

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

Md. Sarwar Jahan¹, Md. Jahirul Islam², Rehana Begum³, Ruhul Kayesh³ and Asma Rahman⁴

¹Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh, ²Department of Chemistry, University of Dhaka, Dhaka, Bangladesh, ³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh, ⁴Drug Analysis and Research Laboratory, Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh.

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 Pharmacopoeia (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 Harmonization (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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Introduction

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug that belongs to arylpropionic acid derivatives and is chemically (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid. Paracetamol or acetaminophen (PARA) is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer), and it

belongs to para-aminophenol derivatives¹ and is chemically N-(4-hydroxyphenyl) acetamide as shown in Figure 1.

Combination of ibuprofen and paracetamol (200/500 mg) tablets is frequently indicated for temporary relief of mild to moderate pain associated with migraine, headache, back-ache, period pain, dental pain, rheumatic and muscular pain,

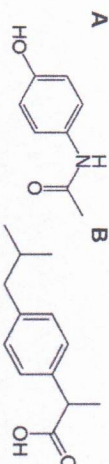


Figure 1. Structure of (A) paracetamol and (B) ibuprofen.

non-serious arthritis pain, cold and flu symptoms, sore throat, and fever.² This product is especially suitable for pain that requires stronger analgesia than ibuprofen or paracetamol alone.³ Development of an analytical method for assessment of drugs in pharmaceutical dosage form is of utmost necessity to confirm the quality of tablets or capsules with respect to assay, content uniformity, and dissolution. There are compendial methods for assessment of these two drugs singly in United States Pharmacopoeia (USP) and British Pharmacopoeia (BP). A number of high-performance liquid chromatography (HPLC) methods have been already stated for determination of ibuprofen and paracetamol in their combination dosage form.^{4–6} But we found some drawbacks in those methods, which are listed below:

- Almost all of those methods have used organic solvents more than 50%, which is not cost effective for routine analysis in pharmaceutical industries.
- Mobile phase containing more than 50% organic phase may be detrimental to HPLC column as at that concentration, buffer salts may precipitate.
- In some methods, shape and tailing do not seem to be satisfactory.
- Stress degradation studies and their chromatograms were not shown properly.

Herein we put an effort to develop a cost-effective, rapid, and robust reversed phase (RP)-HPLC method with enough data of validation parameters. First, pKa of drugs was investigated. pKa of paracetamol and ibuprofen was 9.5 and 4.85, respectively. As a rule of thumb, pH of mobile phase is selected two units above or below the pKa value of drug. If we consider pKa of paracetamol, then we cannot choose the pH above 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 around 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

Table 1. Newly developed RP-HPLC method.

Mobile phase: Phosphate buffer (pH 6.8); Acetonitrile = 65:35, v/v
Column: C18 (150 × 4.6 mm, 5 µm, Phenomenex Inc.)
Flow rate: 0.7 mL/min
Wave length: 222 nm
Retention time: Paracetamol: 2.8 ± 0.1 min, Ibuprofen: 4.7 ± 0.1 min
Tailing factor: Paracetamol: 1.3, Ibuprofen: 1.1

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 summarized in Table 1. Typical chromatogram is shown in Figure 2. This study was validated according to the guidelines of International Conference on Harmonisation (ICH) and USP.^{9,10}

Materials and Methods

Materials. Working standards of paracetamol and ibuprofen were a kind gift of Healthcare Pharmaceuticals Limited, Rajendrapur, Bangladesh. HPLC grade acetonitrile was purchased 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 µm, 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.