1. INTRODUCTION

1.1 Scope of the method

The method allows the quantitative determination of residues of CGA 245704 in soil (See section 6.1, Figure 1 for structure and chemical name).

The lower practical level of quantitation by this method is 0.02 mg/kg.

1.2 Principle of the method

CGA 245704 is extracted by shaking a homogenized subsample with water and acetonitrile. An aliquot of the extract is cleaned up by passage through a solid phase extraction (SPE) C_{18} cartridge. The eluate containing CGA 245704 is diluted with water and saturated sodium chloride solution and the a.i. is partitioned into n-hexane. The reextract is cleaned up by passage through a silica cartridge. CGA 245704 is determined by HPLC with UV-detection.

2. MATERIALS AND METHODS

Standard laboratory equipment is not listed. All equipment and chemicals mentioned herein can be substituted by suitable products of any origin. Prove suitability of reagents by analyzing reagent blanks.

2.1 Equipment

2.1.1 Rotating evaporator, Büchi, Rotavapor RE, Büchi AG, Flawil, CH.
2.1.2 Vacuum system, Terno Duo 500 S, Terno AG, Küblis, CH.
2.1.3 Circulation cooler, mgw Lauda WK 450, Meßgeräte-Werke-Lauda, Dr. R. Wobser KG, Lauda-Königshofen, FRG.
2.1.4 Vortex mixer, Model Vortex-Genie, Scientific Industries Inc., Springfield, Massachusetts, USA, represented in Switzerland by Dr. Bender & Hobein AG, Zürich.
2.1.5 Lab-shaker, A. Kühner AG, CH-Basel.
2.1.6 Folded filter paper, 15 cm diameter, Macherey-Nagel, 5160 Düren, FRG, Cat. No.: MN 713 1/4.
2.1.7 Borosilicate glass tube, 8 mL volume, J. T. Baker Inc., Phillipsburg, N.J. 08865, USA, represented in Switzerland by P. H. Stehelin and Cie AG, 4003 Basel, Cat. No. 7328-06.
2.1.8 PTFE frits for 8 mL borosilicate glass tubes, J. T. Baker Inc., Cat. No. 7329-06.
2.1.9 Glass reservoirs of about 25 mL and 250 mL content to be attached to the cartridges.
2.1.10 Vacuum manifold to accomodate solid-phase extraction cartridges, built in-house or commercial equivalent, e.g. VISIPREP, Supelco Inc., Bellefonte, Pennsylvania, USA, represented in Switzerland by Supelco SA, Gland, CH, Cat. No. 5-7030.
2.1.11 Ultrasonic bath, Bransonic 220, Branson Cleaning Equipment Co., Parrot Drive, Shelton, CT 06484-0768, USA, represented in Switzerland by Dr. Bender & Hobein, Zürich.
2.1.12 High Performance Liquid Chromatograph: refer to section 2.4.1.

2.2 Reagents and standards

Main suppliers' addresses: E. Merck AG, 6100 Darmstadt, FRG.
Fluka Chemie AG, 9470 Buchs, CH.

2.2.1 Water, HPLC-grade, prepared in house.
2.2.2 Acetonitrile for extraction, distilled in house.
2.2.3 tert.-Butyl methyl ether (TBME), HPLC grade, Fluka, Cat. No 20247
2.2.4 n-Hexane Lichrosolv, chromatography grade, Merck, Cat. No. 4391.
2.2.5 Acetonitrile, LiChrosolv, chromatography grade, Merck, Cat. No. 14291
2.2.6 Silica gel for flash chromatography, J. T. Baker Inc., Cat. No. 7024-01
2.2.7 Bakerbond octadecyl C_{18}, J. T. Baker Inc., Cat. No. 7425-00
2.2.8 Sodium chloride, analytical grade, Merck, Cat. No. 6404. Prepare a saturated solution of sodium chloride in water for HPLC (brine).
2.2.9 Sodium sulfate, anhydrous, analytical grade, Merck, Cat. No. 6649
2.2.10 CGA 245704 reference substance for standardization and recovery experiments. Prepare a stock solution of CGA 245704 in acetonitrile Lichrosolv (e.g. 200 µg a.i./mL acetonitrile). Store the stock solution at a temperature of 5°C or lower.

