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Research Paper

Development and Validation of a Spectrophotometric Method for Determination of Zaltoprofen in Bulk Drug and Pharmaceutical Formulation

[1]Manish Kumar Thimmaraju, [2]G.Priyanka,[3]Srikanth Gurrala, [4]K.Pavan Kumar, [5]G.Surya Prakash, [6]N.Raghunandan

[1]Head, Department of Pharmaceutical Analysis Balaji Institute of Pharmaceutical Sciences. Narsampet, Warangal, Andhra Pradesh, India-506331
[2,3,4]Department of Pharmaceutical Analysis Gland Institute of Pharmaceutical Sciences. Narsapur, Medak Andhra Pradesh, India
[5]Principal Balaji Institute of Pharmaceutical Sciences Narsampet, Warangal, Andhra Pradesh, India-506331

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ABSTRACT: A simple, sensitive and accurate spectrophotometric method has been developed for the determination of Zaltoprofen in bulk and pharmaceutical formulation. The λ max of the Zaltoprofen was found to be 335nm in ACN: 0.1N HCL [PH 1.2]. The method shows high sensitivity with linearity 10 to 80µg/ml (regression equation: Y=0.013X + 0.002; r^2 = 0.999). The apparent molar absorptivity was found to be 0.369x10^{4}lmol−1 cm−1 in ACN: 0.1N HCL (PH 1.2). This method was tested and validated for various parameters according to ICH guidelines and USP. The Detection limit and quantitation limit were found to be 0.150 µg ml–1 and 0.158 µg ml–1 in ACN: 0.1 HCL (pH 1.2) respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 1%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of Zaltoprofen in bulk and pharmaceutical formulation.

***Keywords*:** Zaltoprofen, ACN: 0.1 N HCL, Spectrophotometry.

I. INTRODUCTION

Zaltoprofen (ZLT), 2-(10, 11dihydro-10-oxodibenzo [b,f]thiepin-2-yl) propionic acid is a potent non-steroidal anti-inflammatory drugs (NSAIDs).[1-2] It has been used clinically for treatment of post-operative pain and low back pain for more than ten years, and has recently been reported to cause potent inhibition ofcyclooxygenase-2 with fewer side effects on the gastro-intestinal tract and to induce apoptosis in a variety of cell lines.[3-4] Zaltoprofen is a unique compound that also has anti-bradykinin activity. Its analgesic effects may be a result of inhibition of bradykinin B2 receptor-mediated bradykinin responses not only of cyclooxygenases but also of bradykinin-induced 12-lipoxygenase inhibitors[5-6]. Literature survey reveals that a simple, rapid, reproducible, selective and sensitive HPLC method was developed and validated for the determination of zaltoprofen from human plasma [7], A simple and rapid RP-HPLC analysis method for direct determination of (+) and (-) zaltoprofen glucuronide in rat hepatic microsomes and from pharmaceutical bulk dosage forms were developed and validated[8-9]. A Lc-ms/ms method for the determination of zaltoprofen in human plasma.[10-11] The pharmacokinetic parameters of zalatoprofen in rat plasma were predicted using a validated column-switching HPLC method.[12]So far to our present knowledge there is no economical method for estimation of zaltoprofen by simple, sensitive and accurate spectrophotometric method in bulk and pharmaceutical formulation. It is felt necessary to develop a simple, sensitive and accurate spectrophotometric method for zaltoprofen. The method was validated as per ICH guidelines.

II. MATERIALS AND METHOD

2.1 Instrument

UV-Vis SpectrophotometerT60 (model), Analytical technologies Limited, connected to the digital system loaded with UV Win software ver.5.1.1. For intermediate precision study a UV-Vis spectrophotometer, LABINDIA Analytical Instruments pvt. Ltd, model UV3092 connected to computer loaded with UVWin5 software ver.5.2.0.1104. Both the instruments have an automatic wavelength accuracy of 0.1nm and matched quartz cells of 10mm path length.

