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Chiral separation of new designer drugs (Cathinones) on chiral ion-exchange type stationary phases

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ABSTRACT

We present the enantioseparation of new designer drugs from the cathinone family on structurally different chiral ion-exchange type stationary phases. A novel strong cation-exchange type chiral stationary phase was synthesized and its performance compared with previously reported ion-exchange type chiral stationary phases. The influence of structural elements of the chiral selectors on their chromatographic performance was studied and the possibilities of tuning chromatographic parameters by varying the polarity of the employed mobile phases were determined. Evidence is provided that a change in mobile phase composition strongly influences the solvation shell of the polarized and polarizable units of the selectors and analytes, as well as ionizable mobile phase additives. Furthermore, the structural features of the selectors (e.g. the size of aromatic units and their substitution pattern) are shown to play a key role in the effective formation of diastereomeric complexes with analytes. Thus, we have achieved the enantioseparation of all test analytes with a mass spectrometry-compatible mobile phase with a chiral strong cation-exchange type stationary phase.

1. Introduction

In recent years, the increasing activity of clandestine chemists has led to a broad spectrum of new designer drugs. These new substances belong to large families of cannabinoids, cathinones, phenethylamines, amphetamines, and tryptamines all of which are known for their psychoactive effects. These designer drugs are available under various disguises, such as "plant-growth fertilizers", "bath-salts" and "spices" not only illegally on the black market, but also legally on the Internet. Due to the increasing consumption of these substances, overdose cases and reports of severe side effects have become more frequent worldwide [1–3].

The rising number of available psychoactive substances makes it essential to identify new compounds. Recently, well-established methods such as gas chromatography (GC) coupled with mass spectrometry (MS) [4] or high-performance liquid chromatography (HPLC) with UV-characterization [5] have been replaced by HPLC-MS methods that use various MS detection techniques [6–14]. Moreover, the structure-properties relationships of the new designer drugs have been intensively studied and summarized in monographs and reviews [15,16]. Indeed, great effort has already been invested into the precise analysis of a number of new designer drugs and their metabolites, but their enantioseparation, especially in LC–MS mode, is far from being routinely available.

New designer drugs, in particular of the amphetamine and cathinone families, are usually available as racemic mixtures. Chiral analysis of these illicit drugs could provide further information on the synthetic route which, in turn, may help to identify the country and place of origin [17]. Furthermore, enantiomers of drugs, licit as well as illicit, can follow different metabolic pathways in the living organism, and, thus, may have also a substantially different pharmacologic effect [18]. Both of these issues make the enantioseparation of the new designer drugs an important topic.

Currently, a plethora of chiral stationary phases (CSPs) can be employed for efficient enantioseparation [19,20]. The methodology for the chiral separation of various designer drugs has already been established; methods involving GC [21,22], capillary electrophoresis [23–25], capillary electrochromatography [26], HPLC,

Abbreviations: AcOH, acetic acid; ACN, acetonitrile; c-SCX, chiral strong cation exchanger; DEA, diethyl amine; FA, formic acid; MeOH, methanol; SA, selectand (analyte); SO, selector; TEA, triethyl amine; TFA, trifluoroacetic acid; ZWIX, zwitterion ion exchange-type stationary phase.

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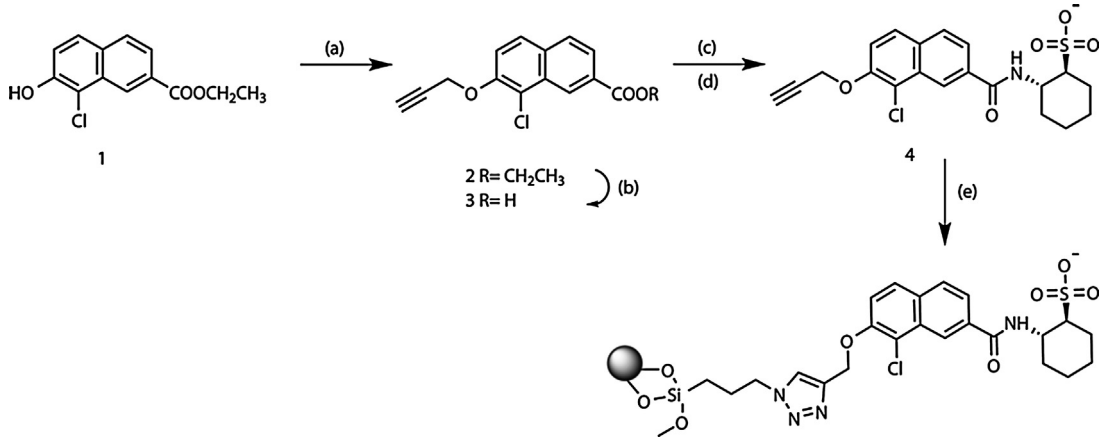

Scheme 1. (a) propargyl bromide, potassium carbonate, dry DMF, 60 °C, 12 h; (b) 1. aqueous EtOH/NaOH, reflux, 1 h, 2. aq. HCl; (c) oxalyl chloride, reflux; (d) (*S,S*)-2-aminocyclohexanesulfonic acid, CH_2Cl_2, stirring 48 h, N_2; (e) CuI, *N,N*-diisopropylethylamine, acetonitrile, 72 h.

and supercritical fluid chromatography [27] have all been reported. In this context, CSPs based on chiral crown ethers [28,29], cyclodextrines [30–32], proteins [33], antibiotics [31], and, more recently, polysaccharides [34] have been mainly used for the enantioseparation of amphetamines and cathinones.

