Dansyl Chloride Derivatization of Methamphetamine: A Method with Advantages for Screening and Analysis of Methamphetamine in Urine

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Abstract
The screening and quantitation of methamphetamine (MP) in urine using dansyl chloride (DNC) as the derivatization reagent were studied. Urinary MP derivatized with DNC could be detected by visual observation of the fluorescence in a solid-phase extraction column such as a Sep-Pak® C18 cartridge to which the whole reaction solution was applied. The DNC-derivatized MP was eluted from the cartridge and then identified and quantitated by gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC). In the GC–MS analysis with the MS detector in the electron-impact mode, DNC-derivatized MP and amphetamine (AP), exhibited diagnostic molecular ion peaks. The intensities of the molecular ions were 15% (DNC-MP) and 35% (DNC-AP) of the base peak (a fragment ion because of the loss of dimethylnaphthalene from M⁺), demonstrating that this method of derivatization has a major advantage for confirming APs by GC–MS. MP derivatized with DNC could be determined by HPLC with ultraviolet detection. Because a good correlation (r = 0.95) between the GC–MS and HPLC method for urinary MP was confirmed, both HPLC and GC–MS appear to be useful tools for determining urinary MP. The intensity of the cartridge fluorescence due to DNC-derivatized MP was approximately related to the urinary content of MP determined by HPLC or GC–MS, although a false positive in the visual fluorescence was observed in some urinary specimens from healthy volunteers. From these results, screening and confirmation/determination following DNC derivatization is proposed as a suitable method for the analysis of MP.

Introduction

Immunoscreening and subsequent analysis by gas chromatography coupled with mass spectrometry (GC–MS) are widely used for the analysis of illicit methamphetamine (MP) in biological samples (1). However, there are some problems which need to be solved. For example, immunoscreening detects amphetamines (APs) and many other related drugs; AP and MP derivatized with reagents routinely used exhibit no, or only a very weak, molecular ion peak during GC–MS analysis.

Our previous study has shown that (1) dansyl chloride (DNC)-derivatized AP and MP (Figure 1) can be retained in a commercial reversed-phase cartridge and (2) the fluorescence of these derivatives in the cartridge is readily apparent under UV light (2). That no fluorescence is seen in the cartridge in the case of urine samples not containing APs suggests that this method is suitable for screening for APs (2). Because this method detects compounds other than AP and MP, such as desipramine and tyramine, the drug screen is not completely specific for APs (2). However, many other drugs of abuse, such as opiates and cocaine, gave negative results in this system. Furthermore, because the described method detects only primary and secondary amines, detection of tertiary amines such as methylephedrine can be ruled out. The DNC-derivatized drugs retained in a cartridge can then be subjected to further examination using systems with high selectivity and sensitivity. If this is possible, a series of screening and confirmation tests can be carried out following a single derivatization. Our previous report demonstrated the usefulness of DNC derivatization for screening, but its applicability to MP confirmation and determination was not reported. To address this possibility, we studied the GC–MS and high-performance liquid
chromatography (HPLC) analyses of urinary APs following DNC-derivatization.

**Experimental**

**Materials**

d-MP hydrochloride was purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). d-AP sulfate was donated by Dr. T. Yanagita (Central Institute of Experimental Animals, Kawasaki, Japan). Methyleneedioxymethamphetamine (MDMA) hydrochloride was supplied by the Japanese Ministry of Health and Welfare. DNC was purchased from Sigma Chemical Co. (St. Louis, MO). Sep-Pak® C18 plus cartridges were obtained from Waters Corp. (Milford, MA). MP-d4 (deuterium-labeled at the methine and methyl moiety) was donated by Dr. Y. Nakahara (National Institute of Health Sciences, Tokyo, Japan). Prenylamine was a gift from Sanwa Chemical Co. (Nagoya, Japan). All other reagents were of the highest quality commercially available.

**DNC---derivatization and screening**

The analytical procedures using DNC as a derivatization reagent for urinary APs are summarized in Figure 1. Derivatization was performed according to the method reported elsewhere (2,3). In standard conditions, 0.5 mL human urine from healthy volunteers spiked with test drug was mixed with 3 mL DNC reagent. In the quantitative determination, MP-d4 (30 ng/0.5 mL urine) for GC–MS or prenylamine lactate (2.1 µg/0.5 mL urine) for HPLC was added to the urine as the internal standard. DNC reagent was prepared by mixing equal volumes of 10mM sodium carbonate (pH 9) and 1mM DNC in acetone, and stored at 4°C. The reaction mixture was heated at 45°C for 1 h and applied to a Sep-Pak C18 cartridge pretreated with 5 mL methanol and 10 mL water. The cartridge was washed with 10-mL quantities of both water and 50% (w/w) acetone. The fluorescence in the cartridge was observed under UV light (366 nm) demonstrating the presence of MP in the sample. The DNC-derivatized drug retained in the cartridge was eluted with 5 mL acetone. After evaporation of the solvent, the residue was reconstituted in 200 µL methanol followed by GC–MS and HPLC analyses. Derivatized MP and AP in solid state could be stored at 4°C without decomposition.

