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Simultaneous enantioseparation of nonsteroidal anti-inflammatory drugs by a one-dimensional liquid chromatography technique using a dynamically coated chiral porous silicon pillar array column

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Abstract

The preparation of a highly efficient chiral liquid chromatography (LC) column is explored by dynamically coating a reversed-phase porous silicon pillar array column with hydroxypropyl-β-cyclodextrin (Hp-β-CD) as the chiral selector. Analyte mixtures composed of non-steroidal anti-inflammatory drugs were tested to reveal the enantioseparation potential of the column. The mechanism of chiral discrimination was investigated. The adsorbed Hp-β-CDs on the column surface experience different interaction with enantiomers. The chiral stationary phase showed satisfying stability and could be easily restored by recovering the selector with sufficient flushing and repeating the loading procedure. The peak capacity of the column was evaluated, and it was found high enough to separate three enantiomer couples using a one-dimensional LC technique.

Keywords: Chiral; Dynamic coating; One-dimensional liquid chromatography; Non-steroidal anti-inflammatory drugs; Porous silicon pillar array column.

1. **Introduction**

Chiral separation has become an important topic, not only for the analytical determination of enantiomeric purity, but also for the isolation of enantiomers. As a result, the demand for stereoselective separation techniques and analytical assays to evaluate the enantiomeric purity of chiral compounds has increased. The relevance of enantioseparation has particularly increased in the pharmaceutical industry and the development of various methods for analytical and preparative chiral separations is therefore recognized as a critical point in pharmaceutical research. This is related to the fact that the human body, with its numerous homochiral compounds such as proteins and amino acids, operates as the chiral environment. It therefore responds differently to each of the racemic drugs enantiomers and enantiomers display different biological activities such as metabolism, toxicology, pharmacokinetics, and so on [1,2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) play an important role in modern therapy. Since most NSAIDs are chiral and each enantiomer shows different biological and therapeutic activities, the enantioselective separation of this main drug family is important [3-5].

To achieve appropriate NSAIDs enantioresolution of individual chiral pairs, several liquid chromatography (LC) methods have been proposed. While LC methods using polysaccharide-based chiral stationary phase (CSP) are most popular [6-8], some methods using various chiral selectors such as vancomycin [9] and hydroxypropyl-β-cyclodextrin (Hp-β-CD) [10, 11] as the chiral mobile phase additive have been developed as well.

In general, the simultaneous chiral separation of analytes encounters several difficulties. The achievement of a high peak capacity is a good measure of the general performance of the column. While it is not common to require the separation of large amounts of enantiomers, the need for high efficiency is very relevant. High efficiency is essential when e.g. aiming at the (rapid) detection of low quantities of enantiomeric impurities. To the best of our knowledge there are no successful one-dimensional (1D) approaches described in literature to separate multiple pairs of NSAIDs.

However, chiral chemically bonded stationary phases may be perceived as the ideal stationary phase format, since they give more stable retention times. Dynamic coating of non-chiral LC columns with a chiral selector represents another viable approach. The latter approach is a simple, reversible and inexpensive way to prepare CSPs. Change of the selector to another one is straightforward if a suitable washing procedure is employed and followed by coating with a new selector.

Advantages and drawbacks of chemical and dynamic coated CSPs are discussed in literature. For instance, chemically and dynamically bonded CSPs were tested for enantioseparation of amino acids, α -hydroxy acids, and dipeptides. The chemically bonded CSP was found to be superior to the dynamically coated CSP in terms of enantioseparation of amino acids and dipeptides. However, α-hydroxy acids were better resolved on the dynamically coated phase [12]. Several

authors have described the successful application of dynamically coated LC phase using different types of chiral selectors on a monolithic support or on packed columns [13-15].

To further increase the attainable peak capacity and column efficiency, pillar array columns (PACs) have been introduced as an alternative column format to packed bed and monoliths. The order and design freedom of the pillar bed makes the PAC unique and offers several advantages compared to conventional columns. The eddy diffusion (the A term in the van Deemter equation) is negligible in this column due to homogeneous flow paths in the separation bed. In addition, the much lower back pressure of PACs allows for the application of very long columns and concomitantly very high plate counts [16, 17]. In terms of loadability, porous PACs are preferred for most applications in LC [18], but also non-porous columns have been used successfully by several groups to perform a wide range of applications [19-22] that can even benefit from the non-porous nature of the column.

