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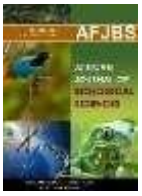

Analytical method validation for related substances of Ibuprofen by HPLC

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ABSTRACT
This work reports on the validation of an analytical technique that uses high-performance liquid chromatography (HPLC) to quantify related ibuprofen compounds. In order to effectively separate ibuprofen from related contaminants, the chromatographic conditions have to be optimized during the technique development process. By examining a conventional combination of ibuprofen and known contaminants, specificity was verified and interference from matrix elements was shown to be absent. Spike-recovery studies were used to evaluate accuracy and linearity across an appropriate concentration range. Studies on precision showed mediocre precision and good repeatability. To evaluate sensitivity, limits of quantification and detection were established. By purposefully introducing differences in chromatographic parameters was assessed. Studies on forced degradation were carried out to verify the method's specificity. The validation results show that the developed HPLC technique is sensitive, specific, accurate, and exact for quantifying related ibuprofen compounds, making it appropriate for use in pharmaceutical analysis and quality control.

Key words: Ibuprofen, HPLC, Specificity, Method development, Validation, ICH guidelines

Introduction

One common nonsteroidal anti-inflammatory medicine (NSAID) that is well-known for its ability to reduce pain and inflammation is ibuprofen. On the other hand, the stability, safety, and effectiveness of pharmaceutical formulations might be impacted by the inclusion of related chemicals or contaminants. To ensure the quality and safety of pharmaceutical goods, it is imperative to design and validate an analytical method for the measurement of associated components of ibuprofen. Because of its high sensitivity, selectivity, and reproducibility, high-performance liquid chromatography (HPLC) is a frequently used technology for the study of pharmaceutical substances. The objective of this investigation was to create and verify an HPLC technique for measuring ibuprofen-related compounds. Under regulatory requirements like those provided by the international council for harmonization (ICH), the method's specificity, linearity, accuracy, precision

and sensitivity must be demonstrated as part of the validation process. The ability of the procedure to precisely distinguish ibuprofen from similar contaminants and other elements in the sample matrix is ensured by specificity. Over a predetermined concentration range, linearity defines the connection between analyte concentration and detector response. Spike-recovery tests are frequently used to determine the true values, from which the accuracy of the measured values is evaluated. Precision assesses the repeatability and intermediate precision of the procedure in various scenarios. The boundaries of detection and quantification define sensitivity.

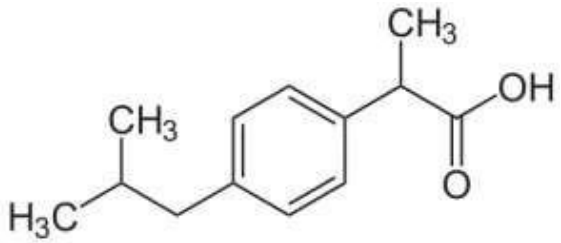

Figure. 1: Chemical structure for Ibuprofen.

Method description

Chromatographic conditions

High performance liquid chromatography systems (Shimadzu) with PDA detector, Analytical Balance, were used. The HPLC (Shimadzu) system equipped with PDA detector, Xterra MS C18, (150 mm x 4.6 mm, 5 µm) was used to achieve chromatographic separation. Mobile phase was composed of orthophosphoric acid, Acetonitrile and sufficient water. Degassed and injected onto the column at 2.0 ml/min flow rate. Load volume of the drug solution was 20 µl and the run time was 85.01min. The column temperature was ambient (25°C).

Preparation

Mobile phase A

About 0.5 ml of orthophosphoric acid, 300 ml of acetonitrile and sufficient water to produce 1000ml with water. This solution was used as mobile phase A.

Mobile phase B: Acetonitrile was used as mobile phase B

Diluent (Blank)

Mobile phase B and mobile phase A in the ratio of (20:80) V/V was mixed. This solution was used as diluent

Gradient programme:

Time	Mobile phase – A (% v/v)	Mobile phase – B (% v/v)
0	100	0
25	100	0
55	15	85
70	15	85
71	100	0
85	100	0
85.01	100	0

Solution standard

Ibuprofen WS/BPCRS was weighed and transferred, 70 ml of diluent was add to the 100 ml volumetric flask. This solution was sonicate for 5 min to dissolve it. Next, more diluent was added to the flask to reach the desired volume and the solution was thoroughly mixed. 5.0 ml diluent was add of the aforementioned solution to 100 ml, then it was thoroughly mixed. Further the solution was dilute 4.0 ml to 50 ml by using diluent and thoroughly mixed.

