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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DETERMINATION OF pK_a VALUES OF TENOXICAM, PIROXICAM AND MELOXICAM BY RP-HPLC AT 25°C AND 37°C IN THF-WATER BINARY MIXTURES

TENOKSİKAM, PİROKSİKAM VE MELOKSİKAM'IN pK_a DEĞERLERİNİN RP-HPLC YÖNTEMİYLE 25°C VE 37°C'DE THF-SU İKİLİ ORTAMINDA TAYİNİ

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ABSTRACT

Objective: *In this study, the ionization constant (pK_a) values of oxicam group drug active ingredients, tenoxicam, piroxicam and meloxicam, were investigated both because of their effectiveness in reducing pain and inflammation and because of their pharmaceutical importance.*

Material and Method: *pK_a values were determined by RP-HPLC method in tetrahydrofuran-water binary mixture (30%-40%(v/v)) at 25°C and 37°C. The pK_a values of these compounds in water were evaluated by mole fraction and Yasuda-Shedlovsky extrapolation methods.*

Result and Discussion: *This study is the first pK_a determination study for tenoxicam, piroxicam and meloxicam in tetrahydrofuran-water media and also at 37°C, which is body physiological temperature. For tenoxicam, piroxicam and meloxicam, the pK_a values calculated by the mole fraction method at 25°C were 5.067 ± 0.037; 5.237 ± 0.065; 4.027 ± 0.144; pK_a values at 37°C are 5.166 ± 0.017; 5.197 ± 0.084; 4.161 ± 0.116. By Yasuda-Shedlovsky extrapolation, pK_a values calculated at 25°C were 5.061 ± 0.035; 5.232 ± 0.063; 4.021 ± 0.141; pK_a values at 37°C are 5.161 ± 0.013; 5.192 ± 0.053; 4.155 ± 0.094. The results are in agreement with previous studies with different methods and different solvents at 25°C.*

Keywords: *Oxicam, RP-HPLC, THF-water binary mixtures*

ÖZ

Amaç: *Bu çalışmada, oksikam grubu ilaç etken maddeleri olan tenoksikam, piroksikam ve meloksikamın hem ağrı ve iltihabı azaltmadaki etkinlikleri hem de farmasötik önemi nedeniyle iyonizasyon sabiti (pK_a) değerleri araştırıldı.*

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Gereç ve Yöntem: *pK_a değerleri, 25°C ve 37°C'de tetrahidrofuran-su ikili karışımında (%30-40(h/h)) RP-HPLC yöntemiyle belirlendi. Bu bileşiklerin sudaki pK_a değerleri, mol kesri ve Yasuda-Shedlovsky ekstrapolasyon yöntemleri ile değerlendirildi.*

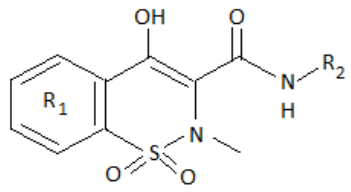
Sonuç ve Tartışma: *Tenoksikam, piroksikam ve meloksikam için 25°C'de mol kesri yöntemiyle hesaplanan pK_a değerleri 5.067 ± 0.037; 5.237 ± 0.065; 4.027 ± 0.144; 37°C'de pK_a değerleri 5.166 ± 0.017; 5.197 ± 0.084; 4.161 ± 0.116. Yasuda-Shedlovsky ekstrapolasyonu ile 25°C'de hesaplanan pK_a değerleri 5.061 ± 0.035; 5.232 ± 0.063; 4.021 ± 0.141; 37°C'deki pK_a değerleri 5.161 ± 0.013; 5.192 ± 0.053; 4.155 ± 0.094. Sonuçlar, 25°C'de farklı yöntemler ve farklı çözücüler ile yapılan önceki çalışmalarla uyumludur. Bu çalışma, tetrahidrofuran-su ortamında ve ayrıca vücut fizyolojik sıcaklığı olan 37°C'de tenoksikam, piroksikam ve meloksikam için yapılan ilk pK_a belirleme çalışmasıdır.*

