Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1070 (2005) 163–170
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

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

Rodger W. Stringham *

Chiral Technologies, 730 Sprindale Drive, Inc., Exton, PA 19341, USA

Received 29 July 2004; received in revised form 29 September 2004; accepted 11 February 2005
Available online 26 February 2005

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

* Tel.: +1 610 594 2100x245; fax: +1 610 594 2324.
E-mail address: rstringham@chiraltech.com.

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.02.044

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.