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Chiral high performance liquid chromotography resolution of ibuprofen esters[1]

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Abstract

Two cellulose-based chiral stationary phases (Chiralcel OD and Chiralcel OJ) were compared on their ability to resolve various aliphatic ibuprofen esters. Chiralcel OJ with hexane as the mobile phase allows for the separation of most of the esters. Observed changes in resolution depending on the solute nature (basicity of the solute, esterified alcohol chain length, presence of a double bond) are discussed. An example of the application of this method for following the kinetic resolution of racemic ibuprofen is presented. Crown copyright © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Chiral HPLC; Tris(4-methylbenzoate) cellulose CSP; Resolution; Ibuprofen ester; Enzymatic esterification

1. Introduction

(*S*)-ibuprofen (2-(4-isobutyl phenyl) propionic acid) is therapeutically important as a non-steroidal antiinflammatory drug, 28 times more physiologically potent than (*R*)-ibuprofen [1]. Therefore, in order to obtain enantioenriched (*S*)-ibuprofen, many efforts have gone into resolving the racemic mixture. Among the different methods used, enzymatic kinetic resolution of (*R*,*S*)-ibuprofen has been reported by carrying out the reaction with an immobilized lipase either in molten substrates or in solvent media [2–4]. Enantioselective esterification of (*R*)-ibuprofen with aliphatic alcohols produces the (*R*)-ester of ibuprofen while leaving the (*S*)-ibuprofen unmodified. The development and optimization of this process depends on a good analytical method allowing the quantification of both enantiomers with high accuracy.

Many chiral stationary phases (CSP) have been developed for HPLC (for an overview see [5]). Enantiomers can form diastereomeric compounds with a chiral molecule bonded to the stationary phase. The separation of enantiomeric compounds on CSP is due to differences in energy between temporary diastereomeric complexes formed between the solute isomers and the CSP. The observed retention and efficiency of a CSP is the total of all the interactions between the solutes and the CSP, including achiral interactions.

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Cellulose CSP generally exist as β-polymeric chains of derivatised D-glucose residues with β-1,4-linkages arranged in an extended helicoidal structure having a certain degree of rigidity. The recognition mechanism is determined by the formation of a diastereomeric solute-CSP complex in the chiral cavities of the cellulose polymers resulting in interactions at the specific derivatives of the glucose unit [6,7]. For the Chiralcel OD and the Chiralcel OJ columns (Fig. 1), the main adsorbing sites are considered to be, respectively, the polar carbamate function and the polar ester function on the phenyl moiety via hydrogen bonding and dipole-dipole interactions. The phenyl moiety can also provide π-π interactions with aromatic groups of the solute. As mentioned above, the chiral resolution process reflects the total of all the interactions between the solute and the CSP.

In this paper, we discuss the use of two commercially chiral cellulose columns, Chiralcel OD and Chiralcel OJ, for the separation of both enantiomers of various aliphatic ibuprofen esters. The observed differences in resolution depending of the solute nature are discussed and compared with the literature. This procedure is compared to a previously published technique which uses an achiral column [8].

2. Materials and methods

2.1. Materials

Novozym 435 (immobilized *Candida antarctica* lipase type B) was a generous gift from Novo Industries (Denmark). Racemic ibuprofen, optically pure (*S*)-ibuprofen and optically pure (*R*)-ibuprofen were generous gifts from Ethyl Corporation (Baton Rouge, LA). Racemic 2-phenyl propionic acid, racemic 2-phenyl butyric acid, 1-decanol 99% (C10:0), 1-dodecanol 98% (C12:0), 1-tetradecanol 96% (C14:0), 1-hexadecanol 99% (C16:0), 1-octadecanol 99% (C18:0) and trifluoroacetic acid (TFA) were purchased from Aldrich Chemical Company (Milwaukee, WI). Oleyl alcohol (C18:1), eicosenol (C20:1) and erucyl alcohol (C22:1) were purchased from Sigma Chemical Company (St. Louis, MO). 2-Propanol, *n*-butanol and HPLC grade hexane were purchased from Fisher Scientific (Nepean, Ontario). Methanol Accusolv and HPLC grade water were purchased from Anachemia (Montreal, Quebec). Ethyl ether was obtained from ACP Chemicals (Montreal, Quebec). The silica gel used for ester purification (4 μm, flash chromatography packing) was purchased from J.T. Baker (Phillipsburg, New Jersey).

