Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1101 (2006) 86–93
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Chiral separation of amines by high-performance liquid chromatography using polysaccharide stationary phases and acidic additives

Rodger W. Stringham *, Yun K. Ye [1]

Chiral Technologies, Inc., 800 North Five Points Road, West Chester, PA 19380, USA

Received 22 August 2005; received in revised form 15 September 2005; accepted 26 September 2005
Available online 17 October 2005

Abstract

A dramatic and beneficial effect of ethanesulfonic acid (ESA) on the chiral HPLC separation of basic compounds was found. Using a single chiral column and a starting mobile phase, more than half of a diverse set of amines was baseline separated. Changing alcohol content and alcohol type increased the success rate. Methanesulfonic acid (MSA) proved even more successful. The mechanism of this unexpected finding appears to be a combination of ion-pair salt formation in the mobile phase and increased binding with the chiral stationary phase (CSP) arising from a localized decrease in pH.

Keywords: Chiral HPLC; Mobile phase additives; Ethanesulfonic acid

1. Introduction

Acidic and basic mobile phase additives are widely used on polysaccharide-based chiral stationary phases with the intent of minimizing interactions with residual silanols, giving better peak shape and increased resolution of chiral compounds. Acidic additives are required to elute carboxylic acid analytes [1]. Recent work [2–4] demonstrated that incorporation of such additives into the mobile phase can also affect selectivity for amino acid analogs. Acidic additives increased selectivity presumably due to their ability to alter non-specific adsorption [2]. Addition of various amines gave a range of selectivity results, with some dramatic increases resulting from increased retention of the second-eluting enantiomer [3]. This was attributed to bulky amine additive restricting the access of modifier to elute of this enantiomer. Subsequent work [5] extended the application of bulky amine additives to supercritical fluid chromatography (SFC). Although the effects were most dramatic with amino acid esters there were observations of separations of small amines only made possible by the use of additive. The utility of this observation is somewhat limited by the need for percent levels of additive.

Further work with amino acid esters [4] showed that ethanesulfonic acid (ESA) could actually increase retention, presumably through additional hydrogen bonding between analytes and ESA bound to the stationary phase. Attempts to verify incorporation of the additive into the CSP proved unsuccessful and increased retention has since been attributed to increased hydrogen bonding [6] likely due to a localized pH effect. A recent report [7] showed that under SFC conditions ESA can act as an ion-pairing agent resulting in the separation of a wide variety of amines. Tang et al. [8,9] reported the beneficial effect of trifluoroacetic acid on the separation of basic compounds in HPLC. Ye et al. [10] reported the use of alkylsulfonic acid additives to separate underivatized amino acids. Clearly the concept of mobile phase additives simply masking interactions with the underlying chromatographic support does not explain the observed effects. Work reported here examines the effect of ESA on the separation of a variety of amines in HPLC.

2. Experimental

2.1. Safety note

Alkyl sulfonic acids may react with alcohols to form alkyl sulfonic esters. Some such esters are known carcinogens. The

* Corresponding author. Tel.: +1 610 594 2100x245; fax: +1 610 594 2325.
E-mail address: rstringham@chiraltech.com (R.W. Stringham).
[1] Present address: Bristol-Myers Squibb Company, 1 Squibb Dr, New Brunswick, NJ, USA.

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.065

low levels of ESA and MSA used in this work should not form measurable levels of the esters at room temperature. However, if these mobile phases are used for preparative separations, the heating in alcohol and increased acid concentration during product isolation supposedly could result in ester formation. Gas chromatographic analysis of old methanol and ethanol solutions (0.1% MSA) and of such solutions heated to dryness and concentrated 10-fold showed no indication (20 ppm detection limit) of ester formation.

2.2. 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, USA). Ethanol was obtained from J.T. Baker (Phillipsburg, NJ, USA) and 2-propanol was from Pharmco (Brookfield, CT, USA). Probe samples were dissolved at ~2 mg/mL in mobile phase.

2.3. Chromatography

Chromatographic studies were performed on Agilent 1100 HPLCs (Agilent, Palo Alto, CA, USA) equipped with autosampler, thermostated-column device and a variable-wavelength UV detector. Chiralpak AD-H columns (250 mm × 4.6 mm) were packed at Chiral Technologies (West Chester, PA, USA). The flow rate used was 1 mL/min with detection via UV at 210 nm unless otherwise specified. Injection volume was 5 μL.

