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## Distribution of chloralose in a fatal intoxication

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## **Abstract**

Chloralose (alpha-chloralose) is a poisonous substance currently used as a rodenticide or avicide. It has primarily been used in Europe since 1893 as a human and veterinary hypnotic agent. Chloralose is a central nervous system depressant also acting as a stimulant on spinal reflexes. In the present case, a 24-year-old man was found dead in his bedroom near vomit residues. Several items were seized from the scene, including an empty bottle of Murex 50 g (alpha-chloralose), sold in Italy as rodenticide. Postmortem examination revealed no evidence of natural disease or trauma. Heart blood, urine, gastric contents, vitreous humour, brain, bile and liver were collected and submitted for toxicological analysis. Several extraction procedures and a specific liquid chromatography–tandem mass spectrometry protocol were purposely developed and validated. Chloralose was found in blood at a concentration of 65.1 mg/L and high levels were also detected in the gastric contents, confirming its ingestion shortly before the man's death. The distribution of chloralose in the body was evaluated by analyzing urine, vitreous humour, brain, bile and liver specimens. Quantitation of chloralose in several body fluids and tissues adds new data about the distribution of this chemical in the human body after massive ingestion.

## Introduction

Chloralose (alpha-chloralose) is a condensation product of chloral hydrate and glucose. It has been used primarily in Europe since 1893 as a human and veterinary hypnotic agent and as avicide and rodenticide for control of birds and rodents. In humans, chloralose has both a depressive effect on the central nervous system, producing sedation and anesthesia, and a stimulant effect on spinal reflexes, producing spontaneous myoclonic movements or generalized convulsions (1–3). Chloralose is no longer used in medical practice, but it has been available until recently as 75–300 mg oral and rectal dosage forms as anesthetic for human administration (4). Adverse reactions associated with chloralose overdose include tachycardia, ataxia potentially progressing to prostration and coma, hypothermia or hyperthermia, rhabdomyolysis, miosis and respiratory depression (5–7). The oral toxic dose of chloralose is approximately 1 g in adults and 20 mg/kg in infants (8). Chloralose is converted to chloral hydrate by hepatic metabolism, then transformed by further reduction to trichloroethanol, which is then oxidized to trichloroacetic acid or glucuronidated to urochloralic acid (4). The presence of these metabolites was not confirmed in previous reports of human poisoning (9).

## Case History

A 24-year-old man was found unconscious in his bedroom shortly after vomiting. The family called the medical rescue, but after 30 min of resuscitation efforts, he was pronounced dead. The family members reported that he had been in treatment for depression since 2007 after a suicide attempt. Two weeks before his death, he had allegedly started taking mesterolone tablets, typically used to increase muscle mass. Several packages of pharmaceuticals were found in his room. Specifically, the police seized one opened box of Tavor (lorazepam) containing seven tablets, one opened box of Largactil (chlorpromazine) containing four tablets, two boxes of Pantoprazolo Mylan Generics (pantoprazole), one opened box of Proviron (mesterolone) containing 16 tablets and one empty bottle of Murex 50 g (chloralose), sold in Italy as rodenticide. Furthermore, several packages of nutritional supplements were also seized, including creatine, amino acids and proteins. The death was reported to the Public Prosecutor who took jurisdiction of the case. At the autopsy, the body appeared wellnourished and the internal examination presented no evidence of natural disease or trauma to account for his death. All the organs were macroscopically normal. General pulmonary edema and multi-visceral congestion were found. Hence, our laboratory was asked to execute the inherent toxicological analyses in order to determine whether a possible massive drug consumption could be taken into account as the cause of the death. The specimens sampled during the autopsy included heart blood, urine, vitreous humour, gastric contents, brain, bile and liver.

