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Spectrophotometric simultaneous determination of mefenamic acid and paracetamol in combined tablet dosage form by dual wavelength, area under curve and absorbance corrected method

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ABSTRACT

Three simple, economical, precise and accurate methods are described for the simultaneous determination of Mefenamic acid (MFA) and Paracetamol (PCT) in combined tablet dosage form. The first method (Method A) is Dual wavelength, second method (Method B) is Area under curve and third method (Method C) is Absorption Corrected . In the First method, MFA was determined by plotting the difference in absorbance at 234.73 and 260.70 nm (difference is zero for PCT) against the concentration of MFA. Similarly for the determination of PCT, the difference in absorbance at 243.5 and 267.79 nm (difference is zero for MFA) was plotted against the concentration of MFA in combined formulation. In second method wavelength ranges of 233.017-236.34 nm and 267.79-271.0 nm were selected to determine PCT and MFA by AUC method in combined formulation. In the third method MFA concentration was determined directly from calibration plot by measuring absorbance at 343 nm and PCT was determined after correction for absorbance of MFA at 233 nm. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH). Beer's law is obeyed in the concentration range of 5-25 µg/mL for MFA and 4.5-22.5 µg/mL for PCT by both the methods.

Key Words: Dual wavelength, Area under curve, Absorbance corrected, Mefenamic acid, Paracetamol.

INTRODUCTION

Mefenamic acid (MA) is 2-[(2, 3-dimethylphenyl) amino]benzoic acid. MFA, an anthranilic acid derivative, is a member of the fenamate group of nonsteroidal anti inflammatory drugs (NSAIDs). It exhibits anti-inflammatory, analgesic, and antipyretic activities. Similar to other NSAIDs, MFA inhibits prostaglandin synthetase. The drug is official in British Pharmacopoeia with estimation of the drug by non-aqueous titrimetric method. Paracetamol (PCT) is chemically N -(4-hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent. It has a narrow therapeutic index – the therapeutic dose is close to the toxic dose. Literature survey reveals that various analytical techniques viz, UV spectrophotometry [1-5] spectroflurometry [6], High performance liquid chromatography (HPLC)[7-13] and High performance thin layer chromatography (HPTLC)[14-15] were reported for the analysis of PCT and MFA in pharmaceuticals. Few HPLC methods have been reported for the simultaneous determination of PCT and MFA. Our aim was to develop proper method which estimates both the analytes in a shorter time and to develop low cost method.

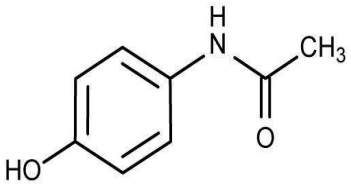

Fig 1 Paracetamol

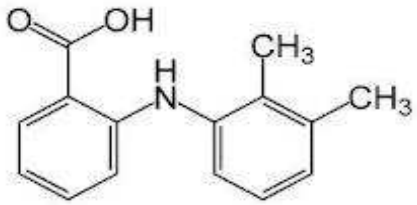

Fig 2 Mefenamic acid

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

Reagents and chemicals

Pure drug sample of MFA (% purity 99.84) and PCT (% purity 99.94) were kindly supplied as a gift sample by Emcure Pharmaceutical Pvt. Ltd. Pune and Nulife Pharmaceuticals Pvt. Ltd Pune, India, respectively and were used without further purification. Tablet used for analysis was Biospas Forte (Batch No. X2458) manufactured by Biochem Pharmaceutical Pvt. Ltd. Pune, containing MFA 500 mg and PCT 450 mg per tablet.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of pure drug containing 1000 µg/mL of MFA and PCT were Prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analytes in the concentration range of 5-25 µg/mL and 4.5-22.5 µg/mL of MFA and PCT, respectively and scanned in the range of 200-400nm (Fig 3).

