

SUMMARY

The method has been developed for the determination of SL-160 in drinking water and was validated in the concentration range from 0.05 µg/l to 1 µg/l. The analytical method employs a simple approach, involves minimum cost and time and require commonly available equipment as requested by the guideline for monitoring purposes.

SL-160 was extracted from 500 or 1000 ml drinking water by partition with ethyl acetate or dichloromethane after acidification to pH 2.5. The organic phase was concentrated in a rotary evaporator and the residue dissolved in 1 ml acetonitrile/water (1+1; v/v). Separation and quantification was performed by high performance liquid chromatography (HPLC) using a reversed-phase column and UV detection.

The analytical method was validated by fortifying drinking water samples at 0.05, 0.1, 0.5 and 1.0 µg/l. Samples were run in triplicate. RCC Umweltchemie AG drinking water was used for the fortification and as control sample.

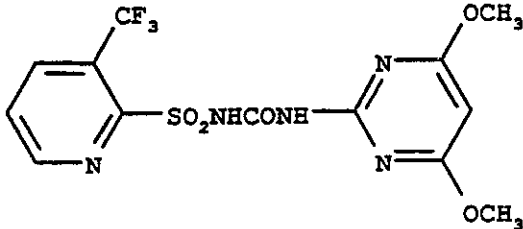
Limit of detection (LDC): 0.02 µg/l
Limit of determination (LDM): 0.05 µg/l

2 MATERIALS AND METHODS

2.1 TEST ARTICLE

SL-160 Analytical Standard (used for fortification and calibration)

Data as provided by the sponsor:

Identity:	SL-160
Chemical Name [IUPAC]:	1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulphonyl)urea
Chemical Structure:	
Lot No.:	8706-S
Purity:	99.7%
Physical Form:	Solid
Solubility in Water (25 °C):	pH 5: 27 mg/l pH 7: 2100 mg/l
Stability in Water:	Stable at least for 2 hours
Solubility in Acetonitrile:	870 mg/100 g at 25 °C
Storage at RCC:	In the original container protected from light, at approx. -20 °C
Expiration Date:	October 31, 1999
Safety Precautions:	Routine hygienic procedures.

2.2 TEST SYSTEM

As control water for the spiking and recovery experiments, drinking water as available at RCC in CH-4452 Itingen/Baselland was used. This drinking water used in the validation tests was characterised according to the parameters described in Section 2.6.

2.3 REAGENTS AND APPARATUS

Reagents and Solvents

All reagents and solvents were of pesticide residue analytical grade, if not specified otherwise.

Water (HPLC-grade): (Millipore)	Purified by Milli-Q Plus 185 Water System
Water, bi-distilled:	RCC Umweltchemie AG
Acetonitrile (HPLC-grade):	Baker no. 9017
Ethyl acetate:	Baker no. 9260-03
Dichloromethane:	Baker no. 9264
Hydrochloric acid (32%):	Baker no. 6070
Sodium Sulphate:	Baker no. 0313
Buffer pH 4:	Baker no. 5657 (for calibration of pH-meter)
Buffer pH 7:	Baker no. 5656 (for calibration of pH-meter)

Equipment

Rotary-evaporator:	Resona Technics Labo rota S300
Ultrasonic bath:	Bender & Hobein, Branson 5200
Analytical balance:	AE 240, Mettler + HL 52, Mettler
Magnetic stirrer:	IKAMAG ICT
pH-meter:	pH 96; WTW
Glass electrode: 104023311	INGOLD U402-57/120 (for pH 0-14); no.
Volumetric flask:	Various sizes
Measuring cylinder:	Various sizes
Round-bottom flask:	250 ml + 500 ml
Separatory funnel:	1000 ml + 2000 ml
Hamilton syringe:	Various sizes
Bulb pipette:	Various sizes
Measuring pipette:	Various sizes
Lyophilisator:	Freeze dryer Modulyo; Fa. Edwards

2.4 SOLUTIONS FOR FORTIFICATION AND CALIBRATION

Stock Solution (A) 1000 µg/ml

An aliquot of 10.0 mg of SL-160 (99.7 %, see Section 2.1) was placed in a volumetric flask and dissolved in 10 ml of acetonitrile.

Solution (B) 10 µg/ml

1 ml of the stock solution (A) was transferred to a 100-ml volumetric flask and filled to the mark with acetonitrile.

Calibration and Fortification Solutions

Aliquots of solution B were transferred to 10-ml volumetric flasks and diluted to the mark with bi-distilled water/acetonitrile (1+1, v/v).

- C) 1 ml of solution B to 10 ml = 1.00 µg SL-160/ml
- D) 5 ml of solution C to 10 ml = 0.50 µg SL-160/ml
- E) 2 ml of solution D to 10 ml = 0.10 µg SL-160/ml
- F) 5 ml of solution E to 10 ml = 0.05 µg SL-160/ml
- G) 4 ml of solution F to 10 ml = 0.02 µg SL-160/ml

2.5 STABILITY OF SOLUTIONS

Standard and fortification solutions were prepared new each time they were needed. Therefore no stability check was needed.

2.6 SAMPLES

2.6.1 Water Parameters

For the validation tests, drinking water as available at RCC Umweltchemie AG was used.

For characterisation of this drinking water the following parameters were determined:

Total Hardness

The total water hardness was determined by complexometric titration with Aquameter®.

The beaker was first rinsed for several times with drinking water and then filled to a volume of 5 ml. The tritrant was added dropwise until the solution turned from red to blue, marking the end point of the titration. The drops were counted. Determination of water hardness was performed in triplicate.

One drop corresponds to 1°dH (German degree of hardness) or 0.18 mmol/l alkaline-earth ions. Hence, the total water hardness was calculated to be 13°dH or 2.34 mmol/l.

