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Forensic Toxicologic Analysis of Methamphetamine and Amphetamine Optical Isomers by High Performance Liquid Chromatography

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Summary. Optical isomers (*d* and *l*) and racemic compounds *(dl)* of methamphetamine (MAMP) and amphetamine (AMP), and biologic materials including those substances, could be analyzed by high performance liquid chromatography. Examining the temperature for the analysis, 40°C was the optimal condition in the reproducibility of separated MAMP-isomers. The reproducibility at the temperature did not vary significantly. The measured values of optical isomers were 0.116 ± 0.012, 1.082 ± 0.070 and 8.984 ± 0.136 for the mixing ratios *(l/d)* of 0.111, 1.000, and 9.000, respectively. The detection limit for both *d*- and *l*-isomers was 25 ng.

The analytic result of hair specimens from two stimulant abusers by the present method indicates that they contained only *d*-MAMP and *d*-AMP, which is believed to have the strongest pharmacologic effect among the optical isomers of MAMP. The coefficient of variation in the analysis of five replicate standards, prepared by adding 1,000 ng each of racemate MAMP and AMP to hair, was less than 4%. The measured value against $l/d = 1.000$ was 1.040 ± 0.040 in MAMP and 0.980 ± 0.030 in AMP. The detection limit for both racemate MAMP and AMP accumulated in hair was 250 ng.

The analysis of the optical isomers by our method would contribute to identifying the smuggling routes or the illicit method.

Key words: Methamphetamine, toxicological analysis – Amphetamine, toxicologic analysis

Zusammenfassung. Es wurde eine neue Methode zur Trennung von optisch aktiven Isomeren (d und l) und der racemischen Verbindung (dl) von Met-

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amphetamin (MAMP) und Amphetamin (AMP) mittels Hochleistungsflüssigkeitschromatographie durch die UV-Methode ausgearbeitet. Wir fanden, daß 40°C in der Auflösung und der Reproduzierbarkeit der MAMP-Isomeren am besten waren. Meßwerte von Isomeren waren 0.116 ± 0.012, 1.082 ± 0.070 und 8.984 ± 0.136 gegenüber den Mischungsverhältnissen (l/d) von 0.111, 1.000 und 9.000. Die Nachweisgrenze von d- und l-Isomeren war jeweils 25 ng.

Die Analysenergebnisse menschlicher Haare von zwei Stimulationsmittelkonsumenten zeigten mittels der oben genannten Methode, daß die Haare nur d-MAMP und d-AMP enthielten, welche unter den Isomeren die stärksten pharmakologischen Wirkungen haben. Die Streuung der Analysen von fünf Standard-Materialien, nach Zusatz von 1,000 ng jeder racemischen MAMP und AMP zu menschlichen normalen Kopfhaaren, war weniger als 4%; die Meßwerte von Isomeren gegenüber den Mischungsverhältnissen $l/d = 1.000$, waren 1.040 ± 0.040 bei MAMP und 0.980 ± 0.030 bei AMP. Die Nachweisgrenze der racemischen Verbindung von MAMP und AMP, die in Haaren angehäuft sind, war jeweils 250 ng.

Die Analyse der Isomeren von MAMP und AMP durch unsere Methode würde der Aufdeckung von Schmuggelungen und -methoden dienen.

Schlüsselwörter: Metamphetamin, toxikologische Analyse – Amphetamin, toxikologische Analyse

Introduction

In Japan, methamphetamine (MAMP) is one of the drugs abused. It is under the strict control of the Stimulants Control Law. There is almost no domestic manufacture of these drugs, and the majority seems to be smuggled from Taiwan, South Korea, Hong Kong, and the Philippines [1].

MAMP was first synthesized by Nagayoshi Nagai in 1893 from ephedrine contained in *Ephedra sinica* Staphf [2]. There are two optical isomers (*d* and *l*) and a racemate *(dl)* of the MAMP [3, 4]. It is not only possible to produce these isomers separately, but also preparations with different *l/d* ratios from the racemates by the use of resolution reagents. However, no study has been carried out for determining the optical purity of the abused preparations and for examining which optical isomers are abused.

