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REVERSE-PHASE HPLC ENANTIOSEPARATION OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS USING POLYSACCHARIDE-BASED CHIRAL SELECTORS

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Abstract: The enantiomers of chiral drugs may differ in pharmacological activity, efficacy, and safety, making their reliable enantioseparation important for pharmaceutical analysis. The enantioseparation of three aryl propionic acid-derived nonsteroidal anti-inflammatory drugs (ibuprofen, ketoprofen, and carprofen) was investigated by high-performance liquid chromatography using polysaccharide-based chiral stationary phases. Two amylose-based and four cellulose-based columns were tested using acidified water-acetonitrile mobile phases. The effects of the chiral selector, mobile-phase composition, column length, and temperature were examined. Lux® Amylose-1 provided baseline separation of ibuprofen and carprofen, while Lux® Amylose-2 separated carprofen and gave only partial separation of ketoprofen. Lux® Cellulose-3 gave the best overall results, with baseline separation of ibuprofen and carprofen on both the 150 and 250 mm columns. Lux® Cellulose-1 separated only carprofen, whereas Lux® Cellulose-2 did not separate any of the three compounds. Ketoprofen could not be baseline resolved under the conditions tested. The results show that enantioselectivity was determined mainly by the chemical structure of the chiral selector rather than by the cellulose or amylose backbone alone.

Keywords: chiral HPLC; enantioseparation; nonsteroidal anti-inflammatory drugs; ibuprofen; ketoprofen; carprofen; polysaccharide-based stationary phases

1. Introduction

Chirality is one of the fundamental concepts in pharmaceutical chemistry as biological macromolecules, including enzymes, proteins, receptors, and nucleic acids, are all chiral. The enantiomers of a chiral drug may differ in their interactions with biological targets, resulting in differences in metabolism, pharmacological activity, and toxicity. In some cases, one enantiomer is more active, while the other may be less active or sometimes associated with adverse effects (Barron, 2008; Ceramella et al., 2022).

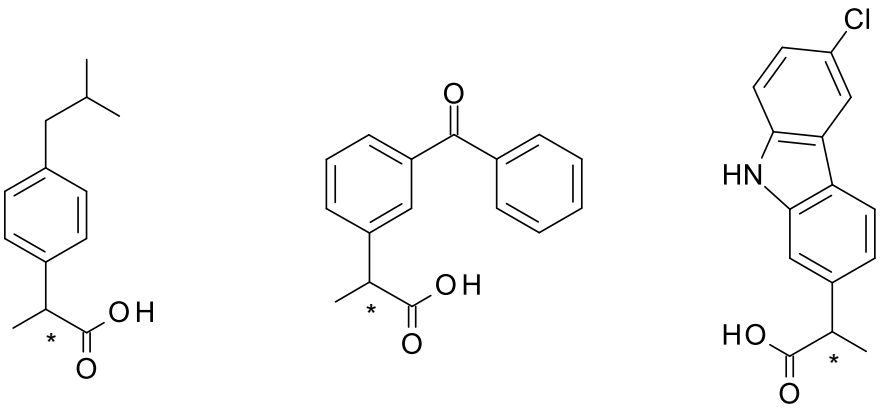
Approximately half of the drugs currently in clinical use contain one or more stereogenic centers, and many are still marketed as racemic mixtures. During the last three decades,

however, pharmaceutical research has increasingly focused on the development of single-enantiomer drugs. This trend has been driven by advances in asymmetric synthesis and chiral separation techniques, together with regulatory requirements that require the pharmacokinetic, pharmacological, and toxicological evaluation of individual enantiomers during drug development. The availability of enantiomerically pure active pharmaceutical ingredients may improve therapeutic efficacy, reduce administered dose, minimize adverse reactions, and result in more predictable pharmacokinetic profiles (Calcaterra & D'Acquarica, 2018; McVicker et al., 2024).

Ibuprofen, ketoprofen, and carprofen are chiral non-steroidal anti-inflammatory (NSAIDs) drugs belonging to the 2-arylpropionic acid class (also called profens), each containing a single stereogenic center. Although their pharmacological activity is primarily associated with the *S*-enantiomer, differences in stereoselective metabolism and chiral inversion make the individual analysis of their enantiomers analytically and pharmacologically relevant. The chemical structures of the analyzed profens are presented in **Figure 1**.

Ibuprofen ((*R*,*S*)-2-(4-isobutyl phenyl) propanoic acid) is a chiral aryl propionic acid derivative marketed primarily as a racemic mixture. The anti-inflammatory activity is mainly attributed to *S*-ibuprofen, whereas *R*-ibuprofen undergoes partial metabolic inversion to the active *S*-ibuprofen in vivo. Owing to this unidirectional chiral inversion, both enantiomers contribute to the overall therapeutic effect, although to different extents. In several countries, the pure *S*-enantiomer is also available as dexibuprofen, with administration of lower doses than the racemic mixture (Evans, 2001; Hao et al., 2005).

Ketoprofen ((*R*,*S*)-2-(3- benzoylphenyl) propanoic acid) is available both as a racemic mixture and as the single-enantiomer drug dexketoprofen. *S*-ketoprofen is responsible for the anti-inflammatory and analgesic effects through cyclooxygenase (COX) inhibition, whereas *R*-ketoprofen exhibits considerably lower pharmacological activity and does not undergo significant chiral inversion in vivo. Dexketoprofen is a well-known example of a successful chiral switch among NSAIDs (Jamali & Brocks, 1990; Ong et al., 2005).



Ibuprofen Ketoprofen Carprofen

Fig. 1. Chemical structures of the investigated aryl propionic acid derivatives (ibuprofen, ketoprofen, carprofen). The asterisk (*) indicates the chiral center.

