



SPECTROPHOTOMETRIC SIMULTANEOUS ANALYSIS OF PARACETAMOL, PROPYPHENAZONE AND CAFFEINE IN TABLET DOSAGE FORMS

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Received: 01 March 2011, Revised and Accepted: 03 April 2011

ABSTRACT

In this proposed study, the simultaneous analysis of Paracetamol, Propyphenazone and Caffeine by UV-Visible spectrophotometry in their ternary mixture and in tablet dosage form is based on the additivity of absorbance of the drugs. The absorption maxima of drugs were found to be at 243 nm for paracetamol, 266 nm for propyphenazone and 272.8 nm for caffeine in double distilled water and these wavelengths were selected for the analysis. Paracetamol obeyed Beer-Lambert's law in the concentration range of 2-16 µg/ml at 243 nm, 4-40 µg/ml at 266 nm and 6-60 µg/ml at 272.8 nm, whereas propyphenazone was observed to be linear in the concentration range of 2-20 µg/ml at all three selected wavelengths. Caffeine was found to be linear in the concentration range of 2-20 µg/ml at 272.8 and 266 nm and 10-70 µg/ml at 243 nm. The standard absorptivity values were calculated at each wavelength and the amount of drugs in the tablet dosage form were calculated by solving matrix using Cramer's rule. This proposed method was statistically validated in accordance with ICH guidelines. Precision was calculated as inter-day and intra-day variations of the drug absorption. The reproducibility, repeatability of the proposed method were found to be satisfactory which was evidenced by low values of RSD (<5%). Recovery of the developed method was found to be in the range of 95-102%. This method can be applied successfully for the routine analysis of Paracetamol, Propyphenazone and Caffeine analgesic, anti-pyretic tablets in pharmaceutical industry.

Keywords: Paracetamol, Propyphenazone, Caffeine, Cramer's rule, Matrix

INTRODUCTION

Tablets containing Paracetamol (PCM), Propyphenazone (PP) and Caffeine (CAF) are emerging as the most widely prescribed combination for pain relief^{1,2}. PCM, PP and CAF in combination causes reduction in the amount of prostaglandin whereas CAF is also known to increase the analgesic effect of PCM and PP, synergistically, providing relief from symptoms like headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, migraine, general pain, toothache and menstrual pain. The combination is also found to be effective in controlling fever originating from bacterial or viral infection. With limited side effects the combination is suitable for all ages³.

PCM is 4-hydroxyacetanilide, sparingly soluble in water and PP is a 4-isopropyl-2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one, slightly soluble in water whereas a central nervous system stimulant, CAF is 3, 7-dihydro-1, 3, 7-trimethyl-1H-purine-2, 6-dione, sparingly soluble in water⁴(Fig. 1).

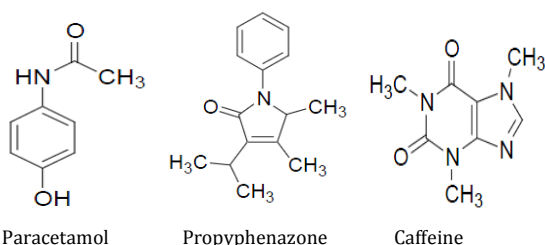


Fig. 1: Structure of drugs.

Currently, the reported methods for simultaneous estimation of PCM, PP and CAF in combined dosage form are single flow-through UV multiparameter sensor⁵, derivative ratio spectra-zero crossing spectrophotometry⁶ and HPLC methods⁷. Despite being a popular combination, there are no simple simultaneous UV-Visible spectrophotometric methods reported.

This study proposes a simple, rapid, accurate and cost effective simultaneous spectrophotometric analysis for the estimation of PCM, PP and CAF in combined dosage forms.

MATERIALS AND METHODS

Instrumentation

A Shimadzu UV-Visible spectrophotometer model 1700 (Japan) with 1 cm matched quartz cells was used along with a Sartorius digital balance.

