Journal of Chromatography A, 1234 (2012) 56–63

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Optimization of the liquid chromatography enantioseparation of chiral acidic compounds using cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector and polar organic mobile phases

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ARTICLE INFO

Article history:
Available online 31 January 2012

Keywords:
Liquid chromatography
Enantioseparation
Chiral acidic compounds
Chlorinated cellulose based chiral stationary phases
Polar organic mobile phases
Optimization

ABSTRACT

The LC enantioseparation of chiral acidic and zwitterionic drugs selected as model compounds was optimized using chlorine containing cellulose based chiral stationary phases and polar organic mobile phases. The main solvent of the mobile phase was acetonitrile, the temperature was settled at 25 °C and a stationary phase with cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector (3-Cl-4-Me-PC) was selected. In the screening step, the nature and concentration of both acidic and basic additives were found to have a significant effect on retention, selectivity and resolution. Acetic acid (AcA) was selected as acidic additive for the optimization step since it could lead to the enantioseparation of more acidic compounds than trifluoroacetic acid (TFA) and formic acid (FA), while among the three basic additives tested, diethylamine (DEA) most often gave better results with respect to enantioresolution and selectivity than butylamine (BuA) and triethylamine (TEA). The optimization was performed using a central composite face-centered design with two factors, namely the concentration of acetic acid (0.1–0.3%) and the concentration of DEA (0.01–0.1%) in the mobile phase. On the basis of the results obtained in the screening and optimization steps, a strategy for the rapid development of methods for the enantioseparation of acidic or neutral compounds was proposed.

1. Introduction

It is well known that many implications of chirality are related to the fact that the enantiomers of chiral drugs can have different pharmacological activities. Indeed, enantiomerically pure natural compounds such as sugars, amino acids and proteins are involved in most biological processes.

Since the tragedy of the sedative drug thalidomide, there has been a particularly high interest in the biological activity, both pharmacological and toxicological, of the enantiomers of chiral drugs [1]. As consequence, it is now a legal requirement to thoroughly assess the racemate as well as each single enantiomer of the potential chiral drug candidates. The production of the enantiomers and the control and determination of the enantiomeric composition of chiral drug substances have become key issues for both the pharmaceutical industry and regulatory agencies [2]. Thus, many analytical and preparative techniques have been developed for the enantioseparation of chiral compounds [3–8]. Among these techniques, liquid chromatography (LC) has become an essential tool for to perform these separations because of the widespread availability of chiral stationary phases (CSPs) for direct enantioresolution.

Even though the majority of enantioseparations can be performed on chiral stationary phases in LC, it should be emphasized that there is no universal CSP [9]. Therefore, the enantioseparation of a target chiral drug depends on the choice of a suitable combination between a CSP and a LC mobile phase. Whatever is the goal of chiral separations, many strategies have been reported in literature to define simple and efficient chromatographic conditions [10–17]. However, these strategies using polysaccharide based CSPs in normal (NP), reversed (RP) or polar organic phase (POSC) mode do not often include the important role of acidic or basic additives in the mobile phase. Indeed, the nature and concentration of these additives have been shown to influence the retention, selectivity and enantioresolution of chiral compounds [14–16,18–22]. Thus, in addition to the use of various CSPs, the selection of different acidic or basic additives could be of great benefit for enantioselectivity as demonstrated in our previous works dealing with the enantioseparation of basic

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doi:10.1016/j.chroma.2012.01.072

compounds using chlorine containing cellulose-based CSPs in POSC [14–16,20,21]. These recently commercialized CSPs were synthesized and evaluated first by Chankvetadze et al [23–25].

The present paper describes the optimization of the enantioseparation of acidic and zwitterionic compounds using a CSP with cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector (3-Cl-4-Me-PC) in POSC. The resolving power of this CSP has been evaluated using twelve acidic or amphoteric drugs with widely different structures and polarities as representative examples and ACN as the main mobile phase component. To achieve this goal, a univariate screening step was first performed to select the factors likely to influence retention, enantioresolution and selectivity in these LC systems. Then a face-centered central composite design (FCCD) was used to deduce optimal LC conditions for the enantioresolution of these compounds.

2. Materials and methods

2.1. Chemicals and reagents

Carprofen, fenoprofen, flurbiprofen, ketoprofen, ketorolac, 2-phenylpropionic acid, 2-phenoxypropionic acid and proglumide were supplied by Sigma–Aldrich (Saint-Louis, MO, USA). Indoprofen, ibuprofen and chlortalidone were gifts from different pharmaceutical companies.

Formic acid (FA) and acetic acid (AcA) were provided by Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) was obtained from Acros Organics (Geel, Belgium). Diethylamine (DEA) and triethylamine (TEA) pro analysi were obtained from Sigma–Aldrich (Steinheim, Germany), butylamine (BuA) pro analysi from Fluka (Buchs, Switzerland) and acetonitrile (ACN) HPLC grade from J.P. Baker (Deventer, Netherlands).

2.2. Instrumentation

The chromatographic system from Agilent Technologies (Waldbronn, Germany) consisted of a quaternary pump, a thermostated column compartment, a diode array detector and an automatic injector, all of 1200 series. The Chemstation software was used for system control and data acquisition. The chiral columns, Sepapak-2 (equivalent to Lux® Cellulose-2 from Phenomenex, Torrance, CA, USA), Sepapak-4 (equivalent to Lux® Cellulose 4 from Phenomenex) and Sepapak-5 (250 mm × 4.6 mm I.D.) were kindly provided by Sepaserve GmbH (Münster, Germany).

