Determination of the chiral status of different novel psychoactive substance classes by capillary electrophoresis and β-cyclodextrin derivatives

Johannes S. Hägele | Eva-Maria Hubner | Martin G. Schmid

Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Correspondence
Martin G. Schmid, Department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, University of Graz, Universitätsplatz 1, A-8010 Graz, Austria.
Email: martin.schmid@uni-graz.at

Abstract
Besides the abuse of well-known illicit drugs, consumers discovered new synthetic compounds with similar effects but minor alterations in their chemical structure. Originally, these so-called novel psychoactive substances (NPS) have been created to circumvent law of prosecution because of illicit drug abuse. During the past decade, such compounds came up in generations, the most popular compound was a synthetic cathinone derivative named mephedrone. Cathinones are structurally related to amphetamines; to date, more than 120 completely new derivatives have been synthesized and are traded via the Internet. Cathinones possess a chiral center; however, only little is known about the pharmacology of their enantiomers. However, NPS comprise further chiral compound classes such as amphetamine derivatives, ketamines, 2-(aminopropyl)benzofurans, and phenidines. In continuation of our project, a cheap and easy-to-perform chiral capillary zone electrophoresis method for enantioseparation of cathinones presented previously was extended to the aforementioned compound classes. Enantioreolution was achieved by simply adding native β-cyclodextrin, acetyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, or carboxymethyl-β-cyclodextrin as chiral selector additives to the background electrolyte. Fifty-one chiral NPS served as analytes mainly purchased from online vendors via the Internet. Using 10 mM of the aforementioned β-cyclodextrins in a 10 mM sodium phosphate buffer (pH 2.5), overall, 50 of 51 NPS were resolved. However, chiral separation ability of the selectors differed depending on the analyte. Additionally, simultaneous enantioseparations, the determination of enantiomeric migration orders of selected analytes, and a repeatability study were performed successfully. It was proven that all separated NPS were traded as racemic mixtures.

KEYWORDS
2-hydroxypropyl-β-cyclodextrin, acetyl-β-cyclodextrin, capillary electrophoresis, carboxymethyl-β-cyclodextrin, native β-cyclodextrin, novel psychoactive substances
1 | INTRODUCTION

Novel psychoactive substances (NPS) gained an enormous popularity during the past decade. As the main reason for the fast and easy distribution of NPS, the technological progress of the Internet can be referred. The substances are mainly synthesized in China or other Asian countries, and they can be purchased from diverse online vendors titulated as “birdcage cleaners,” “plant food,” or “research chemicals” via the World Wide Web. Their labels, for example, “Not for human consumption” and doubtful purity and identity data online, guarantee low risk of prosecution for online vendors. Dubious information about ways of consumption and the effectiveness of the compounds consumers gain, for example, via YouTube channels or drug fora.\(^1,2\) The United Nations Office on Drugs and Crime (UNODC) reported an increase of NPS in 111 countries worldwide by the end of 2017. However, Asia, Europe, and North America have the lead in the number of substances reported.\(^3\) The second important institution for European concerns, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), reported in their annual review meanwhile more than 730 different NPS derivatives and give a special emphasize to four main categories: synthetic cannabinoids, stimulants, opioids, and antidepressants.\(^2\) NPS are mainly synthesized by more or less simple modifications of molecular structures of well-known classic synthetic illicit drugs. Lead substances like, for example, native amphetamine, are therefore chemically modified to bypass national and international drug controls. Consumers replace prohibited illegal compounds with this legal “imitations.” A huge number of them possess a chiral center yielding in two possible enantiomeric forms. Their pharmacological effects like, that is, their potencies and effects, may differ as it is well known from diverse active pharmaceutical ingredients. Referred examples of lead substances showing different effects are, for example, methcathinone, mephedrone, amphetamine, and methamphetamine.\(^4–7\) The fact of a potentially different pharmacological behavior of NPS makes chiral method development indispensable. A further goal is the development of chiral separation methods to check the enantiomeric status of real-life samples. Up to now, some articles report enantiomeric separations of NPS. Separation techniques like high-performance liquid chromatography (HPLC),\(^8–20\) capillary electrophoresis (CE),\(^21–31\) gas chromatography (GC),\(^32–36\) and supercritical fluid chromatography (SFC)\(^37–41\) are cited in literature. These separation techniques are used to check the compounds as solid samples or in biological matrices.\(^42–45\) Among them, CE turned out to be a cheap, easy-to-perform, and reliable separation technique. Advantageously, the chiral selector is added to the electrolyte. Successful use of cyclodextrins (CDs), macrocyclic antibiotics, or chiral crown ethers as chiral selectors has been shown. Particularly native CDs and diverse substituted derivatives turned out to be used successfully for this purpose. As chiral separation principle, formation of inclusion complexes and additional interactions of the moieties of its derivatives have to be taken into account. Different complex stability constants of the CD-analyte complexes and consequently differing electrophoretic mobilities are responsible for chiral discrimination.

The goal of this study was the continuation of our project to extent a cheap and easy-to-perform chiral CE method for enantioseparation of cathinones presented previously to enantioseparation of amphetamine derivatives, ketamines, 2-(aminopropyl)benzofurans, and phe-nidines.\(^27\) Four different β-CDs, namely, native β-CD, acetyl-β-CD, 2-hydroxypropyl-β-CD, and carboxymethyl-β-CD, served as chiral selectors. Additionally, this method should give information about the enantiomeric status of real-life samples and possibly the origin of the substances.

2 | MATERIALS AND METHODS

2.1 | Chemicals and solutions

Native β-CD and 2-hydroxypropyl-β-CD (degree of substitution: 0.6) were from Fluka Chemika AG (Buchs, Switzerland). Acetyl-β-CD (degree of substitution: 1.0) and carboxymethyl-β-CD (degree of substitution: 0.5) were purchased from Wacker-Chemie GmbH (Salzburg, Austria). Sodium phosphate and diluted phosphoric acid were bought from Merck KGaA (Darmstadt, Germany). Milli-Q-Water (HiPerSolv CHROMANORM) was from VWR International (Vienna, Austria). All reagents were of analytical grade.

