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Study Title

Residue Analytical Method for the Determination of YRC 2894
Residues in Plant Materials by HPLC

Data Requirements

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Performed By

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Page 1a of 55
(Includes pages 1a, 1b, 1c and 1d for a total of 59 pages)

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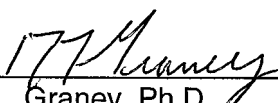
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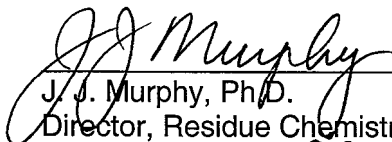
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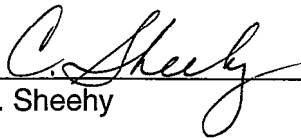
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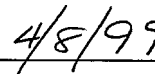
Except as noted below, the pages of this report are presented as originally produced. Page 6 has an English translation of the German summary given on page 7.

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2. Addition of Data Confidentiality Statement, page 1b
3. Addition of Certification of Good Laboratory Practice, page 1c
4. Addition of Certification of Authenticity, 1d
5. Addition of Bayer Report Number
6. Pages reduced to the standard 8 1/2 x 11 inch page size

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Study Title

**Residue Analytical Method for the Determination of YRC 2894
Residues in Plant Materials by HPLC**

Purpose

**Determination of Recoveries and Blank Values of Control Samples
and the Detectorlinearity for Validation of the Analytical Method
for YRC 2894**

Author

Dr. F.-J. Placke

Report Completion Date

April 29, 1996

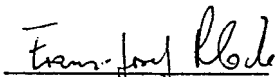
Testing Facility

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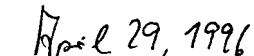
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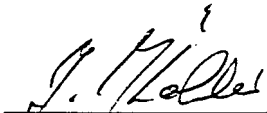
Certification of Authenticity


(Dr. Placke)


Study Director and Author


(Date)

Signature of Management


(Dr. Köhler)

Manager Testing
Facility


(Date)

Archiving

All raw data pertaining to this study and the original report will be stored in the central GLP archive PF-F, Bayer AG, D-51368 Leverkusen, for at least as long as required by GLP-principles. Test- and reference substances are stored in the archives of PF-E/FT-EA, Bayer AG, D-51368 Leverkusen as long as their quality still guarantees an evaluation.

Time Schedule

The experimental work was performed during the following time period:

Start of Experimental Phase: February 8, 1996 (1st recovery set)
End of Experimental Phase: March 7, 1996 (last recovery set)
April 4, 1996 (last storage stability determination)

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Table of Contents	Page
Certification of Authenticity	2
Archiving	2
1. Summary	6
1. Zusammenfassung	7
2. Introduction	8
3. Safety Instructions	9
4. Sampling	9
5. Experimental	9
5.1 Materials	9
5.1.1 Apparatus	9
5.1.2 Reagents	10
5.1.3 Reference Substance	10
5.1.4 Standard Solutions Required	11
5.2 Analytical Method	12
5.2.1 Extraction and Sample Clean-up	12
5.2.2 ChemElut® Column Clean-up	12
5.2.3 Column Chromatography on Florisil®	13
5.2.3.1 Florisil® Column Preparation	13
5.2.3.2 Florisil® Column Clean-up	13
5.3.1 HPLC UV-Determination of YRC 2894 Residues	14
5.3.2 Confirmatory Method for YRC 2894 Residues	15
6. Calculation	16
6.1 Calculation of YRC 2894 Residues	16
6.2 Calculation of YRC 2894 Recoveries	16
7. Results and Discussion	17
7.1 Linearity of the detector	17
7.2 Extraction Efficiency	17
7.3 Recovery Data and Statistical Evaluation	17
7.4 Stability of Analytical Solution	19
8. References	19

Table of Contents (cont'd)

	Page
Tables	20
Table 1: Recovery Rates for YRC 2894	20
Table 2: Recovery Rates for YRC 2894 with Confirmatory Method	21
Table 3: Stability of YRC 2894 in Analytical Solutions	22
Figures	23
Figure 1: Flowdiagram of Method	23
Figure 2: Detector Linearity with HPLC-UV	24
Figure 3: Detector Linearity with HPLC-UV Confirmatory Method	25
Appendices 1 - 15: Chromatograms with Primary Method	26
Appendix 1: Chromatograms of YRC 2894 Recoveries from Apple, Fruit	26
Appendix 2: Chromatograms of YRC 2894 Recoveries from Apple, Dried Fruit	27
Appendix 3: Chromatograms of YRC 2894 Recoveries from Apple, Dry Pomace	29
Appendix 4: Chromatograms of YRC 2894 Recoveries from Apple, Juice	28
Appendix 5: Chromatograms of YRC 2894 Recoveries from Apple, Sauce	30
Appendix 6: Chromatograms of YRC 2894 Recoveries from Cucumber, Fruit	31
Appendix 7: Chromatograms of YRC 2894 Recoveries from Melon, Peel	32
Appendix 8: Chromatograms of YRC 2894 Recoveries from Melon, Pulp	33
Appendix 9: Chromatograms of YRC 2894 Recoveries from Paprika, Fruit	34
Appendix 10: Chromatograms of YRC 2894 Recoveries from Peach, Fruit	35
Appendix 11: Chromatograms of YRC 2894 Recoveries from Peach, Preserve	36
Appendix 12: Chromatograms of YRC 2894 Recoveries from Tomato, Fruit	37
Appendix 13: Chromatograms of YRC 2894 Recoveries from Tomato, Juice	38
Appendix 14: Chromatograms of YRC 2894 Recoveries from Tomato, Paste	39
Appendix 15: Chromatograms of YRC 2894 Recoveries from Tomato, Preserve	40
Appendices 16-30: Chromatograms with Confirmatory Method	41
Appendix 16: Chromatograms of YRC 2894 Recoveries from Apple, Fruit	41
Appendix 17: Chromatograms of YRC 2894 Recoveries from Apple, Dried Fruit	42
Appendix 18: Chromatograms of YRC 2894 Recoveries from Apple, Dry Pomace	43
Appendix 19: Chromatograms of YRC 2894 Recoveries from Apple, Juice	44
Appendix 20: Chromatograms of YRC 2894 Recoveries from Apple, Sauce	45

Table of Contents (cont'd)	Page
Appendix 21: Chromatograms of YRC 2894 Recoveries from Cucumber, Fruit	46
Appendix 22: Chromatograms of YRC 2894 Recoveries from Melon, Peel	47
Appendix 23: Chromatograms of YRC 2894 Recoveries from Melon, Pulp	48
Appendix 24: Chromatograms of YRC 2894 Recoveries from Paprika, Fruit	49
Appendix 25: Chromatograms of YRC 2894 Recoveries from Peach, Fruit	50
Appendix 26: Chromatograms of YRC 2894 Recoveries from Peach, Preserve	51
Appendix 27: Chromatograms of YRC 2894 Recoveries from Tomato, Fruit	52
Appendix 28: Chromatograms of YRC 2894 Recoveries from Tomato, Juice	53
Appendix 29: Chromatograms of YRC 2894 Recoveries from Tomato, Paste	54
Appendix 30: Chromatograms of YRC 2894 Recoveries from Tomato, Preserve	55

1. Summary

This method describes the determination of residues of the insecticide YRC 2894 (Thiacloprid, proposed common name) in plant material.

YRC 2894 is extracted from plant material with a mixture of acetone/water. After filtration an aliquot of the extract is concentrated to the aqueous remainder. The residues are dissolved in water and partitioned against cyclohexane/ethyl acetate using a ChemElut® column.

Further clean-up is performed by column chromatography on Florisil® and elution with acetonitrile.

The residues are quantified by reversed phase HPLC with UV-detection. If necessary, HPLC on a cyano phase column can be used as the confirmatory method.

The method was validated by conducting recovery experiments with commodities relevant for the intended use of YRC 2894. Control samples were amended with YRC 2894 at fortification levels of 0.02 and 0.2 mg/kg. The lowest fortification level corresponds to the limit of quantitation (LOQ) and was 0.02 mg/kg for all matrices tested.

All samples were analysed with the primary method, i.e. chromatography on a reversed phase column. Additionally, the samples at the LOQ were analysed using the confirmatory method, i.e. chromatography on a cyano phase column.

Recoveries of YRC 2894 with the primary method ranged from 72 to 105 % (mean: 95 %, relative standard deviation (RSD): 5.8, n= 97) and with the confirmatory method from 68 to 115 % (mean: 94 %, RSD: 9.0 %, n= 51).

The recoveries were not corrected for interferences. Matrix interference was minimal as illustrated in the control sample chromatograms shown in the Appendix (<10 % of LOQ). Well resolved peaks were obtained with all sample commodities at all fortification levels. This shows the high specificity of the method for the compound of interest.

The repeatability was determined for apple fruit and melon peel running five recoveries at 0.02 and 0.2 mg/kg each, simultaneously. The samples spiked at the LOQ were also analysed with the confirmatory method.

The RSD of the repeatability determination for each recovery set with the primary method ranged from 1.9 to 6.2 %. With the confirmatory method the RSD was about 8 % with the mean recovery identical with the primary method.

These results show excellent repeatability with both methods and a very good agreement between primary and confirmatory method.

1. Zusammenfassung

Die vorliegende Methode beschreibt die Bestimmung von Rückständen des Insektizids YRC 2894 (Thiacloprid, vorgeschlagener Common Name) in pflanzlichen Probenmaterialien. Die Rückstände werden mit einer Mischung von Aceton/Wasser aus Pflanzenmaterial extrahiert. Nach Filtration wird ein Aliquot des Extraktes bis zum wäßrigen Rest eingeengt. Die Rückstände werden in Wasser gelöst und einer Phasenverteilung mit Cyclohexan/Ethylacetat auf einer ChemElut-Säule unterzogen. Die weitere Aufreinigung erfolgt durch Säulenchromatographie über Florisil und Elution mit Acetonitril.

Die Rückstände werden durch Reversed Phase HPLC mit UV-Detektion quantifiziert. Sofern erforderlich, kann HPLC auf einer Cyano-Phase als Bestätigungsmethode verwendet werden.

Die Methode wurde durch Bestimmung von Wiederfindungsraten mit den für die Anwendung von YRC 2894 relevanten Probenmaterialien validiert. Dazu wurden Kontrollproben mit YRC 2894 aufgestockt. Die Zusatzniveaus betragen 0,02 und 0,2 mg/kg. Das niedrigste Zusatzniveau entspricht der Bestimmungsgrenze und betrug für alle getesteten Probenmaterialien 0,02 mg/kg. Alle Proben wurden mit der 'Primärmethode', d. h. RP-HPLC, analysiert. Außerdem wurden die Proben an der Bestimmungsgrenze mit der Bestätigungsmethode, d.h. HPLC auf CN-Phase, analysiert.

Die Ausbeuten für YRC 2894 mit der 'Primärmethode' lagen zwischen 72 und 105 % (Mittelwert: 95 %, relative Standardabweichung (RSD): 5,8 %, n= 97) und für die Bestätigungsmethode zwischen 68 und 115 % (Mittelwert: 94 %, RSD: 9 %, n= 51).

Für die Ausbeuten wurde keine Blindwertkorrektur durchgeführt.

Wie aus den Chromatogrammen der Kontrollproben ersichtlich, waren die Interferenzen aus der Matrix minimal (<10 % der Bestimmungsgrenze). Für alle Matrices und Zusatzniveaus waren die Peaks gut von den Begleitstoffen abgetrennt.

Diese Ergebnisse zeigen die hohe Selektivität der Methode für YRC 2894.

Die Wiederholbarkeit der Methode wurde mit Apfel, Frucht und Melone, Schale überprüft. Dazu wurden jeweils fünf Ausbeuten mit Zusätzen von 0,02 und 0,2 mg/kg parallel bestimmt. Die Proben an der Bestimmungsgrenze wurden auch mit der Bestätigungsmethode analysiert. Die RSD für die einzelnen Probensätze lag zwischen 1,9 und 6,2 %. Mit der Bestätigungsmethode betrug die RSD 8 %.

Die Ergebnisse zeigen eine exzellente Wiederholbarkeit mit beiden Methoden und eine hervorragende Übereinstimmung zwischen den Ergebnissen der 'Primärmethode' und Bestätigungsmethode.

2. Introduction

YRC 2894 is a new experimental insecticide belonging to the chloronicotinyl group. The metabolism of the compound has been investigated in apples and tomatoes following spray application [1, 2]. Further studies with cotton and rice are in progress. From the apple and tomato metabolism study it is evident that the relevant residue is the parent compound which represents about 90 % of the total radioactive residue.

The compound has the following chemical and physical properties:

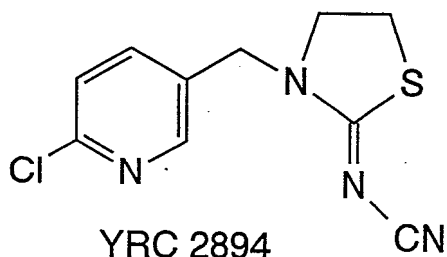
Chemical designation according to

CAS: [3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene] cyanamide

Chemist code: YRC 2894

Proposed common name: Thiacloprid

Structural formula:



Empirical formula:	$C_{10}H_9ClN_4S$
Molecular weight:	252.7 g/mole
Appearance:	yellowish, crystalline powder
Hydrolytic stability: in Acetonitrile/Buffer (1:1)	half life period at 50° C pH 4: >500 h pH 7: >500 h pH 9: >500 h
Melting point:	136 °C
Solubility:	Water 0.18 g/l (pH 4, 7, 9 and unbuffered water) n-Heptane <0.10 g/l Xylol 0.30 g/l 1-Octanol 1.30 g/l 2-Propanol 2.90 g/l Ethyl acetate 9.40 g/l Polyethylene glycol 41.0 g/l Acetonitrile 52.0 g/l Acetone 64.0 g/l Dimethylsulfoxide 50 g/l Dichloromethane 150 g/l
Partition coefficient: (n-octanol/water)	at 20 °C: 18 log (P _{ow}): 1.25
Thermal stability:	YRC 2894 is thermally stable at ambient temperature under air
Dissociation constant:	no basic or acidic properties pK-value not determinable

3. Safety Instructions

For conduct of this method no specific safety measures are required. The German guidelines for laboratories of the Trade Cooperative Associations (e.g. Bulletin M006) or comparable guidelines in other countries must be taken into consideration when working according to this method.

The pertinent safety instructions must be observed (see "R- und S-Sätze") when working with all compounds mentioned in this method.

4. Sampling

Analytical samples are taken and prepared according to the instructions of Chapter VIII of the DFG method collection for the analysis of pesticide residues (Deutsche Forschungsgemeinschaft = German Association for the Advancement of Scientific Research, 1991) to ensure a representative sampling [3].

If the samples cannot be analysed immediately, they are stored in the deep freezer at -18°C or below.

5. Experimental

5.1 Materials

5.1.1 Apparatus

Blender, e.g.: Ultra-Turrax T50 (Janke + Kunkel, IKA-Labortechnik)

Büchner funnel, 90 mm i.d.

Calibrated pipettes, 1 ml, 2 ml, 5 ml, 10 ml

Chromatography column, length 25 cm, 10 mm i.d., with glass frit

Glass beaker, 100 ml, 200 ml

Graduated cylinder, 50 ml, 100 ml, 250 ml

Graduated pipettes, 3.0 ml

HPLC instrument with UV-detector, e.g.: Hewlett Packard 1090 M with diode array detector
or Spectra Physics UV 1000

Integrator or laboratory data system, e.g.: Hewlett Packard LAS 3350

Pear-shaped flask, 100 ml

Round-bottomed flask, 250 ml

Storage bottle, 500 ml, wide-necked with ground joint

Vacuum filter flask, 500 ml

Vacuum rotary evaporator with water bath

Vial, 1.5 ml with safety cap and low-temperature septum

5.1.2 Reagents

Acetone for residue analysis, e.g. Promochem, 46469 Wesel, Germany,
Acetonitrile for chromatography, e.g. LiChrosolv, E. Merck, 64271 Darmstadt, Germany
ChemElut® CE 1020 cartridges (diatomaceous earth) fitted with a disposable stainless steel
needle, e.g. Varian, 24201 Frampton Avenue, Harbor City, CA 90710, USA
Cyclohexane for residue analysis, e.g. Promochem, 46469 Wesel, Germany,
Ethyl acetate for residue analysis, e.g. Promochem, 46469 Wesel, Germany,
Hydrochloric acid, fuming, 37 %, for analysis, E. Merck, 64271 Darmstadt, Germany
Water pure, e.g. from Millipore Milli Q Unit
Filter paper, 90 mm i.d., e.g.: Schwarzband, Schleicher+Schuell, D-3354 Dassel, Germany
Filter aid, e.g.: Celite 545, E. Merck, 64271 Darmstadt, Germany
Florisil®, particle size 0.150 - 0.250 mm, e.g. E. Merck, 64271 Darmstadt, Germany
Sodium sulphate anhydrous, for analysis, e.g. E. Merck, 64271 Darmstadt, Germany
HPLC column, e.g. LiChrospher 60, Select B (5 µm), length 25 cm x 0.4 cm i.d., E. Merck,
64271 Darmstadt, Germany
HPLC column: e.g.: Zorbax SB-CN, Stablebond (5 µm), length 25 cm x 0.46 cm i.d., Bischoff,
71229 Leonberg, Germany
Solvent mixtures:
acetone/water 3/1, v/v
cyclohexane/ethyl acetate 1/1, v/v
acetonitrile/water 1/1, v/v

5.1.3 Reference Substance

The following reference substance was used during this study:

Name of Substance:	YRC 2894 (Thiacloprid)
Certificate of Analysis:	941013ELB01, dated November 14, 1994
Certified Assay:	99.7 %
Expiry Date:	November 1996

5.1.4 Standard Solutions Required

Stock solution of YRC 2894:

1000 mg/l YRC 2894 in acetonitrile

Weigh 0.100 g of YRC 2894 into a 100-ml volumetric flask. Dilute the chemical to volume with acetonitrile.

Secondary Standards: Prepare additional solutions from the primary standard as follows:

- | | |
|---------------|---|
| (A) 100 mg/l | Pipette 10.0 ml of the primary standard into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (B) 10 mg/l | Pipette 10.0 ml of the secondary standard (A) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (C) 2.5 mg/l | Pipette 2.5 ml of the secondary standard (A) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (D) 1 mg/l | Pipette 10.0 ml of the secondary standard (B) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (E) 0.5 mg/l | Pipette 5.0 ml of the secondary standard (B) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (F) 0.25 mg/l | Pipette 2.5 ml of the secondary standard (B) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (G) 0.1 mg/l | Pipette 10.0 ml of the secondary standard (D) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |
| (H) 0.05 mg/l | Pipette 5.0 ml of the secondary standard (D) into a 100-ml volumetric flask, and dilute the solution to volume with acetonitrile/water (1/1, v/v). |

Storage of Standard Solutions

Store the standard solutions under refrigerator conditions (6 +/- 3°C) protected from light; under these conditions the standard solutions are stable for at least 6 months.

5.2 Analytical Method

5.2.1 Extraction and Sample Clean-up

1. Place 25.00 g [G, weight of the sample to be used in the residue calculation] of the plant material in a 500-ml storage bottle. Add 150 ml of acetone/water (3/1, v/v) and allow the sample to soak for 30 min.
2. Blend the sample using an ultra-turrax blender (or equivalent) for approximately 3 min.
3. Vacuum filter the suspension through 5 g of Celite filter aid using Schwarzband filter paper supported on a Büchner funnel into a 500-ml vacuum filter flask.
4. Wash the filtered solids with a total of 70 ml of acetone/water (3/1, v/v). Press residual solvent from the solids using rubber damming. Discard the filtered solids.
5. Transfer the filtrate and wash to a 250-ml graduated cylinder. Add sufficient acetone to bring the total volume of the extracts to 250 ml. Mix the solution well, and transfer 50 ml (5 g sample equivalent) to a 250-ml round-bottom flask.
6. Concentrate the aliquot to an aqueous remainder of 5 to 10 ml using a rotary evaporator with a max. bath temperature of 60 °C.
7. Proceed to ChemElut column clean-up step 5.2.2

5.2.2 ChemElut® Column Clean-up

1. Add 10 to 15 ml water to the aqueous solution from 5.2.1 step 7 to bring the total volume of the extracts to 20 ml.
2. Place the aqueous solution on the top of the ChemElut® CE 1020 (20 ml volume) column and wait for 15 minutes to achieve an equal distribution of the liquid on the column.
3. Elute the YRC 2894 residues from the column with 60.0 ml of cyclohexane/ethyl acetate (1/1, v/v). Collect the eluate in a 250-ml round-bottom flask.
4. Evaporate the eluate from step 3 to dryness using a vacuum rotary evaporator and a max. bath temperature of 60 °C.
5. Dissolve the residue in 2.00 ml of ethyl acetate and proceed to step 5.2.3.2.

5.2.3 Column Chromatography on Florisil®

5.2.3.1 Florisil® Column Preparation

1. Apply 50 ml ethyl acetate into a chromatography column (i.d. 10 mm) and allow 10 g Florisil® (deactivated with 5 % water) to trickle into the column.
2. Compact the column packing by carefully knocking on the glass column and cover the packing with a ca. 0.5 cm layer of sodium sulphate.
3. Allow the solvent to drain down to the sodium sulphate layer.

5.2.3.2 Florisil® Column Clean-up

1. Apply the organic solution from 5.2.2 step 5 onto the column. Allow the solution to pass through the column at a flow rate of 1 ml/min. Rinse the 250-ml round-bottom flask with 2.0 ml of ethyl acetate and apply the solution onto the column, too.
2. Wash the column with 80.0 ml of ethyl acetate at a flow rate of 1-2 ml/min. Discard the eluate.
3. Elute the YRC 2894 residues with 30.0 ml of acetonitrile at a flow rate of 1-2 ml/min. Collect the eluate in a 100-ml pear-shaped flask.
4. Evaporate the eluate from step 3 to dryness using a vacuum rotary evaporator and a max. bath temperature of 60 °C. Dissolve the residue in 1.00 ml of acetonitrile/water (1/1, v/v) and proceed to step 5.3.1.

NOTE

1. *The volumes to be used for flushing the column with ethyl acetate and for elution with acetonitrile must be newly determined for each batch of deactivated Florisil®!*
2. *The flow rate should not be too high, since otherwise losses of the residues in step 2 may occur with recoveries below 70 % and the clean-up in step 3 is less effective.*

5.3.1 HPLC UV-Determination of YRC 2894 Residues

Measuring equipment and HPLC conditions for the primary method:

Instrument: Hewlett Packard 1090 M *
 Column: e.g.: LiChrospher 60, RP-Select B (5 µm)
 25 cm length x 0.4 cm i.d.
 Injection volume: 25 µl
 Oven temperature: 40°C
 Detection: UV-detector, 242 nm; e.g.: Spectra Physics UV 1000*
 Eluant: A) Milli-Q-water with 0.4 ml hydrochloric acid (35 %) / 1 water
 B) Acetonitrile
 Flow rate: 1.5 ml/min

* = or equivalent instrument

Gradient:

Time [min]	A [% v/v]	B [% v/v]
0	80	20
10	65	35
12	10	90
15	10	90
16	80	20
20	80	20

Retention time: ca. 9.2 min

Total run time: 20 min

For information concerning the linearity of the detector response see Figure 2.

5.3.2 Confirmatory Method for YRC 2894 Residues

If necessary, the residues as determined according to 5.3.1 can be confirmed using the procedure described below.

