

1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of cyprodinil (CGA 219417) and its metabolites CGA249287, CGA275535 and CGA321915 (Figures 1 to 4) in soil using an external standardisation procedure. The limit of quantification of the method is 0.01 mg kg^{-1} . This method supersedes REM 141.8.

Figure 1

Compound : CGA219417
Common Name : Cyprodinil
CAS Number : 121552-61-2
IUPAC Name : N-(4-cyclopropyl-6-methyl-pyrimidi-2-yl)-aniline

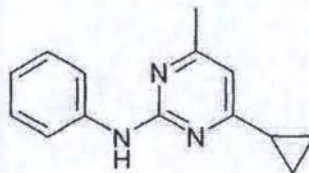


Figure 2

Compound : CGA249287
CAS Number : 92238-61-4
IUPAC Name : 4-cyclopropyl-6-methyl-pyrimidin-2-ylamine

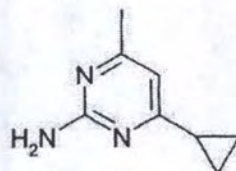
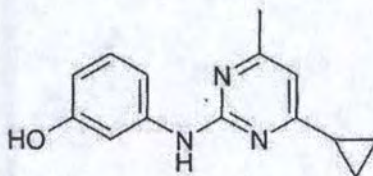
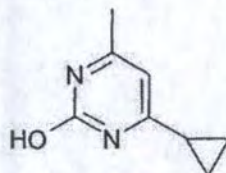


Figure 3

Compound : CGA275535
CAS Number : Not Known
IUPAC Name : N-(4-cyclopropyl-6-methyl-pyrimidi-2-yl)-3-hydroxy-aniline

**Figure 4**

Compound : CGA321915
CAS Number : 1221553-48-8
IUPAC Name : 4-cyclopropyl-6-methyl-pyrimidin-2-ol

**1.2 Method Summary**

A 10 g sub sample of soil is extracted by reflux with methanol/water solution (80:20, v/v) for one hour. After centrifugation, an aliquot of the soil extract is evaporated to remove the methanol prior to analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS). The limit of quantification of the method is 0.01 mg kg⁻¹.

1.3 Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

1.4 Apparatus

See Appendix 1 for a list of apparatus used during this method.

1.5 Reagents

All solvents and other reagents must be of high purity, i.e. pesticide grade solvents and analytical grade reagents. Extreme care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

1.6 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a five figure balance, sufficient cyprodinil CGA249287, CGA275535 and CGA321915 analytical standard to allow dilution in methanol to give $200 \mu\text{g mL}^{-1}$ stock solutions in volumetric flasks. These standards should then be diluted by serial dilution to $0.01 \mu\text{g mL}^{-1}$ with methanol. If required, mixed standards can also be prepared to $0.01 \mu\text{g mL}^{-1}$ in methanol.

When not in use, always store the standard solutions in a refrigerator at $\leq 7^\circ\text{C}$ to prevent decomposition and/or concentration of the standard. Analytical standards should be replaced with freshly prepared standards after four months.

1.7 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate safety manual (e.g. Syngenta Laboratory Safety Manual) which contains recommendations and procedures for handling chemicals or a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Reference 1).

Solvent Hazards

	Methanol	Acetic acid
Harmful Vapour	✓	✓
Highly Flammable	✓	✗
Harmful by Skin Absorption	✗	✓
Syngenta Divisional Toxicity Class	3	3
OES Short Term (mg m ⁻³)	310	37

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

Cyprodinil has been assigned a Syngenta toxicity classification of 3. At present there is insufficient data available to assign a Syngenta toxicity classification for CGA249287, CGA275535 and CGA249287. They should be treated as a Class 3 compounds until further information indicates otherwise. The toxicity classification scale rates highly toxic chemicals as class 1 and non toxic chemicals as Class 5.

1.8 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples over the course of 1 day.

1.9 Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure. Acceptable external standard recoveries will validate the work stoppages. Samples should be stored in sealed vessels at a temperature of $\leq 7^{\circ}\text{C}$.

2. Analytical Procedure

2.1 Sample Preparation

Samples should be prepared using an approved method of sample preparation for residue analysis, such as Syngenta standard operating procedure ESJH/911/-- (Reference 2).

2.2 Extraction

- a) Weigh a representative amount of soil (10 g) into a round-bottomed flask (250 ml size). At least one untreated control and two control samples fortified with known amounts of cyprodinil, CGA249287, CGA275535 and CGA321915 in methanol should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

- b) Add methanol/water solution [80:20 v/v, 100 mL] to the sample and place the round-bottomed flask on a heating mantle and heat the sample under reflux for 1 hour.
- c) Allow the sample to cool to room temperature, decant the soil extract into a plastic centrifuge bottle (250 mL size) and centrifuge at a speed that separates the solid and liquid phase e.g. 3500 rpm for five minutes.
- d) Carefully decant the sample into a measuring cylinder (100 mL) and adjust to 100 mL volume with methanol or methanol/water (80:20). Pour the sample back into the centrifuge bottle. This solution is likely to be cloudy and so it is necessary to re-centrifuge. Centrifuge the sample at a speed that separates the particulate matter from the liquid e.g. 3500 rpm for five minutes. A 1 mL aliquot of this extract is equivalent to 0.1 g soil.

