CLEAN-UP AND SAMPLING TECHNIQUES FOR GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS

F. I. Onuska

F.I. ONUSKA, M.E. COMBA
ANALYTICAL METHODS RESEARCH SECTION,
CANADA CENTRE FOR INLAND WATERS,
BURLINGTON, ONTARIO. L7R 4A6

INTRODUCTION

At the Analytical Methods Research Section, CCIW Branch, Inland Waters Directorate, a lot of effort has been directed to the development of suitable analytical techniques for the quantitative determination and structural elucidation of organochlorine bridged pesticides, their photo-alteration products, chlorinated biphenyls, 1,4-oxathiins, dithio-carbamates, organophosphates and sulphur containing pesticides.

The instrument mainly responsible for evaluating such determinations is the gas chromatograph (GC). This instrument is used routinely for the separation of complex mixtures. However, when used for trace analyses, the amount of material present often does not allow confirmatory tests to be undertaken to definitely establish the identity of a component. In order to overcome this limitation and add to the instrument's versatility in the area of trace analysis, particularly structural characterization, a mass spectrometer (MS) is often used in conjunction with the GC.

In general, mass spectrometry is a powerful technique for the quantitative analysis of gases and volatile liquids and the elucidation of organic molecular structures. The substantial growth in this aspect of mass spectrometry is a direct result of the enormous improvement in vacuum and electronics technology which often allows information to be obtained on a few micrograms of material. Basically an electron impact mass spectrometer ionizes vaporized molecules which undergo fragmentation and are observed and measured as relative abundances versus mass to charge ratios. The information, which is presented by a mass

spectrum generally shows the molecular ion and concurrent ion fragments resulting from losses of an atom, molecule or rearrangement of the compound. The mass spectrometer is unique in its ability to provide a primary piece of structural information - the exact mass of a particular compound.

The fragments produced from the molecular ion indicate the moiety from which the molecule was composed, and their interpretation is used to deduce how they relate to the original molecular structure.

GC and MS share two important characteristics; in both cases, samples in the vapor phase and in microgram quantities can be used. The GC separates substances in pure form for the MS-detector and also acts as an inlet system for the MS, especially for small samples. Thus, samples need not be isolated or rigorously purified. For components of unknown structure or class, information ranging from molecular weights to total structures may frequently be realized. But it must be kept in mind that samples should be investigated in conjunction with classical methods such as spot tests, melting points, elemental analyses and expanded with such techniques as GC, TLC, infrared, NMR or atomic absorption where possible.

The AMRS-mass spectrometry lab has a powerful tool in computerized GC/MS. This system is capable of semi-automatic pollutant identification, and can be extended to quantitative analysis through the use of a multiple peak monitor, (M.I.D.). Using this technique samples which are invariably complex mixtures need not be highly purified as the results are not affected by the degree of GC-separation of the components. This could save both time and loss of valuable samples in the purification processes.

The detection limit of the MS can be extended conservatively by a factor of 100 using the MID system when individual ion peaks in the spectrum are monitored and effectively integrated. These individual peaks are selected as being unique to the compound studied and the detection of their presence in the correct abundant ratios with respect to retention time, provides a means of identifying compounds. The compound under study and its mass spectrum must, of course, be known and there is a multitude of applications where chemists are looking for small quantities of a known substance in complex environmental mixtures.

Mass spectrometry alone, cannot readily provide the answer to the complete unkown. It is vital that sample characteristics have been defined in order to obtain effective and beneficial results. The incorporation of sampling procedures, source information, preliminary analysis, extraction and derivitization techniques are an integral part of the analytical process.

Wash Procedure for 40 oz. Glass Bottle

1. Rinse with cold tap water.
2. Wash with chromic acid, contacting all inner surfaces.
3. Rinse 6-8 times with hot tap water.
4. Wash with heavy duty detergent (Sparkleen will suffice), scrubbing vigorously with brush.
5. Rinse at least 3 times with hot tap water, making sure all traces of detergent are removed.
6. Rinse 3 times with regular grade acetone, contacting all inner surfaces.
7. Rinse twice with pesticide grade acetone, contacting all inner surfaces.
8. Rinse twice with pesticide grade petroleum ether, contacting all inner surfaces.
9. Blow dry with pre-purified nitrogen
10. Place in oven at 300°C for a minimum of 6 hours.

Wash Procedure for Aluminum, for Liners for Above

1. Dip roll of Aluminum Foil (N.B. removed centre cardboard cylinder) several times in pesticide grade acetone using solvent cleaned forceps.
2. Repeat the above using pesticide grade petroleum ether.
3. Allow to dry or blow dry with pre-purified nitrogen.
4. Again using the forceps tear off a square (or cut using solvent cleaned scissors) of approximately 2" and place over mouth of bottle, then cap.

SAMPLING

Lake, river and industrial waters are not homogenous. The primary objective of most sampling is to obtain samples which truly represent the characteristics of the stream from which they were taken. Manual collection of samples is practical for exploratory and short-term surveys.

The volume of the sample collected is usually one or two liters. Compounds present in a one-liter sample at a concentration of 5 µg/l or greater will generally give better quality spectra when processed by extraction, preconcentration, and GC/MS techniques. For greater sensitivity larger samples are required.

COLLECTION

Manual collection of samples, should be taken in accordance with the methods specified in the booklet, "Instructions for Taking and Shipping Water Samples for Physical and Chemical Analysis," (1) with the precautions related to each method of analysis being applied.

