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Quantification of ketoprofen in film-coated tablets using hydrophilic interaction liquid chromatography-Ultraviolet spectrometry

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Abstract

A simple zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) method was developed and applied to film-coated tablets to determine ketoprofen. Separation was performed using gradient elution with a mixture of acetonitrile and acetate buffer at an Ultraviolet (UV) detection wavelength of 255 nm on self-constructed ZIC-HILIC-1 and ZIC-HILIC-4 columns. The hydrophilicity of ketoprofen was validated by examining its behaviour with varied acetate buffer, acetonitrile proportions, and pH values. The mechanism of separation is based on ketoprofen partitioning in the hydrophobic interaction. The two available approaches are a viable alternative to the present ketoprofen separation methods. The developed ZIC-HILIC methods had an excellent precision (0.61%), linear ranges of 0.05-10 ppm, and detection limits of 0.01-0.008 ppm with a coefficient of 0.9995 0.9997 for the ZIC-HILIC-1 and ZIC-HILIC-4 columns, respectively. The methods' findings were compared to the British Pharmacopoeia procedure using statistical testing, which revealed no difference in accuracy between the methods.

Keywords: ZIC-HILIC, Pharmaceutical formulation, ketoprofen, NSAIDs, HPLC

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used worldwide for analgesic, anti-inflammatory, and antipyretic properties [1]. Globally, NSAIDs are the most frequently prescribed drugs, accounting for around 5–10% of all medications prescribed each year [2]. Because the elderly are frequent users of NSAIDs [3-5] and are heavily involved in prescription and non-prescription drugs [6], they are particularly vulnerable to polypharmacy, drug-drug interactions, and eventually drug-related problems and death [7-9]. Serious/fatal gastrointestinal issues, including ulcers and bleeding, have frequently been documented with prolonged NSAID use [10], and hence, co-prescribing gastroprotective medications is critical for avoiding such hazards [11]. In older adults, it was estimated that NSAIDs were likely responsible for 29% of fatal peptic ulcer complications [12]. Ketoprofen (Fig.1) is a nonsteroidal anti-inflammatory and analgesic (NSAID) medication used to treat osteoarthritis. In patients with rheumatoid arthritis, it lowers joint swelling. Additionally, it was compared to nonsteroidal anti-inflammatory drugs such as ibuprofen, aspirin, and indomethacin. Ketoprofen is frequently prescribed to treat a variety of ailments, most notably epicondylitis, frozen shoulder, and tendinitis [13]. It has analgesic, anti-inflammatory, and antipyretic actions. It is a racemic combination where only the S-isomer has cyclo-oxygenase inhibitory activity, whilst the R-isomer is significantly less effective [14]. The abstract of the literature survey used to estimate Ketoprofen with HPLC is shown in Table 1.

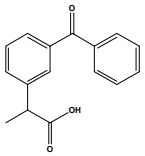

Fig. 1. Structure of ketoprofen.

Hydrophilic interaction chromatography (HILIC) is liquid chromatography (LC) approach that combines a polar stationary phase with a mobile phase with high water content while increasing the amount of a less polar solvent. Separations usually are carried out with 5–40% water; the method is also compatible with gradient elution. Alpert proposed the name HILIC in 1990 to describe its concepts and their significant uses [15]. In HILIC, hydrophilic and polar molecules are retained preferentially over hydrophobic neutral chemicals, unlike RP-HPLC.

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Table 1. Review of the literature on the determination of ketoprofen using HPLC techniques.

