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RESEARCH ARTICLE

Comparative studies on enantioseparation of New Psychoactive Substances using cyclodextrin-assisted capillary electrophoresis with UV detection

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Abstract: New psychoactive substances (NPS) count as psychoactive substances, which are slightly modified compared to illicit drugs regarding their chemical structure to circumvent law. Compared to classical drugs such as heroin, cocaine, or amphetamine, they show similar psychoactive effects, however, because of their novelty there is few knowledge about their side effects or toxicity. NPS are available as different chemical substance classes, among them chiral novel derivatives of amphetamine, cathinone, and ketamine. Since in most cases no clinical studies are available about the possibly different effects of the two enantiomers, there is a big demand for enantioseparation method development. Besides high-performance separation techniques such as gas chromatography or HPLC, capillary electrophoresis has turned out to be a powerful alternative for chiral separation development. The addition of chiral additives such as cyclodextrins to the background electrolyte often results in successful attempts. The present study compares the chiral separation power of different previously used non-charged ß-cyclodextrins, among them native ß-cyclodextrin as well as some of its derivatives such as acetyl-, and 2-hydroxypropyl-β-cyclodextrin, with the negatively charged derivatives carboxymethyl-, carboxyethyl- and succinyl-β-cyclodextrin by capillary zone electrophoresis. A total of 136 chiral NPS were investigated with these cyclodextrins, 122 of them were resolved in their enantiomers successfully by means of a simple electrolyte composition consisting of 10 mM aqueous sodium hydrogen phosphate buffer, pH 2.5 and 10 mM of the chiral selector. Furthermore, the presented method turned out to be useful to distinguish between positional isomers and examples for both enantiomer order and positional order for seized samples are given.

Keywords: native β-cyclodextrin, acetyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, carboxymethyl-β-cyclodextrin, carboxyethyl-β-cyclodextrin, succinyl-β-cyclodextrin, novel psychoactive substances, chiral

1 Introduction

Besides classic illicit drugs such as cannabis, amphetamine, cocaine or heroin, the so-called New Psychoactive Substances (NPS) came up in generations since about two decades [1] because of high prices along with poor quality of certain illicit drugs at that time. Minor structural changes in the chemical structure of several classic drugs resulted in an emerge of a huge number of novel derivatives. The main intention was to circumvent law against their abuse and additionally to compete price and quality. Since the middle of the 2000s [2] the worldwide drug market was flooded with such designer drugs with aim to mimic the psychoactive effects of classic drugs and to avoid violation of drug laws.

The United Nations Single Convention on Narcotic Drugs of 1961 provided a base for the specific regulation for production, use, possession, and distribution of illicit drugs [3]. Herein, narcotic plants, substances and preparations are listed, for example cannabis, opium, cocaine, methadone, and heroin [4]. In 1972, psychotropics such as amphetamine (Speed), methamphetamine (Crystal meth), LSD and MDMA (Ecstasy) were included [5]. This law also comprises derivatives like isomers, salts, esters, and ethers of the listed substances. Although a big variety of these compounds is covered by this convention and the corresponding national laws, drug chemists found creative attempts to produce and trade legal alternatives. For example, structural alterations such as introduction of halogen substituents, carbonyl-, or carboxy-groups led to new derivatives with similar psychoactive effects. Contrary to their parent structures, they were and are partially still legal. Marketed as “bath salts”, “bird cage cleaners”, “research chemicals” these so-called “legal highs” are distributed mostly over the internet. The distribution

of drugs, not solely via the dark net, is a concerning trend that rose to a new peak during the ongoing pandemic [2]. Figure 1 shows offers of cathinones as crystals or tablets available in the clearnet.

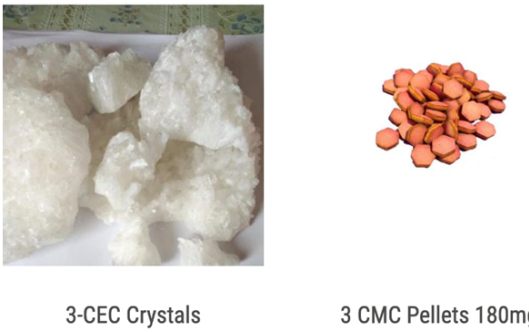


Figure 1 Cathinone crystals and tablets offered in an online shop

According to the latest European Drug Report currently 830 NPS are monitored by the European Monitoring Centre for Drugs and Drug Addition (EMCDDA), 46 of them were reported for the first time in 2020. Though the number of newly reported substance is declining in the last years, it is still on a high level [2].

Many of these novel designer drugs contain an asymmetric centre with the consequence that two enantiomers are possible. In general, chiral substances might exhibit different pharmacological characteristics for each enantiomer as pharmaceutical drug substances do. It is already well-known that the psychoactive effect of some classic chiral addictive substances is restricted mainly to one distinct enantiomer, as it applies e. g. for S-(+)-amphetamine, S-(+)-methamphetamine or S-(+)-ketamine [6–9]. This fact leads to the conclusion that derivatives of the afore mentioned compound classes might exhibit pharmacological differences with respect to their enantiomers as well, however, besides some exceptions [10–15], there is little or no knowledge about it.

Therefore, the development of chiral separation techniques for chiral NPS is of huge interest. It serves to determine the chiral status of samples as well as to allocate positional isomers. Furthermore, changes within the illegal drug market can be monitored more precisely. The knowledge of both psychoactive effects and unwanted side effects restricted to different enantiomers might be useful to understand strategy of synthesis. In this context, mostly racemic mixtures are produced, however, there are few exceptions. In-house analysis of real-life samples seized by Austrian police revealed that pure enantiomers of certain products are a result of educts as pure enantiomers, as it is the case for the production of crystal meth from ephedrine available as over-the-counter tablets [16]. These findings also correlate with a recent publication [17].

Enantioseparation of chiral NPS for analytic purposes are mainly performed via HPLC by means of various chiral columns, among them cellulose derivatives or cyclodextrins [18] as chiral selectors. Further successful work was done in this field by means of gas chromatography (GC). A comprehensive overview of reported methods for enantioseparation of NPS is given in a survey article [18]. Capillary electrophoresis (CE) counts as a complementary technique to HPLC and represents a powerful alternative for separation of enantiomers by means of simple method development and low sample and electrolyte consumption combined with inexpensive UV detection. In this field, previous work has already shown the potential of cyclodextrins as chiral additives in CE [19–21]. This chiral selector class comprises 6 (α-), 7 (β-) or 8 (γ-cyclodextrin) cyclic glycopyranose units forming a truncated cone. Chiral recognition takes place by host-guest interaction via inclusion complexation as well as by additional hydrogen bondings or dipol-dipol interactions. Each enantiomer is intended to form a differently migrating complex with the chiral selector leading to two separated peaks in the electropherogram. Also, native cyclodextrins can undergo derivatisation of the hydroxy groups in position 2, 3 and 6 resulting in derivatives, which are determined by their degree of substitution (DS) and varying cone depths. Moreover, they possess low UV-absorption preventing detection problems. This makes cyclodextrins to the most often applied chiral selectors for CE [22].

First, cyclodextrin-assisted chiral separations of the parent compounds amphetamine and its derivatives as well as cathinone and methcathinone were reported by Lurie *et al.* already in 1994 [23]. Later, further amphetamine derivatives were separated using β-cyclodextrins but also sulfated γ-cyclodextrins [24–35]. In the sequel, successful enantioseparation of various cathinones [36–39] and benzofurans [40] as new compound classes of NPS was reported. Chiral separation of the parent compound ketamine was performed first in 1992 [41].

The present work ties in with the work of Hägele *et al.* [42, 43], presenting also successful

results of latest NPS involving further chiral substance classes by means of the non-charged native, acetyl- and 2-hydroxypropyl-β-CD as well as the negatively charged carboxymethyl-β-CD (DS 0.5). The aim is to extend the number of chiral separations of NPS. In total, 136 NPS were tested by carboxymethyl-β-CD (DS 3.5), carboxyethyl-β-CD and succinyl-β-CD. Because of their novelty, these NPS are partially not yet commercially available and were purchased from various internet shops or seized by Austrian police.

2 Materials and Methods

2.1 Chemicals

Carboxymethyl-, succinyl- and carboxyethyl-β-CD (degree of substitution 3.5 each) were bought from CycloLab Ltd. (Budapest, Hungary). Di-sodium hydrogen phosphate and diluted phosphoric acid were from Merck KGaA (Darmstadt, Germany) and water from Fisher Scientific (Loughborough, UK). All components were of analytical grade.

2.2 Samples

Analytes already commercially available were purchased from LGC Standards GmbH (Wesel, Germany) or Lipomed AG (Arlesheim, Switzerland). The majority of NPS were either bought from different online shops or represented real-life samples seized by Austrian police and provided for research purposes. Prior to experiments, their identity was confirmed by GC-MS. Chemical structures and names of tested NPS are listed in Table 1. They were mainly available as hydrochloric acid salts.