The majority of these compounds are basic in nature and, thus, can be easily ionized. This behaviour makes them ideal analytes for chiral cation exchange type stationary phases. Generally, chiral ion exchangers operate under polar (hydro) organic mode conditions with weakly acidic mobile phases and volatile buffers [35]. Thus, they are fully compatible with MS detection techniques [36]. Moreover, because the optimization of the mobile phase composition is more flexible, ion exchangers often offer striking advantages for enantiomeric resolution over conventional phases [37]. Despite these inherent advantages, chiral ion exchangers have not yet been used for the enantioseparation of new designer drugs.

In this contribution, we present the chiral HPLC separation of a series of cathinones using three types of CSPs: a commercially available chiral zwitterion ion exchanger [Chiralpak ZWIX (+)]; a chiral strong cation exchanger (c-SCX) based on syringic acid [38]; and a novel naphthalene-based c-SCX. To compare the chromatographic performance of the naphthalene-based c-SCX with the other two CSPs, it is also tested for chiral separation of β-blockers and antimalarials. It should be stressed that, for enantioseparation and chiral recognition on an ion exchanger, it is essential that, apart from the non-directional electrostatic forces, additional, directional intermolecular interactions between the chiral selector (SO) and the chiral selectands (SAs) come into play [19]. To maximize such SO–SA interactions, the optimization of a selector structure is an essential tool in the development of new CSPs. We discuss the structural features necessary for the successful enantioseparation of the studied cathinones.

2. Materials and methods

2.1. Materials

Solvents and chemicals for synthesis were purchased either from VWR (Vienna, Austria), Carl Roth GmbH (Karlsruhe, Germany) or Sigma Aldrich (Vienna, Austria). Silica gel for flash chromatography was purchased from Merck (Darmstadt, Germany). Silica gel from Daiso Fine Chem GmbH (Düsseldorf, Germany) was azidopropyl-modified for the novel CSP [39]. Reaction monitoring was performed with thin-layer-chromatography (TLC) (Silica gel, 60 F_{254} 20 × 20). NMR solvents were purchased from Deutero GmbH (Kastellaun, Germany) and the spectra were analyzed with SpinWorks software. HPLC grade solvents were purchased from Fischer Scientific (Pardubice, Czech Republic). Mobile phase additives were obtained from Sigma Aldrich (Prague, Czech Republic).

CSP I (150 × 4 mm, 5 μm) was synthesized as described previously [38] (selector loading 180 μmol/g) and packed in house. CSP II is similar to the commercially available column Chiralpak ZWIX (+). However, in order to directly compare the performance of the chiral ZWIX column (CSP II) with CSP I, the chiral zwitterionic selector was immobilized onto 5 μm spherical silica (selector loading 240 μmol/g) and packed in house into a stainless steel column (150 × 4 mm). CSP III (150 × 4 mm, 5 μm) with the selector loading of 120 μmol/g represents a novel c-SCX-type CSP.

2.1.1. Synthesis of CSP III

The synthetic concept of the selector to be bound to the pre-activated silica is depicted in Scheme 1. The substituted naphthalene ring **1**, which represents the central unit of CSP III, was synthesized according to literature [40]. Similarly, the protected 2-aminocyclohexanesulfonic acid was prepared following a reported method [41]. The immobilization of the selector onto azidopropyl-modified silica by means of Huisgen cycloaddition reaction (click chemistry) was performed in accord with a previously described method for cation exchangers [42].

2.1.2. Ethyl 8-chloro-7-propargyloxynaphthalene-2-carboxylate (*2*)

To a suspension of ester **1** (1.43 g, 5.7 mmol) and dry potassium carbonate (1.38 g, 9.98 mmol) in dry DMF (80 mL), propargyl bromide (80% solution in toluene, 1.12 mL, 9.98 mmol) was added under inert atmosphere. The reaction mixture was heated up to 60 °C and stirred overnight. Then it was cooled down, diluted with distilled water (120 mL) and the resulting suspension was extracted with EtOAc (4 × 50 mL). The collected organic solution was washed with water (2 × 30 mL), brine (20 mL) and dried with magnesium sulphate. The solvent was evaporated, the structure of the product was verified by ^{1}H NMR and it was immediately subjected to the following reaction.

^{1}H NMR: 1.46 (t, 3 H, J = 6.7 Hz, CH_3), 3.65 (t, 1 H, ^4J = 2.3 Hz, HC≡C), 4.47 (q, 2 H, J = 6.7, CH_2) 4.95 (d, 2 H, J = 2.3 Hz, CH_2O), 7.34 (d, 1 H, J = 9.0 Hz, H-6), 7.74 (d, 1 H, J = 9.0 Hz, H-5), 7.80 (d, 1 H, J = 8.2 Hz, H-4), 7.98 (dd, 1 H, ^3J = 8.2 Hz, ^4J = 1.6 Hz, H-3), 8.97 (bs, 1 H, H-1). ^{13}C NMR: 166.6 (C=O), 152.2 (C_{Ar}), 131.5 (C_{Ar}), 131.3 (C_{Ar}), 129.2 (C_{Ar}), 128.3 (CH_{Ar}), 127.5 (CH_{Ar}), 126.5 (CH_{Ar}), 124.0 (CH_{Ar}), 118.1 (CH_{Ar}), 117.5 (C_{Ar}), 79.5 (C_q), 79.2 (CH), 61.2 (CH_2), 59.2 (CH_2), 14.4 (CH_3). Elemental analysis for $C_{16}H_{13}ClO_3$, calculated: C 66.56, H 4.54, Cl 12.28%; found: C 66.81, H 4.55, Cl 12.09%.