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Analysis of 4-methylthioamphetamine in clinical specimens

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SUMMARY. There has been much publicity, particularly in Europe, regarding a new phenylethylamine-based compound called 4-methylthioamphetamine (4-MTA), also known as para-methylthioamphetamine (p-MTA), MTA or 'Flatliner'. Chemically, 4-MTA is an amphetamine derivative and is a potent, non-neurotoxic serotonin-releasing agent and reversible inhibitor of rat monoamine oxidase-A. Its effects, therefore, appear different from those of amphetamine.

Analysis of various plasma and urine specimens in three clinical cases implicating 'Ecstasy' ingestion revealed the presence of 4-MTA. Presumed metabolites were also detected, with one compound identified as being 4-MTA sulphoxide. The concentrations of 4-MTA measured in the plasma ranged from 0·131 mg/L to 0·760 mg/L. In one patient the 4-MTA concentration was determined in a series of plasma samples and this allowed a presumptive half-life of approximately 7 h to be estimated. This paper describes the first reported data regarding possible pharmacokinetics of 4-MTA in humans and presents the first reported non-fatal instances of 4-MTA intoxication in the UK.

INTRODUCTION

4-methylthioamphetamine (4-MTA), also known as para-methylthioamphetamine (p-MTA) or MTA was first synthesized in 1992 by Nichol *et al.* Chemically, 4-MTA is an amphetamine derivative (*see* Fig. 1) and potent psychoactive compound, but it exhibits different effects from those of amphetamine and LSD.[1,2] Studies also indicated that 4-MTA is a potent, dose-dependent serotonin-releasing agent and reversible inhibitor of rat monoamine oxidase-A (MAO-A). At behaviourally relevant doses in rats it appeared to be non-neurotoxic, but at high doses serotoninergic behaviour was observed. Additional animal studies suggested it also had a delayed reaction compared to the 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstacy')-related compounds studied.[3]

Tablets containing 4-MTA were first seized in Europe in 1997, with the street-name 'Flatliner' or 'S5'; however, they are usually sold as Ecstasy. Nevertheless, 4-MTA-containing tablets comprise only a very small proportion of illicitly available Ecstasy tablets (King L, Groombridge C, personal communication, 1998, 1999). It is common for tablets (especially Flatliners) to contain only 4-MTA, but some S5 tablets have been found to contain caffeine as well.[4] Since 1997, various numbers of tablets containing 4-MTA have been seized in sporadic batches across Europe and also in Australia, with most having been seized in the UK (King L, Groombridge C, personal communication, 1998, 1999).

Users have noted that 4-MTA produces a greater 'high' and has a delayed reaction compared to 'normal' Ecstasy, thereby supporting the animal data; the latter characteristic can therefore result in accidental overdose if the user takes additional tablets while waiting for an effect[5] (Thomas, personal communication 1998, 1999). It is believed to have been implicated in seven deaths worldwide (five in the UK, one in the Netherlands and one in France) and at least 10 reported cases of non-fatal intoxication (four in the UK, five in Belgium and one in the Netherlands) since 1997[5–7] (European Monitoring Centre for Drugs and Drug Addiction, personal communication, 1999, 2000). 4-MTA alone has only been detected in one of the deaths reported, and both fatal and non-fatal intoxications invariably involve additional drugs.[7,9,10] Therefore, owing to the limited number of instances where 4-MTA has been encountered,

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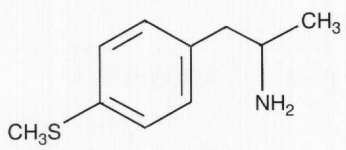

FIGURE 1. *Chemical structure of 4-methylthioamphetamine.*

the exact mechanism of 4-MTA poisoning has yet to be ascertained. However, in most cases the patient/deceased experienced convulsions and respiratory depression.

