**Research Article**

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# Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Paracetamol and Aceclofenac in marketed tablets

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## Abstract

The aim of the study was to develop UV-spectrophotometric and RP-HPLC methods for the simultaneous analysis of paracetamol and aceclofenac in marketed tablets. The methods were validated in terms of linearity, accuracy (% Recovery), precision (inter day, intraday and reproducibility) and robustness. Both the methods were linear ($R^2$ = 0.997-0.999 for UV method and 0.999 for RP-HPLC method) and accurate (% recovery was 99.19% - 100.14%). The method was also found precise (% RSD< 2%) and robust. Potency of three marketed brands was determined by both the methods and no statistically significant difference was noticed between the potency obtained by two methods by paired $t$ test at 5% significance level. Paracetamol released from marketed products was found to comply compendia specification but inter brand variation in case of aceclofenac release was observed by ANOVA (Sig. 0.000). Any one of the validated methods can be used for the analysis of paracetamol and aceclofenac tablets.

**Key-Words:** Paracetamol, Aceclofenac, UV, RP-HPLC, Method validation

## Introduction

Paracetamol is one of the most popular over-the-counter drugs. It has analgesic and antipyretic properties with weak anti-inflammatory activity and it is used in the symptomatic management of moderate pain and fever. When taken at recommended doses it has an excellent safety profile. It is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories[1]. The drug is official in different pharmacopeia[2-3]. Paracetamol is often combined with other drugs (caffeine, aceclofenac) for greater patient acceptability, increased potency, multiple activity, fewer side effects and quick relief. Aceclofenac, [(2-{2,6-dichlorophenyl)amino} phenylacetooxyacetic acid] is a non-steroidal anti-inflammatory drug (NSAID) indicated for the symptomatic treatment of pain and inflammation with a reduced side effect profile, especially gastro-intestinal events that are frequently experienced with NSAID therapy.

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Aceclofenac is practically insoluble in water with good permeability (calculated log P = 2.170) and belongs to biopharmaceutics classification system (BCS) class II (low solubility, high permeability). Therefore, AC shows dissolution rate limited absorption that gives rise to difficulties in pharmaceutical formulations for oral delivery, which may lead to variable bioavailability. Therefore constant surveillance on marketed paracetamol and aceclofenac tablets by the government, manufactures and independent research groups is essential to ensure availability of quality medicines.

Selective and sensitive analytical method for quantitative determination of drugs and their metabolites are essential for successful evaluation of clinical pharmacology, pharmacokinetics (PK), bioavailability (BA) and bioequivalence (BE) studies. Paracetamol is official in BP and USP. Analysis methods are described in these pharmacopeias. But neither aceclofenac tablet nor the combination of paracetamol and aceclofenac is official in BP or USP. So, official analytical methods are not available for simultaneous determination. A number of methods have been reported for the analysis of aceclofenac either by UV or HPLC [4-6]. Both UV and HPLC

methods for simultaneous estimation of paracetamol and aceclofenac have been reported[7-9].

Pawar V. T et al., 2010 described a method where they used methanol and water as solvent for analysis[7]. As Phosphate buffer is generally used as the dissolution medium it is better to develop the method in the phosphate buffer. Gajanand et al., 2010 reported a simultaneous method where they used Hydrotropic Solubilization Technique[8]. Uttam et al., 2009 described an HPLC method where retention time of aceclofenac is 19.48 min which is not suitable for analysis of large number of samples due to higher solvent consumption[9]. So development of analysis method for the simultaneous estimation of paracetamol and aceclofenac is still required. In this study we have validated both UV spectroscopic methods in by using dissolution media as solvent and we used simultaneous equation method for the calculation. We also validate RP-HPLC method by using simple solvent system and compare these two methods by paired *t* Test, so that one can test aceclofenac-paracetamol tablet with their available facility. The proposed methods were validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines[10].

