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Enantiomeric resolution of ketoprofen using validated thin layer chromatographic method involving frovatriptan as chiral selector

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ABSTRACT: The importance of developing chiral chromatographic methods to monitor and ensure the purity of enantiomers is underscored by the distinct biological properties exhibited by the different forms of ketoprofen. We employed a thin-layer chromatographic approach to separate ketoprofen from commercially available tablet formulations. Our method utilized frovatriptan as the chiral selector, which was incorporated into the stationary phase through a silica gel slurry. The mobile phase consisted of a combination of acetonitrile, methanol, and triethylamine in a ratio of 6:2:2, with a pH of 9. Frovatriptan also served as the chiral selector in the stationary phase, maintaining a pH of 5. The detection and quantitation limits for the ketoprofen enantiomers were determined to be 8.4 µg spot^{-1} and 25.2 µg spot^{-1}, respectively. Notably, this study marks the first-ever application of frovatriptan as a chiral selector in thin-layer chromatography to resolve ketoprofen enantiomers.

KEYWORDS: Enantioresolution; pharmaceuticals; ketoprofen; NSAIDs; Frovatriptan; Chiral chromatography; method validation.

1. INTRODUCTION

Understanding the specific pharmacological profiles of enantiomers is crucial in developing safe and effective drugs. In many cases, a drug compound may have multiple enantiomers, and each enantiomer can have different pharmacological properties, including differences in potency, efficacy, toxicity, and metabolism [1,2]. In some cases, enantiomerically pure drugs may be developed and marketed. In contrast, in others, a racemic mixture with a specific ratio of enantiomers may be used if both enantiomers contribute to the desired therapeutic effect or if the inactive enantiomer has minimal toxicity [3-5]. The growing interest in single-enantiomer production reflects the recognition of the potential benefits derived from understanding and utilizing the distinct properties of enantiomers in drug development and therapy [6]. Therefore, isolating and studying each enantiomer separately is crucial to understand their characteristics and effects. Various analytical techniques, such as chiral chromatography, capillary electrophoresis, and spectroscopic methods, have achieved enantiomeric separation and quantification.

Ketoprofen (KET) (Fig. 1) is a non-steroidal anti-inflammatory drug (NSAID) that possesses analgesic, anti-inflammatory, and antipyretic properties [7]. Its chemical name is (±)-(*RS*)-2-(3-benzoyl phenyl)propionic acid, and it is often administered as a racemic mixture containing both (*R*)-KET and (*S*)-KET. (*S*)-KET and (*R*)-KET have been found to display significantly different pharmacological activities. Studies have shown that (*S*)- KET is the more active enantiomer and is responsible for most of the pharmacological effects of KET [8]. It exhibits better anti-inflammatory and analgesic properties than (*R*)-KET. It has been reported that the (*R*)-enantiomer of KET can convert into its antipode, meaning it can undergo a process in which it transforms into the (*S*)-enantiomer in the bodies of humans and animals [9]. This enantiospecific metabolic inversion of (*R*)-KET to the (*S*)-form can occur in vivo [10-13]. Due to this difference in the pharmacological profile of enantiomers of (R)- and (*S*)-KET, it becomes evident to develop a validated method for separating KET for its better therapeutic efficacies.

Frovatriptan (FRV) is a member of the triptan class of drugs and used for treating migraines. It is administered as a single enantiomer, specifically the (*R*)-enantiomer, and it is also commercially available as a pure enantiomer [14]. It acts as a selective serotonin receptor agonist, targeting the 5-HT1B and 5-HT1D

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receptors. By binding to these receptors, frovatriptan helps to constrict blood vessels and inhibit the release of inflammatory peptides, thus relieving the symptoms of migraines [15]. (*R*)-enantiomer of frovatriptan is responsible for its therapeutic effects, while the (*S*)-enantiomer is typically less active or inactive. FRV is a carbazole derivative containing an indole ring in its structure. It also contains a methylamino group (CH_3-NH-) and a carboxamide group (-$CONH_2$) in its structure. Considering the reactive functionalities present in FRV and its commercial availability as a single enantiomer, (*R*)-FRV can be used as a chiral selector for impregnating TLC plates and allowing the resolution of enantiomers.