Chemicals and reagents

PCM, PP and CAF were kindly supplied by Juggat Pharma, Bangalore, India. Tablets containing PCM (300 mg), PP (150 mg) and CAF (50 mg) (Dart tablets, Juggat Pharma, Bangalore) were used as marketed formulation. Freshly prepared double distilled water was used throughout the experiment.

Experimental

Preparation of standard stock solution

Standard stock solution of PCM was prepared by dissolving 100 mg PCM in double distilled water, sonicated for 10 minutes and made up the volume up to 100 ml whereas standard stock solution of PP and CAF were prepared by dissolving 10 mg in double distilled water, sonicated for 10 minutes and made up the volume up to 100 ml.

Determination of wavelength of maximum absorbance

The stock solutions of PCM, PP and CAF were further diluted with double distilled water to get concentration of 30 µg/ml. This solution was scanned in the range 200-400 nm. The wavelengths of maximum absorbance of PCM, PP and CAF were found to be at 243, 266 and 272.8 nm respectively (Fig. 2).

Preparation of calibration curve

The stock solution of PCM was appropriately diluted with double distilled water to obtain concentration range of 2-16 µg/ml, 4-40 µg/ml and 6-60 µg/ml and were measured at 243, 266 and 272.8 nm respectively (Fig. 3).

The stock solution of PP was appropriately diluted to obtain concentration range of 2-20 µg/ml with double distilled water and measured at 266, 243 and 272.8 nm (Fig. 4).

The stock solution of CAF was appropriately diluted to obtain concentration range of 2-20 µg/ml and absorbance of the solutions were measured at 272.8 and 266 nm whereas this stock solution was also diluted to 10-70 µg/ml with double distilled water and absorbance were measured at 243 nm (Fig. 5).

