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*

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Abstract

Enantiomers of amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) exhibit different pharmacological properties. This may be important for the interpretation of analytical results. Plasma samples were analyzed using validated negative ion chemical ionization gas chromatography–mass spectrometry procedures. The results for clinical toxicology cases, divided into screening (SCR) and intoxication (ITX) cases, and those of driving under the influence of drugs (DUID) cases were compared. The concentrations of all enantiomers, except R-(-)-MDA and R-(-)- and S-(+)-MA, in the SCR samples were lower than in ITX and DUID samples. Differences between concentrations in ITX and DUID samples were only significant for both enantiomers of AM (DUID higher). These findings suggested impairment in drugged drivers. Different enantiomer ratios (R vs. S) were found for AM between DUID and SCR samples, for MDMA between ITX and SCR samples, and for MDA between DUID and ITX and DUID and SCR samples. Higher MDMA enantiomer ratios in SCR compared to ITX samples are in accordance with a previously described increase of those ratios over time, possibly allowing differentiation of recent from nonrecent ingestion. Pharmacokinetic analysis of a MDMA poisoning yielded elimination half-lives of 6.0 h for R-(-)-MDMA and 4.1 h for S-(+)-MDMA. The enantiomer ratios rose exponentially over time.

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Introduction

Amphetamine (AM), methamphetamine (MA), and the amphetamine-derived designer drugs 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) are widely abused recreational drugs. AM and MA are mainly taken because of their potent stimulant effects (1,2), whereas the main desired effects of the so-called entactogens (3) MDA, MDMA, and MDEA include increased alertness and endurance, as well as a sense of euphoria, closeness to other people, and greater sociability (4–6). This has made them especially popular among young people as so called “rave drugs”. However, all of the above-mentioned compounds also exhibit many undesired effects, and their abuse is not without risk. Acute side effects include tachycardia, hypertension, increased muscle tension, hyperpyrexia, nausea, blurred vision, and ataxia (4–7), and many severe or even fatal intoxications have been described (2,4–6). Concerning chronic toxicity, it is well documented that these compounds can cause irreversible neuronal damage in the central nervous system (4–6,8–11). Furthermore, in recent years, the above mentioned drugs have also become increasingly important in the context of driving under the influence of drugs (DUID) (12–14). In 54% of DUID cases in Belgium from the period 2000–2001, AM and/or MDMA were detected in plasma in concentrations above the legal limit (15). Undesired effects like blurred vision and ataxia can impair the ability to drive a car. Increased risk taking and the decreased ability to estimate and predict movement have been reported. Detailed accounts on this issue, including reviews of the literature, have been published (2,7,16).
In clinical toxicology (CT) and in forensic toxicology, it can be important to estimate possible relationships between plasma concentrations of AM, MA, MDA, MDMA, and MDEA and symptoms of intoxication and/or driving impairment. In this context, the chirality of these drugs is often neglected. However, their S-(+)-enantiomers are known to be five to ten times more potent than the R-(-)-enantiomers, and their toxicokinetics are known to be different (4,6,17–19). In the cases of AM and MA, information on the enantiomer composition of AM and MA in clinical and forensic plasma samples is important for interpretation of the results because both compounds are available on the illegal drug market as racemic mixtures and as optically pure S-(+)-enantiomers. Furthermore, both drugs are metabolites of several (racemic or optically pure) therapeutic drugs or are even used as such themselves [e.g., R-(-)-MA in cold medications like Vicks Inhaler and S-(+)-AM within withdrawal treatment programs] (17,20–23). For these reasons, enantioselective analysis can help to differentiate therapeutic from illicit ingestion of AM, MA, or one of their precursor drugs (1,17,23). In the cases of MDA, MDMA, and MDEA, there are no legal precursor drugs. However, the toxicokinetics of the individual enantiomers of these compounds are known to differ considerably (18,19,24), so that the concentrations of individual enantiomers of these drugs cannot be estimated from their total concentrations. So far, only few data have been published on enantiomer concentrations of AM, MA, MDA, MDMA, and MDEA in plasma samples (25–28). Only Peters et al. (28) had analyzed a great number of CT cases, whereas in the other references, only case reports on fatal poisonings were documented.

The aim of the study presented here was to determine the concentrations of AM, MA, MDA, MDMA, and MDEA enantiomers in a large number of CT and DUID cases, to compare the results, and to discuss the implications of the results in clinical and forensic toxicology. The data from reference 28 were included in these evaluations.