## Material and methods

### Reagents and Chemicals

Methanol was of HPLC grade and was purchased from E. Merck, Darmstadt, Germany. Potassium di-hydrogen phosphate and other reagents were of analytical-reagent grade and purchased from E. Merck, India. Water was deionised and double distilled. Working standard of paracetamol and aceclofenac were collected from Eskayef Bangladesh Ltd as gift samples. Marketed tablets containing paracetamol 500 mg and aceclofenac 100 mg were purchased from local drug store in Dhaka city after checking their manufacturing license number, batch number, production and expiry date. All the brands were imported. Paracetamol – aceclofenac tablets manufactured by local pharmaceutical plant were not available.

### Validation of UV Method

**Instrumentation:** A double-beam UV-Visible spectrophotometer (Model UV-1700 PC, Shimadzu, Japan) equipped with wavelength accuracy of +0.5 nm (with automatic wavelength correction) was used. The drug analyses data were acquired and processed using UV Probe software (Version 2.0, Shimadzu, Japan) running under Windows XP on a Pentium PC.

**Preparation of standard solution and derivation of simultaneous equation:** Stock solution of paracetamol (100 mcg/ml) and aceclofenac (100 mcg/ml) prepared in phosphate buffer pH 6.8 were diluted to get standard solution of paracetamol and aceclofenac across the range of 2-20 mcg/ml. Solution containing mixture of paracetamol and aceclofenac (10, 12, 15, and 18 mcg/ml paracetamol along with 10 mcg/ml aceclofenac and vise versa) were also prepared by diluting standard solutions. Standard solutions of paracetamol and aceclofenac were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption and absorbtivity for both the drugs. Paracetamol and aceclofenac showed absorbance maxima at 243.5 nm and 273 nm respectively. Absorptivity values for paracetamol at 243.5 nm and 273 nm were 660 ($ax_1$) and 150 ($ax_2$) while respective values for aceclofenac were 150 ($ay_1$) and 250 ($ay_2$). Spectra for both the drugs are shown in Fig 1.

Now simultaneous equations were derived for determination of paracetamol and aceclofenac in mixed standard solution and in its pharmaceutical formulation by replacing the absorptivity values of paracetamol ($ax_1$= 660 and $ax_2$= 150) and aceclofenac ($ay_1$= 150 and $ay_2$= 250) in the following equations:

$$C_x = A2\ ay1 – A1\ ay_2\ /\ ax_2 ay_1\text{-}ax_1\ ay_2 \ldots\ldots\ldots\ldots(1)$$
$$C_y= A1\ ax2 –A2\ ax_1\ /\ ax_2\ ay_1\text{-}ax_1\ ay_2 \ldots\ldots\ldots\ldots(2)$$

where, A1 and A2 are absorbance of sample solution at λmax of paracetamol (243.5nm) and λmax of aceclofenac(273nm) respectively; $ax_1$ and $ax_2$ are the absorptivities of paracetamol at 243.5nm and 273 nm respectively and $ay_1$ and $ay_2$ are the absorptivities of aceclofenac at the two wavelengths respectively.

**Validation:** The proposed method was validated for the parameters like linearity, accuracy, precision and robustness as per ICH guidelines [10].

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. Linearity of the method was determined by constructing calibration curves. Absorbance of standard solutions of paracetamol and aceclofenac of different concentrations level (4-20 mcg/ml) were measured in six replicates at 243.5 nm and 273 nm. The absorbance was plotted against the concentrations to obtain the calibration curves and correlation coefficients.

The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments. Absorbance of solution containing known concentration of paracetamol and aceclofenac was measured at two selected wave length. Then potency was calculated and accuracy was assessed from the test results as the percentage of the drug recovered by the assay.

The precision of the method was investigated with respect to repeatability (inter assay precision),

intermediate precision (inter day precision) and reproducibility. Repeatability was determined by performing three repeated analysis of the three standard solutions (4, 6, 8 µg/ml paracetamol along with 10 mcg/ml aceclofenac and vise versa) of standard mixture solution on the same day, under the same experimental conditions. Intermediate precision of the method was assessed by carrying out the analysis of standard solutions on three different days (inter-day) in the same laboratory. For reproducibility analysis was carried out in another lab.