From the aspects of forensic medicine and forensic chemistry, we established a method for detecting the optical isomers of MAMP and AMP by high performance liquid chromatography (HPLC) and attempted to detect the optical isomers from biologic material (hair) of stimulant abusers.

Materials and Methods

MAMP and AMP

The *d*- and *l*-isomers of MAMP produced by Dai Nippon Pharmaceutical Co., Ltd. (Japan) were used, and the racemate *(dl)* was synthesized by the method of Caldwell et al. [5]. The

two optical isomers (*d* and *l*) and the racemate *(dl)* of AMP were synthesized by the method of Blackburn et al. [6].

Standard Solutions of Calibration Curves

Each optical isomer of MAMP and AMP was diluted with distilled water, and the concentration was prepared between 0.125 μg/ml and 5.000 μg/ml.

Reagents for HPLC

The volume ratio of n-hexane (He) and isopropanol (Ip) (Merck, Japan) was adjusted to He/Ip = 9/1 v/v and 19/1 v/v.

Internal standard Compound

N-n-propylaniline (NnPA) was adjusted to 2.500 μg/ml.

Biological Material (Human Hair)

The hair (250 mg) from a non-MAMP user was taken for control, and that (200–250 mg) from abusers as specimens.

Analysis

Sample Preparation. The above mentioned materials were extracted by the routine procedure [7] with organic solvents and converted into acetyl (Ac) derivatives. These derivatives were dissolved in 100 μl n-hexane, and 20 μl samples were analyzed by HPLC.

Calibration Curves. Absolute calibration curves were made on the basis of the ratio of the peak area of each *d*- and *l*-Ac-MAMP and *d*- and *l*-Ac-AMP in various concentrations to that of the internal standard (NnPA-Ac).

Analytic Conditions. We used HPLC devices (UV-8000, CCPN, FBR-2) manufactured by Toyo Soda Co., Shimadzu Digital Integrator (ITG-4A) and two types of columns, one containing an optically inactive carrier (Toyo Soda Co.: TSK gel/ODS-80 TM, 25 cm × 4.6 mm i.d.) and the other containing an optically active carrier (Daicel Ind. Co.: Chiralcel OB, 25 cm × 4.6 mm i.d.). The temperatures in the thermostat were set at 4°C, 22°C, and 40°C.

The solvent system ratio and flow rate of the mobile phase in the analysis of the optical purity of MAMP were He/Ip = 9/1 v/v and 1.0 ml/min, respectively, and those in the analysis of biologic material (hair), He/Ip = 19/1 v/v and 1.0 ml/min or 1.1 ml/min using a set of the same optically active column. The wavelength of UV used for the determination was 215 nm.

Results

Determination of the Optical Purity of MAMP

Examination of Analytic Condition for MAMP. When the *dl*-Ac-MAMP was analyzed at 22°C using an optically inactive column, it was not separated into the *d*- and *l*-Ac-MAMP, and they were detected together at the same retention time.

Thus, we examined the optimum temperature for the analysis of *dl*-Ac-MAMP using the optically active column. When the temperature was raised to 40°C, the resolution improved markedly, with excellent quantitative results as shown in Fig. 1. The resolution of *dl*-Ac-MAMP at 4°C was inferior to that at 40°C or 22°C.