Experimental

Chemicals and reagents

Methanolic solutions (1000 mg/L) of racemic AM-d_{11} and MA-d_{5} and methanolic solutions (100 mg/L) of racemic MDA-d_{5} and MDMA-d_{5} were obtained from Promochem (Wesel, Germany). Hydrochlorides of racemic AM, MA, MDA, MDMA, and MDEA were obtained from Lipomed (Arlesheim, Switzerland). Isolute Confirm HCX cartridges (130 mg, 3 mL) were obtained from Separtis (Grenzach-Wyhlen, Germany). Sodium hydrogen carbonate was obtained from Fluka (Steinheim, Germany). All other chemicals were obtained from E. Merck (Darmstadt, Germany). All chemicals were of analytical grade or the highest purity available. The derivatization reagent S-(−)-heptafluorobutyrylprolyl chloride (S-HFBPCI) was synthesized in the authors’ laboratory according to reference 28.

Plasma samples

Pooled human blank plasma samples were used for preparation of calibration samples. They were tested for absence of AM, MA, MDA, MDMA, and MDEA before use by a gas chromatographic-mass spectrometric (GC–MS) procedure with a limit of detection (LOD) below 1 μg/L (29). Authentic human plasma samples from DUID cases had been taken during routine roadside controls in Belgium. All these drivers had been suspected of being impaired. Authentic human blood samples from CT cases had been submitted by various hospitals for toxicological analysis. Only samples positive for one or more of the analytes were used after completion of routine analysis and anonymization.

Apparatus

The samples were analyzed using an Agilent Technologies (AT, Waldbronn, Germany) 6890 series GC system combined with an AT 5973 network mass selective detector, an AT 7683 series injector, and an AT enhanced ChemStation G1701CA version C.00.00 21-Dec-1999. The GC conditions were as follows: splitless injection mode; column, 5% phenyl methyl siloxane (HP-5MS, 30 m x 0.25-mm i.d., 250-nm film thickness); injection port temperature, 250°C; carrier gas, helium; flow rate, 1 mL/min; column temperature program for AM and MA analysis, 100°C raised to 180°C at 30°C/min, to 230°C at 5°C/min, to 310°C at 30°C/min; and temperature program for MDA, MDMA, and MDEA analysis, 100°C raised to 200°C at 30°C/min, to 260°C at 5°C/min, and to 310°C at 30°C/min. The MS conditions were as follows: transfer line heater, 280°C; NICI, methane (2 mL/min); source temperature, 150°C; solvent delay, 9 min for analysis of AM and MA analysis, 11 min for MDA, MDMA, and MDEA analysis; and selected-ion monitoring (SIM) mode, parameters for AM and MA analysis given in Table I, parameters for MDA, MDMA, and MDEA analysis given in Table II.

Sample preparation

Aliquots (0.025–0.2 mL) of plasma or serum were diluted

<table>
<thead>
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<th>Time Window</th>
<th>Analyte</th>
<th>Monitored Ions* (m/z)</th>
</tr>
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<tbody>
<tr>
<td>9–11 min</td>
<td>AM-d_{11}</td>
<td>379, 399, 439</td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>368, 388, 428</td>
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<tr>
<td>11–13 min</td>
<td>MA-d_{5}</td>
<td>387, 407, 447</td>
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<tr>
<td></td>
<td>MA</td>
<td>382, 402, 442</td>
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* Target ions underlined.

Timed event: electron multiplier voltage raised by 400 V+.

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<tr>
<th>Time Window</th>
<th>Analyte</th>
<th>Monitored Ions* (m/z)</th>
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<tr>
<td>11–13 min</td>
<td>MDA-d_{5}</td>
<td>432, 457, 477</td>
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<tr>
<td></td>
<td>MDA</td>
<td>432, 452, 472</td>
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<tr>
<td>13–15 min</td>
<td>MDMA-d_{5}</td>
<td>431, 451, 491</td>
</tr>
<tr>
<td></td>
<td>MDMA</td>
<td>426, 446, 486</td>
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<tr>
<td></td>
<td>MDEA</td>
<td>460, 480, 500</td>
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* Target ions underlined.
with 2 mL of purified water. After addition of 0.1 mL of a methanolic solution of the racemic internal standards (IS) MDA-d$_5$ (0.04 mg/L), AM-d$_{11}$, MA-d$_5$, and MDMA-d$_5$ (0.2 mg/L each) 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 0.01M 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, v/v) 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 (sodium bicarbonate/sodium carbonate, 7:3; 5%, w/v, pH 9) and 6 µL of derivatization reagent (0.1M S-HFBPCI in dichloromethane), the reaction vials were sealed and left on a rotary shaker at room temperature for 30 min. Thereafter, 0.1 mL of cyclohexane was added, the reaction vials were sealed again, and left on a rotary shaker for 1 min. The phases were separated by centrifugation (10,000 x g, 1 min), and the cyclohexane phase (upper) was transferred to autosampler vials. Aliquots (3 µL) were injected into the GC–MS system.

