Drug Testing in Blood: Validated Negative-Ion Chemical Ionization Gas Chromatographic-Mass Spectrometric Assay for Enantioselective Determination of the Designer Drugs MDA, MDMA (Ecstasy) and MDEA and Its Application to Samples From a Controlled Study with MDMA

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Drug Testing in Blood: Validated Negative-Ion Chemical Ionization Gas Chromatographic–Mass Spectrometric Assay for Enantioselective Measurement of the Designer Drugs MDEA, MDMA, and MDA and Its Application to Samples from a Controlled Study with MDMA

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Background: **The enantiomers of the designer drugs 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) differ in their pharmacologic and toxicologic potency. The aim of this study was to develop an assay for measuring these enantiomers in small plasma volumes and to analyze samples from a controlled study with MDMA.**

Methods: **The analytes were extracted from ≤0.2 mL of plasma by mixed-mode solid-phase extraction. After derivatization with *S*-(−)-heptafluorobutyrylprolyl chloride, the resulting diastereomers were separated by gas chromatography (HP-5MS) within 17 min and detected by mass spectrometry in the negative-ion chemical ionization mode. The method was fully validated and applied to samples from a controlled study in which a single dose of racemic MDMA (75 mg) was administered.**

Results: **The derivatized enantiomers were well separated and detected with good sensitivity. The assay was linear (per enantiomer) at 1–50 μg/L for MDA and 5–250 μg/L for MDMA and MDEA. Analytical recovery, accuracy, repeatability, and intermediate precision data were within required limits. Extraction yields were 82.1%–95.3%. In the study samples, concentrations of *R*-(−)-MDMA significantly exceeded those of *S*-(+)-MDMA. Their ratios (*R* vs *S*) were always >1.0 and increased over time. Concentrations of *S*-(+)-MDA exceeded those of *R*-(−)-MDA, their ratios (*R* vs *S*) also increasing over time but remaining <1.0.**

Conclusions: **This assay enables sensitive, reliable, and fast enantioselective measurement of MDA, MDMA, and MDEA in small volumes of plasma. The controlled study data confirm previous findings of MDMA and MDA enantiomer ratios (*R* vs *S*) increasing over time after ingestion of racemic MDMA.**

The designer drugs 3,4-methylenedioxyamphetamine (MDA),[4] 3,4-methylenedioxymethamphetamine (MDMA; ecstasy; adam), and 3,4-methylenedioxyethylamphetamine (MDEA; eve) are recreational drugs that are par-

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[4] Nonstandard abbreviations: MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; NICI, negative-ion chemical ionization; GC-MS, gas chromatography–mass spectrometry; *S*-HFBPCl, *S*-(−)-*N*-heptafluorobutyrylprolyl chloride; LOD, limit of detection; IS, internal standard(s); SPE, solid-phase extraction; AT, Agilent Technologies; QC, quality control; ACR, above calibration range sample; LQS, limit of quantification sample; AM, amphetamine; MA, methamphetamine; CI, confidence interval; LOQ, limit of quantification; and HFBP, heptafluorobutyrylprolyl.

ticularly popular among young people in the so-called "rave scene". The desired effects of these drugs include increased alertness and endurance as well as a sense of euphoria, closeness to other people, and greater sociability *(1–4)*. For these reasons they are also called entactogens *(5)*. However, they also exhibit many undesired effects, and their abuse is not without risk. Acute side effects include increased muscle tension, hyperpyrexia, nausea, blurred vision, and ataxia *(1–4)*. Many severe or even fatal intoxications have been described *(1, 2, 4)*. Concerning chronic toxicity, data from animal experiments strongly suggest that these compounds can cause irreversible damage to serotoninergic nerve terminals in the central nervous system *(1–4, 6)*. Decreased concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid *(7)* and serotonin transporters *(8)* found in recreational MDMA users compared with control individuals with no history of MDMA exposure point in the same direction. However, because the history of drug abuse of the studied MDMA users relied on self-reports, it cannot be excluded that these findings were, in part, attributable to concomitant abuse of other drugs of abuse.

In recent years, the above-mentioned drugs have also become increasingly important in the context of driving under the influence of drugs *(9–11)*. In 35% of cases in Belgium of driving under the influence of drugs for the period 2000–2002, MDMA was detected in blood plasma in a concentration exceeding the legal limit of 50 μg/L (Nele Samyn, personal communication). It is obvious that undesired effects such as altered sensory perception, attention, and risk-taking in decision-making behavior, as well as psychomotor effects can impair the ability to safely drive a car. A detailed account on this issue, including a review of the literature, was published by Logan and Couper in 2001 *(12)*. At that time, it was impossible to reliably estimate the possible relationship between plasma concentrations of the designer drugs and driving impairment. In the majority of cases, plasma concentrations were either not available or not interpretable because of additional involvement of other drugs or missing data on driving performance of the respective individuals. Meanwhile, a controlled study has been conducted to evaluate impairment of driving-related attention and psychomotor skills after a single recreational dose of MDMA, the most widely abused designer drug. The achiral analytical data from 4 biological matrices *(11)* as well as the results on cognitive and psychomotor performance tests have been published *(13)*. The study also investigated the influence of MDMA on several physiologic and mood indices *(14)*. However, to date, the chiral character of MDMA has not been taken into account, although the pharmacologic, toxicologic, and toxicokinetic properties of its enantiomers are known to differ considerably *(2, 4, 15–17)*. Whereas *S*-(+)-MDMA has been described as the more potent stimulant, *R*-(−)-MDMA seems to exhibit more mescaline-like effects *(2)*. Furthermore, elimination of the *S*-(+) enantiomer is faster than elimination of the *R*-(−) enantiomer *(2, 4, 15–18)*.

The aim of the present study was to develop and validate an assay for measurement of MDA, MDMA, and MDEA enantiomers in small volumes of blood plasma. This assay was used to investigate enantiomer concentrations and ratios (*R* vs *S*) of MDMA and its metabolite MDA in the samples from the above-mentioned controlled study and to compare them with previously published data after lower *(16)* or higher *(18)* doses of MDMA. Such data should also be a useful basis for further studies on correlations between enantiomer concentrations of MDMA in plasma and changes in physiologic variables as well as psychomotor performance.

Because only minimal specimen volumes remained from the controlled study, a sensitive assay for the measurement of these enantiomers from small sample volumes was necessary. To allow further application of the present assay to enantioselective analysis of clinical and forensic samples, MDEA was also included in the method development and validation. None of the previously published assays for enantioselective analysis of MDA, MDMA, and MDEA in human plasma samples could be used because they required 0.5 mL *(18)* or 1.0 mL of plasma *(16, 17, 19, 20)*. Therefore, this report describes a highly sensitive negative-ion chemical ionization (NICI) gas chromatographic–mass spectrometric (GC-MS) assay, its validation, and its application to analysis of the plasma samples from the above-mentioned controlled study.

Materials and Methods

MATERIALS

Chemicals and reagents. Methanolic solutions (100 mg/L) of racemic MDA-d_5 and MDMA-d_5 were obtained from Promochem. Hydrochlorides of racemic MDA, MDMA, and MDEA were obtained from Lipomed. Aqueous solutions (10 mg/L) of *S*-(+)-MDA, *S*-(+)-MDMA, and *S*-(+)-MDEA were kindly provided by Prof. Dr. Kovar (Tuebingen, Germany). Isolute Confirm HCX cartridges (130 mg; 3 mL) were obtained from Separtis. Sodium hydrogen carbonate was obtained from Fluka. All other chemicals were obtained from E. Merck. All chemicals were of analytical grade or the highest purity available. The derivatization reagent *S*-(−)-*N*-heptafluorobutyrylprolyl chloride (*S*-HFBPCl) was synthesized in our laboratory according to a previously described method *(21)*.

