Research Article

Chiral separation of cathinone derivatives using β-cyclodextrin-assisted capillary electrophoresis—Comparison of four different β-cyclodextrin derivatives used as chiral selectors

In the past decade, more than 100 different cathinone derivatives slopped over entire Europe due to their enormous popularity. Generally, these novel psychoactive substances are easily available via the internet. This fact leads to various social problems, since cathinones are substances with consciousness-changing effects and are mainly misused for recreational matters by their consumers. Cathinones possess a chiral center including two enantiomeric forms with potentially different pharmacological behavior. This fact makes analytical method development regarding their chiral separation indispensable. In this study, a chiral capillary zone electrophoresis method for the enantioseparation of 61 cathinone and pyrovalerone derivatives was developed by means of four different β-cyclodextrin derivatives. As chiral selectors, native β-cyclodextrin as well as three of its derivatives namely acetyl-β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and carboxymethyl-β-cyclodextrin were used. The cathinone and pyrovalerone derivatives were either purchased in internet stores or seized by police. As a result, overall 58 of 61 studied substances were partially or baseline separated by at least one of the four chiral selectors using 10 mM of β-cyclodextrin derivative in a 10 mM sodium phosphate buffer (pH 2.5). Furthermore, the method was found to be suitable for simultaneous enantioseparations, for enantiomeric purity checks and to differentiate between positional isomers. Moreover, an intra- and an interday validation was performed successfully for each chiral selector to prove the robustness of the method.

Keywords:
2-Hydroxypropyl-β-cyclodextrin / Acetyl-β-cyclodextrin / Capillary electrophoresis / Carboxymethyl-β-cyclodextrin / Cathinones / Native β-cyclodextrin

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1 Introduction

The European Monitoring Centre for Drugs and Drug Addiction monitored more than 670 novel psychoactive substances (NPS) on Europe’s drug market by 2018, including the main categories of synthetic cannabinoids, opioids, stimulants, and depressants. NPS are substances that are produced via molecular structure modification of already existing compounds and mimic illicit drugs such as amphetamine (speed), 3,4-methyldioxy-N-methamphetamine (ecstasy) and N-methamphetamine (crystal). NPS are often not covered by international drug controls, particularly in Europe, legislation differs a lot. After cannabimimetics, the second largest group of NPS represents synthetic cathinones with a count of above 130 different derivatives detected in total. The European Monitoring Centre for Drugs and Drug Addiction also stated that some classes of NPS, especially cathinone derivatives, are becoming more evident in the European drug market [1–3].

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Abbreviations: 4-MEC, 4-methylethcathinone; EMO, enantiomeric migration order; NPS, novel psychoactive substances

Color online: See article online to view Figs. 1 and 2 in color.
Plants such as khat (*Catha edulis*), cannabis (*Cannabis sativa*), coca (*Erythroxylum coca*), and opium (*Papaver somniferum*) were used for hundreds of years due to their consciousness-changing and stimulating effects. The main psychoactive component of the khat plant represents the alkaloid cathinone. Cathinone has a close chemical relationship to amphetamine and bears an additional beta-keto group in its phenethylamine structure. Cathinones are also called beta-keto amphetamines. Khat plants are mainly cultivated in the Arabian Peninsula and eastern Africa where the residents consume the leaves as naturally occurring stimulants. The central nervous system stimulating effect is mainly based on an increased dopamine- and norepinephrine release and can range from slight euphoria to distinct deliria.

Synthetic cathinone derivatives are mainly produced in China or other Asian countries. They are sold as for example, “research chemicals” or “plant food” with an easy and cheap access for nearly everyone and are mainly distributed via the internet [2, 4–8].

The two parent compounds of cathinone derivatives, namely cathinone and methcathinone, are prohibited substances in the majority of the European countries and in the United States. In the years 2008–2012, the para methylated analog 4-MMC (mephedrone, 4-methyl-methcathinone) obtained enormous popularity and floated the European drug market. This derivative shows nearly the same stimulating effects as its pharmaceutical lead. To counteract this “mephedrone—boom” the countries had to react and control the substance under their legislation. As a consequence, further derivatives like its ortho-(2-methyl-methcathinone, 2-MMC) and meta-(3-methyl-methcathinone, 3-MMC) analogues were sold as mephedrone. Additionally, halogenated derivatives as well as different ethcathinones like 4-MEC (4-methyl-ethcathinone) emerged on the drug markets as “legal” replacements for prohibited drugs. In parallel, an increased advent of pyrovalerone derivatives, especially descendants of α-pyrrolidinopentiophenone (α-PVP, Flakka), was observed during the last years [2, 9].