Ketoprofen, naproxen and ibuprofen are frequently used profens. While the (S)-naproxen enantiomer binds to the cyclooxygenase enzyme to decrease prostaglandin production at the site of injury for decreasing pain, (R)-naproxen isomer does not inhibit inflammation properties and is furthermore toxic to the liver [23]. Also, (S)-ibuprofen is the only active form and (R)-ibuprofen even causes side effects [24]. The (R) and (S)-ketoprofen enantiomers produce different therapeutic actions. The (S)-enantiomer is used to relieve pain, while the(R)-enantiomer is applied to prevent periodontal disease. In addition, the (R)-enantiomer of the ketoprofen has been reported to convert into its antipode in human and animals' bodies [25]. Therefore, optical purity of these drugs is a critical point and their enantioseparation is necessary.

In the present study, we prepared a dynamically coated reversed-phase -porous silicon pillar array column (RP-PAC) using Hp-β-CD as chiral selector and used it to simultaneously enantioseparate selected NSAIDs (naproxen, ketoprofen, ibuprofen) and to get some insight in the separation mechanism. The main goal of this work was to evaluate the chiral separation potential of the pillar array column.

2. Experimental

2.1. Apparatus

A nano-LC (UltiMate 3000, Thermo Fischer Scientific, Massachusetts, USA) equipped with a UV-detector system and column oven was used for (dynamic) chiral coating and chromatography evaluation. The UV system was set at wavelength equal to 214, 254 and 230 nm. The sample injection was performed using an automated 4 nl valve system injections (C4N-4004) obtained from Valco (Schenkon, Switzerland). RP-PACs were acquired from PharmaFluidics (Zwijnaarde, Belgium). The chips contain channels with a length of 2 m and a width of 315 µm. The channels are filled with cylindrical pillars (5 µm diameter, 18 µm deep, 2.5 µm interpillar distance, 59% bed channel porosity, 450 nm porous layer thickness) and were

fabricated in the same way as already described in detail by Callewaert et al. [26]. A typical SEM image of the pillar array is depicted in Fig .1.

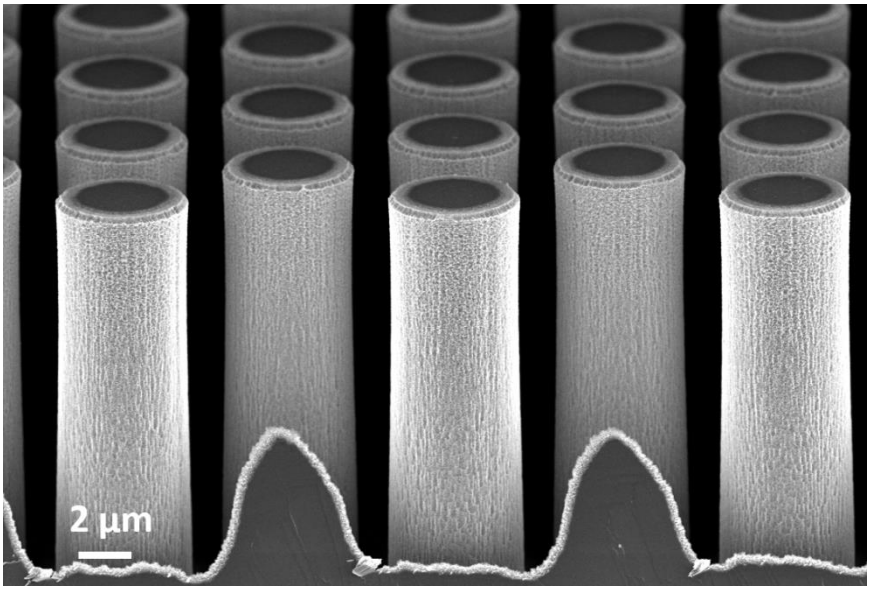

Fig. 1. SEM picture of the pillar array

A pH-meter (WTWinoLab, Weilheim, Germany) was used for pH adjustments of buffer solutions.

