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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF ZALTOPROFEN AND PARACETAMOL IN THEIR COMBINED SOLID DOSAGE FORM BYRP-HPLC METHOD

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Keywords:

Zaltoprofen, Paracetamol, RP-HPLC, Validation.

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ABSTRACT: A simple, sensitive, linear, precise and accurate RP-HPLC method for simultaneous estimation of Zaltoprofen and Paracetamol in bulk and tablet formulation was developed and validated. The proposed RP-HPLC method utilizes Phenomenex C18 column (150 ×4.6 mm i.d., 5 µm), optimum mobile phase consisted of isocratic run of Phosphate buffer (pH-4.2): acetonitrile (40:60V/V) with the effluent flow rate of 1.0ml/min, and UV detection wavelength 260 nm. The developed method was statistically validated for linearity, precision, robustness, ruggedness and specificity. The method was linear over the range of 2-10 µg/ml for Zaltoprofen and 8-40 µg/ml for Paracetamol. The mean recovery was 99.27% and 99.76% for Zaltoprofen and Paracetamol respectively. The intermediate precision data obtained under different experimental setup was quite concurrent with less critical %RSD. The proposed method can be useful in the quality control of Zaltoprofen and Paracetamol in their combined dosage form.

INTRODUCTION: Zaltoprofen, 2-(10, 11-dihydro-10-oxodibenzo [b, f] thiepin-2- yl) pro-pionic acid **(Figure 1)** is a potent non-steroidal anti-inflammatory drug (NSAID). It has powerful anti inflammatory and analgesic effects on inflammatory pain, which preferentially inhibits COX- 2 activity. It inhibits bradykinin-induced pain responses without blocking the receptors [1.] Paracetamol (PCM), N-(4-hydroxyphenyl) acetamide **(Figure 2),** is a non-opiate, non-salicylate, centrally and peripherally acting analgesic agent.

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Comparative effect of Paracetamol and NSAID or their combination in post operative pain management, rheumatoid arthritis and short term treatment of cancer pain has been reported.[2-4] Combined Paracetamol treatment may increase the effect and decrease the dose dependent side effects of NSAID[5] Paracetamol is official in Indian pharmacopoeia and describes UV-visible spectrophotometric titration method for its estimation[6]

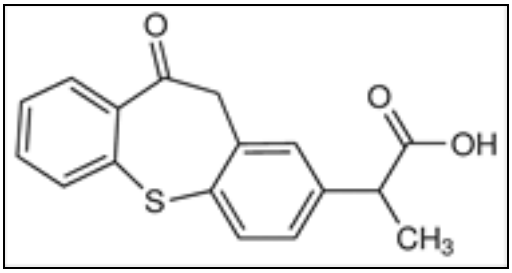

FIGURE 1: ZALTOPROFEN (ZLT)

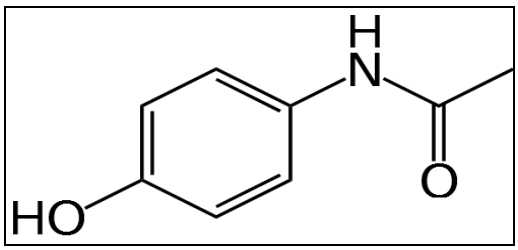

FIGURE 2: PARACETAMOL (PCM)

Addition of Paracetamol to NSAID is well tolerated and effective in the treatment of osteoarthritis. Therefore a novel combination of Zaltoprofen with Paracetamol will be a potent analgesic and anti inflammatory drug for future in the pain management.[8]

Objective of study: Survey of literature revealed that numbers of method have been reported in literature for the individual analysis of Zaltoprofen and Paracetamol by UV spectrophotometric and RP-HPLC method. UV spectrophotometric method available in literature for simultaneous determination of Paracetamol with other drugs like Etodolac, Domperidon, Ibuprofen, and Aspirin.[8-11] RP-HPLC method available in literature for simultaneous determination of Paracetamol with Etoricoxib, Lornoxicam, tramadol hydrochloride and Drotaverine.[12-15]

Lirerature survey shows very few analytical testing methods are available for Zaltoprofen. The drug was estimated by HPLC in plasma.[16-18] There is a chiral HPLC method for enantioselective analysis.[19] Zaltoprofen is official in JP. There are no HPLC methods available for determination of Zaltoprofen and Paracetamol in fixed dose combination formulation. The present study describes simple, precise and accurate reverse phase HPLC method for simultaneou determination of Zaltoprofen and Paracetamol in tablet formulation.