Column	Mobile phase	Detection	Statistical data analysis	Application	Ref.
C18	ACN- potassium phosphate acid (0.05 M, pH 4.5)- H_20 (43: 2: 55, v/v/v)	UV at 233 nm	LOD = 2.67 ng ml−1 LOQ = 4 ng ml−1 (R.S.D.=0.456 < 2%)	Pharmaceutical formulation	[28]
C18	methanol, ACN and 1.5 % sodium acetate solution (15:35:50 v/v/v) as mobile phase	UV-detection at 240 nm.	LOD = 0.2 µg/ml LOQ = 1.0 µg/ml	Gel formulation	[29]
C18	((1%) Triethylamine aqueous buffer adjust pH=2 by H_3PO_4 (85%): Methanol: ACN); (35: 20: 45 v\v%)	UV-Vis detector at 220 nm	(correlation coefficient=0.999) accuracy was > 99%. LOD µg/ml= 0.04 LOQµg/ml = 0.06	Pharmaceutical Drugs	[30]
C18	mixture of ACN: 0.2 % formic acid (60:40, v:v)	Diode array 255 nm	LOD/µg L–1 = 0.08 LOQ/µg L–1 = 0.26	River water and wastewater	[31]
HS C18	methaol: H_20 (70:30) adjusted to pH 3.3 with phosphoric acid	UV detector at 260 nm	R^2= 0.9999 LOD = 0.122µgmL^{-1} LOQ= 0.244µgmL^{-1}	Human Plasma	[32]
C18 mBondapack	ACN—0.05 M KH_2PO_4 buffer (60:40, v/v) as mobile phase	UV detection at 254 nm	(r . 0.9999) Recovery = 100.2% RSD = 0.6% LOD = 0.016 mg/mL LOQ = 0.08 mg/mL	Pharmaceutical form.	[33]
Zorbax SB-C18	55:45 (v/v) mixture of ACN and 1% trifluoroacetic acid	UV at 257 nm	r =0.9997 precision= 6.0 %) accuracy =< 4.8 %) LLOQ = 153 ng/mL	Human plasma	[34]
C18	Methanol: H_20 (50:50v/v)	photodiode diode array at 233nm	correlation coefficient = 0.999 LOD = 0.087µg/mL LOQ = 0.77µg/mL	Gel formulation	[35]
Zorbax Eclipse plus C18	ACN –acetate buffer (pH 3.2, 25 mM) (60:40) (v/v)	ultraviolet detector 230 nm	LOQ = 0.21 µg/ L LOD = 0.63 µg/ L	Pharmaceutical drug	[36]
chiral columns	n-hexane:ethanol: formic acid, 95:5:0.1 (v/v/v)	UV detection at 254 nm	LOD = 0.033µg/ml LOQ = 0.10µg/mL (R2) = 0.9999	Dexketoprofen Formulations	[37]

Despite numerous investigations for the separation and determination of ketoprofen in HPLC (Table 1), there is no published literature in this subject of estimating and separating ketoprofen utilizing ZIC-HILIC methods. Thus, Rasheed and colleagues have recently observed a remarkable rise in the estimate of pharmaceuticals, nucleosides, carboxylic acids, inorganic ions, and amino acids using HILIC technology [16-26]. Additionally, the influence of the chain longitude of the ZIC-HILIC columns on ketoprofen retention behaviour has not been examined previously. Rasheed and colleagues [19, 27] have investigated the influence of chain length on HILIC columns and its effect on the Ranitidine and esculin behaviour. They discovered that the longer the chain length, the more interaction between the Ranitidine and esculin and the ZIC-HILIC column occurs, resulting in a longer retention time. The final goal was to develop a simple method for quantifying ketoprofen in pure and pharmaceutical samples.

Experimental

Chemicals and reagents

Ketoprofen ≥98% (TLC) purchased by Sigma-Aldrich. Acetic acid was purchased from BDH. Sodium acetate was obtained for Fluka. From Sigma-Aldrich obtained acetonitrile (HPLC-grade). Millipore filters (0.45 µm) were employed to purify solutions. Millipore water conductivity of 18.2 MΩ (System-US Millipore) was used. Film-coated tablets of three different commercial companies (100 mg) were purchased from the local pharmacy: Profenid-Sanofi Winthrop Industrie-France, Keto-ACME Laboratories Ltd.-Bangladesh, Ketoprofen-Novell pharmaceutical laboratories-Indonesia

Preparation of stock solution for ketoprofen

A ketoprofen stock solution (50 µg/mL) was made by dissolving exactly the ketoprofen amount (2.5 mg) in 50 mL of acetonitrile. The stock solution was filtered through a 0.45 µm filter.

