NWRI CONTRIBUTION 88-101

TECHNIQUES AND PROCEDURES FOR PREPARATION OF AQUATIC SAMPLES FOR CHROMATOGRAPHIC ANALYSES

by

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November 1988

MANAGEMENT PERSPECTIVE

This manuscript reviews analytical sample preparation, isolation and cleanup techniques as related to environmental organic trace analysis by gas chromatography and gas chromatography-mass spectrometry.

It provides the environmental chemist with a systematic protocol for the quality control of analytical procedures. It also serves as a guide to the analysts who employ techniques which are available for the analysis of trace contaminants in different sample matrices.

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PERSPECTIVE - GESTION

Le présent document examine les techniques de préparation d'échantillons analytiques, d'isolement et de purification qui concernent le dosage d'éléments organiques, présents à l'état de traces dans l'environnement, par chromatographie en phase gazeuse et par chromatographie en phase gazeuse couplée d'une spectrométrie de masse.

Ce document fournit aux chimistes qui travaillent dans le domaine de l'environnement un protocole systématique leur permettant de contrôler la qualité des méthodes analytiques. Il sert également de guide aux analystes qui utilisent des techniques disponibles pour doser des contaminants à l'état de traces dans différentes matrices d'échantillons.

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ABSTRACT

This review article discusses objectives for collecting environmental samples, their isolation and preconcentration procedures based on the US EPA Priority Pollutant Protocol and the broad spectrum analytical protocol for non-target pollutants.

Several isolation and concentration techniques, among them, headspace analysis, liquid-liquid extraction, liquid-solid and supercritical fluid extractions have been discussed.

Cleanup techniques based on their practicality, time, instrumental availability and cost to assure that they allow detection and quantification of analytes at the required sensitivity level.

The last important part of the review describes derivatization to enhance detectability of contaminants and to improve the limit of detection and thermal stability.

The author was asked by the Editorial Board of the Journal of High Resolution Chromatography and Chromatography Communications published in Heidelberg, Germany, to prepare this authoritative article for a wide audience of chromatographers.

RÉSUMÉ

Le présent article examine les objectifs relatifs au prélèvement d'échantillons de l'environnement, à leur isolement et aux méthodes de préconcentration basées sur le Priority Polluant Protocol de l'EPA des É.-U. et sur le protocole analytique à large spectre destiné à des polluants non visés.

On a examiné plusieurs techniques d'isolement et de concentration, entre autres l'analyse dite de "l'espace de tête", l'extraction liquide-liquide, l'extraction liquide-solide et l'extraction sous pressions hypercritiques.

Les techniques de purification sont basées sur leurs qualités pratiques, leur durée, sur la disponibilité et le coût du matériel car on veut s'assurer qu'il est possible de déceler et de quantifier les substances à analyser au degré de sensibilité voulu.

La dernière partie importante de l'étude décrit la dérivatisation destinée à favoriser la décelabilité des contaminants et à améliorer la détection et la stabilité thermique.

Le comité de rédaction du Journal of High Resolution Chromatography and Chromatography Communications, publié à Heidelberg en Allemagne, a demandé à l'auteur de préparer cet article qui fait autorité à l'intention d'une vaste audience d'experts en chromatogaphie.

1.0 INTRODUCTION

The measurement of low levels of organic compounds in environmental samples presents the researcher with a number of possible pathways to effectively obtain and analyze samples. Numerous sampling methods, sample preparation techniques and extraction procedures are available. A number of different approaches to sampling strategy, isolation, concentration, cleanup and fractionation will be discussed.

Objectives for collecting environmental samples differ from those for many other types of samples because reliable measurements at very low levels are frequently required. Often, specific analytes need to be measured at the ug/kg and even ng/kg levels in complex matrices. Advances in analytical methodology continue to lower the levels at which reliable measurements can be made. At these levels, many factors that are of little or no concern in other analytical measurements are of critical importance in influencing the outcome and reliability of environmental analyses.

Analytical measurements are used for determining the composition and the quantities of analytes in the defined system at various concentration levels. Environmental analytical measurements provide data about the transportation and transformation of an environmental contaminant and for determination of its concentration in a sample.

2.0 SAMPLING

The first prerequisite in any sampling program is to obtain samples that are truly representative of the quality of analytes

that are present in them. Therefore, the sampling procedure should be clearly and precisely formulated to achieve required objectives of the program. Based on this request, the sampling should be truly representative of the quality that is present at the particular sampling site. Before deciding on the frequency and type of sampling, it is necessary to define the solutes of interest, analytical methods which are to be employed, and how samples are to be handled and delivered to a testing laboratory. Beside these important points, it is necessary to clarify how results are to be reported and how these data will be used to provide maximum information to fulfil objectives of the analytical quantitative measurements. It must be realized from the beginning of the process that obtained data represent estimates of the true value, and thus involve some level of uncertainty. For this reason, they must be evaluated by means of statistical methods. A detailed treatment can be found in the literature (1,2,3).

Environmental trace analysis can achieve best results only by a well designed and consistently implemented quality assurance program. In general, quality assurance covers activities whose purpose is to provide data that meet defined standards of quality with clearly indicated level of confidence. This definition of quality assurance consists of two activities; a) a mechanism to assure the proper implementation of quality assurance, and b) quality control that is adequate and economical for the user and for quality assessment. This provides qualitative information of the data produced and considers the performance of

the analytical laboratory (1).

The reliability of any analytical measurement is directly connected to the uncertainties of the sampling process, storage and preservation of collected samples, isolation of analytes from a sample matrix and the cleanup procedure prior to analysis. In any of these stages of the analytical process, errors may be introduced.

