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Validation of a Stability-Indicating Assay Method for the Determination of Ibuprofen in a Tablet Formulation

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Abstract

Ibuprofen, a derivative of propionic acid, is an anti-inflammatory and analgesic frequently used in the treatment of pain. However, ibuprofen is not immune to counterfeiting. The main objective of this work was to validate a stability-indicating HPLC method for ibuprofen and to apply it to the quality control of a tablet formulation. The chromatographic conditions were a C18 column (125 mm × 4.6 mm, 5 µm), a mobile phase consisting of a mixture of acetonitrile/triethylamine buffer at pH 7.05 (60:40, v/v) at a flow rate of 1.5 ml/min, an injection volume of 10 µl, and UV detection at 220 nm. Validation included linearity, repeatability, accuracy (n = 3) by spiked additions, limit of detection (LOD), and limit of quantification (LOQ). The validated method was used for the analysis of an ibuprofen tablet formulation. Forced degradation studies were performed by exposing the ibuprofen standard solution to acid (HCl; 1 N), base (NaOH; 1 N), and an oxidizing agent (10% H2O2) for 24 to 48 hours. The calibration curve showed a linearity range between 5.33 µg/ml and 16 µg/ml of ibuprofen. The LOD and LOQ were 0.447 µg/ml and 1.356 µg/ml, respectively. The recovery rate expressing accuracy was 100.45%. The repeatability CVs were all less than 2%. The analyzed sample had a compliant ibuprofen content. Forced degradation studies showed degradation of the molecule under basic conditions, with the identification of impurity C. The ibuprofen molecule was stable under acidic and oxidative conditions. The method applied to the analysis of a tablet formulation showed a compliant ibuprofen content of 103.51% and the absence of degradation products.

Keywords

Ibuprofen, Validation, HPLC, Stability-Indicating

1. Introduction

Ibuprofen is a derivative of propionic acid (**Figure 1**) with anti-inflammatory and analgesic properties [1]. It is used as a blood thinner to treat muscle pain, dysmenorrhea, fever, and headaches [2], and in the management of infection with COVID-19 [3] [4]. Ibuprofen is one of the NSAIDs to have received marketing authorization (MA) for the treatment of mild to moderate migraine attacks [5].

Pharmaceutical forms of ibuprofen are widely used for long-lasting pain relief, sometimes lasting up to 8 hours [6] [7]. While generally well-tolerated [8] and considered a safe drug because the human body can metabolize it into its main metabolites and excrete them in the urine [6], it can pose risks when it is of inferior quality or counterfeit. Indeed, like most medications in Africa, ibuprofen is not immune to counterfeiting. In fact, according to the WHO, at least 1 in 10 medicines in low- and middle-income countries are of inferior quality or falsified [9]. This proportion can reach 15% to 30% in Africa, where control and regulatory systems are often fragile [10].

Several analytical techniques have been used in the literature to study this molecule. These studies have been carried out on biological or simulated matrices [11]-[13], in the environment and in drug products alone or in combination with other molecules [14].

The implementation of accurate, reliable, and rapid methods for analyzing ibuprofen-based medications could effectively combat the circulation of counterfeit or substandard drugs. The main objective of this work was to validate an HPLC method indicative of ibuprofen stability and to apply it to the quality control of a tablet formulation.

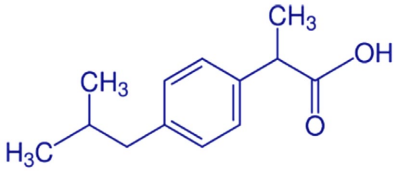

Figure 1. Chemical structure of ibuprofen.

2. Materials and Methods

2.1. Reference Substance and Reagents

The reagents used were analytical grade. They consisted of: Ibuprofen BP purchased from Palghar, India, with a purity of 98%; acetonitrile (≥99.8%); triethylamine (95.5%); orthophosphoric acid (85%); 1N sodium hydroxide; 1N hydrochloric acid; 10% hydrogen peroxide and ultrapure water.

The analyses focused on a specialty containing ibuprofen tablets dosed at 400 mg marketed in Ivory Coast.

2.2. Equipment and Operating Conditions

The apparatus consisted of a HPLC (Waters) system and a precision balance (Mettler

Toledo), a pH meter (seven compact), an oven (prolabo), a UV lamp, an ultrasonic bath (cleaner-TH), millipore filters (Millex-HN nylon 0.45 µm) and classic laboratory glassware.