Table 1.1 Structures and names of investigated NPS

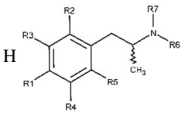
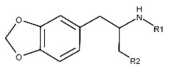
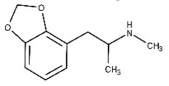
Amphetamines	
A0: All R = H	 Amphetamine, (±)-1-Phenylpropan-2-amine
A1: R1 = Cl	4-Chloroamphetamine, 4-CA, (±)-1-(4-Chlorophenyl)propan-2-amine
A2: R2 = F	2-Fluoroamphetamine, 2-FA, (±)-1-(2-Fluorophenyl)propan-2-amine
A3: R3 = F	3-Fluoroamphetamine, 3-FA, (±)-1-(3-Fluorophenyl)propan-2-amine
A4: R1 = F	4-Fluoroamphetamine, 4-FA, (±)-1-(4-Fluorophenyl)propan-2-amine
A5: R1 = SH	4-Methylthioamphetamine, MTA, (±)-1-[4-(Methylsulfanyl)phenyl]propan-2-amine
A6: R1 = OCH_3	4-Methoxyamphetamine, (±)-1-(4-Methoxyphenyl)propan-2-amine
A7: R2 = R4 = OCH_3	2,5-Dimethoxyamphetamine, 2,5-DMA, (±)-1-(2,5-Dimethoxyphenyl)propan-2-amine
A8: R1 = R4 = OCH_3	3,4-Dimethoxyamphetamine, 3,4-DMA, (±)-1-(3,4-Dimethoxyphenyl)propan-2-amine
A9: R1 = Br, R2 = R4 = OCH_3	4-Bromo-2,5-dimethoxyamphetamine, DOB, (±)-4-Bromo-2,5-dimethoxyamphetamine
A10: R1 = Cl; R2 = R4 = OCH_3	2,5-Dimethoxy-4-chloroamphetamine, DOC, (±)-1-(4-Chloro-2,5-dimethoxyphenyl)propan-2-amine
A11:R1 = R4 = R5 = OCH_3	3,4,5-Trimethoxyamphetamine, 3,4,5-TMA, (±)-1-(3,4,5-Trimethoxyphenyl)propan-2-amine
N-Methamphetamines	
A12: R6 = CH_3	N-Methamphetamine, (±)-N,α-dimethylphenethylamine
A13: R3 = R6 = CH_3	3-Methylmethamphetamine, 3-MMA, (±)-1-(3-Methylphenyl)-N-methylpropan-2-amine
A14: R2 = F; R6 = CH_3	2-Fluoromethamphetamine, 2-FMA, (±)-1-(2-Fluorophenyl)-N-methylpropan-2-amine
A15: R3 = F; R6 = CH_3	3-Fluoromethamphetamine, 3-FMA, (±)-1-(3-Fluorophenyl)-N-methylpropan-2-amine
A16: R1 = F; R6 = CH_3	4-Fluoromethamphetamine, 4-FMA, (±)-1-(4-Fluorophenyl)-N-methylpropan-2-amine
Other N-substituted Amphetamines	
A17: R6 = C_2H_5	N-Ethylamphetamine, (±)-N-Ethyl-1-phenylpropan-2-amine
A18: R6 = C_3H_6Cl	Mefenorex, (±)-3-Chloro-N-(1-methyl-2-phenylethyl)propan-1-amine
A19: R6 = R7 = CH_3	N,N-Dimethylamphetamine, (±)-N,N-dimethylphenethylamine
A20: R2 = F, R6 = C_2H_5	2-Fluoroethamphetamine, 2-FEA, (±)-1-(2-Fluorophenyl)-N-ethylpropan-2-amine
A21: R3 = F, R6 = C_2H_5	3-Fluoroethamphetamine, 3-FEA, (±)-1-(3-Fluorophenyl)-N-ethylpropan-2-amine
A22: R1 = F, R6 = C_2H_5	4-Fluoroethamphetamine, 4-FEA, (±)-1-(4-Fluorophenyl)-N-ethylpropan-2-amine
A23: R6 = C_3H_7	N-Propylamphetamine, (±)-N-Propyl-1-phenylpropan-2-amine
Methylenedioxy-N-amphetamines	
B0: all R = H	 3,4-Methylenedioxyamphetamine, MDA, (±)-1-(2H-1,3-benzodioxol-5-yl)propan-2-amine
B1: R1 = CH_3	3,4-Methylenedioxymethamphetamine, 3,4-MDMA, (±)-1-(Benzo [1,3]dioxol-5-yl)-N-methyl-propan-2-amine
B2: R1 = C_2H_5	Methylenedioxyethamphetamine, MDEA, (±)-1-(Benzo [1,3]dioxol-5-yl)-N-methyl-propan-2-amine
B3: R1 = R2 = CH_3	Methylenebenzodioxolbutylamine, MBDB, (±)-1-(Benzo [1,3]dioxol-5-yl)-N-methyl-butyl-2-amine
B4:	 2,3-Methylenedioxymethamphetamine, 2,3-MDMA, (±)-1-(Benzo [1,3]dioxol-4-yl)-N-methyl-propan-2-amine