In the case of Sepapak-2 (3-Cl-4-MePC), selected for most experiments reported in this paper, the chiral selector adsorbed on aminopropylsilanized silica (nominal particle size 5 μm and nominal pore diameter 100 nm) was cellulose tris(3-chloro-4-methylphenylcarbamate) in the amount of 25% (w/w). For Sepapak-4 (4-Cl-3-MePC) and Sepapak-5 (3,5-diClPC) the chiral selectors consisted of cellulose tris(4-chloro-3-methylphenylcarbamate) and cellulose tris(3,5-dichorophenylcarbamate), respectively.

The experimental design and the statistical calculations were performed using the MODDE® software version 6.0 from Umetrics AB (Umea, Sweden).

2.3. Solutions for method development

The mobile phases used of the different experiments were prepared by mixing the required proportions (v/v) of acetonitrile, acidic additives (TFA, FA or AcA) and basic additives (BuA, DEA or TEA). Analytical solutions of racemic compounds of nearly 100 μg/mL were prepared by dissolving the appropriate amount of the substance in the required volume of mobile phase.

2.4. Chromatographic conditions

The mobile phases made up of acetonitrile containing acidic and basic additives were pumped at a constant flow-rate of 1.0 mL min^{-1}. The injection volume was 20 μL. The analytes were detected at 240 nm and the temperature was settled at 25 °C for all experiments.

3. Results and discussion

In previous studies, the recognition ability of chlorine containing cellulose based CSPs in POSC was found to be influenced by the nature and the concentration of the acidic additive in the mobile phase, such as TFA, FA or AcA [14–16]. In these works, only basic chiral compounds were tested and three rapid screening conditions (mobile phases) were retained for method development, namely ACN/0.1% TFA/0.1% DEA, ACN/0.2% FA/0.1% DEA and ACN/0.2% AcA/0.1% DEA.

In order to extend these studies to acidic and zwitterionic compounds, twelve pharmaceuticals with widely different structures and polarities (the first twelve compounds in Fig. 1) were used as representative examples. All of them possess a carboxylic group except chlorthalidone, which has a much less acidic character and ofloxacin, which has also an alkylamino group besides its carboxylic function. After preliminary tests, the CSP with cellulose tris(3-chloro-4-methylphenylcarbamate) as selector, 3-Cl-4-MePC, was selected among the chiral columns tested (3-Cl-4MePC, 4-Cl-3-MePC and 3,5-diClPC), because significantly higher enantioresolution values were obtained with this CSP using the three mobile phases mentioned above for rapid screening of chiral basic drugs. The chiral recognition ability of this CSP was then evaluated toward the nature and concentration of acidic (TFA, FA, AcA) and basic (BuA, DEA, TEA) additives in the mobile phase. To achieve this objective, a screening of these factors by means of a univariate approach was first performed.

3.1. Screening

3.1.1. Effect of the nature of the acidic additive

In order to study the influence of the nature of the acidic additive, the three mobile phases selected for rapid method development for chiral basic compounds were tested using 3-Cl-4-MePC as CSP. As can be seen in Table 1, the nature of the acidic additive, namely FA, TFA and AcA, in the mobile phase strongly influenced the retention as well as the enantioselectivity and enantioresolution of the chiral compounds studied.

It is worth noting that the retention of the analytes seems to be related to the acidic character of the additive. Indeed, the highest retention was always obtained with AcA (pKa 4.8) while TFA (pKa 0.5) gave rise to the lowest retention. This was not the case, however, with basic compounds, for which retention with the TFA containing mobile phase was found to be the lowest while retention with FA was higher than with AcA [16]. Ion-pair formation between cationic compounds and acetate is more likely to occur in these non-aqueous mobile phases than with formate, which might explain the lower retention obtained for basic compounds using AcA as acidic additive.

As can be seen in Table 1 the nature of the acidic additive has also a great impact on the selectivity and enantioresolution of acidic and zwitterionic chiral compounds. The selectivity values were often higher with AcA than with the other acidic additives except for the less acidic compound chlortalidone and for 2-phenoxypropionic acid, for which the highest selectivity was obtained using TFA as acidic additive in the mobile phase. The

