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Exploration of the separation mechanism of flurbiprofen and nimesulide utilizing RP-HPLC

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Abstract

This study aims to create an easy method for simultaneously measuring nimesulide and flurbiprofen in pharmaceutical formulations. This research provides insight into how variations in buffer concentration, pH levels, and acetonitrile content influence the separation mechanism of two non-steroidal anti-inflammatory drugs (NSAIDs). The retention time of two NSAIDs was increased with an increase of eluent pH value from 3 to 5.5. When acetonitrile content increased from 5 % to 50 %, the retention of two target drugs decreased, indicating hydrophobic and ionic interactions. The RP - HPLC system with UV detection accomplished separation (250 x 4.60 mm, 130Å, and 5) using a C8 Hyper Clone BDS column. The acetonitrile and acetate buffer mixture is used as the mobile phase gradient elution at a detection wavelength of 254 nm and a 1 mL/min flow rate. The linear ranges were 0.05-12.50 and 0.03-17.35 $\mu g.ml^{-1}$ for nimesulide and flurbiprofen, respectively. LOD 0.030, 0. 020$\mu g.ml^{-1}$ and LOQ 0.091, 0.060$\mu g.ml^{-1}$ for nimesulide and flurbiprofen, respectively. The verification findings demonstrate the suitability of the proposed methods for quantifying NSAIDs in pharmaceutical formulations.

Keywords: Flurbiprofen, Nimesulide, NSAIDs drugs, Pharmaceutical formulations, RP-HPLC.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been employed with success for over 3500 years to alleviate pain, fever, and inflammation [1]. Currently, NSAIDs are widely utilized as over-the-counter medications globally, making up 5% of all prescribed pharmaceuticals [2]. NSAIDs are primarily employed in the therapy of patients suffering from pain and inflammatory conditions, including arthritis and various other rheumatic diseases [3, 4]. Additionally, epidemiological research has demonstrated that the extended use of NSAIDs decreases the chances of developing Alzheimer's disease and delays its initiation [5, 6]. Long-term use of NSAIDs may lead to the emergence of severe side effects, including gastrointestinal bleeding and elevated cardiovascular risks [7]. NSAIDs were traditionally classified based on their chemical properties, wherein the majority of NSAIDs are classified as significant derivatives including acetic acid, salicylic acid, anthranilic acid, enolic acid, or propionic acid [8]. Flurbiprofen (FBN) is an NSAID and is distinguished by its considerable anti-inflammatory, analgesic, and antipyretic effects Fig. 1 [9]. It displays an effectiveness similar to that of other NSAIDs, such as aspirin, ibuprofen, naproxen, and diclofenac, which are commonly employed in the treatment of rheumatoid arthritis [10]. FBN exhibits rapid and almost complete absorption when administered orally [11]. Nimesulide (NIM) is an NSAID that exhibits a selective inhibition of cyclooxygenase-2 (COX-2) with a promising choice for treating different inflammatory conditions [12]. The COX-2 selectivity of this NSAID medication enhances its analgesic and anti-inflammatory therapeutic properties without the negative gastrointestinal and renal effects often associated

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with other NSAIDs such as indomethacin or ibuprofen [13, 14]. NIM is prescribed for the treatment of rheumatoid arthritis and its antipyretic efficacy [15].

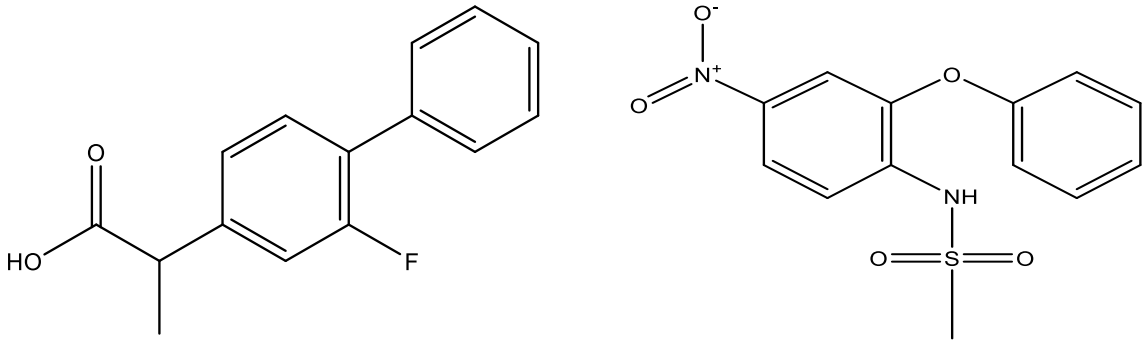

Figure 1. Chemical structure of Flurbiprofen and Nimesulide[16]

