

1 Analytical Toxicology: Overview

1.1 Introduction

Analytical toxicology is concerned with the detection, identification and measurement of drugs and other foreign compounds (xenobiotics) and their metabolites in biological and related specimens. The analytical toxicologist can play a useful role in the diagnosis, management and, in some cases, the prevention of poisoning, but to do so a basic knowledge of clinical and forensic toxicology is essential. Moreover the analyst must be able to communicate effectively with clinicians, pathologists, coroners, police and, possibly, others. In addition, a good understanding of clinical chemistry, pharmacology and pharmacokinetics is desirable.

1.1.1 *Historical development*

The use of physicochemical techniques in the analysis of drugs and other poisons in body fluids or tissues and related specimens has its origins in the development of forensic toxicology. Important contributions came later from work to improve food safety and from occupational toxicology.

The trial of Mary Blandy at Oxford in March 1752 for the murder of her father is the first reported use of chemical tests to detect a poison, in this case arsenic, in a criminal trial (Mitchell, 1938; Watson, 2004). Mathieu Joseph Bonaventure Orfila (1787–1853) in Paris, following the work of the physician François Emmanuel Fodéré (1764–1835), divided poisons into six classes: ‘corrosives, astringents, acrids, stupifying or narcotics, narcotico-acrids and septic or putrefiants’ (Orfila, 1821), and pioneered systematic study of the role of chemical analysis in the diagnosis of poisoning. This work was carried on by his pupils, notably Sir Robert Christison (1797–1882) in Edinburgh, Alfred Swaine Taylor (1806–1880) at Guy’s Hospital in London (Coley, 1998), and Jöns Jacob Berzelius (1779–1848) in Sweden (Jones, 1998).

Plenck (1781) had observed that ‘the only certain sign of poisoning is the botanical character of a vegetable poison or the chemical identification of a mineral poison found in the body’. However, this was not accepted by British toxicologists, notably Christison, who held that the medical probability, in conjunction with the general evidence, might be so strong that a diagnosis of poisoning was undoubted. Nevertheless, given this caveat the importance of chemical tests in confirming a suspicion of poisoning was clear and the partnership between clinical and analytical (chemical) toxicology has developed over the last 250 years.

Sensitive and selective chemical methods for the detection of metallic poisons such as arsenic (Marsh, 1836), antimony, bismuth and mercury (Reinsch, 1841) in biological specimens were introduced in the 1830s. Solvent extraction of alkaloids such as nicotine from biological specimens to facilitate their detection and identification using chemical or physiological tests was introduced by Stas in the 1850s, and developed into a systematic method for the extraction of nonvolatile organic compounds from body fluids by Otto. By the late nineteenth century, chemical and spectroscopic methods for the measurement of carbon monoxide and

chemical methods for the measurement of ethanol in blood had also been developed (Niyogi, 1981).

Further relevant advances in chemical toxicology included the introduction of a colorimetric technique for the measurement of barbiturates (1933) and the use of ammonium sulfate to 'salt out' alkaloids (1937). An alternative approach to the purification of basic drugs from biological specimens, the precipitation of protein with trichloroacetic acid followed by kaolin adsorption and subsequent elution of the drugs of interest, was described in 1937, and sodium tungstate protein precipitation was introduced in 1946. Florisil, a synthetic magnesium silicate, was introduced in 1949 for the adsorption of basic drugs such as narcotics from biological specimens in place of kaolin. Microdiffusion methods (Conway, 1947) were developed for volatile analytes.

Major advances in analytical methodology followed the introduction and application of refined physicochemical techniques such as spectrophotometry and chromatography in the late 1940s. In particular, ultraviolet (UV) and infrared (IR) spectrophotometry, together with visible spectrophotometry (colorimetry), and paper and ion-exchange column chromatography were widely used. More recently, paper chromatography has been largely superseded by thin-layer chromatography (TLC) as this latter technique offers advantages of speed of analysis and lower detection limits. Improved instrumentation for UV spectrophotometry (including facilities for derivative spectroscopy), spectrophotofluorimetry, atomic absorption spectrometry, anodic stripping voltammetry, electrochemistry, X-ray diffraction, mass spectrometry, nuclear magnetic resonance and neutron activation analysis has led to these techniques being widely applied to particular problems.

1.2 Modern analytical toxicology

The last 25 years have seen many advances in methods for detecting, identifying and measuring drugs and other poisons in biological fluids with consequent improvement in the scope and reliability of analytical results. The value of certain emergency assays and their contribution to therapeutic intervention has been clarified. Some such assays are performed for clinical purposes, but have overt medicolegal implications and require a high degree of analytical reliability. Examples include 'brain death' and child abuse screening, and instances of suspected iatrogenic poisoning. In addition, demand for the measurement of plasma drug and sometimes metabolite concentrations to aid treatment [therapeutic drug monitoring (TDM)], for drugs of abuse and laxative/diuretic screening, and for laboratory analyses to monitor occupational exposure to certain chemicals, has increased.

Nowadays, a range of powerful analytical methods, typically chromatographic methods, ligand immunoassays and other techniques (Table 1.1) are available to the analytical toxicologist. However, it remains impossible to look for all poisons in all samples at the sensitivity required. It is vital therefore that the reason for any analysis is kept clearly in view. Although the underlying principles remain the same in the different branches of analytical toxicology, the nature and the amount of specimen available can vary widely, as may the time-scale over which the result is required and the purpose for which the result is to be used. All these factors may, in turn, influence the choice of method(s) for a particular analysis.

Gas-solid chromatography (GSC) and, more commonly, gas-liquid chromatography (GLC) have made a notable contribution to both the qualitative and quantitative analysis of drugs and other organic poisons, especially since the introduction of sensitive detectors such as the

1.2 MODERN ANALYTICAL TOXICOLOGY

3

Table 1.1 Some methods for the analysis of drugs and other organic poisons in biological samples

