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Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Pamabrom in Pharmaceutical Dosage Form

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical second derivative spectrophotometric method for the simultaneous determination of Paracetamol and Pamabrom in dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The second order derivative spectra were obtained in dist. water and the determinations were made at 268.2 nm (ZCP of Pamabrom) for Paracetamol and 225.0 nm (ZCP of Paracetamol) for Pamabrom. The linearity was obtained in the concentration range of 4-18 µg/ml for Paracetamol and 2-16 µg/ml for Pamabrom The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of Paracetamol and Pamabrom in pharmaceutical tablet dosage form.

Keywords: Paracetamol (PCM), Pamabrom (PBM), recovery, second order derivative spectrophotometric method, validation.

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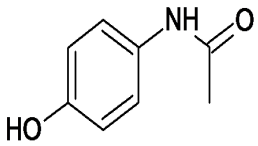
INTRODUCTION

Paracetamol (N- 4-hydroxyphenyl acetamide) is an Analgesic-antipyretic drugs with poor Anti-inflammatory action, belongs to Para-aminophenol derivative categories of NSAIDs. The Main mechanism of action of paracetamol is considered to be the inhibition of cyclooxygenase (COX), recent findings suggest that it is highly selective for COX-2.Because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes.[1]

Pamabrom is a xanthine derivative drug and it might increase the renal blood flow by virtue of their cardiac stimulant property and vasodilator action which promote filtration of fluid by the glomeruli. They also produce dieresis by diminishing the tubular reabsorption of water. The chief mechanism seems to be the interference in tubular reabsorption of Na+ and Cl- , perhaps by acting on the enzyme concerned with the transport of these ions. This eventually leads to less absorption of water and favours its excretion.

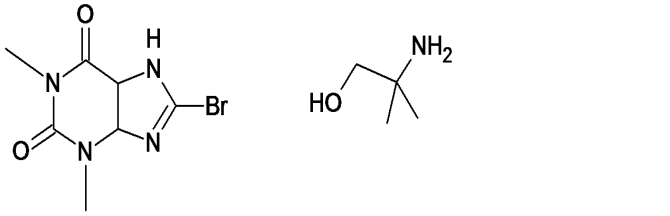
Paracetamol and Pamabrom tablet is use for menstrual pain. Paracetamol is a official in Indian Pharmacopoeia, British Pharmacopoeia, and United state Pharmacopoeia. Many methods like spectroscopy, HPLC, and HPTLC method are reported for estimation paracetamol[5]. Pamabrom is a Official in United state Pharmacopoeia[15]. Only HPLC method is reported for estimation Pamabrom.

Paracetamol and Pamabrom combination is approved by CDSCO. But no method Reported for estimation of Combination of Paracetamol and Pamabrom in pharmaceutical dosage form.



N-(4-hydroxy phenyl)-ethanamide

Figure. 1: Paracetamol



1:1 Mixture of 2-amino-2-methyl-1-propanol and 8-bromotheophyllinate

Figure. 2: Pamabrom

MATERIALS AND METHODS:

Instrument:

A Shimadzu model UV-1800 double beam UV-visible Spectrophotometer, attached to a computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched quartz cells was used. Simadzu analytical balance (Sartorius, Gottingen, Germany), and Ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used throughout the practical. Class 'A' volumetric glassware were used.

Reagents and Materials:

Paracetamol and Pamabrom bulk powder was kindly gifted by reputed Pharmaceutical Company in, Hyderabad, India.

Preparation of Standard Stock Solutions:

Accurately weighed portions of PCM (100mg) and PBM (100mg) were transferred to a separate 100ml volumetric flask and dissolved and diluted to the mark with Dist. Water to obtain standard solution having concentrations of PCM (1000µg/ml) and PBM (1000µg/ml). From the above stock solution transferred 10 ml to 100 ml of volumetric flask and diluted to the mark with Dist. water to obtain standard solution having concentrations of PCM (100µg/ml) and PBM (100µg/ml).

Preparation of Sample Solution:

Take 100mg of PBM and 250 mg of PCM and transferred in to a 100 ml volumetric flask and sonicated for 20 min. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with Dist. water. This solution is expected to contain 1000µg/ml PBM and 2500µg/ml PCM. This solution (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with Dist. Water to get a concentration of PBM (100µg/ml) and PCM (250 µg/ml). From this solution(2 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with Dist. water to get a final concentration of PBM (2µg/ml) and PCM (5 µg/ml).

DEVLOPMENT OF METHOD:

The spectrum of the standard solution was taken in the range of 200 nm to 400 nm. This spectrum of the drugs was converted to second derivative forms and ZCP'S (Zero Crossing Points) were determined and it were found to be 225.0 nm, 258.0 nm and 278.0 nm for Paracetamol and 219.0 nm, 268.2 nm and 293.0 nm for Pamabrom.

