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Identification and characterization of 4-chloromethamphetamine (4-CMA) in seized ecstacy - a risk to public health

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Abstract

This paper reports the structure elucidation and characterization of 4-chloromethamphetamine (4-CMA), a compound never previously reported outside of laboratory settings in seized drug samples or substances actively being used at large dance festivals.

Identification of 4-CMA was obtained by liquid chromatography with diode array detector (HPLC-PDA) and gas chromatography mass spectrometry (GC-MS). Further structure elucidation was performed by fragment pattern analysis of the trimethylsilyl and trifluoroacetyl derivatives with GC-MS. The regio-isomeric assignment of the chloro-substituent was performed by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR). HPLC-PDA was used for quantitation of 4-CMA in the seized tablet to obtain an indication of the potency.

A literature review of the toxic effects of 4-CMA and potential widespread harm to the public in events where similar substances or tablets start appearing and circulating on a larger scale in the general population is discussed.

Keywords: 4-chloromethamphetamine (4-CMA), identification, elucidation, NPS, (neuro)toxic, ecstasy

1. Introduction

Large changes have occurred in the recreational drug market in Europe over the last decade, which can be roughly divided in two general phenomena: high dose ecstasy, and arrival of new psychoactive substances (NPS).

First, since the end of the ‘90s and the beginning of 2000, the appearance of a plethora of NPS designed to evade current international drug legislation was observed. Examples include the advent of mephedrone around 2010 and synthetic cannabinoids since approximately 2005; the latter still play a predominant role in the rapidly evolving ‘legal highs’ market and are responsible for a large increase in the detected number of new substances.

Second, as a result from legislation and precursor shortages due to increased seizures, the average MDMA content in ‘ecstasy’ tablets was historically low around the early 2000’s in Belgium, resulting in low-dosed ecstasy tablets, which were often contaminated with other substances such as piperazines (e.g. mCPP). As illustrated in Figure 1, mean MDMA dosages of around 60 mg (expressed as MDMA base) were observed until 2009, followed by an increase to 92 mg. Dosage further increased and reached a mean of 108 mg in 2013. The highest recorded dosage was observed in 2014 (125 mg) (1).

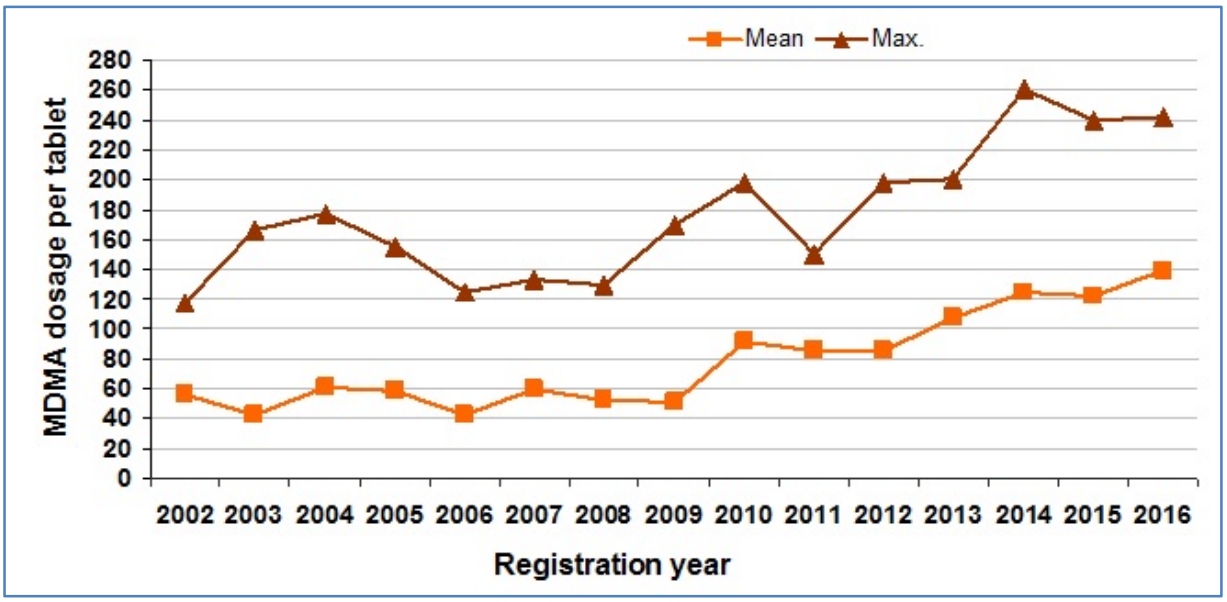


Figure 1. Evolution of MDMA dosage in ecstasy tablets in Belgium (2005 – 2016).

These historically low dosages coincided with the arrival of NPS (Figure 2). Partly because of the scheduling of substances such as MDMA, derivatives were designed with similar chemical structure that retained the psychoactive properties of the original molecule, but remained legal to manufacture and sell. Most of these substances are sold in smart shops or online as “bath salts”, “carpet cleaner”, “plant food”, “legal highs”, and “research chemicals”, and labeled not to be suited for human consumption.