2.2. Equipment

All HPLC analyses were performed using a Waters Millennium 2010 liquid chromatography system purchased from Waters Scientific (Mississauga, Ontario) and equipped with one Waters M590 solvent delivery system, one Waters WISP sample processor 712, one Waters M481 (UV) spectrophotometer and a Digital Celebris 590 computer. The desired column temperature was maintained with a column heater from Croco-Cil™.

2.3. Chemical synthesis of ibuprofen esters

The esterification was carried out by reacting racemic (*R*,*S*)-ibuprofen and aliphatic alcohol (methyl, butyl, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C20:1 or C22:1) in equimolar ratio (2.42 mmol) in the presence of 2–3 fold excess of trimethylchlorosilane at room temperature for 24 h. After evaporation of the reaction mixture un-

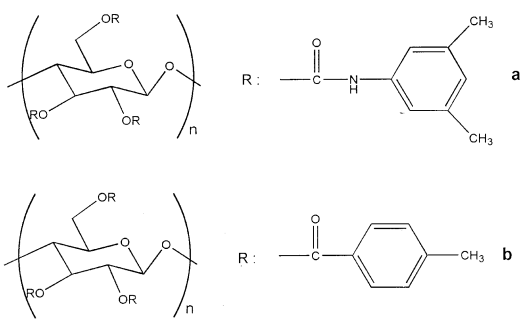

Fig. 1. Structure of the chiral stationary phases. (a) Chiralcel OD: cellulose tris(3,5-dimethyl phenyl carbamate) on a 10 μm silica-gel substrate. (b) Chiralcel OJ: cellulose tris(4-methyl benzoate) on a 10 μm silica-gel substrate.

der vacuum, the samples were diluted in ether, extracted twice with 1 M $NaHCO_3$, washed with water, dried with $MgSO_4$ and the solvent removed under vacuum. Purified yields for the racemic (*R*,*S*)-esters were between 80 and 95%. The same procedure has been used for the synthesis of methyl, butyl, eicosenyl and erucyl (*S*)-ibuprofen esters leading to purified yields in the same order of magnitude.

2.4. Enzymatic synthesis of ibuprofen esters

The reactions were carried out by reacting 4.85 mmol of (*R*)-ibuprofen with 5.33 mmol of aliphatic alcohol (C10:0, C12:0, C14:0, C16:0 or C18:0 alcohol) at 70°C under vacuum in the presence of 0.33 g Novozym 435 for 1 day. After filtering the reaction mixtures individually with 100 ml ether, the solvent was evaporated and the resulting product redissolved in 2–3 ml hexane. The individual (*R*)-esters were obtained after purification by chromatography on silica gel using an eluent solvent of hexane/ether (90:10, v/v). The purified yields of the (*R*)-esters were between 65 and 75%. The esters of (*R*,*S*)-2-phenyl propionic acid and (*R*,*S*)-2-phenyl butyric acid were prepared using the same procedure. The order of elution of the esters under (*R*) and (*S*) forms has been deduced from enzymatic enantioselective esterification kinetics carried out from racemic acid: the two enantiomers are not esterified at the same rate and the observed decrease of the enantiomer under the acidic form corresponds to the increase of the same enantiomer under the ester form.

2.5. HPLC analysis on a Chiralcel OD column

Analysis are performed as described previously [8]. A volume of 1 μl of each product (acid or ester) was dissolved in 1000 μl of the mobile phase (hexane/2-propanol/TFA (90:1:0.1, v/v/v)). Chiral resolution of the samples was accomplished with a HPLC system equipped with a Chiralcel OD column (25 cm × 4.6 mm) (Chiral Technologies Inc., Exton) connected to a UV detector. The chiral stationary phase is characterized by a tris(3,5-dimethyl phenyl carbamate) cellulose layer on a silica-gel substrate. The flow rate was maintained constant at 1.0 ml min^{-1} and the column temperature was 20°C. A total of 5 μl injections of each sample were used. All HPLC chromatograms were monitored at 210 nm.

2.6. HPLC analysis on a Chiralcel OJ column

In a 1000 μl of the mobile phase (hexane), 1 μl of each pure ester was dissolved. Chiral resolution of the ester enantiomers was accomplished with a HPLC system equipped with a Chiralcel OJ column (25 cm × 4.6 mm) (Chiral Technologies, Exton) connected to a UV detector. The chiral stationary phase is characterized by a tris(4-methyl benzoate)cellulose layer adsorbed on to macroporous silica-gel substrate. The flow rate was maintained constant at 0.4 ml min^{-1} or 1.0 ml min^{-1} (see Section 3) and the column temperature was controlled at 8°C. Injections of 5 μl of each sample were used. All HPLC chromatograms were monitored at 210 nm.

