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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ZALTOPROFEN AND PARACETAMOL IN BULK AND TABLET FORMULATION

***Annasaheb S Gaikwad, Yogesh R Thombare, Nisha S Mhaske and Mahendra B Datir**

Department of Quality Assurance Technique, Pravara Rural Education Society's College of Pharmacy, Sinnar , Dist- Nashik, M.S. India, Sp, Pune University, Pune, M.S. India

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**Corresponding author:*

ABSTRACT

A simple, sensitive, linear, precise and accurate RP-HPLC method for simultaneous estimation of Zaltoprofen and Paracetamol in bulk and tablet formulation as developed and validated. Chromatographic conditions used are stationary phase Grace C^{18} column (250mm × 4.6mm, 5µ particle size. The mobile phase Methanol: Phosphate buffer (PH 3.0) in the ratio 75:25 v/v and flow rate was maintained 0.8ml/min, detection wavelength was 241nm. The retention times were 3.101min and 5.838min for Zaltoprofen and Paracetamol respectively. Calibration plot were linear R^2 =0.9994 over the concentration range 10-18µg/ml for Zaltoprofen, R^2 = 0.9994 for the Paracetamol 40-72µg/ml. No interference from any component of pharmaceutical dosage form was observed. The proposed method has been validated as per ICH guidelines, validation studies revealed that method id specific, rapid, reliable and reproducible. The developed method successfully employed for routine quality control analysis in the combined pharmaceutical dosage form.

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INTRODUCTION

Zaltoprofen 2-(10, 11-dihydro-10-oxdibenzo (b, f) thiepin-2-yl propionic acid is a potent NSAID. Zaltoprofen is a preferential COX-2 inhibitor and selectively inhibits prostaglandin E_2 (PGE_2) production at inflammatory sites. Paracetamol is 4-hydroxy phenyl acetamide. The central analgesic action of paracetamol is like aspirin, i.e. it raise pain threshold, but has weak peripheral anti-inflammatory action. It is poor inhibitor of PG synthesis in peripheral tissues, but more active on COX inhibitor in the brain.

The ability of paracetamol to inhibit COX-3 could also account for its analgesic, antipyretic action. The combined paracetamol treatment may increase the effect and decrease the dose dependent side effect of NSAIDs and combination of Zaltoprofen with Paracetamol will be potent analgesic and anti-inflammatory drug for future in the pain management. ZAL is marketed in the combination with PCM under the trade name ZOTT ® P by Aeon Formulations Pvt. Ltd. Literature reveals that there are many methods for the individual determination of ZAL and PCM; but few methods are cited for determination of combined dosage form so, it was proposed to develop an economical, rapid and simple RP-HPLC method for simultaneous estimation of these drugs in combined dosage form.

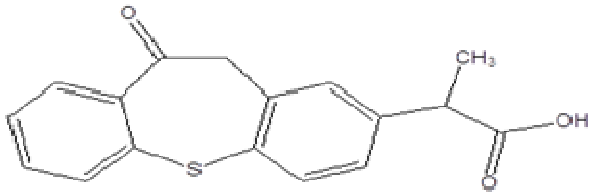
MATERIAL OF METHODS

Chemicals: HPLC grade Methanol, HPLC grade Water, Potassium dihydrogen phosphate AR grade. All other chemicals were of analytical grade.

Instrumentation:HPLC3000 series instrument, P-3000-M Reciprocating pump 40M pal. RP-HPLC Binary gradient system with grace C_{18}column (250mm × 4.6 mm id, particle size 5µ) equipped with UV 3000 –M series detector use, Wenser high precision balance (PGB 100).

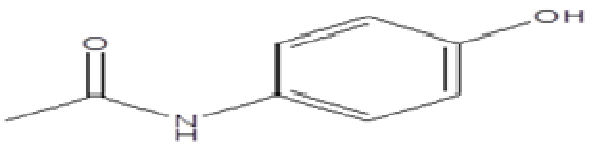
Chromatographic Conditions: The mobile phase ratio was optimized in isocratic mode for analysis of Zaltoprofen and Paracetamol. Different ratio was studied such as, 70:30, 80:20, 75:25. Of Methanol: Phosphate Buffer for Zaltoprofen and Paracetamol. The final mobile consisted 75:25 and mobile phase was clarified by filtration through nylon filter paper with pore size 0.45µm and degassed through sonicator then pumped at flow rate 08ml/min, in gradient mode on grace C_{18} column. The peak response was monitored at 241nm wave length. The sample solution was injected (20µl) HPLC system and data was acquired LC solution workstation software.

