PESTICIDE ANALYTICAL MANUAL

FOR

BCF CONTRACTING AGENCIES

by

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This compilation of procedures was prepared to promote uniformity among contracting laboratories in the analysis of organochlorine pesticide residues in the marine environment.

The methods are adaptations of techniques described in the literature of pesticide residue methodology. We have successfully employed these methods for the past three years and have obtained adequate recovery rates with a minimum of time and effort.

I have tried to describe the procedures in detail so that a technician with a minimum of experience should be able to perform the analysis with little difficulty.

It is beyond the scope of this manual to discuss the theory and operation of Gas-Liquid chromatography. A brief section is devoted to operational hints which the beginner will find useful.

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EQUIPMENT

The following list of equipment is based on the analysis of six samples at a time.

Sample Preparation:

Osterizer with six cutting heads

6 1-pint Mason jars

Sample Extraction:

Six place Soxhlet apparatus complete with extraction assemblies for 123 mm x 43 mm Whatman extraction shells

6 Whatman extraction shells, 123 mm x 43 mm, single thickness

6 3-ball Snyder columns (Kontes Glass Company, Catalog number K-50300)

Sample Clean-up:

6 250-ml separatory funnels with teflon stopcocks

12 crystallizing dishes, 70 mm x 50 mm

12 chromatographic tubes, 400 mm x 20 mm

12 powder funnels, 100 mm diameter

12 500-ml Erlenmeyer flasks with 24/40 joint

6 250-ml vacuum flasks

12 funnels, 65 mm diameter

12 25-ml glass stoppered graduated cylinders

Miscellaneous Equipment:

Slide warming table (Ranson)

Freezer

Water bath, eight hole

Fume hood

Glass wool

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Miscellaneous Equipment (contd.):

2 Vacuum pumps

Wash bottles

Pipettes, volumetric, transfer (1, 2, 3, 4, 5, 10 ml)

Volumetric flasks (25, 50, 100, 250, 500 ml)

Separatory funnel, 2000 ml

2 oz. Amber bottles with Teflon lined caps for standards (A. H. Thomas)

Pesticide standards: Obtain reference grade material direct from manufacturers or from U. S. Public Health Service, Pesticides Repository, P. O. Box 490, Perrine, Florida 33157.

REAGENTS

Sodium sulfate, anhydrous, powder (J. T. Baker #3898)

Quso - G30 Unreductionized (Philadelphia Quartz Co.)

Sand, Sea (Fisher Scientific #S-25)

Petroleum ether, ethyl ether and acetonitrile (Burdick and Jackson redistilled solvents)

Acetonitrile: Saturate in a separatory funnel with petroleum ether. Vent pressure by opening stopcock.

6% eluting mixture: dilute 60 ml of ethyl ether to 1000 ml with petroleum ether.

15% eluting mixture: dilute 150 ml of ethyl ether to 1000 ml with petroleum ether.

Florisil PR Grade (Floridin Co., 3 Penn Center, Pittsburgh, Pa. 15235): for use, heat at least 5 hours at 130°C. Store in desiccator at room temperature. If stored more than 2 days after heating, it must be reheated and cooled to room temperature before use.

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Celite 545 (Johns-Manville Co.)

Magnesium oxide (Fisher Scientific, Sea Sorb #43): Treat as follows: Prepare a distilled water slurry of about 500 grams, heat on boiling water bath about 30 minutes and filter with suction. Dry overnight at 130°C., let cool and pulverize with Osterizer. Store in closed jar.

Magnesium oxide - Celite mixture: Mix treated MgO with Celite 1:1 by weight.

SAMPLE PREPARATION

Cleanliness is essential during all operations. Gas-liquid chromatography permits the detection of organochlorine pesticides at the 10^{-10} gram level and less. All glassware and utensils must be thoroughly cleaned and rinsed with acetone to prevent cross contamination of samples.

Samples for bioassay should be prepared as soon as possible after collection. If necessary, they may be held over for 1 or 2 days maintained as you would fresh food; they need to be refrigerated but not frozen. They may be frozen for longer storage but care must be taken on thawing them, so that all of the drained tissue fluids are saved and blended with the sample. Mollusks should be shucked before freezing.

Oysters, Clams, and Mussels

1. Open by cracking hinge. Remove free water by shaking out 3 times after shell has been opened but before the adductor muscle is cut. Then, shuck 12 individuals into clean Mason jar.

2. Blend for 1 minute or until sample is well homogenized.

3. Place 30 $\pm$ 5 grams of the homogenate in a tared, numbered, 1-pint standard Mason jar and record all weights on data sheet.

4. Cap with Osterizer Cutting Assembly and place in freezer to chill - about 1/2 hour - DO NOT FREEZE. Use separate cutting head for each sample to save time.