The reversed-phase liquid chromatography method was performed on a stationary phase consisting of a Nucleodur column octadecylsylanized (125 mm × 4.6 mm, 5 µm). The mobile phase consisted of an acetonitrile/buffer solution mixture at pH 7.05 (60:40, v/v). The buffer solution was composed of HPLC-grade water, triethylamine, and orthophosphoric acid (1000:1:0.5 v/v/v).

The flow rate was set at 1.5 ml/min for an injection volume of 10 µl, the column temperature was maintained at 25˚C and the detection of ibuprofen was carried out in UV at 220 nm.

The analyses were carried out at the National Public Health Laboratory (LNSP).

2.3. Preparation of Solutions

2.3.1. Mobile Phase

The mobile phase consisted of a mixture of acetonitrile and triethylamine buffer solutions at pH 7.05. The buffer solution triethylamine was prepared in a 1000 ml volumetric flask by adding 1 ml of triethylamine to 400 ml of milliliter water, followed by 0.5 ml of orthophosphoric acid. After manual stirring for 30 seconds, the solution was filled to the mark with milliliter of water and then homogenized using ultrasound for 15 minutes. The pH of the solution was then measured using a pH meter and adjusted to 7.05 with orthophosphoric acid. The mobile phase was prepared in a 1000 ml volumetric flask by mixing 600 ml of acetonitrile with 400 ml of pH 7.05 buffer solution (60:40 v/v).

2.3.2. Reference Solution

A quantity of 40.01 mg of ibuprofen was weighed and introduced into a 100 ml volumetric flask to which 70 ml of diluent-1, consisting of a buffer/acetonitrile mixture (20:80 v/v), was added. The mixture was dissolved ultrasonically for 15 min and made up to volume with diluent-1. After homogenization, 5 ml of this preparation (stock solution) was transferred into a 25 ml volumetric flask and made up to the mark with diluent-2, consisting of a buffer/acetonitrile mixture (40:60 v/v).

2.3.3. Preparation of the Solution to Analyze

Twenty tablets were weighed and pulverized using a mortar and pestle. The mass of powder equivalent to 400 mg of ibuprofen was dissolved in a 250 ml volumetric flask with 175 ml of diluent-1 and placed in an ultrasonic bath for 30 minutes with intermittent stirring. The mixture was then made up to volume with diluent-1 and homogenized. Approximately 25 ml of the mixture was centrifuged at 3000 rpm for 5 minutes. Five ml of the supernatant was transferred to a 25 ml volumetric flask, made up to 25 ml with diluent-2, homogenized, filtered, and injected for HPLC analysis.

2.4. Validation Criteria

The process was validated by determining specificity, linearity, precision, accuracy,

limits of detection (LOD) and limits of quantification (LOQ) according to the guidelines of the International Conference on Harmonisation (ICH) [15] and the United States Pharmacopeia (USP) guidelines [16].

The linearity study (n = 5) was performed with reference solutions of concentrations 5.33 µg/ml, 6.857 µg/ml, 9.6 µg/ml, 12 µg/ml, and 16 µg/ml. Two statistical tests were subsequently carried out. These were, on the one hand, the Student's t-test comparing the y-intercept with the null hypothesis (H0: the y-intercept is equal to zero at the risk α = 5%) and, on the other hand, the test for the existence of a significant slope with the null hypothesis (H0 = the slope is significantly different from zero at the risk α = 5%). Repeatability (n = 6) was performed on solutions of concentrations 8 µg/ml and 12 µg/ml. The accuracy of the method was performed on (n = 3) increasing quantities of ibuprofen powder: 800 µg, 1000 µg, and 1200 µg/ml. µg) were added to the initial quantity of 1000 µg.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the analytical curve as defined by ICH [15] [17]. Its formula is as follows:

$$LD = 3.3\sigma/S \text{ and } LQ = 10\sigma/S$$

σ = the standard deviation of the response;

S = the slope of the calibration curve.

2.5. Studies of Forced degradation

Forced degradation studies were undertaken to deliberately degrade the active drug. These studies served to evaluate the analytical method's ability to measure the active ingredient and its degradation products without interference. These degradation studies were performed under acidic, basic, and oxidative conditions.

