**Appendix 5.5: Technical procedure (non-GLP) (continued)**



# 1 INTRODUCTION

BAS 351 H is a herbicide, which is used in different crops (e.g., corn, cereals, potatoes, beans, peas). For registration and monitoring purposes, a residue analytical method for the active ingredient BAS 351 H and its metabolite N-Methyl-bentazon (BH 351-N-Me; Reg. No. 79520) in soil and sediment with a limit of quantitation of 0.01 mg/kg is needed.
The described method L0136/01 allows the determination of BAS 351 H and BH 351-N-Me with the required limit of quantitation in soil and sediment.
This method was developed at BASF SE, Agricultural Center Limburgerhof, Germany.

# 2 PRINCIPLE OF THE METHOD

A 5 g soil sample is extracted with 50 mL methanol/water (50/50, v/v) by mechanical shaking for 60 min at 225 rpm. A 5 mL aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol/water (50/50, v/v) to the appropriate final volume and measured by Ultra Performance Liquid Chromatography (UPLC)-MS/MS or LC-MS/MS.

The method has a limit of quantitation of 0.01 mg/kg in soil.

# 3 TEST AND REFERENCE ITEMS

## 3.1 Test Items

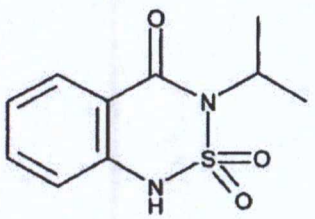
(used for fortifications)

### 3.1.1 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

**Appendix 5.5: Technical procedure (non-GLP) (continued)**

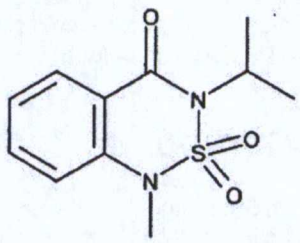


### 3.1.2 BH 351-N-Me (N-Methyl-bentazon)

Reg-No.: 79520

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

Structural formula:



Empirical formula: $C_{11}H_{14}N_2O_3S$

Molecular weight: 254.31 g/mol

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

### 3.2 Reference Items

(used for calibration)

Same items as test items, see 3.1

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



# 4 MATERIALS AND INSTRUMENTS

## 4.1 Equipment for Extraction and Sample Clean-up

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

| Equipment | Size, Description | Manufacturer/ Supplier | Catalog Number |
|---|---|---|---|
| Balance | Top load, LP 5200 P | Sartorius (Germany) | |
| Balance | Analytical, AT261 Delta Range | Mettler (Germany) | |
| Mechanical shaker | SM25 | Edmund Buehler (Germany) | |
| Centrifuge, refrigerated | Eppendorf Centrifuge 5810R | Eppendorf (Germany) | |
| Rotary Evaporator | Laborota 4003 | Heidolph | |
| Vacuum Pump/ Controller | CVC2 | Vacuubrand | |
| Radiator | WK2400 | Lauda | |
| Glass beaker/vial with screw top | 150 mL, | | |
| Test tubes | 125mm*16mm with 15mm screw joint. Volumina=ca.15 mL | | |
| Amber Glass Bottle with screw top | 2500mL | | |
| Cylinders, graduated | 1000 mL, 2000mL | | |
| Glass funnels | 150 mm | | |
| Amber Vials with Teflon®-lined screw caps | 15mL | Sigma-Aldrich/Supelco (Germany) | 27003 |
| Vials/microvials | 2 mL, 350 µL | | |
| Vial caps | Teflon®-lined snap-caps | | |
| Dispenser Fortuna Optifix Basic | 50mL | | |
| Handy Step electronic | | Brand | |
| Microman Pipet | M1000 | Gilson (France) | |
| Microman Pipet | M250 | Gilson (France) | |
| Microman Pipet | M100 | Gilson (France) | |
| Microman Pipet | M50 | Gilson (France) | |
| Capillary + piston | CP1000 | Gilson (France) | |
| Capillary + piston | CP250 | Gilson (France) | |
| Capillary + piston | CP100 | Gilson (France) | |
| Capillary + piston | CP50 | Gilson (France) | |
| Lab spoon | | | |

