

## 2.0 INTRODUCTION

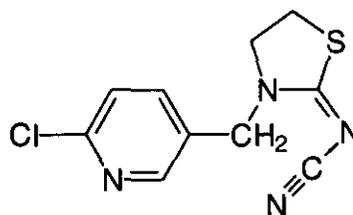
The objective of this study was to evaluate the ruggedness, usability, and any potential weakness of the analytical method in Bayer Report Number 107809 for the determination of YRC 2894 and two metabolites in soil from field dissipation studies.

## 3.0 MATERIALS

### 3.1 TEST SUBSTANCES

The six analytical standards were received in frozen condition on November 1, 1997. The standards were stored at -20 °C from their arrival at Ricerca to the completion of the study. Standard solutions were prepared on November 17, 1997 and November 18, 1997.

Common name:	YRC 2894
Chemical name:	[3-[(6-Chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]cyanamide
ID No.:	K-722
Reference No.:	941013ELB01
GLP Purity %:	99.3
Date Assayed:	8/13/96
Molecular Weight:	252.80
Weight, (g):	0.0839
CAS No.:	111988-49-9
Expiration Date:	8/1/98
Empirical Formula:	C <sub>10</sub> H <sub>9</sub> ClN <sub>4</sub> S
Structure:	

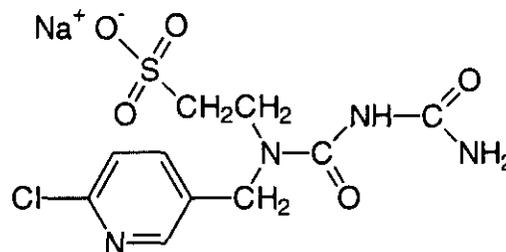


Common name:	YRC 2894-amide
Chemical name:	(Z)-[3-[(6-Chloro-3-pyridinyl)methyl]-2-thiazolidinylidene]urea
ID No.:	K-723
Reference No.:	960529ELB01
GLP Purity %:	99
Date Assayed:	5/29/96
Molecular Weight:	270.80
Weight, (g):	0.0761
Expiration Date:	5/1/01
Empirical Formula:	C <sub>10</sub> H <sub>11</sub> ClN <sub>4</sub> OS
Structure:	

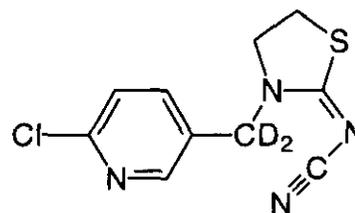


Common name:	WAK 6999 (sodium salt), YRC 2894-sulfonic acid
Chemical name:	Sodium 2- [[[(aminocarbonyl)amino]carbonyl][(6-chloro-3-pyridinyl)methyl]amino] ethanesulfonate
ID No.:	K-656
Reference No.:	950208ELB02
GLP Purity %:	91
Date Assayed:	1/31/97
Molecular Weight:	358.74
Weight, (g):	0.0766
Expiration Date:	2/1/99
Empirical Formula:	[C <sub>10</sub> H <sub>12</sub> ClN <sub>4</sub> O <sub>5</sub> S] <sup>-</sup> Na <sup>+</sup>

Structure:



Common name:	WAK 7091, YRC 2894- <i>d</i> <sub>2</sub>
Chemical name:	[3-[(6-Chloro-3-pyridinyl)methyl- <i>d</i> <sub>2</sub> ]-2-thiazolidinylidene]cyanamide
ID No.:	K-737
Reference No.:	950808ELB01
GLP Purity %:	96
Date Assayed:	7/22/97
Molecular Weight:	254.50
Weight, (g):	0.0900
Expiration Date:	7/1/99
Empirical Formula:	C <sub>10</sub> H <sub>7</sub> D <sub>2</sub> CIN <sub>4</sub> S
Structure:	

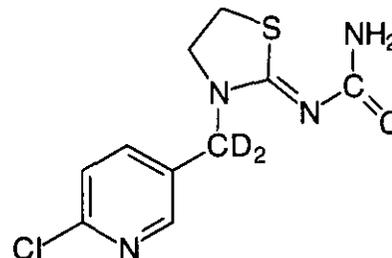


Common name:	YRC 2894-amide- <i>d</i> <sub>2</sub>
Chemical name:	( <i>Z</i> )-[3-[(6-Chloro-3-pyridinyl)methyl- <i>d</i> <sub>2</sub> ]-2-thiazolidinylidene]urea
ID No.:	K-738
Reference No.:	M00113
GLP Purity %:	94
Date Assayed:	11/26/96
Molecular Weight:	272.80
Weight, (g):	0.8000
Expiration Date:	11/1/98

Empirical Formula:

 $C_{10}H_9D_2ClN_4OS$ 

Structure:



Common name:

WAK 7295 d2

YRC 2894-sulfonic acid- $d_2$ 

Chemical name:

Sodium 2-  
[[[(aminocarbonyl)amino]carbonyl][(6-  
chloro-3-pyridinyl)methyl- $d_2$ ]amino]  
ethanesulfonate

ID No.:

K-739

Reference No.:

960611ELB01

GLP Purity %:

97

Date Assayed:

4/22/96

Molecular Weight:

360.75

Weight, (g):

