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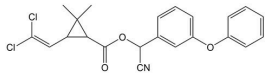
Standard Operating Procedure (SOP) for the Analysis of α-Cypermethrin in Biological Samples by Gas Chromatography with Electron Capture Detection



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Chemistry Method Devel...

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We use this protocol and it's working

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Abstract

This standard operating procedure uses the QuEChERS extraction method for the determination of α-cypermethrin levels in serum and other samples from animal studies using gas chromatography with electron capture detection.

Attachments

XLSX

Example quantificati...

33KB

Materials

Accessories and supplies

Crimp-top GC-vial (Fisher, 03-391-6)
Eppendorf centrifuge 5810 R (Eppendorf)
Furnace (Thermolyne F30400 Furnace)
Disposable culture tube, size 12*125 mm (Fisher, 1495935A)
Pasteur pipettes (Fisher, 22-183632)
Pipette sizes: 20-200 µL, 100-1000 µL
Pipette tips 100-1000 µL
QuEChERS tube, 800 mg $MgSO_4$/200 mg NaCl (United Chemical, ECQUUS115CT)
QuEChERS dSPE tube, 150 mg $MgSO_4$/50 mg CEC 18 (United Chemical, CUMC182CT)
Screw caps, thread 14-415 mm (Fisher, 14-930-15E)
Vortex mixer (Fisher)

Chemicals

α-Cypermethrin (Sigma-Aldrich; C2237) CAS number = 67375-30-8; PubChem CID: 2912
Acetonitrile, pesticide grade (Fisher, A999-4)
Cis-permethrin (Toronto Research Chemical; P288560) CAS number = 554774-45-7; Pubchem CID: 40463
Deltamethrin (Crescent Chemical, C1212000) CAS number = 52918-63-5; PubChem: 40585
Ethyl Acetate (Fisher, E145-4)
Hexane, pesticide grade (Fisher, H300-4)
Magnesium sulfate (Sigma-Aldrich, 208094-500G) *(Note: grind the $MgSO_4$ to a fine powder using a mortar and pestle)*
Milli-Q water (20 mΩ)
Sodium chloride (ACS grade; Research Products International, S23025-500.0) *(Note: grind the sodium chloride to a fine powder using a mortar and pestle)*
Trans-permethrin (Toronto Research Chemical; P288550) CAS number = 51877-74-8; PubChem CID: 43859

Instruments

Agilent Technologies 7890A gas chromatograph equipped with a ^{63}Ni-micro electron capture detector (µECD), an Agilient 7693 Autosampler, and a SPB-1 capilary column (60 m length, 250 μm inner diameter, 0.25 μm film thickness; Supelco)

Standard Solutions

α-Cypermethrin, 1µg/mL in acetonitrile
Cis/trans-permethrin, 1 µg/mL in acetonitrile as surrogate recovery standard
Deltamethrin, 0.125 µg/mL in ethyl acetate as internal standard

Preparation and cleaning of glassware: After each use, glassware is washed with tap water and combusted in a furnace (program: 3, max temp 400 °C).

Troubleshooting

Safety warnings

Cypermethrin SDS.pdf 0 µL

Before start

Pyrethroids readily degrade in the inlet. The inlet temperature of the gas chromatograph needs to be kept relatively low and the glass liner (e.g., inlet liner, splitless type; Supelco, 2046605) should not contain glass wool.

1 Label disposable culture tubes and GC vials. Tubes and vials that need to be labeled include quality assurance/quality control (QA/QC) samples (i.e., water and matrix blank samples), experimental samples and the Reference Standard.

Important: Use unique sample numbers that include your initials and identifiers for the experiment (e.g., notebook number) and the sample. The goal is to avoid identical sample numbers across experiments.

1.1 At least one tube for method blanks (water only).

1.2 At least one tube for sample blanks (matrix sample from unexposed or vehicle-exposed animals).

1.3 Three tubes for Ongoing Precision and Recovery (OPR) samples (matrix samples from unexposed or vehicle-exposed animals spiked with known amounts of the target analyte(s)).

1.4 Tubes for all samples (depends on number of samples to be analyzed; it is recommended that 20% of the samples are QA/QC samples; increase the number of blank samples accordingly).

