

Determination of Pyrethroids in Blood Plasma and Pyrethroid/Pyrethrin Metabolites in Urine by Gas Chromatography–Mass Spectrometry and High-Resolution GC–MS

Gabriele Leng and Wolfgang Gries

Summary

In this chapter, two analytical methods are presented suitable for the determination of pyrethroids in blood plasma and pyrethroid/pyrethrin metabolites in urine. As pyrethroids such as cyfluthrin, cypermethrin, deltamethrin, permethrin, and bioallethrin are metabolized very fast, they can only be detected within about 24 h after exposure; that is, the method shown should only be applied in case of intoxication. After solid-phase extraction, the sample is analyzed by high-resolution gas chromatography–negative chemical ionization mass spectrometry (HRGC–NCIMS) with a detection limit of 5 ng/L blood plasma. In all other cases of exposure (occupational surveillance, environmental, biological monitoring programs, etc.), the determination of metabolites in urine by gas chromatography–mass spectrometry (GC–MS) or HRGC–MS should be preferred. The urine method is adequate for the simultaneous determination of the pyrethroid metabolites *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, 3-phenoxybenzoic, and 4-fluoro-3-phenoxybenzoic acid as well as of the pyrethrin/bioallethrin-specific metabolite *trans*-chrysanthemumdicarboxylic acid (-CDCA). After acid hydrolysis and sample extraction with tert-butyl-methylether, the residue is derivatized with 1,1,1,3,3,3-hexafluoroisopropanol and analyzed by HRGC–MS (detection limit 0.1 µg/L urine).

Key Words: Bioallethrin; biomonitoring; blood plasma; cyfluthrin; cypermethrin; deltamethrin; derivatization; insecticide; GC–MS; hexafluoroisopropanol; HRGC–NCIMS; metabolites; permethrin; pyrethroids; pyrethrum; solid-phase extraction; -chrysanthemum-dicarboxylic acid; urine.

1. Introduction

Synthetic pyrethroids such as cyfluthrin, cypermethrin, deltamethrin, and permethrin originate from the botanical insecticide pyrethrum, an extract obtained from the flowers of *Chrysanthemum cinerariaefolium*. Pyrethrins as one of the natural

esters of pyrethrum, and the synthetic pyrethroids are among the insecticides most often used worldwide.

In mammals, pyrethroid esters are rapidly detoxified by ester hydrolysis and hydroxylation, partially conjugated, and finally eliminated, mainly in the urine (**Fig. 1**). The main metabolites are *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA and *trans*-DCCA), *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (-DBCA), 3-phenoxybenzoic acid (3-PBA), and 4-fluoro-3-phenoxybenzoic acid (FPBA). The biological half-lives of the different pyrethroids vary between 2.5 and 12 h in blood plasma (**1–3**). Half-lives of 6.44 h were found for the urinary excretion of the metabolites *cis*-DCCA, *trans*-DCCA, and FPBA after oral or inhalation exposure to cyfluthrin in volunteers. Of the metabolites, 94% were excreted renally during the first 48 h after exposure (**4**).

Chrysanthemate insecticides like natural pyrethrins or (S)-bioallethrin are also metabolized by hydrolysis, oxidation and finally conjugation with the major metabolite eliminated in the urine (**5,6**). **Figure 2** shows that the major metabolite is -(E)-chrysanthemumdicarboxylic acid (*trans*-CDCA). Interestingly, *cis*-CDCA as well as *trans*-chrysanthemic acid are not found in humans. Following (S)-bioallethrin exposure, maximum peak excretion of *trans*-CDCA was within the first 24 h after exposure, and 72 h later the concentration of *trans*-CDCA was below the limit of detection (**6**).

In humans, a variety of reversible symptoms, such as paraesthesia, irritations of the skin and mucosa, headache, dizziness, and nausea, are reported following pyrethroid/pyrethrin exposure (**1,7,8**). For these adverse health effects, the original pyrethroid/pyrethrin and not the detoxified metabolites is responsible. Therefore, from the medical point of view, it is useful to determine the pyrethroid/pyrethrin in plasma. Exposure to high pyrethroid doses, as seen in cases of acute intoxication, leads to detectable pyrethroid concentrations in blood plasma during the first hour after exposure, rapidly decreasing within 24 h (**9**). In persons occupationally exposed to pyrethroids as well as in persons exposed in their private surroundings, pyrethroid plasma levels are always below the detection limit, although detectable amounts of metabolites can be found in urine (**10–12**). Therefore, for routine biological monitoring of persons exposed to pyrethrins or pyrethroids, the determination of the corresponding metabolites in urine is most often described in literature (**4,9–16**).

1.1. Determination of Pyrethroids in Blood Plasma

With the method described here, all relevant pyrethroids (i.e., cyfluthrin, cypermethrin, deltamethrin, permethrin, and bioallethrin) can be determined in 1 mL blood plasma (see **Note 1**). After cleanup, sample enrichment with solid-phase extraction and elution with hexane/dichloromethane, the sample is analyzed by high-resolution gas chromatography–negative chemical ionization mass spectrometry (HRGC–NCIMS) (5 ng/L blood plasma detection limit) (see **Note 2**). The analysis in negative chemical ionization (NCI or CI⁻) mode is more sensitive than the most-often-used positive electron impact (EI⁺) mode. This is based on the weaker ionization process in NCI and results in lower mass fragmentation, which enables lower detection limits.

