The Identification of a Chlorinated MDMA

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Abstract
The abuse of the designer amphetamines such as 3,4-methylenedioxymethamphetamine (MDMA) is increasing throughout the world. They have become popular drugs at all night techno dance parties, and their detection is an important issue. The objective of the presented study was to identify an unknown compound detected by thin-layer chromatography (TLC) in the urine of an illicit drug abuser. The compound was isolated by TLC and analyzed by gas chromatography–mass spectrometry (GC–MS) in electron ionization (EI) and positive ion chemical ionization (PICI) mode to elucidate its chemical structure. Based on EI-MS and PICI-MS mass spectral data, the unknown compound was indicated to be a structure similar to MDMA, substituted by a single chlorine atom—a chlorinated MDMA (CI-MDMA). To confirm the CI-MDMA structure, the unknown compound was silylated, trifluoroacetylated, acetylated, heptafluorobutyrylated, and analyzed by GC-MS. The position of the chlorine atom cannot be assigned exactly from the mass spectral data presented here; however, we believe that the unknown compound could be 6-CI-MDMA.

Introduction

The current wave of popularity of recreational “rave drugs” among young teenagers has spread around the world. These drugs are produced in illicit laboratories and sold illegally in drug market. The most popular has become 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), an amphetamine derivative, for its entactogenic and empathogenic effects. The majority of published papers on designer drugs address the pharmacologic effects and metabolism of MDMA (1–23). Experimental studies, both animal and human, as well as human epidemiologic data and individual case reports indicate significant health and social risks connected with the abuse of MDMA. Serotonin syndrome, hepatotoxicity, neurotoxicity, psychopathology, and life-threatening effects have been associated with the use of MDMA. There are hundreds of possible modifications to the basic amphetamine structure that retain or modify the stimulant effect of the parent compound. Their manufacturing methods and syntheses are available on the World Wide Web and through Internet discussion groups, and websites describing procedures for the synthesis of MDMA are numerous (24–27). Although MDMA is included in the Schedule of the Controlled Substances in Europe and the United States, illicit laboratories producing amphetamine analogues make small structural changes so that they might circumvent existing controlled substance laws. These laboratories produce various tablet forms with different logos and do not guarantee their composition. The most recent trend in structural alterations for illicit amphetamine analogues has been the substitution of the phenyl ring pattern (28). At present, many modifications of the phenyl ring-substituted amphetamine derivatives exist. The resulting compounds include 3,4-methylenedioxy-N-ethylamphetamine; N-methylbenzodioxylbutanamine; 4-methylthioamphetamine; 4-methoxymethylamphetamine; 2,5-dimethoxy-4-bromophenethylamine; 2,5-dimethoxy-4-bromoamphetamine; and 2,5-dimethoxy-4-methylamphetamine (29–38).

Aim

The aim of the presented study was to identify an unknown compound detected by thin-layer chromatography (TLC) in the urine of an illicit drug abuser. The compound was isolated by TLC and analyzed by gas chromatography–mass spectrometry (GC–MS) in electron ionization (EI) and positive ion chemical ionization (PICI) mode to elucidate its chemical structure.

Case History

At a party, a 29-year-old male ingested cocaine, marijuana, four Ecstasy pills, and alcoholic drinks. The following day, at about 6:00 in the morning, he was transported by his friends to the hospital. He was unconscious and hypoxical. A short time later, bradycardia developed, and he was hypoventilated. The
patient was intubated and received intravenous fluids. Two hours after regaining consciousness, he was extubated, and at about 6:00 in the afternoon on the same day, he was discharged from the hospital. A urine specimen collected from him in the hospital yielded positive EMIT immunoassay screenings for cannabinoids, amphetamines, and cocaine. Instrument Du Pont aca IV, Medical Products (Wilmington, DE) was used. A routine analysis of urine by TLC using a fractional diethyl ether liquid-liquid extraction identified ephedrine, MDMA, and an unknown compound.

Methods

Materials

The TLC glass plate (Kieselgel G 60 10 × 20 cm) was produced by Merck (Darmstadt, Germany). The following standards were obtained from UNDCP (Vienna, Austria): atropine sulfate, codeine phosphate, phenmetrazine hydrochloride, aminophenazone, diazepam, amphetamine sulfate, methamphetamine hydrochloride, mephentermin sulphate, phentermine hydrochloride, ephedrine hydrochloride, and MDMA hydrochloride. N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and N-methyl-bis-trifluoroacetamide (MBTFA) were obtained from Macherey-Nagel, (Düren, Germany). Heptfluorobutyric anhydride (HFBA), acetic anhydride (ACA), and stabilizer 1,2-ethanedithiobis(trimethylsilane) (EDTBTS) were obtained from Fluka (Buchs, Switzerland). Support chemicals such as hydrochloric acid, tartaric acid, sodium carbonate, sodium phosphate, and ammonium iodide were obtained from Lachema (Brno, Czech Republic). Ethylacetate and cyclohexane were obtained from Scharlau (Barcelona, Spain), and methanol was obtained from Penta (Chrudim, Czech Republic). All chemicals were of analytical grade unless otherwise stated.

