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Strong cation exchange-type chiral stationary phase for enantioseparation of chiral amines in subcritical fluid chromatography

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ABSTRACT

A new strong cation exchange type chiral stationary phase (SCX CSP) based on a syringic acid amide derivative of *trans*-(*R*, *R*)-2-aminocyclohexanesulfonic acid was applied to subcritical fluid chromatography (SFC) for separation of various chiral basic drugs and their analogues. Mobile phase systems consisting of aliphatic alcohols as polar modifiers and a broad range of amines with different substitution patterns and lipophilicity were employed to evaluate the impact on the SFC retention and selectivity characteristics. The observed results point to the existence of carbonic and carbamic acid salts formed as a consequence of reactions occurring between carbon dioxide, the alcoholic modifiers and the amine species present in the sub/supercritical fluid medium, respectively. Evidence is provided that these species are essential for affecting ion exchange between the strongly acidic chiral selector units and the basic analytes, following the well-established stoichiometric displacement mechanisms. Specific trends were observed when different types of amines were used as basic additives. While ammonia gave rise to the formation of the most strongly eluting carbonic and carbamic salt species, simple tertiary amines consistently provided superior levels of enantioselectivity. Furthermore, trends in the chiral SFC separation characteristics were investigated by the systematic variation of the modifier content and temperature. Different effects of additives are interpreted in terms of changes in the relative concentration of the transient ionic species contributing to analyte elution, with ammonia-derived carbamic salts being depleted at elevated temperatures by decomposition. Additionally, in an effort to optimize SFC enantiomer separation conditions for selected analytes, the impact of the type of the organic modifier, temperature, flow rate and active back pressure were also investigated.

1. Introduction

In the last decades, chromatographic separation of chiral compounds has developed into the preferred technology for analytical assessment of stereochemical purity and preparative separation of enantiomers [1]. In context with these applications, HPLC and in particular SFC have gained considerable popularity, and are routinely employed to address separation challenges in academic and industrial settings [2,3]. Supercritical fluid was first applied as a mobile phase for chromatographic separation in early 1960s [4]; however, the method was in the following decades outperformed by HPLC. For quite some time SFC was considered as a niche separation technique. The current rapid development of SFC can be mainly ascribed to the development of a new generation of reliable and compact instruments, which enable to take the main advantage of SFC over HPLC – its environmental friendliness (less organic solvents and short analysis times) and high separation efficiency. Generally, supercritical fluids have lower viscosities and better diffusion properties than usual liquids, thus considerably reducing the elution time [5].

Currently, carbon dioxide (CO_2) is the most popular medium employed in SFC, due to its conveniently accessible critical parameters (31 °C, 74 bar). As a serious limitation, it usually lacks sufficient solvent strength to elute medium to highly polar compounds. Thus the addition of polar organic modifiers is usually required to achieve adequate elution strength. The presence of these co-solvents, however, shifts the inherent critical parameters to higher pressure/temperature figures. Therefore, the majority of today's separations in SFC mode are actually performed under subcritical

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conditions (subFC), yet the term SFC used also for subcritical applications is generally accepted. Operation in the subcritical regime is comparably convenient as phase separation hardly occurs [6]. In case of ionizable compounds, such as bases and acids, additives may be required to facilitate reasonable retention and elution times, respectively. Basic additives are frequently employed to mask residual silanol groups present on the silica-based stationary phase thus suppressing the interaction of basic analytes with these groups and improving the overall peak shape [7,8]. Occasionally, improved selectivity has been achieved upon addition of basic additives [9,10].

Other than in conventional HPLC, chromatographic parameters in SFC are also influenced by pressure [11]. This behaviour is a consequence of pronounced variations in fluid density, viscosity and polarity occurring upon pressure changes, affecting the interactions of analyte with the stationary phase. Under extreme conditions, these phenomena are claimed to lead for SFC separations to two distinct chromatographic regimes: (i) the so-called GC-like mechanism, in which retention is brought about exclusively by interaction of the analyte with a stationary phase and only little contribution of the mobile phase; and (ii) the LC-like mechanism, which involves both the interaction of analyte with a solid stationary phase in concert with the mobile phase accompanied by a change of solvent strength [12]. An additional complication in SFC resulting from the compressible nature of the medium is the fact that the diminishing pressure drop along the column leads to an expansion of the mobile phase [13].

