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Determination of the enantiomeric composition of amphetamine, methamphetamine and 3,4-methylendioxy-*N*-methylamphetamine (MDMA) in seized street drug samples from southern Germany

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Abstract

Amphetamine (*speed*), methamphetamine (*crystal meth*), and 3,4-methylenedioxy-N-methylamphetamine (MDMA, *ecstasy*) represent the most frequently abused amphetamine-type stimulants (ATS). Differences in pharmacological potency and metabolism have been shown for the enantiomers of all three stimulants. Legal consequences in cases of drug possession may also differ according to the German law depending on the enantiomeric composition of the seized drug. Therefore, enantioselective monitoring of seized specimens is crucial for legal and forensic casework.

Various kinds of samples of amphetamine ($n = 143$), MDMA ($n = 94$), and methamphetamine ($n = 528$) that were seized in southern Germany in 2019 and 2020 were analyzed for their chiral composition using different chromatographic methods.

Whereas all samples of amphetamine and MDMA were racemic mixtures, the chiral composition of the methamphetamine specimens was diverse. Although the vast majority ($n = 502$) was present as (S)-methamphetamine, also specimens containing pure (R)-methamphetamine ($n = 7$) were found. Furthermore, few samples ($n = 8$) were of racemic nature or contained non-racemic mixtures of both enantiomers ($n = 10$).

Because methamphetamine appears in varying enantiomeric compositions, any seizure should be analyzed using an enantioselective method. Amphetamine and MDMA, on the other hand, currently appear to be synthesized exclusively via racemic pathways and are not chirally purified. Nevertheless, regular monitoring of the chiral composition should be ensured.

KEYWORDS

amphetamines, chiral chromatography, enantiomers, forensic toxicology, seizures

1 | INTRODUCTION

Over the past decade, global seizures of amphetamine-type stimulants (ATS) have continuously increased.[1] In addition to amphetamine (*speed*) and methamphetamine (*crystal meth*), the two major representatives of the ATS group, MDMA (3,4-methylenedioxy-*N*-methylamphetamine), commonly known as ecstasy, poses another frequently abused member of this group.[2] Use of ATS is on the rise in Europe and especially in Germany, where amphetamine is the most commonly abused ATS.[1,3] At the same time, an increasing trend in the use of *ecstasy* can be observed, whereas the MDMA content of tablets is rising and other high-purity forms such as powder and crystals have also become available.[1,4] The use of methamphetamine is also becoming more widespread in many European countries.[1,3,4]

Amphetamine, methamphetamine, and MDMA each possess one stereogenic center and therefore exist in two enantiomeric forms. For all three substances, studies suggest a higher stimulant activity of the (S)-(+)-enantiomer.[5–9] Furthermore, pharmacokinetic studies showed metabolic preferences for the respective (S)-enantiomers.[7,10–13] Due to the different potencies, for amphetamine and methamphetamine, the threshold of the *non-small amount* in the sense of the German narcotics act (*Betäubungsmittelgesetz*, BtMG) differs depending on the enantiomeric composition of the seized drugs. Consequently, the *non-small amount* for (R)-amphetamine is twice and for (R)-methamphetamine 10 times as high as for the more potent (S)-enantiomers.[14] According to § 31a BtMG, in the case of production, possession, acquisition, etc. of drugs for personal use in amounts below this threshold (*small amount*), the public prosecutor may refrain from prosecution.[15]

The methods used for the synthesis of the stimulants differ both in their precursors and in the chiral composition of the products and are subject to dynamic change due to limitations of the precursor compounds.[1,16,17]

In order to enable assessment of legal and forensic cases, it is therefore necessary to investigate and monitor the enantiomeric composition of seized ATS and to consider possible variations in routine casework. In the present study, two methods of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and one method of liquid chromatography coupled with diode array detector (HPLC-DAD) were used to enantioselectively analyze seized ATS samples from southern Germany. Specimens of confiscated amphetamine, methamphetamine, and MDMA from the years 2019 and 2020 were collected and analyzed. The aim of this study is the assessment of the enantiomeric composition of the seized samples, and the evaluation regarding legal and forensic implications.

2 | MATERIAL AND METHODS

2.1 | Amphetamine and MDMA

2.1.1 | Material

(RS)-Amphetamine and (RS)-MDMA-d_5 were obtained from LGC (Wesel, Germany). (RS)-MDMA was purchased from Merck (Darmstadt, Germany). (RS)-Amphetamine-d_{11}, (S)-amphetamine, and (R)-amphetamine were obtained from Lipomed (Weil am Rhein, Germany). All analytical standards were provided as methanolic solutions with concentrations of 0.1 mg/mL (internal standards) and 1 mg/mL (all other solutions). The standards labeled with "RS" were racemic according to the manufacturer's certificates. Methanol (HPLC grade) was acquired from Thermo Fisher Scientific (Dreieich, Germany). Water (LC-MS grade) and acetonitrile were obtained from Carl Roth (Karlsruhe, Germany). LC-MS-grade ammonia solution (25%) and ammonium bicarbonate were purchased from Merck (Darmstadt, Germany).

