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# *Master's dissertation in Chemistry*

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fulfillment of the requirements for a master degree in Chemistry
Analysis**

# Dosage Method Validation Using UV for Prednisone Active ingredient in 5 mg, PRECORTYL® Generic pill. Princeps Dissolution Kinetic Study

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# Introduction

GLUCOCORTICOIDS are a class of steroid hormones [1] that turns immune activity so, the inflammation down. They are used in medicine to treat diseases caused by an overactive immune system, such as allergies, asthma and autoimmune diseases [2], Prednisone is one of the synthesis molecule used to treat these kind of diseases. It is produced as CORTANCYL® or PRECORTYL®. The second is the generic drug of the CORTANCYL® princeps their active ingredient is the Prednisone, which is presented with different dosages: 5, 10 and 20 mg. PRECORTYL® is produced by the national laboratory SAIDAL, which produces generic drugs. The latters has no research and development. Therefore, the cost and reimbursement of generics is 30% lower than the specialty (princeps), which makes it worthwhile.
As it is vital, the pharmaceutical industry is one of the most reglemented and must be accompagnied with rigourous controls in each level.

For any project to be launched on the market, the validation process should be carried out according to the following steps:

- Validate the control methods if necessary;
- Prepare 3 pilot batchs;
- Do a comparative dissolution kinetic between the generic and the princeps study to determinate the percentage of the released active ingredient of the pills versus time;
- Study the stability on three batches under accelerated conditions.

One of the control methods is the validation as it is checking and verrifying the analysis method. The validation is based on statistical analysis that uses probability criteria and logic arguments. It represents an important objective that is used in all analysis procedures. It is used in raw material, in-process and intermediate and finished products controls, as well as for stability tests of all pharmaceuticals.

This dissertation is the logical continuation of the works done in Process engineering department at Bejaia university that consisted in generic drugs physicochemical and pharmacotechnical controls and validation using HPLC[3].

In this thesis we will concentrate on the validation method using UV-Spectrophotometry and we will study the comparative dissolution kinetic.

The validation method is used by SAIDAL to control the production of the PRECORTYL®. A new assay method, cheeper, faster and environmentally friendly than the its predecessors has been validated in order to be used in routine and daily basis. It is the UV spectrophotometery method.

To achieve this study, we will structurate our work as follows:

We will present, at first, the generalities about the drug, then physico-chemical methods of analysis and pharmaco-technical controls. After that, analytical method validation thechniques will be exposed, then we will come to experimental aspect and we will expose the results and discuss them. In the end we will close our work with a general conclusion.

Chapter

# 1 The drug

The European pharmacopoeia [4] defines the medical product as any substance or combination of substances presented as having properties for treating or preventing diseases in human beings and/or animals; or 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.

## 1.1 Chemistry of drugs

Drugs may be classified into four categories: basic, acid, neutral and amphoteric compounds. The concept is based on the ionic stat of the compound at various pH values.
A drug that contains a basic functional group, such as an amine, is able to accept a proton at low pH and become a cation thus, basic drugs are cations at a certain pH range.

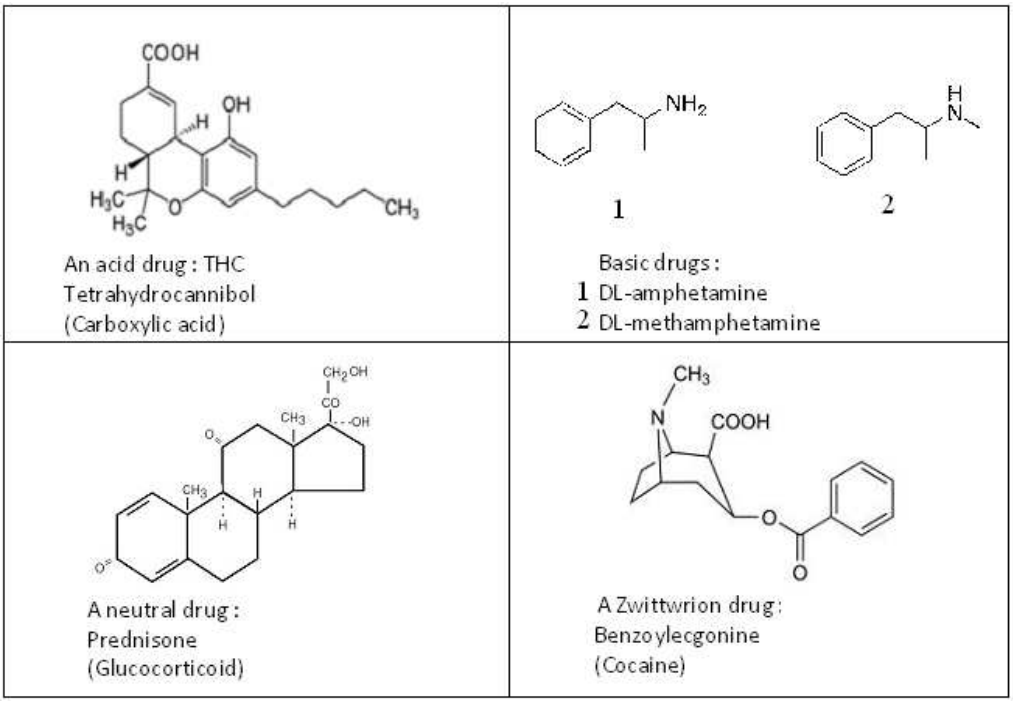


**Figure 1 : Four classes of drugs or pharmaceuticals**

A drug that contains an acid functional group, such as a carboxylic acid, can donate a proton at pH above 5 and become an anion. These are acid drugs.
Drugs that contain neither acid nor basic functional groups are neutral drugs because they are not ionic at any pH.
Finally, the fourth category are drugs that contain both acid and basic functional groups, they are called amphoteric drugs. They may be either cationic, ionic, or zwittwrions (both positively and negatively) charged at the same time, depending on the pH. Figure 1 shows an example of each of the four classes of drugs.

## 1.2 Composition of drug

A medical product contains one or more substances that are pharmacologically active and that substances are called the active ingredients (AI) or actives pharmaceutical ingredients (API) and an inactive portion made of one or more excipients.

### 1.2.1 Active pharmaceutical ingredient

Any substance intended to be used in the manufacture of a medicinal product and, 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 [5].

### 1.2.2 Excipient

It is a component other than the active ingredient, which is present in the drug or used for its manufacture. Its function is to serve as a vehicle for the active ingredient [5]. It must be inert in relationship to the active ingredient, body and the conditionnement articles. The main functions of excipients must ensure that:

- The preparation has a shape and size that is easy to use for the patient,
- The active substance is optimally absorbed by the patient,
- The preparation has an acceptable shelf life,
- The preparation doesn't have an unpleasant taste or odor,
- The production is easy.

## 1.3 Bioavailability

In pharmacology, bioavailability (BA) is a subcategory of absorption and is the fraction of an administered dose of unchanged drug that reaches the systemic circulation, one of the principal pharmacokinetic properties of drugs. By definition, when a medication is administered intravenously, its bioavailability is 100%. However, when a medication is administered via other routes (such as orally), its bioavailability generally decreases (due to incomplete absorption and hepatic first-pass) or may vary from patient to patient. Bioavailability is one of the essential tools in pharmacokinetics [6].

## 1.4 Bioequivalence

Bioequivalence is no significant difference in the bioavailability of an active ingredient, from an equivalent dosage form administered at the same dose under similar conditions in an appropriate study [7].

## 1.5 Patent

A patent is a legal tool that provides a temporary monopoly for inventors of a new product. In exchange, they must share their knowledgebase with scientific communities. This device allows inventors to recoup their investments and realize a reasonable profit. At the expiration of the patent,this knowledge falls into the public domain and then becomes usable by all [8].

## 1.6 Princeps or reference drug

The term drug reference means that this drug was the first of its kind to be marketed. New products are often patented to give to developers, exclusive right to produce them.
A laboratory discovers a new molecule (princeps), it patented it, for about 20 years. It is estimated that it takes 10 years to develop a princeps (medicine specialty) since its discovery until its marketing. There is then an average of 10 years to recoup its investment since marketing until the end of the patent.

Those that are not patented are called generic drugs since they can be produced by other companies without restrictions or licence from the patent holder.

## 1.7 Generic drugs

A generic drug is considered to be substantially similar to the original drug, when it has the same qualitative and quantitative composition in active ingredient, and the same pharmaceutical dosage form whose bioequivalence with the first product has been demonstrated by appropriate bioavailability studies [9][10].
Generic drugs are copies of brand-name drugs that no longer benefit from market exclusivity (lifting of the patent). They are intended to replace the princeps because their price is cheaper [11].

The manufacturing company has no research and development. Therefore, the cost and reimbursement of generics is lower than the specialty (princeps), which makes it worthwhile [11].

### 1.7.1 Registration procedure of generic drugs

The registration procedure of generic drugs is simplified to the extent that they are exempt from pharmaco-toxico-clinical studies already performed for the originator. The generic drug quality and bioequivalence with the originator are approved by the Ministry of Health

that garants it a registration decision, before marketing it.
Generic drugs are evaluated by the Algerian National Laboratory for the Control of Pharmaceuticals (NLCP) in the same manner and according to the same criteria as other drugs.

### 1.7.2 Advantage of generic drugs

- Benefit to the population:

Tool mastery of spending in countries where there exists a health insurance system, it can also be a wider intrument of access to treatment for the poor of the third world, and patients save money.

- Interest for prescribers:

More choices for prescribers and it guarantees the purchase and use of drugs.

- Advantages for the pharmacist:

Less expensive prescriptions, so drugs are more purchased [12].

### 1.7.3 Comparison between the princeps and the generic

The comparison [13] between princeps and generics must be carried out, compared with the following criteria, table (1.1):

Table 1.1: Comparison criteria between the princeps and the generic

| PRINCEPS | GENERIC |
|---|---|
| Active ingredient | + |
| Administration route | + |
| Posology | + |
| Dosage | + |
| Therapeutic effects | + |
| Dosage form | 0 |
| Stability | 0 |
| Name | - |
| Marketing autorisation | - |
| Pachaging | - |
| Price | - |

+: Similar, 0: Alike, -: Differents.

Drug substances are administered very rarely as the pure active substance. Typically the active substance and excipients (auxiliary substances) are combined into dosage forms to produce the final medical product.

## 1.8 Dosage form

Dosage form can be, for example, a tablet, a capsule or syrup to be administered orally, injections that are for parenteral administration. Figure 2 shows the typical dosage forms.

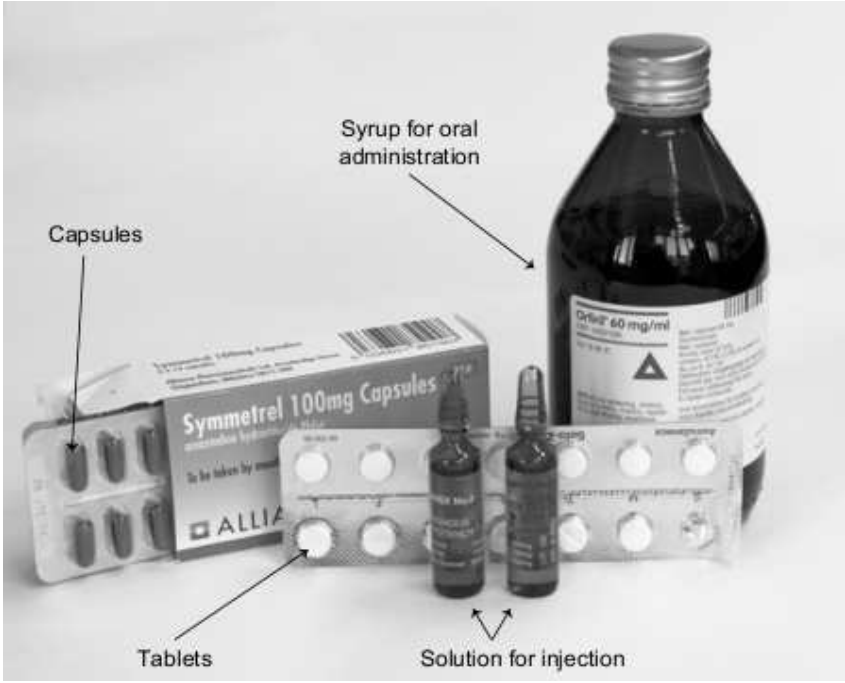


**Figure 2: Different dosage forms**.

In our case, we focus our attention on the solid dosage forms pillls and tablets.

### 1.8.1 Pills

Pills are an outdated dosage form seldom encountered in modern pharmacy practice. Technically, a pill is a small rounded mass, usually handmade, and is intended to be swallowed whole. However, the word is in common usage as a generic term for all solid dose forms, e.g. extasy pills, slimming pills or contraceptive tablets ('the pill'). Some branded products still use the word, but usually referring to small sugar-coated tablets[14].

### 1.8.2 Tablets

Tablets are the compressed dosage form that contain both active ingredients and excipients, which include diluents, adhesives, binders, fillers, lubricants, disintegrants and colours. Tablets need to be strong enough to withstand transport and handling yet still disintegrate and release the active ingredients when consumed. The behaviour of the tablet depends on the production methods, the various excipients and their effect on the active ingredient. The physical form of the active ingredient (particle size) and the particular salts are also important. We can notice that tablets are bigger than pills [14].

Several categories of tablets for oral use may be distinguished:

- Uncoated tablets:

Soluble tablets, dispersible tablets, orodispersible tablets, effervescent tablets, sublungual tablets, oral lyophilisates.

- Coated tablets:

Gastro-resistant tablets, modified-release tablets,

Formulation is the process in which different chemical substances, including the active ingredient and excipients are combined to produce a final medical product. It involves developing a preparation of the drug that is both stable and acceptable to the patient.

## 1.9 Glucocorticoids

Glucocorticoids (GC) are a class of steroid hormones that bind to the glucocorticoid receptor (GR), which is present in almost every vertebrate animal cell. The name glucocorticoid (pertaining to glucose + cortex ) derives from its role in the regulation of the metabolism of glucose, its synthesis in the adrenal cortex, and its steroidal structure [15]. In technical terms, corticosteroid refers to both glucocorticoids and mineralocorticoids (as both are mimics of hormones produced by the adrenal cortex), but is often used as a synonym for glucocorticoid.
Glucocorticoids are part of the feedback mechanism in the immune system that turns immune activity (inflammation) down. They are therefore used in medicine to treat diseases caused by an overactive immune system, such as allergies, asthma, autoimmune diseases. Glucocorticoids have harmful side effects. They cause their effects by binding to the glucocorticoid receptor (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of proinflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus (transrepression) [16].

Cortisol (or hydrocortisone) is the most important human glucocorticoid. It is essential for life, and it regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions. Various synthetic glucocorticoids are available; these are used either as replacement therapy in glucocorticoid deficiency or to suppress the immune system.
There are two kinds of glucocorticoids, the natural glucocorticoids and synthetic glucocorticoids. The first one includes the cortisol (hydrocortisone) and cortisone, meanwhile the second includes prednisone, prednisolone, methyl prednisolone, dexamethasone, betamethasone, triamcinilone, beclometasone, fludrocortisone, deoxycorticosterone, aldosterone. All of them differ by their actions (glucocorticoid potency) and their pharmaceutical form etc, but they all have a common particularity that connects them, that is the nucleus, that confers them the anti-inflammatory effect, which is known as Pregnan nucleus. It consists of a molecule whose squelton is composed of a linking of 21 atoms of carbon (shows in figure3).