Quantitation procedure

The enantiomers of AM, MA, MDA, MDMA, and MDEA were quantitated 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 spiked calibration standards had been plotted versus their concentrations using a weighted (1/x$^2$) least-squares regression model. Calibration samples enriched with racemic AM, MA, MDA, MDMA, and MDEA were used to construct the calibration curves. Assuming a 1:1 ratio between the enantiomers of each analyte, the calibration standards contained 1, 10, 20, 30, 40, and 50 µg/L per enantiomer of MDA, as well as 5, 50, 100, 150, 200, and 250 µg/L per enantiomer of AM, MA, MDMA, and MDEA.

Comparative studies

Samples from a total of 172 cases were analyzed as described. Of these samples, 73 were from DUID cases and the other 99 samples were from CT cases. The latter were divided up into different groups. One group represented intoxication (ITX, n = 48) cases with the patients showing typical symptoms of intoxication with one of the analytes like tachycardia, hypertension, agitation, or hallucinations. The second group (ITXe, n = 32) represented a part of the ITX cases. However, in this second group, all ITX cases were excluded in which the observed symptoms might also have been attributable to other drugs detected in a previous screening analysis (e.g., cocaine). The third group represented screening cases (SCR, n = 51), in which the analytes had been found during routine analysis, but no typical symptoms had been observed in the patients at the time of sampling. The results obtained for these four different groups of samples (DUID, ITX, ITXe, and SCR) were compared by the nonparametric Kruskal-Wallis test followed by Dunn’s post-test for multiple comparisons. These statistics were calculated for total concentrations, individual enantiomer-concentrations, and enantiomer ratios (R vs. S) of each analyte.

Pharmacokinetic analysis of a severe poisoning case

One of the CT cases was a severe poisoning, in which an unknown dose of MDMA had been ingested in an attempt to commit suicide. Blood samples were taken 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 26, 28, 30, 37, and 50 h after ingestion of MDMA. The concentrations of total MDMA and its metabolite MDA were plotted against sampling time. The same was done for the individual enantiomers and the enantiomer ratios (R vs. S) of both compounds. Nonlinear curve fitting was applied to the data points of each plot. Furthermore, the elimination half-lives of total MDMA, R-(+)-MDMA, and S-(+)-MDMA were estimated from the respective data sets.

Results and Discussion

Sample analysis

Samples were analyzed according to a previously published procedure for the enantiomers of AM and MA in plasma (28),...
which was adapted for the analysis of MDA, MDMA, and MDEA (30,31). Both assays had been validated according to internationally accepted criteria. They had proven to be selective. Linearity ranged from 1 to 50 µg/L for each enantiomer of MDA and from 5 to 250 µg/L for each enantiomer of AM, MA, MDMA, and MDEA. Samples with concentrations above these ranges were reanalyzed using reduced sample volumes. Analytical recoveries ranged from 88.8% to 106.9%, and precision was between 1.3% and 14.9% relative standard deviation. The elution order of the diastereomeric derivatives had been determined by analysis of optically pure standards. For illustration of the applicability of these methods for the presented study, merged mass fragmentograms of an authentic sample containing AM and MA (Figure 1A) and of an authentic sample containing MDA, MDMA, and MDEA (Figure 1B) after SPE and derivatization with S-HFBPCI are shown in Figure 1.

**Comparative studies**

Samples from 73 DUID and 99 CT cases were analyzed as described. The results for total and single enantiomer concentrations of AM, MA, MDA, MDMA, and MDEA in the plasma samples from the four groups (DUID, ITX, ITXe, and SCR), as well as their corresponding enantiomer ratios (R vs. S), were compared. Non-parametric statistical testing procedures (Kruskal-Wallis test followed by Dunn’s test) were applied to check for significant differences between the groups because prerequisites for parametric analysis of variance (normally distributed values, homogeneity of variances) were not fulfilled for most comparisons. Furthermore, application of these robust statistical tests allowed inclusion of semiquantitative results for concentrations below the limit of quantitation (LOQ). Although this might seem questionable from a strict analytical point of view, exclusion of results below the LOQ would have eliminated the lower part of the distribution of concentration values, especially in the SCR samples, leading to overestimation of the corresponding median and to a bias in the results of the comparative studies. However, for the comparison of enantiomer ratios, the statistical analysis only included values that had been calculated from enantiomer concentrations above the LOQ.

