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Case Report

Suicide by self-injection of chlormequat trademark C5SUN®

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ABSTRACT

Chlormequat is a quaternary ammonium used as plant growth regulating agent. We report here the first suicide case involving a 45 year-old farmer man who intentionally self-injected C5SUN®, containing chlormequat and choline. An original liquid chromatography high resolution mass spectrometry method (LC-HR-MS), using a hybrid quadrupole-orbitrap mass spectrometer, was developed for qualitative and quantitative analysis of chlormequat in different biological matrices. Toxicological analyses of post-mortem samples highlighted the presence of chlormequat in the blood (2.25 mg/L) and the urine (4.45 mg/L), in addition to ethanol impregnation blood (1.15 g/L). The route of administration (subcutaneous injection) was confirmed by the detection of chlormequat in the abdominal fat sample (chlormequat: 10.04 mg/g) taken from the traumatic injury location, as well as in the syringe found at the death scene, close to the victim's body. Based on the results of these post-mortem investigations, the cause of death was determined to be consecutive to cardiac dysrhythmia and cardiac arrest following chlormequat self-injection.

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1. Introduction

Chlormequat (2-chloro-*N*,*N*,*N*-trimethylethanaminium) is a plant growth regulator. It is a quaternary ammonium compound, widely used to promote flower formation, to improve fruit setting in fruits and vegetables and reduce vegetative growth and the inhibition of sprouting [1–3]. Chlormequat is commonly applied in the form of the salt chlormequat-chloride or chlorocholine chloride (CCC). CCC is known to be a competitive inhibitor of cholinesterase in animals [4]. This anticholinesterase chemical induces a cholinergic syndrome throughout the central and peripheral nervous system [4]. Administration of anticholinesterase agents may cause cholinergic crisis manifested by symptoms such as sweating, increased salivation, visual disturbance, pulmonary edema, bradycardia, cardiac dysrhythmias, seizures, and eventually ventricular fibrillation and cardiac arrest [5]. Neurological disorders may include confusion and coma [5].

An illegal use of CCC has been reported especially in the case of animals' euthanasia [6,7], and the ingestion of CCC as a method of suicide is circulating through Internet social networks. Until now, at least 10 fatal cases of CCC poisoning have been published [6,8–10], and all of these cases were reputed from ingestion of the plant growth regulator (CCC). A main limitation of all the published cases is the lack of analytical and forensic results, especially the quantification of the target substance in the different biological samples.

In the present report, an original case of a suicide after subcutaneous self-injection of CCC is presented for a 45 year-old man, with complete autopsy findings and toxicological results. Toxicological analysis of the different post-mortem samples is carried out after development of a specific and original liquid chromatography high resolution mass spectrometry (LC-HR-MS) method for the identification and quantification of CCC in biological matrices. Therefore, the outlines of the analytical method are described and the measurement of CCC concentrations is used for determining the cause of death.

2. Case report

2.1. Case history

A 45 year-old Caucasian farmer man was found dead at home, lying on his bed in a supine position. The victim was covered, fully

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clothed and a belt loosened. The deceased's clothes were intact and did not reveal any evidence of struggle or violence, no bleeding wounds were observed at any site. A 20 mL Terumo® Syringe with a 1.2 mm × 40 mm needle and a plastic 10 L canister of C5SUN® were found in the immediate vicinity of the body. Emergency services attempted performing cardiopulmonary resuscitation without success. The victim had no specific medical history and did not undertake any treatment. The macroscopic external examination of the body at the death scene essentially revealed a 10 cm × 5 cm bruise of the right iliac fossa with a circa 1 mm diameter injection site (Fig. 1).

2.2. Autopsy findings

Autopsy was performed 17 h post-mortem. No lesions were found in the external examination of the body except the bruise with injection site previously described. Autopsy showed congestion of internal organs and pulmonary edema. No organs were injured, and although the cause and manner of death were not established, a traumatic cause was definitely excluded. No pathological previous state was noted. Femoral blood, urine and abdominal subcutaneous fat underlying the injection site were sampled for toxicological analysis. The heart was taken for histopathological analysis, which showed no macroscopic or microscopic abnormalities. One hundred millilitre of the solution contained in the C5SUN® canister and the syringe were also sent for toxicological analysis.

3. Toxicological analysis

3.1. Volatile compounds analysis

Ethanol as well as other volatile compounds (acetone, isopropanol, and methanol) was determined by gas chromatography-flame ionisation detection-headspace (GC-FID-HS) in the C5SUN® canister liquid contents and blood sample.