Biosamples. Pooled human blank plasma samples were used for validation of the procedure and were obtained from a local blood bank. The samples were tested for absence of MDA, MDMA, and MDEA before use by a GC-MS procedure with a limit of detection (LOD) <1 μg/L *(22)*. A certified reference sample (Medidrug® BTMF 2/99-B S-plus) was obtained from Medichem. The study blood samples were taken from 12 study participants 1, 2, 3, 4, and 5 h after administration of 75 mg of racemic MDMA to each. For details, see Ref. *(11)*. Unfor-

tunately, plasma samples were not available from all volunteers at each sampling time; therefore, the numbers of samples (respective sampling times) were as follows: n = 11 (1 h), n = 12 (2 h), n = 11 (3 h), n = 9 (4 h), and n = 9 (5 h).

SAMPLE PREPARATION

The volume of plasma samples used for analysis was 0.2 mL. If less than this volume of plasma was available, 0.1 mL was used. After dilution with 2 mL of purified water and addition of 0.1 mL of a methanolic working solution of the racemic internal standards (IS) MDA-d_5 and MDMA-d_5 (0.04 and 0.2 mg/L, respectively), the samples were mixed (15 s) on a rotary shaker and loaded on solid-phase extraction (SPE) cartridges previously conditioned with 1 mL of methanol and 1 mL of purified water. After extraction, the cartridges were washed with 1 mL of purified water, 1 mL of 0.01 mol/L hydrochloric acid, and 2 mL of methanol. Reduced pressure was applied until the cartridges were dry, and the analytes were eluted with 1 mL of methanol–aqueous ammonia (98:2 by volume) into 1.5-mL reaction vials. The eluates were evaporated to dryness under a stream of nitrogen at 56 °C. After addition of 0.2 mL of aqueous carbonate buffer (35 g/L sodium bicarbonate–15 g/L sodium carbonate, pH 9) and 6 μL of derivatization reagent (0.1 mol/L *S*-HFBPCl in dichloromethane), the reaction vials were sealed and left on a rotary shaker at room temperature for 30 min. Cyclohexane (0.1 mL) was then added, and the reaction vials were sealed again and left on a rotary shaker for 1 min. The phases were separated by centrifugation (10 000 *g* for 1 min), and the cyclohexane phase (upper) was transferred to autosampler vials. Aliquots (3 μL) were injected into the GC-MS system.

ENANTIOSELECTIVE GC-NICI-MS QUANTIFICATION

The samples were analyzed in an Agilent Technologies (AT) 6890 Series GC system combined with an AT 5973 network mass selective detector, an AT 7683 series injector, and an AT enhanced Chem Station G1701CA, Ver. C.00.00 21-Dec-1999. The GC conditions were as follows: splitless injection mode; column, 5% phenyl methyl siloxane [HP-5MS; 30 m × 0.25 mm (i.d.); 250 nm film thickness]; injection port temperature, 280 °C; carrier gas, helium; flow rate, 1 mL/min; column temperature, 100 °C increased to 200 °C at 30 °C/min, to 260 °C at 5 °C/min, and to 310 °C at 30 °C/min. The NICI-MS conditions were as follows: transfer line heater, 280 °C; NICI, methane (2 mL/min); source temperature, 150 °C; solvent delay, 11 min; selected-ion monitoring mode. The time windows and monitored ions as given in Table 1.

The enantiomers of MDA, MDMA, and MDEA were quantified by comparison of their peak-area ratios (enantiomer of analyte vs corresponding enantiomer of the IS; MDMA-d_5 was also used as IS for MDEA) to calibration curves in which the peak-area ratios of enriched calibrators had been plotted vs their concentrations by a weighted ($1/x^2$) least-squares linear regression model.

ASSAY VALIDATION

Preparation of solutions. Separate aqueous stock solutions of racemic hydrochlorides of MDA (20 mg/L, free base), MDMA (100 mg/L, free base), and MDEA (100 mg/L, free base) were prepared. Methanolic working solutions of the racemic IS MDA-d_5 and MDMA-d_5 (0.04 and 0.2 mg/L, respectively) were prepared from the commercially available methanolic solutions. Aqueous working solutions containing racemic MDA (0.004, 0.04, 0.08, 0.12, 0.16, and 0.2 mg/L) as well as racemic MDMA and racemic MDEA (0.02, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L each) were prepared. Aqueous enrichment solutions containing racemic MDA (0.2, 0.4, 4.0, and 8.0 mg/L), racemic MDMA, and racemic MDEA (1.0, 2.0, 20.0, and 40.0 mg/L each) were prepared for racemic quality-control (QC) samples. Methanolic enrichment solutions containing racemic MDA (0.016, and 0.32 mg/L), racemic MDMA, and racemic MDEA (0.08 and 1.6 mg/L each) were prepared for the extraction efficiency experiments. All solutions were stored at 4 °C.

Preparation of QC samples. For preparation of QC samples, 0.1-mL aliquots [0.5 mL for the above-calibration range

Table 1. Time windows and selected ions (with relative intensities) used in the selected-ion monitoring method.

		Target ion		Qualifier ion 1		Qualifier ion 2	
Time window	**Analyte**	***m/z***	**Relative intensity,[a] %**	***m/z***	**Relative intensity, %**	***m/z***	**Relative intensity, %**
11–13 min	*R*-(−)-MDA-d_5	437	100	457	23	477	72
	R-(−)-MDA	432	100	452	21	472	61
	S-(+)-MDA-d_5	437	100	457	9	477	78
	S-(+)-MDA	432	100	452	9	472	68
13–15 min	*R*-(−)-MDMA-d_5	451	100	431	45	491	28
	R-(−)-MDMA	446	100	426	44	486	22
	S-(+)-MDMA-d_5	451	100	431	43	491	27
	S-(+)-MDMA	446	100	426	48	486	25
	R-(−)-MDEA	460	100	480	84	500	28
	S-(+)-MDEA	460	100	480	92	500	24

[a] Relative intensity$_{Ion}$ = (intensity$_{Ion}$/intensity$_{Target\ ion}$) × 100%.

(ACR) sample] of the corresponding enrichment solutions were transferred to volumetric flasks. Blank plasma was added stepwise to reach a final volume of 10.0 mL. Before each addition step and after the final volume had been reached, the samples were thoroughly vortex-mixed to obtain homogeneous samples. Thus, the following QC samples containing racemic MDA, MDMA, and MDEA were prepared [enantiomer concentrations (μg/L) of each analyte, respectively, are given in parentheses]: limit of quantification sample (LQS, 1/5/5), LOW (2/10/10), MED (20/100/100), HIGH (40/200/200), and ACR (160/800/800). The QC samples were divided into aliquots (0.45 mL) and stored at −20 °C.

Selectivity. Blank plasma samples from 10 different sources were prepared as described above to check for peaks that might interfere with the detection of the analytes or the IS. A zero sample (blank sample + IS) was analyzed to check for absence of analyte ions in the respective peaks of the IS. Blank plasma samples enriched with other sympathomimetic amines [ephedrine, pseudoephedrine, norephedrine (phenylpropanolamine), norpseudoephedrine, phentermine, gepefrine, and pholedrine (1000 μg/L each)] and authentic plasma samples containing other drugs of abuse [amphetamine (AM), methamphetamine (MA), cannabis, and cocaine] were also checked for interfering peaks. In addition, LQS samples (n = 5) enriched with the above-mentioned sympathomimetic amines (1000 μg/L each) were analyzed to determine whether accurate and precise quantification would be possible even in the presence of possible interferents.

