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Research Article

Determination of the Lipophilicity of Ibuprofen, Naproxen, Ketoprofen, and Flurbiprofen with Thin-Layer Chromatography

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The lipophilicity is an important parameter that influences the activity of the drugs in the human body. The reversed phase high performance thin layer chromatography was applied to determine the Log P values of ibuprofen, ketoprofen, naproxen, and flurbiprofen. The stationary phase used in the study was silica-gel coated plates. The mobile phase was the mixture of acetonitrile and water in different proportions. The content of acetonitrile varied in 5% increments from 50% to 80%. The R_{m0} values were determined for the compounds with a known Log P and for the analyzed substances (ibuprofen, naproxen, ketoprofen, and flurbiprofen). The Log P values were calculated for the analyzed compounds using the regression curve $R_{m0} = f(\text{Log } P)$ parameters for the compounds with the known lipophilicity. Flurbiprofen is characterized by the highest Log P value: 3.82. The lowest one is noted for ketoprofen: 2.66. The determined Log P values of tested compounds were similar to the values calculated by the software.

1. Introduction

Lipophilicity is defined as the affinity of a molecule or moiety to a lipophilic environment. It is an important parameter described by the partition coefficient (P). It describes the partition of the compound between aquatic and nonpolar solvent. It is commonly assessed by the distribution in a liquid-liquid system (with the shake-flask method) or liquid-solid (using chromatographic techniques). Lipophilicity is a complex effect of molecular interactions both between solute and solvent as well as interactions between solvent molecules in each phase. The substance, when dissolved in the solvent, disrupts its structure, which causes the breakdown of intermolecular bonds. The substance must create a free space in a given volume of solvent. It requires a certain amount of energy and results in a clear correlation of the lipophilicity with the volume or surface of the dissolved molecule [1].

Lipophilicity affects the biological activity of a drug because it plays a significant role in drug interactions with the receptor, the pharmacokinetics of the molecule, its toxic action, and in the pharmaceutical aspect—the solubility of the substance [2]. The chemical substance undergoes a series of biophysical and biochemical transformations in a human body. They can be divided into three phases: pharmaceutical (drug form and its release), pharmacokinetic (drug transport), and pharmacodynamic phase (interaction with the receptor). The lipophilicity affects the pharmacokinetic processes in living organisms especially the distribution process. All substances that penetrate into the body's cell encounter lipophilic barriers (cell membranes). Only molecules with appropriate affinity for lipophilic biological membranes will pass through them. It results in the distribution of the drug in the human body, and the substance will reach the receptors. This feature is highly correlated with passive transport across cell membranes.

Various extraction systems can be used to determine lipophilicity. The n-octanol/water system is often used to determine the partition coefficient which is usually denoted as the logarithm of P (Log P). The n-octanol/water system is used in a classic shake-flask method [3]. The determination of the Log P in the n-octanol/water system is widely used in chemistry, medicine, pharmacology, and toxicology

to describe the transport through biological membranes, solubility, toxicity, and absorption of substances [4]. However, it has some disadvantages—it is time-consuming and requires relatively more amount of the reagents when compared with chromatographic methods. Chromatographic division of the compounds with reversed-phase thin-layer chromatography (RP-TLC) between the nonpolar stationary phase and the aqueous mobile phase is similar to the division in the biological system. The stationary phase RP-18 (octadecylsilanized silica gel) is stable and resistant to many eluents. It is the most common stationary phase for RP-TLC and RP-HPLC. Its surface has anisotropic properties, similar to biological membranes, which cannot be attributed to the octanol phase in extraction methods. Long hydrocarbon chains on the surface of the adsorbent, forming a hydrophobic film, allow the analyte's molecule to penetrate to various depths, which more accurately corresponds to interactions with the body membranes. For this reason, reverse-phase chromatography should better correspond to biological lipophilicity than the static and isotropic extraction system [1].

