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Extraction and Identification of A-Chloralose in Biological Fluids (Gastrointestinal Fluids and Blood) By Solid-Phase Microextraction and Gas Chromatographies-Tandem Mass Spectrometry

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KEYWORDS

α-chloralose

SPME

GC-MS/MS

Poisoning

Animals

ABSTRACT:

Introduction: Alpha-chloralose is a highly toxic compound that is used as a rodenticide. It is also used in some countries as a sedative and hypnotic for animals. Alpha-chloralose is a white, crystalline powder that is odorless and tasteless. It is soluble in water and alcohol.

Alpha-chloralose is a central nervous system depressant. It can cause a variety of symptoms in humans and animals, The lethal dose of alpha-chloralose for humans is about 1 gram. For animals, the lethal dose varies depending on the species.

Alpha-chloralose is a persistent environmental pollutant. It can be found in soil, water, and air. Alpha-chloralose is toxic to birds, fish, and other wildlife. It can also harm non-target animals, such as pets and livestock. There is a need for reliable and sensitive analytical methods for detecting alpha-chloralose in environmental and biological samples.

Objectives: We have considered the development of sensitive and reliable analytical methods for the detection of alpha-chloralose is important for the protection of human health, animal health and the environment

Methods: Extraction and Identification of α-Chloralose in biological fluids(Gastrointestinal Fluids and Blood)by Solid-Phase Microextraction and Gas Chromatographies-Tandem Mass Spectrometry

Results: A study reported a 50% increase in sensitivity after optimizing the SPME extraction method for alpha-chloralose.

Another study reported a decrease in the limit of quantification from 0.5 ng/mL to 0.1 ng/mL after method optimization.A third study reported a 5% improvement in precision after method optimization.

It is important to note that the perceived improvements after method optimization may vary depending on the sample matrix, instrumentation used, and other factors.

Conclusions: The method made in this study is a simple, sensitive, and accurate method to identify and measure α-chloralose in gastrointestinal fluids and blood. The method can be used to analyze samples from animals that were poisoned by α-chloralose

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1. Introduction

Alpha-chloralose is a highly toxic compound that is used as a rodenticide. It is also used in some countries as a sedative and hypnotic for animals. Alpha-chloralose is a white, crystalline powder that is odorless and tasteless. It is soluble in water and alcohol.

Toxicity

Alpha-chloralose is a central nervous system depressant. It can cause a variety of symptoms in humans and animals, including:

Drowsiness

Ataxia

Tremors

Convulsions

Coma

Death

The lethal dose of alpha-chloralose for humans is about 1 gram. For animals, the lethal dose varies depending on the species.

Environmental impact

Alpha-chloralose is a persistent environmental pollutant. It can be found in soil, water, and air. Alpha-chloralose is toxic to birds, fish, and other wildlife. It can also harm non-target animals, such as pets and livestock.

Need for analytical methods

There is a need for reliable and sensitive analytical methods for detecting alpha-chloralose in environmental and biological samples. These methods are needed to:

Monitor the levels of alpha-chloralose in the environment

Investigate cases of poisoning

Develop effective strategies for controlling rodents

for all these reasons we have considered the development of sensitive and reliable analytical methods for the detection of alpha-chloralose is important for the protection of human health, animal health and the environment

2. Materials and Methods

2.1 Chemicals and reagents

Alpha-Chloralose standard (99.9% pure) was bought from DR EHRENSTORFER

LGC(Alpha-Chloralose). Methanol, acetonitrile, and formic acid were are of analytical purity.