Linearity. Aliquots of blank plasma (0.2 mL) were enriched with 0.1 mL of the corresponding working solutions to obtain calibration samples containing 1, 10, 20, 30, 40, or 50 μg/L of each enantiomer of MDA as well as 5, 50, 100, 150, 200, or 250 μg/L of each enantiomer of MDMA and MDEA. Replicates (n = 6) at each concentration were analyzed as described above. The regression line was calculated by use of a weighted ($1/x^2$) least squares linear regression model. Daily calibration curves using the same concentrations (single measurements per concentration) were prepared with each batch of validation and authentic samples. The back-calculated concentrations of all calibration samples were required to fall within an acceptance interval of 100% ± 15% of the respective nominal concentrations according to Shah et al. *(23)*.

Analytical recovery, accuracy, and precision. QC samples (LQS, LOW, MED, and HIGH) were analyzed as described above in duplicate on each of 8 days. The ACR QC samples were analyzed in the same way, but only 0.05 mL of sample was used instead of 0.2 mL. The concentrations of MDA, MDMA, and MDEA enantiomers in the QC samples were calculated from the daily calibration curves. Analytical recovery was calculated for each enantiomer as the percentage of the mean calculated concentration from the theoretical concentration. Repeatability (within-day precision) and intermediate precision (combined within- and between-day effects) were calculated according to Massart et al. *(24)* and Clinical and Laboratory Standards Institute (formerly NCCLS) document EP5-A *(25)* by one-way ANOVA with the grouping variable "day". For evaluation of accuracy, the certified external reference sample (Medidrug® BTMF 2/99-B S-plus) was analyzed, and the calculated concentrations of MDA, MDMA, and MDEA were compared with the certified confidence range.

Stability. For estimation of stability of processed samples under the conditions of GC-MS analysis, LOW and HIGH QC samples (n = 10 each) were extracted and derivatized as described above. The extracts obtained at each concentration were pooled. Aliquots (n = 10) of these pooled extracts at each concentration were transferred to autosampler vials and injected under the conditions of a regular analytical run at time intervals of 2 h and 40 min. Stability of the derivatives was tested by regression analysis plotting absolute peak areas corresponding to each enantiomer of MDA, MDMA, and MDEA at each concentration vs injection time. Instability of processed samples would be indicated by a negative slope significantly different from zero ($P \leq 0.05$).

For evaluation of freeze–thaw stability, QC samples (LOW and HIGH) were analyzed before (control samples, n = 6) and after 3 freeze–thaw cycles (stability samples, n = 6). For each freeze–thaw cycle, the samples were frozen at −20 °C for 21 h, thawed, and kept at ambient temperature for 3 h. The concentrations of the control and stability samples were calculated from daily calibration curves. For the ratio of the stability sample means vs the corresponding control sample means, an acceptance interval of 90%–110% was applied. In addition, an acceptance interval of 80%–120% of the control sample means was applied for the 90% confidence interval (CI) of the stability samples.

The experimental design and procedure for evaluation of long-term stability were similar to those used for freeze–thaw stability. QC samples (LOW and HIGH) were analyzed before (control samples, n = 6) and after storage at −20 °C for 6 months (stability samples, n = 6).

Limits. The lowest points of the calibration curves were 1 μg/L for both enantiomers of MDA and 5 μg/L for each enantiomer of MDMA and MDEA. An independent QC sample at these concentrations (LQS) was included in the analytical recovery and precision experiments (see the section on analytical recovery, accuracy, and precision). The results obtained for this sample were compared with the acceptance criteria established for the limit of quantification [LOQ; analytical recovery within 100% ± 20% of nominal value; CV <20% *(23)*]. The LOD was not systematically evaluated.

Extraction efficiency. Extraction samples (n = 5) at low (2 μg/L for each enantiomer of MDA and 10 μg/L for each enantiomer of MDMA and MDEA) and high (40 μg/L for each enantiomer of MDA and 200 μg/L for each enantiomer of MDMA and MDEA) concentrations were prepared by enriching blank plasma (0.2 mL; previously diluted with 2 mL of purified water) with 0.05 mL of the methanolic enrichment solutions of racemic MDA, MDMA, and MDEA. Samples were loaded on SPE columns and extracted. Before evaporation, 0.1 mL of the IS solution was added to each eluate. For the control samples (n = 5), 0.2 mL of blank plasma was diluted with 2 mL of purified water, loaded on SPE columns, and extracted. Before evaporation, 0.05 mL of the methanolic enrichment solutions of MDA, MDMA, and MDEA and 0.1 mL of IS solution were added to the eluates. After evaporation, the residues were derivatized and analyzed as described above. Extraction efficiencies (mean and 95% CI) were estimated by comparison of the peak area ratios (analytes vs IS) from extraction samples and control samples for each enantiomer of MDA, MDMA, and MDEA at each concentration.

APPLICATION

The samples from the controlled study were assayed as described above. The plasma concentrations of the *R*-(−) and *S*-(+) enantiomers of MDMA in individual samples were compared visually and by 2-tailed paired *t*-tests at each sampling time. In addition, the mean enantiomer ratios (*R* vs *S*) for MDMA at each sampling time were compared with the theoretic ratio of 1.0 for racemic mixtures by 1-tailed *t*-tests. The same statistics were calculated for the concentrations and ratios of MDA enantiomers.

Results

SAMPLE PREPARATION AND GC-NICI-MS QUANTIFICATION

The mixed-mode (reversed phase C_8 and strong cation exchange) SPE procedure used to isolate MDA, MDMA, and MDEA from plasma samples led to very clean extracts. The extracted analyte enantiomers readily reacted with the derivatization reagent *S*-HFBPCl under the applied aqueous alkaline conditions, yielding the corresponding *R,S* and *S,S* diastereomers. Extraction of these derivatives from the aqueous phase with 0.1 mL of cyclohexane provided a further cleanup of the final extract because only rather lipophilic compounds were extracted into the organic phase.

The diastereomeric derivatives of MDA, MDMA, and MDEA could be separated on an HP-5MS GC column. The elution order was determined by analysis of aqueous solutions of the respective *S*-(+) enantiomers. For all 3 analytes, the derivatives corresponding to the *S*-(+) enantiomers were found to elute after those of the corresponding *R*-(−) enantiomers. In the present study, the initial temperature and the oven ramp temperature program were optimized to achieve baseline separation of the enantiomers of all 3 analytes within a 17-min program. The peak corresponding to *S*-(+)-MDMA could not be fully separated from the one corresponding to *R*-(−)-MDEA. Nevertheless, these 2 analytes could be differentiated because of their different MS properties.

The heptafluorobutyrylprolyl (HFBP) derivatives of MDA, MDMA, and MDEA enantiomers were readily ionized in the NICI mode, and the observed noise was low, allowing sensitive detection of the MDA, MDMA, and MDEA enantiomers, even in a small sample volume (≤0.2 mL). Shown in Fig. 1 are merged mass fragmentograms of a zero sample (Fig. 1A) and an enriched calibration sample containing 10 μg/L of each enantiomer of MDA and 50 μg/L of each enantiomer of MDMA and MDEA (Fig. 1B) after SPE and derivatization with *S*-HFBPCl.

ASSAY VALIDATION

Selectivity. None of the analyzed blank and zero samples contained any interfering peaks. As already mentioned, typical mass fragmentograms of a zero sample after SPE and derivatization are shown in Fig. 1A. The peaks corresponding to the analytes and IS were clearly separated from the few matrix peaks present in the fragmentograms. No interference was detected in any of the enriched samples containing other sympathomimetic amines or in any of the authentic samples tested that contained the other drugs of abuse (AM, MA, cannabis, and/or cocaine). The analytical recoveries for the LQS samples enriched with other sympathomimetic amines were 96.3%–113.6%, and the CVs were 3.3%–9.7%.

