1
Introduction to Pharmaceutical Analytical Chemistry

1.1 Introduction

In daily conversation, words like pills and drugs are typically in use. However, when entering the pharmaceutical world, wording becomes very important and correct terms should be used. A pharmaceutical preparation contains a substance that is pharmacologically active, which is called the active pharmaceutical ingredient (or active ingredient). The active pharmaceutical ingredient is often abbreviated to API. A large number of APIs exists and two examples are shown in Figure 1.1, namely paracetamol (acetaminophen), which is used against pain, and insulin aspart, which is used in the treatment of diabetes. Paracetamol is a small molecule API (or small molecule drug) produced by organic synthesis and with a molecular mass of 151 Da. Insulin aspart, on the other hand, is a two-chain peptide produced by recombinant DNA technology. It is a large molecule drug with a molecular mass of 5826 Da and is termed a biopharmaceutical due to its biological origin.

An active ingredient is not given (administered) to the patient as a pure substance, but is combined with excipients (synonymous with inactive ingredients) into a dosage form in order to be able to give an exact dose to the patient. The excipients are not
pharmacologically active. The dosage form can be a tablet, a capsule, or a syrup for oral administration, an injection for parenteral administration, or an ointment for topical administration. The excipients used in pharmaceutical preparations serve several functions, and these can be summarized as follows:

- Ensure that the dosage form has a shape and size that is easy to use for the patient.
- Ensure that the API is released and delivered to the patient in the correct amount.

### Table 1.1 Excipients in paracetamol tablets and paracetamol syrup (example)

<table>
<thead>
<tr>
<th>Content</th>
<th>Amount (mg)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tablet (mass 285 mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>250</td>
<td>Active ingredient</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose</td>
<td></td>
<td>Binder</td>
</tr>
<tr>
<td>Maize starch</td>
<td></td>
<td>Disintegrant</td>
</tr>
<tr>
<td>Talcum</td>
<td></td>
<td>Glidant</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td></td>
<td>Lubricant</td>
</tr>
<tr>
<td><strong>Syrup (volume 1 mL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>24</td>
<td>Active ingredient</td>
</tr>
<tr>
<td>Sorbitol</td>
<td></td>
<td>Sweetener</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td>Sweetener</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone</td>
<td></td>
<td>Thickenning agent</td>
</tr>
<tr>
<td>Saccharine sodium salt</td>
<td></td>
<td>Sweetener</td>
</tr>
<tr>
<td>Methylparaben</td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td>Ethylparaben</td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td>Propylparaben</td>
<td></td>
<td>Preservative</td>
</tr>
<tr>
<td>Sodium metabisulfite</td>
<td></td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Citric acid</td>
<td></td>
<td>pH regulator</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td></td>
<td>pH regulator</td>
</tr>
<tr>
<td>Strawberry aroma</td>
<td></td>
<td>Flavouring agent</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>Solvent</td>
</tr>
</tbody>
</table>

Figure 1.1  Paracetamol (small molecule drug) and insulin aspart (biopharmaceutical)
• Ensure that the pharmaceutical preparation has an acceptable stability.
• Ensure that the pharmaceutical preparation does not have an unpleasant taste or odour.
• Facilitate production of the pharmaceutical preparation.

The excipients vary widely for different preparations. To exemplify this, Table 1.1 shows the excipients of tablets and syrup containing *paracetamol* as the active ingredient. Paracetamol has both an *analgesic* and an *antipyretic* effect, which means that it is used against pain and fever. Each paracetamol tablet has a total mass of 285 mg. Paracetamol constitutes 250 mg, while the remaining 35 mg is made up of excipients. The excipients include a disintegrating agent, a lubricant, a glidant, and a binder. *Binders, lubricating agents,* and *gliding agents* are added to facilitate manufacture. The *disintegrating agent* ensures rapid disintegration of the tablet in the stomach of the patient and rapid release of paracetamol.

