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Determination of Ibuprofen in Pharmaceutical Preparations by UPLC-MS/MS Method

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Keywords
Ibuprofen, UPLC-MS/MS, Pharmaceutical analysis

Abstract: Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug that is extensively prescribed. For the determination of IBU in pharmaceutical formulations, a sensitive, simple, accurate, and rapid ultra-performance liquid chromatography method in combination with tandem triple quadruple mass spectrometry (UPLC-MS/MS) has been used and validated. The chromatographic separation was accomplished using a C_{18} UPLC column, 95 Å, 2.1 x 50 mm, 1.8 µm, and 0.1 percent formic acid in conjunction with acetonitrile (25:75 v/v. The flow rate was 0.15 ml min^{-1}, with a run duration of 2.0 minutes. The injection volume was 5 µL and the column temperature was held constant at 40 °C.The mass transitions of İbuprofen and IS were m/z 205.0 → 159.0 and 249.9 → 228.9, respectivelty. According to ICH guidelines, the approach was thoroughly verified. The linear range 1-5000 ng mL^{-1} calibration curve has a strong correlation coefficient (0.9921). Within and between days precision were expressed as relative standard deviation and were lower than 6.24%. This method has been used to determine IBU in both pure form and pharmaceutical formulations with great success.

İbuprofenin Farmasötik Preperatlarda UPLC-MS/MS Yöntemiyle Tayini

Anahtar Kelimeler
İbuprofen, UPLC-MS/MS, Farmasötik analiz

Öz: İbuprofen (IBU), yaygın olarak reçete edilen, steroid olmayan bir anti-inflamatuar ilaçtır. Farmasötik formülasyonlarda IBU'nun belirlenmesi için tandem üçlü dörtlü kütle spektrometrisi ile kombinasyon halinde hassas, basit, doğru ve hızlı ultra performanslı sıvı kromatografi yöntemi (UPLC-MS/MS) yöntemi kullanılmış ve doğrulanmıştır. Kromatografik ayırma, bir C18 UPLC kolonu, 95 Å, 2.1 x 50 mm, 1.8 µm ve asetonitril ile birlikte yüzde 0.1'lik formik asit (25:75 v/v) kullanılarak gerçekleştirildi. Akış hızı, 2.0 dakikalık bir çalışma süresi ile 0.15 ml dak^{-1} idi. Enjeksiyon hacmi 5 µL ve kolon sıcaklığı 40 °C'de sabit tutuldu. İbuprofen ve IS kütle geçişleri sırasıyla m/z 205.0 → 159.0 ve 249.9 → 228.9 dur. ICH yönergelerine göre, yaklaşım tamamen doğrulandı. 1-5000 ng mL^{-1} kalibrasyon eğrisi doğrusal aralığı, güçlü bir korelasyon katsayısına (0.9921) sahipti. Gün içi ve günler arası kesinlik, bağıl standart sapma olarak ifade edildi ve %6.24'ten düşüktü. Bu yöntem, hem saf formda hem de farmasötik formülasyonlarda IBU'yu büyük bir başarıyla belirlemek için kullanılmıştır.

1. INTRODUCTION

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug that is commonly used. By inhibiting the cyclooxygenase-2 enzyme, it primarily acts as an anti-inflammatory, antipyretic, and analgesic drug [1, 2]. IBU is commonly used to alleviate fever, pain, and inflammation in premature newborns, as well as to treat patent ductus arteriosus [3]. IBU takes 1-3 hours to achieve its apparent maximum concentration (Tmax), which is rapidly absorbed after oral intake, and maximum concentrations (Cmax) vary from 50-100 µg mL^{-1}. [4]. Ibuprofen has the chemical formula $C_{13}H_{18}O_2$ and the name (RS)-2-[4-(2-methylpropyl) phenyl] propanoic acid [5]. The chemical structure of IBU is shown in Figure 1. [6].

