



Page 1  
Report No : RJ1352B

**ICI AMERICAS INC**  
**Agricultural Products**  
**Wilmington, Delaware 19897**

**Study Title**

**ACETOCHLOR : Storage Stability of the (N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field, Corn, Grain, Forage and Fodder.**

**Data Requirement**

**Residue Chemistry Guidelines**  
**Subdivision 0, Series 171-4(c)**

**Author(s)**

**S J Crook**

**Report Completed On**

**3 December 1992**

**Performing Laboratory**

**ICI AGROCHEMICALS**  
**JEALOTT'S HILL RESEARCH STATION**  
**BRACKNELL, BERKSHIRE, RG12 6EY, UK**

**Laboratory Project ID**

**91JH223**

**Page 1 of 25 Pages**

Study No : 91JH223  
Report Title : ACETOCHLOR : Storage Stability of the (N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field, Corn, Grain, Forage and Fodder.

**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

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

Company : ICI Americas Inc.

Company Agent : Cindy I. Faulkner

Date: March 23, 1993

Regulatory Product Manager  
Title

*Cindy I. Faulkner*  
Signature


Study No : 91JH223  
Report Title : ACETOCHLOR : Storage Stability of the (N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field, Corn, Grain, Forage and Fodder.

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

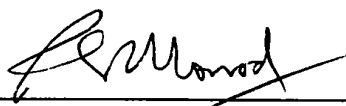
The work on this study to the date of this report has been conducted in compliance with the Principles of Good Laboratory Practice (GLP) laid down in the United Kingdom Department of Health Compliance Programme (1989).

Under the Memorandum of Understanding signed by both the United States of America and the United Kingdom the work on this study to the date of this report is considered to satisfy the requirement that it be conducted in accordance with 40 CFR Part 160.

I believe that the work on this study to the date of this report is valid for the purposes for which it was conducted and that this report is a true reflection of the raw data generated.

  
\_\_\_\_\_  
Study Director

D A French  
ICI Agrochemicals

  
\_\_\_\_\_  
Sponsor

R S Morrod  
Head of Department  
ICI Agrochemicals

  
\_\_\_\_\_  
Submitter

Study No : 91JH223

Report Title : Acetochlor : Storage Stability of the N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field Corn, Forage and Fodder.

### QUALITY ASSURANCE STATEMENT

In accordance with ICI Agrochemicals policy and procedures for Good Laboratory Practice, the conduct of this study to date has been inspected/audited by the Quality Assurance Unit at Jealotts Hill Research Station, Bracknell, Berks, RG12 6EY, UK.

Date of Inspection	Inspection/Audit	Date of Inspection Report
15 Jul 1991	Protocol	15 Jul 1991
06 Aug 1991	Study Conduct	09 Aug 1991
22 Nov 1992	Interim Report	27 Nov 1992

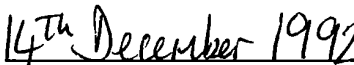
In addition, the following facility and procedure inspections associated with this type of study have been carried out.

01 Nov 1991	Laboratory Facilities	09 Dec 1991
-------------	-----------------------	-------------

So far as can be reasonably established, the methods described and results incorporated in this report accurately reflect the raw data produced during the study.

  
Quality Assurance Officer

J A Vickers

  
Date

**AUTHENTICATION**

Study No : 91JH223  
Report Title : ACETOCHLOR : Storage Stability of the (N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field, Corn, Grain, Forage and Fodder.

I, the undersigned, declare that this study was performed under my direction and that this report represents a true and accurate record of the results obtained.

N D Simmons was Study Director from 1st August 1991 to 9th January 1992.

*DA French* D A French 3/12/92  
Study Director Date

The following personnel carried out work on this report.

*S. J. Crook* S J Crook 3/12/92  
Principal Scientist Date

Authorised for management by :

*D W Bewick* D W Bewick 07.12.92  
Section Manager Date

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Study No : 91JH223  
Report Title : ACETOCHLOR : Storage Stability of the (N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid Metabolite in Deep Frozen Field, Corn, Grain, Forage and Fodder.  
Author : S J Crook

#### **SUMMARY**

The stability of residues of the acetochlor metabolite N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid in deep frozen field corn; grain, forage and fodder has been established for a period of approximately 12 months. These data represent interim results, the study is continuing.

1

## INTRODUCTION

It is necessary to provide adequate data on the possible effects of freezer storage on the magnitude of any residue in commodities during the interval between sample collection and sample analysis. This data requirement is outlined in the Residue Chemistry Guidelines Subdivision 0, Series 171-4(c).

This study was initiated to provide storage stability data on the N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid metabolite, to support the registration of the chloroacetanilide herbicide, acetochlor.

20 g samples of prepared control field corn; grain, forage and fodder; lettuce; turnip roots and tops; soya seed and hay were fortified at approximately 0.10 mg/kg with <sup>14</sup>C-phenyl radiolabelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid metabolite and then deep frozen at <-10°C. Duplicate samples were taken and analysed for N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid residues at predetermined intervals of 0, 6 and 12 months. The analyses were carried out at the Residue Laboratories of the Environmental Sciences Department, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK.

