Study the Effect of Zwitterionic Hydrophilic Interaction for Simultaneous Determination of Non-Steroidal Anti-Inflammatory Drugs in Dosage Forms

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Summary: Flurbiprofen and ketorolac are non-steroidal anti-inflammatory drugs (NSAIDs) that include both hydrophilic and hydrophobic functional groups in their structures. By connecting sulfobetaine molecules to polystyrene-divinylbenzene particles, two ZIC-HILIC stationary phases (ZIC-SB1 and ZIC-SB4) were developed for chromatographic separation of NSAIDs with varying chain lengths using Ultraviolet as detection. The different chain lengths of the stationary phases are utilized to investigate the retention behaviour of NSAIDs. Simultaneous quantification methods for ZIC-HILIC were developed. Flurbiprofen and ketorolac exhibited high precision with a small relative standard deviation (RSD) value of 0.48%, linear ranges of 0.015-6 and 0.06-4.5 ppm and detection limits of 0.009-0.006 and 0.01-0.008 ppm with a coefficient of determination (R^2) of 0.9995-0.9997 for the ZIC-SB1 and ZIC-SB4 stationary phases, respectively. Statistical testing was used to compare the approaches to the British Pharmacopoeia protocol, and no difference in accuracy was discovered.

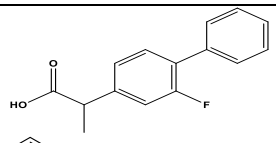
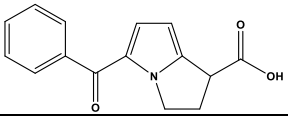
Keywords: Flurbiprofen, Ketorolac, NSAIDs, HILIC, Dosage forms.

Introduction

NSAIDs are widely prescribed around the world. They are used to treat inflammation, chronic pain, and acute pain [1]. The anti-inflammatory efficacy of NSAIDs and the majority of their other pharmacological actions result from their inhibition of arachidonic acid conversion to prostaglandins, which operate as mediators of the inflammatory process [2]. The increased demand for NSAIDs necessitates tighter control over the quality of these medicinal drugs and preparations. As a result, new analytical methods for detecting and quantifying NSAIDs and their combination medications are required. Flurbiprofen (FBP) and ketorolac (KETO) display acidic properties classified as aryl-propionic acids and hetero-aryl acetic acids, respectively. The physicochemical properties and molecular structures of the two model pharmaceuticals are summarized in Table-1.

Numerous RP-HPLC methods for individual analysis of FBP and KETO and some of their combinations are described in the literature [3-14]. Still, so far, few studies for the simultaneous determination of NSAIDs using HILIC/MS [15, 16]. Nonetheless, no method for simultaneously quantifying the specified NSAIDs (FBP and KETO) using zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) with Ultraviolet detection has been published, and this is the first paper reporting the use of HILIC/UV. ZIC-HILIC is a liquid chromatography approach that combines a polar stationary phase with a mobile phase with high water content while increasing the amount of a less polar solvent. There is currently considerable interest in using ZIC-HILIC to study a wide variety of polar medicines, metabolites, and physiologically significant chemicals [17]. ZIC-HILIC is a suitable alternative to HPLC for analyzing various chemicals.

Table-1:Chemical structures and physicochemical properties of flurbiprofen and ketorolac [36].

NSAID drug	Molecular structure	pKa	Log P_{ow}
Flurbiprofen		**4.42**	**3.944**
Ketorolac		**3.84**	**2.283**

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Additionally, ZIC-HILIC can be used with charged substances [18]. It is also critical to note that when hydrophilic and ionizable medicines are analyzed using this method, mass spectrometry (MS) can considerably boost the sensitivity of the results [19]. Using the ZIC-HILIC method, they recently found a tremendous increase in the estimation of medicines, amino acids, inorganic ions, carboxylic acids, and nucleosides [20-32]. There has been no prior study of the effect of the different length methylene chains of the sulfobetaine stationary phases on the retention behaviour of FBP and KETO. Recently [23, 33, 34], researchers examined the effect of chain length on ZIC-HILIC stationary phases and how this affects the behaviour of ranitidine, simvastatin, and esculin. They discovered that as the chain length increased, the interaction between ranitidine, simvastatin, esculin, and the ZIC-HILIC stationary phases increased, leading to a longer retention time. The established methodologies enabled the determination of the FBP and KETO medication concentrations in various commercial pharmaceutical formulations.