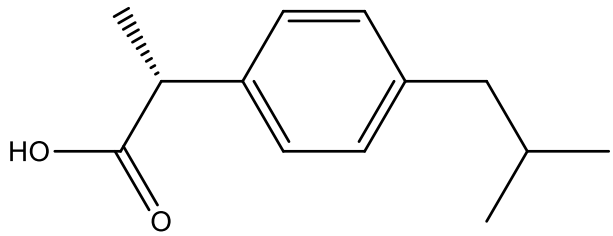

Figure 1. The chemical structure of Ibuprofen

According to a survey of the literature, no research have been done utilizing the UPLC-MS/MS technique to determine the quantity of IBU in pharmaceutical formulations. There have been research that used the LC-MS/MS approach to determine IBU from plasma. To determine the amount of IBU in pharmaceutical preparations, analytical studies have been conducted using the high-performance liquid chromatography (HPLC) method [7-9], capillary electrophoresis [10], HPTLC [2, 11, 12], and spectrofluorimetry [13, 14]. However, the analysis time in these research are lengthy, and the pre-analytical preparation procedures are time-consuming.

Ultra-high performance liquid chromatography (UPLC) is an upgraded derivative of HPLC systems with high-quality tiny porous packing material and the capacity to operate at extremely high pressures. Higher pressure capability and smaller particles in the stationary phase allow for enhanced efficiency and sensitivity, as well as quicker chromatographic analysis, thanks to sharper and higher peaks. As a result, the key benefits of UPLC systems include improved resolution and, more importantly, a large decrease in processing time [15-18]. Furthermore, compared to HPLC, mass spectrometric approaches can give higher sensitivity and specificity [3].

The study's goal was to develop a fast and sensitive ultra-performance liquid chromatography tandem mass spectrometer (UPLC-MS/MS) method for determining IBU in pharmaceutical preparations and pure form with high recovery and short run-time, as well as to validate the method according to ICH Q2(R1) guideline. The developed method was successfully applied in the analysis of IBU-containing tablet dosage forms after test scenarios were completed.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Novagenix Company (Ankara, Turkey) provided IBU with a purity of >99 percent and Erdostein (IS, purity>99 percent). Methanol and acetonitrile hyper grade for LC-MS systems were being provided by Merck (Darmstadt, Germany). Synergy® UV Water Purification System was used to make deionized water on a regular basis (Merck Millipore, Darmstadt, Germany). As a result, all of the other compounds were analytical grade and could be utilized without additional purification.

2.2. Instrumentation and Operation Conditions

An ultra-high-performance liquid chromatography system (UHPLC, 1290 Series, Agilent Technologies, Santa Clara, CA, USA) was employed to analyze the samples, which was linked to an Agilent 6490 Triple Quadrupole mass spectrometer (Agilent Technologies), which contained a triple quadrupole mass spectrometer, a degasser, an autosampler, a column compartment, as well as a binary pump. The autosampler tray temperature was kept at 10 degrees Celsius, and the reserved-phase C18 UPLC column (ZORBAX RRHD Eclipse Plus C18, 95, 2.1 x 50 mm, 1.8 m, Agilent Technologies, Loveland, CO, USA) was kept at 40 degrees Celsius was used to achieving Chromatographic separation. The samples were separated using isocratic elution with a mobile phase of 0.1 percent formic acid solution and acetonitrile (25:75, volume/volume). The mobile phase was processed using a 0.45 µm Millipore membrane filter before use. With a run time of 2.0 minutes, the flow velocity was 0.15 mL min^{-1}. The injection volume was 5 µL and the column temperature was kept fixed at 40°C for symmetrical peaks.

A mass spectrometer with a Jet Stream electrospray ion source interface was used in both negative and positive ionization mode in the mass range of 50–250 Da. The desolvation gas (1000 L h^{-1}) and cone gas (50 L h^{-1}) were both nitrogen. Capillary voltage of 2.0 kV, source temperature of 250 °C, and nebulizer pressure of 35 psi were used to determine the ion monitoring conditions. To conduct quantitative analysis, multiple reaction monitoring (MRM) modes of m/z 205.0→ 159.0 for IBU and 249.9→ 228.9 m/z for IS were used.

In methanol, stock solutions of IBU (1000 µg mL^{-1}) and IS (1000 µg mL^{-1}) were made. The IS 5000 ng/mL working standard solution was prepared by dilution with methanol from the IS stock solution; working solutions for calibration and controls were prepared similarly from stock solutions using methanol diluent. All of the solutions were kept at -20 degrees Celsius and warmed to room temperature before being used.

2.3. Selection of Internal Standard

We employed erdosteine as IS for IBU in a prior investigation. For both materials, mass and chromatographic settings were optimized [19]. We used erdosteine as an internal standard for IBU in this study and validated the approach we developed for IBU.