2.2. Material

Ketoprofen, ketorolac, indoprofen, fenoprofen, suprofen, ibuprofen, S-ibuprofen, flurbiprofen, benzoic acid, naphthalene, and uracil standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). Naproxen reference standard was kindly provided by Temad faculty (Tehran, Iran). The chemical structures of solutes have been summarized in Fig. 1S. Some chemical and physical properties of tested solutes are collected in table 1. Hp-β-CD with the substitution degree of 3.5-5.5 was obtained from Cyclodextrin-Shop (Tilburg, Netherlands). Purified water from a Milli-Q reagent water system (Millipore, Bedford, MA, USA) was used to prepare the buffer and reagent solutions. The analytical grade H_3PO_4, NaH_2PO_4, NaOH were purchased from Merck (Darmstadt, Germany). LC/MS grade acetonitrile, ethanol and methanol were also purchased from Biosolve (Valkenswaard, Netherland).

2.3. Preparation of stock and standard Solution

The standard stock solution of the chemicals was prepared in organic solvent (methanol, ethanol, acetonitrile) at a concentration of 1000 µg mL^{-1}. Standard working solutions were diluted with water/organic solvent (50/50) to get adequate concentration.

2.4. Chromatographic conditions

All separations were carried out at 30 ºC at a flow rate of 0.25 µL/min. LC/MS-grade methanol, acetonitrile, ethanol, deionized water were used as components of the mobile phase.

For the chromatographic evaluation of chiral and non- chiral columns, peak capacity, retention coefficient, and resolution were calculated using following equations:

Peak capacity as the number of peaks that can fit into a chromatogram between the first and the 'last peaks, can calculate based on retention time (n_r):

$$(n_r) = (T_R - T_0)/W_b \quad (1)$$

Also, peak capacity based on gradient time (n_G) can calculate as:

$$(n_G) = 1 + (T_G/W_b) \quad (2)$$

The retention coefficient and resolution were calculated by:

$$\text{Retention coefficient} = (T_R - T_0)/T_0 \quad (3)$$

$$\text{Resolution} = 1.18\ (T_{R,2} - T_{R,1})/(W_{2,50\%} - W_{1,50\%}) \quad (4)$$

with T_R, T_0, T_G, $T_{R,2}$, $T_{R,1}$, $W_{2,50\%}$, $W_{1,50\%}$ and W_b the retention time of a retained compound, the retention time of a non- retained compound, the gradient time, the retention time of more retained compound, the retention time of less retained compound, the peak width of more retained compound at 50% height, the peak width of less retained compound at 50% height and the baseline peak width, respectively.

2.5. Chiral selector immobilization via physical absorption

The dynamic chiral coating process consists of the attachment of the Hp-β-CD chiral selector on a PAC column coated with C_{18} groups through non-covalent coating. To immobilize the selector, the 10mM Hp-β-CD was dissolved in phosphate buffer (25 mM, pH 4) and pumped through the column [15,28] at a column temperature that was fixed at 30 ºC. The chiral selector solution was recycled through the column at a constant flow rate of 0.25 µl/min Changes in the absorbance of the eluent were followed by UV detection. Equilibrium in the coating process was indicated by an initial abrupt in the UV baseline and then stabilizes to the new baseline [29], which occurred after 15 h. In practice the column was flushed for 28h with feed solution, which is equivalent to 50 column volumes.

Thereafter, the column was washed with water and organic solvent

Table 1 Some properties of the tested solutes [27].

Analyte	Molar mass (g/mol)	Log D	pk_a	Van der Waals volume (A^{o3})
Ketorolac	255.27	2.28	3.84	223.07

Indoprofen	281.31	2.86	3.74	250.7
Suprofen	260.31	3.53	4.01	225.21
Ketoprofen	254.28	3.61	3.88	233.86
Naproxen	230.23	2.99	4.19	213.06
Fenoprofen	242.27	3.65	3.96	223.4
Flurbiprofen	244.26	3.94	4.42	219.19
Ibuprofen	206.28	3.84	4.85	211.80
Naphthalene	128.27	2.96	-	125.17
Benzoic acid	122.12	1.63	4.08	109.73

3. Results and discussion

Before performing the dynamic coating, first two sample sets of the NSAID family were separated on the RP-PAC . In a next step, a chiral column was simply prepared by pumping a solution of Hp-β-CD through the column. This column was then used to simultaneously separate ketoprofen, naproxen and ibuprofen enantiomers.