2.1.2 | Seized drug samples

143 amphetamine samples from a total of 79 seizures and 94 MDMA samples from a total of 60 seizures were available for investigation. All samples were seized by the state police on the territory of Rhineland-Palatinate in the years 2019–2020. Amphetamine was almost exclusively present as powder, with four samples being present in liquid form. MDMA specimens were seized either as tablets of various colors ($n = 63$), in crystalline form ($n = 30$), or as powder ($n = 1$). Some seizures contained both tablets and crystalline samples. Quantitative determination of the sample purity was conducted by achiral HPLC-DAD. Ecstasy pills contained a median of 37.0% (19.3%–50.0%, mean [SD] 36.3 ± 6.0%) MDMA base, whereas the crystalline form exhibited significantly higher purity (median 79.8%, 76.5%–82.7%, mean [SD] 79.6 ± 1.6%), which corresponds to a purity of at least 90.9%, assuming the hydrochloride form. One specimen that was seized as purple powder, was of significantly lower purity (7.1%), and contained traces of amphetamine. Amphetamine powder contained a median of 16.2% (0.4%–73.1%, mean [SD] 21.6 ± 14.9%) amphetamine base, with two samples representing almost pure (>99%) amphetamine, assuming the sulfate form. One powder sample (24.6%) also contained traces of MDMA. Liquid amphetamine samples contained 62.7%, 60.6%, 47.1%, and 2.6% amphetamine base, respectively. The latter one was of blue color and also contained MDMA, ethanol, and gamma-butyrolactone (GBL). Boxplots in Figure 1 illustrate the distribution of purity for the various types of samples seized.

2.1.3 | Sample preparation

Sample preparation was carried out according to a forensic guideline.[18] In brief, the preparation for the different sample types was conducted as follows: At first, all samples were dried to a constant weight. For seizures of tablets in a single packaging unit, at least 30% of the samples were homogenized and diluted in water. For seizures of tablets in multiple packaging units, at least 30% of each unit were homogenized. Subsequently, at least one mixture of equal proportions of the homogenisates was prepared and diluted in water. For powder and crystalline samples in a single packaging unit, a composite sample containing at least 30% of the material was taken from at least three

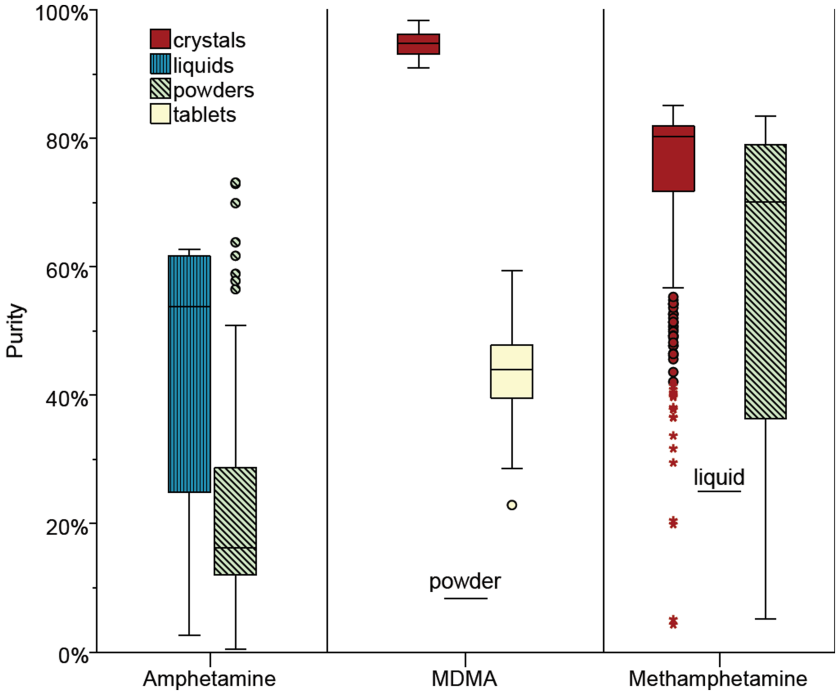

FIGURE 1 Boxplots for purity of the different sample types of amphetamine, MDMA, and methamphetamine. Horizontal lines represent the median, and boxes represent the range between lower and upper quartile of the subgroups (inter quartile range). Whiskers represent all samples within ±1.5 times the interquartile range. Outliers > median ±1.5 times the interquartile range are shown as circles. Extreme values with >median ±3 times the interquartile range are shown as asterisk. For each MDMA powder and methamphetamine liquid, only one sample was available, which is presented as single line

spots of the specimen and subsequently diluted in water. For powder and crystalline samples in multiple packaging units, at least one mixture of equal proportions of all units was prepared and diluted in water. All liquid samples were stored sealed until the day of analysis. For analysis, all aqueous solutions were diluted with methanol to reach a final racemic concentration of 100–500 ng/mL. The solutions were transferred into sampling vials, and racemic internal standard solution was added (racemic amphetamine-d_{11} and MDMA-d_5, respectively; final concentration 500 ng/mL).