REMARK : CGA 245704 solutions in acetonitrile have been demonstrated to rapidly degrade if exposed to day light at room temperature. Therefore prepare solutions of CGA 245704 in brown flasks. However, if a stock solution of 200 µg a.i./mL acetonitrile is stored in the dark at -20°C, the a.i. is stable at least for 6 months.

2.3 Analytical procedure

REMARK : During the analysis, it should be kept in mind that CGA 245704 degrades, specially in diluted solutions, when exposed to daylight. Therefore, after beginning, the analysis has to be continued without delay and exposition to sunlight has to be avoided. If an interruption of the analysis is unavoidable (for instance over night), all extracts and solutions involved in the analysis have to be stored at a temperature of 5°C or lower in the dark.

2.3.1 Preparation of samples and subsamples

Remove big stones from field sample. Homogenize laboratory samples (1 kg or more) by following suitable procedures. Analyze the samples immediately after preparation or store at about - 20 °C until analysis.

For analysis weigh a subsample corresponding to 10 g dry soil into a 250 mL wide mouth jar (e.g. weigh 12.1 g for a sample having a 82.4% dry matter content).

2.3.2 Fortification

To regularly check the performance of the method, analyze also at least two fortified control samples with each series of analyses. To prepare these samples, add known amounts of CGA 245704 to control samples prior to extraction.

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Select fortification levels to be two and ten times the lower practical level of determination or in the range of the expected residue levels. Make sure that control samples neither are contaminated nor show interfering signals.
To fortify samples with 0.04 and 0.2 mg/kg, prepare solutions of CGA 245704 containing 0.4 and 2.0 µg per mL acetonitrile (Lichrosolv) by appropriate dilution of the stock solution (cf. section 2.2.10). Add 1 mL of the 0.4 µg/mL solution or 1 mL of the 2.0 µg/mL solution to untreated subsamples to obtain fortification levels of 0.04 or 0.2 mg/kg, respectively. Proceed as described in section 2.3.3.

REMARK : The 0.4 µg/mL solution was determined to be chemically stable for at least 12 days if stored in a refrigerator at about 4°C.

2.3.3 Extraction

Add as much HPLC-water to the weighed subsample (cf. section 2.3.1) as to achieve a total water volume of 20 ml taking into account the soil moisture content (e.g. for a sample having 82.4% dry soil content weigh 12.1 g wet soil and add 17.9 mL water). Swirl the mixture manually for a few seconds. Add 80 mL (79 mL to fortified samples) of distilled acetonitrile to the mixture. Shake the tightly sealed jar for about 30 minutes. Total volume of the extract is 100 mL. Allow solids to settle for about 5 minutes. Filter about 50 mL extract through a folded filter paper of 15 cm diameter into a 100 mL Erlenmeyer flask. Transfer 20 mL of clear extract, corresponding to 2 g dry soil to a 250 mL Erlenmeyer flask.

2.3.4 Cleanup by SPE C_{18} cartridge

Remark: To pack the cartridges used for this cleanup proceed as follows : Insert a PTFE frit on the bottom of a borosilicate glass tube of 8 mL content, fill 1 g of Bakerbond octadecyl into the tube and place a second frit on the top of the filling. Vibrate for 1 minute for the material to settle, using a Vortex mixer and press the upper frit on the top of the adsorbent.
The cartridge is ready for use.

Attach an SPE C_{18} cartridge packed as described above, to the vacuum manifold. Tightly connect a 250 mL glass reservoir to the top of the cartridge. Precondition the cartridge by successively passing through 5 mL acetonitrile LiChrosolv and 5 mL of water by suction. Add 100 mL of water to the aliquot of the extract in the Erlenmeyer flask (section 2.3.3), shake and transfer to the reservoir. Pass the mixture through the cartridge at a rate of 5 - 7 mL per minute by suction. Wash the flask with 10 mL of a solvent mixture of water + acetonitrile Lichrosolv 7 vol. + 3 vol., transfer the wash to the reservoir and pass it through the cartridge. Disconnect the reservoir from the cartridge and elute CGA 245704 with 7 mL of a solvent mixture of acetonitrile LiChrosolv + water 6 vol. + 4 vol. into a 25 mL test tube by means of a 10 mL syringe or by suction. Add 6 mL water and 5 mL saturated sodium chloride solution to the eluate in the test tube.

