

2 OBJECTIVE

The aim of the study was to validate an analytical method for the determination of SL-160 (Flazasulfuron), DTPU and TPSA in surface water.

Limit of Quantification (LOQ):

Following the guideline recommendations for surface water, the LOQ must not exceed a concentration which has an impact on non-target organisms deemed to be unacceptable according to the requirements of Annex VI. In agreement with the guidelines the following limits of quantitation (LOQ) were validated:

- SL-160: The LOQ is 0.1 µg/l
- DTPU: The LOQ is 1.0 µg/l well below the lowest value of the aquatic toxicology of 9.0 mg/l (= E_bC₅₀)*.
- TPSA: The LOQ is 10 µg/l well below the lowest value of the aquatic toxicology of 60.2 mg/l (= EC₅₀)*.

* data as provided by the sponsor

3 MATERIALS AND METHODS

3.1 TEST ITEMS

The test items and the information concerning the test items were provided by the sponsor.

3.1.1 SL-160

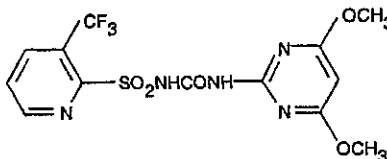
Identity:

SL-160

Chemical name [IUPAC]:

1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulphonyl)urea

Chemical structure:



Molecular formula:

C₁₃H₁₂F₃N₅O₅S

Molecular weight:

407.3

Batch no.:

8706-S

Purity:

99.7% (method of analysis: HPLC)

Expiration date:

October 31, 1999

Solubility in water:

pH 5 buffer: 27 mg/L at 25 °C

pH 7 buffer: 2100 mg/L at 25 °C

(in acetonitrile 8.7 g/kg at 25 °C)

Stability:

The item is unstable in methanol and upon 24 hours storage in water

Physical state:

Solid

Appearance:

Crystalline powder

Melting point:

147 - 150 °C

Storage conditions:

In the original container protected from light, in a refrigerator at approx. 4 °C

Safety Precautions:

Routine hygienic procedures are sufficient to assure personnel health and safety.

3.1.2 DTPU

Identity:	DTPU
Batch no.:	Lot. No. 9403
Expiration date:	May 31, 2001
Purity:	99%
Stability in water:	Stable during the course of this study
Solubility in water:	According to RCC-D Project 562902 at least 200 mg/L ¹
pH in aqueous solution:	Unknown
Aggregate state under storage conditions:	Solid / powder
Colour:	White
Vapour pressure:	Not indicated by the sponsor
Octanol/water partition coefficient:	Not indicated by the sponsor
Storage conditions:	Freezer, approx. -20° C, in the dark
Safety precautions:	Routine hygienic procedures are sufficient to assure personnel health and safety.

3.1.3 TPSA

Identity:	TPSA
Batch no.:	Lot No. 930523
Expiration date:	May 31, 2001
Purity:	98.4%
Stability in water:	Stable during the course of this study
Solubility in water:	According to RCC-D Project 564002 at least 200 mg/L ²
pH in aqueous solution:	Not indicated by the sponsor
Aggregate state under storage conditions:	Solid / powder
Colour:	Light brown
Vapour pressure:	Not indicated by the sponsor
Octanol/water partition coefficient:	Not indicated by the sponsor
Storage conditions:	Freezer, approx. -20° C, in the dark
Safety precautions:	Routine hygienic procedures are sufficient to assure personnel health and safety.

¹ Nominal concentration as tested during a fish toxicity test (mean recovery rate was found to be 87%)

² Nominal concentration as tested during a fish toxicity test (mean recovery rate was found to be 97%)

3.2 REFERENCE ITEM

The reference item and the information concerning the reference item were provided by the sponsor.

Identity:	DTPP
Batch no.:	Lot No. 9403
Expiration date:	May 31, 2001
Purity:	99.0%
Molecular weight:	300.2
Stability in water:	Stable during the course of this study
Solubility in water:	According to RCC-D Project 565102 at least 200 mg/L ³
Storage conditions:	Freezer, approx. -20° C, in the dark

3.3 TEST SYSTEM

The surface water was collected from a small stream called "Hoellenbach" in D-79685 Häg, Germany, near house no. 42 on February 26, 1999. The "Hoellenbach" is a typical rural surface water stream in the area of the black-forest in Germany. The sampled water was stored at ambient conditions for three days. Upon arrival at the analytical laboratory of RCC Ltd. the water was stored at room temperature until analysis.