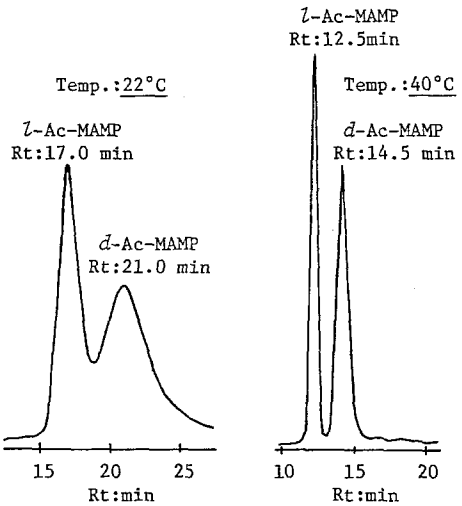

Fig. 1. Resolution difference of HPLC analyses of racemic acetyl-methamphetamine [*dl*-Ac-MAMP] at temperatures of 22°C and 40°C using an optically active column. *d*- or *l*-Ac-MAMP: *d*- or *l*-acetyl-methamphetamine. Conditions: mobile phase: He/Ip (9:1 v/v), flow rate: 1.0 ml/min. Detector UV (215 nm), range (ABU/FS: 0.08)

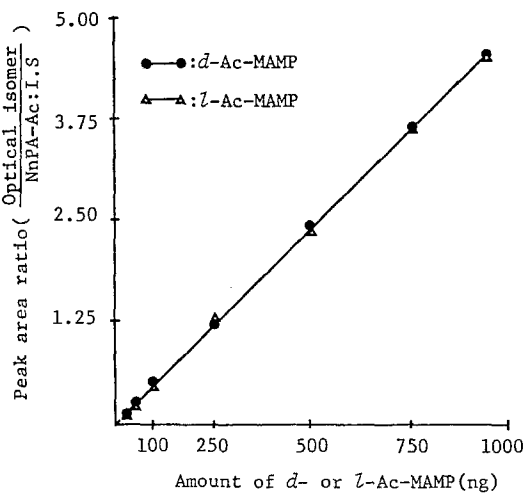

Fig. 2. Calibration curve of optical isomers of MAMP (*d*- or *l*-Ac-MAMP) at 40°C

These results led us to set the temperature for the subsequent analysis to 40°C.

Recovery Rate, Analytic Accuracy and Detection Limit. The recovery rate (%) from the *d*- and *l*-MAMP solutions (5,000 ng/ml) was 95.4 ± 1.3% ($n = 5$) and 94.8 ± 1.8% ($n = 5$), respectively. The detection limit for both *d*- and *l*-Ac-MAMP was 25 ng as absolute quantity. Their calibration curves were linear, passing through the origin (Fig. 2). For the analytic accuracy of the analysis of *d*- and *l*-Ac-MAMP, a measured value of 0.116 ± 0.012 ($n = 5$) was obtained against 0.111, the mixing ratio (theoretical value) of the optical isomers, 1.082 ± 0.070 ($n = 5$) against 1.000 and 8.984 ± 0.136 ($n = 5$) against 9.000. An analytic example is shown in Fig. 3.

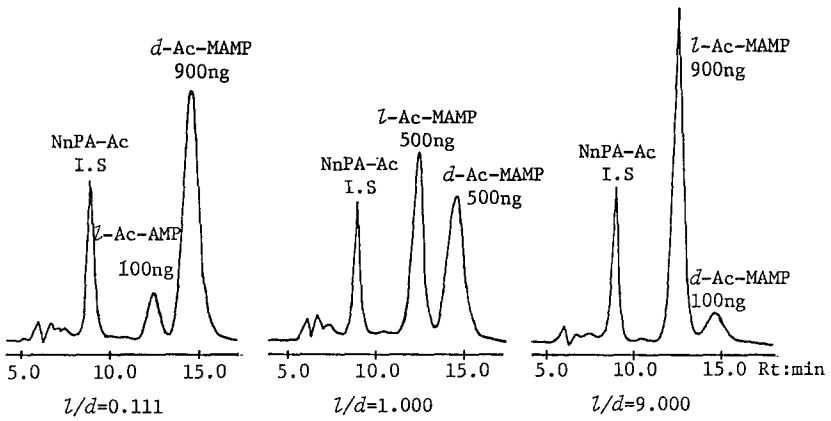

Fig. 3. HPLC chromatograms of optical isomers of MAMP (*d*- and *l*-Ac-MAMP) with three different *l*/*d* ratios (0.111, 1.000 and 9.000) at 40°C. Conditions: same as in Fig. 1

