Report Title

EPTC: Determination of the EPTC Hydroxy Metabolites and their Conjugates in Crops by Gas Chromatography

Data Requirement

Guideline § 171-4 (c)

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Table of Contents

Page No.

Report Title Page ... 1

Statement of No Data Confidentiality Claims ... 2

Good Laboratory Practice Compliance Statement ... 3

Table of Contents ... 5

1 Summary/Introduction ... 8

2 Materials and Methods ... 9

- 2 1 Apparatus ... 9
- 2.2 Reagents ... 11
- 2.3 Analytical Reference Standards ... 11

3 Analytical Procedure ... 13

- 3.1 Fortification ... 13
- 3.2 Extraction ... 13
  - 3.2.1 Most Solid Samples (including water-insoluble samples high in starches or sugars) ... 13
  - 3.2.2 Water-soluble Samples High in Sugars ... 14
  - 3.2.3 Oil Samples ... 14
  - 3.2.4 Oily Solids ... 14
- 3 3 Hydrolysis ... 15
- 3.4 Partition ... 15
- 3 5 Pre-derivatization Column Cleanup ... 16
- 3.6 Derivatization ... 16
  - 3.6.1 Column-cleaned-up Samples ... 16
  - 3.6.2 Crude Samples ... 17
- 3.7 Post-derivatization Column Cleanup ... 17
- 3.8 Derivatization of Calibration Solutions ... 18

4 Instrumentation ... 19

- 4.1 Operating Conditions ... 19
- 4.2 Calibration Procedures ... 19
- 4.3 Analysis ... 20

Table of Contents (continued)

Page No.

4.4 Matrix Effects 20
4.5 Confirmatory Techniques 20
4.6 Time Required for Analysis 21
5 Calculations 21
5.1 Calibration Factor 21
5.2 Analyte in Sample 22
6 Results and Discussion 22
6.1 Precision and Accuracy 22
6.2 Limit of Detection and Quantitation 23
6.3 Extraction Efficiencies 23
6 4 Method Notes 24
6.5 Safety Precautions 24
7 Conclusions 25
8 Tables and Figures 25
Table I. Commodities Analyzed for Residues of the Hydroxy Metabolites of EPTC 27
Table II. Percent Recoveries of the Hydroxy EPTCs from Green Bean Vines, Hay, and Seeds/Pods 28
Table III. Percent Recoveries of the Hydroxy EPTCs from Snap Bean Vines, Hay, and Seeds/Pods 28
Table IV. Percent Recoveries of the Hydroxy EPTCs from Cottonseed and Processed Cottonseed Products (Cotton Meal and Cotton Hulls) 28
Table V. Percent Recoveries of the Hydroxy EPTCs from Dehydrated Sugar Beet Pulp 29
Table VI. Percent Recoveries of the Hydroxy EPTCs from Sugar Beet Refined Sugar 29
Table VII. Percent Recoveries of the Hydroxy EPTCs from Sunflower Seed Crude Oil 29
Table VIII Percent Recoveries of the Hydroxy EPTCs from Almond Nutmeats 29
Figure 1. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Vines 30
Figure 2. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Hay 31
Figure 3. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Pods and Seeds 32
Figure 4. GC/NPD Chromatograms the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Vines 33

Table of Contents (continued)

Page No.

Figure 5. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Hay 34

Figure 6. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Pods and Seeds 35

Figure 7 GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Cotton Hulls 36

Figure 8. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Dehydrated Pulp 37

Figure 9. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Refined Sugar 38

Figure 10. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Crude Sunflower Oil 39

Figure 11. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Almond Nuts 40

Figure 12. GC/MSD Full Scans of the Trifluoroacetate Esters of the Hydroxy EPTCs 41

Figure 13. Confirmation GC/MSD SIM Chromatograms of 2-Hydroxy EPTC (R248722) in Green Bean Hay 42

Figure 14. Calibration Curves: Data Obtained from Calibration Solutions of the Hydroxy EPTC Trifluoroacetate Esters (concentrations correspond to 0 01, 0 02 and 0.10 µg hydroxy EPTC/mL) 43

9 References 44

1 Summary/Introduction

This method is intended for the determination of residues of the free and conjugated hydroxy metabolites of EPTC (S-ethyl dipropylcarbamothioate). EPTC is the active herbicidal ingredient in the formulated products marketed by Zeneca Ag Products, a business unit of Zeneca Inc. (formerly ICI Americas Inc.), under the trade name EPTAM® and ERADICANE®. The compounds, 2-hydroxypropyl EPTC (R248722), 2-hydroxyethyl EPTC (R245255) and 3-hydroxypropyl EPTC (R248723) are plant metabolites of EPTC (References 1-3).

For the purposes of this method, the agricultural commodities can be divided into five classes:

1) most solid commodities
2) water insoluble commodities high in starches or sugars (e.g., cornstarch and sugar beet dehydrated pulp)
3) water soluble commodities high in sugars (e.g., molasses and sugar)
4) oils
5) oily solids (e.g., nutmeats)

A summary of the method follows.

> For samples other than oil and oily solid samples, residues of free and conjugated 2-hydroxypropyl EPTC (R248722), 2-hydroxyethyl EPTC (R245255) and 3-hydroxypropyl EPTC (R248723) are extracted from a weighed sample with acetone/water. The extract is filtered and the acetone is evaporated. (Water-soluble samples high in sugars are dissolved in water).
>
> For oil samples, the oil is taken up in hexane, and residues of free and conjugated 2-hydroxypropyl EPTC (R248722), 2-hydroxyethyl EPTC (R245255), and 3-hydroxypropyl EPTC (R248723) are partitioned into methanol/water. The methanol is then evaporated. For oily solid samples residues of free and conjugated 2-hydroxypropyl EPTC (R248722), 2-hydroxyethyl EPTC (R245255) and 3-hydroxypropyl EPTC (R248723) are extracted with methanol/water, the extracts are filtered, and the filtrate is partitioned with hexane to remove the oil. The methanol/water phase is then evaporated to remove the methanol.
>
> In all cases the aqueous extract is heated in 1 N HCl for one hour under reflux in order to hydrolyze the conjugates. The hydroxy metabolites of EPTC are then

partitioned into 1:1 (v:v) ether:hexane, and the solvent is evaporated. (In the case of water-insoluble samples high in starches or sugars or water-soluble samples high in sugars, an aliquot is cleaned up on a Cyano solid-phase extraction column to remove co-extractives.) The residuum is dissolved in dichloromethane, and trifluoroacetic anhydride (TFAA) is then added to convert the hydroxy metabolites to their trifluoroacetate ester derivatives. The solvent is evaporated and the residuum is taken up in hexane. Except in the case of oil samples, a portion of the final extract is passed through a Cyano solid-phase extraction column to remove co-extractives and give a sample suitable for analysis by GC.

The validated limit of quantitation for the method is 0.01 mg of each hydroxy EPTC per kg of crop.

The method has been used to analyze a wide variety of agricultural commodities as shown in Table I.

2 Materials and Methods

Equipment and reagents of comparable function and purity can be substituted for those listed below.

2.1 Apparatus

1. Gas Chromatograph: Hewlett-Packard 5890 II, equipped with an Hewlett-Packard (HP) Model 7673 automatic sampler, nitrogen-phosphorous detector, and data acquisition system HP 3350

2. Analytical Column: J&W DB-5.625: 30 meter x 0.25 millimeter x 0.25 microns (J&W #122-1032)

3. Inlet Liner: double restrictor, single piece (Restek #20784)

4. Fused-silica Wool: fused-silica wool for packing the inlet liner (Restek #20790; inserter tool, Restek #20114)

5. Syringes, Gas-chromatography Injection: 10 µL capacity (Hamilton 701N) for HP 7673 autosampler

6. Syringes, Sample Handling: 10, 25, 100, 250 µL capacity (Hamilton gas-tight 1700 series)

7. Analytical Balances: Mettler PM 2000 or Mettler PC 4400 for weighing of samples and Mettler AE 200 for preparing stock solutions

8. Glass Pipets: 2, 5, and 10 mL disposable, graduated glass pipets for general use

9. Glass Test Tubes: 15 mL glass screw capped test tubes with caps containing polytetrafluoroethylene (PTFE) liners (Fisher #14-933-1A)

10. Buchner Funnel, Rubber Adapter: filter funnel approximately 100 mm wide (Fisher #10-356D), neoprene rubber adapter to interface to the vacuum filter adapter

11. Filter Paper: Whatman #1 , 9 cm diameter, (Fisher #09-805D)

12. Vacuum Filter Adapter: glass adapter to connect the Buchner funnel to the 500 mL round bottomed flask (Fisher #15-323C)

13. Graduated Cylinder: 100 mL graduated glass cylinder (Fisher #08-554E)

14. Filter/Distillation Flask: flat bottomed 500 mL flask (Fisher #09-559D)

15. Ultrasonic Cleaner: stainless Steel tank 2.8 liter capacity, tank size 5"L x 6"W x 4"D (Branson Model No. 1200)

16. Nitrogen Evaporator: used for evaporating samples in the screw topped test tubes (N-EVAP, Model 112, Organomation Associates, Inc. PO Box 159, South Berlin, MA)

17. Solvent Distillation Assembly: rotary evaporation system, Buchi Rotovapor Model 121 (Buchi Water Bath Model 461)

18. Sorvall Mixer: mixer/blender for macerating crop into solvent, Model no. 17105 (E.I. Du Pont De Nemours & Co., Inc. Newton, CT 06470)

19. Mason Jar: 12 ounce Mason jar which screws into the Sorvall mixer

20. Separatory Funnels: 500 mL partitioning funnels (Fisher #10-437-5D)

21. Filter Funnel: Coors 100 mm i.d. (Fisher #10-356D)

22. Glass Wool: glass wool for filter funnel (Fisher #11-390)

23. Glass Micropipets: glass micropipets for small volumes (Fisher #13-678-20C)

24. Vortex/mixer: vortexer for mixing liquid extracts (Fisher #12-814-5)

25. Cyano SPE Columns: 3 mL capacity for sample cleanup (Bakerbond SPE Extraction Column, Cyano, J. T. Baker product number 7021-03)

26. Centrifuge Tubes: 15 mL screw capped, KIMAX (Fisher #05-538-32B)

2.2 Reagents

1. Water: deionized water or equivalent

2. Acetone: HPLC grade, Fisher Optima or equivalent

3. Extraction Solvent: 70:30 (v:v) acetone:water or 60:40 (v:v) methanol:water; for example, combine a volumetric ratio 700 mL of acetone and 300 mL of water

4. Dichloromethane: Baker-analyzed for organic residue or Fisher Optima

5. Ethyl Ether: Fisher reagent ACS grade

6. Hydrochloric Acid: Fisher reagent ACS grade

7. Hexane: EM Omnisolve or Fisher Optima

8. Partition Solvent: a volumetric ratio 1:1 (v:v) of ethyl ether and hexane; for example, 500 mL of hexane added to 500 mL of ether

9. Methanol: Fisher Optima

10. Trifluoroacetic Anhydride: 99% Janssen Chimica or Acros #14.781.37 from Spectrum

11. Sodium Sulfate: anhydrous and granular, 500 g (Fisher #S418-500)

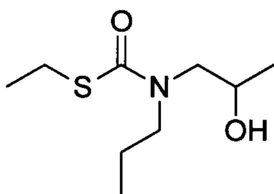
2.3 Analytical Reference Standards

Analytical reference grade standards are available from Zeneca Ag Products, 1200 South 47th Street, Box 4023, Richmond, CA 94804-0023. The compounds available are 2-hydroxypropyl EPTC, 2-hydroxyethyl EPTC and 3-hydroxypropyl EPTC.

The chemical names are given below.

