WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 6, 685-692 Research Article ISSN 2278 – 4357

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, IBUPROFEN AND CHLORZOXAZONE IN BULK AND COMBINED TABLETS DOSAGE FORMS

Dnyanda Dalvi* and Dr. Snehalatha Boddu

Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi-Mumbai.

Article Received on
14 April 2017,

Revised on 04 May 2017,
Accepted on 24 May 2017,

DOI: 10.20959/wjpps20176-9326

***Corresponding Author**
Dnyanda Dalvi
Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi-Mumbai.

ABSTRACT

This method describes a procedure to quantify the assay of Paracetmol, Ibuprofen and chlorzoxazone tablet using a mobile phase containing mixture of acetonitrile: 0.02M potassium dihydrogen phosphate buffer (60:40) adjusted to PH 3with Orthophosphoric acid, at flow rate 1ml/min in isocratic mode. The detector was set at 221nm. Paracetamol, Chlorzoxazone and ibuprofen is subsequently analyzed by reverse phased HPLC Prontosil C18 column (250 x 4.6mm, 5µm). The retention time of Paracetamol, Chlorzoxazone and Ibuprofen peaks are about 2.263, 3.773 and 8.897minutes respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity and robustness. The linearity for Paracetamol, Chlorzoxazoneand Ibuprofen was in the range of 39-104µg/mL, 30-80 µg/ml and 40-140µg/ml respectively. The method was successfully applied to the estimation of paracetamol ibuprofen and chlorzoxazone in combined dosage form.

KEYWORDS: Paracetamol, Chlorzoxazone, Ibuprofen, Validation, RP-HPLC.

INTRODUCTION

This combination of drugs was found to be more effective in relieving mild to moderate pain from certain muscle problems. It may also be used for other conditions as determined by your doctor. Paracetamol, Chlorzoxazone and Ibuprofen is a muscle relaxant analgesic and antipyretic combination. It works by decreasing pain and inflammation, which helps muscles

to relax. This HPLC method determines assay of Paracetamol, Chlorzoxazone and Ibuprofen tablet formulation.

Ibuprofen (IPB), α-methyl-4-(2-methylpropyl)-benzene acetic acid, is one of the most common non-steroidal anti-inflammatory drugs (NSAIDs). It is widely used as an analgesic in mild to moderate pain and in the treatment of rheumatoid arthritis and osteoarthritis.[1] The conventional daily dose of this NSAID is 600–1200 mg per day.[2]

Ibuprofen appears to exert its pharmacologic actions by inhibiting cyclo-oxygenase and thus blocking the first step in the synthesis of prostaglandins.[3] Furthermore, high concentrations of ibuprofen inhibit the migration, adherence, swelling, and aggregation of neutrophils and the release of lysosomal enzymes.[4-7]

Paracetamol is an acetanilide derivative chemically 4-hydroxy acetanilide having analgesic, antipyretic and weak anti-inflammatory action.[8-9]

Chlorzoxazone (5-chloro-2(3*H*)-benzoxazolone) is a compound with skeletal muscle relaxant property. It is used to decrease muscle tone and tension and thus to relieve spasm and pain associated with musculoskeletal Disorders.[10 11]