Due to the presence of an asymmetric carbon atom, two different enantiomeric forms with potentially different pharmacological behavior exist. Chiral analytical method development to determine the enantiomeric status of real life samples is therefore indispensable. Up till now, it is well known that for example the $S(\pm)$-enantiomers of the illicit drugs amphetamine and methamphetamine show higher potencies than their corresponding $R(-)$ forms [10,11]. Also, for the already mentioned pharmaceutical lead substances cathinone and methcathinone, it is of common knowledge that the $S(-)$ enantiomers cause higher central nervous system-stimulating effects [12].

In literature, several articles deal with the enantioseparation of cathinone related compounds and further NPS. Chiral separations were carried out via different separation techniques including HPLC [13–26], supercritical fluid chromatography [27–29], GC [20, 22, 30–33], and also CE and CEC [19, 26, 27, 32–51]. Electrophoretically driven methods have great advantage that no expensive chiral stationary phases are needed. Consumption of both analytes and chiral selector as buffer additive is low. Generally, for CE, various chiral selectors such as CDs, polysaccharides, macrocyclic antibiotics, proteins, chiral crown ethers, chiral surfactants, and chiral metal complexes have been used in the past. However, among them, all forms of native CDs consist of six to eight glucopyranose subunits as well as their substituted derivatives turned out to be a very frequently applied chiral selector class. Generally, CDs are used as an additive to the BGE for direct chiral separations, since UV detection is not affected [52]. A scheme of all CDs applied in this study is pictured in Fig. 1.

The chiral separation principle of CDs is based on the formation of inclusion complexes with the analytes. In this

![Chemical structures of the four investigated β-CD derivatives.](https://www.electrophoresis-journal.com)

Substitution pattern:

- Native β-cyclodextrin: $R = -H$
- Acetyl-β-cyclodextrin: $R = -H$ or $-\text{CO-CH}_3$
- (2-Hydroxypropyl)-β-cyclodextrin: $R = -H$ or $-\text{CH}_2\text{-CHOH-CH}_3$
- Carboxymethyl-β-cyclodextrin: $R = -H$ or $-\text{CH}_2\text{-COO}^-\text{Na}^+$

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case the substances interact with their hydrophobic cavity. Additionally, the effect of their hydrophilic surface has to be taken into account. Diverse modifications on their exterior surface area can have enormous impact on the formation and the stability of the inclusion complexes. Due to additional polar interactions with the analytes on the one hand and changing the sizes of the hydrophobic cavities of the CDs on the other side, chiral separation selectivity can be modulated. The different complex stability constants of these inclusion complexes with the molecules and further with their R- and S-enantiomers in combination with their different electrochromatographic mobilities are the reason for successful chiral separation results [52, 53].

During the past years, plenty successful chiral separation methods for cathinones by CE using different CDs were presented. Lurie et al. used different sulfobutylerether β-CDs already in the year 1998 for the chiral separation of NPS derivatives by CE [34]. In 2012, Mohr et al. presented a CE–UV method applying sulfated β-CD for the enantioseparation of 19 cathinone analogs being available at this time [42]. Furthermore, Merola et al. who used native β-CD and sulfated β-CD [46], Taschwer et al. who employed sulfobutylerether β-CD [45] and sulfated β-CD [32], Baciu et al. also working with native β-CD [47], and latest Nowak et al. who used 2-hydroxyethyl-β-CD [49] for successful enantioseparation of cathinones have to be referred.

The aim of this work was to create an inexpensive chiral CE method using and comparing four different β-CDs, namely native β-CD, acetyl-β-CD, 2-hydroxypropyl-β-CD, and carboxymethyl-β-CD as chiral selectors. Their applicability for the enantioseparation of a broad spectrum of cathinone analogs containing novel derivatives should be proven. Furthermore, special attention was given to pyrvaleron derivatives, especially the new subfamily that arised from α-PVP, where an increased appearance in internet stores was detected.

2 Materials and methods

2.1 Chemicals and solutions

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

Because of the novelty of the substances, they were mostly non-commercially available from official suppliers. As a consequence, they were bought from diverse online stores. Additionally, some analytes were real life samples seized by Austrian police. The origin of each substance is listed in Supporting Information Table 1. However, some of the stated online stores are already closed. Pure enantiomers for enantiomeric elution order experiments were prepared via a semipreparative HPLC method (unpublished results) in our laboratory in a small scale for scientific purposes.

Prior to experiments, all substances were characterized by GC–MS and NMR, if necessary.

