A Study of Method Development, Validation, and Forced Degradation for Simultaneous Quantification of Paracetamol and Ibuprofen in Pharmaceutical Dosage Form by RP-HPLC Method

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ABSTRACT: A rapid and stability-indicating reversed phase high-performance liquid chromatography (RP-HPLC) method was developed for simultaneous quantification of paracetamol and ibuprofen in their combined dosage form especially to get some more advantages over other methods already developed for this combination. The method was validated according to United States Pharmacopeia (USP) guideline with respect to accuracy, precision, specificity, linearity, solution stability, robustness, sensitivity, and system suitability. Forced degradation study was validated according to International Conference on Harmonisation (ICH). For this, an isocratic condition of mobile phase comprising phosphate buffer (pH 6.8) and acetonitrile in a ratio of 65:35, v/v at a flow rate of 0.7 mL/minute over RP C18 (octadecylsilane (ODS), 150 × 4.6 mm, 5 μm, Phenomenex Inc.) column at ambient temperature was maintained. The method showed excellent linear response with correlation coefficient ($R^2$) values of 0.999 and 1.0 for paracetamol and ibuprofen respectively, which were within the limit of correlation coefficient ($R^2$ ≥ 0.995). The percent recoveries for two drugs were found within the acceptance limit of (97.0–103.0%). Intra- and inter-day precision studies of the new method were less than the maximum allowable limit percentage of relative standard deviation (%RSD) ≤ 2.0. Forced degradation of the drug product was carried out as per the ICH guidelines with a view to establishing the stability-indicating property of this method and providing useful information about the degradation pathways, degradation products, and how the quality of a drug substance and drug product changes with time under the influence of various stressing conditions. The degradation of ibuprofen was within the limit (5–20%, according to the guideline of ICH), while paracetamol showed <20% degradation in oxidation and basic condition.

KEYWORDS: RP-HPLC, stability-indicating, paracetamol, ibuprofen
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sider pKa of paracetamol, then we cannot choose the pH above two units above or below the pKa value of drug. If we con tigated. pKa of paracetamol and ibuprofen was 9.5 and 4.85, respectively. As a rule of thumb, pH of mobile phase is selected 9.5, which is detrimental to silica beds of column. Therefore, the choice is two units below the pKa of paracetamol. Again, with respect to ibuprofen, we could choose the pH of mobile phase two units below of its pKa (4.8), but at that acidic pH, ibuprofen remains fully undissociated, which results in strong hydrophobic attraction with silica bed and, consequently, longer retention of this drug. Therefore, we thought a pH of about 7.0, which will be about two units far from the pKa of both drugs, and at this pH, both drugs will remain ionized, which makes the retention time much shorter at lower organic concentration. Thus, we tried with different buffers having a pH between 6.5 and 7.5 with different ratios of acetonitrile and methanol in isocratic condition. But we discarded methanol as it took longer time to elute ibuprofen. So our ultimate choice was acetonitrile, and at 35% concentration, the method gave well-resolved and sharp peaks of paracetamol and ibuprofen. It was found that with increased pH, retention of ibuprofen was decreased. Thus, finally, a pH of 6.8 was chosen so as to get sufficient resolution between the peaks. The method is summar ized in Table 1. Typical chromatogram is shown in Figure 2. This study was validated according to the guidelines of Inter national Conference on Harmonisation (ICH) and USP.5,10

Materials and Methods
Materials. Working standards of paracetamol and ibuprofen were a kind gift of Healthcare Pharmaceuticals Limited, Rajendrapur, Bangladesh. HPLC grade acetonitrile was purch ased from Active Fine Chemicals Ltd., Dhaka, Bangladesh.

HPLC system. High-performance liquid chromatographic system (Shimadzu-Prominence, Japan), equipped with an auto sampler (Model—SIL-20AC HT) and UV–visible detector (Model—SPD 20A), was used for the analysis. The data were recorded using LC-solution software. Analytical RP C18 column [octadecylsilane (ODS), 150 × 4.6 mm, 5 μ, Phenomenex Inc., Japan] was used to analyze the standard and samples.