2.1.4 | Chiral LC-MS/MS instrumentation and analytical parameters

Enantiomers of amphetamine and MDMA were analyzed using a LC-MS/MS system from Agilent (Waldbronn, Germany). Chromatography was conducted using a 1290 Infinity II LC system, coupled via Jet Stream interface (ESI) to a 6495C triple quadrupole mass spectrometer. Chromatographic separation of both enantiomeric pairs was achieved using a polysaccharide-based chiral Lux® 3-µm AMP 150 × 3.0 mm analytical column, guarded with a Lux AMP® 4 × 2.0 mm security guard cartridge (both Phenomenex; Aschaffenburg, Germany). Injection volume was 1 µL. Mobile Phase A consisted of 5 mM ammonium bicarbonate solution adjusted to pH 11 by addition of ammonia solution. Composition of mobile Phase B and gradient elution differed between the determination of amphetamine and MDMA. For enantiomeric separation of amphetamine, methanol was used as organic mobile phase (B), and for enantiomeric separation of MDMA, a mixture of methanol/acetonitrile (1/1, v/v) was used. Holding a temperature of 30°C and a flow rate of 0.35 mL/min, the following gradients were used for separation of amphetamine (if applicable different percentages for MDMA in parentheses): holding B at 60% (50%) for 13 min, increasing it to 95% within 0.5 min, holding for 3 min, and decreasing to 60% (50%) within 0.5 min. With an additional post-time of 2 min, total run time for both gradients was 19 min. Baseline separation was achieved for all analytes and ISTD. Electrospray parameters were as follows: gas flow 11 L/min at 200°C; nebulizer 15 psi, sheath gas flow 12 L/min at 400°C; capillary voltage +3500 V. Analytes were detected using multiple reaction monitoring (MRM) mode using the following transitions (*m/z*, collision energy in parentheses, target ion underlined): amphetamine 136.1 → 91.0 (17 eV), 119.0 (5 eV), 65.0 (45 eV); amphetamine-d_{11} 147.2 → 98.0 (21 eV), 130.1 (5 eV), 70.1 (45 eV); MDMA 194.1 → 163.0 (9 eV), 105.0 (25 eV), 51.1 (80 eV); MDMA-d_5 199.2 → 165.0 (13 eV), 107.0 (29 eV), 135.0 (21 eV). The two LC-MS/MS methods used for this study also allowed simultaneous enantioselective detection of 3,4-methylenedioxyamphetamine (MDA) and methamphetamine. Data evaluation was done using Agilent MassHunter Workstation Software (Version B.09.00). Identification of the elution order for amphetamine enantiomers was conducted by injection of enantiopure standard solutions. For all other experiments, a racemic amphetamine solution was used as analytical standard, because the monoenantiomeric solutions (especially for (R)-amphetamine, which eluted first) were not completely pure. Due to the lack of enantiopure standards for MDMA, elution order could not be determined but was deduced. The present chromatographic method was also validated for quantitative detection of MDMA enantiomers in serum samples. Studies show that (S)-MDMA is metabolized more rapidly than (R)-MDMA in humans, and therefore, (R)/(S)-concentration ratios greater than 1 can be found in serum samples.[10,12,13] Because the first eluting enantiomer consistently showed a larger area under the curve (AUC) when serum samples were

measured, it was identified as (R)-MDMA. For each sample, (R)/(S)-concentration ratio was calculated as the quotient of the AUC of the analyte relative to the AUC of the respective ISTD according to Equation 1. To assess the precision of the method, two methanolic solutions of certified racemic amphetamine and MDMA were prepared to reach a final racemic concentration of 250 and 500 ng/mL, respectively. Analogous to the procedure for the seized samples, the solutions were transferred into sampling vials, and racemic internal standard solution was added (final concentration 500 ng/mL). Both low and high quality control (QC) samples were injected 10 times each, and the (R)/(S) ratio was calculated.

$$(R)/(S)-\text{ratio}=\frac{AUC_{(R)-\text{enantiomer}}}{AUC_{(R)-\text{enantiomer of ISTD}}}/\frac{AUC_{(S)-\text{enantiomer}}}{AUC_{(S)-\text{enantiomer of ISTD}}} \quad (1)$$

2.2 | Methamphetamine

2.2.1 | Material

Water was obtained from Merck (Darmstadt, Germany). Methanol, phosphoric acid (85%), potassium dihydrogen phosphate, and hydrochloric acid (37%) were purchased from VWR (Darmstadt, Germany). (RS)-Methamphetamine, (S)-methamphetamine, and (R)-methamphetamine were obtained from Lipomed (Weil am Rhein, Germany). All analytical standards were provided as methanolic solutions with concentrations of 1 mg/mL. (RS)-Methamphetamine was racemic according to the manufacturer's certificate. All chemicals and solvents were of analytical grade.