Experimental

Materials and Reagents

Active pharmaceutical ingredients (flurbiprofen and ketorolac) and HPLC-grade acetonitrile (ACN) were obtained from Sigma-Aldrich. Acetic acid and sodium acetate were purchased from BDH. Milli-Q gradient ultrapure water system was used to create ultrapure water (Billerica, MA, USA).

Preparation of stock and calibration curves

In a volumetric flask, 10 mg of FBP and KETO were dissolved in 100 mL acetonitrile to prepare drug stock solutions. Serial dilutions of stock solution were prepared for FBP (0.015, 0.05, 0.5, 2.5, 5, and 6 ppm) and KETO (0.06, 0.3, 0.9, 1.5, 2, 3, and 3 ppm) to construct calibration curves of FBP and KETO. Three commercial companies' Tablets and ampoules (KETO) were obtained from a local pharmacy: Derelax (10 mg)-Eurowin-France, Ketro (30 mg/mL)-Huons co. ltd-Korea and Ketorom (30 mg/mL)-Rompharm-Romania. Three commercial companies' Tablet (FBP-100 mg) were obtained from a local pharmacy. Fortine-BİLİM İLAÇ SANAYİİ VE TİCARET A.Ş-Turkey, Zero-p-Deva Holding A.Ş-Turkey and maximus-Drogsan-Turkey.

Preparation of NSAIDs drugs formulation

Ten Tablets were collected for Tablet forms. About 100 and 10 mg of FBP and KETO were dissolved in an appropriate volume of acetonitrile and diluted to the desired concentration in a 100-mL volumetric flask with acetonitrile. Ten ampoules were collected for two ampoule forms. About 30 mg of KETO was transferred and diluted to the desired concentration in a 100-mL volumetric flask with water. Next, the solution was filtered using Millipore filters (0.22 µm). By diluting the stock solution, other standard solutions were developed.

Chromatography

The studies were done on the Merck-Hitachi HPLC module with L-4200 Ultraviolet (UV) detector and gradient pump L-6200. The chromatographic and the integrated data were acquired using N2000 Data Workstation software. Chromatographic separations were done on ZIC-SB1 and ZIC-SB4 self-made stationary phases (100× 4.6 mm, 3 μm) according to procedures by Rasheed and coworkers [25, 26]. The SB1 represents one-methylene groups between the charged sites in ZIC-SB1 stationary phase, while SB4 indicates four-methylene groups. Using a mobile phase consisting of acetonitrile and acetate buffer at a flow rate of 0.75 mL/min at 25 ◦C. Detection of FBP and KETO was performed at 254 nm.

Validation of method

According to the ICH [35], the method was validated in terms of recovery, precision, the limit of detection (LOD), the limit of quantification (LOQ), and linearity. It is hypothesized that the HPLC response is linearly proportional to the standard concentration throughout a limited range of concentrations for linear calibration curves. The model is used to compute the LOD, and LOQ can be expressed as LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the intercept and S is the slope of the calibration curves. Inter-day precisions were represented in terms of relative standard deviation (RSD). Five replicates (n = 5) of the analysis of FBP and KETO were performed on five different days (n = 5). Recovery and relative standard deviation (%RSD) experiments using three concentrations (0.8, 1, and 3 ppm for FBP and KETO 0.7, 0.9, and 1.3 ppm) and five replicates (n = 5) for each concentration administered determined the suggested methods' accuracy and precision, respectively.

Results and Discussion

ZIC-HILIC-UV conditions optimization

During the development and optimization of ZIC-HILIC conditions to determine FBP and KETO

from bulk and dosage forms, acetonitrile proportion, pH, and buffer acetate concentration were investigated.

Effect of acetonitrile proportion on NSAIDs retention

The acetonitrile concentration in the mobile phase is significant for ZIC-HILIC separation. It considerably affects the retention time, peak shapes, sensitivity, and separation efficiency. In initial examinations, the impact of acetonitrile proportion on the separation of FBP and KETO was investigated using ZIC-SB1 and ZIC-SB4 stationary phases at a proportion ranging from 60% to 95% during the gradient. Meanwhile, the acetate buffer (40 mM, pH = 4.75) was maintained constant. FBP and KETO exhibited typical reversed-phase (RP) behaviours of decreased retention with increased ACN proportion in the eluent (Fig. 1) on the SB_{1-4}-polystyrene-divinylbenzene (PS/DVB) stationary phases. There are two reasons for this, the low polarity of the columns and the low hydrophilicity (log P_{ow}) of FBP and KETO, as shown in Table-1.

