2. BACKGROUND

BYI 08330 is an insecticide currently being developed by Bayer CropScience with potential uses in several crops including vegetables and orchards.

The purpose of this study was to demonstrate that the "Analytical Method 00836 for the Determination of BYI 08330 and BYI 08330-enol in Drinking and Surface Water by HPLC-MS/MS and HPLC-UV[1], (Appendix 5), can be performed with acceptable recoveries at an independent laboratory having no prior experience with the method. The method was developed by Bayer CropScience AG, Development-Residues, Operator and Consumer Safety, at their laboratory in Monheim, Germany and reported by Bjoern Brumhard[1].

On initially reviewing the analytical method it was noted that parts of the method did not meet the criteria in OPPTS 860.1340[2] (Residue Analytical Method), specifically: there were no estimates of the practical MDL and LOQ, and the method as written required the use of untreated control matrix as a blank for use in preparation of the matrix-matched standards.

As OPPTS 850.7100 (d)(2)(i)[3] states, the laboratory conducting the ILV must use the method exactly as written, and therefore initially the analyses were performed as described in the method (i.e. using matrix-matched standards). Then the same samples were reanalyzed using the calibration solutions prepared in deionized water. With calibration solutions prepared in deionized water, recovery of BYI 08330-enol in drinking water was unacceptable. Therefore, a second trial was conducted using the calibration solutions prepared in deionized water which also contained isotopically labeled internal standards of BYI 08330 and BYI 08330-enol.

Method detection limit (MDL) and LOQ were calculated using the data generated from the analyses using matrix-matched calibration solutions and calibration solutions with internal standards prepared in deionized water.

3. EXPERIMENTAL DETAILS

Study initiation date: March 13, 2006

The following personnel were involved in the conduct of this study.

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3.1 Test Substances

The test substances for this study were BYI 08330 and BYI 08330-enol. See Appendix 3 for complete nomenclature and chemical structures.

3.2 Analytical Reference Substances

The test substances also served as the analytical reference substances. See Appendix 3 for complete nomenclature, chemical structures and reference information for the reference substances. The test and reference substances were stored in a freezer until used to prepare fortification and calibration solutions. All stock and working solutions were stored in a refrigerator set at less than 10°C when not in use.

3.3 Test System

The test systems were sub-samples of drinking (finished water) and surface water (raw water) obtained from Bayer CropScience Study Number 04RAOAY001[4], Surface Water Monitoring for Residues of Oxadiazon in High Use Areas in the United States. Characterization data for the water is presented in Appendix 4. The samples to be analyzed in this study were identified by the Bayer CropScience study number followed by a numeric suffix assigned sequentially.

The control water was stored refrigerated until spiked.

3.4 Method Summary

Each analytical set included one reagent blank, two unfortified control samples, five samples fortified at the LOQ (0.05 µg/L or ppb) and five samples fortified at 10x LOQ (0.5 µg/L)

Sixteen milliliters of the water sample was transferred to a 20-mL disposable glass vial. Fortified samples were prepared by adding 0.08 mL of a mixed standard for each fortification level: 0.05 and 0.5 µg/L. Samples were then diluted to 19.2 mL by transferring 3.2 mL of acetonitrile and 0.004mL acetic acid into each glass vial. For the samples run with the deionized water standards with internal standard, 0.0384mL internal standard solution was added to the final volume to achieve a final concentration of 0.24 µg/L in the sample. After mixing well, an aliquot of the sample was transferred to an HPLC vial for analysis by electrospray LC/MS/MS.

3.5 Instrumentation

- Sciex API 4000 LC/MS/MS System (Applied Biosystems)
- Shimadzu LC-10AD$_{VP}$ HPLC Pumps (2) with a high pressure mixer and Shimadzu SCL-10A$_{VP}$ Pump Controller
- Shimadzu SIL-20A Autosampler

3.6 HPLC Conditions

Note that the injection volume and flow rates listed below are different from the original method, but found to be optimum for the LC/MS/MS instrument used in this study.