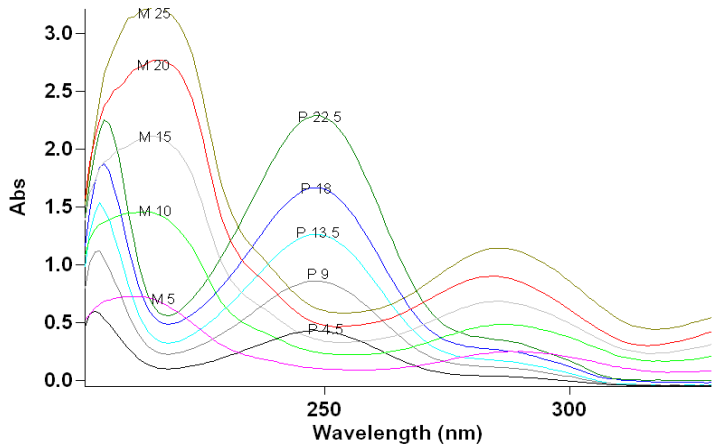

Fig 3: Overlay spectra of PCT (4.5-22.5 µg/mL) and MFA (5-25 µg/mL) in methanol.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 500 mg of MFA (PCT 450 mg) was weighed and dissolved in the 70 mL of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with solvent,

adding washings to the volumetric flask, volume was made up to the mark with methanol. The solution was suitably diluted with methanol to get 15 µg/mL MFA and 13.5 µg/mL of PCT.

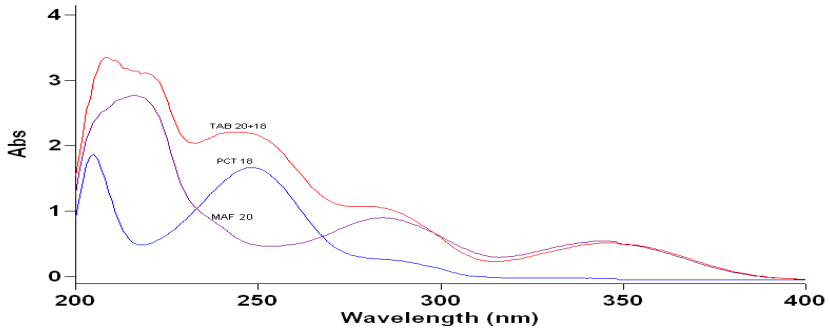

Fig 4.: overlay spectra of PCT (18 µg/mL),MFA (20 µg/mL) and Formulation (20+18 µg/mL)

Theoretical Aspects

Method A : Dual wavelength method

In this method difference in absorbance at two selected wavelengths were calculated. The differene in absorbance at 237.73 and 260.70 nm was found to be zero for PCT. Hence these two wavelengths were selected for the determinat ion of MFA. Similarly, 243.50 and 267.79 nm were selected for the determination of PCT, where the difference in a bsorbance was found to be zero for MFA. Zero order spectra were recorded for solutions of different concentration of MFA and

PCT between 200:400nm. The difference in absorbance at 243.50 and267.79 nm were plotted against the concentrati on of MFA and that 237.73 and 260.70 nm were plotted against the concentration of PCT to construct two separate calibration curves for MFA and PCT. The equations of line obtained to determine concentrations of MFA and PCT.

Method B: Area under curve

For the simultaneous determination using the area under the curve method, suitable dilutions of the standard stock solutions (1000 µg/mL) of MFA and PCT were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm. For Area

Under Curve method, the sampling wavelength ranges selected for estimation of MFA and PCT were 233.01-236.34 nm (λ1-λ2) and 269.79-271 nm (λ3-λ4). Mixed standards were prepared and their Area under the Curve were measured at the selected wavelength ranges.

Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and (2).

$$\mathbf{C_{MFA}} = A_2 \times a_{X2} - A_1 \times a_{y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2} \quad \text{..... (1)}$$

$$\mathbf{C_{PCT}} = A_2 - a_{X2} \times C_{MFA} / a_{Y2} \quad \text{..... (2)}$$

Method C: Absorption Corrected Method

The solutions were scanned in UV Spectrophotometer in the range 200 - 400 nm at 0.5 band width and 600 nm/min scan speed for the determinations of λmax of MFA & PCT and was found to be at 343 nm and 233 nm respectively. MFA showed absorbance at 343 nm, while PCT did not show any interference at 343 [Fig 3]. To construct Beer's plot for MFA and PCT, stock solutions of 1000 µg/mL of both the drugs were prepared in methanol and working standard dilutions were made in methanol using stock solution of 1000 µg/mL. Also Beer's plot was constructed for MFA and PCT in solution mixture at different concentration (5:4.5, 10:9, 15:13.5, 20:18, 25:22.5 µg/ml) levels. Both the drugs followed linearity individually and in mixture within the concentration range 5-25 µg/mL and 4.5-22.5 µg/mL for MFA and PCT, respectively.

Recovery studies
The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drug standard solution was 15 µg/mL of MFA and 13.5 µg/mL of PCT for all the methods.

Solution stability
Method stability was checked by analyzing solution kept in fridge and at room temperature by both methods. Solution at room temperature was stable for 12 hours and solution in fridge was stable for 30 days.

Method sensitivity (LOD and LOQ)
The values of LOD and LOQ were calculated by using σ (standard deviation of response) and b (slope of the calibration curve) and by using equations, LOD = (3.3 × σ)/b and LOQ = (10 × σ)/b.

Precision of the Method
Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined (Table 1). Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Specificity
Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness:
The robustness of the proposed methods was tested by changing parameters such as wavelength range, slit width, temperature, shaking time etc. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

Ruggedness:
Ruggedness of the proposed methods were determined by analyzing aliquots from homogenous lot in different under graduate laboratories using similar operational and environmental conditions; data is presented in (Table 1).

Table 1: Optical characteristics, Precision study and result of formulation analysis

Parameter		Mefenamic acid			Paracetamol		
		Method A	Method B	Method C	Method A	Method B	Method C
Wavelength (nm)		300.00	244.14	343	268.022	373.45	233
Beer's law limit (µg/mL)		5-25	5-25	5-25	4.5-22.5	4.5-22.5	4.5-22.5
Regression Equation*	Slope (m)	0.04935	0.0960	0.02542	0.09701	0.2203	0.06308
	Intercept(c)) (c)	-0.03401	-0.1652	-0.03972	-0.06018	-0.1473	-0.06694
Correlation coefficient (r)		0.999	0.999	0.998	0.997	0.999	0.998
Precision (%RSD)	Repeatability (n=5)	0.51	0.76	0.86	0.79	0.89	0.65
	Intra-day	0.65	0.78	0.98	1.00	0.85	0.74
	Inter-day	0.71	0.65	0.85	0.65	1.65	1.10
Formulation Analysis (% Assay, %RSD), n=6		100.24 ±0.45	100.81 ± 0.46	99.91 ± 0.80	99.81 ± 0.81	99.21 ± 0.29	100.21 ± 0.70
LOD (µg/mL)		0.621	0.3227	0.6734	0.3199	0.2979	0.4912
LOQ (µg/mL)		1.881	0.9778	2.0407	0.9696	0.9029	1.4886
Ruggedness (%RSD)	Analyst I	0.55	0.92	1.12	0.45	0.63	0.56
	Analyst II	0.56	0.75	1.25	0.83	0.48	0.89

RSD = Relative Standard Deviation, Y = mX + c, where Y is the absorbance and X the concentration in micrograms per milliliter*

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. Using appropriate dilutions of standard stock solution the two solutions were Scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in (Table 1). As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and Precision. Beer's law is obeyed in the concentration range of 5-25 µg/mL and 4.5-22.5 µg/mL for MFA and PCT, respectively. Correlation coefficient was greater than 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets

Containing MFA and PCT. Percent labeled claim and standard deviation (S.D) was calculated and the results are presented in (Table 1). Results of accuracy study are presented in (Table 2). The accuracy is evident from the data as results are close to 100 % and standard deviation is low. All the statistical data analysis was performed by using MIP Pharmasoft 1.0, software developed and validated in the Institute.