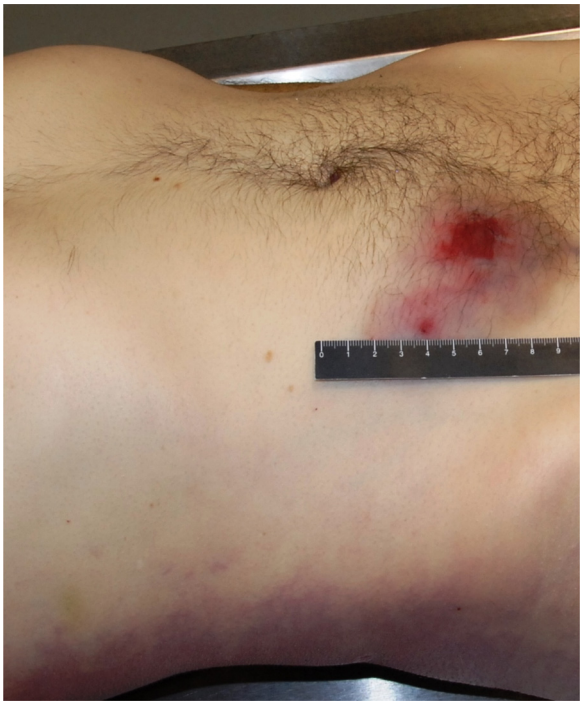

Fig. 1. Bruise and injection site of the abdominal wall.

3.2. Chlormequat analysis

Both qualitative and quantitative analyses of chlormequat were performed using liquid chromatography high resolution mass spectrometry detection (LC-HR-MS), equipped with a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific™, San Jose, USA). Data were acquired in both full-scan and targeted MS^2 (t-MS^2) modes. Chromatographic and spectrometric conditions, as well as retention times and t-MS^2 parameters of chlormequat, choline and acetylcholine (I.S) are reported respectively in Tables 1 and 2. C5SUN® canister liquid and of syringe contents have been diluted in methanol before injection into LC-HR-MS. For biological sample purification, blood (200 μL) and urine (200 μL) samples were added in 300 μL of 0.1 M zinc sulphate solution and supplemented with 500 μL of methanol containing the internal standard (0.2 mg/L). The solution was mixed on vortex for 2.0 min, kept for 10 min at 4.0 °C, and then centrifuged for 10 min at 3000 × *g*. Supernatants were evaporated to dryness at 50 °C under a stream of nitrogen. Residues were dissolved in 200 μL of LCMS grade water, vortexed and again centrifuged for 5 min at 3000 × *g*, before transferring supernatants into chromatographic vials for LC-HR-MS analysis. Calibration curves were obtained by fortifying chlormequat-free human biological fluids (blood and urine) with working solution of CCC at final concentrations of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ng/mL. Fat sample (1 g) was diluted in 5 mL of hexane, and then finely homogenized for 10 min with a Polytron PT-1200 homogenizer (Kinematica AG). The resulting solution was then mixed on vortex for 2.0 min, sonicated for 2 h, and again homogenized by turning for 1 h. For purification, 200 μL of this liquid solution were added in 300 μL of 0.1 M zinc sulphate solution and supplemented with 500 μL of methanol containing the internal standard (0.2 mg/L). The solution was mixed on vortex for 2.0 min, kept for 10 min at 4.0 °C, homogenized by turning for 2 h and then centrifuged for 10 min at 3000 × *g*. The aqueous layer was removed and evaporated to dryness at 50 °C under a stream of nitrogen. Residues were dissolved in 200 μL of LC-MS grade water, vortexed and again centrifuged for 5 min at 3000 × *g*, before transferring

Table 1
Liquid chromatography and mass spectrometry conditions.

Liquid chromatography	Mass spectrometry
•Column: C18 (15 mm × 2.1, 2.6 μm)	•Source: HESI-II
•Temperature: 20 °C	•Probe: 300 °C
•Mobile phase:	•Mode: positive
A: 60% CH_3COONH_4 (10 mM)	•Spray voltage: 3 kV
B: 40% ACN with 0.1% (v/v) formic acid	•Sheath gas and auxiliary gas: N_2
	•Capillary temperature: 300 °C
•Mode: isocratic	•Source lens: 60 V
•Run time: 3 min	•Acquisition data: Full scan HR + Targeted MS^2
•Flow rate: 0.3 mL/min	•Resolution: 70,000 FWHM
•Tray temperature: 15 °C	•C-trap capacity: 10^6 charges
•Injected volume: 5 μL	•Maximum injection time: 100 ms
	Mass range: 50–150 *m/z*

Table 2
Multiple reaction monitoring parameters for chlormequat, choline and acetylcholine.

Compounds	Chemical formula	RT (min)	Precursor ion $[M+H]^+$ (*m/z*)	NCE (%)	Product ion (*m/z*)
Chlormequat	$C_5H_{13}ClN$	1.57	122.0733	70	58.0659
Choline	$C_5H_{14}NO$	1.42	104.1773	70	60.0815
Acetylcholine (I.S)	$C_7H_{16}N_2$	1.52	146.1253	35	87.0446

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