This paper focuses on the determination of the lipophilicity of ibuprofen ((RS)-2-(4-(2-methylpropyl) phenyl)propionic acid), ketoprofen ((RS)-2-(3-benzoylphenyl)-propionic acid), naproxen ((S)-(+)-2-(6-methoxy-2-naphthyl) propionic acid), and flurbiprofen (2-(2-fluoro-4-biphenylyl) propionic acid) by reversed-phase high-performance thin-layer chromatography (RP-HPTLC). They are the nonsteroidal anti-inflammatory drugs. They are the most commonly used drugs due to their vast availability. Their pharmacological activity is based on the inhibition of the COX-1 and COX-2 activity. They might be applied as antipyretic and analgesic agents. They are also applied topically in the treatment of traumatic, overload, or inflammatory changes in periarticular tissues and muscles as well as for the treatment of inflammations in the oropharyngeal cavity or the dental pain. Due to the analgesic activity, the investigated drugs are used in the treatment of muscular pains, after tooth extraction, after surgery, in neuralgia, in root syndromes, in discopathy, and also in migraine [5–7].

2. Materials and Methods

2.1. The Chromatographic Procedure. The HPTLC separation was carried out on the HPTLC RP-18, F254s glass plates (10×10 cm, Merck, Germany). The mixture of acetonitrile-water (v/v) was used as the mobile phase. The content of acetonitrile (POCH, Gliwice, Poland) varied in 5% increments from 50% to 80% [6]. All experiments were performed at the room temperature, 21 ± 1 (°C). All measurements were performed in triplicate.

The concentration of the investigated solutions was 1%. The analyzed substances were dissolved in methanol. 10 μL was transferred onto the plate. The chromatograms were developed in the horizontal chamber and visualized with the UV254 lamp.

2.2. The Calculation of the Data. Using the following equation, the R_f values were calculated:

$$R_f = \frac{x}{y}, \tag{1}$$

where x is the distance from baseline traveled by solute and y is the distance from baseline traveled by the solvent.

The R_f were used to calculate the parameter according to Bate-Smith and Westall equation [8,9]:

$$R_m = \left(\frac{1 - R_f}{R_f}\right). \tag{2}$$

The R_m values were extrapolated to pure water (R_{m0}) by the equation for the following linear function $R_m = f(c)$ [10]:

$$R_m = aC + R_{m0}, \tag{3}$$

where a is the slope of the curve which indicates the rate at which the solubility of the compound increases in the mobile phase [8], C is the concentration of the acetonitrile, R_{m0} is the R_m of the compound when the concentration of ACN is zero (extrapolated value). The equations are listed in Table 1.

2.3. The Calculation of the Lipophilicity. The lipophilicity expressed as $\log P$ is described with the linear function $R_{m0} = f(\log P)$ and calculated with the following equation:

$$R_{m0} = B + a \log P, \tag{4}$$

where B is the intercept, a is the slope, and R_{m0} is the R_{m0} value for the compounds with known lipophilicity. The compounds with known lipophilicity were isatin (Fluka, Germany), *N*-(2,6-dichlorophenyl)acetamide (Sigma-Aldrich), *N*-(2,4-dichlorophenyl)acetamide (Sigma-Aldrich), 3,4-dichloroaniline (Aldrich, UK), 2,6-dichloroaniline (Aldrich, UK), and *p*-nitrophenol (POCH, Gliwice, Poland) [11].

The precision was within the range 2.92–8.76% and 3.65–8.42 for the compounds with known lipophilicity and the analyzed ones. The method was linear ($|r| > 0.989$).

The final equation for calculation of the lipophilicity was $R_{m0} = 0.5718\ \mathrm{Log}\,P + 0.3262$ ($|r| = 0.9963$).

The R_{m0} and $\log P$ values for the compounds with the known lipophilicity are listed in Table 2. The calculated values for the inflammatory drugs are listed in Table 3.

3. Results and Discussion

All the equations listed in Table 1 have a negative slope. It implies that with the increase of the organic modifier concentration in the mobile phase, the R_m value for less lipophilic compounds decreases more slowly than for more lipophilic derivatives. This means that lipophilic compounds are more sensitive to changing the polarity of the mobile phase. The negative value of the slope in TLC equation suggests an increase in the migration of substances per unit of growth in the content of the organic solvent. The highest values of the slope characterize the compounds that are most susceptible for the change of the content of organic solvent in the mobile phase. The highest values are observed for flurbiprofen and ibuprofen, whereas the lowest values were observed for isatin (Table 1).

