Effect of Basic and Acidic Additives on the Separation of Some Basic Drug Enantiomers on Polysaccharide-Based Chiral Columns With Acetonitrile as Mobile Phase

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ABSTRACT The separation of enantiomers of 16 basic drugs was studied using polysaccharide-based chiral selectors and acetonitrile as mobile phase with emphasis on the role of basic and acidic additives on the separation and elution order of enantiomers. Out of the studied chiral selectors, amylose phenylcarbamate-based ones more often showed a chiral recognition ability compared to cellulose phenylcarbamate derivatives. An interesting effect was observed with formic acid as additive on enantiomer resolution and enantiomer elution order for some basic drugs. Thus, for instance, the enantioseparation of several β-blockers (atenolol, sotalol, toliprolol) improved not only by the addition of a more conventional basic additive to the mobile phase, but also by the addition of an acidic additive. Moreover, an opposite elution order of enantiomers was observed depending on the nature of the additive (basic or acidic) in the mobile phase. *Chirality 27:228–234, 2015.*

KEY WORDS: separation of enantiomers; polysaccharide-based chiral selectors; basic chiral drugs; enantiomer elution order; effect of minor additives

INTRODUCTION

Polysaccharide-based chiral selectors (columns) are established as the most useful materials for analytical- and preparative-scale separation of enantiomers in liquid chromatography[1,2] and several related techniques, such as super-/subcritical fluid chromatography,[3] nano-liquid chromatography,[4,5] and capillary electrochromatography.[5–7] In spite of the wide application of polysaccharide phenylcarbamates and esters in liquid-phase separation of enantiomers, the chiral recognition mechanism of these materials is still poorly understood. Although many efforts involving various experimental[8–14] and computation techniques[14–16] have been made in the past, at present we are still far from being able to develop a tailor-made chiral selector for the separation of the enantiomers of a given chiral analyte, or from predicting the most effective separation mode or mobile phase (including its additives), not to mention from predicting the enantiomer elution order (EEO).

Polysaccharide phenylcarbamate-based chiral selectors were initially proposed for the separation of enantiomers in high-performance liquid chromatography (HPLC) in combination with hydrocarbon-alcohol mobile phases. In the very first article on this topic published 30 years ago, Okamoto and colleagues[17] reported on separations conducted in a water-ethanol mixture as a mobile phase but primarily emphasized the usefulness of hydrocarbon-alcohol-made mobile phases, thus assuming that hydrogen bonding between the chiral selector and chiral analytes to be the most important contributor to chiral recognition. Initially, polysaccharide phenylcarbamate-based chiral selectors were not recommended to be used in combination with pure polar organic solvents (such as alcohols or acetonitrile) as the mobile phase, although an earlier article on the application of cellulose triacetate as a useful chiral selector for liquid chromatographic separation of enantiomers reported the successful use of pure ethanol as mobile phase.[18] A few articles have been published on the application of polysaccharide phenylcarbamates for HPLC separation of enantiomers in combination with some alcohols in the 1980s and 1990s.[19–21] However, these studies do not stress any potential advantages of polar-organic mobile phases for HPLC separation of enantiomers. More systematic studies in this area were published since early 2000 and at present the polar-organic mobile phase mode (PO) has been well established for analytical, as well as for preparative-scale enantioseparations.[22–33] Major advantages of this separation mode include short analysis times, high plate numbers, favorable peak shape, and commonly higher solubility of the analyte in the mobile phase. The last advantage is important in preparative-scale separations of enantiomers. The potential of pure acetonitrile,[34] acetonitrile with minor basic or acidic additives,[24–26,29,34] or acetonitrile in mixture with hydrocarbons (e.g., n-hexane)[27,28,30–33] as a mobile phase for HPLC separations of enantiomers using polysaccharide-based chiral selectors has been evaluated in several studies. Interesting effects of basic and acidic additives in this separation mode were reported for some basic drugs in our previous study.[34]

In the present study the separation of enantiomers of 16 basic drugs was investigated on six different polysaccharide-type chiral columns and acetonitrile as the bulk mobile phase. The emphasis was on the effect of minor basic and acidic

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additives present in the mobile phase on the resolution and elution order of enantiomers.

