Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name:	Clothianidin
MRID # :	454226-02
Matrix:	Soil
Analysis:	LC/MS/MS

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Study Title

Analytical Method for the Determination of TI-435 and the Degradates, TZNG, TZMU, MNG and TMG in Soil by Liquid Chromatography with APCI MS/MS-Detection

Data Requirement

USEPA – Subdivision N, 164-1; Soil Dissipation - Supplemental
Canada PMRA – DACO No. 8.3.2 Soil Method

454226-02

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Completion Date

February 6, 2001

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Bayer Report No.

110263

Statement of Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of it falling within the scope of FIFRA section 10(d)(1) (A), (B) or (C).

Company: Bayer Corporation
Agriculture Division
Research and Development Department
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These data are the property of the Agriculture Division of Bayer Corporation, and as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

Good Laboratory Practice Certification

The study was not conducted in compliance with EPA Good Laboratory Practice Standards (40 CFR 160, August 17, 1989). The data presented in the report has been verified by the Sponsor and Ricerca LLC, as being accurate. However, a GLP audit of the data by Ricerca LLC, QAU was not performed.

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Certification of Availability of Raw Data

It is hereby certified that the registrant possesses or has access to all raw data for this report. All raw data or certified copies of raw data which was acquired but has not been included in this report has been archived by Bayer Corporation, Agriculture Division, P.O. Box 4913, Kansas City, Missouri, 64120-0013.

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P. A. Toll
Supervisor, Quality Assurance

Quality Assurance Statement

Because this study was not performed under GLP (Good Laboratory Practice regulations of FIFRA, Part 160, August 17, 1989) no QAU audits were performed during the study.

Certification of Authenticity

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Analytical Method for the Determination of TI-435 and the Degradates, TZNG, TZMU, MNG and TMG in Soil by Liquid Chromatography with MS/MS-Detection

1.0 Summary

This report describes the determination of the active ingredient TI-435 and its degradates TZNG, TZMU, MNG and TMG in soil. The method is a modification of the soil residue method described in Bayer report 109586.

Soil samples of 20 g are extracted on an ASE 200 extractor with approximately 45 mL acetonitrile/water/acetic acid/guanidine hydrochloride (25/100/0.1/1; v/v/v/w). Following the extraction, 1 mL of an internal standard solution is added and the volume is adjusted to 50 mL with acetonitrile/water (1:4). Identification and quantification of the active ingredient and the degradates are performed by high performance liquid chromatography using atmospheric pressure chemical ionization (APCI) tandem mass spectroscopy (MS/MS) detection. Isotopically labeled internal standards (d_3-TI-435; ^{13}C,^{15}N-TZNG, d_3-TZMU, d_3-MNG and d_3-TMG) are used to compensate for possible matrix effects in the MS/MS-detector.

A loam soil was fortified at two levels (5 and 50 µg/kg) for determining the accuracy of the method. The mean recoveries of the method, determined for the 5.0 µg/kg fortification (n=5) level were 100.4% for TI-435 (relative standard deviation (RSD) = 5.1%), 100.9% for TZNG (RSD = 8.2%), 97.3% for TZMU (RSD = 4.9%), 91.9% for MNG (RSD = 5.5%) and 91.7% for TMG (RSD = 3.0%).

The mean recoveries of the method, determined for the 50.0 µg/kg fortification level (n=5) were 100.3% for TI-435 (RSD = 3.1%), 93.4% for TZNG (RSD = 5.9%), 95.9% for TZMU (RSD = 4.5%), 111.1% for MNG (RSD = 2.0%) and 101.1% for TMG (RSD = 7.9%).

Duplicate control samples and one reagent blank sample were also analyzed and did not show any interferences that would inhibit the analysis of the five compounds in this study.

A calibration curve based on nine separate standard concentrations was used to quantify the concurrent recoveries. Standards from 1 µg/L to 500 µg/L were used (equivalent to 2.5 µg/kg to 1250 µg/kg samples), and the correlation coefficient for each compound ranged from 0.997 to 0.999.

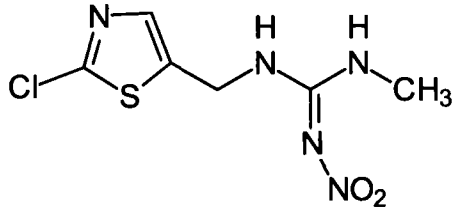
The limit of quantification (LOQ) of the method is 5 µg/kg for TI-435 and its degradates. The Method Detection Limit (MDL) of the method was 0.95, 1.55, 0.90, 0.96 and 0.51 µg/kg for TI-435, TZNG, TZMU, MNG and TMG, respectively. The MDL was based upon the standard deviation of five replicate analyses of fortifications made at the LOQ.

2.0 Introduction

The active ingredient TI-435 is an insecticide. A soil residue method is necessary to analyze soil samples collected from soil dissipation studies required for registration of the product. Analysis was targeted for TI-435 and degradates, TZNG, TZMU, MNG and TMG, which were identified at significant concentrations (>10%) in the environmental fate studies conducted with TI-435. The chemical structure of TI-435 and its degradates are:

- **TI-435**

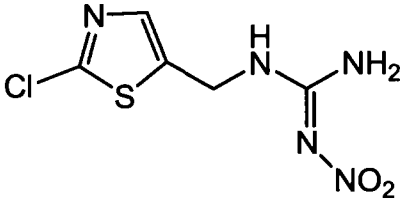
Chemical Name (CAS): (*E*)-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine



CAS Number:	210880-92-5 (previously 205510-53-8)
Formula:	$C_6H_8ClN_5O_2S$
Molar Mass:	249.7 g/mol
Bayer Reference No.:	M00343
Purity:	99.8%
Expiration Date:	August 2001

- **TZNG (thiazolyl-nitroguanidine)**

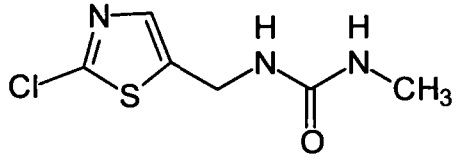
Chemical Name (CAS): *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine



Formula:	$C_5H_6ClN_5O_2S$
Molar Mass:	235.7 g/mol
Reference No.:	M00674
Purity:	99.7%
Expiration Date:	February 2002

- **TZMU (thiazolyl-methylurea)**

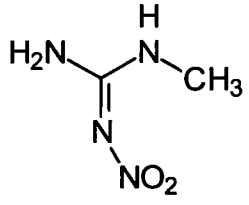
Chemical Name (CAS): *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-methylurea



Formula:	$C_6H_8ClN_3OS$
Molar Mass:	205.7 g/mol
Reference No.:	M00886
Purity:	98.0%
Expiration Date:	August 2002

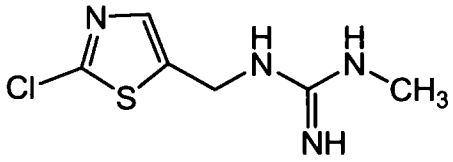
- **MNG (methyl-nitroguanidine)**

Chemical Name (CAS): *N*-Methyl-*N'*-nitroguanidine



Formula:	$C_2H_6N_4O_2$
Molar Mass:	118.1 g/mol
Reference No.:	M00500
Purity:	99.0%
Expiration Date:	February 2002

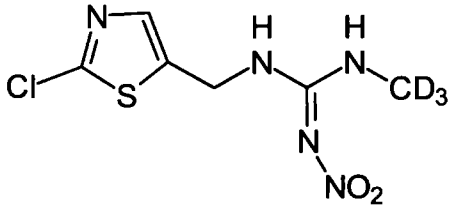
- **TMG (thiazolyl-methylguanidine)** **Chemical Name (CAS):** *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-methylguanidine



Formula:	$C_6H_9ClN_4S$
Molar Mass:	204.7 g/mol
Reference No.:	M01822
Purity:	91.0%
Expiration Date:	March 2001

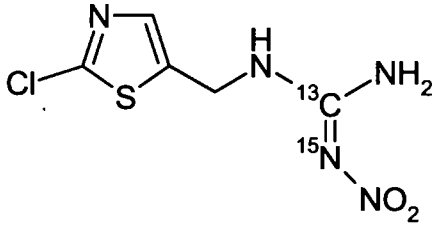
The Reference Substances (Internal Standards) have the following structures:

- **TI-435-d_3**



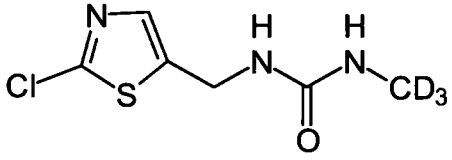
Formula:	$C_6H_5ClD_3N_5O_2S$
Molar Mass:	252.7 g/mol
Reference No.:	M01288
Purity:	99.0%
Expiration Date:	October 2002

- **TZNG-^{13}C,^{15}N**



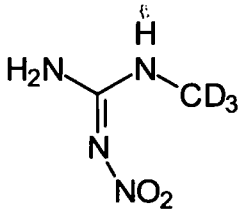
Formula:	$C_4{}^{13}CH_6ClN_4{}^{15}NO_2S$
Molar Mass:	237.7 g/mol
Reference No.:	M01322
Purity:	99.0%
Expiration Date:	February 2001

- **TZMU-d_3**



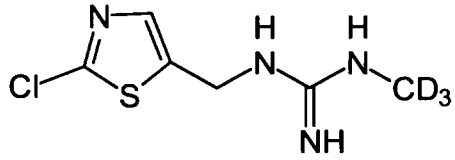
Formula:	$C_6H_5ClD_3N_3OS$
Molar Mass:	208.7 g/mol
Reference No.:	M01897
Purity:	99.2%
Expiration Date:	October 2002

- **MNG-d_3**



Formula:	$C_2H_3D_3N_4O_2$
Molar Mass:	121.1 g/mol
Reference No.:	M01289
Purity:	99.0%
Expiration Date:	November 2002

- **TMG-d_3**



Formula:	$C_6H_6ClD_3N_4S$
Molar Mass:	207.7 g/mol
Reference No.:	M01898
Purity:	99.4%
Expiration Date:	October 2002

2.1 Test Facility and Study Dates

All modifications and validation of the method was performed at Ricerca LLC (7528 Auburn Road, Painesville, Ohio, 44077-1000). Work associated with method development and validation occurred in July of 2000.