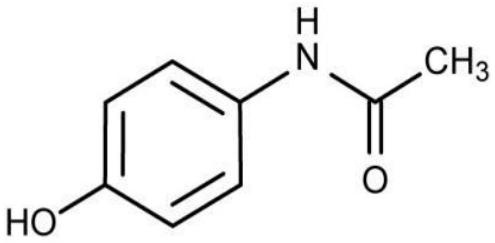

Fig 1: Structure of Paracetamol

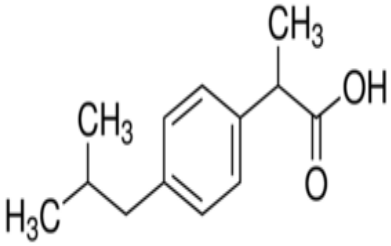

Fig 2: Structure of Ibuprofen

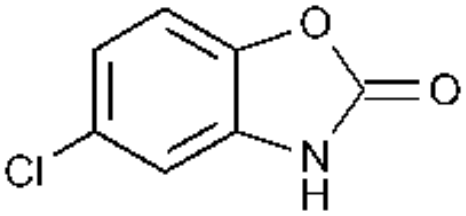

Fig 3: Structure of Chlorzoxazone

EXPERIMENTAL

Materials and reagents

Paracetamol, Chlorzoxazone was obtained from Yarrowchem Pvt Ltd and Ibuprofen was obtained as gift sample from Strides Shasun Limited, Navi Mumbai. A commercial preparation (FLEXON MR TABLET) used for analysis was procured from pharma market. Each tablet contains 325mg of PARA, 250mg CHLRZ and 400mg IBU, HPLC grade acetonitrile (Thomas Baker) and water, Potassium dihydrogen phosphate (LOBA CHEM), Orthophosphoric Acid, Triethyl Amine (Thomas Baker).

Instrumentation

RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD, rheodyne sample injection port with 20 microlitre loop, SPD-20A UV-Detector, Spinchrom software, column used was Prontosil C18(250 x 4.6mm, 5µm), Weighing was done on Contech CA-123 balance and pH was adjusted using PCI analytics Digital pH meter 111.

Chromatographic Conditions

A reverse phase column [Prontosil C18 (250 x 4.6mm, 5µm particle size)], equilibrated with mobile phase consisting of acetonitrile: 0.02M potassium dihydrogen phosphate buffer (60:40) adjusted to pH 3with Orthophosphoric acid was used. Mobile phase flow rate was maintained at 1mL/min and effluents were monitored at 221nm. The sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 13 min.

Preparation of 0.02 M Potassium dihydrogen orthophosphate (pH 3.0)

About 2.7218 g of Potassium dihydrogen orthophosphate was accurately weighed and transferred to 1000ml volumetric flask and dissolved in 900 ml of water. The pH with o-phosphoric acid to 3.0 ± 0.05, volume was made upto 1000ml using mobile phase. The solution was then filtered using 0.45 µ membrane filter.

Mobile Phase Preparation

Potassium dihydrogen orthophosphate (0.02 M) pH was adjusted to 3.0 with Orthophosphoricacid and mixed with Acetonitrile in the ratio 40:60 and was sonicated.

Standard solution preparation

100 mg of Ibuprofen, 100mg of Paracetamol and 100 mg of Chlorzoxazone standard were accurately weighed and transferred into 100 ml volumetric flask respectively. About 70 ml of

mobile phase was added, sonicated to dissolve and diluted to 100ml using mobile phase. Final concentraionof Paracetamol, Chlorzoxazone and Ibuprofen were made to 65 µg/ml and 50 µg/ml and 80 µg/ml, respectively by suitable dilutions.

Sample solution preparation

10 tablets were weighed and powdered. The quantity of powder equivalent to 400 mg of Ibuprofen, 325mg of Paracetamol and 250 mg of Chlorzoxazone were transferred into a 1000 ml volumetric flask. The volume was made up using the mobile phase, mixed and filtered through 0.45µ PVDF filter. Final concentration of Paracetamol, Chlorzoxazone and Ibuprofen were made to 65µg/ml and 50 µg/ml and 80 µg/ml, respectively by suitable dilutions.

Validation of HPLC method

The proposed RP-HPLC method was validated as per ICH guidelines.

Assay

The amounts of PARA, CHLRZ and IBU tablet were determined by using the %assay formula. Results are reported in Table 1.

Selectivity and Specificity

To assess the selectivity of the developed method solutions of all three drugs were injected into the system then observe three sharp peaks of PARA, CHLRZ and IBU were obtained at retention time of 2.263 min, 3.3733 and 8.897 mins respectively in reference to standard solution. Specificity was determined by comparison of the chromatogram of mixed standards and sample solutions. As the retention time of standard drugs and the retention time of the drugs in sample solutions were same, so the method was specific. The parameters like resolution (Rs) and asymmetric factor were calculated. Good correlation was found between the results of mixed standards and sample solutions. Results are shown in the Table 2.

Linearity

Linearity of PARA, CHLRZ and IBU was performed using a standard solution in the range of 39-104µg/ml, 30-80 µg/ml and 40-140µg/ml respectively. Results are table no shown in 3.

