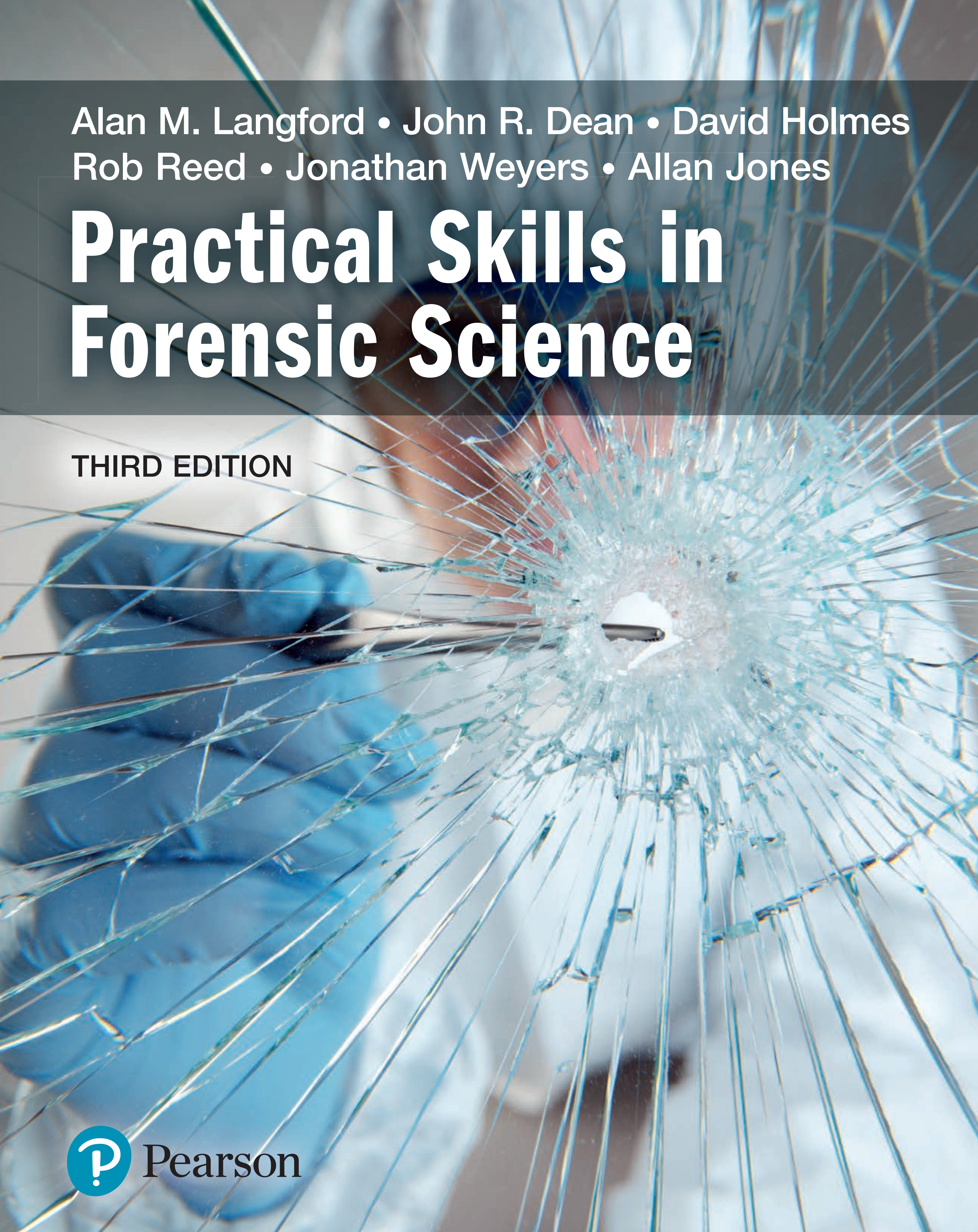

Alan M. Langford • John R. Dean • David Holmes
Rob Reed • Jonathan Weyers • Allan Jones

Practical Skills in Forensic Science

THIRD EDITION

Pearson

Practical Skills in

Forensic Science

Practical Skills in Forensic Science

ALAN M. LANGFORD
JOHN R. DEAN
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Preface

'Forensic Science is defined as the application of science to serve the purposes of the law. The sciences used in the analysis of physical evidence include many aspects of chemistry, biology, physics, mathematics and statistics. This multidisciplinary nature is a core feature of forensic science.'

QAA for HE Subject Benchmark Statement for Forensic Science (2012).

Practical skills form the cornerstone of forensic science. However, the diversity of skills required in the laboratory means that a student's experience may be limited. While some techniques do require specific skills, many of them are transferable generic skills that are required throughout the subject area.

Limited time constraints of the modern curriculum often preclude or minimise laboratory time. It is the aim of this book to provide a general guidance for use in and out of practical sessions and also to cover a range of techniques from the basic to the more advanced.

In creating the third edition of *Practical Skills in Forensic Science*, we have maintained the approach of the previous editions, with the aim of providing support to students taking forensic science based courses in a concise and user-friendly manner. Key points, definitions, illustrations, 'how to' boxes, checklists, worked examples, tips and hints are included where appropriate. However, we have also used this opportunity of the new edition to restructure the layout, to literally start at the beginning of the laboratory process and progress to the end, with the dissemination of results.

In updating and thoroughly revising the book to include a 'taste' of the latest developments in methodology, we have considered carefully the Quality Assurance Agency UK Subject Benchmarking statements for Forensic Science, reviewed and updated in 2012, and have attempted to cover all the generic skills, along with the practical aspects of the subject specific topics in forensic science. With that in mind we have carefully arranged sections to cover the following themes: crime scene investigation; forensic biology; and, forensic chemistry. We have also been mindful to support one of the QAA's aims for forensic science degrees (under- and postgraduate) programmes in the context of practical skills. Specifically, "to develop a sound knowledge of science and of laboratory and other transferable skills which are of value in areas of employment other than forensic science, such as schools, hospitals, analytical science-based companies, the pharmaceutical industry, the Home Office and other government agencies".

To students who buy this book, we hope you will find it useful in the laboratory during your practical classes and in your project work – this is not a book to be left on the bookshelf.

We would like to take this opportunity to thank our wives (Jules, Lynne, Polly, Gill, Mary and Angela) and families for their continued support, and to recognise the following colleagues and friends who have provided assistance, comment and food for thought at various points during the production of all editions: James Abbott, Gary Askwith, Chris Baldwin, Dave Bannister, Jon Bookham, Samantha Bowerbank, Susan Carlile, Michelle Carlin, Jim Creighton, Sarah Cresswell, Martin Davies, Mike Deary, Sylvain Denieul, Les Dix, Marcus Durrant, Jackie Eager, Gordon Forrest, Laura Heath, Kris Heath, Derek Holmes, Helen Hooper, Alan Jones, Ed Ludkin, Ton Nelson, Tom Marshall, Dave Osborne, Justin Perry, Lee Rounds, Jane Shaw, Tony Simpson, Dave Wealleans and Ian Winship. We would also like to thank the staff of Pearson Education for the friendly support over the years, and would wish to acknowledge Richelle Zakrewski, Rufus Cornow, Pat Bond, Owen Knight, Simon Lake, Alex Seabrook and Pauline Gillett.

As with previous editions, we would be grateful to hear of any errors you might notice, so that these can be put right at the earliest opportunity.