The chosen BGEs were prepared by dissolving 10 mM of the particular β-CD. 10 mM sodium phosphate adjusted with diluted phosphoric acid in Milli-Q-Water (pH 2.5). Before measurements, solutions were degassed by ultrasonification and filtered through a 0.45 μm pore size nylon filter (Carl Roth, Karlsruhe, Germany).

2.2 Instrumentation

A fully automated 3DCE system (Waldbronn, Germany) equipped with a DAD was used for the measurements. All experiments were carried out at ambient temperature. The sample tray and the capillary were thermostated at 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. Detection was performed via on-column measurements of UV absorption at 209 nm. Before and after each run, the capillary was rinsed with 0.2 M sodium hydroxide, water and BGE, respectively. All samples were injected dynamically by applying a pressure of 10 mbar × 5 s on the inlet vial if not stated otherwise.

2.3 Sample preparation

Each sample was dissolved in Milli-Q-Water in a concentration of 1.0 mg/mL if not stated otherwise. The samples consisted mainly of hydrochloric acid salts. To accelerate the dissolving processes the samples were put on an ultrasonic bath for 1 min prior to filtration. Afterward, they were filtered through a 0.45 μm pore size filter (Carl Roth, Karlsruhe, Germany).

3 Results and discussion

Starting from the hype designer drug mephedrone, during the past 10 years more than 100 derivatives were designed for drug consumption circumventing law. For this reason, samples of cathinones were collected in our lab since 2010 either via internet purchase or because of seizures by Austrian police. All chemical structures of the investigated analytes are listed in Supporting Information Table 1.

As already stated, the application of different CD-derivatives for enantiopereation of diverse NPS via different
was used since this pH value was already found to be optimal by Merola et al. [46]. No further pH optimization was carried out. At constant pH, the effect of different chiral selector concentrations as well as different BGEs was investigated for optimal enantioresolution with respect to acceptable migration times. During the first experiment, the optimal electrolyte composition was tested by sodium phosphate buffer concentrations from 5 to 40 mM. The obtained results are given in Table 1.

Buffer baseline separations were obtained for each BGE consisting of the amount of sodium phosphate shown in Table 1. With respect to the acceptable resolution $R_s$ of 2.5 in combination with the fast migration time for further experiments 10 mM of sodium phosphate buffer was chosen. Furthermore, the effect of the selector concentration should be studied. The obtained results are shown in Table 2.

Therefore, β-CD concentrations ranged from 5 to 18 mM. Optimal results were obtained with a selector concentration of 10 mM β-CD. The application of both more and less than 10 mM β-CD led to worse separation. For this reason, 10 mM β-CD in a 10 mM sodium phosphate buffer (pH 2.5) and a voltage of 30 kV to the cathode were used as final conditions to test a set of 61 different cathinone analogs. Furthermore, the method was transferred without any additional optimization to the chiral selectors 2-hydroxypropyl-β-CD, acetyl-β-CD, and carboxymethyl-β-CD. However, the applied voltage was chosen individually for each β-CD derivative individually to create the fastest possible separation results in combination of a stable current during the measurements.

Under the above-mentioned conditions a set of 61 cathinone derivatives was tested. Overall 58 of the substances were partially or baseline separated with at least one of the different CD-electrolytes within max. 40 min. All separation data are shown in Supporting Information Tables 2a and 2b in detail.

Additionally, a comparison between the four β-CD derivatives and their separation ability for cathinone derivatives is given in Fig. 2.

Only for the analytes 2-CMC, 4-MPrC, and TH-PVP no chiral separation with any of the tested β-CD derivatives

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**Table 1.** Effect of the background electrolyte on migration time and enantioresolution using the model substance pentedrone

| Sodium phosphate (mM) | Applied voltage (kV) | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ |
|-----------------------|----------------------|-------------|-------------|---------|-------|
| 40                    | 20 to cathode         | 19.53       | 20.27       | 1.038   | 3.2   |
| 20                    | 25 to cathode         | 12.62       | 14.09       | 1.035   | 3.1   |
| 10                    | 30 to cathode         | 8.93        | 9.19        | 1.030   | 2.5   |
| 5                     | 30 to cathode         | 8.27        | 8.55        | 1.028   | 1.6   |

Conditions: 10 mM β-CD, sodium phosphate, pH 2.5 adjusted with diluted phosphoric acid, cassette temperature: 25°C, injection: 10 mbar for 5 s, sample: 1 mg/mL in water

**Table 2.** Effect of the selector concentration on enantioresolution investigated by the model substance pentedrone

| β-CD (mM) | $t_1$ (min) | $t_2$ (min) | $\alpha$ | $R_s$ |
|-----------|-------------|-------------|---------|-------|
| 5         | 8.94        | 9.15        | 1.023   | 1.6   |
| 10        | 8.93        | 9.19        | 1.030   | 2.5   |
| 15        | 10.99       | 11.37       | 1.035   | 2.3   |
| 18        | 11.78       | 12.19       | 1.035   | 2.1   |