The aim of the present work is to develop easy, economic, accurate, specific and precise RP-HPLC methods for simultaneous determination of Zaltoprofen and Paracetamol in combined solid dosage form and validate the newly developed method.

MATERIALS AND METHODS:

Reagents and equipments: A gratuitous sample of pure Zaltoprofen was obtained from Swapnroop pharmaceutical LTD, (Aurangabad) and Paracetamol from Yarrow chem. Laboratories. (Mumbai). HPLC grade acetonitrile, orthophosphoric acid and water were procured from Merck (Mumbai, India). Milipore 0.45µ Nylon filters for solvent filtration and 0.22 µ Nylon filters for sample filtration were used. Fixed dose combination tablet formulation of Zaltoprofen and Paracetamol (REDUCIN-A, containing 80 mg of Zaltoprofen and 325 mg of Paracetamol; Sunglow pharmaceuticals (P) Ltd. Puducherry.) was purchased from the local market.

Chromatographic Conditions:

A High Performance Liquid Chromatography system, with LC solutions data handling system (Shimadzu-LC2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with Octadecylsilane chemically bonded to porous silica particles of 5mm diameter (Phenomenex C18, 5m, 150mm x 4.6 mm,) with the mobile phase containing acetonitrile and phosphate buffer (pH 4.2) in the ratio of 60:40 v/v at ambient temperature. Flow rate was kept at 1.0 ml/min and the elution was monitored at 260 nm.

Preparation of Mobile Phase: Mixed a mixture of 0.05M Phosphate buffer(pH4.2) 400ml and 600ml of HPLC grade Acetonitrile of HPLC grade, and degassed in ultrasonic water bath for 15 minutes, filtered through 0.45µm membrane filter.

Diluent Preparation*:* Mobile phase is used as diluents.

Preparation of standard solution

Stock solutions of standard drugs ZALTO and PCM were prepared by weighing accurately 25 mg of ZALTO and 100 mg of PCM into a 100 ml volumetric flask. About 70 ml of the mobile phase was added and sonicated to dissolve the drugs completely. The volume was made upto 100 ml with the mobile phase and filtered through 0.45 µm membrane filter. From the above prepared standard stock solution, 5 ml was taken to 50 ml volumetric flask and the volume was made up with the mobile

phase to obtain a concentration of 25µg/ml and 100µg/ml for ZALTO and PCM respectively.

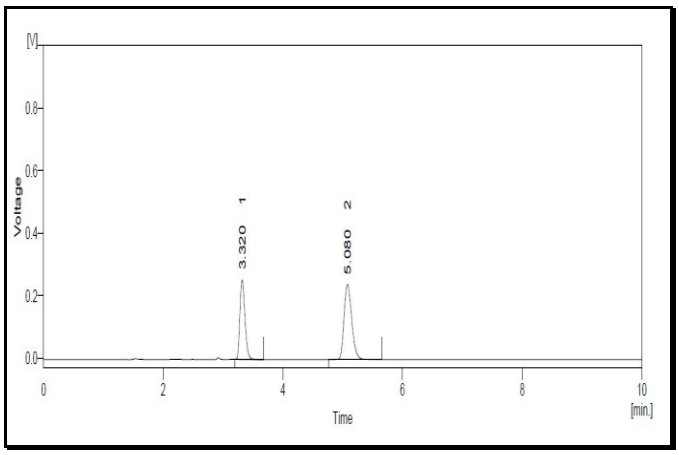

FIGURE 3: CHROMATOGRAM OF ZALTOPROFEN (3.320min), PARACETAMOL (5.080 Min), RESPECTIVELY

Estimation of Zaltoprofen and Paracetamol in tablet formulation:

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 100mg PCM and 25mg ZALTO was sonicated with 60ml diluent for 15minutes and the volume was made to 100ml with diluent. The solution was filtered and 5ml of clear filtrate was diluted to 50ml with diluent. The resultant solution (0.4ml) was further diluted to 10.0ml with diluent, so that final concentration of 10µg/ml for Zaltoprofen and 40µg/ml for Paracetamol on the basis of labeled claim was obtained. Five replicate sample solutions were prepared in similar manner.

