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Attachment II

STUDY TITLE

Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection

DATA REQUIREMENT

171-4(c)

AUTHOR

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STUDY COMPLETED ON

August 31, 1994

PERFORMING LABORATORY

North American Environmental Chemistry Laboratory
DowElanco
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LABORATORY STUDY ID

RES94025

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: XDE-105

Title: Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection

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

Company: DowElanco

Company Agent: R. F. Bischoff

Title: Product Registration Manager

Signature: R. F. Bischoff

Date: 8/2/94

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection

Study Initiation Date: 04-Mar-1994 Study Completion Date: 31-August-1994
Experimental Start Date: 07-Mar-1994 Experiment Termination Date: 23-May-1994

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

All phases of this study were conducted in compliance with the above Good Laboratory Practice Standards with the following exceptions: (1) The method development aspects of Residue Method GRM 94.02 were not subject to GLP regulations and were not conducted under protocol. Those method development aspects not conducted according to GLP included the generation of the UV spectra (Figure 8) and the study on the potential interference from other pesticides. (2) The untreated control samples (cottonseed, meal, hulls, crude oil, refined oil, and soapstock) that were used for method validation were collected for a method development project that was not conducted under a GLP protocol.

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DowElanco Quality Assurance Unit
Good Laboratory Practice Statement Page

Study: RES94025

Compound: XDE-105

Title: Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection

Study Initiation Date: 3/4/94 **Study Completion Date:** 8/31/94

GLP Quality Assurance Inspections

Date of GLP Inspection(s)	Date Reported to the Study Director and to Management	Phases of the Study which received a GLP Inspection by the Quality Assurance Unit
3/7/94	3/7/94	Protocol
4/28/94	4/29/94	Sample Fortification, Analysis, Weighing, Extraction Procedures, Balance Calibration/Log, and HPLC Log
8/8-10/94	8/12/94	Final Report and Raw Data

QUALITY ASSURANCE STATEMENT:

The Quality Assurance Unit has reviewed the final study report and has determined the report reflects the raw data generated during the conduct of this study.

D I Haynes — August 31, 1994

D. I. Haynes
DowElanco, Quality Assurance

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Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection

ABSTRACT

A residue method is described for the determination of XDE-105 in cottonseed and five processed commodities (cottonseed meal, hulls, crude oil, refined oil, and soapstock). The method determines the total toxic residue of XDE-105, which consists of the two active ingredients (factors A and D), at a validated limit of quantitation of 0.01 µg/g. Samples are extracted with an organic solvent. The extracts are purified and then analyzed by high performance liquid chromatography with UV detection.

GRM: 94.02
EFFECTIVE:
SUPERSEDES: GRM 93.02.R1

Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography With Ultraviolet Detection

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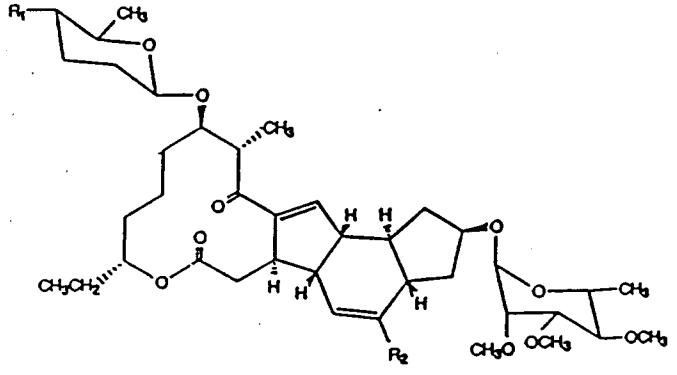
A. Scope

This method is applicable for the quantitative determination of residues of XDE-105 insecticide in cottonseed and cottonseed processed commodities (meal, hulls, crude oil, refined oil, and soapstock). The method has been validated over the concentration range of 0.01-0.1 µg/g with a limit of quantitation of 0.01 µg/g. The method determines the "total toxic residue," which consists of XDE-105 factors A[a] and D[b].



XDE-105
factor A (compound number 232105), $R_1 = N(CH_3)_2$ and $R_2 = H$
factor D (compound number 275043), $R_1 = N(CH_3)_2$ and $R_2 = CH_3$

[a] Factor A is 2-((6-deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number 131929-60-7).

[b] Factor D is 2-((6-deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1H-as-Indaceno(3,2-d)oxacyclododecin-7,15-dione (CAS Number 131929-63-0).

B. Principle

Residues of the active ingredients in XDE-105 insecticide (factors A and D) are extracted from samples using appropriate organic solvents. The extracting solvent is 60% hexane/40% acetone (for cottonseed, meal, or hulls), hexane (for cottonseed oil), or methylene chloride (for soapstock). An aliquot of the extract is purified by liquid-liquid partitioning and a silica solid phase extraction (SPE) cartridge. Factors A and D are determined simultaneously in the purified extracts by reversed-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 250 nm. Confirmation of residue identity may be accomplished by reinjecting the same final solution into the chromatograph while utilizing a different column, mobile phase, and/or UV wavelength.

C. Safety Precautions

1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE AND OTHER RELATED DATA. Safety information on non-DowElanco products may be obtained from the container label or from the supplier. Disposal of reagents and solvents must be in compliance with local, state, and federal laws and regulations.

2. Volatile and flammable organic solvents must be used in well-ventilated areas away from ignition sources.

3. Hydrochloric acid is corrosive and sodium hydroxide is caustic. Both of these reagents can cause severe burns, and proper eye protection must be worn when handling these reagents. When working with concentrated solutions, long laboratory coats and rubber gloves must be worn.

4. Erlenmeyer flasks under vacuum are susceptible to implosion. Use polypropylene flasks or glass flasks covered with electrical tape. Evaporations under vacuum must be conducted behind appropriate shields while wearing eye protection.

D. Equipment (Note N.1.)

1. Balance, analytical, Model AE-160, Mettler Instrument Corporation, Hightstown, NJ 08520.

2. Balance, toploading, Model P-1200 or BB2240, Mettler Instrument Corporation.

3. Centrifuge, Model CU-5000, International Equipment Company, Needham Heights, MA 02194.

4. Filtration apparatus for HPLC solvents, catalog number 5-8061M, Supelco, Inc., Bellefonte, PA 16823.

5. Grinder, Homoloid Model J, with a screen of approximately 3-5 mm, The Fitzpatrick Company, Chicago, IL 60126.

6. High performance liquid chromatograph, Model 1050, with a UV detector, and a recording integrator, Model 33396 Series II, Hewlett-Packard, Wilmington, DE 19808.

7. Rotary vacuum evaporator, Model 1007-4 IN, Rinco Instrument Company, Inc., Greenville, IL 62246.

8. Sep-Pak cartridge rack and reservoirs, part number 22030, Waters (Division of Millipore), Milford, MA 01757.

9. Shaker, gyratory, New Brunswick Model G-33, Fisher Scientific, Pittsburgh, PA 15219.

10. Water bath.

11. Water purifier, Milli-Q UV Plus, Millipore Corporation, Milford, MA 01730.

E. Glassware and Materials (Note N.1.)

1. Bottles, glass, 8-oz (237-mL), catalog number 03-321-1B, Fisher Scientific.

2. Cartridges, Sep-Pak silica, part number 51900, Waters.

3. Culture tubes, borosilicate glass, 13-mm x 100-mm, disposable, catalog number 14-961-27, Fisher Scientific.

4. Filter paper, 15-cm, pre-pleated, Number 588 (0.19-mm thickness), catalog number 03710, Schleicher and Schuell, Keene, NH 03431.

5. Glass wool, Pyrex® fiberglass, catalog number 3950, Fisher Scientific.

   Purify by completely submerging approximately 100 g of glass wool in 400 mL of methanol for at least five minutes. Vacuum filter to remove the methanol. Repeat the procedure with 400 mL of methylene chloride. Dry the glass wool in a hood for approximately two hours. (See Note N.2.a.)

6. High performance liquid chromatography column, ODS-AQ, catalog number MCQ-110, 5 µm, 120 Å, 100 mm x 2.0 mm i.d., YMC, Inc., Wilmington, NC 28403.

7. High performance liquid chromatography column, RP-8 Cation, Mixed Mode, 5 µm, 150 mm x 2.1 mm i.d., catalog number C-600B, Alltech/Applied Science, Deerfield, IL 60015.

8. pH test paper, pHydrion Insta-Chek 0-13, catalog number 14-850-1, Fisher Scientific.

9. Membrane filters for HPLC solvents, Nylon-66, 47-mm i.d., 0.45µm pore size, catalog number 5-8067M, Supelco, Inc.

F. Reagents and Prepared Solutions (Note N.1.) (Critical Step--See Note N.2.)

1. Reagents

a. Acetic acid, glacial, Analytical Reagent, catalog number 2504-05, Mallinkrodt Specialty Chemicals Company, Paris, KY 40361.

b. Acetone, OmniSolv®, catalog number AX0116-1, EM Science, Gibbstown, NJ 08027.

c. Acetonitrile, ChromAR HPLC grade, catalog number 2856-09, Mallinkrodt Specialty Chemicals Company.

d. Ammonium acetate, HPLC grade, catalog number A639-500, Fisher Scientific.

e. Hexane, OmniSolv®, catalog number HX0296-1, EM Science.

f. Hydrochloric acid, concentrated, Reagent A.C.S., catalog number A144-500, Fisher Scientific.

g. Methanol, ChromAR HPLC grade, catalog number 3041-09, Mallinkrodt. Specialty Chemicals Company.

h. Methylene chloride (dichloromethane), OPTIMA HPLC grade, catalog number DX0831-1, Fisher Scientific.

i. Nitrogen, refrigerated liquid, catalog number LQNI-230, Airco Gas and Gear, Indianapolis, IN 46241.

j. Sodium chloride crystals, EM Science, catalog number SX0420-5, Cherry Hills, NJ 08034.

k. Sodium hydroxide pellets, certified ACS, catalog number S-318, Fisher Scientific.

l. Sodium sulfate, anhydrous granular, catalog number S421-3, Fisher Scientific.

Purify by rinsing approximately 800 g of sodium sulfate with approximately 1000 mL of hexane. (Critical Step--See Note N.2.a.)

m. Standard:

Obtain pure active ingredients or analytical standards of XDE-105 factors A and D from Test Substance Coordinator, DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268-1053.

n. Water, ultra-pure, purified using Milli-Q UV Plus.

2. Prepared Solutions

a. Acetic acid, 0.1N

Dilute 5.75 mL of glacial acetic acid to 1000 mL with ultra-pure water.

b. 2% Ammonium acetate/acetonitrile (67:33)

Prepare by dissolving 20.0 g of ammonium acetate per liter of ultra-pure water. Mix 670 mL of 2% ammonium acetate and 330 mL of acetonitrile. Filter through a 0.45µm membrane filter.

c. 60% hexane/40% acetone (v/v):

Prepare by mixing 2400 mL of hexane and 1600 mL of acetone.

d. 0.04 N hydrochloric acid/5% sodium chloride (v/v)

Prepare by adding 3.3 mL of concentrated hydrochloric acid per liter of aqueous 5% sodium chloride solution. (See Step F.2.i. and Safety Precaution C.3.)

e. Methanol/5% sodium chloride (70:30) (v/v)

Prepare by mixing 2100 mL of methanol with 900 mL of 5% aqueous sodium chloride.

f. 50% methanol/50% acetonitrile (v/v)

Prepare by mixing 500 mL of methanol and 500 mL of acetonitrile.

g. Methanol/acetonitrile/2% ammonium acetate (1:1:1) (v/v/v)

Prepare by mixing 300 mL of each.

h. 75% methylene chloride/25% methanol (v/v)

Mix 2250 mL of methylene chloride with 750 mL of methanol.

i. 5% sodium chloride, aqueous (w/v)

Dissolve 150 g of sodium chloride in 3000 mL of ultra-pure water.

j. Sodium hydroxide, 1.0 N

Dissolve 40 g of sodium hydroxide pellets in ultra-pure water and dilute to 1000 mL.