Sample Information

Samples submitted for mass spectrometric analysis should be accompanied by a sheet giving pertinent data regarding the samples background. This can be summarized as follows;

1. Sample location
2. Sample type: (ie. water extract, fish extract, acid esterification extract etc.)
3. Sample concentrations (if known).

4. Environmental effect. (ie. toxic to fish, birds, mammals, odorous, high concentration in plants etc.)
5. Type (s) of compounds expected. (based on preliminary investigations).
6. Samples should be accompanied by an F.I.D. chromatogram.

CLEAN-UP

When extracts of water are analyzed by GC, the extract usually must be cleaned-up to remove interferences (i.e., large hydrocarbon background), in such a manner that the procedures used are directly related to the sample information required. The techniques most widely used are fluorisil or silica gel column chromatography, sometimes in combination with acetonitrile partitioning. Dry-column chromatography is also recommended as a clean-up technique in connection with thin-layer chromatography.

If preliminary gas chromatographic runs show that clean-up of the extract is necessary, the first approach should be to clean-up the concentrated extract on activated fluorisil. As another approach, after solubility separation of the crude extract, the neutral fraction may be further separated on a silica gel column. Elution with isooctane, benzene, methanol-chloroform (1:1) separates organics into aliphatics, aromatics and oxygen-containing organics fractions, respectively (2). Fortunately, the mass spectrum of a compound is much more definitive than its retention time, extracts for GC-MS analysis may not need further clean-up when solubility class separation has been used during sample extraction.

The sampling procedures described have been taken in part from EPA-

Report R2-73-277 (3) and the Analytical Methods Manual, Inland Waters Directorate, (4) modified to suit mass spectrometric requirements.

ORGANOCHLORINATED PESTICIDE AND PCB's IN WATERS

Collect samples in all glass containers, and store at $5^{o}C$

Extraction of Samples

1. (a) Add approximately 25 ml of benzene to one liter of water sample, in the original sample bottle. Stir the mixture with a magnetic stirrer so that the vortex formed at the surface almost reaches the bottom of the bottle. After 30 min., transfer the content quantitatively to a one-liter separatory funnel. Rinse the empty water sample bottle with 2 x 30 ml benzene making sure that the glass surface which is exposed to the water sample is well washed by benzene. This can be achieved by putting the bottle horizontally or almost so on a table and rotating the bottle back and forth several times. Transfer the benzene rinsings to the same separatory funnel. NOTE: For PCB analysis, extract at least two identical 1-liter water samples or one 2-liter water sample.

   (b) Shake contents in the separtory funnel vigorously and allow the organic layer to separate. If an emulsion forms, add a few drops of one of the following: saturated sodium sulfate solution, methanol, isopropanol or 2-octanol. Gently agitating contents may further help to break the emulsion. Caution: Do not add too much alcohol, otherwise a large solvent peak may result; 5 to 10 drops should be sufficient.

(c) Transfer the aqueous layer back to the empty sample bottle and dry the organic layer under rapid suction through a filter funnel containing 50 gm of sodium sulfate into a 300-ml round-bottom flask.

(d) Repeat extraction procedure, twice using 25 ml of benzene and stirring for 10 min. rinsing sample bottle once for each extraction with 20 ml benzene. Dry extracts as described previously.

(e) To the combined organic extracts add 1 ml or so iso-octane and concentrate in rotary evaporator to approximately 3 ml. During evaporation the water bath temperature should not exceed 40^{o}C. When the extract is concentrated to 10-12 ml, finish the concentration step by letting the flask rotate in the airway from the water bath. This step is critical. Severe loss of pesticide will result if the extract is allowed to dry, particularly if the water bath is too warm.

(f) Sample extracts from duplicate collection procedures may be combined for higher concentrations.

Florisil Fractionation

2. (a) If PCB's are present and/or the extract contains many pesticides to make GLC interpretation difficult, pass the concentrated extract through a florisil column prepared as follows:

(b) In a 20 cm x 400 cm chromatographic column with coarse sintered disk near the bottom, filled with hexane (3/4 full), add 2 gm pretreated sodium sulfate, followed by 10 gm of florisil in portions. Tap the column gently while adding the florisil to the column; this prevents channelling. Drain some hexane to

settle the florisil layer. Add 3 gm of pretreated sodium sulfate ensuring minimum disturbance of the florisil layer.

(c) Pre-wash the column with 50 ml benzene, followed by 2 successive additions of 75 ml hexane. Allow column to drain and discard eluates.

(d) Dilute the concentrated extract (0.5 ml) to about 2 ml with hexane. Quantitatively transfer the hexane extract to the column. Allow the extract to sink just to the surface of the sodium sulfate layer. Wash the round-bottom flask with 3 ml hexane and transfer the wasing solution to the column. Let the extract run down as before. Repeat twice with 3ml hexane.

(e) Pour with care 100 ml of hexane into the column. Make sure that the florisil layer is not is not disturbed.

(f) Run the eluate into a 200-ml round-bottom flask and concentrate under vacuum to approximately 3 ml ensuring that precautions during the evaporation are taken. Transfer quantitatively the concentrated eluate to a 15-ml graduated centrifuge tube. Wash the flask twice, each time with 2 ml to 3 ml petroleum ether, and transfer to the same tube. Add ½ ml of iso-octane or toluene as a keeper. Concentrated to 1 ml under nitrogen as described above. Label fraction.