To determine the robustness different solvent composition was used. Percent recovery was calculated for both the drug. Analytical method is generally known as robust if percent recovery is within 98-102%

**Validation of HPLC Method**

**HPLC Instrumentation:** A Shimadzu (Japan) HPLC system consisting of a CMB-20 Alite system controller, two LC-20AT pumps, SIL-20A auto-sampler and CTO-10ASVP column oven were used. Ultraviolet detection was achieved at 273 nm with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analyses data were acquired and processed using LC solution (Version 1.3, Shimadzu, Japan) software running under Windows XP on a Pentium PC. The mobile phase, water (pH 3.5 with acetic acid): methanol (50:50 v/v) pumped at a flow rate of 1.0 ml/min through the column ($C_{18}$; 250 mm X 4.6 mm, 5µ shim-pack, Japan) at $30^0C$. The mobile phase was filtered through a 0.2µ nylon membrane filter and degassed prior to use under vacuum. Elusions were analyzed by UV detector at a sensitivity of 0.0001.

**Preparation Standard solution of for HPLC analysis:** Different concentrations (80%, 90%, 100% 110% and 120% of target concentration) of paracetamol and aceclofenac were prepared in mobile phase from stock solution of paracetamol (100µg/ml) and aceclofenac (100µg/ml). Solution containing mixture of paracetamol and aceclofenac of five different concentrations (80%, 90%, 100% 110%, and 120% of target concentration) were also prepared by dilution as required.

**Validation:** The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision and robustness.

The system suitability was assessed by six replicate analyses of standard solution at a 100% (50 mcg/ml paracetamol and 10 mcg/ml aceclofenac) level to verify reproducibility of retention time, tailing factor and theoretical plates (Tangent) of the column.

To determine the selectivity of the method standard samples and placebo formulation of paracetamol and aceclofenac were injected one after another. Then the chromatograms were analyzed for retention time, peak area and peak shape to determine selectivity of the method.

For linearity, calibration graph was constructed from peak area obtained by injecting increasing amount of standard solutions (80%, 90%, 100%, 110% and 120% of target concentration).

For accuracy determination standard solution containing paracetamol and aceclofenac were injected and percent recoveries were calculated from peak area. The accuracy was calculated from the test results as the percentage of the drug recovered by the assay.

The precision of the method was investigated by performing four repeated analysis of the three standard solutions (90%, 100% and 110% of target concentration) of standard solution on the same day (for repeatability) and different day for inter day precision. For reproducibility analysis was carried out in another lab. The relative standard deviation (% RSD) was determined in order to assess the precision of the method.

The robustness of the method was assessed by altering the some experimental conditions such as by changing the flow rate from 0.9 to1.1 ml/min, amount of methanol (48% to 52%) and the temperature of the column (28 °C to 32 °C).

**Determination of potency of paracetamol and aceclofenac tablets**

Average weight of paracetamol and aceclofenac tablet was calculated. Then the tablets were grinded separately to fine powder with the help of mortar and pestle. Powder containing 50 mg paracetamol and 10 mg aceclofenac was dissolved in phosphate buffer pH 6.8, shaken for about 10 minutes and filtered through filter paper. The filtered solution was further diluted in phosphate buffer pH 6.8 and used for absorbance measurement in a double-beam Shimadzu (Kyoto, Japan) UV-Visible spectrophotometer (Model UV-1700 PC) to find out the potency. The samples were also prepared in mobile phase in the same way (50 mcg/ml paracetamol and 10 mcg/ml aceclofenac) and injected in Shimadzu (Japan) HPLC system. Potency was calculated from peak area.

**Dissolution test**

The dissolution test was undertaken using tablet dissolution tester (TDT-08L, Electrolab, India) in 5 replicates for each brand. Dissolution media were USP buffer solutions at pH 6.8 (phosphate buffer solution). The medium was maintained at 37 ± 0.5°C. In all the experiments, 5 ml of dissolution sample was withdrawn at 0, 10, 20, 30, 40, 50 and 60 min and replaced with equal volume to maintain sink condition. Samples were filtered and assayed by UV

spectroscopic method. The concentration of each sample was determined from a calibration curve obtained from pure samples of paracetamol and aceclofenac.

