Analytical Method for the Determination of YRC 2894 and Two Metabolites in Soil by High Performance Liquid-Chromatography Electrospray Tandem Mass Spectrometry (LC-ESI/MS/MS)

1.0 SUMMARY

An analytical method was developed to quantitate YRC 2894, YRC 2894-amide and YRC 2894-sulfonic acid in soil using Dionex Accelerated Solvent Extractor (ASE) and electrospray LC/MS-MS. Soil (25-g) was extracted with methanol : 5% acetic acid in water (4:1, v/v) using ASE extractor at temperature of 90 °C for 10 min. After extraction, deuterated internal standards of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid were added to the soil extract. The extracts were concentrated to about 10 mL in a Turbo Vap LV (Zymark). The concentrated extract was centrifuged and a 2-mL aliquot of the extract was removed for analysis by electrospray LC/MS/MS.

2.0 *INTRODUCTION*

This method was developed to support the YRC 2894 soil dissipation studies as set forth in the EPA guidelines for Pesticide Registration, Subdivision N, Section 166-1. This method is a modification of Bayer AG method 00440, MR-368/96[2] and MR-21/97[3] and employs a new extraction technique -- accelerated solvent extraction (ASE). This technique allows faster extractions than classical methods. In addition, it consumes less solvent and thus generates less waste after extraction.

3.0 EXPERIMENTAL

3.1 *Location*

The development and validation of the method was conducted at Bayer Research Park (BRP), Bayer Corporation, Agriculture Division, Stilwell, Kansas from May to July 97. The study report and raw data (notebook number 97B075) are archived at the Bayer Research Park, Stilwell, Kansas.

3.2 Equipment

- Volumetric flasks (10- and 100-mL)
- Volumetric pipettes (Baxter or equivalent)
- Graduated cylinders (50- and 100-mL)
- Centrifuge tubes (13-mL)
- Syringes, gas-tight type (25-, 50-, 100-, 250- and 500-µL)
- Pipetman (Gilson or equivalent)
- Pasteur pipettes (Kimble or equivalent)
- Autosampler vials (2-mL, Wheaton #223682 or equivalent)
- Vials, 60-mL VOA (volatile organic analysis, I-Chem S236-0060 or equivalent)
- Analytical Balance (Mettler A163 or equivalent)
- Balance, Top loader, capable of weighing to the nearest 0.01 g
- Turbo Vap LV (Zymark)
- Centrifuge (IEC DPR-6000)
- Accelerated Solvent Extractor ASE 200 (Dionex)
- Purosper RP18 endcapped, 125 x 2 mm, 5-µm column (Merck)
- TSQ 7000 LC/Tandem Mass Spectrometer with Electrospray interface and gradient HPLC, or equivalent (Finnigan Corp)
- Solvent degasser (Phenomenex Degassex DG-4000 or equivalent)
- Column heater (Eppendorf CH-30 column heater or equivalent)

3.3 Reagents and Solvents

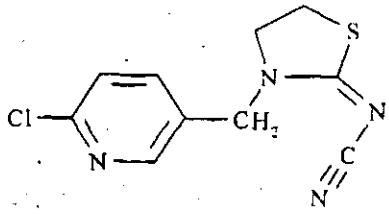
- Methanol (MeOH; HPLC Grade, Burdick & Jackson or equivalent)
- Acetonitrile (ACN; HPLC Grade, Burdick & Jackson or equivalent)
- Water (HPLC Grade, Burdick & Jackson or equivalent)
- Glacial Acetic Acid (Mallinckrodt or equivalent)
- Hydromatrix (0019-8003, Varian)
- 5% acetic acid (v/v) in water. Add 10 mL acetic acid and dilute to 200 mL with HPLC grade water.
- Extraction solvent, methanol : 5% acetic acid (4:1, v/v) . Add 800 mL methanol to 200 mL of 5% acetic acid in water.
  The above extraction solvent is also the same as 1% acetic acid in [methanol : water 4:1] Add 800 mL methanol to 200 mL water. Add 10 mL of acetic acid into the 1000 mL mixed solvents.
- Mobile Phase A: 1% (v/v) acetic acid in water. Add 10 mL acetic acid and dilute to 1 liter with water.
- Mobile Phase B: 1% (v/v) acetic acid in acetonitrile. Add 10 mL acetic acid and dilute to 1

liter with acetonitrile.

