Chiral Separation of Cathinone and Amphetamine Derivatives by HPLC/UV Using Sulfated ß-Cyclodextrin as Chiral Mobile Phase Additive

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ABSTRACT In the last years the identification of new legal and illegal highs has become a huge challenge for the police and prosecution authorities. In an analytical context, only a few analytical methods are available to identify these new substances. Moreover, many of these recreational drugs are chiral and it is supposed that the enantiomers differ in their pharmacological potency. Since nonenantioselective synthesis is easier and cheaper, they are mainly sold as racemic mixtures. The goal of this research work was to develop an inexpensive method for the chiral separation of cathinones and amphetamines. This should help to discover if the substances are sold as racemic mixtures and give further information about their quality as well as their origin. Chiral separation of a set of 6 amphetamine and 25 cathinone derivatives, mainly purchased from various Internet shops, is presented. A LiChrospher 100 RP-18e, 250 x 4 mm, 5 μm served as the stationary phase. The chiral mobile phase consisted of methanol, water, and sulfated ß-cyclodextrin. Measurements were performed under isocratic conditions in reversed phase mode using UV detection. Four model compounds of the two substance classes were used to optimize the mobile phase. Under final conditions (methanol:water 2.5:97.5 + 2% sulfated ß-cyclodextrin) enantiomers of amphetamine and five derivatives were baseline separated within 23 min. In all, 17 cathinones were completely or partially chirally separated. However, as only 3 of 25 cathinones were baseline resolved, the application of this method is limited for cathinone analogs. Additionally, the results were compared with an RP-8e column. *Chirality 00:000–000, 2014.*

KEY WORDS: cathinone; amphetamine; chiral separation; sulfated ß-cyclodextrin

INTRODUCTION

In the last years the spectrum of legal and illegal drugs has increased enormously. Further derivatives or even new unknown substances entered the market nearly every week. According to the EU drug market report of the European Monitoring Centre for Drugs and Drug Addiction, 73 new psychoactive substances showed up in 2012, 49 in 2011, and 41 in 2010. Since 2005, more than 200 new compounds have been reported in the EU.[1] 4-Methylmethcathinone, better known as mephedrone, is the most popular compound traded via the Internet as plant fertilizer or bath salt in the last years. As it was a legal alternative to the amphetamines or ecstasy, it caused much hype in Europe. In 2009, the hype was at its peak and the authorities were forced to react. Since the 3rd of December 2010 there has been an EU-wide prohibition of mephedrone. As a consequence, the drug market replaced them with further psychoactive cathinone derivatives to circumvent the law. In Austria, e.g., the New Psychoactive Substances Act and the New Psychoactive Substances Regulation was introduced in January 2012. This law regulates the distribution or trade of these new compounds.

The new drugs are synthesized in clandestine laboratories and are sold worldwide on the Internet as research compounds, bath salts, room odorizers, or fertilizers for plants. The progressively worldwide black market of these legal and illegal highs represents a big problem for the police and prosecution authorities. Some of these new drugs are chiral amphetamine and cathinone derivatives. Syntheses of these drugs are sometimes simple and cheap, but not enantiopure. As the enantiomers can differ in their pharmacological activity, metabolic, and pharmacokinetic characteristics, one of the enantiomers might show a stronger effect, while the second one might exhibit a weaker or even toxic effect. The S-(−)-enantiomer of methcathinone and the S-(+)-enantiomer of amphetamine, for example, show more stimulating effects than their R-(+)- and R-(−)-antipodes.[2–4]

The drug market has changed completely in recent years. In order to circumvent the law, illegal drugs are replaced by legal alternatives instead. Amphetamines, for example, are replaced by legal cathinones, cannabis by legal incense, and LSD by legal phenetylamines or legal tryptamines. In contrast to the well-known illegal drugs such as heroin and cocaine, the new substances do not have common names. They are called NRG-1 or synthacaine, for instance. Besides the problem that the addicted do not know what they consume, there is additionally the risk of unknown impurities. Recently, drug dealers publish product analyses by mass spectroscopy (MS) and nuclear magnetic resonance (NMR) on their Internet pages in order to prove the purity of the offered substances. However, neither the addicted understand the information, nor can they check its correctness. Therefore, there is a need for development of analytical methods for identification of these new drugs. Since to our knowledge no rapid tests to identify

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these new substances are available, it is reasonable to use chromatographic methods.

A further problem of these legal and illegal highs is that there are only few data available about the pharmacology, toxicology, and the long-term damage they cause. Most of the information about the effects and side effects are based on progress reports of users, which are discussed on Internet forums.

In the literature, there are some articles dealing with the determination and chiral separation of cathinone, amphetamine, and related substances by high-performance liquid chromatography (HPLC),[5–8] capillary electrophoresis (CE),[9–12] and gas chromatography (GC).[13–15]

Nhujak's group reported on the chiral separation by CE using a dual cyclodextrin system consisting of ß-cyclodextrin and dimethyl-ß-cyclodextrin; the improved enantioseparation of amphetamine and drug enantiomers could be achieved with a single chiral selector.[16]

For HPLC, chiral stationary phases based on crown ether showed very successful separation results; however, they are limited by the presence of a primary amine group at the analyte.[17–19]

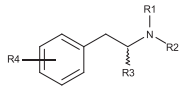
Previously, our group succeeded in resolving 19 of 25 cathinone derivatives into their enantiomers using the CHIRALPAK AS-H column as stationary phase.[20] Another success was the chiral separation of cathinone derivatives by CE using sulfated ß-cyclodextrin as an added chiral selector.[21] As these results of the enantioseparation were satisfactory, the attempt was to use sulfated ß-cyclodextrin as a chiral additive in the mobile phase to achieve enantioseparation with HPLC. Therefore, the aim of this work was to develop an inexpensive and easy to perform method for the chiral separation of cathinone and amphetamine derivatives using a common reversed-phase column under isocratic conditions.

