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Analytical Method Development and Validation of High-Performance Liquid Chromatography for Simultaneous Determination of Ibuprofen and Baclofen

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Abstract

Combining baclofen with ibuprofen offers synergistic pain relief by targeting both muscle spasms and inflammation, enhancing efficacy in managing musculoskeletal conditions. This study aims to develop and validate an analytical method for the separation and simultaneous determination of ibuprofen and baclofen in combination using high-performance liquid chromatography (HPLC) in pharmaceutical dosage forms. An HPLC analytical method was developed to simultaneously quantify ibuprofen and baclofen. The optimized method was validated in accordance with the International Conference on Harmonization (ICH) guidelines. The method was tested for validation parameters, including specificity, accuracy, precision, linear range, limit of detection, lower limit of quantification, and robustness. A simple HPLC method was developed to separate two active ingredients using a C18 stationary phase column (25 cm × 0.46 cm, 5 µm). The mobile phase consisted of methanol: buffer (pH 3 ± 0.05), and acetonitrile in a ratio of 5:30:65 (%v/v). The analysis was performed at 25 °C with a 1.5 mL/min flow rate. A 20 µL sample volume was injected and detected at 265 nm. The validation method was specific, precise, and accurate. The average recovery of the two ingredients was 100.5% [99.1-102.0]. The repeated tests of the two compounds at different concentrations showed a percentage relative standard deviation (%RSD) of less than 1%. The method demonstrated linearity for both ibuprofen and baclofen, with an R^2 value of 0.99. The linearity range was 12.5-150 µg/mL for baclofen, and was 100-1800 µg/mL for ibuprofen. The lower limit of quantification (LLOQ) was 7 and 6 µg/mL for ibuprofen and baclofen, respectively. The limit of detection (LOD) was 2 µg/mL for both ingredients. The developed method is simple, rapid, precise, and accurate. It can be used for routine analysis of a combination of ibuprofen and baclofen in pharmaceutical dosage forms.

Keywords: HPLC, Ibuprofen, Baclofen, Validation, ICH, Pharmaceutical dosage form

Introduction

Ibuprofen, also known as [-methyl-4-(2-methylpropyl)-benzene acetic acid], is a nonsteroidal anti-inflammatory medication (NSAID). It is commonly used as an analgesic in treating mild to moderate pain and rheumatoid arthritis [1]. Ibuprofen is a crystalline substance with a melting point of 77–78 °C and typically appears in a needle-

shaped crystal form. It is a slightly polar, lipophilic compound with a LogP value of approximately 3.5, reflecting its strong affinity for lipids and limited solubility in water. Its water solubility is very low, measured at around 0.0684 mg/mL, indicating it is practically insoluble in aqueous environments [2, 3].

Baclofen is a synthetic anti-spastic or muscle relaxant whose chemical name is (amino methyl)-4-chlorobenzene propanoic acid. Baclofen reduces spasticity in neurological disorders, including multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injuries, and spasticity associated with flexor spasms. Still, it is relatively ineffective in stroke, cerebral palsy, rheumatic and traumatic muscle spasms, and Parkinsonism [4-6]. Baclofen is a moderately polar compound with a LogP value of around 1.3, indicating lower lipophilicity and greater water solubility compared to more lipophilic drugs. Its chemical formula is $C_{10}H_{12}ClNO_2$, with a molecular weight of 213.67 g/mol. Baclofen is readily soluble in water (~0.712 mg/mL), as well as in 0.1 N HCl and 0.1 N NaOH, and is only slightly soluble in methanol and ethanol. It has a relatively high melting point, ranging from 206 to 208 °C [7, 8].