Precision

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of PARA, CHLRZ and IBU were carried out by estimating the

correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of PARA, CHLRZ and IBU and the results are reported in terms of % relative standard deviation (%RSD) however, all results fall within acceptance limits (RSD < 2), as shown in Table 4.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 75%, 100% and 125%. The recovery studies were carried out by adding known amounts of standard PARA, IBU and CHLRZ were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries results of PARA, IBU and CHLR in pharmaceutical preparation are shown in the Table 4.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ±1% change in mobile phase ratio, column temperature and ±1% change in pH. There was no s significant impact on the retention time and tailing factor.

Table 1: Analysis of tablet Formulation

Brand		% Amount Found (%assay)
FLEXON MR (Para 325mg + CHLRZ 250mg+ IBU 400 mg)	**PARA**	98.975%
	CHLRZ	99.076%
	IBU	98.946%

Table 2: System suitability parameters

System Suitability Parameters	PARA	CHLRZ	IBU
Retention time (min)	2.260	3.3773	8.890
Resolution	2.4	2.7	16.2
Theoretical plates	5775	6862	6546
Asymmetric factor	1.00	0.93	0.963

Table 3: Linearity studies

PARAMETERS	PARA	CHLRZ	IBU
Linearity range	39-104µg/ml	30-80 µg/ml	40-140 µg/ml
Slope	12.265	18.093	18.473
Intercept	1.1525	0.884	5.022
Correlation coefficient	0.9996	0.9993	0.9999

Table 4: Results of precision and LOD & LOQ

Parameters	PARA	CHLRZ	IBU
	Precision (%RSD)		
Intra-day (n=3)	0.76	0.53	0.47

Inter-day (n=3)	0.62	0.77	0.84
Limit of detection	1.85	1.88	1.31
Limit of quantitation	5.62	5.71	3.99

Table 5: Results of Recovery studies

Pre-analyzed sample solution[µg/ml]	Sample concentration [µg/ml]	Excess drug added [µg/ml]	Amount recovered [µg/ml]	% Recovery
Para	32.5	16.25	48.90	99.30%
	32.5	32.5	64.99	98.98%
	32.5	48.75	80.95	98.63%
CHLRZ	25	12.5	37.69	99.50%
	25	25	49.86	98.72%
	25	37.5	62.83	99.52%
IBU	40	20	60.25	99.41%
	40	40	80.48	99.59%
	40	60	99.83	98.83%