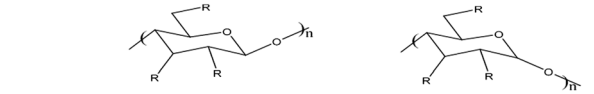
EXPERIMENTAL

Pharmaceuticals, Chemicals, and Chiral Columns

Diethylamine, formic acid, and commercially available chiral basic drugs, acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, bupranolol hydrochloride, bunitrolol hydrochloride, celiprolol, isoproterenol, mabuterol, metoprolol, metipranolol, nifenalol, oxprenolol, sotalol, and toliprolol were purchased from Sigma Aldrich (Taufkirchen, Germany). The structures of these compounds are shown in Figure 1. Enantiomerically pure fractions of each studied drug, if not commercially available, were collected by using analytical columns and multiple sequential injections of the racemate and manual collection of effluents corresponding to each enantiomer. The eluent was evaporated at room temperature and the enantiomerically pure residue used for spiking experiments. Acetonitrile of HPLC quality was acquired from Carl Roth (Karlsruhe, Germany). The amylose tris(3,5-dimethylphenylcarbamate) (ADMPC)-based column was an experimental column provided by Enantiosep (Münster, Germany), while the other chiral columns, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, and Lux Cellulose-4, were kindly provided by Phenomenex (Torrance, CA). The schematic structures of the chiral selectors of these chiral columns are shown in Figure 2. All columns had the dimensions 250 x 4.6 mm and were packed with 5-μm particles.

HPLC Separations

All HPLC experiments were performed with an Agilent 1200 HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a G1367C HiP ALS-SL autosampler, a G1316B TCC-SL temperature controller, a G1311A quaternary pump, and a G1314D VWD variable wavelength detector. The Chemstation software (v. B.03.02-SR2) was used for instrument control, data acquisition, and data handling. If not stated otherwise, the samples were dissolved in the mobile phase used for the respective separation at a concentration of 0.2 mg/mL. HPLC separations were performed at 20°C with 1.00 ml/min mobile phase flow rate and detection at 220 nm. The elution order of enantiomers was determined in each case by spiking racemic sample with enantiomerically pure isomers and/or by correlation with previous studies. Diethylamine (DEA) additive to the mobile phase was added in 0.1% amount (v/v) and formic acid (FA) in its equivalent amount to 0.1% of DEA.



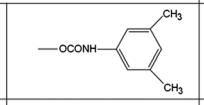
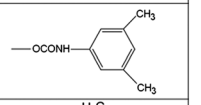
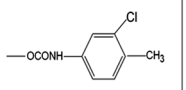
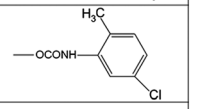
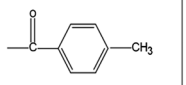
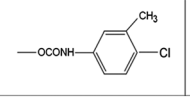
Cellulose Derivatives, R=		Amylose Derivatives, R=	
Cellulose-1		ADMPC	
Cellulose-2		Amylose-2	
Cellulose-3			
Cellulose-4			

Fig. 2. The schematic structures of the chiral selectors part of the chiral columns used in this study.

RESULTS AND DISCUSSION

General Overview of Separation of Basic Drug Enantiomers

An interesting tendency was observed when screening six polysaccharide-based chiral columns for their separation ability towards the enantiomers of 16 chiral basic drugs (Table 1). Thus, both amylose-based columns, Lux Amylose-2, and ADMPC were more successful than cellulose-based columns (Table 1, Fig. 3). Based on their suitability these columns ranked in the order Lux Amylose-2>ADMPC>Lux Cellulose-2=Lux Cellulose-4>Lux Cellulose-1. No separation of enantiomers was observed on the cellulose ester type column, Lux Cellulose-3. The above-mentioned higher success rate of amylose-based columns compared to cellulose-based columns with ACN as the mobile phase is in agreement with the results obtained on a larger set of chiral analytes earlier.[25,26,29] The two cellulose-based chiral columns with very