2.2.2 | Seized drug samples

528 methamphetamine (MA) samples from a total of 237 seizures from the years 2019 and 2020 were collected. All samples were seized by the state police in the Free State of Bavaria. The samples originated from the following governmental districts: Upper Franconia ($n = 125$), Middle Franconia ($n = 298$), Lower Franconia ($n = 1$), Upper Palatinate ($n = 94$), Upper Bavaria ($n = 1$), and Lower Bavaria ($n = 9$). Methamphetamine was mostly present in crystalline form ($n = 498$, 94.3%), less frequently as powder ($n = 29$, 5.5%), and in one case, a liquid was seized. Some seizures contained both crystalline and powder samples. Quantitative determination of the sample purity was conducted by achiral HPLC-DAD. Crystals contained a median of 80.3% (4.3%–85.1%, mean [SD] 74.5 ± 12.5%) MA base, whereas MA powder contained a median of 70.1%, (5.2%–83.4%, mean [SD] 56.3 ± 28.1%), and the liquid had a purity of 25% and a total volume of only 250 μL. Boxplots in Figure 1 illustrate the distribution of purity for the various types of methamphetamine samples seized. Traces of amphetamine (nine samples) and MDMA (one sample) were found in both crystalline and powder samples. The relative amphetamine content in these samples was between 3.9% and 1474% (median 37.1%). The sample with MDMA contained more MDMA than methamphetamine (258%).

2.2.3 | Sample preparation

Sample preparation was based on a forensic guideline.[18] All samples were dried at a temperature of 70°C to a constant weight and subsequently homogenized. Up to a sample weight of 500 g, 2 × 25 mg of the homogenized material were taken. Above a weight of 500 g, 3 × 25 g were prepared, and above 1000 g, 4 × 25 mg were used. For analysis, homogenisates were diluted in 25 mL diluted hydrochloric acid (1/99, v/v) to reach a final racemic below 1 mg/mL.

2.2.4 | Chiral LC-DAD instrumentation and analytical parameters

Chromatography was conducted using a 1100 series HPLC-DAD system from Agilent (Waldbronn, Germany). Methamphetamine enantiomers were separated on a cyclodextrin-based ChiraDex® 5 μm (250 × 4.0 mm Merck, Darmstadt, Germany) chiral column. Injection volume was 2 μL and column temperature was 40°C. The mobile phase was prepared by adding 49.75 g phosphate buffer solution (pH 6.8) and 2.5 g methanol to 447.75 g water. Separation was achieved by isocratic elution at a flow rate of 0.8 mL/min. The total run time was 10 min. Spectra were detected at 210, 220, 230, 240, and 280 nm, whereas quantification was done at a wavelength of 210 nm. Data evaluation was done using Agilent ChemStation software. Elution order of methamphetamine enantiomers was conducted by injection of enantiopure standard solutions. When enantiomerically pure methamphetamine or racemate was detected by the chiral method, quantitative results of the achiral HPLC-DAD were applied. When non-racemic mixtures were detected, the enantiomers were quantified separately using the chiral method. For both enantiomers, linearity of calibration was tested within a range of 0.001–1 mg/mL. The method's specificity was examined with respect to the also detected analytes amphetamine, MDMA, MDA, MDE, creatine, paracetamol, caffeine, piracetam, and ephedrine. Traces of other phenethylamines were detected up to an absolute content of 0.5%.

3 | RESULTS

3.1 | Enantiomeric composition of amphetamine and MDMA

Baseline separation was achieved for both analytes and internal standards (cf. Figure 2). Validation experiments showed satisfactory precision for the determination of (R)/(S)-ratios. Low and high amphetamine QC samples showed ratios between 0.96 and 1.06

