Method for the Analysis of Cyclanilide (RPA090946) Residues: Common Moiety Method

(Version 2.2 for Bovine Milk, Muscle, Kidney, Liver, and Fat)

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I. INTRODUCTION AND SUMMARY

A. Scope

Cyclanilide (aka. RPA090946)[1] is an Ethephon synergist developed by the Rhône-Poulenc Ag Company (RPAC) for use on cotton. Applications of RPA090946 to cotton plants may result in RPA090946 residues in/on the mature plant and cotton bolls[2]. During the ginning process, cotton fiber is separated from associated plant material (gin trash) which may subsequently be incorporated into the diet of dairy cows[3]. Hence, a method is required for analysis of RPA090946 residues in dairy cow milk, muscle, kidney, liver, and fat.

RPA090946 is a 2,4-dichloroaniline derivative (amide) of cyclopropane-1,1-dicarboxylic acid. The compound can be hydrolyzed by hot alkali to 2,4-dichloroaniline which will react with acid chlorides, yielding stable amide derivatives easily analyzed by gas chromatography techniques. Using this strategy, this method determines RPA090946 residues in a variety of dairy cow matrices, including milk, muscle, kidney, liver, and fat.

B. Principle

RPA090946 residues are hydrolyzed by reflux with hot, aqueous alkali. The hydrolysis product, 2,4-dichloroaniline, is distilled from the reaction mixture, partitioned into methylene chloride (DCM), then reacted with 2-chloropropionyl chloride (CPC) to yield N-(2,4-dichlorophenyl)-2-chloropropylamide (2,4-DCPA). Further

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[1]The terms cyclanilide and RPA090946 are used interchangeably throughout this document.

[2]Prior metabolism studies have shown that RPA090946-related residues in cotton are comprised solely of parent compound [1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid].

[3]Up to 20% of the diet. From U.S. Environmental Protection Agency. Pesticide Assessment Guidelines Subdivision O. Residue Chemistry, EPA 540/9-82-023, Document PB83-153981, Washington, DC, 1982.

purification is effected by Florisil® chromatography. Quantification of 2,4-DCPA in the final extract is performed by GC with electron capture detection. For milk, kidney, and liver, this procedure has approximate detection and quantification limits (LOQ) for RPA090946 of 0.002 ppm and 0.005 ppm, respectively; for muscle, the approximate detection and quantification limits are 0.001 ppm and 0.003 ppm, respectively; for fat, the approximate detection and quantification limits are 0.004 ppm and 0.011 ppm, respectively.

Figure 1 presents the chemical structures of RPA090946 and 2,4-DCPA.

II. MATERIALS AND METHODS

A. Equipment

Equipment	Suggested Manufacturers:[4]
Analytical Balance	Ohaus GA110
Boiling Stones, Charcoal	Fisher Scientific
Distillation Glassware	Fisher Scientific (See Figure 2)
Gas Chromatograph with Electron Capture Detector Wide-bore Capability Split-Splitless Injector	Hewlett-Packard Model 5890 Series II
Gas Chromatograph Column: Supelco "Sup-Herb"	Supelco, 15 M X 0.53 mm i.d., 0.5 μM film thickness
General Laboratory Glassware	Various
Glass Columns: 11 mm i.d. X 25 cm, equipped with a teflon stopcock and a 200-mL reservoir	Fisher Scientific, #K420280-0213
Glass Wool	Fisher Scientific
Heating Mantles, 1 L	Fisher Scientific
pH Paper	Fisher Scientific
Rotary Evaporator	Fisher Scientific
Single Pan Balance	Ohaus E400

B. Reagents and Standards

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[4]Equivalent sources of the listed equipment and reagents may be used.

Antifoam B®: Sigma Chemical Company, Catalog #A-5757
Acetone: Fisher Optima Grade

2-Chloropropionyl Chloride (CPC): Aldrich Chemical Company, 98% Catalog #15,713-9. **Caution: Severe lachrymator.** See Note 5.

50% CPC in DCM: When prepared as described in Note 5, the reagent contains 50% CPC in methylene chloride.

Cyclohexane: Fisher HPLC Grade

Diethylether (EtOEt): Fisher Anhydrous

6% Diethylether in Hexane: Dilute 30 mL of diethylether to 500 mL with hexane.

Dry Ice™: Local Supplier
Ethyl Acetate: Fisher Optima Grade

Florisil®: 100-200 mesh, Fisher Scientific

Activate Florisil® overnight at 150°C. Cool and store in a desiccator; the reagent is stable for 12 months.

Hexane: Fisher Optima Grade

Igepal® CO-660: Rhône-Poulenc, Inc. Princeton, NJ.

10% (w/w) aqueous Igepal®: Dissolve 10 g of Igepal® in 90 g of distilled water.

Methanol: Fisher Optima Grade

Methylene Chloride (DCM): Fisher Optima Grade

Potassium Hydroxide: Fisher Scientific

3N Aqueous KOH: Dissolve 168 g KOH pellets in 1 L of distilled water. Cool to room temperature.

Sodium Chloride: Fisher Scientific
Sodium Bicarbonate: Fisher Scientific

Sodium Sulfate (anhydrous): Fisher Scientific (ACS grade)

Water: Distilled and/or Deionized

Cyclanilide (aka. RPA090946 and 1-(2,4-dichlorophenyl-aminocarbonyl)-cyclopropane carboxylic acid), available from Rhône-Poulenc Ag Company (RPAC).

N-(2,4-dichlorophenyl)-2-chloropropylamide (2,4-DCPA), available from RPAC.

C. Analytical Procedure

C.1 Sample Preparation

For muscle, liver, kidney, and fat, homogenize the sample thoroughly in a grinder, then freeze pending analysis. For milk, mix thoroughly and freeze pending analysis. Just prior to subsampling for analysis, milk should be thawed, then vigorously mixed again to homogenize the cream and milk.

C.2 Hydrolysis/Distillation

a. Weigh a 10 g sample (5 g for fat) into a 1-L boiling flask. Quality control fortifications are made at this point; for untreated control (UTC) samples analyzed parallel with the fortified samples, add neat methanol equivalent to the largest volume used for fortification.

b. Add 6 mL of 10% Igepal®, 32 drops of Antifoam B®, and charcoal boiling stones to the sample (Note 1).

c. Add ca. 300 mL of 3N aqueous KOH to the sample. Swirl to mix. Securely attach the sample flask to a distillation apparatus (Figure 2), then bring the extract to a rolling boil (Note 2). Collect ≥100 mL of distillate (≥200 mL for fat samples, Note 3) in a beaker or Erlenmeyer flask.

C.3 Dichloromethane Partition

a. Transfer the distillate into a 125-mL separatory funnel (250-mL to 1-L for fat samples) using ca. 15 mL of dichloromethane (DCM). Add ca. 0.25 g of sodium chloride (Note 4).

b. Mix the phases vigorously (ca. 30 seconds). After phase separation, percolate the lower DCM layer through a tightly-packed glass-wool plug (pre-washed with DCM) into a flask. Repeat the partition sequence two more times with ca. 15 mL of DCM each time. Pool the DCM extracts; discard the aqueous phase. Rinse the glass wool plug with 2 x ca. 15 mL of DCM.

c. Add 5-10 drops of 50% 2-chloropropionyl chloride (CPC) reagent to the DCM solution (**Caution: Severe lachrymator!**, also, see Note 5). Allow to react at room temperature for 30 minutes. The reaction product is N-(2,4-dichlorophenyl)-2-chloropropylamide (2,4-DCPA).

d. Rotary-evaporate the DCM extract to near-dryness. Add ca. 10 mL of cyclohexane, then rotary-evaporate to dryness. Repeat the cyclohexane addition once more, then rotary-evaporate the sample to dryness (Note 6).

C.4 Florisil® Chromatography

a. Prepare a Florisil® clean-up column as follows: Seat a small plug of glass wool in a 11 mm i.d. glass column. Rinse the column and glass wool with diethylether and allow to air dry. Add 1 gram of fully-activated Florisil® to the column. Top the Florisil® with a small amount (ca. 0.5 cm) of anhydrous sodium sulfate. **Do not pre-equilibrate the column with solvent.** The column is now ready for use.

b. Dissolve the dry residue from C.3.d in 10 mL of hexane. Transfer 4 mL of the solution to the top of the Florisil® column, taking care not to disturb the column surface. Percolate the sample into the column (ca. 2 drops/second), then wash the column sides with ca. 40 mL of hexane. Percolate the rinse through the column. Discard all eluants to this point (Note 7).

c. Elute 2,4-DCPA from the column with 50 mL of 6% EtOEt in hexane (Note 8).

d. Rotary-evaporate the eluant to dryness. Dissolve the dry residue in a known volume of ethyl acetate (for LOQ residues, generally 2

mL for milk, liver, kidney, and muscle, and 1.5 mL for fat). Dilute with ethyl acetate as necessary to maintain the analyte concentration within the standard curve range. Submit the sample for GC/ECD analysis as described in Step D.

D. Gas Chromatographic Analysis

D.1 Equipment

A gas chromatograph equipped with an Electron Capture Detector is required. Split-splitless injection and wide-bore capabilities are suggested.

GC Column:	Supelco Sup-Herb® (Catalog #2-5322), 15 M length, 0.53 mm i.d., 0.5 μM film. Other columns may be substituted if they give satisfactory resolution between the 2,4-DCPA analyte and any interferences.

D.2 Suggested Operating Conditions

Temperatures:

Injector: 230°C, 2 mm dia. open glass insert

Detector: 300°C

Column:

Initial:	95°C, hold 1 min.
Ramp Rate 1:	40°C/min to 180°C, hold 5 min.
Ramp Rate 2:	40°C/min to 280°C, hold 3.00 min.

Gas flows:

Carrier Gas:	He, 7.9 mL/min at 95°C, head pressure = 3.0 psi. Constant flow off.
Injector Purge:	He @ 3.5 mL/min.
Split Vent:	He @ 20→75 mL/min, on @ 0.75 min.

Detector make up: N_2 @ 65→70 mL/min.

Injection: 2 µl, Split/Splitless

D.3 Confirmatory Column

A DB-5 capillary column may be used to confirm RPA090946 residues in sample extracts. Suggested operating parameters are as noted below:

GC Column: DB-5, 30 M X 0.53 mm, 1.5 µM film thickness, J & W Scientific, 91 Blue Ravine Road, Folsom, CA 65630-4714.

Temperatures:

Injector: 230°C, 2 mm dia. open glass insert.

Detector: 300°C

Column:

Initial:	95°C, hold 1 min.
Ramp Rate 1:	40°C/min to 250°C, hold 5 min.
Ramp Rate 2:	40°C/min to 280°C, hold 3.00 min.

Gas flows:

Carrier Gas:	He, 4.7 mL/min at 95°C, head pressure = 3.0 psi. Constant flow off.
Injector Purge:	He @ 4.0 mL/min.
Split Vent:	He @ 29 mL/min, on @ 0.75 min.
Detector make up:	N_2 @ 65 mL/min.

Injection: 2 µl, Split/Splitless

E. Calibration Procedures

E.1 Preparation of Standard Solutions

a. Stock solutions of RPA090946 are made in methanol and are stored in a freezer; stock concentrations are approximately 1.0 mg/mL.

b. Dilutions of RPA090946 are made at appropriate concentrations for fortification standards. These dilutions are made in methanol[5]. Stability of these solutions during freezer storage in the dark is at least 4 months.

c. Stock solutions of N-(2,4-dichlorophenyl)-2-chloropropylamide are made in ethyl acetate and are stored in a freezer. GC standards are prepared in ethyl acetate from the stock solutions. Stability of this analyte during freezer storage is at least 4 months.

E.2 Detector Calibration

The sensitivity of the ECD detector is monitored by injecting 2,4-DCPA standards before, between, and after the samples. The suggested mass range is 10 pg to 240 pg injected. 2,4-DCPA must be detectable at the chosen minimum concentration. A minimum standard of 36.7 pg 2,4-DCPA injected (24.5 pg for fat) and a final dilution volume of (a) 2 mL for a 10 gram milk, kidney, liver, or muscle sample, or (b) 1.5 mL for a 5 gram fat sample, results in a calculated residue of 0.01 ppm RPA090946.

Under the conditions of this assay, 2,4-DCPA elutes from the GC column at 5 to 6 minutes after injection. The GC/ECD limit of detection for 2,4-DCPA (injected as a solution of pure standard) is 5→10 pg injected.

F. Methods of Calculation

F.1 Injection Sequence

Run sequences are started and ended with one or two standards; standard injections are made throughout the run, generally with no more than two sample injections between each standard. A standard curve of 2,4-DCPA concentration (ng/mL) versus peak height or area is constructed using a method of

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[5]Acetone was used as the diluent for fortification standards during methods development research. However, acetone should be avoided since alkali from the hydrolysis/distillation step catalyzes formation of numerous acetone condensation products. These products are not removed by the Florisil® clean-up step, are detected by the GC/ECD, and may confound quantification of the 2,4-DCPA analyte.

curve generation appropriate for the GC/ECD instrumentation. The construction may be linear, quadratic or logarithmic.

F.2 Calculations

Calculate ppm values for RPA090946 residues using the following equation:

(1) For milk, liver, kidney, and muscle:

$$ppm = \frac{(ng/mL\ Final\ Extract)\ X\ (mL\ Final\ Extract)}{} \times \frac{10\ mL\ Florisil\ Total\ Volume}{4\ mL\ Florisil\ Aliquot\ Volume} \times \frac{1}{10\ grams} \times \frac{1\ ug}{1000\ ng} \times 1.09$$

(2) For fat:

$$ppm = \frac{(ng/mL\ Final\ Extract)\ X\ (mL\ Final\ Extract)}{} \times \frac{10\ mL\ Florisil\ Total\ Volume}{4\ mL\ Florisil\ Aliquot\ Volume} \times \frac{1}{5\ grams} \times \frac{1\ ug}{1000\ ng} \times 1.09$$

The "1.09" conversion factor corrects for molecular weight differences between 2,4-DCPA and RPA090946. The molecular weights for RPA090946 and 2,4-DCPA are 274.1 g/mole and 252.5 g/mole, respectively.

For fortified-control samples, use the following equation to calculate the percent recovery:

$$\%\ Recovery = \left(\frac{(ppm\ Found) - (ppm\ Control)}{ppm\ Fortified}\right) X\ 100$$

G. Interferences

G.1 Sample Matrices

In bovine matrices, there are only minor interferences at the target quantification limit of 0.01 ppm. There are two peaks near the retention time for 2,4-DCPA, one before and one after the analyte, and the GC parameters should be optimized to maximize resolution between 2,4-DCPA and these potential interferences. The interferences are from the CPC reagent and are not removed by the Florisil® cleanup column.