Measuring equipment and HPLC conditions:

Instrument: Hewlett Packard 1090 M *
Column: e.g.: Zorbax SB-CN, Stablebond (5 µm)
25 cm length x 0.46 cm i.d.
Injection volume: 25 µl
Oven temperature: 40°C
Detection: UV-detector, 242 nm; e.g.: Spectra Physics UV 1000*
Eluant: A) Milli-Q-water
B) Acetonitrile
Flow rate: 1.5 ml/min

* = or equivalent instrument

Gradient:

Time [min]	A [% v/v]	B [% v/v]
0	85	15
11	60	40
13	10	90
16	10	90
17	85	15
20	85	15

Retention time: ca. 9.6 min #
Total run time: 20 min

For information concerning the linearity of the detector response under conditions of the confirmatory procedure see Figure 3.

Note

The retention time differed about 1 min. between individual columns. A better reproducibility of the retention time may be achieved by addition of buffer to the mobile phase.

6. Calculation

6.1 Calculation of YRC 2894 Residues

1. Calculate the average of the standard responses to be used for calibration.
2. Determine the peak area or peak height for the corresponding peak from the chromatogram of the analysed sample.
3. Calculate the residue level in mg/kg as follows:

$$R = \frac{F_A * V_{END} * W_{ST}}{F_{ST} * V_{INJ} * G * Al}$$

where:

- R = Determined amount of residue in mg/kg
- F_A = Peak area for the analytical solution obtained from V_{INJ}
- F_{ST} = Peak area for the standard solution obtained from W_{ST}
- V_{END} = Final volume of the respective sample solution in ml
- V_{INJ} = Injection volume in µl
- W_{ST} = Amount of YRC 2894 in ng contained in V_{INJ} of the standard solution
- G = Weighed amount of analytical sample in g
- Al = Aliquotation factor

6.2 Calculation of YRC 2894 Recoveries

1. Calculate the average of the residue in the recovery sample according to 6.1.
2. Calculate the percent recovery as follows:

$$\text{Recovery} = \frac{\text{Mean Residue} * 100}{\text{Fortification Level}}$$

- Recovery = Recovered amount of YRC 2894 found in fortified sample in %
- Mean Residue = Average residue in fortified sample determined according to 6.1 in mg/kg
- Fortification Level = Fortified concentration of YRC 2894 in mg/kg

7. Results and Discussion

7.1 Linearity of the detector

The linearity of the detector response was tested for YRC 2894 with the primary and confirmatory method (see Figures 2 and 3). The injected amount ranged from 1.25 ng to 62.5 ng of YRC 2894. An excellent linear relation between injected amount and peak area was observed with a correlation coefficient of the regression line of 1.0000 and a negligible intercept. This justifies using the single point calibration method as described in chapter 6 for calculation of the residues.

7.2 Extraction Efficiency

In the apple and tomato metabolism study 84 % of the total radioactive residue (TRR) was removed by surface washing with dichloromethane or methanol. Further 13 to 14 % of the TRR were extracted by blending the fruits with methanol and only 2-3 % of the TRR remained in the solids. From these data it can be extrapolated that the extraction procedure with acetone/water used in this method should be capable of extracting the residues of YRC 2894 from fruits after spray application.

7.3 Recovery Data and Statistical Evaluation

The method was validated by conducting recovery experiments with all commodities relevant for the intended use of YRC 2894. Control samples were amended with YRC 2894 at fortification levels of 0.02 and 0.2 mg/kg. For apple and tomato juice recoveries were performed at 0.02 mg/kg only, since for juice no concentration of residues is expected to occur during processing.

The lowest fortification level corresponds to the limit of quantitation (LOQ) and was 0.02 mg/kg for all matrices tested.

All samples were analysed with the primary method, i.e. chromatography on a reversed phase column. Additionally, the samples at the LOQ were analysed using the confirmatory method, i.e. chromatography on a cyano phase column.

Table 1 lists the recoveries of YRC 2894 which were obtained with the primary method. The recoveries determined using the confirmatory method are presented in Table 2.

The recoveries were not corrected for interferences. Matrix interference was minimal as illustrated in the control sample chromatograms shown in the Appendix (<10 % of LOQ). For most sample materials two control samples of different origin were analysed. Well resolved peaks were obtained with all sample commodities at all fortification levels. This shows the high specificity of the method for the compound of interest.

For the primary method the overall mean recovery for YRC 2894 with all commodities at different fortification levels was 95 % with a relative standard deviation (RSD) of 5.8 % calculated from a total of 97 individual results. The minimum recovery was 72 %, while the maximum recovery was 105 %.

A more detailed evaluation of the results of the recovery experiments with the primary method is summarised in the following table:

Results of Recovery Testing for YRC 2894

Spiking Level (mg/kg)	Recovery Results			
	Mean (%)	Range Min-Max	RSD (%)	N
0.02*	95	72-105	6.0	51
0.2	95	79-104	4.9	46
Overall	95	72-105	5.8	97

RSD: relative standard deviation, N: number of recovery experiments

*: lowest spiking level corresponding to LOQ

The repeatability was determined for apple fruit and melon peel running five recoveries at 0.02 and 0.2 mg/kg each, simultaneously. The samples spiked at the LOQ were also analysed with the confirmatory method. The results are tabulated below:

Results of Repeatability Testing for YRC 2894 with Primary Method

Sample Material	Spiking Level (mg/kg)	Recovery Results (%)	Mean (%)	RSD (%)	N
Apple, Fruit	0.02	98, 100, 101, 102, 103	101	1.9	5
	0.2	90, 94, 96, 97, 99	95	3.6	5
Melon, Peel	0.02	84, 85, 93, 93, 97	90	6.2	5
	0.2	92, 97, 99, 99, 101	98	3.5	5

RSD: relative standard deviation, N: number of recovery experiments

Results of Repeatability Testing for YRC 2894 with Confirmatory Method

Sample Material	Spiking Level (mg/kg)	Recovery Results (%)	Mean (%)	RSD (%)	N
Apple, Fruit	0.02	94, 97, 99, 100, 114	101	7.7	5
Melon, Peel	0.02	80, 85, 91, 93, 99	90	8.2	5

RSD: relative standard deviation, N: number of recovery experiments

The RSD of the repeatability determination for each recovery set with the primary method ranged from 1.9 to 6.2 %. With the confirmatory method the RSD was about 8 % with the mean recovery identical with the primary method.

These results show excellent repeatability with both methods and a very good agreement between primary and confirmatory method.

7.4 Stability of Analytical Solution

The stability of YRC 2894 in the analytical solutions of recovery experiments with various sample materials (apple fruit, melon peel, tomato, fruit and paste) at 0.02 and 0.2 mg/kg was investigated.

After initial analysis within 1 day after extraction, the analytical solutions were stored in a refrigerator (4 to 8 °C) and analysed after storage periods of 1 and 4 weeks. The results obtained are given in Table 3. These data demonstrate that the residues in the analytical solutions are stable for at least 4 weeks under refrigerator conditions.

In general the stability of the residues during the whole analytical procedure is proven by performing concurrent recovery experiments with each sample set.

8. References

- [1] T. Clark and W. Bornatsch (1996)
Metabolism of [Pyridinyl-¹⁴C-Methyl]YRC 2894 in Apples
Bayer AG, report in preparation .
- [2] P. Babczinski and W. Bornatsch (1996)
Metabolism of [Pyridinyl-¹⁴C-Methyl]YRC 2894 in Tomatoes
Bayer AG, internal report PF-4118, report in preparation
- [3] Deutsche Forschungsgemeinschaft (1991):
(German Society for the Advancement of Scientific Research):
Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für
Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel, Methodensammlung
der Arbeitsgruppe "Analytik", (Residue analysis of plant protectants, announcement VI
of the Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und
Vorratsschutzmittel, method collection of the working group "Analytics"), 1st to 11th
instalment.
VCH Verlagsgesellschaft, Weinheim; Deerfield Beach, Florida; Basel.

Tables

Table 1: Recovery Rates for YRC 2894

Column: Merck, LiChrospher 60, RP-Select B, 25 cm, 0.4 cm i.d., 5 µm

Crop	Sample material	Fortification level [mg/kg]	Recovery rate [%]		RSD [%]
			Individual values	Mean	
Apple	Fruit	0.02	100, 102, 98, 101, 103	101	1.9
		0.20	94, 97, 96, 99, 90	95	3.6
	Dried apple	0.02	72, 86, 88	82	10.6
		0.20	86, 91, 93	90	4.0
	Juice	0.02	99, 94, 100	98	3.3
		Pomace, dry	0.02	84, 91, 98	91
	Sauce		0.20	79, 94, 84	86
		0.02	95, 100, 98	98	2.6
0.20	92, 98, 93	94	3.4		
Cucumber	Fruit	0.02	89, 94, 94	92	3.1
		0.20	83, 92, 96	90	7.4
Melon	Peel	0.02	93, 97, 85, 93, 84	90	6.2
		0.20	92, 99, 99, 97, 101	98	3.5
	Pulp	0.02	92, 95, 97	95	2.7
		0.20	91, 100, 96	96	4.7
Paprika	Fruit	0.02	97, 99, 99	98	1.2
		0.20	92, 99, 95	95	3.7
Peach	Fruit	0.02	91, 103, 100	98	6.4
		0.20	96, 98, 102	99	3.1
	Preserve	0.02	97, 99, 100	99	1.5
		0.20	95, 96, 93	95	1.6
Tomato	Fruit	0.02 *	#42, 92, 98		
		0.02 **	100, 102, 105	99	4.9
		0.20 *	100, 94, 96	97	3.2
		0.20 **	99, 97, 104	100	3.6
	Paste	0.02	98, 97, 99	98	1.0
		0.20	97, 95, 97	96	1.2
	Juice	0.02	89, 94, 97	93	4.3
		Preserve	0.02	90, 95, 98	94
	0.20		90, 94, 90	91	2.5
				Overall	95

* First sample set, ** Second sample set, #: Value not used for evaluation

Table 2: Recovery Rates for YRC 2894 with Confirmatory Method

Column: Bischoff, Zorbax SB-CN, 25 cm, 0.46 cm i.d., 5 µm

Crop	Sample material	Fortification level [mg/kg]	Recovery rates (%)		RSD
			Individual values	Mean	
Apple	Fruit	0.02	114, 99, 94, 100, 97	101	7.7
	Dried apple	0.02	68, 80, 80	76	9.1
	Juice	0.02	97, 109, 98	101	6.6
	Pomace, dry	0.02	82, 115, 94	97	17.2
	Sauce	0.02	84, 90, 88	87	3.5
Cucumber	Fruit	0.02	88, 96, 90	91	4.6
Melon	Pulp	0.02	88, 91, 93	91	2.8
	Peel	0.02	91, 93, 85, 99, 80	90	8.2
Paprika	Fruit	0.02	90, 94, 97	94	3.7
Peach	Fruit	0.02	92, 102, 103	99	6.1
	Preserve	0.02	95, 96, 102	98	3.9
Tomato	Fruit	0.02 *	#41, 90, 99		
		0.02 **	102, 99, 102	98	5.0
	Paste	0.02	99, 95, 99	98	2.4
	Juice	0.02	88, 93, 90	90	2.8
	Preserve	0.02	85, 95, 96	92	6.6
Overall				94	9.0

* First sample set, ** Second sample set, #: Value not used for evaluation

Table 3: Stability of YRC 2894 in Analytical Solutions

Sample material	Days of Storage	Recovery after Storage	
		Fortification Level / Sample ID	
		0.02 mg/kg / A, B, C, D, E	0.2 mg/kg / A, B, C, D, E
Apple, Fruit	0	100, 102, 98, 101, 103	94, 97, 96, 99, 90
	7	98, 100, 96, 100, 98	94, 97, 95, 99, 89
	28	94, 97, 93, 96, 95	93, 95, 95, 98, 88
Melon, Peel	0	93, 97, 85, 93, 84	92, 99, 99, 97, 101
	7	95, 96, 89, 100, 80	91, 103, 102, 97, 107
	28 #	119, 99, 106, 129, 82	89, 115, 109, 97, 134
Tomato, Fruit	0	92, 42, 98	100, 94, 96
	7	91, 41, 98	99, 94, 96
	28	93, 42, 100	99, 95, 95
Tomato, Paste	0	98, 97, 99	97, 95, 97
	7	99, 91, 104	98, 96, 97
	28	113, 100, 94	97, 97, 97

#: Increase in residues is due to evaporation of solvent during storage, because sample vials (0.02 mg/kg sample A, C, D and 0.2 mg/kg sample B, C, E) were not capped tightly

Figures

Figure 1: Flowdiagram of Method

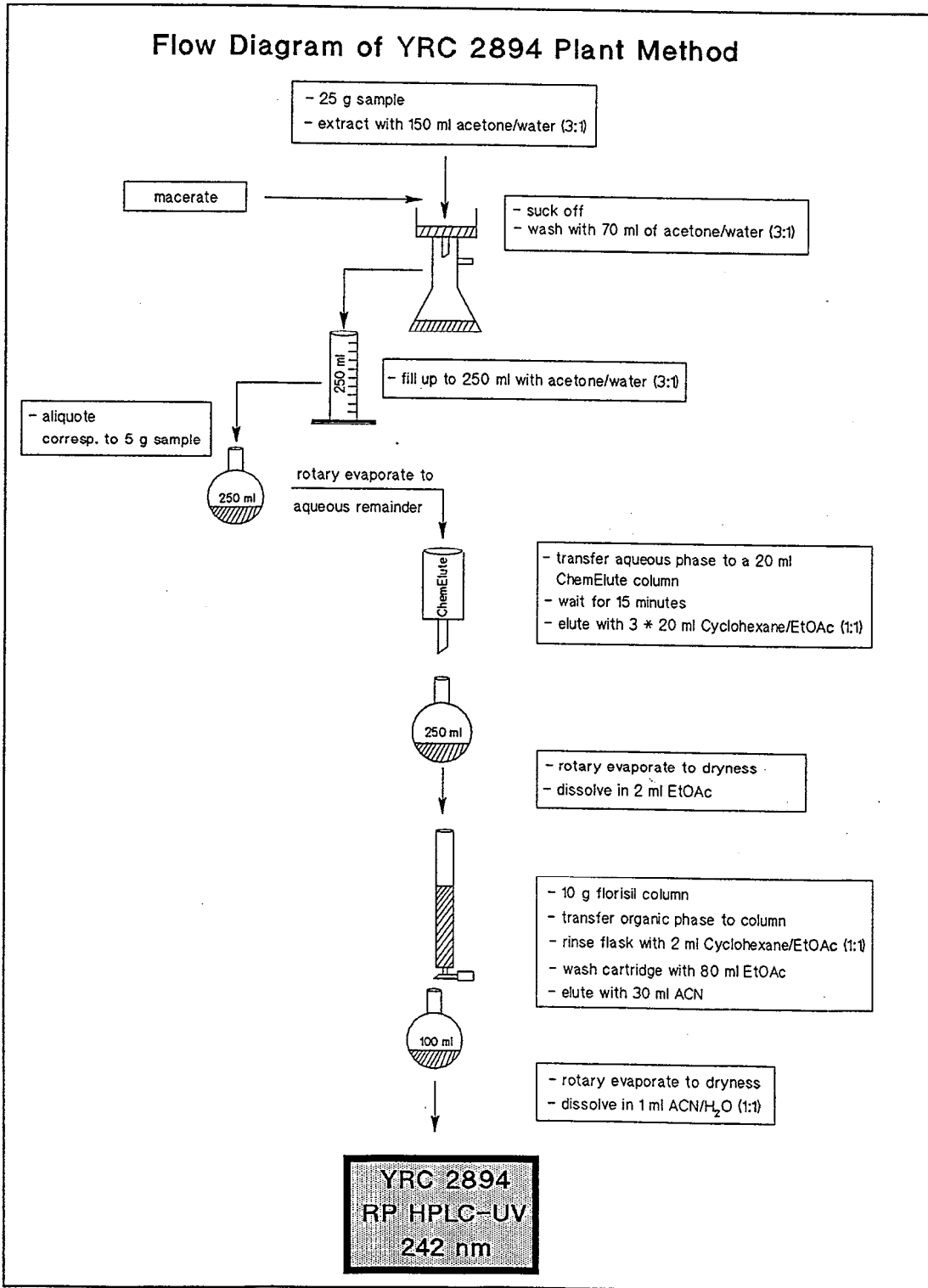
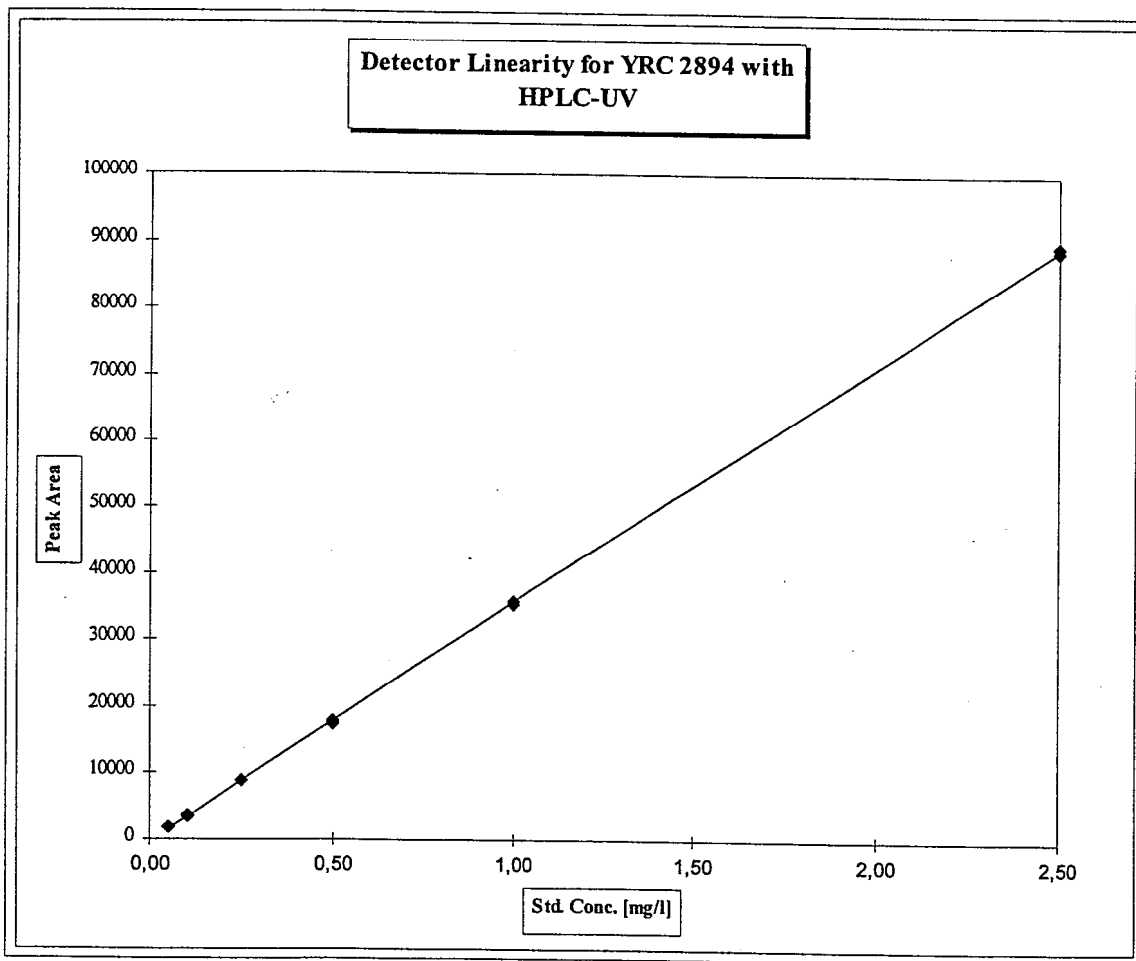


Figure 2: Detector Linearity with HPLC-UV



Data for Linearity Determination of YRC 2894

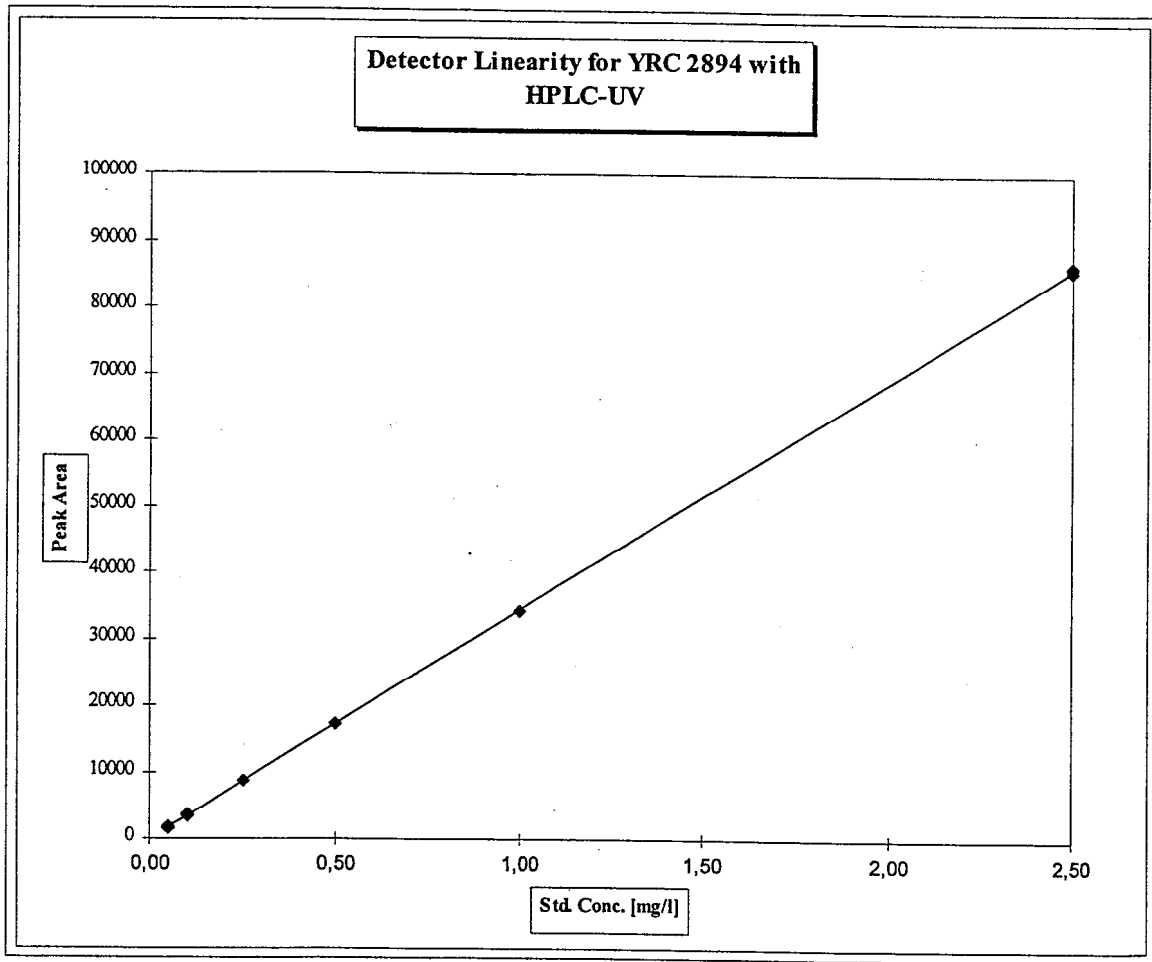
Instrument: HP 1090
 Column: Merck, Select-B, 25 cm, 0.4 cm i.D., 5 um, 25 ul injected
 Detector: Spectra Physics Analytical, UV-1000, Range 0.02

