1

The Analytical Approach

LEARNING OBJECTIVES

After completing this chapter, students should be able to:

- Contextualize an environmental problem.
- Comprehend the implications of persistent organic pollutants in the environment.
- Undertake a COSHH assessment.
- Develop a strategy for effective practical work.

1.1 Introduction

Environmental analysis does not start in the laboratory but outside (e.g. in a field, river, lake, urban environment or industrial atmosphere). Therefore, environmental analysis requires more than just knowledge of the analytical technique to be used (e.g. chromatography). It requires a consideration of the vast array of extraction techniques that are available pre-analysis. The focus of these pre-analysis extraction techniques (covered in Chapters 3–15) is to recover the organic compounds of interest from a matrix. The matrices can be diverse in their form but generically can be considered as solid, liquid or gas. The purpose of the extraction techniques is to, therefore, recover the organic compounds from the matrices and allow pre-concentration/matrix clean-up to take place.

In addition, it is important to place the extraction and subsequent analysis in its context. Important aspects, therefore, are as follows:

- Consideration of the appropriate health and safety aspects in the laboratory (and the external environment).
- What do you know already about the site to be investigated?
- What are the expectations about the results?
- What type of sampling regime is planned?
- How might the collected samples be stored and preserved?
- What type of sample preparation methodologies are appropriate to the sample?
- How might the analysis be done?
- What are the quality control procedures to be used (including calibration strategies and the use of certified reference materials)?

Extraction Techniques for Environmental Analysis, First Edition. John R. Dean.

- How will the knowledge of the results and their interpretation, contextualization and subsequent action be considered?

While all of these are covered to some extent in this book, the reader should also consult other resources, e.g. books, scientific journals and the web.

1.2 Environmental Organic Compounds of Concern

The range of potential organic compounds to be identified and quantified is vast. Their sources are equally diverse and varied. The Stockholm Convention is a global initiative, established in 2001 from former international collaborators, by the United Nations Environmental Programme (UNEP) and requires its signatories to take measures to eliminate or reduce the release of persistent organic pollutants (POPs) into the environment to protect human health where exposure is often via the food chain. UNEP identified 12 (initial) POPs that cause adverse effects on humans and the ecosystem (Table 1.1). There chemical structures, molecular formulae and molecular weights are shown in Figure 1.1.

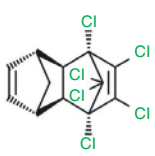
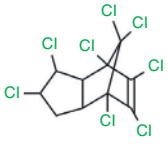
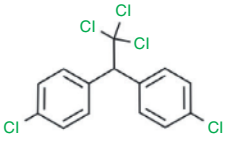
Aldrin: A pesticide applied to soils to kill termites, grasshoppers, corn rootworm and other insect pests; it can also kill birds, fish and humans. In humans, the fatal dose for an adult male is estimated to be about 5 g. Human exposure is mostly through dairy products and animal meats.

Chlordane: It is used to control termites and as a broad-spectrum insecticide on a range of agricultural crops. It can remain in the soil for an extended time and has a reported half-life of one year. Chlordane may affect the human immune system and is therefore classified as a possible human carcinogen.

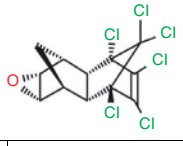
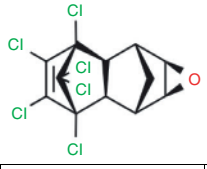
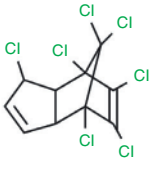
Table 1.1 Stockholm Convention: The 12 initial persistent organic pollutants (POPs).

Category	Chemical[a]
Pesticides	Aldrin
	Chlordane
	DDT
	Dieldrin
	Endrin
	Heptachlor
	Hexachlorobenzene
	Mirex
	Toxaphene
Industrial	Hexachlorobenzene
	Polychlorinated biphenyls (PCBs)
Byproducts	Hexachlorobenzene
	Polychlorinated dibenzo-p-dioxins (PCDD)
	Polychlorinated dibenzofurans (PCDF)

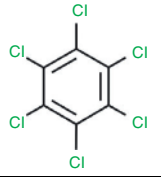
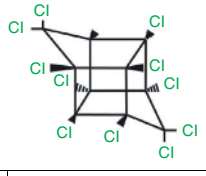
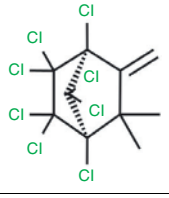
[a] Note: Hexachlorobenzene appears under all three categories.

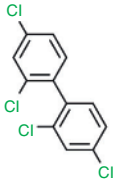
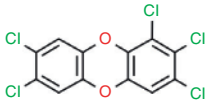
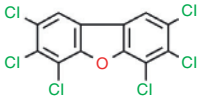
Name	Aldrin	Chlordane	Dichlorodiphenyl trichloroethane (DDT)
Molecular formula	$C_{12}H_8Cl_6$	$C_{10}H_6Cl_8$	$C_{14}H_9Cl_5$
Molecular weight	364.91	409.78	354.49

Name	Dieldrin	Endrin	Heptachlor
Molecular formula	$C_{12}H_8Cl_6O$	$C_{12}H_8Cl_6O$	$C_{10}H_5Cl_7$
Molecular weight	380.91	380.91	373.32

Name	Hexachlorobenzene	Mirex	Toxaphene
Molecular formula	C_6Cl_6	$C_{10}Cl_{12}$	$C_{10}H_5Cl_7$
Molecular weight	284.78	545.54	373.32

Name	Example, 2, 2′, 4, 4′ - PCB	Example, 1, 2, 3, 7, 8- Pentachlorodibenzo- p-dioxin	Example, 2, 3, 4, 6, 7, 8- Hexachlorodibenzofuran
Molecular formula	$C_{12}H_6Cl_4$	$C_{12}H_3Cl_5O_2$	$C_{12}H_2Cl_6O$
Molecular weight	291.99	356.42	374.86

Figure 1.1 Chemical structures of the 12 initial persistent organic pollutants.

Dichlorodiphenyltrichloroethane (DDT): It was widely used during World War II to protect soldiers and civilians from malaria, typhus and other diseases spread by insects. Subsequently, it has continued to be used to control disease in crops (e.g. cotton) and insects (e.g. mosquitoes). DDT continues to be applied against mosquitoes in developing countries to control malaria. DDT has long-term soil persistence (10–15 years) after application. It has been used extensively, and so its residues can be found everywhere.

Dieldrin: It has been used mainly to control termites and textile pests, as well as to control insect-borne diseases and insects living in agricultural soils. It has a half-life in soil of approximately five years. Aldrin (see earlier) can rapidly convert to dieldrin, so higher concentrations of dieldrin than expected can be found in the environment. Dieldrin is highly toxic to fish and other aquatic animals (e.g. frogs). Residues of dieldrin can be found in air, water, soil, fish, birds and mammals, including humans.

Endrin: It is sprayed on the leaves of crops (e.g. cotton and grains) to protect from insects. It can also be used to control rodents (e.g. mice and voles). It has a long half-life (persisting up to 12 years in soils). In addition, endrin is highly toxic to fish.

Heptachlor: It is used to kill soil insects and termites, as well as cotton insects, grasshoppers, other crop pests and malaria-carrying mosquitoes. Heptachlor is classified as a possible human carcinogen.

Hexachlorobenzene (HCB): It is used to kill fungi that affect food crops (e.g. to control wheat bunt). It is also a byproduct from the manufacture of industrial chemicals and can occur as an impurity in several pesticide formulations. In high doses, HCB is lethal to some animals and, at lower levels, adversely affects their reproductive success.

Mirex: It is used to control ants and termites. In addition, it has also been used as a fire retardant in plastics, rubber and electrical goods. Direct exposure to Mirex does not appear to cause injury to humans; however, the results of animal studies have led it to be classified as a possible human carcinogen. It has a half-life in soil of up to 10 years.

Toxaphene: It is used to protect cotton, cereal grains, fruits, nuts and vegetables from insects. It has also been used to control ticks and mites in livestock. It has a half-life, in soil, of up to 12 years. It has been listed as a possible human carcinogen due to its effects on laboratory animals.

Polychlorinated biphenyls (PCBs): These compounds (209 different types of which 13 exhibit a dioxin-like toxicity) are used in industry as heat exchange fluids, in electric transformers and capacitors, and as additives in paint, carbonless copy paper and plastics. Their persistence in the environment corresponds to the degree of chlorination, and half-lives can vary from 10 days to 1.5 years.

Polychlorinated dibenzo-p-dioxins (PCDDs): These compounds (75 different types of which 7 are of concern) are produced unintentionally due to incomplete combustion, as well as during the manufacture of pesticides and other chlorinated substances. They are emitted mostly from the burning of hospital waste, municipal waste and hazardous waste, as well as automobile emissions, peat, coal and wood. They can have a half-life in soil of up to 10–12 years. They are associated with a number of adverse effects in humans, including immune and enzyme disorders and chloracne, and they are classified as possible human carcinogens.

Polychlorinated dibenzofurans (PCDFs): These compounds (135 different types) are produced unintentionally from many of the same processes that produce dioxins, as well as during the production of PCBs. They have been detected in emissions from waste

incinerators and automobiles. Furans are structurally like dioxins and share many of their toxic effects. They are persistent in the environment for long periods and are classified as possible human carcinogens.

In addition, in subsequent revisions of the original Stockholm Convention, another 16 POPs have been added to the listings (Table 1.2).

α-Hexachlorocyclohexane and β-hexachlorocyclohexane: The technical mixture of hexachlorocyclohexane (HCH) contains mainly five forms of isomers, namely α-, β-, γ-, δ- and ε-HCH. Lindane is the common name for the γ isomer of HCH. The α- and β-HCH are highly persistent in water in colder regions and may bioaccumulate and biomagnify in biota and arctic food webs. They are subject to long-range transport, are classified as potentially carcinogenic to humans and adversely affect wildlife and human health in contaminated regions. The use of α- and β-HCH as insecticides has been phased out but are produced as byproducts of lindane. For each ton of lindane produced, around 6–10 tons of α- and β-HCH are also produced. This has led to large stockpiles, which can cause site contamination.

Chlordecone: It is chemically related to Mirex (Table 1.1). It is highly persistent in the environment, has a high potential for bioaccumulation and biomagnification and based on physico-chemical properties and modelling data, chlordecone can be transported for long distances. It is classified as a possible human carcinogen and is very toxic to aquatic organisms. Chlordecone is a synthetic chlorinated organic compound, which was mainly used as an agricultural pesticide. While it was commercially introduced in 1958, it has now been banned for sale and use in many countries.

