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Evaluation of non-conventional polar modifiers on immobilized chiral stationary phases for improved resolution of enantiomers by supercritical fluid chromatography

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ABSTRACT

An evaluation of the use of non-conventional polar modifiers for the supercritical fluid chromatographic separation of enantiomers on immobilized chiral stationary phases is presented. The resolution of a group of nine commercially available racemates is studied on the Chiralpak IA, IB, IC, ID, IE, and IF chiral stationary phases using CO_2-based eluents containing non-conventional polar modifiers such as dichloromethane, chloroform, tetrahydrofuran, 2-methyl tetrahydrofuran, methyl *tert*-butyl ether, cyclopentyl methyl ether, acetone, ethyl acetate, toluene, 2,2,2-trifluoroethanol, and *N*,*N*-dimethylformamide. Screening experiments and method development for the commercial racemates on the immobilized columns with the non-conventional solvents demonstrated an ability to adjust the retention and improve resolution. From these results we were able to assign a general eluotropic relationship between the non-conventional solvents and methanol. A general ability to selectively adjust chromatographic retention while improving analyte solubility can lead to improved preparative chromatographic performance.

1. Introduction

The vast majority of drug candidates are chiral, and most are produced and evaluated as single enantiomers, as the undesired enantiomer can often have very different pharmacological activity and/or toxicity [1]. Developing an asymmetric synthesis can be both very labor and time consuming and, in the early stages of drug development, a chromatographic chiral separation can sometimes be a faster way to access the pure enantiomer, allowing savings in time, resources and money [2,3]. Supercritical fluid chromatography (SFC) using carbon dioxide (CO_2) based eluents has become the preferred technique for the resolution of racemic molecules to aid in the rapid development of drug candidates [4]. While the technique is nearly universal, some drug candidates and intermediates are not well suited for SFC resolution, most notably those compounds that are poorly soluble in the conventional co-solvents used in chiral SFC (methanol, ethanol, 2-propanol, and acetonitrile) and/or when the carbon dioxide is added to the sample during separation. Chlorinated solvents have sometimes been used to aid in the dissolution of samples with poor solubility characteristics; however, this is a somewhat dangerous game, as too much chlorinated solvent in the diluent or eluent can lead to leaching of many of the adsorbed stationary phases that are routinely used for chiral SFC separations [5].

In recent years, immobilized analogs of these coated stationary phases, as well as some novel phases, have been introduced, widely expanding the range of solvents that can be used, and virtually eliminating the problem of stationary phase leaching due to injection solvent or eluent [6–8]. The resulting columns, Chiralpak IA, IB, IC, ID, IE, and IF, have significantly expanded the range and performance of chiral SFC [9]. Some research has been published evaluating the use of non-conventional solvents with HPLC [10,11]. While these columns are routinely used in chiral SFC analysis, information on the methodical evaluation of the use of these new column/solvent combinations in the SFC mode remains somewhat scarce [12–14].

In this report we describe the systematic investigation of a group of nine commercially available racemates on six immobilized chiral stationary phase columns, using eleven non-conventional solvents and solvent mixtures. The utility of these non-conventional polar modifiers for increasing/decreasing retention and addressing solubility and/or stability issues for both

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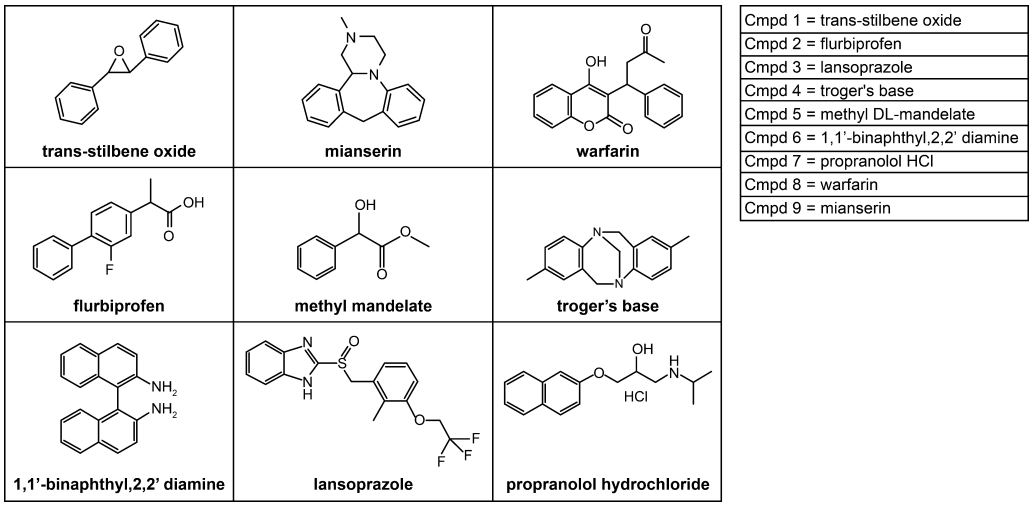

Fig. 1. Nine commercial racemates used in the study.

analytical and preparative supercritical fluid chromatography is demonstrated.

