

Chapter 1

Pre- and Post-Extraction Considerations

Learning Objectives

- To appreciate the wide ranging types of organic compounds that are investigated in environmental and food matrices.
- Using an example, to be aware of pre-sampling issues associated with a contaminated land site.
- To be aware of the information required for a desk-top study (in a contaminated land situation).
- To understand the different sampling strategies associated with solid, aqueous and air samples.
- To be aware of the different types of contaminant distribution on a site.
- To understand the practical aspects of soil and sediment sampling.
- To understand the practical aspects of water sampling.
- To understand the practical aspects of air sampling.
- To be aware of the different analytical techniques available to analyse organic compounds.
- To understand and explain the principle of operation of a gas chromatography system.
- To understand and explain the principle of operation of a high performance liquid chromatography system.
- To be able to understand the principles of quantitative chromatographic analysis.

- To be aware of the approaches and limitations for sample pre-concentration in the analysis of organic compounds.
- To appreciate the importance of quality assurance in quantitative analysis.
- To understand the health and safety aspects of performing laboratory work and the consequences for non-compliance.

1.1 Introduction

This book is concerned with the removal of organic compounds, principally persistent organic compounds (POPs), from a range of sample matrices including environmental matrices (soil, water and air samples), but also some other matrices including foodstuffs. The book is designed to be an informative guide to a range of extraction techniques that are used to remove organic compounds from various matrices. The use of discussion questions (DQs) and self-assessment questions (SAQs) throughout the text should allow you (the reader) to think about the main issues and to allow you to consider alternative approaches.

1.2 Organic Compounds of Interest

The range of organic compounds of interest in the environment and in other matrices varies enormously. They range from simple aromatic cyclic structures, for example, benzene, toluene, ethylbenzene and xylene(s) (collectively known as BTEX), to larger molecular weight compounds, such as polycyclic aromatic hydrocarbons (PAHs), and more complicated structures, e.g. pesticides and polychlorinated biphenyls (PCBs). A list of organic compounds that are measured in environmental (and other) matrices is shown in Table 1.1.

SAQ 1.1

What are the important physical and chemical properties of these organic compounds that are useful to know when extracting them from sample matrices?

1.3 Pre-Sampling Issues

Prior to sampling it is necessary to consider a whole range of issues that are directly/indirectly going to influence the quality of the final data that is produced after what is often a long and costly process. Therefore it is imperative to think

Table 1.1 Potential organic contaminants in the environment

Class of compound	Name of specific compound
<i>Aromatic hydrocarbons</i>	Benzene
	Chlorophenols
	Ethylbenzene
	Phenol
	Toluene
	<i>o</i> -xylene
<i>Chlorinated aliphatic hydrocarbons</i>	<i>m,p</i> -xylene
	Polycyclic aromatic hydrocarbons
	Chloroform
	Carbon tetrachloride
	Vinyl chloride
	1,2-Dichloroethane
	1,1,1-Trichloroethane
	Trichloroethene
	Tetrachloroethene
	Hexachlorobuta-1,3-diene
	Hexachlorocyclohexanes
<i>Chlorinated aromatic hydrocarbons</i>	Dieldrin
	Chlorobenzenes
	Chlorotoluenes
	Pentachlorophenol
	Polychlorinated biphenyls
	Dioxins and furans

about the ‘whole picture’ before any sampling is started. In reality a range of individuals will be involved in the process. To illustrate some of the steps involved a simple generic approach is presented to allow you to think about the overall process.

DQ 1.1

Is a former industrial site suitable for building domestic houses?

Answer

[In order to answer this it is appropriate to consider yourself as the individual responsible for overseeing this work on behalf of the current owner of the land.]

Initial thoughts should revolve around carrying out a desk-top study. A desk-top study, as the name suggests, involves gathering information that is readily

available without necessarily having to analyse anything (at least at this point in time). A desk-top study may contain the following information:

- Physical setting.
 - Site details including a description of location, map reference, access to site, current land use and general description of site.
- Environmental setting.
 - Site geology including a description of surface and below-surface geology, e.g. coal seam.
 - Site hydrogeology including details of river or stream flows and whether groundwater is abstracted and for what purpose.
 - Site hydrology including known rainfall and river/stream/pond locations.
 - Site ecology and archaeology including whether the site has any known scheduling, e.g. site of special scientific interest (SSSI); any features of archaeological significance.
 - Mining assessment, e.g. evidence of former quarrying activity.
- Industrial setting and recent site history. Information available via historic and modern ordnance survey maps including (aerial) photographs of the site.
- Qualitative risk assessment including development of a site-specific conceptual model that seeks to assess the following:
 - Source of contaminants.
 - The pathway by which a contaminant could come into contact with a receptor, e.g. people.
 - The characteristics and sensitivity of the receptor to the contaminant.
- Site walkover, i.e. by visiting the site it is possible to identify key issues, major features, position of walkways, etc.
- Any previous site investigations.
- Conclusions and recommendations.

Useful information can be gathered about a former industrial site by obtaining detailed historic ordnance survey maps. By studying these maps it will be evident what building infrastructure will have been present at set times in history. For example, Figure 1.1(a) shows a historic map (1898) from a site which is largely marsh land and was underdeveloped in 1898, while Figures 1.1(b–d) illustrate the growth of the industrial aspects of the site from 1925 (Figure 1.1(b)) through

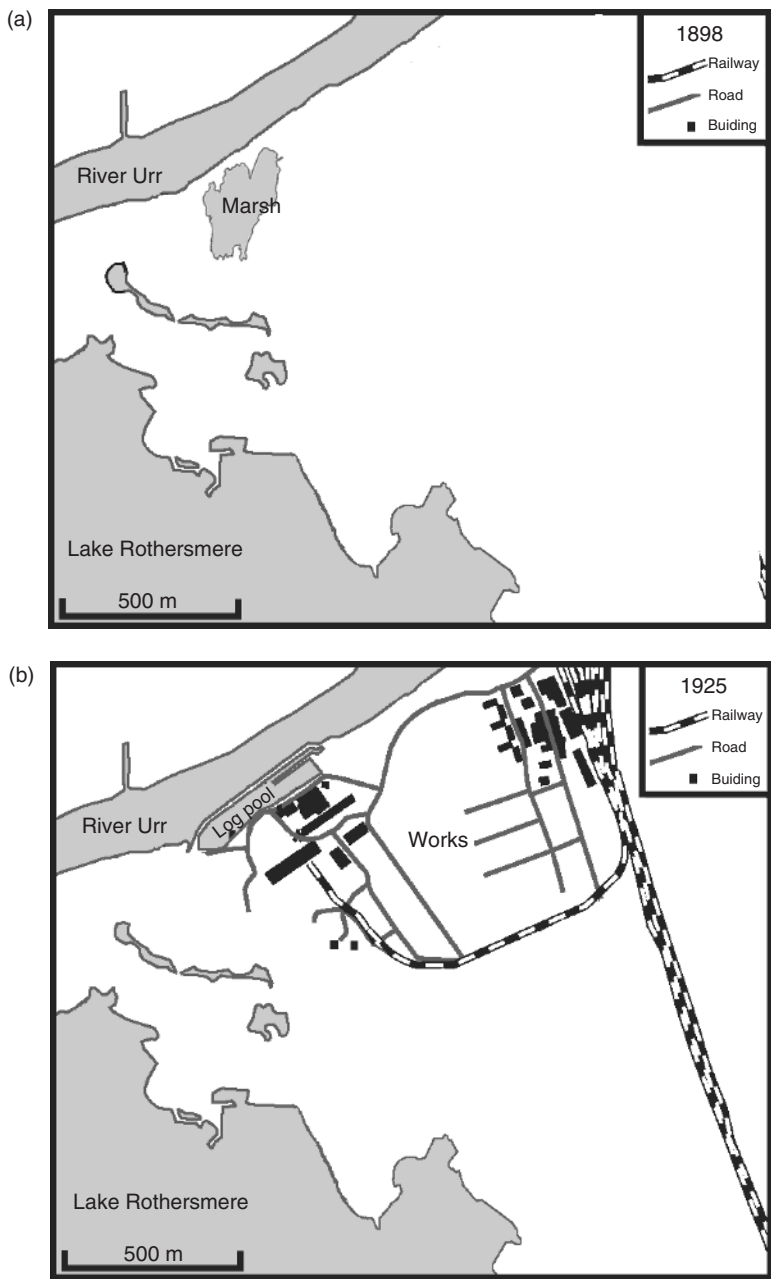


Figure 1.1 Historic maps of a selected site: (a) 1898; (b) 1925; (c) 1954; (d) 1990. Reproduced by permission of Dr M. Deary, Northumbria University, Newcastle, UK.