5. Remove from freezer and add exactly three times the sample's weight of the desiccant mix.

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The desiccant mix is made up of about 10% Quso (precipitated silica) and 90% anhydrous sodium sulfate. It is convenient to make the mix by weighing out exactly 9 grams of Quso and adding enough Na_2SO_4 to bring the Mix to the required weight of precisely 3X the amount of sample homogenate, (2X for fish samples, up to 9X for plankton).

6. Mix desiccant and sample in jar thoroughly with a spatula being sure to incorporate all tissue adhering to sides and bottom of jar.

7. Recap with Cutting Assembly and place in freezer. Allow mixture to freeze (about 1 hour).

8. Remove from freezer and grind until mixture is relatively free flowing. Cover jar with can or plastic beaker while grinding as a safety measure.

9. Refreezing and regrinding may be necessary to obtain a free flowing mixture.

10. Remove Cutting Assembly and seal with standard Mason jar cover. Store sample in freezer.

Fish

1. Fish samples: 15 fish are blotted dry and passed through a meat grinder three times, mixing between grinding. A 35-gram (approximately) aliquot is added to a tared, number 1-pint Mason jar and the sample prepared as for oysters, except that exactly 2 times the sample weight of desiccant mix is added.

2. Small fish (15) - total weight less than 60 grams; blot fish dry and freeze. Remove from freezer, separate fish and place in tared, numbered Mason jar. Add exactly 2 times the sample weight of desiccant mix and grind immediately. Cover jar with can or plastic beaker while grinding. Return sample to freezer for 1 hour or longer: remove sample and regrind. Refreezing and regrinding may be necessary to obtain a free flowing sample. Do not use high speed until sample is partially homogenized.

Snails

Crack shell and remove entire body. Proceed with Step 2 under oysters.

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Lobster and Large Crabs

Remove and discard dorsal carapace of cephalothorax after cutting free any attached soft tissues. Proceed as with fish.

Plankton

Drain sample on reagent grade filter paper; remove jelly fish, ctenophores, obvious small fish and pieces of trash, and transfer to clean glass dish such as a watch glass. Mix well with spatula, remove and weigh a small sample (5 gms. or less). Dry this sample at 90°C. for 24 hours and weigh for determination of moisture content. Proceed with the remainder of the sample as for oysters, starting with Step 3. Because of varying amount of moisture, residues in plankton are recorded on a dry-weight basis and therefore appear to be relatively higher.

If 30 gram samples cannot be obtained, a minimum of 10 grams can be analyzed. When preparing samples of less than 30 grams, adjust the weight of desiccant mix added so that sample plus desiccant will be about 100 grams.

(Example:

Sample weight	10.4 grams (wet weight)
Desiccant factor	9
Desiccant weight	93.6
Gross weight	104.0)

SAMPLE EXTRACTION

1. Write the sample number in pencil on the extraction shell.
2. Weigh shell and record on sample data sheet.
3. Using a clean powder funnel, transfer prepared sample from Mason jar to shell and record weight of sample plus shell on sample data sheet.
4. Place about 1/4" thick a wad of glass wool on top of each shell.

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5. Number a 300 ml extraction flask with sample number, add 1/4 teaspoon full of sand and approximately 250 ml of petroleum ether.

6. Place shell containing sample in Soxhlet apparatus and join the corresponding numbered extraction flask. Turn on heater and water and extract sample for 4 hours. Heaters should be adjusted to permit solvent to completely cycle once every 6 to 7 minutes.

7. To obtain weight of sample extracted, divide net weight of sample by a number one greater than the Na_2SO_4 factor used to prepare sample.

Example (oyster sample):

Weight of prepared sample + shell --- 238.3 grams
Weight of shell ---------- 113.5 "
Weight of prepared sample ---------- 124.8 "

Na_2SO_4 factor is 3 thus: $\frac{124.8}{4}$ = 31.2 grams extracted.

SAMPLE CLEAN-UP

A. Acetonitrile - petroleum ether partition

1. Turn off heaters on extractors after 4 hours.

2. After boiling has completely stopped, remove extraction flask from Soxhlet apparatus and discard shell containing sample.

3. Add more sand to flask, attach Snyder column and evaporate to about 10 ml on steam table in a fume hood. Wash Snyder column with about 5 ml of petroleum ether and let drain into extraction flask.

4. Remove Snyder column and transfer entire extract to a 250 ml separatory funnel equippped with teflon stopcock. Dilute extract to 25 ml. Before analysis, add 25 ml of petroleum ether to each 250 ml separatory funnel and etch at the 25 ml level.

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5. Add 50 ml of acetonitrile saturated with petroleum ether and shake for 1 minute.