Table 1: The chromatographic equations of the $R_m = f(c)$ function for the analyzed compounds.

Compound	Equation
Isatin	$R_m = -0.0214c + 0.8308$
2,6-Dichloroaniline	$R_m = -0.0274c + 2.0028$
p-Nitrophenol	$R_m = -0.0234c + 1.1115$
3,4-Dichloroaniline	$R_m = -0.0258c + 1.8251$
N-(2,4-Dichlorophenyl)-acetamide	$R_m = -0.0230c + 1.5421$
N-(2,6-Dichlorophenyl)-acetamide	$R_m = -0.0230c + 1.0612$
Ketoprofen	$R_m = -0.0306c + 1.8491$
Naproxen	$R_m = -0.0301c + 1.8623$
Ibuprofen	$R_m = -0.0319c + 2.3091$
Flurbiprofen	$R_m = -0.0366c + 2.5076$

Table 2: The R_{m0} and Log *P* values of the compounds with a known lipophilicity.

Compound	$R_{m0} \pm$ SD	Log *P* [11]
Isatin	0.8308 ± 0.0285	0.83
2,6-Dichloroaniline	2.0028 ± 0.0820	2.82
p-Nitrophenol	1.1115 ± 0.0973	1.38
3,4-Dichloroaniline	1.8251 ± 0.0841	2.69
N-(2,4-Dichlorophenyl)-acetamide	1.5421 ± 0.0984	2.18
N-(2,6-Dichlorophenyl)-acetamide	1.0612 ± 0.0486	1.32

Table 3: The R_{m0}, Log *P*, and Log P_{calc} values of the investigated compounds.

Compound	$R_{m0} \pm$ SD	Log *P*	Log P_{calc} [12]
Ketoprofen	1.8491 ± 0.1226	2.66	2.86
Naproxen	1.8623 ± 0.0736	2.69	2.79
Ibuprofen	2.3091 ± 0.1944	3.47	3.48
Flurbiprofen	2.5076 ± 0.1392	3.82	3.77

Lipophilicity is a valuable parameter of the drug which affects its activity in the human body. The Log *P* value of the compound indicates the permeability of the drugs to reach the target tissue in the body. All the investigated compounds are lipophilic because the Log $P > 0$ (or $P > 1$) (Table 3). It implies that they prefer the organic phase, and it reflects in their physical properties—they are practically insoluble in water. According to PubChem database, the lowest solubility in water is reported for flurbiprofen (8 mg/l) for which the highest Log P_{TLC} value is reported in our study (Table 3). Ibuprofen and naproxen have similar solubility values which are 21.5 mg/l and 15.9 mg/l, respectively. The highest solubility was noted for ketoprofen (51 mg/l), and the least Log P_{TLC} value is reported in our study. All the solubility values are reported for room temperature [13]. The most lipophilic compounds are flurbiprofen and ibuprofen. Lower Log *P* values of ketoprofen and naproxen result from the presence of an additional oxygen atom, which is an acceptor of hydrogen bonds. It lowers the lipophilicity of these compounds compared to ibuprofen and flurbiprofen, which are free of additional oxygen. Ibuprofen is more lipophilic because of the presence of 2-methylpropyl moiety. Flurbiprofen has the highest Log P_{TLC} value due to the presence of a fluorine atom. Flurbiprofen is more lipophilic than ketoprofen because it possesses the fluorine atom and the phenyl rings which are not bonded together by an oxygen atom (Figure 1). Li et al. [14] determined the lipophilicity of flurbiprofen in *n*-octanol/water system. Its Log K_{oct} value was 4.24. Pyka et al. reported the following Log *P* values for ketoprofen, naproxen, ibuprofen, and flurbiprofen: 3.12, 3.18, 3.97, and 4.16, respectively [12]. They were determined also for the *n*-octanol/water technique. The Log P_{TLC} values determined in our study are lower (Table 3). However, the strong linear and statistically significant correlation between these values is observed and is shown in Figure 2. The TLC technique is based on the interaction with the stationary phase (C-18). The hydrogen bonds play a significant role in the dissolution process. Substances which molecules can form hydrogen bonds (in this case with *n*-octanol) will be preferred in the partitioning process. An important conclusion can be drawn that the experimentally determined lipophilicity parameters depend on the applied system [1].

