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ESTIMATION OF PARACETAMOL AND ACECLOFENAC IN TABLETS BY A NOVEL RATIO DIFFERENCE METHOD

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ABSTRACT: The ratio difference spectrophotmetric method is employed for the simultaneous estimation of Paracetamol and Aceclofenac in tablet dosage form. For the estimation of Paracetamol wavelengths 245nm and 270nm were chosen as λ_1 and λ_2 and for the estimation of Aceclofenac wavelengths 214nm and 242nm were chosen as λ_1 and λ_2.The drug obeys Beer's law in concentration range of 3-40µg/ml and 3-10µg/ml for Paracetamol and Aceclofenac respectively. Limit of Detection for Paracetamol and Aceclofenac were found to be 0.1449µg/ml and 0.156µg/ml respectively. The method was validated with other ICH validation parameters also like precision and accuracy. The result shows that the method can be used for routine quality control analysis of the tablet formulations containing Paracetamol and Aceclofenac. The proposed method is simple yet rapid, accurate and cost effective.

KEYWORDS: Paracetamol, Aceclofenac, NSAID, ratio difference method, ICH guideline.

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1.INTRODUCTION

Paracetamol (PCM) is chemically *N*-(4-Hydroxyphenyl)acetamide. It is crystalline powder, sparingly soluble in water, freely soluble in alcohol and very slightly soluble in methylene chloride. Aceclofenac (ACF) is chemically [[[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic

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acid. It is crystalline powder, practically insoluble in water, freely soluble in acetone and ethanol [1]. ACF, a phenylacetic acid derivative, is an NSAID related to diclofenac. It is used in the management of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Aceclofenac is well absorbed from the gastrointestinal tract; peak plasma concentrations are reached 1 to 3 hours after an oral dose [2]. PCM is now recognised to be an inhibitor of PG synthesis in cellular systems under specific conditions and has an apparent selectivity for one of the cyclooxygenase (COX) enzymes, namely COX-2. Chemically, PCM is a phenol and, like many phenols, it is easily oxidised. This oxidation is central to its postulated mechanism of action as a substrate and an inhibitor of the peroxidase function of COX-1 and COX-2. Paracetamol is also oxidised by and inhibits other haem peroxidases, including myeloperoxidase [3]. ACF is a non-steroidal anti-inflammatory drug (NSAID) indicated for the symptomatic treatment of pain and inflammation with a reduced side effect profile, especially gastro-intestinal events that are frequently experienced with NSAID therapy. It is a pro-drug of Diclofenac [4]. The European pharmacopoeia supplementreported HPLC method for the determination of aceclofenac in presence of diclofenac [5]. There are manyreported analytical methods likespectrofluorimetric[6], colorimetric[7], spectrophotometric [8,9] HPLC [10], [11,12,13] and HPTLC [14,15] for estimation of ACF in bulk drug and pharmaceutical dosage form. Severalmethods have also been reported for estimation of PCMby GC [16] spectrophotometry [17], [18,19] titrimetry [20,21] HPLC [22,23] and HPTLC [24,25] as single component as well as in combination with other drugs. But till now any ratio difference spectrophotometric method have been reported. Thus the aim of the present work is to develop simple, economical, reproducible and rapid method for estimation of PCM and ACF in combined tablet dosage form using ratio difference spectrophotometric method.The ratio difference spectrophotometric method (RDSM) is a method in which the amplitude difference between two points on the ratio spectra of a mixture is directly proportional to the concentration of the component. For a mixture of the two drugs (X) and (Y), X can be determined by dividing the spectrum of the mixture by a known concentration of Y as a divisor (Y'). The division will give a new curve that represents:

$$(X + Y)/Y' = X/Y' + Y/Y' = X/Y' + \text{constant}.$$

By selecting two wavelengths (λ_1 and λ_2) on the obtained ratio spectrum and subtracting the amplitudes at these two points the constant Y/Y' will be cancelled along with any other instrumental error or any interference from the sample matrix.