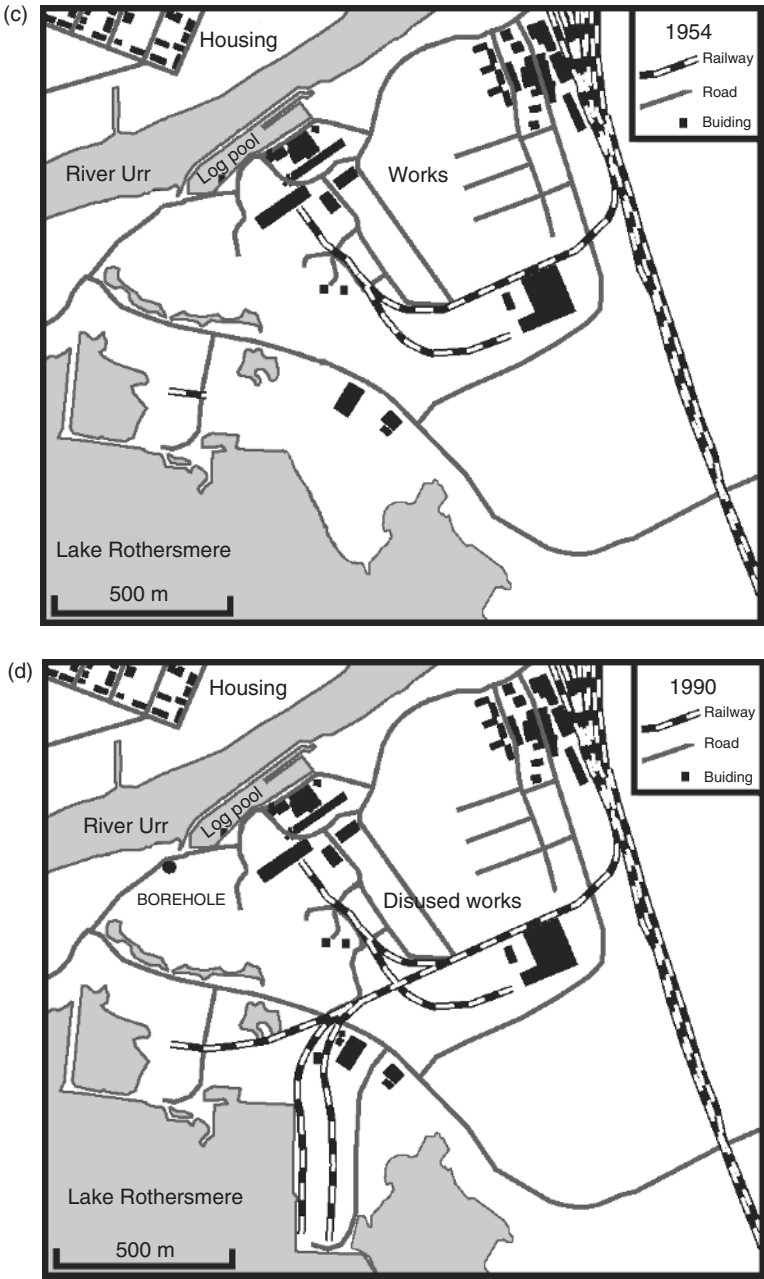


Figure 1.1 (continued)

to 1954 (Figure 1.1(c)) and its subsequent decline by 1990 (Figure 1.1(d)). The emergent development of housing is noted in Figure 1.1(d). In addition, information about the use of the former buildings can be obtained from local archivists, e.g. city/town councils and history societies, who will retain records on historic activities. By gathering this detailed information it is possible to build up a picture of possible organic contaminants that may still be present on the site (not necessarily amenable on the surface but buried beneath other material).

DQ 1.2

What other contaminants may be present on the site?

Answer

As well as organic compounds other contaminants may be present, including heavy metals, asbestos etc.

With regard to carrying out some specific sampling it is necessary to obtain answers, in advance, about the following:

- (1) Do you have permission to obtain samples from the site?
- (2) Is specialized sampling equipment required? If so, do you have access to it? If not can you obtain the equipment and from whom?
- (3) How many samples (including replicates) will it be necessary to take?
- (4) What soil/water/air testing is required?
- (5) What instrumentation is available to do the testing on?
- (6) Is the instrumentation limited with respect to sample size (mass or volume)? Does sample size constrain the analytical measurement?
- (7) What quality assurance procedures are available? Has a protocol been developed?
- (8) What types of container are required to store the samples and do you have enough of them?
- (9) Do the containers require any pre-treatment/cleaning prior to use and will this be done in time?
- (10) Is any sample preservation required? If so what is it and how might it impact on the analysis of the contaminants?

1.4 Sampling Strategies: Solid, Aqueous and Air Samples

Ideally, all sample matrices should be analysed at or on-site without any need to transport samples to a laboratory. Unfortunately in most cases this does not happen and samples are transported back to a laboratory and analysed. The exception is where a preliminary assessment takes place on site, for example, by using a photoionization detector to assess the level of volatile organic compounds in the atmosphere. The issue in most instances is to consider how many samples should be taken and from which location. Therefore significant consideration needs to be given to the sampling protocol as to whether the sample is solid, liquid or gaseous in order that the data that are obtained at the end of the analytical process has meaning and can be interpreted appropriately. Two main types of sampling can be undertaken: random or purposeful sampling. The former is the most important as it infers no selectivity in the sampling process.

The sampling process involves the following:

- selection of the sample points;
- the size of the sample area;
- the shape of the sample area;
- the number of sampling units in each sample.

It is advantageous before sampling to consider information, e.g. location of former buildings on the site, to potentially assess the likely distribution of the contaminants. Any distribution of contaminants can be generally described as:

- random;
- uniform (homogenous);
- patchy or stratified (homogenous within sub-areas);
- present as a gradient.

Examples of these potential likely distributions of contaminants are shown in Figure 1.2.

In practice, however, the site to be sampled can be hindered by the occurrence of modern building, footpaths and other infrastructure obstacles (e.g. stanchions for bridges).

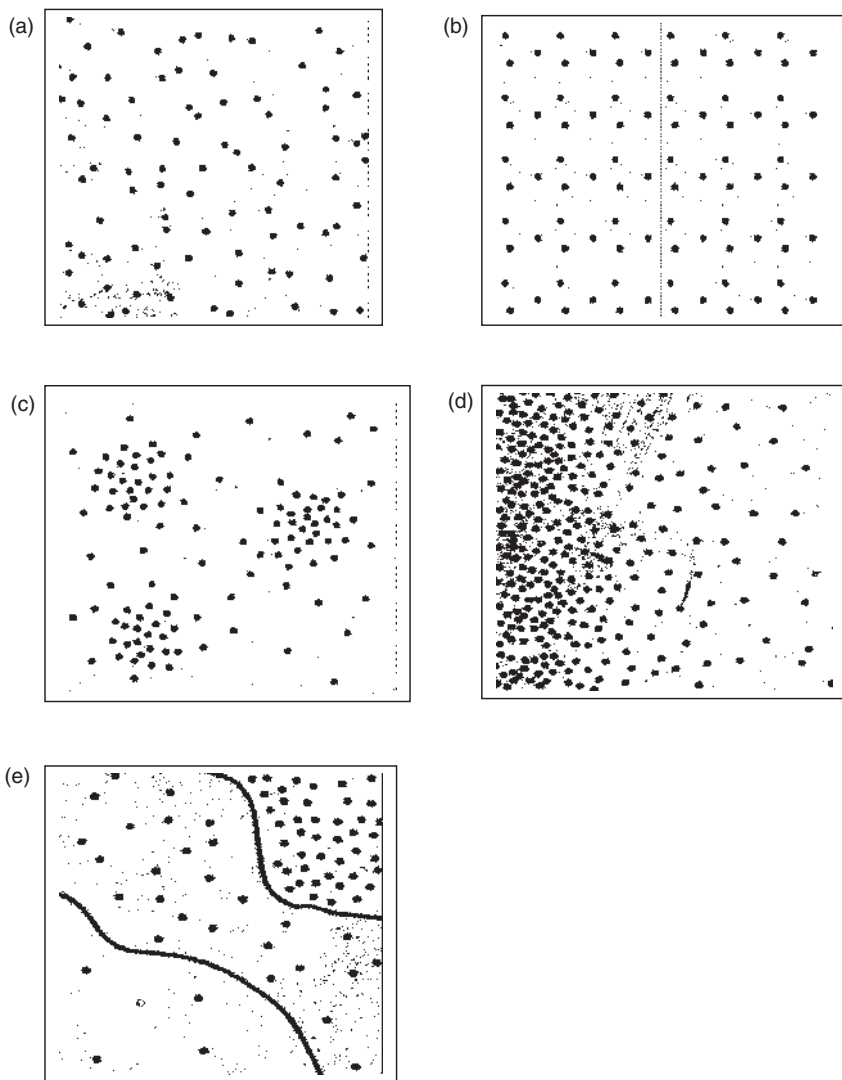


Figure 1.2 Different distributions of inorganic and organic contaminants: (a) random; (b) uniform (homogeneous); (c) patchy; (d) stratified (homogeneous within sub-areas); (e) gradient. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

DQ 1.3

Consider the map outline shown in Figure 1.3(a). Based on the current site where might you choose to sample?

Answer

A suggestion of particular sampling locations is shown in Figure 1.3(b). Note that it is not always possible to maintain the numerical sequence of the sampling points due the presence of permanent structures.

