Appendix III

ANALYSIS OF ENVIRONMENTAL MATERIALS FOR POLYCHLORINATED BIPHENYLS

PREFACE

The analytical procedures currently used to estimate environmental polychlorinated biphenyl (PCB) levels generally include the five following steps[1-6]:

1. Isolation of the PCBs and other "PCB like" residues from the bulk of the sample matrix via solvent extraction.

2. Chemical and/or physical separation of the PCBs from the other "PCB like" residues and extractable matrix components.

3. Gas chromatographic separation of the PCB residue into components and electron capture detection of these components.

4. Structural confirmation of the presence of PCBs in the extracts via an alternate chemical, physical, or instrumental technique.

5. Estimation of the level of PCBs present in the sample via electron capture gas chromatography.

In order to illustrate the scope and limitations of the basic analytical procedure, the steps required to analyze a typical wildlife tissue sample for PCBs will be reviewed.

The weighed excised tissue sample is combined with enough anhydrous, sodium sulfate to absorb the water and a suitable volume of a water insoluble organic solvent such as hexane to dissolve the fat, PCBs, and other "PCB like" hydrocarbon residues. The mixture is homogenized and the extracts (organic solvent + the dissolved materials) filtered into an evaporative concentrator. This process is repeated several times and the combined extracts evaporated until only the hexane soluble fat and residues remain. The fat is then weighed and a portion of this taken for analysis.

Environmental residue levels are generally calculated and reported on the basis of both the fresh tissue weight and hexane extractable fat weight, the fat values are believed, by most researchers[7], to be of more interest from an ecological point of view because of the fact that fat is most important in the transport of energy and as a carrier of residues between the species in the food chain. For this reason, although both values are generally reported, most discussion is based on residue levels in extractable fat.

The extracted fat is now redissolved in a minimum volume of hexane and passed through a column filled with a suitable solid adsorbant. The solid absorbant selectively retains the fat and most of the "PCB like" hydrocarbon residues from the hexane while allowing the PCBs and some of the "PCB like" hydrocarbons to pass. The hexane which elutes from the column is collected and evaporated to a small known volume. This solution is then examined by electron capture gas chromatography to verify the effectiveness of the isolation and separation procedures and to determine if PCBs and/or "PCB like" materials are present.

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Electron capture gas chromatography (EC/GC) simply involves converting the components retained in the treated extracts into the gaseous state and sweeping them with an inert carrier gas into a column packed with a material which retains these components for different periods of time. The components then elute from the column in the order of their retention times into an electron capture detector. The electron capture detector senses the components eluting from the column on the basis of their ability to absorb or capture electrons. The ability to capture electrons is called electron affinity and depends on the composition and structure of the component. Electron affinity can vary over a wide range. This response variation is advantageous from a selectivity viewpoint in that chlorinated hydrocarbons, such as PCBs and DDT, are very good electron absorbers while a number of other constituents often present in the extracts in gross amounts are not. The source used to produce a constant electron flow in the detector is generally a radioactive foil which continuously emits beta particles (electrons). The degree to which this constant electron current is attenuated by component eluting from the column is proportional to its concentration in the carrier gas and in turn to its level in the original sample. The end product of this is a recorder trace or electron capture chromatogram which when interpreted by an experienced analyst indicates if matrix interferences have been eliminated and if PCBs or "PCB like" hydrocarbons are present in the sample extracts. Examples of typical electron capture chromatograms of the unaltered Monsanto Aroclor or PCB products are shown in Figure I.

Such chromatograms are used to confirm the presence and estimate the level of PCBs in environmental samples. However, there are problems. These problems are caused by (1) incomplete gas chromatographic separation of the individual PCB components and "PCB like" hydrocarbons retained in the "cleaned up" extracts, (2) the limitations of the alternate techniques available for confirmation of PCBs in the extracts, and (3) quantitative determination of the exact level of PCBs present in the extracted sample.

Incomplete gas chromatographic resolution of the individual PCB components and "PCB like" hydrocarbons isolated from environmental samples is caused by both the low relative efficiency of packed columns as well as the complexity of the extracted residues[6,14]. Figure II illustrates this point. In it is shown a typical packed column electron capture chromatogram of a commercial PCB, Aroclor 1242, and a high resolution chromatogram of the same material using the most efficient gas chromatographic columns available. Inspection of these chromatograms readily shows that a pure PCB which appears to have only 15 components has in reality at least 55 or more components. The complexity of the situation is further increased by the fact that other "PCB like" multi-component residues are not always removed by the chemical and physical separation procedures normally employed. Examples of materials which can cause interference of this sort are DDT and its metabolites, toxaphene, strobane, chlordane, and chlorinated naphthalenes, to name a few. This means that great care must be taken in the identification of PCBs in environmental samples on basis of packed column electron capture chromatograms alone. Further confirmatory analytical procedures must always be employed except in the case where the chromatogram of the residue duplicates that of one of the commercial PCB products.

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Alternate chemical and/or physical procedures are available which, in the hands of an experienced analyst, will permit differentiating PCBs from other components. However, most of these procedures are time consuming and for this reason not always employed.

The only absolute confirmatory technique currently available is gas chromatography with a mass spectrometer as a detector[1,6,12]. Unfortunately, this absolute detection system is both less sensitive and less selective than the electron capture detector and is, therefore, not always suitable. It also requires expensive equipment and expertise which is not normally available to the smaller laboratories carrying out the bulk of the PCB analyses.

Determination of the absolute level (quantitation) of PCBs in wildlife samples is also presently not possible by electron capture gas chromatography[5,6,12]. The reason for this lies in the fact that most of the major components present in the commercial PCB products which were released to the environment are not found in PCB residues isolated from wildlife samples[1,2,5,6,15-17]. The result is that an exact calibration standard is no longer available.

If the electron capture detector responded in an equivalent fashion to each of the PCB components, this would, of course, not be a problem. Unfortunately, it does not and in fact, its response is dependent on both the number and position of the chlorine atoms in each PCB component. This, in turn, means that the level of PCBs presently being reported in environmental samples is an empirical estimate and not an absolute determination of the amount present. This situation is further complicated by the fact that the empirical estimates being reported are not calculated in the same manner in all laboratories. This means that the levels currently being reported are not generally comparable from laboratory to laboratory[2,3,5,6,12,14].

The current status of PCB methods can be best summarized by quoting a statement from an article published by Dr. S. Jensen[7] who in 1966 was first to identify PCBs in environmental samples. He states the following regarding quantitation of PCB residues under the most ideal of conditions with the most sophisticated instrumentation available:

> "An attempt to quantitate PCB has been made in Sweden, but because the method is still rather rough the values may be correct only within a factor of 2. The method is indirect because all the PCB components have not been isolated yet, and it is based on a combination of mass spectrometry, microcoulombmetric and electron capture detection."

Details of the procedures used by Monsanto Industrial Chemicals Company for the measurement of PCBs in environmental samples follow.

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Analytical Chemistry
Method 71-35

(Consolidated Revision of
Analytical Chemistry
Methods 69-13 and 70-1)

ANALYSIS OF ENVIRONMENTAL MATERIALS FOR POLYCHLORINATED BIPHENYLS

SCOPE

This methodology was developed for the determination of the amount and type of polychlorinated biphenyls (PCB's) in environmental materials. Absolute confirmation of PCB structures is not obtained with this method. Structure proof can be obtained using additional techniques such as mass spectrometry to further identify the GC fractions.

PRINCIPLE

The PCB's present in environmental and laboratory exposed samples are isolated from the bulk of the sample matrix by solvent extraction. Interfering matrix components and endogenous residues are then removed from the sample by a suitable combination of clean up procedures and the amount and type of PCB's present determined by electron capture gas chromatography (EC/GC).

REAGENTS

Hexane	Nanograde, Mallinckrodt Chemical Works, Catalog No. 4159.
N,N-Dimethyl Formamide	Certified, Fisher Scientific Company, Catalog No. D-119. (Saturated in a separatory funnel with hexane.)
Ethyl Ether	Anhydrous, AR grade, Mallinckrodt Chemical Works, Catalog No. 0848.
Acetonitrile	Nanograde, Mallinckrodt Chemical Works, Catalog No. 2442.
Sodium Sulfate	Anhydrous, granular: AR grade, Mallinckrodt Chemical Works, Catalog No. 8042. Heat at 400°C for one hour prior to use.
Alumina Adsorption	(For chromatographic analysis) 80/200 mesh, Fisher Scientific Co., Catalog No. A540. Heat at 400°C for a minimum period of 4 hours and deactivate with 5% (w/w) distilled water.
	Alumina column preparation: Fill a chromatographic column with hexane up to the point where the reservoir joins the column and push a glass wool plug to the bottom with a glass rod. In a 50 ml beaker measure 35 ml of deactivated alumina (∿30g), and pour this

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REAGENTS (Cont'd)

Alumina Adsorption (Cont'd)	slowly into the column. Tap or vibrate the column to settle the alumina and top the alumina with 2-3 cm of anhydrous sodium sulfate. Wash the column with 50-100 ml of hexane prior to the addition of the sample.
Distilled Water	Extracted with hexane to remove hexane soluble electron capturing impurities.
Sulfuric Acid	Analytical Reagent Grade, SG = 1.84.
Potassium Oxalate	Analytical Reagent Grade.
Potassium Hydroxide	Analytical Reagent Grade.
Ethanol	Formula 2B.
2.5% (w/v) Alcoholic Potassium Hydroxide	Dissolve ∿12.5 grams of AR grade KOH in 500 ml of ethanol.
5% (w/v) Aqueous Potassium Oxalate	Dissolve ∿5 grams of AR grade potassium oxalate in 100 ml of distilled water.
PCB Standards	Production Grade - Aroclor 1221, Aroclor 1242, Aroclor 1248, Aroclor 1254 and Aroclor 1260.

APPARATUS

1. Separatory funnels equipped with ground glass stoppers and Teflon stopcocks: 125, 250, 500, 1000 and 2000 ml capacities.

2. Kunderna-Danish Evaporative Concentrators, 500 ml capacity equipped with 3-ball Snyder columns and graduated 5 ml capacity vials: Ace Glassware Company, Catalog No. 6707.

3. Chromatographic columns, glass, 10" X 20 mm (OD) with a 5" X 50 mm (OD) reservoir at the top, equipped with Teflon stopcocks.

4. Sintered glass filter funnels, 600 ml capacity, 90M.

5. Flat bottomed boiling flasks, 125 ml capacity: Ace Glassware Company, Catalog No. 6896, Code - 04.

6. Liebig Condenser, 200 mm in length: Ace Glassware Company, Catalog No. 5915, Code - 12.

7. Hot plates, Corning PC-100: Fisher Scientific Company.

8. Water bath, Thelco, Precision Scientific, Model No. 84, Fisher Scientific Company.

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APPARATUS (Cont'd)

9. Reciprocating variable speed shaker, Eberbach Corporation, Fisher Scientific Company.

10. 10 µℓ Hamilton Syringes, Catalog No. 701 RN.