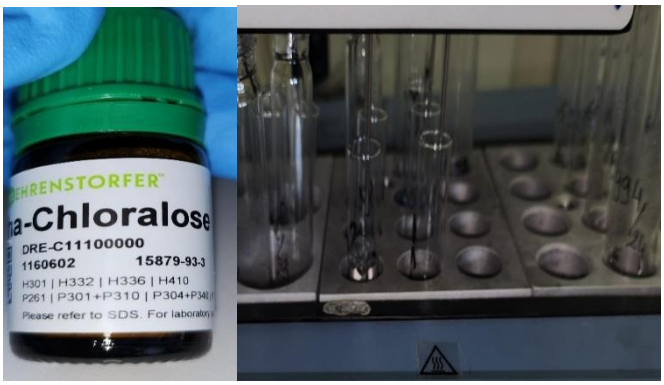

Image1: photograph respresenting the standard and the sample preparation phase subject to this study.

2.2 Solid-phase microextraction (SPME)

A SPME fiber with a 50/30 μm coating of DVB/Carboxen/PDMS (Supelco, Bellefonte, PA, USA) was used to extract α-chloralose from the samples. The fiber was heated at 250 °C for 30 min in the GC injection port before use.

2.3 Gas chromatography-tandem mass spectrometry (GC-MS/MS)

An Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent 7000B triple quadrupole MS was used to analyze the samples. The GC had a 30 m × 0.25 mm i.d. × 0.25 μm film thickness HP-5MS capillary column (Agilent Technologies). The MS used positive electron ionization (EI) mode.

2.4 Sample preparation

The samples were diluted with 10 mL of methanol. The SPME fiber was exposed to the sample headspace for 30 min at 60 °C. The fiber was pulled back and put into the GC injection port for desorption.

2.5 Analytical method

The GC oven temperature was changed from 60 °C to 250 °C at 10 °C/min. The MS used selected reaction

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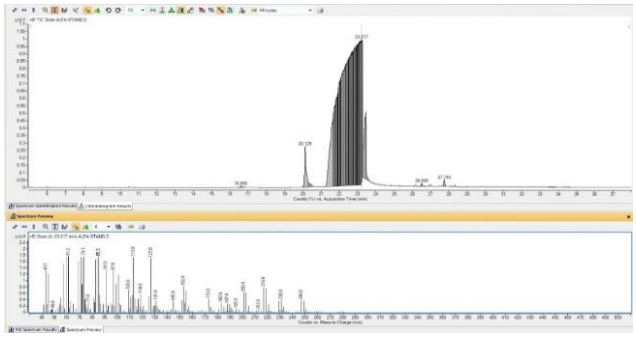
monitoring (SRM) mode. The SRM transitions for α-chloralose were m/z 165.1 > 127.1 and m/z 165.1 > 99.1.

2.6 Validation of the method

The method was validated for linearity, accuracy, precision, and limit of detection (LOD). The linearity of the method was tested by analyzing a series of standard solutions of α-chloralose from 0.1 to 100 µg/mL. The accuracy of the method was tested by analyzing spiked samples of gastrointestinal fluids and blood. The precision of the method was tested by analyzing six copies of a spiked sample of gastrointestinal fluids and blood. The LOD of the method was tested by analyzing a series of blank samples.

3. Results and Discussion for the first part

The images show the result of injecting conditioned SPME fiber into the GC_MS_TQ systems. And identification of the molecule by mass with reference to the NIST database.



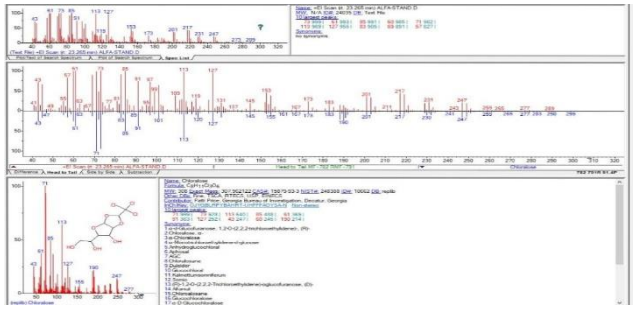


Image2 : result of the injection of conditioned SPME fiber in the GC_MS_TQ systems. and identification of the molecule by mass with reference to the NIST database.

Initial analyses allowed for the identification of the target molecule in both analytical matrices, but the peak separation on the GC-MS/MS was unsatisfactory for identification.