Preparation of Standard Solution: Weigh accurately 10mg of Zaltoprofen and Paracetamol was transferred into 10ml volumetric flask it was dissolved with methanol from this



Zaltoprofen

Fig No.1 Zaltoprofen



paracetamol

Fig No. 2 Paracetamol

solution 1ml was diluted to 10ml to give the stock solution containing 100µg/ml of Zaltoprofen and Paracetamol. Preparation of sample solution: these were labeled contain 80mg of Zaltoprofen and 325mg of Paracetamol as an active ingredient per four tablets. Containing 562.5mg Zaltoprofen and Paracetamol accurately weighed and powdered. And powdered equivalent to 325mg of Paracetamol as weigh 17.2mg and transferred to a 50ml volumetric flask. The volume was adjusted 50ml with solution and filter through whatman filter paper. From this filtrate 1ml was transferred to 10ml volumetric flask and diluted in order to obtain final concentration.

Experimental

Linearity: A calibration curve is the relationship between instrument response and known concentration of the analyte. Linearity was established by analyzing five concentrations of ranging between 10-18µg/ml and 40-72µg/ml respectively, by plotting the peak area ratio against corresponding concentration.

Precision: The precision of an analytical procedure express the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed condition. The inter-day (Day-I and Day-II) and intra-day (Morning and Evening) precision was studied.

Accuracy: The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy of the method was determined by Standard Addition Method. The accuracy method expressed the mean and precision expressed the relative standard deviation.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions as per ICH guidelines, change in mobile phase flow rate 1ml/min.

Sensitivity: (LOD and LOQ): The lowest standard on the calibration curve was identified as the lower limit of quantitation as the analyte peak was identifiable.

RESULTS AND DISCUSSION

Method Validation: The chromatographic method was validated using ICH guidelines.