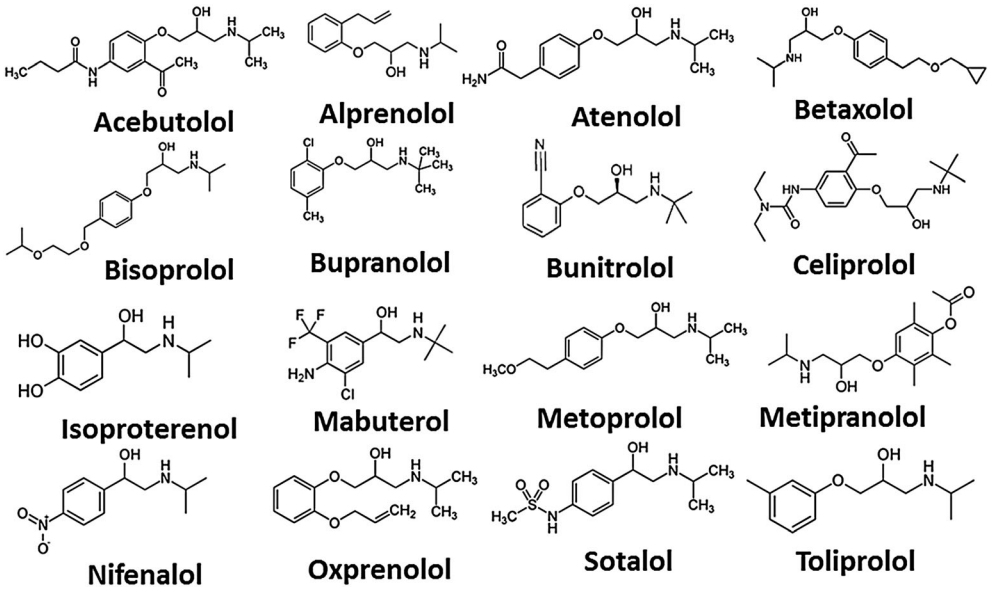


Fig. 1. Structure of studied basic drugs.

TABLE 1. Retention factor of the first peak (k′) and separation factor of enantiomers (α) of 16 basic analytes on 6 polysaccharide-based chiral columns in acetonitrile and acetonitrile +0.1% diethylamine (v/v) as mobile phases