The literature offers various analytical methods for determining KET enantiomers in biological samples and pharmaceutical formulations. High-performance liquid chromatography [16-20], liquid chromatography coupled with tandem mass spectrometry [21,22], and UV-vis spectroscopy [23] capillary electrophoresis [24-27] are commonly employed techniques for this purpose. On the other side, Thin-layer chromatography (TLC) is a widely used method for the separation and analysis of racemates as well as for assessing the enantiomeric purity of enantiomeric drugs. TLC offers several advantages viz simplicity, low cost, and rapid investigation. It is widely used in research laboratories, quality control settings, and educational institutions. It is a valuable chromatographic tool that can provide important information in drug development, quality control, and chemical analysis. However, there is very little literature available on the resolution of KET enantiomers involving TLC using L-(−)-serine [28] and L-(−)-threonine [29] as chiral selectors for the enantioresolution of KET.

Considering the advantages of TLC-based methods, we designed a direct TLC approach involving commercially available (*R*)-FRV as a chiral selector for impregnating silica TLC plates. To the best of the author's knowledge, this is the first report on the resolution of KET enantiomers using direct TLC plates coating with (*R*)-FRV; the resulting CSPs can selectively interact with enantiomers present in a sample, allowing for chiral separation and analysis. The developed TLC method offers several advantages, including simplicity, low cost, and rapid analysis, and can be suitably applied in research laboratories and quality control settings.

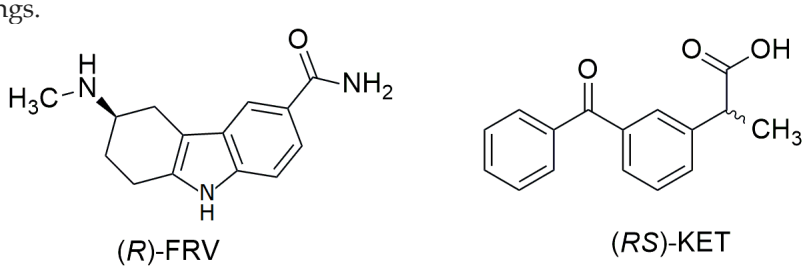

Figure 1. Structures of (*R*)-Frovatriptan and (*RS*)-Ketoprofen

2. EXPERİMENTAL

2.1. Reagents and materials

The reagents and solvents mentioned below were acquired from Merck (India): (*RS*)-ketoprofen (>97% HPLC), (*R*)-Frovatriptan, (*S*)-(+)-ketoprofen silica gel G (with 13% CaSO4 as a binder), and solvents of analytical grade including n-hexane, n-heptane, n-butanol, 2-butanol, isopropanol, methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), dichloromethane (DCM), and chloroform ($CHCl_3$). Ketofen tablets (Pharma Corp Inc.), each containing 100 mg of ketoprofen were procured from the local market. The instruments employed in the study were as follows: FT-IR spectrometer: FT-IR spectrometer 1600 (Boardman, OH, USA), elemental analyzer: Vario EL III (Hanau, Germany), polarimeter: Krüss P3001RS (Hamburg, Germany), pH meter: Cyberscan 510 (Singapore).

2.2. Sample preparation

Four tablets of Ketofen containing 100 mg of KET were weighed and ground in a mortar. The resulting powder, equivalent to 400 mg of KET, was measured and transferred into a 100 mL volumetric flask. Subsequently, 50 mL of ethanol was added to the flask and subjected to 15 minutes of sonication. After that, 30 mL of mobile phase was added and sonicated for 10 minutes. Finally, the volume was adjusted to the mark using the mobile phase, and the solutions were filtered using syringe filters with a pore size of 0.2 microns. The resulting filtrate was considered a standard solution of KET with a concentration of 100 mM and further diluted to get working solutions in the range of 10 mM to 100 mM. Similarly, a solution of FRV (50 mM) was also prepared by dissolving its appropriate quantity in ethanol.