2.4. Preparation of Standards and Quality Control (QC) Samples

In methanol, IBU (1000 µg mL^{-1}) and IS (1000 µg mL^{-1}) stock solutions were produced. By diluting the stock solutions with methanol, the IBU and IS working standard solutions were created. Using methanol, further dilutions of working solutions for calibration and controls were generated from stock solutions. Working solutions were kept at -20 °C until they were needed and

then raised to room temperature. The solutions for quality control were produced on a daily basis.

2.5. Method Validation

Validation was conducted out in accordance with ICH Q2(R1) guidelines to ensure the analytical method's performance [20].

2.5.1. Selectivity and specificity

Figure 2 shows representative MRM chromatograms of solutions derived from tablet formulations. It demonstrated that decent separation was acquired both standard and real sample conditions and no interfering peaks were discovered at the retention time of IBU and IS. [21] (Figure 2.).

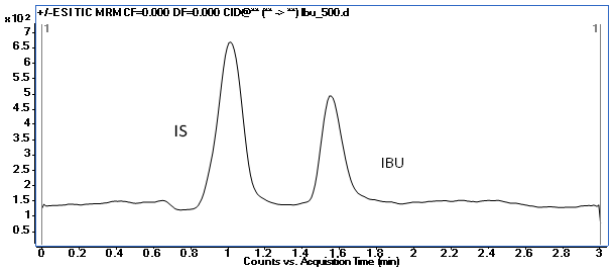

Figure 2. UPLC-MS/MS total ion current (TIC) chromatogram of IBU (500 ng mL^{-1}) and IS (500 ng mL^{-1})

2.5.2. Linearity and sensitivity

Standard working solutions at seven different concentrations of IBU and IS (500 ng mL^{-1}) were generated triplicate to obtain a calibration graph in the range of 0.1–5000 ng mL^{-1}. These samples were evaluated using the established approach, and the equation was constructed using least-squares weighted (1/x2) linear regression analysis. Continuous lower accumulations of standard solution were evaluated in sensitivity experiments. The LOD level was chosen as the lowest discernible and distinct peak. According to ICH recommendation, the detection and quantification limits were calculated based on the signal-to-noise ratio of 3:1 and 10:1, respectively [22].

2.5.3. Precision and accuracy

The accuracy and precision of the UPLC-MS/MS technique were determined by analyzing quality control samples (QC's) in low, medium, and high concentrations within the calibration curve (4, 400, and 4000 ng mL^{-1}). Intraday precision and accuracy were determined by analyzing QC samples three times in one day, and inter-day precision and accuracy were determined by analyzing the same samples three times in three days following the intraday analysis. To calculate precision and accuracy, the RSD and RE are employed [22].

2.5.4. Analysis of pharmaceuticals and recovery

The applicability of the methods for the determination of IBU in solid dosage forms (tablets) was examined by analyzing marketed medicinal products of BRUFEN (400 mg Abbott Pharmaceuticals Inc) and DOLVEN (600 mg Sanofi Pharmaceuticals Inc). The contents of each medication were extracted and weighed individually. Weighed and dissolved in methanol in an amount equivalent to one tablet. To ensure thorough dissolution, the solutions were sonicated for 15 minutes. The solutions were diluted with an optimum amount (150 and 400 ng mL^{-1}) and transmitted to an autosampler vial after being filtered through a microfilter. For analysis, 5 µL was injected into the UPLC-MS/MS system. By comparing the observed concentration to the notional concentration, average recoveries were computed.

3. RESULTS AND DISCUSSION

3.1. UPLC–MS/MS Method Development and Optimization

To attain the highest abundances of product and fragment ions, mass spectrometric settings were optimized. Direct injection of IBU and IS solutions into the mass spectrometer in both positive and negative ionization modes with an ESI source in the mass range of 80–300 Da resulted in full scan mass spectra and product ion scan spectra at 1000 ng mL^{-1}. IS had a greater peak intensity in negative ion mode than in positive mode, but IBU had a substantially higher peak intensity in negative mode. Using the multiple reaction monitoring mode, data for the two highest intense and/or different product ions for each precursor ion was obtained. MRM is a sensitive targeted mass spectrometry technique for identifying and quantifying particular chemicals by screening specific precursor molecule-to-fragment ion transitions [23]. IS transitioned to product at m/z 249.9→ 228.9, whereas IBU transitioned to product at m/z 205.0→ 159.0. However, there was only one product ion present for ibuprofen (IS), and there was no extra fragmentation product that could be chosen as the predictive ion (Figure 3.). For IBU and IS, the optimal collision energies were determined to be 2 and 1 eV, respectively. Further details about the optimum mass spectrometric conditions were shown in Table 1.

