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Chiral separations of cathinone and amphetamine-derivatives: Comparative study between capillary electrochromatography, supercritical fluid chromatography and three liquid chromatographic modes☆

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ABSTRACT

The screening part of an earlier defined chiral separation strategy in capillary electrochromatography (CEC) was used for the separation of ten cathinone- and amphetamine derivatives. They were analyzed using 4 polysaccharide-based chiral stationary phases (CSPs), containing cellulose *tris*(3,5-dimethylphenylcarbamate) (ODRH), amylose *tris*(3,5-dimethylphenylcarbamate) (ADH), amylose *tris*(5-chloro-2-methylphenylcarbamate) (LA2), and cellulose *tris*(4-chloro-3-methylphenylcarbamate) (LC4) as chiral selectors. After applying the screening to each compound, ADH and LC4 showed the highest success rate. In a second part of the study, a comparison between CEC and other analytical techniques used for chiral separations i.e., supercritical fluid chromatography (SFC), polar organic solvent chromatography (POSC), reversed-phase (RPLC) and normal-phase liquid chromatography (NPLC), was made. For this purpose, earlier defined screening approaches for each technique were applied to separate the 10 test substances. This allowed an overall comparison of the success rates of the screening steps of the 5 techniques for these compounds. The results showed that CEC had a similar enantioselectivity rate as NPLC and RPLC, producing the highest number of separations (9 out of 10 racemates). SFC resolved 7 compounds, while POSC gave only 2 separations. On the other hand, the baseline separation success rates for NPLC and RPLC was better than for CEC. For a second comparison, the same chiral stationary phases as in the CEC screening were also tested with all techniques at their specific screening conditions, which allowed a direct comparison of the performance of CEC versus the same CSPs in the other techniques. This comparison revealed that RPLC was able to separate all tested compounds, and also produced the highest number of baseline separations on the CSP that were used in the CEC screening step. CEC and NPLC showed the same success rate: nine out of ten substances were separated. When CEC and NPLC are combined, separation of the ten compounds can be achieved. SFC and POSC resolved eight and three compounds, respectively. POSC was the least attractive option as it expressed only limited enantioselectivity toward these compounds.

1. Introduction

Amphetamine, a potent central nervous system (CNS) stimulant, is used to treat attention deficit hyperactivity disorder (ADHD) syndrome [1,2]. Its derivatives have been abused as recreational drugs and utilized as illegal euphorients. The risk of dependence is high, so many countries issued strict regulations to control their consumption and reduce amphetamines abuse. For example, amphetamine is categorized as class B in the United Kingdom, while it is in class I in the Canadian legislations. Also, in the European Union, these substances are classified as illicit drugs [3–5]. Amphetamine and its derivatives formed the classical, illegal drugs of abuse for many years, but are recently replaced by a new legal alternative substance

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class, the cathinone-derivatives, to subvert drug regulatory law [6]. Cathinone is a natural beta-keto amphetamine analogue that forms the main component of the leaves of *Catha edulis* "Khat" plant [6]. Because of their amphetamine-like stimulant effects, cathinone-derivatives abuse has grown significantly worldwide [7–9]. The increased abuse of such substances may refer to the unspecified places of their production; which usually are clandestine laboratories that could be organized anywhere. These new substances lack of officially recognized names in different regulations because most of them are new and often unknown, hence facilitating their illicit use by individuals [10–12]. Cathinone-derivatives are considered "legal highs", as they are usually sold under different names such as "plant fertilizer", "bath salts" or "research compounds" along with the indication "not for human use", to circumvent the law [13].

Stereochemical features of amphetamine and cathinone derivatives make most of them chiral and their enantiomers may exhibit different pharmacological effects and different potencies on living organisms. For example, the S(+)-enantiomer of amphetamine is a more potent stimulant than its R(−)-enantiomer [14,15]. Many amphetamine derivatives used as illicit drugs also are chiral molecules [16,17] but only little is known about the pharmacology of racemates and enantiomeric forms. For cathinone derivatives, also limited toxicological and pharmacological data are available because of the their novelty [18]. Performing enantioseparations of amphetamine derivatives and the relatively new class of cathinone derivatives may provide preparative methods that allow obtaining substantial amounts of individual enantiomers, which can then be used in further pharmacological studies. Such chiral separation methods can also be used to indicate the synthesis pathway, which in turn may provide information about the lab of origin and facilitates their tracking. Consequently, developing chiral separation methods for amphetamine and their derivatives may be recommended [19]. Different enantioselective methods for the enantioseparation of amphetamine and its derivatives have been developed using several analytical techniques, such as gas chromatography (GC) [20,21], capillary electrophoresis (CE) [22–24], high-performance liquid chromatography (HPLC) [18,25] and more recently capillary electrochromatography [26].