11. 32 oz. all glass mortars and pestles.

12. 8 X 12 X 2" (2-1/2 qt.) Pyrex baking dishes.

13. U.S. Standard Sieve, No. 30, Fisher Scientific Company.

14. Polytron Tissue Homogenizer, PT 10-20-3500, Brinkman Instruments Co.

15. Grinding Mills, Catalog No. 8-450, and Grinding Unit, Catalog No. 8-455, Fisher Scientific Company.

16. Usual laboratory glassware.

PROCEDURES

Sampling

It is to be assumed that a rather wide variety of sampling techniques were employed in collecting the samples submitted to us for analysis. In general the procedures used were probably selected for ease of adaptation to the local situation. For this reason, a brief description of the manner in which we treat the samples submitted will usually precede the sample preparation and extraction sections.

Sample Preparation and Extraction

Water

Where possible, the entire water sample, including the container in which it was collected, is extracted with hexane. With larger samples, where this is not physically possible, the containers are simply agitated and a 250 ml portion used for analysis.

Extraction of Water Samples

1. Extraction of water samples - after agitating, transfer the entire aqueous sample or a 250 ml aliquot into a graduated glass cylinder. Record the volume of the sample and quantitatively transfer it to a separatory funnel with distilled water.

2. Rinse the sample container and graduated cylinder with two 50 ml portions of hexane and add each to the separatory funnel.

3. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

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PROCEDURES (Cont'd)

4. Extract the water sample a second time with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous layer to the original separatory funnel.

5. Repeat the extraction with a third 50 ml portion of hexane. Discard the aqueous layer and combine the hexane extracts.

6. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Sediment and Soil

Any excess water is decanted and the entire sediment or soil sample transferred to a glass baking dish to air dry at room temperature. The dried material is transferred from the dish into a mortar and pestle and ground. The ground sediment is sieved, remixed, and a 250g portion taken for analysis.

Extraction of Sediment and Soil Samples

1. Weigh 250g (to the nearest 0.01g) into a 16 oz. narrow neck screw cap (aluminum foil liner) glass bottle.

2. Moisten the soil with water (∿10 ml) and add 150 ml of acetonitrile. Cap the bottle tightly, and mechanically shake for a minimum period of 1 hour.

3. Quantitatively transfer the acetonitrile extract into a 600 ml sintered glass filter funnel containing a 1/4" layer of anhydrous sodium sulfate. Collect the filtrate in a 600 ml beaker (vacuum filtration may be necessary).

4. After the acetonitrile has completely drained into the beaker, wash the bottle twice with 50 ml portions of acetonitrile, adding each wash to the funnel after the previous has completely percolated through the sediment.

5. Quantitatively transfer the extract to a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

6. Carefully evaporate the acetonitrile extracts to dryness with the aid of a gentle stream of dry nitrogen. Re-dissolve the residue in maximum of 5 ml of nanograde hexane.

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PROCEDURES (Cont'd)

Whole Animal, Poultry and Fish and Individual Tissue (Muscle, Liver, Fat, Kidney, Heart, Spleen, Lungs, Fat, Etc.) Samples

All individual and composite tissue samples exceeding 75 grams (25 grams for liver) must initially be homogenized by passing through a meat grinder. The tissue mass is re-ground, with mixing between grindings, until the tissue appears homogeneous. A 50-60 gram portion of the ground tissue (25-30 grams for liver) is transferred to a tared 500 ml Erlenmeyer flask for extraction.

Samples weighing less than 75 grams (25 grams for liver) are cut into small chunks (1-2 cm^3) and the entire sample transferred to a tared 500 ml Erlenmeyer flask for extraction.

Extraction of Tissue Samples

1. Weigh the tared Erlenmeyer flask containing the tissue to be extracted and record sample weight. Add ∿100g of anhydrous sodium sulfate and ∿150 ml of hexane to the flask. Insert the Polytron tissue homogenizer probe and blend, while rotating the flask, until the mixture is well homogenized (∿60-90 seconds).

2. Carefully decant the supernate (hexane extract) through a 4" funnel plugged with glass wool and covered with a layer of anhydrous sodium sulfate, collecting the supernate in a 500 ml Kunderna-Danish evaporative concentrator or a 250 ml volumetric flask.

3. Re-extract the homogenate with a second portion of hexane, and again decant the supernate into the filtering funnel.

4. Wash the Polytron* shaft with several small portions of hexane and transfer the homogenate and washings into the filtering funnel with the aid of several small portions of hexane. Press the homogenate with a spatula to remove as much hexane as possible and complete the filtration by washing the funnel and tissue cake with several small portions of hexane.

5. (a) If the extracts are collected in a volumetric flask, dilute to volume with hexane and mix.

   (b) Transfer a suitable aliquot to a tared 50 ml beaker and evaporate the solvent with the aid of heat and a gentle stream of clean dry nitrogen. Re-weigh the beaker and record the lipid weight.

*The Polytron tissue homogenizer is cleaned between each sample by operating it with the shaft immersed in distilled water followed by nanograde acetone and hexane.

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PROCEDURES (Cont'd)

6. (a) If the extracts are collected in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place and reduce the solvent volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED INTO A HOOD.)

   (b) After cooling, remove the 5 ml graduated tube, dilute to volume with hexane, cap and mix thoroughly. The entire concentrate or an aliquot is used to determine the lipid weight in manner described above.

Milk

1. Weigh ∿50 grams of whole milk in a 150 ml beaker and quantitatively transfer it to a 500 ml separatory funnel with the aid of several portions of water.

2. Add 5 ml of the 5% aqueous potassium oxalate solution and swirl to mix.

3. Add 100 ml of diethyl ether, shake for several minutes, add 50 ml of hexane and shake for several more minutes.

4. Allow the mixture to stand for 10-15 minutes and discard the lower aqueous layer.

5. Filter the remaining solvent layer through a 4" funnel plugged with glass wool covered with anhydrous sodium sulfate into a tared 400 ml beaker.

6. Evaporate the solvent, allow the beaker to cool, re-weigh, and record the lipid weight.

Sample Clean Up

DMF/Hexane Partition, Chemical Treatment

1. Place an appropriately sized aliquot of the hexane extracts, containing a maximum of 3.5g of fat and not exceeding 25 ml, in a 500 ml separatory funnel. If the aliquot taken is some volume less than 25 ml, add enough additional hexane to yield a final volume of 25 ml.

2. Add 50 ml of DMF (hexane saturated) to the separatory funnel, stopper the funnel, and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower DMF layer to a second separatory funnel.

3. Repeat the extraction of the hexane layer with two more 50 ml portions of DMF (hexane saturated) collecting each in turn in the second separatory funnel. Discard the remaining hexane layer.

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PROCEDURES (Cont'd)

4. Add 100 ml of distilled water to the combined DMF extracts, swirl to mix, without excessive agitation, allowing the hexane to layer out.

5. Transfer the lower aqueous-DMF layer to another 500 ml separatory funnel.

6. Extract the aqueous DMF sample with a 50 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous-DMF layer to the original separatory funnel.

7. Re-extract the aqueous-DMF with two more 50 ml portions of hexane. Discard the aqueous-DMF layer and combine the hexane extracts.

8. Filter the combined extracts through a 4" funnel plugged with glass wool which is covered with sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Saponification with Alcoholic Potassium Hydroxide and Extraction with Sulfuric Acid

1. Quantitatively transfer the concentrated extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

2. After cooling, remove the 5 ml graduated tube and transfer the concentrate of extracts to a 125 ml extraction flask with the aid of several small portions of solvent.

3. Evaporate the extract just to dryness with a gentle stream of dry filtered air and add 25 ml of 2.5% alcoholic potassium hydroxide.

4. Add a boiling chip, put a water condenser in place, and allow the solution to reflux for 45 minutes.

5. After cooling, transfer the solution to a 250 ml separatory funnel with the aid of 25 ml of distilled water.

6. Rinse the extraction flask with 25 ml of hexane and add it to the separatory funnel.

7. Stopper the separatory funnel and shake vigorously for at least 1 minute. Allow the layers to separate and transfer the lower aqueous phase to a second separatory funnel.

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PROCEDURES (Cont'd)

8. Extract the saponification solution with a second 25 ml portion of hexane. After the layers have separated, add the first hexane extract to the second separatory funnel and transfer the aqueous alcohol layer to the original separatory funnel.

9. Repeat the extraction with a third 25 ml portion of hexane. Discard the saponification solution and combine the hexane extracts.

10. CAREFULLY add 25 ml of concentrated sulfuric acid, in small portions, to the hexane extracts.

11. Stopper the separatory funnel and shake vigorously for at least one minute. Allow the layers to separate and discard the lower aqueous -acid layer. Repeat this step until the acid layer is colorless.

12. Wash the hexane with 25 ml portion of water. Discard the water wash.

13. Filter the hexane extract through a 4" funnel plugged with glass wool which is covered with a layer of sodium sulfate. Collect the filtrate in a Kunderna-Danish evaporative concentrator, add a small boiling chip, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Liquid/Solid Chromatographic Clean-Up With Alumina

1. After preparation of the alumina column, described in the "Reagents" section, quantitatively transfer the hexane extracts (5 ml or less) on to the column with the aid of several 5 ml portions of hexane (reducing the solvent level to the adsorbant level after each addition of solvent).

2. Carefully add 125 ml of hexane to the column reservoir and collect the total eluent in a Kunderna-Danish evaporative concentrator.

3. Add a small boiling chip to the Kunderna-Danish evaporative concentrator, put the Snyder column in place, and reduce the hexane volume to less than 5 ml by heating the apparatus in a 80-90°C water bath. (CAUTION: SOLVENT VAPORS MUST BE VENTED TO A HOOD.)

Electron Capture Gas Chromatography

Instrument(s): Hewlett-Packard 5750 Research Chromatograph and/or 402 High Efficiency Gas Chromatograph

Detector: High Temperature Ni^{63} Electron Capture Cell

Column: Glass, Spiral and/or "U", 2M x 4mm, 4% XE-60 on 80/100 Mesh Chromosorb W, HP, AW-DMCS

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Column Temperature: 170°C-220°C (specific temperatures employed for each Aroclor are shown on attached chromatograms)

Detector Temperature: 250°-300°C

Injection Port: 220°C

Pulse: 50 μsec

Flow Rates

Carrier: ∿60 ml/min He

Purge: ∿120 ml/min AR/CH_4 (10% AR)

The electron capture gas chromatographic system can be effectively employed to guide the isolation, separation and clean up procedures. Sample extracts should be checked for the presence of PCB's and/or matrix and other chlorinated hydrocarbon interferences by injecting microliter portions of the extracts at various points in the extraction and concentration scheme. In this manner, it can be determined if the sample must be concentrated or diluted and what, if any, confirmatory and/or clean-up procedures are needed. The approaches routinely employed in our laboratory are shown schematically in Figure I.