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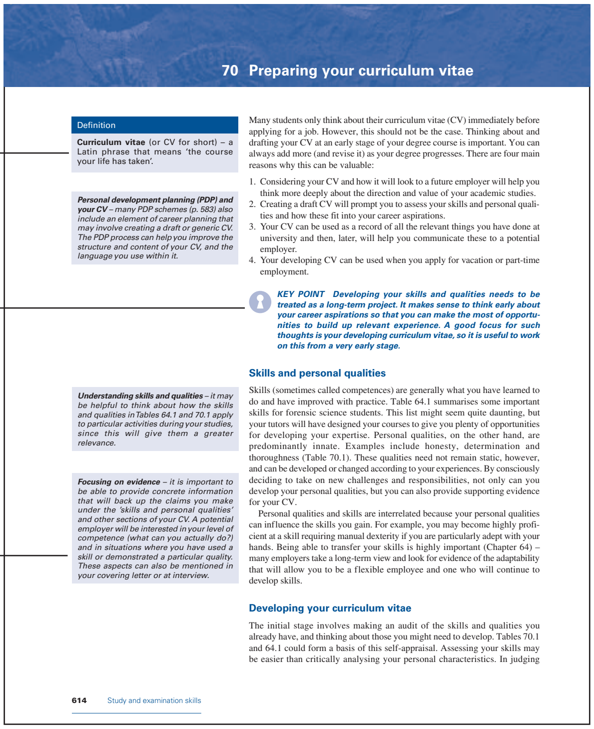
Guided tour

Definitions of key terms and concepts are highlighted in the text margin.

Key points highlight critical features of methodology.

Tips and hints provide useful hints and practical advice, and are highlighted in the text margin.


70 Preparing your curriculum vitae

Definition

Curriculum vitae (or CV for short) – a Latin phrase that means 'the course your life has taken'.

Personal development planning (PDP) and your CV *– many PDP schemes (p. 583) also include an element of career planning that may involve creating a draft or generic CV. The PDP process can help you improve the structure and content of your CV, and the language you use within it.*

Many students only think about their curriculum vitae (CV) immediately before applying for a job. However, this should not be the case. Thinking about and drafting your CV at an early stage of your degree course is important. You can always add more (and revise it) as your degree progresses. There are four main reasons why this can be valuable:

1. Considering your CV and how it will look to a future employer will help you think more deeply about the direction and value of your academic studies.
2. Creating a draft CV will prompt you to assess your skills and personal qualities and how these fit into your career aspirations.
3. Your CV can be used as a record of all the relevant things you have done at university and then, later, will help you communicate these to a potential employer.
4. Your developing CV can be used when you apply for vacation or part-time employment.

KEY POINT Developing your skills and qualities needs to be treated as a long-term project. It makes sense to think early about your career aspirations so that you can make the most of opportunities to build up relevant experience. A good focus for such thoughts is your developing curriculum vitae, so it is useful to work on this from a very early stage.

Skills and personal qualities

Understanding skills and qualities *– it may be helpful to think about how the skills and qualities in Tables 64.1 and 70.1 apply to particular activities during your studies, since this will give them a greater relevance.*

Skills (sometimes called competences) are generally what you have learned to do and have improved with practice. Table 64.1 summarises some important skills for forensic science students. This list might seem quite daunting, but your tutors will have designed your courses to give you plenty of opportunities for developing your expertise. Personal qualities, on the other hand, are predominantly innate. Examples include honesty, determination and thoroughness (Table 70.1). These qualities need not remain static, however, and can be developed or changed according to your experiences. By consciously deciding to take on new challenges and responsibilities, not only can you develop your personal qualities, but you can also provide supporting evidence for your CV.

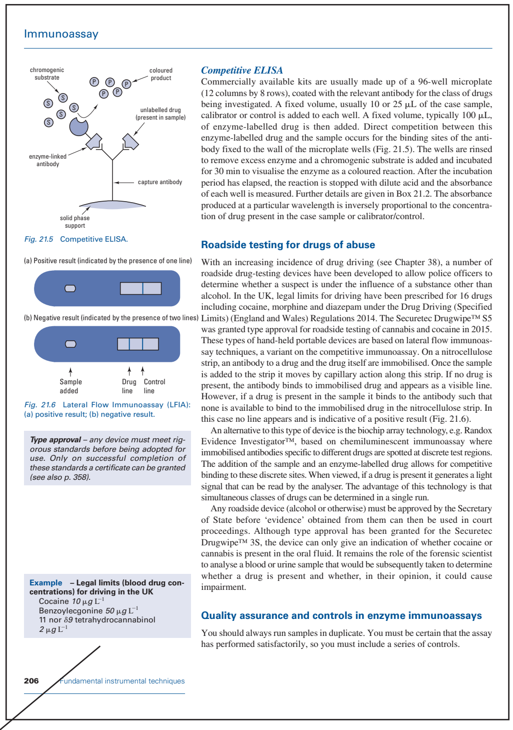
Personal qualities and skills are interrelated because your personal qualities can influence the skills you gain. For example, you may become highly proficient at a skill requiring manual dexterity if you are particularly adept with your hands. Being able to transfer your skills is highly important (Chapter 64) – many employers take a long-term view and look for evidence of the adaptability that will allow you to be a flexible employee and one who will continue to develop skills.

Focusing on evidence *– it is important to be able to provide concrete information that will back up the claims you make under the 'skills and personal qualities' and other sections of your CV. A potential employer will be interested in your level of competence (what can you actually do?) and in situations where you have used a skill or demonstrated a particular quality. These aspects can also be mentioned in your covering letter or at interview.*

Developing your curriculum vitae

The initial stage involves making an audit of the skills and qualities you already have, and thinking about those you might need to develop. Tables 70.1 and 64.1 could form a basis of this self-appraisal. Assessing your skills may be easier than critically analysing your personal characteristics. In judging

614 Study and examination skills


Immunoassay

chromogenic substrate
coloured product
unlabelled drug (present in sample)
enzyme-linked antibody
capture antibody
solid phase support

Fig. 21.5 Competitive ELISA.

Competitive ELISA

Commercially available kits are usually made up of a 96-well microplate (12 columns by 8 rows), coated with the relevant antibody for the class of drugs being investigated. A fixed volume, usually 10 or 25 μL of the case sample, calibrator or control is added to each well. A fixed volume, typically 100 μL, of enzyme-labelled drug is then added. Direct competition between this enzyme-labelled drug and the sample occurs for the binding sites of the antibody fixed to the wall of the microplate wells (Fig. 21.5). The wells are rinsed to remove excess enzyme and a chromogenic substrate is added and incubated for 30 min to visualise the enzyme as a coloured reaction. After the incubation period has elapsed, the reaction is stopped with dilute acid and the absorbance of each well is measured. Further details are given in Box 21.2. The absorbance produced at a particular wavelength is inversely proportional to the concentration of drug present in the case sample or calibrator/control.

(a) Positive result (indicated by the presence of one line)
(b) Negative result (indicated by the presence of two lines)
Sample added
Drug line
Control line

Fig. 21.6 Lateral Flow Immunoassay (LFIA): (a) positive result; (b) negative result.

Roadside testing for drugs of abuse

With an increasing incidence of drug driving (see Chapter 38), a number of roadside drug-testing devices have been developed to allow police officers to determine whether a suspect is under the influence of a substance other than alcohol. In the UK, legal limits for driving have been prescribed for 16 drugs including cocaine, morphine and diazepam under the Drug Driving (Specified Limits) (England and Wales) Regulations 2014. The Securetec Drugwipe™ S5 was granted type approval for roadside testing of cannabis and cocaine in 2015. These types of hand-held portable devices are based on lateral flow immunoassay techniques, a variant on the competitive immunoassay. On a nitrocellulose strip, an antibody to a drug and the drug itself are immobilised. Once the sample is added to the strip it moves by capillary action along this strip. If no drug is present, the antibody binds to immobilised drug and appears as a visible line. However, if a drug is present in the sample it binds to the antibody such that none is available to bind to the immobilised drug in the nitrocellulose strip. In this case no line appears and is indicative of a positive result (Fig. 21.6).