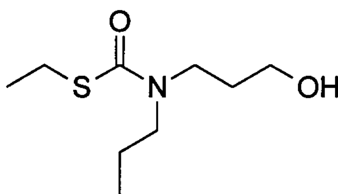
- The Chemical Abstracts name for 2-hydroxypropyl EPTC is: S-ethyl (2-hydroxypropyl)propylcarbamothioate [CAS Reg. No. 65109-69-5].
- The Chemical Abstracts name for 2-hydroxyethyl EPTC is: S-(2-hydroxyethyl) dipropylcarbamothioate [CAS Reg. No. 65109-71-9].
- The Chemical Abstracts name for 3-hydroxypropyl EPTC is: S-ethyl (3-hydroxypropyl)propylcarbamothioate [CAS Reg. No. 65109-70-8].

The chemical structures and their molecular weights (MW) are shown below.



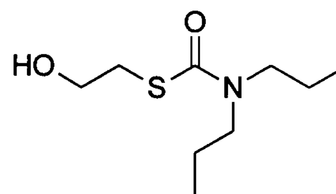
R-248722

MW 205.3



R-248723

MW 205.3



R-245255

MW 205.3

Stock fortification and calibration solutions, 1000 µg /mL, are independently prepared for each analyte. To prepare each of these solutions weigh approximately 0.05 ± 0.01 g of the reference standard into a 4 ounce narrow mouthed bottle on a 5 figure balance. Add a measured weight of the solvent to the bottle to produce a solution of approximately 1000 µg/mL. Close the bottle with a Polyseal® cap and mix the contents thoroughly to dissolve the standard. Acetone is the solvent used for the fortification standard, and dichloromethane is the solvent used for the calibration standard. Aliquots of all three of the fortification stock solutions are mixed together and diluted to 100, 10, and 1 mg/mL concentrations in acetone. Likewise, aliquots of all three of the calibration stock solutions are mixed together and diluted to 100, 10, and 1 mg/mL concentrations in dichloromethane.

Store the solutions in a refrigerator when not in use. Standard solutions are stable for at least 6 months if kept at 10 °C.

3 Analytical Procedure

3.1 Fortification

If possible, analyze fortified and unfortified control samples with each sample set to demonstrate method recovery. For example, add 0.10 mL of a working fortification solution (1 mg/mL) to an untreated control sample (10 g) to produce a fortification level of 0.01 mg/kg. Extract the sample as detailed in Section 3.2. Additional higher fortification levels may be necessary depending on expected residue levels.

3.2 Extraction

3.2.1 Most Solid Samples (including water-insoluble samples high in starches or sugars)

Weigh 10 g (20 g for samples with high moisture content) of the ground sample into a Mason jar and attach to the Sorvall mixer. Add 100 mL of 70:30 (v:v) acetone:water and macerate for 5 minutes at low speed. Using a Buchner funnel and minimal vacuum, filter the extract through a Whatman #1 filter paper into a tared 500 mL flat bottomed flask. Wash the funnel with an additional 15 mL of acetone.

Return the solids (paper included) to the jar and extract with a fresh 100 mL of 70:30 (v:v) acetone:water. Filter the contents through a Whatman #1 filter paper into the flask. Wash the funnel with an additional 35 mL of acetone.

Concentrate the extract under vacuum on a rotary evaporator at a bath temperature of 30 °C to remove the acetone. (The rotary evaporator pressures are initially minus 580 Torr for 10 minutes and are reduced slowly to minus 680 Torr for 20 minutes.) Reduce the mass to approximately 60 to 85 g (a guide only). The actual mass will be somewhat greater for crops high in water content and somewhat less for drier crops. Add enough distilled water to the concentrated extract to increase the mass to 100 ± 1 g.

3.2.2 Water-soluble Samples High in Sugars

Dissolve 10 g of the sample in enough water to give 100 ± 1 g of solution.

3.2.3 Oil Samples

Weigh out 10 g of the oil and transfer it to a 500 mL separatory funnel. Add 100 mL of hexane and 50 mL of distilled water and swirl to mix thoroughly. Drain the resulting lower (aqueous) phase into a tared 500 mL flat bottomed flask. Add 50 mL of 60:40 (v:v) methanol:water to the upper (hexane) phase and swirl to mix thoroughly. Drain the resulting lower phase into the flask. Add 50 mL of 60:40 (v:v) methanol:water to the upper (hexane) phase and swirl to mix thoroughly. Drain the resulting lower phase into the flask.

Concentrate under vacuum on a rotary evaporator at a bath temperature of 40 °C to remove most of the methanol and reduce the mass to between 95 and 100 g. (The rotary evaporator pressures are gradually reduced to minus 680 Torr and maintained at this level.) Add water, if necessary, to increase the mass to 100 ± 1 g.

3.2.4 Oily Solids

Weigh 10 g of the ground sample into a Mason jar and attach to the Sorvall mixer. Add 80 mL of 60:40 (v:v) methanol:water and macerate for 5 minutes at low speed. Using a Buchner funnel and minimal vacuum, filter the extract through a Whatman #1 filter paper into a tared 500 mL round bottomed flask. Return solids (paper included) to the jar and extract with a fresh 80 mL of 60:40 (v:v) methanol:water. Filter the jar's contents through a Whatman #1 filter paper into the flask. Rinse the jar with two 40-mL portions of hexane, filtering each rinse through the crop solids in the Buchner funnel, and combining with the crop extract. Transfer the combined filtrates to a 500-mL separatory funnel. Swirl to mix, avoiding aeration which may result in formation of emulsions. Allow the phases to separate, return the lower phase to the tared round bottomed flask, and discard the upper phase.

Concentrate the resulting mixture under vacuum on a rotary evaporator at a bath temperature of 40 °C to remove most of the methanol. The rotary evaporator pressures are initially minus 580 Torr and are decreased slowly to minus 660-680 Torr for approximately 20 minutes. Reduce the mass to approximately 69 to 79 g. Add enough water to increase the mass to 100 ± 1 g.

3.3 Hydrolysis

Add 9.1 mL of 12 *N* HCl; this will produce an HCl concentration of about 1 *N*. Using a water condenser, heat under reflux for one hour. Remove the heating mantle from the reflux assembly immediately after the one hour reflux period, and allow the contents of the flask to cool to room temperature. Then, rinse down the condenser with 10 mL of hexane:ether, 1:1 (v:v).

Note: The hydrolysis for one hour duration has been shown to cleave the conjugates of the hydroxy metabolites of EPTC. Prolonged heating of the extract has been shown to cause degradation of 2-hydroxypropyl EPTC (R248722).

3.4 Partition

Transfer the hydrolysate and the hexane/ether mixture into a 500 mL separatory funnel. Rinse the 500 mL flat bottomed flask with 100 mL of 1:1 (v:v) hexane:ether, and transfer to the separatory funnel. Shake the funnel for about 30 seconds.

After allowing the phases to separate, drain the lower (aqueous) phase back into a hydrolysis flask. Drain the upper phase through a funnel plugged with glass wool and filled with about 40 g of anhydrous granular sodium sulfate. Collect the filtrate in a clean and dry 500 mL flat bottomed flask.

Return the aqueous phase to the separatory funnel, and rinse the hydrolysis flask with 100 mL of 1:1 (v:v) hexane:ether. Use the rinse to extract the aqueous phase once again.

After allowing the phases to separate, drain and discard the lower (aqueous) phase. Drain the upper phase through the sodium sulfate into the same flat bottomed flask.

Wash the sodium sulfate with 10 mL of 1:1 (v:v) hexane:ether. Concentrate the extract just to dryness under vacuum on a rotary evaporator at a bath temperature of 30 °C. Operate the rotary evaporator by slowly lowering the pressure (suggested pressure is from minus 500 Torr to minus 620 Torr for 10-20 minutes). Please note that, although it is not measured, the final volume is usually less than 0.5 mL. For certain crops such as sunflower seeds, which contain some oil but can still be extracted by the procedure of Section 3.2.1, the volume can be much higher (e.g., 4 to 5 mL).

3.5 Pre-derivatization Column Cleanup (To be used on extracts of water-insoluble samples high in starches or sugars or of water-soluble samples high in sugars)

Transfer the residuum quantitatively to a graduated tube with 1:1 (v:v) ether:hexane and dilute to 10 mL with 1:1 (v:v) ether:hexane.

Condition the column (Bakerbond SPE* Extraction Column, Cyano, J. T. Baker product number 7021-03) by passing 2 x 3 mL of 1:1 (v:v) hexane:ether through it under gentle pressure. Discard the wash.

Load a 2.0 mL portion (equivalent to 2 g) of the sample solution onto the column under gentle pressure. Collect the eluate in a graduated tube. Pass 5 mL of 1:1 (v:v) hexane:ether through the column under gentle pressure. Collect this eluate in the same tube and evaporate just to dryness in a gentle stream of nitrogen.

3.6 Derivatization

3.6.1 Column-cleaned-up Samples

Dissolve the residuum in 5 mL of dichloromethane, add 1 mL of trifluoroacetic anhydride (TFAA), vortex mix for 0.5 minute, and allow the solution to stand for 20 minutes. Evaporate just to dryness in a gentle stream of nitrogen. Dissolve in 2 mL of hexane. The sample-to-solvent ratio at this point is 1 g/mL.

3.6.2 Crude Samples

Dissolve the residuum in the flat bottomed flask in dichloromethane. (Sonication may be necessary to remove solids from the wall of the flask.) Using a micropipet, carefully transfer the contents of the flask to a 15 or 25 mL test tube with a screw cap containing a PTFE liner. Add further portions of dichloromethane to the residuum in the flat bottomed flask, swirl the mixture, and transfer it to the same 15 or 25 mL test tube . Altogether, these two portions of dichloromethane and the residuum should make up a total volume of 10 to 12 mL. Agitate the tube to ensure that the solution is mixed.

Add 1 mL of TFAA and vortex mix for 0.5 minute. Leave the capped tube standing for 20 minutes. Evaporate the extract in a gentle nitrogen stream until about 0.5 mL of oil or crop material remains. Make up to a total volume of 10 mL using hexane. Vortex mix for 0.5 minutes. Sonication may be necessary. The sample-to-solvent ratio at this point is 1 g/mL if the size of the original sample was 10 g, or 2 g/mL if the size of the original sample was 20 g.

Suspended solids may be present in the hexane solution; centrifugation is advisable. These solids may hinder the column cleanup procedure.

Note: Typically, samples are held at room temperature for 20 minutes with the trifluoroacetic anhydride, and the calibration solutions are held for 10 minutes. Using longer times of up to one hour does not hinder the derivatization.

3.7 Post-derivatization Column Cleanup (not applicable in the case of oil samples)

Condition the column (Bakerbond SPE* Extraction Column, Cyano, J. T. Baker product number 7021-03) by passing 5 to 6 mL of methanol followed by 9 to 10 mL of hexane through it under gentle pressure. Discard the wash.

Transfer 2.0 mL (equivalent to 2 or 4 g of the original sample; see Section 3.6.2) of the derivatized extract to the column by using a pipet (or, in the case of a sample that has been

subjected to the pre-derivatization cleanup, use a minimum of hexane to transfer the derivatized extract quantitatively to the column), and collect the eluate in a 15 mL graduated centrifuge tube.

Elute the column with 6 mL of hexane using gravity or gentle pressure. Collect the eluate into the same 15 mL graduated centrifuge tube.

Evaporate the eluate in a gentle stream of nitrogen to less than 2 mL. Allow to warm to room temperature, and carefully adjust to a total volume of 2.0 mL with hexane. Vortex mix for 0.5 minute, and transfer a sample of the resulting solution to an autosampler vial for analysis by gas chromatography (GC). The crop-to-solvent ratio is 1.0 g/mL if the original sample size was 10 g, or 2.0 g/mL if the original sample size was 20 g.

Variations in elution characteristics may exist between lots of the Cyano SPE* columns. In addition, specific matrices may affect the elution of the analytes from the column. To test or confirm the recommended elution scheme with a new lot of columns or a specific matrix, the control extract is fortified to a known level by addition of a small aliquot of derivatized (see Section 3.8) analyte standard, just prior to the SPE cleanup step.

If recoveries are not satisfactory, adjust the SPE elution scheme to eliminate analyte losses. Once the elution profile is determined, adjustments to the elution scheme can be made to increase the recovery of the analytes.