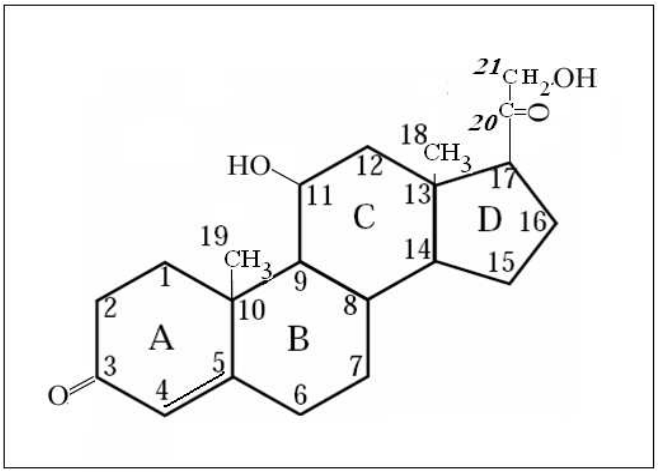


**Figure 3 : Pregnane nucleus**.

These molecules are generally characterized by:

- An insatured A cycle (ethylenic link in $C_4 - C_5$);
- An acetonic function in $C_3$;
- A hydroxide group or an acetone in $C_{11}$;
- An acetonic group or an alcohol in $C_{17}$.

These groupings and structural particularities, form the essential basis for the glucocorticoids activities. We find them in all products, including natural drifts except the $OH$ in $C_{17}$.
Starting from the basic nucleus, different chemical modifications have allowed to adjust the action of molecules. The aim of these modifications is to increase the effect of the anti-inflammatory by reducing the effect of the mineralo-corticoids which are mainly responsible of water and soda retention.

### 1.9.1 A cycle

A double link between the carbon 1 and 2 decreases the soda retention and boosters the metabolism of glucids activity (the increase of the glucocorticoid effect).

### 1.9.2 B cycle

The addition of methyl group in $C_\alpha$ increases at once the glucocorticoid effect and mineralocorticoid effect for certain molecules (cortisol) where the mineralocorticoid effect decreases

by a quite augmentation of the anti-inflammatory effect (prednisolone). A $9\alpha$ fluoridation exacerbates all the corticoids activities, probably by the increasing the electronic density next to the $11\beta$ hydroxid group.

### 1.9.3 C cycle

The oxygen atom brought by the carbon 11 is necessary for the anti-inflammatory activity. It exists in the form of a hydroxyl grouping in the case of hydrocortisone as well as the form of acetone for the cortisone and prednisone.

### 1.9.4 D cycle

The addition of methyl group or hydratation of the C16 reduces the mineralocorticoid effect and modifies just a bit of the anti-inflammatory action. The hydroxylation in $17\alpha$ is not necessary but, it seems to increase the anti-inflammatory effect.

Chapter 2

# Physico-chemical methods of analysis and pharmaco-technical controls

Before marketing a generic drug or princeps, producing company must obtain a marketing authorization (MA). For this, the drug in question must be produced at the pilot scale in order to go through a series of tests to ensure its compliance. If these tests are compliant, the drug may obtain authorization for placing on the market to be produced on an industrial scale. From these testing methods, we are going to mention some physico-chemical methods of analysis and pharmaco-technical controls.

## 2.1 Physico-chemical methods of analysis

The physico-chemical methods of analysis, becomes with time essential to check the composition of the (chemical, pharmaceutical, agroalimentary, water,...) products. They are as varied as their scope application, and they constitute today an obligatory passage in the industry (control, checking, and dosage) and an unequalled utility in research.

### 2.1.1 High Performance Liquid Chromatography (HPLC)

Liquid chromatography presents popularity results from its convenient separation of a wide range of sample types, exceptional resolving power, and nanomolar detection levels. It is presently used in biological and pharmaceutical research and development[17]:

- To purify synthetic or natural products.
- To assay active ingredients, impurities, degradation products and in dissolution assays.
- In pharmacodynamic and pharmacokinetic studies.

Improvements made in HPLC in recent years include[17]:

- Changes in packing material, such as smaller particle size, new packing and column materials.

- Micro-HPLC, automation and computer-assisted optimisation.
- Improvements in detection methods.

### Principle of method

The process of chromatography can be defined as a technique of separation including a mass transfer between the mobile phase and the stationary phase. The HPLC uses a liquid mobile phase to separate the components from a mixture, and a stationary phase which can be liquid or solid. It is thus a technique which makes it possible to detect and to quantify compounds. The principle rests on the separation of these compounds in a column containing generaly silica gel, called stationary phase, by pumping of a solvent, called mobile phase, through the column. According to the single affinity of each component which exists between the mobile phase and the stationary phase, the compounds migrate along the column at various speeds and arise at various times, thus establishing a separation of the mixture.The compounds which have a great affinity towards the mobile phase migrate more quickly to the bottom of the column, while those which have a great affinity towards the stationary phase migrate slowly[18].

### Hardware

HPLC instrumentation includes a pump, an injector, a column, a detector and a recorder or data system (Figure 4). The heart of the system is the column in which separation occurs. Since the stationary phase is composed of micrometer-sized porous particles, a high-pressure pump is required to move the mobile phase through the column. The chromatographic process begins by injecting the solute on to the top of the column. Separation of components occurs as the analytes and mobile phase are pumped through the column. Eventually, each component elutes from the column and is registered as a peak on the recorder. Detection of the eluting components is important; this can be either selective or universal, depending upon the detector used. The response of the detector to each component is displayed on a chart recorder or computer screen and is known as a chromatogram. To collect, store and analyse the chromatographic data, computers, integrators and other data-processing equipments are used frequently[19].

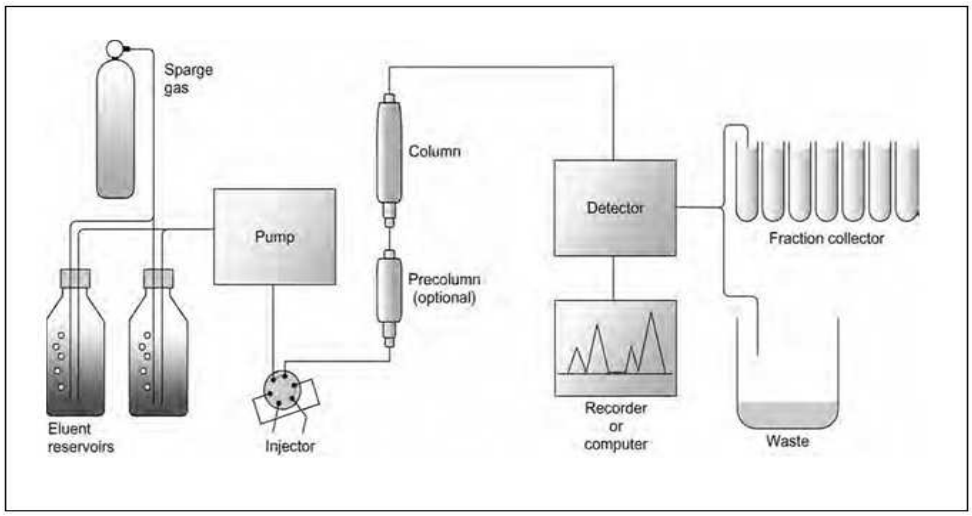


**Figure 4 : A Tipical HPLC System**

### 2.1.2 Thin-layer chromatography (TLC)

Thin layer chromatography is a separation technique in which a stationary phase, made up of an adapted material, is widespread on a thin and uniform layer on a support (plate) of glass, metal or plastic. Solutions of analytes are applied to the plate before the development.

#### Principle of method

In a thin layer chromatography silica is fixed on a plate. Eluant (solvent used to make migrate a mixture through a mobile phase) goes up on the silica plate and involves with it the mixture. The various compounds of the mixture have not the same interactions. There is thus a separation of the mixture which can be seen under a UV lamp because the silica plate contains a substance sensitive to these rays. Generally, silica being polar, the most unpolar substances migrate more quickly than the polar ones.

#### Hardware

In thin layer chromatography, the installation [19] is composed of a plate, a chromatographic tank, a stationary phase, a mobile phase, adsorbents and binders.

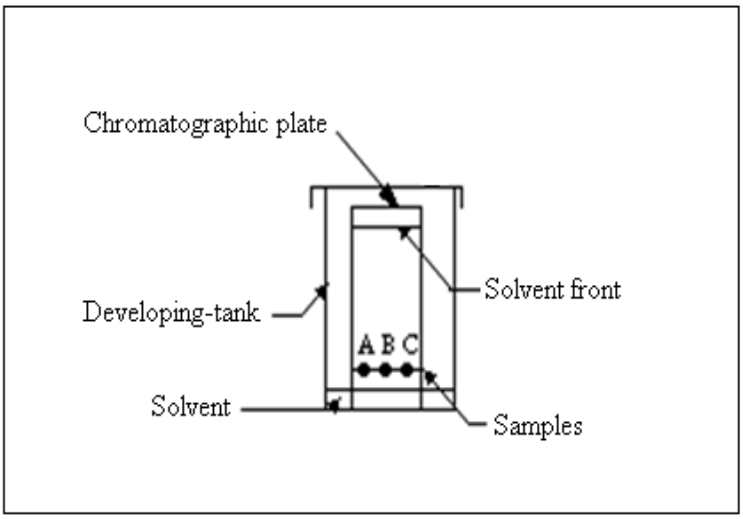


**Figure 5 : A typical TLC system**

### 2.1.3 UV spectrophotometry

This section reviews the principles of quantitative derermination and identification based on UV spectrophotometry, which is an official method in the European pharmacopeia. It is used for quality control of active ingredients, excipients and pharmaceutical procucts. Instrumentation is discussed briefly.
UV spectrometry is a key method in pharmaceutical analysis and is used both for identification of raw materials and pharmaceutical products, and for quantitative determinations. In quantitative determinations, the absorbance is read at a given wavelength, based on beer's law the absorbance is converted to a concentration. The identification is based on absorbance spectra and substances are identified by comparison of their absorbance spectras with reference spectra.
The wavelength span is conventionally divided into two ranges: the UV extends from 200 nm to about 400 nm; the visible range extends from about 400 nm to 800 nm [20].
A molecular grouping specifically responsible for absorption is described as a chromophore (-OR, -NR2, -SR, $-C_6H_5$,..), and is usually a conjugated system with extensive delocalisation of electron density.

#### Principle of quantitative determination

When monochromatic electromagnetic radiation (radiation with only one wave length) with the intensity of $I_0$ passes $I$ through a solution of an analyte, some of the radiation is absorbed by the analyte while the rest passes right through. When the intensity of the

transmitted monochromatic radiation, which is measured at the backside of the solution, is $I$ (equation 2.1.1) the absorbance [20] of the radiation ($A$) is defined as:

$$A = \log_{10} 1/T = \log_{10} I/I_0, \tag{2.1.1}$$

where $T$ is the transmittance ($I/I_0$).
Beer's law governs the measurement of absorbance of radiation by a solution of molecules. According to beer's law, the absorbance ($A$) is proportional to the path length through which passes ($l$) and the concentration of the substance in the solution ($c$).

$$A = \varepsilon lc. \tag{2.1.2}$$

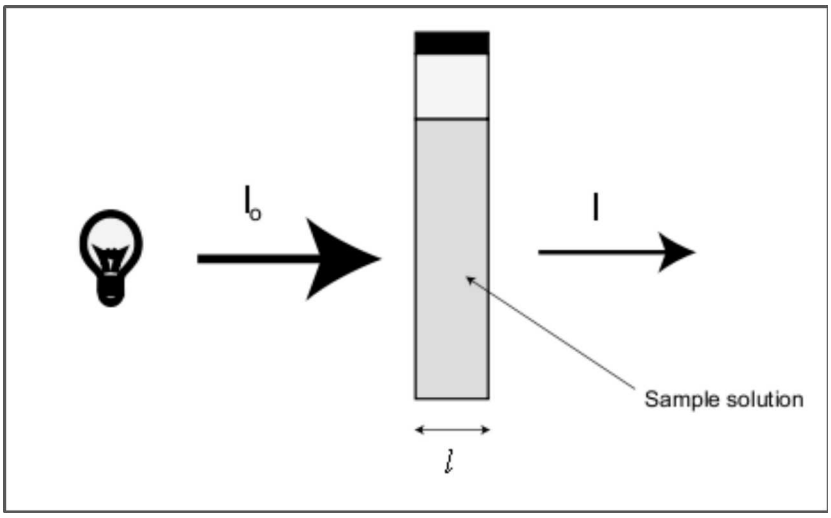


**Figure 6 : Scheme of the principle of the beer's law** .

When $l$ is expressed with centimeters and c in moles per liter (M), $\varepsilon$ is called the molar absorptivity, which is constant for that particular substance. Beer's law states that the absorbance is proportional to the molar absorptivity, the path length and the analyte concentration in the solution. When $\varepsilon$ and l are known from before, the unknown molar concentration of a drug can be determined by reading the absorbance.
The path length is specified for the equipment used and measurements are normally done at the path of 1 cm.
The absorbance of an analyte must be determined after calibration. In calibration, the absorbance of standard solution of known concentrations is measured.
The absorbance is dependent on:

- Wavelength
- Solvent
- pH when the substance is an acid or base
- Temperature (with relatively small extent)

**Instrumentation**

A spectrophotometer is an instrument which is capable of isolating 'monochromatic' radiation; or that which specifically contains a dispersing element: a prism or a grating. It is pertinent to mention here that there are a plethora of commercially available spectrophotometers of varying design i.e, single-beam (simple), double-beam (more precise and accurate). In this particular section, the following two types of spectrophotometers shall be discussed briefly [19]:
Single-beam Spectrophotometer, and Double-beam Spectrophotometer.

- Single beam spectrophotometer:

The desired wavelength is isolated by using a prism or grating and auxiliary mirrors and slits hat collectively from a microchromator of the instrument. The wavelength dial on a spectrophotometer is adjusted to a specific value, but the radiation leaving the exit-slit is found to be rarely monochromatic.

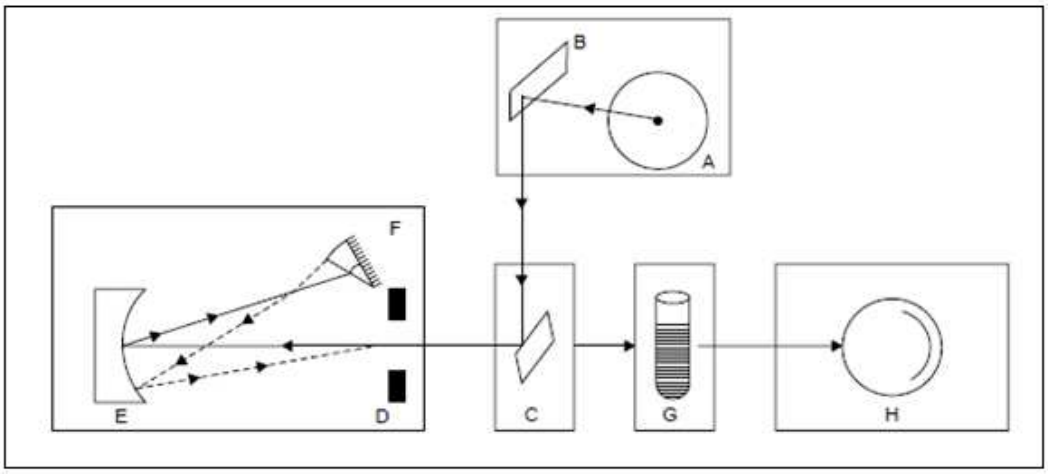


**Figure 7 : Schematic representation of the optical system in a single beam spectrophotometer**
**Model: Beckman DU-Spectrophotometer**

.

The various components of Figure 7 are given below:

- A = Source of light
- B = Condensing mirror
- C = Slit-entrance mirror
- D = Adjustable slit
- E = Collimator mirror
- F = Prism (Reflecting)
- G = Cuvette containing sample
- H = Phototube

Light from the source (A) is focused on the condensing mirror (B) and directed in a beam to the 45° slit-entrance mirror (C). The slit-entrance mirror subsequently deflects the beam through the adjustable slit (D) and into the monochromator to the collimator mirror (E). As a result the light falling on the collimator mirror is rendered parallel and reflected to the prism (F), where it undergoes refraction. The back surface of the prism is aluminized, so that the light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges. The desired wavelength of light is selected by rotating the wavelength selector fixed on top of the monochromator case. This control, in fact, adjusts the position of the prism. The spectrum from the prism is directed back to the collimating mirror which canters the chosen wavelength of light on the slit and the sample (G). Light passing through the sample strikes the phototube (H), causing a voltage to appear across a load-resistor. The voltage is duly amplified and registered on either the strip-chart recorder or the null-meter.
Two different sources of light, namely:

(Single beam spectrophotometer) H2 or D2 Lamp-for measurement in UV-region, and (Double beam spectrophotometer) Tungsten Lamp-for measurement in visible region, thereby permitting measurements from 190-1000 nm. A computer system has also been provided to enable automatic spectrochemical measurements and to perform calculations simultaneously.