Absolute confirmation of the presence of PCB's in a sample is obtained by GC/Mass analysis of the residue. Alternate chemical and physical confirmatory techniques not routinely employed in this laboratory are listed in the reference section.

Quantitation

Quantitative determinations employing the electron capture detector are non-stoichiometric measurements made by comparing peak heights or areas for known concentrations with those for unknown compositions. Four variations of the peak height or area quantification procedures have been employed.

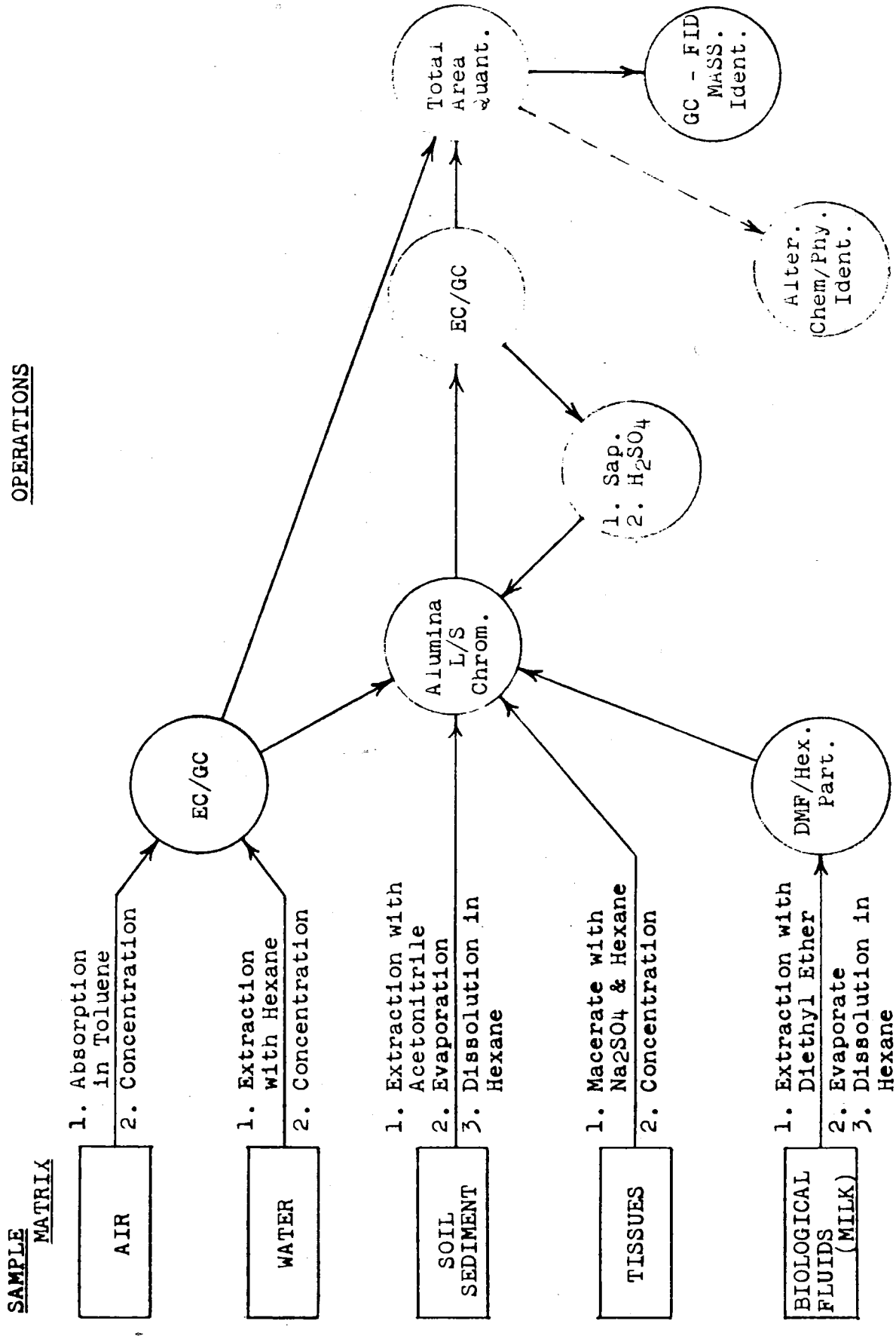
Case I EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with no evidence of interferences.

Case II EC gas chromatogram of PCB unknown altered with respect to standard PCB with no evidence of interference.

Case III EC gas chromatogram of PCB unknown unchanged with respect to standard PCB with evidence of interference.

Case IV EC gas chromatogram of PCB unknown altered with respect to standard PCB with evidence of interference.

The PCB level in a Case I sample can be determined by comparison of the height of the major peak in the unknown with a calibration curve prepared by plotting the height of the major peak in the electron capture chromatogram of the corresponding standard vs the number of ng of the standard injected. With a Case II sample because of the alteration, the maximum PCB level is estimated by comparison of the total area of the electron capture envelope of the unknown to a calibration curve prepared by plotting the total area of the electron capture envelope of the most similar standard vs the number of ng of the standard



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PROCEDURES (Cont'd)

injected. Case III and IV samples are initially subjected to the chemical clean-up procedure followed by chromatography as outlined on alumina. If the interferences are removed by this treatment, the PCB levels are calculated for Case III and IV in the same manner as Case I and II, respectively. If dominant interference(s) is (are) still present, then the height of a different analytical peak, free from obvious interference, is employed to calculate the PCB level in Case III. With Case IV samples, the maximum PCB level is estimated as in Case II after correcting the total area for that of the interfering peak(s).

In all cases, the response of the electron capture must be linear for quantitative analysis.

Detection Limits

Sample Matrix	Sample Size	Final Extract Volume	Injection Volume	Detection* Lim
Water	250 ml	5 ml	5 µl	2 ppb
Tissues	25-50g	5 ml	5 µl	10-20 ppb
Milk	50g	5 ml	5 µl	20 ppb

*Greater sensitivity can be achieved as needed by increasing the sample size, decreasing the final extract volume, or increasing the injection volume.

DISCUSSION

Extraction

The extraction of PCB's from water employing hexane as the extractant was found to be quantitative and to be sufficiently simple and rapid for use as a routine procedure.

The evaluation of this method was based on spiking water samples with standard acetone solutions of PCB's. The spiking method consisted of adding the PCB's in acetone (25-50 µl) to 500 ml of tap water in a 32 oz. narrow neck screw cap jar. After thoroughly mixing, duplicate 225-250 ml aliquots were taken and subjected to the proposed sample preparation and work up as outlined. The results were quantified by preparing a calibration curve using standard hexane solutions of the PCB's used to spike the water samples. The total area of the electron capture chromatogram was used to construct the calibration plot.

The average recovery and deviation achieved substantiated the applicability of the method for the quantitative recovery and analysis of PCB's from water at the ppb-ppm level.

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DISCUSSION (Cont'd)

No PCB recovery experiments from spiked sediment and soil samples have been performed. Instead, several of the residual solids representative of some of the types of sediment or soil analyzed were re-extracted with hexane/acetone (40/60) in a soxhlet extractor to test for the efficiency of the acetonitrile extraction step. The hexane, after isolation by dilution with distilled water was then carried through the purification steps. Recoveries by soxhlet extraction have indicated that the acetonitrile extraction of PCB's was essentially quantitative in the cases checked.

Based on the assumption that fat or fatty organs generally yield the lowest recoveries a series of experiments were conducted with hexane solutions of chicken fat fortified with known amounts of Aroclor. The spiked fat samples were cleaned up (DMF/Hexane partition and alumina chromatography) and the amount of PCB determined by EC/GC. All recoveries fell in the range of 98-102%; for this reason recovery experiments were not carried out with each individual tissue. While a more realistic evaluation of the overall recoveries might produce recoveries in the range of 70-90%, it was felt that this would be more than sufficient for our purposes, especially in light of the quantitation problems which exist.

Sample Concentration

Concentration of sample extracts is necessary, prior to clean up by chromatographic or chemical means, to reduce sample size and increase sensitivity. The preferred method of concentrating allows minimum loss through volatilization or chemical decomposition and requires a minimum time. The three methods of solvent volume reduction most commonly used are evaporation by exposure to a stream of air, evaporation employing a Kunderna-Danish evaporative concentrator equipped with a Snyder column, and evaporation under reduced pressure. We have used all three techniques and have not encountered any significant losses from volatilization or chemical alternation. However, the Kunderna-Danish evaporative concentrator and the stream of air were employed because of the ease of use.

Column Adsorption Chromatography and Chemical Clean-Up

Silica gel, Florisil and Alumina deactivated with 0, 1.0, 1.5, 2.0 and 5% water were investigated as adsorbants for the elimination of interferences. Alumina (5% water) was found to be effective and more reproducible than either silica gel or Florisil. The activity of alumina varies with age and lot, therefore, 5% water was added to the alumina, after heating for a minimum of 4 hours at 400°C, to insure a reproducible activity.

Saponification and subsequent extraction of the sample with sulfuric acid is an effective way to remove a number of chlorinated hydrocarbon interferences as well as other matrix interferences. Nevertheless, several pesticides survive the chemical clean-up procedures (e.g. strobane, toxaphene, DDE, chlordane, etc.) demanding careful interpretation of the EC chromatograms or alternate chemical and separation procedures referenced in the appendix. PCB's are not affected.

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DISCUSSION (Cont'd)

Electron Capture Gas Chromatography

Columns

Column performance is the key to effective gas chromatographic analysis and as such the choice of column materials is particularly important. Ideally, the support employed should be inert, mechanically strong, and of high surface area. For these reasons, Chromosorb W, HP, AW-DMCS was used in all of our work.

A variety of polar and non-polar liquid phases were investigated. The following columns were found to provide adequate separation, etc., for use in PCB analysis by electron capture: 4% (w/w) DC-200, SF-96, OV-17, SE-30, SE-54, XE-60, Apiezon L, and 6% QF-1. DC-200 and XE-60 or QF-1 have been found to be the most suitable of these liquid phases.

Another important consideration when working with an extremely sensitive detector and consequently low levels of materials is column conditioning. With polar phases such as XE-60 and QF-1, we have found that operating a new column overnight at a temperature 25-50°C higher than to be used during analysis results in a more stable column. A no-flow conditioning technique is employed to condition non-polar columns. The column is purged with carrier gas, heated for 30 minutes at an elevated temperature without carrier flow and then cooled to room temperature. At the end of this cycle, the carrier flow is resumed and the conditioning is completed as in the case of the polar liquid phase. Two precautions: During conditioning, the column should not be connected to the detector and one should not exceed the maximum safe temperature of the liquid phase.

Since all liquid substrates bleed to one degree or another and columns eventually degrade, we characterize all new columns with two column performance indicators - the number of theoretical plates (N) and a tailing factor (T). p,p'-DDT is employed to check these parameters because it is known to degrade on "poor" columns. In this manner, we can determine if the performance of a new column is satisfactory and when the column performance begins to fall off. We consider a column good if the number of theoretical plates per foot is on the order of 400-500 with tailing factors of 1.0-1.3. Calculation of these parameters is shown in the appendix. Additionally, there should be no significant extraneous peaks upon injection of a pure p,p-DDT standard.