6. Drain lower layer (Acetonitrile) into a crystallizing dish.

7. Repeat Step 5.

8. Drain second 50 ml lower layer into the same crystallizing dish as in Step 6. Discard upper ether layer remaining in separatory funnel.

9. Evaporate acetonitrile extract just to dryness in hood on a slide warming table (approximately 40°C.) with draft over top of dish.

B. Florisil column

1. Prepare 400 mm x 20 mm chromatographic tube with 4 inches (after settling) of Florisil, topped with 1/2 inch of Na_2SO_4. Tap or vibrate column to settle material. Before analysis, check each lot of Florisil with a mixture of pesticide standards to assure that separation is correct, complete and the recoveries are quantitative. All monitored pesticides should elute from the column with 200 ml of the 6% eluting mixture, except dieldrin and endrin which will elute with 200 ml of the 15% eluting mixture. These volumes may have to be adjusted from batch to batch of Florisil.

2. Wash column with 35 ml of petroleum ether and discard wash.

3. Place a 500 ml Erlenmeyer flask equipped with 24/40 joint under column and transfer entire extract from crystallizing dish to column with petroleum ether. When all of extract has past through Na_2SO_4 layer, elute column with 200 ml of 6% ethyl ether in petroleum ether.

4. When the last of this elute has passed through Na_2SO_4 layer, remove receiving flask and replace with another. Then elute column with 200 ml of <u>15%</u> ethyl ether in petroleum ether.

5. After the column has been eluted, add sand to each receiving flask and fit Snyder column. Evaporate to approximately 10 ml on a steam table. Rinse Snyder column with about 5 ml of petroleum ether.

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6. Quantitatively transfer 6% eluate to a 25 ml glass-stoppered graduated cylinder with petroleum ether and dilute to the 25 ml mark. The 6% extract is now suitable for gas chromatography.

7. The 15% extract needs further clean-up on an MgO - Celite column. Attach a 400 mm x 20 mm chromatograph tube to a 250 ml vacuum flask with a rubber stopper. Attach vacuum hose to side arm. With full vacuum, add 10 grams of a 1:1 mixture of MgO - Celite. Bleed vacuum line and wash column with 35 ml petroleum ether so that the elution rate is approximately 15 - 20 ml/min. It is not necessary to discard wash. Quantitatively transfer the 15% eluate to this column. When last of sample just reaches top of MgO - Celite mixture, elute column with 100 ml petroleum ether. Quantitatively return eluate to 500 ml Erlenmeyer flask and evaporate on steam table as in Step 5.

8. Quantitatively transfer MgO - Celite eluate to a glass-stoppered graduated cylinder with petroleum ether and dilute to the 25 ml mark. The 15% extract is now ready for gas chromatography.

GAS LIQUID CHROMATOGRAPHY

Be thoroughly familiar with the operation and theory of your gas chromatograph before attempting to evaluate your extraction and clean-up procedures. Read and understand the manufacturer's instruction manual.

The following list of suggestions will be helpful to those using the Model 610D Varian Aerograph Gas Chromatograph.

1. If only one column is used, obtain 3% DC 200 on Gas Chrom Q 80/100 mesh from Applied Science Laboratories, State College, Pennsylvania. Pack this material in a 5' x 1/8" Pyrex column and condition for 24 hours at 225°C. The second recommended column packing material is 5% QF-1 on Gas Chrom Q 80/100 mesh. This material is also packed in 5' x 1/8" Pyrex column, but must be conditioned 3 days at 225°C. The latter column provides different retention

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times for pesticides and thus aids in identification. Use silane treated glass wool (Applied Science Laboratories) to cap ends of column.

2. Bake all silicone "O" rings and injector septa overnight at 200°C. before use.

3. Replace injector septa and clean glass injector inserts with solvent at the end of each working day.

4. Replace 13X molecular sieve in gas line filter when nitrogen cylinder is changed.

5. Oil oven fan every 30 days.

6. Remove EC detector from column effluent overnight and store in heated EC gold cylinder. (This cuts down on the column bleed to the tritium foil, thus prevents frequent cleaning.)

7. Standards made up at the following concentrations (nanogram per microliter) will provide an on scale chromatogram of each standard at 10X to 32X attenuations, depending upon detector sensitivity. Inject 5 microliters. See appendix for sample chromatograms.

Standard A		Standard B	
Lindane	0.008 ng/µl	Dieldrin	0.05 ng/µl
Heptachlor	0.02 "	Endrin	0.05 "
Aldrin	0.02 "		
Heptachlor epoxide	0.02 "	Standard C	
p,p' DDE	0.04 "	Methoxychlor	0.3 ng/µl
p,p' DDD (TDE)	0.08 "		
p,p' DDT	0.12 "		

8. At chart speeds of 30 inches/hour, the ideal retention distance for DDT is about 120 mm. This is obtained with the above column packing materials at oven temperature around 185°C. and N_2 flow of 40 ml/min. These parameters may vary depending on the batch of column packing material obtained.