2.3 Sample Evaporation

- a) Transfer an aliquot of the soil extract to be analysed equivalent to 0.1 g soil (1 mL) into a graduated test tube (10 mL size) and add 10 mM ammonium acetate solution (0.7 mL). Evaporate the sample to 1.0 mL volume under a stream of clean, dry air in a heating block with the temperature set to 40 °C.
- b) Transfer the sample to an autosampler vial ready for final determination by LC-MS/MS.

2.4 Preparation of LC-MS/MS Calibration Standard

LC-MS/MS calibration standards should be prepared in methanol/10 mM ammonium acetate solution (10:90 v/v) with each analysis batch. For example, to prepare a $0.01 \mu\text{g mL}^{-1}$ calibration standard, transfer 1 mL of a $0.1 \mu\text{g mL}^{-1}$ cyprodinil, CGA249287, CGA275535 and CGA321915 mixed standard to a volumetric flask (10 mL), and dilute to 10 mL volume with 10 mM ammonium acetate solution.

3. Final Determination by LC-MS/MS

The following instrumentation and conditions have been found to be suitable for this analysis in this laboratory. Other instrumentation can also be used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

3.1 Instrument Description

Pump	: Agilent 1100 series quaternary pump model number G1311A
Degasser	: Agilent 1100 series model number G1322A
Column Oven	: Agilent 1100 series model number G1316A fitted with column switching valve
Detector	: Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software version 1.3.1
Autosampler	: CTC HTS PAL
Gas Supply	: Peak Scientific NM20ZA gas station

3.2 Chromatography Conditions

Column	: Kromasil KR100 ODS 5µm 50 mm x 3.2 mm i.d.
Column Oven Temperature	: 40°C
Flow rate	: 1.0 mL min ⁻¹
Injection volume	: 20 µL
Stop Time	: 4.0 minutes
Injection protocol	: Analyse calibration standard after 3 to 4 sample injections
Mobile phase	: Solvent A = methanol Solvent B = 10 mM ammonium acetate

Mobile Phase Gradient

Time (min)	% A	% B
0.0	10	90
2.0	95	5
2.9	95	5
3.0	10	90
4.0	10	90

3.3 API 4000 Mass Spectrometer Conditions

Interface	:	TurboIonSpray			
Polarity	:	Positive			
Curtain gas (CUR)	:	Nitrogen set at 10 (arbitrary units)			
Gas 1	:	Air set at 50 (arbitrary units)			
Gas 2	:	Air set at 50 (arbitrary units)			
Interface Heater	:	On			
Temperature (TEM)	:	500°C			
Ionspray voltage	:	5500 V			
Collision gas setting (CAD)	:	Nitrogen set at 6 (arbitrary units)			
Scan type	:	MRM			
		Cyprodinil	CGA249287	CGA275535	CGA321915
Q1 mass	:	226.0	150.0	241.9	151.0
Q3 mass	:	93.1	118.1	93.1	93.1
Dwell time	:	75 ms	75 ms	75 ms	75 ms
Resolution Q1	:	Unit	Unit	Unit	Unit
Resolution Q3	:	Unit	Unit	Unit	Unit
Declustering potential (DP)	:	81 V	71 V	106 V	56 V
Entrance potential (EP)	:	10 V	10 V	10 V	10 V
Collision energy (CE)	:	53 V	37 V	53 V	36 V
Collision cell exit potential (CXP)	:	6 V	6 V	6 V	6 V

Typical chromatograms are shown in Appendix 4.

4. Calculation of Results

Residues may be calculated using an external standardisation procedure.

Cyprodinil, CGA249287, CGA275535 and CGA321915 residues may be calculated in mg kg^{-1} for each sample using a mean standard response from each of the injections bracketing the sample as follows.

- a) Make repeated injections of a standard containing cyprodinil, CGA249287, CGA275535 and CGA321915 at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for cyprodinil, CGA249287, CGA275535 and CGA321915.
- b) Make an injection of each sample solution and measure the peak areas of the peaks corresponding to cyprodinil, CGA249287, CGA275535 and CGA321915.
- c) Re-inject the standard solution after a maximum of four injections of sample solutions.
- d) Calculate the cyprodinil, CGA249287, CGA275535 and CGA321915 residues in the sample, expressed as mg kg^{-1} , using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard ($\mu\text{g mL}^{-1}$)
Sample Conc. = Sample concentration (g mL^{-1})

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \text{ (mg kg}^{-1}\text{)}$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

5. Control and Recovery Experiments

Control experiments should be completed as Section 3 for each set of samples analysed to verify that samples are free from cyprodinil, CGA249287, CGA275535 and CGA321915 contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery experiments (untreated samples accurately fortified with a known amount of cyprodinil, CGA249287, CGA275535 and CGA321915 prior to extraction) should also be completed alongside each batch of samples. Provided the recovery values are acceptable they may be used to correct any cyprodinil, CGA249287, CGA275535 and CGA321915 residues found. The recovery levels should be appropriate to the residue levels expected.