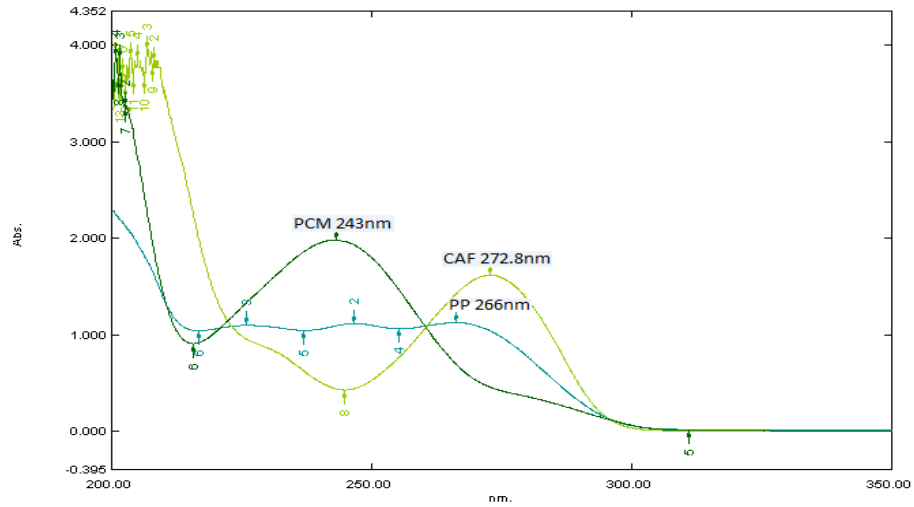


Fig. 2: Overlay spectra PCM, PP and CAF in double distilled water

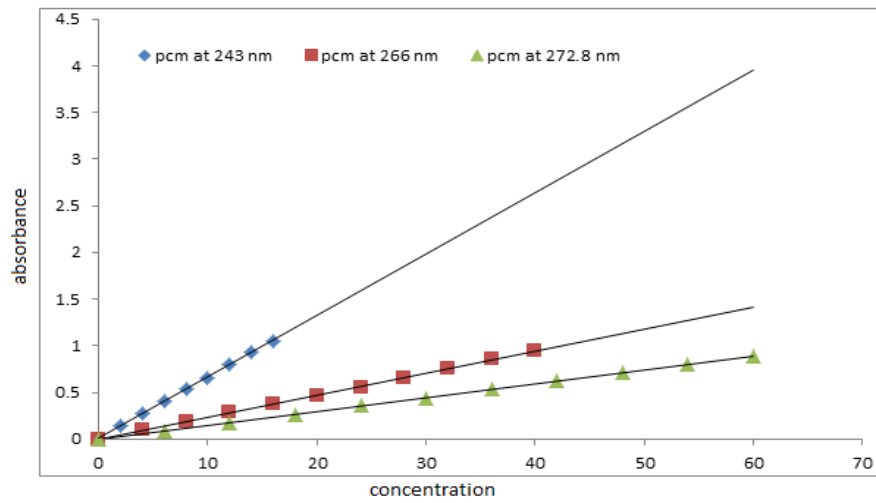


Fig. 3: Calibration curve for PCM at 243, 266 and 272.8nm.

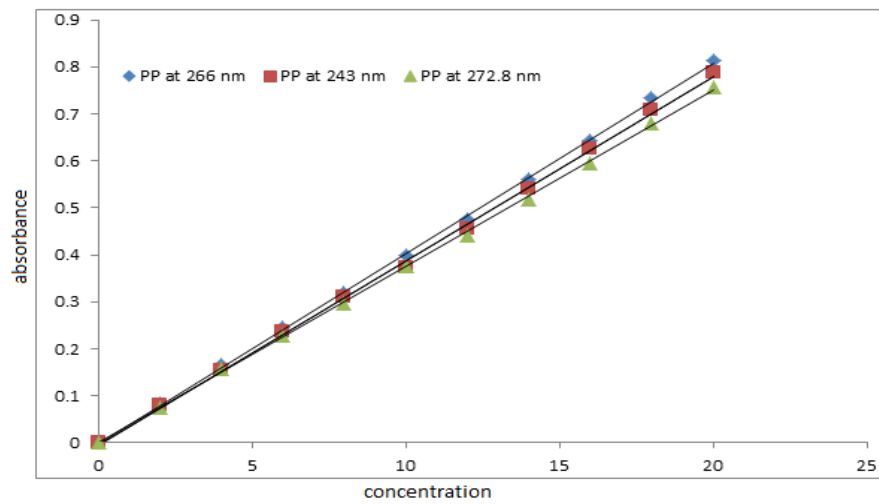


Fig. 4: Calibration curve for PP at 266, 243 and 272.8nm

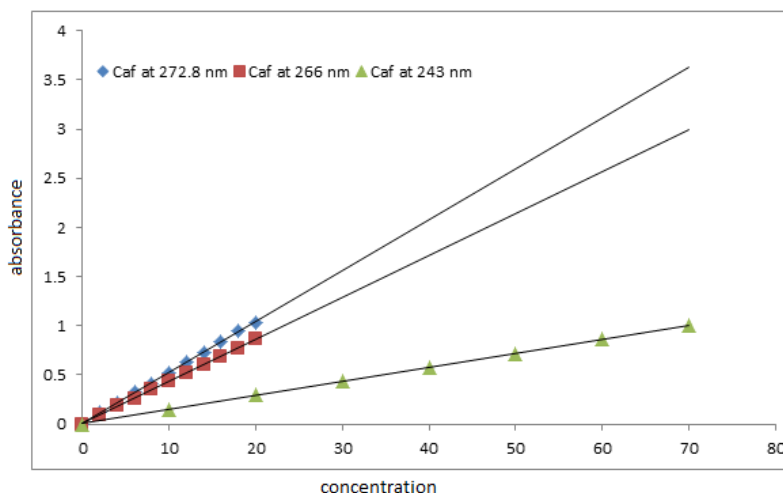


Fig. 5: Calibration curve for CAF at 272.8, 266 and 243nm.

Assay of marketed formulation

Twenty tablets were weighed and crushed to a fine powder. Powder equivalent to 60 mg of PCM, 30 mg of PP and 10 mg of CAF was accurately weighed and transferred in a 100 ml volumetric flask. Approximately 20 ml of double distilled water was added and sonicated for 15 minutes and then volume was made up to 100 ml

with double distilled water. Insoluble excipients were separated by filtration using whatman filter paper no. 41.