Principle	Technique
Chemical	Colour test
Electrochemical	Biosensors
	Differential pulse polarography (DPP)
Spectrometric	Mass spectrometry (MS), also known as mass fragmentography (MF)
	Nuclear magnetic resonance (NMR)
	Spectrophotofluorimetry (SPFM)
	Ultraviolet/visible absorption spectrophotometry (UV/Vis)
Chromatographic	Gas chromatography (GC), includes gas–solid chromatography (GSC) and gas–liquid chromatography (GLC)
	(High performance) liquid chromatography [(HP)LC]
	(High performance) thin-layer chromatography [(HP)TLC]
	Super(critical) fluid chromatography [S(C)FC]
Electrophoretic	Capillary (zone) electrophoresis [C(Z)E]
	Capillary electro-chromatography (CEC)
	Micellar electrokinetic capillary chromatography (MECC)
Immunoassay	Cloned enzyme donor immunoassay (CEDIA)
	Enzyme linked immunosorbent assay (ELISA)
	Enzyme multiplied immunoassay technique (EMIT)
	Fluorescence polarization immunoassay (FPIA)
	Latex agglutination tests (LAT)
	Microparticle enzyme immunoassay (MEIA)
	Radioimmunoassay (RIA)
Enzyme-based assay	Alcohol dehydrogenase – ethanol
	Aryl acylamide amidohydrolase – paracetamol

flame-ionization detector (FID) and the electron-capture detector (ECD). These detectors are often complementary because the ECD shows a selective response to certain compounds or derivatives of compounds, in practice those containing either a halogen or some other electronegative or 'electron-capturing' species such as a nitro moiety, while the FID responds to most organic compounds. More recently, the introduction of nitrogen-selective detectors (NSD), also known as alkali flame-ionization (AFID) and nitrogen–phosphorus (NPD) detectors, which show an enhanced and selective response to compounds containing C–N bonds or phosphorus, further extended the scope of GLC. However, the use of gas chromatography in combination with mass

spectrometry (GC-MS), sometimes referred to as an example of a 'hyphenated technique', provides high sensitivity together with unparalleled selectivity and can identify unequivocally many compounds using only nanogram quantities of material, and has largely supplanted the use of the NPD, certainly as far as qualitative work is concerned.

High performance (originally high pressure) liquid chromatography (HPLC) has achieved wide application in analytical toxicology since the early 1970s. Gases and very volatile solvents excepted, most analytes are amenable to analysis by HPLC or a variant of the basic procedure, in contrast to GC, which is restricted to the analysis of compounds which are both stable and volatile at temperatures up to approximately 350 °C. However, the use of HPLC in the qualitative analysis of drugs is restricted to a certain extent by the lack of a sensitive universal detector analogous to the FID in GC, although a range of sensitive and reliable detectors [notably UV absorption, fluorimetric, electrochemical (ED) and MS] of varying sensitivities and selectivities are now available. In addition, fractions of column effluent corresponding to the chromatographic peaks of interest may be collected and analyzed off-line, for example, by immunoassay.

Immunoassays have also found wide application, whether radioimmunoassay (RIA) or more recent variants, for example, enzyme-multiplied immunoassay technique (EMIT) and cloned enzyme donor immunoassay (CEDIA), and are often highly sensitive. Enzyme-based assays, such as that for paracetamol (acetaminophen), have also been described. However, all of these assays have the disadvantage that antibodies, enzymes, or specific binding proteins have to be prepared for each analyte or group of analytes before an analysis is possible. On the other hand, these and similar assays may often be used directly in small volumes of aqueous media ('homogenous assay'), in contrast to chromatographic methods which often require some form of purification procedure, for example, solvent extraction, prior to the analysis. Although, immunoassays can be very sensitive, some may be poorly selective, that is the antibody may recognize several structurally similar molecules. Sometimes this cross-reactivity can be exploited, as in screening for classes of abused drugs such as opiates.

1.2.1 Drugs and pesticides

Capillary GC, often with MS detection, is widely used both in systematic toxicological analysis (STA) and in the assay of specific analytes, although packed column GC may still find a place for certain applications. HPLC, often nowadays in conjunction with MS, is used to analyze specific compounds or groups of compounds, although STA procedures based on diode-array detectors (DAD) and wavelength ratioing techniques are also used.

The problem in STA (poisons screening, drug screening, unknown screening) is simply to detect reliably as wide a range of compounds as possible in as little sample (plasma/serum/whole blood, urine, vitreous humour, stomach contents or vomit, or tissues) as possible at high sensitivity, but with no false positives. Ideally some sample should be left to permit confirmation of the results using another technique and also quantitation of any poison(s) present to aid clinical interpretation of the results.

When screening for unknown substances it is important to adopt a systematic approach in order to eliminate possible contenders and to 'home in' on the compound(s) present. STA can be divided into three key stages (Figure 1.1). The aim of the sample preparation step is to retain all the toxicologically important substances whilst removing potentially interfering sample matrix components. Thus, as wide a range as possible of analytes of interest, including lipophilic and

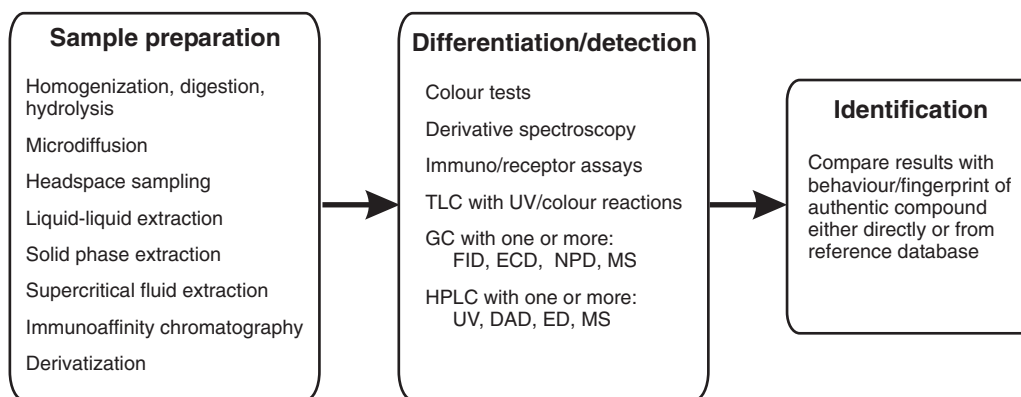


Figure 1.1 The three key steps in systematic toxicological analysis.

moderately polar, acidic, basic and neutral species, should be isolated. To increase the yield of analyte(s), the sample may be treated with β -glucuronidase/arylsulfatase to hydrolyze conjugated metabolites.

The aim of the differentiation/detection step is identify the relevant compounds in the minimum amount of time. This requires a combination of relatively nonspecific ('universal') assays with highly specific methods. Immunoassays, particularly if the antibody has wide cross-reactivity, are useful for identifying classes of drugs. TLC has the advantage that all the nonvolatile materials in the extract remain on the plate, whereas with GC and HPLC there is always the possibility that compounds have not been eluted from the column. Obviously, one analytical technique cannot separate and identify all the possible compounds of interest; for example, only a finite number of compounds can be resolved on a single TLC plate.