One significant advantage of combining baclofen with ibuprofen is the potential for synergistic pain relief in musculoskeletal conditions. While baclofen primarily targets muscle spasms by acting on the central nervous system, ibuprofen alleviates inflammation and pain by inhibiting the synthesis of prostaglandins. They may offer enhanced efficacy in managing conditions such as muscle strains, sprains, or chronic musculoskeletal pain, providing a dual-action approach to symptom relief. This combination has been reported to result in improved pain control and functional outcomes compared to monotherapy with either drug alone. Furthermore, the combination may allow for reduced dosages of each drug, potentially mitigating adverse effects associated with high doses of either medication [9, 10].

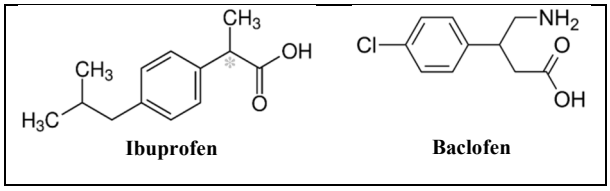

Figure 1: **Chemical structure of ibuprofen and baclofen**

Several analytical and bioanalytical approaches were developed and validated to determine ibuprofen and baclofen independently. The methods were used to determine concentrations in pharmaceutical items and biological matrices. These approaches employed several detection modalities. Canaparo et al. employed HPLC to analyze ibuprofen in plasma using a spectrofluorometric detector [11]. Meanwhile, Sochor et al. and employed HPLC and UV spectrophotometry, respectively [12]. Baclofen was also analyzed using different techniques; Tosunoğlu et al. developed an HPLC method with a fluorimetry detector [13]. Moreover, an HPLC system equipped with a UV spectrophotometer was used to analyze baclofen in plasma [14, 15].

The development and validation of HPLC methods are crucial in the discovery, development, and manufacture of pharmaceutical products. The development of an analytical HPLC method begins with selecting the HPLC technique and starting the system, followed by the selection of initial conditions, and lastly, the optimization of system parameters [16]. An optimal chromatogram is one in which all peaks are symmetrical and well-separated within a short time frame. Changes in chromatographic conditions impact the following parameters:

number of theoretical plates (N), resolution, selectivity, and capacity factor [17].

A mobile phase composed of methanol (MeOH), buffer, and acetonitrile (ACN) demonstrated effective separation performance. These conditions served as the starting point for optimizing chromatographic parameters on an HPLC system. The optimal separation was achieved by adjusting the mobile phase to an acidic pH, along with optimizing the column temperature and flow rate. The sample was injected at a volume of 20 µL and analyzed at a wavelength within the UV range [18].

Method validation is the process of establishing documented evidence that a specific activity consistently produces a desired result or product that meets its predetermined specifications and quality characteristics. The functioning of HPLC must be confirmed, according to the International Conference on Harmonization (ICH) [19], the Food and Drug Administration (FDA) [20], and the United States Pharmacopeia (USP) [7]. The specificity, accuracy, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), and robustness of the method are all studied [21-23].

This research aims to develop and validate a simple and feasible analytical method utilizing the HPLC chemotrophic technique. This method tests ibuprofen in combination with baclofen ingredients in minor amounts. The technique can also be applied in biological systems, tablet dosage forms, or pharmaceutical preparations.

Materials and Methods

Chemicals and reagents

All the reagents used were purchased from reliable sources; ibuprofen was provided as a gift from Al-Quds Pharmaceutical Company, Ramallah, Palestine. The following reagents were purchased from Sigma Aldrich: baclofen (Sigma Aldrich-UK), acetic acid glacial (Sigma-Aldrich-USA), and chloroacetic acid (Sigma Aldrich, USA). Other reagents were also used in research, including acetonitrile (Imperial Chemical Industries UK), ammonium hydroxide (Sigma-Aldrich, USA), methanol (HPLC grade, Alfa Aesar), and hydrochloric acid (Alfa Aeser, UK).