3. THİN-LAYER CHROMATOGRAPHY

3.1 Preparation of Thin-Layer Plates

The TLC plate comprises a flat surface often made of glass coated with a thin layer of absorbent material like silica gel. Glass plates measuring 10 x 5 cm x 0.5 mm were utilized to prepare planar TLC plates. These plates were coated by spreading a silica gel slurry which contained the chiral selector FRV, onto the glass plates using a "Stahl-type applicator." The planar plates were activated by placing them in an incubator overnight at a temperature of 60°C. Subsequently, the TLC plates underwent impregnation with a chiral selector (FRV) at a concentration of 20 mM. For this study, a slurry of silica gel G was prepared by dispersing 25 g of silica gel in a 50 mL solution containing the chiral selector FRV at a concentration of 20 mM. The chiral selector was incorporated into the stationary phase of the TLC plate while maintaining a pH of 5 in the stationary phase. The chiral selector interacts differently with the enantiomers in the sample mixture, allowing for their separation based on these distinct interactions. The chiral selector's concentration was adjusted to achieve optimal separation and resolution. To optimize the separation conditions, additional TLC plates were prepared by varying the amount of the chiral selector in the silica gel slurry, ranging from 10 to 50 mM.

3.2 Development of chromatograms

The sample spot is carefully applied to the plate and positioned in a developing chamber containing an appropriate mobile phase system for the separation. Using a Hamilton syringe, a 1.0 µL droplet of (*RS*)-KET solution was carefully placed onto a TLC plate 1 cm above the lower edge of the plate to ensure proper separation. The upward movement of the sample (on TLC plates) was carried out in a rectangular glass chamber with a suitable mobile phase. Capillary action facilitates the upward migration of the enantiomers with solvent for their separation. This separation is achieved based on the distinct affinities of the sample components for both the absorbent material and the solvent. The chamber temperature was rigorously maintained at 25±2 °C to ensure consistent and reliable results. After allowing the chromatograms to develop, the TLC plates were immersed in an iodine chamber to visualize the separated components. These components manifest as distinct brown spots due to the reaction between iodine and specific functional groups of the analyte molecule.

To optimize the separation process in TLC, chromatographic factors like the composition of the mobile phase, pH level, temperature, and the quantity of the chiral selector were adjusted. By modifying these parameters, the separation efficiency in TLC can be enhanced, leading to improved resolution between individual components of the mixture. The composition of the mobile phase containing various solvents was carefully adjusted to achieve the desired separation. Different solvents or solvent combinations can affect the interactions between the sample components, the stationary phase (absorbent material), and the mobile phase. The pH also plays a role in the separation process, as it can influence the ionization and charge properties of the sample components, thereby affecting their migration on the TLC plate.

3.3 Method validation

The International Conference on Harmonization (ICH) has set the guidelines for validating analytical methods used in the pharmaceutical industry [32,33]. These guidelines are specifically developed to ensure that the methods employed are accurate, reliable, and consistent in their performance. The validation process includes the determination of linearity, accuracy, and precision and determining the limits of detection (LOD) and quantification (LOQ).

3.3.1 Linearity

To evaluate the linearity of the method, a series of samples with concentrations ranging from 10 mM to 100 mM were analyzed, and a calibration curve was constructed. The determination coefficient (R^2) of the calibration curve was employed as a measure to assess the linearity of the method.

3.3.2. Accuracy

Accuracy refers to the degree of agreement between the results obtained from a method and the true value of the substance being analyzed in a sample. To evaluate the accuracy of the method, three quality control samples were prepared, representing low (10 mM), medium (50 mM), and high (100 mM) concentrations. The accuracy was determined by calculating the percentage of the known amount of the substance that was effectively recovered by the method.

3.3.3 Precision

Precision refers to the level of consistency and reproducibility of results obtained from a method when conducted under identical conditions. The replicate samples spanning concentrations from 10 mM to 100 mM were prepared. Each portion was subsequently analyzed using the TLC method. This evaluation enabled the measurement of the method's precision by quantifying the variation and reproducibility of results across the replicate samples.

3.3.4 LOD and LOQ

The terms LOD and LOQ represent the minimum concentrations at which the analyte can be reliably detected and accurately quantified, respectively. LOD and LOQ were evaluated by analyzing samples containing low concentrations of the analyte and calculating the signal-to-noise ratio (S/N) based on the obtained results.