The ability of a given analytical method to identify a compound from a given set of test compounds is known as the identification power. One approach in quantifying identification power is the use of discriminating power (DP):

$$DP = 1 - \frac{2M}{N(N-1)} \quad (1.1)$$

where M is the number of pairs of compounds which are not resolved and N is the number of compounds examined. The concept of discriminating power was introduced by Moffat *et al.* (1974) with the aim of quantifying the ability of paper chromatography, TLC and GLC to give unequivocal identification of unknowns. When this approach was applied to an investigation of the separation of 34 neutral compounds in 15 TLC systems, it was shown that one system had the greatest DP (0.75). However, by combining the results from two of the systems, the DP could be increased to 0.88 (Owen *et al.*, 1978). As the identification power increases so the DP increases towards 1.0.

A second approach to define the identification power is the mean list length (MLL). A list length is defined as the number of feasible candidates for a particular analytical parameter, for example, the retention index in a GC system (Section 5.3). The average of all list lengths gives the MLL for that set of compounds in that system. As in the example above, MLLs can be calculated for a combination of systems. MLLs are >1.0 , but will approach 1.0 as identification power increases. In both cases (DP and MLL), examination of a low number of test

compounds will give an overestimation of the identification power of the method (Boone *et al.*, 1999).

The greater the number and range of techniques that are available to the analyst, the greater the probability that unknown substance(s) will be identified correctly. Investigation of the responses of various analytes to different detectors, for example FID/ECD, can provide valuable information about the nature of a compound. HPLC-DAD not only provides spectral information, but also can confirm peak purity via multiple scanning of an eluting peak (Section 8.8.3). Hyphenated techniques such as GC-MS can provide robust analyte identification, particularly when combined with computerized libraries of electron ionization (EI) fragmentation data that can be searched rapidly to confirm compound identity. In addition, chemical ionization (CI) MS can be used to obtain the M_r of a substance.

Analytes may be chemically modified to improve their chromatographic properties or 'detectability', but derivatization can also give useful qualitative information. One old, but classic, example is the so-called 'acetone-shift' (reaction of acetone with a primary amine to give the corresponding Schiff's base). Amphetamine, for example, reacts with acetone to form *N*-(1-methyl-2-phenylethyl)propanimine (Figure 1.2).

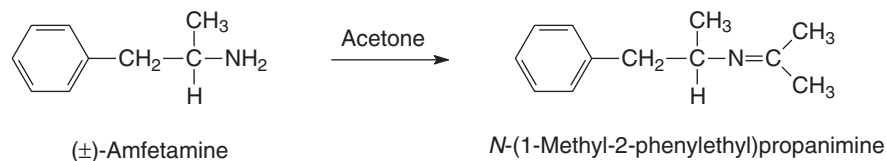


Figure 1.2 Reaction of amphetamine with acetone.

The third step in STA is to compare the observed data with validated database information. Clearly, databases used in compound identification need to be regularly updated, and must include information on not only patent compounds, but also metabolites, common interferences, and contaminants. It is important that the analytical techniques used in establishing such databases are reproducible, both within and between laboratories.

1.2.2 Ethanol and other volatile substances

Enzymatic methods for blood ethanol using alcohol dehydrogenase with spectrophotometric measurement of a coenzyme are available in kit form such as that available for the Abbott TDx/ADx. GC analysis of ethanol either by direct injection of blood or urine diluted with deionized water (Curry *et al.*, 1966), or by static headspace sampling (Machata, 1975), is also widely used, particularly in forensic work. GC is advantageous because methanol, 2-propanol and acetone may be separated and measured simultaneously. Methanol poisoning from ingestion of synthetic alcoholic drinks is one of the few causes of acute poisoning 'epidemics' and measurement of blood methanol is important in confirming the diagnosis and in monitoring treatment.

More than 20 additional volatile compounds may be encountered in acute poisoning cases arising, for example, from deliberate inhalation of vapour in order to become intoxicated ['glue sniffing', solvent abuse, inhalant abuse, volatile substance abuse (VSA)]. Some of these volatile compounds have metabolites that may be measured in urine in order to assess exposure, notably hippuric and methylhippuric (toluric) acids (from toluene and the xylenes, respectively) and

trichloroacetic acid (from trichloroethylene). However, most volatile substances are excreted unchanged in exhaled air, and thus whole blood is the best sample in which to detect and identify these compounds (Flanagan *et al.*, 1990).

1.2.3 Trace elements and toxic metals

In order to help diagnose chronic poisoning, where elevations of only a few $\mu\text{g L}^{-1}$ (parts per billion, ppb, i.e. parts per thousand million) of blood or serum can be important, good accuracy and reproducibility are essential (Braithwaite and Brown, 1988). Sample contamination during collection (e.g. from sample tubes, or even from syringe needles in the case of chromium and manganese) and within the laboratory itself can be serious sources of error. This applies particularly to common elements such as lead and aluminium. Modern methods for measuring toxic metals in biological materials (Table 1.2) vary enormously in terms of complexity, cost, accuracy and sensitivity. Some techniques (isotope dilution MS, neutron activation analysis) are in reality reference methods. Atomic absorption spectrometry with either flame or electrothermal atomization using a graphite furnace has been employed widely, but is being superseded by inductively coupled plasma-mass spectrometry (ICP-MS). In the case of serum iron, however, reliable kits based on the formation of a coloured complex remain widely used in clinical chemistry.

Table 1.2 Methods for the analysis of toxic metals in biological materials

Technique	Mode	Variant
Electrochemical	Potentiometric	Ion selective electrodes
	Coulometric	(Differential pulse) polarography
		Anodic/cathodic stripping voltametry (A/CSV) ^a
Spectrophotometric	Atomic emission (AE)	Flame emission photometry (FEP) ^b
		DC plasma
		Inductively coupled plasma (ICP-AES)
	Atomic absorption (AA)	Flame
		Hydride generation
		Electrothermal
		Cold vapour
X-Ray	Fluorescence	
Nuclear	Neutron activation	
	Proton activation	
Mass spectrometry		Inductively coupled plasma (ICP-MS)

^a Also known as potentiometric stripping analysis (PSA)

^b Normally refers to the use of filters to select the emission wavelength – used mainly for potassium, lithium and sodium assay

ICP-MS is a multi-element technique that can detect and measure elements with detection limits of $\mu\text{g L}^{-1}$ to ng L^{-1} . Different isotopes of an element can also be measured. For some elements, the relative abundance of the isotopes depends upon the source of the metal. Therefore, by measuring the isotope ratios of an element such as lead in a sample from a chronically poisoned patient with those found in material present in the patient's immediate environment it may be possible to localize the source of exposure (Delves and Campbell, 1988). Ethnic cosmetics such as surma may contain from 0 to 80 % elemental lead as either the oxide or sulfide and such products are important causes of lead poisoning. So-called 'traditional' medicines may also contain toxic doses of salts of lead or other toxic metals (Braithwaite and Brown, 1988).

