Journal of Chromatography A, 1217 (2010) 1063–1074

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Lipase-mediated enantioselective kinetic resolution of racemic acidic drugs in non-standard organic solvents: Direct chiral liquid chromatography monitoring and accurate determination of the enantiomeric excesses

Ashraf Ghanem[a,*], Mohammed Nabil Aboul-Enein[b], Aida El-Azzouny[b], Mohammed F. El-Behairy[b]

[a] Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, Private Bag 75, Hobart, Tasmania 7001, Australia
[b] Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (NRC), Dokki-Cairo, Egypt

ARTICLE INFO

Article history:
Available online 31 October 2009

Dedicated to Professor Volker Schurig on the occasion of his 70th birthday.

Keywords:
Chiralpak IB
Enantiomeric excess
Enantioselective HPLC
Direct monitoring
Kinetic resolution
Lipase
Racemic acidic drugs

ABSTRACT

The enantioselective resolution of a set of racemic acidic compounds such as non-steroidal anti-inflammatory drugs (NSAIDs) of the group arylpropionic acid derivatives is demonstrated. Thus, a set of lipases were screened and manipulated in either the esterification or hydrolysis mode for the enantioselective kinetic resolution of these racemates in non-standard organic solvents. The accurate determination of the enantiomeric excesses of both substrate and product during such reaction is demonstrated. This was based on the development of a direct and reliable enantioselective high performance liquid chromatography (HPLC) procedure for the simultaneous baseline separation of both substrate and product in one run without derivatization. This was achieved using the immobilized chiral stationary phase namely Chiralpak IB, a 3,5-dimethylphenylcarbamate derivative of cellulose (the immobilized version of Chiralcel OD) which proved to be versatile for the monitoring of the lipase-catalyzed kinetic resolution of racemates in non-standard organic solvents.

1. Introduction

The importance of chirality has constantly increased in the last decades and it is nowadays a central issue in organic and pharmaceutical industry especially in the development of new drugs. The market value for chiral platform technologies has reached around 2.7 billion US$ in 2007 with an average annual growth rate of 10.8% [1]. This fact demonstrates the increased demand to generate enantiomers in pharmaceutical research and discovery. In pharmaceutical industry, often only one enantiomer of a racemic chiral drug is the effective agent and has a therapeutically useful action, while the second enantiomer does not or may be less effective, totally ineffective, or in the worst case even toxic. Legislation now requires both enantiomers of a racemic drug to be pharmacologically investigated [2]. Accordingly, a great deal of effort has been developed over the years to render the enantioselective access to enantiomerically pure drugs more appealing to the large demand of the market [1].

The modern and most sensitive methods used in the determination of enantiomeric excess (ee) and hence enantiomeric ratio or enantioselectivity (*E*) of the outcome of kinetic resolution reactions are enantioselective gas chromatography (GC) and high performance liquid chromatography (HPLC) methods. These methods allow a detection as little as 0.1% of one enantiomer in the presence of another. GC has been widely used for monitoring biochemical transformation reactions. However, it is not convenient for non-volatile compounds and in most cases derivatization is required. HPLC might be the method of choice. However, for the enantioselective transformations catalyzed by enzymes in non-standard HPLC solvents such as dichloromethane (DCM), ethyl acetate (EtOAc), methyl *tert*-butyl ether (MtBE) and tetrahydrofuran (THF), HPLC analysis is time consuming and labor intensive. Thus, these reactions are usually monitored offline since such harmful solvents might cause irreversible damage of the CSP by dissolving or swelling of the chiral selector [3].

In a typical offline HPLC monitoring of enzymatic reaction, an aliquot of the supernatant reaction mixture is withdrawn at several time intervals during the course of a biotransformation, solvent is evaporated, and the residue is dissolved in mobile phase eluent and analyzed by HPLC equipped with an appropriate chiral stationary phase and a UV-detector. Yet, a new technology has been introduced by making the HPLC CSPs with a silica support onto which the polymer chiral selector (polysaccharide derivative) has been bonded or immobilized [4]. Thus, Chiralpak IA, a 3,5-dimethylphenylcarbamate derivative of amylose,

* Corresponding author. Tel.: +61 03 6226 1074; fax: +61 03 6226 2858.
E-mail addresses: aghanem@utas.edu.au, ashraf_ghanem@yahoo.com, ashraf.ghanem@utas.edu.au (A. Ghanem).

0021-9673/$ – see front matter.
doi:10.1016/j.chroma.2009.10.080

immobilized onto silica (the immobilized version of Chiralpak AD) was the first launched in this series followed by Chiralpak IB, a 3,5-dimethylphenylcarbamate derivative of cellulose (the immobilized version of Chiralcel OD) and Chiralpak IC (cellulose tris 3,5-dichlorophenylcarbamate) immobilized onto silica-gel [4]. Such immobilization of the polymeric chiral selectors on the silica support is considered as an efficient approach to confer a universal solvent compatibility to this kind of CSP, thereby broadening the choice of solvents able to be used as mobile phase eluent or diluents. This is of particular interest in monitoring reactions performed in non-standard HPLC organic solvents.

Non-steroidal anti-inflammatory drugs (NSAIDs) of the 2-arylpropionic acid (2-APA) class represent one of the most commercially successful and important classes of analgesic anti-inflammatory drugs. Their enantioselective resolutions were reported using gas chromatography GC, high performance liquid chromatography HPLC and capillary electrochromatography (CEC) [5]. However, most of these assays are indirect thus racemization, partial or complete chiral conversion might occur. In fact, none of the reported procedure used to access enantiomerically pure/enriched acidic drugs did include a straightforward enzymatic resolution with direct monitoring of the enantiomeric excesses of both substrate and product. This is due to the difficulty associated with the simultaneous baseline resolution of the free acid (enzyme substrate) and its corresponding ester (product) in one run without derivatization. Such baseline resolution is mandatory for the accurate determination of the enantiomeric excesses. Therefore, in the present work we report on the first straightforward lipase-catalyzed enantioselective access to enantiomerically enriched acidic drugs among which are the well-known non-steroidal anti-inflammatory drugs (NSAIDs) of the 2-arylpropionic acid (2-APA) class. These enzymatic reactions were directly monitored by enantioselective HPLC using non-standard HPLC organic solvents as diluents to dissolve the enzyme substrate and eluent to analyze both substrate and product in one run without further derivatization. The simultaneous baseline separation of both substrate and product in one run allows the successful and accurate determination of their enantiomeric excesses.

2. Experimental

2.1. Instrumentation

The mobile phase for LC was filtered through a Millipore membrane filter (0.45 μm) from Nihon Millipore (Yonezawa, Japan) and degassed via Waters in-line degasser AF. The LC system consisted of a Waters binary pump, Model 1525 (Milford, MA, USA), equipped with a dual λ absorbance detector model 2487, an autosampler model 717plus and an optical rotation detector (IBZ MESSTECHNIK GMBH, Hannover, Germany) operating at room temperature. The UV-detector was set at drug specific wavelength. The collection of data was performed using Empower Software® from Waters. The Chiralpak IB (4.6 mm × 250 mm ID immobilized on 5 μm silica-gel) and Chiralcel OD-H (4.6 mm × 250 mm ID coated on 5 μm silica-gel) columns were purchased from Chiral Technologies Europe (Illkirch, France). Mass spectral data were recorded as electron impact technique (EI) from a Finnigan Mat SSQ-7000 Spectrophotometer. ^{1}H-NMR spectra were recorded using either on a Bruker AC 250, WM400 or AMX600 FT NMR spectrometer (Karlsruhe, Germany), at a sample temperature of 300 K. The Rotating Frame Overhauser Experiments (ROESY) was performed using the pulse program roesyprtp with an applied mixing time of 225 ms. ^{1}H-NMR, AC250, transmitter frequency 250 MHz Silica gel 60 F_{254} neutral plates for TLC (MERCK) were used for thin layer chromatography. Visualization was performed by illumination with UV-light source (254 nm).

2.2. Materials

All solvents were LC-grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Trifluoroacetic acid was purchased from E. Merck (Darmstadt, Germany). Carprofen (**1**) was purchased from Pfizer Co. Etodolac (**2**) was purchased from Wyeth-Ayerst (NJ, USA). Fenoprofen (**3**), flurbiprofen (**4**), ibuprofen (**5**), indoprofen (**6**), *o*-methoxymandelic acid (**7**) and 2-phenoxy propionic acid (**9**) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Naproxen (**8**) was purchased from Syntex Lab. Tiaprofenic acid (**10**) was extracted from the readily marketed tablets according to literature procedure [6]. The structures of all compounds are shown in Fig. 3. Lipases from *Asperigillus niger* (lipase AS), *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) free (lipase PS) and immobilized on ceramic particles (lipase PS-C) together with *Candida rugosa* (lipase AYS) were gifts from Amano (Nagoya, Japan). Immobilized *Candida antarctica* lipase B (Novozym435), its free form (Novozym525), and immobilized *Rhizomucor miehei* lipase (lipozyme RM IM) were from Novo Nordisk (Bagsvaerd, Denmark). Lipase type VII from *C. rugosa* (CRL) was from Sigma (Steinhiem, Germany). Lipase from *Candida cylinderacea* (CCL) and lipase immobilized in sol–gel-Ak on sintered glass from *Mucor miehei* (MML) were purchased from Fluka (Buchs, Switzerland).

2.3. Chromatographic conditions

The mobile phase consisted of either n-hex/EtOAc (90:10 v/v, 80:20 v/v, 70:30 v/v, 50:50 v/v and 40:60 v/v), MtBE/THF (90:10 v/v), n-hex/DCM (90:10 v/v), n-hex/2-PrOH/TFA (80:20:0.1 v/v/v), MtBE (100 v) or DCM (100 v). The pump and UV-detector were set at different flow rates and wavelengths (depending on the analyte), respectively. The columns were operating at room temperature (24 °C). 100 μl of 10 μg ml^{-1} samples was injected for the enantioselective separation of substrates and synthesized products.

2.4. General method for the chemical synthesis of n-butyl esters (*1a* and *3a–10a*)

To a solution of 0.01 mol of the racemic acid in 50 ml benzene, 0.03 mol n-butanol was added followed by few drops of conc. sulphuric acid and the mixture was stirred under reflux over night, the solvent was evaporated under vacuum and the residue was neutralized with 10% sodium hydrogen carbonate. The ester was extracted twice with diethyl ether (30 ml). The collected ethereal extract was dried over anhydrous sodium sulphate, filtered and evaporated under vacuum to afford the racemic n-butyl esters and were used as such.