Instruments

The following equipment was used in the chromatographic separation:

HPLC System: A Hitachi Elite La Chrom HPLC System, consisting of an L-2130 Pump, was used to deliver the mobile phase at a specified flow rate. The L-2200 Autosampler was used to inject samples into the HPLC system automatically. L-2300 Column Oven was employed to maintain a constant temperature for the column during separation. L-2450 Diode Array Detector was used to detect and measure the absorbance of the analytes in the eluent. EZ Chrome Elite software was utilized for peak integration and data processing. A stationary phase column (Hypersil BDS C18, Thermo) was used. The column has dimensions of 25 cm in length and an inner diameter of 0.46 cm. The particle size of the stationary phase was 5 µm.

An Elmasonicultra sonicator (Elma S120 H) was employed to enhance the dissolution or dispersion of samples in solvents. Milli-Q water (Veolia Water Technologies) was used to generate the mobile phase and prepare other HPLC solutions.

Additional equipment, such as a pH meter (Mettler Toledo S220), was used to measure the pH of solutions. An electronic

balance (Kern ABT 120/4NM) was used to weigh samples and reagents accurately.

Mobile Phase Preparation for HPLC

The preparation methods for the mobile phase, buffer, standard solutions, and sample solutions are shown below:

Mobile Phase: The composition and ratio were 5% methanol, 30% buffer, and 65% acetonitrile (v/v).

Buffer Solution: Dilute 4 mL of glacial acetic acid with 400 mL of Milli-Q water. The mixture was adjusted with acetic acid to pH 3 ± 0.5. The solution was filtered after the pH adjustment.

Stock and working standard Solutions: Ibuprofen standard was prepared by dissolving 40 mg of standard ibuprofen in a mixture of 12.5 mL (100 mL H_2O: 1 mL acetic acid). The volume was completed with acetonitrile up to 25 mL. The final concentration of the solution was 1600 µg/mL.

Baclofen standard was prepared by dissolving 50 mg of baclofen in a mixture of 12.5 mL (100 mL H_2O: 1 mL acetic acid). The volume was then completed with acetonitrile up to 25 mL. The final concentration of the solution was 200 µg/mL.

A serial solution of a mixture consisting of an 8:1 ratio of ibuprofen and baclofen was prepared. The prepared solution has different concentrations for ibuprofen (1200, 800, 400, and 100 µg/mL) and baclofen (150, 100, 50, and 12.5 µg/mL). The solutions were used to prepare standard calibrant concentrations and test different validation parameters.

Sample ibuprofen and baclofen tablet mixture solution: A tablet mixture of the two active ingredients was prepared by mixing two tablet solutions of ibuprofen and baclofen. The detailed recreation was as follows: Ten 200 mg ibuprofen tablets were crushed and ground into a powder. An amount equivalent to 20 mg of the drug substance was dissolved in a mixture of 12.5 mL (100 mL of H_2O: 1 mL of acetic acid). The volume was completed with acetonitrile up to 25 mL. The concentration of the solution was 800 µg/mL (100% standard).

Baclofen (10 tablets) was crushed, and 2.5 mg of the baclofen drug substance was dissolved in a mixture of 12.5 mL (100 mL H_2O: 1 mL acetic acid). The volume was completed with acetonitrile up to 25 mL. The concentration of the solution is 100 µg/mL (100% standard). Finally, the two prepared solutions of ibuprofen and baclofen tablets were mixed to achieve a total volume of 50 mL.

Development and Validation of Analytical Methods

Initially, we used the active ingredients ibuprofen and baclofen to determine the absorption wavelength using the UV spectrophotometer. The UV spectra indicate that both ingredients exhibit their best absorption at 265 nm (Fig. 2).