G. Preparation of Standards (Note N.3.)

1. Preparation of XDE-105 Standard Curve Solutions

a. Stock Solution: Weigh 10.0 mg of XDE-105 factor A analytical standard or pure active ingredient (corrected for purity), and separately weigh 10.0 mg of XDE-105 factor D analytical standard or pure active ingredient (corrected for purity). Quantitatively transfer both compounds to a 200-mL volumetric flask and dissolve in 50% methanol/50% acetonitrile. Mix or shake until the solids completely dissolve. Dilute to volume to obtain a mixture containing 50.0 µg/mL each of factors A and D.

b. Transfer a 20.0-mL aliquot of the Stock Solution to a 100-mL volumetric flask, and dilute to volume with methanol/acetonitrile/2% ammonium acetate (1:1:1) to obtain a mixture containing 10.0 µg/mL each of factors A and D.

c. Standard Curve Solutions: Transfer the following aliquots of the 10.0-µg/mL solution from Step G.1.b. to 100-mL volumetric flasks, and dilute to volume with methanol/acetonitrile/2% ammonium acetate (1:1:1):

Initial Volume (mL)	Final Volume (mL)	Final Concentration (µg/mL)
0.0	100.0	0.0
0.5	100.0	0.05
1.0	100.0	0.1
2.0	100.0	0.2
5.0	100.0	0.5

2. Preparation of Fortification Standard Solutions

   a. Transfer a 10.0-mL aliquot of the 50.0 µg/mL Stock Solution to a 50-mL volumetric flask and dilute to volume with the appropriate solvent to obtain a mixture containing 10.0 µg/mL each of factors A and D. For cottonseed, meal, hulls, and oil, dilute with 60% hexane/40% acetone. For soapstock, dilute with methylene chloride.

   b. Fortification Standard Solutions: Transfer appropriate aliquots of the 10.0-µg/mL Stock Solution to 100-mL volumetric flasks and dilute to volume with 60% hexane/40% acetone (for seed, meal, or hulls), hexane (for oil), and methylene chloride (for soapstock). Examples are listed below:

Initial Volume (mL)	Final Volume (mL)	Final Concentration (µg/mL)	Equivalent Sample Concentration (µg/g)[a]
0.5	100	0.05	0.005
1.0	100	0.1	0.01
2.0	100	0.2	0.02
4.0	100	0.4	0.04
5.0	100	0.5	0.05
6.0	100	0.6	0.06
8.0	100	0.8	0.08
10.0	100	1.0	0.1

[a] The equivalent sample concentration is the concentration that would result from fortifying a 10-g sample with 1.0 mL of the appropriate Fortification Standard Solution (or from fortifying a 5-g sample with 0.5 mL).

H. High-Performance Liquid Chromatography

1. Instrumentation: Hewlett-Packard Model 1050 with a UV detector, an autosampler capable of injecting 100 µL, and a recording integrator.

2. Column: ODS-AQ, MCQ-110, S-5, 120 Å, 100 mm x 2.0 mm i.d., YMC, Inc.

3. Typical Conditions (Note N.4.):

   Column Temperature: 30 °C

Mobile Phase: 41% reservoir A/41% reservoir B/18% reservoir C (isocratic), with reservoir A = methanol; reservoir B = acetonitrile; reservoir C = 2% ammonium acetate/acetonitrile (67:33).

Flow Rate: 0.4 mL/min

Injection Volume: 100 µL

Attenuation: 2^2 or 4 (adjust to yield a peak response of approximately 30%-50% of full-scale deflection for the 0.2 µg/mL standard)

Chart Speed: 0.2 cm/min

UV Wavelength: 250 nm

Run Time: 20 minutes per sample (use longer time if late-eluting peaks occur)

4. Calibration Curve: Demonstrate that the calibration curve is linear over the range of standard curve concentrations specified, with a least squares correlation coefficient (r^2) equal to or greater than 0.997. A typical calibration curve for factor A is contained in Figure 1. Calibration curves for factor D are similar to those for factor A.

5. Typical Chromatograms: Typical chromatograms obtained under the conditions in Step H.3. are contained in Figures 2-7.

6. UV Spectra: UV spectra are contained in Figure 8.

7. Column and Typical Conditions for Confirmation of XDE-105 Residues

   Column: RP-8 Cation, Mixed Mode, 5µm, 150 mm x 2.1 mm i.d., Alltech/Applied Science, catalog number C-600B.

   Mobile Phase: 33% reservoir A/33% reservoir B/20% reservoir C/14% reservoir D (isocratic), with reservoir A = methanol; reservoir B = acetonitrile; reservoir C = 2% ammonium acetate/acetonitrile (67:33); reservoir D = 0.1 N acetic acid

   UV Wavelength: 250 nm, 235 nm or 275 nm (See section L.)

   Other Parameters: Same as in Step H.3.

8. Typical chromatograms under the conditions in Step H.7. are contained in Figure 9.

I. Determination of Recovery of XDE-105

1. Preparation of Recovery Samples

   a. Cottonseed, Meal, or Hulls

      (1) Prepare the cottonseed, meal, or hulls samples for analysis by freezing with liquid nitrogen and grinding them through a Homoloid Model J grinder with

approximately a 3-5 mm screen. Mix to produce a homogeneous sample. (Oil and soapstock samples typically do not require sample preparaton.)

(2) Weigh duplicate 10.0-g samples of an untreated control into 8-oz (237-mL) glass bottles. Using a pipet, add 1.0 mL of the 0.1 µg/mL Fortification Standard prepared in 60% hexane/40% acetone from Step G.2.b. to result in recovery samples containing 0.01 µg/g each of XDE-105 factors A and D. (If other recovery levels are desired, add 1.0 mL of the appropriate Fortification Standard from Step G.2.b.)

(3) Add 60 mL of 60% hexane/40% acetone, seal the jar with a lid, and shake the samples on a gyratory shaker at approximately 250 rpm for at least 30 minutes.

(4) Centrifuge the sample bottles at approximately 2250 rpm for at least 5 minutes. Decant a 30-mL aliquot of the supernatant solvent through a funnel containing pre-pleated filter paper into a 50-mL graduated cylinder. (If necessary, press the cottonseed samples in the bottle with a spatula while decanting to obtain a sufficient volume for the 30-mL aliquot.)

(5) Transfer each 30-mL aliquot to a 250-mL evaporating flask by pouring through a long-stemmed funnel. Prior to evaporating the sample, rinse the rotary vacuum evaporators with hexane and then methanol. (See Note N.2.b.) Evaporate the solvent using the rotary vacuum evaporators with a water bath heated to approximately 35-50 °C. (A small amount of cottonseed oil will remain in the flask.)

(6) Dissolve the residue in 50 mL of hexane, and transfer the 50-mL hexane extract to a clean 250-mL separatory funnel. Rinse the evaporating flask with 25 mL of methanol/5% sodium chloride (70:30) and transfer the solution to the separatory funnel. Rinse the flask with 25 mL of an acidic salt solution containing 0.04 N hydrochloric acid and 5% sodium chloride, and transfer the solution to the separatory funnel. (Critical Step-See Note N.5.)

(7) Gently shake the separatory funnel for approximately 20-30 seconds. (Vigorous shaking can sometimes increase the formation of emulsions.) Allow at least 15 minutes for the layers to separate. After the aqueous and organic layers have separated, drain the aqueous (lower) layer, including the slight emulsion, into a 250-mL beaker. Discard the hexane (upper) layer.

If necessary, use a stirring rod to help break emulsions. If emulsions still form that prevent an adequate separation of layers during any of the partitioning steps, drain the emulsified solutions into a clean centrifuge bottle and centrifuge at approximately 2250 rpm for approximately 5-10 minutes. (The use of a stirring rod or centrifugation is seldom required.) After centrifugation, carefully return the separated layers to the separatory funnel, drain the aqueous layer into a 250-mL beaker and discard the hexane layer.

(8) For all sample types except crude cottonseed oil, proceed to Step I.1.a.(9). For crude cottonseed oil samples, return the aqueous phase in the 250-mL beaker to the separatory funnel, add 50 mL of hexane, and conduct a second partitioning step as described in Step I.1.a.(7).

(9) Return the aqueous phase in the 250-mL beaker to the separatory funnel and add 4.0 mL of 1 N sodium hydroxide. (Critical Step--See Note N.6.) Extract XDE-105 from the aqueous phase by shaking with three 50-mL aliquots of hexane for approximately 20-30 seconds each. After each partitioning, wait until the layers have adequately separated. Drain the aqueous (lower) phase (including the slight emulsion) into a 250-mL beaker. Combine the three hexane extracts in a 500-mL evaporating flask by draining through a funnel containing a small plug of glass wool and approximately 25 mL (approximately 40 g) of hexane-washed sodium sulfate. After draining the hexane from the third partitioning step, rinse the sodium sulfate with an additional 15 mL of hexane.

(10) Prior to evaporating the samples, rinse the rotary vacuum evaporators with hexane and then methanol. Evaporate the samples to dryness using the rotary vacuum evaporators with a water bath heated to approximately 35-50 °C. Dissolve the residue in 10 mL of hexane.

(11) Purify the samples using the following silica SPE cartridge procedure (Section K):

Attach an SPE cartridge reservoir to a silica SPE cartridge and attach the cartridge to the cartridge rack. Place the hollow discard tube in position "A" and the culture tube for sample collection in position "B". Prior to adding the sample, adjust the cartridge rack to the "A" (discard) setting and condition the cartridge under vacuum using the following sequence of eluants: 10 mL of 75% methylene chloride/25% methanol, then 10 mL of acetonitrile, followed by 20 mL of hexane. (Full vacuum may be used during Steps I.1.a.(11) through I.1.a.(13), but use dropwise elution in Step I.1.a.(14).) Turn off the vacuum between solvent additions.)

(12) Add the sample from Step I.1.a.(10) to the SPE cartridge in 10 mL of hexane. After the hexane has eluted, rinse the evaporating flask with 10 mL of hexane, add the hexane to the cartridge, and elute the solvent. Repeat with an additional 10 mL of hexane. Rinse the evaporating flask with an additional 40 mL of hexane, add the rinsate to the cartridge, and elute the solvent.

(13) Rinse the evaporating flask with two separate 6-mL aliquots of acetonitrile and add both rinsates separately to the cartridge.

(14) After the second 6-mL acetonitrile rinse has eluted, adjust the cartridge rack to the "B" (collection) setting. Rinse the evaporating flask with 5 mL of 75% methylene chloride/25% methanol and add the rinsate to the cartridge. Collect the eluate in a clean culture tube using dropwise elution to prevent the sample solutions from bubbling out of the collection tube.

