Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

DEVELOPMENT AND VALIDATION OF RATIO SPECTRA DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF TERNARY MIXTURE OF IBUPROFEN, PARACETAMOL, CHLORZOXAZONE IN FORMULATION

Hable Asawaree Anand*[1] and Rokade Jaydip Jaywant[2]

[1*]Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune, India.

[2]Division of Avian Genetics and Breeding, ICAR-Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India.

ABSTRACT

A simple and economical ratio spectra derivative spectrophotometric method for Ibuprofen (IBU), Paracetamol (PAR), and Chlorzoxazone (CLR) combination on laboratory prepared tablet formulation has been developed. The method is also precise and accurate. The principle of method is to obtain first derivative of ratio spectra and measuring amplitude at selected wavelength. The first derivative amplitudes of ratio spectra measured at 215.18 nm, 241.70 nm and 274.43 nm for measuring the response of IBU, PAR, CLR, respectively. Beer's law is obeyed in the concentration range of 4-20 µg ml^{-1} for IBU, 3.2-16 µg ml^{-1} for PARA and 2.5-12.5 µg ml^{-1} for CLR. The % assay was found to be in the range 98.85 - 101.0 % for IBU and 98.91 - 101.72 % for PAR and 98.71-101.2 % for CLR by the proposed method. The method was validated with respect to linearity, precision and accuracy. Drug recovery for IBU, PAR and CLR was found in the range of 98.72 - 101.2 %, 98.65 - 100.64 % and 98.32-101.32 %, respectively. % RSD was found in the range of 0.65-0.93, 0.87-1.02, 0.57-1.06 for IBU, PAR, CLR, respectively.

KEYWORDS

Ibuprofen, Paracetamol, Chlorzoxazone and Ratio Spectra Derivative spectrophotometric Method.

Author for Correspondence:

Hable Asawaree Anand,
Department of Pharmaceutics,
MAEER's Maharashtra Institute of Pharmacy,
Kothrud, Pune, India.

Email: asawaree.hable@gmail.com

INTRODUCTON

The determination and analysis of every single component from complex multicomponent systems without separation of the constituting analytes is a difficult task. The zero-crossing measurements based on the analysis of binary mixtures from compounds having overlapped spectra by derivative technique, frequently[1,2]. A spectrophotometric method for resolving binary mixtures for Ibuprofen and Paracetamol, Paracetamol and Chlorzoxazone has been

reported[3,4]. The method is based on the use of the first derivative of the ratio of the spectrum[5-7].

Ibuprofen (IBU) is chemically 2 [4-(2-methyl propyl) phenyl] propanoic acid (Figure No.1). It is non-steroidal anti-inflammatory drug (NSAID) and used to get relief from symptoms of arthritis i.e. joint pain, inflammation and primary dysmenorrheal i.e. painful cramps during menstruation. It also shows an anti-platelet blood-thinning effect, antipyretic as reduces fever and analgesic as relives from pain. It inhibits the biosynthesis of prostaglandins because of its pro-inflammatory and immunosuppressive properties. Thus it is utilized to treat coccidiosis in broiler chickens[8].

Paracetamol (PAR) has chemical composition as N-(4-hydroxyphenyl) acetamide (Figure No.1). It is non-opioid analgesic and antipyretic which acts a centrally and peripherally. It is effective in treating mild to moderate pain such as headache, along with the course of bones, joints, muscles, ligaments, or nerves. It stimulates growth hormone activity, so it influences weight gain and nutrition utilization in chickens[9].

Chlorzoxazone (CLR) (5-chloro-2(3H) - benzoxazolone) is a compound (Figure No.1) with skeletal muscle relaxant property. It is used to relieve muscle tone, tension, spasm and pain associated with musculoskeletal disorders.

The combination of IBU, PAR and CLR is being prescribed for pain associated with musculoskeletal disorders. Literature survey revealed that there are various spectrophotometric analytical method reported for binary mixtures of paracetamol or ibuprofen with various different drugs in tablet or capsule dosage forms[10-18]. A HPLC method for ternary estimation of Paracetamol, Chlorzoxazone and Aceclofenac has been reported[19]. Since no spectrophotometric method has been reported for ternary estimation IBU, PAR, and CLR combination, therefore the combination was selected for the study.