**Data analysis**

Dissolution profiles were analyzed by difference factor (f1), similarity factor (f2), dissolution efficiency (%DE) and ANOVA.

## Results and Conclusion

**Validation of UV method**

The result of validation parameters like linearity, accuracy, precision and robustness of UV method was found with in limit. The proposed method was linear with correlation coefficient 0.997-0.999. (Fig 2 and 3). Results of accuracy, precision and robustness are summarized in table 1. Percent recovery was found 99.42% ± 0.31 for paracetamol and 99.19%± 0.72 for aceclofenac. % RSD all precision study were less than 2%. No significant effect was observed in the recovery of drugs due to change of pH of phosphate buffer. So the proposed UV spectroscopic method is accurate, precise and robust.

**Validation of HPLC Method**

Table 2 represents system suitability tests results of this method. The system is found suitable in respect of retention time (% RSD 0.019-0.024) mean theoretical plate count (more than 4500) and tailing factor (less than 1.5).

Peaks of paracetamol and aceclofenac from standard placebo solution were on same time (Fig 4). On the other hand no additional peaks other than drugs were found within 7 min run time. Excipients did not change the retention time or interfere the analysis results. So the method is highly selective.

Results of linearity, accuracy and precision (repeatability, inter day and reproducibility) are summarized in table 3. Correlation coefficient of calibration curve was 0.999 for both paracetamol and aceclofenac.

% recovery was found 100.14 ± 0.62 with % RSD value 0.062 for paracetamol and 99.15± 0.49 with % RSD value 0.494. % RSD was less than 2% for all precision study. All the results indicate that the method is linear, accurate and precise.

Robustness study was performed by making a slight variation in flow rate, amount of methanol and column temperature. No significant effect was observed in the recovery of drugs. % recovery was 98% to 102%. On the other hand changes in retention time, theoretical plate and resolution were also negligible. So we can say that the method is robust.

**Potency of tablets**

The validated UV spectroscopic and HPLC methods were used to determine the potency of commercially available paracetamol and aceclofenac tablets (table 4). Potency calculated from UV method and HPLC method was compared by paired *t* Test at 0.05 significance level. The P-value was greater than the significance level, indicating that there was no statistically significant difference between the two methods.

***In vitro* drug release study and Comparison of dissolution data**

The release profiles of paracetamol and aceclofenac tablets (A-C) are shown in Fig 5. All dissolution data are based on the actual drug content of the test tablets as calculated from the assay results.

USP specification for paracetamol immediate tablet is that not less than 80% (Q) of the labeled amount of paracetamol ($C_8H_9NO_2$) should dissolve in 30 minutes. All the brands complied with the USP specification. More than 80% drug was released with in 30 min and around 100% drug was released with in 40 min from all the immediate release brands.

Aceclofenac tablet is not official in BP or USP. There is no official specification regarding the drug release of aceclofenac. Aceclofenac release from different brands was not uniform. Inter brand variation was observed. The following statistical analysis of dissolution data also proves the inter brand variation of aceclofenac release.

Difference factor (f1), similarity factor (f2) and dissolution efficiency (%DE) were calculated and compare for dissolution data obtained from two dissolution media. Difference factor f1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f2) 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 equations were used to calculate difference factor f1 and similarity factor f2.

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100$$

$$f_2 = 50 \log \left\{ \left( 1 + \frac{1}{n} \sum_{i=1}^{n} (R_t - T_t)^2 \right)^{-0.5} X100 \right\}$$

where n is the number of time points, Rt is the dissolution value of reference product at time t and Tt is the dissolution value for the test product at time t. Dissolution efficiency (DE) was also employed to compare the drug release from various brands. Dissolution efficiency is the area under the dissolution curve within a time range (t1 - t2). %DE was calculated by using the following equation:

$$AUC = \sum_{i=1}^{i=n} \frac{(t_i - t_{i-1})(y_{i-1} + y_i)}{2}$$

Where *y* is the percentage dissolved at time *t*
Table 5 shows the f1, f2 and % DE of paracetamol and aceclofenac tablet. Brand A was used as reference product to calculate f1 and f2. Paracetamol release from the tablet was found similar with reference brand as f2 were greater than 50 and f1 were less than 15. But aceclofenac release was not similar with reference brand as f2 were smaller than 50. Again dissolution efficiency for paracetamol was higher than aceclofenac.
Similarity of paracetamol release and inter brand variation of aceclofenac release can also be proved by ANOVA of drug release (Table 6). P value is less than the defined significance level in case of aceclofenac release that indicates statistically significant difference in drug release.
Aceclofenac is practically insoluble in water. So it shows poor dissolution. Dissolution rate may be increased by optimizing formulation and manufacturing technique.
From this validation study we can conclude that the developed UV and RP-HPLC methods are accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviation. Any one of the methods can be used for simultaneous determination of paracetamol and aceclofenac in bulk or pharmaceutical dosage form (individual or combine). Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method. So this method can be used in the quality control department for potency and dissolution study. On the other hand aceclofenac release from each of these samples varied greatly from tablet to tablet indicating lack of homogeneity in the

selected batches. Although the study was performed on limited in vitro experiments, the dissolution data of aceclofenac reported in this paper clearly indicate a substantial probability of bioavailability problems with the use of the test products.

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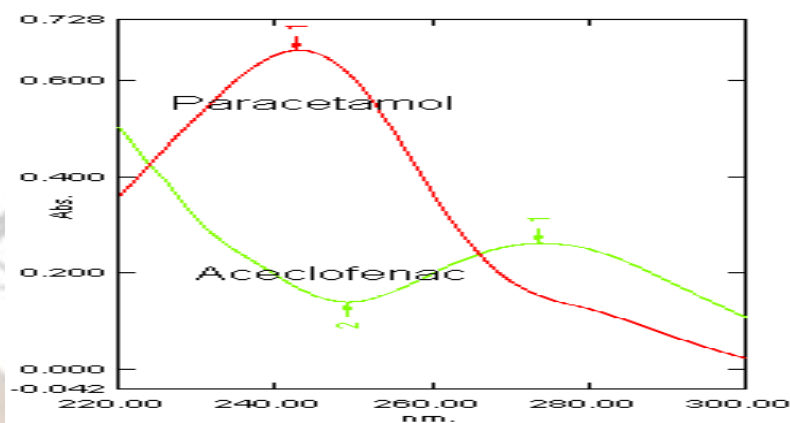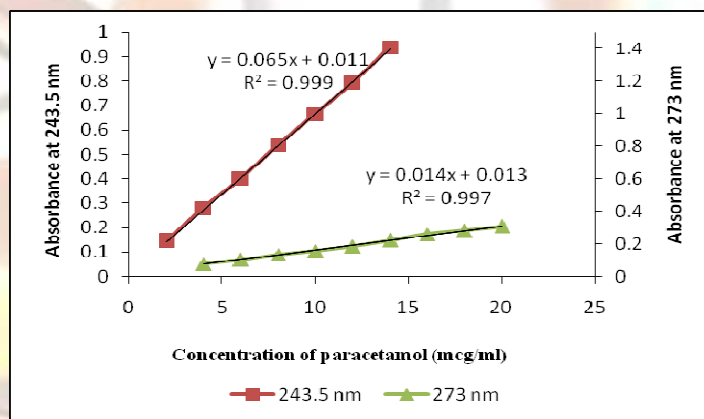
**Fig. 1: Spectrum of paracetamol and aceclofenac**