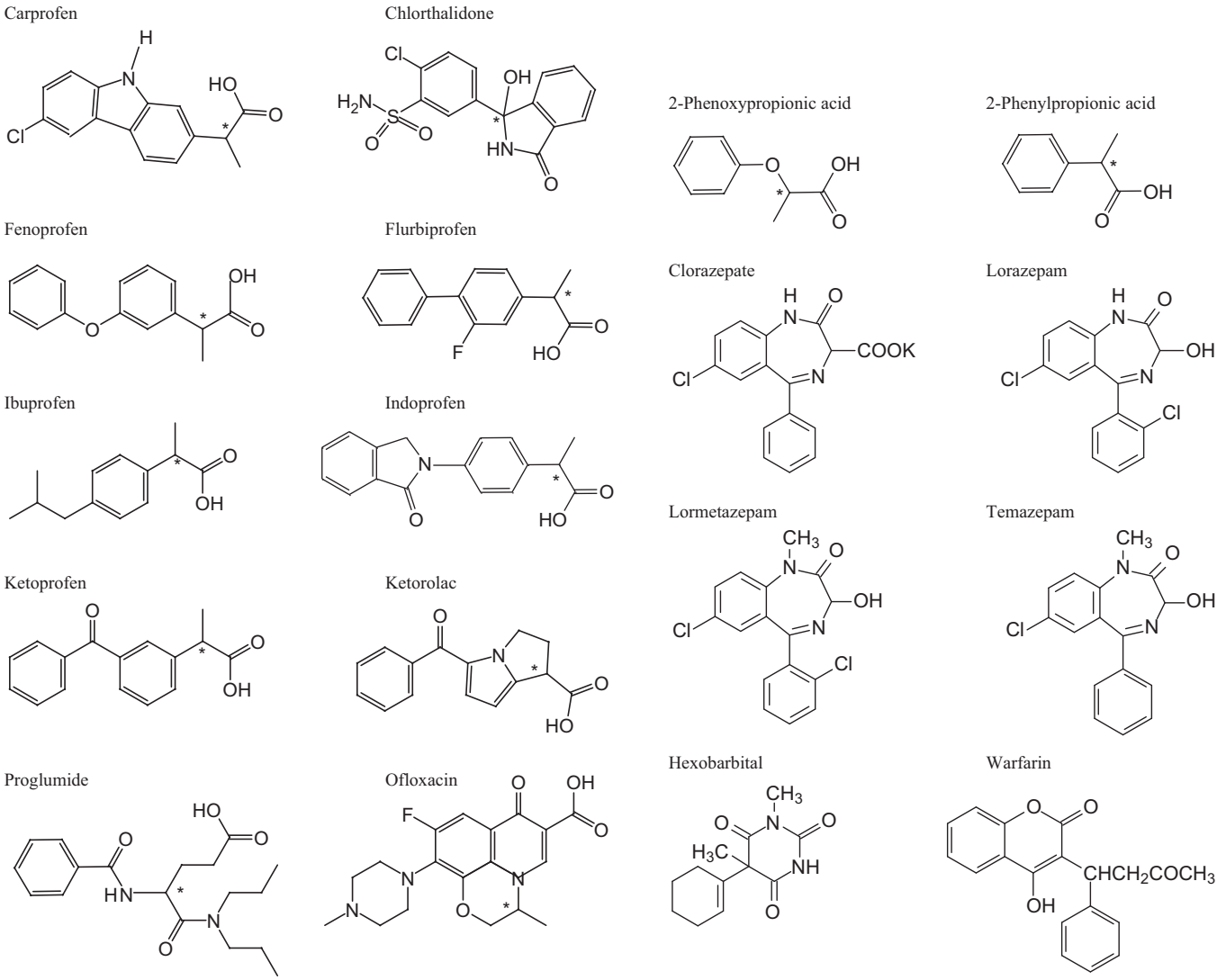

Fig. 1. Structures of the studied compounds.

enantioresolution values showed the same tendency. Fig. 2A presents a typical chromatogram of the enantioseparation of ketorolac showing that the replacement of TFA by FA or AcA gives rise to a significant increase on retention, selectivity and enantioresolution using 3-Cl-4-MePC as CSP. Therefore, AcA could be considered as the most useful acidic additive for the enantioseparation of acidic compounds using this CSP and was then selected for the optimization step.

3.1.2. *Effect of the concentration of acetic acid in the mobile phase*

If the acidic additive with the highest acidic character gives rise to the lowest retention of the analyte enantiomers, it is

Table 1
Influence of the nature of the acidic additive in the mobile phase on the retention factor of the first enantiomer (k'_1), enantioresolution (R_s) and selectivity (α).

	TFA			FA			AcA		
	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α
Carprofen	0.27	–	–	1.13	–	–	7.6	–	–
Fenoprofen	0.13	–	–	0.43	0.60	1.08	2.6	2.9	1.19
Flurbiprofen	0.12	–	–	0.54	–	–	3.1	0.60	1.02
Indoprofen	0.73	–	–	1.49	–	–	7.0	0.63	1.05
Ibuprofen	0.15	–	–	0.43	–	-	2.6	1.77	1.13
Ketoprofen	0.20	–	–	0.64	–	–	3.6	0.82	1.07
Ketorolac	0.37	0.63	1.15	1.70	1.73	1.15	7.3	2.4	1.21
Proglumide	0.67	1.96	1.32	1.43	2.9	1.32	10.0	5.7	1.65
2-Phenylpropionic acid	0.09	1.05	1.55	0.35	–	–	2.1	0.82	1.13
2-Phenoxypropionic acid	0.05	2.1	3.2	1.37	2.4	1.21	4.8	1.72	1.11
Chlorthalidone	0.47	1.30	1.24	0.78	1.07	1.14	1.30	0.60	1.03
Ofloxacin	3.5	–	–	/	/	/	7.5	8.0	2.3

/: no peak obtained within 60 min; –: $R_s \leq 0.5$.
Mobile phase: ACN/0.1% DEA/0.2% AcA or FA or 0.1% TFA. For other conditions, see Section 2.