Table 1.2 Structures and names of investigated NPS

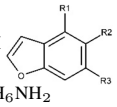
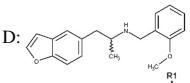
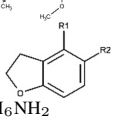
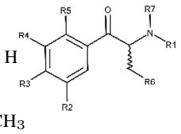
Benzofuranes	
C0: All R = H 	
C1: R1 = $C_3H_6NH_2$	4-(2-Aminopropyl)-benzofurane, 4-APB, (±)-1-(1-Benzofuran-4-yl)propan-2-amine
C2: R2 = $C_3H_6NH_2$	5-Aminopropylbenzofurane, 5-APB, (±)-1-(1-Benzofuran-5-yl)propan-2-amine
C3: R3 = $C_3H_6NH_2$	6-Aminopropylbenzofurane, 6-APB, (±)-1-(1-Benzofuran-6-yl)propan-2-amine
C4: R2 = $C_5H_{11}NH$	5-EAPB, (±)-1-(Benzofuran-5-yl)-N-ethylpropan-2-amine
C5: R3 = $C_5H_{11}NH$	6-EAPB, (±)-1-(Benzofuran-6-yl)-N-ethylpropan-2-amine
C6: R2 = C4H9NH	5-MAPB, (±)-1-(Benzofuran-5-yl)-N-methylpropan-2-amine
D: 	N-MOB-5-APB, (±)-1-(Benzofuran-5-yl)-N-(2-methoxybenzyl)-propan-2-amine
E0: All R = H 	
E1: R1 = $C_3H_6NH_2$	5-APDB, (±)-1-(2,3-Dihydro-1-benzofuran-5-yl)propan-2-amine
E2: R2 = $C_3H_6NH_2$	6-APDB, (±)-1-(2,3-Dihydro-1-benzofuran-6-yl)propan-2-amine
Cathinones:	
G0: all R = H 	
G1: R3 = CH_3	4-Methylcathinone, 4-MC, nor-Mephedrone, (±)-2-Amino-1-(4-methylphenyl)propan-1-one
Methcathinones	
G2: R1 = CH_3; R5 = CH_3	2-Methylmethcathinone, 2-MMC, (±)-2-(Methylamino)-1-(2-methylphenyl)propan-1-one)
G3: R1 = CH_3; R4 = CH_3	3-Methylmethcathinone, 3-MMC, (±)-2-(Methylamino)-1-(3-methylphenyl)propan-1-one)
G4: R1 = CH_3; R3 = CH_3	4-Methylmethcathinone, 4-MMC, Mephedrone, (±)-2-(Methylamino)-1-(4-methylphenyl)propan-1-one)
G5: R1 = CH_3; R3 = R4 = CH_3	3,4-Dimethylmethcathinone, 3,4-DMMC, (±)-1-(3,4-Dimethylphenyl)-2-(methylamino)propan-1-one
G6: R1 = CH_3; R3 = R5 = CH_3	2,4-Dimethylmethcathinone, 2,4-DMMC, (±)-1-(2,4-Dimethylphenyl)-2-(methylamino)propan-1-one
G7: R1 = CH_3; R5 = OCH_3	2-Methoxymethcathinone, 2-MeOMC, (±)-1-(2-Methoxyphenyl)-2-(methylamino)propan-1-one
G8: R1 = CH_3; R4 = OCH_3	3-Methoxymethcathinone, 3-MeOMC, (±)-1-(3-Methoxyphenyl)-2-(methylamino)propan-1-one
G9: R1 = CH_3; R3 = OCH_3	4-Methoxymethcathinone, 4-MeOMC, Methedrone, (±)-1-(4-Methoxyphenyl)-2-(methylamino)propan-1-one
G10: R1 = CH_3; R5 = Cl	2-Chloromethcathinone, 2-CMC, (±)-1-(2-Chlorophenyl)-2-(methylamino)propan-1-one
G11: R1 = CH_3; R4 = Cl	3-Chloromethcathinone, 3-CMC, (±)-1-(3-Chlorophenyl)-2-(methylamino)propan-1-one
G12: R1 = CH_3; R3 = Cl	4-Chloromethcathinone, 4-CMC, Clephedrone, (±)-1-(4-Chlorophenyl)-2-(methylamino)propan-1-one
G13: R1 = CH_3; R4 = C_2H_5	3-Ethylmethcathinone, 3-EMC, (±)-1-(3-Ethylphenyl)-2-(methylamino)propan-1-one
G14: R1 = CH_3; R3 = C_2H_5	4-Ethylmethcathinone, 4-EMC, (±)-1-(4-Ethylphenyl)-2-(methylamino)propan-1-one
G15: R1 = R3 = CH_3; R6 = OCH_3	Mexedrone, (±)-3-Methoxy-2-(methylamino)-1-(4-methylphenyl)propan-1-one
G16: R1 = CH_3; R5 = F	2-Fluoromethcathinone, 2-FMC, (±)-1-(2-Fluorophenyl)-2-(methylamino)propan-1-one
G17: R1 = CH_3; R4 = F	3-Fluoromethcathinone, 3-FMC, (±)-1-(3-Fluorophenyl)-2-(methylamino)propan-1-one
G18: R1 = CH_3; R3 = F	4-Fluoromethcathinone, 4-FMC, Flephedrone, (±)-1-(4-Fluorophenyl)-2-(methylamino)propan-1-one
G19: R1 = CH_3; R3 = Br	4-Bromomethcathinone, 4-BMC, Brephedrone, (±)-1-(4-Bromophenyl)-2-(methylamino)propan-1-one
G20: R1 = R6 = CH_3	Buphedrone, (±)-2-(Methylamino)-1-phenylbutan-1-one
G21: R1 = R3 = R6 = CH_3	4-Methylbuphedrone, (±)-2-(Methylamino)-1-4-mehtylphenylbutan-1-one)
G22: R1 = CH_3; R6 = C_2H_5	Pentedrone ((±)-1-Phenyl-2-(methylamino)pentan-1-one
Ethcathinones	
G23: R1 = C_2H_5	Ethcathinone, (±)-1-(Phenyl)-2-(ethylamino)propan-1-one
G24: R1 = C_2H_5, R5 = F	3-Fluoroethcathinone, 3-FEC, (±)-1-(3-Fluorophenyl)-2-(ethylamino)propan-1-one
G25: R1 = R7 = C_2H_5	Amfepramone, (±)-2-Diethylamino-1-phenylpropan-1-one
G26: R1 = C_2H_5; R5 = Cl	3-Chloroethcathinone, 3-CEC, (±)-1-(3-Chlorphenyl)-2-(ethylamino)propan-1-one
G27: R1 = C_2H_5; R3 = Cl	4-Chloroethcathinone, 4-CEC, (±)-1-(4-Chlorphenyl)-2-(ethylamino)propan-1-one
G28: R1 = C_2H_5; R6 = C_3H_7	N-Ethylhexedrone, (±)-2-(Ethylamino)-1-phenylhexan-1-one
G29: R1 = C_2H_5; R6 = CH_3	N-Ethylbuphedrone, (±)-2-(Ethylamino)-1-phenylpentan-1-one
G30: R1 = C_2H_5; R6 = C_2H_5	N-Ethylpentedrone, (±)-2-(Ethylamino)-1-phenyl-1-pentanone
G31: R1 = C_2H_5; R3 = CH_3	4-Methylethcathinone, 4-MEC, (±)-2-(Ethylamino)-1-(4-methylphenyl)propan-1-one
G32: R1 = C_2H_5; R4 = CH_3	3-Methylethcathinone, 3-MEC, (±)-2-(Ethylamino)-1-(3-methylphenyl)propan-1-one
G33: R4 = R3 = OCH_3; R1 = C_2H_5; R6 = C_2H_5	DL-4662, (±)-1-(3,4-Dimethoxyphenyl)-2-(ethylamino)pentan-1-one
G34: R1 = R5 = C_2H_5	2-Ethylethcathinone, 2-EEC, (±)-2-(Ethylamino)-1-(2-ethylphenyl)propan-1-one
G35: R1 = R4 = C_2H_5	3-Ethylethcathinone, 3-EEC, (±)-2-(Ethylamino)-1-(3-ethylphenyl)propan-1-one
G36: R1 = R3 = C_2H_5	4-Ethylethcathinone, 2-EEC, (±)-2-(Ethylamino)-1-(4-ethylphenyl)propan-1-one
Other N-substituted Cathinones	
G37: R4 = Cl; R1 = $C(CH_3)_3$	Bupropione, (±)-1-(3-Chlorphenyl)-2-tert-butylamino-propan-1-one
G38: R1 = C_3H_7, R3 = CH_3	4-Methylpropcathinone, 4-MPC, (±)-2-(Propylamino)-1-(4-methylphenyl)propan-1-one)
G39: R1 = C_3H_7	N-Propcathinone, NiPP, (±)-2-(Propylamino)-1-(phenyl)propan-1-one)
G40: R1 = C_3H_7, R3 = Cl	4-Chlorpropcathinone, 4-CPRC, (±)-2-(Propylamino)-1-(4-chlorphenyl)propan-1-one)
G41: R1 = $CH(CH_3)_2$, R3 = F	4-FNPP, (±)-1-(4-Fluorophenyl)-2-(isopropyamino)-pentan-1-one
G42: R1 = $CH(CH_3)_2$, R3 = Cl	4-ClC, (±)-1-(4-Chlorophenyl)-2-(isopropylamino)-propan-1-one
G43: R1 = C_4H_9	4-Chlorobutcathinone, 4-CBC, (±)-1-(4-Chlorphenyl)-2-(butylamino)-propan-1-one
G44: R1 = R7 = CH_3, R3 = Cl	4-CDC, (±)-1-(4-Chlorphenyl)-2-(dimethylamino)-propan-1-one
G45: R1 = $CH(CH_3)_2$, R6 = C_4H_9	NDH, (±)-2-[(2-methylpropyl)amino]-1phenylhexan-1-one
G46: R1 = R5 = OCH_3; R7 = CH_3	2,5-Dimethoxy-4-methylcathinone, DOMC, (±)-2-[Methoxy(methyl)amino]-1-(2-methoxyphenyl)-propan-1-one
G47: R1 = benzyl; R3 = CH_3	Benzedrone, 4-MBC, (±)-2-(benzylamino)-1-(4-methylphenyl)propan-1-one