AM was present in 51 DUID, 40 ITX (including 24 ITXe), and 46 SCR cases. The data for all groups are summarized as box plots in Figure 2. In all of these cases, both AM enantiomers were present. The concentrations in SCR samples were significantly lower than in DUID, ITX, and ITXe samples, which is in line with expectations because of the absence of AM-like effects in the SCR cases. Furthermore, the concentrations in the DUID samples were significantly higher than those in the ITX samples, but the difference was no longer significant if only

![Figure 2](image-url)  
**Figure 2.** Box plots and statistical comparisons of plasma concentrations of R-(-)-AM, S-(+)-AM, total AM, and of R vs. S ratios of AM enantiomers in DUID, ITX, ITXe, and SCR cases. Horizontal bars represent median values, boxes interquartile ranges, whiskers highest/lowest nonextreme values, and open circles extreme values. Tables below the box plots give the results from pairwise statistical comparisons of the different groups. Single stars indicate significant differences at \( p = 0.05 \), double stars at \( p = 0.01 \), and triple stars at \( p = 0.001 \). Nonsignificant differences are indicated by “ns”.

![Figure 3](image-url)  
**Figure 3.** Box plots and statistical comparisons of plasma concentrations of R-(-)-MA, S-(+)-MA, total MA, and of R vs. S ratios of MA enantiomers in DUID, ITX, ITXe, and SCR cases. Horizontal bars represent median values, boxes interquartile ranges, whiskers highest/lowest nonextreme values, and open circles extreme values. Tables below the box plots give the results from pairwise statistical comparisons of the different groups. Single stars indicate significant differences at \( p = 0.05 \), double stars at \( p = 0.01 \), and triple stars at \( p = 0.001 \). Nonsignificant differences are indicated by “ns”.

555
ITXe samples were compared to the DUID samples. For the ratio between enantiomer concentrations (R vs. S) in the different study groups, significant differences were observed between DUID and SCR samples, with higher ratios in the SCR samples. The latter is in line with reports on faster elimination of S-(+)-AM compared to R-(-)-AM (23). However, the majority of the studied samples had an enantiomer ratio close to the theoretical value of 1.0 for racemic mixtures. Therefore, this parameter is not very helpful for interpretation. However, both racemic and optically pure enantiomers are available as medications or illicit drugs, and only enantioselective analysis can provide information about which form of AM is present in a sample. This information may be important because observed symptoms should be attributable mainly to the more potent S-(+)-enantiomer. Furthermore, it may help to differentiate between therapeutic ingestion of AM precursors, like the antiparkinson drug selegiline, and illicit ingestion of AM or MA (17,23).

MA was present in 7 DUID, 13 ITX (including 4 ITXe), and 12 SCR cases. The data for all groups are summarized as box plots in Figure 3. No significant differences were detected between any of the groups. This might in part be due to a lack of statistical power because of small sample numbers in the different groups. Nevertheless, some interesting results were obtained for MA. All but two cases contained both enantiomers of MA, and their ratios (R vs. S), with only one exception, were greater than 1.0, which is in line with the faster elimination of S-(+)-MA (23). The finding of only R-(-)-MA in one of the DUID samples might also be explained by the faster elimination of S-(+)-MA. Because R-(-)-MA was present at a rather low concentration of 5.1 µg/L in this sample, one would assume that it had been taken a rather long time after ingestion of MA, so S-(+)-MA might have been completely eliminated from plasma by that time. However, detection of only R-(-)-MA would also be in accordance with therapeutic use of a cold medication containing R-(-)-MA (e.g., Vicks Inhaler) or with therapeutic ingestion of the precursor drug selegiline. This must always be considered in such cases. In another DUID sample, only S-(+)-MA was found to be present. This finding might be explained by ingestion of optically pure S-(+)-MA, which can be synthesized from ephedrine and is available on the illicit drug market. Ingestion of both racemic and optically pure S-(+)-MA might also explain the finding of an enantiomer ratio below 1.0.