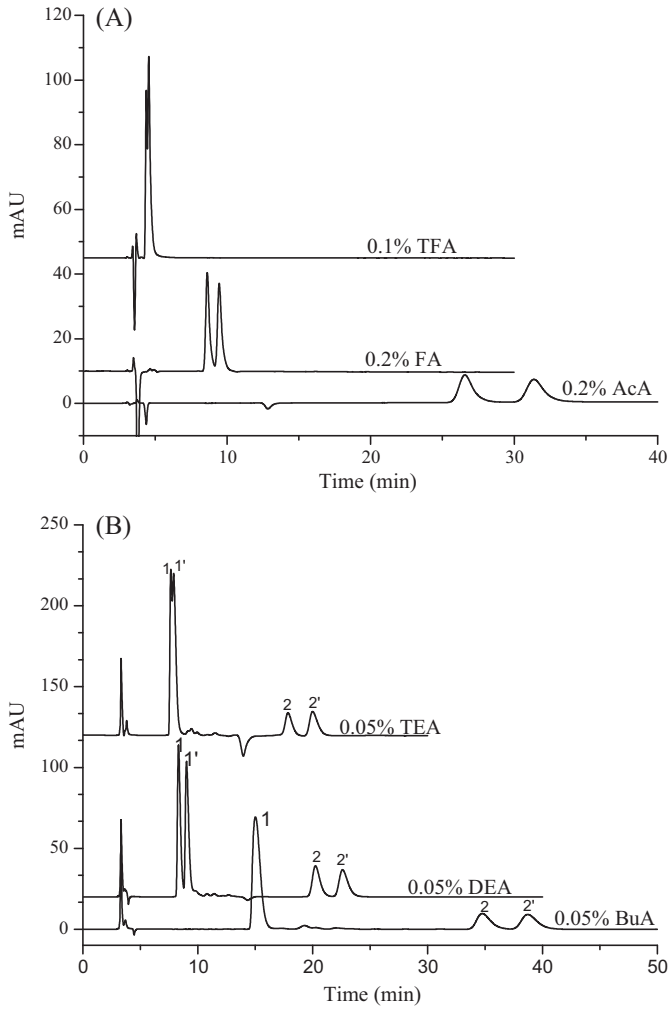

Fig. 2. Chromatograms of: (A) ketorolac enantiomers illustrating the effect of the nature of the acidic additive and (B) 2-phenylpropionic acid (1,1′) and 2-phenoxypropionic acid (2,2′) enantiomers illustrating the effect of the nature of the basic additive Mobile phases: (A) ACN/0.1% DEA/0.1% TFA, 0.2% AcA or 0.2% FA and (B) ACN/0.2% AcA/0.05% BuA, DEA or TEA. Other conditions: see Section 2.

reasonable to expect that an increase in acetic acid concentration (from 0.05 to 0.2%) in mobile phase would induce a decrease in retention. This assumption was verified for all studied compounds except the less acidic compound chlortalidone and the zwitterionic compound ofloxacin (Table 2). Moreover, the enantioresolution values were slightly improved for most tested compounds with increasing AcA concentration in the mobile phase. However, this increase seems to be essentially related to an improvement in peak efficiency since the selectivity values were almost unchanged. Clearly the concentration of acetic acid in the mobile phase is an important factor to be taken into account in the optimization step. A percentage range of 0.1–0.3% for AcA was selected to obtain enantioseparations in reasonable analytical times.

3.1.3. Effect of the nature and concentration of the basic additive in the mobile phase

As can be seen in Table 3, the retention, selectivity and the enantioresolution values were found to strongly depend on the nature of the basic additive (0.05% BuA, DEA or TEA) in a mobile phase containing 0.2%AcA. It is noteworthy that the highest retention was always obtained with BuA and the lowest with TEA except for ofloxacin. The retention of the latter compound, which is the only one that contains a basic alkylamino group in its molecule, seems to be little influenced by the change of the nature of the basic additive in the mobile phase.

Table 3 also shows that BuA seems to be particularly useful for the enantioseparation of the studied profens using 3-Cl-4-MePC as CSP. Even though only partial enantioresolution was observed in most cases, it should be emphasized that indoprofen (R_s 2.6) and carprofen (R_s 3.0) were baseline separated with BuA. Moreover, the complete enantioresolution of ketoralac, proglumide, 2-phenoxypropionic acid and ofloxacin was obtained using any of the three basic additives. Fig. 2B presents the chromatograms of 2-phenylpropionic and 2-phenoxypropionic acids obtained with the three basic additives. As can be seen in this figure, the general trend for retention was observed for both acidic compounds (BuA > DEA > TEA) while the behavior of these two compounds with respect to enantioresolution and selectivity is completely different. The enantioresolution of 2-phenylpropionic acid is strongly influenced by the nature of the basic additive, being almost completely lost in the presence of BuA or TEA. On the contrary, the nature of the basic additive has almost no effect on the enantioseparation of 2-phenoxypropionic acid, a complete resolution of its enantiomers being obtained with the three basic additives, as mentioned above.

With DEA as basic additive in the mobile phase, six compounds (fenoprofen, ibuprofen, ketorolac, ofloxacin, proglumide and 2-phenoxypropionic acid) enantiomers were completely enantioresolved while partial enantioresolution was obtained for flurbiprofen, indoprofen, ketoprofen and 2-phenylpropionic acid. On the contrary, only four chiral acidic compounds (ofloxacin, ketorolac, proglumide and 2-phenoxypropionic acid) were completely resolved when TEA was used as basic additive. The use of TEA did not improve significantly selectivity and resolution compared to DEA since for most chiral compounds resolved using TEA, better enantioresolution values with DEA were obtained. Therefore DEA was selected as basic additive for the optimization step.

Moreover, the concentration of DEA in the mobile phase was also found to influence the enantioseparation of the acidic compounds studied. Indeed, when comparing results obtained with the mobile phase made up of ACN/0.1%DEA/0.2%AcA (Table 1) and those found with that consisting of ACN/0.0.05%DEA/0.2%AcA (Table 3), significant differences in enantioresolution and retention factor can be observed indicating that the proportion of DEA in the mobile phase is an important factor to be optimized.