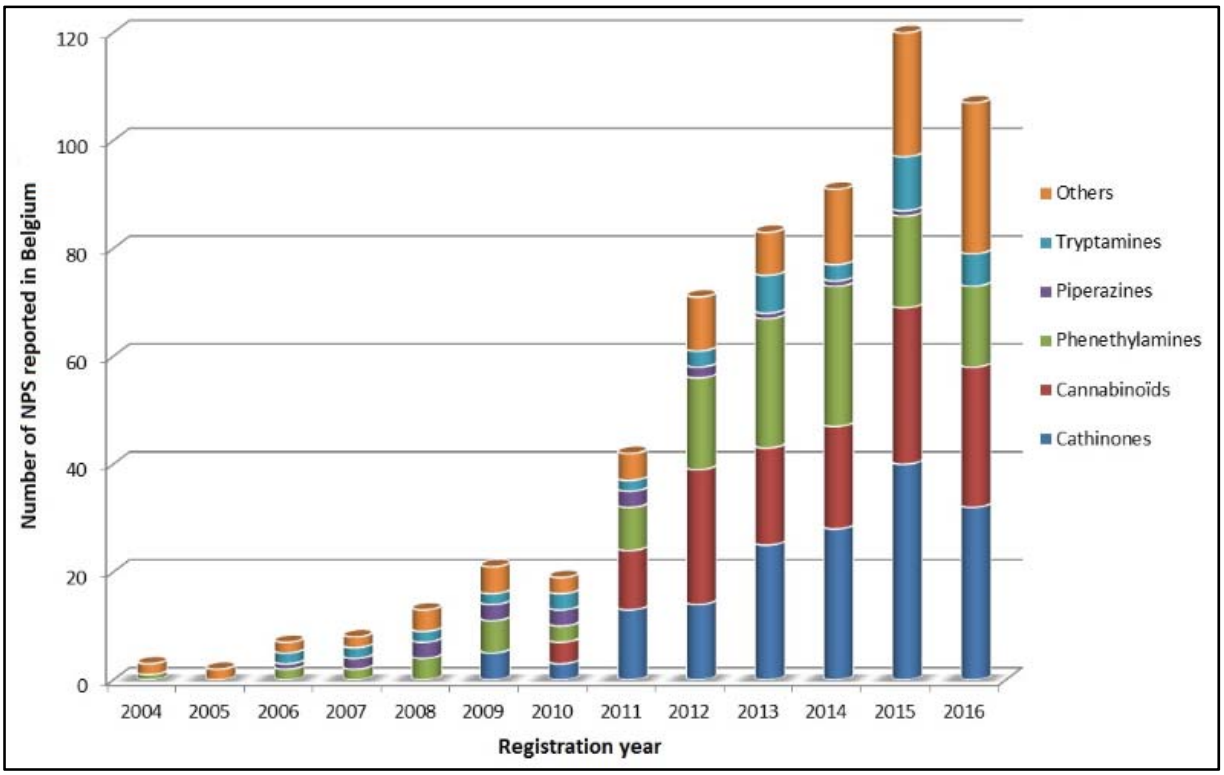
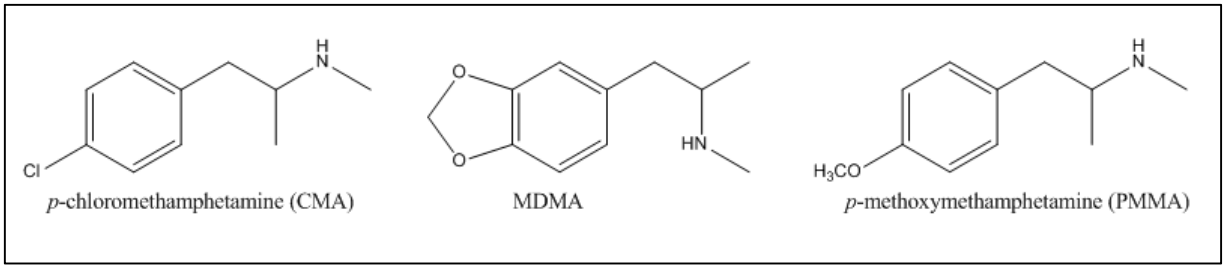


Figure 2. Evolution of number of NPS detected in Belgium each year (2004 – 2016).

NPS encompass a wide range of psychoactive compounds, including synthetic phenethylamines, cathinones and synthetic cannabinoids. By the end of 2016 more than 500 unique NPS compounds had been identified by the Early Warning System of the European Centre for Drugs and Drug Addiction (EMCDDA) (2). Since most of these substances have never been studied formally, and (toxic) effects in humans are mostly unknown, NPS constitute a real danger to public health, especially in the last three years when derivatives of fentanyl ("fentalogs") have started to appear on the market. Also, due to the lack of analytical reference standards, identification and general analysis of these substances poses a significant challenge in forensic toxicology.