- Double beam spectrophotometer:

The quantum leap amalgamated with qualified success in the advancement of Analytical Instruments are necessary for more rapid and precise and accurate measurements in UV and visible spectroscopy. It could be accomplished with the help of the following two cardinal modifications, namely: the single beam spectrophotometer need for a continuous change in wavelength so that light through the blank and through the sample may be monitored continuously, and the double beam spectrophotometer measurements done with a recording spectrophotometer. The above two modifications have been duly incorporated in a double-beam spectrophotometer. Figure 8, depicts the schematic diagram of the optical system involved in a Lambda-2 microcomputer-controlled UV-VIS Spectrophotometer (Perkin-Elmer).

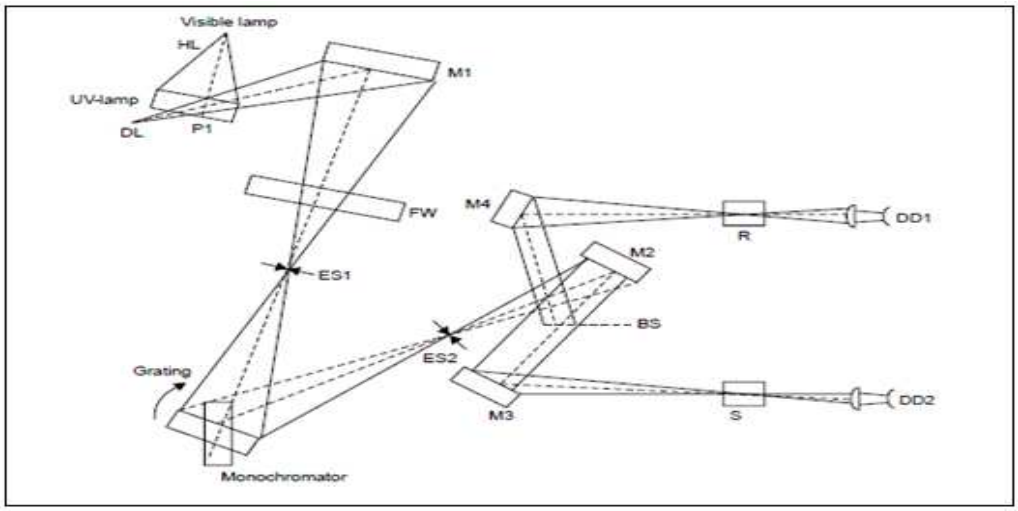


**Figure 8 : Schematic representation of the optical system in a double-beam spectrophotometer**
**Model lambda-2 micrometer controlled UV-Visible spectrophotometer (Perkin-Elmer)**

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The various components of Figure 8 are stated below:
VIS-LAMP = Tungsten Lamp (TL). UV-LAMP = Hydrogen Lamp (HL), Deuterium Lamp (DL), P1 = Movable source-selection mirror, M1, M2, M3, M4 = Mirrors, FW = Filter wheel, ES 1 = Entrance slit, ES 2 = Exit slit, BS = Beam Splitter, R = Reference Sample Holder, S = Sample holder (Test), and DD 1, DD 2 = Diode detectors.

## 2.2 Pharmaco-technical controls

All the characteristics of the product that are likely to vary with time must be determined. We can quote some examples of pharmaco-technical controls, according to standards of the European Pharmacopeia $6^{th}$ Ed, 2009[4].

### 2.2.1 Aspect and form

This test consists in controlling the form (to measure the size) and the aspect (evaluation of the color of compressed or powders contained in the capsule, granulometric aspect of the powder contained in the capsule) like with conditioning (swelling of the cells of blister,...).

### 2.2.2 Mass uniformity

This test consists in making a follow-up of the profit or loss of the weight during the study by comparing the calculated averages starting from the results of the weighing of 20 tablets. The value of the standard deviation allows the calculation of the coefficient of variation which informs about the homogeneity of the weighings compared to the average. Moreover, it is checked that the average weight respects the criteria of the European Pharmacopeia.

### 2.2.3 Friability

The friability test makes it possible to check the loss of mass of a tablet after rotation in a drum during 4 min at the speed of 25 turns per minute. This test does not apply to the capsules.

### 2.2.4 Disintegration time

The deletion time or disintegration is the phase which in general precedes dissolution. The deletion test measures the time taken by the pharmaceutical solid form to disaggregate in the given environment.
Disintegration consists in making goes and comes a tablet in a basket until complete disintegration from the drug. According to the European pharmacopoeia, the test is done on six tablets, the time limit for the tablets is 15 minutes.

### 2.2.5 Dissolution

The solid drugs intended for the oral route see their active ingredient absorbed only provided that it is dissolve in the gastro-intestinal liquids. Consequently, it is significant to know in vitro the quantity of active ingredient released and dissolved starting from the dosage form. Dissolution test is provided to determine compliance with the dissolution requirements for solid dosage forms, administered orally. In this test, a dosage unit is defined as one tablet or one capsule or the amount specified equivalent to minimum dose.

**Apparatus**

- Apparatus for Basket Method (figure 9)

Apparatus of basket method is used only for the capsules, solid dosage forms. The assembly consists of the following: a vessel which may be covered , made of glass or other inert, transparent material; a motor ; a drive shaft and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at $35 \pm 0.5^{\circ}C$ during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element durin the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 Liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to delay evaporation. The shaft is positionned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly. Adjust a speed-regulating device to maintaine the shaft rotation speed at a specified rate, within ±4%.

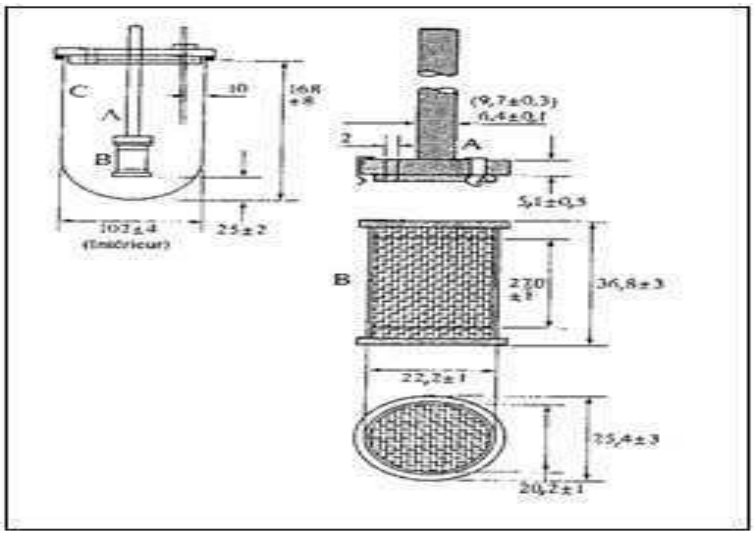


**Figure 9 : Basket Apparatus**

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All dimensions are expressed on mm.

- Apparatus for Paddle Method (figure 10)

Apparatus of paddle method is used only for pills and tablets. Use the assembly from the apparatus of basket method except that the basket formed from a blade and a shaft is used as a stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly. The distance of $25 \pm 2mm$ between the bottom of the blade and the inside vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-parts detachable design may be used provided the assembly remains firmly engaged during the test.
The paddle blade and shaft may be coat with a suitable coated so as to make them inert. The dosage unit is allowed to sink in the bottom of the vessel before rotation of blade start.

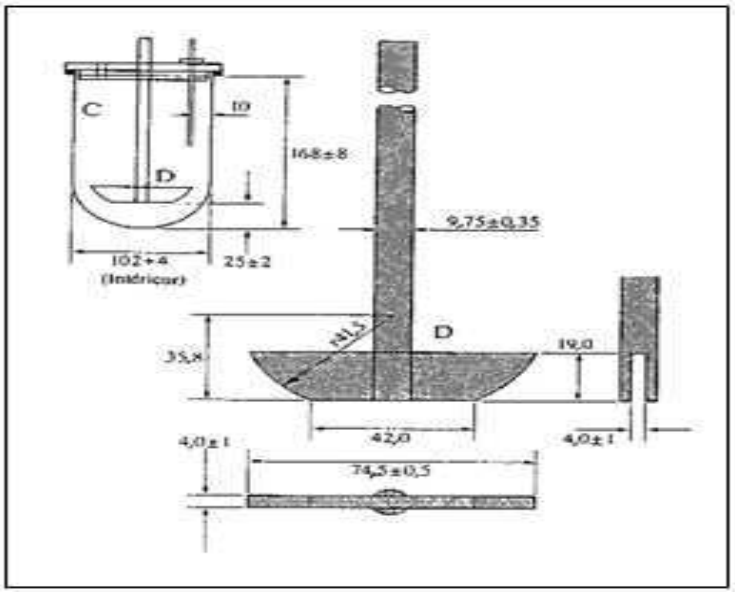


**Figure 10 : Paddle Apparatus**

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All dimensions are expressed in mm.

Chapter

# 3 Analytical Validation Method

In order to market a generic drug, the producing firm must develop a dosage method to quantify and identify the active ingredient, and this method must be validated according to international standards. Validation is the ultimate step in the development of a new analytical method prior to its application in routine analysis. Based on scientific and regulatory requirements (European Directives, ICH, ISO, ...), it must evaluate the performance of the method by studying a number of parameters known as Validation Criteria using appropriate statistical tools.

## 3.1 Definition

Validation processes are a preventive concept, therefore an analytical method should be validated prior to use to reduce the unordinary production. The purpose of method validation is to demonstrate that an analytical method is suitable for its intended purpose and ensures that a given analytical method will give sufficiently reliable and reproducible results. Experiments are conducted to ensure that factors that would cause variability are under control [21].

Validation of an analytical method is necessary when it comes to changes:

- The drug substance synthesis;
- In the analytical procedure;
- In the composition of a finished product.

### 3.1.1 Definition of analysis type to validate

There are three types of analysis method to validate:

- Identification: The tests used to verify the identity of the analyte in the sample;
- Impurity analysis: Evaluation of the sample purity accurately.

- Quantification of substances with their contents or concentrations: they are used to measure an active ingredient in a given sample (assay or dosage).

## 3.2 Unfold of the validation

For the validation of each analysis we refer to [22] the following table:

| Type of analytical procedure<br><br>characteristics | IDENTIFICATION | TESTING FOR IMPURITIES<br><br>quantitat. limit | | ASSAY<br>- dissolution (measurement only)<br>- content/potency |
|---|---|---|---|---|
| Accuracy | - | + | - | + |
| Precision | | | | |
| Repeatability | - | + | - | + |
| Interm.Precision | - | + (1) | - | + (1) |
| Specificity (2) | + | + | + | + |
| Detection Limit | - | - (3) | + | - |
| Quantitation Limit | - | + | - | - |
| Linearity | - | + | - | + |
| Range | - | + | - | + |

**Figure 11: Unfold of validation**

− signifies that this characteristic is not normally evaluated.
+ signifies that this characteristic is normally evaluated.
(1) in cases where reproducibility has been performed, intermediate precision is not needed.
(2) lack of specificity of one analytical procedure would be compensated by other supporting analytical procedure.
(3) may be needed in some cases.

### 3.2.1 Specificity

The analytical method to be properly suitable to the analyzed substance in presence of other components must have the garantee that the result of the analysis method comes only from the analyte, and not from other substances [23].
The study allows to verify the absence of interference due to excipients. This test is used to verify the identity of the analyte in the sample. The study is carried out on the following solutions :

- Placebo : Mixture of all the components of the drug at different doses without the active ingredient;
- Standard solutions;
- The blank.

### 3.2.2 Linearity

Linearity is the capacity of an analysis method, inside a specific interval, to provide an instrumental response or results proportional to the amount of analyte in the sample to be assayed [24]. The study is carried out on the standard solution and the finished product from a concenration of 100 per cent. It must determine whether a linearity in the defined domain exists, using the analytical method.

### 3.2.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. [25].

### 3.2.4 Fidelity

The fidelity of the method expresses the closeness of agreement between a series of measurements taken from multiple testing of the same sample, under prescribed conditions. It is expressed by measuring repeatability and reproducibility. Repeatability is evaluated by analyzing the same samples several times by the same operator within a short time interval, with constant operating conditions. The reproducibility is verified by carrying out manipulations by different operators on the same equipment at different times with the same method on a homogeneous initial sample [26].

## 3.3 Statistics

The validation is performed using a UV spectrophotometer according to validation criteria which are illustrated in the following table:

Table 3.1: Validation criteria

| | |
|---|---|
| Linearity | Test of existence of a straight line fit.<br>Comparison test of the intercept with "0", Student test.<br>Homogeneity of variances test, Cochran test.<br>The existence of a significant slope test, $F_1$ Fisher test.<br>Test validity of the straight line fit, $F_2$ Fisher test. |
| Accuracy | Homogeneity of variances test, Cochran test.<br>Test of validity of the averages, $F_3$ Fisher test.<br>Average recovery valuation. |
| Fidelity | Homogeneity of variances test, Cochran test.<br>Study of intergroup variations of the repeatability and the reproducibility. |

$$y = mx + b. \tag{3.3.1}$$

Where: y is the dependent variable, x is the independent variable, m is the slope of the curve, and b is the intercept on the ordinate (y axis); y is usually the measured variable, plotted as a function of changing x. In a spectrophotometric calibration curve, y would represent the measured absorbances and x would be the concentrations of the standards. Our problem, then, is to establish values for m and b.
It can be shown statistically that the best straight line through a series of experimental points is that line for which the sum of the squares of the points deviation from the line is minimum. This is known as the least squares method. If x is the fixed variable (concentration), and y is the measured variable (absorbance), than the deviation of y vertically from the line at a given value of x ($x_l$) is of interest. If $y_l$ is the value on the line, it is equal to $mx_{ij} + b$. The square of the sum of the differences, S, is then

$$S = \sum_{j=1}^{k} \sum_{i=1}^{nj} (y_{ij} - y_l)^2 = \sum_{j=1}^{k} \sum_{i=1}^{nj} [y_{ij} - (mx_{ij} + b)]^2 \tag{3.3.2}$$

This equation assumes no error in x, the independant variable.
The best straight line occurs when S goes through a minimum. This is obtained by use of differential calculations by setting the derivative of S with respect to m and b equal to zero and solving for m and b. The result is

$$m = \frac{\sum_{j=1}^{K} \sum_{i=1}^{nj} (x_{ij} - \overline{x})(y_{ij} - \overline{y})}{\sum_{j=1}^{K} \sum_{i=1}^{nj} (x_{ij} - \overline{x})^2} \tag{3.3.3}$$

$$b = \overline{y} - m\overline{x} \tag{3.3.4}$$

Where: $\overline{x}$ is the mean of all the values of $x_{ij}$, and $\overline{y}$ is the mean of all the values of $y_{ij}$; j is the index of the group, k is the number of groups; i is the index of individual values in the j group; nj is the number of observations of the j group; $x_{ij}$ is the independant variable (concentration); $y_{ij}$ is the dependante variable (absorbance).

The use of differences in calculations is cumbersome, and equation (3.3.3) can be transformed into an easier to use form,

$$m = \frac{\sum_{i=1}^{N} x_i y_i - [(\sum_{i=1}^{N} x_i \sum_{i=1}^{N} y_i)/n]}{\sum_{i=1}^{N} (x_i)^2 - [\sum_{i=1}^{N} (x_i)^2/N]} \tag{3.3.5}$$

Where: N is the total number of data points.