Placebo solution

About 336.9 mg of placebo or 0.2 g of ibuprofen were weighed,, into a 100 ml volumetric flask. Then, 20 ml of acetonitrile was added and the solution was thoroughly shake. After discarding 1 ml, mobile phase A was added to made up the volume and filter through whatman GF/C.

Solution sample

20 capsules were taken, split them open and place the medication in a dried, clean petri dish. the ingredients was combined, weigh approximately 536.9 mg (or 0.2 g of Ibuprofen) of medication in a 100 ml volumetric flask, 20 ml of acetonitrile was added and thoroughly shake. After 1 ml was discarding and mobile phase A was add to made up the volume and filter through whatman GF/C.

Impurity B stock solution for ibuprofen

In a 100 ml volumetric flask, precisely 6.0 mg of ibuprofen impurity B WS/BPCRS was weighed. Then, add 50 ml of acetonitrile was added and shake thoroughly to dissolve. Acetonitrile was used to made up for the volume.

System suitability solution

Accurately 20.0 mg of Ibuprofen WS/BPCRS was weighed, then dissolve it with 2 ml of acetonitrile in a 10 ml volumetric flask. Then add 1 ml of the Ibuprofen Impurity B WS/BPCRS stock solution was shake. Use mobile phase A to bring the volume up to par.

Specificity

Specificity is the ability of the method to measure the analyte in the presence of matrix components. Demonstrate the specificity by identification of analyte, blank and placebo interference and peak purity of analyte. The following solutions were prepared and injected into HPLC system to establish specificity blank, placebo, standard solution, sample solution and spiked sample solution.

Table 1: Known impurities for unspiked sample preparation

System suitability parameters		
Observation	Retention time	Peak purity index
Impurity-1	36.5	1.000
Impurity-E	35.8	1.000
Impurity-A	33.6	0.999
Impurity-J	7.2	1.000
Impurity-N	11.3	0.999
Ibuprofen sorbitol ester	8.0	1.000
Ibuprofen sorbitol ester	15.5	0.999
Acceptance criteria	NA	NLT0.99

Table 2: Known impurities for spiked sample preparation

System suitability parameters		
Observation	Retention time	Peak purity index
Impurity-1	36.4	1.000
Impurity-E	35.7	1.000
Impurity-A	33.5	1.000
Impurity-J	7.2	1.000
Impurity-N	11.4	1.000
Ibuprofen sorbitol ester	8.0	1.000
Ibuprofen sorbitan ester	15.5	1.000
Acceptance criteria	NA	NLT0.99

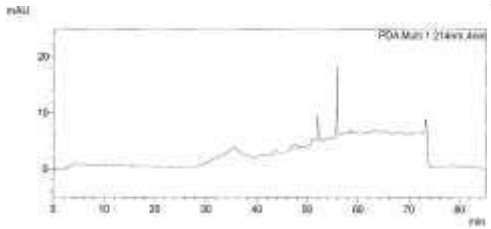

Figure. 2: Specificity Blank.

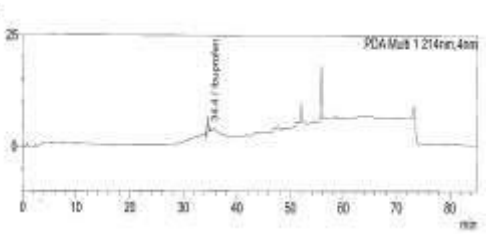

Figure. 3: Specificity Standard.

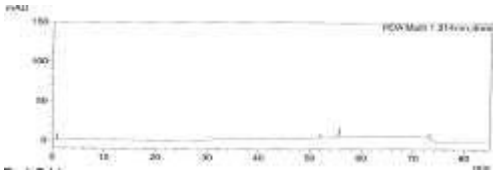

Figure. 4: Specificity Placebo.

Linearity and range

Linearity

The capacity of an analytical method to produce results that are exactly proportionate to the concentration (amount) of the analyte in the sample, within a specified range, is known as linearity.

Range

It is the range between the maximum and minimum analyte concentration for which the analytical method's precision, accuracy, and linearity have been shown to be appropriate. LOQ, 50.0%, 75.0%, 100.0%, 125.0%, and 150.0% of the working concentration of impurity-A, J, N, and E, as well as ibuprofen, ibuprofen sorbitol ester, and ibuprofen sorbitan ester, were all performed linearly. calculated the slope, y-intercept, and correlation coefficient after recording the average area for each level. Plotted the graph with the area response on the Y-axis and the corresponding analyte peak concentration on the X-axis.

