

METHOD

General principle

Samples were extracted with acetonitrile:water and cleaned-up by liquid-liquid partition with hexane before liquid-liquid partition into ethyl acetate. The ethyl acetate was removed and the sample reconstituted in methanol:water. Quantitation was by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

Apparatus, glassware etc

Polyethylene bottles (250 ml)
 Polypropylene tubes (50 ml)
 Pipettes (various sizes)
 Syringes (various sizes)
 Volumetric flasks (various)
 Rotary film evaporator
 Vacuum pump
 Round bottom flasks (100 ml)
 Glass funnels
 Measuring cylinders (various)
 Mistral 3000E centrifuge
 Reciprocating shaker

Materials	Grade	Supplier
Acetic acid (glacial)	HPLC	Fisher Scientific, Loughborough, England
Acetonitrile	HPLC	Fisher Scientific, Loughborough, England
Ammonium acetate	AR	Fisher Scientific, Loughborough, England
Ethyl acetate	HPLC	Fisher Scientific, Loughborough, England
Hexane	HPLC	Fisher Scientific, Loughborough, England
Methanol	HPLC	Fisher Scientific, Loughborough, England
Water	UP	Fisher Scientific, Loughborough, England

Test substance solutions

A weighed amount (corrected for purity if required) of LGC-30473 was dissolved in acetonitrile in an amber volumetric flask to produce a stock standard solution. Aliquots of the stock standard solution were diluted with acetonitrile to give standard fortification solutions. LGC-30473 was further diluted in methanol:water (50:50 v:v) to produce a series of calibration solutions in the range 1 ng/ml to 50 ng/ml.

Preparation of Reagents

Preparation of extraction solvent - acetonitrile:water (70:30 v:v)

Acetonitrile (700 ml) was added to UP water (300 ml) and mixed thoroughly.

Preparation of final solution - methanol:water (50:50 v:v)

Methanol (500 ml) was added to UP water (500 ml) and mixed thoroughly.

Preparation of mobile phase A - water:acetonitrile (80:20 v:v) containing 0.01 M ammonium acetate and 0.1 % acetic acid

Ammonium acetate (0.8 g), acetic acid (1 ml) and acetonitrile (200 ml) were added to UP water (800 ml). The bottle was capped and shaken thoroughly until all dissolved.

Preparation of mobile phase B water:acetonitrile (20:80 v:v) containing 0.01 M ammonium acetate and 0.1 % acetic acid

Ammonium acetate (0.8 g), acetic acid (1 ml) and acetonitrile (800 ml) were added to UP water (200 ml). The bottle was capped and shaken thoroughly until all dissolved.

Procedure

A sub-sample (20 g) of soil was weighed into a 250 ml polyethylene bottle. The sample was fortified with the test substance at this stage if required. An aliquot (80 ml) of acetonitrile:water (70:30 v:v) was added and the sample shaken for approximately 30 minutes. The sample was centrifuged and the extract transferred to a 250 ml polyethylene bottle through a glass funnel plugged with glass wool. Another aliquot (80 ml) of acetonitrile:water (70:30 v:v) was added to the remaining residue and the sample was shaken, centrifuged and combined with the first extract. The combined extract was made up to 200 ml volume with more acetonitrile:water (70:30 v:v) and a 10 ml aliquot was transferred to a 50 ml polypropylene tube. Aliquots (20 ml) of water and hexane (10 ml) were added, the samples shaken well (using centrifugation to separate the phases if necessary). The hexane (upper) layer was removed and discarded. A further aliquot (10 ml) of hexane was added and the procedure repeated, again discarding the hexane. An aliquot (10 ml) of ethyl acetate was added, the samples shaken well (using centrifugation to separate the phases if necessary). The ethyl acetate (upper) layer was transferred into a 100 ml round bottomed flask. A further aliquot (10 ml) of ethyl acetate was added and the procedure repeated, combining the ethyl acetate layers in the flask. The sample was evaporated to dryness by rotary film evaporation at approximately 40 °C and reconstituted in an appropriate amount of methanol:water (50:50 v:v) before quantitation by LC-MS/MS.