Pyka et al. [12] reported also the Log *P* values for flurbiprofen, ibuprofen, naproxen, and ketoprofen calculated with the various numeric methods such as A Log *P*s, IA Log *P*, C Log *P*, Log P_{Kowwin}, x Log *P*, and MI Log *P*. These techniques take into consideration the impact of the atoms or group of atoms on the lipophilicity. IA Log *P* and Log P_{Kowwin} are based on the molecular structures with measured lipophilicity. Having compared the values obtained in our study (Log P_{TLC}) with the computational Log *P* values reported in the study of Pyka et al. [12], the strong linear correlation is observed for A Log *P*s, C Log *P*, and Log P_{Kowwin}. The correlation coefficients (R^2) are 0.9427, 0.9614, and 0.9970 for Log $P_{Kowwin,}$ C Log *P*, and A Log *P*s, respectively. The closest theoretical Log *P* values to the values Log P_{TLC} determined in our study are observed for C Log *P* (Table 4). C Log *P* is the technique that estimates the interactions of the new fragments. It is more accurate than statistical methods because it is based on the established chemical interactions [12]. The linear correlation is also observed for x Log *P* (R^2 0.8354). The weaker correlation is observed for the IA Log *P* (R^2 0.6451). The lowest one is noted for mi Log *P* (R^2 0.4859). The values of Log *P* determined with the *n*-octanol/water system are similar to computational ones. It might be caused by the fact that some of the computational techniques are useful for the prediction of the partitioning between *n*-octanol/water (A Log *P*s or Log P_{Kowwin}).

The lipophilicity of the compounds has the impact on the chromatographic analysis. Increase in the lipophilicity results in the longer retention of the compounds in the reversed-phase system both on the column and on the TLC plate. Ketoprofen and naproxen are the drugs that are characterized with lower Log *P* values which implies the faster elution than flurbiprofen and ibuprofen. In HPLC method reported by Gallo et al. [15], ketoprofen and naproxen were eluted from the column after ca. 16 minutes; however, ketoprofen was eluted first. Faster elution of ketoprofen than naproxen was reported also by Zakeri-Milani et al. [16]. Flurbiprofen and ibuprofen were eluted after 21–23 minutes. Guo et al. [17] reported also faster elution of ketoprofen enantiomers than flurbiprofen.

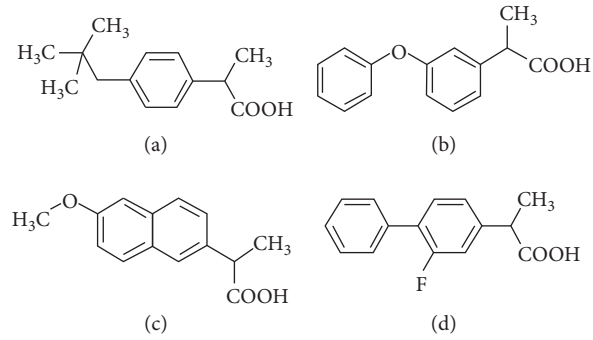

Figure 1: The molecular structures of the analyzed compounds.

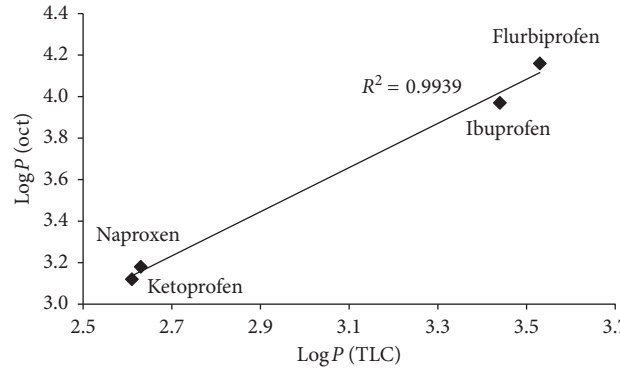

Figure 2: The correlation between the Log P determined with the n-octanol/water (Log P (oct)) [14] and the HPTLC (Log P (TLC)) methods of the analyzed compounds.

