Z Rechtsmed (1988) 100:231–236

Zeitschrift für
Rechtsmedizin

Simultaneous Quantitative Analysis of Methamphetamine and 4-Hydroxymethamphetamine in Body Fluids by Gas Chromatography/Mass Spectrometry

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Summary. Pentodeuterated 4-hydroxymethamphetamine(HMAMP-d5),1-(4-hydroxyphenyl)-2-(methyl-d_3-amino)-propane-1,2-d_2 of high quality was prepared and proved to be a most useful internal standard for quantitative analyses of 4-hydroxymethamphetamine(HMAMP), a main metabolite of methamphetamine(MAMP). Highly reliable results were obtained by gas chromatography/mass spectrometry. Simultaneously we determined also MAMP, amphetamine(AMP), 4-hydroxyamphetamine(HAMP), and HMAMP in body fluids, using two internal standards, HMAMP-d5 for HMAMP and HAMP and pentodeuterated methamphetamine, 1-phenyl-2-(methyl-d_3-amino)-propane-1,2-d_2, for MAMP and AMP.

Key words: 4-Hydroxymethamphetamine, metabolite of methamphetamine – Analysis in body fluid, methamphetamine and 4-hydroxymethamphetamine

Zusammenfassung. Pentadeuteriertes 4-Hydroxymethamphetamin (HMAMP-D5),1-(4-Hydroxyphenyl)-2-(methyl-D3-amino)propan-1,2-D2 wurde in hoher Ausbeute synthetisiert und als brauchbarer interner Standard im Rahmen der quantitativen Analyse von 4-Hydroxymethamphetamin, einem Hauptmetaboliten des Methamphetamins (MAMP) eingesetzt. Die gaschromatographisch-massenspektrometrische Untersuchung lieferte gut reproduzierbare Resultate. Weiterhin gelang die simultane Bestimmung von MAMP, Amphetamin(AMP), 4-Hydroxymethamphetamin(HAMP) und HMAMP in Körperflüssigkeiten bei Verwendung zweier interner Standards: HMAMP-D5 für HMAMP und HAMP, bzw. pentadeuteriertem Methamphetamin(1-Phenyl-2-(methyl-D3-amino)-propan-1,2-D2) für MAMP und AMP.

Schlüsselwörter: 4-Hydroxymethamphetamin – Amphetamin – Methamphetamin

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Introduction

The determination of methamphetamine(MAMP) and its metabolites in body materials is required to obtain evidence for drug addiction and to estimate related toxic aspects. We reported that pentodeuterated methamphetamine(MAMP-d5), 1-phenyl-2-(methyl-d_3-amino)-propane-1,2-d_2, can be used as an internal standard for quantitative analyses of MAMP and amphetamine(AMP) in body materials, using gas chromatography/mass spectrometry (GC/MS) [1]. While there are the reports [2, 3] on the GC analysis of 4-hydroxymethamphetamine (HMAMP), one of main metabolites of MAMP [4], use of the internal standard seemed to require further consideration. As the nature of HMAMP differs from that of MAMP, other compounds should be given attention when attempting to acquire an internal standard for quantitative analyses of HMAMP.

We prepared pentodeuterated 4-hydroxymethamphetamine(HMAMP-d5), 1-(4-hydroxyphenyl)-2-(methyl-d_3-amino)-propane-1,2-d_2, for use as an internal standard for assay of HMAMP by GC/MS. The simultaneous determination of MAMP, AMP, HMAMP, and 4-hydroxyamphetamine(HAMP) in body fluids can also be done. As HAMP and HMAMP could not be extracted using the previous method, the method of Terada [2] was modified.

Materials and Methods

Agents

Dimethyl-d_6 sulfate and aluminum oxide (Aluminum oxide 90 active, neutral, for column chromatography) were obtained from E. Merck (Darmstadt, FRG), 47% HBr was from Wako Pure Chemical Industries Ltd., and methamphetamine(MAMP) hydrochloride was from Dainippon Seiyaku Co. Ltd. Amphetamine(AMP) sulfate was prepared by the method of Lindeke and Cho [5]. 4-Hydroxyamphetamine(HAMP) hydrobromide (m.p. 195°–196°C) was prepared by heating 4-methoxyamphetamine [5] in 47% HBr at 125°C and recrystallization from isopropanol-diethyl ether [6]. 4-Hydroxymethamphetamine (m.p. 169.5°–171°C, HMAMP) was synthesized through a similar process to the following pathway for 4-hydroxymethamphetamine-d_5(HMAMP-d5). Pentodeuterated methamphetamine(MAMP-d5) hydrochloride was prepared as described [1]. Standard solutions including amines were prepared in similar manner as described [1].