This interim report includes data generated between August 1991 and October 1992 and details results only from analyses of field corn; grain, forage and fodder samples stored for up to 12 months. Results from the analyses of lettuce; turnip roots and tops; soya seed and hay are not reported here and will be detailed in the final report. The study is continuing.

2

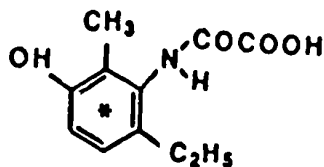
## MATERIALS

2.1

### Test Substance

Chemical Name : N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid

**Figure 1 : Structure of N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid and position of <sup>14</sup>C-radiolabel**



Molecular Formula : C<sub>11</sub>H<sub>13</sub>NO<sub>4</sub>  
Code Number : ICIA5676/57  
Molecular Weight : 223

<sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid, Reference 91-33, was prepared by Western Research Center, Richmond, California and had a specific activity of 1.968 GBq mmol<sup>-1</sup>.

Confirmation of identity using nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS); were carried out at WRC, Richmond, California. NMR confirmation was carried out at ICI Agrochemicals, Jealott's Hill, Bracknell, Berkshire, RG12 6EY.

## 2.2 Test Commodity

The control samples used to set up this study were taken from magnitude of residues trials and crop rotation trials from various locations in the U.S. Full details of field corn control fractions are given in Table 1.

**TABLE 1 : Control Field Corn; Grain; Forage and Fodder Fractions Used in Study 91JH223**

Crop Fraction	Study Number	Trial	Sample Number	Location	Variety	Growth Stage at Harvest
Grain	5676-88-MR-01	IITX-88-235	11355/88	Luling, Texas	Pioneer 3165	Mature Plant
Forage	5676-88-MR-01	21IL-88-226	1601/89	Monticello, Illinois	Agrigold A6445	Milk Dough Stage
Fodder	5676-88-MR-01	17CA-88-233	11331/88	Stockton, California	Funk's G-4507	Mature Plant

Control lettuce; turnip roots and tops; soya seed and hay fractions were taken from Study Number 5676-88-CR-01. Full details for these fractions will be given in the final report.

## 3 ANALYTICAL PROCEDURES

### 3.1 Experimental Design

Control samples used in this study were pre-prepared and had been stored deep frozen at a temperature of <-18°C for a period of approximately two years prior to the commencement of this study.

For the investigation of the storage stability of N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid residues in field corn, untreated field corn; grain, forage and fodder samples (20 g) were weighed into individual screw cap glass storage jars.

The samples were fortified at 0.09 mg kg<sup>-1</sup> with <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid by the addition of 40 µl of a radiolabelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid standard in acetonitrile containing 441 000 Bq ml<sup>-1</sup>; equivalent to approximately 50 µg ml<sup>-1</sup>. (0 month samples were spiked 4 days after the samples for the other time intervals). All caps were secured, the samples fully labelled and stored at <-10°C.

At predetermined intervals, duplicate samples were taken and analysed for N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid residues.

### 3.2 Analytical Techniques

#### 3.2.1 Liquid Scintillation Counting (LSC)

Liquid samples were radioassayed by liquid scintillation counting, using an LKB 1219 Rackbeta spectral liquid scintillation spectrometer. This instrument utilises the spectral quench parameter method of sample quench correction and is used in conjunction with an appropriate quench correction curve.

Suitable sample aliquots were prepared by mixing with 'Optiphase Safe' scintillation solution (LKB). Each batch of samples was preceded by two suitable background samples, from which a mean was calculated and automatically subtracted from subsequent measurements. Samples were counted in duplicate.

Full details and an example of an LSC output are presented as Appendix 3.

#### 3.2.2. Thin Layer Chromatography (TLC)

Normal phase silica gel chromatography was carried out using 0.25 mm Sil S-25 UV<sub>254</sub> plates (Machery Nagel). Plates were eluted in saturated vapour conditions by lagging the inside of the tank with absorbent paper. The following solvent systems were used:

Solvent system A : ethyl acetate:propan-1-ol:water  
60:40:10 (v/v/v)

Solvent system B : chloroform:methanol:water:formic acid  
75:25:3:3 (v/v/v/v)

Fractions were applied to the chromatoplates admixed with standard reference compounds. The reference compounds were detected by the quenching of gel fluorescence under short wave ultraviolet light. To ensure the correct assignments on the chromatoplates, the standards were also chromatographed alongside fractions of plant extracts.

#### 3.2.3 Detection of Radioactive Areas on Thin Layer Chromatograms

The radioactive areas on thin layer chromatograms were detected by radioscanning.

Radioscanning was carried out using Isomess IM3000 and 68000 Automatic TLC Linear Analysers (Appendix 4) and was used to quantify the amount of radioactivity associated with each area on the chromatogram. The quantification was carried out by computer integration of the areas on the chromatograms after background subtraction, using the Apple or 68000 data systems.