Column: Synergi™ 4µ Fusion-RP 80, Length 150 mm x 4.6 mm i.d., Particle size 4µm, Part. No. 00F-4424-E0

Column oven temperature: 40 °C

Injection volume: 95 µL
Mobile phase: A: HPLC water / acetonitrile / acetic acid (900/100/0.1; v/v/v)
B: acetonitrile / acetic acid (1000/0.1; v/v)

Flow rate (column): 0.800 mL/min

Retention times: BYI 08330: approx. 7.8 min
BYI 08330-enol: approx. 5.9 min

3.7 HPLC Gradient Parameters

Time [min]	% A	% B
0.10	75	25
1	75	25
7	10	90
10	10	90
10.1	75	25
14.0	System Controller	Stop

3.8 Valco Valve Method Properties

Note that the setting listed below are different from the original method, but found to be optimum for the LC/MS/MS instrument used in this study.

Step	Total Time (min)	Position
1	0.0	B- To Waste
2	2.0	A- To MS

3.9 MS/MS Conditions

CAD Gas Setting [L/min]	5
Curtain Gas Setting [L/min]	11
GS1 Setting [L/min]	35
GS2 Setting [L/min]	50
Source Temperature [°C]	500
ihe	ON
Resolution of Q1 and Q3	Q1 Unit, Q3 Low
Scan type	MRM
Polarity	Positive
Ion Source	Turbo Spray

Compound dependent:	BYI 08330	BYI 08330-enol	BYI 08330-$^{13}C_3$	BYI 08330-enol-cis-$^{13}C_3$
Q1 Mass [amu]	374.23	302.30	377.49	305.29
Q3 Mass [amu]	215.95	216.00	305.10	219.1
Dwell [msec]	500	600	500	500
Ionization Mode	Positive	Positive	Positive	Positive
Ion Spray Voltage (IS) [V]	5500	5500	5500	5500
Entrance Potential (EP) [V]	10	10	10	10
Declustering Potential (DP) [V]	76	75	71	96
Collision Energy (CE) [V]	47	39	23	39
Collision Cell Exit Potential (CXP) [V]	16	15	10	16

Detector Parameters	
CEM	2200 V
DF	-50 V

3.10 Calculations

Matrix-Matched Calibration Standards (External Standards Method)

An example calculation for BYI 08330 for drinking water sample spiked at LOQ (sample ID: RAFNX019-021) is shown below. This sample was fortified with 0.05 ppb each of BYI 08330 and BYI 08330-enol.

The standards were fit to the linear equation: $Y = MX + B$

where: X is the concentration of the reference standard in µg/L (ng/mL)
M is the slope
B is the intercept
Y is the peak area

The analyte concentrations were weighted 1/x. The example shown below is for the calculation of BYI 08330 residues. BYI 08330-enol residues are calculated in a similar fashion.

After regression coefficients were calculated, the residue in parts per billion was determined. The parts per billion (ppb) of BYI 08330 in the water was calculated using the following equation,

$$\text{BYI 08330 found (ppb)} = \frac{(Y-B) \times D}{M}$$

$$\text{Where Dilution Factor (D)} = \frac{\text{Final volume } (V_2)}{\text{Initial volume } (V_1)}$$

V_1	V_2	Y	M	B
16mL	19.2mL	12840	323,490	(-490.4)

From the above equations:

Dilution Factor (D) = 19.2/16 = 1.2

$$\text{BYI 08330 Found (ppb)} = \frac{(12840 + 490.4) \times 1.2}{323490} = 0.0494\ \mu g/L$$

Calibration Standards with Internal Standards (Internal Standards Method)

An example calculation for BYI 08330 for surface water sample spiked at LOQ (sample ID: RAFNX019-034) is shown below. This sample was fortified with 0.05 ppb each of BYI 08330 and BYI 08330-enol and internal standards at 0.24 ppb each of BYI 08330-$^{13}C_3$ and BYI 08330-enol-$^{13}C_3$.