Paracetamol syrup (liquid preparation) is a 24 mg/mL solution of paracetamol in water. In addition, several excipients are added. *Sweetening* and *flavouring agents* are added for

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**Box 1.1 Official European Pharmacopoeia definitions**

**Medicinal product:**
(i) Any substance or combination of substances presented as having properties for treating or preventing disease in human beings and/or animals or (ii) any substance or combination of substances that may be used in or administered to human beings and/or animals with a view either to restoring, correcting, or modifying physiological functions by exerting a pharmacological, immunological, or metabolic action, or to making a medical diagnosis.

**Pharmaceutical preparation:**
Pharmaceutical preparations are medicinal products generally consisting of active substances that may be combined with excipients, formulated into a dosage form suitable for the intended use, where necessary after reconstitution, presented in a suitable and appropriately labelled container.

*Example: Paracetamol tablets as received from the pharmacy*

**Dosage form:**
Physical manifestation of a product that contains the active ingredient(s) and/or excipient(s) that are intended to be delivered to the patient.

*Examples: Tablets, syrups*

**Active pharmaceutical ingredient:**
Any substance intended to be used in the manufacture of a medicinal product and that, when so used, becomes an active ingredient of the medicinal product. Such substances are intended to furnish a pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure and function of the body.

*Example: Paracetamol*

**Excipient:**
Any constituent of a medicinal product that is not an active substance. Adjuvants, stabilizers, antimicrobial preservatives, diluents, antioxidants, for example, are excipients.

*Example: Hydroxypropyl cellulose*
better taste. *Antimicrobial agents* are added to prevent bacterial growth and *antioxidants* are added to reduce chemical degradation of the API. The latter aspect is particularly important with liquid pharmaceutical preparations, because chemical compounds in solution are more sensitive to oxidative degradation. In addition, agents are added to increase the viscosity of the syrup and to control pH. Controlling pH is important in order to keep the dissolved active substance stable and high viscosity makes the syrup easier to handle by the patient.

The terms pharmaceutical preparation, dosage form, API, excipient, and *medicinal product* have strict definitions; these are summarized in Box 1.1. The definitions are important to have in mind when reading this textbook.

Pharmaceutical preparations may be divided into ‘over the counter drugs’ (OTC drugs), which may be sold directly to the consumer in pharmacies and supermarkets without restrictions, and ‘prescription only medicine’ (POM) that must be prescribed by a licensed practitioner (normally a medical doctor). Paracetamol tablets are a typical OTC drug, whereas paroxetine tablets used against serious depression is a typical POM.

### 1.2 Pharmaceutical Analytical Chemistry

#### 1.2.1 A Brief Definition

This textbook is about pharmaceutical analytical chemistry (or pharmaceutical analysis as a short name). Pharmaceutical analysis is the scientific discipline of analytical chemistry applied to pharmaceuticals. This textbook teaches how to *identify* and *quantify* (measure the content of) drug substances in a given sample. The sample can be a pharmaceutical ingredient, a pharmaceutical preparation, or a biological fluid such as blood and urine. The following sections briefly explain where such samples are examined by pharmaceutical analysis, to give an understanding of the importance of the subject of the current textbook.

#### 1.2.2 Manufacture of Pharmaceuticals

Most pharmaceutical preparations are produced industrially by *pharmaceutical manufactures*, but some small-scale production also occurs in hospitals and pharmacies. Figure 1.2 outlines the work-flow for a typical industrial production of a pharmaceutical preparation. Production starts by ordering the *pharmaceutical ingredients*, namely the API and the necessary excipients. In some cases, the manufacturer produces some of these ingredients in-house, but most commonly they are produced elsewhere by different industrial suppliers. The pharmaceutical ingredients arrive in large quantities and are typically packed in cardboard drums or in large plastic containers.