3.8 Derivatization of Calibration Solutions

Transfer a 1 mL aliquot of an appropriate calibration solution (prepared according to Section 2.3) to a 15 mL screw topped test tube and add 0.3 mL of trifluoroacetic anhydride (TFAA). Put on a cap with a PTFE liner and shake the contents. Hold at room temperature for ten minutes. Evaporate just to dryness under a stream of nitrogen, and take up in 10 mL of hexane.

4 Instrumentation

The following conditions have been found suitable for gas chromatographic analysis of the trifluoroacetates of the hydroxy metabolites. Alternative conditions may be used if necessary.

4.1 Operating Conditions

Gas Chromatograph:

Model:	Hewlett Packard 5880 or 5890 Series II
Column:	J&W DB-5.625, 30 meter, 0.25 mm i.d., 0.25 mm film thickness
Carrier:	Helium, 17.5 psig at column head
Injector Temp:	240 °C
Detector:	NPD

Oven Temperature Profile:

Initial Oven Temperature:	100 °C
Initial Time:	0.5 min
Program Rate:	6 °C/min, to 180 °C
Final Oven Temperature:	250 °C
Final Time:	8 min

Other Conditions:

Volume Injected:	2 mL
Purge On:	0.8 min
Injection Solvent:	hexane
Total Run Time:	20 min

Expected Retention Times:

Trifluoroacetate ester of R248722	*ca.* 10.2 min
Trifluoroacetate ester of R245255	*ca.* 12.3 min
Trifluoroacetate ester of R248723	*ca.* 12.6 min

4.2 Calibration Procedures

Calibrate the gas chromatograph by using the calibration solutions derivatized and diluted according to Section 3.7.

4.3 Analysis

Standards should be injected after analysis of about every 3 sample extracts. Quantitation is based upon the responses of the standard calibration solutions bracketing the sample extracts. The average response of the standards bracketing the sample injections is used to calculate the response factor.

4.4 Matrix Effects

No matrix effects have been seen in any of the analyses completed to date.

4.5 Confirmatory Techniques

Confirmation of peaks can be achieved by quantitation using a gas chromatograph with mass-selective detection, trying different ions of the analyte or comparing the ratios of two or more ions. Ions for the derivatives of the hydroxy EPTCs are listed below.

Compound	Ion (m/z)	Abundance (%)*
2-hydroxypropyl EPTC (R248722):	301	1.3
	240	22
2-hydroxyethyl EPTC (R245255):	301	1.1
	244	20
3-hydroxypropyl EPTC (R248723):	301	1.0
	240	19

* approximate % relative abundance

The use of an alternate column with a different stationary phase may help to confirm the presence of the analytes, or to distinguish the analytes from interferences.

4.6 Time Required for Analysis

The analysis can be completed by one person in three eight-hour days if adequately ground samples are available.

5 Calculations

The concentrations of the analytes in the original sample are calculated by the external standard method; i.e., the responses obtained for the analytes resulting from injection of the sample extract are compared to the responses obtained from separate injections of known amounts of the analytes (calibration solutions). To use the calculations shown below, the injection volumes for all calibration solutions and sample extracts must be fixed at the same volume. The average of responses for each analyte in the standard injections before and after the samples of interest is used to determine the calibration factor for that analyte.

5.1 Calibration Factor

Calculate the response factor, F, for injection of a calibration solution as follows:

$$F = \frac{C}{R}$$

Where:

F = response factor

C = concentration of calibration solution, μg/mL

R = average of response units (e.g., peak height, area) from detector for bracketing calibration solutions

5.2 Analyte in Sample

Calculate the analyte concentration, A, in the original sample as follows:

$$A = \frac{F \times R}{C}$$

Where:

A = concentration of analyte in original sample (µg/g or mg/kg or ppm)

F = response factor, (µg/mL)/response unit

R = sample response units from detector

C = concentration of crop in final extract (g/mL)

6 Results and Discussion

This method is suitable for the determination of free or conjugated 2-hydroxypropyl EPTC, 2-hydroxyethyl EPTC, and 3-hydroxypropyl EPTC in crops. Recovery data given in Tables II through VIII (Section 8) reflect the methodology described herein.

6.1 Precision and Accuracy

The range of recoveries from fortified samples of a large variety of raw and processed agricultural commodities (References 4-25) was:

- 2-hydroxypropyl EPTC (R248722) 65 to 132%
- 2-hydroxyethyl EPTC (R245255) 61 to 131%
- 3-hydroxypropyl EPTC (R248723) 56 to 120%

If the results for the problem matrices potato chips (Reference 23), corn starch (Reference 5), dry bean vines (Reference 7), tomatoes (Reference 24), tomato wet pomace (Reference 24), refined cottonseed oil (Reference 22), sugar beet sugar (Reference 25), and sugar beet molasses (Reference 25) are eliminated, the ranges for all the hydroxy metabolites taken together become

69 to 121%. Statistical analyses of the recoveries observed with the commodities studied by us have been reported (References 4-25).

6.2 Limit of Detection and Quantitation

The detection limit for a specific analyte in a specific crop is based on the minimum detectability of the analyte and the crop concentration in the extract. The minimum detectable amount has been established as a response large enough that a 25% change can be distinguished. A signal to noise ratio of at least 10 is required. The detection limit for a specific crop is obtained by dividing the minimum detectable concentration by the crop concentration in the extract. The detection limit was 0.01 mg/kg.

The lower limit of quantitation (LOQ) is defined as the lowest concentration at which a method has been verified. It may differ from the detection limit. LOQ values of 0.01 mg/kg for the hydroxy-EPTCs were obtained for these crops.

6.3 Extraction Efficiencies

2-hydroxy EPTC (R248722), free and/or conjugated, is the hydroxy EPTC found at the highest levels in a recently completed series of magnitude-of-the-residue studies on crops and processed commodities (References 4-25). The efficiency of extraction of R248722 was shown by a radiovalidation experiment (Reference 26) on pinto bean vines grown for a metabolism study (Reference 2). The pinto bean vines had been harvested 90 days after the first of two treatments with radiolabelled EPTC. Application of the extraction procedure (see Section 3.2.1) and the hydrolysis procedure (see Section 3.3) to these vines was followed by quantitative HPLC analysis for R248722. The total concentration of free and conjugated R248722 was found to be 0.43 ppm (expressed as µg EPTC/g vine), whereas the concentration determined by the metabolism study was 0.21 ppm. The hydrolysis procedure in Section 3.3 was responsible for the increased recovery of the acid-sensitive R248722; it calls for a shorter acid treatment than that used in the metabolism study.

6.4 Method Notes

The period of reflux in the hydrolysis step (Section 3.3) is critical. As noted in Section 6.3, it has been shown that R248722 is excessively degraded by extension of the hydrolysis period beyond one hour.

There can be volatile loss of all of the hydroxy EPTCs, especially R248722, in the evaporation steps (Sections 3.2, 3.4, and 3.5). The durations of evaporation should not be extended past those given in the method steps.

There can be volatile loss of the EPTC hydroxy trifluoroacetate esters, especially that of R248722, in the evaporation steps (Sections 3.6, 3.7, and 3.8) using the nitrogen stream. The duration of evaporation steps should be just to dryness, or when the solvent level ceases to decrease in the drying tubes and only the oil remains.

Low-volume inserts should not be used in the GC vials. We found that our supplier of low-volume inserts manufactured them from a grade of glass that was less inert than that used to make the vials. This resulted in degradation of the hydroxy EPTC trifluoroacetate esters, particularly the esters of primary alcohols, R245255 and R248723, and particularly of esters in very dilute solution, i.e., those in the 0.01 µg/mL GC calibration standard.

The procedures of the method can be stopped at any point, and the extracts can be retained at room temperature overnight. However, it is advisable to reconstitute the low volumes of the sample extract in the required solvent for overnight storage.

6.5 Safety Precautions

Personnel untrained in good laboratory practices and the routine safe handling of chemicals must not attempt to use this procedure. Information on first aid procedures can be found in the Material Safety Data Sheets accompanying the chemical or are available from the chemical supplier. In general, always wear safety glasses with side shields, work in a well ventilated area,

avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

Acetone, methanol, ethyl ether, dichloromethane and hexane are flammable. HCl and trifluoroacetic anhydride are corrosive.

7 Conclusions

The method has been shown to be functional for the analysis and the determination of free and conjugated residues of 2-hydroxypropyl EPTC, 2-hydroxyethyl EPTC and 3-hydroxypropyl EPTC in crops. Only commercially available laboratory equipment and reagents are required.

8 Tables and Figures

Table I. Commodities Analyzed for Residues of the Hydroxy Metabolites of EPTC

Table II. Recoveries of the Hydroxy EPTCs from Green Bean Vines, Hay, and Seeds/Pods

Table III. Recoveries of the Hydroxy EPTCs from Snap Bean Vines, Hay, and Seeds/Pods

Table IV. Recoveries of the Hydroxy EPTCs Cottonseeds and Processed Products (Cotton Meal and Cotton Hulls)

Table V. Recoveries of the Hydroxy EPTCs from Sugar Beet Dehydrated Pulp

Table VI. Recoveries of the Hydroxy EPTCs from Sugar Beet Refined Sugar

Table VII. Recoveries of the Hydroxy EPTCs from Sunflower Seed Crude Oil

Table VIII. Recoveries of the Hydroxy EPTCs from Almond Nutmeats

Figure 1. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Vines

Figure 2. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Hay

Figure 3. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Pods and Seeds

Figure 4. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Vines

Figure 5. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Hay

Figure 6. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Pods and Seeds

Figure 7. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Cotton Hulls

Figure 8. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Dehydrated Pulp

Figure 9. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Refined Sugar

Figure 10. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sunflower Seed Crude Oil

Figure 11. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Almond Nutmeats

Figure 12. GC/MSD Full Scans of the Trifluoroacetate Esters of the Hydroxy EPTCs

Figure 13. Confirmation GC/MSD SIM Chromatograms of 2-Hydroxy EPTC in Green Bean Hay

Figure 14. Calibration Curves. Data Obtained from Calibration Solutions of the Hydroxy EPTC Trifluoroacetate Esters (concentrations correspond to 0.01, 0.02 and 0.10 µg hydroxy EPTC/mL).

Table I. Commodities Analyzed for Residues of the Hydroxy Metabolites of EPTC

Part A. Raw Agricultural Commodities	
Crop	Raw Agricultural Commodity Analyzed
Alfalfa	forage, hay
Almond	hulls, nutmeats
Clover	forage, hay
Corn	fodder, forage, grain
Corn (sweet)	forage, ears
Cotton	forage, seeds
Dry bean	dried seeds, vines, hay
Green bean	pods and seeds, vines, hay
Potato	tubers
Safflower	seeds
Snap bean	pods and seeds, vines, hay
Sugar beet	roots, tops
Sunflower	seeds
Tomato	fruit
Walnut	nutmeats

Part B. Processed Commodities	
Raw Agricultural Commodity	Processed Commodity Analyzed
Corn grain	meal, starch, grits, flour, refined oil
Cottonseed	meal, hulls, crude oil, refined oil
Potato tubers	chips, dry peels, granules, wet peels
Tomato fruit	wet pomace, dry pomace, puree, juice
Sugar beet roots	dehydrated pulp, molasses, sugar
Sunflower seeds	meal, hulls, crude oil, refined oil

Table II. Percent Recoveries of the Hydroxy EPTCs from Green Bean Vines, Hay, and Seeds/Pods

Commodity	Sample No.	Fortification Level (mg/kg)	R248722	R245255	R248723
Vines	J161-1	0.01	89.2	87.8	85.5
Hay	J161-3	0.01	74.3	86.9	69.8
Seed/Pods	J161-6	0.01	77.1	85.0	84.2

Table III. Percent Recoveries of the Hydroxy EPTCs from Snap Bean Vines, Hay, and Seeds/Pods

Commodity	Sample No	Fortification Level (mg/kg)	R248722	R245255	R248723
Vines	H231-1	0.01	84.5	70.1	75 2
	H231-1	0.10	75.3	89 1	77.2
	H232-1	0.01	76.3	86 4	71.2
Hay	H232-3	0 01	78.6	85.4	70.2
	H231-3	0.01	71 8	82.9	80.8
Seeds/Pods	H231-7	0.01	88.0	97.8	94.3