Table 1.2 Stockholm convention: the additional 16 persistent organic pollutants (POPs).

The 16 new additional POPs
α-Hexachlorocyclohexane
β-Hexachlorocyclohexane
Chlordecone
Hexabromobiphenyl
Hexabromocyclododecane
Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether)
Hexachlorobutadiene
Lindane
Pentachlorobenzene
Pentachlorophenol and its salts and esters
Perfluorooctane sulphonic acid (PFOS), its salts and perfluorooctane sulphonyl fluoride (PFOSF)
Polychlorinated naphthalenes
Technical endosulphan and its related isomers
Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether)
Decabromodiphenyl ether (commercial mixture, cDecaBDE)
Short-chain chlorinated paraffins (SCCPs)

Hexabromobiphenyl: It belongs to the group of polybrominated biphenyls (i.e. brominated hydrocarbons formed by substituting hydrogen with bromine in biphenyl). It is highly persistent in the environment, highly bioaccumulative and has a strong potential for long-range environmental transport. It is classified as a possible human carcinogen and has other chronic toxic effects. It has historically been used as a flame retardant. It is no longer produced or used in most countries due to restrictions under national and international regulations.

Hexabromocyclododecane (HBCD): It has a strong potential to bioaccumulate and biomagnify. It is persistent in the environment and has a potential for long range environmental transport. It is very toxic to aquatic organisms. It is particularly harmful to humans as a neuroendocrine carcinogen. It was used as a flame-retardant additive on polystyrene materials (in the 1980s) and as part of safety regulation for articles, vehicles and buildings.

Hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether): These are the main components of commercial octabromodiphenyl ether. The commercial mixture of octaBDE is highly persistent, has a high potential for bioaccumulation and food-web biomagnification, as well as for long-range transport. The only degradation pathway is through debromination and producing other bromodiphenyl ethers.

Hexachlorobutadiene: It is a halogenated aliphatic compound, mainly created as a byproduct in the manufacture of chlorinated aliphatic compounds. It is persistent, bioaccumulative and very toxic to aquatic organisms and birds. It can be long-range transported leading to significant adverse human health and environmental effects, and it is classified as a possible human carcinogen. It is mainly used as a solvent for other chlorine-containing compounds. It occurs as a byproduct during the chlorinolysis of butane derivatives in the large-scale production of both carbon tetrachloride and tetrachloroethene.

Lindane: It is persistent, bioaccumulates easily in the food chain and bioconcentrates rapidly. There is evidence for long-range transport and toxic effects (immunotoxic, reproductive and developmental effects) in laboratory animals and aquatic organisms. It has been used as a broad-spectrum insecticide for seed and soil treatment, foliar applications, tree and wood treatment and against ectoparasites in both veterinary and human applications. Its production has decreased.

Its production has decreased rapidly in the last few years due to the introduction of regulations in several countries.

Pentachlorobenzene (PeCB): It belongs to a group of chlorobenzenes that are characterized by a benzene ring in which the hydrogen atoms are substituted by one or more chlorines. It is persistent in the environment, highly bioaccumulative and has a potential for long-range environmental transport. It is moderately toxic to humans and very toxic to aquatic organisms. Previously, it was used in PCB products, in dyestuff carriers, as a fungicide and a flame retardant. It is produced unintentionally during combustion, thermal and industrial processes, and can occur in the form of impurities in solvents or pesticides.

Pentachlorophenol (PCP) and its salts and esters: It can be found in two forms: PCP itself or as its sodium salt (which dissolves easily in water). It is detected in the blood, urine, seminal fluid, breast milk and adipose tissue of humans. It is likely, because of its long-range environmental transport, to lead to significant adverse human health and/or environmental effects. It has been used as a herbicide, insecticide, fungicide, algaecide, disinfectant and as an ingredient in antifouling paint. Its use has significantly declined due to the high toxicity of PCP and its slow biodegradation; its main contaminants include other polychlorinated phenols, polychlorinated dibenzo-pdioxins and polychlorinated dibenzo furans.

Perfluorooctane sulphonic acid (PFOS), its salts and perfluorooctane sulphonyl fluoride (PFOSF): PFOS is a fully fluorinated anion, which is commonly used as a salt or incorporated into larger polymers. It is extremely persistent and has substantial bioaccumulations and biomagnifying properties; however, it does not partition into fatty tissues but instead binds to proteins in the blood and the liver. It has a capacity to undergo long-range transport. PFOS is both intentionally produced (for use in electric and electronic parts, firefighting foam, photo imaging, hydraulic fluids and textiles), and an unintended degradation product of related anthropogenic chemicals.

Polychlorinated naphthalenes (PCNs): They are mixtures (up to 75 chlorinated naphthalene congeners plus byproducts) often described by the total fraction of chlorine. While some PCNs can be broken down by sunlight and, at slow rates, by certain microorganisms, many PCNs persist in the environment. Bioaccumulation has been confirmed for tetra- to heptaCNs. Chronic exposure can lead to increased risk of liver disease. PCNs make effective insulating coatings for electrical wires; they are also used as wood preservatives, as rubber and plastic additives, for capacitor dielectrics and in lubricants. Intentional production of PCN is assumed to have ended; however, they can be formed during high-temperature industrial processes in the presence of chlorine.

Technical endosulphan and its related isomers: It occurs as two isomers: α- and β-endosulphan. They are both biologically active. Technical endosulphan (CAS No: 115-29-7) is a mixture of the two isomers along with small amounts of impurities. It is persistent in the atmosphere, sediments and water. Endosulphan bioaccumulates and has the potential for long-range transport. It is toxic to humans and has been shown to have adverse effects on a wide range of aquatic and terrestrial organisms. The use of endosulphan is banned or will be phased out in 60 countries that, together, account for 45% of current global use. It has been used as an insecticide to control crop pests, tsetse flies and ectoparasites of cattle and as a wood preservative.

Tetrabromodiphenyl ether and pentabromodiphenyl ether (commercial pentabromodiphenyl ether): Tetrabromodiphenyl ether and pentabromodiphenyl ether are the main components of commercial pentabromodiphenyl ether. They belong to a group of chemicals known as 'polybromodiphenyl ethers' (PBDEs). The commercial mixture of penta-BDE is highly persistent in the environment, bioaccumulative and has a potential for long-range environmental transport (it has been detected in humans throughout all regions). There is evidence of its toxic effects in wildlife, including mammals. Polybromodiphenyl ethers including tetra-, penta-, hexa- and hepta-BDEs inhibit or

suppress combustion in organic materials and therefore are used as additive flame retardants. The production of tetra- and penta-BDEs has ceased in certain regions of the world, while no production of hexa- and hepta-BDEs is reported.

Decabromodiphenyl ether (commercial mixture, cDecaBDE): The commercial mixture consists primarily of the fully brominated decaBDE congener in a concentration range of 77.4–98%, and smaller amounts of the congeners of nona-BDE (0.3–21.8%) and octa-BDE (0–0.04%). The deca-BDE is highly persistent, has a high potential for bioaccumulation and food-web biomagnification, as well as for long-range transport. Adverse effects are reported for soil organisms, birds, fish, frog, rat, mice and humans. Deca-BDE is used as an additive flame retardant and has a variety of applications including plastics/polymers/composites, textiles, adhesives, sealants, coatings and inks. Deca-BDE-containing plastics are used in housings of computers and TVs, wires and cables, pipes and carpets. Commercially available deca-BDE consumption peaked in the early 2000s, but c-deca-BDE is still extensively used worldwide.

Short-chain chlorinated paraffins (SCCPs): Chlorinated paraffins (CPs) are complex mixtures of certain organic compounds. Their degree of chlorination can vary between 30 and 70 wt%. They are sufficiently persistent in air for long-range transport to occur and appear to be hydrolytically stable. Many SCCPs can accumulate in biota. They are likely, because of their long-range environmental transport, to lead to significant adverse environmental and human health effects. They are used as a plasticizer in rubber, paints, adhesives, flame retardants for plastics, as well as an extreme pressure lubricant in metal working fluids. They are produced by chlorination of straight-chained paraffin fractions. The carbon chain length of commercial chlorinated paraffins is usually between 10 and 30 carbon atoms; however, the short-chained chlorinated paraffins vary between C10 and C13. The production of SCCPs has decreased globally as jurisdictions have established control measures.

In addition, a whole range of other organic pollutants are investigated in the environment including some classes of compounds, e.g. volatile organic compounds (e.g. BTEX: benzene, toluene, ethylbenzene and xylenes); solvents (e.g. carbon tetrachloride, chloroform) and polycyclic aromatic hydrocarbons (e.g. naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, indeno(1,2,3-c,d)pyrene and dibenzo(a,h)anthracene). In addition, a whole range of emerging pollutants (EPs) are now of concern (Table 1.3). The term emerging is used as a descriptor not because these compounds are new, but because their environmental impact is raising cause for concern. While EPs are an increasing list of organic compounds with diverse sources and include antibiotics, analgesics, anti-inflammatory drugs, psychiatric drugs, steroids and hormones, contraceptives, fragrances, sunscreen agents, insect repellents, microbeads, microplastics, antiseptics, pesticides, herbicides, surfactants and surfactant metabolites, flame retardants, industrial additives and chemicals, plasticizers and gasoline additives, among others, and often enter the environment via waste water discharge into rivers, lakes etc. Within this identifier of EPs, a sub-group of organic pollutants has been identified and are often named pharmaceuticals and personal care products (PPCPs). Often the exposure to these EPs is at low concentration, so concern is focused on chronic low-level exposure and mixtures of these compounds with additive or unexpected effects.

Table 1.3 Emerging pollutants in the environment.