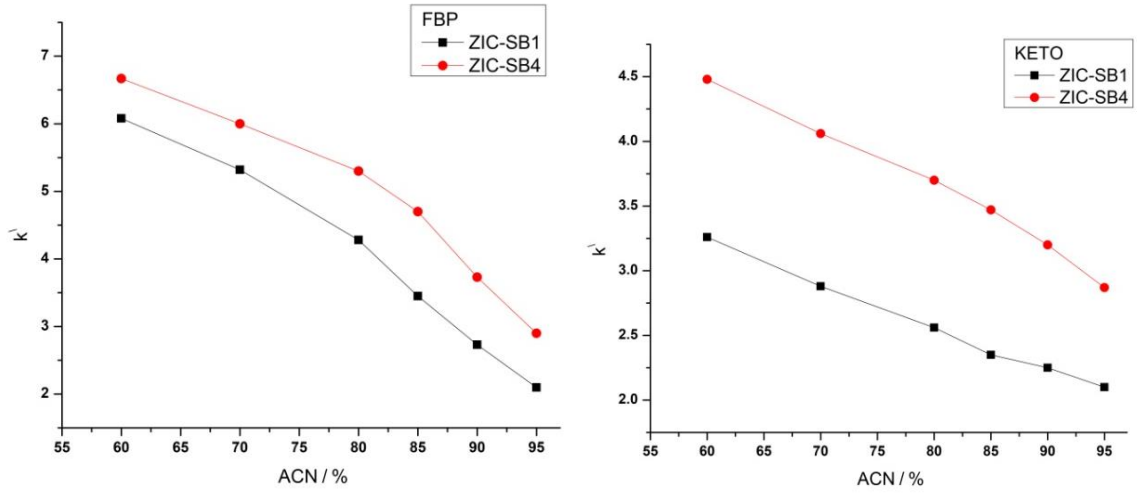

Fig. 1: Effect of ACN content on the FBP and KETO.

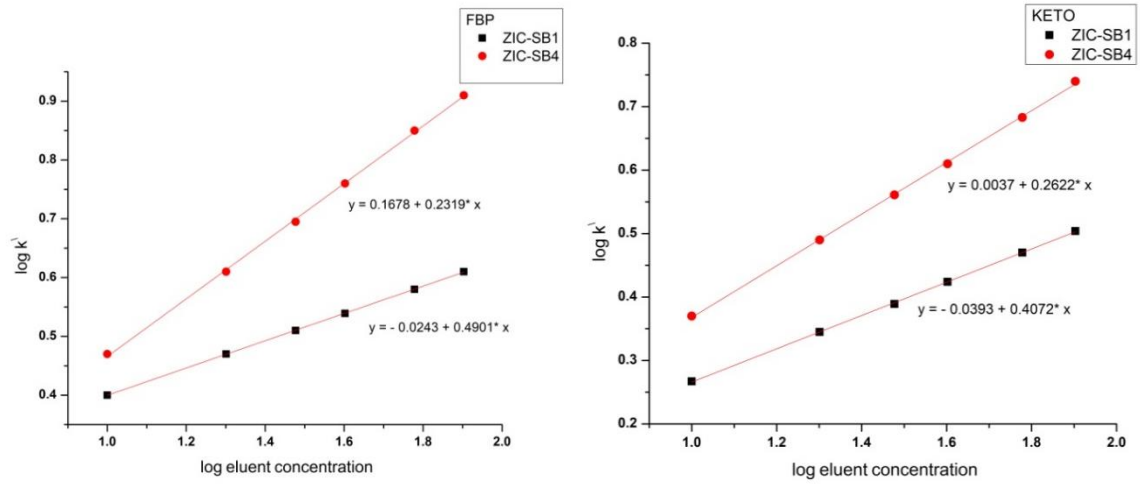

Fig. 2: Effect of eluent strength on the FBP and KETO.