3.0 Test System

The method was validated using soil from a dissipation site in Watsonville, California (USA). The soil was selected to represent a "difficult" matrix. The soil samples were classified according to USDA specifications. Soil textural characterization is summarized in Table 1.

Table 1: Soil Characteristics

Parameter	Value
sand (%)	38.8
silt (%)	38.4
clay (%)	22.8
texture	loam
Organic Matter (%)	2.0
pH	5.4
Cation Exchange Capacity (meq/100g)	8.6
Water Holding Capacity-1/3 bar (%)	21.5
Water Holding Capacity-15 bar (%)	6.3
Bulk Density (g/mL)	1.57
Soil Series Classification	Elder

4.0 Principle of the Method

With the following method, the active ingredient TI-435 and the degradates TZNG, TZMU, MNG and TMG can be determined in soil down to a limit of quantification of 5 µg/kg (LOQ). The method is a modification of the soil residue method described in Bayer Report 109586. Some difficulties encountered with the LC-MS/MS analyses required modification of the existing method, and are described here. All solvent, standard, and extraction conditions are the same as those described in Bayer Report 109586, which also provides additional information regarding mass spectra of the analytes, and linear response curves.

Soil samples of 20 g are extracted on an ASE 200 extractor with approximately 45 mL of a mixture of acetonitrile/water/acetic acid/guanidine hydrochloride (25/100/0.1/1; v/v/v/w). Following the extraction, 1 mL of an internal standard solution is added and the volume is

adjusted to 50 mL with acetonitrile/water. Identification and quantification of the active ingredient and the degradates are performed by high performance liquid chromatography using atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (MS/MS) detection. Isotopically labeled internal standards (d_3-TI-435, ^{13}C,^{15}N-TZNG, d_3-TZMU, d_3-MNG and d_3-TMG) are used to compensate for possible matrix effects in the MS/MS-detector.

5.0 Standards and Sample Preparation

Calibration Standards and the samples analyzed by LC-MS/MS were composed of the same ratio of acetonitrile and water (1:4). Additionally, the amount of internal standard was kept constant at 50 µg/L (125 µg/kg soil equivalents) in all samples analyzed. The ratio of native material to internal standard was plotted for each of the calibration standards in a straight line fit, and used to determine the concentration of native material in the recovery samples.

5.1 Standard Preparation

Calibration standards were prepared according to Bayer Report 109586 at the following concentrations: 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 µg/L (native standard). These concentrations correspond to soil equivalents of 2.5 to 1250 µg/kg. The amount of internal standard was constant in all standards at 50 µg/L in each of these calibration standards.

5.2 Equipment

Extraction Equipment:	Accelerated Solvent Extractor (ASE): ASE 200 Dionex Corporation, USA Sunnyvale, California
Cellulose Filters:	(for ASE 200 extraction cell caps) Part No. 049458 , Dionex Corporation
Extraction Solvent:	acetonitrile/water/acetic acid/guanidine HCl (25/100/0.1/1; v/v/v/w) 400 mL Acetonitrile (HPLC Grade, Fisher) 1600 mL Water (HPLC Grade, Fisher) 1.6 mL Acetic Acid (ACS Grade, Fisher) 16 grams Guanidine Hydrochloride (>99%, Fluka)

5.3 Extraction Procedure

1. Weigh 4 g of hydromatrix and 20 g of a soil sample into a weigh dish.
2. Mix the contents thoroughly using a spatula.
3. Place a cellulose filter at the bottom of a 33 mL stainless steel cartridge (ASE sample cell).
4. Place the soil/hydromatrix mixture into the ASE cell using a funnel.
5. Extract the samples on the ASE 200 extractor at 140 °C with approximately 45 mL of extraction solvent (see 5.4 for ASE conditions).

6. After extraction, add 1 mL of the internal standard solution and adjust the volume to 50 mL with 1:4 acetonitrile:water.

5.4 ASE Conditions

Preheat:	0 min
Heat:	7 min
Static:	10 min
Flush volume:	35 % of cell
Purge:	5 min
Cycles:	3
Pressure:	150 bar
Temperature:	140 °C

6.0 LC Conditions

Column: Phenomenex AQUA C_{18} ; length 25.0 cm, i.d. 4.6 mm

Injection volume: 50 µL

Oven temperature: Column at room temperature

Mobile phase: A: water
B: methanol

Run time: 20 min

Flow rate (column): 1.0 mL/min
Flow rate (interface): 1.0 mL/min

Retention times:

TI-435:	approx.	9:82 min.
TZNG:	approx.	9:37 min.
TZMU:	approx.	8.92 min.
TMG:	approx.	8.91 min.
MNG:	approx.	3.87 min.

Table 2: HPLC-Gradient

Time [min]	0	1	2	7	7	11	11.1	
% A	90	90	65	40	20	20	90	
% B	10	10	35	60	80	80	10	[1)]

[1)]The gradient can be changed to optimize the separation.

Table 3: HPLC-Timetable

Time	Setting	Value	
14.0	Column Switching Valve	Column 1	1)

1) switching eluent stream to waste

7.0 Mass Spectroscopy

Mass Spectrometer: PE Sciex API-III with APCI Interface and heated nebulizer.

7.1 Principle of Measurement

Substances introduced into the mass spectrometer are ionized using an APCI interface. Sample ions are accelerated by an adequate voltage regulation and separated by mass in the first quadrupole (Q1). The most abundant ions of the analyte (parent ions) are impulsed with nitrogen in the collision cell (Q2). Fragments of these ions (daughter ions) are separated by mass in the third quadrupole (Q3) and detected. The mass spectroscopic parameters for the analytes and the selected ions are listed in Table 4 to 8 on the following pages.

7.2 Mass Spectroscopic Parameters

The reported parameters are examples for an optimal adjustment of the mass spectrometer. From time to time these parameters have to be checked and adjusted, if necessary, depending on the instrument used. Abbreviations used in Tables 4 to 8 are as follows:

(DI)	Discharge Needle Current	(RE3)	Quad 3 Resolution
(OR)	Orifice Plate	(RX)	Lens Element RX
(R0)	Quad 0 Rod Offset	(R3)	Quad 3 Rod Offset
(RE1)	Q1 Resolution	(L9)	Lens Element 9
(DM1)	Q1 Delta Mass	(FP)	Faraday Plate Voltage
(R1)	Quad 1 Rod Offset	(MU)	Channel Electron Multiplier
(L7)	Lens Element 7	(DM3)	Quad 3 Delta Mass
(R2)	Quad 2 Rod Offset		

Table 4: MS/MS-Parameters of TI-435 and d_3-TI-435

Auxiliary gas (N_2): 4 L/min, temperature: 400 °C
Curtain Gas (N_2): 1.2 L/min
Nebulizer Gas (N_2): 80 PSI
Collision Gas (N_2): 236 (setting)

Experiment Information

Scan Type : Multiple Reaction Monitoring
Mass Range Information

Mass Range 1	TI-435	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
250	169	300

Mass Range 1	d_3-TI-435	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
253	172	100

Positive Ion Mode

Parameter	Value [volt]
DI	5µA
OR	54
RO	30
REI	120
DMI	0.2
R1	26
L7	21
R2	16
RE3	117
DM3	0.14
RX	1.0
R3	11.0
L9	-50
FP	-50
MU	-4800

Table 5: MS/MS-Parameters of TZNG and ^{13}C, ^{15}N-TZNG

Auxiliary gas (N_2): 4 L/min, temperature: 400 °C
Curtain Gas (N_2): 1.2 L/min
Nebulizer Gas (N_2): 80 PSI
Collision Gas (N_2): 236 (setting)

Experiment Information

Scan Type : Multiple Reaction Monitoring
Mass Range Information

Mass Range 1	TZNG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
236	132	300

Mass Range 1	^{13}C, ^{15}N-TZNG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
240	134	100

Positive Ion Mode

Parameter	Value [volt]
DI	5µA
OR	54
RO	30
REI	120
DMI	0.2
R1	26
L7	21
R2	16
RE3	117
DM3	0.14
RX	1.0
R3	11.0
L9	-50
FP	-50
MU	-4800

Remark: Due to the fact, that the m/z 238 ion derives from both the native and internal standard, due to the chlorine isotopes (^{37}Cl-TZNG and ^{35}Cl-^{13}C,^{15}N-TZNG), the not superimposed ions m/z 236 (^{35}Cl-TZNG) and m/z 240 (^{37}Cl-^{13}C,^{15}N-TZNG) were used for the identification and quantification of the two compounds.

