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Chiral separation of amines in subcritical fluid chromatography using polysaccharide stationary phases and acidic additives

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Abstract

The chiral separation of basic compounds by subcritical fluid chromatography (SFC) is often unsuccessful, due possibly to multiple interactions of the analyte with the mobile and stationary phase. Incorporation of a strong acid, ethanesulfonic acid (ESA), into the sample diluent and mobile phase modifier gives a dramatic improvement in these separations. Screening with ethanol containing 0.1% ESA on CHIRALPAK® AD-H gave separation of 36 of 45 basic compounds previously not separated in SFC. The mechanism appears to involve the separation of an intact salt pair formed between the basic compound and ESA. Other modifiers, other acids and one additional stationary phase were examined and found to yield additional separations.

Keywords: Chiral separation; Amines; Subcritical fluid chromatography

1. Introduction

Recent work [1–4] has shown mobile phase additives used to improve peak shapes in chiral HPLC may also affect enantioselectivity on polysaccharide chiral stationary phases (CSPs). An examination of the effects of various acidic additives on the separation of phenylalanine analogs indicated the involvement of both ion suppression and ion pair formation effects [1]. Separations of phenylalanine analogs with free amine functionalities [2] were altered by the inclusion of amine additives. In many cases, additives gave slight increases in selectivity through a larger decrease in retention of the first eluting enantiomer than of the second. Decreased retention is viewed as arising from competition for binding opportunities between the amine additive and the analytes. There were also observations of increased retention in response to inclusion of cyclic alkyl amine additives, often giving dramatic increases in selectivity. The size and shape of the additive strongly influenced the retention increase, leading to the suggestion that the amine was preventing access of modifier seeking to displace tightly bound enantiomer. This observation has been recently extended to subcritical fluid chromatography (SFC) [5].

Acidic mobile phase additives are required to achieve elution of acidic analytes from polysaccharide CSPs in HPLC. These additives are not required in SFC, which is usually attributed to the "acidic" nature of carbon dioxide. It is worth noting that a protic modifier is required and that inclusion of an amine additive prevents elution of acidic analytes. These results corroborate an acid–base equilibrium in SFC mobile phases whereby the acidity of carbon dioxide is sufficient to transfer a proton from the alcohol modifier to the acidic analyte. An amine additive is basic enough to prevent this transfer.

Amine additives have been used in SFC occasionally with the intent of improving peak shape [6–9] of amine analytes. The common interpretation is that amine additives mask silanols that contribute to non-specific retention of such amines. Diminishing non-specific interactions would decrease retention but should also increase observed selectivity. Amine additives would also be expected to compete with amine analytes for specific binding sites giving decreased retention but mixed effects on selectivity. This is the typical

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observation for a broad range of amine analytes [9]. Admittedly, amine additives have not been examined in depth in SFC. This may be due to the relative lack of success of the technique with amine analytes. Amines often fail to elute, or give peaks so distorted that optimization is not attempted.

Poor peak shapes for amines in SFC may be attributed to the possibility of carbon dioxide forming transient complexes with amine groups [7,10–13]. The formation of such complexes has been proffered as an explanation for different selectivity for amine analytes between SFC and HPLC. Spectroscopic evidence [10,12] is compelling. The acid–base equilibria in carbon dioxide should also be considered. It is possible that distorted amine peaks arise in SFC from a protonation–deprotonation equilibrium induced by the acidic nature of the mobile phase. Addition of an amine additive could force deprotonation and improved peak shape would result from simplification of the equilibrium. It is unlikely that the effects of amine additives can be interpreted this simply. Primary, secondary and tertiary amines would be expected to have different effects on this equilibrium. This is rarely observed to be true [9].

The protonation–deprotonation equilibrium of amine additives might also be simplified by addition of acidic additive. A recent report [3] described increased retention and enantioselectivity for amino acid esters in HPLC arising from the incorporation of ethanesulfonic acid (ESA) into the mobile phase. This effect was attributed to incorporation of the additive into the stationary phase creating additional interaction sites for the underivatized amino group. This work describes the effect of alkylsulfonic acids on chiral separations of amine compounds in SFC.

2. Experimental

2.1. Reagents

All reagents used in this study were reagent grade or better. Probe molecules and acid additives were obtained from Sigma-Aldrich (St. Louis, MO). Ethanol was obtained from J.T. Baker (Phillipsburg, NJ) and methanol and 2-propanol were from Pharmco (Brookfield, CT). Probe samples were dissolved at ∼2 mg/mL in ethanol containing 0.1% additive.