2.4.1. Carprofen butyl ester (*1a*): brown oil (66%)

IR presence of carbonyl ester band at 1720 cm^{-1}, MS (EI) *m*/*z* 273.

2.4.2. Chemical synthesis of etodolac butyl ester (*2a*)

One gram of etodolac (0.0034 mol) was added to a solution of 0.75 g dicyclohexylcarbodiimide (DCCDI) and 0.25 g n-butanol (0.0034 mol, 0.32 ml) in THF. The mixture was stirred at room temperature for 1.5 h then at 55 °C for 1.5 h, then cooled, filtered and evaporated under vacuum. The residue was neutralized with 10% sodium hydrogen carbonate and extracted with diethyl ether (30 ml × 2). The ethereal extract was dried over anhydrous sodium

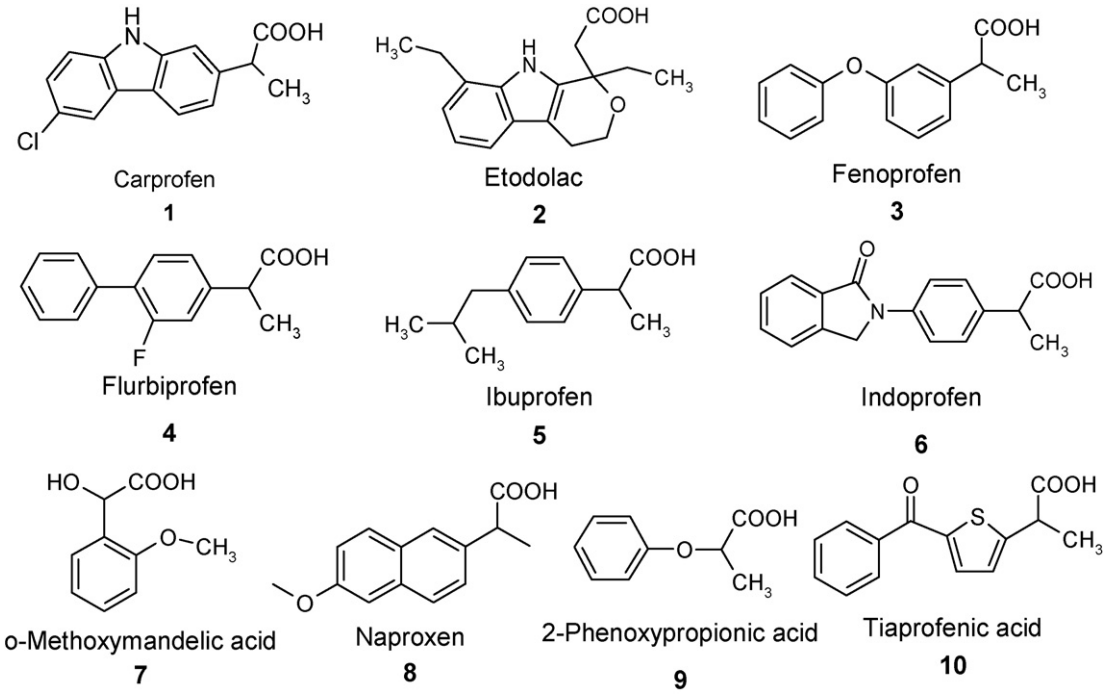

Fig. 1. A list of the chemical structures of the investigated acidic compounds (**1**–**10**).

sulphate, filtered and evaporated under vacuum to afford yellowish oil of the n-butyl ester **7a** (84%): IR presence of carbonyl ester band at 1718 cm^{-1}, MS (EI) *m*/*z* 287.

2.4.3. Fenoprofen butyl ester (*3a*)

Dark brown liquid (87%): ^{1}H NMR ($CDCl_3$) δ 6.7–7.2 (m, 9H, aromatic), 3.9 (t, 2H, **OCH$_2$**$CH_2CH_2CH_3$), 3.5 (q, 1H, **CH**CH_3), 1.5 (m, 2H, OCH_2**CH$_2$**CH_2CH_3), 1.3 (d, 3H, CH**CH$_3$**), 1.1 (m, 2H, OCH_2CH_2**CH$_2$**CH_3), 0.8 (t, 3H, $OCH_2CH_2CH_2$**CH$_3$**). ^{13}C NMR (CDCl3) δ 187.2, 174.2, 157.4, 147.2, 142.7, 129.7, 128.2, 127.0, 125.7, 123.2, 122.3, 118.8, 118.2, 117.4, 64.7, 45.5, 41.7, 31.2, 30.7, 21.8, 18.3, 13.6, 12.2.

2.4.4. Flurbiprofen butyl ester (*4a*)

Viscous liquid (76%): ^{1}H NMR ($CDCl_3$) δ 6.8–7.7 (m, 8H, aromatic), 3.9 (t, 2H, **OCH$_2$**$CH_2CH_2CH_3$), 3.6 (q, 1H, **CH**CH_3), 1.5 (m, 2H, OCH_2**CH$_2$**CH_2CH_3), 1.4 (d, 3H, CH**CH$_3$**), 1.2 (m, 2H, OCH_2CH_2**CH$_2$**CH_3), 0.8 (t, 3H, $OCH_2CH_2CH_2$**CH$_3$**). ^{13}C NMR (CDCl3) δ 130.7, 128.9, 128.4, 127.6, 126.9, 123.5, 115.4, 115.0, 64.8, 45.1, 30.6, 19.0, 18.3, 13.6, 12.2.

2.4.5. Ibuprofen butyl ester (*5a*)

Pale yellowish oil (78%): ^{1}H NMR ($CDCl_3$) δ 6.9–7.2 (dd, 4H, aromatic), 3.9 (t, 2H, **OCH$_2$**$CH_2CH_2CH_3$), 3.6 (q, 1H, **CH**CH_3), 2.3 (d, 2H, **CH$_2$**$CH(CH_3)_2$), 1.7 (m, 1H, **CH**$(CH_3)_2$), 1.4 (m, 2H, OCH_2**CH$_2$**CH_2CH_3), 1.3 (d, 3H, CH**CH$_3$**), 1.1 (m, 2H, OCH_2CH_2**CH$_2$**CH_3), 0.8 (d, 6H, CH(**CH$_3$**)$_2$), 0.7 (t, 3H, $OCH_2CH_2CH_2$**CH$_3$**). ^{13}C NMR ($CDCl_3$) δ 179.9, 174.8, 140.7, 140.4, 137.9, 137.2, 129.2, 127.3, 65.8, 64.1, 45.1, 41.3, 30.6, 30.2, 22.3, 19.0, 18.4, 15.2, 13.6.

2.4.6. Indoprofen butyl ester (*6a*)

Gray white solid (87%): ^{1}H NMR ($CDCl_3$) δ 7.1–7.8 (m, 8H, aromatic), 4.7 (s, 2H, NCH_2), 4.0 (t, 2H, **OCH$_2$**$CH_2CH_2CH_3$), 3.6 (q, 1H, **CH**CH_3), 1.5 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.4 (d, 3H, CH**CH$_3$**), 1.2 (m, 2H, OCH_2CH_2**CH$_2$**CH_3), 0.8 (t, 3H, $OCH_2CH_2CH_2$**CH$_3$**). ^{13}C NMR ($CDCl_3$) δ 174.5, 140.1, 138.4, 136.8, 133.2, 132.1, 128.4, 128.2, 124.1, 122.6, 119.6, 64.7, 45.0, 30.7, 19.0, 18.6, 13.6, 1.0.

2.4.7. o-Methoxymandelic butyl ester (*7a*)

Dark brown oil (78%): IR presence of carbonyl ester band at 1720 cm^{-1}, MS (EI) *m*/*z* 182.

Table 1
Separation condition of racemic acidic drugs **1**–**10** and their corresponding butyl esters **1a**–**10a**.

Drug	Mobile phase v/v/v/v				λ (nm)	Flow rate (ml/min)	Acid		Ester	
	n-Hexane	2-PrOH	MtBE	TFA			*α*	Rs	*α*	Rs
1	90	/	10	0.1	254	1.0	1.1	2.0	1.1	1.7
2	80	/	20	0.1	280	1.0	1.7	5.8	2.3	10.7
3	95	/	5	0.2	280	1.0	1.1	2.2	1.1	3.2
4	85	0.4	15	0.1	254	1.0	1.1	1.3	1.1	2.8
5	90	/	10	0.1	214	1.0	1.2	1.2	1.1	4.0
6	90	10	/	0.1	281	1.3	1.1	3.7	1.2	7.5
7	95	5	/	0.2	258	1.0	1.1	12	1.1	9.6
8	98	2	/	0.1	230	1.0	1.2	1.3	1.2	4.0
9	87	13	/	0.5	254	1.0	1.4	5.5	1.3	4.8
10	90	1	10	0.1	300	1.3	1.2	2.3	1.1	2.1

*2.4.8. Naproxen butyl ester (**8a**)*

Gray white solid, m.p. 68.1 °C (83%): ^{1}H NMR ($CDCl_3$) δ 7.0–7.6 (m, 6H, aromatic), 4.0 (t, 2H, $OCH_2CH_2CH_2CH_3$), 3.8 (s, 3H, OCH_3), 3.7 (q, 1H, $CHCH_3$), 1.5 (m, 2H, $OCH_2CH_2CH_2CH_3$), 1.4 (d, 3H, $CHCH_3$), 1.2 (m, 2H, $OCH_2CH_2CH_2CH_3$), 0.7 (t, 3H, $OCH_2CH_2CH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ 174.7, 157.6, 135.9, 133.7, 129.3, 129.0, 127.0, 126.3, 125.9, 118.9, 105.7, 64.6, 55.3, 45.5, 30.6, 19.0, 6.

*2.4.9. Phenoxypropionic butyl ester (**9a**)*

Yellowish oil (85%). ^{1}H NMR ($CDCl_3$) δ 6.8–7.2 (m, 5H, aromatic), 4.7 (q, 1H), 4.1 (t, 2H), 1.8 (d, 3H), 1.5 (m, 2H), 1.2 (m, 2H), 0.8 (t, 3H).