Calibration model, analytical recovery, accuracy, and precision. The slopes and *y*-intercepts (including 95% CIs of both variables) and coefficients of determination for each enantiomer of MDA, MDMA, and MDEA as obtained in the linearity experiments are listed in Table 2.

The results of the analytical recovery and precision experiments are given in Table 3. All analytical recovery values fulfilled the acceptance criteria for this parameter *(23)*, lying within 100% ± 15% (100% ± 20% at the LOQ) of the nominal concentrations. Accuracy was evaluated by analysis of a certified reference sample (Medidrug BTMF 2/99-B S-plus). The calculated concentrations, with certified target values (confidence ranges) given in brackets, were 80.4 μg/L [80.7 μg/L (61.8–99.6 μg/L)] for total MDA, 84.6 μg/L [83.6 μg/L (64.1–103.1 μg/L)] for total MDMA, and 57.1 μg/L [61.7 μg/L (46.6–76.8 μg/L)] for total MDEA. All 3 measured values lay well within the certified confidence ranges, demonstrating the accuracy of the described procedure. In addition, all 3 compounds were found to be present in the sample as racemic mixtures. Repeatability and intermediate precision were calculated according to Massart et al. *(24)* and Clinical and Laboratory Standards Institute (formerly NCCLS) document EP5-A *(25)*. With the exception of the intermediate precision estimate for *S*-(+)-MDA in the LQS sam-

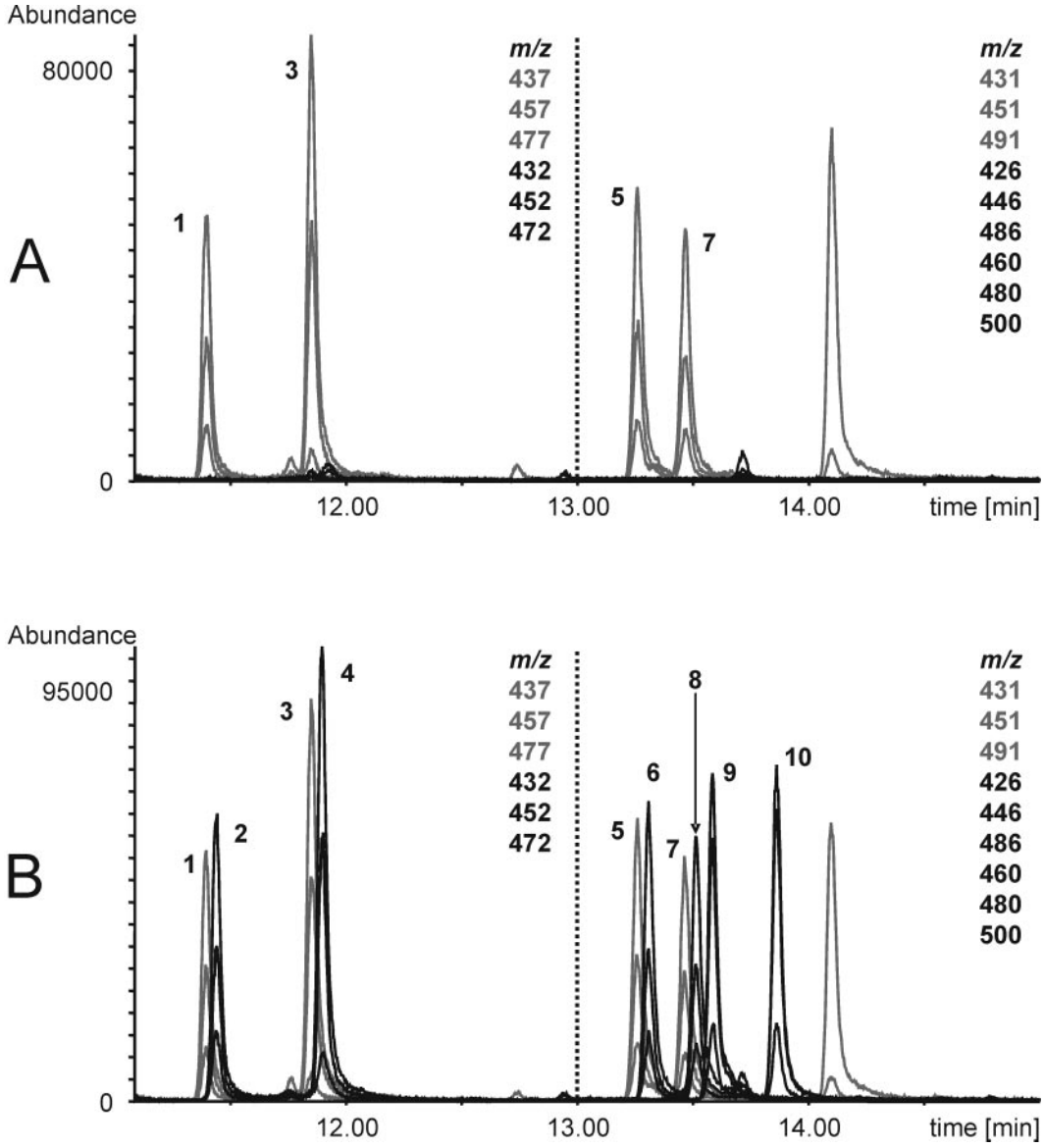

Fig. 1. Merged mass fragmentograms of plasma samples after SPE and derivatization with *S*-HFBPCl.

The *dotted lines* separate two time windows; the *m/z* values corresponding to the monitored ions are given for each time window. (*A*), zero sample (blank sample + IS). (*B*), blank sample enriched with 10 μg/L each of *R*-(−)-MDA (*peak 2*) and *S*-(+)-MDA (*peak 4*) as well as 50 μg/L each of *R*-(−)-MDMA (*peak 6*), *S*-(+)-MDMA (*peak 8*), *R*-(−)-MDEA (*peak 9*), and *S*-(+)-MDEA (*peak 10*). *Peaks* 1 and *3* correspond to 10 μg/L *R*-(−)-MDA-d_5 and *S*-(+)-MDA-d_5, respectively. *Peaks 5* and *7* correspond to 50 μg/L *R*-(−)-MDMA-d_5 and *S*-(+)-MDMA-d_5, respectively.

Table 2. Intercepts, slopes (with corresponding 95% CI), and coefficients of determination of weighted ($1/x^2$) linear calibration models for MDA, MDMA, and MDEA enantiomers at calibration ranges of 1–50 μg/L (MDA) and 5–250 μg/L (MDMA and MDEA).

	y-Intercept		Slope, L/μg		Coefficient of determination (R^2)
Analyte	Best-fit value	95% CI	Best-fit value	95% CI	
R-(−)-MDA	−0.004	−0.010 to 0.002	0.105	0.103–0.110	0.999
S-(+)-MDA	0.029	0.024–0.034	0.108	0.106–0.110	0.999
R-(−)-MDMA	−0.012	−0.019 to −0.006	0.0197	0.0191–0.0202	0.999
S-(+)-MDMA	−0.008	−0.014 to −0.003	0.0203	0.0198–0.0207	0.999
R-(−)-MDEA	−0.012	−0.016 to −0.007	0.0206	0.0202–0.0210	0.998
S-(+)-MDEA	−0.017	−0.023 to −0.011	0.0249	0.0244–0.0254	0.998

Table 3. Analytical recovery, repeatability, and intermediate precision data for the determination of MDA, MDMA, and MDEA enantiomers in human plasma.