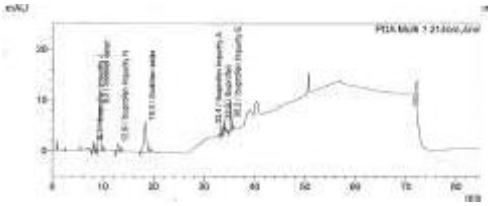

Figure. 5: Linearity-50%.

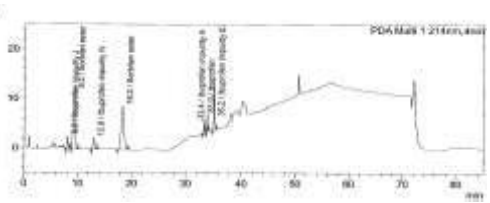

Figure. 6: Linearity-75%.

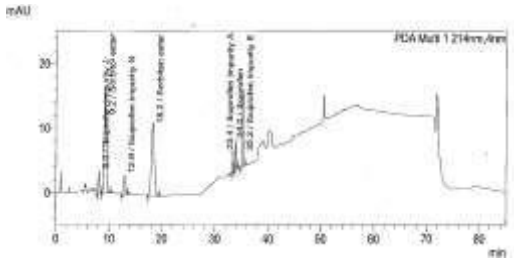

Figure. 7: Linearity-100%

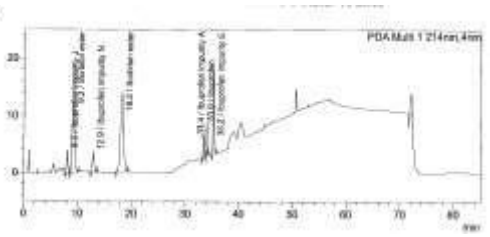

Figure. 8: Linearity-125%.

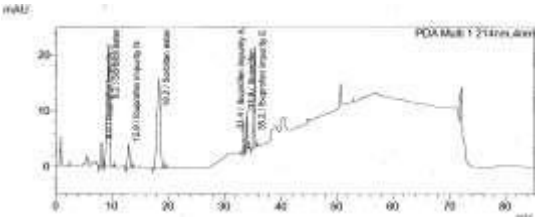

Figure. 9 Linearity-150%.

Accuracy

The degree to which test results produced by the method closely resemble the genuine value is known as accuracy. The percentage recovery by the content of known, introduced amounts of analyte is a common way to express accuracy. A measure of an analytical method's exactness is its accuracy. The '4' concentration (LOQ, 50.0%, 100.0%, and 150.0%) was used to evaluate accuracy. Standard and spiked sample solutions are made according to protocol, with concentrations of 50%, 100%, 150%, and LOQ. The area acquired for every concentration is used to compute the percentage of recovery. The information is provided below.

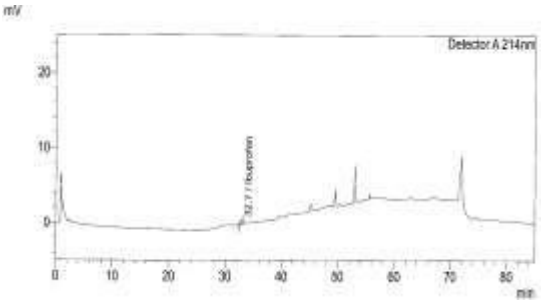

Figure.10: Accuracy LOQ (Ibuprofen).

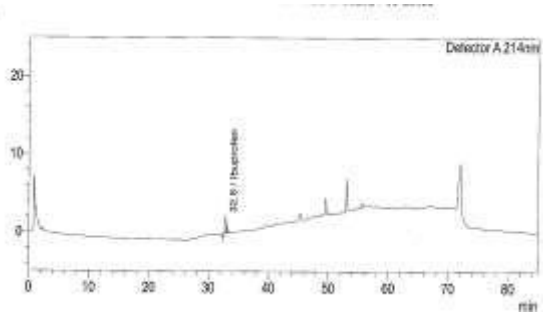

Figure. 11: Accuracy 50% (Ibuprofen).

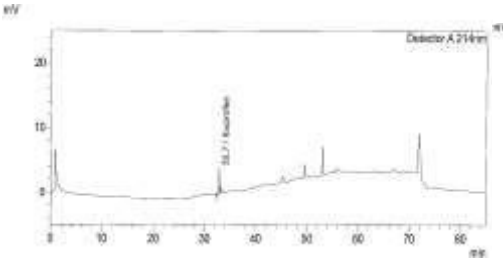

Figure.12: Accuracy 100% (Ibuprofen).