NPS were mostly not commercially available from official suppliers due to their novelty. As a consequence, they were bought from diverse online stores like, for example, www.purechemicals.net, www.Get-RC.to or www.rc-supply.to. Additionally, some analytes represent real-life samples seized by Austrian police or were synthesized in microscale amounts in our laboratory. Pure enantiomers were prepared via a semipreparative HPLC method (unpublished results) in our laboratory in milligram scale for scientific purposes.

All analytes were characterized by GS-electron impact mass spectrometry (GC–MS) and, if necessary, nuclear magnetic resonance (NMR) prior to experiments.

BGEs were prepared by dissolving 10 mM of β-CD or β-CD derivative, 10 mM sodium phosphate adjusted with
TABLE 1  Psychoactive compounds and their chemical structure investigated in this study

A

| Compound | Chemical Structure |
|----------|-------------------|
| A0: All R = H | Amphetamine ((±)-1-Phenylpropan-2-amine) |
| A1: R1 = Br | 4-Bromoamphetamine (4-BA, (±)-1-(4-Bromophenyl)propan-2-amine) |
| A2: R2 = Cl | 2-Chloroamphetamine (2-CA, (±)-1-(2-Chlorophenyl)propan-2-amine) |
| A3: R1 = Cl; R2 = R4 = OCH3 | 2,5-Dimethoxy-4-chloroamphetamine (DOC, (±)-1-(4-Chloro-2,5-dimethoxyphenyl)propan-2-amine) |
| A4: R1 = F | 4-Fluoroamphetamine (4-FA, (±)-1-(4-Fluorophenyl)propan-2-amine) |
| A5: R3 = F | 3-Fluoroamphetamine (3-FA, (±)-1-(3-Fluorophenyl)propan-2-amine) |
| A6: R2 = F | 2-Fluoroamphetamine (2-FA, (±)-1-(2-Fluorophenyl)propan-2-amine) |
| A7: R1 = NO2 | 4-Nitroamphetamine (4-NA, (±)-1-(4-Nitrophenyl)propan-2-amine) |
| A8: R1 = SH | 4-Methylthioamphetamine (MTA, (±)-1-[4-(Methylsulfanyl)phenyl]propan-2-amine) |
| A9: R2 = R4 = OCH3 | 2,5-Dimethoxymethylamphetamine (2,5-DMA, (±)-1-(2,5-Dimethoxyphenyl)propan-2-amine) |
| A10: R1 = R4 = OCH3 | 3,4-Dimethoxymethylamphetamine (3,4-DMA, (±)-1-(3,4-Dimethoxyphenyl)propan-2-amine) |
| A11: R1 = OCH3 | 4-Methoxymethylamphetamine ((±)-1-(4-Methoxyphenyl)propan-2-amine) |
| A12: R1 = Br; R2 = R4 = OCH3 | 4-Bromo-2,5-dimethoxymethylamphetamine (DOB, (±)-4-Bromo-2,5-dimethoxymethylamphetamine) |
| A13: R6 = C2H5 | N-Ethylamphetamine ((±)-N-Ethyl-1-phenylpropan-2-amine) |
| A14: R6 = C3H6Cl | Mefenorex ((±)-3-Chloro-N-(1-methyl-2-phenylethyl)propan-2-amine) |
| A15: R6 = CH3 | N-Methamphetamine ((±)-N,Nα-diethylphenethylamine) |
| A16: R1 = Br; R6 = CH3 | 4-Bromomethamphetamine (4-BMA, (±)-1-(4-Bromophenyl)-N-methylpropan-2-amine) |
| A17: R2 = Cl; R6 = CH3 | 2-Chloromethamphetamine (2-CMA, (±)-1-(2-Chlorophenyl)-N-methylpropan-2-amine) |
| A18: R1 = Cl; R6 = CH3 | 4-Chloromethamphetamine (4-CMA, (±)-1-(4-Chlorophenyl)-N-methylpropan-2-amine) |
| A19: R2 = F; R6 = CH3 | 2-Fluromethamphetamine (2-FMA, (±)-1-(2-Fluorophenyl)-N-methylpropan-2-amine) |
| A20: R1 = F; R6 = CH3 | 4-Fluromethamphetamine (4-FMA, (±)-1-(4-Fluorophenyl)-N-methylpropan-2-amine) |

B

N-Methyl-3,4-methylendioxy-amphetamine (MDMA, (±)-1-(Benzo[1,3]dioxol-5-yl)-N-methyl-propan-2-amine)

C

(±)-N-(1-(2,3-Dihydro-benzo[b][1,4]dioxin-6-yl)propan-2-yl)-N-methylhydroxylamine (EFLEA)

D

(Continues)
### Table 1 (Continued)

| D0: All R = H          | 4-(2-Aminopropyl)-benzofurane (4-APB, (±)-1-(1-Benzofuran-4-yl)propan-2-amine) |
|-----------------------|--------------------------------------------------------------------------------|
| D1: R1 = C₃H₆NH₂     | 4-(2-Aminopropyl)-benzofurane (4-APB, (±)-1-(1-Benzofuran-4-yl)propan-2-amine) |
| D2: R2 = C₃H₆NH₂     | 5-(2-Aminopropyl)-benzofurane (5-APB, (±)-1-(1-Benzofuran-5-yl)propan-2-amine) |
| D3: R3 = C₃H₆NH₂     | 6-(2-Aminopropyl)-benzofurane (6-APB, (±)-1-(1-Benzofuran-6-yl)propan-2-amine) |
| D4: R4 = C₃H₆NH₂     | 7-(2-Aminopropyl)-benzofurane (7-APB, (±)-1-(1-Benzofuran-7-yl)propan-2-amine) |
| D5: R2 = C₃H₁₁NH     | (±)-1-(Benzofuran-5-yl)-N-ethylpropan-2-amine (5-EAPB) |
| D6: R3 = C₃H₁₁NH     | (±)-1-(Benzofuran-6-yl)-N-ethylpropan-2-amine (6-EAPB) |
| D7: R2 = C₄H₉NH     | (±)-1-(Benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) |

| E                      | (±)-1-(Benzofuran-5-yl)-N-(2-methoxybenzyl)-propan-2-amine (N-MOB-5-APB) |

