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“Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of PARACETAMOL and PAMABROM in Bulk and Pharmaceutical Dosage Form”.

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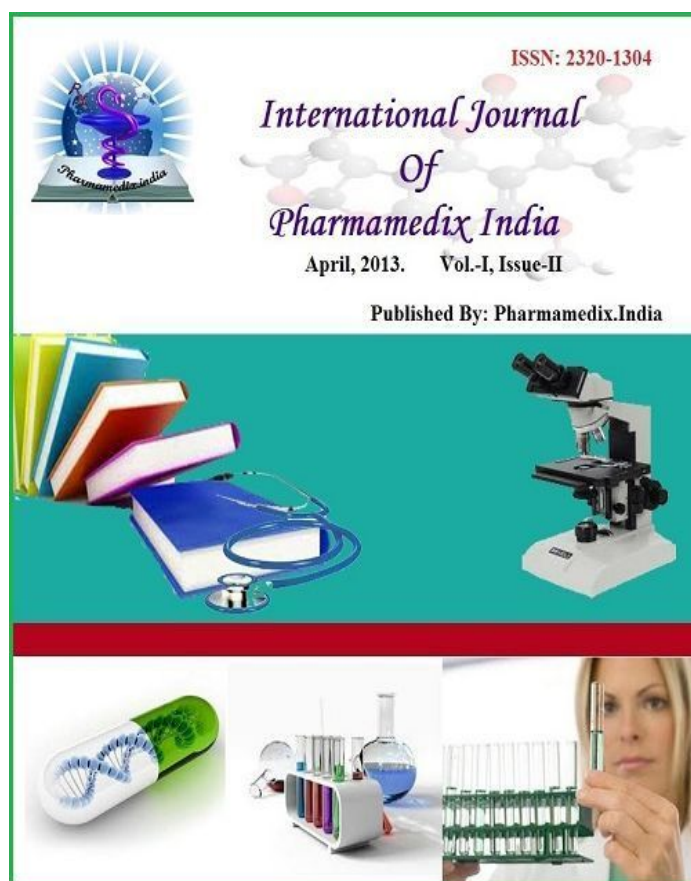
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Abstract:

Two methods for simultaneous estimation of Paracetamol and Pamabrom in combined tablet dosage form have been developed using Dist. water as a solvent. The first UV spectrophotometric method was a determination using the simultaneous equation method at 245.0 nm and 279.0 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 265.0 nm (iso absorptive point) and at 279.0 nm the maximum absorption of Pamabrom. The linearity ranges for Paracetamol and Pamabrom were 4-14 µg/ml and 2-12 µg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 98.06% and 98.95 for simultaneous equation method and 98.65% and 99.54% for Q analysis (absorption ratio) method for Paracetamol and Pamabrom respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keyword: Absorption Ratio Method, Paracetamol (PCM), Pamabrom (PBM), Simultaneous equation method, Validation.

Introduction:

Paracetamol (N- 4-hydroxyphenyl acetamide) is a 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 diuresis 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^[2].

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. Pamabrom is a Official in United state Pharmacopoeia. 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.

MATERIALS AND METHODS:

Instrument:

A Shimadzu model UV-1700 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. The commercial fixed dose combination product (containing 25 mg PBM and 325 mg PCM).

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).

DEVELOPMENT OF THE METHOD:

Method 1 :(Simultaneous equation method)

The standard solutions of PCM (10 µg/ml) and PBM (10 µg/ml) were scanned separately in the UV range of 200-400 nm to determine $\lambda_{\max}$ of both the drugs. The $\lambda_{\max}$ of PCM and PBM were found to be 245.0 nm and 279.0 nm respectively (Fig.1). Five standard solutions having concentration 4, 6, 8, 10, 12 and 14 µg/ml for PCM and 2, 4, 6, 8, 10 and 12 µg/ml for PBM were prepared in distilled water using the solutions having concentration 100 µg/ml. The absorbance of resulting solutions was measured at 245.0 nm and 279.0 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of PCM and PBM in sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of PCM and PBM at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration^[3].

$$C_x = (A_1a_{x2} - A_2a_{x1}) / (a_{x2}a_{y1} - a_{x1}a_{y2}).$$

$$C_y = (A_2a_{y1} - A_1a_{y2}) / (a_{x2}a_{y1} - a_{x1}a_{y2}).$$

Where,

C_x = Concentration of Paracetamol,

C_y = Concentration of Pamabrom,

A_1 = absorbance of sample at 245.0 nm,

A_2 = absorbance of sample at 279.0 nm,

a_{x1} = absorptivity of Paracetamol at 245.0nm,

a_{x2} = absorptivity of Paracetamol at 279.0 nm,

a_{y1} = absorptivity of Pamabrom 245.0nm and

a_{y2} = absorptivity of Pamabrom at 279.0 nm.