In some cases NPS "cross-over" from the online scene is observed, meaning NPS entering the "classic" illicit drugs market where they are sold by the same dealers; for example 4-

fluoroamphetamine is frequently encountered in this fashion (3). Other NPS have been found in ecstasy tablets or in powders sold as heroin and amphetamine (4–6). In almost all cases the user is unaware of their presence, thinking instead he or she received a “weak” batch of the wanted drug. This carries great risk; one example in Belgium and The Netherlands was the contamination of amphetamine/speed with 4-methylamphetamine, a NPS with strong serotonergic action. At least six people have died in Belgium as a result of the consumption of this amphetamine mixture (7). Another widespread example is the presence of CMA and PMMA in ecstasy tablets (Figure 3), which has led to dozens of deaths worldwide (8,9).



p-chloromethamphetamine (CMA) MDMA *p*-methoxymethamphetamine (PMMA)

During the summer of 2015 an ecstasy tablet was submitted to the laboratory for identification. In this paper, firstly we present the identification and structure elucidation of the unknown molecule in the confiscated tablet using GC-MS, HPLC-PDA and ^{1}H-NMR. Secondly, we will report on potential (neuro)toxic effects and potential widespread harm to the public in events where similar substances or tablets start appearing and circulating on a larger scale in the general population.

2. Materials and methods

2.1 Samples and case histories

Several tablets confiscated by federal police in the framework of an international electronic music festival in Belgium were submitted to the laboratory for toxicological analysis. Based on

physical properties, eight different tablets could be distinguished. One of the tablets was rectangular, yellow/brown (non-uniform colour), ‘durex’-logo on one side and single-scored on the other side (weight: 427.1 mg; length:12.51 mm; width: 4.29 mm; height: 8.44 mm). The other tablets in the seizure (with different physical characteristics) were found to contain MDMA, MDMA, sildenafil/tadalafil, MDMA, MDMA, DOB and MDMA, respectively.

2.2 Materials

Certified reference components and general chemical reagents were obtained from Cayman Chemical (Michigan, USA). Solvents used for GC-MS and HPLC-PDA were of analytical grade. Methanol, acetonitrile and hydrochloric acid (37 %) were obtained from Fisher Chemical (Fisher Bioblock, Belgium). Water was purified by a Milli-Q system obtained from Merck Millipore (Darmstadt, Germany). Triethylammonium (TEA) phosphate 1 M was purchased from Sigma (Zwijndrecht, Belgium) and was diluted 1/40 immediately before use. The external standard diphenylamine was obtained from VWR International (Leuven, Belgium). N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and N-methyl-bis(trifluoroacetamide) (MBTFA) were purchased from Machery-Nagel (Germany). NMR analysis and associated sample preparation: deuterated solvents for NMR were purchased from Euriso Top (St. Aubain, France). Tetramethylsilane was of NMR grade and was acquired from Acros Organics (Geel, Belgium). Dichloromethane was purchased from Sigma-Aldrich and was of HPLC grade. Hydrochloric acid and sodium hydroxide were purchased from Acros Organics (Geel, Belgium) and were of ACS grade. Ultrapure water was obtained from a Millipore Synergy UV apparatus (Billerica MA, USA).

2.3 Sample preparation and instrumentation

2.3.1 Gas chromatography-mass spectrometry (GC-MS)

A fresh sample solution of 4-CMA in methanol (containing 200 µg/ml diphenylamine as external standard) was prepared. A mass spectrum was recorded by injecting a sample aliquot on an Agilent 6890 N gas chromatograph in combination with an Agilent 7683 injector and an Agilent 5973 inert mass selective detector (Agilent Technologies, California, USA). Mass spectra were recorded using a Varian CP-SIL 8 CB low bleed capillary column (30 m x 0.25 mm, 0.25 µm film thickness) connected to a fused silica retention gap (2.5 m x 0.25 mm). The used carrier gas was helium at a constant flow of 1.1 ml/min. The temperature gradient was applied: starting at 70 °C with 2 min holding time; increase to 310 °C at 8 °C/min with a 9 min holding time. Total runtime was 41 min. Injection port and detector temperatures were set at 300 °C and 230 °C respectively; transfer line temperature was set at 280 °C. An injection volume of 1 µL was used in split less injection mode. Mass spectra were recorded in the range *m/z* 40-550.

For further structure elucidation, trimethylsilyl- (TMS) and trifluoroacetyl- (TFA) derivatives were prepared by evaporation of the methanolic extract at 40 °C under a gentle stream of nitrogen and subsequent heating in a sealed glass vial at 70 °C for 30 minutes in the presence of 100 µl MSTFA or 100 µl MBTFA, respectively. Obtained derivatives were evaporated to dryness and reconstituted in acetonitrile. Subsequent analysis was performed using the procedures and protocols outlined in the GC-MS methods mentioned above.

2.3.2 Liquid chromatography with photo-diode array detection (HPLC-PDA)

The tablet was extracted with a freshly prepared methanol and subsequent evaporation of the methanol under a gentle nitrogen taking care stream not to let temperature rise higher than 40° C. A 50 µl aliquot was evaporated to dryness at 40 °C under nitrogen, and the powder was

reconstituted in 1.0 ml of mobile phase A. Mobile phases consisted of 25 mM TEA-phosphate buffer (A) and 100% acetonitrile (B). The gradient used during elution consisted of 95% A at time 0, changing to 30% A in 30 min and held there for another 5 min.

