Appendix 5.4: Technical procedure (non-GLP) (continued)

Introduction

1.1 Scope of the Method

Determination of BAS 351 H (Bentazon) in water is currently archieved by method 423/0 [1], using GC-MS. The present method L0141/01 applies LC-MS/MS, which is also accepted as confirmatory technique.
BAS 351 H is a herbicide typically used in corn, cereals, potatoes, beans, and peas. For registration of the compound and for monitoring purposes a residue analytical method for the active ingredient BAS 351 H in water with a limit of quantitation of 0.03 µg/kg is needed.
The described method L0141/01 allows the determination of BAS 351 H with the required limit of quantitation in surface water and groundwater.
This method was developed at BASF SE, Agricultural Center Limburgerhof, Germany.

1.2 Principle of the Method

A 10 g aliquot of the water sample is adjusted to pH 2 and extracted by SPE. The analyte is eluted with methanol. After evaporation to dryness the residues are dissolved in water/methanol (50 + 50, v + v). An aliquot of the final volume is measured using LC-MS/MS.

The method has a limit of quantitation of 0.03 µg/kg in water.

1.3 Specificity

BAS 351 H is identified and quantified as individual compound.

1.4 Safety

(1) Normal laboratory precautions are sufficient for safe handling of BAS 351 H.
(2) Methanol is flammable and should not be used near heat, sparks or open flames. Methanol is toxic. Formic and hydrochloric acid are corrosive and irritating.
(3) All solvents should be used only in well ventilated laboratories.
(4) Protective glasses and clothing should be worn during all laboratory procedures.
(5) Disposal of samples and standards must be done in compliance with on-site safety policies and procedures.

Appendix 5.4: Technical procedure (non-GLP) (continued)

2 TEST AND REFERENCE ITEMS

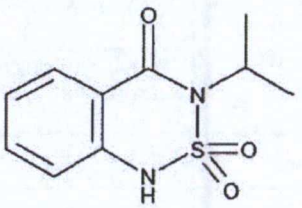
2.1 Test Items

2.1.1 BAS 351 H

Reg-No.: 51929

Chemical name: 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide

Structural formula:



Empirical formula: $C_{10}H_{12}O_3N_2S$

Molecular weight: 240.28 g/mol

Storage: at room temperature (+25°C) or cooler

2.2 Reference Items

Compound described in chapter 2.1 was used.

2.3 Stability of Calibration Solutions and Residues in Water

Standard solutions are kept refrigerated at 4°C. Standard stability of BAS 351 H is tested in the study 334544.

Note: Materials, chemicals and equipment specified below were used for method development. They are specified as examples only and may be substituted with supplies of similar specifications. If the use of supplies other than those stated is intended, applicability to this method must be confirmed prior to method validation and/or routine analysis.

Appendix 5.4: Technical procedure (non-GLP) (continued)

3 MATERIALS AND METHODS

3.1 Equipment for Extraction and Sample Clean-up

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Balance	Top load, PM 4800	Mettler (Germany)	
Balance	Analytical, AT261 Delta Range	Mettler (Germany)	
pH meter	Type 530, series 500	Knick (Germany)	
Processing Station	VacMaster Sample	International Sorbent Technology	
SPE columns	Bond Elut-ENV 200 mg, 3 mL	Varian	12105015
SPE column dryer (N_2)	see Attachment 1		
Evaporator	TurboVapLV Evaporator	Zymark	
Vacuum pump/ controller	CVC 2	Vacuubrand (Germany)	
Beaker	50 mL		
Culture tubes (with screwing tops)	10 mL		
Volumetric pipets and tips	Various sizes, 25 – 1000 µL	Microman Abimed	
Pasteur pipets	L =150 mm	Fortuna (Germany)	3.525
Amber bottles	15 mL	Sigma-Aldrich/Supelco (Germany)	27003
Teflon®-lined screw caps		Sigma-Aldrich/Supelco (Germany)	27163
Vials/microvials	2 mL, 350 µL		
Vial caps	Teflon®-lined snap-caps		

3.2 Reagents

Note: Equivalent chemicals from other suppliers may be substituted but all chemicals used must be at least of "analytical grade" or must meet equivalent specifications.