**GC–MS analysis**

A GC (Japan Electron Optics, Tokyo, Japan) equipped with a semi-capillary column (DB-5, 15 m × 0.25-mm i.d., 0.25-µm film thickness) was used. The operating conditions were as follows: column temperature, linear gradient from 80°C to 280°C (20°C/min) and then held 280°C for 6 min; injection port temperature, 280°C; carrier gas (N2), 1 mL/min; ion source temperature, 280°C; and ionizing voltage, 70 eV.

**HPLC analysis**

HPLC analysis was carried out under the following conditions: instrument, Hitachi L-6200 pump equipped with an UV detector (Hitachi L-7420); column, NOVA Pak C18 cartridge (0.8 × 10 cm, 4-µm particle size, Waters Corp.); mobile phase, solvent A [1mM imidazole (pH 7)/acetonitrile (3:7, v/v)] for 5 min, switching to solvent B [1mM imidazole/acetonitrile (1:9, v/v)] for 1 min, solvent B for 15 min; detection, UV absorption at 340 nm. Under these conditions, DNC-derivatized AP, MP, and prenylamine, an internal standard, were separately detected at retention times of 10.6, 12.4, and 19.8 min, respectively. The calibration curves for MP and AP were linear in the range between 20 to 800 ng/mL urine (data not shown).

**Results**

**GC–MS analysis of DNC-derivatized APs**

GC–MS chromatographic profile of DNC-derivatized MP is shown in Figure 2. MP was added to urine from a healthy volunteer, derivatized with DNC, and extracted using a Sep-Pak cartridge under the conditions described in the Experimental section. GC analysis (total ion monitoring) showed a peak of DNC-derivatized MP at about 12.7 min that was not present in the control urine. DNC-derivatized AP and MDMA were detected at 12.4 and 15.8 min, respectively (chromatograms not shown), and there were no peaks of urinary components that interfered with the detection of these drugs.

The electron-impact mass spectra of DNC-derivatized APs that were eluted from the GC are shown in Figure 3. Interest-
ingly, diagnostic molecular ion peaks were observed in the spectra of DNC-derivatized MP and AP, although DNC-derivatized MDMA exhibited no molecular ion in its mass spectrum. As shown in each spectrum, a fragment ion due to the loss of a benzyl moiety was detected. For example, DNC-derivatized MP and AP exhibited fragment ions of \( m/z \) 291 and 277, respectively. The base peak of all the drugs was the fragment ion due to the formation of \( N,N \)-dimethylaminonaphthalene (\( m/z \) 170).

In the quantitative determination of MP by GC–MS, the selected ion of \( m/z \) 291 together with \( m/z \) 295 of DNC-derivatized MP-d\(_4\), an internal standard, were monitored. For the quantitation of AP, a fragment ion of \( m/z \) 277 was monitored. The calibration curve obtained using this method was linear at least over the range 20 to 800 ng MP and AP per milliliter of urine (Figure 4).

**Determination of MP in drug abusers**

The urinary content of MP and AP in 16 drug abusers was analyzed by DNC-derivatization followed by GC–MS and HPLC methods (Table I). Although the values listed in Table I are the mean of two determinations, reproducibility of GC–MS method was confirmed: when four different samples with MP content of 2–27 ng/mL urine were analyzed in triplicate, the relative standard deviation was less than 13%. In separate experiments, the urine diluted 10-, 30-, and 100-fold with water as well as

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**Figure 2.** Gas chromatogram of dansyl chloride-derivatized MP. Human urine spiked with MP (2 ng/mL) (A) and control urine (B) was treated with DNC, and subjected to extraction with a Sep-Pak cartridge, followed by GC–MS (total ion) analysis. DNC-derivatized MP purified by cartridge extraction was dissolved into 200 \( \mu L \) methanol, and an aliquot (1 \( \mu L \)) was subjected to GC. Arrow indicates the peak of DNC-derivatized MP. Experimental section gives the details of the chromatographic conditions.