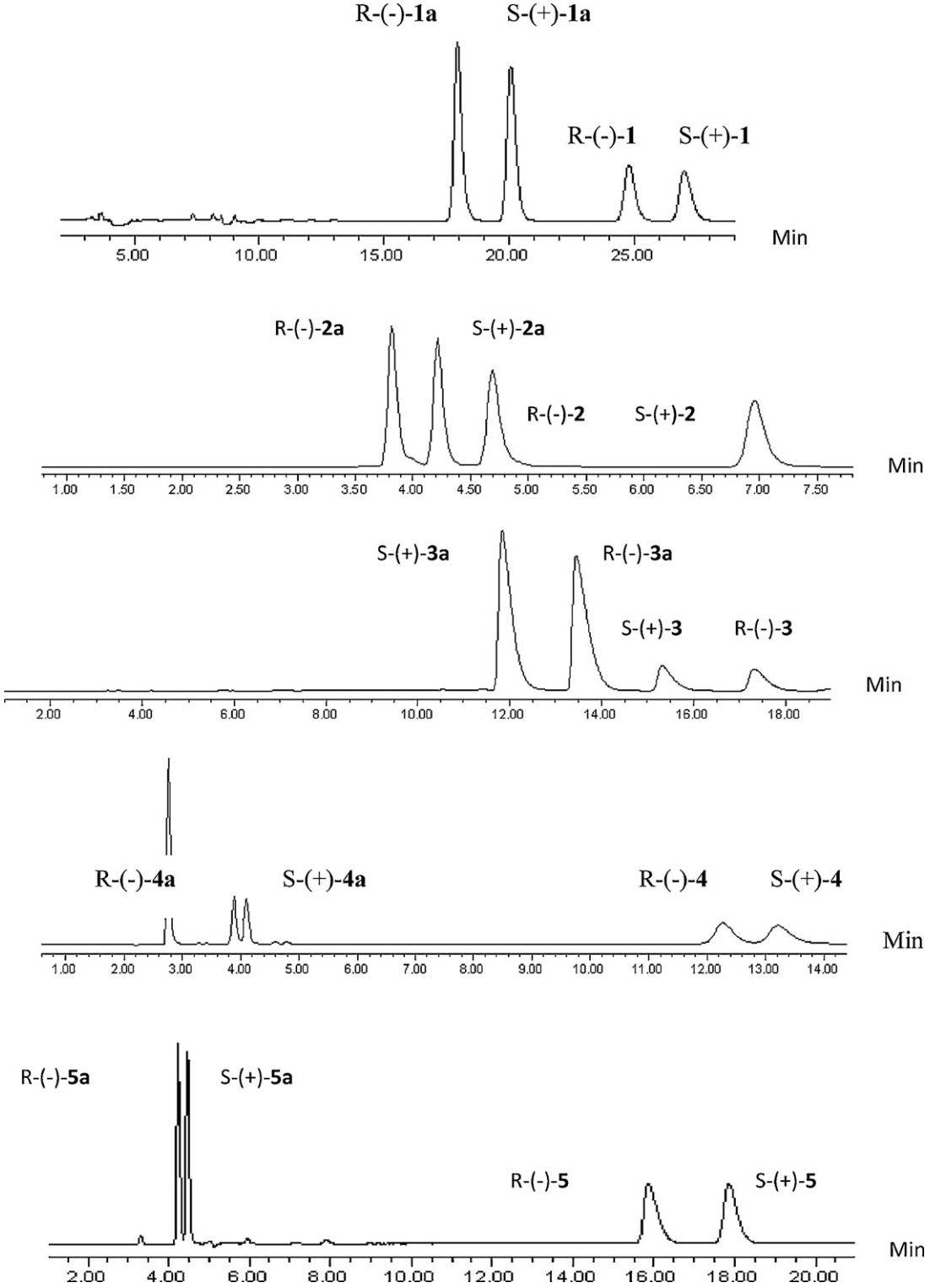

Fig. 2. UV traces of the simultaneous enantioselective LC baseline separation of synthesized racemic carprofen (**1**), etodolac (**2**), fenoprofen (**3**), flurbiprofen (**4**), ibuprofen (**5**), indoprofen (**6**) *o*-methoxymandelic acid (7), naproxen (**8**), 2-phenoxypropionic acid (**9**), tiaprofenic acid (**10**) and their corresponding n-butyl ester **1a**, **2a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a**, **9a** and **10a**, respectively on Chiralpak IB. Chromatographic parameters including the separation factor (α) and the resolution (Rs) and chromatographic conditions are shown in Table 1.

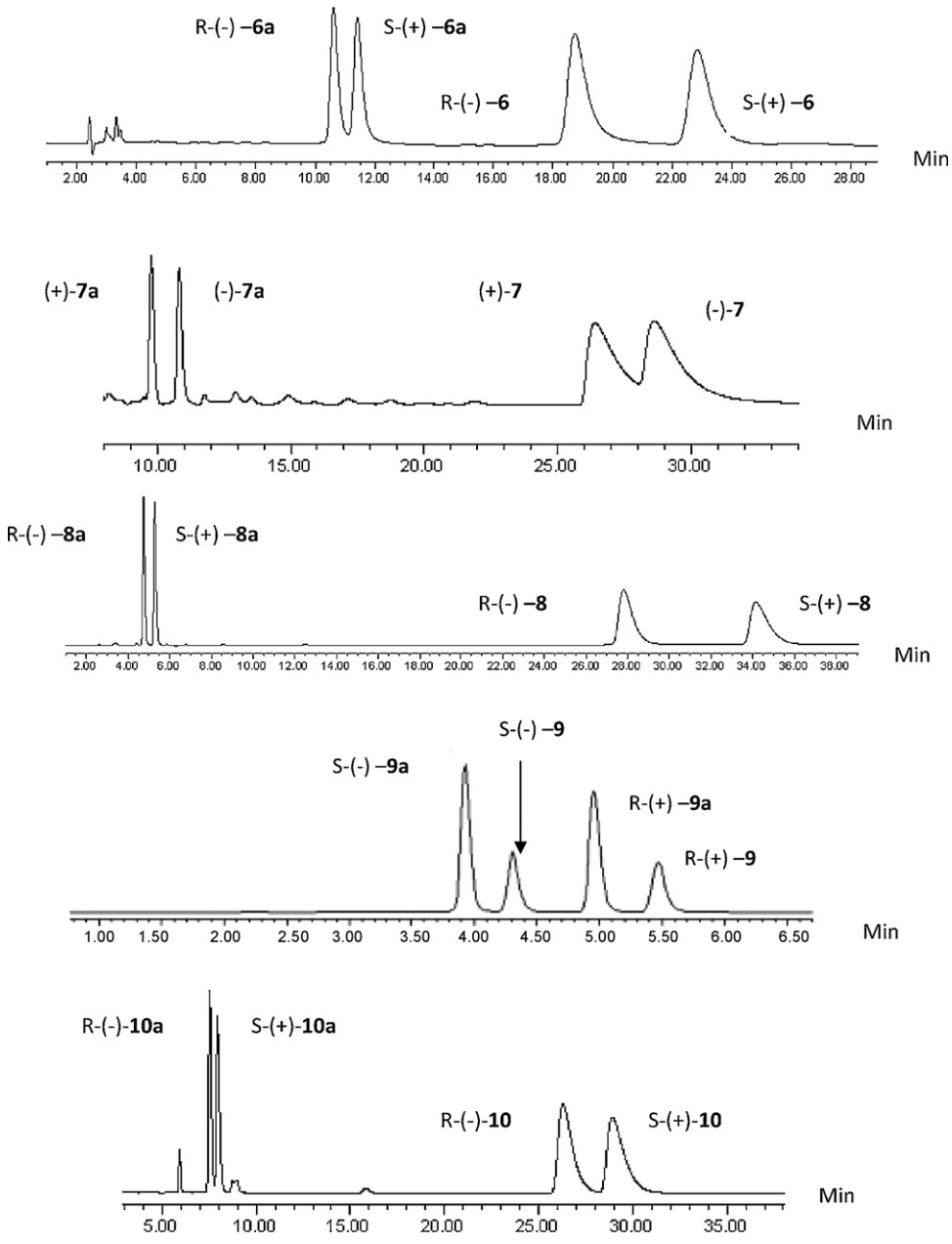

Fig. 2. (*Continued*).

13C NMR ($CDCl_3$) d 13.6, 18.6, 18.9, 30.5, 65.0, 72.6, 115.1, 121.5, 129.5, 157.7, 172.3. ^{13}C NMR (CDCl3) δ 172.3, 157.7, 129.5, 121.5, 115.1, 65.0, 30.5, 18.9, 18.6, 13.6.

*2.4.10. Tiaprofenic acid butyl ester (**10a**)*

Pale yellowish oil (73%): ^{1}H NMR ($CDCl_3$) δ 6.9–7.8 (m, 7H, aromatic), 4.0 (t, 2H, $\mathbf{OCH_2}CH_2CH_2CH_3$), 3.4 (q, 1H, $\mathbf{CH}CH_3$), 1.6 (d, 3H, $CH\mathbf{CH_3}$), 1.4 (p, 2H, $OCH_2\mathbf{CH_2}CH_2CH_3$), 1.2 (m, 2H, $OCH_2CH_2\mathbf{CH_2}CH_3$), 0.8 (t, 3H, $OCH_2CH_2CH_2\mathbf{CH_3}$). ^{13}C NMR ($CDCl_3$) δ 172.5, 152.8, 142.2, 134.8, 132.1, 129.1, 128.4, 125.9, 65.8, 65.3, 41.7, 30.5, 19.1, 19.0, 15.2, 13.6.

2.5. General procedures for the lipase-catalyzed enantioselective esterification of acidic drugs

In a 4 ml reaction vial, the appropriate racemic acid (0.04 mmol) and n-butanol (0.12 mmol, 3 equiv.), were dissolved in 2 ml organic solvent (non-standard HPLC organic solvent) in presence of molecular sieves. The mixture was stirred and heated at 40 °C. Thereafter, 50 μl sample was withdrawn and injected at zero time (control). Lipase (40 mg) was added and 50 μl sample of the supernatant was withdrawn and directly injected to HPLC without dilution or workup at several time intervals.