Total Mineral Content

The total mineral content was determined by a gravimetric method.

Two aliquots of 500 ml drinking water were lyophilised in 1000 ml round bottom flasks. The dried residue was weighed to be 0.55 g/l.

pH-Value

The pH-value of the drinking water, measured by means of a calibrated pH-meter with glass electrode, was pH 7.25.

2.6.2 Set of Samples

For the validation of the analytical method (see section 2.7) untreated water samples were amended in triplicate by addition of separate standard aliquots of SL-160 at the following levels:

1.0 µg SL-160/l

500 ml water aliquots (triplicate) were fortified with 500 µl of standard solutions C.

0.5 µg SL-160/l

500 ml water aliquots (triplicate) were fortified with 500 µl of standard solutions D.

0.1 µg SL-160/l

500 and 1000 ml water aliquots (each in triplicate) were fortified with 500 µl or 200 µl of standard solutions E and D, respectively.

0.05 µg SL-160/l

500 and 1000 ml water aliquots (each in triplicate) were fortified with 500 µl of standard solutions F and E, respectively.

For each series one control sample (not amended) was worked up similarly as the amended samples.

2.7 ANALYTICAL METHOD

500 ml samples were worked up first in one set by using ethyl acetate as extraction solvent. The results showed some interference of the matrix at the lowest concentration of 0.05 µg/l (Figure 8). Therefore, the analysis for the lowest concentration 0.05 µg/l and 0.1 µg/l was repeated by working up 1000 ml and using dichloromethane as extraction solvent. Dichloromethane extracted less matrix than ethyl acetate and only half the amount as ethyl acetate were used.

Extraction Procedure

A volume of drinking water (Vs) was first transferred to a 1- or 2-l separatory funnel depending on the sample volume to be worked up (500 ml or 1000 ml). Afterwards, the solution was adjusted to pH 2.5 with about 0.45 ml (500 ml sample) or 0.9 ml (1000 ml sample) of hydrochlorid acid (32%). The acidified sample solution was extracted once with 200 ml ethyl acetate or 100 ml dichloromethane by partitioning for about 1 min.

Thereafter, the organic phase was filtered over anhydrous sodium sulphate into a round-bottom flask. The organic phase was totally evaporated in a rotavap at about 30 °C and any residual solvent was removed in a stream of nitrogen at room temperature, if necessary.

The residue was dissolved in 1.0 ml (VR) of acetonitrile/water (1+1, v/v) by means of an ultrasonic bath for HPLC-analysis.

Conditions for HPLC-Analysis

The concentrations of SL-160 in the extracts were quantified by High Performance Liquid Chromatography (HPLC) under the following conditions:

Instrument:	Pump: Merck-Hitachi L-6200 A Autosampler: Merck-Hitachi AS-2000 A UV-Detector: Merck-Hitachi L-4000
Column:	Beckman Ultraspher ODS (C-18) Endcapped, 5 µm (250 mm x 4.6 mm) (same guard column 4 x 4 mm)
Mobile Phase:	A = 0.05% H ₃ PO ₄ B = Acetonitrile
Gradient:	Isocratic
Flow:	1 ml/min
Injection:	200 µl (autosampler)
Detection:	UV-detector 254 nm

The typical retention time of SL-160 was about 8 minutes. Representative HPLC chromatograms are shown in Figures 1 to 11.

2.8 EVALUATION OF RESULTS

Injected samples were quantified by peak area with reference to the calibration curve. The latter was obtained by correlation of the peak areas of the analytical standards with their corresponding concentrations of SL-160 injected ($\mu\text{g/ml}$), using a linear regression (see equation (1)). The stability of the analytical system was tested by injection of standard solutions alternating with the sample solutions. The calibration curves of SL-160 are shown on page 20.

$$Y = a + b \cdot X \quad (1)$$

where: X = Concentration of SL-160 in injected sample ($\mu\text{g/ml}$)
Y = Detector response (peak area)
a = Y-axis intercept
b = Slope

Therefore, the concentration ($\mu\text{g/ml}$) of SL-160 in the injected sample was calculated by:

$$X = \frac{(Y - a)}{b} \quad (2)$$

The residue concentration ($\mu\text{g/l}$) of SL-160 was calculated using the following equation:

$$C = \frac{X \cdot V_R}{V_S} \cdot 1000 \quad (3)$$

where C = Concentration of SL-160 in drinking water samples ($\mu\text{g/l}$)
X = Concentration ($\mu\text{g/ml}$) of SL-160 in the injected sample derived from equation (2)
V_R = Reconstitution volume before injection into HPLC (1.0 ml)
V_S = Volume of drinking water Sample (0.5 l or 1 l)
1000 = Conversion factor to l drinking water

The recovery rate was calculated using the following equation (4):

$$R = \frac{C}{F} \times 100\% \quad (4)$$

where: R = Recovery in %
C = Total concentration found in $\mu\text{g/l}$
F = Fortification level in $\mu\text{g/l}$

Limit of Detection (LDC)

The limit of detection was estimated as the concentration corresponding the lowest calibration concentration. Comparing the peak response of the concentration of the lowest calibration point 0.02 µg/ml (Figure 1) with the matrix background noise observed in the control sample chromatogram around the retention time of the analyse (Figure 4) and the volume of drinking water worked up, the limit of detection was estimated as 0.02 µg/l.

Limit of Determination (LDM)

The limit of determination was 0.05 µg/l and was derived from the lowest fortification level (0.05 µg/l) at which acceptable recoveries in the range of 70 - 110 % with a relative standard deviation not exceeding 20 % were obtained.

Precision of HPLC-Analysis

The precision of the HPLC analysis was determined by multiple injection of the calibration solutions and duplicate injection of the sample solutions. The mean values of the peak areas of the samples were calculated.