(15) Transfer the eluate in the test tube to a clean 125-mL evaporating flask. Rinse the culture tube with 5 mL of 75% methylene chloride/25% methanol and add the rinse to the evaporating flask. Rinse the neck of the evaporating flask with 5 mL of 75% methylene chloride/25% methanol.

(16) Prior to evaporating the samples, rinse the rotary vacuum evaporators with hexane and then methanol. Evaporate the eluate in the evaporating flask to

dryness using a rotary vacuum evaporator and a water bath heated to approximately 35-50 °C.

(17) Dissolve the residue in 1.0 mL of methanol/acetonitrile/2% ammonium acetate (1:1:1). Cap the flask. Tilt the flask to a horizontal position and slowly rotate the flask so that the solvent dissolves any residue on the side of the flask.

(18) Using a disposable Pasteur pipet, transfer the solution to an HPLC sample vial and seal the vial with a cap and crimper. Do not filter the final solution. (See Note N.2.c.)

(19) Analyze the standard and sample solutions by HPLC as described in Steps H.1.-H.3. Determine the suitability of the chromatographic system using the following performance criteria:

(a) Standard curve linearity: Determine that the correlation coefficient (r^2) equals or exceeds 0.997 for the least squares equation which describes the detector response as a function of standard curve concentration.

(b) Peak resolution: Determine that baseline resolution has been achieved for XDE-105 factors A and D.

(c) Appearance of chromatograms: Determine that the chromatograms resemble those shown in Figures 2-7 in terms of peak response, baseline, and background interference. A signal-to-noise ratio of approximately 5:1 to 10:1 should be attainable for the 0.05 µg/mL standard curve solution.

(20) If the peak response for any of the samples exceeds the range of the standard calibration curve, dilute the samples with methanol/acetonitrile/2% ammonium acetate (1:1:1) to yield a response within the range of the calibration curve.

(21) Proceed to the calculations in Step I.2.

b. Crude or Refined Cottonseed Oil

(1) Weigh duplicate 5.0-g samples of an untreated control into 8-oz (237-mL) glass bottles. Using a pipet, add 1.0 mL of the 0.05 µg/mL Fortification Standard prepared in hexane from Step G.2.b. to result in recovery samples containing 0.01 µg/g each of XDE-105 factors A and D. (If other recovery levels are desired, add 1.0 mL of the appropriate Fortification Standard from Step G.2.b.)

(2) Proceed as described in Steps I.1.a.(6) through I.1.a.(21) for cottonseed.

c. Cottonseed Soapstock

(1) Weigh duplicate 10.0-g samples of an untreated control into 8-oz (237-mL) glass bottles. Using a pipet, add 1.0 mL of the 0.1 µg/mL Fortification Standard prepared in methylene chloride from Step G.2.b. to result in recovery samples containing 0.01 µg/g each of XDE-105 factors A and D.

(If other recovery levels are desired, add 1.0 mL of the appropriate Fortification Standard from Step G.2.b.)

(2) If the soapstock sample is in a solid form, add 100 mL of methylene chloride and shake the samples at approximately 250 rpm for approximately 30 minutes. Filter a 50-mL aliquot of the methylene chloride through pre-pleated filter paper and proceed to Step I.1.c. (5).

If the soapstock sample is in a liquid form, add 100 mL of methylene chloride and shake the samples on a gyratory shaker table at approximately 250 rpm for approximately 5 minutes. (Shaking the samples at a higher speed or for a longer time often increases the formation of emulsions that will not adequately separate during centrifugation.)

(3) Centrifuge the samples in the glass bottles at approximately 2250 rpm for approximately 5-10 minutes.

(4) Carefully decant more than half (i.e., more than 50 mL) of the methylene chloride through a funnel into a 250-mL separatory funnel. Decant only a minimal amount of soapstock into the separatory funnel. (Due to its viscosity, the soapstock will decant more slowly than the methylene chloride.) Open the stopcock on the separatory funnel and drain approximately 51-55 mL of the methylene chloride (lower phase) into a graduated cylinder. Try not to drain the soapstock into the graduated cylinder. Discard the soapstock and any methylene chloride that remain in the separatory funnel. Using a disposable Pasteur pipet, remove any soapstock particles from the graduated cylinder and reduce the volume of methylene chloride to 50 mL in the graduated cylinder.

(5) Transfer the 50-mL aliquot of methylene chloride through a clean, long-stemmed funnel into a 250-mL evaporating flask. Prior to evaporating the samples, rinse the rotary vacuum evaporators with hexane and then methanol. (See Note N.2.b.) Evaporate the methylene chloride using a rotary vacuum evaporator and a water bath heated to approximately 35-50 °C, and dissolve the residue in 50 mL of hexane.

(6) Proceed as described in Steps I.1.a.(6) through I.1.a.(21) for cottonseed.

2. Calculation of Net Percent Recovery of XDE-105

a. Inject the calibration standards described in Section G.1.c. into the HPLC and determine the HPLC peak response for XDE-105 factors A and D.

b. Prepare separate standard curves for factors A and D by plotting the concentrations on the abscissa (x-axis) and the resulting peak responses on the ordinate (y-axis) as shown in Figure 1. Using regression analysis, determine the equation for the curve with respect to the abscissa.

The general equation for calculating the least squares line for the standard curve is as follows:

$$PR = mC + b$$

where: PR = peak response
m = slope of the line
C = concentration
b = y-axis intercept

Rearranging the above equation, the concentration (C) of the analyte in the final solution can be calculated from the standard curve as:

$$C = \frac{(PR - b)}{m}$$

For example, the following equation results from a least squares regression analysis with the data in Figure 2:

$$C = \frac{[PR - (-0.01)]}{20.44}$$

c. Determine the net concentration in each recovery sample by first subtracting the response in the corresponding control sample from that of the recovery sample. Substitute the net peak response obtained into the above equation and solve for concentration.

For example, using the data for factor A in the 0.010 µg/g recovery sample in Figure 2:

The net peak response = 1.0 - 0.0 = 1.0, and

$$C = \frac{[1.0 - (-0.01)]}{20.44} = 0.0494 \text{ µg/mL}$$

d. Determine the residue concentration (µg/g) of the analyte in the fortified recovery sample as follows:

$$\text{µg/g} = \frac{C \times AF \times V}{W}$$

where: C = concentration (µg/mL) as determined above
AF = aliquot factor (extraction volume divided by aliquot volume)
V = final volume (normally 1.0 mL unless further diluted)
W = weight of sample extracted (normally 5.0 g or 10.0 g)

For the example above, the µg/g concentration of factor A is calculated as:

$$\frac{0.0494 \text{ µg/mL} \times 2.0 \times 1.0 \text{ mL}}{10 \text{ g}} = 0.0099 \text{ µg/g}$$

e. Determine the net percent recovery (R) by dividing the net concentration (µg/g) by the theoretical concentration added:

$$R = \frac{\mu g/g \text{ Found}}{\mu g/g \text{ Added}} \times 100\%$$

For the above example,

$$\frac{0.0099}{0.010} \times 100\% = 99\%$$

J. Determination of XDE-105 in Cottonseed and Processed Commodities

1. Prepare treated samples, a system (reagent) blank, fortified recovery samples; and an untreated control (if available) as described in Section I.1.

2. Prepare separate standard calibration curves for XDE-105 factors A and D and determine the percentage recovery of both factors as described in Section I.2.

3. Determine the concentrations (µg/mL) of factors A and D from their respective standard calibration curves, and calculate the uncorrected residue result as follows:

$$\text{Uncorrected Result } (\mu g/g) = \frac{\mu g/mL \text{ (from std. curve)} \times AF \times V}{W}$$

Where: AF = aliquot factor (extraction volume divided by aliquot volume)
V = final volume (normally 1.0 mL unless further diluted)
W = weight of extracted sample (normally 5.0 g or 10.0 g)

For example, using the same data from the same sample in section I.2., the uncorrected residue (µg/g) is calculated as:

$$\mu g/g \text{ (uncorrected)} = \frac{0.0494 \ \mu g/mL \times 2.0 \times 1.0 \ mL}{10 \ g} = 0.0099$$

4. If desired, correct the residue result for the recovery obtained as follows:

$$\text{Corrected residue } (\mu g/g) = \text{uncorrected residue } (\mu g/g) \times \frac{100\%}{R_a}$$

where: R_a = average % recovery from fortified samples (Section I.2.)

For the sample above, the residue corrected for recovery is calculated as:

$$\text{Corrected residue } (\mu g/g) = 0.0099 \ \mu g/mL \times \frac{100\%}{99\%} = 0.01 \ \mu g/g$$

K. Standardization of Silica Sep-Pak Elution Profile

Variation in the silica SPE cartridges can influence the elution profile of XDE-105. It is necessary to obtain an elution profile for each lot of SPE cartridges used to ensure optimum recovery and clean-up efficiency. The following procedures may be used:

1. Prior to using a new lot of silica SPE cartridges, determine the elution profile for XDE-105 factors A and D. Prepare a silica SPE cartridge as described in Step I.1.a.(11).

2. Transfer 0.5 mL of the 1.0 µg/mL standard solution (Step G.2.b.) to a 125-mL evaporating flask and evaporate to dryness using a rotary vacuum evaporator and a water bath heated to approximately 35-50 °C. Dissolve the sample in 10 mL of hexane.

3. Add the sample to the SPE cartridge. Rinse the evaporating flask with two separate 10-mL portions of hexane and add both rinsates separately to the SPE cartridge.

   Rinse the evaporating flask with an additional 40-mL of hexane, add the rinsate to the cartridge, and discard all of the eluate collected thus far.

4. Rinse the evaporating flask with two separate 6-mL portions of acetonitrile, add them separately to the SPE cartridge, and collect both of the eluates in separate culture tubes.

5. Add at least eight 1-mL volumes of 75% methylene chloride/25% methanol to the SPE cartridge and collect each 1 mL of eluate in separate culture tubes using dropwise elution.

6. Proceed as described in Steps I.1.a.(15) through I.1.a.(21).

7. Calculate separate percentage recoveries of XDE-105 factors A and D as described in Step I.2.

8. If the elution pattern for XDE-105 differs from that described in Steps I.1.a.(13) to I.1.a.(14), adjust the volume of acetonitrile forerun that is to be discarded (Step I.1.a.(13)) or the volume of 75% methylene chloride/25% methanol to collect (Step I.1.a.(14)).

9. A typical elution profile is contained in Figure 10.

L. Confirmation of XDE-105 Residues

1. If necessary, confirm the identity of XDE-105 factors A and/or D by analyzing the same final solution (from Step I.1.a.(18)) under different chromatographic conditions. Use the conditions in Step H.7. for the confirmation of XDE-105 residues.

2. For maximum sensitivity, maintain the UV wavelength at 250 nm and utilize the alternative column and mobile phase conditions listed in Step H.7. Compare the resulting concentrations with those obtained using the conditions in Step H.3. to determine if they are similar.

3. To utilize different wavelengths, inject the standard and sample solutions with the UV detector set at 235 nm. Repeat at 275 nm. Compare the resulting concentrations with those obtained at 250 nm (Step H.3.) to determine if they are similar. The sensitivity will be decreased at 235 mm or 275 nm (Figure 8).