2.6. General procedures for lipase-catalyzed enantioselective hydrolysis of esters

In a 4 ml reaction vial, the appropriate racemic ester 0.04 (mmol) was dissolved in 1 ml organic solvent (non-standard HPLC organic solvent) followed by addition of 1 ml of 0.1 M phosphate buffer pH 7. The reaction mixture was stirred at 40 °C and 50 μl sample of the non-standard HPLC organic solvent aliquot was withdrawn and injected at zero time (control). Lipase (50 mg) was added and 50 μl samples of the non-standard HPLC organic solvent aliquot

were withdrawn at several time intervals. The samples were filtered and used directly without dilution for direct enantioselective HPLC analysis.

3. Results and discussion

The accurate determination of the enantiomeric excess of substrates and products is a crucial prerequisite for the detailed investigation of the enzyme-catalyzed enantioselective resolution of racemic acidic drugs. For this purpose, the racemic acids (**1**–**10**, Fig. 1) and their corresponding synthesized racemic esters (**1a**–**10a**) should be simultaneously baseline separated. Thus, each mixed individual racemic acid and its corresponding butyl ester should be ultimately separated in one run without further derivatization. This is to prevent any possible racemization that may lead to a misleading calculation of the enantiomeric excess and hence the enantiomeric purity of the resulting substrate and/or product. *N*-butyl esters were particularly selected since previous studies showed that 1-butanol provides the highest enzymatic activity for lipase-catalyzed esterification [7].

The simultaneous enantioselective HPLC separation of both synthesized racemic acids (**1**–**10**) and their corresponding butyl esters (**1a**–**10a**) were first investigated using either coated 3,5-dimethylphenylcarbamate derivative of cellulose-based chiral stationary phase namely Chiralcel OD or its immobilized version namely Chiralpak IB. This was done using standard organic solvents consisting of *n*-hexane/2-propanol with different volume ratios. In most cases, only the acids (**1**–**10**) were baseline separated using the above-mentioned mobile phase composition with volume ratio 90/10 v/v. However, their corresponding esters (**1a**–**10a**) have never been simultaneously separated with the acids in the same run on both columns. Because of the limited solvent choice restricted to standard organic solvents in coated Chiralcel OD, this phase was excluded from our investigation which has been continued on its immobilized version, namely, Chiralpak IB. The latter showed solvents' versatility including non-standard organic solvents [3]. Non-standard organic LC solvents such as dichloromethane (DCM), ethyl acetate (EtOAc), terahydrofuran (THF), methyl-t-butyl ether (MtBE) enable the dissolution of the chiral selector in the physically coated CSP. Consequently, their use was restricted to immobilized CSP (Chiralpak IA, IB and IC) which can withstand such harmful solvents. Starting with non-standard LC organic solvents in mobile phase composition, solvents like n-hex/EtOAc in different volume ratios (40/60, 50/50, 70/30, 80/20 and 90/10 v/v), n-hex/DCM (90:10 v/v), MtBE/THF (90:10 v/v), DCM (100 v) and MtBE (100 v) were investigated as eluents at fixed flow rate (1 mL min^{-1}) for the simultaneous enantioselective separation of the above-mentioned acidic drugs and their corresponding butyl esters on Chirapak IB.

The best results were achieved using *n*-hexane associated with MtBE and/or 2propanol in different ratios (Table 1 and Fig. 2). Trifluroacetic acid (TFA) was a permanent component in all used mobile phases in order to mask the free OH groups of silica and hence avoid tailing. This is the first simultaneous baseline enantiomeric separation of both racemic acids **1**–**10** and their corresponding esters **1a**–**10a** on Chiralpak IB in one run and without derivatization. It is worth to mention that Zhang et al. [8] previously separated the enantiomers of carprofen (**1**) using the immobilized Chiralpack IB column and mobile phase composition ($CHCl_3$/EtOH/TFA 98/2/0.1 v/v/v) with a separation factor $\alpha = 1.2$ and resolution Rs = 2.1. However, the simultaneous enantiomeric separation of racemic carprofen (**1**) and its n-butyl ester (**1a**) has not been so far reported. By the achievement of the enantioselective LC baseline separations of the above-mentioned acidic drugs and their corresponding *n*-butyl esters, the enzymatic reaction can, yet, be easily and directly HPLC monitored in either standard or non-standard organic solvents.

3.1. Lipase-catalyzed enantioselective esterification of acidic drugs

The screening of 10 different lipases for the enantioselective esterification of acidic drugs **1**–**10** (Figs. 1 and 3) was first performed to select the most appropriate enzymes and organic solvents required to achieve high enantioselectivity *E* at maximum conversion (up to 50%). Albeit the existence of a correlation between the solvent hydrophobicity (expressed as log *P* and defined as the ratio of concentration of a substance in two immiscible phases at equilibrium namely octanol and water) and enzyme activity, the role of solvents in controlling the activity/enantioselectivity of lipases is still a controversial area. However, such effect might be due to conformational change of the lipase arising from specific interaction between the solvent and the lipase itself [9].

Based on a previous reported data [10], isooctane (log *P* 4.5) and *n*-hexane (log *P* 3.5) as standards organic solvents were the most suitable organic solvents for the lipase-catalyzed enantioselective esterification reactions. However, out of the current investigated acidic drug, only fenoprofen (**3**) and ibuprofen (**5**) were soluble in isooctane. Duan and Ching [11] reported that acetonitrile might be an alternative for drugs which are hardly soluble in hydrophobic solvents. Consequently, acetonitrile was used as a solvent for the lipases screening. However, during the experimental manipulations, lipases did not show better selectivity in acetonitrile. Therefore, the non-standard HPLC organic solvents reported above were used as an organic reaction medium for lipase-catalyzed enantioselective resolution of acidic drugs, most of which are non-steroidal anti-inflammatory drug of the group arylpropionic acid derivatives. The direct monitoring was performed using Chiralpak IB.

3.1.1. Carprofen (**1**) (R,S-2-(6-chloro-9H-carbazol-2-yl) propanoic acid) (NSAIDs)

The commercially available products of carprofen contain a racemic mixture of the two enantiomers *S*-(+) and *R*-(−). An *in vitro* study showed some evidences that the *S*-(+)-enantiomer of carprofen is the anti-inflammatory eutomer, being 100 times more active than the *R*-(−)-distomer. Furthermore, the conversion of one enantiomer to another does not appear to occur to any significant degree in rats, dogs, horses and human beings [12], while plasma concentration of each enantiomer is not affected by concurrent administration of the racemic mixture.

The resolution of carprofen enantiomers has been reported through diastereomeric crystallization [13]. However, the lipase-catalyzed esterification of carprofen has never been reported. In the present study, an attempt to resolve racemic carprofen via lipase-catalyzed enantioselective esterification was achieved. Poor conversions and enantioselectivities were observed during the lipases screening in non-standard solvents (Table 2), thus no further investigations have been carried out.

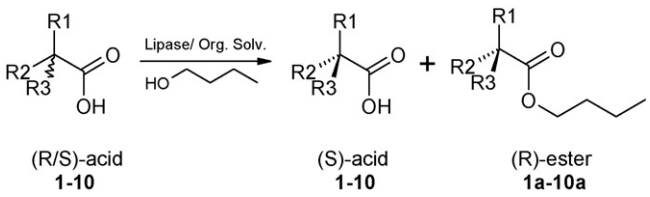

Fig. 3. General scheme for the lipase-catalyzed enantioselective esterification of racemic acidic drugs.

Table 2
Enzymatic parameters of the lipase-catalyzed enantioselective esterification in organic solvents.

Drugs	Lipase	Solvent	Time	ee_s (%)	ee_p (%)	Conv. %	*E*
1	Novozym525	Toluene	144 h	2.4	28.8	7.7	1.8
2	Novozym435	Toluene	72 h	6.4	70.4	8.4	6.1
3	Novozym435	MtBE	24 h	51.7	61.4	45.7	6.9
4	Novozym525	MtBE	120 h	75.7	98.2	43.5	263.5
5	Novozym525	MtBE	72 h	20.1	73.3	21.5	7.9
6	Novozym435	MtBE	12 d	20.1	84.3	19.3	14.3
7	Novozym525	Ethylacetate	9 d	26.1	95.9	21.4	62.5
8	Novozym435	MtBE	168 h	58.3	58.7	49.8	6.8
9	Novozym435	Ethylacetate	49 h	89	83	51.5	33.2
10	Novozym435	Toluene	0.66 h	70.9	59.6	54	263.5

*3.1.2. Etodolac (**2**) (R,S-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid) (NSAIDs)*

Used as analgesic and antipyretic drug, with stereogenic carbon atom at position one. All the effects of etodolac *in vivo* were due to *S*-(+)-enantiomer while *R*-(−)-enantiomer shows little activity [14]. Despite this fact, etodolac still marketed as racemic mixture. The resolution of etodolac enantiomers was reported using diastereomeric crystallization [15]. However, the lipase-catalyzed esterification of etodolac has not been reported yet. This might be due to the hard solubility of this drug in conventional standard organic solvents that were conventionally used in the lipase-catalyzed esterification reactions. The possibility of using non-standard organic solvents in HPLC encourages us to perform the first lipase-catalyzed approach to access enantiopure etodolac. However, poor conversions and enantioselectivities (Table 2 and Fig. 4) were observed during the lipases and solvents screening of etodolac.

Despite the achieved poor results of both etodolac and carprofen, the new analytical baseline separations of both racemic drugs and their corresponding n-butyl esters will be of great importance with respect to future biocatalytical investigations on these drugs.

*3.1.3. Fenoprofen (**3**) (R,S-2-(3-phenoxy phenyl) propionic acid) (NSAIDs)*

Fenoprofen (**3**) (*R,S*-2-(3-phenoxy phenyl) propionic acid) (NSAIDs): is an antipyretic and analgesic drug. Of its two enantiomers, the *S*-form is about 35 times more potent than the *R*-isomer [16]. The lipases screening was first conducted in isooctane (Table 2). Novozym435 and Novozym525 were selected and further investigated in non-standard organic solvents. In terms of the enantioselectivity (*E*) and conversion (Conv. %), Novozym435 showed the best performance in MtBE having log *P* 1.3 (ee_s = 51%, ee_p = 61%, *E* = 6.9 and Conv. % = 46 after 24 h).

