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Chemical extraction of sulfachloropyridazine from liver samples

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ABSTRACT

Analytical methodology for the detection of sulfachloropyridazine (SCP) in samples of feathers via LC-MS/MS was implemented based on techniques previously published by other authors:

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- 4- Pang G, Cao YZ, Zhang JJ, Jia GQ, Fan CL, Li XM, Liu YM, Li ZY, Shi YQ. Determination of sulfonamides in honey by liquid chromatography- tandem mass spectrometry. *J AOAC Int* 2005; 88:1304-11.
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The method is based on a solid-liquid extraction with organic solvents. The analyte was concentrated using a water bath at 40-50°C under a mild nitrogen flow. For the instrumental analysis, a Symmetry C8 analytical column of 3.5µm and 2.1 x 100mm (Waters®) was fitted in an Agilent 1290 infinity series liquid-chromatograph equipment, coupled to an API 3200 (AB Sciex, Darmstadt, Germany) triple-quadrupole mass-spectrometer. The analytical data was then integrated using the Analyst® version 1.5 software package (SCIEX, Framingham, Massachusetts).

MATERIALS

MATERIALS

- ✘ 1.5 mL Eppendorf tubes **Contributed by users**
- ✘ Falcon Tube (50 mL) **Fischer Scientific**
- ✘ Sulfamethazine-(phenyl-13C6) hemihydrate **Sigma Aldrich Catalog #32519**
- ✘ Sulfachloropyridazine **Sigma Aldrich Catalog #S9882**
- ✘ 1 ml syringe **Contributed by users Catalog #JD+01T2713**
- ✘ 10 mL syringe **Contributed by users Catalog #JD+10L2125-WEI**
- ✘ Glass wool **Merck Millipore Catalog #1040860250**
- ✘ Millex Syringe Filter **Merck Millipore Catalog #SLGVX13NK**

STEP MATERIALS

- ✘ Sodium hydroxide **Merck Millipore (EMD Millipore) Catalog #1064981000**
- ✘ Hydrochloric acid fuming 37% **Merck Millipore (EMD Millipore) Catalog #1003172500**
- ✘ Ethyl Acetate **J.T. Baker Catalog #9280-03**
- ✘ Ethyl Acetate **J.T. Baker Catalog #9280-03**
- ✘ Methanol **J.T. Baker Catalog #9093-03**
- ✘ Water **Merck Millipore (EMD Millipore) Catalog #1153334000**
- ✘ Formic Acid 98-100% **Merck Millipore (EMD Millipore) Catalog #100264**
- ✘ Water **Merck Millipore (EMD Millipore) Catalog #1153334000**
- ✘ Sodium hydroxide **Merck Millipore (EMD Millipore) Catalog #1064981000**
- ✘ Hydrochloric acid fuming 37% **Merck Millipore (EMD Millipore) Catalog #1003172500**
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SAFETY WARNINGS

- ! Protect hands, eyes and face from organic solvents and reactivities during all the extraction steps with all the necessary materials.

BEFORE START INSTRUCTIONS

Liver samples must be ground in a food processor before proceeding to extract the analytes to ensure their homogeneity of the samples.

Weigh 2 ± 0.02 g of muscle sample in a 50-mL polypropylen..

1

Fortifie the samples with the internal standard solution Sul..

2

Samples must be rested before extraction.

3

00:15:00

Add 15 mL of Water.

4

Water Merck Millipore (EMD Millipore) Catalog #1153334000

Stirr the sample on a vortex-mixer.

5

00:10:00

Add 15 μ L of NaOH 1M to the mixture.

6

Sodium hydroxide Merck Millipore (EMD Millipore) Catalog #1064981000

Stirr the sample again.

7

00:15:00

Sonicate the sample.

8

00:05:00

Adjust the pH of the sample to 7.8 - 8.0 with 10% v/v HCl.

9

Hydrochloric acid fuming 37% Merck Millipore (EMD Millipore) Catalog #1003172500

Add 10 mL of ethyl acetate.

10

Ethyl Acetate J.T. Baker Catalog #9280-03

Stirr on a vortex-mixer.

11

00:10:00

Centrifuge at 2,790 g

12

00:02:00

To dissolve the resulting gel, shook gently the sample by h...

13

00:10:00

Transferre the supernatant to a clean 50 mL polypropylene...

14

Ethyl Acetate J.T. Baker Catalog #9280-03

Evaporate resulting supernatant using a water bath at 40-5..

15

Reconstitute the sample with 500 µL of a mixture of mobile..

16

Note

Mobile phase A: 0.1% formic acid in methanol (pH 2.9 ± 0.3).

Mobile phase B: 0.1% formic acid in water (pH 2.7 ± 0.2).

Methanol J.T. Baker Catalog #9093-03 Water Merck Millipore (EMD Millipore) Catalog #1153334000 Formic Acid 98-100% Merck Millipore (EMD Millipore) Catalog #100264

Stirr on a vortex-mixer the reconstituted solution.

17

00:05:00

Sonicate the reconstituted solution.

18

00:05:00

Transfer the reconstituted solution into an Eppendorf tube ...

19

00:10:00

Filter the sample through 13 mm millex filters with 0.22 μm ...

20