MATERIALS AND METHODS

Chromatographic Conditions

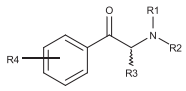
Chiral separation experiments were carried out with an HP Hewlett Packard (Corvallis, OR) Series II; 1090; Liquid Chromatograph, equipped with an autosampler and a diode array detector. Ultraviolet (UV)-detection was performed at 220, 240, and 270 nm. The measurements were carried out

TABLE 2. Chemical structures of the tested amphetamine analogs



Compound	R1	R2	R3	R4
Amphetamine	H	H	CH_3	H
3-Fluoroamphetamine (3-FA)	H	H	CH_3	3-F
4-Fluoroamphetamine (4-FA)	H	H	CH_3	4-F
4-Fluoromethamphetamine (4-FMA)	H	CH_3	CH_3	4-F
N-Methamphetamine	H	CH_3	CH_3	H
3,4-Methylendioxymethamphetamine (MDMA)	H	CH_3	CH_3	3,4-methylendioxy

TABLE 1. Chemical structure of the tested cathinone derivatives



Compound	R1	R2	R3	R4
Benzedrone (4-MBC)	H	Benzyl	CH_3	4-CH_3
4-Bromomethcathinone (4-BMC)	H	CH_3	CH_3	4-Br
Buphedrone	H	CH_3	C_2H_5	H
Butylone	H	CH_3	C_2H_5	3,4-methylendioxy
N,N-Dimethylbutylone	CH_3	CH_3	C_2H_5	3,4-methylendioxy
3,4-Dimethylmethcathinone (3,4-DMMC)	H	CH_3	CH_3	3,4- dimethyl
Ethylbuphedrone	H	C_2H_5	C_2H_5	H
4-Ethylmethcathinone (4-EMC)	H	CH_3	CH_3	4-C_2H_5
Ethylone	H	C_2H_5	CH_3	3,4-methylendioxy
2-Fluoromethcathinone (2-FMC)	H	CH_3	CH_3	2-F
3-Fluoromethcathinone (3-FMC)	H	CH_3	CH_3	3-F
4-Fluoromethcathinone (4-FMC)	H	CH_3	CH_3	4-F
Mephedrone (4-MMC)	H	CH_3	CH_3	4-CH_3
Methedrone	H	CH_3	CH_3	4-OCH_3
4-Methyl-alpha-Pyrrolidinopropiophenone (MPPP)	pyrrolidinyl		CH_3	4-CH_3
4-Methylbuphedrone	H	CH_3	C_2H_5	4-CH_3
Methylendioxypyrovalerone (MDPV)	pyrrolidinyl		C_3H_7	3,4-methylendioxy
4-Methylethcathinone (4-MEC)	H	C_2H_5	CH_3	4-CH_3
3-Methylmethcathinone (3-MMC)	H	CH_3	CH_3	3-CH_3
Methylone	H	CH_3	CH_3	3,4-methylendioxy
Naphyrone	pyrrolidinyl		C_3H_7	ß-naphtyl instead of phenyl
Pentedrone	H	CH_3	C_3H_7	H
Pentylone	H	CH_3	C_3H_7	3,4-methylendioxy
α-Pyrrolidinopentiophenone (α-PVP)	pyrrolidinyl		C_3H_7	H
α-Pyrrolidinopropiophenone (α-PPP)	pyrrolidinyl		CH_3	H

under isocratic conditions at ambient temperature with a flowrate of 1 ml/min and an injection volume of 10 µl. Data were collected with Chemstation Rev. A. 0903 (Agilent Technologies, Waldbronn, Germany) software.

A LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, from Merck (Darmstadt Germany) and a LiChrospher 100 RP-8e, 250 x 4 mm, 5 µm, from Macherey-Nagel (Düren, Germany) served as stationary phases.

Chemicals and Solutions

As the compounds (benzedrone, 4-BMC, buphedrone, butylone, 3,4-DMMC, N,N-dimethylbutylone, 4-EMC, ethylone, ethylbuphedrone, 2-FMC, 3-FMC, 4-FMC, methedrone, MDMC, MDPV, 4-methylbuphedrone, 4-MEC, MPPP, 4-MMC, 3-MMC, naphyrone, pentedrone, pentylone, α-PPP, α-PVP, amphetamine, 3-FA, 4-FA, 4-FMA, MDMA, methamphetamine) were not available at official suppliers, they were purchased from various Internet shops over the last years. In Tables 1 and 2 the abbreviations of the compounds are listed.

The identity of these substances was proven by GC-MS and if necessary by NMR.

Methanol and sulfated ß-cyclodextrin (degree of substitution: 7–11 $mol*mol^{-1}$) were from Sigma-Aldrich (St. Louis, MO). Nanopure water was produced in our lab.

All chemicals were of analytical grade.