Despite the availability of different types of chiral stationary phases, occasionally with broad chiral discrimination ability, developing a chiral separation is still not an easy task [27,28]. Small changes in solute structure or/and in the chromatographic/electrophoretic environment often have large effects on the chiral resolution ability of many CSPs. In addition, it is difficult to predict which CSP could be suitable for the enantioseparation of a given chiral molecule and most of the time one relies on a trial-and-error approach, which is a time-, labor- and money-consuming process [29]. To facilitate the development of a chiral separation method in a systematic manner, a generic chiral separation strategy may be proposed. In a first step, called screening, such strategy gives rapidly an idea about the enantioselectivity of a limited set of chromatographic systems for a given compound. Based on the outcome from the screening step, an optimization step could follow, either to optimize the analysis time or to produce a baseline separation. These strategies are intended to be generic, i.e., applicable on diverse molecules. Different strategies have been defined for many separation techniques, such as normal phase liquid chromatography [30], reversed phase liquid chromatography [31], polar organic solvents chromatography [32], supercritical fluid chromatography [33] and also for capillary electrochromatography [34].

The considered chiral separation strategies are all based on utilizing polysaccharide-based CSPs, which are characterized by a broad enantioselectivity and are applicable under CEC, SFC and HPLC conditions [35–37]. These phases provide different sites that contribute to the interaction with the analyte during separation [38]. Enantiomers are discriminated by enantioselective inclusion into chiral cavities, by formation of hydrogen-bonding, dipole–dipole and dipole-induced dipole interactions, steric and π–π interactions [39]. Chlorinated polysaccharides-based CSPs have been used more recently to update existing strategies [31–34,40]. The presence of a chlorine atom increases the number of possible interaction sites available for chiral analysis, due to modifications in the higher order structure of the polymeric selector. Furthermore, more interaction sites are provided through an increased polarity of the stationary phase.

In this study, a set of ten cathinone- and amphetamine derivatives were separated into their enantiomers using five techniques, i.e., supercritical fluid chromatography, polar organic solvents chromatography, reversed-phase and normal-phase liquid chromatography, and capillary electrochromatography, according to the screening approaches defined earlier [30–34]. To our knowledge, this is the first time that the enantiomers of these compounds are separated using all above mentioned approaches. This allows the comparison of the screening step results of the 5 techniques for the separation of these amphetamine derivatives. Since these protocols do not always contain the same CSPs as those tested in CEC, the screening phases of CEC which were not included in the other screening steps, were also tested using their specific screening conditions. This allowed a fair comparison between all techniques, i.e., how the chiral phases perform in CEC compared to other analytical techniques, on the one hand, and how the different techniques/strategies perform for a given set of compounds on the other. The success rates achieved after applying the screening steps of the five techniques will be compared. Finally, it will be evaluated how one could come to the chiral separation of the ten cathinone and amphetamine derivatives in the most economic/rational way.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade *n*-heptane was purchased from BDH (Poole, UK). HPLC-grade acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), 2-propanol and 0.1 N hydrochloric acid were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). 0.1 M sodium hydroxide solution, thiourea, disodium hydrogen phosphate and sodium dihydrogenphosphate monohydrate (analytical reagent grade) were obtained from Merck (Darmstadt, Germany). Sodium tetraborate decahydrate (borax, $Na_2B_4O_7{\cdot}10H_2O$), trifluoroacetic acid (TFA), potassium hexafluorophosphate (KPF_6), and isopropylamine (IPA) were obtained from Sigma–Aldrich (Steinheim, Germany). Diethylamine (DEA) was from UCB (Brussels, Belgium). Carbon dioxide (CO_2) quality 4.5 (purity $\geq$ 99.995%) was obtained from Messer (Sint-Pieters-Leeuw, Belgium). Ultra-pure water for the preparation of the mobile phases was produced in-house by an Arium® Pro UV instrument (Sartorius Stedim Biotech, Vilvoorde, Belgium).

The polysaccharide-based CSPs used were Chiralcel ODRH (ODH), Chiralcel OZH and Chiralpak ADRH (ADH) obtained from Chiral Technologies (Illkirch, France). Lux Amylose 2 (LA2), Lux Cellulose 4 (LC4), Lux Cellulose 2 (LC2), Lux Cellulose 1 (LC1), Lux Cellulose 3 (LC3) and Sepapak 5 (SP5) were purchased from Phenomenex (Utrecht, The Netherlands).

The same column dimensions, 25 cm × 4.6 mm i.d, with 5 μm particle size, were used in all SFC and HPLC experiments. In CEC, the chiral stationary phases were packed in fused-silica capillaries (total length 33.5 cm; 25 cm packed) with dimensions of 100 μm i.d. and 375 μm o.d (Polymicro Technologies, supplied by CM Scientific, Worcestershire, UK).