Standard Amount		Peak Area
[ng]	[mg/l]	
1.25	0.05	1781, 1802, 1793
2.50	0.10	3602, 3553, 3536
6.25	0.25	9011, 8947, 8911
12.5	0.50	17803, 17537, 17695
25.0	1.00	35728, 35374, 35398
62.5	2.50	89629, 88938, 89630

Regression parameters

y-axis intercept: -71
 slope: 35754
 correlation coefficient: 1.0000

Figure 3: Detector Linearity with HPLC-UV Confirmatory Method



Data for Linearity Determination of YRC 2894 with Confirmatory Method

Instrument: HP 1090
 Column: Bischoff, Zorbax SB-CN, 25 cm, 0.46 cm i.D., 5 um, 25 ul injected
 Detector: Spectra Physics Analytical, UV-1000, Range 0.02

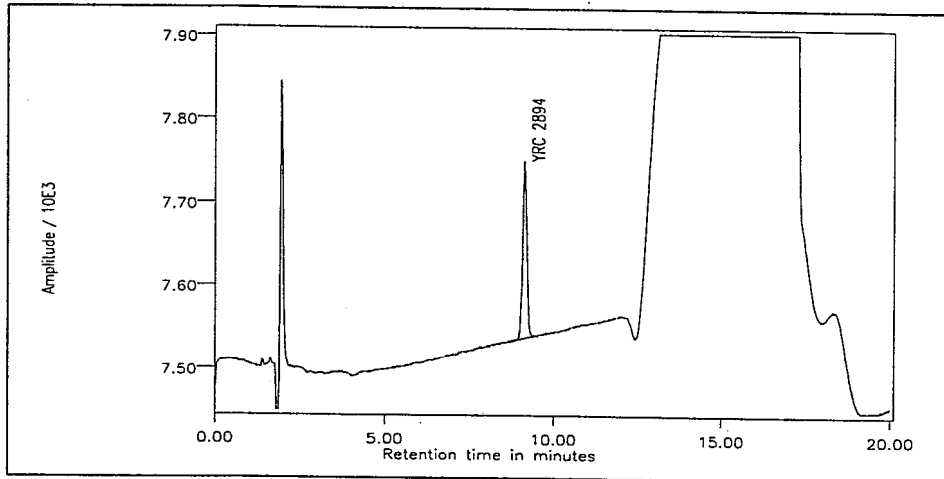
Standard Amount		Peak Area
[ng]	[mg/l]	
1.25	0.05	1711, 1876, 1913
2.50	0.10	3489, 3565, 3569
6.25	0.25	8627, 8807, 8713
12.5	0.50	17264, 17140, 17132
25.0	1.00	34181, 34145, 34358
62.5	2.50	86695, 86402, 85868

Regression parameters
 y-axis intercept: 20
 slope: 34476
 correlation coefficient: 1.0000

Appendices 1 - 15: Chromatograms with Primary Method

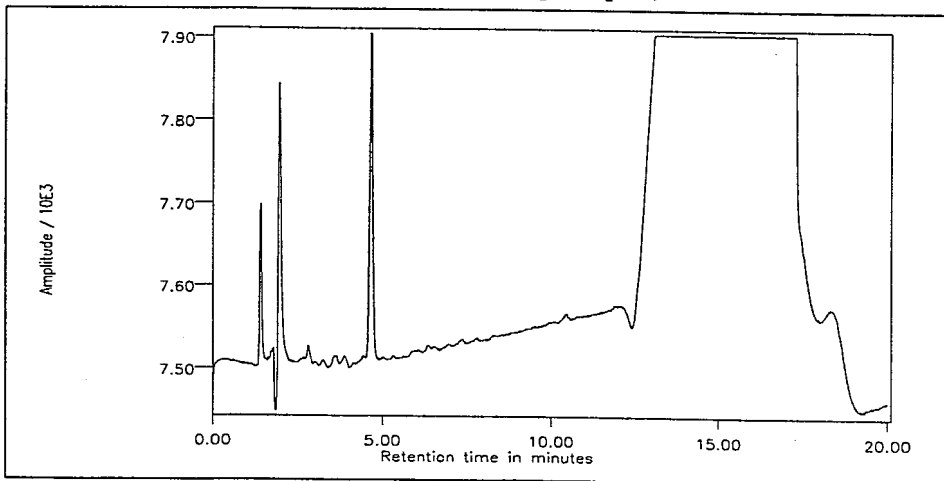
Appendix 1: Chromatograms of YRC 2894 Recoveries from Apple, Fruit

Standard YRC 2894, 0.1 mg/l



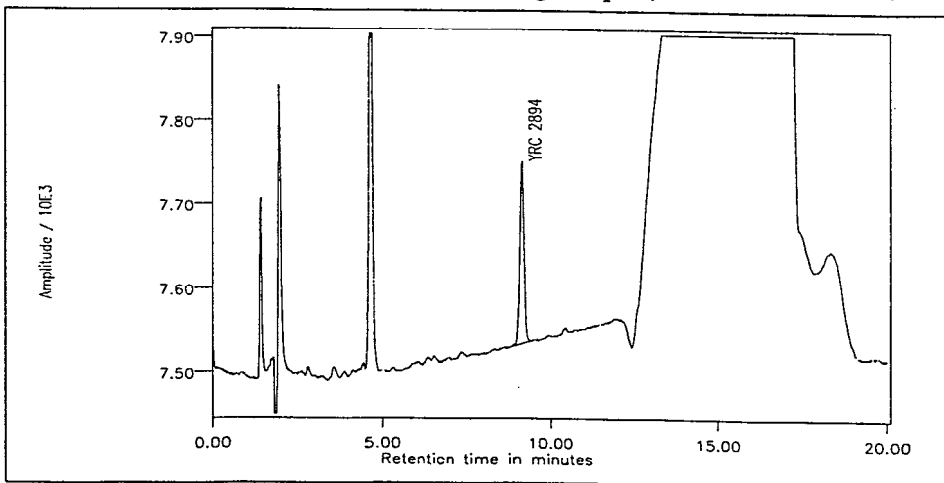
Apple, Fruit, Control,

5 g Aliquot, 1 ml Final Volume



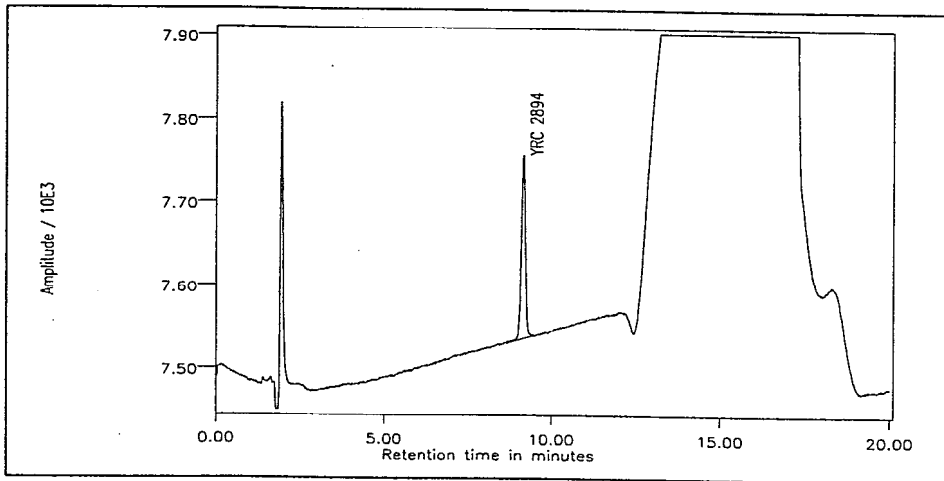
Recovery YRC 2894, Apple, Fruit

Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

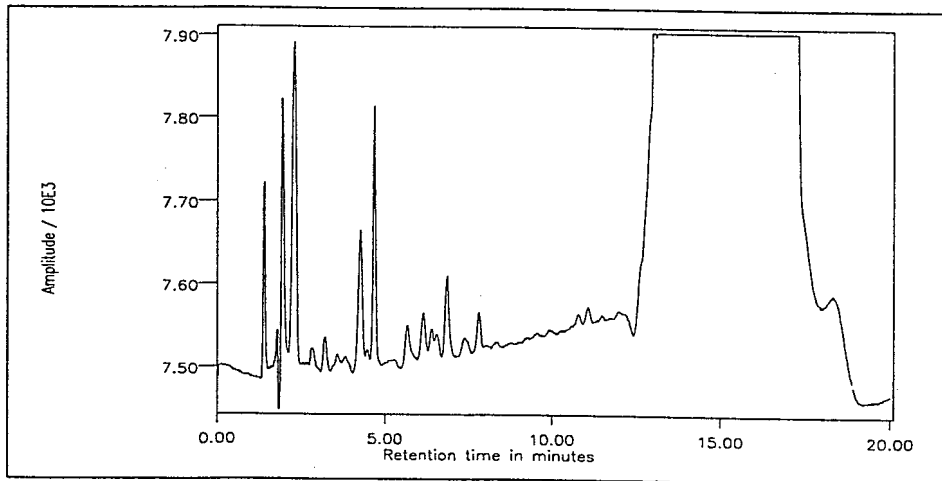


Appendix 2: Chromatograms of YRC 2894 Recoveries from Apple, Dried Fruit

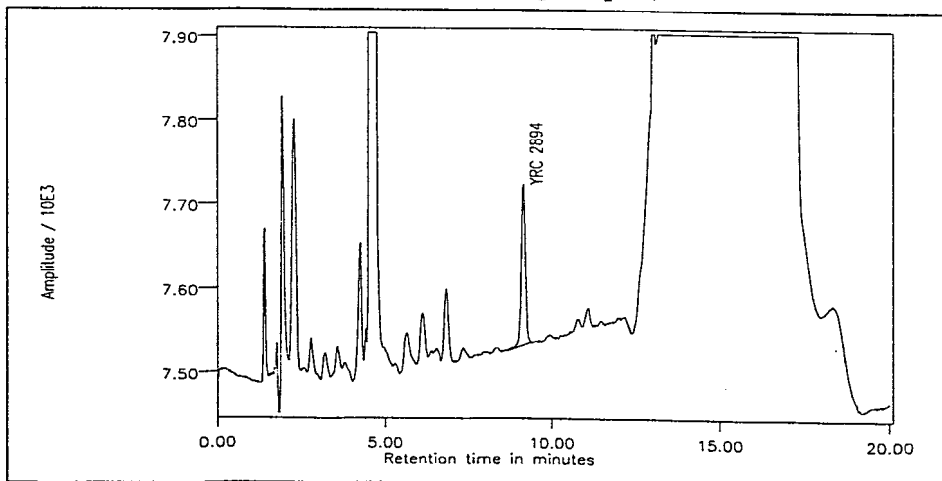
Standard YRC 2894, 0.1 mg/l



Apple, Fruit, Dried, 5 g Aliquot, 1 ml Final Volume

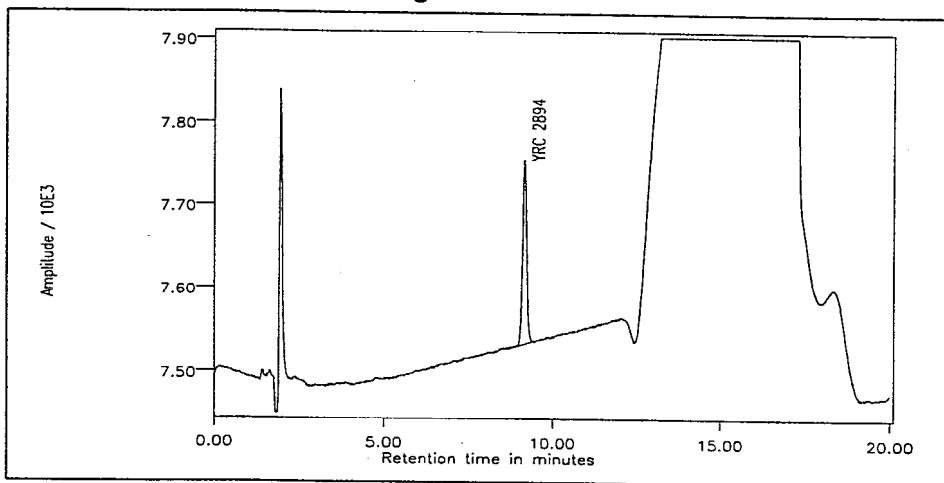


Recovery YRC 2894, Apple, Dried
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume



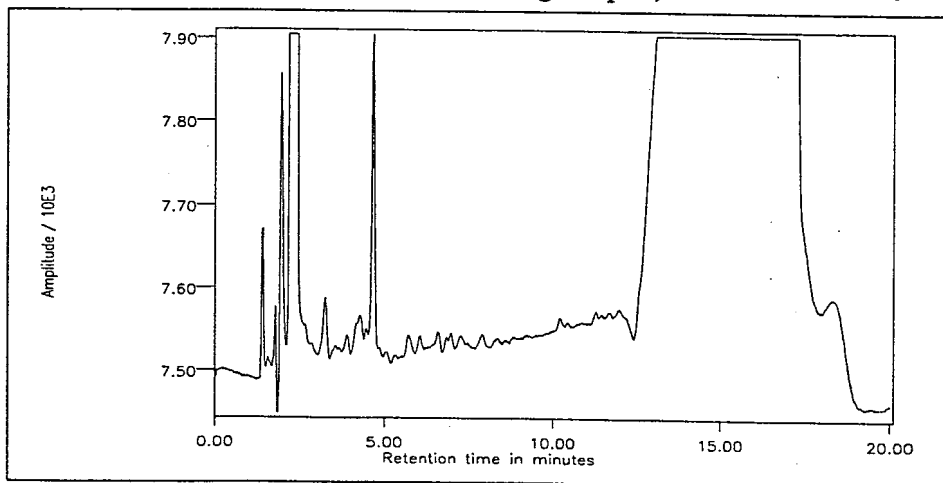
Appendix 3: Chromatograms of YRC 2894 Recoveries from Apple, Dry Pomace

Standard YRC 2894, 0.1 mg/l



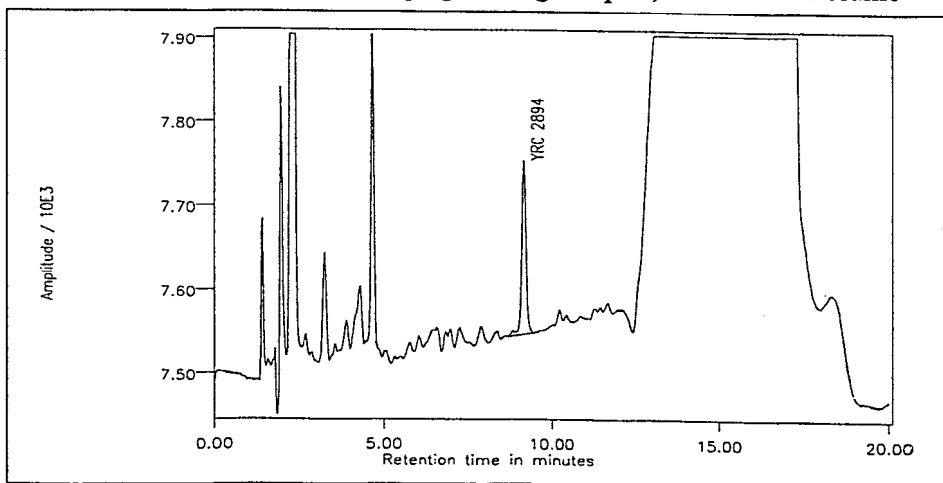
Apple, Fruit, Draff,

5 g Aliquot, 1 ml Final Volume



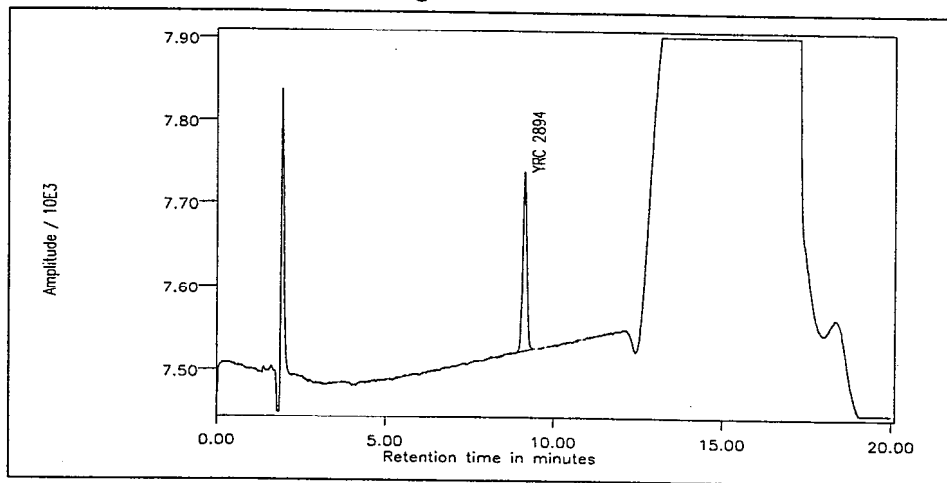
Recovery YRC 2894, Apple, Draff

Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

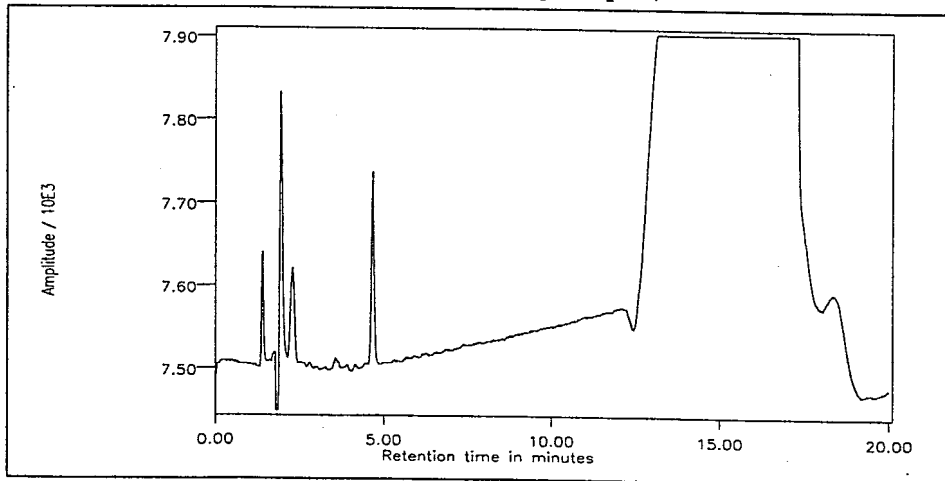


Appendix 4: Chromatograms of YRC 2894 Recoveries from Apple, Juice

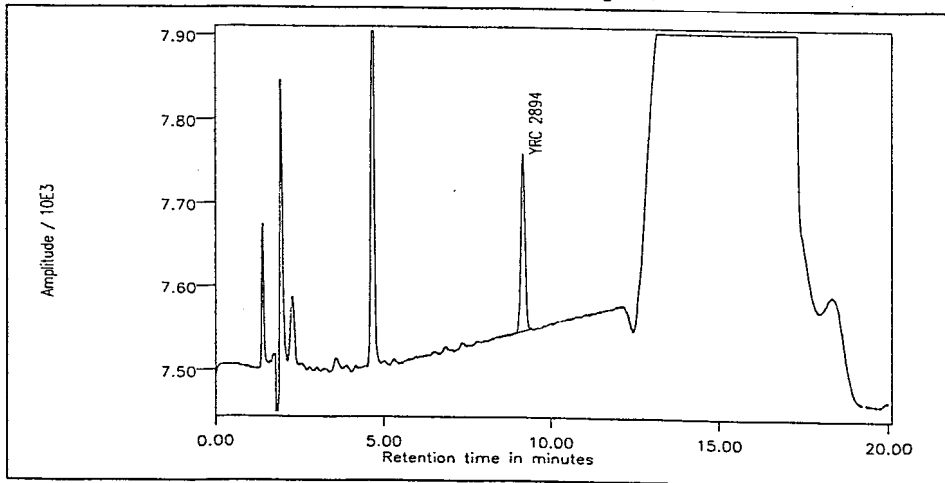
Standard YRC 2894, 0.1 mg/l



Apple, Juice, Control, 5 g Aliquot, 1 ml Final Volume

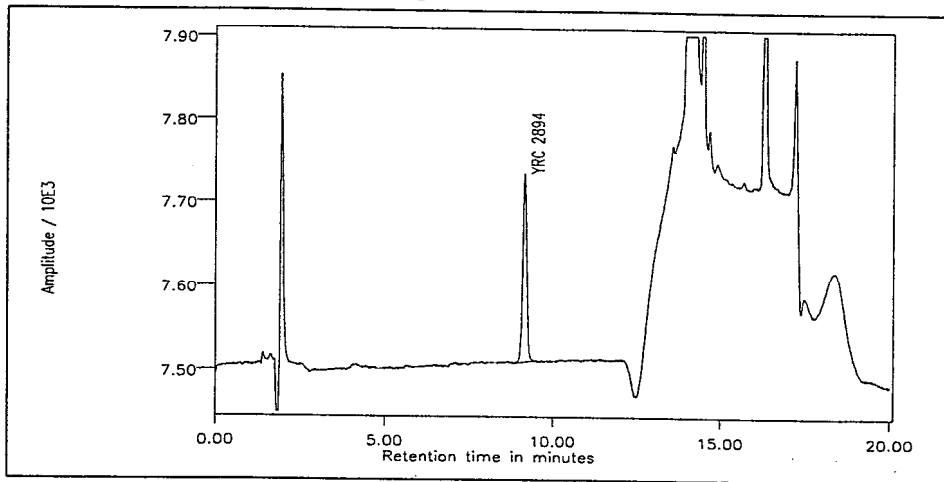


Recovery YRC 2894, Apple, Juice
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