Table 6: MS/MS-Parameters of TZMU and d_3-TZMU

Auxiliary gas (N_2): 4 L/min, temperature: 400 °C
Curtain Gas (N_2): 1.2 L/min
Nebulizer Gas (N_2): 80 PSI
Collision Gas (N_2): 236 (setting)

Experiment Information

Scan Type : Multiple Reaction Monitoring
Mass Range Information

Mass Range 1	TZMU	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
206	175	600

Mass Range 1	d_3-TZMU	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
209	175	300

Positive Ion Mode

Parameter	Value [volt]
DI	5µA
OR	54
RO	30
REI	120
DMI	0.2
R1	26
L7	21
R2	16
RE3	117
DM3	0.14
RX	1.0
R3	11.0
L9	-50
FP	-50
MU	-4800

Table 7: MS/MS-Parameters of MNG and d_3-MNG

Auxiliary gas (N_2): 4 L/min, temperature: 400 °C
Curtain Gas (N_2): 1.2 L/min
Nebulizer Gas (N_2): 80 PSI
Collision Gas (N_2): 236 (setting)

Experiment Information

Scan Type : Multiple Reaction Monitoring
Mass Range Information

Mass Range 1	MNG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
117	60.7	250

Mass Range 1	d_3-MNG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
120.0	60.7	250

Negative Ion Mode

Parameter	Value [volt]
DI	-3µA
OR	-54
RO	-30
REI	-120
DMI	0.19
R1	-20
L7	-26
R2	-21
RE3	119
DM3	0.1
RX	-6.0
R3	-16.0
L9	50
FP	50
MU	4800

Table 8: MS/MS-Parameters of TMG and d_3-TMG

Auxiliary gas (N_2): 4 L/min, temperature: 400 °C
Curtain Gas (N_2): 1.2 L/min
Nebulizer Gas (N_2): 80 PSI
Collision Gas (N_2): 236 (setting)

Experiment Information

Scan Type : Multiple Reaction Monitoring
Mass Range Information

Mass Range 1	TMG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
205	132	300

Mass Range 1	d_3-TMG	
Q1 Mass (amu)	Q3 Mass (amu)	Dwell (msec)
208	132	100

Positive Ion Mode

Parameter	Value [volt]
DI	5µA
OR	54
RO	30
REI	120
DMI	0.2
R1	26
L7	21
R2	16
RE3	117
DM3	0.14
RX	1.0
R3	11.0
L9	-50
FP	-50
MU	-4800

Table 9: MS/MS-Timetable

Detection time [min]	Principle	Dwell time [msec]	m/z Parent	m/z Product	Substance
0 – 5	$APCI^-$	250	117	60.7	MNG
0 – 5	$APCI^-$	250	120	60.7	d_3-MNG
5 – 12.8	$APCI^+$	150	205	132	TMG
5 – 12.8	$APCI^+$	150	208	132	d_3-TMG
5 – 12.8	$APCI^+$	150	206	175	TZMU
5 – 12.8	$APCI^+$	150	209	175	d_3-TZMU
5 – 12.8	$APCI^+$	150	236	132	TZNG
5 – 12.8	$APCI^+$	150	240	134	^{13}C, ^{15}N-TZNG
5 – 12.8	$APCI^+$	150	250	169	TI-435
5 – 12.8	$APCI^+$	150	253	172	d_3-TI-435

$APCI^+$ = positive ion mode, i.e. production of positive ions
$APCI^-$ = negative ion mode, i.e. production of negative ions

8.0 Calculations

For calculation of the concentrations, nine point calibration curves are used. These curves are calculated using linear regression, automatically after each sequence run, with the Perkin Elmer quantification software *MacQuan Version 1.5*.

The linear equation is expressed as:

$$y = \text{Intercept} + \text{Slope} \cdot x$$

y = Area, x = Concentration

For the calculation of standard/internal standard ratios:

$$y = \frac{\text{Area}_{\text{Standard}}}{\text{Area}_{\text{Internal Standard}}} = \text{Int Ratio} \quad \text{and} \quad x = \frac{\text{Conc}_{\text{Standard}}}{\text{Conc}_{\text{IS}}} = \text{Conc}_{\text{ratio}}$$

Int.Ratio = Intensity ratio
$Conc_{Standard}$ = Concentration of standard solution [µg/L]
$Conc_{IS}$ = Concentration of internal standard solution [µg/L]
$Conc_{ratio}$ = Concentration ratio

Because the concentrations of the isotopically labeled internal standards were the same in all solutions that were injected into the HPLC instrument, they do not need to be taken into consideration. However, the concentrations of the internal standard solutions should be in the range of the concentration of the sample solutions.

By means of the linear equation (calibration curve), the compound concentration in soil can be calculated as follows:

$$\text{Dilution}_{\text{Factor}} = \frac{\text{Volume}_{\text{final}}}{\text{Weight}}$$

$$\text{Conc}_{\text{Analyte}} = \frac{A_i}{A_{iSi}}, \quad \text{Int.Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

$$\text{Conc}_{\text{Soil}} = \text{Conc}_{\text{Analyte}} \cdot \text{Dilution}_{\text{Factor}}$$

$Volume_{final}$ = Final volume of the sample solution [L]
Weight = Weight of the soil sample [kg]
$Conc_{Analyte}$ = Concentration of the analyte in the sample solution [µg/L]
$Conc_{Soil}$ = Concentration of the analyte in soil [µg/kg]

The recovery is calculated according to the following equation:

$$\text{Recovery} = \frac{\text{Conc}_{\text{Soil}} \cdot 100\%}{\text{Conc}_{\text{Soil Spiked}}}$$

$Conc_{Soil\ Spiked}$ = Concentration of the reference substance spiked [µg/kg]

Example calculation for recovery of TI-435, 50 µg/kg (sample ID 98-0109-10):

$$\text{Dilution}_{\text{Factor}} = \frac{0.05\,\text{L}}{0.02\,\text{kg}} = 2.5\,\text{L/kg} \qquad \text{Dilution}_{\text{Factor}} = \frac{\text{Volume}_{\text{final}}}{\text{Weight}}$$

$$Conc_{Analyte} = \frac{0.391 - (-0.000159)}{0.019450} = 20.1\,\mu g/L$$

$$Conc_{Analyte} = \frac{Int.Ratio - Intercept}{Slope}$$

$$Conc_{Soil\,Wet} = 20.1\,\mu g/L \cdot 2.5\,L/kg = 50.3\,\mu g/kg$$

$$Conc_{Soil\,Wet} = Conc_{Analyte} \cdot Dilution_{Factor}$$

$$Recovery = \frac{50.3\,\frac{\mu g}{kg} \cdot 100\%}{50.0\,\frac{\mu g}{kg}} = 101\%$$

$$Recovery = \frac{Conc_{Soil} \cdot 100\%}{Conc_{Soil\,Spiked}}$$

9.0 Detector Linearity

Standard solutions of TI-435, TZNG, TZMU, MNG and TMG were measured in a concentration range of 1.0 to 500 µg/L (equivalent to 2.5 to 1250 µg/kg samples). The concentrations of the internal standard solutions were constantly kept at 50 µg/L. In this concentration range, the mass spectrometric detector showed a linear correlation between concentration and intensity ratio (Table 10), with correlation coefficients ranging from 0.997 to 0.999.

Table 10: Correlation Coefficients for each Compound

Substance	Correlation Coefficient
TI-435	0.998
TZNG	0.997
TZMU	0.999
MNG	0.998
TMG	0.999

10.0 Control and Reagent Blank Samples

The analytical results of duplicate control samples were below 1.5 µg/kg for TI-435, TZNG, TZMU, MNG and TMG. Figure 2 shows a representative chromatogram of the analyte ions of control soil.

11.0 Limit of Quantification and Limit of Detection

The limit of quantification (LOQ) of the method , as determined from this validation, is 5 µg/kg for TI-435 and its degradates.

The method detection limit (MDL) of the method is 0.95, 1.55, 0.90, 0.96, and 0.51 µg/kg for TI-435, TZNG, TZMU, MNG and TMG, respectively (Tables 11-15). The MDL was calculated by multiplying the standard deviation by the appropriate Student's *t* value at a 99% confidence level (for n = 5, *t* = 3.747), as described in 40CFR Ch. 1, Part 136, App. B, (7-1-94 Edition).

12.0 Determination of the Recoverability

Soil from Watsonville, California was fortified with the active ingredient TI-435 and its degradates. Fortification levels of the different soils of TI-435, TZNG, TZMU, MNG and TMG as well as recoveries and relative standard deviations are presented in the following table (also see Table 11-15).