*3.1.4. Flurbiprofen (**4**) (R,S-2-(3-fluoro-4-phenyl) phenyl propionic acid) (NSAIDs)*

Its *S*-(+)-enantiomer exhibits most of its anti-inflammatory activity, while the presence of the *R*-(−)-enantiomer enhances its gastrointestinal toxicity [17]. Furthermore, the *R*-(−)-flurbiprofen blocks nociception in rats, indicating a different mechanism of analgesia and suggesting the use of this enantiomer, and possibly other *R*-(−)-aryl propionic acids, as analgesics. Flurbiprofen is less prone to undergo chiral inversion *in vivo*. This renders the resolution of the enantiomers of this drug clinically interesting. ^{1}H NMR was the method of choice in the previously reported reaction mon-

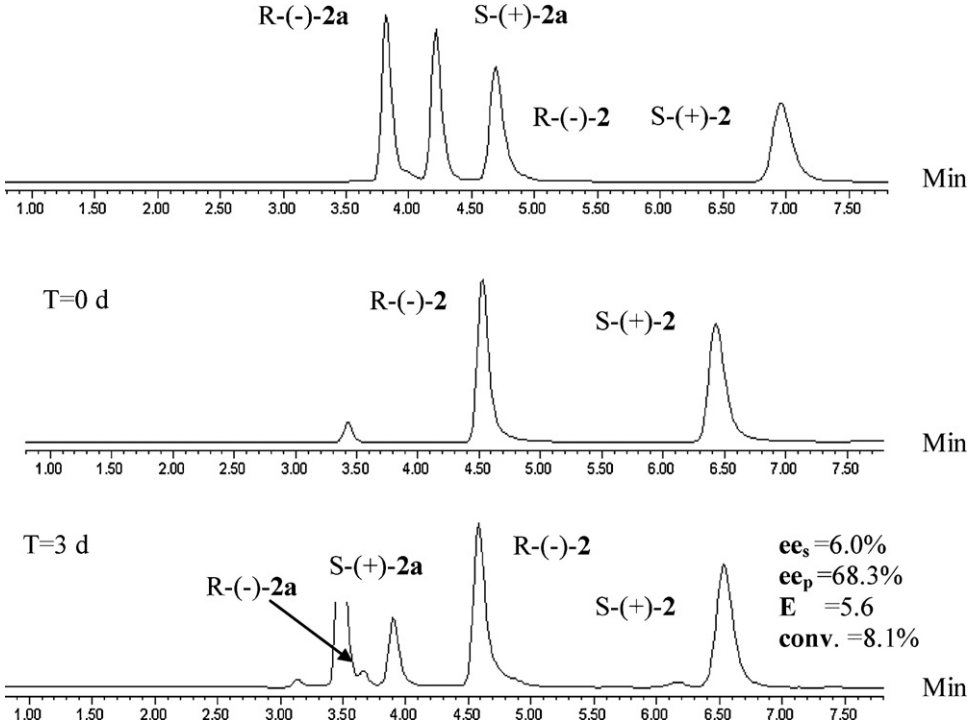

Fig. 4. HPLC chromatograms (from *T* = 0 to *T* = 3 d) showing the UV traces of both etodolac (**2**) and etodolac butyl ester (**2a**) during the direct HPLC monitoring of the reaction progress of the Novozym435-catalyzed enantioselective esterification of etodolac (**2**) using 1-butanol in toluene.

itoring of the lipase-catalyzed enantioselective esterification by Morrone et al. [18] as well as Sinisterra and Arroyo [19]. Thus, using Novozym435 in acetonitrile and isobutyl methyl ketone (iBMK), the enantioselectivities were $E = 12.3$ and $E = 2.0$, respectively. Enantioselective HPLC was also used for reactions' monitoring using Novozym435 in acetonitrile (ee = 93%) [11].

Despite the low activity of lipases in polar solvents (characterized by low log *P*) [9], the reported lipase-catalyzed esterifications of flurbiprofen were performed in polar solvents (acetonitrile log *P* −0.34 and isobutyl methyl ketone log *P* 1.2). This was due to the poor solubility of flurbiprofen in hydrophobic solvents (isooctane and *n*-hexane). Yet, with the launching of the new immobilized Chiralpak IB column, the lipase-catalyzed esterification of flurbiprofen in non-standard solvents aiming to have better enzyme activity and enantioselectivity becomes achievable. The lipases screening for the enantioselective esterification of flurbiprofen was performed in order to select the suitable enzyme for such reaction. *C. antarctica* lipase B in both immobilized (Novozym435) and free (Novozym525) forms were the most fitting lipases for the esterification of flurbiprofen (Table 2). Subsequently, both forms were further investigated in non-standard organic solvents. In terms of the enantioselectivity (E), enantiomeric excesses of the unreacted substrate (ee_s), resulting product (ee_p) and the conversion (Conv. %), Novozym525 showed the best performance in MtBE having log *P* 1.3 (ee_s = 75%, ee_p = 98%, $E = 263$ and Conv. % = 43 after 120 h) and in toluene (ee_s = 96%, ee_p = 81%, $E = 39$ and Conv. % = 54 after 16 h).

3.1.5. Ibuprofen (**5**) (R,S-2-(isobutylphenyl)propionic acid) (NSAIDs)

Ibuprofen (**5**) (*R,S*-2-(isobutylphenyl)propionic acid) (NSAIDs): is used as a racemic mixture in the treatment of arthritis and other similar diseases. The (S)-(+)-enantiomer is 160 times more potent than the (R)-(−)-enantiomer in inhibiting prostaglandin synthesis *in vitro* [20]. Consequently, there is an increasing interest in getting pure enantiomeric form of this therapeutically active drug. The enantioselective esterification of ibuprofen (**5**) was reported by Carvalho et al. using Novozym435 ($E = 6.7$), *A. niger* ($E = 4.8$) and *C. rugosa* lipases ($E = 12$) in isooctane [21]. In biphasic systems (isooctane/ionic liquids) *A. niger*, *Aspergillus terreus*, *C. rugosa*, *Candida antarcatica* and *R. miehei* lipases showed low enantioselectivities ($E = 1.9$–8.5) [22]. Moreover, Hongwei et al. [23] used *C. rugosa* lipase for the esterification of ibuprofen (**5**) in isooctane ($E = 13$) and ionic liquids ($E = 1.1$–24.1). ^{1}H NMR was used for the monitoring of lipase-catalyzed esterification of ibuprofen by Llama and coworkers [24] using Novozym435 in toluene and several additives such as (benzo-[18]-crown-6 and porphyrins) aiming to enhance the reaction rate. However; poor enantiomeric excess was obtained (ee = 53%). Furthermore, poor enantioselectivity ($E = 2.5$) has been disclosed by Sinisterra and Arroyo [19] when using Novozym435 in isobutyl methyl ketone. Despite the possibility of tuning the enantioselectivity by changing the reaction medium i.e. solvent [25], most of the reported lipase-catalyzed esterification of ibuprofen was performed using *C. rugosa* lipase in isooctane. Thus, more investigations were needed to test the versatility of lipases in non-standard organic solvents for the enantioselective esterification of ibuprofen. Surprisingly, *C. antarctica* lipase B, both the immobilized (Novozym435) and the free (Novozym525) forms, showed better activity (Conv. %) and enantioselectivity (*E*) (Table 2). Thus, these two enzymes were selected for subsequent esterification of the drug in non-standard organic solvents namely toluene, MtBE, ethyl acetate, THF and DCM. These reactions were directly monitored using HPLC equipped with the immobilized Chiralpak IB column. In the present study, in terms of enantioselectivity (*E*), Novozym525 showed the best performance in MtBE having log *P* 1.3 (ee_s = 25%, ee_p = 71%, $E = 7.6$ and Conv. % = 26 after 144 h). While in term of conversion, Novozym525 showed the best performance in toluene having log *P* 2.5 (ee_s = 46%, ee_p = 34%, $E = 3.0$ and Conv. % = 57 after 29 h).

3.1.6. Indoprofen (**6**) (R,S-2-[4-(1-oxo-2-isoindolinyl) phenyl] propionic acid) (NSAIDs)

It is used as pain reliever but withdrawn from the market since 1983 due to its carcinogenicity towards animal models (rodents) and gastrointestinal toxicity. Recently, it has been suggested that an off-the-market pain reliever may be a lead compound and a starting point for finding a new drug to treat, the devastating childhood neurological disorder spinal muscular atrophy (SMA) [26]. Indoprofen enantiomers are characterized by different degrees of interaction with serum proteins and have different pharmacokinetic properties. The anti-inflammatory effect is entirely due to the *S*-(+)-isomer and no stereospecific inversion of this enantiomer to the inactive one occurs [27]. This renders the resolution of enantiomers of indoprofen highly crucial. The lipase-catalyzed esterification of indoprofen (**6**) was reported by Nicolosi and coworkers [28] using Novozym435 in dioxane–toluene (1:1) as the solvent system (ee of the R-ester product 89%, ee of the S-acid 44% and $E = 26$). The reaction was monitored using ^{1}H NMR. Due to the sparing solubility of indoprofen (**6**) in hydrophobic solvents [28] such as standard (isooctane) or non-standard (toluene), the reported lipase-catalyzed esterification was performed in solvent mixtures consisted of dioxane to enhance the drug solubility and hydrophobic solvent to enhance the lipases activity in 1:1 ratio. In this study, trials to enhance the lipases activity and enantioselectivity by increasing the reaction media hydrophobicity (log *P*) were performed. Thus, the solubility enhancer (dioxane log *P* −1.1) was replaced by a more hydrophobic solvent namely THF (log *P* 0.49) and the ratio of hydrophobic solvents in the solvent mixture was increased from 1:1 to be 2:1. In terms of the enantioselectivity (*E*) and conversion (Conv. %), Novozym435 showed the best performance in toluene/THF having log *P* 2.5 (ee_s = 6%, ee_p = 93%, $E = 31$ and Conv. % = 6.4). However upon doubling the enzyme amount to enhance the conversion, Novozym435 showed better conversion in MtBE (ee_s = 20%, ee_p = 84%, $E = 19$ and Conv. % = 14) (Table 2 and Fig. 5).

