DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMET METHOD FOR SIMULTANEOUS ESTIMATION OFPARACETAMOL AND CAFFEINE IN PURE AND TABLET DOSAGE FORM

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ABSTRACT

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The present work describes two methods for simultaneous estimation of Paracetamol and Caffeine in pure and solid dosage forms, Based on the simultaneous equation method at two selected wavelengths, 257nm and 273nm, respectively and also the absorbance ratio method at two selected wavelengths, 269.0nm (Iso-absorptive point) and 257.0nm (λmax of Paracetamol). Calibration curves were linear over the concentration ranges of 5-25 μg/ml for Paracetamol and 3-15 μg/ml for Caffeine.These methods are simple and accurate, and the analysis results have been validated statistically and by recovery studies.

KEYWORDS

Paracetamol(PARA), Caffeine (CAFF), Simultaneous equation method, Absorbance ratio method.

INTRODUCTION:

Paracetamol is 4-Hydroyacetanilide. It is antipyretic and analgesic. Caffeine, combined with Paracetamol, is an analgesic adjunct to enhance pain relief, although it has no analgesic activity. Acute consumption of Caffeine in combination with over-the-counter (OTC) analgesics such as Aspirine (Acetylsalicylic Acid) or Paracetamol (Acetaminophen) increases their activity by as much as depending on the specific type of pain involved. It is likely due to the ability of Caffeine to cause constriction of the cerebral blood vessels and facilitate the absorption of other drugs. The observed synergism of Paracetamol and Caffeine o the inhibition of PEG $_2$ synthesis in microglial cells, a standard model for the COX-2 inhibiting the activity of non-steroidal anti-inflammatory drugs.

An extensive literature survey reveals none of the available methods based on the estimation of Paracetamol and Caffeine simultaneously by absorption ratio UV-spectrophotometric method.

The present work aimed to develop simple, precise, accurate and economical spectrophotometric methods for the simultaneous determination of binary drug formulation.

International Conference on Harmonization (ICH) guidelines optimised and validated the proposed method.

EXPERIMENTAL PROCEDURE

Instrumentation: A double-beam Jasco U.V.- 630; U.V. Visible spectrophotometer, the spectral bandwidth of 2nm, wavelength accuracy ±0.5nm and a pair of 1-cm matched quartz cells has used to measure the absorbance of the resulting solution.

Materials: Standard samples of Paracetamol and Caffeine were taken. The commercially available tablet, Pacimol Active (Label claim: Paracetamol 650 mg, Caffeine 50 mg), was procured from the local market.

Selection of common solvent: After assessing the solubility of drugs in different solvents, 0.1N NaOH has been selected as a common solvent for developing spectral characteristics.

Preparation of Standard Stock Solutions: Paracetamol and Caffeine (10mg each) were accurately weighed and dissolved separately in 100ml of 0.1 N NaOH to give stock (100μg/ml). From the standard stock solution, 1 ml of PARA and CAFF were taken in a 10 ml volumetric flask, and the volume was made up to mark with 0.1N NaOH. The aliquot portion was appropriately diluted with 0.1 N NaOH to get a final concentration of 5-25μg/ml (PARA), and 3-15μg/ml (CAFF) prepared respectively to give last attention and scanned between 200-400nm.

METHOD:

Application of the Proposed Method for the determination of PARA and CAFF in Tablet Dosage Form:

Simultaneous Equation Method: Twenty tablets were weighed, and the average weight was calculated. The tablet was crushed into fine powder. Tablet powder equivalent to 10mg of PARA was transferred to a 100ml volumetric flask and sonicated for 10 min. The volume is made up to the mark of 0.1N NaOH. The resulting solution was then filtered through a Whatman filter paper (No.41). Aliquot portion was appropriately diluted with 0.1N NaOH to get a final concentration of 20μg/ml. The concentration of both PARA and CAFF was determined by measuring the absorbance of the sample at 257.0nm and 273.0nm in spectrum mode (**Fig 1**), and values were substituted in respective formulae to obtain the concentration.

$$C_x = A_2a_{y1} - A_1a_{y2} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$$

$$C_Y = A_1a_{x2} - A_2a_{x1} / a_{x2}\, a_{y1} - a_{x1}\, a_{y2}$$

Where,

Cx = Concentration of PARA

Cy = Concentration of CAFF

A1 = absorbance of mixture at 257nm;

A2 = absorbance of mixture at 273nm;

ax1= Absorptivity of PARA at 257nm;

ax2 = Absorptivity of PARA at 273nm;

ay1 = Absorptivity CAFF at 257nm;

ay2 = Absorptivity CAFF at 273nm.