2. Experimental

2.1. Chemicals

Food grade (99.8%) carbon dioxide was purchased from Praxair (Woodbridge Township, NJ, USA). Fourteen different solvents were used for the solvent screening. Methanol, acetone, methyl *tert*-butyl ether, and tetrahydrofuran were purchased from Fisher Scientific (Waltham, MA, USA). Isopropanol, dichloromethane, chloroform, ethyl acetate, toluene, cyclopentyl methyl ether, 2-methyl tetrahydrofuran, 2,2,2-trifluoroethanol, and *N*,*N*-dimethylformamide were purchased from Acros Organics (Geel, Belgium). Nine commercially available racemates were used for the racemic compound screening set. *Trans*-stilbene oxide was obtained from Acros Organics (Geel, Belgium). Flurbiprofen, Troger's Base, methyl DL-mandelate, 1,1′-binaphthol-2,2-diamine, and propranolol hydrochloride were obtained from Sigma–Aldrich (St. Louis, MO, USA). Lansoprazole and mianserin were obtained from CNW Technologies GmbH (Düsseldorf, Germany). Warfarin was obtained from Pfaltz & Bauer (Waterbury, CT, USA). 1,3,5-Tri-*tert*-butyl benzene, our void marker [15], was obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Chiral stationary phases

The analytical columns that were used for this study (Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak ID, Chiralpak IE, and Chiralpak IF) were purchased from Chiral Technologies (West Chester, PA, USA). Dimensions and particle size for all columns were 4.6 mm × 250 mm I.D., 5 μm. Before each column was used, it was flushed with 40% 2-propanol/CO_2 for 15 min to remove the solvents that it contained during shipment. See Ref. [9] for the structure of the selectors for each bonded column.

2.3. Racemic sample preparation

A stock solution of each racemic compound was prepared by dissolving 250 mg of racemate and 25 mg of 1,3,5-tri-*tert*-butyl benzene (TTBB) to 25 mL of methanol in a volumetric flask to obtain a concentration of 10 mg/mL racemate:1 mg/mL TTBB. For warfarin, 7 mL of acetone was also added to completely solubilize the racemate to obtain a stock solution with a concentration of 7.7 mg/mL warfarin: 0.77 mg/mL TTBB. For 1,1′-binaphthol-2,2-diamine, 8 mL of dichloromethane was also added to completely solubilize the racemate to obtain a stock solution with a concentration of 7.5 mg/mL 1,1′-binaphthyl-2,2-diamine: 0.75 mg/mL TTBB.

2.4. Instrumentation

The SFC instrumentation used in the studies was an Aurora Fusion A5 coupled to an Agilent 1100 series HPLC. The instrument consists of a CO_2 booster, an automatic nozzle back pressure regulator, a CO_2 pump, a modifier pump, an autosampler, a column oven with a six position column selection valve, a two position solvent switching valve, a degasser, a photodiode array UV detector and Chemstation™ software which controls the instrument and data processing.

Non-Conventional Solvent	Є° on Silica
Methanol	0.70-0.73
Dichloromethane	0.30-0.32
Chloroform	0.26
Methyl *tert*-butyl ether	0.48
Acetone	0.47-0.53
Ethyl Acetate	0.38-0.48
Tetrahydrofuran	0.53
Methyl Tetrahydrofuran	--
2,2,2 Trifluoroethanol	--
Cyclopentyl methyl ether	--
Toluene	0.22
N,N-Dimethylformamide	--

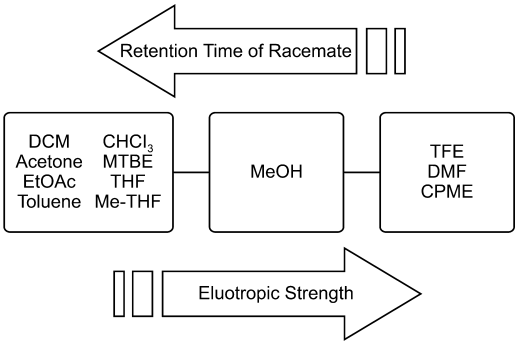

Fig. 2. Eluotropic strength values on silica for HPLC (Ref. [21]) and relative eluotropic strength of non-conventional polar modifiers for chiral SFC.