Other chromatographic conditions that can be adjusted are column temperature and flow rates. Although resolution of a mixture increases with decreasing temperature, a temperature should be chosen that allows the elution of all components within a convenient time period. The flow rates are optimum for our instrument, column and detector system and, of course, should be adjusted if better results can be achieved.

Two gas chromatographic systems have been used for PCB analysis - HP Model 402 and 5750. We find that any system of instrument and column suitable for chlorinated pesticides is satisfactory for PCB analysis.

DISCUSSION (Cont'd)

The bulk of analyses in our laboratories was carried out using the systems outlined. The use of the high temperature Ni^{63} electron capture cell is highly recommended. The ability to operate at higher temperatures prevents maintenance problems due to contamination from high boiling components.

CONTAMINATION

In determining PCB's by electron capture gas chromatography, laboratory sources of contamination can be a major problem. The samples and extracts should never be allowed to come in contact with materials other than glass, Teflon or metal. Laboratory glassware should be thoroughly washed with hot, soapy water, rinsed with distilled water, acetone and then hexane. All equipment should also be rinsed again with hexane just prior to use and blanks should be frequently carried through all steps of the procedures to insure against the possibility of contamination.

db

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A P P E N D I X

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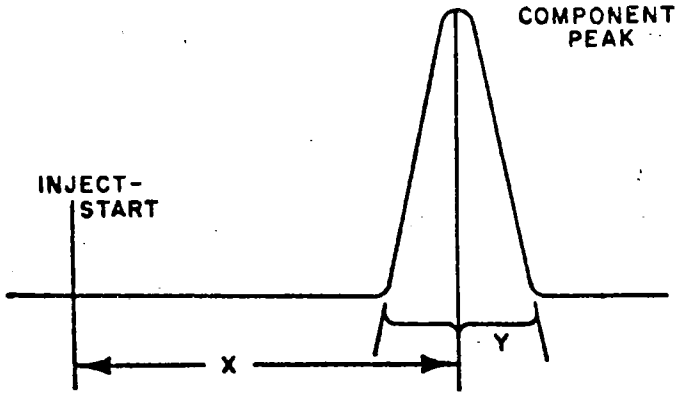
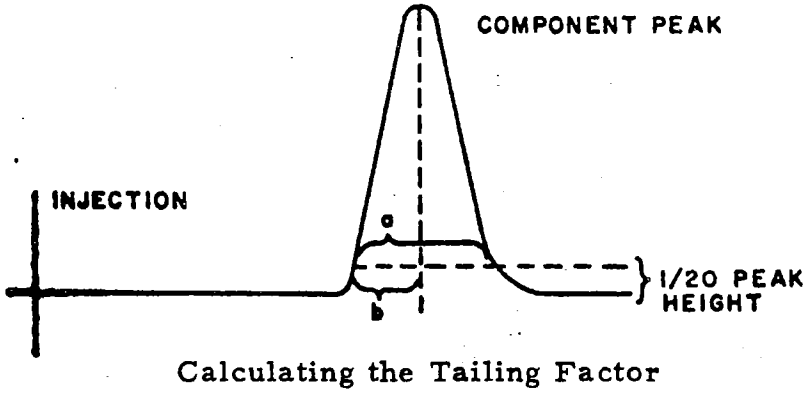
COLUMN PERFORMANCE INDICATORS



Calculating Column Efficiency

1. **Theoretical Plates, N**

$$N = 16(x/y)^2$$



Calculating the Tailing Factor

2. **Tailing, T**

$$T = a/2b$$

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REFERENCES

ALTERNATE CHEMICAL/PHYSICAL SEPARATION AND IDENTIFICATION PROCEDURES

1. "Method for Separating Polychlorinated Biphenyls from DDT and Its Analogs", J. A. Armour and J. A. Burke, J. of the AOAC, 53, (4), 1970.

2. "Column Chromatographic Separation of Polychlorinated Biphenyls from Chlorinated Hydrocarbon Pesticides, and Subsequent Gas Chromatographic Quantitation in Terms of Derivatives", O. W. Berg, P. L. Diosady and G. A. V. Rees, Division of Laboratories, Ontario Water Resources Commission. J. H. Neil, Director - private communication, submitted for publication.

3. "Behavior of Chlorinated Naphthalenes in Analytical Methods for Organochlorine Pesticides and Polychlorinated Biphenyls", J. A. Armour and J. A. Burke, J. of the AOAC, 54 (1), 1971.

4. "Structural Identification of Polychlorinated Biphenyls in Commercial Mixtures by Gas-Liquid Chromatography, Nuclear Magnetic Resonance and Mass Spectrometry", D. Sissions and D. Welti, J. Chromatogr., 60, 15-32 (1971).

FIGURES

Figure I	Composite electron capture chromatograms - demonstrating overlap regions for technical Aroclor products.
Figure II	Aroclor 1221 electron capture reference chromatogram at analysis conditions.
Figure III	Aroclor 1242 electron capture reference chromatogram at analysis conditions.
Figure IV	Aroclor 1248 electron capture reference chromatogram at analysis conditions.
Figure V	Aroclor 1254 electron capture reference chromatogram at analysis conditions.
Figure VI	Aroclor 1260 electron capture reference chromatogram at analysis conditions.
Figure VII	Support Coated Open Tubular flame ionization chromatogram - demonstrating the complexity of the isomer distribution of Aroclor 1242.
Figure VIII	Typical alteration of Aroclor 1242 observed after metabolism and/or excretion by Albino rats.
Figure IX	Typical alteration of Aroclor 1254 observed after metabolism and/or excretion by Albino rats.
Figure X	Typical alteration of Aroclor 1260 observed after metabolism and/or excretion by Albino rats.
Figure XI	Typical alteration of Aroclor 1242 after metabolism and/or excretion by White Leghorn chickens.
Figures XII - XIX	Electron capture chromatograms of some commercial pesticide mixtures before and after treatment on XE-60 and DC-200.

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FIGURE I

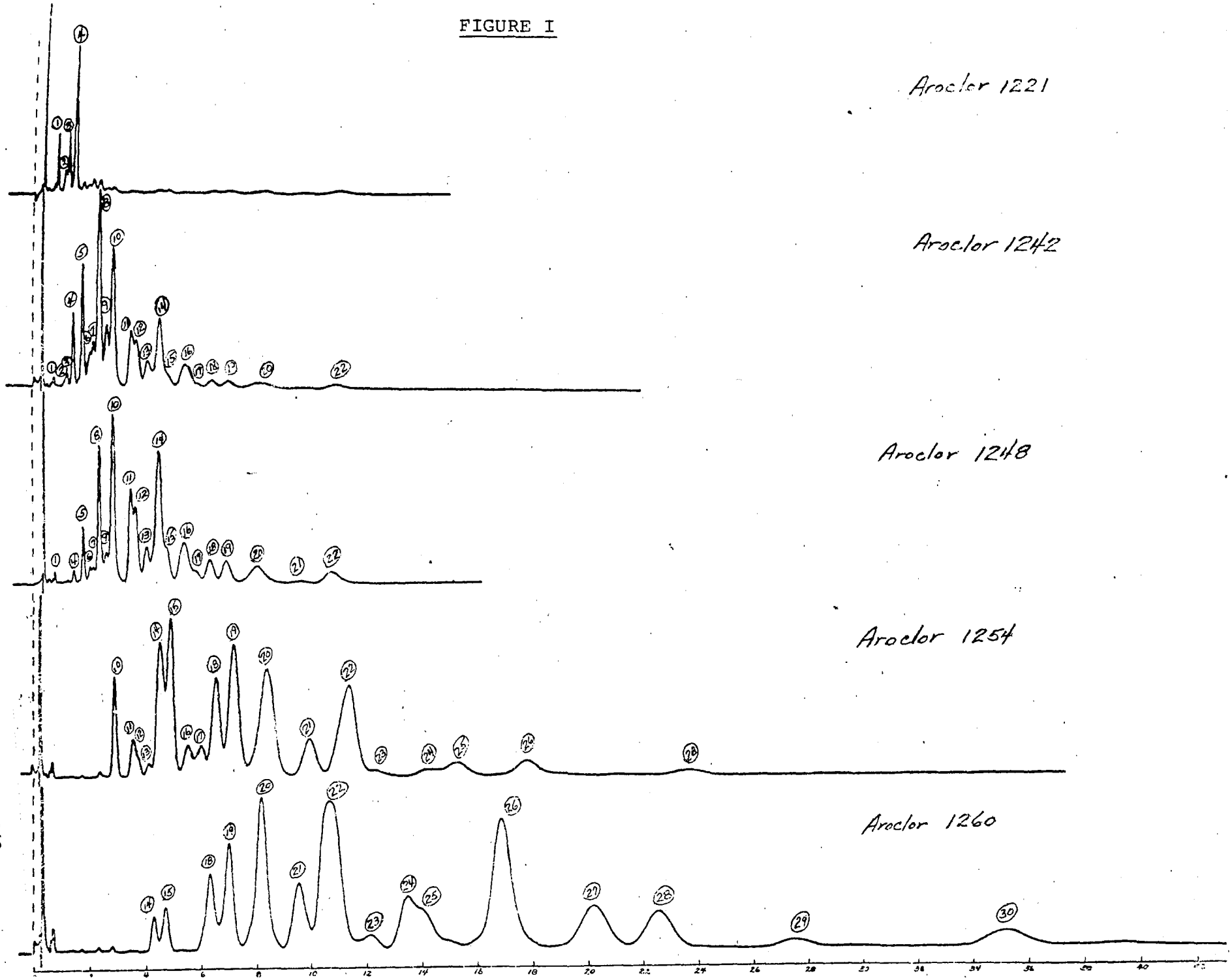

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FIGURE II

AROCLOR 1221

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 170°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.48 X 10^{-6} g/ml
Range - 10
Attn. - 4