1.3 Provision of analytical toxicology services

The stages in processing analytical work if poisoning is suspected can be divided into preanalytical, analytical and postanalytical phases (Table 1.3).

Table 1.3 Steps in undertaking an analytical toxicological investigation

Pre-analytical	Obtain details of current (suspected) poisoning episode, including any circumstantial evidence of poisoning, and the results of biochemical and haematological investigations, if any. Also obtain the patient's medical and occupational history, if available, and ensure access to the appropriate sample(s) Decide the priorities for the analysis
Analytical	Perform the agreed analysis
Postanalytical	Interpret the results in discussion with the physician looking after the patient or the pathologist. Perform additional analyses, if indicated, using either the original samples or further samples from the patient. Save any unused or residual samples in case they are required for further tests

1.3.1 Samples and sampling

In analytical toxicology, clinical chemistry and related fields, the words 'sample' and 'specimen' are used to denote a portion of a body fluid, tissue, incubation medium, and so on obtained under defined conditions. The samples encountered may range from relatively pure solutions of a drug to a putrefying piece of tissue. Liquids, such as blood, saliva, urine and cerebrospinal fluid (CSF), are generally easier to sample and to analyze than solids and semisolids, which require homogenization or digestion prior to analysis. Blood plasma is used in clinical work if quantitative measurements are needed in order to assess dosage or monitor treatment as in TDM. Urine is commonly used in qualitative work such as drugs of abuse screening as collection is noninvasive and the concentrations of many drugs and other poisons, and their metabolites, tend to be higher than in blood, thereby facilitating analyte detection. Further aspects related to samples and sampling are discussed in Chapter 2.

1.3.2 Choice of analytical method

In responding to a given analytical problem many factors must be considered (Peng and Chiou, 1990). It may seem self-evident that the method used should be appropriate for the intended

analysis. In practice, the choice of method depends on several factors including: the circumstances under which an analysis is requested (i.e. the question being asked), the sample to be analyzed, the nature of the analyte (if known), the expected concentration of any analyte(s), the time available for the analysis, the apparatus available, and the training and experience of the analyst.

The nature of the sample and the expected concentration of any analyte(s) are obvious influences on the choice of method. It may be possible to measure the concentration of a known substance in a relatively pure solution directly using a simple technique such as UV spectrophotometry. However, if the sample is a piece of postmortem tissue such as liver then a wholly different approach will be required. Typically, a representative portion of the tissue will have to be homogenized and the analyte obtained in a relatively pure form by liquid-liquid solvent extraction of the homogenate at an appropriate pH. Further purification or extract concentration steps may be needed prior to instrumental analysis. In the case of organic poisons this will usually be by a chromatographic method such as GC or HPLC because both qualitative and quantitative information can be obtained during the course of the analysis. The choice of instrument may influence the choice of sample preparation procedure, although this is not always the case.

For optically active (chiral) drugs (Table 1.4), the desired clinical activity often resides predominantly in one isomer. The other isomer may be pharmacologically inactive or have different properties from its enantiomer, so administration of a racemate (a 50:50 mixture of enantiomers, Table 1.5) is the same as giving different compounds as far as the body is concerned. The supply of optically active compounds as pure enantiomers (optical isomers) is sometimes indicated by the name used (dexamfetamine, dextropropoxyphene, levorphanol), but this does not always apply (hyoscyne, morphine, physostigmine). Moreover, it is thought that up to 25 % of currently used drugs are chiral and are supplied as racemates, usually without any indication of the fact. Atropine is the approved name for (\pm)-hyoscinamine, for example. Whilst there are few clear indications for providing chiral methodology for routine analytical toxicology at present, it should be noted that chromatographic methods have made a major contribution to the development of pharmacology and therapeutics by providing methods to separate enantiomers on a preparative scale and in biological samples.

1.3.3 *Method implementation and validation*

Whatever method is used for a given analysis it must be validated, that is it must be shown to be 'fit for purpose'. Understanding method validation is clearly important not only when developing a method, but also when implementing a method for routine use. A number of terms important in understanding method validation are given in Table 1.6. A fundamental starting point in any assay is obtaining certified pure reference material, or at least the best approximation to such material that can be attained. When preparing primary standards, particular attention should be paid to the M_r of salts and their degree of hydration (water of crystallization). Analytical results are normally reported in terms of free acid or base and not of a salt.

Obviously the method must possess adequate sensitivity for the task in hand. The limit of sensitivity is a term often used to describe the limit of accurate measurement, but this is better defined as the lower limit of quantification (LLoQ). The limit of detection (detection limit, 'cut-off value') is a better term for limit of sensitivity.

Quantitative methods must also have good precision (reproducibility) and accuracy (the results must reflect the true concentration of the analyte). Selectivity (freedom from interference, specificity) is important when a single species is to be measured, but broad specificity may be useful when screening for the presence of a particular class of compounds as discussed above.

Table 1.4 Summary of chiral nomenclature

Number of chiral centres	If n = number of optical centres there will be 2^n isomers. Molecules with 2 optical centres can exist as four molecules: two diastereoisomers (diastereomers), each consisting of two enantiomers, (i.e., there are <i>two pairs of enantiomers</i>). The exception to this is if two molecules have a <i>plane of symmetry</i> (a plane that divides a molecule into two parts, each a mirror image of the other) and therefore cancel out their net optical rotation. In such cases they are known as meso forms
Nomenclature	Enantiomers possess a unique property in that they rotate plane-polarized light in equal and opposite directions. This is the basis of the (+)/(−) or d/l notation, the former being preferred as it avoids confusion with D/L, however it does not unequivocally distinguish between enantiomers because some molecules may change rotation on forming salts. The notation tells nothing about the absolute configuration (i.e., the spatial arrangement) of the atoms
Rotation of plane polarized light	Rotates to the right: dextrorotatory (+ or d); rotates to the left: levorotatory (− or l).
Fischer	The absolute stereochemistry in the Fischer notation gives the absolute spatial arrangement by reference to D-glyceraldehyde. The letters D or L are used (not to be confused with lower case d or l). The Fischer convention is still used for carbohydrates and amino acids. The original choice of D-glyceraldehyde was arbitrary, but was proved correct by X-ray crystallography
Cahn–Ingold–Prelog (CIP)	The CIP system is the definitive method of assigning absolute configuration. The letters <i>R</i> and <i>S</i> indicate spatial arrangements as follows: <ol style="list-style-type: none"> 1. Assign values to the substituent groups by highest atomic number^a 2. Point the lowest value away 3. If the remainder go from high to low clockwise then <i>R</i> (rectus) 4. If the remainder go from high to low anticlockwise then <i>S</i> (sinister) 5. In case of a tie go to the next atoms along <p>The Fischer convention cannot be simply converted to the CIP system, that is <i>R</i> does not always equate to D. All naturally occurring α-amino acids in mammalian proteins are L. Using the CIP system, cysteine and cystine are <i>S</i> the others, without sulfur, are <i>R</i></p>