3.2.1 Chemicals

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
HCl (hydrochloric acid)	conc., min. 37%	Riedel-de Haen (Germany)	30721
HCOOH (formic acid)	conc.	Merck (Germany)	1.00264
CH_3OH (methanol)	High Purity	Merck (Germany)	1.06011
Ultra pure water, in this method referred to as H_2O	High Purity	prepared with Millipore apparatus Milli-Q plus 185 (in-house system)	Millipore (France)

Appendix 5.4: Technical procedure (non-GLP) (continued)

3.2.2 Solutions and Solvent mixtures

Code	Solvent Mixture
S 1	methanol / water (50 + 50, v + v)
S 2	6 mol/L hydrochlorid acid, prepared with H_2O and conc. HCl
S 3	water pH 2.0, prepared with H_2O and 6 mol/L HCl
S 4	water pH 2.0, prepared with H_2O and conc. HCOOH
HPLC eluents	mobile phase A: H_2O + HCOOH (1000 + 1, v + v) mobile phase B: CH_3OH + HCOOH (1000 + 1, v + v)

3.2.3 Solutions for fortification purposes

Stock solution for fortifications
Prepare a 1.0 mg/mL stock solution of BAS 351 H in methanol.

Diluted standard solutions for fortifications
Prepare a standard solution containing 100 ng/mL of Bentazon by appropriately diluting the corresponding stock solution with **S 1**. Suggested concentrations of standard solutions are 10 ng/mL (for 0.03 µg/kg spiking) and 100 ng/mL (for 0.3 µg/kg spiking).

3.2.4 Standard solutions for calibration

Starting from the 100 ng/mL solution described under 3.2.3 working solutions are prepared by dilution with **S 1** as needed.
Suggested concentrations of standards for calibration are 0.025, 0.05, 0.10, 0.15, 0.25, 0.5, and 1.0 ng/mL. If required, other concentration schemes, and different or additional standard concentrations may be used.

4 Analytical Procedure

4.1 Sample Storage

Samples are not filtered in order to include analytes sorbed to floating particles and to avoid losses due to filter sorption. Until analysis, water samples are stored in clean amber glass bottles in a refrigerator at ca. +4 °C or in plastic bottles ca. -20°C, respectively.

4.2 Sample Preparation and Fortification

10 g of untreated water samples are weighed into a beaker/bottle. 30 µL of the spiking solution with analyte concentrations of 10 or 100 ng/mL is added to the samples. The correlation between the concentration of the spiking solution and the resulting final analyte concentration in the sample is shown below:

Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
10 g	0.00 ng/mL	30 µL	0.00 µg/kg*
10 g	10 ng/mL	30 µL	0.03 µg/kg **
10 g	100 ng/mL	30 µL	0.3 µg/kg

* control sample
** proposed limit of quantification

Appendix 5.4: Technical procedure (non-GLP) (continued)

4.3 Extraction of Sample Material

4.3.1 SPE Column Conditioning

Mount the Bond Elut-ENV columns onto the Processing Station and rinse the columns with 3 x 3 mL methanol and 3 x 3 mL with **S 3**.
Keep care that the columns do not run dry. Discard the wash solutions.

4.3.2 Water Extraction

Weigh 10 g of the water sample into a beaker/bottle. For the fortification samples, add the appropriate amount of standard spiking solution. Adjust sample to pH = 2 with 30 µL of **S 2**. Pour the water sample onto the preconditioned SPE column. Percolate the whole water sample through the SPE column (suported by vacuum if necessary). Considering a flowrate of about 1 mL/min and the amount of water sample the column extraction takes about 10 min.
Rinse beaker and add 3 mL **S 4** to the SPE column. Percolate the liquid through the column (suported by vacuum if necessary).

4.3.3 SPE-Column Drying

Air dry the column for 5 min under a vacuum (400 mbar). After that mount the column onto the SPE column dryer. Dry the column with a stream of N_2 at a temperature of 40 °C for 30-45 min.

4.3.4 SPE-Column Elution

Provide the sample collector rack of the Processing Station with culture tubes. Mount the dry SPE columns from section 4.3.3 onto the Processing Station. Elute the analytes from the column using 3 x 3 mL methanol.

4.3.5 Preconcentration and Preparation for LC-MS/MS Quantitation

The collected eluates are evaporated to dryness in the evaporator at 40 °C water bath temperature. The residues are dissolved with an appropriate volume of **S 1** (= final volume, e.g. 2 mL for concentrations at LOQ, see also below).

4.4 Quantitation

From the final volume V_{End} an aliquot of 50 µL is injected into the LC-MS/MS instrument for quantitation.

The LC system is coupled to a triple quadrupole mass spectrometer operated in MS/MS mode. The instrument is equipped with an ESI interface.

