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REGULAR ARTICLE

WILEY

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

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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. Enantioresolution 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 phenidines.[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

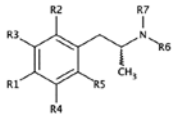
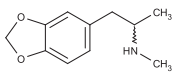
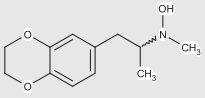
Native β-CD and 2-hydroxypropyl-β-CD (degree of substitution: 0.6) were from Fluka Chemika AG (Buchs, Switzerland). Acetyl-β-CD (degree of substitution: 1.0) and carboxymethyl-β-CD (degree of substitution: 0.5) were purchased from Wacker-Chemie GmbH (Salzburg, Austria). Sodium phosphate and diluted phosphoric 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	
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 = OCH_3	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 = NO_2	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 = OCH_3	2,5-Dimethoxyamphetamine (2,5-DMA, (±)-1-(2,5-Dimethoxyphenyl)propan-2-amine)
A10: R1 = R4 = OCH_3	3,4-Dimethoxyamphetamine (3,4-DMA, (±)-1-(3,4-Dimethoxyphenyl)propan-2-amine)
A11: R1 = OCH_3	4-Methoxyamphetamine ((±)-1-(4-Methoxyphenyl)propan-2-amine)
A12: R1 = Br; R2 = R4 = OCH_3	4-Bromo-2,5-dimethoxyamphetamine (DOB, (±)-4-Bromo-2,5-dimethoxyamphetamine)
A13: R6 = C_2H_5	*N*-Ethylamphetamine ((±)-*N*-Ethyl-1-phenylpropan-2-amine)
A14: R6 = C_3H_6Cl	Mefenorex ((±)-3-Chloro-*N*-(1-methyl-2-phenylethyl)propan-1-amine)
A15: R6 = CH_3	*N*-Methamphetamine ((±)-*N*,α-dimethylphenethylamine)
A16: R1 = Br; R6 = CH_3	4-Bromomethamphetamine (4-BMA, (±)-1-(4-Bromophenyl)-*N*-methylpropan-2-amine)
A17: R2 = Cl; R6 = CH_3	2-Chloromethamphetamine (2-CMA, (±)-1-(2-Chlorophenyl)-*N*-methylpropan-2-amine)
A18: R1 = Cl; R6 = CH_3	4-Chloromethamphetamine (4-CMA, (±)-1-(4-Chlorophenyl)-*N*-methylpropan-2-amine)
A19: R2 = F; R6 = CH_3	2-Fluoromethamphetamine (2-FMA, (±)-1-(2-Fluorophenyl)-*N*-methylpropan-2-amine)
A20: R1 = F; R6 = CH_3	4-Fluoromethamphetamine (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)

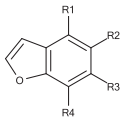
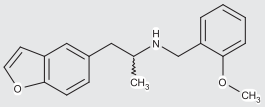
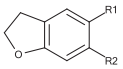
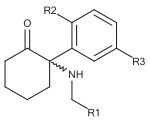
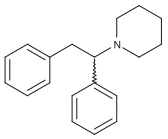
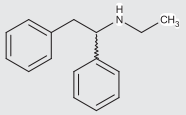
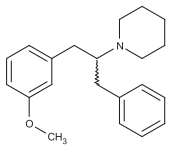
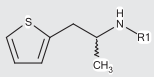
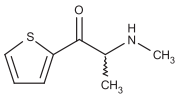
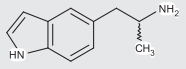
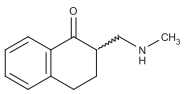
	