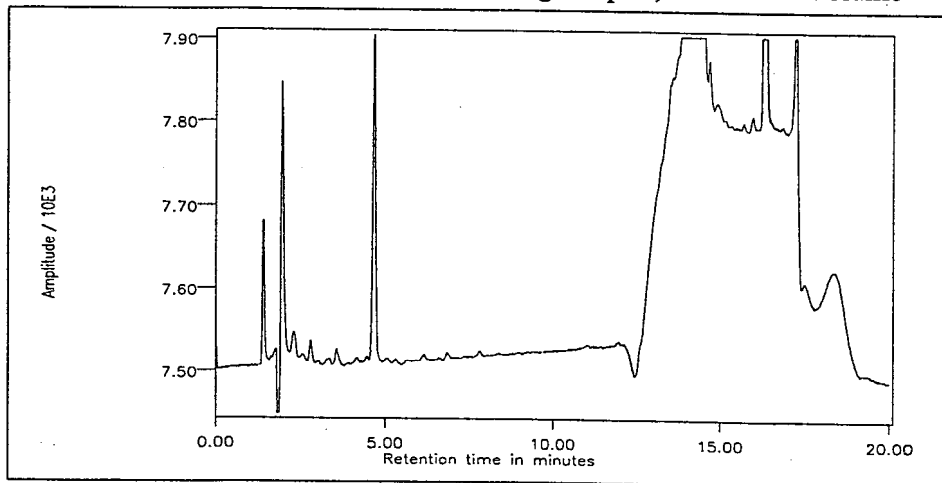


Appendix 5: Chromatograms of YRC 2894 Recoveries from Apple, Sauce

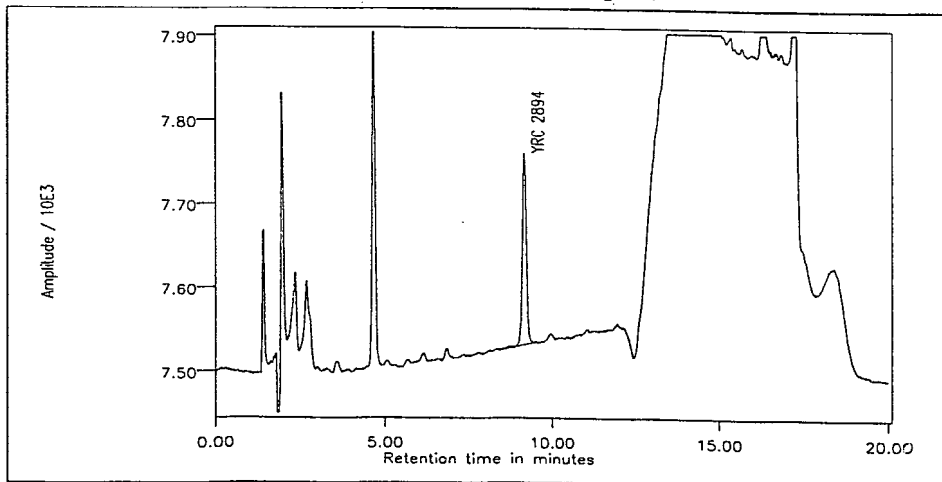
Standard YRC 2894, 0.1 mg/l



Apple, Sauce, Control, 5 g Aliquot, 1 ml Final Volume

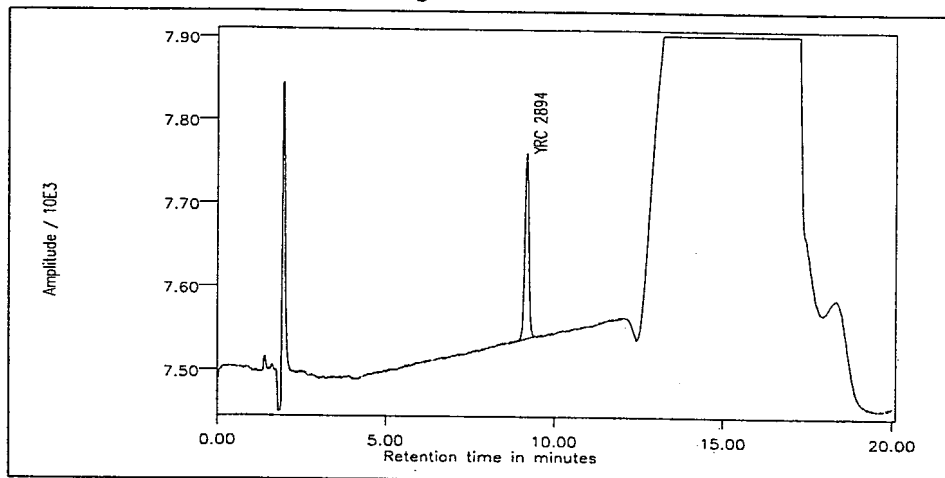


Recovery YRC 2894, Apple, Sauce
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

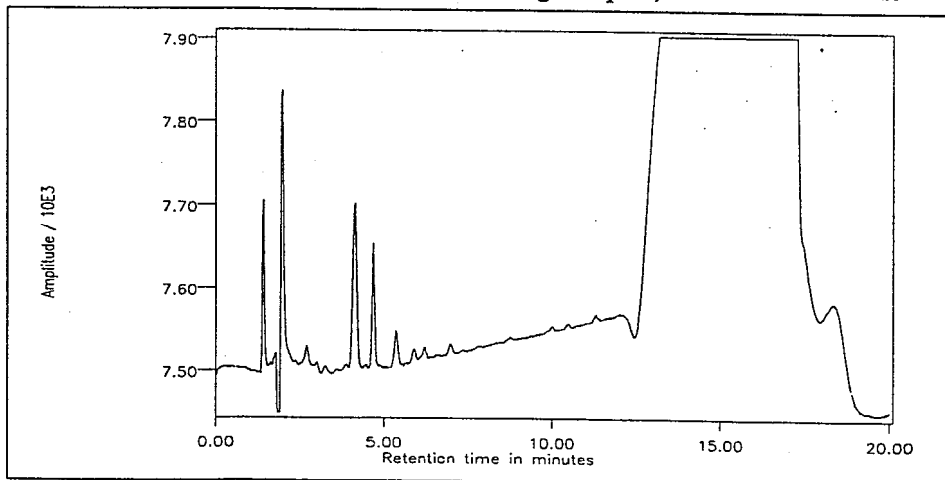


Appendix 6: Chromatograms of YRC 2894 Recoveries from Cucumber, Fruit

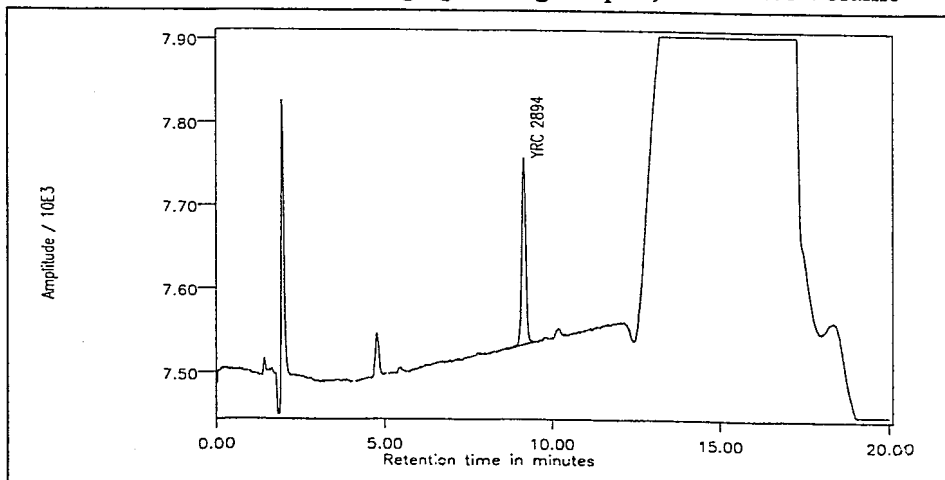
Standard YRC 2894, 0.1 mg/l



Cucumber, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

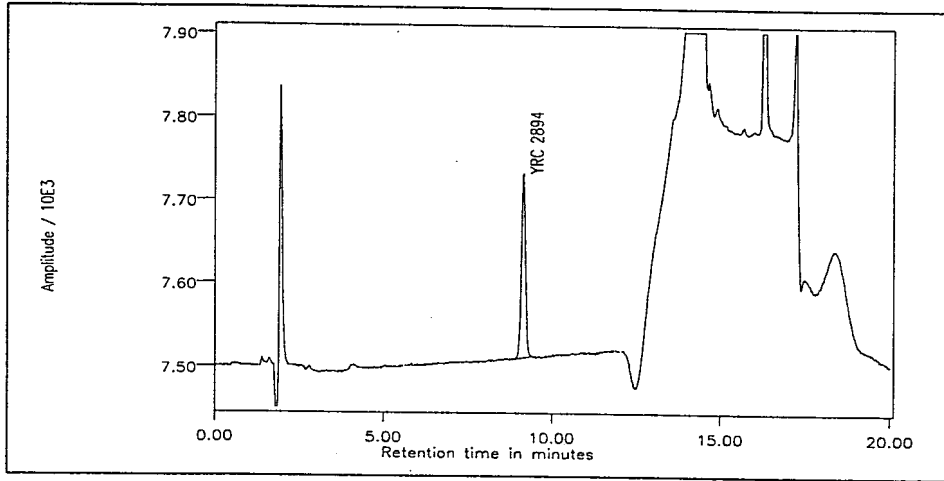


Recovery YRC 2894, Cucumber, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

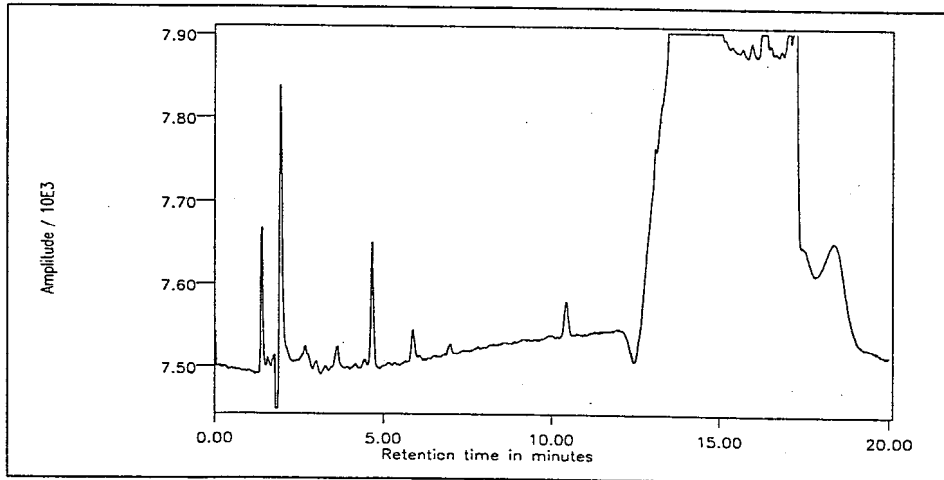


Appendix 7: Chromatograms of YRC 2894 Recoveries from Melon, Peel

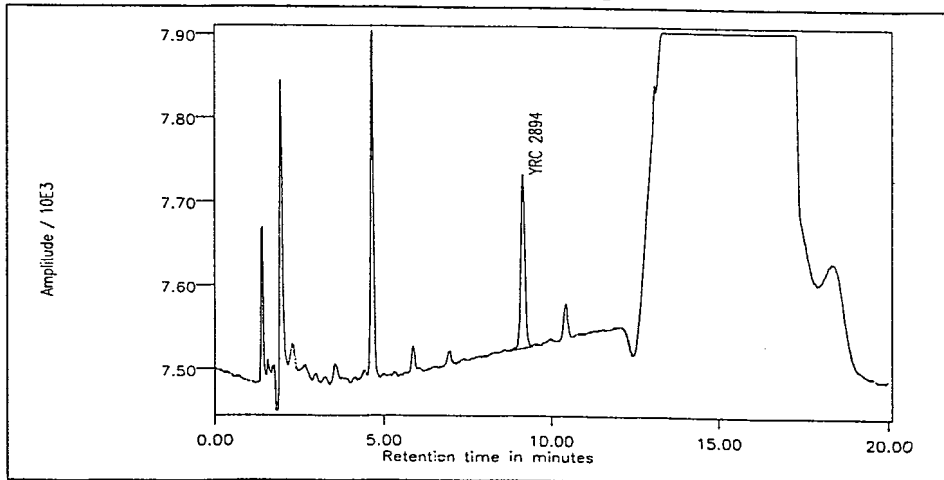
Standard YRC 2894, 0.1 mg/l



Melon, Peel, Control, 5 g Aliquot, 1 ml Final Volume

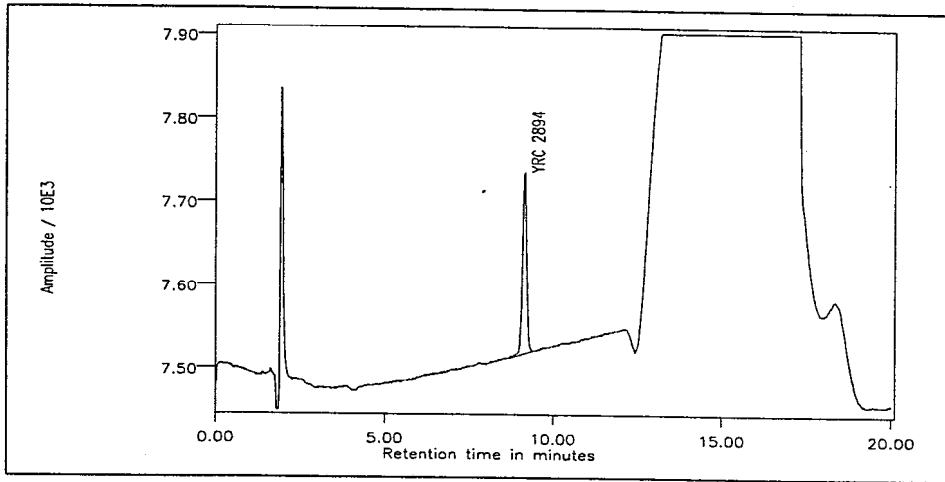


Recovery YRC 2894, Melon, Peel
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

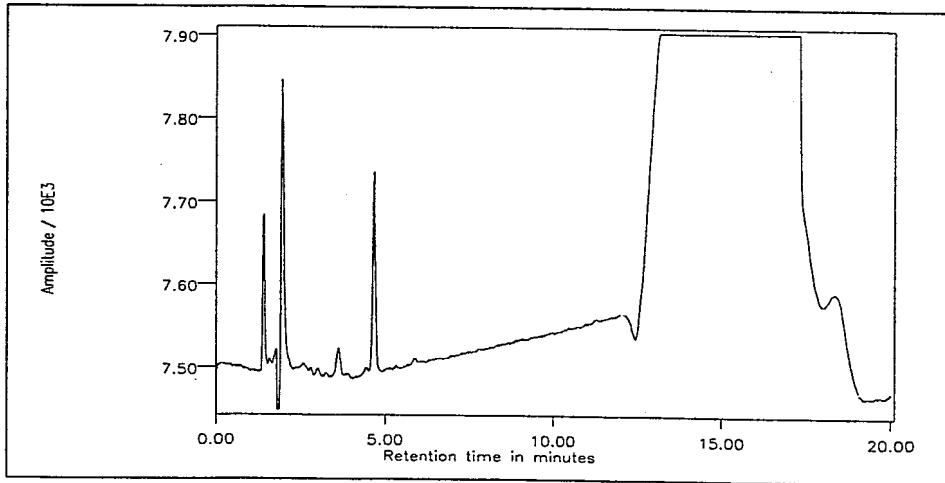


Appendix 8: Chromatograms of YRC 2894 Recoveries from Melon, Pulp

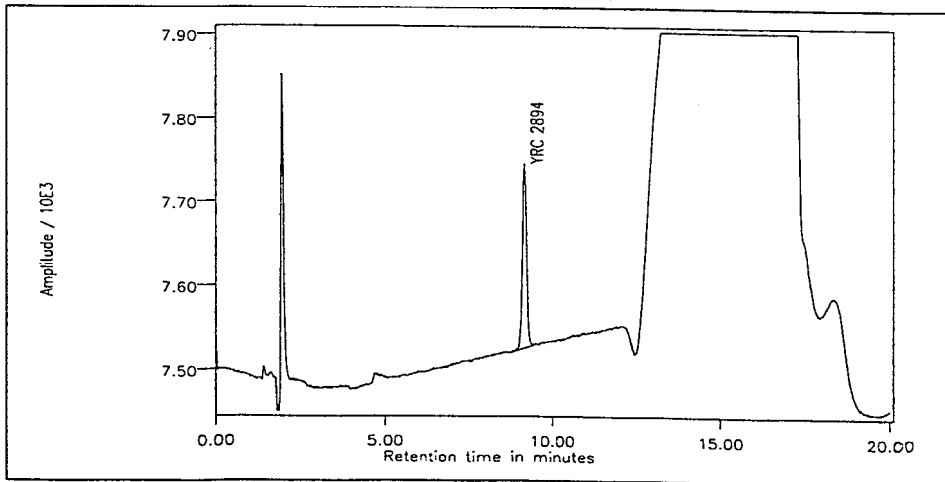
Standard YRC 2894, 0.1 mg/l



Melon, Pulp, Control, 5 g Aliquot, 1 ml Final Volume

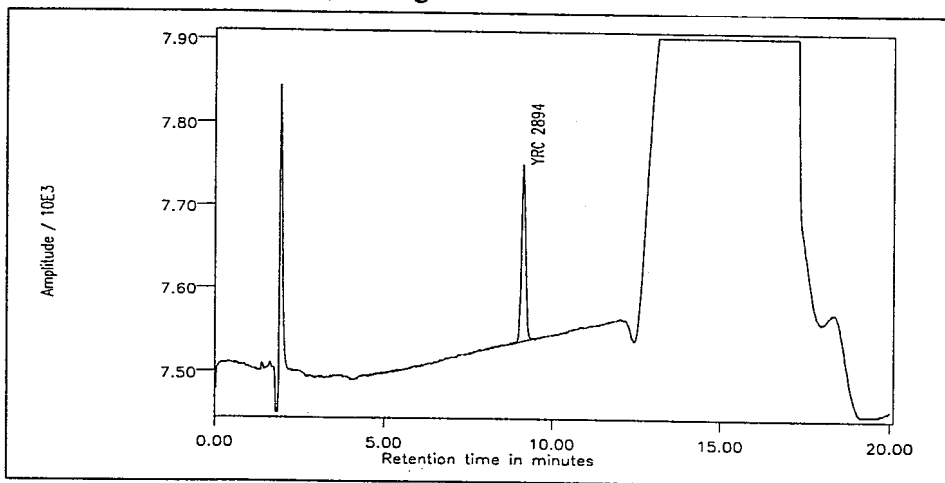


Recovery YRC 2894, Melon, Pulp
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