(g) Elute the same column with 100 ml of 15% ethyl ether in petroleum ether or hexane concentrate eluate or if β-endosulfan is present, elute column with chloroform or 50% ethyl ether in

pet. ether and concentrate as previously described.

NOTE: The hexane or petroleum ether fraction will elute α-BHC, heptachlor aldrin p,p'-DDE and PCB's (Aroclor 1248, 1254 and 1260). The 6% ethyl ether in hexane or petroleum ether fraction will elute p,p'-DDD, p,p'-DDT, o,p'-DDT, lindane cis-and trans-chlordane, methoxychlor and heptachlor epoxide. The 15% ethyl ether in hexane a petroleum ether will elute aldrin, α-endosulfan and dieldrin. The last fraction will elute β-endosulfan.

PHENOXY ACID HERBICIDES IN WATER

Sampling Procedure and Storage

Water samples should be collected in an all-glass system, kept in the dark at $5^{o}C$ and extracted within 12 hours because degradation of 2,4-D is rapid in an aqueous environment. The sample should be acidified to pH 2 with sulfuric acid immediately after collection.

Extract of Sample

3. (a) Transfer acidified water sample quantitatively to a 2000-ml separatory funnel.

   (b) Add 50 ml of chloroform to the separatory funnel and shake the mixture thoroughly for 1 minute. Occasionally emulsions are formed, but can usually be broken by adding small portions of 2-propranol, acetone or a saturated NaCl solution.

   (c) Allow at least 5 minutes for complete separation of the layers and draw off the bottom chloroform layer into a clean 500 ml separatory funnel.

(d) Extract the sample twice more and combine the extracts in the 500 ml separatory funnel. The chloroform extracts are then washed with 100 ml of glass-distilled water and the aqueous layer is removed, making sure the glass-distilled water is slightly acidic.

(e) The combined chloroform extracts are dried over acidified anhydrous sodium sulfate for about ten minutes. Caution: The extract should not remain in contact with the sodium sulfate for any period exceeding one-half hour.

(f) The residues are concentrated on a rotary evaporator to about 5 ml; 10 ml of methanol is added and evaporated again to 5 ml. Continue this procedure until all traces of chloroform are removed and transfer the methanol solution to a graduated centrifuge tube (15 ml) and concentrate to 1 ml under a gentle stream of nitrogen.

Esterification and Clean-up

4. (a) To the graduated centrifuge tube add 0.5 ml of the BF_3-methanol complex, cap tube and heat in a water bath at $50^{o}C$ for 30 minutes. The reaction mixture is then allowed to cool to room temperature.

(b) About 5 ml of 5% aqueous sodium sulfate solution is added to the tube and extraction of the methyl esters is carried out with two 2 ml portions of hexane. The hexane extract is concentrated to 1 ml under a stream of dry nitrogen.

(c) The hexane phase containing the methyl esters of the phenoxy acid herbicides is passed through a small column, prepared by plugging a disposable pipette with glass wool and packing with 2 cm of neutral anhydrous sodium sulfate over 2 cm of florisil. The herbicide esters are eluted with 10 ml of benzene.

NOTE:

Strict attention is required of the analyst to obtain reproducible and satisfactory recovery. In the steps where solvents are evaporated, extreme care must be exercised, especially when working with the methyl esters. The extracts should never be taken to dryness as the esters are extremely volatile.

Care must be taken to ensure that the tubes are tightly capped and remain so after introduction of the BF_3-methanol reagent. The temperature should be about $50^{o}C$ for good yields. The methylation is a very crucial step in the procedure.

Extracts should be labelled and analyzed as soon as possible.

ORGANOCHLORINATED PESTICIDES AND PCB'S IN FISH AND SEDIMENTS

Sampling Procedure

Fish and sediment samples should be collected and frozen immediately in an all-glass system or metal container. Clean aluminum foil may be used to wrap frozen fish for storage.

Grind the entire frozen fish sample in a large electric food grinder such as the Hobart Grinder Model 1812. If the fish is too large, it may be cut into several pieces before grinding. The ground fish is mixed thoroughly and a subsample (homogenate) taken for analysis. The unused portion should be stored immediately in a glass jar or an aluminum can below $0^{o}C$.

Line the cap of the sample bottle with cleaned aluminum foil to prevent sample from contacting the glue lining of the sample top. If an all-aluminum can is used, this procedure is not necessary.

Never use plastic utensils during analysis for storage or transferring.

Extraction

(a) Transfer 10 grams of the homogenate into a glass jar of a Waring Blender with a Bakelite top. (Do not use a rubber or a plastic top). Add 120 ml of acetonitrile and blend at medium-high speed for fifteen minutes. Allow solid particles to settle somewhat. Pour the acetonitrile extract, which may contain some suspened particles, into an Ahlihn filter tube containing prewashed celite covering the sintered glass.

(b) To the residue in the blender, add another 120 ml of acetonitrile and 40 ml of distilled water and blend for 10 minutes. Filter as before.

NOTE: If the residue in the Ahlihn filter tube becomes excessive, it should be scraped out with a spoon-type spatula and combined with that in the blender, before the second blending discussed above.