Fortification [µg/kg]		Mean Values [%]				
	N	TI-435	TZNG	TZMU	MNG	TMG
5.0	5	100.4	100.9	97.3	91.9	91.7
50.0	5	100.3	93.4	95.9	111.1	101.1

Figures 2 to 4 show representative chromatograms of the analysis of control soil, fortifications made at 5 µg/kg and fortifications made at 50 µg/kg.

13.0 Storage Stability of the Extracts

The stability of the analytes in the extracts was proven by the acceptable recoveries achieved for the sample extracts, after storage in a freezer at <0 °C for the period from extraction on April 4, 2000 to analysis on July 20, 2000 (107 days).

14.0 References

1. Schramel, O., "Residue Analytical Method 00521 (MR-343/98) for Determination of TI-435 and the Metabolites TZNG, TZMU, MNG and TMG in Soil by Liquid Chromatography with Electrospray MS/MS-Detection," Bayer Report 109586, 1999.

Table 11: Analysis of Controls and Recovery Samples Fortified with TI-435.

Sample	Fortified Amount µg/kg	µg/L	µg/kg	% Recovery	Average Recovery	% Relative Std. Dev.	MDL (StdDev*3.747)
TI-435							
98-0109-1	5.0	2.054	5.14	102.7			
98-0109-2	5.0	2.056	5.14	102.8			
98-0109-3	5.0	2.031	5.08	101.6			
98-0109-4	5.0	1.828	4.57	91.4			
98-0109-5	5.0	2.072	5.18	103.6	100.4	5.1	
		Average =	**5.02**	**Std Dev =**	**0.254**		**0.95**
TI-435							
98-0109-6	50.0	20.310	50.78	101.6			
98-0109-7	50.0	20.363	50.91	101.8			
98-0109-8	50.0	20.558	51.40	102.8			
98-0109-9	50.0	18.969	47.42	94.8			
98-0109-10	50.0	20.117	50.29	100.6	100.3	3.1	
		Average =	**50.16**	**Std Dev =**	**1.579**		

Table 12: Analysis of Controls and Recovery Samples Fortified with TZNG.

Sample	Fortified Amount µg/kg	µg/L	µg/kg	% Recovery	Average Recovery	% Relative Std. Dev.	MDL (StdDev*3.747)
TZNG							
98-0109-1	5.0	2.237	5.59	111.9			
98-0109-2	5.0	1.970	4.93	98.5			
98-0109-3	5.0	2.138	5.35	106.9			
98-0109-4	5.0	1.893	4.73	94.7			
98-0109-5	5.0	1.847	4.62	92.4	100.9	8.2	
		Average =	**5.04**	**Std Dev =**	**0.414**		**1.55**
TZNG							
98-0109-6	50.0	17.378	43.45	86.9			
98-0109-7	50.0	17.631	44.08	88.2			
98-0109-8	50.0	19.809	49.52	99.0			
98-0109-9	50.0	19.351	48.38	96.8			
98-0109-10	50.0	19.212	48.03	96.1	93.4	5.9	
		Average =	**46.69**	**Std Dev =**	**2.740**		

Table 13: Analysis of Controls and Recovery Samples Fortified with TZMU.

Sample	Fortified Amount µg/kg	µg/L	µg/kg	% Recovery	Average Recovery	% Relative Std. Dev.	MDL (StdDev*3.747)
TZMU							
98-0109-1	5.0	1.914	4.79	95.7			
98-0109-2	5.0	2.094	5.24	104.7			
98-0109-3	5.0	1.881	4.70	94.1			
98-0109-4	5.0	1.987	4.97	99.4			
98-0109-5	5.0	1.855	4.64	92.8	97.3	4.9	
		Average =	**4.87**	**Std Dev =**	**0.241**		**0.90**
TZMU							
98-0109-6	50.0	19.768	49.42	98.8			
98-0109-7	50.0	19.190	47.98	96.0			
98-0109-8	50.0	19.905	49.76	99.5			
98-0109-9	50.0	17.750	44.38	88.8			
98-0109-10	50.0	19.265	48.16	96.3	95.9	4.5	
		Average =	**47.94**	**Std Dev =**	**2.137**		

Table 14: Analysis of Controls and Recovery Samples Fortified with MNG.

Sample	Fortified Amount µg/kg	µg/L	µg/kg	% Recovery	Average Recovery	% Relative Std. Dev.	MDL (StdDev*3.747)
MNG							
98-0109-1	5.0	1.764	4.41	88.2			
98-0109-2	5.0	1.828	4.57	91.4			
98-0109-3	5.0	2.016	5.04	100.8			
98-0109-4	5.0	1.806	4.52	90.3			
98-0109-5	5.0	1.780	4.45	89.0	91.9	5.5	
		Average =	**4.60**	**Std Dev =**	**0.255**		**0.96**
MNG							
98-0109-6	50.0	22.180	55.45	110.9			
98-0109-7	50.0	22.959	57.40	114.8			
98-0109-8	50.0	21.945	54.86	109.7			
98-0109-9	50.0	22.096	55.24	110.5			
98-0109-10	50.0	21.880	54.70	109.4	111.1	2.0	
		Average =	**55.53**	**Std Dev =**	**1.085**		

Table 15: Analysis of Controls and Recovery Samples Fortified with TMG.

Sample	Fortified Amount µg/kg	µg/L	µg/kg	% Recovery	Average Recovery	% Relative Std. Dev.	MDL (StdDev*3.747)
TMG							
98-0109-1	5.0	1.850	4.63	92.5			
98-0109-2	5.0	1.757	4.39	87.9			
98-0109-3	5.0	1.832	4.58	91.6			
98-0109-4	5.0	1.825	4.56	91.3			
98-0109-5	5.0	1.910	4.78	95.5	91.7	3.0	
		Average =	**4.59**	**Std Dev =**	**0.137**		**0.51**
TMG							
98-0109-6	50.0	22.431	56.08	112.2			
98-0109-7	50.0	18.817	47.04	94.1			
98-0109-8	50.0	18.873	47.18	94.4			
98-0109-9	50.0	19.703	49.26	98.5			
98-0109-10	50.0	21.295	53.24	106.5	101.1	7.9	
		Average =	**50.56**	**Std Dev =**	**3.971**		

Figure 1: Flow Diagram of Analysis Procedure

Mix 20 g of soil with 4 g of Hydromatrix in a beaker,
fill into an ASE 200 extraction cell

⇩

Extract with approx. 45 mL of acetonitrile:water:acetic acid:guanidine hydrochloride
(25:100:0.1:1; v:v:v:w), on an ASE Extractor at 140 °C

⇩

Add 1 mL of internal standard and adjust the volume
to 50mL with 1:4 acetonitrile:water

⇩

Fill into a HPLC vial

⇩

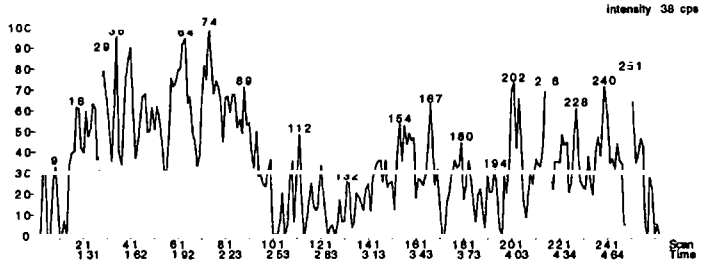
HPLC-MS/MS (APCI)

Figure 2: LC-MS/MS Chromatogram of Control sample, 98-0109-C.

MacQuan, version 1 5
Printed: Thu, Jul 20, 2000 19 44
Calibration File: CFA072000 Path. LS-038 MAC #1 Data Bayer/Takeda.A072000.
Comments: No Comments

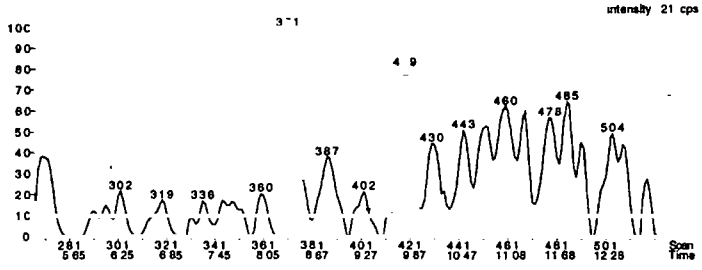
A072000009 A072000009 Thu, Jul 20, 2000 12 29
98-0109 C

13 03 in 2 periods
MNG
Internal Standard MNG-d3
Use Area
Absolute Retention Time
1 4 00 MRM, 268 scans
117 0->80 7
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 1
Expected RT 3 87
Area 0
Height 0
Start Time -0 00
End Time -0 00
Integration Width -0 00
Retention Time 0 00
Integration Type



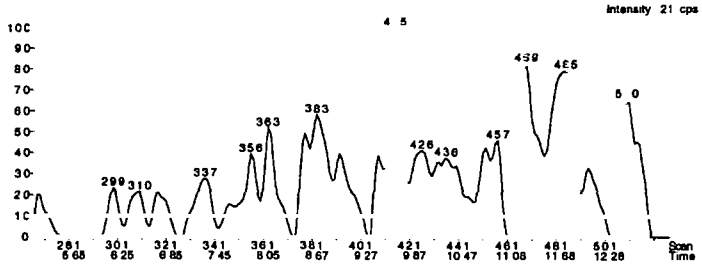
A072000009 A072000009 Thu Jul 20 2000 12 29
98 0109-C