Carprofen ((*R*,*S*)-2-(6-chloro-9H-carbazol-2-yl)propanoic acid) is another chiral NSAID used primarily in veterinary medicine for the treatment of pain and inflammation. It is marketed as a racemic mixture despite stereoselective differences in the pharmacokinetics, and pharmacological activity of its enantiomers. *S*-carprofen is considered the principal COX inhibitor, while both enantiomers contribute to the clinical response through different pharmacological mechanisms (McKellar et al., 1994).

Reliable analytical methods are required for the separation and quantification of individual drug enantiomers. High-performance liquid chromatography (HPLC) is widely used for chiral analysis, with cellulose- and amylose-based chiral stationary phases (CSPs) being among the most applied stationary phases. Chiral recognition on these CSPs involves hydrogen bonding, π–π and dipole interactions, as well as steric effects. Enantioselectivity is also influenced by the mobile-phase composition and chromatographic conditions (Yashima, 2001; Papp et al., 2024).

Previous studies have demonstrated that polysaccharide-based CSPs provide good enantioselectivity for 2-arylpropionic acid derivatives. Okamoto et al. first reported the direct HPLC enantioseparation of ibuprofen, ketoprofen, flurbiprofen, and tiaprofenic acid using cellulose- and amylose-based tris(3,5-dimethylphenylcarbamate) CSPs under normal-phase (NP) conditions. This work established polysaccharide-based CSPs as highly efficient CSs for profens and represented a major step in the development of direct chromatographic methods for their enantioseparation (Okamoto et al., 1989).

Subsequently, Van Overbeke et al. evaluated two cellulose-based CSPs for the separation of ten profens under both NP and reversed-phase (RP) conditions. Chiralcel OJ showed superior enantioselectivity for the direct separation of underivatized profens in NP mode, whereas the experimental Tolylcellulose CSP exhibited limited performance under RP conditions and, in many cases, required derivatization of the carboxylic acid group to achieve satisfactory resolution (Van Overbeke et al., 1995).

A systematic comparison of commercially available polysaccharide-based CSPs was reported by Matarashvili et al., who investigated the enantioseparation of ten profens on six cellulose- and amylose-based columns under NP conditions. Their results demonstrated that enantiomeric recognition is strongly influenced by the polysaccharide backbone, substituents of the CS, mobile phase composition, and column temperature. The study also highlighted several cases of enantiomer elution order reversal resulting from changes in chromatographic conditions (Matarashvili et al., 2013). In a subsequent study, the same group extended this approach to fourteen chiral carboxylic acid derivatives using polar organic (PO) mobile phases (methanol, ethanol, acetonitrile). The authors compared six polysaccharide-based CSPs and identified amylose tris(3,5-dimethylphenyl-carbamate) as the most versatile selector, while also confirming the influence of mobile-phase composition and temperature on enantioselectivity (Matarashvili et al., 2015).

Cao et al. developed an RP-HPLC method for the enantioseparation of six profens using an amylose tris(3-chloro-5-methylphenyl-carbamate) CSP. Ibuprofen, ketoprofen, naproxen, and loxoprofen were baseline separated after optimization of the mobile phase and column temperature. The method was validated and applied to pharmaceutical formulations (Cao et al., 2021).

Camilo & Foley developed an RP-HPLC method for the simultaneous achiral and chiral separation of ketoprofen, ibuprofen,

flurbiprofen, and naproxen using an amylose tris(3,5-dimethylphenylcarbamate) CSP. The method allowed the simultaneous separation of the four profens, although ketoprofen enantiomers remained unresolved under the investigated RP conditions (Camilo & Foley, 2021).

Recently, Dobó et al. developed a RP HPLC method for the simultaneous determination of dexketoprofen enantiomeric purity and related organic impurities using polysaccharide-based CSPs. Among four screened amylose- and cellulose-derived columns, only the amylose tris(5-chloro-2-methylphenylcarbamate) CSP provided baseline enantioseparation under RP conditions, highlighting the strong influence of both the CS and the mobile phase composition on ketoprofen enantiorecognition (Dobó et al., 2024).

Despite these advances, studies comparing the performance of multiple commercially available cellulose- and amylose-based CSPs under RP conditions remain limited. Most published investigations have focused on NP or PO separation modes or have evaluated only a single CSP. A systematic comparison of different polysaccharide-based CSPs under identical RP conditions would therefore facilitate column selection and method development for the enantioseparation of aryl propionic acid derivatives.

The present study compares six commercially available cellulose- and amylose-based CSPs for the RP-HPLC enantioseparation of ibuprofen, ketoprofen, and carprofen. The effects of CSP, mobile-phase composition, column dimensions, and temperature on enantioseparation were investigated.

2. Materials and Methods

2.1. Instrumentation

Chromatographic determinations were performed using a Merck-Hitachi L-7400 HPLC system equipped with a quaternary pump, online degasser, thermostated autosampler, column oven, and UV-Vis detector.

The following polysaccharide-based CSPs (Phenomenex, Torrance, CA, USA) were evaluated: Lux® Amylose-1 (150 × 4.6 mm, 5 µm); Lux® Amylose-2 (150 × 4.6 mm, 5 µm); Lux® Cellulose-3 (150 × 4.6 mm, 5 µm); Lux® Cellulose-1 (250 × 4.6 mm, 5 µm); Lux® Cellulose-2 (250 × 4.6 mm, 5 µm); Lux® Cellulose-3 (250 × 4.6 mm, 5 µm).

Samples were weighed using an Ohaus Discovery analytical balance (Greifensee, Switzerland). Ultrapure water was produced with a Direct-Q purification system (Millipore, France). Sample and mobile-phase solutions were sonicated using a Transsonic T700H ultrasonic bath (Elma, Germany).

2.2. Chemicals and Reagents

Pharmaceutical grade ibuprofen (Novartis, Switzerland), ketoprofen (Italian Society of Medicine, Scandicci, Italy), and carprofen (Billion Team Enterprise Ltd., Hong Kong) were used as reference standards. HPLC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from Merck KGaA (Darmstadt, Germany), while formic acid was obtained from Tovarni Chemických Výrobků (Czech Republic). Ultrapure water was produced in-house using a Direct-Q purification system.