		Analytical recovery,[a] %						Repeatability (CV),[b] %						Intermediate precision (CV),[c] %					
		MDA		MDMA		MDEA		MDA		MDMA		MDEA		MDA		MDMA		MDEA	
QC sample	Nominal concentration, μg/L	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)	*R*-(−)	*S*-(+)
LQS	1.0	102.5	100.0					3.4	8.7					6.8	14.9				
	5.0			104.1	106.9	97.5	98.8			2.1	3.3	3.6	4.8			7.9	5.5	6.4	7.3
LOW	2.0	95.3	98.7					2.3	3.1					3.6	9.0				
	10.0			96.1	97.9	92.2	94.3			3.6	3.0	5.6	6.6			4.1	3.8	5.6	6.6
MED	20.0	98.4	99.8					1.6	1.4					2.6	3.0				
	100.0			97.3	98.0	95.7	97.7			1.4	1.5	1.7	1.6			3.2	2.5	4.1	2.8
HIGH	40.0	99.0	98.1					1.2	1.0					1.6	2.5				
	200.0			99.4	100.6	98.0	100.3			1.1	1.1	2.5	0.9			1.7	1.8	3.1	2.9
ACR	160.0	100.1	100.4					2.2	2.2					3.2	3.1				
	800.0			103.2	100.4	100.7	99.9			3.6	2.4	4.3	3.4			4.2	4.0	5.6	6.2

[a] Analytical recovery = (mean calculated concentration/nominal concentration) × 100; n = 16.
[b] *df* = 8.
[c] *df* = 8 to 15; calculated according to the Satterwaithe equation *(25)*.

ple, all CVs were <10% with the majority of CVs <5% (Table 3).

Stability, limits, and extraction efficiency. Regression analysis of absolute peak areas of MDA, MDMA, and MDEA enantiomers plotted vs injection time indicated no instability of processed samples during a time interval of 24 h. The ratios of means (stability vs control samples) as well as the 90% CI of the freeze–thaw and long-term stability samples fulfilled the acceptance criteria.

Analytical recovery values within 100% ± 20% and precision CV ≤20% have been proposed as acceptance limits for the LOQ *(23)*. The corresponding data for the LQS sample (Table 3), which contained the analytes at concentration corresponding to the lowest points of the calibration curves, easily fulfilled these criteria.

The results for extraction efficiencies, including 95% CI, are listed in Table 4. As expected, the results were similar for the corresponding enantiomers of MDA, MDMA, and MDEA. The narrow CI indicated that the extraction was highly reproducible.

APPLICATION

The described GC-MS method was applied to 52 plasma samples taken during a controlled study with 12 volunteers who had each ingested 75 mg of racemic MDMA. Samples were taken at 1-h intervals after ingestion. Typical merged mass fragmentograms of plasma samples after SPE and derivatization with *S*-HFBPCl are shown in Fig. 2. The sample in Fig. 2A was taken 1 h after administration of 75 mg of racemic MDMA. Even at this early sampling time, a considerable difference of the concentrations of *R*-(−)-MDMA (58.2 μg/L) and *S*-(+)-MDMA (49.8 μg/L) could be observed. The concentrations of the corresponding metabolites *R*-(−)-MDA and *S*-(+)-MDA were below LOQ and near the LOQ, respectively, because only small amounts of metabolites had been produced at this early stage. The sample in Fig. 2B was taken 4 h after administration. The difference in concentrations of *R*-(−)-MDMA (105.8 μg/L) and *S*-(+)-MDMA (66.9 μg/L) had increased at this later stage of the study, and more *R*-(−)-MDA (1.4 μg/L) and *S*-(+)-MDA (6.7 μg/L) had been produced.

Table 4. Extraction yields (mean and 95% CI) of the SPE procedure for extraction of MDA, MDMA, and MDEA enantiomers from human plasma at high and low concentrations.

		Extraction yield,[a] %									
		MDA				MDMA				MDEA			
		R-(−)		*S*-(+)		*R*-(−)		*S*-(+)		*R*-(−)		*S*-(+)	
QC sample	Nominal concentration, μg/L	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
LOW	2.0	91.6	88.3–95.0	91.7	87.5–95.9								
	10.0					95.3	92.9–97.6	92.7	89.1–96.3	83.5	77.5–89.4	82.1	82.1–91.6
HIGH	40.0	95.1	94.5–95.8	93.5	92.1–94.9								
	200.0					95.2	94.4–96.0	92.8	91.5–94.0	92.6	90.8–94.4	90.7	89.1–92.2

[a] n = 5 each for MDA, MDMA, and MDEA.

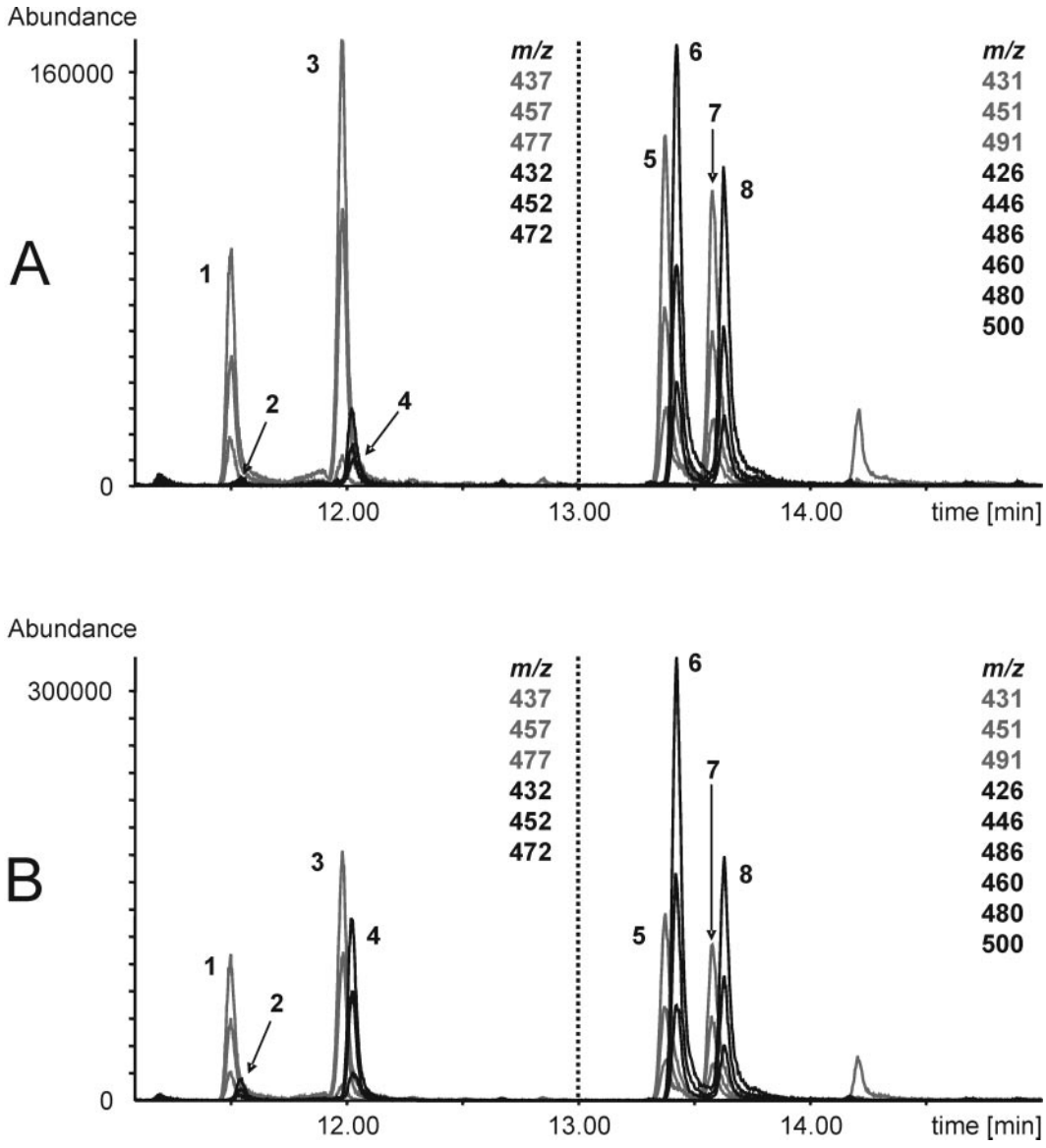

Fig. 2. Merged mass fragmentograms of plasma samples after SPE and derivatization with *S*-HFBPCl.