## Experimental

### Materials

Alpha-chloralose, coumatetralyl, sodium hydrogen carbonate ( $\text{NaHCO}_3$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), sodium phosphate dibasic dehydrate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ), potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ), methanol, formic acid, acetonitrile, methyl t-butyl ether (MTBE), acetic acid (purity  $\geq 99.7\%$ ), sodium acetate ( $\text{CH}_3\text{COONa}$ ), ammonium acetate ( $\text{CH}_3\text{COONH}_4$ ) and  $\beta$ -glucuronidase (from *Escherichia coli*) were purchased from Sigma–Aldrich (Milan, Italy).  $\beta$ -Glucuronidase/arylsulfatase (from *Helix pomatia*) was obtained from Roche Diagnostic (Milan, Italy). Chloroform was purchased from Lab-Scan/Analytical Sciences (Dublin, Ireland). All solutions and buffers were prepared using deionized water obtained from Milli-Q system (Millipore, Billerica, MA). Acetate buffer 0.1M was prepared by dissolving 17.12 g of  $\text{CH}_3\text{COONa}$  and 6.4 mL of acetic acid in 1 L of water. Phosphate buffer was prepared by dissolving 4.63 g of  $\text{KH}_2\text{PO}_4$  and 11.75 g of  $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$  in 1 L of water, whereas carbonate buffer was prepared by dissolving 2.12 g of  $\text{Na}_2\text{CO}_3$  and 6.72 g of  $\text{NaHCO}_3$  in 1 L of water.

### Sample preparation

For general screening analysis, 2 mL (or 1 g) of samples were extracted under alkaline conditions (pH 9.6) by adding 2 mL of a 0.1M carbonate buffer and then 10 mL of MTBE. After shaking the mixture in a multimixer for 10 min, the organic layer was separated and then dried under a gentle flow of nitrogen. The resulting residue was reconstituted with 50 mL of methanol. Finally, a 1-mL aliquot was injected (split ratio of 10:1) into the gas chromatography–mass spectrometry (GC–MS) system. Before liquid–liquid extraction, urine samples were buffered at pH 7.4 with 2 mL of a 0.1M phosphate buffer and deconjugated by adding 30 mL of  $\beta$ -glucuronidase before incubating the mixture at 55°C for 1 h. For chloralose quantitation, coumatetralyl was chosen as the internal standard (1 mg/mL final concentration). Three extraction procedures were developed for the detection and quantitation of chloralose in blood, urine and tissues that are briefly reported in Table I. Ten microliters of the final extract was injected into the liquid chromatography–tandem mass spectrometry (LC–MS–MS) instrument.

### Apparatus and methods

Preliminary screening analyses for amphetamines, tricyclic antidepressants, barbiturates, benzodiazepines, cannabinoids, methadone, cocaine and opiates were performed by enzyme multiplied immunoassay technique (EMIT; Abbott Laboratories, IL), while the ethanol concentration in blood, urine and gastric content was determined by a headspace-gas chromatography–mass spectrometry (HS-GC–MS) method. Screening analysis for unknown substances was performed using a 6890N gas chromatograph (Agilent Technologies, Milan, Italy) equipped with a 17-m fused-