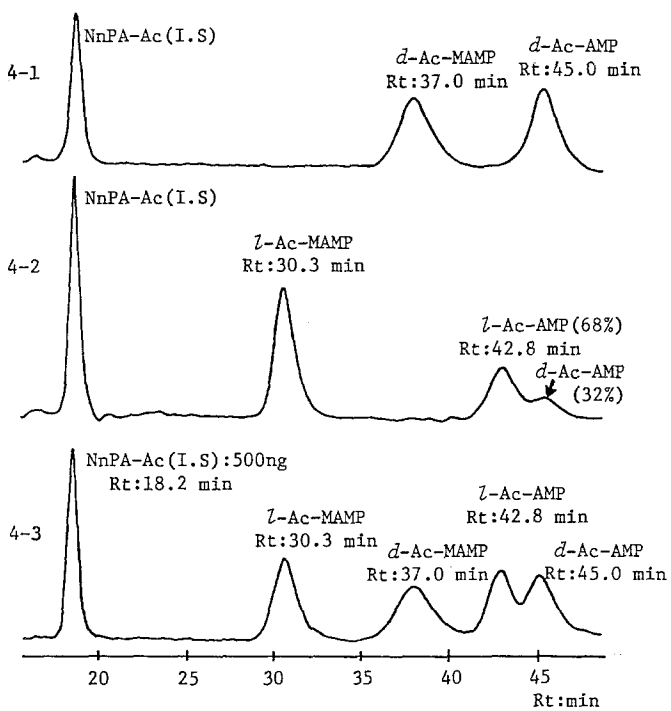

Fig. 4. HPLC analyses of biologic materials using a set of the same optically active column at 40°C. 4–1, 4–2: Standard substance (*d, l* of MAMP and AMP). *d*- and *l*-Ac-AMP: *d*- and *l*-acetyl-AMP. 4–3: Non-abuser's hair fortified with 1,000 ng of both racemic MAMP and AMP. Conditions: mobile phase: He/Ip (19:1 v/v), flow rate: 1.1 ml/min. Detector UV (215 nm), range (ABU/FS: 0.08)