3.1. Separation of NSAIDs on the RP-PAC

Two different sample sets containing 1. naproxen, ibuprofen, ketoprofen and 2. ketorolac, suprofen, indoprofen, ketoprofen, naproxen, ibuprofen, fenoprofen, flurbiprofen were injected and separated using acetonitrile/water in the gradient elution as the mobile phase. The obtained chromatograms are shown in Fig. 2 and Fig. 3. The chromatographic parameters are summarized in table 2 and table 3.

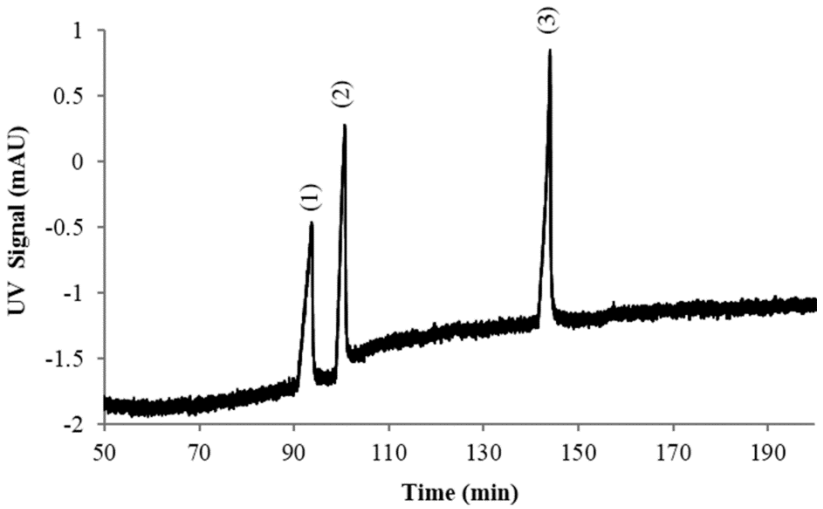

Fig. 2. Separation of some profens on the studied RP-PAC. Components: 1. ketoprofen 2. naproxen 3. Ibuprofen. Chromatographic conditions: column length 200cm. mobile phase: gradient elution of acetonitrile–water (15/85–80/20 v/v), flow rate: 0.25 µl/min, injection volume: 4 nL, column temperature: 30ºC and detection: UV at 214 nm.

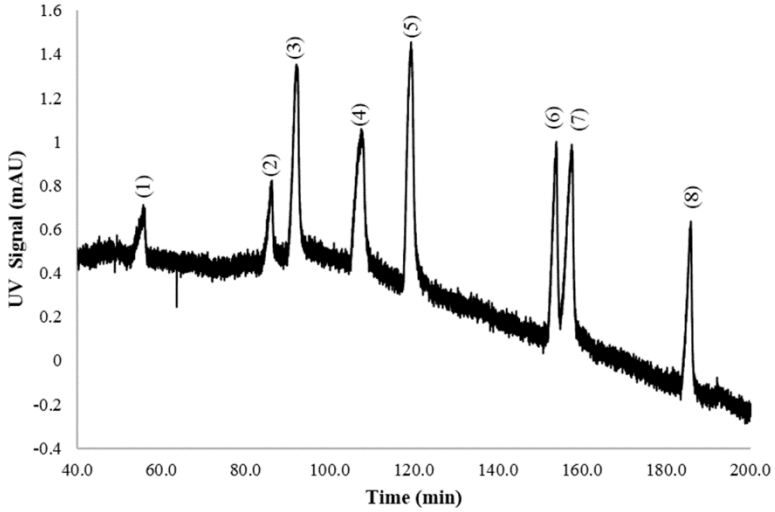

Fig. 3. Separation of some profens. Components: 1. ketorolac, 2. indoprofen, 3. suprofen, 4. ketoprofen, 5. naproxen, 6. fenoprofen, 7. flurbiprofen, 8. ibuprofen. Chromatographic conditions: column: 200 cm RP-PAC. mobile phase: gradient elution of acetonitrile–water (15/85–40/60 v/v), flow rate: 0.25 µl//min, injection volume: 4 nL, column temperature: 30 ºC and detection: UV at 214 nm.