Effect of buffer concentration on NSAIDs retention

The concentration of the aqueous mobile phase buffer is another parameter that affects the retention characteristics of ZIC-HILIC. When the log eluent strength increased from 1.3 to 1.9 mM (pH 4.75) at 80% ACN constant, the retention of FBP and KETO increased (Fig. 2) due to increased electrostatic interaction between the positively charged sulfobetaine stationary phase and the negatively charged FBP and KETO. Due to the complexity of the separations, it is impossible to examine the separation mechanisms individually. However, it can be shown that hydrophilicity and electrostatic interactions observed in the ZIC have a role. In addition, it appears that separating the mobile and pseudo-stationary phases influences chromatographic separations. The separation of NSAID models essentially depends on forming a pseudo-stationary water layer above the stationary phase, between which the rich organic solvent mobile phase rapidly partitions.

Effect of NSAIDs retention on pH buffer

The buffer pH varied between 3 and 5.5 in a 40 mM acetate buffer and 80% ACN constant to maximise chromatographic separation. Due to the positively charged quaternary amino groups in the sulfobetaine stationary phases. As seen in (Fig. 3, the retention factors for FBP and KETO increased with increasing pH values. The carboxylic acid functional groups in FBP and KETO examined are negatively charged in a solution with a pH range of 3 to 5.5 because the FBP and KETO of interest are weak acids with pKa values ranging between 4.42 and 3.84, respectively [36].

Optimum separation of NSAIDs

The percentages of acetonitrile, the concentration of acetate buffer, and the pH in the mobile phase were all factors in optimizing the ZIC-HILIC method on the ZIC-SB1 and ZIC-SB4 stationary phases. The chromatograms of FBP and KETO are shown in (Fig. 4. Chromatograms were carried out in a solution of 80% ACN and 60 mM acetate buffer (pH 4.75). The ZIC-SB4 stationary phase retains substantially more target drugs than ZIC-SB1 stationary phase. The presence of methylene groups between charged groups in ZIC-SB stationary phases is unavoidably the reason for this [23, 25, 26, 33]. The highest retention of target pharmaceuticals in the ZIC-SB4 stationary phase is often due to the geometrically arranged sulfobetaine stationary phases.

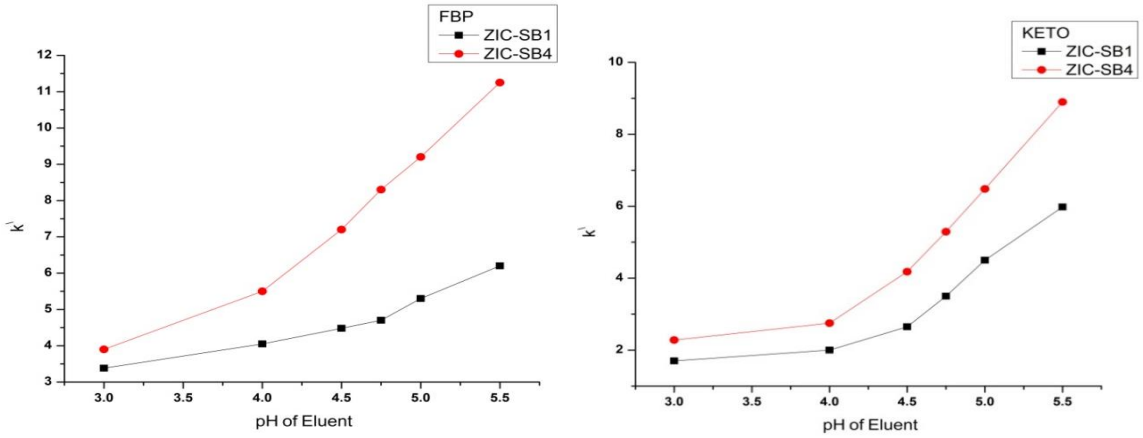

Fig. 3: Effect of eluent pH on the FBP and KETO.

Table-2: Linearity, regression equation, determination coefficient (R2), LOD, and LOQ of FBP and KETO using ZIC-SB1 and ZIC-SB4 stationary phases.

