Separation and Sample Preparation

There are millions of organic compounds that have been characterized in the chemical literature, with still millions more that are waiting for attention. The biggest problem in organic analysis is isolating the single compound of interest from the rest of the organic soup.

Take for example the simple and very common compound hexane, C_6H_{14}. Hexane does not exist as a single unique compound. There are five structural isomers of hexane (Table 1-1), all with the same molecular formula. The mixture of hexanes available in a bottle for laboratory use are not homogeneous for the molecular weight 86 from the formula C_6H_{14}. There are additional compounds for each of these structural isomers that include the naturally occurring isotopes of hydrogen (deuterium) and carbon (carbon-13). Consider the simple substitution of one hydrogen atom with a deuterium. Among the isomers of the hexanes there are 18 unique compounds with just one deuterium. And for the substitution of a single carbon-13 atom there are 18 compounds.

Table 1-1. Structural isomers of hexane.

Compound	CAS No.	Structure
n-Hexane	110-54-3	
2-Methylpentane	107-83-5	
3-Methylpentane	96-14-0	
2,2-Dimethylbutane	75-83-2	
2, 3-Dimethylbutane	79-29-8	

In that bottle of hexanes, there are also contributions from closely related relatives of the hexanes. For the six-carbon relatives, there are the family of compounds with the formula C_6H_{12}, which includes molecules with a single double bond (13 compounds) or a single ring (seven compounds). These are also subject to having isotopic substitution, generating more than 100 unique compounds. If the five- and seven-carbon relatives are added, the number of compounds increases dramatically.

So when the task is to determine the presence of hexane in a sample, one of the objectives to be answered prior to any beginning of analysis, is how fine a discrimination is to be made in this determination. Is n-hexane, C_6H_{14}, Mol. Wt. 86 the target analyte, or will any compound that exhibits the same properties as generic hexane in the analytical system be accepted as hexane?

The above is a completely valid consideration that has to be applied to the design and implementation of organic analytical methods and to the evaluation of test results for any set of organic analytes. Analyzing a bottle of hexanes for specific isotopically

substituted structural isomers is possible. However, the analysis of environmental samples is something of a Catch-22 in that the methods have been designed to be very robust so that they can handle all the complex crud associated with the sample, yet at the same time provide a measure of selectivity and specificity for isolation and identification of regulated compounds. It's akin to mixing the contents of the hexanes bottle with equal portions of gasoline and spent solvents, then being given the task of determining the isotopic distribution of the contents as well as the gasoline and spent solvents. It simply can not be done with a single analysis.

A. Analyte Isolation and Concentration

The first task in any analysis is to separate the analytes from the bulk of the sample. The traditional liquid extraction is the most common means employed. A portion of the sample is mixed with an organic solvent into which the analyte is preferentially partitioned. The idea of partitioning is key to the success of this procedure. No organic analyte is completely removed from a sample by a single washing with an organic solvent.

Partitioning is thermodynamically controlled. To achieve an equilibrium where, for example, 80% of the analyte molecules are in the organic solvent and 20% remain in the sample matrix, requires a certain amount of intimate contact time between the sample matrix, the analyte molecules, and the organic solvent. Gently sloshing together 1000 mL of aqueous sample and 60 mL of methylene chloride is not intimate contact nor is it efficient for achieving a thermodynamic equilibrium. The equilibrium is achieved by mixing the two phases together as thoroughly (violently) as possible for as long as possible, then allowing the phases to separate. Then repeat the procedure with additional portions of solvent. If equilibrium is established, then the first extraction recovers 80% of the analyte, the second extraction recovers an additional 16% (80% of the remaining 20% in the sample), and the third extraction recovers a further 3.2% for a total recovery of 99.2%. If equilibrium is not allowed to become established, the efficiency of the extraction procedure can be much lower.

All organic molecules have an affinity for the sample matrix to one degree or another, otherwise the organic molecules would not be present in the sample. The affinity may take the form of an actual absorption on the surface of sample particles or interaction with the water or other matrix molecules in a solvation phenomenon. Or it may take the form of an occlusion, where individual molecules of the analyte do not exhibit any attractive interaction with the substrate but are completely surrounded by substrate and have no means of exit from the cage.

It is necessary in an extraction process to overcome these interactions with the sample substrate. As a first attempt, and it must be stressed that, an attempt should be the operative concept, the sample is mixed with methylene chloride if the sample is aqueous, or, if the sample is solid, methylene chloride or a mixture of acetone and hexane. These are very powerful organic solvents and have great ability to partition analytes and other organic materials out of the sample. For some samples the great strength of these solvents actually works against the idea of separating the analytes from the matrix, particularly when the matrix contains non-analyte organic materials. When too much organic material ends up in the extraction solvent it is sometimes useful to switch to a solvent of lesser ability, such as hexane, pentane, or petroleum ether. In other cases, particularly when the sample is largely organic, such as an oil, the opposite tack can be useful, for instance, use of the polar solvent methanol to extract polar organic materials like the phenols from the matrix.

The organic acids (phenols) and organic bases (amines and pyridines) present other opportunities for sample isolation, where the pH of the sample can be adjusted to

control the direction of partitioning. Acidification of the sample converts the organic bases into salts that preferentially partition into the water phase. Adjusting the pH of the sample to values >11 with a suitable base neutralizes the basic analytes and changes the direction of partitioning to the organic phase, while at the same time converting the organic acids to hydrophilic salts.