Appendix 5.4: Technical procedure (non-GLP) (continued)

> **Note:** It is advisable to verify the retention time and the sensitivity of the analyte on the chromatography system prior to each analytical series. For this, appropriate standard solutions can be injected into the chromatography system to verify peak retention time, resolution, and sensitivity of the reference substance and show the stability of the system. The retention time depends strongly on type and dimensions of the chromatography system.
> The equipment and the conditions listed were used for the test of the method in water. They may be substituted, however, by comparable ones, if the applicability was proven before.

4.4.1 Chromatographic Conditions

LC system:	Agilent 1100 LC Binary Pump
Autosampler:	CTC PAL
Injection volume:	50 µL
LC column:	Betasil C18, 100 x 2.1 mm ID, 5 µm
Column temperature:	RT
Mobile phase:	Solvent A – Water/formic acid, (1000/1, v/v) Solvent B – Methanol/formic acid, (1000/1, v/v)

Gradient:

Time (min)	Composition (% A)	(%B)
0	50	50
2.5	35	65
4.0	35	65
4.1	0	100
6.0	0	100
6.1	50	50
9.0	50	50

Flow rate:	0.5 mL/min	
Retention times:	BAS 351 H (51929):	approx. 2.6 min
Run time:		approx. 9.0 min

4.4.2 Mass Spectrometric Conditions

Mass spectrometer:	AB Sciex API 3000 triple stage quadrupole	
Interface:	ESI	
Ion mode:	BAS 351 H (51929):	(-) MRM
Transitions:	BAS 351 H (51929):	239 -> 132 and 239 -> 197

Appendix 5.4: Technical procedure (non-GLP) (continued)

4.4.3 Calibration Procedures

Calibration curves are generated by plotting peak area or height versus the concentration of the analytes measured by direct injection of reference standards containing known amounts of BAS 351 H. The linear least squares working curve in the form y = bx + c is used for the construction of the calibration curve.
A typical curve could cover a range from 0.025 to 1 ng/mL. In a given analytical series, the same injection volume is used for all samples and standards.
In a measuring series standards and samples are injected alternately to show the stability of the detection response during the whole series.
For each series, the set should begin and end with standard injections. Each standard level should be injected at least in duplicate.

4.4.4 Determination of Instrumental Recovery with QCSs

Within each analytical series at least one quality control sample is analysed to check for potential matrix effects.
For this purpose, an untreated water sample is extracted as described in chapter 4.3.1 - 4.3.5. For example, 0.5 mL of the final extract is reduced to dryness (water bath, ~40°C). The residual matrix is reconstituted in 0.5 mL of standard solution containing 0.15 ng/mL of each analyte.
The concentration is determined from the calibration curve and related to the nominal concentration of 0.15 ng/mL (equals to LOQ).

5 Calculation of Residues

5.1 Principle

Calculation of results is based on calibration curves recorded within each analytical series. Peak area or peak height is plotted versus the concentration of analyte. The residue of BAS 351 H is calculated from its calibration curve and the equations are shown in section 5.2.

5.2 Equation

The residue (R) in the water sample in µg/kg is calculated as shown in the following equation:

$$R = \frac{V_{End} \times C_B}{S_M}$$

R = Residue in the water sample [µg/kg]
V_{End} = End volume of the extract after all dilution steps [mL]
C_B = Conc. of analyte in the injection volume as read from the calibration curve [ng/mL]
S_M = Weight of water sample extracted [g]

Appendix 5.4: Technical procedure (non-GLP) (continued)

If residue data are to be corrected for loss of analyte during sample extraction and clean-up procedures the residue [R] has to be corrected with the results of the procedural recoveries as shown in the following equation:

$R_{RC} = R \times R_{FE}$

R_{RC} = Residue concentration of the analyte in the sample corrected with the procedural recovery of the analyte in fortification experiments [µg/kg sample material]