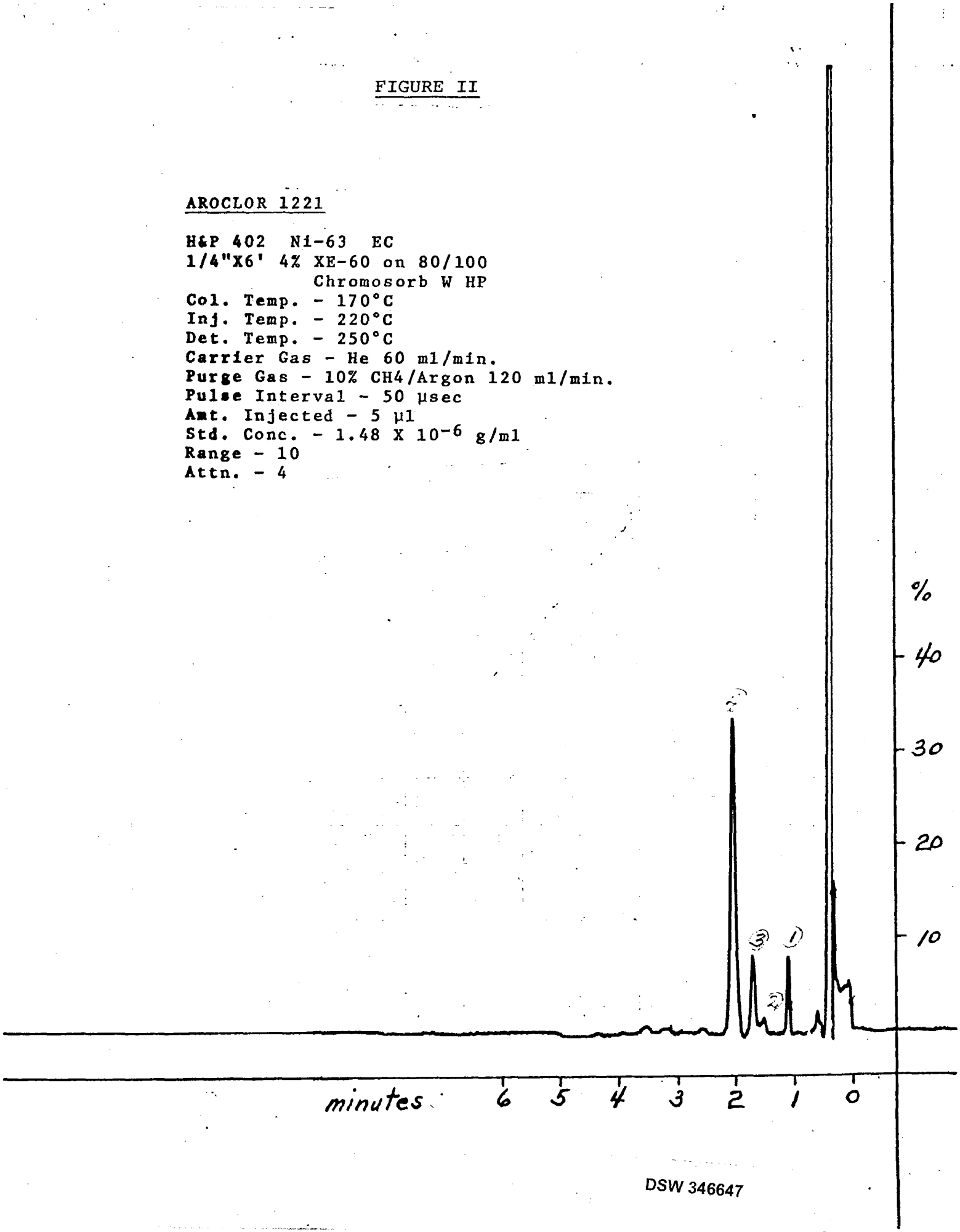

FIGURE III

AROCLOR 1242

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 190°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.03 X 10^{-6} g/ml
Range - 10
Attn. - 8

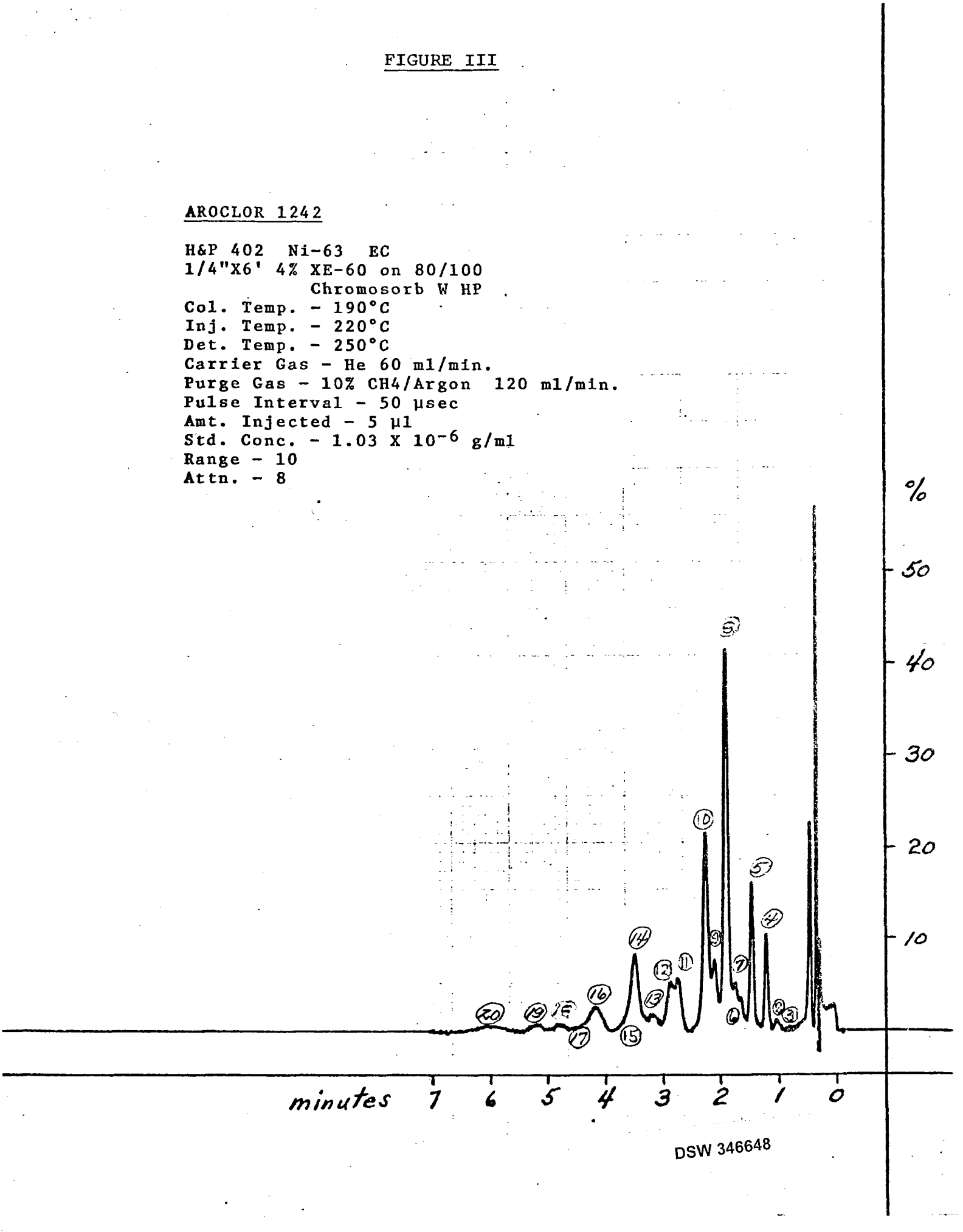

FIGURE IV

AROCLOR 1248

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 190°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min.
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.31 X 10^{-6} g/ml
Range - 10
Attn. - 4

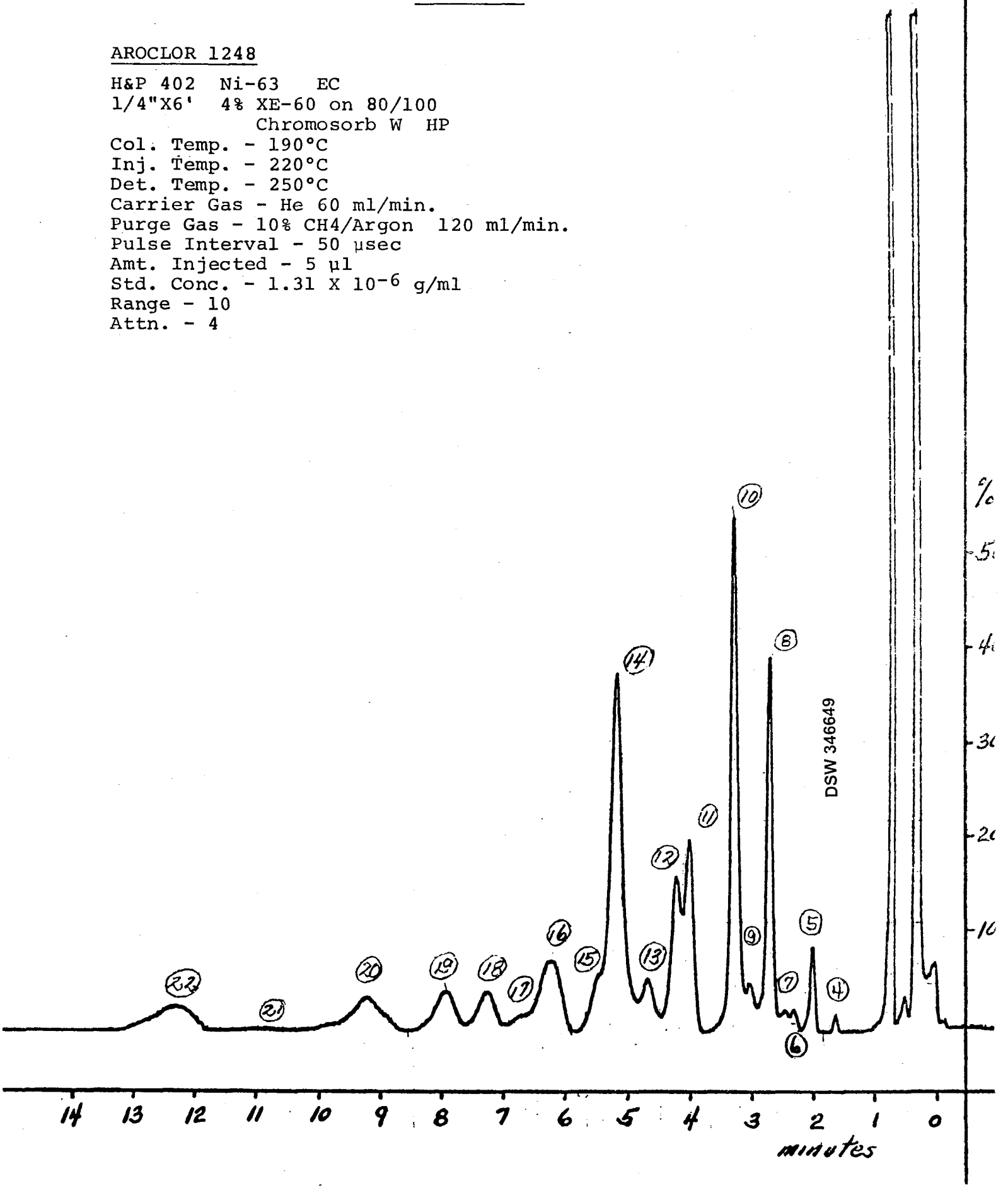

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FIGURE V

AROCLOR 1254

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 205°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min
Purge Gas - 10% CH4/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 1.02 X 10^{-6} g/ml
Range - 10
Attn. - 8