Table 1.3 Structures and names of investigated NPS

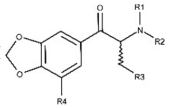
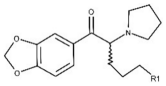
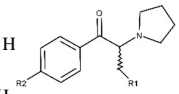
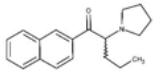
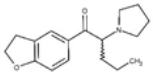
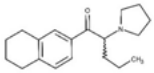
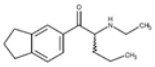
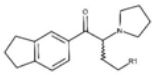
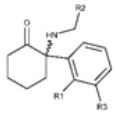
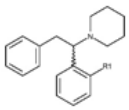
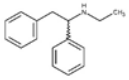
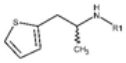
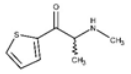
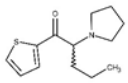
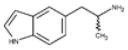
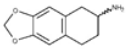
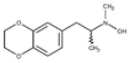
Methylenedioxycathinones	
H0: All R = H 	
H1: R1 = CH_3	3,4-Methylenedioxymethcathinone, Methylone, MDMC, (±)-1-(1,3-Benzodioxol-5-yl)-2-(methylamino)propan-1-one
H2: R1 = CH_3; R4 = OCH_3	5-Methoxymethylone, 2-AIMP, (±)-1-(7-Methoxy-1,3-benzodioxol-5-yl)-2-(methyl-amino)propan-1-one
H3: R1 = R2 = CH_3	Dimethylone, (±)-1-(1,3-Benzodioxol-5-yl)-2-(dimethylamino)-propan-1-one
H4: R1 = R3 = CH_3;	Butylone, (±)-1-(1,3-Benzodioxol-5-yl)-2-(methylamino)-butan-1-one
H5: R1 = R2 = R3 = CH_3	N,N-Dimethylbutylone, (±)-1-(1,3-Benzodioxol-5-yl)-2-(dimethylamino)-butan-1-one
H6: R1 = CH_3; R3 = C_2H_5	Pentylone, (±)-*1*-(1,3-Benzodioxol-5-yl)-2-(methylamino)-pentan-1-one
H7: R1 = C_2H_5	Ethylone, (±)-1-(1,3-Benzodioxol-5-yl)-2-(ethylamino)propan-1-one
H8: R1 = R3 = C_2H_5	N-Ethylpentylone, Bk-Ethyl-K, (±)-1-(7-Ethyl-1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one
H9: R1 = C_2H_5; R4 = CH_3	5-Methylethylone, 5-ME, (±)-1-(7-Methyl-1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one
H10: R1 = benzyl, R3 = CH_3	N-Benzylnorbutylone, (±)-N-Benzyl-(3,4-methylenedioxyphenyl)-2-aminobutan-1-one
H11: R1 = $C(CH_3)_3$	MDPT, (±)-1-(2H-1,3-benzodioxol-5-yl)-2-(tertbutylamino)propan-1-one
Methylenedioxypyrovalerones	
I0: All R = H 	3,4-Methylenedioxypyrovalerone, MDPV, (±)-1-(Benzo[d]-[1,3]-dioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one
I1: R1 = CH_3	3,4-Methylenedioxy-α-pyrrolidinohexiophenone, MD-PHP, (±)-1-(Benzo[d]-[1,3]-dioxol-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one
I2: R1 = C_2H_5	MDPEP, (±)-1-(Benzo[d]-[1,3]-dioxol-5-yl)-2-(pyrrolidin-1-yl)heptan-1-one
Pyrovalerones	
J0: All R = H 	α-PPP, (±)-1-Phenyl-2-(pyrrolidin-1-yl)propan-1-one
J1: R2 = CH_3	M-PPP, (±)-1-(4-Methylphenyl)-2-(pyrrolidin-1-yl)propan-1-one
J2: R1 = C_2H_5	α-PVP, (±)-1-Phenyl-2-(1-pyrrolidinyl)-1-pentan-1-one
J3: R2 = F; R1 = C_2H_5	4-F-PVP, (±)-1-(4-Fluorophenyl)-2-(pyrrolidin-1-yl)pentan-1-one
J4: R2 = Cl; R1 = C_2H_5	4-Cl-PVP, (±)-1-(4-Chlorophenyl)-2-(pyrrolidin-1-yl)pentan-1-one
J5: R2 = CH_3; R1 = C_2H_5	4-MPrC, (±)-1-(4-Methylphenyl)-2-(pyrrolidin-1-yl)pentan-1-one
J6: R2 = OCH_3; R1 = C_2H_5	4-MeO-α-PVP, (±)-1-(4-Methoxyphenyl)-2-(1-pyrrolidinyl)pentan-1-one
J7: R2 = CH_2, R1 = C_3H_7	4-MPHP, (±)-1-(4-Methylphenyl)-2-(1-pyrrolidinyl)pentan-1-one
J8: R1 = F; R2 = C_3H_7	4-F-PHP, (±)-1-(4-Fluorphenyl)-2-(pyrrolidin-1-yl)hexan-1-one
J9: R2 = C_4H_9	PV8, (±)-1-phenyl-2-(1-pyrrolidinyl)-1-heptanone
J10: R1 = F; R2 = C_4H_9	4-F-PV8, (±)-1-(4-Fluorophenyl)-2-(1-pyrrolidinyl)-1-heptanone
J11: R1 = C_5H_{11}	PV9, (±)-1-Phenyl-2-(1-pyrrolidin-1-yl)octan-1-one
J12: R1 = C_6H_{13}	PV10, (±)-1-Phenyl-2-(1-pyrrolidin-1-yl)nonan-1-one
J12: R1 = $CH(CH_3)_2$	α-PIHP, (±)-4-Methyl-1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one
K 	Naphyrone, (±)-1-Naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one
L 	5-Dihydrobenzofuranepyrovalerone, 5-DBFPV, (±)-1-(2,3-Dihydrobenzofuran-5-yl)-2-(pyrrolidin-1-yl)-pentan-1-one
M 	TH-PVP, (±)-2-(Pyrrolidin-1-yl)-1-(5,6,7,8-tetrahydronaphthalen-2-yl)pentan-1-one
Other Cathinones	
N 	bk-iVP, (±)-1-(2,3-Dihydro-1H-inden-5-yl)-2-(ethylamino)pentan-1-one
O0: R1 = H 	5-PPDi, (±)-1-(2,3-Dihydro-1H-inden-5-yl)-2-(pyrrolidin-1-yl)-butan-1-one
O1: R1 = C_2H_5	5-BPDi, (±)-1-(2,3-Dihydro-1H-inden-5-yl)-2-(pyrrolidin-1-yl)-hexan-1-one
Ketamines	
P0: All R = H 	Deschlorketamine, 2-Oxo-PCM, (±)-2-(Methylamino)-2-phenylcyclohexan-1-one
P1: R1 = Cl	Ketamine, (±)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexan-1-one
P2: R1 = F	2-Fluoroketamine, (±)-2-(2-Fluorophenyl)-2-(methylamino)cyclohexan-1-one)
P3: R2 = CH_3	N-Ethyldeschloroketamine, 2-Oxo-PCE, (±)-2-(Ethylamino)-2-phenylcyclohexan-1-one
P4: R2 = CH_3, R1 = Cl	N-Ethylketamine, (±)-2-(2-Chlorophenyl)-2-(ethylamino)cyclohexan-1-one
P5: R2 = CH_3, R1 = OCH_3	2-MeO-Ketamine, (±)-2-(2-Methoxyphenyl)-2-(methylamino)cyclohexan-1-one
P6: R1 = CH_3; R3 = OCH_3	Methoxetamine, (±)-2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexan-1-one

Table 1.4 Structures and names of investigated NPS

Phenidines	
Q0: R1 = H 	Diphenidine, (±)-1-(1,2-Diphenylethyl)piperidine
Q1: R1 = OCH_3	Methoxphenidine, (±)-2-Methoxy-1-(1,2-Diphenylethyl)piperidine
R: 	Ephenidine, (±)-N-Ethyl-1,2-diphenylethylamine
Thiophenes	
S0: R1 = H 	Thiopropamine, (±)-1-(Thiophen-2-yl)-2-aminopropane
S1: R1 = CH_3	Methiopropamine, MPA, (±)-1-(Thiophen-2-yl)-2-methylaminopropane
T: 	Thiothinone, (±)-2-(Methylamino)-1-(thiophen-2-yl)propan-1-one
U: 	α-Pyrrolidinopentiothiophenone, α-PVT, (±)-2-(Pyrrolidin-1-yl)-1-(thiophen-2-yl)pentan-1-one
Other Chiral NPS	
V: 	5-API, (±)-5-(2-Aminopropyl)-indole
W: 	MDAT, (±)-6,7-Methylenedioxy-2-aminotetraline
X: 	EFLEA, (±)-N-(1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)propan-2-yl)-N-methylhydroxylamine

2.3 Preparation of samples and background electrolyte

Samples were dissolved in water (1 mg/ml) using an ultrasonic bath. Background electrolyte (BGE) consisted of aqueous 10 mM di-sodium hydrogen phosphate and 10 mM of the respective cyclodextrin. pH was adjusted to 2.5 with diluted phosphoric acid. Both, samples and BGE were filtered through a 0.45 μm single-use syringe filter (Carl Roth, Karlsruhe, Germany).

2.4 Instrumentation

Measurements were carried out on a DAD equipped Agilent 7100 Capillary Electrophoresis using UV-detection at 209 nm. The used voltage to cathode was set to 22 kV for the BGE containing carboxymethyl- and carboxyethyl-β-CD and 26 kV for the experiments with succinyl-β-CD. Measurements using the succinyl-β-CD electrolyte were set to an autozero after 3 minutes due to absorption interference probably from succinate. Cassette temperature was set to 25°C. Measurements were performed in fused silica capillaries (ID 50 μm) purchased from MircoQuartz (Munich, Germany) with total length of 68.5 cm and effective length of 60.0 cm. Sample injection was conducted dynamically by pressure set to 10 mbar for 5 s at the inlet vial.

3 Results and Discussion

3.1 Comparison of cyclodextrins

Overall, all three negatively charged β-cyclodextrins showed excellent separation ability for all tested substances groups. Rs values and migration times were comparable to the results of CM-β-CD (DS 0.5) in previous work. CM-, CE- and succinyl-β-CD represent negatively charged cyclodextrin derivatives. Apparently, the positively charged samples tend to show better interaction with these types of cyclodextrins thus leading to higher resolution. In addition, measurements using buffers containing negatively charged cyclodextrins were performed with lower voltage to secure an acceptable amount of Joule heat, however, resulting in longer migrations times. Migration times ranged from 4.7 to 17.6 min with native, acetyl- or

hydroxypropyl-β-cyclodextrin. The use of negatively charged cyclodextrins produced migration times from 7.8 to 24.4 min with a few exceptions up to 47 min. Table 2 provides migration times and Rs values for each analyte investigated.

In Table 3 the total number of successful enantioseparations by means of each cyclodextrin is compared. Table 3 combines the data from Hägele *et al.* [42, 43] with new measurements of the present work. Negatively charged cyclodextrins are shown to be superior particularly in terms of baseline separation (RS value >2). Mostly baseline separations were obtained with carboxymethyl-β-cyclodextrin. The comparison of the two carboxymethyl-β-cyclodextrins with different DS revealed differences in their enantioselectivity.