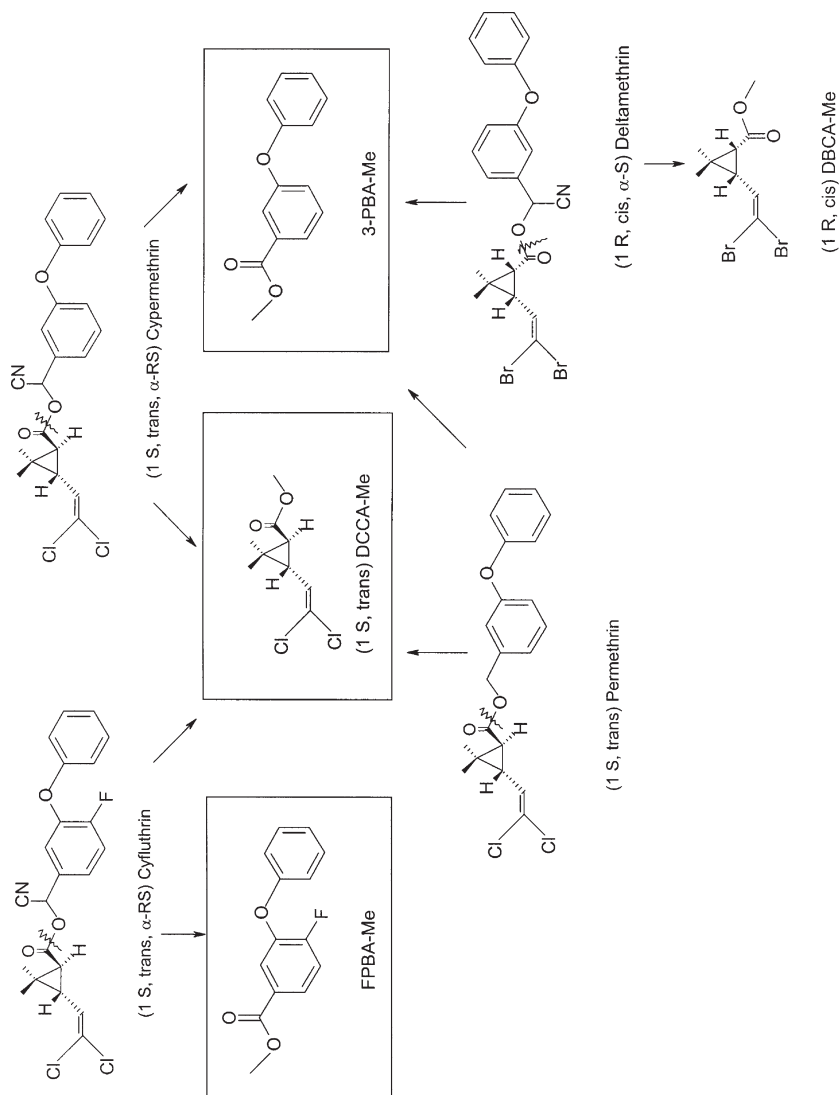


Fig. 1. Metabolism of the pyrethroids cyfluthrin, cypermethrin, deltamethrin, and permethrin in humans. The corresponding metabolites found in urine are shown in brackets. *cis*-DCCA and *trans*-DCCA: *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; *cis*-DBCA: *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid; 3-PBA: 3-phenoxybenzoic acid; FPBA: 4-fluoro-3-phenoxybenzoic acid.

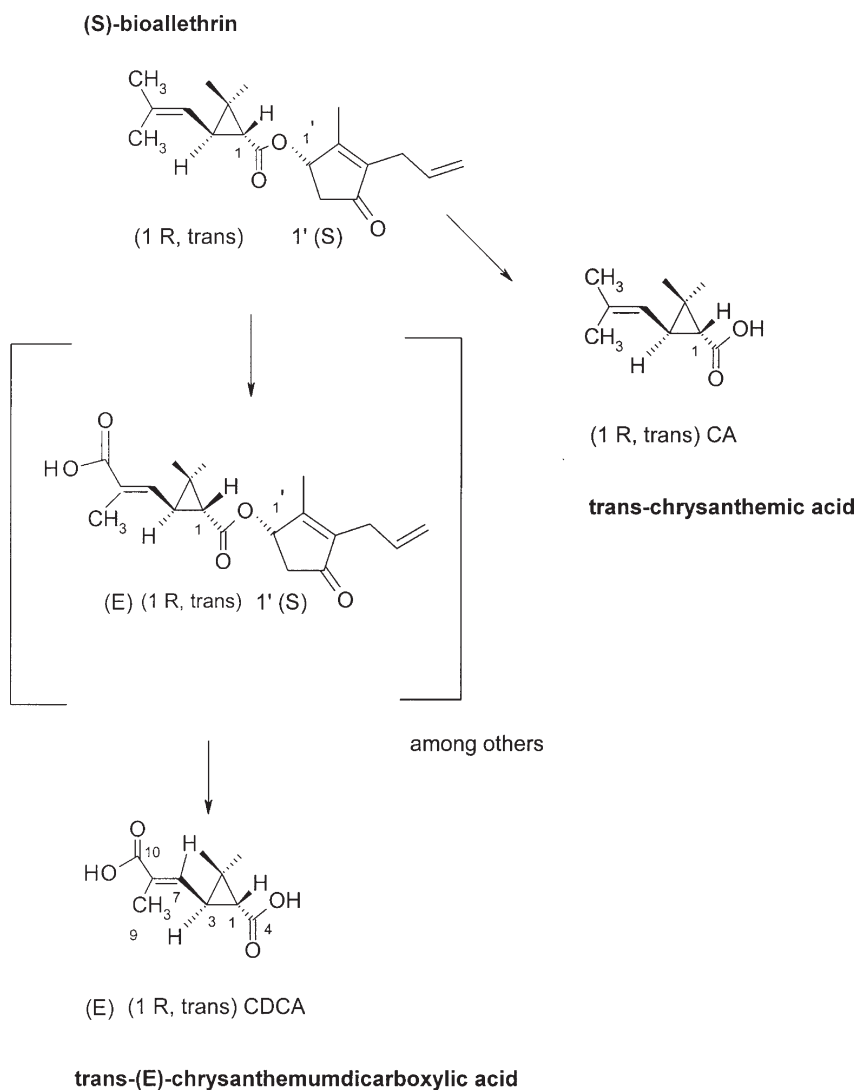


Fig. 2. Metabolism of (S)-bioallethrin in humans.