Full details are presented as Appendix 4.

3.3 **Analytical Method**

Analysis for the acetochlor metabolite, N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid was carried out using the methodology described below.

Prepared, fortified field corn grain, and forage samples (20 g) were extracted by maceration with an acetonitrile/water mixture (50 ml, 1:1 (v/v)) until well homogenised. Fodder samples were extracted using the same procedure but acetonitrile/water (100 ml, 1:1 (v/v)) was used.

Samples were filtered through two Whatman No. 1 filter papers into an appropriate sized round bottomed flask. At this stage samples were transferred to volumetric flasks and made to a known volume; aliquots were taken for liquid scintillation counting (LSC) to assess the percentage of radioactivity, hence the percentage of N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid, extracted from the crop.

Extracts were then rotary evaporated to dryness and redissolved in acetone (4 ml).

Characterisation of the radioactive residue in the extracts was made by thin layer chromatography (TLC) using two separate solvent systems, described in Section 3.2.2, with radioscanning detection.

4 **RESULTS**

The results of the analyses of the field corn samples are detailed in Table 2.

An example calculation is presented in Appendix 2.

**TABLE 2 : Storage Stability in Frozen Field Corn Stored at <-18°C**

Field Corn Fraction	Date of Analysis	Storage Duration (Months)	Mean Residue (mg kg <sup>-1</sup> )
Grain	12th August 1991	0	0.06
	10th February 1992	6	0.07
	14th August 1992	12	0.06
Forage	12th August 1991	0	0.07
	10th February 1992	6	0.08
	14th August 1992	12	0.07
Fodder	12th August 1991	0	0.07
	10th February 1992	6	0.08
	14th August 1992	12	0.07

5           **DISCUSSION**

In the fortified field corn, grain, forage and fodder samples, the N-(6-ethyl-3-hydroxy-2-methylphenyl)-oxamic acid metabolite residue showed no decrease after 12 months of freezer storage compared to zerotime analysis. These data represent interim results, the study is continuing.

6           **RAW DATA**

All raw data for the study from which the field corn fractions originated, together with a copy of the interim report, are stored in the ICI Agrochemical GLP Archive, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY under study numbers 5676-88-MR-01 and 91JH223 respectively.

Details of synthesis and spectral data for <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid are stored in the ICI Agrochemical GLP Archive, Jealott;s Hill Research Station, Bracknell, Berkshire, RG12 6EY under file number 91-33.

All raw data for this interim report are stored with S J Crook in lab 111/26, Environmental Sciences Building, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY.

7           **REFERENCES**

1.       Laboratory notebook D7615, S J Crook.
2.       Report RJ0818B for Field information and preparation details of samples from Study 5676-88-MR-01.

Report No :    RJ1352B  
Disc No :      WP/92/CL/63  
Reference :    SJC/CL  
Date :         2 December 1992

**APPENDIX 1**

**Example Chromatograms (T.L.C)**

**Grain Extract After 12 Months Deep Frozen Storage  
Solvent System A**

RAYTEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
Measurement D76157EA

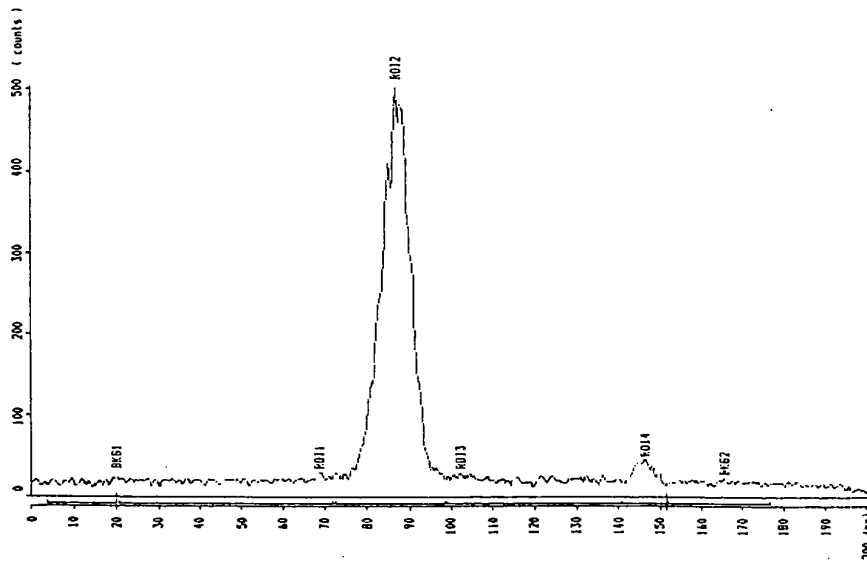
Date of run: Sep 26, 92, 11:12

Run time 10000 s  
Origin 20.0mm Solvent front 151.6mm  
Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROI	%Total	rf
1	BKG1	4.0	20.2	---	---	---	---	---
2	ROI1	20.7	72.1	68.9	483.6	2.76%	2.76%	0.3715
3	ROI2	72.6	98.8	86.9	15863.8	90.69%	90.53%	0.5084
4	ROI3	99.3	140.7	102.5	574.9	3.29%	3.28%	0.6266
5	ROI4	141.2	151.6	146.4	569.6	3.26%	3.25%	0.9606
6	BKG2	152.1	176.8	---	---	---	---	---
Remainder					32.3	0.18%	0.18%	---