Selective isolation of analytes through pH adjustment has numerous applications. For instance, the isolation of phenolic compounds from ash and cement kiln clinkers is complicated by the high pH of the matrix. One solution is to use the basic pH as an aid to the isolation[1]. The solid sample is mixed with a large volume of water, 50 g to 1000 mL. The resulting water suspension exhibits a pH greater than 10 for most of these matrices. The slurry is extracted with methylene chloride to recover the neutral and basic organic analytes. Then the solids are allowed to settle from the water. The supernatant liquid is separated from the solids and acidified with sulfuric acid, then extracted with the organic solvent to recover the phenols.

The simple expedient of separate analysis of the acid and basic extractions, rather than combining the extracts into a single sample extract, often serves to reduce matrix interference to a manageable level. As illustrated later in Figures 1-4 and 1-5, the interference can be significantly reduced by this approach.

No two samples are identical. Each sample is a unique combination of matrix-analyte interactions. Adding surrogate compounds to each sample and then determining the recovery of the surrogates is used as a yardstick for gauging the success of the extraction procedure. The best surrogates are those that are most like the target analytes. Isotopically labeled versions of each of the target molecules are the ideal solution. Phenol-d5 and 1,2-dichloroethane-d4 are perfect surrogates for phenol and 1,2-dichloroethane. The isotopic dilution techniques are the ultimate extension of this idea, where the recovery of each labeled analyte is used as an internal correction for the efficiency of the analyte isolation procedure in the quantitation of each target compound. For most purposes, this level of confidence (and expense) in the analysis is not warranted.

As an alternative, a short list of representative surrogates is frequently used. The choice of the surrogates can limit or it can maximize the interpretational utility of the recoveries. For most analyses, it is possible to choose surrogates that will always generate excellent recoveries, regardless of the complexity of the sample. Choosing surrogates to produce "acceptable" QC is counterproductive. The idea behind surrogates is to obtain information about the weak points of the analytical procedure. In most organic analyses the sample preparation factors that need to be monitored through the surrogates are the chemical and physical behaviors of the target analytes. For samples that are to be analyzed by gas chromatography, the key physical behavior is the range of vapor pressures exhibited by the analytes. Important chemical behaviors include acidic and basic properties of the analytes, the range of polarity exhibited by the analytes, reactivity in derivatization procedures, and finally, the sensitivity of the analytes to decomposition caused by extremes of chemical or physical environment. Surrogates chosen to monitor any of these areas should ideally bracket the range of the property. For vapor pressure, a surrogate compound with a high vapor pressure used in conjunction with a surrogate with a low vapor pressure provides the maximum range of information. Examples of surrogates that generate these types of information are found in a variety of the EPA organic methods. However, it should be pointed out that very few individual methods specify surrogates that provide information on all these areas, let alone the idea of bracketing the property. Under the Performance Based

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[1] Jackson, C., 1996. *Analysis of phenolic acid compounds in calcareous soils by SW-846 Method 8270,* 19th Annual EPA Conference on Analysis of Pollutants in the Environment, Norfolk VA 15-16 May, 1996.

Measurement System (PBMS) paradigm, additional surrogates beyond those specified in the relevant reference method can and should be used to extract the maximum amount of information about the sample.

Sample extract concentration is the one area in the isolation procedure that has the greatest potential for loss of analytes. The operative physical concept during concentration is vapor pressure, not boiling point. Boiling point is a discontinuous function: if the sample temperature is below the boiling point, it does not boil, if the sample is at the boiling point, it boils, and faster boiling is achieved by the addition of more heat. Boiling is uncontrolled: all the molecules are attempting to convert from liquid to gas at the same time. Equating sample concentration to boiling point leads to very erroneous conclusions about what is appropriate practice and what is not. Vapor pressure is a continuous function that relates to the rate of evaporation, and evaporation is what is desired during sample concentration. At low ambient temperatures vapor pressure is low, and evaporation is slow. At higher temperatures vapor pressure is higher and evaporation is faster. The conversion of a substance from a liquid state to a gaseous state requires heat, and the heat is generally extracted from the surroundings. On a hot day, if you wet your skin and then expose the skin to the air, your skin temperature is reduced due to the evaporation of the water, and you feel cooler. Obviously, the temperature of neither you nor your surroundings is at the boiling point of water, but the water still makes the transition from liquid to gaseous state.

These same processes are functioning during sample extract concentration. Conversion of solvent molecules from liquid state to gas is the desired objective, but the target analyte molecules are doing the same thing. It's just that, hopefully, the rate of conversion is slower for target analytes than it is for solvent. The proper technique is to control the rate of solvent evaporation while minimizing analyte loss. A completely successful sample extraction can be performed that is completely negated by an over-exuberant sample extract concentration.

Guidelines are normally presented in the methods, such that concentration of an extract below 1.00 mL is unacceptable because there is a substantial probability for loss of target analytes. As a rule this is useful, but it does not encompass all sample situations. There are samples that contain considerable amounts of co-extracted organic material, and even attempted reduction of the extract volume to 10.00 mL can result in complete loss of all potential target analytes.

The use of a surrogate that is more volatile than any of the target analytes is a very useful monitoring tool for concentration problems. Examples of surrogates that perform this function are tetrachloro-m-xylene (TCMX) in the chlorinated pesticides and PCB analyses, phenol-d$_5$ and 2-fluorophenol in the GC-MS analysis of semivolatile organics, and n-nonane in diesel fuel determinations (DRO). Low recoveries of these surrogates, in the presence of otherwise acceptable surrogate information, are almost always diagnostic for sample concentration problems. In this situation, recovery of the more volatile target analytes is regarded as similarly low, and data should be qualified as probably lower than the true values in the sample.