1.2. Determination of Pyrethrin/Pyrethroid Metabolites (cis/trans CDCA, cis/trans-DCCA, cis-DBCA, FPBA, 3-PBA) in Urine

This method is developed for the simultaneous determination of the metabolites of synthetic pyrethroids (*cis*-DCCA, *trans*-DCCA, *cis*-DBCA, 3-PBA, and FPBA) together with the metabolite of pyrethrin chrysanthemumdicarboxylic acid (*trans*-CDCA) (*see Note 3*). After acid hydrolysis, the sample is derivatized with 1,1,1,3,3,3-

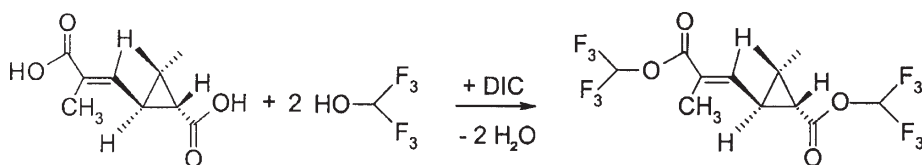


Fig. 3. Esterification of CDCA with 1,1,1,3,3,3-hexafluoroisopropanol.

hexafluoroisopropanol (HFIP) in the presence of *N,N'*-diisopropylcarbodiimide (DIC). Detection is done by HRGC–MS after separation on a Rtx 65 fused silica capillary column (0.1 µg/L urine detection limit) (*see Note 4*).

The reaction scheme of CDCA esterification with hexafluoroisopropanol is shown in Fig. 3.

2. Materials

2.1. Determination of Pyrethroids in Blood Plasma

1. Microliter pipets, adjustable between 1 and 1000 µL (e.g., Eppendorf, Hamburg, Germany).
2. 10-mL tubes with Teflon-sealed screw caps.
3. Nitrogen evaporator.
4. Microvials (e.g., Agilent, Palo Alto, CA).
5. Microevaporator unit.
6. Solid-phase extraction (SPE) column station with column drying option (e.g., Supelco, Bellefonte, PA).
7. Oasis HLB [hydrophilic–lipophilic balance] cartridges, 6 mL/200 mg (Waters, Milford, MA).
8. GC–MS system with NCI equipment (e.g., AutoSpec Ultima, Micromass/Waters, Milford, MA).
9. Helium 5.0.
10. Capillary column, 30 m × 0.25 mm × 0.1 µm DB5 (Durabond 5; Agilent).
11. Cyfluthrin (e.g., Dr. Ehrenstorfer GmbH, Augsburg, Germany).
12. Deltamethrin (e.g., Dr. Ehrenstorfer).
13. Cypermethrin (e.g., Dr. Ehrenstorfer).
14. Permethrin (e.g., Dr. Ehrenstorfer).
15. Bioallethrin (e.g., Dr. Ehrenstorfer).
16. Fenvalerat (e.g., Dr. Ehrenstorfer), used as internal standard (ISTD).
17. Dichloromethane (Supra-Solv).
18. Hexane (Supra-Solv).
19. Methanol (Supra-Solv).
20. For conditioning of Oasis HLB cartridges, First wash each column with 4 mL methanol at atmospheric pressure. After methanol is rinsed through the column, repeat the same procedure with 6 mL water.
21. To prepare the standard solutions, about 10 mg of each compound (or proportionally more if purity < 100%) is weighed into separate 10-mL flasks. Each flask is diluted to volume with acetonitrile. The concentration of these standard starting solutions is 1000 mg/L. The following dilutions were performed with these starting solutions.

Table 1
Necessary Fortification Levels for Pyrethroids in Blood Plasma

Concentration ($\mu\text{g/L}$)	Stock solution	Spike volume (μL) in 1 mL plasma	Spike volume ISTD dilution 0.1 mg/L (μL)
Blank value	—	—	10
0.01	4	10	10
0.02	4	20	10
0.05	4	50	10
0.10	3	10	10
0.20	3	20	10
0.50	3	50	10
1.00	2	10	10

- For stock solution 1 of 1.0 mg/L, 100 μL of each standard starting solution is added to a 100-mL flask, which is filled to volume with acetonitrile (1:1000 dilution).
- For stock solution 2 of 0.1 mg/L, 1000 μL of stock solution 1 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:10,000 dilution).
- For stock solution 3 of 0.01 mg/L, 100 μL of stock solution 1 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:100,000 dilution).
- For stock solution 4 of 0.001 mg/L, 100 μL of dilution 2 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:1,000,000 dilution).
- For ISTD solution of 0.1 mg/L, preparation is done with separate dilutions in comparison to stock solution 2 described in **a** above.

For the calibration experiment, defined volumes of dilution 1, 2, 3, or 4 are added to 1 mL plasma. The dilutions for necessary concentrations are shown in **Table 1**.

2.2. Determination of Pyrethrin/Pyrethroid Metabolites (cis-/trans-CDCA, cis-/trans-DCCA, cis-DBCA, FPBA, 3-PBA) in Urine

1. Microliter pipets, adjustable between 1 and 2000 μL (e.g., Eppendorf).
2. 20-mL tubes with Teflon-sealed screw caps.
3. Microvials (e.g., Agilent).
4. Centrifuge.
5. Block heater for hydrolysis.
6. Shaker.
7. Nitrogen evaporator.
8. GC-MS system (e.g., AutoSpec Ultima).
9. Helium 5.0.
10. Capillary column, 30 m \times 0.25 mm \times 0.25 μm Rtx 65.
11. HFIP (e.g., Aldrich, Poole, UK).
12. DIC (e.g., Aldrich).
13. 3-Phenoxybenzoic acid (e.g., Aldrich)
14. 2-Phenoxybenzoic acid (e.g., Aldrich) used as ISTD.
15. *cis*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (e.g., Dr. Ehrenstorfer).

