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Analysis and purification of alcohol-sensitive chiral compounds using 2,2,2-trifluoroethanol as a modifier in supercritical fluid chromatography☆

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ABSTRACT

2,2,2-Trifluoroethanol (TFE) is evaluated as an alternative modifier for the analysis and purification of alcohol-sensitive chiral compounds using supercritical fluid chromatography (SFC). Four chiral compounds, selected for their sensitivity to alcohols, in addition to a variety of standard chiral compounds were analyzed by SFC using TFE with polysaccharide and Pirkle-type chiral stationary phases (CSPs) to produce selectivities (α) and resolutions (R_s) as high as 1.4 and 7.2. A preparative isolation of 2-phenylglutaric anhydride was achieved using TFE as the mobile phase modifier to produce clean enantiomers.

1. Introduction

SFC has become a preferred method of choice for purification of many compounds on both small and large scale, including chiral separations [1–4]. The technique generally involves an eluent of liquid CO_2 and 0–70% of a modifier, such as methanol, ethanol, isopropanol or acetonitrile. While these modifiers are convenient and effective, for samples containing alcohol-sensitive groups, they may lead to side reactions such as ester exchange, nucleophilic cleavage, or substitution reactions. Common acyl derivatives such as simple esters (especially sensitive cases such as trifluoroacetates), anhydrides, thiol esters, phenol esters and certain amides often undergo significant reaction with, for example, methanol during the separation and recovery process. Substrates that are sensitive to nucleophilic substitution reactions, such as allylic halides, alkyl phosphates, or other activated alkyl species are also susceptible to reactions with alcohols; in particular when acid or base modifiers are added to the mobile phase.

One alternative involves the use of immobilized polysaccharide CSPs along with non-alcoholic modifiers. These phases have added many possibilities for optimizing sample dissolution and separation selectivity; however, in many cases the non-alcoholic solvent is primarily effective to increase the solubility of the solute and often requires an alcohol co-solvent to enhance the selectivity and resolution of the separation [5].

We recognized that TFE might offer a useful alternative. The electron withdrawing effect of the β-fluoro substituents serves to increase the acidity of the hydroxyl group (pK_a for TFE is ca. 10) while strongly reducing the nucleophilic reactivity of the oxygen electron pairs. TFE should serve as a polar H-bond donor while being less reactive as a nucleophile compared to ethanol or methanol. The solvent is relatively expensive but recyclable, with a boiling point slightly lower (+73.6 °C) than that of ethanol. Encouraged by the findings of Kagan [6] wherein a fluorinated solvent (ethoxynonafluorobutane) is used in chiral HPLC applications without apparent detrimental effects to the phases, we evaluated TFE as a chromatographic solvent for nucleophile-sensitive functionality and typical solid supports. Its general separation properties including applications in chiral SFC separations are demonstrated.

Our prototype case is 2-phenylglutaric anhydride with the reactive anhydride functional group. Monitoring by ^{1}H NMR spectroscopy, we showed that a solution of 2-phenylglutaric anhydride in ethanol underwent cleavage of the anhydride ring slowly on standing at room temperature. In contrast, a solution of 2-phenylglutaric anhydride in TFE at room temperature showed no change. We proceeded to examine TFE as a modifier for chiral SFC. Twelve racemates were selected for chiral method development using TFE, methanol, ethanol, isopropanol, acetonitrile, dichloromethane, ethyl acetate and tetrahydrofuran using a variety of CSPs. Four of the racemates were selected for their sensitivity to

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hydrolysis: 2-phenylglutaric anhydride (**1**), isradipine (**2**), felodipine (**3**), and 2,2,2-trifluoro-*N*-(phenylethyl)-*N*-tosylacetamide (**4**) (Fig. 1). A preparative SFC separation was carried out on 500 mg of the anhydride (**1**) to test the feasibility of using TFE for preparative-scale chiral separations.

2. Experimental

2.1. Reagents and solvents

All compounds (except for 2,2,2-trifluoro-*N*-(phenylethyl)-*N*-tosylacetamide, **4**) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Compound (**4**) was synthesized as described by Moussa et al. [7] Structures of hydrolytically sensitive compounds are shown in Fig. 1. Carbon dioxide (industrial grade) was obtained from Air Gas (Radnor, PA, USA). All HPLC grade solvents were obtained from EMD Chemicals (Gibbstown, NJ, USA) except for ethanol, which was obtained from Pharmco-Aaper (Brookfield, CT, USA) and TFE obtained from TCI America (Portland, OR, USA).

