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# Chemical extraction of sulfachloropyridazine from feather samples

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**Protocol status:** Working
**We use this protocol and it's working**

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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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2- Renew JE, Huang CH. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. J Chromatogr A 2004; 1042: 113-21.
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6- Bedendo GC, Jardim IC, Carasek E. A simple hollow fiber renewal liquid membrane extraction method for analysis of sulphonamides in honey samples with determination by liquid chromatography-tandem mass spectrometry. J Chromatogr A 2010; 1217:6449-54.
7- Yu H, Tao Y, Chen D, Wang Y, Huang L, Peng D, et al. Development of a high-performance liquid chromatography method and a liquid chromatography-tandem mass spectrometry method with the pressurized liquid extraction for the quantification and confirmation of sulphonamides in the foods of animal origin. J Chromatogr B 2011; 879:2653-62.

The method is based on a solid-liquid extraction with organic solvents. The clean up is carried out throught aromatic sulfonic acid (Bakerbond spe™) disposable extraction columns of 6 mL. The analyte is 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 × 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
- Falcon Tube (50 mL) **Fischer Scientific**
- BAKERBOND spe™ Aromatic Sulfonic Acid (C6H5SO3H) Disposable Extraction Columns **J.T. Baker Catalog #**7090-29
- Sulfamethazine-(phenyl-13C6) hemihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**32519
- Sulfachloropyridazine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S9882
- 1 ml syringe **Catalog #**JD+01T2713
- 10 mL syringe **Catalog #**JD+10L2125-WEI
- Glass wool **Merck Millipore (EMD Millipore) Catalog #**1040860250
- Millex Syringe Filter **Merck Millipore (EMD Millipore) Catalog #**SLGVX13NK

STEP MATERIALS

- Ethyl Acetate **J.T. Baker Catalog #**9280-03
- BAKERBOND spe™ Aromatic Sulfonic Acid (C6H5SO3H) Disposable Extraction Columns **J.T. Baker Catalog #**7090-29
- Ethyl Acetate **J.T. Baker Catalog #**9280-03
- n-Hexane **Merck Millipore (EMD Millipore) Catalog #**1037014000
- Methanol **J.T. Baker Catalog #**9093-03
- Water **Merck Millipore (EMD Millipore) Catalog #**1153334000
- Methanol **J.T. Baker Catalog #**9093-03
- Ammonia solution 25% **Merck Millipore (EMD Millipore) Catalog #**1054322500
- Formic Acid 98-100% **Merck Millipore (EMD Millipore) Catalog #**100264
- Methanol **J.T. Baker Catalog #**9093-03
- Water **Merck Millipore (EMD Millipore) Catalog #**1153334000
- Ethyl Acetate **J.T. Baker Catalog #**9280-03
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Methanol **J.T. Baker** **Catalog #**9093-03

Ammonia solution 25% **Merck Millipore (EMD Millipore)** **Catalog #**1054322500

Formic Acid 98-100% **Merck Millipore (EMD Millipore)** **Catalog #**100264

Methanol **J.T. Baker** **Catalog #**9093-03

Water **Merck Millipore (EMD Millipore)** **Catalog #**1153334000

## Protocol materials

- Methanol **J.T. Baker** **Catalog #**9093-03
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- Water **Merck Millipore (EMD Millipore)** **Catalog #**1153334000
- 1 ml syringe **Catalog #**JD+01T2713

Glass wool **Merck Millipore (EMD Millipore)** **Catalog #**1040860250

Ammonia solution 25% **Merck Millipore (EMD Millipore)** **Catalog #**1054322500

Water **Merck Millipore (EMD Millipore)** **Catalog #**1153334000

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Methanol **J.T. Baker** **Catalog #**9093-03

Ammonia solution 25% **Merck Millipore (EMD Millipore)** **Catalog #**1054322500

Formic Acid 98-100% **Merck Millipore (EMD Millipore)** **Catalog #**100264

Methanol **J.T. Baker** **Catalog #**9093-03

Water **Merck Millipore (EMD Millipore)** **Catalog #**1153334000

## Safety warnings

Protect hands, eyes and face from organic solvents and liquid nitrogen during al the extraction steps with all the necessary materials.

## Before start

Feather samples must be cryogenically treated with liquid nitrogen and then ground in an industrial Robot Coupe® R4 table-top cutter food processor (Burgundy, France) to ensure their homogeneity.

Weigh 2 ± 0.02 g of feather sample in a 50-mL polypropylene tube.

1

Fortifie each sample with the internal standard solution, Sulfamethazine-(phenyl-13C6) hemihydrate (SMZ-13C6) and the positive controls with the analyte Sulfamethazine .

2

Samples must be rested before extraction.

3 00:15:00

Add 40 mL of ethyl acetate.

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

Stirr the samples on a vortex-mixer.

5 00:15:00

Sonicate the sample.

6 00:05:00

Centrifuge at 1,800 g

7 00:10:00

Filter the supernatant through glass wool, using a syringe of 10 mL. Pass the filtrate to a fresh 50-mL polypropylene tube.

8

Concentrate down to 15 mL using a water bath at 40-50 °C under a mild nitrogen flow.

9

Condition Aromatic sulfonic acid (Bakerbond spe™) disposable extraction columns with 6 mL of hexane and 6 mL of ethyl acetate.

10

- BAKERBOND spe™ Aromatic Sulfonic Acid (C6H5SO3H) Disposable Extraction Columns **J.T. Baker Catalog #**7090-29
- Ethyl Acetate **J.T. Baker Catalog #**9280-03
- n-Hexane **Merck Millipore (EMD Millipore) Catalog #**1037014000

FIlter the samples through the columns.

11

Wash the column with 2 mL of water and 2 mL of methanol.

12

- Methanol **J.T. Baker Catalog #**9093-03
- Water **Merck Millipore (EMD Millipore) Catalog #**1153334000

Elute the column with 10 mL of a mixture of methanol and ammonia solution (97/3)

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- Methanol **J.T. Baker Catalog #**9093-03
- Ammonia solution 25% **Merck Millipore (EMD Millipore) Catalog #**1054322500

Evaporate the samples using a water bath at 40-50°C under a mild nitrogen flow.

14

Reconstitute the sample with 300 µL of a mixture of mobile phase A and B (15/85)

15

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).

Formic Acid 98-100% **Merck Millipore (EMD Millipore) Catalog #**100264

Methanol **J.T. Baker Catalog #**9093-03

Water **Merck Millipore (EMD Millipore) Catalog #**1153334000

Stirr the reconstituted solution on a vortex-mixer.

16 00:05:00

Sonicate the reconstituted solution.

17 00:05:00

Transfer the reconstituted solution into an Eppendorf tube and centrifuge at 17,000 g

18 00:10:00

Filter the sample through 13 mm millex filters with 0.22 µm polyvinylidene fluoride (PVDF) membranes and transfer into a glass vials.

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