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A Screening Process for Development of Enantioselective Supercritical Fluid Chromatography Separation Methods

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The selection of mobile phase modifiers in enantioselective supercritical fluid chromatography using the new generation of immobilized polysaccharide chiral stationary phases (CSPs) is discussed. A method development protocol and the identification of the most useful solvents are addressed.

The choice of mobile phase modifier in enantioselective SFC has now expanded dramatically due to the solvent stability of the new generation of immobilized polysaccharide phases. In order to determine which of the many possible solvents are most useful in rapid development of separation methods, screening of a variety of solutes with most of the possible solvents was carried out.

Experimental

Columns packed with 5 micron CHIRALPAK® IA™, CHIRALPAK IB™ and CHIRALPAK IC™, all 4.6 mm i.d. × 250 mm long (Daicel Chemical Industries, Ltd.), were used throughout. A flow rate of 5 ml/min with back pressure of 150 bar at 35 °C were used. A Berger Minigram SFC system fitted with autosampler and diode array detector was employed.

A set of 39 compounds were studied, chosen such that additives were not required in order to simplify these first experiments.

Results

The solvents studied were methanol (MeOH), 2-propanol (IPA), acetonitrile (ACN), tetrahydrofuran (THF), methyl tertiary butyl ether (MTBE), chloroform (CHCl₃), methylene chloride (CH₂Cl₂) and methyl acetate (MeOAc). Several of these required addition of methanol to allow elution of the more polar compounds in the sample set. Table I shows a summary of the separation success rates for the various solvents, while Table II shows a summary of success rates for the different CSPs. In these tables, partial separation refers to those separations with resolution less than 1.5 but with a measurable selectivity. Complete separations are to baseline. 93% of the compounds studied could be separated.

Since many SFC units are not equipped with solvent selection valves to allow automated screening, it was of interest to determine the smallest number of solvents and in which order they should be screened to maximize the chance of finding a separation quickly. The conclusions are shown in Table III. THF was found to be the most useful solvent, followed by MTBE (containing some methanol to adjust the solvent strength) and then by the alcohols, MeOH and IPA.

Table I: Separation Success Rates (%) — Solvents

Solvent	Full	Partial	Total
MeOH	41	22	63
IPA	42	36	78
MeOAc	24	52	76
THF	47	33	80
ACN	30	41	71
MTBE	45	39	84
CH ₂ Cl ₂	46	22	68
CHCl ₃	37	34	71

Table II: Separation Success Rates (%) — CSPs

CSP	Full	Partial	Total
CHIRALPAK IA	45	37	82
CHIRALPAK IB	21	45	66
CHIRALPAK IC	47	34	81
Global	61	32	93

Table III: Screening Solvents

Co-Solvent	CO ₂ (%)
THF	25
20% MeOH in MTBE	25
MeOH	20
IPA	20

Conclusions

Separation screening success rates in enantioselective SFC can be maximized by the use of four solvents and three immobilized chiral stationary phases. Enantioselective recognition was achieved with 93% of the samples studied. The most useful solvents for initial screening were identified.

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