Ann Toxicol Anal. 2012; 24(4): 159-163
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DOI: 10.1051/ata/2012021

Available online at:
www.ata-journal.org

ANNALES DE TOXICOLOGIE ANALYTIQUE

Original article / Article original

Spectroscopic characterization of 4-methylamphetamine: formaldehyde adduct formation hindered the identification by mass spectrometry

Caractérisation spectroscopique de 4-methylamphétamine : la formation d'adduit de formaldéhyde entrave l'identification par spectrométrie de masse

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Abstract – A powder was submitted to the author's laboratory for analysis on the presence of illegal drugs. Based on the structure elucidation performed by nuclear nuclear magnetic resonance spectroscopy and analysis of the trimethylsilyl and trifluoroacetyl derivatives with gas chromatography mass spectrometry, the powder was found to contain the designer drugs 4-methylamphetamine, besides amphetamine and caffeine. The electron impact mass spectrum of 4-methylamphetamine obtained from the analysis of a methanolic sample solution showed a base ion of m/z 56 rather than the expected m/z 44, therefore no hit was found with an electron impact mass spectrum present in the mass spectral library and literature. The increase of 12 atomic mass units was explained by formaldehyde-adduct formation.

Key words: 4-methylamphetamine, formaldehyde, mass spectrometry

Résumé – Une poudre a été soumise au laboratoire de l'auteur pour analyse quant à la présence de drogues illégales. Sur base de l'explication de structure exécutée par la spectroscopie de résonance magnétique nucléaire et l'analyse du trimethylsilyl et les dérivés du trifluoroacétyle par la chromatographie en phaze gazeuse avec la spectrométrie de masse comme détection, il a été découvert que la poudre contenait de la drogue synthétique 4-methylamphétamine, en plus de l'amphétamine et de la caféine. Le spectre de masse d'impact électronique de 4-methylamphétamine obtenu par l'analyse d'une solution d'échantillon méthanolique a démontré un ion de base de m/z 56 plutôt que le m/z 44 attendu. Par conséquent, aucun résultat n'a été trouvé contenant un spectre de masse d'impact électronique présent dans la bibliothèque spectrale de masse et littérature. L'augmentation de 12 unités atomiques de masse a été expliquée par la formation d'adduit de formaldéhyde.

Mots clés : 4-methylamphétamine, formaldéhyde, masse spectrométrie

Received 7 June 2012, accepted after revision 23 November 2012
Published online 29 January 2013

1 Introduction

In 1952, the stimulant 4-methylamphetamine (1-(4-methylphenyl)propan-2-amine) was investigated as an anorexigenic agent to gain weight loss but was never completely developed and commercialized [1]. In 2010, 4-methylamphetamine was reported as a new designer drug found in an amphetamine mixture in Germany [2]. The structure elucidation by gas chromatography mass spectrometry (GC/MS) and nuclear nuclear magnetic resonance spectroscopy (NMR) was described by Westphal *et al.* [2]. The GC/MS analysis and gas chromatography-infrared detection of 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine was reported by Davis *et al.* [3].

A confiscated powder related with an overdose death was subjected to the authors systematic toxicology identification scheme based on analysis of a freshly prepared methanolic sample solution on reversed-phase liquid chromatography with

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a photodiode array UV detector (HPLC/PDA) and GC/MS (electron impact, EI). The off-white powder was found to contain an amphetamine mixture; amphetamine, methamphetamine (minor), caffeine, di-(phenyl isopropyl)amine, one major and three minor unidentified compounds. The structure of the major unknown was elucidated by analysis of the trimethylsilyl and trifluoroacetyl derivatives with GC/MS and NMR. The synthetic drug 4-methylamphetamine was identified. Formaldehyde-adduct formation hindered the identification by electron ionization GC/MS. In literature, few reports were find concerning the condensation of a parent drug with the extraction solvent or contaminant, hindering the identification or leading to the misidentification by computer based library search [4–9].

2 Materials and methods

2.1 Chemicals and reagents

The reference material 4-methylamphetamine was obtained from Promochem (Hertfordshire, England) [10]. The standard compound was diluted in methanol. Methanol was obtained from Sharlau Chemie S.A. (TMC, Belgium). Solvents were of analytical grade. N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and N-methyl-bis (trifluoroacetamide) (MBTFA) were purchased from Marchery-Nagel (Germany). The external standard diphenylamine was obtained from Alfa Aesar (Karlsruhe, Germany).