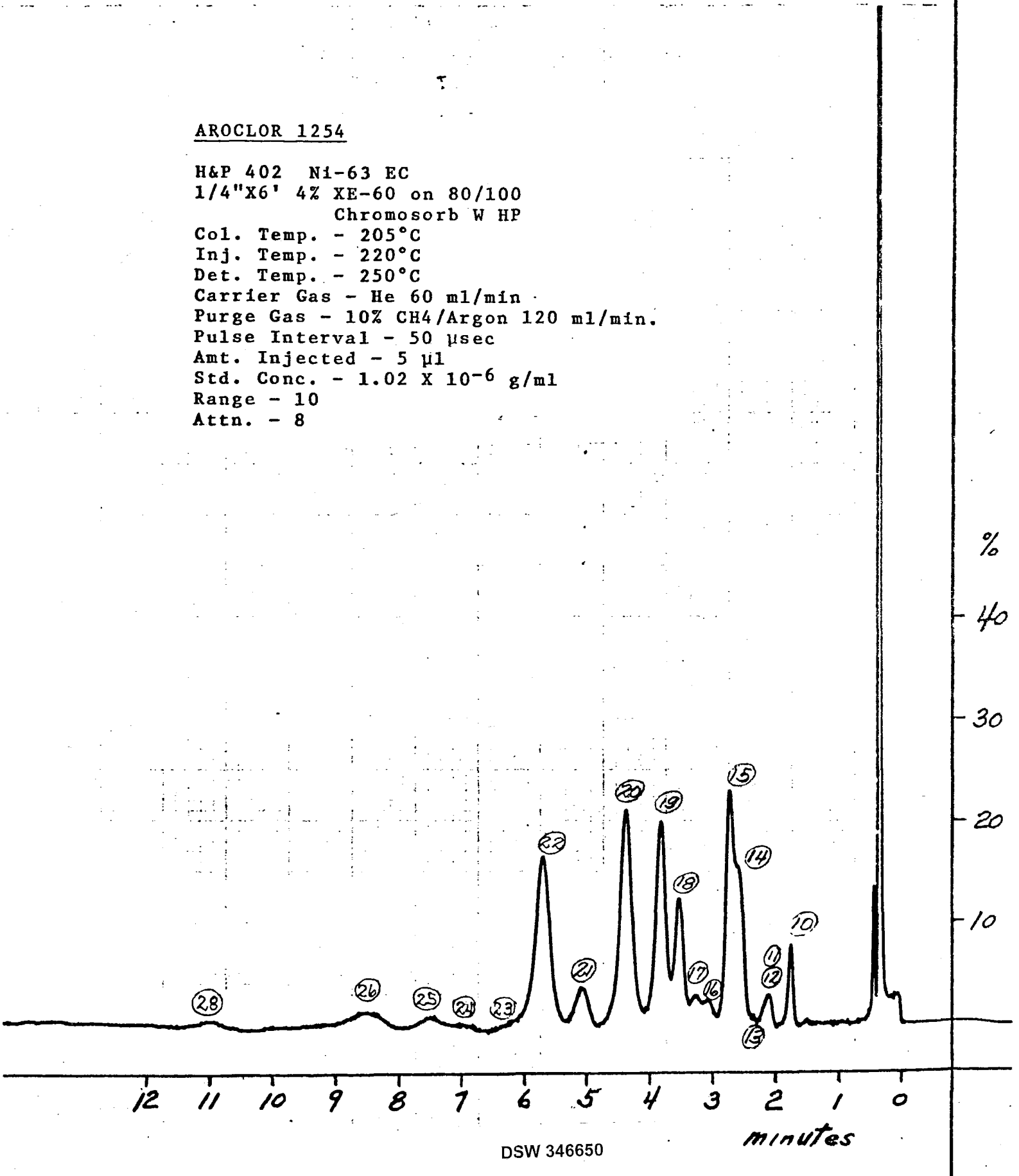

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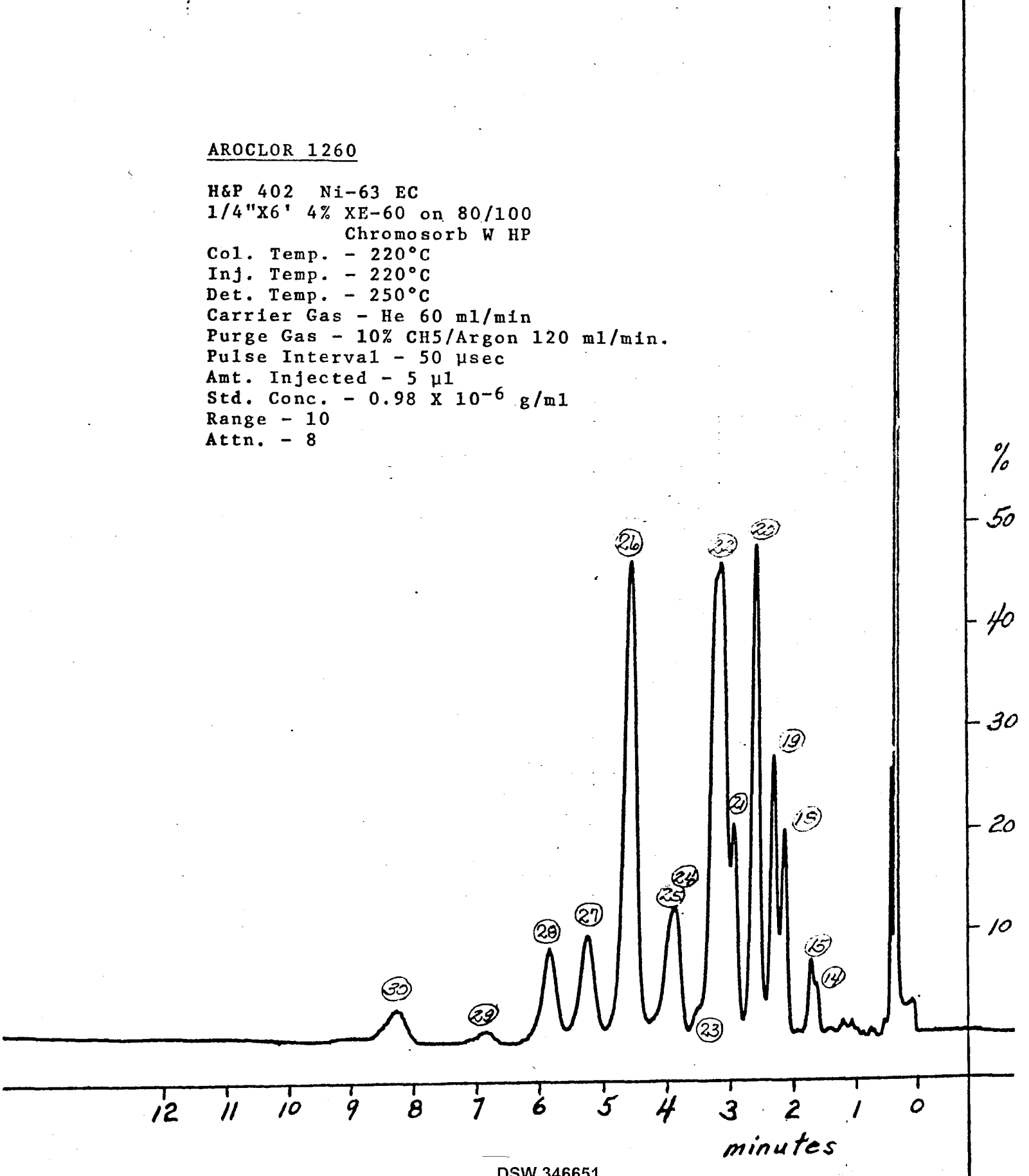
FIGURE VI

AROCLOR 1260

H&P 402 Ni-63 EC
1/4"X6' 4% XE-60 on 80/100
Chromosorb W HP
Col. Temp. - 220°C
Inj. Temp. - 220°C
Det. Temp. - 250°C
Carrier Gas - He 60 ml/min
Purge Gas - 10% CH5/Argon 120 ml/min.
Pulse Interval - 50 µsec
Amt. Injected - 5 µl
Std. Conc. - 0.98 X 10^{-6} g/ml
Range - 10
Attn. - 8



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CHROMATOGRAPHIC CONDITIONS

FIGURE VII

Instrument: Perkin-Elmer Model 800

Type Detector: Flame Ionization

Column: 100' X 0.02" I.D. FFAP S.C.O.T. (Support Coated Open Tubular) Capillary (Perkin-Elmer Part No. 008-0804 for each 50' length)

Column Temperature: 230°C

Detector Block Temperature: 285°C

Injection Port Temperature: 330°C

Sample Volume Injected: 0.002 ml
Split 30 to 1

Flow Rates

Column Helium: 5 ml/min
(7 psig)

