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THE DEVELOPMENT AND VALIDATION OF A NOVEL GAS CHROMATOGRAPHIC TECHNIQUE FOR THE SIMULTANEOUS DETECTION OF IBUPROFEN

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ABSTRACT:

Analgesic, anti-inflammatory, and antipyretic effects are all present in the NSAID ibuprofen. Ibuprofen prescriptions are frequently given in clinical settings. The initial work reporting is here. For the purpose of determining ibuprofen, a high-performance liquid chromatography method was created and approved. Ibuprofen-containing compounds may be subject to quality control using this technique. By carefully tuning chromatographic parameters such flow rate, column, detection wavelength, column temperature, buffer solution, and gradient elution, this method was effectively developed. The separation effectiveness and robustness of an Agilent Optima-17ms (250 x 4.6mm, 0.5 m particle size) column were taken into consideration. For detection, a photodiode-array detector was employed. In accordance with the recommendations of the International Conference on Harmonization (ICH), this method was validated to establish its superiority and stability.

***Keywords**: Liquid chromatography, Mass spectrometry, Ibuprofen, Method development and validation.*

INTRODUCTION

Ibuprofen is a commonly used over-the-counter medication that works to reduce pain, fever, and inflammation. It belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs), which are used to treat a variety of conditions related to pain, fever, and inflammation, such as arthritis, menstrual cramps, headaches, and muscle aches. It is also used to reduce fever, which can be caused by illnesses like the common cold, flu, and other infections [1]. Ibuprofen is available in many different forms, including tablets, capsules, liquid suspensions, and gels. It is generally well-tolerated, but it can cause side effects, including stomach upset, dizziness, and headache [2].

Ibuprofen is a chemical compound that is 2[4-(2-methyl propyl) phenyl] propanoic acid. $C_{13}H_{18}O_2$ is the structural formula, and the molecular weight is 206 [3]. Figure 1 depicts the structure and other characteristics of Ibuprofen.

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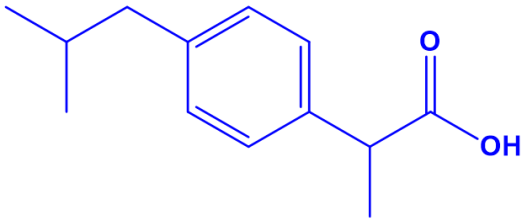

Chemical Name	2-(4-isobutylphenyl)propanoic acid
Chemical formula	$C_{13}H_{18}O_2$
Molecular weight	206.28
Melting Point	76 °C
Solubility	Methanol
Elemental Analysis	C: 75.69; H: 8.80; O: 15.51

Figure 1. Chemical structure and other details of Ibuprofen.

Various analytical techniques, including HPLC, GC, GCMS, and LCMS, have been used to quantify enantiomers of ibuprofen, other structurally related NSAIDs such as ketoprofen, and their metabolites in biological and environmental samples. Pentafluorobenzyl bromide (PFB-Br) has been used to derivatize the carboxylic group of both endogenous and exogenous compounds such as fatty acids and acetylsalicylic acid. PFB-Br has also been used to esterify ibuprofen in human serum and analyze it with GC and electron capture detection [4, 5]. Ibuprofen in environmental samples such as surface water and sewage has been esterified with PFB-Br and analysed by GCMS in electron-capture negative-ion chemical ionization mode. In addition, ibuprofen and other NSAIDs have been analysed in biological samples by various methods, including LCMS[6].

This research study aimed to explore the critical steps that can influence the data quality when developing a LCMS method to identify and validate Ibuprofen drug. Quality by Design was emphasized to ensure the dependability and robustness of the method once applied in practice for pharmacokinetic and bioequivalent studies. In order to have good recovery values and clean extracts, the sample preparation step was critically assessed. Finally, the EMA guideline for bioanalytical method validation was used to validate the method. This was confirmed by determining the concentrations of ibuprofen in human plasma after a healthy volunteer ingested an ibuprofen capsule.

EXPERIMENTAL

Chemicals and materials

Acetonitrile, Methanol, Ethanol, and Acetic Acid from Merck India Limited Company were utilised as HPLC grade solvents. The reference standard for ibuprofen was bought from Sigma-Aldrich. Additionally, SRL Limited employed formic acid of the analytical grade.

Solution Preparation

Ibuprofen stock solution was made by combining 10 mg of the drug with 963 litres of methanol to form an initial solution of 2.1 mM ibuprofen in methanol. This produced a 50 mM ibuprofen solution, of which an aliquot was evaporated in acetonitrile to produce a 2.1 mM ibuprofen solution [7]. Both the 2.1 mM ibuprofen solution in acetonitrile and the 50 mM ibuprofen solution in

methanol were kept at -25°C until they were needed. As needed, the 2.1 mM ibuprofen solution was diluted with acetonitrile.

Instrumentation

A Shimadzu LCMS system with a 515 pump, photodiode-array detector, and a 5 µm particle size column was employed to enhance separation and quantification of impurities. The Optima-17ms column was 250 mm long and 4.6 mm wide and the mobile phase consisted of 90% methanol and 10% water containing 0.1% (v/v) acetic acid.