Pathway of the Synthesis of HMAMP-d5

3.1 g of 1-(4-methoxy-phenyl)-2-amino-propane-1,2-d_2 hydrochloride, m.p. 218°–220.5°C, prepared by the method of Lindeke and Cho [5], was dissolved and alkalized in distilled water, and extracted with diethyl ether. The ether dehydrated with potassium carbonate was transferred to a flask and condensed in a water bath at 50°C. Next, 6 ml benzaldehyde was poured into the flask, and the preparation was heated in a boiling water bath for 2 h. A Schiff base of the primary amine and benzaldehyde was formed [6, 7]. The base was mixed with 6 ml dimethyl-d_6 sulfate in 20 ml of benzene and heated in a steam bath for 1 h. The resulting salt was extracted into 30 ml of distilled water. The water phase was continuously heated on the steam bath and washed with diethyl ether until the odor of benzaldehyde disappeared. The cooled water solution was alkalized with 10 N NaOH, extracted with diethyl ether, and dehydrated with potassium carbonate. The solution was applied on a column of aluminum oxide, 30 cm × 2.2 cm i.d. glass tube with a glass filter. After absorption, the column was washed with ethanol-diethyl ether (2% and 4%, 50 ml each), and the N-methylamine was eluted with ethanol-

diethyl ether (6%, 10%, and 20%, 50 ml each). A few milliliters of conc. HCl was added into the solution condensed to a suitable volume and pentodeuterated 4-methoxymethamphetamine [MOMAMP-d5,1-(4-methoxy-phenyl)-2-(methyl-d_3-amino)-propane-1,2-d_2] was crystallized as a hydrochloride salt. The salt was recrystallized from ethanol-diethyl ether. The purified material is a white fine powder with m.p. 183.5°–184.5°C, and the yield was 1.2 g. When MAMP-d5 was synthesized by modifying the above process, this method was superior to our previous one [1], with regard to yield.

One gram of MOMAMP-d5 hydrochloride was dissolved in 5 ml 47% HBr by the heating at 125°C. The solution was then heated at 125°C for 2 h, evaporated to remove excess HBr in vacuo, and diluted with a small amount of distilled water. As we were not able to obtain the crystalline salt, the method of Buzas and Dufour [8] was modified to obtain a base crystal. HMAMP-d5 was extracted with ethyl acetate-dichloromethane (3:1) after the solution had been saturated with ammonium carbonate. The extract was dried in vacuo and the powder recrystallized from ethanol. The crystal is a white powder with m.p. 168°–169°C, yield 0.27 g.

Preparation of Sample

Two milliliters of body fluid was placed into a centrifuging glass tube with a glass stopper. Five micrograms of each of internal standards, 2 ml 20% Na_2CO_3, and 2 g NaCl were added and the solution was extracted with 8 ml ethyl acetate. The organic phase was transferred into another tube, and back-extracted into 2.2 ml of 0.1 N HCl. In an extracting glass tube, 2 ml aqueous phase was mixed with 2 ml 20% Na_2CO_3 and 2 g NaCl, and re-extracted in 1.5 ml ethyl acetate. Of the organic phase 1.2 ml was collected in a glass container with a plastic stopper and evaporated to the volume of 0.5 ml for the trifluoroacetylation. Into the extracted solution ca. 0.1 ml trifluoroacetic anhydride was added, and the mixture was heated at 50°C for 30 min. Finally, the resulting solution was evaporated to ca. 0.05 ml, under a stream of nitrogen at 40°C and injected onto a GC/MS.