Actually establishing the distribution of contaminants on the site does require some actual preliminary testing of the site, i.e. a pilot study. This allows the

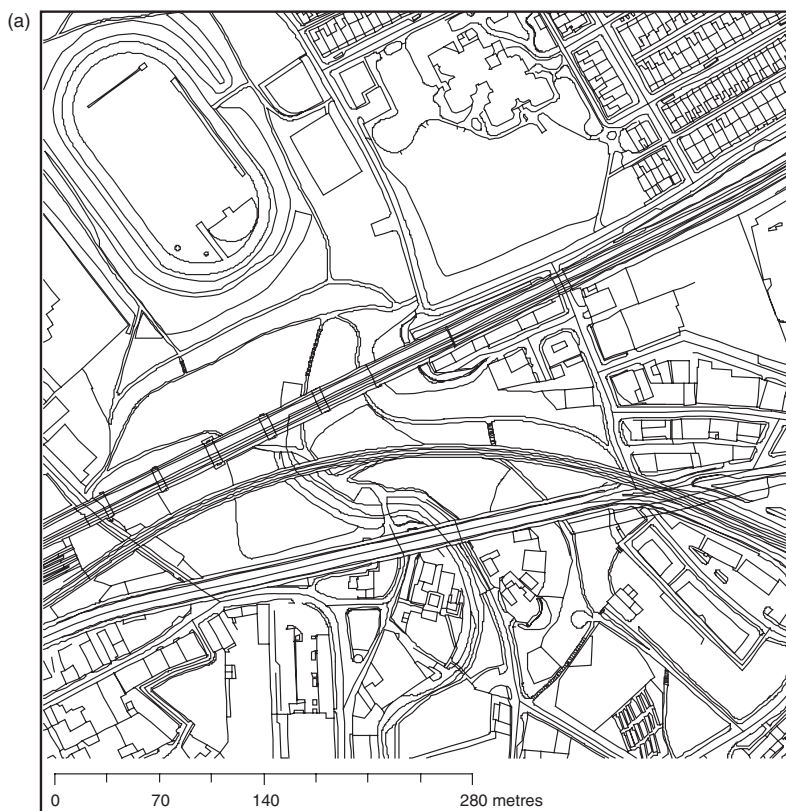


Figure 1.3 An example of a potential contaminated land site for investigation. (a) Consider the options for locating a sample grid. (b) Sampling grid and selected sites (numbered). © Crown Copyright Ordnance Survey. An EDIMA Digimap/JISC supplied service.

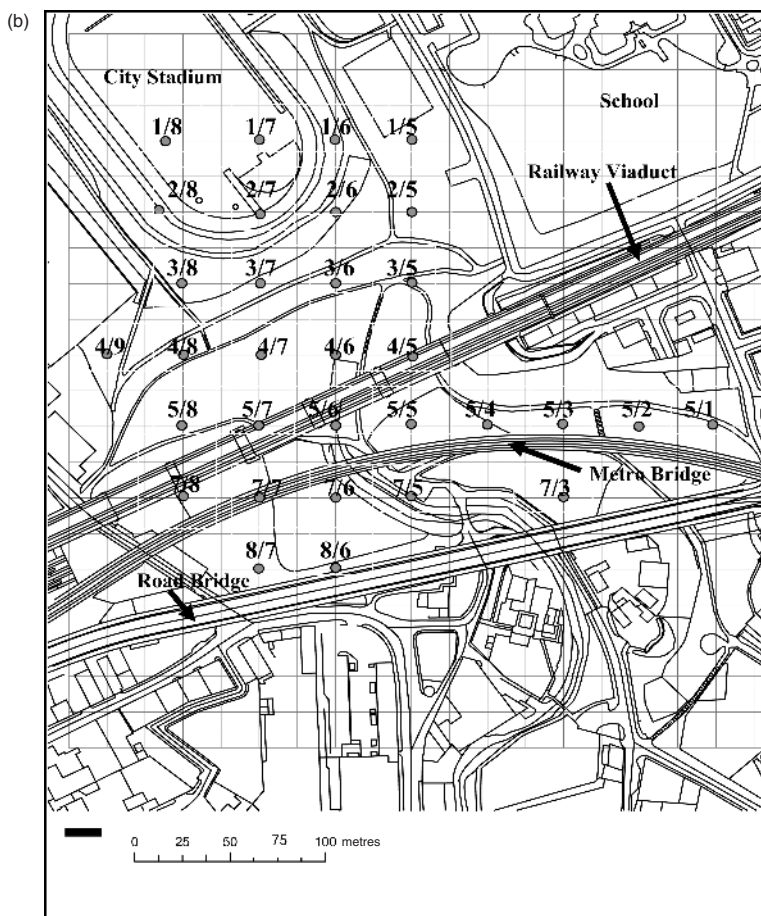


Figure 1.3 (continued)

level and distribution of contaminants to be assessed. The sampling position can be assessed by overlaying a 2-dimensional coordinate grid on the site to be investigated (see for example, Figure 1.3(b), and then deciding to sample, for example, from either every grid location or every other grid location. This approach to sampling is appropriate in the context of contaminants which are likely to be homogeneously distributed about the site.

1.4.1 Practical Aspects of Sampling Soil and Sediment

This sample type is often characterized by its heterogeneity and hence diversity of chemical and physical properties. Samples are usually taken with an auger,

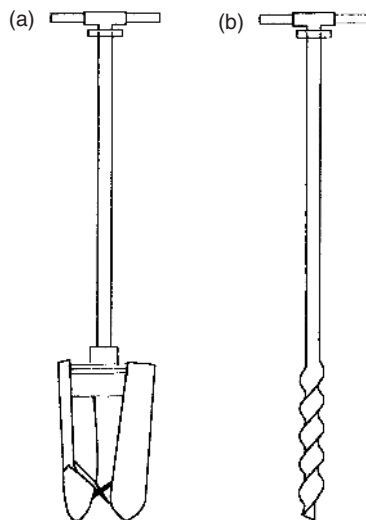


Figure 1.4 Types of augers used for soil sampling: (a) twin blade; (b) corkscrew. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

spade and a trowel. The auger is a hand-held device that can penetrate the soil in a screw-like manner which acts to bring the soil to the surface (Figure 1.4). A trowel is often used for surface (e.g. 0–10 cm depth) gathering of previously disturbed material, a spade to access lower levels (e.g. 0–100 cm depth) and an auger for deeper levels (e.g. >100 cm depth). Soil samples, once gathered, should be placed in a geochemical soil bag (e.g. a ‘Kraft bag’) or polythene bag, sealed and clearly labelled with a permanent marker pen. When the soil sample has been gathered any unwanted soil should be placed back in the hole and covered with a grass sod, if appropriate. The samples are then transported back to the laboratory and dried. In the case of the geochemical soil bag the sample can be left *in-situ* and dried. Drying is normally done by placing the sample in a special drying cabinet that allows air flow at a temperature $<30^{\circ}\text{C}$.

DQ 1.4

Why should a higher temperature *not* be used for organic compounds?

Answer

Higher temperatures should not be used for samples containing organic compounds to prevent premature loss of the compounds under investigation.

Depending on the sample moisture content the drying process may be complete with 48 h. The air dried sample is then sieved (2 mm diameter holes) through a pre-cleaned plastic sieve to remove stones, large roots and any other unwanted material. The sieved sample can then be sub-sampled and analysed. Sometimes it is appropriate to reduce the sample size further. For example, samples may be sieved through a pre-cleaned 250 μm sieve such that two size fractions are available for analysis, i.e. the $>250\mu\text{m}$ and $<250\mu\text{m}$ fractions. The prepared soil samples can then be further sub-sampled using the process of coning and quartering to obtain a representative sample for extraction and subsequent analysis.

SAQ 1.2

What is coning and quartering?

1.4.2 Practical Aspects of Sampling Water

Water can be classified into many types, e.g. surface waters (rivers, lakes, runoff, etc.), groundwaters and springwaters, wastewaters (mine drainage, landfill leachate, industrial effluent, etc.), saline waters, estuarine waters and brines, waters resulting from atmospheric precipitation and condensation (rain, snow, fog, dew), process waters, potable (drinking) waters, glacial melt waters, steam, water for sub-surface injections, and water discharges including waterborne materials and water-formed deposits.

Water is often an heterogeneous substance with both spatial and temporal variation.

DQ 1.5

Why might spatial variation occur in natural water?

Answer

Spatial variation occurs due to stratification within lakes due to variations in flow, chemical composition and temperature.

DQ 1.6

Why might temporal variation occur in natural water?

Answer

Temporal variation, i.e. variation with respect to time occurs, for example, because of heavy precipitation (i.e. snow, rain) and seasonal changes.

A schematic of a typical manual water sampling device is shown in Figure 1.5. The device consists of an open tube with a known volume (e.g. 1 to 30 l) fitted with a closure mechanism at either end. The device is usually made of stainless-steel or PVC. The sample is taken by lowering the device to a pre-determined depth and then opening both ends for a short time. Then, both ends are closed and sealed. By this process the water is sampled at a specified depth. The sampled water is then brought to the surface and transferred to a suitable glass container with a sealable lid.