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



### 4.2 Reagents

#### 3.2.1 Chemicals

**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.

| Chemical | Grade | Manufacturer/ Supplier | Catalog Number |
|---|---|---|---|
| Methanol | High Purity | Merck, Germany | 106,011 |
| Ultra pure Water, in this method referred to as $H_2O$ | High Purity | prepared with Millipore apparatus Milli-Q plus 185 | Millipore (France) |

#### 4.2.2 Solvent Mixtures

| Code | Solvent Mixture |
|---|---|
| S 1 | methanol / water (50/50, v/v) |

### 4.3 Standard Solutions

#### 3.3.1 Standard Solution Storage and Stability

Standard solutions are kept refrigerated at 4°C. Standard stability of BAS 351 H and BH 351-N-Me will be tested in the validation study.

**Note:** Preferably, use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed below. A different concentration scheme may be used and additional standards may be prepared as needed.

#### 3.3.2 Standard Solutions for Fortifications and Calibration

**Stock Solution**
Prepare a 1.0 mg/mL stock solution of each test item in methanol.

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



**Diluted Standard Solutions for Fortifications**

Prepare a solution containing e.g. 100 µg/mL of each test item by pipetting 1.0 mL of the corresponding stock solution into a 10 mL volumetric flask. Dilute to mark with S1. Prepare serial solutions of this solution with S1 as needed. Suggested concentrations of solutions are 10 µg/mL (for 1.0 mg/kg spiking), 1.0 µg/mL (for 0.1 mg/kg spiking) and 0.1 µg/mL (for 0.01 mg/kg spiking), in S1.

**Standard Solutions for Calibration**

Starting from the 1000 µg/mL stock solution described above working and standard solutions are prepared by dilution with S1 as needed. For example, first a mixed standard solution of 5 µg/L BAS 351 H and 100 µg/L BH 351-N-Me is made from 50 µL BAS 351 H stock solution and 1 mL BH 351-N-Me stock solution filled up to 10 mL. This solution is then further diluted to the levels of the calibration curve.
For BAS 351 H, suggested concentrations of standards for calibration are 0.005, 0.010, 0.025, 0.05, 0.1, and 0.25 ng/mL. For BH 351-N-Me, suggested concentrations of standards for calibration are 0.1, 0.2, 0.5, 1, 2, and 5 ng/mL.

## 5 ANALYTICAL PROCEDURE

### 5.1 Sample Preparation and Storage

Prepare homogeneous soil samples. Store samples in a freezer at ~20°C.

### 5.2 Spiking of Samples for Recovery Experiments

5 g of untreated soil samples are weighed into a 150 mL glass vial with screw cap. E.g. 0.5 mL of the spiking solutions with analyte concentrations of e.g. 0.1, 1.0 and 10.0 µg/mL are added to the samples. The correlation between the suggested concentration of the spiking solution and the resulting final analyte concentration in the sample are shown below:

| Sample Weight [g] | Concentration of Spiking Solution [µg/mL] | Volume of Spiking Solution [mL] | Level of Fortification [mg/kg] |
|---|---|---|---|
| 5 | | | 0.00 |
| 5 | 0.1 | 0.5 | 0.01* |
| 5 | 1.0 | 0.5 | 0.1 |
| 5 | 10.0 | 0.5 | 1.0 |

* proposed limit of quantification

### 5.3 Extraction of the Sample Material

A 5 g aliquot ($S_M$) of the soil sample is weighed into a 150 mL glass vial with screw cap and extracted with 50 mL S1 by shaking on a mechanical shaker for 60 min at 225 rpm.
A 5 mL aliquot of the suspension is centrifuged for 5 min at 4000 rpm (20°C). For determinations at the LOQ of BAS 351 H, the extract is diluted 20-fold with S1, while for determination at the LOQ of BH 351-N-Me the extract is not diluted. An aliquot of the final volume is measured using LC-MS/MS.

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



## 5.4 Quantitation

The quantitation of the residues is performed with an UPLC-MS/MS system and alternatively with an HPLC-MS/MS system. Conditions are described below.

For analysis at the limit of quantitation of the method (0.01 mg/kg) a final volume (=$V_{End}$) of 50 mL and 1000 mL should be used for BH 351-N-Me and BAS 351 H, respectively. In case of higher residues dilute with appropriate amounts of S1.