Net counts in regions : 17491.9  
Net total counts : 17524.2  
BKG1 (counts/mm) : 71.81  
BKG2 (counts/mm) : 72.98

RAYTEST RITA-3200 Radio-TLC-Analyser  
User S\_CROOK  
Measurement D76157EA - Peak D7615/47/11  
Date of run: Sep 26, 92 11:12  
Run time: 10000 s



**Grain Extract After 12 Months Deep Frozen Storage  
Solvent System B**

RAI TEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
Measurement D76157EB

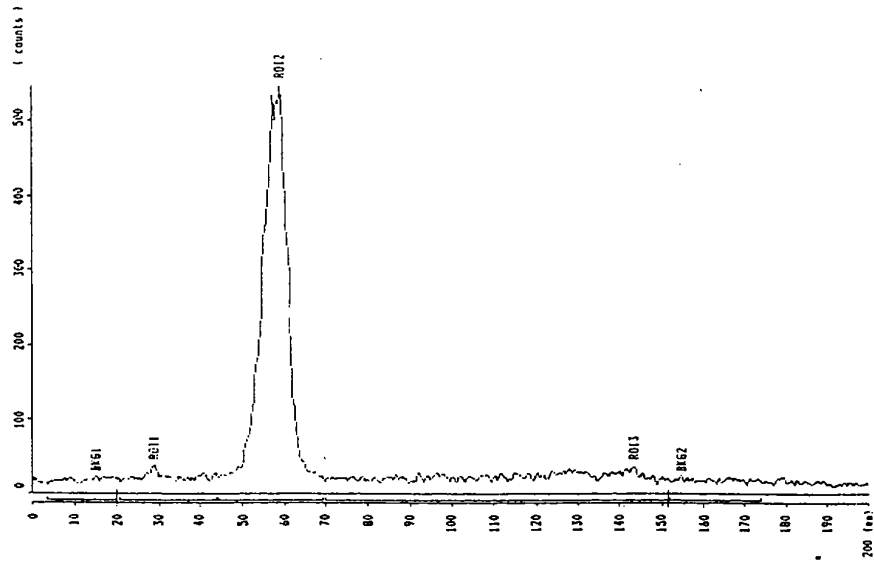
Date of run: Sep 29, 92, 11:47

Run time 10000 s  
Origin 20.0mm Solvent front 151.6mm  
Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROI	%Total	rf
1	BK61	3.5	20.2	---	---	---	---	---
2	ROI1	20.7	44.0	29.1	383.0	2.29%	2.25%	0.0694
3	ROI2	44.4	69.6	58.8	14734.5	88.19%	88.17%	0.2946
4	ROI3	70.1	151.6	143.7	1589.5	9.51%	9.51%	0.9400
5	BK62	152.1	173.8	---	---	---	---	---
Remainder					5.3	0.03%	0.03%	---

Net counts in regions : 16706.9  
Net total counts : 16712.3  
BK61 (counts/mm) : 74.66  
BK62 (counts/mm) : 73.31

RAI TEST RITA-3200 Radio-TLC-Analyser  
User S\_CROOK  
Measurement D76157EB  
Date of run: Sep 29, 92 11:47  
Run time: 10000 s  
*RAI D76157EB / 48/61*