Today, HPLC is commonly used for separation and purification in diverse fields, including pharmaceuticals [17-19] environment [20-22], and human plasma [23-25]. Over the past decade, HPLC has established itself as the favored approach for analyzing a wide range of compounds [26]. Its primary advantage over GC is the capacity to analyze analytes that are not volatile, making HPLC a suitable choice for analyzing macromolecules [25]. Numerous analytical techniques have been employed for the analysis of Flurbiprofen and Nimesulide, including spectrophotometric methods [27-29] and RP-HPLC [30-32], have been reported. The suggested technique has advantages: it exhibits higher sensitivity than previously reported techniques for both drugs. It is more eco-friendly because it utilizes minimal ACN compared to these methods. However, no analytical methods have been published to elucidate the separation mechanism of Flurbiprofen and Nimesulide on RP-HPLC. This study addressed issues that have not been extensively investigated in previous methods by examining the influence of factors such as pH, buffer concentration, and the type of buffer on the separation mechanism of two NSAID models. This investigation provides a rapid, accurate, and simple RP-HPLC method for quantifying Flurbiprofen and Nimesulide in pharmaceutical formulations. The established method successfully quantified the selected drugs in various commercial dosage forms. This technique would be helpful for simultaneously quantitating two NSAIDs in pharmaceutical preparations.

Materials and Methods

Chemicals and reagents

Flurbiprofen (98.5%) and nimesulide (98%) were supplied by Sigma-Aldrich. Acetonitrile (HPLC grade) and all other chemicals were purchased from Merck (Germany). Ultra-pure water is acquired by Purifying deionized water using a Milli-Q system (Millipore, USA). Flurbiprofen tablets were obtained from Bilimilac Sanayii Ve Ticaret A. S (100 mg, Fortine, Turkey, Sample 1), Drogsan ilaclari San .ve. Tic. A.S (100 mg, Maximus, Turkey, Sample 2), and Deva Holding A.S (100 mg, Zero-p, Turkey, Sample 3), respectively. Nimesulide tablets were obtained from Deva Holding A.S Kapakli (100 mg, Nimelid, Turkey, Sample 1), Brawn Laboratories Limited (100 mg, Solide-p, Indian, Sample 2), and Excel Biolife Pvt. Ltd (100 mg, ECH-OFF, Indian, Sample 3), respectively.

Chromatographic conditions

The analysis was performed on a Merck-Hitachi HPLC (Germany-Japan), which featured a T-6300 separation center with injection valves and a column oven. This setup had an L-4200 UV/Vis detector and an L6200 gradient pump. Data collection and analysis were performed using the N2000 Photographic Data Workstation Module Integrator. Chromatographic separation of Flurbiprofen and Nimesulide was performed on a C8 Hyper Clone BDS column (250 x 4.60 mm, 130Å, and 5). UV absorbance at 254 nm was employed to detect Flurbiprofen and Nimesulide. The optimal wavelength for determination of the two drugs was

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chosen based on the British Pharmacopeia. several experiments were carried out by increasing and decreasing the wavelengths in the British States Pharmacopeia by 10-50 nm to identify the optimal wavelength. The flow rate was established at 1 mL/min, and a 10 µL injection volume was used[16].

Preparation of working standard solution

Stock standard solutions of flurbiprofen and nimesulide (500 µg.ml^{-1} and 100 µg.ml^{-1}, respectively) were prepared in ACN. To prepare working solutions, a series of dilutions were performed on the individual stock standard solutions using the mobile phase to achieve a final concentration of 30µg/ml and 10µg/ml for Flurbiprofen and Nimesulide, respectively[33].

Preparation of sample solution

Fourteen tablets were weighed, and the mean weight was determined. The tablets were then crushed, and the quantity of powder was equivalent to a single tablet's content. The finely powdered was moved into a 50 mL volumetric flask with 15 mL of ACN. The flask was then degassed for 10 minutes in an ultrasonic bath and filtered through a 0.45 µm Millex® Syringe filter. Finally, the volumetric flask was topped up to the mark with ACN.

Results and Discussion

To get a closer view into the mechanisms of the separation of pharmaceuticals, eluent conditions are changed systematically by starting with a variation of acetonitrile content, eluent ionic strength, and eluent pH.