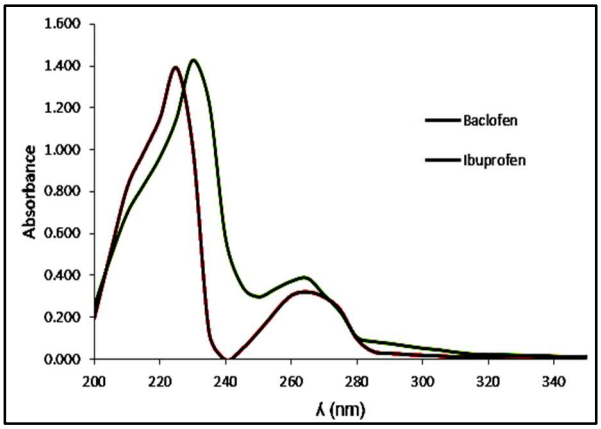

Figure 2. **UV spectra of ibuprofen and baclofen**

Various mobile phases and HPLC conditions were employed to inject the

ibuprofen-baclofen mixture using stationary phases, flow rates, and mobile phases. The optimal HPLC chromatographic conditions were determined based on achieving the most effective separation. Additionally, method selectivity was assessed by injecting a placebo sample containing common excipients in oral solid dosage forms.

The procedure was validated in accordance with international standards, including ICH and USP [24]. To assess the precision of the assay method for ibuprofen and baclofen, six samples were prepared within the concentration ranges of 100–1200 µg/mL for ibuprofen and 12.5–100 µg/mL for baclofen. Each sample was injected in triplicate for both intra-day and inter-day precision evaluations.

The Relative Standard Deviation (RSD) percentage was calculated for each set of triplicate injections, aiming for RSD values of less than 2.0%.

To evaluate the linearity of the assay procedure, a series of standards at different concentrations for baclofen (12.5-150 µg/mL) and ibuprofen (100-1200 µg/mL) was prepared, corresponding to 50%-150% relative to the measuring concentration in the standard solution. Following triplicate chromatography of each preparation, linear regression analysis was performed on the average peak ratios versus the concentrations of the levels studied. A correlation coefficient of 0.998 or greater was required for the test to pass.

Accuracy for ibuprofen was assessed at four concentration levels: high (1200 µg/mL, 150%), intermediate (800 µg/mL, 100%), and low (400 µg/mL, 50%). Three replicates of each concentration were injected for assessment.

The standard error (SE) of the regression and the slope of the calibration curve were utilized to calculate the LOD and LOQ. The LOD was determined using the formula: LOD = (3.3 × SE) / Slope, where SE refers to the standard error of the y-intercept from the regression analysis, and the slope represents the gradient of the calibration curve. Similarly, the LOQ was calculated with the formula: LOQ = (10.0 × SE) / Slope. These formulas adhere to the guidelines set by the ICH, ensuring the reliability of detection and quantification limits. The multiplicative factors of 3.3 for LOD and 10 for LOQ are statistical constants that take into account the desired signal-to-noise ratio.

Accuracy was evaluated based on three concentrations around the test concentration (80%, 100%, and 120%), with three replicates of each concentration injected. The percentage of recovery and RSD were calculated for each set of repeated samples.

Results and Discussion

Method Development

A mobile phase composed of MeOH: Buffer: ACN (10:30:60) %v/v showed separation capabilities. These mobile phase constituent values served as the starting point for investigating and optimizing chromatographic settings on an HPLC device. The best separation was obtained using a mobile phase composed of MeOH: Buffer: ACN (5:30:65) %v/v with pH = 3 ± 0.5, temperature = 25 ºC, and a 1.5 mL/min flow rate. At a volume of 20 µL, the samples and standards were examined at 265 nm. Ibuprofen was eluted after 6.58 ± 0.03 min (mean SD) under optimal conditions, while baclofen was eluted earlier (3.11 ± 0.05 min). Fig. 3 shows a typical chromatogram of a combined solution of ibuprofen and baclofen standards.