Upon arrival, all the pharmaceutical ingredients are registered in the manufacturer’s documentation system, tagged with internal labels, and stored in a separate area. Here the ingredients are temporarily in *quarantine*. Then, samples of the pharmaceutical ingredients are collected and analysed to ensure that they have the correct *identity* and are of high *purity*. Such testing involves pharmaceutical analysis and this is discussed in details in Chapter 18 for small molecule drugs and in Chapter 21 for biopharmaceuticals. The results from testing are compared with the *specifications* (requirements) of the manufacturer, and provided that the results comply with the specification, the pharmaceutical ingredients are labelled as
Arrival of starting and packaging materials
Sampling of starting materials
Manufacturing
Filling
Labelling
Packaging
Documentation and control of finished product and product release

**Figure 1.2** Illustration of a typical workflow for pharmaceutical manufacturing

released material and transferred to production. Production starts with weighing or measuring the different ingredients in appropriate amounts for the subsequent production. Then, the ingredients are transferred to the manufacture. Manufacture of tablets uses several types of equipment such as machinery for granulation, drying, and tablet pressing. The manufacture of liquid preparations is carried out in large tanks, while the production of ointments and creams are carried in large pots with agitation and heating. During the manufacturing process, critical process parameters are measured to ensure the quality of the pharmaceutical preparation, and systems for this are defined as process analytical technology (PAT). When the pharmaceutical preparation leaves the production site, samples are taken for the final testing. This testing again involves pharmaceutical analysis, and is intended to confirm that the API is identified and is present in the correct amount in the pharmaceutical preparation. Such final testing of pharmaceutical preparations is discussed in detail in Chapter 19. The pharmaceutical preparation is then filled in appropriate containers (filling), the containers are marked with labels (labelling), and the containers are packed in large units (packaging). The pharmaceutical preparation is in quarantine until the final assessment. Here, the results from pharmaceutical analysis need to be in compliance with the specification. The assessment also embraces many other factors, including production conditions, results of in-process testing, a review of the manufacturing (including packaging), documentation, compliance with finished product specifications, and examination of the final finished pack. After leaving the manufacturer, the pharmaceutical preparations are sent to pharmaceutical wholesalers, and from here the preparations are distributed to pharmacies, hospitals, or other retailers where they become available to the patients.

The industrial manufacture of pharmaceutical preparations is a complicated process involving many different steps. Typically, production is a batch process, which means
that the products are made in limited batches. Each time a batch is produced, a new
manufacturing process is started from the beginning with new ingredients. Between
each manufacture of a given pharmaceutical preparation, the equipment is often used
for the production of other preparations. Consequently, the production facility must be
cleaned thoroughly between each batch to prevent earlier production ingredients from
contaminating the next preparation (this is termed cross-contamination).

1.2.3 Development of New Drugs

New APIs are developed by pharmaceutical companies, based on drug discovery research
and subsequent preclinical development. Drug discovery recalls innovation and heavy
research activities. In this work, new chemical/biochemical substances are identified
and tested for their pharmacological activity. A successful drug candidate is then tested
in animals for its effect and for its toxicity. At this stage the absorption, distribution,
metabolism, and excretion (abbreviated to ADME) of the drug candidate are studied and
the appropriate dose is settled. Pharmaceutical analysis is involved in all parts of these
processes for characterization, identification, and quantitation of the drug candidate as a
pure substance, in preparations and in blood, urine, and tissue samples.

New APIs are patented to give the pharmaceutical companies an exclusive right to pro-
duce and market them for a certain number of years. Before entering the market, new APIs
first have to enter clinical trials on humans (phases I, II, and III) to ensure efficacy and
safety. Again pharmaceutical analysis is involved, and a large number of blood samples
from the clinical trials are analysed to quantify the new API. Then, all data from drug dis-
covery, preclinical development, and clinical trials I, II, and III are combined into a Market-
ing Application (MA, for Europe) or a New Drug Application (NDA, for the United States)
and submitted to the regulatory authorities. In Europe, the MA is examined and approved
by the European Medicines Agency (EMA), while in the US the examination and approval is
performed by the US Food and Drug Administration (FDA). Active pharmaceutical ingre-
dients with no patent protection, or with expired patents, can be produced and marketed as
generic drugs by other pharmaceutical companies without restrictions or licences.