4. If the retention times of the sample peaks do not match those of the standard, consider the residue to be due to compounds other than XDE-105. If the retention times

match, but significantly different concentrations are obtained using the primary and confirmatory chromatographic conditions, consider the detected residue to be due at least in part to interfering compounds and not to XDE-105.

M. Results and Discussion

1. Method Validation

a. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the residue method; the results are summarized for the various commodities in Tables I-VI. For the six commodities combined, average recoveries ranged from 90-100% for Factor A with one standard deviation ranging from 4-14%. For Factor D, average recoveries ranged from 85-102% with one standard deviation ranging from 4-11%.

b. Standard Curve Linearity

The average correlation coefficient (r^2) for the least squares equations describing the detector response as a function of standard curve concentration was 0.9999 for both XDE-105 factors A and D.

c. Extraction Efficiency

In a previous study (1), duplicate control cottonseed samples fortified with 0.2 µg/g each of ^{14}C XDE-105 factors A and D were aged in a freezer for more than three months and then analyzed. During the current method validation, control samples were fortified with ^{14}C XDE-105 factors A and D at 1.0 µg/g (soapstock) or 2.0 µg/g (crude cottonseed oil). These samples were aged in a freezer for nearly two months before analysis. The results obtained by the residue procedure with HPLC-UV detection compared favorably with those obtained by radiochemical analysis (Table VII).

2. Calculated Limits of Detection and Quantitation

The limits of detection and quantitation were calculated using the standard deviation from the 0.01 µg/g recovery results. Following a published technique (2), the limit of detection (LOD) was calculated as 3X the standard deviation, and the limit of quantitation (LOQ) was calculated as 10X the standard deviation. For factor A, the calculated LOD ranged from 0.001-0.002 µg/g, and the calculated LOQ ranged from 0.004-0.008 µg/g for the six different commodities. For factor D, the calculated LOD ranged from 0.002-0.004 µg/g, and the calculated LOQ ranged from 0.005-0.012 µg/g (Table VIII). However, the validated LOQ was 0.010 µg/g for both analytes in all six commodities, and numerical values should be reported as <0.01 µg/g for residues found at levels below the validated limit of quantitation.

3. Assay Time

A set of 10-15 samples can be prepared for analysis during a typical workday, with overnight injection of the samples using an autosampler. If desired, completion of the sample analysis may be delayed after Step I.1.a.(9) or I.1.a.(10) if the sample solutions are protected from light.

4. Interference from Other Pesticides

Several pesticides commonly used on cotton have been tested for potential interference with XDE-105. Using 10 µg/mL solutions, the following 38 pesticides were tested for interference by direct injection into the HPLC. Only avermectin, dicofol, propargite, thiodicarb, and tralomethrin produced interference peaks. However, none of these five pesticides interfered when carried through the entire analytical procedure and analyzed using the conditions described in Step H.3. A representative chromatogram for interference by direct injection is contained in Figure 11, along with a chromatogram demonstrating the removal of the interference with the cleanup procedures in the method. (The interference study was conducted previously using a 4.6-mm i.d. column instead of the 2.0-mm column that is presently recommended. The use of a minibore column is unlikely to affect the potential interference because most of the compounds listed below are unretained and elute with the solvent front.)

Acephate
Aldicarb
Avermectin
Azinphos-methyl
Bifenthrin
Butifos
Chlorpyrifos
Cyanazine
Cyhalothrin
Cypermethrin
Dicofol
Dimethoate
Endosulfan
Ethephon
Fenvalerate
Fluazifop-butyl
Flumetsulam
Fluometuron
Malathion
Mepiquat chloride
Metalyxil
Methomyl
Methyl parathion
MSMA
Norflurazon
Oxamyl
PCNB
Pendamethalin
Permethrin
Profenofos
Propargite
Prometryn
Sethoxydim
Sulprofos
Terbufos
Thiodicarb
Tralomethrin
Trifluralin

N. Notes

1. Equipment, glassware, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are therefore not listed here.

2. Because it is necessary to use a nonselective UV wavelength (250 nm) to obtain adequate sensitivity, certain precautions must be taken to avoid interferences that can result from the reagents or equipment. When following the procedures as described, interferences in the chromatograms of system (reagent) blanks have not occurred. However, if interferences occur, individual reagents and chemicals must be tested for purity by treating them as they are used in the procedure and then analyzing the resulting solutions by HPLC to isolate the source(s) of the interferences. Those reagents or equipment found to be a source of interferences must be suitably purified or replaced with different sources of materials that do not produce interferences.

Certain equipment and reagents have been previously determined to cause interferences, and the following recommendations should be followed:

a. Sodium sulfate and glass wool: To prevent interferences, prepare the glass wool and sodium sulfate as specified in Steps E.5. and F.1.1., respectively.

   Essentially quantitative recoveries have been obtained with sodium sulfate supplied by Fisher Scientific (catalog number S421-3). Sodium sulfate supplied by Merck (catalog number SX0760-3) has produced recoveries as low as 27% due to adsorption of the analytes.

b. Rotary vacuum evaporators: Thoroughly rinse rotary vacuum evaporators as described to prevent contamination of samples.

c. 0.45µm filters: Do not remove particulates by filtering the final solutions through 0.45µm filters prior to injection into the HPLC unless it has been demonstrated that the filters do not produce interferences under the HPLC conditions specified. (Failure to clarify the final solutions has not resulted in a noticeable chromatographic problem after several months of daily operation.)

d. Glassware: Rinse dirty glassware with deionized water before machine washing. After washing, rinse the glassware with acetone.

3. If desired, prepare standard solutions at other concentrations by making appropriate dilutions.

4. If necessary, modify the typical HPLC conditions to obtain optimum performance.

5. The pH of the aqueous phase in the separatory funnel in Steps I.1.a.(6)-I.1.a.(8) must be acidic to prevent loss of the analytes during the partitioning with hexane.

6. The pH of the aqueous phase in Step I.1.a.(9) must be basic to ensure partitioning of the analytes into hexane. The pH should be approximately 10-12 as indicated by pH paper.

O. References

1. West, S. D.; Turner, L.G., "Determination of XDE-105 Insecticide in Cottonseed by High Performance Liquid Chromatography with Ultraviolet Detection", GRM 93.02.R1, 1994, unpublished method of DowElanco.

2. Keith, L. H., Crummett, W.; Deegan, J.; Libby, R. A.; Taylor, J. K.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.* 1983, 55, pp. 2210-2218.

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Table I. Recovery of XDE-105 Factors A and D from Cottonseed

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
12815501	14-Mar-1994	0.0	ND[b]	ND	ND	ND
12815501	28-Mar-1994	0.0	ND	ND	ND	ND
12815501	25-Apr-1994	0.0	ND	ND	ND	ND
12815501	25-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
12815501	25-Apr-1994	0.003	<0.010	<0.010	NA	NA
12815501	14-Mar-1994	0.010	0.0119	0.0105	119	105
12815501	14-Mar-1994	0.010	0.0099	0.0082	99	82
12815501	14-Mar-1994	0.010	0.0119	0.0117	119	117
12815501	14-Mar-1994	0.010	0.0109	0.0094	109	94
12815501	14-Mar-1994	0.010	0.0109	0.0105	109	105
12815501	14-Mar-1994	0.010	0.0099	0.0082	99	82
12815501	14-Mar-1994	0.010	0.0109	0.0105	109	105
12815501	14-Mar-1994	0.010	0.0109	0.0105	109	105
12815501	28-Mar-1994	0.020	0.0147	0.0166	74	83
12815501	28-Mar-1994	0.020	0.0156	0.0155	78	78
12815501	28-Mar-1994	0.040	0.0314	0.0327	79	82
12815501	28-Mar-1994	0.040	0.0323	0.0327	81	82
12815501	28-Mar-1994	0.060	0.0611	0.0606	102	101
12815501	28-Mar-1994	0.060	0.0620	0.0617	103	103
12815501	28-Mar-1994	0.080	0.0741	0.0735	93	92
12815501	28-Mar-1994	0.080	0.0741	0.0746	93	93
12815501	28-Mar-1994	0.100	0.1020	0.1020	102	102
12815501	28-Mar-1994	0.100	0.1040	0.1040	104	104
				$\bar{x}$ =	99	95
				s =	14	11

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

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Table II. Recovery of XDE-105 Factors A and D from Cottonseed Meal

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
11382401	21-Mar-1994	0.0	ND[b]	ND	ND	ND
11382401	25-Apr-1994	0.0	ND	ND	ND	ND
11382401	25-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
11382401	25-Apr-1994	0.003	<0.010	<0.010	NA	NA
11382401	21-Mar-1994	0.010	0.0081	0.0085	81	85
11382401	21-Mar-1994	0.010	0.0091	0.0073	91	73
11382401	21-Mar-1994	0.010	0.0091	0.0096	91	96
11382401	21-Mar-1994	0.010	0.0091	0.0085	91	85
11382401	21-Mar-1994	0.010	0.0100	0.0096	100	96
11382401	21-Mar-1994	0.010	0.0091	0.0073	91	73
11382401	21-Mar-1994	0.010	0.0091	0.0085	91	85
11382401	21-Mar-1994	0.010	0.0091	0.0085	91	85
11382401	21-Mar-1994	0.100	0.0953	0.0935	95	94
11382401	21-Mar-1994	0.100	0.0795	0.0796	80	80
				$\bar{x}$ =	90	85
				s =	6	8

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

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Table III. Recovery of XDE-105 Factors A and D from Cottonseed Hulls

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
11381601	25-Mar-1994	0.0	ND[b]	ND	ND	ND
11381601	25-Apr-1994	0.0	ND	ND	ND	ND
11381601	25-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
11381601	25-Apr-1994	0.003	<0.010	<0.010	NA	NA
11381601	25-Mar-1994	0.010	0.0091	0.0099	91	99
11381601	25-Mar-1994	0.010	0.0091	0.0099	91	99
11381601	25-Mar-1994	0.010	0.0100	0.0110	100	110
11381601	25-Mar-1994	0.010	0.0100	0.0087	100	87
11381601	25-Mar-1994	0.010	0.0110	0.0110	110	110
11381601	25-Mar-1994	0.010	0.0100	0.0099	100	99
11381601	25-Mar-1994	0.010	0.0110	0.0110	110	110
11381601	25-Mar-1994	0.010	0.0091	0.0087	91	87
11381601	25-Mar-1994	0.100	0.0891	0.0874	89	87
11381601	25-Mar-1994	0.100	0.1160	0.1080	116	108
				$\bar{x}$ =	100	100
				s =	10	10