Table 2. Chromatographic parameters for ketoprofen, naproxen and ibuprofen using the RP-PAC. Experimental conditions are the same as in Fig. 2.

Analyte	Retention time (min)	Retention coefficient	Width (50%)	Resolution
Ketoprofen	93.70	1.94	1.59	3.04
Naproxen	100.68	2.16	1.12	25.27
Ibuprofen	144.04	3.52	0.91	/

Table 3. Chromatographic parameters for eight profens on the RP-PAC. Experimental conditions are the same as in Fig. 3.

Analyte	Retention time (min)	Retention coefficient	Width (50%)	Resolution
Ketorolac	55.68	0.68	1.11	16.98
Indoprofen	86.29	1.61	1.01	2.79
Suprofen	92.09	1.78	1.44	5.10
Ketoprofen	107.49	2.25	2.12	3.70
Naproxen	119.38	2.61	1.67	14.19
Fenoprofen	153.92	3.65	1.20	1.53
Flurbiprofen	157.59	3.76	1.62	12.03
Ibuprofen	185.90	4.62	1.16	/

3.1.1. Separation mechanism

To gain more understanding in the separation mechanism, first the elution order of eight separated profens is investigated. The main interaction in reverse phase columns is hydrophobic interaction. The octanol/water distribution coefficient (D), defined as the ratio of the molar concentration of the solute in octanol or water at a specified temperature, is usually used to represent molecular hydrophobicity. According to the chemicals' log D the analytes should elute in this order: 1. ketorolac (2.28), 2. indoprofen (2.86) 3. naproxen (2.99) 4. suprofen (3.53) 5. ketoprofen (3.61) 6. fenoprofen (3.65), 7. ibuprofen (3.84), 8. flurbiprofen (3.95). However, the obtained results show another elution order: 1. ketorolac, 2. indoprofen, 3. suprofen, 4. ketoprofen, 5. naproxen, 6. fenoprofen, 7. flurbiprofen, 8. ibuprofen.

This result indicates that hydrophobic partitioning interaction does not control the separation process solely and that at least another retention mechanism plays a role. The steric hindrance of solute molecules can be considered as an influencing factor in retention of the analytes. It is suspected that steric hindrance made interactions less strong and that the order of elution was therefore, to some extent, scrambled. For example, ibuprofen has a small volume and is expected to experience less steric hindrance, resulting in high retention in comparison to e.g. flurbiprofen, which is more hydrophobic but voluminous.

The elution order of the separated eight profens based on the size of compounds merely can be estimated according to their volume (table 1): 1. indoprofen (250.7 A^{o3}) 2. ketoprofen (233.86

A^{o3}) 3. suprofen (225.21 A^{o3}) 4. fenoprofen (223.4 A^{o3}) 5. ketorolac (223.07 A^{o3}) 6. flurbiprofen (219.19 A^{o3}) 7. naproxen (213.06 A^{o3}) 8. ibuprofen (211.80 A^{o3}).

When the elution order is interpreted as a function of hydrophobicity and the volume of chemicals it is observed that the separation remains mainly controlled by hydrophobic interaction, however, the volume of molecules is clearly important too. A good example is naproxen, its retention is the combination of both effects.

To get more insight in the separation order and mechanism, another sample set consisting of benzoic acid, ketoprofen, naproxen, naphthalene and ibuprofen was injected.

By considering log D, the solutes should elute according to the following order: 1. benzoic acid, 2. ketoprofen 3. naproxen 4. naphthalene 5. ibuprofen. However, a different order was observed: 1. ketoprofen, 2. naproxen, 3. benzoic acid, 4. ibuprofen, 5. naphthalene.

To explain this order, we again considered the chemicals' volume. According to this parameter and size-based separation assumption, the elution order should be: 1. ketoprofen, 2. naproxen, 3. ibuprofen, 4. naphthalene, 5. benzoic acid. As can be seen, both factors (hydrophobicity and size) are effective on the solutes retention and real elution order is a combination of the order according size and hydrophobicity.