2.2. Stability of 2-phenylglutaric anhydride as monitored by ^{1}H NMR

A sample of racemic 2-phenylglutaric anhydride (>95% pure by ^{1}H NMR) was dissolved in ethanol and allowed to stand at room temperature. Aliquots were removed at various time points, concentrated by rotary evaporation, and analyzed by ^{1}H NMR spectroscopy. The appearance of a triplet at δ 1.25 and quartet at 3.70 ppm indicated the incorporation of ethanol into the anhydride. The concentration of 2-phenylglutaric anhydride decreased steadily over 48 h (Fig. 2). A parallel experiment with TFE in place of ethanol showed no change in the ^{1}H NMR spectrum after 44 h at room temperature.

2.3. Chiral SFC screens

Compounds were analyzed on six (25 cm × 0.46 cm) chiral columns: Chiralcel OD-H and OJ-H, Chiralpak AS-H and AD-H from Chiral Technologies (Exton, PA, USA), Whelk-O1 (S,S) column from Regis (Morton Grove, IL, USA) and Sepapak-3 from Sepaserve (Münster, Germany). Samples were analyzed on a Berger Analytical SFC System equipped with a dual pump (FCM-1200), an auto sampler (ALS 3100), a column oven (TCM-200) and a diode-array detector (DAD-4100) (Mettler-Toledo, Newark, DE, USA). The screen was carried out under isocratic conditions using 20 and 40% of organic modifier in CO_2 at a backpressure of 100 bars with a flow rate of 3 mL/min, a temperature of 40 °C for 15 min and detection at 220 nm. The organic modifiers include methanol, ethanol, isopropanol, acetonitrile and TFE. The four alcohol-sensitive compounds (listed in Fig. 1) were also analyzed using an 'alcohol-free' screen, in addition to three bonded phase columns: Chiralpak IA and IC (Chiral Technologies, Exton, PA, USA) and Whelk-O1 (S,S). Samples were analyzed on a Berger Analytical SFC system under isocratic conditions (described above). The organic modifiers used include dichloromethane, ethyl acetate, tetrahydrofuran and TFE. Samples containing free amines were analyzed with the addition of 0.1% diethylamine (v/v) to the mobile phase. Alcohol-sensitive compounds (**1**–**4**) were dissolved in TFE while all other compounds were dissolved in ethanol. A total of 10 μL of 5–10 mg/mL solutions were injected for each analysis.

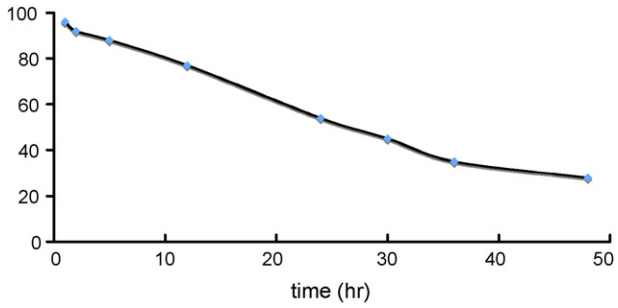

Fig. 2. Mol% of (**1**) versus time (h) monitored by ^{1}H NMR spectroscopy.

2.4. Preparative SFC separation of 2-phenylglutaric anhydride

SFC separation was carried out on a Berger Multigram II SFC equipped with two SD-1 Varian pumps, a Knauer K-2501 Spectrophotometer, Knauer K-1900 pump (Mettler-Toledo, Newark, DE,