The following parameters were measured

- t_0, solvent front
- t_i, retention times of both enantiomers (*R*) and (*S*)
- k', capacity factor: $k' = (t_{i\_} t_0)/t_0$
- α, selectivity factor: $\alpha = k'_2/k'_1$
- R_s, resolution factor: $R_s = 2(t_2 - t_1)/(\omega_1 + \omega_2)$, where ω = peakwidth at baseline.
- R_p, Kaiser's peak separation index: the ratio of the mean valley height between two peaks and the mean peak height, which rises to 1 for perfectly separated peaks.

3. Results and discussion

The enantiomeric separation of ibuprofen and similar molecules has been first performed on a Chiralcel OD column known to give a good resolution of such products under their acidic form. As can be seen in Table 1, good resolution has been obtained for ibuprofen, 2-phenyl propionic acid and 2-phenyl butyric acid under 30 min. Under these analytical conditions, esters of such products which cannot form hydrogen bonds with the CSP, have very low retention times and in most cases, both isomers co-elute. Complete enan-

Table 1
Separation obtained on a Chiralcel OD column

	tr_R	tr_S	k'_R	k'_S	α	R_S	R_P
Ibuprofen	15.95	18.55	3.31	4.01	1.21	2.50	1.00
2-Phenylpropionic acid	28.35	34.58	7.42	9.27	1.25	3.62	1.00
2-Phenylbutyric acid	24.98	36.25	5.72	8.75	1.53	6.53	1.00
Me-ibuprofen ester	5.17	5.55	0.53	0.64	1.21	1.20	0.85
Bu-ibuprofen ester	4.55	4.75	0.35	0.41	1.17	0.63	0.21
C10:0-ibuprofen ester	4.20	4.33	0.25	0.29	1.16	ND	0.05
C12:0-ibuprofen ester	4.18	4.18	0.25	0.25	1.00	0.00	0.00
C14:0-ibuprofen ester	4.07	4.07	0.21	0.21	1.00	0.00	0.00
C16:0-ibuprofen ester	3.98	3.98	0.18	0.18	1.00	0.00	0.00
C18:0-ibuprofen ester	3.90	3.90	0.16	0.16	1.00	0.00	0.00
C18:1-ibuprofen ester	4.00	4.00	0.19	0.19	1.00	0.00	0.00
2-Ph-propionic-C10:0-ester	5.15	5.15	0.53	0.53	1.00	0.00	0.00
2-Ph-butyric-C10:0-ester	4.73	5.47	0.27	0.47	1.72	2.12	1.00

Mobile phase, hexane/2-propanol/TFA (99:1:0.1, v/v/v); flow rate, 1 ml min^{-1} at 20°C.

tiomeric separation could only be obtained for 2-phenyl butyric C10:0-ester, partial separation was observed for Me- and Bu-ibuprofen esters, and a sharp decrease in resolution resulted when the ester chain length was increased.

The use of a Chiralcel OJ column with hexane as the mobile phase allows the separation of most of the esters (Table 2). In our analytical conditions, free acids remain tightly bound on the column and are not eluted, probably due to strong hydrogen bonds between the ester function of the CSP and the acid function of the solute. In order to elute the acids, the solvent polarity must be increased by the addition of a modifier such as 2-propanol, which can compete for achiral and chiral binding on the CSP [9]. Under the ester form, retention times are much lower because the interactions between the solute and the support are weaker since no labile hydrogen is available on the solute for the formation of hydrogen bond interactions with the CSP.

3.1. Change in the basicity of the solute

The π basicity of the solute has been reported to increase the π-π interaction with the benzoyl moiety of the CSP, which can improve the resolution [10,6]. 2-Phenyl propionic C10:0 ester and C10:0-ibu ester only differ by the presence of an iso-butyl group para on the benzyl substituent, which increases the relative π basicity of the aromatic ring of the C10:0-ibu ester. As can be seen in Table 2, this leads to a dramatic decrease of the k' of both enantiomers and, in addition, to a reversal of selectivity. This marked effect could be due to strict steric requirements in the chiral cavity rather than electronic effects. Wainer et al. have also reported such observations in the separation of various alcohols on a Chiralcel OB column (tris(benzoyl cellulose CSP)) [9].