Environmental samples are usually heterogeneous, and thus a large number of samples should be analyzed to obtain meaningful data. The number of samples and the quality of the sampling protocol must be well documented to achieve reliable results. Environmental trace analysis is performed on samples, where the standard deviation of the individual samples is not known in advance and where measurement error cannot be predicted nor can it be assumed to be negligible. There are very few analytical methods where the sample is directly accessible to measurement. Even chromatographic methods employing selective and specific detectors do not have this sensitivity. Organic contaminants in environmental samples are generally present in trace amounts at the nanogram (10^{-9}g) to microgram (10^{-6} g) per kilogram levels as a part of a complex matrix. The matrix can be water, sediment or tissue. For gaseous samples of ambient air or industrial stack emissions the solutes can be present in either the gaseous or particulate forms. Due to the wide variety of contaminants present, no single extraction technique is capable of isolating all organic components from these matrices.

In general, the ideal isolation technique should have a high

selectivity, thus the analytes should be separated from unwanted matrices and other contaminants present in the sample. After separation, detection should be specific enough to provide information about the identity and the quantity of an analyte.

3.0 ISOLATION AND CONCENTRATION PROCEDURES

During last ten years many attempts have been made to set a protocol for isolation and preconcentration of many target compounds that are considered to be toxic. Among these attempts, a comprehensive qualitative and quantitative protocol to include inorganic and organic industrial chemicals, purgeable volatiles, extractables (base-neutral and acidic fractionation), and pesticides has been described by Garrison *et al.*(4). It is known as the U.S. EPA Master Analytical Scheme. Details were also discussed by Pellizzari (5).

Another approach to analyze trace organic chemicals in water based on the largest possible number of contaminants contained in the sample at one time is the broad spectrum analysis procedure developed by Gibs and Suffet (6). This technique is applicable to samples needing a minimum pretreatment and samples containing a large molecular weight range of organic contaminants. Its principle is based upon being able to observe the changes in analyzed data illustrated by differences between two chromatograms. It requires extremely high quality assurance for the sample, and uniform instrumental and working conditions. It seems that broad spectrum analytical schemes can expand U.S. EPA Priority Pollutant Protocol, since the target contaminants specified in the protocol examines all non-target components in

the chromatogram and deductions can be made about the types of contaminants present. This approach allows a chromatogram to be divided into arbitrary windows covering polarity, volatility and molecular weight distribution in such windows. In fact this methodology can be used on a sophisticated computer-based pattern recognition algorithms to yield highly specific information required for identifying sources and predicting trends for modelling purposes and advising the user on the next course of action (see Table 1).

Several isolation and concentration techniques have been used for organic pollutant residue analysis. Among them, head-space analysis, liquid-liquid extraction, gas-liquid extraction and liquid-solid extraction in water analysis will be discussed.

3.1 Direct Injection

Nicholson *et al.* (7) reported the determination of trihalomethanes in water using a direct aqueous injection technique. The water sample (up to 10 uL) is injected into a GC injector equipped with a packed column and an electron capture detector (ECD). Trihalomethane results from the direct injection of a sample onto a column provided much higher results than those for the purge and trap technique.

Pfaender *et al.*(8) showed that a value close to the true trihalomethane concentration could be obtained if the sample was reinjected after purging it for 30 minutes with purified nitrogen, and subtracting this value from the first value determined before purging. The method detection limit is about

1 ug/L and reproducibility is in the 5 % range. The head-space analysis can be performed in static mode or in dynamic mode of operation.

In general the headspace analysis can be performed under static conditions, where the sample comes into equilibrium with its vapor at a defined temperature. Dynamic headspace analysis is performed using an inert gas to strip the volatile components from the water sample and this extracting medium is passed through a sorbent or cryogenic trap. Experimental conditions that affect the results are related to the sample withdrawal and the temperature.

3.2 Headspace Analysis (HSA)

Gas chromatography is an ideal separation technique for analyzing volatiles in water because gas chromatography can handle gaseous mixtures directly. Of course, it would be very desirable to inject water samples directly onto an open tubular column. This approach seems to be very simple but until now there are difficulties with the stability of the inner coating of the fused silica wall or glass capillary columns. The only acceptable stationary phase that can withstand direct water samples is OV-1701 a cyanopropyl-phenyl-methyl polysiloxane phase coating.

3.3 Static Headspace Analysis

Direct injection analysis of vapor above the water sample requires little sample preparation and shows a minimum number of artifacts during analysis. Most commonly, the water sample is placed in a closed container and the head space from the closed container is introduced directly onto a gas chromatographic column. In the static mode, the sample comes into equilibrium with its vapor at a defined temperature and at this point a known amount of vapor is taken from the container and injected onto the OTC. One drawback to a static head-space analysis is that the concentrations of contaminants of interest are usually very low so their partial pressures are significantly lower than the partial pressure of water. Sometimes the concentration is too low to be detected by available detectors. To overcome this problem, many modifications for concentrating head space samples have been suggested. An aqueous solution with some headspace is equilibrated in a thermostat for up to 30 minutes and a certain volume of headspace gas over the water is withdrawn with a gas-tight syringe. This headspace sample is directly introduced onto the GC column for analysis. The theoretical aspects of the technique have been described by Drozd and Novak (9,10) and Vitenberg (11). Reproducibility of quantitative methodology for volatile halocarbons using static headspace analysis has been improved by employing internal and external standards (12). Bertsch *et al.* (13) showed that the volatile components can be significantly enriched to provide an adequate signal for a detector. They determined the breakthrough volumes for various

analytes in water using Tenax as a sorbent. Elution of the volatiles from the trap are performed with a small amount of organic solvents.

3.4 Dynamic Headspace Analysis

Recently, a programmed cryofocusing capability in automated systems for headspace HRGC has been developed (14,15) which improves the sensitivity of GC analysis. This dynamic headspace technique can be used for the both liquid and solid samples. The following description shows the simplicity of operation. Liquid samples in the sparger vessel are continuously purged with sparge gas during selected time period. Volatiles are thus extracted and transferred to the trap where they are adsorbed and preconcentrated. When the purge period is completed the trap is automatically backflushed and pulse-heated to desorb the trapped volatiles for analysis.