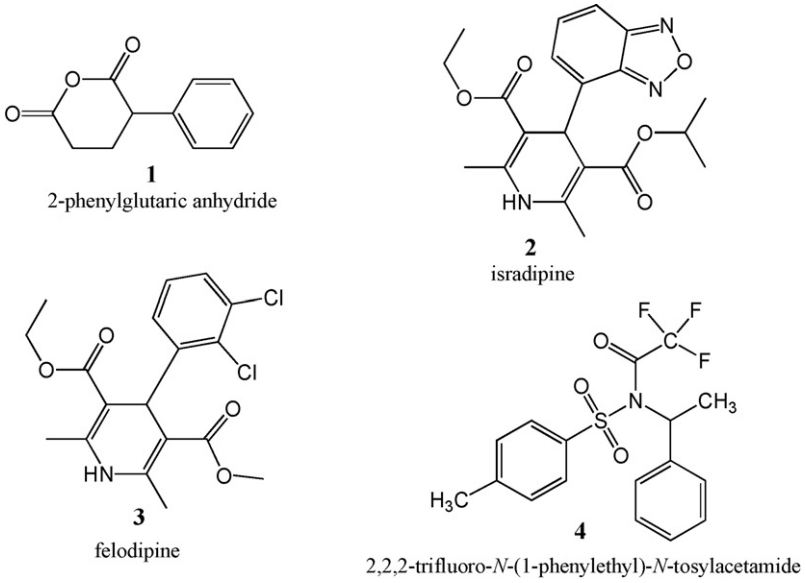

Fig. 1. Examples of alcohol-sensitive compounds.

USA) and a Vatran condenser SGP-50-100 (Vatran, Chula Vista, CA, USA). A 500-mg sample was dissolved in 10 mL of TFE. Injection volume was 1 mL (50 mg/injection) and sample was injected at intervals of 60 s onto a (2 cm × 15 cm) Chiralpak AD-H column (Chiral Technologies, Exton, PA, USA). Sample peaks were collected using 'threshold' at a wavelength of 220 nm. Total sample recovery was 97.5%.

3. Results and Discussion

Retention factors, selectivity and resolution of all chiral compounds are given in Table 1. Retention factors, selectivity and resolution of alcohol-sensitive chiral compounds are given in Table 2.

Analysis of both alcohol-sensitive chiral compounds and a variety of chiral pharmaceutical compounds, shows that TFE is a useful substitute for the alcohol modifiers typically used in SFC. For 9 of the 12 racemates analyzed, separation conditions were developed producing resolutions ranging from 3.2 to 7.2 and selectivities ranging from 1.2 to 1.4. Three of the four alcohol-sensitive compounds analyzed yielded separations adequate for preparative-scale isolations (Fig. 3). While none of the columns were stored in the solvent for extended periods of time, both analytical and preparative columns were periodically exposed to the solvent over a 1-year period, without any signs of adverse effects to the coated polysaccharide stationary phases.

Compound **1** was chosen for its sensitivity to anhydride ring-opening in the presence of alcohols. NMR experiments in which solutions of 2-PGA in ethanol and in TFE were compared, demon-