The separation mechanism on the RP-PAC for small molecules appears to be a combination of partition and steric hindrance effects, with the hydrophobic interaction the most effective. A similar conclusion has been reported for the separation of some NSAIDs on a C_{18} packed column [30].

3.2. Evaluation of the Separation Potential of a Dynamically Coated chiral RP-PAC

A simple and straightforward procedure was used to prepare the chiral stationary phase. A 25 mM phosphate buffer at pH 4.0 containing 10mM HP−β−CD was pumped through the column for 28h.

Hp-β-CD is a suitable and commonly used chiral selector for enantioseparation of NSAIDs in different analytical methods due to its ability to form inclusion complexes with analytes in its hydrophobic cavity [10,11,31,32].

3.2.1. Effect of HP-β-CD concentration

The influence of the concentration of HP-β-CD on the resolution of the selected NSAIDs enantiomers and performance of the chiral column was evaluated by adding different concentrations of HP-β-CD (2, 5, 10 and 20 mM) to the coating solution. At concentrations below 10 mM not enough chiral sites seemed to be available to achieve the enantioseparation of selected profens. The coating solutions with higher concentrations (10 & 20 mM) modified the

surface column successfully as well as indicated by a successful chiral separation of analytes. 10mM was selected as the optimum concentration.

3.2.2. Suitable mobile phase

To obtain the best enantioselectivity the organic solvents methanol, acetonitrile and ethanol as organic modifier in aqueous mobile phase were evaluated (water/methanol- water/ethanol-water/acetonitrile). No considerable difference between selected organic solvents was observed. The resolution was almost unaltered when shifting from methanol to ethanol, while applying acetonitrile decreased the resolution slightly (around 0.05). A similar influence was observed for the retention time. Methanol and ethanol caused an increase in retention times of around 0.5 min in comparison to acetonitrile. In addition, the application of TFA (0.1%) in organic or/ and aqueous solvents was investigated. As only a minor decrease in chromatographic parameters were observed (differences in retention time and resolution below 1 min and 0.2, respectively), all experiments were performed with the same mobile phase (water/acetonitrile)

3.2.3. Chiral separation of some NSAIDs on a dynamically coated RP-PAC

After dynamic chiral coating of the column, the potential of the column was evaluated for enantioseparation of the sample set containing ketoprofen, naproxen and ibuprofen, individually or simultaneously.

Hence, determination and examination of chiral drugs s by single-run analysis needs sufficient chiral and achiral sites on the stationary phase surface to enable adequate resolution between enantiomers and other analytes. The simultaneous chiral separation of a sample set containing ketoprofen, naproxen and ibuprofen was set as a key objective in this study.

The stability of column was evaluated. he racemic standard of naproxen was injected three times during 6000 min running time and the relative standard deviation (RSD) of retention time of each enantiomer data was less than 1.88 and 1.32%. This result showed that the dynamic coating is adequately stable.

Fig. 4. shows the successful application of a dynamically coated column in enantioselectivity of ketoprofen, naproxen and ibuprofen simultaneously. The accumulated chromatographic parameters in table 4 indicate that retention of all enantiomers on the column is sufficient (retention coefficient > 5 in all cases).