- Correlation coefficient (r)

The correlation coefficient is used as a measure between two variables. When x and y are correlated rather than being functionally related (Relationship between x and y)

$$r = \frac{\sum_i (x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_i (x_i - \overline{x})^2 \sum_i (y_i - \overline{y})^2}} \tag{3.3.6}$$

Where r is the correlation coefficient; N is the number of observations; $x_i$ and $y_i$ are the individuals values of the variable x and y, respectively; and $\overline{x}$ and $\overline{y}$ are their means. The equation can be transformed to a more convenient form:

$$r = \frac{\sum_i x_i y_i - n\overline{xy}}{(\sum_i x_i^2 - N\overline{x}^2)(\sum_i y_i^2 - N\overline{y}^2)} \tag{3.3.7}$$

We can, also, simplify the equation (3.3.7), and obtain

$$r = \frac{N \sum_i x_i y_i - \sum_i x_i \sum_i y_i}{\sqrt{[N \sum_i x_i^2 - (\sum_i x_i)^2][N \sum_i y_i^2 - (\sum_i y_i)^2]}} \tag{3.3.8}$$

The maximum value of r is 1, when this occurs, there is exact correlation between the two variables. When the value of r is zero (when x and y equal to zero), there is complete independence of the variables.
As a general rule, $0.90 < r < 0.95$ indicate a fair curve, $0.95 < r < 0.99$ a good curve, and $r > 0.99$ indicates excellent linearity.
The regression coefficient is $r^2$, when it is equal or tend to 1, it also indicates the existence of a linear connection between x and y.

### Comparison test of the intercept with "0", Student test

This test verifies if the value of the ordinate at the origin is not significantly different from zero. We have to calculate the value of t(a) and compare it with a known theoretical value according to the following condition of validity: $t_{calculated} \leq t_{theoritical}$ at a chosen risk .
The value of t(a) is determined by the following equation

$$t(a) = \frac{\mid a \mid}{S_a} \tag{3.3.9}$$

$S_a$, is the standard deviation of the intercept

$$S_a = S_r \sqrt{\frac{\sum_{j=1}^{N} x_j^2}{\sqrt{N \sum_{j=1}^{N} x_j^2 - (\sum_{j=1}^{N} x_j)^2}}} \tag{3.3.10}$$

where $S_r$ is:

$$S_r = \sqrt{S/(N-2)} \tag{3.3.11}$$

**Variances homogeneity test, Cochran test**

This test checks (at a $\alpha$ risk selected ) the homogeneity of variances of the individual values. In an other term, it permits to decide on the repeatability of intergroup trials, thus verifying that these variances are not different between them.
It involves comparing the criterion of Cochran, with a theoretical value read from the table:
$c_{calculated} \leq c_{theoritical}$ at a chosen risk
the value of $c_{calculated}$ is determined by the following equation

$$c_{calculated} = \frac{S_j^2 max}{\sum_{j=1}^{K} S_j^2} \tag{3.3.12}$$

Where, $S_j^2 max$ is the highest variance of the j group, and $S_j^2$ is the variance of the j group, K is the number of groups.

- Variation ($S^2$)

variation is the square of the standard deviation which is given in the equation (3.4.2). With simplifying calculations we obtain the following equation:

$$S^2 = var(y_{ij}) = \frac{1}{N-1} \sum_{i=1}^{N} (y_i - \overline{y})^2 \tag{3.3.13}$$

$y_{ij}$ are values of $y_i$ in the same j group.

**Test for the existence of a significant slope, $F_1$ Fisher test**

The aim of this test is to compare the variations due to the regression, the experimental errors and adjustment errors. For it, we have to prove that the straight-line fit slope is not only due to the residual variance, but the straight-line possesses its own slope
(regression variance > residual variance).

Some variation source must be considered:

- Variance due to linear regression $S_i^2$;
- Residual variance $S_r^2$, which represents the difference between the theoretical value and the real value;
- Total variance $S_T^2$.

The verification of the existence of a significant slope is established using the Fisher test $F_1$, by showing that the variances $S_i^2$ and $S_r^2$ are significantly different to the chosen risk.

$$F_1 = \frac{S_i^2}{S_r^2} \tag{3.3.14}$$

$S_i^2 > S_r^2$, or if $S_r^2 > S_i^2$, we must reverse the numerator and denominator because $F_1 > 1$.

- Regression variance:

$$S_i^2 = \sum I^2 \tag{3.3.15}$$

$$\sum I^2 = m^2 \sum_{j=1}^{k} n_j (x_j - \overline{x})^2 \tag{3.3.16}$$

n: number of asseys by group.

- Residual variance:

$$S_r^2 = \sum R^2 \tag{3.3.17}$$

$$\sum R^2 = \sum T^2 - \sum I^2 \tag{3.3.18}$$

- Total variance:

$$\sum T^2 = \sum_{j=1}^{k} \sum_{i=1}^{nj} y_{ij}^2 - \frac{\sum_{j=1}^{k} \sum_{i=1}^{nj} y_{ij}^2}{N} \tag{3.3.19}$$

N: total number of assays.

**Test validity of the straight line fit, $F_2$ Fisher test**

The objective of this test is to check that the variances $S_L^2$ of the regression error and $S_E^2$ the experimental error (variation inside each level), are not significantly differents in a certain $\alpha$ chosen risk.

$$F_2 = \frac{S_L^2}{S_E^2} \tag{3.3.20}$$

If $F_2$ is very big, we reverse the numerator and denominator. The variances of the two errors experimental and of the regression are given respectively by the following equations:

$$S_E^2 = \frac{\sum E^2}{N-K} \tag{3.3.21}$$

$$S_L^2 = \frac{\sum L^2}{K-2} \tag{3.3.22}$$

Where N is the total number of essay and K is the number of groups.

- Experimental error:

$$\sum E^2 = \frac{\sum_{i=1}^{K}[\sum_{i=1}^{nj}(y_{ij} - \overline{y})^2]}{N-K} \tag{3.3.23}$$

- Regression error:

$$\sum L^2 = \frac{\sum T^2 - \sum I^2 - \sum_{j=1}^{K}[\sum_{i=1}^{nj}(y_{ij} - \overline{y})^2]}{K-2} \tag{3.3.24}$$

### 3.3.1 Accuracy

**Test of homogeneity of variances, Cochran test.**

It is the same test which is that of the linearity. See equation (3.4.12).

**Test of validity of the averages, $F_3$ Fisher test**

It is the same test which is of the linearity. See equation (3.4.14).

- Inter groups variation: equation (3.4.16)
- Intra goup variations: equation (3.4.18)
- Total variation: equation (3.4.19)

**Estimation of the confidence interval and the average recovery**

This test allows to say if the recovery in all the interval of measurements (80 to 120) is good or not, calculated from the following equation:

$$CI_L = \overline{\overline{y}} \pm S_r/\sqrt{N} \tag{3.3.25}$$

Where t is the Student coefficient $= 2,160$; N total number of assays, $S_r$ is the standard deviation of $y_{ij}$ recovery; $\overline{\overline{y}}$ is the average recovery.

### 3.3.2 Fidelity

**Test of homogeneity of variances, Cochran test**

It the same test which is that of the linearity, See equation (3.4.12), but here we have to compare the intergroup variations of the repeatability ($CV_r$) and the reproducibility ($CV_R$).

**Study of intergroup variations of the repeatability** ($CV_r$) **and the reproducibility** ($CV_R$)

- Variation coefficient of repeatability $CV_r$:

$$CV_r = 100S_r/\overline{\overline{y}} \tag{3.3.26}$$

- Variation coefficient of reproducibility $CV_R$:

$$CV_R = 100S_R/\overline{\overline{y}} \tag{3.3.27}$$

Where $\overline{\overline{y}}$ is the mean of the averages of y values of the j groups

$$\overline{\overline{y}} = \sum_{j=1}^{k} \overline{y}_j/k \tag{3.3.28}$$

$$S_g^2 = (\sum_{j=1}^{k}(y_j - \overline{y}^2/k - 1) - \frac{S_r^2}{n} \tag{3.3.29}$$

$$S_R^2 = S_r^2 + S_g^2 \tag{3.3.30}$$

## 3.4 Statistical analysis of the comparative dissolution study of the PRECORTYL® and its princeps

The maen ($\overline{x}$) the Standard deviation (S) and the Standard error of the mean (SEM) of the both of the drug PRECORTYL® and CORTANCYL® are determined in order to compare them using the Student-Fisher test.

### 3.4.1 Mean

$$\overline{x} = \sum_{i=1}^{n}(x_i)/n \tag{3.4.31}$$

$x_i$: is the individual value and n : is the number of tests.

### 3.4.2 Standard deviation

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \overline{x})^2} \tag{3.4.32}$$

### 3.4.3 Student-Fisher test

The comparison between the two averages $\overline{x}_1$ and $\overline{x}_2$ of two series of samples $n_1$ and $n_2$ ($n_1 < 30$, $n_2 < 30$), having respectively $S_1^2$ and $S_2^2$ variances is performed using the Student-Fisher test. The calculated $t_c$ value of Student is given by the equation (3.4.33).

$$t_c = \frac{\overline{x}_2 - \overline{x}_1}{S\sqrt{1/n_1 + 1/n_2}} \tag{3.4.33}$$

Where:

$$\Delta S = \sqrt{\frac{n_1 S_1^2 - n_2 S_2^2}{(n_1 + n_2) - 2}} \tag{3.4.34}$$

For a degree of freedom $(n_1 + n_2) - 2$, the $t_c$ value gives the P value read from the Student table. The compared gap between the two averages $\overline{x}_1 and \overline{x}_2$ is significant else if $P < 5\%$:

$P > 0,05$:non significant difference, $P < 0,05$:significant difference.

Chapter

# 4 Experimental

My training course was carried out at the national laboratory SAIDAL, which produces generic drugs. Among them, the drug which is the main object of my present study, PRECORTYL® 5mg prednisone, that its princeps is CORTANCYL®). SAIDAL is a society by shares with a capital of 2.5 billion Algerian Dinars. 80% of the SAIDAL group capitals are held by the Algerian state and 20% of the remaining capital in 1999 were sold through the stock exchange to institutional investors and individuals. Made up of several subsidiary companies (see flowsheet in the appendix 1), as well as development and research center, DRC-SAIDAL. My task in this laboratory consisted in making a study of comparative dissolution kinetic between the generic (PRECORTYL®) and its princeps (CORTANCYL®), which were carried out at the pharmal subsidiary company. I also carried out a validation dosage of the active ingredient, prednisone, in the finished product by spectrophotometry UV, in the development and research center (DRC-SAIDAL). We can see the description of the premises (Pharmal and DRC-SAIDAL) in the appendix 1. The High Performance Liquid Chromatography (HPLC) is precise but it can have a lot of desavantages, such as the cost (Equipments, Solvents,..), calibration, time and environmental pollution (Release of large amount of solvents,..). This is why a assay method using UV was developed at the development and resaerch center DRC-SAIDAL, this method is faster, less expenssive and environmentally friendly (unlike the HPLC method which is given in the United State Pharmacopoeia, USP), which makes it possible to used in the routine tests. This method, before to be used in the industry, must be Validated by the methods given in the Chapter3.

## 4.1 Description of the PRECORTYL® drug, Prednisone 5mg pill

PRECORTYL® is a steroidal anti-inflammatory (SAI) glucocorticoid's family, whose reference specialty is CORTANCYL®.


**Figure 12 : PRECORTYL®** .

It is presented as a 7mm diameter white color and odorless biconvex pill, composed of an active ingredient: Prednisone, and five excipients. The table (4.1) shows the different excipients of the PRECORTYL® pill and their role.

Table 4.1: Different excipients of the PRECORTYL® pill and their role

| EXCIPIENTS | FUNCTION |
|---|---|
| Lactose | Diluent (thinner) |
| Maize starch | Diluent (thinner), desintegrant |
| Polyvinylpyrollidone PVK 90 | Thickening agent (binder) |
| Magnesium stearate | Lubricant |
| Water | Solvent |

Diluent : A diluent is used primarily to obtain a sufficient volume of powder to produce a pill of the desired size.
Desintegrant : The role of the disintegrant is to accelerate the disintegration of the pill so the dispersion of the active ingredient in water or in the digestive juices.
Thickening agent : It is a substance which is able of forming physical bonds thus meets all the components of the pill.
Lubricant: A lubricant is a product used to reduce friction.

**Prednisone**

Prednisone (1.4-prednadiene-17. 21-diol-3.11.20-trione), $C_{21}H_{26}O_5$ is a synthetic glucocorticoid commonly used for the treatment of immunologic, allergic and inflammatory diseases, including asthma and arthritis [27], figure 13 shows the prednisone molecule.

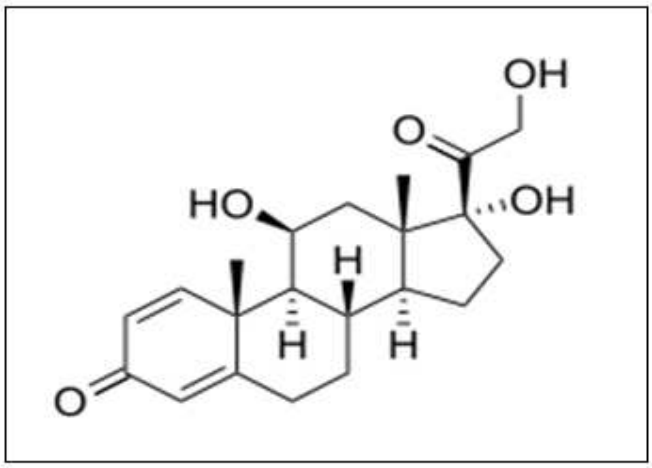


**Figure 13 : Prednisone** $C_{21}H_{26}O_5$

- Chemical properties of prednisone [4]:

Molecular formula: $C_{21}H_{26}O_5$.

Molecular weight
MW ($C_{21}H_{26}O_5$)=358.4 g per mole.

Appearance
White or almost white photosensitive, crystalline powder.

Solubility
Practically insoluble in water at room temperature, slightly soluble in ethanol 96° and in methylene chloride[28].

### Side effects

The following side effects may occur when taking prednisone for even a short period of time and will go away as the prednisone is reduced or stopped[29]:

- Mood changes, sometimes feeling full of energy and having difficulty sleeping, or feeling depressed or irritable.
- Upset stomach, nausea or indigestion. Take prednisone with food to avoid an upset stomach.
- Increased appetite.
- Weight gain from increased appetite or fluid retention.
- Fluid retention causing swelling of the face, upper part of back and ankles, this is why we do not add salt to food.
- Increased blood pressure.
- Blurry vision.
- Lower resistance to fighting infections.
- Changes in the menstrual cycle.
- A rise in blood sugar levels and an increased risk of diabetes.

When prednisone is used for a long time, other side effects that can occur are[29]:

- Skin changes such as acne, hair growth, easy bruising or wounds take longer to heal.
- Thinning of the bones (osteoporisis) can occur due to a loss of calcium from the bones which may cause a fracture.
- Weakness of the hip and shoulder muscles and damage to bones.

## 4.2 Materials and methods

This section deals with materials and methods used during our manipulations, we go to present at first an overview of the finished product control, then we will detail the operative mode of the comparative dissolution kinetic with the princeps, and we finally give the operative mode for the achievement of the assay method validation. We worked with the lot number: 0340. Date of manufacture: 22/02/2013. Expiry date: 02/2016 (03 years validity).

### 4.2.1 Pharmacotechnical controls

In order to reduce the probabilities of errors that can taint our results and calculations concerning the validation and the comparative study of the dissolution kinetics, we will check first some pharmacotechnical parameters of the finished product, namely: aspect and form; mass uniformity; friability; deletion time. Once these tests reveal conforming results to the lot of the finished Product, we shall finally proceed to the manipulations in question.

#### Mass uniformity

This control allows to know the weight of pills.

- Operative mode:

Weigh individualy twenty pills and record their weight and average.

- Result:

The average weight should be within a range given in the Marketing Authorisation File (MAF). The uniformity of mass of pills must be within the range of the average weight plus or minus an percentage ($\pm 7,5\%$ for PRECORTYL®, 5mg) according to the European Pharmacopoeia. We assume only two values exceeding this range, ie the average weight $\pm 7,5\%$, but no value of weight must exceed the fork of the average weight, more or less the double of the percentage. ($\pm 15\%$ for the PRECORTYL, 5mg)

#### Friability

The pills must remain intact, even under aggressive conditions. Using its drum, the friabilimeter can present adverse conditions to the pills.