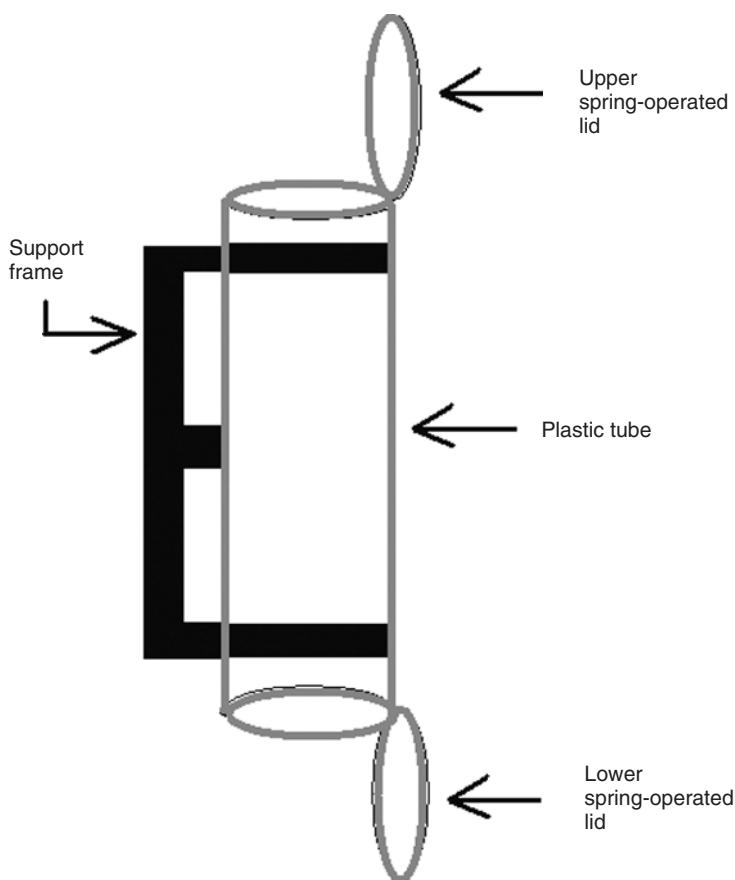


Figure 1.5 A schematic of a typical manual device used for water sampling. Figure drawn and provided by courtesy of Naomi Dean.

SAQ 1.3

Why is it often not advisable to use a plastic container for organic compounds?

Fortunately the methods of preservation are few for organic compounds and intended to fulfil the following criteria: to retard biological action, to retard hydrolysis of chemical compounds and complexes, to reduce volatility of constituents and to reduce adsorption effects. For organic compounds the normal process is to store the water samples for the shortest possible time, in the dark and at 4°C. Suggested storage conditions for selected organic compounds are shown in Table 1.2.

1.4.3 Practical Aspects of Air Sampling

Air sampling can be classified into two distinct themes: vapour/gas sampling or particulate sampling. In the case of the latter, particles are collected on filters (e.g. fibreglass, cellulose fibres) which act as physical barriers whereas in the former case air-borne compounds are trapped on a sorbent (e.g. ion-exchange resins, polymeric substrates) which provide active sites for chemical/physical retention of material.

In sorbent tube sampling (Figure 1.6), volatile and semi-volatile organic compounds are pumped from the air and trapped on the surface of the sorbent (Figure 1.6 (a)). Quantitative sampling is possible by allowing a measured quantity of air (typical volumes of 10–500 m³) to pass through the sorbent. The sorbent tube is then sealed and transported back to the laboratory for analysis. As the organic compounds collected are either volatile or semi-volatile they will be analysed by gas chromatography (see Section 1.5.1). First however, they need to be desorbed by either the use of organic solvent (solvent extraction) or heat (thermal desorption). The latter approach can be done in a fully automated manner using commercial instrumentation and is therefore the preferred analytical approach.

1.5 An Introduction to Practical Chromatographic Analysis

Organic compounds can be analysed by a variety of analytical techniques including chromatographic and spectroscopic methods. However, in this book the main emphasis is on the use of chromatographic approaches. A brief overview of some of the most important chromatographic techniques is provided together with some practical information.

Table 1.2 Selected examples of preservation techniques for water samples^a

Compound	Storage container	Preservation	Maximum holding time
Pesticides (organochlorine)	Glass	1 ml of a 10 mg ml ⁻¹ HgCl ₂ or adding of extraction solvent (500 ml of water)	7 days, 40 days after extraction
Pesticides (organophosphorus)	Glass	1 ml of a 10 mg ml ⁻¹ HgCl ₂ or adding of extraction solvent (500 ml of water)	14 days, 28 days after extraction
Pesticides (chlorinated herbicides)	Glass	Cool to 4°C, seal, add HCl to pH < 2 (500 ml of water)	14 days
Pesticides (polar)	Glass	1 ml of a 10 mg ml ⁻¹ HgCl ₂ (500 ml of water)	28 days
Phenolic compounds	Glass	Cool to 4°C, add H ₂ SO ₄ to pH < 2 (500 ml of water)	28 days

^a As recommended by different agencies (USEPA and ISO).

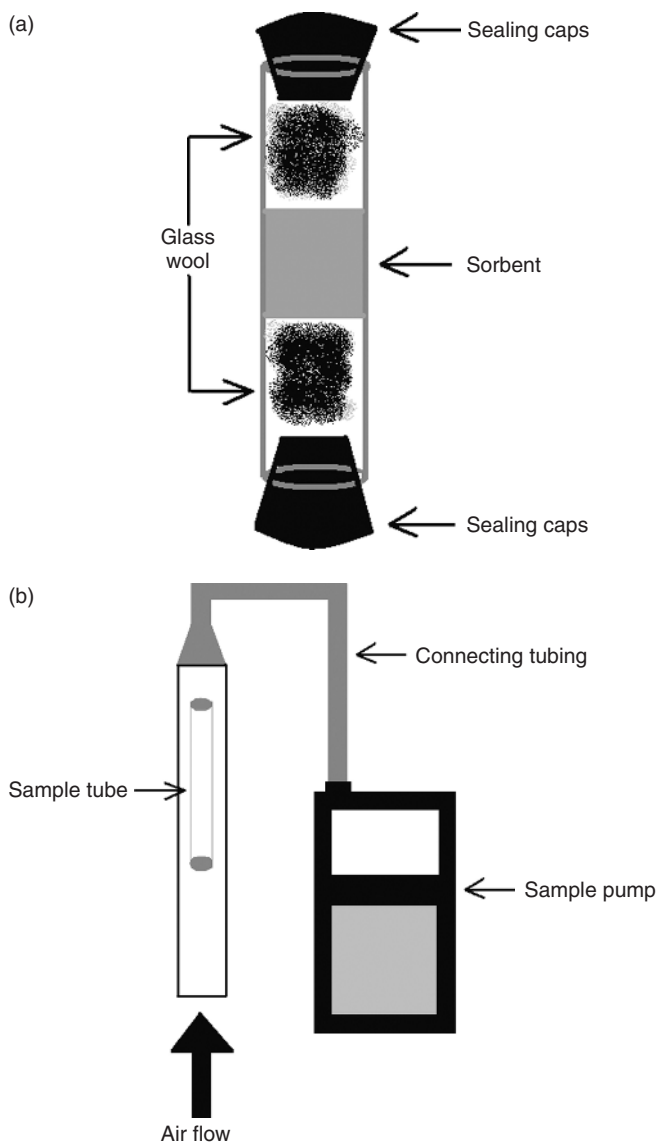


Figure 1.6 Air sampling: (a) schematic of a typical sorbent tube; (b) schematic of the system used to carry out measurements. Figure drawn and provided by courtesy of Naomi Dean.

1.5.1 Gas Chromatography

Gas chromatography (GC) is used to separate samples that contain volatile organic compounds. A schematic diagram of a gas chromatograph is shown in Figure 1.7.

1.5.1.1 Sample Introduction in GC

A volatile liquid is injected, via a 1 μl syringe, through a rubber septum in to the heated injection port, where the sample is volatilized. The most common injector is the split/splitless injector (Figure 1.8) which can operate in either the split or splitless mode. In the splitless mode all of the injected sample is transferred to the column whereas in the split mode only a portion of the sample (typically 1 part in either 50 or 100) passes onto the column. Alternate sample introduction systems for GC include the *programmed temperature vaporizer* (PTV) injector in which a large volume of sample (typically 30–50 μl) is introduced onto the column. The PTV injector allows a larger sample volume to be injected by means of a temperature programme within the injection port itself. This allows solvent to be vented and a more concentrated sample to be introduced onto the column. Another alternative is when a gaseous sample can be introduced directly into the injection port of the gas chromatograph (see Chapter 11). Split/splitless injection can be done either manually, by hand or via an autosampler which is computer-controlled to introduce consecutive samples/standards.

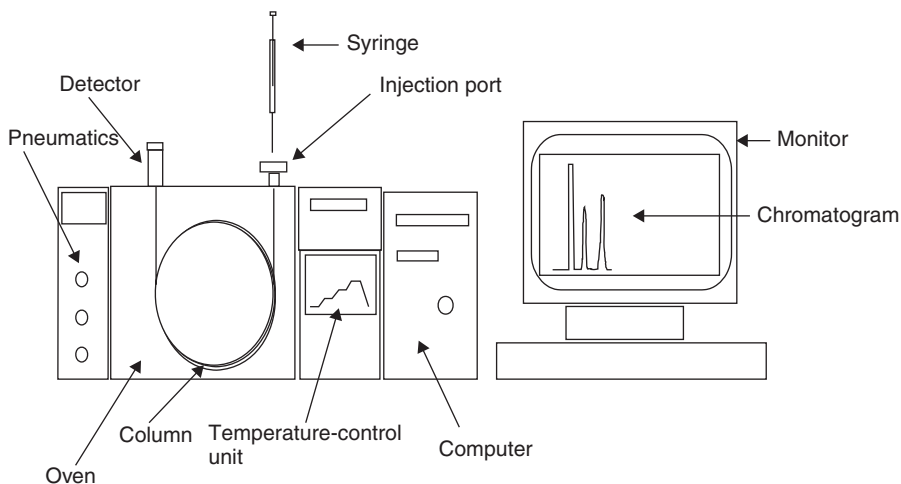


Figure 1.7 Schematic diagram of a typical gas chromatograph. Reproduced by permission of Mr E. Ludkin, Northumbria University, Newcastle, UK.