The mobile phase was prepared by mixing methanol and water in the required ratios prior to adding 2% sulfated ß-cyclodextrin. To accelerate the dissolving process of the cyclodextrin, a magnetic stirrer was used. Then the solution was degassed with helium for 2 min and filtered through a 0.45-µm pore size cellulose filter (Carl Roth, Karlsruhe, Germany). For adjusting the pH a diluted sulfuric acid was used.

Sample Preparation

There was no special sample preparation required; 0.5 mg of each compound was dissolved in 1 ml nanopure water.

RESULTS AND DISCUSSION

In Tables 1 and 2 the structures of the investigated cathinone and amphetamine derivatives (tabulated by structure) are given, respectively.

TABLE 3. Enantioseparation of some test compounds using 10% methanol added to the mobile phase

Compound	t_1 (min)	t_2 (min)	α	R_s
4-FMC	8,95	9,54	1,065	0,61
4-MMC*	13,65	—	—	—
3-FA	3,64	4,71	1,290	2,84
MDMA*	1,99	2,77	1,395	2,31

Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (10:90) + 1% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV:
*240 nm, 270 nm, injection: 10 µl.

TABLE 4. Chiral separation results of a set of 6 amphetamines using an RP-18e column

Compound	t_1 (min)	t_2 (min)	α	R_s
Amphetamine	7.68	10.02	1.404	3.30
3-FA**	7.84	9.80	1.327	2.70
4-FA**	7.88	9.86	1.328	2.68
4-FMA**	18.59	22.04	1.206	1.98
MDMA*	5.75	7.42	1.433	1.80
N-Methamphetamine	10.82	13.79	1.333	2.96

Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm,
*240 nm,
**270 nm, injection: 10 µl.

Based on previous results of our group, a LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm was chosen as the stationary phase. According to the information by the manufacturer, a LiChrospher

TABLE 5. Chiral separation results of a set of 25 cathinone derivatives using an RP-18e column

Compound	t_1 (min)	t_2 (min)	α	R_s
Benzedrone	57.81	61.37	1.064	0.51
4-BMC	39.42	40.16	1.020	0.20
Buphedrone	27.23	28.87	1.065	0.78
Butylone	24.35	26.03	1.075	0.73
3,4-DMMC	73.31	83.99	1.149	0.95
N,N-Dimethylbutylone	37.02	45.08	1.229	2.73
4-EMC	100.24	103.94	1.038	0.54
Ethylbuphedrone	44.37	47.00	1.062	0.84
Ethylone	17.29	-	-	0
2-FMC*	10.00	11.05	1.108	1.26
3-FMC	14.15	14.62	1.033	0.40
4-FMC	15.09	16.31	1.092	0.96
4-MEC	57.75	61.16	1.061	0.51
4-Methylbuphedrone	82.04	93.53	1.143	1.61
MDPV	n.d.	n.d.	-	-
3-MMC	35.79	-	-	0
Mephedrone*	6.13	7.40	1.205	0.55
MPPP	73.64	80.04	1.089	0.64
Methedrone	15.70	-	-	0
Methylone	9.63	-	-	0
Naphyrone	53.36	-	-	0
α-PVP	n.d.	n.d.	-	-
α-PPP	22.07	24.02	1.069	0.93
Pentedrone	104.22	120.58	1.160	3.09
Pentylone	104.37	-	-	0

Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: *240 nm, 270 nm, injection: 10 µl.

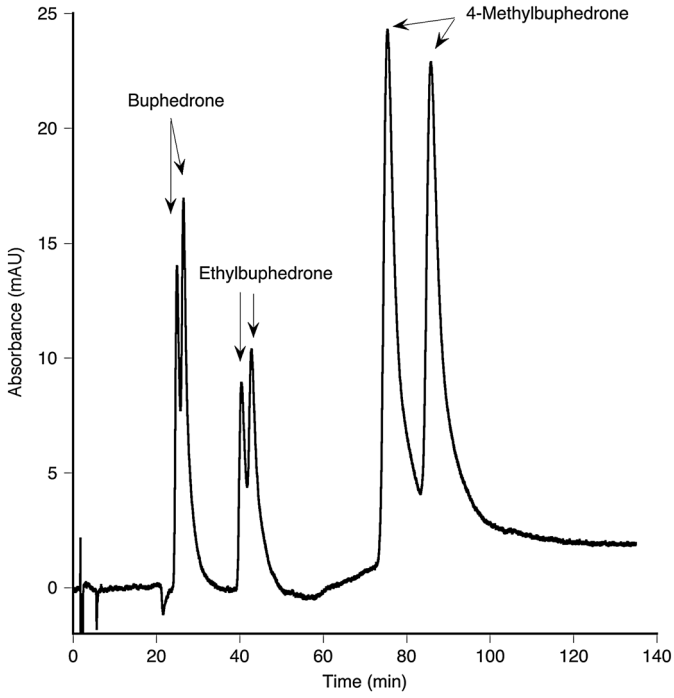


Fig. 1. Simultaneous chiral separation of buphedrone, ethylbuphedrone and 4-methylbuphedrone. Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 µl.