- Operative mode:

Weigh twenty pills ($P_0$). Start the apparatus (the drum turns during 4 min at 100 turns per minute). Remove the pills, wipe and weight them ($P_i$). Calculate the coefficient of the friability, which is given by:

$$CF = \frac{(P_0 - P_i)}{P_0} X100 \tag{4.2.1}$$

Where $P_0$ represents the mass of the 20 pills before their passage by the friabilimeter and $P_i$ is the mass of the 20 pills after their passage by the friabilimeter.

- Result:

The coefficient must be $< 1\%$.

**Disintegration time**

The purpose of this control is to check the time of disintegration of pills, using a desagregator, latter is composed of a cylinder provided with six tubes (6 stations) undergoing a vertical reciprocating motion, the unit is plunged in water with $37.0 \pm 2^{\circ}C$

- Operative mode:

Put six compress in each of the six stations, then pose on a pill a disc to prevent them from going up on the surface. To place the assembly in the cylindrical mud containing the liquid medium, water at a temperature of $37.0 \pm 2^{\circ}C$, switch on the apparatus. The test is satisfied, if all the samples are disintegrated at the prescribed time. Finally, note the time of disintegration.

- Result:

The time of disintegration must be included in an interval of time given in the marketing autorisation file of the drug, for the PRECORTYL® 5 mg the time of disintegration must be $< 30min$.

### 4.2.2 PRECORTYL® and CORTANCYL® dissolution kinetic

This study is intended to determine the percentage of the released active ingredient of the pills, in a neutral medium (distilled water), in the course of time.

**Outfit (Appendix2)**

- Agitation plate.
- Analytical balance (Metteler Toledo).
- Filter: Acrodis MILLIPORE MILLEX $0,45\mu m$ Nylon.
- Dissolutest SOTAX, Paddle apparatus.
- UV-Visible spectrophotometer (Perkin Elmer $\lambda 25$).

The identification and quantification of prednisone after dissolution will be done by a spectrophotometer UV-Visible.

**Operating conditions**

- Reactif and solutions:

Medium: 500 ml of distilled water.
Apparatus: Paddle apparatus.
Time: 30 min.
Speed: 50 trs/min.
Temperature: $37 \pm 0.5^{\circ}C$.

- Standards and reactifs:

Prednisone: 200 mg.
Ethanol 96°: 200 ml x 1/100 ml (dilution).

- Necessary quantity of sample:

6 PRECORTYL® pills.
6 CORTANCYL® pills.

- Standard solution:

Weigh 200 mg of prednisone, dissolve it in 10 ml of ethanol 96°, (agitation and heating during 2min), then cool and supplement until the volume of 200 ml with the distilled water than do a dilution of 1/100 ml with the same solvent.

- Test solution:

Put one pill in each mud which contains as a preliminary, 500 ml of distilled water, the temperature and the rotation speed of each paddle are fixed at $37 \pm 0.5^{\circ}C$ and 50trs/min, respectively. Take 10 ml of solution in each mud, at different time (samplig time:10min, 20min and 30min), then let the solutions cool to room temperature. Filter through $0.45\mu m$ porosity filter.

- Reading the optical density (OD):

Achieve the reading by measuring the absorbance of the reference solution and the test solution at a wavelength of 242nm, (specific wavelength of measurement of prednisone), with a UV spectrophotometer.

- Calculations:

$$Q = \frac{OD_{test} P_{e.m.st} V_t}{OD_{st} P_{e.m.t} 5} 100 \tag{4.2.2}$$

Q:Dissolved amount of prednisone.
$OD_{test}$: Optical density of the test solution.
$P_{e.m.st}$: Experimental mass of the pills used in the standard solution (mg).
$OD_{st}$: Optical density of the standard solution.
$V_t$: total volume.
$P_{e.m.t}$: Experimental mass of the pills used in the test solution (mg).

### 4.2.3 Assay method validation of the prednisone in the PRECORTYL® 5mg pill by UV-spectrophotometry

The active ingredient dosage is performed with a UV / Vis spectrophotometer (Perkin Elmer $\lambda 25$).
Handling Precautions: Handle away from light.

#### Reactifs

Ethanol 96°.

#### Equipment

A UV-Visible spectrophotometer is needed, 1 cm quartz cuvette, an analytical balance, a water bath and a magnetic stirrer.

#### Operating conditions

Wavelength: 239 nm.
Blank: Ethanol 96°.

#### Operating mode

- Standard solution:

Weigh exactly 50 mg of prednisone (raw material), and introduce it into a 100 ml flask and add 50 ml of ethanol at 96° and stir for 2 min. Bring in a water bath while continuing to stir for 10 minutes, let cool, then supplement to the volume with the same solvent. Take 1 ml of this solution and dilute to 50 ml with ethanol 96°, and then filter the solution with a filter paper.

- Test solution:

Weigh exactly 150 mg of PRECORTYL®, 5 mg pill and introduce it into a 100 ml flask and add 50 ml of ethanol at 96° and stir for 2 min. bring in a water bath while continuing to stir for 10 minutes, let cool, then supplement to the volume with the same solvent, filter the solution with a filter paper (Mn 1670Φ125$mm$, thickness 0,13mm). Take 5 ml of this solution and dilute it with to 25 ml of ethanol 96°.

- Placebo solution:

Weigh exactly 150 mg of placebo powder (which is previously prepared by the galenic laboratory), and introduce it into a 100 ml flask and add 50 ml of ethanol at 96° and stir for 2 min. bring in a water bath while continuing to stir for 10 minutes, let cool, then

supplement to the volume with the same solvent, filter the solution with a filter paper (Mn 1670Φ125$mm$, thickness 0,13mm). Take 5 ml of this solution and dilute it with 25 ml of ethanol 96°.

Measuring the absorbance of the solutions at a wavelength of 239 nm.

**Calculations**

- Amount of active ingredient in percentage:

$$T_{\%} = \frac{OD_{test}}{OD_{st}} \frac{(P_{e.m.st}/100)(1/50)}{(P_{e.m.t}/100)(5/25)} \overline{AW} T_{rm} 100 \tag{4.2.3}$$

Standards: $108\% > T\% > 92\%$

- Amount of active ingredient in mg by pill:

$$T_{mg/pill} = \frac{OD_{test}}{OD_{st}} \frac{(P_{e.m.st}/100)(1/50)}{(P_{e.m.t}/100)(5/25)} \overline{AW} T_{rm}/5 \tag{4.2.4}$$

Standards: $5,4mg/pill > T(mg/pill) > 4,6mg/pill$

$\overline{AW}$: Average weight.
$T_{rm}$: Purity of the raw material=$100,82\%$.
$OD_{test}$: Optical density of the test solution.
$OD_{st}$: Optical density of the standard solution.
$P_{e.m.st}$: Experimental mass of the pills used in the standard solution (mg).
$P_{e.m.t}$: Experimental mass of the pills used in the test solution (mg).

**Parameters and tests of statistical controls of the prednisone assay**

- Specificity:

We have to prepare a placebo and standard solutions and the blanck (solvent). In order to validate this criterion, we must demonstrate that there is no interference between the standard and excipients, the condition being that the absorbance of the placebo should be negligible at about 239nm (specific wave length of prednisone).

- Linearity:

The study is performed on the active ingredient alone (control solution) and the finished product in order to check the linearity. A range of calibration has been prepared in different concentrations (80%, 90%, 100%, 110%, 120%). Where 100% represents the 5 mg of

prednisone at 0,010 mg/ml, so we can deduce the concentrations in mg/ml (0,008mg/ml, 0,009mg/ml, 0,010mg/ml, 0,011mg/ml, 0,012mg/ml).

-Preparation of standard solutions at different dosages:

Introduce exactly 40, 45, 50, 55 and 60mg of prednisone (raw material), into a 100 ml volumetric flasks and add 50 ml of ethanol at 96° in each of them and stir for 2 min. Bring in a water bath while continuing to stir for 10 minutes, let cool, then supplement to the volume with the same solvent. Take 1 ml of this solution and dilute to 50 ml with ethanol 96°, and then filter the solutions with a filter paper.
Each solution is analyzed on three consecutive days. The straight line fit is determined by the least squares method, where: x =Concentrations, y= Absorbances. The coefficient correlation (r) is calculated to define if it is close to 1, which characterizes a good correlation between the concentration and the absorbance.

- Accuracy:

It is determined using the results of the linearity, and is evaluated using the following statistical tests: homogeneity of variances, of validity of the averages and estimation of the average recovery.

- Fidelity:

It is assessed at two levels:

-Repeatability:
It allows to characterize the variability of the method during the day. A standard solution of Prednisone at a concentration of 0.010 mg/ml (100%) is prepared and analyzed 07 times during the same day and under the same operating conditions.

-Reproducibility:
It allows to characterize the variability of the method over several days. in order to assess it, a standard solution of Prednisone at a concentration of 0.010 mg/ml (100%) was prepared and analyzed 07 times over three days.

Chapter

# 5 Results and discussion

In this chapter we go to present the results of our study, which includes the results of three parts: pharmaco-technical controls, PRECORTYL® dissolution kinetic study with its princeps and validation of the assay method.

## 5.1 Pharmacotechnical controls

The results of the pharmacotechnical parameters are shown in the following:

### 5.1.1 Mass uniformity

| Pill | $P_1$ | $P_2$ | $P_3$ | $P_4$ | $P_5$ | $P_6$ | $P_7$ | $P_8$ | $P_9$ | $P_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Mass (mg) | 151,7 | 147,6 | 151,0 | 149,8 | 150,1 | 149,0 | 144,5 | 149.0 | 148,5 | 149,6 |

| pill | $P_{11}$ | $P_{12}$ | $P_{13}$ | $P_{14}$ | $P_{15}$ | $P_{16}$ | $P_{17}$ | $P_{18}$ | $P_{19}$ | $P_{20}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Mass (mg) | 147,4 | 152,0 | 146.9 | 147,2 | 149,2 | 150,2 | 149,0 | 149.9 | 149,1 | 148,9 |

**Table 5.1: Results of mass uniformity control** .

$P_1, P_2, P_3, ..., P_{20}$ are masses of each pill, taken by mg. After calculs we found an average weight: $\bar{X} = 149,03mg$ Average weight interval: [138,75mg-161,25mg], (150mg±5%). Mass uniformity interval: [137,78mg-160,28mg], ($\bar{X} \pm 7,5\%$) in this case all the obtained pill weights must be compliant (00/20pills), in an other case: [126,53mg-171,53mg], ($\bar{X} \pm 15\%$), two errors are acceptable (02/20pills).

- Discussion:

The average weight is 149,03mg, this weight is within the interval [138,75mg-161,25mg], and the weight of each pill is within the mass uniformity interval [137,78mg-160,28mg], so the results are compliant according to the Marketing Autorisation Folder.

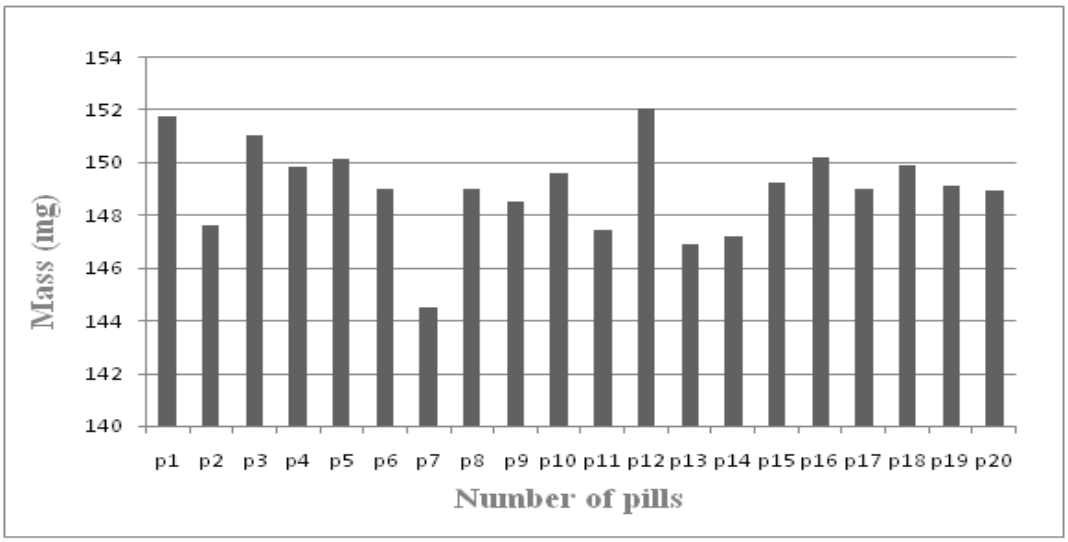


**Figure14: Histogram of results control of weight of PRECORTYL®5mg**

### 5.1.2 Friability

- Initial mass of twenty pills: $P_0$ = 2999,0 mg
- Final mass of twenty pills: $P_i$ =2993,0 mg

$$CF = \frac{(P_0 - P_i)}{P_0} X100 = 0,002\% \tag{5.1.1}$$

The obtained result is <1%, so, we have a compliance.

### 5.1.3 Time of disintegration

After the test. The obtained disintegration time is 1min4s, this result is compliant according to the Marketing Autorisation Folder ($<30min$).

## 5.2 PRECORTYL® and CORTANCYL® dissolution kinetic

The aim of this study is to determine the percentage of the active ingredient released in pills, in a neutral solution (distilled water), in the course of time. After that a dissolution kinetic statistical study is done. The obtained optical density values are presented in the appendix 3. These values allows to study the dissolution kinetic of the active ingredient (water release) of the two drugs PRECORTYL® and CORTANCYL®. The obtained raw data are also given in the appendix 3.

- PRECORTYL® 5mg prednisone (Generic)

The following table summarizes the dissolution kinetic results of the PRECORTYL® 5mg:

| Time (min) | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| % of dissolution | 0 | 68,58 | 98,37 | 108,15 |

**Table 5.2 : PRECORTYL® dissolution kinetic**

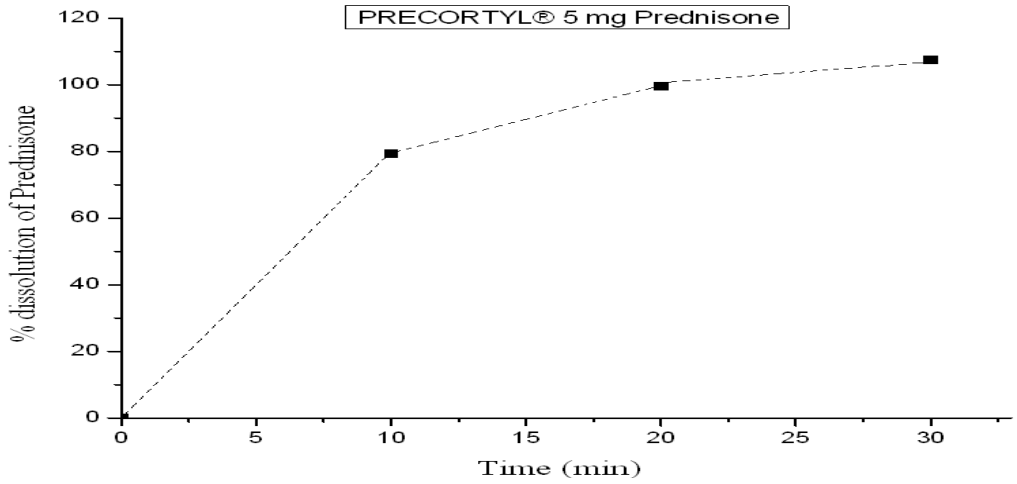


**Figure15 : PRECORTYL® dissolution kinetic profile**

- CORTANCYL® 5mg prednisone (Princeps)

The following table summarizes the dissolution kinetic results of the CORTANCYL® 5mg:

| Time (min) | 0 | 10 | 20 | 30 |
|---|---|---|---|---|
| % of dissolution | 0 | 79,27 | 99,59 | 107,42 |

