

Dissolution Method Development and Validation of Paracetamol – Aceclofenac Tablets

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ABSTRACT

A dissolution method and an analytical procedure by UV spectrophotometry were developed and validated for evaluation of the dissolution behavior of tablet dosage form containing aceclofenac and paracetamol as there was no official method available. Four different commercially available products were selected for this study. The analytical method developed by UV spectrophotometry was based on the application of Vierodts equation which involved the formation and solving of simultaneous equations at two wavelengths 243 nm as the λ_{\max} of paracetamol and 273 nm as λ_{\max} of aceclofenac. The method was validated according to International Conference on Harmonisation (ICH) guidelines which include accuracy, precision, specificity, linearity, and analytical range. In addition, stability and solubility of both the drugs in different media i.e., water, 0.1 N HCl, acetate buffer and phosphate buffer of different pH were studied. Based on this, dissolution medium containing 0.02 M phosphate buffer, pH 6.8, was found suitable to ensure sink conditions and chemical stability for both the drugs. The established dissolution conditions were 900 ml dissolution medium at temperature $37 \pm 0.5^\circ\text{C}$, using USP apparatus II at stirring rate of 50 rpm for 40 min. The corresponding dissolution profiles were constructed and all the selected brands showed more than 80% drug release within 30 min. The *in vitro* release profiles were compared for the similarity using the f_2 test. Thus, the proposed dissolution method can be applied successfully for the quality control of aceclofenac and paracetamol tablets.

Keywords: Aceclofenac, Dissolution, Paracetamol, Simultaneous equation method.

INTRODUCTION

The dissolution test is a simple and useful *in vitro* tool that can provide valuable information about drug release similarity among different batches and brands. It describes about manufacturing reproducibility, product performance similarity and biological availability of drug from its formulation. Therefore, it is considered as one of the most quality control test of solid pharmaceutical dosage forms.¹

Chemically, Paracetamol (PCM) (fig. 1a) is N-(4-hydroxyphenyl) ethanamide. It has analgesic and antipyretic properties with weak anti-inflammatory activity and used in the symptomatic management of moderate pain and fever. This action of PCM is due to inhibition of cyclo oxygenase (COX), COX-1 and COX-2 (analgesic activity) and direct action on heat regulating centres of the hypothalamus (anti-pyretic activity). PCM is often combined with other drugs (caffeine, aceclofenac) for greater patient acceptability, increased potency, multiple activity, fewer side effects and quick relief. Aceclofenac (ACE)

(fig. 1b), 2-[2-(2,6 Dichlorophenyl)aminophenyl]acetyl}oxyacetic acid is a non steroidal anti-inflammatory drug (NSAID) indicated for symptomatic treatment of pain and inflammation with a reduced side effect profile, especially gastro intestinal events that are frequently associated with NSAID therapy. It is practically insoluble in water and belongs to Biopharmaceutics classification system (BCS) class II (low solubility, high permeability).²

Dissolution testing of formulations containing poorly soluble drugs has experienced increasing interest in recent years for finding proper conditions for the routine quality control.³ PCM is official in British Pharmacopoeia (BP) and United States Pharmacopoeia (USP).^{4,5} ACE is official in BP⁴ but the combination of PCM and ACE is unofficial in any of the pharmacopoeia. A number of methods have been reported for the analysis of PCM and ACE either by UV or HPLC.⁶⁻⁸ The literature search revealed lack of validated dissolution test for the combination of PCM and ACE tablets. Therefore, the

method development and validation of dissolution test for the tablets of PCM – ACE combination were developed.

MATERIALS AND METHODS

All experiments were performed by pharmaceutical grade PCM and ACE (Aurobindo pharmaceuticals Ltd, Hyderabad), analytical grade reagents and solvents. Buffer solutions were prepared by double distilled water. All dilutions were performed in standard volumetric flasks. Solvents and solutions were filtered through 0.45 µm nylon filters before use. The pharmaceutical preparations containing 500 mg PCM and 100 mg ACE were procured from local drug stores.