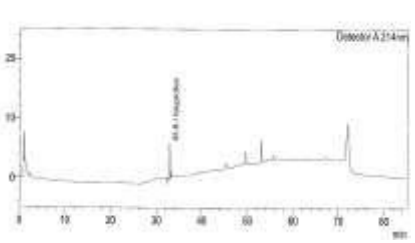

Figure.13: Accuracy 150% (Ibuprofen).

Table 3: Accuracy result

Sample	Amount taken		Area	Amount added (in %)	Amount recovered (in %)	% Recovery	Average	%RSD
	Ibuprofen taken for standard stock (in mg)	Placebo added (in mg)						
LOQ -1	50.29	172.46	7290	0.0240	0.0257	107.0833	111.2	3.2
LOQ -2		167.50	7686		0.0271	112.9166		
LOQ -3		171.29	7729		0.0273	113.7500		

50%		167.97	30009	0.1001	0.1061	105.9940	106.3	0.5
100%		170.94	52501	0.2003	0.1856	92.6610	93.5	1.5
150%		166.45	79152	0.3005	0.2798	93.1114	93.4	0.5

Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samples of homogeneous sample. The precision of the analytical method is determined by estimating impurities for 6 aliquots of homogeneous sample.

System Precision

Ibuprofen Standard Solution was produced according to protocol and injected into the HPLC system in six repetitions. The system suitability parameters were determined in accordance with protocol, and the tabulated results are displayed below.

Table 4: For Ibuprofen

No of Injection	RT (Minute)	Area
01	33.7	106468
02	33.7	105969
03	33.7	106763
04	33.7	107341
05	33.7	105591
06	33.7	107208
Average	33.7	106557
SD	0	688.1443
%RSD	0	0.6

Method precision

Six replicated spiked and two replicated unspiked sample solutions of 200 mg ibuprofen capsules were produced and injected into the HPLC system in accordance with protocol. The precision parameters of the method are determined in accordance with the protocol, and the tabulated results are displayed below.

6. Stability of analytical solution

By injecting the standard and sample solutions for up to 48 h, the stability of the solution was shown. The area of the standard and sample solutions' percentage RSD was computed.

Table 5: Stability report

Time interval	Area		
	Standard	Unspiked Sample	Spiked Sample
Initial	100305	81924929	79178151
12th Hours	100678	82104162	79124695
24th Hours	101579	81983054	79196715
36th Hours	101846	82182110	79312418

48th Hours	100624	82164995	79271824
60th Hours	100759	82232230	79312903
72th Hours	101456	82168198	79195218
84th Hours	101399	82330358	78857799
96th Hours	102961	82349723	79414479
Average	101289	82159973	79207134
SD	813.5	141643	157865
%RSD	0.8	0.1	0.1

Discussion

The creation and approval of analytical techniques for the quantification of pharmaceutical compounds are essential steps in ensuring the reliability and accuracy of drug analysis. In this review, we discuss the method development and validation processes specifically pertaining to the analysis of ibuprofen, a widely used non-steroidal anti-inflammatory drug (NSAID), in various matrices. Method development for ibuprofen analysis typically involves optimizing chromatographic conditions, including the selection of stationary and mobile phases, column dimensions, and detection parameters. High performance liquid chromatography (HPLC) is the most commonly employed technique due to its sensitivity, selectivity, and versatility. Overall, the development and validation of analytical methods for ibuprofen analysis are critical for ensuring the accuracy, reliability, and reproducibility of drug quantification in pharmaceutical formulations, biological samples, and other matrices. By employing rigorous method development and validation protocols, researchers and analysts can confidently assess the quality and potency of ibuprofen-containing products, contributing to safe and effective drug therapy for patients.

Conclusion

Although it works well, use cautious because of possible side effects, especially gastrointestinal irritation, ulceration, and bleeding. Vulnerable groups should be given extra attention, including the elderly, people with a history of gastrointestinal problems, and people who are taking other medications that could interact with ibuprofen. To maximize ibuprofen's advantages and minimize its hazards, proper dose, adherence to specified treatment durations, and knowledge of contraindications and drug interactions are crucial. When it comes to monitoring for potential negative effects and educating patients on the safe and appropriate use of ibuprofen, healthcare professionals are essential. Overall, when taken sensibly and in accordance with medical advice, ibuprofen continues to be a useful tool for treating pain and lowering temperature. Its ongoing use emphasizes how crucial it is to weigh treatment advantages against possible hazards in order to provide patients with the best possible results.

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