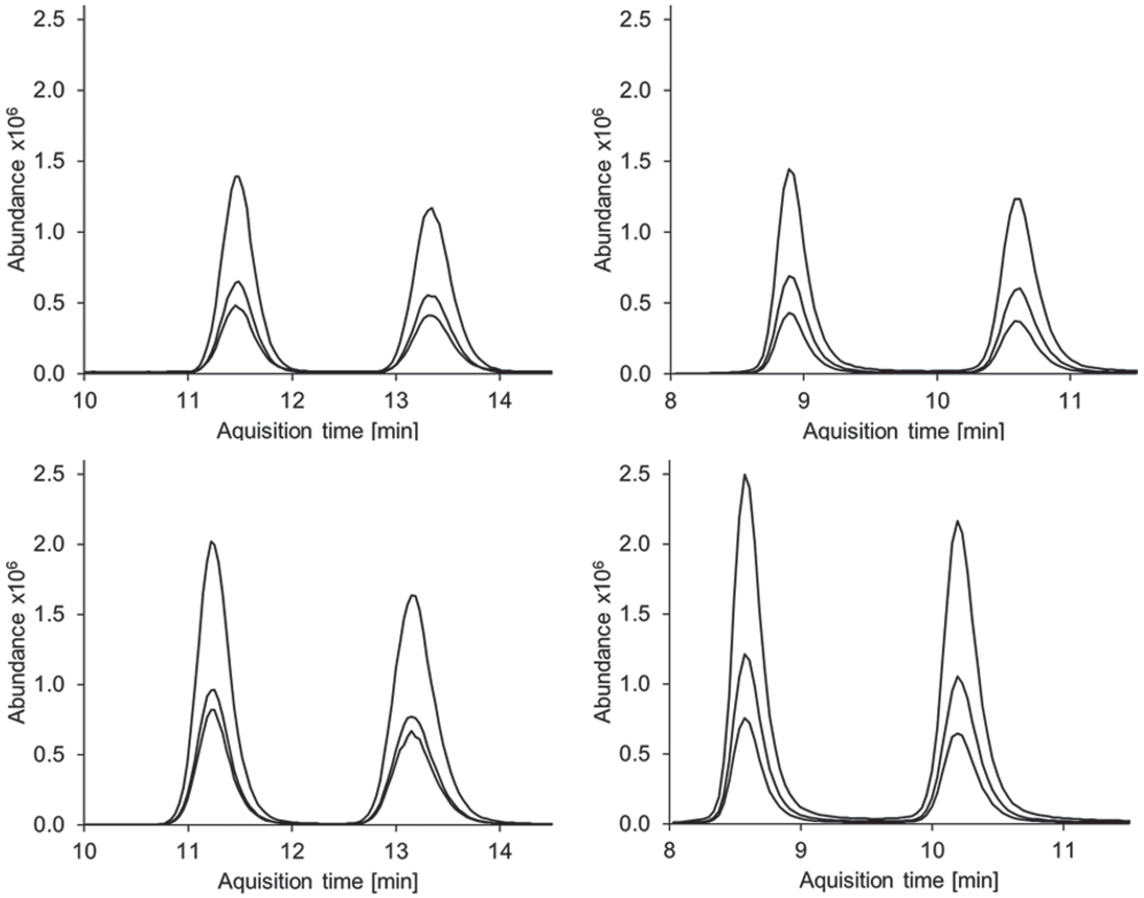

FIGURE 2 Multiple reaction monitoring (MRM) chromatograms for two authentic seized drug samples of MDMA (left plots, (R)/(S)-ratio 1.02) and amphetamine (right plots, (R)/(S)-ratio 0.99). Chiral chromatographic separation is shown for each the analytes (top) and their internal standards (bottom). Elution times were as follows (with the (R)-enantiomer always eluting first): MDMA 11.3 and 13.3 min, amphetamine 8.9 and 10.6 min, MDMA-d_5 11.1 and 13.1 min, amphetamine-d_{11} 8.6 and 10.2 min. The following ion transitions are displayed in decreasing order of intensity (target ion underlined): MDMA 194.1 → 163.0, 105.0, 51.1; amphetamine 136.1 → 91.0, 119.0, 65.0; MDMA-d_5 199.2 → 165.0, 107.0, 135.0; amphetamine-d_{11} 147.2 → 98.0, 130.1, 70.1

(mean 1.00), and ratios for low and high MDMA QC samples were between 0.94 and 1.06 (mean 1.01). (R)/(S)-ratios for seized amphetamine were between 0.97 and 1.04 (mean 1.00). Also, both enantiomers of amphetamine (very minor concentration) were found in the one MDMA sample, which was seized as purple powder.

(R)/(S)-ratios for seized MDMA were between 0.96 and 1.03 (mean 1.00) for all three different sample types (crystals, liquid, powder). MDMA (R)/(S)-ratio was 1.01 for the adulterated amphetamine powder and 1.00 for the blue amphetamine liquid. Boxplots of the determined ratios for seized amphetamine and MDMA samples are shown in Figure 3. Contaminations of methamphetamine in amphetamine samples ($n = 67$, 47%) and MDA in MDMA samples ($n = 4.4\%$) were always below a relative content of 1%. In all cases of MDA and methamphetamine trace detection, both enantiomers were found in roughly equal proportions; however, in one case, there was an approximately 10-fold excess of (S)-methamphetamine. Due to the low concentration of MDA and methamphetamine traces, a more precise determination was not possible.

3.2 | Enantiomeric composition of methamphetamine

Sufficient separation of methamphetamine enantiomers was achieved (cf. Figure 4). The method also showed specificity and linearity for both methamphetamine enantiomers. Traces of enantiomers below the lower limit of quantification (LLOQ, 0.1% of the total mass) were considered as incidental impurities and therefore neglected. The one liquid sample as well as 28 out of 29 powder samples contained enantiopure (S)-methamphetamine. In contrast, the remaining one powder sample contained enantiopure (R)-methamphetamine. The enantiomeric composition of the crystalline samples was more diverse. The vast majority ($n = 473$, 95.0%) of crystalline samples contained enantiopure (S)-methamphetamine, whereas only seven samples (1.4%) contained pure (R)-methamphetamine. Another 10 samples (2.0%) contained racemic methamphetamine. Furthermore, there was a total of 10 samples containing a non-racemic mixture of both enantiomers. Seven out of these 10 samples originated

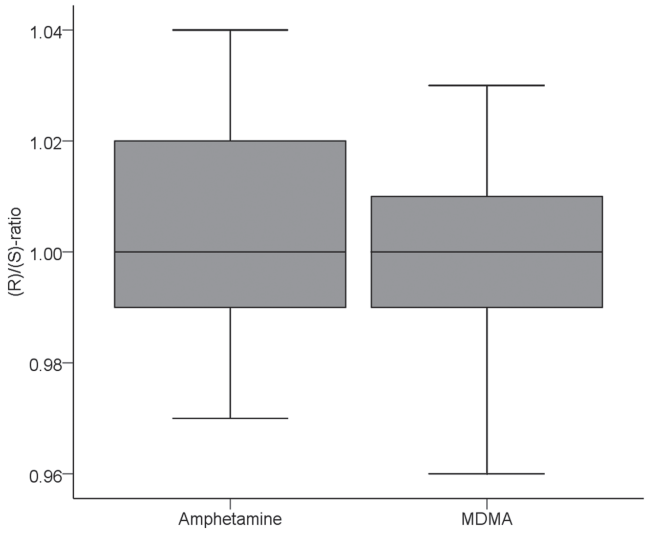

FIGURE 3 Boxplots of calculated (R)/(S)-ratios for all amphetamine ($n = 143$) and MDMA ($n = 94$) samples. Horizontal lines represent the median, and boxes represent the range between lower and upper quartile of the subgroups (interquartile range). Whiskers represent all samples within ±1.5 times the interquartile range. No outliers or extreme values with >median ±1.5 times the interquartile range were present