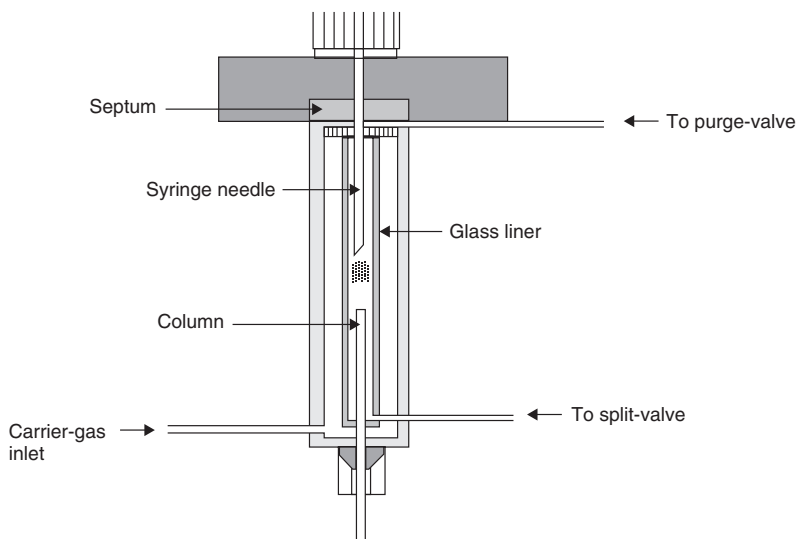


Figure 1.8 Schematic diagram of a split/splitless injector used in gas chromatography. Reproduced by permission of Mr E. Ludkin, Northumbria University, Newcastle, UK.

DQ 1.7

How might you manually inject a sample/standard into the gas chromatograph?

Answer

In the manual injection mode the sample/standard is introduced as follows:

- The syringe is filled (1.0 μl) with the sample/standard solution; this is achieved by inserting the needle of the syringe into the solution and slowly raising and then rapidly depressing the plunger. After several repeats of this process the plunger is raised to the 1.0 μl position on the calibrated syringe.
- The outside of the syringe is then wiped clean with a tissue.
- Then, the syringe is placed into the injector of the gas chromatograph and the plunger on the syringe is rapidly depressed to inject the sample.

A gaseous carrier gas (nitrogen or helium) transports the sample from the injection port to the column.

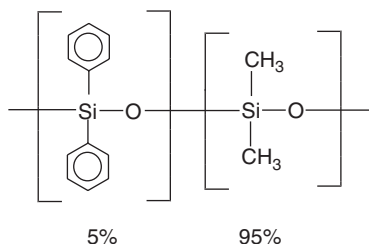


Figure 1.9 The stationary phase of a DB-5 GC column, consisting of 5% diphenyl- and 95% dimethylpolysiloxanes. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

1.5.1.2 GC Column

A typical capillary GC column is composed of polyimide-coated silica with dimensions of between 10 and 60 m (typically 30 m) long with an internal diameter between 0.1 and 0.5 mm (typically 0.25 mm), and a crosslinked silicone polymer stationary phase (for example, 5% polydiphenyl–95% polydimethylsiloxane – generically known as a DB-5 column), coated as a thin film on the inner wall of the fused silica (SiO_2) capillary of thickness 0.1–0.5 μm (typically 0.25 μm) (Figure 1.9).

The column is located within an oven, capable of accurate and rapid temperature changes, allowing either isothermal or temperature programmed operation for the separation of organic compounds. In the isothermal mode the temperature of the oven, and hence the column environment, is maintained at a fixed temperature (e.g. typically in the range 70–120°C), while in the temperature programmed mode a more complex heating programme is used. This approach is often necessary for the separation of complex mixtures of organic compounds. A typical oven temperature programme could be as follows: start at an initial temperature of 70°C for 2 min, then a temperature rise of 10°C/min up to 220°C, followed by a ‘hold time’ of 2 min. In order for the next sample to be introduced the oven must cool back to 70°C prior to injection; this process is rapid, taking approximately 1–2 min.

1.5.1.3 Detection in GC

After GC separation the eluting compounds need to be detected. The most common detectors for GC are the universal detectors, as follows:

- the flame ionization detector (FID);
- the mass spectrometer (MS) detector.

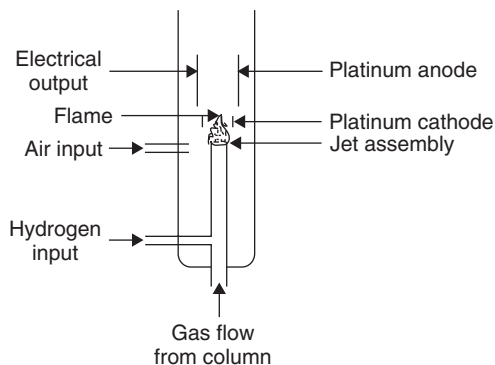


Figure 1.10 Schematic diagram of a flame-ionization detector. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

In the case of the FID (Figure 1.10) the exiting GC carrier gas stream, containing the separated organic compounds, passes through a (small) hydrogen flame that has a potential (>100 V) applied across it. As the organic compounds pass through the flame they become ionized, producing ions and electrons. It is the collection of these electrons that creates a small electric current that is amplified to produce a signal response proportional to the amount of organic compound. The FID is a very sensitive detector with a good linear response over a wide concentration range.

In the case of the mass spectrometer detector, compounds exiting the column are bombarded with electrons from a filament (electron impact or EI mode) (Figure 1.11) causing the compound to fragment with the production of charged species. It is these charged species which are then separated by a mass spectrometer (typically a quadrupole MS) based on their mass/charge ratio. Upon exiting the quadrupole the ions are detected by an electron multiplier tube which converts the positive compound ion (cation) into an electron, which is then multiplied and collected at an anode, resulting in a signal response which is proportional to the amount of organic compound. The MS can collect data in two formats: total ion current (TIC) (or full scan) mode and single (or selected) ion monitoring (SIM) mode.

SAQ 1.4

What is the difference in output between the TIC and SIM modes and how is it achieved?

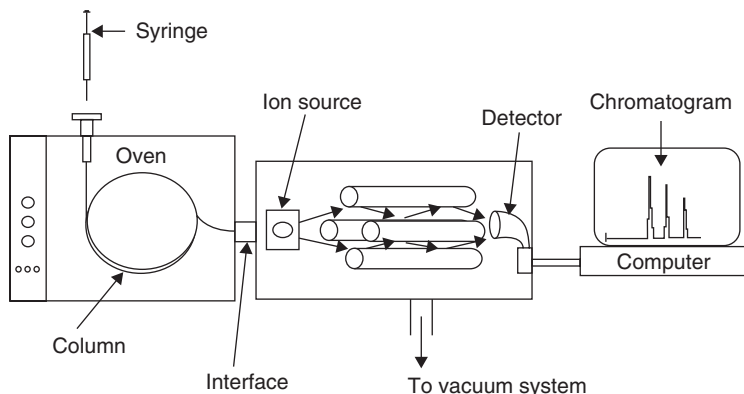


Figure 1.11 Schematic diagram of a capillary gas chromatography–mass spectrometry hyphenated system. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

1.5.2 High Performance Liquid Chromatography

In high performance liquid chromatography (HPLC) a mobile phase, into which the sample is introduced, passes through a column packed with micrometre-sized particles. HPLC allows rapid separation of complex mixtures of non-volatile compounds. A schematic diagram of an HPLC system is shown in Figure 1.12.

1.5.2.1 Mobile Phase for HPLC

The mobile phase for HPLC consists of an organic solvent (typically methanol or acetonitrile) and water (or buffer solution). The mobile phase is normally filtered (to remove particulates) and degassed (to remove air bubbles) prior to being pumped to the column by a reciprocating piston pump. The pumping system can operate in one of two modes allowing either isocratic or gradient elution of the non-volatile organic compounds. In the isocratic mode the same solvent mixture is used throughout the analysis while in the gradient elution mode the composition of the mobile phase is altered using a microprocessor-controlled gradient programmer, which mixes appropriate amounts of two different solvents to produce the required gradient. Gradient elution allows the separation of more complex organic compound mixtures rather than isocratic elution. Also, at the end of the gradient, elution time has to be allowed for a re-equilibration of the system to the initial mobile phase conditions. A typical gradient elution approach may consist of the following: start at an initial mobile phase composition of 30:70 vol/vol methanol:water for 2 min, then a linear gradient to 90:10 vol/vol methanol:water in 20 min, followed by a ‘hold mobile phase composition’ for 2 min. In order for the next sample to be introduced, the mobile phase composition

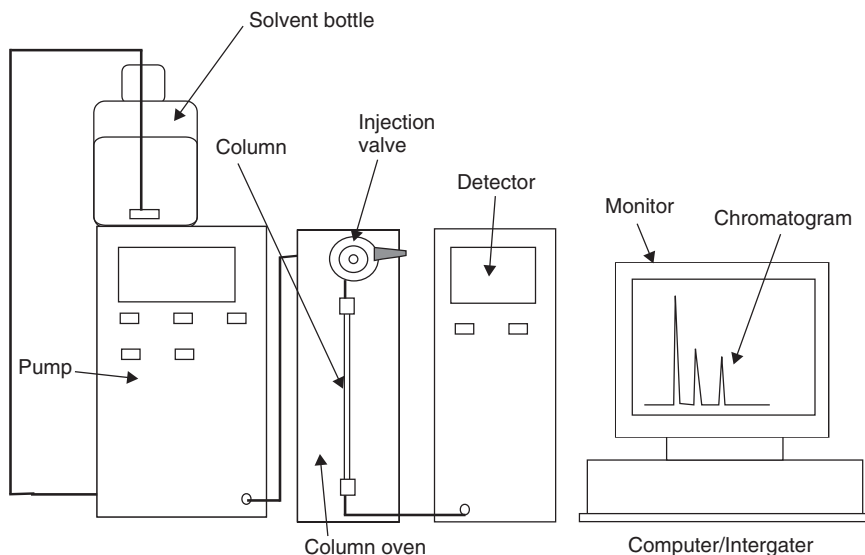


Figure 1.12 Schematic diagram of an isocratic high performance liquid chromatograph. Reproduced by permission of Mr E. Ludkin, Northumbria University, Newcastle, UK.

must return to the initial conditions, i.e. 30:70 vol/vol methanol:water prior to injection; this process is relatively rapid taking approximately 5–10 min.