1.5 One tube for the Reference Standard (will include known amounts of all analytes, including the surrogate recovery standards and internal standard)

(Note: Solutions of all analytes are added to the tube with the Reference Standard at the same time when the samples are spiked)

2 Prepare the samples by adding the following to the prelabeled tubes:

2.1 Method blanks: Add 200 µL of water to the method blank tubes (each experiment needs to include at least one method blank).

2.2 Sample blanks: Add 200 mg of clean sample matrix (from unexposed or vehicle-exposed animals).

2.3 OPR: Add 200 mg of clean sample matrix the each of the three OPR tubes (from unexposed or vehicle-exposed animals).

2.4 Experimental samples from exposed animals: Add 200 mg of each samples to the corresponding vial (from exposed animals).

3 Spike 100 µL of 1 µg/mL of cis/trans-permethrin in acetonitrile (100 ng each per sample) to every tube, including the tube with the Reference Standard.

4 Spike 100 µL of 1 µg/mL α-cypermethrin in acetonitrile (100 ng per sample) to the three OPR tubes and the Reference Standard.

(Note: Do not spike blanks or the experimental samples)

5 Add 1.9 mL of acetonitrile to the method blanks and 1.8 mL of acetonitrile into the Reference Standard, OPR tubes, and experimental samples.

(*Note: The total amount of acetonitrile in a sample should be 2 mL including the solvent of standard solutions)*

6 Add 1 mL of Milli-Q water (20 mΩ) into the tube.

7 Cap and vortex each sample for 1 min.

8 Centrifuge at 3,000 rpm (1811 x g) for 5 min to facilitate the phase separation.

9 Transfer 1 mL of the acetonitrile phase (top layer) to a dSPE tube (150 mg $MgSO_4$/50 mg CEC 18, UCT, CUMC182CT).

(Note: Make sure that no solids are transferred during this step)

10 Cap, vortex for 1 min, and centrifuge at 3,000 rpm (1811 x g) for 5 mins.

11 Transfer 500 µL of each sample to the corresponding prelabeled GC vials and store capped vials at ambient temperature overnight.

12 Evaporate samples to dryness under a gentle stream of nitrogen.

13 Add 200 µL of 0.125 µL/mL deltamethrin in ethyl acetate (internal standard) and 800 µL ethyl acetate to all GC vial (25 ng per sample).

*(Note: other solvents, such as **isooctane and acetonitrile**, are not suitable solvents at this stage of the analysis)*

14 An ethyl acetate solvent blank and the samples generated are analyzed on and Agilent GC with a µECD using the following temperature program: 50 °C, hold for 1 min, 15 °/min to 220 °C, 1 °/min to 240 °C, hold for 12 min, 15 °/min to 280 °C, and hold for 10 min. Helium (carrier gas) flow rate :1.0 mL/min. Inlet temperature: 240 °C. Detector temperature: 300 °C. Make-up flow: 60 mL/min.

15 **Sample quantification:**

15.1 Integrate all chromatograms from the experiment, including the reference standard, using ChemStation software and export the data from the respective QUANTTAB.CSV file into the MS Excel document (*Example quantification of cypermethrin.xlsx*) attached to this protocol for quantification. Create additional worksheets for additional experimental samples or QA/QC standards as needed.

Key formulas for the calculation of the relative retention time [RRT], relative response factor [RRF], the mass of the analyte in the sample, and the recovery of the surrogate standard [SURR recovery] are shown in the next step.

(Note: The MS Excel document does not correct for the recovery of the surrogate recovery standard [SURR recovery])

15.2 **Formulae for analysis:**

$$\mathrm{RRT} = \frac{\text{RT of deltamethrin-A (IS)}}{\text{RT of a sample}}$$

Formula 1, Relative retention time (RRT)

$$\mathrm{RRF} = \frac{(\text{Injected [ng] of a sample}) \times (\text{Response of IS})}{(\text{Response of a sample}) \times (\text{Injected ng of IS})}$$

Formula 2, Relative Response Factor

$$\text{Mass [ng] of a sample} = \text{RRF of a sample} \times \text{Response of a sample} \frac{(\text{Mass[ng] of IS})}{(\text{Response of IS})}$$

Formula 3, Calculation of the mass [ng] of an analyte in a sample

$$\text{SURR recovery} = \frac{\text{Mass [ng] of a sample (obtained from calculation)}}{\text{Spike mass ng of a sample}}$$

Formula 4, Recovery of the surrogate recovery standard (SURR recovery) added to a sample. If appropriate, the mass of the target analyte can be adjusted for the SURR recovery.