4 RESULTS AND DİSCUSSİON

4.1 Impregnation of TLC plates with chiral selector

The TLC plates with varying concentrations (10 to 50 mM) of the chiral selector (FRV) were prepared. Impregnation involves the incorporation of the chiral selector onto the TLC plates, allowing them to function as chiral stationary phases. This process enhances the ability of the TLC plates to separate enantiomers based on their interactions with the chiral selector. During impregnation, a solution or slurry of the chiral selector is applied to the TLC plates. The chiral selector may be dissolved or dispersed in a suitable solvent or suspension medium. The TLC plates are typically coated or spread with the chiral selector solution. The plate with varying concentrations of the chiral selector from 10 mM and 50 mM was prepared to get the optimum quantity of chiral in the stationary phase. It was observed that the TLC plates with 20 mM of chiral selector provided the best results in terms of the shape of chromatographic spots. In the case of a lower quantity of chiral selector in the stationary phase, no resolution spots were observed; with higher concentration >20 mM, the spots of irregular shape and elongated tailing were observed.

Impregnation ensures that the chiral selector is uniformly distributed and adheres to the stationary phase, typically made of absorbent material like silica gel. The chiral selector selectively interacts with the enantiomers in the sample mixture, leading to their differential migration and separation during the TLC process and providing valuable information about their individual properties and characteristics. The investigation revealed that TLC plates containing a chiral selector concentration of 20 mM yielded the most favorable outcomes for spot shape and better resolution values (R_s>2.6). Conversely, when different concentrations of the chiral selector were employed in other experiments, either no resolution or inadequate resolution was observed. Consequently, based on these findings, the authors opted to utilize TLC plates prepared with 20 mM concentrations of LRV as their preferred method for separating KET enantiomers.

4.2 Optimization of separation conditions through TLC

A comprehensive evaluation of multiple mobile phase systems was conducted to achieve effective separation of enantiomers. The objective was to identify the most suitable combination of solvents that enable the successful separation of the enantiomers. Several mobile phase systems containing various solvents in different concentrations were tested, including n-hexane, n-heptane, n-butanol, 2-butanol, isopropanol, methanol, ethanol, acetonitrile, DCM, chloroform, and water. The choice of mobile phase was based on the specific characteristics of the enantiomers being separated; the chiral selector employed in the TLC plates. Each solvent system was carefully evaluated to optimize the separation process and achieve a well-defined resolution of the enantiomers. The mobile phase system composed of ACN, MeOH, and aqueous TEA in a specific ratio of 6:2:2 at pH 9 demonstrated successful resolution of KET enantiomers.

4.2.1 Effect of pH

The pH of the mobile and the stationary phase was recognized as a critical factor in optimizing the separation of components within the sample. The selection of a ternary mixture, rather than a binary or quaternary mixture, provided better control over the properties of the mobile phase; resulting in an enhanced separation of the KET enantiomers. Figure 2 illustrates chromatograms that visually show the successful resolution of KET enantiomers using FRV as the chiral selector.

Figure 2. The photograph of chromatograms representing the resolution of enantiomers of (*RS*)-KET using FRV as chiral selector in stationary phase. Chromatographic conditions: mobile phase: ACN-MeOH-TEA (6:2:2, pH 9); development time, 10 min; temperature, 25±2 °C, chiral selector FRV in stationary phase, pH 5.0. The spots were visualized in iodine vapor.

The pH was adjusted by adding dilute hydrochloric acid (HCl) or triethylamine (TEA) to the mobile and stationary phases. The pH values were systematically varied from 3 to 10, with intervals of 1 unit. The findings indicated that maintaining a neutral or acidic pH in the mobile phase did not yield a satisfactory resolution of enantiomers. However, as the pH of the mobile phase was increased to 9, an improvement in separation was observed. Beyond pH 9, the improvement in resolution reached a plateau, suggesting that further increases in pH did not significantly enhance the separation process. These observations highlight the influence of pH on the interaction between the chiral selector and the analyte. The pH of the mobile phase affects the strength and nature of this interaction, thereby impacting the separation of enantiomers. The results emphasize the importance of carefully selecting and controlling the pH of the mobile phase to optimize the separation and achieve the desired resolution of enantiomers in chromatography.