D0: All R = H	
D1: R1 = $C_3H_6NH_2$	4-(2-Aminopropyl)-benzofurane (4-APB, (±)-1-(1-Benzofuran-4-yl)propan-2-amine)
D2: R2 = $C_3H_6NH_2$	5-(2-Aminopropyl)-benzofurane (5-APB, (±)-1-(1-Benzofuran-5-yl)propan-2-amine)
D3: R3 = $C_3H_6NH_2$	6-(2-Aminopropyl)-benzofurane (6-APB, (±)-1-(1-Benzofuran-6-yl)propan-2-amine)
D4: R4 = $C_3H_6NH_2$	7-(2-Aminopropyl)-benzofurane (7-APB, (±)-1-(1-Benzofuran-7-yl)propan-2-amine)
D5: R2 = $C_5H_{11}NH$	(±)-1-(Benzofuran-5-yl)-*N*-ethylpropan-2-amine (5-EAPB)
D6: R3 = $C_5H_{11}NH$	(±)-(1-(Benzofuran-6-yl)-*N*-ethylpropan-2-amine) (6-EAPB)
D7: R2 = C_4H_9NH	(±)-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 = $C_3H_6NH_2$	(±)-1-(2,3-Dihydro-1-benzofuran-5-yl)propan-2-amine (5-APDB)
F2: R2 = $C_3H_6NH_2$	(±)-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 = CH_3; R2 = Cl	*N*-Ethyl-ketamine ((±)-2-(2-Chlorophenyl)-2-(ethylamino)cyclohexanone)
G3: R1 = CH_3	2-Oxo-PCE ((±)-2-(Ethylamino)-2-phenylcyclohexan-1-one)
G4: R2 = F	4-Fluoroketamine ((±)-2-(2-Fluorophenyl)-2-(methylamino)cyclohexanone)
G5: R2 = OCH_3	2-MeO-Ketamine ((±)-2-(2-Methoxyphenyl)-2-(methylamino)cyclohexanone)
G6: R1 = CH_3; R3 = OCH_3	Methoxetamine ((±)-2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone)
H	

TABLE 1 (Continued)

	
	Diphenidine ((±)-1-(1,2-Diphenylethyl)piperidine)
I	
	
	Ephenidine ((±)-*N*-Ethyl-1,2-diphenylethylamine)
J	
	
	Methoxphenidine ((±)-2-Methoxy-1-(1,2-Diphenylethyl)piperidine)
K	
	
K1: R1 = H	Thiopropamine ((±)-1-(Thiophen-2-yl)-2-aminopropane)
K2: R1 = CH_3	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-2*H*-naphthalen-1-one))
O	

(Continues)

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 ^{3D}CE 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 measurement, 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).

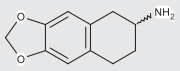
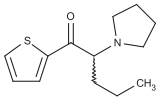
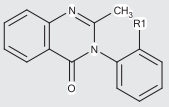
3 | RESULTS AND DISCUSSION

All NPS and pure NPS enantiomers comprising different compound classes were collected since 2010. They were purchased via the Internet, produced by chemical synthesis 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 experiments. 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)
P	 α-Pyrrolidinopentiothiophenone (α-PVT, (±)-2-(Pyrrolidin-1-yl)-1-(thiophen-2-yl)pentan-1-one)
Q	
Q1: R1 = H	Methaqualone ((±)-3-(2-Methylphenyl)-2-methylquinazolin-4-one)
Q2: R1 = CH_3	Ethaqualone ((±)-3-(2-Ethylphenyl)-2-methylquinazolin-4-one)

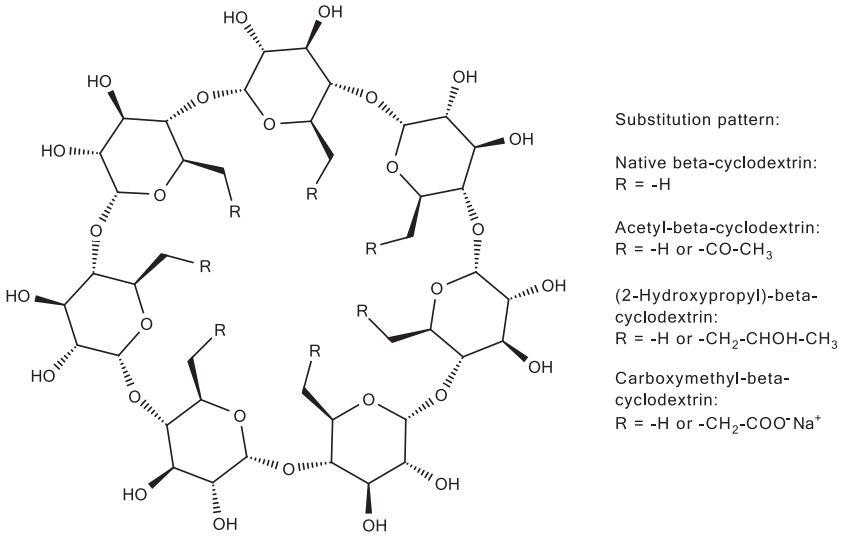


FIGURE 1 Chemical structures applied β-cyclodextrin derivatives (already published in Hägele et al.[27])