TLC

Glass TLC plates (Kieselgel G 60 10 × 20 cm) were used with a mobile phase of ethyl acetate/ethanol/ammonia (36:2:2 v/v/v). A sample of urine was prepared by fractional diethyl ether liquid–liquid extraction. Fifty milliliters of urine was extracted with 100 mL diethyl ether after adding 5 mL of concentrated hydrochloric acid, evaporated to dryness. The aqueous phase was extracted with 100 mL diethyl ether again after adding 5 mL of sodium carbonate to adjust the pH to 9.0. The organic phase (basic urine extract) was transferred to a dish and, after adding 50 μL of concentrated hydrochloric acid, evaporated to dryness. Reconstituted basic urine extract (50 μL), 10 μL of 1 mg/mL drug standards for identifying unknown compounds (AKFAD: atropine sulfate, codeine phosphate, phenmetrazine hydrochloride, aminophenazone, and diazepam), and 10 μL MDMA standard (1 mg/mL in ethanol) were spotted on a TLC plate, eluted in ethyl acetate/ethanol/ammonia (36:2:2, v/v/v), and detected with Dragendorff, Marquis, and Fast Black K color reagents. Ten microliters of amphetamines standard (amphetamine, methamphetamine, mephenetermine, phentermine, norephedrine, and MDMA) and 50 μL of the basic urine extract were spotted on a TLC plate and eluted in benzene/methanol/isopropanol/ammonia (35:5:5:1, v/v/v/v). The bands were visualized under an ultraviolet light (254 nm) and detected with reagents giving colored chromatograms.
GC–MS

**Instrument 1.** Analyses were performed using a Hewlett-Packard HP 5890 series II GC with HP 7673 autosampler coupled to a Hewlett-Packard HP 5972 quadrupole mass selective detector. The capillary column used was a 15-m HP5-MS (5% phenyl-methylsilicone, 0.25-mm i.d. and 0.25-μm film thickness). The following temperature program was used: 100°C, increased to 300°C at 15°C/min, and held 4 min. Helium was the carrier gas at a constant flow rate of 1.0 mL/min. The injections were made in splitless mode with a splitless time of 1.0 min and an injector temperature of 250°C. The transfer line temperature was 280°C. Scan time was 1.0 s. Full scan spectra were obtained in EI mode, recording mass-to-charge ratios from 35 to 500. Ionization gas was methanol vapor.

**Instrument 2.** Analyses were performed using a Finnigan MAT Magnum, which included a Varian 3400 GC with an A200S GC autosampler coupled to a Finnigan ITD ion trap detector. The capillary column used was a 30-m HP5-MS (5% phenyl-methylsilicone; 0.25-mm i.d. and 0.25-μm film thickness). The following temperature program was used: held at 85°C for 1 min, increased to 150°C at 30°C/min, increased to 250°C at 15°C/min, and held 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injections were made in septum-equipment programmable injector (SPI) mode with a temperature program from 85°C to 230°C. The injection volume was 1.0 μL. The transfer line temperature was 280°C and the scan time was 0.5 s. Full scan spectra were obtained in EI mode or in the PICI mode, recording mass-to-charge ratios from 35 to 500. Ionization gas was methanol vapor.

**Derivatization**

The unknown compound band from TLC plate was scraped and extracted with 1 mL methanol. The extract was divided to five aliquots and evaporated before derivatization.

For TMS derivatization (trimethylsilylation), the extract was derivatized with 50 μL MSTFA/ammonium iodide (NH₄I)/1,2-ethanedithiois (trimethylsiline) (1000:2.5:3, v/w/v) at 70°C for 20 min. This reagent mixture is routinely used in doping control. After cooling to laboratory temperature, the mixture was transferred to a new vial and injected into the GC–MS column.

For TFA derivatization (trifluoroacetylation), the extract was derivatized with 50 μL MBTFA at 70°C for 30 min (39). After cooling, the mixture was transferred to a new vial and injected into the GC–MS column.