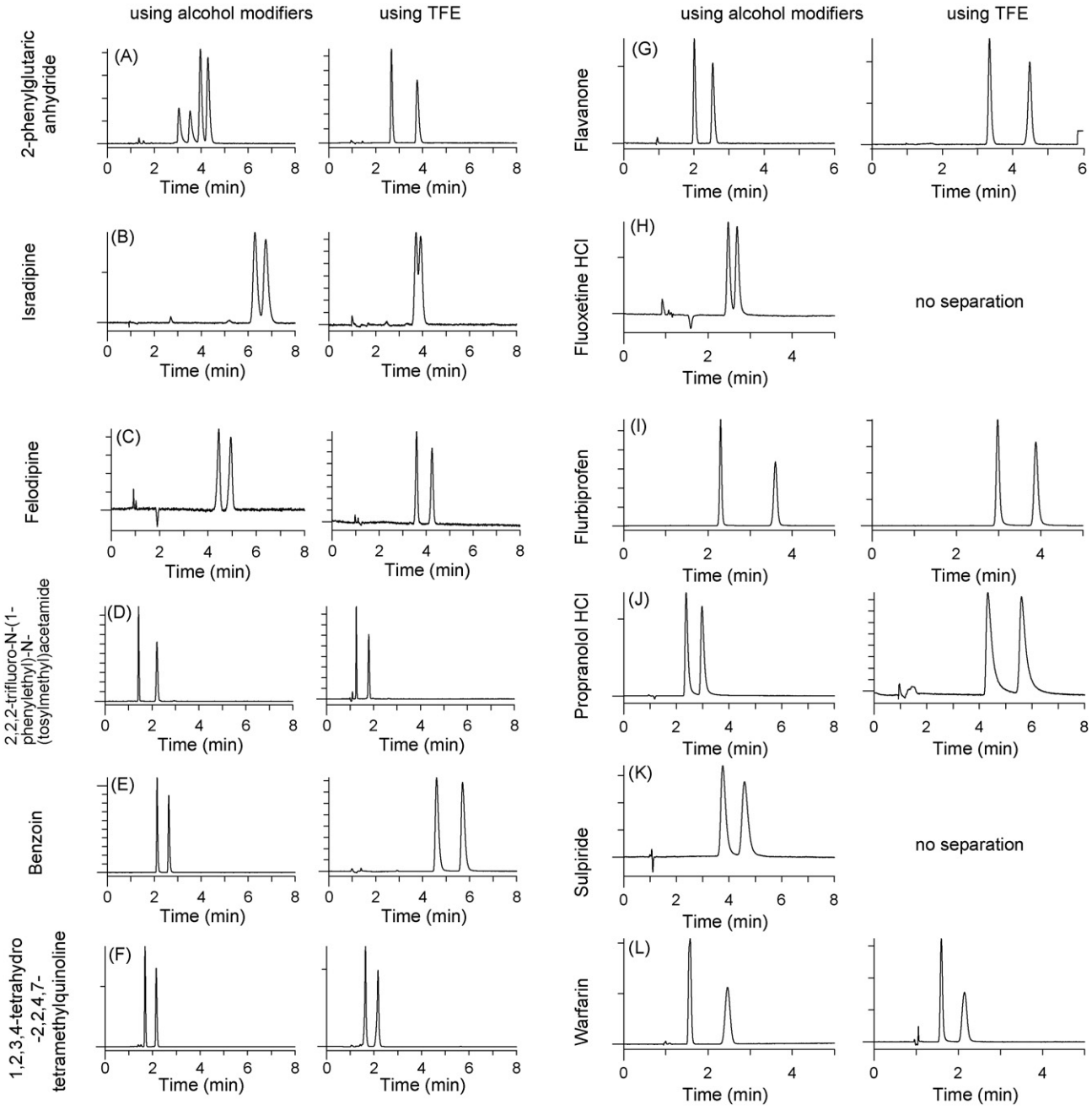

Fig. 3. SFC separations of alcohol-sensitive compounds and chiral standards using alcohols and TFE.

Table 1
Retention factors, selectivity and resolution of all chiral compounds

Compound	Using alcohol modifier				Using TFE			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
(1) 2-Phenyl glutaric anhydride	3.0	3.3	1.1	1.6	1.7	2.8	1.4	6.6
(2) Isradipine	5.3	5.7	1.1	1.4	2.7	2.9	1.1	0.7
(3) Felodipine	3.5	4.0	1.1	2.4	2.6	3.3	1.2	4.0
(4) 2,2,2-Trifluoro-*N*-(1-phenyl-ethyl)-*N*-(tosylmethyl)acetamide	0.4	1.2	1.6	8.6	0.3	0.8	1.4	6.9
(5) Benzoin	1.1	1.6	1.2	5.6	4.0	4.7	1.2	4.3
(6) 1,2,3,4-Tetrahydro-2,2,4,7-tetramethylquinoline	0.7	1.2	1.3	5.0	0.6	1.2	1.3	5.0
(7) Flavanone	1.0	1.5	1.3	4.4	2.2	3.5	1.3	7.2
(8) Fluoxetine HCl	1.5	1.7	1.1	1.5	No separation			
(9) Flurbiprofen	1.3	2.6	1.6	11.0	2.0	2.9	1.3	6.0
(10) Propranolol HCl	1.4	2.0	1.3	2.9	3.3	4.6	1.3	3.2
(11) Sulpiride	2.8	4.0	1.2	2.2	No separation			
(12) Warfarin	0.6	1.5	1.6	5.1	0.6	1.2	1.3	3.5

Conditions are described in Fig. 3.

Table 2
Retention factors, selectivity and resolution of alcohol-sensitive chiral compounds

Compound	Using non-alcohol modifiers				Using TFE			
	k_1	k_2	α	R_s	k_1	k_2	α	R_s
(1) 2-Phenyl glutaric anhydride	3.5	4.9	1.3	3.8	1.7	2.8	1.4	6.6
(2) Isradipine	No separation				2.7	2.9	1.1	0.7
(3) Felodipine	10.6	11.6	1.1	1.4	2.6	3.3	1.2	4.0
(4) 2,2,2-Trifluoro-*N*-(1-phenyl-ethyl)-*N*-(tosylmethyl)acetamide	No separation				0.3	0.8	1.4	6.9

Conditions are described in Fig. 5.

strated the sensitivity of the sample to ethanol, with gradual ring-opening over a period of 48 h at room temperature (Fig. 2). The anhydride degrades at an even faster rate when exposed to ethanol under SFC conditions. A fresh sample of 2-PGA dissolved in TFE decomposed within 2 min of exposure to 15% ethanol/CO_2 following injection (Fig. 4). The same sample remains intact when injected on the same column using 15% TFE/CO_2 as the eluent, and a selectivity and resolution of 1.4 and 6.6 on a Chiralpak AD-H column is observed.