Despite the substantial clean-up procedures employed in this method, extensive sample injections do cause some undesirable chromatographic effects, most notably reduced instrument sensitivity towards 2,4-DCPA. This problem is corrected by cleaning the injector insert and cutting 10 → 20 cm from the proximal end of the column.

G.2 Other Pesticides

A specificity study has been conducted for this method using compounds with tolerance levels established for cotton matrices and/or bovine tissues. The compounds were analyzed as reagent spikes at fortification levels of ≈10 or ≈100 ppm. Only one compound (profluralin, 0.011 ppm equivalent) interfered with the quantification of RPA090946 on the Sup-Herb® analytical column. Injection of the sample obtained from this compound on the DB-5 confirmatory column indicated that adequate separation between RPA090946 and the interference peak could be achieved for accurate quantification of RPA090946.

G.3 Solvents and Reagents

The solvents specified in this procedure do not present any interferences at the target LOQ. However, **do not use cotton in place of glass wool where the latter is indicated unless the cotton has been tested for interferences;** numerous ECD sensitive compounds which interfere with 2,4-DCPA analysis may be co-extracted from cotton in the assay.

G.4 Glassware

No interferences are detected from the labware at the stated LOQ. Glassware used for sample analysis is pre-rinsed with acetone, then dried prior to use. Glass vessels are recommended for all steps without substitutions with plastic.

III. METHODS VALIDATION

A. Experimental Design

A complete validation study for this method was performed. The method was validated in cow milk, muscle, liver, kidney, and fat. The RPA090946 fortification levels for each matrix were 0.00 ppm (5 samples), 0.01

ppm (7 samples), 0.05 ppm (5 samples), and 0.50 ppm (5 samples) analyzed over two days as follows:

1. Three UTC samples, 4 UTC samples fortified at 0.01 ppm RPA090946, 2 UTC samples fortified with 0.05 ppm RPA090946, and 2 UTC samples fortified with 0.50 ppm RPA090946 were analyzed on day 1 for each substrate.

2. Two UTC samples, 3 UTC samples fortified at 0.01 ppm RPA090946, 3 UTC samples fortified with 0.05 ppm RPA-090946, and 3 UTC samples fortified with 0.50 ppm RPA090946 were analyzed on day 2 for each substrate.

3. The average apparent residue (ppm) of RPA090946 in the five UTC samples analyzed in steps 1 and 2 above was determined. The standard deviation (ppm) of the seven UTC samples that were fortified at 0.01 ppm RPA090946 in steps 1 and 2 was determined.

4. The minimum detectable limit (MDL) for RPA090946 was calculated by summing the average residue of the UTC samples and 3-times the sample standard deviation of the UTC samples that were fortified at 0.01 ppm RPA090946.

The MDL is defined as the minimum concentration of RPA090946 that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

5. The limit of quantification (LOQ) for RPA090946 was calculated by summing the average residue of the UTC samples and 10-times the sample standard deviation of the UTC samples that were fortified with 0.01 ppm RPA090946.

The LOQ corresponds to an uncertainty of plus-or-minus 30% in the measured residue value at the 99% confidence level.

6. Precision: The standard deviation of the recovery at each fortification level divided by the mean recovery at that level, times 100. Precision was also estimated by analysis of variance (ANOVA) statistical techniques, which estimated method precision in the absence of matrix, fortification level, day-of-analysis, and interaction main effects.

7. Accuracy: The net residue[6] divided by the fortification level, times 100.

B. Analytical Reference Materials

Specific reference materials used during the conduct of the method validation study are described in Table 1.

C. Control Matrices

The method was validated using untreated Holstein milk. Liver, kidney, fat, and muscle tissues of unknown variety were obtained from a local beef market.

D. Extraction Efficiency

The extraction efficiency of the method was tested by analyzing goat tissues (liver and kidney, Hazelton Europe study number 198/72) containing incurred ^{14}C-RPA090946 residues. Aliquots of appropriate fractions derived from the method were analyzed for radioactivity; the final extract was also assayed by GC/ECD.

E. Validation Results

Analyte recoveries obtained from the method validation studies are presented in Tables 2 through 6. By matrix, the mean recoveries over all fortification levels were (a) milk; 98.1%, (b) muscle; 108.1%, (c) kidney; 108.1%, (d) fat; 99.8%, and (e) liver; 99.2%.

The recovery data were analyzed by analysis of variance (ANOVA) as presented in Table 7. The ANOVA error mean square (8.6) estimates σ^2, the recovery population variance of the method. Thus, the estimated method standard deviation is $\sqrt{8.6} = 2.93$, giving an estimated method recovery performance of 102.7% ± 2.93% (COV = 2.9%), independent of any matrix, fortification level, or interaction main effects.

The ANOVA data indicated significant main effects on recovery of RPA090946 due to matrix, fortification level, day-of-analysis, and interaction. Main effects due to matrix were significant because recoveries from kidney and muscle tended higher than for liver, milk, and fat. Similarly, recovery at the 0.50 ppm (93.4%) fortification level was significantly lower than at 0.01 ppm (107.6%)

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[6]Net residue = ppm found minus average ppm measured in the associated controls.

and 0.05 ppm (105.0%). There was a small significant effect ($p < 0.05$) due to day of-analysis (Day 1 = 103.4% and Day 2 = 102.0%). However, during routine applications of the method at other laboratories, acceptance criteria for individual data sets should be based upon independently-derived evaluations of method performance.

From the results of analysis of the control samples and 0.01 ppm fortifications, the MDL for each matrix was calculated at 0.0021, 0.0008, 0.0013, 0.0022, and 0.0036 ppm for milk, muscle, kidney, liver and fat, respectively. The calculated LOQ for each matrix was 0.0058, 0.0026, 0.0042, 0.0063, and 0.0113 ppm for milk, muscle, kidney, liver and fat, respectively (Table 8).

The recovery results also indicate the method has a high degree of precision and accuracy (Table 9). Mean recoveries of RPA090946 from all matrices at all fortification levels ranged from 88.1 to 114.2% while the coefficients of variation varied from 1.3 to 10.3%.

A total of 11-12 chemist-hours is required for hydrolysis, distillation, partitioning, derivatization, and cleanup of a set of 11 samples. Chromatographic analysis is unattended and automated.

F. Extraction Efficiency Results

Extraction of ^{14}C-RPA090946 residues from radiolabeled dairy goat liver and kidney by the method was highly efficient; the method recovered 89.1% and 94.9%, respectively (average of duplicate analyses), of the radiolabeled residues in the terminal GC fractions. Residue recoveries measured from the same extract by GC/ECD were 101.1% and 105.0% for liver and kidney, respectively.

G. Conclusions

Based upon data obtained from the method validation studies, the performance of this method is summarized as follows:

(1) Recoveries of RPA090946 from kidney and muscle averaged 108.1%.

(2) Recoveries of RPA090946 from milk, liver, and fat averaged 98.1%, 99.2, and 99.8%, respectively.

(4) Recovery of RPA090946 from bovine matrices was dependent upon matrix, fortification level, day-of-analysis, and interaction. However, the dif-

IV. NOTES

Note 1: Foaming is controlled by liberal use of antifoam reagents during distillation (also see Note 2 below).

Note 2: All joints in the distillation apparatus must be firmly sealed to prevent loss of the volatile 2,4-dichloroaniline. Foam and/or boiling extract should not "bump" or reflux over into the collection flask. If this occurs excessively (>2 mL), the distillate may be transferred back into the cooled reflux flask and re-distilled. The distillation requires ca. 1 hour to collect 100 mL. The pH of the distillate ranges from near-neutral to slightly basic. The 2,4-dichloroaniline product is recovered quantitatively in the condensate; it is not necessary to trap the aniline as the salt via addition of acid to the collection flask.

Kidney samples must be distilled gently. **Use low heat!**; otherwise, excessive foaming occurs in spite of the antifoam reagents.

Note 3: More distillate must be collected from fat samples for acceptable recovery of analyte. The reason is not known, but distillation-pot residuals do contain a precipitate (probably soap) which may sequester the 2,4-dichloroaniline product and slow its distillation.

Note 4: The sodium chloride prevents emulsions.

Note 5: Each lot of CPC should be pre-qualified prior to use since substandard lots have been noted by developers of this method. To pre-qualify the reagent, pipet 10-15 µg of 2,4-dichloroaniline (in acetone or another suitable solvent) into ca. 45 mL of DCM. Add 5-10 drops of CPC reagent and allow to react for 30 minutes. Evaporate the sample as noted in Step C.3.d, dissolve the product in 250 mL of ethyl acetate, then analyze by GC/ECD. The yield should exceed 90%.

Substandard lots of CPC probably contain water. Water slowly hydrolyzes CPC, yielding acetic and hydrochloric acids. When added to sample extracts, these acids form salts with 2,4-dichloroaniline, rendering the latter unavailable for reaction with CPC.

Substandard lots of CPC may be cleaned up as follows: **Caution: Perform all operations in a fume hood with proper self-protective equipment!**. In a separatory funnel, dilute ca. 100 mL of CPC with ca. 100 mL of DCM. Wash the solution twice with ca. 100 mL of 5% aqueous

ferences were small and have no practical meaning regarding routine performance of the method since mean recoveries were always well-within established EPA guidelines for recovery of analytes (70% → 120%).

(5) Inherent method precision for bovine matrices is estimated (by ANOVA) at 2.9% of the mean recovery (μ = 102.7%).

(6) Recovery of ^{14}C-RPA090946 residues by the method from radiolabeled goat liver and kidney tissues is nearly quantitative as measured by scintillation analysis (92.0%), and quantitative by GC/ECD analysis (103.0%).

Original raw data and the original report for the methods validation research are archived at the RPAC archives in Research Triangle Park, NC.

sodium bicarbonate each time (**Caution: Do not cap the separatory funnel. Instead, vigorously swirl the reagents in the un-capped separatory funnel. The reaction between bicarbonate and acids contained in the CPC reagent yields carbon dioxide which can rapidly pressurize and cause failure of a sealed separatory funnel!**). The pH of the aqueous bicarbonate layer should remain neutral to slightly-alkaline (pH paper). Wash the DCM with distilled water (100 mL), then percolate the DCM/CPC layer through a cone of anhydrous sodium sulfate; discard the aqueous layer. **The final product should be a clear, colorless solution. If the solution is cloudy, dehydrate the product through a second cone of sodium sulfate.** Pre-qualify the product as noted above and store the solution in a tightly-capped, amber bottle over granular sodium sulfate (anhydrous) in the refrigerator. Periodically check the sodium sulfate; it should remain free-flowing with no lumps, which would indicate the presence of water.

Note 6: Addition of cyclohexane and subsequent rotary-evaporation helps remove excess CPC from the sample. Limited studies have indicated up to a 10% loss of 2,4-DCPA during rotary-evaporation at elevated water-bath temperatures. Thus, rotary-evaporations should be conducted at-or-below room temperature.

Note 7: The pre-wash with hexane elutes several matrix- and reagent-related chromatographic interferences (especially from CPC) from the sample. **Caution: The 2,4-DCPA analyte can be eluted from the column if rinsed with excess hexane.**

Note 8: Fractionation parameters for each batch of Florisil® must be independently evaluated.

V. TABLES

Table 1. Standard Reference Materials.

STANDARD NAME	HL STANDARD NUMBER	LOT NUMBER	PURITY	PHYSICAL CHARACTERISTICS
RPA090946, 1-(2,4-dichlorophenyl-aminocarbonyl)-cyclopropane carboxylic acid, CAS #113136-77-9	HL0186	45EAR111	99.6%	White Powder
2,4-DCPA, N-(2,4-dichlorophenyl)-2-chloropropylamide	HL0167	MW012794	100%	White Powder

Table 2. Recovery of RPA090946 Residues from Bovine Milk.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
UTC Samples					
10123-1R	Whole Milk	0.00 ppm	0.00047	---	---
10123-2R	Whole Milk	0.00 ppm	0.00044	---	---
10123-3R	Whole Milk	0.00 ppm	0.00049	---	---
10123-12R	Whole Milk	0.00 ppm	0.00042	---	---
10123-13R	Whole Milk	0.00 ppm	0.00048	---	---
		Average	0.00046	---	---
		Std. Dev.	0.00003	---	---
0.01 ppm Fortifications					
10123-4R	Whole Milk	0.01 ppm	0.01140	0.01094	109.4
10123-5R	Whole Milk	0.01 ppm	0.01106	0.01060	106.0
10123-6R	Whole Milk	0.01 ppm	0.01017	0.00971	97.1
10123-7R	Whole Milk	0.01 ppm	0.01036	0.00990	99.0
10123-14R	Whole Milk	0.01 ppm	0.00995	0.00949	94.9
10123-15R	Whole Milk	0.01 ppm	0.01031	0.00985	98.5
10123-16R	Whole Milk	0.01 ppm	0.01017	0.00971	97.1
				Average	100.3
				Std. Dev.	5.32
				COV (b)	5.3%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.

(b) COV = Coefficient of Variation.

