Interpretation and Enantiomer Analysis of Methamphetamine Abusers' Urine and Illegally Brewed Methamphetamine Crystals

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Abstract

This study deals with the high-performance liquid chromatographic identification of methamphetamine (MAMP) and amphetamine (AMP) enantiomers (d- and l-forms) in five illicit MAMP crystals and in urine specimens from 30 Japanese MAMP abusers. The analysis revealed that two of the types of crystals have a different optical purity ratio (I/d) and the other three have a single crystal of either the d- or l-enantiomer. The I/d ratios of two types of crystals were 0.04 and 49.4, and no racemic form (I/d = 1.00) was found. The urinary analysis showed that nonmetabolized MAMP and its demethylated metabolite, AMP, were present in urine specimens of all addicts. The stereoisomeric profiles in urine can be classified roughly into five groups according to the detected amount and the I/d ratio of MAMP and AMP enantiomers collected at one time point. In the first group, only d-MAMP and d-AMP were detected (in 16 cases). In the second group, only l-MAMP and l-AMP were detected (in one case). In the third group, the amount of the l-enantiomer detected, for both MAMP and AMP, was less than that of the d-enantiomer, and the I/d ratio was between 0.004 and 0.54 for MAMP and between 0.01 and 0.07 for AMP (in five cases). In the fourth group, the l-enantiomer of MAMP and AMP was found to be more abundant than the d-enantiomer, and the I/d ratio was between 2.63 and 30.11 for MAMP and between 1.23 and 31.30 for AMP (in four cases). In the fifth group, the amount of l-MAMP detected was greater than that of d-MAMP, and less l-AMP than d-AMP was detected. The I/d ratios were between 1.13 and 8.82 for MAMP and between 0.17 and 0.82 for AMP (in four cases). These results might be suitable for identification and the forensic toxicological investigation of AMP analogues.

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Introduction

Methamphetamine (MAMP) and amphetamine (AMP), which have psychostimulant actions, are widely used in various countries of East Asia, Europe, and North America. Consumption of MAMP is extremely high in Japan, South Korea, Taiwan, and Hong Kong. Abusers of MAMP have been increasing in number (1), and most of them are aged between 13 and 59. AMP is little misused, and it was said that only d-enantiomer of MAMP would be misused in Japan (2). One of the reasons may be that d-enantiomer has stronger stimulant effects on the central nervous system than the l-enantiomer. Another possibility is that the acquisition of l-ephedrine, a starting material, is easy and the synthesizing method is simple. Nevertheless, the d- and the l-enantiomers and their racemic (dl) form can be synthesized through the reduction and the condensation of ephedrine and phenylacetonit, respectively (3). Several methods for the identification of these chemicals have already been reported, including gas chromatography (GC), immunoassay, high-performance liquid chromatography (HPLC), and capillary electrophoresis (4–7). These chemicals are also detected in human and animal urine specimens as metabolites of pharmaceuticals such as selegline (8,9), Vicks inhaler (10,11), and famprofazone (12,13). There are few reports, if any, on the simultaneous detection and the stereoisomeric profiles of MAMP and AMP eliminated in human urine after dosing of racemic MAMP or the complex of d- and l-MAMP (11).

The present study deals with recent information on the analysis of MAMP and AMP enantiomers in the urine specimens of MAMP addicts who were arrested in Japan. An analysis of some confiscated MAMP crystals is also reported.
**Experimental**

**Materials**

Urine samples from 30 MAMP-abusing Japanese subjects who had been arrested under the strict Stimulant Drug Law Control of Japan were obtained, and five illegal MAMP crystals from five Japanese subjects were confiscated over a one-month period in an area of Tochigi Prefecture in Japan. These trial samples were obtained from the Scientific Crime Detection Laboratory, Tochigi Prefectural Police Headquarters in Japan, and the work was done in cooperation with Dokkyo University and the Tochigi Police.