TABLE 1: ASSAY RESULT FOR MARKET FORMULATION

Formulation (Tablet)	Amount of drug taken(mg)		Amount of drug found(mg)		% Assay Mean* ± S.D. (n=3)	
	PCM	ZALTO	PCM	ZALTO	PCM	ZALTO
REDUCIN-A	40	10	39.27	9.83	98.17±0.38	98.3±0.46

Validation of proposed method:

Specificity No interference was detected at the retention time of ZALTO and PCM in sample solution.

Linearity and Range:

Linearity of developed HPLC method was studied by obtaining calibration curves of ZALTO and PCM at five different concentration levels ranging from 2-10µg/ml for Zaltoprofen and 8-40µg/ml Paracetamol. **Table 2** shows the linearity data of ZALTO and PCM. The equation for regression line was y = 13071X+62147 (R^2= 09996) for Zaltoprofen and y = 49963X+84052 (R^2= 0.9999) for Paracetamol. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

TABLE 2: LINEARITY DATA OF ZALTOPROFEN AND PARACETAMOL

Zaltaprofen		Paracetamol	
Conc. (µg/ml)	Peak Area	Conc. (µg/ml)	Peak Area
2	881346	8	1231710
4	1141183	16	1651594
6	1407549	24	2039052
8	1679492	32	2439685
10	1919383	40	2836196

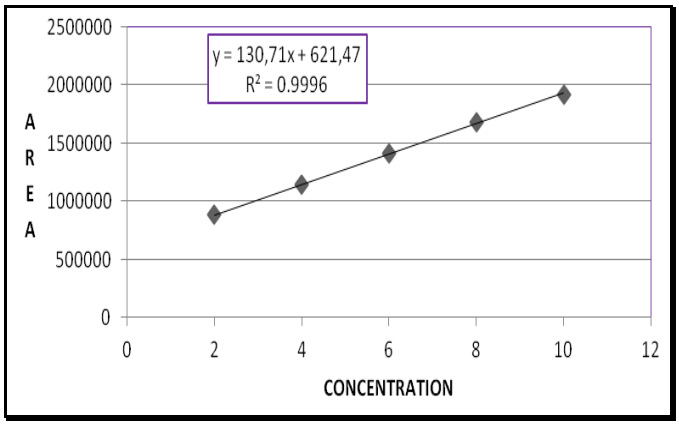

FIGURE 4: CALIBRATION CURVE OF ZALTOPROFEN

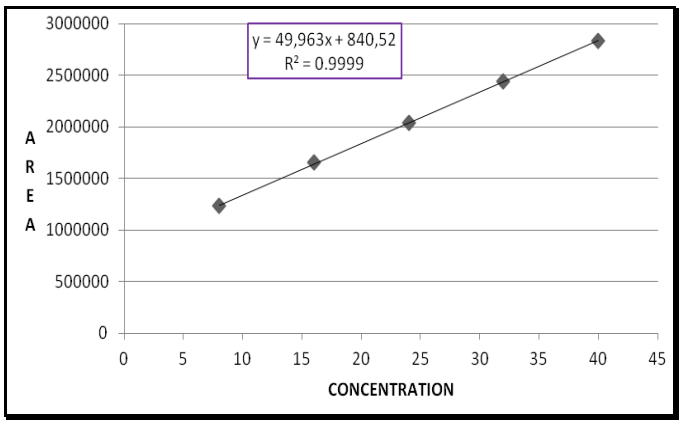

FIGURE 5: CALIBRATION CURVE OF PARACETAMOL

Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% in triplicate and the percentage of recovery was calculated shown in **Table 3**. The mean recovery of the drugs was found to be in the range of 98- 102% and % of RSD is less than 2, indicating a high degree of accuracy for the developed method.