2.3. Standard Solutions and Mobile Phases

Individual stock solutions of ibuprofen, ketoprofen, and carprofen were prepared in MeOH at a concentration of 10 mg mL^{-1}. The appropriate amount of each analyte was accurately weighed, transferred to a 10 mL

volumetric flask, dissolved in MeOH, and diluted to volume. All solutions were sonicated for 15 min before use and stored at 8-12°C until analysis.

Two RP mobile-phase systems were evaluated:

- system A: water containing 0.1% (v/v) formic acid (solvent A) and MeOH containing 0.1% (v/v) formic acid (solvent B);
- system B: water containing 0.1% (v/v) formic acid (solvent A) and ACN containing 0.1% (v/v) formic acid (solvent B).

All mobile phases were prepared daily, filtered through a 0.45 µm membrane filter, and degassed by ultrasonication for 15 min before use.

3. Results and Discussions

The chromatographic screening was carried out using six polysaccharide-based CSPs under RP conditions. Preliminary experiments with MeOH-based mobile phases resulted in broader peaks and higher system backpressure. ACN was therefore selected as the organic modifier for subsequent experiments. The proportion of the organic modifier, column temperature, and column length were adjusted according to the retention and separation observed for each analyte. The acidic additive was used to suppress the ionization of the carboxylic groups and to improve peak shape and interaction with the chiral selector.

The physicochemical data of the analytes presented in **Table 1** indicate that the three compounds are moderately weak lipophilic acids. Their similar logP values suggest comparable hydrophobic interactions under RP conditions, whereas differences in pKa, molecular size, aromatic structure, and conformational flexibility may account for their different retention and enantioselective behavior. Ketoprofen is the most acidic compound, while ibuprofen has the lowest molecular weight. Carprofen contains a rigid chlorinated carbazole system, which may favor more defined stereoselective interactions with the stationary phase.

Table 1. Physicochemical properties of ibuprofen, ketoprofen, and carprofen.

Chemical compound	pKa	log P	Molecular weight (g/mol)
Ibuprofen	4.85	3.84	206.28 g/mol
Ketoprofen	3.88	3.61	254.28 g/mol
Carprofen	4.3	3.8	273.71 g/mol

3.1. Evaluation of Amylose-Based CSP

The initial screening was performed on the Lux® Amylose-1 column (150 × 4.6 mm, 5 µm) using a mobile phase consisting of 55% water containing 0.1% formic acid and 45% ACN containing 0.1% formic acid. The chromatographic conditions were as follows: flow rate 1.0 mL min^{-1}, column temperature 25 °C, injection volume 5 µL, and UV detection at 220 nm. The chromatogram of a ibuprofen-carprofen mixture obtained on the Lux® Amylose-1 column is presented in **Figure 2**. Under these chromatographic conditions, ibuprofen and carprofen exhibited chiral separation, whereas ketoprofen did not show enantiomeric separation and is therefore not included in the figure. Ibuprofen eluted at 6.74 and 7.38 min, while the carprofen enantiomers eluted at 13.77 and 16.77 min. The longer retention of carprofen may be related to its rigid aromatic structure and to stronger non-stereoselective and stereoselective interactions with the CSP. Both analytes were baseline separated, with carprofen showing higher resolution than ibuprofen.

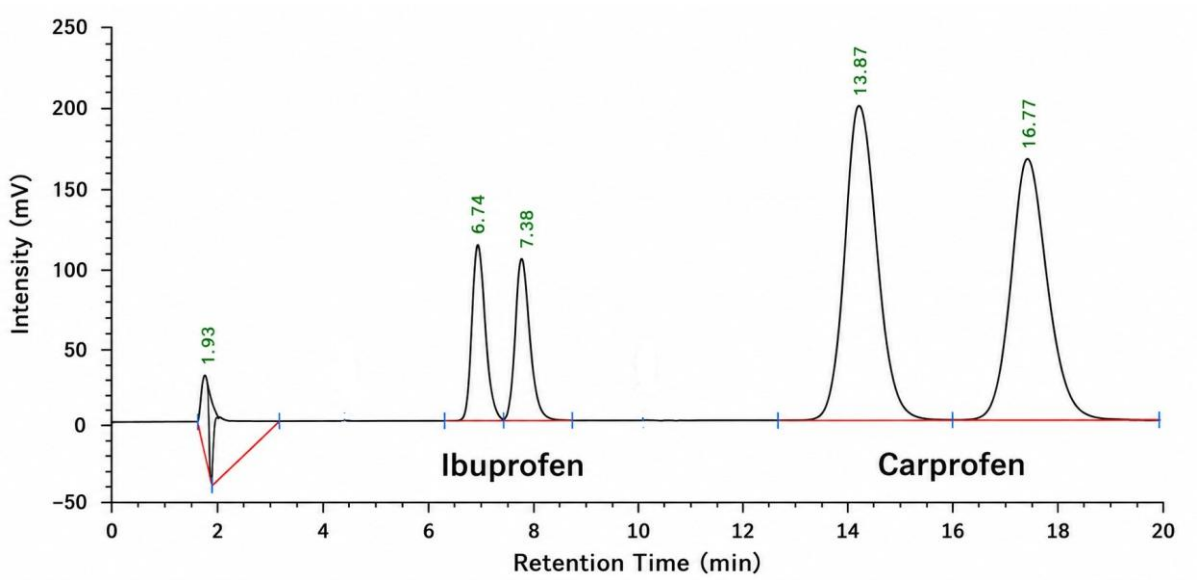


Fig. 2. Chromatogram of a mixture of ibuprofen and carprofen obtained on a Lux® Amylose-1 CSP (150 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase, 55% water containing 0.1% formic acid (A) and 45% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