An alternative to this type of device is the biochip array technology, e.g. Randox Evidence Investigator™, based on chemiluminescent immunoassay where immobilised antibodies specific to different drugs are spotted at discrete test regions. The addition of the sample and an enzyme-labelled drug allows for competitive binding to these discrete sites. When viewed, if a drug is present it generates a light signal that can be read by the analyser. The advantage of this technology is that simultaneous classes of drugs can be determined in a single run.

Type approval *– any device must meet rigorous standards before being adopted for use. Only on successful completion of these standards a certificate can be granted (see also p. 358).*

Any roadside device (alcohol or otherwise) must be approved by the Secretary of State before 'evidence' obtained from them can then be used in court proceedings. Although type approval has been granted for the Securetec Drugwipe™ 3S, the device can only give an indication of whether cocaine or cannabis is present in the oral fluid. It remains the role of the forensic scientist to analyse a blood or urine sample that would be subsequently taken to determine whether a drug is present and whether, in their opinion, it could cause impairment.

Example – Legal limits (blood drug concentrations) for driving in the UK
Cocaine *10* $\mu g\ L^{-1}$
Benzoylecgonine *50* $\mu g\ L^{-1}$
11 nor δ*9* tetrahydrocannabinol
2 $\mu g\ L^{-1}$

Quality assurance and controls in enzyme immunoassays

You should always run samples in duplicate. You must be certain that the assay has performed satisfactorily, so you must include a series of controls.

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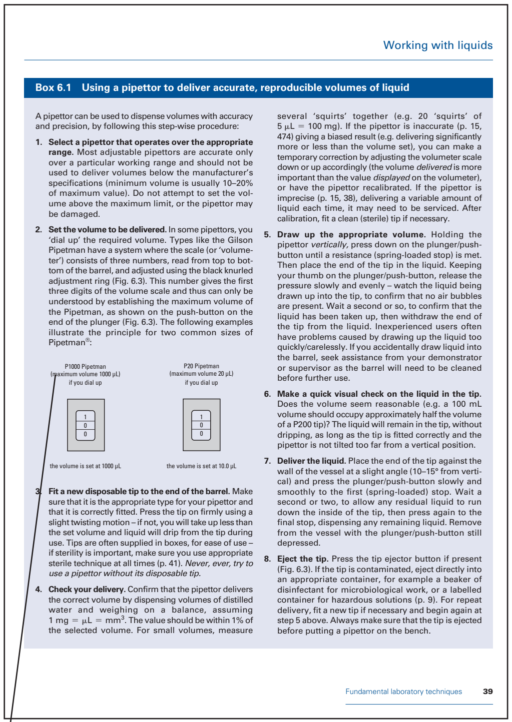
Working with liquids

Box 6.1 Using a pipettor to deliver accurate, reproducible volumes of liquid

A pipettor can be used to dispense volumes with accuracy and precision, by following this step-wise procedure:

1. **Select a pipettor that operates over the appropriate range.** Most adjustable pipettors are accurate only over a particular working range and should not be used to deliver volumes below the manufacturer's specifications (minimum volume is usually 10–20% of maximum value). Do not attempt to set the volume above the maximum limit, or the pipettor may be damaged.
2. **Set the volume to be delivered.** In some pipettors, you 'dial up' the required volume. Types like the Gilson Pipetman have a system where the scale (or 'volumeter') consists of three numbers, read from top to bottom of the barrel, and adjusted using the black knurled adjustment ring (Fig. 6.3). This number gives the first three digits of the volume scale and thus can only be understood by establishing the maximum volume of the Pipetman, as shown on the push-button on the end of the plunger (Fig. 6.3). The following examples illustrate the principle for two common sizes of Pipetman®:

P1000 Pipetman (maximum volume 1000 μL) if you dial up
1 0 0
the volume is set at 1000 μL

P20 Pipetman (maximum volume 20 μL) if you dial up
1 0 0
the volume is set at 10.0 μL

3. **Fit a new disposable tip to the end of the barrel.** Make sure that it is the appropriate type for your pipettor and that it is correctly fitted. Press the tip on firmly using a slight twisting motion – if not, you will take up less than the set volume and liquid will drip from the tip during use. Tips are often supplied in boxes, for ease of use – if sterility is important, make sure you use appropriate sterile technique at all times (p. 41). *Never, ever, try to use a pipettor without its disposable tip.*
4. **Check your delivery.** Confirm that the pipettor delivers the correct volume by dispensing volumes of distilled water and weighing on a balance, assuming 1 mg = μL = mm^3. The value should be within 1% of the selected volume. For small volumes, measure several 'squirts' together (e.g. 20 'squirts' of 5 μL = 100 mg). If the pipettor is inaccurate (p. 15, 474) giving a biased result (e.g. delivering significantly more or less than the volume set), you can make a temporary correction by adjusting the volumeter scale down or up accordingly (the volume *delivered* is more important than the value *displayed* on the volumeter), or have the pipettor recalibrated. If the pipettor is imprecise (p. 15, 38), delivering a variable amount of liquid each time, it may need to be serviced. After calibration, fit a clean (sterile) tip if necessary.
5. **Draw up the appropriate volume.** Holding the pipettor *vertically,* press down on the plunger/push-button until a resistance (spring-loaded stop) is met. Then place the end of the tip in the liquid. Keeping your thumb on the plunger/push-button, release the pressure slowly and evenly – watch the liquid being drawn up into the tip, to confirm that no air bubbles are present. Wait a second or so, to confirm that the liquid has been taken up, then withdraw the end of the tip from the liquid. Inexperienced users often have problems caused by drawing up the liquid too quickly/carelessly. If you accidentally draw liquid into the barrel, seek assistance from your demonstrator or supervisor as the barrel will need to be cleaned before further use.
6. **Make a quick visual check on the liquid in the tip.** Does the volume seem reasonable (e.g. a 100 mL volume should occupy approximately half the volume of a P200 tip)? The liquid will remain in the tip, without dripping, as long as the tip is fitted correctly and the pipettor is not tilted too far from a vertical position.
7. **Deliver the liquid.** Place the end of the tip against the wall of the vessel at a slight angle (10–15° from vertical) and press the plunger/push-button slowly and smoothly to the first (spring-loaded) stop. Wait a second or two, to allow any residual liquid to run down the inside of the tip, then press again to the final stop, dispensing any remaining liquid. Remove from the vessel with the plunger/push-button still depressed.
8. **Eject the tip.** Press the tip ejector button if present (Fig. 6.3). If the tip is contaminated, eject directly into an appropriate container, for example a beaker of disinfectant for microbiological work, or a labelled container for hazardous solutions (p. 9). For repeat delivery, fit a new tip if necessary and begin again at step 5 above. Always make sure that the tip is ejected before putting a pipettor on the bench.

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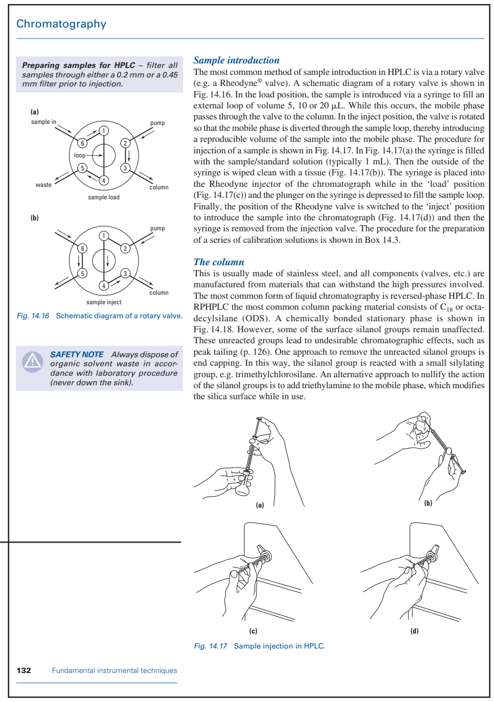
Examples are included in the margin to illustrate important points without interrupting the flow of the main text.

Worked examples and **'How to' boxes** set out the essential procedures in a step-by-step manner.