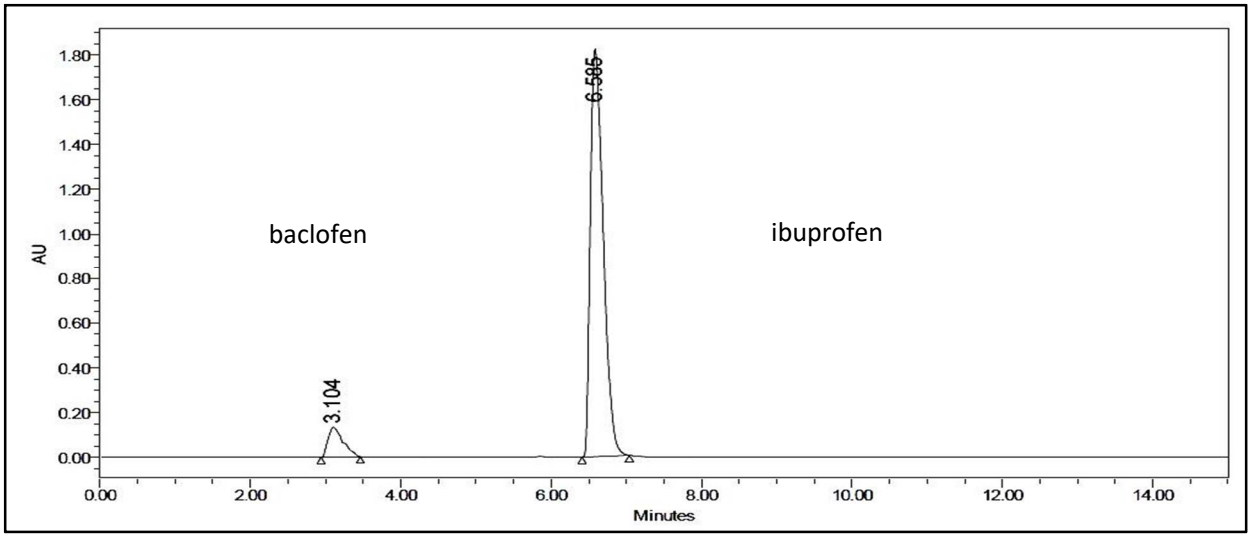

Figure 3. **Chromatogram of a mixture of ibuprofen (400 µg/mL) and baclofen (50 µg/mL) standard solution**

Method Validation

The specificity parameter test was performed by injecting a placebo sample of the excipients used in the formulated tablet oral solid dosage form (i.e., starch, lactose, magnesium stearate, and avicel) along with the active ingredients. The results demonstrate selectivity with no interfering peaks in the retention times of ibuprofen (retention time = 6.65 min) or baclofen (retention time = 3.10 min). The active ingredients in the chromatogram were well separated. The absorbance of the placebo was very low (0.002–0.010), while the chromatogram's absorbance for ibuprofen and baclofen ranged from 0.2 to 1.8.

Accuracy results for ibuprofen were assessed at four different concentration levels: high, 1200 µg/mL (150%); intermediate, 800 µg/mL (100%); and low, 400 and 100 µg/mL (50 and 12.5%). The injections were done in three replicates of each concentration. The average recovery observed was 100.6% [99.1-102.0]. The result demonstrates that the method was accurate and consistent with the ICH guidelines, which set limits for accuracy of ± 2 of the theoretical amount. Table 1 presents the detailed accuracy results.

Moreover, the accuracy of baclofen was performed. Table 1 shows the accuracy values for baclofen at four different concentrations: high concentrations of 150 µg/mL (150%), intermediate 100 µg/mL (100%), and low 50 and 12.5 (50 and 12.5%). The injections were performed in triplicate for each concentration. They are within the accepted value range, with a value of [98.2-102.2], indicating high accuracy.

Precision is a crucial parameter for assessing the reliability and repeatability of an analytical method. It measures the agreement or closeness between multiple measurements of the same sample or sample mixture. In this study, precision testing was carried out using multiple sample mixtures containing varying concentrations of ibuprofen and baclofen, prepared at different times. Two samples were prepared each day (intra-day) over three consecutive days (inter-day), resulting in a total of six samples per concentration level. Each sample was analyzed in triplicate. The method's precision was quantified using the %RSD value.