Anahtar Kelimeler: *Oksikam, RP-HPLC, THF-su ikili karışımı*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the best-selling over-the-counter medicines in the world, accounting for 5% of prescription medicines used for the treatment of patients suffering from chronic pain and inflammatory conditions [1]. They cover a wide range of drugs according to their structural and functional properties. NSAIDs are classified according to their chemical properties as major derivatives of salicylic acid, acetic acid, enolic acid, anthranilic acid or propionic acid. In general, they show weak organic acid properties because they have an acidic part such as carboxylic acid or enol attached to an aromatic functional group [1, 2]. Ionization constants (pK_a) range from 3 to 6 [2, 3].

Oxicams are enolic acid derivatives and are widely used NSAID drugs in the pain and inflammation of rheumatic diseases [2, 4]. Tenoxicam, meloxicam and piroxicam are oxicam group drugs, which are enolic acid derivatives with similar structural properties [2, 5] (Figure 1).



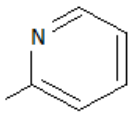
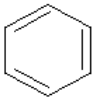
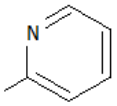
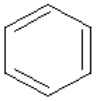
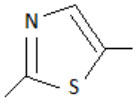
Compound Name	R_1	R_2
Tenoxicam		
Piroxicam		
Meloxicam		

Figure 1. Chemical structure of tenoxicam, meloxicam and piroxicam

When their chemical structures are examined, it is seen that while R_1 groups are different in tenoxicam and piroxicam, R_2 groups are different in meloxicam and piroxicam. Although they have different therapeutic activities depending on these similarities and differences in their structures, their mechanisms of action are similar. At the same time, due to their high lipophilicity, they cause side effects such as various gastrointestinal complications [1, 4]. Absorption, distribution, metabolism and excretion (ADME) mechanisms of these drugs in the body need to be improved in order to reduce their side effects and facilitate their use. In order to improve ADME properties, it is necessary to know the pK_a value, which affects on the properties of drugs such as solubility, lipophilicity, permeability and protein binding [6, 7]. With the pK_a value, it is possible to comment on the effect on the biological behavior of the molecule, depending on the pH of the media [6]. In particular, it is necessary to know the pK_a value in order to develop and design new drug active molecules, because with this value, it can be interpreted about the movement of drugs into cells and other membranes [8].

When the literature studies are examined, it is seen that there are comprehensive studies that include the methods used for the determination of pK_a [8, 9, 10, 11, 12]. In these methods, pK_a can be calculated based on the measurement of a physical property of the analyte. At the same time, there are studies on computer programs developed for the estimation of pK_a and their comparison with experimental results [9, 13, 14, 15, 16]. Among these methods, HPLC method is one of the preferred methods, especially in determining the pK_a of drugs that are poorly soluble in water, in addition to its features such as obtaining fast results with a small sample, high sensitivity and precision [8, 9, 17]. An organic solvent-water mixture is generally used to determine the pK_a of poorly water-soluble drugs [18, 19]. By performing several experiments in different organic solvent-water mixtures, the pK_a values in these media ($^{s}_{s}pK_a$) are determined and the pK_a values in water ($^{w}_{w}pK_a$) can be estimated by an extrapolation method such as Yasuda-Shedlovsky [8, 12, 14, 20, 21, 22]. The selected organic solvents consist of alcohols such as dimethyl sulfoxide, dimethylformamide, acetonitrile, acetone, dioxane, tetrahydrofuran (THF), which are widely used with this extrapolation method and have the ability to dissolve ionizable compounds [12]. Although there is no universal organic solvent-water binary mixture for the determination of pK_a, the most preferred solvent mixtures are methanol-water, acetonitrile-water and THF-water mixtures [12, 23].

When the literature is examined for the determination of pK_a values of oxicams, it is stated that the pK_a values of the compounds are determined by different methods. In these literatures, it was generally studied at 25°C for the determination of pK_a. The biorelevant temperature in humans is 37°C. Therefore, it is known that studies at this temperature are more beneficial in biologically occurring mechanical dissolution studies, and in elucidating intercellular transmission mechanisms by ionizable molecules [24].

