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Enantioselective analysis of the methamphetamine precursors ephedrine and pseudoephedrine by capillary electrokinetic chromatography using cyclodextrins as chiral selectors

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ABSTRACT

Ephedrine and pseudoephedrine can be used as precursors for the illicit synthesis of stimulant drugs, such as methamphetamine and methcathinone. For this reason, an enantioselective capillary electrokinetic chromatography method was developed for the separation of ephedrine and pseudoephedrine enantiomers. After testing two different charged or chargeable cyclodextrins (*β*-cyclodextrin sulphate and carboxymethyl-*β*-cyclodextrin) and a few mixtures as possible chiral selectors, a mixture of 3 mM carboxymethyl-*β*-cyclodextrin and 3 mM heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin in pH 3.4 acetate buffer was used as the enantioselective background electrolyte and the separation was carried out in an uncoated silica capillary (75 μm internal diameter, 48.6 cm total length, 40.0 cm effective length). Analytical method performance was then evaluated in terms of linearity, precision, selectivity and accuracy with good results. Proof of concept application to mixtures simulating illicit methamphetamine (containing small amounts of ephedrine and pseudoephedrine as possible residual synthesis impurities) provided satisfactory results for the identification and quantitation of the four analyte diastereomers.

1. Introduction

In the long-term fight against the production, trafficking and dealing of drugs of abuse in modern-day societies, the need to monitor national and international movements of possible drug "precursors" has been recognised relatively late. In fact, the first mention of precursors within the frame of official international conventions dates back to 1988, in the first "United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances" [1], almost 30 years after the seminal 1961's Single Convention on Narcotic Drugs [2]. Even in the 1988 convention, the inclusion of precursors was debated and the first draft of the document did not mention them. Since then, however, all updates to the 1988 Convention up to the latest in 2020 [3] have included an annex with two distinct tables listing two kinds of precursors over which strict controls are mandated: "immediate precursors" (Table I of the Convention document) and "essential chemicals" (Table II, including solvents and other reagents). Among those only six "immediate precursors" listed in the 1988 Convention, both ephedrine (EPH, *eryhtro*-2-(methylamino)-1-phenylpropan-1-ol, Fig. 1a,b) and pseudoephedrine (ΨEPH, *threo*-2-(methylamino)-1-phenylpropan-1-ol, Fig. 1c,d) were already present [1].

The basic structure of 2-(methylamino)-1-phenylpropan-1-ol includes 2 stereogenic centres, potentially producing two pairs of diastereomers. Commonly, the term EPH corresponds to the (1*R*,2*S*)-(–)- and (1*S*,2*R*)-(+)- enantiomers, while ΨEPH refers to the (1*R*,2*R*)-(–)- and (1*S*,2*S*)-(+)- enantiomers. In nature, both ephedrines (EPHS), EPH and ΨEPH, are mainly found in plants of the *Ephedra* genus, whence the name, but also in some species of the *Sida* and *Pinellia* genera. These plants only produce the (1*R*,2*S*)-(–)-EPH and (1*S*,2*S*)-(+)-ΨEPH isomers, however some degree of racemisation can occur during storage of the corresponding products [4]. Nowadays most EPH and ΨEPH are synthesised and racemic mixtures are the norm from this source.

The 2-phenylethylamine structure of EPHS gives them significant *α*- and *β*-adrenergic activity when administered to humans: for example, EPH has been used therapeutically as a bronchodilator and ΨEPH is still present in nasal decongesting preparations [5]. Both have been also used in weight loss dietary supplements, but this use has been prohibited in most countries (e.g. since 2004 in the USA, since 2015 in the EU [6]) due to their intrinsic toxicity and potential for abuse. In fact, their structure closely resembles those of amphetamine ((*S*)-1-phenylpropan-

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Fig. 1. Chemical structures of: (a) (1*R*,2*S*)-(–)-ephedrine, (b) (1*S*,2*R*)-(+)-ephedrine, (c) (1*R*,2*R*)-(–)-pseudoephedrine and (d) (1*S*,2*S*)-(+)-pseudoephedrine.