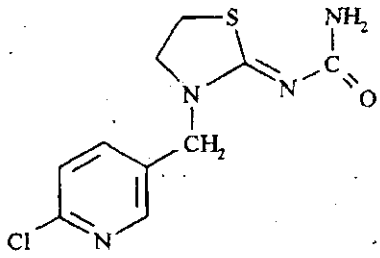
800:200:1 water:methanol:acetic acid (v/v/v). Add 800 mL water into solution containing 200 mL methanol and 1 mL acetic acid.

3.4 Analytical Standards

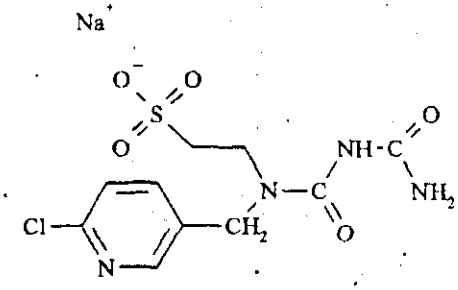
Common Name: **YRC 2894**
Standard Ref.#: K-722
Empirical Formula: $C_{10}H_9ClN_4S$
Molecular Weight: 252.73
Chemical Purity: 99.3%
Expiration Date: 8/98
Chemical Name: [3-[(6-Chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]cyanamide
CAS Number: 111988-49-9



Common Name: **YRC 2894-amide**
Standard Ref.#: K-723
Empirical Formula: $C_{10}H_{11}ClN_4OS$
Molecular Weight: 270.74
Chemical Purity: 99%
Expiration Date: May 2001
Chemical Name: (Z)-[3-[(6-Chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]urea



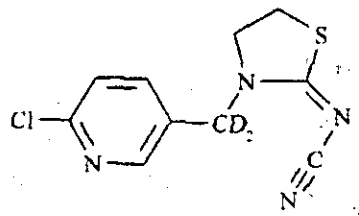
Common Name: **YRC 2894-sulfonic acid**
Standard Ref.#: K-656
Empirical Formula: $[C_{10}H_{12}ClN_4O_5S]^- Na^+$
Molecular Weight: 358.74
Chemical Purity: 91% (calculated as sulfonic acid)
97% (sodium salt)
Expiration Date: 2/99
Chemical Name: Sodium 2-[[[(aminocarbonyl)amino] carbonyl][(6-chloro-3-pyridinyl]amino] ethanesulfonate



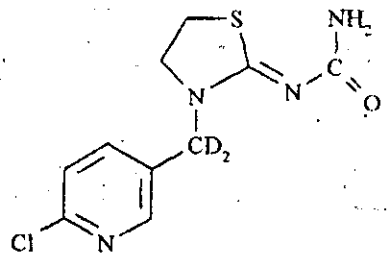
The following are the internal standards used in this study:

Common Name: **YRC 2894-d_2**
Standard Ref.#: K-737

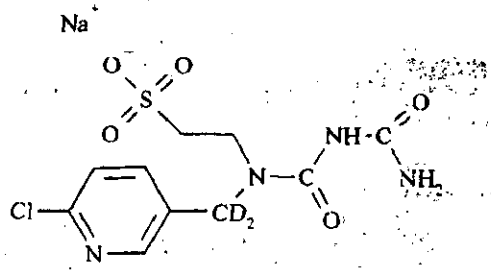
Empirical Formula: $C_{10}H_7D_2ClN_4S$
Molecular Weight: 254.7
Chemical Purity: 96%
Expiration Date: 8/97