(c) Pour 60 ml of acetonitrile into the blender and blend the homogenate for 10 min. Transfer all the residue, if necessary with 2 x 20 ml acetonitrile, into the Allihn tube and filter. Apply strong suction so that the residue in the tube contains little solvent.

(d) Transfer, with petroleum ether rinsing, the combined acetonitrile extracts into a 1-litre funnel and dilute with distilled water to adjust the aqueous content to 20%. Extract the resulting mixture with 150 ml and then twice with 75 ml petroleum ether.

(e) Wash the combined petroleum ether extracts with approximately 200 ml distilled water. Discard water washing and pass the

organic extract under suction or with air pressure, through an anhydrous sodium sulfate (10-15 gm) column using a 500 ml round-bottom flask as a receiver.

(f) In a rotary evaporator, evaporate the contents in the 500 ml flask to 2 or 3 ml. (Do not let contents get dry) and do not use a water bath temperature over 40°C for evaporation; otherwise, there will be possible loss of pesticides and PCB's.

Clean-up

(a) Transfer the concentrated petroleum ether extract with a clean disposable pipette onto a 30 gm Florisil column with ½" of anhydrous sodium sulfate on the top of the florisil. Use a 300-ml round-bottom flask as a receiver. (See procedures for water analysis for the precaution and discussion of Florisil column).

(b) Allow the extract to sink down just to the sodium sulfate layer. Rinse the round-bottom flask with 2 or 3 ml of petroleum ether and transfer the rinsing with the same disposable pipette onto the column. Let the rinsing solvent again sink down just to the sodium sulfate layer. Rinse the round-bottom flask again with 2 or 3 ml of petroleum ether and transfer the rinsing onto the column.

(c) Again rinse the round-bottom flask. This time with 20-30 ml petroleum ether. Carefully pour the petroleum ether onto the column so that the sodium sulfate layer is not disturbed. Elute the column with a total of 200 ml (including the above rinsings) of petroleum ether.

(d) Concentrate eluate with a rotary evaporator to 1 or 2 ml and transfer, with benzene rinsings, to a 10 ml volumetric flask. Make up to 10 ml with benzene.

(e) Change receiver and elute column with 200 ml of 6% diethyl ether containing 2% ethanol. Concentrate eluate on rotary evaporator and make up to 10 ml with benzene as previously described.

(f) With a third 300-ml round-bottom flask as a receiver, elute the column with 200 ml of 15% ether in petroleum ether. Concentrate to 10 to 20 ml with a rotary evaporator. Add 50-60 ml of benzene and concentrate to 1-2 ml. Make up to 10 ml with benzene in a cleaned glass vial.

(g) Use 200 ml of chloroform or 50% diethyl ether in petroleum ether to elute β-endosulfan from the column. Concentrate eluate to 2 or 3 ml. Add 50-60 ml benzene and concentrate to 2 or 3 ml. Repeate the last step once more before making up to 10 ml in a cleaned glass vial. Ship extracts by air freight in a frozen state.

NOTE: The petroleum ether fraction contains: PCB's, heptachlor, aldrin, p,p'-DDE and α-BHC.

The 6% diethyl ether in petroleum ether fraction contains: lindane, heptachlor epoxide, p,p'-DDT, p,p'-DDD, methoxychlor, o,p'-DDT, cis-and trans-chlordanes.

The 15% diethyl ether in petroleum ether fraction contains: dieldrin, α-endosulfan and endrin.

The last fraction contains: β-endosulfan.

EXTRACTION OF INDUSTRIAL ORGANIC CHEMICALS IN EFFLUENTS AND STREAMS

This procedure has been used for the extraction of a wide variety of organic chemicals including hydrocarbons, aromatics, organochlorines, organophosphates, sulfur compounds, amines, ureas, alcohols, ketones, aldehydes, ethers, isothiocyanates, phenols, and carboxylic acids. The extraction efficiency of the method will vary with the different chemical classes; therefore, any concentration value should be considered a minimum value unless the extraction efficiency of the compound quantitated has been determined. The solvent system specified in this procedure is for a general organic scan and is designed to provide as much separation as possible of neutral organics from weak and strong acids while still providing acceptable extraction efficiency (60-100%) for the broad range of organics. Therefore, it may be necessary to select an entirely different solvent or solvent mixture if you desire to extract a specific organic compound with 80-100% efficiency.

A gas chromatograph equipped with a flame ionization detector is used for preliminary analysis of the prepared extracts. If organochlorine, organophosphate, or organosulfur compounds are suspected, specific detectors such as electron capture, electrolytic conductivity, microcoulometric, flame photometric, or thermionic detectors may be used in addition to the flame ionization detector.

If the sample extract is too complex for accurate qualitative and quantitative analysis, the extract should be simplified by column chromatography, a solubility separation technique or possibly by high speed liquid chromatography.

PROCEDURE FOR EXTRACTION NON-COMPOSITED SAMPLES

1. Equipment should be set up to extract each sample in duplicate (one for esterification).
2. (a) Determine and record the sample pH.

   (b) Blend the sample with a blender to get a uniform suspension of any particular matter present and immediately transfer to a graduated cylinder. Record the volume. Divide the sample into duplicate portions making sure the particulate matter is equally divided. Transfer to a 2 l erlenmeyer flask if the pH is 5-14 or to a 2 l separatory funnel if the pH is 1->5. Divide the rinse solvent into two equal portions and add one portion to each sample duplicate.