	Cellulose-1				Cellulose-2				Cellulose-3				Cellulose-4				Amylose-2				ADMPC			
	ACN		ACN +DEA		ACN		ACN +DEA		ACN		ACN +DEA		ACN		ACN +DEA		ACN		ACN +DEA		ACN		ACN +DEA	
Chiral Analyte	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α	k_1'	α
Acebutolol	0.60	1.00	0.72	1.00	1.03	2.99	1.16	3.21	0.32	1.00	0.21	1.00	2.66	1.00	2.79	1.00	0.89	1.00	1.73	2.37	2.15	1.00	1.67	1.39
Alprenolol	0.59	1.00	0.63	1.00	0.94	1.00	0.98	1.00	0.25	1.00	0.23	1.00	2.67	1.00	2.72	1.00	0.78	1.00	0.79	1.13	2.09	1.00	2.11	1.00
Atenolol	0.70	1.00	0.74	1.00	039	1.00	1.05	1.00	0.23	1.00	0.23	1.00	2.81	1.00	2.83	1.00	4.60	1.12	1.51	1.33	2.62	1.00	3.80	1.18
Betaxolol	0.80	1.00	1.74	1.29	1.10	1.00	1.31	1.00	0.29	1.00	0.24	1.00	2.55	1.00	3.25	1.04	0.91	1.00	1.04	1.39	2.10	1.00	2.13	1.00
Bisoprolol	1.10	1.00	1.73	1.10	1.40	1.00	1.44	1.00	0.23	1.00	0.27	1.00	2.45	1.00	3.06	1.00	0.86	1.00	0.94	1.00	1.89	1.00	1.78	1.00
Bupranolol	1.10	1.00	1.12	1.00	1.04	1.00	1.00	1.00	0.23	1.00	0.28	1.00	2.55	1.00	3.05	1.00	0.67	1.00	0.72	1.00	1.02	1.91	2.04	1.79
Bunitrolol	0.80	1.00	0.70	1.00	1.08	1.00	1.00	1.00	0.24	1.00	0.23	1.00	2.56	1.00	3.32	1.00	0.71	1.00	0.73	1.00	2.12	1.00	2.14	1.00
Celiprolol	0.60	1.00	0.80	1.00	0.82	1.00	0.87	1.00	0.23	1.00	0.21	1.00	3.01	1.00	1.04	1.66	0.82	1.00	0.85	1.00	2.13	1.00	2.18	1.00
Isoproterenol	1.10	1.00	0.78	1.00	0.76	1.00	0.77	1.00	0.24	1.00	0.23	1.00	2.74	1.00	2.81	1.00	0.84	1.00	0.91	1.00	1.44	1.00	1.56	1.00
Mabuterol	0.86	1.00	0.74	1.00	0.23	1.67	0.67	1.98	0.21	1.00	0.22	1.00	2.54	1.00	2.77	1.00	0.81	1.00	1.12	1.00	1.79	1.00	1.77	1.00
Metoprolol	0.91	1.00	1.87	1.15	0.78	1.00	0.56	1.00	0.27	1.00	0.24	1.00	2.56	1.00	2.72	1.00	0.86	1.00	0.98	1.00	1.51	1.00	1.55	1.00
Metipranolol	0.78	1.00	0.93	1.00	0.81	1.00	0.77	1.00	0.22	1.00	0.23	1.00	2.63	1.00	2.42	1.05	0.91	1.00	0.83	1.00	1.53	1.00	1.56	1.00
Nifenalol	0.50	1.00	0.51	1.00	1.35	1.00	1.16	1.00	0.21	1.00	0.21	1.00	2.63	1.00	1.66	1.00	0.66	1.00	0.69	1.00	1.56	1.00	1.52	1.00
Oxprenalol	0.54	1.00	0.64	1.00	0.68	1.00	0.69	1.00	0.24	1.00	0.22	1.00	2.78	1.00	2.39	1.07	0.66	1.00	0.67	1.00	1.33	1.00	1.66	1.00
Sotalol	0.82	1.00	1.98	1.00	1.33	1.00	0.87	1.54	0.23	1.00	0.21	1.00	2.83	1.00	2.85	1.00	0.63	1.37	1.05	1.46	0.89	1.00	1.08	1.47
Toliprolol	0.89	1.00	1.82	1.00	1.56	1.00	0.87	1.22	0.23	1.00	0.21	1.00	2.79	1.00	2.83	1.00	0.67	1.00	1.05	1.37	0.92	1.00	1.11	1.51

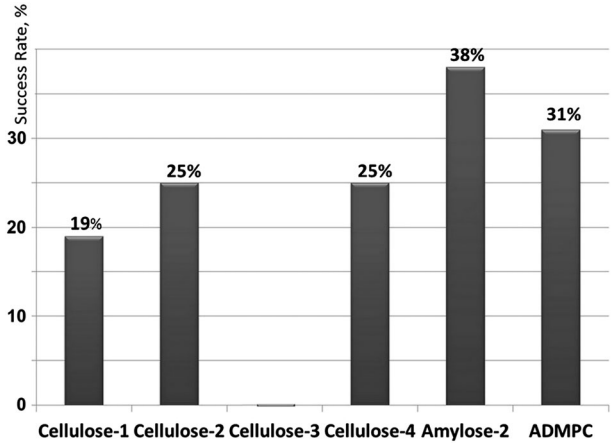


Fig. 3. Success rate of separation of basic drug enantiomers on various chiral columns with ACN+0.1%DEA as the mobile phase.

similar chiral selector chemistry, namely Lux Cellulose-2 and Lux Cellulose-4, exhibited similar overall success but were quite complementary to each other. Thus, the enantiomers of acebutolol and mabuterol were well resolved on Lux Cellulose-2 but not resolved at all on Lux Cellulose-4. Contray to this, the enantiomers of betaxolol, celiprolol, metipranolol, and oxprenolol were not resolved on Lux Cellulose-2 column but baseline or partially resolved on Lux Cellulose-4 column (Table 1). Out of the various mobile phases used, ACN with 0.1% DEA was most successful with all columns studied (Table 1). The success rate with ACN+FA and ACN+DEA +FA was studied with Lux Amylose-2 and ADMPC and was very similar with both chiral selectors (data not shown). DEA was more successful as an additive compared to FA. No complementarity was observed between these two additives, i.e., no new separation was observed with FA as additive. However, some difference was observed in terms of EEO, which will be discussed in the next subsection.