Common Name: **YRC 2894-amide-d_2**
Standard Ref.#: K-738
Empirical Formula: $C_{10}H_9D_2ClN_4OS$
Molecular Weight: 272.8
Chemical Purity: 94%
Expiration Date: 11/98



Common Name: **YRC 2894-*sulfonic acid*-d_2**
Standard Ref.#: K-739
Empirical Formula: $[C_{10}H_{10}D_2ClN_4O_5S]^- Na^+$
Molecular Weight: 360.8
Chemical Purity: 97%
Expiration Date: 6/98



3.5 Safety and Health

The toxicity or carcinogenicity of all reagents used in this method have not been precisely determined, and thus each chemical compound must be treated as a potential health hazard. Therefore, exposure to these chemicals must be reduced to the lowest possible level.

3.6 Procedures

3.6.1 Preparation of Standards and Reagents

3.6.1.1 Native Analyte Solutions

Prepare 100-ppm stock solution (nominally 0.1 mg/mL) of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid (see section 3.3 for standard reference numbers) by weighing 10-20 mg of each using an analytical balance and adding the corresponding volumetric amount of acetonitrile (for YRC 2894 sulfonic acid, prepare it in 1:1 acetonitrile:water (v/v)). If the purity of a standard is < 99%, weigh the appropriate amount to correct for the purity.

E.g.: Add 10 mg of YRC 2894 to 100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of YRC 2894 amide to 100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of YRC 2894 sulfonic acid to 100-mL volumetric flask and dilute to the mark with 1:1 acetonitrile:water (v/v).

Appropriately label these solution to reflect the actual concentration of the analyte. Store all solutions in a freezer when not in use (< -7 °C).

3.6.1.2 Mixed Native Analyte Solutions

Prepare mixed solutions from the stock solutions of the individual native analytes (Section 3.6.1.1) as follows (Allow the stock solutions to equilibrate to room temperature prior to use):

E.g. 10-ppm mixed native solution
Add 10 mL of each solution of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid from Section 3.6.1.1 to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

E.g. 5-ppm mixed native solution
Add 5 mL of each solution of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid from Section 3.6.1.1 to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

E.g. 1-ppm mixed native solution
Add 1 mL of each solution of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid from Section 3.6.1.1 to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

E.g. 0.2-ppm mixed native solution
Add 2 mL of mixed solutions of 10-ppm of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

E.g. 0.04-ppm mixed native solution
Add 4 mL of mixed solutions of 1-ppm of YRC 2894, YRC 2894 amide and YRC 2894 sulfonic acid to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

Store all solutions in a refrigerator (< 8 °C) when not in use.

3.6.1.3 Internal Standard Stock Solutions

Prepare 100-ppm stock solution (nominally 0.1 mg/mL) of YRC 2894-d_2, YRC 2894 amide-d_2 and YRC 2894 sulfonic acid-d_2 (see section 3.3 for standard reference numbers) by weighing 10-20 mg of each using an analytical balance and adding the corresponding volumetric amount of acetonitrile (for YRC 2894 sulfonic acid-d_2, prepare it in 1:1 acetonitrile:water (v/v)). If the purity of a standard is < 99%, weigh the appropriate amount to correct for the purity.

E.g. Add 10 mg of YRC 2894-d_2 to 100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of YRC 2894 amide-d_2 to 100-mL volumetric flask and dilute to the mark with acetonitrile.
Add 10 mg of YRC 2894 sulfonic acid-d_2 to 100-mL volumetric flask and dilute to the mark with 1:1 acetonitrile:water (v/v).

Appropriately label these solution to reflect the actual concentration of the analyte. Store all solutions in a freezer when not in use (< -7 °C).

3.6.1.4 Internal Standard Mixed Solutions

Prepare internal standard mixed solutions from the stock solutions of the individual internal standard Section (3.6.1.3) as follows (Allow the stock solutions to equilibrate to room temperature prior to use):

E.g. 5-ppm internal standard mixed solutions
Add 5 mL of each solution of YRC 2894-d_2, YRC 2894 amide-d_2 and YRC 2894 sulfonic acid-d_2 from 3.6.1.3 to a 100-mL volumetric flask and dilute to the mark with 800:200:1 water:methanol:acetic acid (v/v/v).