Solubility Studies

Solubility data was used as the basis for the selection of the best medium for dissolution of PCM - ACE tablets. For equilibrium solubility studies, excess of both the drugs, PCM and ACE was placed separately in 25 ml beakers containing different media: distilled water, 0.1 N HCl (pH 1.2), pH 4.5 acetate buffer and phosphate buffer of pH 6.8, pH 7.2 and pH 7.4. The samples were gently rotated in water bath shaker at 37.0 ± 0.5 °C for 24 h. An aliquot (2 ml) was removed from each beaker after 12 h and 24 h and filtered using 0.45 µm syringe filter. 1 ml of the filtered sample was diluted with corresponding medium and analyzed spectrophotometrically in triplicate to determine the absorbance at their respective λ_{\max} . The amount of drug dissolved was calculated using the calibration curve.

Stability Studies

Stability studies were performed by preparing solutions of pure drug mixture and marketed formulation of both the drugs and preserving it for 2 days. An accurately weighed quantity of pure drug mixture and tablet powder (10 mg) each was transferred to 100 ml volumetric flask, dissolved in sufficient quantity of phosphate buffer pH 6.8. The volume was made up to the mark with phosphate buffer pH 6.8 to get the concentration 100 µg/ml. An aliquot (1 ml) of this solution was diluted with phosphate buffer pH 6.8 in a 10 ml volumetric flask up to mark to get final concentration 10 µg/ml. All the solutions were prepared in three replicates. The solutions were kept at 37 ± 0.5 °C for 1 h under light shaking; later being left at room temperature for 48 h. Aliquots of samples were analyzed spectrophotometrically after 1 h, 24 h and 48 h.

UV method development and validation

Simultaneous equation method⁹

Stock solutions of PCM and ACE (100 µg/ml) each prepared in phosphate buffer pH 6.8 were diluted to get standard solution across the range of 2 - 16 µg/ml for PCM and 2 - 44 µg/ml for ACE. Standard solutions of PCM and ACE were scanned separately in the range of 200-400 nm using UV spectrophotometer (UV model 1700, Shimadzu, Japan) to determine the wavelength of maximum absorption and absorptivity for both the drugs. PCM and ACE showed absorbance maxima at 243 nm and 273 nm respectively. Absorptivity values for PCM at 243 nm and 273 nm were 636.87 (a_{x1}) and 146.4 (a_{x2}) while respective values for ACE were 183.9 (a_{y1}) and 248.3 (a_{y2}). Now simultaneous equations were derived for determination of PCM and ACE in mixed standard solution and in its tablet formulation by replacing the absorptivity values of PCM and ACE in the following equations:

$$C_x = A_2 a_{y1} - A_1 a_{y2} / a_{x2} a_{y1} - a_{x1} a_{y2}$$

$$C_y = A_1 a_{x2} - A_2 a_{x1} / a_{x2} a_{y1} - a_{x1} a_{y2}$$

where A_1 and A_2 are absorbance of sample solution at λ_{\max} of PCM (243 nm) and λ_{\max} of ACE (273 nm) respectively; a_{x1} and a_{x2} are the absorptivities of PCM at 243 nm and 273 nm respectively and a_{y1} and a_{y2} are the absorptivities of ACE at the two wavelengths respectively. In a similar manner, sample solutions of PCM (2 – 16 µg/ml) and ACE (2 – 10 µg/ml) were prepared from the stock solution of the marketed tablets.

Validation

The proposed method was validated for the parameters like linearity, accuracy, specificity and precision as per International Conference on Harmonization (ICH) guidelines¹⁰.

Linearity of the method was determined by constructing calibration curves. Absorbance of standard solutions of PCM (2 – 16 µg/ml) and ACE (2 – 10 µg/ml) were measured in six replicates at 243 nm and 273 nm. The absorbance was plotted against the concentrations to obtain the calibration curves and correlation coefficients.

The accuracy of the proposed method was determined by performing recovery studies. It was carried out at 50, 100 and 150% of the test concentration as per ICH guidelines. The sample solutions were spiked with known amount of pure drugs and the recovery was performed. All the solutions were prepared in triplicate.

The specificity of test method was established by comparing the spectra of the sample solutions of same concentration of both the

drugs in pure drug mixture and marketed formulation.