The filtrate was further diluted to get final concentration of 6, 3 and 1 µg/ml of PCM, PP and CAF respectively. Absorbance was measured at three selected wavelengths and concentrations were determined by solving matrix by using a Cramer's rule and results shown in Table 1.

Table 1: Results of the assay of marketed tablets

Drug	Label claim mg/tab	Mean Amount found (mg/tab)	Mean Amount found (%)	R.S.D. (%) n=3
PCM	300	305	101.66	1.6868
PP	150	145	101.65	3.275
CAF	50	53	106	4.2316

Table 2: Results of recovery studies

Drug	Percent of amount of the drug found in preanalysed tablet powder(n=3)		
	50 % Addition	100% Addition	150 % Addition
PCM	99.89 ± 1.7713	100.39 ± 0.4752	99.71 ± 0.7742
PP	98.66 ± 0.9555	98.44 ± 0.6931	100.30 ± 1.2043
CAF	95.22 ± 1.4682	102.83 ± 1.9490	102.07 ± 2.5795

RESULTS

Method validation

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and expressed as relative standard deviation (RSD). Intra-day precision was evaluated by analysis of three concentration levels in triplicate of all drugs (For PCM 2,8,16 µg/ml, PP and CAF 2, 10, 20 µg/ml) at three different time intervals under the same experimental conditions on the same day. Intermediate precision (inter-day precision) was determined by analysing above mentioned concentrations of each drug in triplicate on three consecutive days.

Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by standard addition of drug at three different levels (50%, 100% and 150%). A known amount of PCM, PP and CAF were added to pre-analysed tablet powder and percentage recovery of each drug was calculated. The results of recovery studies are presented in Table 2.

DISCUSSION

To start with, the overlain spectra of PCM, PP and CAF were studied in traditional solvents like 0.1N sodium hydroxide, 0.1N hydrochloric acid, methanol, ethanol, dimethyl formamide and double distilled water. Except for minor differences in PP spectrum in water, the overlain spectra of PCM and CAF were found to be almost identical due to which it was not feasible to analyse the mixture by using traditional simultaneous equation and absorption ratio method. As a result, it was concluded that double distilled water was the best suited for this study as it offered advantages of common solubility and lower cost of analysis over the above solvents. All the three drugs absorb at each other's wavelength of maximum absorbance. An absorbance of mixture at each wavelength is the combined absorbance of all the drugs. By applying the Beer-Lambert's equation ($A=abc$ when pathlength is 1 cm, $A=ac$), the absorbance of mixture at each wavelength is derived as follows.

$$a_1 C_x + b_1 C_y + c_1 C_z = A_1 \quad \text{at } \lambda_1 \text{ (243, } \lambda_{\text{max}} \text{ of PCM)}$$

$$a_2 C_x + b_2 C_y + c_2 C_z = A_2 \quad \text{at } \lambda_2 \text{ (266, } \lambda_{\text{max}} \text{ of PP)}$$

$$a_3 C_x + b_3 C_y + c_3 C_z = A_3 \quad \text{at } \lambda_3 \text{ (272.8, } \lambda_{\text{max}} \text{ of CAF)}$$

A_1 , A_2 and A_3 are the absorptions of the mixture of the drugs at λ_1 , λ_2 and λ_3 respectively where Cx, Cy and Cz indicates the concentration of PCM, PP and CAF. In the above matrix, a_1 , a_2 and a_3 are the absorptivity of PCM, b_1 , b_2 and b_3 are the absorptivity of PP and c_1 , c_2 and c_3 are the absorptivity of CAF at λ_1 , λ_2 and λ_3 respectively.

Absorptivity of all the drugs at all the wavelengths was determined from the calibration curves and above equations is derived as follows:

$$659Cx + 236Cy + 149Cz = A_1 \dots\dots (\lambda_1)$$

$$392Cx + 405Cy + 375Cz = A_2 \dots\dots (\lambda_2)$$

$$143Cx + 426Cy + 518Cz = A_3 \dots\dots (\lambda_3)$$

Since the above equations have 3 unknowns, Matrix methodology was used as it easily solves the system of equations with three unknowns. The above 3 equations were drawn in a matrix form and then Cramer's Rule was used to solve the matrix. The result was the concentration of PCM, PP and CAF in the sample solution.