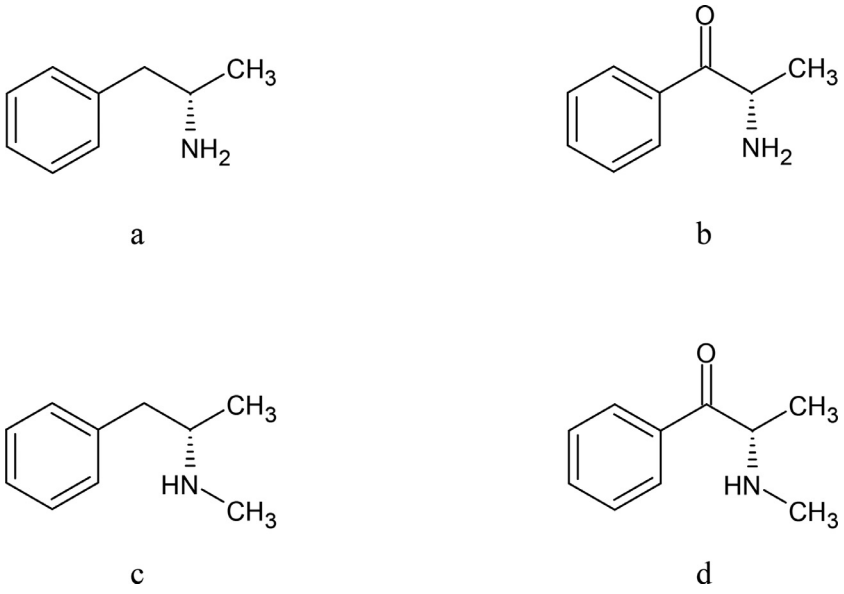

Fig. 2. Chemical structures of: (a) (*S*)-amphetamine, (b) (*S*)-cathinone, (c) (*S*)-methamphetamine and (d) (*S*)-methcathinone.

2-amine, Fig. 2(a) and cathinone ((*S*)-2-amino-1-phenylpropan-1-one, Fig. 2(b), and thus they can produce similar, albeit less potent, psychostimulant effects [7]. However, EPHS are even more important as precursors since their structural similarity and easy availability make them ideal candidates for the production of illicit stimulants. Simple elimination of the hydroxyl group in the natural (1*R*,2*S*)-(–)-EPH and (1*S*,2*S*)-(+)-ΨEPH products directly leads to the formation of the most potent enantiomer (*S*)-(+)-methamphetamine (Fig. 2c; there is only one product since one chiral carbon loses its stereogenicity in the reaction), while oxidation of the same function brings about (*S*)-(+)-methcathinone (Fig. 2d).

Clandestine methamphetamine synthesis is relatively simple and is based on six major routes of production, using as precursor either phenyl-2-propanone (P2P) or EPH/ΨEPH. In the latter case, methods of illicit production involve protonation of the hydroxyl group on the EPH or ΨEPH molecule (Fig. 3). The “Nagai” route (Fig. 3a) involves red phosphorus and hydrogen iodide to reduce either EPH or ΨEPH to methamphetamine. Hydrogen iodide is replaced by iodine and water in the “Moscow” route (Fig. 3b). Some criminal groups have been known to substitute red phosphorus with either hypophosphorous acid or phosphorous acid (the "Hypo” route, Fig. 3c). The conceptually similar “Emde” route involves reduction of EPH to chloroephedrine followed by catalytic hydrogenation with palladium or platinum (Fig. 3d). Similarly, the “Rosenmund” route also uses hydrogen gas and a palladium catalyst (Fig. 3e). The Birch reduction, also called the “Nazi” or “Birch-Nazi” route, became popular in the mid-1990s and involves the reaction of ΨEPH with liquid anhydrous ammonia and an alkali metal such as sodium or lithium (Fig. 3f) [8].

Acknowledging this fact, since 2015 EPHS are included in EU’s precursor tables twice, both as the pure compounds themselves, and as medicinal or veterinary products containing them or their salts. In fact, the latter constitute the only entries of Category 4 of regulation UE n. 1259/2013 as products whose export outside the EU is strictly regulated [9].

Given the high potential of illicit EPHS production, trafficking and use, there is wide interest in their identification and quantification, also including their enantiomeric separation. Not only EPHS enantiomers themselves have considerably different stimulant potency, but the same do the corresponding amphetamines and cathinone enantiomers that can be synthesised starting from them. Moreover, interesting information on illicit drug synthetic strategies and drug sources or provenance can be gleaned from complete enantioselective analysis of either bulk EPHS, or their mixtures as such or as impurities in seized amphetamines or cathinones.

