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Development and Validation of Stability Indicating Spectrophotometric Method for Simultaneous Quantitative Estimation of Analgesic, Antipyretic and Anti-Inflammatory Drugs

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ABSTRACT:

Two simple, rapid, precise and accurate UV-spectrophotometric methods have been developed for quantitative estimation of Paracetamol (PCM) and Aceclofenac (ACF) by simultaneous equation method and stability study method in Pharmaceutical tablet dosage form. The simultaneous equation method is based on measurement of absorbance at 247 nm and 275 nm as two wavelengths selected for determination of Paracetamol and Aceclofenac. The method obeyed Beer's law in the concentration range of 30-80 µg/ml for PCM and 10-60 µg/ml for ACF. Paracetamol and Aceclofenac were subjected to forced or stress degradation under different conditions recommended by ICH. The proposed methods were validated and can be applied successfully for routine quality control analysis of Paracetamol and Aceclofenac in bulk drugs and pharmaceutical dosage form.

KEYWORDS: Simultaneous Equation Method, λ_{max} , Validation, Paracetamol (PCM), Aceclofenac (ACF), Stability Study, Stress Degradation.

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INTRODUCTION:

Paracetamol, N-(4-Hydroxyphenyl) acetamide, is an analgesic and antipyretic drug. Paracetamol has negligible anti-inflammatory action.^[1] It is a poor inhibitor of PG synthesis in peripheral tissues, but more active on COX in the brain.^[2] Melting point of Paracetamol is 169-171 °C. Paracetamol having molecular formula $C_8H_9NO_2$ and molecular weight 151.163 g/mol. It is official in IP, BP, EP and USP^[3] (**Fig. No. 1**).

Aceclofenac, 2-[2-[2-[(2,6-dichlorophenyl) amino]phenyl] acetyl]oxyacetic acid, is a non-steroidal anti-inflammatory drug (NSAID).^[4] It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac works by blocking the action of a substance in the body called cyclo-oxygenase.^[5] Melting point of Aceclofenac is 149-153 °C. Aceclofenac having molecular formula $C_{16}H_{13}Cl_2NO_4$ and molecular weight 354.18472 g/mol. It is official in IP, BP and USP^[6] (**Fig. No. 2**).

The literature review reveals that few analytical techniques like UPLC, RP-HPLC, HPTLC, HPLC, Spectroscopic methods have been reported for the quantitative estimation of Paracetamol and its combination with other drugs in pharmaceutical preparations.^[7,8] Estimation of Aceclofenac in combination with other drugs by spectrophotometric methods, LC-MS and HPLC has been reported.^[9,10] But there are no methods reported in any pharmacopoeia for

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simultaneous quantitative estimation of Paracetamol and Aceclofenac by stability indicating method in combined tablet dosage form.

MATERIALS AND METHODS

Instruments:

- Double Beam UV- Visible Spectrophotometer with 1 cm matched quartz cell Model SL 218- Elico.
- Hot Air Oven- Dolphin.
- Electronic Balance- Shimadzu.
- Sonicator- Dolphin.

Reagents and Chemicals:

Paracetamol and Aceclofenac reference standard were kindly provided by Wockhardt Limited Aurangabad. 0.1 N Sodium Hydroxide of analytical grade was used as a solvent throughout the analysis.

Marketed Preparation:

The brand name of marketed combined tablet formulation is ACP Plus containing Paracetamol 500 mg and Aceclofenac 100 mg manufactured by NG Life Sci. Pvt. Ltd.

Preparation of standard stock solution:

(a) Paracetamol:- Standard stock solution of Paracetamol was prepared by dissolving 100 mg Paracetamol in 50 ml of 0.1N sodium hydroxide and sonicated for 20 min and then diluted upto 100 ml to produce a concentration of 1000 µg/ml which was standard stock solution.

(b) Aceclofenac:- Standard stock solution of Aceclofenac was prepared by dissolving 100 mg of Aceclofenac in 100 ml of 0.1N sodium hydroxide to produce a concentration of 1000 µg/ml which was standard stock solution.