The *dotted lines* separate two time windows; the *m/z* values corresponding to the monitored ions are given for each time window. (*A*), plasma sample taken 1 h after administration of 75 mg of racemic MDMA. *Peaks 2*, *4*, *6*, and *8* correspond to *R*-(−)-MDA below the LOQ: 1.2 μg/L *S*-(+)-MDA, 58.2 μg/L *R*-(−)-MDMA, and 49.8 μg/L *S*-(+)-MDMA, respectively. (*B*), plasma sample taken 4 h after administration of 75 mg of racemic MDMA. *Peaks 2*, *4*, *6*, and *8* correspond to 1.4 μg/L *R*-(−)-MDA, 6.7 μg/L *S*-(+)-MDA, 105.8 μg/L *R*-(−)-MDMA, and 66.9 μg/L *S*-(+)-MDMA, respectively. In *both panels*, *peaks 1* and *3* correspond to 10 μg/L *R*-(−)-MDMA-d_5 and S-(+)-MDMA-d_5 and *peaks 5* and *7* to 50 μg/L *R*-(−)-MDMA-d_5 and S-(+)-MDMA-d_5, respectively.

In Fig. 3, the plasma concentrations of the enantiomers of MDMA (panel A) and of its metabolite MDA (panel B) as well as the enantiomer ratios (*R* vs *S*) of MDMA (panel C) and MDA (panel D) have been plotted vs sampling time. The data points for MDMA represent means (SE) of the n = 7 datasets for which samples were available at all time points. Those for MDA represent means (SE) of the n = 5 datasets for which samples were available, and MDA enantiomer concentrations were above LOQ at all time points.

Concentrations of *R*-(−)-MDMA in all samples ranged from 11.2 to 119.2 μg/L and those of *S*-(+)-MDMA from 7.8 to 93.7 μg/L. The concentrations of *R*-(−)-MDMA always exceeded those of *S*-(+)-MDMA, the differences being statistically significant at all sampling times ($P < 0.0001$). Furthermore, the enantiomer ratios (*R* vs *S*) increased steadily over time and were significantly >1.0, the theoretical value ratio for racemic mixtures, for all sampling times ($P < 0.0001$).

Concentrations of *R*-(−)-MDA in all samples ranged from below the LOQ to 1.8 μg/L and those of *S*-(+)-MDA from 1.1 to 8.0 μg/L. For this analyte, the concentrations of the *S*-(+) enantiomer always exceeded those of the *R*-(−) enantiomer, which can be explained by enantioselective N-dealkylation of MDMA to MDA. The differences were significant for sampling times 2 to 5 h

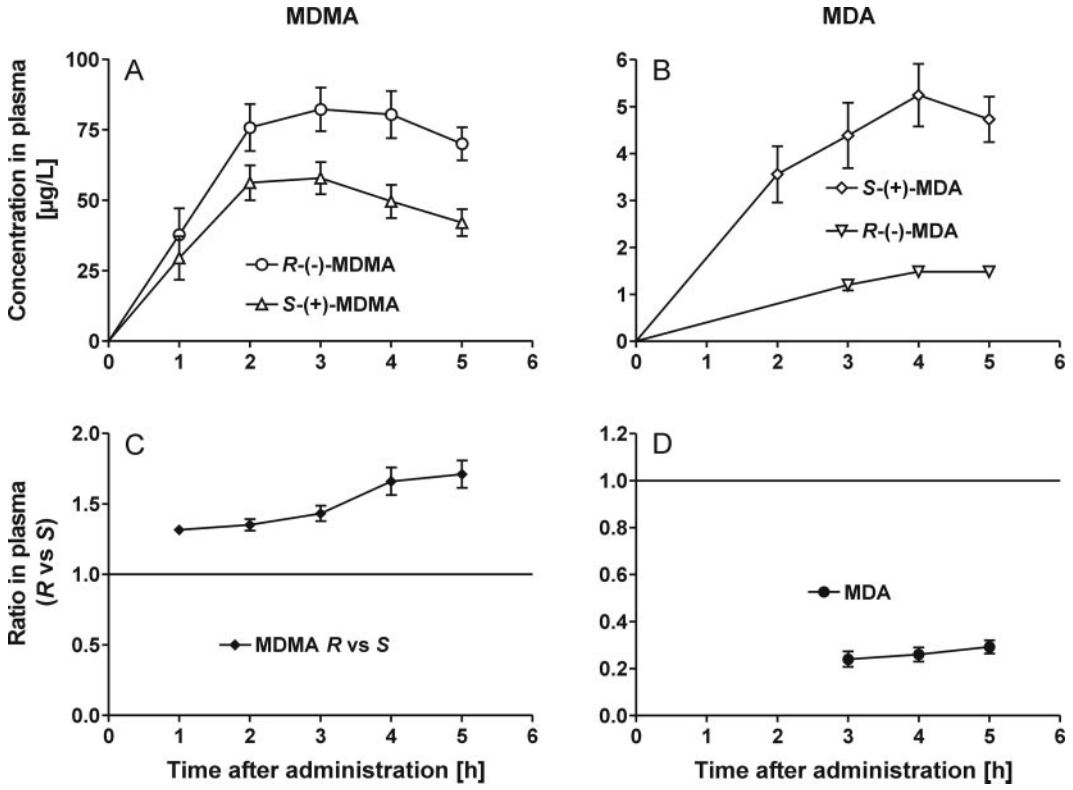

Fig. 3. Plots of enantiomer concentrations (*A* and *B*) and enantiomer ratios (*C* and *D*) of MDMA and its metabolite MDA, respectively, vs time after administration of 75 mg of racemic MDMA.

A and *C*, data points are the means (SE; *error bars*) of 7 values; *B* and *D*, *A* and *C*, data points are the means (SE; *error bars*) of 5 values.

($P \leq 0.0006$). Accordingly, the MDA enantiomer ratios (*R* vs *S*) ranged from 0.17 to 0.38. They were significantly different from 1.0 for sampling times 2 to 5 h ($P \leq 0.0004$) and also showed a steady increase over time. Statistical evaluations of MDA enantiomer differences and ratios were not possible at sampling time 1 h because all values of *R*-(−)-MDA were below the LOQ. The same was true for several samples at the other sampling points, but there were always at least 3 values above the LOQ.