The pH-value, the total organic carbon content and the residue on evaporation were determined to characterize the water.

³ Nominal concentration as tested during a fish toxicity test (mean recovery rate was found to be 99%)

3.4 REAGENTS AND APPARATUS

All reagents were of analytical, residue analytical or HPLC grade.

REAGENTS	SIZE	SUPPLIER	ARTICLE NO.
Water, Milli-Q		in-house prepared by a Milli-Q water purification unit (Millipore)	
Bi-distilled water		RCC	
Acetonitrile		Baker	9017-54
Dichloromethane		Baker	9264
Ethyl acetate		Baker	9260
Toluene		Baker	9336
Hydrochloric acid (32%)		Baker	6070
Sodium sulfate powder (anhydrous)		Baker	0313
Sodium hydroxide (1 M)		Baker	7097
Sodium chloride		Baker	0278
Phosphoric acid (85%)		Baker	6024
		0.05%: 1250 µL H ₃ PO ₄ was dissolved in 2.5 L ELGA water	
Silica gel column (Bakerbond) (500 mg/3 mL)		Baker	7086-03
Buffer solution pH 7.0		Metrohm	6.2307.110
Buffer solution pH 4.0		Metrohm	6.2307.100
Buffer solution pH 9.0		Metrohm	6.2307.120
Assorted glassware		General laboratory	
Hamilton syringes	various sizes		
Disposable syringes	2, mL, 20 mL	Becton Dickinson	
PTFE-microfilter	0.45 µm	BGB	
HPLC-vials (brown)		BGB	
Water bath		Heraeus	
pH-meter		Metrohm	
Ultrasonic bath		Bender & Hobein	220
Lab shaker		A. Kühner	
Rotary evaporator		Büchi	011
Analytical balance		Sartorius	L2200P
Analytical balance		Mettler	UM-3

3.5 WATER PARAMETERS

For characterization of surface water the following parameters were determined:

3.5.1 Total Organic Carbon

The total organic carbon in the surface water was determined by means of a Shimadzu TOC-500 analyser. The TOC was calculated to be **2.3 mg C/L water**.

3.5.2 Total Weight of Residues

The total weight of residues was determined by a gravimetric method.

One liter of surface water was transferred into a tared 500-mL round-bottom flask and rotary-evaporated to dryness at 50-60 °C. The dried residue was stored in a drying oven at about 90 °C overnight. Finally, the residue was weighed to be **0.32 g**.

3.5.3 pH-Value

The pH-value of the surface water, measured by means of a calibrated pH-meter (Heraeus), was **pH 8.0**.

3.6 STANDARD SOLUTIONS

DTPP:

A stock solution of the reference item DTPP in acetonitrile with a concentration of 1000 µg/mL was prepared by dissolving 9.2316 mg (99%), corresponding to 9.139 mg (100%), of reference item was dissolved in 9.139 mL of acetonitrile using an ultrasonic bath. This stock solution was further diluted with acetonitrile to prepare a 10-µg/mL solution. Calculated volumes of the latter solution were further diluted with bi-distilled water/acetonitrile (7+3 v/v) to obtain standard solutions in the range from 0.01 µg/mL to 1.0 µg/mL. These standard solutions were used for calibration of the HPLC. The stock solution and the 10-µg/mL solution were stored deep-frozen, and all standard solutions were stored in a refrigerator (about 4 °C) during the analytical procedure.

DTPU:

A stock solution of the test item DTPU in acetonitrile with a concentration of 2000 µg/mL was prepared by dissolving 18.6578 mg (99%), corresponding to 18.4712 mg (100%), of test item in 9.236 mL of acetonitrile using an ultrasonic bath. This stock solution was further diluted with acetonitrile to prepare a 100-µg/mL solution. Calculated volumes of the latter solution were further diluted with bi-distilled water/acetonitrile (7+3 v/v) to obtain standard solutions in the range from 0.1 µg/mL to 10.0 µg/mL. These standard solutions were used for calibration of the HPLC. The stock solution and the 100-µg/mL solution were stored deep-frozen, and all standard solutions were stored in a refrigerator (about 4 °C) during the analytical procedure.