MATERIAL AND METHODS

Reagents and chemicals

The spectrometric analysis was carried out on an UV-Visible double beam spectrophotometer (Varian Cary - 100) with 10 mm matched quartz cells were used. All weighing Shimadzu AUW-220D balance was used. Pure drug sample were kindly supplied as a gift sample of IBU by Vapi Care Pharma Private Ltd, Vapi Gujarat, PAR by Zest Pharma, Indore, India and CLR by Sunmour Pharmaceutical Private Ltd, Thane, India. These samples were used as such with no further purification. Methanol was of analytical grade.

Preparation of standard solutions and Construction of calibration graphs

Stock solutions of 1 mg/ml for all the three drugs were prepared separately in methanol. Further dilutions were also done using methanol. Then different aliquots of the standard solutions of IBU, PAR, and CLR within the concentration range in Table No.1, was then transferred into three sets of volumetric flasks. Further these solutions were then made up to the volume of 10 ml with methanol. The absorption spectrum was recorded and stored for each solution. For construction of calibration curve, solution 1- 5 of ternary standard mixture containing IBU, PAR, CLR in increasing concentration and solution 6-8 of binary mixture IBU+PAR, PAR+CLR and IBU+CLR have prepared in methanol as given in Table No.1. For estimation of IBU, the binary mixture of PAR+CLR used as devisor. For estimation of PAR, the binary mixture of IBU+CLR used as devisor and similarly for estimation of CLR, the binary mixture of IBU+PAR used as devisor.

METHODOLOGY

Spectrophotometric measurements

The formulation containing the combination of drugs IBU+PARA+CLR in ratio of 4:3.2:2.5 respectively was selected for analysis. The dilutions of standard drug and formulation mixture were made in the ratio 4:3.2:2.5 μg ml^{-1} for IBU + PARA + CLR, respectively and such five replicates were prepared in increasing concentration. The absorbance's of the standard solutions within the wavelength range 200–400 nm were documented and stored.

Preparation of Sample Solution and Formulation Analysis

Accurately weighed powder of 20 tablets equivalent to 12 mg of IBU (9.6 mg of PAR and 7.5 mg of CLR) was dissolved in the 25 ml of methanol, sonicated and filtered. Filter paper was washed with methanol,

washings were added. Further dilutions were made with methanol upto a 100 ml. Resulting solution was further diluted using methanol to acquire solution having concentration 12 µg/ml, 9.6 µg/ml and 7.5µg/ml of IBU, PAR and CLR, respectively. The scanning of sample solutions was done in the wavelength range of 200-400 nm. The estimation of sample was done according to procedure given above.

RESULTS AND DISCUSSION

Assay conditions

This mixture contains IBU, PAR and CLR. The absorption spectra of the three components are strongly overlapped. There was no zero-crossing point for determination of any component in presence of other two (Figure No.2). On the other hand, for demonstration of the resolving power of the proposed method, this spectral overlapping was sufficiently enough. The Figure No.3, 4, 5 shows first derivative of ratio spectra of IBU, PAR, CLR, respectively.

Ratio spectra first derivative spectrophotometry

The method involves the obtaining five UV-spectrum of the formulation in increasing concentration in the same ratio selected for study and dividing the spectra of mixture by spectrum of two analytes other than the target analyte having middle concentration (e.g. 9.6+7.5 µg of PAR+CLR for determination of IBU, latter obtaining the first derivative of ratio spectra and measurement of derivative amplitude at 215.18 nm). Likewise ratio spectra were obtained for PAR and CLR and derivative amplitude was measured at selected wavelengths (Table No.1).