Discussion

We chose the mixed-mode SPE procedure for isolating the analytes from plasma samples because it had already been shown to be useful for extraction of the same and related compounds *(21, 22)*. It was also suitable for the extraction of MDA, MDMA, and MDEA from plasma samples, which is emphasized by extraction efficiencies between 83.5% and 95.3% and by their narrow 95% CIs. Derivatization of MDA, MDMA, and MDEA before GC-MS analysis is commonly used to improve their chromatographic properties *(26)*. It was, therefore, an obvious choice to use an optically pure chiral reagent for the derivatization step in the present method, thus allowing separation of the resulting diastereomers on a standard achiral column. *S*-HFBPCl was chosen for this chiral derivatization because this reagent had been used successfully by the same and other authors for analysis of the same and structurally related analytes *(21, 27–32)*. In addition, the derivatives were readily ionized in the NICI mode because of the electronegativity of the heptafluorobutyryl moiety *(21, 29, 33)*. Derivatization with *S*-HFBPCl under aqueous alkaline conditions had already been described by Lim et al. *(27)* to have advantages over derivatization in organic solvents: little or no racemization occurred, and excess reagent is destroyed, eliminating GC-MS interference. The latter fact also improved column life. No loss of column performance was observed in the present study after hundreds of sample analyses.

The HP-5MS GC column was chosen for its exceptionally low bleed characteristics, which are advantageous for sensitive MS detection. The elution order of the corresponding *S*-(+) enantiomers on this column was the same as had been found in our previous study with AM and MA *(21)*. In the present study, baseline separation of the enantiomers of all 3 analytes was achieved within 17 min. However, the peak corresponding to *S*-(+)-MDMA could not be fully separated from the one corresponding to *R*-(−)-MDEA. This caused problems in the early stages of method development, when MDEA-d_5 was used as a third IS, because the fragments ions of MDEA-d_5, with exception of the molecular ion, had the same *m/z* values as those

of MDMA, causing interference with the quantification of the latter. Therefore, MDEA-d_5 could not be used as IS, and MDMA-d_5 had to be used as IS for MDEA.

As already mentioned, the *S*-HFBP derivatives of MDA, MDMA, and MDEA were readily ionized in the NICI mode. Operating the MS in the selected-ion monitoring mode further increased the high sensitivity of this ionization technique. In the validation samples, the abundances of MDA derivatives were comparable to those of the MDMA and MDEA derivatives, although the concentrations of MDA in these samples were 5 times lower than those of MDMA and MDEA. This indicated that the ionization properties of the MDMA and MDEA derivatives were not quite as good as those of the MDA derivatives. A similar phenomenon had already been observed for *S*-HFBP derivatives of AM and MA *(21)*. In that study, despite equal concentrations of AM and MA, comparable abundances were obtained only after the electron multiplier voltage in the time window of MA had been increased by 400 V. The different ionization properties of MDA derivatives on one hand and MDMA and MDEA derivatives on the other hand should not be considered a disadvantage of the method. On the contrary, because of this phenomenon, the linearity ranges of all analytes fell within the linear dynamic range of the apparatus without further adjustment of the electron multiplier voltage during a sample run.

The homologous fragment ions *m/z* 432, 446, and 460 were chosen as target ions for quantification of MDA, MDMA, and MDEA, respectively. The corresponding fragments *m/z* 437 and 451 were chosen as target ions for the IS MDA-d_5 and MDMA-d_5, respectively. All of these target ions were the most or second most abundant fragment ions in the respective full-scan NICI mass spectra. In addition, for each compound, the molecular ion and another fragment ion were chosen as qualifier ions (Table 1). Because of the high *m/z* values of these ions, the observed noise was low, as can be seen in Fig. 1. This low noise, combined with the excellent ionization properties in the NICI mode, was the basis for the sensitive detection of the MDA, MDMA, and MDEA enantiomers, even in the small sample volumes used in this study.

The experimental design used for the validation experiments was similar to the one proposed by Wieling et al. *(34)* with the modifications introduced by Peters et al. *(21)*. Calibration samples were prepared at 6 concentrations spread evenly from 1 to 50 μg/L for each enantiomer of MDA and from 5 to 250 μg/L for each enantiomer of MDMA and MDEA. In our experience, this range covers concentrations that are to be expected for most authentic samples *(35)*. The inverse of the squared concentration ($1/x^2$) was an appropriate weighting factor to account for unequal variances (heteroscedasticity) over the calibration range. Evaluation of weighted linear and second-order regression models indicated a slight curvature in the data for *R*-(−)-MDA and those for both enantiomers of MDMA. This indicated a better fit of the second-order model. However, Hartmann et al. *(36)* have proposed that simpler linear models be accepted if the data for precision and analytical recovery are within required limits. Because this was the case for the presented data, we decided to accept the weighted ($1/x^2$) linear regression model. The narrow CI of the regression statistics as obtained in the linearity experiments justified confinement to single measurements for daily calibration curves, which were prepared with each batch of samples. The back-calculated concentrations of all calibration samples fulfilled the criteria established by Shah et al. *(23)*.

QC samples for analytical recovery and precision experiments were prepared at 3 concentrations (LOW, MED, and HIGH) covering the calibration range, as has become the international standard *(23, 34, 36, 37)*. To account for the high concentrations expected in toxicology cases, a QC sample containing the analytes at a concentration above the highest points of the calibration curves (ACR) was also prepared. Another QC sample containing the analytes at concentrations equal to those of the lowest point of the calibration curve (LQS) was prepared to determine whether the criteria for analytical recovery and precision were fulfilled even at these concentrations, which corresponded to the practical LOQ. The QC samples were used to evaluate the analytical recovery and precision for the described assay. The experimental design used to determine these parameters was based on the proposal by Hartmann et al. *(36)*. The results easily fulfilled the acceptance criteria established by Shah et al. *(23)*. The fact that the majority of the CVs were <5% (Table 3) further shows that possible variations during sample preparation were well compensated by the use of MDA-d_5 and MDMA-d_5 as IS and that the latter was also a suitable IS for MDEA. Accuracy was determined with an external certified reference sample, and the measured values lay well within the certified confidence ranges. This, along with the favorable analytical recovery and precision data, demonstrated the reliability of the described procedure. In the stability experiments, there was no indication of instability under any of the applied conditions. This was of special importance for the described method because the samples from the controlled study had already been frozen and thawed during achiral analysis. The tested 24-h interval for processed samples was ~2 to 3 h longer than the maximum run time needed for analysis of one batch of samples in the present study. Therefore, no instability problems of processed samples are to be expected during routine analysis. These findings are in line with those obtained for AM and MA *(21)*. During the freeze–thaw experiments, the QC samples were frozen for only 21 h instead of 24 h *(36)* and were left at ambient temperature for 3 h instead of 1 h *(36)* to allow simultaneous evaluation of benchtop stability, i.e., stability of analyte in matrix at ambient temperature over the expected maximum period of time needed for preparation of a batch of samples. The observed stability of the analytes during long-term storage is in accordance with

the findings of Clauwaert et al. *(38)*. A systematic evaluation of the LOD of the described method did not seem reasonable because even small optical impurities in the derivatization reagent would make the LOD of one enantiomer dependent on the concentration of the other enantiomer. In other words, if one enantiomer is present in the sample at a rather high concentration, small impurities in the derivatization reagent would cause a signal at the retention time of the optical antipode even if it was not present in the sample.

The described assay was applied to enantioselective analysis MDMA and its metabolite MDA in samples from a controlled study, in which 75 mg of MDMA had been taken by volunteers. The respective results are in good agreement with our other findings and those of other authors *(16–18, 35)*, who also found that *R*-(−)-MDMA concentrations always exceeded those of *S*-(+)-MDMA. Fallon et al. *(16)* were also able to establish a mathematical model to estimate the time since ingestion of 40 mg of racemic MDMA based on the steady increase in enantiomer ratios over time. A similar observation has been reported by Peters et al. *(35)* in a case of severe self-poisoning with MDMA. Of course such findings are of special interest for forensic toxicologists because they might help to differentiate between recent and nonrecent ingestion of MDMA. Unfortunately, in the present study, such a model could not be confirmed or established because there were insufficient data available for nonlinear curve fitting. However, it would have been interesting to compare the results obtained with such mathematical models after administration of 40 and 75 mg of MDMA because differences could be expected because of nonlinear MDMA pharmacokinetics *(39)*.