Chromatography

Preparing samples for HPLC – filter all samples through either a 0.2 mm or a 0.45 mm filter prior to injection.

(a) sample in, pump, loop, waste, column, sample load

(b) pump, column, sample inject

Fig. 14.16 Schematic diagram of a rotary valve.

SAFETY NOTE Always dispose of organic solvent waste in accordance with laboratory procedure (never down the sink).

Sample introduction

The most common method of sample introduction in HPLC is via a rotary valve (e.g. a Rheodyne® valve). A schematic diagram of a rotary valve is shown in Fig. 14.16. In the load position, the sample is introduced via a syringe to fill an external loop of volume 5, 10 or 20 μL. While this occurs, the mobile phase passes through the valve to the column. In the inject position, the valve is rotated so that the mobile phase is diverted through the sample loop, thereby introducing a reproducible volume of the sample into the mobile phase. The procedure for injection of a sample is shown in Fig. 14.17. In Fig. 14.17(a) the syringe is filled with the sample/standard solution (typically 1 mL). Then the outside of the syringe is wiped clean with a tissue (Fig. 14.17(b)). The syringe is placed into the Rheodyne injector of the chromatograph while in the 'load' position (Fig. 14.17(c)) and the plunger on the syringe is depressed to fill the sample loop. Finally, the position of the Rheodyne valve is switched to the 'inject' position to introduce the sample into the chromatograph (Fig. 14.17(d)) and then the syringe is removed from the injection valve. The procedure for the preparation of a series of calibration solutions is shown in Box 14.3.

The column

This is usually made of stainless steel, and all components (valves, etc.) are manufactured from materials that can withstand the high pressures involved. The most common form of liquid chromatography is reversed-phase HPLC. In RPHPLC the most common column packing material consists of C_{18} or octadecylsilane (ODS). A chemically bonded stationary phase is shown in Fig. 14.18. However, some of the surface silanol groups remain unaffected. These unreacted groups lead to undesirable chromatographic effects, such as peak tailing (p. 126). One approach to remove the unreacted silanol groups is end capping. In this way, the silanol group is reacted with a small silylating group, e.g. trimethylchlorosilane. An alternative approach to nullify the action of the silanol groups is to add triethylamine to the mobile phase, which modifies the silica surface while in use.

(a) (b) (c) (d)

Fig. 14.17 Sample injection in HPLC.

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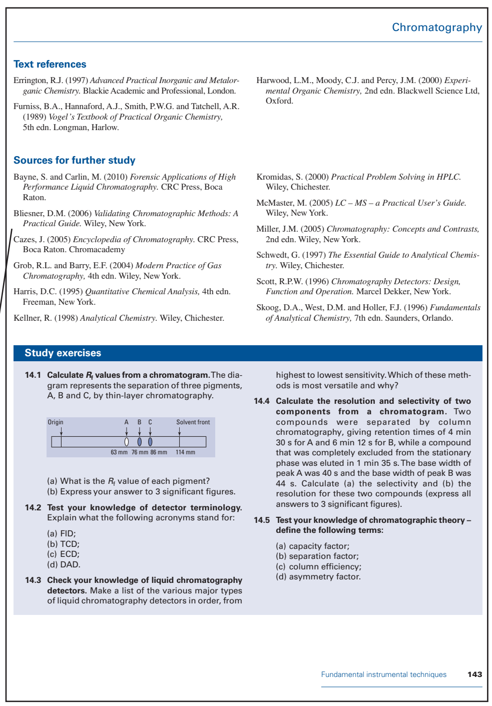
Figures are used to illustrate key points, techniques and equipment.


Chromatography

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Study exercises

14.1 Calculate R_f values from a chromatogram. The diagram represents the separation of three pigments, A, B and C, by thin-layer chromatography.

Origin A B C Solvent front
63 mm 76 mm 86 mm 114 mm

(a) What is the R_f value of each pigment?
(b) Express your answer to 3 significant figures.

14.2 Test your knowledge of detector terminology. Explain what the following acronyms stand for:

(a) FID;
(b) TCD;
(c) ECD;
(d) DAD.

14.3 Check your knowledge of liquid chromatography detectors. Make a list of the various major types of liquid chromatography detectors in order, from highest to lowest sensitivity. Which of these methods is most versatile and why?

14.4 Calculate the resolution and selectivity of two components from a chromatogram. Two compounds were separated by column chromatography, giving retention times of 4 min 30 s for A and 6 min 12 s for B, while a compound that was completely excluded from the stationary phase was eluted in 1 min 35 s. The base width of peak A was 40 s and the base width of peak B was 44 s. Calculate (a) the selectivity and (b) the resolution for these two compounds (express all answers to 3 significant figures).

14.5 Test your knowledge of chromatographic theory – define the following terms:

(a) capacity factor;
(b) separation factor;
(c) column efficiency;
(d) asymmetry factor.

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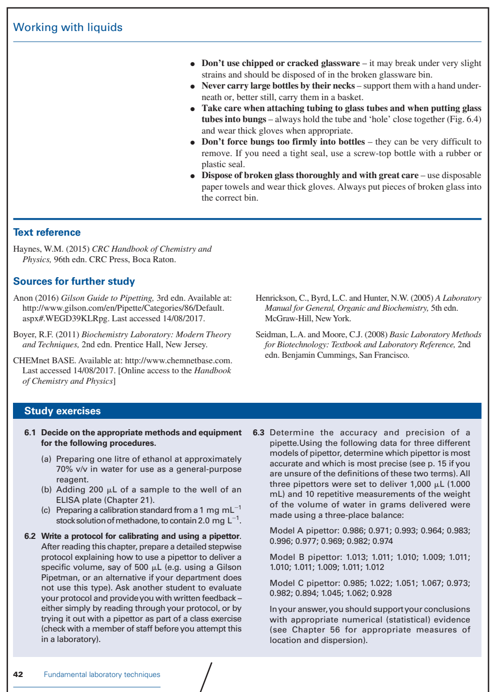
Working with liquids

- **Don't use chipped or cracked glassware** – it may break under very slight strains and should be disposed of in the broken glassware bin.
- **Never carry large bottles by their necks** – support them with a hand underneath or, better still, carry them in a basket.
- **Take care when attaching tubing to glass tubes and when putting glass tubes into bungs** – always hold the tube and 'hole' close together (Fig. 6.4) and wear thick gloves when appropriate.
- **Don't force bungs too firmly into bottles** – they can be very difficult to remove. If you need a tight seal, use a screw-top bottle with a rubber or plastic seal.
- **Dispose of broken glass thoroughly and with great care** – use disposable paper towels and wear thick gloves. Always put pieces of broken glass into the correct bin.

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Study exercises

6.1 Decide on the appropriate methods and equipment for the following procedures.

(a) Preparing one litre of ethanol at approximately 70% v/v in water for use as a general-purpose reagent.
(b) Adding 200 μL of a sample to the well of an ELISA plate (Chapter 21).
(c) Preparing a calibration standard from a 1 mg mL^{-1} stock solution of methadone, to contain 2.0 mg L^{-1}.

6.2 Write a protocol for calibrating and using a pipettor. After reading this chapter, prepare a detailed stepwise protocol explaining how to use a pipettor to deliver a specific volume, say of 500 μL (e.g. using a Gilson Pipetman, or an alternative if your department does not use this type). Ask another student to evaluate your protocol and provide you with written feedback – either simply by reading through your protocol, or by trying it out with a pipettor as part of a class exercise (check with a member of staff before you attempt this in a laboratory).