It was noticed that all drugs obey the Beer-Lambert's law in different concentration range and the range of concentration was selected factoring the relative error. Precision was calculated as inter-day and intra-day variations of drug absorption. The reproducibility, repeatability of the proposed method were found to be satisfactory which was evidenced by low values of RSD (<5%) shown in Table 3. Recovery of the developed method was found to be in the range of

95-102%. The uncoated tablets containing 300 mg of PCM, 150 mg of PP and 50 mg of CAF (DART Tablets) were analysed (n=6), the amount of drugs were found to be satisfactory and in the limits of official pharmacopoeia.

The study established that by adopting this method, the time taken for analysis can be reduced as this method involves very limited steps when compared to the traditional methods. While in the traditional method, the PCM, PP and CAF were need to be separated before analysis, by adopting the proposed methodology the sample solution of tablets was needs to be prepared as per the assay procedure and measured at 243, 266 and 272.8 nm. Matrices should be drawn using the values of absorption of the sample solution and then solved using Cramer's rule to determine the amount of each drug in the sample solution. Results of all validation aspects are shown in Table 3.

Thus the developed spectrophotometric method in the present investigation was found to be simple, sensitive, accurate and precise and can be successfully applied for the routine analysis of Paracetamol, Propyphenazone and Caffeine in tablet dosage form.

ACKNOWLEDGEMENT

The authors shall remain grateful to Juggat Pharma Ltd., Bangalore, India for providing PCM, PP and CAF in the form of 'Gift sample' and Nargund College of Pharmacy, Bangalore, for providing lab and research facilities to complete this work.

Table 3: Analytical validation parameters

Drugs name	Parameters	Observations			
PCM	Wavelengths (nm)	243	266	272.8	
	Beer's law range($\mu\text{g/ml}$)	2-16	4-40	6-60	
	Standard regression equations (n=9)	$Y=0.065863x + 0.007256$	$Y=0.023599x - 0.000289$	$Y=0.014939x - 0.003920$	
	Standard absorbance value, A [1%, 1cm]	658.6 ~ 659	235.99 ~ 236	149.39 ~ 149	
	Correlation Coefficient	0.9997	0.999630	0.999844	
	LOD($\mu\text{g/ml}$)	0.131623	0.092152	0.299317	
	LOQ($\mu\text{g/ml}$)	0.398858	0.279249	0.907022	
	PP	Wavelengths (nm)	266	243	272.8
		Beer's law range($\mu\text{g/ml}$)	2-20	2-20	2-20
		Standard regression equations (n=9)	$Y=0.040459x - 0.001091$	$Y=0.039220x - 0.003645$	$Y=0.037518x - 0.000245$
Standard absorbance value, A [1%, 1cm]		404.59 ~ 405	392.20 ~ 392	375.18 ~ 375	
Correlation Coefficient		0.9996	0.999310	0.999431	
LOD($\mu\text{g/ml}$)		0.050896	0.093312	0.076963	
LOQ($\mu\text{g/ml}$)		0.15423	0.282764	0.233221	
CAF		Wavelengths (nm)	272.8	266	243
		Beer's law range($\mu\text{g/ml}$)	2-20	2-20	10-70
		Standard regression equations (n=9)	$Y=0.051788x + 0.007601$	$Y=0.042631x + 0.009640$	$Y=0.01432x + 0.004106$
	Standard absorbance value, A [1%, 1cm]	517.88 ~ 518	426.31 ~ 426	143.2 ~ 143	
	Correlation Coefficient	0.9997	0.999670	0.999633	
	LOD($\mu\text{g/ml}$)	0.076657	0.132136	0.594092	
	LOQ($\mu\text{g/ml}$)	0.232293	0.400413	1.800279	

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