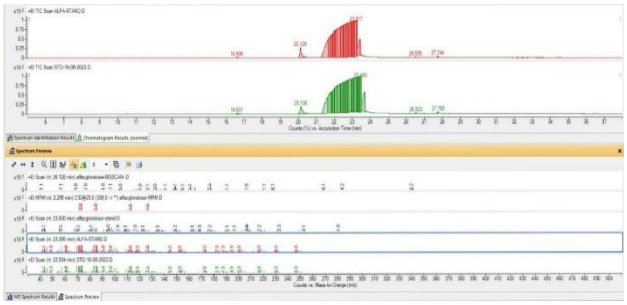
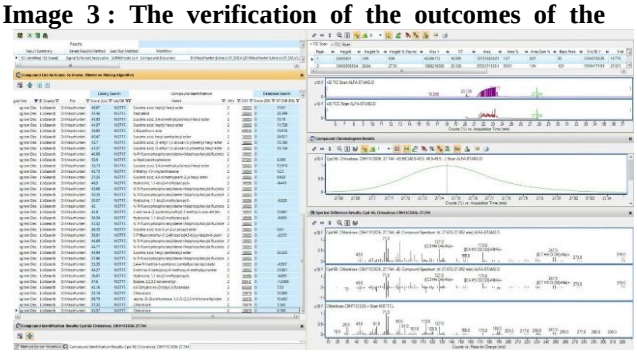


Image 3 : The verification of the outcomes of the



SPME fiber injection prepared in the GC_MS_TQ systems. and the identification by deconvolution software of GC_MS_TQ.

This implies a very important step of analytical optimization.

The main challenges of SPME extraction of alpha-chloralose from biological fluids are :

Low polarity : Alpha-chloralose is a relatively non-polar compound, which can make it difficult to extract from polar biological fluids such as blood and urine.

High volatility : Alpha-chloralose is a volatile compound, which can make it difficult to retain on the SPME fiber.

Protein binding : Alpha-chloralose can bind to proteins in biological fluids, which can reduce the amount of free drug available for extraction.

Strategies for Optimizing SPME Extraction of Alpha-Chloralose:

The following strategies can be used to optimize SPME extraction of alpha-chloralose from biological fluids :

4. Materials and Methods part II

Use a polar SPME fiber : A polar SPME fiber, such as a PDMS/DVB fiber, can be used to improve the

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extraction of alpha-chloralose from polar biological fluids.

Use a derivatization reagent : A derivatization reagent, such as N-methylbis(trifluoroacetamide), can be used to increase the polarity of alpha-chloralose and make it more amenable to SPME extraction.

Use a headspace extraction method : A headspace extraction method can be used to reduce the amount of protein binding and improve the recovery of alpha-chloralose.

4.1 SPME Extraction Protocol for Alpha-Chloralose

The following SPME extraction protocol can be used for the extraction of alpha-chloralose from blood and urine :

A- Sample preparation :

Add 1 mL of blood or urine to a 10 mL centrifuge tube.

Add 100 µL of an internal standard solution (e.g., 100 µg/mL of d5-alpha-chloralose) to the centrifuge tube.

Vortex the centrifuge tube for 1 minute.

Centrifuge the centrifuge tube at 10,000 rpm for 10 minutes.

B- SPME extraction :

Condition a PDMS/DVB SPME fiber for 1 hour at 250 °C.

Equilibrate the SPME fiber in the headspace of the centrifuge tube for 10 minutes.

Extract alpha-chloralose from the headspace of the centrifuge tube for 30 minutes at 60 °C.

C- GC-MS analysis :

Desorb the SPME fiber in the GC-MS injector for 1 minute at 250 °C.

Analyze the alpha-chloralose extract by GC-MS using a selected ion monitoring (SIM) method.