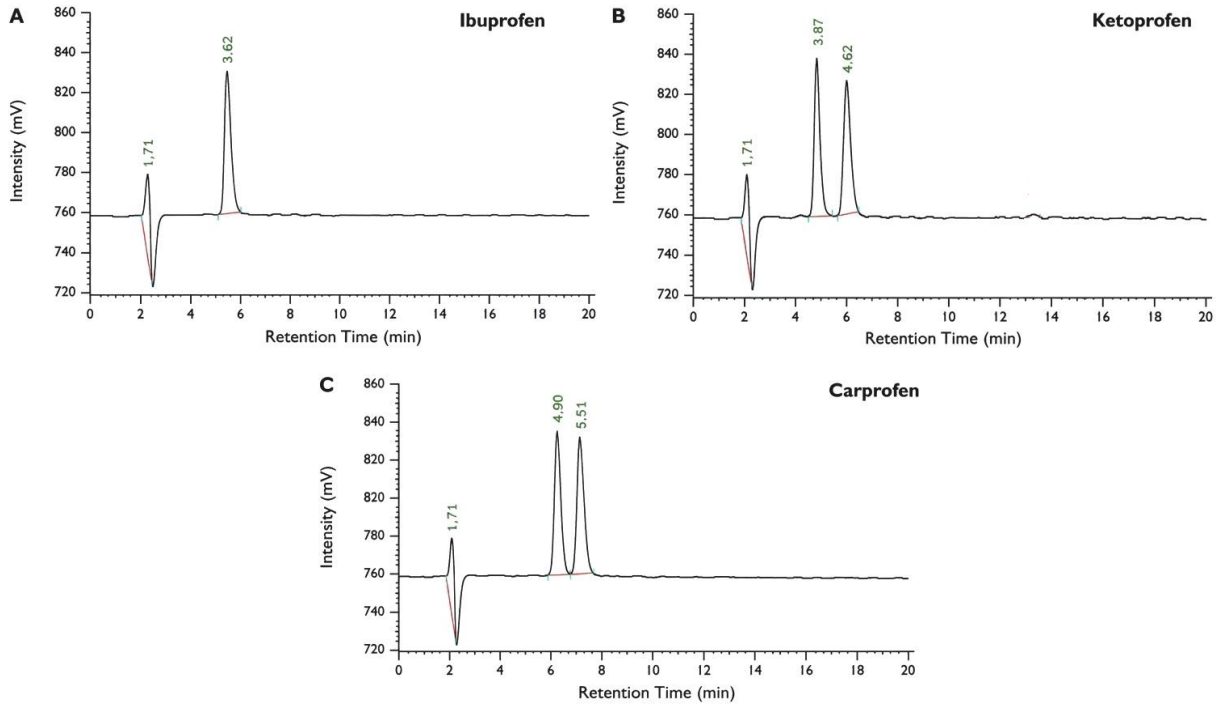


Fig. 3. Chromatograms of ibuprofen (A), ketoprofen (B), and carprofen (C) obtained on a Lux® Amylose-2 CSP (150 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase: 50% water containing 0.1% formic acid (A) and 50% acetonitrile containing 0.1% formic acid (B); flow rate, 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

The performance of the Lux® Amylose-2 column (150 × 4.6 mm, 5 μm) was evaluated using a mobile phase composed of 50% water containing 0.1% formic acid and 50% ACN containing 0.1% formic acid. The chromatographic conditions included a flow rate of 1.0 mL min^{-1}, a column temperature of 25 °C, an injection volume of 5 μL, and UV detection at 220 nm.

Representative chromatograms obtained on the Lux® Amylose-2 column are shown in **Figure 3**. Ibuprofen eluted as a single peak (3.62 min), indicating the absence of chiral recognition under these analytical conditions. Ketoprofen produced two partially resolved peaks at 3.87 and 4.62 min, while carprofen was baseline separated with retention times of 4.90 and 5.51 min. These results indicate that the Amylose-2 CSP provided sufficient enantioselectivity only for carprofen, whereas chiral recognition of ketoprofen remained incomplete and ibuprofen was not resolved.

3.2. Evaluation of Cellulose-Based Sationary Phases

Amylose and cellulose selectors contain related glucose-based structures but differ in their higher-order conformation. Amylose generally adopts a more flexible helical arrangement, whereas cellulose derivatives have a more rigid and extended structure. These conformational differences modify the accessibility and spatial arrangement of the interaction sites responsible for chiral recognition (Wang & Chen, 1999).

Following the evaluation of the amylose-based columns, the chromatographic behavior of the analytes was investigated on the Lux® Cellulose-3 CSP (150 × 4.6 mm, 5 μm). The separation was performed using a mobile phase consisting of 50% water containing 0.1% formic acid and 50% ACN containing 0.1% formic acid. The chromatographic conditions included a flow rate of 1.0 mL min^{-1}, a column temperature of 25 °C, an injection volume of 5 μL, and UV detection at 220 nm. Representative chromatograms obtained on the Lux® Cellulose-3 column are shown in **Figure 4**.

Ibuprofen and carprofen were baseline separated, with retention times of 8.68/9.21 min and 6.14/6.49 min, respectively. Ketoprofen eluted as a single peak at 4.41 min. Compared with the amylose-based columns, the Cellulose-3 stationary phase provided improved enantioselectivity for ibuprofen while maintaining excellent separation of carprofen.

After the individual analytes had been evaluated, a mixture containing ibuprofen and carprofen was analyzed under the same chromatographic conditions (**Fig. 5**). Four well-resolved peaks corresponding to the two pairs of enantiomers were obtained without overlap between the compounds. This experiment confirmed that the selected chromatographic system allowed the simultaneous enantioseparation of both analytes within a single run. To evaluate the effect of column length on chromatographic performance, the Lux® Cellulose-3 column (250 × 4.6 mm, 5 μm) was investigated under RP conditions. The mobile phase consisted of 60% water containing 0.1% formic acid and 40% acetonitrile containing 0.1% formic acid. The analyses were performed at a flow rate of 1.0 mL min^{-1}, a column temperature of 25 °C, an injection volume of 5 μL, and UV detection at 220 nm.