**Table 5.3 : CORTANCYL® dissolution kinetic**

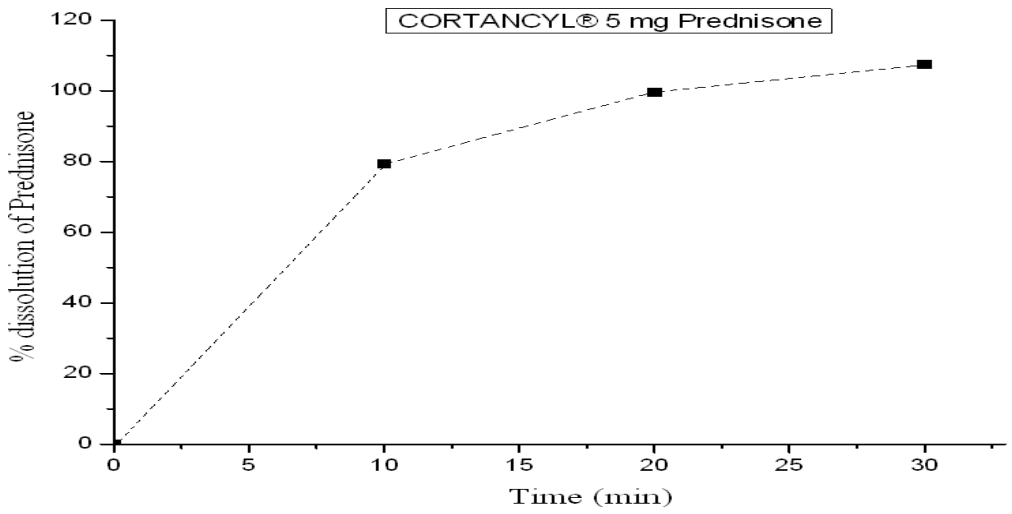


**Figure16 : CORTANCYL® dissolution profile**

- Discussion and interpretation

According to the obtained results of the dissolution kinetic of both molecules, we notice that the solutions optical densities increase versus time, with dissolution and liberation in the solution of an increasingly significant concentration of active ingredient until it stabilizes. The variation curves of the dissolution kinetic, which represents the optical density virsus time, are increasing and starts to be stabilized with time bordering 20 min for the two curves. This is a good sign because it corresponds the USP (United State Pharmacopoeia) standards, which stipulates that the dissolution rate must be equal or exceeds 80% in 30 minutes. This time corresponds to the liberation of the active ingredient in the organism, but in our case we cannot see the stabilization of the curve which corresponds to the liberation of the active ingredient, because of lack dissolution results at 25 minutes, fault of material and reagents,but this does not prevent us from concluding that the dissolution difference of the generic (PRECORTYL®) and its specialty (CORTANCYL®) is not significant. Thus the generic drug has the same dissolution profile as the princeps.

### 5.2.1 Statistical results of the dissolution kinetic

The comparison of the average values is carried out using the Student-Fisher test. The formulas are given in the chapter 3.
The obtained raw data are given in the appendix 3. The table 5.4 illustrate the obtained numerical results.

| Dissolution test (%) | | | | | | |
|---|---|---|---|---|---|---|
| $\overline{X}_1$ | $\overline{X}_2$ | $S_1$ | $S_2$ | $\triangle S$ | $t_c$ | DF |
| 88,7 | 95,42 | 19,793 | 14,52 | 11,64 | 0,70 | 4 |
| **Conclusion** Accorrding to the Student table P=0,4142 >0,05, so, the result is no significant | | | | | | |

**Table 5.4 : Statistical results of the kinetic dissolution**

$\overline{X}_1$ is the average of PRECORTYL® dissolution results.
$\overline{X}_2$ is the average of CORTANCYL® dissolution results.

- Discussion and interpretation

The difference between the dissolution of the generic drug PRECORTYL® and its princeps CORTANCYL® is no significat so, that proves that the generic has the same dissolution profit than its princeps.

## 5.3 Statistical results of the assay method validation

### 5.3.1 Specificity

The UV spectrum obtained with the placepo solution presents an not significant absorbance in the range of active ingredient absorbance so, the method is specific. The obtained spectra are given in the appendix 4.

### 5.3.2 Linearity

- Determination of the straight line fit:

The following table shows all the raw data obtained from three series of assay.

| Tests | Range | Concentration (mg/ml) | Absorbance (UA) |
|---|---|---|---|
| 1 | 80% | 0,008 | 0,3454 |
| 2 | | 0,008 | 0,3483 |
| 3 | | 0,008 | 0,3514 |
| 1 | 90% | 0,009 | 0,3923 |
| 2 | | 0,009 | 0,3916 |
| 3 | | 0,009 | 0,3952 |
| 1 | 100% | 0,010 | 0,4458 |
| 2 | | 0,010 | 0,4386 |
| 3 | | 0,010 | 0,4354 |
| 1 | 110% | 0,011 | 0,4917 |
| 2 | | 0,011 | 0,4808 |
| 3 | | 0,011 | 0,4933 |
| 1 | 120% | 0,012 | 0,5292 |
| 2 | | 0,012 | 0,5319 |
| 3 | | 0,012 | 0,5314 |

**Table 5.5: Raw data obtained from three series of assays**

The linearity of the assay method is studied by the regression line, which is obtained by the least square method.
The obtained responses at different concentrations are determined as absorbances. In order to confirm that the assay method is linear, we have to check five parameters namely:

### Straight line fit

The parameters of the linearity equation are calculated according to the formulas given in the Chapter 3. The obtained results are shown in the Table 5.6. The correlation coefficient (r) is optimal, and it is estimated at 0,9984 it is very close to 1.

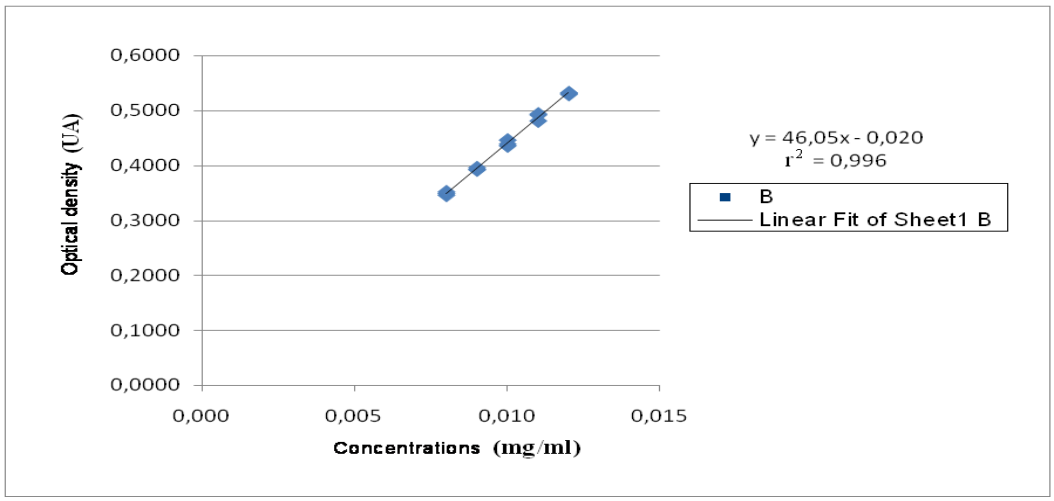


**Figure 17: Variation of the optical density in function of concentration**

| y = m x + b | |
|---|---|
| Slope (b) | 46,0500 |
| Intersept (m) | -0,020 |
| Correlation coefficient (r) | 0,9984 |
| Regression coefficient ($r^2$) | 0,9968 |

**Table 5.6: Numerical values of the straight line fit**

Equation of the straight line fit is $y = 45,0500x - 0,020$

- Discussion and interpretation:

The correlation coefficient r is within 0 and 1 ($0 < r < 1$), so, there is a dependence between x and y variables. The regression coefficient $r^2$ shows that there is a link between x and y distributions which can be represented with the straight line $y = mx+b$. The value of the correlation coefficient is close to 1 ($r = 0,9984$), so, the optical density variation in function of sample concentrations is considered as linear.

**Comparison of the intercept with zero, Student test:**

The comparison of the intercept with zero is performed with the Student test according to the formulas given in the Chapter 3. This test allows to check if the intercept is not significantly different to zero. The obtained resuts are presented in the table 5.7.

| | |
|---|---|
| Intercept (b) | -0,020 |
| Standard deviation of the intercept | 0,0073 |
| N (degrees of freedom) | 15 |
| $t_{calculated}$ | 2,77 |

**Table 5.7: Numerical results of the Student test**

- Discussion and interpretation:

$t_{th5\%} = 2,16$, $t_{th1\%} = 3,01$ and $t_{cal} = 2,77$, the condition is so that $t_{cal} < t_{th}$, we conclude that this test is valid at 1% risk, so, the intercept of the regression line is not significantly different from zero at the probability of p = 99%.

**Homogeneity of variances test, Cochran test:**

The homogeneity of variances test is performed with the Cochran test according to the formulas given in the Chapter 3. The obtained resuts are shown in the table 5.8.

| | |
|---|---|
| $S^2max$ | 0,00005 |
| Sum of variances | 0,00009 |
| C calculated | 0,5178 |
| K (nb tests) | 5 |
| n (nb repetition by tests) | 3 |

**Table 5.8: Numerical results of the Cochran test**

- Discussion and interpretation:

According to the results $C_{cal} = 0,5178 < C_{th5\%} = 0,68$, the homogeneity of variances analysis at each concentration shows that all the variances of the different groups can be considered homogeneous at 5% risk.

**Existance of a significant slope test, $F_1$ Fisher test:**

Test of existance of a significant slope is performed with the Fisher $F_1$ test according to the formulas given in the Chapter 3. this test allows to check if the linear relation between x and y. The obtained resuts are represented in the table 5.9.

| | DF | Sum of squares | Variance | F1 value |
|---|---|---|---|---|
| Totale variation | 14 | 0,06382 | | |
| Variation caused to regression | 1 | 0,06362 | 0,06362 | 4017,42 |
| Residual variation | 13 | 0,00021 | 0,00002 | |

**Table 5.9:Numerical results of Fisher test $F_1$**

- Discussion and interpretation:

$F_{1cal} = 4017,42 > F_{th1\%} = 9,07$, the $F_{1cal}$ value is significant, it is concluded that there is a slope, and therefore a linear dependency probability threshold p = 99%.

**Validity test of the straight line fit, $F_2$ Fisher test:**

The validity test of the straight line fit is performed by the Fisher $F_2$ test according to the formulas given in the Chapter 3. this test allows to check validity test of the straight line fit by comparing the power of the experimental error due to other components than the error linked directly to the active ingredient assay. the obtained resuts are represented in the table 5.10.

| Source of variation | DF | Sum of squares | Variance | $F_2$value |
|---|---|---|---|---|
| Expérimental error | 10 | 0,00018 | 0,00002 | 0,51 |
| Erreur caused to regression | 3 | 0,00003 | 0,00001 | |

**Table 5.10: Numerical results of Fisher test $F_2$**

- Discussion and interpretation:

$F_{2cal} = 0,51 < F_{th5\%} = 3,71$ The adjustment is considered valid at at 95% probability level.

**Summary of the linearity:**

| | |
|---|---|
| • Slope | 46,0500 |
| • Intercept (b) | -0,020 |
| • Regression coefficient ($r^2$) | 0,9968 |

**Table 5.11: Results obtained with the straight line fit**

At the end, the hole linearity numerical results are summarized in the next table:

Comparison of the intercept with 0:

| $t_{calculated}$ | $T_{theoritical}$ 5% | $T_{theoritical}$ 1% | Condition | Conclusion |
|---|---|---|---|---|
| 2,77 | 2,16 | 3,01 | $t < t_{th}$ | Valid at 1% risk |

Homogeneity of Variances:

| $C_{calculated}$ | $C_{theoritical}$ 5% | $C_{theoritical}$ 1% | Condition | Conclusion |
|---|---|---|---|---|
| 0,5178 | 0,68 | 0,79 | $C < C_{th}$ | Valid at 5% risk |

Existence of a significant slope:

| $F_{1calculated}$ | $F_{theoritical}$ 5% | $F_{theoritical}$ 1% | Condition | Conclusion |
|---|---|---|---|---|
| 4017,42 | 4,67 | 9,07 | $F_1 > F_{th}$ | Valid at 1% risk |

Validity of the straight line fit :

| $F_{2calculated}$ | $F_{theoritical}$ 5% | $F_{theoritical}$ 1% | Condition | Conclusion |
|---|---|---|---|---|
| 0,51 | 3,71 | 6,55 | $F_2 < F_{th}$ | Valid at 5% risk |

**Table 5.12: Summarized of the linearity numerical results**

The five parameters of the linearity are satisfied, so, the linearity criterion is validated.

### 5.3.3 Accuracy:

The results of the linearity are used to calculate the accuracy tests.

**Recovery:**

The table 5.13 presents all obtained results of the recovery.

| Test | Range | Introduced Amount (mg/ml) | Optical density (AU) | Found amount (mg/ml) | Recovery (%) | nj | $S^2$ |
|---|---|---|---|---|---|---|---|
| 1 | 80% | 0,008 | 0,3454 | 0,00775 | 96,87 | 3 | 4,06939 |
| 2 | | 0,008 | 0,3483 | 0,00794 | 99,26 | | |
| 3 | | 0,008 | 0,3514 | 0,00807 | 100,88 | | |
| 1 | 90% | 0,009 | 0,3923 | 0,00880 | 97,78 | 3 | 2,3688 |
| 2 | | 0,009 | 0,3916 | 0,00893 | 99,20 | | |
| 3 | | 0,009 | 0,3952 | 0,00908 | 100,85 | | |
| 1 | 100% | 0.010 | 0,4458 | 0,0100 | 100,00 | 3 | 0 |
| 2 | | 0,010 | 0,4386 | 0,0100 | 100,00 | | |
| 3 | | | 0,4354 | 0,0100 | 100.00 | | |
| 1 | 110% | 0,011 | 0,4917 | 0,01103 | 100,27 | 3 | 3,1659 |
| 2 | | 0,011 | 0,4808 | 0,01096 | 99,66 | | |
| 3 | | 0,011 | 0,4933 | 0,01133 | 103,00 | | |
| 1 | 120% | 0,012 | 0,5292 | 0,01187 | 98,92 | 3 | 2,1226 |
| 2 | | 0,012 | 0,5319 | 0,01213 | 101,08 | | |
| 3 | | 0,012 | 0,5314 | 0,01220 | 101,66 | | |

**Table 5.13: Recovery results**

**Homogeneity of variances test, Cochran test:**

The homogeneity of variances test is performed with the Cochran test according to the formulas given in the Chapter 3. The obtained results are represented in the table 5.14.

| | |
|---|---|
| S2max | 4,06939 |
| Sum of variances | 11,736 |
| Ccalculated | 0,3501 |
| Ccalculated 5% | 0,68 |
| Ctheoritical 1% | 0,79 |
| Conclusion | Valid at 5% risk |

**Table 5.14: Numerical results of the Cochran test for the accuracy**

- Discussion:

According to the results $C_{cal} = 0,3501 < C_{th5\%} = 0,68$ the homogeneity of variances analysis at each concentration shows that all the variances of the different groups can be considered homogeneous at 5% risk.

**Test validity of the averages, $F_3$ Fisher test:**

The validity of the averages test is performed with the Fisher test according to the formulas given in the Chapter 3. The obtained resuts are represented in the table 5.15.

| | DF | Sum of squares | Variance | $F_{calculated}$ | $F_{th5\%}$ | $F_{th1\%}$ | Conclusion |
|---|---|---|---|---|---|---|---|
| Total variation | 14 | 31,91536 | 2,27967 | 0,89 | 3,48 | 5,99 | |
| Intra-group variation | 10 | 23,56459 | 2,35646 | | | | Valid at 5% risk |
| Inter-group variation | 4 | 8,35078 | 2,08769 | | | | |

**Table 5.15: Numerical results of Fisher test $F_3$ for the accuracy**

- Discussion:

The calculated $F_3$ is not significant: $F_{3cal} = 0,89 < F_{3th5\%} = 3,48$ so, the variantions between different groups are due to experimental errors.
The method is accurate at 5% risk.