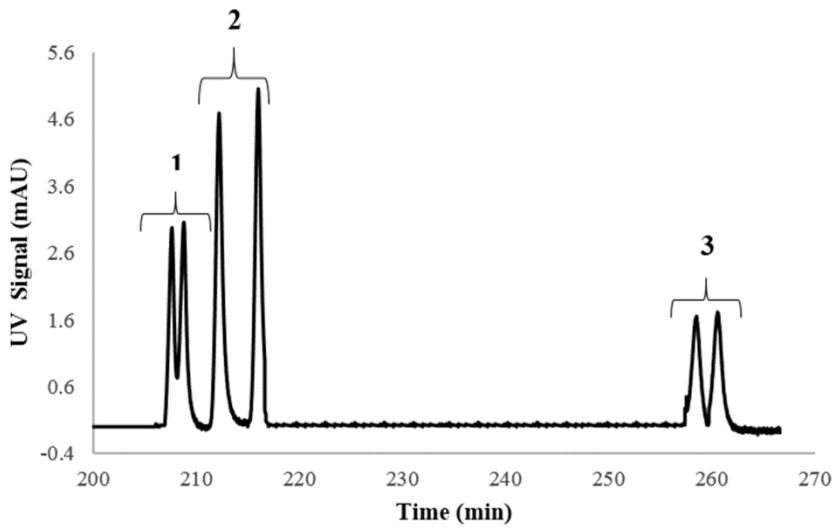

Fig. 4. Simultaneous enantioseparation of profens on a dynamical chiral coated RP-PAC with Hp-β-CD. Components: 1. ketoprofen enantiomers (R & S) 2. naproxen enantiomers (R & S) 3. ibuprofen enantiomers (R & S). Chromatographic conditions: column: 200 cm dynamically chiral coated RP-PAC. mobile phase: gradient elution of acetonitrile–water (15/85–80/20 v/v), flow-rate: 0.25 µl/min, injection volume: 4 µnL, column temperature: 30 ºC and detection: UV at 214 nm

Table 4. Chromatographic parameters for ketoprofen, naproxen and ibuprofen enantiomers using the dynamically chiral coated RP-PAC. Experimental conditions are the same as in Fig. 4.

Analyte	Retention time (min)	Retention coefficient	Width (50%)	Resolution
Ketoprofen	207,63	5,27	0,54	1,27
Ketoprofen	208,79	5,31	0,54	3,28
Naproxen	212,21	5,41	0,68	3,37
Naproxen	216,01	5,52	0,65	34,01
Ibuprofen (R)	258,53	6,81	0,83	1,45
Ibuprofen (S)	260,57	6,87	0,83	/

Re-injection of the separated enantiomers of ibuprofen indicated that the second peak is related to (S)-enantiomer and that the (R)-enantiomer has less interaction with the chiral column. This order of elution has been observed in chiral separation of ibuprofen by β-cyclodextrin based [33,34] other chiral HPLC columns e.g. ((R)-1-naphthylglycine and 3,5-dinitrobenzoic acid amide) [35, 36].

The LOQs of each enantiomer (defined as the analyte concentration producing a signal that is ten times greater than the noise signal [37]) for naproxen, ketoprofen and ibuprofen are 0.18 ppm, 0.55 ppm and 1.98 ppm, respectively. These different LOQs are related to the nature of the compound, e.g. ibuprofen is less efficiently detected with UV in comparison to naproxen. When injecting e.g. 50 ppm of sample, it can be extrapolated that the analysis of fraction values down

to 0.5, 1 and 4% enantiomeric impurity of naproxen, ketoprofen and ibuprofen would be possible with the present setup

3.2.4. Chiral separation mechanism

After evaluation of chiral discrimination of some NSAIDs in the column, the separation mechanism using chromatographic parameters was investigated. During flushing of the column with buffer that contains chiral selector, Hp-β-CD, chiral selector can adsorb on the surface of the stationary phase. The adsorbed chiral selector molecules on the surface make different interaction with enantiomers and formed diastereomers. The host-guest inclusion complexation mainly controls the chiral separation of analytes on the dynamic chiral coating column [38].

Adsorption of the chiral selector was suspected due to the following observation. In our procedure, first the column was flushed with buffer phosphate containing Hp-β-CD for 28h and then mobile phase was switched to water/organic solvent (without chiral selector). In this situation enantioseparation of analytes was observed. If Hp-β-CD would not be adsorbed on the surface of stationary phase, the chiral separation would not be possible. This separation indicates that the chiral selector is most likely adsorbed on the stationary phase surface. Flushing the column with the mobile phase for a long time (more than 100 h) leads to a decrease of the resolution between two enantiomers of analytes (enatioselectivity), which is a result of removing chiral selector from the surface. Also, the retention time of analytes started to decrease substantially after 100h due to desorption of chiral selector from column surface. Flushing with 6 column volumes (after 100h) led to a decrease 2.6 % retention time of naproxen enantiomers. Continued flush up to 18 column volumes further decreased retention times to 5.5 %.