Table 4: The experimental values of Log P and the theoretical values of Log P [14] of the investigated compounds.

Compound	Log P_{TLC}	Log $P^{a}_{oct/water}$	A Log Ps[a]	IA Log P[a]	C Log P[a]	mi Log P[a]	Log P^{a}_{Kowwin}	x Log P[a]
Ketoprofen	2.66	3.12	3.28	2.78	2.76	3.59	3.00	3.22
Naproksen	2.69	3.18	3.29	3.32	2.82	3.38	3.10	2.84
Ibuprofen	3.47	3.97	3.50	3.54	3.68	3.46	3.79	3.64
Flurbiprofen	3.82	4.16	3.57	3.59	3.75	4.05	3.81	3.76

[a]Values reported by Pyka et al. [12]; Log P_{TLC}, the Log P determined in our study with TLC technique; Log $P_{oct/water}$, the Log P value determined with the n-octanol/water method.

The lipophilicity is a significant factor that influences the distribution of the substance in the human body. The values of Log P_{TLC} for the analyzed compounds were also compared with their volume of distribution. It can be noticed that with the increase in the Log P_{TLC} of the analyzed compounds, their volume of distribution increases. For ketoprofen which has the lowest Log P_{TLC} value, the volume of distribution is 0.10 l/kg. The highest values are noticed for ibuprofen and flurbiprofen: 0.18 l/kg and 0.17 l/kg, respectively [18–20]. The lipophilic character of the compounds is also confirmed by the affinity of the drugs to bind with plasma proteins. All investigated compounds are the weak acids, and it results in binding with albumins [21]. For ketoprofen, ibuprofen, flurbiprofen, and naproxen, the 98–99.9% of the drug is bound to the plasma proteins [21–24]. The other barrier that drugs encounters in human body is blood-brain barrier (BBB). Ajome-Cat et al. reported that nonsteroidal anti-inflammatory drugs crossed the BBB efficiently and they interact with microglial cell. They inhibit the COX activity but also repress the expression of the genes associated with the microglial activation [25]. Novakova et al. reported in *in vitro* tests that ibuprofen passes the barrier easily; however, it depends on the presence on the plasma proteins. The presence of 7.5% serum in *in vitro* model reduced the permeability of the drug [26].

The high lipophilicity of the investigated profens reflects also their use in the clinical practice. They may be administered topically on the skin (naproxen, ibuprofen, and ketoprofen) as well on the mucous membranes (flurbiprofen). The topical administration of the drug provides the targeted therapy, and the substance penetrates in the site of action and might be used by the patients which do not tolerate the nonsteroidal anti-inflammatory drugs orally [27].

The nonsteroidal anti-inflammatory drugs are divided into three categories due to their affinity to COX-1 and COX-2 [28]. Knights et al. [28] reported that ibuprofen competitively binds to COX active site but dissociates rapidly from the active site of the enzyme. Its action towards

COX-1 and COX-2 is rapid and reversible [29]. On the other hand, flurbiprofen is a slow tight-binding molecule. It competes poorly at the beginning with the arachidonic acid but then binds tightly in a time-dependent manner. Both flurbiprofen and ibuprofen have the same conformation in the active site. However, they are in the different categories. The factor that differentiates them is the speed and efficiency binding to the aminoacids of the active site of COX [28]. These two drugs have the highest Log P values (Table 3) which may cause the high affinity to COX binding sites created by Arg120, Tyr355, and Glu524 [30]. The investigated drugs possess the carboxyl group in their structure contrary to the selective COX-2 inhibitors which do not possess carboxyl moiety and the interaction with Arg120 is not necessary [28]. The most lipophilic flurbiprofen has the highest affinity to COX-1 and COX-2 described by IC_{50}. The reported affinity of the investigated drugs to COX-1 and COX-2 is different, and its activity differs between used cell lines [28].