MDMA was present in 52 DUID, 16 ITX (including 8 ITXe), and 12 SCR cases. The data for all groups are summarized as box plots in Figure 4. The concentrations in SCR samples were significantly lower than those in DUID, ITX, and ITXe samples. These findings are similar to those for AM. However, whereas for

**Figure 4.** Box plots and statistical comparisons of plasma concentrations of R-(-)-MDMA, S-(+)-MDMA, total MDMA, and of R vs. S ratios of MDMA enantiomers in DUID, ITX, ITXe, and SCR cases. Horizontal bars represent median values, boxes interquartile ranges, whiskers highest/lowest nonextreme values, and open circles extreme values. Tables below the box plots give the results from pairwise statistical comparisons of the different groups. Single stars indicate significant differences at p = 0.05, double stars at p = 0.01, and triple stars at p = 0.001. Nonsignificant differences are indicated by "ns".

**Figure 5.** Box plots and statistical comparisons of plasma concentrations of R-(-)-MDA, S-(+)-MDA, total MDA, and of R vs. S ratios of MDA enantiomers in DUID, ITX, ITXe, and SCR cases. Horizontal bars represent median values, boxes interquartile ranges, whiskers highest/lowest nonextreme values, and open circles extreme values. Tables below the box plots give the results from pairwise statistical comparisons of the different groups. Single stars indicate significant differences at p = 0.05, double stars at p = 0.01, and triple stars at p = 0.001. Nonsignificant differences are indicated by "ns".
AM the level of significance (p-values) were similar no matter if R(-)-, S(+)-, or total AM concentrations in the different groups were compared, for MDMA the p-values for the comparisons of ITX and ITXe samples with SCR samples were higher for the concentrations of R(-)-MDMA (p < 0.05) than for the concentrations of S(+)-MDMA (p < 0.001). The corresponding p-values for total MDMA lay in between (p < 0.01). The explanation for these results can be found in the absolute values of the enantiomer ratios (R vs. S) of MDMA, which were considerably higher than those for AM and reached values of up to 8.11. Enantiomer ratios of MDMA in SCR cases were significantly higher than those in ITXe cases, but not significantly different from those obtained for ITX and DUID samples. Taking into account a steady increase of MDMA enantiomer ratio over time after ingestion (18,31), these differences indicated recent ingestion of MDMA in the ITXe cases and a rather long time after ingestion in the SCR cases. Accordingly, the times after ingestion in DUID and ITX cases obviously lay between these two extremes, as indicated by nonsignificant differences to any of the other groups. At this point, it should also be mentioned that in one of the DUID samples S(+)-MDMA was no longer detectable. However, R(-)-MDMA was still present at a concentration of 65.8 µg/L. This was above the legal cutoff value of 50 µg/L for total MDMA in DUID samples (15). However, impairment of the respective driver seems unlikely, because only the less potent R(-)-enantiomer was present.

MDA was present in 52 DUID, 17 ITX (including 8 ITXe), and 12 SCR cases. The data for all groups are summarized as box plots in Figure 5. For concentrations of R(-)-MDA, no significant differences were detected between any of the four groups. This might be because of possible saturation kinetics of R(-)-MDA formation from R(-)-MDMA, even at low concentrations of R(-)-MDMA. For S(+)-MDA, significant differences of concentrations were only detected between DUID and SCR samples. Similar findings were obtained for total MDA. For the MDA enantiomer ratios, significant differences were obtained between ITX and DUID samples, as well as between SCR and DUID samples. However, interpretation of these results is virtually impossible because high MDA enantiomer ratios might either be caused by a rather long time since ingestion of MDMA or else caused by (additional) ingestion of racemic MDA.

MDEA was present in only one of the DUID samples. Merged mass fragmentograms of this sample after SPE and derivatization with S-HFBPCI are shown in Figure 1B. The enantiomer ratio (R vs. S) was 1.62, which is accordance with Brunnenberg et al. (19), who reported faster elimination of S(+)-MDEA compared to its R(-)-enantiomer after ingestion of racemic MDEA.

Pharmacokinetic analysis of a severe poisoning case

In a severe self-poisoning case, a 27-year-old male had ingested an unknown dose of MDMA approximately 2 h before he was brought to hospital. The patient was unconscious and suffering from severe seizures and massive jaw clenching. Therefore, gastric lavage and/or application of activated charcoal were not performed. Although the seizures leveled off after application of clonazepam, the jaw clenching persisted unchanged during the first hours of treatment.