silica capillary column (J&W Scientific HP-5) of 0.2-mm inner diameter and 0.33-mm film thickness, for GC separation. Helium was employed as the carrier gas at a constant pressure of 23.24 psi. The gas chromatograph oven temperature was set at 90°C for 1 min and then raised to 180°C with a 30°C/min heating rate. The oven temperature was maintained at 180°C for 7 min and then raised to 315°C with a 15°C/min heating rate. The gas chromatograph injector and transfer line were maintained at 280°C. Full scan spectra in the interval 50–500 amu were acquired using a 5975 inert mass-selective detector (Agilent) operating in the EI mode at 70 eV. For chloralose confirmation analyses, a specific LC–MS–MS procedure was developed and validated. Chromatographic separation was performed using an Agilent 1100 series liquid chromatograph equipped with a Merck LiChroCart Purospher STAR-RP C18 column (4.6 × 150 mm, 5 mm) protected by a guard column. The elution solvents were ammonium acetate 10 mM solution (component A) and methanol (component B). The mobile phase eluted under the following linear gradient conditions: (A:B; v:v) from 60:40 to 10:90 in 18 min, with 7 min for re-equilibration. Each chromatographic separation was run at a flow rate of 500 mL/min. Detection was carried out by an Applied Biosystems API 3200 triple quadrupole mass spectrometer (ABSCIEX, Foster City, CA) equipped with turbo ion spray source, operating in the negative ionization mode. Optimal results were obtained using a source block temperature of 350°C. Data were recorded in the selected reaction monitoring (SRM) mode. To establish appropriate SRM conditions, the analyte and the internal standard (IS) were individually infused into the electrospray ionization (ESI) capillary and the cone voltage (CV) was adjusted to maximize the intensity of the deprotonated molecular species. Collision energy voltage (CE) was selected to preserve approximately 10% of precursor ion. Nitrogen was employed as the collision gas. Precursor ion and the corresponding product ions for chloralose and IS are presented in Table II. Qualitative and quantitative analysis for chlorpromazine, mesterolone (17 $\beta$ -hydroxy-1 $\alpha$ -methyl-5 $\alpha$ -androstane-3-one), mesterolone metabolites (met 1: 3 $\alpha$ -hydroxy-1 $\alpha$ -methyl-5 $\alpha$ -androstane-17-one; met 2: 1 $\alpha$ -methyl-5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol) and lorazepam were performed in blood and urine specimens by means of analytical methods used in our laboratory and described elsewhere (10, 11).

#### Validation of the LC–MS–MS confirmation methods for chloralose quantitation

The method was validated by investigating the following parameters: selectivity, linearity, identification and quantitation limits (limit of detection, LOD, and limit of quantitation, LOQ), precision, accuracy and recovery. Matrix effect phenomena were also evaluated (12, 13). Blood, urine and liver were chosen as the target matrices for method validation. The linear calibration models were checked by analyzing (three replicates) blank samples (blood, urine and liver) spiked with chloralose standard solution at final concentrations of 0, 0.75, 1, 5, 10, 50, 100, 150 and 200 mg/L (or mg/kg). Whenever the effective drug concentration exceeded the calibration range, the samples were diluted to fit the quantitation interval considered in the curve. Dilution integrity was

evaluated spiking the matrix at concentration 1.5 times the highest calibration point (300 mg/L or mg/kg) and diluting them twice and ten times with blank matrix. These samples were analyzed along the calibration curve and the accuracy was considered satisfactory in the interval +15%.

For each matrix, ten different blank samples were prepared as described in Table I to test the selectivity of the whole analytical procedure. The occurrence of possible interferences from endogenous substances was checked by monitoring the signal-to-noise ratio (S/N) for the chloralose SRM transitions at the expected retention time. Matrix effects were evaluated by comparing the signal obtained when the analyte was added to the matrix extract with the response obtained from a methanolic solution containing the analyte at the same concentration. LOD values were estimated as the analyte concentration whose response provided an S/N value equal to 3, as determined from the least abundant transition. LOD values were extrapolated from S/N recorded at the concentration of 0.375 mg/L or mg/kg, whereas the lowest calibration point was 0.75 mg/L. The calculated LOD was then experimentally confirmed by analyzing spiked samples at LOD concentration of chloralose. LOQ values were estimated as the analyte concentration whose response provided an S/N value equal to 10. LOQ generally corresponds to the lowest concentration that provides a useful signal along the calibration curve. Relative extraction recoveries were determined by comparing the responses of the extracted samples (five replicates for blood, urine and liver) initially spiked with the analytes at a final concentration of 10 and 100 mg/L (or mg/kg) with the responses of blank samples in which chloralose was added at the same concentration after the extraction step. Within-batch precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias percent), were assessed by extracting and analyzing, for each tested matrix, a series of five samples fortified at 10 and 100 mg/L (or mg/kg).