3.2. Optimization

As demonstrated above, the proportion of the acidic and basic additives in the mobile phase has a significant influence on the enantioresolution and selectivity of acidic compounds using 3-Cl-4-MePC as CSP. The optimization of enantioresolution for these compounds was therefore carried out with two quantitative factors, namely the AcA proportion (0.1–0.3%, v/v) and the DEA proportion (0.01–0.1%, v/v) in the mobile phase.

Of the twelve compounds tested in the screening step, nine were selected for the optimization step. Carprofen and chlorthalidone, which did not show any enantioseparation using a mobile phase made up of ACN/0.05%DEA/0.2%AcA (Table 3), were discarded, as well as indoprofen, the enantioresolution of which was very low under these conditions. However, it is worth recalling that carprofen and indoprofen were completely enantioseparated using AcA and BuA as additives (Table 3) while a good separation of chlorthalidone enantiomers was obtained using TFA and DEA as additives (Table 1).

Table 2
Influence of the acetic acid proportion in the mobile phase on the retention factor of the first enantiomer (k'_1), enantioresolution (R_s) and selectivity (α).

	0.05%			0.1%			0.2%		
	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α
Carprofen	/	/	/	13.2	0.6	1.04	7.6	–	–
Fenoprofen	5.8	2.4	1.17	4.5	2.4	1.18	2.6	2.9	1.19
Flurbiprofen	6.4	–	–	5.0	–	–	3.1	0.60	1.02
Indoprofen	16.1	1.12	1.09	12.3	1.02	1.09	7.0	0.63	1.05
Ibuprofen	6.8	1.75	1.12	5.0	1.64	1.11	2.7	1.77	1.13
Ketoprofen	7.9	0.60	1.01	6.1	0.60	1.04	3.6	0.82	1.07
Ketorolac	/	/	/	10.1	2.5	1.21	7.3	2.4	1.21
Proglumide	/	/	/	17.4	6.2	1.7	10.0	5.7	1.65
2-Phenylpropionic acid	5.3	1.58	1.10	3.9	1.65	1.12	2.1	0.82	1.13
2-Phenoxypropionic acid	7.8	1.74	1.13	6.5	1.01	1.07	4.8	1.72	1.11
Chlorthalidone	1.3	0.60	1.04	1.6	0.69	1.11	1.3	0.60	1.03
Ofloxacin	7.8	6.8	2.8	6.7	7.1	2.5	7.5	8.0	2.3

/: no peak obtained within 60 min; –: $R_s \leq 0.5$.
Mobile phase: ACN/0.1% DEA/0.05–0.2% AcA. For other conditions, see Section 2.

3.2.1. Multivariate approach

The optimization of the selected quantitative factors was performed using a face-centered central composite design (FCCD) with eleven experimental points with one replicate resulting in 22 randomized runs (with three replicates in the center). It was necessary to select three levels for each factor in order to estimate the quadratic effects. For that purpose, a quadratic regression model was applied in order to observe possible quadratic and interaction effects besides the main effects. The model was assumed to be expressed by the following second order polynomial equation:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon$$

where y is the response, enantiomeric resolution or selectivity; β_1 and β_2 (coefficients for linear effects), β_{11} and β_{22} (coefficients for quadratic effects), β_{12} (coefficient for the interaction effect), β_0 (intercept) and ε (error term) are the different coefficients of the model; X_1 and X_2 are the two factors (AcA and DEA proportion in the mobile phase, respectively).

The quality of fit of the model was assessed by the coefficient of determination (R^2), which is the fraction of response variation predicted by the model. All values of R^2 obtained were in the range 0.85–0.99, which demonstrates the quality of fit. The part of the variation of enantioresolution that can be predicted by the model, namely Q^2, always remained higher than 0.69, meaning that at least 69% of the variability of enantioresolution could be predicted.

The p-value is the probability of getting a result as extreme or more extreme than the one observed if the proposed null hypothesis is correct. The effect of a factor is considered as significant if its p-value is lower than 0.05. Here the main effect of the proportion of DEA was found to be significant for all studied compounds (p-values below 0.009 for enantioresolution and 0.013 for selectivity) as well as that of the proportion of AcA (p-values below 0.033 for R_s and 0.038 for α). A significant quadratic effect of DEA concentration was found for all compounds (p-values below 0.009) except for ofloxacin. On the contrary, no significant quadratic effect of AcA concentration was found for any of the tested compounds. However, there is significant interaction effect between the studied factors for all compounds (p-values below 0.015 for enantioresolution and 0.004 for selectivity).

The corresponding response surfaces were plotted in order to obtain a better visualization of the results. The response surface is a three dimensional view that may provide a clear picture of the response (enantioresolution) against the two studied variables (proportions of AcA and DEA in the mobile phase).

Since flurbiprofen, ketoprofen and 2-phenoxypropionic acid exhibited a similar behavior, only the response surface obtained for one compound (flurbiprofen) is presented in Fig. 3A. Fig. 3B illustrates the response surface for ibuprofen, which is comparable to that obtained for fenoprofen, ketorolac, proglumide and 2-phenylpropionic acid, whereas the response surface for the bifunctional compound ofloxacin is depicted in Fig. 3C.

Table 3
Influence of the nature of the basic additive in the mobile phase on the retention factor of the first enantioemer (k'_1), enantioresolution (R_s) and selectivity (α).