### 3.4.1 UPLC-MS/MS instrument

#### 5.4.1.1 Chromatographic Conditions

| | |
|---|---|
| LC system: | ACQUITY Binary Solvent Manager (Waters) |
| Autosampler: | ACQUITY Sample Manager (Waters) |
| Injection volume: | 5.0 µL |
| LC column: | Acquity BEH C18 50x2.1mm 1.7um |
| Column temperature: | RT |
| Mobile phase: | Solvent A – Water/formic acid, (1000/1, v/v)<br>Solvent B – Acetonitrile/formic acid, (1000/1, v/v) |

Gradient:

| Time (min) | Composition (% A) | (%B) |
|---|---|---|
| 0 | 60 | 40 |
| 0.2 | 60 | 55 |
| 0.9 | 45 | 55 |
| 0.91 | 0 | 100 |
| 1.3 | 0 | 100 |
| 1.31 | 60 | 40 |
| 1.7 | 60 | 40 |

| | | |
|---|---|---|
| Flow rate: | 0.8 mL/min | |
| Retention times: | BAS 351 H (51929): | approx. 0.49 min |
| | BH 351-N-Me (79520): | approx. 0.85 min |
| Run time: | approx. 2.05 min | |

#### 5.4.1.2 Mass Spectrometric Conditions

| | | |
|---|---|---|
| Mass spectrometer: | AB Sciex API 5000 triple stage quadrupole | |
| Interface: | ESI | |
| Ion mode: | BAS 351 H (51929): | (-) MRM |
| | BH 351-N-Me (79520): | (+) MRM |
| Transitions: | BAS 351 H (51929): | 239 -> 132 and 239 -> 197 |
| | BH 351-N-Me (79520): | 255 -> 134 and 255 -> 213 |

## Appendix 5.5: Technical procedure (non-GLP) (continued)



### 3.4.2 HPLC-MS/MS instrument

#### 5.4.2.1 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)<br>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. 2.7 min |
| | BH 351-N-Me (79520): | approx. 3.7 min |
| Run time: | approx. 9.0 min | |

#### 5.4.2.2 Mass Spectrometric Conditions

| | | |
|---|---|---|
| Mass spectrometer: | AB Sciex API 3000 triple stage quadrupole | |
| Interface: | ESI | |
| Ion mode: | BAS 351 H (51929): | (-) MRM |
| | BH 351-N-Me (79520): | (+) MRM |
| Transitions: | BAS 351 H (51929): | 239 -> 132 and 239 -> 197 |
| | BH 351-N-Me (79520): | 255 -> 134 and 255 -> 213 |

**Note:** The equipment listed above may be substituted by instruments with similar specifications. Columns with equivalent stationary phases and similar specifications may be available from other sources. If the use of material with specifications other than those stated is intended, applicability of the new equipment for this method must be confirmed.

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



### 3.4.3 Calibration Procedures

Calibration curves are generated by plotting peak area or height versus the amount of the analytes measured by direct injection of reference standards containing known amounts of the analytes. 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 0.005 to 0.25 ng/mL for BAS 351 H, and 0.1 to 5 ng/mL for BH 351-N-Me. 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.

### 3.4.4 Determination of Instrument Recovery

Within each analytical series one or two quality control samples are analysed to check for potential matrix effects.
For this purpose an untreated soil sample is extracted as described in chapter 5.3. Due to the different $V_{End}$ of the analytes, instrument recoveries should then be prepared separately for each analyte as follows:

- For BAS 351 H, 100 µL of the 0.5 ng/mL BAS 351 H standard solution and 850 µL of S1 are added to 50 µL of the extract of the untreated soil sample. This results in a concentration of 0.05 ng/mL in the instrument recovery sample.
- For BH 351-N-Me, 0.5 mL of the extract of the untreated soil sample are reduced to dryness and reconstituted in 0.5 mL of 1 ng/mL BH 351-N-Me standard solution.

The concentration is determined from the calibration curve and related to the nominal concentration of 0.05 and 1.0 ng/mL for BAS 351 H and BH 351-N-Me, respectively.