Emerging pollutants	Compounds
Perfluorinated compounds (PFCs)	Perfluorooctanesulphonate (PFOS), perfluorooctanoic acid (PFOA) (see also Table 1.2), and their salts are the most essential representative PFCs and are widely used in fire-fighting foams, lubricants, metal spray plating and detergent products, inks, varnishes, coating formulations (for walls, furniture, carpeting and food packaging), waxes and water and oil repellents for leather, paper and textiles
Water disinfection byproducts	Disinfection chemicals used in swimming pool and drinking water purification. Disinfection byproducts (DBPs), particularly chlorinated DBPs (CDBPs) in purified water, and nearly all humans are exposed to these chemicals in developed regions through swimming pools and drinking water. More than six hundred DBPs have been discovered, including iodinated trihalomethanes (THMs), aldehydes, ketones, halomethanes, hydroxy acids, carboxylic acids, alcohols, keto acids, esters and even nitrosamines
Gasoline (petrol) additives	Gasoline encompasses more than five hundred components, such as the known or suspected carcinogenic substances benzene, 1,3-butadiene and methyl tert-butyl ether (MTBE) (an unleaded petrol additive)
Manufactured nanomaterials	Manufactured nanomaterials (with a particle size of approximately 1–100 nm) include amorphous silicon dioxide (SiO_2), carbon nanotubes (CNTs) and titanium dioxide (TiO_2), which due to their large surface area can adsorb organic compounds, e.g. PAHs
Human and veterinary pharmaceuticals	Pharmaceuticals are emerging contaminants in the environment because of their increasing applications in humans and animals. Approximately three thousand different chemicals involved in human medicine, including lipid regulators, anti-inflammatory drugs, analgesics, contraceptives, neuroactive medicine, antibiotics and beta-blockers, exist. The main pathway through which pharmaceuticals enter the surface water is human intake, followed by subsequent excretion in municipal wastewater, hospitals, pharmaceutical waste and landfills. Veterinary Antibiotics (VAs) are being increasingly used in many regions to protect the health of animals and treat diseases to improve the feed efficiency of livestock, poultry, pets, aquatic animals, silkworms, bees etc. The VAs are mainly divided into several pharmacological types: antimicrobial, anthelmintic, steroidal and nonsteroidal, anti-inflammatory, antiparasitic, astringent, estrus synchronization, nutritional supplement and as growth promoters
UV-filters	Sunscreens/ultraviolet filters (UV-filters) are mainly used in personal care products, such as lipsticks, perfumes, hairsprays, hair dye and moisturizers, skin care products, shampoos and makeup, as well as in non-cosmetic products, including furniture, plastics, carpets and washing powder. Organic sunscreens absorb photons of UV and include 3-(4-methylbenzylidene) camphor (4-MBC), benzophenone-3 (BP-3), 2-ethylhexyl 4-methoxycinnamate (OMC), 2-ethylhexyl 4-dimethylaminobenzoate (OD-PABA), 3-benzylidene camphor (3- BC), homosalate (HMS) and 4-aminobenzoic acid (PABA). These compound types enter the aquatic environment by bathing, washing clothes and swimming
Personal care products	Personal care products encompass many different substances that are in regular use, such as fragrances and cosmetic ingredients. They are often released in raw sewage water, then often inefficiently treated in sewage treatment plants and finally discharged in rivers, streams or lakes. They can also be directly released into water during swimming and bathing in lakes or rivers

1.3 Essentials of Practical Work

It is important to develop a good understanding of the underlying principles of good laboratory practice (GLP) and apply them from the start to the end of the process. The effective recording of all relevant data and information at the time of obtaining the scientific information is essential. Therefore, a systematic and appropriate method of recording all information accurately is essential.

All experimental observations, and data, should be recorded in either an A4 notebook or electronically (and backed up on an external hard drive or USB stick). Example templates are shown for sample collection (Table 1.4), sample treatment (Table 1.5) and sample preparation (Table 1.6). Some key reminders include the recording of the sampling geographical location, the date the sample was collected, the total weight or volume of each individual sample, sample storage conditions, sample treatment and sample preparation. In addition, the quality assurance used to ensure that the data is fit for purpose, and the actual recording of the results and their initial interpretation. It is important to remember to record all information at the point of collection; it is easy to forget it later if not written down.

Important factors to remember when recording information:

- Record data correctly in tabular format and legibly if by hand, even you may not be able to read your own writing later.
- Include the date and title of individual experiments and/or areas of investigation.

Table 1.4 Example template for use electronically or in notebook form: sample collection.

Template: Sample collection
• Date of sample collection: day / month / year • Location of sampling site: Address: .. .. Grid reference: .. .. • Whom was permission obtained to obtain samples: Name: . .. . Tel. no. / mobile: . .. . Email: . .. . • Weather (and source of information) when samples collected: e.g. dry, sunny, overcast. Temperature, wind direction etc. . .. . Temperature: . .. . °C Wind Direction: . .. . Precipitation: . .. . mm Source of information: . .. . • Method of obtaining samples: . .. . • Number of samples obtained: . .. . Unique sample code added to each container Yes / No Was the date added Yes / No

Table 1.5 Example template for use electronically or in notebook form: sample treatment.

Template: Sample Treatment (e.g. solids)

- Grinding and sieving

Grinder used (model/type) . . ..
Particle size (sieve mesh size) . . ..

- Mixing of the sample

Manual shaking yes / no
Mechanical shaking yes / no . . .. rpm
Other (specify) . . ..

- Sample storage

Fridge yes / no Temperature . . .. °C
Frieezer yes / no Temperature . . .. °C
Other (specify) . . ..

- Other comments

pH . . ..

Table 1.6 Example template for use electronically or in notebook form: sample preparation.

- Sample weight(s)

(record to 4 decimal places)
sample 1 . . .. .g sample 2. . .. . .g

- Soxhlet extraction method

Drying agent added and weight (specify) . . ..
Type of solvent(s) used . . ..
Volume of solvent(s) used. . .. . .ml
Any other details. . .. .

- Other method of sample extraction e.g. SFE, PLE, MAE, and operating conditions (specify)

. . ..
. . ..
. . ..

- Sample clean-up yes / no

Specify . . ..

. . ..

- Pre-concentration of the sample yes / no

Method of solvent reduction . . ..
Final volume of extract. . .. .

(Continued)

Table 1.6 (Continued)

- Sample derivatization

Specify . .. .

. .. .

Reagent concentration . .. . .mol l^{-1}

Reagent volume used . .. . .ml

Heat required (specify) . .. .

- Sample dilution

Specify with appropriate units the dilution factor involved.

. . ..

. .. .

- Addition of an internal standard

Specify . .. .

Added before extraction yes / no

Added after extraction yes / no

- Sample and reagent blanks

Specify . .. .

. .. .

- Recovery

Specify . .. .

Added before extraction yes / no

Added after extraction yes / no

- Briefly outline the purpose of the investigation/experiment, i.e. what you hope to achieve by the end.
- Identify and record the hazards and risks associated with the chemicals/equipment being used. It is a requirement to complete a Control of Substances Hazardous to Health (COSHH) form for each chemical and a risk assessment (see Section 1.4).
- Refer to the method/procedure being used (undergraduate laboratory) or write a full description of the method/procedure and its origins (postgraduate research).
- Record your observations (and note your interpretation at this stage), e.g. accurate weights, volumes, how standards and calibration solutions were prepared and instrumentation settings (and the actual operating parameters).
- Record data with the correct units, e.g. mg, μg g^{-1}, mmol l^{-1} and to an appropriate number of significant figures, e.g. 26.3 mg and 0.48 μg g^{-1} (and not 26.3423 mg and 0.4837 μg g^{-1}).
- Interpret data in the form of tables, graphs (including calibration graphs) and spectra.
- Record initial conclusions.
- Identify any actions for future work.

1.4 Health and Safety

It is a legal requirement for institutions to provide a working environment that is both safe and without risk to health. In the United Kingdom, the Health and Safety at Work Act 1974 provides the legal framework for health and safety. The introduction of the COSHH regulations in 2002 imposed specific legal requirements for risk assessment when hazardous chemicals (or biological agents) are used. In the European Union (EU), the system for controlling chemicals is the Registration, Evaluation, Authorization and restriction of Chemicals (REACH). While in the United States, the Environmental Protection Agency (EPA) is responsible for chemical safety relating to human health and the environment. Adherence to health and safety requirements is often evidenced by the provision of training and information on safe working practices in the laboratory (and external environment). For the student, this is often done by attending an appropriate safety briefing, the reading (and subsequent signing) of a safety guide or booklet acknowledging an understanding of safety and their role in protecting themselves and other students, as well as receiving appropriate training in the use of scientific equipment.

In all cases, however, it is important to understand the definitions applied to hazard and risk.

- A hazardous substance is one that has the ability to cause harm.
- A risk is about the likelihood that the substance may cause harm.

Prior to undertaking any laboratory work, a risk assessment must be undertaken by an appropriately identified person (e.g. supervisor, academic or technical staff). The purpose of the risk assessment is to identify laboratory activities that could cause injury to people and then to provide control measures to ensure that the risk is reduced. Important considerations are:

- substance hazards;
- how the substance is to be used;
- how it can be controlled;
- who is exposed;
- how much exposure and its duration.

It is important to distinguish between the hazard of a substance and its risk from exposure. This can be done by doing a Risk Matrix Analysis (RMA). The RMA allows a prioritization of the likelihood and severity, to the individual, from the hazard identified. All manufacturers of hazardous chemicals are required to provide a Material Safety Data Sheet (MSDS) for the stated chemical. The MSDS will contain information about the chemical including:

- manufacturer;
- name of chemical;
- chemical components;
- hazards associated with the product (including a Hazard Statement and a Precautionary Statement);
- first aid measures;
- firefighting measures;
- handling and storage;
- accidental release procedures;

- exposure control and personal protection;
- physical and chemical properties;
- stability and reactivity;
- toxicological and ecological information;
- disposal practices;
- other miscellaneous information.

With this information, the user must then complete a COSHH form (an example is shown in Table 1.7). As part of the COSHH process, specific details of the Hazard Statement

Table 1.7 An example of Control of Substances Hazard to Health (COSHH) form.

Section 1: Overview

Names of chemicals to be used:	*Enter the name of each hazardous chemical to be used*		
Title of activity:	*Enter the title of the activity*		
Brief description of theactivity:	*Briefly describe the activity to be undertaken*		
Responsible person:	*Enter name of the member of staff responsible for your work e.g. supervisor*		
Faculty / Department	*Enter the name of your Faculty / Department*		
Date of assessment	*Enter the date*	**Date of Re-assessment**	*Enter the date one year from now*
Location of work:	*Enter the name of Building / Laboratory in which the work will be carried out.*		

Section 2: Emergency Contacts (e.g. project supervisor).