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9. Check all operating parameters (temperature, gas flow, and standing current) frequently until you are familiar with identifying malfunctions in these areas.

10. Quantitate by the following equation:

$$ppm = \frac{Vwd_2}{Wvd_1}$$

W = Weight of sample in grams

V = Volume of extract in milliliters

w = Weight of pesticide in standard sample injected (nanogram)

v = Volume of extract injected (microliters)

d_1 = Peak height in mm. for w

d_2 = Peak height in mm. for v

11. To insure reliability, inject a standard solution after at least every third sample.

12. When sample concentration is high and peak is off scale on recorder, dilute extract by an appropriate factor to bring peak height on scale.

13. Inject 5 microliters of standards and sample with a 10 microliter Hamilton syringe equipped with guide.

14. Confirmation of analysis is most easily obtained by the use of two columns of different polarity such as DC-200 and QF-1. Thin-layer chromatography and other chemical techniques may also be used.

15. The o,p' isomers of DDT, although not frequently found, are difficult to separate on the above column packings.

(1) p,p' DDE and o,p' DDD and (2) p,p' DDD and o,p' DDT are not resolved on a DC-200 column. o,p' DDD and o,p' DDT are not resolved on a QF-1 column. As experience is gained, the analyst should experiment with mixtures of these column packing materials in an attempt to separate these isomers. With our particular lot of these packings, we have found that a 1:1 mixture of 3% DC-200 and 5% QF-1 on Gas Chrom Q 80/100 mesh will separate these isomers.

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APPENDIX

References:

Burchfield, H. P. and Donald E. Johnson.
Guide to the Analysis of Pesticide Residues, Volume I and II.
U. S. Department of Health, Education and Welfare Public Health Service, Office of Pesticides, Washington, D. C. 20201.

Mills, P. A., J. Onley and R. Gaither.
Rapid Method for Chlorinated Pesticide Residues in Nonfatty Foods. Journal of the Association of Official Analytical Chemist, 46:186 (1963).

Johnson, L.
Collaborative Study of a Method for Multiple Chlorinated Pesticide Residues in Fatty Foods. Journal of the Association of Official Analytical Chemist, 48:668 (1965.)

PCB off in 620 Et-O Et in Pet.

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PESTICIDE ANALYSIS REPORT
BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL LABORATORY

Sample No.: Date collected: Collector:

Description:

Locality:

Treatment:

Date received: Date analyzed: Analyst:

Method of analysis:

	a	b
Gross weight:		
Tare:		
Sample weight:		
Na_2SO_4 factor:		
Na_2SO_4 weight:		
Tare:		
Gross weight:		
Gross thimble weight:		
Tare:		
Sample weight:		

Weight extracted:

SAMPLE DATA SHEET

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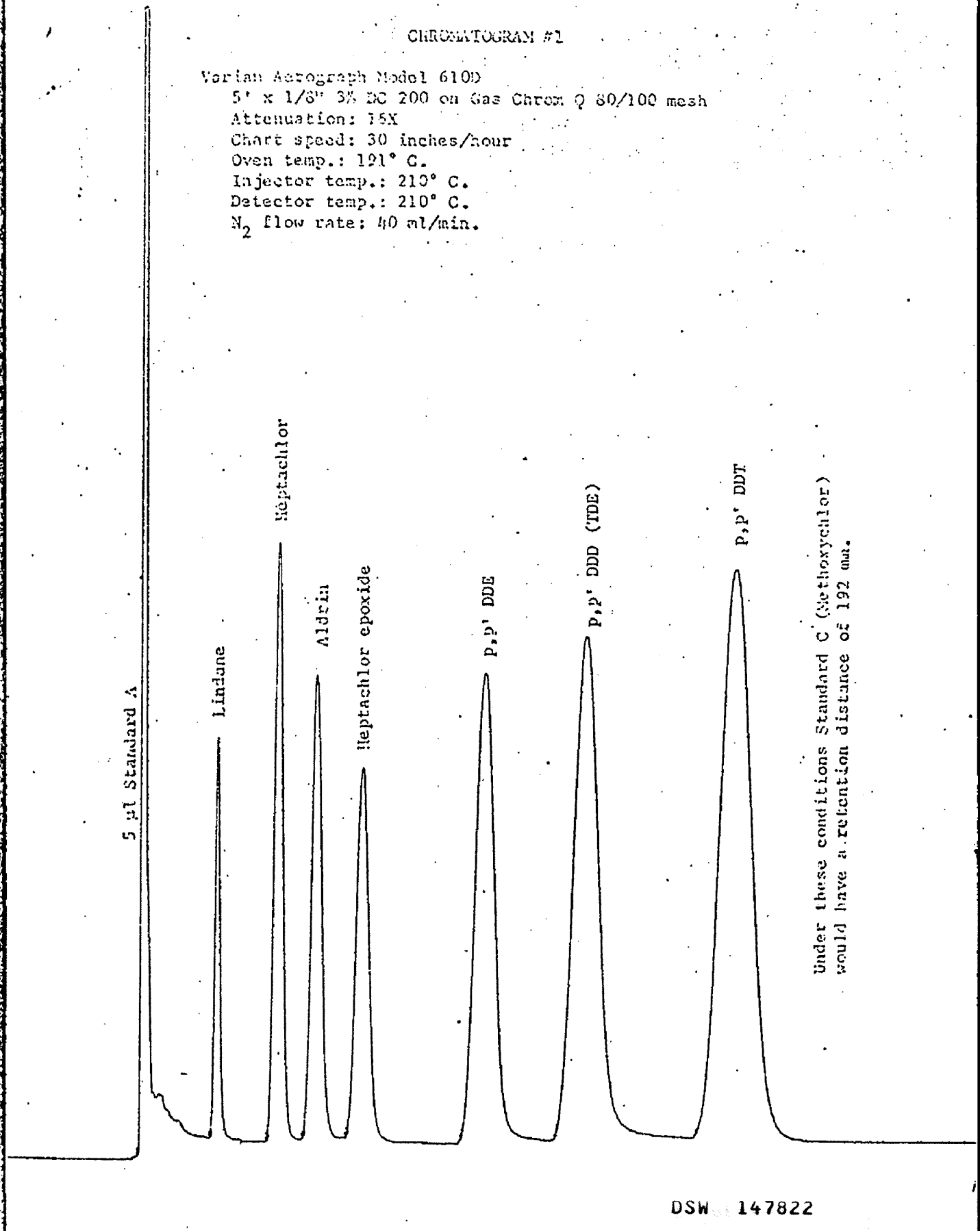
CHROMATOGRAM #1

Varian Aerograph Model 610D
5' x 1/8" 3% DC 200 on Gas Chrom Q 80/100 mesh
Attenuation: 16X
Chart speed: 30 inches/hour
Oven temp.: 191° C.
Injector temp.: 210° C.
Detector temp.: 210° C.
N_2 flow rate: 40 ml/min.



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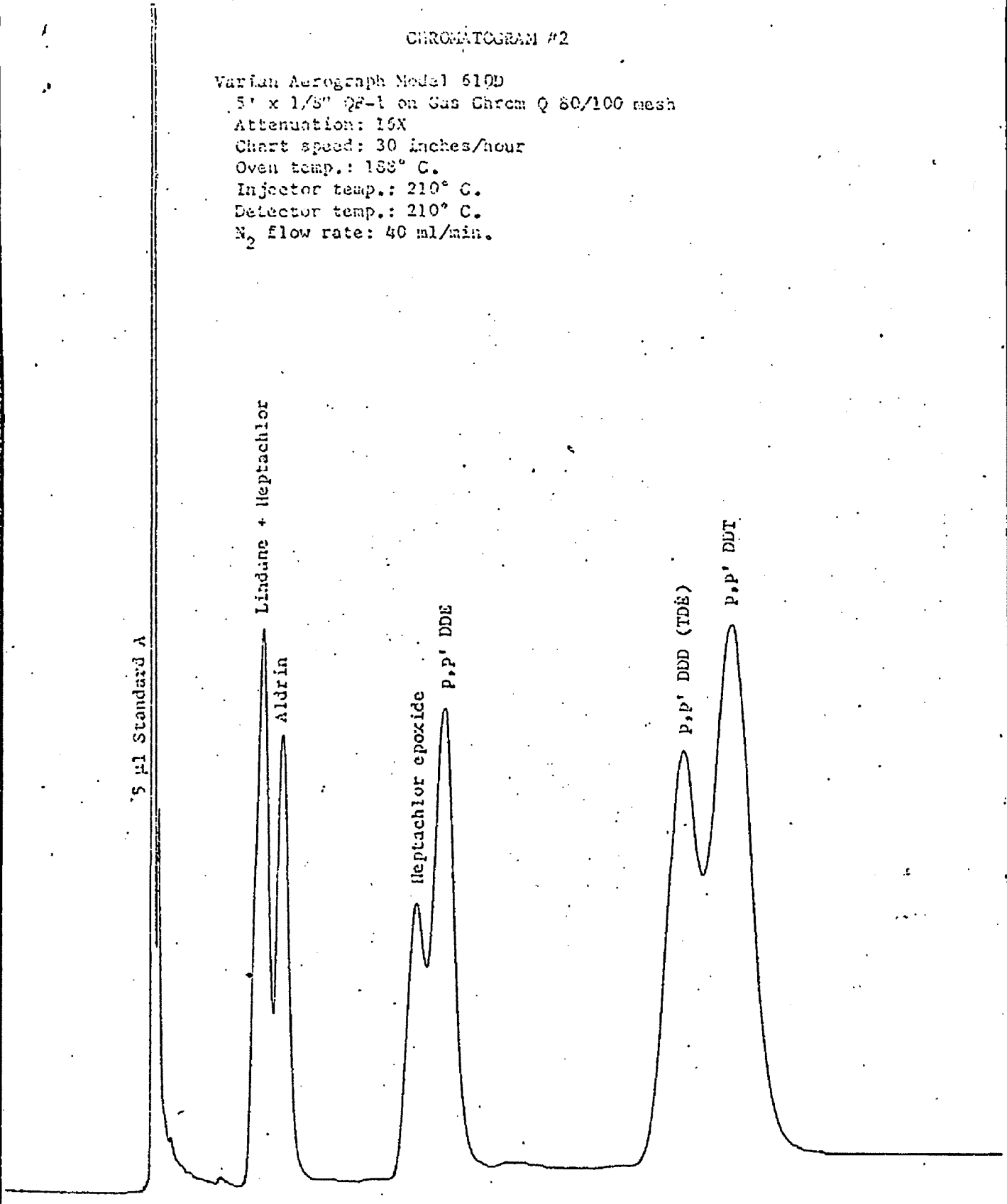
CHROMATOGRAM #2