Figure 6 shows the chromatograms obtained on the 250 mm Lux® Cellulose-3 column. Increasing the aqueous content of the mobile phase increased the retention of all analytes, as expected under RP conditions. Higher retention and improved enantiomeric separation were observed on the 250 mm column using a mobile phase containing 60% aqueous phase.

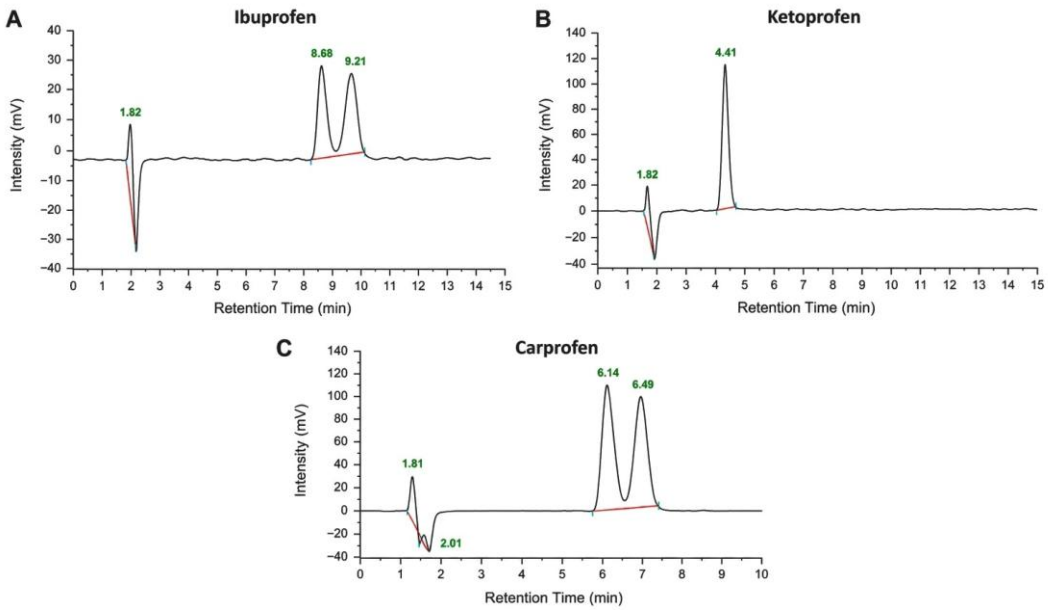


Fig. 4. Chromatograms of ibuprofen (A), ketoprofen (B), and carprofen (C) obtained on a Lux® Cellulose-3 CSP (150 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase, 50% water containing 0.1% formic acid (A) and 50% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

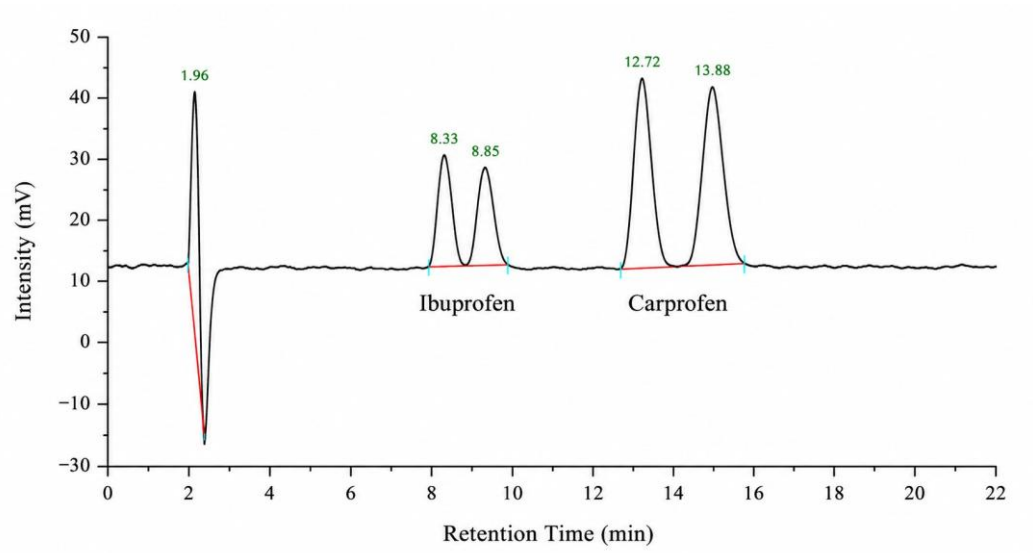


Fig. 5. Chromatogram of a mixture of ibuprofen and carprofen obtained on a Lux® Cellulose-3 CSP (150 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase 50% water containing 0.1% formic acid (A) and 50% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