Influence of the acetonitrile content

Mobile phase compositions were changed systematically by varying the acetonitrile content from 5 % to 50 % (v/v) with a constant concentration of the buffer 0.01 M at pH 4.75 Fig. 2. The flurbiprofen and nimesulide showed increased retention of choosing NSAIDs with increasing aqueous phase (acetate buffer). Reducing the polarity of the mobile phase by increasing the proportion of the acetonitrile enhances the hydrophobic interaction between the stationary phase and solutes, hence facilitating solute elution. The hydrophilicity of the chosen NSAIDs is responsible for hydrophobic interaction. The values of the NSAIDs are evident from the log Pow. Log Pow flurbiprofen and nimesulide values explain this (4.42 and 6.7).

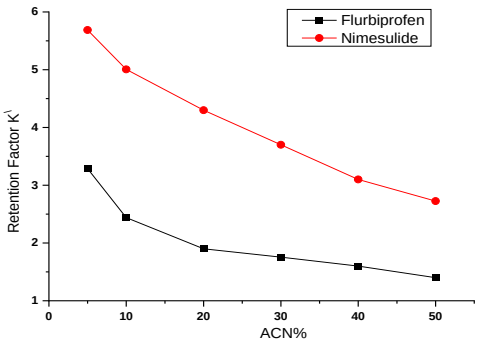

Figure 2. Effect of acetonitrile fraction used in the mobile phase

Influence of the eluent pH

As illustrated in Fig. 3, the retention factors for nimesulide and flurbiprofen increased as the pH value was increased. Simultaneously, the other mobile phase composition was maintained at 50 % acetonitrile and 50 % acetate buffer 10 mM. Nimesulide and flurbiprofen have a pKa range of 1.78-3.54 and were negatively charged, and the negative charge increased as the eluent pH increased from 3 to 5.5. Thus, NSAID model deprotonation increased as the pH increased from 3 to 5.5, increasing retention factors [34].

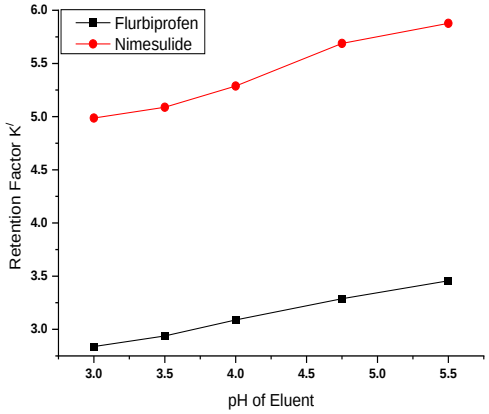

Figure 3. Effect of the eluent pH used in the mobile phase

Influence of the eluent ionic strength

At the end of optimization conditions, the buffer concentration was changed from 10 to 30 mM with a constant ACN content of 50 % at pH 4.75; therefore, we found no significant alteration in Fig. 4.

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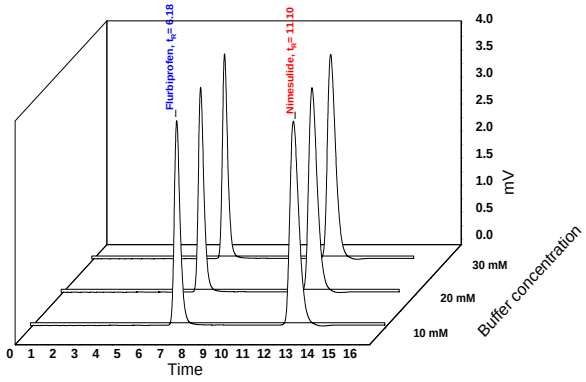

Figure 4. Effect of the eluent ionic strength used in the mobile phase

Validation of the method

The method's analytical validation parameters were determined following the International council on Harmonization (ICH) guidelines [35] .Calibration curves were established by employing linear regression to analyze the relationship between the peak area of the selected drugs and their concentrations, Fig. 5. The correlation coefficients (r^2) achieved for the two NSAID drugs with values greater than 0.999 demonstrate excellent linearity. The statistical findings for the calibration graphs of flurbiprofen and nimesulide were acquired using the RP method and are presented in Table 2. Table 3 illustrates the %RSD and %recovery measurements assessed within the same day(intra-day) and various days (inter-day).