Table 1
SFC screening results on a CHIRALPAK® AD-H using 20% ethanol containing 0.1% ESA

Compound	Class	*t*1	*t*2	*α*	Rs
Tyrosine-methyl ester	Amino acid ester	3.00	15.7	9.44	13.3
Leucine-benzyl ester	Amino acid ester	2.32	2.71	1.54	2.68
Phenylalanine-methyl ester	Amino acid ester	2.29	4.87	4.26	8.64
Phenylalanine	Amino acid	2.20	2.74	1.77	3.13
Proline	Amino acid	2.09	2.34	1.60	1.38
Tyrosine	Amino acid	2.47	3.75	1.50	4.74
2-Phenylglycine	Amino acid	2.52	2.83	1.30	1.80
Metoprolol	β-Blocker	4.15	4.65	1.19	1.85
Atenolol	β-Blocker	10.2	13.2	1.34	4.59
Alprenolol	β-Blocker	2.77	3.12	1.27	2.40
2-Amino-3-phenyl-1-propanol	1° amine	3.21	3.55	1.20	1.71
α-Methylbenzylamine	1° amine	4.78	5.43	1.20	1.80
Chloramphetamine	1° amine	3.01	3.72	1.47	3.99
2-Amino-1-phenylethanol	1° amine	6.18	6.59	1.09	1.21
Norephedrine	1° amine	3.05	3.42	1.24	2.07
Tranylcypromine	1° amine	2.77	3.31	1.42	3.35
Octopamine	1° amine	7.25	9.51	1.39	3.80
Baclofen (25% modifier)	1° amine, acid	2.90	5.62	2.93	7.31
Ephedrine	2° amine	3.12	3.40	1.18	1.43
Epinephrine	2° amine	7.00	8.36	1.25	2.35
Ketamine	2° amine	3.26	4.24	1.56	4.93
Fluoxetine	2° amine	2.29	2.40	1.14	0.91
Terbutaline	2° amine	3.81	4.44	1.27	1.81
FTMQ[a]	2° amine	3.14	3.23	1.05	0.72
Nomifensine	2°, 3° amine	3.71	5.03	1.60	1.49
Nicardipine	2°, 3° amine	8.27	9.22	1.14	1.31
Bupivacaine	3° amine	2.33	2.83	1.61	1.09
Atropine	3° amine	8.62	9.43	1.11	1.61
Homatropine	3° amine	10.6	15.8	1.57	8.68
Laudanosine	3° amine	4.76	4.93	1.05	0.77
Tolperisone	3° amine	3.52	4.14	1.31	3.20
Phenoxybenzamine	3° amine	8.17	13.1	1.74	8.71
Trimebutine	3° amine	5.78	6.82	1.24	2.82
Trihexyphenidyl	3° amine	5.48	6.02	1.13	1.62
Promethazine	di-3° amine	8.52	9.29	1.11	1.95
Trimipramine	di-3° amine	5.61	6.13	1.13	1.85

[a] 6-Fluoro-1,2,3,4-tetrahydro-2-methylquiniline.

2.2. Chromatography

Chromatographic studies were performed on Berger supercritical fluid chromatographs (Berger, Newark, DE) equipped with autosampler, thermostated-column device and a variable-wavelength UV detector. Retention factors, selectivity and resolution values were calculated by the supplied Berger software using USP definitions. The void volume was taken to be 3.0 mL which has been found to be consistent for these columns. CHIRALPAK® AD-H® and CHIRALCEL® OD-H® columns (250 × 4.6 mm) were packed at Chiral Technologies (Exton, PA). Chromatographic screening studies were performed at room temperature with a 2.0 mL/min flow rate, 180 bar back pressure, 20% ethanol modifier containing 0.1% ESA additive. Alternative conditions are described in the text.

3. Results and discussion

Amino acid esters, the first samples tested with these conditions, were found to be very well separated. This initial success led to the testing of a much wider variety of basic analytes, which had not been previously separated in SFC. Results of screening with 20% ethanol containing 0.1% ESA are given in Table 1. Of 45 basic compounds screened some separation was observed for 36, including amino acids, amino acid esters, β-blockers, and 1°, 2° and 3° amines. Compounds not showing separation (tryptophan, DOPA, propanolol, oxyprenolol, napropamide, haloperidol, chlorpheniramine, methocarbamol, ethopropazine) were similarly diverse. Baseline resolution was obtained for 30 probes with these screening conditions. Fig. 1 shows an under 4 min separation of chloramphetamine hydrochloride with a resolution of 3.99, compared to the single tailing peak observed without additive. Phenoxybenzamine, a tertiary amine shows a resolution of 8.71 in Fig. 2, and phenylalanine methyl ester is separated to a resolution of 8.64 in slightly more than 5 min (Fig. 3). In these figures a possibly split distorted peak is observed without additive. Changing the amount of modifier changes retention times as expected with very little effect on selectivity. Decreasing the ethanol level to 15% gave baseline separation of proline and bupivacaine. Nadolol, a β-blocker expected to give four stereoisomers showed two peaks in initial screening. Increasing modifier level to 30% gave the separation shown in Fig. 4. All four isomers are resolved in