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

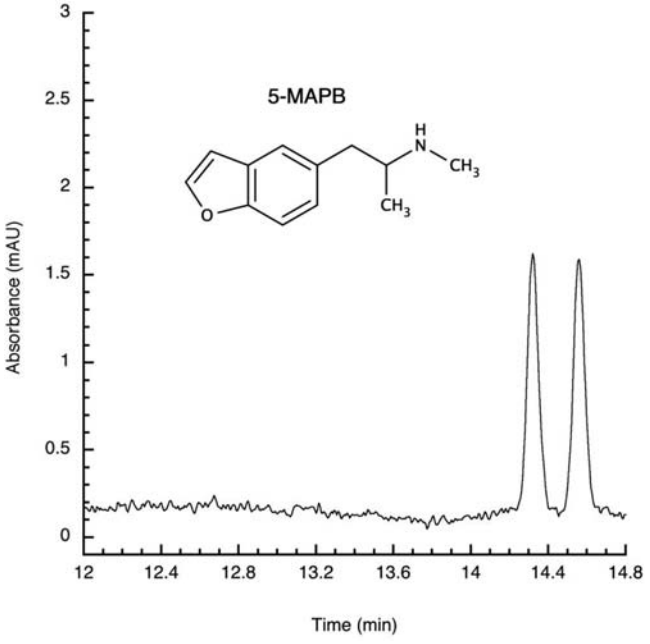


FIGURE 2 Single chiral separation of 5-MAPB. Conditions: 10 mM carboxymethyl-β-cyclodextrin, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltage: 22 kV to cathode, injection: 10 mbar for 5 s, sample: 1 mg/ml in water

TABLE 2 Chiral separation data of 23 amphetamine and methamphetamine derivatives

Compound	t_1 (min)	t_2 (min)	α	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

TABLE 2 (Continued)

Compound	t_1 (min)	t_2 (min)	α	R_s	Chiral selector	Applied voltage (kV)
	15.17	n.d.	-	-	CM-β-CD	+22
3,4-DMA	7.56	n.d.	-	-	β-CD	+30
	8.56	n.d.	-	-	Acetyl β-CD	+29
	7.71	n.d.	-	-	HP-β-CD	+29
	13.14	13.23	1.007	1.0	CM-β-CD	+22
4-MeO-amphetamine	10.50	10.58	1.008	0.7	β-CD	+30
	12.78	n.d.	-	-	Acetyl β-CD	+29
	8.88	8.96	1.009	0.7	HP-β-CD	+29
	21.54	22.19	1.030	2.3	CM-β-CD	+22
N-Methamphetamine	8.91	9.02	1.012	0.9	β-CD	+30
	12.38	12.55	1.013	0.9	Acetyl β-CD	+29
	8.07	8.22	1.018	1.4	HP-β-CD	+29
	15.99	16.49	1.031	4.8	CM-β-CD	+22
4-BMA	12.12	12.24	1.009	0.7	β-CD	+30
	15.27	n.d.	-	-	Acetyl β-CD	+29
	11.24	11.39	1.014	1.0	HP-β-CD	+29
	26.56	26.96	1.015	1.5	CM-β-CD	+22
2-CMA	9.33	9.64	1.033	2.2	β-CD	+30
	11.00	11.38	1.035	1.2	Acetyl β-CD	+29
	8.58	8.87	1.034	2.1	HP-β-CD	+29
	18.84	20.22	1.073	6.0	CM-β-CD	+22
4-CMA	9.62	9.69	1.008	0.9	β-CD	+30
	13.55	n.d.	-	-	Acetyl β-CD	+29
	9.26	9.34	1.009	0.6	HP-β-CD	+29
	23.20	23.84	1.028	2.0	CM-β-CD	+22
2-FMA	9.14	9.37	1.025	1.8	β-CD	+30
	11.21	11.35	1.013	0.5	Acetyl β-CD	+29
	8.18	8.36	1.023	1.6	HP-β-CD	+29
	18.12	19.19	1.059	5.5	CM-β-CD	+22
4-FMA	8.61	8.68	1.009	0.8	β-CD	+30
	12.64	12.86	1.017	1.1	Acetyl β-CD	+29
	7.78	7.85	1.008	0.8	HP-β-CD	+29
	15.42	15.73	1.020	1.9	CM-β-CD	+22
MDMA	11.02	11.16	1.013	0.9	β-CD	+30
	14.67	n.d.	-	-	Acetyl β-CD	+29
	10.57	10.83	1.024	2.2	HP-β-CD	+29
	25.66	26.45	1.031	2.9	CM-β-CD	+22
N-Ethylamphetamine	9.74	9.85	1.011	0.8	β-CD	+30
	13.24	13.42	1.014	0.9	Acetyl β-CD	+29
	9.30	9.48	1.019	1.3	HP-β-CD	+29
	17.64	18.20	1.032	4.2	CM-β-CD	+22
Mefenorex	9.99	10.10	1.010	0.8	β-CD	+30
	14.16	14.35	1.014	1.0	Acetyl β-CD	+29