^aThe rules are in fact more detailed: Highest atomic number > highest atomic mass > *cis*- prior to *trans*- > like pairs (RR) or (SS) prior to unlike > lone pairs which are considered an atom of atomic number 0. [N.B. R* indicates a single enantiomer obtained, but with unknown stereochemistry].

Table 1.5 Some terms used in stereochemistry

Absolute stereochemistry	The absolute spatial configuration of the atoms of a molecule
Chiral	Hand-like, that is left- and right-handed mirror images
Enantiomer	One mirror image form of a pair of optically active compounds
Epimers	Optically active molecules with more than two chiral centres differing at only one chiral centre
Epimerization	Partial racemization of one chiral centre in a molecule with two or more chiral centres
Diastereoisomers	Structures with more than one chiral centre such that they are not mirror images (enantiomers)
Inversion	Conversion of one enantiomer to the other
Meso	Optically inactive isomer in which the optical activity of chiral centres are balanced
Racemate	Equimolar mixture of both enantiomers of an optically active compound
Racemization	Conversion of a single enantiomer to a racemate

The recovery of the analyte, that is how much of the compound of interest is recovered from the sample matrix during an extraction, for example, is important if sensitivity is limiting, but need not be an issue if the LLoD, accuracy and precision of the assay are acceptable.

Ideally, whatever the methodology employed, quantitative assay calibration should be by analysis of standard solutions of each analyte (normally 6–8 concentrations across the calibration range) prepared in the same matrix and analyzed as a batch along with the test samples. A graph of response against analyte concentration should be prepared and used to calculate the analyte concentration in the sample (so-called ‘external standard’ method).

Any quantitative analysis is a measurement and, in common with all measurements, has associated errors, both random and systematic. In chromatographic and other separation methods the ‘internal standard’ method is often used to reduce the impact of systematic errors such as variations in injection volume or evaporation of extraction solvent during the analysis. Thus, a known amount of a second compound (the internal standard) that behaves similarly to the analyte during the analysis, but elutes at a different place on the chromatogram or is otherwise detected independently of the analyte is added at an appropriate stage in the analysis. Subsequently, the detector response of the analyte relative to the response of the internal standard is plotted against analyte concentration when constructing a calibration graph.

1.3.4 Quality control and quality assurance

Once an analytical method has been validated and implemented it is important to be able to show that the method continues to perform as intended. In qualitative work, known positive and negative specimens should normally be analyzed at the same time as the test sample. A negative control (‘blank’) helps to ensure that false positives (owing to, e.g. contaminated reagents or glassware)

Table 1.6 Terms used when reporting method validation

Term	Notes
Accuracy	The difference between the measured value and the accepted ('true') value
Calibration range	The range of concentrations between the highest and lowest calibration standards. This should encompass the range of concentrations found in the test samples
Coefficient of variation (CV)	An obsolete term for RSD
Higher limit of quantification (HLoQ)	The highest concentration that can be quantified. Not always quoted, but important in assays with a clear upper 'cut-off', for example immunoassays and fluorescence assays
Internal standard	A second compound, not the analyte, added at an appropriate stage in the assay to correct for systematic errors in the analysis
Limit of detection (LoD)	The smallest amount of analyte that can be detected. Usually defined as some multiple (e.g. 5) of the baseline noise (signal to noise ratio = 5) or multiple of the SD of the blank signal
Linearity	A definable and reproducible relationship between a physicochemical measurement (e.g. UV absorption) and the concentration of the analyte. Not necessarily a straight line
Lower limit of quantification (LLoQ)	The lowest concentration that can be measured within defined limits. Usually a concentration for which the precision and accuracy have been set arbitrarily, for example RSD < 20 %
Precision	The scatter of measured values about a mean value Usually quoted as RSD – within-assay and between-assay precision is commonly given
Relative standard deviation (RSD)	The standard deviation of replicate measurements expressed as a percentage of the mean value: $\text{RSD} = \text{SD}/\text{Mean} \times 100 \%$ Useful when comparing precision at different concentrations
Selectivity	The ability to distinguish between the analyte and some other compound
Signal-to-noise (S/N) ratio	Strictly, the response to the analyte divided by the amplitude of random electronic noise of the detection system. In practice, the background signal due to interfering compounds is often greater than the electronic noise

are not obtained. Equally, inclusion of a true positive serves to check that the reagents have been prepared properly and have remained stable.

In quantitative work, assay performance is monitored by the systematic analysis of internal quality control (IQC) samples, independently prepared standard solutions of known composition prepared in the same matrix as the samples and not used in assay calibration. Plotting the results for the IQC samples on a chart allows the day-to-day performance of the assay to be monitored and gives warning of any problems as they arise. When new batches of calibration and IQC samples are prepared it is prudent to ensure comparability of the results obtained with those given by an earlier batch, or with the results obtained using external QC material.

Participation in appropriate external quality assessment (EQA) or proficiency testing (PT) schemes is also important (Wilson, 2002). In such schemes, (sometimes lyophilized) plasma, serum, whole blood, or urine specimens are sent to a number of participating laboratories. After reconstitution in deionized water if appropriate, the specimens are analyzed as if they were real samples and the results are reported before the true or target concentrations are made known.

1.4 Applications of analytical toxicology

Cases in which toxicological analyses are requested tend to fall into: (i) emergency and general hospital toxicology, including 'poisons screening' and (ii) more specialized categories such as forensic toxicology, screening for drugs of abuse, therapeutic drug monitoring (TDM) and occupational/environmental toxicology. However there is considerable overlap between all of these areas.