Table 2: Accuracy results

Recovery Level	Analyte name	Amount Spiked (µg/mL)	% Mean Recovery, % RSD, n=6		
			Method A	Method B	Method C
80%	MFA	12	100.39 0.79	99.45, 0.38	99.89,0.56
	PCT	10.8	99.45, 0.97	98.75, 1.05	100.44,0.54
100%	MFA	15	99.90, 1.03	98.63, 0.92	99.14,0.12
	PCT	13.5	98.89, 1.57	99.13, 1.72	101.12,0.57
120%	MFA	18	100.05, 0.19	101.75, 0.74	98.34,0.45
	PCT	16.2	98.93, 1.34	99.66, 0.93	100.30,0.94

CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of MFA and PCT in tablet dosage form.

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REFERENCES

[1] Satyanarayana; Dondeti; Kannan; Kamarajan; Manavalan; Rajappan, Journal of the Serbian Chemical Society (**2006**), 71(11), 1207-1218.
[2] Dinc; Erdal; Yucesoy; Cem; Onur, Feyyaz, J.Pharma Biomed Anal (**2002**), 28(6), 10911100.
[3] Thai; Duy Thin; Nguyen; Tuong Vy; Tran, Viet Hung. Tap Chi Duoc Hoc (**2006**), 46(2), 27-31.
[4] Gangwal; Shrenik; A. K. Sharma, Indian J. Pharma Sci. (**1996**), 58(5), 216-218.
[5] Das, Sukomal; Sharma; C Suresh; Talwar; K Santosh; P. D Sethi, Analyst (Cambridge, United Kingdom) (**1989**), 114(1), 101-3.
[6] Madrakian; Tayyebeh; Afkhami; Abbas; Mohammadnejad; Masoumeh; Analytica Chimica Acta (**2009**), 645(1-2), 25-29.
[7] Hung; Chin-Yin; Hwang; Ching-Chiang, J. Chroma. Sci. (**2008**), 46(9), 813-818.
[8] Jaiswal; Yogini; Talele; Gokul; Surana; Sanjay, Journal of Liquid Chromatography & Related Technologies (**2007**), 30(8), 1115-1124.
[9] E. Mikami; T Goto; T. Ohno; H Matsumoto; K Inagaki; H.Ishihara; M.Nishida, J. Chromatogr B: Biomed Sci and Applications (**2000**), 744(1), 81-89.
[10] Rau; L. Harish; A. R Aroor; Rao; P Gundu, Indian Drugs (**1991**), 28(12), 563-5.
[11]Madhukar.A; V. Sudhirkumar;P.Anand; C.H Samrat, J.Chem. Pharma. Res. (**2011**), 3(3), 464-469
[12] D.K.Mandloi, P.K.Tyagi, V.K.Rai, S. Dey, R.K Ashada and P.Mohanraj J.Chem. Pharma. Res. (**2009**),1(1), 286-296

[13] S. R. Pattan, S. G. Jamdar, R. K. Godge, N. S. Dighe, A.V. Daithankar, S. A. Nirmal and M.G.Pai , J. Chem. Pharma. Res. (**2009**),1(1), 329-335
[14] Maliye; N Amit; Walode; G Sanjay; Kasture; V Avinash; Wadodkar; G Sudhir, Asian J Chemistry (**2005**), 18(1), 667-672.
[15] A.P.Argekar; J G Sawant, Journal of Planar Chromatography— Modern TLC(**1999**), 12(5), 361-364.