Effect of Chiral Selector and Basic and Acidic Additive in the Mobile Phase on the Enantiomer Elution Order of Chiral Basic Analytes

EEO represents an important aspect of chiral separations from both a practical and theoretical viewpoint. Understanding

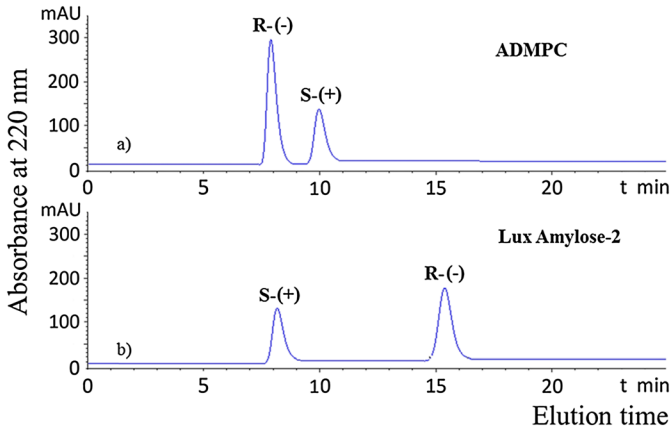


Fig. 4. Separation of enantiomers of acebutolol (1:2 ratio of S- and R-enantiomers, respectively) on ADMPC (**a**) and Lux Amylose-2 (**b**) columns. The mobile phase was ACN+0.1%DEA.

of the molecular mechanisms of EEO reversal may bring us closer to an understanding of chiral recognition mechanisms. It has been well established that EEO may be the opposite with various polysaccharide-based chiral columns.[1,33,34] However, molecular mechanisms bringing about reversals in EEO are currently not well understood. Therefore, as many as possible examples of this kind of EEO reversal must be accumulated and discussed systematically in order to get a clear idea about the origin of this phenomenon, which definitely may be different from case to case. As mentioned in the previous subsection, amylose-based columns exhibited a chiral recognition ability in more cases than cellulose-based ones for the chiral basic drugs part of this study in combination with ACN as mobile phase. In order to follow EEO, the R-(−) and S-(+)-enantiomers of sotalol were available for our experiments. The stereochemical configuration of each eluted fraction of acebutolol was assigned based on correlations with the elution order of its enantiomers described in Ref. 35. Similarly, the absolute stereochemical configuration of each eluted fraction of atenolol was assigned based on the elution order reported in the paper by Agustian et al.[36] Such a correlation was not possible for toliprolol enantiomers due to the absence (at least to our knowledge) of any earlier HPLC publication on the separation of toliprolol enantiomers with a reliable description of experimental conditions and a clear determination of EEO.

An interesting example of reversal in EEO was observed for the enantiomers of acebutolol between Amylose-2 and ADMPC columns (Fig. 4). Thus, R-acebutolol eluted as the first peak off the ADMPC column while second off the