Determination of λ max of Paracetamol and Aceclofenac:

The aliquot portion of stock standard solutions of PCM and ACF were diluted appropriately with 0.1 N sodium hydroxide to obtain concentration 500 µg/ml and 100 µg/ml respectively. The solutions were scanned in the range of 200-400 nm in 1 cm cell against blank. The λ_{max} was determined on double beam UV – Visible Spectrophotometer using 0.1 N sodium hydroxide as blank. The λ_{max} was found to be 247 nm and 275 nm

respectively. The Overlain UV absorbance spectrum of PCM and ACF is shown in **Fig. No. 3**.

Preparation of Calibration Curve or study of Beer-Lambert's Law:

The aliquot portion of stock standard solutions of PCM and ACF were diluted appropriately with 0.1 N sodium hydroxide as solvent to get a series of concentration between 30-80 µg/ml of PCM and 10-60 µg/ml of ACF. The absorbance of each solution was measured at 247 nm and 275 nm in 1 cm cell against 0.1 N sodium hydroxide as blank. The graphs plotted as concentration Vs absorbance at selected wavelengths for PCM and ACF are shown in **Fig. No. from 4 and 5**.

Determination of A (1%, 1 cm) of drugs at selected wavelengths:

Aliquot portions of PCM from stock solution were transferred to five 10 ml volumetric flasks; volume was adjusted to mark to obtain the concentration of 500 µg/ml. Similarly, aliquot portions from ACF stock solution were transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain concentration of 100 µg/ml. Absorbance of these solutions were recorded at two wavelengths 247 nm and 275 nm. A (1%, 1 cm) values of drugs were calculated using following formula-

$$A (1\%, 1 \text{ cm}) = \text{Absorbance} / \text{Concentration (g / 100 ml)}$$

Results of A (1 %, 1 cm) of drugs are given in **Table No. 1**.

$$C_x = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \text{ ----- (I)}$$

$$C_y = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \text{ ----- (II)}$$

Where,

A₁ and A₂ are the absorbance of the sample solution measured at 247 nm and 275 nm respectively.

C_x and C_y are concentration of PCM and ACF respectively.

a_{x1} and a_{x2} are absorptivity of PCM at 247 nm and 275 nm respectively.

a_{y1} and a_{y2} are absorptivity of ACF at 247 nm and 275 nm respectively.

Simultaneous Estimation of drugs in standard laboratory mixture:

In order to study the practicability of proposed method for simultaneous estimation of PCM and ACF in marketed pharmaceutical formulations, the method was first tried

for estimation of drugs in standard laboratory mixture. Accurately weighed 100 mg PCM and 100 mg ACF were transferred to 100 ml volumetric flask individually containing 40 ml 0.1 N sodium hydroxide, shake manually for 10 minute and the volume was adjusted to the mark with the same solvent. Appropriate aliquot portion of these solutions were mixed to get the concentration 500 µg/ml PCM and 100 µg/ml of ACF. Absorbance was measured at 247 nm and 275 nm against 0.1 N sodium hydroxide as blank. Amount of each drug was estimated using (I) and (II) equation and results are given in **Table No. 2.**

Simultaneous Estimation of drugs in tablets:

Twenty tablets each containing 500 mg of PCM and 100 mg of ACF were weighed and average weight was calculated. The tablets were crushed to fine powder. The powder equivalent to 500 mg of PCM and 100 mg of ACF was transferred to 100 ml volumetric flask containing 70 ml of 0.1 N sodium hydroxide by intermittent shaking followed by sonication for 10 min and then the volume was made upto 100 ml with 0.1 N sodium hydroxide. The solution was diluted further with 0.1 N sodium hydroxide to obtain 500 µg/ml of PCM and 100 µg/ml of ACF. The solution was filtered through a Whatman filter paper (No. 41). The absorbances were recorded. The concentrations of two drugs in sample were determined using equation No. (I) and (II), results are given in **Table No. 3.**

Recovery Study:

In recovery study to the preanalysed sample solutions (500 µg/ml PCM and 100 µg/ml of ACF) a known amount of standard solutions of pure drugs (PCM and ACF) was added at different level. The % recovery was calculated by using formula,

$$\% \text{ Recovery} = (A / B + C) \times 100$$

Where,

A = Total amount of drug estimated,

B = Amount of drug found on preanalysed basis and

C = Amount of pure drug added.