In this study first time, pK_a values were calculated at both 25℃ and 37℃ by RP-HPLC method using THF-water organic solvent mixture (30%-40% (v/v)), which has not been used before in the pK_a determination of meloxicam, piroxicam and tenoxicam compounds. From the ${}^{s}_{s}pK_a$ values calculated for each media, ${}^{w}_{w}pK_a$ values were calculated using the Yasuda–Shedlovsky equation and the mole fraction-pK_a extrapolation method.

MATERIAL AND METHOD

Chemicals and Reagents

Meloxicam, piroxicam and tenoxicam, whose pK_a was determined by RP-HPLC, were obtained from Sigma Aldrich (Darmstadt, Germany). THF, the organic solvent used for the mobile phase, and uracil, the unretained species in the column, were supplied from Sigma Aldrich (Darmstadt, Germany). Sodium hydroxide used for adjusting the mobile phase pH was purchased from Merck (Darmstadt, Germany), and ortho-phosphoric acid was obtained from Riedel-de Haën (Seelze, Germany). Potassium hydrogen phthalate (KHP) used for pH meter calibration, were supplied from Merck (Darmstadt, Germany).

Apparatus

Agilent 1260 Infinity (Santa Clara, United States) brand High Performance Liquid Chromatograph device was used to determine the pK_a values of the studied compounds. The system includes 1260 Quat Pump VL pump, 1260 DAD VL detector, 1260 ALS automatic sample injection part.

Hanna HI 221 pH meter (Carrollton, Texas) device was used for pH measurements of RP-HPLC mobile phase and Hanna HI 1131 glass electrode was used for measurements. During the adjustment of the mobile phase pH, the temperature was kept constant at 25 °C.

As a column, ACE C18 (5 µm, 4.6 x 250 mm) column with low silanol activity was used. Thanks to this feature, it removes the negative effects on the chromatographic separation of silanols, and is preferred for the separation of both acidic and basic compounds. In addition, due to its high stability, its repeatability and peak shapes are also very good. The fact that it can work up to 100℃ in the pH range of 2.0-8.0 is one of the other reasons for preference of the column.

Chromatographic Procedures

The electrode was calibrated by keeping it in the working water-organic solvent mixture for 15-20 minutes so that the readings could be stable. THF-water binary mixtures were prepared with pH in the range of 3.0-7.0 for each water-organic solvent mixture. In these adjustment processes, 50 mM

phosphoric acid was used, taking into account the buffer range.

100 ppm stock solutions were prepared by weighing 1 mg of each of the compounds provided in analytical purity and dissolving them in 10 ml of mobile phase. 20 µl of these prepared solutions were injected into the liquid chromatography device. In the RP-HPLC study, the required to (dead time) value for the determination of the capacity factors was determined using uracil. In this study, the average retention factors of both uracil and compounds were determined by injecting twice for each compound. For the determination of the ionization constants of the compounds, THF-water binary mixture was used, the column temperature was determined as 25°C and 37°C, and the flow rate was 1 ml/min. The studied wavelenghts were 355 nm for compounds and 254 nm for uracil. For the determination of the ionization constant of the compounds, 30%, 35% and 40% (v/v) THF-water binary mixtures were used.

Data Treatment

The t_o value used to calculate the capacity factors of the compounds was calculated with the formula $k=(t_R-t_0)/t_0$ using uracil. The variation of the capacity factor values of the compounds with pH shows sigmoidal behavior. The pH value at the midpoint of this sigmoidal gives the pK_a value of that compound on the working media. The ${}^{s}_{s}pK_a$ values of the compounds were calculated by evaluating the capacity factor –pH relationship in 30%, 35% and 40% (v/v) THF-water binary media with the NLREG program. NLREG is a nonlinear regression program specially developed for calculating pK_a values from capacity factors and pH values [25]. Mole fraction extrapolation method and Yasuda–Shedlovsky equation were used for the transition from ${}^{s}_{s}pK_a$ values calculated from THF-water mixtures to ${}^{w}_{w}pK_a$ values in water.