6.3 Determine the accuracy and precision of a pipette. Using the following data for three different models of pipettor, determine which pipettor is most accurate and which is most precise (see p. 15 if you are unsure of the definitions of these two terms). All three pipettors were set to deliver 1,000 μL (1.000 mL) and 10 repetitive measurements of the weight of the volume of water in grams delivered were made using a three-place balance:

Model A pipettor: 0.986; 0.971; 0.993; 0.964; 0.983; 0.996; 0.977; 0.969; 0.982; 0.974

Model B pipettor: 1.013; 1.011; 1.010; 1.009; 1.011; 1.010; 1.011; 1.009; 1.011; 1.012

Model C pipettor: 0.985; 1.022; 1.051; 1.067; 0.973; 0.982; 0.894; 1.045; 1.062; 0.928

In your answer, you should support your conclusions with appropriate numerical (statistical) evidence (see Chapter 56 for appropriate measures of location and dispersion).

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Sources for further study – every chapter is supported by a section giving printed and electronic sources for further study.

Study exercises are included in every chapter to reinforce learning with problems and practical exercises.

For the student

This book aims to provide guidance and support over the broad range of your undergraduate course, including laboratory classes, project work, lectures, tutorials, seminars and examinations, as outlined below:

Chapters 1–44 cover a wide range of specific practical skills required in forensic science

These are based on the authors' experience of the questions students often ask in practical classes, and the support that is needed in order to get the most out of particular exercises. The text includes tips, hints, definitions, worked examples and 'how to' boxes that set out the key procedures in a step-by-step manner, with appropriate comments on safe working practice. The material ranges from basic laboratory procedures, such as experimental design (Chapter 5) and preparing solutions (Chapters 6–8), through the fundamentals of crime scene investigation and scientific support (Chapters 23–29) to the more advanced practical procedures that you might use during a final-year project, for example analytical methods such as chromatography (Chapter 14) and spectroscopy (Chapters 16–20).

Chapters 30–44 cover the major sub-disciplines within forensic chemistry and forensic biology

As with the chapters on specific skills and techniques, these chapters are designed to provide practical guidance and advice on the various aspects of forensic analysis from a student's perspective. Many of the chapters contain 'how to' boxes and worked examples along with specific case examples, to illustrate how the individual disciplines operate in relation to particular criminal cases.

Chapters 45–49 deal with IT and library resources

These chapters will help you get the most out of the resources and information available in your library, and online resources and the Internet, as well as providing helpful guidance on the use of software packages for data analysis.

Chapters 50–57 explain data analysis and presentation

This will be an important component of your course and you will find that these chapters guide you through the skills and techniques required, ranging from the presentation of results as graphs or tables through to the application of statistical tests. Worked examples are used to reinforce the numerical aspects wherever possible.

Chapters 58–63 deal with evaluating and communicating data

These chapters will help you with preparing assignments, essays and laboratory reports, alongside support in relation to oral, visual and written forms of communication. The ability to evaluate information is an increasingly important skill in contemporary society, and practical guidance is provided here, as well as more specific advice, for example on preparing and presenting a forensic report.

Chapters 64–70 cover general skills

These include a number of transferable skills that you will develop during your course, for example self-evaluation, time management, teamwork, preparing for examinations and creating a CV.

We hope that you will find this book a helpful guide throughout your course, and beyond.

Acknowledgements

Figures

Figure 2.3 from http://www.sigmaaldrich.com/safety-center/understanding-the-label.html#67-548-ec-pictograms, Sigma-Aldrich. Used with permission of Merck KGaA, Darmstadt Germany and/or its affiliates; Figure 22.10 from http://www.sciex.com/Documents/brochures/MDQPlus_brochure.pdf page 5, Image provided by SCIEX © 2017; Figure 23.3 adapted from *Digital Imaging Procedure. V2.1. Publication number: 58/07* Home Office Scientific Development Branch (Cohen, N. and Maclennan-Brown, K. 2007) p.36, https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/378451/DIP_2.1_16-Apr-08_v2.3—Web_2835.pdf, © Crown copyright. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/; Figure 23.4 from Image Authentication for Digital Image Evidence, 5, pp. 1–11 (2006), Figure 8, *Forensic Science Journal*, 5, pp1–11 (Wen, C. and Yang, K. 2006), Central Police University Taiwan, ROC Taiwan Academy of Forensic Sciences, Taiwan, R.O.C; Figure 30.1 from Laura Barnes, With permission of L. Barnes; Figure 36.3 after *Phytoplankton* 2ed., Edward Arnold (Boney, D.A 1989) Reprinted by permission of Cambridge University Press.

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Tables

Table 38.3 from Therapeutic and toxic blood concentrations of nearly 1000 drugs and other xenobiotics, *Critical care* 16(4), R136 (Schulz, M., Iwersen-Bergmann, S., Andresen, H and Schmoldt, A. 2012), https://doi.org/10.1186/cc11441 © Schulz et al.; licensee BioMed Central Ltd. 2012 https://creativecommons.org/licenses/by/2.0/legalcode; Table on page 383 from https://www.gov.uk/government/statistics/seizures-of-drugs-in-england-and-wales-financial-year-ending-2015, © Crown copyright. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/; Table 44.1 from www.gov.uk/government/collections/fire-statistics, © Crown copyright. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/; Table 66.1 from http://www.belbin.com/belbin-for-teams/, © Belbin® 2012

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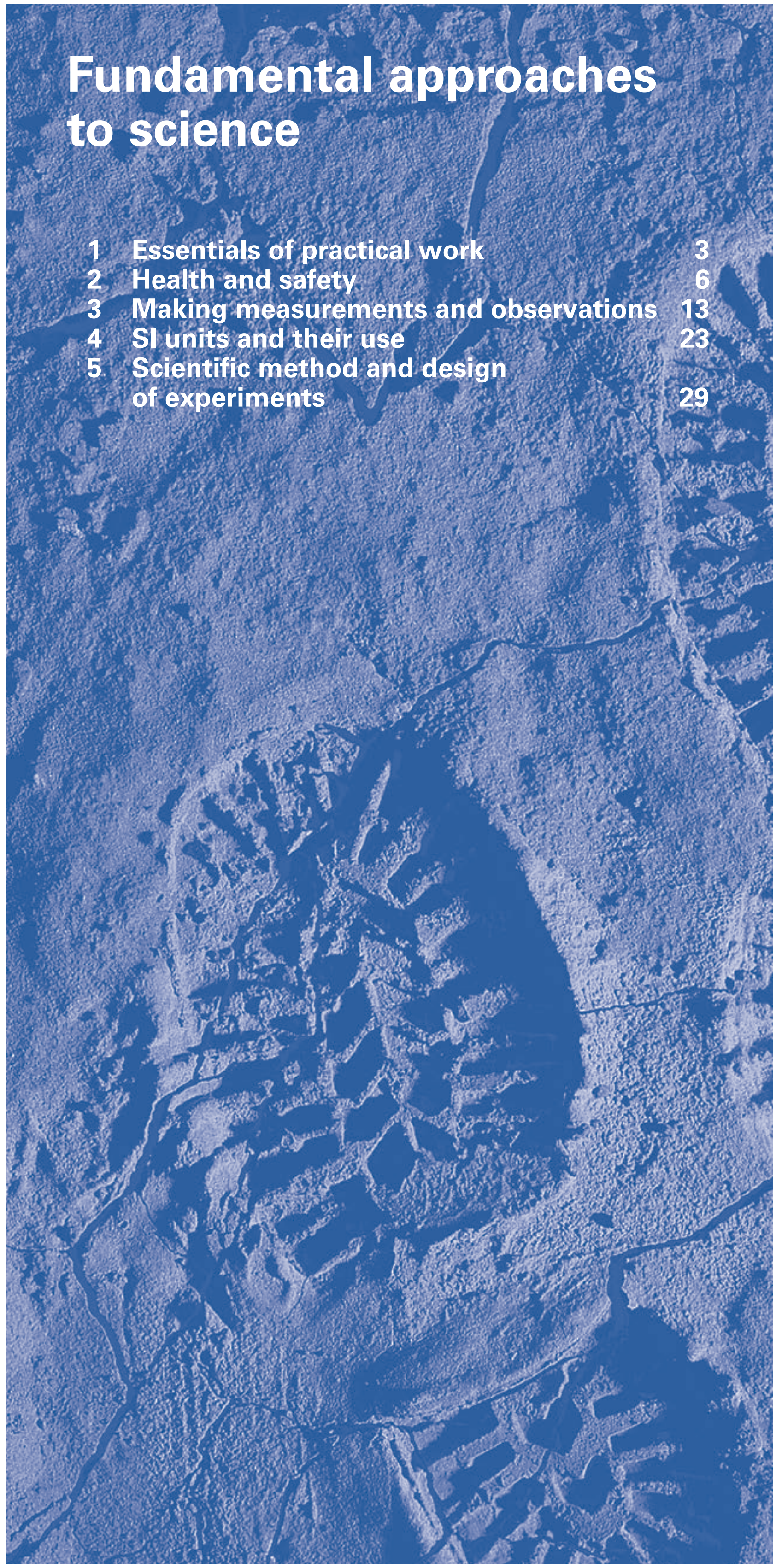
Abbreviations