The equation for calculating residues in the samples is as follows.

$$X = \frac{(Y - B)}{M} \times IS$$

where

X = concentration of analyte in sample (µg/L)
Y = ratio of analyte response (area or height) to internal standard response (area or height)
B = intercept from linear regression analysis
M = slope from linear regression analysis (area ratio per conc. ratio)
IS = concentration of internal standard (µg/L) in the starting sample:

$$IS = \frac{V \times c}{S}$$

V = volume of internal standard solution added to sample, 0.0384 mL
c = concentration of internal standard solution, 100 µg/L
S = volume of starting sample, 16 mL

From above equation, $IS = \frac{0.0384 \times 100}{16}$

$= 0.24\ \mu g/L$

$$\text{BYI 08330 Found (ppb)} = \frac{(0.175 - 0.011361)}{0.78244} \times 0.24$$

$= 0.503\ \mu g/L$

Calculation of Percent Recovery

As the sample was fortified with known amounts of analyte prior to extraction, the percent recovery was determined using the following equation.

$$\% \text{ Recovery} = \frac{\text{(analyte found (ppb))} \times 100}{\text{analyte added (ppb)}}$$

Using the residue value for the example RAFNX019-034 above, the BYI 08330 recovery is calculated as follows.

$$\% \text{ Recovery} = \frac{(0.0503 \times 100)}{0.05} = 101\%$$

The % relative standard deviation (RSD) was calculated as follows.

$$\text{RSD} = \frac{\text{Standard Deviation}}{\text{Average Recovery}}$$

Using the percent recovery values for surface water spiked at 0.05 ppb BYI 08330 (Table 1), the %RSD is calculated as follows

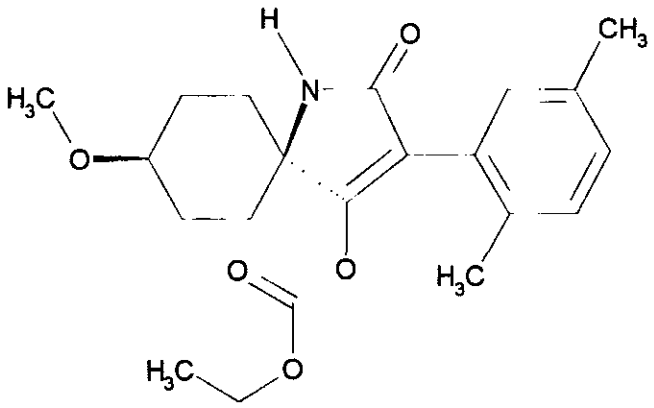
$$\text{RSD} = \frac{2}{90} = 2\%$$

Remark: Example calculations shown above were performed using the LC/MS/MS software *Analyst (version 1.4.1)*. The example calculation was performed using the area values reported by the instrument. The instrument software carries additional figures not shown in the intermediate results. Therefore, instrument software calculated values will differ slightly from the results derived using a calculator.

Appendix 3. Identity and Purity of the Test and Reference Materials Used

BYI 08330

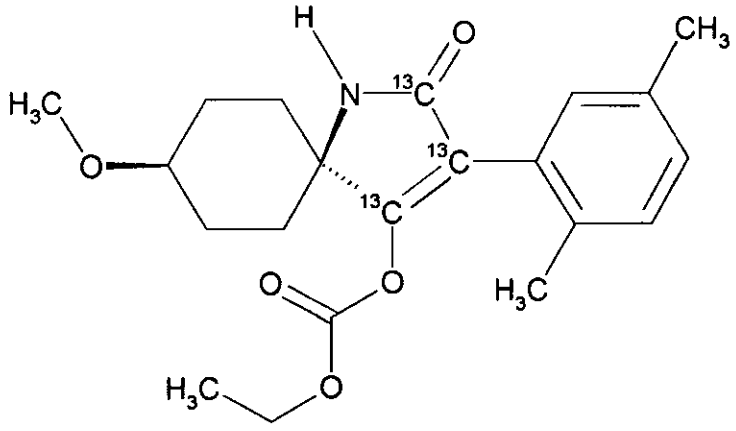
Structural formula:



CAS Number:	203313-25-1
Common name:	Spirotetramat
Code name:	BYI 08330
Chemical name:	*cis*-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1- azaspiro[4.5]dec-3-en-4-yl *ethyl carbonate*
Empirical formula:	$C_{21}H_{27}NO_5$
Molecular weight:	373.45 g/mol
Batch:	K-1451
Purity	99.2%
Expiration date	5/02/2008

BYI 08330-cis-$^{13}C_3$ (BYI 08330 cis-azaspirodecenyl-2,3,4-^{13}C)

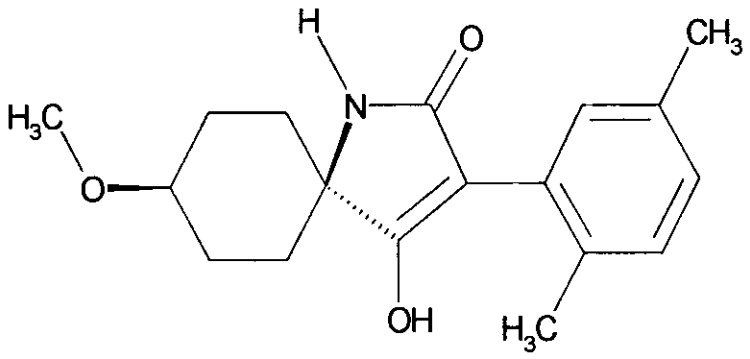
Structural formula:



CAS Number:	Unavailable
Chemical name:	*cis*-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl -*2,3,4-*$^{13}C_3$ ethyl carbonate
Empirical formula:	C_{21} H_{27} N O_5
Molecular weight:	376.41 g/mol
Batch:	K-1542
Purity	99.6%
Expiration date	6/21/2015

BYI 08330-enol

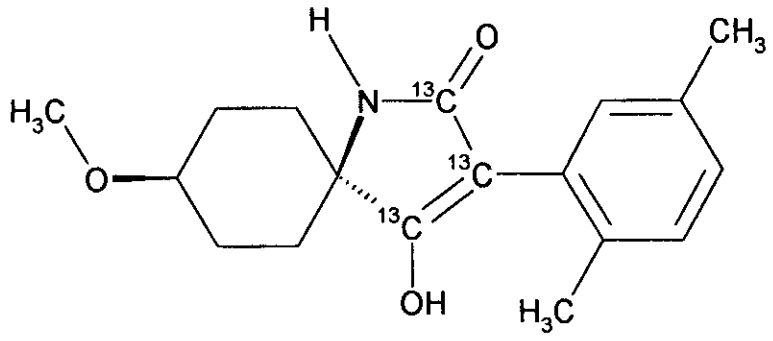
Structural formula:



CAS number:	203312-38-3
Code name:	BYI 08330-enol
Chemical name:	cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8- methoxy-1-azaspiro[4,5]dec-3-en-2-one
Empirical formula:	$C_{18}H_{23}NO_3$
Molecular weight:	301.38 g/mol
Batch	K-1450
Purity	99.4%
Expiration date	2/08/2008

BYI 08330 enol-cis-$^{13}C_3$

Structural formula:



CAS number:	Unavailable
Chemical name:	*cis*-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4,5]dec-3-en-2-one-*2,3,4*-$^{13}C_3$
Empirical formula:	C_{18} H_{23} N O_3
Molecular weight:	304.35 g/mol
Batch	K-1543
Purity	98.3%
Expiration date	6/21/2015