1.5.2 Validation of a Method

In actual practice, a great care should be taken that accurate and precise results are duly accomplished in a **pharmaceutical analysis.** It is, however, pertinent to mention here that the best way and means to **validate a method (technique)** is to analyze a **standard reference substance (material)*** of known composition.

One may critically observe that each and every measurement essentially possesses some sort of imprecision associated with it, that predominantly gives rise to **certain array of random distribution of results,** such as:

(*a*) **Gaussian Distribution:** The number of individual sample increments needed to accomplish a given level of confidence in the **'analytical results'** is usually estimated by the following expression:

$$n = \frac{t^2 S^2_s}{r^2 x^{-2}} \qquad ...(i)$$

where, t = Student t value for the desired confidence level,

S^2_s = Sampling variance,

r = **Acceptable** relative standard deviation of the average of the analytical results, $\bar{x}$;

S_s = **Absolute** standard deviation, in the same unit as $\bar{x}$; and hence, x is unitless.

S_s and $\bar{x}$ = Values are invariably obtained from the **preliminary measurements** or from the **prior knowledge.**

Equation (*i*) actually holds good for a **Gaussian Distribution** of analyte concentrations very much with in the bulk material, *i.e.,* it would be centered around x having **68%** of the values falling within **one standard deviation** or even upto **95%** very much within **two standard deviations.**

Therefore, the experiment may be carefully planned and designed in such a fashion so as to narrow its range, but it cannot be eliminated completely.

(*b*) **Systematic Error:** A **systematic error** refers to one that specifically **biases a result consistently in one direction.**

(*c*) **Sample Matrix:** The **sample matrix** (*i.e.,* and environment in which a sample gets developed) may usually suppress the signal emanated by an instrument.

(*d*) The **weight of an analytical balance** may be in error, made biased either high or low.

(*e*) The sample may not be dried sufficiently.

***BPCRS:** British Pharmacopoea Chemical Reference Substance;

EPCRS: Europeon Pharmacopoea Chemical Reference Substance;

Ca (Calcium), C (Carbon), and O (Oxygen) are present actually. Bearing in mind the fact that $CaCO_3$ (Calcium Carbonate) may be considered as a combination of CaO (Calcium Oxide) together with CO_2 (Carbonic Anhydride), the ultimate composition of this particular salt (*i.e.*, $CaCO_3$) is also frequently expressed in terms of the corresponding percentages of the *two* **oxides** CaO and CO_2 respectively.

1.2 IMPORTANCE OF QUANTITATIVE ANALYSIS IN QUALITY CONTROL

Quantitative **pharmaceutical analysis** is of enormous importance and relevance not only confined to the ever expanding knowledge in various disciplines of science, but also in industry, particularly the pharmaceutical industry for the critical analysis of raw materials, intermediates, and final dosage forms (*i.e.*, the secondary pharmaceutical products).

Salient Features

The various salient features with respect to the **quantitative analysis in quality control** are as enumerated under:

1. **Chemical Formula of an Unknown Substance:** The chemical formula of an '**unknown substance**' either synthesized in the laboratory or isolated from naturally occurring plant sources is invariably determined and established from the percentage contents of its constituents found by actual analysis.
2. In fact, the '**chemical analysis**' represents the backbone of a plethora of most important method of investigation; and, therefore,employed extensively and profusely in practically all branches of science that are intimately associated to chemistry.

   Examples: It finds its abundant utility in pharmaceutical analysis of both inorganic and organic chemical substances, microbiology, biochemistry, biotechnology, pharmaceutical technology, besides, certain highly specific disciplines *e.g.*, physiology, geology, minerology, medical, and agricultural sciences.
3. The '**chemical analysis**' possesses an enormous potential in a **pharmaceutical industry** in particular, and other **allied industries** in general. On a rather more specific note—a '**pharmacist**' should be fully aware at each and every step of the adopted **production process** the **qualitative** as well as the **quantitative** chemical composition of the materials (substances) that have undergone expected and desired **conversion** (or modification).

   Example: **Thermogravimetric Analysis (TGA) of Calcium Oxalate Monohydrate:** It has been duly observed that a large number of chemical substances usually get decomposed upon heating. In fact, this very idea and concept of heating a particular given sample to observe carefully the ensuing weight variations is the underlying principle of thermogravimetric analysis (TGA). Interestingly, the TGA of Ca-oxalate monohydrate represents the '**dynamic thermogravimetric analysis**' wherein the said sample is specifically subjected to conditions of predetermined and controlled continuous increase in temperature which is mostly observed to be '**linear with time**'.

Method

The '**thermogram**' for calcium oxalate monohydrate [$CaC_2O_4.H_2O$] is duly represented in Figure 1.1. One may, however, distinctly observe the **successive plateaus,** namely:

(*i*) Correspond to the **anhydrous oxalate** (100-250°C),

(*ii*) Correspond to the **anhydrous calcium carbonate** (400-500°C), and

(*iii*) Correspond to the **calcium oxide** (700-850°C).

