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Method Development and Validation of Dual Wavelength UV Spectrophotometric Method for Simultaneous Estimation of Paracetamol and Caffeine in Combined Dosage Form by Internal Standard Method

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A simple, accurate and precise dual wavelength spectrophotometric method has been developed for simultaneous determination of paracetamol and caffeine in combined pharmaceutical dosage form. The absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. During selection of two wavelengths, the interfering component shows same absorbance while the component of interest shows significant difference in absorbance with concentration. The literature review reveals that there is no dual wavelength method was developed for this combination of drugs, hence this method was developed. The wavelengths selected for determination of paracetamol were 260 nm and 281 nm, whereas, the wavelengths selected for determination of caffeine were 234 nm and 249 nm. The concentration of caffeine is very less in formulation, hence standard caffeine API is added to make it detectable in terms of absorbance. Methanol was taken as a solvent. Regression analysis of Beer's plots showed good correlation in concentration range of 10-60 µg/mL for paracetamol and 3-18 µg/mL for caffeine. Accuracy of method was found between 98-102 %. The precision (intra-day, inter-day and analyst to analyst) of method was found within limits (% CV < 2). The proposed method was successfully applied to determination of these drugs in commercial tablets.

Keywords: Paracetamol, Caffeine, Dual wavelength method, UV spectrophotometric method.

INTRODUCTION

Paracetamol is chemically, N-(4-hydroxyphenyl)acetamide or N-(4-hydroxyphenyl)ethanamide[1], is an analgesic, antipyretic agent, use as pain reliever and a fever reducer. It is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds and fevers[2]. It is official in IP, BP & USP[3]. Literature survey also reveals spectrophotometric[4,5], HPLC[6,7], HPTLC[8] methods for determination of paracetamol with other drugs. Caffeine is chemically, 1,3-trimethyl-1H-purine-2,6(3H,7H)-dione 3,7-dihydroxy-1,3,7-trimethyl-1H-purine-2,6-dione[9], is a CNS stimulating agent, used to restore mental alertness or wakefulness during fatigue or drowsiness. Caffeine is also found in some headache and migraine medications and in many popular energy drinks[10]. It is official in IP, BP and USP. Literature survey also reveals spectrophotometric methods[11], HPLC[12,13] has been developed for both paracetamol and caffeine. The combination of these two drugs available in pharmacopoeia, but literature survey does not reveal simple spectrophotometric for simultaneous estimation of paracetamol and caffeine in combined dosage forms by dual wavelength method. The aim of the study is to develop a new UV spectrophotometric method for the simultaneous estimation of paracetamol and caffeine in combined tablet dosage form by dual wavelength method and to validate the develop method by using various parameters as per ICH guidelines. The concentration of caffeine is very less in formulation, hence standard caffeine API is added to make it detectable in terms of absorbance.

EXPERIMENTAL

A double beam UV/visible spectrophotometer (Lab India 3000+) using photo diode UV enhance wide range solid state photodiode as detector with spectral width of 2 nm, wavelength accuracy of 1 nm, wavelength read out at 0.1 nm per interval, was used to measure absorbance of all the solutions. An analytical

balance (Mettler Toledo); an ultrasonic bath (Janki Impex Pvt. Ltd., Ahmedabad, Gujarat, India) was used in the study.

Paracetamol and caffeine API were gifted from HETERO LABS. HYDERABAD, India. The marketed formulations, tablet(s), were procured from Insight Pharmaceuticals with the brand name of Anacin which had a label claim of 500 mg for paracetamol and 30 mg for caffeine.

Preparation of standard stock solutions: Standard stock solution of paracetamol and caffeine were prepared in methanol (1000 µg/mL) (1^0 stock). From this solution 1 mL was taken and diluted to 10 mL with distilled water (100 µg/mL) (2^0 stock). Calibration standards at six levels were prepared by diluting 2^0 stock standard solutions in the concentration range of 10-60 µg/mL and 3-18 µg/mL for paracetamol and caffeine respectively. Samples in triplicates were made for each concentration. Calibration graph was plotted by taking concentration on x-axis and absorbance on y-axis.