## **Results and Discussion**

On the basis of the mass-spectrometric data, three diagnostic transitions for chloralose and one characteristic transition for the internal standard were acquired. The SRM chromatograms from 10 negative samples of blood, urine and liver showed no interfering signals (i.e., S/N ratio lower than 3) at the corresponding retention time for the three matrixes tested. This demonstrated the selectivity of the methods and that they are free from interferences from matrix components. The LODs for chloralose were estimated from the previously described calculations at 0.02 and 0.16 mg/L in blood and urine, respectively, and 0.04 mg/kg in liver, resulting from S/N ratios of 60, 7 and 26 in blood, urine and liver, respectively. Estimated LODs were experimentally confirmed by analyzing in triplicate a blank matrix sample (blood, urine and liver) spiked with chloralose at the LOD concentration. All the observed S/N ratios exceed the critical value of 3, as expected. The estimated LOQ values for chloralose were 0.06 and 0.54 mg/L in blood and urine, respectively, and 0.14 mg/kg in liver. These values are smaller than the lowest calibration point, which was arbitrarily

fixed at 0.75 mg/L in blood and urine and 0.75 mg/kg in liver. Calibration curves (eight points plus a zero sample) were generated by linear regression and exhibited squared correlation coefficients ( $R^2$ ) of 0.9915 (blood), 0.9953 (urine) and 0.9934 (liver). At 10 mg/L (or mg/kg) concentration, extraction recoveries were 103.3, 26.0 and 12.8% in blood, urine and liver, respectively, while at 100 mg/L (or mg/kg), the extraction recoveries were 97.1, 35.2 and 18.6% in blood, urine and liver. The within-batch data on precision and accuracy are reported in Table III. The results show a satisfactory repeatability, because the CV% is lower than 10% for chloralose spiked in blood, urine and liver samples at both 10 and 100 mg/L (or mg/kg). In the three tested matrices, the accuracy (expressed as percent bias) varies from  $\pm 1.1$  to 29.2% and all experimental bias values were below the acceptable limit of +15% at 10 and 100 mg/L (or mg/kg) concentration. At the two tested concentrations, no significant matrix effects were found for chloralose in the different matrix (values between 21.6% and  $\pm 6.0\%$ ). However, to prevent matrix effects, possibly present when a specific matrix has to be analyzed, all calibrations and validation tests were conducted on the target matrices spiked with the analyte standard solution. All validation results are summarized in Table III. In the real case reported, the presence of chloralose was confirmed in all specimens; all the toxicological results are reported in Table IV. Therapeutic levels for chloralose are not reported in literature. In four reported poisoning cases involving chloralose, the plasma concentrations ranged from 13.7 to 41.3 mg/L (mean 25.4 mg/L) (9), whereas in seven comatose patients, the drug was found at an average level of 50 mg/L (range, 6.8– 180 mg/L) (4). In three fatal cases due to massive ingestion of chloralose, postmortem peripheral blood concentrations were between 151 and 410 mg/L (4, 14, 15). In the case presented here, the heart blood concentration of chloralose was 65.1 mg/L. The LC–MS–MS profile resulting from the detection of chloralose in blood and the profile of blank blood samples are shown in Figure 1. This concentration is lower than those previously reported, but it can be considered highly toxic. Indeed, the diagnosis of chloralose intoxication is frequently immediate, so that a rapid treatment can be carried out (i.e., gastric lavage, activated charcoal, sedation with benzodiazepines or mechanical ventilation), leading to complete recovery. The prolongation of a comatose state without an early emergency remedy can lead to acute respiratory failure resulting in cardiac arrest (3). Free and total chloralose were also identified in urine. Glucuronide conjugate exceeded the free parent drug by 7.3-fold. This finding confirms that chloralose is excreted primarily as the glucuronide metabolite, as previously reported (9, 16). Other analytical findings indicate the recent intake of lorazepam, chlorpromazine and mesterolone, consistent with scene investigation. Due to their low blood levels, their presence was considered non-influential in the acute intoxication episode.

## Conclusions

This report presents a fatal case involving acute intoxication by alpha-chloralose. After preliminary GC–MS screening, a specific LC–MS-MS procedure was developed and validated to specifically and accurately detect the drug in various human fluids and tissues. The high concentration in the gastric content proved the recent massive ingestion of chloralose. Chloralose was measured in bile, liver, brain and vitreous humour, all of which showed a significant presence of the drug. However, vitreous humour was not a major site of alpha-chloralose deposition, as previously described in fatal cases involving an acute consumption of chloralose (15). Finally, the heart blood concentration was in the range considered to be highly toxic, although lower than that found in previous cases. In conclusion, the agreement between the postmortem examination and the toxicological findings were consistent with fatal chloralose intoxication.

