Chapter

Spectrophotometric/Titrimetric Drug Analysis

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Abstract

The importance of pharmaceuticals comes from their direct connection to human life. Therefore, many analysis techniques such as chromatography, spectroscopic methods, and others have been developed for one goal, which is to ensure that the drug reaches humans with high quality. Spectrophotometric and titrimetric methods have been in general use for the last 40 years and over this period have become the most important analytical instrument in the modern-day laboratory. In many applications, other techniques could be employed in pharmaceutical analysis, but none rival UV–visible spectrometry as well as titrimetry, for their simplicity, versatility, speed, accuracy, and cost-effectiveness. This chapter highlights the spectroscopic methods in the ultraviolet and visible regions, as well as the titration methods that are still widely used in the field of pharmaceutical analysis. The types of titrations, as well as the most important reactions used in spectrophotometric methods, are presented. Examples of the most important applications in the field of pharmaceutical analysis are also presented.

Keywords: analysis, spectrophotometric, titrimetric, pharmaceutical, electromagnetic radiation

1. Introduction

The majority of the methods currently available used in pharmaceutical analysis are high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), high-performance thin layer chromatography (HPTLC), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), capillary electrophoresis (CE), voltammetry, HPLC/NMR, etc., all require highly sophisticated instruments which are very expensive, involve tedious multiple extraction steps and are time-consuming. Therefore, there is a constant need for developing analytical methods, such as titrimetry and spectrophotometry that are simple, sensitive, rapid, accurate, precise, and inexpensive and that can be easily adapted by the pharmaceutical industry. In recent years, the assay methods in the monographs including titrimetric and spectrometric analytical methods can be seen in the literature for pharmaceutical analysis.

Titrimetric methods have maintained their great value as an analytical tool despite the steadily growing resort to purely physical methods which often necessitate very sophisticated and expensive instrumentation.

The titrimetric techniques are still widely used in the analysis for the assay of bulk drug materials and their share in the European Pharmacopeia (EP) is almost 70%. Also, in the United States Pharmacopeia (USP) more than 40% of low molecular weight organic compounds are determined by aqueous or non-aqueous titration [1]. In fact, titrimetric methods are still as widely used as ever in pharmaceutical analysis, especially since the development of physicochemical assays of measurement, as well as spreading of non-aqueous titration method and potentiometric end point detection, expanding and improving the field of application of titrimetric methods, especially in the pharmaceutical analysis. The European and United States Pharmacopeias are adopting many analytical methods to ensure the quality of the drug, such as titration, spectrometry, chromatography, and others. These methods and their respective proportions are included in **Table 1** according to the edition of European (The European Pharmacopeia and Council of Europe, 2002) and US (United States Pharmacopeia, 2004) pharmacopeias [2, 3]. It is noted from the table that spectroscopic and titration methods are still widely used in the pharmaceutical analysis. To name a few, in the literature survey, titrimetric methods have been used for the determination of terbinafine [4–8], ethionamide [9–13], and Amoxicillin [14–20] in pharmaceutical formulations. In addition to many drug formulations that were estimated using titration methods.

Further, among the various instrumental methods available for trace analysis, UV–visible spectrophotometry continues to be one of the most popular, because of its simplicity and cost-effectiveness. UV–visible spectrophotometry is one of the most widespread techniques were used in analytical chemistry for drug analysis, capable of producing accurate and precise results. For these reasons, procedures using this technique are found in analytical, pharmaceutical, and research laboratories. Specially, in the field of pharmaceutical analysis, spectrophotometric offers the best detection sensitivity, accuracy, and reproducibility of drug analysis in the bath of drug research, development, and laboratories quality control testing of marketed drug products.

In the United States Pharmacopeia (USP), UV–visible spectrophotometric methods still provide the majority of the spectrophotometric procedures, there are still over 200 specific monographs containing UV–visible spectrophotometric measurements in the current version of (USP36-NF31). As well as, the number of UV–visible spectrophotometric assays used in the pharmaceutical analysis is increasing more than other spectrophotometric techniques, such as IR and fluorescence.