4. Conclusions

The HPTLC technique is suitable for the determination of the lipophilicity of ibuprofen, ketoprofen, naproxen, and flurbiprofen. There is a strong correlation between the Log P values obtained with n-octanol/water and chromatographic method and also with the Log P values calculated with a software. The lipophilicity of the compounds influences the physicochemical and biological properties.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The author declares that there are no conflicts of interest.

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References

[1] K. Jóźwiak, H. Szumiło, and E. Soczewiński, "Lipofilowość, metody wyznaczania i rola w działaniu biologicznym substancji chemicznych," *Wiad Chem*, vol. 55, no. 11-12, pp. 1047–1074, 2001.

[2] R. Margabandu and K. Subramani, "Experimental and theoretical study on lipophilicity and antibacterial activity of biphenylamine derivatives," *International Journal of ChemTech Research*, vol. 2, no. 3, pp. 1501–1506, 2010.

[3] M. Kostecka, *Comparison of Methods Used for Estimation of Lipophilicity of Biologically Active Phenylthioamides*, UMCS Lublin, Poland, 2009.

[4] E. P. Andreeva and O. A. Raevsky, "Lipophilicity of organic compounds calculated using structural similarity and molecular physicochemical descriptors," *Pharmaceutical Chemistry Journal*, vol. 43, no. 5, pp. 258–262, 2009.

[5] N. Moore and J. M. Scheiman, "Gastrointestinal safety and tolerability of oral non-aspirin over-the-counter analgesics," *Postgraduate Medicine*, vol. 130, no. 2, pp. 188–199, 2018.

[6] S. Karacabey, E. Sanri, S. Yalcinli, and H. Akoglu, "Which is more effective for the treatment of acute migraine attack: dexketoprofen, ibuprofen or metoclopramide?," *Pakistan Journal of Medical Sciences*, vol. 34, no. 2, pp. 418–423, 2018.

[7] A. Hussain, M. A. Syed, N. Abbas et al., "Development of an ANN optimized mucoadhesive buccal tablet containing flurbiprofen and lidocaine for dental pain," *Acta Pharmaceutica*, vol. 66, no. 2, pp. 245–256, 2016.

[8] A. Czyrski and B. Kupczyk, "The determination of partition coefficient of 6-mercaptopurine derivatives by thin layer chromatography," *Journal of Chemistry*, vol. 2013, Article ID 419194, 4 pages, 2013.

[9] A. Czyrski, T. Hermann, B. Rubiś, M. Rybczyńska, and D. Sledź, "Biopharmaceutical characterization of some new papaverine decomposition products," *Pharmazie*, vol. 66, no. 3, pp. 165–167, 2011.

[10] F. L. Andrić, J. Đ. Trifković, A. D. Radoičić, S. B. Šegan, Ž. L. Tešić, and D. M. Milojković-Opsenica, "Determination of the soil–water partition coefficients (logKOC) of some mono- and poly-substituted phenols by reversed-phase thin-layer chromatography," *Chemosphere*, vol. 81, no. 3, pp. 299–305, 2010.

[11] C. Hansch, A. Leo, and D. Hoekman, *Exploring QSAR Hydrophobic, Electronic and Steric Constants*, Professional Reference Book, ACS, Washington, DC, USA, 1995.

[12] A. Pyka, M. Babuśka, and M. Zachariasz, "A comparison of theoretical methods of calculation of partition coefficients for selected drugs," *Acta Poloniae Pharmaceutica*, vol. 63, no. 3, pp. 159–167, 2006.

[13] https://pubchem.ncbi.nlm.nih.gov/.

[14] Q. Li, H. Tsuji, Y. Kato, Y. Sai, Y. Kubo, and A. Tsuji, "Characterization of the transdermal transport of flurbiprofen and indomethacin," *Journal of Controlled Release*, vol. 110, no. 3, pp. 542–556, 2006.

[15] P. Gallo, S. Fabbrocino, F. Vinci et al., "Multi-residue determination of non-steroidal anti-inflammatory drug residues in animal serum and plasma by HPLC and photo-diode array detection," *Journal of Chromatographic Science*, vol. 44, no. 10, pp. 585–590, 2006.