100 RP-18e column is suitable for the separation of acidic, neutral, and weak basic compounds. Due to the endcapping, the column is especially suitable for basic compounds. As the cathinone and amphetamine derivatives show a basic character, suitable interactions can be expected. Thus, a mobile phase consisting of methanol:water (10:90) + 1% sulfated ß-cyclodextrin was tested. To find the optimal composition of the mobile phase, 3-FA, MDMA, 4-FMC, 3-MMC, and 4-MMC were used as model compounds of the two substance classes. With the given mobile phase, retention times were quite short and only amphetamine derivatives were separated. While 4-FMC was partially separated, 3-MMC was not determined and with 4-MMC no separation was achieved. However, the two amphetamine derivatives eluted quickly and were baseline separated (Table 3). To reduce the elution power of the mobile phase the ratio methanol/water was changed to 2.5:97.5. As expected, the retention times increased but also the separation got worse. In the next step, the amount of sulfated ß-cyclodextrin was increased to a final concentration of 2%. Cyclodextrins represent commonly used chiral selectors. They are shaped like a truncated cone with a lipophilic central cavity and a hydrophilic outer surface. They may form inclusion complexes with analytes in aqueous solutions. The chiral

TABLE 6. Enantioseparation of some test compounds using an RP-18e column and 2% methanol added to the mobile phase

Compound	t_1 (min)	t_2 (min)	α	R_s
Benzedrone	n.d.	n.d.	—	—
Buphedrone	36.80	38.85	1.060	0.60
Butylone	32.63	34.76	1.064	0.65
3,4-DMMC	n.d.	n.d.	—	—
N,N-Dimethylbutylone	n.d.	n.d.	—	—
4-FMC	19.51	21.12	1.083	0.60
4-MEC	n.d.	n.d.	—	—
Pentedrone	n.d.	n.d.	—	—
Naphyrone	n.d.	n.d.	—	—

Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2:98) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 µl.

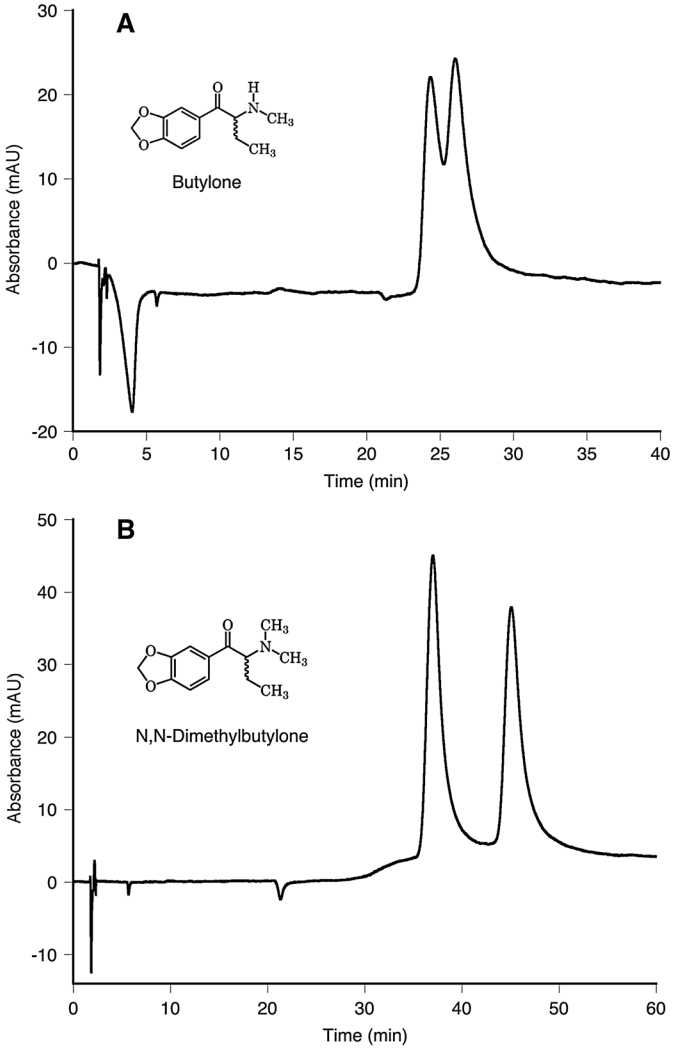


Fig. 2. Comparison of (**a**) butylone and (**b**) N,N-dimethylbutylone. Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 µl.