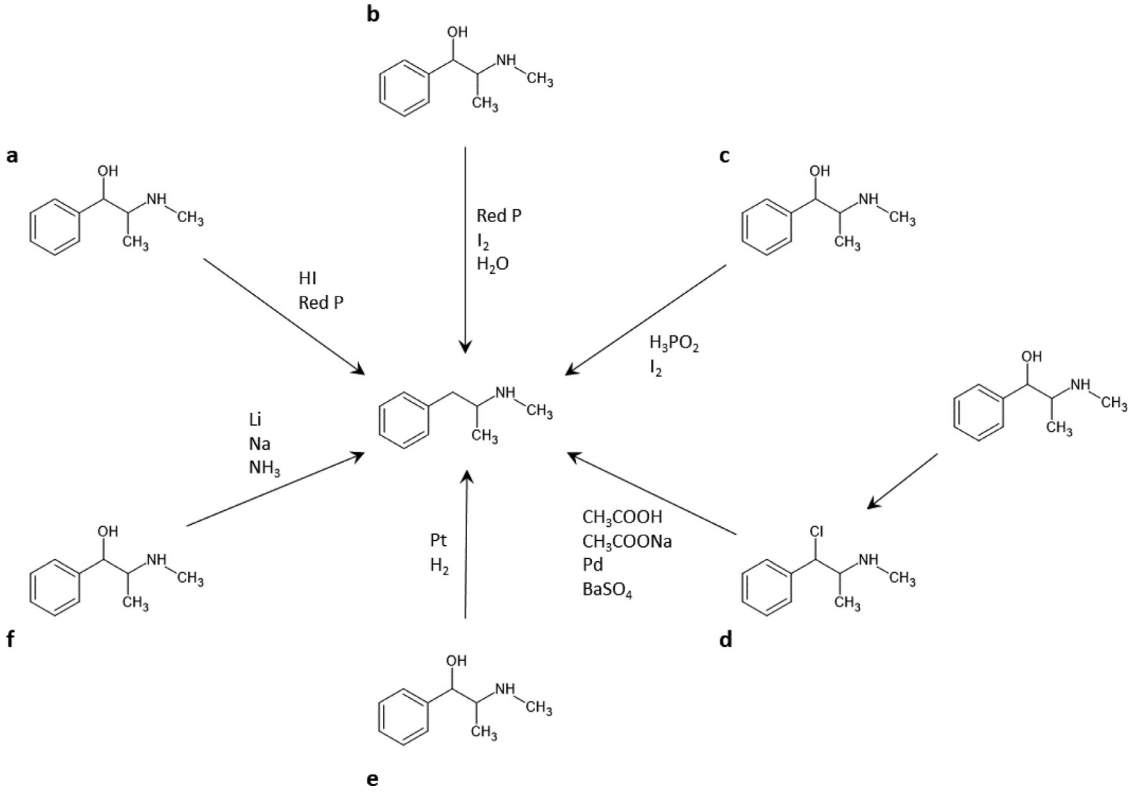

Fig. 3. Synthetic route of methamphetamine from EPH/ΨEPH: (a) Nagai route, (b) Moscow route, (c) Hypo route, (d) Emde route, (e) Rosenmund and (f) Nazi-Birch route.

Given this interest in the topic, it is no surprise that several scientific papers have been published on the enantioseparation and analysis of EPHS enantiomers as such or as illicit drug impurities. Chromatographic methods include LC-MS/MS with dedicated chiral carbohydrate-modified columns [10,11] and GC-MS with a γ-cyclodextrin (γ-CD)-modified capillary column [12]. As could be expected, electrodriven techniques are also well represented, as detailed in two recent reviews [13,14], including the use as chiral selectors of different kinds of native (α- [15,16], β- [16,17]) or modified (*O*-acetylated [17,18], *O*-methylated [16,19,20]) CDs, modified-surface (sulfonated) capillary wall [21], bovine serum albumin (BSA) [22].

Herein we report the study of different modified CDs than previously reported (namely, β-CD sulphate, carboxymethyl-β-CD and their mixtures with other CDs) for the chiral separation of EPH and ΨEPH enantiomers through capillary electrokinetic chromatography (CEKC), the performance evaluation of the resulting optimised method and its application to simulated illicit amphetamine samples.