**Reagents**

Racemic MAMP and AMP were prepared as previously reported (2). Normal-hexane and 2-propanol used as HPLC solvents and benzoyl chloride were purchased from Wako Pure Chemical (Osaka, Japan). 4-Phenylbutylamine was obtained from Aldrich Chemical (Milwaukee, WI). All other chemicals were of special grade and were obtained from commercial sources.

**Extraction and determination**

MAMP and AMP enantiomers in urine specimens were extracted with an Extrelut 1 column (Merck): 10 μL of 4-phenylbutylamine (internal standard, I.S., 100.0 μg/mL), 0.2 mL of 1.5N NaOH, and 0.6 mL of distilled water were added to 0.2 mL of urine. The mixture was transferred to an Extrelut 1 column. Each enantiomer of MAMP and AMP was eluted from the column 20 min later with 6.0 mL of n-hexane, followed by back-extraction with 1.0 mL of 0.1M H₂SO₄. The lower layer was then stirred vigorously in 3.0 mL of 0.05N NaOH and 10 μL of benzoyl chloride for 3.0 min. The benzoyl derivatives of MAMP and AMP were extracted with 3.0 mL of n-hexane, washed twice with distilled water, and evaporated to dryness in a water bath at 40°C with N₂ gas. The residue was dissolved in 200 μL of n-hexane/2-propanol (88:12, v/v), and a 20-μL aliquot was injected for HPLC. MAMP crystals were examined by diluting them to the concentration of 10 μg/mL with distilled water. The extraction and the benzoylation of MAMP from the crystals were done the same as those of urine specimens. Determinations were performed using a calibration curve based on the peak-area ratio of each enantiomer in the concentration range 0-10 μg/mL to an internal standard.

**Instrumental analyses**

HPLC was carried out with a CCPM pump and CO-8020-type column oven (TOSO Co., Tokyo, Japan). The column was a Chiralcel OB-H (25 cm x 4.5-mm i.d., Daicel Chemical Ind., Tokyo, Japan). The detectors used were an UV-8000 ultraviolet spectroscope (TOSO Co., Tokyo, Japan) and a Shodex OR-2 polarimeter (Showa Electric, Tokyo, Japan). The detection wavelength was set at 220 nm for the ultraviolet (UV) detector and 450 nm for the optical rotation (OR) detector. The UV wavelength, 220 nm, corresponds to the maximum absorption of the benzoyl derivatives of MAMP and AMP, and the OR detector wavelength is fixed at 450 nm because of the use of a xenon lamp. The column temperature was maintained at 50°C. The mobile phase was n-hexane/2-propanol (88:12, v/v), and the flow rate was 1.0 mL/min. This analytical method was used for the determination of MAMP and AMP enantiomers in confiscated crystals and urine specimens.

GC was performed with a Hewlett-Packard (Palo Alto, CA) model 5890 series GC equipped with a flame ionization detector. The fused-silica column was a DB-5 (30 m x 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA). The column temperature was programmed to rise from 50 to 250°C at the rate of 20°C/min. The injection temperature was set at 250°C. The carrier gas was helium, and the flow rate was 1.0 mL/min. This analytical method was used for the identification of MAMP in confiscated MAMP crystals.

GC–MS was performed with a JEOL AUTO-MASS 150 GC–MS (JEOL Co., Tokyo, Japan) equipped with a DB-5ms fused-silica capillary column (30 m x 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific). The operating temperatures were as follows: injection port, 270°C; column, 50–250°C (20°C/min); and ion source, 180°C. The flow rate of the carrier gas, helium, was 1.0 mL/min. The mass spectra were obtained by electron impact (EI). The ionization voltage was 75 eV. This analytical method was used for the demonstration of MAMP after HPLC analysis.