Acid-base properties of the surrogate must mimic those of the target analytes, otherwise the surrogate may be lost during sample manipulation. As an example, if the analytes are phenols, the surrogates must be phenols or other organic acids. A polyaromatic hydrocarbon such as phenanthrene is completely unsuited as a surrogate for phenols. A variety of isotopically labeled phenols are available for the mass spectral techniques, phenol-d$_5$ being the most common. EPA Method 8041 (determination of phenols) suggests use of 2,4-dibromophenol as surrogate for the non-mass spectral technique; however, any phenols that are not analytes, and are not present in the sample, can be used. Phenols are not the only organic acids that are subject to analysis. EPA Method 552 (determination of haloacetic acids) uses

3,5-dichlorobenzoic acid, 2,3-dichloropropionic acid, 2-bromopropionic acid, and 2,3-dibromopropionic acid as surrogates, depending on which version of the method is being followed.

When allowed choices in surrogates the analyst should make selections based on obtaining the maximum amount of information from the surrogates. In the case of the phenols, the compounds exhibit a range of ionization abilities, quantitatively expressed as the pK_a of the substance. Highly acidic phenols such as the nitrophenols have low pK_a values (2,4,6-trinitrophenol pK_a 0.42) and are more completely transferred to the water phase as salts than are the less easily ionized phenols (3-methylphenol pK_a 10.00). The nitrophenols are also less favorably partitioned into the organic solvent than the less acidic materials. In choosing surrogates for a phenols analysis, at least one surrogate should have a low pK_a while another has a high pK_a.

At present there are very few organic bases that are target analytes in environmental samples, as compared to the number of organic acids. Organic compounds that are classified as organic bases almost always contain nitrogen at the center of the ionization properties of the compound. However, not all nitrogen-containing compounds exhibit basic properties. True amines such as triethylamine and triethanol amine, are moderately strong bases. Aromatic amines (anilines) behave more like neutral compounds than as bases, and, although there are exceptions (4-nitroaniline, the benzidines, and the aniline dyes), are not partitioned into acidified water to any significant degree. Nitrogen-containing aromatic rings, such as pyridine, the quinolines, and other naturally occurring and synthetic alkaloids (caffeine and nicotine), are strong bases. Amides do not behave as bases. The strength of organic bases as bases can be expressed as either pK_a, or more commonly as pK_b, and these values can be used as a guide for selecting appropriate compounds as surrogates. Pyridine-d$_5$ has seen considerable use as a base surrogate, particularly in the analysis of wastewater effluents, but few other organic bases are employed for such purposes.

Organic analytes exhibit a wide range of polarity. Hexane and methanol are both organic compounds, but a mixture of the two solvents will form two distinct layers. There should be a polarity-matching between surrogates that are used in the analysis and the target analytes, particularly if any sample clean-up procedures are going to be used. One of the best tuned examples of this matching is the use of 1-chlorooctadecane in the Massachusetts extracted petroleum hydrocarbons (EPH) procedure. A silica gel column is used to separate the aliphatic hydrocarbons from the aromatic hydrocarbons. 1-Chlorooctadecane exhibits a polarity that is between that of the aliphatic and the aromatic hydrocarbons. The surrogate is monitored to insure that it ends up in the aliphatic fraction and not in the aromatic fraction. This assures that a complete separation of the aliphatics has been achieved.

In general, if separations are being used for sample clean-up, the best procedure is to use at least two surrogates. One should be similar in polarity to the target analytes, while the other more closely mirrors the interferents that are removed. This way both recovery and removal can be quantitatively monitored.

When derivatization procedures are used in an analysis, surrogates should be employed that can monitor the success of the reaction. The most common example is the use of a derivatization reaction to convert phenols to methyl ethers and organic acids to methyl esters. Diazomethane, trimethylsilyldiazomethane, boron trifluoride-methanol, and simply acidified methanol have been used for these conversions. Other derivatizations that are used include the conversion of phenols to pentafluorobenzylbromoethers (EPA Method 8041) and the conversion of biphenyl and chlorinated biphenyls to decachlorobiphenyl (EPA Method 508A). This last procedure is particularly finicky, and difluorobiphenyl is a satisfactory surrogate to use to

demonstrate complete reaction. The surrogate product is octachlorodifluorobiphenyl, which is chromatographically distinct from decachlorobiphenyl.

Not all target analytes exhibit the same degree of reactivity in these procedures. A surrogate should be used that first and foremost generates information that the derivatization has, in fact, occurred successfully. Second, a surrogate should be chosen that represents the more difficult analytes to successfully derivatize. Using a surrogate that is instantaneously and quantitatively derivatized tells little about the more sluggish analytes. An example of such is the use of pentafluorophenol as a reaction monitor for the derivatization of pentachlorophenol, a compound that is difficult to derivatize in most procedures.

Hydrolysis reactions, such as those used in the isolation of chlorinated acid herbicides, are potential points for analyte loss. A surrogate compound that is added to the sample in a form that is difficult to hydrolyze can provide useful information about the success of the hydrolysis reaction. For the chlorinated acid herbicides, target analytes in the form of esters are the most difficult to recover, even though in agriculture the esters are a frequently applied form of the compounds. The use of a surrogate like an ester of 2,4-dibromophenoxyacetic acid can provide the needed information. The ester that is chosen can be simple like the methyl ester, or it can be complex and very representative, such as the wettable ester from propylene glycol butyl ether (CAS 1928-45-6).