2. Experimental

2.1. Chemicals and standard solutions

Stock solutions (1 mg/mL) of (*1S,2R*)-EPH hydrochloride, (*1R,2R*)-ΨEPH, (*1S,2S*)-ΨEPH, (*S*)-(+)-methamphetamine; carboxymethyl-β-CD (CM-β-CD, degree of substitution ≅ 3), heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), β-CD sulphate (S-β-CD, 12–15 mol per mol β-CD) sodium salt, all pure (> 99%) powders; 2-propanol (99.9%), sodium hydroxide pellets (> 97%), phosphoric acid (> 85%), glacial acetic acid (> 99%) were purchased from Merck (Milan, Italy). Methanol and acetonitrile (HPLC grade) as well as (*1R,2S*)-EPH and β-phenylethylamine (internal standard, IS) powder (> 99%) were obtained from Carlo Erba (Milan, Italy). Ultrapure water (18.2 MΩ cm) was obtained by means of a Milli-Q apparatus from Millipore (Milford, MA, USA). Sodium dihydrogen phosphate (> 97%) was bought from Fluka (Buchs, Switzerland). Stock solutions of (*1R,2S*)-EPH and the IS (1 mg/mL) were prepared by dissolving suitable amounts of pure powders in methanol. All standard working solutions were prepared daily by dilution with water. All solutions were stored protected from light in stoppered amber glass vials from Waters. All rinsing and conditioning solutions were filtered through a nylon syringe filter (pore size 0.45 μm) from Agilent (Santa Clara, CA, USA).

2.2. Instrumentation and conditions

All CEKC experiments were carried out using an Agilent ^{3D}CE apparatus. An uncoated, fused silica capillary (75 μm I.D., 375 μm O.D., 48.6 cm total length, 40.0 cm effective length) from Supelco (Bellefonte, PA, USA) was used. The separation of EPH and ΨEPH enantiomers was performed using a background electrolyte (BGE) composed of 150 mM, pH 3.4 aqueous acetate buffer containing 3.0 mM CM-β-CD and 3.0 mM DM-β-CD. Injection was carried out by pressure at the anodic end at 50 mbar for 10 s. The applied voltage was set at +25 kV and the capillary was thermostatted at 15.0 °C. The detection was carried out using a diode array detector (DAD) operating at 195±4 nm and 207±4 nm (the latter was used for quantitative purposes).

Before use, the new capillary was conditioned with 1 M sodium hydroxide, water, and then with the BGE for 10 min each. Before each run, the capillary was rinsed with BGE for 2.5 min; after each run, it was rinsed with water for 4 min and with BGE for 4 min. For storage overnight, the capillary was washed with water, 1 M sodium hydroxide and water again (rinsing time 5 min each).

2.3. Analytical characteristics of the method

The tested parameters were linearity (including limits of detection and limit of quantitation), precision, selectivity, stability and accuracy.

2.3.1. Linearity

Standard solutions of the four analytes at seven different concentrations, containing the IS at a constant concentration, were injected into the CEKC-DAD system. The analysis was carried out in triplicate for each concentration. The obtained analyte enantiomer / IS peak area ratios were plotted against the corresponding concentrations and the calibration curves were obtained by means of the least-squares method. LOQ and LOD were determined experimentally as the analyte concentrations which gave rise to peaks whose areas were 10 and 3 times the baseline noise, respectively.

2.3.2. Retention time and peak area reproducibility

Reproducibility was evaluated by repeatedly preparing and analysing standard solutions at four different, known concentrations (LOQ, 0.25 μg/mL, 1 μg/mL and 10 μg/mL) and containing IS at 1 μg/mL. The solutions were prepared six times in the same day to obtain intraday precision and six times over six different days to obtain interday precision, expressed as percentage relative standard deviation (RSD%), of both analyte retention times and peak areas.

2.3.3. Stability

To test analyte stability, standard solutions of racemic EPH and ΨEPH at two concentration levels (high and low concentrations with respect to the calibration curve), were stored protected from light in stoppered amber glass vials at 4 °C. At regular intervals, aliquots were analysed in triplicate. The measured analyte concentrations were compared to those of the same samples analysed immediately after preparation. Samples were considered stable when% bias from the nominal concentrations was within ± 15%.