Table IV. Percent Recoveries of the Hydroxy EPTCs from Cottonseed and Processed Cottonseed Products (Cotton Meal and Cotton Hulls)

Commodity	Sample No	Fortification Level (mg/kg)	R248722	R245255	R248723
Cottonseed	H251-1	0.02	82.8	104.7	94.7
	H251-101	0 01	93 2	102.5	90.2
Cotton Meal	H251-102	0 02	72.5	79.0	72.3
Cotton Hulls	H251-103	0.02	75.5	81.0	80.0

Table V. Percent Recoveries of the Hydroxy EPTCs from Dehydrated Sugar Beet Pulp

Commodity	Sample No.	Fortification Level (mg/kg)	R248722	R245255	R248723
Sugar Beet Dehydrated Pulp	H227-102	0.05	87.9	99.6	91.8

Table VI. Percent Recoveries of the Hydroxy EPTCs from Sugar Beet Refined Sugar

Commodity	Sample No.	Fortification Level (mg/kg)	R248722	R245255	R248723
Sugar Beet Refined Sugar	H227-104	0.02	65 8	103	71.6

Table VII. Percent Recoveries of the Hydroxy EPTCs from Sunflower Seed Crude Oil

Commodity	Sample No.	Fortification Level (mg/kg)	R248722	R245255	R248723
Sunflower Seed Crude Oil	H262-105	0.02	76.7	86 8	76.1

Table VIII. Percent Recoveries of the Hydroxy EPTCs from Almond Nutmeats

Commodity	Sample No.	Fortification Level (mg/kg)	R248722	R245255	R248723
Almond Nutmeats	J113-5	0.02	80.9	69.8	71.9

Figure 1. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Vines

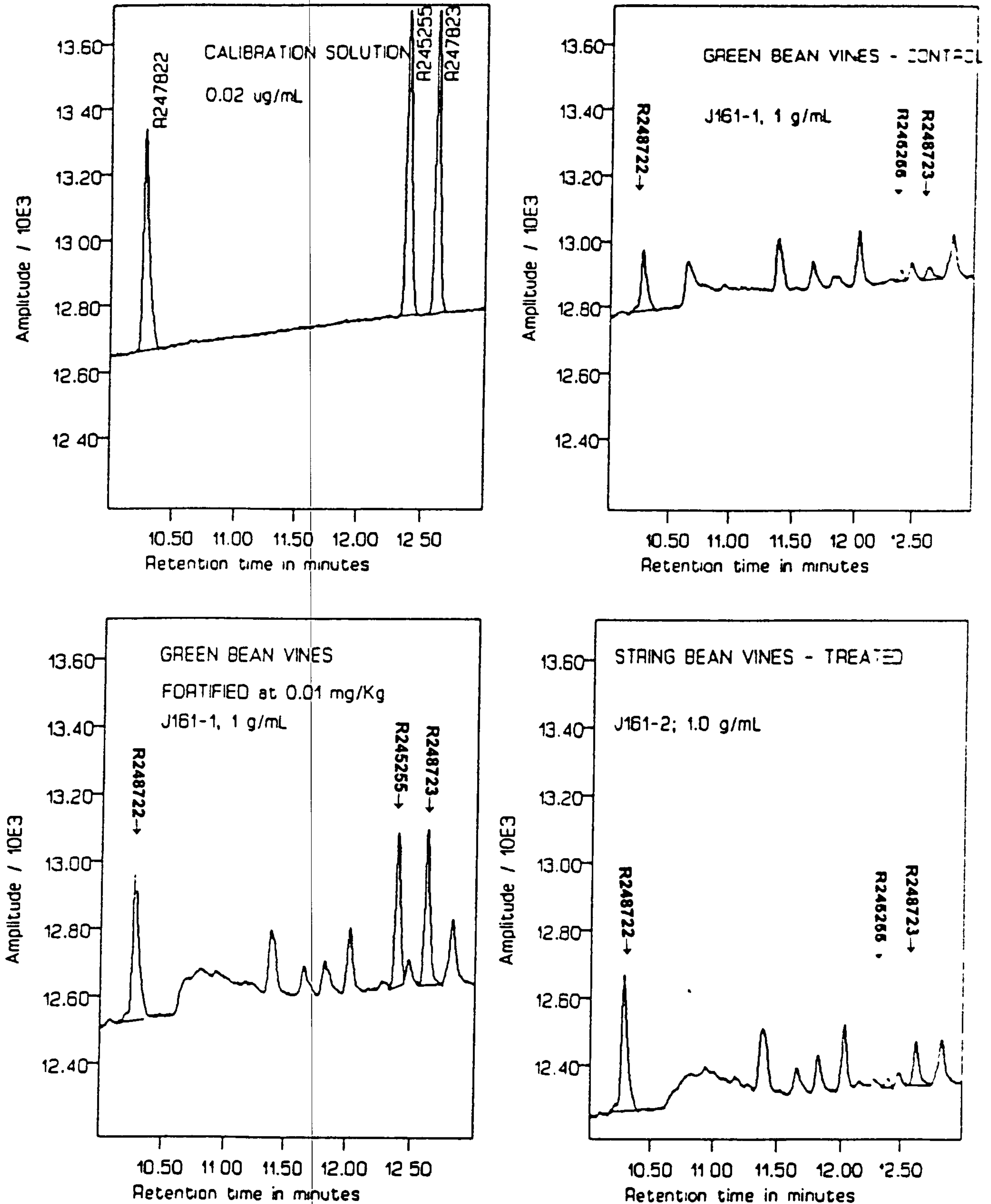
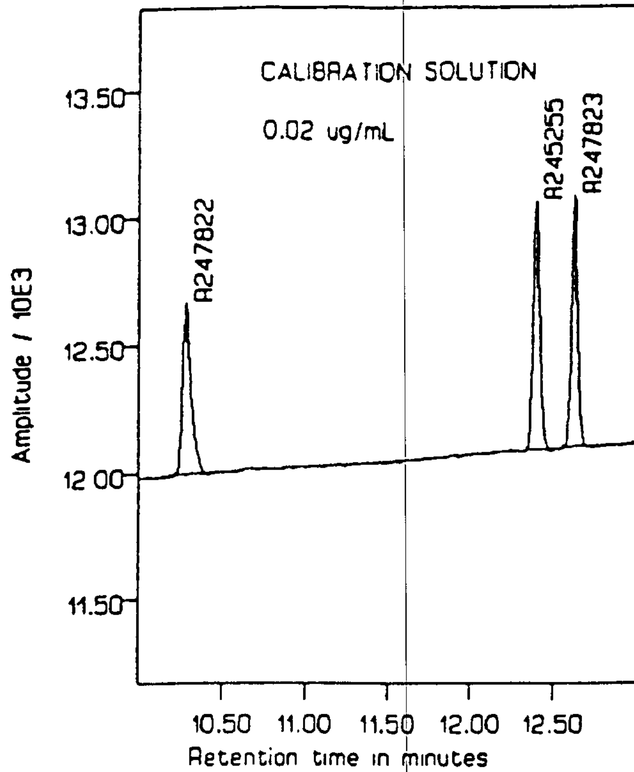
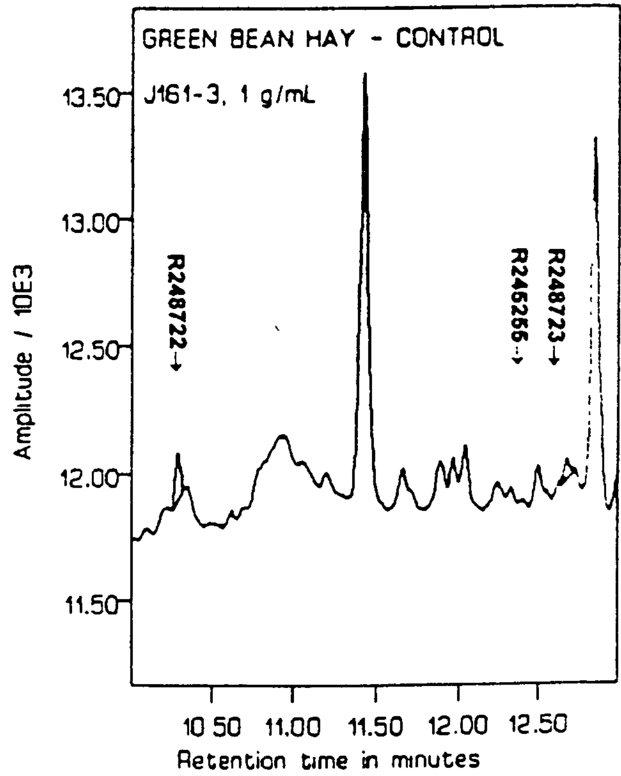
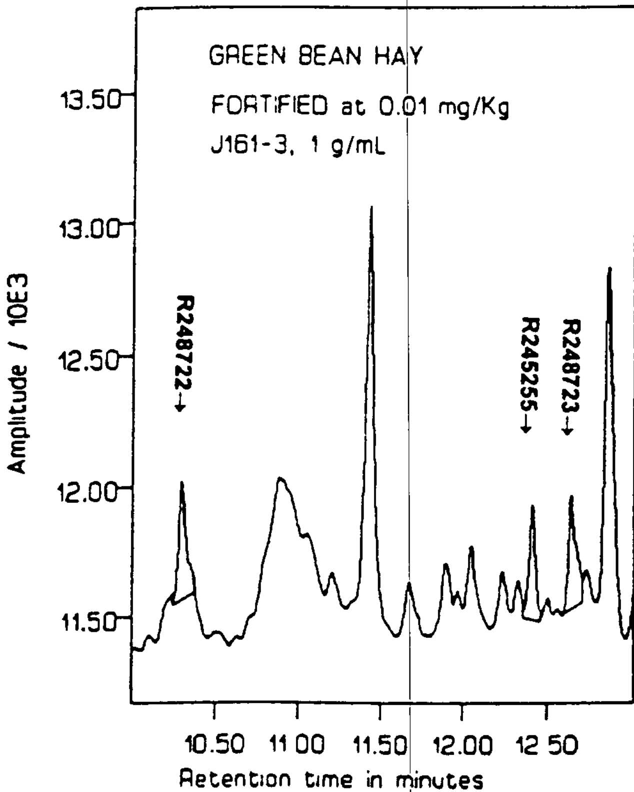


Figure 2. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Hay







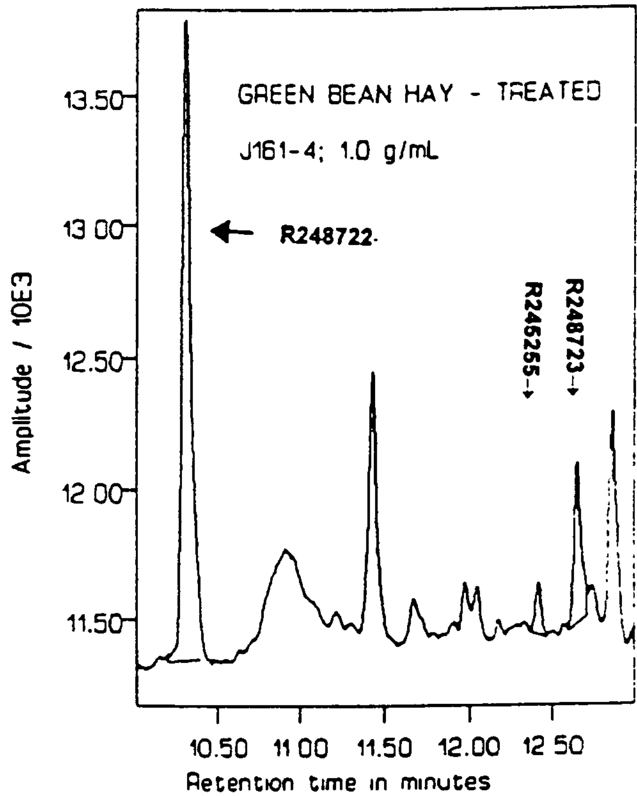


Figure 3. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Green Bean Pods and Seeds