In the mole fraction extrapolation method, a linear equation is obtained by plotting the pK_a values against the mole fraction values calculated depending on the volume percent of the organic solvent used in the mobile phase. The ${}^{s}_{s}pK_a$ value is calculated from the intercept value of this equation. Mole fraction values for THF-water mixtures were obtained from Barbosa, 1999.

Yasuda-Shedlovsky equation is another extrapolation method that helps to calculate ${}^{s}_{s}pK_a$ value from different organic solvent-water mixtures with Equation 1 based on Born electrostatic model [12, 21, 22].

$$ {}^{s}_{s}pK_a + \log[H_2O] = a_\varepsilon\, \varepsilon^{-1} + b_\varepsilon \qquad \text{Equation 1} $$

Here ${}^{s}_{s}pK_a$ gives the pK_a value of the mobile phase, ε the dielectric constant of the organic solvent in the water-organic solvent binary mixture, aε the slope value obtained from the linear equation, and bε the intercept value obtained from the linear equation. In order to calculate the ${}^{s}_{s}pK_a$ values in each media, the dielectric (ε) and autoprotolysis (pK_w) constants must be known. These values were obtained from literature sources [23, 26].

RESULT AND DISCUSSION

${}^{s}_{s}pK_a$ Values Determined by RP-HPLC

Acetonitrile-water, methanol-water and THF-water media, which are more preferred by RP-HPLC method in pK_a determination, were tested with both Agilent Zorbax Eclipse Plus C18 column and ACE C18 column at 25°C and 37°C. ACE C18 column, peak shapes and retention times in THF-water media were found suitable when evaluated in terms of both temperature conditions, since it provides a short retention time and the peak shapes are good.

In this study, 30%, 35% and 40% (v/v) THF-water mobile phase composition was studied. Considering the pK_a values of the enolic acid group in the structure of the compounds, the retention factors were determined in the pH range of 3.0-7.0. Chromatograms showing the retention times of the compounds at pH 3.0 and 7.0 are given in Figure 2. The relationship between the pH value and capacity factors in 30% (v/v) THF-water media obtained with the NLREG program gives a sigmoidal curve, as can be seen in Figure 3.

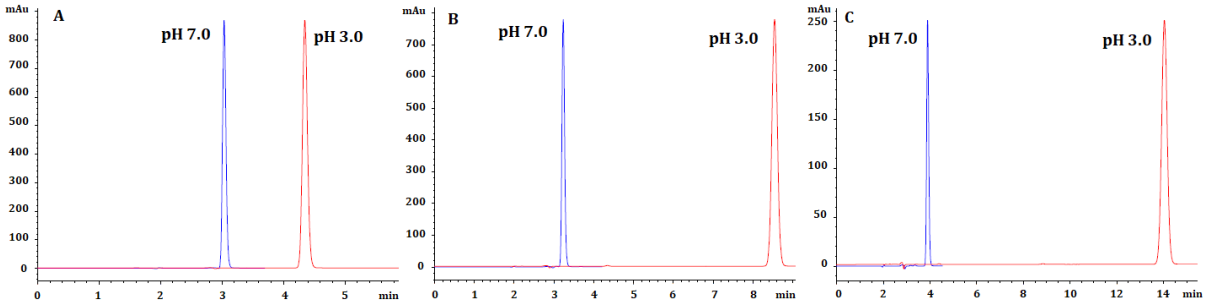


Figure 2. Chromatograms showing the retention times of the compounds at pH 3.0 and 7.0 in 35% (v/v) THF-water media at 37°C (A: tenoxicam, B: piroxicam, C: meloxicam)

The condensation of the benzene ring or thiophene ring in the structure of tenoxicam, meloxicam and piroxicam compounds with the heterocyclic system and also the presence of the amide group in the structure gives the enolic group acidic properties [5]. While tenoxicam has a thiophene ring that replaces the benzene ring in piroxicam; meloxicam is an analog of piroxicam and has 5 methylthiazole groups in the amidic part of the molecule instead of the pyridyl ring. (Figure 1). In this study, pK_a values of enolic acid group were determined for tenoxicam, piroxicam and meloxicam. The pK_a values of the compounds obtained from this study in THF-water media are given in Table 1. As can be seen from Table 1, an increase was observed in pK_a values as THF content increased. This can be explained by the increase in THF content, which has a low dielectric constant (Ɛ (H_2O)=78.36 and Ɛ (THF)=7.6), a decrease in the dissociation of electrolytes and a decrease in conductivity [26].