Similarly, sediment or fish tissue sample can be placed in a sample tube thermal desorber. Volatile organics are desorbed and concentrated in the trap during an appropriate time of continuous heating and purging of the headspace around the solid sample. When the cryogenic traps are used, even the low molecular weight compounds are effectively collected for automatic direct HRGC analysis.

3.5 Closed-Loop Stripping

Trace contaminants of headspace gas can be concentrated by trapping them in a short column packed with charcoal. During the stripping process, concentration of the components in the gas leaving the system is continuously decreasing. Measurements of volatile and semi-volatile, intermediate molecular weight organic pollutants are analyzed by this method in water samples at ng/L level (25). The stripping apparatus employing a closed loop inert gas recirculation consists of the sample flask (1 to 4 litre volume) and a pump for recirculating the inert gas. The headspace gas is recirculated by a pump and passes through the sintered glass trap containing activated carbon. As the gas passes through the sample, organics are purged from water into the headspace gas. The trap then adsorb these components from the gas. The water bath temperature is controlled at 30 °C and the preheater temperature is kept at 80 °C to prevent condensation of water in the carbon trap.

The reliability of the quantitative results depends on the efficiency of stripping the compounds from the water and this process is time dependent. The trap is extracted with approx. 10 uL of carbon disulphide. Because all the volatile solutes are injected into the GC, this technique provides up to 200-fold enhancement in sensitivity (from a 4 L sample) over the purge and trap method. Unfortunately, recoveries of the more volatile components such as chloroform are low, so this technique cannot be used for their determination. For compounds in the volatility range between benzene and hexachlorobiphenyl recoveries are very

good (26,27). This technique is recommended for medium volatility solutes (28). A thorough discussion of the contamination and the limitations of the technique has been described by Wegman and Melis (29).

3.6 Liquid - Liquid Extraction (LLE)

Liquid-liquid extraction of organic compounds has been an effective method for removing contaminants from environmental samples. It has become a very important technique for concentrating trace contaminants and for removing interfering components from a sample. For extraction, certain analytes in water can be transformed directly into an organic solvent which is not miscible with water, such as hexane, carbon disulphide, chloroform or trichlorotrifluoroethane. A selected solvent or mixture of two solvents may be used for extraction, provided the extraction is at least 80 % efficient. It should be selective enough to require a minimum cleanup, and not to interfere with the final determination. Optimum extraction conditions are found by recovery studies for each type of analysis. Hexane and hexane-acetone mixtures are typical solvents for nonpolar, fat-soluble contaminants such as organochlorine pesticides. Dichloromethane, chloroform and ethyl acetate are suitable for more polar compounds such as N-methyl carbamates and organophosphorous compounds. Acetonitrile is an excellent general solvent used for partitioning of unknown residues of a wide polarity range. The distribution of a compound between two immiscible liquid phases can be expressed in terms of its partition coefficient. However, a more convenient term is the p-value which describes the

fractional amount of compound present in the nonpolar or less polar phase after partitioning between two phases of equal volume (30).

Apart from the choice of solvent, other parameters such as pH, ionic strength, water-solvent ratio, number of extractions, type of analytes and their concentration must be considered. If emulsions are a problem, they may be broken in one of the following ways: a) transfer emulsion to centrifuge bottles and centrifuge it at high speed (e.g. 4000 rpm) for 5 minutes; or b) salt out organics by adding sodium sulfate or saturated sodium chloride solution and small amount of sodium lauryl sulfate to a sample. Many types of liquid-liquid extractors perform well and they are commercially available. Recently, a large-sample extractor for determining organic contaminants in water has been developed at the National Water Research Institute by Goulden *et al.* (31).

One approach to greater sensitivity could be the use of larger samples combined with concentration at the sampling site. Such concentration processes as adsorption on urethane foam (32) or on resins, and solvent extractions have been used with varying degrees of success. McCrea and Fischer designed the APLE sampler, where APLE is an acronym for Aqueos Phase Liquid-Liquid Extractor. This extractor consists of a 250 L drum in which 200 L of water is extracted with 8 L methylene chloride using a centrifugal pump and solvent spray bar for agitation. Work with this system has shown that the organic contaminants can be determined to low ng/L level with standard analytical techniques

and essentially complete extraction of organic solutes can be obtained with a single-stage process. However, the drum must be thoroughly cleaned and blanks run periodically (33).

A more efficient and versatile sampler allowing for both, batch and the composite sampling has been designed and tested at the NWRI (31). The composite sampling is recommended when a limited number of samples are taken at a sampling site. Samples are grouped on the basis of time (e.g. temporal monitoring of effluents from industry) or zones (e.g. the hypolimnion or epilimnion of a lake). The large-sample extractor is basically a mixer-settler, extracting water at up to 1 L/min. The water is further extracted in a packed column by the pure solvent used to make up the solvent lost by solubility in the effluent water. Figure 1 shows a flow diagram of the extractor. The extractor is made from a Pyrex glass. The stirrer is a 4-blade turbine type rotor connected to the motor and is mounted on a stand. The rest of the equipment is hung from a rod. The bottom of the support is fixed in the stand. The upper end is held in an aluminum spacer block. The extractor is held by two chain clamps which hold a sheet metal sleeve with a teflon liner around the mixing chamber. The rest of the glassware is supported by jaw-style laboratory clamps. The heater is set to heat the water to 20-22 °C before entering the mixing chamber by a variable rheostat. The water supply pump, solvent make-up pump, and spiking pump are manufactured by Fluid Metering Inc., Oyster Bay, N.Y., U.S.A. All connections are made with glass or teflon tubing and stainless steel fittings. In order to overcome the water-hammer effect from

the pump, the water supply pump inlet and outlet are fitted with small vertical closed end glass tubes.