HPLC-PDA analysis was performed using a Varian Prostar solvent delivery module in combination with a Varian Prostar 410 autosampler and Varian Prostar photodiode array detector. Data acquisition and analysis were performed with the Varian Star and Polyview software. A LiChrospher® 100 RP-18 (5 µm) (Merck, Darmstadt, Germany) was used as saturation column. Separation of compounds was performed in gradient mode using a Microsorb C18 column (150 mm x 4.6 mm, 5 µm particle size, Agilent, California, USA) connected to a C18 guard column (4 mm x 3.0 mm, 3.5 µm particle size). Oven temperature was set at 35 °C. Scan range was 220-340 nm and the chromatogram was monitored at 220 nm and 254 nm for 35 minutes. The injection volume was 50 µl.

For quantitative analysis a stock solution of 1mg/ml in methanol was prepared immediately prior to use. A 30 mg aliquot of the homogenized powder of the tablet was weighted in a 10 ml volumetric flask and made up to volume with methanol: respectively 25 µl, 50 µl and 75 µl of methanolic extracts (sonicated for 30 min, homogenized and centrifuged) were transferred into an autosampler vial, dried under a stream of nitrogen after addition of 50 µl 10 % hydrochloric acid in methanol, and finally reconstituted in 1.0 ml initial mobile phase containing an additional 20 µg/ml diphenylamine as external standard. A calibration series was used by transferring 10 µl, 20 µl, 50 µl, 75 µl and 100 µl of stock solution into an auto sampler vial, which were subsequently dried under a stream of nitrogen (after addition of 50 µl 10 % hydrochloric acid in methanol) and reconstituted in 1.0 ml initial mobile phase containing 20 µg/ml diphenylamine as external standard.

2.3.3 ^{1}H nuclear magnetic resonance spectroscopy (NMR)

For the NMR analysis, the active component needed to be isolated from the yellow tablet. Since amphetamines are amines with a pKa of approximately 10, an acid/base extraction procedure was estimated to be suitable. To this end, a fragment of the tablet was placed in a glass test tube to which 10 mL water was added. The tube was sealed with a polypropylene cap and agitated until the material was mostly dissolved and only minor amounts of precipitate persisted. This suspension was transferred to a separatory funnel. The tube was rinsed thoroughly with 2 mL water. The pH of the solution in the separatory funnel was adjusted to 2-3 (Merck indicator paper) using 2.0 M HCl. The remaining solids of the suspension did not go into solution upon the addition of HCl. The aqueous suspension was washed with dichloromethane (3 x 20 mL). These dichloromethane fractions were discarded. The pH of the (acidic) aqueous fraction was adjusted to 12-13 by the dropwise addition of 4.0 M NaOH. At this pH the amine group in the amphetamine is expected to be in the free base form, which allows extraction into an organic solvent. The aqueous layer was thoroughly extracted with dichloromethane (5 x 20 mL). The dichloromethane fractions were pooled, dried using Na_2SO_4, filtered and concentrated *in vacuo*. A minimal amount of an oily residue was obtained. This material was stored under vacuum (~ 1 mBar) in an attempt to remove all volatile impurities. The residue was dissolved in 1 mL $CDCl_3$ (+ 0.5% v/v TMS (tetramethylsilane)). From this solution 750 µL was transferred to an NMR tube which was closed with a polypropylene cap.

All NMR spectra were recorded at 25 °C on a Varian Mercury-300BB (300/75 MHz) and processed using the Varian VNMRJ 3.2 software package.2.3. A solution of the material (± 20 mg) in $CDCl_3$ + 0.5% v/v TMS (750 µL) was prepared in an NMR tube (5 mm diameter, VWR-

300 MHz grade) and sealed using a polypropylene cap. The spectrum was recorded at 300 MHz using 32 scans and was referenced to the signal of TMS at 0 ppm.

3. Results

3.1. GC-MS

A methanolic sample solution of the tablet was analyzed by GC-MS and the major peak was identified as 4-chloromethamphetamine (4-CMA) by means of computer-based library search and matching with the SWGDRUG Mass Spectral Library (Version 3.1). The mass spectra are shown in Figure 5.

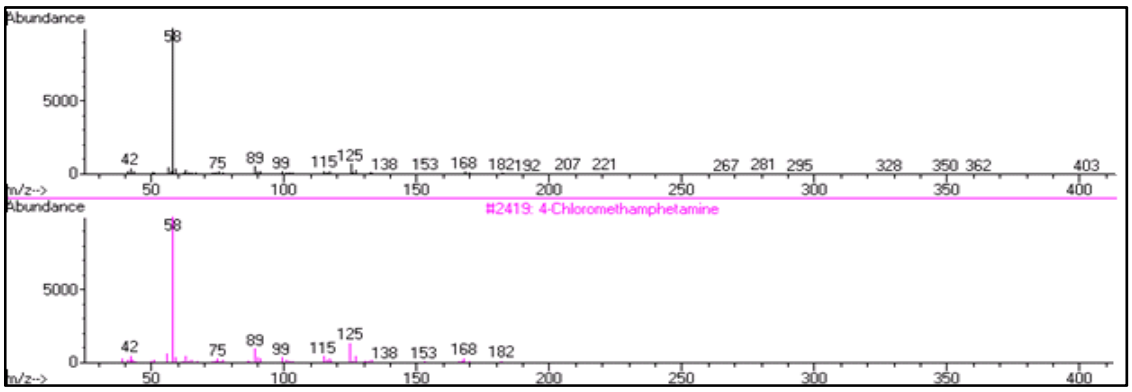


Figure 5. Mass spectrum of 4-CMA in the chromatogram from the tablet (upper) and reference mass spectrum of 4-CMA present in the SWGDRUG library (lower).