Method validation

Method was initially applied to mixtures of standard drug solutions, after getting satisfactory results it was then applied for marketed (T1) and laboratory formulated (T2) tablet formulation. In order to test the accuracy of this method, different proportions of synthetic mixtures of each combination were prepared. The assay of resulting mixtures were done as per above procedure. The results were calculated to find percentage of analyte recovered for every component separately. The good recovery values of the standard deviation assure the high accuracy of this method. The linearity of this method was calculated by analyzing different concentrations of IBU, PAR, and CLR. The assay was performed according to the previously stated conditions. The corresponding first derivative amplitude for each drug component was measured at the specific wavelength and graph of amplitude vs wavelength plotted (Table No.2). For each component, a straight line was obtained. Statistical analysis of these graphs showed excellent linearity for calibration graph and follow Beer's law. The slope was free from control of the concentration of each component in the mixture. Statistical data in the result shows precision, recovery and %RSD was always less than 2% indicate precision and accuracy of the method.

By preparing and analyzing mixtures of various concentrations within the linearity range, method selectivity was detected. The mixture contains variable quantities of one component and constant amounts of the other two components. The mixtures were then analyzed as per procedures stated above and the first derivative ratios were obtained. Statistical analysis of data showed that the slope of the calibration graph for each drug is free from control of the concentration of the other components present (Table No.2). It is observed that the first derivative amplitudes were only a function of the concentration of the specific component at the particular wavelength. Consequently, the verification of the high selectivity of this method and its potential for simultaneous estimation of single component from this mixture was done by the results obtained.

Table No.1: Solutions used for acquiring Spectra of three drug mixture and two drug mixture for the method

S.No	Drug conc. in formulation/standard mixture (µg/ml)			Ratio Spectra obtained by dividing spectra obtained of solution 1 - 5 with…		1st order derivative Amplitude measured at
	IBU	PARA	CLR	Target Analyte	Divisor Analyte mixture (conc. µg mL^{-1})	
1	4	3.2	2.5	IBU	PARA+CLR(9.6+7.5) (solution- 6)	215.18 nm
2	8	6.4	5	PAR	IBU + CLR (12+7.5) (solution -7)	241.70 nm
3	12	9.6	7.5			
4	16	12.8	10	CLR	IBU+ PAR (12+9.6) (solution -8)	274.43 nm
5	20	16	1.5			

Table No.2: Study showing result of precision, recovery and regression equation

S.No	Parameter		Name of Drug		
			IBU	PAR	CLR
1	Recovery Study (% mean recove1ry, % RSD) at Recovery Level of	50%	100.15, 0.65	98.65, 1.02	99.67, 0.57
		100%	98.72, 0.89	100.64, 0.74	101.32, 1.06
		150%	101.2, 0.73	99.51, 0.87	98.32, 1.02
2	Precision (%RSD)	Repetition	0.65	0.97	1.02
		Intra Day	0.85	1.02	0.78
		Inter Day	0.93	1.32	1.51
3	Regression equation (Y=ax+b)*	a (SE)**	1.78 (0.263044655)	0.043 (0.131522328)	0.095 (0.003965547)
		b (SE)	0.105 (0.003965547)	0.0176 (0.003965547)	0.48 (0.013152233)
4	Correlation coefficient (r)		0.999	0.999	0.999
5	Formulation Analysis (% assay, %RSD)	T1	99.89, 0.87	101.62, 0.54	99.26, 1.02
		T2	100.08, 0.73	98.34, 0.43	98.51, 1.1

