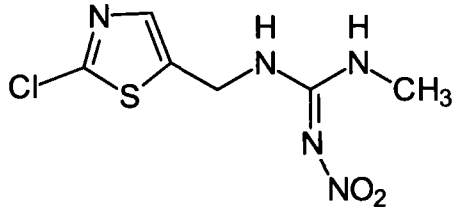
2.0 Introduction

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**

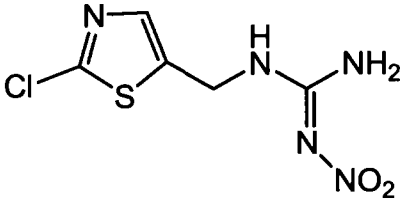
Chemical Name (CAS): (*E*)-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine



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)**

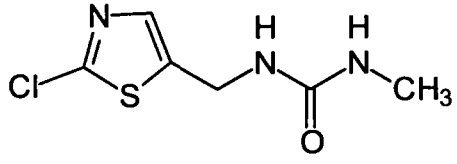
Chemical Name (CAS): *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine



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)**

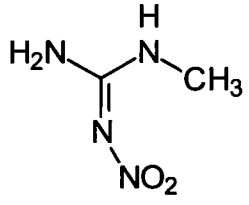
Chemical Name (CAS): *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-methylurea



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

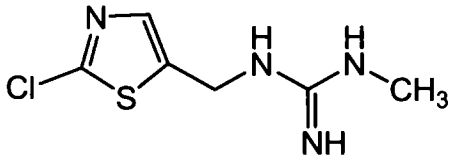
- **MNG (methyl-nitroguanidine)**

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

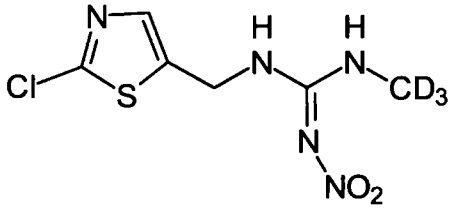
- **TMG (thiazolyl-methylguanidine)** **Chemical Name (CAS):** *N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-methylguanidine



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

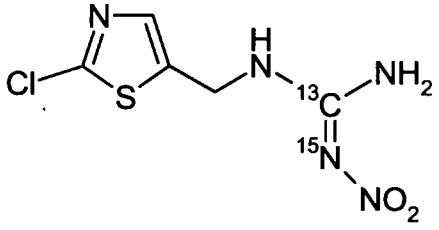
The Reference Substances (Internal Standards) have the following structures:

- **TI-435-d_3**



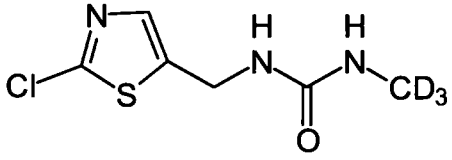
Formula:	$C_6H_5ClD_3N_5O_2S$
Molar Mass:	252.7 g/mol
Reference No.:	M01288
Purity:	99.0%
Expiration Date:	October 2002

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



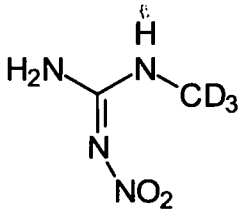
Formula:	$C_4{}^{13}CH_6ClN_4{}^{15}NO_2S$
Molar Mass:	237.7 g/mol
Reference No.:	M01322
Purity:	99.0%
Expiration Date:	February 2001

- **TZMU-d_3**



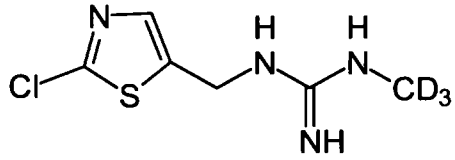
Formula:	$C_6H_5ClD_3N_3OS$
Molar Mass:	208.7 g/mol
Reference No.:	M01897
Purity:	99.2%
Expiration Date:	October 2002

- **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):

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

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

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

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

$$\text{Conc}_{\text{Analyte}} = \frac{\text{Int.Ratio} - \text{Intercept}}{\text{Slope}}$$

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

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

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)

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.