The sensitivity of target analytes to chemical decomposition can be monitored. The chlorinated hydrocarbon pesticides and the nitrogen-phosphorus pesticides are very sensitive to sample extraction and clean-up procedures. Chemically labile surrogates are monitored to insure that the analytes have not been subjected to abusive conditions. Dibutylchlorendate (DBC), a surrogate used in the chlorinated pesticide procedures, is very sensitive to pH conditions during the sample processing and will easily hydrolyze when the pH exceeds the 5-9 range, giving low recoveries. Low recoveries of the surrogate then are related to low recoveries of any of the labile target analytes (endrin, the DDT family, the endosulfan family). An alternate use for this particular compound is to monitor the acid-permanganate clean-up that is used in the PCB analysis (EPA Method 8082). If the sample extract has been adequately treated during the clean-up there should be no trace of DBC in the chromatogram.

The nitrogen-phosphorus analysis (EPA Method 8141A, September, 1994 version) suggests use of tributyl- and triphenyl-phosphates as surrogates. These compounds are essentially inert to all the abusive conditions that could completely prevent any meaningful recovery of target analytes from the sample. To monitor these aspects of sample preparation, an additional labile surrogate should be added to the analysis.

Related to chemical decomposition is absorption and loss of the target analytes to the glassware and other equipment that is used during the processing. The chlorinated acid herbicides are famous for exhibiting low recoveries due to absorption to glass surfaces. Although it is difficult to choose a surrogate that is specifically oriented toward the absorption problem, the use of a hydrolysis surrogate that is wettable (esters that contain one or more hydroxyethyl functions) can serve a dual function.

B. Resolution

After the sample has been extracted and the extract been through clean-up, the next step is the separation of the individual components through gas chromatography (GC) or use of liquid chromatography (LC). The idea is to have all components in the sample extract presented as single, completely resolved peaks to the detector.

Resolution is the degree of separation between any two sequentially eluting peaks in an analysis. There are quantitative expressions for resolution. It can be expressed as

the average of the widths of the peaks (W_a and W_B in Figure 1-2) divided into the difference in retention times (Figure 1-1):

$$\text{Resolution} = \frac{2[RT\ B - RT\ A]}{W_A + W_B}$$

However, few analysts will sit down and actually calculate resolution. Instead it's an eyeball evaluation. If two peaks are separate and distinct, they are said to be resolved. If the peaks partially overlap they are partially resolved, and if they substantially overlap, they are not resolved. See Figures 1-1 and 1-2.
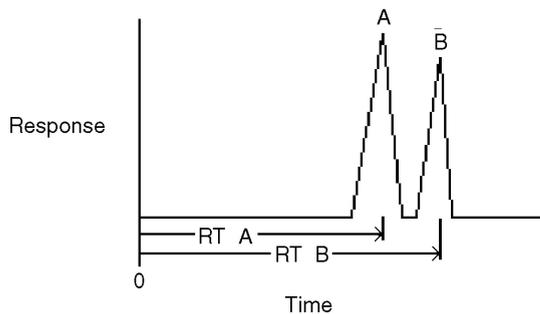
Figure 1-1. Retention times of two peaks in a generic chromatogram.
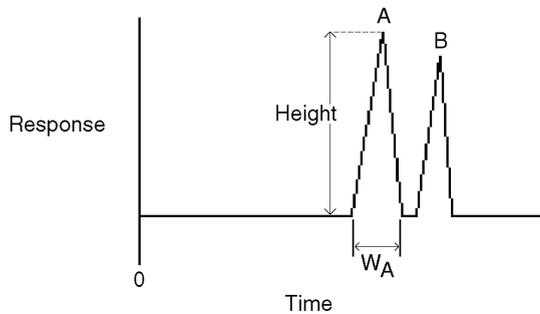

Figure 1-2. Characteristic properties of peaks in chromatography.

Peaks that overlap present problems in quantitation. The quantitation problems exist in the initial calibration of the instrument and in the analysis of samples.

Although the desired state is to have each and every component exiting the column as a single unique peak, real samples often contain co-extracted non-target compounds that co-elute with the calibrated analytes. Most times the analyst has to settle for completely isolated calibrated compounds. This can be difficult to achieve for 100% of the target analytes, particularly when some of the analytes are structural isomers of each other. Well known examples of representative analyte pairs are benzo(b)- and benzo(k)fluoranthene in the PAH and BN/A analysis, and 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene in the explosives residue determinations (Figure 1-3).

benzo(k)fluoranthene

benzo(b)fluoranthene

2-amino-4,6-dintrotoluene

4-amino-2,6-dinitrotoluene
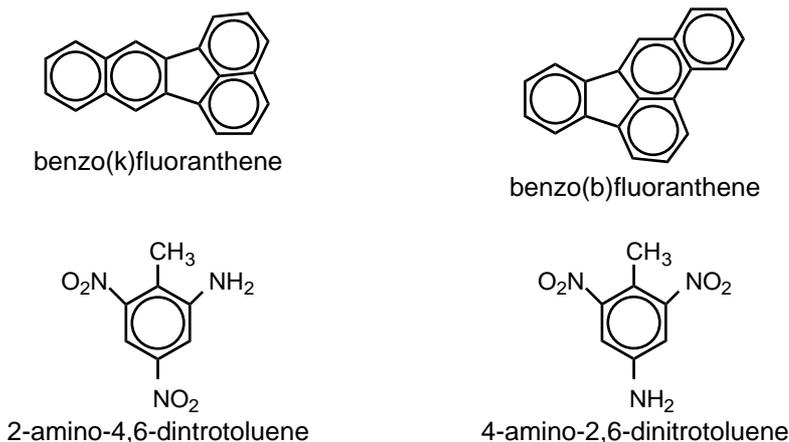
Figure 1-3. Examples of analyte pairs that are difficult to resolve.