Precision was determined as repeatability, inter day and intraday precision. To check repeatability, the sample solutions were analyzed at 243 nm and 273 nm on same day and under same experimental conditions. The intraday precision was determined by analyzing the sample solutions in two different laboratories on the same day. The inter day precision was determined by analyzing the sample solutions on different days in the same laboratory.

Dissolution method development and validation

One tablet was placed in each of the six vessels of the dissolution apparatus USP type II (Tablet dissolution tester, USP model: TDT-06P, Electrolabs, India), containing 900 ml of 0.02 M Phosphate buffer (pH 6.8), preheated at $37 \pm 0.5^\circ\text{C}$ and the dissolution medium was stirred at 50 rpm. Aliquots of the dissolution medium (5 ml) were withdrawn at 5, 10, 20, 30 and 40 min and filtered, discarding the first portions of the filtrate; 2 ml of the filtrates were transferred to 50 ml volumetric flasks and made up to the mark with buffer. The amount of drug dissolved was determined by UV using the simultaneous equation method.

Validation

The dissolution method was validated for precision and robustness as per the ICH guidelines.

The interday precision was determined by analysis of two sets of six tablets each, from the same lot on two different days. The robustness of the dissolution method was established by performing dissolution at two different paddle speed (50 and 75 rpm).

Data analysis¹¹

The dissolution profiles were analyzed by similarity factor. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. The following equation was used to calculate the similarity factor f_2 .

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^t Wt (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where, n is the number of time points, R_t is the dissolution value of reference product at time t and T_t is the dissolution value for the test product at time t. According to FDA, the dissolution profiles are found to be similar if f_2 is between 50 and 100.

RESULTS AND DISCUSSION

Solubility Studies

The solubility of PCM increases with the increase in pH. Solubility of ACE was poor in water and 0.1 N HCl. At lower pH, the solubility was less and as pH increases from acidic to 6.8, the solubility drastically increases.¹² The solubility data of both drugs was depicted in Table 1. Phosphate buffer pH 6.8 was found suitable for dissolution studies.

Stability studies

Both PCM and ACE were found to be stable under dissolution test conditions and the measured absorbance of the standard and sample solutions was similar with deviation of ± 0.002 . There was no evidence of degradation of the drugs under these conditions. Thus, the solutions were stable for more than 48 h.

UV method development and Validation

From the overlain spectra (fig. 2), the λ_{max} of PCM and ACE were found to be 243 nm and 273 nm. The calibration curves of standard PCM (fig. 3) and ACE (fig. 4) were constructed and found to be linear. The absorptivity values of both the drugs are given in Table 2.

The proposed method was found to be linear with range of 2 – 16 $\mu\text{g/ml}$ for PCM ($r^2 = 0.999$) and 2 – 10 $\mu\text{g/ml}$ for ACE ($r^2 = 0.998$). The recovery studies were performed and the recovery of all the four brands is under the acceptance criteria. The method was found to be specific as there was no interference of the excipients. The method precision was evaluated for repeatability, inter day and intraday precision and the % RSD was found to be less than 2%. The data indicating the validation parameters is given in Table 3.

Dissolution method development and validation

The experimental results revealed that the dissolution rate of PCM and ACE increased with time irrespective of the paddle speed. More than 80% of the drug was released within 30 min. The dissolution profiles of PCM and ACE are shown in fig. 5 and 6 respectively.

The dissolution method precision was evaluated by performing inter day precision and all the four brands showed % RSD less than 2%. Thus, the method was precise.

The robustness of the dissolution method was performed employing different paddle speed of 50 and 75 rpm. As there was not much difference in the release rates, the method was found to be robust.

Data Analysis

The release of both drugs, PCM and ACE was found similar with the reference as f_2 was greater than 50 for all the brands.