Method 2:(Q-analysis method)

The standard solutions of PCM (10 µg/ml) and PBM (10 µg/ml) were scanned in the UV range of 200-400 nm to determine isoabsorptive point. The isoabsorptive point was found to be 265 nm (Figure.1). Five standard solutions having concentration 4, 6, 8, 10, 12 and 14 µg/ml for PCM and 2, 4, 6, 8, 10 and 12 µg/ml for PBM were prepared in distilled water using the solutions having concentration 100 µg/ml. The absorbance of resulting solutions was measured at 265 nm (isoabsorptive point) and 279 nm ($\lambda_{\max}$ of PBM) and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of PCM and PBM in sample solution was determined by solving the respective Q-analysis equations generated by using absorptivity coefficients and absorbance values of PCM and PBM at these wavelengths. The absorbance and absorptivities values at the particular

wavelength were substituted in the following equations to obtain the concentration [3].

$$C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times a_{X1}} \text{ AND } C_Y = \frac{A_1}{a_{X1} - C_X}$$

Where, A1& A2 are the absorbance of the mixture at 265.0 nm & 279.0 nm respectively; aX1 and aY1 are absorptivities of PCM and PBM respectively at 265.0 nm; aX2 and aY2 are absorptivities of PCM and PBM respectively at 279 nm; $Q_M = A_2/A_1$, $Q_X = a_{X2}/a_{X1}$ and $Q_Y = a_{Y2}/a_{Y1}$.

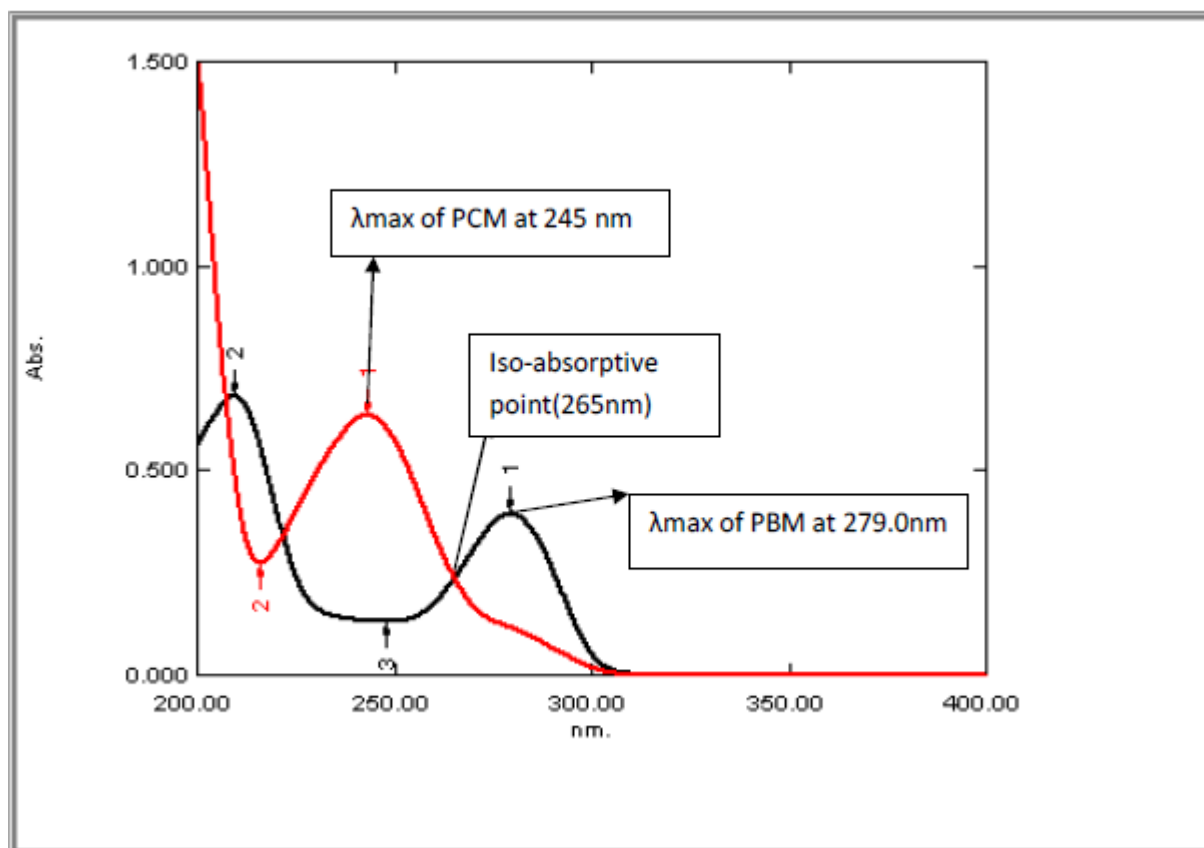
VALIDATION OF THE PROPOSED METHOD:

The proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines ^{[4][6-15]}.