This paper describes the detection and quantification of 4-MTA in human plasma by high-performance liquid chromatography with diode array UV detection (HPLC-DAD), in three clinical investigations involving intoxication with this drug, in which the patients survived. The presence of additional drugs and the plasma concentrations of other amphetamine derivatives (i.e. amphetamine and MDMA) detected during toxicological screening are also presented. Although these cases occurred 2 years ago there are still very few published data regarding non-fatal instances of 4-MTA intoxication and resultant plasma concentrations, in particular preliminary human pharmacokinetic data. Therefore, the author believes these to be the first reported data regarding the possible pharmacokinetics of 4-MTA in humans, and also to represent the first reported non-fatal instances of 4-MTA intoxication in the UK.

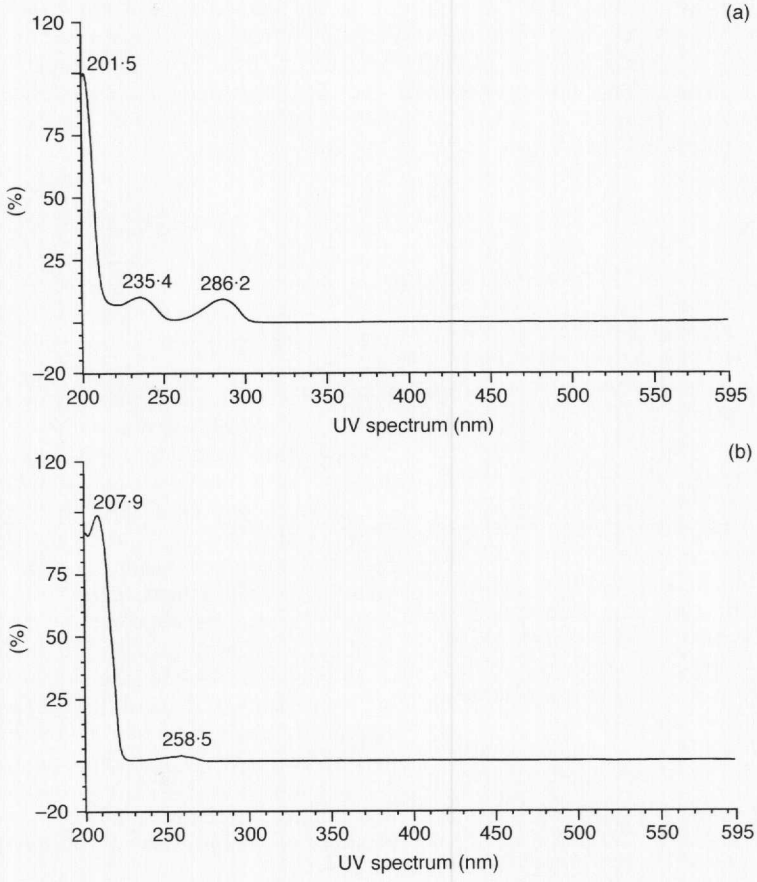

FIGURE 2. *UV spectra of (a) 3,4-methylenedioxymethamphetamine (MDMA), (b) amphetamine, (c) 4-methylthioamphetamine (4-MTA) and two associated compounds, (d) 4-MTA associated compound 1 and (e) 4-MTA sulphoxide.*