The pH of the stationary phase was carefully maintained at pH 5, while the chiral selector existed in the form of a cation (-NH^+). This indicates that the chiral selector possesses a functional group with a positive charge, allowing ionic interactions with the analyte. At pH 9, the analyte (KET) likely undergoes deprotonation, generating a carboxylate ion. This carboxylate ion can interact with the positively charged chiral selector through ionic interactions. The increased strength of the ionic interaction between the chiral selector and the analyte contributes to the enhanced resolution of the analyte during the separation process.

4.2.3 Effect of temperature

An investigation was carried out to examine the effects of temperature on the resolution of enantiomers in chromatography. The experiment involved varying the temperature from 10 to 40 ^oC with an accuracy of 25±2 ^oC. The tailing, shape, and diameter of spots were closely monitored to get a successful resolution. It was observed that at temperatures exceeding 25 ^oC (±2 ^oC), significant tailing of spots occurred. Conversely, lower temperatures resulted in the formation of irregular spots. Based on the findings, the optimized temperature for achieving optimal resolution was 25±2 ^oC.

4.3 Mechanism of TLC resolution

The ability to separate enantiomers in chiral environments is based on the principle of three-point interactions. To selectively interact with one enantiomer and discriminate against the other, a chiral molecule must establish three types of interactions with the target molecule. These interactions can manifest in various ways, such as steric interactions, dipole-dipole interactions, hydrophobic interactions, hydrogen bonding, pi-pi interactions, or ionic interactions [34].

Steric interactions result from the spatial arrangement of atoms and groups within a molecule, leading to steric hindrance or complementarity. Hydrophobic interactions minimize contact between nonpolar groups and polar solvents. Pi-pi interactions occur between aromatic rings, while ionic interactions arise from charged groups such as cations and anions. Chiral drugs exhibit distinct three-point interactions with the chiral environment, forming transient diastereomeric species. These interactions contribute to the unique properties and behavior of enantiomers.

To determine the elution order of enantiomers using the chiral selector (FRV), a spot of (*S*)-KET was applied alongside the racemic drug on the TLC plates. The enantiomer shows better retention to the chiral selector will elute later, while the one with weaker retention will elute earlier compared to (*S*)-KET. Including a reference standard like (*S*)-KET allows for establishing the elution order of enantiomers. In Figure 3, the chromatograms illustrate that (*R*)-KET eluted earlier, while (*S*)-KET had a longer retention time. This suggests that the (*S*)-enantiomer formed more robust interactions with the FRV in the stationary phase. Therefore, the disparate elution times of (*S*)-KET and (*R*)-KET indicate distinct interactions between the enantiomers and the TLC plate's stationary phase.

As mentioned earlier, the pH played a crucial role in achieving effective enantioresolution of the enantiomers of KET. Optimal separation was attained by maintaining an acidic pH (=5) for the stationary phase and a basic pH (= 9) for the mobile phase. The stationary phase contained positively charged chiral selectors (-NH+), while the mobile phase caused the analyte molecules to become negatively charged (-COOH). This difference in charge facilitated favorable interactions between the chiral selectors and analyte molecules, leading to improved separation of the enantiomers.

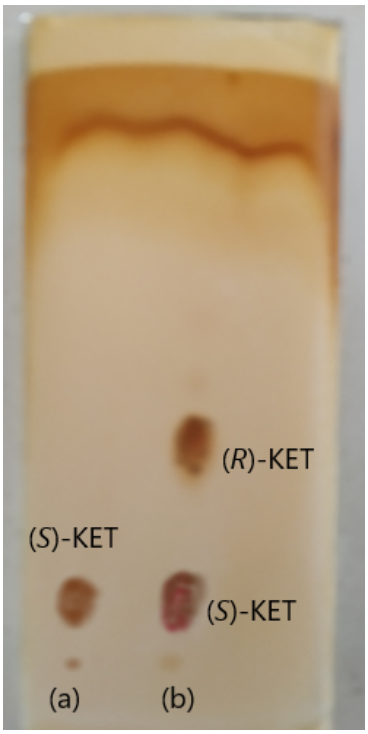

Figure 3. The photograph of chromatograms representing the resolution of enantiomers of (*S*)-KET (a) and (*RS*)-KET using FRV as a chiral selector in the stationary phase. Chromatographic conditions: mobile phase: ACN-MeOH-TEA (6:2:2, pH 9); development time, 10 min; temperature, 25±2 °C, chiral selector FRV in stationary phase, pH 5.0. The spots were visualized in iodine vapor.