1.4.1 *Clinical toxicology*

The specialized nature of analytical toxicological investigations dictates that facilities are concentrated in centres that are often remote from the patient. Frequently routine clinical chemical tests will be performed at one site, whilst more complex toxicological analysis will be performed by a different department, possibly at a different location. The toxicology laboratory will usually undertake a range of analyses in addition to emergency toxicology. Despite this, the importance of direct liaison between the physician treating the patient and the analytical toxicologist cannot be overemphasized. Ideally, this liaison should commence before any specimens are collected as some analytes, toxic metals, for example, require special precautions in specimen collection (Section 11.2). At the other extreme, residues of samples held in a clinical chemistry laboratory or by other departments, for example in the emergency department (ED) refrigerator, can be invaluable if the possibility of poisoning is raised in retrospect.

Nowadays, toxicology screening is normally performed using immunoassays and/or TLC and temperature programmed capillary GC-MS. The concept of STA (Section 1.2.1) is valuable, but sometimes overstates the case for absolute reproducibility of retention data. In real life many factors (clinical and circumstantial evidence, availability of a particular poison, past medical history, occupation, number of peaks present on the chromatogram, selective detector responses, etc.) are considered before reporting results.

The range of analyses that can be offered by specialized laboratories, sometimes on an emergency basis, usually encompasses several hundred poisons. 'Poisons screens' must use reasonable amounts of commonly available samples (20–30 mL urine, 2–5 mL plasma). If any tests are to influence immediate patient management, the (preliminary) results should be available within 2–3 h

of receiving the specimens (1 h in the case of paracetamol). In some cases the presence of more than one poison, for example, may complicate the analysis and examination of further specimens from the patient may be required.

A quantitative analysis carried out on whole blood or plasma is usually needed to confirm poisoning unequivocally, but this may not be possible if laboratory facilities are limited, or if the compound is particularly difficult to measure. It is important to discuss the scope and limitations of the tests performed with the clinician concerned and to maintain high standards of laboratory practice, especially when performing tests on an emergency basis. It may be better to offer no result rather than misleading data based on unreliable tests. Clinicians often treat poisoned patients on the basis of suspicion and history rather than await the results of a laboratory test, but may change their approach once they have the result. The treatment of paracetamol poisoning is an example.

Circumstantial evidence of the compound(s) involved in a poisoning episode is often ambiguous and thus, on the rare occasions when an analysis for 'poisons' is indicated, it is advisable to perform a 'poisons screen' routinely in all but the simplest cases. Similarly, the analysis should not end after the first positive finding because additional, hitherto unsuspected compounds may be present. One exception is provided by sublethal carbon monoxide poisoning, which can be difficult to diagnose even if carboxyhaemoglobin measurements are available – circumstantial evidence of poisoning may prove invaluable in such cases. Of course, a 'positive' result on a 'poisons screen' does not of itself confirm poisoning because such a result may arise from incidental or occupational exposure to the poison in question or the use of drugs in treatment.

Blood is often the easiest specimen to obtain from an unconscious patient and is needed for many quantitative measurements. Urine is also a valuable specimen as drug or metabolite concentrations tend to be higher than in blood and relatively large volumes are usually available. However, some compounds, such as many benzodiazepines, are extensively metabolized prior to excretion and then blood plasma is the specimen of choice for detecting the parent compound. Quantitative measurements in urine are generally of little use in emergency toxicology. All poisons screens have limitations (Wiley, 1991). Thus, of the drugs commonly used to treat depression, lithium has to be looked for specifically, whilst those monoamine oxidase inhibitors (MAOIs) which act irreversibly, such as phenelzine, have a prolonged action in the body even though plasma concentrations are very low after overdosage. Any drug that is not bound to the enzyme may be excreted rapidly and may be difficult to detect except in a urine specimen obtained soon after the event. Tricyclic antidepressants are very lipophilic and thus urinary concentrations, even after fatal poisoning, may be below the LLoD of the analytical method if death has occurred relatively soon after the ingestion.

1.4.2 Forensic toxicology

Toxicological investigations of deaths (including fatal road traffic accidents) are often undertaken if there is a possibility that drugs or other poisons may have been involved. These include instances where deliberate poisoning, including self-poisoning, is a possibility, especially if death has occurred in children or whilst in police custody, or when decomposition has taken place to such an extent that it is difficult to glean much information as to the cause of death from a conventional postmortem examination. Non-fatal incidents where toxicological investigations may be useful include collapse whilst in custody, alleged offences under Road Traffic Acts involving ethanol or other drugs, allegations of poisoning of relatives or pets, doping in sex offences and other cases of assault. It may also be important to analyze samples from a suspect for the presence of drugs

such as ethanol, which may have altered his or her perception or behaviour during the course of a crime.

The specimens available may range from fresh blood to decomposing tissues recovered from a partial skeleton, while the quantity available may range from a kilogram of liver to a dried bloodstain. Breath analysis was introduced in the United Kingdom for the detection of the intoxicated motorist in 1967, initially in the form of indicator tubes based on the reduction of potassium dichromate and more recently in the form of evidential breath ethanol instruments. Difficult areas still include screening for a wide range of compounds which could affect driving performance in, say, 2 mL of whole blood while leaving sufficient sample for a quantitative measurement, and detecting drugs used in sexual or other assaults (drug facilitated sexual assault, DFSA).

The primary role of the Coroner in England and Wales and of the Procurator Fiscal in Scotland is to exclude criminal acts as a possible cause of death. The data derived from such courts may be invaluable in monitoring the incidence of fatal poisoning. The importance of adequately documenting all acute poisoning incidents both in the hospital notes and in the laboratory records becomes clear when it is remembered that even an apparently trivial case may eventually be reviewed in detail in a coroner's court. Required documentation includes correctly recorded patient and sample details, the date and time of collection of samples, details of physical examination, nature and timing of treatment, particularly drug treatment, results of investigations (including units), and conversations with poisons information services and the laboratory. The laboratory should fully document all analyses and keep copies of all the reports issued. Samples should be kept, appropriately stored, for example at -20°C , until the conclusion of the case.

In assessing the evidence of the analytical toxicologist the courts are concerned especially with the experience of the analyst, the origin and condition of the samples, and the analytical methods used. The ability to prove continuous and proper custody of the specimen is important. Normally, a minimum of two unrelated analytical methods should be employed before a tentative identification is accepted. The results should be presented together with sufficient information to ensure accurate interpretation of the findings by a coroner, magistrate, judge and/or jury. There is always the possibility of an independent examination by a further expert instructed by another party in the case.