Auxiliary Helium: 50 ml/min

Hydrogen: 50 ml/min

Air: 400 ml/min

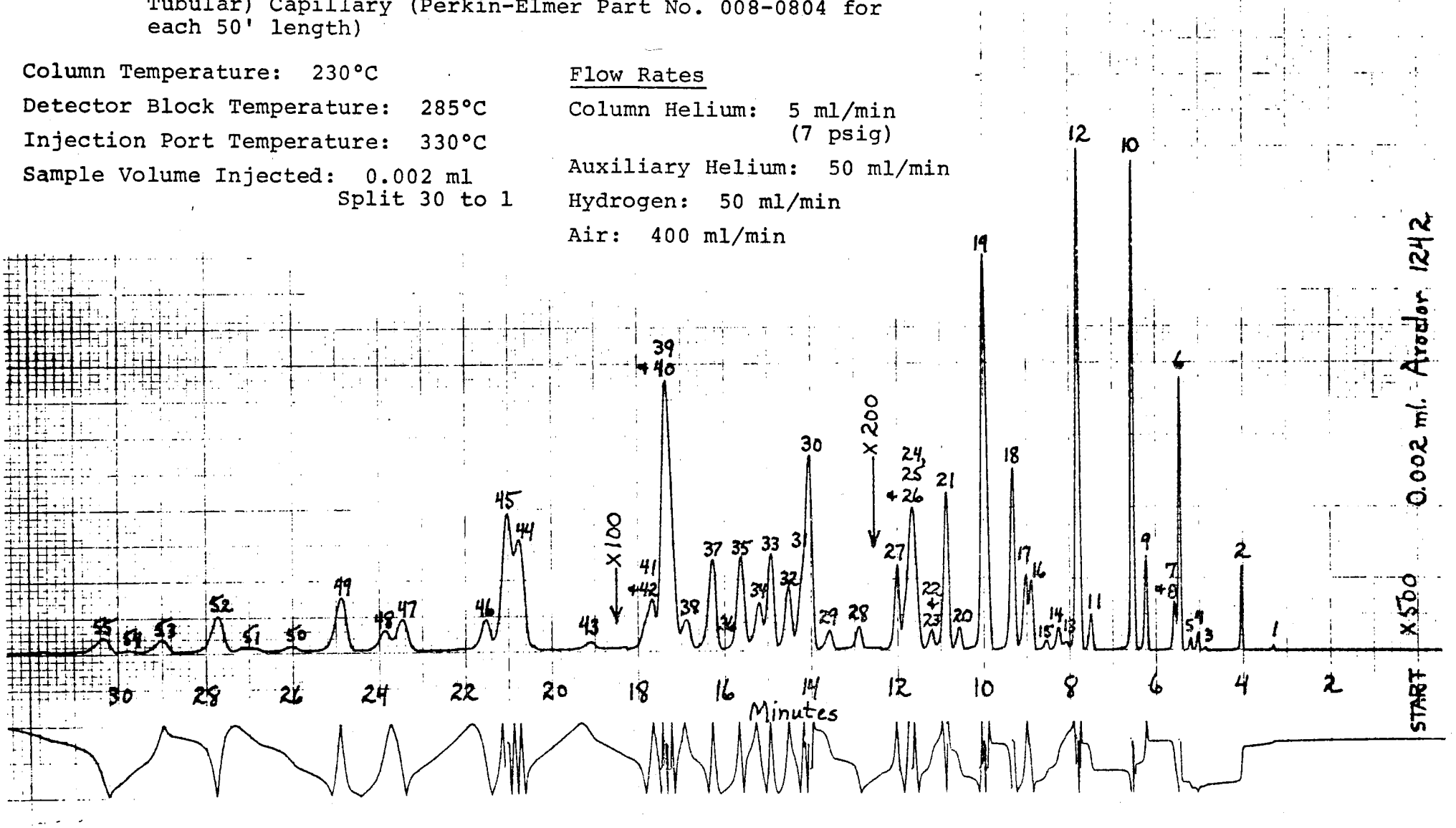

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FIGURE VIII

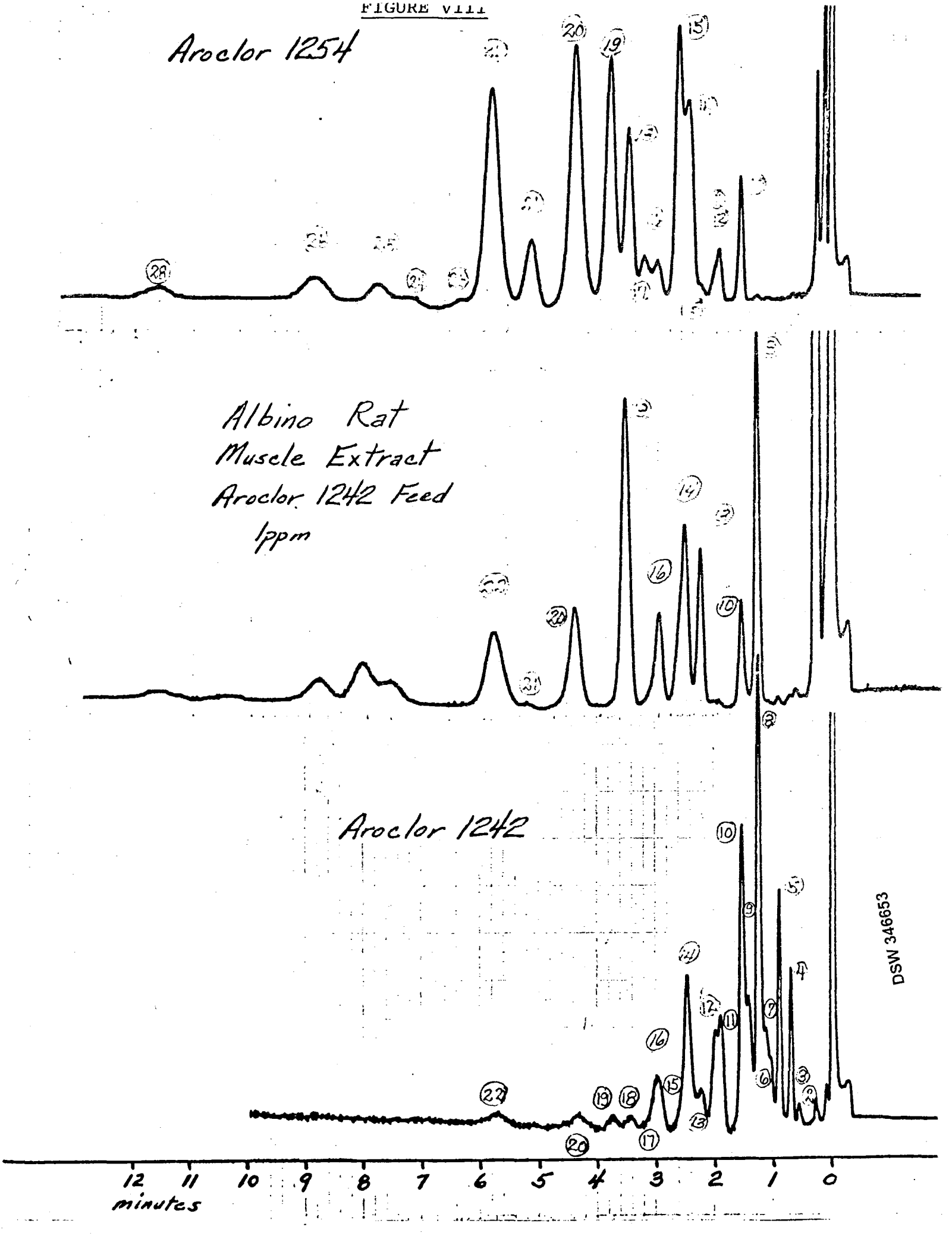

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FIGURE IX

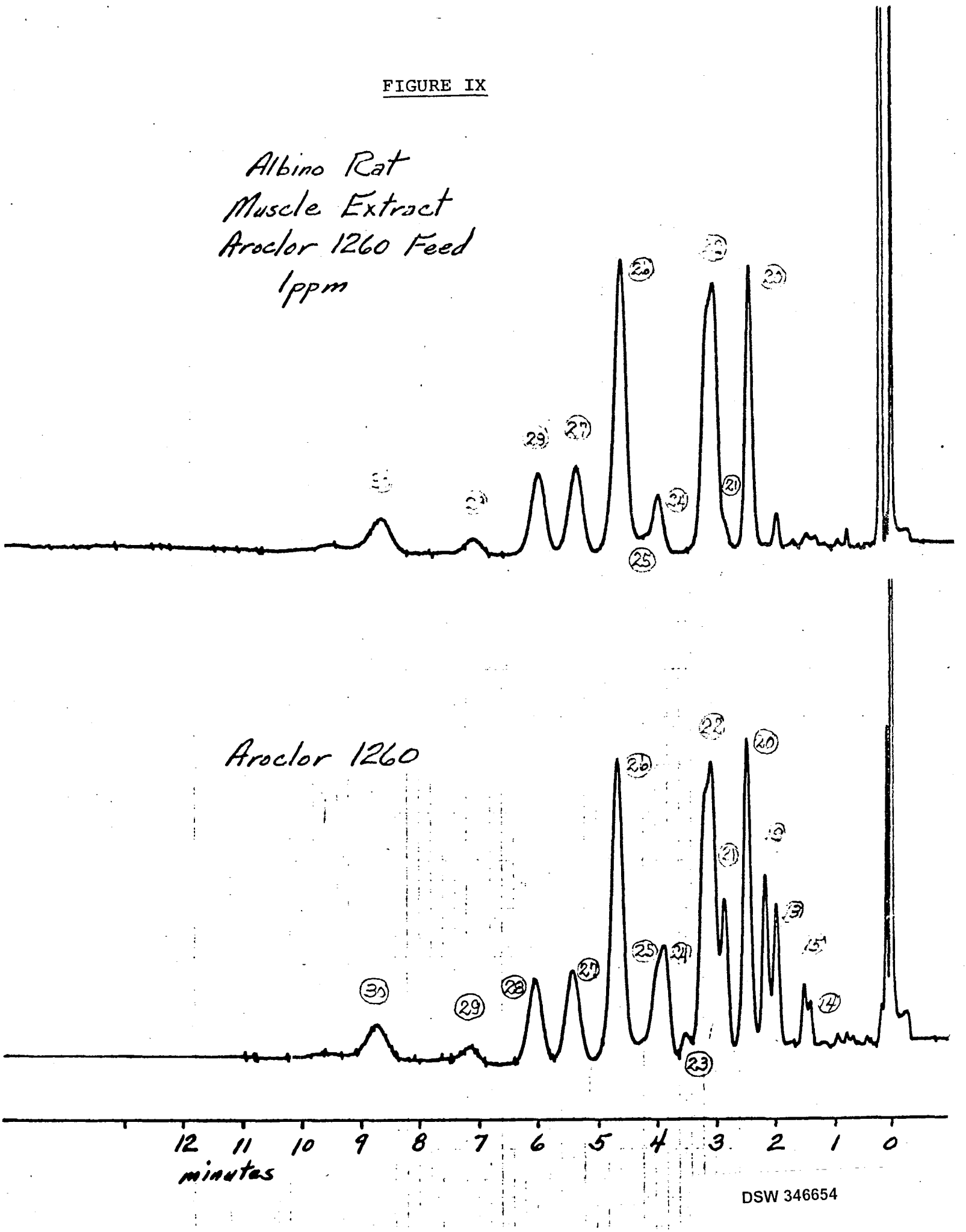
Albino Rat
Muscle Extract
Aroclor 1260 Feed
1ppm
Aroclor 1260
12 11 10 9 8 7 6 5 4 3 2 1 0
minutes
DSW 346654