2.2 Materials

Zaltoprofen (JP), Formulations containing Zaltoprofen: ***Zaltokin-80*** tablets, labelled to contain 80mg of Zaltoprofen per tablet (Ipca Laboratories Ltd., India) and ***Zalto*** tablets, labelled to contain80 mg of Zaltoprofen per tablet (Intas Pharmaceuticals, India) were collected from local Indian market. All other chemicals and reagents used were of analytical grade.

2.3 Selection of wavelength

In order to ascertain the wavelength of maximum absorption (λmax) of the drug, Five different concentrations were prepared in the range of 10-80µg/ml of Zaltoprofen in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium and absorbance found at 335nm. The standard graph was plotted (***Figure - 4)*** and the calibration data are presented in (***Table – 1)***

2.4 Preparation of stock solution

10 mg of Zaltoprofen was accurately weighed and dissolved in 100 ml ofACN: 0.1 N HCL (pH 1.2) in 100ml volumetric flask to get the concentration about100 µg ml^{-1} stock solution.

Preparation of 0.1N Hydrochloric acid solvent

Accurately measured 8.5ml of concentrated hydrochloric acid and made up to 1000ml with triple distilled water to obtain 0.1N HCL solvent

2.5 Preparation of calibration curve

Different stock solutions of 100µg/ml of Zaltoprofen were prepared in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system by dissolving 10mg of Zaltoprofen in 100ml Solvent. For preparation of different concentrations, aliquots of stock solutions were transferred into a series of 10ml standard flasks. Five different concentrations were prepared in the range of 10-80µg/ml of Zaltoprofen in ACN: 0.1N HCl (pH 1.2) and absorbance are found at 335nm. The standard graph was plotted (***Figure- 4)*** and the calibration data are presented in (***Table – 1)***

III. METHOD VALIDATION

3.1 Specificity

Zaltoprofen solutions (5µg/ml) were prepared in both the selected media along with and without common excipients (dicalcium phosphate, lactose, starch, methyl cellulose, hydroxyl propyl methyl cellulose, microcrystalline cellulose, dextrose, titanium oxide, magnesium stearate, talc, aerosol and benzalkonium chloride) separately. All the solutions were scanned from 400-200 nm and checked for change in the absorbance at respective wavelengths. In a separate study, drug concentration of 5µg/ml was prepared independently from pure drug stock and commercial sample stock in selected media and analyzed (N=5).

3.2 Accuracy

As a part of determining accuracy of the proposed methods, Zaltoprofen were prepared from independent stock solution and analyzed (N = 9). Accuracy was assessed as the percentage recovery. ***(Table -3)***

3.3 Precision

Precision determined by studying repeatability and intermediate precision. Repeatability was determined by using different concentrations of pure drug: 15, 50, 75µg/ml and analyzed (N = 27) at 335nm in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium. Inter-day and intra-day variation and instrument variation were taken to determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (N = 27). ***(Table -4)***

3.4 Linearity

To establish linearity of the proposed method, nine separate series of solutions of the Zaltoprofen in solvent systems i.e., in the range of 10-80µg/ml at 335nm in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium were prepared from the stock solutions and analyzed. (***Figure- 4)***

3.5 Detection limit (DL) and Quantitation limit (QL)

The DL and QL of Zaltoprofen by the proposed methods were determined by using calibration standards. DL and QL were calculated as 3.3 σ/S and 10 σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (***Table – 2***).

3.6 Robustness

Robustness of the proposed method was determined by changing concentration of acetonitrile by ±0.2 units in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium and. Mean percentage recovery was determined (***Table - 5***).

3.7 Analysis of Zaltoprofen in Pharmaceutical formulations

Applicability of the method was tested by analyzing the commercially available two brands i.e., ***Zaltokin-80*** tablets (Ipca Laboratories Ltd., India) and ***Zalto*** tablets (Intas Pharmaceuticals, India), both brands are labeled to contain 80mg of Zaltoprofen per tablet. Twenty tablets of ***Zaltokin-80*** and ***Zalto*** tablets of Zaltoprofen were individually weighed and pulverized. Amount of the powder equivalent to 10mg of Zaltoprofen from both brands was taken and extracted with selected solvent system i.e., ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium for 30min to get the concentration 100µg/ml and filtered through Whatman filter paper number 40 then filtrate was suitably diluted to obtain 10µg/ml concentration and the samples were analyzed using proposed methods. The concentration of Zaltoprofen from tablet formulations in selected media was determined by linear regression equation (***Table - 6***).