LCMS conditions

At 25°C, a chromatographic separation was carried out using the Phenomenex-purchased Optima-17ms (250 x 4.6mm, 0.5µm particle size). The mobile phase contained acetic acid at a concentration of 0.1% (v/v), methanol at an 80:20 ratio, and water. The autosampler temperature was set at 15°C, the flow rate was set at 0.5 mL/min, the injection volume was 15 µL, and the chromatographic run time was 18 minutes. Methanol and water were used as the sample solvent in a 60:40 (v/v) ratio.

The mass spectrometer was set in the selected reaction monitoring mode by utilizing negative electrospray ionization. The parameters for this analysis included an oven temperature program with 1 minute at 60°C, followed by an increase to 280°C at a rate of 20°C/min, and a hold of 2 minutes at 280°C. The interface and ion-source were maintained at 280°C and 160°C, respectively, with electron energy at 65 eV and electron current at 280 A.

Method validation

The method validation for detecting Ibuprofen was done referring to the United State (US) Food and Drug Administration (FDA) guidelines on chemical method validation. The linearity, accuracy, precision, specificity, robustness, and detection limit of the analytical method were all evaluated as part of the validation study. Ibuprofen standards were examined at various concentrations to determine the linearity of the procedure. Analyzing Ibuprofen samples at three different concentrations allowed researchers to evaluate the method's accuracy, precision, and specificity.

RESULTS AND DISCUSSION

A LCMS method was developed for the determination of Ibuprofen drug. To make this method optimal, parameters such as column, temperature, flow rate, pH of buffer, and detection wavelength were examined. The goal of this method was to be precise, accurate, reproducible, specific, and robust enough to be used in a quality control laboratory. Optima-17ms (250 x 4.6mm, 0.5µm particle size) column provided the best results. Ammonium formate was used as a

component of mobile phase in ESI(-) mode, and the addition of acetic acid in methanol/water mobile phase allowed for the separation of Ibuprofen drug.

The ESI results showed that ibuprofen could be ionized in both positive and negative modes, with the latter providing the highest sensitivity. The LC analysis of the mixture indicated that negative ion compounds eluted in the 8–15-minute timeframe. Figure 2 shows the chromatogram for the compounds' separation, and the method was able to analyze the compounds without interference from other sources. An automated report was generated with the optimal settings for the SRM method, and the compound's mass spectra is seen in Figure 3.

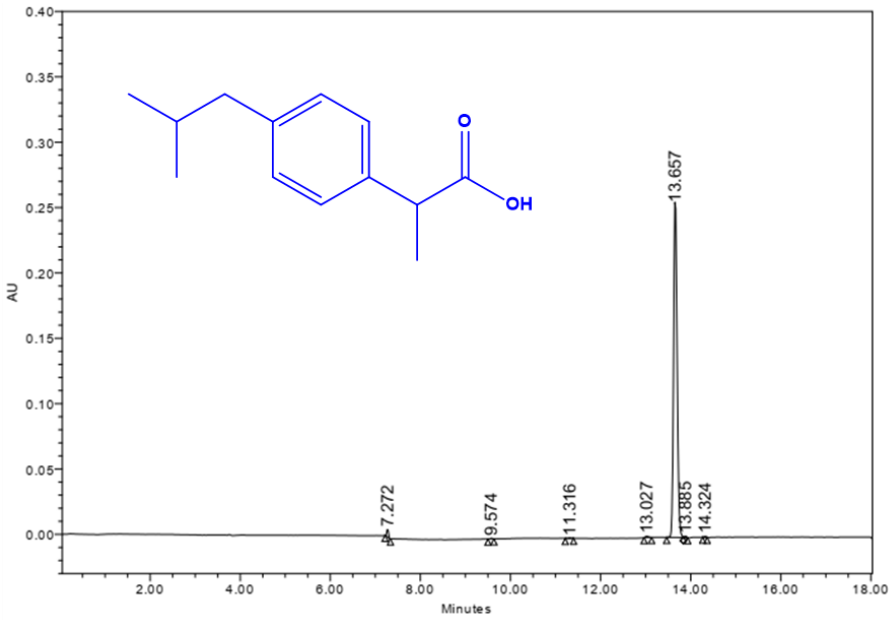

Figure 2. Chromatogram of Ibuprofen.