Investigation of the analyte retention times provides valuable data and supports the proposed mechanism. The retention time of the analytes at the dynamically chiral coated column is higher than with the native reversed phase column (compare table 2 and 4), related to the fact that more active sites in the column (adsorbed Hp-β-CD) interact with the analytes.

3.3. Comparison of RP-PAC with and without chiral coating

Comparison of chromatographic parameters of analytes using the same chromatography conditions before and after application of the chiral coating shows the effect the chiral selector. Four relevant factors were used to compare between two columns: 1. peak capacity 2. retention time 3. retention coefficient 4. resolution between two analytes.

The peak capacities before and after chiral dynamic coating was calculated by eq. 1 & 2 and collected in table 5. The peak capacity of the column decreases after dynamically chiral coating, however, both columns provided high peak capacities, related to the inherent high plate count of the column [17]. The peak capacity is affected by the packing structure and quality, which was not altered during the present study. But it is also determined by the chemical nature of the interaction between analytes and stationary phase, there with affecting dispersion and the elution

window. The latter parameter has changed when switching from (single mode) reversed phase mode to multimode interaction in the dynamically chiral bonded column.

Table 5. Peak capacity for profens on RP-PAC before and after applying the chiral coating

Coating type in column	n_r	n_G
C_{18}	345	582
C_{18}+ Hp-β-CD	162	179

Inspection of table 2 and 4 parameters reveals that the column shows a higher retention time and retention coefficient after application of the dynamic chiral coating, due to the adsorbed chiral selector.

The observed resolutions reveal an interesting observation. The resolution between the second enantiomer of ketoprofen and the first enantiomer of naproxen was 3.28, while the resolution between ketoprofen and naproxen on the non-chiral column was 3.04. Also, the resolution between the second enantiomer of naproxen and the first enantiomer of ibuprofen was 34.01, while the resolution between ibuprofen and naproxen on non-chiral column was 25.27. These results indicate that chiral interactions can improve the resolution of two different compounds.

3.4. Comparison of the developed method with other methods

The current method was compared with another method, which is one of the scarce methods available in literature for the simultaneous chiral separation of profens. In these studies, LC/MS/MS coupled instruments were used with (R)-1-naphthylglycine 3,5-dinitrobenzoic acid [36] and amylose tris(3,5-dimethylphenylcarmabate) [39] as the packed separation column to simultaneously achieve enantioselectivity of the selected NSAIDs. The dynamically coated RP-PAC column approach from the present study provides sufficiently high peak capacities to allow for enantioseparation of the selected profens using a single separation operation.

The prepared chiral column shows a similar, even in some case better, separation capacity and enantioresolution as compared to the optimized commercial column. Appropriate enantioresolution for naproxen and ibuprofen using polysaccharide- base column was achieved, with a resolution of 1.0 and 1.1, respectively [39]. The obtained resolutions by another stationary phase for naproxen and ibuprofen were 2.8 and 1.4 [36]. The dynamically coated RP-PAC showed better resolutions (3.37 and 1.45 for naproxen and ibuprofen enantiomers, respectively).

The peak capacity of current chiral column was compared with a variety of commercial chiral stationary phases used for enantioseparation of different chemicals (table 6).

The peak capacity of these cases was less than 25, while the new chiral column provides a value of more than 160.

Table 6. Comparison of the peak capacity of commercial columns with proposed chiral column

Stationary phase	Peak capacity	Ref.
Amylose tris(3,5-dimethylphenylcarmabate)	8	39
(R)-1-Naphthylglycine 3,5-dinitrobenzoic acid	10	36
Amylose tris-((S)- α-methylbenzylcarbamate)	22	40
Ovomucoid	10	41

4. Conclusion

A temporary and stable chiral column was prepared via a dynamic coating approach on a reverse- phase pillar array column. Some profens were separated, individually and simultaneously. To better understand the chiral separation mechanism, the separation of some profens before applying a chiral coating was investigated and possible mechanisms have been discussed. Our data show that the developed method is flexible and simple over the use of chiral stationary phases and shows good chromatography properties such as retention coefficient, enantioresolution and peak capacity, which makes the simultaneous chiral separation of three and more diastereomer compounds possible by a one-dimensional technique.

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Conflicts of interest

Wim De Malsche is co-founder of PharmaFluidics and has shares of the company.

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