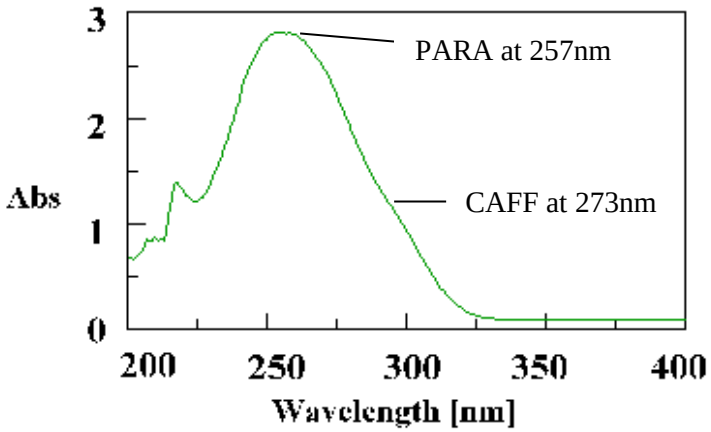

Fig 1: SIMULTANEOUS SPECTRA FOR PARA AND CAFF

2) Absorbance Ratio Method: In the absorbance ratio method, from the overlay spectra of drugs (**Fig.2**), wavelengths 269.0nm (Iso-absorptive point) and 257.0nm (λmax of Paracetamol) were selected for analysis. The calibration curves for Paracetamol and Caffeine were plotted in the concentration range of 5-25µg/ml and 3-15µg/ml at both wavelengths, respectively. The absorptivities values were determined for both the drugs at both wavelengths (fig.2). From the following set of equations, the concentration of each component in the sample is calculated,

$C\times = Qm-Qy/Qx-Qy. A_1/ax_1$ ... (1) Cy $= Qm-Qx/Qy-Qx. A_1/ay_1$ ……. (2)

Where

Cx= concentration of Paracetamol,

Cy= concentration of Caffeine,

A_1= absorbance of the sample at wavelength 257.0nm,

ax_1=Absorptivity of Paracetamol at 257.0nm,

ay_1= Absorptivity of Caffeine at 273.0nm,

Qm = ratio of absorbance of sample solution at 269.0nm and 257.0nm

Qx= ratio of absorptivities of Paracetamol at 269.0nm and 257.0nm and

Qy= ratio of absorptivities of Caffeine at 269.0nm and 273.0nm.

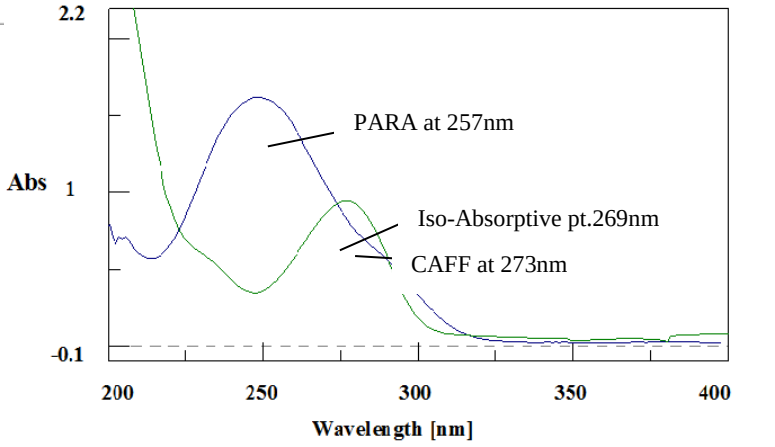

FIG 2: OVERLAIN SPECTRA FOR PARA AND CAFF

VALIDATION PARAMETER

Linearity: For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lamberts concentration range is 5-25µg/ml and 3-15µg/ml for Paracetamol and Caffeine, respectively. The linearity data for both methods are presented in **Fig.3** and **4.**