Results are shown in **Table No. 4.**

Validation of Proposed Method:

Validation of method was done as per ICH guidelines. Method was validated for various parameters such as

accuracy, precision, linearity, repeatability and ruggedness.

Accuracy: Accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. The results of recovery studies are shown in **Table No. 4.**

Precision: Precision was determined by intra-day and inter-day precision. Intra-day precision was determined by analyzing the 400, 500, 600 µg/ml of PCM and 80, 100, 120 µg/ml of ACF drug solution for three times in the same day. Inter-day precision was determined by analyzing the same concentration at three different days. The results are given in **Table No.5.**

Linearity: The study of linearity and range was performed as per ICH guidelines. PCM and ACF was found to be linear at a concentration range of 30-80 µg/ml and 10-60 µg/ml respectively with $R^2 = 0.99$ at selected wavelength for the proposed method.

Repeatability: Repeatability was determined by analyzing PCM (500 µg/ml) and ACF (100 µg/ml) of drug solutions for five times and results are given in **Table No. 6.**

Ruggedness: Analysis of aliquots from homogenous slot by two analyst using same operational and environment conditions was performed for determination of Ruggedness of proposed method. The results are given in **Table No. 7.**

Method for Performing Stability Study (Stress or Forced Degradation Study):

Stress degradation by hydrolysis under acidic condition:

To each 1 ml of standard stock solutions of PCM (500 µg/ml) and ACF (100 µg/ml), 5 ml of 1N HCl was added separately in 10 ml of volumetric flasks and kept for 3 hours. After 3 hours solutions were diluted with 0.1 N sodium hydroxide up to the mark and the solutions were taken in cuvette and analysed in UV spectrophotometer at 247 nm and 275 nm of PCM and ACF respectively.

Stress degradation by hydrolysis under basic condition:

To each 1 ml of standard stock solutions of PCM (500 µg/ml) and ACF (100 µg/ml), 5 ml of 1 N NaOH was added separately in 10 ml of volumetric flasks and kept for 3 hours. After 3 hours solutions were diluted with 0.1 N sodium hydroxide up to the mark and the solutions were taken in cuvette and analysed in UV spectrophotometer at 247 nm and 275 nm of PCM and ACF respectively.

Oxidative degradation: To each 1 ml of standard stock solutions of PCM (500 µg/ml) and ACF (100 µg/ml), 5 ml of 6% Hydrogen Peroxide (H₂O₂) was added separately in 10 ml of volumetric flasks and kept for 3 hours. After 3 hours solutions were diluted with 0.1 N sodium hydroxide up to the mark and the solutions were taken in cuvette and analysed in UV spectrophotometer at 247 nm and 275 nm of PCM and ACF respectively.

Dry heat induced degradation: 500 mg standard PCM and 500 mg standard ACF were taken separately in a Petri plates and exposed to a temperature of 50°C for 48 hours in an oven. After 48 hours, 100 mg of PCM and 100 mg of ACF were diluted separately with 0.1 N NaOH in order to make the volume upto 100 ml. From this solution, dilutions were made (500 µg/ml of PCM & 100 µg/ml of ACF) and the solutions were taken in cuvette and analysed in UV spectrophotometer at 247 nm and 275 nm of PCM and ACF respectively.

Determination of % Degradation:-

Record the absorbance of stressed sample then compare it with absorbance of unstressed sample to determine the % degradation.

$$\% \text{ Degradation} = \frac{(\text{Response of unstressed sample}) - (\text{Response of stressed sample})}{\text{Response of unstressed sample}} \times 100$$

Response of unstressed sample

Results of the Stability studies are given in **Table No. 8**.