Recovery data is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq 20\%$.

6. Specificity

6.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the soil matrices tested has not been observed.

6.2 Reagents and Solvents

Using high purity solvents and reagents no interference has been found, however it is recommended that each batch of reagents or solvent is checked for contamination prior to use.

6.3 Labware

The method uses disposable labware, which minimises the possibility of cross contamination.

Appendix 1 : Apparatus

UK Suppliers

Polypropylene centrifuge bottles (250 mL), available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

General laboratory glassware e.g. round-bottomed flasks, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Heating mantle for reflux of samples, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK

Laboratory centrifuge, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

10 mL graduated centrifuge tubes, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

Techne Dri-block DB3-A heating block for evaporation of samples, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

Crimp cap autosampler vials and caps available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

API4000 LC-MS/MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 120 Birchwood Boulevard, Warrington, Cheshire WA3 7PB, UK.

Agilent 1100 HPLC system equipped with quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies UK Limited, Chemical Analysis Group, Lakeside Heath, Cheadle Royal Business Park, Stockport, Cheshire SK8 3GR.

CTC HTS PAL autosampler, available from Presearch Ltd, System House, 59-61 Knowlpiece, Hitchin, Herts SG4 0TY, UK.

HPLC column, Kromasil KR100 ODS, 50 mm × 3.2 mm i.d., 5 µm particle size, available from Phenomenex, Queens Avenue, Hurdsfield Industrial Estate, Macclesfield, Cheshire SK10 2BN, UK.

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments Ltd., Fountain Crescent, Inchinnan Business Park, Inchinnan, Renfrew PA9 4RE, Scotland.

US Suppliers

Polypropylene centrifuge bottles (250 mL), available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

General laboratory glassware e.g. round-bottomed flasks, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Heating mantle for reflux of samples, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Laboratory centrifuge, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Techne Dri-block DB3-A heating block for evaporation of samples, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

10 mL graduated centrifuge tubes, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Crimp cap autosampler vials and caps available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

API4000 LC-MS-MS system equipped with a TurboIonSpray source, available from Applied Biosystems, 850 Lincoln Center, Foster City, CA 94404-1128, USA.

Agilent 1100 HPLC system equipped with quaternary pump, vacuum degasser and column compartment with column switching valve, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

CTC HTS PAL autosampler, available from LEAP Technologies Inc., P.O. Box 969, Carrboro, NC 27510 USA.

HPLC column, Kromasil KR100 ODS, 50 mm × 3.2 mm i.d., 5 µm particle size, available from Phenomenex Inc. 2320 W. 205th Street, Torrance, CA 9051-1456, USA.

Peak Scientific NM20ZA gas station, available from Peak Scientific Instruments, 1300 West Belmont Ave., Chicago IL 60657, USA.

Appendix 2 : Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

UK Suppliers

Ammonium acetate, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK.

Methanol, pesticide grade, available from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK.

Ultra pure water from a laboratory water purification system e.g. Elga Maxima available from Elga Ltd., High Street, Lane End, High Wycombe, Bucks HP14 3JH, UK.

Cyprodinil, CGA249287, CGA275535 and CGA321915 analytical standards, available from Syngenta Crop Protection, Jealott's Hill Research International Research Centre, Bracknell, Berkshire RG42 6EY, UK.

US Suppliers

Ammonium acetate, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Methanol, pesticide grade, available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

Ultra-pure water from a laboratory water purification system available from Waters Corporation, Milford, MA, USA.

Cyprodinil, CGA249287, CGA275535 and CGA321915 analytical standards, available from Syngenta Crop Protection, Inc., P. O. Box 18300, Greensboro, NC 27419-8300.

Appendix 6 : API 4000 MS/MS Tuning Procedure

Calibration of Instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturers instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning Instrument for Cyprodinil, CGA249287, CGA275535 and CGA321915

Infuse a standard solution of cyprodinil, CGA249287, CGA275535 and CGA321915 (0.01 to $1.0 \mu\text{g mL}^{-1}$) in mobile phase (see section 4.2) directly into the mass spectrometer interface at a rate of about $10 \mu\text{L min}^{-1}$. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at $m/z = 226.0$, $m/z = 150.0$, $m/z = 241.9$ and $m/z = 151.0$

Using the Analyst software quantitative optimisation routine, tune the instrument for cyprodinil, CGA249287, CGA275535 and CGA321915, ensuring that the correct ions are selected (initial Q1 $m/z = 226.1$ and product ion $m/z = 93.1$ for cyprodinil, initial Q1 $m/z = 149.9$ and product ion $m/z = 118.1$ for CGA249287, initial Q1 $m/z = 241.7$ and product ion $m/z = 93.1$ for CGA275535 and initial Q1 $m/z = 150.9$ and product ion $m/z = 93.1$ for CGA321915). If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of cyprodinil, CGA249287, CGA275535 and CGA321915 standards using mobile phase at the flow rate to be used and at the intended split ratio. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.