Table 2.1 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Amphetamines					
	10.296	10.481	1.0180	1.0	Succinyl
Amphetamine sulfate	12.694	12.884	1.0150	1.0	CE
	16.043	16.457	1.0258	1.5	CM 3.5
	17.086	17.386	1.0176	1.2	Succinyl
4-Chloramphetamine	22.740	23.172	1.0190	1.2	CE
	23.060	23.512	1.0196	1.4	CM 3.5
	12.803	13.071	1.0209	1.0	Succinyl
2-F-Amphetamine	15.727	16.088	1.0230	1.2	CE
	17.316	17.999	1.0394	2.0	CM 3.5
	13.309	13.523	1.0161	1.0	Succinyl
3-F-Amphetamine	15.934	16.210	1.0173	1.1	CE
	17.178	17.531	1.0205	1.4	CM 3.5
	11.872	12.085	1.0179	1.1	Succinyl
4-F-Amphetamine	15.590	15.812	1.0142	1.1	CE
	15.140	15.378	1.0157	1.0	CM 3.5
	13.112	n.d.	-	-	Succinyl
MTA (4-Methylthioamphetamine)	19.564	n.d.	-	-	CE
	28.472	28.681	1.0073	0.5	CM 3.5
	9.462	n.d.	-	-	Succinyl
DOB (4-Br-2,5-DiMeO-Amphetamine)	12.619	n.d.	-	-	CE
	15.366	n.d.	-	-	CM 3.5
	12.510	n.d.	-	-	Succinyl
DOC (2,4-DiMeO-4-Cl-Amphetamine)	15.510	n.d.	-	-	CE
	15.446	n.d.	-	-	CM 3.5
	8.622	n.d.	-	-	Succinyl
3,4-Dimethoxyamphetamine	11.223	n.d.	-	-	CE
	12.487	12.579	1.0074	0.1	CM 3.5
	11.448	11.637	1.0165	1.1	Succinyl
4-MeO-Amphetamine	15.008	15.151	1.0095	0.7	CE
	20.067	20.473	1.0202	1.3	CM 3.5
	9.305	9.404	1.0106	0.9	Succinyl
2,5-Dimethoxyamphetamine	12.893	13.063	1.0132	1.0	CE
	15.729	16.030	1.0191	1.4	CM 3.5
	8.771	n.d.	-	-	Succinyl
3,4,5-Trimethoxyamphetamine	11.472	n.d.	-	-	CE
	12.401	n.d.	-	-	CM 3.5
N-Methamphetamines					
	13.335	13.679	1.0258	1.5	Succinyl
N-Methamphetamine	13.480	13.758	1.0206	1.4	CE
	16.581	17.004	1.0255	1.9	CM 3.5
	12.295	12.611	1.0257	1.4	Succinyl
2-FMA	13.574	14.003	1.0316	1.9	CE
	19.563	20.604	1.0532	4.4	CM 3.5
	13.599	13.903	1.0224	1.3	Succinyl
3-FMA	17.525	17.900	1.0214	1.4	CE
	17.544	17.918	1.0213	1.6	CM 3.5
	11.054	11.237	1.0166	0.9	Succinyl
4-FMA	12.477	12.640	1.0131	0.9	CE
	17.600	17.898	1.0169	1.2	CM 3.5
	14.078	14.513	1.0309	2.2	Succinyl
3-MMA	18.096	18.698	1.0333	2.6	CE
	18.078	18.680	1.0333	2.5	CM 3.5
Cathinones					
4-Methylcathinone (4-MC)	11.293	n.d.	-	-	Succinyl
	13.891	n.d.	-	-	CE
	16.170	16.352	1.0113	0.9	CM 3.5