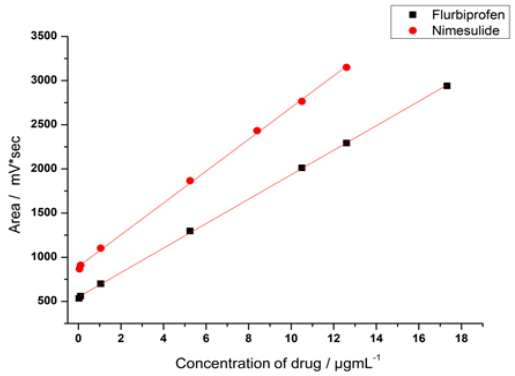

Figure 5. Calibration curve of flurbiprofen and nimesulide using C8 stationary phase

Table 1. Linearity, regression equation, determination coefficient (R2), LOD, and LOQ of flurbiprofen and nimesulide using C8 stationary phase

Parameter	Flurbiprofen	Nimesulide
Y=a+b*x	y= 548.67+138.59 *x	Y=893.84+180.10* x
Linearity µg/ml	0.03-17.35	0.05-12.50
R^2	0.9997	0.9991
LOD µg/ml	0.020	0.030
LOQ µg/ml	0.060	0.091

Table 2. Accuracy and precision of proposed RP-HPLC technique for the determination of flurbiprofen and nimesulide

	Same-Day Analysis n=5			Day-to-Day Analysis n=5		
Taken (µg ml^{-1})	**Found (µg ml^{-1})**	**% Rec.**	**%RSD**	**Found (µg ml^{-1})**	**% Rec.**	**%RSD**
		Flurbiprofen				
5.00	4.98	99.60	0.20	4.96	99.20	0.31
7.00	7.05	100.71	0.18	7.05	100.71	0.29
		Nimesulide				
5.00	5.03	100.60	0.55	5.05	101.00	0.44
7.00	6.95	99.28	0.36	6.93	99.00	0.27

System suitability

The obtained correlation coefficient values were 0.9997 for flurbiprofen and 0.9991 for nimesulide, indicating a robust linear correlation between the average area and different concentrations on the calibration curve. The linear regression equations were determined to be y = 548.67 + 138.59x for flurbiprofen and Y = 893.84 + 180.10x for nimesulide. Tables 1 demonstrate the linear correlation between concentration and response for the two NSAIDs.

Detection limit and limit of quantification (LOD and LOQ)

The LOD values for flurbiprofen and nimesulide were determined to be 0.020 and 0.030 µg/mL, respectively. The LOQ values were 0.060 and 0.091 µg/mL for flurbiprofen and nimesulide, respectively.

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These findings suggest that the values of LOD and LOQ for flurbiprofen are lower than those for nimesulide, suggesting that flurbiprofen can be determined at lower concentrations with greater accuracy. Table 1 illustrates the LOD and LOQ values for flurbiprofen and nimesulide.

Precision

A two-level precision analysis was performed on each drug to evaluate the precision of the proposed method. Repeatability was determined by injecting five replicates of a standard preparation containing 100 µg/mL of FLU and NIM. Intermediate precision was evaluated by analyzing five replicates of solutions at the identical concentration level as in the repeatability tests. These solutions were prepared on different days and by various analysts. Table 2 illustrates the %RSD value on the same and different days. These findings confirm the excellent precision of the technique, as the RSD% values were below 2%.

Optimization

The study focused on optimizing separation conditions by examining the eluent concentration, elution gradient, and detection wavelength. Different concentrations of the mobile phase components were evaluated, and the study examined various pH levels, ranging from 3 to 5.5, and buffer concentrations from 10 to 30. The best conditions for separating flurbiprofen and nimesulide were achieved using a 0.01 mM acetate buffer with a pH of 4.75, with a mixture of ACN and acetate buffer in 30:70 ratios. The ideal sensitivity for two NSAIDs was achieved at 254 nm. Chromatographic separation was carried out at a 1.0 mL/min flow rate. The suggested method demonstrated high resolution, sensitivity, recovery, stability, accuracy, and precision for the selected analyte, with complete elution of two NSAIDs within 12 min, as illustrated in Fig. 5.

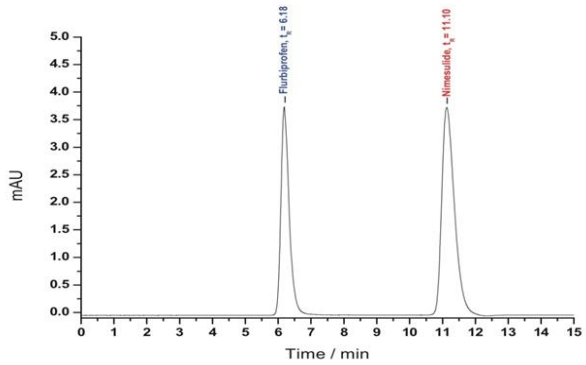

Figure 5. the chromatogram shows the retention time of flurbiprofen and nimesulide

Determination of flurbiprofen and nimesulide in pharmaceutical preparations

The suggested RP-HPLC technique was employed to analyze flurbiprofen and nimesulide in three pharmaceutical formulations. The quantification results for flurbiprofen and nimesulide within their pharmaceutical formulations provide evidence that the novel developed and validated method is appropriate for analyzing this analyte without any interference caused by the excipients. The results are illustrated in Table 3. The comparison method (British Pharmacopeia) results were used to evaluate the competence and effectiveness of the suggested RP-HPLC methods [16]. Statistical analyses used t and F-test variance ratios at 95% confidence. The t and F values obtained do not exceed the theoretic value indicated in Table 4, suggesting no significant difference in the accuracy of determining FBN and NIM in the pharmaceutical formulations between the two methods.