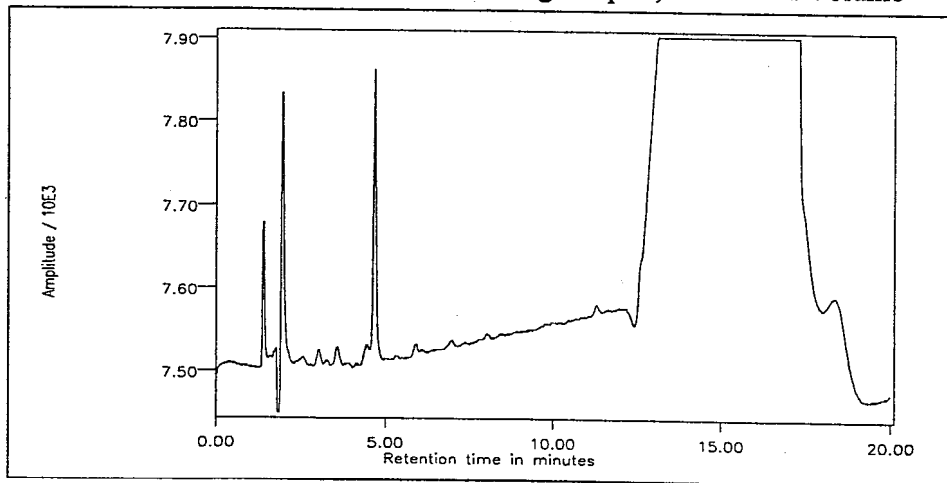


Appendix 9: Chromatograms of YRC 2894 Recoveries from Paprika, Fruit

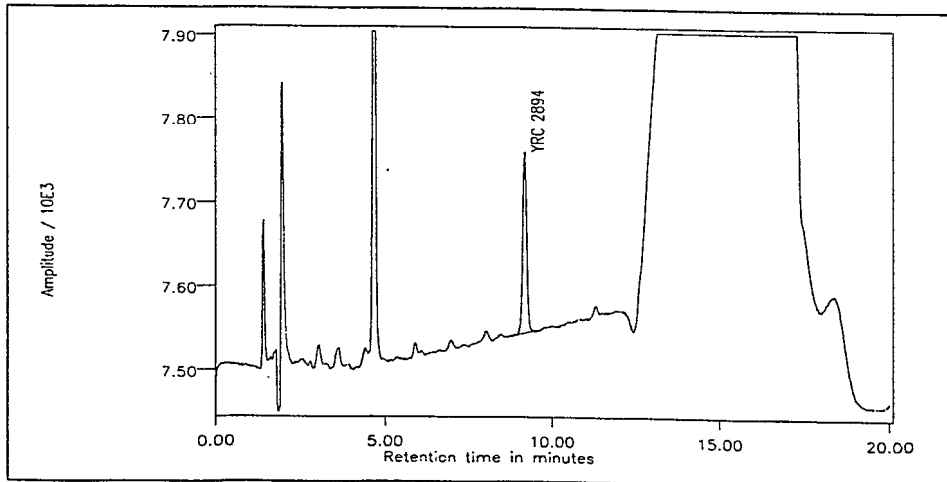
Standard YRC 2894, 0.1 mg/l



Paprika, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

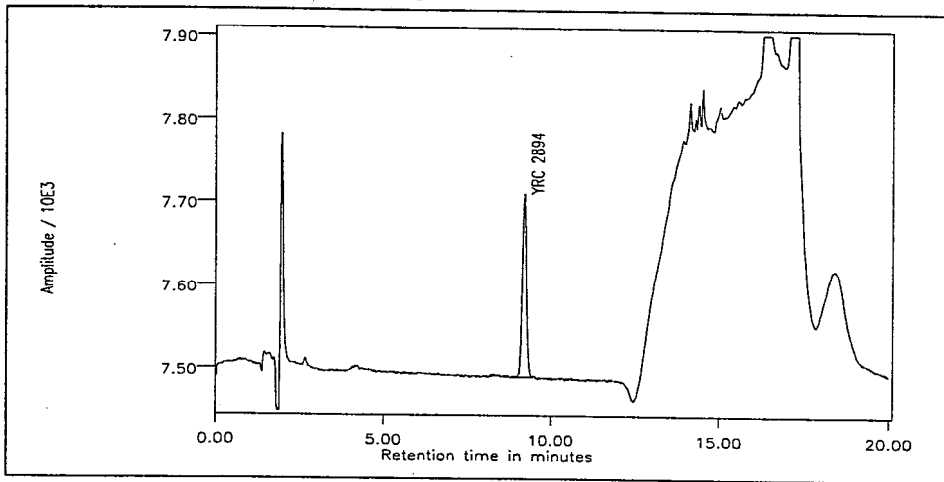


Recovery YRC 2894, Paprika, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

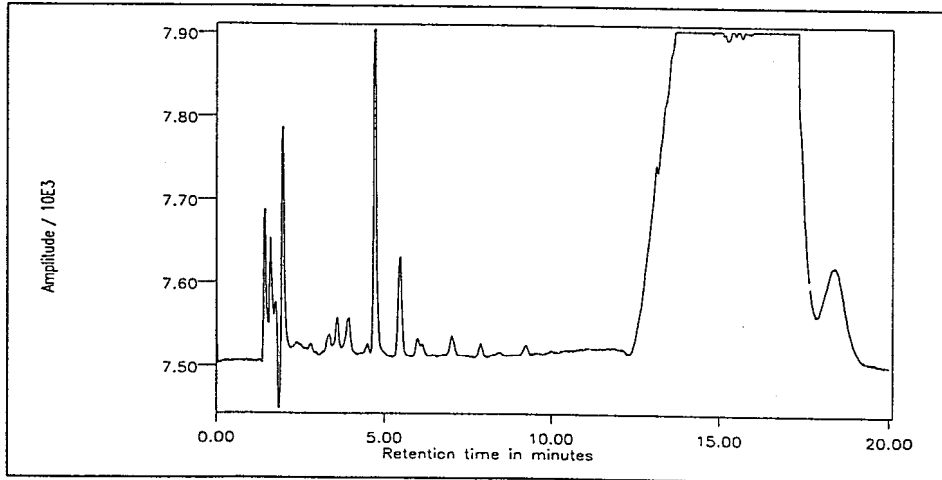


Appendix 10: Chromatograms of YRC 2894 Recoveries from Peach, Fruit

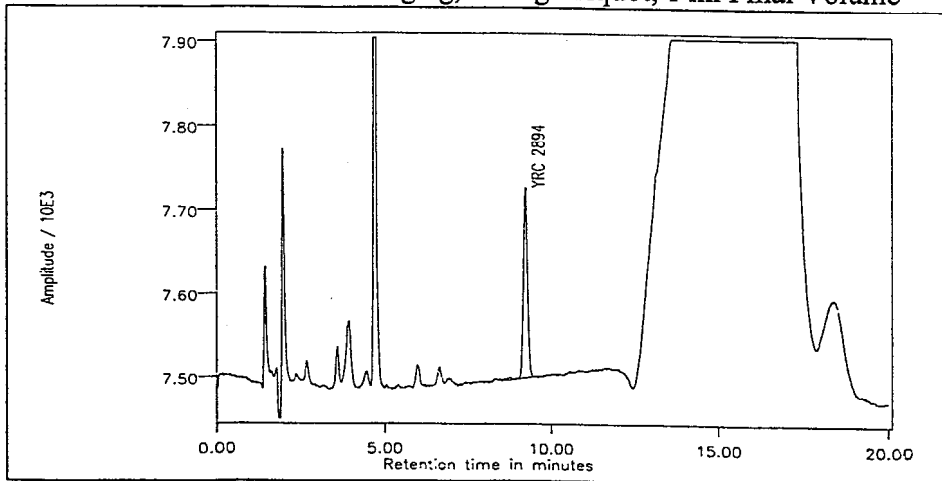
Standard YRC 2894, 0.1 mg/l



Peach, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

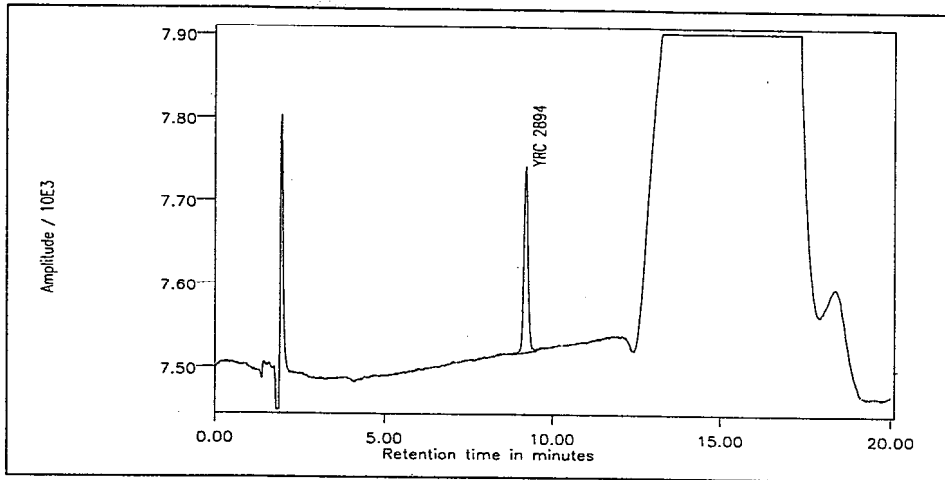


Recovery YRC 2894, Peach, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

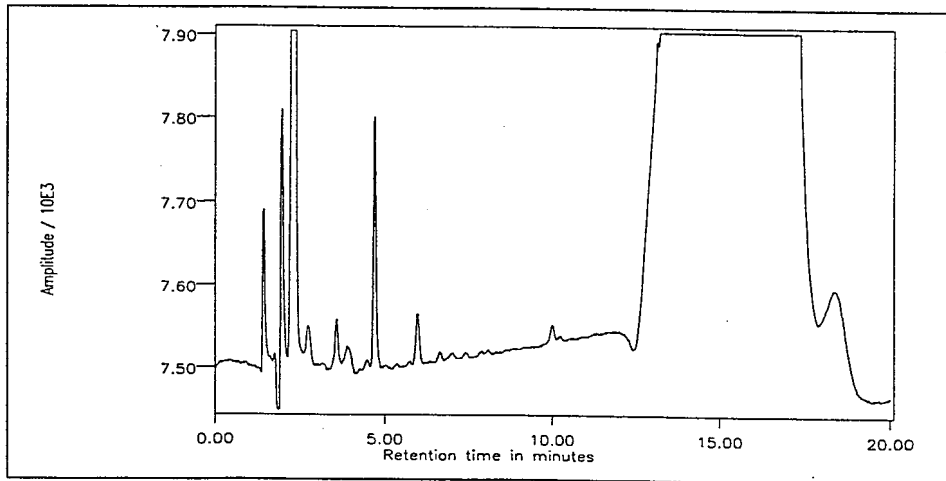


Appendix 11: Chromatograms of YRC 2894 Recoveries from Peach, Preserve

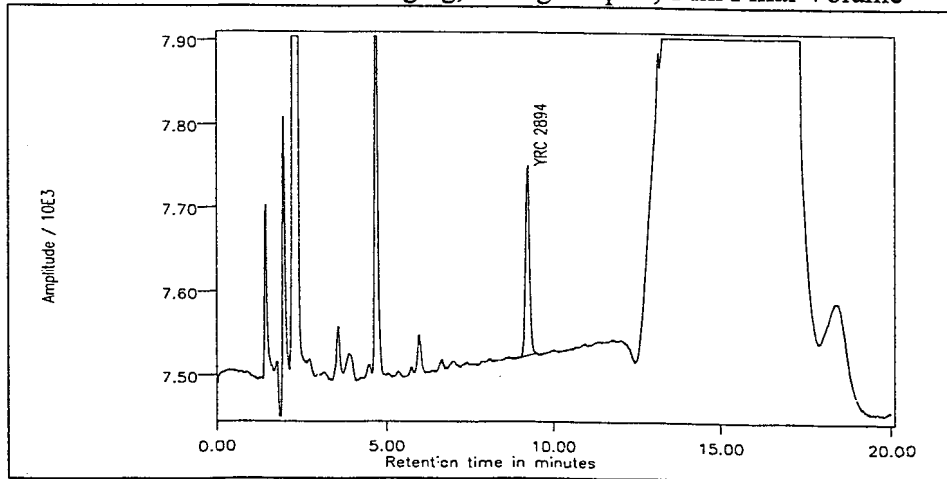
Standard YRC 2894, 0.1 mg/l



Peach, Preserve, Control; 5 g Aliquot, 1 ml Final Volume

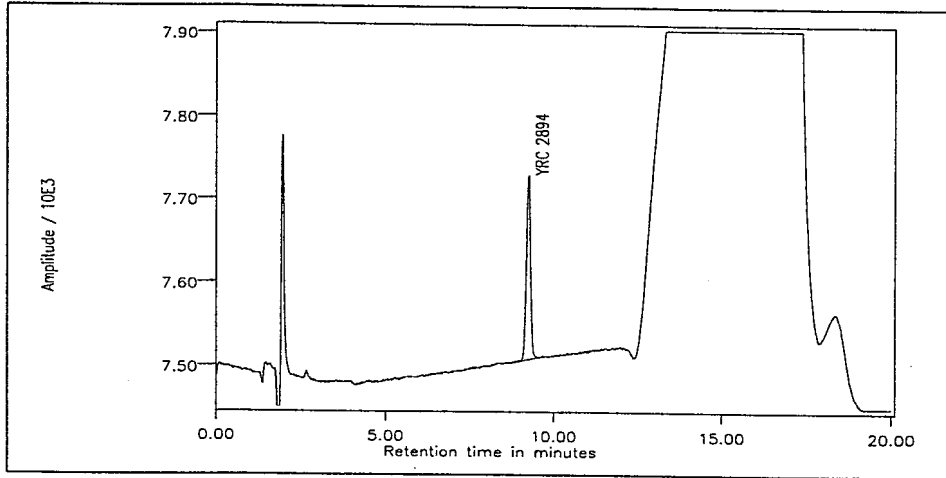


Recovery YRC 2894, Peach, Preserve
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

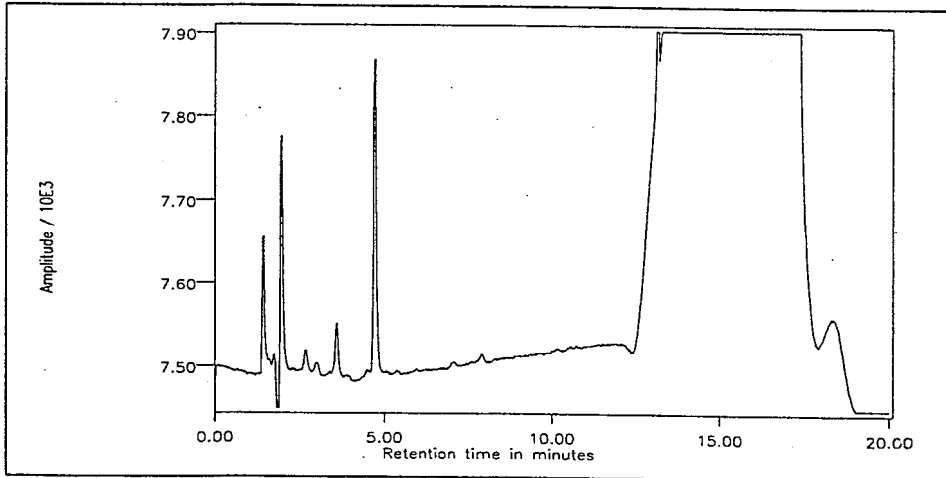


Appendix 12: Chromatograms of YRC 2894 Recoveries from Tomato, Fruit

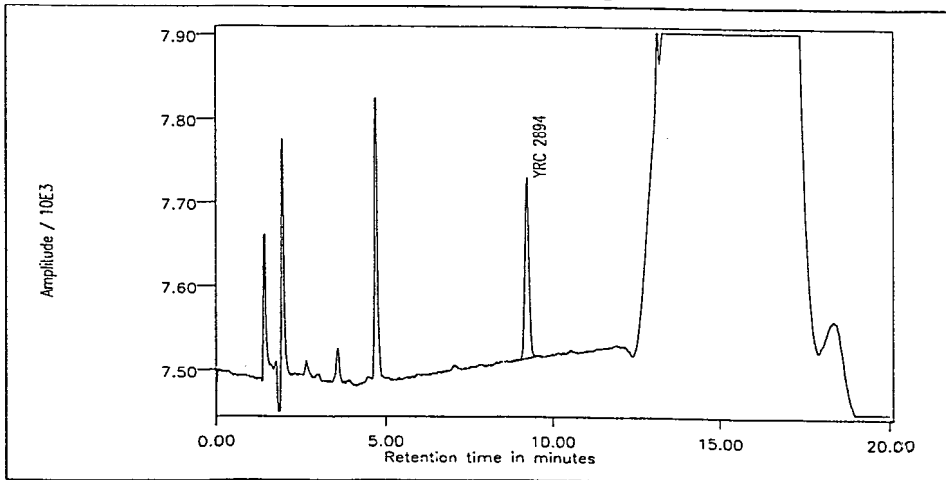
Standard YRC 2894, 0.1 mg/l



Tomato, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

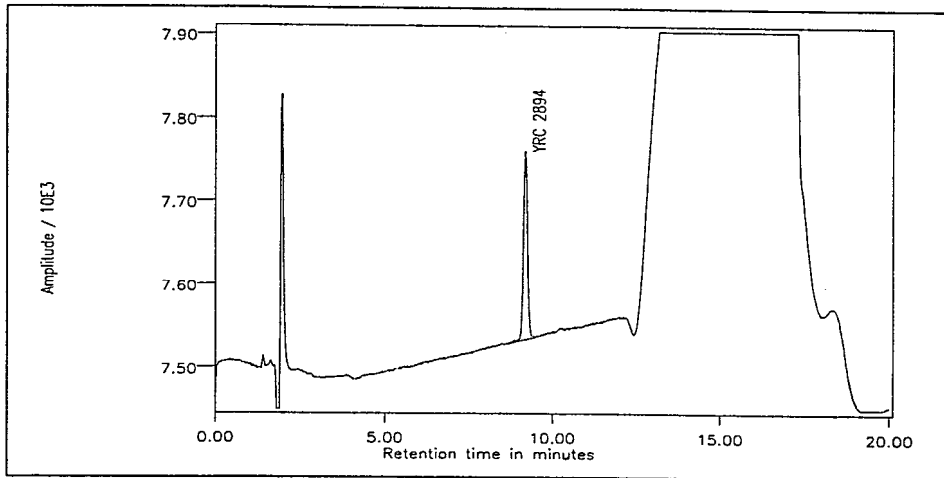


Recovery YRC 2894, Tomato, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

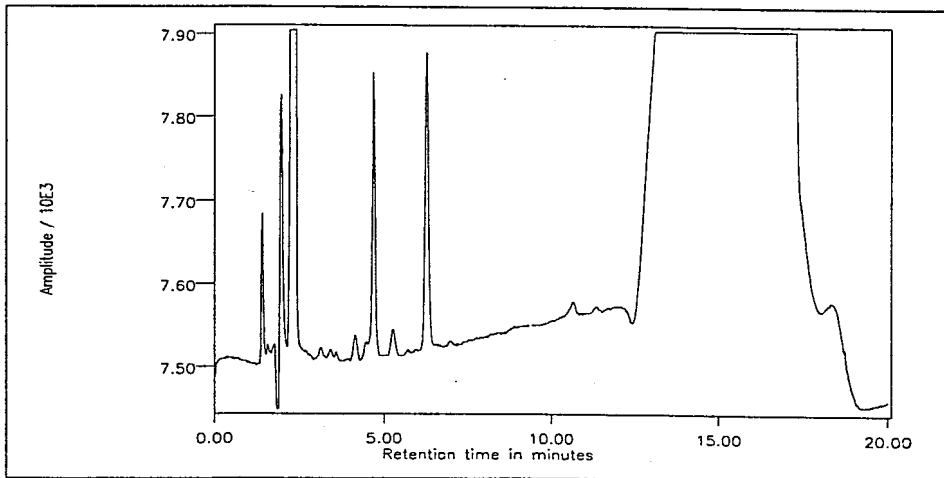


Appendix 13: Chromatograms of YRC 2894 Recoveries from Tomato, Juice

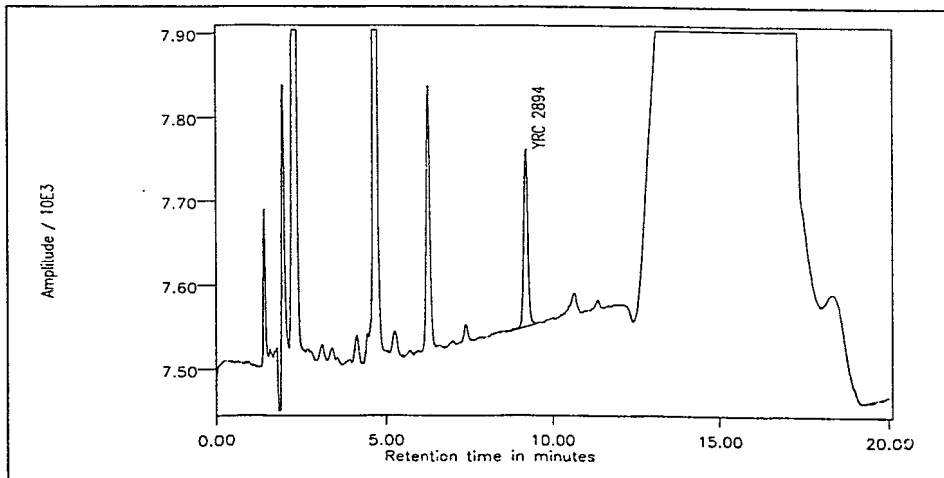
Standard YRC 2894, 0.1 mg/l



Tomato, Juice, Control, 5 g Aliquot, 1 ml Final Volume

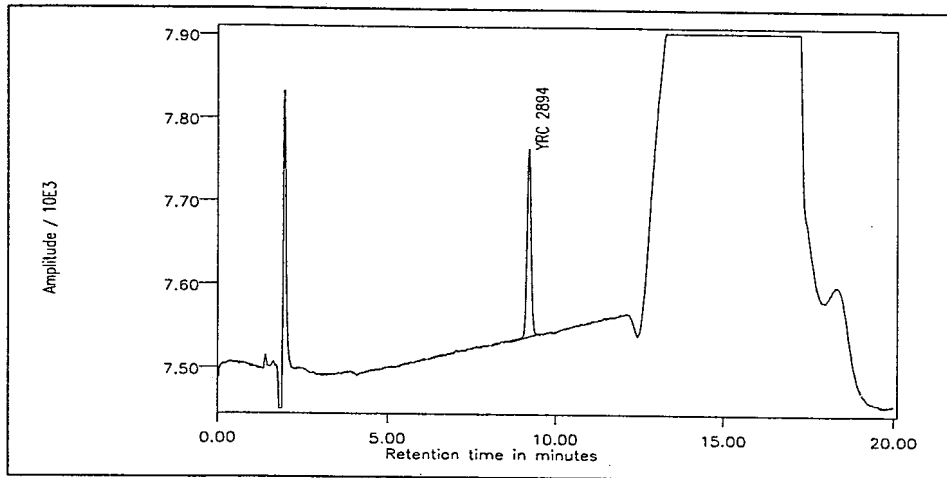


Recovery YRC 2894, Tomato, Juice
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

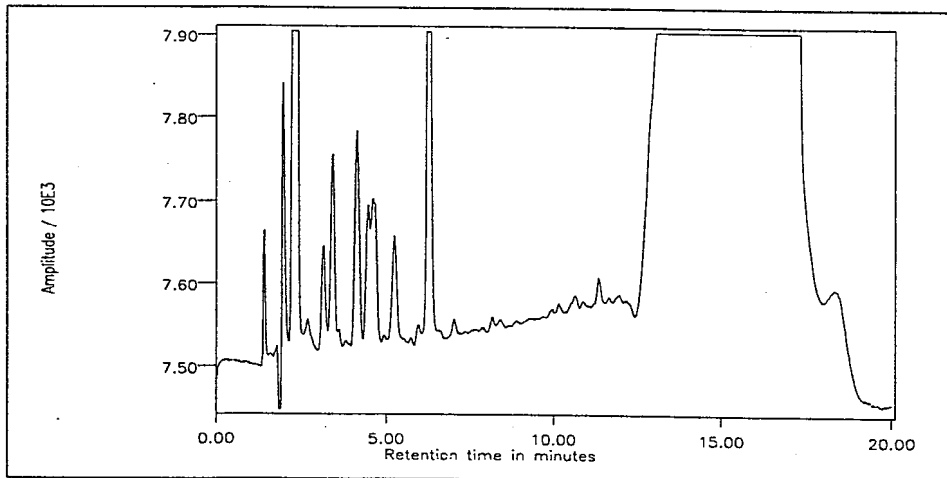


Appendix 14: Chromatograms of YRC 2894 Recoveries from Tomato, Paste

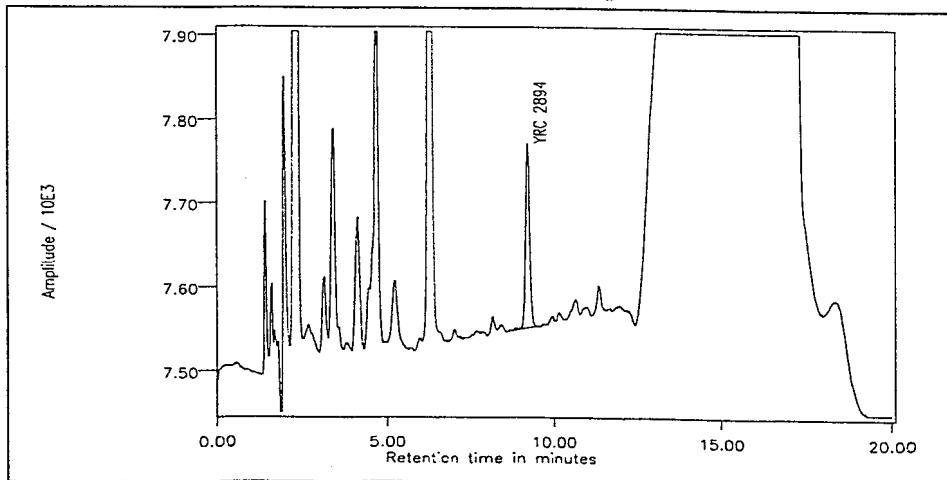
Standard YRC 2894, 0.1 mg/l



Tomato, Paste, Control, 5 g Aliquot, 1 ml Final Volume

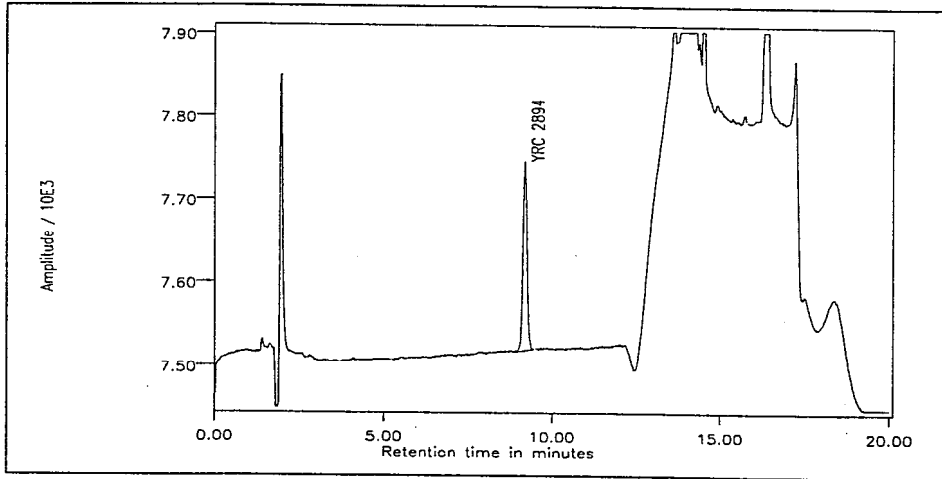


Recovery YRC 2894, Tomato, Paste
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