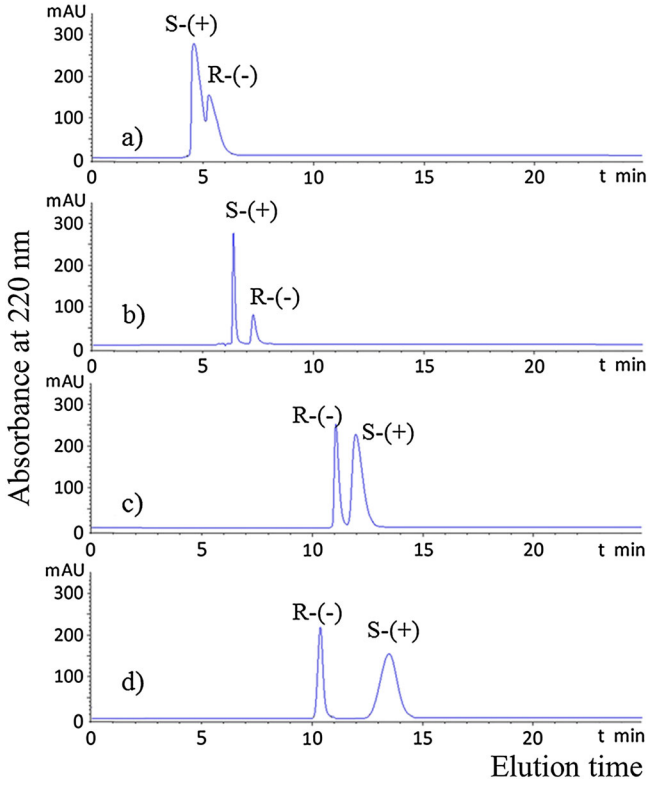


Fig. 5. Separation of enantiomers of sotalol (1:2 ratio of R- and S-enantiomers, respectively) on Lux Amylose-2 column with the following mobile phases: ACN (**a**), ACN+0.1% DEA (**b**), ACN+ eq. FA (**c**), and ACN+0.1% DEA+ eq. FA (**d**).

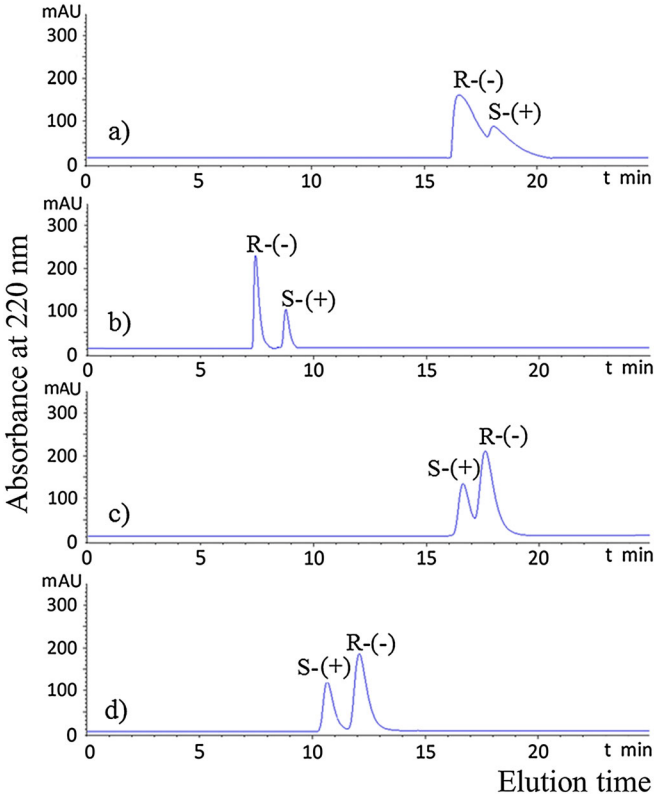


Fig. 6. Separation of enantiomers of atenolol (1:2 ratio of S- and R-enantiomers, respectively) on Lux Amylose-2 column with the following mobile phases: ACN (**a**), ACN+0.1% DEA (**b**), ACN+ eq. FA (**c**), and ACN+0.1% DEA+ eq. FA (**d**).

Amylose-2 column. Also, enantioresolution was significantly better with the latter column. The EEO of sotalol enantiomers was again different on Amylose-2 and ADMPC columns, with S-(+)-sotalol eluting first off Lux Amylose-2 and second off ADMPC with ACN + 0.1% FA mobile phase (data not shown). Reversal in EEO based on the choice of chiral selector is a useful approach but lacks expediency, first because it is not predictable and, second because it requires at least two chiral columns. From this viewpoint bringing about a reversal in EEO by varying the mobile phase composition seems more cost-effective. Although, this latter approach to reversing EEO is also unpredictable because of our limited understanding of chiral recognition mechanisms with polysaccharide-based chiral selectors, at least it requires no more than a single column. Several examples of EEO reversal based on the nature of the mobile phase additive were observed in the current study. For example, the separation of sotalol enantiomers was unsatisfactory on Lux Amylose-2 with ACN as the mobile phase without any additive (Fig. 5a). This separation improved significantly when 0.1% (v/v) of DEA was added to the mobile phase, with S-(+)-sotalol eluting before R-(−)-sotalol (Fig. 5b). Surprisingly, the separation of sotalol enantiomers was also possible when the acidic additive FA was present instead of DEA in mobile phase ACN (at the concentration level equivalent to 0.1% DEA; Fig. 5c). Even more surprisingly, the EEO inverted and R-(−)-sotalol eluted before S-(+)-sotalol when FA was used. This is one of the very few examples reported in the literature when the EEO can be adjusted based on the nature of the mobile phase additive. Although the improvement in enantiomer separation of basic chiral analytes with the use of acidic additives, or of a combination of acidic and basic additives, has been reported previously,[33,37–39] reversals in EEO based on the alternative use of basic or acidic additives was reported for the first time in our recent study.[34] Interestingly, the resolution of sotalol enantiomers with acidic additive or with a combination of acidic and basic additives was superior to that with basic additive only (Fig. 5d). The significantly different peak shape of S-(+)-sotalol in the presence of an acidic additive or a combination of acidic and basic additives in the mobile phase also deserves attention and is the subject of further studies.