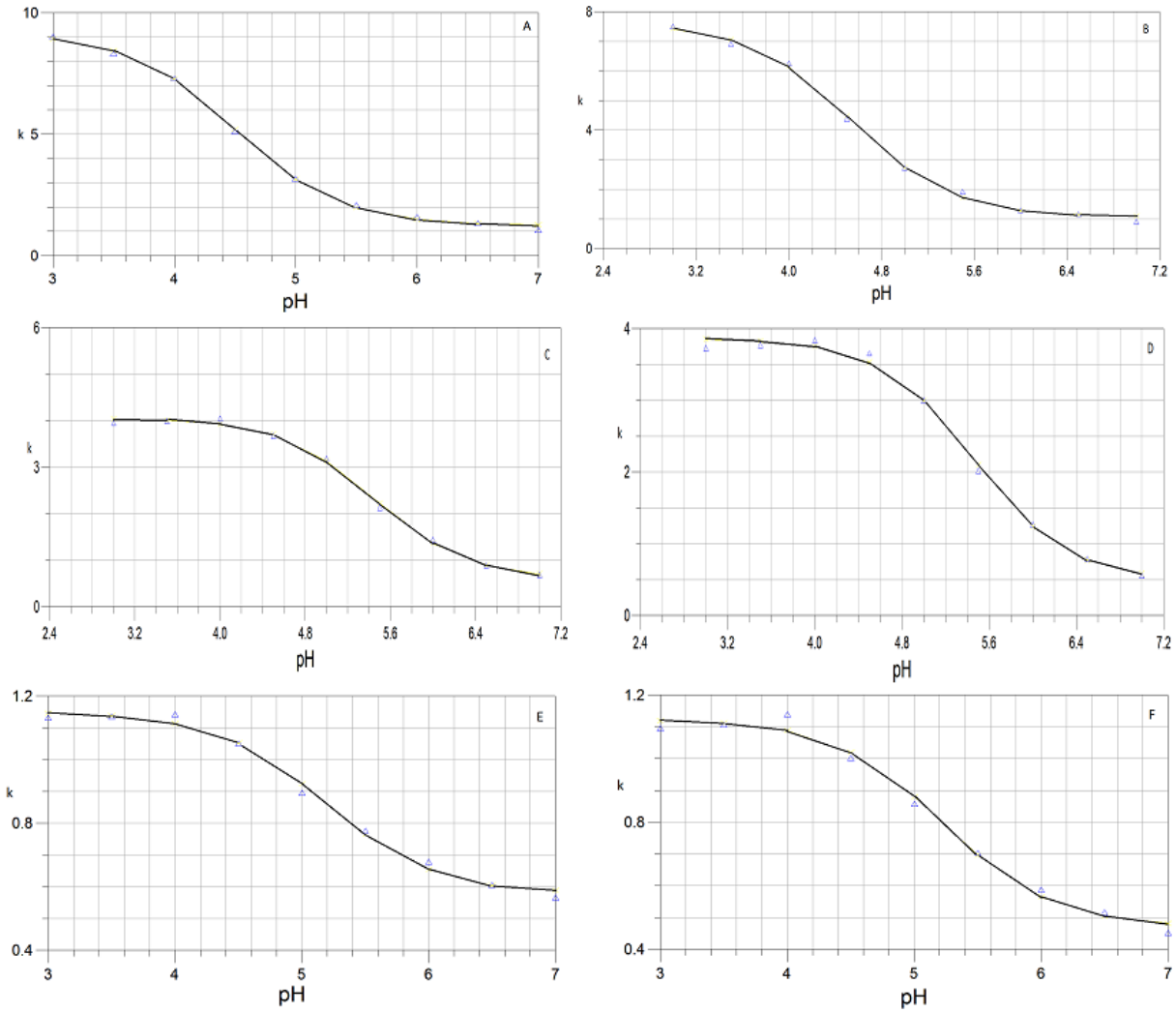


Figure 3. NLREG plots of compounds in 30% THF-water media of compounds (A: meloxicam at 25°C, B: meloxicam at 37°C, C: piroxicam at 25°C, D: piroxicam at 37°C, E: tenoxicam at 25°C, F: tenoxicam at 37°C.)

