1. INTRODUCTION

Background and Objective:

The objective of this study was to validate an analytical method for the determination of S-2200 in water. The target limit of quantification (LOQ) is 0.1 µg/L.

Data Requirements and Guidelines:

EC Guidance document on residue analytical methods (SANCO/825/00 rev. 8.1 16/11/2011).

Method Principles and Method Validation:

Residues of S-2200 were determined in surface water by direct injection with LC-MS/MS in the positive ion mode, using 2 transition ions for quantitation and confirmation.

For method validation the surface water specimens were fortified (5 replicates per fortification level) at 0.10 µg/L (LOQ) and at 1.0 µg/L (10xLOQ) of S-2200. Additional two specimens were kept untreated as blank controls.

2. EXPERIMENTAL

2.1 Test System

Water was collected on 17-Feb-11 from the Waldsee in Senden (Southern Germany). The water was characterized for physical and chemical properties as follows: pH 8.03, total water hardness: 3.3 mmol/L corresponding to 18.2 °dH. The water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following:

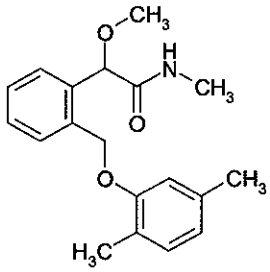
DOC (dissolved organic carbon, EN 1484:1997):	0.70 mg/L
Silt content (EN 872 Whatman GF 6):	0.3 mg/L
Electric conductivity (at 25 °C):	644 µS/cm

The surface water was kept refrigerated at approximately 4 °C and in the dark after collection and prior to extraction.

2.2 Analytical Test and Reference Items

The following standard provided by the Sponsor (see Appendix 1) was used as test / reference item:

- S-2200



Empirical Formula: $C_{19}H_{23}NO_3$ Molar Mass: 313.4 g/mol

The analytical test / reference item was stored refrigerated when not in use.

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

Mettler-Toledo XP205DR analytical balance for analytical standards.
Ultrasonic bath Transsonic 460, Schmidbauer. Vortex mixer Assistent Reamix, Transsonic.
pH Meter Denver Instrument
Typical glassware and laboratory equipment.
All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

2.3.1.2 LC-MS System

Agilent 1200 SL Series HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.
Column: Phenomenex, Luna C_{18}, 5 µm particle size, 50 mm length, 2.0 mm i.d. Pre-column: Phenomenex C_{18}, 4 mm length, 3.0 mm i.d.

Applied Biosystems MDS Sciex API 4000 triple quadrupole LC-MS/MS system with Turbo IonSpray ESI source. Analyst 1.4.2 Instrument control and data acquisition software.

2.3.2 Solvents and Chemicals

HPLC water (HPLC grade) and acetonitrile (≥ 99.9 %), Fluka
Formic acid (≥ 99.9 %), Promochem. Total Hardness Test, Merck.

2.3.3 Preparation of Standard Solutions

A stock solution of S-2200 was prepared by accurately weighing 10.06 mg of the analytical standard (purity of 100 %) and dissolving it in 10.0 mL of acetonitrile to obtain a concentration of 1.0 mg/mL.

This solution was further diluted volumetrically into acetonitrile to obtain fortification solutions at 5.0, 0.50, 0.010 and 0.0010 µg/mL.

For fortification of recoveries at 0.10 µg/L, the fortification solution with the concentration of 0.0010 µg/mL and for recoveries at 1.0 µg/L, the fortification solution with a concentration of 0.010 µg/mL was used.

Intermediate solutions at concentrations of 20000, 400 and 20 ng/mL were prepared by volumetric dilution of the stock solution. Further dilutions into acetonitrile/water (1/9, v/v) were prepared to obtain calibration solutions with 0.0175 to 2.0 ng/mL.

2.3.4 Stability of Standard Solutions and Extracts

Fortification and standard solutions were stored refrigerated in amber glass bottles when not in use. Stability of standard solutions during the course of the study was demonstrated by consistent LC-MS/MS results. Consistent fortification results demonstrate stability of extracts (at ambient conditions) during the duration of the analysis (approximately 1 day).