Felodipine (**2**) and isradipine (**3**), two dicarboxydihydropyridines, are each rendered chiral by differentially substituted esters. Both compounds suffered ester exchange under chiral HPLC conditions using a 1:1 mixture of hexane and ethanol (data not shown). A baseline separation of the enantiomers of isradipine could not be obtained by SFC under any of the conditions attempted (Figs. 3 and 5). Felodipine, however, demonstrated baseline resolution under three sets of conditions (Figs. 3 and 5) with greatest selectivity and resolution using TFE/CO_2 as the eluent.

Trifluoroacetate **4** is an example of a chiral compound harboring a delicate but useful protecting group in which the *N*-trifluoroacetate group is sensitive to cleavage in the presence of alcohol. Ideal conditions for analysis and isolation were obtained on a Chiralpak AD-H column using 10% TFE/CO_2 as the eluent. A resolution of 6.9 and selectivity of 1.4 were obtained.

The 'non-alcoholic' SFC screen using the immobilized polysaccharide chiral stationary phases, Chiralpak IA and IC, as well as the Whelk-01 column produced baseline separations for two of the four alcohol-sensitive compounds using THF/CO_2 as the mobile phase.

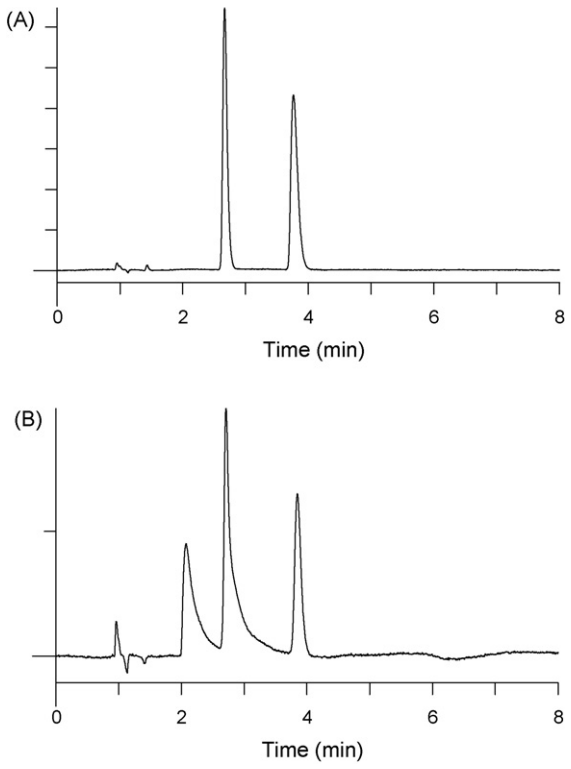

Fig. 4. Analysis of 2-phenylglutaric anhydride to determine stability in ethanol versus TFE.