	BuA			DEA			TEA		
	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s	α
Carprofen	11.5	3.0	1.26	6.2	–	–	5.6	–	–
Fenoprofen	5.1	0.77	1.06	2.1	2.5	1.20	1.99	1.1	1.09
Flurbiprofen	6.8	1.06	1.08	2.7	0.60	1.05	2.6	1.02	1.08
Indoprofen	13.7	2.6	1.25	5.6	0.60	1.02	4.8	0.65	1.06
Ibuprofen	4.8	0.60	1.04	1.85	1.81	1.15	1.65	0.60	1.05
Ketoprofen	6.9	0.60	1.04	3.0	1.22	1.10	2.9	–	–
Ketorolac	16.4	2.3	1.17	7.7	2.2	1.18	7.4	2.3	1.19
Proglumide	14.8	3.0	1.31	7.5	4.5	1.48	6.2	3.8	1.43
2-Phenylpropionic acid	3.7	–	–	1.65	1.38	1.14	1.39	0.60	1.06
2-Phenoxypropionic acid	9.9	1.64	1.13	5.3	1.79	1.14	4.6	1.81	1.15
Chlorthalidone	1.3	–	–	1.04	–	–	0.99	0.60	1.04
Ofloxacin	8.7	7.9	2.4	9.2	8.5	2.5	10.5	9.2	2.5

–: $R_s \leq 0.5$.
Mobile phase: ACN/0.05% BuA, DEA or TEA/0.2% AcA. For other conditions, see Section 2.

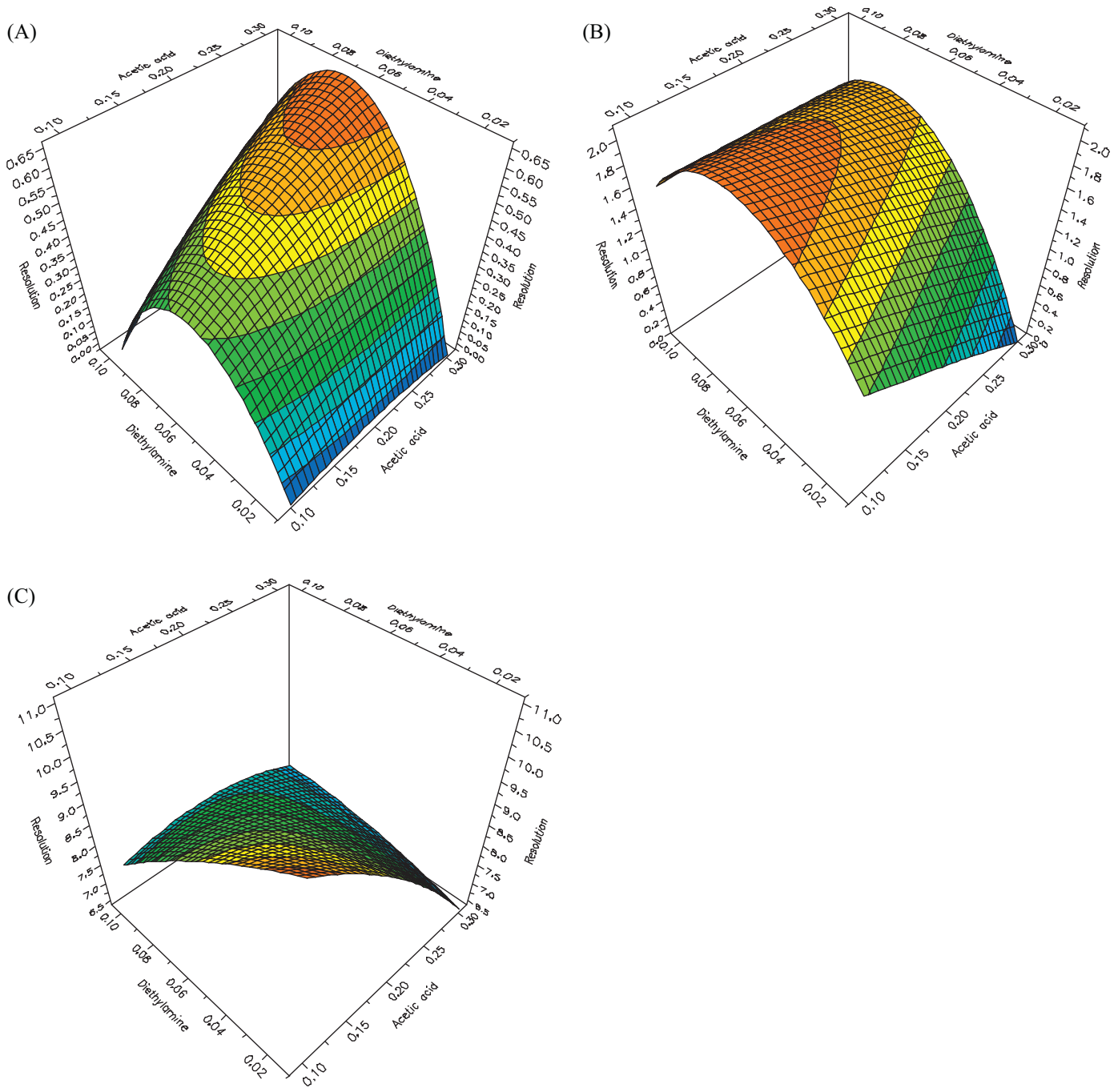

Fig. 3. Response surfaces for: (A) flurbiprofen, (B) ibuprofen and (C) ofloxacin.