**Forage Extract After 12 Months Deep Frozen Storage  
Solvent System A**

RAYTEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
Measurement D7615BFA

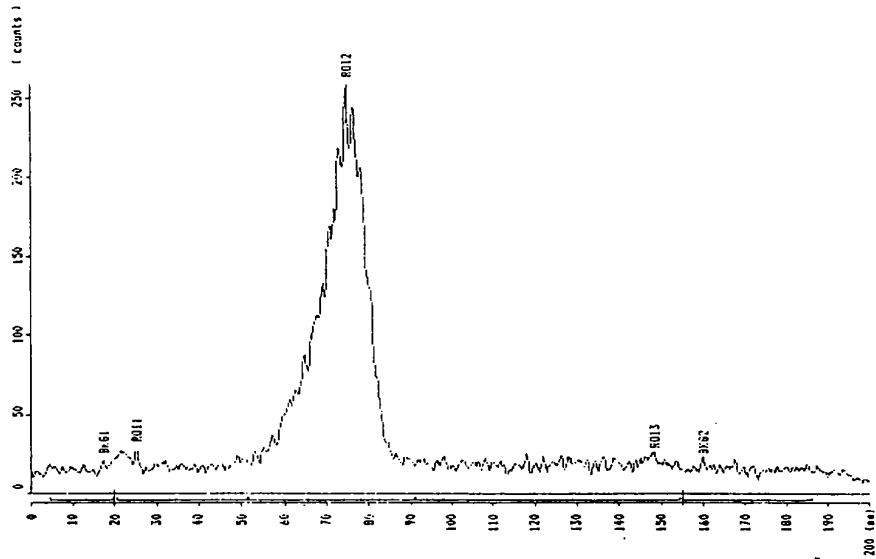
Date of run: Sep 26, 92, 19:33

Run time 10000 s  
Origin 20.0mm Solvent front 155.1mm  
Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROIs	%Total	rf
1	BK61	4.4	19.8	---	---	---	---	---
2	RO11	20.7	51.4	22.0	344.7	2.64%	2.65%	0.0146
3	RO12	51.9	90.9	75.1	11881.6	91.02%	91.19%	0.4077
4	RO13	91.4	154.6	148.1	826.9	6.33%	6.35%	0.9468
5	BK62	155.1	186.2	---	---	---	---	---
Remainder				---	-23.4	-0.16%	-0.16%	---

Net counts in regions : 13053.2  
Net total counts : 13029.9  
BK61 (counts/mm) : 64.03  
BK62 (counts/mm) : 64.48

RAYTEST RITA-3200 Radio-TLC-Analyser  
User S\_CROOK  
Measurement D7615BFA - Plate D7615/4  
Date of run: Sep 26, 92 19:33  
Run time: 10000 s



**Forage Extract After 12 Months Deep Frozen Storage  
 Solvent System B**

RAYTEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
 Measurement D76158EB

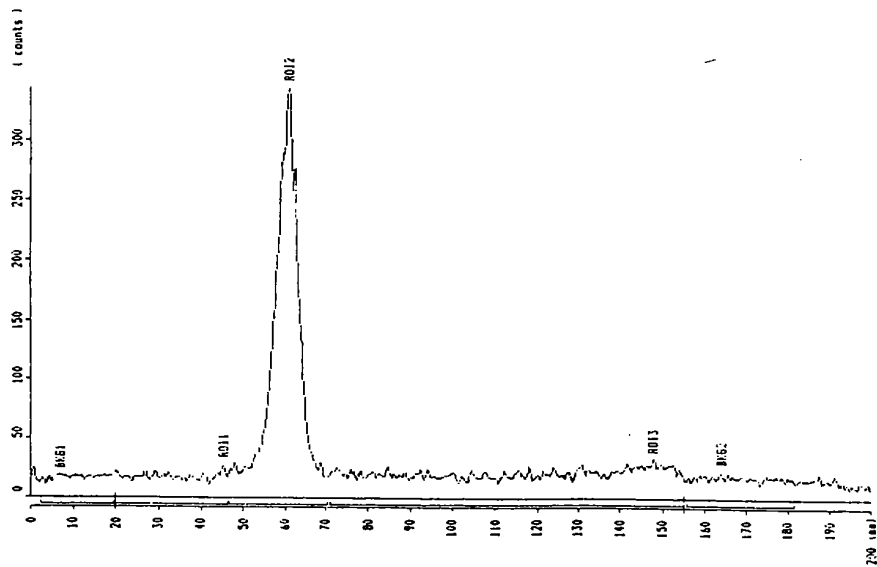
Date of run: Sep 29,92, 17:21

Run time 10000 s  
 Origin 20.0mm Solvent front 155.1mm  
 Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROIs	%Total	rf
1	BK61	2.5	19.8	---	---	---	---	---
2	RO11	20.2	46.4	45.4	62.2	0.64%	0.64%	0.1883
3	RO12	46.9	70.6	61.0	8274.5	85.85%	85.75%	0.3035
4	RO13	71.1	155.1	147.9	1301.3	13.50%	13.49%	0.9476
5	BK62	156.0	181.7	---	---	---	---	---
Remainder				---	11.1	0.11%	0.11%	---

Net counts in regions : 9638.0  
 Net total counts : 9649.0  
 BK61 (counts/mm) : 69.25  
 BK62 (counts/mm) : 72.28

RAYTEST RITA-3200 Radio-TLC-Analyser  
 User S\_CROOK  
 Measurement D76158EB - Plate D7615/4  
 Date of run: Sep 29,92 17:21  
 Run time: 10000 s