**Figure 3.** Electron-impact MS spectra of DNC-derivatized MP (A), AP (B), and MDMA (C). Postulated assignment of fragmentation is shown inside the figures.

**Figure 4.** Calibration curves for MP (closed symbols) and AP (open symbols) obtained for selected ion monitoring in the GC–MS analysis. For the determination by GC–MS, the ions at \( m/z \) 291 (MP) and \( m/z \) 277 (AP) were monitored, respectively. Internal standard (MP-d\(_4\)) was monitored, using an ion at \( m/z \) 295.
undiluted urine were treated with DNC reagent, applied to cartridges and then the cartridge fluorescence was observed. The degree of fluorescence was classified as strong positive (++), positive (+), quasi-positive (±), or negative (−) (Table I, see also Figure 5 for typical photograph). The HPLC method showed that the MP content of the samples used in this study was 0.35 to 352 μg/mL. Although all samples were not analyzed by the GC–MS method, the content obtained by the two methods was correlated (Figure 6). This observation suggests that conventional HPLC methods as well as the GC–MS method can be used to determine urinary MP.

On the other hand, analysis of AP by the HPLC method has some problems; for example, the HPLC method showed an extremely high content of AP in samples 2, 3, and 6 in comparison with the values obtained by GC–MS (Table I). Generally, the AP content determined by HPLC tends to be higher than that obtained by GC–MS (Table I). The reason for this inconsistency was not further clarified in this study, but substances that interfere with the AP analysis are assumed to be present in some urine samples.

The fluorescence of the cartridge charged with undiluted urine was strongly positive except for one sample (#16), and the intensity decreased as the urine was diluted (Table I). Many samples required 30- or 100-fold dilution to reduce the fluorescence intensity in the cartridges. Some urine specimens from healthy volunteers were weakly positive or quasi-positive, although a 10-fold dilution made them quasi-positive or negative, respectively (Table I). To investigate the relationship between cartridge fluorescence and the content of urinary MP, the drug abusers shown in Table I were grouped according to the fluorescence intensity in the cartridges (Figure 7). Figure includes fluorescence data from diluted urine as well as undiluted samples, and the MP content in diluted samples was calculated after dividing the MP content of the undiluted sample by the dilution factor. The mean values of the MP content in the ±, +, and ++ groups are shown in Figure 7. In the + group, two values (28.6 and 0.003) were much higher and lower than other values observed. If these data were omitted as abnormal values, a mean of 0.53 μg/mL was obtained. This correction is likely to be acceptable, and taking the MP content in the ± group (see Figure 7) into consideration, the practical limit necessary for a weak positive cartridge fluorescence appears to be about 0.5 μg/mL. On the other hand, in the ++ group consisting of 42 items of data, a mean of 24.9 was obtained (not shown in Figure 7). However, it is not helpful to know the MP content needed for a strong positive, because this group contains many samples having a very high MP content, such as 154 and 352 μg/mL (see Figure 7). A large number in this group (31 items of data, about 74% of the total) contained less than 20 μg/mL of MP, and they covered a relatively narrow range of MP content. When calculated using this subgroup (< 20 μg/mL), a mean of 3.69 was obtained. Therefore, the MP content required

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* The samples with Arabic numerals are abusers' urine, and those of A-C are urine from healthy volunteers.

* In this experiment, urine was diluted 10 (× 10), 30 (× 30), and 100- (× 100) times with de-ionized water, and the aliquot (0.5 mL) was treated with DNC reagent followed by application to cartridge. Urine was 40- or 80-times diluted with de-ionized water, and the 0.5 mL was subjected to the assay. Each value represents the mean of two determination.

* N.A.: not analyzed; N.D.: not detected.
for strong fluorescence in the cartridge appears to be about 4 μg/mL. This accords with our previous work indicating that a few micrograms of MP per milliliter of urine exhibits distinct fluorescence in the cartridge (2).