Suppose the amplitudes at the two selected wavelength are P_1 and P_2 at λ_1 and λ_2, respectively; by subtracting the two amplitudes the interfering substance Y shows no interference; then;

$$P_1 - P_2 = [(X/Y')_1 + \text{constant}] - [(X/Y')_2 + \text{constant}] = (X/Y')1 - (X/Y')_2$$

The concentration of X is calculated by using the regression equation representing the linear

relationship between the differences of ratio spectra amplitudes at the two selected wavelengths versus the corresponding concentration of drug (X). Similarly, Y could be determined by the same procedure using a known concentration of X as a divisor X'[26].

2. MATERIALS AND METHODS

Shimadzu UV-VIS Spectrophotometer (UV 1800, Shimadzu, Japan) with fixed slit width 2 nm was used for absorbance measurements. All weighing was done on digital electronic balance (Sartorius CP 225D). Both Paracetamol and Aceclofenac standards were kindly provided by Yarrow Chem Products (Mumbai, India) which was used as such without purification. Zerodol – P tablets (labeled to contain aceclofenac 100 mg and paracetamol 325 mg) from IPCA Laboratories Ltd were purchased from the local pharmacy store. Methanol of HPLC grade was from S.d. Fine Chemicals Ltd., Mumbai, India.

Preparation of Standard solution

Accurately weighed 25mg of standard PCM, transferred it in a clean and dry 50 ml volumetric flask. Dissolved and finally volume was made up to 50ml by adding methanol.Similarly, accurately weighed 25mg of standard ACF, transferred it in a clean and dry 50ml volumetric flask. Dissolved and finally volume was made up to50ml by adding methanol.

Preparation of Sample solution

Weighed and transferred 50mg equivalent of PCM tablet powder to a clean and dry 50ml volumetric flask. Methanol was added and shook it for 5 minutes.Final volume was made by adding methanol up to the mark. Mixed, filtered and pipetted out 2.0ml from the filtrate and transferred in a 100ml volumetric flask and final volume was made with distilled water.

Method: Ratio Difference Spectrophotometric Method

The Drugs were scanned in the UV range of 190-400nm to observe their λ_{max}i.e the wavelength at which the drug show maximum absorbance and their respective spectra. From the spectrum two specific wavelengths were chosen where the drug shows same absorbance. PCM showed λ_{max} at 245nm after dividing it by the spectra of ACF (20µg/ml) whereas ACF showed λ_{max} at 214nm after dividing it by the spectrum of PCM(20µg/ml). For the estimation of PCM wavelengths 245 and 270nm were chosen as λ_1 and λ_2 and for the estimation of ACF wavelengths 214 and 242nm were chosen as λ_1 and λ_2.