CONCLUSION

Dissolution is a characterization test commonly used by the pharmaceutical industry to guide formulation design and control product quality. It is used as a quality control tool. The analytical method for the combination of paracetamol and aceclofenac developed was UV spectrophotometric method involving the simultaneous equation method for the simultaneous estimation of PCM and ACE. The two wavelengths employed in this method were 243 nm (λ_{\max} of PCM) and 273 nm (λ_{\max} of ACE). The method was validated for various parameters like linearity, accuracy, precision and specificity. All the parameters were found to be under the acceptance

criteria. The *in vitro* dissolution profile was obtained using 900 ml of dissolution medium containing 0.2 M phosphate buffer pH 6.8 maintained at $37^\circ \pm 0.5^\circ\text{C}$ with paddle apparatus at 50 rpm. More than 85% of the drug, both PCM and ACE were released within 30 min. The drug release rates were compared using the similarity factor and were found similar. Thus, the dissolution method developed was precise, accurate and reproducible and can be employed as a quality control method.

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Table 1: Solubility of Paracetamol and Aceclofenac

Solvent	Solubility \pm SD ($\mu\text{g/ml}$)	
	PCM	ACE
Distilled water	14.7 \pm 0.2	0.18 \pm 0.2
0.1 N HCl	89.4 \pm 0.1	0.045 \pm 0.1
Acetate buffer pH 4.5	6.1 \pm 0.4	5.2 \pm 0.3
Phosphate buffer pH 6.8	5.6 \pm 0.1	9.6 \pm 0.1
Phosphate buffer pH 7.2	5.3 \pm 0.2	6.2 \pm 0.3
Phosphate buffer pH 7.4	4.9 \pm 0.1	6.7 \pm 0.1

Table 2: Absorptivities of Paracetamol and Aceclofenac

Absorptivity	PCM		ACE	
	At 243 nm	At 273 nm	At 243 nm	At 273 nm
	636.85	146.43	183.89	248.30
	636.89	146.48	183.88	248.35
	636.86	146.44	183.93	248.31
Mean \pm SD	636.87 \pm 0.02	146.45 \pm 0.04	183.90 \pm 0.02	248.32 \pm 0.02

Table 3: Accuracy and Precision Data for PCM and ACE in Marketed Formulations

Brand	% Recovery \pm SD	Repeatability (%RSD)	Intra Day Precision (%RSD)		Inter Day Precision (%RSD)	
			Lab1	Lab2	Day1	Day2
PCM						
A	99.74 \pm 0.46	0.5	0.5	0.4	0.5	0.3
B	100.83 \pm 0.45	0.5	0.5	0.3	0.3	0.3
C	99.58 \pm 0.51	0.4	0.4	0.4	0.4	0.4
D	99.86 \pm 0.34	0.2	0.2	0.3	0.1	0.3
ACE						
A	99.21 \pm 0.26	0.5	0.5	0.4	0.5	0.3
B	99.64 \pm 0.41	0.5	0.5	0.4	0.5	0.4
C	98.56 \pm 0.58	0.4	0.4	0.5	0.4	0.4
D	99.71 \pm 0.51	0.4	0.4	0.4	0.4	0.3