[16] P. Zakeri-Milani, M. Barzegar-Jalali, H. Tajerzadeh, Y. Azarmi, and H. Valizadeh, "Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 39, no. 3-4, pp. 624–630, 2005.

[17] C.-C. Guo, Y.-H. Tang, H.-H. Hu, L.-S. Yu, H.-D. Jiang, and S. Zeng, "Analysis of chiral non-steroidal anti-inflammatory drugs flurbiprofen, ketoprofen and etodolac binding with HSA," *Journal of Pharmaceutical Analysis*, vol. 1, no. 3, pp. 184–190, 2011.

[18] T. Ishizaki, T. Sasaki, T. Suganuma et al., "Pharmacokinetics of ketoprofen following single oral, intramuscular and rectal doses and after repeated oral administration," *European Journal of Clinical Pharmacology*, vol. 18, no. 5, pp. 407–414, 1980.

[19] D. J. Greenblatt, D. R. Abernethy, R. Matlis, J. S. Harmatz, and R. I. Shader, "Absorption and disposition of ibuprofen in the elderly," *Arthritis and Rheumatism*, vol. 27, no. 9, pp. 1066–1069, 1984.

[20] A. Qayyum, M. H. Najmi, and Z.-R. Farooqi, "Determination of pharmacokinetics of flurbiprofen in Pakistani population

using modified HPLC method," *Journal of Chromatographic Science*, vol. 49, no. 2, pp. 108–113, 2011.

[21] K. D. Rainsford, "Ibuprofen: pharmacology, efficacy and safety," *Inflammopharmacology*, vol. 17, no. 6, pp. 275–342, 2009.

[22] P. J. Hayball, R. L. Nation, F. Bochner, J. L. Newton, R. A. Massy-Westropp, and D. P. Hamon, "Plasma protein binding of ketoprofen enantiomers in man: method development and its application," *Chirality*, vol. 3, no. 6, pp. 460–466, 1991.

[23] G. J. Szpunar, K. S. Albert, and J. G. Wagner, "Pharmacokinetics of flurbiprofen in man. II. Plasma protein binding," *Research Communications in Chemical Pathology and Pharmacology*, vol. 64, no. 1, pp. 17–30, 1989.

[24] M. V. Calvo and A. Dominguez-Gil, "Binding of naproxen to human albumin. Interaction with palmitic acid," *International Journal of Pharmaceutics*, vol. 16, no. 2, pp. 215–223, 1983.

[25] M. A. Ajmone-Cat, A. Bernardo, A. Greco, and L. Minghetti, "Non-steroidal anti-inflammatory drugs and brain inflammation: effects on microglial functions," *Pharmaceuticals (Basel)*, vol. 3, no. 6, pp. 1949–1964, 2010.

[26] I. Novakova, E.-A. Subileau, S. Toegel et al., "Transport rankings of non-steroidal antiinflammatory drugs across blood-brain barrier in vitro models," *PLoS One*, vol. 9, no. 1, Article ID e86806, 2014.

[27] M. Hagen and M. Baker, "Skin penetration and tissue permeation after topical administration of diclofenac," *Current Medical Research and Opinion*, vol. 33, pp. 1623–1634, 2017.

[28] K. M. Knights, A. A. Mangoni, and J. O. Miners, "Defining the COX inhibitor selectivity of NSAIDs: implications for understanding toxicity," *Expert Review of Clinical Pharmacology*, vol. 3, no. 6, pp. 769–776, 2010.

[29] B. J. Orlando, M. J. Lucido, and M. G. Malkowski, "The structure of ibuprofen bound to cyclooxygenase-2," *Journal of Structural Biology*, vol. 189, no. 1, pp. 62–66, 2015.

[30] B. S. Selinsky, K. Gupta, C. T. Sharkey, and P. J. Loll, "Structural analysis of NSAID binding by prostaglandin H2 synthase: time-dependent and time-independent inhibitors elicit identical enzyme conformations," *Biochemistry*, vol. 40, no. 17, pp. 5172–5180, 2001.