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

Table IV. Recovery of XDE-105 Factors A and D from Crude Cottonseed Oil

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
11378601	16-Mar-1994	0.0	ND[b]	ND	ND	ND
11378601	31-Mar-1994	0.0	ND	ND	ND	ND
11378601	22-Apr-1994	0.0	ND	ND	ND	ND
11378601	22-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
11378601	22-Apr-1994	0.003	<0.010	<0.010	NA	NA
11378601	16-Mar-1994	0.010	0.0086	0.0085	86	85
11378601	16-Mar-1994	0.010	0.0086	0.0085	86	85
11378601	16-Mar-1994	0.010	0.0096	0.0096	96	96
11378601	16-Mar-1994	0.010	0.0086	0.0085	86	85
11378601	16-Mar-1994	0.010	0.0096	0.0096	96	96
11378601	16-Mar-1994	0.010	0.0096	0.0096	96	96
11378601	16-Mar-1994	0.010	0.0096	0.0096	96	96
11378601	16-Mar-1994	0.010	0.0096	0.0096	96	96
11378601	31-Mar-1994	0.020	0.0184	0.0172	92	86
11378601	31-Mar-1994	0.020	0.0184	0.0183	92	92
11378601	31-Mar-1994	0.040	0.0407	0.0387	102	97
11378601	31-Mar-1994	0.040	0.0453	0.0387	113	97
11378601	31-Mar-1994	0.060	0.0546	0.0527	91	88
11378601	31-Mar-1994	0.060	0.0602	0.0581	100	97
11378601	31-Mar-1994	0.080	0.0824	0.0796	103	99
11378601	31-Mar-1994	0.080	0.0824	0.0796	103	99
11378601	31-Mar-1994	0.100	0.0945	0.0925	94	92
11378601	31-Mar-1994	0.100	0.0954	0.0925	95	92
				$\bar{x}$ =	96	93
				s =	7	5

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

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Table V. Recovery of XDE-105 Factors A and D from Refined Cottonseed Oil

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
11380801	23-Mar-1994	0.0	ND[b]	ND	ND	ND
11380801	22-Apr-1994	0.0	ND	ND	ND	ND
11380801	28-Apr-1994	0.0	ND	ND	ND	ND
11380801	22-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
11380801	22-Apr-1994	0.003	<0.010	<0.010	NA	NA
11380801	23-Mar-1994	0.010	0.0098	0.0092	98	92
11380801	23-Mar-1994	0.010	0.0098	0.0092	98	92
11380801	23-Mar-1994	0.010	0.0088	0.0080	88	80
11380801	23-Mar-1994	0.010	0.0098	0.0092	98	92
11380801	23-Mar-1994	0.010	0.0098	0.0080	98	80
11380801	23-Mar-1994	0.010	0.0098	0.0092	98	92
11380801	23-Mar-1994	0.010	0.0098	0.0092	98	92
11380801	23-Mar-1994	0.010	0.0098	0.0103	98	103
11380801	28-Apr-1994	0.100	0.0701	0.0682	70	68
11380801	28-Apr-1994	0.100	0.0771	0.0732	77	73
				$\bar{x}$ =	92	86
				s =	10	11

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

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Table VI. Recovery of XDE-105 Factors A and D from Cottonseed Soapstock

Control Sample No.	Date of Analysis	XDE-105 Added, µg/g[a]	XDE-105 Found, µg/g		Percent Recovery	
			Factor A	Factor D	Factor A	Factor D
11379401	29-Mar-1994	0.0	ND[b]	ND	ND	ND
11379401	29-Mar-1994	0.0	ND	ND	ND	ND
11379401	22-Apr-1994	0.0	ND	ND	ND	ND
11379401	22-Apr-1994	0.003	<0.010	<0.010	NA[c]	NA
11379401	22-Apr-1994	0.003	<0.010	<0.010	NA	NA
11379401	29-Mar-1994	0.010	0.0093	0.0110	93	110
11379401	29-Mar-1994	0.010	0.0093	0.0100	93	100
11379401	29-Mar-1994	0.010	0.0102	0.0100	102	100
11379401	29-Mar-1994	0.010	0.0102	0.0100	102	100
11379401	29-Mar-1994	0.010	0.0102	0.0100	102	100
11379401	29-Mar-1994	0.010	0.0093	0.0100	93	100
11379401	29-Mar-1994	0.010	0.0102	0.0110	102	110
11379401	29-Mar-1994	0.010	0.0093	0.0100	93	100
11379401	29-Mar-1994	0.020	0.0203	0.0197	101	98
11379401	29-Mar-1994	0.020	0.0203	0.0218	101	109
11379401	29-Mar-1994	0.040	0.0397	0.0392	99	98
11379401	29-Mar-1994	0.040	0.0397	0.0392	99	98
11379401	29-Mar-1994	0.060	0.0602	0.0598	100	100
11379401	29-Mar-1994	0.060	0.0592	0.0598	99	100
11379401	29-Mar-1994	0.080	0.0787	0.0793	98	99
11379401	29-Mar-1994	0.080	0.0787	0.0793	98	99
11379401	29-Mar-1994	0.100	0.1040	0.1030	104	103
11379401	29-Mar-1994	0.100	0.1030	0.1030	103	103
				$\bar{x}$ =	99	102
				s =	4	4

[a]The indicated amounts were added for both XDE-105 factors A and D.
[b]None detected at a detection limit of 0.003 µg/g.
[c]Not applicable. (The residue was below the 0.010 µg/g limit of quantitation.)

Table VII. Comparison of HPLC-UV Results and Radiochemical Results for Aged Residues of ^{14}C-XDE-105 Factors A and D in Representative Commodities

Commodity	XDE-105 Added, µg/g	Factor A Found, µg/g		Factor D Found, µg/g	
		Radiochemical	HPLC-UV	Radiochemical	HPLC-UV
Cottonseed[a,b]	0.2	0.17	0.16	0.16	0.16
	0.2	0.19	0.18	0.15	0.15
Crude Oil[c]	2.0	2.10	2.00	2.03	2.04
Soapstock[c]	1.0	0.94	0.95	0.93	0.94

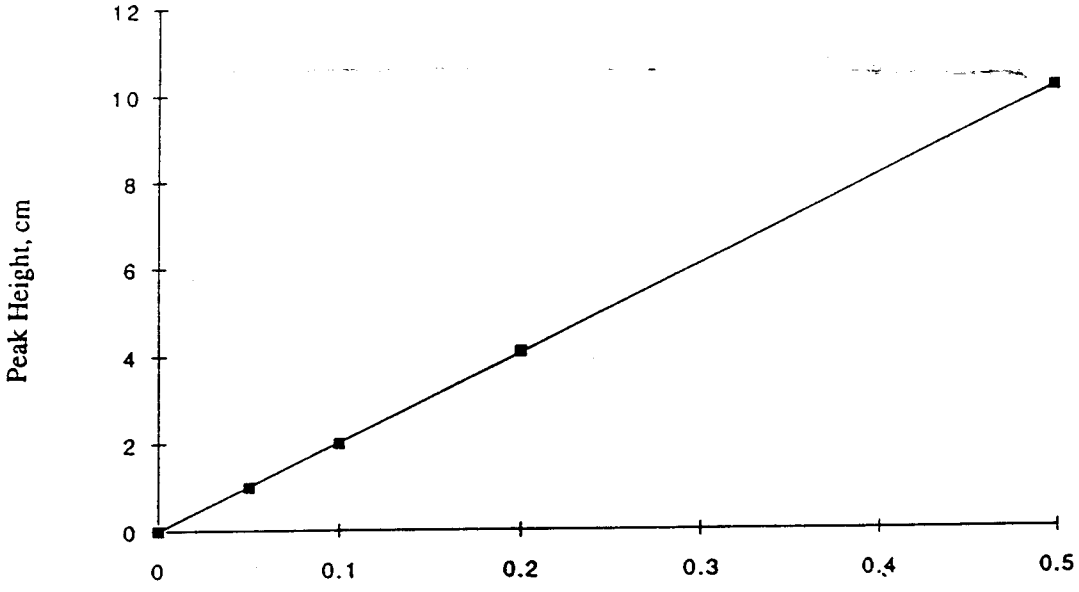
[a]The cottonseed was analyzed during a previous validation (1).
[b]The cottonseed sample results are not corrected for a procedural recovery because a radiolabeled procedural recovery was not conducted.
[c]The crude cottonseed oil and soapstock samples are corrected for procedural recoveries.

Effective Date: GRM 94.02

Table VIII. Calculated Limits of Detection and Quantitation (µg/g) for XDE-105 Factors A and D

	µg/g					
	Cottonseed	Meal	Hulls	Crude Oil	Refined Oil	Soapstock
Factor A						
$\bar{x}$[a]	0.0109	0.0091	0.0099	0.0092	0.0097	0.0098
s[b]	0.0008	0.0005	0.0008	0.0005	0.0004	0.0005
LOD ($3s$)[c]	0.002	0.002	0.002	0.002	0.001	0.002
LOQ ($10s$)[d]	0.008	0.005	0.008	0.005	0.004	0.005
Factor D						
$\bar{x}$	0.0099	0.0085	0.0100	0.0092	0.0090	0.0103
s	0.0012	0.0009	0.0010	0.0006	0.0007	0.0005
LOD ($3s$)	0.004	0.003	0.003	0.002	0.002	0.002
LOQ ($10s$)	0.012	0.009	0.010	0.006	0.007	0.005

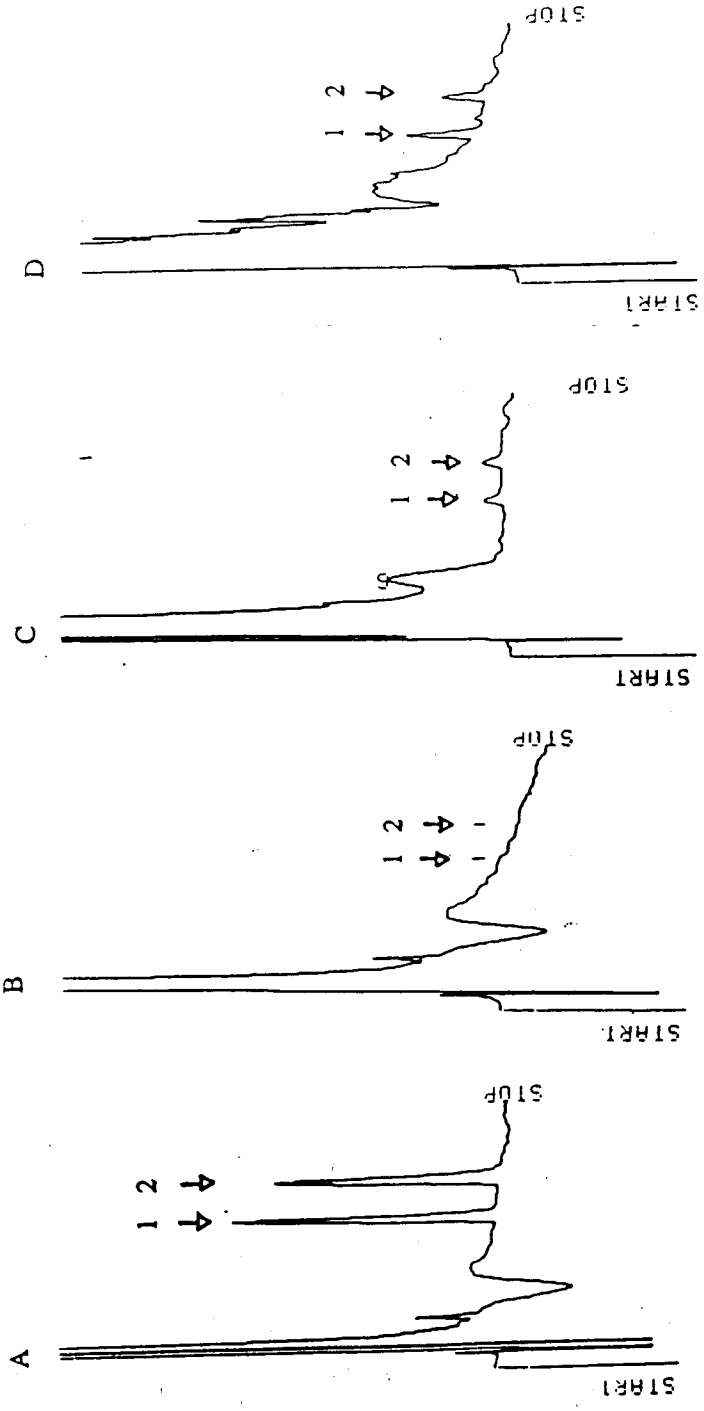
[a]Mean value of the µg/g results for the 0.010 µg/g recoveries listed in Tables I-VI.
[b]Standard deviation of the µg/g results for the 0.010 µg/g recoveries in Tables I-VI.
[c]Calculated limit of detection, calculated as $3s$.
[d]Calculated limit of quantitation, calculated as $10s$.