ACA derivatization (acetylation) was achieved by mixing the extract with 50 μL of ACA/pyridine 10:1 (v/v) at 60°C for 30 min. This was followed by the addition of 10 μL of 0.01% tartaric acid in ethyl acetate (18–21). The vial was removed from the heating block, allowed to cool to laboratory temperature, and evaporated to dryness. The residue was reconstituted in 100 μL ethyl acetate and injected into the GC–MS column.

For HFB derivatization (heptafluorobutyrylation), the extract was derivatized with 50 μL HFAA at 70°C for 30 min. After cooling, redistilled cyclohexane (100 μL) and an aqueous 0.5 mol/L sodium phosphate solution (200 μL) were added (22). The vial was vortex mixed, and the top phase was transferred to a new vial before injection into the GC–MS column.

**Results and Discussion**

**TLC**

The calculated Rf value for the MDMA standard (Rf = 0.39) corresponded to one of the separated bands of the basic urine.
extract. The Rf value of the unknown compound was calculated to be 0.52 and did not allow for identification of the compound. The unknown compound band from each plate was scraped and extracted with methanol for subsequent derivatization prior to GC-MS studies.

**GC-MS**

GC-MS analyses of an unknown compound were performed using two instruments. The compound of interest was analyzed alone (Figure 1) and after derivatizations with MSTFA (Figure 2) and MBTFA (Figure 3) on instrument 1 (Hewlett-Packard) with EI and after derivatizations with ACA (Figure 4) and HFBA (Figure 5) on instrument 2 (Finnigan MAT Magnum) with electron ionization. To determine the molecular weight of the unknown compound, instrument 2 was used with positive ion chemical ionization (Figure 6).

The EI-MS spectrum of MDMA contains the following ions: m/z 193, 177, 135, 77, and 58 (40). Closer scrutiny of the EI-MS and PICI-MS mass spectral data of the unknown compound indicated a structure similar to MDMA, with the substitution of a single chlorine atom—a chlorinated MDMA (Cl-MDMA). The MS spectrum of the unknown compound in EI exhibited the characteristic A+2 chlorine isotopic cluster for m/z 169 (135 - 1 + 35) and for the M+1 ion at m/z 226. The MS spectrum produced by the unknown compound in PICI exhibited a characteristic A+2 chlorine isotopic cluster at m/z 228 (MH+). The molecular weight of the unknown compound is 227 amu (193 - 1 + 35). To confirm the Cl-MDMA structure, the unknown compound isolated by TLC was silylated, trifluoroacetylated, acetylated, heptafluorobutyrylated, and analyzed by GC-MS. The EI-MS spectra of derivatized Cl-MDMA exhibited the characteristic A+2 isotopic cluster for m/z 169 (135 - 1 + 35) for all used derivatization methods mentioned. Another isotopic A+2 cluster was created in all M+ and M+1 ions of each derivatized chlorinated MDMA: acetylated Cl-MDMA at m/z 270 (235 - 1 + 35), silylated Cl-MDMA at m/z 284 (250 - 1 + 35), trifluoroacetylated Cl-MDMA at m/z 328 (293 - 1 + 35) and heptafluorobutyrylated Cl-MDMA at m/z 423 (389 - 1 + 35). The joint cluster at m/z 196 (162 - 1 + 35) occurred in the acetylated, trifluoroacetylated, and heptafluorobutyrylated derivatives, but not the silylated derivative. The position of the chlorine atom cannot be exactly determined from the mass spectral information presented here; however, we believe that the unknown compound could be 6-CI-MDMA. Position 2 and 5 would also be possible, but 6-CI-MDMA has been listed as a new synthetic drug in Europe and in the USA since the mid-1990s (41) making it the most likely candidate. Our assumption can be supported by a paper published by Lewis and co-workers (42), in which 6-CI-MDMA has been identified in an illicit drug seizure, which included 26 off-white tablets. 6-CI-MDMA has been identified by GC-MS using EI after derivationization with heptafluorobutyric anhydride and 1H-NMR spectroscopy. In our case, we isolated the unknown compound from a biological matrix and did not have enough material for a 1H-NMR study.

**Conclusions**

A chlorinated MDMA was identified after derivatization of an unknown compound that had been isolated by TLC. Using a literature source (42), and through interpretation of our mass spectra of heptafluorobutyrylated Cl-MDMA, it was possible to surmise that the unknown compound could be 6-CI-MDMA. To support this claim, we used additional derivatization techniques such as acetylation, trifluoroacetylation, and silylation. For unambiguous proof
that the compound is 6-Cl-MDMA, synthesis and analysis of all three possible structure should be done in future research. 6-Cl-MDMA is listed in New Synthetic Drugs reported in Europe and the U.S. since the mid-1990s in the joint action of The European Monitoring Centre for Drugs and Drug Addiction (41).

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References