(Continues)

TABLE 2 (Continued)

Compound	t_1 (min)	t_2 (min)	α	R_s	Chiral selector	Applied voltage (kV)
	10.04	10.22	1.018	1.6	HP-β-CD	+29
	21.47	22.27	1.037	3.7	CM-β-CD	+22
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.

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.

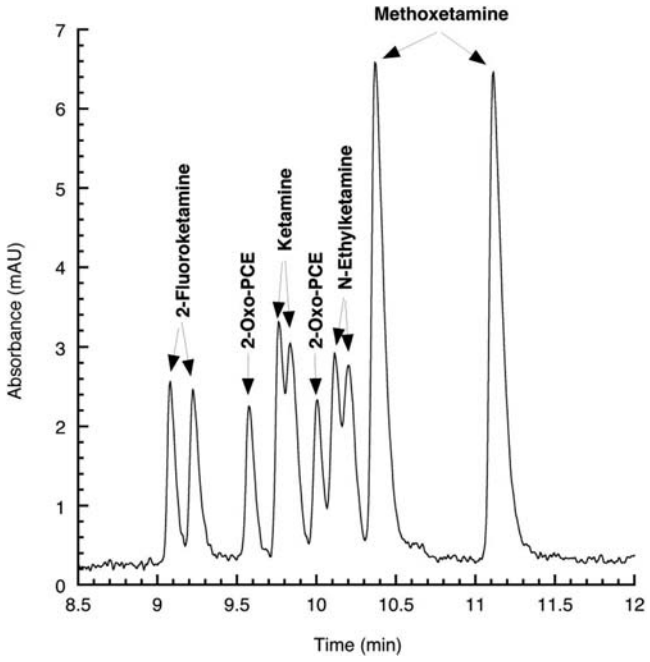


FIGURE 3 Simultaneous enantioseparation of five different ketamine derivatives. Conditions: 10 mM acetyl-β-cyclodextrin, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltage: 29 kV to cathode, injection: 10 mbar for 5 s, sample: 1 mg/ml in water

TABLE 3 Chiral separation results of a set of 10 benzofuran derivatives

Compound	t_1 (min)	t_2 (min)	α	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)	α	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)	α	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
Ephenidine	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.

TABLE 6 Chiral separation results of a set of nine other psychoactive substances

Compound	t_1 (min)	t_2 (min)	α	R_s	Chiral selector	Applied voltage (kV)
Metaqualone	20.63	n.d.	-	-	β-CD	+30
	25.35	25.77	1.017	1.7	Acetyl β-CD	+29
	17.25	n.d.	-	-	HP-β-CD	+29
	28.04	n.d.	-	-	CM-β-CD	+22
Etaqualone	10.26	n.d.	-	-	β-CD	+30
	19.82	19.99	1.009	1.0	Acetyl β-CD	+29
	19.33	n.d.	-	-	HP-β-CD	+29
	26.41	26.83	1.016	2.6	CM-β-CD	+22
α-PVT	9.96	10.07	1.011	1.1	β-CD	+30
	10.11	n.d.	-	-	Acetyl β-CD	+29
	8.80	n.d.	-	-	HP-β-CD	+29
	19.98	20.64	1.033	3.8	CM-β-CD	+22
Thiothinone	7.62	7.68	1.007	0.6	β-CD	+30
	8.96	n.d.	-	-	Acetyl β-CD	+29
	7.08	7.14	1.008	0.8	HP-β-CD	+29
	12.49	12.65	1.012	1.4	CM-β-CD	+22
Methiopropamine	8.10	8.30	1.025	2.1	β-CD	+30
	10.37	10.46	1.008	0.6	Acetyl β-CD	+29
	7.41	7.48	1.009	0.7	HP-β-CD	+29
	12.72	12.99	1.021	2.1	CM-β-CD	+22
Thiopropamine	8.10	8.16	1.007	0.7	β-CD	+30
	11.36	n.d.	-	-	Acetyl β-CD	+29
	7.13	n.d.	-	-	HP-β-CD	+29
	13.95	14.26	1.022	2.2	CM-β-CD	+22
5-APi	12.27	12.61	1.027	2.5	β-CD	+30
	14.58	n.d.	-	-	Acetyl β-CD	+29
	10.42	10.70	1.027	1.7	HP-β-CD	+29
	26.43	28.28	1.070	6.4	CM-β-CD	+22
MTTA	9.51	9.62	1.012	1.2	β-CD	+30
	10.65	10.82	1.015	1.6	Acetyl β-CD	+29
	8.54	8.60	1.007	0.5	HP-β-CD	+29
	18.71	18.98	1.014	1.4	CM-β-CD	+22
MDAT	4.72	n.d.	-	-	β-CD	+30
	5.36	n.d.	-	-	Acetyl β-CD	+29
	4.77	n.d.	-	-	HP-β-CD	+29
	8.06	n.d.	-	-	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