1.2.4 Use of Pharmaceuticals

At the start of any medication, it is common to treat patients with a standard dose, but it
is well known that different patients may exhibit large variations in response to a given
pharmaceutical product. In such cases it is important to adjust the dose. One example is the
treatment of hypertension. The dose may be reduced when blood pressure is too low and the
dose may be increased when blood pressure is too high. For other types of treatment, such
as depression, psychosis, and epilepsy, the efficacy of the medication is more challenging to
evaluate, and in those cases therapeutic drug monitoring (TDM) is advised. In TDM a blood
sample is collected from the patient and analysed to ensure that the drug level is appropriate.
The analysis of drugs in biological fluids is termed bioanalysis. In addition to TDM, and the
previously mentioned ADME studies, bioanalysis is crucial in drug development programs
(clinical trials) and for the detection of drugs of abuse in biological samples (blood, urine,
saliva) from humans (forensic investigations and doping control). Bioanalysis is another
major area of pharmaceutical analysis, which is discussed in Chapter 20.
1.3 This Textbook

From the discussions above, it appears that pharmaceutical analysis plays a major role in the life cycle of pharmaceuticals. Thus, pharmaceutical analysis is important for people working in the pharmaceutical industry, hospital laboratories, contract analytical laboratories, pharmaceutical and medical research institutions, and institutions investigating cases of drug abuse and doping in sports (forensic and doping laboratories).

The textbook is especially written for pharmacy students. In Europe, the training of pharmacists has to be in compliance with Directive 2005/36/EC of the European Parliament and of the Council. Box 1.2 summarizes some of the requirements of this directive.

**Box 1.2 Part of directive 2005/36/EC on the recognition of professional qualifications in article 44: training as a pharmacist**

(Subjects related to pharmaceutical analysis are given in bold.)

Training for pharmacists shall provide an assurance that the person concerned has acquired the following knowledge and skills:

(a) Adequate knowledge of medicines and the substances used in the manufacture of medicines;
(b) Adequate knowledge of pharmaceutical technology and the physical, chemical, biological and microbiological testing of medicinal products;
(c) Adequate knowledge of the metabolism and the effects of medicinal products and the action of toxic substances, and of the use of medicinal products;
(d) Adequate knowledge to evaluate scientific data concerning medicines in order to be able to supply appropriate information on the basis of this knowledge;
(e) Adequate knowledge of the legal and other requirements associated with the pursuit of pharmacy.

Course of training for pharmacists

- Plant and animal biology
- Physics
- General and inorganic chemistry
- Organic chemistry
- **Analytical chemistry**
  - Pharmaceutical chemistry, including analysis of medicinal products
- General and applied biochemistry (medical)
- Anatomy and physiology; medical terminology
- Microbiology
- Pharmacology and pharmacotherapy
- Pharmaceutical technology
- Toxicology
- Pharmacognosy
- Legislation and, where appropriate, professional ethics
The general teaching in ‘Analytical chemistry’ as defined by the DIRECTIVE is covered by Chapters 3 to 17. Basically, this can be found in textbooks in analytical chemistry as well, but the content in the current textbook has been carefully selected to cover the analytical techniques and concepts most relevant for pharmaceutical analysis. The level of detail is less than in comprehensive analytical chemistry textbooks to fit the subject into the broad pharmacy curriculum. In some cases, the reader may require more technical details, but they are easily found in analytical chemistry textbooks or Internet resources based on the fundamental understanding from reading the current textbook.

The teaching in ‘chemical testing of medicinal products’ and ‘analysis of medicinal products’ as defined by the DIRECTIVE is covered by Chapters 18 to 21. These chapters focus on key pharmaceutical issues, including:

- Chemical analysis of pharmaceutical ingredients
- Chemical analysis of pharmaceutical preparations
- Chemical analysis of biopharmaceuticals
- Chemical analysis of drug substances in biological fluids

Also in this part, the level of detail has been selected to fit the subject into the broad curriculum of pharmaceutical sciences. Readers looking for more details can find these in pharmacopoeias and Internet resources.