| F                      |                                                                  |
|------------------------|-------------------------------------------------------------------|
| F0: All R = H          |                                                                  |
| F1: R1 = C₃H₆NH₂     | (±)-1-(2,3-Dihydro-1-benzofuran-5-yl)propan-2-amine (5-APDB)       |
| F2: R2 = C₃H₆NH₂     | (±)-1-(2,3-Dihydro-1-benzofuran-6-yl)propan-2-amine (6-APDB)       |

| G                      |                                                                  |
|------------------------|-------------------------------------------------------------------|
| G0: All R = H          |                                                                  |
| G1: R2 = Cl            | Ketamine ((±)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone)    |
| G2: R1 = CH₃; R2 = Cl  | N-Ethyl-ketamine ((±)-2-(2-Chlorophenyl)-2-(ethylamino)cyclohexanone) |
| G3: R1 = CH₃           | 2-Oxo-PCE ((±)-2-(Ethylamino)-2-phenylcyclohexan-1-one)           |
| G4: R2 = F             | 4-Fluoroketamine ((±)-2-(2-Fluorophenyl)-2-(methylamino)cyclohexanone) |
| G5: R2 = OCH₃          | 2-MeO-Ketamine ((±)-2-(2-Methoxyphenyl)-2-(methylamino)cyclohexanone) |
| G6: R1 = CH₃; R3 = OCH₃| Methoxetamine ((±)-2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone) |

| H                      |                                                                  |
**TABLE 1** (Continued)

|   | Compounds                                                                 |
|---|--------------------------------------------------------------------------------|
| I | Diphenidine ((±)-1-(1,2-Diphenylethyl)piperidine)                            |
| J | Ephenidine ((±)-N-Ethyl-1,2-diphenylethylamine)                             |
| K | Methoxphenidine ((±)-2-Methoxy-1-(1,2-Diphenylethyl)piperidine)              |
|   | K1: R1 = H Thiopropamine ((±)-1-(Thiophen-2-yl)-2-aminopropane)              |
|   | K2: R1 = CH₃ Methiopropamine ((±)-1-(Thiophen-2-yl)-2-methylaminopropane)    |
| L | Thiothinone ((±)-2-(Methylamino)-1-(thiophen-2-yl)propan-1-one)              |
| M | (±)-5-(2-Aminopropyl)-indole (5-API)                                         |
| N | Mephtetramine (MTTA, ((±)-2-(Methylaminomethyl)-3,4-dihydro-2H-naphthalen-1-one)) |
diluted phosphoric acid in Milli-Q-Water (pH 2.5). Prior to
the chiral separation studies, solutions were degassed
by ultrasonification and filtered through a 0.45-μm pore
size nylon filter (Carl Roth, Karlsruhe, Germany).

2.2 | Instrumentation

For CE measurements, a fully automated 3DCE system
(Agilent Technologies, Waldbronn, Germany) equipped
with a diode array detector was used. All experiments
were carried out at ambient temperature (25°C). CE was
performed in 50 μm ID-fused silica capillaries
(MicroQuartz, Munich, Germany) with a total length of
68.5 cm and an effective length of 60 cm. UV absorption
was measured at 209 nm. Before and after each measure-
ment, the capillary was flushed with 0.2 M sodium
hydroxide, water, and BGE, respectively. All samples
were injected by applying a pressure of 10 mbar * 5 s on
the inlet vial.

2.3 | Sample preparation

Because the samples consisted mainly of hydrochloric
acid salts, each sample was dissolved in Milli-Q-Water
in a concentration of 1.0 mg/ml. To accelerate the dissolving
processes, the samples were given in an ultrasonic bath
for 1 min before filtration. After ultrasonification, they
were also filtered through a 0.45-μm pore size filter (Carl
Roth, Karlsruhe, Germany).

3 | RESULTS AND DISCUSSION

All NPS and pure NPS enantiomers comprising different
compound classes were collected since 2010. They were
purchased via the Internet, produced by chemical synthe-
sis and by semipreparative methods (unpublished
results), or were seized by Austrian police. The chemical
structures of the analyzed substances are given in
Table 1.

Based on the work of Merola et al.22 and a further
method optimization of our group,27 10 mM β-CD in a
10 mM sodium phosphate buffer (pH 2.5) was found out
to be appropriate as final BGE. A voltage of 30 kV to the
cathode was applied during the chiral separation experi-
ments. The electrolyte conditions of native β-CD were
additionally transferred to the derivatives 2-hydroxypropyl-β-CD, acetyl-β-CD, and carboxymethyl-
β-CD. However, the applied voltages were adjusted to
29 kV to the cathode for 2-hydroxypropyl-β-CD and
acetyl-β-CD and 22 kV to the cathode for carboxymethyl-
β-CD to create a stable current under the fastest possible
separation conditions. A scheme of all used CDs is given
in Figure 1.

Using the stated conditions, a set of 51 NPS including
23 amphetamine derivatives, 10 2-(aminopropyl)

| TABLE 1 (Continued) |
|----------------------|

| (±)-6,7-Methylendioxy-2-aminotetraline (MDAT) |

| α-Pyrrolidinopentiothiophenone (α-PVT, (±)-2-(Pyrrolidin-1-yl)-1-(thiophen-2-yl)pentan-1-one) |

| Q1: R1 = H Methaqualone ((±)-3-(2-Methylphenyl)-2-methylquinazolin-4-one) |
| Q2: R1 = CH3 Ethaqualone ((±)-3-(2-Ethylphenyl)-2-methylquinazolin-4-one) |
benzofurans, six ketamine derivatives, three phenidines, and nine other NPS was tested. Overall, 50 of the 51 analytes were partially or baseline separated by at least one of the different CD-electrolytes. Measurements did not exceed 48 min. A complete overview of all chiral separation data is shown in Tables 2–6 in detail. An electropherogram of a single chiral separation of (±)-1-(benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) using the chiral selector carboxymethyl-β-CD is given in Figure 2. Chiral separation data within the different compound classes were satisfactory. All substances except one, namely, MDAT (6,7-methylenedioxy-2-aminotetraline), were resolved in their enantiomers partially or completely.