3.2. Effect of the esterified alcohol

It can be seen that the less sterically hindered methyl ester is much more strongly retained on the column than the esters with longer chain length. However, as an exception, enantiomers of Bu-ibuprofen ester are not separated on this column (while they were on a Chiralcel OD column). Looking at the saturated ibuprofen fatty ester series, optimal separation can be achieved with a chain length of C14 and a noticeable loss of resolution is observed by increasing or decreasing the chain length (Fig. 2). It can be noted that, whereas saturated C18:0-ibuprofen esters are well resolved, no separation is achieved in the presence of a double bond. For the unsaturated series, resolution increases with the ester chain length (Fig. 3). The double bond of the C18:1-ibuprofen ester is located on the ninth carbon from the

Table 2
Separation obtained on a Chiralcel OJ column

	tr_R	tr_S	k'_R	k'_S	α	R_S	R_P
Me-ibuprofen ester[a]	56.23	22.50	16.39	5.96	2.75	7.66	1.00
Bu-ibuprofen ester[b]	25.47	25.47	2.19	2.19	1.00	0.00	0.00
C10:0-ibuprofen ester[b]	19.82	22.33	1.49	1.81	1.21	1.09	0.83
C12:0-ibuprofen ester[b]	19.13	22.38	1.41	1.82	1.29	1.41	0.95
C14:0-ibuprofen ester[b]	17.57	21.57	1.20	1.70	1.42	1.75	0.98
C16:0-ibuprofen ester[b]	16.33	18.88	1.05	1.37	1.30	1.21	0.89
C18:0-ibuprofen ester[b]	15.12	16.93	0.90	1.13	1.25	0.92	0.67
C18:1-ibuprofen ester[b]	21.47	21.47	1.62	1.62	1.00	0.00	0.00
C20:1-ibuprofen ester[b]	18.85	21.12	1.42	1.71	1.20	0.77	0.51
C22:1-ibuprofen ester[b]	16.90	23.47	1.14	1.97	1.73	2.19	1.00
2-Ph-propionic-C10:0-ester[a]	23.90	15.88	6.39	3.91	1.63	2.72	1.00
2-Ph-butyric-C10:0-ester[b]	29.30	29.30	2.69	2.69	1.00	0.00	0.00

[a] Mobile phase, hexane with flow rate of 1 ml min^{-1} at 8°C.
[b] Mobile phase, hexane with flow rate of 0.4 ml min^{-1} at 8°C.

carbonyl group and the cis configuration imparts a certain rigid folding to the molecule. This rigidity probably leads to more steric hindrance in the case of the unsaturated C18:1-ibu ester compared to the equivalent saturated molecule, which disturbs the interactions of the solute with the support, leading to no resolution. For the C20:1-ibuprofen ester, the double bond is now located at the carbon 11 after the carbonyl group, and the steric hindrance due to the folding of the molecule related to the cis configuration has less effect on the interactions with the support. As a result, there is an increased separation of both enantiomers without complete resolution. As expected, the double bond of the C22:1-ibuprofen ester (located at the carbon 13 after the carbonyl group) has less influence and complete resolution of both enantiomers is attained. This results show that small changes in the structure of the solute have great impact on the resolution which involves a tight fit between the solute and the CSP.

With one OJ column, saturated fatty aliphatic esters of ibuprofen are not completely resolved, as shown by the inability of the HPLC peaks to

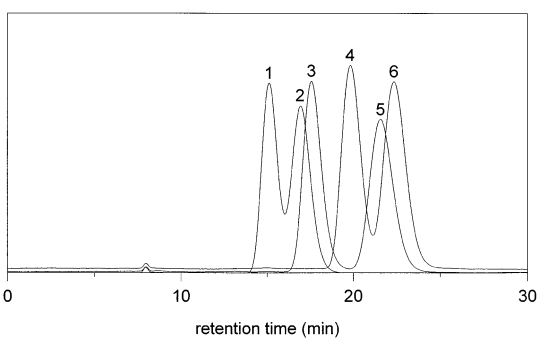

Fig. 2. Examples of separation of saturated racemic (*R*,*S*)-ibuprofen esters on a Chiralcel OJ column. Mobile phase, hexane; flow rate, 0.4 ml min^{-1} at 8°C. 1, (*R*)-C18:0 ibuprofen ester; 2, (*S*)-C18:0 ibuprofen ester; 3, (*R*)-C14:0 ibuprofen ester; 4, (*R*)-C10:0 ibuprofen ester; 5, (*S*)-C14:0 ibuprofen ester; 6, *(S)*-C10:0 ibuprofen ester.