Name	Position	Contact Telephone Number
Enter the name	*Enter their position*	*Enter their telephone number*

Section 3: Hazard Identification

3.1 For hazardous substances in this activity, click all that apply.

	☐ Toxic		☐ Severe Health Hazards		☐ Health Hazards
	☐ Explosive		☐ Flammable		☐ Oxidising
	☐ Corrosive		☐ Gases Under Pressure		☐ Environmental

3.2 Select the hazard phrases (H-phrases) for each hazardous substance.

1.	*Select a Hazard phrase*	e.g.	H302-Harmful if swallowed
e.g.	H226-Flammable liquid and vapour	e.g.	EUH014-Reacts violently with water

Section 4: Hazard Properties

Name of substance	Physical form	Quantity	Frequency	Route of exposure
Enter substance name	*Enter physical form*	*Enter the quantity*	*Enter the frequency*	*Select route*
e.g. *Chemical name*	solid dust	1 g	weekly	Ingestion

Section 5: Identifying Those at Risk

5.1 Who might be at risk? Select all that apply.

☐ Staff/PGRs ☐ Taught Students ☐ Young persons (under 18 years old)

☐ New or expectant mothers ☐ Others:

Table 1.7 (Continued)

5.2 Assessment of risk to human health before control measures are in place. Select the likelihood and severity of harm in the presence of the identified hazards **before** the control measures outlined above are implemented. Calculate the risk rating and act accordingly.		
Likelihood of harm	**Severity**	**Risk Rating and Outcome (likelihood x Severity)**
e.g. 2. Unlikely	*2. Minor Injury*	*5. Good lab practice required.*

Section 6: Control Measures (Specify control procedures to each hazardous substance identified in section 4.)

6.1.Physical or engineering controls				
☐ Laboratory	☐ Controlled area	☐ Total containment	☐ Glove Box	☐ Fume cupboard
☐ Microbial safety cabinet	☐ Local exhaust ventilation	☐ Access control	☐ Other: *Enter details*	
You must also specify below at which point in the work activity they are to be used.				
Specify at which point the control measures should be implemented				
6.2 Administrative controls.				
Describe administrative controls				
6.3 Personal protective equipment (PPE).				
 ☐ Eyewear protection (Minimum standard CE EN166)		 ☐ Disposable lab coat		
 ☐ Lab coat		 ☐ Chemical suit		
 ☐ Specialised footwear. (Minimum standard EN ISO 20345) State type: *Enter details here*		 ☐ Hearing protection (Minimum standard EN352-1) State type: *Enter details here*		
 ☐ Gloves State minimum standard: ☐ BS EN455 – single use for chemical hazards. ☐ BS EN374 – single use for chemicals hazards and microorganisms. State type used: *Enter details here*		 ☐ Respirator State type: ☐ Disposable P3 (Minimum standard EN149) ☐ Replaceable filter (Minimum standard EN140) ☐ Powered respirator. State type used: *Enter details here*		
 ☐ Full-face visor		 ☐ Other State: *Enter details here*		
6.4 Storage requirements.				
Describe storage conditions				
6.5 Transport of hazardous substances.				
Describe how you will transport the hazardous substances				
6.6 Disposal of waste. If specialised waste is to be generated, you must discuss this with a member of technical staff and consult the university waste policy.				

(*Continued*)

Table 1.7 (Continued)

Waste type	Waste subtype	Detail method of disposal
Select waste type e.g. liquid	*Select a waste sub-type e.g. Inorganic waste*	*Describe the method of disposal e.g. down the sink with plenty of water*

6.7 Emergency procedures.

Minor spillage (for less than 250 mL / 250 g of materials with a low-medium risk rating).	**Major spillage (for greater than 250 mL / 250 g of materials with a low-medium riskrating, or any high risk materials).**
☐ Secure the spill area.	☐ Evacuate and secure the laboratory/area.
☐ Inform a competent person (e.g. a member of technical staff or your supervisor).	☐ Inform a competent person (e.g. a member of technical staff or your supervisor).
☐ Other *Describe other emergency procedures*	☐ Evacuate the building using the fire alarm.

In the event of fire, assuming you are trained in the handling of extinguishers and it is safe to do so, specify which types of fire control may be used:

☐ Carbon dioxide	☐ Water	☐ Dry powder	☐ Foam	☐ Fire blanket	☐ Automatic fire suppression

☐ Other *Describe other fire control measures here, if applicable*

In the event of an accident requiring first aid, seek assistance as soon as possible.
Detail below any specific considerations, which must be made for the hazardous substances in use.

☐ If hazardous material comes into contact with skin, remove any affected clothing and wash the area with copious amounts of water. ☐ For large areas rinse the skin using the emergency shower.	☐ If hazardous material comes into contact with the eyes, rinse the eyes using an eye wash station. ☐ For serious eye burns, use diphoterine station.
☐ For phenol burns, wash with copious amounts of water and apply polyethylene (PEG) 300 to the area.	☐ For hydrofluoric acid burns, wash with copious amounts of water and apply calcium gluconate gel to the area.
☐ If cyanide has been inhaled, move the victim to fresh air.	☐ Other. Please state: *Enter details here*

6.8 Assessment of risk to human health once control measures are in place.
Select the likelihood and severity of harm in the presence of the identified hazards **after** the control measures outlined above are implemented. Calculate the risk rating and act accordingly. Guidance may be found in the appendix by clicking here.

Likelihood of harm	Severity	Risk Rating and Outcome (Likelihood x Severity)
Enter likelihood e.g. 2. Unlikely	*Enter severity e.g. 1. Delay only*	*Enter risk rating e.g. 2. Good laboratory practice only required*

6.9 Instruction, training and supervision.
In consultation with the approver, specify the level of training and supervision required to safely carry out the work described. **Select all that apply.**

☐ Special instructions are required to safely carry out the work.

☐ Special training is required to safely carry out the work.

☐ Work may be carried without direct supervision.	☐ Work may be carried without indirect supervision.

Table 1.7 (Continued)

☐ Work may not be started without the advice and approval of the approver.	☐ Work may not be carried out without close supervision.

Section 7: Approval

I hereby confirm that the above is a suitable and sufficient risk assessment for the work activity described.

7.1 The assessor.		
Name	**Signature**	**Date**
7.2 The approver (if required).		
Name	**Signature**	**Date**

and Precautionary Statement, for each chemical, must be included (Table 1.8). Then, an assessment of the likelihood of harm coming to pass given the amount/nature of the chemical to be used and the environment/manner it is to be used in; at this stage, the likelihood is assessed on the basis that no specific control measures are being taken. The likelihood therefore assesses the highest risk. After assessing the likelihood, the next stage is to consider the severity of the risk. This is done by considering the substance-specific risk (rather than the activity specific risk). Again, like the likelihood, this considers the highest severity. By then performing the RMA (Risk = Likelihood × Severity) (Table 1.9), you arrive at the risk for using the chemical.

The individual working in the laboratory is also a major source of contamination. Therefore, as well as the normal laboratory safety practices of wearing a laboratory coat

Table 1.8 Examples of (a) Hazard[a] and (b) Precautionary[b] Statements.

(a)

Letter	Type of hazard	Intrinsic properties of the substance	Example
H	2 = physical	e.g. explosive properties for codes 200–210; flammability for codes 220–230; etc.	H302 harmful if swallowed.
H	3 = health		
H	4 = environmental		

[a] There are 72 individual and 17 combined Hazard statements.

(b)

Letter	Type of precaution	Examples
P	1 = general precaution	P102 Keep out of the reach of children
P	2 = prevention precaution	P281 Use personal protective equipment as required
P	3 = response precaution	P301 If swallowed:
P	4 = storage precaution	P404 Store in a closed container
P	5 = disposal precaution	P501 Dispose of contents/container to . . .

[b] There are 116 individual and 33 combined Precautionary statements.

Table 1.9 Risk matrix analysis[a].

			Severity					
			6 multiple fatalities	5 single fatality	4 major injury	3 lost time injury	2 minor injury	1 delay only
Likelihood	6	certain	36	30	24	18	12	6
	5	very likely	30	25	20	15	10	5
	4	likely	24	20	16	12	8	4
	3	may occur	18	15	12	9	6	3
	2	unlikely	12	10	8	6	4	2
	1	remote	6	5	4	3	2	1

[a] **Note: Low risk:** numerical score 1–10. Good laboratory practice (including Personal Protective Equipment of a laboratory coat and safety glasses) required.
High risk: numerical score 12–18. Specific identified control measures must be used.
Very high risk: numerical score 20+. Trained personnel only.

and safety glasses, it may be necessary to take additional steps such as the wearing of 'contaminant-free' gloves, a close-fitting hat or mask (COVID-19) as well as working in a fume cupboard.

The basic generic rules for laboratory work (and as appropriate for associated work outside the laboratory using chemicals) are as follows:

- Always wear appropriate protective clothing, typically, this involves a clean laboratory coat fastened up, eye protection in the form of safety glasses or goggles, appropriate footwear (open toed sandals or similar are inappropriate) and ensure long hair is tied back. In some circumstances, it may be necessary to put on gloves, e.g. when using concentrated acids.
- Never eat or drink in the laboratory.[1]
- Never work alone in a laboratory.[2]
- Make yourself familiar with fire regulations in your laboratory and building.[3]
- Be aware of accident/emergency procedures in your laboratory and building.[3]
- Use appropriate devices for transferring liquids, e.g. pipette, syringe, Gilson.
- Only use/take the minimum quantity of chemical required for your work. This can prevent cross-contamination, as well as reducing the amount to be disposed.
- Use a fume cupboard for hazardous chemicals, e.g. volatile organic compounds. Check that the fume cupboard is functioning properly (i.e. has an air flow that takes fumes away from the worker) before starting your work.

1 Smoking is banned in public buildings in the United Kingdom.
2 This is strictly enforced with undergraduate students; however, postgraduate researchers often work in the proximity of others to ensure some safety cover is available. Universities will have procedures in place to allow such work to take place, and it will always involve notifying others of your name and location. In the case of postgraduate researchers, the proximity of a (mobile) telephone is additionally beneficial to alert others.
3 This might involve additional training.

- Clear up spillages and breakages as they occur; for example in the undergraduate laboratory, notify the demonstrator/technician immediately to ensure that appropriate disposal takes place, e.g. broken glass in the glass bin.
- Always work in a logical and systematic manner; it saves time and can prevent a waste of resources, e.g. only weighing out the amount of chemical required when it is required.
- Always think ahead and plan your work; accordingly, this involves reading the laboratory script before you enter the laboratory, as well as checking that you are following the script while undertaking the experiment.

1.5 Considerations for Data Presentation

1.5.1 Useful Tips on Presenting Data in Tables

Tables are a useful method for recording numerical data in a readily understandable form. Tables provide the opportunity to summarize data and to allow comparisons between methods. Typically, the data is shown in columns (running vertically) and rows (running horizontally). Columns may contain details of the sample (a sample code identifier), concentration (with units), names of compounds, as well as the properties measured, while rows contain the written or numerical information for the columns. An example is shown in Table 1.12.