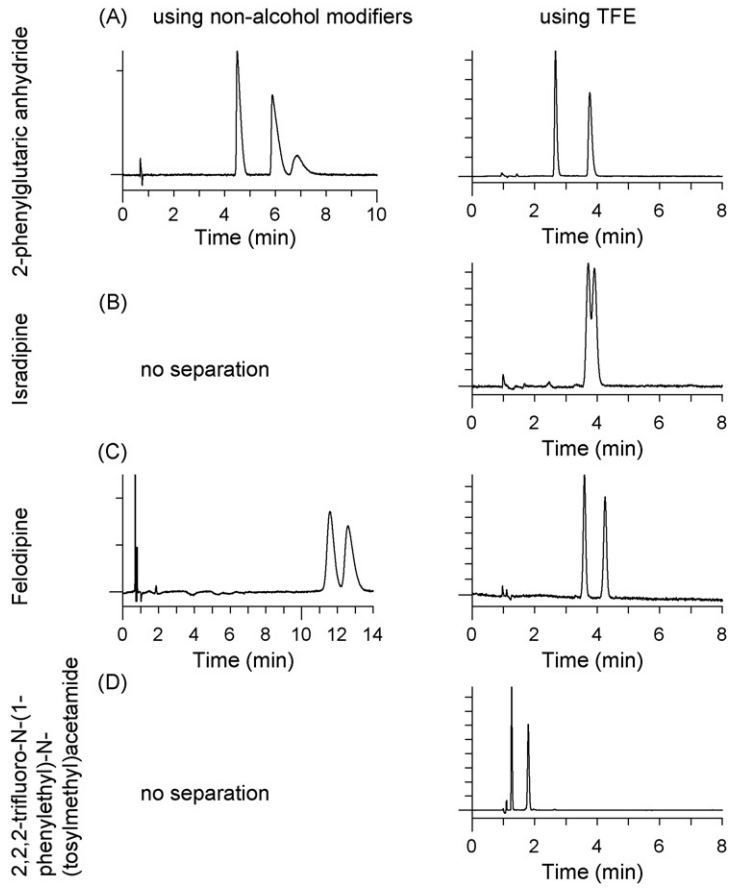

Fig. 5. SFC separations of alcohol-sensitive compounds on bonded chiral phases using non-alcoholic modifiers.

The experiments were carried out to determine the usefulness of the aprotic solvents as alcohol alternatives and are not designed to determine the resolving ability of the bonded stationary phases. The bonded phases, in general, offer great alternatives using a great variety of solvent combinations and were not tested in combination with TFE in the current study. Aprotic solvents such as those used in the 'non-alcoholic' screen appear to be somewhat weaker in elution power in SFC than in HPLC and alcohols are often added to increase the solvent strength sufficiently to allow elution of the solutes [8]. The addition of TFE to the aprotic solvents, although

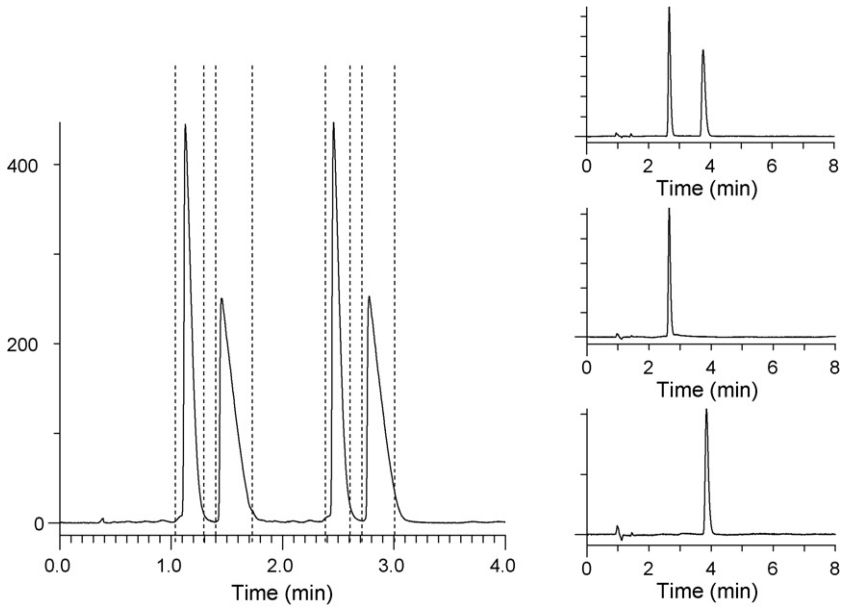

Fig. 6. Preparative SFC separation and analysis of the enantiomers of 2-phenylglutaric anhydride.

not tested here, might be a viable alternative to alcohol addition to increase the elution power of the mobile phase.

In addition to the applicability to compounds sensitive to alcohols, TFE is effective at lower percentage composition in the CO_2 mobile phases compared to alcohols conferring similar resolutions of those enantiomers. To address the scalability of the analytical method developed; a preparative separation was carried out on compound **1**. A total of 500 mg of the sample was dissolved in 10 mL of TFE and 1-mL injections were made onto a 2 by 15 cm AD-H column. The mobile phase consisted of 15% TFE/CO_2 at 100 bar and 100 mL/min. The preparative separation scaled perfectly, although the conditions presented in Fig. 6 (3 g/h) were not optimized to maximize throughput. Enantiomers 1 and 2 were collected, concentrated and analyzed. The separation produced two fractions with enantiomeric excesses greater than 99% and recovery of 97.5% (Fig. 6).

4. Conclusion

TFE is worth consideration by virtue of its ability to resolve a variety of enantiomers while remaining compatible with the coated polysaccharide chiral stationary phases and is especially suitable when conventional alcohol modifiers should not be used for the analytical application or the preparative separation.

Acknowledgements

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