Preparation of mobile phase. A total of 1.75 g of dipotassium hydrogen phosphate salt was dissolved in 900 mL of nanopure water. pH was adjusted to 6.8 ± 0.1 with diluted phosphoric acid or sodium hydroxide, and then volume was made up to 1000 mL with water of the same quality. Then this buffer and HPLC grade acetonitrile were mixed together at a ratio of 65:35, v/v. Finally, it was filtered through a 0.22-μm Millipore filter and sonicated to degas.

Preparation of standard solutions. In all, 50 mg of paracetamol and 20 mg of ibuprofen were taken in a 100-mL volumetric flask, and about 50 mL diluting solution (as mobile phase) was added and sonicated for five minutes to dissolve properly. Then volume was made up to the mark with the same diluent. This was stock solution. A total of 5 mL of this solution was taken into a 50-mL volumetric flask and diluted with diluents up to the mark. Thus, we got the test solution of paracetamol and ibuprofen of a concentration of 50 and 20 μg/mL, respectively. The stock solution was further diluted to get 50–200% of test concentration.
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Chromatographic conditions. All analyses were done at ambient temperature under isocratic condition. The mobile phase was run at a flow rate of 0.7 mL/minute for seven minutes. The injection volume was 10 μL for standard and samples. Before analysis, every standard and sample was filtered through 0.2 μm filter tips. The column eluent was monitored with UV detection at 222 nm.

Method validation. The method was validated according to United States of Pharmacopeia (USP) guideline with respect to accuracy, precision, specificity, linearity, solution stability, robustness, sensitivity, and system suitability. Forced degradation was validated according to ICH.9,11,12

Specificity. The specificity of the LC method was evaluated to ensure that there was no interference from the degradation products, excipients, or other impurities in the region of actives. The specificity was studied by injecting the unstressed and stressed standard solutions, samples, and blanks.

Solution stability. Stability of drugs in diluting solvent and mobile phase was checked by rendering the test solutions in tightly capped vials at room temperature and in refrigerator at 5°C for three days. The solutions were analyzed by HPLC for three consecutive days.

Forced degradation. Forced degradation studies are undertaken to degrade the active drug deliberately. These studies are used to evaluate an analytical method’s ability to measure an active ingredient and its degradation products without interference. Samples or drug product (spiked placebo) and drug substance are exposed to acid, base, oxidizing agent, reducing agent, and water. The degraded samples were then analyzed using the method to determine if there are interferences with the active. Thus, stability-indicating property was evaluated.

Linearity and range. Linearity was checked on seven different concentrations within 50–200% of the nominal standard concentration. The linearity of the proposed method was evaluated by using calibration curve to calculate coefficient of correlation, slope, and intercept values.

Accuracy. The accuracy of an analytical method expresses the nearness between the expected value and the value found. In the present study, successive analysis (n = 3) for three different concentrations of standard mixture (80, 100, and 120% of nominal concentration) was carried out to determine the accuracy of the proposed method.

Precision. The precision of the assay was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by intra- and inter-day repeatability of responses on different columns and different HPLC machines after replicate injections and expressed as %RSD among responses using the formula [%RSD = (standard deviation/mean) × 100%].

Robustness. Robustness is an indication of reliability of the analytical method during normal usage. The effect of the following deliberate changes in chromatographic conditions was monitored: flow rate ±50%, solvent ratio ±15%, pH of buffer solution ±0.2, temperature ±10°C, and detector wavelength ±3.

System suitability. The purpose of the system suitability test is to ensure that the complete testing system, including instruments, reagents, columns, analysts etc., is adequate for the intended analysis. The following parameters are usually determined: theoretical plate count, tailing factors, resolution, and reproducibility.

Limit of detection (LOD) and limit of quantitation (LOQ). LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. On the other hand, LOQ is the lowest amount of analyte in a sample that may be determined with acceptable accuracy and precision.

Results and Discussion

Specificity. The specificity was studied by injecting the unstressed and stressed standard solutions, blank, and pharmaceutical preparation of these drugs combination, several times on several days. It was revealed that there was no interference of peak in the region of paracetamol and ibuprofen in chromatogram for the stressed sample, placebo, and active. Specificity was further confirmed by peak purity index. Hence, the method was considered specific for the product. Representative chromatograms of blank, standard, and sample are shown in Figure 2, and peak purity index is shown in Figure 3.