Conditions of GC/MS

Apparatus: Shimadzu LKB 9000 Gas Chromatograph/Mass Spectrometer [electron impact (EI) mode] controlled through GCMSPAC 500D. Ionization energy: 20 eV. Accelerating voltage: 3,500 V. Ion source temperature: 270°C. Monitoring ions for selected ion monitoring (SIM): m/z 140 for AMP and HAMP, m/z 154 for MAMP and HMAMP, and m/z 158 for MAMP-d5 and HMAMP-d5. Column: 2 m × 3 mm i.d. glass tube packed with 5% OV 17 on Chromosorb W, HP (80/100 mesh). Temperatures: Injection port 240°C, column oven 190° to 220°C (programmed at 7°C/min), separator 250°C. Carrier gas: Helium at a flow rate of 30 ml/min.

Results and Discussion

Confirmation of HMAMP-d5

HMAMP-d5 was trifluoroacetylated and checked using mass spectrometry. The EI (electron impact) and CI (chemical ionization) mass spectra of HMAMP-d5 are shown in Figs. 1 and 2, in parallel with those of HMAMP and trideuterated 4-hydroxymethamphetamine(HMAMP-d3,N-methyl-d_3) prepared using a similar process. Quasi molecular ions with an additional proton were found in three CI spectra taken under the same conditions as in the previous report [1] and they showed numbers of additional deuterium on the derivatives. The fragmentation of these derivatives was similar to one of the MAMP derivatives in the EI [9]. The ion at m/z 158 of HMAMP-d5 ressults from β-cleavage, and the ion at m/z 231 or m/z 232 from α-cleavage with deuteron or protein transfer.

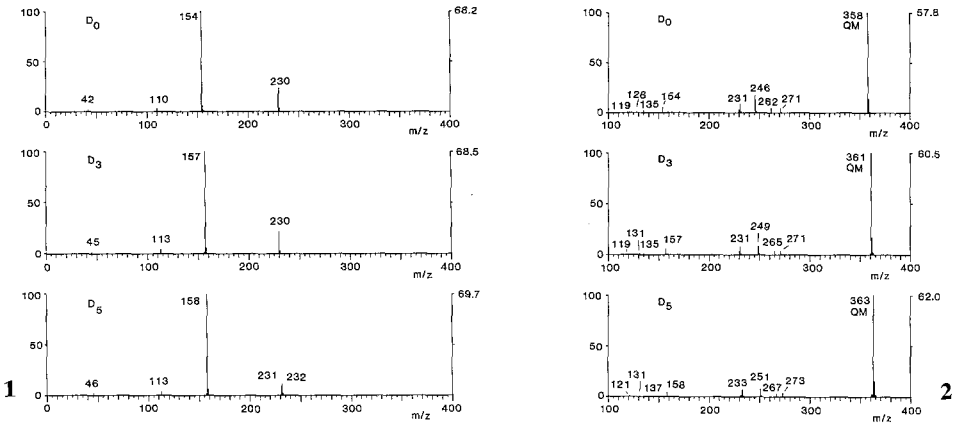

Fig. 1. Mass spectra of HMAMP(D_0), HMAMP-d3(D_3), and HMAMP-d5(D_5) in the EI mode

Fig. 2. Mass spectra of HMAMP(D_0), HMAMP-d3(D_3), and HMAMP-d5(D_5) in the CI mode

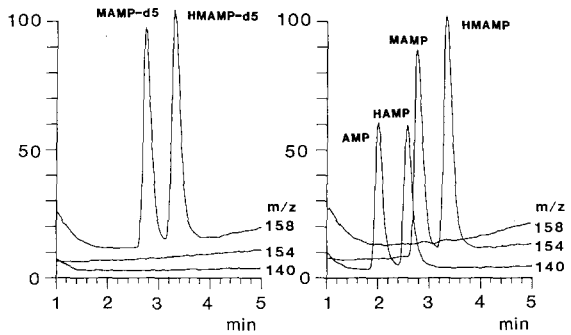

Fig. 3. Chromatograms of internal standards (MAMP-d5 and HMAMP-d5) and standards (AMP, MAMP, HAMP, and HMAMP) in the GC/SIM

Quantitative Analysis

GC/SIM in the EI system was used for the quantitative analysis. The ions from β-cleavage were selected as monitoring ones, m/z 140 for AMP and HAMP, m/z 154 for MAMP and HMAMP and m/z 158 for MAMP-d5 and HMAMP-d5. No contamination was observed between MAMP and MAMP-d5, or HMAMP and HMAMP-d5, as shown in Fig. 3. No interference peaks appeared in connection with the urine and blood. A chromatogram from the extract sample of the amine mixture is shown in Fig. 4. Six peaks were identifiable with these six compounds.