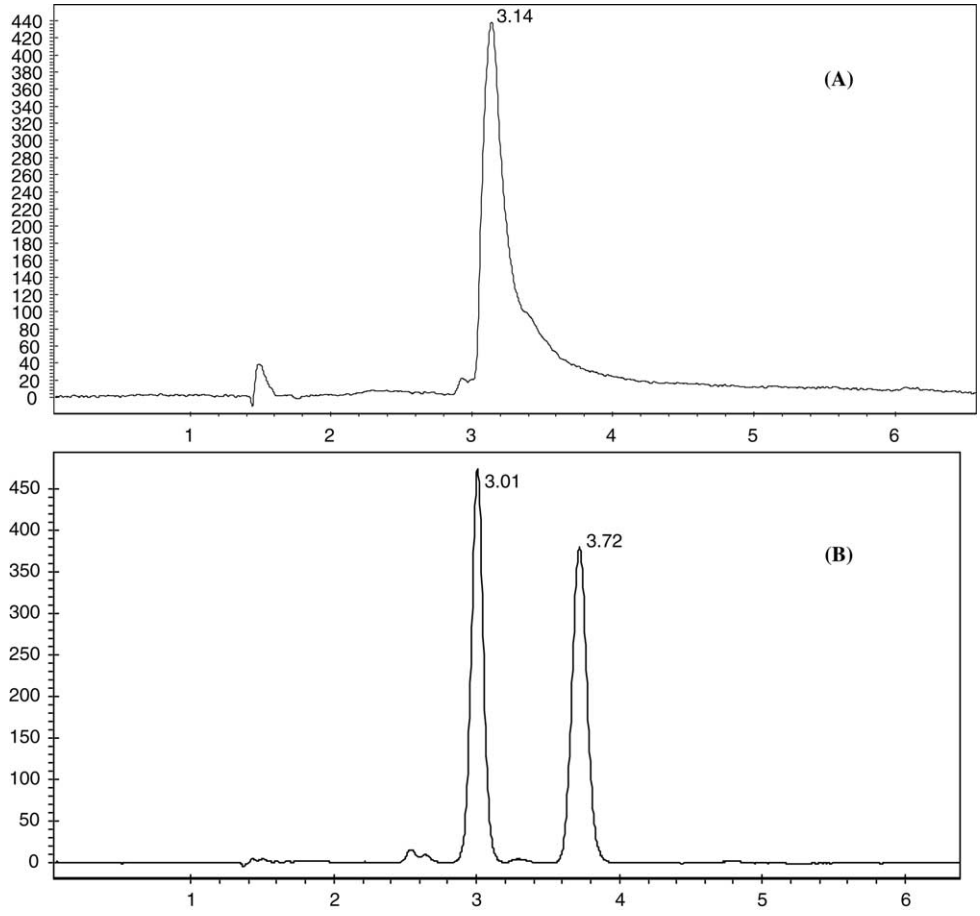

Fig. 1. SFC chromatogram of chloramphetamine hydrochloride salt on a CHIRALPAK® AD-H column using (A) 20% ethanol and (B) 20% ethanol containing 0.1% ESA modifier. Sample dissolved in modifier.