0.0729

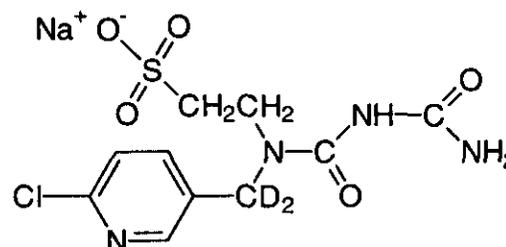
Expiration Date:

6/1/98

Empirical Formula:

 $[C_{10}H_{10}D_2ClN_4O_5S]^- Na^+$ 

Structure:



### 3.2 SOIL

The control soil sample used for the study was from a plot near Arena, Wisconsin. An 800 g sample of this soil identified by study number Y4022102 was shipped by Bayer Corporation on November 11, 1997 and received by Ricerca, Inc. on November 13, 1997 frozen in dry ice. It was given Ricerca code number 97-0829 for identification purposes.

### 3.3 REAGENTS

Fisher Optima grade solvents were used and Fisher Reagent ACS grade acetic acid.

### 3.4 APPARATUS

3.4.1. TurboVap Model LV modified with ASE upgrade Kit (Zymark Part Number 60911)

3.4.2. ASE 200 Accelerated Solvent Extractor

3.4.3. P.E. Sciex API III Plus LC-MS/MS System using Turbo Ion Spray

## 4.0 PRELIMINARY QUALITY CONTROL

Duplicate control soil samples were processed through the procedure after verification of the ASE extractor and turbovap operating parameters. These controls were analyzed with a 4-point calibration curve run in triplicate. Criteria for the method to be "under control" were 1)  $r^2 > 0.98$ , 2) no response greater than 20% of the LOQ observed about the retention time in the unfortified blank control samples, 3) all procedures are followed as written in the method. The  $r^2$  values for YRC 2894, YRC 2894-amide and YRC 2894-sulfonic acid were 1.000, 0.999 and 0.997, respectively. No measurable response was observed for the control samples. Greater instrument sensitivity was observed so a 5  $\mu$ L injection was satisfactory instead of the 50  $\mu$ L injection recommended in the method. Based on these findings, the method validation was performed.

## 5.0 PROCEDURE

### 5.1 METHOD VALIDATION

The method validation set consisted of two control (unfortified) soil samples, one method (reagent) blank sample, 5 replicate fortifications at the LOQ (0.01  $\mu$ g/g) and 5 replicate fortifications at 10 times the LOQ (0.1  $\mu$ g/g). The extraction was performed on December 2, 1997 and the analysis by LC-MS/MS was performed on December 3 and December 4, 1997. Sample calculations and chromatograms are found in Appendix C.

## **5.2 ANALYTICAL METHOD**

The analytical method, Bayer number 107809 was used as written. It is provided for reference purposes in Appendix B.

## **5.3 DESCRIPTION OF INSTRUMENTS AND OPERATING PARAMETERS**

**5.3.1** Ricerca's TurboVap Model LV was modified with a TurboVap LV ASE upgrade kit (Zymark Part Number 60911) so that ASE extraction tubes could be concentrated in the TurboVap without transfer. Thus a modification of the procedure was not required at this step.

**5.3.2** The LC MS/MS system used was a P.E. Sciex API III Plus System using Turbo IonSpray. This system appears to be more sensitive than the Finnigan Corp. TSQ 7000 used in the method. Consequently, a 5  $\mu$ L injection volume was used rather than the 50  $\mu$ L recommended in Section 3.6.3.1 of the method. Instrument parameters describing the LC-MS/MS are detailed with the chromatograms in Appendix C.

## **7.0 METHOD MODIFICATION**

The method called for a 100  $\mu\text{L}$  injection (50  $\mu\text{L}$  sample reaching the column). Due to increased instrument sensitivity for the Sciex API III Plus LC-MS/MS system it was necessary to inject only 5  $\mu\text{L}$  for suitable sensitivity. No other method modifications were made.

## **8.0 GENERAL DISCUSSIONS**

### **8.1 TIME**

The sample set of 13 was extracted during one 8-hour day. The LC-MS/MS run required 11 hours but was run over night. Set up and data calculations required most of a second day.

### **8.2 PROBLEMS ENCOUNTERED**

No significant problems were encountered with the method.

### **8.3 CLARITY OF THE METHOD**

The following sections of the method could be changed as follows to make the method easier for a new analyst to follow:

**8.3.1 Solvents**

Page 11, 12, 14, 15:

The extraction solvent and the solvent for dilution of standards can be confused easily. It is recommended that their composition ratios be expressed in the same manner for clarity.

Extraction Solvent:

MeOH/H<sub>2</sub>O/HOAc 800/200/10

Solvent for dilution of standards:

MeOH/H<sub>2</sub>O/HOAc 200/800/1

**8.3.2 Page 16, Step 8**

The solutions are hot when they first come off the extractor. It does not specify if they should be allowed to cool prior to addition of the internal standard. (The method was validated based on addition to cool solutions.)

**8.3.3 Page 20, Step 5**

Add "of analyte" after "No fortification." The internal standard is still added.

**8.3.4 Section 3.6.3.1**

"100  $\mu$ L (using 50  $\mu$ L loop)" is not as clear as "50  $\mu$ L injection."

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