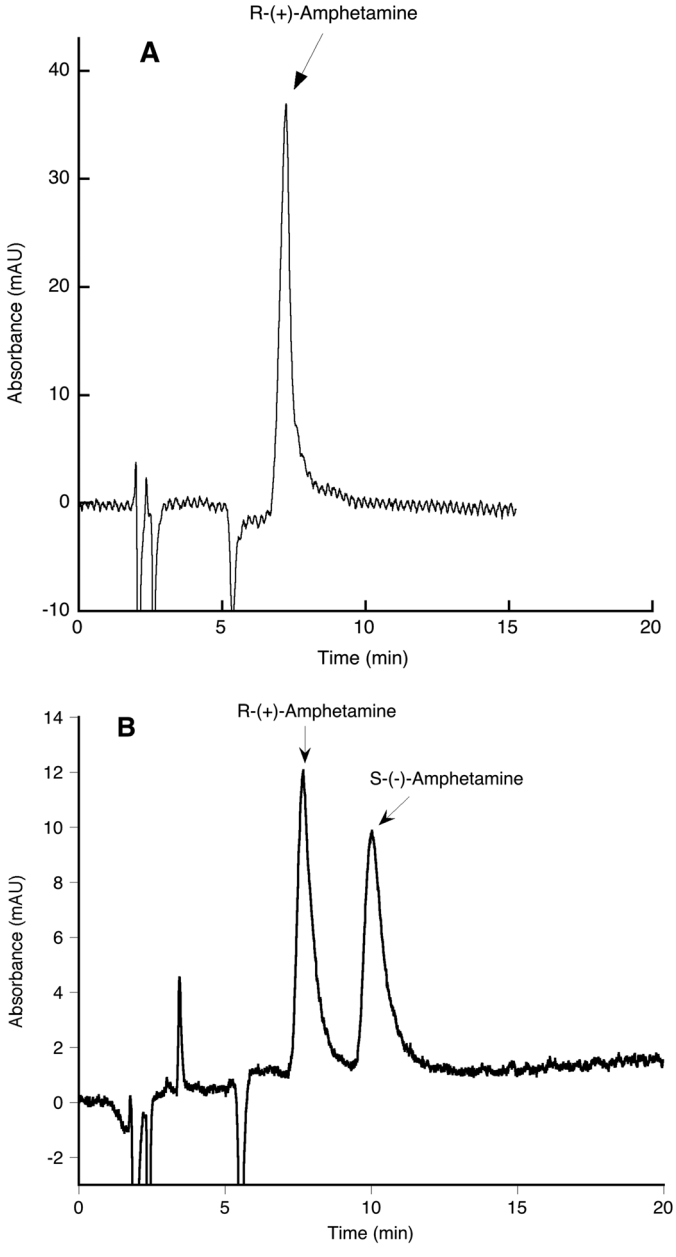


Fig. 3. Determination of the enantiomer elution order by means of amphetamine. Conditions: Column: LiChrospher 100 RP-18e, 250 x 4mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm, injection: 10 µl. A: R-(+)-amphetamine, B: racemic amphetamine.

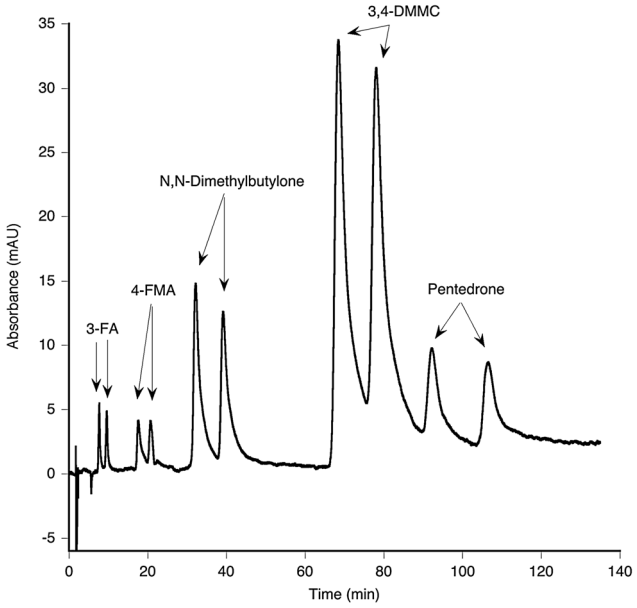


Fig. 4. Simultaneous chiral separation of 3-FA, 4-FMA, N,N-dimethylbutylone, 3,4-DMMC, pentedrone. Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 μm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 μl.

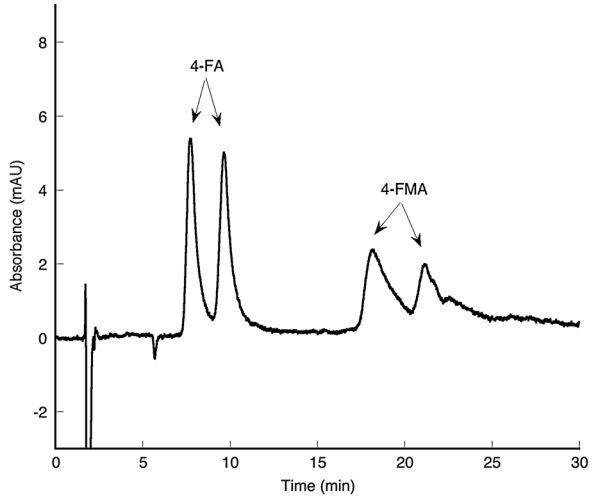


Fig. 5. Simultaneous chiral separation of 4-FA and 4-FMA. Conditions: Column: LiChrospher 100 RP-18e, 250 x 4 mm, 5 μm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 μl.

TABLE 7. Chiral separation results of a set of 6 amphetamines using an RP-8e column

Compound	t_1 (min)	t_2 (min)	α	R_s
Amphetamine	8.85	7.30	1.336	2.38
3-FA**	6,86	8,43	1.315	2,78
4-FA**	12.20	13.90	1.165	2.07
4-FMA**	16.72	19.67	1.199	2.31
MDMA*	4.51	5.50	1.376	0.98
N-Methamphetamine	7.82	9.67	1.312	2.37

Conditions: Column: LiChrospher 100 RP-8e, 250 x 4 mm, 5 μm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm,
*240 nm,
**270 nm, injection: 10 μl.

separation mechanism is based on the formation of diastereomeric selector-enantiomer complexes, which can be separated with an achiral stationary phase due to their different physical and chemical properties. Besides inclusion interactions, dipol–dipol interactions, hydrogen bondings and hydrophobic