Table 2(Cont'd). Recovery of RPA090946 Residues from Bovine Milk.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
0.05 ppm Fortifications					
10123-8R	Whole Milk	0.05 ppm	0.05041	0.04995	99.9
10123-9R	Whole Milk	0.05 ppm	0.04988	0.04942	98.8
10123-17R	Whole Milk	0.05 ppm	0.05239	0.05193	103.9
10123-18R	Whole Milk	0.05 ppm	0.05128	0.05082	101.6
10123-19R	Whole Milk	0.05 ppm	0.05192	0.05146	102.9
				Average	101.4
				Std. Dev.	2.07
				COV (b)	2.0%
0.50 ppm Fortifications					
10123-10R	Whole Milk	0.50 ppm	0.45054	0.45008	90.0
10123-11R	Whole Milk	0.50 ppm	0.46098	0.46052	92.1
10123-20R	Whole Milk	0.50 ppm	0.45830	0.45784	91.6
10123-21R	Whole Milk	0.50 ppm	0.49020	0.48974	97.9
10123-22R	Whole Milk	0.50 ppm	0.43784	0.43738	87.5
				Average	91.8
				Std. Dev.	3.87
				COV (b)	4.2%
				Grand Average	98.1
				Std. Dev.	5.77
				COV (b)	5.9%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 3. Recovery of RPA090946 Residues from Bovine Liver.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
UTC Samples					
10123-67	Liver	0.00 ppm	0.00000	---	---
10123-68	Liver	0.00 ppm	0.00062	---	---
10123-69	Liver	0.00 ppm	0.00080	---	---
10123-78	Liver	0.00 ppm	0.00055	---	---
10123-79	Liver	0.00 ppm	0.00000	---	---
		Average	0.00039	---	---
		Std. Dev.	0.00037	---	---
0.01 ppm Fortifications					
10123-70	Liver	0.01 ppm	0.01094	0.01055	105.5
10123-71	Liver	0.01 ppm	0.01087	0.01048	104.8
10123-72	Liver	0.01 ppm	0.01152	0.01113	111.3
10123-73	Liver	0.01 ppm	0.01132	0.01093	109.3
10123-80	Liver	0.01 ppm	0.01014	0.00975	97.5
10123-81	Liver	0.01 ppm	0.01011	0.00972	97.2
10123-82	Liver	0.01 ppm	0.01014	0.00975	97.5
				Average	103.3
				Std. Dev.	5.94
				COV (b)	5.8%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 3(Cont'd). Recovery of RPA090946 Residues from Bovine Liver.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
0.05 ppm Fortifications					
10123-74	Liver	0.05 ppm	0.05164	0.05125	102.5
10123-75	Liver	0.05 ppm	0.05218	0.05179	103.6
10123-83	Liver	0.05 ppm	0.04915	0.04876	97.5
10123-84	Liver	0.05 ppm	0.04852	0.04813	96.3
10123-85	Liver	0.05 ppm	0.05177	0.05138	102.8
				Average	100.5
				Std. Dev.	3.37
				COV (b)	3.4%
0.50 ppm Fortifications					
10123-76	Liver	0.50 ppm	0.47246	0.47207	94.4
10123-77	Liver	0.50 ppm	0.46859	0.46820	93.6
10123-86	Liver	0.50 ppm	0.45466	0.45427	90.9
10123-87	Liver	0.50 ppm	0.44851	0.44812	89.6
10123-88	Liver	0.50 ppm	0.45923	0.45884	91.8
				Average	92.1
				Std. Dev.	1.97
				COV (b)	2.1%
				Grand Average	99.2
				Std. Dev.	6.38
				COV (b)	6.4%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 4. Recovery of RPA090946 Residues from Bovine Kidney.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
UTC Samples					
10123-45	Kidney	0.00 ppm	0.00000	---	---
10123-46	Kidney	0.00 ppm	0.00000	---	---
10123-47	Kidney	0.00 ppm	0.00000	---	---
10123-56	Kidney	0.00 ppm	0.00000	---	---
10123-57	Kidney	0.00 ppm	0.00000	---	---
		Average	0.00000	---	---
		Std. Dev.	0.00000	---	---
0.01 ppm Fortifications					
10123-48	Kidney	0.01 ppm	0.01170	0.01170	117.0
10123-49	Kidney	0.01 ppm	0.01148	0.01148	114.8
10123-50	Kidney	0.01 ppm	0.01168	0.01168	116.8
10123-51	Kidney	0.01 ppm	0.01192	0.01192	119.2
10123-58	Kidney	0.01 ppm	0.01087	0.01087	108.7
10123-59	Kidney	0.01 ppm	0.01146	0.01146	114.6
10123-60	Kidney	0.01 ppm	0.01081	0.01081	108.1
				Average	114.2
				Std. Dev.	4.23
				COV (b)	3.7%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 4(Cont'd). Recovery of RPA090946 Residues from Bovine Kidney.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
0.05 ppm Fortifications					
10123-52	Kidney	0.05 ppm	0.05659	0.05659	113.2
10123-53	Kidney	0.05 ppm	0.05803	0.05803	116.1
10123-61	Kidney	0.05 ppm	0.05516	0.05516	110.3
10123-62	Kidney	0.05 ppm	0.05303	0.05303	106.1
10123-63	Kidney	0.05 ppm	0.05263	0.05263	105.3
				Average	110.2
				Std. Dev.	4.60
				COV (b)	4.2%
0.50 ppm Fortifications					
10123-54	Kidney	0.50 ppm	0.50944	0.50944	101.9
10123-55	Kidney	0.50 ppm	0.49572	0.49572	99.1
10123-64	Kidney	0.50 ppm	0.48158	0.48158	96.3
10123-65	Kidney	0.50 ppm	0.48848	0.48848	97.7
10123-66	Kidney	0.50 ppm	0.46661	0.46661	93.3
				Average	97.7
				Std. Dev.	3.19
				COV (b)	3.3%
				Grand Average	108.1
				Std. Dev.	8.12
				COV (b)	7.5%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 5. Recovery of RPA090946 Residues from Bovine Muscle.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
UTC Samples					
10123-23	Muscle	0.00 ppm	0.00000	---	---
10123-24	Muscle	0.00 ppm	0.00000	---	---
10123-25	Muscle	0.00 ppm	0.00000	---	---
10123-34	Muscle	0.00 ppm	0.00000	---	---
10123-35	Muscle	0.00 ppm	0.00000	---	---
		Average	0.00000	---	---
		Std. Dev.	0.00000	---	---
0.01 ppm Fortifications					
10123-26	Muscle	0.01 ppm	0.01163	0.01163	116.3
10123-27	Muscle	0.01 ppm	0.01130	0.01130	113.0
10123-28	Muscle	0.01 ppm	0.01151	0.01151	115.1
10123-29	Muscle	0.01 ppm	0.01094	0.01094	109.4
10123-36	Muscle	0.01 ppm	0.01126	0.01126	112.6
10123-37	Muscle	0.01 ppm	0.01135	0.01135	113.5
10123-38	Muscle	0.01 ppm	0.01172	0.01172	117.2
				Average	113.9
				Std. Dev.	2.61
				COV (b)	2.3%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 5(Cont'd). Recovery of RPA090946 Residues from Bovine Muscle.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
0.05 ppm Fortifications					
10123-30	Muscle	0.05 ppm	0.05475	0.05475	109.5
10123-31	Muscle	0.05 ppm	0.05476	0.05476	109.5
10123-39	Muscle	0.05 ppm	0.05540	0.05540	110.8
10123-40	Muscle	0.05 ppm	0.05696	0.05696	113.9
10123-41	Muscle	0.05 ppm	0.05521	0.05521	110.4
				Average	110.8
				Std. Dev.	1.82
				COV (b)	1.6%
0.50 ppm Fortifications					
10123-32	Muscle	0.50 ppm	0.48827	0.48827	97.7
10123-33	Muscle	0.50 ppm	0.44549	0.44549	89.1
10123-42	Muscle	0.50 ppm	0.50241	0.50241	100.5
10123-43	Muscle	0.50 ppm	0.49420	0.49420	98.8
10123-44	Muscle	0.50 ppm	0.50283	0.50283	100.6
				Average	97.3
				Std. Dev.	4.76
				COV (b)	4.9%
				Grand Average	108.1
				Std. Dev.	7.89
				COV (b)	7.3%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 6. Recovery of RPA090946 Residues from Bovine Fat.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
UTC Samples					
10123-89	Fat	0.00 ppm	0.00182	---	---
10123-90	Fat	0.00 ppm	0.00000	---	---
10123-91	Fat	0.00 ppm	0.00000	---	---
10123-100R	Fat	0.00 ppm	0.00000	---	---
10123-101R	Fat	0.00 ppm	0.00000	---	---
		Average	0.00036	---	---
		Std. Dev.	0.00081	---	---
0.01 ppm Fortifications					
10123-92	Fat	0.01 ppm	0.01059	0.01023	102.3
10123-93	Fat	0.01 ppm	0.01005	0.00969	96.9
10123-94	Fat	0.01 ppm	0.01008	0.00972	97.2
10123-95	Fat	0.01 ppm	0.00997	0.00961	96.1
10123-102R	Fat	0.01 ppm	0.01178	0.01142	114.2
10123-103R	Fat	0.01 ppm	0.01246	0.01210	121.0
10123-104R	Fat	0.01 ppm	0.01221	0.01185	118.5
				Average	106.6
				Std. Dev.	10.94
				COV (b)	10.3%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.

(b) COV = Coefficient of Variation.

Table 6(Cont'd). Recovery of RPA090946 Residues from Bovine Fat.

Horizon Laboratory Number	Control Matrix	Fortification Level	ppm Found	Corrected ppm (a)	% Recovery
0.05 ppm Fortifications					
10123-96	Fat	0.05 ppm	0.05026	0.04990	99.8
10123-97	Fat	0.05 ppm	0.04748	0.04712	94.2
10123-105R	Fat	0.05 ppm	0.05213	0.05177	103.5
10123-106R	Fat	0.05 ppm	0.05196	0.05160	103.2
10123-107R	Fat	0.05 ppm	0.05518	0.05482	109.6
				Average	102.1
				Std. Dev.	5.64
				COV (b)	5.5%
0.50 ppm Fortifications					
10123-98	Fat	0.50 ppm	0.44315	0.44279	88.6
10123-99	Fat	0.50 ppm	0.43224	0.43188	86.4
10123-108R	Fat	0.50 ppm	0.43957	0.43921	87.8
10123-109R	Fat	0.50 ppm	0.44168	0.44132	88.3
10123-110R	Fat	0.50 ppm	0.44836	0.44800	89.6
				Average	88.1
				Std. Dev.	1.17
				COV (b)	1.3%
				Grand Average	99.8
				Std. Dev.	10.83
				COV (b)	10.9%

(a) ppm found are corrected for the average RPA090946 residue found in the companion control samples.
(b) COV = Coefficient of Variation.

Table 7. Analysis of Variance for Recovery of RPA090946 Residues from Bovine Matrices.

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F	PROBABILITY
MATRIX	4	1707.3	426.8	49.8	<<0.01
LEVEL	2	3149.6	1574.8	183.9	<<0.01
DAY OF ANALYSIS	1	40.4	40.4	4.7	<0.05
INTERACTION	22	1456.6	66.2	7.7	<0.01
ERROR	55	471.1	8.6		

Table 8. Minimum Detection Limit (MDL) and Limit of Quantification (LOQ) for Recovery of RPA090946 from Bovine Matrices.

Matrix	Average Residue in UTCs (ppm)	Standard Deviation of UTCs at 0.01 ppm (a)	MDL (ppm) (b)	LOQ (ppm) (c)
Whole Milk	0.00046	0.00053	0.0021	0.0058
Muscle	0.00000	0.00026	0.0008	0.0026
Kidney	0.00000	0.00042	0.0013	0.0042
Liver	0.00039	0.00059	0.0022	0.0063
Fat	0.00036	0.00109	0.0036	0.0113

(a) Standard deviations were calculated from gross recovered residues (also see Tables 2 through 6).
(b) MDL = The average residue of UTC samples plus 3-times the sample standard deviation of UTC samples fortified at 0.01 ppm.
(c) LOQ = The average residue of UTC samples plus 10-times the sample standard deviation of UTC samples fortified at 0.01 ppm.

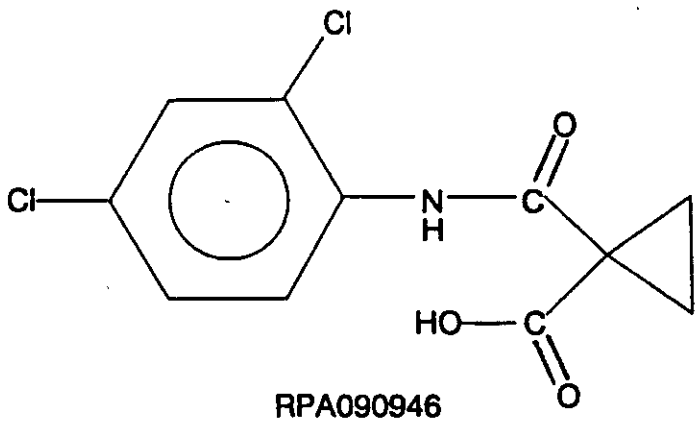
Table 9. Precision and Accuracy for Recovery of RPA090946 from Bovine Matrices.

Matrix	Fortification Level (ppm)	Number of Samples	Mean Net Recovery	Standard Deviation	COV (%) (a)
Whole Milk	0.01	7	100.3	5.32	5.3
	0.05	5	101.4	2.07	2.0
	0.50	5	91.8	3.87	4.2
Muscle	0.01	7	113.9	2.61	2.3
	0.05	5	110.8	1.82	1.6
	0.50	5	97.3	4.76	4.9
Kidney	0.01	7	114.2	4.23	3.7
	0.05	5	110.2	4.60	4.2
	0.50	5	97.7	3.19	3.3
Liver	0.01	7	103.3	5.94	5.8
	0.05	5	100.5	3.37	3.4
	0.50	5	92.1	1.97	2.1
Fat	0.01	7	106.6	10.94	10.3
	0.05	5	102.1	5.64	5.5
	0.50	5	88.1	1.17	1.3

(a) COV = Coefficient of variation = (standard deviation/mean recovery) X 100.

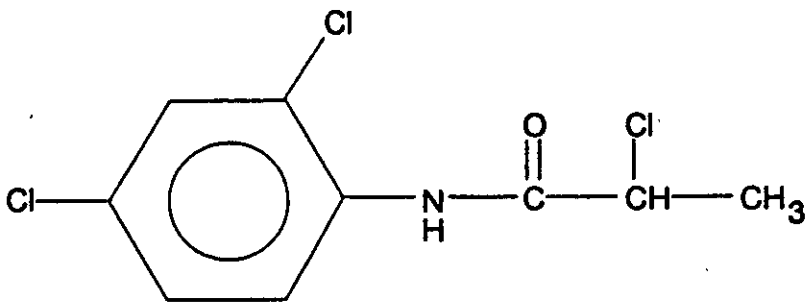
VI. FIGURES

Figure 1. Chemical Structures of RPA090946 and 2,4-DCPA.