Fragment pattern analysis of 4-CMA, TMS and TFA derivates confirmed the presence of a chloromethamphetamine regioisomer. Proposed fragmentation patterns of 4-CMA, TMS and TFA derivates are given in Figure 6.

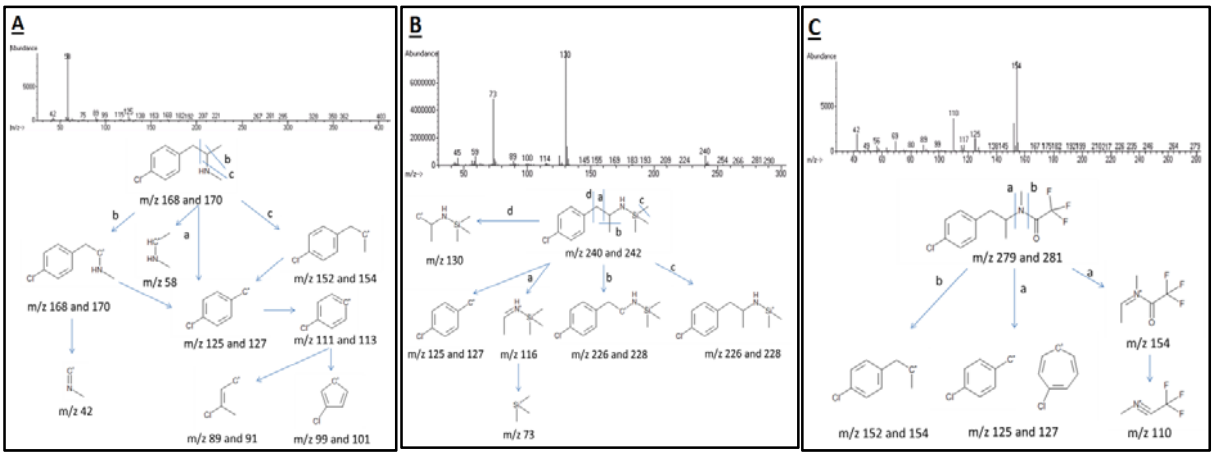


Figure 6: Proposed fragmentation pattern of 4-CMA (A), 4-CMA TMS derivate (B) and 4-CMA TFA derivate (C).

3.2. HPLC-PDA

In the chromatograms of the HPLC-PDA analysis, a peak was observed with the same retention time (± 2%) and UV spectrum (similarity index > 0.995) as 4-CMA. The HPLC-PDA chromatogram of the tablet with UV-spectrum of 4-CMA is shown in Figure 7. Calibrators and sample extracts were analyzed in one batch. A six-point calibration curve was made by plotting the ratio of the observed peak area of 4-CMA to this of the external standard diphenylamine to the amount of 4-CMA in the autosampler vial. The calibration curve was linear over the concentration range investigated (r: 0.9969). Residual plots were evaluated, confirming that the used calibration model was appropriate (criteria: 10%). Two aliquots were extracted. All results were within the calibration range and concentrations were calculated from the linear regression equation, taking in account the amount of aliquot extracted. The following mean concentration was measured: 98 mg 4-CMA (as base)/tablet (n= 6; range: 86 – 106 mg/tablet). A blank was analysed before every sample. No carry-over was observed.