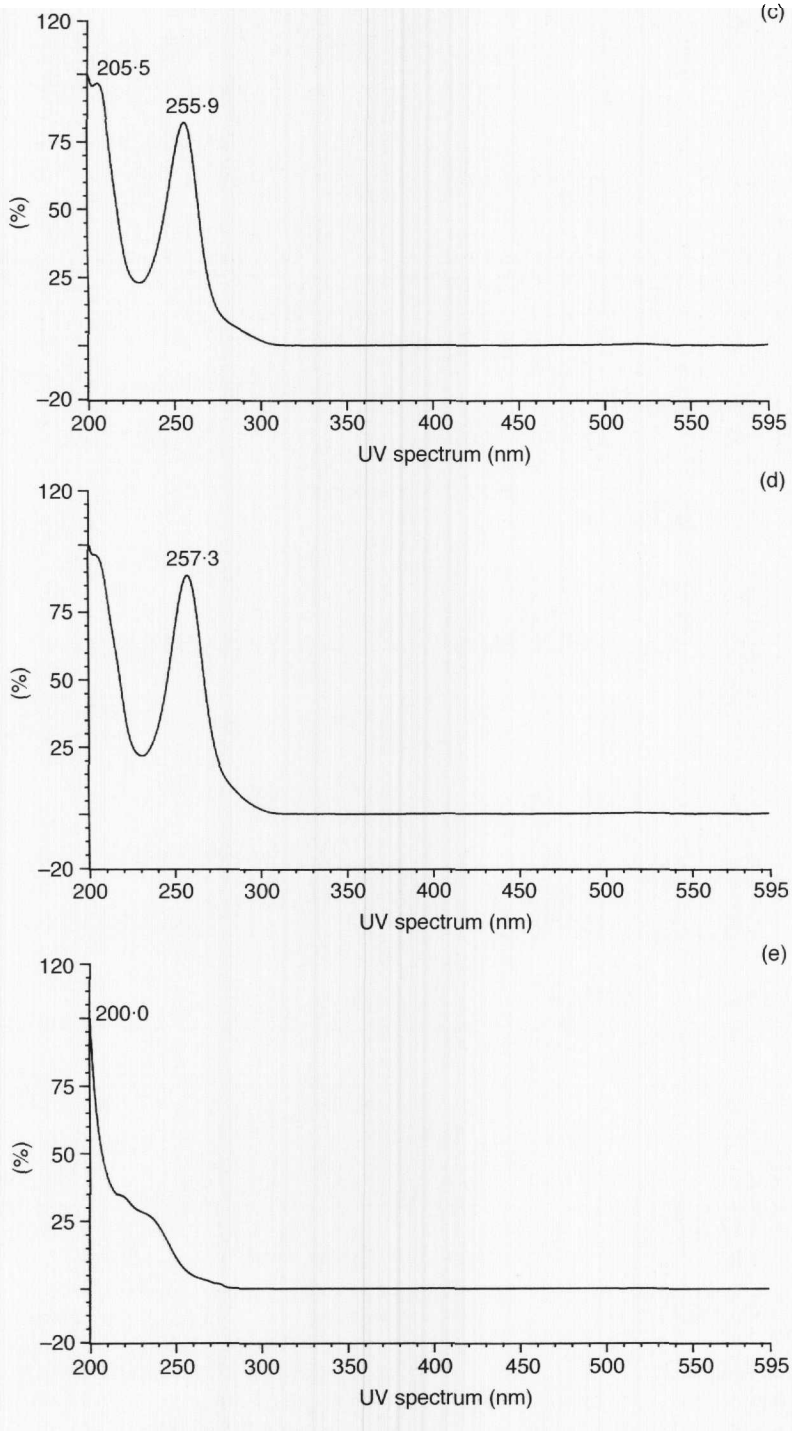

FIGURE 2. *continued.*

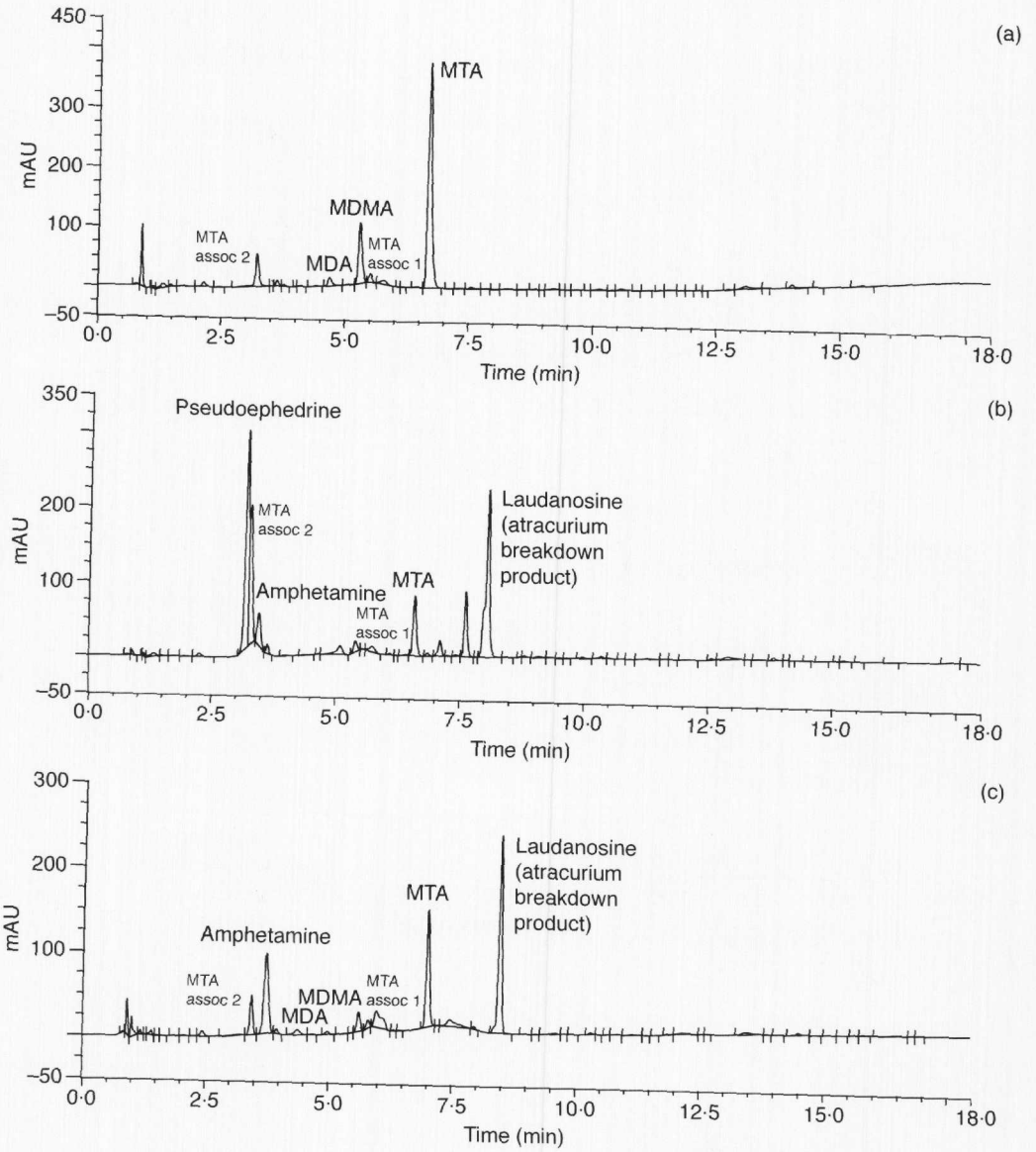

FIGURE 3. *Chromatograms obtained from (a) case 1, (b) case 2 and (c) case 3 following qualitative high-performance liquid chromatography with diode array UV detection analysis of 0·5 mL of human urine at pH 10 after solvent extraction and acid back extraction. Injection volume was 30 µL. MTA = methylthioamphetamine; assoc = associated compound; MDMA = 3,4-methylenedioxymethamphetamine.*

MATERIALS AND METHODS

Chromatographic equipment

HPLC-DAD analysis was performed using a M480 high-precision pump, a column oven, a Gina 50 autosampler and a UVD340S diode array detector, all from Dionex UK (Macclesfield, Cheshire, UK), with an X-Act 4-channel degasser from Jour Research. A Waters Spherisorb S5OD/CN 4·6 mm × 150 mm cartridge column (Watford, UK), protected by a 4 mm × 10 mm guard column of Spherisorb S5ODS2, was used for the analysis. Data acquisition was handled by a Dionex Chromeleon software package running on an Elonex MMX Pentium PC (233 MHz, 128 Mb RAM), with the diode-