2.2 Mass spectrometry

Freshly prepared methanol sample solutions were analyzed using a Varian Star 3400 gas chromatograph used in combination with a Varian 8200 auto sampler and Varian Saturn 2000 mass spectrometer. A Varian CP-SIL 8 CB Low bleed capillary column (30 m × 0.25 mm *i.d.*, 0.25-µm film thickness) was used connected to a fused silica retention gap (2.5 m × 0.25 mm). Carrier gas was helium at a constant pressure of 12 psi. The GC oven was programmed to an initial temperature of 70 °C and hold time of 2 min, followed with an 8 °C/min ramp to a final temperature of 310 °C, and hold time of 7 min. The temperatures of the injection port and detector were set at 300 °C and 230 °C respectively. The transfer line temperature was set at 260 °C. The injection volume was 2 µL using the split less injection mode. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV of electron energy. Mass spectra were recorded in the range m/z 40–500.

For further structure elucidation, trimethylsilyl (TMS) and trifluoroacetyl (TFA) derivatives were prepared by evaporation of the methanolic sample extract at 40 °C under a gentle stream of nitrogen and subsequently heating in a sealed glass vial at 70 °C for 30 minutes in the presence of 100 µL MSTFA or MBTFA, respectively. Derivates were dried and reconstituted in acetonitrile and were analyzed on a quadrupole GC/MS using an Agilent 6890 N gas chromatograph in combination with a Agilent 7683 injector and an Agilent 5973 inert mass selective detector. A HP5-MS column (30 m × 0.25 mm *i.d.*, 0.25-µm film thickness) purchased from J&W Scientific was used connected to a fused silica retention gap (2.5 m × 0.25 mm). Carrier gas was helium at a constant flow of 1.1 mL/min. The temperature program was started at 70 °C held for 2 min, increased to 310 °C at 8 °C/min and held for 7 min. The temperatures of the injection port and the detector were set at 300 and 230 °C respectively. The transfer line temperature was set at 280 °C. The injection volume was 1 µL using the split less injection mode. Mass spectra were recorded in the range m/z 40-650.

3 Results

In the chromatogram obtained from the HPLC/PDA, a peak was observed with the same retention time (±2%) and UV spectrum (similarity index > 0.995) as 4-methylamphetamine.

A freshly prepared methanolic sample solution was analyzed on iontrap GC/MS. The full-scan chromatogram is shown in figure 1. The following compounds were detected: amphetamine, methamphetamine (minor), one major unknown, caffeine, di-(phenyl isopropyl) amine and three minor unidentified compounds. The structure confirmation and regioisomer determination of the major compound identified as a methyl ringsubstituted amphetamine by GC/MS analysis of the TMS and TFA derivates [2] was driven by the ^{1}H-NMR spectrum and confirmed by the Heteronuclear Multiple Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectra.

4 Discussion

The condensation of pseudoephedrine or ephedrine with aldehydes and ketones to form oxazolidines was previously reported [4,5]. The adduct formation with formaldehyde present as contaminant in the solvent resulted in the misidentification by library search [5]. In this case the EI-mass-spectrum obtained from the injection of a freshly prepared methanolic sample solution showed a base ion of m/z 56 rather than the expected m/z 44 as shown in figure 1. No hit was found by computer based library search (NIST MS Search 2.0). The proposed formation pathway of the 4-methylamphetamine-formaldehyde adduct is given in figure 2 and resulted in the increase of 12 amu. The adduct had almost the same retention times as the parent drugs. The condensation reaction was driven by the injector temperature. Lowering the temperature from 300 °C to 200 °C resulted in the detection of an EI-MS-spectrum with a base ion of m/z 44 revealing 4-methylamphetamine as first hit by NIST computer library search.

The mass spectra of 4-methylamphetamine obtained from the GC/MS analysis of the TFA and TMS derivates of the powder were in accordance with the findings of Westphal *et al.* [2] and mass spectra of the reference compound. The probable fragmentation pathways of 4-methylamphetamine TMS and TFA derivates are shown in figures 3 and 4 [11].