**Results**

**Authentic substances**

Two HPLC chromatograms in Figure 1 (A and B) show the optical activity identification and the simultaneous analysis of the two enantiomers of MAMP and AMP. With the OR detector, the d-enantiomer and the l-enantiomer of AMP analogues had the opposite number degrees of optical rotation (Figure 1A). As shown in Figure 1B, the retention time of each enantiomer matched that of its analysis with the UV detector. The peak resolution was more than 1.0 for both MAMP and AMP, and the four enantiomers and an internal standard were distinctly separated. Each enantiomer shows 10.0-μg chromatographic peak for UV detector as absolute amount. The detection limits were approximately 2.5 μg per 20-μL injection for the OR detector and a 125-ng chromatographic peak for UV detector. The analysis time was approximately 30 min. The ratios (l/d) of racemic MAMP and AMP in the optical purity, which were calculated from the peak areas of each enantiomer, were close to their theoretical values (l/d = 1.00). The average l/d ratios for five determinations were 1.00 ± 0.01 for MAMP and 0.99 ± 0.01 for AMP. The coefficients of variation were within ±1.0%.

**Confiscated MAMP crystals**

Table I shows the results of HPLC analysis of MAMP enantiomer in five crystals that were confiscated from five Japanese men aged from 28 to 52. Among the five crystals, crystals 1 and 2 consisted of the d- and l-enantiomers of MAMP; crystals 3 and 4 were d-enantiomer only, and crystal 5 was l-enantiomer only. The content rate of l-enantiomer was 98.0% in crystal 1 and 4.3% in crystal 2. The l/d ratio calculated from the contents of two enantiomers were 49.35 in crystal 1 and 0.04 in crystal 2.
Crystals 3, 4, and 5 contained a single enantiomer; therefore, the $l/d$ ratio of these crystals could not be calculated. The racemic form ($l/d = 1.00$) of MAMP was not found in the five confiscated MAMP crystals. All five of these crystals were found to contain a single substance, that is, MAMP, which was identified in GC analysis equipped with an achiral column (DB-5), and the purity was more than 99% in each crystal. Figures 1 and 2 show the HPLC analyses of crystals 1 and 2. Two chromatographic peak-area values in HPLC were different in crystals 1 and 2. Moreover, the two peaks after the HPLC fractionation had a structure in EI spectrum similar to that of GC–MS analysis, and they corresponded to the $d$- and the $l$-enantiomers of authentic MAMP in HPLC.

**MAMP abusers’ urine**

Table II shows the concentrations of MAMP and AMP enantiomers in urine specimens obtained from 30 Japanese MAMP abusers using HPLC analysis. In all the urine specimens, non-metabolized MAMP and its demethylated metabolite, AMP, were detected. Stereisomeric patterns in urine samples were classified into five groups on the basis of the amounts of the two enantiomers detected, that is, (I) only $d$-MAMP and the AMP enantiomer were detected (addicts 1–16); (II) only $l$-MAMP and the AMP enantiomer were detected (addict 17); (III) the amount of the $l$-enantiomer of MAMP and AMP detected was less than that of the $d$-enantiomer (addicts 18–22). The $l/d$ ratios were between 0.004 and 0.54 for MAMP and were between 0.01 and 0.07 for AMP. (IV) The amount of the $l$-enantiomer of MAMP and AMP detected was more than that of the $d$-enantiomer (addicts 23–26). The $l/d$ ratios calculated from the amounts of both enantiomers in each urine specimen were between 2.63 and 30.11 for MAMP, and were between 1.23 and 31.30 for AMP, and the $l/d$ ratios were completely reversed when compared to III. (V) More MAMP $l$-enantiomer was detected than $d$-enantiomer, and less AMP $l$-enantiomer was detected than its $d$-enantiomer (addicts 27–30). The $l/d$ ratios were between 1.13 and 8.82 for MAMP and between 0.17 and 0.82 for AMP. Figure 3 shows the three HPLC chromatographic patterns of MAMP abusers’ urinalysis.