2.3.4. Selectivity

Standard solutions of the simulated illicit mixture components other than EPHS, i.e. mannitol and (*S*)-(+)-methamphetamine, were injected at the concentration of 100 µg/mL to check for possible interference.

2.3.5. Accuracy and matrix effect

Recovery assays were carried out in order to evaluate method accuracy and possible matrix effect: standard solutions containing known amounts of racemic EPHS (corresponding to a low, an intermediate and a high value of the calibration curves) and a constant amount of IS were added to simulated illicit samples. The obtained fortified samples were then analysed, and the recovery of spiked EPHS enantiomers was calculated with respect to the nominal concentrations. Matrix effect was evaluated by comparing the recovery of EPHS enantiomers added to a blank sample extract with that of standard solutions at the same nominal concentrations.

2.4. Proof-of-concept application to simulated illicit mixtures

Solid samples were prepared by weighing 10 mg of mannitol and adding appropriate volumes of standard methanol solutions of (*S*)-methamphetamine, (*1R,2R*)-(−)-ΨEPH, (*1S,2R*)-(+)-EPH, (*1R,2S*)-(−)-EPH, (*1S,2S*)-(+)-ΨEPH (1 mg/mL). The final (*S*)-methamphetamine concentration in the mixture was 5% (w/w), while analyte concentrations ranged from 0.1 to 1% (exact concentration levels are reported in Table 2).

The wet mixture was then left to dry at room temperature for one hour under mixing to ensure complete solvent evaporation and homogeneity of the mixture.

Aliquots of 1 mg of the previously described mixtures were dissolved in an amber vial with 1 mL of ultrapure water containing the IS at a concentration of 1 μg/mL and were subjected to ultrasonic bath for 10 min, before being injected as such into the CEKC-DAD system.

3. Results and discussion

3.1. Development of the CEKC-DAD method

3.1.1. Optimisation of non-enantioselective electrophoretic conditions

As a starting point for method development, different experimental conditions for the CEKC method were studied and optimised to achieve good non-enantioselective resolution of EPH and ΨEPH.

As starting conditions, a fused silica capillary was employed (48.6 cm total length, 40 cm effective length, and 50 μm i.d.). BGE was composed of phosphate buffer adjusted to pH 2.50, adequate to ensure sufficient protonation of the amino group of the analytes (pKa of EPH and ΨEPH: 9.65). Standard methanolic solutions of the analytes were injected hydrodynamically at the anodic side using a pressure of 50 mbar for 5 s. Constant voltage (+25 kV) was applied throughout the analysis.

However, under these conditions it was not possible to detect any significant electrophoretic signal. Thus, capillary i.d. was increased to 75 μm and injection was carried out at 50 mbar for 20 s, in order to increase the injected sample volume. Despite these changes, no appreciable results could be achieved. BGE composition was then modified to a 150 mM, pH 3.4 acetic acid solution, leading to satisfactory electrophoretic peaks for both EPH and ΨEPH. To reduce background noise, injection time was decreased from 20 s to 10 s.

3.1.2. Optimisation of β-CDs as chiral selectors

Two different CDs potentially suitable for the analytes were initially tested for the separation of enantiomers: S-β-CD and CM-β-CD. They are negatively charged (the former) or chargeable (the latter) CDs, thus they are suitable for establishing strong interactions with the possible positive charge of the EPHS' amine group as well as counter-current separation mechanisms, possibly leading in turn to high enantioselectivity.

The first tested CD was S-β-CD, added to BGE at concentrations of 0.03, 8.81, 22.03, 44.27 mM (0.005%, 1.6%, 4.1%, 8.2%, respectively). Under these conditions, no appreciable results were obtained: at very low CD concentration it was not possible to observe any peak, while at higher concentrations the baseline was unstable and lacked reproducibility. These results are compatible with very strong analyte-selector interactions, which can cause extreme delay in analyte retention, since the selector tends to go toward the anode.