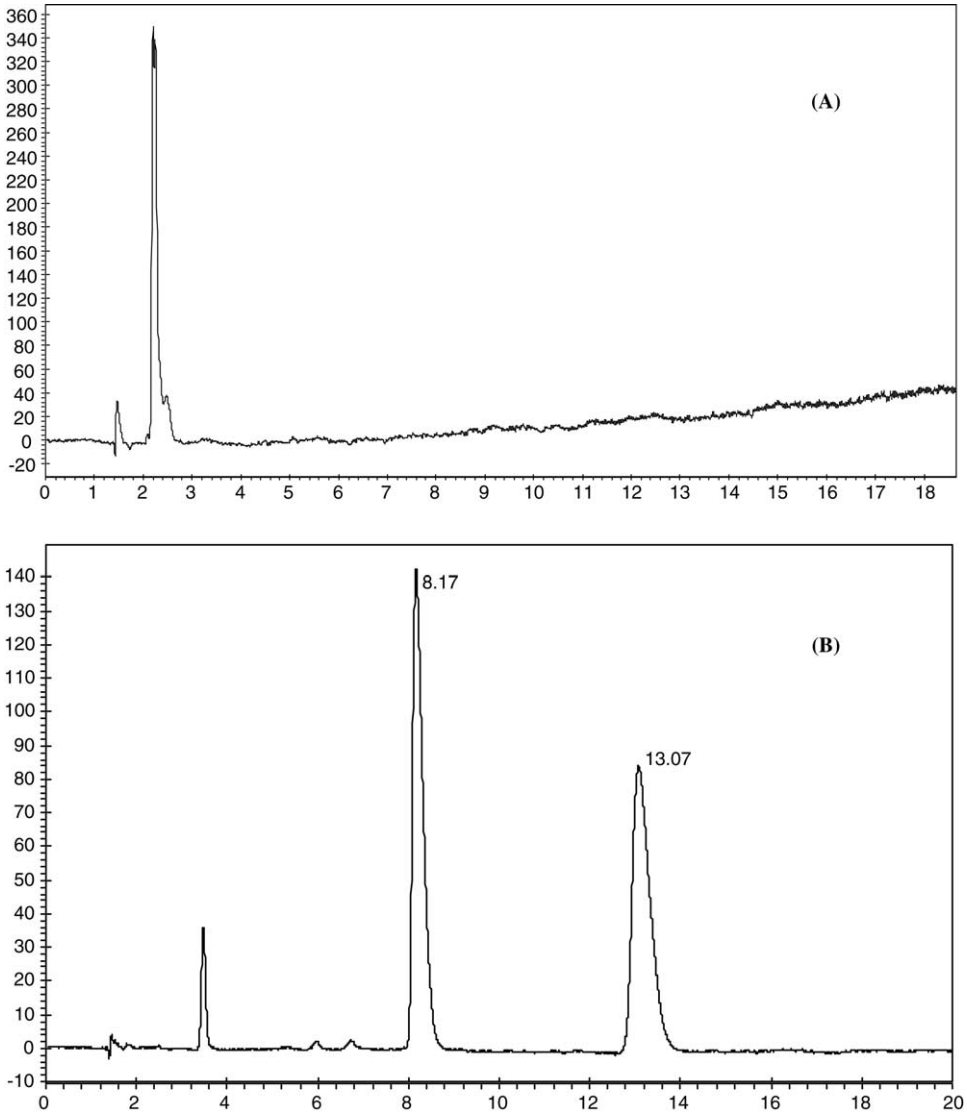

Fig. 2. SFC chromatogram of phenoxybenzamine hydrochloride salt on a CHIRALPAK® AD-H column (A) 20% ethanol and (B) 20% ethanol containing 0.1% ESA modifier. Sample dissolved in modifier.

less than 7 min. Without additive no peaks were observed. This material was purchased as a free base. In general, amine compounds as free bases did not give peaks in SFC when ESA was not used while amine compounds as hydrochloride salts gave results similar to those shown in Figs. 1A, 2A and 3A. It is likely that the hydrochloride salts elute intact in SFC [14,15]. Peak distortion may result from partial separation or a slow dissociation of the salt pair.

Substitution of methanol and 2-propanol for ethanol typically gave different selectivity. In general these alcohols did not yield as many separations as ethanol and there was no clear trend between selectivity for a particular compound and modifier size. Methanol gave the best selectivity about 25% of the time as did 2-propanol. Of the nine compounds not separated in ethanol screening, four were baseline resolved and a fifth partially resolved with either methanol or 2-propanol containing ESA. Methanol gave a good separation of tryptophan which was not separated with ethanol or 2-propanol. DOPA separated well with 2-propanol but not with either ethanol or methanol.

It was observed during screening work that the acid needed to be included in the sample diluent for separation to occur. Further it was observed that upon removal of acid from the mobile phase separations quickly collapsed and peaks did not elute. These observations suggest a mechanism where the ESA forms a salt with the basic compound which then separates in SFC. It has been well demonstrated [14,15] that hydrochloride salts elute as intact salts through SFC. If the salt is the specie being separated, then changing the acid should change the salt structure and give different chromatog-