The absorbance of the Paracetamol solution was measured at 268.2 nm (ZCP of Pamabrom) and for the Pamabrom solution absorbance was measured at 225.0 nm (ZCP of Paracetamol). The linearity was found in the range of 4-18 µg/ml and 2-16 µg/ml for Paracetamol and Pamabrom respectively. The calibration curves for derivative spectroscopy were constructed by plotting drug absorbance at ZCP Vs concentration and regression equations were computed.

Validation of the proposed method:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity:

Calibration curves were plotted over a concentration range of 4-18µg/ml and 2-16µg/ml for PCM and PBM respectively. Accurately measured standard working solutions of PCM (4.0, 6.0, 8.0, 10.0, 12.0, 14.0 ,16.0 and 18.0ml) and PBM (2.0, 4.0, 6.0, 8.0, 10.0,12.0,14.0 and 16.0 ml) were transferred to a series of 100ml of volumetric flasks and diluted to the mark with Dist. water, and absorbance were measured at 268.2 nm(ZCP of PBM) and 225.0 nm(ZCP of PCM) for PCM and PBM.

The calibration curves were constructed by plotting absorbances at ZCP Vs concentrations.

Method precision (repeatability):

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of PCM and PBM (12µg/ml and 10 µg/ml) without changing the parameters for the method.

Intermediate precision (reproducibility):

The intraday and interday precisions of the proposed methods were determined by analyzing the corresponding responses 3 times on the same day and on 3 different days with 3 different concentrations of standard solutions of PCM and PBM (4, 12 and 18 µg/ml and 2, 10, 16µg/ml respectively). The results were reported in terms of relative standard deviation (RSD).

Limit of detection and limit of quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the drug wee derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Accuracy (recovery study):

The accuracy of the methods was determined by calculating recoveries of PCM and PBM by the standard addition method. Known amounts of standard solutions of PCM and PBM were added at 80, 100 and 120% levels to pre-quantified sample solutions of PCM and PBM(5 and 2µg/ml respectively). The amounts of PCM and PBM were estimated by applying the obtained values to the equation.

Analysis of PCM and PBM in synthetic mixture:

Binary mixture was prepared for combination of both drug in ratio of 5:2(PCM and PBM). The absorbance was measured at 268.2 and 225.0 nm for quantification of PCM and PBM,

respectively. The amounts of PCM and PBM present in sample solutions were determined by fitting the response into the equation for PCM and PBM. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Paracetamol and Pamabrom in mixture.

RESULTS AND DISCUSSION:

In this method, the overlain spectra of drugs showed the absorbance of the Paracetamol solution was measured at 268.2 nm (ZCP of Pamabrom) and for the Pamabrom solution absorbance was measured at 225.0 nm (ZCP of Paracetamol)(Figure 1). Both the drugs obeyed linearity range 4-18 µg/ml and 2-16µg/ml respectively and correlation coefficient (r2) were found to be <1 in both cases(Figure 2,3&4 Table-1).