**Fodder Extract After 12 Months Deep Frozen Storage  
Solvent System A**

RAYTEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
Measurement D76159FA

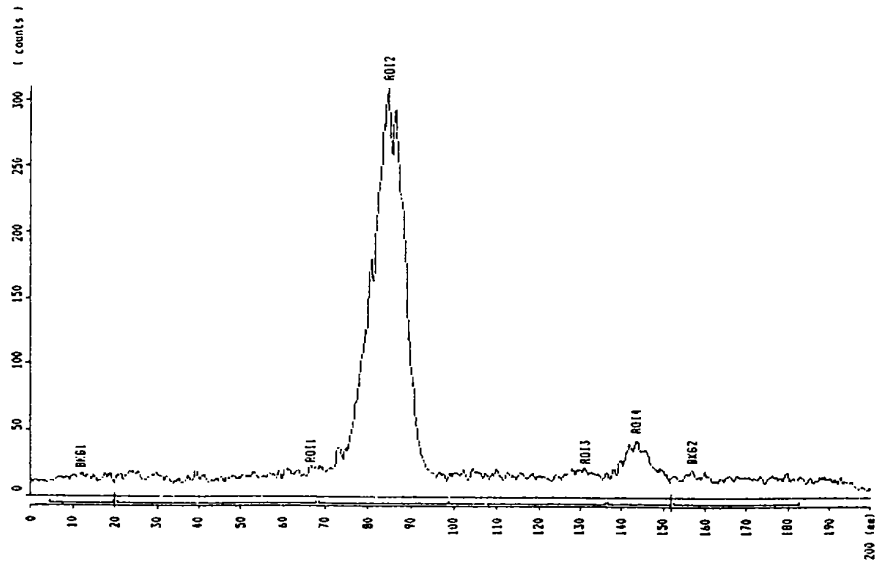
Date of run: Sep 27,92, 01:07

Run time 10000 s  
Origin 20.0mm Solvent front 152.1mm  
Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROI	%Total	rf
1	BKG1	4.4	19.8	---	---	---	---	---
2	ROI1	20.7	68.1	67.2	160.5	1.35%	1.36%	0.3570
3	ROI2	68.6	98.8	84.7	10653.5	89.82%	90.15%	0.4897
4	ROI3	99.3	136.3	131.1	334.0	2.82%	2.83%	0.8411
5	ROI4	136.8	152.1	143.7	713.5	6.02%	6.04%	0.9364
6	BKG2	152.6	182.7	---	---	---	---	---
Remainder				---	-43.7	-0.37%	-0.37%	---

Net counts in regions : 11861.5  
Net total counts : 11817.8  
BKG1 (counts/mm) : 60.49  
BKG2 (counts/mm) : 64.31

RAYTEST RITA-3200 Radio-TLC-Analyser  
User S\_CROOK  
Measurement D76159FA - Plate D7615/47/11  
Date of run: Sep 27,92 1:07  
Run time: 10000 s



**Fodder Extract After 12 Months Deep Frozen Storage  
Solvent System B**

RAYTEST RITA-3200 Radio-TLC-Analyser

User S\_CROOK  
Measurement D76159EB

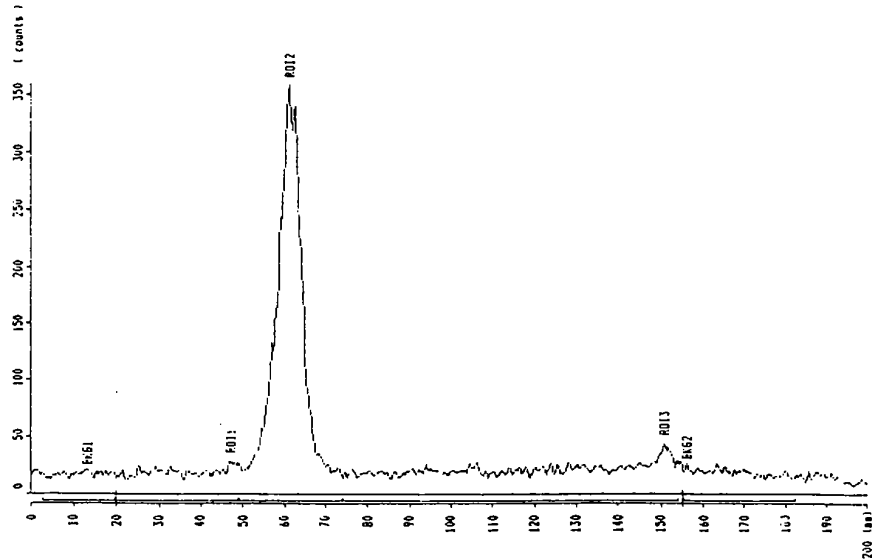
Date of run: Sep 29,92, 22:55

Run time 10000 s  
Origin 20.0mm Solvent front 155.1mm  
Setting 3 : manual

No	Name	from	to	Max.	Contents	%ROI's	%Total	rf
1	BKG1	3.0	19.8	---	---	---	---	---
2	ROI1	20.2	48.9	47.4	192.6	1.82%	1.81%	0.2029
3	ROI2	49.4	74.1	61.2	9325.8	88.05%	87.59%	0.3053
4	ROI3	74.6	154.1	151.4	1073.5	10.14%	10.08%	0.9726
5	BKG2	155.6	182.2	---	---	---	---	---
	Remainder	---	---	---	54.9	0.52%	0.52%	---

Net counts in regions : 10591.9  
Net total counts : 10646.8  
BKG1 (counts/mm) : 69.55  
BKG2 (counts/mm) : 75.91

RAYTEST RITA-3200 Radio-TLC-Analyser  
User S\_CROOK  
Measurement D76159EB *Plc. # D7615/48/82*  
Date of run: Sep 29,92 22:55  
Run time: 10000 s



## APPENDIX 2

### Example Calculations of Mean Residue Figure

#### 0 Month Fodder

Specific activity of  $^{14}\text{C}$ -phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid =  $1.968 \text{ GBq mmol}^{-1}$ .