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 4-14 µg/ml and 2-12 µg/ml for PCM and PBM respectively for Simultaneous equation and Q-analysis. Accurately measured standard solutions of PCM (4, 6, 8, 10, 12 & 14 ml) and PBM (2, 4, 6, 8,10 and 12 ml) were transferred to a series of 100 ml of volumetric flasks and

FIGURE 1: Overlain Spectra of Paracemol and Pamabrom.



diluted to the mark with distilled water for simultaneous equation Method and Q-analysis Method. The absorbances of the solutions were measured at 245.0 and 279.0 nm against distilled water as blank for simultaneous equation Method. The absorbances of the solutions were measured at 265.0nm and 279.0 nm against distilled water as blank for Q-analysis Method. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision

The intraday and interday precision of the proposed methods was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of PCM and PBM for both methods.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of PCM and PBM by the standard addition method. Known amounts of standard solutions of PCM and PBM were added at 75, 100 and 125 % level to prequantified sample solutions of PCM and PBM. (5 µg/ml for PCM and 2µg/ml for PBM) The amounts of PCM and PBM were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times for both methods.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the Intercept of Calibration curve and S = slope of the calibration curve.

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 245.0 and 279.0 nm for Simultaneous equation method and 265.0 nm and 279.0 nm for Q-Analysis Method. 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.

RESULT AND DISCUSSION:

The proposed methods for simultaneous estimation of Paracetamol and Pamabrom in combined tablet dosage form were found to be simple accurate economical and rapid. The % RSD was found to be less than 2% in the

developed method. Hence proposed method may be used for routine analysis of these drugs in combined dosage forms.

TABLE-1 Regression Analysis Data and Summary of Validation Parameter of the Calibration Curves.

Parameter	Method-1				Method-2			
	PCM		PBM		PCM		PBM	
Wavelength(nm)	245	279	245	279	265	279	265	279
Beer's law limit($\mu\text{g/ml}$)	4-14	4-14	2-12	2-12	4-14	4-14	2-12	2-12
Molar Absorptivity($1 \text{ mole}^{-1} \text{cm}^{-1}$)	10114.48	1976.0	4242.67	13124.35	15644.63	7246.14	6197.58	10626.08
Regression equation Slope(b) Intercept(a)	0.0659 0.0025	0.0114 0.0087	0.0124 0.0059	0.0375 0.0144	0.0242 0.0028	0.0116 0.0037	0.0237 0.0085	0.0405 0.0162
Correlation Coefficient(r^2)	0.9997	0.995	0.9986	0.9989	0.9993	0.9997	0.9997	0.9981
LOD ^a ($\mu\text{g/ml}$)	0.18	0.37	0.36	0.24	0.31	0.38	0.17	0.19
LOQ ^b ($\mu\text{g/ml}$)	0.55	1.10	1.10	0.74	0.94	1.17	0.51	0.58
Repeatability(RSD ^c , n=6)%	1.039	1.47	1.78	1.24	1.56	1.49	1.81	1.34

a LOD = Limit of detection.

b LOQ = Limit of quantitation.

c RSD = Relative standard deviation, %

TABLE-2 Results of the Recovery Studies

Level of recovery	Amount of pure drug added (ml)		Simultaneous equation method % recovery		Q-Absorbance method % Recovery	
	PCM (ppm)	PBM (ppm)	PCM	PBM	PCM	PBM
75	5	2	97.14	98.14	98.34	99.18
100	5	2	98.24	99.87	97.28	100.87
125	5	2	98.81	98.86	100.31	98.56
Mean % recovery			98.06	98.96	98.64	99.54
SD			0.84	0.87	1.54	1.19

TABLE-3 Results of Analysis of Mixture

Drugs	Simultaneous equation method % $\pm$ SD(n=6)	Q-Absorbance method % $\pm$ SD(n=6)
PCM	100.6 $\pm$ 1.78	100.21 $\pm$ 1.14
PBM	99.33 $\pm$ 3.54	101 $\pm$ 2.79

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