1. INTRODUCTION

Background and Objective:

The objective of this study was to develop and to validate an analytical method for the determination of dimethenamid-P and its metabolites M23, M27 and M31 in surface and drinking water with a target limit of quantitation (LOQ) of 0.03 µg/L per analyte.

2. EXPERIMENTAL

2.1 Test System

Drinking (Tap) Water

Water was collected from a PTRL Europe laboratory tap located in Ulm, in Southern Germany. The appearance of the water was clear and without any odor. The water was characterized for physical and chemical properties as follows: pH 7.72, total water hardness: 2.33 mmol/L (Deutsche Härtegrade, 13.0°d) by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods, non-GLP).

Surface (River) Water

Water was collected on 04-Oct-12 from the Brenz River in Herbrechtingen, located in Southern Germany. The water was characterized for physical and chemical properties by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

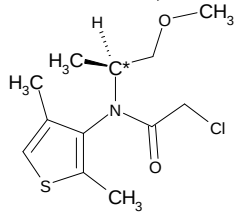
Total water hardness:	1.26 mmol/L (Deutsche Härtegrade, 7.1°d)
TOC (total organic carbon, EN 1484:1997):	1.30 mg/L
DOC (diluted organic carbon, EN 1484: 1997):	1.0 mg/L
pH (DIN 38 404-C5):	8.09

Water was stored at room temperature in the dark when not used.

2.2 Analytical Test and Reference Item

The following standards provided by the sponsor (see Appendix 1) were used as test / reference items:

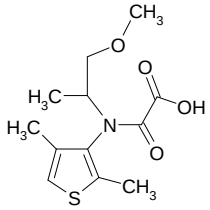
Dimethenamid-P (BAS 656 H, Reg. No. 363851)



IUPAC name:(S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide

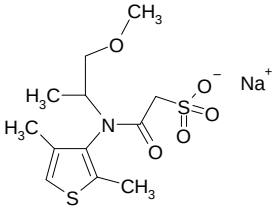
Empirical formula: $C_{12}H_{18}ClNO_2S$ Molar mass: 275.8 g/mol

Dimethenamid-P Metabolite M23 (Reg. No. 360715)



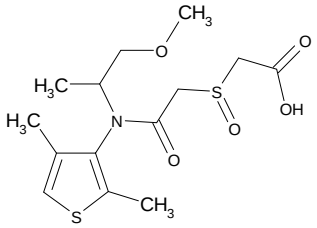
IUPAC name:*N*-(2,4-dimethyl-thiophen-3-yl)-*N*-(2-methoxy-1-methyl-ethyl)-oxalamic acid
Empirical formula: $C_{12}H_{17}NO_4S$ Molar mass: 271.3 g/mol

Dimethenamid-P Metabolite M27 (Reg. No. 360714)



IUPAC name:Sodium [(2,4-dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonate
Empirical formula: $C_{12}H_{18}NO_5S_2$ Na Molar mass: 343.4 g/mol

Dimethenamid-P Metabolite M31 (Reg. No. 360712)



IUPAC name:[[(2,4-Dimethyl-thiophen-3-yl)-(2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfinyl]-acetic acid
Empirical formula: $C_{14}H_{21}NO_5S_2$ Molar mass: 347.5 g/mol

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

XP205DR balance, Mettler Toledo.
Transsonic 460 bath, Elma Hans Schmidbauer.
Vortex mixer REAX top, Heidolph.
Typical glassware and laboratory equipment.
All 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: Agilent Zorbax SB-C18, 5 µm particle size, 150 mm length, 4.6 mm i.d.
Pre-column: Phenomenex C_{18}, 4 mm length, 3.0 mm i.d.

Applied Biosystems MDS Sciex API 5500 triple quadrupole LC-MS/MS system with Turbo IonSpray ESI source. Analyst 1.5.2 Instrument control and data acquisition software.

2.3.2 Solvents and Chemicals

Millipore Water (PTRL Europe)
Methanol, HPLC Grade (≥ 99.9 %), Promochem.
Formic acid (98-100%), Sigma Aldrich.

2.3.3 Preparation of Standard Solutions

Stock solutions of dimethenamid-P and its metabolites M23, M27 and M31 were prepared in methanol, such as described in the following table (e.g.):

Substance name	Weight [mg]	Dissolve in [mL]	Obtain [mg/mL] (*)
Dimethenamid-P (purity 96.4 %)	11.92	11.50	1.0
M23 (purity 98.4 %)	10.21	10.05	1.0
M27 (purity 97.4 %)	10.33	10.06	1.0
M31 (purity 98.7 %)	10.15	10	1.0

(*): Purity taken into account.

Fortification solutions containing all analytes with concentrations of 10 and 1.0 µg/mL (intermediate solutions), 0.015 µg/mL and 0.0015 µg/mL were prepared in methanol, by accurate dilution of the stock solutions. Calibration solutions containing all analytes were prepared by volumetric dilution in water containing 0.1% formic acid to obtain concentrations ranging from 0.009 ng/mL to
10 µg/mL.
Calibration solutions in extracts from blank control samples used to demonstrate matrix effect were prepared by accurate dilution of a selected calibration solution in solvent to obtain the concentration of 0.10 ng/mL.

All standard solutions were stored refrigerated in amber glass bottles when not in use.

2.3.4 Stability of Standard Solutions and Extracts

Stability of solutions is proven by comparing two calibration solutions diluted from an old and a freshly prepared stock solution (refrigerated storage for five days; see Table 3).

Deviation was < 20 %.

Stability in extracts is demonstrated by re-injection of selected samples after about 4 days of refrigerated storage as shown in Table 4. As the recoveries were all still within the acceptable range of 70-120 % of the primary concentration, stability was considered sufficiently proven.