**Estimation of the average recovery:**

The estimation of the average recovery is performed with the interval confidance calculations according to the formulas given in the Chapter 3. The obtained resuts are represented in the table 5.16.

| | |
|---|---|
| Average recovery | 99,963 |
| Confidance interval | 99,13 à 100,80 |

**Table 5.16: Average recovery**

- Discussion:

The average recovery is close to 100% so, the theoritical amount of active ingredient is satisfactory.
The confidance limits are within 80% and 120% for all concentration levels and the obtained confidance interval is within the latter.

Based on the obtained results, the procedure can be considered correct on all the determined assay.

### 5.3.4 Fidelity

The obtained raw data of the repeatability and reproducibility are given in the tables (5.17) and (5.18). These results are used to check the faithfulness of the assay method the latter must be repeatable an reproducible.

- Repeatability:

Table (5.17) shows all the raw data obtained by assaying seven Prednisone solutions, with a concentration of 0,01mg/ml, during the same day.

| Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Concentration (mg/ml) | 0,01 | 0,01 | 0,01 | 0,01 | 0,01 | 0,01 | 0,01 |
| Absorbance (AU) | 0,4308 | 0,4359 | 0 ,4367 | 0,4439 | 0,4348 | 0,4382 | 0,4389 |

**Table 5.17:Raw data for the repeatability**

- Reproducibility:

Table (5.18) shows all the raw data obtained with assaying three series of seven Prednisone solutions, with a concentration of 0,01mg/ml, during three days.

| | | Absorbances (AU) | |
|---|---|---|---|
| Essais | J | J+1 | J+2 |
| 1 | 0,4308 | 0,4343 | 0,4430 |
| 2 | 0,4359 | 0,4304 | 0,4402 |
| 3 | 0,4367 | 0,4341 | 0,4411 |
| 4 | 0,4439 | 0,4324 | 0,4398 |
| 5 | 0,4348 | 0,4405 | 0,4356 |
| 6 | 0,4382 | 0,4388 | 0,4321 |
| 7 | 0,4389 | 0,4386 | 0,4344 |

**Table 5.18:Raw data for the reproducibility**

**Recovery:**

The table 5.19 presents all obtained fidelity recovery results.

| Test | Introduced Amount (mg/ml) | Optical density (AU) | Found amount (mg/ml) | Recovery (%) | nj | $S^2$ |
|---|---|---|---|---|---|---|
| 1 | 0.01 | 0,4308 | 0,0097 | 97,00 | | |
| 2 | 0.01 | 0,4359 | 0,0098 | 97,78 | | |
| 3 | 0.01 | 0,4367 | 0,0098 | 97,96 | | |
| 4 | 0.01 | 0,4439 | 0,0100 | 99,57 | 7 | 0,664571 |
| 5 | 0.01 | 0,4348 | 0,0098 | 97,53 | | |
| 6 | 0.01 | 0,4382 | 0,0098 | 98,30 | | |
| 7 | 0.01 | 0,4389 | 0,0098 | 98,45 | | |
| 1 | 0.01 | 0,4343 | 0,0099 | 99,02 | | |
| 2 | 0.01 | 0,4304 | 0,0098 | 98,13 | | |
| 3 | 0.01 | 0,4341 | 0,0099 | 98,97 | | |
| 4 | 0.01 | 0,4324 | 0,0099 | 98,59 | | |
| 5 | 0.01 | 0,4405 | 0,0100 | 100,43 | 7 | 0,731824 |
| 6 | 0.01 | 0,4388 | 0,0100 | 100,05 | | |
| 7 | 0.01 | 0,4386 | 0,0100 | 100,00 | | |
| 1 | 0.01 | 0,4430 | 0,0102 | 101,75 | | |
| 2 | 0.01 | 0,4402 | 0,0101 | 101,10 | | |
| 3 | 0.01 | 0,4411 | 0,0101 | 101,31 | | |
| 4 | 0.01 | 0,4398 | 0,0101 | 101,01 | 7 | |
| 5 | 0.01 | 0,4356 | 0,0100 | 100,05 | | 0,845885 |
| 6 | 0.01 | 0,4321 | 0,0099 | 99,24 | | |
| 7 | 0.01 | 0,4344 | 0,0100 | 99,77 | | |

**Table 5.19: Fidelity recovery results**

**Homogeneity of variances test, Cochran test:**

The homogeneity of variances test allows to know if the method is precize or not, it is performed with the cochran test according to the formulas given in the chapter3. The obtained resuts are represented in the table 5.20.

| | |
|---|---|
| $S^2$max | 0,8459 |
| Sum of variances | 2,24228 |
| Ccalculated | 0,3535 |
| k (nb tests) | 3 |
| n (nb repetitions by test) | 7 |
| Ctheoritical 5% | 0,68 |
| Ctheoritical 1% | 0,76 |
| Conclusion | Valid at 5% risk |

**Table 5.20:Numerical results of the Cochran test for the fidelity**

- Discussion:

According to the results $C_{cal} = 0,3535 < C_{th5\%} = 0,68$ the homogeneity of variances analysis at each concentration shows that all the variances of the different groups are homogeneous at 5% risk so, the method is precize.

### Study of intergroup variations of the repeatability and the reproducibility:

The Study of intergroup variations of the repeatability and the reproducibility is performed with the coefficient of the repeatability and the reproducibility calculations according to the formulas given in the chapter3. The obtained results are illustrated in the table 5.21.

| | |
|---|---|
| Repeatability variance | 0,79771394 |
| Intergroups Variance | 1,538926299 |
| Reproducibility variance | 2,336640239 |
| Average | 99,31629739 |
| VC repeatability | 0,9% |
| VC reproducibility | 1,54% |

**Table 5.21:Intergroup variations of the repeatability and the reproducibility**

- Discussion:

The repeatability and reproducibility coefficients are $< 2\%$, which proves that the method is repeatable and reproducible so the assay method is precize and faithful.

- Conclusion:

According to the obtained results during the validation tests, we can conclude that the method is valid for the Prednisone assay, in the finished product, PRECORTYL®, 5mg pill in the defined measurement interval.

### 5.3.5 Results of the prednisone active ingredient dosage in the finished product PRECORTYL® 5 mg with UV-visible spectrophotometry:

Name of the product: PRECORTYL® 5 mg pill
Lot number: 0340
Date of manufacture: 02/22/2013
Expiry date: 02/2016
Validity: 03 years
Result in mg/pill: $T = 5,05 mg/pill$
Standards: $4,6 mg/pill < T < 5,4 mg/pill$
Result in %: $T = 101,075\%$
Standards: $92,00\% < T < 108,00\%$

According to the obtained results we concludes that the amount of prednisone assayed is compliant.

# Conclusion

FOR more safety, to reduce costs and to be in compliance with international regulations, the physicochimical and pharmacotechnical controls of the finished product and the validation of its analysis method as well as a comparative kinetic study between the generic and its princeps are a must in the pharmaceutical industry.

PRECORTYL® is a generic of CORTANCYL® drug. It is a glucocorticoid which is used in endocrine problems to treat a large range of hormonal disturbances such as allergies, asthma and autoimmune diseases this is why it is widespread in the pharmaceutical market and this is what makes it very interresting as generic. That implies that a very tight control should be carried to ensure that this medicine is efficient and not over-under dosed.

According to the obtained results, we can conclude that:

- The pharmacotechnical controls (mass uniformity, friability, time deletion) gives compliant results.

- In other hands, the assay method validation using UV performed by checking validation criteria using statistical studies (STUDENT, COCHRAN and FISHER tests) allows to validate the assay method. The assay method is linear, accurate, reproducible and repeatable with acceptable variation coeficient.
  The amount of the Prednisone is within the standards of the marketing authorisation file, so, the used assay method was validated with high degree of confidence.

- The dissolution profile of the generic is the same as the princeps and it is kinitically reproducible.

At the end of this work, we can say that the assay mathod using UV Spectrophotometry for the Prednisone active ingredient in the PRECORTYL® 5mg pill could be used in the routine tests.

It is good to notice that the PRECORTYL® should not be used in bacterian, viral or phongic or other affections that makes the immunitary system react to fight the desease, as the immunitary system is weakened by this kind of molecules.

# Appendix

## Appendix 1

## Description of premises

We are going to present initially the pharmal subsidiary company.

### Pharmal subsidiary company

Pharmal has three production unit and a laboratory of quality control that also provides benefits for its units and the external customers. Factory Dar El Beida: In the industrial area of Algiers, the plant produces a very wide range of drugs in various dosage forms (pasty, liquid and solid). Its production capacity exceeds 45 million sales unit (SU).

- Constantine factory:

Located in Constantine in the east of the country it has two (02) workshops syrups with a production capacity of 10 million sales unit (SU).

- Insulin Unit:

This unit is specialized in the production of human insulin in three types, a rapid-acting insulin (Rapid), a slow-acting insulin (Basal) and a combined insulin (Comb 25). These insulins are in suspended form.

- Annaba factory:

The factory is specialized in the manufacture of solid dosage forms with a production capacity of over 05 million sales unit (SU).

### Research and Developement Center, RDC-SAIDAL

It is located at the 35 AV BENYOUCEF KHETTAB El-Mohammadia, at 16Km of the Algiers capital.
The center of research and development (CRD) is responsible for the following:

- The development of policy and the development of research areas related to strategic missions SAIDAL in the medical sciences, particularly in pharmaceutical innovation.
- Participation in policy making drug development of SAIDAL group.
- The industrial design and development of generic drug in benefit of the SAIDAL group.
- Technical assistance to production subsidiaries.
- The Meeting of the material and technical resources and the mobilization and development of human resources to enable it to provide a technological and a prospective approach for the benefit of the group.
- The collection, processing and funding of scientific and technical information related to their field of activity and tasks of the group, it ensures the preservation and dissemination and facilitates consultation.
- Participation in training and re-training of staff technical development and scientific group.
- The testing of physico-chemical, pharmaco-technical, toxicological and microbiological drug.
- Promotion, enhancement and dissemination of technical and scientific work and research results.
- The completion of exploration work and studies to SAIDAL to establish alliances or strategic partnerships and profitable.

**Organization and operation**

Activities are carried out through DRC laboratories, technical facilities, structures and organs of support, project teams and research teams.

- Laboratory of pharmaceutics:

Primarily responsible for the formulation of generic drugs in various forms (dry, liquid, paste and injectable).

- Analytical Chemistry Laboratory:

Appendix

Responsible for monitoring compliance of raw materials (active ingredients, excipients, packaging ...), developing analytical and bio-analytical and monitoring stability studies of drugs developed.

- Laboratory pharmacotoxicology:

Responsible for monitoring compliance of raw materials and finished products by reactive animal toxicity studies, local tolerance and pharmacodynamics studies.

- Microbiology Laboratory:

Responsible for monitoring compliance of raw materials and finished products testing antibacterial activity and sterility tests, purity and efficiency.

- Center for Scientific Documentation and Technical Information:

Charged of building a reliable network of information necessary to the achievement of development objectives and its update permanently.

- Quality Assurance:

Responsible for the implementation of a quality system and its maintenance in accordance with international standards, and to report to management review as a basis for improvement.

- Regulatory Affairs:

Responsible for the development of pharmacy records and business name registration at the National Institute of Industrial Property (INAPI).

The percentage of the turnover of the group dedicated to research SAIDAL is 3%, equivalent to 150 million AD.

**RDC's workforce**

2nd cycle graduates (pharmacy, chemistry, biology, veterinary medicine, agronomy): 35%; Postgraduate (Pharmacy, Chemistry, and Agronomy): 18%; Licensees (finance, letters, library): 12%; Senior technicians: 5%; Maintenance: 3%.

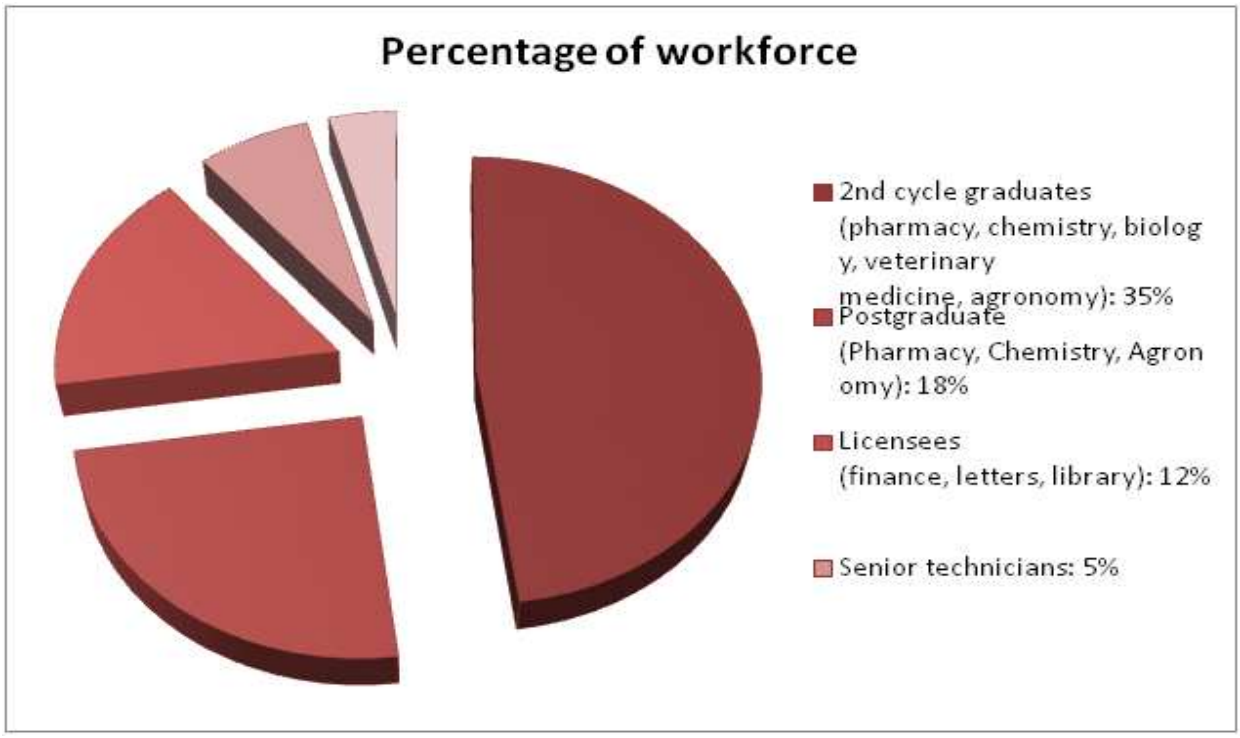


**Figure A: RDC's Workforce**

- SAIDAL flowsheet:

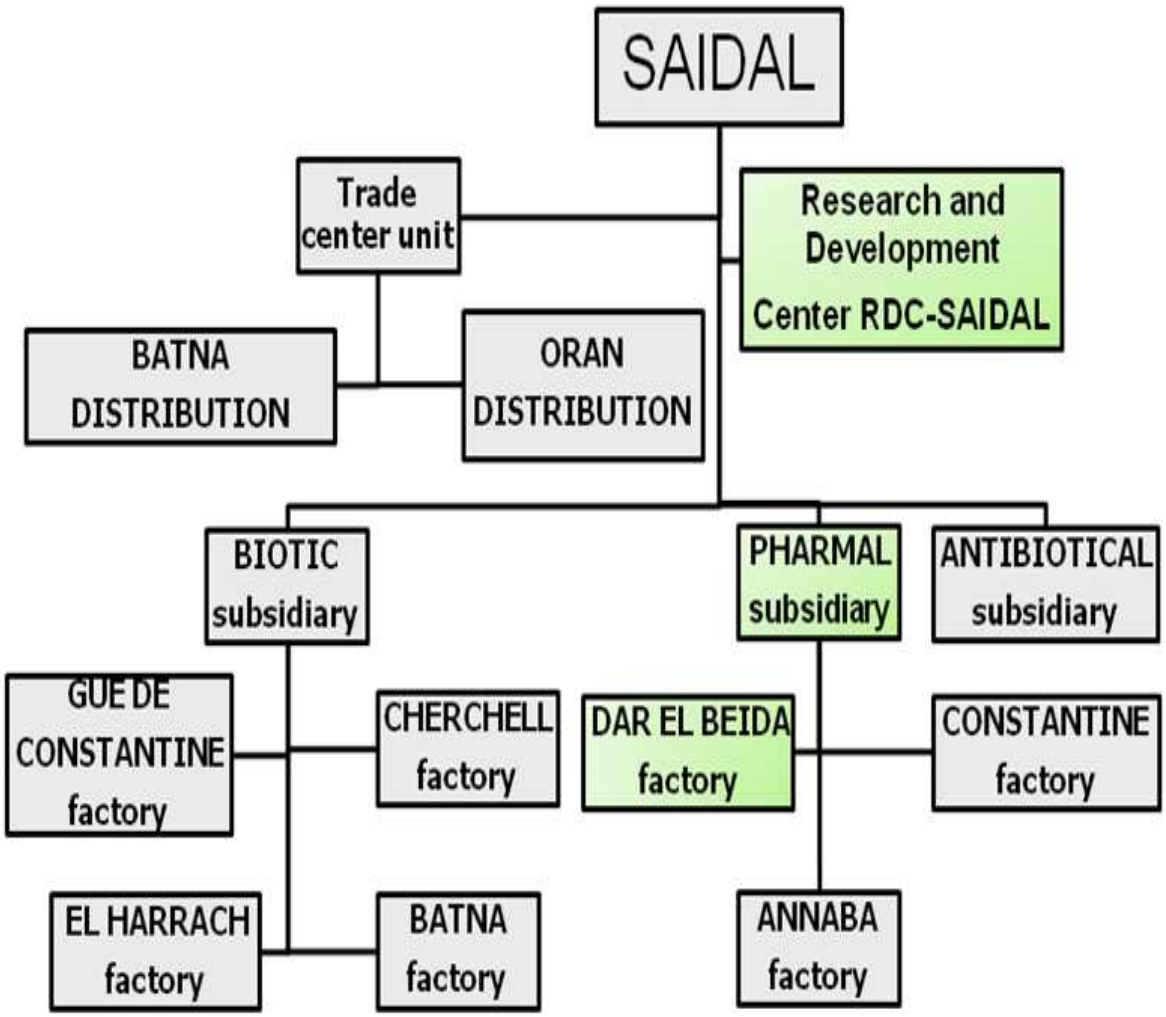


**Figure B: SAIDAL flowsheet**

# Appendix 2

# Hardware


**Figure C: Analytical balance, Sartorius**

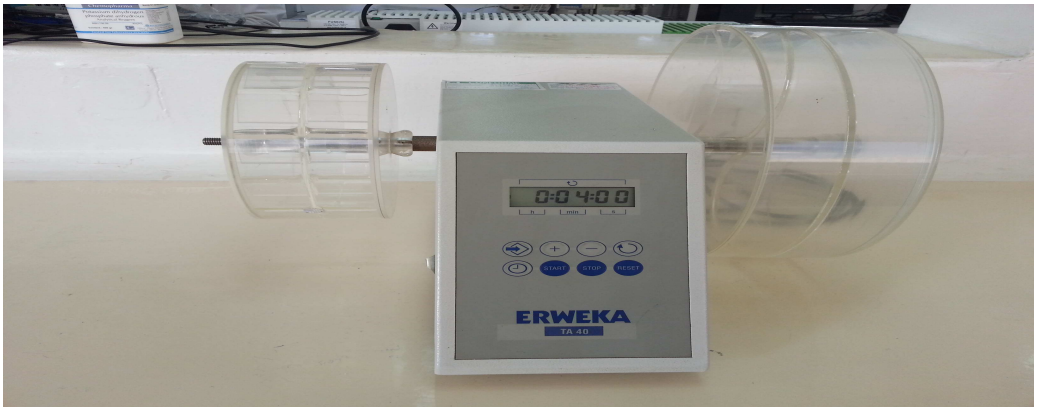


**Figure D: Friabilimeter, ERWAKA TA40**

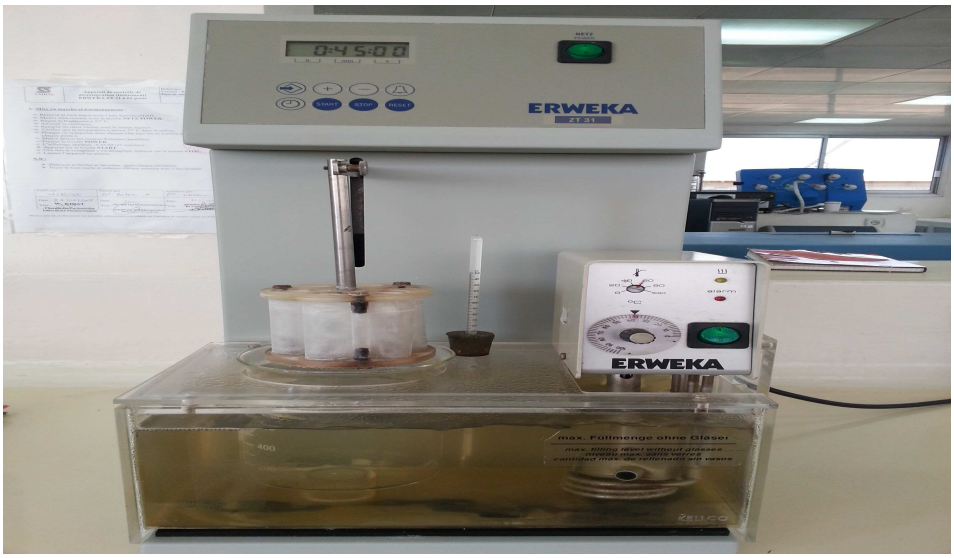


**Figure E: Deletion apparatus, ERWEKA ZT31**


**Figure F: UV-Spectrophotometer**


**Figure G: Simple Dissolutest, SOTAX**


**Figure H: Dissolutest autosampling, SOTAX**

# Appendix 3

# Dissolution kinetic results

- PRECORTYL®, 5mg prednisone (Generic)

The PRECORTYL®, 5mg prednisone Optical Densities obtained (OD) at 10, 20, 30 min are illustrated in the following tables:

- Results at 10mn:

| RSD | ODT | Absorbance 242nm | T (%) |
|---|---|---|---|
| | 0,3356 | 0,1885 | 56,83 |
| 0,39 | 0,3379 | 0,2638 | 79,53 |
| | 0,3378 | 0,2254 | 67,96 |
| Mean t | 0,3371 | 0,2081 | 62,74 |
| | | 0,2291 | 69,07 |
| | | 0,2499 | 75,34 |
| Pet(mg) | 200,6 | Mean | 68,58 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table I: Obtained OD at 10min**

- Results at 20mn:

| RSD | ODT | Absorbance 242nm | T (%) |
|---|---|---|---|
| 0,39 | 0,3356 | 0,2806 | 84,60 |
| | 0,3379 | 0,2896 | 87,31 |
| | 0,3378 | 0,3300 | 99,49 |
| Mean t | 0,3371 | 0,3394 | 102,33 |
| | | 0,3314 | 99,92 |
| | | 0,3866 | 116,56 |
| Pet(mg) | 200,6 | Mean | 98,37 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table II: Obtained OD at 20min**

- Results at 30mn:

| RSD | ODT | Absorbance 242nm | T (%) |
|---|---|---|---|
| 0,39 | 0,3356 | 0,3419 | 103,08 |
| | 0,3379 | 0,3242 | 97,74 |
| | 0,3378 | 0,3587 | 108,15 |
| Mean t | 0,3371 | 0,3682 | 111,01 |
| | | 0,3985 | 120,15 |
| | | 0,3607 | 108,75 |
| Pet(mg) | 200,6 | Mean | 108,15 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table III: Obtained OD at 30min**

- Obtained OD raw tada at 10, 20 and 30 min:

**Wavelength program - Lambda 25 lundi 1 avril 2013 16:15 Paris, Madrid (heure d'été)**

Data Table

| Sample ID | Description | 242.0 |
|---|---|---|
| T1 | | 0.3356 |
| T2 | | 0.3379 |
| T3 | | 0.3378 |
| G1-10 | | 0.1885 |
| G2-10 | | 0.2638 |
| G3-10 | | 0.2254 |
| G4-10 | | 0.2081 |
| G5-10 | | 0.2291 |
| G6-10 | | 0.2499 |
| G1-20 | | 0.2806 |
| G2-20 | | 0.2896 |
| G3-20 | | 0.3300 |
| G4-20 | | 0.3394 |
| G5-20 | | 0.3314 |
| G6-20 | | 0.3866 |
| G1-30 | | 0.3419 |
| G2-30 | | 0.3242 |
| G3-30 | | 0.3587 |
| G4-30 | | 0.3682 |
| G5-30 | | 0.3985 |
| G6-30 | | 0.3607 |

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Printed by: Administrator
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Print Date and Time: avril 01, 2013 16:30:54 Paris, Madrid (heure d'été)

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- CORTANCYL®, 5mg prednisone (PRINCEPS)

The CORTANCYL®, 5mg prednisone Optical Densities (OD) obtained at 10, 20, 30 min are illustrated in the following tables:

- Results at 10mn:

| RSD | ODT | Absorbance 242nm | T (%) |
|---|---|---|---|
| 22,18 | 0,2267 | 0,1945 | 65,98 |
| | 0,3153 | 0,2523 | 85,59 |
| | 0,3568 | 0,2572 | 87,25 |
| Mean t | 0,2996 | 0,2362 | 80,13 |
| | | 0,2373 | 80,50 |
| | | 0,2245 | 76,16 |
| Pet(mg) | 200,6 | Mean | 79,27 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table IV: Obtained OD at 10min**

- Results at 20mn:

| RSD | ODT | Absorbance 242nm | T (%) |
|---|---|---|---|
| 22,18 | 0,2267 | 0,2516 | 85,35 |
| | 0,3153 | 0,2702 | 91,66 |
| | 0,3568 | 0,2904 | 98,51 |
| Mean t | 0,2996 | 0,3136 | 106,38 |
| | | 0,3212 | 108,96 |
| | | 0,3145 | 106,69 |
| Pet(mg) | 200,6 | Mean | 99,59 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table V: Obtained OD at 20min**

- Results at 30mn:

| RSD | ODT | Absorbance 242 nm | T (%) |
|---|---|---|---|
| 22,18 | 0,2267 | 0,3103 | 105,26 |
| | 0,3153 | 0,3164 | 107,33 |
| | 0,3568 | 0,3298 | 111,88 |
| Mean t | 0,2996 | 0,3195 | 108,38 |
| | | 0,3023 | 102,55 |
| | | 0,3216 | 109,10 |
| Pet(mg) | 200,6 | Mean | 107,42 |
| TITRE (%) | 101,33 | | |
| Pe E (mg) | 5 | | |

**Table VI: Obtained OD at 30min**

- Obtained OD raw data at 10, 20 and 30 min:

**Wavelength program - Lambda 25 lundi 1 avril 2013 13:15 Paris, Madrid (heure d'été)**

Data Table

| Sample ID | Description | 242.0 |
|---|---|---|
| T1 | | 0.2267 |
| T2 | | 0.3153 |
| T3 | | 0.3568 |
| G1-10 | | 0.1945 |
| G2-10 | | 0.2523 |
| G3-10 | | 0.2572 |
| G4-10 | | 0.2362 |
| G5-10 | | 0.2373 |
| G6-10 | | 0.2245 |
| G1-20 | | 0.2616 |
| G2-20 | | 0.2702 |
| G3-20 | | 0.2904 |
| G4-20 | | 0.3136 |
| G5-20 | | 0.3212 |
| G6-20 | | 0.3145 |
| G1-30 | | 0.3103 |
| G2-30 | | 0.3164 |
| G3-30 | | 0.3298 |
| G4-30 | | 0.3195 |
| G5-30 | | 0.3023 |
| G6-30 | | 0.3216 |

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Printed by: Administrator
Template: Default-Wavelength Program
Print Date and Time: avril 01, 2013 14:30:54 Paris, Madrid (heure d'été)

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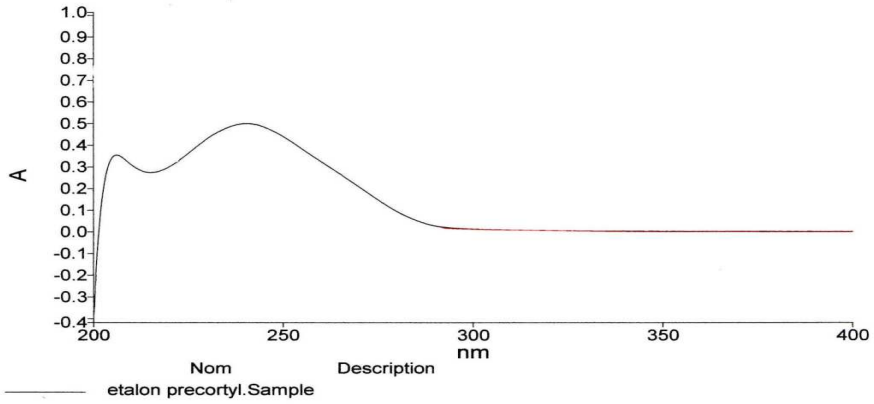
# Appendix 4

# Specificity results

- Standard solution spectrum:

**Scan - Lambda 25 dimanche 28 avril 2013 15:28 Paris, Madrid**

Raw Spectra




Nom Description

etalon precortyl.Sample

Instrument Settings

| | | |
|---|---|---|
| Scan Range Start | 400 | nm |
| Scan Range End | 200 | nm |
| Scan Speed | 480 | nm/min |
| Data Interval | 1 | nm |
| Cycle Count | 1 | |
| Cycle Time | 1 | s |
| Ordinate Type | A | |
| Slit Width | 1 | nm |
| UV Lamp On | Yes | |
| Visible Lamp On | Yes | |
| Lamp Change-over Wavelength | 326 | nm |

| | |
|---|---|
| Method | Scan - Lambda 25 |
| Analyst | Analyst |
| Time | avril 28, 2013 15:31 Paris, Madrid |

PerkinElmer precisely

User: Analyst
Template: Default-Scan
Date: 04/28/2013 15:35 Paris, Madrid (heure d'été)

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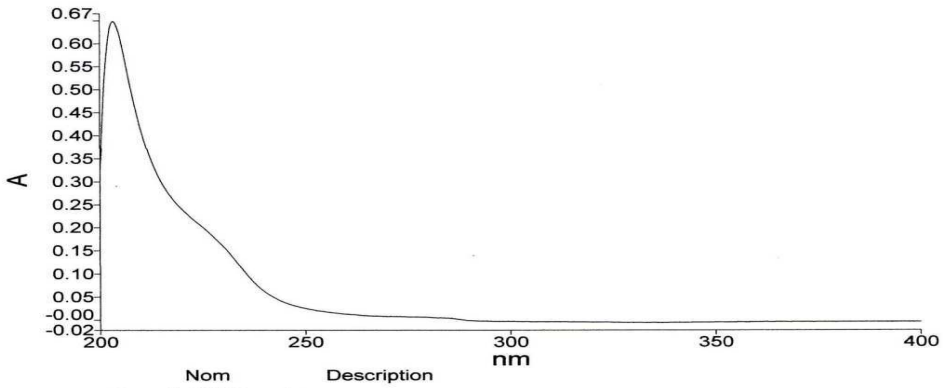
- Placebo solution spectrum:

**Scan - Lambda 25 dimanche 28 avril 2013 15:28 Paris, Madrid**

Raw Spectra




| Nom | Description |
|---|---|
| Sample393.Sample | |

Instrument Settings

| | | |
|---|---|---|
| Scan Range Start | 400 | nm |
| Scan Range End | 200 | nm |
| Scan Speed | 480 | nm/min |
| Data Interval | 1 | nm |
| Cycle Count | 1 | |
| Cycle Time | 1 | s |
| Ordinate Type | A | |
| Slit Width | 1 | nm |
| UV Lamp On | Yes | |
| Visible Lamp On | Yes | |
| Lamp Change-over Wavelength | 326 | nm |

| | |
|---|---|
| Method | Scan - Lambda 25 |
| Analyst | Analyst |
| Time | avril 28, 2013 15:52 Paris, Madrid |

PerkinElmer precisely

User: Analyst
Template: Default-Scan
Date: 04/28/2013 15:53 Paris, Madrid (heure d'été)

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