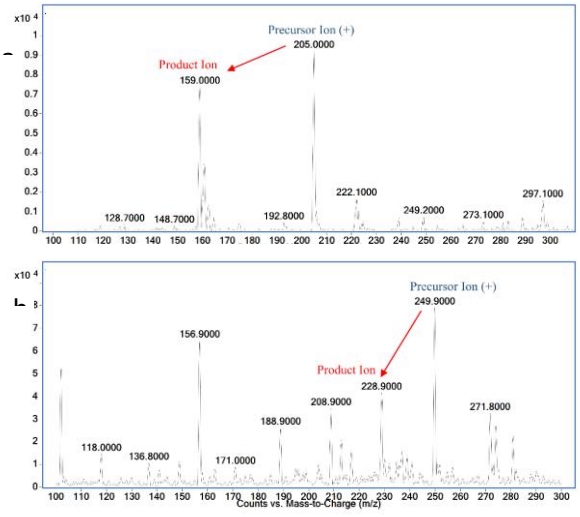

Figure 3. Product İon Mass Spectra for (A) Ibuprofen (m/z 205.0 → 159.0), (B) IS (Erdosteine) (m/z 249.9 → 228.9)

Table 1. Optimized MS/MS Parameters of the Method

	IBU (Product Ion)	IS (Product Ion)
Ionization Mode	ESI+Agilent Jet Stream	
MRM Transitions (m/z)	205.0 → 159.0	249.9 → 228.9
Fragmentor Voltage (V)	60	23
Collision Energy (V)	1	2
Polarity	Negative	Positive
Dwell Time	165	165
Gas Temp (°C)	250	250
Gas Flow (l/min)	8	8
Nebulizer (psi)	35	35
SheathGasHeater	250	250
SheathGasFlow	10	10
Capillary (V)	2000	2000

The mobile phase is optimized by testing a variety of solvents, ratios and flow programs to achieve good separation with good resolution of IBU and IS peaks in a short analysis time. It was found that the non-polar properties of IS became dominant during chromatographic separation and remained on the C18 column, requiring the use of acetonitrile for its elution. It was also found that the acidic aqueous solution significantly improved the resolution of IBU and minimized peak tailing compared to other solvents. Therefore, the optimal mobile phase consisting of water (0.1% formic acid) and acetonitrile was finally used at a flow rate of 0.15 mL min^{-1}. Under these specified conditions, IBU and IS retention times were approximately 1.554 and 1.017 for IBU and IS, respectively. Total analysis time was 2.0 minutes. This is a significantly faster execution time than the previous survey [24-28].

3.2. Selectivity and Specificity

Figure 5 shows representative MRM chromatograms of solutions derived from tablet formulations (150 and 400 ng mL^{-1}). It demonstrated that good separation was acquired both standard and real sample conditions and no intruding peaks were discovered at the retention time of IBU and IS [21].

3.3. Linearity and Sensitivity

The calibration curve was created by plotting the peak area ratio of IBU to IS to the concentration. The calibration curve was created by analysis in 7 different concentrations of IBU, the developed method was linear in the range of 1-5000 ng mL^{-1}, and the coefficient of determination (R2) exceeded 0.996 (Figure 4). LOD was calculated by injecting a continuous small accumulation of standard solution using the developed method, and values of 0.3 ng $mL^{-1}$and 3:1 S/N values were observed. The LOQ corresponding to the 10: 1 signal-to-noise ratio was determined to be 1 ng / mL. This shows that this method is sensitive enough.

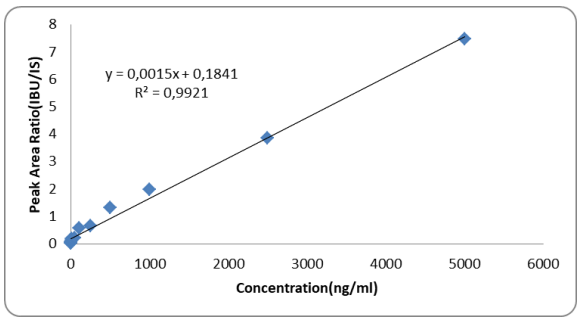

Figure 4. The Calibration Curve of the Method Obtained From Linear Regression Analysis of the Method (n=6).