Table 3. Determination of flurbiprofen and nimesulide in pharmaceutical formulations using C8 stationary phase.

Formulations	Present (mg)	Get it (mg)	%Rec	%RSD
Flurbiprofen				
Fortine-FBP	100	99.00	99.00	0.28
Zero-p-FBP	100	100.40	100.40	0.22
Maximus-FBP	100	98.00	98.00	1.55
Nimesulide				
Nimelid	100	98.60	98.60	0.81
Solide-p	100	100.60	100.60	0.76
ECH-OFF	100	98.60	98.60	0.87

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Table 4. The comparison of the proposed techniques with the reference method for flurbiprofen and nimesulide analysis by using t-and F-statistical tests.

Applications	RP method	British Pharmacopeia method	t-Test (theor.)	F-Test (theor.)
Flurbiprofen				
Fortine-FBP	99.00	100.50	0.7679 (2.7764)	0.4122 (19.000)
Zero-p-FBP	100.40	98.50		
Maximus-FBP	98.00	99.26		
Nimesulide				
Nimelid	98.60	99.22	0.8711 (2.7764)	0.0780 (19.000)
Solide-p	100.60	98.78		
ECH-OFF	98.60	99.44		

Conclusion

A simple, sensitive, rapid HPLC approach has been developed to simultaneously quantify two NSAIDs in pharmaceutical preparations. The retention mechanism was studied by altering the parameters affecting chromatographic selectivity. The retention behavior of both drugs on the reverse phase demonstrated characteristic hydrophobic and ionic interactions. The two NSAIDs were effectively separated and quantified in a short duration (within 12 minutes) using a minimal quantity of ACN (30%). The investigation's statistical results demonstrated exceptional linearity, precision, accuracy, and specificity. As indicated, the recommended approach's satisfactory analytical performance improves its suitability for standard drug analysis in quality control laboratories. The suggested methods were validated according to the ICH guidelines, yielding acceptable results. Statistical comparisons between the suggested and reference methods showed no significant variance. Therefore, the suggested RP-HPLC approach can be used to evaluate NSAIDs in therapeutic dosages regularly.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and Images, which are not ours, have been given permission for re-publication attached with the manuscript.
- Al-Nahrain University
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Al-Nahrain University

Authors' Contribution Statement

B. A. A.and M. J. M. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

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استكشاف الية فصل الفلوربيبروفين و النيميسوليد باستخدام كروموتوغرافيا السائل عالي الاداء ذو الطور المعكوس

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الخلاصة

تهدف هذه الدراسة إلى إنشاء طريقة بسيطة لتقدير النيميسوليد والفلوربيبروفين في المستحضرات الصيدلانية. بالمقارنة مع الأساليب الأخرى، ولا سيما الطيفية، توفر كروموتوغرافيا السائل عالي الأداء ذوالطور العكسي (RP - HPLC) حساسية ودقة أكبر . التحقق من تركيز الوقاء المثالي، مستوى الحموضة (pH)، ومحتوى الأسيتونيتريل كان جزءًا من تحسين وتطوير تقنية RP-HPLC لتأكيد فصل وتقدير الدوائين المضادين للالتهابات غير ستيرويدية تقديرا كميا. حُقق الفصل من خلال استخدام كروموتوغرافيا السائل عالي الاداء ذو الطور المعكوس مع كاشف الاشعه فوق البنفسجية والعمود المستخدم كان من نوع C8 Hyper Clone BDS ذو الابعاد(250×4.6mm,160A°,5).تم استخدام مزيج من الاسيتونيتريل ووقاء اسيتات كطور متحرك عند طول موجه مكشاف254 نانومتر وبمعدل جريان 1 مل/دقيقة. وقد بينت النتائج ملائمه الطريقه المقترحة لتقدير الدوائين المضادين للالتهابات غير ستيرويدية في المستحضرات الدوائية.

الكلمات المفتاحية: كروموتوغرافيا السائل عالي الاداء ذو الطور المعكوس , الادوية المضادة للالتهابات غير ستيرويدية, فلوربيبروفين نيميسوليد,المستحضرات الصيدلانية.