Preparation of pharmaceutical dosage forms

Thirteen film-coated tablets were collected for each of the three pharmaceutical dosage forms. About

100 mg ketoprofen was dissolved in a suitable volume of acetonitrile and diluted with acetonitrile to the mark in a 100 mL volumetric bottle. Millipore filters (0.45 µm) were then used to filter the solution. Other standard solutions were created by diluting the stock solution.

Chromatographic condition and instrumentation:

The Merck-Hitachi HPLC has a 10 µL injection loop equipped with a gradient pump L-6200 and a UV detector L-4200. The chromatographic analysis data was monitored using the N2000 Data Workstation program. The UV area with a wavelength of 255 nm was employed to identify ketoprofen. For the separation of ketoprofen, the ZIC-HILIC-1 and ZIC-HILIC-4 homemade columns were created on the poly (styrene-divinylbenzene), utilizing a grafted sulfobetaine monomer (100 mm x 4 mm I.D) in PEEK columns [22]. The numbers 1 and 4 in the ZIC-HILIC-1 and ZIC-HILIC-4 columns indicate the presence of methylene groups between the charged groups in sulfobetaine monomers. Raskop et al. [37] devised a systematic cycle for the grafting reaction. The capacity of ZIC-HILIC-1 and ZIC-HILIC-4 handmade columns was 432 and 488 μeqg^{-1} [22], respectively.

Results and Discussion

Effect of ketoprofen retention on acetonitrile proportion

The effect of acetonitrile proportion variation on ketoprofen retention was investigated using an acetate buffer (pH 4.75-30 mM). Ketoprofen retention typically decreases from 60% to 95% in the acetonitrile percentage (Fig. 2). It is attributed to ketoprofen's hydrophilicity; the hydrophobic behaviour of ketoprofen as shown in two handmade columns (ZIC-HILIC-1 and ZIC-HILIC-4), as a result of the ketoprofen $LogP_{OW}$ value (2.82).

Eluent concentration impact on retention of ketoprofen

The effect of acetate buffer modification on the retention behaviour of ketoprofen has been documented in the mobile phase of ketoprofen at a concentration of 10-80 mM (pH 4.75) in 85% acetonitrile. Fig. 3 illustrates the results of this effect on ZIC-HILIC-1 and ZIC-HILIC-4 columns. By increasing the buffer concentration in the eluent, the ketoprofen retention factor is decreased. The cause for ketoprofen's behaviour is the drug's low hydrophilicity.

Eluent pH effect on ketoprofen retention

With a change in eluent pH, the following enhanced eluent composition can be used. The eluent pH must be adjusted to achieve complete ketoprofen separation in ZIC-HILIC mode. At a constant buffer concentration of 30 mM and an acetonitrile proportion of 85, the pH increased from 3 to 5.5. As illustrated in Fig. 4, the retention factor for ketoprofen increases on ZIC-HILIC-1 and ZIC-HILIC-4 columns. That is because ketoprofen has a deprotonated hydroxyl group. That depicts the anticipated physicochemical properties of ketoprofen. Ketoprofen has a pKa value of 3.88, indicating an anionic compound.