   Analysts have often resorted to the band-aid solution of using two or more sets of analytes to achieve accurate calibration of overlapping target analytes. One member of the pair is in one set of calibration solutions, and the other member is in a separate set of solutions. The overly optimistic hope is that both compounds do not appear in the same sample extract. The more correct approach is to closely examine and optimize the separation technique during the initial set-up of the analysis to avoid co-eluting target analytes.

   There are a number of factors that will affect any given resolution problem. They are the:

   • Mass of analytes on the column
   • Temperature of the separation
   • Chemical selectivity of the separatory column (stationary phase)
   • Choice of mobile phase
   • Rate of mobile phase flow.

   The mass of analytes on the column is a corollary to the idea that for any given amount of liquid phase on column, there is a maximum amount of analyte that can interact with the liquid phase in a completely regular manner. The ideal operation of the separation is achieved when the molecules of the analyte will display a statistically normal distribution between being dissolved in the liquid phase and being in the vapor phase. As more molecules of the analyte are added to the system, they interact with each other, and the attraction to the liquid phase is as pairs or larger groupings of molecules rather than as single molecules. This changes the dynamics of the interaction with the liquid phase. Eventually the molecules' attraction for each other exceeds the interactive forces with the liquid phase and fronting or tailing occurs depending on the vapor pressure of the analyte-analyte interaction as compared to the analyte-liquid phase.

   The solution is to inject no more analyte than that amount which will produce completely symmetrical peaks in the chromatogram. When completely symmetrical peaks are achieved, the mid-point of the peak is the ideal elution time (retention time) for the analyte, and the width of the peak is related to the amount of analyte on the column. As less analyte is placed on the column, the width of the peak decreases. When two analytes overlap, then one answer is to place lesser amounts of the two on

the column and decrease the width of the individual peaks such that resolution increases, and the signals separate. This is easily achieved as a quick-fix by either decreasing the amount of sample extract solution injected into the instrument (a very poor choice due to non-linear discriminations in the injector port of the instrument) or diluting the sample extract with solvent and re-injecting the same volume of solution. The major consequence is that the lower limit of quantitation rises as the amount of analytes on-column decreases. More permanent fixes are to either use a longer column or use a column with a greater liquid phase thickness.

The temperature of the separation affects resolution. The vapor pressure (distribution profile) for the analyte-liquid phase is temperature dependent. At lower temperatures there is more interaction between the analyte and the liquid phase. When resolving two peaks, the lower temperature allows greater liquid phase discrimination over tiny differences between the analytes. The ideal situation is to keep the temperature of the analysis constant (isothermal operation), which allows the same level of interaction of the analytes with the liquid phase over the entire length of the column. A secondary effect of lower temperature is that the peaks become wider; however, the resolution increases faster than the broadening of the peaks.

Well, that's the ideal. In practical operation, the analyst is faced with a number of target compounds in the analysis that exhibit a range of vapor pressures. To complete the analysis in a reasonable amount of time, the column oven is temperature programmed, meaning that the temperature increases during the analysis. This allows analysis of a greater range of compounds in the GC run and also sharpens the peaks. However, it decreases the resolution as compared to an isothermal analysis. The rate of temperature increase affects resolution. Very fast temperature ramping severely degrades resolution. Slower rates of temperature increase give better resolution. It also makes the analysis time longer.

I once had the task of separating double bond positional isomers in straight chain hydrocarbons that ranged from C_{23} to C_{43} in length. The key compounds were those with double bonds in the 9-10 position as compared to those with the 10-11 double bond. A temperature program with a 1 ℃/min increase from 200 ℃ to 320 ℃ achieved the needed resolution, but the analysis took 2 hours for each sample.[2] The point is that resolution of overlapping analytes can be achieved, it's just a matter of time.

The analyst needs to carefully consider the beginning of the GC analysis. The first signal out of the GC is the solvent front. Complete separation between the solvent front and the first eluting target compound must be achieved. Quantitation of early eluting peaks that are sitting on the downslope of the solvent peak is poor analytical practice and leads to a lack of precision in the determination. Many samples will exhibit early eluting co-extractables, and these can complicate identification and quantitation of the early surrogates and target compounds. A good practice is to have at least five minutes of separation between the last portion of the solvent and the first eluting calibrated compound. Again the trade-off is time.