Conditions: β-CD, 10 mM sodium phosphate, pH 2.5 adjusted with diluted phosphoric acid, cassette temperature: 25°C, applied voltage: 30 kV to the cathode, injection: 10 mbar for 5 s, sample: 1 mg/mL in water

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Figure 2. Cumulative and comparative trend: Chiral separation ability of the introduced chiral selectors for cathinone derivatives as analytes. Conditions: 10 mM chiral selector, 10 mM sodium phosphate, pH 2.5 adjusted with phosphoric acid, cassette temperature: 25°C, applied voltages: 30 kV to cathode for β-CD, 29 kV to cathode for acetyl-β-CD and hydroxypropyl-β-CD, 22 kV to cathode carboxymethyl-β-CD, injection: 10 mbar for 5 s, sample: 1 mg/mL in water.
could be observed. A potential reason for this finding could be a too weak chiral interaction of the enantiomers with the chosen selectors. Furthermore, the enantiomeric migration order (EMO) of the analytes 4-MEC and ethylone was tested by spiking a sample of the racemic analyte with pure enantiomers. In both cases, the pure enantiomers were prepared in-house via a semipreparative HPLC method (unpublished results). For the EMO determination of 4-MEC, the chiral selector carboxymethyl-β-CD and for ethylone the chiral selector 2-hydroxypropyl-β-CD were chosen. In both cases, the (−)-enantiomer migrated faster than the corresponding (+)-enantiomer. An electropherogram of a 4-MEC sample spiked with its (−)-enantiomer is shown in Fig. 3. Due to the lack of further pure cathinone enantiomers further the EMO determination was not feasible.

A further goal of this study was to check simultaneous enantioseparations. This was found to be suitable for a broad range of analytes and analyte combinations. An example out of various successful simultaneous chiral separations is given in Fig. 4, showing the enantiomeric separation of buphedrone, 4-methylbuphedrone, and N-ethylbuphedrone. Acetyl-β-CD was chosen as chiral selector for this analyte composition.

Additionally, the presented method is also applicable to distinguish between different positional isomers. An example of a positional isomer discrimination is shown in Fig. 5 by means of three different fluorinated methcathinone derivatives and carboxymethyl-β-CD as chiral selector. The analytes show different migration times although the only difference in their chemical structure is the position of the fluorine atom. Due to the fact that these substances show the same molecular weight, it is difficult to distinguish them via GC–MS. This represents a further benefit of the presented method.

Finally, an intra- and an interday validation to prove the robustness of the method was performed. For each chiral selector acceptable values were found. As model compound pentedrone was chosen again due to the fact that the analyte was resolved with each chiral selector. The number of the intra- as well as the interday measurements was \( n = 5 \) each. Detailed validation results are given in Supporting Information Table 3. Regarding the RSD of the migration times for the intraday measurements, no chiral selector showed values higher than 1.5%, and respectively, 7.7% as the resolution factor. RSD of the interday measurements for the migration times was less than 2.5% and for the resolution factor less than 12.4% for each chiral selector.
Figure 4. Simultaneous chiral separation of three different cathinone derivatives (1: buphedrone, 2: N-ethylbuphedrone, 3: 4-methylbuphedrone). Conditions: 10 mM acetyl-β-CD, 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.

Figure 5. Separation of three positional isomers of fluorinated methcathinone. Conditions: 10 mM carboxymethyl-β-CD, 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.
4 Concluding remarks

During the last few years, cathinones have gained enormous popularity in entire Europe and the number of further upcoming derivatives is constantly growing.

The introduced method represents a reliable and easy chiral CE method for the enantioseparation of a broad spectrum of this chiral compound class.

Under optimized conditions, 58 of 61 tested cathinones were resolved in their enantiomers successfully within 40 min. The effect of different chiral selectors, the electrolyte and the selector concentration on migration time and enantioresolution was demonstrated. Resolution factors with respect to all tested β-CD derivatives ranged from 0.3 to 6.2. Furthermore, the presented technique was found to be suitable for simultaneous enantioseparations, enantiomeric purity checks, and for positional isomer discrimination.

In future, the investigated method might become an additional useful technique for enantio- and positional isomer separation of further upcoming cathinones as well as a reliable tool to clarify the enantiomeric status of real life samples. Additionally, the presented technique can be transferred to further NPS substance classes.

The authors have declared no conflict of interest.

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