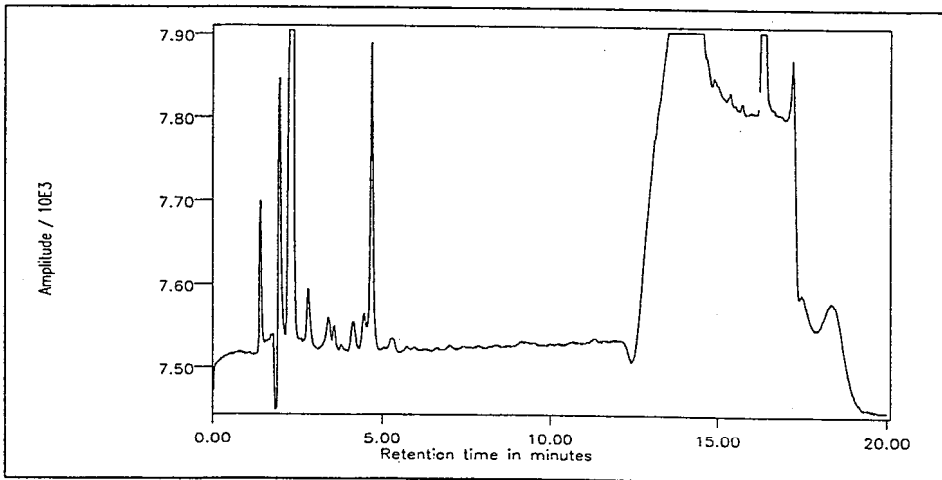


Appendix 15: Chromatograms of YRC 2894 Recoveries from Tomato, Preserve

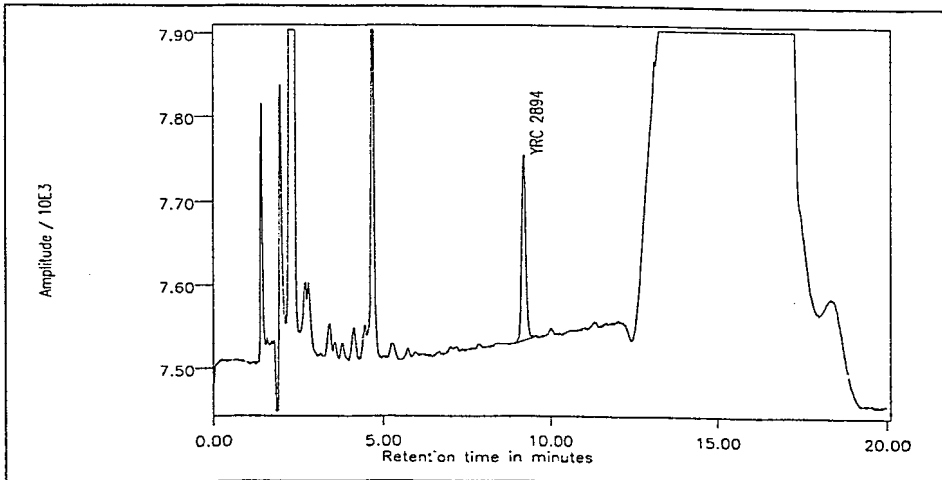
Standard YRC 2894, 0.1 mg/l



Tomato, Preserve, Control, 5 g Aliquot, 1 ml Final Volume



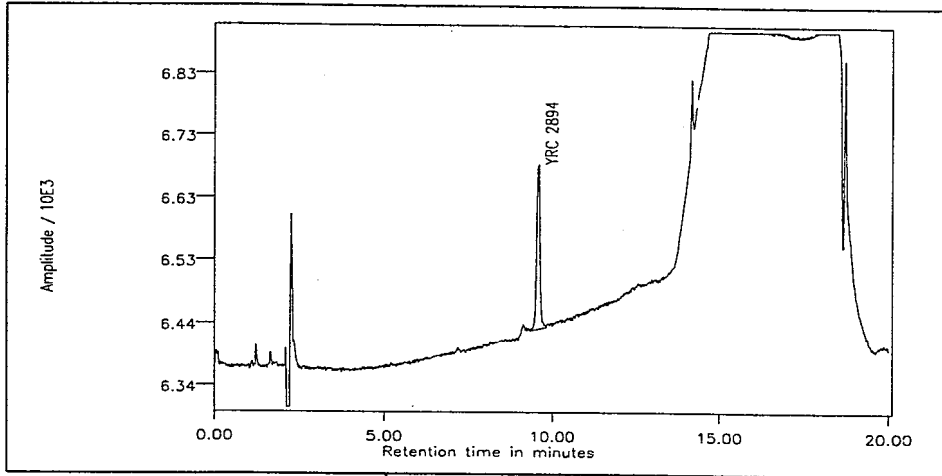
Recovery YRC 2894, Tomato, Preserve
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume



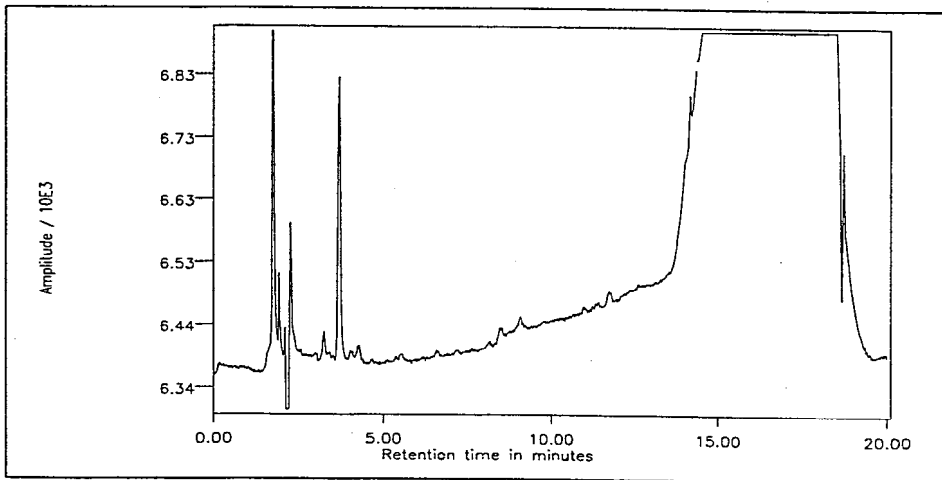
Appendices 16-30: Chromatograms with Confirmatory Method

Appendix 16: Chromatograms of YRC 2894 Recoveries from Apple, Fruit

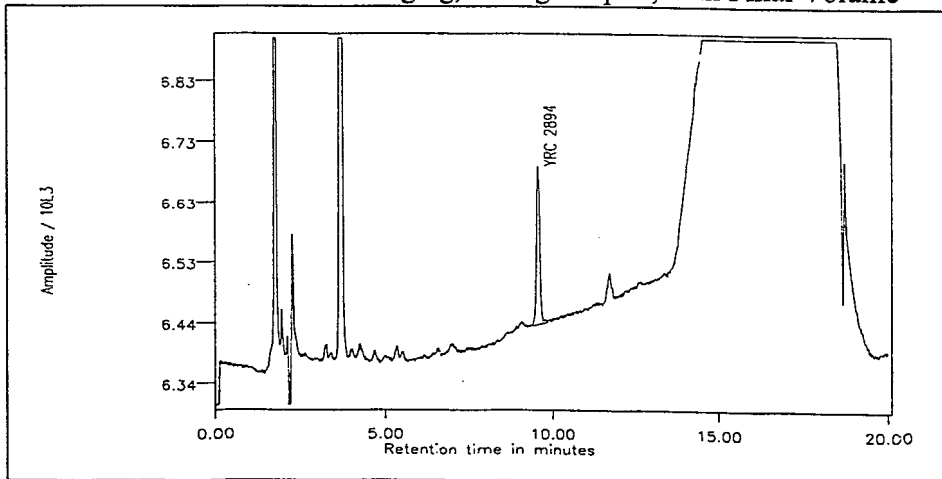
Standard YRC 2894, 0.1 mg/l



Apple, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

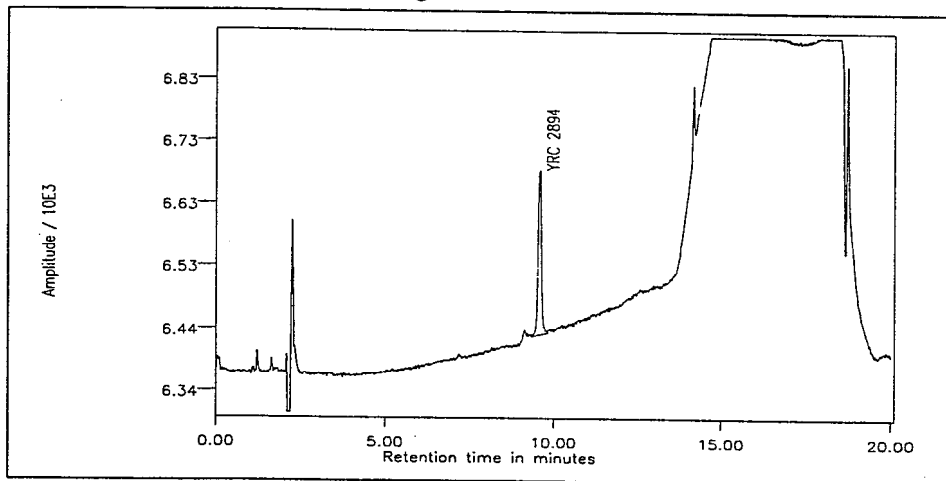


Recovery YRC 2894, Apple, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume



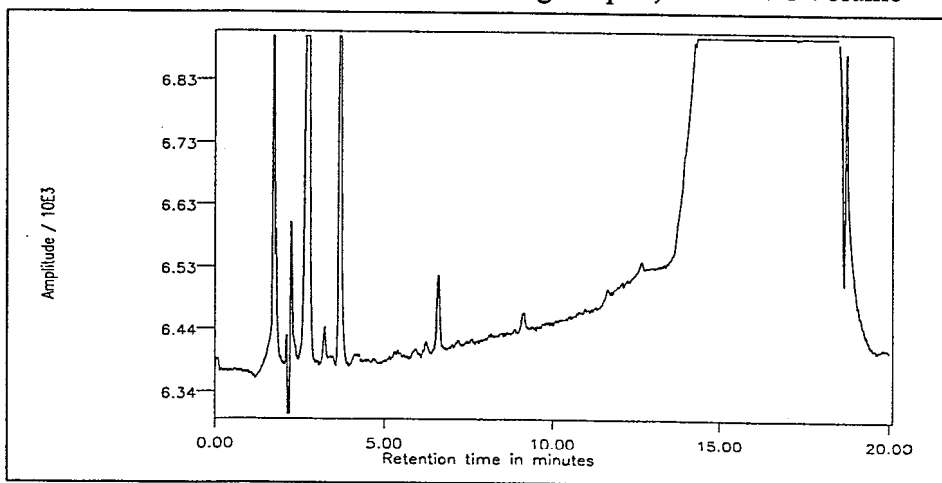
Appendix 17: Chromatograms of YRC 2894 Recoveries from Apple, Dried Fruit

Standard YRC 2894, 0.1 mg/l



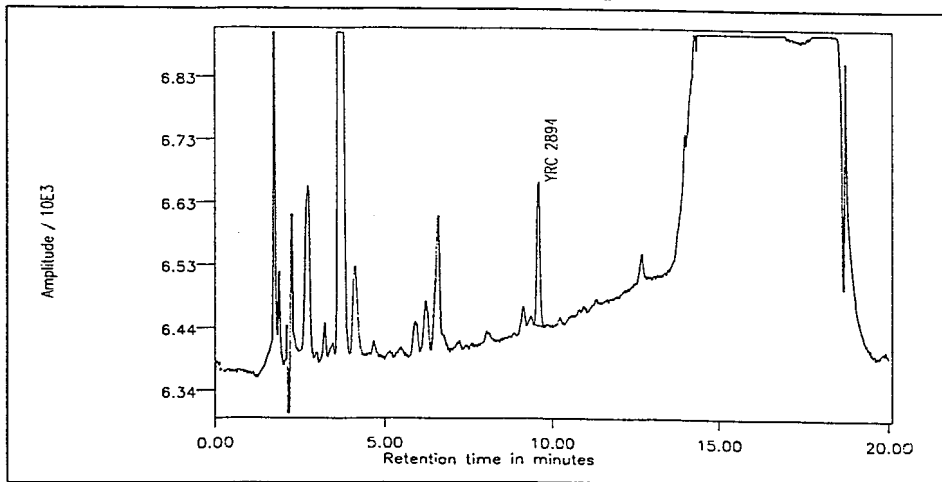
Apple, Fruit, Dried,

5 g Aliquot, 1 ml Final Volume



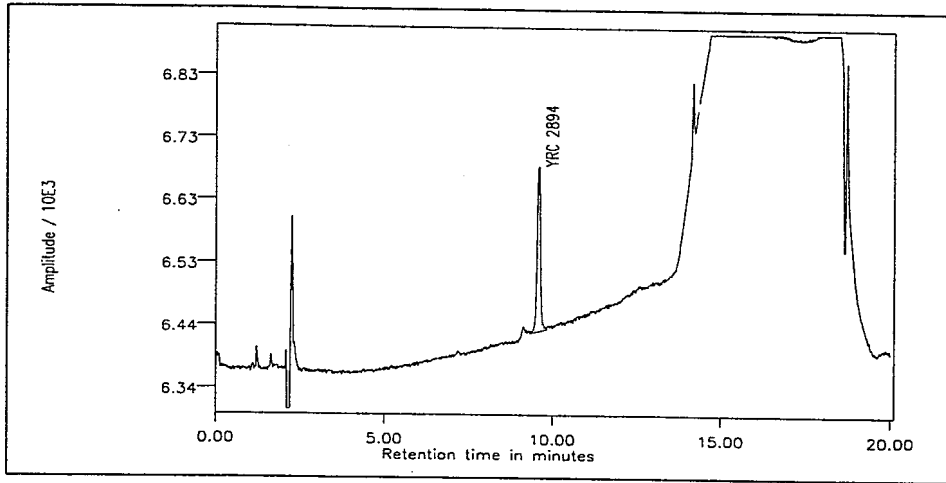
Recovery YRC 2894, Apple, Dried

Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

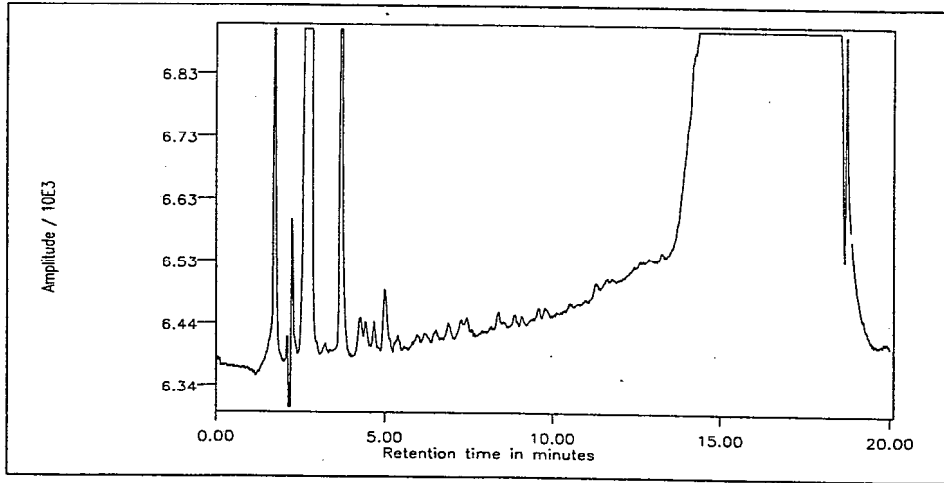


Appendix 18: Chromatograms of YRC 2894 Recoveries from Apple, Dry Pomace

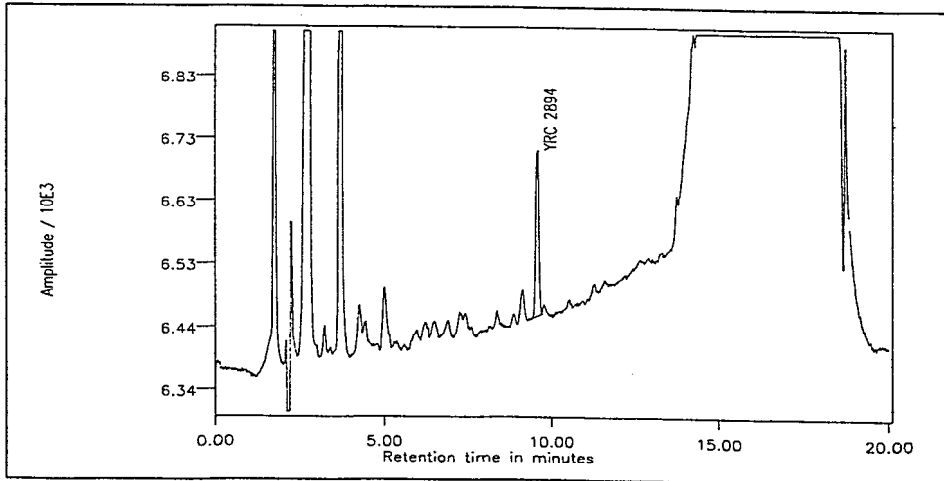
Standard YRC 2894, 0.1 mg/l



Apple, Pomace Dry, Control, 5 g Aliquot, 1 ml Final Volume

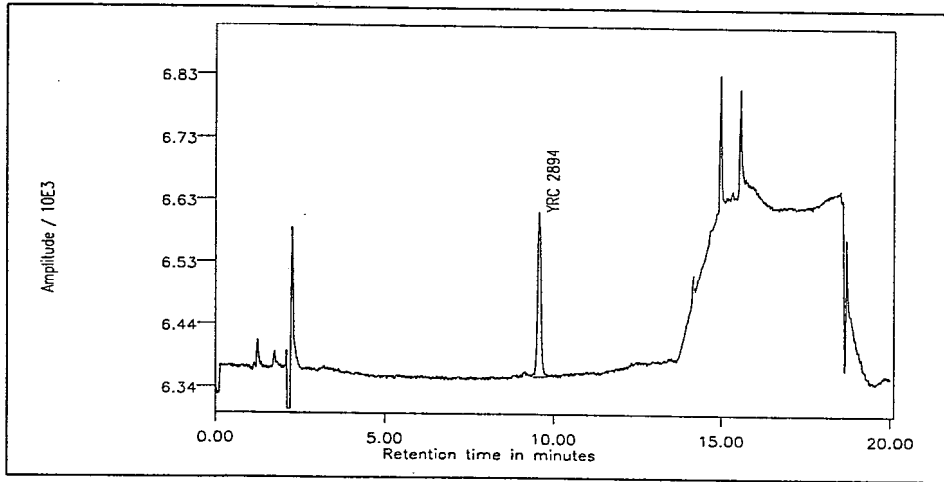


Recovery YRC 2894, Apple, Pomace Dry
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

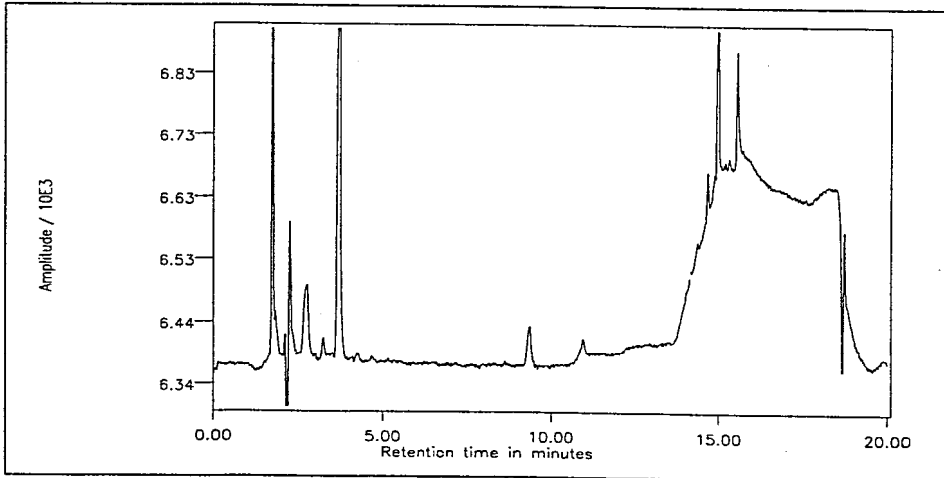


Appendix 19: Chromatograms of YRC 2894 Recoveries from Apple, Juice

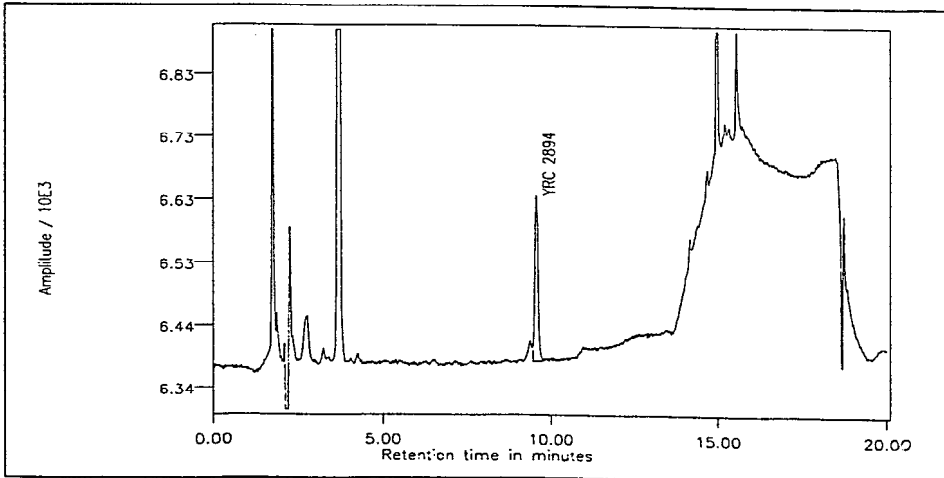
Standard YRC 2894, 0.1 mg/l



Apple, Juice, Control, 5 g Aliquot, 1 ml Final Volume

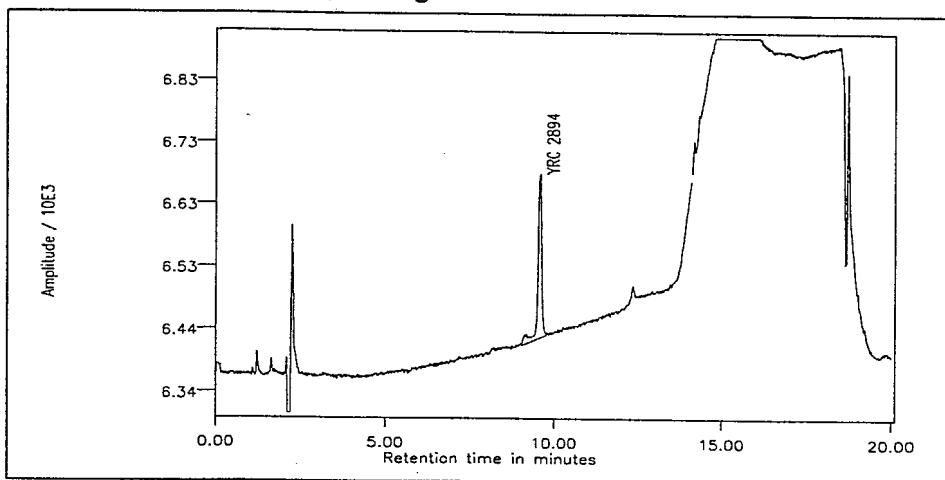


Recovery YRC 2894, Apple, Juice
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