1.5.2.2 Sample Introduction for HPLC

The most common method of sample introduction in HPLC is via a rotary 6-port valve, i.e. a Rheodyne® valve. A schematic diagram of a rotary 6-port valve is shown in Figure 1.13. Injection of a sample (or a standard) can be done either manually, by hand, or via a computer-controlled autosampler.

DQ 1.8

How might you manually inject a sample/standard into the chromatograph?

Answer

In the manual injection mode a sample/standard is introduced as follows:

- The syringe is filled (1.0 ml) with the sample/standard solution; this is achieved by inserting the needle of the syringe into the solution and slowly raising the plunger, taking care not to introduce any air bubbles.

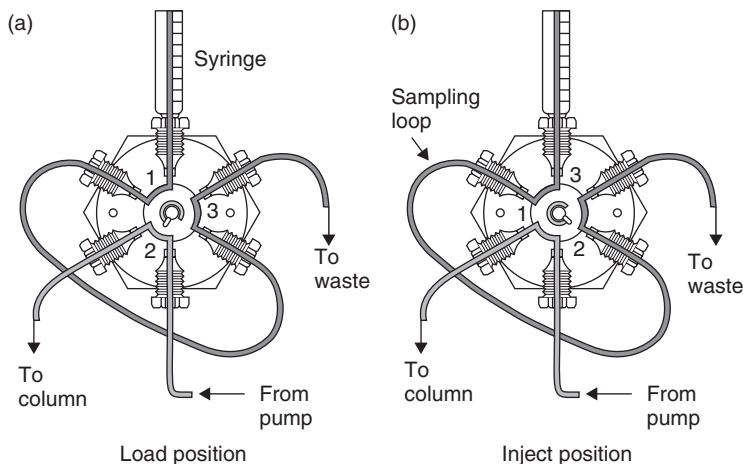


Figure 1.13 Schematic diagram of a typical injection valve used for high performance liquid chromatography: (a) load position; (b) inject position. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

- The outside of the syringe is then wiped clean with a tissue.
- Then, the syringe is placed into the 6-port valve which is located in the 'load' position and the plunger depressed (but not all the way) to introduce the sample into an external loop of fixed volume (typically 5, 10 or 20 μl). While this is occurring the mobile phase passes through the 6-port valve to the column.
- Then, the 6-port valve is rotated into the 'inject' position. This causes the mobile phase to be diverted through the sample loop, thereby introducing a reproducible volume of the sample into the mobile phase.

The mobile phase transports the sample from the 6-port valve to the column.

1.5.2.3 HPLC Column

An HPLC column is made of stainless steel tubing with appropriate end fittings that allow coupling to connecting tubing (either stainless steel or PEEK). Typical column lengths vary between 1 and 25 cm (typically 25 cm) with an internal diameter of <1.0 mm to 4.6 mm (typically 4.6 mm). The stationary phase is bonded to silica particles (typically 3 or 5 μm diameter). Based on the composition of the mobile phase, described above, the chemically bonded stationary phase is typically C_{18} (also known as octadecylsilane (ODS)) (Figure 1.14). Other stationary

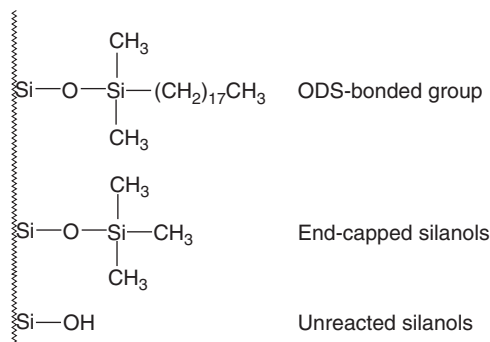


Figure 1.14 Silica particles coated with octadecylsilane (ODS) for reversed phase high performance liquid chromatography. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

phases include C_8 , C_6 , C_2 and C_1 . The presence of unreacted silanol groups on the stationary phase can lead to detrimental compound separation.

SAQ 1.5

How might this detrimental separation be evident?

To compensate for these issues it is possible to obtain end-capped C_{18} ; in this situation the silanol groups are blocked with C_1 entities. The column is often located within an oven which is used to stabilize peak elution. The temperature of the oven is maintained at a fixed temperature (e.g. typically in the range $23\text{--}35^\circ\text{C}$).

1.5.2.4 Detectors for HPLC

After HPLC separation the eluting compounds need to be detected. The most common detectors for HPLC are the universal detectors, as follows:

- the ultraviolet/visible detector (UV/visible);
- the mass spectrometry (MS) detector.

In the case of the UV/visible detectors they are widely used and have the advantages of versatility, sensitivity and stability. They are available in three forms:

- fixed wavelength;

- variable wavelength;
- as a diode array detector.

A fixed wavelength detector is simple to use with low operating costs. It contains a mercury lamp as a light source and operates at fixed, known wavelengths.

DQ 1.9

What are the common wavelengths that a fixed UV/visible detector can operate at?

Answer

Typically one of the following: 214, 254 or 280 nm.

Variable-wavelength detectors use a deuterium lamp and a continuously adjustable monochromator for wavelength coverage between 190 to 600 nm. The use of a diode array detector incorporates the advantage of multi-wavelength coverage with the ability to run a UV/visible spectrum for any compound detected. This 3-dimensional image of absorbance (i.e. the signal) versus compound elution time (i.e. the chromatogram) and a UV/visible spectrum is invaluable in chromatographic method development. The sensitivity of the UV/visible detector is influenced by the pathlength of the 'z-shaped' flow cell (typically 10 mm) which maximizes signal intensity (Figure 1.15).

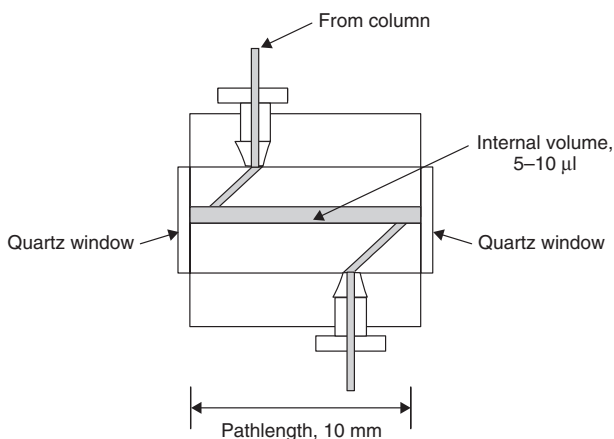


Figure 1.15 Schematic diagram of a UV/visible detector cell for high performance liquid chromatography. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

In the case of the mass spectrometry (MS) detector, compounds exiting the column are ionized at atmospheric pressure (i.e. external to the MS detector). The two major interfaces are:

- electrospray (ES) ionization;
- atmospheric pressure chemical ionization (APCI).

In ES ionization (Figure 1.16) the mobile phase is pumped through a stainless-steel capillary tube held at a potential of between 3 to 5 kV. This results in the mobile phase being sprayed from the exit of the capillary tube, producing highly charged solvent and solute ions in the form of droplets. Applying a continuous flow of nitrogen carrier gas allows the solvent to evaporate, leading to the formation of solute ions. These ions are introduced into the spectrometer via a 'sample-skimmer' arrangement. By allowing the formation of a potential gradient between the electrospray and the nozzle, the generated ions are introduced into the mass spectrometer.