2.3.5 Cleanup by partition and silica cartridge

Remark: To pack the cartridges used for this cleanup proceed as described under 2.3.4 substituting silica for Bakerbond octadecyl and add 2 g of anhydrous sodium sulfate.

Attach a silica cartridge, packed as described above, to the vacuum manifold. Tightly connect a 25 mL glass reservoir to the top of the cartridge. Precondition the cartridge by passing 5 mL of n-hexane through at a rate of about 4 - 6 mL/min.
Add 7 mL n-hexane to the aqueous phase in the test tube (section 2.3.4), stopper the test tube and shake the mixture for about 30 seconds. After separation of phases, transfer the upper n-hexane phase into the glass reservoir by means of a Pasteur pipette. Pass through the cartridge by suction and discard the eluate. Repeat the extraction once more with a second portion of 7 mL n-hexane. Separate and pipette the n-hexane phase into the cartridge. Discard the eluate. Add another 7 mL n-hexane to the aqueous phase, shake vigorously and after separation, transfer the upper phase into the glass reservoir. Pass through the cartridge by suction and collect the eluate in a 25 mL round bottom flask. Discard the aqueous phase. Elute with 10 mL n-hexane and collect the eluate in the 25 mL round bottom flask.

2.3.6 Preparation of the final solution

Evaporate the eluate to dryness under reduced pressure using a rotating evaporator (water bath temp. : ~30°C). Dissolve the residue in 4 mL n-hexane (final solution).

REMARK : Analyse the samples immediately after dissolution or store the final solutions at 5°C or lower.

2.4 Instrumentation

2.4.1 High Performance Liquid Chromatographic system (HPLC)

Use a high performance liquid chromatograph equipped with a UV-detector, e.g. a Kratos Spectroflow 783 programmable UV/VIS-HPLC-detector (Applied Biosystems, Ramsey, New Jersey 07446, USA), a Shimadzu solvent delivery module LC-9A (Shimadzu Corporation, analytical instrument division, Kyoto, Japan), a Spark Promis II Kingsize programmable autosampler/injector (Spark Holland, 7825 VE Emmen, the Netherlands), a strip chart recorder SE 120 dual channel (ABB, Goertz Metrawatt, 1101 Vienna, A), and optional (for system automation) a control and data collection unit HP 3350A laboratotory Automation system (Hewlett-Packard, Paolo Alto, CA 94304, USA).

REMARK : To avoid degradation of the a.i. in the course of a long sequence (for instance more than 6 hours), the vials must be protected from direct sunlight. For example, start the sequence to run over night or darken the laboratory (blinds down) or use dark vials.

Use a column and conditions as follows or suitable equivalent ones :

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Column : Stainless steel tube, 10 cm length, 2 mm i.d., packed with normal phase Nucleosil 100, particle size 5 µm (Dr. H. Knauer KG, 6370 Oberursel, FRG, Cat. No. : B45 - Y54).

Mobile phase : n-hexane LiChrosolv + TBME 9 vol. + 1 vol.

Flow rate : 0.2 mL/min

Retention time : 5 min.

Injection vol. : 25 µL

Recorder : 10 mV full scale

Chart speed : 1 cm/min

Detector : Wave length: 324 nm
sensitivity: 0.005 aufs

2.4.2 Preparation of Standard Solutions

Standardize the chromatographic system each time a series of samples is to be quantitated. The range of the concentrations is depending on the range of residues to be determined, in particular, the lowest standard concentration is depending on the lower practical level. Calculate the lowest standard concentration (C) as follows:

$$C = \frac{L \cdot A}{V_f} \quad [\mu g/ml]$$

L = lower practical level [mg/kg] or [µg/g]

V_f = volume of the final solution [mL]

A = aliquot of crop cleaned up [g]

With the values proposed in this method, the lowest standard concentration is 0.01 µg/mL as presented below :

$$C = \frac{0.02 \times 2}{4} = 0.01 \ \mu g/mL$$

Prepare at least four standard solutions of different concentrations by appropriately diluting the stock solution of the reference compound (section 2.2.10) with n-hexane.
Select the concentrations as required ; typical values are 0.20, 0.08, 0.02 and 0.01 µg/mL. 25 µL of these solutions correspond to 5, 2, 0.5 and 0.25 ng.