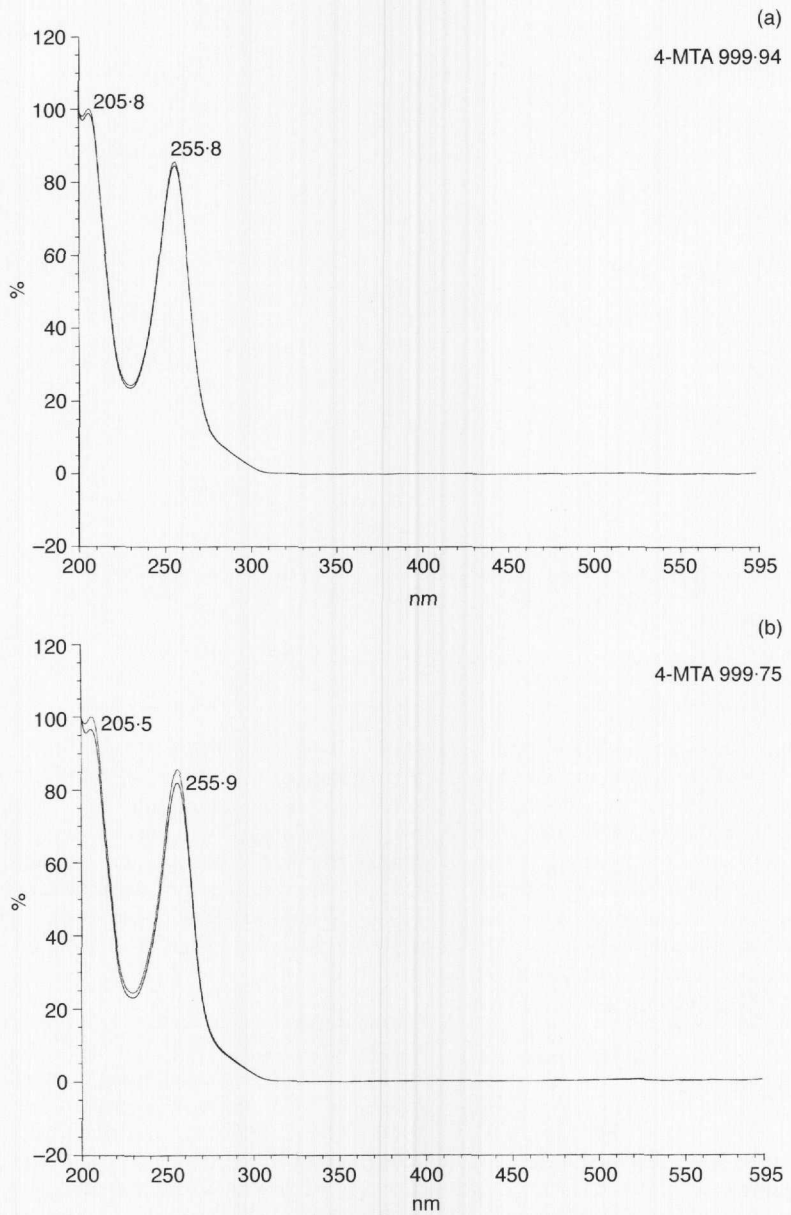

FIGURE 4. *Comparison of UV spectra obtained from patient urine and UV spectral library entry for 4-methylthioamphetamine (4-MTA; from pure standard). (a) 4-MTA 999·94; (b) 4-MTA 999·75; (c) 4-MTA 999·77. The software-derived spectral match (expressed out of 1000) with 4-MTA is indicated in the top right-hand corner of each graph.*

array detector recording spectral data between 200 nm and 595 nm.

Materials

The 1·0 mol/L triethylammonium phosphate (TEAP) buffer (pH 3·0) was supplied by Fluka, Gillingham, Dorset, UK, and the HPLC-grade acetonitrile was supplied by Rathburn Chemicals Ltd, Walkerburn, Scotland. The HPLC-grade 1-chlorobutane was obtained from Fisher Scientific International, Loughborough, UK. MDMA, fenfluramine hydrochloride and amphetamine