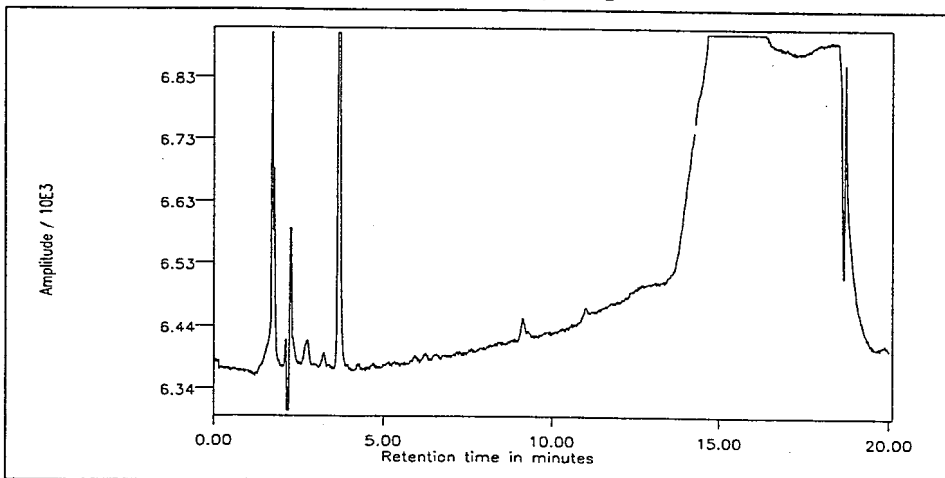


Appendix 20: Chromatograms of YRC 2894 Recoveries from Apple, Sauce

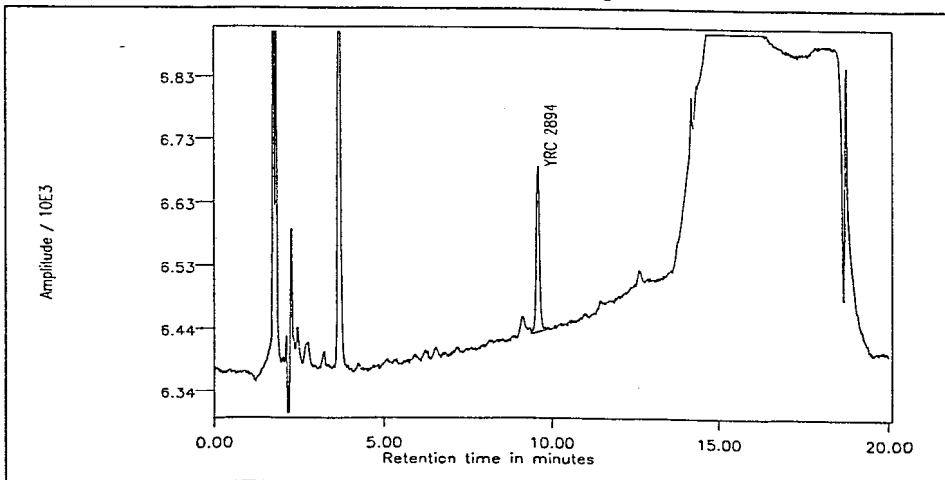
Standard YRC 2894, 0.1 mg/l



Apple, Sauce, Control, 5 g Aliquot, 1 ml Final Volume

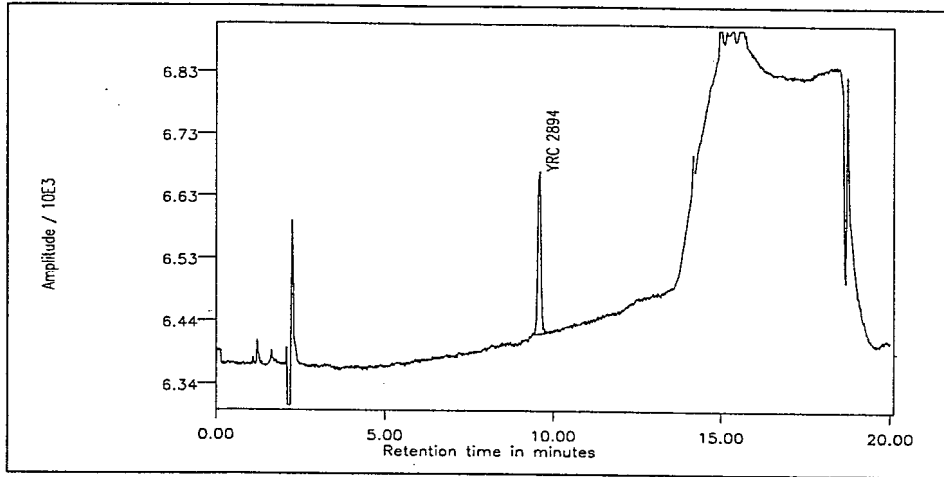


Recovery YRC 2894, Apple, Sauce
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

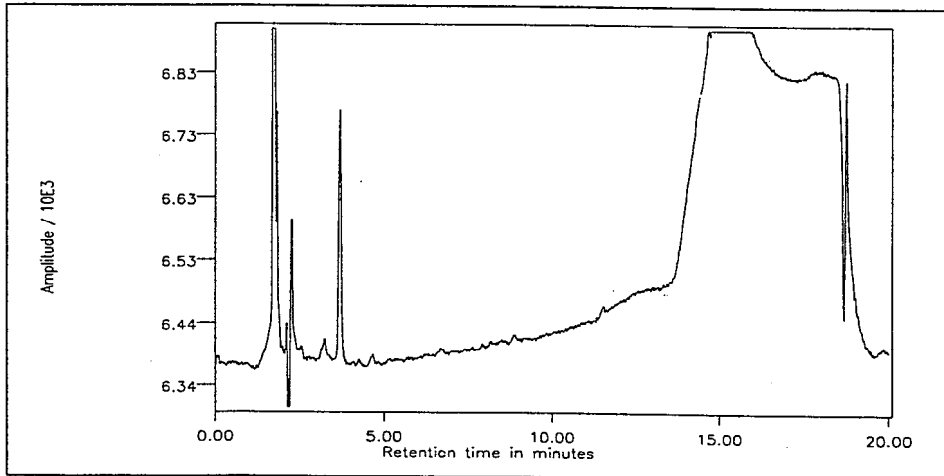


Appendix 21: Chromatograms of YRC 2894 Recoveries from Cucumber, Fruit

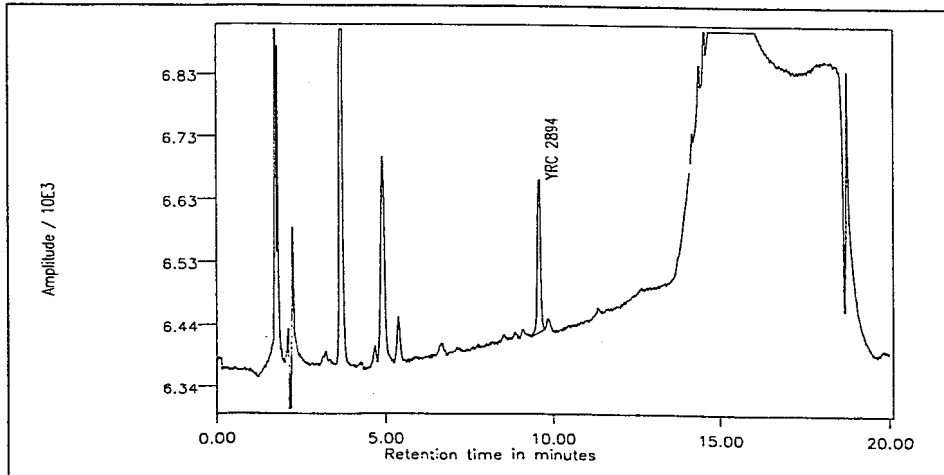
Standard YRC 2894, 0.1 mg/l



Cucumber, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

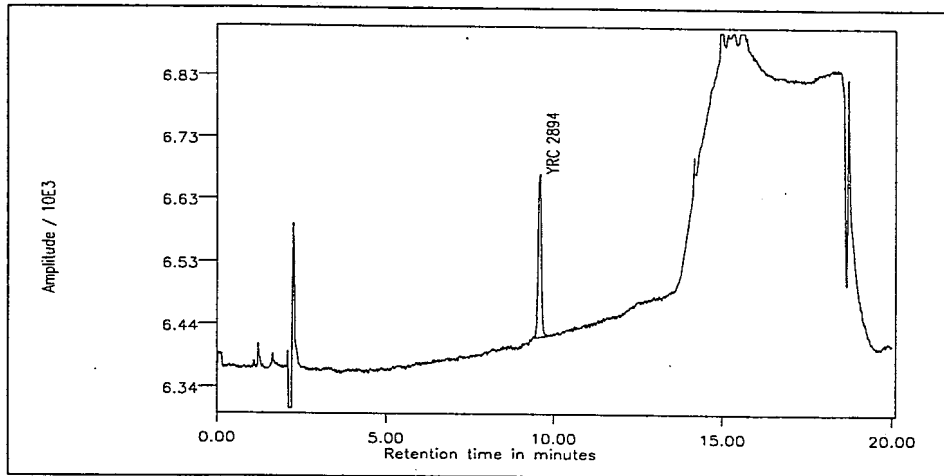


Recovery YRC 2894, Cucumber, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

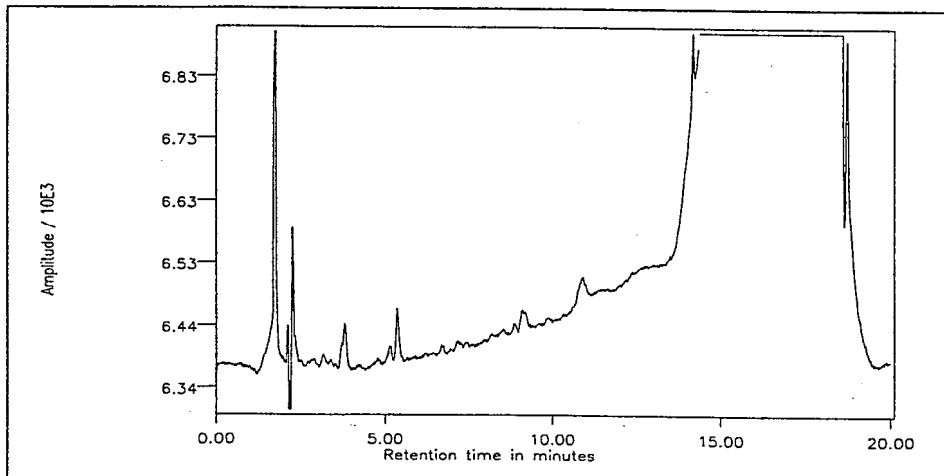


Appendix 22: Chromatograms of YRC 2894 Recoveries from Melon, Peel

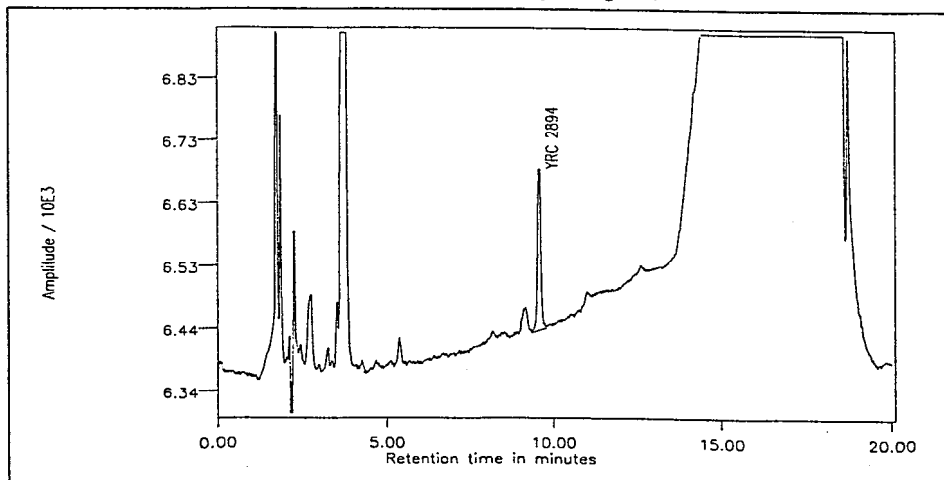
Standard YRC 2894, 0.1 mg/l



Melon, Peel, Control, 5 g Aliquot, 1 ml Final Volume

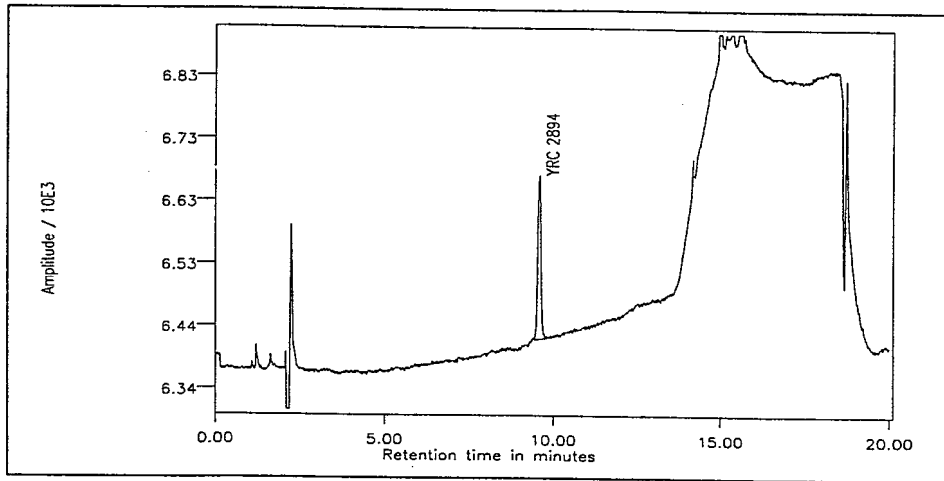


Recovery YRC 2894, Melon, Peel
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

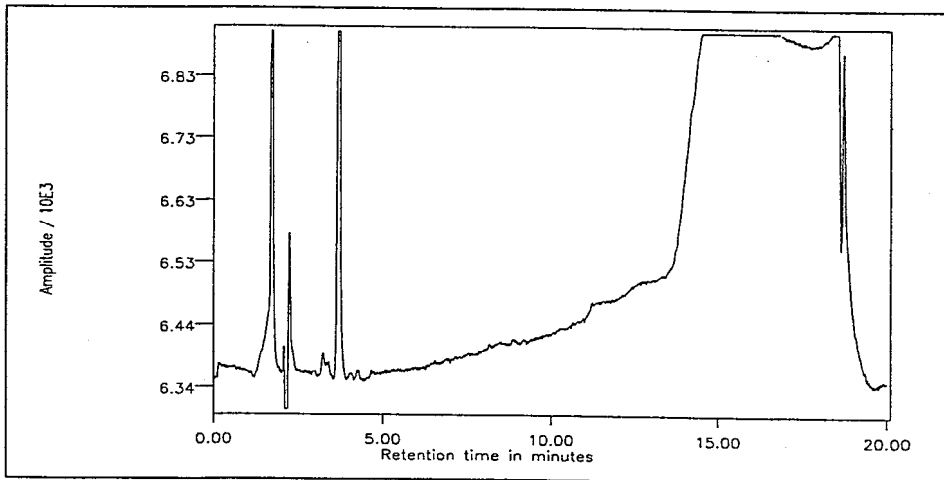


Appendix 23: Chromatograms of YRC 2894 Recoveries from Melon, Pulp

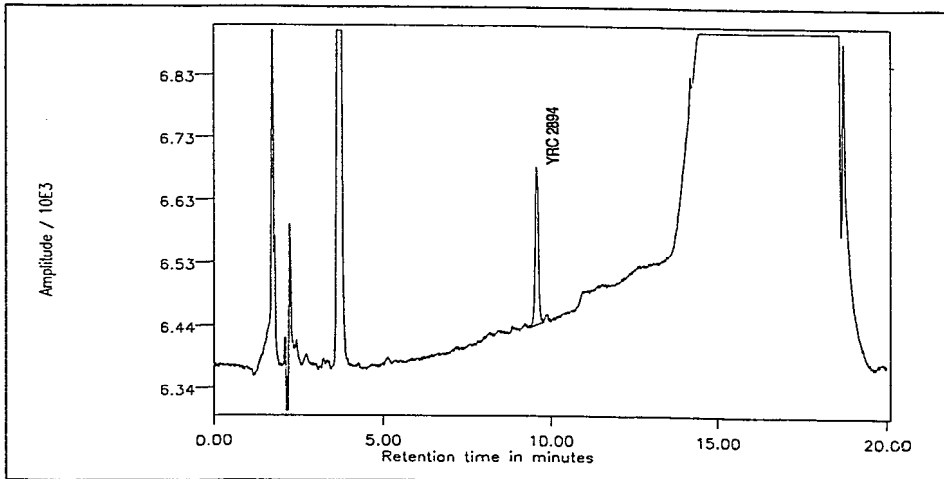
Standard YRC 2894, 0.1 mg/l



Melon, Pulp, Control, 5 g Aliquot, 1 ml Final Volume

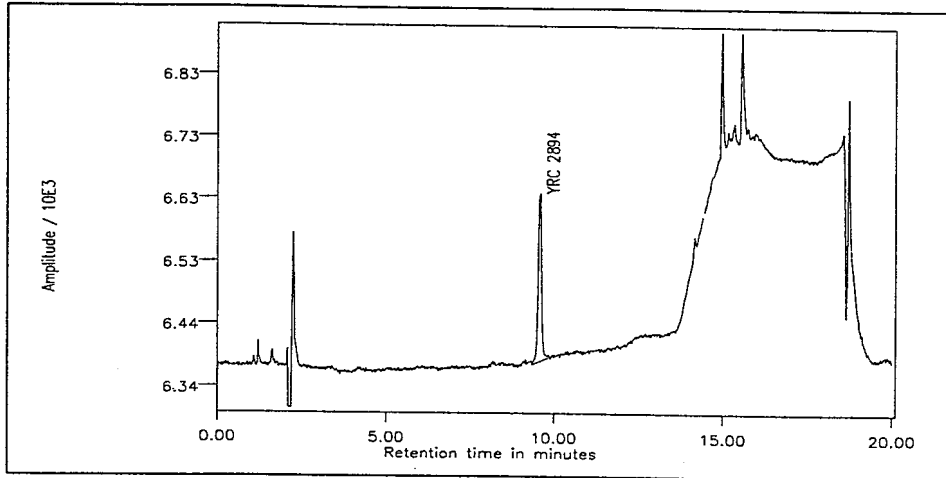


Recovery YRC 2894, Melon, Pulp
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