   (d) Add the appropriate solvent (hexane or methylene chloride) to each sample duplicate to bring the total volume of solvent to 60 ml per liter of sample.

EXTRACTION PROCEDURE FOR COMPOSITED SAMPLES

3. (a) Determine the total volume of composited sample needed to extract in duplicate and then the volume needed from each sample to be composited. (If sample size permits, the total volume should be at least 2000 ml so that 1000 ml duplicates can be extracted). NOTE: It is again very important to have particulate matter in uniform suspension prior to measuring out the duplicates and to transfer all particular matter to the extraction containers.

   (b) Blend the sample to get a uniform suspension of any particular matter and immediately transfer to a clean dry container being

sure all particulate matter is transferred. Measure, record and discard the volume remaining in the original sample container. Repeat the above on the samples remaining to be composited.

(c) Shake the composited sample and determine and record the pH.

(d) Split the composited sample into duplicate portions and transfer to a 2 l erlenmeyer flask if the pH is 5-14 or to a 2 l separatory funnel if the pH is 1->5.

(e) Rinse the original sample container with 25 ml of hexane if the pH is 5-14 or with 25 ml of methylene chloride if the pH is 1->5. Split the rinse solvent in the same proportions as the sample was split. Discard the rinse portion that represents the portion of the samples that were composited. Divide the rinse composites into two equal portions and add one portion to each sample duplicate.

(f) Add the appropriate solvent (hexane or methylene chloride) to each sample duplicate to bring the total volume of solvent to 60 ml per liter of sample.

For Samples with pH 1 >5

4. (a) Adjust the pH of the water to strongly acid (pH>2) with phosphoric acid.

(b) Add 100 ml of diethyl ether and shake the sample for 2 minutes. Allow the layers to separate and drain the upper (diethyl ether) layer into a filter tube containing acid washed glass wool prewet with 50 ml diethyl ether. Collect the dried extract in a 400 ml beaker.

(c) Extract the water sample twice more with 60 ml portions of methylene chloride. Shake the sample each time for two minutes. Pass the extract through glass wool and collect in the beaker with the ether extract. If the sample is suspected to contain basic compounds retain the water for extraction as outlined in 6.

(d) Transfer the acid extract to a rotary evaporator and concentrate on a hot water bath to 1-2 ml in a concentrator tube. Disconnect the calibrated concentrator tube and place in a 150 ml beaker containing water maintained at 30-35°C. Reduce the extract volume to 1.0 ml using a gentle stream of clean, dry nitrogen.

(e) Transfer 0.5 ml of sample extract to a calibrated tube, add about 1 ml of carbon tetrachloride and reduce the volume back to 1.0 ml. Transfer to a vial and label "acid" extract.

For Samples with pH 5-14

5. (a) Add 5 gm of sodium chloride and a magnetic stiring pellet to the flask, place on a magnetic stirrer and adjust the stirring rate so that the swirling pellet disperses the solvent vortex as bubblets into the water. Stir in this manner for 15 minutes.

(b) Remove flask from stirrer and pour contents into a 2000 ml separatory funnel. Allow layers to separate for several minutes, drain lower (water) layer back into flask and drain the solvent extract into a filter tube packed with a glass wool pad that has been pre-wet first with 50 ml of methylene chloride then 50 ml of hexane. Collect the extract in a 400 ml beaker or a pint bottle.

NOTE: If an emulsion is present it can be broken by forcing it through the glass wool using some air pressure. This will probably necessitate passing the sample extract through a clean glass wool pad to remove any droplets of water that were forced through the first filter pad.

(c) Extract the water twice more with additional 60 ml portions of hexane per liter of sample as outlined in Steps (a) and (b). Retain the water for acid extraction in the 2 1 separatory funnel and proceed as described in step 4.

(d) Transfer the extract to a rotary evaporator and concentrate on a steam bath until the hexane ceases to actively distill. Disconnect the calibrated concentrator tube, add about 2 ml of iso-octane and place in a 150 ml beaker containing water maintained at 30-35^{o}C. Reduce the extract volume to 1.0 ml using a gentle stream of clean, dry, nitrogen. Transfer to a vial and label "neutral" extract.

Samples Suspected of Containing Basic Organic Compounds

6. (a) Adjust the sample pH to >11 with the KOH solution.

(b) Extract the sample three times with 60 ml portions of methylene chloride by shaking it for 2 minutes. Pass the methylene chloride extract through pre-rinsed Pyrex glass wool to dry the sample and combine the dried extracts in a 400 ml beaker.

(c) Transfer the dried extract to a rotary evaporator. Add a small boiling stone and reduce the volume to 1-2 ml using a hot water bath. Disconnect the concentrator tube, add 1-2 ml

of carbon tetrachloride and reduce the volume to 1.0 ml as outlined in 5 (d). Transfer to a vial and label "basic".

Esterification

Diazomethane and dimethyl sulfate are used routinely to prepare the methyl esters of carboxylic acids and the methyl esters of phenols and give the highest yield. Both methods are outlined.

Diazomethane Methylation

7. (a) Evaporate the methylene chloride in the other 0.5 ml portion (step 4) just to dryness using nitrogen and placing the tube in a beaker of warm water. Immediately redissolve the residue in approximately 3 ml of 10% methanol in diethyl ether. Mix on a Vortex Genie to dissolve any residue adhering to the walls of the tube. Stopper until ready to esterify. A small amount of methylene chloride may be reatined, but the presence of chloroform may produce artifacts.