Because of the severe symptoms, which also prevented effective decontamination, the plasma concentrations of MDMA were closely monitored. Plots of total and single enantiomer concentrations and of enantiomer ratios of MDMA and MDA versus time are shown in Figure 6. Exponential curves were fitted into the data sets of concentrations of total MDMA (coefficient of determination, R^2 = 0.9926), R(-)-MDMA (R^2 = 0.9905), S(+)-MDMA (R^2 = 0.9953), total MDA (R^2 = 0.9474), and S(+)-MDA (R^2 = 0.9497). The R^2 values indicated very good fits of these curves, especially for total MDMA and its enantiomers. This was also confirmed by runs tests, which revealed no significant deviations of the data from the assumed exponential models (p > 0.05). These findings indicate first-order elimination kinetics for each total MDMA and both enantiomers in the reported case. From the corresponding elimination constants, plasma half-lives of 5.0 h for total MDMA, 6.0 h for R(-)-MDMA, and 4.1 h for S(+)-MDMA were calculated, which are in accordance with those reported by Fallon et al. (18). For the exponential fits for total MDA and S(+)-MDA concentrations, the first values were excluded, as they had not reached a maximum by that time and exponential fits could only be expected for the elimination phase. The fits were not quite as good as those for MDMA, which is probably because of the fact that the plasma concentration time curves of these compounds were not only dependent on elimination, but also on their metabolic formation from MDMA. The situation for R(-)-MDA was rather different. After a concentration of about 10–11 µg/L had been reached approximately 6–8 h after ingestion of MDMA, the plasma concentrations for R(-)-MDA remained virtually constant until approximately 26–28 h after ingestion. However, the concentration of R(-)-MDMA had dropped by approximately two thirds in the same time interval. These results might possibly be explained by saturation of the enzymes responsible for metabolic formation of R(-)-MDA. Such saturation effects have also been discussed by de la Torre et al. (32), who reported nonlinear toxicokinetics of MDMA. Furthermore, possible interactions between clonazepam and MDMA have to be considered. Clonazepam has been described to be an in vivo inducer and an in vitro inhibitor of cytochrome P450 enzymes of

![Figure 6. Plots of total and single enantiomer concentrations and of enantiomer ratios of MDMA and MDA versus time after ingestion in a severe self-poisoning with MDMA. The lines in the data sets represent exponential curve fits. The solid lines at y = 1.0 correspond to the enantiomer ratios in racemic mixtures.](image-url)
the CYP2B family in rats (33). The corresponding human enzyme CYP2B6 is known to be involved in MDMA metabolism in humans (34), so that metabolic interactions might be possible. However, even if clonazepam would induce CYP2B enzymes also in humans, this should not be relevant in the described case because induction of enzymes is a rather slow process that becomes only relevant after days. In case of relevant inhibition of CYPB6 by clonazepam, one would have expected much longer elimination half-lives.

Interesting results were also obtained for the enantiomer ratios (R vs. S) of MDMA and MDA. Exponential curves fitted well into the plots of both parameters versus time after ingestion of MDMA. The \( R^2 \) values were 0.9931 and 0.9968, respectively, and runs tests revealed no deviations from the assumed models (\( p > 0.05 \)). Whereas MDMA enantiomer ratios rose from values close to 1.0 to approximately 10 because of faster elimination of S-(+)-MDMA; the curve for MDA enantiomer ratios started at approximately 0.15 and rose to approximately 2.5 because of enantioselective formation in favor of S-(+)-MDA. Equal concentrations of MDA enantiomers were reached approximately 30 h after ingestion of MDMA. Fallon et al. (18) had proposed a mathematical model for the estimation of the time after ingestion based on MDMA enantiomer concentrations. However, although these authors chose a more complicated model stating that “there is no a priori reason to believe that the best predictor is the simple ratio rather than the ratio of differing powers on the enantiomeric concentrations”, the monoexponential increase of MDMA enantiomer ratios over time presented here (Figure 6, lower left) may suggest estimations of the times after ingestion of MDMA could also be based on simple enantiomer ratios.

Conclusions

Our studies showed that the plasma concentrations of AM and MDMA in ITX and DUID cases were comparable and significantly higher than those in SCR cases. This finding is in accordance with the suspected driving impairment in the selected drivers. MDMA enantiomer ratios were generally higher than 1.0, and they might well be relevant to estimate the time since ingestion of this drug. MDMA enantiomer ratios greater than 1.0 implied a rather long time since ingestion of MDMA or an (additional) ingestion of racemic MDA. In contrast to this, AM enantiomer ratios were of little interpretative value unless only single enantiomers were present in the samples.

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