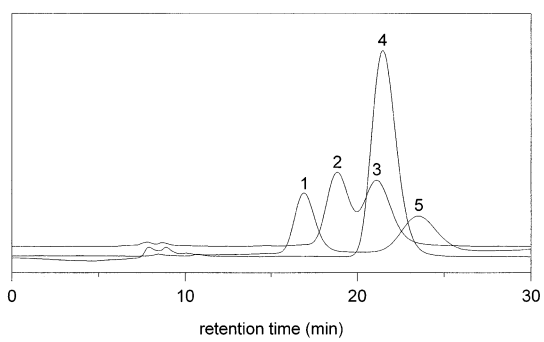

Fig. 3. Examples of separation of unsaturated racemic (*R*,*S*)-ibuprofen esters on a Chiralcel OJ column. Mobile phase, hexane; flow rate, 0.4 ml min^{-1} at 8°C. 1, (*R*)-C22:1 ibuprofen ester; 2, (*R*)-C20:1 ibuprofen ester; 3, (*S*)-C20:1 ibuprofen ester; 4, (*R*,*S*)-C18:1 ibuprofen ester; 5, (*S*)-C22:1 ibuprofen ester.

Table 3
Separation obtained on two Chiralcel OJ columns in series

	tr_R	tr_S	k'_R	k'_S	α	R_S	R_P
C10:0-ibuprofen ester	38.83	43.42	1.45	1.73	1.20	1.49	0.95
C12:0-ibuprofen ester	37.32	43.37	1.35	1.73	1.28	1.91	0.99
C14:0-ibuprofen ester	34.37	41.87	1.17	1.64	1.41	2.35	1.00
C16:0-ibuprofen ester	31.98	37.00	1.02	1.33	1.31	1.70	0.99
C18:0-ibuprofen ester	29.70	33.30	0.87	1.10	1.26	1.32	0.92

Mobile phase, hexane with flow rate of 0.4 ml min^{-1} at 8°C.

return to the baseline and R_p values inferior to 1.00. However, as one would expect, using two Chiralcel OJ columns in series improves significantly the resolution (Table 3).

This simple and easy HPLC method has been applied in our laboratory in the study of the stereoselectivity of lipases for the enzymatic resolution of (*R*,*S*)-ibuprofen in non-aqueous media. Several kinetics have been performed by carrying the esterification of the racemate with the dodecyl alcohol in various initial conditions [11]. With very high concentration of (*R*,*S*)-ibuprofen and low concentration in alcohol, kinetics cannot be monitored by measuring both concentrations of (*R*)- and (*S*)-ibuprofen on a Chiralcel OD column. The concentrations of both ester enantiomers have to be measured to obtain accurate results. An existing HPLC method also allows the quantification of both enantiomers of ibuprofen esters by using a reversed phase column connected to a refractive index detector and a chiral monitor [8]: the individual enantiomers are not physically separated, but through a simple calculation, quantification of each enantiomer is achieved. However, the major drawback with this method is that high specific rotations are required for high sensitivity on the chiral monitor and for this reason, this method cannot be used for monitoring the current reaction. The HPLC method described in this paper is very sensitive and allows the quantification of small amounts of both ibuprofen esters. As can be seen in Fig. 4, this method permits an accurate and convenient monitoring of the kinetics of the enantioselective esterification of (*R*,*S*)-ibuprofen.

4. Conclusion

This study shows that most of the esters of ibuprofen can be resolved on a Chiralcel OJ column with hexane as the mobile phase. The recognition mechanism for this type of molecules seems to be very sensitive to sterical hindrance of the solute, at once by the presence of substituents on the benzyl group of the solute, and also by the nature of the esterified alcohol. In particular, the presence of a double bond, even distant from the carbonyl group has great impact on the recognition mechanism.

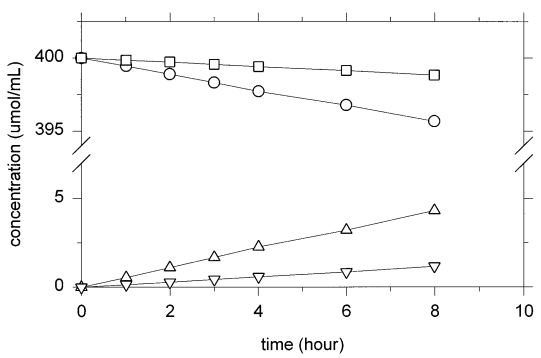

Fig. 4. Kinetics of (*R*,*S*)-ibuprofen esterification catalyzed by *Candida antarctica* type B lipase in toluene. [(*R*,*S*)-ibuprofen] = 800 mM; [dodecanol] = 10 mM; temperature, 22°C; $a_W = 0.09$. □, (*S*)-C12:0 ibuprofen ester; ○: (*R*)-C12:0 ibuprofen ester; △, (*R*)-C12:0 ibuprofen ester; ▽: (*S*)-C12:0 ibuprofen ester.

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