(b) Set up apparatus as shown in Figure 1.

(c) Add about 5 ml of distilled-in-glass ether to the first tube of the apparatus to saturate the nitrogen carrier gas with ether. Add 0.7 ml of ether, 0.7 ml of carbitol, 2-(2-ethoxy-ethoxy) ethanol, 1.0 ml of 37% aqueous KOH (not over 2 days old), and 0.1-0.2 g of N-methyl-N-nitrouso-p-toluenesulfonamide ("Diazald", Aldrich Chemical Co.) to the second tube. The base immediately begins to release diazomethane from the sulfonamide.

(d) Immediately position the second test tube and adjust the nitrogen flow to about 10 ml per minute. CAUTION! Diazomethane is an extremely toxic and explosive gas. A good fume hood

and safety glasses are mandatory. No chipped glassware should be used, as rough glass surfaces catalyze decomposition of Diazomethane.

(e) Position the third tube (a safety trap to prevent reagent carry-over) and the sample tube (4) to bubble the nitrogen and diazomethane gas mixture through the sample. Continue the reaction until the slight yellow color of diazomethane persists in the sample solution (from a few seconds to 30 minutes, depending upon the sample concentration). In the case of dark colored extracts in which the diazomethane is not visible, a reaction time of 30 minutes is recommended.

(f) Allow the esterified sample to stand unstoppered in the hood for 15 to 30 minutes to allow excess diazomethane to escape from the ether solution. Discard all waste from the reaction with care and rinse the apparatus with acetone. Add 1-2 ml of carbon tetrachloride to the sample tube, mix on a Vortex Genie and concentrate to 1.0 ml using N_2 as previously outlined. Transfer to a vial and lable "acid esterified" extract.

DIMETHYL SULFATE METHYLATION

8. (a) Set up apparatus as shown in Figure 2.

(b) Bring 300 ml of the original sample to pH 11 and extract with chloroform to remove neutral and basic compounds.

(c) A 500 ml 3-neck (standard taper 24/40) round bottom flask, equipped with a fourth neck for a thermometer, is fitted with two pressure-equalizing addition funnels, the probe of a single-probe pH meter, and a magnetic stirrer.

(d) Nitrogen is introduced into the top of the first addition funnel and exits from the top of the second one. Place forty ml of Eastman reagent grade dimethyl sulfate into the first addition funnel and a 50% solution of sodium hydroxide (80 ml) into the second.

(e) Pour the sample into the flask and flush the system with nitrogen.

(f) After raising the temperature to 85°C, begin dropwise addition of both the dimethylsulfate and the sodium hydroxide solution. Maintain temperature between 80-90° (Caution--exothermic reaction. Have ice available to add to water bath). and the pH between 10.5-11. Since dimethylsulfate is not readily soluble in water vigorous stirring must be used. The addition time is about 1 hour.

(g) After all the dimethylsulfate is added, maintain the reaction vessel at 85-90° for an additional 15-2- minutes and then cool to room temperature.

(h) Add 5 ml concentrated ammonium hydroxide to destroy excess dimethylsulfate and re-extract the reaction mixture with chloroform to remove the methyl esters of acids and the methyl ethers of phenols.

(i) Dry the chloroform extract and evaporate to 1 ml.

DERIVIATIVE FORMATION BY SILYLATION

Many compounds are not volatile enough to be transformed to the gas phase without decompostion. Such polar compounds as amino acids, sulfonic acids, nucleic acids and carbohydrates are not directly gas chromatographable. Some steroids, carboxylic acids, and phenols can be directly chromatographed in their free form, but require special columns. Phenols fail on most GC columns.

Derivatization reagents are now available for all these classes of compounds. To be useful for GC/MS analysis, a derivative should fulfil the following criteria:

(a) be formed quantitatively from the free precursor by a rapid reaction with a readily obtainable reagent;

(b) be volatile enough for vaporization in the GC inlet;

(c) be thermally stable in the GC/MS system

Trimethylsilyl (TMS) and dimethylsilyl ether (DMS) are widely used for derivatization reactions, requiring little time and effort. The selection of a derivatizing agent and the methods employed are numerous and unfeasible to present here. For proper selection, reference should be made to the booklet offered by Pierce (5). A short summary of some of the more common reagents is listed.

(a) BSA, N_1O-bis (trimethylsilyl) acetanide

reacts far more rapidly than the hexamethyldisilazane (HMDS)-trimethylchloro-silane (TMCS) mixture and does not yield HCl and NH_4Cl as a by-product.

(b) Sylon BT

This is a mixture of BSA and TMCS (5:1). It will convert unhindered hydroxyl groups and moderately hindered ones.

(c) Trimethylsilylimidazole

The silylation is accomplished with the reagent at 100°C for two hours without removing the excess.

(d) Sylon BTZ

This is one of the most potent sylylating agents and will apparently derivatize all hydroxyl groups in any position. It consists of BSA, TMCS and trimethylsilylimidazole.

Reagents

(a) All solvents must be of pesticide quality or nanograde and must be checked before use.

(b) Potassium hydroxide - 60 gm dissolved in 100 ml of H_2O.

(c) Phosphoric acid.