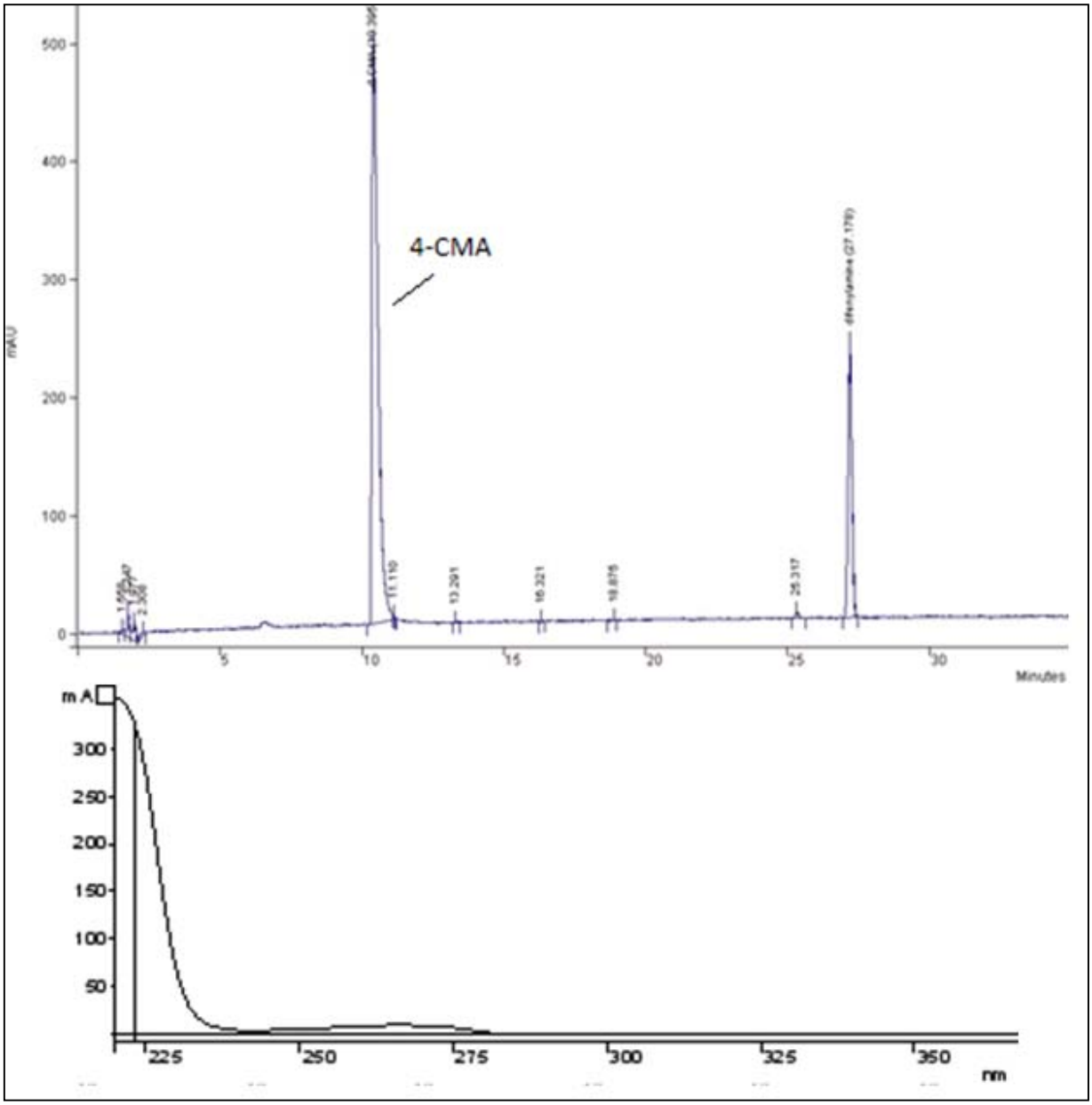


Figure 7: HPLC-PDA chromatogram of the tablet (upper) with UV-spectrum of 4-CMA (lower).

3.3. ^{1}H NMR

For chloromethamphetamine, with respect to the position of the chlorine atom, three region-isomers are possible, respectively 2-chloromethamphetamine, 3-chloromethamphetamine and 4-chloromethamphetamine. The signals for the protons of the phenyl group can be found in the area between 6.5 - 8.0 ppm in the ^{1}H-NMR spectrum. The peaks are shown to be two doublets, each with an integral of approximately 2, indicating the presence of a clear para-substitution. A full assignment of all the peaks of the 4-CMA structure can be found in Figure 8 and Figure 9.

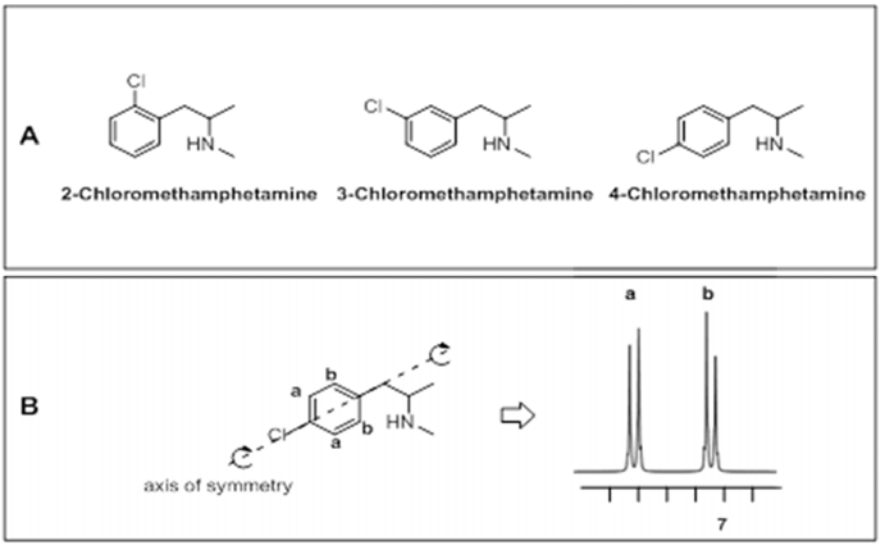


Figure 8: A: Three regioisomeric forms of chloroamphetamine B: Only 4-CMA has the required symmetry to yield the two doublets at 6.6 - 8.0 ppm.