The sampler was used on board ship to confirm the applicability of this type of equipment to a shipboard laboratory. Water samples of approximately 50 L volume were extracted with dichloromethane during monitoring cruises on the Great Lakes. Both unfiltered and filtered water samples were extracted. The dichloromethane extracts were later analyzed for organochlorine pesticides, chlorinated benzenes, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. A solution containing surrogate standards was continuously metered into the water being extracted. The recoveries of these surrogates provide a continuous measure of the extraction efficiency and reproducibility of the analytical process, and confirm that the processes employed are valid. Surrogate standards spike recoveries were between 82.4 to 150 percent.

3.7 Supercritical Fluid Extraction

Supercritical fluids such as carbon dioxide, ammonia, nitrous oxide, ethylene and some fluorochlorocarbons, have been used to extract thermolabile natural products. A symposium was held on the topic of extraction with supercritical fluids (34). The solubility behavior of various natural products was examined by Stahl *et al.* (35). Ehntholt *et al.*(36) studied the use of supercritical carbon dioxide to extract low levels of organic substances from water. They found that compounds of high partial pressure and low aqueous solubility were readily extracted under the conditions used (2500 psi; 45-50 °C). Compounds of

higher water solubility were only partially extracted. The extraction and recovery of organic contaminants from sediments and fly-ash is a critical and often limiting step in analysis schemes used to identify and determine organic pollutants. Recent studies have demonstrated that the use of supercritical fluids for analytical extractions can provide a powerful alternative to traditional extraction techniques (37-42). These reports have described the ability to extract polycyclic aromatic hydrocarbons, polychlorinated biphenyls, 2,3,7,8-tetrachloro dibenzo-p-dioxin, phthalates, nitrogen-, sulfur-, and oxygen-containing PAHs using both steady-state or static extraction and non-steady state or dynamic extraction with different supercritical fluids and modifiers added to these fluids. Supercritical fluid extraction can be performed at relatively low temperatures and no sample handling or concentration procedures are required between extraction and HRGC analysis, thus reducing the potential for loss of analytes and eventual thermal degradation. Although the supercritical fluid extraction with tandem chromatographic techniques (gas, liquid and supercritical fluid chromatography) does not allow for class-fractionations yet, many laboratories are working on this concept. Improvements in detection sensitivity would speed-up developments in this novel analytical approach in environmental organic trace analysis.

3.8 Solid Phase Extraction

Classical sample extraction and subsequent cleanup, involving separatory funnels or similar apparatus, uses expensive chemicals and is often time consuming. Based on data derived from an HPLC analysis, it is possible to clean samples by means of solid phase extraction (SPE).

In general, using solid phase extraction, conditions are arranged to retain analytes of interest as the sample is passed through a short bed of silica based packing which may contain different functional groups and polarity. The bed is then washed with an eluant. All contaminants more weakly retained than the analytes of interest are washed out. The important analytes are then selectively eluted in a small volume of an appropriate solvent. Alternatively, sample contaminants can be retained in the tube, providing a one step cleanup. Manufacturers claim up to 100 percent recoveries at microgram level (43, 44). A typical example of a cost effective way to monitor triazine herbicides in pond water using Supelclean LC-18 SPE tubes indicates high recovery of simazine, atrazine and promazine from water samples (45). Selected references are given in Table 2.

The diol, amino, cyano, hydroxyl and florisil silica cartridges are polar in character, while the octadecyl-, octyl-, ethyl-, and cyclohexyl phases are nonpolar. The nature of the sorbent should be selected to exploit differences between the analyte and other components in the sample.

4.0 ADSORPTION

All materials have some affinity for binding on solid surfaces. Common adsorbents are alumina, charcoal, silica gel, molecular sieves, ion exchanging resins and porous polymers (46). The adsorptive capacity of a given adsorbent depends in part on the treatment or manufacturing conditions and on the composition of the adsorbent. Selective desorption can be controlled by the solvent used. Desorption is usually accomplished by heat or by use of solvents. Selected references are given in Table 3.

Usually the water sample is pumped through a column packed with an adsorbent. The adsorbed components are then desorbed from the adsorbent and analyzed by chromatographic methods.

An excellent adsorbent for sampling airborn contaminants is graphitized carbon black studied and developed by Kiselev *et al.*(47). This adsorbent is commercially available by Supelco as Carbotrap. It has no surface ions or functional groups on its surface. The entire surface can interact with an analyte solely on dispersion interaction based on London forces. The graphitized carbon is more hydrophobic then either of the resins on the market. It can be used very effectively to adsorb many air pollutants such as hydrocarbons, polycyclic aromatic hydrocarbons alcohols, amines, ketones and various pesticides.

4.1 Miscellaneous Sample Procedures

In addition to extraction and adsorption procedures there are a few others miscellaneous sample preparation techniques suitable for environmental samples.

Freeze-drying technique provides a selective partial removal of water by crystallizing out the water in the form of ice. Some of the problems of this method include partial uncontrolled loss of volatiles by evaporation during long periods of freeze-drying and also losses due to occlusion in the ice crystals if the concentration step is carried too far.

Distillation is often selected as the initial concentration step. All liquids have a vapor pressure that is constant at a given temperature. When the temperature is raised so that the vapor pressure of the liquid equals that of external pressure, the liquid starts to boil. Distillation can be used in environmental analysis only for removal of water when preconcentration of high boiling components is of concern. Distillation can be performed as a *fractional distillation* and as *steam distillation*. Rijks *et al.*(48) investigated a theoretical model describing the recovery of different classes of organic compounds as a function of the process time. The quantitative performance of steam distillation was reported for different classes of organic compounds at 10^{-7} to 10^{-9} g levels. Acceptable results were obtained within 20 minutes.