UV spectrophotometric method for estimation paracetamol and caffeine was carried by the dual wavelength method. Paracetamol and caffeine solubility were tested in different organic and aqueous solvents. Both are soluble in methanol and water.

Selection of suitable wavelength: The working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. The maximum absorbance at λ_{max} for paracetamol and caffeine was 248 and 271 nm respectively in methanol. From the overlain spectrum (Fig. 1) the wavelengths selected for estimation of paracetamol were 260 and 281 nm, where caffeine has no absorbance difference at those wavelengths and for caffeine they were 234 nm and 249 nm, where caffeine has no absorbance difference. Different concentrations of paracetamol and caffeine were prepared and then run in entire range from 200 to 400 nm. The drugs obey the Beer's law in the concentration range of 10-60 µg/mL, 3-18 µg/mL for paracetamol and caffeine respectively.

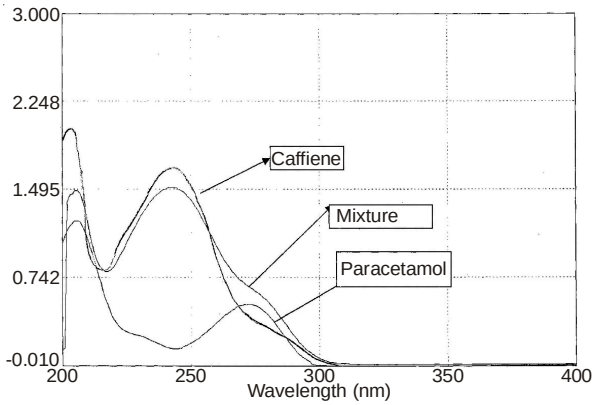

Fig. 1. Overlay spectra of paracetamol, caffeine and mixture

Method validation: The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[13]. Method validation was performed in terms of linearity, accuracy, precision, range, LOD, LOQ, Sandal sensitivity and system suitability.

Linearity: The linearity of calibration curves in pure solution was checked over the concentration ranges of about range of 10-60 and 3-18 µg/mL for paracetamol and caffeine respectively. The regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained: $Y = 0.021x - 0.001$; $R^2 = 0.998$ for paracetamol and $Y = 0.005x - 0.002$; $R^2 = 0.998$ for caffeine respectively. The concentration of caffeine is very less in combination so 6 µg/mL standard caffeine is added to make it 7.5 µg/mL. The mean and correlation coefficient of standard curves (N = 3) were calculated. The represented data was shown in Table-1.

TABLE-1
LINEARITY OF PARACETAMOL AND CAFFEINE

S. No.	Paracetamol		Caffeine	
	Conc. (µg/mL)	Absorbance at 260-281 nm	Conc. (µg/mL)	Absorbance at 234-249 nm
1	10	0.227	3	0.011
2	20	0.416	6	0.0411
3	30	0.639	9	0.050
4	40	0.828	12	0.086
5	50	1.089	15	0.109
6	60	1.282	18	0.132
Regression value R^2	0.998		0.998	
Regression equation	Y = 0.021x - 0.001		Y = 0.005x - 0.002	

Precision: The precision of the developed method was assessed in terms of repeatability and intermediate precision by analysing replicate QC standard sample of 20 µg/mL, 40 µg/mL, 60 µg/mL for paracetamol and 6 µg/mL, 12 µg/mL, 18 µg/mL for caffeine. The % RSD values of the results corresponding to the absorption values were expressed for intraday precision and on 3 days for inter-day precision. The intra and inter-day accuracy and precision were calculated and results were presented in the Table-2. Precision of the analytical method was found to be reliable based on % RSD.

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at spiking levels of 80, 100 and 120 % of test concentration. Results of recovery were presented in the Table-3.

Limit of detection (LOD), limit of quantification (LOQ): Limit of detection was found to be 0.468 µg/mL for paracetamol and 0.286µg/mL for caffeine respectively and limit of quantification was found to be 1.41 µg/mL for paracetamol and 0.863 µg/mL for caffeine. Results were listed in Table-4.