Store all solutions in a refrigerator (< 8 °C) when not in use.

3.6.2 Extraction -- Accelerated Solvent Extractor (ASE)

Figure 1 shows the analytical scheme for the extraction of YRC 2894 and its metabolites from soil. The detailed stepwise procedure is summarized as follows:

1. Screw an end cap onto the end of the 33-mL extractor body which is closest to the Dionex logo. (This is designated as the bottom of the extractor cell)

2. Insert a disposable cellulose filter in the bottom of the 33-mL extractor cell with an insertion tool.

3. Weigh approximately 2 g hydromatrix and load it into the 33-mL extractor cell with a funnel.

4. Weigh 25 ± 0.1 g of sample and load it into the 33-mL extractor cell with a funnel.

5. Label and put the corresponding 60-mL collection vial into the vial tray.

6. Operate the ASE under the following conditions:

Solvent:	methanol : 5% acetic acid (4:1, v/v) (This is the same as 1% acetic acid in [methanol : water 4:1])
Pressure:	1500 psi
Temperature:	90 °C
Static Time:	5 min
Cycle:	1
Flush Volume:	50%
Purge Time:	60 s

7. Remove the collection vials from the vial tray and use a marker to mark a line which is about 2 cm from the bottom of the vial. (This is roughly equal to 8-9 mL solvent)

8. Add 0.5 mL of 5-ppm mixed internal standard solutions (Section 3.6.1.4) into each collection vial and mix.

9. Evaporate the extract to slightly below the mark under nitrogen in a Turbo Vap LV evaporator maintained at < 45 °C.

10. Transfer the extract from the collection vial into a 13-mL centrifuge tube. Rinse the collection vial with ≈1 mL HPLC-grade water. Transfer the rinsate into the centrifuge tube.

11. Dilute the extract in the centrifuge tube to 10 mL with HPLC-grade water.

12. Centrifuge the diluted extract for ≈7 min at ≈2000 rpm.

13. Transfer about 2 mL of the extract from the centrifuge tube into an autosampler vial for LC/MS/MS analysis.

3.6.3 HPLC/MS/MS Analysis

3.6.3.1 HPLC Conditions

Column:	Purospher RP 18 endcapped, 125 x 2 mm, 5 microns
[1]Column Oven:	40 °C
[2]Flow (column):	300 µL/min
Split ratio (interface):	1:1
Flow (interface):	150 µL/min
Injector Volume:	100 µL (using 50-µL loop)
Flush solvent (Autosampler):	MeOH
Mobile Phase:	A: 1% (v/v) acetic acid in water B: 1% (v/v) acetic acid in acetonitrile

Time (min)	% Solvent A	% Solvent B
0	100	0
1	100	0
12	60	40
14	40	60
16	10	90
20	10	90
20.5	100	0
26	100	0

Approx. retention times:

YRC 2894	=	12.4 min
YRC 2894 amide	=	10.5 min
YRC 2894 sulfonic acid	=	9.39 min

If column heater is not used and the flow rate through the column is equal to 200 µL/min, the retention times will change as follows:

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[1]*It is very important to maintain column temperature at 40 °C*

[2]*Solvent degassing is absolutely necessary to ensure reproducibile HPLC column retention times.*

Approx. retention times:	YRC 2894	=	15.2 min
	YRC 2894 amide	=	13.3 min
	YRC 2894 sulfonic acid	=	12.7 min

3.6.3.2 MS/MS Conditions

The mass spectrometer (MS) begins in the negative mode for the analysis of YRC 2894 sulfonic acid and then is switched to the positive mode for the analysis of YRC 2894 amide and YRC 2894. The desired ions are accelerated by voltage and separated by mass in the first quadrupole (MS1). The most intensive ions (the protonated or de-protonated ions) of the analytes (parent ions) are impulsed with argon in the collision cell (MS2). Fragments of these ions (daughter ions) are separated by mass in the third quadrupole (MS3) and detected. The analytes and the selected ions are shown in Table 1. The general operation conditions of MS is as follows:

CID Pressure:	2.4 mtorr/argon
Capillary Temperature:	275 °C
Sheath Gas Pressure:	80 psi /N_2
Auxiliary Gas Flow (N_2):	0 mL/min

3.6.4 Detector Linearity

Prepare seven-point data solvent linearity curves using 0-, 0.004-, 0.01-, 0.1-, 0.5-, 1.0- and 2.5-ppm standards (concentration in sample equivalent), each containing 0.25 µg/mL (0.1-ppm equivalent for a 25-g sample) of internal standards as shown in Table 2. Analyze each standard solution by LC/MS/MS in **duplicate** (two injections for each solution).

Solution for 0-ppm sample equivalent standard (0 µg/mL): Prepare by adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 to a 10-mL volumetric flask, and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 0.004-ppm sample equivalent standard (0.01 µg/mL): Prepare by adding 500 µL of the 0.2-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 0.01-ppm sample equivalent standard (0.025 µg/mL): Prepare by adding 1.25 mL of the 0.2-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 0.1-ppm sample equivalent standard (0.25 µg/mL): Prepare by adding 500 µL of the 5-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5- ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 0.5-ppm sample equivalent standard (1.25 µg/mL): Prepare by adding 2.5 mL of the 5-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 1.0-ppm sample equivalent standard (2.5 µg/mL): Prepare by adding 2.5 mL of the 10-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

Solution for 2.5-ppm sample equivalent standard (6.25 µg/mL): Prepare by adding 6.25 mL of the 10-ppm mixed native solution from Section 3.6.1.2 to a 10-mL volumetric flask, adding 500 µL of the 5-ppm internal standard from Section 3.6.1.4 and bringing to volume with 800:200:1 water:methanol:acetic acid (v/v/v).

3.6.5 Calibration Curve

Prepare four-point calibration curves using 0.004-, 0.01-, 0.1- and 0.5-ppm standards (concentration in sample equivalent), each containing 0.25 µg/mL (0.1 ppm equivalent for a 25-g sample) of internal standards as shown in Table 2. Analyze each standard solution by LC/MS/MS in **triplicate** (three injections for each solution).

3.7 Method Validation

3.7.1 Linearity in Soil Matrix

Prepare seven-point data matrix linearity curves using 0-, 0.004-, 0.01-, 0.1-, 0.5-, 1.0- and 2.5-ppm standards (concentration in sample equivalent), each containing 0.25 µg/mL (0.1 ppm equivalent for a 25-g sample) of internal standards as shown in Table 3. Analyze each standard solution by LC/MS/MS in **duplicate** (two injections for each solution).

1. Extract seven 25-g control soil according to Section 3.6.2 step 1-6.

2. Remove collection vials from ASE extractor. Add 0-, 0.004-, 0.01-, 0.1-, 0.5-, 1.0- and 2.5-ppm standards (concentration in sample equivalent), each containing 0.25 µg/mL (0.1 ppm equivalent for a 25-g sample) of internal standards as shown in Table 3.

3. Follow steps 8-12 according to Section 3.6.2. .

3.7.2 Recovery Validations

1. Take fourteen 33-mL extractor cells. Designate seven cells each as 0.01 ppm and five cells each as 0.1 ppm. Designate the remaining two cells as controls (unspiked).

2. Follow step 1-4 in Section 3.6.2

3. Fortify the soil at 0.01-ppm level (seven replicates) by adding 250 µL of 1-ppm mixed native solution from Section 3.6.1.2 into a 25-g soil.

4. Fortify the soil at 0.1-ppm level (five replicates) by adding 500 µL of 5-ppm mixed native solution in Section 3.6.1.2 into a 25-g soil.