The chemical selectivity of the column for the analyte is a key component in achieving resolution. It is also the most expensive component to adjust. Walter Jennings, the founder of J & W Scientific, a major supplier of capillary columns for GC, is reputed to have said that he could separate any two compounds with a dimethylsilicone liquid phase. This may or may not be true with regard to separations,

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[2] Smith, R.-K., 1990. Chemotaxonomy of honey bees (*Apis mellifera* L.). Part 1: European and African workers. *Bee Science* 1(1):23-32; Smith, R.-K., 1991. Chemotaxonomy of honey bees (*Apis mellifera* L.). Part 2: Africanized workers. *Bee Science* 1(2):82-94; Smith, R.-K., M. Spivak, O.R. Taylor, Jr , C. Bennett, and M.L. Smith, 1992. Chemotaxonomy of honey bees (*Apis mellifera* L.). Part 3: Identification of Africanization in honey bee queens (Hymenoptera: Apidae). *Bee Science* 2(2): 93-105.

but the job can be made a lot simpler with a judicious choice of liquid phase. There are a lot of different liquid phases available for GC, and the situation is even more diverse on the LC side of the laboratory. If there is any one single item in regulatory methods that quickly becomes obsolete information, it is the column specifications in the method. Although one of the more current books on LC or GC technique may offer an idea of what is available, the better sources of information are the catalogs that are published by the suppliers. Most include sections on applications, and the analyst may find the exact solution in one of the applications. Time spent perusing column manufacturer's catalogs is not time wasted; it's time well spent. However, the best source of information is the technical support group of the supplier. These are the experts in matching columns with needed separations. When I used a GC regularly I was constantly on the phone to the various tech support centers of the major suppliers. Today you can contact the tech people by e-mail and do not have to sit on hold for extended periods of time. Do not limit yourself to a single supplier since no two companies offer exactly the same product line of columns. By asking the same question of a variety of people you improve your chances of getting the correct answer.

LC offers a wider selection of columns than GC does. Aside from the standard range of reverse phase octyl, octadecyl, and cyanopropyl columns, there are many quite unique columns whose like is not seen in the GC selection. These include the size exclusion, chiral, and immunochemical columns, one of which may be exactly suited for the needed separation. LC columns can also be connected in series to achieve particularly difficult determinations.

The mobile phase in GC is the carrier gas, and the gas selected has a bearing on the resolution. Nitrogen has very poor resolution ability, while helium or hydrogen are better choices. Hydrogen is actually the best carrier gas for resolution; however, it is reactive and may not be compatible with all sets of target analytes. There is an optimum flow rate for each carrier gas to achieve maximum resolution. As the temperature of the GC oven increases, the flow rate of the gas changes due to thermal expansion of the gas. Not so long ago (20 years) the analyst had to choose the temperature where he/she needed the maximum resolution and then set the carrier gas flow to optimize at that temperature. Most modern GC are equipped with constant flow devices that change the gas valve settings as the temperature in the oven changes, so changing flow rates are no longer a concern. Once the flow is optimized at one temperature it is optimized for all temperatures.

In LC there are as many or more choices of mobile phases as there are columns. Further the composition and flow rate of the mobile phase can be altered (gradients) during the analysis. The true sign of the expert LC operator is the ability to successfully choose the best combination of mobile and stationary phases to achieve needed resolutions.

C. Matrix Interferences

Many samples analyzed in the environmental laboratory display trivial-to-minor amounts of interfering substances, and the analysis is fairly simple. This is true for most wastewater samples and many groundwater samples, particularly those that are being collected as part of a long term monitoring program such as those associated with municipal wastewater treatment plants or landfills.

However, samples collected from sources that are heavily laden with organic matter present problems. A chromatogram of a wastewater sample extract from a chemical manufacturing plant is illustrated in Figure 1-4. The chromatogram exhibits a major amount of co-extracted interference that is localized in the 17- to 22- minute elution time window, and then a lot of minor interference that exists in all parts of the chromatogram. It should be obvious that identification and quantitation is going to be

difficult for target compounds that would normally elute in the 17 to 22 minute range. The proper approaches to this type of problem are either to perform some sort of sample clean-up that effectively removes the interference from the sample extract or to use an alternate analytical instrument that is not affected by the interference. There are many clean-ups that are possible, although no one technique can be promoted as a universal solution to interference problems. Several useful techniques are discussed later.

Unfortunately the most common remedy selected by analysts is to dilute the sample extract by a factor of 5 to 10 or even up to 1000 in some cases depending on the amount of interference present, and re-analyze the diluted extract. The load on the detector of the instrument has been lessened by this technique, but the problem of sample interference is completely dodged. A significant side-effect of this technique is that when detection limits for target analytes are multiplied by the dilution factor a non-detect for an analyte can be at levels above regulatory limits, frequently viewed as evidence of a permit violation by regulatory officials.
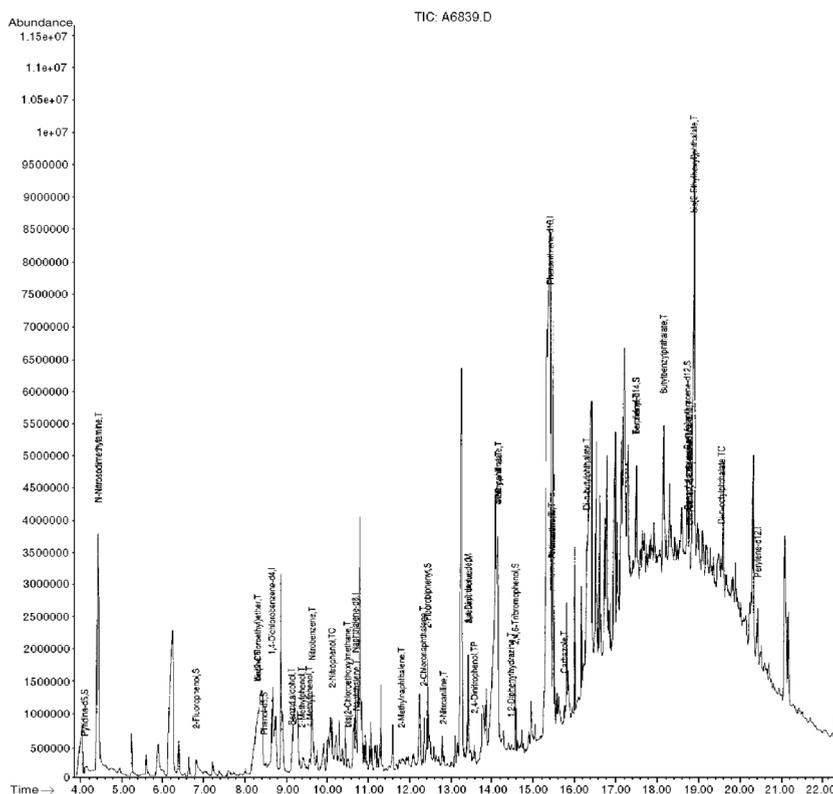
Figure 1-4. Acid extract chromatogram of a wastewater effluent sample.