AAS	atomic absorption spectrometer
AES	atomic emission spectrometer
AC	affinity chromatography
ACS	American Chemical Society
AMPFLP	amplified fragment-length polymorphisms
ANOVA	analysis of variance
AP	acid phosphatase
APCI	atmospheric pressure chemical ionisation
A_r	relative atomic mass
ASO	allele specific oligonucleotide
ATP	adenosine triphosphate
BMI	body mass index
b.pt.	boiling point
Cb	measured blood or breath alcohol concentration
CDT	carbohydrate-deficient transferrin
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
CO	carbon monoxide
CoA	calculated alcohol concentration in blood or breath
CODIS	combined DNA index system
COSHH	control of substances hazardous to health
CoV	coefficient of variation
CRM	certified reference material
CSM	crime scene manager
CZE	capillary zone electrophoresis
DAD	diode array detection
DCM	dichloromethane
DFSA	drug-facilitated sexual assault
DMAC	p dimethylaminocinnamaldehyde
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
EI	electron impact (ionisation)
ELISA	enzyme-linked immunosorbent assay
EMR	electromagnetic radiation
en	ethylenediamine
EOF	electro-osmotic flow
ESDA	electrostatic detection apparatus
ESI	electrospray ionisation
FAAS	flame atomic absorption spectrometer
FID	flame ionisation detector
FOA	first officer attending
FSS	Forensic Science Service
FT	Fourier transform
FT–IR	Fourier transform – infrared spectroscopy
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
GFC	gel filtration chromatography
GGT	γ glutamyl transferase
GHB	γ hydroxy butyrate
GPC	gel permeation chromatography
GRIM	glass refractive index measurement
GSR	gunshot residue

HASAW	hazards at work
H&E	haemotoxylin and eosin
HCB	hexachloro-1,3-butadiene
HCL	hollow cathode lamp
HFBA	heptafluorobutyric anhydride
HIC	hydrophobic interaction chromatography
HPLC	high-performance liquid chromatography
HV	hypervariable region
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma–mass spectrometry
IEC	ion exchange chromatography
IEF	isoelectric focusing
IR	infrared (radiation)
ISE	ion selective electrode
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
KM	Kastle Meyer
LC–MS	liquid chromatography–mass spectrometry
LCN	low copy number
LGC	Laboratory of the Government Chemist
LMG	leuco malachite green
LSD	lysergic acid
m	mass
MDL	minimum detectable level
MDMA	3,4-methylenedioxymethylamphetamine (ecstasy)
MEKC	micellar electrokinetic chromatography
MEL	maximum exposure limit
m.pt.	melting point
M_r	relative molecular mass
MS	mass spectrometry
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
mtDNA	mitochondrial DNA
NCA	National Crime Agency
NDNAD	National DNA Database
NH	null hypothesis
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
NP-HPLC	normal phase high-performance liquid chromatography
ODS	octadecylsilane
OEL	occupational exposure standard
PAGE	polyacrylamide gel electrophoresis
PCIA	phenol/chloroform/isoamyl alcohol
PCR	polymerase chain reaction
PDT	pyridyldiphenyl triazine
PFA	perfluoroalkoxyvinylether
PTFE	polytetrafluoroethylene
PLOT	porous layer open tubular (column)
PMT	photomultiplier tube
PPE	personal protection equipment
r	Widmark factor
R_f	relative frontal mobility
RNA	ribonucleic acid
RP-HPLC	reversed phase high-performance liquid chromatography
rpm	revolutions per minute
SAX	strong anion exchange
SCOT	support coated open tubular (column)
SCX	strong cation exchange
SDS	sodium dodecyl sulphate

SE	standard error (of the sample mean)
SEM	scanning electron microscopy
SGM	second generation multiplex
SI	Système International d'Unités
SIO	senior investigating officer
SLR	single lens reflex
SNP	single nucleotide polymorphism
SOCO	scene of crime officer
SOP	standard operating procedure
STR	short tandem repeat
TCA	trichloroacetic acid
TCD	thermal conductivity detector
TE	Tris/EDTA
TEA	thermal energy analyser
TG	thermogravimetry
TLC	thin-layer chromatography
TMS	tetramethylsilane
TRIS	tris(hydroxymethyl)aminomethane or 2-amino-2-hydroxymethyl-1,3-propane-diol
UK	United Kingdom
UKAS	United Kingdom Accreditation Services
URL	uniform resource locator
USEPA	United States Environmental Protection Agency
UV	ultraviolet (radiation)
Vd	volume of distribution
VNTR	variable number of tandem repeats
WCOT	wall-coated open tubular (column)

Fundamental approaches to science



1 Essentials of practical work

Developing practical skills *– these will include:*

- *designing experiments;*
- *observing and measuring;*
- *recording data;*
- *analysing and interpreting data;*
- *reporting/presenting.*

All knowledge and theory in science has originated from practical observation and experimentation – this is equally true for disciplines as diverse as chromatography and molecular genetics. Practical work is an important part of most courses and often accounts for a significant proportion of the assessment marks. The abilities developed in practical classes will continue to be useful throughout your course and beyond, some within science and others in any career you choose.

Being prepared

***KEY POINT** You will get the most out of practicals if you prepare well in advance. Do not go into a practical session assuming that everything will be provided, without any input or involvement on your part.*

The main points to remember are:

- **Read any handouts in advance** – make sure you understand the purpose of the practical and the particular skills involved. Does the practical relate to, or expand on, a current topic in your lectures? Is there any additional preparatory reading that will help?
- **Take along appropriate textbooks,** to explain aspects of the practical.
- **Consider what safety hazards might be involved,** and any precautions you might need to take before you begin (p. 6).
- **Listen carefully to any introductory guidance and note any important points** – adjust your schedule/handout as necessary.
- **During the practical session, organise your bench space** – make sure your lab book is adjacent to, but not within, your working area. You will often find it easier to keep clean items of glassware, etc. on one side of your working space, with used equipment on the other side.
- **Write up your work as soon as possible** and submit it on time, or you may lose marks.
- **Catch up on any work you have missed as soon as possible** – preferably, before the next practical session.

Using textbooks in the lab *– take this book (or photocopies of relevant pages) along to the relevant classes, so that you can make full use of the information during your practical sessions.*

SAFETY NOTE ***Using mobile phones*** *– these should never be used in a lab class, as there is a risk of contamination from hazardous substances. Always switch off your mobile phone before entering a laboratory.*

Ethical and legal aspects of laboratory work

You will need to consider the ethical and legal implications of forensic science work throughout your degree studies:

- **Safe working in the laboratory** means following a code of safe practice, supported by legislation, alongside a moral obligation to avoid harm to yourself and others, as discussed in Chapter 2.
- **Any laboratory work that involves working with animal or human tissues** must be considered carefully and must be performed in accordance with the relevant rules/legislation, including appropriate disposal after use.