RPA090946

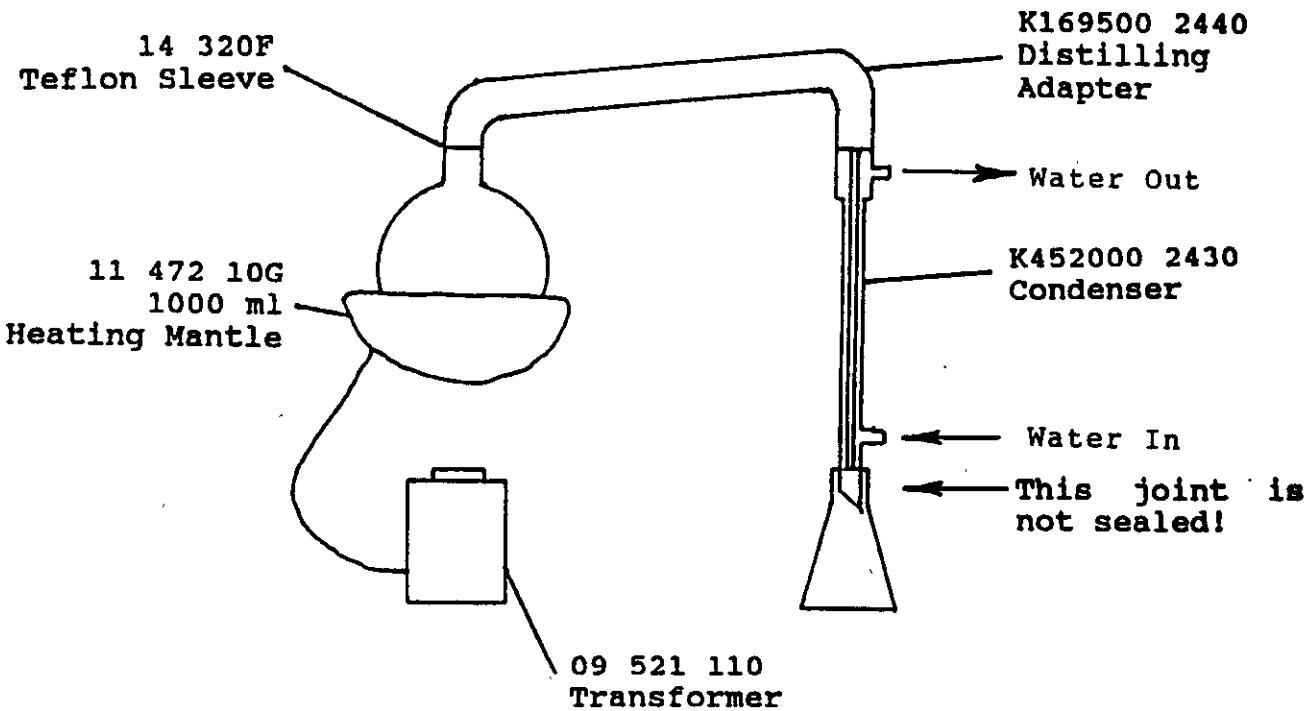
1-(2,4-DICHLOROPHENYLAMINOCARBONYL)-
CYCLOPROPANE CARBOXYLIC ACID



2,4-DCPA

N-(2,4-DICHLOROPHENYL)-2-CHLOROPROPYLAMIDE

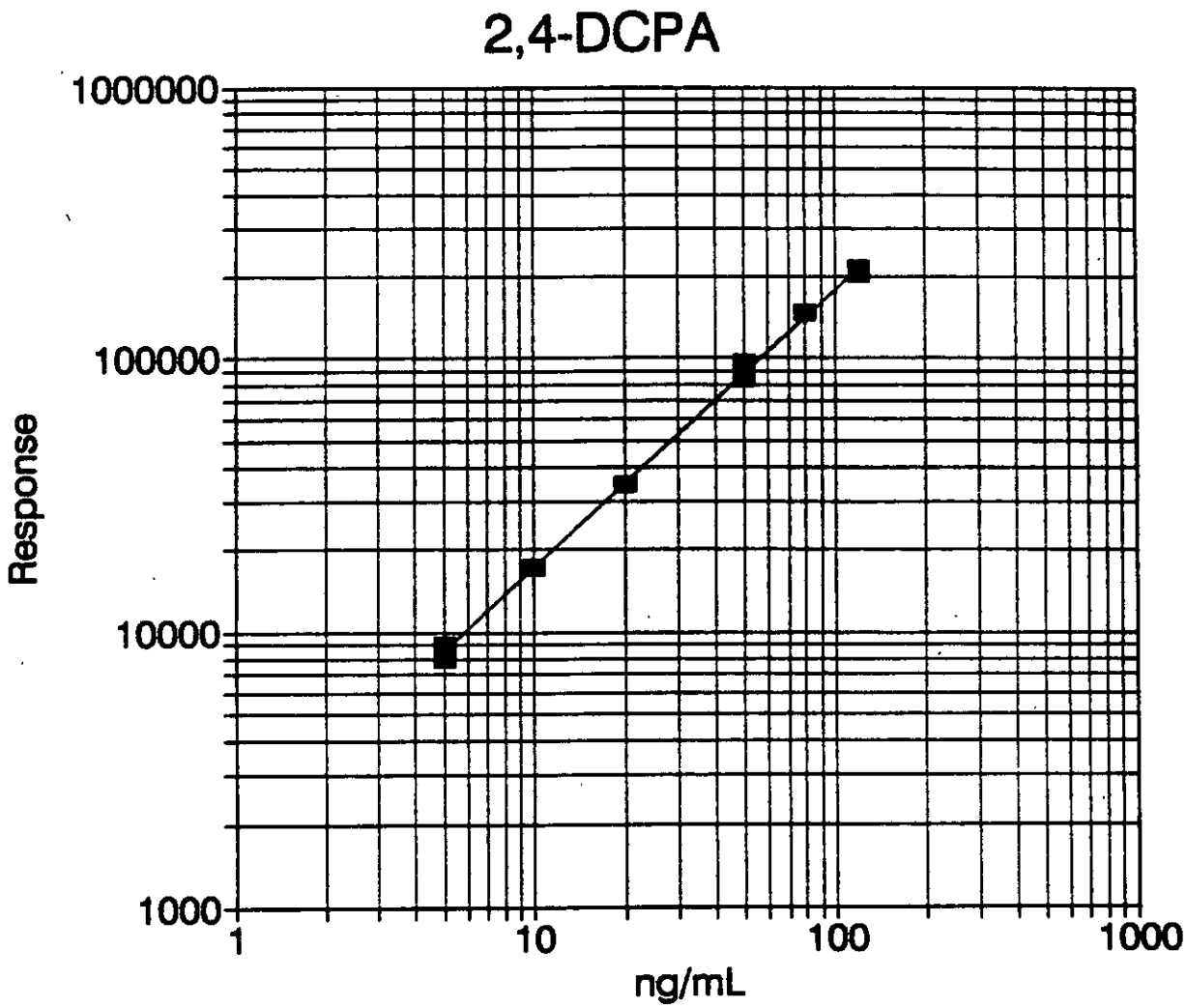
Figure 2. Distillation Unit Setup



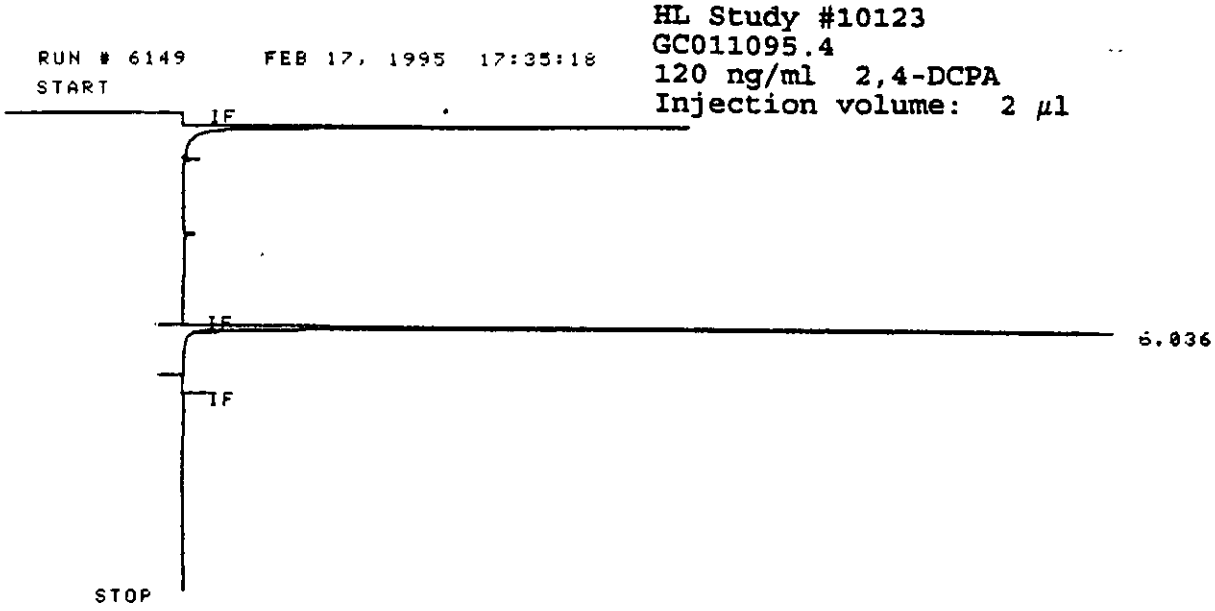
Note: Fisher Scientific part numbers.

Figure 3. Example Chromatography

Example Standard Curve



HL Study #10123
GC011095.4
120 ng/ml 2,4-DCPA
Injection volume: 2 µl



RUN# 6149 FEB 17, 1995 17:35:18

SAMPLE# 6

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

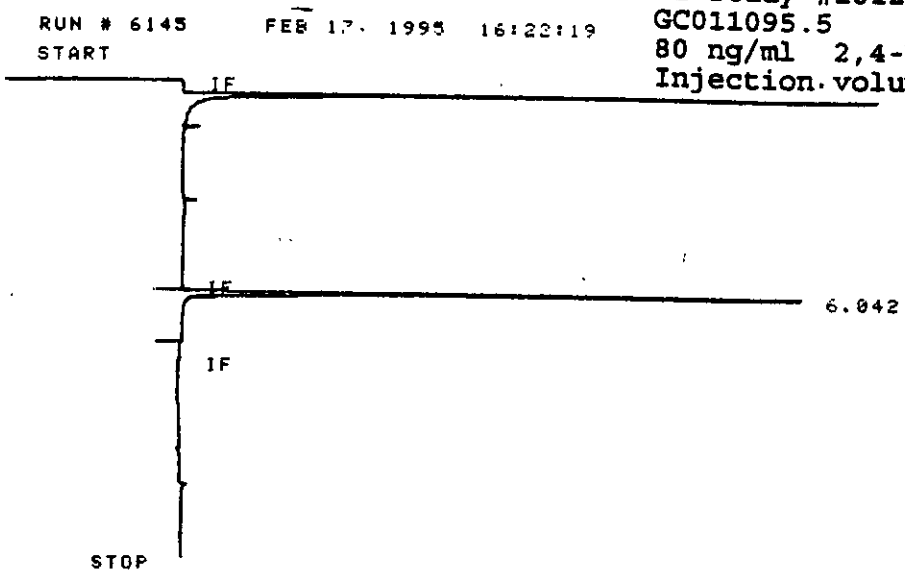
Y

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.036	BB	886799	.066	223072	1R	.002	2,4-DCPA

TOTAL HEIGHT= 223072
MUL FACTOR=1.0000E+00

HL Study #10123
GC011095.5
80 ng/ml 2,4-DCPA
Injection.volume: 2 µl



RUN# 6145 FEB 17, 1995 16:22:19

SAMPLE# 2

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

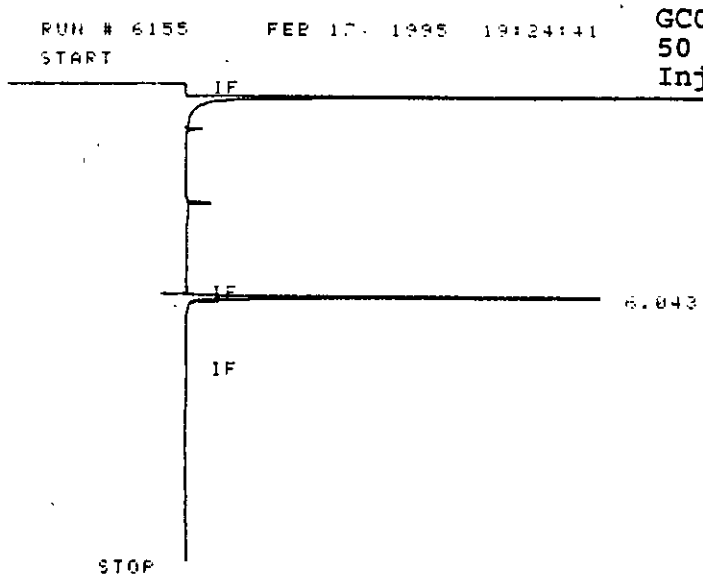
Y

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.042	BB	593543	.066	148822	1R	.001	2,4-DCPA

TOTAL HEIGHT= 148822
MUL FACTOR=1.0000E+00

HL Study #10123
GC011095.6
50 ng/ml 2,4-DCPA
Injection volume: 2 µl



RUN# 6155 FEB 17, 1995 19:24:41

SAMPLE# 12

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

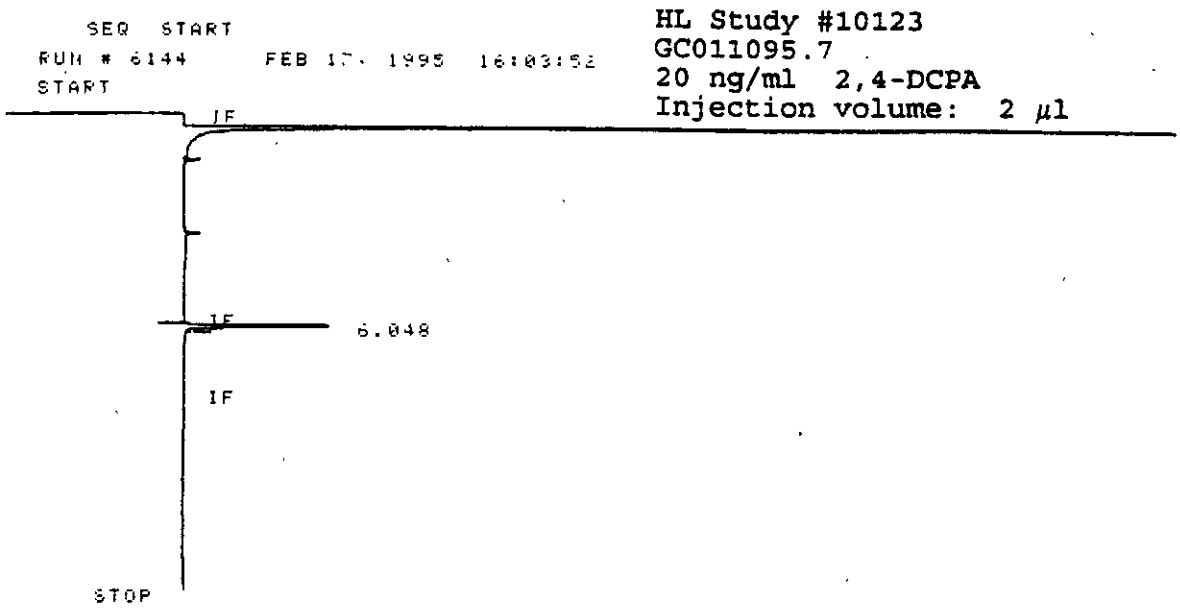
V

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.043	BB	386992	.065	99065	1R	.001	2,4-DCPA