The standard curves for quantitative analysis were prepared from 2 ml water samples containing standards at 0.01, 0.05, 0.10, 0.50, and 1.0 μg each and internal standards at 1.0 μg each. MAMP-d5 was the internal standard for MAMP and AMP, and HMAMP-d5 for HMAMP and HAMP. The curves obtained by plotting the peak area ratios between standard amines and internal standards were linear in the range from 0.01 μg to 1.0 μg for MAMP and

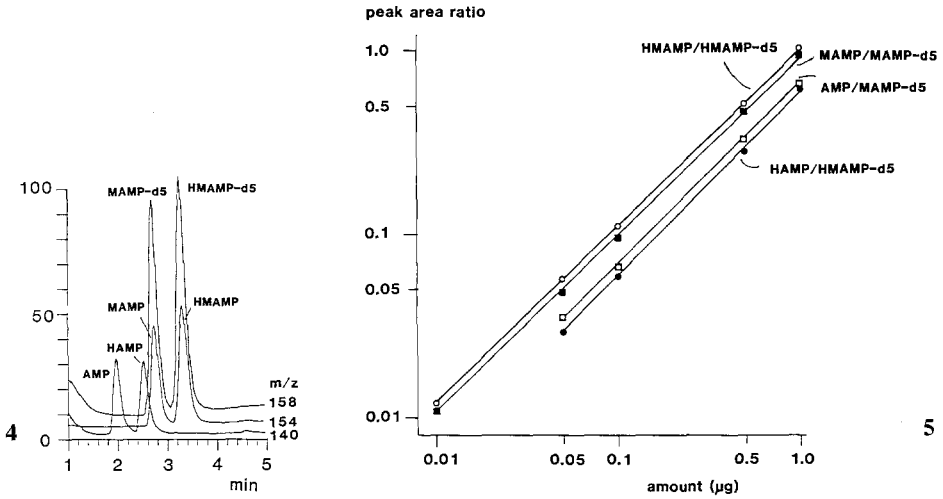

Fig. 4. Chromatogram of the blood sample containing 0.5 μg each of four standards and 1.0 μg each of two internal standards in the GC/SIM

Fig. 5. Standard curves for quantitative analysis of AMP, MAMP, HAMP, and HMAMP

Table 1. Reproducibilities

	Blood	Urine
AMP	0.494 ± 0.010 (2.0)	0.483 ± 0.009 (1.9)
MAMP	0.499 ± 0.005 (1.0)	0.494 ± 0.008 (1.7)
HAMP	0.451 ± 0.024 (5.3)	0.481 ± 0.011 (2.3)
HMAMP	0.499 ± 0.009 (1.8)	0.488 ± 0.005 (1.0)

Value (μg) = mean ± SD (cv %)

Table 2. Relative recoveries of HMAMP to MAMP

	Peak area ratio HMAMP/MAMP
Water	0.901 ± 0.129 (14.3)
Urine	1.057 ± 0.118 (11.2)
Blood	0.802 ± 0.088 (11.0)

Value = mean ± SD (cv %)

HMAMP, and from 0.05 μg to 1.0 μg for AMP and HAMP (Fig. 5). The reproducibilities of values ($n = 8$) at 0.5 μg in the curves were 2.0% for AMP, 1.3% for MAMP, 3.2% for HAMP, and 1.9% for HMAMP, as coefficients of variation (cv). These amines were detectable in a sample including 0.005 μg each, but the ratios to the internal standards were not related to the standard curves.

To test the reliability in this method, samples in five series were prepared from 2 ml body fluids, each of which included 0.5 μg each of AMP, MAMP, HAMP, and HMAMP. The results determined on the standard curves are

shown in Table 1, with standard deviations (SD) and cv. The apparent recoveries of these amines from urine and blood were 96%–100%, and 90% for HAMP from blood. The relative recoveries of HMAMP to MAMP are given in the ratios of peak areas in Table 2. These results suggest the requirement of two internal standards for simultaneous analyses of MAMP and HMAMP.

Acknowledgments. We express their thanks to Prof. T. Nagata, Kyushu University, for his pertinent advice and M. Ohara, Kyushu University, for his comments on the manuscript.

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Received July 21, 1987