IV. RESULTS AND DISCUSSION

Zaltoprofen showed pH dependent UV absorption spectra. This implies pH of the analytical media is very important for estimation of Zaltoprofen. Addition of acetonitrile/methanol in various proportions with various aqueous media did not improve the sensitivity of the method. The final decision of using ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system as a media was based on criteria like; sensitivity of the method, cost, ease of preparation and applicability of the method to dissolution studies.

The absorption maxima of Zaltoprofen in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium were found at 335nm. At these wavelengths absorbance of Zaltoprofen in respective media was noted. Apparent molar absorptivity of drug was found to be 0.369×10^4 L/mol/cm in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium. (***Table - 2)***.

The results showed an excellent correlation between absorbance and concentration of the Zaltoprofen. Validation parameters like accuracy, precision and linearity found low percent RSD values which indicates that the method is sensitive.

In ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium, the linear regression equation was obtained at 335nm: $y = 0.013x + 0.002$ with a regression coefficient of 0.999 (***Figure – 4)***

The UV spectrum of Zaltoprofen was not changed in the presence of common excipients in both the selected media. Absorption spectrum of pure drug sample was matching with the marketed formulation sample in both the selected media. Therefore proposed methods are specific and selective for the drug.

The excellent mean percent recovery values (nearly 100%) and their low standard deviation values (SD<1.0) of Zaltoprofen in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium represent accuracy (***Table -3***). This result revealed that any small change in the drug concentration in the solution can be accurately determined by these proposed methods.

Precision determined by studying repeat Precision determined by studying repeatability and intermediate precision. In both the selected media, the percent RSD were found to be below 1.0% at all three levels of concentrations (***Table - 4***). The percent RSD values were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision. DL and QL of Zaltoprofen in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system, were found to be 0.150µg/ml, DL and QL were found to be 0.158µg/ml (***Table - 2***).

Variation of acetonitrile by ±0.2 units in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium did not have any significant effect on absorbance (***Table-5***) and therefore the proposed method is robust.

In ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system, the assay (±SD) values of Zaltoprofen in ***Zaltokin-80*** and ***Zalto*** tablet formulations were found to be 99.98(±0.0005) and 100.02(±0.0063) respectively,the assay values of Zaltoprofen in ***Zaltokin-80*** and ***Zalto*** tablet formulations were found to be 99.98(±0.0011) and

99.99(±0.0010) respectively. Assay values of formulations were same as mentioned in the label claim, this indicated that the interference of excipient matrix is insignificant in estimation of Zaltoprofen by proposed methods (***Table - 6***). The estimated drug content with low values of standard deviation established the precision of the proposed methods.

4.1. Analytical Validation

4.2. Specificity and selectivity:

Zaltoprofen solutions (5µg/ml) were prepared in both the selected media along with and without common excipients (dicalcium phosphate, lactose, starch, methyl cellulose, hydroxypropylmethyl cellulose, microcrystalline cellulose, dextrose, titanium oxide, magnesium stearate, talc, erosol and benzalkonium chloride) separately. All the solutions were scanned from 400-200 nm and checked for change in the absorbance at respective wavelengths. In a separate study, drug concentration of 5µg/ml was prepared independently from pure drug stock and commercial sample stock in selected media and analysed (N = 5).

4.3. Accuracy

As a part of determining accuracy of the proposed methods, Zaltoprofen were prepared from independent stock solution and analyzed (N = 9). Accuracy was assessed as the percentage recovery. In ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system, the percentage recoveries (±SD) for Zaltoprofen were found to be within acceptable limit. The high percentage recovery and their low standard deviation values indicate that method is accurate (***Table - 3***). This result revealed that any small change in the drug concentration in the solution can be accurately determined by these proposed methods.