TOTAL HEIGHT= 99065
MUL FACTOR=1.0000E+00

HL Study #10123
GC011095.7
20 ng/ml 2,4-DCPA
Injection volume: 2 µl



RUN# 6144 FEB 17, 1995 16:03:52

SAMPLE# 1

METHOD NAME: M*DCPA.MET

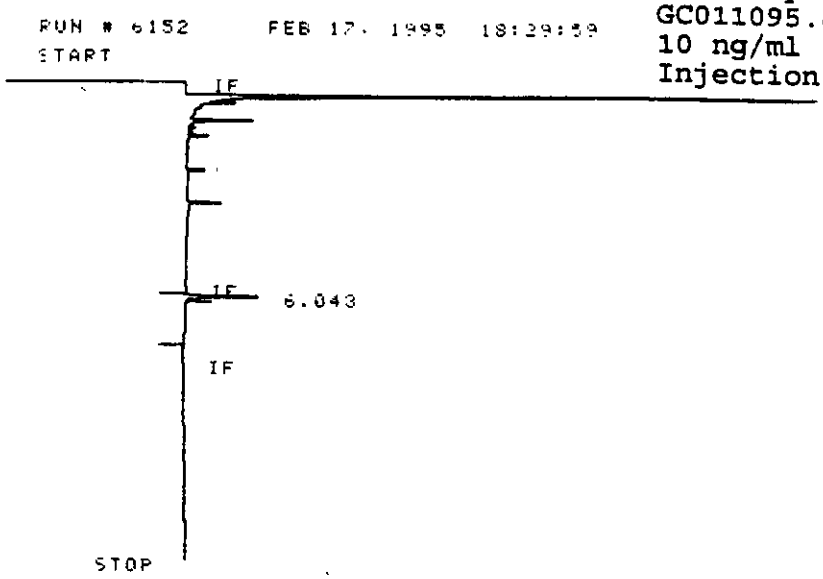
IDENTIFIER : INST HL #529

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.048	BB	142479	.066	35798	1R	.000	2,4-DCPA

TOTAL HEIGHT= 35798
MUL FACTOR=1.0000E+00

HL Study #10123
GC011095.8
10 ng/ml 2,4-DCPA
Injection volume: 2 µl



RUN# 6152 FEB 17, 1995 18:29:59

SAMPLE# 9

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

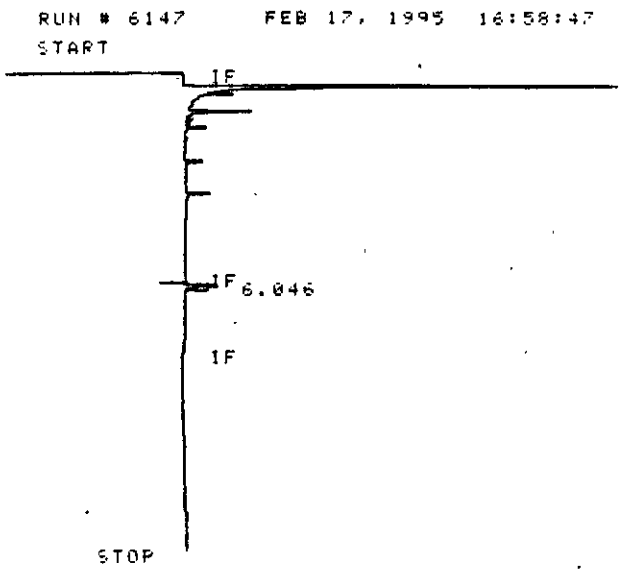
Y

ESTD-HEIGHT

PT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.043	BB	69159	.065	17648	1P	.000	2,4-DCPA

TOTAL HEIGHT= 17648
MUL FACTOR=1.0000E+00

HL Study #10123
GC011095.9
5 ng/ml 2,4-DCPA
Injection volume: 2 µl



RUN# 6147 FEB 17, 1995 16:58:47

SAMPLE# 4

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

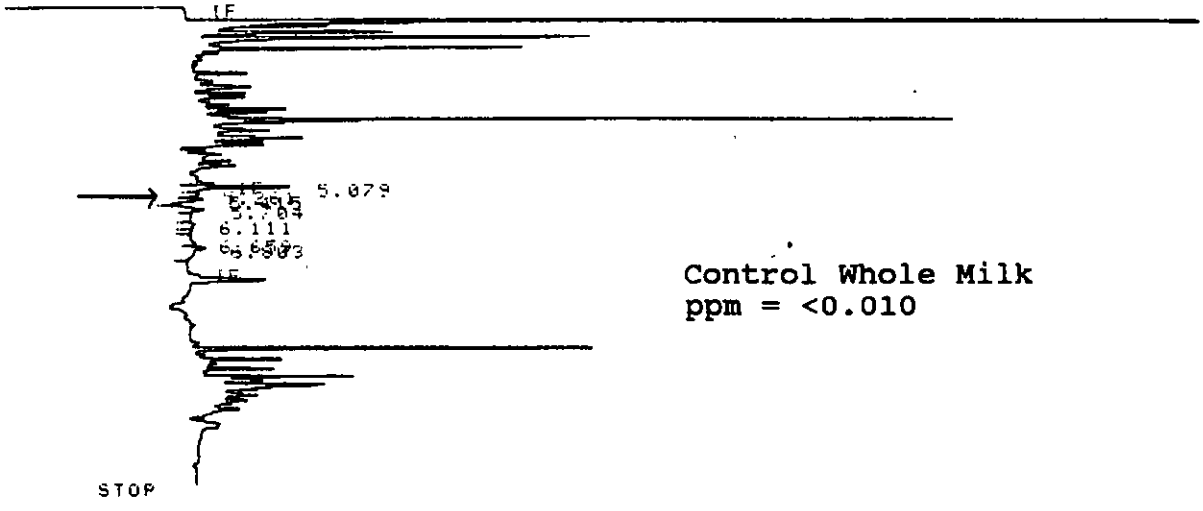
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.046	BB	31598	.068	7708	1R	.000	2,4-DCPA

TOTAL HEIGHT= 7708
MUL FACTOR=1.0000E+00

HL Study #10123
10123-1R 2 ml
Whole Milk UTC
Injection volume: 2 µl

RUN # 5138 JAN 17, 1995 13:27:25
START



Control Whole Milk
ppm = <0.010

RUN# 5138 JAN 17, 1995 13:27:25

SAMPLE# : 35

METHOD NAME: M*DCPA.MET

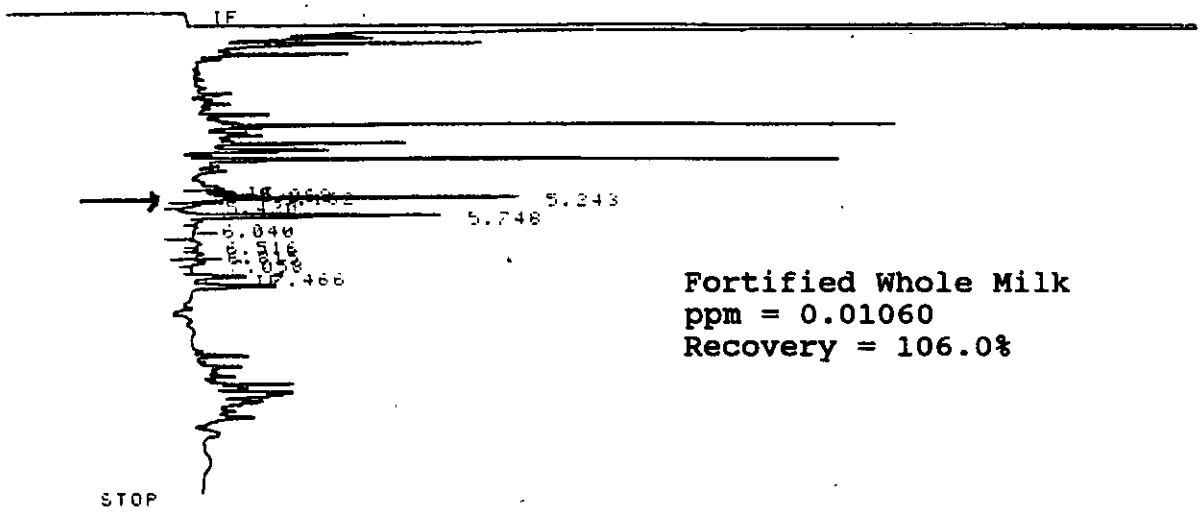
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.079	BV	17311	.054	5355		.000	
5.261	VV	4226	.116	608	1	.000	2,4-DCPA
5.415	VP	8516	.095	1500		.000	
5.704	PV	14329	.119	2000		.000	
6.111	VV	14008	.200	1167		.000	
6.659	VV	15181	.283	895		.000	
6.803	VV	16874	.190	1477		.000	

TOTAL HEIGHT= 13002
MUL FACTOR=1.0000E+00

HL Study #10123
10123-5R 2 ml
Whole Milk UTC + 0.01 ppm
Injection volume: 2 µl

RUN # 5145 JAN 17, 1995 21:34:19
START



Fortified Whole Milk
ppm = 0.01060
Recovery = 106.0%

RUN# 5145 JAN 17, 1995 21:34:19

SAMPLE# 43

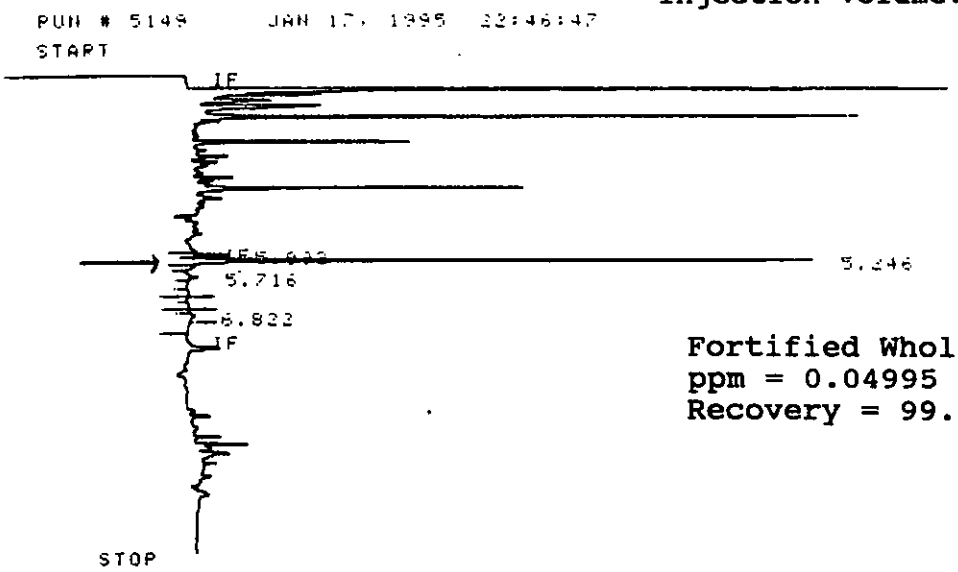
METHOD NAME: M*OCPH.MET

IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.088	BV	5337	.050	1779		.000	
5.162	VV	9457	.047	3334		.000	
5.243	VB	58643	.050	19370	1	.000	2,4-DCPH
5.420	BP	3843	.087	740		.000	
5.748	PV	67835	.072	15756		.000	
6.040	VB	3674	.112	549		.000	
6.516	BV	4390	.120	609		.000	
6.815	PB	2475	.073	566		.000	
7.058	BP	4712	.090	870		.000	
7.466	I PH	12894	.067	3186		.000	

TOTAL HEIGHT= 46759
MUL FACTOR=1.0000E+00

HL Study #10123
10123-8R 5 ml
Whole Milk UTC + 0.05 ppm
Injection volume: 2 µl



Fortified Whole Milk
ppm = 0.04995
Recovery = 99.9%

RUN# 5149 JAN 17, 1995 12:46:47

SAMPLE# 46

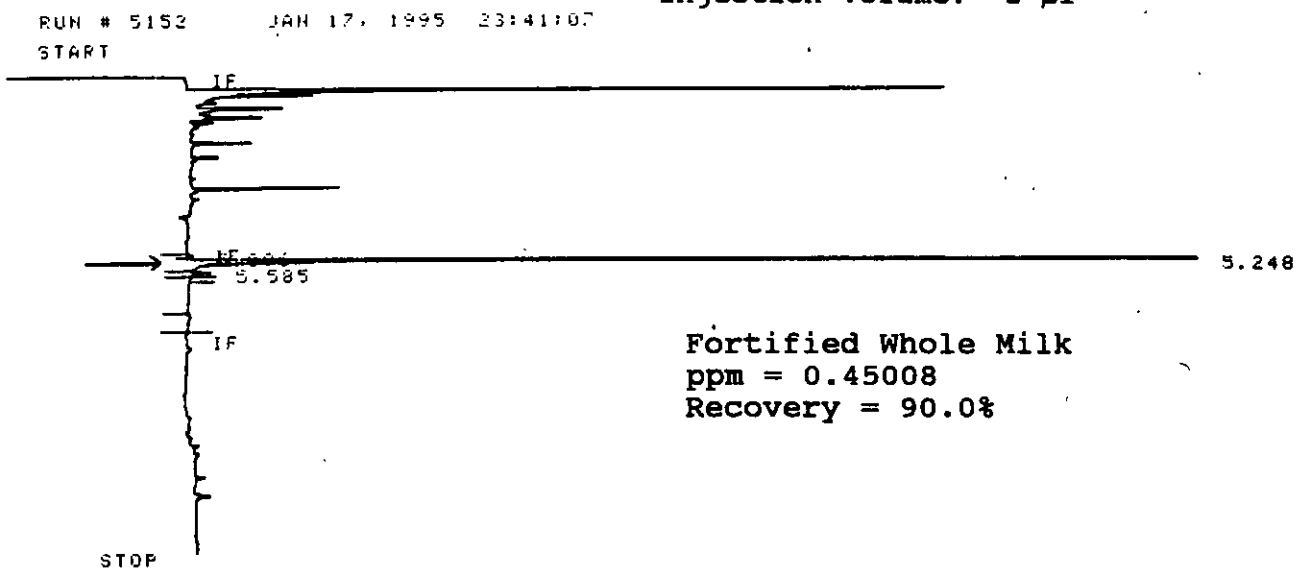
METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.092	BP	7675	.055	2323		.000	
5.246	PB	109017	.049	37376	1	.000	2,4-DCPA
5.716	PV	6286	.117	894		.000	
6.822	PB	3212	.083	442		.000	