In other words, the ***three*** observed plateaus (in Fig. 1.1) clearly designate the following vital aspects of the decomposition curve, namely:

(*a*) clear indication of **constant weight**, and

(*b*) stable phases encountered within a specified temperature interval.

The various distinct **chemical reactions** that are actually involved may be summarized as follows:

$$\underset{\text{Calcium oxalate monohydrate}}{CaC_2O_4 \cdot H_2O} \xrightleftharpoons{100\text{-}250^\circ C} \underset{\text{STAGE-1}}{} \underset{\text{Calcium oxalate}}{CaC_2O_4\ [+H_2O\uparrow]} \xrightleftharpoons{400\text{-}500^\circ C} \underset{\text{STAGE-2}}{} \underset{\text{Calcium carbonate}}{CaCo_3\ [+CO\uparrow]} \xrightleftharpoons{700\text{-}850^\circ C} \underset{\text{STAGE-3}}{} \underset{\text{Calcium oxide}}{CaO\ [+CO^{2}\uparrow]}$$

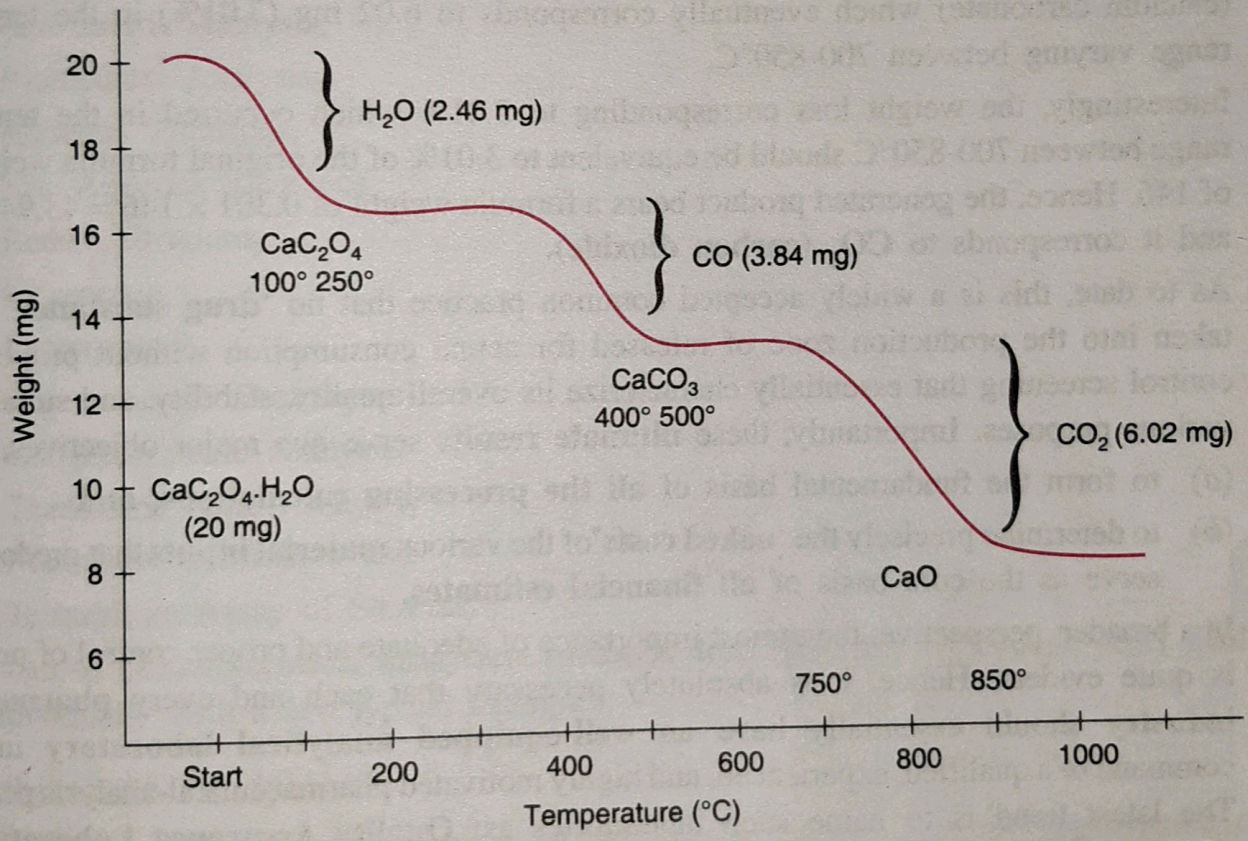

Fig. 1.1 Thermogravimetric Analysis (TGA) of Calcium Oxalate Monohydrate at the Rate of 6°C per Minute.

Explanations

The adequate explanations of the *three* cardinal stages intimately encountered in Fig. 1.1 are as given below:

STAGE-1

The inherent water of crystallization (or hydration) from calcium oxalate monohydrate is virtually lost that corresponds to 2.46 mg (12.3%) equivalent to 1 mole of water (H_2O) between the temperature range 100-250°C.