Table 2.2 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Methylendioxy-/Methylendioxy-N-Amphetamines					
	14.991	15.152	1.0107	0.8	Succinyl
3,4-MDA (3,4-Methylendioxyamph.)	16.098	16.359	1.0162	0.9	CE
	25.924	26.299	1.0145	1.0	CM 3.5
	13.217	13.476	1.0196	1.2	Succinyl
3,4-MDMA	16.825	17.156	1.0197	1.1	CE
	24.045	24.512	1.0194	1.5	CM 3.5
	9.260	9.363	1.0111	1.0	Succinyl
2,3-MDMA	10.932	11.036	1.0095	0.9	CE
	12.466	12.647	1.0145	1.4	CM 3.5
	16.710	17.072	1.0217	1.1	Succinyl
MDEA	18.705	19.016	1.0166	1.0	CE
	26.239	26.613	1.0143	1.0	CM 3.5
	17.226	17.346	1.0070	0.7	Succinyl
MBDB	18.943	n.d.	-	-	CE
	26.188	n.d.	-	-	CM 3.5
Other N-substituted Amphetamines					
	13.404	13.786	1.0285	1.4	Succinyl
N-Ethylamphetamine	14.715	14.987	1.0185	1.0	CE
	18.518	19.011	1.0266	1.7	CM 3.5
	14.462	14.793	1.0229	1.3	Succinyl
Mefenorex	15.717	15.987	1.0172	1.2	CE
	20.266	20.753	1.0240	1.8	CM 3.5
	11.695	12.088	1.0336	2.5	Succinyl
DMA (N,N,-Dimethylamphetamine)	13.699	14.087	1.0283	1.9	CE
	16.875	17.423	1.0325	2.0	CM 3.5
	13.811	14.203	1.0284	1.5	Succinyl
2-F-Ethamphetamine	15.074	15.525	1.0299	1.9	CE
	18.531	19.340	1.0437	3.0	CM 3.5
	14.109	14.449	1.0241	1.4	Succinyl
3-F-Ethamphetamine	14.612	14.880	1.0183	1.1	CE
	18.057	18.455	1.0220	1.8	CM 3.5
	12.443	12.730	1.0231	1.5	Succinyl
4-F-Ethamphetamine	13.750	13.928	1.0129	1.0	CE
	17.907	18.180	1.0152	1.4	CM 3.5
	11.666	11.939	1.0234	1.4	Succinyl
N-Propylamphetamine	15.032	15.484	1.0301	1.9	CE
	18.387	19.188	1.0436	3.3	CM 3.5
Benzofuranes					
	8.397	8.508	1.0132	1.2	Succinyl
4-APB	11.277	11.436	1.0141	0.9	CE
	13.955	14.265	1.0222	1.6	CM 3.5
	14.355	n.d.	-	-	Succinyl
5-APB	20.192	20.409	1.0107	1.0	CE
	15.645	15.999	1.0226	2.9	CM 3.5
	10.850	11.036	1.0171	1.3	Succinyl
5-APDB	15.061	15.317	1.0170	1.3	CE
	14.053	14.331	1.0198	2.7	CM 3.5
	16.930	17.296	1.0216	1.4	Succinyl
5-EAPB	19.377	19.658	1.0145	1.3	CE
	28.557	29.105	1.0192	1.6	CM 3.5
	16.055	16.458	1.0251	1.7	Succinyl
5-MAPB	18.298	18.625	1.0179	1.6	CE
	24.805	25.322	1.0208	2.5	CM 3.5
	14.366	n.d.	-	-	Succinyl
N-MOB-5-APB	20.648	n.d.	-	-	CE
	27.070	n.d.	-	-	CM 3.5
	14.812	n.d.	-	-	Succinyl
6-APB	20.985	21.217	1.0111	1.3	CE
	15.014	15.333	1.0212	3.4	CM 3.5
	14.379	n.d.	-	-	Succinyl
6-APDB	18.773	19.022	1.0133	0.9	CE
	28.518	29.051	1.0187	1.3	CM 3.5
	15.045	n.d.	-	-	Succinyl
6-EAPB	21.439	21.632	1.0090	0.7	CE
	30.787	n.d.	-	-	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 2.3 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Methcathinones					
	9.244	n.d.	-	-	Succinyl
2-MMC	11.588	n.d.	-	-	CE
	13.965	n.d.	-	-	CM 3.5
	10.890	11.073	1.0168	1.1	Succinyl
3-MMC	13.204	13.464	1.0197	1.2	CE
	17.440	17.884	1.0255	2.1	CM 3.5
	11.389	n.d.	-	-	Succinyl
Mephedrone (4-MMC)	14.239	14.348	1.0077	0.6	CE
	20.811	21.234	1.0203	1.4	CM 3.5
	13.459	13.747	1.0214	1.2	Succinyl
3,4-DMMC	16.649	16.958	1.0186	1.1	CE
	23.395	24.080	1.0293	2.0	CM 3.5
	8.735	n.d.	-	-	Succinyl
2,4-DMMC	12.852	n.d.	-	-	CE
	14.390	14.585	0.2815	1.4	CM 3.5
	12.671	12.799	1.0101	0.7	Succinyl
3-MeO-MC	14.688	15.104	1.0283	2.1	CE
	17.446	18.162	1.0410	3.5	CM 3.5
	8.272	n.d.	-	-	Succinyl
2-MeO-MC	11.449	11.510	1.0053	0.5	CE
	12.698	12.983	1.0224	1.8	CM 3.5
	10.291	10.414	1.0120	0.9	Succinyl
Methedrone (4-MeO-MC)	14.429	n.d.	-	-	CE
	19.399	n.d.	-	-	CM 3.5
	16.609	n.d.	-	-	Succinyl
2-CMC	n.d.	n.d.	-	-	CE
	28.025	n.d.	-	-	CM 3.5
	12.078	12.303	1.0186	1.6	Succinyl
3-CMC	14.540	14.685	1.0100	0.7	CE
	18.848	19.016	1.0089	0.6	CM 3.5
	11.978	12.166	1.0157	1.1	Succinyl
4-CMC	14.814	n.d.	-	-	CE
	18.938	19.114	1.0093	0.7	CM 3.5
	11.171	11.499	1.0294	2.0	Succinyl
3-EMC	16.516	16.745	1.0139	1.0	CE
	21.026	21.346	1.0152	1.1	CM 3.5
	14.035	14.197	1.0115	0.9	Succinyl
4-EMC	17.762	17.898	1.0077	0.6	CE
	24.169	24.626	1.0189	1.6	CM 3.5
	11.072	11.217	1.0131	0.8	Succinyl
Mexedrone	14.029	14.105	1.0054	0.6	CE
	19.399	n.d.	-	-	CM 3.5
	17.769	n.d.	-	-	CM 3.5
	7.877	n.d.	-	-	Succinyl
2-FMC	12.187	12.413	1.0185	2.8	CE
	15.033	15.637	1.0402	4.0	CM 3.5
	9.452	n.d.	-	-	Succinyl
3-FMC	11.552	11.607	1.0048	0.5	CE
	14.192	14.372	1.0127	1.2	CM 3.5
	9.037	9.120	1.0092	0.7	Succinyl
4-FMC	11.251	n.d.	-	-	CE
	13.568	n.d.	-	-	CM 3.5
	13.417	13.604	1.0139	0.9	Succinyl
4-BMC	16.913	n.d.	-	-	CE
	22.520	22.717	1.0087	0.8	CM 3.5
	10.913	11.359	1.0409	2.3	Succinyl
Buphedrone	13.623	n.d.	-	-	CE
	16.275	n.d.	-	-	CM 3.5
	13.115	13.721	1.0462	2.9	Succinyl
4-Methylbuphedrone	16.493	n.d.	-	-	CE
	21.165	n.d.	-	-	CM 3.5
	11.644	11.837	1.0166	1.1	Succinyl
Pentedrone	13.902	14.413	1.0368	2.8	CE
	16.552	17.319	1.0463	3.9	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 2.4 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Ethcathinones					
	10.200	10.274	1.0073	0.6	Succinyl
Ethcathinone	11.137	11.237	1.0090	0.7	CE
	14.184	14.403	1.0154	1.2	CM 3.5
	12.153	12.326	1.0142	1.2	Succinyl
Amphepramone	13.434	13.827	1.0293	2.2	CE
	17.860	18.592	1.0410	3.6	CM 3.5
	12.236	12.491	1.0208	1.3	Succinyl
3-CEC	13.886	14.008	1.0088	0.6	CE
	21.697	21.908	1.0097	0.6	CM 3.5
	12.291	12.539	1.0202	1.3	Succinyl
4-CEC	13.733	n.d.	-	-	CE
	21.827	21.927	1.0046	0.4	CM 3.5
	10.776	10.957	1.0168	1.5	Succinyl
DL-4662	12.294	12.355	1.0050	0.5	CE
	14.982	n.d.	-	-	CM 3.5
	12.130	12.488	1.0295	2.0	Succinyl
N-Ethylhexedrone	14.066	14.407	1.0242	1.7	CE
	18.317	18.951	1.0346	2.9	CM 3.5
	10.952	11.100	1.0135	0.8	Succinyl
3-MEC	12.719	12.909	1.0149	1.2	CE
	16.035	16.321	1.0178	1.4	CM 3.5
	11.912	n.d.	-	-	Succinyl
4-MEC	13.611	13.706	1.0070	0.6	CE
	17.803	18.071	1.0151	1.2	CM 3.5
	11.703	12.143	1.0376	2.7	Succinyl
N-Ethylbuphedrone	13.692	n.d.	-	-	CE
	16.887	n.d.	-	-	CM 3.5
	10.539	10.711	1.0163	1.3	Succinyl
N-Ethylpentedrone	12.374	12.439	1.0053	0.6	CE
	14.651	n.d.	-	-	CM 3.5
	9.698	9.748	1.0052	0.6	Succinyl
2-Ethylethcathinone	12.266	n.d.	-	-	CE
	15.399	n.d.	-	-	CM 3.5
	12.707	12.966	1.0204	1.7	Succinyl
3-Ethylethcathinone	15.630	15.734	1.0067	0.6	CE
	21.661	21.834	1.0080	0.9	CM 3.5
	12.067	12.299	1.0192	1.5	Succinyl
4-Ethylethcathinone	14.238	n.d.	-	-	CE
	17.724	n.d.	-	-	CM 3.5
	12.240	12.349	1.0089	0.8	Succinyl
3-F-Ethcathinone	15.208	15.379	1.0112	0.9	CE
	14.993	15.162	1.0113	0.9	CM 3.5
Other N-substituted Cathinones					
	14.159	15.226	1.0754	4.7	Succinyl
Bupropione	15.902	16.227	1.0204	1.3	CE
	22.277	22.896	1.0278	1.6	CM 3.5
	13.952	14.316	1.0261	1.6	Succinyl
4-MPC	16.505	n.d.	-	-	CE
	23.394	n.d.	-	-	CM 3.5
	12.511	n.d.	-	-	Succinyl
N-Propcathinone (NiPP)	15.152	15.454	1.0199	1.4	CE
	19.305	19.790	1.0251	1.8	CM 3.5
	14.379	14.760	1.0265	2.0	Succinyl
4-CPRC	17.036	17.393	1.0210	1.6	CE
	23.014	n.d.	-	-	CM 3.5
	11.559	11.674	1.0099	0.8	Succinyl
4-FNPP	14.019	14.425	1.0290	2.2	CE
	16.736	16.833	1.0058	0.6	CM 3.5
	13.558	13.857	1.0221	1.6	Succinyl
4-Cl-C	15.766	n.d.	-	-	CE
	20.576	n.d.	-	-	CM 3.5
	13.162	13.502	1.0258	1.4	Succinyl
4-CBC	15.081	n.d.	-	-	CE
	18.966	n.d.	-	-	CM 3.5
	11.960	12.231	1.0227	1.5	Succinyl
4-CDC	13.478	n.d.	-	-	CE
	15.918	n.d.	-	-	CM 3.5
	12.972	n.d.	-	-	Succinyl
NDH	14.825	15.226	1.0270	2.1	CE
	18.205	18.868	1.0364	3.0	CM 3.5
	11.873	n.d.	-	-	Succinyl
DOMC	16.274	16.380	1.0065	0.6	CE
	20.141	20.896	1.0375	2.0	CM 3.5
	11.737	n.d.	-	-	Succinyl
4-MBC (Benzedrone)	14.943	15.070	1.0085	0.9	CE
	17.715	17.984	1.0152	1.3	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 2.5 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Pyrovalerone					
	14.945	15.295	1.0234	1.5	Succinyl
PV8	16.704	n.d.	-	-	CE
	26.789	27.106	1.0118	0.7	CM 3.5
	14.319	14.531	1.0148	1.3	Succinyl
4-F-PV8	15.750	n.d.	-	-	CE
	20.938	21.33	1.0187	1.4	CM 3.5
	13.554	13.976	1.0311	2.1	Succinyl
α-PVP	14.561	15.140	1.0398	2.8	CE
	23.086	24.614	1.0662	4.3	CM 3.5
	15.807	16.797	1.0626	4.3	Succinyl
4-Cl-PVP	17.039	17.406	1.0215	1.6	CE
	24 438	n.d.	-	-	CM 3.5
	16.198	16.571	1.0230	1.7	Succinyl
4F-PVP	18.856	n.d.	-	-	CE
	26.792	27.008	1.0081	0.7	CM 3.5
	15.423	15.906	1.0313	2.5	Succinyl
4-MeO-α-PVP	17.046	17.570	1.0307	2.4	CE
	28.181	29.391	1.0429	3.2	CM 3.5
	18.032	n.d.	-	-	Succinyl
4-MPrC	21.093	n.d.	-	-	CE
	41.095	41.693	1.0146	1.0	CM 3.5
	16.266	16.660	1.0242	1.6	Succinyl
PV9	18.699	n.d.	-	-	CE
	32.237	32.379	1.0044	0.3	CM 3.5
	10.986	11.580	1.0541	3.2	Succinyl
α-PPP	11.946	12.215	1.0225	1.4	CE
	15.933	16.372	1.0276	1.0	CM 3.5
	13.147	13.731	1.0444	2.7	Succinyl
M-PPP	14.199	14.448	1.0175	1.2	CE
	20.317	20.813	1.0244	1.3	CM 3.5
	15.513	15.786	1.0176	1.1	Succinyl
α-PIHP	17.031	17.182	1.0089	0.6	CE
	28.810	29.610	1.0278	1.7	CM 3.5
	12.641	12.833	1.0152	1.1	Succinyl
4F-PHP	14.038	14.343	1.0217	1.6	CE
	19.670	19.868	1.0101	0.9	CM 3.5
	17.015	17.232	1.0128	0.9	Succinyl
Naphyrone	18.530	18.834	1.0164	1.2	CE
	31.496	32.304	1.0257	1.8	CM 3.5
	15.560	15.832	1.0175	1.3	Succinyl
4-MPHP	17.152	17.306	1.0090	0.7	CE
	23.193	23.574	1.0164	1.1	CM 3.5
	17.144	17.383	1.0139	1.0	Succinyl
PV10	20.596	n.d.	-	-	CE
	29.053	n.d.	-	-	CM 3.5
	14.630	15.082	1.0309	2.6	Succinyl
5-DBFPV	21.930	22.207	1.0126	1.3	CE
	24.211	24.625	1.0171	1.7	CM 3.5
	16.752	n.d.	-	-	Succinyl
TH-PVP	23.491	n.d.	-	-	CE
	28.836	n.d.	-	-	CM 3.5
Methylenedixoyethcathinones					
	11.794	n.d.	-	-	Succinyl
Ethylone	15.590	15.743	1.0098	0.9	CE
	18.933	19.208	1.0145	1.2	CM 3.5
	14.716	n.d.	-	-	Succinyl
N-Ethylpentylone (Bk-Ethyl-K)	18.553	18.974	1.0227	1.8	CE
	24.010	24.807	1.0332	2.8	CM 3.5
	12.662	n.d.	-	-	Succinyl
5-ME	15.570	15.724	1.0099	1.0	CE
	21.623	22.037	1.0191	1.3	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 2.6 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Ketamines					
	10.036	n.d.	-	-	Succinyl
Ketamine	14.288	14.369	1.0057	0.5	CE
	15.700	n.d.	-	-	CM 3.5
	10.736	10.890	1.0143	1.3	Succinyl
N-Ehtylketamine	14.746	n.d.	-	-	CE
	16.446	n.d.	-	-	CM 3.5
	11.220	11.665	1.0397	2.5	Succinyl
Methoxetamine	15.758	15.956	1.0126	1.0	CE
	17.576	18.006	1.0245	1.3	CM 3.5
	10.076	10.378	1.0300	2.0	Succinyl
2-Oxo-PCE	13.412	13.449	1.0028	0.3	CE
	14.905	15.051	1.0098	0.9	CM 3.5
	9.877	10.351	1.0480	3.6	Succinyl
2-Oxo-PCM	12.571	12.755	1.0146	1.1	CE
	13.610	13.855	1.0180	1.4	CM 3.5
	10.157	10.313	1.0154	1.3	Succinyl
2-F-Ketamine	12.993	13.038	1.0035	0.4	CE
	15.672	15.767	1.0061	0.6	CM 3.5
	11.278	11.97ß	-	-	Succinyl
2-MeO-Ketamine	15.751	15.966	1.0136	1.0	CE
	17.296	17.709	1.0239	1.5	CM 3.5
Phenidines					
	16.457	n.d.	-	-	Succinyl
Diphenidine	21.485	n.d.	-	-	CE
	25.215	n.d.	-	-	CM 3.5
	16.643	n.d.	-	-	Succinyl
Methoxyphenidine	22.384	n.d.	-	-	CE
	27.772	n.d.	-	-	CM 3.5
	15.498	15.928	1.0277	2.0	Succinyl
Ephenidine	20.465	20.669	1.0100	0.9	CE
	30.075	n.d.	-	-	CM 3.5
Thiophenes					
	12.931	13.175	1.0189	1.7	Succinyl
α-PVT	14.091	14.380	1.0205	1.4	CE
	17.559	18.094	1.0305	2.6	CM 3.5
	8.783	n.d.	-	-	Succinyl
Thiothinone	10.521	10.548	1.0026	0.3	CE
	12.069	12.236	1.0138	1.2	CM 3.5
	11.068	11.254	1.0168	1.4	Succinyl
Methiopropamine	11.943	12.116	1.0145	1.2	CE
	13.832	14.098	1.0192	1.4	CM 3.5
	8.824	8.922	1.0111	0.5	Succinyl
Thiopropamine	11.397	11.493	1.0084	0.7	CE
	13.056	13.281	1.0172	1.6	CM 3.5
Other Chiral Substances					
	15.076	15.166	1.0060	0.6	Succinyl
5-APi	17.973	18.515	1.0302	2.4	CE
	25.210	26.355	1.0454	3.3	CM 3.5
	10.402	10.63	1.0219	1.2	Succinyl
MTTA	14.094	14.230	1.0096	0.9	CE
	18.003	18.247	1.0136	1.1	CM 3.5
	5.179	n.d.	-	-	Succinyl
MDAT	6.797	n.d.	-	-	CE
	7.366	n.d.	-	-	CM 3.5
	15.038	n.d.	-	-	Succinyl
EFLEA	22.512	n.d.	-	-	CE
	30.731	n.d.	-	-	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 2.7 Results of 136 investigated NPS