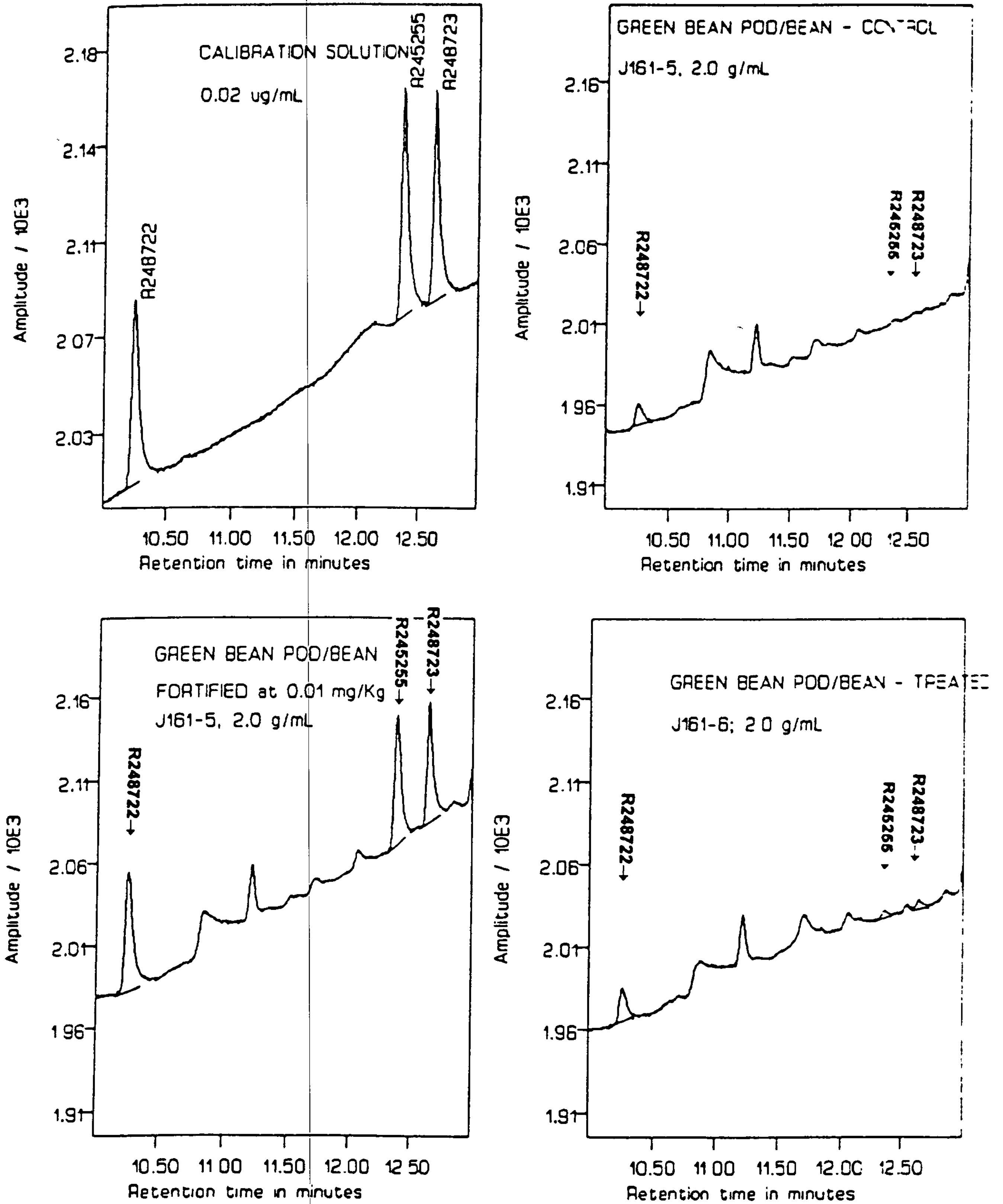


Figure 4. GC/NPD Chromatograms the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Vines

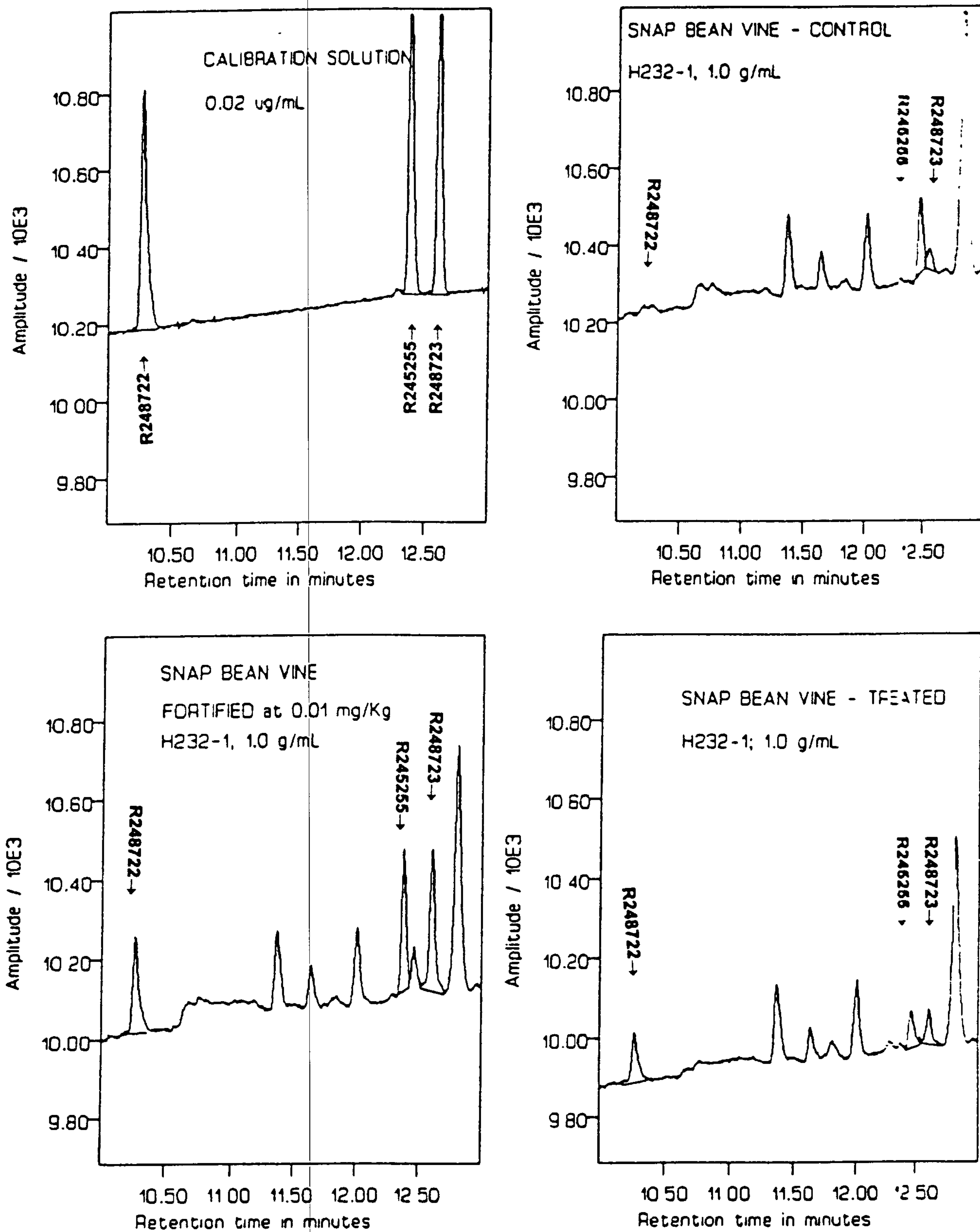


Figure 5. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Hay

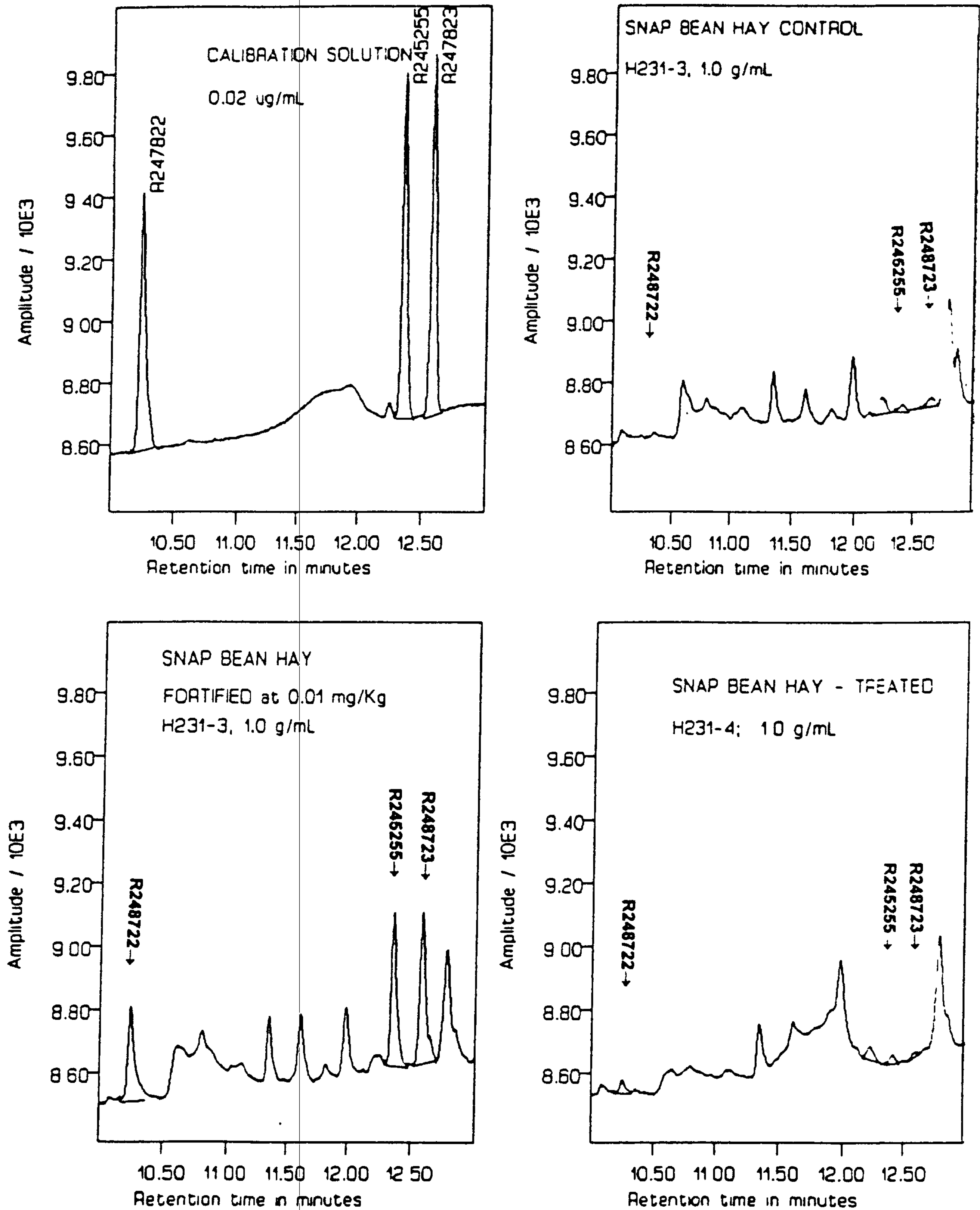


Figure 6. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Snap Bean Pods and Seeds