2.3.5 Residue Analysis

1. An aliquot of 1.0 mL (V_{Sample}) water is dosed into an autosampler vial.
2. 100 µL of the corresponding fortification solution is added, if necessary.
3. 100 µL of acetonitrile is added to blank control samples.
4. The sample is mixed using a vortexer.

Determination of the analyte is done by using LC-MS/MS.

2.4 LC-MS/MS Analysis

Specimen extracts and calibration solutions in solvent were analyzed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS):

LC System	Agilent 1200 SL HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler
LC Column	Phenomenex Luna C_{18} column: Length: 50 mm, i.d.: 2.0 mm, particle size: 5 µm, temp.: 40 °C.
LC Injection Volume	20 µL.
LC Method	Solvent A: Water with 0.1 % formic acid Solvent B: Acetonitrile with 0.1 % formic acid Mobile Phase Composition: Time (min) / Flow rate (mL/min) / % A / % B 0.00 / 0.500 / 90 / 10 0.20 / 0.500 / 90 / 10 0.70 / 0.500 / 5 / 95 3.80 / 0.500 / 5 / 95 4.00 / 0.500 / 90 / 10 6.00 / 0.500 / 90 / 10
Retention time	≈ 2.9 min for S-2200.
MS/MS System	Applied Biosystems MDS Sciex API 4000 triple quadrupole LC-MS/MS system with TurboIonspray (ESI) source.
Ion Source Conditions ESI Positive Polarity	Source temperature: 450 °C Nebulizer Gas supply (GS 1): 40 (arbitrary units) Turbo Gas supply (GS 2) 70 (arbitrary units) Curtain gas: 20 (arbitrary units) CAD gas: 5 (arbitrary units) Entrance potential: 10 V IonSpray voltage: 5000 V Resolution: Q1: Unit, Q3: Unit
MS/MS Conditions for chlorpyrifos-methyl	MS/MS transition for quantification: 314 m/z > 192 m/z Collision energy (CE): 15 V Cell exit potential (CXP): 14 V Dwell time: 100 ms Declastering potential (DP): 36 V MS/MS transition for confirmation: 314 m/z > 160 m/z Collision energy (CE): 27 V Cell exit potential (CXP): 16 V Dwell time: 400 ms Declastering potential (DP): 36 V

See Figure 5 for the product ion spectrum of S-2200 obtained.

The quantitative determination was carried out by external standardization using calibration standards in solvent. Calibration functions ranging from 0.0175 to 2.0 ng/mL were used to evaluate the final dilutions (exemplified in Figure 1 and Figure 2).

Representative LC-MS/MS ion chromatograms of calibration solutions in solvent and of final dilutions of fortified and control specimens are presented in Figure 3 and Figure 4.

2.5 Calculations

Results derived from LC-MS/MS and calculations are shown in detail in Table 1.

The following equation was used to calculate the individual residues R in µg/L:

$R = c_{End} \times (V_{End} / V_{Sample})$

$= c_{End} \times$ Multiplier M

R: Analyte residue in µg/L.

c_{End}: Final concentration of S-2200 in specimen extract, in ng/mL.

V_{Sample}: Sample volume: 1.0 mL.

V_{End}: Volume of final extract: 1.1 mL.

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$Rec. = (R / R_{fortified}) \times 100\ \%$

Example for Calculation:

The calculation is exemplified with the surface water specimen P2376-25 fortified at 0.10 µg/L (LOQ). The final extract was examined by LC-MS/MS in run file P2376API#024 (Figure 4, middle) to give a peak area of 36700 counts for S-2200 isomer in the transition 314 m/z to 192 m/z. Using the respective calibration curve (see Figure 1) a final concentration of 0.0886 ng/mL is calculated (see Table 1).

Thus:

$R = c_{End} \times (V_{End} / V_{Sample})$

$= c_{End} \times$ Multiplier M

$= 0.0886$ ng/mL x (1.1 mL / 1.0 mL)

$= 0.0886$ ng/mL x 1.1

$= 0.097$ ng/mL or µg/L

$Rec. = (R / R_{fortified}) \times 100\ \%$

$= (0.097\ \mu g/L / 0.10\ \mu g/L) \times 100\ \% = 97\ \%.$

Calculations were performed with full precision. Thus discrepancies may arise when recalculated.