RESULTS AND DISCUSSION

The λ_{max} of Paracetamol and Aceclofenac were found to be 247 nm and 275 nm respectively in 0.1 N Sodium Hydroxide as a solvent. In this method drugs obeyed Beer's law in the concentration range of 30-80 µg/ml of PCM and 10-60 µg/ml of ACF. The results showed an excellent correlation between absorbance's and concentration of the drugs. Standard calibration curves for PCM and ACF were linear with correlation coefficients R² =0.99 at all the selected wavelengths. The results of analysis of the marketed combined tablet formulation by Simultaneous Equation Method are shown in **Table No.3**. Method validations were done as per ICH Validation parameters like Accuracy, Precision, Linearity, Repeatability and Ruggedness. The method showed accuracy in the range of 97-100 %. Results of Validation parameters are shown in **Table No. from 4-7**. The stress degradation studies showed that Paracetamol and

Aceclofenac undergoes degradation in oxidative and dry heat conditions whereas it is relatively stable when exposed to acidic and basic conditions. Results of stress degradation studies of PCM and ACF are shown in **Table No.8**.

CONCLUSION

A simple, accurate, precise and rapid UV-Spectrophotometric method has been developed for simultaneous estimation of PCM and ACF in combined tablet dosage form. The method was validated for PCM and ACF in pharmaceutical dosage form. The present study was concluded to understand the degradation behavior of PCM and ACF under ICH recommended stress conditions. These drugs showed degradation in oxidative and dry heat conditions. The drugs were stable when exposed to acidic and basic conditions. The method developed for simultaneous quantitative estimation of PCM and ACF is rapid, precise, accurate and selective. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of PCM and ACF in bulk drugs and pharmaceutical dosage form.

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TABLES AND FIGURES

Table No. 1: Absorptivity values of PCM and ACF at 247 nm and 275 nm

Sr. No.	PCM (247 nm)	PCM (275 nm)	ACF (247 nm)	ACF (275 nm)
1	121.2	91	98.2	131
2	118	96.3	105.3	129.4
3	121.6	85	110	127
4	117.6	86.1	107.9	133
5	119	84.8	110	136.11
Mean	119.48	88.64	106.28	131.302

Table No. 2: Analysis of PCM and ACF in Standard Laboratory Mixture

Sr. No.	Drugs	Conc. of std (µg/ml)	Amount found (µg/ml)	% Amount found	% RSD (n=5)
1	PCM	500	486	97.2	0.95
2	ACF	100	98.10	98.10	0.81

Table No. 3: Analysis of PCM and ACF in Tablets

Sr. No.	Drugs	Conc. of tablet (µg/ml)	Mean amount found (mg/tab)	% Amount found (mean)	% RSD (n=5)
1	PCM	500	490	98	1.01
2	ACF	100	99.1	99.1	0.21

Table No. 4: Results of Recovery Studies

Sr. No.	Pre-analysed samples (µg/ml)		Pure drug added (µg/ml)		Drug recovered (µg/ml)		% Recovery		% RSD (n=5)	
	PC M	AC F	PC M	AC F	PCM	ACF	PC M	ACF	PC M	AC F
1	500	100	400	80	388	78.0	97	97	1.2	0.83
2	500	100	500	100	495.05	99.01	98	99	0.6	0.78
3	500	100	600	120	589.8	117.96	98	98	1.0	1.52

Table No. 5: Results of Precision Studies (Intra-day and Inter-day)

Sr. No.	Drugs	Conc. (µg/ml)	Intra-day amount found (n=5)		Inter-day amount found (n=5)	
			Mean	% RSD	Mean	% RSD
1	PCM	400	379	1.31	381	0.65
		500	482	0.39	490	0.82
		600	575	0.76	584	1.29
2	ACF	80	77.35	1.55	79.05	0.19
		100	97.19	0.97	98.28	0.44
		120	116.80	0.88	118	1.50

Table No. 6: Results of Repeatability Studies

Sr. No.	Drugs	Conc. (µg/ml)	Mean conc. found (µg/ml)	% RSD (n=5)
1.	PCM	500	492	1.14
2.	ACF	100	98.90	0.76