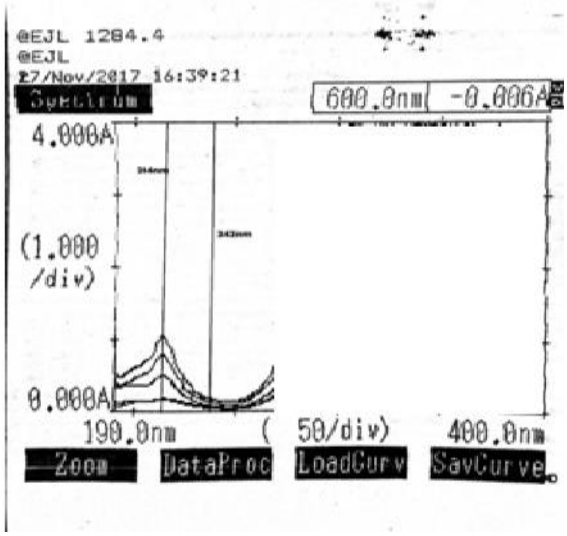

Fig 1: Linearity Spectra of Aceclofenac

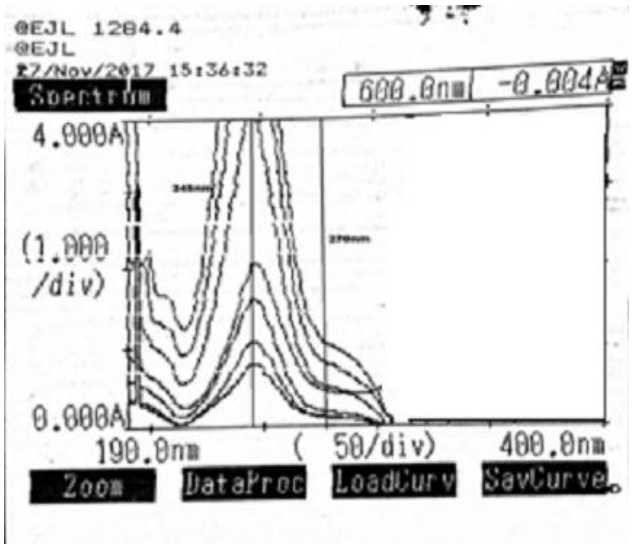

Fig 2: Linearity Spectra of Paracetamol

3. RESULTS AND DISCUSSION

The method was validated as per ICH guidelines[27].Under experimental conditions described, linearity was found in the range 3-40 µg/ml for PCM and 3-10µg/ml for ACF with correlation coefficients value of 0.997 and 0.998 respectively. Six replicate weights of tablet powder were taken to perform the precision parameter. The method showed good intra-day and inter-day precision having RSD value of 1.14 and 1.20 for PCM and 1.48 and 1.47 for ACF. Limit of detection of PCM was 0.1449µg/ml and ACF was 0.156µg/ml. Similarly, Limit of Quantitation for PCM and ACF were found to be 0.4393µg/ml and 0.4734µg/ml respectively. For recovery study, different volumes of standard solution of PCM and ACF were spiked to known concentration of PCM and ACF tablet solutions. The recoveries were found between 90-104% for both the drugs.The proposed method is found to be simple yet rapid, accurate and cost effective for estimation of PCM and ACF in combined tablet dosage form. It can be used for routine quality control of PCM and ACF containing tablet dosage form.

Table 1: Linearity, LOD and LOQ of Paracetamol and Aceclofenac

Parameters	Paracetamol	Aceclofenac
Linearity	3-40µg/ml	3-10µg/ml
Slope	0.1691	0.1373
Standard Deviation of response	0.007429	0.0065
Correlation coefficient	0.997	0.998
LOD	0.1449 µg/ml	0.156µg/ml
LOQ	0.4393 µg/ml	0.4734 µg/ml

Table 2: Results of Intra-day Precision Studies of Paracetamol and Aceclofenac

Parameters	Paracetamol	Aceclofenac
Precision(% purity)	100.5%	97.5%
	97.6%	96.4%
	96.76%	99.7%
	97.8%	95.5%
	97.87%	97.9%
	97.8%	97.8%
Mean % recovery	98.05%	97.6%
Standard Deviation(SD)	3.85	1.44
Relative SD	1.20	1.47

Table 3:Results of Inter-day Precision Studies of Paracetamol and Aceclofenac

Parameters	Paracetamol	Aceclofenac
Precision(% purity)	100.67%	98.17%
	98.07%	97.58%
	97.3%	100.95%
	98.15%	96.68%
	98.02%	98.79%
	98.46%	98.95%
Mean % recovery	98.43%	98.52%
Standard Deviation(SD)	3.67	1.46
Relative SD	1.14	1.48

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Table 4: Accuracyresult of Paracetamol and Aceclofenac

Drug	Sample conc	Spiked conc	Total conc	% recovery
Paracetamol	20µg /ml	5 µg /ml	25 µg /ml	90%
	20 µg /ml	10 µg /ml	30 µg /ml	102%
	20 µg /ml	15 µg /ml	35 µg /ml	90.13%
Aceclofenac	6.5 µg /ml	2 µg /ml	8.5 µg /ml	97%
	6.5 µg /ml	2.5 µg /ml	9 µg /ml	103.2%
	6.5 µg /ml	3.5 µg /ml	10 µg /ml	99.14%

4. CONCLUSION

The developed ratio difference method is very simple to use in terms of day to day use in quality control laboratory as it does not involve difficult and lengthy calculations of other such multicomponent analytical methods. At the same time accuracy and precision of our method is very high, which is comparable to highly expensive chromatographic methods like HPLC and HPTLC. The very low level of LOD and LOQ obtained is also a good indicator that our method can give results as good as any other chromatographic methods available for analysis of PCM and ACF.

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CONFLICT OF INTEREST

None

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