4.4 Method validation

The linearity of the method was assessed by analyzing enantiomer samples at concentrations ranging from 10 mM to 100 mM and constructing a calibration curve. The linearity range was determined by plotting

the absorbance (y-axis) against the enantiomer concentration (x-axis) for the first and second eluting enantiomers. The resulting calibration plots can be described by the equations given as y = 36.7x + 2.58 and y = 42.6x + 3.74, respectively. The high R^2 values of 0.969 and 0.987 indicate excellent linearity, demonstrating the method's ability to determine enantiomer concentration within the covered range. Accuracy was determined by calculating the percent recovery of the known quantity of enantiomers in the samples. The average percent recovery of the enantiomers exceeded 94%, indicating better accuracy.

The precision of the method was assessed by calculating the RSD. The RSD values for the developed method ranged from 2.78% to 3.84%. These values indicate that the developed method exhibits relatively low variability and can be considered precise. A low RSD signifies that the measurements obtained using the method are consistent and reproducible, further emphasizing the reliability of the method's results. The narrow range of RSD values demonstrates the method's consistency in delivering accurate and precise measurements across the analyzed samples.

The reported LOD and LOQ values demonstrate the effectiveness of FRV as a chiral selector in facilitating the separation and detection of the (*RS*)-KET enantiomers. With the LOD of 8.4 µg $spot^{-1}$, the method can detect even trace amounts of the enantiomers in the analyzed samples. The LOQ of 25.2 µg $spot^{-1}$ signifies the method's ability to quantify the enantiomers at concentrations above this threshold. These results affirm the suitability of FRV as a chiral selector for achieving sensitive and accurate analysis of the (*RS*)-KET enantiomers, making it a valuable component of the chromatographic method utilized in this study.

5. CONCLUSİONS

The present study introduces a novel and direct TLC method for the efficient separation of KET enantiomers present in commercial tablet formulations. Silica gel was impregnated with FRV, serving as the chiral selector. The LOD and LOQ values for the method were determined as 8.4 µg $spot^{-1}$ and 25.2 µg $spot^{-1}$, respectively. These values indicate the method's sensitivity and capability to detect and quantify the KET enantiomers at low concentrations. The low RSD value (<3.84%) signifies the method's ability to consistently deliver accurate results with precise measurements, ensuring the robustness of the developed TLC approach. The utilization of FRV as a chiral selector in the TLC system represents an innovative aspect of this study. This approach demonstrates the efficacy of FRV in achieving the enantioresolution of KET enantiomers, highlighting its potential as a valuable tool in chiral compound analysis. The results obtained from this study have significant implications for advancing analytical techniques targeting the separation and identification of chiral compounds in pharmaceutical formulations. Such methods are essential for improving the understanding and quality control of chiral compounds within the pharmaceutical industry. The findings of this study contribute to the ongoing efforts for developing efficient and reliable techniques for the separation and analysis of chiral compounds, ultimately benefiting the pharmaceutical field as a whole.

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REFERENCES

[1] Shimazawa R, Nagai N, Toyoshima S, Okuda H. Present state of new chiral drug development and review in japan. J Health Sci. 2008; 54(1):23–29. http://dx.doi.org/10.1248/jhs.54.23.

[2] Gunjal P, Singh SK, Kumar Rajesh, Kumar Rajan, Gulati M. Role of chromatograph-based analytical techniques in quantification of chiral compounds: An update. Curr Anal Chem. 2021; 17(3):355–373. http://dx.doi.org/10.2174/1573411016999200525144506.

[3] Kumari Rayala VVSP, Kandula JS, Radhakrishnanand P. Advances and challenges in the pharmacokinetics and bioanalysis of chiral drugs. Chirality. 2022; 34(10):1298–1310. http://dx.doi.org/10.1002/chir.23495.

[4] Bernreuther A, Epperlein U, Koppenhoefer B. Enantiomers: Why they are important and how to resolve them. In: Techniques for Analyzing. CRC Press. 2020; p. 143–207.