Table 1. **Accuracy results of ibuprofen and baclofen at different concentrations.**

No	Ibuprofen 100 (µg/mL) AUP* (% Accuracy)	400 (µg/mL) AUP* (% Accuracy)	800 (µg/mL) AUP* (% Accuracy)	1200 (µg/mL) AUP* (% Accuracy)
1	291149 (99.1)	1221606 (107.7)	23338319 (99.1)	34999720 (99.4)
2	291737 (99.31)	1214399 (101.13)	23644473 (100.44)	34929298 (99.22)
3	292912 (99.70)	1225209 (102.01)	23479621 (99.71)	35034931 (99.50)
Average	292031 (99.40)	1221606 (101.72)	23479621 (99.70)	34999720 (99.40)
Accuracy %	100.61			
No	**Baclofen 12.5 (µg/mL) AUP* (% Accuracy)**	**50 (µg/mL) AUP* (% Accuracy)**	**100 (µg/mL) AUP* (% Accuracy)**	**150 (µg/mL) AUP* (% Accuracy)**
1	30895 (95.90)	118405 (101.31)	219277 (99.01)	333679 (99.90)
2	32345 (100.42)	118522 (101.41)	226364 (102.22)	333011 (99.71)
3	32828 (101.91)	118405 (98.32)	217726 (98.34)	332342 (99.51)
Average	32023 (99.41)	118405 (99.80)	221049 (99.80)	333011 (99.70)
Accuracy %	100.51			

*** Area under the peak**

Table 2 reports the %RSD values for the two compounds at various concentrations. All the %RSD values were less than 1. This indicates that the method demonstrates high precision for both ibuprofen and baclofen within the studied concentration range. The %RSD values being less than 1 indicate high agreement and consistency between replicate measurements.

Table 2. **Results for ibuprofen and baclofen precision at various concentrations.**

Ibuprofen				
Concentration (µg/mL)	Area under the peak (AUP)			
	100	400	800	1200
Average	293794	1201186	23550272	35210986
SD	8833	18285	165233	49945
%RSD	0.30	0.15	0.70	0.14
Baclofen				
Concentration (µg/mL)	Area under the peak (AUP)			
	12.5	50	100	150
Average	32217	116886	221492	334013
SD	342	526	1449	654
%RSD	0.16	0.05	0.65	0.19

A generally accepted criterion for precision is a %RSD value below a certain threshold, often set at 2% (standard deviation). Therefore, in this study, the observed %RSD values below 1 suggest that the method exhibits excellent precision for both ibuprofen and baclofen.

Linearity is a crucial parameter in method validation, as it evaluates the relationship between the analyte concentration and the corresponding detector response. This study evaluated the linearity of ibuprofen and baclofen using calibration curves spanning a range of concentrations. For ibuprofen, the concentration range was 100-1800 µg/mL. The calibration linear regression equation was $Y = 21901x + 53414$. For baclofen, the concentration range was 12.5-150 µg/mL. The regression line equation was: $Y = 29312x - 117665$. The calibration curves for both

gradients showed excellent linearity, with R^2 values of 0.9998 for both ibuprofen and baclofen.

The sensitivity validation parameter was assessed using the SE and the slope of the HPLC system's calibration curves. The LOQ was determined to be 7 µg/mL for ibuprofen, while it was found to be 6 µg/mL for baclofen. Additionally, the LOD was set at 2 µg/mL for both ibuprofen and baclofen.

The robustness of an analytical procedure was evaluated to assess its ability to withstand minor, intentional variations in method parameters. These parameters included flow rate, temperature, concentration, percentage of organic matter, reaction time, purity of reagents, and pH. The purpose was to determine the quantitative influence of these variables and evaluate the procedure's reliability under normal conditions. In this study, the robustness of the method was assessed by calculating %RSD with deliberate variations in three specific parameters: flow rate (± 0.1 mL), composition of the mobile phase (± 10 mL), and temperature (± 5 ºC). The % RSD values obtained for these deliberate variations were less than 1 (Table 3). These findings indicate that the method is robust and capable of producing reliable and consistent results, even when subjected to slight variations in the specified parameters.