The standard mixture was exposed to acid (HCl, 1 N), a base (NaOH, 1 N) and an oxidizing agent (H_2O_2, 10%) for 24 to 48 hours [18] [19]. Samples were taken for each type of degradation at 24 hours and 48 hours of exposure.

3. Results and Discussion

The objective of this work was to validate an HPLC method indicative of the stability of ibuprofen and to apply it to the assay of a tablet specialty.

3.1. Validation of the Analysis Method

Ibuprofen was identified (**Figure 2**), using a Nucleodur C18 stationary phase (125 mm × 4.6 mm, 5 µm), with a retention time of 1.28 min; this time is shorter than that obtained with a column Hypersil BDS (150 × 4.6 mm, 5 µm), which was approximately 3.29 minutes [20]. This time difference is due to the column length, which is longer than that used in our study. This time saving will reduce the amount of mobile phase and consequently, the cost of the analysis. The method was specific because no degradation peak interfered with that of ibuprofen, as shown in the chromatogram of the standard ibuprofen solution (**Figure 2**).

Furthermore, a linearity domain was identified for ibuprofen concentrations

between 5.33 µg/ml and 16 µg/ml, with a straight-line equation.

Y = 82320X + 23142, and a coefficient of determination R^2 = 0.9995 (Figure 3). The test for the y-intercept showed that it is not significantly different from 0 at the probability level α = 5% (Table 1). Similarly, the test for the existence of a significant slope showed that the slope b is significantly different from zero (Table 2). The coefficients of variation reflecting repeatability, obtained for the solutions concentrated at 8 µg/ml and 12 µg/ml were less than 2% with respective values of 0.23 and 0.30%. The repeatability of the method is in accordance with ICH guidelines [15].

The limits of detection and quantification were 0.447 µg/ml and 1.356 µg/ml, respectively. The mean percentage recovery, reflecting accuracy, was 100.45% (Table 3). The validation criteria all met ICH requirements and demonstrated satisfactory linearity, precision, and sensitivity for the assay of ibuprofen in tablet dosage forms [17].

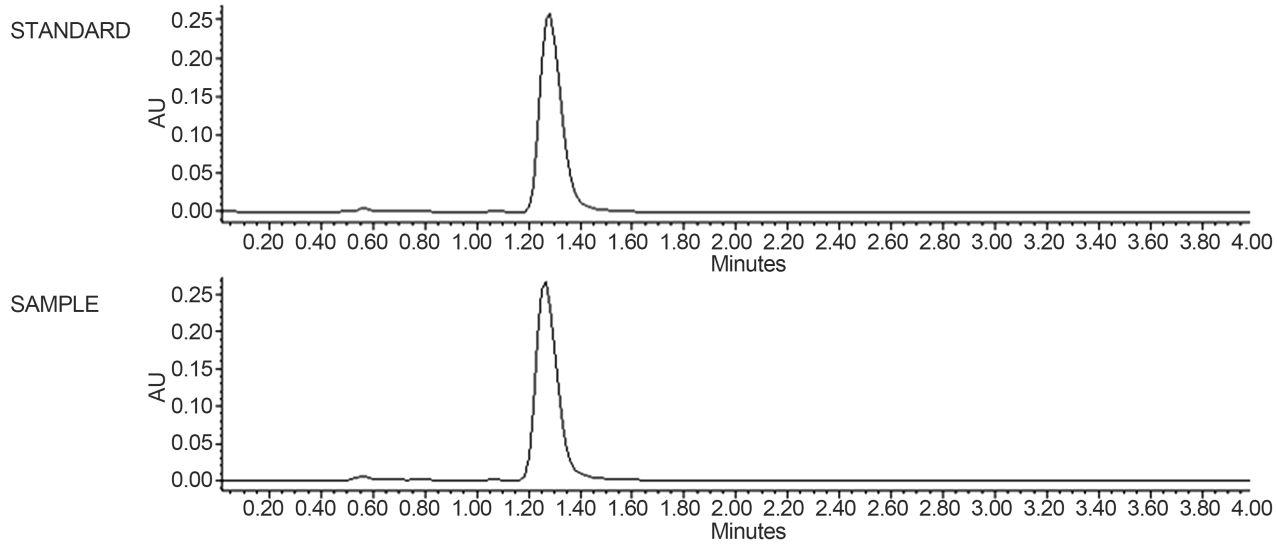

Figure 2. Chromatogram of standard ibuprofen (9.6 µg/ml)/sample ibuprofen.