1.5.2 Useful Tips on Presenting Data in Graphical Form

Graphs are used, normally, to represent a relationship between two variables, x and y. It is normal practice to identify the x-axis as the horizontal axis (absicca axis) and to use this for the independent variable e.g. concentration ($\mu g\ ml^{-1}$). The vertical or ordinate axis (y-axis) is used to plot the dependent variable, e.g. signal response (mV). An example is shown in Figure 1.2a.

1.6 Use and Determination of Significant Figures

A common issue when recording data from practical work is the reporting of significant figures. The issue is important as it conveys, to the reader, an understanding of the underlying practical work. A few examples will illustrate the issues and how they can be interpreted.

Example 1.1 When asked to *accurately* weigh out approximately 0.5 g of sample, how many decimal places should be reported?

Response 1.1 In this situation, it would be expected that a four decimal place analytical balance would be used to accurately weigh out the sample. On that basis, the sample would be recorded as, for example 0.5026 g. In practice, the sample would have been weighed by difference, i.e. a sample container would be first weighed, then the sample placed inside the container and the weight again recorded, and finally, the sample transferred to an extraction vessel, and the sample container re-weighed. By taking the weights of the container with/without the sample allows an accurate recording of the weight of sample transferred to the container.

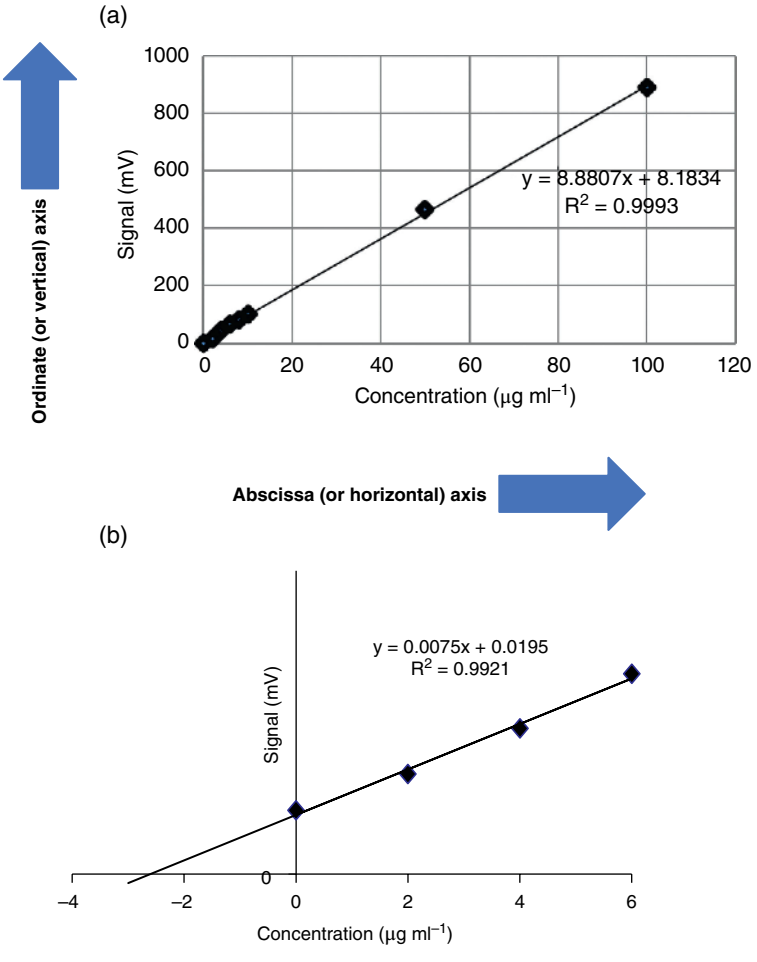


Figure 1.2 Calibration graphs. (a) A direct calibration graph and (b) a standard additions method calibration graph.

Example 1.2 Is it appropriate to round up/down numbers?
Response 1.2 Yes, for example if you have a numerical value, representing a weight or concentration, of 276.643, it would be reasonable to represent this as 276.6 or even 277. If the value was 0.828, then it may be reasonable to round up to 0.83. Whereas for a value of 12 763, it would be reasonable to report as 12 763 or, in some circumstances 12 760.

Example 1.3 In calculating a result in a spreadsheet, for the concentration (mg kg^{-1}) of an organic compound in a solid sample, a numerical value of 25.21 345 678 is obtained. Is this correct?
Response 1.3 No, this is a totally unrealistic representation in terms of the number of decimal places, in terms of the actual determination of the concentration, its interpretation as a concentration and demonstrates a lack of understanding of the data. A more appropriate, and realistic, reporting of the concentration would be 25.2 mg kg^{-1}.

In general terms, the following guidance is provided:

- When rounding up numbers, add one to the last figure if the number is greater than 5, e.g. 0.54 66**7** would become 0.5467.
- When rounding down numbers, remove one to the last figure if the number is less than 5, e.g. 0.54 66**2** would become 0.5466.
- For a number 5, round to the nearest even number, e.g. 0.955 would become 0.96 (to two significant figures) OR if the value before 5 is even, it is left unchanged, e.g. 0.9**4**5 would become 0.94 (to two significant figures) OR if the value before 5 is odd, its value is increased by one, e.g. 0.9**5**5 would become 0.96 (to two significant figures).
- Zero is not a significant figure when it is the first figure in a number, e.g. 0.0067 (this has two significant figures **6** and **7**). In this situation, it is best to use scientific notation, e.g. $6.7 \times \mathbf{10^{-3}}$.

1.7 Units

The Systeme International d'Unites (SI) is the internationally recognized system for measurement (Table 1.10). The most used SI derived units are shown in Table 1.11. It is also common practice to use prefixes (Table 1.12) to denote multiples of 10^3. This allows numbers to be kept between 0.1 and 1000. For example, 1000 ppm (parts per million) can also be expressed as 1000 μg ml^{-1} or 1000 mg l^{-1} or 1000 ng μl^{-1}.

Table 1.10 Some commonly used base SI Units.

Measured quantity	Name of SI unit	Symbol
Length	Metre	m
Mass	Kilogram	kg
Amount of substance	Mole	mol
Time	Second	s
Thermodynamic temperature	Kelvin	K

Table 1.11 Some commonly used derived SI units.

Measured quantity	Name of unit	Symbol	Definition in base units	Alternative in derived units
Electric charge	Coulomb	C	A s	$J\,V^{-1}$
Energy	Joule	J	$m^2\,kg\,s^{-2}$	N m
Force	Newton	N	$m\,kg\,s^{-2}$	$J\,m^{-1}$
Frequency	Hertz	Hz	s^{-1}	—
Pressure	Pascal	Pa	$kg\,m^{-1}\,s^{-2}$	$N\,m^{-2}$
Power	Watt	W	$m^2\,kg\,s^{-3}$	$J\,s^{-1}$

Table 1.12 Commonly used prefixes.

Multiple	Prefix	Symbol
10^{15}	peta	P
10^{12}	tera	T
10^{9}	giga	G
10^{6}	mega	M
10^{3}	kilo	k
10^{-3}	milli	m
10^{-6}	micro	μ
10^{-9}	nano	n
10^{-12}	pico	p
10^{-15}	femto	f

1.8 Calibration and Quantitative Analysis

Quantitative analysis is the cornerstone of environmental analysis with all analyses requiring some form of calibration associated with the determination of contaminants. To assess the level of contamination requires the determination of a concentration; this is achieved using a calibration graph. To perform a calibration graph requires the preparation of calibration solutions from a stock solution, as well as the practical skills inherent in weighing, dilutions and quantitative transfer of solids and liquids. Figure 1.3 shows the manual dexterity required to quantitatively remove a known volume of stock solution from a volumetric flask. All these skills require knowledge of balances, pipettes and volumetric flasks, as well as the correct choice of standards (e.g. pentachlorophenol, benzo(a)pyrene) and their associated grades (i.e. purity), including the use of solvents (including grades of acetone, dichloromethane).

1.9 Terminology in Quantitative Analysis

In doing any quantitative analyses, it is important to be consistent in the terminology used. A concise description of some of the key terms is shown below:

Accuracy: The closeness of agreement between a test result (i.e. measured in the laboratory) and the accepted reference value (i.e. from a certified reference material; see Section 1.16).

Error (of measurement): The result of a measurement minus the true value of the measurand.

Random error *(of a result):* A component of the error which, in the course of a number of test results for the same characteristic, varies in an unpredictable way; it is not possible to correct for random error.

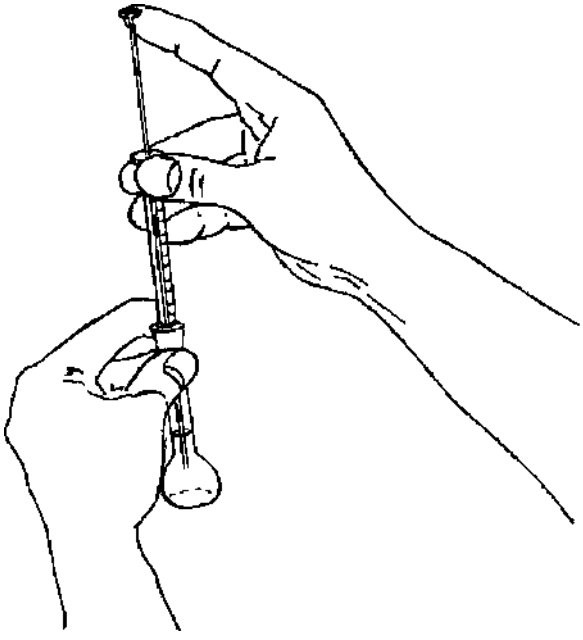


Figure 1.3 Quantitative transfer of a known volume of stock solution for preparation of calibration solutions, using a microsyringe. Note the insertion of the syringe containing the organic solvent/extract in the receiving solution.

Systematic error: A component of the error which, in the course of a number of test results for the same characteristic, remains constant or varies in a predictable way; systematic errors and their causes may be known or unknown.

Precision: The closeness of agreement between independent test results obtained under stipulated conditions.

Repeatability: Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test items in the same laboratory, by the same operator, using the same equipment within short intervals of time.