3. Results and discussion

Initial screening of 42 structurally diverse amines was performed on the AD-H column using a mobile phase consisting of hexane/ethanol/ESA with an 85/15/0.1 composition. Results are given in Table 1. Baseline resolution was obtained for 25 compounds. Fig. 1 shows the separation obtained for three compounds. Partial separation was observed for nine additional compounds with five showing no sign of selectivity. Peaks were not observed for three of the probes. There does not appear to be any relationship between probe structure and separation success. Not included in this table are results for nadolol and labetolol, β-blockers expected to give four stereoisomers. An excellent separation of nadolol was obtained (peaks at 17.1, 21.4, 29.6 and 38.4 min) while labetolol gave only partial resolution.

Retention times were too long for some probes and others did not elute. Increasing the level of ethanol in the mobile phase gave the expected decline in retention for 11 probes. In general, selectivity did not change with increased ethanol content but there were several examples where selectivity declined significantly. The run time for thioridazine decreased from 105 to 15 min when the ethanol content was increased to 50%. Very well-resolved sulpiride peaks were observed with this mobile phase (Fig. 2). The excellent separation of nadolol was maintained (Fig. 3) in a usable analysis time. Use of neat alcohol containing 0.1% ESA gave unretained peaks for all probes tested.

Changing the alcohol modifier may give dramatic changes in chiral separations on polysaccharide columns [11–16]. Twenty-

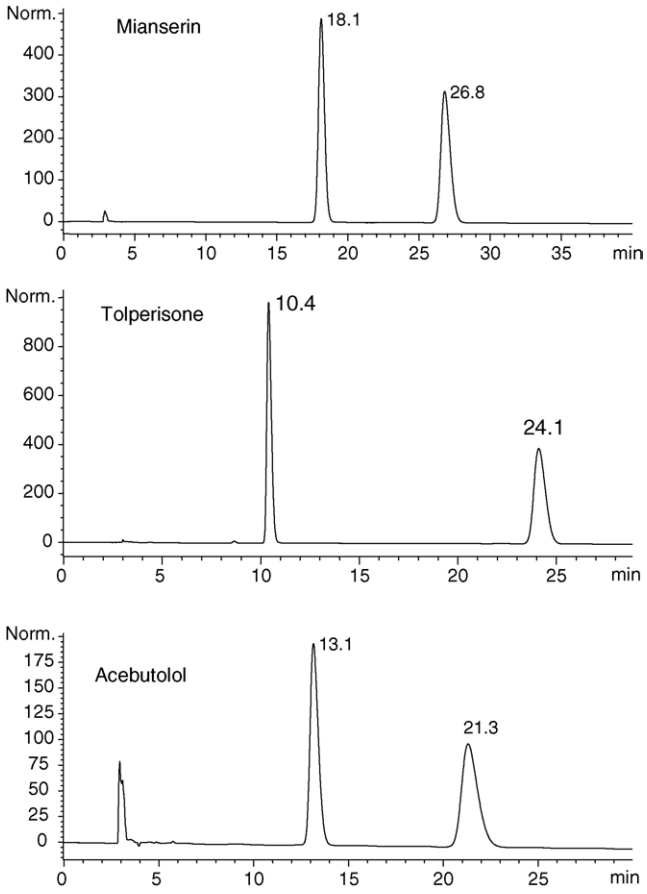

Fig. 1. Example chromatograms obtained from screening on an AD-H column using a hexane/ethanol/ESA (85/15/0.1) mobile phase.

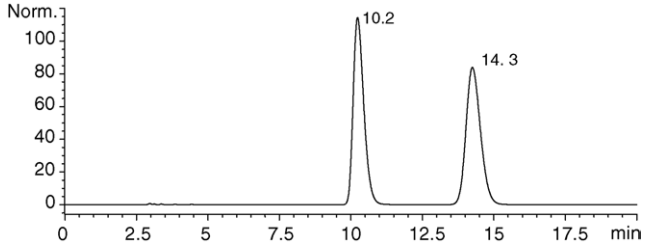

Fig. 2. Chromatogram of sulpiride on an AD-H column using a hexane/ethanol/ESA (50/50/0.1) mobile phase.

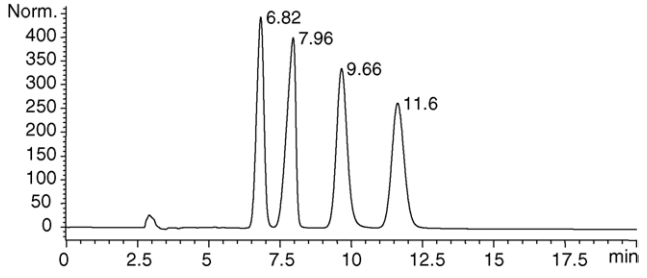

Fig. 3. Chromatogram of nadolol on an AD-H column using a hexane/ethanol/ESA (75/25/0.1) mobile phase.