REMARK : Prepare these solutions just before the determination. Solutions were demonstrated to be chemically stable for at least 7 days if stored in a refrigerator at 4°C.

2.4.3 Quantitation of residues

Inject 25 µL of standards and final solutions. Measure the response of the analyte at the characteristic retention time and calculate response function and residues as detailed in REM 119.04 (Ciba, plant protection division, residue analysis, 1991).

2.5 Interferences

None observed, so far.

2.6 Confirmatory techniques

If peaks with same retention time as CGA 245704 appear, results may be confirmed by HPLC using reverse phase chromatography. If during use of the confirmative method a peak appears in the control sample, the peak to be confirmed should be at least three times the height of the control peak.**
Proceed as follows:
Pipette an appropriate aliquot of the final solution e.g. 2 mL (section 2.3.6) into a 10 mL round bottom flask and evaporate to dryness (water bath : - 30°C)
Dissolve the residue in an appropriate volume of a solvent mixture of acetonitrile LiChrosolv + water 4 vol. + 6 vol., e.g. 2 mL, using an ultra sonic bath

2.6.1 High Performance Liquid Chromatographic conditions

Use the same high performance liquid chromatograph as described in section 2.4.1 under the following conditions or suitable equivalent ones

Column :	Stainless steel tube, 10 cm length, 2 mm i.d., packed with Nucleosil 100 C_{18}, particle size 5 µm (Dr. H. Knauer KG, 6370 Oberursel, FRG, Cat. No.: B45 - Y76).
Mobile phase :	Acetonitrile LiChrosolv + water 1 vol. + 1 vol.
Flow rate :	0.2 mL/min
Retention time :	7 min.
Injection vol. :	50 µL
Recorder :	10 mV full scale
Chart speed :	1 cm/min
Detector :	Wave length: 324 nm sensitivity: 0.005 aufs
Standards :	Prepare solution of CGA 245704 in a solvent mixture of acetonitrile LiChrosolv + water 4 vol. + 6 vol. containing 0.2, 0.08, 0.02 and 0.01 µg/mL. Inject 50 µL of each solution corresponding to 10, 4, 1 and 0.5 ng of CGA 245704.

** One of three control samples analyzed using reverse phase was found to contain an interference peak corresponding to about 0.01 mg/kg CGA 245704

2.7 Time required for analysis

A total of about 16 hours is required to work up a set of 12 samples to the point of HPLC injection. Automated HPLC analysis can be performed overnight.

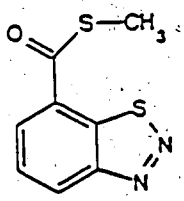
2.8 Modifications and potential problems

Wrong results are obtained if samples or standard solutions have been exposed unprotected to sunlight at room temperature (1/2 day or more).

6. FIGURES AND TABLES

6.1 Figures 1 : Structure and chemical name

CGA 245704



$C_8H_6N_2OS_2$

Molecular mass: 210.28

Benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester

Figure 2: Procedure flow diagram

10 g homogenized soil + 20* ml water +
80 ml acetonitrile ; shake 30 minutes

↓

Filter and take an aliquot of 20 mL of the extract (2 g dry soil)

↓

Add 100 mL water and shake. Pass the solution through a preconditioned cartridge packed with 1 g Bakerbond octadecyl C_{18}

↓

Wash with 10 mL water + acetonitrile 7 vol. + 3 vol.
Elute CGA 245704 with 7 mL water + acetonitrile 4 vol. + 6 vol.

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Add 6 mL water and 5 mL saturated sodium chloride solution

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Extract two times with 7 mL n-hexane, each and pass each reextract through a preconditioned silica cartridge packed with 1 g adsorbent and 2 g anhydrous sodium sulfate.
Discard the eluates

↓

Reextract the aqueous phase again with 7 mL of n-hexane
Pass the reextract through the cartridge and collect the eluate in a 25 mL round bottom flask.

↓

Continue the elution of CGA 245704 by passing 10 mL of n-hexane through the cartridge

↓

Evaporate the eluate to dryness and dissolve the residue in 4 mL of n-hexane

↓

Quantitate by HPLC

* The moisture content of the soil is to be taken in consideration (see section 2.3.3).