1 mmol N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid = 223 mg

$$\begin{aligned}\therefore 1 \text{ dps} &= \frac{223}{1.968 \times 10^9} = 1.133 \times 10^{-7} \text{ mg} \\ &= 1.133 \times 10^{-4} \mu\text{g}\end{aligned}$$

$$\therefore 1 \mu\text{g} = \frac{1}{1.133 \times 10^{-4}} = 8826 \text{ dps}$$

20 g crop fortified at  $0.1 \mu\text{g g}^{-1} = 2.0 \mu\text{g}$  N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid to be applied.

Actual amount  $^{14}\text{C}$ -phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid applied

$$= 15889 \text{ dps}$$

$$= 1.8 \mu\text{g}$$

$$= 0.09 \mu\text{g g}^{-1}$$

% radioactivity extracted in duplicate samples = 92% and 96%

$\therefore$  Mean Recovery = 94%

% radioactivity co-chromatographing with the N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid marker from duplicate samples in each solvent system.

= System A : 83% and 82%  
System B : 89% and 76%

$$\therefore \text{Mean from all systems} = \frac{83 + 82 + 89 + 76}{4} = 83\%$$

It therefore follows that :

Of the 94% of radioactivity extracted, 83% was <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid.

∴ Total radioactivity as N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid

$$= \frac{94 \times 83}{100} = 78\%$$

∴ As Sample fortified at 0.09 mg kg<sup>-1</sup>, mean residue

$$= \frac{0.09 \times 78}{100} = 0.07 \text{ mg kg}^{-1}$$

### 12 Month Fodder

Specific activity of <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid = 1.968 GBq mmol<sup>-1</sup>.

1 mmol N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid = 223 mg

$$\begin{aligned} \therefore 1 \text{ dps} &= \frac{223}{1.968 \times 10^9} = 1.133 \times 10^{-7} \text{ mg} \\ &= 1.133 \times 10^{-4} \mu\text{g} \end{aligned}$$

$$\therefore 1 \mu\text{g} = \frac{1}{1.133 \times 10^{-4}} = 8826 \text{ dps}$$

20 g crop fortified at 0.1 μg g<sup>-1</sup> = 2.0 μg N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid to be applied.

Actual amount <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid applied

$$= 15889 \text{ dps}$$

$$= 1.8 \mu\text{g}$$

$$= 0.09 \mu\text{g g}^{-1}$$

% radioactivity extracted in duplicate samples = 91% and 88%

∴ Mean Recovery = 90%

% radioactivity co-chromatographing with the N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid marker from duplicate samples in each solvent system.

= System A : 90% and 90%  
System B : 88% and 88%

$$\therefore \text{Mean from all systems} = \frac{90 + 90 + 88 + 88}{4} \\ = 89\%$$

It therefore follows that :

Of the 90% of radioactivity extracted, 89% was <sup>14</sup>C-phenyl labelled N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid.

$$\therefore \text{Total radioactivity as N-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid} \\ = \frac{89 \times 90}{100} = 80\%$$

\therefore As Sample was fortified at 0.09 mg kg<sup>-1</sup>, mean residue

$$= \frac{0.09 \times 80}{100} = 0.07 \text{ mg kg}^{-1}$$

**APPENDIX 3 :**

**Measurement of Radioactivity by Liquid Scintillation Counting Using the LKB 1219 Spectral**

The LKB Rackbeta 'Spectral' is linked to an LKB 442OVDU with an LKB4510 printer and an option of data storage in an LKB two-speed data logger. Counting was carried out using a fixed window setting (5-650 ev for  $^{14}\text{C}$ ), a CPM/DPM counting mode, and a sample quench monitoring with the external standard spectrum generated from a  $^{226}\text{RA}$  source (SQP[E]). Quench correction curves (Efficiency vs SQP[E]) to allow automatic calculation of DPM values were set up using a series of quenched standards and a present smoothed spline fitting method.

Counting errors due to colour quenching are compensated for by an automatic adjustment of the SQP[E] values. CPM's due to chemiluminescence are accurately monitored and subtracted to give a net CPM value.

Each operator has a unique identification code number for counting samples.

Each batch of samples are preceded by two suitable background samples from which a mean is calculated. This is automatically subtracted from subsequent count rates in the batch to provide net counts per minute value. The computer then calculates the percentage error in the background corrected count rate due to the statistical nature of the radioactive decay using the following equation:

$$\Delta \frac{R}{R} (\%) = 100 \times \frac{\left[ 60 \times \frac{I}{t} \right]^{1/2}}{R}$$

Where R = count rate corrected for background  
t = counting time in seconds  
I = uncorrected count rate

Example LSC Output From The LKB 1219 Spectral

PARAMETER GROUP: 7  
ID: ~~N. PENBELLY~~ S. J. Crook.