3.4. Precision and Accuracy

The accuracy and precision values for QC solutions (4, 400, and 4000 ng mL^{-1}) were determined using percent relative error (percent RE) and percent relative standard deviations (percent RSD) over the same day (intra-day, n=3) and three consecutive days (inter-day), respectively. Table 2 shows the accuracy and precision findings. In every case, the intra-day and interday accuracy and precision values were within acceptable limits. With RE % (lower than 4.25 %) and % RSD (lower than 6.24 %) values, the approach demonstrated high accuracy and precision.

Table 2. Precision and Accuracy of the Method (n=6). x̄: Mean of the Six Replicated Analysis, SD: Standard Deviation, RE%: Relative Error, RSD: Relative Standard Deviations

	Intra-day			Inter-day		
Added (ng/mL)	Found x̄ (ng mL^{-1}) ± SD	Accuracy RE %	Precision RSD %	Found x̄ (ng mL^{-1}) ± SD	Accuracy RE %	Precision RSD %
4	4.23 ± 0,13	5.75	3.07	4.17 ± 0.26	4.25	6.24
400	416.76 ± 14.85	4.19	3.56	395.40 ± 15.22	-1.15	3.85
4000	3982.60 ± 84.59	-0.44	2.02	3979.52 ± 77.20	-0.51	1.94

3.5. Analysis of Pharmaceuticals and Recovery

The determination of IBU in tablet formulations was conducted to measure the applicability of the UPLCMS/MS method (Figure 5.). The amount of IBU in the selected commercial pharmaceuticals was found to be in good agreement with the IBU content of these formulations in experiments. For IBU six different lots of each drug, the drug content was found to be between 99.07 percent and 102.66 percent (Table 3.). These findings indicate that the method could be used to analyze IBU-containing tablet formulations on a regular basis.

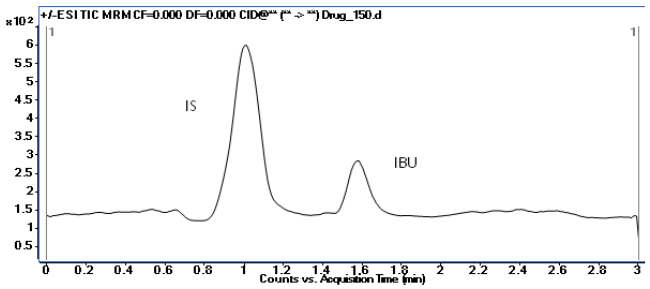

Figure 5. A Representative UPLC-MS/MS Chromatogram of IBU (150 ng mL^{-1}) Solutions Prepared from Tablet Formulations

Table 3. The Assay Results and Recovery of Pharmaceuticals Containing IBU in Two Different Concentration Levels (n=6)

	BRUFEN 400 mg IBU		DOLVEN 600 mg IBU	
	150 ng mL^{-1}	400 ng mL^{-1}	150 ng mL^{-1}	400 ng mL^{-1}
$\bar{x}$ (ng mL^{-1})	147.11	403.22	153.20	410.65
SD	5.04	7.49	7.47	9.78
RSD %	3.42	1.85	4.87	2,38
Average Recovery %	98.07	99.20	102.13	102.66

4. CONCLUSION

In the literature, the present approach was developed and validated for the determination of IBU from pharmaceutical formulations and pure form using the UPLC-MS/MS method. The linear range 1-5000 ng mL^{-1} calibration curve has a strong correlation coefficient (0.9921). Within and between days precision were expressed as relative standard deviation and were lower than 6.24%. The findings showed that the suggested UPLC-MS/MS technique is a simple, precise, quick, accurate, and low-cost approach. This approach also offers a fast analytical time and a high sensitivity. In conclusion, this approach may be utilized to determine IBU in pure form and pharmaceutical formulations, routine analysis, pharmaceutical industry quality control laboratories, and stability monitoring.

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Conflict of Interest

There are no conflicts of interest declared by any of the writers.

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