced Degradation Studies:

In order to establish a stability indicating assay method ZPF was purposefully degraded as given under section 1.5.4. The analysis was carried out by HPLC with a PDA detector. 20 µl of each forced degradation sample was injected at regular intervals and the final stress conditions were established in such a way that 10-20 % degradation of ZPF was occurred.

a) Oxidative Degradation:

100 mg of ZPF sample was taken into a 100 ml round bottom flask, 10 ml of 10 % hydrogen peroxide solution was added, contents were mixed well and kept for constant stirring for 6 h. at 80^{o}C. 1.0 ml of this solution was diluted to 10 ml with diluent.

b) Acid Degradation:

100 mg of ZPF sample was taken into a 100 ml round bottom flask, 10 ml of 1 N hydrochloric acid solution was added, contents were mixed well and kept for constant stirring for 6 h. at 80^{o}C. 1.0 ml of this solution was diluted to 10 ml with diluent.

c) Base Degradation:

100 mg of ZPF sample was taken into a 100 ml round bottom flask, 10 ml of 1 N sodium hydroxide solution was added, contents were mixed well and kept for constant stirring for 2 hrs. at 80 ^{o}C. 1.0 ml of this solution was taken in 10 ml volumetric flask, neutralized with 1.0 ml of 1 N HCl and diluted to 10 ml with diluent.

d) Hydrolytic Degradation:

100 mg of ZPF sample was taken into a 100 ml round bottom flask, 10 ml of milli Q water was added, the contents were mixed well and kept for constant stirring for 6 h. at 80 °C. 1.0 ml of this solution was diluted to 10 ml with diluent.

e) Thermal Degradation:

1.0 g of ZPF sample was taken in to a petridish and kept in oven at 80°C for 7 days. 10.0 mg of this sample was taken in to a 10 ml volumetric flask, dissolved in diluent and diluted to volume with diluent.

f) Photolytic Degradation:

1 g of ZPF sample was taken in to a petri dish and kept into photo stability chamber / 200 Watt hours/ sq.metre in U.V light and 1.2 million lux hours in Visible light for 7 days. 10.0 mg of this sample was taken in to a 10 ml volumetric flask, dissolved in diluent and diluted to volume with diluent.

iii. Results of forced degradation studies:

Significant degradation of ZPF was observed in base degradation. The molecule was found to be stable in all other degradation conditions. Photodiode array detector was employed to check and ensure the homogeneity and purity of ZPF peak in all the stressed sample solutions. Assay studies were carried out for stress samples against ZPF qualified reference standard and the mass balance (% assay + % sum of all compounds+ % sum of all degradants) was calculated. The assay of ZPF was unaffected by the presence of its impurities and degradation products and thus confirms the stability-indicating power of the developed method. Since all the impurities were eluting before 20 min, the run time was reduced to 30 min.

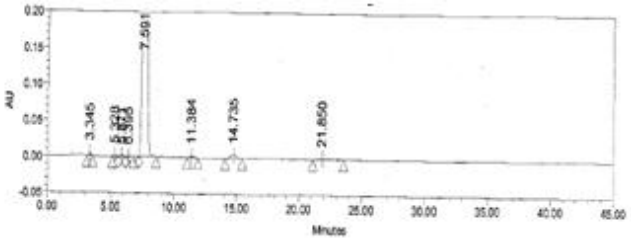

Fig. 4: HPLC Chromatogram Representing ZPF Oxidative Degradation

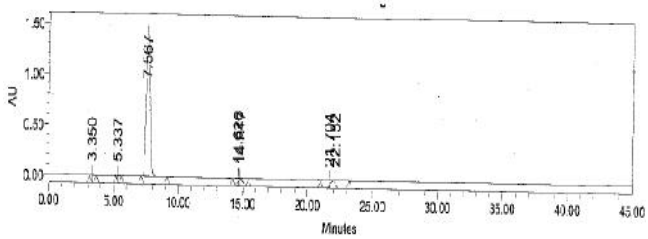

Fig. 5: HPLC Chromatogram Representing ZPF Acid Degradation

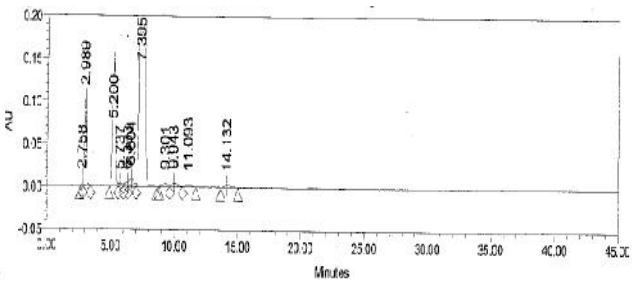

Fig 6. HPLC Chromatogram Representing ZPF Base Degradation

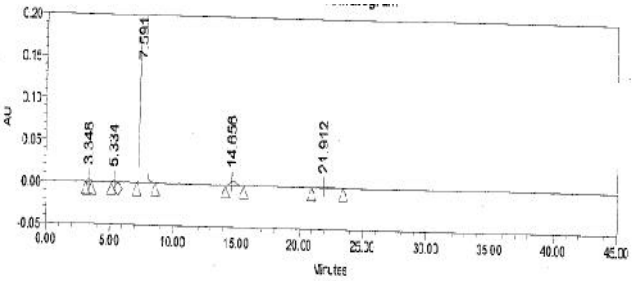

Fig. 7: HPLC Chromatogram Representing ZPF Hydrolytic Degradation

MSUI: Maximum Single Unknown Impurity

iv. HPLC Method Validation:

The proposed method was validated as per ICH guidelines.

a) Specificity:

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The specificity of the developed LC method was checked in the presence of its impurities and forced degradation products. For this purpose, all the stressed samples of ZPF were spiked with its three process impurities (0.15 % with respect to ZPF concentration). All the impurities and degradants are well resolved from one another and ZPF peak indicating the specificity of the proposed method to assay of ZPF.

b) Linearity:

Linearity test solutions for ZPF assay were prepared from stock solution at five concentration levels from 50 to 150 % of assay analyte concentration (0.1 mg/ml of ZPF). The peak area versus concentration data was performed by least-squares linear regression analysis. The correlation coefficient of the respective calibration curve was calculated.

c) Precision:

Precision was evaluated by carrying out six independent assays of test sample of ZPF at 0.1 mg/ml level against qualified reference standard. The intermediate precision of the assay method was evaluated by different days. The % RSD values were calculated.

d) Accuracy:

The accuracy of the ZPF assay was evaluated in triplicate at three concentration levels *viz.* 50, 100 and 150 % with respect to 0.1 mg/ml of ZPF test concentration.

e) Robustness:

The robustness was illustrated by getting the resolution between any two compounds to be greater than 2.0, when

mobile phase flow rate (± 0.2 ml/min), pH (± 0.2), organic solvent ratio (± 5 %) and column temperature (± 2 °C) were deliberately varied.

f) Solution Stability:

The solution stability of ZPF assay (in diluent) was determined by leaving 0.1 mg/ml ZPF solution in tightly capped volumetric flasks at room temperature for 48 h during which they were assayed at 6 h intervals and comparing the results with those obtained from freshly prepared solution. The mobile phase was prepared at the beginning of the study period and not changed during the experiment. The % RSD values for solution stability experiments was calculated and found to be 1.35. All the samples were found to be stable up to 48 hours.

g) System Suitability:

1. The % RSD for five replicate injections from standard preparation-2 should not more than 2.0.
2. The number of theoretical plates should not be less than 3000 from standard preparation-2.
3. The tailing factor should not be more than 2.0 from standard preparation-2.

Table 1: Validation Data of ZPF Assay by HPLC

Parameter	Result
Linear Range (mg/ml)	0.05-0.15
Slope	2568.5
Intercept	205.6
Correlation Coefficient	0.999
Repeatability#	0.75
Intermediate Precision#	0.91
Accuracy*	
4 µg/ml level	99.5 ± 0.91
5 µg/ml level	99.1 ± 0.65
6 µg/ml level	99.0 ± 0.98

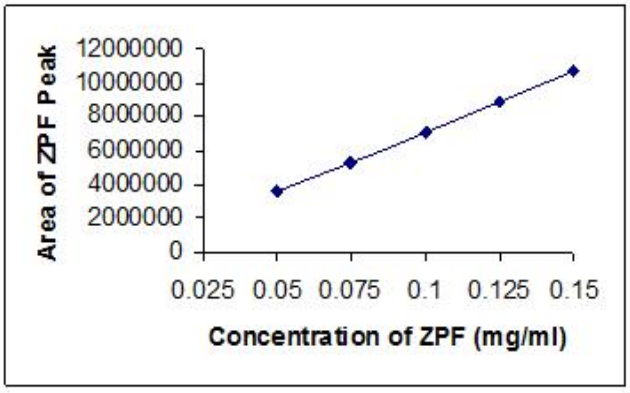

Fig. 8: Linearity Curve for ZPF Assay by HPLC

4. Conclusion

In HPLC, an isocratic method was developed. Assay was determined by using ZPF reference standard. The method was proven to be stability indicating as it separates three process related impurities and degradants obtained by performing forced degradation. The assay on dried basis was found to be 99.5 %.

Table 2: Summary of Forced Degradation Studies

Stress Conditions	% Degradants formed					% Assay	Mass Balance
	Imp-1	Imp-2	Imp-3	MSUI	Total Impurities		
Unstressed	ND	0.02	ND	0.05	0.48	-	-
Oxidative Degradation	ND	0.02	ND	0.06	0.52	99.1	99.6
Acid Degradation	ND	0.02	ND	0.05	0.63	99.1	99.7
Base Degradation	ND	0.02	ND	6.98	13.34	85.5	98.8
Hydrolytic Degradation	ND	0.02	ND	0.06	0.51	99.0	99.5
Thermal Degradation	ND	0.02	ND	0.06	0.49	99.2	99.7
Photolytic Degradation	ND	0.02	ND	0.05	0.48	99.2	99.8

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