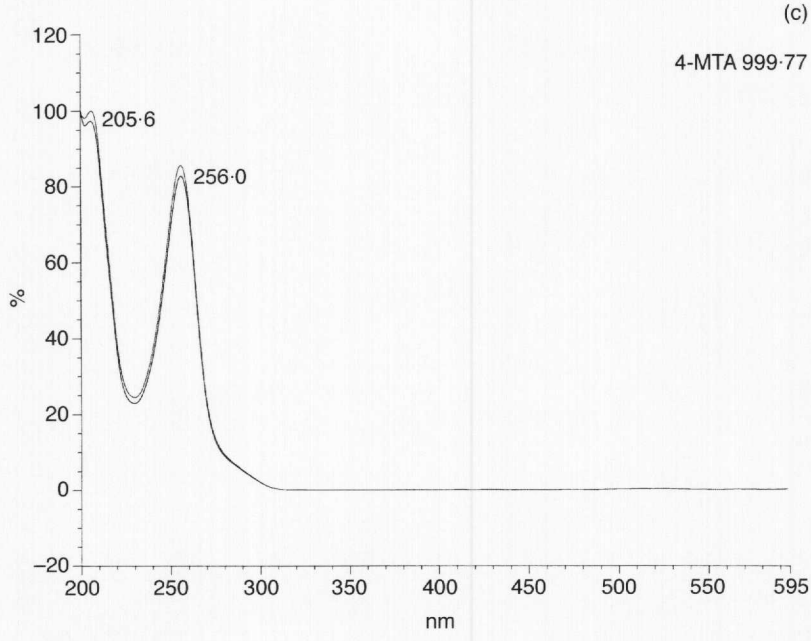

FIGURE 4. *continued.*

sulphate were supplied by Sigma-Aldrich, Poole, Dorset, UK. Pure 4-MTA hydrochloride was kindly supplied by Drs Huizer and Poortman (Forensic Science Laboratory, Rijswijk, The Netherlands) and was used to prepare reference and calibration standards for the formal identification and quantification of 4-MTA in the specimens analysed. Calibration standards with assigned values of 0·50, 1, 2·5 and 5 mg/L 4-MTA were prepared in blank equine plasma. Two further standards with values of 0·05 mg/L and 0·02 mg/L were also produced to determine the limit of detection of the assay.

Extraction methods for biological specimens

HPLC-DAD screening

Five hundred microlitres of Na_2CO_3, 200 mmol/L pH 10, were added to 500 μL of blood/urine followed by 5 μmL of 1-chlorobutane in a 12 mL polypropylene tube. After shaking for 3 min and following centrifugation at 4500 rpm for 3 min, the supernatant was transferred to a second tube. Drugs were extracted into 100 μL 50 mmol/L H_2SO_4, shaken for 3 min, centrifuged at 3500 rpm for 3 min, and then the supernatant was aspirated and the acid layer transferred into a vial for injection. The injection volume was 30 μL.

HPLC-DAD quantification

Five hundred microlitres of 10 mg/L fenfluramine internal standard (in 200 mmol/L Na_2CO_3 solution) was added to 500 μL of sample/standard, followed by extraction with 5 mL 1-chlorobutane and back extraction with 100 μL 50 mmol/L H_2SO_4, as for the screening procedure detailed above. The injection volume was 20 μL.

Chromatography conditions

For HPLC-DAD screening a single-step gradient elution was performed using a mixed mobile phase. Mobile phase A consisted of 700 mL 99·9% HPLC-grade acetonitrile together with 25 mL 1 mol/L TEAP buffer (pH 3·0) diluted with 275 mL HPLC-grade water to give a final concentration of 25 mmol/L buffer and 70% acetonitrile. Mobile phase B consisted of 25 mL 1 mmol/L TEAP buffer diluted with 975 mL HPLC-grade water to give a final concentration of 25 mmol/L buffer. The elution conditions for the gradient were achieved using a 2 mL/min flow rate of 0–70% acetonitrile for 15 min, and then holding at 70% acetonitrile for 3 min.[8–10] The column temperature was maintained at 25°C.

The 4-MTA quantification procedure was based on 20% acetonitrile (in 25 mmol/L TEAP