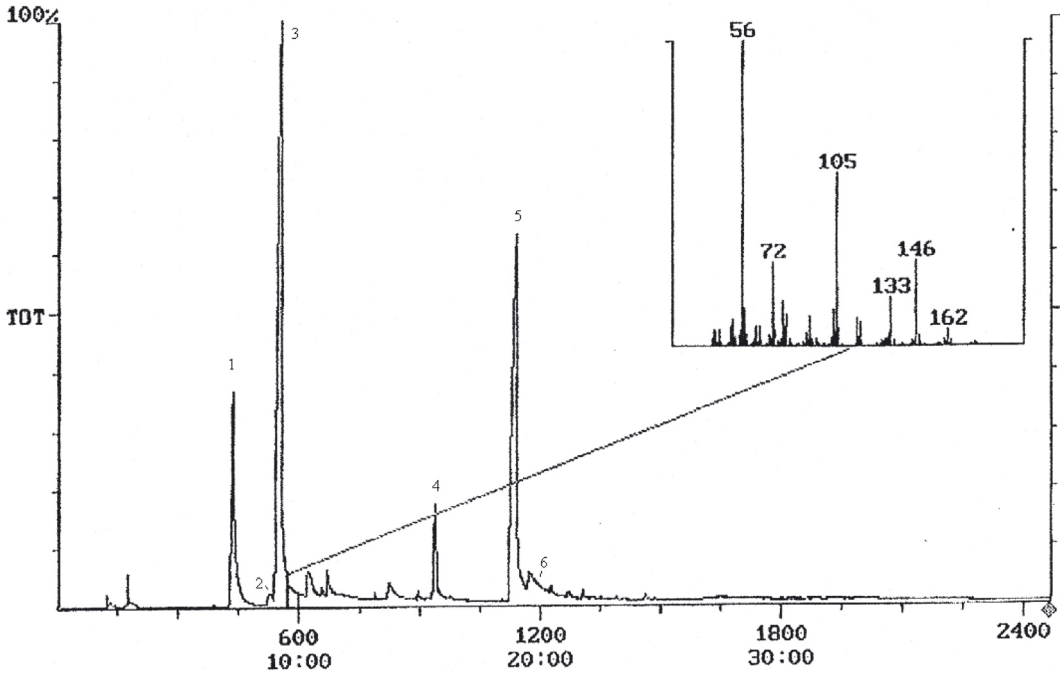

Fig. 1. Full-scan chromatogram of a methanolic sample solution with the mass spectrum of 4-methylamphetamine-formaldehyde adduct (1: amphetamine, 2: metamphetamine, 3: 4-methylamphetamine-formaldehyde adduct, 4: difenylamine, 5: caffeine, 6: di-(phenyl isopropyl) amine).

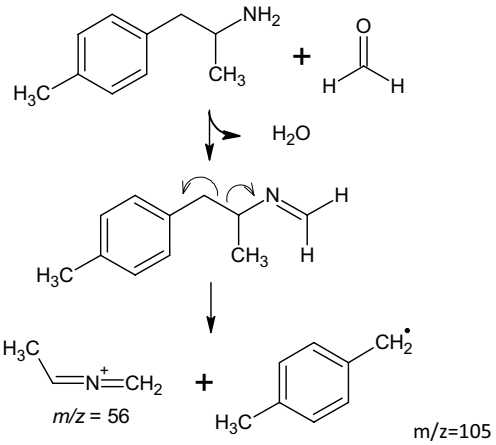

Fig. 2. Proposed formation pathway of the 4-methylamphetamine-formaldehyde adduct.

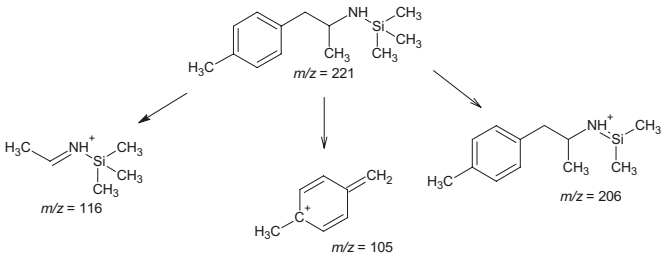

Fig. 3. Proposed fragmentation pathway of 4-methylamphetamine-TMS.

These pathways indicate that the regioisomers 2- and 3-methylamphetamine present the same mass spectral fragments. Davis *et al.* studied the mass spectrum of 2-, 3- and 4-methylamphetamine and their N-acetyl derivatives and concluded that other analytical techniques are needed to identify the correct regioisomer of a methyl ringsubstituted amphetamines [3]. This is a drawback when identification of the specific regioisomers is a legislative requirement. In this case, the position of the methyl group was determinated by NMR measurements. In the 1H-NMR spectrum the signals corresponding to the aromatic protons show a AA'BB' pattern which is characteristic for 1-,4-disubstituted aromatic rings.