Table No. 7: Results of Ruggedness studies

Sr. No.		Amount found (%)		% RSD (n=3)	
		PCM	ACF	PCM	ACF
1	Analyst-I	97.80	98.18	1.35	0.86
2	Analyst-II	98.24	99.03	0.65	0.14

Table No. 8: Results of Stability Studies

Sr. No.	Degradation Parameters	Abs. of Stressed samples		Abs. of Unstressed samples		% Degradation	
		247 nm	275 nm	247 nm	275 nm	PC M	ACF
1	Acidic Degradation (1 N HCl, 3 Hours)	0.858	0.688	0.880	0.712	2.5	3.3
2	Basic Degradation (1 N NaOH, 3 Hours)	0.864	0.698	0	0	1.8	1.9
3	Oxidative Degradation (6% H ₂ O ₂ , 3 Hours)	0.785	0.632	0.785	0.632	10.7	11.1
4	Dry Heat Induced Degradation (50 °C, 48 Hours)	0.761	0.637	0.761	0.637	13.4	10.5

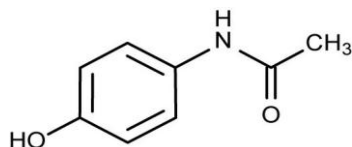


Figure No. 1: Chemical Structure of Paracetamol

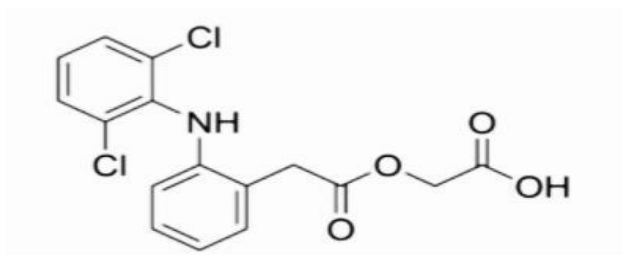


Figure No. 2: Chemical Structure of Aceclofenac

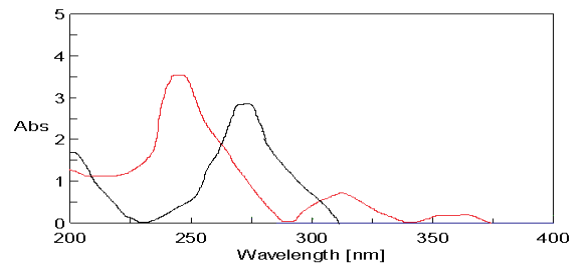


Figure No. 3: Overlain spectra of PCM and ACF

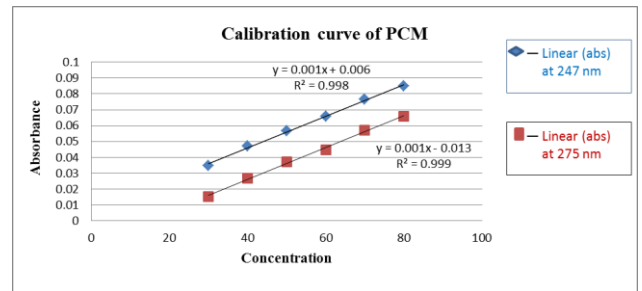


Figure No. 4: Plot of Beer- Lambert's study for PCM at 247 nm and 275 nm

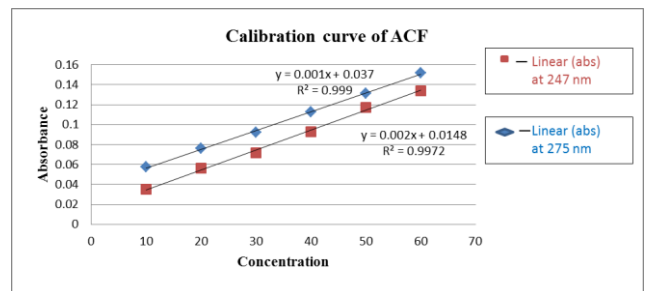


Figure No. 5: Plot of Beer- Lambert's study for ACF at 247 nm and 275 nm