As can be seen in Fig. 3A, the DEA concentration in the mobile phase has a quadratic effect on flurbiprofen enantioresolution since the response surface passes through a maximum near 0.07% DEA. On contrary, the AcA concentration in the mobile phase has a fairly linear effect on enantioresolution and an increase of the proportion of this acidic additive has a positive effect. Therefore maximum resolution for flurbiprofen can be obtained with the highest proportion of AcA and nearly 0.07% DEA. A similar tendency is observed for ketoprofen and 2-phenoxypropionic acid.

From the response surface presented in Fig. 3B, it appears clearly that the DEA proportion has also a quadratic effect on the enantioresolution of ibuprofen but this effect is less pronounced compared to that observed in Fig. 3A. However, an increase in AcA concentration has a slightly negative effect on enantioresolution, i.e. an opposite effect compared to that observed with the first group (Fig. 3A). Therefore, it results from the analysis of the response surfaces of the five compounds of this group (ibuprofen, fenoprofen, ketorolac, proglumide and 2-phenylpropionic acid) that maximum enantioresolution values can be obtained using the lowest proportion of AcA and nearly 0.07% DEA. I should be noted, however, that as expected, the use of low AcA concentrations leads to rather high retention times (Tables 2 and 4). For these two groups of compounds the response surfaces for selectivity are similar to those for enantioresolution indicating that changes in R_S values are highly correlated to those in α values.

Concerning ofloxacin, an increase of the DEA concentration in the mobile phase has a negative effect on enantioresolution, as well

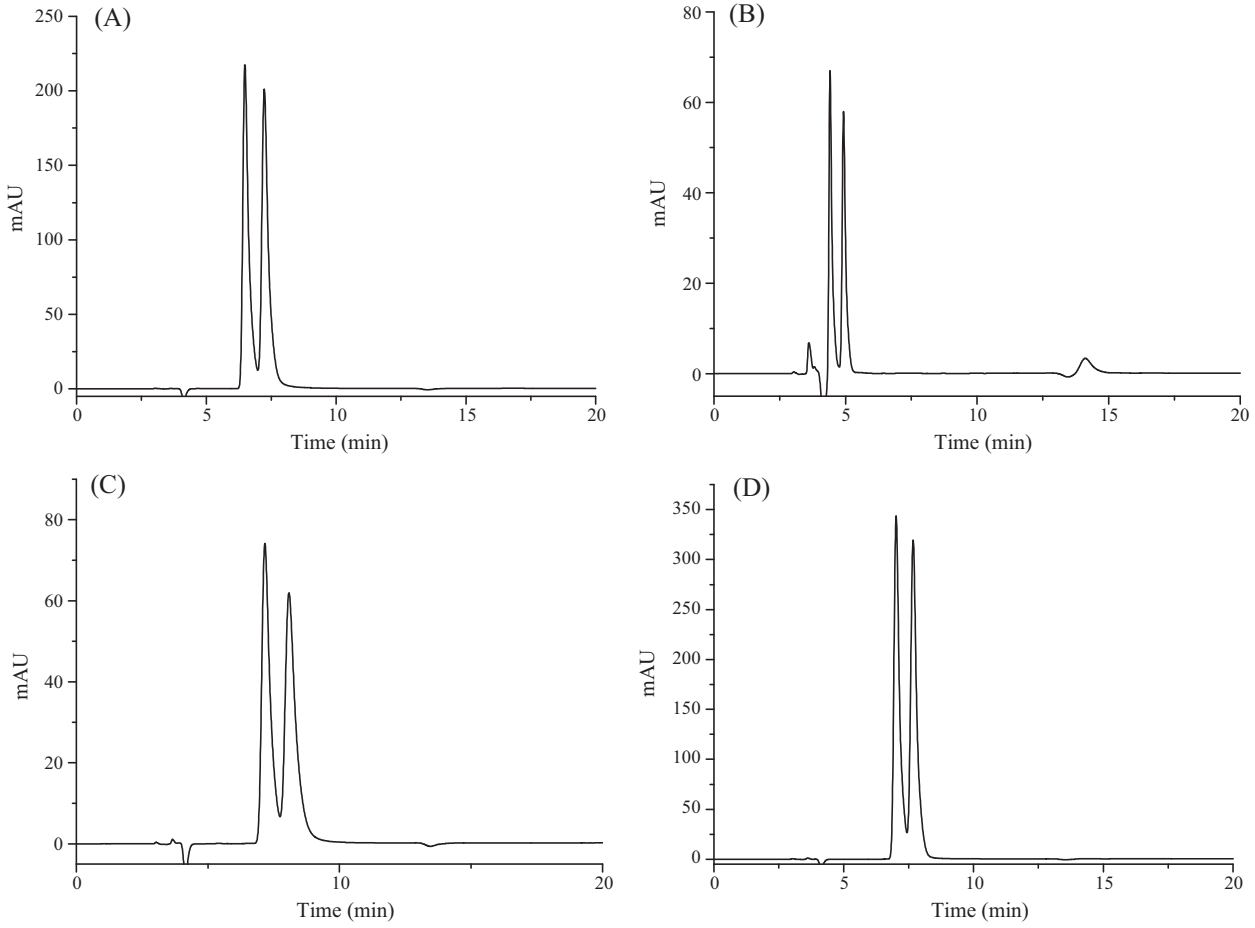

Fig. 4. Chromatograms obtained using the proposed mobile phase for rapid method development: (A) lorazepam; (B) hexobarbital; (C) warfarin; (D) temazepam. Mobile phase: ACN/0.07% DEA/0.2% AcA; temperature: 25 °C. Other conditions: see Section 2.

as an increase in AcA proportion (Fig. 3C). Therefore, the optimum enantioresolution for ofloxacin can be obtained with the lowest proportions of the two additives in the mobile phase. However, the response surface for selectivity is somewhat different from that for enantioresolution but similar in fact to that observed for ibuprofen.