2.3.5 Sample Analysis

1. Place 1.0 mL of drinking or surface water containing 0.1% of formic acid into an autosampler vial.
2. Fortify the water sample, with 0.020 mL of 1.5 ng/mL or with 0.020 mL of 15 ng/mL fortification solution to obtain the LOQ (0.03 µg/L) and higher level (0.30 µg/L), respectively.
3. Vortex the sample.
4. Analyse the sample by LC-MS/MS.

2.4 LC-MS/MS Analysis

Specimens 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	Agilent Zorbax SB-C_{18} column: Length: 150 mm, i.d.: 4.6 mm, particle size: 5 µm, oven temp.: 40°C.
LC Injection Volume	100 µL.
LC Method	Solvent A: Water containing 0.1 % formic acid Solvent B: Methanol containing 0.1 % formic acid Program: Time (min) / Flow rate (mL/min) / % A / % B 0.00 / 1.0 / 80 / 20 5.00 / 1.0 / 20 / 80 10.00 / 1.0 / 20 / 80 10.10 / 1.0 / 80 / 20 15.00 / 1.0 / 80 / 20
Retention time	≈ 6.5 min for dimethenamid-P, ≈ 5.4 min for M23, ≈ 5.1 min for M27, ≈ 5.2 min for M31
MS/MS System	Applied Biosystems MDS Sciex API 5500 triple quadrupole LC-MS/MS system with TurboIonspray (ESI) source.
Ion Source Conditions ESI Positive or Negative Polarity	Source temperature: 550°C Gas supply (GS 1): 40 (arbitrary units) Gas supply (GS 2) 70 (arbitrary units) Curtain gas: 30 (arbitrary units) CAD gas: Medium Entrance potential: -10 V / 10 V IonSpray voltage: -4500 V / 4500 V Resolution: Q1: Unit, Q3: Unit

MS/MS Conditions	Dimethenamid-P (positive ion mode)	
	MS/MS transition for quantification:	276 *m/z* > 244 *m/z*
	Collision energy (CE):	19 V
	Cell exit potential (CXP):	22 V
	Dwell time:	500 ms
	Declustering potential (DP):	111 V
	MS/MS transition for confirmation:	276 *m/z* > 168 *m/z*
	Collision energy (CE):	33 V
	Cell exit potential (CXP):	18 V
	Dwell time:	500 ms
	Declustering potential (DP):	111 V
	M23 (negative ion mode)	
	MS/MS transition for quantification:	270 *m/z* > 198 *m/z*
	Collision energy (CE):	-14 V
	Cell exit potential (CXP):	-21 V
	Dwell time:	150 ms
	Declustering potential (DP):	-70 V
	MS/MS transition for confirmation:	270 *m/z* > 166 *m/z*
	Collision energy (CE):	-22 V
	Cell exit potential (CXP):	-7 V
	Dwell time:	250 ms
	Declustering potential (DP):	-70 V
	M27 (negative ion mode)	
	MS/MS transition for quantification:	320.1 *m/z* > 121 *m/z*
	Collision energy (CE):	-30 V
	Cell exit potential (CXP):	-11 V
	Dwell time:	150 ms
	Declustering potential (DP):	-205 V
	MS/MS transition for confirmation:	320.1 *m/z* > 80 *m/z*
	Collision energy (CE):	-62 V
	Cell exit potential (CXP):	-11 V
	Dwell time:	200 ms
	Declustering potential (DP):	-205 V
	M31 (negative ion mode)	
	MS/MS transition for quantification:	346 *m/z* > 240 *m/z*
	Collision energy (CE):	-16 V
	Cell exit potential (CXP):	-13 V
	Dwell time:	250 ms
	Declustering potential (DP):	-80 V
	MS/MS transition for confirmation:	346 *m/z* > 198 *m/z*
	Collision energy (CE):	-28 V
	Cell exit potential (CXP):	-13 V
	Dwell time:	250 ms
	Declustering potential (DP):	-80 V

See Figure 41 to Figure 44 for the product ion spectra of dimethenamid-P and its metabolites M23, M27 and M31.

The quantitative determination was carried out by external standardization using calibration standards in solvent. Calibration functions ranging from 0.009 to 0.40 or 1.0 ng/mL (≥ 5 levels) were used to evaluate the final sample volumes (exemplified in Figure 1 to Figure 8). For evaluation of the stability in solutions and extracts a calibration function ranging from 0.009 to 1.0 with 3 levels each injected in duplicate was used.

Representative LC-MS/MS ion chromatograms of calibration solutions in solvent and of final sample volumes of fortified and control specimens are presented in Figure 9 to Figure 40.

2.5 Calculations

Recovery results derived from LC-MS/MS analysis and calculations are shown in details in Table 1 to Table 2.

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

$R = c_{End}$

R: Analyte residue in µg/L.

c_{End}: Concentration of analyte in final sample volume, in ng/mL.
(where multiple injections were evaluated: mean).

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

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

The calculation is exemplified with the drinking water specimen P2711-31 fortified at 0.30 µg/L (10xLOQ). The final sample volume was examined by LC-MS/MS in run file P2711#021 to give a final concentration C_{End} of 0.31 ng/mL for 320 *m/z* -> 121 *m/z*. The following calculation is demonstrated for the fragment ion 121 m/z:

Thus:

$R = c_{End}$
$= 0.31$ ng/mL or µg/L

$Rec. = (R / R_{fortified}) \times 100\ \%$
$= (0.31\ µg/L / 0.31\ µg/L) \times 100\ \% = 103\ \%$

Calculations were performed with full precision by computer software (Excel). Thus slight discrepancies may arise when recalculated using a pocket calculator.