## References

1. Leikin, J.B., Paloucek, F.P. (2007) Poisoning and toxicology handbook, 4th edition. Informa Healthcare, New York, NY.
2. Federici, S., Claudet, I., Laporte-Turpin, E., Marcoux, M.O., Cheuret, E., Marechal, K. (2006) Intoxication se´ve`re par le chloralose chez un nourisson. *Archives of Pediatrics*, 13, 364–366.
3. Kouraichi, N., Brahmi, N., Elghord, H., Be´ji, O., Thabet, H., Amamou, M. (2010) Intoxication par le chloralose: Facteurs pronostiques et prise en charge. *Reanimation*, 19, 581–586.
4. Baselt, R.C. (2004) Disposition of toxic drugs and chemicals in man, 7th edition. Biomedical Publications, Foster City, CA.
5. Manzo, L., Richelmi, P., Crema, A., Allaria, B., Caputo, E. (1979) Electrocerebral changes in acute alphachloralose poisoning: A case report. *Veterinary and Human Toxicology*, 21, 245–247.
6. Thomas, H.M., Simpson, D., Prescott, L.F. (1988) The toxic effects of alpha-chloralose. *Human Toxicology*, 7, 285–287.
7. Tracqui, A., Gibey, S., Marchal, A., Geraut, A., Ludes, B. (2001) Intoxication aigue“ se´ve`re par a-chloralose: Donne´es cliniques et toxicologiques a` propos d’un cas. *Journal de Medecine Legale, Droit Medical*, 44, 497–504.
8. Clarke, E.G.C. (2005) Clarke’s isolation and identification of drugs in pharmaceutical, body fluids and post-mortem materials, 3<sup>rd</sup> edition. The Pharmaceutical Press, London.
9. Kintz, P., Jamey, C., Mangin, P. (1996) Trichloroethanol is not a metabolite of alpha chloralose. *International Journal of Legal Medicine*, 108, 191–193.
10. Salomone, A., Gerace, E., Brizio, P., Gennaro, M.C., Vincenti, M. (2011) A fast liquid chromatography-tandem mass spectrometry method for determining benzodiazepines and

analogues in urine. Validation and application to real cases of forensic interest. *Journal of Pharmaceutical and Biomedical Analysis*, 56, 582–591.

11. Gerace, E., Salomone, A., Abbadessa, G., Racca, S., Vincenti, M. (2012) Rapid determination of anti-estrogens by gas chromatography/ mass spectrometry in urine: Method validation and application to real samples. *Journal of Pharmaceutical Analysis*, 2, 1–11.

12. Peters, F.T., Maurer, H.H. (2002) Bioanalytical method validation and its implications for forensic and clinical toxicology—A review. *Accreditation and Quality Assurance*, 7, 441–449.

13. Peters, F.T., Drummer, O.H., Musshoff, F. (2007) Validation of new methods. *Forensic Science International*, 165, 216–224. 14. Schmid, M., Iten, P.X. (1994) A fatal poisoning involving a-chloralose. Presented at the International association of Forensic Toxicologists meeting, Tampa, FL.

15. Kintz, P., Doray, S., Cirimele, V., Ludes, B. (1999) Testing for alphachloralose by headspace-GC/MS. A case report. *Forensic Science International*, 104, 55–63.

16. Savin, S., Cartigny, B., Azaroual, N., Humbert, L., Imbenotte, M., Tsouria, D. et al. (2003) <sup>1</sup>H NMR Spectroscopy and GC-MS analysis of a-chloralose. Application to two poisoning cases. *Journal of Analytical Toxicology*, 27, 156–161.