5.0 CLEANUP TECHNIQUES

Cleanup procedures are chosen based on their practicality, time, reagent and instrumental availability and cost involved. The methods chosen must be tested to assure, they allow detection and determination of contaminants at the required sensitivity level. The cleanup required prior to the final determination of analytes depends upon the selection of previously described

extraction procedure and the analytical method employed.

Very elaborate cleanup procedures are required for samples that are at very low concentration in fish, sediment and biota matrices. As an example, the florisil cleanup will be discussed for organochlorine pesticides, organophosphorous compounds as well as triazines and carbamates (49) and approaches to comprehensive analyses of persistent environmental contaminants as suggested by Stalling *et al.*(50).

Florisil column chromatography cleanup is used for fractionation of organochlorine pesticides from various matrices, by elution with solvents of increasing polarity. The solvent system consists of n-hexane and its mixture with 6, 15 and 50 percent of diethyl ether in n-hexane.

Before using a florisil separation, it is important to standardize the florisil to get the correct elution order of the pesticides or PCBs.

Florisil cleanup is performed in a glass column 22 mm I.D. x 30 cm long with a solvent reservoir at the top. The outlet should have a coarse glass frit and a stopcock to regulate flow. The column is packed with a slurry of florisil in n-hexane to about 10 cm of column length when settled, or with an exact amount as recommended in a procedure. About 1 cm of anhydrous sodium sulfate is placed over the florisil to take up traces of water that may be left over from the sample. Kuderna-Danish assemblies are placed under each florisil column. Two hundred mL portions of elution solvent of n-hexane- diethyl ether (94+6 % v/v);n-hexane+ diethyl ether (85+15 %) and n-hexane-diethyl ether (50+50 %) are

employed at a rate 5 mL/min. Each eluate is collected in two separate Kuderna-Danish assemblies. At the instant the solvent level reaches top of sodium sulfate level, another portion of new mixture is used. Eluents are concentrated on a steam bath to approximately 5 mL. Compounds contained in fractions eluted from a florisil column are shown in Table 4.

Another more sophisticated approach which can be fully automated has been developed at the Columbia National Fisheries Research Laboratory by Stalling *et al.* (50). A series of chromatographic processes have been integrated into an automated sequential procedure that has been specifically developed for uninterrupted cleanup and fractionation of multiclass organic contaminants from environmental samples. The effectiveness and general applicability of this sequential cleanup and fractionation procedure were demonstrated by the recovery of phenols, pesticides, polychlorinated biphenyls, chlorinated dibenzo-p-dioxins and dibenzofurans representing a broad range of residue classes from various matrices such as fish, sediment and biota. A chromatographic controller carries out the numerous sample and solvent manipulations in an automated and continuous manner. Alkali metal hydroxide treated silica gel effectively separates phenols and acids from neutral fraction. The combination of gel permeation chromatography, carbon chromatography, and cesium hydroxide-treated silica gel columns were combined and operate as a single system. A commercial system is available from the Analytical Biochemistry Company, Columbia, Missouri.

6.0 DERIVATIZATION

These techniques include procedures in which the analyte chemical structure is changed using various derivatizing agents to enhance detectability or improve separation efficiency. Derivatization is also employed to improve the limit of detection of an analytical procedure. In addition, the separation of the analytes is often easier to achieve not only because the reaction is selective and the formed derivatives may be detected selectively but also the derivatization can neutralize activity of polar functional groups. Among other advantages, derivatization often improves thermostability and volatility of thermally labile and low volatile compounds.

Some derivatization reagents are described below.

6.1 Diazomethane

Is a powerful derivatizing agent for methylating acidic compounds for GC and GC-MS analysis. It is prepared by reaction of N-methyl-N'-nitro-N-nitrosoguanidine in diethyl ether with aqueous 5 N NaOH solution at subambient temperatures (78).

6.2 Acetylation Reagents

Amines, amides, alcohols, phenols, thiols, enols, glycols, unsaturated compounds and moieties with aromatic rings may react with trifluoromethyl-, pentrafluoropropyl- or heptafluorobutyl anhydrides to form stable, highly volatile compounds. By using fluorinated anhydride derivatives the response to ECD is significantly increased.

6.3 Fluoro Acyl Imidazoles

The N-acylimidazoles offer advantages over the use of anhydrides. The imidazole reaction is releasing no acids into the system to hydrolyze samples. They will acylate hydroxyl groups and both primary and secondary amines and they have also been used for bifunctional derivatizations (73).

6.4 Pentafluorobenzyl Bromide

Has been used for an extractive alkylation of carboxylic acids, phenols, mercaptans and sulfonamides using a potassium carbonate catalyst with the ECD analysis (74).

6.5 Dimethyl Acetal (Alkyl-8)

This reagent offers speed and one step procedure for preparing methyl esters of fatty acids for HRGC (75).

Many silylating reagents are employed to derivatize hydroxyl, carboxyl, thiol and primary and secondary amino groups. For a particular silyl reagent the easy of reaction follows the order : prim. alcohols > sec. alcohols > phenols and carboxylic acids > prim. amines > sec. amines > amides.

Silyl donor ability decreases in the order of the following reagents :

6.6 N,O-bis(Trimethylsilyl) trifluoroacetamide (BSTFA)

BSTFA is a powerful trimethylsilyl (TMS) donor which reacts quantitatively under relatively mild conditions with acids and aminoacids to form volatile derivatives (76).

6.7 N-Methyl-N-(tert. Butyldimethylsilyl) trifluoroacetamide

The tert. butyldimethylsilyl moiety derivatizes hydroxyl, carboxyl, thiol as well as primary and secondary amines (77).

6.8 N-Trimethylsilylimidazole (TMSI)

TMSI reacts preferentially with -OH groups but not with amine groups, thus it is used in the derivatization of alcohols, phenols, organic acids, steroids, hormones, glycols, nucleotides and narcotics (78).