TPSA:

A stock solution of the test item TPSA in acetonitrile with a concentration of 1000 µg/mL was prepared by dissolving 10.4604 mg (98.4%), corresponding to 10.293 mg (100%), of test item in 10.293 mL of acetonitrile using an ultrasonic bath. Calculated volumes of this stock solution were further diluted with bi-distilled water to obtain standard solutions in the range from 1.0 µg/mL to 100 µg/mL. These standard solutions were used for calibration of the HPLC. The stock solution was stored deep-frozen, and all standard solutions were stored in a refrigerator (about 4 °C) during the analytical procedure.

3.7 FORTIFICATION SOLUTIONS

SL-160 solution:

A stock solution of the test item SL-160 in acetonitrile with a concentration of 1000 µg/mL was prepared by dissolving 10.9740 mg (99.7%), corresponding to 10.941 mg (100%), of test item in 10.941 mL of acetonitrile using an ultrasonic bath. This stock solution was further diluted with acetonitrile to prepare a 10-µg/mL solution.

Fortification solution I (SL-160: 1.0 µg/mL; DTPU: 10 µg/mL):

1.0 mL of the 10-µg/mL SL-160 solution, 1.0 mL of the 100-µg/mL DTPU solution, and 1.0 mL of the 1000-µg/mL TPSA stock solution* were filled to 10 mL with acetonitrile.

Fortification solution II (SL-160: 0.1 µg/mL; DTPU: 1.0 µg/mL):

1.0 mL of the fortification solution I was filled to 10 mL with acetonitrile.

Fortification solution III (TPSA: 50 µg/mL):

0.5 mL of the 1000-µg/mL TPSA stock solution (cf. Section 3.6) was filled to 10 mL with bi-distilled water.

Fortification solution IV (TPSA: 5.0 µg/mL):

1.0 mL of the fortification solution III was filled to 10 mL with bi-distilled water.

3.8 SET OF SAMPLES

This confirmatory trial was performed on one set of samples per test item. Each set consisted of two control samples and one control sample used for fortification at levels of 0.1 µg SL-160/L water, 1.0 µg SL-160/L water, 1.0 µg DTPU/L water, 10.0 µg DTPU/L water, 10.0 µg TPSA/L water, and 100 µg TPSA/L water, respectively.

For determination of the recovery rate, defined volumes of surface water were fortified with calculated volumes of SL-160, DTPU, or TPSA in acetonitrile and were worked-up according to the analytical methods.

Fortification level (µg/L)	Volume of surface water used (mL)	Fortification solution used ¹ (µg/mL)	Volume of fortif. solution added (mL)
SL-160			
1.0	1000	I	1.0
0.1	1000	II	1.0
DTPU			
10.0	1000	I	1.0
1.0	1000	II	1.0
TPSA			
100	500	III	1.0
10.0	500	IV	1.0

¹ cf. Section 3.7

* negligible since TPSA was determined separately (cf. fortification solutions III and IV)

4 ANALYTICAL METHOD FOR SL-160 (AS DTPP) AND DTPU

The analytical method described below was developed in pretests which were not performed in compliance with the GLP guidelines and which were not described in this report. The raw data will be archived under this project.

4.1 ABSTRACT

The alkalified water sample was extracted with dichloromethane. The organic phase was concentrated to dryness. The residue was dissolved in dichloromethane and cleaned-up on a silica gel column [elution of DTPU with dichloromethane/ethyl acetate (1+1 v/v)]. The eluate was concentrated to dryness. The residue was dissolved in bi-distilled water/acetonitrile and DTPU was quantified by HPLC with UV-detector. The aqueous phase remaining after the first partitioning step was acidified and extracted with dichloromethane. The organic phase was concentrated to dryness. The dried residue was dissolved in acetonitrile. After derivatization with sodium hydroxide (1 molar), the derivative was extracted with dichloromethane. The organic phase was concentrated to dryness. The residue was dissolved in dichloromethane and cleaned-up on a silica gel column [elution of DTPP with dichloromethane/ethyl acetate (9+1 v/v)]. The eluate was concentrated to dryness. The residue was dissolved in bi-distilled water/acetonitrile and DTPP was quantified by HPLC with UV-detector.

4.2 LIQUID-LIQUID PARTITION

After fortification of 1000 mL of surface water, 1 mL of sodium hydroxide (1 molar) was added and the sample was transferred into a 1-L separatory funnel. The sample solution was successively shaken with 100 mL and 50 mL of dichloromethane. The organic phases were dried over anhydrous sodium sulfate and the filter cake was washed with 20-30 mL of dichloromethane. The filtrate was rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 10 mL of dichloromethane.