Table 1. The pK_a values of the studied compounds in THF-water mixture at 25°C and 37°C.

Temperature	THF%	Tenoxicam	Meloxicam	Piroxicam
25°C	30%	5.184 ±0.066[a]	4.497 ±0.032	5.444 ±0,040
	35%	5.225 ±0.052	4.609 ±0.041	5.512 ±0.067
	40%	5.258 ±0.176	4.782 ±0.015	5.574 ±0.064
37°C	30%	5.222 ±0.084	4.538 ±0.039	5.463 ±0.047
	35%	5.236 ±0.063	4.633 ±0.045	5.554 ±0.066
	40%	5.256 ±0.066	4.768 ±0.057	5.631 ±0.063

[a]: Standart deviation

David et al. obtained decreased pK_a values (belonging to the pyridinyl group) despite the increasing methanol percentage of oxicams in 40-60% methanol-water media by RP-HPLC method [27]. They concluded that this situation is related to the keto/enol equilibrium state of oxicam drugs in

the methanol-water media. In another study, 30-40% acetonitrile-water with RP-HPLC method and 30-45% acetonitrile-water media with potentiometric titration method, pK_a values (belonging to the enol group) increased with increasing acetonitrile percentage [20, 28]. Syatesh et al., with the spectrophotometric method, concluded that the pK_a values of both the enol group and the pyridinyl group of meloxicam increased with increasing ethanol percentage in 20-80% ethanol [29]. In this study, the pK_a values obtained in THF media are slightly higher than the values obtained in different solvent-water media in the literature. This is because in aprotic solvents such as THF, cationic forms adhere more than anionic form and pK_a values are higher in water-organic solvent media [30]. Kütt et al. show that pK_a values in THF-water media may differ by about 2-3 units [18].

Unlike other studies, the pK_a values were studied both at 25°C and at 37°C, which is the biorelevant temperature. The effect of temperature on pK_a depends on the structure of the functional group. With the increase in temperature, basic groups tend to protonate more easily, so a decrease was observed in the pK_a values of basic groups while an increase was observed in the pK_a values of acidic groups [24, 31, 32]. The pK_a values of the enolic acid group for tenoxicam, piroxicam and meloxicam increased with temperature, as seen in Table 1.

${}^{w}_{w}pK_a$ Values Obtained Using The Yasuda–Shedlovsky Equation and the Mole Fraction (X_{THF} – pK_a) Extrapolation Method

Yasuda-Shedlovsky mathematical equation and X_{THF}-pK_a extrapolation method were used to calculate ${}^{w}_{w}pK_a$ values from ${}^{s}_{s}pK_a$ values obtained from three different THF-water solvent media. The linear equations obtained from these methods are given in Table 2 and Table 3.

As can be seen in Table 2 and Table 3, positive slope values were obtained in the equations obtained by both extrapolation methods. This is because Tenoxicam, piroxicam and meloxicam are weakly acidic, so the acidity decreases with increasing THF content in the solution. Due to the similarity of the R_2 groups in Tenoxicam and Piroxicam, the slope values gave close results. Table 4 shows the ${}^{w}_{w}pK_a$ values of the compounds obtained from the X_{THF}–${}^{s}_{s}pK_a$ extrapolation method, the Yasuda Shedlovsky method, and the ${}^{w}_{w}pK_a$ values in the literature. It is seen that the ${}^{w}_{w}pK_a$ values obtained by both extrapolation methods are compatible with each other and the results are close to the literature values. The pK_a value of the enolic structure was found to be close to each other with the effect of the pyridinyl group in Tenoxicam and Piroxicam.