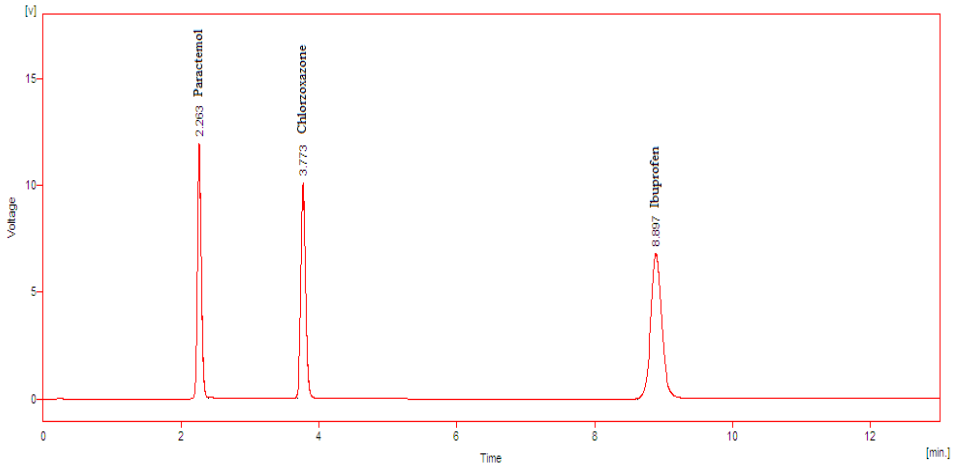

Fig 4: Chromatogram of standard solution

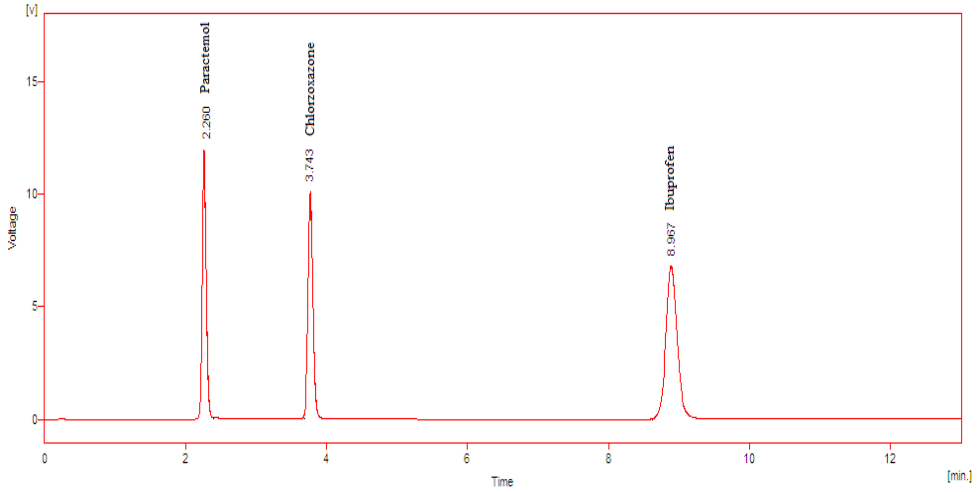

Fig 5: Chromatogram of sample solution

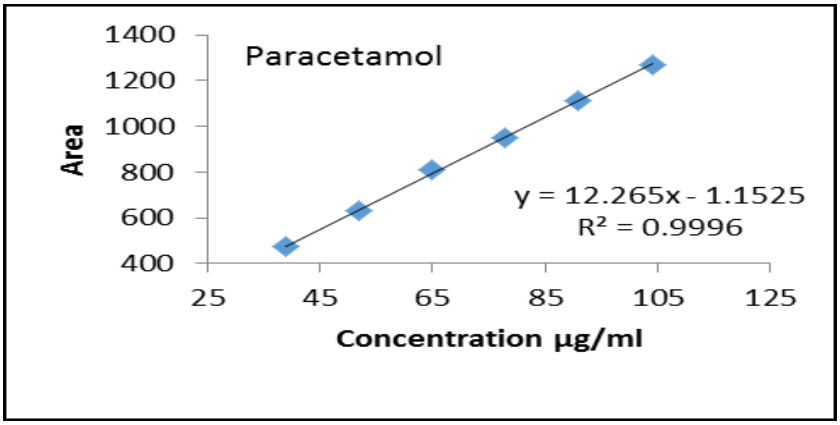

Fig 6: Calibration curve of Paracetamol

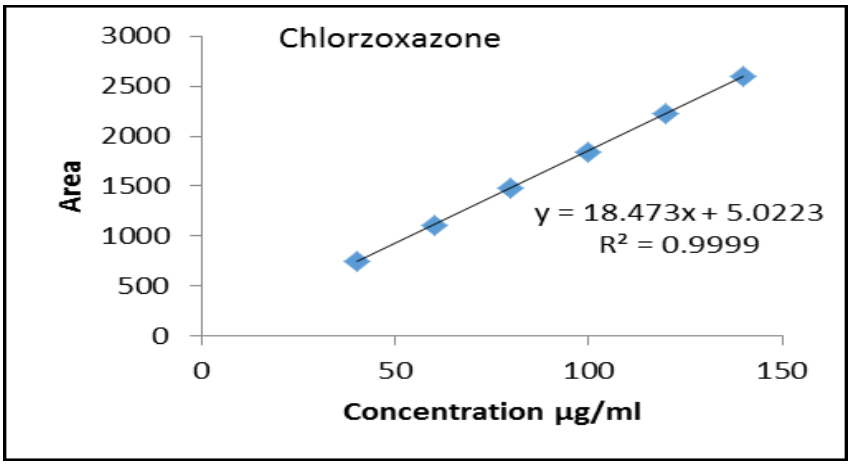

Fig 7: Calibration curve of Chlorzoxazone

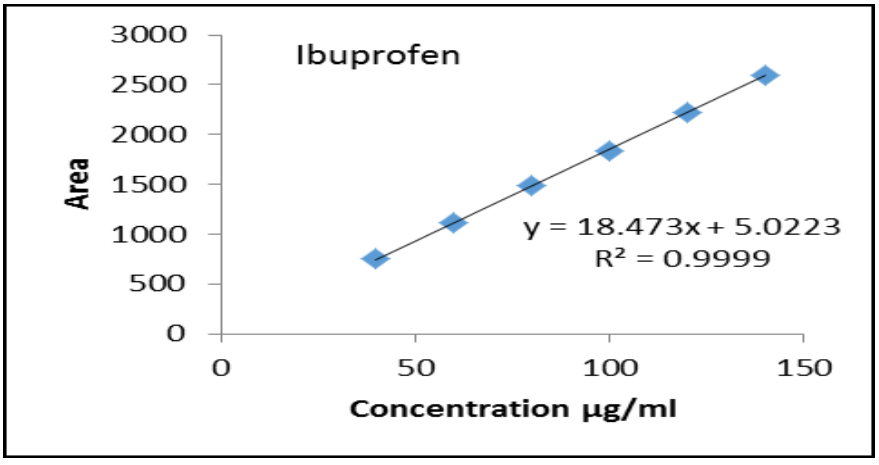

Fig 8: Calibration curve of Ibuprofen

CONCLUSION

Rapid separation of PCM, CHLRZ and IBU was successfully attained with a relatively short retention time, provides outstanding resolution, good peak shape, gives reliable and highly reproducible results on C18 HPLC column. Separation of PCM, CHLRZ and IBU mixture

was achieved with a total run time of 13mins. Excellent values for precision, recovery and linearity were achieved together with low LOD and LOQ. The ease in preparation of mobile phase and economy of the components of mobile phase show explicitly the applicability of this method the best choice in routine analysis of PCM, CHLRZ and IBU in pharmaceutical quality control departments in short period and even in low concentration levels.

REFERENCES

1. Zhao XG, Wei DZ, Song QX. A facile enzymatic process for the preparation of ibuprofen ester prodrug in organic media. J Mol Catal B: Enzym 2005; 36(1-6): 47-53.
2. De Brabander C, Vervaet C, Van Bortel L, Remon JP. Bioavailability of ibuprofen from hot-melt extruded mini-matrices. Int J Pharm 2004; 271(1-2): 77-84.