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References

1. Hegadoren KM, Baker GB, Bourin M. 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans [Review]. Neurosci Biobehav Rev 1999;23:539–53.
2. Kalant H. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs [Review]. CMAJ 2001;165:917–28.
3. de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M, et al. Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition [Review]. Ther Drug Monit 2004;26: 137–44.
4. Steele TD, McCann UD, Ricaurte GA. 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): pharmacology and toxicology in animals and humans [Review]. Addiction 1994;89:539–51.
5. Nichols DE. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J Psychoactive Drugs 1986; 18:305–13.
6. Monks TJ, Jones DC, Bai F, Lau SS. The role of metabolism in 3,4-(+)-methylenedioxyamphetamine and 3,4-(+)-methylenedioxymethamphetamine (ecstasy) toxicity [Review]. Ther Drug Monit 2004;26:132–6.
7. McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after (+/−)3,4-methylenedioxymethamphetamine (MDMA; "Ecstasy"): a controlled study in humans. Neuropsychopharmacology 1994;10:129–38.
8. McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 1998;352:1433–7.
9. Moeller MR, Kraemer T. Drugs of abuse monitoring in blood for control of driving under the influence of drugs [Review]. Ther Drug Monit 2002;24:210–21.
10. Samyn N, van Haeren C. On-site testing of saliva and sweat with Drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users. Int J Legal Med 2000;113:150–4.
11. Samyn N, de Boeck G, Wood M, Lamers CTJ, De Waard D, Brookhuis KA, et al. Plasma, oral fluid and sweat wipe ecstasy concentrations in controlled and real life conditions. Forensic Sci Int 2002;128:90–7.
12. Logan BK, Couper FJ. 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) and driving impairment. J Forensic Sci 2001;46: 1426–33.
13. Lamers CT, Ramaekers JG, Muntjewerff ND, Sikkema KL, Samyn N, Read NL, et al. Dissociable effects of a single dose of ecstasy (MDMA) on psychomotor skills and attentional performance. J Psychopharmacol 2003;17:379–87.
14. Riedel WJ, Lamers CTJ. A pilot study of impairment testing of MDMA with a computer-based task. Project deliverable DR2b, certified EU research project (contract no RO-98-RS. 3054). Leeds, United Kingdom: School of Psychology, University of Leeds, 2000.
15. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, of amphetamine, methamphetamine and their N-alkyl derivatives [Review]. Ther Drug Monit 2002;24:277–89.
16. Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, Hutt AJ. Stereospecific analysis and enantiomeric disposition of 3,4-methylenedioxymethamphetamine (Ecstasy) in humans. Clin Chem 1999;45:1058–69.
17. Pizarro N, Llebaria A, Cano S, Joglar J, Farre M, Segura J, de la Torre R. Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 2003;17: 330–6.
18. Pizarro N, Farre M, Pujadas M, Peiro AM, Roset PN, Joglar J, de la Torre R. Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). Drug Metab Dispos 2004;32:1001–7.
19. Brunnenberg M, Kovar KA. Stereospecific analysis of ecstasy-like *N*-ethyl-3,4-methylenedioxyamphetamine and its metabolites in humans. J Chromatogr B Biomed Sci Appl 2001;751:9–18.
20. Buechler J, Schwab M, Mikus G, Fischer B, Hermle L, Marx C, et al. Enantioselective quantitation of the ecstasy compound (*R*)- and (*S*)-*N*-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma and urine. J Chromatogr B Analyt Technol Biomed Life Sci 2003;793:207–22.
21. Peters FT, Kraemer T, Maurer HH. Drug testing in blood: validated negative-ion chemical ionization gas chromatographic-mass spectrometric assay for determination of amphetamine and metham-

phetamine enantiomers and its application to toxicology cases. Clin Chem 2002;48:1472–85.
22. Peters FT, Schaefer S, Staack RF, Kraemer T, Maurer HH. Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom 2003;38:659–76.
23. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, et al. Bioanalytical method validation—a revisit with a decade of progress. Pharm Res 2000;17:1551–7.
24. Massart DL, Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J. Internal method validation. In: Vandeginste BGM, Rutan SC., eds. Handbook of chemometrics and qualimetrics: part A, 1st ed. Amsterdam: Elsevier, 1997:379–440.
25. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS Document EP5-A. Wayne, PA: NCCLS, 1999.
26. Kraemer T, Maurer HH. Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine [Review]. J Chromatogr B Biomed Sci Appl 1998;713:163–87.
27. Lim HK, Hubbard JW, Midha KK. Development of enantioselective gas chromatographic quantitation assay for DL-threo-methylphenidate in biological fluids. J Chromatogr 1986;378:109–23.
28. de Boer D, Tan LP, Gorter P, van-de-Wal RM, Kettenes-van den Bosch JJ, de-Bruijn EA, et al. Gas chromatographic/mass spectrometric assay for profiling the enantiomers of 3,4-methylenedioxymethamphetamine and its chiral metabolites using positive chemical ionization ion trap mass spectrometry. J Mass Spectrom 1997;32:1236–46.
29. Lim HK, Su Z, Foltz RL. Stereoselective disposition: enantioselective quantitation of 3,4-(methylenedioxy) methamphetamine and three of its metabolites by gas chromatography/electron capture negative ion chemical ionization mass spectrometry. Biol Mass Spectrom 1993;22:403–11.
30. Segura J, Ventura R, Jurado C. Derivatization procedures for gas chromatographic-mass spectrometric determination of xenobiotics in biological samples, with special attention to drugs of abuse and doping agents [Review]. J Chromatogr B 1998;713:61–90.
31. Srinivas NR, Hubbard JW, Cooper JK, Midha KK. Enantioselective gas chromatographic assay with electron-capture detection for DL-fenfluramine and DL-norfenfluramine in plasma. J Chromatogr 1988;433:105–17.
32. Srinivas NR, Hubbard JW, Hawes EM, McKay G, Midha KK. Enantioselective gas chromatographic assays with electron-capture detection for methoxyphenamine and its three primary metabolites in human urine. J Chromatogr 1989;487:61–72.
33. Maurer HH. The role of gas chromatography-mass spectrometry with negative ion chemical ionization (GC-MS-NCI) in cinical and forensic toxicology, doping control and biomonitoring [Review]. Ther Drug Monit 2002;24:247–54.
34. Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B, et al. Rational experimental design for bioanalytical methods validation. Illustration using an assay method for total captopril in plasma. J Chromatogr A 1996;730:381–94.
35. Peters FT, Samyn N, Kraemer T, de Boeck G, Maurer HH. Concentrations and ratios of amphetamine, methamphetamine, MDA, MDMA, and MDEA enantiomers determined in plasma samples from clinical toxicology and driving under the influence of drugs cases by GC-NICI-MS. J Anal Toxicol 2003;27:552–9.
36. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of bioanalytical chromatographic methods [Review]. J Pharm Biomed Anal 1998;17:193–218.
37. Peters FT, Maurer HH. Bioanalytical method validation and its implications for forensic and clinical toxicology—a review [Review]. Accred Qual Assur 2002;7:441–9.
38. Clauwaert KM, Van Bocxlaer JF, De Leenheer AP. Stability study of the designer drugs “MDA, MDMA and MDEA” in water, serum, whole blood, and urine under various storage temperatures. Forensic Sci Int 2001;124:36–42.
39. de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, et al. Non-linear pharmacokinetics of MDMA (‘ecstasy’) in humans. Br J Clin Pharmacol 2000;49:104–9.