Concentration (µg/mL)	Peak Height (cm)
0.0	0.0
0.05	1.0
0.1	2.0
0.2	4.1
0.5	10.2

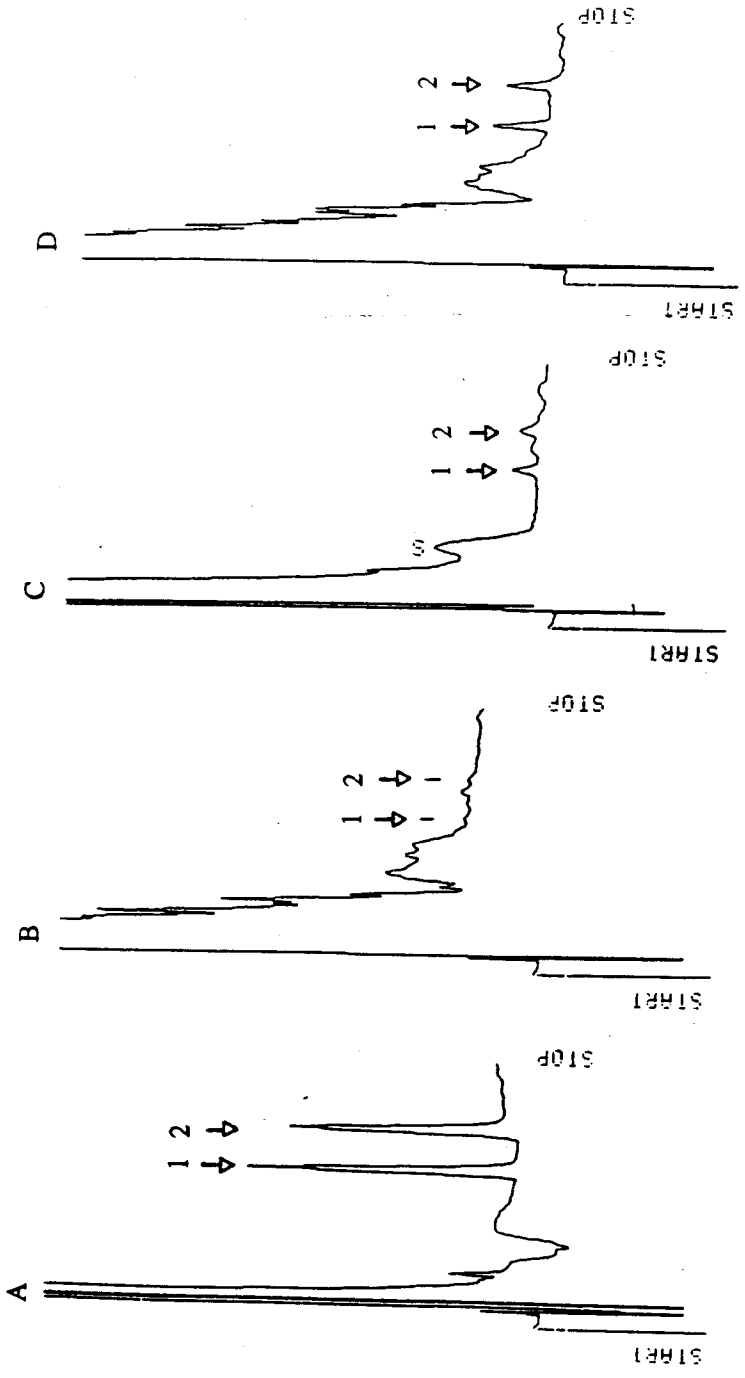
Slope (m) = 20.44
Y-axis intercept (b) = -0.01
Correlation coefficient (r^2) = 0.99999

Figure 1. Typical Calibration Curve for the Determination of XDE-105 Factor A



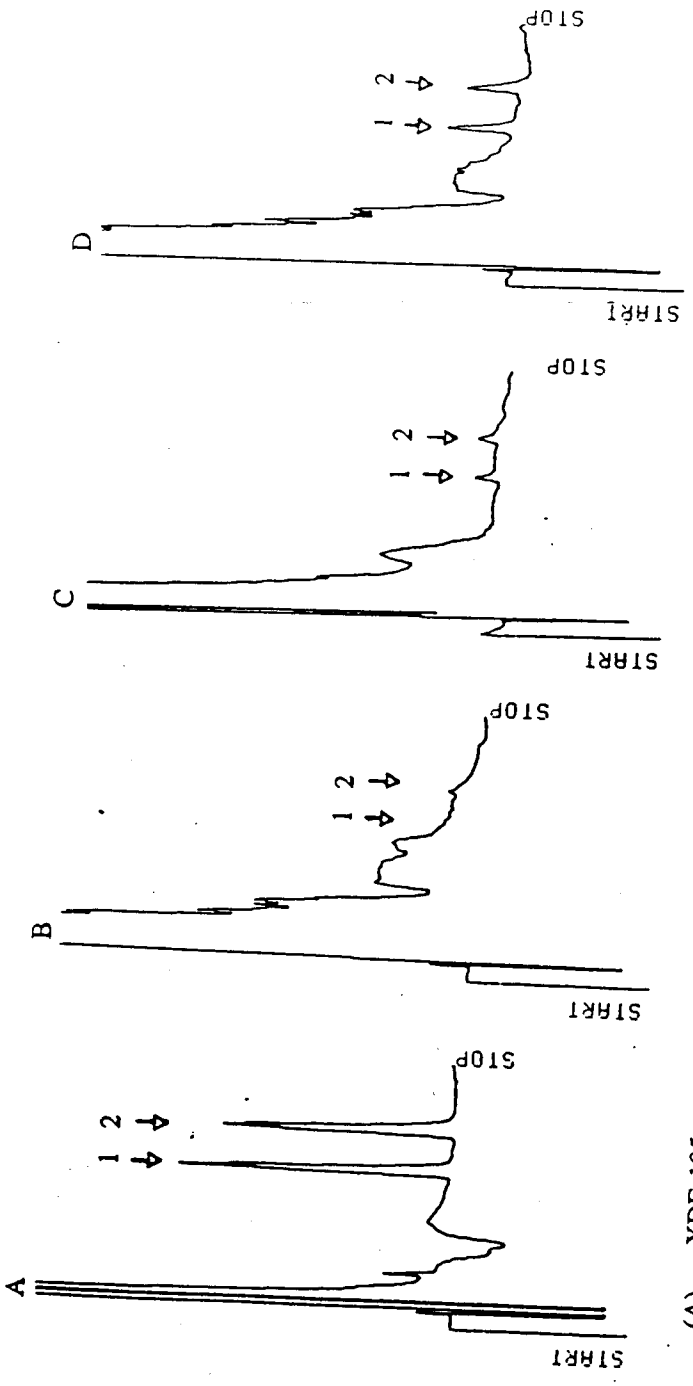
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control cottonseed containing no detectable residue.
(C) Untreated control cottonseed fortified with 0.003 µg/g of XDE-105 factors A and D (limit of detection). Factor A peak height = 0.3 cm; Factor D peak height = 0.3 cm.
(D) Untreated control cottonseed fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to a 99% recovery for factor A and an 82% recovery for factor D. Factor A peak height = 1.0 cm; Factor D peak height = 0.7 cm.

Figure 2. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Cottonseed (1=Factor A, retention time=11.3 min; 2=Factor D, retention time=14.2 min)



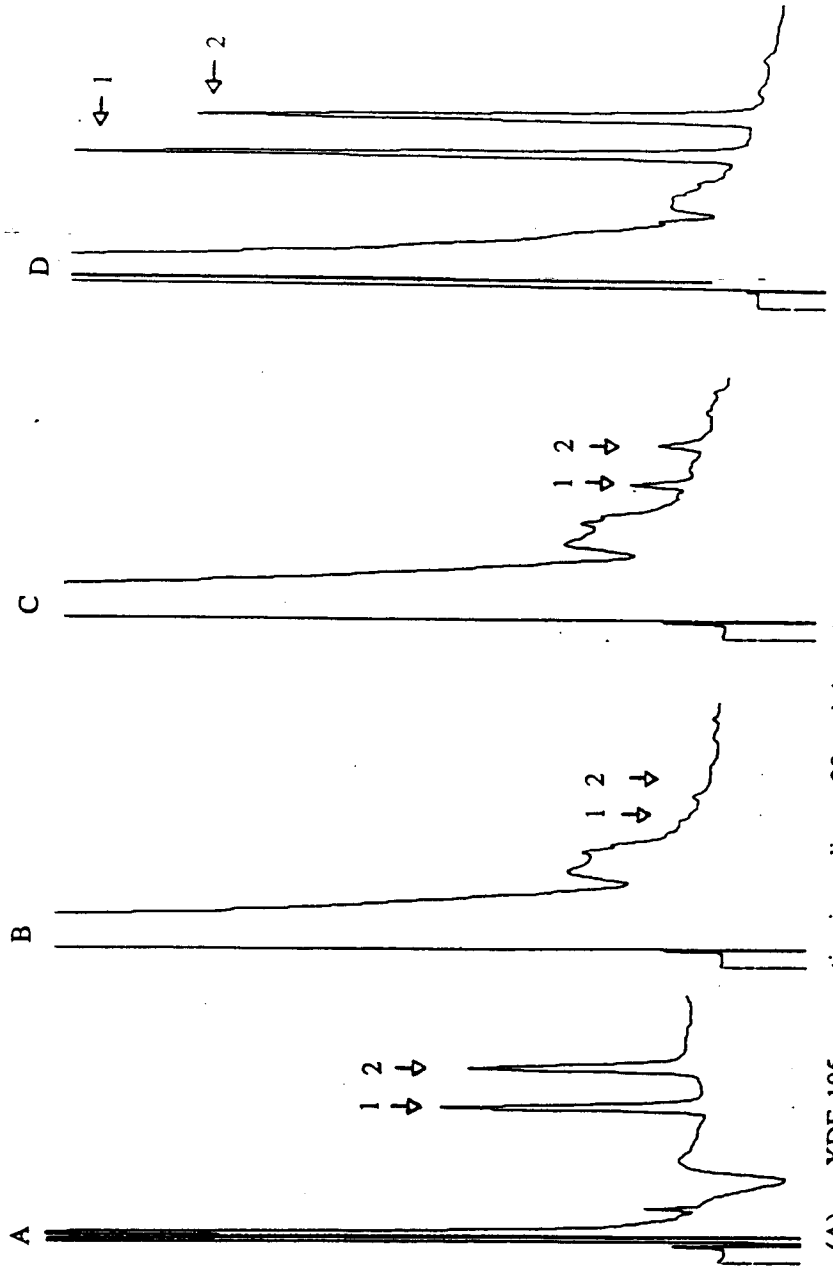
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control cottonseed meal containing no detectable residue.
(C) Untreated control cottonseed meal fortified with 0.003 µg/g of XDE-105 factors A and D (limit of detection). Factor A peak height = 0.4 cm; Factor D peak height = 0.3 cm.
(D) Untreated control cottonseed meal fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to a 91% recovery for factor A and a 96% recovery for factor D. Factor A peak height = 0.9 cm; Factor D peak height = 0.8 cm.