16. *trans*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (e.g., Dr. Ehrenstorfer).
17. *cis*-3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (e.g., Roussel-Uclaf, Romainville Cedex, France).
18. 4-Fluoro-3-phenoxybenzoic acid (e.g., Bayer Industry Services, Leverkusen, Germany).
19. *cis*-CDCA (e.g., Bayer Industry Services).
20. *trans*-CDCA (e.g., Bayer Industry Services).
21. Acetonitrile (Supra-Solv).
22. Tert.-Butyl-methylether (Supra-Solv).
23. Iso-octane (Supra-Solv).
24. For preparation of the standard solutions, about 10 mg of each compound (or proportionally more if purity < 100%) is weighed into separate 10-mL flasks. Each flask is diluted to volume with acetonitrile. The concentration of these standard starting solutions is 1000 mg/L. The following dilutions are performed with these starting solutions:
 - a. For stock solution 1 of 10.0 mg/L, 100 μ L of each standard starting solution is added to a 10-mL flask, which is filled to volume with acetonitrile (1:100 dilution).
 - b. For stock solution 2 of 1.0 mg/L, 1000 μ L of dilution 1 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:1000 dilution).
 - c. For stock solution 3 of 0.1 mg/L, 100 μ L of dilution 1 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:10,000 dilution).
 - d. For stock solution 4 of 0.01 mg/L, 1000 μ L of dilution 3 is added to a 10-mL flask, which is filled to volume with acetonitrile (1:100,000 dilution).
 - e. For the ITSD solution of 1.0 mg/L, the preparation is done with separate dilutions as for dilution 2 described in.

For the calibration experiment, defined volumes of dilution 1, 2, 3, or 4 are added to 2 mL urine. The dilutions for necessary concentrations are shown in **Table 2**.

3. Methods

3.1. Determination of Pyrethroids in Blood Plasma

3.1.1. Sample Preparation

1. Put the conditioned Oasis column on the SPE station.
2. Dilute 1 mL plasma with 1 mL ultrapure water in a test tube.
3. Add 10 μ L of ITSD (e.g., Fenvalerat) (*see Note 5*).
4. Mix the sample slightly to get a homogeneous solution.
5. Trickle sample slowly on the Oasis column.
6. Let sample elute through the column under atmospheric pressure.
7. Rinse column with 2 mL ultrapure water.
8. Dry column 30 s under vacuum on the SPE station.
9. Dry column under nitrogen steam on the SPE station with drying option (approx 30 min) at room temperature.
10. Rinse column with 2 mL n-hexane (*see Note 6*).
11. Elute pyrethroids with 3 mL hexane:dichloromethane 1:1 (v/v).
12. Evaporate solution in a nitrogen evaporator (e.g., Pierce) down to approx 200 μ L.
13. Transfer sample in a microvial and narrow carefully with nitrogen down to dryness (*see Note 7*).
14. Resolve sample in 25 μ L toluene (analysis sample).

Table 2
Necessary Fortification Levels for Pyrethroid Metabolites (HFIP Method) in Urine

Concentration ($\mu\text{g/L}$)	Stock solution	Spike volume (μL) in 2 mL urine	Spike volume ISTD dilution 1 mg/L (μL)
Blank value	—	—	20
0.05	4	10	20
0.1	4	20	20
0.2	4	40	20
0.5	4	100	20
1.0	3	20	20
2.0	3	40	20
5.0	2	100	20
10.0	2	20	20
20.0	2	40	20
50.0	2	100	20
100.0	1	20	20

3.1.2. Operational Parameters for GC and MS

3.1.2.1. GC PARAMETERS

Use HP 5890II with SSL-Injector and CTC A 200S Autosampler.

DB5 (30 m \times 0.25 mm \times 0.1 μm) column

He 80 kPa for 1 min, 5 kPa/min, 100-kPa gas pressure

1 min off purge time

40-mL/min split

3-mL/min septum purge

60°C 1 min > 15°C/min > 320°C > 10 min

300°C injection temperature (*see Note 8*)

1 μL injection volume

3.1.2.2. MS PARAMETERS

Use an Micromass AutoSpec Ultima.

250°C interface

200°C source

NCI mode inner source

Ammonia CI gas, 2×10^{-5} KPa source pressure (*see Note 9*)

0.5-mA filament

100 eV electron energy

Maximum 8000 accelerating voltage

350-V multiplier

10,000 resolution

Perfluorokerosin calibration gas

3.1.3. Analytical Determination

Inject into the GC–MS system 1 μL of the sample (*see Subheading 3.1.1., step 14*). If the instrument parameters are set as described in in selected ion monitoring

Table 3
SIM Masses and Retention Time for MS Detection of Pyrethroids

Pyrethroid	Target mass (m/z)	Retention time (min)
Bioallethrin	167.107	10:45
Permethrin (sum of 2 isomers)	206.998	14:13/14:18
Cyfluthrin (sum of 4 isomers)	206.998	14:36/14:45
Cypermethrin (sum of 4 isomers)	206.998	14:48/14:58
Deltamethrin	296.895	15:55
Fenvalerat (ITSD)	211.053	15:25/15:34

(SIM) mode (**Table 3**), a stereoselective resolved chromatogram of each pyrethroid can be obtained as shown in **Fig. 4**.

3.1.4. Method Validation

The calibration curve and the samples for precision control are prepared with plasma of persons not exposed to pyrethroids. Necessary fortification levels for this procedure are prepared in agreement with the fortification levels in **Table 1**. The linearity of all pyrethroids is tested in a range between 5 and 1000 ng/L blood plasma, with correlation coefficients more than 0.995. If the method is working correctly, quality criteria for precision in series can be achieved as shown in **Table 4**. The average recovery of all compounds reached 90%.

3.1.5. Storage Stability

The starting solutions can be stored in a deep freezer at -18°C for at least 6 mo. Longer times were not tested. We prefer fresh (monthly) preparation of stock solutions 2, 3, and 4. It was found that pyrethroids in plasma are not stable if they are stored at $+4^{\circ}\text{C}$ (**II**). If analysis cannot start in about a day after blood plasma sampling, the plasma samples must be stored in a deep freezer at -70°C . Then, they are stable for more than a year.