![Figure 1. High-performance liquid chromatographic separation profiles of racemic methamphetamine (MAMP), racemic amphetamine (AMP), and 4-phenylbutylamine (I.S., internal standard) in non-drug users’ urine specimens by two different detectors, polarimetry (A) and ultraviolet microscope (B).](https://academic.oup.com/jat/article-abstract/24/2/140/853738)

<table>
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<tr>
<th>Crystal</th>
<th>Gender</th>
<th>Age</th>
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<th>$l$-form</th>
<th>$l/d$ (%)$^t$</th>
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<tr>
<td>1</td>
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<td>52</td>
<td>+</td>
<td>+</td>
<td>49.35 98.0</td>
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<tr>
<td>2</td>
<td>male</td>
<td>35</td>
<td>+</td>
<td>+</td>
<td>0.04 4.3</td>
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<tr>
<td>3</td>
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<td>28</td>
<td>+</td>
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<td>N.C.$^5$ &lt;1.0</td>
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<tr>
<td>4</td>
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<td>32</td>
<td>+</td>
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<td>N.C.$^5$ &lt;1.0</td>
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<tr>
<td>5</td>
<td>male</td>
<td>37</td>
<td>N.D.$^4$</td>
<td>+</td>
<td>&gt;99.0</td>
</tr>
</tbody>
</table>

$^*$ MAMP, methamphetamine.

$^t$ Percentages are the amount of $l$-form to the sum of the amount of $d$- and $l$-form contained in a crystal.

$^4$ Not detected, that is, less than the detection limit.

$^5$ Not calculated, that is, either $d$-form or $l$-form was not detected.

![Figure 2. High-performance liquid chromatographic profiles of $d$-MAMP and $l$-MAMP mixtures found in illicit MAMP crystals confiscated. A, crystal 1 consists of $l$-MAMP of 98% and $d$-MAMP of 2.0%; B, crystal 2 consists of $l$-MAMP of 4.3% and $d$-MAMP of 95.7%.](https://academic.oup.com/jat/article-abstract/24/2/140/853738)
Discussion

The abusers of stimulants have not decreased in number, even though MAMP and AMP are strictly controlled in Japan (1). Most of those who misuse MAMP, which circulates in Japan, range in age from their teens to their fifties. The abuse of MAMP is much more common than that of illicit drugs such as narcotics, hallucinogens, and opiates. Most of the illicitly manufactured or smuggled MAMP is of the d-enantiomer type. Nevertheless, MAMP and AMP are strictly controlled in Japan and are not metabolites derived from deprenyl (8,9), Vicks inhaler (10,11), or famprazezone (12,13). Enantiomer analysis has become necessary in forensic chemistry and toxicology in recent years.

In this study, d-MAMP, l-MAMP, and a mixture of d- and l-MAMP were detected in MAMP addicts' urine and in confiscated MAMP crystals obtained in one region of Japan. No racemic form was found in the five illicit MAMP crystals. Among the types of crystals examined, two had a different optical purity ratio (I/d); the I/d ratios were 49.35 and 0.04, and the content rates of l-enantiomer were 98.0% and 4.3%, respectively. It is not yet known whether the difference in the values was caused at two enantiomer separations from the racemate by optical activity reagents such as manderic acid and tartaric acid or simply caused by the mixture of d-enantiomer and l-enantiomer. Others contained only d-enantiomer or only l-enantiomer of MAMP. In urinalysis, the stereoisomeric profile of nonmetabolite MAMP and its demethylated metabolite, AMP, has enabled us to classify 30 Japanese MAMP addicts into five groups on the basis of the detected amount of each enantiomer of MAMP and AMP at one time point, though accurate information on the dosing time, the time postdose, and the method of administration could not be obtained from each MAMP abuser examined. The first group was for those who had misused conventional d-MAMP (2), and the second group was for those who had misused d-MAMP alone. The third group was for the subjects whose urine contained an amount of l-enantiomer of both MAMP and AMP that was lower than that of d-enantiomer at the collection time. The fourth group's criteria were the opposite of the third group's: in both MAMP and AMP, more l-enantiomer than the d-enantiomer was excreted by the collection time. In the fifth group, samples contained higher amounts of l-MAMP than of d-MAMP, and l-MAMP amounts were lower than d-MAMP amounts.