If we follow the international refereed journals that are concerned with publishing scientific research in the field of drug analysis, we will find that the majority of

USP	European Pharmacopeia	Method
44	16	HPLC
41	70	Titration
9	10	UV–visible spectrophotometry
6	4	Other

Table 1.
Proportion of titrimetric/spectrophotometric methods used for the assay of bulk drug according to European and Unites States pharmacopeias [00,000].

published research has used the spectrophotometric technique in analysis, directly or indirectly. To name a few, in the estimation of some anti-infective agents in pharmaceuticals, we will find that most of the published research about it used spectroscopic methods [21–30]. We also find that browsing through the majority of pharmaceutical analysis books finds that all of them give more space in that books to talk about spectrophotometric techniques and their various applications, especially in the field of drug analysis.

The above proves beyond any doubt that titrimetric and spectrophotometric methods are considered to present and future as the most important methods used in the analysis of pharmaceutical formulations with their accuracy in measurements.

2. Titrimetric techniques and their applications in pharmaceutical analysis

Although about 200 years have elapsed since the publication of the first papers dealing with titrimetric analysis [31], the technique is still as widely used as ever in pharmaceutical analysis because of its robustness, cheapness, and capability for high precision, with also many advantages associated with these methods which include saving time and labor, and no need of using reference standards. In fact, titrimetric methods to these days are still widely used in pharmaceutical analysis simultaneously with the development of physicochemical methods for different measurements, as well as the spreading of non-aqueous titration method and potentiometric end point detection, expanding and improving the field of application of titrimetric methods, especially in the pharmaceutical analysis.

Titrimetry is the volumetric procedure for the determination of the concentration of the drug sample by adding a known concentration of the standard drug substance. This reacts quantitatively with the sample solution. Then a chemical reagent is used to detect the endpoint by the color change, the precipitate, or complex formation at the equivalent point of the titration. This reagent is known as the indicator.

2.1 Types of titrations commonly used in the pharmaceutical analysis

- Acid-base reactions: These reactions are based upon the titrations of the acidic or basic compounds by the consequent acids or bases. In addition, many drugs can be classified as acids or bases based on the presence of some functional group in the drug and these drugs can be analyzed using this type of reaction. In this type of reaction, H^+ reacts with OH^- to form H_2O as in the following examples: These reactions are mainly based upon the reactions of the hydrogen ion and hydroxide ion to form water.

$$H^+ + OH^- \rightarrow H_2O \tag{1}$$

A classic application of this type of titration is the determination of aspirin (acetylsalicylic acid) [32].

- Complexometric reactions: These types of titrations are based on the complexation reactions by using the complexing agent such as ethylenediaminetetraacetic acid (EDTA). these reactions are carried out by

complex formation by combining ions by using complexing agents like EDTA. The endpoint is detected by using metal ion detectors.

- Precipitation reactions: These titrations are based on the precipitate formation.

Example: AgCl titrations: these reactions are carried out by the formation of the precipitate by combining the ions by using the precipitating reagents.

A precipitation reaction is a titration in which the reaction between the analyte and the titrants forms an insoluble precipitate. Most precipitation titrations involve standard Ag^+ as a titrant and Cl^-, SCN^- as an analyte. An example is a titration of chloride ions with silver nitrate solution to form a silver chloride precipitate. This type of reaction is used in pharmaceutical assays of many drugs, especially the drugs that are found as chloride salts such as bupropion hydrochloride (antidepressant drug) [33].

- Redox reactions: Redox reactions are more widely used in titrimetric pharmaceutical analysis than other types of reactions. The ions may exist in different oxidation states resulting in the possibility of a very large number of redox reactions. Many of these reactions satisfy the requirements for use in titrimetric analysis and applications are numerous. These reactions also are important for some basic functions of life, such as photosynthesis [34].

A good example of a redox reaction is the thermite reaction, in which iron atoms in ferric oxide lose (or give up) O atoms to Al atoms, producing Al_2O_3 [3].

$$Fe_2O_{3(s)} + 2Al_{(s)} \rightarrow Al_2O_{3(s)} + 2Fe_{(l)} \quad (2)$$

The successful application of a redox reaction to titrimetric analysis requires, among other things, the means for detecting the equivalence point. Therefore, it is worth examining the changes that occur in variations that are most pronounced in the region of the equivalence point.