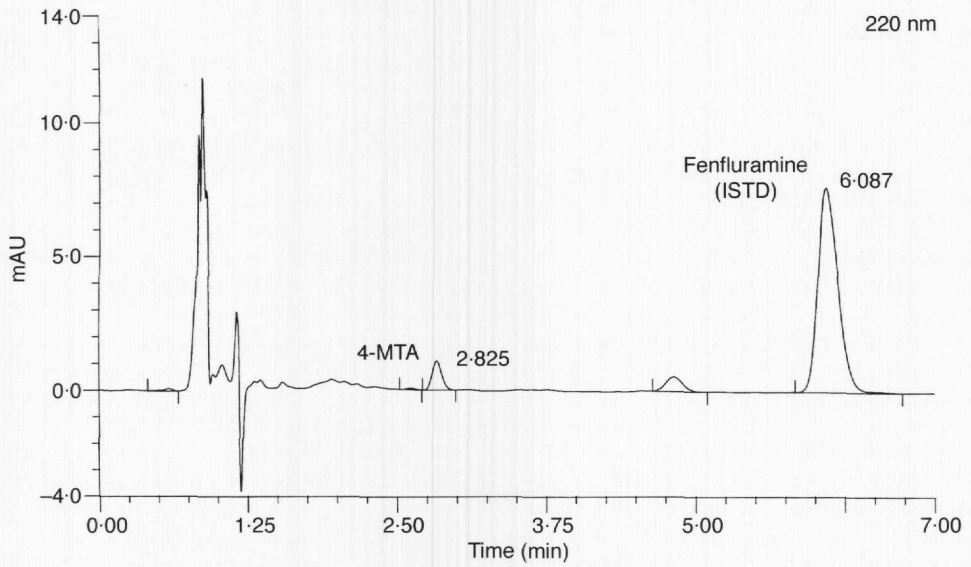

FIGURE 5. *Case 2. Chromatogram showing elution of 4-methylthioamphetamine (4-MTA) and fenfluramine (internal standard) under 20% acetonitrile isocratic conditions. 500 μL of human plasma was extracted using 1-chlorobutane and back-extracted into 100 μL of 50 mmol/L H_2SO_4. Injection volume was 20 μL. ISTD = internal standard.*

buffer) isocratic elution at a flow rate of 2 mL/min. For amphetamine and MDMA quantitative analysis, 10% acetonitrile isocratic conditions at a flow rate of 2 mL/min were used. The column temperature was maintained at 25°C.

RESULTS AND DISCUSSION

Qualitative analyses

4-methylthioamphetamine obtained from the Netherlands was used to obtain gas chromatography-nitrogen phosphorus detection (GC-NPD), GC–mass spectrometry and HPLC-DAD analytical data, including retention index (RI) values for GC and HPLC, mass spectral data (molecular ion = 181) and UV spectral data (UV maxima = 205 and 255 nm).[7] An in-house HPLC UV spectrum and retention index database and GC retention index database was used for the identification of other drugs and drug metabolites present.[8]

In 1998, three patients presented to local hospitals exhibiting atypical symptoms of Ecstasy overdose. Physicians requested emergency drug screening in order to assist in diagnosis and possible treatment. Comprehensive drug screening of the plasma and urine specimens by GC-NPD, EMIT II immunoassay and HPLC-DAD was performed. In all cases, 4-MTA and two associated compounds were detected by GC-NPD and HPLC-DAD, in addition to other stimulants, MDMA, amphetamine and pseudoephedrine. An atracurium breakdown product was also detected owing to the use of atracurium while intubating the patient. At present there is no information regarding 4-MTA pharmacokinetics and the author believes these to be the first reported data concerning probable metabolites.

Using HPLC-DAD, 4-MTA was identified by retention index value (RI) and by UV spectral matching. The UV spectrum of 4-MTA is different from that of MDMA and other amphetamine-related compounds and has a UV maximum at 255 nm (*see* Fig. 2). Figure 3 shows the results of qualitative HPLC-DAD analysis of the urine specimens obtained at 210 nm. Figure 4 shows the results of spectral matching.

One of the observed associates of 4-MTA (4-MTA associate 1) has a spectrum virtually identical to that of 4-MTA, and *may* correspond to a hydroxy derivative of the parent compound. This would be consistent with the metabolism of amphetamine and other amphetamine derivatives, where hydroxylated metabolites are also produced.[10]

The UV spectrum of 4-MTA associate 2 suggests that the chromophore has been disrupted, possibly by sulphoxidation (i.e. 4-MTA associate 2 could be 4-MTA sulphoxide). This theory was supported in part by oxidation of 4-MTA with hydrogen peroxide. Sulphoxidation is a common and relatively rapid reaction and observations of 4-MTA standards in various storage environments have indicated that 4-MTA is readily oxidized and hence unstable. It is therefore recommended that specimens containing 4-MTA be collected in fluoridated containers and kept at −20°C prior to analysis.