TABLE 8. Chiral separation results of a set of 25 cathinone derivatives using an RP-8e column

Compound	t_1 (min)	t_2 (min)	α	R_s
Benzedrone	50.33	53.50	1.065	0.92
4-BMC	32.54	—	—	0
Buphedrone	25.85	27.33	1.062	0.93
Butylone	22.34	23.88	1.075	0.64
3,4-DMMC	63.87	72.76	1.143	1.45
N,N-Dimethylbutylone	36.52	44.31	1.225	2.67
4-EMC	80.65	83.64	1.038	0.70
Ethylbuphedrone	42.58	45.12	1.062	0.97
Ethylone	15.75	—	—	—
2-FMC*	11.27	—	—	—
3-FMC	13.99	—	—	—
4-FMC	14.06	15.11	1.086	0.82
4-MEC	48.51	51.62	1.067	0.84
4-Methylbuphedrone	70.86	80.83	1.145	1.98
MDPV	47.23	—	—	—
3-MMC	32.12	—	—	—
Mephedrone*	26.87	—	—	—
MPPP	63.24	68.77	1.090	0.92
Methedrone	13.57	—	—	—
Methylone	9.09	—	—	—
Naphyrone	n.d.	n.d.	—	—
α-PVP	47.44	—	—	—
α-PPP	20.27	22.13	—	—
Pentedrone	85.72	99.03	1.159	2.97
Pentylone	85.03	110.80	1.310	4.59

Conditions: Column: LiChrospher 100 RP-8e, 250 x 4 mm, 5 μm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV:
*240 nm, 270 nm, injection: 10 μl.

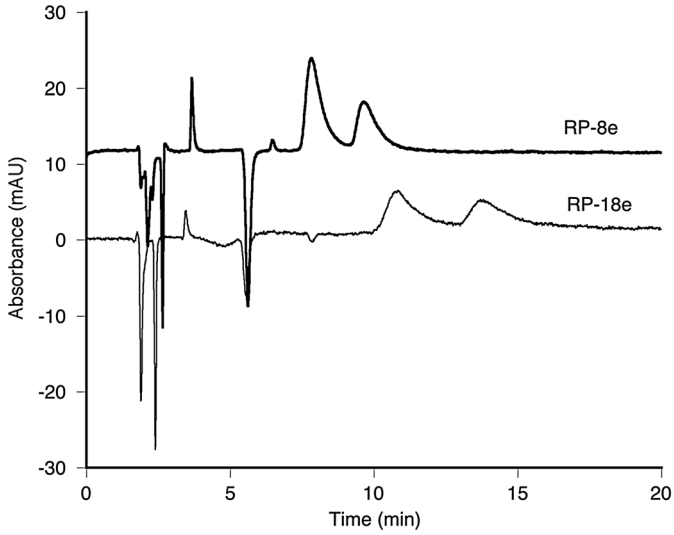


Fig. 6. Comparison of the elution of methamphetamine with two different reversed-phase LiChrospher columns. Conditions: Columns: LiChrospher 100 RP-18e and RP-8e, 250 x 4 mm, 5 μm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm, injection: 10 μl.

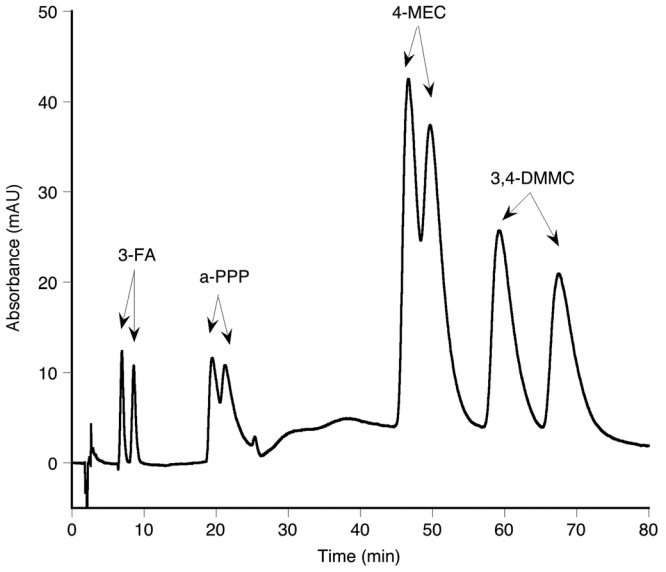


Fig. 7. Simultaneous chiral separation of 3-FA, α-PPP, 4-MEC, 3,4-DMMC. Conditions: Column: LiChrospher 100 RP-8e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 270 nm, injection: 10 µl.