In addition to the above, forensic science throws up some moral and legal dilemmas, and students are increasingly likely to be asked to reflect on ethical topics, for example in group discussions on current issues or recent cases in the media. For many topics, you will find that there are not always 'right' or 'wrong' answers, and it is important to be able to consider these issues in a rational and logical manner, and to provide reasoned argument in support of a particular viewpoint.

Basic requirements for laboratory work

Recording practical results

An A4 loose-leaf ring binder offers flexibility, since you can insert laboratory handouts or lined and graph paper at appropriate points. The danger of losing one or more pages from a loose-leaf system is the main drawback. Bound books avoid this problem, although those containing alternating lined/graph or lined/blank pages tend to be wasteful – it is often better to paste sheets of graph paper into a bound book as required.

All of your forensic examination notes should be written in ink. Any mistakes should simply be scored out and initialled. Buy a black, spirit-based (permanent) marker for labelling lab glassware, etc. Fibre-tipped fine line drawing/lettering pens are useful for preparing final versions of graphs and diagrams for assessment purposes. Use a see-through ruler (with an undamaged edge) for graph drawing, so that you can see data points and information below the ruler as you draw.

Presenting results *– although you don't need to be a graphic designer to produce work of a satisfactory standard, presentation and layout are important and you will lose marks for poorly presented work.*

Calculators

These range from basic machines with no pre-programmed functions and only one memory, to sophisticated programmable minicomputers with many memories. The following may be helpful when using a calculator:

- **Power sources** – choose a battery-powered machine, rather than a mains-operated or solar-powered type. You will need one with basic mathematical/scientific operations, including powers, logarithms (p. 503), roots and parentheses (brackets), together with statistical functions such as sample means and standard deviations (Chapter 55).
- **Mode of operation** – the older operating system used by, for example, Hewlett-Packard calculators, is known as the reverse Polish notation. To calculate the sum of two numbers, the sequence is 2 [enter] 4 + and the answer 6 is displayed. The more usual method of calculating this equation is as 2 + 4 =, which is the system used by the majority of modern calculators. Most newcomers find the latter approach to be more straightforward. Spend some time finding out how a calculator operates, for example does it have true algebraic logic (√ then number, rather than number then √)? How does it deal with scientific notation (p. 502)?
- **Display** – some calculators will display an entire mathematical operation (e.g. '2 + 4 = 6'), while others simply display the last number/operation. The former type may offer advantages in tracing errors.
- **Complexity** – in the early stages, it is usually better to avoid the more complex machines, full of impressive-looking but often unused pre-programmed functions. Go for more memory, parentheses or statistical functions rather than engineering or mathematical constants. Programmable calculators may be worth considering for more advanced studies. However, it is important to note that such calculators are often unacceptable for exams.

Using inexpensive calculators *– many unsophisticated calculators have a restricted display for exponential numbers and do not show the 'power of 10', e.g. displaying 2.4×10^{-5} as 2.4^{-05}, or 2.4E–05, or 2.4–05.*

Using calculators for numerical problems *– Chapter 54 gives further advice.*

Presenting more advanced practical work

In some practical reports and in project work, you may need to use more sophisticated presentation equipment. Computer-based graphics packages can be useful – choose easily-read fonts such as Arial or Helvetica for posters and

Presenting graphs and diagrams *– ensure these are large enough to be easily read – a common error is to present graphs or diagrams that are too small, with poorly chosen scales.*

Printing on acetates *– standard overhead transparencies are not suitable for use in laser printers or photocopiers – you need to make sure that you use the correct type.*

consider the layout and content carefully (p. 573). Alternatively, you could use fine-line drawing pens and dry-transfer lettering/symbols, such as those made by Letraset, although this approach can be more time-consuming than computer-based systems.

To prepare overhead transparencies for spoken presentations, you can use spirit-based markers and acetate sheets. An alternative approach is to print directly from a computer-based package, using a laser printer and special acetates, or use a digital projector with, for example, PowerPoint (p. 547). You can also photocopy on to special acetates. Advice on content and presentation is given in Chapter 59.

Sources for further study

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Study exercises

1.1 Consider the value of practical work. Spend a few minutes thinking about the purpose of practical work within a specific part of your course (e.g. a particular first-year module) and then write a list of the six most important points.

1.2 Make a list of items required for a particular practical exercise. This exercise is likely to be most useful if you can relate it to an appropriate practical session on your course, e.g. bulk drug examination.

1.3 Check your calculator skills. Carry out the following mathematical operations, using either a hand-held calculator or a PC with appropriate 'calculator' software.

(a) $5 \times (2 + 6)$
(b) $(8.3 \div [6.4 - 1.9]) \times 24$ (to four significant figures)
(c) $(1 \div 32) \times (5 \div 8)$ (to three significant figures)
(d) $1.2 \times 10^5 + 4.0 \times 10^4$ in scientific notation (see p. 44)
(e) $3.4 \times 10^{-2} - 2.7 \times 10^{-3}$ in 'normal' notation (i.e. conventional notation, not scientific format) and to three decimal places.

(See also the numerical exercises in Chapter 54.)

2 Health and safety

***Health and Safety legislation** – In the UK, the **Health and Safety at Work etc. Act 1974** provides the main legal framework for health and safety. **The Control of Substances Hazardous to Health (COSHH) Regulations 2002** impose specific legal requirements for risk assessment wherever hazardous chemicals or biological agents are used, with approved codes of practice for the control of hazardous substances, carcinogens and biological agents, including pathogenic microbes.*

Health and safety legislation requires institutions to provide a working environment that is safe and without risk to health. Where appropriate, training and information on safe working practices must be provided. Students and staff must take reasonable care to ensure the health and safety of themselves and of others, and must not misuse any safety equipment.

***KEY POINT** All practical work must be carried out with safety in mind, to minimise the risk of harm to yourself and to others – safety is everyone's responsibility.*

Risk assessment

A risk assessment is a systematic approach to hazard identification and control. It is essential to consider what aspects of a laboratory or crime scene investigation activity can cause injury (to people) and then to control measures that will reduce the risk of injury to an acceptable level. Important aspects to consider are:

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

***KEY POINT** It is important to distinguish between the HAZARD of a substance and the RISK resulting from exposure.*

The risk assessment process

The five-step process requires you to:

1. **Identify the hazards and risk**: One way to do this is by using 'PEME', i.e. People, Equipment, Materials and Environment:
   (a) **'People' hazards** can cover a range of issues including the individual themselves and the systems that people have to use. In this 'people' context, consider the following terms: training, capabilities/restrictions, supervision, communication, adequate numbers and human error.
   (b) **'Equipment' hazards** relate to the equipment to be used, e.g. injection port of a gas chromatograph (GC) is typically 270°C (Chapter 14); it will also consider related aspects of the equipment including repair, maintenance, handling, storage, cleaning and operation of the equipment.
   (c) **'Materials' hazards** cover any liquid, solid or gas associated with the task, e.g. using controlled drugs to determine their concentration in blood (Chapter 38). This aspect also covers any by-products or waste generated by the activity.
   (d) **'Environment' hazards** relate to the surrounds you are working in, e.g. in crime scene investigation you may encounter poor lighting, heating and ventilation, poor access and egress, tripping/slipping hazards, restricted space/visibility and other activities taking place nearby.

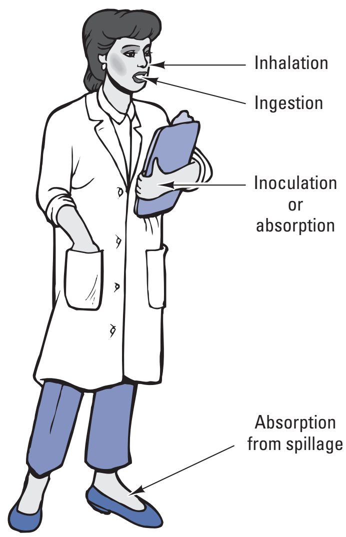

Fig. 2.1 Major routes of entry of harmful substances into the body.