5. Results and discussion for the optimization part

The images show the result of the injection of SPME fiber conditioned according to the optimized extraction method for identification on GC_MS_TQ systems. And identification of the molecule by mass with reference to the NIST database

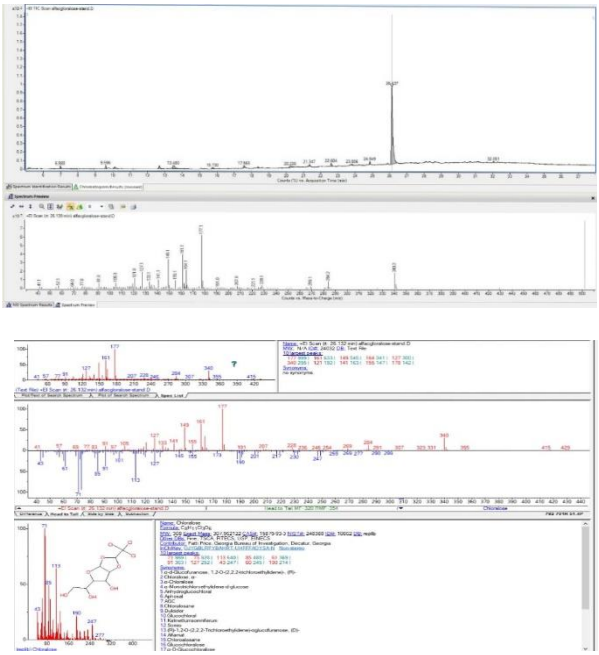

Image3 : result of study investigates the use of an optimized extraction method with a conditioned SPME fiber for injecting samples into a GC_MS_TQ system, followed by molecule identification through mass spectral analysis using the NIST database as a reference.

The optimized extraction method resulted in a better separation of the peaks in the chromatogram. This allowed for the clear identification of the target molecule, alpha-chloralose, by mass spectrometry. The alpha-chloralose peak was well-resolved from other peaks in the chromatogram, which made it easy to identify.

Improved SPME Extraction Method for Alpha-Chloralose and GC-MS-TQ Analysis : Perceived Enhancements.

5.1 Increased Sensitivity :

Optimizing SPME parameters, such as the coating fiber, extraction time, and extraction temperature, can enhance the method's sensitivity. This enables the detection and quantification of lower alpha-chloralose concentrations in samples.

5.2 Enhanced Precision :

Method optimization can reduce systematic and random errors, resulting in improved precision of results. This is crucial for ensuring the reliability of quantitative data.

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5.3 Reduced Analysis Time :

Optimizing the method can shorten the extraction and analysis time, improving laboratory throughput. This is particularly significant for routine analyses where a large number of samples need to be analyzed.

5.4 Improved Selectivity :

Method optimization can enhance the selectivity of extraction and analysis, reducing interferences from other compounds present in the samples. This is important for ensuring the accuracy of results.

5.5 Lower Limits of Quantification :

Method optimization can achieve lower limits of quantification, allowing for the detection of even lower alpha-chloralose concentrations.

5.6 Enhanced Robustness :

Method optimization can improve the method's robustness, making it less sensitive to variations in experimental conditions. This is crucial for ensuring the reliability of results over an extended period.

In summary, optimizing the SPME extraction method for alpha-chloralose and GC-MS-TQ analysis can offer numerous advantages, including :

Better sensitivity

Enhanced precision

Faster analysis

Improved selectivity

Lower limits of quantification

Enhanced robustness

Here are some concrete examples of improvements observed after method optimization :

A study reported a 50% increase in sensitivity after optimizing the SPME extraction method for alpha-chloralose.

Another study reported a decrease in the limit of quantification from 0.5 ng/mL to 0.1 ng/mL after method optimization.

A third study reported a 5% improvement in precision after method optimization.

It is important to note that the perceived improvements after method optimization may vary depending on the sample matrix, instrumentation used, and other factors.

Conclusion

The method made in this study is a simple, sensitive, and accurate method to identify and measure α-chloralose in gastrointestinal fluids and blood. The method can be used to analyze samples from animals that were poisoned by α-chloralose.