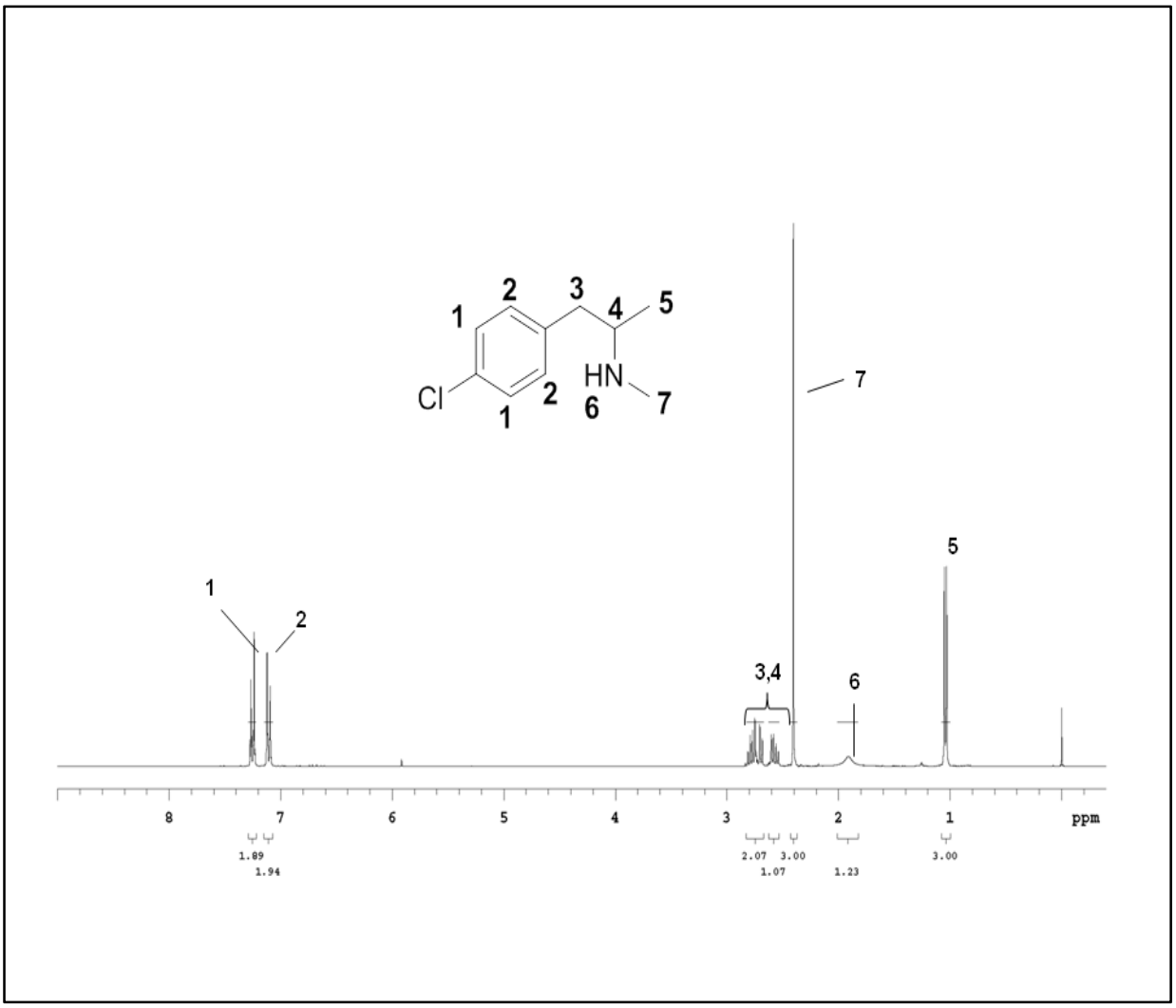


Figure 9. [1]H-NMR spectrum of 4-CMA with full structural assignment.

4. Discussion

The combination of all applied techniques HPLD-PDA, GC-MS and [1]H-NMR allowed to unequivocally identify the unknown substance as 4-CMA in a tablet confiscated at an international music festival. Since this seizure, which happened in August of 2015 (Figure 10, part A), several other cases were reported in the EU where 4-CMA was identified in seized drug samples: in November and December of 2015 respectively , light yellow tablets with a turtle logo containing 4-CMA were identified in respectively Romania and Austria (Figure 10, part B).

Finally, in March 2016 a larger quantity of these tablets with turtle logo was identified by police services in Croatia.

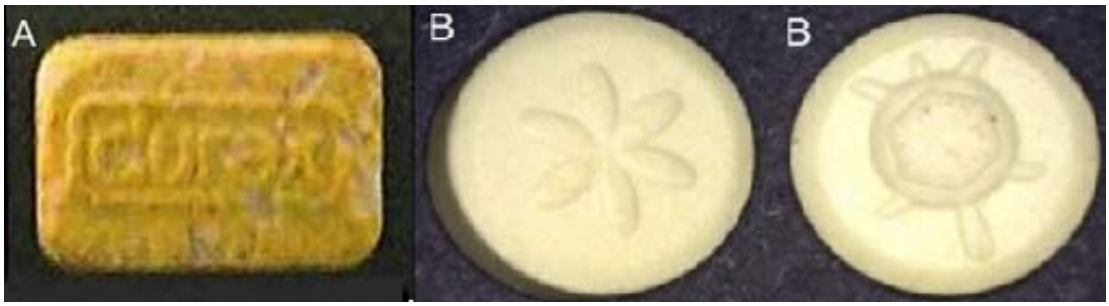


Figure 10. Ecstasy tablets containing 4-CMA, found in 2015 in Belgium (A) and elsewhere in Europe (B).