01A PROGRAM MODE 1 ->  
01B COUNT MODE: FIXED WINDOW: 5- 650  
SOP(E) SINGLE LABEL  
C-14  
01C COLOUR CORRECTION Y ->  
GLASS OR PLASTIC VIALS 6 ->  
02 LISTING Y ->  
03 TIME 300 ->  
04 COUNTS 1 20000 ->  
07 NUMBER OF WINDOWS 1 ->  
08 WINDOW 1 5- 650 ->  
11 EXTERNAL STD TIME 15 ->  
12 EXTERNAL STD COUNTS 900000 ->  
13A SAMPLE QUALITY MONITOR N ->  
14 PRINT 1,2,10,11,12,19,29,30,36,37  
->  
\*\*\*STD. CURVE FITTING\*\*\*

POS	CTIME	COUNTS1	CPM1	CPM1%	EFF1	EFF2	SOP(E)	CLM%	DPS1
001	00300	162	35.34	7.527	93.58	.00	468.08	.000	.629! - Blank.
002	00300	184	34.83	7.582	93.67	.00	469.58	.000	.620! - Blank.
REPLICATE: BACKGROUND SAMPLE									
		MEAN	THEOR. ERROR(%)	OBS. ERROR(%)	CHI SQUARED	PROBABILITY(%)			
CPM1		35.1	5.342	.727	.019	89.18			
SOP(E)		466.83		.160					
POS	CTIME	COUNTS1	CPM1	CPM1%	EFF1	EFF2	SOP(E)	CLM%	DPS1
003	00300	1276	219.01	3.024	89.35	.00	402.59	.000	4.085 - D7615/42/1
004	00300	1224	208.59	3.098	89.37	.00	402.84	.000	3.890 - D7615/42/2
005	00300	17768	3524.48	.754	91.17	.00	429.63	.000	64.434 - D7615/42/3
006	00300	17705	3512.39	.755	91.28	.00	431.39	.000	64.133 - D7615/42/4
007	00300	19050	3782.66	.728	90.41	.00	418.01	.000	69.733 - D7615/42/5
008	00300	19151	3803.44	.726	90.52	.00	419.71	.000	70.029 - D7615/42/6
009	00300	19787	3930.61	.714	90.40	.00	417.92	.000	72.466 - D7615/42/7
010	00300	19835	3940.30	.713	90.54	.00	419.94	.000	72.537 - D7615/42/8
POS	CTIME	COUNTS1	CPM1	CPM1%	EFF1	EFF2	SOP(E)	CLM%	DPS1
011	00300	19142	3801.75	.726	90.31	.00	416.49	.000	70.164 - D7615/42/9
012	00300	19479	3689.11	.720	89.59	.00	411.84	.000	71.656 - D7615/42/10
013	00299	20141	4014.90	.709	89.64	.00	406.80	.000	74.646 - D7615/42/11
014	00296	20099	4047.57	.708	89.62	.00	406.40	.000	75.233 - D7615/42/12
015	00289	20120	4150.02	.709	87.03	.00	372.61	.000	79.474 - D7615/42/13
016	00297	20066	4168.35	.709	86.64	.00	370.61	.000	80.000 - D7615/42/14
017	00293	20100	4089.53	.708	87.29	.00	382.02	.000	77.635 - D7615/42/15
018	00298	20113	4163.51	.708	87.78	.00	381.87	.000	79.050 - D7615/42/16
019	00287	20144	4185.67	.707	91.58	.00	436.09	.000	76.178 - D7615/42/17
020	00282	20088	4247.83	.708	91.54	.00	435.50	.000	77.341 - D7615/42/18

#### **APPENDIX 4 :**

##### **TLC Analysis by Linear Analysers**

###### **The Isomess (68000) Automatic Linear Analyser**

The design of the TLC-Linear Analyser is such that the head remains stationary above the chromatogram and accumulates counts from the radioactive areas on the chromatogram (with a resolution of approximately 2 mm). The length of accumulation time depends on the amount of radioactivity on the chromatogram, the lower the amount of radioactivity, the longer the required accumulation time. Immediately after the chromatogram has been scanned, the head is positioned over a non-radioactive area on the TLC plate and allowed to accumulate a background chromatogram for the same length of time as the sample chromatogram. At the end of this accumulation time, the background chromatogram is subtracted, channel by channel, from the sample chromatogram using a background subtraction computer programme present in the data processing module of the analyser software. Using another part of the data processing facility, the background subtracted chromatogram is then divided, by the operator, into regions of interest, that is areas in which the different radioactive compounds are located.

The counts in the different regions of interest are summed and the percentage of radioactivity found in each region of interest is calculated.

###### **The Isomess Automatic (IM3000) Linear Analyser**

The Isomess Automatic (IM3000) Linear Analyser operated in exactly the same way as the Isomess 68000 Automatic Linear Analyser, except background subtraction and allocation of regions of interest are performed on Apple II computer, using programs written for this computer and implemented by the operator.