Discussion

Analysis using GC–MS is routinely used for the confirmation of urinary MP (1,4). For this purpose, MP is usually derivatized, prior to analysis. A number of reagents, trifluoroacetic anhydride (5,6), pentafluoropropionic anhydride (7), heptafluorobutyric anhydride (6), perfluorooctanoyl chloride (8), trichloroacetic anhydride (6), 4-carbethoxyhexafluorobutyryl chloride (9), and chloroformates (10,11), have been used for derivatization. However, MP derivatized by the above methods shows no, or very weak, molecular ion peaks in its mass spectrum. This study found that DNC-derivatized MP and AP exhibited diagnostic molecular ion peaks in the mass spectra obtained in conventional electron-impact mode. Detection of the molecular ion or quasi-molecular ion peak of MP has already been reported. For example, the following technology can achieve this purpose: GC–chemical impact MS (12,13), HPLC equipped with thermospray MS (14), and capillary electrophoresis coupled with time-of-flight MS (15); however, GC–MS in electron-impact mode is now used much more widely than these methods. Taking this into consideration, it is possible to demonstrate that the DNC derivatization method has the advantage that it offers improved MP/AP analysis. The analysis with DNC derivatization needs purification with an extraction column. However, this procedure combines cleanup with screening. On the other hand, widely employed analytical methods require extraction of MP and AP before derivatization. Therefore, the method reported here can be performed in an amount of time similar to that needed for routinely used methods including acylation and other derivatization.

As reported previously (2), fluorescence in a cartridge containing DNC-treated urine could be used as a screen for the presence of MP. This study examined the validity of such cartridge fluorescence, and it seems to be a possible screen because of the observed correlation between the fluorescence intensity and MP content. However, it should be noted that, in some cases, control urine exhibited a positive fluorescence. As indicated, the AP content of urine from some MP abusers determined by the HPLC–UV method was much greater than

![Figure 5. Fluorescence of cartridges following application of DNC-treated urine from MP abusers. A urine sample (#15, also see Table I) was diluted several-fold as indicated in the figure, and the diluted and undiluted samples were applied to cartridges. Although no photographs are shown, control urine showing negative fluorescence appears very similar to the cartridge at the extreme right in the figure.](image)

![Figure 6. Correlation of the content of urinary MP between HPLC and GC–MS methods.](image)

![Figure 7. Distribution of methamphetamine content in groups classified by observation of cartridge fluorescence. See text and Table I for grouping MP abusers due to cartridge fluorescence. Solid horizontal bars indicate the mean values. In the + group, two values (28.6 and 0.003) much higher and lower than other values were observed. The corrected value indicated by the dashed bar was calculated by omitting the above two values. The MP content needed for a strong positive (++ group) is assumed by using the subgroup indicated by the arrow with an MP content less than 20 μg/mL.](image)
that assayed by GC–MS. One possible reason for this discrepancy seems to be the presence of materials that are chemically similar to AP and capable of derivatization with DNC. If this is the case, the cartridge fluorescence in control urine may be rationalized. In this study, we did not identify the compound responsible. Our previous study indicated that DNC-derivatized tyramine exhibited cartridge fluorescence even after washing the cartridge with 50% acetone (2). Thus, cartridge fluorescence as a screening and analytic procedure for AP following DNC-treatment of urine is not absolutely trustworthy. However, this does not affect the advantages of the DNC derivatization, that is, molecular ion formation in EI-MS spectroscopy and easy analysis of MP in HPLC. Forensic chemists need preliminary information about the MP content, prior to carrying out a quantitative analysis to confirm and determine the drug. Concerning this point, it is likely that monitoring the fluorescence of cartridges which contain diluted as well as undiluted urine can be used to obtain this information. If four cartridges are observed as shown in Table I, the dilution factor required for the disappearance of the fluorescence can be obtained. This information will help in determining the approximate MP content.

In this study, we derivatized MP with DNC for 1 h. However, the reaction time can be shortened to 5 min by heating (2). Detection sensitivity for visual observation of cartridge fluorescence is the same as the immuno-screening method such as Triage™ (2). The present method requires testing with diluted sample for fine screening, and this may be a shortcoming of this method. However, this procedure gives useful information that suggests the dilution factor needed for subsequent GC–MS and HPLC analysis. As described, DNC derivatization has the advantage of exhibiting molecular ion peak in GC–MS. Taken together, a series of screening and analysis using DNC derivatization is a practical and useful method for the confirmation of urinary MP.

Conclusions

A diagnostic molecular ion peak was confirmed in the electron-impact MS spectra of DNC-derivatized MP/AP, suggesting that this method of derivatization is useful for the analysis of MP and AP in GC–MS. MP derivatized with DNC was determined by HPLC with UV detection with accuracy equivalent to GC–MS analysis. Relationship between the intensity of cartridge fluorescence and MP content in urine was determined. Although determination of DNC-derivatized AP by HPLC lacks reliability, screening and analysis using DNC derivatization is shown to be an useful method for detecting MP in urine.

Acknowledgment

This work was supported by Health Science Research Grants FY 1998–2000 for Comprehensive Narcotic Control Research from the Ministry of Health and Welfare, Japan.

References


Manuscript received February 22, 2001; revision received June 22, 2001.