4.4 Precision

Precision determined by studying repeatability and intermediate precision. Repeatability was determined by using different concentrations of pure drug: 15, 50, 75µg/ml and analysed (N = 27) at 335nm in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium. Inter-day and intra-day variation and instrument variation were taken to determine intermediate precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (N = 27). One set of different levels of the concentrations were re analysed using LABINDIA Analytical instrument by proposed methods (N= 9). The percent RSD values were found to be below 2% which indicating that these methods have excellent repeatability and intermediate precision (***Table - 4***).

4.5 Linearity

To establish linearity of the proposed method, nine separate series of solutions of the Zaltoprofen in selected solvent system i.e., in the range of 10-80µg/ml at 335nm in ACN: 0.1N HCl (pH 1.2) were prepared from the stock solutions and analyzed (***Figure - 3 and 4***).

4.6 Detection limit (DL) and Quantitation limit (QL)

The DL and QL of Zaltoprofen by the proposed methods were determined by using calibration standards. DL and QL were calculated as 3.3 σ/S and 10 σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (***Table – 2***).

4.7 Robustness

Robustness of the proposed method was determined by changing concentration of acetonitrile by ±0.2 units in ACN: 0.1N HCl (pH 1.2) [25:75, v/v] medium and. Mean percentage recovery was determined (***Table - 5***).

4.8 Estimation of formulations

In ACN: 0.1N HCL (pH 1.2) [25:75, v/v] the assay values of Zaltoprofen for tablet ranged from 99.98 ±0.005 % with Relative standard deviation <1%. Assay values of formulations were same as mentioned in the label claim, this indicated that the interference of excipient matrix is insignificant in estimation of Zaltoprofen by proposed methods. The estimated drug content with low values of standard deviation established the precision of the proposed methods. **(Table 6).**

V. CONCLUSION

Simple UV Spectroscopic methods were developed for the quantification of Zaltoprofen using ACN: 0.1N HCl (pH 1.2) [25:75, v/v] solvent system in bulk and pharmaceutical formulation. To the best of my knowledge, the present study is the first report for the purpose. The present method succeeded in adopting a

simple sample preparation and achieved satisfactory percentage recovery and therefore it can be concluded that use of this method can be save analysis time and money. The proposed method is rapid, selective, accurate, precise, robust and inexpensive for the determination of Zaltoprofen in bulk and tablet dosage form. Hence, it can be employed for routine analysis in Quality Control Laboratories.

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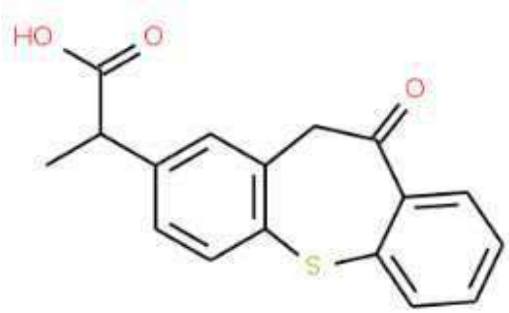