Confiscated illicit MAMP crystals were therefore classified into four groups, and the excretion profiles of enantiomers in the abusers' urine were classified into five. This is the first time that l-enantiomer and a mixture of d- and l-enantiomer have been detected in Japan. They are not metabolites derived from deprenyl (8,9), Vicks inhaler (10,11), or famprazezone (12,13). None of these drugs has been admitted into Japan as a pharmaceutical. No

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Table II. Analytical Data on Enantiomers of Nonmetabolite Methamphetamine and its Demethylated Metabolite, Amphetamine, in Urine Specimens from 30 Japanese Methamphetamine Abusers*

<table>
<thead>
<tr>
<th>Addict Group</th>
<th>no.</th>
<th>Gender</th>
<th>Age (years)</th>
<th>MAMP</th>
<th>AMP</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(µg/mL)</td>
<td>(µg/mL)</td>
</tr>
<tr>
<td>I</td>
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<td>28</td>
<td>36.75</td>
<td>41.59</td>
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<td>21.29</td>
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<tr>
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<td>15.47</td>
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<tr>
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<td>4.92</td>
<td>5.75</td>
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<td>4.92</td>
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<td>15.47</td>
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<td>19</td>
<td>15.25</td>
<td>15.47</td>
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</table>

* Accurate information on the dosing time, the time postdose, and the method of administration could not be obtained from each methamphetamine abuser listed.

Abbreviations: MAMP, methamphetamine; AMP, amphetamine.
smuggling of these three drugs has been reported. If they are used, the discrimination between illicit MAMP itself and MAMP derived from these drugs is possible only by analyzing the optical activity. It would be necessary to examine the optical activity of the parent drugs and the metabolites. In the third and fourth groups, the urinalysis profiles on nonmetabolized MAMP and its demethylated metabolite, AMP, revealed that the l/d ratio of both MAMP and AMP were 1.00 or less in the third group and that the ratios were 1.00 or more in the fourth group. This result was different distinctly compared with the analytical result of nonmetabolite MAMP and its demethylated metabolite, AMP, in human urine specimens after racemic MAMP administration as shown by Cody et al. (11): the l/d ratio of MAMP was 1.00 or more, and the ratio of AMP was 1.00 or less. It suggested that d-MAMP is metabolized more rapidly than l-MAMP. Taken all together, in the third group of our results, more d-enantiomer than l-enantiomer may have been taken, and in the fourth group more l-enantiomer may have been used. In fact, some MAMP crystals contained more l-enantiomers and others contained more d-enantiomers in our presented data. Further, the excretion profiles of the third and fourth groups were not similar to those of racemic ethylamphetamine (EAMP) and AMP, which have a similar structure and pharmacological action (15,16). The excretion profile of nonmetabolized MAMP and its demethylated metabolite, AMP, in the fifth group was similar to that of racemic MAMP (11), AMP (16), and EAMP (15,17) in humans, and it was also similar to that of human urine after a single administration of d- or l-enantiomer of MAMP (18); more d-enantiomer than l-enantiomer of MAMP was detected in human urine. Perhaps the optical purity ratio of the MAMP crystals used resembles that of the racemic form in the fifth group. In particular, the urinary excretion patterns of nonmetabolized MAMP and its demethylated metabolite AMP obtained from addicts 27 and 28 were nearly the same as that reported by Cody et al. (11). Their stereoisomeric profiles suggested the stereoselective disposition in humans. The stereoisomeric profiles were not the same in the third and fourth groups or in rat urine (2). In addition, our data suggested that the human has no isomerase, which catalyzes metabolic chiral inversion of d- or l-MAMP.

The results of our present study suggest that illicit MAMP in d-form, l-form, and in a mixture of d-form and l-form have already been introduced and sold in various places in Japan. In the future, analysis of the optical activity form will become necessary in order to differentiate between illicit MAMP itself and MAMP derived from other drugs (8–13), and it will be useful in forensic chemistry and toxicology.

References

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