Precision: The precision at 100 % concentration of the assay method was evaluated by six replicate injections and measurement of peak areas by determining the % RSD of Zaltoprofen and Paracetamol. The calculated values of % RSD for Zaltoprofen and Paracetamol are mentioned in **Table 4**. The results indicated a high degree of repeatability.

TABLE 3: ACCURACY RESULTS

% level of recovery	% mean recovery		%RSD	
	ZALTO	PCM	ZALTO	PCM
80%	99.22	100.004	0.72	0.99
100%	98.97	99.63	0.85	0.66
120%	99.63	99.66	0.25	0.45

TABLE 4: RESULTS FOR PRECISION

Conc. (µg/ml)	Zaltoprofen			Conc. (µg/ml)	Paracetamol		
	Peak area	Mean* ± S.D.	%RSD		Peak area	Mean* ± S.D.	%RSD
6	140573			24	2038543		
6	140597			24	2023452		
6	140952			24	2014562		
6	140578	140678 ±716.014	0.50	24	2019453	2021668 ±8081.58	0.39
6	142394			24	2015432		
6	140678			24	2018564		

LOD and LOQ:

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows.

LOD=3.3 * SD/slope of calibration curve
LOQ=10 * SD/slope of calibration curve
SD = Standard deviation of intercepts

TABLE 5: LOD AND LOQ DATA OF ZALTO AND PCM

Parameter	ZALTO (µg/ml)	PCM(µg/ml)
LOD (µg/ml)	0.68	1.70
LOQ (µg/ml)	1.08	5.16

Robustness: To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like of mobile phase composition, flow rate and pH which bare shown in **Table 6.**

TABLE 6: RESULTS OF ROBUSTNESS PARAMETER

S. No.	Parameters	%RSD for Zaltoprofen	%RSD for Paracetamol
01	Change in the Mobile Phase Composition(± 2ml in organic Phase)	0.205	0.839
02	Change in flow rate (± 0.2 ml/min)	0.376	0.239
03	Change in pH (± 0.2)	0.246	0.306

TABLE 7: VALIDATION AND SYSTEM SUITABILITY STUDIES.

Parameters	ZALTO	PCM
Beer's range(µg/ml)	1-5	4-20
Regerssion equation	Y=13071X+62147	Y=49963X+84052
Correlation coefficient	0.9970	0.999
Retention time	5.01	3.32
Theoretical plates	15503	8080
Tailing factor	1.17	1.27
Resolution	4.35	
Reapeatibility (%RSD, n=6)	0.50	0.39
Intraday(%RSD)	0.28	0.45
Interday(%RSD)	0.97	1.62
LOD(µg/ml)	0.68	1.70
LOQ(µg/ml)	2.08	5.16

RESULTS AND DISCUSSION:

The system suitability parameters and precision are evaluated and found within the limits. A plot is drawn between concentration of component and instrument response. It is found to be linear in the concentration range 2-10µg/ml and 8-40µg/ml for ZALTO and PCM respectively with good correlation coefficient (R2 = 0.999). Precision and accuracy from the developed method are expressed in % RSD and % of recovery of active pharmaceutical ingredient respectively. All system suitability parameters were found within the limit as shown in table 7.

CONCLUSIONS: The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of ZALTO and PCM in bulk as well as in tablet formulation.