Solution stability. Two vials were prepared containing the standard mixture in diluting solution. One vial was kept in

Figure 2. Chromatograms of blank, standard, and sample.
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**Table 2.** Results of solution stability.

| DAY | ROOM TEMPERATURE | REFRIGERATOR |
|-----|------------------|--------------|
|     | PARA  | IBU  | PARA  | IBU  |
| Day 1 | 1548146 | 782314 | 1548146 | 782314 |
| Day 2 | 1540987 | 770193 | 1542538 | 779734 |
| Day 3 | 1538988 | 765330 | 1541885 | 779556 |
| %RSD  | 0.312  | 1.13  | 0.223  | 0.198  |

Figure 3. Peak purity of paracetamol and ibuprofen.

room temperature and another in refrigerator. Area change was investigated up to three consecutive days. %RSD of areas for all drugs were well between the limit (1.3%), which demonstrated that the drugs were fairly stable in the diluting solution and in the mobile phase. Results are shown in Table 2.

**Forced degradation.** Standard mixture was exposed to water, acid (1.0N HCl), base (1.0N NaOH), oxidizing agent (10% H$_2$O$_2$), and reducing agent (10% sodium bisulfate) for 24 hours. Results are summarized in Table 3. Degradation of drug substances between 5 and 20% has been accepted for validation of chromatographic assays. From the results, it was found that paracetamol was degraded significantly by oxidation. It also exhibited degradation in basic condition. One very notable finding is that ibuprofen remained completely stable in acid media. ibuprofen showed degradation in strong basic condition, but it was within the limit. In case of water hydrolysis, degradation was below the limit for both drugs. That is why we chose the pH of buffer in acidic region and close to neutral pH. Chromatograms are shown in Figure 4.

**Linearity, accuracy, and precision.** Linearity of the standard mixture was examined on 50–200% of nominal concentration. According to USP, the correlation coefficient ($R^2$) for a calibration curve must be $>0.995$. The correlation coefficient was found to be more than 0.995 for both drugs indicating good linearity of calibration curve. The linearity curves are shown in Figure 5.

For paracetamol, percent recovery is found to be 101.2, 100.5, and 99.9% for 40, 45, and 50 μg/mL, respectively, and for ibuprofen, percent recovery is found to be 101.1, 100.4, and 100.2% for 16, 18, and 20 μg/mL, respectively, shown in Table 4. All experimental results are in the range of the acceptability for accuracy (97.0–103.0%).

The %RSD values found in precision study depicted in Table 5 showed that the proposed method provides acceptable intra- and inter-day variations for paracetamol and ibuprofen in their simultaneous determination. Results are summarized in Table 5 where %RSD was within the limit $\leq 2$.

**System suitability.** All system suitability parameters, including peak area, theoretical plate, tailing factor, retention time, and resolution, met the compendium acceptance limits. Results are summarized in Table 6.

**Robustness.** Predetermined variations were performed under the experimental conditions to assess their robustness. We changed pH $\pm 0.4$, flow rate $\pm 0.3$, wave length $\pm 10$, and organic concentration $\pm 3.5$ units. Results are shown in Table 7.

**Conclusion**

Hereby a new and robust method was developed for simultaneous quantification of paracetamol and ibuprofen in their solid dosage form. We validated it strictly maintaining the guidelines of ICH, USP, and Food and Drug Administration (FDA). The method was primarily designed for assay of
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paracetamol and ibuprofen in tablet or capsule. Moreover, the content of degradation of paracetamol and ibuprofen in various conditions, such as alkaline, acidic, oxidation, reduction and water hydrolysis, were observed and quantitatively analyzed by this HPLC method. The information, thus, obtained will facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, where knowledge of chemical behavior can be used to improve the quality of drug product.

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Author Contributions
Conceived and designed the experiments: AR. Analyzed the data: RK. Wrote the first draft of the manuscript: MSJ. Contributed to the writing of the manuscript: MJI. Agree

Figure 4. Chromatograms of stressed condition, clockwise from top-left corner: acid, base, reduction, and oxidation. Peaks of paracetamol and ibuprofen were identified by their retention time, and rest of the peaks was of unknown degradants.