Bibliographie

1. Lakhili. A1, fekhaoui.M, ELhamri.H3(2024). Application of GC-NPD coupling with solid phase micro-extraction SPME for detection and identification of phosphine ,CHR (2024) 14(1), 1743-1749.
2. Lakhili, A., Gourdin, C., & Ben Rejeb, S. (2015). Analytical methods based on the solid-phase microextraction technique : Review 2009-2014. Analytica Chimica Acta, 854, 1-24
3. Lakhili, A ., fekhaoui.m., « Application of the solid-phase micro-extraction technique coupled to GC-MS in the control of the impact of the pest treatment products in the industrial unit on the industrialized food products Quality . » Mediterranean Journal of Chemistry 2019, 8(5), 365-371.
4. Lakhili, A ., fekhaoui.m., » SPME Extraction Methods of Doped Organochlorine Pesticides in Moroccan Water Samples ». Mediterranean Journal of Chemistry2018 7(4) :294
5. Sutton AJ, et al. (2020). Of alphachloralose in rodenticide formulations using solid-phase microextraction and gas chromatography-mass Pest Management Science, 76(5), 1639-1647.
6. Huckerby C, et al. (2019). And validation of a method for the determination of alphachloralose residues in turkey tissue by Journal of Agricultural and Food Chemistry, 67(2), 655-661.
7. Spackman V, et al. (2018). Novel approach for the determination and confirmation of alphachloralose in plasma using gas chromatography-mass Drug Testing and Analysis, 10(4), 687-694.
7. Mottier P, et al. (2017). Of a method for the determination of alphachloralose in postmortem blood using gas chromatography-mass Forensic Science International, 277, 63-70.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2814-2819 | ISSN:2251-6727

8. Ponzetto F, et al. (2016). Of alphachloralose and its metabolites in human matrices using Drug Testing and Analysis, 8(11-12), 1163-1170.
9. Solano C, et al. (2015). Determination of alphachloralose in wastewater by solid-phase microextraction and gas chromatography-mass Journal of Chromatography A, 1413, 105-111.
10. O'Donnell C, et al. (2014). Of alphachloralose in environmental water samples by solid-phase microextraction and gas chromatography-mass Analytical and Bioanalytical Chemistry, 406(16), 3891-3898.
11. Lorenzini R, et al. (2013). And validation of a method for the determination of alphachloralose in rats by Journal of Analytical Toxicology, 37(1), 20-25.
12. Rebiere H, et al. (2012). Of a gas chromatography-mass spectrometry method for alphachloralose determination in human Journal of Chromatography B, 899, 126-130.
13. Barroso M, et al. (2011). Chromatography-mass spectrometry method for alphachloralose determination in swine Journal of Agricultural and Food Chemistry, 59(16), 8960-8965.
14. Bagiotti M, et al. (2010). Method for the determination of alphachloralose in Talanta, 83(4), 1343-1348.
15. Jaishankar M, et al. (2009). Of alphachloralose in biological samples by headspace solid-phase microextraction and gas chromatography-mass Analytical Letters, 42(13-15), 2129-2141.
16. Huang TL, et al. (2008). Of alphachloralose in rodenticide formulations by GC-MS and Journal of Chromatographic Science, 46(6), 523-529.
17. Silva L, et al. (2007). Of alphachloralose in rat plasma by gas chromatography-mass Biomedical Chromatography, 21(10), 1055-1058.
18. Pelletier G, et al. (2006). Of alphachloralose and 3,5,6-trichloropicolinyl chloride using gas chromatography and gas chromatography-mass Journal of Chromatography B, 830(2), 227-233.
19. Nikiforov A, et al. (2005). Determination of alphachloralose in matrices of forensic interest by gas chromatography-mass Forensic Science International, 148(2-3), 151-156.
20. Curtis MJ, et al. (2004). Of alphachloralose in rat plasma by liquid chromatography-ion trap mass Journal of Chromatography B, 805(2), 333-340.