13 03 in 2 periods
TMG
Internal Standard TMG-d3
Use Area
Absolute Retention Time
2 7 80 MRM, 260 scans
205 0->132 0
Noise Thres 5 0
Quant Thres 0 2
Min Width 6
Mult Width 9
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 8 91
Area 0
Height 0
Start Time -0 00
End Time -0 00
Integration Width -0 00
Retention Time 0 00
Integration Type



A072000009 A072000009 Thu, Jul 20, 2000 12 29
98-0109-C

13 03 in 2 periods
TZMU
Internal Standard TZMU-d3
Use Area
Absolute Retention Time
2 7 80 MRM, 260 scans
206 0 >175 0
Noise Thres 5 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 8 92
Area 0
Height 0
Start Time -0 00
End Time -0 00
Integration Width -0 00
Retention Time -0 00
Integration Type



A072000009 A072000009 Thu, Jul 20, 2000 12 29
98-0109 C

13 03 in 2 periods
TZNG
Internal Standard TZNG-C13 N15
Use Area
Absolute Retention Time
2 7 80 MRM, 260 scans
236 0->132 0
Noise Thres 10 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 9 37
Area 0
Height 0
Start Time 0 00
End Time 0 00
Integration Width 0 00
Retention Time -0 00
Integration Type

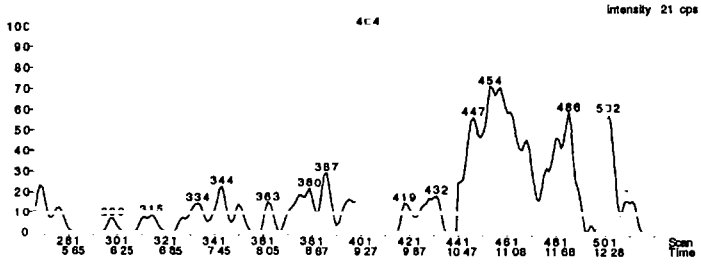
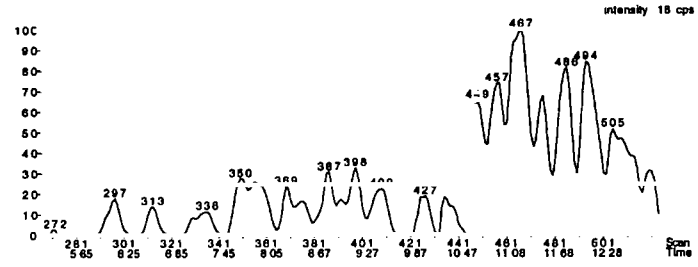


Figure 2: LC-MS/MS Chromatogram of Control sample, 98-0109-C. (Continued)

MacQuan, version 1 5
Printed: Thu, Jul 20, 2000 19:44 TK
Calibration File CFA072000 Path LS-038 MAC #1 Data Bayer/Takeda A072000:
Comments. No Comments

A072000009 A072000009 Thu, Jul 20 2000 12 29
98 0109-C

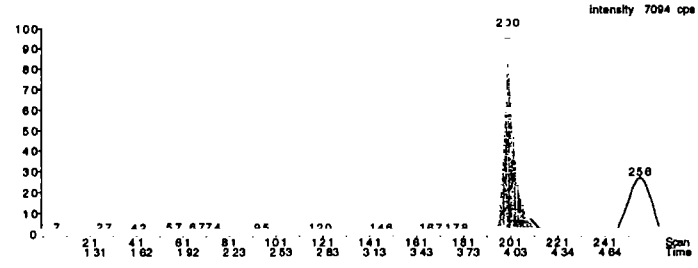
13 03 in 2 periods
TI-435
Internal Standard TI-435-d3
Use Area
Absolute Retention Time
2 7 80 MRM, 260 scans
250 0->169 0
Noise Thres 5 0
Quant Thres 0 5
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 2 0
Smooth 5
Expected RT 9 82
Area 0
Height 0
Start Time 0 00
End Time -0 00
Integration Width -0 00
Retention Time 0 00
Integration Type



A072000009 A072000009 Thu, Jul 20 2000 12 29
98 0109-C

13 03 in 2 periods
MNG-d3
use as Internal Standard

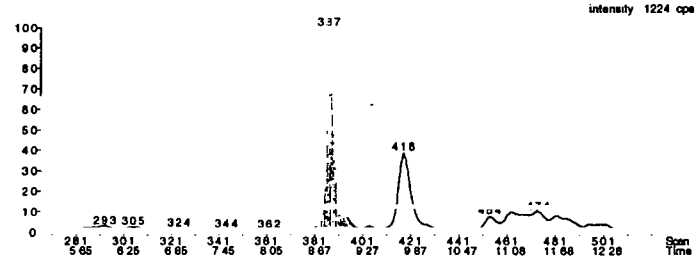
1 4 00 MRM, 266 scans
120 0->80 7
Noise Thres 10 0
Quant Thres 0 5
Min Width 6
Mult Width 8
Base Width 80
RT Win (secs) 2 0
Smooth 5
Expected RT 3 88
Area 46274
Height 7094
Start Time 3 91
End Time 4 29
Integration Width 0 38
Retention Time 4 02
Integration Type A - BB



A072000009 A072000009 Thu, Jul 20 2000 12 29
98-0109-C

13 03 in 2 periods
TMG-d3
use as Internal Standard

2 7 80 MRM 260 scans
208 0->132 0
Noise Thres 10 0
Quant Thres. 1 0
Min Width 6
Mult Width 8
Base Width 80
RT Win (secs) 2 0
Smooth 5
Expected RT 8 66
Area 12899
Height 1220
Start Time 8 64
End Time 9 45
Integration Width 0 81
Retention Time 8 85
Integration Type A BB



A072000009 A072000009 Thu, Jul 20 2000 12 29
98-0109-C

13 03 in 2 periods
TZMU-d3
use as Internal Standard

2 7 80 MRM, 280 scans
209 0->175 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 8
Base Width 80
RT Win (secs) 2 0
Smooth 5
Expected RT 8 89
Area 26981
Height 2443
Start Time 8 70
End Time 9 39
Integration Width 0 69
Retention Time 8 88
Integration Type A - BV

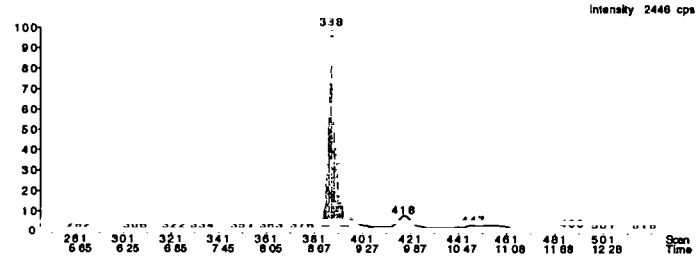


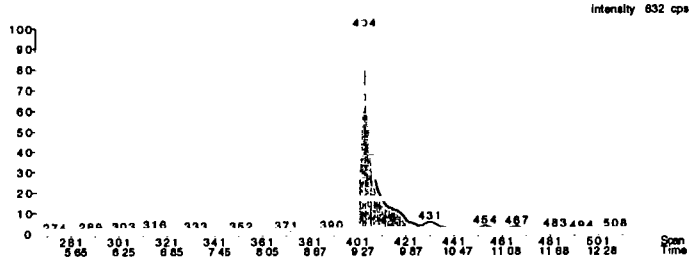
Figure 2: LC-MS/MS Chromatogram of Control sample, 98-0109-C. (Continued)

MacQuan, version 1 5
Printed. Thu, Jul 20, 2000 19:44
Calibration File CFA072000 Path. LS-038 MAC #1:Data Bayer/Takeda A072000:
Comments: No Comments

A072000009 A072000009 Thu Jul 20, 2000 12 29
98-0109-C

13 03 in 2 periods
TZNG-C13 N15
use as Internal Standard

2 7 80 MRM, 260 scans
240 0->134 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 9 37
Area 7934
Height 629
Start Time 9 18
End Time 10 02
Integration Width 0 84
Retention Time 9 36
Integration Type A - BV



A072000009 A072000009 Thu, Jul 20 2000 12 29
98-0109 C

13 03 in 2 periods
TI-435-d3
use as Internal Standard

2 7 80 MRM, 260 scans
253 0->172 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 9 79
Area 53285
Height 4368
Start Time 9 57
End Time 11 14
Integration Width 1 56
Retention Time 9 76
Integration Type A - BV

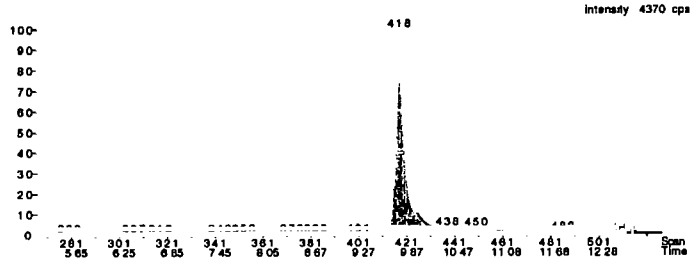
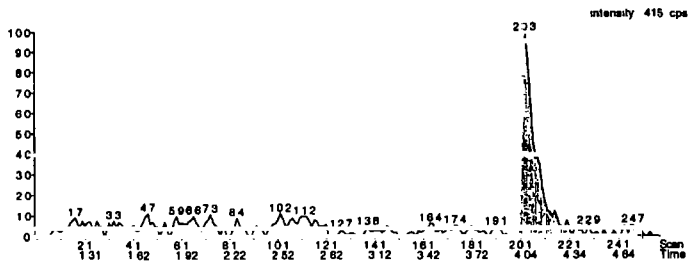


Figure 3: LC-MS/MS Chromatogram of 5 ppb fortified sample, 98-0109-1.