7.0 SUMMARY

Because of the range and variety of samples encountered in environmental organic trace analysis and because of the diversity of analytical techniques used, no general sample preparation procedure can be outlined. Therefore, the analytical chemist must rely on fundamental principles for guidance as to what procedures must be used under what conditions. The importance of sample preparation cannot be overemphasized because many of the experimental difficulties encountered result from improperly prepared samples presented for highly sophisticated and expensive instrumental analyses. Quality assurance and statistical methods should be more intensively used together with pattern recognition methods, such as chemometrics to increase qualitative and quantitative precision and accuracy in environmental measurements.

REFERENCES

1 J.K. Taylor, *Quality Assurance of Chemical Measurements*, Lewis Publishers, Inc., Chelsea, Mich., 1987

2 C.J. Kirchmer, Environ. Sci.Technol., *17*, (1983) 174A

3 F.I. Onuska, F.W. Karasek, *Open Tubular Column Gas Chromatography in Environmental Analysis*, Plenum Press, New York, N.Y., 1984

4 A.W. Garrison, A.L. Alford, J.S. Craig, J.J. Ellington, A.F. Haeberer, J.M. McGuire, J.D. Pope, W.M. Schackelford, E.D. Pellizzari, J.E. Gebhart, in *Advances in the Identification and Analysis of Organic Pollutants in Water*,Vol.1., L.H. Keith, Ed., Ann Arbor Science, Ann Arbor, Mich. 1981

5 E.D. Pellizzari, *Experimental Development of the Master Analytical Scheme for Organic Compounds in Water. Part 1.* U.S. EPA: Athens, GA, 1985, EPA-600/4-85-007a.

6 J. Gibs, Ph.D. Thesis, Drexel University, Philadelphia,PA,1983

7 A.A. Nicholson, O. Meresz, B. Lenyk, Anal.Chem. *49*,(1977) 814

8 F.K. Pfaender, R.B. Jonas, A.A. Stevens, L.Moore, J.R. Haas, Environ.Sci.Technol. *12*, (1978) 438

9 J. Drozd, J. Novak, J. Chromatogr., *165*, (1979) 141

10 J. Drozd, J. Novak, J.A. Rijks, J. Chromatogr. *158*, (1978) 471

11 A.G. Vitenberg, J. Chromatogr.Sci. *22*, (1984) 122

12 B. Kolb, M. Auer, P. Pospisil, J. Chromatogr. *279*, (1983) 341

13 W. Bertsch, E. Anderson, G. Holzer, J. Chromatogr. *112,(1975)* 701.

14 CDS Information Bulletin CO287, 1985

15 R. Otson, Anal.Chem. *53*, (1981) 929

16 B.V. Stolyarov, Chromatographia *14*, (1981) 699

17 B. Kolb, P. Pospisil, T. Borath, M. Auer, J. High Resolut. Chromatogr.Chromatogr. Commun. *2*, (1979) 283

18 K.L.E. Kaiser, I. Valdmanis, J.Great Lakes Res. *5*,(1979) 160

19 M.E. Comba, K.L.E. Kaiser, Intern.J. Environ.Anal.Chem. *16*, (1983) 17

20 W.D. Snyder, Hewlett-Packard Tech.Paper GC-71, 1978

21 T. Ramstad, L.W. Nicholson, Anal.Chem. *54*, (1982) 1191

22 J.F. Pankow, L.M. Isabelle, T.J. Kristensen, Anal.Chem. *54*, (1982) 1815

23 M.M. Thomason, W. Bertsch, J. Chromatogr. *279*, (1983) 383

24 T.A. Bellar, J.J. Lichtenberg, J.Am.Water Works Assoc. *66*,(1974) 739

25 K. Grob, F. Zuercher, J.Chromatog.,*117*, (1976) 285

26 R.G. Melton, W.E. Coleman, R.W. Slater, F.C. Kopfler, W.K. Allen, T.A. Aurand, D.E. Mitchell, S.J. Voto, in *Advances in the Identification and Analysis of Organic Pollutants in Water*,Vol. 2., L.H. Keith, Ed., Ann Arbor Science, Ann Arbor,MI, 1981