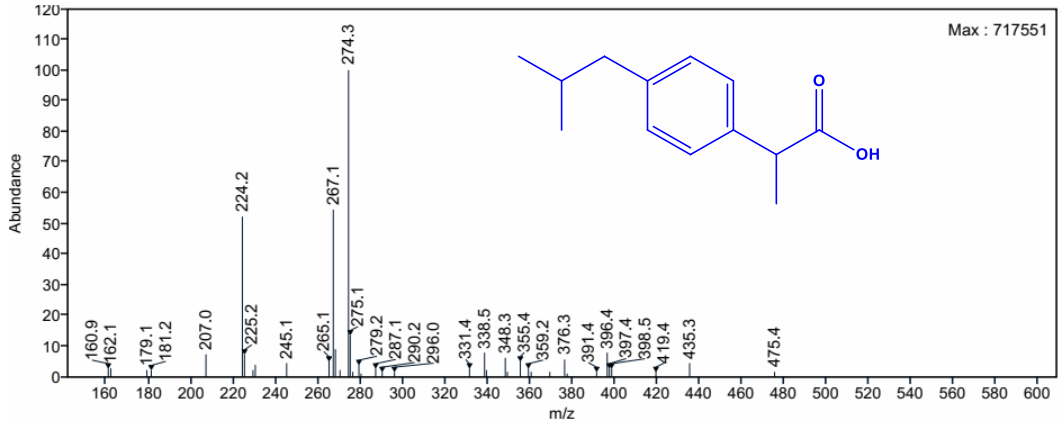

Figure 3. Mass spectra of Ibuprofen.

The method's linearity was determined at various concentration levels, and the optimum findings for ibuprofen are shown in Table 1. The calibration curves were linear, and the correlation coefficient was calculated to be 0.999. The calibration curve's slope and intercept were y = 0.0083x + 0.003. The results reveal that there is an excellent association between response factor and drug concentration within the concentration range specified above.

Table 1. Method validation Results

Parameters	**Ibuprofen drug**
Equation y= mx + c	y=0.0083x + 0.003
Correlation coefficient	0.999
LOD	1.3471
LOQ	4.2536
Accuracy	0.46
Precision	97.86

The developed LCMS method was used to inject standard solutions at progressively lower concentrations in order to ascertain the Limit of Detection (LOD) and Limit of Quantification (LOQ) of the method. According to Table 1's LOD and LOQ, all examined substances are observable and measurable at the microgram per millilitre level. Ibuprofen's LOD and LOQ were determined to be 1.3471 micrograms and 4.2536 micrograms per millilitre, respectively.

The technique exhibits good chromatographic selectivity and specificity. All analytes in the matrix had no detectable interference peaks at the RT. Ibuprofen's intra-day accuracy and precision were discovered to be 0.46% and 97.86%, respectively. These results for accuracy and precision demonstrated that the devised approach is dependable for quantifying ibuprofen and fell within acceptable standards.

After the development and validation of the liquid chromatographic method, the ^{1}H-NMR spectroscopy was used to confirm the structure of ibuprofen. Ibuprofen's NMR spectra are displayed in Figure 4. The Figure demonstrates that the results are in good agreement with the literature reports that supported Ibuprofen's structural integrity.

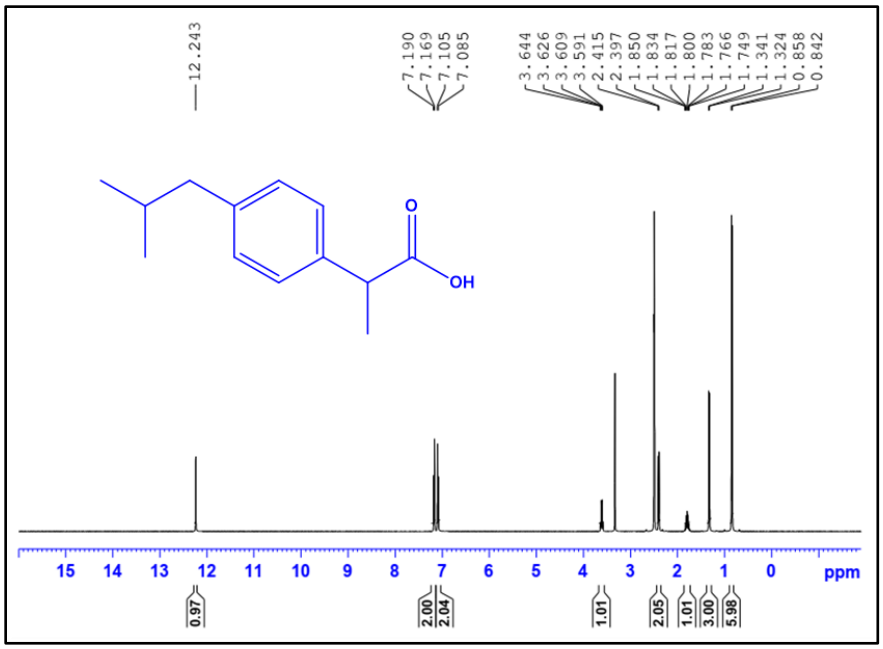

Figure 4. ^{1}H-NMR spectra of Ibuprofen.

CONCLUSIONS

In the current study, we created and validated a straightforward gas chromatographic method for determining the medication Ibuprofen. This method was successfully created by optimizing chromatographic parameters such flow rate, column, detection wavelength, column temperature, buffer solution, and gradient elution. It was sensitive, accurate, precise, and robust for the determination of the medication ibuprofen. In accordance with ICH recommendations, this approach was validated. Both the pharmaceutical sector and research can make use of this technique.

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