Parameter		ZIC-SB1	ZIC-SB4
y = a + b *x	FBP	y = 203.76 + 409.75*x	y = 1040.68 + 557.61*x,
	KETO	y = 149.65 + 216.05*x	y = 344.90 + 247.93*x
Linearity (ppm)	FBP	0.015-6.0	0.015-.6.0
	KETO	0.06-4.5	0.06-4.5
R^2	FBP	0.9998	0.9996
	KETO	0.9991	0.9997
LOD (ppm)	FBP	0.009	0.006
	KETO	0.010	0.0080
LOQ (ppm)	FBP	0.027	0.018
	KETO	0.030	0.0242

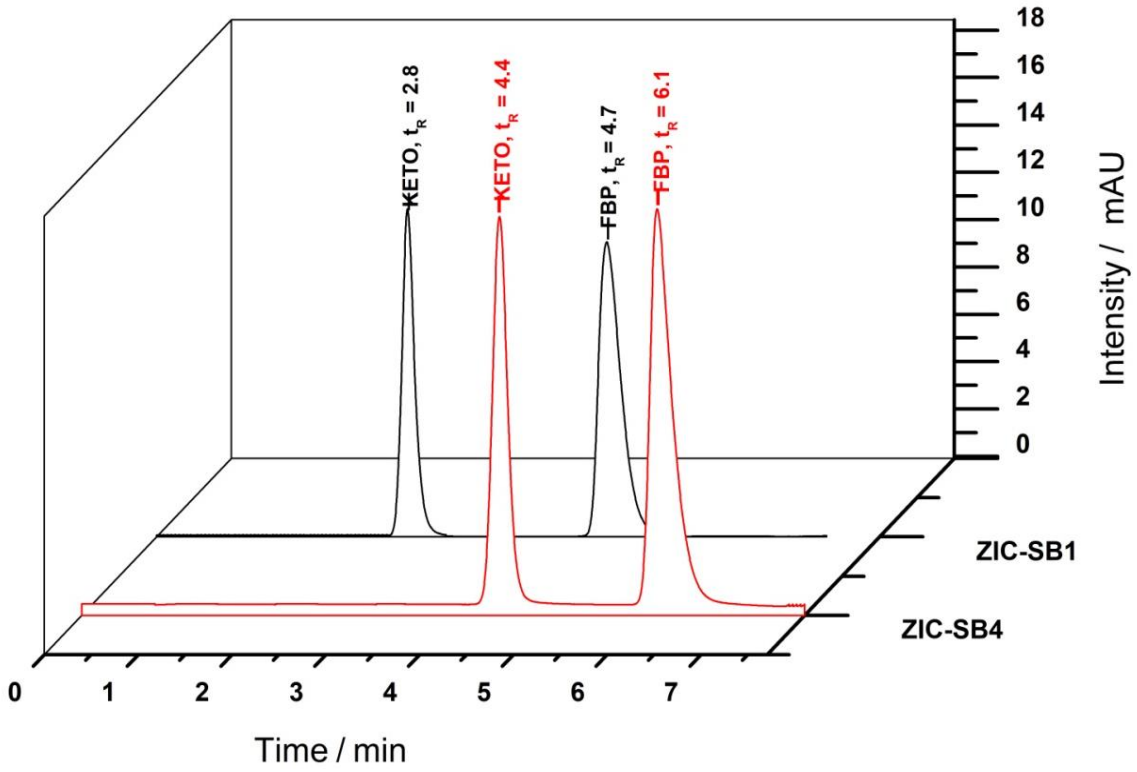

Fig. 4: Chromatogram for the separations of FBP and KETO using ZIC-SB1 and ZIC-SB4 stationary phases.

Table-3: Precision and accuracy of the developed ZIC-HILIC methods.

	Intra-Day Analysis n=5			Interday Analysis n=5		
FBP Taken (ppm)	**FBP Found (ppm)**	**% Rec.**	**%RSD**	**FBP Found (ppm)**	**% Rec.**	**%RSD**
			ZIC-SB1			
0.800	0.795	99.37	0.45	0.798	99.75	0.48
1.000	0.990	99.00	0.48	0.990	99.00	0.52
3.000	2.988	99.60	0.40	2.990	99.66	0.42
			ZIC-SB4			
0.800	0.790	98.75	0.38	0.790	98.75	0.42
1.000	0.994	99.40	0.40	0.996	99.60	0.40
3.000	2.990	99.66	0.46	2.992	99.73	0.55
KETO Taken (ppm)	**KETO Found (ppm)**	**% Rec.**	**%RSD**	**KETO Found (ppm)**	**% Rec.**	**%RSD**
			ZIC-SB1			
0.700	0.693	99.00	0.32	0.695	99.28	0.34
0.900	0.905	100.55	0.35	0.903	100.33	0.40
1.300	1.292	99.38	0.21	1.295	99.61	0.29
			ZIC-SB4			
0.700	0.706	100.85	0.38	0.705	100.71	0.34
0.900	0.891	99.00	0.41	0.893	99.22	0.43
1.300	1.290	99.23	0.30	1.290	99.23	0.33