3.1.7. o-Methoxymandelic acid

(*R*)-Mandelic acid plays a key role in the production of semi-synthetic cephalosporins and penicillins. Furthermore, it is used as a chiral resolving agent and chiral synthon in the synthesis of anti-tumor and anti-obesity agents [29]. The access to enantiomerically pure mandelic acid was previously reported using diastereomeric crystallization [30], asymmetric synthesis and kinetic resolution [31], and chemo-enzymatic approaches [32]. The deracemization process for chiral mandelic acid was previously demonstrated through dynamic kinetic resolution (DKR) process by enzyme-metal combination catalysis [33] and lipase–racemase two-enzyme system [34]. However, none of the reported procedure did include a straightforward enantioselective HPLC monitoring of the lipase-catalyzed resolution of mandelic acid derivative (**7**) in non-standard organic solvents [35]. In the present work, a simultaneous baseline separation for the free *o*-methoxymandelic acid and it corresponding butyl ester (**7a**) was first achieved and followed by the direct enantioselective HPLC monitoring in non-standard organic solvents. The enzymatic parameters of the enzymatic reaction in ethyl acetate were ee_s = 26.1%, ee_p = 95.9%, $E = 62.5$ and Conv. % = 21.4 after 9 d (cf Table 2 and Fig. 6).

3.1.8. Naproxen (**8**) (R,S-2-(6-methoxy-2-naphthyl) propionic acid) (NSAIDs)

Naproxen (**8**) (*R,S*-2-(6-methoxy-2-naphthyl) propionic acid) (NSAIDs) is widely used as a drug for human connective tissue dis-

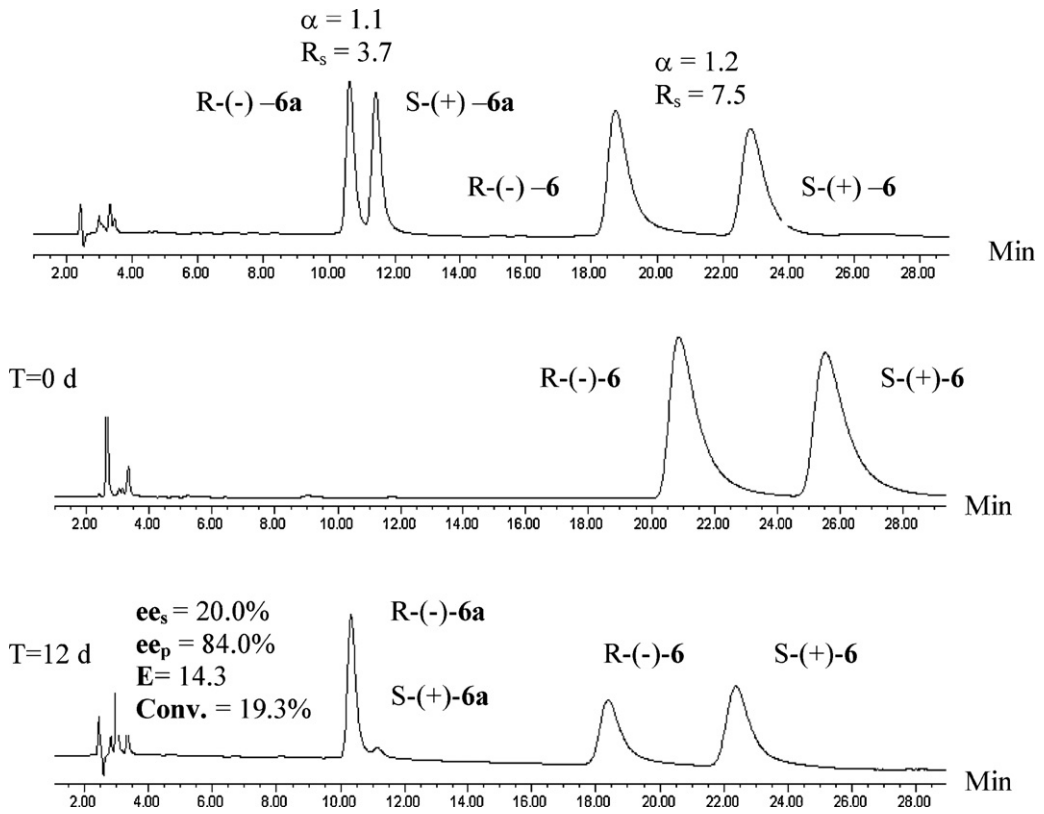

Fig. 5. HPLC chromatograms (from $T = 0$ to $T = 12$ d) showing the UV traces of both indoprofen (**6**) and indoprofen butyl ester (**6a**) during the direct HPLC monitoring of the reaction progress of the Novozym435-catalyzed enantioselective esterification of indoprofen (**6**) using 1-butanol in MtBE as non-standard HPLC solvent.

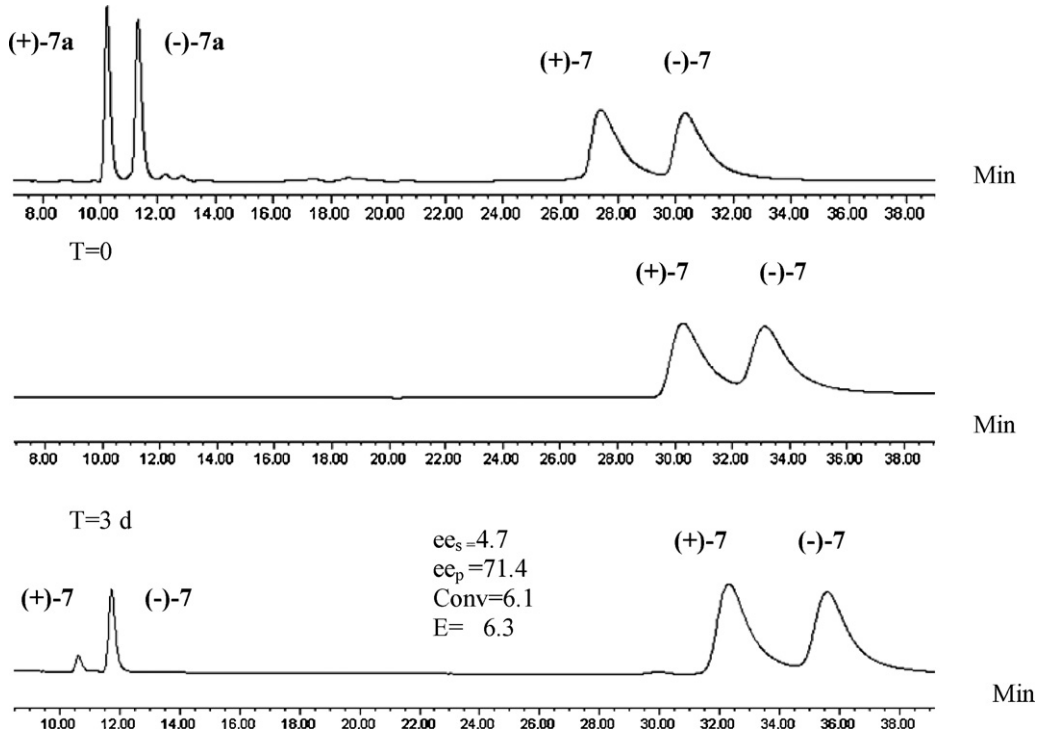

Fig. 6. HPLC chromatograms (from $T = 0$ to $T = 3$ d) showing the UV traces of both *o*-methoxymandelic acid (**7**) and 0-meyhoxymandelic acid butyl ester (**7a**) during the direct HPLC monitoring of the reaction progress of the Novozym435-catalyzed enantioselective esterification of 0-meyhoxymandelic acid (**7**) using 1-butanol in MtBE as non-standard HPLC solvent.

eases. The physiological activity of the *S*-(+)-form is 28-fold greater than that of the *R*-(−)-form [36] hence, only S-(+)-form is used as a clinical drug for humans. The lipase-catalyzed esterification of naproxen (**8**) was reported by Sinisterra and Arroyo [19] using Novozym435 in isobutyl methyl ketone showing enantioselectivity ($E = 1.3$) and ^{1}H NMR for reaction monitoring.

In this study, better enantioselectivity was observed for Novozym435 and Novozym525 in both toluene and MtBE. However, enzyme inhibition occurred leading to poor conversion. Thus, higher enzyme/naproxen ratio was used in an attempt to overcome low enzyme activity. In terms of the enantioselectivity (E) and conversion (Conv. %), Novozym435 showed the best performance in MtBE having log P 1.3 ($ee_s = 58\%$, $ee_p = 58\%$, $E = 6.8$ and Conv. % = 49 after 168 h) (Table 2).

3.1.9. 2-Phenoxy propionic acid (**9**)

2-Phenoxy propionic acid (**9**) is a well-known biologically and industrially important compound. Most of this importance is arising from the (R)-enantiomer responsible for the herbicidal activity and used as precursor for R-p-2-hydroxy phenoxy propionic acid, an intermediate in the synthesis of agrochemically and pharmaceutically active chiral aryloxypropionic acids. Furthermore, enantiomers of 2-phenoxy propionic acid are used as chiral resolving agent of amines such as 2-methylpiperazine [9].

A baseline separation of both racemic 2-phenoxy propionic acid (**9**) and its corresponding butyl ester (**9a**) was achieved using *n*-hexane/2-propanol/trifluoroacetic acid (87:13:0.5 v/v/v). The screening of different lipases in the enantioselective esterification of 2-phenoxy propionic acid was performed in isooctane as standard organic solvent. Of all tested lipases, only the immobilized lipase from *C. antarctica* lipase B CAL-B (Novozyme 435), its unimmobilized form (Novozyme525) and immobilized lipase from *R. miehei* (Lipozyme RM IM) showed better enantioselectivity *E* in a shorter time (up to 48 h). The rest did not show any promising results after 176 h. In most cases of the enantioselective esterification of (R,S)-**9**, (S)-**9** was the faster reacting enantiomer which was selectively esterified to afford (S)-**9a** leaving the second enantiomer of the substrate (R)-**9** in enantiomerically enriched form. This was achieved when using different lipases in the presence of *n*-butanol as alkyl donor in isooctane as organic solvent. However, this was not the case when using CAL-B immobilized and its free form where the preference of the lipase was towards the (R)-substrate (R)-**9** to afford (R)-**9a** leaving (S)-**9** in enantiomerically enriched form.