A: Aceclo Plus, B: Afenak Plus, C: Dolokind Plus and D: Zerodol Plus

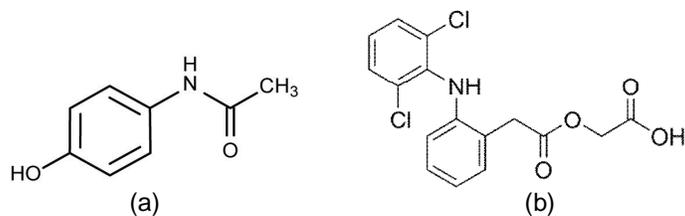


Fig. 1: Chemical structure of (a) Paracetamol, (b) Aceclofenac

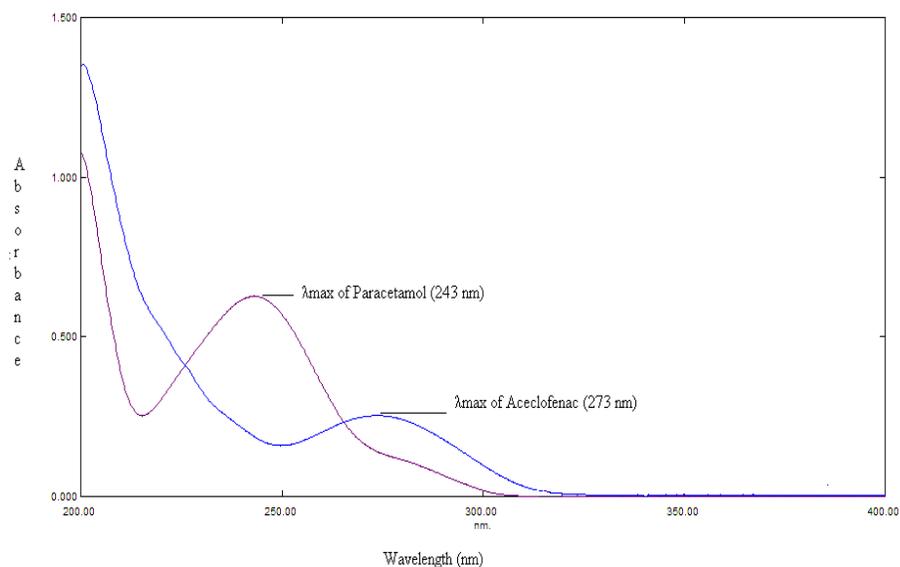


Fig. 2: Overlain Spectra of Paracetamol and Aceclofenac

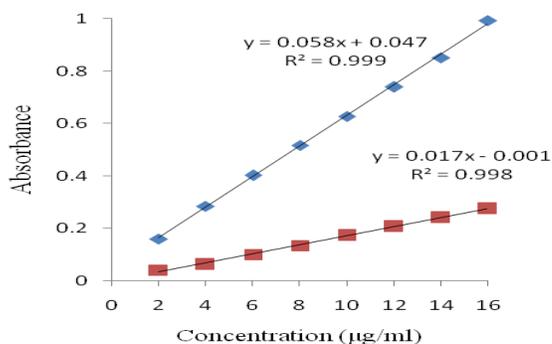


Fig. 3: Calibration curve of Paracetamol

◆ Absorbance at 243 nm ■ Absorbance at 273 nm

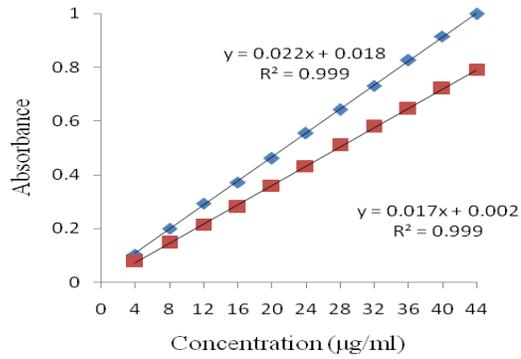


Fig. 4: Calibration curve of Aceclofenac

◆ Absorbance at 273 nm ■ Absorbance at 243 nm

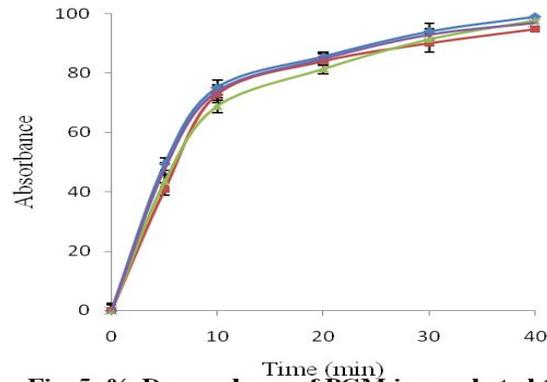


Fig. 5: % Drug release of PCM in marketed tablets

◆ Aceclo Plus ■ Afenak Plus
▲ Dolokind Plus × Zerodol Plus

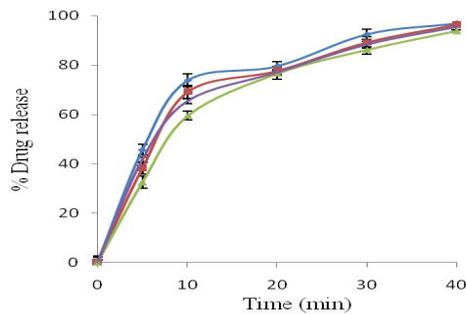


Fig. 6: % Drug release of ACE in marketed tablets

◆ Aceclo Plus ■ Afenak Plus
▲ Dolokind Plus × Zerodol Plus

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