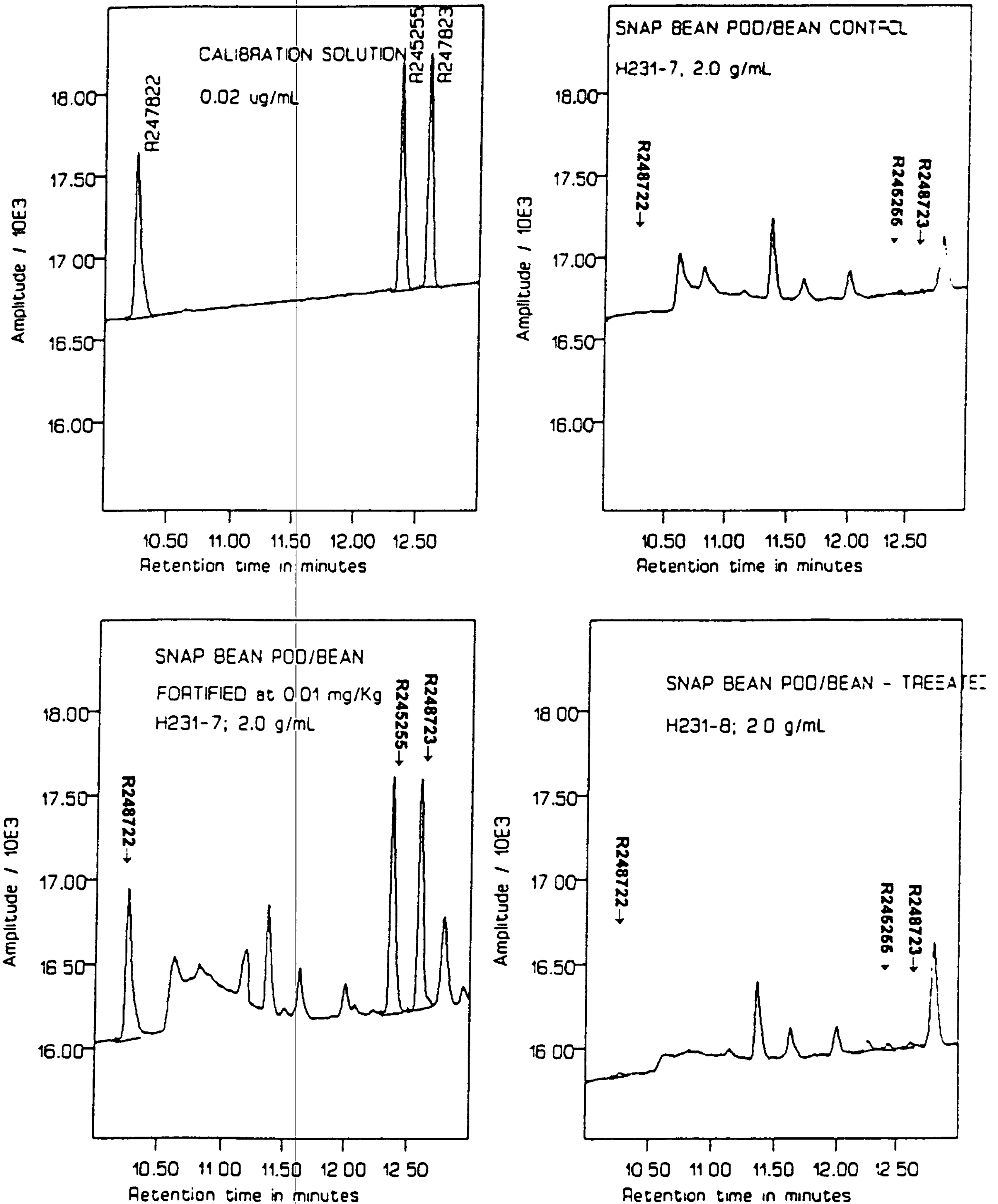


Figure 7. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Cotton Hulls

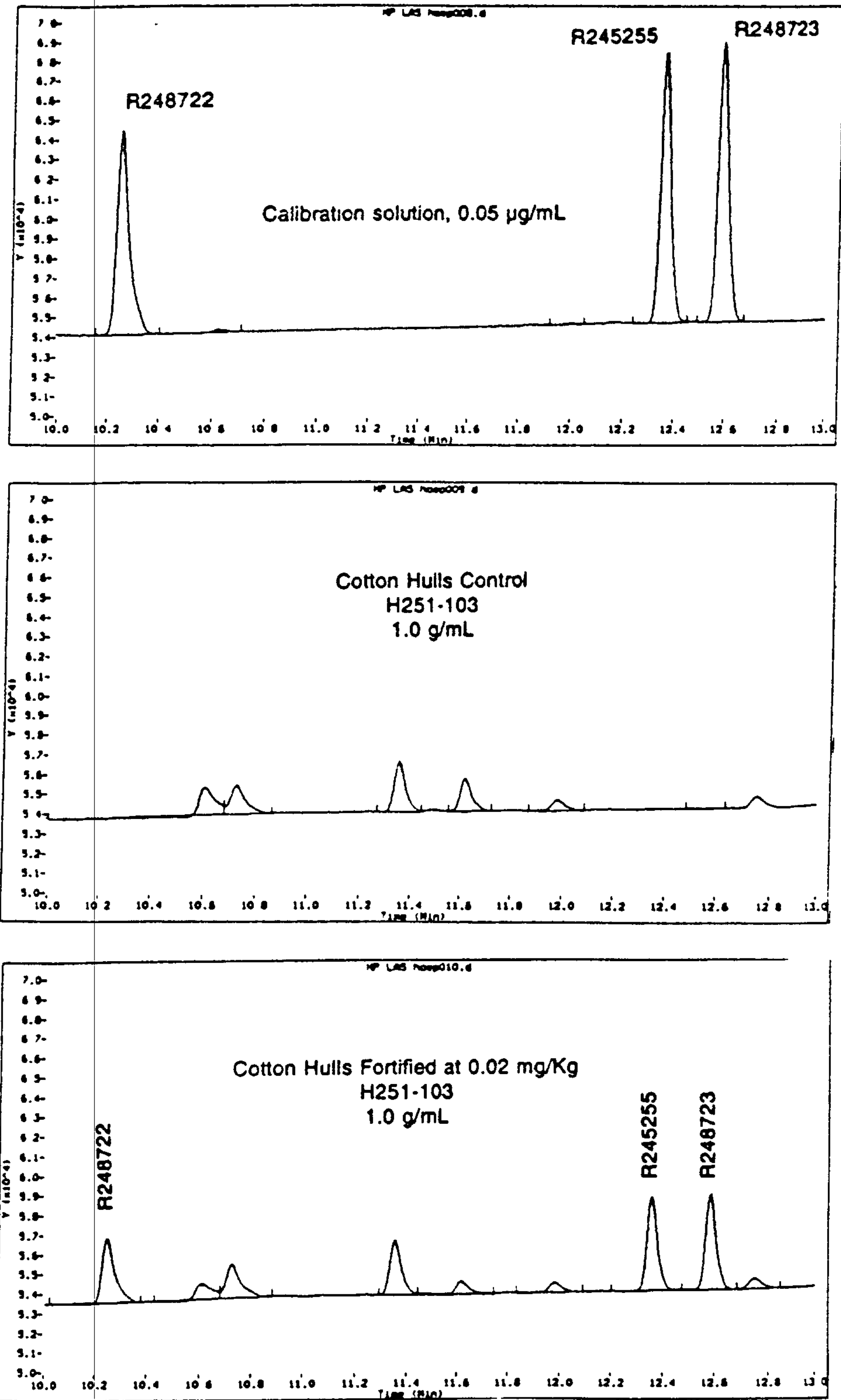


Figure 8. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Dehydrated Pulp

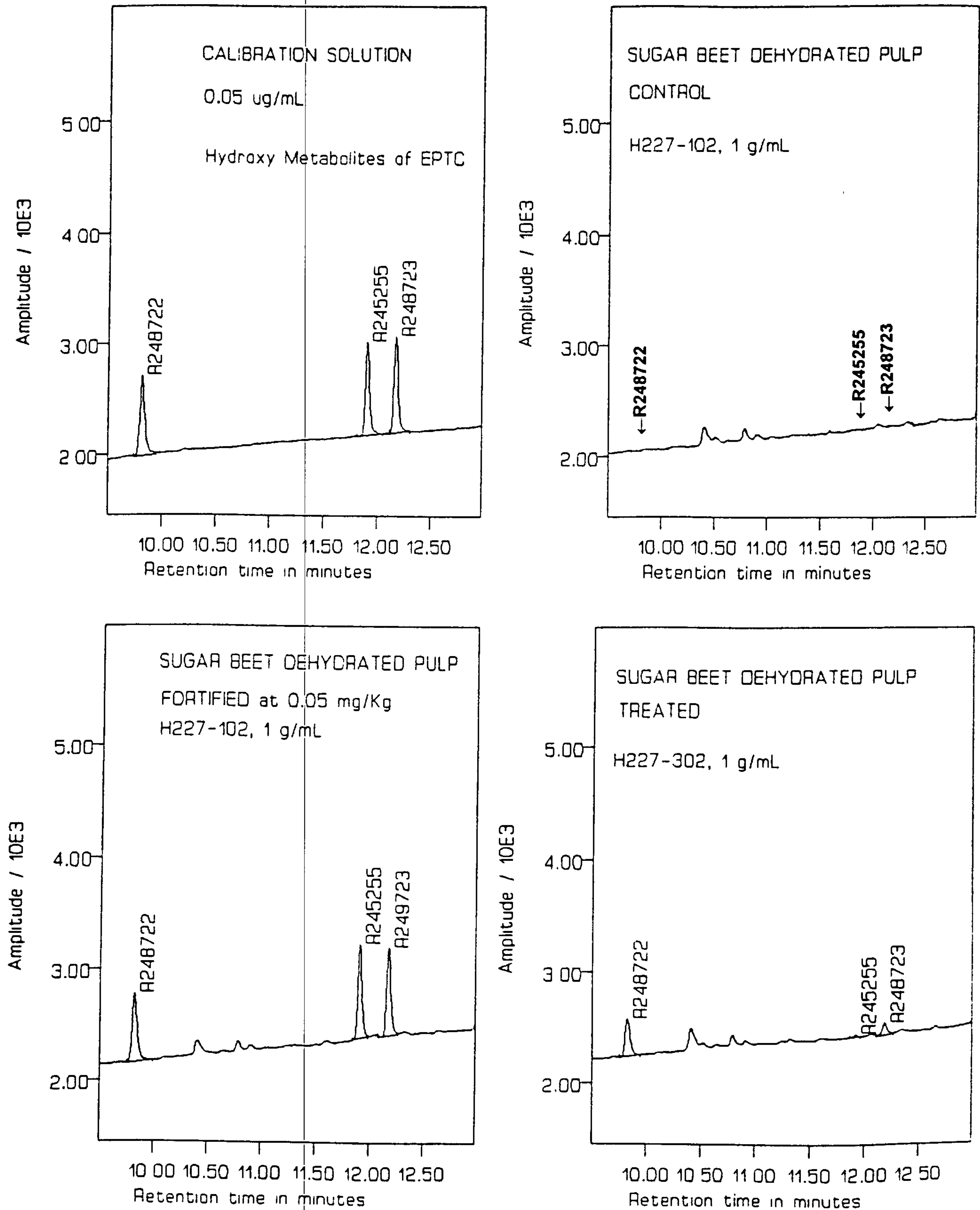


Figure 9. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Sugar Beet Refined Sugar