Similar to the above-mentioned case of sotalol enantiomers, unsatisfactory separation was obtained for the enantiomers of atenolol on Amylose-2, again when ACN was used as a mobile phase without any additive (Fig. 6a). While the separation was improved, the EEO was not affected when 0.1% DEA was added to ACN (Fig. 6b). Again, the separation was also improved and the EEO reverted when FA was added instead of DEA to the ACN mobile phase (Fig. 6c). This is an additional example of EEO adjustment based on the alternative use of basic or acidic additives. In both examples discussed, the presence of FA was the determining factor for EEO: it was the same with only FA or with both FA and DEA present in the ACN mobile phase. The exception from this pattern was the case of toliprolol enantiomers. The EEO was opposite for this analyte on the Amylose-2 column when the two additives, DEA and FA, were used alternatively (Fig. 7a,c), but no separation of enantiomers was observed when both of these two additives were used simultaneously (Fig. 7b). Significant widening of the second peak was observed along with EEO reversal whenever FA was used as additive for all three analytes shown in Figures 5–7.

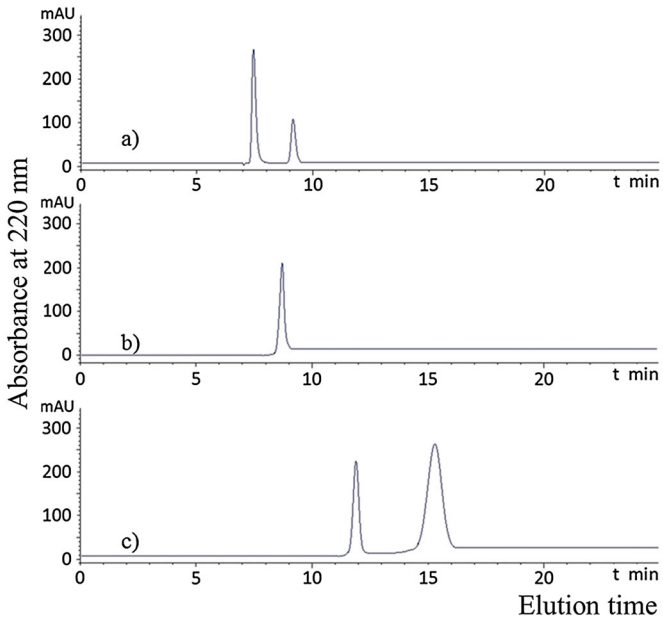


Fig. 7. Separation of enantiomers of toliprolol (1:2 ratio of enantiomers) on Lux Amylose-2 column with the following mobile phases: ACN+0.1% DEA (**a**), ACN+0.1% DEA+ eq. FA (**b**), and ACN+ eq. FA (**c**).

CONCLUSION

Based on a study on the enantioseparation of 16 basic drugs on 4 cellulose- and 2 amylose-based chiral HPLC columns with acetonitrile as mobile phase, the enantiomer-resolving ability of amylose-based columns was found to be better than that of cellulose-based columns. In addition, some interesting examples of reversal in enantiomer elution order were observed as a function of the chemistry of the chiral selector and the nature (basic or acidic) of the mobile phase additive. Of special significance seems to be the possibility of enantiomer elution order adjustment for atenolol, sotalol, and toliprolol by alternative use of diethylamine or formic acid as additives.

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