Assay of tablets (combined formulation of paracetamol and caffeine): Twenty tablets of Anacin were weighed and finely powdered and tablet powder equivalent to 123 mg of were weighed and extracted with methanol in a 100 mL volumetric flask which contains respective labelled claim of both the drugs. The flask was sonicated for 15 min and volume was made up to the mark with methanol. 2.5 mL was transferred into a 10 mL volumetric flask and the volume was made up to the mark with water finally the solution is filtered by using syringe filter to obtain 25 µg/mL of paracetamol and caffeine (1.5 µg/mL).The absorbance of the solution was measured under UV spectrophotometer. The assay procedure was made triplicate and weight of sample taken for assay was calculated. The percentage of drug found in formulation, mean and standard deviation in formulation were calculated. The concentration of caffeine is very less in combination so 6 µg/mL standard caffeine is added to make it 7.5 µg/mL (Table-5).

TABLE-2
PRECISION STUDIES

Intra-day precision				Inter-day Precision			
Paracetamol at 260-281 nm		Caffeine at 234-249 nm		Paracetamol at 260-281 nm		Caffeine at 234-249 nm	
Conc. (µg/mL)	RSD (%)	Conc. (µg/mL)	RSD (%)	Conc. (µg/mL)	RSD (%)	Conc. (µg/mL)	RSD (%)
20	0.177	6	0.323	20	0.120	2	0.325
40	0.416	12	0.314	40	0.421	4	0.320
60	1.244	18	1.298	60	1.325	6	1.380

TABLE-3
RECOVERY STUDIES

Drug name	Amount of sample (µg/mL)	Recovery level (%)	Amount of drug added (µg/mL)	Amount found (µg/mL) (N = 3)	Recovery (%) (N = 3)
Paracetamol	25	80	20.0	44.6	99.11 ± 0.75
		100	25.0	49.2	98.40 ± 0.50
		120	30.0	54.15	98.45 ± 0.45
Caffeine	1.5	80	1.2	2.65	98.14 ± 0.30
		100	1.5	3.02	100.66 ± 0.40
		120	1.8	3.28	99.39 ± 0.67

TABLE-5
ASSAY (COMBINED FORMULATION OF PARACETAMOL AND CAFFEINE)

Formulation	Drug	Label claim (mg)	Amount found ± SD (mg)	Assay (%)	RSD (%)
Anacin	Paracetamol	500	493.32 ± 0.94320	98.60	0.3870
	Caffeine	30	30.40 ± 0.28284	101.33	0.9384

TABLE-4
LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Parameter	Paracetamol	Caffeine
LOD (µg/mL)	0.468	0.286
LOQ (µg/mL)	1.41	0.863

RESULTS AND DISCUSSION

The standard solutions of paracetamol and caffeine were scanned separately in the UV range 200-400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 234 nm (λ1) and 249 nm (λ2) wavelengths was found to be with same absorbance for paracetamol. The difference in absorbance at these two wavelengths (A_{234}–A_{249}) cancels out the contribution of absorbance of paracetamol. These two selected wavelengths were employed to determine the concentration of caffeine. Similarly, the absorbance at 260 nm (λ3) and 281 nm (λ4) wavelengths was found to be with same absorbance for caffeine. The difference in absorbance at these two wavelengths (A_{260}–A_{281}) cancels out the contribution of absorbance of caffeine. These two selected wavelengths were employed to determine the concentration of paracetamol.

The linearity range for paracetamol and caffeine was 10-60 and 3-18 µg/mL, with R^2 value of 0.998 and 0.998 respectively. The % RSD for intraday and interday precision was < 2 %.

The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with % recovery of 98.14-100.66 % (*i.e.*, within acceptable range 98-102 %). The assay results were found to be 98.60 and 101.33 % (within acceptable limits).

Conclusion

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 10-60 and 3-18 µg/mL for paracetamol and caffeine respectively. This method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation such as extraction steps prior to the drug analysis.

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