1.4.3 Drug abuse screening

The value of blood, breath or urinary measurements in the diagnosis of ethanol abuse and in monitoring abstinence is clear. Screening for drugs of abuse in urine is also valuable in monitoring illicit drug taking in dependent patients and guards against prescribing controlled drugs for patients who are not themselves drug dependent. Some substances disappear from biological samples very rapidly and, depending on the time between administration and sample collection, the parent compound may not be detectable. Sometimes, however, metabolite identification can be used to demonstrate that a particular drug has been taken. Other samples, such as saliva (oral fluid) and sweat, are being used increasingly to test for drugs of abuse. Analysis of hair, long used to assess chronic exposure to toxic metals, can also provide a history of exposure to illicit drugs and other organic poisons (Baumgartner *et al.*, 1979; Kintz, 2004).

Testing for drugs of abuse may also be valuable in the psychiatric assessment of patients presenting with no overt history of drug abuse. In addition, the diagnosis of maternal drug abuse, either during pregnancy or postpartum, can be important in the management of the neonate. The need for drug abuse screening of personnel in sensitive positions (armed forces, security services,

pilots, drivers) or those applying for such positions ('employment' and 'pre-employment' screening, respectively), has become accepted in recent years. The detection of illicit or performance-enhancing drug use in sport has also assumed importance. In animal sports the definition of an illicit compound is much easier than in man and can include any substance not normally derived from feedstuffs.

Urine is the specimen of choice in many cases, not only because the concentrations of the compounds of interest tend to be higher than in blood, but also because it is by far the easiest specimen to obtain, especially from patients likely to have damaged veins. Moreover, human urine presents less of a hazard than blood to laboratory staff. The illicit drugs encountered most commonly in the United Kingdom are opiates [mainly heroin (diacetylmorphine, diamorphine)], benzodiazepines, cocaine, amfetamines including metamfetamine and methylenedioxymetamfetamine (MDMA, 'ecstasy') and cannabis. In the United States, abuse of cocaine either as the hydrochloride or as the free base ('crack') is relatively common, and a range of additional compounds may also be encountered, including dextropropoxyphene (propoxyphene), fentanyl and phencyclidine (PCP, 'angel dust').

The purity of 'street' drugs varies widely – heroin may be between 2 and 95 % pure, for example. Overdosage, either with excessively pure 'street' drug or with drug 'cut' with a particularly toxic compound, is a further cause of acute poisoning 'epidemics'. Compounds such as atropine, strychnine, lidocaine, chloroquine, quinine and barbiturates may be used to 'cut' street drugs. Serious acute poisoning may occur if tolerance to heroin or methadone has been reduced through abstinence. Methadone is widely used to treat opioid addiction, although buprenorphine is becoming more commonly employed in this role. Other opioids such as codeine, dihydrocodeine and pethidine (meperidine) also occur.

The availability of a variety of immunoassay kits has proved invaluable, especially in employment and pre-employment screening when large numbers of negative results are to be expected and high sensitivity is required. However, confirmation of positive results with MS linked either to capillary GC or HPLC is essential. In clinical samples TLC can be used to resolve drugs such as morphine from compounds such as codeine and pholcodine that are available in over-the-counter preparations. TLC requires a minimum of apparatus and is generally cost effective. It is also amenable to batch sample processing, but is labour intensive, analyte capacity is low, and interpretation of results can be anything but straightforward. Capillary GC, GC-MS, or HPLC-MS is used to detect and identify amfetamines, and increasingly to confirm TLC and immunoassay results.

Ingestion of laxatives and diuretics in order to produce weight loss is not uncommon and can be difficult to diagnose. Collection of serial urine samples over several days is advisable. Detection of the abuse of osmotic laxatives such as lactulose and bulk-formers such as bran is not possible analytically. The covert ingestion or administration of anticoagulants is well documented but difficult to diagnose.

1.4.4 Therapeutic drug monitoring (TDM)

The measurement of plasma concentrations of drugs given in therapy is useful in assessing adherence and for compounds for which pharmacological effects cannot be assessed easily and for which the margin between adequate dosage and overdosage is small (Hallworth and Watson, 2007). The availability of a variety of immunoassay and other kits means that many TDM assays can be performed more conveniently by such means than by chromatographic methods. However, chromatographic assays are still important in the case of antipsychotic, antiretroviral

and immunosuppressant drugs, for amiodarone, where it has proved impossible to produce an antibody which does not cross react significantly with thyroxine and tri-iodothyronine, and in general where active metabolites should be measured as well as the parent compound. Examples include carbamazepine/carbamazepine-10,11-epoxide, clozapine/norclozapine, procainamide/*N*-acetylprocainamide and amitriptyline/nortriptyline.

1.4.5 Occupational and environmental toxicology

The monitoring of occupational or environmental exposure to toxic substances is an important area. Metal ions such as lead and also some organochlorine pesticides such as chlordane and dieldrin have long half-lives in the body and thus accumulation can occur with prolonged exposure to relatively low concentrations. The manufacture of drugs can also present a hazard to those involved via dermal or inhalational absorption. The abuse of alcohol and of controlled drugs is of much current concern in occupational medicine, especially as regards screening for drug or substance abuse amongst potential employees and amongst, for example, operators of heavy machinery and pilots, as discussed above.

Control of occupational exposure to toxic metals, volatile solvents and some other poisons is an integral part of industrial hygiene and has been achieved, in part, by monitoring ambient air concentrations of the compound(s) under investigation. However, an individual's work pattern and attention to safety procedures may greatly influence exposure and 'biological effect' monitoring, where clinical chemical parameters such as blood zinc protoporphyrin are measured as an indicator of lead exposure, is required practice in certain occupations. Not all poisons are amenable to effect monitoring and so 'biological' monitoring is performed widely. This involves measuring blood, urinary, or breath concentrations of a compound and possibly of its metabolites. The American Conference of Governmental Industrial Hygienists (ACGIH) has set guidance values for 38 substances in the United States (ACGIH, 2005), and the Deutsche Forschungsgemeinschaft (DFG) in Germany has introduced values for 63 chemicals (DFG, 2005).

The investigation of the accidental release of chemicals into the workplace or into the environment (so-called chemical incidents) is a topic of current interest. Examples include the Bhopal disaster in India when methyl isocyanate was released into the atmosphere and the Camelford incident in the United Kingdom, in which aluminium sulfate was accidentally added to the local drinking water supply. Toxicological analyses can be valuable, not only in providing evidence of the nature and magnitude of an exposure, but also in demonstrating that no significant exposure has occurred, thereby allaying public apprehension. Clearly, the early collection of appropriate biological samples is essential. In the absence of information to the contrary it is wise to collect 10 mL whole blood (2×5 mL EDTA) and at least 25–50 mL urine (no preservative) from exposed or possibly exposed individuals. The time and date of sampling and the patients' full names should be recorded on the samples and also on a separate record sheet. The samples should be stored at either 4 °C or –20 °C until the appropriate analyses can be arranged. If the incident is investigated in retrospect then samples may exist in a local hospital laboratory, for example.