Table-4: Determination of FBP and KETO in pharmaceutical formulations using ZIC-SB1 and ZIC-SB4 columns

Formulations	Present (mg)	Get it (mg)	%Rec. n=5	%RSD
		ZIC-SB1		
Fortine-FBP-Tablet	100	99.00	99.00	0.28
Zero-p-FBP-Tablet	100	100.40	100.40	0.22
Maximus-FBP-Tablet	100	98.00	98.00	1.55
		ZIC-SB4		
Fortine-FB--Tablet	100	98.60	98.60	0.32
Zero-p-FBP-Tablet	100	100.60	100.60	0.38
Maximus-FBP-Tablet	100	98.60	98.60	1.22
		ZIC-SB1		
Derelax-KETO-Tablet	10	10.07	100.70	0.81
Ketro-KETO-ampoule	30	29.70	99.00	0.76
Ketorom-KETO-ampoule	30	29.63	98.76	0.87
		ZIC-SB4		
Derelax-KETO-Tablet	10	10.05	100.50	0.73
Ketro-KETO-ampoule	30	29.55	98.50	0.82
Ketorom-KETO-ampoule	30	29.78	99.26	0.76

Table-5: Comparing the suggested methods to the British pharmacopoeia protocol [37].

Formulations	ZIC-SB1	ZIC-SB4	Standard method [37]	t-Test (theor.)	F-Test (theor.)
Fortine-FB--Tablet	99.00	98.60	100.50	0.4461* (2.7764)	0.6145* (19.000)
Zero-p-FBP-Tablet	100.40	100.60	98.50		
Maximus-FBP-Tablet	98.00	98.60	99.26	0.5159** (2.7764)	0.6708** (19.000)
Derelax-KETO-Tablet	100.70	100.50	100.77		
Ketro-KETO-ampoule	99.00	98.50	99.00	0.9725* (2.7764)	1.1016* (19.000)
Ketorom-KETO-ampoule	98.76	99.26	98.63	0.9574** (2.7764)	1.2815** (19.000)

ZIC-SB1
****ZIC-SB4***

Validation of the method

The developed methods were validated concerning the following parameters: linearity, regression equation, determination coefficient (R^2), LOD, and LOQ (Table 2). The proposed method's linearity was evaluated by evaluating FBP and KETO working standard solutions at six to seven concentrations within the working range. Five times each concentration was injected. The results provide high correlations within the concentration ranges evaluated, implying the proposed methods are linear. Accuracy and precision were determined using three different concentrations of FBP and KETO. Each drug was injected five times at three different concentrations. The accuracy and precision of intra- and interday measurements were determined using the Recovery and RSD percentages (Table-3).

Determination of flurbiprofen and ketorolac in market formulations

The developed methods (ZIC-SB1 and ZIC-SB4) were successfully used to determine the flurbiprofen and ketorolac contents in pharmaceutical formulations; the findings are shown in Table 4. The results matched data collected while evaluating the ZIC-HILIC methods' competence and efficacy in the British pharmacopoeia protocol [37]. The t-test and F-test variance ratios with a confidence level of 95 percent are used (Table 5). The t and F values for the measurements were kept within theoretical limits to guarantee that the accuracy of the two methods for determining flurbiprofen and ketorolac in pharmaceutical formulations was not considerably different.

Conclusion

Two ZIC-HILIC methodologies have been designed and effectively applied to quantify two NSAIDs (flurbiprofen and ketorolac) under customised elution circumstances and in pharmaceutical formulations. The ZIC-HILIC methodologies explored thus far have demonstrated a mixed-mode retention mechanism. To our knowledge, there are no documented methods for determining NSAIDs concurrently, such as flurbiprofen and ketorolac in dosage forms using different chain lengths on sulfobetaine stationary phases with UV detection. This method demonstrates that an appropriate level of sensitivity was achieved and that the approach is highly accurate and reproducible. The proposed methods were assessed by comparing their results to

those obtained from the official method (British pharmacopoeia) using a variety of pharmaceutical formulations. The results were highly accepTable, demonstrating the study's development of robust analytical techniques. It should be mentioned that when flurbiprofen and ketorolac were used, the ZIC-SB4 stationary phase with a long chain length demonstrated a longer retention time and a lower detection and limit of quantification than the ZIC-SB1 stationary phase.

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