4.3 CLEAN-UP ON SILICA GEL

The dissolved residue was added to the column and allowed to percolate into the packing. The round-bottom flask, that contained the sample solution, was rinsed with 10 mL of dichloromethane and the rinsing was added on the packing as well. The latter was rinsed with 10 mL of dichloromethane/ethyl acetate (7+3 v/v). All eluates were discarded. Afterwards, DTPU was eluted with 20 mL of dichloromethane/ethyl acetate (1+1 v/v) and rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 2.0 mL of bi-distilled water/acetonitrile (7+3 v/v) using an ultrasonic bath. Before quantification by HPLC, the sample was filtered through a 0.45-µm microfilter.

4.4 LIQUID-LIQUID PARTITION AND DERIVATIZATION

The aqueous phase remaining after the first liquid-liquid partition (see above) was acidified with 5 mL of hydrochloric acid (32%) and successively shaken with 100 mL and 50 mL of dichloromethane. The organic phases were collected in a 250-mL round-bottom flask and rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 1 mL of acetonitrile. After adding 5 mL of bi-distilled water and 5 mL of sodium hydroxide (1 molar), the sample solution was shaken for 10 min at 150 min⁻¹. Afterwards, 100 mL of bi-distilled water and 5 mL of hydrochloric acid were added. The sample solution was transferred into a 250-mL separatory funnel and extracted twice with 50-mL portions of dichloromethane. The organic phases were filtered over anhydrous sodium sulfate and the combined filtrates were evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 10 mL of dichloromethane.

4.5 CLEAN-UP ON SILICA GEL

The dissolved residue was added to the column and allowed to percolate into the packing. The round-bottom flask, that contained the sample solution, was rinsed with 10 mL of dichloromethane and the rinsing was added onto the packing as well. The latter was rinsed with 10 mL of dichloromethane/ethyl acetate (95+5 v/v). All eluates were discarded. Afterwards, DTPP was eluted with 20 mL of dichloromethane/ethyl acetate (9+1 v/v). The eluate was rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 2.5 mL of bi-distilled water/acetonitrile (7+3 v/v) using an ultrasonic bath. Before quantification by HPLC, the sample was filtered through a 0.45-µm microfilter.

4.6 HPLC CONDITIONS

Operating Conditions:

Apparatus:	pump: Merck-Hitachi L-6200
	UV-detector: Merck-Hitachi L-4000
	sampling unit: Merck-Hitachi AS-2000
	integrator: Merck-Hitachi D-2500
Column:	Zorbax Phenyl (SB); 5 µm; 250 mm x 4.6 mm
Eluent:	DTPP: 0.05% phosphoric acid/acetonitrile (80+20 v/v)
	DTPU: 0.05% phosphoric acid/acetonitrile (75+25 v/v)
Flow:	1.0 ml/min
Column temperature:	Room temperature
Injection volume:	100 µL
Wavelength:	DTPP: 261 nm
	DTPU: 254 nm
Retention time:	DTPP: 25.6-27.5 min
	DTPU: 25.1-27.7 min

4.7 EVALUATION OF RESULTS

Concentration of Residues

Injected samples were quantified by peak height or area with reference to the calibration curve. The latter was obtained by correlation of the peak height or area (in counts) of the analytical standards with their corresponding concentration in µg/mL. The correlation is performed using the power regression (equation 1).

$$Y = a \cdot x^b \quad (1)$$

where

- Y = Peak height or area of injected sample [counts]
- x = Concentration of SL-160 (as DTPP) or DTPU (µg/mL)
- a = y-axis intercept
- b = slope

The residue of SL-160 and DTPU in the samples is calculated according to equations 2 and 3.

SL-160:

$$R_{SL-160} = \frac{X \cdot V_F \cdot F}{V_S} \quad (2)$$

DTPU:

$$R_{DTPU} = \frac{X \cdot V_F}{V_S} \quad (3)$$

where

- R = Residue of SL-160 (as DTPP) or DTPU in sample material [µg/L]
- X = Concentration of injected sample [µg/mL] calculated from equation 1
- V_F = Final volume [mL]
- F = Conversion factor DTPP → SL-160 = 1.357*
- V = Volume of sample [L]

The recovery of SL-160 and DTPU in the samples is calculated according to equation 4.

$$Rec = \frac{R \cdot 100}{F} \quad (4)$$

where

- Rec = Recovery of SL-160 and DTPU [%]
- R = Residue of SL-160 or DTPU in sample material [µg/L]
- F = Fortification level [µg/L]

* Conversion factor = molecular weight SL-160 / molecular weight DTPP = 407.3 / 300.2 = 1.357

5 ANALYTICAL METHOD FOR TPSA

The analytical method described below was developed in pretests which were not performed in compliance with the GLP guidelines and which were not described in this report. The raw data will be archived under this project.