**Table 1.** Sample preparation procedures for the detection of chloralose in blood, urine and tissue specimens

<b>Blood samples</b>	<b>Urine samples</b>	<b>Tissue samples (validated as liver)</b>
1. 300 $\mu$ L blood + 15 $\mu$ L IS (20 $\mu$ g/mL solution)	1. 300 $\mu$ L urine + 15 $\mu$ L IS (20 $\mu$ g/mL solution)	1. 2 g of tissue (homogenate) + 40 $\mu$ L IS (40 $\mu$ g/mL solution)
2. Adding 1.5 mL of acetonitrile and mixing for 5 minutes	2. Adding 40 $\mu$ L of enzyme <sup>#</sup> and incubating for 1 h at 37°C	2. Adding 10 mL of Acetate buffer (0.1 M at pH 5) and mixing for 5 minutes
3. Centrifuging at 3000 rpm for 5 minutes and taking the supernatant layer	3. Adding 1.5 mL of chloroform and mixing for 5 minutes	3. Centrifuging at 3000 rpm for 5 minutes and taking the aqueous layer
4. Drying the solvent under nitrogen at 50°C	4. Centrifuging at 3000 rpm for 5 minutes and taking the organic layer	4. Adding 10 mL of chloroform and mixing for 5 minutes
5. Reconstituting the dry residue with 300 $\mu$ L of mobile phase*	5. Drying the solvent under nitrogen at 50°C	5. Centrifuging at 3000 rpm for 5 minutes and taking 1 mL of organic solvent
	6. Reconstituting the dry residue with 300 $\mu$ L of mobile phase*	6. Drying the solvent under nitrogen at 50°C
		7. Reconstituting the dry residue with 50 $\mu$ L of mobile phase*

\* mobile phase = ammonium acetate 10 mM:methanol 60:40 v/v

<sup>#</sup>  $\beta$ -glucuronidase/arilsulfatase from *Helix pomatia*

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**Table 2.** Mass Chromatographic Conditions for Chloralose and Coumatetralyl (IS)

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<b>Compound</b>	<b>RT (min)</b>	<b>SRM transitions (m/z)</b>	<b>CV* (V)</b>	<b>CE* (V)</b>	<b>CXP* (V)</b>
Chloralose	9.89	307.2 → 71.0	-40	-38	-2
		307.2 → 100.8	-40	-23	-2
		307.2 → 85.0	-40	-32	-1
Coumatetralyl	11.80	291.0 → 141.2	-65	-37	-4

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\*CV = cone voltage, CE = collision energy and CXP = Collision Cell Exit Potential.