The second tested CD was the ionisable CM-β-CD in the 0.1–8 mM range. This CD was used at acidic pH in order to keep its ionisation at a minimum (without completely suppressing it), since the results of the permanently charged cyclodextrin were unsatisfactory. CM-β-CD provided promising, but still not entirely satisfactory, results: good between-pair separation was obtained, but with complete coelution of the EPH enantiomers. In an attempt to improve separation, increasing concentrations (9 and 12 mM) of CM-β-CD were tested, as well as the use of an organic additive (5–15% methanol or acetonitrile) to the BGE, however without appreciable results.

As an additional attempt, the most promising CM-β-CD was tested in combination with uncharged CDs (native β-CD and DM-β-CD). DM-β-CD provided the best results, and when both CM-β-CD and DM-β-CD were used at a 3 mM concentration in the BGEs it was possible to separate all four diastereomers, leading to the following enantiomeric elution order (EMO): (*1R,2R*)-(−)-ΨEPH, (*1S,2R*)-(+)-EPH, (*1R,2S*)-(−)-EPH, (*1S,2S*)-(+)-ΨEPH.

The final conditions of the developed CEKC-DAD method exploit a combined CD system as chiral selectors (namely DM-β-CD + CM-β-CD), allowing to obtain satisfactory results in terms of enantiomeric separation (R_s > 4.3 for all analytes) and analysis time (less than 11 min). Peak shape was also considered acceptable when analysing aqueous solutions.

Finally, β-phenylethylamine was selected as the internal standard because it is structurally similar to the analytes, has a comparable pKa value, and did not interfere with any of the enantiomers target of this study. An example of an electropherogram obtained under the final optimised conditions is shown in Fig. 4, related to a standard mixture

Table 1
Linearity and precision results.

Compound	Linearity range (μg/mL)	r^2	Concentration (μg/mL)	Peak area		t_R (min)	t_R	
				Intraday precision (%RSD)[a]	Interday precision (%RSD)[a]		Intraday precision (%RSD)[a]	Interday precision (%RSD)[a]
(*1S,2R*)-(+)-EPH	0.1–10	0.9998	0.1	4.9	5.3	8.41	1.14	1.32
			0.25	4.2	4.6			
			1	3.4	3.9			
			10	2.1	2.5			
(*1R,2S*)-(−)-EPH	0.1–10	0.9996	0.1	5.3	5.6	8.78	1.08	1.40
			0.25	4.7	5.2			
			1	3.7	4.1			
			10	2.5	3.0			
(*1S,2S*)-(+)-ΨEPH	0.1–10	0.9998	0.1	5.2	5.6	9.72	0.98	1.23
			0.25	4.5	5.0			
			1	3.5	3.8			
			10	2.4	2.8			
(*1R,2R*)-(−)-ΨEPH	0.1–10	0.9996	0.1	5.5	5.8	8.16	1.21	1.49
			0.25	5.0	5.3			
			1	3.9	4.3			
			10	2.7	3.2			
IS	/	/	1	2.0	2.2	6.96	0.83	0.98

[a] $n = 6$.

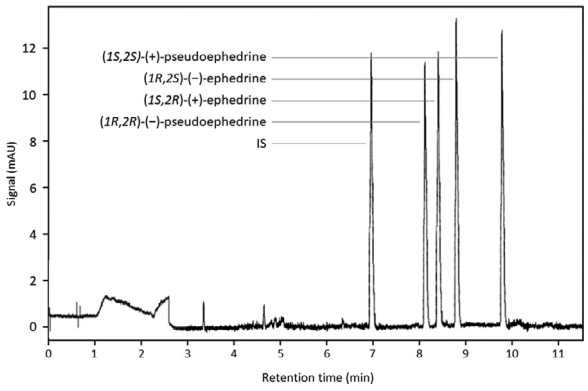

Fig. 4. CEKC-DAD electropherogram of a standard mixture containing (*1R,2R*)-(−)-ΨEPH, (*1S,2R*)-(+)-EPH, (*1R,2S*)-(−)-EPH, (*1S,2S*)-(+)-ΨEPH and IS at the concentration of 1 μg/mL.

containing the 4 target analytes and IS at the nominal concentration of 1 μg/mL.