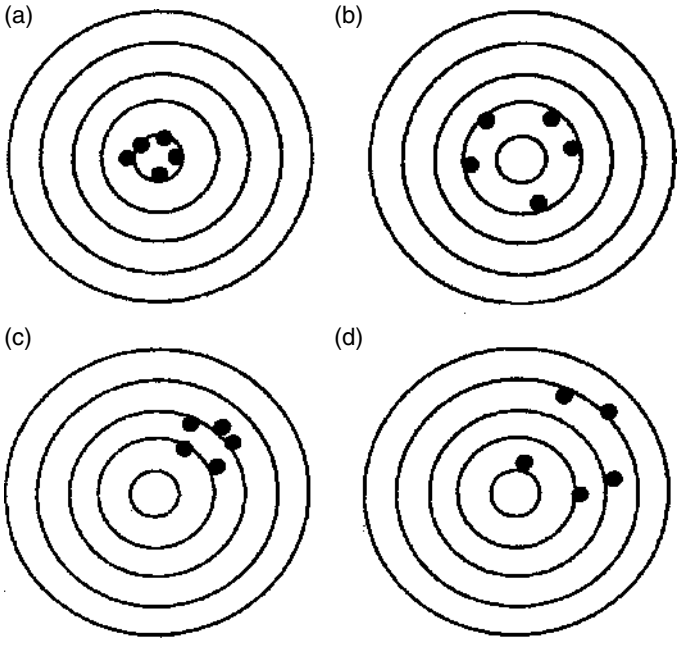
Reproducibility: Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories, with different operators, using different equipment.

Uncertainty: Parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the compound.

A pictorial representation of the terms accuracy and precision is given in Figure 1.4, with explanatory notes.

1.10 Preparing Solutions for Quantitative Work

The basis of any quantitative work is that you start with a known concentration of the substance (e.g. pentachlorophenol, benzo(a)pyrene) as a stock solution. Serial dilutions are then required to produce a set of calibration solutions, which are then run through a specific



The centre of the bullseye represents the 'true' value.

NOTES: (a) the data points would be classed as accurate and precise, (b) the data points would be classed as accurate but imprecise, (c) the data points would be classed as inaccurate but precise and (d) the data points would be classed as inaccurate and imprecise.

Figure 1.4 A pictorial representation of the term's accuracy and precision.

analytical instrument and the responses recorded. The generated data is then used to produce a calibration graph (see, Section 1.11) against which other unknown samples can then be compared.

Solutions are usually prepared in terms of their molar concentrations, e.g. mol l^{-1}, or mass concentrations, e.g. μg ml^{-1}. Both units refer to an amount per unit volume, i.e. concentration = amount/volume. It is important to use the highest (purity) grade of chemicals (liquids or solids) for the preparation of solutions for quantitative analysis, e.g. TraceCERT® or a specified purity, e.g. 97%.

Example 1.4 Prepare a 1000 μg ml^{-1} solution of benzo(a)pyrene (BaP) in a 10 ml volumetric flask. The CAS number for benzo(a)pyrene is 50-32-8.
Response 1.4 For a 1000 μg ml^{-1} solution BaP, dissolve 10.00 mg of BaP in acetone (or another solvent, e.g. dichloromethane) and dilute to 10.0 ml in acetone. This will give you a 1000 μg ml^{-1} solution of BaP.

Example 1.5 Prepare a 0.1 mol l^{-1} solution of benzo(a)pyrene (BaP) in a 10 ml volumetric flask. The CAS number for benzo(a)pyrene is 50-32-8. The molecular weight of benzo(a)pyrene ($C_{20}H_{12}$) is 252.31.

Response 1.5 For a 0.1 mol l^{-1} solution of BaP, dissolve 252 mg of BaP in acetone and dilute to 10 ml in acetone. This will give you a 0.1 mol l^{-1} solution of BaP.

Example 1.6 Prepare a 1000 µg ml^{-1} solution of pentachlorophenol (C_6HCl_5O) (PCP), from the pure compound, in a 10 ml volumetric flask. The CAS number of PCP is 87-86-5.
Response 1.6 For a 1000 µg ml^{-1} solution of PCP, dissolve 10.00 mg of PCP in methanol and dilute to 10.0 ml in methanol. This will give you a 1000 µg ml^{-1} solution of PCP.

Example 1.7 Prepare a 0.1 mol l^{-1} solution of pentachlorophenol (C_6HCl_5O) (PCP), from the pure compound, in a 10 ml volumetric flask. The CAS number of PCP is 87-86-5, and its molecular weight is 266.34.
Response 1.7 For a 0.1 mol l^{-1} solution of PCP: Dissolve 266 mg of PCP in methanol and dilute to 10 ml in methanol. This will give you a 0.1 mol l^{-1} solution of PCP.

1.11 Calibration Graphs

Calibration graphs can be done in two different formats, typically a 'direct' plot or a standard additions method plot (Figure 1.2). In the normal 'direct' calibration graph, the most common type of calibration graph, a plot of signal response (Y on the ordinate axis) versus increasing concentration (x on the abscissa axis) of the compound is made (Figure 1.2a). It is then possible to estimate the concentration of a compound in an unknown sample by interpolation, either graphically or by regression (using, for example Microsoft excel) (see, further reading section at the end of this chapter). Assuming a linear response allows a plot of the line of regression of y on x to be made:

$$y = m.x + c \tag{1.1}$$

where y is the signal response, e.g. absorbance, signal (mV); x is the concentration of the calibration solution (in appropriate units, e.g. µg ml^{-1} or ppm); m is the slope of the graph and c is the intercept on the x-axis.

By simple re-arrangement allows the determination of the unknown sample concentration (x):

$$\left(y - c\right) / m = x \tag{1.2}$$

Alternatively, the method of standard additions can be used; this approach is useful if the sample is known to contain a potentially interfering matrix. In this approach, a known (and fixed) volume of the sample is added to each of the calibration solutions. The volume of sample to be added to the calibration solutions needs to be estimated; this can be done by first running the unknown sample and interpolating from the direct plot. By again plotting the signal response against concentration of compound (as above), a different format of graph is obtained (Figure 1.2b). The graph no longer passes through zero on either axis; by extending the graph toward the x-axis (extrapolation) until it intercepts, it allows the concentration of the analyte in the unknown sample to be estimated. It is essential that the standard additions plot is linear over its entire length, otherwise considerable error will be introduced; it may be therefore necessary to either add a smaller volume of sample to

the calibration solutions or alter the concentration range used. The term *linearity* is used to define the ability of the method to obtain test results proportional to the concentration of the analyte, whereas the *linear dynamic range* is the concentration range over which the analytical working calibration curve remains linear.

Example 1.8 What is the linear dynamic range of the calibration plot shown in Figure 1.5a?
Response 1.8 The linear dynamic range extends from 0 to 160 mg l^{-1} (Figure 1.5b).

1.12 The Internal Standard

Sometimes a chemical compound is added in equal amounts to all samples, and this is referred to as the internal standard. One consequence of adding an internal standard is that it influences the linearity of measurement with an analytical technique. In these situations,

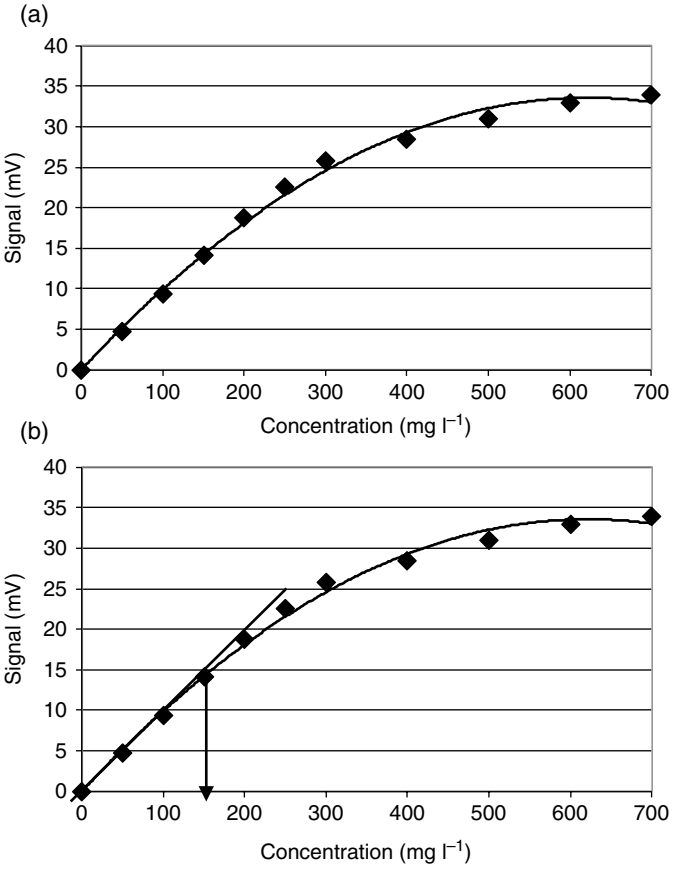


Figure 1.5 An investigation of linear dynamic range. (a) Calibration graph, and (b) interpretation of linear dynamic range.

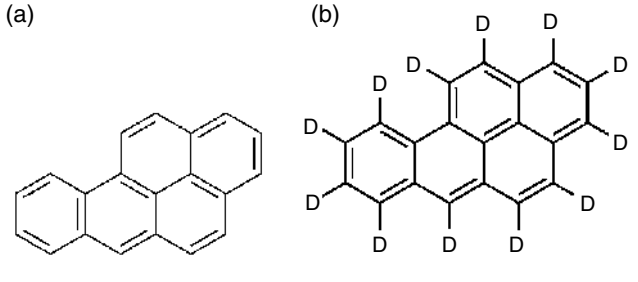
the y-axis (response) is plotted as the ratio of the compound to the internal standard signal versus the compound concentration. The benefits of adding an internal standard are multi-fold, as it allows correction for the following:

- Compound loss during sample preparation
- Non-quantitative transfer of samples between containers
- Compound adsorption loss on storage
- Evaporation loss during pre-concentration (Chapter 15)
- Variation in injection volume (e.g. in gas chromatography)
- Variation in mass spectrometer response due to ion suppression or enhancement (e.g. HPLC-MS).

However, selection of an appropriate compound to act as an internal standard requires consideration. Ideally, it should have similar physicochemical properties and display similar behaviour to the compound or compounds for which it is acting as the internal standard. The two generic types of internal standard that are used are either structural analogues or stable isotope-labelled compounds. In the case of stable isotope-labelled compounds, the label is often ^{13}C, ^{15}N or ^{2}H (D) (Figure 1.6). In the case of selecting a structural analogue compound as an internal standard, the proposed compound ideally has similar structure and functionalities, e.g. COOH, -CHO and -Cl etc. with differences only being with C–H moieties (Figure 1.7).