LC-MS conditions

Instrument:	Quattro LC															
Mode:	Electrospray positive (ESP+)															
Source temperature:	120°C															
API gas flow:	Approximately 400 l/hour															
Ion monitoring details:	LGC-30473 – MRM m/z 321.0>200.0															
Column:	Phenomenex Luna C ₈ (15 cm x 2.0 mm)															
Mobile phase A:	water:acetonitrile (80:20 v:v) containing 0.01 M ammonium acetate and 0.1 % acetic acid															
Mobile phase B:	water:acetonitrile (20:80 v:v) containing 0.01 M ammonium acetate and 0.1 % acetic acid															
Gradient:	<table><thead><tr><th>Time</th><th>%A</th><th>%B</th></tr></thead><tbody><tr><td>0</td><td>60</td><td>40</td></tr><tr><td>6</td><td>0</td><td>100</td></tr><tr><td>12</td><td>0</td><td>100</td></tr><tr><td>13</td><td>60</td><td>40</td></tr></tbody></table>	Time	%A	%B	0	60	40	6	0	100	12	0	100	13	60	40
Time	%A	%B														
0	60	40														
6	0	100														
12	0	100														
13	60	40														
Injection volume:	20µl															
Flow rate:	0.2 ml/min															
Cycle time:	25 min															
Retention times:	LGC-30473: approx. 7.2 minutes															

Calculation of results

Test samples were quantified using the following equation:

$$\text{Residue found (mg/kg)} = x \times \frac{1}{M} \times D$$

Where x (residue concentration in final solution) was calculated using the linear regression

$$y = m x + c \quad \text{where } x \text{ (concentration in ng/ml)} = \frac{y - c}{m}$$

- c = intercept
- m = slope
- y = peak area
- M = matrix concentration (g/ml)
- D = dilution factor

Example calculation of LGC-30473 residue detected in clay loam soil fortified at 0.05 mg/kg (sample identification 03/00/0307 F0.05 D). The primary data for this sample is presented in Table 6, Appendix 1.

Linear regression $y = m x + c$
 $y = 14.44x + 0.42$

where $y = 66.944$
 $m = 14.44$
 $c = 0.42$

Therefore, concentration of LGC-30473 (x) = $\frac{66.944 - 0.42}{14.44} = 4.61 \text{ ng/ml}$

Matrix concentration = 0.2 g/ml

Dilution factor = 2

LGC-30473 detected (mg/kg) = $\frac{4.61 \text{ ng/ml} \times 2}{0.2 \text{ g/ml}} = 46.1 \text{ ng/g}$

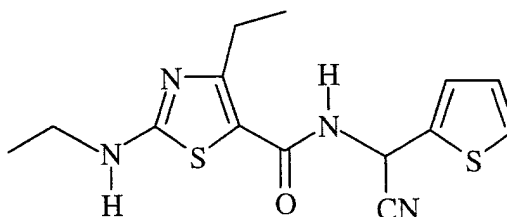
= 0.046 mg/kg

Recovery of LGC-30473 = $\frac{0.046 \text{ mg/kg}}{0.05 \text{ mg/kg}} \times 100 \% = 92\%$

MATERIALS**Test substance**

Identity: LGC-30473
Chemical name (IUPAC): N-(α -cyano-2-thenyl)-4-ethyl-2-(ethylamino)-5-thiazolecarboxamide

Structure:



Molecular formula: $C_{14}H_{16}ON_4S_2$
Molecular weight: 320.7
Appearance: Off white powder
Storage conditions: Approximately 4°C
Batch number: P980622
Receipt date: 8 July 1998
Supplier: LG Life Sciences Ltd
Purity: 99 %