Table 3. **Results of analytical method robustness for ibuprofen and baclofen.**

No	Ibuprofen (800 µg/mL) Flow rate mL/mint			Baclofen (12.5 µg/mL) Flow rate (mL/mint)		
	1.4	**1.5**	**1.6**	**1.4**	**1.5**	**1.6**
1	21688726	23389931	21818052	202549	319126	139798
2	21775647	23719998	21795321	202138	321497	139335
3	21564789	23540886	21556469	202089	325868	139142
Ave	21676387	23550271	21723280	202258	322163	139425
SD	105969	165233	144909	252	3420	337
RSD	0.50	0.70	0.70	0.12	0.16	0.24
No	**pH**			**pH**		
	pH 2.7	**pH 3**	**pH 3.3**	**pH 2.7**	**pH 3**	**pH 3.3**
1	23180453	23389931	23106733	133265	319126	281752
2	23154130	23719998	23221046	133461	321497	280874
3	23191566	23540886	23289461	132789	325868	280893
Ave	23175383	23550271	23205746	133171	322163	281173
SD	19226	165233	92319	345.0	3420	501
RSD	0.08	0.70	0.30	0.30	0.16	0.17
No	**Temp (°C)**			**Temp (°C)**		
	22	**25**	**27**	**22**	**25**	**27**
1	23112676	23389931	23092356	178947	319126	166852
2	23149502	23719998	23158006	178561	321497	166474
3	22987503	23540886	23260691	179141	325868	169304
Ave	23083227	23550271	23170351	178883	322163	167543
SD	84919	165233	84843	295	3420	1536
RSD	0.30	0.70	0.36	0.16	0.16	0.90

Lastly, locally formulated tablets containing 200 mg of ibuprofen and 25 mg of baclofen were tested using the developed method, applying the detailed chromatographic conditions explained in the results section under the method development section. The sample was initially dissolved in 50 mL. The solution was further diluted to achieve concentrations of 1 mg/mL for ibuprofen and 0.125 mg/mL for baclofen before injection. The calculated percentage recovery demonstrates the high accuracy of the developed method; the recovered concentrations of the injected samples were 1.01 and 0.013 mg/mL, with percentage accuracies of 101% and 104% for ibuprofen and baclofen, respectively. The HPLC chromatogram demonstrates the system suitability of the developed method with well-separated peaks and acceptable theoretical plates (Fig. 4).

The results of the developed method of this research are comparable to those of existing methods, which identify only one of the active ingredients in the pharmaceutical dosage forms. Nevertheless, Sanchaniya et al. developed a technique for combining active ingredients of chlorpheniramine maleate, ibuprofen, and phenylephrine hydrochloride. However, the LOD was 120 µg/mL, which indicates that our developed method was more sensitive [25]. Similarly, baclofen was analyzed in its pharmaceutical dosage form alone. Bhushan et al. employed a derivatization procedure to separate the enantiomers [26]. Moreover, the HPLC chromatographic method utilizing a UV spectrophotometer was developed and validated [27, 28]. Our method has comparable LOD and LOQ, but it also offers the advantage of analyzing two active ingredients in a single pharmaceutical dosage form.