27 W.E. Coleman, J.W. Munch, R.W. Slater, R.G. Melton, F.C. Kopfler, Environ.Sci.Technol. *17*, (1983) 571

28 W.E. Coleman, R.G. Melton, R.W. Slater, F.C. Kopfler, J. Am. Water Works Assoc.,*73*, (1981) 119

29 R.C.C. Wegman, P.H.A.M. Melis, CRC-Crit. Reviews Anal.Chem., *16 (4)*, (1986) 281

30 M.C. Bowman, M. Beroza, J.Assoc.Off.Anal.Chem. *48*,(1965) 943

31 P.D. Goulden, D.H.J. Anthony, NWRI Contribution No. 85-121,

1985 and IWD-Technical Series, Bulletin No. 157, 1987

32 F.I. Onuska, K.A. Terry,Water Poll.Res.J.Canada,*17*,(1982) 103.

33 R.C. McCrea, J.D. Fischer, IWD-Technical Bull. No. 138, 1985

34 Anon (1978) Angew. Chem. Int.Ed. Engl.*17*, *(1978)701*

35 E.Stahl, W. Schilz, E. Schultz, and E. Willing, Angew. Chem. Int. Ed. Engl. *17*, (1978) 731

36 D.J. Ehntolt, Ch. Eppig, K.E. Thrun, Int. J. Environ. Anal.Chem., *13*,(1983) 219

37 S.B. Hawthorne, D.J. Miller, M.S. Krieger, Fresenius Z.Anal.Chem. *330*, (1988) 211

38 S.B. Hawthorne, D.J. Miller, J. Chromatogr. *403*, (1987) 63

39 S.B. Hawthorne, D.J. Miller, J.Chromatogr.Sci. *24*, (1986) 258

40 S.B. Hawthorne, D.J. Miller, Anal.Chem. *58*, (1987) 1705

41 M. Saito, T. Hondo, Yukagaku *35*, (1986) 273

42 F.I. Onuska, K.A. Terry, J.High Resol. Chromatogr. Chromatogr. Commun. submitted for publication

43 SUPELCO, Catalog No. 25, 1987, p.176

44 ANSPEC, Catalog No. 14, 1987,p. 142

45 The SUPELCO Reporter, *7 (3)*, (1988) 8

46 M. Dressler, J.Chromatogr. *165*,(1979) 167

47 A.V. Kiselev, Y.I. Yashin, *Gas Adsorption Chromatography*, Nauka Publ., Moscow, 1965

48 J. Rijks, J. Curves, Th. Noy, C. Cramers, J.Chromatogr. *279*,(1983) 395

49 *Analytical Methods Manual 1979*. Inland Water Directorate, Water Quality Branch, Environment Canada, Part 5, Naquadat 18165.

50 D.L. Stalling, L.M. Smith, J.D. Petty, *ASTM Spec. Technical Publication 686*, 1979.

51 R.A. Neal, Environ.Sci.Technol., *17*(3),(1983) 113A.

52 E.D. Pellizzari, A.W. Garrison, Prep.Pap.Am.Chem.Soc. Div. Environ. Chem., *23* (2), (1983), 427.

53 J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, Environ. Sci Technol. *15*, (19881) 1426.

54 T.A. Bellar, J.J. Lichtenberg, J.Am.Water Works Assoc., *66,(1974)* 739.

55 *Evaluation of EPA 609 Method* : EMSL-US-EPA, Cincinnati,OH,1984

56 *Interlaboratory Methods Studies for EPA Methods 601, 602, 604-613 and 624 and 625.* Studies 14-24. EMSL US-EPA: Cincinnati,OH

57 *The Analysis of Trihalomethanes in Drinking Water by Liquid-Liquid Extraction*; EMSL US-EPA : Cincinnati, OH, 1979.

58 Fed. Regist. *48*(29), 1983.

59 J. Gibs, L. Brenner, L. Cognet, I.H. Suffet, in *Organic Pollutants in Water, Ser. 214.* I.H. Suffet and M. Malaiyandi, eds., Am.Chem.Soc., Washington, D.C., 1987, p.267.

60 J. Gibs, I.H. Suffet, in *Organic Pollutants in Water, Ser.124.* I.H. Suffet, M. Malaiyandi eds., ACS, Washington, D.C.,1987,p.327

61 H. Boren, A. Grimvall, R. Savenhed, J. Chromatogr. *252,*(1982) 139.

62 J.W. Graydon, K. Grob, F. Zuercher, W. Giger, J. Chromatogr., *285,(1984) 907.*

63 G.T. Hunt, N. Pangaro, S.Zelenski, Anal. Lett. *19(A7),1980, 521.*

64 G.T. Hunt, N. Pangaro, Anal.Chem. *54*,(1982) 369.

65 F.C. Koepfer, H.P. Ringhand, R.G. Miller, in *Organic Pollutants in Water, Ser.214.* I.H. Suffet, M. Malaiyandi, eds., ACS, Washington, D.C. 1987,p. 425.

66 R.J. Baker, I.H. Suffet, in *Organic Pollutants in Water, Ser. 214.* I.H.Suffet, M. Malaiyandi,eds., ACS,Washington,D.C.,1987, p. 571.

67 B.S. Middleditch, A. Zlatkis, R.D. Schwartz, J.Chromatogr.Sci. *26*, (1988) 150.

68 E. Pellizzari, B. Demian, K. Krost, Anal.Chem. *56*, (1984) 793.

69 A. Zlatkis, L. Ghaoui, S. Weisner, H. Shanfield, Chromatographia *20*, (1985) 343.

70 H.J. Kool, C. FF. van Kreijl, M. Verlaan-de Vries, in *Organic Pollutants in Water, S er.214.* I.H. Suffet, M. Malaiyandi, eds., ACS, Washington, D.C., 1987, p. 605.

71 C.D. Watts, B. Crathorne, R.I. Crane, M. Fielding, in *Identification and Analysis of Organic Pollutants in Water,* Keith L.H., ed., Vol.1, Ann Arbor Science, Ann Arbor,MI, 1981.

72 L.K. Marble, J.J. Delfino, Am. Lab., *20* (11), (1988) 23.

73 H. Miyazuki, M. Ishibashi, C. Mori, N. Ikekawa, Anal.Chem., *45*, (1973) 1164.

74 F.K. Kawahara, Environ.Sci. Technol., *10*(8), (1976) 761.

75 PIERCE Co., 1988 Handbook and General Catalog, p. 162.

76 F. Klebe, H. Finkbeiner, D.M. White, JACS, *88*, (1966) 3390.

77 A.C. Bazan, D.R. Knapp, J. Chromatogr. *236*, (1982) 201.

78 H.M. Fales, *et al.* Anal. Chem., *45*, (1973) 2302.

TABLE 1

Analytical Protocols - Selected References

Protocol	EPA-Method	Ref.
1 U.S. EPA Protocol - GC :		
Purgeable Halocarbons	601	4,5,,58
Purgeable Aromatics	602	
Acrolein and Acrylonitrile	603	
Phenols	604	
Benzidines	605	
Phthalates	606	
Nitrosoamines	607	
Organochlorine Pesticides and PCBs	608	
Nitroaromatics and Isophorone	609	
Polycyclic Aromatic Hydrocarbons	610	
Haloethers	611	
Chlorinated Hydrocarbons	612	
2,3,7,8- Tetrachlorodibenzo-p-dioxin	613	
GC-MS Methods :		
Purgeable Hydrocarbons	624	
Base-Neutral, Organochlorines & PCBs	625	
Isotopic Dillution	1624 and 1625	
2 Broad Spectrum Analysis Protocol covers all compounds in methods 624 & 625 (target compounds) and non-target compounds.		6,59