(d) 10% (V/V) methanol in diethyl ether - prepare just prior to use

(e) 2-(β-Ethoxyethoxy) ethanol (Carbitol), Eastman P2848 or the equivalent.

(f) N-methyl-N-nitroso-p-toluene-sulfonamide (Diazald) solution. Eastman 7066 or the equivalent. Dissolve 1500 mg in 5 ml of diethyl ether. Prepare just prior to use.

(g) Sodium chloride, reagent grade. Heat at 500°C for two hours.

(h) Florisil, 60-100 mesh, calcined at 650°C (factory treated) and kept at 130°C until use. Before the use and standardization of florisil, check any contaminations or intereferences by passing 100 ml of 15% ethyl ether in hexane through 10gm* of florisil column and analyze the concentrated eluate (1 ml or ½ml)** by GLC. The amount and type of solvent used for this test should be the same as used in the analysis. In cases where more than one kind of solvent in column clean-up is used, always use the more polar solvent for this test. Thus, if hexane, 6%

ethyl ether in hexane, and 15% ethyl ether in hexane are used as eluants in the analysis, as in Reynold's extension of Mill's procedure, use the last-mentioned solvent mixture for this test.

*The exact amount can be determined by standardizing the florisil by elution with standard mixture.

**The concentration factor does not have to exceed that used in practice.

(i) Neutral alumina, Woelm, Activity Grade I deactivated with 5% water. 14% Boron trifluoride-methanol complex, esterification reagent, available from Analabs.

(j) Sodium Sulfate- ACS grade or better, anhydrous. The heat treated material is divided, and one part is labelled "neutral sodium sulfate" and stored at 130^{o}C. The other part is slurried with enough ether to cover the crystals and acidified to pH 4 by adding a few drops of sulfuric acid.

To determine the pH a small quantity of slurry is removed, the ether evaporated, water is added to cover the crystals, and the pH is measured on a pH meter. The ether is removed by vacuum from the acidified sodium sulfate. This fraction is labelled "acidified sodium sulfate" and stored at 130^{o}C.

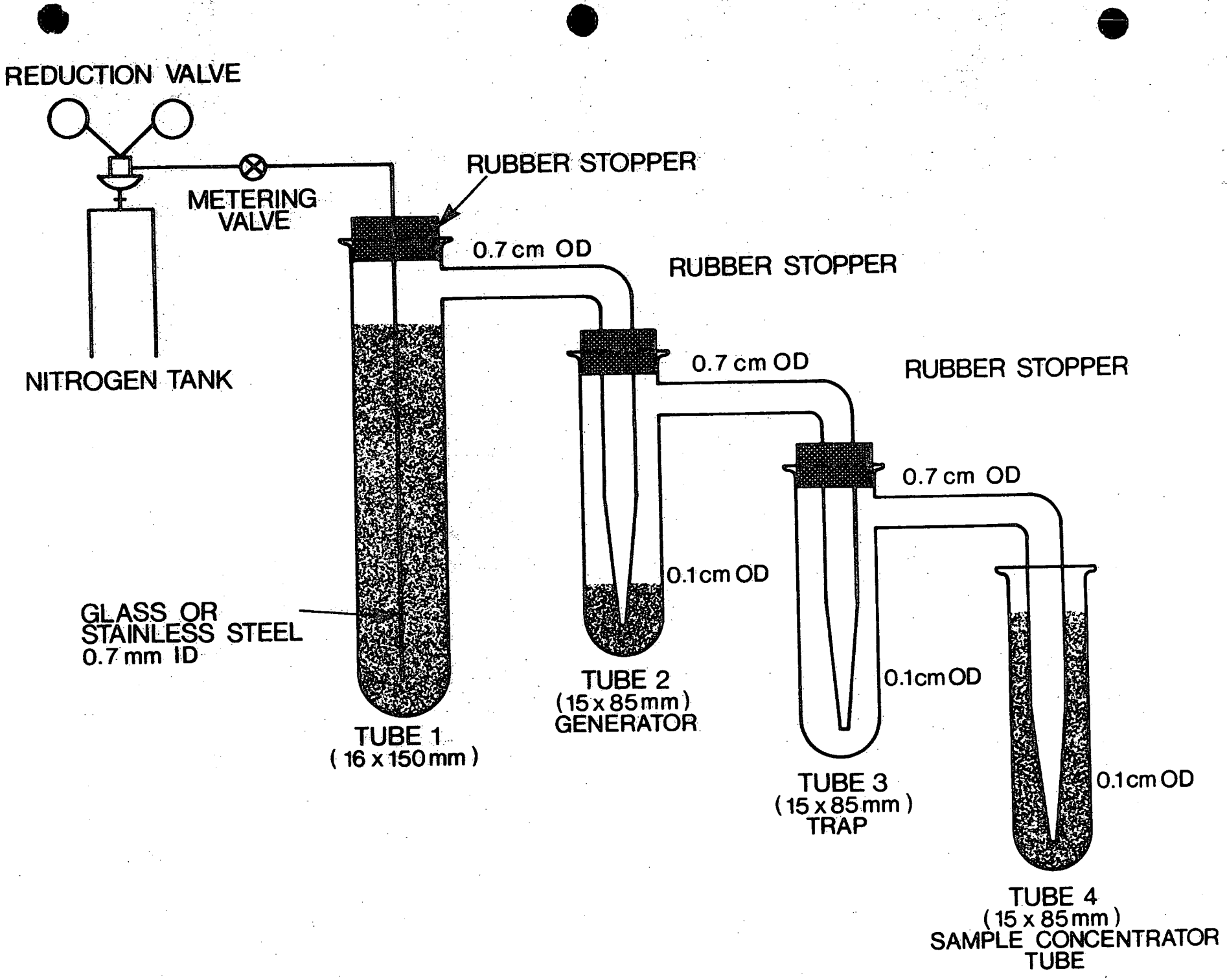
REDUCTION VALVE
METERING VALVE
RUBBER STOPPER
0.7 cm OD
RUBBER STOPPER
NITROGEN TANK
0.7 cm OD
RUBBER STOPPER
0.7 cm OD
0.1 cm OD
GLASS OR STAINLESS STEEL 0.7 mm ID
TUBE 1 (16 x 150 mm)
TUBE 2 (15 x 85 mm) GENERATOR
0.1 cm OD
TUBE 3 (15 x 85 mm) TRAP
0.1 cm OD
TUBE 4 (15 x 85 mm) SAMPLE CONCENTRATOR TUBE