Enantioseparation of chiral basic compounds under SFC conditions has been reported with many different chiral stationary phases ranging from polysaccharide-type [14,15], Pirkle-type [16] and chirobiotic-type [17] to cyclodextrin-type [18] CSPs, and chiral selectors derived from chiral synthetic polymers [3,19,20]. Concerning the class of enantiomers separated, chiral bases are of particular interest because of their frequent applications as fine chemicals, catalysts and pharmaceutically relevant ingredients. Resolution of chiral bases in the HPLC mode has been achieved with a broad variety of chiral stationary phases [21–26], with polysaccharide-type phase showing the widest scope of application. However, basic compounds are ionizable and generally require basic additives to suppress protonation. In contrast, chiral stationary phases operating on cation exchange principles (SCX) are suitable to recognize and separate basic analytes in their protonated form, offering for certain application striking advantages over conventional CSPs [27]. In context with SFC applications, however, ion exchange-type CSPs have been considered being a poor choice, due to the perceived limitation of realizing mobile phases exerting sufficient elution strength for the ionic species. In a recent report, our group has demonstrated that SFC separation of acidic chiral analytes can indeed be achieved also with anion exchange-type CSPs [28].

In this contribution we present enantiomer separations of basic amine-type compounds in SFC mode, applying a strong cation exchange-type CSP (Fig. 1). We provide evidence that on this CSP analyte retention and elution under SFC conditions using alcohols as polar modifiers and amines as additives is based on classical ion exchange principles, following a stoichiometric displacement model. The specific trends found with various basic additives in SFC are discussed. The impact of temperature and flow rate on the enantioseparation characteristics of some model compounds is presented. Based on the combined experimental evidence emerging from these studies, the nature of *in situ* formed transient carbonic and carbamic acid salt species will be discussed, along with their crucial contributions to the elution of basic analytes from the investigated SCX type CSP.

Upfront it should be stressed that for enantiomer separation based on an ion-pairing mechanism it is essential that besides the electrostatic interactions also additional intermolecular binding forces between the chiral selector (SO) and the chiral selectands (SAs) must come into play [29].

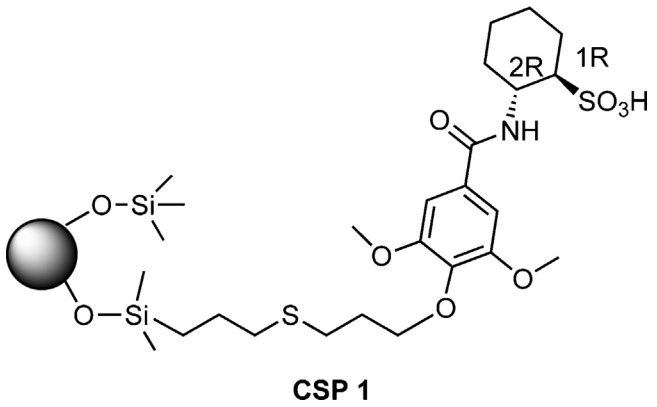

Fig. 1. Strong cation-exchanger (SCX) chiral stationary phase (CSP) used in this study.

2. Materials and methods

2.1. Materials

The HPLC grade solvents were purchased from VWR (Darmstadt, Germany) or from Sigma–Aldrich (Vienna, Austria). Standard quality carbon dioxide was obtained from Air Liquide Austria (Schwechat, Austria). Mobile phase additives (formic acid and amines) were either from Sigma–Aldrich or Fluka. Chemicals used for synthesis were purchased from Sigma–Aldrich (Vienna, Austria). Solvents used for synthesis were of HPLC grade or technical grade from VWR (Darmstadt, Germany). Analytes were either commercially available or prepared in house in frame of other projects.

2.2. Instrumentation and chromatography

2.2.1. HPLC

The chromatographic measurements with HPLC were carried out on an 1100 Series HPLC from Agilent Technologies equipped with a binary pump, a degasser, an auto sampler, a solvent tray and a multiple wavelength detector. The flow rate was usually 1 mL/min and the injection volume was 5 or 10 μL. The sample concentration was 1–2 mg/mL and the void volume was determined by injecting a methanolic solution of acetone.

2.2.2. SFC

The chromatographic measurements with SFC were carried out on a Thar Instruments Method Station System equipped with a Fluid Delivery Module (FDM-10), an auto sampler, a column oven, a VWD-detector and a Back Pressure Regular (BPR-20) (Waters). Additional measurements were done on an ACQUITY UltraPerformance Convergence Chromatography (UPC^2) System equipped with a binary solvent manager, a sample manager with fixed loop, a convergence manager, a column manager (CM-A) and a PDA detector (Waters). The injection volume was 5–10 μL. If not stated otherwise the back pressure was set to 150 bar and the temperature to 40 °C. The sample concentration was 2–3 mg/mL. The void volume in SFC was calculated from the first disturbance of the base line on injection of pure methanol. The detector wavelength was 254 nm in both HPLC and SFC modes.