3.2. Determination of Pyrethrin/Pyrethroid Metabolites (cis/trans CDCA, cis/trans-DCCA, cis-DBCA, FPBA, 3-PBA) in Urine

3.2.1. Sample Preparation

1. Transfer 2 mL urine in a screw cap test tube.
2. Add 20 μL ITSD solution (10 $\mu\text{g/L}$ 2-PBA).
3. Add 500 μL concentrated hydrochloric acid.
4. Cover test tube with screw cap.
5. Hydrolyze sample for 2 h at 100°C in a block heater.
6. Add 3 mL tert.-butyl-methylether to the cold sample.
7. Cover test tube with screw cap and shake urine sample vigorously for 5 min.
8. Centrifuge sample for 5 min at 2000 .
9. Separate organic layer in a new screw cap test tube.
10. Add 2 mL tert.-butyl-methylether to sample.
11. Cover test tube with screw cap and shake urine sample vigorously for 5 min.
12. Centrifuge sample for 5 min at 2000 .

File:GR PY 270900 #1-700 Acq:27-SEP-2000 22:54:58 GC CI- Voltage SIR AutoSpec
 Sample#15 File Exp:GR_NCI_PY_10K

167.1072 S:15

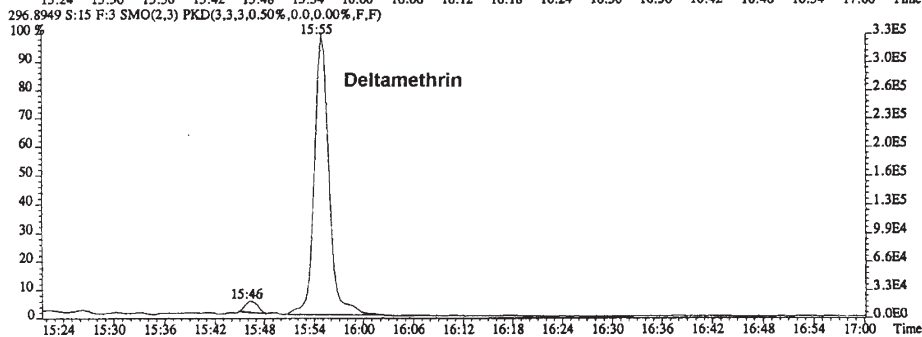
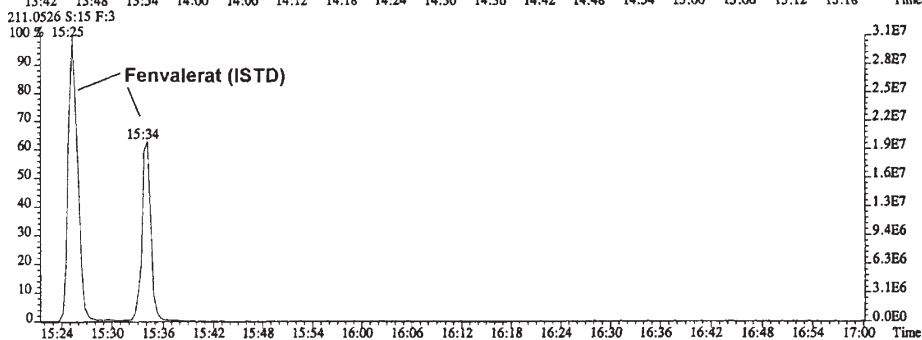
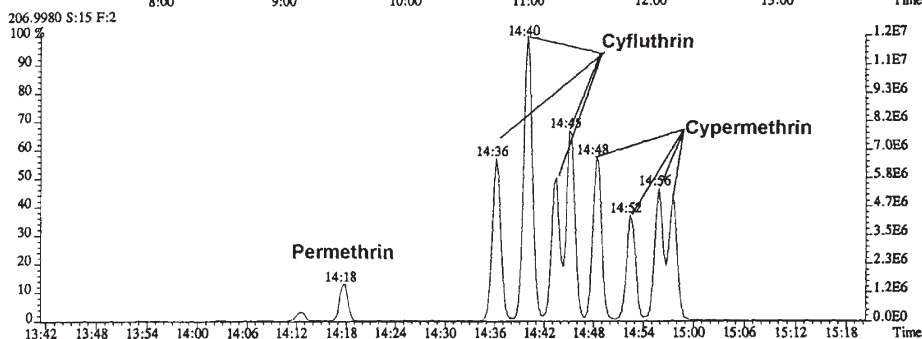
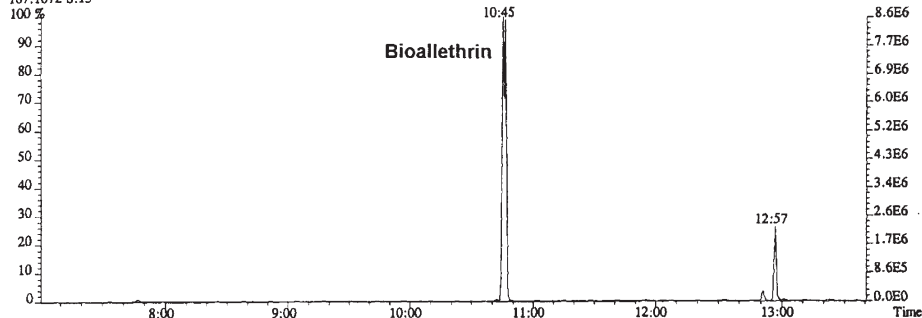


Fig. 4. High-resolution CI-MS chromatogram of pyrethroids in blood plasma separated on a 30 m \times 0.25 mm \times 0.1 μ m DB5 capillary column.

Table 4
Precision in Series and Detection Limits of Pyrethroids

Pyrethroid	Plasma 0.1 μg/L R.S.D. (%)	Plasma 1.0 μg/L R.S.D. (%)	Detection limit (ng/L)
Bioallethrin	10.5	17.5	5
Permethrin (sum of 2 isomers)	8.4	16.9	5
Cyfluthrin (sum of 4 isomers)	9.9	9.4	5
Cypermethrin (sum of 4 isomers)	6.4	10.8	5
Deltamethrin	15.1	10.3	20

RSD, relative standard deviation.