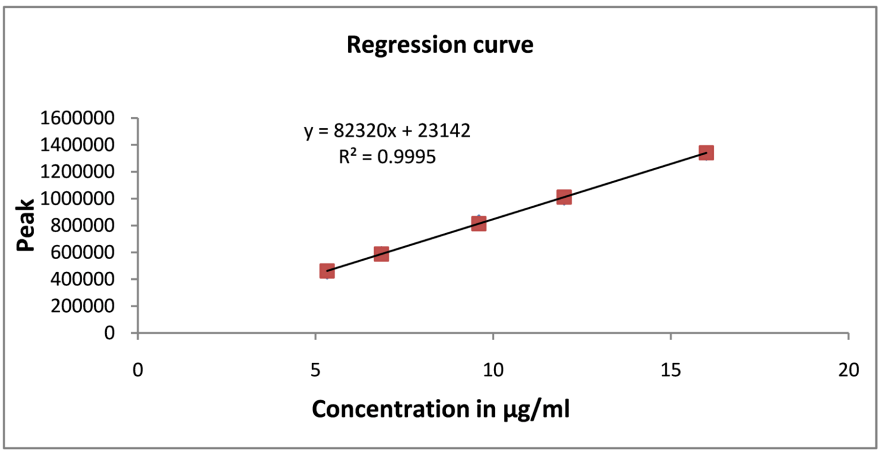

Figure 3. Ibuprofen regression curve.

Table 1. Intercept test.

HAS	Its	t a calculated = 0 − a/S a	t tabulated (0.025; N-2)	t tabulated (0.975; N-2)
12193	6010.63	1.98	2.16	−2.16

t tabulated (0.975; 13) < calculated < tabulated (0.025; 13).

Table 2. Test for the existence of a significant slope.

b	S b	t b calculated = 0 − b/S b	t tabulated (0.025; N-2)	t tabulated (0.975; N-2)
83017	564.14	−143.53	2.16	−2.16

t b calculated < tabulated t (0.975; 13).

Table 3. Accuracy parameters.

Initial ibuprofen quantity (µg)	Quantity of ibuprofen added (µg)	Total measured quantity of ibuprofen (µg)	Recovery rate (%)
1000.0	800.0	1797.3	99.85
1000.0	1000.0	2037.6	101.88
1000.0	1200.0	2191.5	99.61
Average recovery percentage			100.45

3.2. Dosage

The validated method was used for the assay of the drug ibuprofen ubi tablets 400 mg. The ibuprofen content was 103.51% (for n = 3). This value is within the USP specifications of 90% to 110% [16].

3.3. Studies of Forced Degradation

The results of the degradation studies showed that ibuprofen degraded under strong basic conditions (NaOH; 1N). Based on the relative retention time, the ibuprofen-related compound C, described in the United States Pharmacopeia 47—NF 42 [16], was identified at a retention time of 1.7 min after 48 h in the presence of NaOH. Similarly, an unidentified peak was observed at a retention time of 1 min under basic conditions. The degradation results in a basic medium are comparable to those obtained under basic conditions (NaOH; 6N) by Raja *et al.* [18].

Ibuprofen remained relatively stable under acidic (HCl; 1N) and oxidative (H_2O_2; 10%) conditions between 24 and 48 hours of exposure. Rahman *et al.* obtained similar results after simultaneous forced degradation studies of paracetamol and ibuprofen [19]. Furthermore, harsher stress conditions with (H_2O_2; 30 %) for a longer exposure time of 9 days, conducted by Sherri *et al.*, led to the identification of the presence of 4-Isobutylacetophenone (4-IBAP) [21].

When applying the stability indicator method to the analysis of ibuprofen 400 mg tablets under normal conditions, no degradation products were observed.