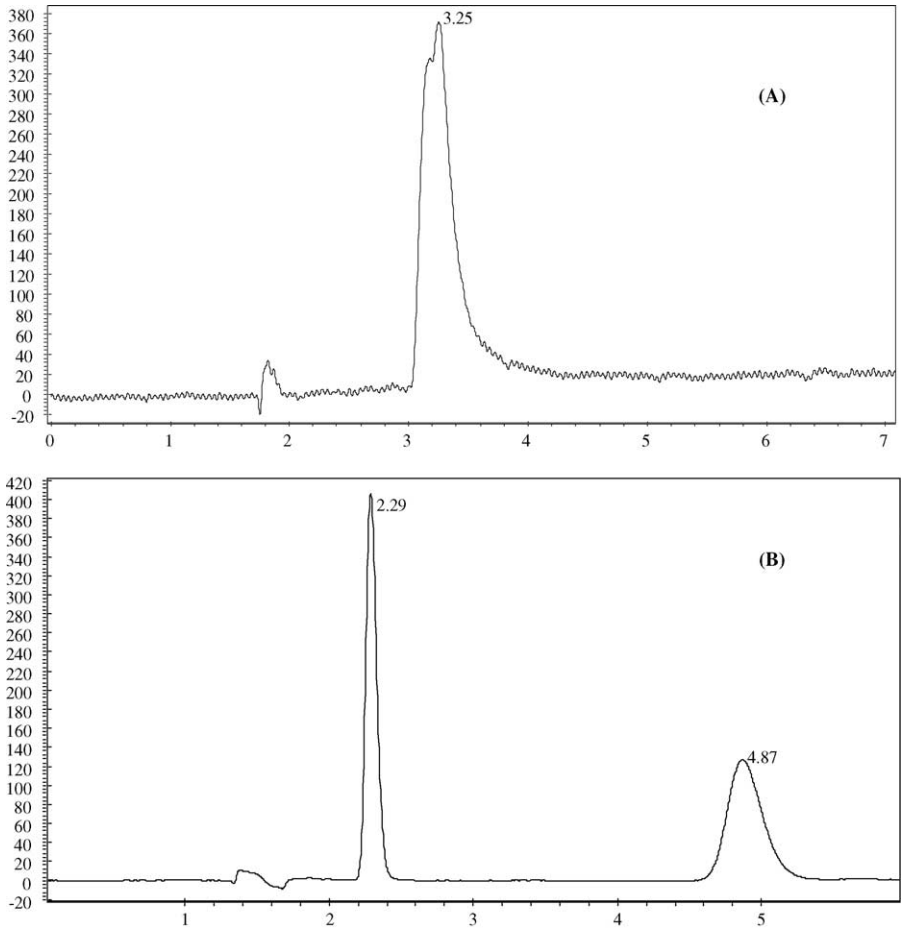

Fig. 3. SFC chromatogram of phenylalanine-methyl ester hydrochloride salt on a CHIRALPAK® AD-H column (A) 20% ethanol and (B) 20% ethanol containing 0.1% ESA modifier. Sample dissolved in modifier.

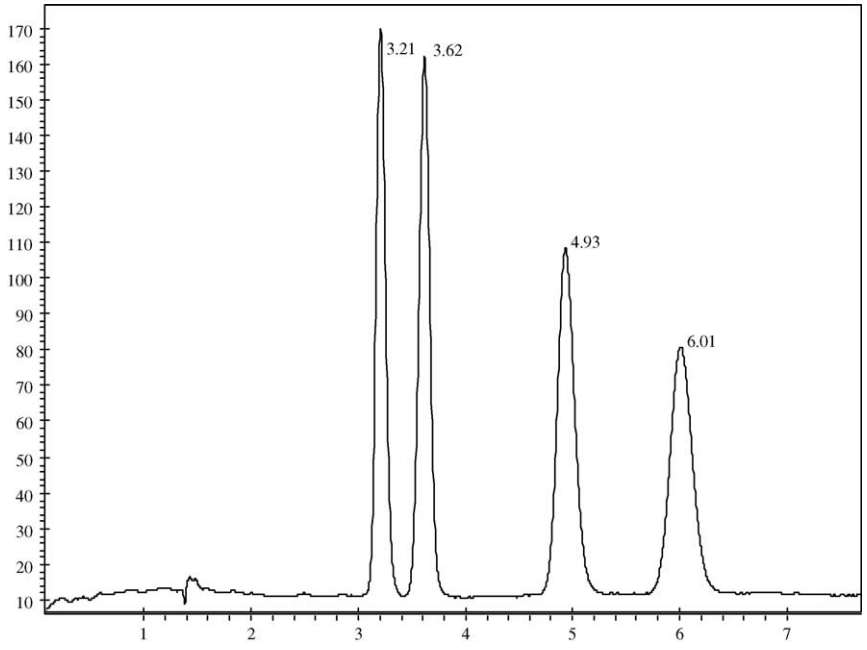

Fig. 4. SFC chromatogram of nadolol free base on a CHIRALPAK® AD-H column using 30% ethanol containing 0.1% ESA modifier.