13. Combine organic layer in the new screw cap test tube (**step 9**).
14. Discard the lower urine phase.
15. Dry organic layer under a gentle stream of nitrogen just to dryness.
16. Dissolve residue in 250 μL acetonitrile.
17. Add 30 μL of HFIP (*see Note 10*).
18. Add 20 μL of DIC.
19. Derivatize solution under slight mixing for 10 min at room temperature.
20. Add 1 mL 1 M sodium hydrogen carbonate solution.
21. Add 250 μL iso-octane.
22. Cover test tube and mix sample 10 min vigorously for extraction.
23. Centrifuge sample 5 min at 2000 for phase separation.
24. Separate iso-octane phase in a microvial.

3.2.2. Operational Parameters for GC and MS

3.2.2.1 GC PARAMETERS

Use HP 5890II with SSL-Injector and CTC A 200S Autosampler.

Rtx 65.30 m × 0.25 mm × 0.25 μm column
 He 120 kPa for 1 min, 100 kPa/min, 80 kPa gas pressure
 1 min off purge time
 40-mL/min split
 3-mL/min septum purge
 60°C 1 min >8°C/min >150°C >30°C/min at 300°C for 20 min
 300°C injection temperature
 1 μL injection volume

3.2.2.2. MS Parameters

Use a Micromass AutoSpec Ultima.

250°C interface.
 250°C source.
 EI mode inner source (*see Note 11*).
 0.3-mA filament.
 70 eV electron energy.
 Maximum 8000 accelerating voltage.
 330-V multiplier.

Table 5
SIM Masses and Retention Time for MS Detection of Pyrethroid Metabolites (HFIP-Ester) in Urine

Pyrethroid metabolite	Target mass (m/z)	Retention time (min)
<i>cis</i> -CDCA	331.077	7:19
<i>trans</i> -CDCA	331.077	6:21
<i>cis</i> -DCCA	323.027	7:34
<i>trans</i> -DCCA	323.027	7:45
<i>cis</i> -DBCA	368.975	10:52
2-PBA (internal standard)	364.053	14:27
3-PBA	364.053	14:39
FPBA	382.044	14:05

10,000 resolution.

Perfluorokerosine calibration gas

3.2.3. Analytical Determination

Inject 1 μ L of the sample (sample preparation, **step 24**) into the GC–MS system. If the instrument parameters are set as described in **Subheading 3.2.2.** in SIM EI⁺ mode (**Table 5**), a stereoselective resolved chromatogram of each pyrethroid metabolite can be obtained as shown in **Fig. 5**. This sample can optionally be analyzed in NCI mode as shown in **Fig. 6** (see **Note 11**).

3.2.4. Method Validation

The calibration curve and the samples for precision control are prepared with urine of persons not exposed to pyrethroids. Necessary fortification levels for this procedure were prepared in agreement with the fortification levels in **Table 2**. The linearity of all pyrethroid metabolites is tested in a range between 0.1 and 100 μ g/L urine, with correlation coefficients more than 0.995. If the method is working correctly, quality criteria for precision in series can be achieved as listed in **Table 6**. The average recovery of all compounds lies between 90 and 100%.

3.2.5. Storage Stability

The starting solutions and stock solutions can be stored in a deep freezer at -18°C for at least 6 mo. Longer times were not tested. Urine samples can be stored for more than a year at -21°C in a deep freezer

4. Notes

1. This method is developed for the determination of very low pyrethroid concentrations in blood plasma, which are caused by the short pyrethroid half-lives in blood plasma. Therefore, this method is useful for the determination of pyrethroids following acute intoxication.
2. Several analytical techniques have been tested previously, but all gave poor reproducibilities or sensitivities. A direct extraction with iso-octane after precipitation with sodium chloride and ethanol also works, but only for a few samples. In such a plasma extract,

File:GR_PY_061103 #1-283 Acq: 7-NOV-2003 02:21:04 GC EI+ Voltage SIR Autospec-Ultima
Sample#28 File
331.0769 S:28 Exp:GR_PY_EIP