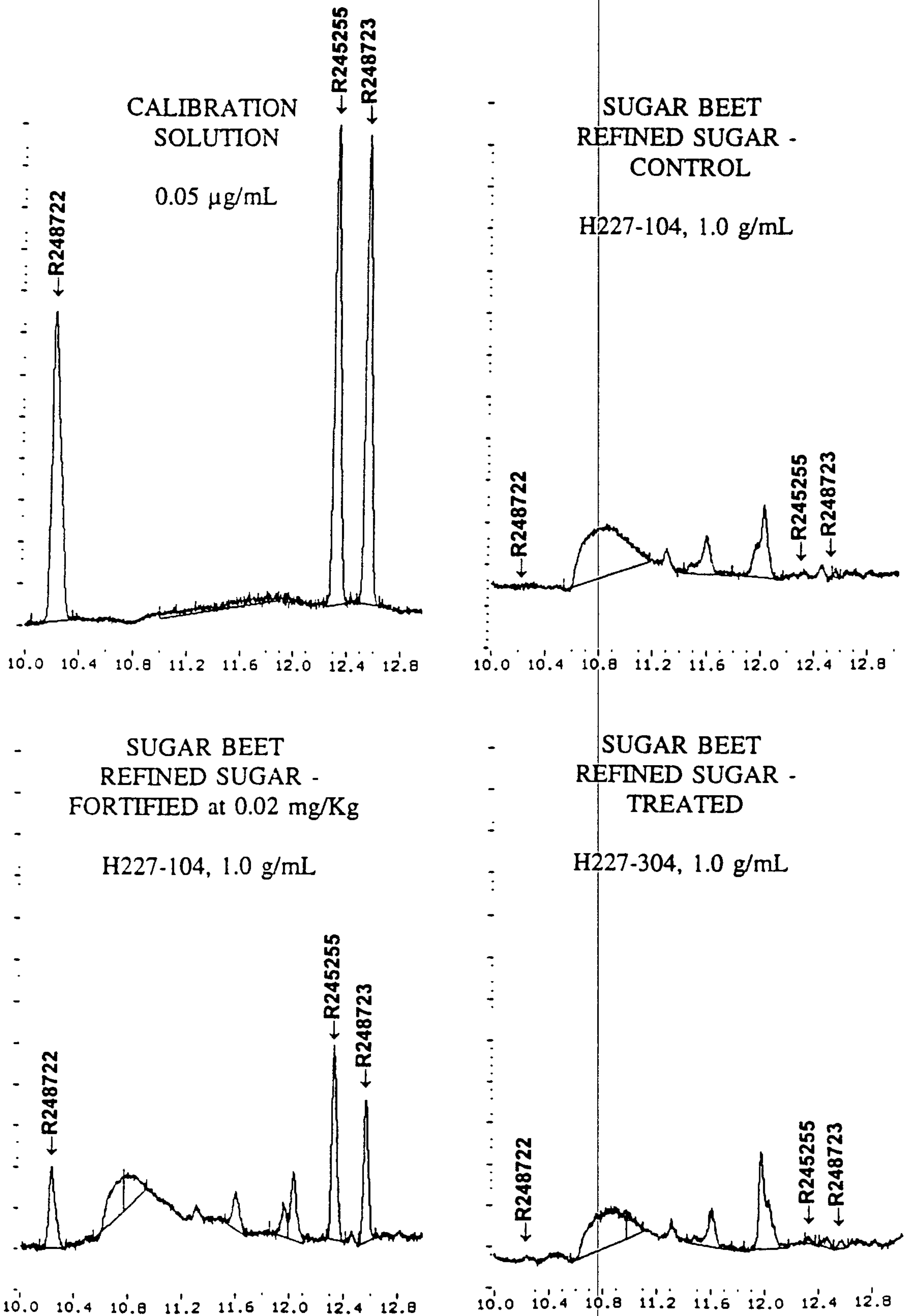


Figure 10. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Crude Sunflower Oil

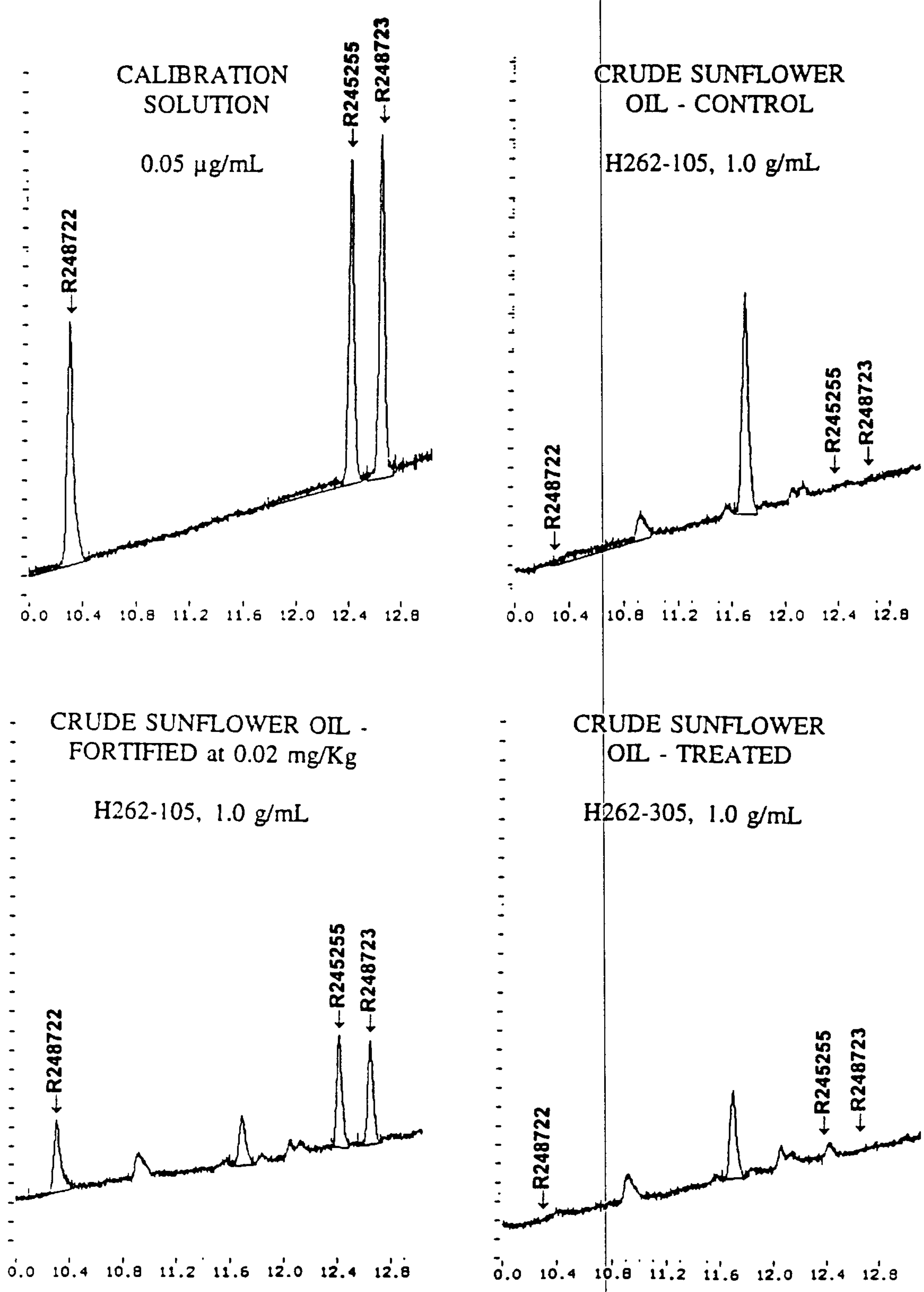


Figure 11. GC/NPD Chromatograms of the Trifluoroacetate Esters of Hydroxy EPTCs in Almond Nuts

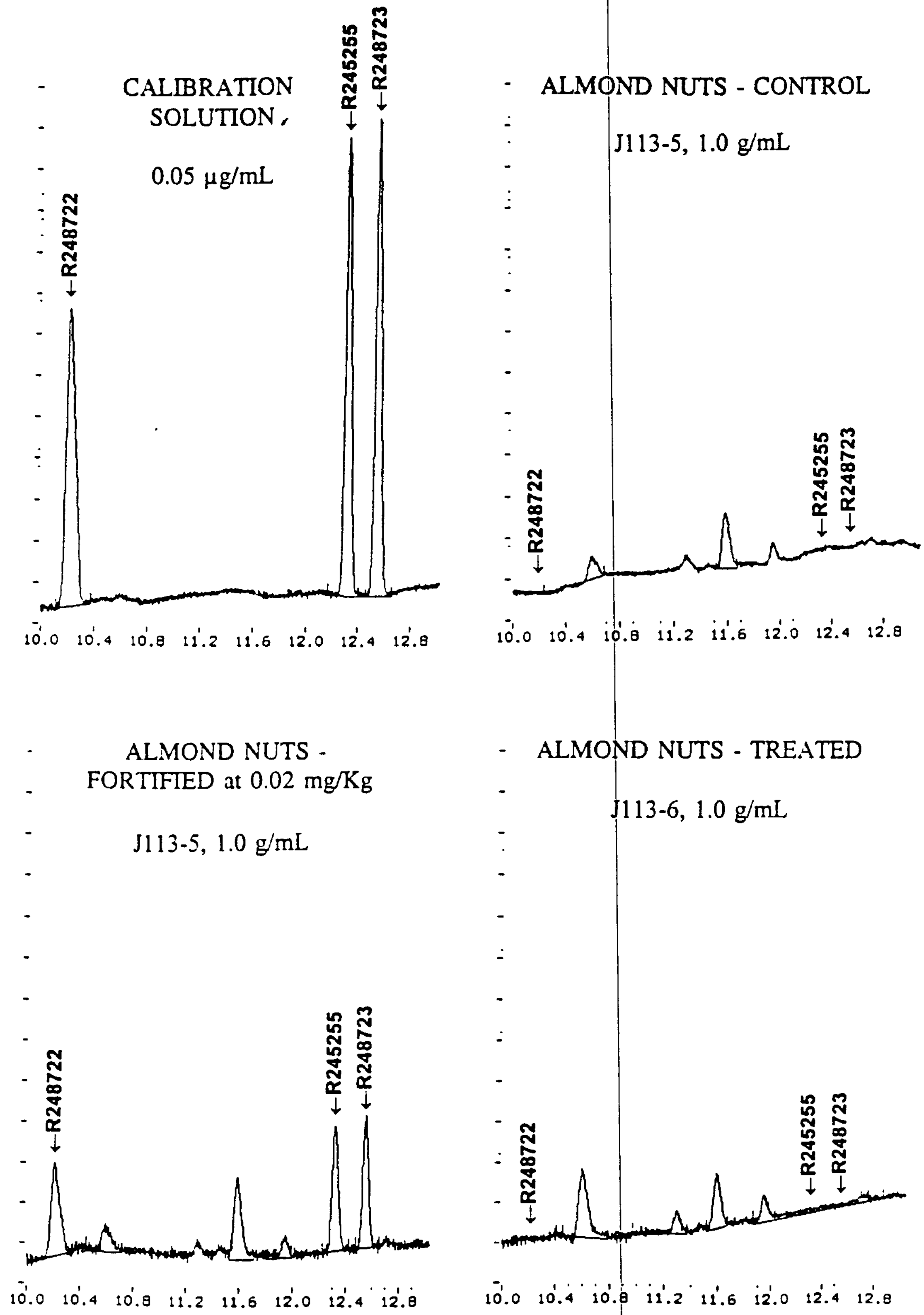
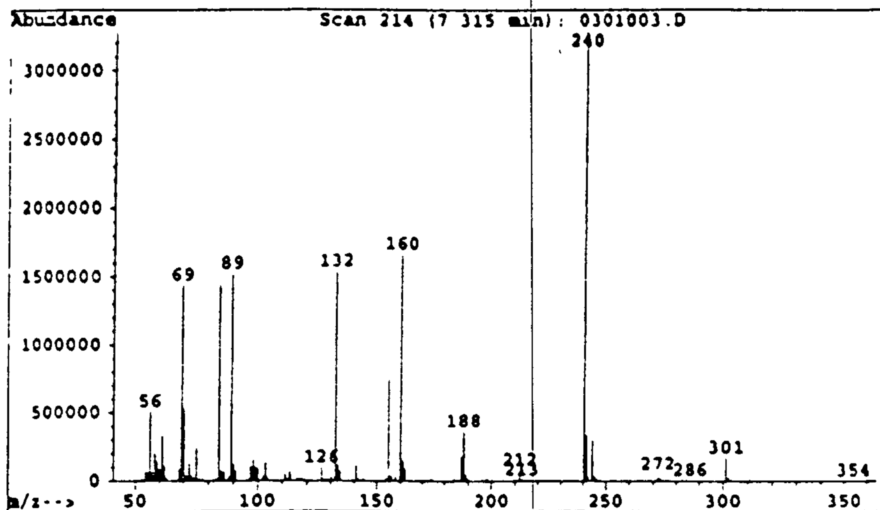
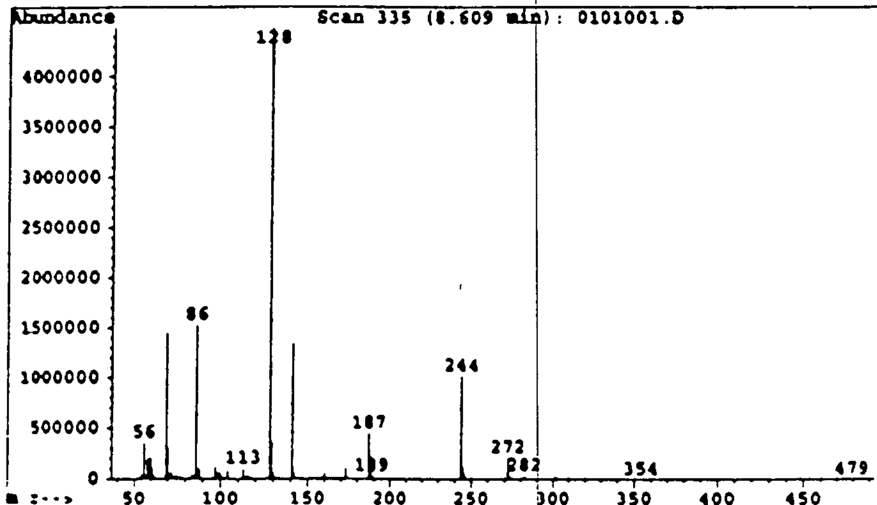