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



# 6 CALCULATION OF RESULTS

## 6.1 Principle

Calculation of results is based on calibration curves recorded within each analytical series. Peak area or peak height is plotted versus the amount of analyte. The residue of BAS 351 H and its metabolite BH 351-N-Me are calculated from its calibration curve and the equations are shown in section 6.2.

## 6.2 Calculation of Residues

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

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

R = Residue in the soil sample [mg/kg]
$V_{End}$ = End volume of the extract after all dilution steps [mL]
$C_B$ = Concentration of analyte in the injection volume as read from the calibration curve [ng/mL]
F = Conversion factor from moist soil to dry soil

$$\left[ \frac{100\ \%}{\text{dry content of the soil } \%} \right]$$

$S_M$ = Weight of the soil sample extracted [g]

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 II:

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

$R_{RC}$ = Residue concentration of the analyte in the sample corrected with the procedural recovery of the analyte in fortification experiments [mg/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.

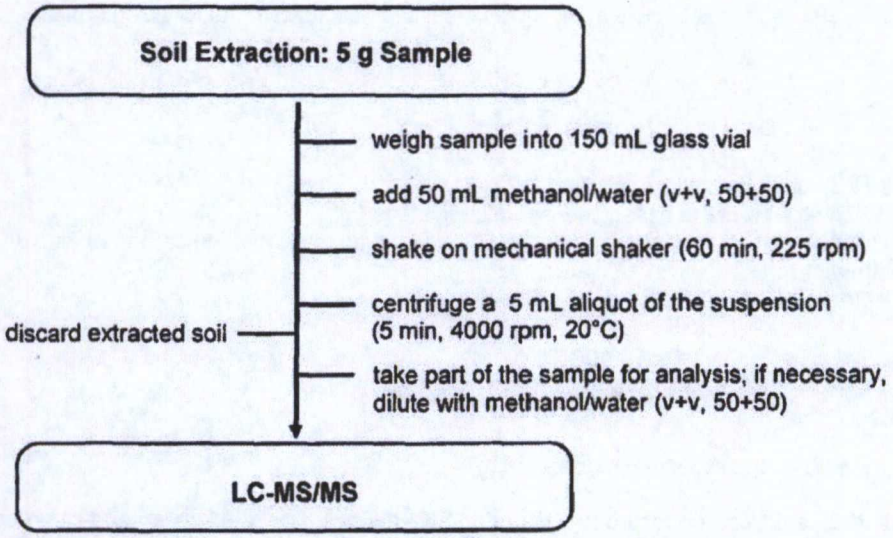
**Appendix 5.5: Technical procedure (non-GLP) (continued)**



### 6.3 Determination of Water Content in Soil

Since the field-moist samples are used for analysis and the residues should be calculated referring to dry soil, it is necessary to determine the respective water content of the samples. Therefore, aliquots of the moist soil samples are dried at about 105°C to constant weight and the water loss is determined by weighing. Alternatively, a moisture analyser can also be used.

## 7 FLOW CHART OF METHOD L0136/01




## 8 RECOVERIES

Recovery data will be provided in the validation part of the analytical method L0136/01.

## 9 LIMIT OF DETERMINATION (LOQ)

The limit of quantitation (LOQ) is defined as the lowest fortification level successfully tested. For soil and sediment, the LOQ is 0.01 mg/kg.

## 10 LIMIT OF DETECTION (LOD)

The limit of detection for BAS 351 H and BH 351-N-Me is 0.25 pg and 5.0 pg, respectively. 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. For a soil sample analysed by the present method, this would equal to a concentration of 0.001 mg/kg (10% of LOQ) for BAS 351 H and BH 351-N-Me.

**Appendix 5.5: Technical procedure (non-GLP) (continued)**



## 11 BLANK VALUES

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

## 12 TYPICAL CHROMATOGRAMS AND CALIBRATION CURVES

Typical chromatograms and calibration curves are given in the validation report.

## 13 CONFIRMATORY TECHNIQUE

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

## 14 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.

## 15 PROBLEMS, SAFETY AND HEALTH CONSIDERATIONS

No potential problems were observed so far. All procedures involving organic solvents should be performed under a well-ventilated hood. Personal protective equipment (gloves, lab coats) should be worn while performing this method. Heed all label statements and precautions.