* a= slope, b = intercept, ** SE= standard error

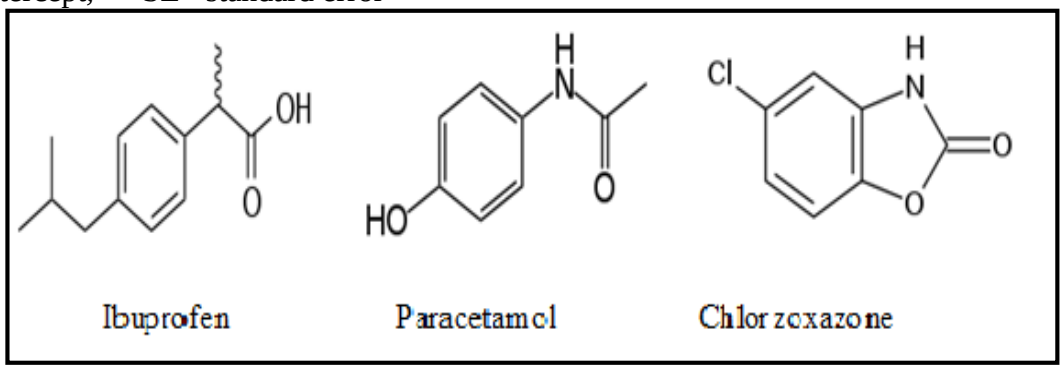

Figure No.1: Chemical structure of drugs

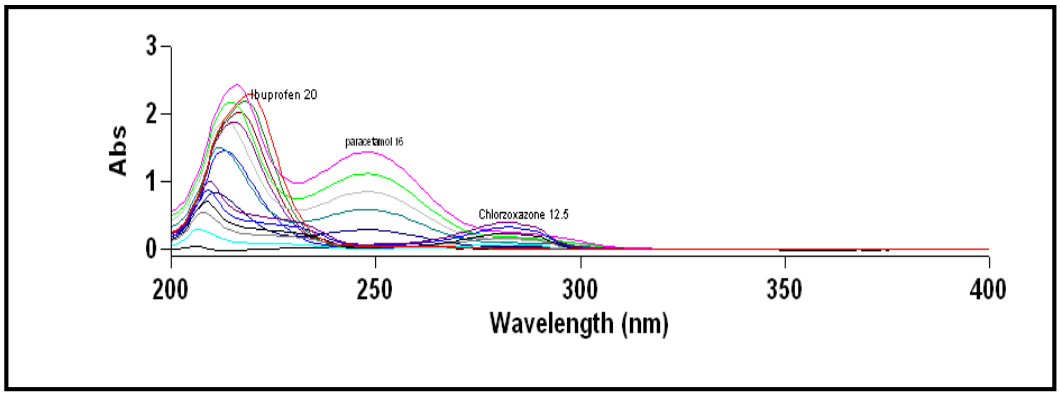

Figure No.2: Zero order spectra of IBU, PAR, CLR

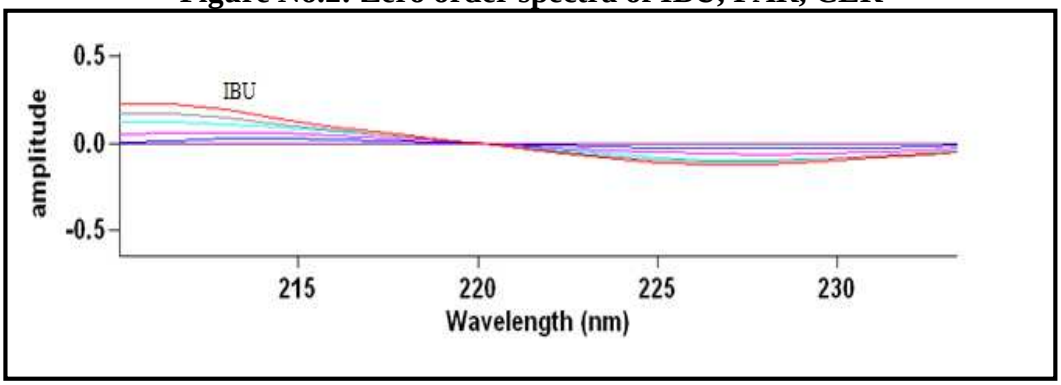

Figure No.3: First derivative of ratio spectra of IBU

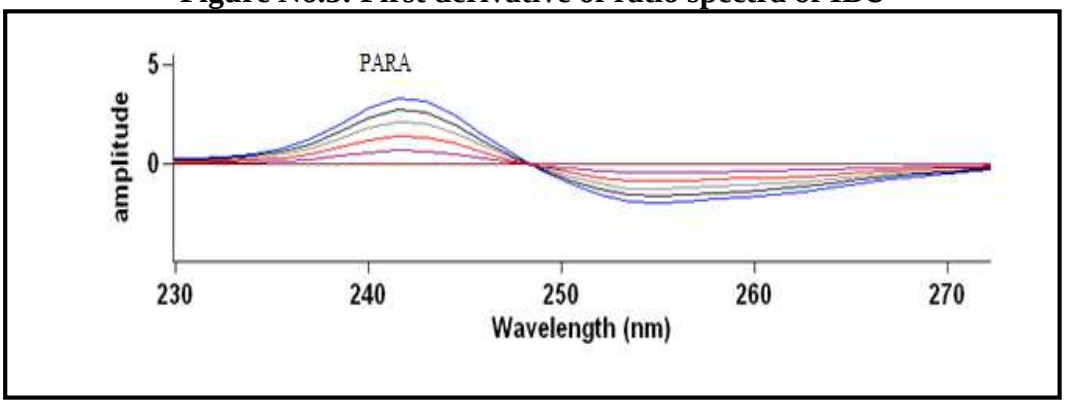

Figure No.4: First derivative ratio spectra of PAR

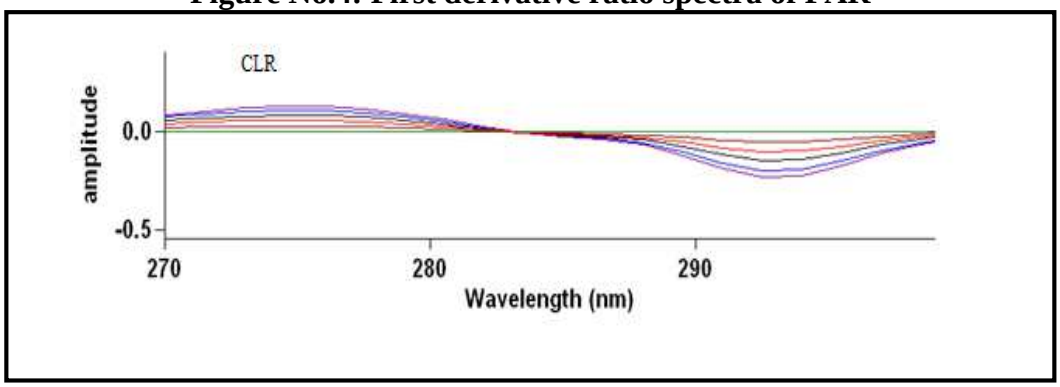

Figure No.5: First derivative ratio spectra of CLR

CONCLUSION

The proposed method is simple, accurate, precise and reproducible for quantitative analysis of IBU, PAR, and CLR as a ternary mixture. There is no need for solvent extraction as this method estimates each component individually, independent of the other components present in mixture. Also the proposed method is rapid, economical and environment friendly. This method can be practiced in quality control laboratories where economy, accuracy, reproducibility and time are essential parameters.