TABLE 2

Isolation Techniques - Selected References

No.	Technique	References
1	Direct Aqueous Injection	7. 8. 67
2	Headspace	9. 10,12,13.14.16.17.18.19.20 21,22,23,24,67
3	Closed-loop Stripping	25,26,27,61,62
4	Liquid-Liquid Extraction	30,31,32,33,66
5	Supercritical Fluid Extraction	34,35,36,37,38,39,40,41,42,65

TABLE 3

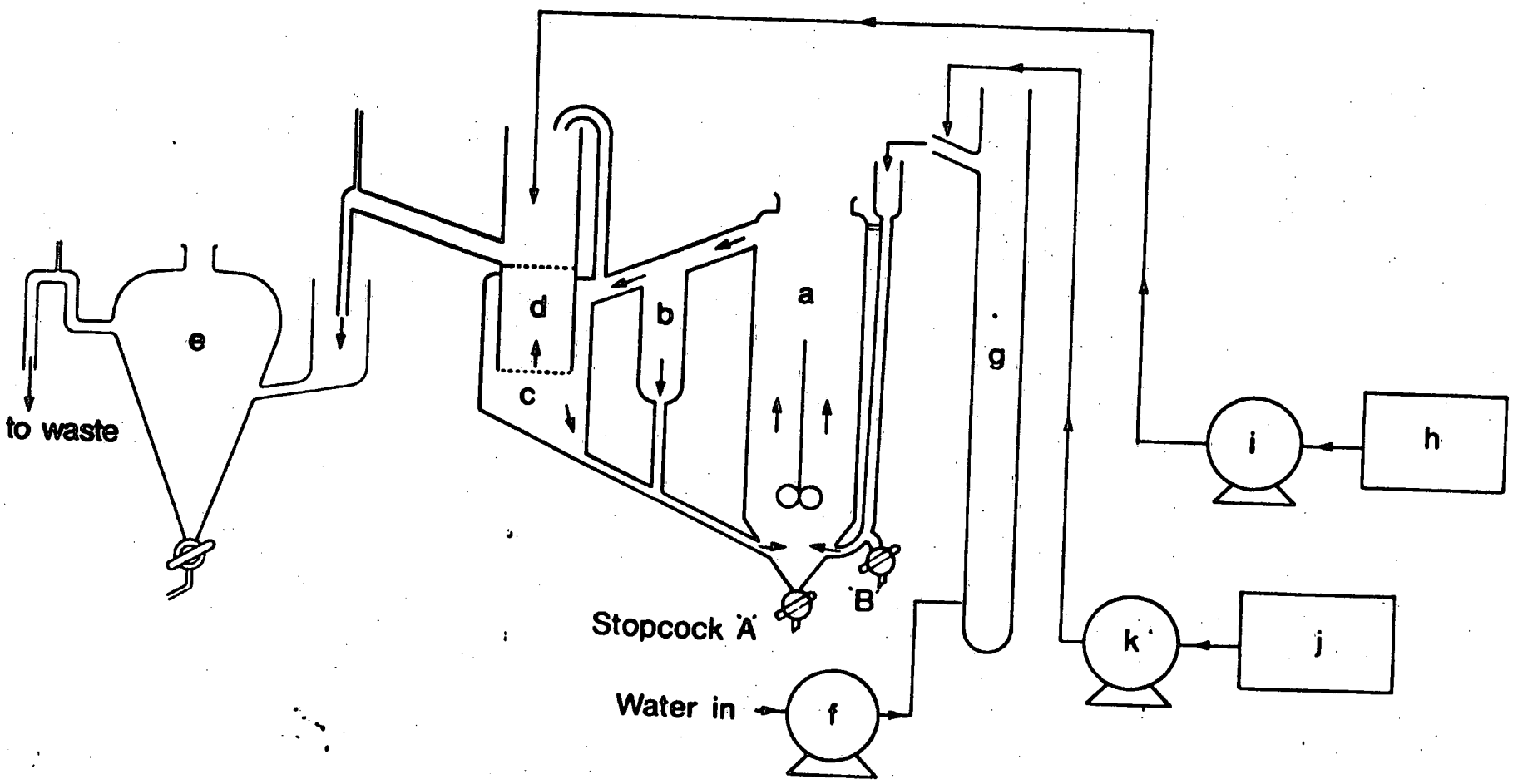
Adsorption Techniques - Selected References

Adsorbent	Reference
Graphitized Carbon	3, 47
Macroreticular Resins	3, 59, 63, 64, 65
Tenax	3, 69

TABLE 4

Elution Patterns and Recovery Data for Florisil

Compound	Elution Increments in mL			
	6% Fraction 0 to 200 mL	15% Fraction 200 to 400 mL	50% Fraction 400 to 600 mL	Recovery %
a-BHC	100			96
b-BHC	100			96
Lindane	100			96
Heptachlor	100			92
Aldrin	100			100
Heptachlor Epox.	100			100
Dieldrin		100		98
Endrin		100		99
p,p'-DDE	100			97
o,p'-DDT	100			99
p,p'-DDT	100			92
Ronnel	100			93
Me-Parathion		100		100
Malathion			100	99
Et-Parathion		100		96
Diazinon		100		85
Trithion	100			



a, mixing chamber; b, first settling chamber; c, second settling chamber; d, packed column; e, separator trap; f, metering pump–water; g, heater tube; h, solvent bottle; i, solvent make–up pump; j, surrogate standards bottle; k, 'spiking' pump.