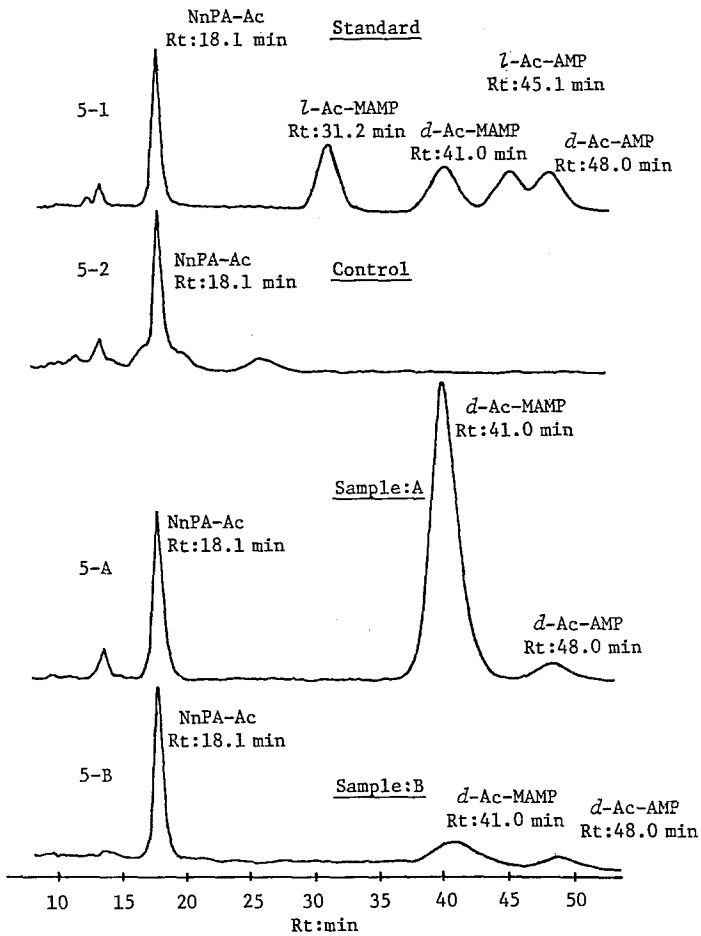

Fig. 5. HPLC analysis of human hair at 40°C. 5–1: Standard substances (racemic MAMP and AMP). 5–2: control hair. 5-A, -B: MAMP abusers' hair. Conditions: mobile phase: He/Ip (19:1 v/v), flow rate: 1.0 ml/min. Detector UV (215 nm), range (ABU/FS: 0.08)

Determination of the Optical Isomers of MAMP and AMP in Biologic Material (Human Hair)

Examination of Analytic Condition for Human Hair. When the *dl*-Ac-AMP was analyzed with the mobile phase of He/Ip = 9/1 v/v at 40°C, *dl*-AMP, a metabolite of MAMP, and *d*-Ac-MAMP overlapped each other. However, the use of the mobile phase of He/Ip = 19/1 v/v resulted in a successful separation; *dl*-Ac-MAMP and *dl*-Ac-AMP were separated into each of the optical isomers as seen in Figs. 4–1, 4–2, and 4–3. Figures 4–1 and 4–2 show the HPLC chromatograms of the standard,*d*-Ac-MAMP, *d*-Ac-AMP, and *l*-Ac-MAMP, and the complex consisted of *l*-Ac-AMP (68%) and *d*-Ac-AMP (32%). Figures 4–3 shows HPLC chromatogram of the mixture in which the standard *dl*-MAMP (1,000 ng) and *dl*-AMP (1,000 ng) are added to the normal human hair (250 mg) from a non-abuser.

The recovery rates of *dl*-MAMP and *dl*-AMP in the human hair were 93.8 ± 1.8% ($n = 5$) and 92.9 ± 2.4% ($n = 5$). When the theoretical *l*/*d* ratio was ad-

justed to 1.000, the measured ratio was 1.040 ± 0.040 ($n = 5$) and 0.980 ± 0.030 ($n = 5$) for the *dl*-Ac-MAMP and *dl*-Ac-AMP, respectively. The detection limit for both *dl*-Ac-MAMP and *dl*-Ac-AMP was 250 ng as absolute quantity.

Analysis of MAMP Abuser's Hairs

In Fig. 5 HPLC analysis of MAMP abuser's hair and controls were demonstrated. Figure 5-A and 5-B show HPLC chromatograms of the optical isomers of MAMP and its metabolite, AMP, in the hair specimens from stimulant abusers. Both *d*-Ac-MAMP at the retention time (Rt) of 41.0 min and its metabolite, *d*-Ac-AMP, at the retention time (Rt) of 48.0 min were detected in the hair of abusers. The concentrations of *d*-MAMP and *d*-AMP in the hair (A) were 14.8 μg/g and 0.7 μg/g, respectively, and those in the hair (B), 2.3 μg/g and 0.1 μg/g, respectively. No *l*-Ac-MAMP or *l*-Ac-AMP was detected. The hair from the normal individual showed no peak as seen in Fig. 5–2. In Fig. 5–1 HPLC chromatogram of the mixture of the standard substance, *dl*-Ac-MAMP (1,000 ng) and *dl*-Ac-AMP (1,000 ng) was illustrated as control.

Discussion

In Japan, the number of arrested MAMP abusers has been increasing annually. In 1985, there were 22,980 persons in custody, and as much as 294.104 kg of this stimulant drug was confiscated from gangsters. The total confiscated quantity of smuggled MAMP was 265.2 kg: 168.1 kg from Taiwan, 55.1 kg from South Korea, 24.3 kg from the Philippines, 8.0 kg from Hong Kong, and 9.7 kg from unknown countries [1].