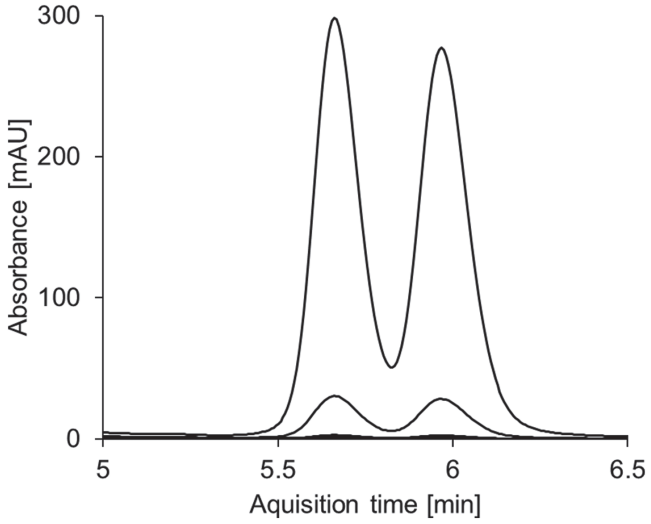

FIGURE 4 HPLC-DAD chromatogram of the separation of methamphetamine enantiomers. Retention time was 5.66 min for (R)-methamphetamine and 5.99 min for (S)-methamphetamine. Quantification was done at 210 nm (absorption maximum), whereas absorption at 220 nm was significantly lower

from a single seizure, which also included one racemic sample. The other three non-racemic mixtures originated from three unrelated seizures. Two samples contained an excess of (R)-methamphetamine, whereas the other eight samples contained an excess of (S)-methamphetamine. The calculated enantiomeric excess (ee) for (S)-methamphetamine ranged from −19% to 91%. Detailed information on composition and ee of the non-racemic mixtures is shown in Table 1. The quantities of the (R)-methamphetamine seizures ranged from 0.45 to 95.6 g (mean 31.3 g, median 14.9 g). For the non-racemic mixtures, the absolute amounts seized ranged from 0.29 to 38.8 g (mean 7.65 g, median 0.36 g). Although larger quantities of (S)-methamphetamine were seized in some cases, the seized quantities of (R)-methamphetamine and non-racemic mixtures were within the usual range. An overview of the enantiomeric compositions of all sample types of seized methamphetamine is given in Figure 5.

TABLE 1 Seizures of non-racemic mixtures of methamphetamine ($n = 10$)

Year	Seizure	$(S)_{rel}$ [%]	$(R)_{rel}$ [%]	ee (S) [%]
2019	1	96	4.3	91
		91	9.3	81
		85	15	71
		78	22	55
		65	35	30
		54	46	8
		41	59	-17
2019	2	92	8.0	84
2020	3	41	59	-19
2020	4	77	23	54

Notes: All samples were present as crystals. The table shows the relative contents of the methamphetamine enantiomers ($(S)_{rel}$ and $(R)_{rel}$) for each sample. Enantiomeric excess (ee) of (S)-methamphetamine is calculated as difference between $(S)_{rel}$ and $(R)_{rel}$. A negative value therefore represents an excess of (R)-methamphetamine (two cases).

4 | DISCUSSION

In the present study, multiple chiral methods were successfully applied to examine a large number of samples of seized amphetamine, methamphetamine, and MDMA. To the best of our knowledge, this is the first study to describe the chiral composition of ATS seized in Germany.

Independent of the sample types, all seized amphetamine and MDMA samples contained both enantiomers. Calculated (R)/(S)-ratios for both substances were within the limits of accuracy determined by the measurement of QC samples containing certified racemic standards. Therefore, all samples can be considered as racemic. MDMA impurities in the one amphetamine powder sample and in the blue amphetamine liquid can also be considered as racemic. Furthermore, the traces of amphetamine in the purple MDMA powder sample also suggest contamination with racemic amphetamine, although a precise determination was not possible in this case.