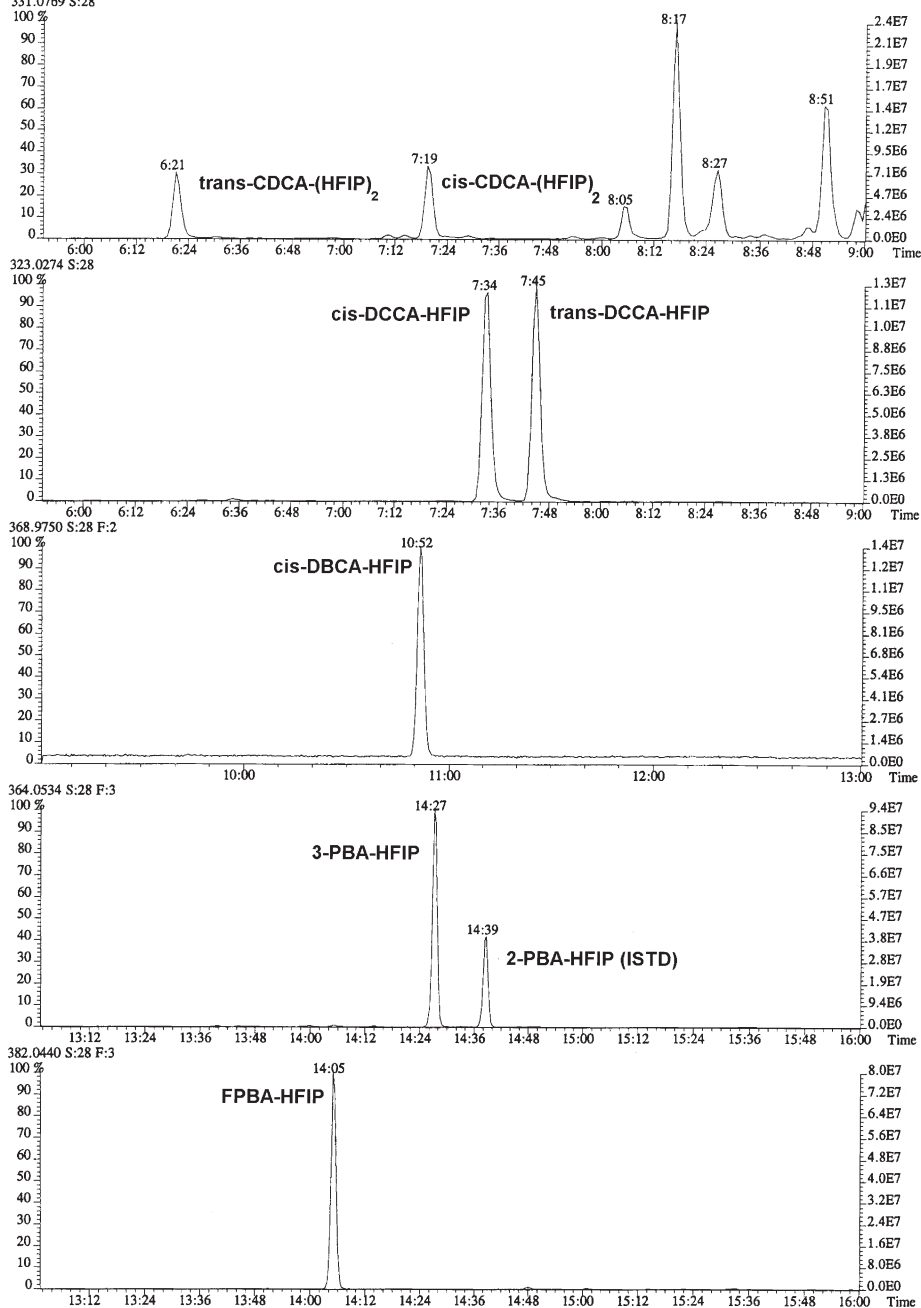


Fig. 5. High-resolution EI⁺ MS chromatogram of pyrethroid metabolites in urine (as HFIP esters) separated on a 30 m × 0.25 mm × 0.25 μm Rtx 65 capillary column.

File:GR_PY_060204 #1-333 Acq: 6-FEB-2004 16:11:34 GC CI- Voltage SIR Autospec-Ultima
Sample#1 File Exp:GR_PY_NCI

330.0691

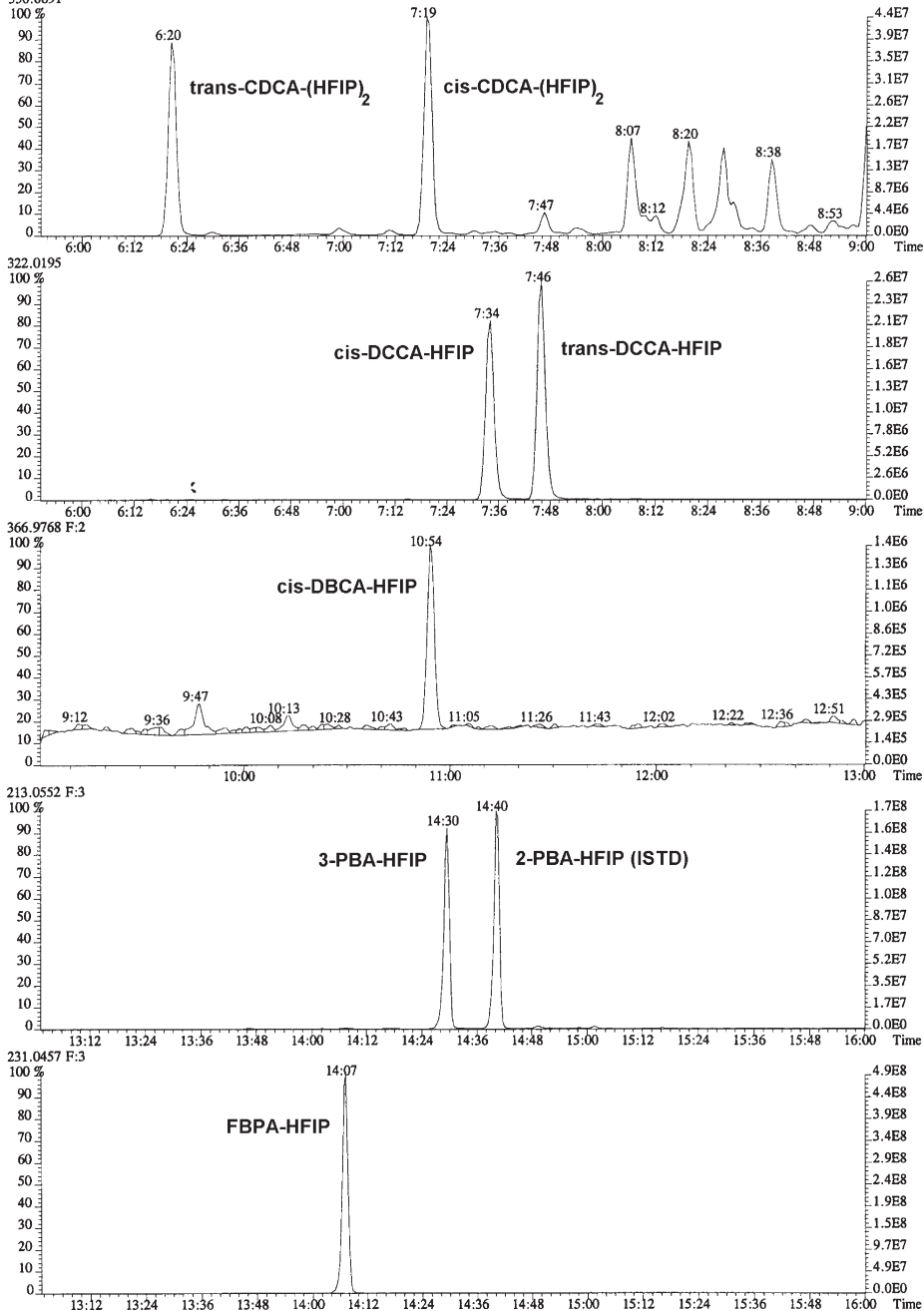


Fig. 6. High-resolution CI-MS chromatogram of pyrethroid metabolites in urine (as HFIP esters) separated on a 30 m × 0.25 mm × 0.25 μm Rtx 65 capillary column.