3.2. Analytical characteristics of the method

3.2.1. Linearity

For the setup of calibration curves, analyte standard racemic mixtures in ultrapure water were prepared at seven concentrations (0.1, 0.15, 0.25, 0.5, 1, 5 and 10 μg/mL) and containing IS at a constant concentration of 1 μg/mL). Method sensitivity for all the four target isomers was 0.1 and 0.03 μg/mL in terms of LOQ and LOD, respectively (corresponding to 0.01% and 0.003% w/w in the starting samples); method linearity was assessed between 0.1 and 10 μg/mL. Good linearity was obtained for all the four target analytes ($r^2 \geq 0.9991$). Complete linearity data are reported in .

3.2.2. Precision

Standard mixtures were prepared at four different concentration levels (LOQ, 0.25 μg/mL, 1 μg/mL and 10 μg/mL) and containing IS at 1 μg/mL. The mixtures were analysed in replicate ($n = 6$) during the same day to evaluate intraday precision and on different days to evaluate interday precision of both analyte retention times and peak areas expressed as%RSD. Complete precision data are reported in Table 1.

3.2.3. Selectivity

Standard solutions at the concentration of 100 μg/mL of methamphetamine and mannitol were injected in the CEKC-DAD system in order to assess selectivity. Potential detection and coelution of interfering compounds were tested with the perspective of method application for the analysis of EPH, ΨEPH and the respective enantiomers in seized illicit samples containing methamphetamine and trace EPHS as possible residual impurities. While mannitol was not detected in the electropherograms, methamphetamine was observed at retention times compatible with the complete resolution and qualitative and quantitative determination of EPHS.

3.2.4. Stability

As assessed by CEKC-DAD analysis, aqueous solutions of the tested EPHS were stable for 48 h when stored protected from light in stoppered amber glass vials at 4 °C (acceptance criterion: analyte loss lower than 15%).

3.3. Proof of concept application and method accuracy

After having evaluated its analytical performance, the method was applied to the analysis of 4 solid samples of mannitol-based powders containing a fixed percentage of (*S*)-(+)-methamphetamine (5%, w/w) and variable percentages of (*1R,2R*)-(−)-ΨEPH, (*1S,2R*)-(+)-EPH, (*1R,2S*)-(−)-EPH, (*1S,2S*)-(+)-ΨEPH ranging between 0.1% and 1% (w/w). This proof of concept application was carried out with the aim of simulating seized illicit samples containing methamphetamine and trace EPHS as possible residual impurities from clandestine production processes. Analyte peak areas were interpolated on the respective calibration curves and the corresponding concentrations of the four analytes were derived.

Fig. 5 shows a representative CEKC-DAD electropherogram of a simulated sample containing 5% (w/w) (*S*)-methamphetamine, 0.1% (w/w) (1R,2R)-(−)-ΨEPH, 0.3% (w/w) (1S,2R)-(+)-EPH, 0.6% (w/w) (1R,2S)-(−)-EPH and 1% (w/w) (1S,2S)-(+)-ΨEPH.

The complete results are reported in Table 2, where the nominal spiked concentrations of the target analytes are reported, together with the results obtained from CEKC-DAD analysis, both expressed as percentage of analyte in the powder (w/w). Analysis was carried out in triplicate.

Moreover, accuracy was calculated by comparing the concentration obtained from the analysis with nominal concentrations and expressed

Table 2
Quantitative analysis and accuracy results.

	Analyte	Nominal concentration (% w/w)	Analysed concentration ± SD (% w/w) [a]	Accuracy (%) [a]
Sample #1	(*1S,2R*)-(+)-EPH	0.10	0.11 ± 0.02	110
	(*1R,2S*)-(−)-EPH	0.30	0.29 ± 0.03	97
	(*1S,2S*)-(+)-ΨEPH	0.60	0.58 ± 0.04	97
	(*1R,2R*)-(−)-ΨEPH	1.00	0.97 ± 0.04	97
Sample #2	(*1S,2R*)-(+)-EPH	1.00	1.01 ± 0.05	101
	(*1R,2S*)-(−)-EPH	0.10	0.09 ± 0.01	90
	(*1S,2S*)-(+)-ΨEPH	0.30	0.32 ± 0.04	107
	(*1R,2R*)-(−)-ΨEPH	0.60	0.63 ± 0.04	105
Sample #3	(*1S,2R*)-(+)-EPH	0.60	0.61 ± 0.03	102
	(*1R,2S*)-(−)-EPH	1.00	1.04 ± 0.03	104
	(*1S,2S*)-(+)-ΨEPH	0.10	0.10 ± 0.02	100
	(*1R,2R*)-(−)-ΨEPH	0.30	0.31 ± 0.03	103
Sample #4	(*1S,2R*)-(+)-EPH	0.30	0.28 ± 0.04	93
	(*1R,2S*)-(−)-EPH	0.60	0.57 ± 0.04	95
	(*1S,2S*)-(+)-ΨEPH	1.00	0.95 ± 0.05	95
	(*1R,2R*)-(−)-ΨEPH	0.10	0.11 ± 0.01	110

[a] $n = 6$.