TOTAL HEIGHT= 41035
MUL FACTOR=1.0000E+00

HL Study #10123
10123-10R 25 ml
Whole Milk UTC + 0.50 ppm
Injection volume: 2 µl



Fortified Whole Milk
ppm = 0.45008
Recovery = 90.0%

RUN# 5152 JAN 17, 1995 23:41:07

SAMPLE# 49

METHOD NAME: M+DCPA.MET

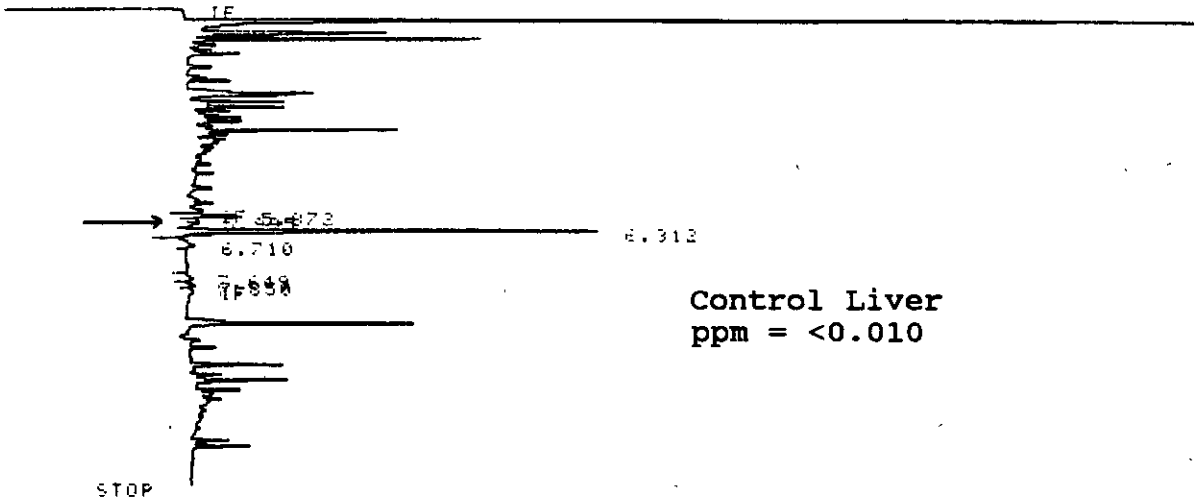
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.096	BP	1134	.054	349		.000	
5.248	PB	208095	.049	70566	1	.000	2,4-DCPA
5.585	BB	4043	.056	1214		.000	

TOTAL HEIGHT= 72129
MUL FACTOR=1.0000E+00

HL Study #10123
10123-67 2 ml
Liver UTC
Injection volume: 2 µl

RUN # 6391 FEB 24. 1995 17:20:18
START



Control Liver
ppm = <0.010

RUN# 6391 FEB 24. 1995 17:20:18

SAMPLE# 3

METHOD NAME: M+DCPH.MET

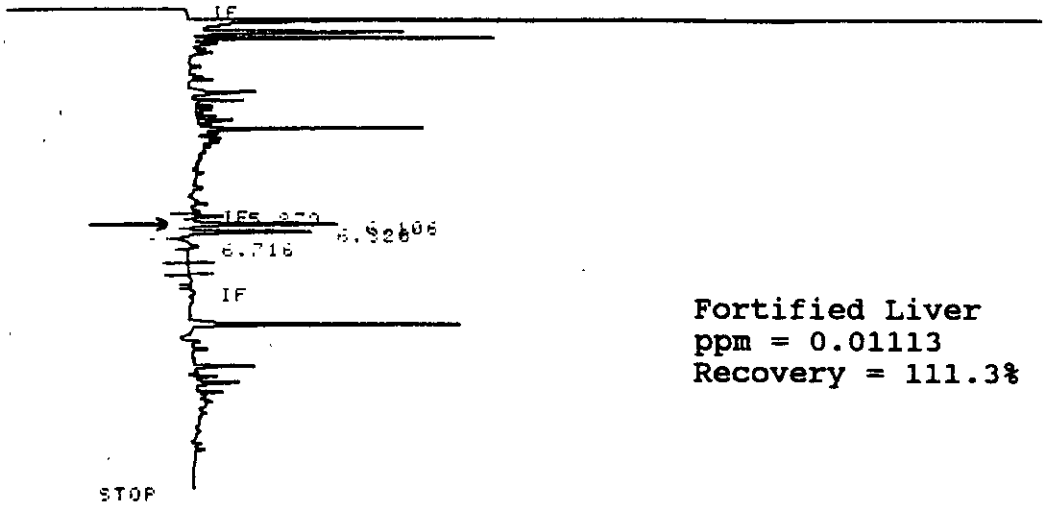
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
5.872	BV	37372	.063	9937		.000	
5.995	VV	5373	.053	1690		.000	
6.312	PB	396093	.067	98937		.000	
6.710	BV	36885	.152	4040		.000	
7.649	VV	44432	.231	3207	1	.000	3,5-DCPH
7.850	VV	28052	.123	3810		.000	

TOTAL HEIGHT= 121621
MUL FACTOR=1.0000E+00

HL Study #10123
10123-72 2 ml
Liver UTC + 0.01 ppm
Injection volume: 2 µl

RUN # 6399 FEB 24, 1995 19:46:09
START



Fortified Liver
ppm = 0.01113
Recovery = 111.3%

RUN# 6399 FEB 24, 1995 19:46:09

SAMPLE# 11

METHOD NAME: M*DCPH.MET

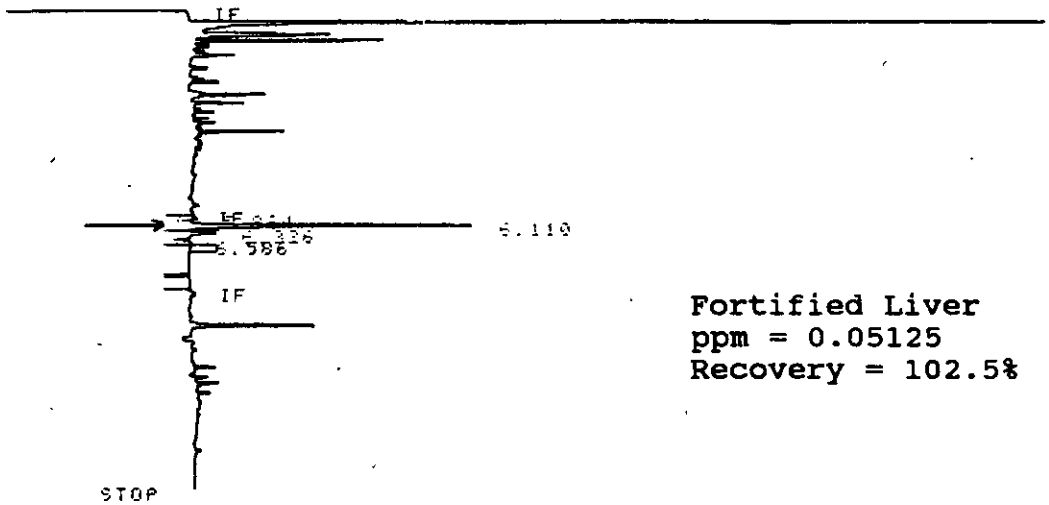
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.879	BV	27443	.063	7293		.000	
6.106	VV	147430	.067	36655	1R	.000	2,4-DCPA
6.320	VP	132055	.071	31120		.000	"
6.716	PV	35836	.148	4037		.000	

TOTAL HEIGHT= 79105
MUL FACTOR=1.0000E+00

HL Study #10123
10123-74 5 ml
Liver UTC + 0.05 ppm
Injection volume: 2 µl

RUN # 6402 FEB 24, 1995 20:40:40
START



Fortified Liver
ppm = 0.05125
Recovery = 102.5%

RUN# 6402 FEB 24, 1995 20:40:40

SAMPLE# 14

METHOD NAME: M*OCPA.MET

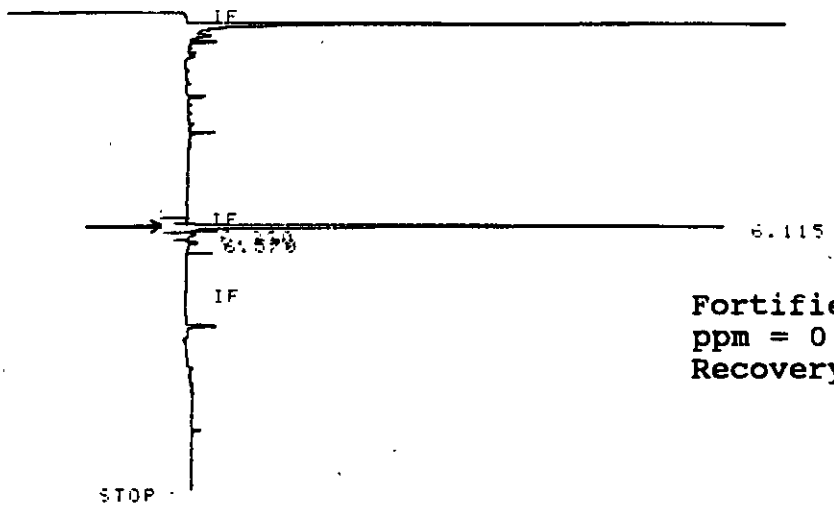
IDENTIFIER : INST HL #529
ESTD-HEIGHT

PT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
5.824	PP	5018	.059	1423		.000	
6.110	PB	256238	.063	67807	1R	.001	2,4-DCPA
6.326	BP	30140	.075	6695		.000	
6.586	PB	5261	.080	1090		.000	

TOTAL HEIGHT= 77103
MUL FACTOR=1.0000E+00

HL Study #10123
10123-76 25 ml
Liver UTC + 0.50 ppm
Injection volume: 2 µl

RUN # 6405 FEB 24, 1995 21:35:16
START



Fortified Liver
ppm = 0.47207
Recovery = 94.4%

RUN# 6405 FEB 24, 1995 21:35:16

SAMPLE# 17

METHOD NAME: M*DCPA.MET

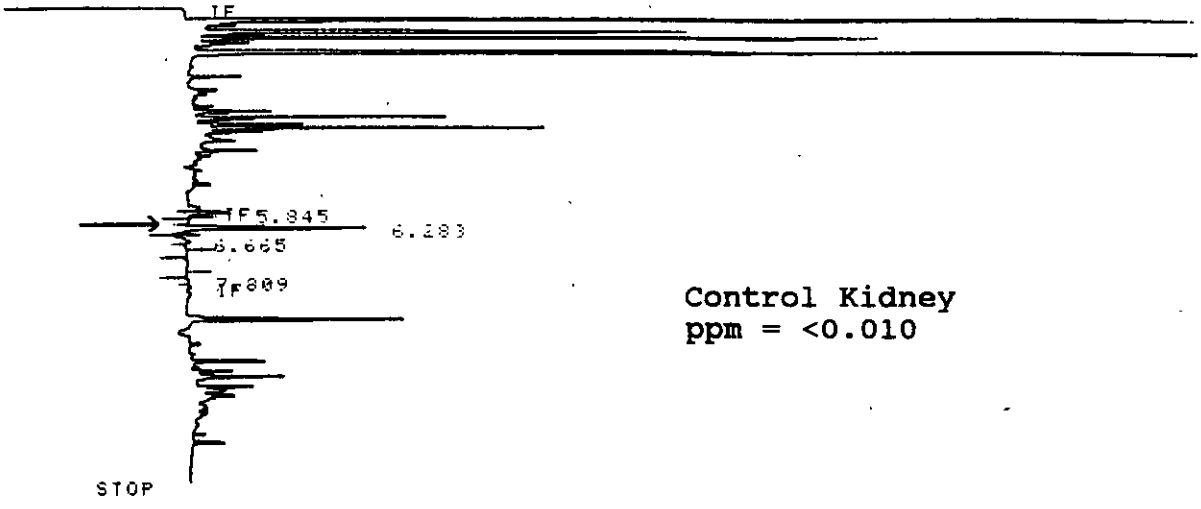
IDENTIFIER : INST HL #529
EXTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.115	PB	500943	.065	123485	1R	.001	2,4-DCPA
6.330	BP	5721	.059	1693		.000	
6.570	PB	14389	.080	2907		.000	

TOTAL HEIGHT= 128085
MUL FACTOR=1.0000E+00

HL Study #10123
10123-46 2 ml
Kidney UTC
Injection volume: 2 µl

RUN # 6301 FEB 22, 1995 20:41:08
START



Control Kidney
ppm = <0.010

RUN# 6301 FEB 22, 1995 20:41:08

SAMPLE# 36

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #539

Y

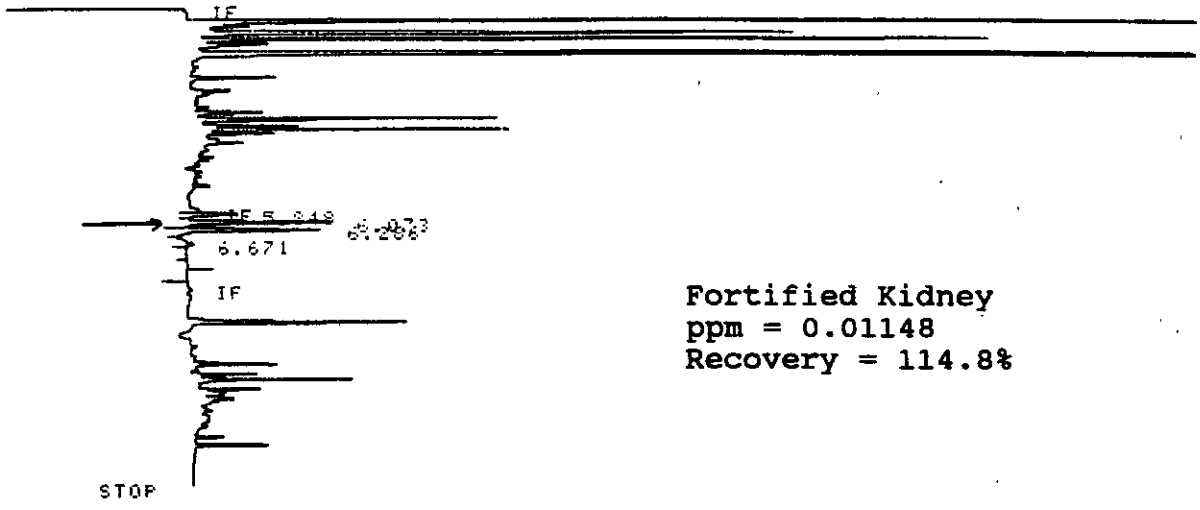
NO CALIB PEAKS FOUND
HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
5.845	6865	BB	.044	12.47501
6.283	44532	VB	.069	80.92314
6.665	2518	PV	.142	4.57569
7.809	1115	PV	.091	2.02617