In Table 2, all chiral separation results of the tested amphetamine derivatives are given. All substances were chirally discriminated with at least one chiral selector within 33 min. The majority of the analytes could be detected within a migration time less than 25 min. Resolution factors ranged from 0.5 to 7.2. Overall, carboxymethyl-β-CD gave the best chiral separation data regarding chromatographic resolution. However, analysis times using this chiral selector were longer than using the other CD derivatives. Additionally, for some analytes like, for example, DOB and DOC, only with acetyl-β-CD chiral separations were observed. A potential reason for this observation could be a higher affinity of their molecular structure to the acetyl moiety of the CD derivative.

Table 3 shows all separation data of the tested 2-(aminopropyl)benzofuran derivatives. Again, all derivatives out of this analyte group could be chirally separated by at least one of the chosen CDs within 48 min. Mainly, the analytes were detected within 15 min. Resolution for the separated 2-(aminopropyl)benzofuran enantiomers varied from 0.5 to 6.4. Again, carboxymethyl-β-CD showed the best chiral separation results regarding resolution in combination with slightly extended migration times.

Regarding the analyzed ketamine derivatives shown in Table 4, resolution ranged from 0.6 to 5.6. All ketamine derivatives were resolved in their enantiomers with at least one of the chosen CDs within 22 min. Only acetyl-β-CD was able to separate all analytes and therefore turned out to be the most potent chiral selector for

Figure 1: Chemical structures applied β-cyclodextrin derivatives (already published in Hägele et al.27)

Substitution pattern:
Native beta-cyclodextrin: 
R = -H
Acetyl-beta-cyclodextrin: 
R = -H or -CO-CH₃
(2-Hydroxypropyl)-beta-cyclodextrin: 
R = -H or -CH₂-CHOH-CH₃
Carboxymethyl-beta-cyclodextrin: 
R = -H or -CH₂-COO⁻Na⁺

Figure 2: Single chiral separation of 5-MAPB. Conditions: 10 mM carboxymethyl-β-cyclodextrin, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltage: 22 kV to cathode, injection: 10 mbar for 5 s, sample: 1 mg/ml in water
| Compound               | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|------------------------|-------------|-------------|----------|-------|----------------|----------------------|
| Amphetamine            | 8.42        | 8.49        | 1.009    | 0.7   | β-CD           | +30                  |
|                        | 11.28       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 7.63        | 7.70        | 1.009    | 0.8   | HP-β-CD        | +29                  |
|                        | 16.81       | 17.30       | 1.029    | 3.7   | CM-β-CD        | +22                  |
| 4-Bromoamphetamine    | 11.16       | 11.25       | 1.009    | 0.8   | β-CD           | +30                  |
|                        | 14.43       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 10.49       | 10.59       | 1.009    | 0.9   | HP-β-CD        | +29                  |
|                        | 25.09       | 25.67       | 1.023    | 2.4   | CM-β-CD        | +22                  |
| 2-Chloroamphetamine   | 9.22        | 9.50        | 1.030    | 2.6   | β-CD           | +30                  |
|                        | 10.09       | 10.28       | 1.018    | 0.6   | Acetyl β-CD    | +29                  |
|                        | 8.24        | 8.44        | 1.024    | 1.4   | HP-β-CD        | +29                  |
|                        | 17.21       | 18.18       | 1.056    | 5.7   | CM-β-CD        | +22                  |
| 2-Fluoroamphetamine   | 8.83        | 8.94        | 1.012    | 1.0   | β-CD           | +30                  |
|                        | 10.59       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 7.80        | 7.88        | 1.010    | 0.7   | HP-β-CD        | +29                  |
|                        | 15.85       | 16.50       | 1.041    | 3.8   | CM-β-CD        | +22                  |
| 3-Fluoroamphetamine   | 8.88        | 8.98        | 1.011    | 1.2   | β-CD           | +30                  |
|                        | 11.52       | 11.60       | 1.007    | 0.6   | Acetyl β-CD    | +29                  |
|                        | 7.62        | 7.68        | 1.009    | 0.8   | HP-β-CD        | +29                  |
|                        | 15.53       | 15.91       | 1.024    | 2.3   | CM-β-CD        | +22                  |
| 4-Fluoroamphetamine   | 8.51        | 8.59        | 1.010    | 0.7   | β-CD           | +30                  |
|                        | 10.05       | 10.13       | 1.008    | 1.4   | Acetyl β-CD    | +29                  |
|                        | 7.83        | 7.90        | 1.009    | 0.9   | HP-β-CD        | +29                  |
|                        | 17.04       | 17.44       | 1.023    | 3.8   | CM-β-CD        | +22                  |
| 4-Nitroamphetamine    | 8.63        | n.d.        | -        | -     | β-CD           | +30                  |
|                        | 13.38       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 8.04        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                        | 18.17       | 18.33       | 1.009    | 1.3   | CM-β-CD        | +22                  |
| MTA                    | 12.60       | n.d.        | -        | -     | β-CD           | +30                  |
|                        | 14.33       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 10.95       | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                        | 27.27       | 27.71       | 1.016    | 1.2   | CM-β-CD        | +22                  |
| 2,5-DMA                | 8.99        | 9.07        | 1.009    | 0.8   | β-CD           | +30                  |
|                        | 10.45       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                        | 8.28        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                        | 18.97       | 19.34       | 1.020    | 3.1   | CM-β-CD        | +22                  |
| DOB                    | 9.25        | n.d.        | -        | -     | β-CD           | +30                  |
|                        | 9.13        | 9.19        | 1.007    | 0.8   | Acetyl β-CD    | +29                  |
|                        | 8.03        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                        | 15.54       | n.d.        | -        | -     | CM-β-CD        | +22                  |
| DOC                    | 8.44        | n.d.        | -        | -     | β-CD           | +30                  |
|                        | 9.80        | 9.86        | 1.007    | 0.6   | Acetyl β-CD    | +29                  |
|                        | 8.30        | n.d.        | -        | -     | HP-β-CD        | +29                  |
| Compound               | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|------------------------|-------------|-------------|----------|-------|----------------|---------------------|
| 3,4-DMA                | 15.17       | n.d.        | -        | -     | CM-β-CD        | +22                 |
| 4-MeO-amphetamine      | 10.50       | 10.58       | 1.008    | 0.7   | β-CD           | +30                 |
| N-Methamphetamine     | 8.91        | 9.02        | 1.012    | 0.9   | β-CD           | +30                 |
| 4-BMA                  | 12.12       | 12.24       | 1.009    | 0.7   | β-CD           | +30                 |
| 2-CMA                  | 9.33        | 9.64        | 1.033    | 2.2   | β-CD           | +30                 |
| 4-CMA                  | 9.62        | 9.69        | 1.008    | 0.9   | β-CD           | +30                 |
| 2-FMA                  | 9.14        | 9.37        | 1.025    | 1.8   | β-CD           | +30                 |
| 4-FMA                  | 8.61        | 8.68        | 1.009    | 0.8   | β-CD           | +30                 |
| MDMA                   | 11.02       | 11.16       | 1.013    | 0.9   | β-CD           | +30                 |
| N-Ethylamphetamine    | 9.74        | 9.85        | 1.011    | 0.8   | β-CD           | +30                 |
| Mefenorex              | 9.99        | 10.10       | 1.010    | 0.8   | β-CD           | +30                 |
ketamines under the stated conditions. Again, the reason for this observation might be a stronger interaction of the analyte enantiomers with the acetyl moieties of this CD derivative.