Figure 1-5. Basic extract chromatogram of a wastewater effluent sample.

Sample extract dilution and re-analysis has a very important but very limited role in the laboratory. The legitimate use occurs when a target analyte is found in the sample, but the amount in the portion analyzed is over the bounds of the calibration curve. The solution is to dilute the extract by a factor necessary to bring the amount of analyte in the analyzed portion within the bounds of the curve so that a proper quantitation can be performed. There are no scientifically valid reasons for performing sample extract dilutions as an alternative to the use of appropriate sample extraction and clean-up.

Some procedures dictate the use of a particular extraction procedure or clean-up prior to analysis that are viewed as minimum steps necessary to obtain reliable data. The use of Florisil® micro-column clean-up of chlorinated pesticide extracts is a required procedure under the Superfund Contract Laboratory Program (CLP). EPA Method 625 dictates the separate preparation and analysis of acid and base/neutral extracts of wastewater samples. The U.S. Army Corps of Engineers recommends use of gel-permeation chromatography for clean-up of soil extracts prior to analysis. Most of the regulatory methods of analysis, particularly the organic methods, contain flexibility so that the analyst can employ alternate methods of extraction and clean-up for problem samples, while still being in legal compliance with the method. The EPA

has published in the methods manual for the solid waste program[3] a large selection of possible extraction and clean-up procedures (Table 1-2). I have discussed a number of these techniques and extensions, or modifications of them, in other publications.[4]

Table 1-2. Organic semivolatile sample preparation and clean-up procedures in SW-846.

3500B	Organic extraction and sample preparation
3510C	Separatory funnel liquid-liquid extraction
3520C	Continuous liquid-liquid extraction
3535	Solid phase extraction (SPE) (3535A in IVB)
3540C	Soxhlet extraction
3541	Automated soxhlet extraction
3542	Extraction of semivolatile analytes collected using modified Method 5 (Method 0010) sampling train
3545	Accelerated solvent extraction (ASE) (3545A in Update IVB)
3550B	Ultrasonic extraction
3560	Supercritical fluid extraction of total recoverable petroleum hydrocarbons (TRPH)
3561	Supercritical fluid extraction of polynuclear aromatic hydrocarbons
3562	Supercritical fluid extraction of PCB and organochlorine pesticides (Update IVB)
3580A	Waste dilution
3585	Waste dilution for volatile organics
3600C	Cleanup
3610B	Alumina cleanup
3611B	Alumina column cleanup and separation of petroleum wastes
3620B	Florisil cleanup
3630C	Silica gel cleanup
3640A	Gel-permeation cleanup
3650B	Acid-base partition cleanup
3660B	Sulfur cleanup
3665A	Sulfuric acid/permanganate cleanup

One clean-up technique in particular can be described as an example of the utility of these procedures. The analysis of polyaromatic hydrocarbons (PAH) is frequently needed on sites that are contaminated with petroleum fuels and oils. The PAH present significant risk to human health, and thus the required reporting limits for some of the compounds are normally quite low, on the order of 1 μg/L or lower for groundwater and 100 μg/kg or lower for soils. The presence of the other petroleum hydrocarbons serve as a significant chromatographic interference, as they are isolated along with the

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[3] *Test Methods for Evaluating Solid Waste - Physical/Chemical Methods*, EPA/SW-846, 3rd Edition, 1986, Update 1, July, 1992, Updates II and IIa, 1994, Update III, 1996, and proposed Updates IVa and IVb, 1998. Available from the Internet at www.epa.gov/osw/methods or by subscription from the Government Printing Office.

[4] Smith, R.-K., 1999. *Handbook of Environmental Analysis, 4th Edition*, Genium Publishing, Schenectady, NY 1-800-243-6486.

PAH in the solvent extraction. Direct analysis by GC-FID or GC-MS of the extract without clean-up generates the familiar "hump-o-gram", from which the analyst will try to pick out the individual target PAH frequently resulting in detection limits above 1000 µg/L for water and 33000 µg/kg for soils, useless values for ascertaining remediation success. One possible remedy is to use a GC-PID for the extract analysis. The PID (photo-ionization detector) is significantly more sensitive to PAH target analytes than the FID (flame ionization detector) or MS (mass spectrometer), while at the same time being less sensitive to saturated petroleum hydrocarbons. This works for some samples; however, the capability of the PID can be exceeded in samples with heavy petroleum loadings.