[5] Coelho MM, Fernandes C, Remião F, Tiritan ME. Enantioselectivity in drug pharmacokinetics and toxicity: Pharmacological relevance and analytical methods. Molecules. 2021; 26(11):3113. http://dx.doi.org/10.3390/molecules26113113.

[6] Ceramella J, Iacopetta D, Franchini A, De Luca M, Saturnino C, Andreu I, Sinicropi MS, Catalano A. A look at the importance of chirality in drug activity: Some significative examples. Appl Sci (Basel). 2022; 12(21):10909. http://dx.doi.org/10.3390/app122110909.

[7] Stinson SC. Chiral drugs: In wake of new FDA guidelines, most drug firms are developing single enantiomers, spawning a "chirotechnology" industry. Chem Eng News Archive. 1992; 70(39):46–79. http://dx.doi.org/10.1021/cen-v070n039.p046.

[8] Cabré F, Fernández MF, Calvo L, Ferrer X, García ML, Mauleón D. Analgesic, antiinflammatory, and antipyretic effects of S(+)-ketoprofen in vivo. J Clin Pharmacol. 1998; 38(S1):3S-10S. http://dx.doi.org/10.1002/jcph.1998.38.s1.3.

[9] Shen Q, Wang L, Zhou H, Jiang H-D, Yu L-S, Zeng S. Stereoselective binding of chiral drugs to plasma proteins. Acta Pharmacol Sin. 2013; 34(8):998–1006. http://dx.doi.org/10.1038/aps.2013.78.

[10] Ossipov MH, Jerussi TP, Ren K, Sun H, Porreca F. Differential effects of spinal (R)-ketoprofen and (S)-ketoprofen against signs of neuropathic pain and tonic nociception: evidence for a novel mechanism of action of (R)-ketoprofen against tactile allodynia. Pain. 2000; 87(2):193–199. http://dx.doi.org/10.1016/S0304-3959(00)00280-3.

[11] Castro E, Soraci A, Fogel F, Tapia O. Chiral inversion of R(-) fenoprofen and ketoprofen enantiomers in cats. J Vet Pharmacol Ther. 2000; 23(5):265–271. http://dx.doi.org/10.1046/j.1365-2885.2000.00280.x.

[12] Igarza L, Soraci A, Auza N, Zeballos H. Chiral inversion of (R)-ketoprofen: influence of age and differing physiological status in dairy cattle, Vet Res Commun. 2002; 26(1):29–37. http://dx.doi.org/10.1023/a:1013301620904.

[13] Campbell DB. Chirality and kinetics. In: Pharmacochemistry Library. Elsevier. 1997; p. 45–60.

[14] Allais G, Benedetto C. A review of the use of frovatriptan in the treatment of menstrually related migraine. Ther Adv Neurol Disord. 2013; 6(2):55–67. http://dx.doi.org/10.1177/1756285612470191.

[15] Balbisi EA. Frovatriptan succinate, a 5-HT1B/1D receptor agonist for migraine: Frovatriptan succinate for migraine. Int J Clin Pract. 2004; 58(7):695–705. http://dx.doi.org/10.1111/j.1368-5031.2004.00218.x.

[16] Ribeiro AE, Gomes PS, Pais LS, Rodrigues AE. Chiral separation of ketoprofen enantiomers by preparative and simulated moving bed chromatography. Sep Sci Technol. 2011; 46(11):1726–1739. http://dx.doi.org/10.1080/01496395.2011.582070.

[17] Péhourcq F, Jarry C, Bannwarth B. Chiral resolution of flurbiprofen and ketoprofen enantiomers by HPLC on a glycopeptide-type column chiral stationary phase: LC resolution of flurbiprofen and ketoprofen enantiomers. Biomed Chromatogr. 2001; 15(3):217–222. http://dx.doi.org/10.1002/bmc.65.

[18] Guo Z, Wang H, Zhang Y. Chiral separation of ketoprofen on an achiral C8 column by HPLC using norvancomycin as chiral mobile phase additives. J Pharm Biomed Anal. 2005; 41(1):310–314. http://dx.doi.org/10.1016/j.jpba.2005.10.045.