MacQuan, version 1 5 Page 34 of 72
Printed: Thu, Jul 20, 2000 19:44
Calibration File CFA072000 Path LS-038 MAC #1 Data Bayer/Takeda A072000
Comments No Comments

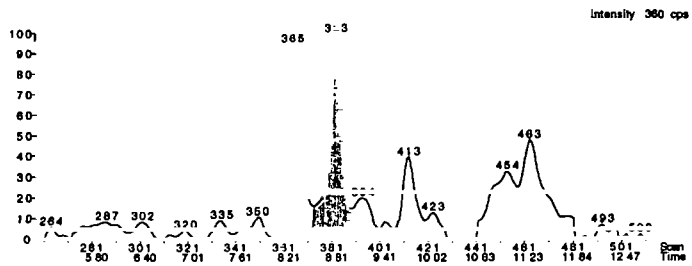
A072000012 A072000012 Thu, Jul 20, 2000 19 27
98-0109-1

12 92 in 2 periods
MNG
Internal Standard MNG-d3
Use Area
Absolute Retention Time
1 3 87 MRM 257 scans
117 0 >60 7
Noise Thres 5 0
Quant Thres 0 5
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 1
Expected RT 3 87
Area 2823
Height 415
Start Time 3 99
End Time 4 34
Integration Width 0 35
Retention Time 4 07
Integration Type A* - BB



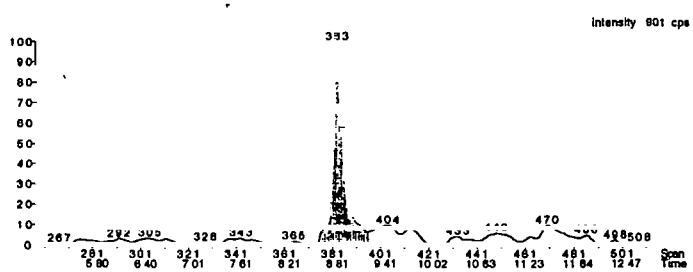
A072000012 A072000012 Thu, Jul 20, 2000 19 27
98-0109 1

12 92 in 2 periods
TMG
Internal Standard TMG-d3
Use Area
Absolute Retention Time
2 7 80 MRM, 259 scans
205 0->132 0
Noise Thres 5 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 40
RT Win (secs) 2 0
Smooth 5
Expected RT 8 91
Area 4087
Height 343
Start Time 8 57
End Time 9 02
Integration Width 0 45
Retention Time 8 87
Integration Type A* VV



A072000012 A072000012 Thu, Jul 20, 2000 19 27
98-0109 1

12 92 in 2 periods
TZMU
Internal Standard TZMU-d3
Use Area
Absolute Retention Time
2 7 80 MRM 259 scans
208 0->175 0
Noise Thres 5 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 5
Expected RT 8 92
Area 10511
Height 900
Start Time 8 63
End Time 9 23
Integration Width 0 60
Retention Time 8 87
Integration Type A - BV



A072000012 A072000012 Thu, Jul 20, 2000 19 27
98 0109-1

12 92 in 2 periods
TZNG
Internal Standard TZNG-C13,N15
Use Area
Absolute Retention Time
2 7 80 MRM, 259 scans
236 0->132 0
Noise Thres 10 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 5
Expected RT 9 37
Area 7417
Height 564
Start Time 9 14
End Time 10 24
Integration Width 1 09
Retention Time 9 32
Integration Type A - BB

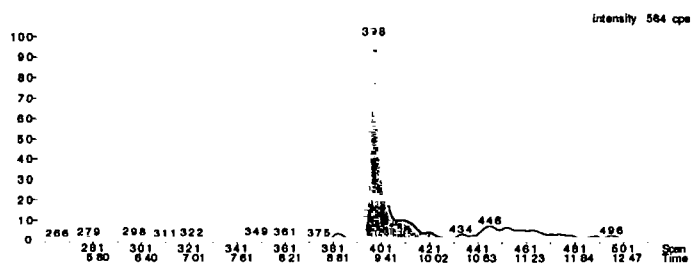
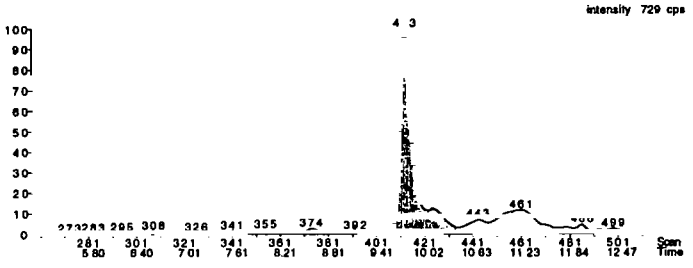


Figure 3: LC-MS/MS Chromatogram of 5 ppb fortified sample, 98-0109-1. (Continued)

MacQuan, version 1.5
Printed: Thu, Jul 20, 2000 19 44
Calibration File CFA072000 Path. LS-038 MAC #1 Data Bayer/Takeda A072000
Comments No Comments

A072000012 A072000012 Thu, Jul 20 2000 19 27
98-0109-1

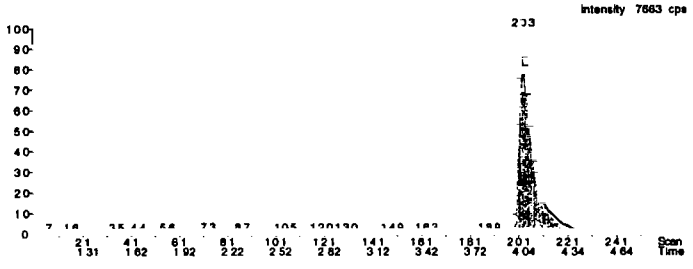
12 92 in 2 periods
TI-435
Internal Standard TI-435-d3
Use Area
Absolute Retention Time
2 7 80 MRM 259 scans
250 0->169 0
Noise Thres 5 0
Quant Thres 0 5
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 9 62
Area 9325
Height 728
Start Time 9 59
End Time 10 39
Integration Width 0 79
Retention Time 9 78
Integration Type A BV



A072000012 A072000012 Thu, Jul 20, 2000 19 27
98-0109-1

12 92 in 2 periods
MNG-d3
use as Internal Standard

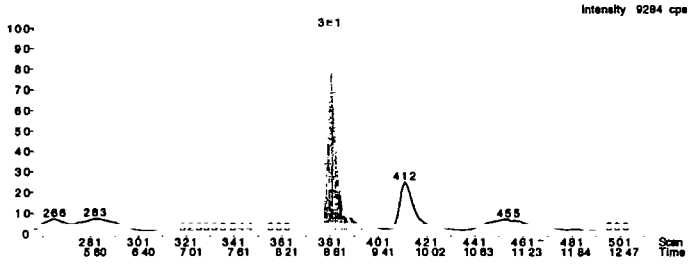
1 3 87 MRM, 257 scans
120 0->60 7
Noise Thres 10 0
Quant Thres 0 5
Min Width 6
Mult Width 8
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 3 88
Area 61503
Height 7663
Start Time 3 96
End Time 4 55
Integration Width 0 59
Retention Time 4 07
Integration Type A BB



A072000012 A072000012 Thu Jul 20, 2000 19 27
98-0109-1

12 92 in 2 periods
TMG-d3
use as Internal Standard

2 7 80 MRM, 259 scans
208 0 >132 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 8
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 8 86
Area 108011
Height 9277
Start Time 8 63
End Time 9 50
Integration Width 0 87
Retention Time 8 81
Integration Type A - BV



A072000012 A072000012 Thu Jul 20, 2000 19 27
98-0109-1

12 92 in 2 periods
TZMU-d3
use as Internal Standard

2 7 80 MRM 259 scans
209 0->175 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 8
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 8 89
Area 249594
Height 21564
Start Time 8 68
End Time 9 58
Integration Width 0 90
Retention Time 8 84
Integration Type A BV