FIG. 1

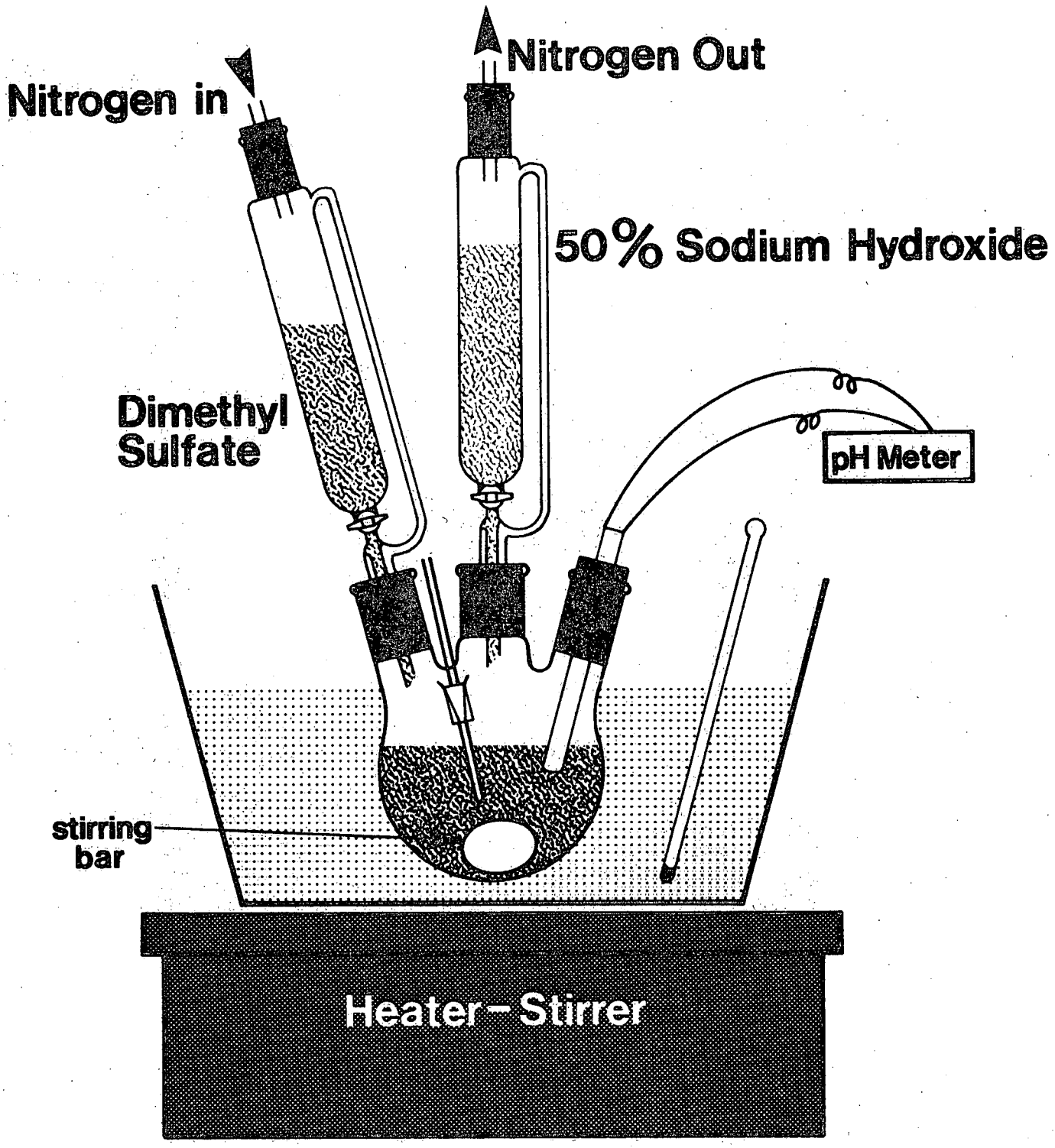
Nitrogen Out
Nitrogen in
50% Sodium Hydroxide
Dimethyl Sulfate
pH Meter
stirring bar
Heater-Stirrer

FIG. 2.

REFERENCES

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5. Pierce Chemical Company, Handbook of Silylation, GPA-3a, 1972.