3. Brune K. Comparative pharmacology of 'non-opioid" analgesics. Med Toxicol, 1986; 1(Suppl 1): 1-9.
4. Higgs GA, Eakins KE, Mugridge KG, Moncada 5, Vane JR. The effects of non-steroid anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. Eur J Pharmacol 1980; 66: 81-6.
5. Kaplan HB, Edelson HS, Korchak HM, et al. Effects of non- steroidal anti-inflammatory agents on human neutrophil functions in vitro and in vivo. Biochem Pharmacol, 1984; 33: 371-8.
6. Maderazo EG, Breaux SP, Woronick CL. Inhibition of human polymorphonuclear leukocyte cell responses by ibuprofen. J Pharm Sci., 1984; 73: 1403-6.
7. Konstan MW, Vargo KM, Davis PB. Ibuprofen attenuates the inflammatory response to Pseudomonas aeruginosa in a rat model of chronic pulmonary infection: implications for anti-inflammatory therapy in cystic fibrosis. Am Rev Respir Dis., 1990; 141: 186-92.
8. Sweetman SC (Ed). Martindale: The complete drug reference, 33rd ed., Pharmaceutical press, London, 2002.
9. Craig RC and Stitzel RE. Modern pharmacology with clinical application, 8th ed., Lippincott Williams and Wilkins Publication, p.314, 2004.
10. The Merck Index, Thirteenth Edition, Merck and Co. Inc. USA, 2001; 379-380.
11. Bailey L C, Remington, The Science and Practice of Pharmacy, Nineteenth edition, Volume II, Mack Publishing Company, Pennsylvania, 1995; 1033.