Based on the above-mentioned results, three lipases were selected for further investigation in non-standard solvents. Thus, lipase from *C. antarctica* lipase B CAL-B (Novozyme435), its free form (Novozyme525) and immobilized lipase from *R. miehei* (Lipozyme RM IM) were used for the enantioselective esterification of racemic phenoxy propionic acid **9** in non-standard HPLC solvents such as toluene, methyl tert butyl ether (MTBE), ethyl acetate (EtOAc), tetrahydrofuran (THF), dichloromethane (DCM) and carbon tetrachloride (CCl_4). The reactions were monitored directly by enantioselective HPLC without further workup. As can be noticed, the preference for lipases used in this investigation was for (R)-**9**, which undergoes the selective esterification to afford (R)-**9a** leaving (S)-**9** in enantioenriched form, however, only in case of ethyl acetate the stereoselectivity of the three lipases investigated was inverted and the preferences were for (S)-**9** rather than (R)-**9** to afford (S)-**9a** leaving (R)-**9** in enantioenriched form. Based on the enantiomeric excesses of unreacted substrate (ee_s), enantiomeric excess of the resulting product (ee_p) and the reaction time at maximum conversion (50%), lipase from *C. antarctica* B (CAL-B) showed the best performance in ethyl acetate ($ee_s = 89\%$, $ee_p = 83\%$, Conv. % = 51.5%, $E = 33.28$ after 49 h) (Table 2).

3.1.10. Tiaprofenic acid (**10**)(R,S-2-(5-benzoylthiophen-2-yl)propanoic acid) (NSAIDs)

It has a potent anti-inflammatory and analgesic properties. Several studies in healthy and arthritic subjects have involved the administration of the racemate. However, tiaprofenic acid has been suggested to exhibit limited pharmacokinetic stereoselectivity [3]. In view of the increasing legislative concern regarding the development and use of single enantiomeric drug in studying the pharmacokinetics and pharmacodynamics of each separate enantiomer, an enantioselective straightforward route to separate enantiomers is required. The analytical enantioselective resolution of tiaprofenic acid was reported using GC, HPLC and CEC [3,5]. Most of these assays are indirect and involve the formation of diastereomers through reaction of the carboxylic acid moiety in tiaprofenic acid with a coupling reagent, 2,2,2-trichloroethyl chloroformate, to form a mixed anhydride. The latter is followed by the formation of an amide using L-leucinamide. However, in addition to racemization, partial or complete chiral inversion might occur [3]. In fact, none of the reported procedures used to give enantiomercially pure/enriched tiaprofenic acid did include a straightforward enzymatic resolution of the racemates. This is probably due to solubility problems of the substrate in conventional standard HPLC organic solvents and the difficulty associated with the simultaneous resolution of the free acid (enzyme substrate) and its corresponding ester (product) in one run without derivatization. The enantioselective transformations catalyzed by enzymes in non-standard HPLC organic solvents might be the method of choice to overcome solubility problem of tiaprofenic acid.

Using the previously developed HPLC baseline separation of tiaprofenic acid and its n-butyl ester (Table 2) the enzymatic reaction can be easily and directly HPLC monitored in either standard or non-standard HPLC organic solvents. Thus, the screening of different lipases for the enantioselective esterification of tiaprofenic acid was first performed in acetonitrile (log P −0.34). Of all the 10 tested lipases, only immobilized lipase from *C. antarctica* lipase B CAL-B (Novozyme435) showed better enantioselectivity (E) in shorter time (up to 48 h). The rest did not show any promising results even after 120 h (Table 2). Thus, *C. antarctica* lipase B CAL-B (Novozyme435) and its free form (Novozyme525) were used for the enantioselective esterification of racemic tiaprofenic acid (**10**) in non-standard HPLC organic solvents. In terms of the enantioselectivity (E), enantiomeric excesses of unreacted substrate (ee_s), and the resulting product (ee_p) and the conversion (Conv. %), Novozym435 showed the best performance in toluene having log P 2.50 ($ee_s = 70.9\%$, $ee_p = 59.6\%$, $E = 8.16$ and Conv. % = 54 after 40 min).

3.2. Lipase-catalyzed enantioselective hydrolysis

Lipase-catalyzed enantioselective hydrolysis is a well established procedure to access enantiomerically pure NSAIDs. Despite the concept that interfacial activation occurs before lipases take part in biochemical transformation reactions [1], most of the reported lipase-catalyzed hydrolysis reactions were carried out in one phase (aqueous phase). In the previous reported lipase-catalyzed hydrolysis reactions, the ester (lipase substrate) was suspended (if solid) or emulsified (if oil) in the aqueous phase (buffer) forming an interface by which activation of the lipase occurs [37]. However, accumulation of the water insoluble hydrolytic reaction product may cause inhibition of the enzyme.

It has been previously demonstrated that butyl ester is one of the most favorable esters to carry out enzymatic hydrolysis [38]. Thus, the enzymatic hydrolysis of the selected NSAIDs butyl esters (**1a**–**10a**) was carried out in phosphate buffer pH 7/solvent (1:1) biphasic systems. Firstly, the lipases screening was performed to select the most fitting lipases. This was followed by solvents screen-

Table 3
Enzymatic parameters of the lipase-catalyzed enantioselective hydrolysis in biphasic solvents consisting of phosphate buffer (pH 7) and non-standard HPLC organic solvents.

Drugs	Lipase	Solvent	Time	ee_s (%)	ee_p (%)	Conv. %	*E*
1	Novozym525	Buffer/Toluene	168	4.1	27.7	12.9	1.8
2	/	/	/	/	/	/	/
3	Novozym435	Buffer/isooctane	120	5.4	32.7	14.2	2.0
4	Novozym435	Buffer/MtBE	72	24.9	22.5	52.4	1.9
5	Novozym435	Buffer/MtBE	120	28.1	25.8	52.1	2.1
6	/	/	/	/	/	/	/
7	Novozym435	Buffer/DCM	5 d	31.31	80.87	27.91	12.81
8	Lipase AYS	Buffer/MtBE	5 d	63	98	39	190
9	/	/	/	/	/	/	/
10	Novozym435	Buffer/MtBE	2	61.6	47.1	56.6	5.0

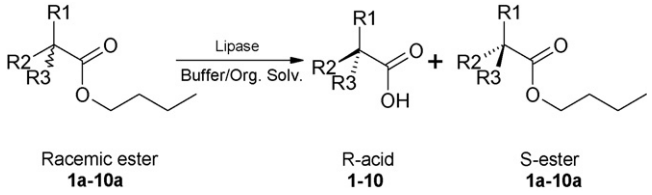

Fig. 7. Lipase-catalyzed enantioselective hydrolysis of n-butyl esters.

ing to test the effect of solvents alteration on the enantioselectivity (*E*) (cf Table 3 and Fig. 7).

These reactions were directly monitored using HPLC equipped with the immobilized Chiralpack IB column. Samples were collected from the organic layer and directly injected onto the HPLC system. In case of *C. antarctica* lipase B (CAL-B) the *R*-(−)-ester was the faster reacting enantiomer affording the *R*-(−)-acid and leaving the *S*-(+)-ester in enantiomerically enriched form (Figs. 8 and 9). While, in case of lipase AYS the *S*-(+)-ester was the faster reacting enantiomer affording *S*-(−)-acid and leaving the *R*-(−)-ester in enantiomerically enriched form.

In case of indoprofen butyl ester (**6a**), it has been excluded from the hydrolytic reaction experiments due to the sparing solubility of the reaction product (indoprofen **6**) in water immiscible solvents.

In case of carprofen (**1**), flurbiprofen (**4**), ibuprofen (**5**), and tiaprofenic acid (**10**), both forms of *C. antarctica* lipase B (Novozym435 and Novozym525) were selected for further investigations in different organic solvents due to their satisfactory hydrolytic activity (Table 3). For fenoprofen (**3**) Novozym435 and lipase PS-C were selected due to the activity of the former and enantioselectivity of the latter (Table 3). In case of naproxen (**8**) Novozym525 and lipase AYS have been selected due to their activity and enantioselectivity (Table 3). Except for naproxen **8**, the lipase-catalyzed esterification (Fig. 3) of the NSAIDs under investigations afforded better results than that of the hydrolysis of the corresponding ester **8a** (Fig. 7). Thus, the lipase-catalyzed enantioselective hydrolysis showed the best results using lipase AYS in phosphate buffer pH 7/MtBE biphasic system (ee_s = 63%, ee_p = 98%,

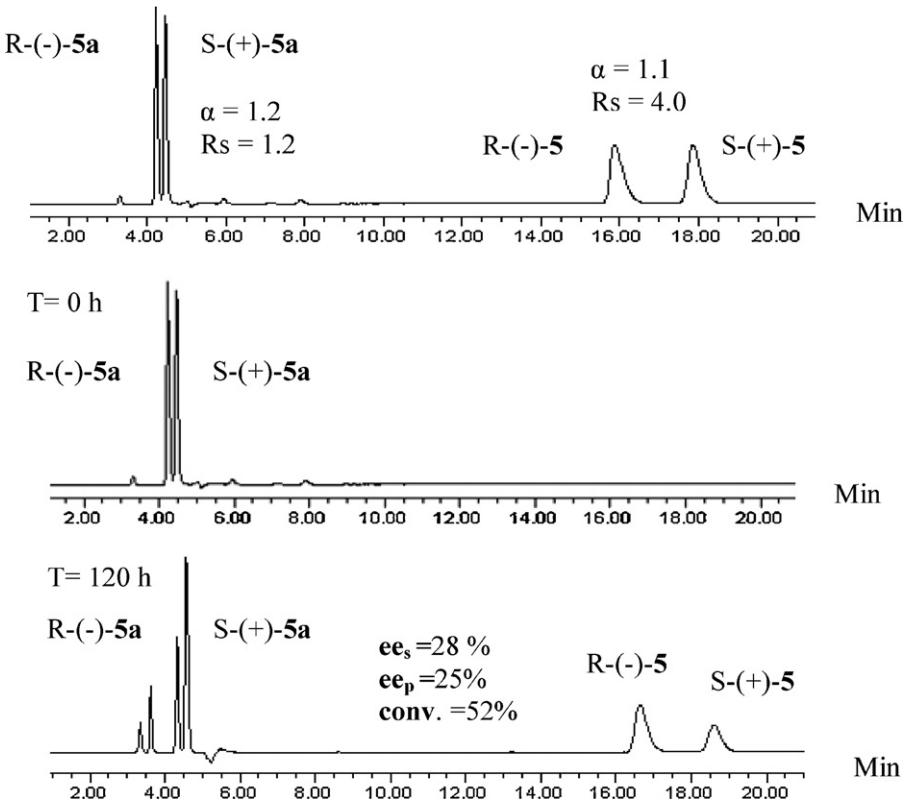

Fig. 8. Selected HPLC chromatograms (from *t* = 0 to *t* = 120 h) showing UV traces of both ibuprofen butyl ester (**5a**) and ibuprofen (**5**) during the direct HPLC monitoring of the reaction progress of Novozym435-catalyzed enantioselective hydrolysis of ibuprofen butyl ester (**5a**) in a biphasic system consisting of MtBE and phosphate buffer pH 7.