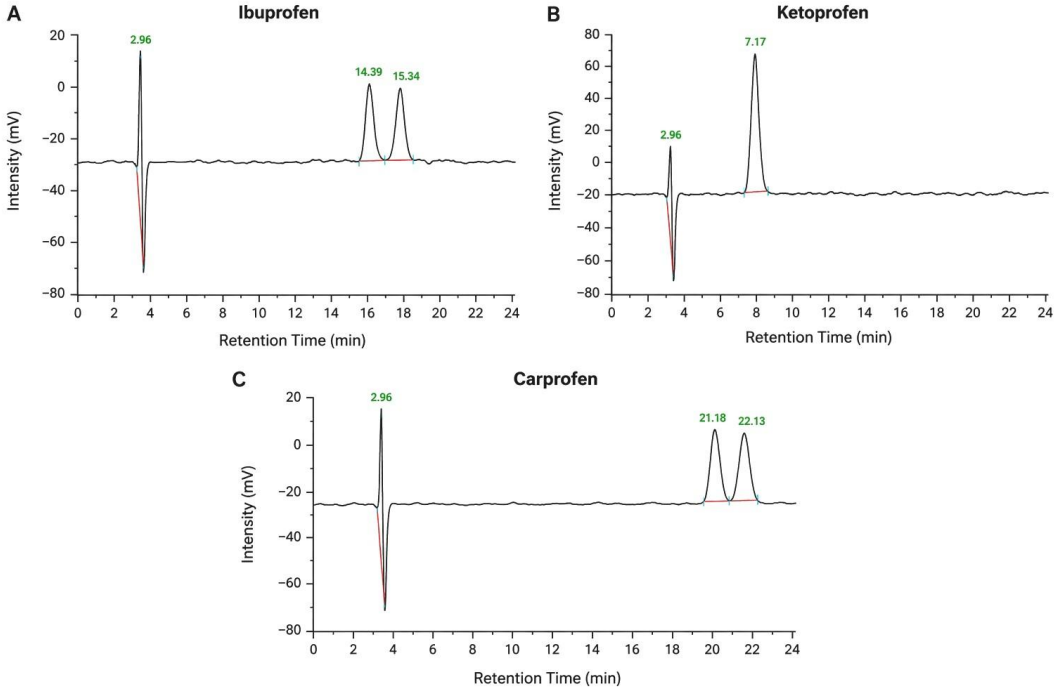


Fig. 6. Chromatograms of ibuprofen (A), ketoprofen (B), and carprofen (C) obtained on a Lux® Cellulose-3 CSP (250 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase, 60% water containing 0.1% formic acid (A) and 40% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

However, because both column length and mobile-phase composition were changed, their individual contributions could not be assessed separately.

In contrast, ketoprofen still eluted as a single peak, indicating that increasing column length and retention alone was insufficient to improve its enantiomeric discrimination.

Figure 7 presents the chromatogram obtained for a mixture containing ibuprofen, ketoprofen and carprofen under the optimized conditions. Ketoprofen eluted first as a single unresolved peak, followed by the baseline-separated enantiomers of ibuprofen and carprofen. No overlap between peaks was observed, demonstrating that the three compounds could be chromatographed in a single run, although the ketoprofen enantiomers remained unresolved.

The chromatographic performance of the Lux® Cellulose-1 column (250 × 4.6 mm, 5 µm) was investigated under the same RP conditions using a mobile phase composed of 50% water containing 0.1% formic acid and 50% ACN containing 0.1% formic acid. The flow rate was 1.0 mL min^{-1}, the column temperature was 25 °C, the injection volume was 5 µL, and UV detection was performed at 220 nm.

The chromatograms obtained on the Lux® Cellulose-1 column are shown in **Figure 8**. Ibuprofen and ketoprofen each produced a single chromatographic peak, indicating the absence of chiral recognition. Carprofen was baseline separated with retention times of 13.28 and 15.21 min. These results indicate that the Cellulose-1 selector exhibited pronounced stereoselectivity only toward carprofen.

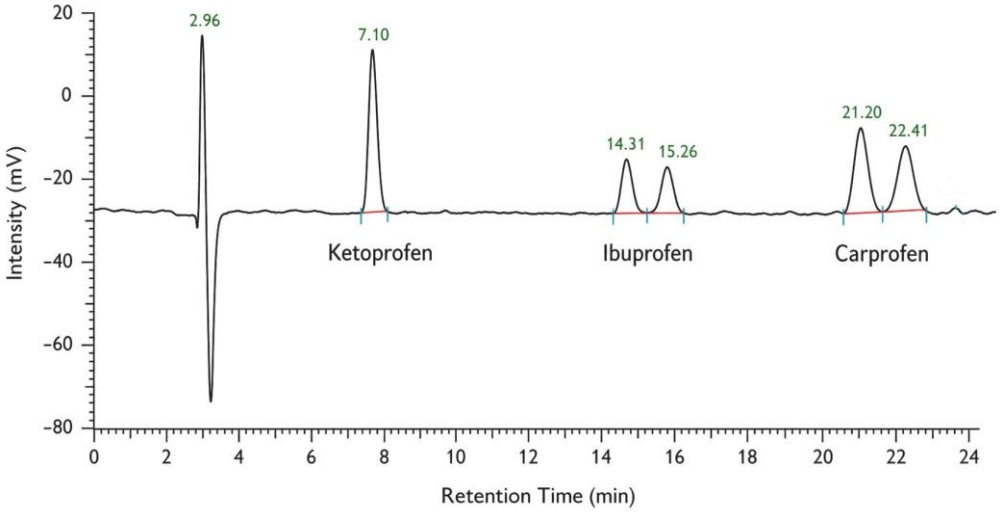


Fig. 7. Chromatogram of a mixture of ibuprofen, ketoprofen and carprofen obtained on a Lux® Cellulose-3 CSP (250 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase 60% water containing 0.1% formic acid (A) and 40% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