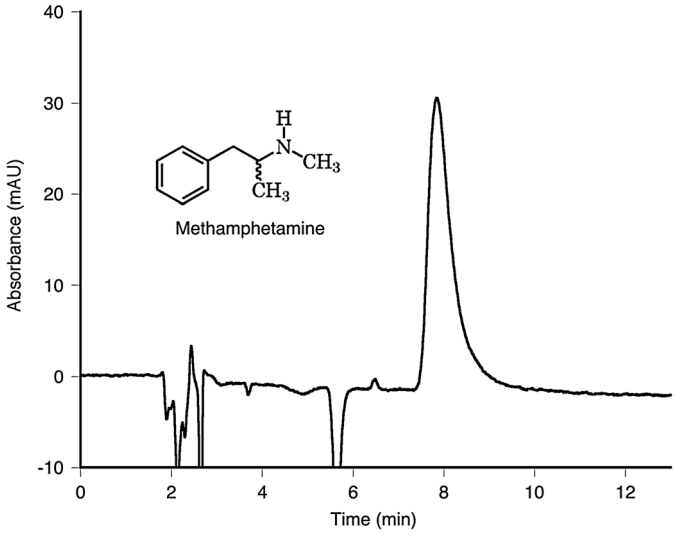


Fig. 9. Enantiomerically pure real life sample: methamphetamine. Conditions: Column: LiChrospher 100 RP-8e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm, injection: 10 µl.

interactions are responsible for the separation of the enantiomers. The added sulfated ß-cyclodextrin shifts the pH of the methanol/water mixture to 6.3. During several measurements a change of the pH was noticed. If the value exceeded 6.3, separation was lost or even no peak appeared. As a consequence, the pH was checked daily and adjusted if necessary.

With the final composition of the mobile phase (methanol: water 2.5:97.5 + 2% sulfated ß-cyclodextrin) the enantiomers of amphetamine and its derivatives were resolved (Table 4). The retention times were below 23 min and the resolution factors ranged from 1.8 (MDMA) to 3.3 (amphetamine).

With this setup, 17 out of 25 cathinone derivatives were partially or baseline separated (Table 5). Retention times were between 15 and 120 min and the resolution factors ranged between 0.20 (4-BMC) and 3.09 (pentedrone). The best results of the cathinone derivatives were achieved with N,N-dimethylbutylone, 4-methylbuphedrone, and pentedrone. Moreover, the side chains of the cathinone derivatives played

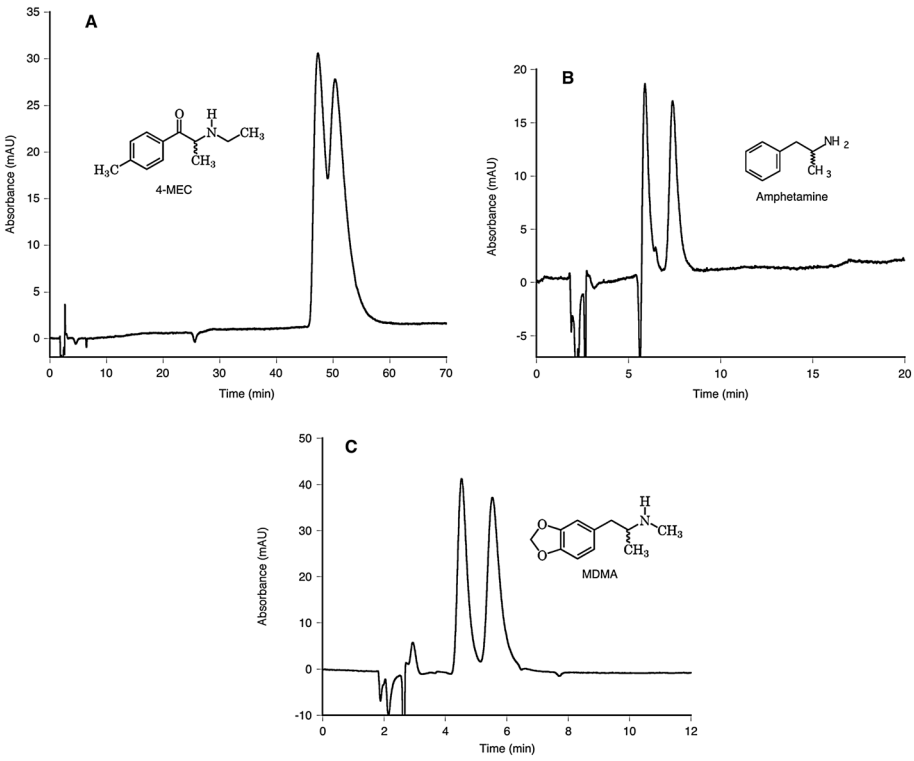


Fig. 8. Racemic real life samples: (**a**) 4-MEC, (**b**) amphetamine, (**c**) MDMA. Conditions: Column: LiChrospher 100 RP-8e, 250 x 4 mm, 5 µm, mobile phase: methanol:water (2.5:97.5) + 2% sulfated ß-cyclodextrin, room temperature, flow: 1 ml/min, UV: 220 nm, 240 nm, 270 nm, injection: 10 µl.

TABLE 9. Repeatability and reproducibility data including retention time and resolution by means of 3-FA using an RP-18e column

Repeatability	t_1 (min)	t_2 (min)	R_s
Intraday n = 5	7.85 ± 0.02, RSD = 0.19%	9.82 ± 0.02, RSD = 0.17%	2,69 ± 0,17, RSD = 6.39%
Interday n = 10	7.99 ± 0.14, RSD = 1.76%	10.00 ± 0.20, RSD = 1.99%	3,03 ± 0,39, RSD = 12.76%
Reproducibility	t_1 (min)	t_2 (min)	R_s
Intraday n = 20	7.79 ± 0.03, RSD = 0.45%	9.71 ± 0.04, RSD = 0.43%	3,54 ± 0,26, RSD = 7.26%

a crucial role regarding resolution. While 4-methylbuphedrone was baseline separated, buphedrone and ethylbuphedrone were only partially separated. Obviously, the presence of a para-methyl group at the phenyl ring improved resolution. Figure 1 shows that the retention times grew significantly with the increasing lipophilic character of the compounds. The inclusion of the molecules in the sulfated ß-cyclodextrin is extremely influenced by the lipophilicity and leads 4-methylbuphedrone to a satisfactory result. Also, separation of butylone and N,N-dimethylbutylone can be compared. While the latter is baseline separated because of the higher lipophilic character, butylone shows only partial resolution (Fig. 2). Comparing 4-MEC and its structural isomer 4-EMC, it can be seen that the ethyl-group at the ring of 4-EMC leads to a better inclusion in the cyclodextrin and a stronger interaction of the stationary phase. The retention times are unexpectedly different. Despite the long retention time of 4-EMC, only a partial separation was achieved.