TOTAL HEIGHT= 55030
MUL FACTOR=1.0000E+00

HL Study #10123
10123-49 2 ml
Kidney UTC + 0.01 ppm
Injection volume: 2 µl

RUN # 6305 FEB 22, 1995 21:54:07
START



Fortified Kidney
ppm = 0.01148
Recovery = 114.8%

RUN# 6305 FEB 22, 1995 21:54:07

SAMPLE# 30

METHOD NAME: M*DCPA.MET

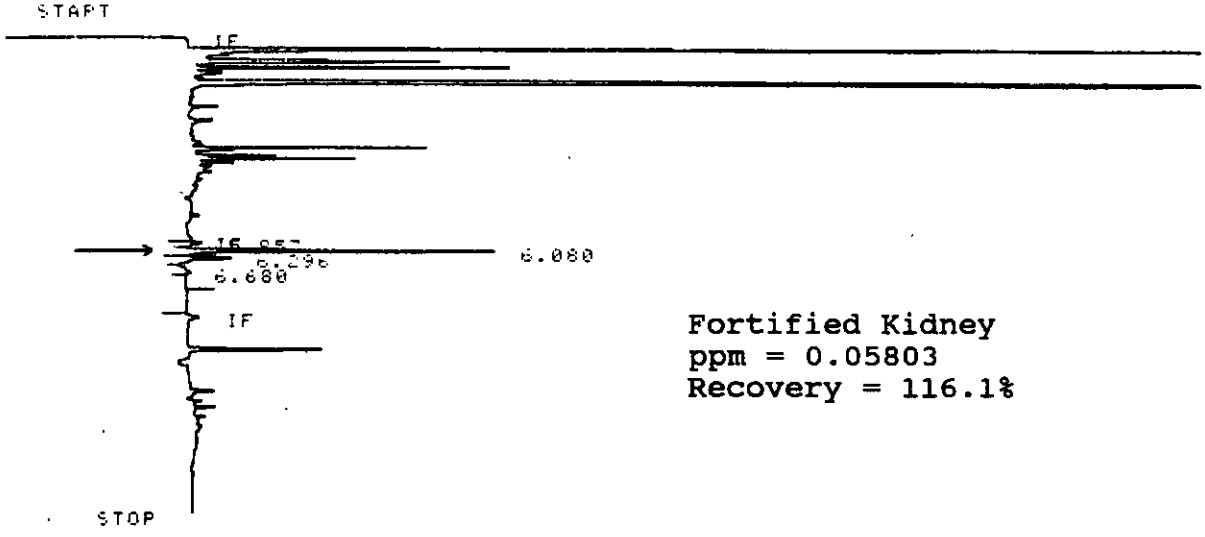
IDENTIFIER : INST HL #529

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.848	BP	28616	.053	8957		.000	
6.073	PB	125631	.060	34651	1R	.000	3,4-DCPA
6.286	BP	131775	.066	33302		.000	
6.671	PV	30524	.151	3372		.000	

TOTAL HEIGHT= 80282
MUL FACTOR=1.0000E+00

HL Study #10123
10123-53 5 ml
Kidney UTC + 0.05 ppm
Injection volume: 2 µl



Fortified Kidney
ppm = 0.05803
Recovery = 116.1%

RUN# 6311 FEB 22, 1995 23:43:40

SAMPLE# 36

METHOD NAME: M*DCPH.MET

IDENTIFIER : INST HL #529

Y

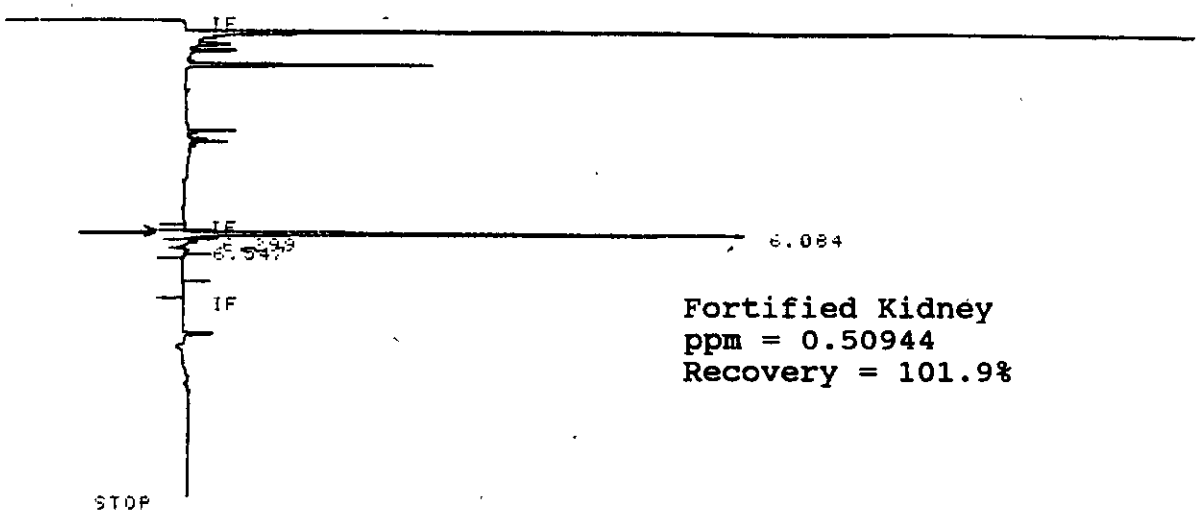
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
5.397	BP	9621	.057	2795		.000	
6.080	PB	271026	.061	73613	1R	.001	2,4-DCPH
6.296	BP	46703	.072	10748		.000	
6.680	PV	25120	.173	3417		.000	

TOTAL HEIGHT= 89573
MUL FACTOR=1.0000E+00

HL Study #10123
10123-54 25 ml
Kidney UTC + 0.50 ppm
Injection volume: 2 µl

RUN # 6313 FEB 23, 1995 00:20:10



Fortified Kidney
ppm = 0.50944
Recovery = 101.9%

RUN# 6313 FEB 23, 1995 00:20:10

SAMPLE# 38

METHOD NAME: M*DCPH.MET

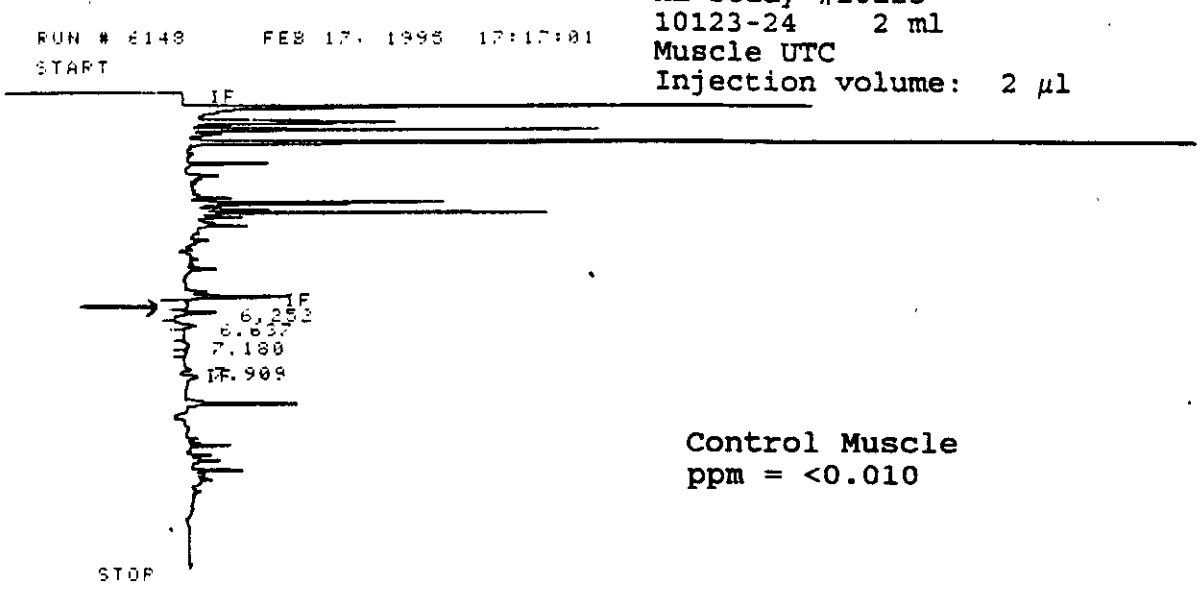
IDENTIFIER : INST HL #529

ESTD-HEIGHT

PT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.094	PB	518096	.064	134483	IP	.001	2,4-DCPA
6.299	BP	15785	.089	2961		.000	
6.547	PB	8965	.077	1934		.000	

TOTAL HEIGHT= 139378
MUL FACTOR=1.0000E+00

HL Study #10123
10123-24 2 ml
Muscle UTC
Injection volume: 2 µl



Control Muscle
ppm = <0.010

RUN# 6148 FEB 17, 1995 17:17:01

SAMPLE# 5

METHOD NAME: M*DCPH.MET

IDENTIFIER : INST HL #529

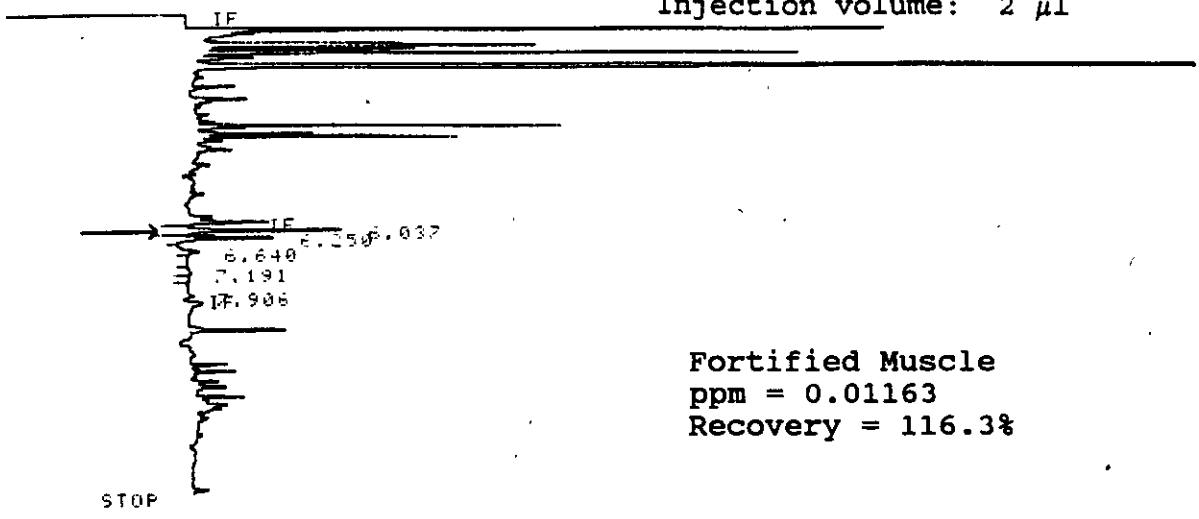
ESTD-HEIGHT

	RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
	6.252	VP	42435	.076	9302	1R	.000	~~2-4-DCPH~~ ①
	6.637	PV	37569	.121	5179		.000	
	7.180	VV	39715	.221	3001		.000	
	7.909	I VP	48234	.310	2593	2	.000	3,5-DCPH

TOTAL HEIGHT= 20075
MUL FACTOR=1.0000E+00

① MIS-IDENTIFIED PEAK 2-23-95 LDC

HL Study #10123
10123-26 2 ml
Muscle UTC + 0.01 ppm
Injection volume: 2 µl



Fortified Muscle
ppm = 0.01163
Recovery = 116.3%

RUN# 6151 FEB 17, 1995 18:11:47

SAMPLE# 8

METHOD NAME: M*DCPA.MET

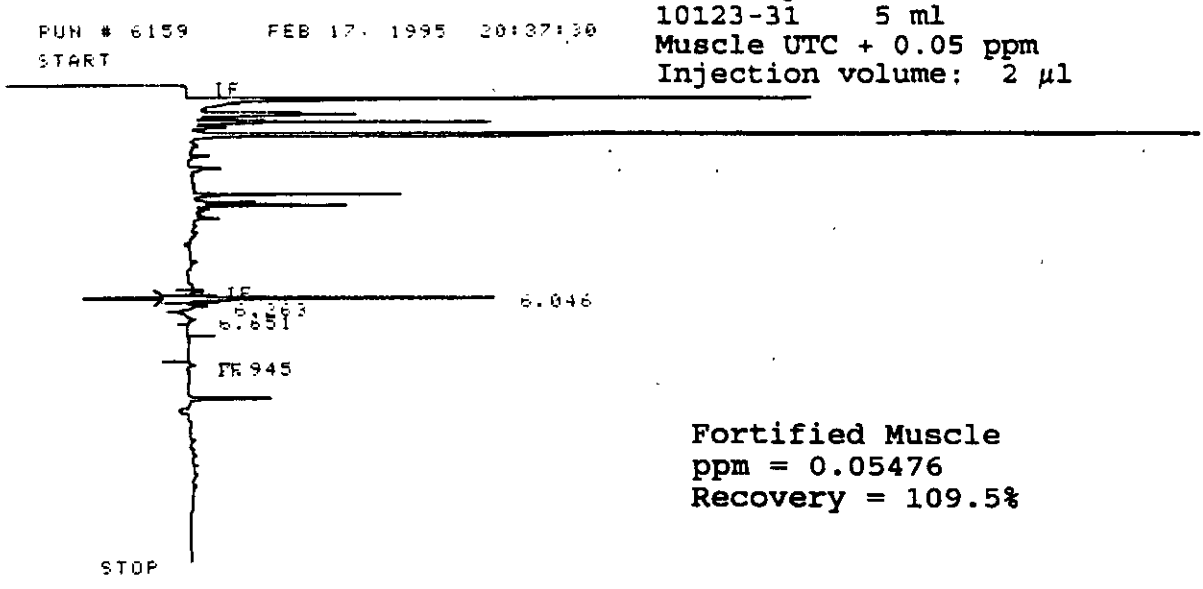
IDENTIFIER : INST HL #529

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.037	BB	138229	.061	37513	1R	.000	3,4-DCPA
6.250	BP	86801	.066	21980		.000	
6.640	PV	40624	.122	5555		.000	
7.191	VV	37393	.225	2764		.000	
7.906	I VP	49166	.317	2585	2	.000	3,5-DCPA