The retention and enantioresolution obtained for the nine tested compounds under optimal chromatographic conditions are given in Table 4. The resolution values observed experimentally were compared to those predicted from the model. Very good agreement was obtained in most cases. Only in the case of ibuprofen and 2-phenylpropionic acid, the enantioresolution values found experimentally were slightly lower than those predicted. This is however not likely to lower significantly the quality of the predictive model.

Only two out of the nine studied compounds (flurbiprofen and ketoprofen) were not baseline enantioseparated using these mobile phases containing AcA and DEA as additives and 3-Cl-4-MePC as CSP. It can be concluded that 3-Cl-4-MePC has high chiral discrimination ability for acidic compounds under these conditions.

3.3. Strategy for rapid method development

Based on the optimal conditions given in Table 4, a single mobile phase can be proposed for rapid development of methods for the enantioseparation of acidic or neutral drugs using 3-Cl-4-MePC, the composition of which is ACN/0.2% AcA/0.07% DEA. A series of six chiral compounds (the last six compounds in Fig. 1), either acidic (clorazepate, warfarin, hexobarbital) or fairly neutral (lorazepam,

Table 4
Retention times, predicted and observed R_s values under the optimal chromatographic conditions.

	Selected conditions	RT (peak 2) (min)	R_s observed	R_s predicted
Fenoprofen		20.4	2.7	2.8 [2.67–2.82]
Ibuprofen		20.5	1.90	2.09 [2.05–2.15]
Ketorolac	ACN/0.1%AcA/0.07%DEA	46.7	2.5	2.56 [2.50–2.62]
2-Phenylpropionic ac.		17.2	1.71	1.89 [1.82–1.98]
Proglumide		98.8	5.6	5.5 [5.27–5.70]
Ofloxacin		64.0	8.4	8.8 [8.26–9.37]
Flurbiprofen		10.4	0.60	0.66 [0.61–0.71]
Ketoprofen	ACN/0.3%AcA/0.07%DEA	11.6	1.10	1.16 [1.06–1.25]
2-Phenoxypropionic ac.		18.9	2.0	2.0 [1.85–2.18]

For other conditions: see Section 2.

Table 5
Retention times and enantioresolution values obtained using the rapid method development strategy.

	Mobile phase	RT (peak 2) (min)	R_s
Clorazepate	ACN/0.07%DEA/0.2%AcA	7.1	–
Warfarin		8.1	1.50
Hexobarbital		4.9	2.5
Lorazepam		7.2	1.85
Lormetazepam		8.0	0.60
Temazepam		7.8	1.72

lormetazepam, temazepam) were tested under these conditions and the results are presented in Table 5. As can be seen in this table, the enantiomers of all compounds except clorazepate were at least partially resolved. Four of them, warfarin, hexobarbital, lorazepam and temazepam, were fairly well enantioresolved confirming the high chiral discrimination ability of 3-Cl-4-MePC and the generic character of the proposed mobile phase. Typical chromatograms showing the enantioseparations of these compounds are presented in Fig. 4.

It is worth noting that very slight structural changes in the 3-hydroxy-1,4-benzodiazepine derivatives can lead to large differences in chiral recognition (Table 5). The methylation of the nitrogen in position 1 on the diazepine ring (lormetazepam compared to lorazepam) suppresses the possibility of hydrogen bonding on this amide group, which might explain the significant decrease in enantioresolution observed for lormetazepam. This nitrogen is also methylated in temazepam but the loss of the electron withdrawing chlorine atom on the benzene ring in position 5 on the diazepine ring (present in lorazepam and in lormetazepam) might have a favorable effect on chiral recognition.

An optimization of AcA and DEA concentrations in the mobile phase would probably lead to a further improvement of enantioresolution for these compounds. Alternative conditions using TFA instead of AcA or BuA instead of DEA as mobile phase additives could also be tested.

4. Conclusion

The resolving power of 3-Cl-4-MePC, a CSP containing cellulose tris(3-chloro-4-methylphenylcarbamate) as chiral selector, was evaluated toward a series of chiral acidic compounds using polar organic mobile phases with acetonitrile as the main component. As already observed in previous studies focused on the enantioseparation of basic compounds, the addition of small amounts of both acidic and basic additives in the mobile phase has a strong impact on retention, enantioresolution, and enantioselectivity and a very fine tuning of the concentration of these additives is essential for such separations. After optimization of the mobile phase composition, the enantiomers of ten out of the twelve acidic compounds tested could be baseline separated. Partial enantioresolution could however be obtained for the two other compounds in these LC systems, clearly showing the very good chiral discrimination ability of this CSP for acidic compounds using this kind of mobile phases. Finally, the results of the optimization process suggested that a single mobile phase consisting of ACN/0.2%AcA/0.07%DEA could be proposed for rapid development of enantioselective methods for chiral acidic and neutral compounds using 3-Cl-4-MePC as CSP. This generic approach was tested on a few chiral drugs and the results are promising. It is obvious, however, that a much larger number of compounds should be examined in order to confirm the usefulness of this approach.

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