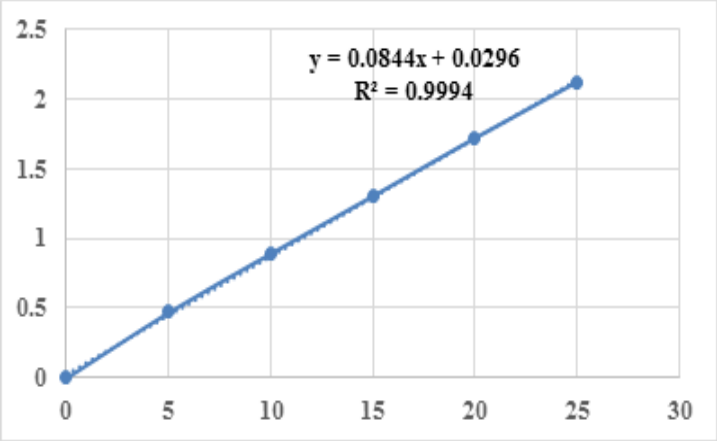

Fig 3: LINEARITY OF PARACETAMOL

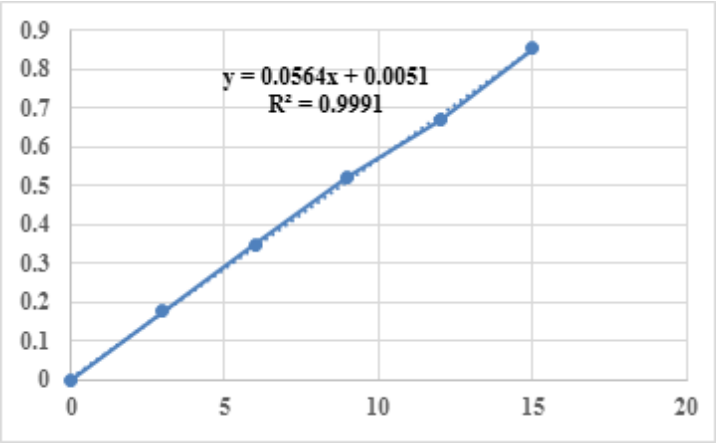

Fig 4: LINEARITY OF CAFFEINE

Accuracy: The recovery study confirmed the accuracy of the developed method as per ICH norms at three different concentration levels of 80 %, 100 %, and 120 % by replicate analysis (n=3). Here to a pre analysed sample solution, standard drug solutions were added and then the percentage drug content was calculated. The result of the accuracy study is reported in Table2. The recovery study indicates that the method is accurate for quantitative estimation of Paracetamol and Caffeine in tablet dosage form as the statistical results are within the acceptance range (S.D.< 2.0).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ of Paracetamol and Caffeine by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S, and ten σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSION

The Beer- Lamberts concentration range is 0-25µg/ml for Paracetamol and 0-15µg/ml for Caffeine at 257.0nm and 273.0nm wavelengths with the coefficient of correlation 0.9994 and 0.9991 respectively. Both the drugs show good regression values at their respective wavelengths, and the recovery study results reveal that the proposed methods could accurately determine any slight change in the drug concentration in the solution. Percentage estimation of the drugs found in the tablet dosage form is 97.4, 99.1 using the simultaneous equation method, whereas98.60, 99.3 using the absorbance ratio method for Paracetamol and Caffeine, respectively, with standard deviation < 2.

Recovery studies assess the validity and reliability of the proposed methods. Sample recoveries for both ways agree with their respective label claims (Table 2). The standard deviation, coefficient of variance and standard error are calculated for tablet formulation (Table 1). Low values of LOD and LOQ indicated good sensitivity to the proposed methods (Table 3).