Varian Aerograph Model 610D
5' x 1/8" QF-1 on Gas Chrom Q 80/100 mesh
Attenuation: 16X
Chart speed: 30 inches/hour
Oven temp.: 188° C.
Injector temp.: 210° C.
Detector temp.: 210° C.
N_2 flow rate: 40 ml/min.



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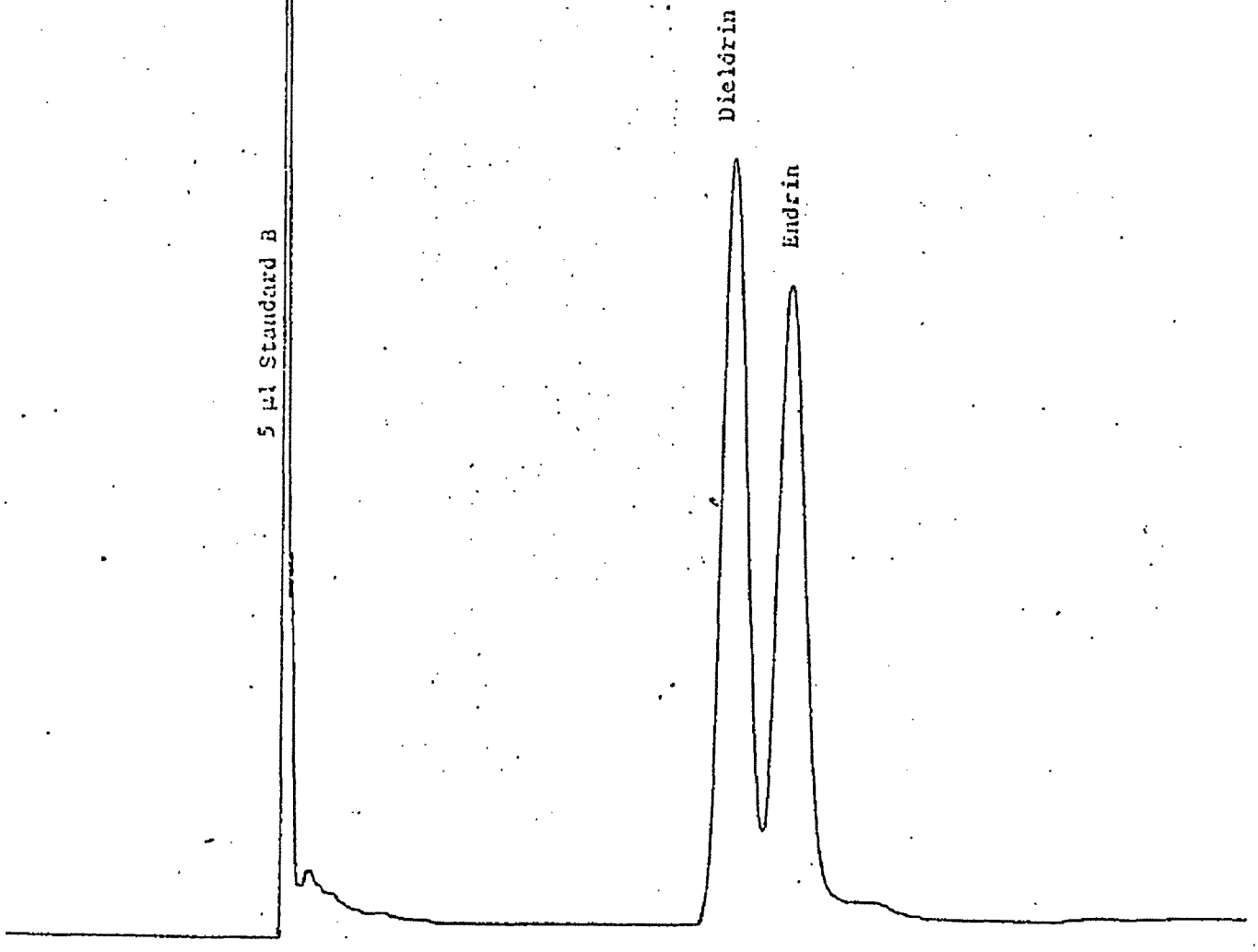
CHROMATOGRAM #3