To estimate the potency of the tablet, a quantitative assessment of the 4-CMA concentration (performed with HPLC-PDA) indicated a dosage of approximately 98 mg 4-CMA (expressed as mg base per tablet).

4-CMA is the para-chlorinated N-methylated derivative of amphetamine and was researched in the 1960's as an appetite suppressant. During these studies, decreased levels of 5-hydroxytryptamine (serotonin, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were observed after the administration of *p*-chloroamphetamine and 4-CMA (10). 4-CMA was described by Patrick et al. and Pletscher et al. Notable was that the substance differed from other amphetamines by exhibiting only a slight central stimulant effect in both animals and humans, and that they acted like an antidepressant rather than a central stimulant. 4-CMA was briefly evaluated clinically as an antidepressant. It was also reported that 4-CMA was found to be a potent and long-lasting depleter of brain serotonin. Further investigation into the long-term effects of chloroamphetamines demonstrated that administration of 4-CMA caused a prolonged reduction in the levels of 5-HT and the activity of tryptophan hydroxylase in the brain. One month after injection of a single dose of the drug, both 5-HT and tryptophan hydroxylase activity

were still maximally reduced (11–14). It has been compared to methamphetamine in normal subjects. No major physiological side effects were noted. Ultimately it was discovered that 4-CMA is neurotoxic, specifically acting at the serotonergic neurotransmission system (15–18). Hence, clinical research in humans was halted. Dosages used in lab animals were 1-2 mg/kg. Human clinical dosages of 4-CMA used during the research as an antidepressant amounted to 80 mg daily (divided into three doses) (19), comparable to what was found in the 4-CMA tablet in Belgium (approximately 98 mg/tablet). Since a specific antidote is lacking treatment of overdoses would be symptomatic.

In the absence of empirical experimental clinical evidence, prof. David Nichols would predict 4-CMA to be a stimulant and hyperthermic agent with a psychopharmacology similar to MDMA, but more potent, and also neurotoxic. 4-CMA might have a longer duration of action compared to MDMA (which lasts 4-5 hours) because it is less susceptible to metabolism. Acute toxicity of this compound (hyperthermia, dehydration) was the first concern of Dr. Nichols (20).

Summarizing the receptor actions of 4-CMA, we estimate that clinical effects of 4-CMA will be a combined result of motor activating effects mediated by NA potentiation, and mood-improving effects caused largely by 5-HT potentiation. In practice, these include stimulant effects (such as increased energy and stimulation, euphoria) and feelings of wellbeing and possibly empathogenic effects comparable to those of MDMA, attributable to the serotonergic properties of 4-CMA (19). Based on rodent data it is believed that 4-CMA will be more potent than MDMA and will likely have a longer duration of action, with a psychopharmacology similar to MDMA (20).

From available literature and expert discussion, we estimate that the health risks for 4-CMA could include both acute and more prolonged long-term effects. Theoretically acute health risks would be comparable to those observed for MDMA, PMMA and 4-MA, and would be mainly due to serotonin release, combined with noradrenergic stimulation. Potentially severe, possibly malignant hyperthermia would be a risk resulting from an induced serotonin syndrome. In addition to acute effects, literature suggests 4-CMA demonstrates neurotoxic properties resulting in permanent destruction of serotonergic neurons. Currently the clinical or biological implications of this neurotoxicity in humans remain unknown. Serotonergic neurotransmission being implicated, it stands to reason that long-term exposure and/or damage could potentially include depression. In addition, no information is available regarding the time of manifestation of these symptoms; late onset of symptoms of induced neurotoxicity is a possibility.

5. Conclusions

To the author's best knowledge, this is the first report in published literature confirming the presence of 4-CMA in a seized ecstasy tablet. Conclusive identification and analytical characterization were performed using HPLC-PDA, GC-MS (including TMS and TFA derivatives) and ^{1}H-NMR. Available literature and discussion with experts suggests neurotoxic properties for 4-CMA, the effects of which on the human body are currently unknown. The drug is typically advertised on the web as a 'research chemical' and offered for sale as either tablets or powder. No intoxications or fatalities involving the use of 4-CMA were found in literature; Considering that after this initial detection several other tablets containing 4-CMA were identified in different parts of Europe, it stands to reason that some people will have consumed

these tablets. In clinical cases with observed neurotoxicity after (prolonged) drug abuse, especially ecstasy, and in the absence of other contributing factors, professionals could consider the potential (past) consumption of tablets containing 4-CMA when assessing patient and case history. After March 2016 no tablets containing 4-CMA were reported again. We estimate that tablets containing 4-CMA were present on the European market for about six months (summer 2015 – spring 2016).

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