FIGURE X

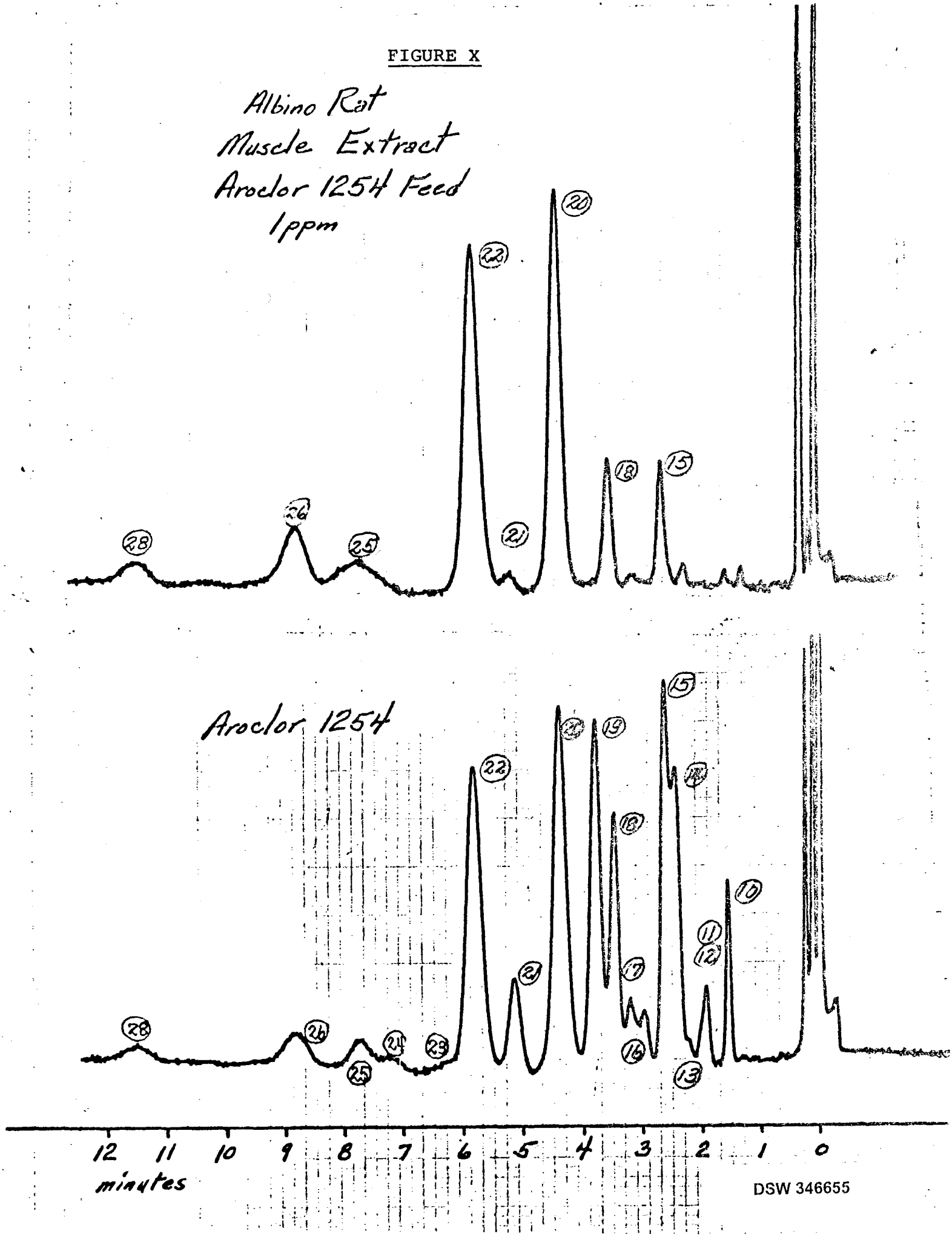

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FIGURE XI

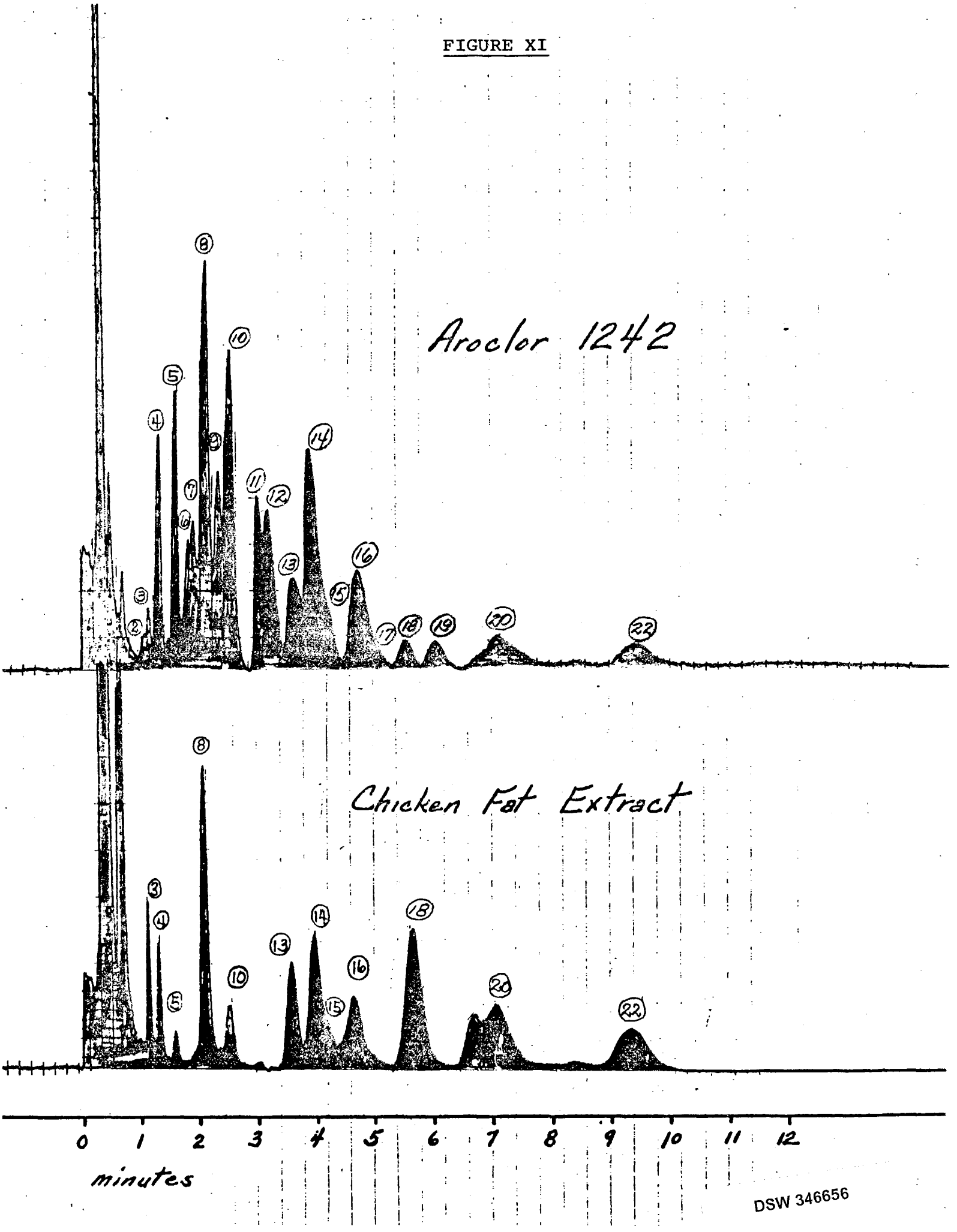

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FIGURE XII

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Figure XIII

5ul Pesticide Mix A Treated
Soap, H_2SO_4, Alumina Col.
G.C. Conditions: Same as XII

5ul Pesticide Mix A TREATED

DSW 346658

Figure XIV

G.C. Conditions - Same as XII

DSW 346659

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Figure XII

5ul Pesticide Mix B Treated
Sp., H_2SO_4, Alumina Gel
G.C. conditions - Same as XII

5 ul Pesticide Mix B Treated

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FIGURE XVI

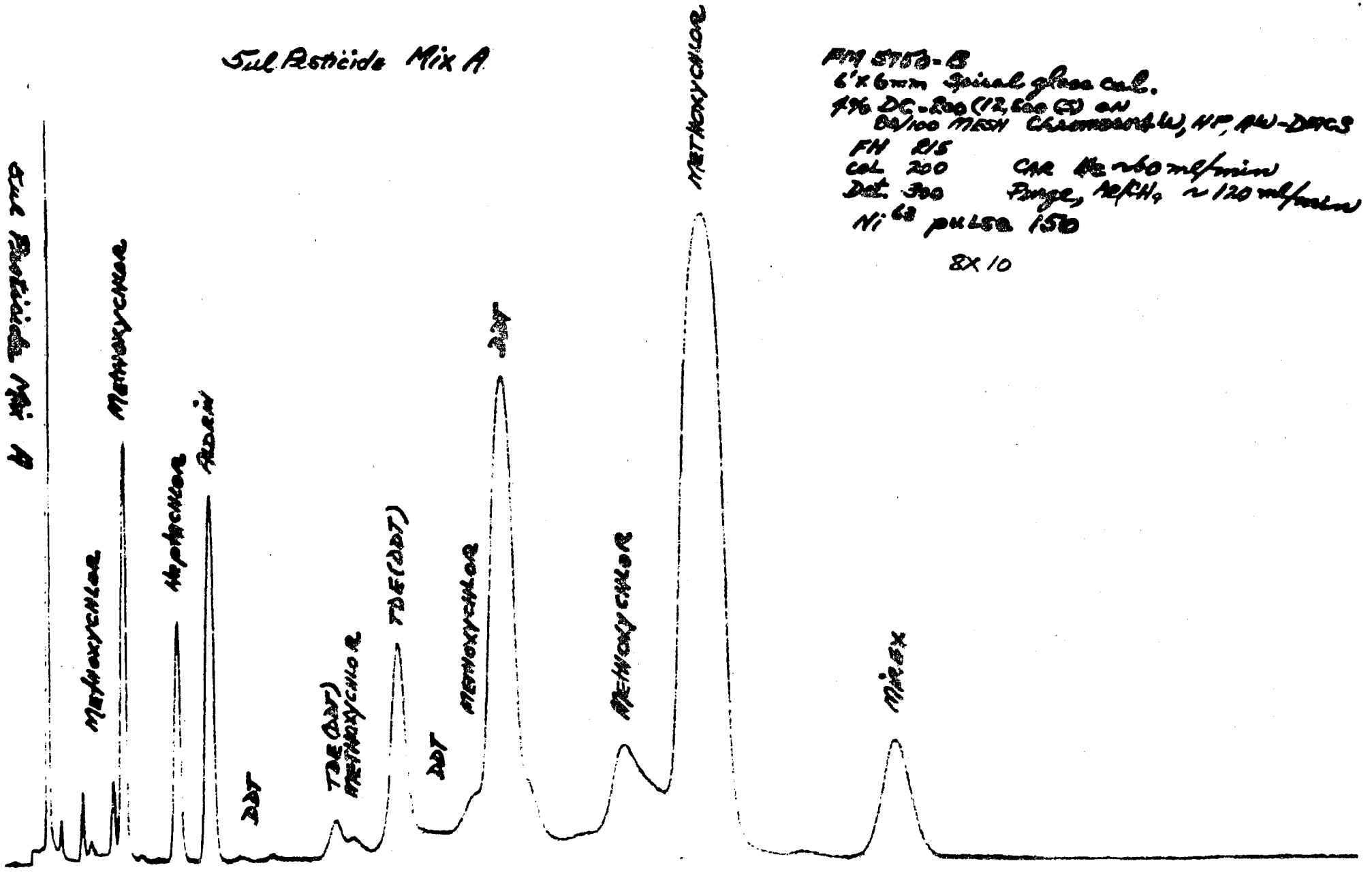

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Figure XIII

Soil Pesticide Mix A Treated (Soil, Florisil, Alumina)
G.C. Conditions - Same as XII

Soil Pesticide Mix A Treated

DSW 346662

FIGURE XVIII

5ug Pesticide Mix B

G.C. conditions — Same as XVI

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Figure XIX

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