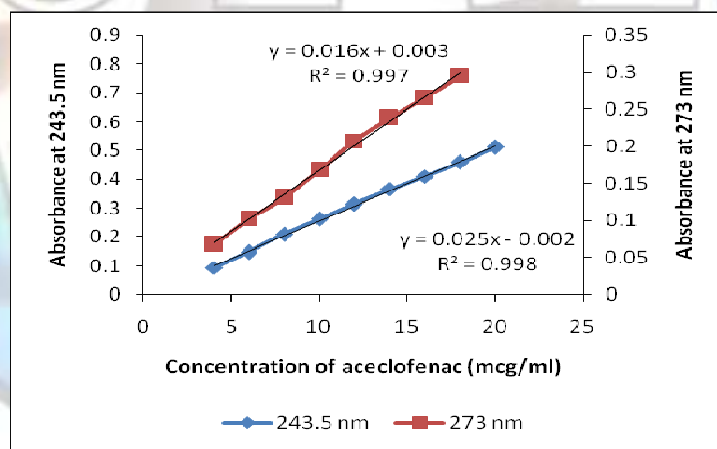**Fig 2: Calibration curve of paracetamol at 243.5 nm and 273 nm**



**Fig 3: Calibration curve of aceclofenac at 243.5 nm and 273 nm**

**Table 1: Accuracy, precision and robustness result of the UV method**

| | Validation parameters | | Paracetamol | Aceclofenac |
|---|---|---|---|---|
| Accuracy | % Recovery ± SD | | 99.42% ± 0.31 | 99.19% ± 0.72 |
| Precision (%RSD) | Repeatability | | 0.46 | 0.28 |
| | Ruggedness | | 0.73 | 0.59 |
| | Reproducibility Lab-I | | 0.74 | 0.95 |
| | Reproducibility Lab-II | | 1.08 | 1.34 |
| Robustness % Recovery ± SD | Phosphate Buffer pH 6.8 | | 99.67 ± 0.49 | 99.52 ± 0.19 |
| | Phosphate Buffer pH 7.2 | | 100.44 ± 0.61 | 99.78 ± 0.35 |

**Table 2: Results of system suitability study**

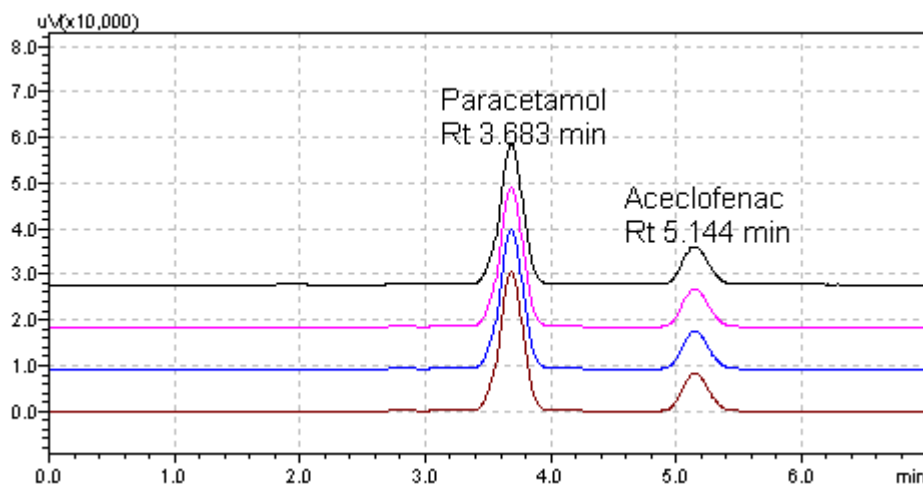| Parameters | Paracetamol (50 mcg/ml) | | Aceclofenac (10 mcg/ml) | |
|---|---|---|---|---|
| | Average ±SD | %RSD | Average ±SD | %RSD |
| Retention time | 3.683±0.001 | 0.024 | 5.144±0.001 | 0.019 |
| Area | 405318±3218 | 0.794 | 121257.33±2263 | 1.867 |
| Theoretical plates | 4523.54±3.141 | 0.069 | 4752.333±3.141 | 0.066 |
| Tailing factor | 1.23±0.003 | 0.275 | 1.1746±0.004 | 0.373 |