5. No fortification is needed for the remaining 2 extractor cells (controls).

6. Allow the soil in the extractor cells to sit at least 15 minutes before extraction.

7. Process and analyze the samples as described from step 5-13 in Section 3.6.2.

4.0 QUANTITATION

Quantitation is based on the use of a four point calibration curve analyzed in triplicate using internal standards to adjust for instrument response. Each analyte is quantitated by using its deuterated analog. The concentration (ppm) of each analyte is calculated by applying the area ratio (native analyte area to internal standard area) to the calibration curve.

4.1 Response Factor

The response factor for each analyte is calculated according to the following formula:

$$RF = \frac{(Area_{NAT})(Conc_{IS})}{(Area_{IS})(Conc_{NAT})}$$

where RF = Response factor
$Area_{NAT}$ = Area of response for the daughter ion from the native standard
$Area_{IS}$ = Area of response for the daughter ion from the internal standard
$Conc_{NAT}$ = Concentration of the native standard (ng/mL)
$Conc_{IS}$ = Concentration of the internal standard (ng/mL)

The average response factor is calculated as follows:

$$RF_{AVG} = \frac{\sum_{i=1}^{12} RF_i}{12}$$

4.2 Analyte Concentration

The analyte concentration is calculated as follows:

$$\text{Analyte Conc (ppb)} = Conc_{NAT} = \frac{(Area_{NAT})(Conc_{IS})}{(RF_{AVG})(Area_{IS})}$$

where RF_{AVG} = Average response factor
$Area_{NAT}$ = Area of response for the native product ion from the extract
$Area_{IS}$ = Area of response for the internal standard product ion from the extract
$Conc_{NAT}$ = Calculated amount (ppm in the sample), uploaded from the MS
$Conc_{IS}$ = Concentration of the internal standard (ng/mL)

4.3 Recovery in Spiked Validation Samples

$$\%\text{Recovery} = \frac{(Conc_{NAT})}{(\text{Spike Level})} \times 100\%$$

where $Conc_{NAT}$ = Calculated amount (ppm in the sample), uploaded from the MS
Spike Level= Concentration (ppm) at which the matrix spike was prepared

5.1 General Considerations

The internal standards used, which have 4 amu higher than the corresponding non-deuterated

analytes, were based on the measurement of the ^{37}Cl isotope of the d_2-analytes. Initially, no column heater and degasser was used and the HPLC mobile phase flow rate was maintained at 200 µL/min. Later, it was found that it is very critical to maintain column temperature at 40 °C and thoroughly degassed solvents to ensure integrity of the HPLC column retention times. To speed up the analysis and increase the column back pressure, the column flow rate was increased to 300 µL/min. The results reported in Section 5.2-5.5 were based on the flow rate of 200 µL/min with no column heater and degasser. However, all the sample analysis in the YRC 2894 soil dissipation study (Report 107900)[1] were changed to flow rate of 300 µL/min with column heater maintained at 40 °C. These changes to the method did not qunatitatively affect the results of the analyses and revalidation of the improved method was not necessary.

5.4 Limit of Detection (LOD) and Quantitation (LOQ)

As demonstrated in the linearity response analyses, the limit of detection (LOD) for YRC 2894 and its metabolites in matrix is 0.01 ppm or less (sample equivalents), which is equivalent to 1.25 ng (i.e. 50 µL of a 0.025-µg/mL solution). Based on the acceptable recoveries (70-120% with a relative standard deviation of 20% or less) from the method validation, the limit of quantitation (LOQ) for YRC 2894 and its metabolites in the soil matrix has been established at 0.01 ppm

7.0 REFERENCES

1. Wood, S. E., Bayer Report No., 107900. *Terrestrial Field Dissipation of YRC 2894 in Wisconsin soil.*

2. Sommer, H, *Method 00110 (MR-368/96) for liquid chromatographic determination of YRC 2894 and the metabolites YRC 2894-amide and YRC 2894-sulfonic acid in soil,* Bayer AG, Jan 20, 1997.