TOTAL HEIGHT= 70397
MUL FACTOR=1.0000E+00

HL Study #10123
10123-31 5 ml
Muscle UTC + 0.05 ppm
Injection volume: 2 µl



Fortified Muscle
ppm = 0.05476
Recovery = 109.5%

RUN# 6159 FEB 17, 1995 20:37:30

SAMPLE# 16

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

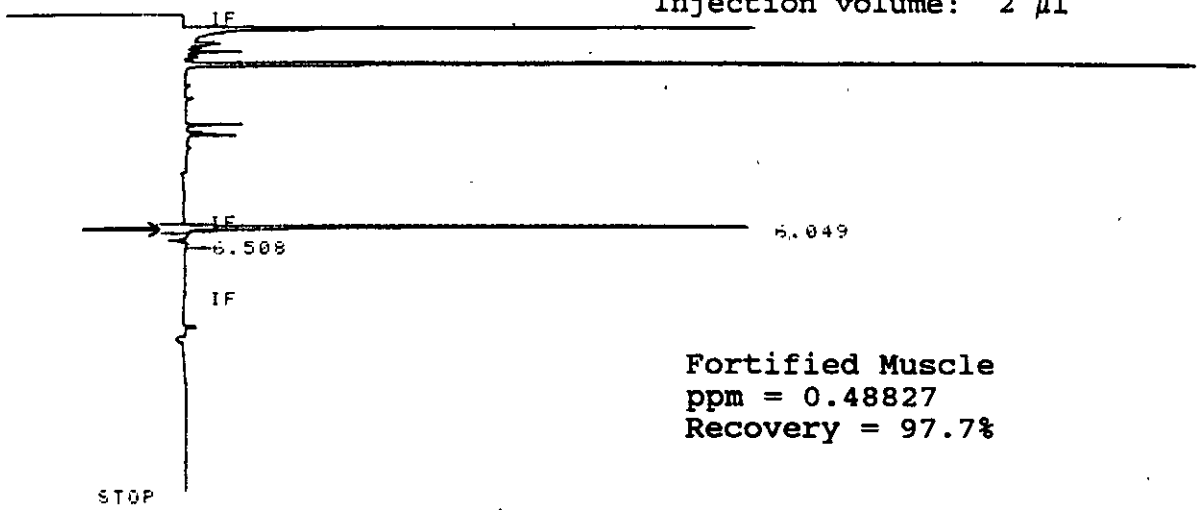
Y

ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.046	PB	270095	.061	73324	1R	.001	2,4-DCPA
6.263	BP	26745	.085	5226		.000	
6.651	PV	29619	.170	2900		.000	
7.945 I	BH	7675	.087	1469	2	.000	3,5-DCPA

TOTAL HEIGHT= 82919
MUL FACTOR=1.0000E+00

HL Study #10123
10123-32 25 ml
Muscle UTC + 0.50 ppm
Injection volume: 2 µl



Fortified Muscle
ppm = 0.48827
Recovery = 97.7%

RUN# 6160 FEB 17, 1995 20:55:39

SAMPLE# 17

METHOD NAME: M*DCPA.MET

IDENTIFIER : INST HL #529

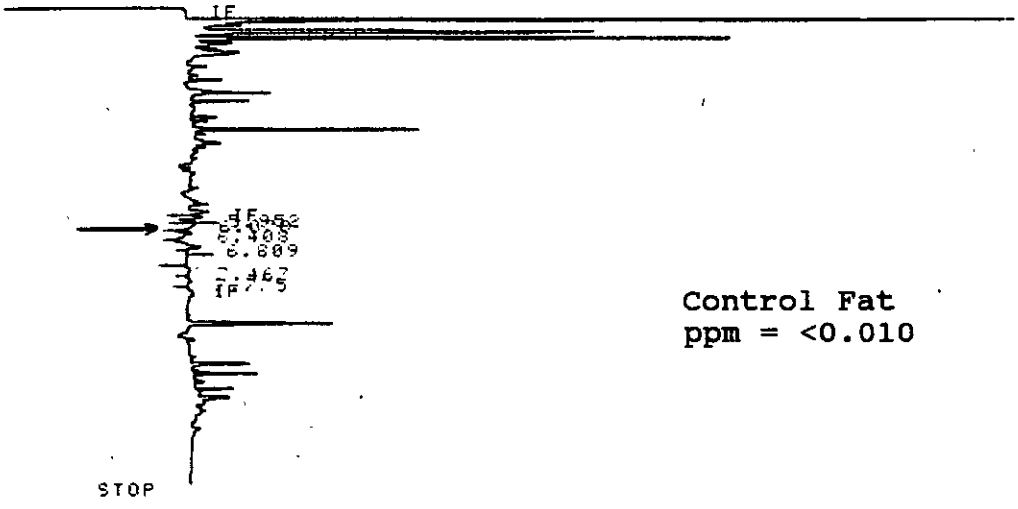
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
6.049	PB	518368	.064	135191	1R	.001	2,4-DCPA
6.508	PB	9597	.082	1943		.000	

TOTAL HEIGHT= 137134
MUL FACTOR=1.0000E+00

HL Study #10123
10123-90 1.5 ml
Fat UTC
Injection volume: 2 µl

RUN # 6569 MAR 1, 1995 04:41:52
START



Control Fat
ppm = <0.010

RUN# 6569 MAR 1, 1995 04:41:52

SAMPLE# 26

METHOD NAME: M*DCPA.MET

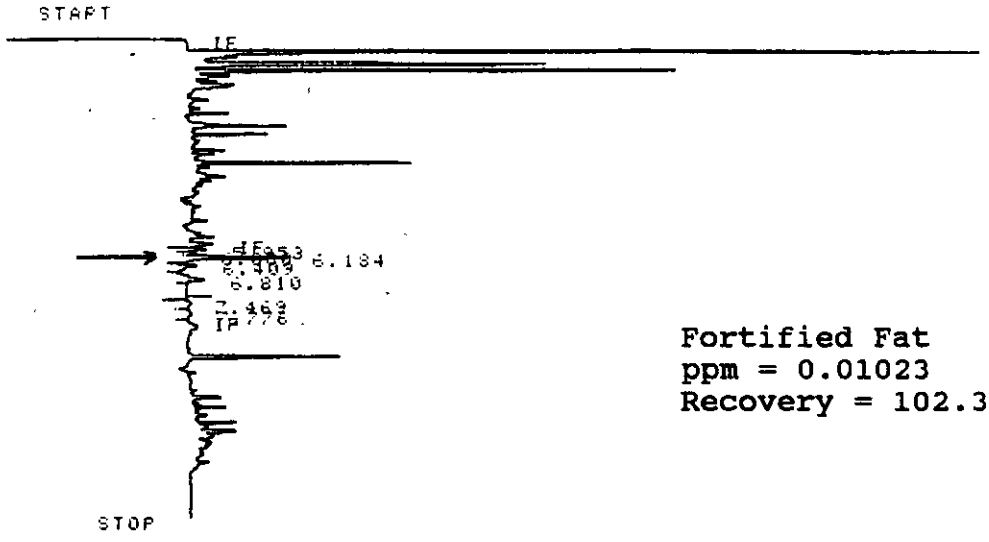
IDENTIFIER : INST HL #529
NO CALIB PEAKS FOUND
HEIGHT%

RT	HEIGHT	TYPE	WIDTH	HEIGHT%
5.952	3214	BP	.059	20.043e6
6.078	881	PB	.044	5.49423
6.408	3927	PP	.102	24.49018
6.809	486E	PV	.128	30.34e11
7.467	1668	BV	.124	10.40224
7.775	1479	VV	.190	9.22357

TOTAL HEIGHT= 16035
MUL FACTOR=1.0000E+00

HL Study #10123
10123-92 1.5 ml
Fat UTC + 0.01 ppm
Injection volume: 2 µl

RUN # 6572 MAR 1, 1995 05:36:01



Fortified Fat
ppm = 0.01023
Recovery = 102.3%

RUN# 6572 MAR 1, 1995 05:36:01

SAMPLE# 29

METHOD NAME: M*DCPH.MET

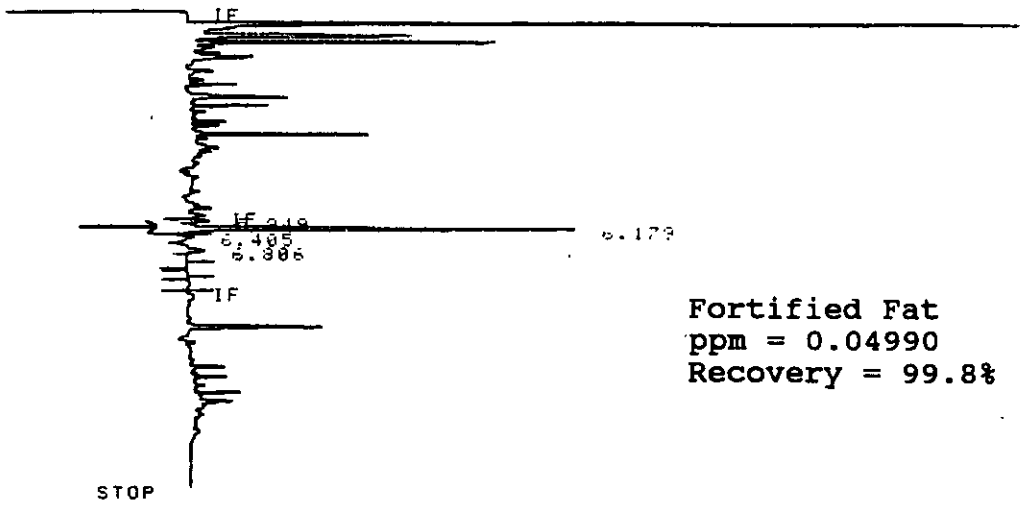
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CAL#	AMOUNT	NAME
5.953	BV	14379	.062	4051		.000	
6.080	VV	8103	.054	2518		.000	
6.184	VP	115609	.075	25541	1F	.000	2,4-DCPH
6.409	PP	26382	.091	4855		.000	
6.810	PV	49583	.135	6133		.000	
7.469	BV	9761	.113	1436		.000	
7.776	VV	17676	.178	1659		.000	

TOTAL HEIGHT= 46192
MUL FACTOR=1.0000E+00

HL Study #10123
10123-96 2 ml
Fat UTC + 0.05 ppm
Injection volume: 2 µl

RUN # 6573 MAR 1, 1995 07:24:17
START



Fortified Fat
ppm = 0.04990
Recovery = 99.8%

RUN# 6573 MAR 1, 1995 07:24:17

SAMPLE# 25

METHOD NAME: M*DCPA.MET

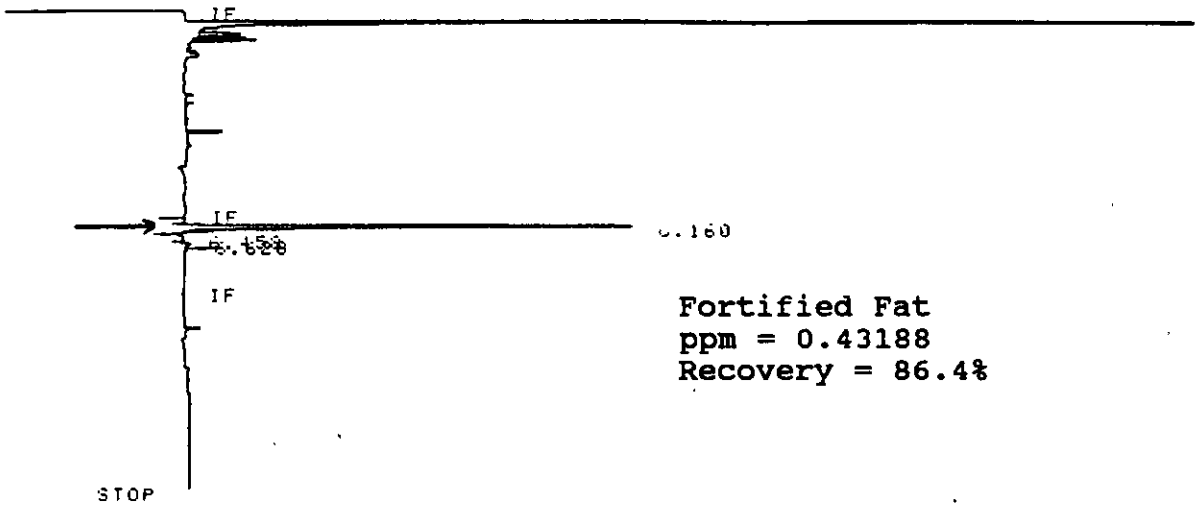
IDENTIFIER : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
5.949	BV	19512	.066	4908		.000	
6.179	VB	380117	.067	94055	1R	.001	2,4-DCPA
6.405	PP	24877	.099	4171		.000	
6.806	PV	50090	.140	5977		.000	

TOTAL HEIGHT= 109111
MUL FACTOR=1.0000E+00

HL Study #10123
10123-99 15 ml
Fat UTC + 0.50 ppm
Injection volume: 2 µl

RUN # 6583 MAR 1, 1995 08:54:49
START



Fortified Fat
ppm = 0.43188
Recovery = 86.4%

RUN# 6583 MAR 1, 1995 08:54:49

SAMPLE# 40

METHOD NAME: M*DCPH.MET

IDENTIFIED : INST HL #529
ESTD-HEIGHT

RT	TYPE	AREA	WIDTH	HEIGHT	CHL#	AMOUNT	NAME
6.160	PB	423630	.065	106262	1R	.001	2,4-DCPH
6.458	BP	8906	.135	1102		.000	
6.628	PB	9082	.072	2106		.000	

TOTAL HEIGHT= 111470
MUL FACTOR=1.0000E+00