5.1 ABSTRACT

The water sample was extracted with ethyl acetate. The sample was further extracted by liquid-liquid partition followed by a clean-up step on a silica gel column [elution with dichloromethane/ethyl acetate (95+5 v/v)]. The eluate was concentrated to dryness. The residue was dissolved in bi-distilled water and determined by HPLC with UV-detector.

5.2 LIQUID-LIQUID PARTITION

500 mL of surface water was placed into a 1-L bottle. After fortification, 200 mL of ethyl acetate and 180 g of sodium chloride was added. This sample mixture was shaken for 10-15 min at 120 min⁻¹ and then transferred into a 1-L separatory funnel. After phase separation, the lower aqueous phase was transferred back into the 1-L bottle. The organic phase was filtered over anhydrous sodium sulfate into a 500-ml round-bottom flask. Afterwards, 200 mL of ethyl acetate was added to the aqueous phase and the sample mixture was shaken as described above. After phase separation, the aqueous phase was discarded and the organic phase was filtered over anhydrous sodium sulfate into the same 500-ml round-bottom flask. The filter cake was washed with 20-30 ml ethyl acetate and the filtrate was rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. The dried residue was dissolved in 10 mL of toluene.

5.3 CLEAN-UP ON SILICA GEL

The dissolved residue was added to the column and allowed to percolate into the packing. The round-bottom flask, that contained the sample solution, was rinsed with 10 mL of toluene and the rinsing was added on the packing as well. The latter was rinsed with 5 mL of dichloromethane. All eluates were discarded. Afterwards, TPSA was eluted with 20 mL of dichloromethane/ethyl acetate (95+5 v/v). The eluate was rotary-evaporated to dryness at about 40 °C. Any residual solvent was removed in a stream of nitrogen. Finally, the dried residue was dissolved in 2.0 mL of bi-distilled water using an ultrasonic bath. Before quantification by HPLC, the sample was filtered through a 0.45-µm microfilter.

5.4 HPLC CONDITIONS

Operating Conditions:

Apparatus:	pump:	Merck-Hitachi L-6200
	UV-detector:	Merck-Hitachi L-4000
	sampling unit:	Merck-Hitachi AS-2000
	integrator:	Merck-Hitachi D-2500
Column:	Zorbax Phenyl (SB); 5 μ m; 250 mm x 4.6 mm	
Eluent:	0.05% phosphoric acid/acetonitrile (85+15 v/v)	
Flow:	1.0 ml/min	
Column temperature:	Room temperature	
Injection volume:	100 μ L	
Wavelength:	254 nm	
Retention time:	13.1-13.9 min	

5.5 EVALUATION OF RESULTS

Concentration of Residues

Injected samples were quantified by peak height with reference to the calibration curve. The latter was obtained by correlation of the peak height (in counts) of the analytical standards with their corresponding concentration in $\mu\text{g/mL}$. The correlation is performed using the linear regression (equation 5).

$$Y = a + bx \quad (5)$$

where

- Y = Peak height of injected sample [counts]
- x = Concentration of TPSA ($\mu\text{g/mL}$)
- a = y-axis intercept
- b = slope

The residue of TPSA in the samples is calculated according to equation 6.

$$R_{TPSA} = \frac{X \cdot V_F}{V_S} \quad (6)$$

where

- R = Residue of TPSA in sample material [$\mu\text{g/L}$]
- X = Concentration of injected sample [$\mu\text{g/mL}$] calculated from equation 5
- V_F = Final volume [mL]
- V_S = Volume of sample [L]

The recovery of TPSA in the samples is calculated according to equation 7.

$$Rec = \frac{R \cdot 100}{F} \quad (7)$$

where

- Rec = Recovery of TPSA [%]
- R = Residue of TPSA in sample material [$\mu\text{g/L}$]
- F = Fortification level [$\mu\text{g/L}$]