3. Sommer, H, *Method 00440, M001 (MR-21/97) for liquid chromatographic determination of YRC 2894 and the metabolites YRC 2894-amide and YRC 2894-sulfonic acid in soil,* Bayer AG, Jan 23, 1997.

4. 40 CFR Part 136 Appendix B; Federal Register 49, 198-199 -- Definition and Procedure for the determination of the Method Detection Limit - Revision 1.11.

Table 1. MS/MS Selected Ions

Substance	Principle	m/z Parent	m/z Daughter	Collision energy (eV)
YRC 2894	ESP+	253	126	-25
YRC 2894-d_2	ESP+	257*	130	-25
YRC 2894 amide	ESP+	271	126	-30
YRC 2894 amide-d_2	ESP+	275*	130	-30
YRC 2894 sulfonic acid	ESP-	335	249	23
YRC 2894 sulfonic acid-d_2	ESP-	339*	253	23

* The mass of 4 amu higher than the non-deuterated analyte is based on the measurement of the ^{37}Cl isotope of the two times deuterated analyte which is used as internal standard.

Table 2. Detector Linearity and Calibration Standards

Combine and dilute to 10 mL with 800:200:1 water:methanol:acetic acid (v/v/v)						
Sample Fortification Level (ppm)[1]	Amount of Native Added per 25-g Sample Equivalent		Native Analyte Conc in 10 mL	Amount of Internal Standard Added per 25-g Sample Equivalent [2]		Internal Standard Conc in 10 mL
	µg	mL Solution	µg/mL	µg	mL Solution [3]	µg/mL
0	0	0	0	2.5	0.5	0.25
0.004	0.1	0.5 [4]	0.01	2.5	0.5	0.25
0.01	0.25	1.25 [4]	0.025	2.5	0.5	0.25
0.1	2.5	0.5 [5]	0.25	2.5	0.5	0.25
0.5	12.5	2.5 [5]	1.25	2.5	0.5	0.25
1.0	25	2.5 [6]	2.5	2.5	0.5	0.25
2.5	62.5	6.25 [6]	6.25	2.5	0.5	0.25

[1] Sample concentration levels in 25-g sample equivalents
[2] Internal standard concentration is 0.1 ppm (sample equivalents) in all samples
[3] 5-ppm mixed internal standard spiking solution from section 3.6.1.4.
[4] 0.2-ppm mixed native solution from section 3.6.1.2.
[5] 5-ppm mixed native solution from section 3.6.1.2.
[6] 10-ppm mixed native solution from section 3.6.1.2.

Table 3. Matrix Linearity

Add the following native and internal standards to the control soil extract in the collection vial. Turbo-vap to about 10 mL						
Sample Fortification Level (ppm)[1]	Amount of Native Added per 25-g Soil Extract		Native Analyte Conc in 10 mL	Amount of Internal Standard Added per 25-g Soil Extract [2]		Internal Standard Conc in 10 mL
	µg	mL Solution	µg/mL	µg	mL Solution [3]	µg/mL
0	0	0	0	2.5	0.5	0.25
0.004	0.1	0.5 [4]	0.01	2.5	0.5	0.25
0.01	0.25	1.25 [4]	0.025	2.5	0.5	0.25
0.1	2.5	0.5 [5]	0.25	2.5	0.5	0.25
0.5	12.5	2.5 [5]	1.25	2.5	0.5	0.25
1.0	25	2.5 [6]	2.5	2.5	0.5	0.25
2.5	62.5	6.25 [6]	6.25	2.5	0.5	0.25

[1] Sample concentration levels in 25-g sample equivalents
[2] Internal standard concentration is 0.1 ppm (sample equivalents) in all samples
[3] 5-ppm mixed internal standard spiking solution from section 3.6.1.4.
[4] 0.2-ppm mixed native solution from section 3.6.1.2.
[5] 5-ppm mixed native solution from section 3.6.1.2.
[6] 10-ppm mixed native solution from section 3.6.1.2.