Furthermore, phenidine derivatives were tested (Table 5). They also serve as NPS and are available at different internet vendors. Again, all compounds were chirally discriminated within 34 min by at least one chiral selector. Mainly, the substances were detected within 16 min. Resolution factors ranged from 0.9 to 2.4.

Table 6 shows the chiral separation results of diverse subcategories of NPS, including, for example, thiophene derivatives. All substances except 6,7-methylenedioxy-2-aminotetraline (MDAT) were separated within 30 min. Resolution factors ranged from 0.5 to 6.4. Carboxymethyl-β-CD turned out to be superior as chiral selector.

In addition to the single chiral separation experiments, attempts of simultaneous enantioseparations were carried out successfully. An example of a simultaneous chiral separation is shown in Figure 3. Five of the six investigated ketamine derivatives were resolved in one single measurement by acetyl-β-CD as chiral selector additive.

Besides ketamine being abused as hallucinogenic, derivatives have entered the NPS market. For example, methoxetamine is available via internet platforms since 2009.

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**Table 2 (Continued)**

| Compound | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|----------|-------------|-------------|----------|-------|----------------|---------------------|
| EFLEA    | 13.19       | n.d.        | -        | -     | β-CD           | +30                 |
|          | 16.10       | n.d.        | -        | -     | Acetyl β-CD    | +29                 |
|          | 14.42       | 14.61       | 1.013    | 1.0   | HP-β-CD        | +29                 |
|          | 30.53       | 32.38       | 1.061    | 7.2   | CM-β-CD        | +22                 |

*Note:* Conditions: 10 mM chiral selector, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, injection: 10 mbar for 5 s, sample: 1 mg/ml in water.

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**Figure 3** Simultaneous enantioseparation of five different ketamine derivatives. Conditions: 10 mM acetyl-β-cyclodextrin, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltage: 29 kV to cathode, injection: 10 mbar for 5 s, sample: 1 mg/ml in water.
| Compound    | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|-------------|-------------|-------------|----------|-------|-----------------|----------------------|
| 4-APB       | 7.79        | 7.85        | 1.008    | 0.7   | β-CD            | +30                  |
|             | 9.61        | 9.71        | 1.011    | 0.9   | Acetyl β-CD     | +29                  |
|             | 8.13        | 8.21        | 1.009    | 0.8   | HP-β-CD         | +29                  |
|             | 12.50       | 12.72       | 1.017    | 1.9   | CM-β-CD         | +22                  |
| 5-APB       | 12.77       | 12.88       | 1.009    | 0.7   | β-CD            | +30                  |
|             | 15.68       | n.d.        | -        | -     | Acetyl β-CD     | +29                  |
|             | 12.62       | 12.85       | 1.018    | 1.6   | HP-β-CD         | +29                  |
|             | 13.45       | 13.66       | 1.016    | 3.3   | CM-β-CD         | +22                  |
| 5-APDB      | 9.79        | 9.89        | 1.011    | 0.8   | β-CD            | +30                  |
|             | 12.60       | 12.72       | 1.009    | 0.8   | Acetyl β-CD     | +29                  |
|             | 10.00       | 10.15       | 1.015    | 1.4   | HP-β-CD         | +29                  |
|             | 13.95       | 14.18       | 1.016    | 3.4   | CM-β-CD         | +22                  |
| 5-EAPB      | 11.46       | 11.61       | 1.013    | 0.9   | β-CD            | +30                  |
|             | 15.50       | n.d.        | -        | -     | Acetyl β-CD     | +29                  |
|             | 12.40       | 12.65       | 1.020    | 1.2   | HP-β-CD         | +29                  |
|             | 14.65       | 14.90       | 1.017    | 6.4   | CM-β-CD         | +22                  |
| 5-MAPB      | 10.83       | 11.00       | 1.016    | 0.9   | β-CD            | +30                  |
|             | 14.89       | 15.04       | 1.010    | 0.7   | Acetyl β-CD     | +29                  |
|             | 11.49       | 11.75       | 1.022    | 1.5   | HP-β-CD         | +29                  |
|             | 14.32       | 14.56       | 1.017    | 3.5   | CM-β-CD         | +22                  |
| N-MOB-5-APB | 12.81       | n.d.        | -        | -     | β-CD            | +30                  |
|             | 15.72       | n.d.        | -        | -     | Acetyl β-CD     | +29                  |
|             | 14.03       | n.d.        | -        | -     | HP-β-CD         | +29                  |
|             | 40.56       | 40.84       | 1.007    | 0.8   | CM-β-CD         | +22                  |
| 6-APB       | 11.52       | 11.63       | 1.009    | 0.8   | β-CD            | +30                  |
|             | 15.71       | 16.07       | 1.023    | 2.1   | Acetyl β-CD     | +29                  |
|             | 13.13       | 13.35       | 1.016    | 2.2   | HP-β-CD         | +29                  |
|             | 14.41       | 14.65       | 1.017    | 3.5   | CM-β-CD         | +22                  |
| 6-APDB      | 11.32       | 11.43       | 1.009    | 0.5   | β-CD            | +30                  |
|             | 15.23       | n.d.        | -        | -     | Acetyl β-CD     | +29                  |
|             | 11.81       | 12.05       | 1.021    | 1.4   | HP-β-CD         | +29                  |
|             | 26.00       | 26.74       | 1.028    | 2.5   | CM-β-CD         | +22                  |
| 6-EAPB      | 13.37       | n.d.        | -        | -     | β-CD            | +30                  |
|             | 16.06       | n.d.        | -        | -     | Acetyl β-CD     | +29                  |
|             | 12.71       | 12.93       | 1.018    | 1.2   | HP-β-CD         | +29                  |
|             | 47.24       | 48.00       | 1.016    | 1.2   | CM-β-CD         | +22                  |
| 7-APB       | 7.67        | n.d.        | -        | -     | β-CD            | +30                  |
|             | 9.41        | 9.47        | 1.006    | 0.5   | Acetyl β-CD     | +29                  |
|             | 8.28        | n.d.        | -        | -     | HP-β-CD         | +29                  |
|             | 13.77       | 13.98       | 1.015    | 1.4   | CM-β-CD         | +22                  |