Figure 12. GC/MSD Full Scans of the Trifluoroacetate Esters of the Hydroxy EPTCs

N-2-hydroxy EPTC
(R248722)



2-hydroxyethyl EPTC
(R245255)



N-3-hydroxy EPTC
(R248723)

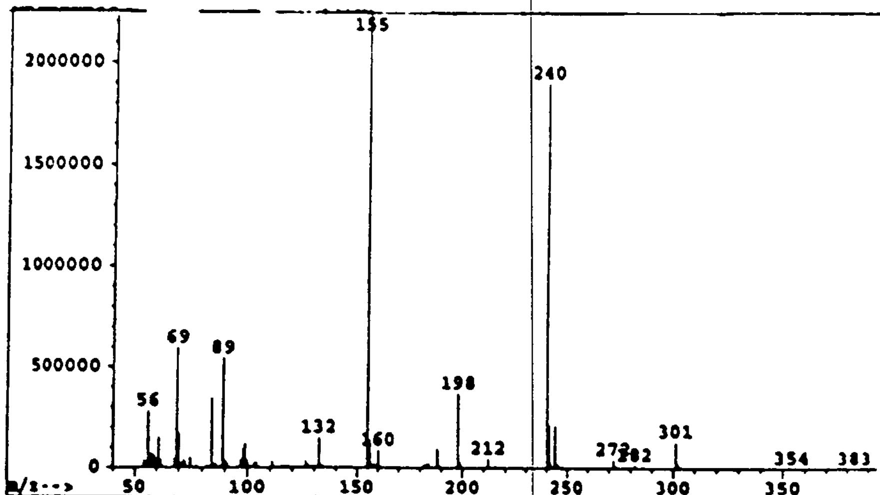


Figure 13. Confirmation GC/MSD SIM Chromatograms of 2-Hydroxy EPTC (R248722) in Green Bean Hay

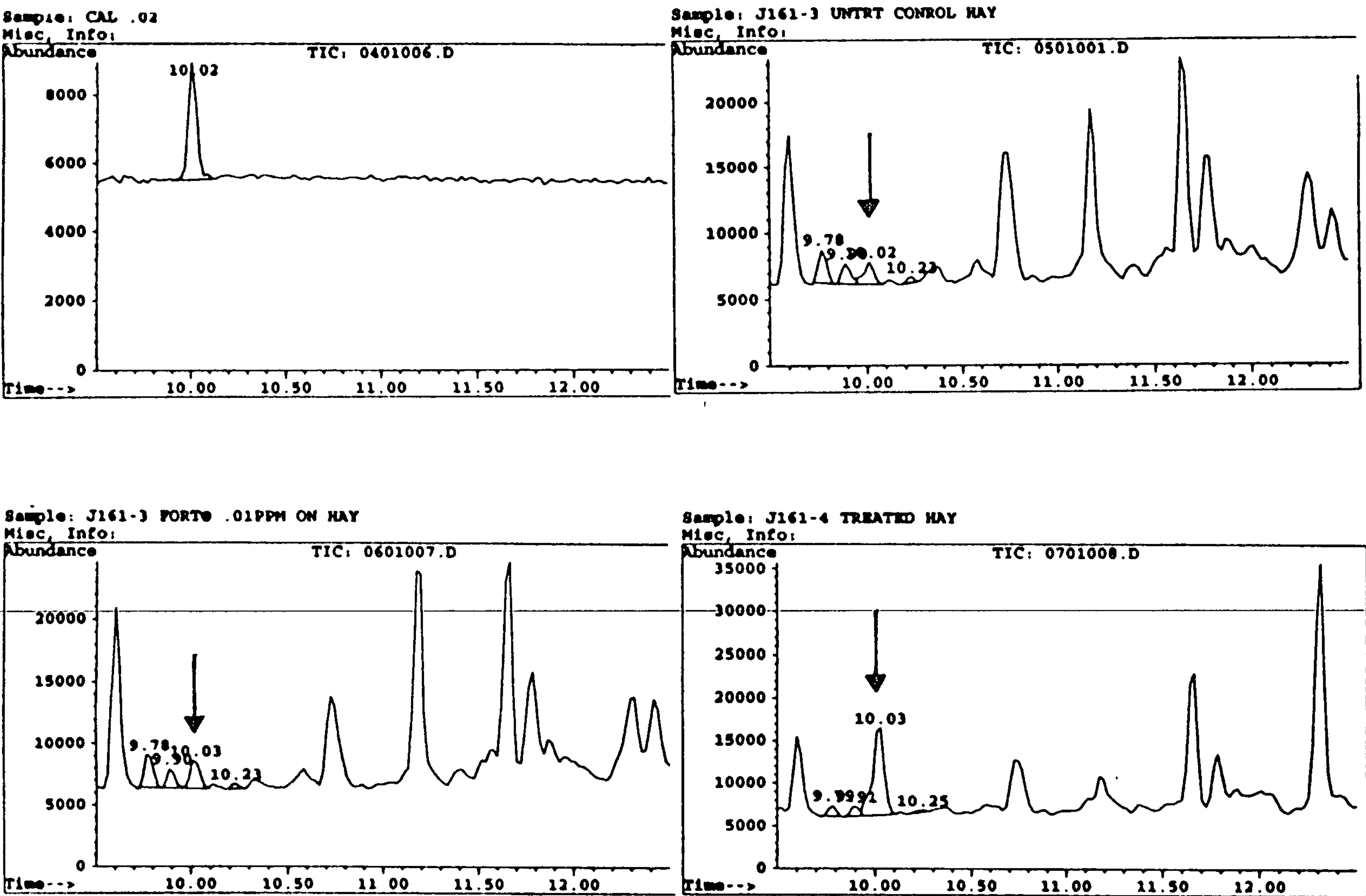
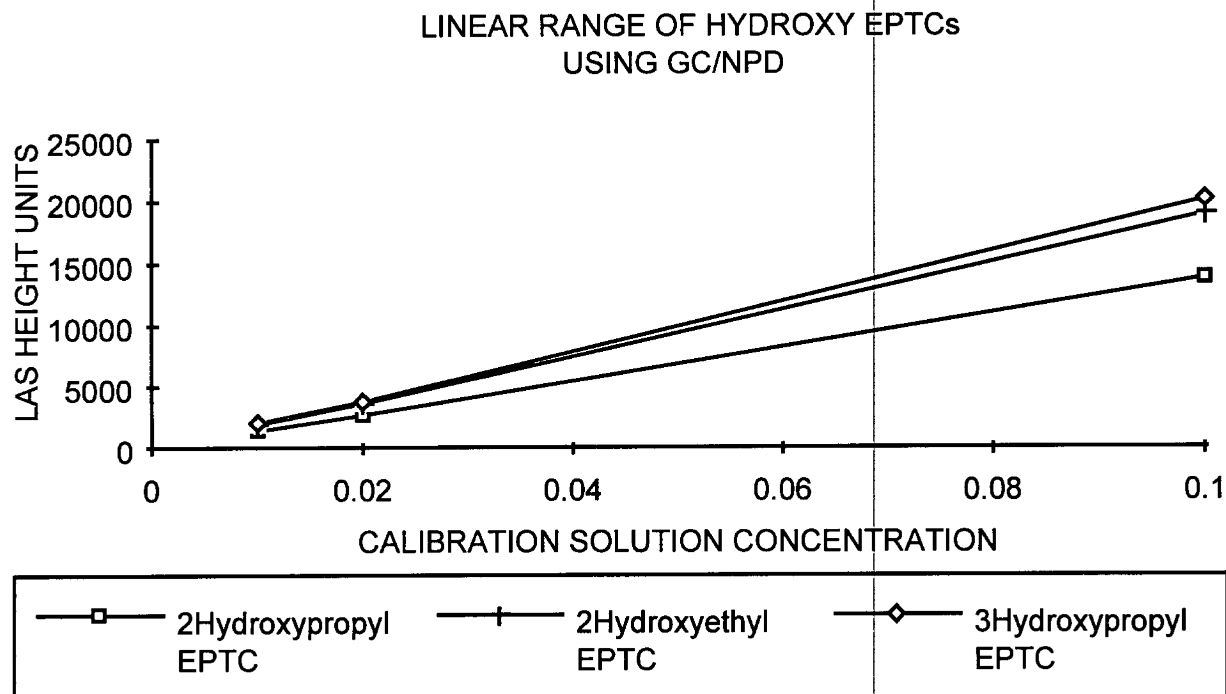


Figure 14. **Calibration Curves: Data Obtained from Calibration Solutions of the Hydroxy EPTC Trifluoroacetate Esters (concentrations correspond to 0.01, 0.02 and 0.10 µg hydroxy EPTC/mL)**



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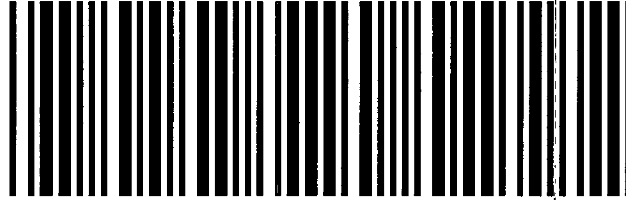
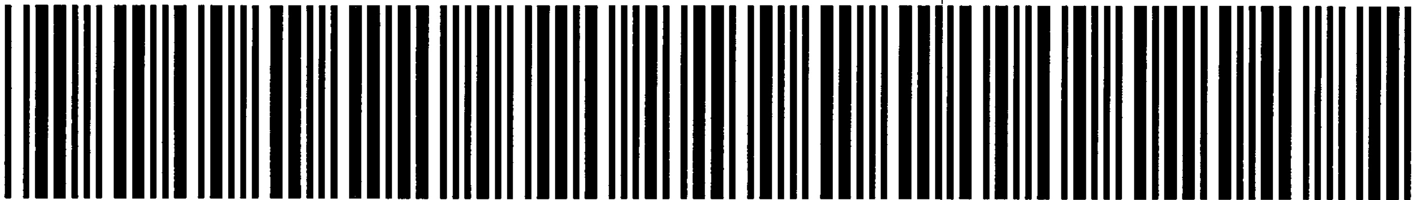


Title

ʾTC: DETERMINATION OF THE EPTC HYDROXY METABOLITES AND THEIR CONJUGATES IN CROPS BY CHROMATOGRAPHY

Author(s)

Francis P., Robbins J., Storoni H.



Method/Validation

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