Figure 3. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Cottonseed Meal (1=Factor A, retention time=11.8 min; 2=Factor D, retention time=14.8 min)



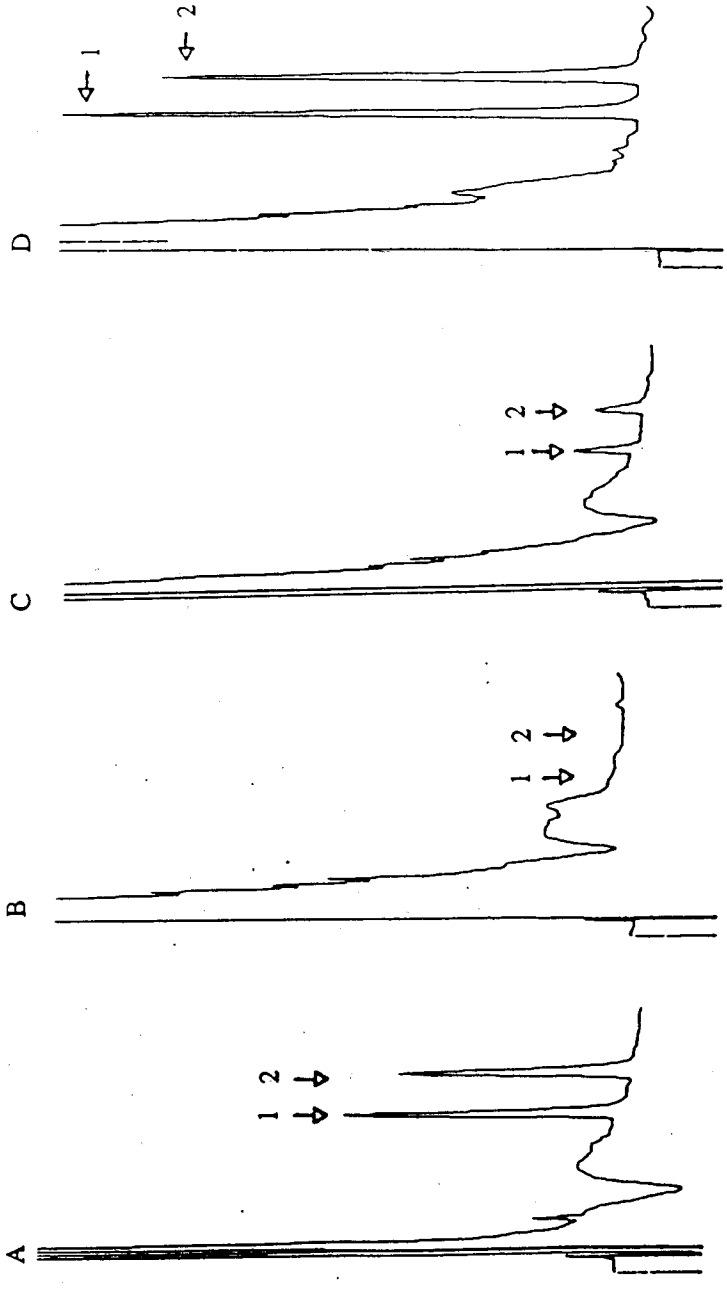
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control cottonseed hulls containing no detectable residue.
(C) Untreated control cottonseed hulls fortified with 0.003 µg/g of XDE-105 factors A and D (limit of detection). Factor A peak height = 0.3 cm; Factor D peak height = 0.3 cm.
(D) Untreated control cottonseed hulls fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to a 100% recovery for factor A and a 110% recovery for factor D. Factor A peak height = 1.0 cm; Factor D peak height = 0.9 cm.

Figure 4. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Cottonseed Hulls (1=Factor A, retention time=11.9 min; 2=Factor D, retention time=15.0 min)



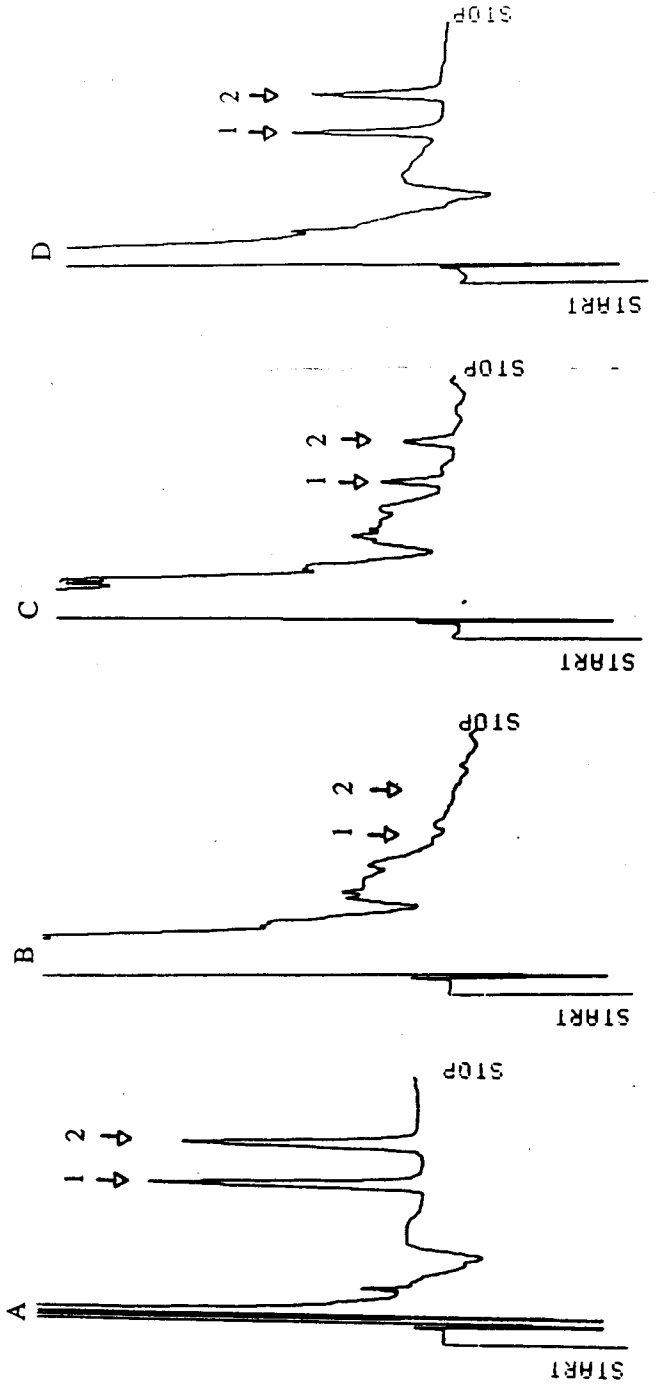
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control crude cottonseed oil containing no detectable residue.
(C) Untreated control crude cottonseed oil fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to an 86% recovery for factor A and an 85% recovery for factor D. Factor A peak height = 0.9 cm; Factor D peak height = 0.7 cm.
(D) Untreated control crude cottonseed oil fortified with 0.100 µg/g of XDE-105 factors A and D, equivalent to a 95% recovery for factor A and a 93% recovery for factor D. Factor A peak height = 10.2 cm; Factor D peak height = 8.6 cm.

Figure 5. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Crude Cottonseed Oil (1=Factor A, retention time=11.5 min; 2=Factor D, retention time=14.4 min)



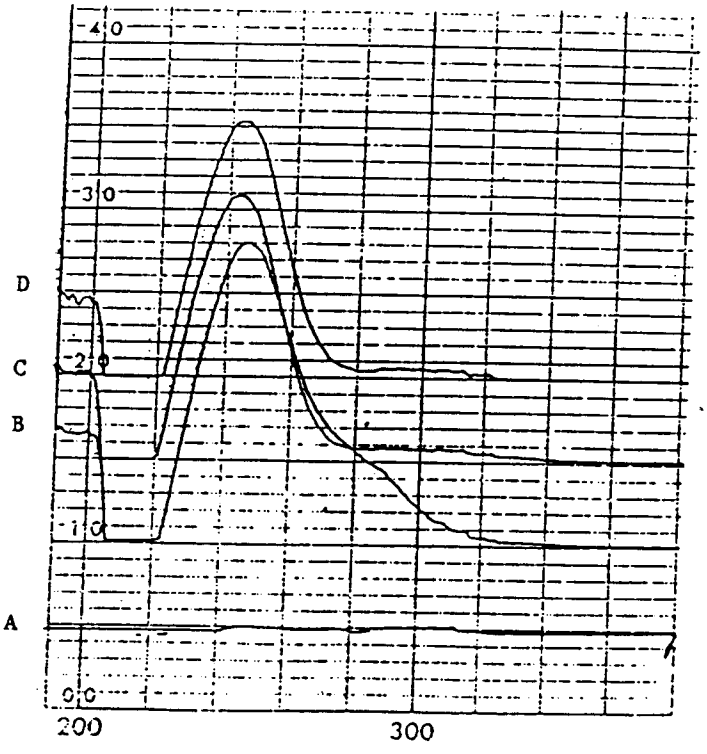
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control refined cottonseed oil containing no detectable residue.
(C) Untreated control refined cottonseed oil fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to an 88% recovery for factor A and an 80% recovery for factor D. Factor A peak height = 0.9 cm; Factor D peak height = 0.7 cm.
(D) Untreated control refined cottonseed oil fortified with 0.100 µg/g of XDE-105 factors A and D, equivalent to a 77% recovery for factor A and a 73% recovery for factor D. Factor A peak height = 8.8 cm; Factor D peak height = 7.3 cm.