Same conditions as Chromatogram #1



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Earlier sample [illegible]

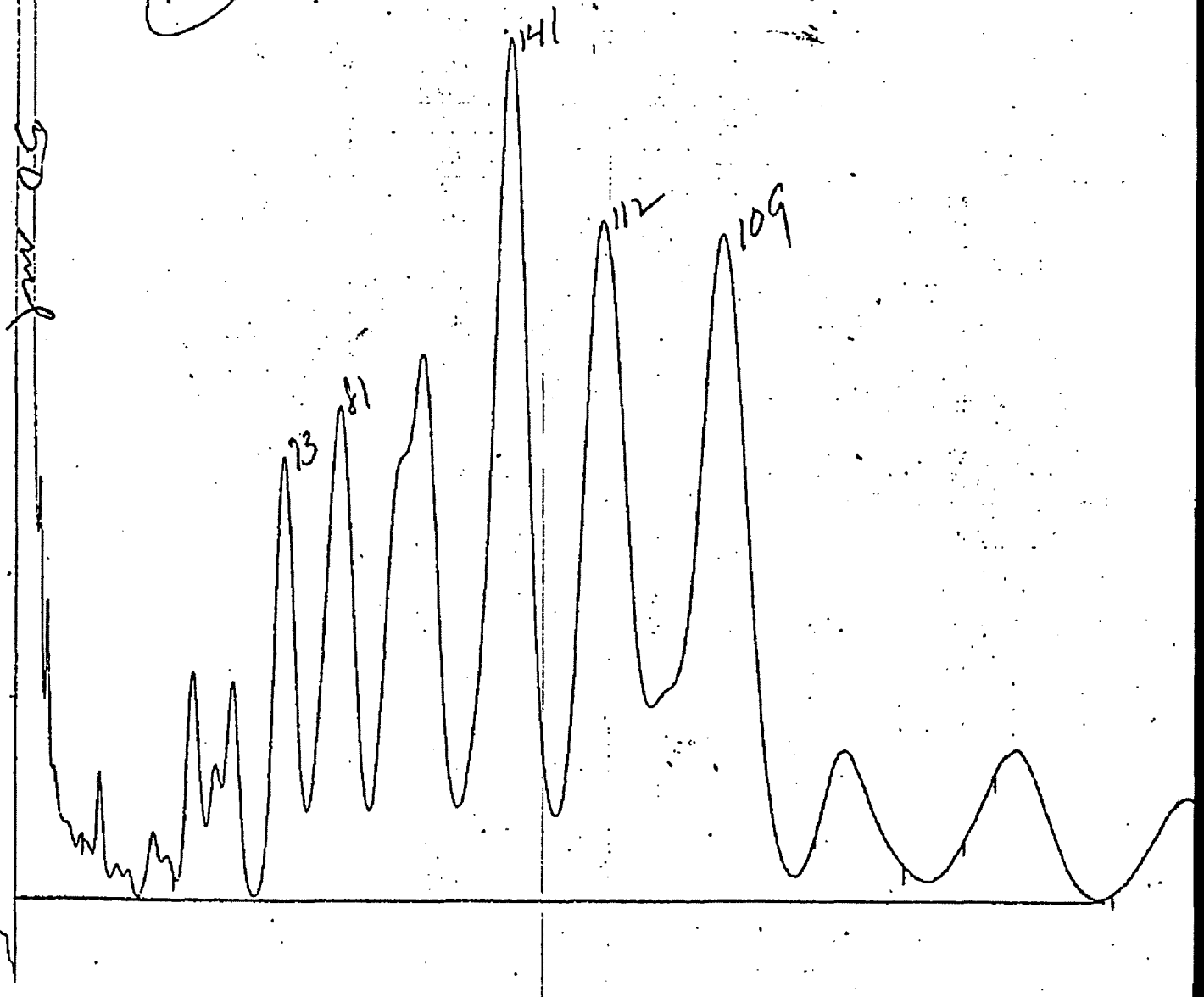
$$\frac{80 \times 100}{8000} \qquad \frac{150 \times 80}{12000} \qquad \frac{103 \times 5}{515}$$