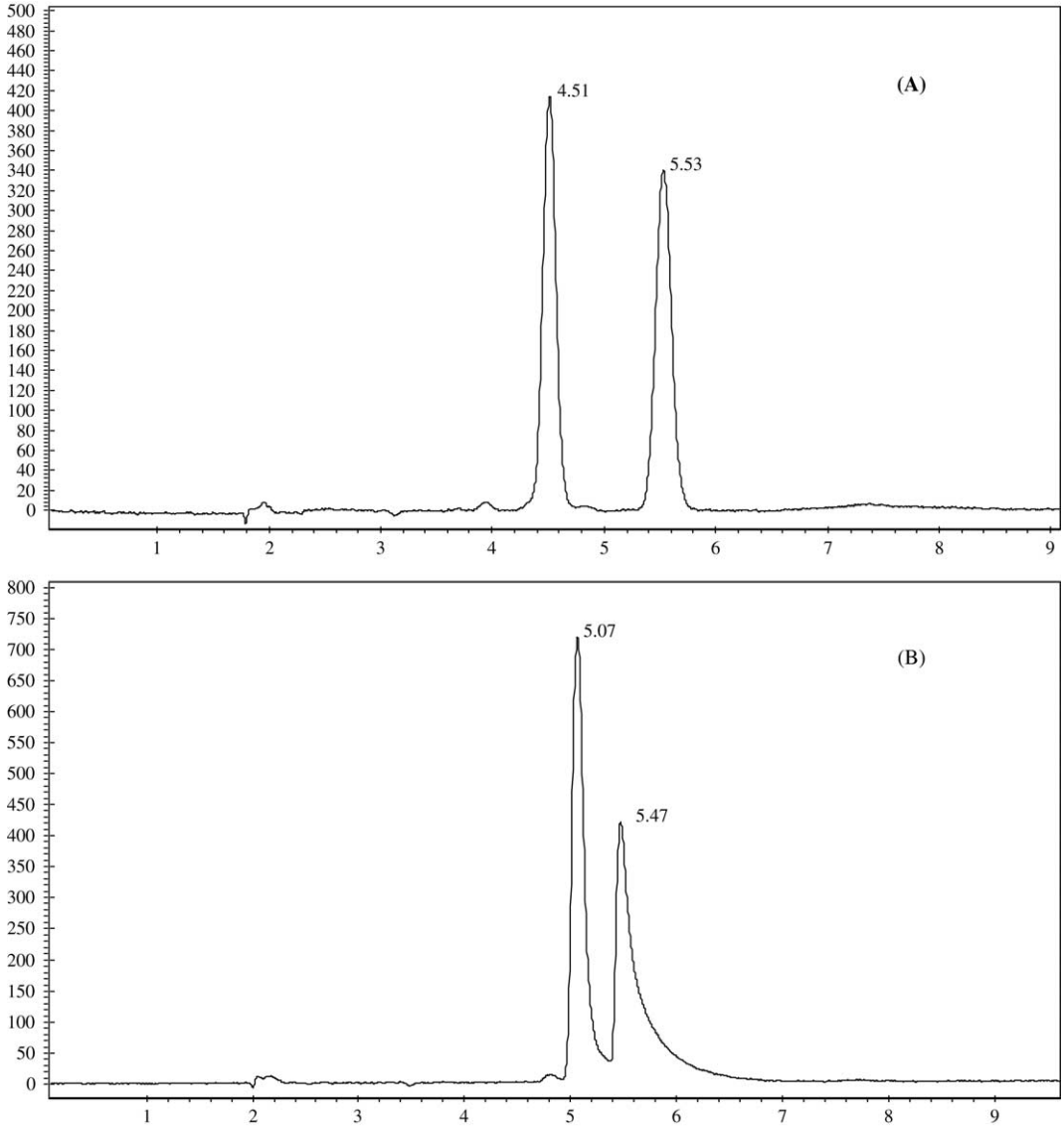

Fig. 5. SFC chromatograms of chloramphetamine on a CHIRALPAK® AD-H column using 15% ethanol modifier containing (A): 0.1% ESA or (B): 0.1% TFA. Samples were prepared in modifier with the corresponding acid.

raphy. The effect of replacing ESA with methanesulfonic acid (MSA) was evaluated for six compounds. Using methanol modifier, MSA consistently gave shorter retention times than ESA. Although the effects of this change on selectivity varied, resolution with MSA was typically lower than with ESA. In method optimization it may be worthwhile to test MSA, but dramatic effects should not be expected. These alkylsulfonic acids are quite similar. Further experimentation tested the effect of a bulkier sulfonic acid, camphorsulfonic acid (CSA), as well as the commonly used trifluoroacetic acid (TFA). Ye, et al. [4] had observed dramatically different selectivity when CSA was substituted for ESA in HPLC separation of amino

Table 2
SFC screening results for β-blockers on a CHIRALCEL® OD-H using 20% alcohols containing 0.1% ESA

Compound	Ethanol		Methanol		2-Propanol	
	α	Rs	α	Rs	α	Rs
Propanolol	1.98	8.56	1.93	8.91	2.23	8.03
Atenolol	2.17	10.54	2.60	13.84	2.57	11.49
Metoprolol	3.35	13.66	2.91	14.32	4.28	16.31
Oxyprenolol	2.56	10.75	2.07	10.17	4.06	15.89
Alprenolol	1.59	4.49	1.40	4.25	2.05	6.73