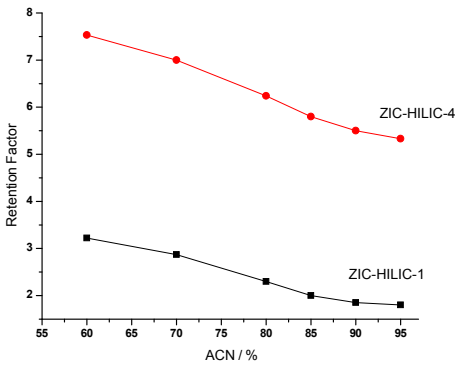

Fig. 2. Acetonitrile proportion impact on ketoprofen retention on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

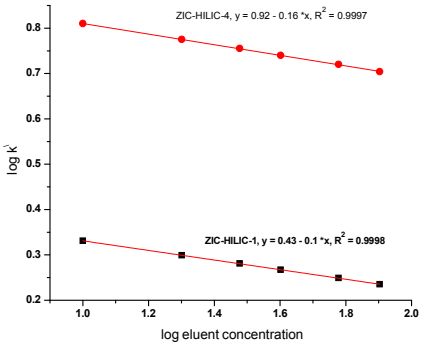

Fig. 3. Acetate buffer impact on ketoprofen retention on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

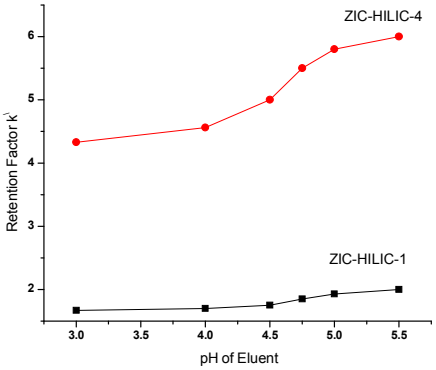

Fig. 4. Acetate buffer pH impact on ketoprofen retention on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

Ketoprofen separation optimization

After determining the optimal conditions for ketoprofen's separation mechanism using ZIC-

HILIC-1 and ZIC-HILIC-4 columns, the chromatograms (Fig. 5) were prepared using 85 acetonitrile proportion and 30 mM-pH 4.75 acetate buffer.

The retention of ketoprofen showed the highest retention in ZIC-HILIC-4 stationary phase compared to ZIC-HILIC-1 stationary phase. The inescapable explanation for this is that the methylene groups in stationary phases between charged groups are likely to be the geometrical arrangement of the sulfobetaine groups.

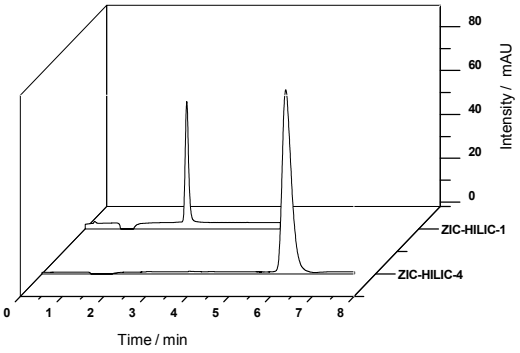

Fig. 5. Chromatograms for the separation of ketoprofen on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

Method validation

As illustrated in Fig. 6, the linearity (0.05-10 ppm) of two methods using ZIC-HILIC-1 and ZIC-HILIC-4 columns with ketoprofen can be observed. For thorough testing of ketoprofen under ZIC-HILIC conditions, the appropriate calibration graphs and statistical data in Table 2 were used. The precision and percentages of recovery and RSD were calculated on the same day. The relatively low default rate and high recovery values indicate a successful method (Table 3).

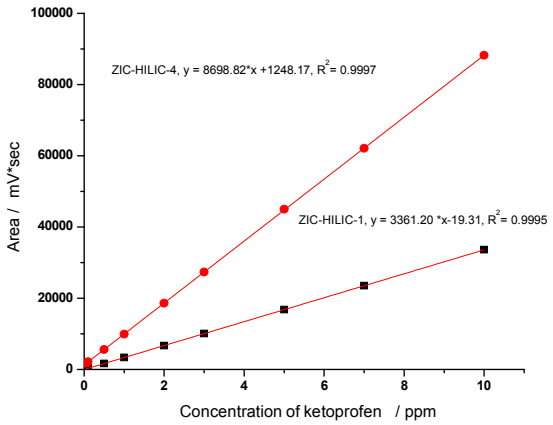

Fig. 6. Calibration graphs for ketoprofen on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

Ketoprofen content determination in film-coated tablets.