A number of procedures is available for synthesizing MAMP. The starting material, according to Frank's report of the procedures for illicit MAMP synthesis [8], is roughly classifiable into three types: (1) phenyl-2-propane (phenyl acetone), (2) benzyl chloride, and (3) ephedrine. Among them (1) and (2) are sources of *dl*-MAMP, and two optical isomers (*d* and *l*) and a racemate *(dl)* are produced from (3) [2–5, 9, 10]. It is also possible to manufacture preparations with different *l*/*d* ratios by resolution of the asymmetric molecules (*d* and *l*) from the racemate, depending on the degree of purification. This was confirmed by the fact that a crystalline substance containing the *d* isomer in the percentage of 32 could be obtained by us in the process of resolving the *l* isomer from *dl*-AMP.

These findings suggested that the determination of the optical purity of the abused drug gave us a clue to know its smuggling route, just as Kishi et al. [11] and Baron et al. [12] assumed the production method of MAMP and the route of smuggling from analysis of impurities in the original material.

The parameter $[\alpha]_D$ and melting point (m.p.) have been used to detect optical activity in the chemical field. However, since the specific rotation of the optical isomers of MAMP is $\pm 14°$ to $\pm 20°$ and in such a small range, the determination of their optical purity *(l/d)* is inadequate. The m.p. of both the *d*- and *l*-MAMP isomers is in the range of 170°C to 175°C, which does not permit to dif-

ferentiate the two isomers. In addition, these parameters demand a large amount of specimens for assay (several hundred micrograms to several tens of milligrams). So they cannot be applied to practical analysis of the optical purity.

On the other hand, gas chromatography (GC) and radioimmunoassay (RIA) have reportedly been applied [13–15]. For GC, however, an optically active liquid phase, which is highly heat stable, and diastereomers must be produced, and for RIA a specific antiserum against the optically different compounds is required. At present, many reports on the assay of MAMP and AMP ignore completely the presence of optical isomers.

The detection sensitivity of our method (optimum resolution temperature; 40°C) for the determination of the optical purity was 25 ng, and that for human hair was 250 ng. Our method showed also a fairly favorable reproducibility. Thus, it would be a most practical procedure for detecting the optical purity of smuggled and abused drug powders, and also identifying optical isomers of MAMP and AMP in biologic materials.

Baumgartner et al. [16], Klug [17] and Nagai et al. [7] reported that hair is one of the sties of in vivo deposition of narcotics and stimulants, and that their accumulation is associated with the history of abuse. The transfer of MAMP and AMP into the hair is not known minutely. However, from the studies of the transfer into urine (Dring et al. [18], Beckett et al. [19] and Caldwell et al. [5]) and the reports concerning hair by Ishiyama et al. [20], Niwaguchi et al. [21] and Suzuki et al. [22], we know that excretion rates into urine and hair are quite different, and the optical isomers may not be converted in vivo. We suggest that both abusers tested in the present study abused *d*-MAMP. Since the concentrations of MAMP and AMP in the hair (A) specimen were about 7 times higher than those in the (B) specimen, it appeared that abuser (A) would consume *d*-MAMP in larger quantities or over a longer period than abuser (B). We regret that in this experiment just two specimens of hair could be examined because they are easily accessible only to police researchers due to the law control in Japan.

As mentioned above, considering the manifestation of the syntheses and purification procedures of MAMP or AMP, as well as presupposing that the conversion of the optical isomers in vivo does not occur, two isomers of MAMP would be detected from abusers' biologic materials in a different rate. Our forthcoming animal experiment would apprecitate the above mentioned presumption.

As mentioned above, the present method allows us to identify optical isomers in the biologic material from abusers and informs us about the optical purity of the smuggled powder.

In further studies the improvement of the detection sensitivity and the shortening of the detection period would be needed.

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