In the period 2011-2012 several fatalities occurred in Belgium due to the simultaneous intake of amphetamine and 4-methylamphetamine which were notified by the Belgian Early Warning System. Several confiscated drugs, off white powders and yellow pastes, were submitted to the laboratory for analysis and were found to contain the same amphetamine mixture. Although 4-methylamphetamine is not a restricted compound in Belgium, it was always found in combination with caffeine and the regulated compound amphetamine.

5 Conclusions

The designer drug 4-methylamphetamine was identified in an amphetamine mixture by HPLC/PDA, NMR spectroscopy and GC/MS analysis. The presence of formaldehyde in a freshly prepared methanolic sample solution analyzed on a iontrap GC/MS caused the formation of an adduct, hindering the identification of 4-methylamphetamine by computer based library search. The condensation was not only driven by the presence of formaldehyde as contaminant in the solvent, but also by the injector temperature. Lowering the temperature

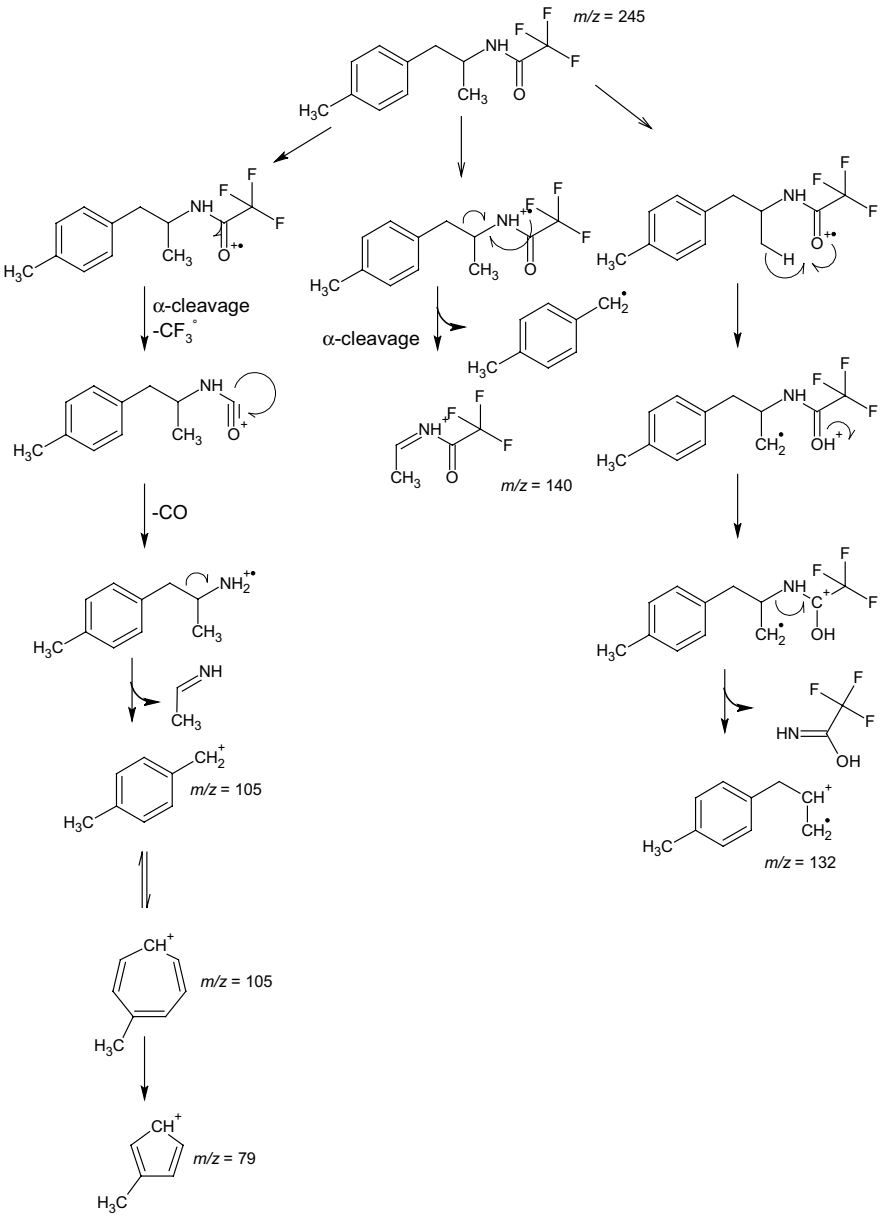

Fig. 4. Proposed fragmentation pathway y of 4-Methylamphetamine-TFA.

resulted in the detection of an EI-MS-spectrum with a base ion of m/z 44 revealing 4-methylamphetamine as first hit.

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