In APCI the voltage (2.5–3.0 kV) is applied to a corona pin which is positioned in front of the stainless-steel capillary tubing through which the mobile phase from the HPLC passes (Figure 1.17). To assist the process the capillary tube is heated and surrounded by a coaxial flow of nitrogen gas. The interaction of the nitrogen gas and the mobile phase results in the formation of an aerosol which enters the corona discharge, producing sample ions. These ions are transported into the mass spectrometer in the same way as described above for ES. Using ES or APCI, organic compounds form singly charged ions by the loss or gain of a proton (hydrogen atom), i.e. $[M + 1]^+$ (typically basic compounds, e.g. amines)

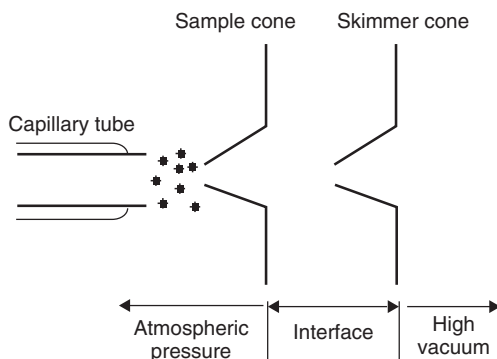


Figure 1.16 Schematic diagram of an electrospray ionization (ESI) source for HPLC–MS. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

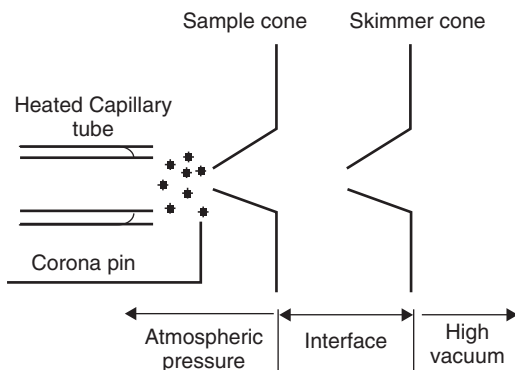


Figure 1.17 Schematic diagram of an atmospheric-pressure chemical ionization (APCI) source for HPLC–MS. From Dean, J. R., *Bioavailability, Bioaccessibility and Mobility of Environmental Contaminants*, AnTS Series, Copyright 2007. © John Wiley & Sons, Limited. Reproduced with permission.

or $[M - 1]^-$ (typically acidic compounds, e.g. carboxylic acids), where M is the molecular weight of the compound allowing the spectrometer to operate in either the positive ion mode or negative ion mode, respectively. Separation of the ions takes place in either a quadrupole mass spectrometer, ion-trap mass spectrometer or time-of-flight mass spectrometer. In order that both positive and negative ions can be detected in MS requires the use of an electron multiplier tube with a conversion dynode prior to the normal discrete dynode. The conversion dynode can be segmented: one segment coated with a material that is responsive to negative ions while a different segment is coated with a material that is responsive to positive ions.

1.5.2.5 Quantitative Analysis in Chromatography

In chromatography the detector output is connected to a computer-based data acquisition and analysis system which results in an output of compound retention time (the time the compound appears in the chromatogram) and its peak height and peak area. Within the working range of the system a linear response of concentration versus signal is produced (a calibration plot) when increasing amounts of the organic compound are introduced. This calibration plot is then used to determine the concentration of unknown compounds.

SAQ 1.6

The data in Table 1.3 have been obtained by a chromatography experiment for the determination of chlorobenzene. Plot the data on a calibration graph using 'Excel'.

Table 1.3 An example of how to record quantitative data from a chromatography experiment

Concentration (mg/l)	Signal
0	23
2.5	2345
5	4543
7.5	6324
10	8456
20	17 843

SAQ 1.7

If the signal response for an unknown sample, containing chlorobenzene, was 1234 what is the concentration of chlorobenzene in the sample?

Often in GC it is necessary to add an internal standard (a substance not present in the unknown sample, but with a similar chemical structure that elutes at a different time to other compounds present) to compensate for variation in injection volumes when introducing sample volumes in GC.

1.5.3 Sample Pre-Concentration Methods

Sometimes when the concentration of the organic compound in the sample extract is expected to be very low it is necessary to reduce the volume of organic solvent present in order to allow a pre-concentration effect. The most common approaches for solvent evaporation are gas blow-down, Kuderna–Danish evaporative concentration, the automated **evaporative concentration system** (EVACS) or rotary evaporation. In all cases, the evaporation method is slow with the risk of contamination from the solvent, glassware and blow-down gas high. Sometimes the sample extract is taken to dryness and reconstituted in a very small volume (e.g. 100 µl) of organic solvent. Often vortex shaking is used to help re-solubilize the extract residue with the organic solvent. This approach is used when the lowest concentration levels are to be determined.

Gas blow-down The typical procedure for gas blow-down is carried out by blowing a stream of nitrogen over the surface of the solution, while gently warming the solution. A schematic diagram of the apparatus is shown in Figure 1.18. The sample is placed in an appropriately sized tube with a conical base. A gentle stream of nitrogen is directed towards the side of the tube so that it flows over the surface of the organic solvent extract which at the same time is being gently heated via a purposely designed aluminium heating block or water bath.

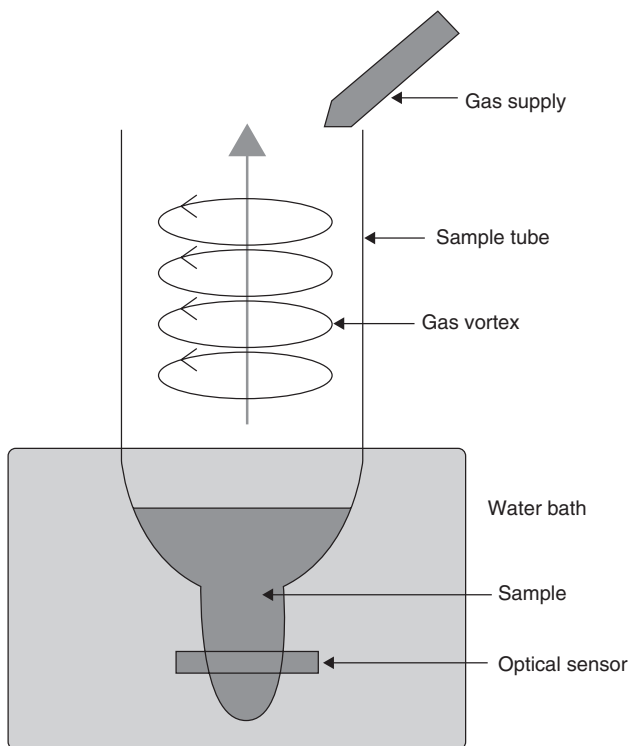


Figure 1.18 Schematic diagram of a typical gas 'blow-down' system (TubovapTM) used for the pre-concentration of compounds in organic solvents. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

SAQ 1.8

How might you speed up the evaporation process?

1.5.3.1 Kuderna–Danish Evaporative Concentration

The Kuderna–Danish evaporative condenser [1] was developed in the laboratories of Julius Hyman and Company, Denver, Colorado, USA [2]. It consists of an evaporation flask (500 ml) connected at one end to a Snyder column and the other end to a concentrator tube (10 ml) (Figure 1.19). The sample containing organic solvent (200–300 ml) is placed in the apparatus, together with one or two boiling chips, and heated with a water bath. The temperature of the water bath should be maintained at 15–20°C above the boiling point of the organic solvent. The

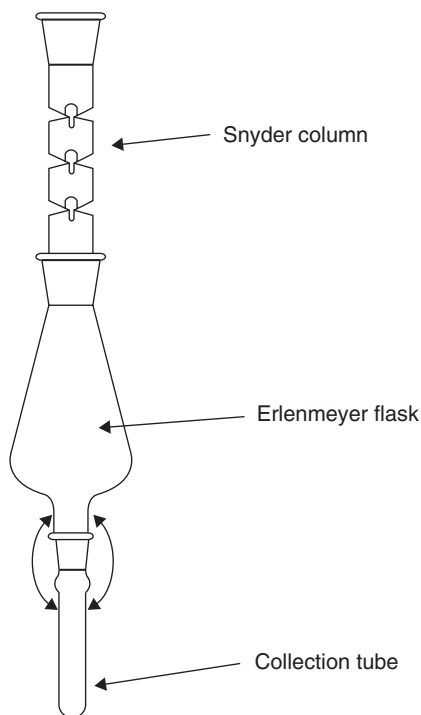


Figure 1.19 Schematic diagram of the Kuderna–Danish evaporative concentration condenser system. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

positioning of the apparatus should allow partial immersion of the concentrator tube in the water bath but also allow the entire lower part of the evaporation flask to be bathed with hot vapour (steam). Solvent vapours then rise and condense within the Snyder column. Each stage of the Snyder column consists of a narrow opening covered by a loose-fitting glass insert. Sufficient pressure needs to be generated by the solvent vapours to force their way through the Snyder column. Initially, a large amount of condensation of these vapours returns to the bottom of the Kuderna–Danish apparatus. In addition to continually washing the organics from the sides of the evaporation flask, the returning condensate also contacts the rising vapours and assists in the process of recondensing volatile organics. This process of solvent distillation concentrates the sample to approximately 1–3 ml in 10–20 min. Escaping solvent vapours are recovered using a condenser and collection device. The major disadvantage of this method is that violent solvent eruptions can occur in the apparatus leading to sample losses. Micro-Snyder column systems can be used to reduce the solvent volume still further.