In the pharmaceutical formulation, a common application of this type of titration involves iodine I_2, potassium permanganate $KMnO_4$, and cerium (VI). The direct titration method against iodine (sometimes termed iodimetry) refers to titrations with a standard solution of iodine.

($I_2 + 2e^- \rightarrow 2I^-$ iodine is oxidizing agent)

Iodine has low solubility in water but the complex I_3^-, is very soluble. So, in the most direct titrations with iodine (iodimetry) iodine solutions are prepared by dissolving I_2 in a concentrated solution of KI (potassium iodide). This type of titration (iodimetry) can be used in the assay of many pharmaceutical compounds such as ascorbic acid (vitamin C), benzylpenicillin, ampicillin, cloxacillin, methicillin, carbenicillin, cefazolin, cephalothin, cephaloglycin, cephalexin, cephalosporin C, 7-aminocephalosporanic acid, and cefoxitin [35, 36].

- The titrant is (I_2 + KI) solution.

  (that can be standardized against $Na_2S_2O_6$; sodium thiosulfate).

- The analyte is ascorbic acid solution, for example.

- The indicator is a starch; the endpoint is the appearance of the blue color.

While the indirect titration method (sometimes termed iodometry) deals with the titration of iodine liberated in chemical reactions.

($2I^- \rightarrow I_2 + 2e^-$ iodine is reducing agent)

The second method (Iodometry) called indirect or back titration that involves an excess of KI being added, reducing the analyte and liberating I_2. The amount of I_2 produced is then determined by a back titration using $Na_2S_2O_3$ as a reducing titrant. The iodometry titration can be used in the assay of many pharmaceutical compounds such as amoxicillin and diethylcarbamazine citrate [14, 37–39].

The titrant is a thiosulfate solution,

- The titration flask contains the analyte and iodide in an acid medium.
- The liberated I_2 is then titrated with thiosulfate using a starch indicator.
- The endpoint is the disappearance of the blue color.

Potassium permanganate ($KMnO_4$) and cerium (IV) also are widely used as an oxidizing titrant in the assay of pharmaceutical compounds such as famotidine citrate, diethylcarbamazine citrate, minoxidil, hydrogen peroxide and Pantoprazole [40–44], Vitamin C, Ofloxacin, and ketotifen [45–47], respectively.

- Non-aqueous reactions: These reactions are based upon the titrations by using the non-aqueous titrants. Non-aqueous titrations are titrations carried out in the absence of water. In potentiometric titrations, absolute potentials or potentials concerning standard half-cells are not usually required, and measurements are made while the titration is in progress. The equivalence point of the reaction will be revealed by a sudden change in the potential in the plot of e.m.f. reading against the volume of the titration solution, and we can determine the end-point graphically. The graphical method (the differential method) involves a plot of the change in potential per unit change in the volume of reagent ($\Delta E/\Delta V$) as a function of the average volume of the reagent added. The end-point is taken as the maximum in the curve and is obtained by extrapolation of the experimental points (**Figure 1**).

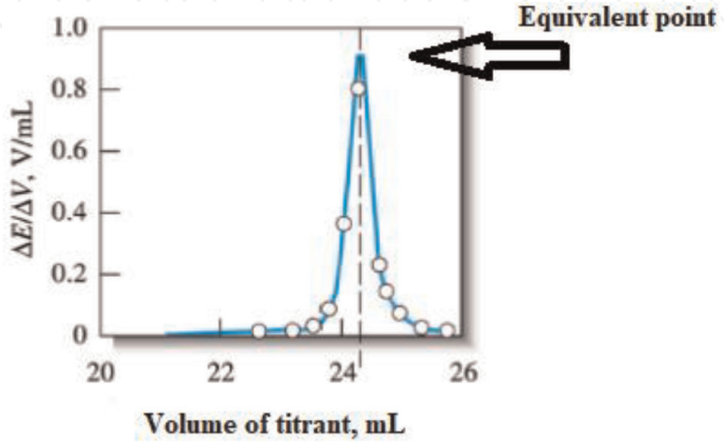

Figure 1.
Potentiometric titration curve.