Method	Drug	Label claim (Mg/tab)	Amount Found	Label Claim (%)	S.D.	S.E.	C.V.
Simultaneous Equation	PARA	650	633.49	97.4	0.3439	0.1404	0.3437
	CAFF	50	49.32	98.64	0.5937	0.4756	0.5948
Absorbance Ratio	PARA	650	626.99	96.46	0.3870	0.3342	0.3898
	CAFF	50	49.16	98.32	0.7809	0.4546	0.7847

Table 1: Analysis data of tablet formulation

PARA: Paracetamol, CAFF: Caffeine, S.D.: Standard Deviation, S.E.: Standard Error, C.V.: Coefficient of Variation, * Average of three estimations of tablet formulation.

Method	Recovery level (Added amount)	Percent recovery ± SD	
		PARA	CAFF
Simultaneous Equation	80 %	97.04±0.64	98.64±0.49
	100 %	97.09±0.54	97.56±0.64
	120 %	98.50±0.63	98.30±0.34
Absorbance Ratio	80 %	96.18±0.75	98.12±0.24
	100 %	96.43±0.83	98.36±0.36
	120 %	98.45±0.48	99.49±0.39

Parameters	Values

Table 2: Result of recovery studies

PARA: Paracetamol, CAFF: Caffeine, S.D.: Standard Deviation, # Average of three estimation

	PARA	**CAFF**
Working λ max	257nm	273nm
Beer's law limit (µg/ml)	0-25	0-15
Absorptive	0.0881	0.0581
Correlation coefficient	0.9994	0.9991
Intercept	0.0296	0.0051
Slope	0.0844	0.0564
LOD(µg/ml)	0.6084	0.4766
LOQ(µg/ml)	1.833	1.4383

Table 3: Optical characteristics data and validation parameter

CONCLUSION: Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible and economical and can be employed for routine quality control of Paracetamol and Caffeine in combined dose tablet formulation.

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REFERENCES:

1.Nogowska M, Muszalska I, Zajac M, Simultaneous spectrophotometric determination of acetylsalicylic acid, Paracetamol and Caffeine in pharmaceutical preparations. Chem. Anal. (Warsaw), 1999, 44 (6), 1041-1048.

2.Argekar AP, Sawant JG, Simultaneous determination of paracetamol and mefenamic acid in tablets by HPTLC. Journal of Planar Chromatography**,** modern TLC, 1999, 12 (5), 361-364.

3.Gopinath, R. Rajan, S., Meyyanathan, S.N., Krishnaveni, N., Suresh, B., A RP-HPLC method forsimultaneous estimation of Paracetamol andaceclofenac in tablets.Indian J. of pharmaceuticalsciences, 2007, 69 (1), 137-140.

4. Senthamil Selvan, P., Gopinath, R., Saravanan, V.S., Gopal, N., Sarvana Kumar, A., periyasamy, K., Simultaneous estimation of Paracetamol and aceclofenac in combined dosage forms by RP-HPLC method. Asian J. of Chemistry, 2007, 19 (2), 1004-1010.

5. Lavorante, A.F., Pires, C.K, Reis, B.F., Multicommuted flow system employing pinch solenoid valves and micro-pumps. Spectrophotometric determination of Paracetamol in pharmaceutical formulations. J. of Pharmaceutical and Biomedical Analysis, 2006, 42 (4), 423-429.

6. Indian Pharmacopoeia, Government of India, Ministry of Health andFamily Welfare, Controller and Publication, Delhi, Vol. 3, pp. 1859-1861 (2022).

7.http://www.drugs.com/paracetamol.html.

8. http://www.drugs.com/caffeine.html.

9. Dokhe M.D., Tarkase M., Bhand SD, Development and Validation of spectrophotometric method for simultaneous estimation of Naproxen and Domperidone in pure and tablet dosage form. Int. Pharm, Sci.Rev.2015;31:72-74

10. International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonised tripartite guidelines, validation of analytical procedure: Text and Methodology Q2 (R1), Current Step 4 version, ICH Geneva, Nov. 2005.