**Table 3.** Validation Data for the detection of chloralose in three different matrixes.

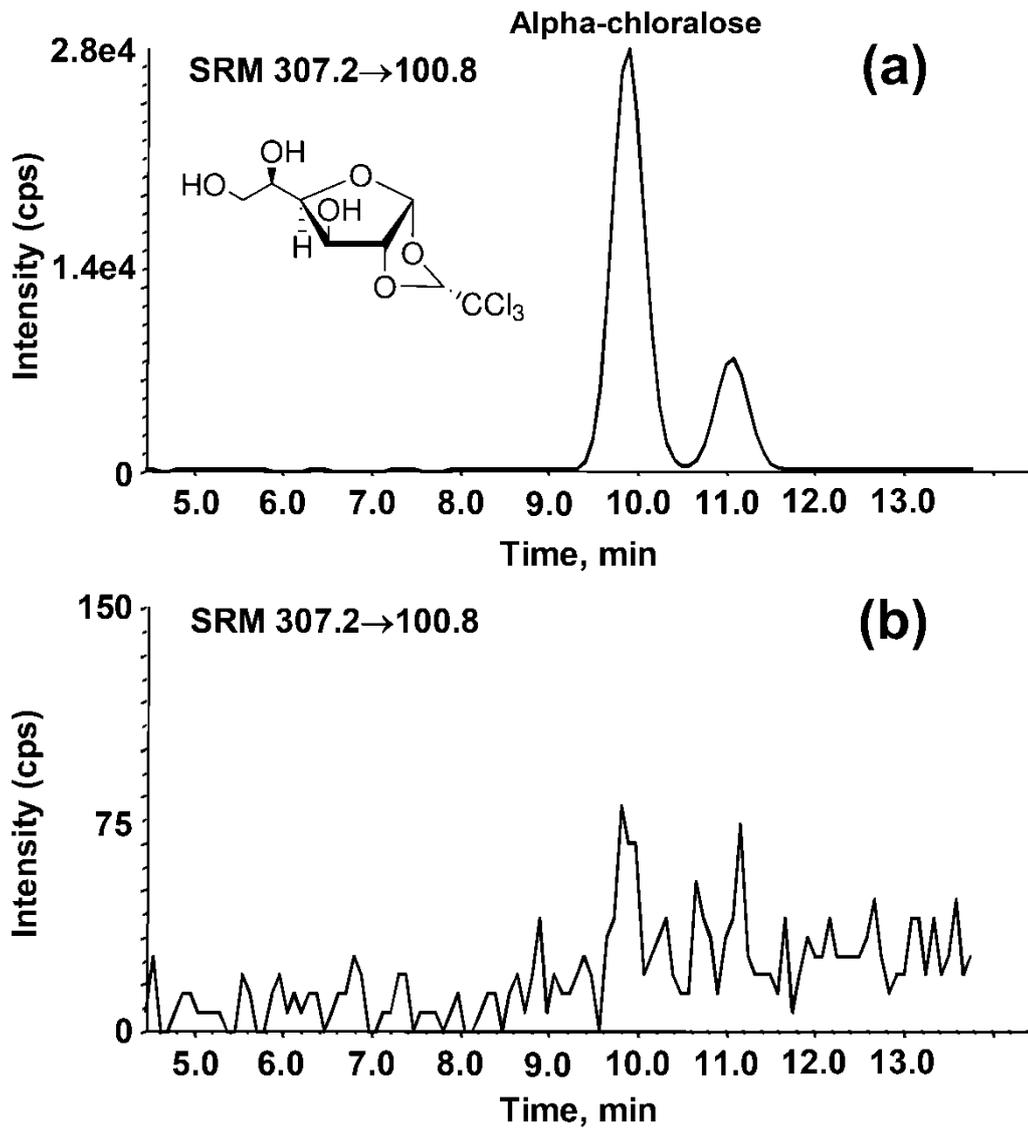
Chloralose												
Matrix	Linearity Range (mg/L)	Correlation coefficient (R <sup>2</sup> )	LOD (mg/L)	LOQ <sup>#</sup> (mg/L)	Precision (CV%)		Accuracy (bias%)		Recovery (%)		Matrix effect (%)	
					10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L
Blood	0.75–200	0.9915	0.02	0.06	8.7	5.2	+1.7	+1.1	103.3	97.1	−0.7	+1.4
Urine	0.75–200	0.9953	0.16	0.54	6.4	4.6	−9.2	−7.6	26.0	35.2	−1.6	+1.5
Liver*	0.75–200	0.9934	0.04	0.14	5.6	5.0	+4.7	+3.2	12.8	18.6	+6.0	+4.8

<sup>#</sup> calculated LOQ

\*all concentrations are expressed as mg/kg

**Table 4.** Chloralose concentrations in post-mortem specimens and other findings.

<b>Sample</b>	<b>Concentration (mg/L)</b>	<b>Other findings</b>
Blood	65.1	Chlorpromazine: 0.19 mg/L Lorazepam: 0.09 mg/L
Urine	635.2 (total) 76.3 (free)	Lorazepam: 1.08 mg/L Mesterolone: 2.22 mg/L Mesterolone met 1: 11.9 mg/L Mesterolone met 2: 0.69 mg/L
Bile	66.6	-
Gastric Content	6750	-
Vitreous Humour	24.7	-
Liver	62.1 (mg/kg)	-
Brain	38.1 (mg/kg)	-



**Figure 1.** Comparison between the extracted ion chromatogram resulting from the detection of chloralose in the victim's blood (a) and a blank blood sample (b).