	t1 (min)	t2 (min)	α (t2/t1)	Rs	Selector
Methylenedioxycathinones					
	11.924	n.d.	-	-	Succinyl
Methylone	19.753	20.032	1.0141	0.9	CE
	22.201	22.615	1.0186	1.3	CM 3.5
	12.540	n.d.	-	-	Succinyl
2-AIMP (5-MeO-Methylone)	16.428	16.533	1.0064	0.7	CE
	23.383	24.374	1.0424	3.3	CM 3.5
	12.317	n.d.	-	-	Succinyl
Dimethylone	14.520	14.641	1.0083	0.8	CE
	18.297	18.502	1.0112	0.9	CM 3.5
	13.725	14.206	1.0350	2.5	Succinyl
Butylone	16.805	n.d.	-	-	CE
	20.994	n.d.	-	-	CM 3.5
	14.374	14.715	1.0237	1.6	Succinyl
N,N-Dimethylbutylone	16.839	n.d.	-	-	CE
	21.916	n.d.	-	-	CM 3.5
	13.205	13.472	1.0202	1.3	Succinyl
Pentylone	16.072	16.325	1.0157	1.5	CE
	20.732	21.222	1.0236	2.1	CM 3.5
Methylenedioxypyrovalerones					
	14.046	14.347	1.0214	2.3	Succinyl
MDPV	19.483	19.941	1.0235	4.0	CE
	24.587	25.277	1.0281	4.2	CM 3.5
	9.953	10.143	1.0191	0.9	Succinyl
MDPHP (3,4-MD-PHP)	14.295	n.d	-	-	CE
	17.036	n.d	-	-	CM 3.5
	12.120	12.293	1.0143	1.0	Succinyl
MDPEP (MD-PV8)	15.076	15.569	1.0327	2.7	CE
	17.664	18.345	1.0386	3.5	CM 3.5
Other Methylenedioxycathinones					
	22.245	22.583	1.0152	1.1	Succinyl
N-Benzylnorbutylone	29.716	30.282	1.0190	1.4	CE
	29.461	30.041	1.0197	1.3	CM 3.5
	11.622	11.884	1.0225	1.4	Succinyl
MDPT	14.365	n.d.	-	-	CE
	17.512	n.d.	-	-	CM 3.5
Other Cathinones					
	15.912	n.d.	-	-	Succinyl
bk-iVP	23.258	23.480	1.0095	0.9	CE
	28.491	28.972	1.0169	1.5	CM 3.5
	16.028	n.d.	-	-	Succinyl
5-PPDi	22.722	n.d.	-	-	CE
	27.685	n.d.	-	-	CM 3.5
	16.052	n.d.	-	-	Succinyl
5-BPDi	22.305	n.d.	-	-	CE
	26.285	n.d.	-	-	CM 3.5

Note: Results of chiral separation attempts using succinyl-, carboxyethyl-, and carboxymethyl-β-CD, degree of substitution each 3.5, n.d. = not detected

Table 3 Total number of chiral separations using each cyclodextrin

	Native	Acetyl	HP	CM 0.5	CM 3.5	CE	Succinyl
Baseline separation (RS $\geq$ 2)	6	13	6	47	28	13	22
Separation (RS $<$ 2)	74	55	75	59	69	81	75
Separation total	80	68	81	106	97	94	97
No separation	56	68	55	30	39	42	39
Measurements total	136	136	136	136	136	136	136

Note: Comparison of Rs values of investigated substances with native-, acetyl-, hydroxypropyl-, carboxymethyl- (DS 0.5 and 3.5), carboxyethyl- and succinyl-β-cyclodextrin.

3.2 Separation of positional isomers

A further look was also taken on the separation of positional isomers with the set of negatively charged cyclodextrins, since distinction of positional isomers by classic achiral chromatography

can be a difficult task. Simple amphetamines or cathinones monosubstituted at their phenyl ring were mainly traded first as para, later as meta and ortho form. They exist as two enantiomers each, leading to the conclusion that *e.g.* methylmethcathinone is present in six different forms. In this context, 17 substances were available as para and/or meta and/or ortho isomers. Successful separations of isomers as well as their isomeric migration order (IMO) is given in Table 4.

Table 4 Separation of positional isomers and their isomeric migration order (IMO)

	CM 3.5		CE 3.5		Succinyl 3.5	
	IMO	separ.	IMO	separ.	IMO	separ.
Amphetamine						
2-/3-/4-FA	3/2/1	bs	2/1/3	o	2/3/1	po
3,4-/2,5-DiMeO-A	1/2	bs	1/2	bs	1/2	bs
2-/3-/4-FMA	3/1/2	po	2/3/1	bs	2/3/1	bs
3,4-/2,3-MDMA	2/1	bs	2/1	bs	2/1	bs
2-/3-/4-FEA	3/2/1	po	3/2/1	bs	2/3/1	po
Benzofuranes						
4-/5-/6-APB	1/3/2	bs	1/2/3	bs	1/2/3	bs
5-/6-APDB	1/2	bs	1/2	bs	1/2	bs
5-/6-EAPB	1/2	bs	1/2	bs	1/2	bs
Cathinones						
2-/3-/4-MMC	1/2/3	bs	1/2/3	bs	1/2/3	bs
3,4-/2,4-DMMC	2/1	bs	2/1	bs	2/1	bs
2-/3-/4-MeO-MC	1/2/3	bs	1/3/2	bs	1/3/2	bs
2-/3-/4-CMC	3/1/2	po	n.d./1/2	po	3/1/2	po
3-/4-EMC	1/2	bs	1/2	bs	1/2	bs
2-/3-/4-FMC	3/2/1	bs	3/2/1	bs	1/3/2	bs
3-/4-CEC	1/2	o	2/1	o	n.d./n.d.	o
3-/4-MEC	1/2	bs	1/2	bs	1/2	bs
2-/3-/4-EEC	1/3/2	bs	1/3/2	bs	1/3/2	bs

Note: bs = baseline separation, po = partial overlapping, o = overlapping, n.d. = not detected

Except for mixtures of CMC (chloromethcathinone) and CEC (chloroethcathinone), baseline separations of the isomers were achieved with at least one of the used cyclodextrins. Most of the mixtures were separated with each negatively charged cyclodextrin. Though some were only partially separated, their migration order can be recognized and used for identification of positional isomers. Figure 2 shows separation and IMO of 2-EEC, 3-EEC and 4-EEC by means of carboxyethyl-β-cyclodextrin within 18 min.