The presence of other 'recreational' drugs is not unexpected owing to the mode of abuse of 4-MTA. Therefore, as 4-MTA tablets invariably only contain 4-MTA, it can be inferred that the other substances detected were due to the ingestion of additional tablets or powder. Furthermore, although amphetamine was detected in two of the three cases analysed, amphetamine does not appear to be a metabolite of 4-MTA, as it was not detected in a related fatality where only 4-MTA was found at high concentrations in all specimens analysed, including postmortem urine.[5] In this case, the peri- and postmortem blood concentrations were reported to be 4.2 mg/L and 4·6 mg/L, respectively.[5]

Quantitative analyses

Using 20% acetonitrile isocratic conditions, 4-MTA eluted at 2·83 min and the 10 mg/L fenfluramine internal standard eluted at 6·09 min (*see* Fig. 5). A linear calibration curve was produced from the 4-MTA plasma standards at 220 nm. MDMA and amphetamine were analysed using alternative isocratic conditions because of their elution characteristics. The identification of all compounds was confirmed using both absolute retention time and UV spectral data. Studies concerning the extraction precision or recovery were not performed.

The results show that the plasma 4-MTA concentration ranged between 0·131 and 0·760 mg/L, and in all cases at the time of specimen collection the patients were in a serious clinical condition requiring ventilation and sedation after suffering convulsions (*see* Table 1). All patients were eventually discharged.

In Case 3, various timed plasma specimens were taken. Shortly after admission the patient was in severe respiratory distress and had a plasma 4-MTA concentration of 0·760 mg/L. The specimen subsequently taken at 07:15 showed a 4-MTA concentration of 0·280 mg/L, a further specimen taken at 14:10 (7 h later) showed that the plasma 4-MTA concentration had virtually halved (0·144 mg/L). The results

TABLE 1. *Results of toxicological analysis of three non-fatal cases involving 4-methylthioamphetamine (4-MTA)*

	Drugs detected (in urine)	Date collected	Time collected	Plasma MDMA concentration	Plasma amphetamine concentration	Plasma 4-MTA concentration	Clinical details
Case 1	4-MTA MDMA	04.07.98	NK	60 μg/L	ND (<20 μg/L)	189 μg/L	Delayed reaction, convulsions, ventilated
Case 2	4-MTA Amphetamine Pseudoephedrine[1]	16.08.09	16:30	ND (<20 μg/L)	50 μg/L	131 μg/L	Ventilated
Case 3	4-MTA Amphetamine	28.09.98? 28.09.98	NK 07:15	NA 40 μg/L	NA 240 μg/L	760 μg/L 280 μg/L	Convulsions, ventilated
	MDMA	28.09.98 28.09.98 30.09.98	14:10 17:15 NK	NA 20 μg/L ND (<20 μg/L)	NA 110 μg/L ND (<20 μg/L)	144 μg/L 154 μg/L ND (<50 μg/L)	

[1]Pseudoephedrine concentration not measured due to insufficient specimen. NK = not known; NA = specimen not analysed (e.g. insufficient sample volume); ND = not detected (limit of detection quoted); MDMA = 3,4-methylenedioxymethamphetamine.

obtained from this particular patient suggest that 4-MTA has a plasma half-life of approximately 7 h. Interestingly, the concentration appeared to be slightly higher 3 h later (0·154 mg/L); this could be due to redistribution but did not appear to be an analytical anomaly. Furthermore, it can be seen that in all three cases there is a relatively low concentration of MDMA and/or amphetamine in the plasma, compared to 4-MTA. The toxicological relevance of this would depend on the pharmacokinetics of 4-MTA and its potential interaction with MDMA or amphetamine at various doses.

The author believes that the data presented here and those obtained from fatalities involving 4-MTA suggest that moderate toxicity may be associated with plasma 4-MTA concentrations of between 0·20 and 0·60 mg/L, severe toxicity with concentrations above 0·60 mg/L, and death with concentrations above 1·50 mg/L. However, further studies are required to confirm whether or not this is the case.

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