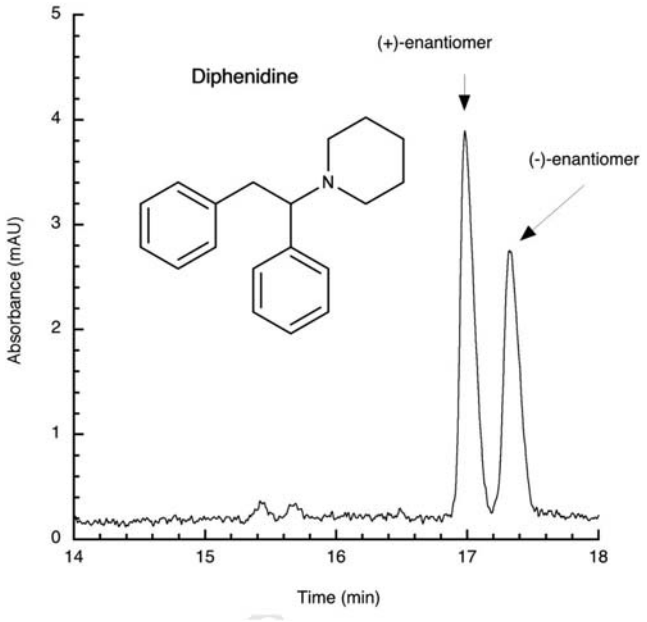


FIGURE 4 Enantiomeric migration order (EMO) determination of diphenidine. Conditions: 10 mM hydroxypropyl-β-cyclodextrin, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltage: 22 kV to cathode, injection: 10 mbar for 5 s, sample: 1 mg/ml in water

TABLE 7 Repeatability data including retention time and resolution

Chiral selector	Model substance	Applied voltage (kV)	Repeatability	t_1 (min)	t_2 (min)	R_s
β-CD	2-FMA	+30	Intraday $n = 5$	9.20 ± 0.07 RSD = 0.5%	9.43 ± 0.09 RSD = 0.7%	1.8 ± 0.1 RSD = 2.5%
			Interday $n = 5$	9.27 ± 0.13 RSD = 0.9%	9.50 ± 0.16 RSD = 1.1%	1.7 ± 0.2 RSD = 4.9%
Acetyl-β-CD	Methoxetamine	+29	Intraday $n = 5$	10.55 ± 0.18 RSD = 1.0%	11.26 ± 0.19 RSD = 1.1%	4.6 ± 0.2 RSD = 2.7%
			Interday $n = 5$	10.59 ± 0.34 RSD = 2.1%	11.31 ± 0.35 RSD = 2.1%	4.5 ± 0.2 RSD = 3.6%
HP-β-CD	Diphenidine	+29	Intraday $n = 5$	16.98 ± 0.06 RSD = 0.3%	17.41 ± 0.0.10 RSD = 0.5%	2.4 ± 0.2 RSD = 4.7%
			Interday $n = 5$	17.04 ± 0.14 RSD = 0.7%	17.48 ± 0.16 RSD = 0.9%	2.4 ± 0.2 RSD = 5.1%
CM-β-CD	2-CMA	+22	Intraday $n = 5$	18.99 ± 0,15 RSD = 0.6%	20.43 ± 0.21 RSD = 0.7%	6.0 ± 0.2 RSD = 2.5%
			Interday $n = 5$	19.04 ± 0.20 RSD = 0.7%	20.51 ± 0.29 RSD = 1.1%	5.9 ± 0.3 RSD = 3.7%

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.

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.[8,10–12,14,21,24,32,46] 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.

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