Table 6

Precision in Series and Detection Limits of Pyrethroid Metabolites (HFIP Ester) in Urine

Pyrethroid metabolite	Urine 1 µg/L R.S.D. (%)	Urine 10.0 µg/L R.S.D. (%)	Detection limit (µg/L)
<i>cis</i> -CDCA	7.4	2.4	0.1
<i>trans</i> -CDCA	5.3	2.7	0.1
<i>cis</i> -DCCA	5.2	3.4	0.1
<i>trans</i> -DCCA	4.4	2.1	0.1
<i>cis</i> -DBCA	3.5	1.9	0.1
3-PBA	3.0	2.1	0.1
FPBA	3.0	2.1	0.1

RSD, relative standard deviation.

pyrethroids and blood plasma fats are extracted together. Then, if this sample is injected into the GC, the blood plasma fat residues cannot be vaporized and build active residues in the injector, which leads to memory effects and adsorption of pyrethroids. These problems can be solved with the described SPE.

3. In contrast to analytical methods covering only pyrethroid metabolites in urine, this method has the advantage that it is possible to determine metabolites of synthetic pyrethroids (*cis/trans*-DCCA, *cis*-DBCA, 4-F-3-PBA, 3-PBA) as well as the metabolite of pyrethrins/bioallethrin (*cis/trans*-CDCA) simultaneously. Another advantage is lower cost because of shorter analysis time.
4. Several analytical techniques were tested previously, but the method chosen is the most adequate. The esterification with methanol gave poor sensitivity for *cis*- and *trans*-CDCA in the lower background level. Furthermore, it is impossible to separate the *cis*- and *trans*-CDCA-methylester on a DB5 capillary column. Esterification with ethanol enables a separation between *cis*- and *trans*-CDCA-ethylester but also shows poor sensitivity in the lower background level. Optimal sensitivity and selectivity are only found with HFIP as an excellent esterification reagent for all tested substances within a quantification level of 0.1 µg/L.
5. The spiked ISTD fenvalerat cannot compensate all different properties of the pyrethroids during the analysis and GC-MS determination. In this method, fenvalerat is used as the ISTD because this pyrethroid is not often used in Germany. Otherwise, it might be useful to work with deuterated or ¹³C-labeled internal pyrethroid standards if they are available.
6. The washing step with n-hexane eliminates the fat residues, and the elution with hexane/dichloromethane (1:1 v/v) is done to get nearly matrix-free extracts.
7. The extract of the Oasis cartridges must be evaporated very carefully to dryness because pyrethroids with lower boiling points evaporate with nitrogen if the nitrogen steam is too high or the evaporating process is not stopped immediately after sample drying. If no fine adjustable nitrogen evaporator is available, this drying step can also be done in a vacuum centrifuge. In this case, 100 µL toluene should be added to the extract before the solvent evaporation process is started. Toluene works as a keeper and minimizes loss of pyrethroids during this step. After sample evaporation to approx 25 µL, this residue can be used for GC-MS analysis.
8. A high temperature of the GC injector is used for optimal evaporation of pyrethroids and reduction of possible memory effects based on injector temperature distribution, which can occur by condensation at cold places in the injector. Therefore, it is advantageous to

use deactivated double-gooseneck injector liners. Matrix residues of samples in injector liners or at the first centimeters of a capillary column can result in lower detection limits, especially for deltamethrin. Deltamethrin is critical to analyze because it shows the lowest response of all pyrethroids based on its unfavorable fragmentation pattern. A possible contamination source is the autosampler syringe itself because residues of pyrethroids are not washed out quantitatively when nonpolar cleaning solvent is used. The best cleaning procedure is a two-step washing process with different polar solvents (e.g., toluene and dichloromethane).

9. Ammonia is described in this method as an NCI reactant gas, but analogous results can be obtained with methane. Notice that not all GC-MS instruments and pumps are equipped for ammonia. If no high-resolution GC-MS system is available, the analyses of pyrethroids can also be done on other GC-MS systems. The only disadvantage is a detection limit that is about a factor of 10 higher.
10. The derivatization with HFIP works only in water-free samples. Therefore, it is important to separate tert.-butyl methylether (t-BME) carefully from the lower water phase. HFIP is a very powerful reagent that reacts spontaneously with carboxylic acids; DIC is used as a catalyzer and water binder (**Fig. 3**).
11. This routine method was developed for analysis in a high-resolution GC-MS system in EI^+ mode and optional CI^- mode. The installation of electronegative fluorine via derivatization with HFIP also enables a very sensitive determination in CI^- mode (**Fig. 6**). By CI^- mode detection, limits in the lower nanogram-per-liter range are possible (**13**). The advantage of high mass resolution (10,000) in both detection techniques enables the accuracy of analytical results. EI^+ mode is used for routine analysis because it is more stable in comparison to CI^- , which is used for verification. The stability or reproducibility in CI^- depends on higher influences of sample matrix to the fragmentation process, which is weaker in CI^- than in EI^+ mode. This problem can be solved with deuterated or ^{13}C -labeled ITSDs. A determination with low mass resolution mass spectrometers was not tested, but is known to work. Possible matrix interferences that occur on these instruments can be solved with longer columns or hydrogen as carrier gas (use caution). Of course, this leads to longer analysis time combined with higher costs.

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