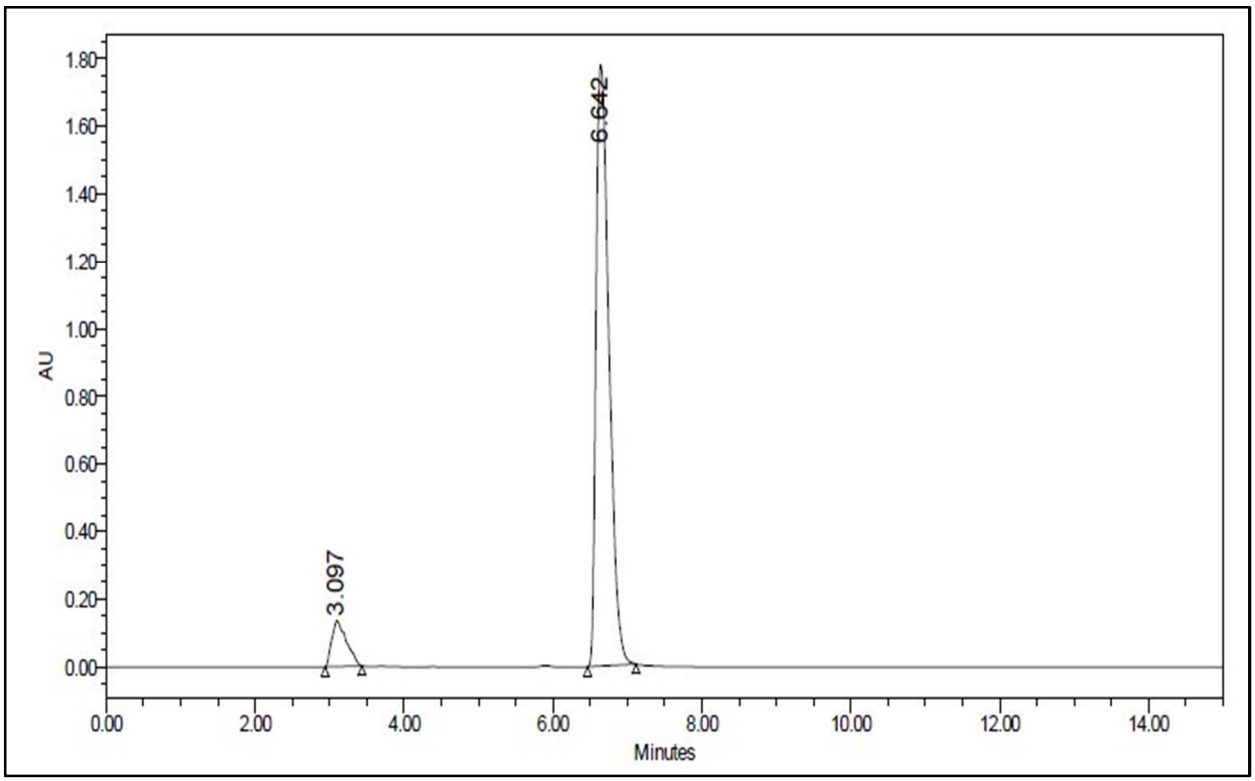

Figure 4. **Chromatogram of the tested products of ibuprofen and baclofen tablets mixture**

Conclusion

A novel reverse-phase ultraviolet high-performance liquid chromatography (UV-HPLC) method has been successfully developed to simultaneously estimate the active pharmaceutical ingredients ibuprofen and baclofen in various dosage forms. This method provides a valuable analytical tool for ensuring the quality and consistency of these drugs in commercial formulations. The development process involved systematically optimizing chromatographic conditions to achieve clear separation, sensitivity, and reproducibility in detecting these compounds. The method was validated rigorously following international guidelines, including linearity, accuracy, precision, robustness, and specificity, demonstrating its reliability for routine quality control analysis. This UV-HPLC method stands out for its simplicity, speed, and efficiency. It offers a robust analytical solution for rapidly and accurately quantifying ibuprofen and baclofen. Its high precision and accuracy make it particularly suitable for quality control laboratories, where time and reliability are critical. This method can be applied to tablets and other pharmaceutical dosage forms, providing comprehensive support for the pharmaceutical industry. This analytical approach opens up exciting possibilities for future research applications. It could be adapted to biological matrices, such as blood plasma.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgment

The manuscript is based on a repository by the authors.

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