4. Conclusion

This work validated a high-performance liquid chromatography method for as-

sessing the stability of ibuprofen. Forced degradation studies showed degradation of the molecule under basic conditions, with the identification of the ibuprofen impurity C and an unknown peak (Figure 4). The ibuprofen molecule proved stable under acidic and oxidative conditions. The method applied to the analysis of a tablet sample yielded a compliant ibuprofen content of 103.51% and the absence of degradation products. This validated method is rapid, reliable, and accurate for routine analysis and high-throughput screening of ibuprofen, particularly in resource-limited settings. Its ease of implementation will enable post-market surveillance and the fight against the circulation of substandard or falsified medicines.

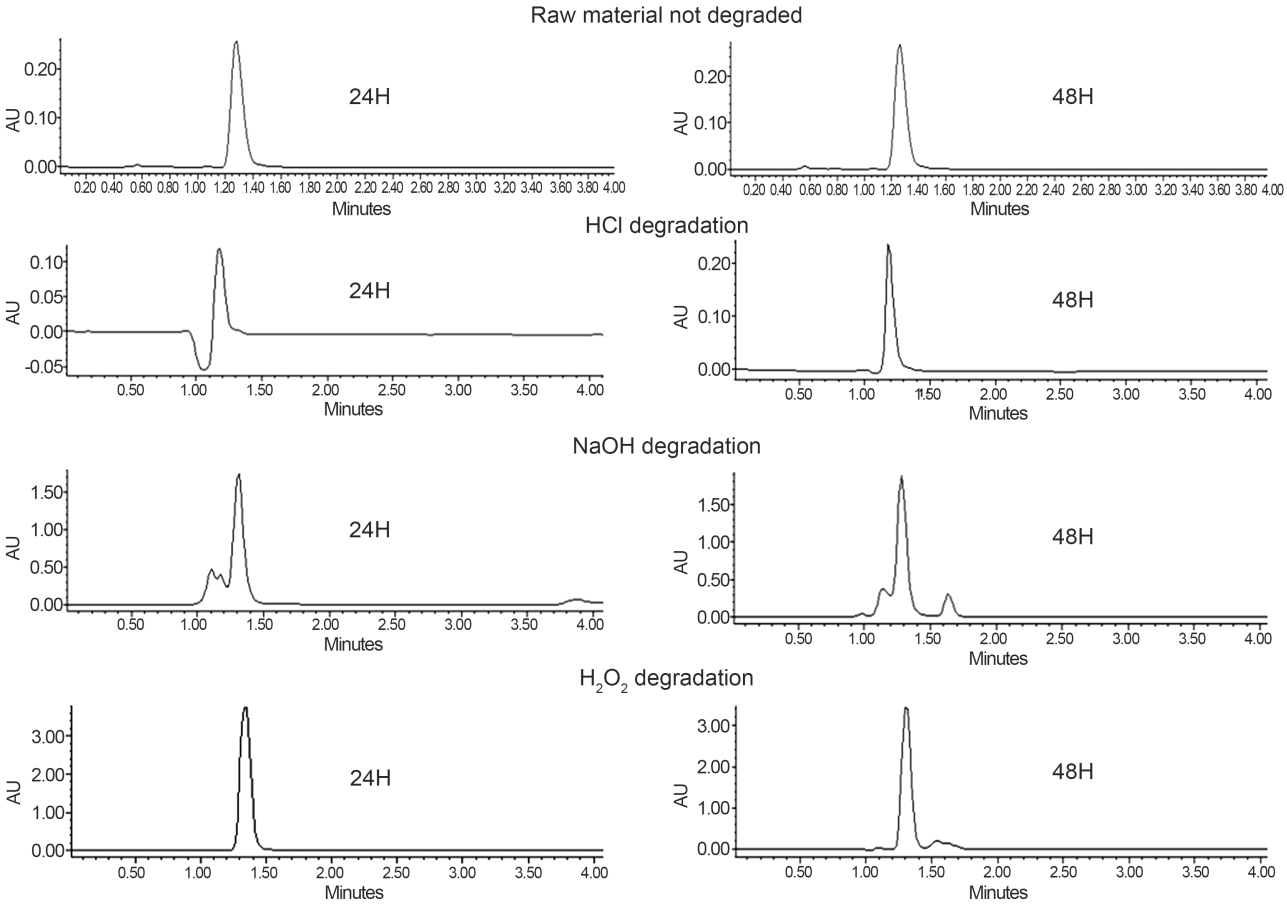

Figure 4. Chromatograms of undegraded ibuprofen/degraded ibuprofen.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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