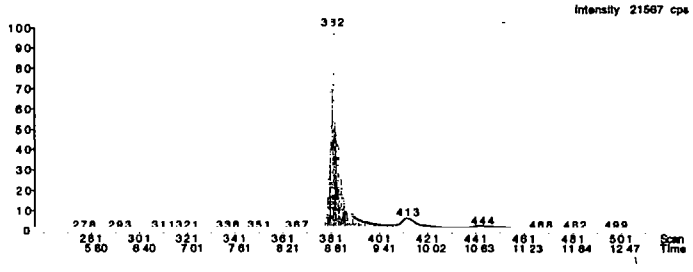
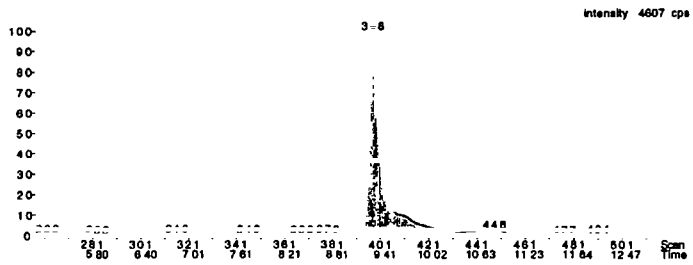


Figure 3: LC-MS/MS Chromatogram of 5 ppb fortified sample, 98-0109-1. (Continued)

A072000012 A072000012 Thu Jul 20, 2000 19 27
98-0109-1

12 92 in 2 periods
TZNG C13,N15
use as Internal Standard

2 7 80 MRM 259 scans
240 0->134 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 6 0
RT Win (secs) 2 0
Smooth 5
Expected RT 9 37
Area 62627
Height 4605
Start Time 9 14
End Time 10 42
Integration Width 1.27
Retention Time 9 32
Integration Type A BV



A072000012 A072000012 Thu Jul 20 2000 19 27
98-0109-1

12 92 in 2 periods
TI-436-d3
use as Internal Standard

2 7 80 MRM, 259 scans
253 0->172 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 6 0
RT Win (secs) 2 0
Smooth 5
Expected RT 9 79
Area 234325
Height 19415
Start Time 9 53
End Time 10 54
Integration Width 1 00
Retention Time 9 75
Integration Type A - BV

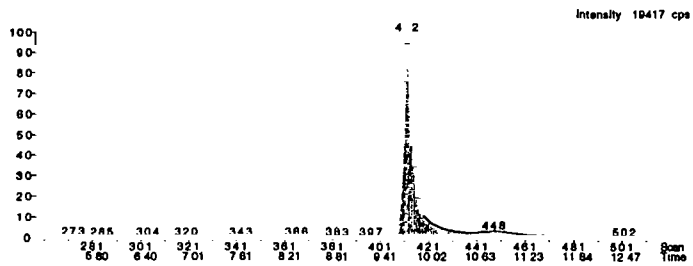
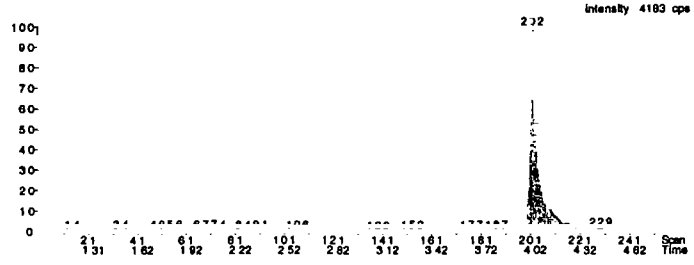


Figure 4: LC-MS/MS Chromatogram of 50 ppb fortified sample, 98-0109-10.

MacQuan, version 1 5
Printed Thu, Jul 20, 2000 19:44
Calibration File CFA072000 Path. LS-038 MAC #1 Data Bayer/Takeda·A072000
Comments· No Comments

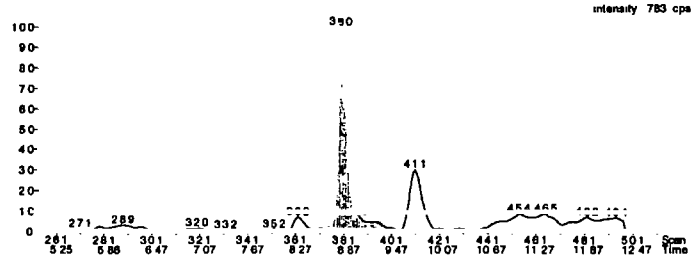
A072000022 A072000022 Thu, Jul 20, 2000 18 19
98 0109-10

12 82 in 2 periods
MNG
Internal Standard MNG-d3
Use Area
Absolute Retention Time
1 3 79 MRM 253 scans
117 0->60 7
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 1
Expected RT 3 87
Area 19909
Height 4183
Start Time 3 96
End Time 4 37
Integration Width 0 41
Retention Time 4 04
Integration Type A - BB



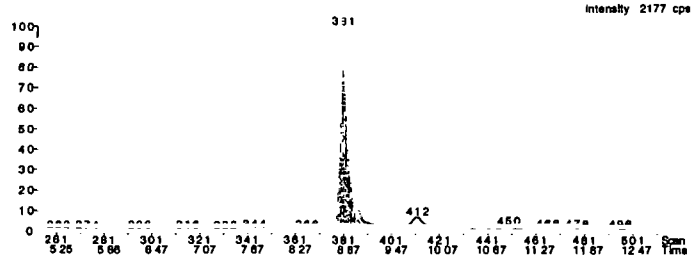
A072000022 A072000022 Thu, Jul 20 2000 18 19
98-0109-10

12 82 in 2 periods
TMG
Internal Standard TMG-d3
Use Area
Absolute Retention Time
2 7 77 MRM, 259 scans
205 0->132 0
Noise Thres 5 0
Quant Thres 0 2
Min Width 6
Mult Width 9
Base Width 60
RT Win (secs) 2 0
Smooth 5
Expected RT 8 91
Area 8622
Height 783
Start Time 8 58
End Time 9 53
Integration Width 0 67
Retention Time 8 84
Integration Type A BB



A072000022 A072000022 Thu Jul 20 2000 18 19
98-0109 10

12 82 in 2 periods
TZMU
Internal Standard TZMU-d3
Use Area
Absolute Retention Time
2 7 77 MRM, 259 scans
206 0 >175 0
Noise Thres 5 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 5
Expected RT 8 92
Area 23425
Height 2176
Start Time 8 69
End Time 9 44
Integration Width 0 75
Retention Time 8 87
Integration Type A - BV



A072000022 A072000022 Thu Jul 20 2000 18 19
98 0109-10

12 82 in 2 periods
TZNG
Internal Standard TZNG-C13,N15
Use Area
Absolute Retention Time
2 7 77 MRM, 259 scans
236 0->132 0
Noise Thres 10 0
Quant Thres 0 2
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 2 0
Smooth 5
Expected RT 9 37
Area 17153
Height 1270
Start Time 9 17
End Time 10 84
Integration Width 1 47
Retention Time 9 32
Integration Type A BV

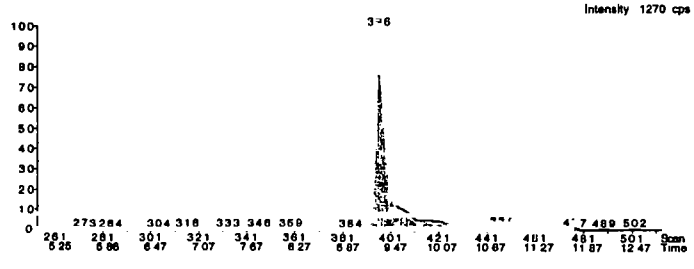
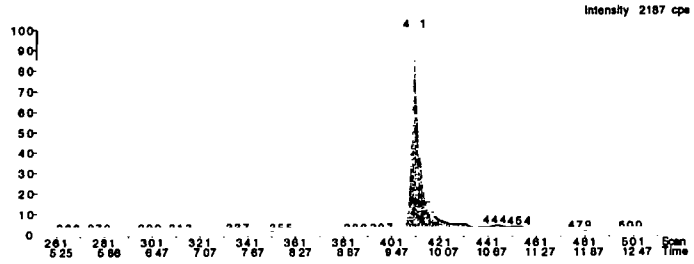


Figure 4: LC-MS/MS Chromatogram of 50 ppb fortified sample, 98-0109-10. (Continued)

MacQuan, version 1 5
Printed Thu, Jul 20, 2000 19 44
Calibration File: CFA072000 Path LS-038 MAC #1.Data Bayer/Takeda A072000
Comments: No Comments

A072000022 A072000022 Thu, Jul 20 2000 18 19
98-0109 10

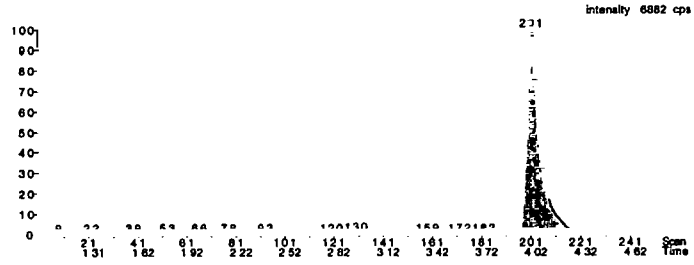
12 82 in 2 periods
TI-435
Internal Standard TI-435-d3
Use Area
Absolute Retention Time
2 7 77 MRM 259 scans
250 0 >169 0
Noise Thres 5 0
Quant Thres 0 5
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 9 82
Area 26526
Height 2188
Start Time 9 56
End Time 10 49
Integration Width 0 93
Retention Time 9 77
Integration Type A BV