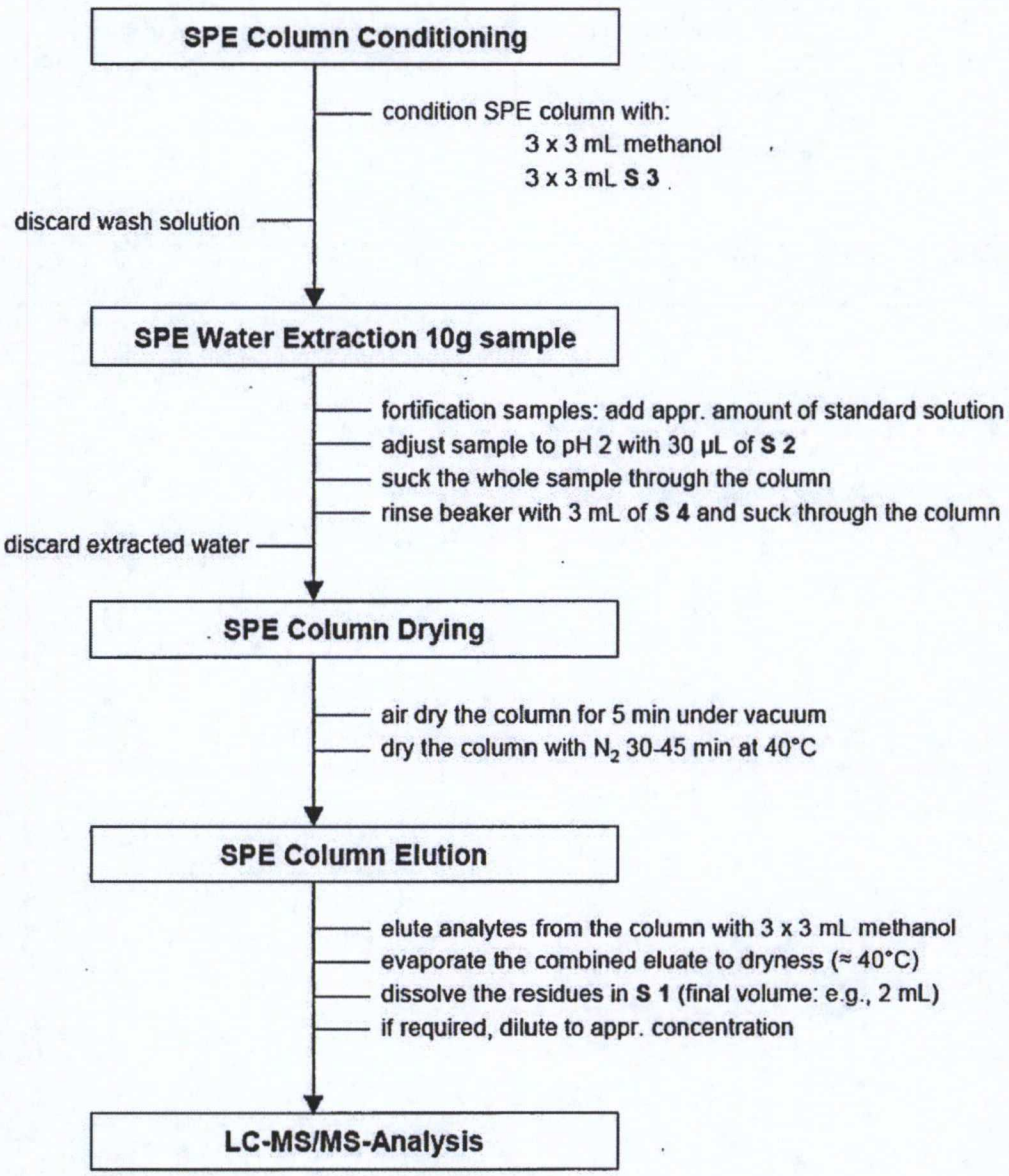
R_{FE} = Procedural recovery of the analyte as determined from fortification experiments performed in parallel to the sample analysis

$$= \frac{100\ \%\ (\text{level of fortification})}{\%\ \text{recovery}}$$

Note: For routine analysis requirements residue data should not be corrected for procedural recoveries. Results of fortification experiments should be listed individually.

Appendix 5.4: Technical procedure (non-GLP) (continued)

6 Flow Chart of Method L0141/01



Appendix 5.4: Technical procedure (non-GLP) (continued)

7 Recoveries, Chromatograms, and Calibration Curves

Recovery data will be provided in the validation part of the analytical method L0141/01.

8 Limit of Determination (LOQ)

The limit of determination (quantitation) is defined as the lowest fortification level successfully tested. For water, the limit of quantitation is 0.03 µg/kg.

9 Limit of Detection (LOD)

The limit of detection for BAS 351 H is 1.25 pg. It is here defined as the absolute amount of analyte injected into the LC-MS/MS instrument using the lowest standard of the calibration curve.

10 Blank Values

The tested untreated water samples showed no significant interferences at the retention time of the analytes.

11 Confirmatory Techniques

Due to the high specificity of LC-MS/MS an additional confirmatory technique is not necessary.

12 Method Management and Time Requirement

The analysis of one series of samples (= 17 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 1.5 working days (12 hours) per laboratory assistant. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

13 REFERENCES

[1] Keller, W.: Validation of Analytical Method No. 423: Determination of Bentazone Residues in Water. Study Code: 46513. BASF DocID: 1998/10079.