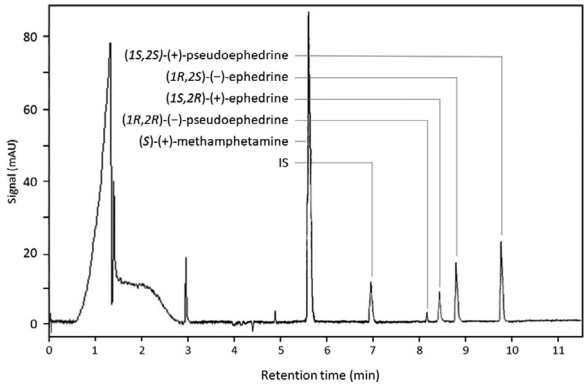

Fig. 5. CEKC-DAD electropherogram of a simulated sample containing 5% (w/w) (*S*)-methamphetamine, 0.1% (w/w) (*1R,2R*)-(−)-ΨEPH, 0.3% (w/w) (*1S,2R*)-(+)-EPH, 0.6% (w/w) (*1R,2S*)-(−)-EPH, 1% (w/w) (*1S,2S*)-(+)-ΨEPH and IS at the injected concentration of 1 μg/mL.

as percent. The calculated accuracy was in the 90–110% range, thus it was deemed satisfactory. Finally, no relevant matrix effect was observed by comparing the recovery of EPHS enantiomers added to a blank sample extract with that of standard solutions at the same nominal concentrations (acceptance criteria: ± 10% of nominal concentration).

Comparing the proposed method with previous papers reporting the chiral separation of EPHS by CEKC, some of them concern mechanical aspects of chiral recognition [16–20] or chiral selector performance [22], so they do not report any application nor the detailed study of quantitative method performances and characteristics. Other papers reported CEKC-UV method application to ephedra plant extracts [23,24], but with higher retention time variability and substantially lower sensitivity. Enantioselective supercritical fluid chromatography – MS (SFC-MS) [25] and bidimensional HPLC-UV (2D-HPLC-UV) [26] have been applied to seized illicit materials. SFC-MS of course provided much better sensitivity than the CEKC-DAD method proposed herein, but with obvious drawbacks such as widely higher costs for equipment and for solvent consumption; method performance evaluation was limited to retention time reproducibility. 2D-HPLC was not enantioselective, thus just diastereomeric separation and quantification were achieved.

4. Conclusion

An enantioselective method based on CEKC employing a combination of DM-β-CD and CM-β-CD as chiral selectors was developed for the analysis of EPH and ΨEPH enantiomers. CEKC analysis is carried out within less than 12 min with satisfactory peak resolution ($R_s > 4.3$). As a proof of concept, the method was applied to powders containing (*S*)-methamphetamine and variable percentages of (*1R,2R*)-(−)-ΨEPH, (*1S,2R*)-(+)-EPH, (*1R,2S*)-(−)-EPH, (*1S,2S*)-(+)-ΨEPH ranging between 0.1% and 1% (w/w) with satisfactory results in terms of accuracy.

The method has demonstrated to be suitable for the analysis of samples containing trace amounts of EPH and ΨEPH racemates as low as 0.1% (w/w). Since chiral aspects are considered crucial in forensic investigations in relation to synthetic processes carried out for the illicit synthesis of methamphetamine, the developed CEKC-DAD method offers a feasible and effective way to acquire chiral information about methamphetamine seizures, which can provide evidence for investigating cases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Michele Protti: Methodology, Investigation, Formal analysis, Validation, Data curation, Writing – original draft. **Roberto Mandrioli:** Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization. **Jose Gonzalez-Rodriguez:** Data curation, Writing – review & editing, Visualization. **Laura Mercolini:** Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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