*Note:* Conditions: 10 mM chiral selector, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, injection: 10 mbar for 5 s, sample: 1 mg/ml in water.
### TABLE 4  Chiral separation results of a set of six ketamine derivatives

| Compound            | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|---------------------|-------------|-------------|----------|-------|----------------|----------------------|
| Ketamine            | 9.95        | 10.04       | 1.009    | 0.8   | β-CD           | +30                  |
|                     | 9.73        | 9.81        | 1.008    | 0.6   | Acetyl β-CD    | +29                  |
|                     | 8.41        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                     | 19.44       | n.d.        | -        | -     | CM-β-CD        | +22                  |
| N-Ethylketamine     | 10.13       | n.d.        | -        | -     | β-CD           | +30                  |
|                     | 10.21       | 10.30       | 1.010    | 0.8   | Acetyl β-CD    | +29                  |
|                     | 8.48        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                     | 20.00       | 20.38       | 1.019    | 2.1   | CM-β-CD        | +22                  |
| Methoxetamine       | 10.74       | 10.87       | 1.012    | 1.0   | β-CD           | +30                  |
|                     | 10.37       | 11.07       | 1.068    | 4.7   | Acetyl β-CD    | +29                  |
|                     | 9.34        | 9.41        | 1.007    | 0.6   | HP-β-CD        | +29                  |
|                     | 21.47       | 22.05       | 1.027    | 2.6   | CM-β-CD        | +22                  |
| 2-Oxo-PCE           | 9.30        | 9.35        | 1.006    | 0.6   | β-CD           | +30                  |
|                     | 9.54        | 10.06       | 1.054    | 4.5   | Acetyl β-CD    | +29                  |
|                     | 8.56        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                     | 15.46       | 15.62       | 1.011    | 1.1   | CM-β-CD        | +22                  |
| 2-F-Ketamine        | 9.17        | n.d.        | -        | -     | β-CD           | +30                  |
|                     | 8.87        | 9.06        | 1.022    | 1.6   | Acetyl β-CD    | +29                  |
|                     | 8.33        | n.d.        | -        | -     | HP-β-CD        | +29                  |
|                     | 14.31       | n.d.        | -        | -     | CM-β-CD        | +22                  |
| 2-MeO-Ketamine      | 10.86       | 11.06       | 1.018    | 1.4   | β-CD           | +30                  |
|                     | 10.10       | 10.92       | 1.081    | 5.6   | Acetyl β-CD    | +29                  |
|                     | 9.21        | 9.28        | 1.007    | 0.7   | HP-β-CD        | +29                  |
|                     | 20.95       | 21.52       | 1.028    | 1.2   | CM-β-CD        | +22                  |

*Note:* Conditions: 10 mM chiral selector, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, injection: 10 mbar for 5 s, sample: 1 mg/ml in water.

### TABLE 5  Chiral separation results of a set of three phenidine derivatives

| Compound          | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ | Chiral selector | Applied voltage (kV) |
|-------------------|-------------|-------------|----------|-------|----------------|----------------------|
| Diphenidine       | 12.49       | n.d.        | -        | -     | β-CD           | +30                  |
|                   | 16.48       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                   | 16.93       | 17.32       | 1.023    | 2.4   | HP-β-CD        | +29                  |
|                   | 33.38       | 33.94       | 1.017    | 1.5   | CM-β-CD        | +22                  |
| Methoxyphenidine  | 13.54       | n.d.        | -        | -     | β-CD           | +30                  |
|                   | 15.48       | n.d.        | -        | -     | Acetyl β-CD    | +29                  |
|                   | 13.74       | 13.91       | 1.012    | 1.0   | HP-β-CD        | +29                  |
|                   | 27.96       | 28.42       | 1.017    | 1.8   | CM-β-CD        | +22                  |
| Ephedrine         | 7.99        | 8.05        | 1.008    | 0.9   | β-CD           | +30                  |
|                   | 15.08       | 15.33       | 1.016    | 1.2   | Acetyl β-CD    | +29                  |
|                   | 12.25       | 12.56       | 1.025    | 1.7   | HP-β-CD        | +29                  |
|                   | 32.31       | 33.00       | 1.022    | 1.9   | CM-β-CD        | +22                  |

*Note:* Conditions: 10 mM chiral selector, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, injection: 10 mbar for 5 s, sample: 1 mg/ml in water.
Furthermore, enantiomeric migration orders (EMOs) and enantiomeric purity checks of the analytes amphetamine, diphenidine, and methoxyphenidine were performed. As chiral selectors, carboxymethyl-β-CD served for amphetamine and methoxyphenidine. Hydroxypropyl-β-CD served for the substance diphenidine. Experiments were carried out by spiking racemic samples with pure enantiomers. In case of
amphetamine, the (−)-enantiomer migrated faster than its corresponding (+)-enantiomer. In contrast to the EMO observation of amphetamine, all other tested substances showed a reversed EMO. In Figure 4, the EMO determination of diphenidine is shown. Racemic sample was spiked with its (+)-enantiomer.

Finally, a repeatability study was carried out. For each chiral selector a representative model compound
was chosen. Five intraday and interday measurements each were performed with satisfactory results. All repeatability data are given in Table 7 in detail.

4 | CONCLUSION

In recent years, the popularity and the number of NPS have been growing constantly worldwide. A lot of these compounds are chiral and may potentially differ in their pharmacological behavior. Therefore, analytical method development regarding chiral separation methods is of great interest.