This is the first study in which the pK_a values of tenoxicam, piroxicam and meloxicam compounds were determined by RP-HPLC method in THF-water solvent media. The pK_a values of the enolic group in the structure of these compounds, which have low water solubility, were evaluated in THF-water solvent mixture in the range of 30-40%. ${}^{s}_{s}pK_a$ values were calculated with the NLREG program. Using these data, the ${}^{w}_{w}pK_a$ values of each compound were determined.

Table 2. Linear equations obtained from Yasuda-Shedlovsky extrapolation method of studied compounds at 25°C and 37°C.

Temperature	Compound	Yasuda Shedlovsky Equation	r
25°C	Tenoxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 25.966(1/Ɛ) + 6.474$	0.995
	Meloxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 105.69(1/Ɛ) + 4.418$	0.997
	Piroxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 46.939(1/Ɛ) + 6.377$	0.997
37°C	Tenoxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 11.111(1/Ɛ) + 6.763$	0.997
	Meloxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 84.871(1/Ɛ) + 4.817$	0.998
	Piroxicam	$\log[H_2O] + {}^{s}_{s}pK_a = 61.087(1/Ɛ) + 6.157$	0.995

Table 3. Linear equations obtained from the X_{THF}–${}^{s}_{s}pK_a$ method of extrapolation of the studied compounds at 25°C and 37°C

Temperature	Compound	X_{THF}–${}^{s}_{s}pK_a$	r
25°C	Tenoxicam	${}^{w}_{w}pK_a = 27.652\ X_{THF} + 4.714$	0.997
	Meloxicam	${}^{w}_{w}pK_a = 107.37\ X_{THF} + 2.657$	0.997
	Piroxicam	${}^{w}_{w}pK_a = 48.624\ X_{THF} + 4.617$	0.997
37°C	Tenoxicam	${}^{w}_{w}pK_a = 12.796\ X_{THF} + 5.003$	0.998
	Meloxicam	${}^{w}_{w}pK_a = 86.557\ X_{THF} + 3.056$	0.998
	Piroxicam	${}^{w}_{w}pK_a = 62.772\ X_{THF} + 4.397$	0.995

Table 4. ${}^{w}_{w}pK_a$ values of studied compounds at 25°C and 37°C and literature values

Temperature	Compound	${}^{w}_{w}pK_a$ values		
		X_{THF}–${}^{s}_{s}pK_a$	Yasuda Shedlovsky	Literature values
25°C	Tenoxicam	5.067 ± 0.037	5.061 ± 0.035	5.34 [33] 5.29 [27] 5.19-5.26 [20] 5.26 [34] 4.97 [35]
	Meloxicam	4.027 ± 0.144	4.021 ± 0.141	4.08 [3, 4, 33] 4.34 [27] 4.17 [34] 4.0 [29]
	Piroxicam	5.237 ± 0.065	5.232 ± 0.063	6.3 [3, 36, 37] 5.3 [4] 5.46 [33] 4.58 [27] 5.98-6.02 [20] 5.3 [38] 5.31 [34]
37°C	Tenoxicam	5.166 ± 0.017	5.161 ± 0.013	-
	Meloxicam	4.161 ± 0.116	4.155 ± 0.094	-
	Piroxicam	5.197 ± 0.084	5.192 ± 0.053	-

RP-HPLC method has been preferred because it gives fast and reliable results in the determination of pK_a in organic solvent-water mixtures. The results obtained showed that the pK_a values of these compounds were dependent on the pH of the capacity factor for different THF contents in the mobile phase.

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AUTHOR CONTRIBUTIONS

Concept: *D.B.D.*; Design: *D.B.D.*; Control: *D.B.D.*; Sources: *D.B.D.*; Materials: *D.B.D.*; Data Collection and/or processing: *D.B.D.*; Analysis and/or interpretation: *D.B.D.*; Literature review: *D.B.D.*; Manuscript writing: *D.B.D.*; Critical review: *D.B.D.*; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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