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REFERENCES:

1. Makoto MM, Ueda IH: NSAID Zaltoprofen possesses novel antinociceptive mechanism through blockage of B2-type bradykinin receptor in nerve endings Neuro. Letters. 2006; 397: 249–253.
2. Hyllested M, Jones S, Pedersen JL: Comparative effect of paracetamol, NSAIDs or their combination in postoperative pain management a qualitative review, BrAnaesth. 2002; 88:199–214.
3. Glowinski J, Boccard E: Placebo-Controlled Study of the Analgesic Efficacy of a Paracetamol 500mg /Codeine 30mg Combination Together with Low-Dose vs High-Dose Diclofenac in Rheumatoid Arthritis. Clin Drug Investig. 1999; 18(3):189–197.
4. McNicol E, Strassels SA, Goudas L, Lau J, Carr DB: NSAIDS or Paracetamol, alone or combined with opioids, for cancer pain, Cochrane Database Syst Rev. 2005;(1):CD005180
5. Seideman P: Paracetamol in rheumatoid arthritis, Agents Actions Suppl. 1993; 44:7–12
6. Indian Pharmacopoeia 2010, Govt. of India Ministry of Health & Family Welfare; 3: 2037-38.
7. Combination tablets for the treatment of osteoarthritis flare pain: a multicenter, outpatient, randomized, double-blind, placebo-controlled, parallel-group,add-on study, (2002) Clin Ther. 2002; 24(2):282–297.
8. Jadav Alpa V, Gohel Bhavika A, Sondagar Mital M, Patel Bhavna A, Parmar Shraddha J:"Method Development And Validation For The Simultaneous Estimation Of Paracetamol And Etodolac By Derivative UV Spectroscopic Method." *Int J PharmaTech Res*. 2013, *5(3)*, 1155-1160
9. Hapse sandip A, Damale pallvai S, Dhumal Virashri A, Kapare Parmeshvar S:"simultaneous estimation and validation of paracetamol and domperidone in bulk and tablet dosage form by using different spectophotoetric method." *Int.Res.j.pharm*. 2013, *4(9)*, 2230-8407.
10. Yousry issa M, sayed I.M zayed, Ibrahim H.I habib: "Simultaneous determination of ibuprofen and Paracetamol using derivatives of the ratio spectra method." *Arabiyan journal of chemistery*. 2011, *4(3)*, 259-263.
11. Murtaza G, Khan SA, Shabbir A: "Development of a UV spectrophotometric method for the simultaneous determination of Aspirin and Paracetmol in tables." *Sci Res & Assays*. 2011, *6(2)*, 417-421.
12. Pattan SR, Jamdar SG, Godge RK, Dighe NS: "RP-HPLC method for simultaneous estimation of Paracetamol and Etoricoxib from bulk and tablets." *J Chem Pharm Res*. 2009, *1(1)*, 329-335.
13. Battu PR, Reddy MS: "RP-HPLC method for simultaneous estimation of Paracetamol and Ibuprofen in tablets." *Asian J Res Chem*. 2009, *2(1)*, 70-73.
14. Jagtap AS, Yadav SS, Rao JR: "Simultaneous determination of Paracetamol and Lornoxicam by RP-HPLC in bulk and tablet formulation." *Int. J. Compre. Pharm*. *2(9)*
15. Kamble M.R, Singh G.S, Singh S.S: "Validated RP-HPLC method for simultaneous estimation of Paracetamol and Tramadol hydrochloride in a commercial tablet." *J. Pham. Res*. 2011, *4(11)*, 4038-4040.
16. Chu VM, Kim KT, Kim SH, Lee W, Lee KC, NguyenVL, Hoang VL, Lee YK, Park KR, Jung SH, Kang JS: Chiral pharmacokinetics of Zaltoprofen in rats by HPLC with solid-phase extraction. J Pharm Biomed Anal. 2012; 70: 567–573.
17. Yang HK, Kim SY, Kim JS, Sah H, Lee HJ: Application of columnswitching HPLC method in evaluating pharmacokinetic parameters of Zaltoprofen and its salt. Biomed Chromatogr.2009; 23: 537-542.
18. Lee HW, Seo JH, Kim YW, Jeong SY, Lee KT: Determination of Zaltoprofen in human plasma by liquid chromatography with electrospray tandem mass spectrometry: application to a pharmacokinetic study. Rapid Commun Mass Spectrom 2006; 20: 2675-2680.
19. Nirogi R. V. S, Kota S, Peruri B. G, Kandikere V. N, Mudigonda K: Chiral high-performance liquid chromatographic method for enantioselective analysis of Zaltoprofen. Acta chromatographica 2006; 17:202-209

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