Figure 6. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Refined Cottonseed Oil (1=Factor A, retention time=12.0 min; 2=Factor D, retention time=15.0 min)



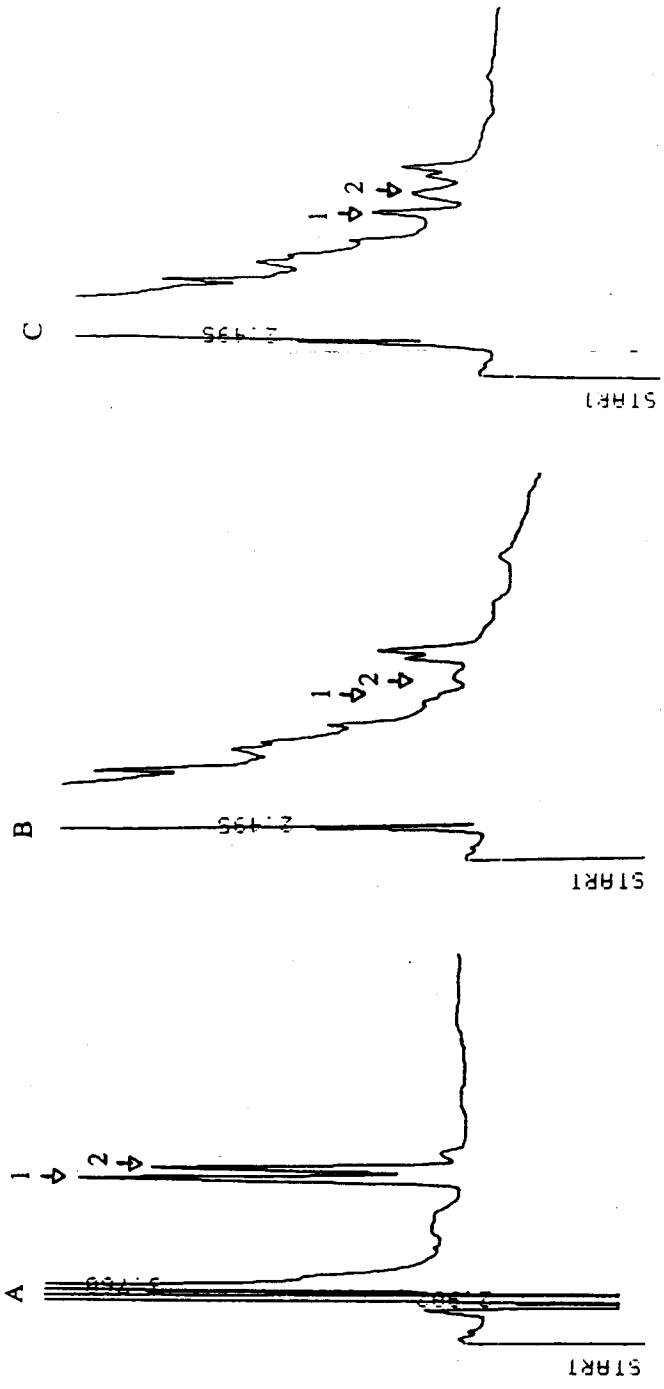
(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control cottonseed soapstock containing no detectable residue.
(C) Untreated control cottonseed soapstock fortified with 0.010 µg/g of XDE-105 factors A and D, equivalent to a 93% recovery for factor A and a 100% recovery for factor D. Factor A peak height = 0.9 cm; Factor D peak height = 0.8 cm.
(D) Untreated control cottonseed soapstock fortified with 0.020 µg/g of XDE-105 factors A and D, equivalent to a 101% recovery for factor A and a 109% recovery for factor D. Factor A peak height = 2.2 cm; Factor D peak height = 2.0 cm.

Figure 7. Typical Chromatograms Demonstrating the Determination of XDE-105 Factors A and D in Cottonseed Soapstock (1=Factor A, retention time=11.8 min; 2=Factor D, retention time=14.8 min)



(A) Solvent blank
(B) XDE-105 Factor D
(C) XDE-105 Factor A
(D) XDE-105 Factor B (an impurity in XDE-105)

Figure 8. UV Spectra for XDE-105 Factors at 10 µg/mL in Methanol/Acetonitrile/2% Ammonium Acetate (44/44/12)



(A) XDE-105 pure active ingredients, 20 ng injected (equivalent to a theoretical residue of 0.04 µg/g).
(B) Untreated control cottonseed meal containing no detectable residue.
(C) Untreated control cottonseed meal fortified with 0.01 µg/g of XDE-105 factors A and D, equivalent to an 85% recovery for factor A and a 92% recovery for factor D. Factor A peak height = 1.1 cm; factor D peak height = 0.8 cm.

Figure 9. Typical Chromatograms for the Confirmation of XDE-105 Factors A and D in Cottonseed on an RP-8/Cation Mixed Mode Column at 250 nm (1=Factor A, retention time=12.2 min; 2=Factor D, retention time=13.0 min)

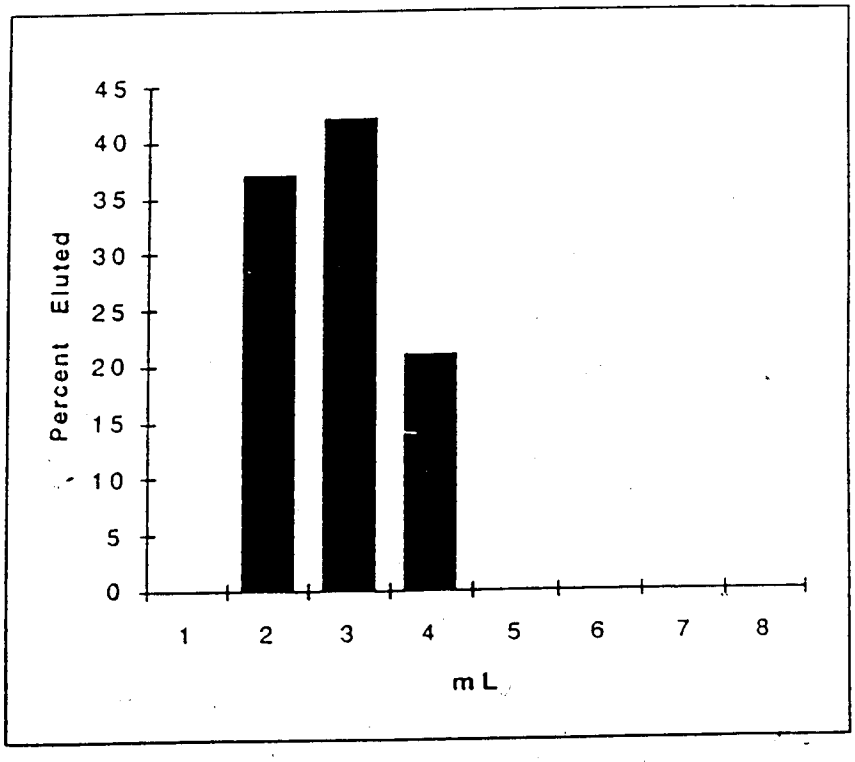
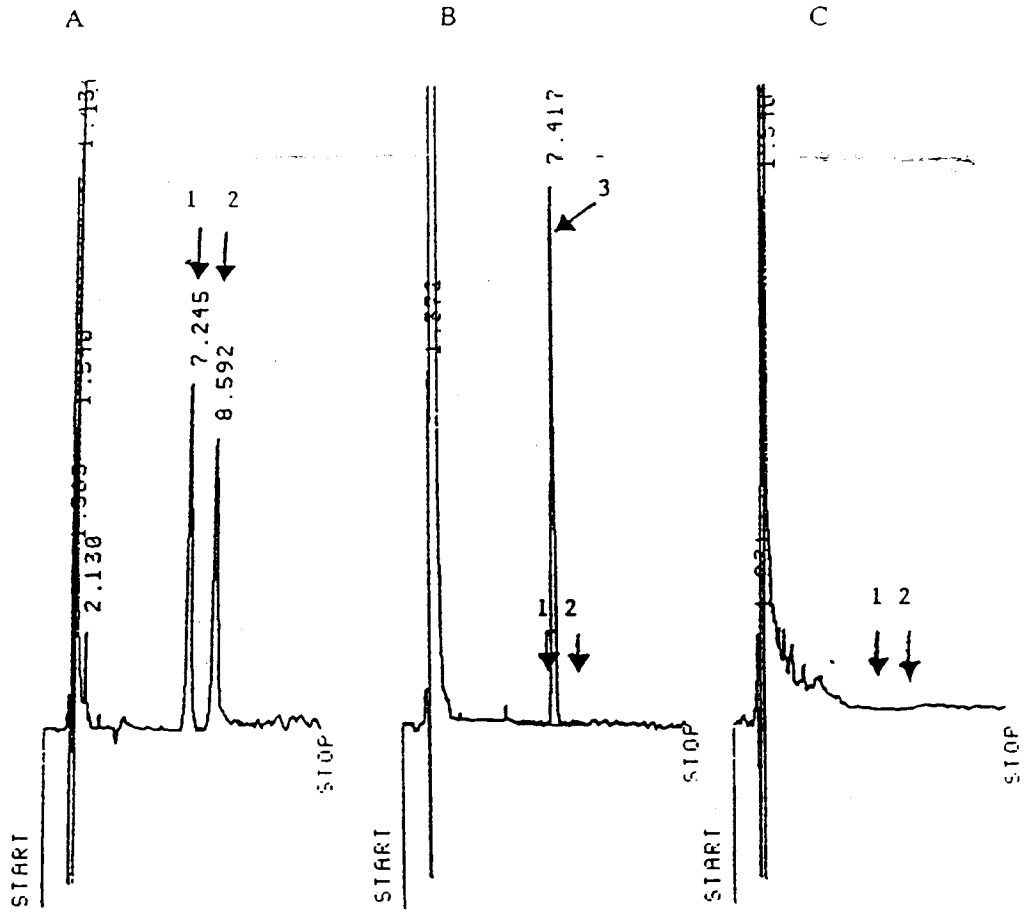


Figure 10. Elution Profile for 5 µg of XDE-105 Factors A and D on Silica Sep-Pak With 8 mL of 75% Methylene Chloride/25% Methanol



(A) XDE-105 pure active ingredients, 0.5 µg injected (equivalent to a theoretical residue of 0.1 µg/g).
(B) Thiodicarb, 5 µg injected directly into the HPLC (equivalent to a theoretical thiodicarb residue of 1.0 µg/g).
(C) 10 µg of thiodicarb carried through the entire cottonseed residue procedure (no interference).

Figure 11. Chromatograms Demonstrating the Presence and Absence of an Interference Peak From Thiodicarb (1=factor A, retention time=7.2 min; 2=factor D, retention time=8.6 min; 3=thiodicarb, retention time=7.4 min)

STATISTICAL METHODS

Statistical methods included the calculation of means, standard deviations, and least squares correlation coefficients.

STANDARDS AND RADIOLABELED MATERIALS

Pure active ingredients of XDE-105 factors A and D were used as standards for the method validation. The pure active ingredient for factor A was lot number V43-A743-196, TSN 100109, with a purity of 98.3% (Report Number A047-3). The pure active ingredient for factor D was lot number B31-A741-58, TSN 100110, with a 93.0% purity (Report Number A047-3). Standard curve and standard fortification solutions were prepared as described in Method GRM 94.02 in this report.

The radiolabeled (^{14}C) factor A was lot number H38-7BH-92A with a radiochemical purity of 95.7% and a specific activity of 1.51 μCi/mg (Notebook reference 9JF-16, Study Number DGS9002). The radiolabeled (^{14}C) factor D was lot number B57-B064-3D with a radiochemical purity of 92.0% and a specific activity of 5.72 μg/g (Notebook reference 7JF-11, Study Number ENV91068).

CONCLUSION

Residue Method GRM 94.02 is capable of determining the total toxic residue of XDE-105 in cottonseed and five processed commodities (meal, hulls, crude oil, refined, oil, and soapstock) at a validated limit of quantitation of 0.01 μg/g.

ARCHIVING

Raw data and the original of the final report are filed in the DowElanco testing facility archives, Indianapolis, Indiana.

ACKNOWLEDGMENTS

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