**Fig 4: Chromatogram of paracetamol and aceclofenac from standard and placebo formulation**

**Table 3: Linearity, accuracy and precision results of HPLC method**

| Validation parameters | | Paracetamol | Aceclofenac |
|---|---|---|---|
| Linearity (regression coefficient-$R^2$) (*Y = mX+C) | $R^2$ (mean±SD) | 0.9997±0.0002 | 0.9993±0.0002 |
| | %RSD ** | 0.021 | 0.021 |
| | Slope (mean±SD) | 8106.36±41.31 | 12125.74.91 ±65.42 |
| Accuracy | % Recovery | 100.14 ± 0.62 | 99.15± 0.49 |
| | %RSD | 0.062 | 0.494 |
| Precision (%RSD) | Repeatability | 0.45 | 0.38 |
| | Inter day | 0.73 | 0.68 |
| | Reproducibility | 1.62 | 1.13 |

\* $R^2$ = regression coefficient

\*\*Y = mX+C; where Y = peak area, m = slope, X = concentration (mcg/ml) and C = intercept.

**Table 4: Potency of the paracetamol and aceclofenac tablets**

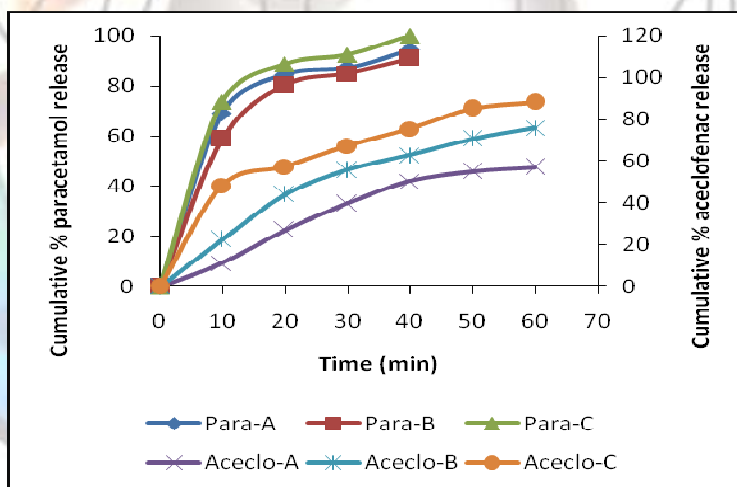| Brand | Paracetamol % ± SD (n = 5) | | | | Aceclofenac % ± SD (n = 5) | | | |
|---|---|---|---|---|---|---|---|---|
| | UV method | HPLC method | t-value | Sig. (2-tailed) | UV method | HPLC method | t-value | Sig. (2-tailed) |
| A | 98.96 ± 0.72 | 97.50 ± 0.47 | | | 97.37 ± 0.82 | 100.96 ± 0.79 | | |
| B | 99.82 ± 0.59 | 98.17 ± 0.99 | 2.008 | 0.182 | 100.52 ± 0.58 | 99.74 ± 0.62 | 0.461 | 0.689 |
| C | 99.48 ± 0.71 | 99.47 ± 0.19 | | | 100.42 ± 0.81 | 99.63 ± 0.95 | | |



**Fig: 5 Drug release from paracetamol and aceclofenac tablets (A-C)**

**Table 5: f1, f2 and % DE of aceclofenac tablets**

| Brand | paracetamol | | | Aceclofenac | | |
|---|---|---|---|---|---|---|
| | f2 | f1 | %DE | f2 | f1 | %DE |
| A | | | 84.51 | | | 41.32 |
| B | 61.18 | 5.99 | 80.07 | 40.60 | 37.82 | 56.54 |
| C | 65.07 | 5.81 | 89.31 | 25.85 | 75.18 | 70.73 |

**Table 6: Results of analysis of variance at 30 time point for paracetamol and aceclofenac**

| Drug | Parameters | Sum of Squares | df | Mean Square | F | P (Sig.) |
|---|---|---|---|---|---|---|
| Paracetamol | Between Groups | 57.90 | 2 | 28.95 | | |
| | Within Groups | 108.60 | 12 | 9.05 | 3.2 | 0.077 |
| | Total | 166.50 | 14 | | | |
| Aceclofenac | Between Groups | 216.30 | 2 | 108.15 | | |
| | Within Groups | 46.80 | 12 | 3.9 | 27.7 | 0.000 |
| | Total | 263.10 | 14 | | | |

Df - Degree of freedom