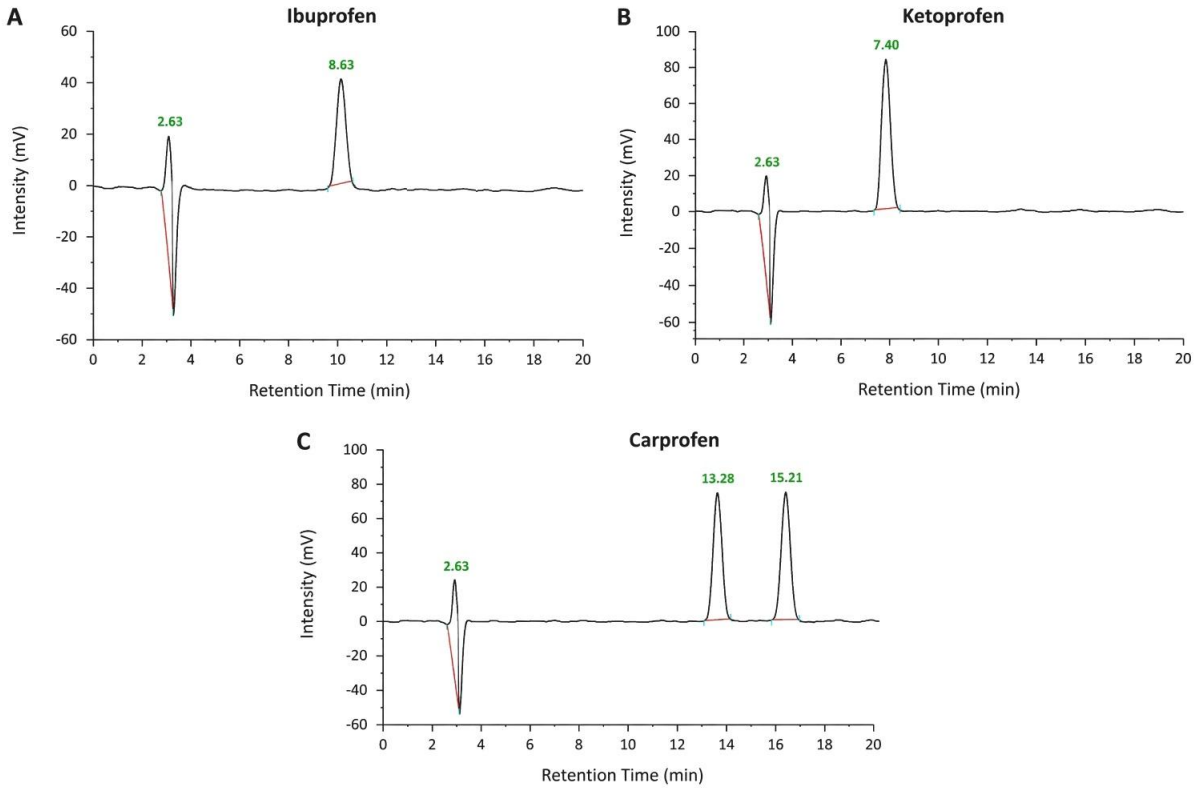


Fig. 8. Chromatograms of ibuprofen (A), ketoprofen (B), and carprofen (C) obtained on a Lux® Cellulose-1 CSP (250 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase, 50% water containing 0.1% formic acid (A) and 50% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

The chromatographic performance of the Lux® Cellulose-2 column (250 × 4.6 mm, 5 µm) was evaluated under RP conditions using the same acidified water-ACN mobile phases. The experiments were performed at a flow rate of 1.0 mL min^{-1}, a column temperature of 25 °C, an injection volume of 5 µL, and UV detection at 220 nm.

Figure 9 shows the chromatograms obtained on the Lux® Cellulose-2 column. Under the investigated conditions, all three analytes eluted as single peaks and no enantiomeric separation was observed. These results indicate that this stationary phase did not provide sufficient stereoselective interactions for the investigated profen derivatives.

The chromatographic conditions and the enantioseparation results obtained for the investigated analytes on the six polysaccharide-based chiral stationary phases are summarized in **Table 2**. The table highlights the influence of the CS and mobile-phase composition on analyte retention and enantioselectivity, providing an overview of the chromatographic performance of the evaluated columns.

Overall, the chromatographic results demonstrated that the enantioselectivity of the investigated NSAIDs depended primarily on the chemical structure of the CS rather than on whether the CSP was cellulose- or amylose-based.