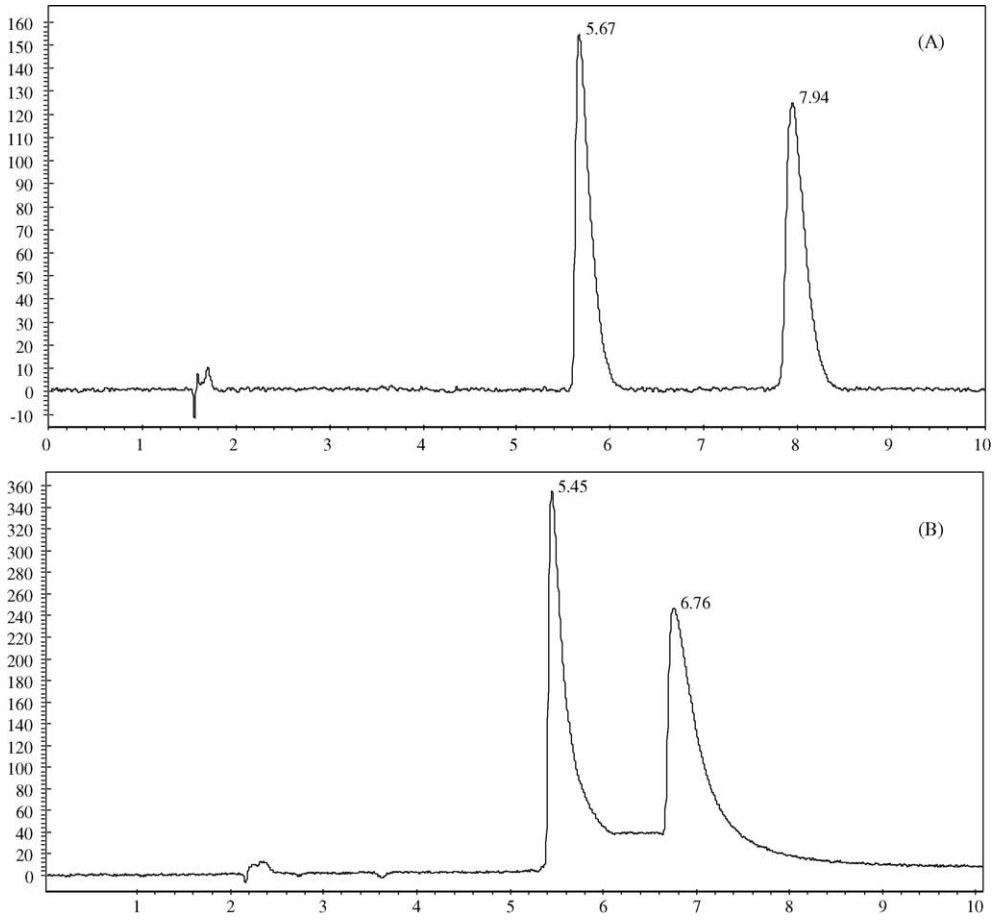

Fig. 6. SFC chromatograms of ketamine on a CHIRALPAK® AD-H column using 15% ethanol modifier containing (A): 0.1% ESA or (B): 0.1% TFA. Samples were prepared in modifier with the corresponding acid.