With the presented study, a reliable and cheap chiral CE method to separate NPS enantiomers out of various different substance classes was presented. As chiral selectors, native β-CD and three of its derivatives (acetyl-β-CD, 2-hydroxypropyl-β-CD, and carboxymethyl-β-CD) were investigated. With the chosen separation conditions, all in all, 50 of 51 tested NPS were resolved in their enantiomers within a maximum of 48 min. Resolution factors with respect to all tested β-CD derivatives ranged from 0.5 to 7.2. In direct comparison, carboxymethyl-β-CD was superior to the other investigated β-CDs regarding chromatographic resolution. Additionally, the presented chiral selectors were found to be applicable for simultaneous enantioseparations and to determine enantiomeric elution orders of the studied NPS classes.

As for cathinones, also for the NPS of other compound classes, it was found that they were traded as racemic mixtures, which was confirmed also by means of other chiral selectors in previous studies. Generally, there are few data, whether the effect of the enantiomers differs.

In future, the investigated method can be an additional useful separation technique of further upcoming NPS derivatives as well as to check the enantiomeric composition of real-life samples.

ORCID

Martin G. Schmid © https://orcid.org/0000-0003-0055-660X

REFERENCES

1. Miliano C, Margiani G, Fattore L, De Luca MA. Sales and advertising channels of new psychoactive substances (NPS): internet, social networks, and smartphone apps. Brain Sci. 2018;8(7):123.
2. EMCDDA. European Drug Report; 2019. http://www.emcdda.europa.eu/system/files/publications/11364/20191724_TDAT19001ENN_PDF.pdf
3. UNODC. Understanding the synthetic drug market: the NPS factor; 2018. https://www.unodc.org/documents/scientific_Global_Smart_Update_2018_Vol.19.pdf
4. Glennon RA, Young R, Martin BR, Dal Cason TA. Methcathinone (“CAT”): an enantiomeric potency comparison. Pharmacol Biochem Behav. 1995;50(4):601-606.
5. Gregg RA, Baumann MH, Partilla JS, et al. Stereochemistry of mephedrone neuropharmacology: enantiomer-specific behavioural and neurochemical effects in rats. Br J Pharmaco. 2015;172(3):883-894.
6. Rasmussen LB, Olsen KH, Johansen SS. Chiral separation and quantification of R/S-amphetamine, R/S-methamphetamine, R/S-MDA, R/S-MDMA, and R/S-MDEA in whole blood by GC-MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;842(2):136-141.
7. Jirovsky D, Lemr K, Sevcik J, Smyrl B, Stransky Z. Methamphetamine—properties and analytical methods of enantiomer determination. Forensic Sci Int. 1998;96(1):61-70.
8. Schmid MG. Optical detection of NPS Internet products via HPLC-DAD systems. Light Forensic Sci Issues Appl. 2017;11:301-332.
9. Taschwer M, Ebner E, Schmid MG. Test purchase of new synthetic tryptamines via the Internet: identity check by GC-MS and separation by HPLC. J Appl Pharm Sci. 2016;6(1):28-34.
10. Taschwer M, Grascher J, Schmid MG. Development of an enantioseparation method for novel psychoactive drugs by HPLC using a Lux® Cellulose-2 column in polar organic phase mode. Forensic Sci Int. 2017;270:232-240.
11. Taschwer M, Seidl Y, Mohr S, Schmid MG. Chiral separation of cathinone and amphetamine derivatives by HPLC/UV using sulfated β-cyclodextrin as chiral mobile phase additive. Chirality. 2014;26(8):411-418.
12. Kadkhodaei K, Forcher L, Schmid MG. Separation of enantiomers of new psychoactive substances by high performance liquid chromatography. J Sep Sci. 2018;41(6):1274-1286.
13. Li L, Lurie IS. Regiosomeric and enantiomeric analyses of 24 designer cathinones and phenethylamines using ultra high performance liquid chromatography and capillary electrophoresis with added cyclodextrins. Forensic Sci Int. 2015;254:148-157.
14. Kadkhodaei K, Kadisch M, Schmid MG. Successful use of a novel lux® i-Amylose-1 chiral column for enantioseparation of “legal highs” by HPLC. Chirality. 2019;32(1):42-52. https://doi.org/10.1002/chir.23135
15. Perera RWH, Abraham I, Gupta S, et al. Screening approach, optimisation and scale-up for chiral liquid chromatography of cathinones. J Chromatogr A. 2012;1269:189-197.
16. Silva B, Fernandes C, Tiritan ME, et al. Chiral enantioresolution of cathinone derivatives present in “legal highs”, and enantioselectivity evaluation on cytotoxicity of 3,4-methylenedioxypyrvalerone (MDPV). Forensic Toxicol. 2016;34(2):372-385.
17. Hägele JS, Seibert E, Schmid MG. A simple HPLC-UV approach for rapid enantioseparation of cathinones, pyrovalerones and other novel psychoactive substances on a 2.5-μm cellulose triis-(3,5-dimethylphenyl-carbamate) column. Chromatographia. 2020;83(3):321-329.
18. Silva B, Pereira JA, Cravo S, et al. Multi-milligram resolution and determination of absolute configuration of pentedrone and methylone enantiomers. J Chromatogr B. 2018;1100–1101:158-164.

19. Spálovská D, Maříková T, Kohout M, Králík F, Kuchař M, Setnička V. Methylene and pentylene: structural analysis of new psychoactive substances. Forensic Toxicol. 2019;37(2):366-377.

20. Doi T, Asada A, Takeda A, et al. Chiral separation of the carboxamide-type synthetic cannabinoids N-(1-amino-3-methyl-1-oxobutan-2-yl)-[1-(5-fluoropentyl)-1H-indazole-3-carboxamide and methyl [1-(5-fluoropentyl)-1H-indazole-3-carbonyl]-valinate in illicit herbal products. J Chromatogr A. 2016;1473:83-89.

21. Hägele JS, Schmid MG. Enantiomeric separation of novel psychoactive substances tetracarboxylic acid as chiral selector. Chirality. 2018;30(8):1019-1026.