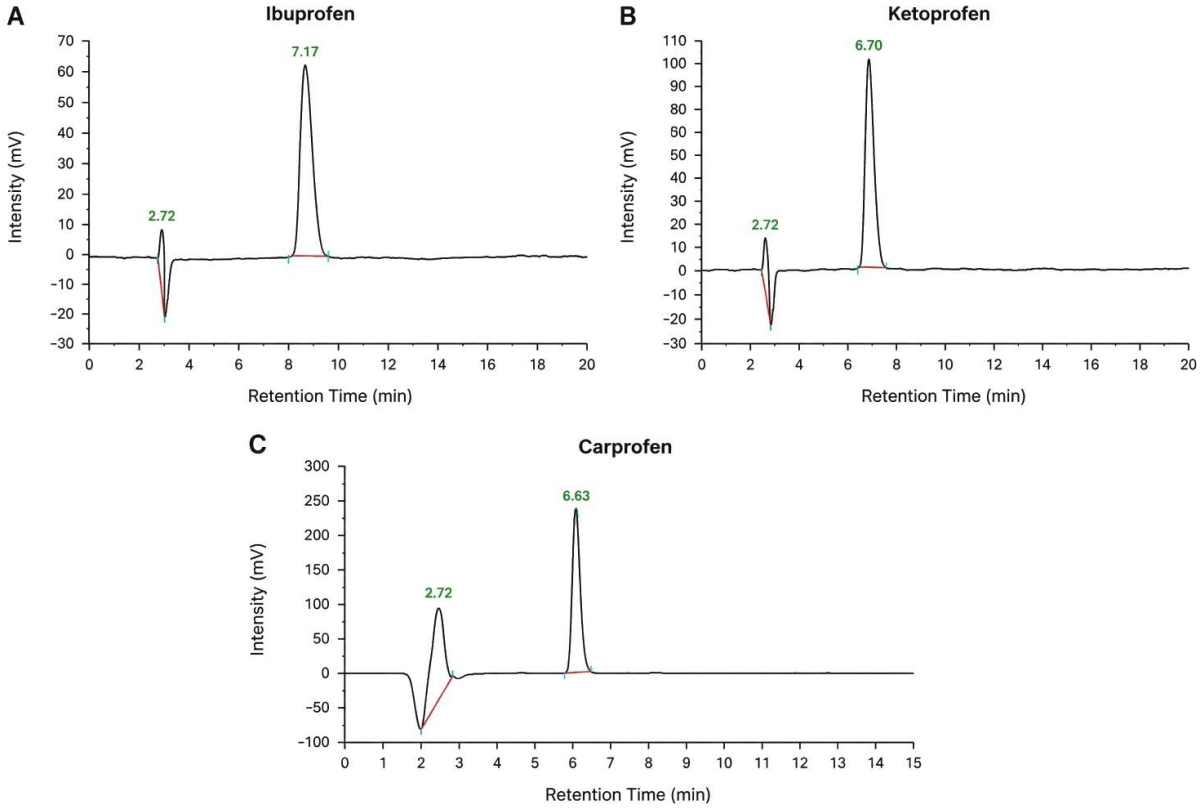


Fig. 9. Chromatograms of ibuprofen (A), ketoprofen (B), and carprofen (C) obtained on a Lux® Cellulose-2 CSP (250 × 4.6 mm, 5 µm). Chromatographic conditions: mobile phase, 50% water containing 0.1% formic acid (A) and 50% ACN containing 0.1% formic acid (B); flow rate 1.0 mL min^{-1}; column temperature 25 °C; injection volume 5 µL; UV detection at 220 nm.

Table 2. Comparison of chromatographic conditions and enantioseparation results obtained on the investigated polysaccharide-based CSPs under RP-HPLC conditions

Column	Chiral selector	Mobile phase	Analyte	Retention time (min)	Enantio-separation
Lux® Amylose-1 (150 × 4.6 mm, 5 µm)	Amylose tris(3,5-dimethylphenylcarbamate)	55% H_2O + 0.1% FA / 45% ACN + 0.1% FA	Ketoprofen	4.38	No
			Ibuprofen	6.74 / 7.38	Yes
			Carprofen	13.77 / 16.77	Yes
Lux® Amylose-2 (150 × 4.6 mm, 5 µm)	Amylose tris(5-chloro-2-methylphenylcarbamate)	50% H_2O + 0.1% FA / 50% ACN + 0.1% FA	Ketoprofen	3.87 / 4.62	Partial
			Ibuprofen	3.62	No
			Carprofen	4.90 / 5.51	Yes
Lux® Cellulose-3 (150 × 4.6 mm, 5 µm)	Cellulose tris(4-methylbenzoate)	50% H_2O + 0.1% FA / 50% ACN + 0.1% FA	Ketoprofen	4.41	No
			Ibuprofen	8.68 / 9.21	Yes
			Carprofen	6.14 / 6.49	Yes
Lux® Cellulose-1 (250 × 4.6 mm, 5 µm)	Cellulose tris(3,5-dimethylphenylcarbamate)	50% H_2O + 0.1% FA / 50% ACN + 0.1% FA	Ketoprofen	7.40	No
			Ibuprofen	9.68	No
			Carprofen	13.28 / 15.21	Yes
Lux® Cellulose-2 (250 × 4.6 mm, 5 µm)	Cellulose tris(3-chloro-4-methylphenylcarbamate)	50% H_2O + 0.1% FA / 50% ACN + 0.1% FA	Ketoprofen	6.70	No
			Carprofen	6.63	No
			Ibuprofen	7.17	No
Lux® Cellulose-3 (250 × 4.6 mm, 5 µm)	Cellulose tris(4-methylbenzoate)	60% H_2O + 0.1% FA / 40% ACN + 0.1% FA	Ketoprofen	7.17	No
			Ibuprofen	14.39 / 15.34	Yes
			Carprofen	21.18 / 22.13	Yes

Note: FA – formic acid, ACN – acetonitrile

Among the evaluated columns, Lux® Cellulose-3 provided the most versatile performance, allowing baseline enantioseparation of both ibuprofen and carprofen under RP conditions, whereas Lux® Cellulose-1 was selective only for carprofen. The two amylose-based columns exhibited different selectivities despite their similar polysaccharide backbone, emphasizing the importance of the phenylcarbamate substituents in chiral recognition.

Conclusions

The present study compared the enantioselective performance of six commercially available polysaccharide-based CSP for the RP HPLC separation of three 2-arylpropionic acid derivatives. The results showed that chiral recognition depended mainly on the chemical structure of the CS rather than on the polysaccharide backbone itself. Even columns derived from the same polysaccharide exhibited markedly different selectivities toward the investigated analytes.

Among the evaluated CSPs, Lux® Cellulose-3 showed the best overall performance, providing baseline enantioseparation of both ibuprofen and carprofen, whereas Lux® Cellulose-1 was suitable only for carprofen. The two amylose-

based columns displayed different selectivities, confirming that the substituents attached to the polysaccharide play a key role in enantiomeric recognition. Lux® Cellulose-2 did not provide useful enantioseparation for any of the investigated compounds.

No single chromatographic system provided baseline enantioseparation of all three analytes. Nevertheless, the results provide practical guidance for the initial selection of polysaccharide-based CSPs in the development of RP-HPLC methods for the enantioselective analysis of profens, potentially reducing the extent of column screening required during method optimization. Future studies should focus on a broader evaluation of mobile-phase composition, temperature, and alternative polysaccharide-based selectors, particularly for ketoprofen, as well as on the validation and application of the most promising separation systems to pharmaceutical formulations and enantiomeric purity assessment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

M.M. (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Preparation, Writing – review & editing), A.G.C. (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing), G.H. (Conceptualization, Funding acquisition, Software, Supervision, Validation, Visualization, Writing – original draft, Preparation, Writing – review & editing).

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Generative AI Statement:

The authors declare that no Artificial Intelligence tools were used in the generation, writing, or editing of this manuscript. The work is entirely original and was developed exclusively by the authors. All data, analyses, and interpretations were independently conducted and critically verified to ensure scientific accuracy and compliance with journal standards.

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