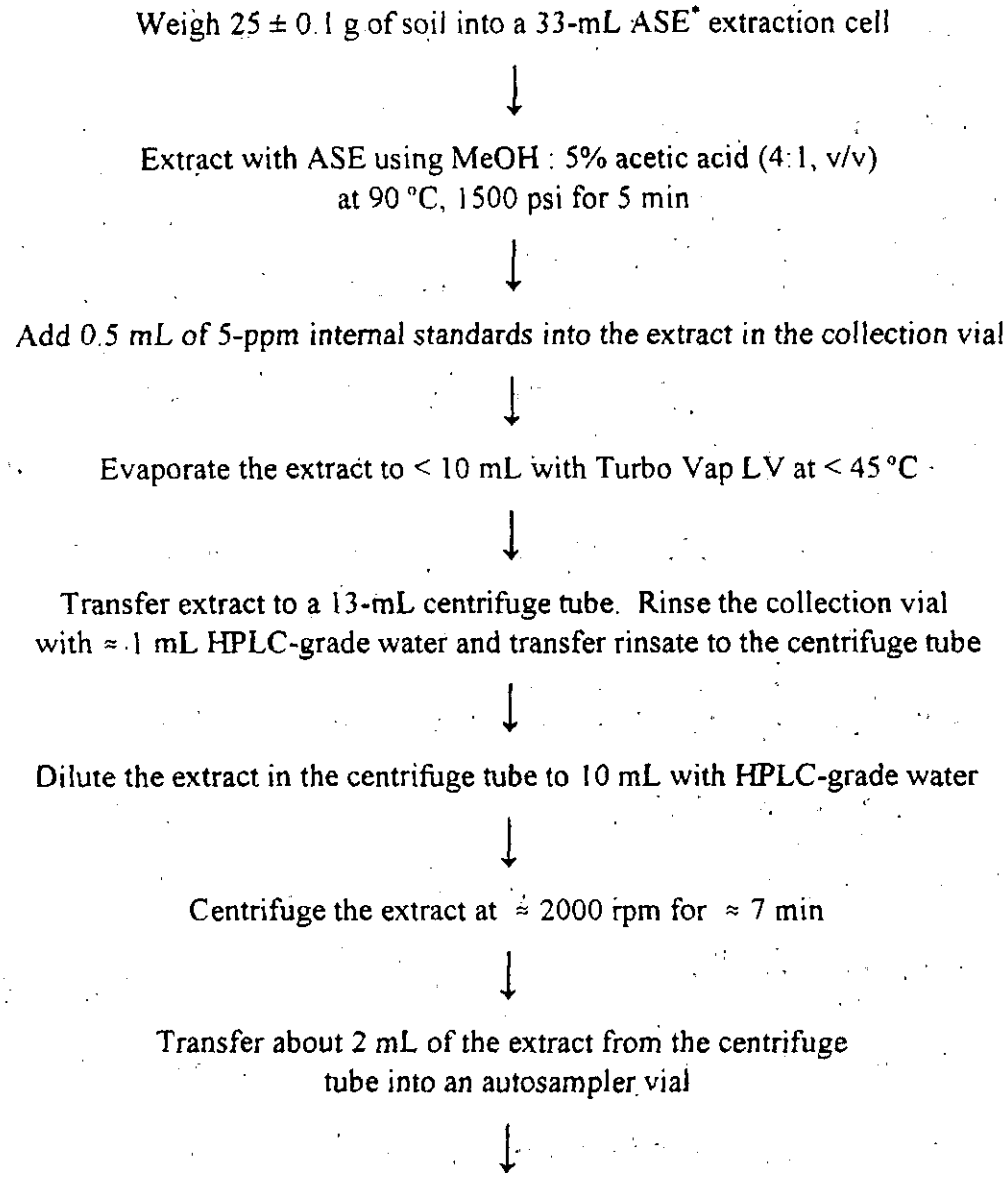

Figure 1. Analytical scheme for sample analyses (See Section 3.6.2 for method details)
*ASE means Accelerated Solvent Extractor.