The proposed methods were effectively applied in analyzing ketoprofen for three film-coated tablets; the results are presented in Table 4. The findings were compared to the data acquired during the assessment of the ZIC-HILIC methodologies' competence and efficacy in the British Pharmaceuticals protocol. The 95% confidence t-test and F-test variance ratio (Table 5) was used for data analysis. The t and F values of the measurements were kept within theoretical values to ensure that the accuracy of ketoprofen determination in three film-coated tablets was not significantly different between the two methods.

Table 2. Linear regression data of ketoprofen on ZIC-HILIC-1 and ZIC-HILIC-4 columns.

Parameter	ZIC-HILIC-1 method	ZIC-HILIC-4 method
Linearity (ppm)	0.05-10	0.05-10
Regression equation	y = 3361.20 *x-19.31	y = 8698.82*x +1248.17
r^2	0.9995	0.9997
LOD (ppm)	0.010	0.008
LOQ (ppm)	0.030	0.024

Conclusion

This article examines the use of ZIC-HILIC methods to evaluate ketoprofen in film-coated tablets. That flexible separation is advantageous since ZIC-HILIC traders with one and four methylene groups exhibit at least two distinct holding modes between charged groups under various conditions, which may result from the ZIC-HILIC-4 column's geometric orientation. The data imply that hydrophobic behaviour is the mechanism behind retention. It's interesting to note that ketoprofen has a longer retention time and a lower LOD and LOQ when used with the ZIC-HILIC-4 stationary phases compared to the ZIC-HILIC-1 stationary phases. The established methods have been successfully applied to film-coated tablets.

Table 3. Accuracy and precision of the proposed methods.

	Intraday-analysis n=5			Interday-analysis n=5		
ZIC-HILIC-1 method						
ketoprofen Taken ppm	**ketoprofen Found ppm**	**%Rec.**	**%RSD**	**ketoprofen Found ppm**	**%Rec.**	**%RSD**
1.00	0.992	99.20	0.37	0.996	99.60	0.41
2.50	2.492	99.68	0.33	2.496	99.84	0.33
3.00	3.050	101.66	0.16	3.010	100.33	0.25
ZIC-HILIC-4 method						
1.00	0.996	99.60	0.54	0.998	99.80	0.61
2.50	2.502	100.02	0.39	2.504	100.16	0.43
3.00	2.992	99.73	0.29	2.994	99.80	0.29

Table 4. The proposed methods of determining ketoprofen in film-coated tablets.

Name of drug	Present (mg)	Get it (mg)	%RSD n=5	%Rec.
	ZIC-HILIC-1 method			
Profenid-Sanofi	100	101.00	0.75	101.00
Keto-ACME	100	99.55	0.39	99.55
Ketoprofen-Novell	100	99.05	0.61	99.05
	ZIC-HILIC-4 method			
	100	100.63	0.81	100.63
	100	100.22	0.51	100.22
	100	99.43	0.55	99.43

Table 5: Comparison of the proposed methods with the British Pharmaceuticals protocol [38].

Name of drug	ZIC-HILIC-1 method	ZIC-HILIC-4 method	Standard method [38]	t-Test (theor.)	F-Test (theor.)
Profenid-Sanofi	101.00	100.63	100.74	0.7969* (2.7764)	2.8804* (19.000)
Keto-ACME	99.55	100.22	99.66		
Ketoprofen-Novell	99.05	99.43	99.76	0.9391** (2.7764)	1.0446** (19.000)

ZIC-HILIC-1 method
****ZIC-HILIC-4 method***

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