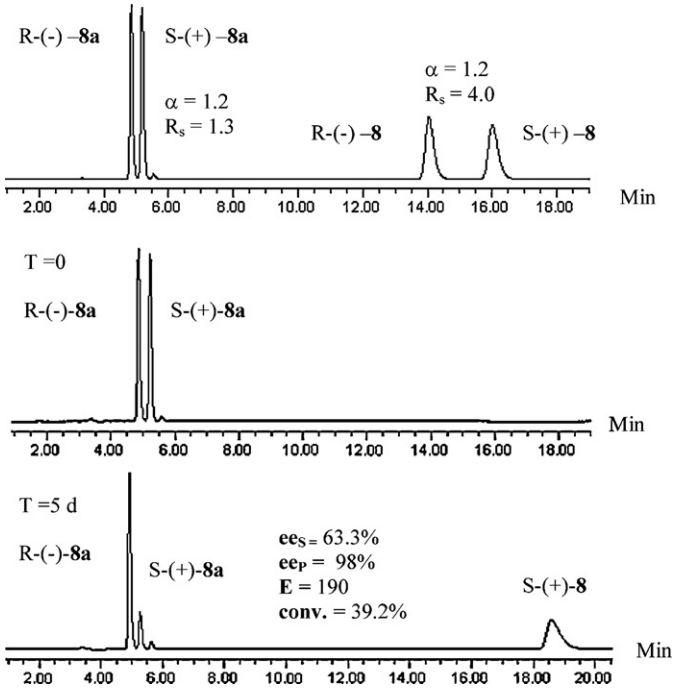

Fig. 9. Selected HPLC chromatograms (from $t = 0$ to $t = 5$ d) showing UV traces of both naproxen butyl ester (**8a**) and naproxen (**8**) during the direct HPLC monitoring of the reaction progress of lipase AYS-catalyzed enantioselective hydrolysis of naproxen butyl ester (**8a**) in a biphasic system consisting of MtBE and phosphate buffer pH 7.

Conv. % = 39 and $E = 190$). The lipase-catalyzed enantioselective hydrolysis of naproxen was reported by Moreno and Sinisterra [38] (ee of *S*-acid = 95%), Xin et al. [39] (ee of *S*-acid = 96%), Giorno and co-workers [40] ($E = 31$), Sih and co-workers [41] ($E = 100$) and Tsai et al. [42] (ee of *S*-acid = 88%) and monitored by adopting HPLC methods using coated chiral columns.

4. Conclusion

The accurate determination of the enantiomeric excesses of both substrate and product during the enantioselective lipase-catalyzed kinetic resolution of racemic acidic drugs is demonstrated. The existence of a direct and reliable analytical procedure for the simultaneous baseline separation of both substrate and product in one run without derivatization is a prerequisite for the success of such procedure. While not all acidic drugs and their corresponding esters are soluble in conventional standard organic HPLC solvents, the use of non-standard organic solvents was in most cases mandatory to achieve a simultaneous baseline separation of the acidic drugs and their corresponding esters. For this purpose, the immobilized chiral stationary phase namely Chiralpak IB, a 3,5-dimethylphenylcarbamate derivative of cellulose (the immobilized version of Chiralcel OD) was used and proved to be versatile for the monitoring of the lipase-catalyzed kinetic resolution of racemates in non-standard organic solvents. The current developed monitoring procedure will render the application of lipase (and other catalysts) to access enantiomerically pure/enriched acidic drugs in non-standard organic solvents feasible and easy to access.

References

[1] A. Ghanem, Tetrahedron 63 (2007) 1721.
[2] FDA's Policy statement for the development of new stereoisomeric drugs, Chirality 4 (1992) 338.
[3] A. Ghanem, M.N. Aboul-Enein, A. El-Azzouny, M.F. El-Behairy, E. Al-Humaidi, A. Alwaleed, K. Amin, M.N. Al-Ahdal, Chirality 20 (2008) 871.
[4] A. Rocco, S. Fanali, J. Sep. Sci. 32 (2009) 1696.
[5] Z. Jiang, M. Bertazzo, R.D. Suessmuth, Z. Yang, N.W. Smith, V. Schurig, Electrophoresis 27 (2006) 1154.
[6] A.M.M. Ali, K.M. Emara, M. Khodari, Analyst (London) 119 (1994) 1071.
[7] A. Zaks, A.M. Klibanov, Science 224 (1984) 1249.
[8] T. Zhang, D. Nguyen, P. Franco, T. Murakami, A. Ohnishi, H. Kurosawa, Anal. Chim. Acta 557 (2006) 221.
[9] A. Ghanem, M.F. El-Behairy, M.N. Al-ahdal, Chromatoghraphia 65 (2007) 681.
[10] A.M. Klibanov, Trends Biochem. Sci. 14 (1989) 141.
[11] G. Duan, C.B. Ching, Biochem. Eng. J. 2 (1998) 237.
[12] A.P. Ricketts, K.M. Lundy, S.B. Seibel, Am. J. Vet. Res. 59 (1998) 1441.
[13] G. Blaschke, K-E. Schulte; US Patent No. 4,973,745 (1990).
[14] D.R. Brocks, F. Jamali, A.S. Russell, J. Clin. Pharmacol. 31 (1991) 741.
[15] E. Brenna, C. Fuganti, D. Fuganti, P. Grasselli, L. Malpezzi, G. Pedrocchi-Fantoni, Tetrahedron 53 (1997) 17769.
[16] A. Rubin, M.P. Knadler, P.P.K. Ho, L.D. Bebechtol, R.L. Wolden, J. Pharm. Sci. 74 (1985) 82.
[17] E.G. Lee, H.S. Won, H.-S. Ro, Y.-W. Ryu, B.H. Chung, J. Mol. Catal. B: Enzym. 26 (2003) 149.
[18] R. Morrone, G. Nicolosi, A. Patti, M. Piattelli, Tetrahedron Asymmetry 6 (1995) 1773.
[19] M. Arroyo, J.V. Sinisterra, J. Org. Chem. 59 (1994) 4410.
[20] S.S. Adams, P. Bresloff, C.G. Mason, J. Pharm. Pharmacol. 28 (1976) 256.
[21] P.O. Carvalho, F.J. Contesini, M. Ikegaki, Braz. J. Microbiol. 37 (2006) 329.
[22] F.J. Contesini, P.O. Carvalho, Tetrahedron Asymmetry 17 (2006) 2069.
[23] Y. Hongwei, W. Jinchuan, C.C. Bun, Chirality 17 (2005) 16.
[24] A. Gradillas, C. Delcampo, J.V. Sinisterra, E.F. Llama, Biotechnol. Lett. 18 (1996) 85.
[25] A. Ducret, M. Trani, R. Lortie, Enzyme Microb. Technol. 22 (1998) 212.
[26] M.R. Lunn, D.E. Root, A.M. Martino, S.P. Flaherty, B.P. Kelley, D.D. Coovert, A.H. Burghes, N.T. Man, G.E. Morris, J. Zhou, E.J. Androphy, C.J. Sumner, B.R. Stockwell, Chem. Biol. 11 (2004) 1489.
[27] V. Tamassia, M.G. Jannuzzo, E. Moro, S. Stegnjaich, W. Groppi, F.B. Nicolis, Int. J. Clin. Pharmacol. Res. 4 (1984) 223.
[28] R. Morrone, G. Nicolosi, M. Piattelli, Chirality 10 (1998) 321.
[29] G.D. Yadav, P. Sivakumar, Biochem. Eng. J. 19 (2004) 101.
[30] E. Wehtje, P. Adlercreutz, B. Mattiasson, Biotechnol. Bioeng. 36 (1990) 39.
[31] S.H. Hsu, S.S. Wu, Y.F. Wang, C.H. Wong, Tetrahedron Lett. 31 (1990) 6403.
[32] J.M. Palomo, G. Fernandez-Lorente, C. Mateo, C. Ortiz, R. Fernandez-Lafuente, J.M. Guisan, Enzyme Microb. Technol. 31 (2002) 775.
[33] M.J. Kim, Y.S. Ahn, J.W. Park, Curr. Opin. Biotechnol. 13 (2002) 578.
[34] U.T. Strauss, K. Faber, Tetrahedron Asymmetry 10 (1999) 1081.
[35] W.J. Choi, K.Y. Lee, S.H. Kanga, S.B. Lee, Sep. Purif. Technol. 53 (2007) 178.
[36] A.L. Margolin, Enzyme Microb. Technol. 15 (1993) 266.
[37] G. Caron, R.J. Kazlauskas, Tetrahedron Asymmetry 4 (1995) 1995.
[38] J.M. Moreno, J.V. Sinisterra, J. Mol. Catal. A: Chem. 98 (1995) 171.
[39] J.Y. Xin, S.B. Li, Y. Xu, J.R. Chui, C.G. Xia, J. Chem. Technol. Biotechnol. 76 (2001) 579.
[40] K. Sakaki, L. Giorno, E. Drioli, J. Membr. Sci. 184 (2001) 27.
[41] S.H. Wu, Z.W. Guo, C.J. Sih, J. Am. Chem. Soc. 112 (1990) 1990.
[42] C.S. Chang, S.W. Tsai, C.N. Lin, Tetrahedron Asymmetry 9 (1998) 2799.