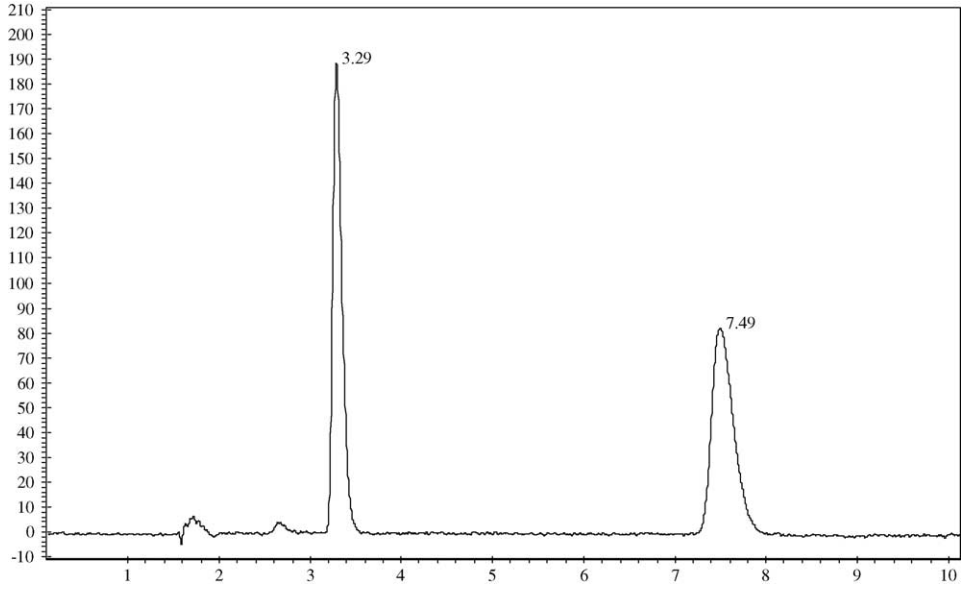

Fig. 7. SFC chromatogram of metoprolol on a CHIRALCEL® OD-H column using 20% ethanol containing 0.1% ESA modifier.

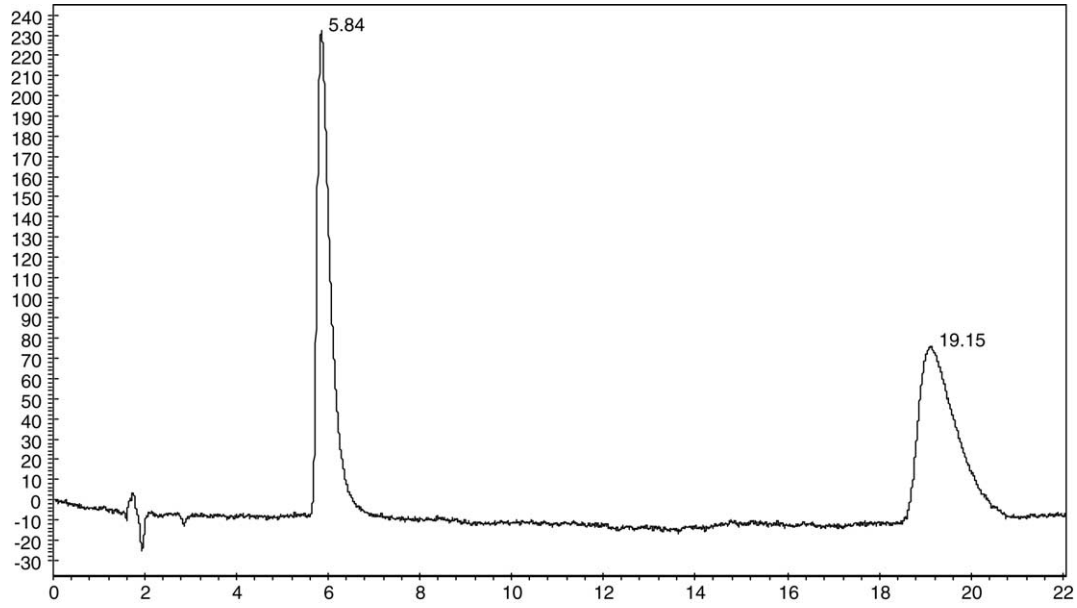

Fig. 8. SFC chromatogram of oxyprenolol on a CHIRALCEL® OD-H column using 20% 2-propanol containing 0.1% ESA modifier.

acids. This sulfonic acid is a bulky bicyclic compound, with an additional ketone functionality and a chiral center. It was expected that adding this much structure would have dramatic effects on chromatography and that possibly each CSA enantiomer would have different effects.

The R enantiomer of CSA was dissolved in ethanol at 0.012 M to approximate the molarity of ESA used. Nine probes were chromatographed with this additive in both the sample diluent and mobile phase. The effects were not dramatic. Retention times were slightly longer and peak shapes were a bit poorer. Selectivity was consistently lower than that observed with ESA. Use of TFA in place of ESA in both diluent and modifier gave broad or absent peaks. Figs. 5 and 6 show this effect for chloramphetamine and ketamine, respectively. TFA is a weaker acid which may account for this result.

The use of ESA in chiral SFC was extended to CHIRALCEL® OD-H. Nineteen compounds were screened using ethanol, methanol and 2-propanol containing 0.1% ESA. At least partial separations were observed for 13 compounds. The best successes in this brief screening were observed with β-blockers (Table 2). Figs. 7 and 8 show the excellent separations obtained for metoprolol and oxyprenolol, respectively.

4. Conclusions

From HPLC findings, incorporation of ethanesulfonic acid into the modifier in SFC separations was originally expected to yield additional opportunities for interaction between amine analytes and additive adsorbed to the stationary phase. It was found instead that this strong acid acts as a counter-ion to a wide range of amines, forming ion-pairs that are stable to SFC separation. A diverse set of amine compounds that had not otherwise been separable by SFC were found to give excellent separations. This approach greatly expands the range of chiral SFC.

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