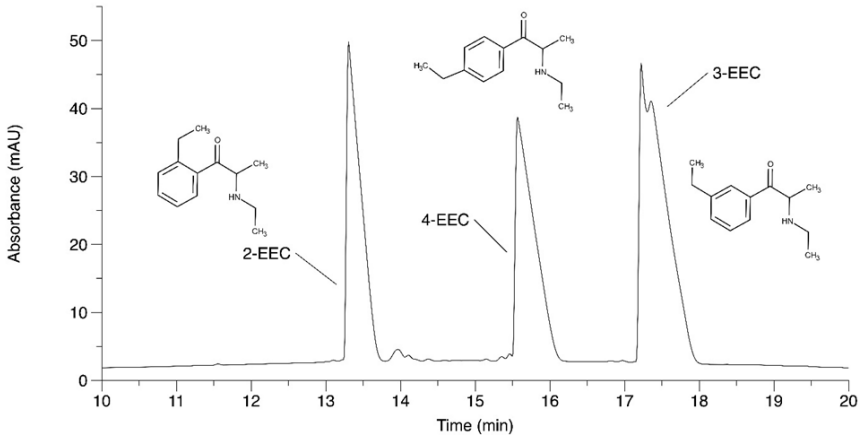


Figure 2 Isomeric migration order of ethylethcathinones, Conditions: 10 mM di-sodium hydrogen phosphate; 10 mM carboxyethyl-β-cyclodextrin; pH 2.5; UV 209 nm; +22 kV; Inj.: 10 mbar/5 sec

3.3 Analysis of real-life samples

To prove the usefulness of chiral separation of NPS with cyclodextrins, three different real-life samples were investigated using the presented method and CM-, CE- and succinyl-β-CD as chiral selectors.

Sample A was a small amount of Crystal meth with an unknown chiral status found with an addicted patient in an Austrian hospital. First, sample A was measured; in contrary to the mostly commercially available racemic mixtures of NPS only one peak was observed and compared with the migration time of D-methamphetamine of a commercially available standard. Also the mixture of both samples revealed one peak, leading to the conclusion that sample A represents D-methamphetamine, which is identical with the eutomer S-(+)-methamphetamine. Additionally, each of the samples was mixed with the standard racemic methamphetamine to determine the enantiomeric migration order. Figure 3 shows an appropriate electropherogram with D-methamphetamine migrating after L-methamphetamine.

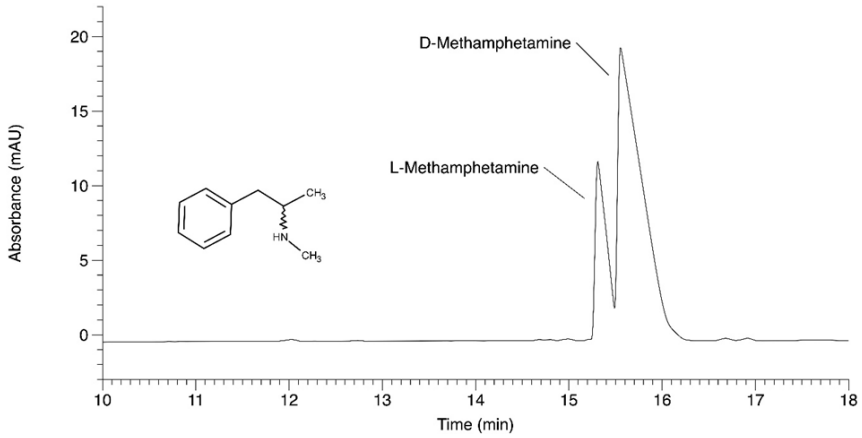


Figure 3 Determination of the enantiomeric status of a methamphetamine containing real-life sample. Mixture of commercially available racemic N-methamphetamine with a methamphetamine real-life sample; Conditions: 10 mM di-sodium hydrogen phosphate; 10 mM carboxymethyl-β-cyclodextrin; pH 2.5; UV 209 nm; +22 kV; Inj.: 10 mbar/5 sec

Sample B was expected to be a crystalline Ecstasy powder seized by police containing methylenedioxymethamphetamine (MDMA). Migration times of the sample, which was present as racemic mixture fit to those of a MDMA standard. A mixture of Sample B and MDMA standard showed no further peaks.

Sample C was assumed to be methylmethcathinone (MMC) of unknown isomeric status. Measurements of sample C showed similar migration times to 4-MMC. To make sure, sample C was added to a mixture of 2-, 3-, and 4-MMC with the expectation of one MMC peak to be spiked. As a result, Figure 4 shows no fit to the MMC peaks but an additional peak in the electropherogram. Therefore, sample C was a different substance.

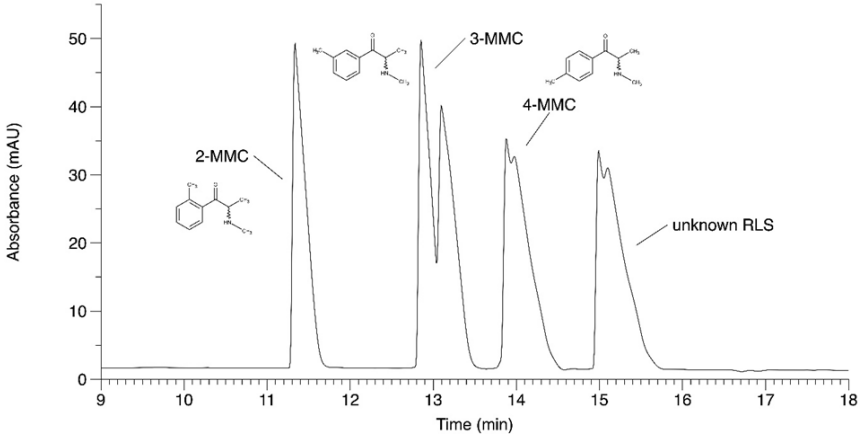


Figure 4 Separation of MMC isomers and an unidentified real-life sample. Mixture of 2-, 3- and 4-MMC with an unidentified real-life sample (RLS); Conditions: 10 mM di-sodium hydrogen phosphate; 10 mM carboxyethyl-β-cyclodextrin; pH 2.5; UV 209 nm; +22 kV; Inj.: 10 mbar/5 sec

In addition, identity of sample A and B was confirmed by mass spectroscopy, and sam-

ple C was identified as 3-methylethcathinone (3-MEC) by mass spectroscopy and infrared spectroscopy.

3.4 Validation data

To prove the robustness of the described method using the negatively charged cyclodextrins, intra- and interday validation was performed, with acceptable values for each chiral selector. As in work of Hägele *et al.* [42,43] pentedrone was chosen as model compound. A summary of the validation data with inter- and intraday measurements n = 5 for each cyclodextrin is given in Table 5.

Table 5 Summary of inter- and intraday validation

		t1	RSD%	t2	RSD%	Rs	RSD%
Intraday validation	CM-β-CD 3.5	16.59 ± 0.54	3.3	17.39 ± 0.59	3.4	4.2 ± 0.4	9.3
	Succinyl-β-CD	11.74 ± 0.08	0.7	11.74 ± 0.07	0.6	0.9 ± 0.0	0.0
	CE-β-CD	13.85 ± 0.88	6.4	14.36 ± 0.95	6.6	2.7 ± 0.2	6.1
Interday validation	CM-β-CD 3.5	16.80 ± 0.74	4.4	17.68 ± 0.82	4.7	4.7 ± 0.3	5.9
	Succinyl-β-CD	11.50 ± 1.04	9.0	11.66 ± 1.05	9.0	1.0 ± 0.1	8.0
	CE-β-CD	13.92 ± 0.11	0.8	14.43 ± 0.12	0.9	2.7 ± 0.1	3.0

4 Conclusion

The present work has shown the ability of β-cyclodextrins for enantioseparation of a huge number of NPS out of different substance classes. There is a clear trend towards the negatively charged CDs like carboxymethyl-, carboxyethyl-, and succinyl-β-cyclodextrin to be superior.

The quick and easy setup of buffer and samples, moderate retention times as well as the usage of common fused silica capillaries promote capillary electrophoresis to a promising alternative for chiral separations. In contrary to HPLC and GC methods, no special chiral columns are necessary, and the chiral selector is low in consumption and is added to the background electrolyte. It can be regarded as a resource saving and environmentally friendly approach because small amounts of solvents, buffer chemicals and analytes are used.

It turned out that NPS are traded as racemic mixtures, only the classic drug Crystal meth represents an exception. Most NPS are traded as hydrochloric salts, which requires no further sample preparation apart of dissolution in water.

In terms of structure elucidation, this method also helps to differ between positional isomers as an additional benefit in contrast to other identification methods. Moreover, the approach was found to be suitable for the investigation of real-life samples seized by police or collected in hospitals.

One simple method turned out to be sufficient for all presented measurements. Separations can be compared easily with respect to choose the optimal selector. Because of the versatile usability of this method, it might be useful for further upcoming NPS in future, both in terms of enantiomeric status as well as distinction of positional isomers.

Conflict of interest

The authors declare no conflict of interest.

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