$$\frac{80 \times 200}{16000}$$

$$\frac{50 \times \cancel{5.0} \times 103}{1500 \times \cancel{5} \times 83} = \frac{515}{12450} = 41.\ \text{PPB}$$

$$12450 \overline{)515.00} = .04$$

103



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Standard 1254

~~Std.~~

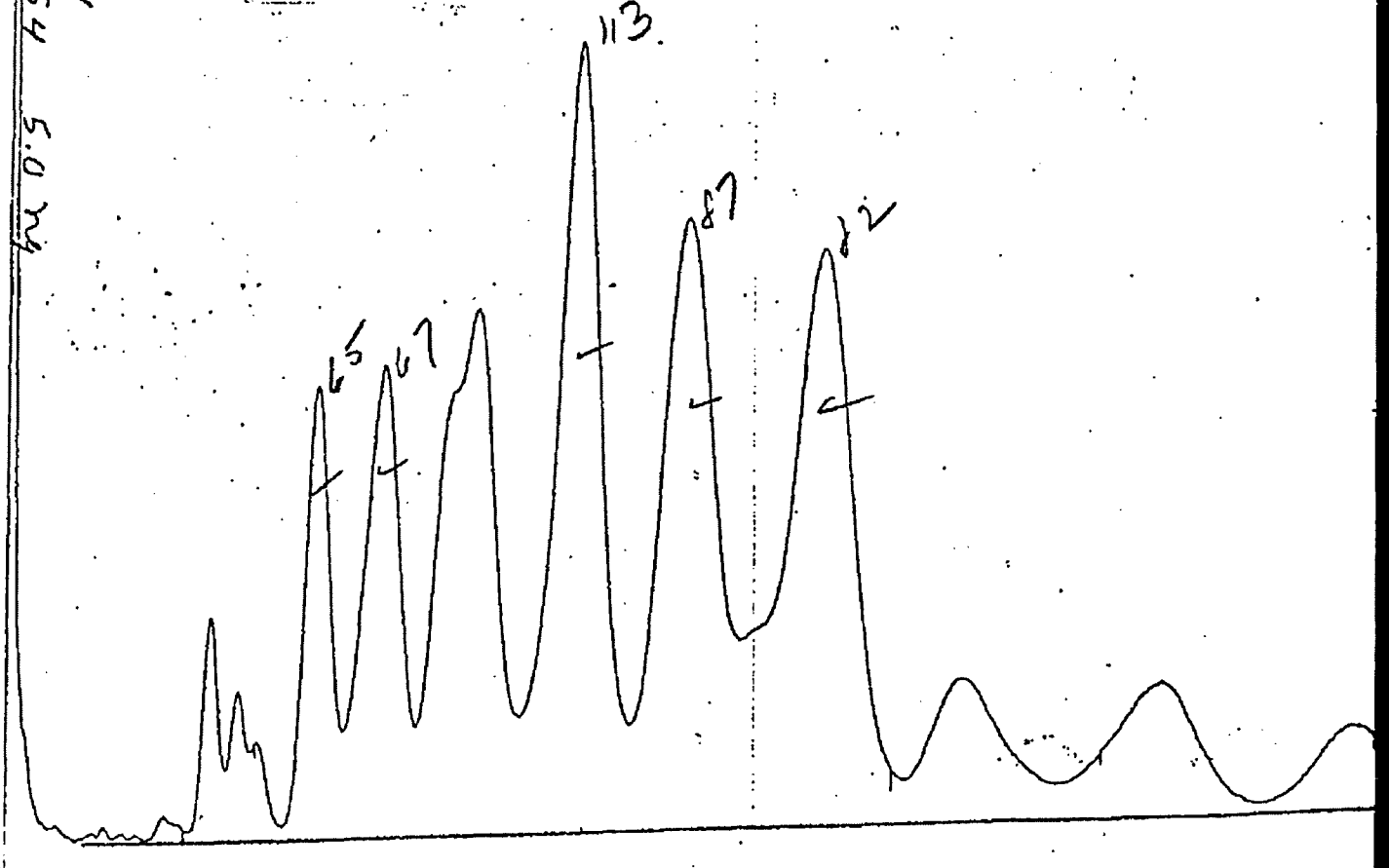
DC-200

Column temp 185°C.

Gas flow rate 60 mL/min

VARIAN AEROGRAPH 1254 5.0 mv

83



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