22. Merola G, Fu H, Tagliaro F, Macchia T, Maccord BR. Chiral separation of 12 cathinone analogs by cyclodextrin-assisted capillary electrophoresis with UV and mass spectrometry detection. Electrophoresis. 2014;35(21–22):3231-3241.

23. Baciu T, Botello I, Borroll F, Calull M, Aguilar C. Capillary electrophoresis and related techniques in the determination of drugs of abuse and their metabolites. Trends Anal Chem. 2015;74:89-108.

24. Taschwer M, Hofer MG, Schmid MG. Enantioseparation of benzofurans and other novel psychoactive compounds by CE and sulfobutylether β-cyclodextrin as chiral selector added to the BGE. Electrophoresis. 2014;35(19):2793-2799.

25. Nowak PM, Olesek K, Woźniakiewicz M, Kościelnia P. Simultaneous enantioseparation of methylone and two isomeric methylmethcathinones using capillary electrophoresis assisted by 2-hydroxyethyl-β-cyclodextrin. Electrophoresis. 2018;39(19):2406-2409.

26. Mohr S, Pilaj S, Schmid MG. Chiral separation of cathinone derivatives used as recreational drugs by cyclodextrin-modified capillary electrophoresis. Electrophoresis. 2012;33(11):1624-1630.

27. Hägele JS, Hubner EM, Schmid MG. Chiral separation of cathinone derivatives using β-cyclodextrin-assisted capillary electrophoresis—comparison of four different β-cyclodextrin derivatives used as chiral selectors. Electrophoresis. 2019;40(14):1787-1794.

28. Řezanková K, Kohoutová R, Kuchař M, Král V, Řezanka P. Enantiomer separation of novel psychoactive chiral amines and their mixture by capillary electrophoresis using cyclodextrins as chiral selectors. Chem Papers. 2018;72(11):2737-2743.

29. Burrai L, Nieddu M, Pirisi MA, Carta A, Briguglio I, Boatto G. Enantiomer separation of 13 new amphetamine-like designer drugs by capillary electrophoresis using modified-β-cyclodextrins. Chirality. 2013;25(10):617-621.

30. Mantim T, Nacapricha D, Wilairat P, Hauser PC. Enantiomer separation of benzofurans and other novel psychoactive compounds by CE and sulfobutylether β-cyclodextrin as chiral selector added to the BGE. Electrophoresis. 2012;33(2):388-394.

31. Fejős I, Varga E, Benkovics G, et al. Characterisation of a single-isomer carboxymethyl-beta-cyclodextrin in chiral capillary electrophoresis. Electrophoresis. 2017;38(15):1869-1877.

32. Weiß JA, Mohr S, Schmid MG. Indirect chiral separation of new recreational drugs by gas chromatography-mass spectrometry using trifluoroacetyl-1-propyl chloride as chiral derivatization reagent. Chirality. 2015;27(3):211-215.

33. Wang SM, Wang TC, Giang YS. Simultaneous determination of amphetamine and methamphetamine enantiomers in urine by simultaneous liquid-liquid extraction and diastereomeric derivatization followed by gas chromatographic-isotope dilution mass spectrometry. J Chromatogr B Anal. 2005;816(1–2):131-143.

34. Taschwer M, Weiß JA, Kunert O, Schmid MG. Analysis and characterization of the novel psychoactive drug 4-chloromethcathinone (clephedrone). Forensic Sci Int. 2014; 244:e56-e59.

35. Alremithi R, Meetani MA, Alaidaros AA, Lanjawi A, Alsumaiti K. Simultaneous quantitative determination of synthetic Cathinone enantiomers in urine and plasma using GC-NCI-MS. J Anal Method Chem. 2018;4396043–4396055.

36. Dhabbah AM. Determination of chiral cathinone in fresh samples of Catha edulis. Forensic Sci Int. 2020;307:110105–110116. https://doi.org/10.1155/2018/4396043.

37. Albals D, van der Heyden Y, Schmid MG, Chankvetadze B, Mangelings D. Chiral separations of cathinone and amphetamine-derivatives: comparative study between capillary electrochromatography, supercritical fluid chromatography and three liquid chromatographic modes. J Pharm Biomed Anal. 2016;121:232-243.

38. Carnes S, O’Brien S, Szewczak A, et al. Comparison of ultra high performance supercritical fluid chromatography, ultra high performance liquid chromatography, and gas chromatography for the separation of synthetic cathinones. J Sep Sci. 2017;40(17):3545-3556.

39. Pauk V, Žihlová V, Borovcová L, Havlíček V, Schug K, Lemr K. Fast separation of selected cathinones and phenylethylamines by supercritical fluid chromatography. J Chromatogr A. 2015;1423:169-176.

40. Geryk R, Kaliková K, Schmid MG, Tesařová E. Enantioselective separation of biologically active basic compounds in ultra-performance supercritical fluid chromatography. Anal Chim Acta. 2016;932:98-105.

41. Folprechtová D, Kozlov O, Armstrong DW, Schmid MG, Kaliková K, Tesařová E. Enantioselective potential of teicoplanin- and vancomycin-based superficially porous particles-packed columns for supercritical fluid chromatography. J Chromatogr A. 2016;1372:145-156.

42. Hädener M, Bruni P, Weinmann W, Frutis M, König S. Accelerated quantification of amphetamine enantiomers in human urine using chiral liquid chromatography and on-line column-switching coupled with tandem mass spectrometry. Anal Bioanal Chem. 2017;409(5):1291-1300.

43. Strano-Rossi S, Odoardi S, Fischella M, Anzillotti L, Gottardo R, Tagliaro F. Screening for new psychoactive substances in hair by ultrahigh performance liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr A. 2014;1372:145-156.

44. Pasin D, Bidny S, Fu S. Analysis of new designer drugs in post-mortem blood using high-resolution mass spectrometry. J Anal Toxicol. 2015;39(3):163-171.
45. Johansen U, Karinen R. Screening of synthetic cannabinoids in preserved oral fluid by UPLC–MS/MS. *Bioanalysis*. 2013;5(18):2257-2268.

46. Schmid MG, Hägele JS. Separation of enantiomers and positional isomers of novel psychoactive substances in solid samples by chromatographic and electrophoretic techniques—a selective review. *J Chromatogr A*. 1624;2020:461256–461271.

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