With the present method, ethylone, 2-FMC, 3-MMC, mephedrone, methylone, naphyrone, and pentylone were not separated into their enantiomers. MDPV and α-PVP, which have a similar structure as α-PPP and MPPP, were not even detected. Among 25 cathinone derivatives, only three were baseline separated; thus, this method is better suitable for the enantioseparation of amphetamines.

Furthermore, it was investigated how the concentration of the organic solvent influences chiral separation. Therefore, a mobile phase with 2% of methanol was prepared, while the concentration of the sulfated ß-cyclodextrin remained unchanged. The higher amount of water resulted in longer retention times, and as a consequence many of the analytes were not determined (Table 6).

To check the enantiomer elution order (EEO), both racemic solutions of amphetamine and R-amphetamine were injected. As shown in Figure 3, the R-enantiomer elutes first, followed by the S-enantiomer. The R-enantiomer is responsible for the main effects such as stimulation, less appetite, and improved performance on working memory. The determination of the enantiomer elution order can be used as a purity check. Moreover, detection of a racemate might be possible evidence of an illegal origin.

Besides single determination of analytes, simultaneous enantioseparation was carried out. An attempt was made to achieve enantioseparation with a couple of compounds. Chiral resolution of five substances was feasible. In Figure 4, enantiomers of 3-FA, 4-FMA, N,N-dimethylbutylone, 3,4-DMMC, and pentedrone were separated simultaneously. The methyl group of 4-FMA makes the molecule more lipophilic and leads to a longer retention time in comparison to 4-FA, which is demonstrated in Figure 5.

A further emphasis of this work was to elucidate the influence of the RP chain length of the column on enantioresolution. It was checked if the C8 chains are hydrophobic enough to obtain the same results as with C18 chains. Generally, the use of an RP-18e column resulted in better enantioseparation compared to an RP-8e column. The separation results of LiChrospher 100 RP-8e column are shown in Tables 7 and 8. One exception was pentylone, which was baseline separated, while with an RP-18e column only one peak appeared. A comparison of the effect of the two different reversed-phase LiChrospher columns on enantioseparation of methamphetamine is illustrated in Figure 6. Moreover, simultaneous chiral separation of 3-FA, α-PPP, 4-MEC, and 3,4-DMMC was performed with this column (Fig. 7).

Furthermore, this new and easy-to-perform chiral separation method was tested by means of real-life samples. Amphetamine, methamphetamine, MDMA, and 4-MEC seized by police in Austria were analyzed to check the ratios of enantiomers. It was discovered that amphetamine, MDMA, and 4-MEC were traded as racemic mixtures (Fig. 8), while methamphetamine was enantiopure (Fig. 9). This might be a hint that the latter compound was synthesized illegally, e.g., by reduction of an enantiopure ephedrine in a clandestine laboratory.

Finally, validation of the new method using the RP-18e column with regard to the reproducibility and the repeatability of the retention time as well as the resolution factor was done with 3-FA as the analyte. The intra- and interday repeatability of the three parameters was satisfactory. For the intraday repeatability, a relative standard deviation (RSD) for the retention times was less than 0.20% and for the resolution factor about 6.39%. Day-to-day repeatability for the retention times was less than 2% and for the resolution factor was 12.76%. This high value can be explained because the efficiency of the peaks varied from day to day. In Table 9 the validation data are shown.

Repeatability data were also collected for the RP-8e column and were found to be similar to those of the RP-18e column.

CONCLUSION

For the majority of the new legal and illegal highs, few analytical methods for their identification are available. The interest in and the importance of the development of new chiral separation methods is increasing, as it is unknown if the enantiomers of the new substances differ in their pharmacological activity, metabolic, and pharmacokinetic characteristics. A simple and easy-to-perform method for the chiral separation of cathinone and amphetamine derivatives was introduced using HPLC with a common RP column and sulfated ß-cyclodextrin added to the mobile phase under isocratic conditions.

Generally, amphetamines showed less interaction with the sulfated ß-cyclodextrins and the LiChrospher 100 RP-18e, 250 x 4 mm, 5 μm column than cathinone derivatives. So their retention times are considerably shorter. While all investigated amphetamines were baseline separated only, some baseline separations of the cathinone derivatives to their enantiomers were achieved.

In addition to the single investigation some simultaneous chiral separations were carried out. Both RP-18e and RP-8e columns can be used. A comparison of an RP-18e and an RP-8e column showed that the chiral separation results suffered partially from a shorter chain length of the RP of the stationary phase. Moreover, a validation of the method through performing intra- and interday repeatability and reproducibility was shown. Also, the presented method was shown to be applicable to real-life samples.

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