The solution is to remove the petroleum hydrocarbon interference from the sample extract, and it is very easily done.[5] The methylene chloride extraction solvent is exchanged for hexane[6], then the sample is passed through a silica gel cartridge. The saturated hydrocarbons pass through the cartridge, while the PAH are retained. The PAH are then selectively eluted with either methylene chloride or ether. Analysis of the eluant by GC-FID or GC-MS gives a chromatogram uncomplicated by the saturated petroleum hydrocarbons, allowing PAH determination at very low detection levels, even when the original sample is soaked with oil.

The use of any sample clean-up in an analysis is always accompanied by the possibility of analyte loss. Procedures that depend upon polarity interactions between the eluting solvent, a solid phase absorbent, and the target analytes to achieve selective isolations, are particularly prone to having the desired compounds ending up in the wrong fraction. Sources of these errors include mistakes in the preparation of the eluting solvent, use of the wrong or a deactivated absorbent, and the presence of traces of polar solvents in the sample solution. Intense attention to detail and procedure are required for successful use of sample clean-up, yet even the most careful technician will occasionally botch the procedure. These fears may be a significant reason why most laboratories do not regularly use sample clean-up procedures.

Use of appropriate quality control procedures can go far toward building confidence about the sample processing. The aspects of the procedure that need to be examined include:

- Lot-wise suitability of the materials to achieve the clean-up
- Introduction of laboratory contamination
- Batch-wise success of the clean-up
- Success of the procedure on the individual sample.

Each new lot of materials, including solvent and solid phase absorbent cartridges must be checked to verify that they can achieve the desired result. Normally, processing a standard mix of the target analytes and a representative matrix interference with each new lot of materials, and measuring the recovery of the analytes and the removal of the interference is sufficient to verify the suitability of the materials. Problems that will be discovered through use of this quality control include manufacturer's pre-packed absorbent mini-columns containing the wrong or a

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[5] This procedure is adapted from the EPH (extracted petroleum hydrocarbon) method for petroleum fractionation from the Massachusetts Department of Environmental Protection. www.state.ma.us/dep/bwsc/vph_eph.htm

[6] Remember that a single addition of hexane to the extract and concentration, leaves traces of methylene chloride in the sample. At least three cycles of addition and concentration are needed to completely remove the lower boiling solvent.

deactivated absorbent, and bottles of solvent that are contaminated with more polar substances.

The introduction of laboratory contamination is a significant concern in sample clean-up. Blanks are the most common quality control used to monitor introduced contamination. A common finding is the presence of phthalates extracted from some plastics that are used for construction of the mini-columns. Other contaminants that have been found include petroleum hydrocarbons in the C_{12} to C_{25} range extracted from the solid absorbent. Solvents can also be contaminated. Blanks should be processed through the sample clean-up each day that the procedure is used. The blank may be specific for the sample clean-up processing, rather than using the normal extraction batch blank; however, in most situations the batch blank will suffice. The blank is examined for the introduction of both target analytes and general contamination.

Batch-wise success of the clean-up can be determined through processing the batch laboratory control sample through the sample clean-up. The recovery of the target analytes is examined along with the introduction of any extraneous materials from the process. Although it may seem that this later function is simply duplicating that of the blank, in fact it is not. The laboratory control sample contains target analytes and exhibits a different polarity from that of the blank. The presence of the target analytes in the laboratory control can serve to displace column contaminants that are unaffected by passage of the blank. The laboratory control also serves as an additional check on random contamination.

Finally, it is desirable to check the success of the clean-up on every sample that is processed. Surrogates are normally used in organic analysis as an individual sample check. Surrogate compounds that are designed and used to monitor sample extraction may not be the most applicable monitors of a clean-up procedure. Take the example of the PAH clean-up described above. If the normal suite of six surrogates used in the Method 8270 analysis are subjected to the silica gel clean-up, four of the surrogates (phenol-d_5, 2-fluorophenol, 1,3,5-tribromophenol, and nitrobenzene-d_5) will probably not be recovered unless a much more polar solvent mixture for the final elution is used. Two surrogates should be recovered, 2-fluorobiphenyl and terphenyl-d_{14}; however, they fail to give information about the success of saturated hydrocarbon removal efficiency. For these reasons, a recovery surrogate, 1-chlorooctadecane is added to the sample extract prior to clean-up. The 1-chlorooctadecane should be completely absent from the PAH fraction, as it is eluted from the column with the saturated hydrocarbons. The recovery standards suggested in the MADEP EPH method, 2-fluorobiphenyl and 2-bromonaphthalene, are used to assure that all the lighter PAH, such as naphthalene and the methylnaphthalenes are retained in the aromatic fraction.

Another example of the use of a recovery surrogate is found in the sulfuric acid clean-up (EPA Method 3660 or 3665) of extracts for PCB analysis (EPA Method 8082). The clean-up procedure is to treat the hexane extract of the sample with concentrated sulfuric acid. In practice, most technicians will add the acid to the hexane extract and then allow the mixture to sit passively for several minutes. At most the technician may shake the mixture once, then let it sit. The only area of actual contact between the acid solution and the extract is at the solvent interface. This is a very ineffective situation. The mixture of the acid and the extract must be vigorously mixed, preferably with a small magnetic stirbar, to achieve a suitable level of contact in the clean-up process. To check that suitable contact has occurred, the addition of an acid-labile surrogate (dibutyl chlorendate, or 4-chloro-3-nitrobenzotrifluoride) to the sample or the sample extract is performed. Again the disappearance of the surrogate is indicative that adequate cleanup has been achieved.