

METHOD

General principle

Water samples were extracted by liquid-liquid partition into dichloromethane. The dichloromethane 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

Pipettes (various sizes)
Separating funnels (250 ml)
Syringes (various sizes)
Volumetric flasks (various)
Rotary film evaporator
Vacuum pump
Round bottom flasks (100 and 250 ml)
Measuring cylinders (various)

Materials	Grade	Supplier
Acetic acid (glacial)	HPLC	Fisher Scientific, Loughborough, England
Acetonitrile	HPLC	Fisher Scientific, Loughborough, England
Ammonium acetate	AR	Fisher Scientific, Loughborough, England
Dichloromethane	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 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.

Procedures

Ground and tap water

A sub-sample (100 ml) of water was transferred into a 250 ml separating funnel. The sample was fortified with the test substance at this stage if required. An aliquot (25 ml) of dichloromethane was added and the sample shaken for approximately 1 minute. The dichloromethane (lower) layer was transferred to a 100 ml round bottom flask. A further aliquot (25 ml) was added, the samples shaken as before, combining the extracts 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.

Surface water

A sub-sample (100 ml) of water was transferred into a 250 ml separating funnel. The sample was fortified with the test substance at this stage if required. An aliquot (50 ml) of dichloromethane was added and the sample shaken for approximately 1 minute. The dichloromethane (lower) layer was transferred to a 250 ml round bottom flask. A further aliquot (50 ml) was added, the samples shaken as before, combining the extracts 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 C8 (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:

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 time: LGC-30473: approx. 7.2 minutes

Calculation of results

Test samples were quantified using the following equation:

$$\text{Residue found } (\mu\text{g/L}) = x \times \frac{1}{M} \times D$$

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 (ml/ml)

D = dilution factor

Example calculation of LGC-30473 residue detected in surface water fortified at 1.0 $\mu\text{g/L}$ (sample identification 3333 F1 C). The primary data for this sample is presented in Table 7, Appendix 1.

$$\begin{aligned} \text{Linear regression } y &= m x + c \\ y &= 16.49x - 0.58 \end{aligned}$$

$$\begin{aligned} \text{where } y &= 74.249 \\ m &= 16.49 \\ c &= -0.58 \end{aligned}$$

$$\text{Therefore, concentration of LGC-30473 } (x) = \frac{74.249 + 0.58}{16.49} = 4.54 \text{ ng/ml}$$

Matrix concentration = 5 ml/ml

Dilution factor = 1

$$\text{LGC-30473 detected } (\mu\text{g/L}) = \frac{4.54 \text{ ng/ml} \times 1}{5 \text{ ml/ml}} = 0.908 \text{ ng/ml}$$

$$= 0.908 \mu\text{g/L}$$

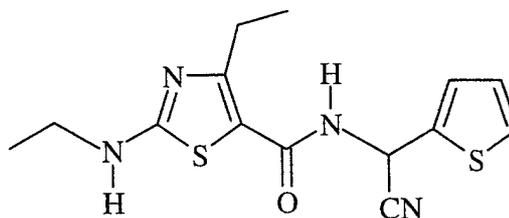
$$\text{Recovery of LGC-30473} = \frac{0.908 \mu\text{g/L}}{1 \mu\text{g/L}} \times 100 \% = 91\%$$

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 %