The detection of racemic amphetamine is consistent with other studies found in literature, although data on enantiomeric composition of seized amphetamine samples are very limited. Van Bocxlaer et al.[19] found both amphetamine enantiomers in almost equal proportions in

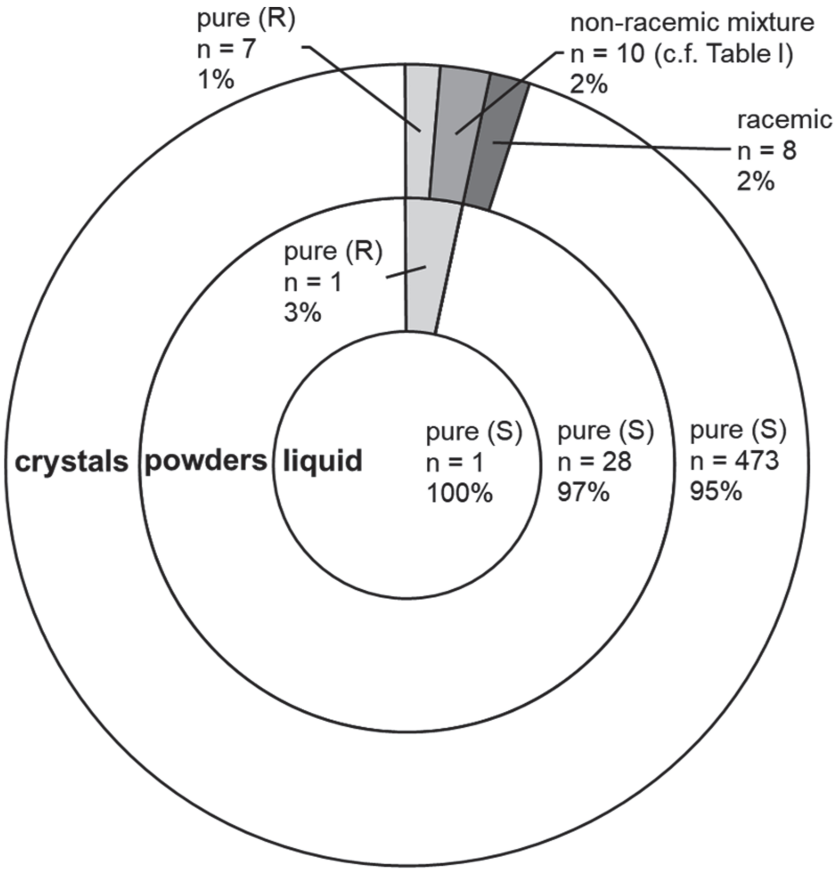

FIGURE 5 Pie chart of the enantiomeric composition of the different methamphetamine sample types. Inner circle represents the liquid sample ($n = 1$), middle annulus represents powder samples ($n = 29$), and outer annulus represents crystalline samples ($n = 498$)

two powder samples that also contained racemic α-phenethylamine. In 2000, George and Braithwaite[20] analyzed 17 crystalline and powder samples and consistently detected an excess of (S)-amphetamine (R/S-ratio 0.72–0.98, mean 0.89), although validation data are not available. More recently, Dhabbah[21] analyzed 12 batches of amphetamine tablets seized in Saudi Arabia and consistently found both enantiomers in similar quantities. Although also in this study a slight excess of (S)-amphetamine up to 9.7% was detected, an adequate interpretation remains impossible due to a lack of precision data.

Data on chiral composition of illegal MDMA are also scarce. However, all available studies consistently found racemic mixtures of MDMA in seized 'ecstasy' tablets,[22–24] whereas corresponding studies on the recently appearing crystalline MDMA are lacking. Recent wastewater analyses in major European cities also indicate the use of racemic amphetamine and MDMA[25] and are therefore also in line with the results of the presented study.

A few of the examined MDMA samples contained very low quantities of MDA. Whereas a few decades ago MDA was sometimes found as the main ingredient in tablets sold as "ecstasy," today ecstasy tablets usually contain MDMA,[26] while the active ingredient content is steadily increasing.[1,4] The occurrence of mixtures of MDMA/MDA[24,27,28] and MDMA/amphetamine[28–30] in tablets sold as "ecstasy" is also documented in literature. MDA can also be found as synthesis-related impurity of MDMA synthesized via reductive amination.[31] Therefore, the detection of both MDA enantiomers was to be expected. Evidence of amphetamine powder samples adulterated with MDMA can, however, not be found in literature.

The vast majority of methamphetamine samples only contained the more potent (S)-enantiomer. Contrarily, one powder sample as well as seven crystalline samples exclusively contained the less potent distomer. Moreover, there were some crystalline samples containing the racemate and non-racemic mixtures of the enantiomers.

Data on the enantiomeric composition of methamphetamine crystals are available mainly from Asia. The majority of the seized crystals there also contained pure (S)-methamphetamine, although different fractions of pure (R)-methamphetamine and racemic and non-racemic mixtures were also found.[32–38] Two of these studies observed a decreasing trend in seizures of the more potent (S)-enantiomer.[32,33] The latter was also observed in an American study from 2005 to 2012, where, in addition to pure (S)-methamphetamine, mainly non-racemic mixtures were found.[39] An investigation of methamphetamine tablets, so-called Thai pills, seized in Switzerland found pure and almost pure (S)-methamphetamine as well as occasional racemic mixtures and one pill mainly containing (R)-methamphetamine.[40]

