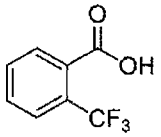
6. MATERIALS

6.1. Test substance

6.1.1. Test substance information

Identification	B-1
Structure	
Molecular formula	$C_8H_5F_3O_2$
Molecular weight	190.12
CAS Number	433-97-6
Description	White powder
Batch	01107BH
Purity	99.7% (GC)
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	28 January 2010 (allocated by NOTOX, 1 year after receipt of the test substance)

The certificate of analysis is shown in Appendix I.

6.1.2. Study specific test substance information

There was no study specific test substance information necessary for this study.

6.2. Water matrixes

Drinking water	Local tap water
Ground water	Water from a local well
Surface water	Pond water taken at the "Schoonrewoerdse Wiel", Leerdam, The Netherlands (51:55:01N; 05:08:05E). This surface water originated as a result of a dike (levee) failure in the 16th century. The force of the water flowing through the dike created a low area behind the dike. After closure of the dike, a small pond remained.
Treatment (at sampling)	sieved through an sieve with a mesh size of 125 µm.
pH (at sampling)	8.2
Temperature (at sampling)	15.4°C
Redox potential (at sampling)	174 mV
Oxygen (at sampling)	7.0 mg/l
Dissolved organic content (DOC)	9.04 mg/l by TOC
Water hardness	150.1 mg/l as $CaCO_3$
Silt content (suspended solids)	2.1 mg/l by gravimetric analysis
Sampling data	05 May 2009
Storage	2-8°C

6.3. Electronic data capture

System control, data acquisition and data processing were performed using the following programmes:
- Analyst version 1.4.2 (Sciex, Toronto, Canada)
- Shimadzu TOC Control V version 1.06.00 (Shimadzu, Kyoto, Japan)

6.4. List of deviations

6.4.1. List of protocol deviations

There were no deviations from the protocol.

6.4.2. List of standard operating procedures deviations

Any deviations from standard operating procedures (SOPs) were evaluated and filed in the study file. There were no deviations from SOPs that affected the integrity of the study.

7. VALIDATION OF AN ANALYTICAL METHOD

7.1. Guidelines

European Community (EC), Commission Directive 96/46/EC of 16 July 1996 amending Council Directive 91/414/EEC concerning the Placing of Plant Protection Products on the Market, Official Journal of the European Communities no. L214, August 23, 1996.

European Commission, SANCO/825/00 revision 7: Guidance Document on Residue Analytical Methods, March 17, 2004.

7.2. Reagents

Water	Tap water purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).
Acetonitrile	Biosolve, Valkenswaard, The Netherlands.
Formic acid, 98-100%	Merck, Darmstadt, Germany.
Ammonium formate	Fluka Chemie, Bucks, Switzerland,

All reagents were of analytical grade, unless specified otherwise.

7.3. Performance of the study

A high performance liquid chromatographic method with tandem mass spectrometric detection (HPLC-MS/MS) for the quantitative analysis of the test substance in water was developed. Validation of the analytical method was performed for the following parameters:

Specificity
A test substance solution and duplicate blank accuracy samples were analysed by single injection. The analytical method was found to be specific if the blank chromatograms showed no response for the test substance or a response of < 30% of the limit of quantification.

According to the guideline, where the HPLC-MS/MS method is highly specific a confirmatory chromatographic method was not required.

Linearity
Calibration solutions were analysed in duplicate. The response of the calibration solutions was correlated with concentration using regression analysis with a $1/concentration^2$ weighting factor. A calibration curve with a coefficient of correlation (r) of > 0.99 and back calculated accuracies of the calibration solutions in the range 85-115% was accepted.

Accuracy and repeatability
Accuracy samples were analysed by single injection into the analytical system. The analytical method was considered applicable for the determination of the test substance if the mean accuracy was in the range 70-110% and the coefficient of variation was ≤ 20%.

Limit of quantification
The limit of quantification (LOQ) is defined as the lowest concentration level at which an accuracy in the range 70-110% and a repeatability of ≤ 20% is demonstrated. The LOQ was obtained from the data of the accuracy- and repeatability test.

Limit of detection
From a chromatogram of a test substance solution the noise level and test substance signal were determined and the limit of detection (LOD) was calculated.

Stability of the analytical system and end solutions
Calibration solutions were injected throughout the validation sequence including the beginning and end. The analytical system and/or end solutions were found to be stable if the coefficient of variation on the responses of the solutions was ≤ 20%.

7.4. Analytical method

7.4.1. Analytical conditions

Instrument	Acquity UPLC system (Waters, Milford, MA, USA)
Detector	API 5000 LC/MS/MS system (Applied Biosystems / MDS Sciex, Toronto, Canada)
Column	Acquity UPLC BEH Shield RP-18, 50 mm × 2.1 mm i.d., dp = 1.7 µm (Waters)
Column temperature	40°C
Injection volume	20 µl
Mobile phase	25/75 (v/v) mobile phase A/mobile phase B A - 2.5 mM ammonium formate in 95/5 (v/v) acetonitrile/water with 0.1% formic acid B - 2.5 mM ammonium formate in 5/95 (v/v) acetonitrile/water with 0.1% formic acid
Flow	0.8 ml/min
MS/MS detection	
Ionisation source	Turbo Ion Spray; positive ion mode
Ion spray voltage	-1500 V
Temperature	450°C
Collision gas	6
Curtain gas	35
Ion source gas 1	50
Ion source gas 2	55
Declustering potential	-50
Entrance potential	-11
Collision energy	-18
Collision cell exit potential	-13
Dwell time	200 msec
Quantitation	*m/z* 189 -> *m/z* 145

7.4.2. Preparation of solutions

Stock- and spiking solutions
Stock solutions of the test substance were prepared in acetonitrile at a concentration of 242 or 258 mg/l.

Spiking solutions were made up from a stock solution and/or dilutions of this solution. The solvent of the spiking solutions was acetonitrile.

Calibration solutions
Five calibration solutions in the concentration range of 0.05 – 1.5 µg/l were prepared from two stock solutions. The end solution of the calibration solutions was 10/90 (v/v) acetonitrile/water containing 0.1% formic acid.

Accuracy samples
4.5 ml blank matrix was spiked with the test substance at a target concentration of 0.1 or 1 µg/l. The accuracy samples were diluted in a 9:1 (v:v) ratio with acetonitrile containing 1% formic acid. The samples prepared with ground water and surface water were filtered through a 0.2 µm Spartan 30/0.2 RC filter (Whatman, Dassel, Germany). The first 10 droplets of the filtrate removed to waste.

The blank accuracy samples were prepared and treated similar to the accuracy samples.

7.5. Formulas

Response (R)	Peak area of the test substance [units]
Calibration curve	$R = a\,C_N + b$
	where: a = slope [units × l/µg] b = intercept [units]
Analysed concentration (C_A)	$C_A = \frac{(R-b)}{a} \times d$ [µg/l]
	where: d = dilution factor
Accuracy	$\frac{C_A}{C_N} \times 100\%$