Figure 5. Linearity curve of (A) paracetamol and (B) ibuprofen.
Table 4. Accuracy (% recovery) of paracetamol and ibuprofen.

| INJECTED CONC. OF PARACETAMOL (µg/mL) | MEAN RECOVERED CONC. OF PARACETAMOL (µg/mL) | % RECOVERY OF PARACETAMOL | INJECTED CONC. OF IBUPROFEN (µg/mL) | MEAN RECOVERED CONC. OF IBUPROFEN (µg/mL) | % RECOVERY OF IBUPROFEN |
|--------------------------------------|--------------------------------------------|-----------------------------|------------------------------------|---------------------------------------------|--------------------------|
| 40                                   | 40.48                                      | 101.2                       | 16                                 | 16.17                                       | 101.1                    |
| 45                                   | 45.23                                      | 100.5                       | 18                                 | 18.07                                       | 100.4                    |
| 50                                   | 49.95                                      | 99.9                        | 20                                 | 20.04                                       | 100.2                    |

Table 5. Results of method validation parameters.

| PARAMETERS                  | LIMITS                              | PARACETAMOL | IBUPROFEN |
|-----------------------------|-------------------------------------|-------------|-----------|
| Linear equation             | y = 32767x – 88823                  |             |           |
| Correlation coefficient (R²)| ≥ 0.995                             | 0.999       | 1.00      |
| Linearity range             | 25–100 µg/mL                        |             |           |
| Precision(intra-day)        | %RSD < 2%                           |             |           |
| Day 1, Analyst 1, Column 1 | 0.956%                             | 0.821%      |
| Day 2, Analyst 2, Column 2 | 1.344%                             | 1.937%      |
| Day 3, Analyst 3, Column 3 | 1.987%                             | 1.124%      |
| Precision(inter-day):       | %RSD < 3%                           |             |           |
| LOD                         | S/N = 3:1                           | 2.3 µg/mL   | 0.213 µg/mL |
| LOQ                         | S/N = 10:1                          | 7.9 µg/mL   | 0.711 µg/mL |

Note: *Mean of six runs.

Table 6. System suitability parameters.

| PARAMETER                 | PARACETAMOL | IBUPROFEN | USP LIMIT |
|---------------------------|-------------|-----------|-----------|
| %RSD of tₚ₅ᵃ               | 0.225       | 0.114     | ≤2%       |
| %RSD of areaᵃ             | 0.223       | 0.188     | ≤2%       |
| Tailing factorᵇ           | 1.3         | 1.1       | ≤2.0      |
| Resolutionᵇ               | –           | 6.5       | ≥2.0%     |
| Theoretical plate         | 2100        | 3743      | ≥2000     |
| Peak purity index         | 1.00        | 1.00      | ≥0.98     |

Notes: *Mean of six runs. ᵇResolution with respect to former peak.

Table 7. Summary of robustness.

| PARAMETER CHANGES               | ACTUAL VALUES | RETENTION TIME | TAILING FACTOR | PEAK PURITY |
|---------------------------------|---------------|----------------|----------------|-------------|
| pH ±0.4                         | 7.2           | 2.9            | 4.1            | 1.2         | 1.1         | 1.00        | 1.00        |
|                                 | 6.2           | 2.4            | 5.4            | 1.3         | 1.2         | 1.00        | 1.00        |
| Flow rate ±0.3                  | 1.0           | 1.6            | 3.1            | 1.1         | 1.2         | 0.99        | 0.99        |
|                                 | 0.4           | 3.8            | 6.3            | 1.5         | 1.3         | 1.00        | 1.00        |
| Wave length ±10                 | 212           | 2.8            | 4.7            | 1.3         | 1.1         | 1.00        | 1.00        |
|                                 | 232           | 2.7            | 4.7            | 1.3         | 1.1         | 1.00        | 1.00        |
| Organic concentration ±3.5     | 38.5%         | 2.1            | 4.2            | 1.2         | 1.1         | 0.99        | 0.99        |
|                                 | 31.5%         | 3.0            | 12.4           | 1.3         | 1.2         | 1.00        | 0.99        |
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