In actual practice, the 12.3% weight loss that usually occur between 100-250°C must correspond to 12.3% of the original formula weight for $CaCo_3.H_2O$ (FW = 146). Therefore, the product which gets lost eventually has a formula weight (FW) of 0.123 × 146 = 17.958 (≃ 18.0), and it definitely corresponds to **H_2O (water).**

STAGE-2

One mole of carbon monoxide (CO) gets evolved from calcium oxalate [CaC_2O_4], corresponding to 3.84 mg (19.2%) between the temperature varying from 400-500°C.

In fact, the 19.2% weight loss which took place between 400-500°C must actually correspond to 19.2% of the original formula weight of 146. Hence, the resulting product being produced bears a formula weight of 0.192 × 146 = 28.0, which corresponds to **CO (carbon monoxide).**

STAGE-3

At this **'final stage'** one mole of CO_2 (carbon dioxide) gets evolved from one mole of $CaCO_3$ (calcium carbonate) which eventually corresponds to 6.02 mg (3.01%) in the temperature range varying between 700-850°C.

Interestingly, the weight loss corresponding to 3.01% which occurred in the temperature range between 700-850°C should be equivalent to 3.01% of the original formula weight (FW) of 146. Hence, the generated product bears a formula weight of 0.301 × 146 = 43.946 (≃ 44), and it corresponds to **CO_2 (carbon dioxide).**

4. As to date, this is a widely accepted common practice that no **'drug substance'** is either taken into the production zone of released for actual consumption without proper quality control screening that essentially characterize its overall quality, stability, and suitability for various purposes. Importantly, these **ultimate results** serve *two* major objectives, namely:
   (*a*) to form the fundamental basis of **all the processing calculations, and**
   (*b*) to determine precisely the **'naked costs'**of the various **material inputs** that predominantly serve as the core basis of **all financial estimates.**
5. In a broader perspective, the utmost importance of adequate and proper control of production is quite evident. Hence, it is absolutely necessary that each and every **pharmaceutical industry** should essentially have an **well-equipped analytical laboratory** under the command of a qualified, experienced, and highly motivated pharmaceutical-analysis personnels. The latest trend is to name such laboratories as: **Quality Assurance Laboratory:** and **Quality Control Laboratory** (a rather older nomenclature), for the chemical control, physical parameters, and microbiological screening of raw materials, intermediates, and finished products.
6. In actuall practice, however, the pharmaceutical analyst's major problem is most commonly simplified to a great extent significantly, by virtue of the fact that the **'qualitative composition'** of majority of the investigated products *viz.,* raw materials, pure drug substances, chemical additives, pharmaceutical adjuvants, intermediates, and finished dosage forms is well-known (as described in the **'official compendia'** *e.g.,* IP, BP, USP, Eur. P., Int. P etc.). Furthermore, the approximate contents of the individual elements are obviously known quite frequently.

Evidently, in such particular instances the usual preliminary qualitative analysis becomes more or less unnecessary absolutely, thereby rendering the proper selection of the most appropriate technique of the **'quantitative analysis'** becomes much more easier and meaningful.

1.3 DIFFERENT TECHNIQUES OF PHARMACEUTICAL ANALYSIS

Over the years, the domain of **'Pharmaceutical Analysis'** have witnessed the emergence of a host of vital and important different techniques that have been used predominantly in the analysis of raw materials, intermediates, and finished dosage forms (*i.e.,* secondary pharmaceutical products). Each individual technique of **'Pharmaceutical Analysis'** is essentially governed by an underlying principle which caters to the dependable and reproducible assay of several **'official drug substances'**, pharmaceutical adjuncts, chemical additives, and preservatives as well.

A few typical variant technique frequently, and abundantly employed in **'Pharmaceutical Analysis'** are as enumerated under:

(*i*) Volumetric Analysis,
(*ii*) Gravimetric Analysis,
(*iii*) Biomedical Analysis,
(*iv*) Aqueous Titrations,
(*v*) Non-Aqueous Titrations,
(*vi*) Redox Titrations,
(*vii*) Iodometry,
(*viii*) Bromometry,
(*ix*) Argentometric Techniques,
(*x*) Complexometric Analysis,
(*xi*) Thermoanalytical Analysis,
(*xii*) Diazotization Method, and
(*xiii*) Tetrazolium Assay of Steroids.

The various **pharmaceutical analytical methods** described from (*i*) through (*xiii*) shall now be treated individually with a few typical examples.

1.3.1 Volumetric Analysis

In general, the **Volumetric Analysis** exhibits an enormous advantage *vis-a-vis* the **gravimetric method** by virtue of the significantly rapid speed of the former. The actual assays are distimctly more rapid due to the fact that instead of weighing the **'reaction product'** (as in **Gravimetric Method**) the actual volume of a reagent solution consumed for the reaction is being measured carefully. Thus, the **Concentration** (termed as the **'titre value'**) of the solution under investigation is determined accurately and precisely.

Titre value

The titre of a solution is normally regarded as the mean number of grammes of substance per 1 ml of solutions.