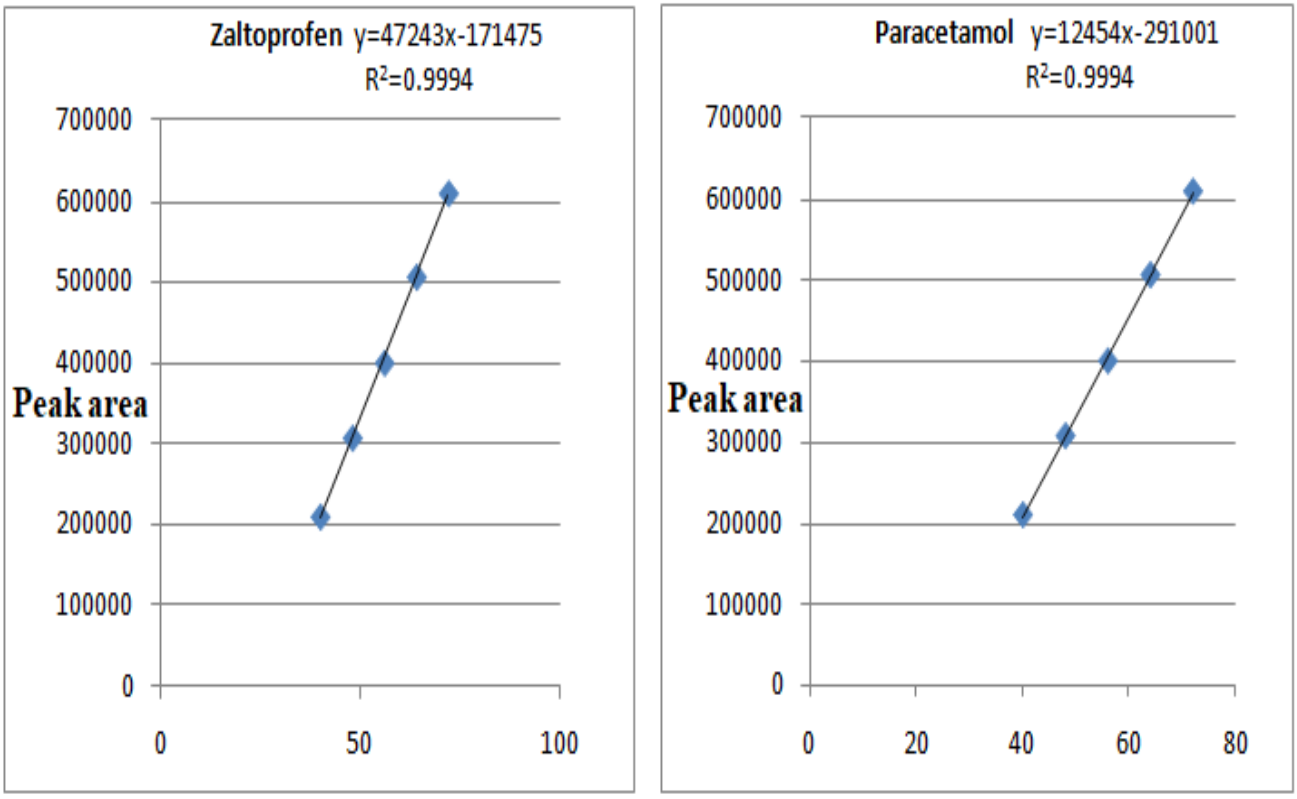


Fig.No.3 Linearity graph for Zaltoprofen and Paracetamol

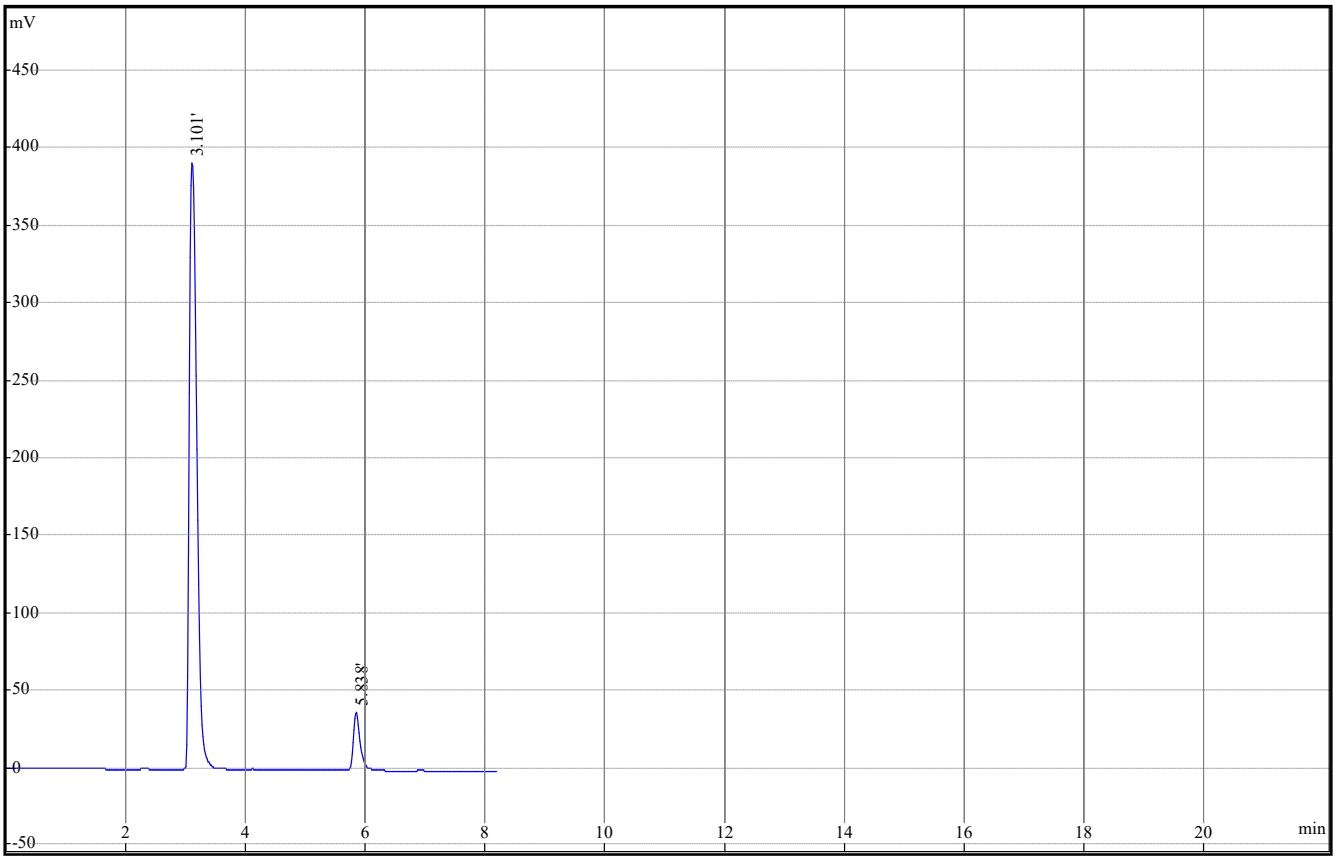


Fig.4. Chromatogram of Zaltoprofen (3.101 min) and Paracetamol (5.838min)

Table No.1 Linearity and System Suitability Parameters

Parameter	Zaltoprofen	Paracetamol
Linearity range (µg/ml)	10-18µg/ml	40-72µg/ml
Correlation coefficient (r^2)	0.9994	0.9994
Slope (m)	47243	12454
Intercept (c)	171475	291001
Theoretical Plate	78694	13282
Tailing Factor	1.23	1.26

Table No.2 Inter-day Precision

Statistical Parameter	ZAL (DAY-I)	PARA (DAY-II)	ZAL (DAY-II)	PARA (DAY-II)
SD	0.0156	0.1559	0.0106	0.0253
%RSD	0.0158	0.1561	0.0108	0.0276

Table No. 3 Intra-day Precision

Statistical Parameters	ZAL (Morning)	PARA (Evening)	ZAL (Morning)	PARA (Evening)
SD	0.0158	0.2765	0.0196	0.0462
%RSD	0.0159	0.2801	0.0198	0.0463

Table No.4 Recovery of Zaltoprofen and Paracetamol (n=3)

Recovery level	Zaltoprofen	Paracetamol
80%	99.97%	99.96%
100%	99.99%	100.00%
120%	99.99%	100.00%
Mean %Recovery	99.98%	99.99%

Table No.5 Robustness Study of Zaltoprofen and Paracetamol

Component	S.D.	R.S.D.
Zaltoprofen	8.7178	0.0012
Paracetamol	9.5393	0.0015

Table No. 6 Limit of Detection, Limit of Quantitation (LOD and LOQ)

Component	LOQ (µg/ml)	LOD (µg/ml)
Zaltoprofen	0.0811	0.0059
Paracetamol	0.0312	0.0103

Validation parameters include linearity, precision, accuracy, robustness, LOD and LOQ.

Chromatography Method: The chromatographic conditions were optimized to provide acceptable resolution of the analytes present in the drugs. The mobile phase selection was based on the peak parameters, run time and ease of preparation. The gradient condition of methanol: phosphate buffer (75:25) proved good resolution of Zaltoprofen and Paracetamol (3.101 and 5.838) fig. 3 shows representative.

Precision

Inter-day and Intra-day Precision: The inter-day (Day-I and Day-II) and intra-day (Morning and Evening) batch precisions were evaluated by assaying the one concentration, three replicate.

Accuracy: The accuracy proposed method was determined on the basis of percent recovery at three concentrations levels 80, 100, and 120 percent. The average percent recovery for Zaltoprofen and Paracetamol was found to be 99.98% and 99.99% respectively. (Table 4).

Robustness: The change in mobile phase flow rate 1ml/min, it was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed and system suitability parameters were found to be acceptable limits.

LOD and LOQ: LOD is the smallest quantity of an analyte that can be detected, and not necessarily determined, in quantitative fashion. It was calculated by the following formula;

LOD= 3.3 ×S.D. ÷ Slope

Where, S.D. = Standard Deviation

LOQ is the lowest concentration of an analyte in a sample that may be determined with acceptable accuracy and precision. It was calculated by the following formula;

Conclusion

The gradient RP-HPLC method for simultaneous determination of Zaltoprofen and Paracetamol is simple, precise, accurate and robust. The results obtained from this method were satisfactory and can be used for the routine quality control analysis of Zaltoprofen and Paracetamol in bulk as well as in tablet formulation.

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