1.5.3.2 Automated Evaporative Concentration System

Solvent from a pressure-equalized reservoir (500 ml capacity) is introduced, under controlled flow, into a concentration chamber (Figure 1.20) [3]. Glass indentations regulate the boiling of solvent so that bumping does not occur. This reservoir is surrounded by a heater. The solvent reservoir inlet is situated under the level of the heater just above the final concentration chamber. The final concentration chamber is calibrated to 1.0 and 0.5 ml volumes. A distillation column is

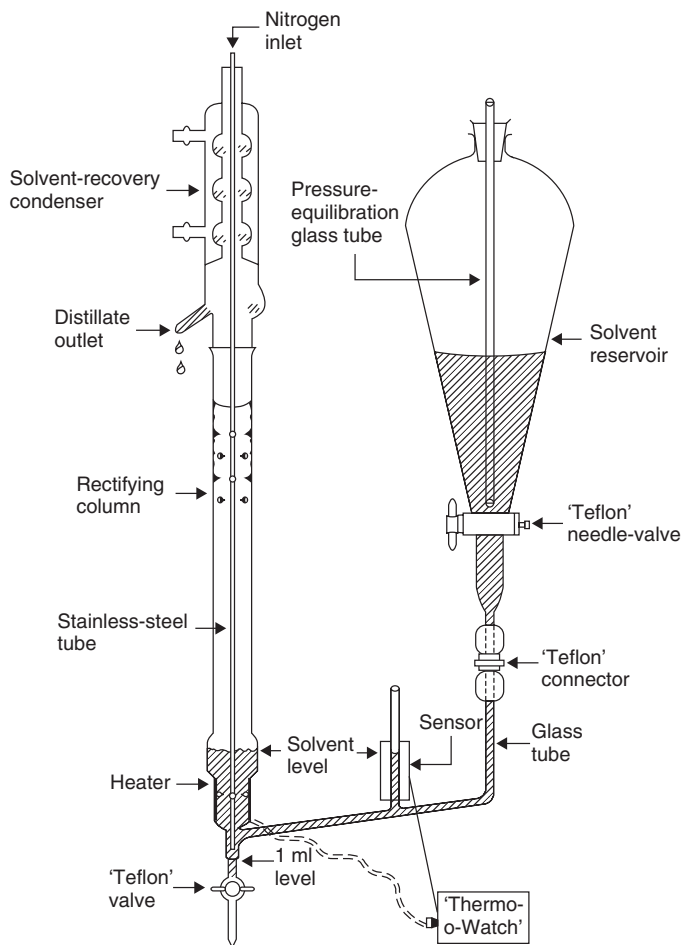


Figure 1.20 Schematic diagram of the automatic evaporative concentration system: ■, solvent; □, vapour. Reprinted with permission from Ibrahim, E. A., Suffet, I. H. and Sakla, A. B., *Anal. Chem.*, **59**, 2091–2098 (1987). Copyright (1987) American Chemical Society.

connected to the concentration chamber. Located near the top of the column are four rows of glass indentations which serve to increase the surface area. Attached to the top of the column is a solvent recovery condenser with an outlet to collect and hence recover the solvent.

To start a sample, the apparatus is operated with 50 ml of high-purity solvent under steady uniform conditions at total reflux for 30 min to bring the system to equilibrium. Then the sample is introduced into the large reservoir either as a single volume or over several time intervals. (NOTE: A boiling point difference of approximately 50°C is required between solvent and analyte for the highest recoveries.) The temperature is maintained to allow controlled evaporation. For semi-volatile analytes this is typically at 5°C higher than the boiling point of the solvent. The distillate is withdrawn while keeping the reflux ratio as high as possible. During operation, a sensor monitors the level of liquid, allowing heating to be switched off or on automatically (when liquid is present the heat is on and vice versa). After evaporation of the sample below the sensor level, the heating is switched off. After 10 min the nitrogen flow is started to give a final concentration from 10 ml to 1 ml (or less). Mild heat can be applied according to the sensitivity of solvent and analyte to undergo thermal decomposition. When the liquid level drops below the tube, 'stripping' nearly stops. The tube is sealed at the bottom, so that the nitrogen is dispersed above the sample and the reduction of the volume becomes extremely slow. This prevents the sample from going to dryness even if left for hours. The sample is drained and the column is rinsed with two 0.5 ml aliquots of solvent. Further concentration can take place, if required.

1.5.3.3 Rotary Evaporation

Organic solvent is removed, under reduced pressure, by mechanically rotating a flask containing the sample in a controlled temperature water bath (Figure 1.21).

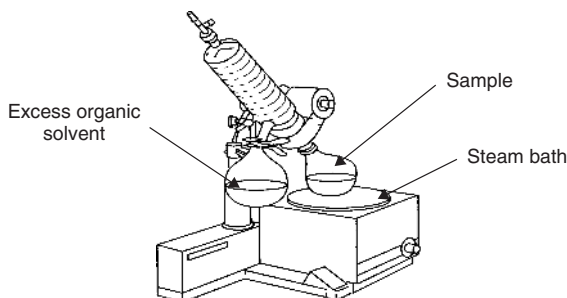


Figure 1.21 A typical rotary evaporation system used for the pre-concentration of compounds in organic solvents. From Dean, J. R., *Methods for Environmental Trace Analysis*, AnTS Series. Copyright 2003. © John Wiley & Sons, Limited. Reproduced with permission.

The waste solvent is condensed and collected for disposal. Problems can occur due to loss of volatile compounds, adsorption onto glassware, entrainment of compounds in the solvent vapour and the uncontrollable evaporation process. The sample residue is re-dissolved in the minimal quantity of solvent, assisted by vortex mixing.

1.6 Quality Assurance Aspects

Quality assurance is about designing laboratory protocols to obtain the correct result for the organic compounds being analysed. In analytical sciences, as we have seen in this chapter, the analytical process has several steps that include: sample collection, pre-treatment and storage which are then followed by extraction and chromatographic analysis.

While it is likely that the final errors in the data are greater from the sampling considerations rather than the laboratory-based aspects it is good practice to assess the laboratory quality assurance protocols. The most important terms in assessing these protocols are accuracy and precision. Accuracy is defined as the closeness of a determined value to its 'true' value, while precision is defined as the closeness of the determined values to each other. It is possible for the extraction and analysis of organic compounds from sample matrices to have combinations of accurate/inaccurate data alongside precise/imprecise data. The skill of the analytical scientist is to assess these variations such that accurate and precise data are obtained on laboratory samples.

The core components of a laboratory-based quality assurance scheme are to:

- select and validate appropriate methods of sample extraction;
- select and validate appropriate methods of chromatographic analysis;
- maintain and upgrade chromatographic instruments;
- ensure good recordkeeping of methods and data;
- ensure the quality of the data produced;
- maintain a high quality of laboratory performance.

An important aspect of establishing a QA scheme is the inclusion within the extraction and chromatographic analysis stages of the use of appropriate certified reference materials. A certified reference material (CRM) is a substance for which one or more analytes have certified values, produced by a technically valid procedure, accompanied with a traceable certificate and issued by a certifying body.

The major certifying bodies for CRMs are the National Institute for Standards and Technology (NIST) based in Washington DC, USA, the Community Bureau

of Reference (known as BCR), Brussels, Belgium and the Laboratory of the Government Chemist (LGC), Teddington, U.K.

Other important procedures to build into any laboratory quality assurance protocols would include:

- Calibration with standards. A minimum number of standards should be used to generate the analytical calibration plot, e.g. 6 or 7. Daily verification of the working calibration plot should also be carried out using one or more standards within the linear working range while the selected standard should be 'sandwiched' between chromatographic runs of unknown sample extracts (typically every 10 unknown sample extracts).
- Analysis of reagent blanks. Analyse reagents whenever the batch is changed or a new reagent introduced. Introduce a minimum number of reagent blanks (typically 5% of the sample load) into the analytical protocol. This allows reagent purity to be assessed and, if necessary, controlled and also acts to assess the overall procedural blank.
- Analysis of precision. Repeat extractions from sub-samples, typically a minimum of three repeats required (ideally 7 repeat extractions of sub-samples should be used).
- Spiking studies on blanks and samples to establish recovery levels.
- Maintenance of control charts for standards and reagent blanks. The purpose is to assess the longer-term performance of the laboratory, instrument, operator or procedure, based on a statistical approach.

1.7 Health and Safety Considerations

All laboratory work must be carried out with due regard to Government legislation and employer guidelines. In the UK while the Health and Safety at Work Act (1974) provides the main framework for health and safety, it is the Control of Substances Hazardous to Health (COSHH) regulations of 1994 and 1996 that impose strict legal requirements for risk assessment of chemicals. Within the COSHH regulations the terms 'hazard' and 'risk' have very specific meanings; a hazardous substance is one that has the ability to cause harm whereas risk is about the likelihood that the substance may cause harm and is directly linked to the amount of chemical being used. For example, a large volume of flammable organic solvent has a greater risk than a small quantity of the same solvent.

All laboratories must operate a safety scheme. Your responsibility is to ensure that you comply with its operation to maintain safe working conditions for yourself and other people in the laboratory. A set of basic generic laboratory rules are described below:

- (1) Always wear appropriate protective clothing, a clean laboratory coat, safety glasses/goggles and appropriate footwear. It may be necessary to wear protective gloves when handling certain chemicals.
- (2) You must never eat or drink in the laboratory.
- (3) You must never work alone in a laboratory.
- (4) You must ensure that you are familiar with the fire regulations in your laboratory and building.
- (5) You should be aware of accident/emergency procedures in your laboratory and building.
- (6) Always use appropriate devices for transferring liquid, e.g. a pipette, syringe, etc.
- (7) Always use a fume cupboard for work with hazardous (including volatile, flammable) chemicals.
- (8) Always clear up any spillages as they occur.
- (9) It is advisable to plan your work in advance; work in a logical and methodical manner.

Summary

This chapter initially summarizes the important considerations necessary in planning the whole analytical protocol, including pre-sampling, sampling, extraction and analysis for organic compounds from solid, aqueous and air samples. The main practical aspects of undertaking gas chromatography and high performance liquid chromatography are described as well as sample extract pre-concentration approaches that may be necessary for pre-analysis. Finally, a general description of quality assurance in an analytical laboratory is described, followed by the important health and safety considerations.

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