1.13 Limits of Detection/Quantitation

The limit of detection (LOD) of an analytical procedure is the lowest amount of compound in an unknown sample which can be detected but not necessarily quantified, i.e. recorded as an exact value. Various definitions exist as to the method of determining the LOD. When quoting concentrations as LOD, it is appropriate to indicate the exact method of determination. The limit of detection, expressed as a concentration (in appropriate units), is derived



Benzo(a)pyrene — MW = 252.31

Benzo(a)pyrene – d12 — MW = 264.38

Figure 1.6 An example of a stable isotope-labelled compound as an internal standard. (a) Analyte compound and (b) labelled compound.

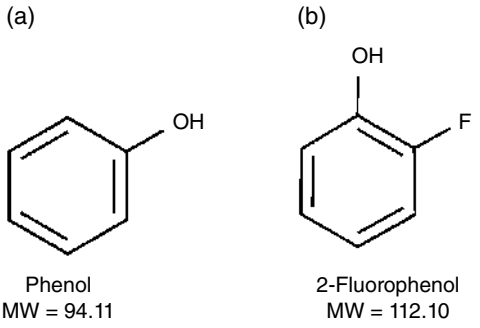


Figure 1.7 An example of a structural analogue compound as an internal standard. (a) Analyte compound and (b) analogue compound.

from the smallest measure, X, that can be detected with reasonable certainty for a given procedure. One approach to determine the LOD is to measure the signal of a known concentration at or near the lowest concentration that is observable (normally at least seven times). The value X is given by the equation:

$$X = X_{LCS} + K.SD_{LCS} \tag{1.3}$$

where X_{LCS} is the mean of the low concentration standard, SD_{LCS} is the standard deviation of the low concentration standard and K is a numerical factor chosen according to the confidence level required (typically 2 or 3).

An alternate approach, useful in chromatography, to calculate the LOD is by determining the concentration of compound providing a signal-to-noise ratio (S/N) of three. In this approach, the signal is measured from decreasing standard solutions until a signal is found whose height is three times taller than the maximum height of the baseline (measured at both sides of the chromatographic peak). The concentration corresponding to that peak is taken as the LOD.

Another approach to calculate the LOD is as follows:

$$LOD = 3.3.\sigma / m \tag{1.4}$$

where σ is the standard deviation of the response and m is the slope of the calibration curve (see Eq. 1.1).

An estimate for the value of σ can be made by either (i) measurement of the magnitude of the analytical background response (i.e. analyse a blank sample (a minimum of seven times) and calculate the standard deviation), or (ii) use the standard deviation of the y-intercept (c term in Eq. 1.2) of multiple regression lines.

As LODs are often not practically measurable, a more realistic value is to use the limit of quantitation (LOQ) of an analytical procedure. The LOQ is the lowest amount of an analyte in a sample, which can be quantitatively determined with suitable uncertainty; the LOQ can be taken as 10 × 'the signal-to-noise ratio' or K = 10 in Eq. (1.3), or substitute 10 (instead of 3.3) in Eq. (1.4).

1.14 Dilution or Concentration Factors

Once the concentration of the compound has been determined from the calibration graph, it is necessary to report its actual concentration in the original starting material, e.g. soil or river water sample. This requires the use of either a dilution or concentration factor. The following examples illustrate the use of these factors.

Example 1.9 Use of the dilution factor. Based on the following information, calculate the concentration of a compound (in units of mg kg^{-1}) in the original soil sample.

An accurately weighed soil sample (2.1189 g) was extracted with dichloromethane. The extract was quantitatively transferred to a 25 ml volumetric flask and made up to the mark in dichloromethane. This solution was then serially diluted by taking 1 ml of the solution and transferring it to a further 10 ml volumetric flask where it is made up to the mark with dichloromethane.

What is the dilution factor?

Response 1.9 The dilution factor is calculated as follows:

$$\left(25\,\text{ml} / 2.1189\,\text{g}\right) \times \left(10\,\text{ml} / 1\,\text{ml}\right) = 119.0\,\text{ml}\,\text{g}^{-1} \tag{1.5}$$

If the solution were then analysed and found to be within the linear portion of the graph (see Figure 1.5b), the value for the dilution factor would then be multiplied by the concentration obtained from the graph. So, if the concentration from the graph was determined to be 15.1 mg l^{-1} (or 15.5 µg ml^{-1}), it would produce a final value, representative of the compound under investigation, i.e. 1797 µg g^{-1}. It is important to consider the number of significant figures quoted; in this case, 1797 µg g^{-1} (or 1797 mg kg^{-1}). The amount of the compound in the original soil sample is therefore 1797 mg kg^{-1}.

Example 1.10 Use of concentration factor. Based on the following information, calculate the concentration of pentachlorophenol (in units of µg l^{-1}) in the original wastewater sample.

A wastewater sample (1000 ml) was extracted into dichloromethane (3 × 5 ml) using liquid–liquid extraction. The extract was then quantitatively transferred to a 25 ml volumetric flask and made up to the mark in dichloromethane. What is the concentration factor?

Response 1.10 The concentration factor is calculated as follows:

$$\left(25\,\text{ml} / 1000\,\text{ml}\right) = 0.025 \tag{1.6}$$

If the solution was then analysed and found to be within the linear portion of the graph (see Figure 1.5b), the value for the concentration factor would then be multiplied by the concentration from the graph. So, if the concentration from the graph was determined to be 58.8 ng ml^{-1}, it would produce a final value, representative of the compound under investigation, i.e. 1.47 ng g^{-1}. It is important to consider the number of significant figures quoted; in this case 1.5 ng ml^{-1} is appropriate; also, be careful with prefixes on units (see, Table 1.12)]. The amount of pentachlorophenol in the original wastewater sample is therefore 1.5 µg l^{-1}.

1.15 Quality Assurance

Quality assurance is all about getting the correct results that are representative of the original sample, i.e. the contaminated land site from which the soil sample was obtained or the river water from which the sample was obtained. In practice, in environmental analyses, this is extremely challenging as it involves multiple steps: sampling, sample collection, sample storage, sample preparation, analytical determination and data interpretation and action. Considerations can be taken to control and inform the sampling, sample collection and storage (see Chapter 2). Subsequently, the samples are prepared and analysed in a laboratory. It is possible to ensure that the laboratory is functioning appropriately by adopting a good quality assurance scheme. The main objectives of a quality assurance scheme are:

- selection and validation of an appropriate method of sample preparation;
- selection and validation of an appropriate method of analysis;
- regular maintenance (and upgrading) of analytical instruments;
- ensure appropriate record of methods and results are maintained;
- ensure that high quality data is produced;
- overall to ensure that a high quality of laboratory performance is maintained.

Examples of important aspects of establishing and maintaining such a QA scheme are as follows:

- Individual performing the analyses
  - Has the individual been trained in the use of the instrumentation and/or procedures? If so by whom (where they trained or experienced)?
  - Was the training formal (formal qualification or certificate of competency obtained) or done in-house?
  - Can the individual use the instrumentation alone or do they require oversight?
- Laboratory procedures and practices
  - Do the procedures use certified reference materials to assess the accuracy of the method? (see Section 1.16)
  - Do the procedures use spiked samples to assess recoveries? The samples are spiked with a known concentration of the analyte under investigation and their recoveries noted; this allows an estimate of analyte matrix effects.
  - Do the procedures include analysis of reagent blanks? Analysing reagents whenever the batch is changed or a new reagent introduced allows reagent purity to be assessed and, if necessary controlled, and also acts to assess the overall procedural blank; typically introduce a minimum number of reagent blanks, i.e. 5% of the sample load).
  - Do the procedures use standards to calibrate instruments? A minimum number of standards should be used to generate the analytical curve, e.g. minimum of 5. Daily verification of the calibration plot should be done using one or more standards within the linear working range.
  - Do the procedures include the analysis of duplicate samples? Analysis of duplicates or triplicates allows the precision of the method to be determined and reported.

- Do the procedures include known standards within the sample run? A known standard should be run after every 10 samples to assess instrument stability; this also verifies the use of a daily calibration plot.

1.16 Use of 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 (Table 1.13) and issued by a certifying body. Examples of certifying bodies include National Institute of Science and Technology NIST), USA and LGC, UK.

Table 1.13 An example of a certificate for a certified reference material[d]

National Institute of Science and Technology Certificate of Analysis
Standard Reference Material 1234: Sandy loam soil Certified Values

Constituent	Certified value[a, b] ($\mu g\ kg^{-1}$)	Uncertainty[a, b, c] ($\mu g\ kg^{-1}$)	Weight of sample (g)[d]
PCB101	57	6	5
PCB118	120	4	5

Certified Values

Constituent	Certified value[a, b] ($mg\ kg^{-1}$)	Uncertainty[a, b, c] ($mg\ kg^{-1}$)	Weight of sample (g)[d]
Phenanthrene	192	6	5
Fluoranthene	320	7	5
Benzo[a]pyrene	31	1	5

Indicative Values

Constituent	Indicative value ($mg\ kg^{-1}$)[a, e]	Number of laboratories
Anthracene	4	8
Chrysene	12	7
Fluorene	8	20

[a] Values expressed on a dry weight basis
[b] The certified values were obtained using procedures involving pressurised liquid extraction.
[c] The uncertainty interval calculated provides a level of confidence of 95%.
[d] Weight of sample taken for homogeneity assessment
[e] Interlaboratory mean of means of the final data set.

It can be seen on the certificate (Table 1.13) that some of the concentration values are 'certified' while others are 'indicative'. The use of the term certified means that the concentrations stated are reliable, whereas the term indicative means that the concentrations stated have some uncertainty (or an insufficient number of methods have been used in their characterization). In addition, other information would be contained on the certificate relating to the actual material supplied, specifically, details of the minimum amount of material that is representative of the whole. If a smaller sample size is taken than recommended on the certificate, then the certified value and its uncertainty are not guaranteed, the expiry date or shelf-life. This is the last date that the material should be used and remains within its certified value and its uncertainty, and moisture correction. Often the material will report its certified value and its uncertainty based on its dry mass. In these situations, correction for the moisture content can be made provided that the dry mass is determined on a separate sub-sample. An extensive range of CRMs are available for organic compounds in different matrices. Typical compounds include dioxins and furans; hydrocarbons and petrochemicals; PCBs and related compounds; pesticides and metabolites, pharmaceutical and veterinary compounds and metabolites; phenols and aromatic compounds; polycyclic aromatic hydrocarbons; and volatile organic compounds, while matrices include waters (e.g. river, waste); sediments (e.g. lake); soils (e.g. loam); sewage sludges (e.g. domestic); and ash, particulate and dusts (e.g. fine).