Studies from Germany are not available for either of the substances investigated here; however, conclusions can be drawn from the production methods used. The presence of solely racemic amphetamine and MDMA samples is consistent with a fully synthetic production. Amphetamine and MDMA on the German market originate mainly from the Netherlands, where it is synthesized from

various non-pharmaceutical precursors.[1,17,41,42] All common routes of synthesis lead to a racemic product.[10,12,16,43,44] For many years, the majority of European methamphetamine originated from the Czech Republic, where it is synthesized from the pharmaceutical precursor (1S,2S)-pseudoephedrine and, more rarely, (1R,2S)-ephedrine.[4,7,16,17,42] Reduction of these chiral compounds yields enantiopure crystalline (S)-methamphetamine.[7,16,17,45] However, due to international restrictions on precursor chemicals, clandestine laboratories are forced to switch to alternative pre-precursors and different (non-stereoselective) pathways of synthesis.[1,16,17] Recently, most methamphetamine seized in Germany originates from the Netherlands.[46] There, it is produced mainly from phenylacetone (P2P, BMK), which, analogous to amphetamine, leads to a racemic product.[7,17,41] Lately, it has been reported that the enantiomers of methamphetamine are separated by chiral resolution using tartaric acid, for example, via the so-called "Pope–Peachey" method.[16,17,47,48] This results in products with different contents of the desired (S)-enantiomer and the (R)-enantiomer, which is considered as less potent by-product.[17,48] However, it is possible to racemize the fraction with the undesired distomer and to subject it to chiral resolution again and again, to virtually yield (S)-methamphetamine only. A mild method for this purpose is the thiyl radical-mediated racemization, for example, with methyl thioglycolate.[49] Although chiral resolution with tartaric acid is less common in Europe,[17] Mexico reports the seizure of several tons of tartaric acid per year, which is linked to methamphetamine production.[16,47] Large quantities of tartaric acid have already been seized in the Netherlands,[16] where Mexican nationals have been involved in the supply and manufacture of methamphetamine.[17,41] The predominance of (S)-methamphetamine observed in this study suggests either stereoselective production from pharmaceutical precursors or extensive use of chiral resolution subsequent to racemic synthesis. Nevertheless, some samples were found that indicate racemic synthesis or incomplete chiral resolution. The pure (R)-methamphetamine found most likely represents the low-quality waste product of chiral resolution. Although chiral resolution would also be possible for amphetamine and MDMA in order to obtain the more potent (S)-enantiomer, this could not be proven in this study either and is also not documented in the literature. One reason could be that although (S)-methamphetamine is suggested to be up to 10 times more potent than the (R)-enantiomer,[6] the efficacy of the eutomers of amphetamine and MDMA is usually estimated to be not more than three times higher than that of the distomers.[5,8] Considering a study by Mendelson et al.,[7] which suggests similar abuse potentials for racemic methamphetamine and for the pure (S)-enantiomer, even in this case, the benefit of a chiral resolution remains questionable. However, quantification of the enantiomers' stimulant activities seems rather unreliable given the complex pharmacodynamic effects of these drugs.

Almost half of the tested amphetamine samples contained traces of methamphetamine. In contrast to the majority of methamphetamine samples, the impurities were almost exclusively racemic, so contamination at the consumer level appears unlikely. An intentional mixing of the two stimulants also does not seem plausible due to the low degree of adulteration. Methamphetamine is also not a by-product of amphetamine synthesis. Contamination in laboratories that manufacture both amphetamine and methamphetamine fully synthetically, on the other hand, would be conceivable.

Because the thresholds for the non-small amount of (S)- and (R)-methamphetamine in the German BtMG differ significantly (5 vs. 50 g) and all kinds of enantiomeric compositions can be found in seized samples, from a legal perspective, each seized sample should be analyzed stereoselectively. For the analysis of powder samples, X-ray powder diffraction (XRPD) offers a time- and cost-saving alternative to chiral chromatography, as it allows racemates to be distinguished from enantiopure samples.[50] A legal distinction by enantiomeric composition, as found in German law, is rather uncommon. For example, Swiss law generally does not distinguish between enantiomers.[40] The corresponding Austrian law (*Suchtgift-Grenzmengenverordnung*, Narcotic Drugs Limit Ordinance) does differentiate between the enantiomers of amphetamine, however, with an identical threshold of 10 mg each.[51] In contrast to methamphetamine, on the basis of this study, it does not appear necessary to examine each seized sample of amphetamine and MDMA enantioselectively. Subject to random control, illicit amphetamine and MDMA can thus be considered racemic. Illegally synthesized amphetamine therefore differs essentially from amphetamine in finished medicinal products approved in Germany, which either contain (S)-amphetamine (Attentin®) or its prodrug lisdexamfetamine (Elvanse®). This allows a forensic distinction of therapeutic use and drug abuse by analyzing blood samples.[43] However, in rare cases, preparations of racemic amphetamine sulfate (syrup or capsules) are made directly in the drug store according to the German *Neues Rezeptur-Formularium* (NRF). In those cases, a distinction from illicit use cannot be made.

5 | CONCLUSION

In the present study, the enantiomeric composition of seized samples of amphetamine, methamphetamine, and MDMA was determined. Whereas all amphetamine and MDMA samples were racemic, methamphetamine samples exhibited diverse compositions, with the pure (S)-enantiomer found most frequently. The production and supply chain of methamphetamine in Europe is currently undergoing changes, so less potent enantiomeric mixtures or even the pure (R)-form are also becoming available. Therefore, seized methamphetamine samples should be routinely analyzed for their enantiomeric composition. The composition of amphetamine and MDMA may also change in this respect, so regular monitoring should be carried out.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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