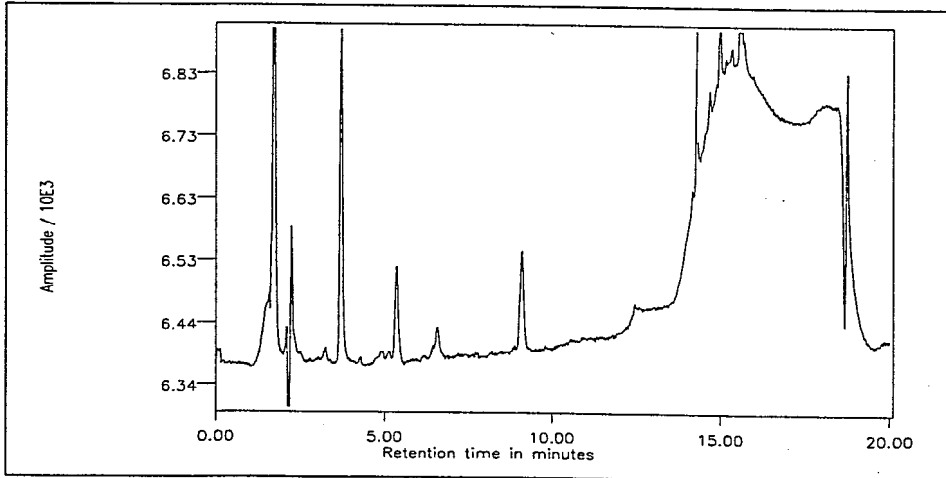


Appendix 24: Chromatograms of YRC 2894 Recoveries from Paprika, Fruit

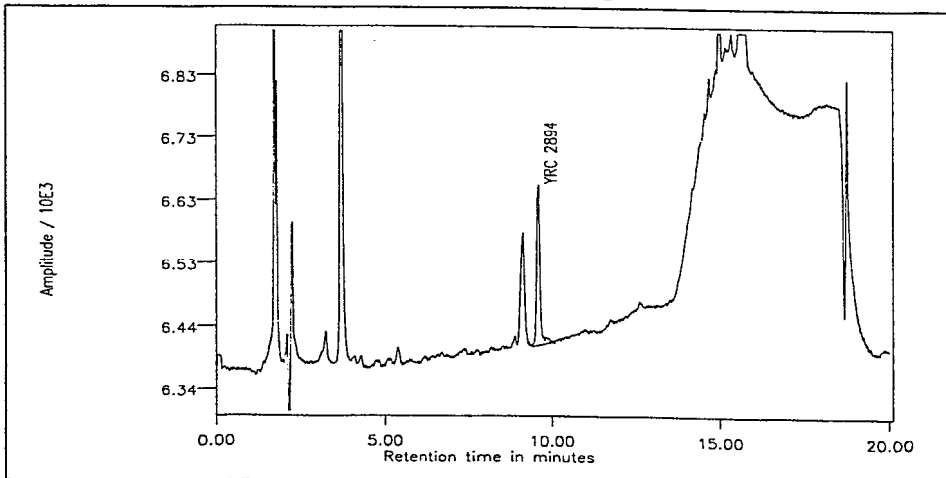
Standard YRC 2894, 0.1 mg/l



Paprika, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

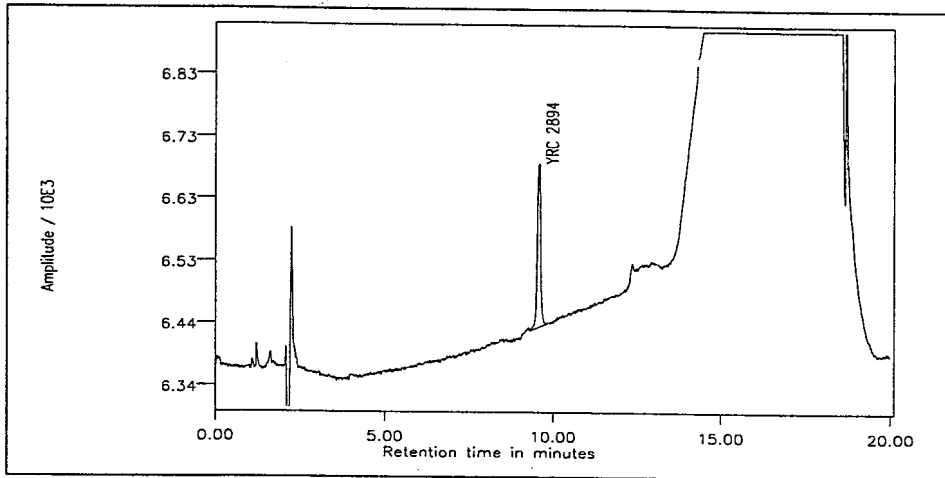


Recovery YRC 2894, Paprika, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

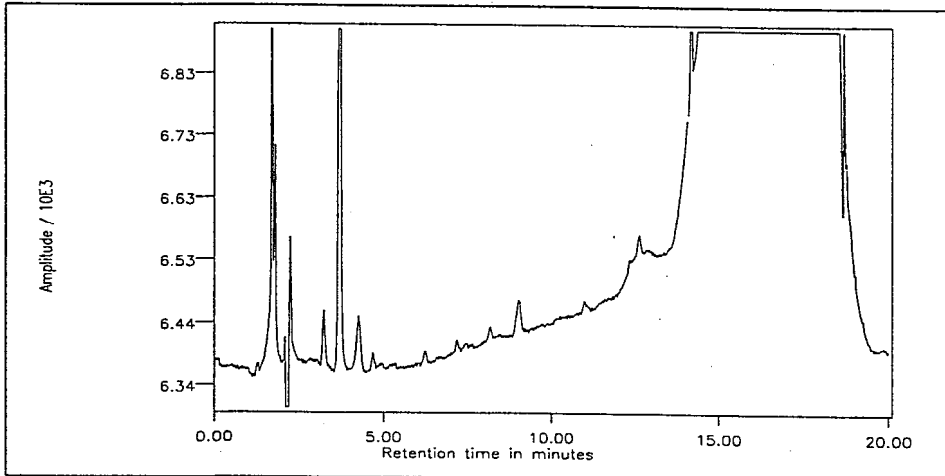


Appendix 25: Chromatograms of YRC 2894 Recoveries from Peach, Fruit

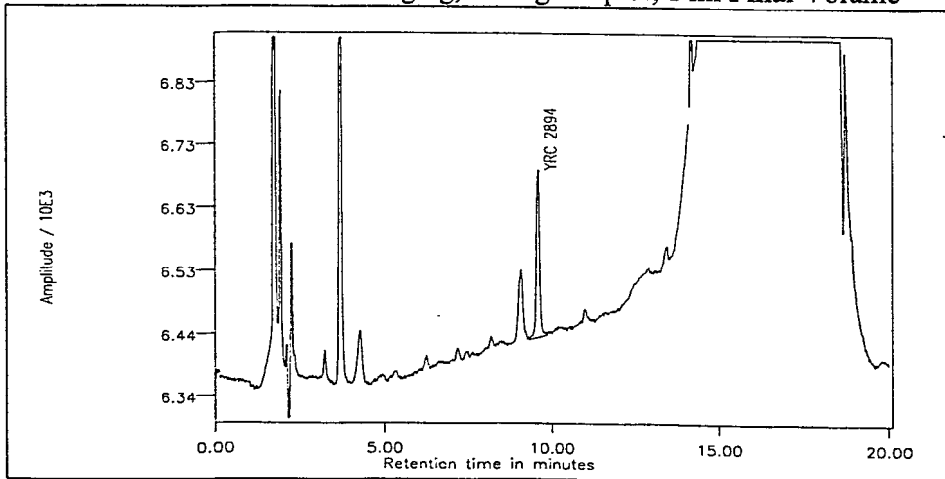
Standard YRC 2894, 0.1 mg/l



Peach, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

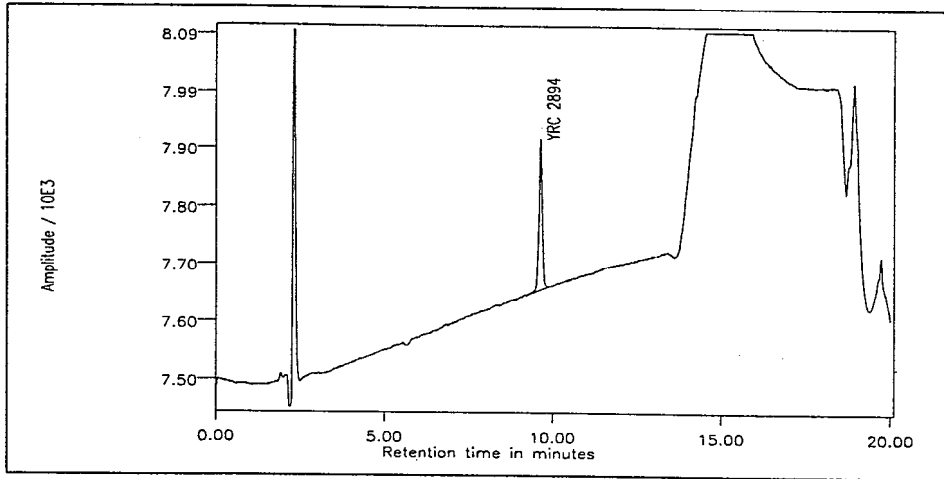


Recovery YRC 2894, Peach, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

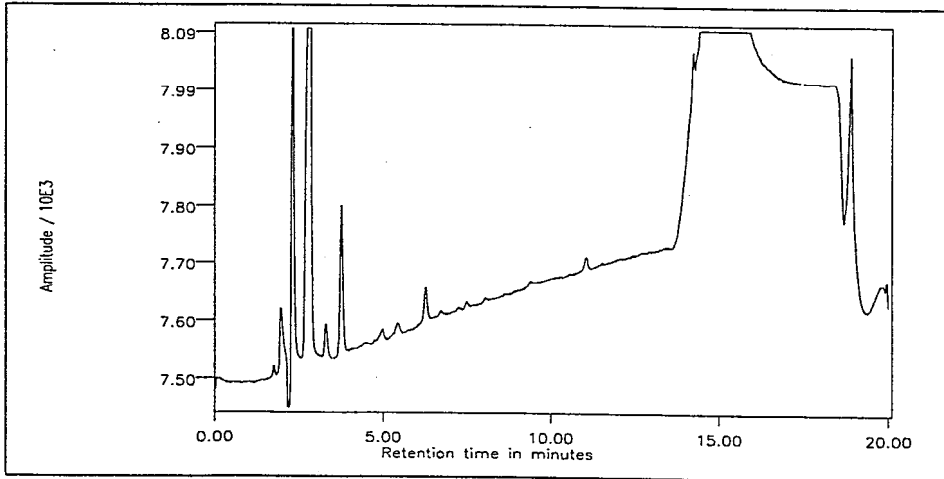


Appendix 26: Chromatograms of YRC 2894 Recoveries from Peach, Preserve

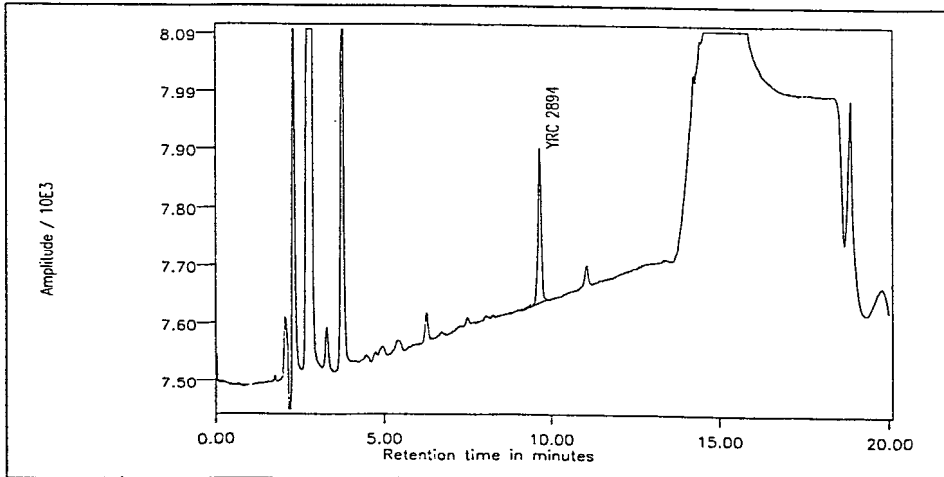
Standard YRC 2894, 0.1 mg/l



Peach, Preserve, Control; 5 g Aliquot, 1 ml Final Volume

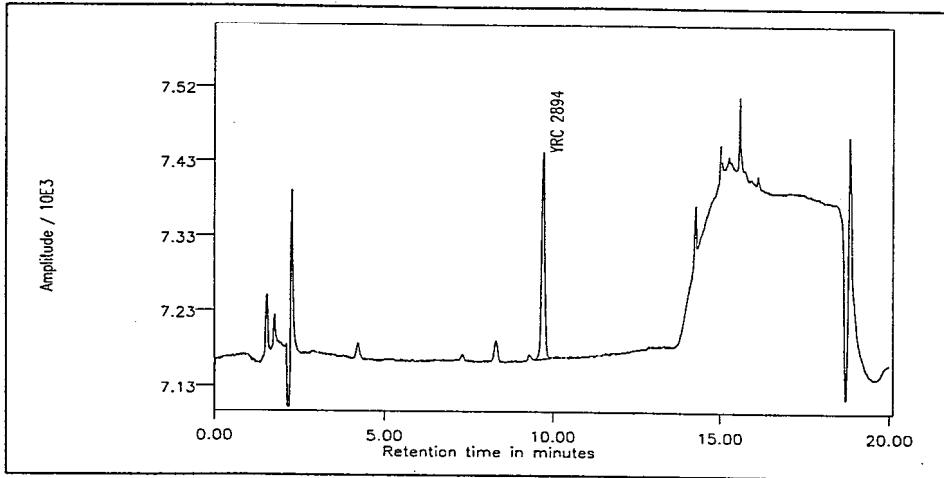


Recovery YRC 2894, Peach, Preserve
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

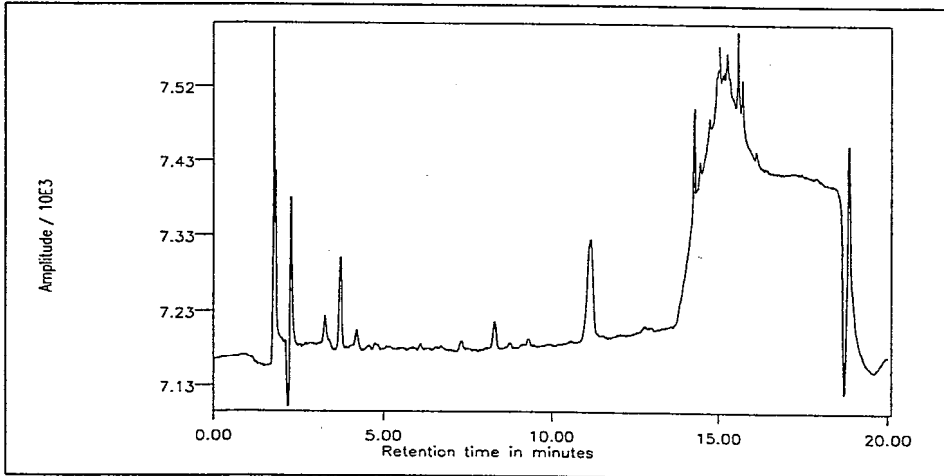


Appendix 27: Chromatograms of YRC 2894 Recoveries from Tomato, Fruit

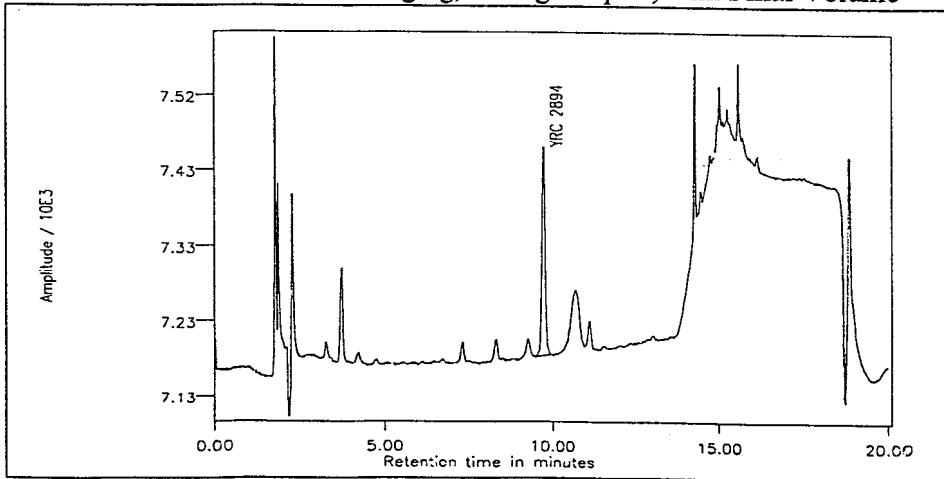
Standard YRC 2894, 0.1 mg/l



Tomato, Fruit, Control, 5 g Aliquot, 1 ml Final Volume

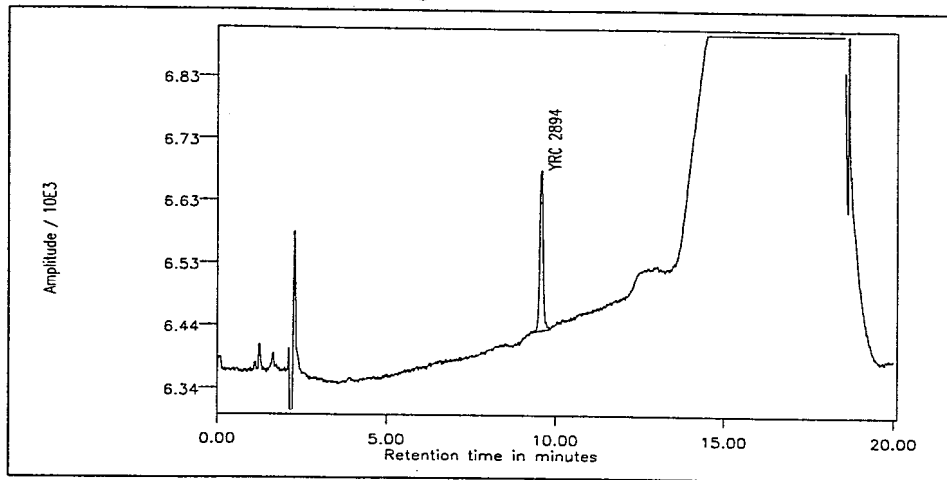


Recovery YRC 2894, Tomato, Fruit
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

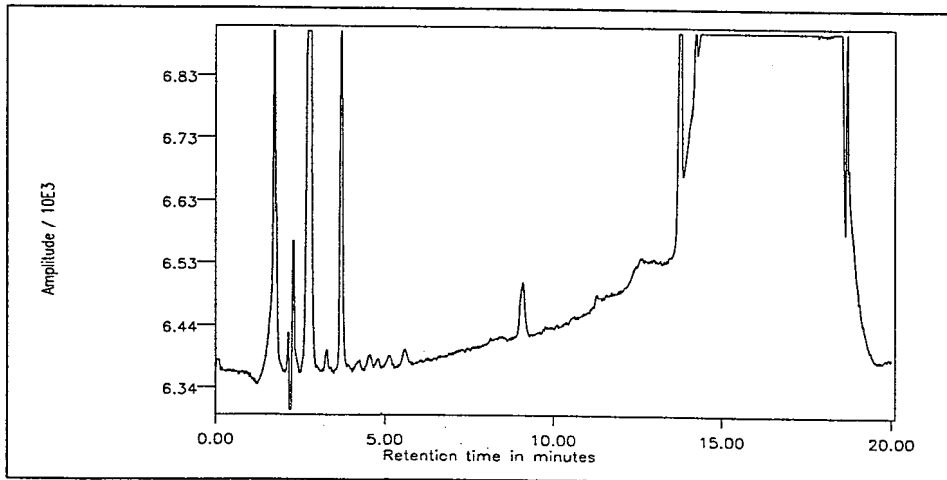


Appendix 28: Chromatograms of YRC 2894 Recoveries from Tomato, Juice

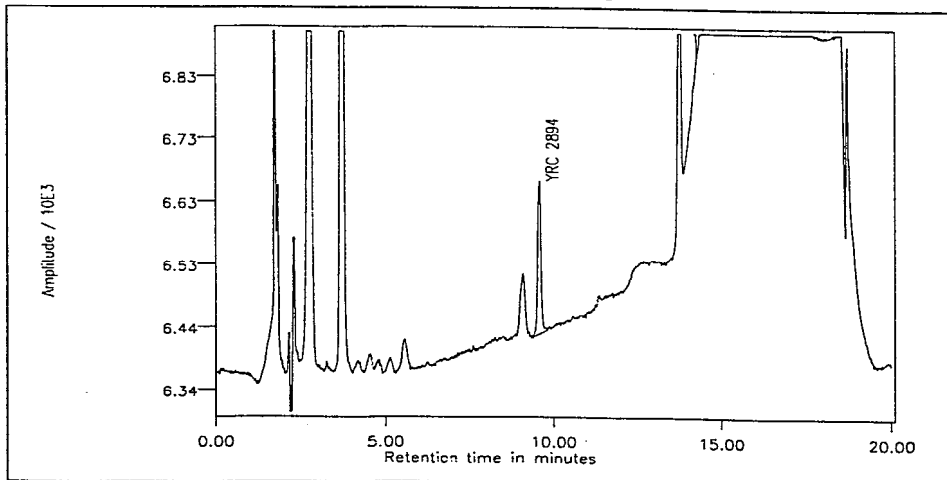
Standard YRC 2894, 0.1 mg/l



Tomato, Juice, Control, 5 g Aliquot, 1 ml Final Volume

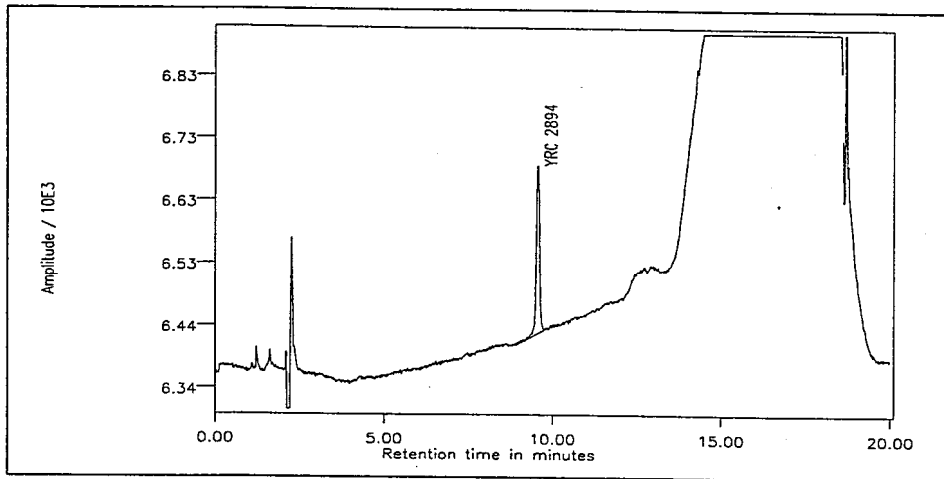


Recovery YRC 2894, Tomato, Juice
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

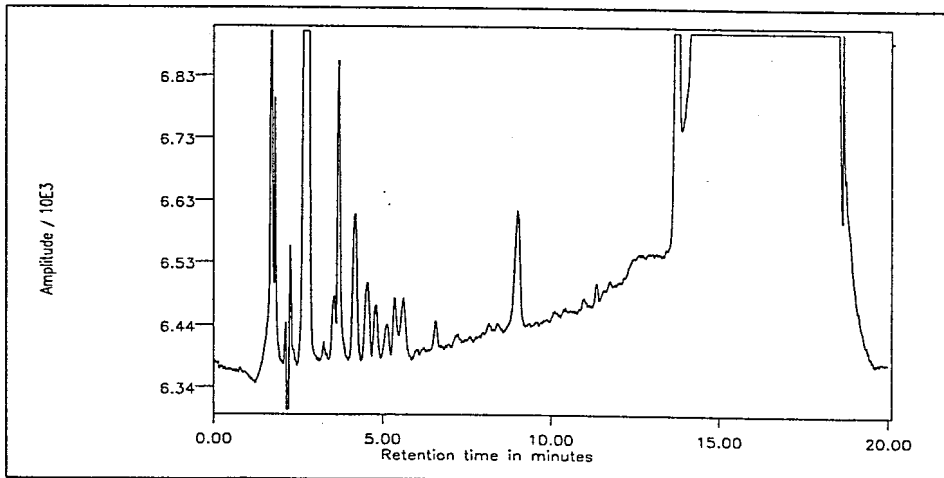


Appendix 29: Chromatograms of YRC 2894 Recoveries from Tomato, Paste

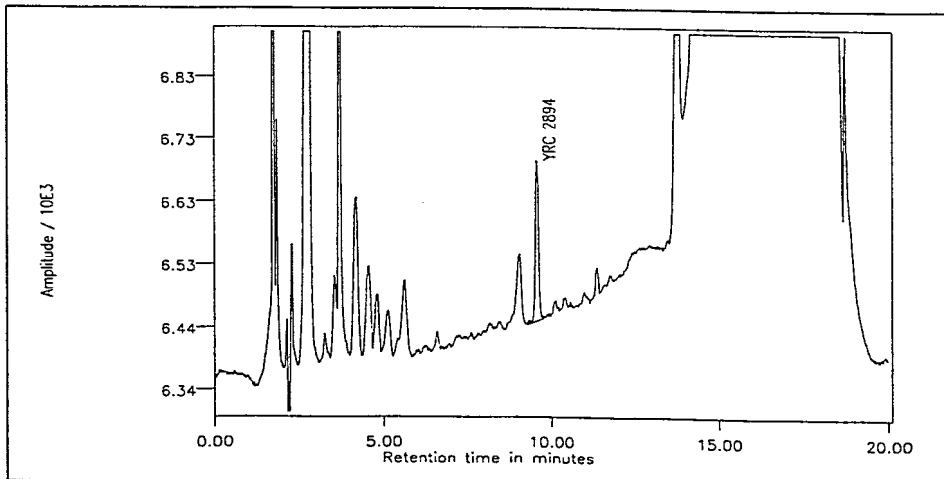
Standard YRC 2894, 0.1 mg/l



Tomato, Paste, Control, 5 g Aliquot, 1 ml Final Volume

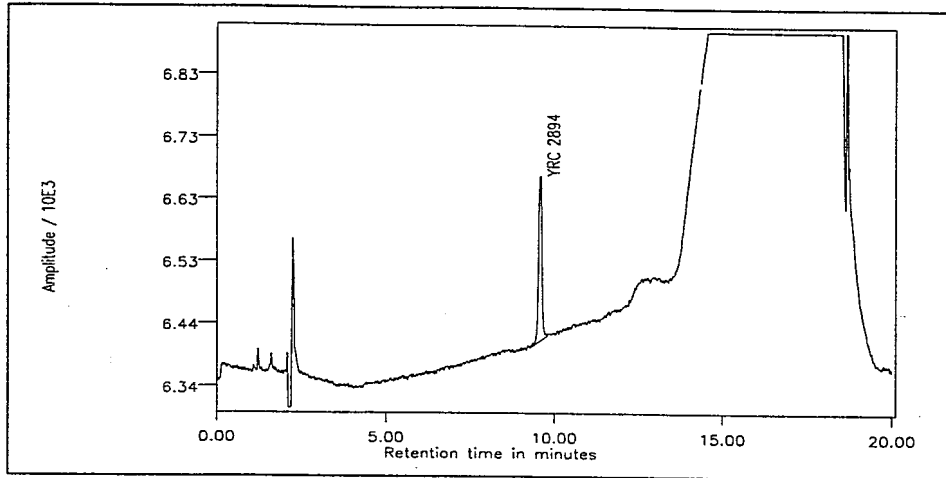


Recovery YRC 2894, Tomato, Paste
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

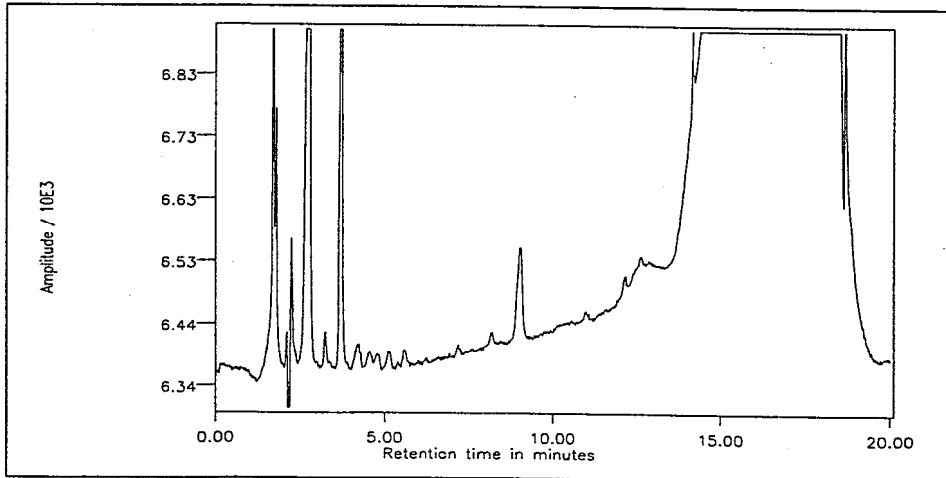


Appendix 30: Chromatograms of YRC 2894 Recoveries from Tomato, Preserve

Standard YRC 2894, 0.1 mg/l



Tomato, Preserve, Control, 5 g Aliquot, 1 ml Final Volume



Recovery YRC 2894, Tomato, Preserve
Fortification Level 0.02 mg/kg, 5 g Aliquot, 1 ml Final Volume