Definitions

Hazard – the potential of a substance or biological agent to cause harm.

Likelihood – the assessment of the likelihood of harm prior to any control measures being in place, given the amount/nature of substance used and the environment/manner it's used in.

Risk – a measure of the likelihood and severity prior to any control measures being in place, calculated by likelihood × severity.

Severity – this is a substance-specific rather than activity-specific measurement that can be indicated on the MSDS. In each instance, the highest numerical assessment should be used to calculate the risk.

2. **Identify who can be harmed and how:**
   (a) **Who** – Although a task may seem to be well managed, if control measures fail then a whole range of people could be injured, e.g. co-workers in the area or people visiting the area. Your risk assessment should consider all those people who could potentially be harmed if the control measures fail.
   (b) **How** – the major routes of chemical exposure (Fig. 2.1) are:
      i **inhalation** – breathing in small particles or chemical vapours is the most common pathway;
      ii **dermal** – some chemicals can be absorbed into the body;
      iii **eye contact** – rubbing your eyes after chemical exposure with your hands (with or without gloves);
      iv **ingestion** – inadvertent hand to mouth transmission;
      v **subcutaneous penetration** – improper use of glass pipettes/syringes and their disposal can lead to injury and exposure of the underlying skin tissue.
3. **Identify the current controls and decide if more is required:**
   (a) **Identify the control measures currently in place** for each hazard you have identified: physical controls (i.e. local exhaust ventilation); procedural controls (i.e. a safe working procedure for the task); and behavioural controls (i.e. adequate supervision and monitoring of behaviour).
   (b) **Identify the risks and decide on precautions** – a risk matrix analysis. A risk analysis is a qualitative estimate of risk associated with each applicable task; it assumes that the planned or existing controls are in place. Box 2.1 shows you how to undertake a risk matrix analysis. The risk matrix evaluates the risk by allocating a numeric risk level and the tolerability of the hazard.
4. **Record your findings** – you will need to record your assessments. You will need to:
   (a) State clearly what task/activity the risk assessment covers.
   (b) Ensure that the hazards and controls are clearly listed.
   (c) Consider all those people who could potentially be harmed.
   (d) Ensure that the appropriate member of staff signs off the assessment.
   (e) Make sure the completed risk assessments are readily available to those who might need them.
5. **Review as necessary**. Risk assessments should be reviewed on a regular basis. The period of review should reflect the hazards: the greater the hazards the more frequent the review.

Box 2.1 How to perform a risk matrix analysis

A risk matrix analysis allows you to prioritise the likelihood and severity of risk to an individual from the hazard identified.

1 **Using the form** in Fig. 2.2 (illustration is for superglue fuming of fingerprints using cyanoacrylate) conduct a COSHH assessment of the chemical to be used in a practical laboratory class.

2 **First consult the Material Safety Data Sheet** (MSDS) supplied; all manufacturers of hazardous chemicals are required to provide one of these sheets for all products that they sell.

3 **Consult the hazard pictograms** (Fig. 2.3) for visible relevant information. In addition, H (hazard) statements and P (precautionary) statements are

Box 2.1 (Continued)

available on the MSDS sheets and/or at http://www.sigmaaldrich.com/help-welcome/hazard-and-precautionary-statements.html (click on the Hazard statement overview or Precautionary statement overview tabs).

4 **Assess the 'likelihood' of harm** prior to any control measures being in place, given the amount/nature of substance used and the environment/manner it is used in (Table 2.1)

5 **Assess the severity** using the MSDS sheets for guidance (Table 2.1).

6 **Calculate the risk using the risk matrix** (Table 2.1) This calculation should quote the highest risk associated with the substance. You should consider additional control measures to further reduce the final risk's numerical value.

Experiment Record - short COSHH record form

COSHH Assessments for **Experiment Title:** Chemical Enhancement of latent fingerprints

Name of Assessor__Alan Langford____ Signed __A Langford__Date__01/10/15 _______

Substance	**H Statement**[1]	**Hazard**[2] Key hazard(s) associated with the substance	Signal Word?[3]	Likelihood[4]	Severity[5]	Risk[6] (before additional control measures)	Specific Risk Control Measures[7]	Controlled Risk[8]
Basic yellow 40	H315, 319, 335	Causes skin, respiratory and serious eye irritation	WARNING	3	4	12	GLP, PPE, gloves safety glasses	4
Ethyl-2-cyanoacrylate	H315, 319, 335	Causes skin, respiratory and serious eye irritation	WARNING	3	4	12	GLP, PPE, gloves safety glasses, used in dedicated fingerprint fuming cabinet	4
Basic yellow working solution Ethanol (100ml+0.2g dye)	H225	Highly flammable liquid and vapour	WARNING (for neat ethanol)	2	3	6	GLP, PPE, use in fume hood	3

Substance	**P Statement**[9]	**Storage**[10]	**Emergency Procedures (in event of spillage, fire etc.)**[11] **Detail**	**Disposal**[12]
Basic yellow 40	P261, 305, 351, 338	Cool, sealed container, dry well ventilated	Fire: wear S/C breathing apparatus if necessary; extinguish; water or CO_2 Spillage: water; do not let enter drain First aid: wash with water for 15 mins	In solvent; to flammable waste for incineration;
Basic yellow working solution	P210, 261, 305, 351, 338	Store in cool place. Keep container tighty closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.	Fire: water, CO_2, powder, foam Spillage: wear gloves, Absorb material, wash area with water First aid: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	Flammable waste for incineration

Fig. 2.2 Risk matrix analysis for chemical enhancement of fingerprints using cyanoacrylate fuming and Basic Yellow 40.

Box 2.1 (Continued)

Description	Pictogram	Hazard class and hazard category:
Exploding Bomb		Unstable explosives Explosives of Divisions 1.1, 1.2, 1.3, 1.4 Self reactive substances and mixtures, Types A,B Organic peroxides, Types A,B
Flame		Flammable gases, category 1 Flammable aerosols, categories 1,2 Flammable liquids, categories 1,2,3 Flammable solids, categories 1,2 Self-reactive substances and mixtures, Types B,C,D,E,F Pyrophoric liquids, category 1 Pyrophoric solids, category 1 Self-heating substances and mixtures, categories 1,2 Substances and mixtures, which in contact with water, emit flammable gases, categories 1,2,3 Organic peroxides, Types B,C,D,E,F Pyrophoric gas (US only)
Flame Over Circle		Oxidizing gases, category 1 Oxidizing liquids, categories 1,2,3
Gas Cylinder		Gases under pressure: - Compressed gases - Liquefied gases - Refrigerated liquefied gases - Dissolved gases
Corrosion		Corrosive to metals, category 1 Skin corrosion, categories 1A,1B,1C Serious eye damage, category 1
Skull and Crossbones		Acute toxicity (oral, dermal, inhalation), categories 1,2,3
Exclamation Mark		Acute toxicity (oral, dermal, inhalation), category 4 Skin irritation, category 2 Eye irritation, category 2 Skin sensitisation, category 1 Specific Target Organ Toxicity – Single exposure, category 3
Health Hazard		Respiratory sensitization, category 1 Germ cell mutagenicity, categories 1A,1B,2 Carcinogenicity, categories 1A,1B,2 Reproductive toxicity, categories 1A,1B,2 Specific Target Organ Toxicity – Single exposure, categories 1,2 Specific Target Organ Toxicity – Repeated exposure, categories 1,2 Aspiration Hazard, category 1
Environment		Hazardous to the aquatic environment - Acute hazard, category1 - Chronic hazard, categories 1,2

Fig. 2.3 Hazard warning pictograms. Sigma Aldrich. Available at: http://www.sigmaaldrich.com/safety-center/understanding-the-label.html#67-548-ec-pictograms.