1.17 Applications

Case Study A Calculation of the Limit of Detection and Limit of Quantitation

Background: The compound atropine (Figure 1.8) is a prescription medicine used to treat the symptoms of low heart rate (bradycardia), reduce salivation and bronchial secretions before surgery or as an antidote for overdose of cholinergic drugs or mushroom poisoning. It is common practice within the determination of the key figures of merit of an analytical technique is to estimate the limit of detection and limit of quantitation. An approach uses the following equations:

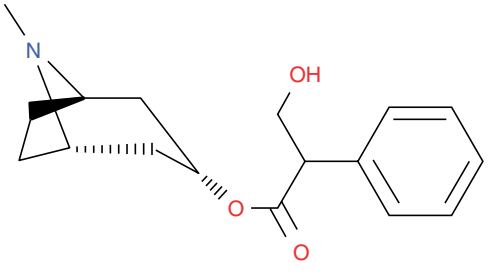


Figure 1.8 Structure of atropine.

$$\text{LOD} = 3.3.\sigma / \text{m} \tag{1.4}$$

$$\text{LOQ} = 10.\sigma / \text{m} \tag{1.7}$$

where σ is the standard deviation of the y-intercepts (c term in Eq. 1.2) of multiple regression lines and m is the (average) slope of the calibration curve (see Eq. 1.1).

Activity: An organic compound (atropine) was analysed using HPLC-UV. To determine the LOD and LOQ, a calibration was produced over the concentration range 0–100 μg ml^{-1} (using 11 data points, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg ml^{-1}). The calibration was run six times to obtain six multiple calibration graphs. Using the data obtained (Table 1.14), calculate the LOD and LOQ.

$$\text{LOD} = \left(3.3 \times 6.533\right) / 11.848$$
$$\text{LOD} = 1.8\,\mu\text{g ml}^{-1} \tag{1.8}$$

and

$$\text{LOQ} = \left(10 \times 6.533\right) / 11.848$$
$$\text{LOQ} = 5.5\,\mu\text{g ml}^{-1} \tag{1.9}$$

Table 1.14 Data for calculation of LOD and LOQ.

Data set	Calibration equation (y = mx + c)	Slope (m)	Intercept (c)
1	y = 11.854x − 28.082	11.854	28.082
2	y = 11.942x − 33.706	11.942	33.706
3	y = 11.640x − 26.002	11.640	26.002
4	y = 11.902x − 34.188	11.902	34.188
5	y = 11.765x − 36.253	11.765	36.253
6	y = 11.987x − 44.464	11.987	44.464
Average		**11.848**	
Standard deviation			**6.533**

Case Study B Some Numerical Worked Examples

Background: By a series of worked examples, the numerical and graph plotting aspects of environmental analysis are considered. The essential stages to the calculations are as follows:

- Determine the concentration of the working solutions.
- Plot the calibration graph (concentration versus signal response). Graph plotting can be done using either a suitable spreadsheet, e.g. Microsoft Excel, or on graph paper.

- Determine the best fit for the calibration data. If we assume that a straight-line graph is obtained, then the following applies. If using a suitable spreadsheet, e.g. Microsoft Excel, then this can be done by selecting 'add trendline' followed by 'display equation on chart' and 'display r squared value on chart'. If using graph paper manually plot the data points, then by using a ruler or flexi curve establish the best fit of the data points to each other. Determine the intercept of the fitted line on the x-axis and calculate the slope of the line. In either case, you should now have the formula for a straight-line equation, i.e. $y = mx + c$, where y is the signal response, m is the slope of the graph, x is the concentration (in appropriate units) and c is the intercept of the line of best fit on the x-axis].
- Calculate, using the equation $y = mx + c$, the concentration (in appropriate units) of the sample based on its generated signal response. This can be done by re-arranging the equation $y = mx + c$ such that the concentration of the sample, x, can be determined as follows: $x = (y - c)/m$].
- Then, establish the dilution/concentration factor associated with the sample preparation (see Section 1.14).
- Calculate the concentration (in appropriate units) based on the dilution/concentration factor (in appropriate units) multiplied by the sample concentration (in appropriate units) as determined from the calibration graph.
- Finally, check that the reported concentration in the original sample is in appropriate units.

Activity 1: An aqueous sample was analysed by GC-FID for pentachlorophenol. The sample was extracted by placing 5 ml of the aqueous sample into a separating funnel with 2 × 5 ml of dichloromethane. The extract was quantitatively transferred to a volumetric flask (25 ml) and made up to volume with dichloromethane (including the addition of internal standard).

A calibration plot was generated by diluting a 500 μg ml^{-1} stock solution of pentachlorophenol. Then, 1 ml of the stock solution was placed in a 10 ml volumetric flask and made up to the mark with acetone (working solution). The working solution is further diluted (Table 1.15) to make the standard solutions.

Table 1.15 Data for worked example 1.

Flask	Pentachlorophenol working solution (ml)	final volume (ml)	GC-FID response (signal)
1	0.00	10	0
2	0.50	10	1523
3	1.50	10	3567
4	3.00	10	6235
5	6.00	10	13 563
Diluted sample			8563

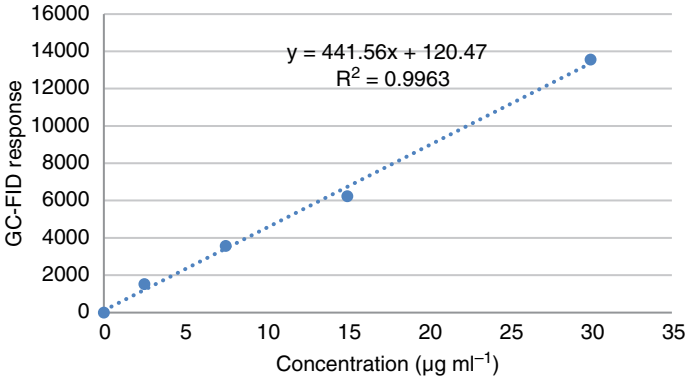


Figure 1.9 Calibration graph for worked example 1, the determination of pentachlorophenol by GC-FID.

By plotting a fully annotated calibration graph of signal response (y-axis) versus concentration (µg ml^{-1}) (x-axis), determine the concentration of pentachlorophenol, in units of µg ml^{-1}, as determined from the graph. Using the dilution/concentration factor and units of the sample extract, determine the concentration of pentachlorophenol, in units of mg l^{-1}, in the original aqueous sample.

The calibration graph is shown in Figure 1.9. The regression coefficient (R^2) indicates the linearity of the graph (i.e. close to a perfect straight-line of 1.0000). Using the equation y = mx+c, the concentration (in appropriate units) was determined.

$$x = (y - c)/m$$
$$x = (8563 - 120.47)/441.56$$
$$x = 19.1\,\mu g ml^{-1}$$

Then, using the dilution/concentration factor, determine the concentration of PCP in the original sample.

$$\text{Dilution/concentration factor is} (25 ml)/(5 ml) = 5$$

The concentration in the original sample was:

$$19.1\,\mu g ml^{-1} \times 5 = 95.5\,\mu g ml^{-1}$$

The concentration of pentachlorophenol in the aqueous sample is 95.5 µg ml^{-1}, which is equivalent to 95.5 mg l^{-1}.

Activity 2: A soil sample was analysed for benzo(a)pyrene as follows. An accurately weighed sample of 2.1351 g was extracted. The extract was quantitatively transferred to a volumetric flask (25 ml) and made up to volume with solvent. A calibration plot was generated by diluting a 1000 µg ml^{-1} stock solution of benzo(a)pyrene. Then, 1 ml of the stock solution was placed in a 10 ml volumetric flask and made up to the mark with solvent (working solution). This solution (Table 1.16) was diluted to make the following standard solutions:

Table 1.16 Data for worked example 2.

Flask	Benzo(a)pyrene working solution (ml)	Final volume (ml)	GC-MS signal
1	0	10	0
2	0.1	10	150
3	0.2	10	290
4	0.3	10	435
5	0.5	10	730
Extracted sample		25	490

Plot a fully annotated calibration graph of signal response (y-axis) versus concentration (μg ml^{-1}) (x-axis). Then, determine the concentration of benzo(a)pyrene, in units of μg ml^{-1}, as determined from the graph. Calculate the dilution/concentration factor and units of the sample extract. Finally, determine the concentration of benzo(a)pyrene, in units of mg kg^{-1}, in the original sample.

The calibration graph is shown in Figure 1.10. The regression coefficient (R^2) indicates the linearity of the graph (i.e. close to a perfect straight-line of 1.0000). Using the equation y = mx + c, the concentration (in appropriate units) was determined.

$$x = (y - c)/m$$
$$x = (490 - 0.8108)/145.5$$
$$x = 3.36\,\mu g\,ml^{-1}$$

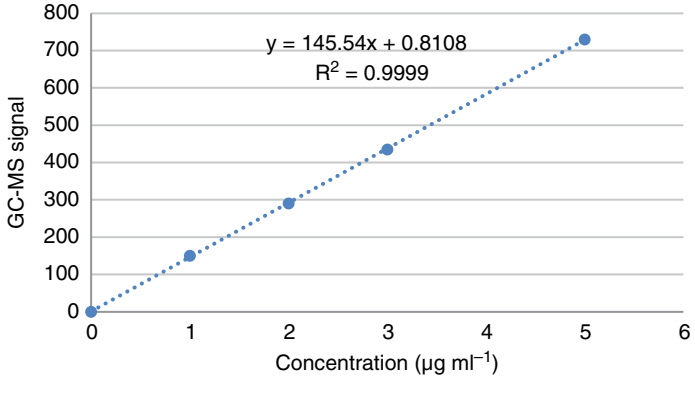


Figure 1.10 Calibration graph for worked example 2, the determination of benzo(a)pyrene by GC-MS.

Then, using the dilution/concentration factor, determine the concentration of PCP in the original sample.

$$\text{Dilution / concentration factor is} \left(25\ \text{ml}\right) / 2.1351\ \text{g} = 11.71\ \text{ml g}^{-1}$$

The concentration in the original sample was:

$$3.36\ \mu\text{g ml}^{-1} \times 11.71\ \text{ml g}^{-1} = 39.3\ \mu\text{g g}^{-1}$$

The concentration of pentachlorophenol in the aqueous sample is 39.3 µg g^{-1}, which is equivalent to 39.3 mg kg^{-1}.

Further Reading

Dean, J.R., Jones, A.M., Holmes, D. et al. (2017). *Practical Skills in Chemistry*, 3e. Harlow, UK: Pearson.