Figure-1- Structure of zaltoprofen

Figure-2- Absorption spectrum of Zaltoprofen

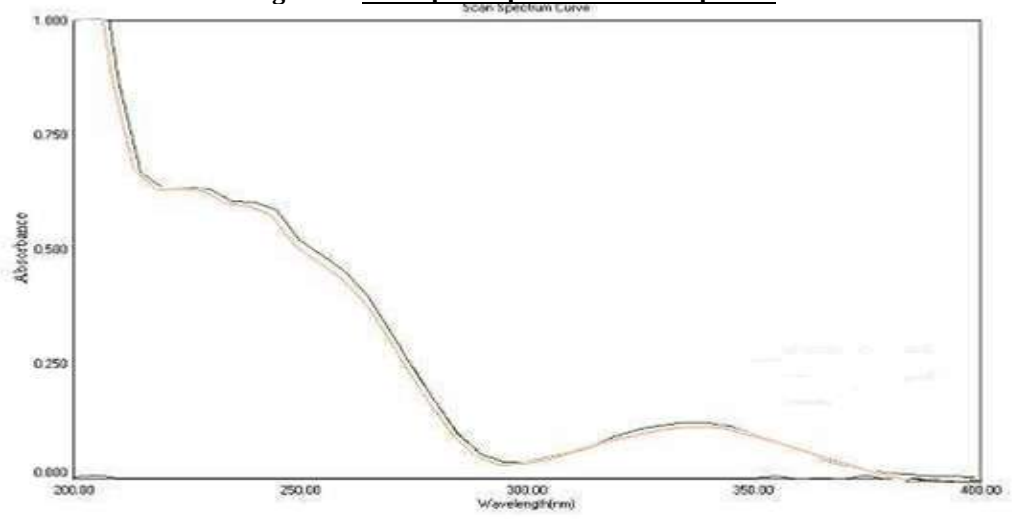

Figure-3 Overlay spectrum of Zaltoprofen at 335 nm

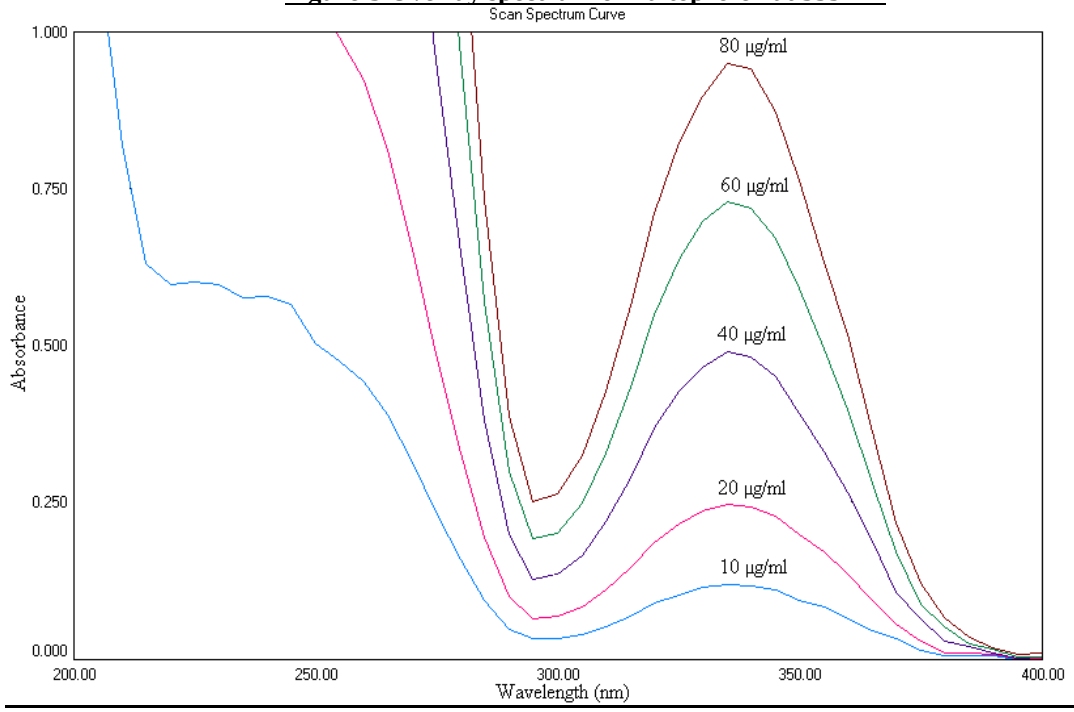

Figure-4 Calibration curve of Zaltoprofen

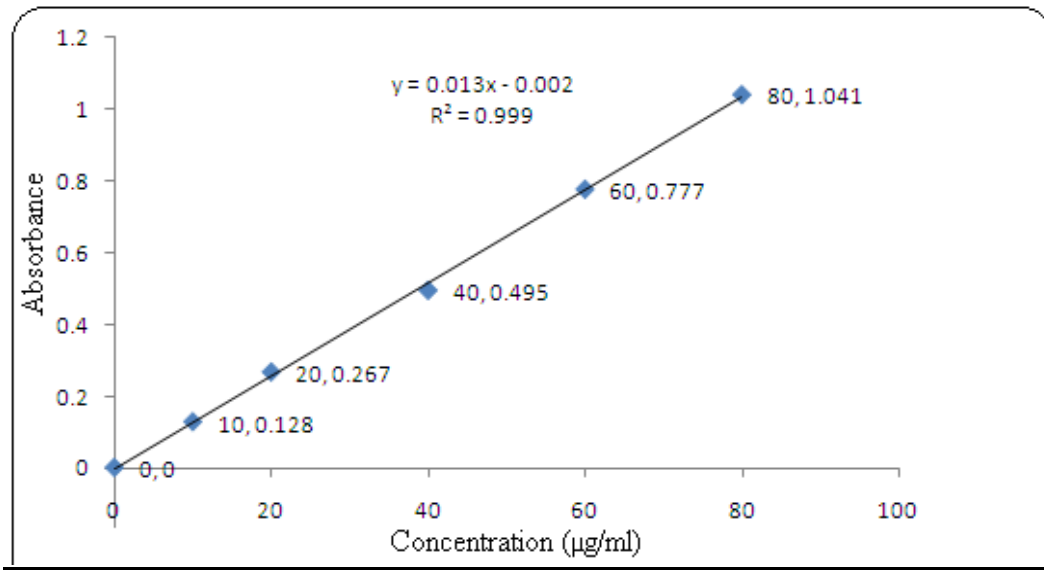

Table-1 Calibration data of Zaltoprofen.

Conc. (µg/ml)	Abs at 335nm (%RSD)	%RSD
10	0.128 ± 0.01	0.7812
20	0.267±0.002	0.749
40	0.495 ±0.004	0.8159
60	0.777 ±0.003	0.4133
80	1.041 ±0.002	0.1999

Table-2 Optical Characteristics and Regression Equation of Zaltoprofen

Wavelength (nm)	**335**
Beer's law limit (µg/ml)	10-80
Molar absorptivity (l/mol/cm)	0.369×10^4
Regression equation*	
Intercept (c)	0.002
Slope (m)	0.013
Regression coefficient (r2)	0.999
DL (µg/ml)	0.150
QL (µg/ml)	0.158

*y = mx+c; where y = absorbance at respective λmax, x = concentration of the analyte; DL – limit of detection, QL – limit of quantification

Table 3 Accuracy data of the developed method