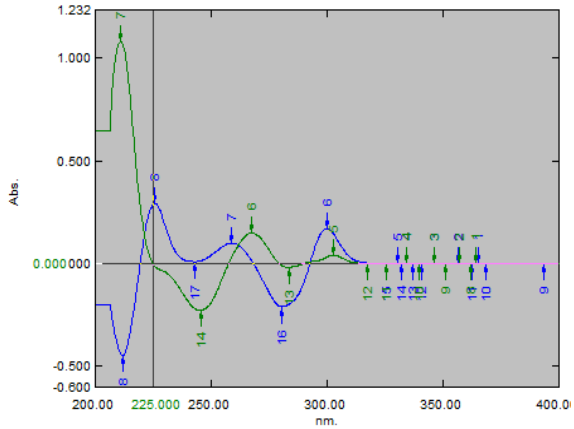


Figure 1: Overlain Second-order spectra of PCM and PBM

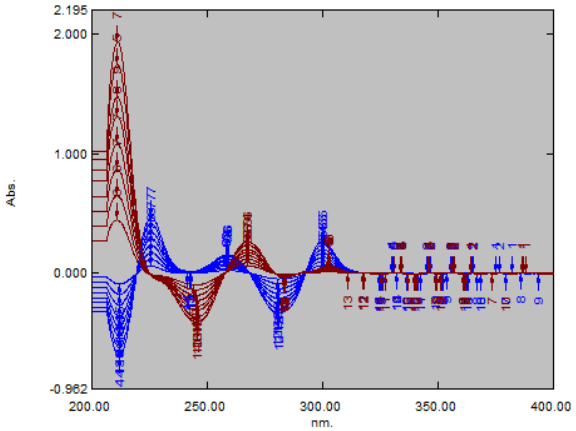


Figure 2: Overlain second order spectra of calibration

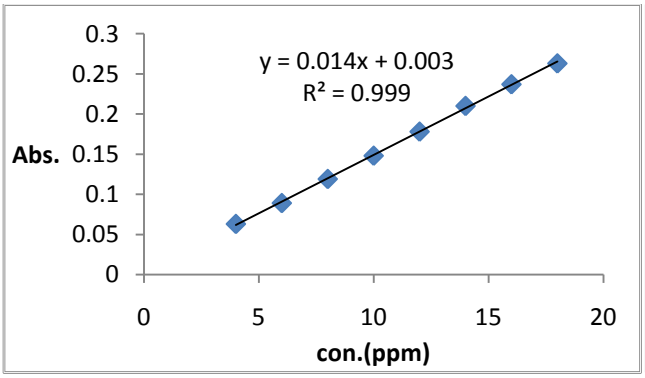


Figure 3: Calibration curve of PCM at 268.2 nm(ZCP of PBM)

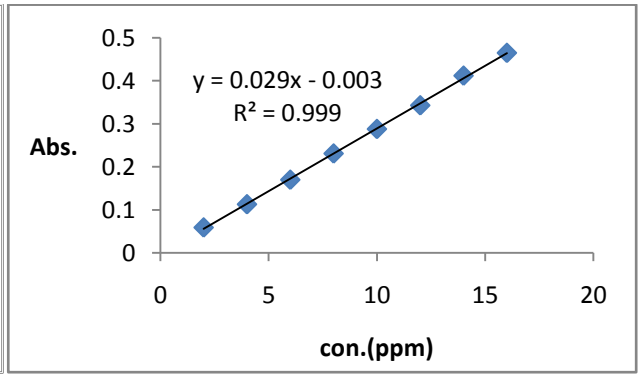


Figure 4: Calibration curve of PBM at 225.0 nm (ZCP of PCM)

The Absorptivity values were calculated and along with absorbances, these values were submitted in equation and to obtain concentration of drugs. The percentage purity of drugs in combined dosage form was found to be 99.60 %for PCM and 98.59 % for PBM (Table-3). The accuracy of the method was determined by performing recovery study by standard addition

method. The % recoveries were found near to 98.65% for PCM and 99.54% for PBM (Table-4). The experiment was repeated six times in a day for precision. The method was found to be precise as % RSD for precision were < 2(Table-2).

Table1. Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Parameter	Paracetamol(PCM)	Pamabrom(PBM)
Analytical wavelength(nm)	268.2 nm	225.0 nm
Linearity range (µg/ml)	4-18	2-16
Regression Equation:	Y=0.0145x+0.0034	Y=0.0292x-0.0031
Slope	0.0145	0.0292
Intercept	0.0034	0.0031
Correlation coefficient	0.9994	0.9995
LOD (µg /ml)	0.144	0.234
LOQ (µg /ml)	0.436	0.7
Repeatability(RSD,n=6), %	1.36%	0.6%

Table 2: Inter-day and Intra-day precision by proposed method

Inter day				Intra day			
Amount taken*		Amount Found** ± %RSD		Amount taken*		Amount Found** ± %RSD	
PCM	PBM	PCM	PBM	PCM	PBM	PCM	PBM
4	2	103.63 ± 0.96	101.19 ± 1.69	4	2	102.75 ± 4.9	101.76 ± 4.23
12	10	101.87 ±0.32	100.14 ±0.86	12	10	103.21±1.47	99.68±1.24
18	16	106.74 ±0.355	101.68 ±0.363	18	16	106.61±0.196	101.76±0.48

*Concentration in µg/ml
** Average of three determinations

Table 3: Analysis of PCM and PBM by Proposed Method

Sample No.	Amount Taken		Amount Found		% Found* ± RSD	
	PCM (mg)	PBM (mg)	PCM (mg)	PBM (mg)	PCM	PBM
1	250	100	249.02	98.59	99.60±1.03	98.59±1.51

*Indicates that each value is mean ± Relative standard deviation of five determinations

Table 4: Recovery Data for the Proposed Method

Sr.No.	Amt present in mixture (µg/ml)		Amt Std Added (%)	% Recovery* ±SD	
	PCM	PBM		PCM	PBM
1	5	2	80	99.29± 0.89	100.65±0.96
2	5	2	100	101.39± 0.97	97.89± 1.64
3	5	2	120	101.88± 0.59	101.47± 0.85

* Indicates that each value is mean ± standard deviation of three determinations

CONCLUSION:

Based on the results, obtained from the analysis of using described method, it can be concluded that the method has linear response in the range of 4 – 18 and 2 - 16µg/ml for Paracetamol and

Pamabrom, respectively with co-efficient of correlation, (r^2) = 0.9994 and (r^2) = 0.9995 for Paracetamol and Pamabrom, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination Paracetamol and Pamabrom. The method can be used for the routine analysis of Paracetamol and Pamabrom in combined dosage form.

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