One area which has been neglected somewhat is that of food-derived poisons. Botulinum toxin and other toxins of microbiological origin are usually considered together with food poisoning. Poisoning from other naturally occurring poisons, which include atropine from *Atropa belladonna*, solanine from potatoes and cyanide from *Cassava* and from apple pips, also occurs (de Wolff, 2004). Here analysis of the foodstuff rather than biological samples can be more helpful in establishing the diagnosis in individual patients. Acute pesticide poisoning sometimes occurs after

ingestion of contaminated produce and again analysis of the foodstuff can be helpful (Stinson *et al.*, 1993). The increasing use of herbal or other 'natural' remedies is an area of especial concern.

1.5 Summary

It is impossible to divorce the study of the analytical methods used in performing toxicological analysis on biological and related samples from the study of toxicology itself, especially clinical and forensic toxicology. By the same token, the laboratory can do nothing to help in the diagnostic process unless someone, be it a clinician, pathologist, or some other person, first suspects poisoning and ensures that specimens are collected and sent for analysis. However, appropriate sample collection and handling is not always straightforward and indeed is a subject in its own right.

References

- ACGIH (American Conference of Governmental Industrial Hygienists) (2005) *TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical and Physical Agents and Biological Exposure Indices 2005*. ACGIH, Cincinnati, OH.
- Baumgartner, A.M., Jones, P.F., Baumgartner, W.A. and Black, C.T. (1979) Radioimmunoassay of hair for determining opiate-abuse histories. *J Nucl Med*, **20**, 748–52.
- Boone, C.M., Franke, J.P., de Zeeuw, R.A. and Ensing, K. (1999) Evaluation of capillary electrophoretic techniques towards systematic toxicological analysis. *J Chromatogr A*, **838**, 259–72.
- Braithwaite, R.A. and Brown, S.S. (1988) Clinical and sub-clinical lead poisoning: A laboratory perspective. *Human Toxicol*, **7**, 503–13.
- Coley, N. G. (1998) Forensic chemistry in 19th century Britain. *Endeavour* **22**, 143–7.
- Conway, E.J. (1947) *Microdiffusion Analysis and Volumetric Error*, 2nd edn, Crosby, Lockwood, London.
- Curry, A.S., Walker, G.W. and Simpson, G.S. (1966) Determination of ethanol in blood by gas chromatography. *Analyst* **91**, 742–3.
- DFG (Deutsche Forschungsgemeinschaft) (2005) *List of MAK and BAT values 2005*. Commission for the investigation of health hazards of chemical compounds in the work area. Report 41. Wiley VCH, Weinheim.
- Delves, H.T. and Campbell, M.J. (1988) Measurement of total lead concentrations and lead isotope ratios in whole blood by use of inductively coupled plasma source mass spectrometry. *J Anal At Spectrom*, **3**, 343–8.
- Flanagan, R.J., Ruprah, M., Meredith, T.J. and Ramsey, J.D. (1990) An introduction to the clinical toxicology of volatile substances. *Drug Saf*, **5**, 359–83.
- Hallworth, M. and Watson, I. (2007) *Therapeutic Drug Monitoring and Laboratory Medicine*, ACB Venture Publications, London.
- Jones, A.W. (1998) Historical developments and present status of forensic toxicology in Sweden. *Nordic Rettsmedisin*, **4**, 35–44.
- Kintz, P. (2004) Hair analysis, in: *Clarke's Analysis of Drugs and Poisons*, 3rd edn (eds A.C. Moffat, D. Osselton and B. Widdop), Pharmaceutical Press, London, pp. 124–133.
- Machata, G. (1975) The advantages of automated blood alcohol determination by head space analysis. *Z Rechtsmed*, **75**, 229–34.
- Marsh, J. (1836) Account of a method of separating small quantities of arsenic from substances with which it may be mixed. *Edin N Philosoph J*, **21**, 229–36.
- Mitchell, C.A. (1938) *Forensic Chemistry in the Criminal Courts*, Institute of Chemistry of Great Britain and Ireland, London.

REFERENCES

19

- Moffat, A.C., Smalldon, K.W. and Brown, C. (1974) Optimum use of paper, thin-layer and gas-liquid chromatography for the identification of basic drugs. I. Determination of effectiveness for a series of chromatographic systems. *J Chromatogr*, **90**, 1–7.
- Niyogi, S.S. (1981) Historical overview of forensic toxicology, in *Introduction to Forensic Toxicology* (eds R.H. Cravey and R.C. Baselt), Biomedical Publications, Davis, pp. 7–24.
- Orfila, M.P. (1821) *A General System of Toxicology, or, A Treatise on Poisons, drawn from the Mineral, Vegetable, and Animal Kingdoms, considered as to their relations with Physiology, Pathology, and Medical Jurisprudence*, Vol 1 & 2, 2nd edn. Trans. Waller JA, E Cox, London.
- Owen, P., Pendlebury, A. and Moffat, A.C. (1978) Choice of thin-layer chromatographic systems for the routine screening for neutral drugs during toxicological analyses. *J Chromatogr*, **161**, 187–93.
- Peng, G.W. and Chiou, W.L. (1990) Analysis of drugs and other toxic substances in biological samples for pharmacokinetic studies. *J Chromatogr*, **531**, 3–50.
- Plenck, J.J. (1781) *Elementa Medicinae et Chirurgiae Forensis*, Graeffer, Wien, p. 36.
- Reinsch, H. (1841) On the action of metallic copper on solutions of certain metals, particularly with reference to the detection of arsenic. *Philosoph Mag*, **19**, 480–83 (first published *J Praktische Chem* 1841; 19).
- Stinson, J.C., O’Gharabhain, F., Adebayo, G., Chambers, P.L. and Feely, J. (1993) Pesticide-contaminated cucumber. *Lancet*, **341**, 64.
- Watson, K. (2004) *Poisoned Lives: English Poisoners and Their Victims*, Hambledon and London, London.
- Wiley, J.F. (1991) Difficult diagnoses in toxicology: Poisons not detected by the comprehensive drug screen. *Pediatr Clin North Am*, **38**, 725–37.
- Wilson, J. (2002) External quality assessment schemes for toxicology. *Forensic Sci Int*, **128**, 98–103.
- de Wolff, F.A. (2004) Natural toxins, in *Clarke’s Analysis of Drugs and Poisons* (eds A.C. Moffat, M.D. Osselton and B. Widdop), 3rd edn, Pharmaceutical Press, London, pp. 189–201.