ACKNOWLEDGEMENT

The authors are sincerely thanks to the Department of Pharmaceutics, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Puranik M, Hirudkar A, Wadher S, Yeole P. Development and Validation of Spectrophotometric Methods For Simultaneous Estimation of Tramadol Hydrochloride and Chlorzoxazone In Tablet Dosage Form, *Indian Journal of Pharmaceutical Sciences,* 68(6), 2006, 737-739.
2. Kale U, Naidu K, Shingare M. Simultaneous Spectrophotometric Estimation of Chlorzoxazone and Nimesulide From Combined Dosage Form, *Indian Journal of Pharmaceutical Sciences,* 64(2), 2002, 168-169.
3. Wafaa H. Determination of Ibuprofen and Paracetamol In Binary Mixture Using Chemometric-Assisted Spectrophotometric Methods, *American Journal of Applied Sciences*, 5(8), 2008, 1005-1012.
4. Abdel-Aziz M. Wahbi, Azza A. Gazy, Omayma Abdel-Razak. Simultaneous Determination of Paracetamol and Chlorzoxazone Using Orthogonal Functions- Ratio Spectrophotometry, *Saudi Pharmaceutical Journal*, 11(4), 2003, 192-200.
5. Sharma R, Gaikwad P, Joshi R. A Novel Application of Hydrotropic Solubilization For Simultaneous Estimation and Validation of Acetaminophen, Chlorzoxazone, and Aceclofenac in Tablet Dosage Form, *Der Pharma Chemica*, 2(3), 2010, 90-99.
6. Garg G, Saraf S. Simultaneous Estimation of Aceclofenac, Paracetamol and Chlorzoxazone in Tablets, *Indian Journal of Pharmaceutical Sciences*, 69(5), 2007, 692-694.
7. Daharwal S. Methods of Estimation of Multi-Component formulations: A Review, *Pharmainfo.Net*, 4(3), 2006, 318-320.
8. Vermeulen B, Peek H, Remon J, Landman W. Effect of ibuprofen on coccidiosis in broiler chickens, *Avian Disease,* 48(1), 2004, 68-76.
9. Dikstein S, Zor U, Ruah D, Sulman F. Stimulatory Effect of Paracetamol on Chicken Growth, *Poultry Science,* 45(4), 1966, 744-746.
10. Chitlange S, Soni R, Wankhede S, Kulkarni A. Spectrophotometric Methods For Simultaneous Estimation of Dexibuprofen and Paracetamol, *Asian Journal Research Chem*, 2(1), 2009, 30-33.
11. Kirtawade R, Seervi C, Kulkarni A, Dhabale P. Validated Spectroscopic Method For Estimation of Ibuprofen From Tablet Formulation, *Arch Pharm Science and Research,* 2(1), 2010, 271-273.
12. Kumar P, Krishna M, Prakash P, Kumar B, Madhusudhan P. Derivative Spectrophotometric Estimation of Ondansetron and Paracetamol, *E-Journal of Chemistry*, 3(12), 2006, 134-136.
13. Gondalia R, Mashru R, Savaliya P. Development and Validation of Spectrophotometric Methods For Simultaneous Estimation of Ibuprofen and Paracetamol in Soft Gelatin Capsule By Simultaneous Equation Method, *International Journal of Chemtech Research*, 2(4), 2010, 1881-1885.
14. Engla G, Maheshwari R, Agrawal M, Agrawal A. Simultaneous Spectrophotometric Estimation of Paracetamol and Aceclofenac in Combined Tablet Formulations Using Hydrotropic Solubilization Technique, *International Journal of Chemical and Analytical Science*, 1(6), 2010, 137-142.

15. Sivasubramanian L, Lakshmi K, Tintu T. Simultaneous Spectro-Photo-metric Estimation of Paracetamol and Lornoxicam in Tablet Dosage Form, *International Journal of Pharm and Pharm Science,* 2(4), 2010, 166-168.
16. Kirtawade R, Salve P, Seervi S, Kulkarni A, Dhabale P. Simultaneous UV Spectrophotometric Method For Estimation of Paracetamol and Nimesulide in Tablet Dosage Form, *International Journal of Chemtech Research*, 2(2), 2010, 818-821.
17. Kalra K, Naik S, Jarmal G, Mishra N. Spectrophotometric Method For Simultaneous Estimation of Paracetamol and Domperidone in Tablet Formulation, *Asian Journal of Research Chem*, 2(2), 2009, 112-114.
18. Sawant R, Bhangale L, Joshi R, Lanke P. Validated Spectrophotometric Methods for Simultaneous Estimation of Paracetamol, Domperidone and Tramadol HCl in Pure and Tablet Dosage Form, *Journal Chemical Metrology*, 4(1), 2010, 21-27.
19. Venkatesh Karthikeyan, Vaidhyalingam, Yuvaraj G, Rajesh Kumar Nema. Simultaneous Estimation of Paracetamol, Chlorzoxazone and Aceclofenac In Pharmaceutical Formulation by Hplc Method, *International Journal of Chemtech Research*, 1(3), 2009, 457-460.

Please cite this article in press as: Hable Asawaree Anand and Roakde Jaydip Jaywant. Development and validation of ratio spectra derivative spectrophotometric method for determination of ternary mixture of ibuprofen, paracetamol, chlorzoxazone in formulation, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 4(2), 2016, 45-51.