A072000022 A072000022 Thu Jul 20, 2000 18 19
98 0109-10

12 82 in 2 periods
MNG-d3
use as Internal Standard

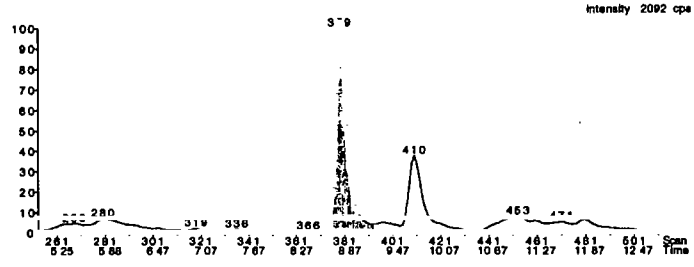
1 3 79 MRM, 253 scans
120 0->80 7
Noise Thres 10 0
Quant Thres 0 5
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 3 88
Area 44318
Height 6882
Start Time 3 95
End Time 4 32
Integration Width 0 38
Retention Time 4 02
Integration Type A BB



A072000022 A072000022 Thu Jul 20, 2000 18 19
98-0109-10

12 82 in 2 periods
TMG-d3
use as Internal Standard

2 7 77 MRM 259 scans
208 0->132 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 8 86
Area 22929
Height 2087
Start Time 8 63
End Time 9 20
Integration Width 0 57
Retention Time 8 81
Integration Type A - BV



A072000022 A072000022 Thu Jul 20 2000 18 19
98-0109-10

12 82 in 2 periods
TZMU-d3
use as Internal Standard

2 7 77 MRM, 259 scans
209 0 >175 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 8 89
Area 54706
Height 4865
Start Time 8 66
End Time 9 47
Integration Width 0 81
Retention Time 8 84
Integration Type A - BV

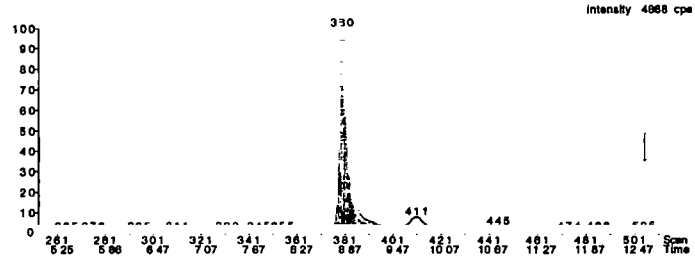


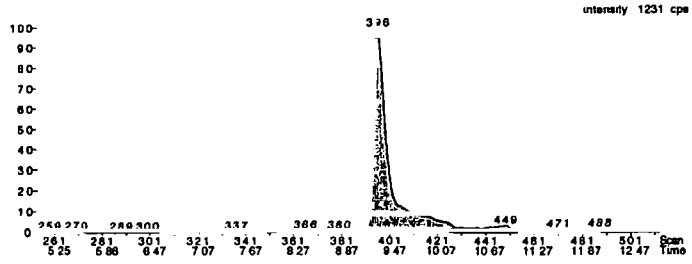
Figure 4: LC-MS/MS Chromatogram of 50 ppb fortified sample, 98-0109-10. (Continued)

MacQuan, version 1 5
Printed: Thu, Jul 20, 2000 19:44
Calibration File. CFA072000 Path: LS-038 MAC #1:Data.Bayer/Takeda:A072000:
Comments No Comments

A072000022 A072000022 Thu, Jul 20, 2000 18 19
98-0109-10

12 82 in 2 periods
TZNG-C13 N15
use as Internal Standard

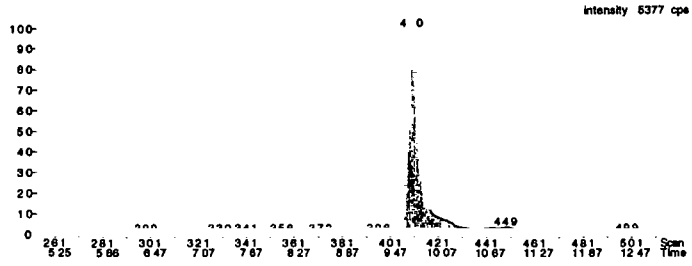
2 7 77 MRM, 259 scans
240 0->134 0
Noise Thres 10 0
Quant Thres 1 0
Min Width 6
Mult Width 6
Base Width 60
RT Win (secs) 20
Smooth 5
Expected RT 9 37
Area 17560
Height 1230
Start Time 9 14
End Time 10 43
Integration Width 1 29
Retention Time 9 32
Integration Type A BV



A072000022 A072000022 Thu Jul 20, 2000 18 19
98-0109-10

12 82 in 2 periods
TI 435-d3
use as Internal Standard

2 7 77 MRM, 259 scans
253 0->172 0
Noise Thres 10 0
Quant Thres. 1 0
Min Width 6
Mult Width 6
Base Width 80
RT Win (secs) 20
Smooth 5
Expected RT 9 79
Area 67822
Height 5375
Start Time 9 56
End Time 10 55
Integration Width 0 99
Retention Time 9 74
Integration Type A - BV



Appendix 1: Standard Preparation

The following are general working procedures for the preparation of standard solutions. Therefore, given weights and volumes do not necessarily correspond exactly to the weights, volumes and concentrations documented in the raw data.

Solvent mixtures
Solvent A: acetonitrile/water (1/1; v/v); **Solvent B**: acetonitrile/water (1/4; v/v)

Standard Stock Solutions

400 mg/L stock solution of TI-435: Weigh approximately 10 ± 0.01 mg of TI-435 into a 25-mL volumetric flask. Dilute to volume with Solvent A.

400 mg/L stock solution of TZNG: Weigh approximately 10 ± 0.01 mg of TZNG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

400 mg/L stock solution of TZMU: Weigh approximately 10 ± 0.01 mg of TZMU into a 25-mL volumetric flask. Dilute to volume with Solvent A.

400 mg/L stock solution of MNG: Weigh approximately 10 ± 0.01 mg of MNG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

400 mg/L stock solution of TMG: Weigh approximately 10 ± 0.01 mg of TMG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

200 mg/L stock solution of d_3-TI-435: Weigh approximately 5 ± 0.01 mg of d_3-TI-435 into a 25-mL volumetric flask. Dilute to volume with Solvent A.

200 mg/L stock solution of ^{13}C, ^{15}N-TZNG: Weigh approximately 5 ± 0.01 mg of ^{13}C, ^{15}N-TZNG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

200 mg/L stock solution of d_3-TZMU: Weigh approximately 5 ± 0.01 mg of d_3-TZMU into a 25-mL volumetric flask. Dilute to volume with Solvent A.

200 mg/L stock solution of d_3-TMG: Aliquot approximately 5 ± 0.01 mg of d_3-TMG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

200 mg/L stock solution of d_3-MNG: Weigh approximately 5 ± 0.01 mg of d_3-MNG into a 25-mL volumetric flask. Dilute to volume with Solvent A.

Remark: Before further use, the standard stock solutions should be ultrasonicated for about one minute to achieve complete dissolution of the compounds.

Internal Standard Solution

(MIX1) 2.5 mg/L of each internal standard:
Pipette 2.5 mL of each of the stock solutions of d_3-TI-435, ^{13}C, ^{15}N-TZNG, d_3-TZMU, d_3-TMG and d_3-MNG into a 200-mL volumetric flask and dilute to volume with Solvent B.

Appendix 1 (cont.): Standard Preparation

Fortification Standard Solution

(MIX2) 1 mg/L of each compound: Pipette 0.25 mL of the stock solutions of TI-435, TZNG, TZMU, TMG and MNG into a 100-mL volumetric flask and dilute to volume with Solvent B.

Mixed Native Standard Solution

(MIX3) 10 mg/L of each compound: Pipette 2.5 mL of the stock solutions of TI-435, TZNG, TZMU, TMG and MNG into a 100-mL volumetric flask and dilute to volume with Solvent B.

Calibration Standard Solutions

(MIX4) 0.5 mg/L of each compound (1250 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 5 mL of the mixed native standard solution MIX3 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX5) 0.25 mg/L of each compound (625 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 2.5 mL of the mixed native standard solutions MIX3 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX6) 0.1 mg/L of each compound (250 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 1 mL of the mixed native standard solutions MIX3 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX7) 0.05 mg/L of each compound (125 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 0.5 mL of the mixed native standard solutions MIX3 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX8) 0.025 mg/L of each compound (62.5 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 0.25 mL of the mixed native standard solutions MIX3 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX9) 0.01 mg/L of each compound (25 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 1 mL of the fortification standard solutions MIX2 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX10) 0.005 mg/L of each compound (12.5 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 0.5 mL of the fortification standard solutions MIX2 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX11) 0.0025 mg/L of each compound (6.25 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 0.25 mL of the fortification standard solutions MIX2 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

(MIX12) 0.001 mg/L of each compound (2.5 µg/kg sample equivalents) and 0.05 mg/L of each internal standard: Pipette 0.1 mL of the fortification standard solutions MIX2 and 2 mL of the internal standard solution MIX1 into a 100-mL volumetric flask and dilute to volume with Solvent B.

All standard solutions should be stored in the freezer.