Amount Added(µg/ml)	**ACN:0.1N HCL at 335 nm**		
	Amount found(µg/ml)	**%Accuracy±SD**	**%RSD**
10	9.999	99.99 ± 0.076	0.765
20	19.999	99.99 ± 0.076	0.3825
40	40.025	100.06 ± 0.117	0.2925
60	59.975	99.95 ± 0.090	0.1501
80	79.999	99.99 ± 0.076	0.0956

S.D-Standard Deviation, %RSD-Relative Standard Deviation

Table 4- Precision data

CONC. (µg/ml)	**Intermediate Precision(µg/ml) (%RSD)**		
	Day1	**Day2**	**Day3**
15	**15.01(0.10)**	**14.98(0.10)**	**14.99(0.10)**
50	**49.99(0.02)**	**49.97(0.04)**	**49.98(0.04)**
75	**74.98(0.02)**	**74.99(0.03)**	**75.02(0.02)**

Table 5-Robostness

Changed Parameter (ACN : 0.1N HCl)	**Zaltoprofen Conc. (µg/ml)**	**Amount found (µg/ml) (±SD)**	**%Recovery**	**%RSD**
pH 6.6	10	10.01	100.1	0.1112
pH7.0	10	10.32	100.32	0.5841

Table 6-Results of Analysis of commercial formulation

Brands	**Drug added (µg/ml)**	**Drug found (µg/ml**	**%Recovery (±SD)**	**%RSD**
Zaltokin-80	**10**	**9.998**	**99.98±0.0005**	**0.005774**
Zalto	**10**	**10.002**	**100.02±0.0063**	**0.063492**