[19] Alkhayer G, Khudr H, Koudsi Y. Enantioselective release behavior of ketoprofen enantiomers from alginate-metal complexes, monitored by chiral HPLC. Anal Bioanal Chem Res. 2020; 7(1):61–76. https://www.analchemres.org/article_91578.html.

[20] Escuder-Gilabert L, Martín-Biosca Y, Perez-Baeza M, Sagrado S, Medina-Hernández MJ. Direct chromatographic study of the enantioselective biodegradation of ibuprofen and ketoprofen by an activated sludge. J Chromatogr A. 2018; 1568:140–148. http://dx.doi.org/10.1016/j.chroma.2018.07.034.

[21] Li M, Liang X, Guo X, Di X, Jiang Z. 2020. Enantiomeric separation and enantioselective determination of some representive non-steroidal anti-inflammatory drug enantiomers in fish tissues by using chiral liquid chromatography coupled with tandem mass spectrometry. Microchem J. 2020; 153(104511):104511. http://dx.doi.org/10.1016/j.microc.2019.104511.

[22] Jin X, Zhang C, Jin D, Lee Y-I. Enantioselective analysis of ketoprofen in human saliva by liquid chromatography/tandem mass spectrometry with chiral derivatization. Microchem J. 2018; 143:280–285. http://dx.doi.org/10.1016/j.microc.2018.08.025.

[23] Obaid A, Jamil AKM, Prabu S, Saharin SM, Mohamad S. Spectroscopic studies for the inclusion complexation of ketoprofen enantiomers with β-cyclodextrin. Spectrochim Acta A Mol Biomol Spectrosc. 2020; 241(118674):118674. http://dx.doi.org/10.1016/j.saa.2020.118674.

[24] Blanco M, González JM, Torras E, Valverde I. Enantiomeric purity determination of ketoprofen by capillary electrophoresis: development and validation of the method. Anal Bioanal Chem. 2003; 375(1):157–163. http://dx.doi.org/10.1007/s00216-002-1629-8.

[25] Blanco M, Coello J, Iturriaga H, Maspoch S, Pérez-Maseda C. Separation of profen enantiomers by capillary electrophoresis using cyclodextrins as chiral selectors. J Chromatogr A. 1998; 793(1):165–175. http://dx.doi.org/10.1016/s0021-9673(97)00893-5.

[26] Abushoffa AM, Fillet M, Hubert P, Crommen J. Prediction of selectivity for enantiomeric separations of uncharged compounds by capillary electrophoresis involving dual cyclodextrin systems. J Chromatogr A. 2002; 948(1–2):321–329. http://dx.doi.org/10.1016/s0021-9673(01)01371-1

[27] Trelli-Seifert LA, Risley DS. Capillary electrophoretic enantiomeric separations of nonsteroidal anti-inflammatory compounds using the macrocyclic antibiotic actaplanin A and 2-methoxyethanol. J Liq Chromatogr Relat Technol. 1998; 21(3):299–313. http://dx.doi.org/10.1080/10826079808000492.

[28] Aboul-Enein HY, El-Awady MI, Heard CM. Enantiomeric resolution of some 2-arylpropionic acids using L-(-)-serine-impregnated silica as stationary phase by thin layer chromatography. J Pharm Biomed Anal. 2003; 32(4–5):1055–1059. http://dx.doi.org/10.1016/s0731-7085(03)00208-5.

[29] Aboul-Enein HY, El-Awady MI, Heard CM. Thin layer chromatographic resolution of some 2-arylpropionic acid enantiomers using L-(-)-serine, L-(-)-threonine and a mixture of L-(-)-serine and L-(-)-threonine-impregnated silica gel as stationary phases. Biomed Chromatogr. 2003; 17(5):325–334. http://dx.doi.org/10.1002/bmc.245.

[30] ICH, Q2B. Validation of analytical procedure: methodology. In: International Conference on Harmonization, Geneva, 1996.

[31] ICH, Q2A. Validation of analytical procedures: text and methodology. In: International Conference on Harmonization, Geneva, 2005.

[32] Dalgliesh CE. The optical resolution of aromatic amino-acids on paper chromatograms. J Chem Soc. 1952; 756: 3940. http://dx.doi.org/10.1039/jr9520003940.