Non-aqueous titration is the most common titrimetric procedure used in the pharmaceutical assays of many drugs [7, 15, 27]. Non-aqueous titrations are widely used in Volumes I and II of the British Pharmacopeia for the assay of drug substances. A large number of drugs are either weakly acidic or weak base. The weak acids are usually titrated with tetrabutylammonium hydroxide (Bu_4NOH) or potassium methoxide (CH_3OK) in dimethyl formamide (DMF) as a solvent. Weak bases are dissolved in glacial acetic acid and titrated with perchloric acid ($HClO_4$). For weak bases, the titration medium usually used for non-aqueous titration of bases is perchloric acid in acetic acid. However, perchloric acid is not a primary standard substance, so it can be standardized using potassium hydrogen phthalate ($KHC_8H_4O_4$) in a glacial acetic acid, and acetous crystal violet as an indicator.

The overall reactions with drug base occurring as follows:

$$HClO_4 + \text{basic drug} \rightarrow \text{basic drug } H^+ + ClO_4^- \quad (3)$$

That is, the perchloric acid acts as a monoprotic acid and 1 mole of perchloric acid is equivalent to 1 mole of the basic drug. British Pharmacopeia (BP) recommends a non-aqueous titration as a reference method for the assay of methyldopa which is a cardiovascular drug using 0.1 M perchloric acid as titrant and crystal violet solution as indicator. In general, the reaction taking place between a primary amine and perchloric acid may be expressed as follows:

$$RNH_2 + HClO_4 \rightarrow [RNH_3]^+ + ClO_4^- \quad (4)$$

Also, several drugs are weakly acidic. Such substances can be titrated against strong bases like potassium methoxide and sodium methoxide, in solvents like toluene-methanol. The principle is similar to the titration of weak bases against perchloric acid. Potassium methoxide and sodium methoxide are not primary standard substances. So, they can be standardized by dimethylformamide (DMF, $H-CON(CH_3)_2$) and benzoic acid using methanolic thymol blue as an indicator. Ethosuximide, for example, is an antiepileptic drug and can be assayed by non-aqueous titration. The drug can be prepared in DMF. The titration can be done with sodium methoxide using azo-violet as an indicator.

3. Spectrophotometric techniques fundamentals, important, and its applications in pharmaceutical analysis

3.1 Fundamentals of spectrophotometric techniques

Ultraviolet–visible spectrophotometry indicates the absorption spectrum in the region between 200 and 800 nm. The absorption in the ultraviolet and visible region depended on the molecules that contain π electrons and non-bonding electrons pairs, which can absorb the energy of ultraviolet or visible light to rise to a higher anti-bonding molecular orbital. The more easily excited the electrons, the longer the wavelength of light they can absorb.

This technique is one of the spectroscopic methods based on the interaction of electromagnetic radiation with the material. Electromagnetic radiation (**Figure 2**) is considered as waves of energy propagated from a source in space and consists of oscillating electric and magnetic fields at right angles to each other. Each

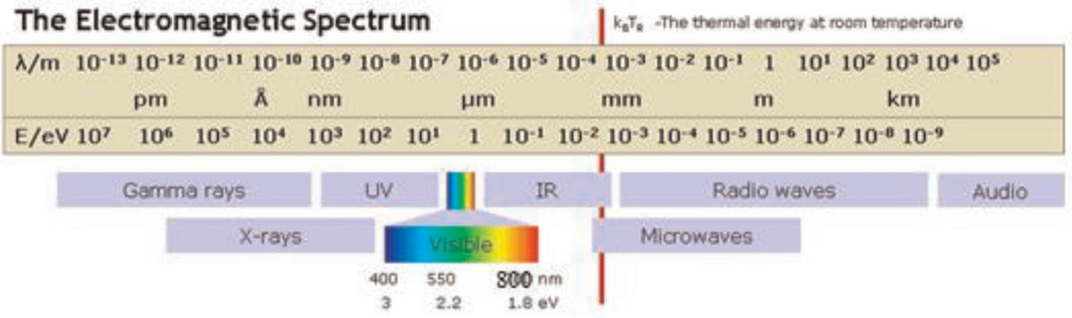

Figure 2.
Electromagnetic spectrum.

Electromagnetic radiation has characteristics of wavelength (λ), frequency (ν), or wave number, ν $[\frac{1}{\lambda}]$. Molecule or ion may absorb energy from Electromagnetic radiation of suitable wavelength (or frequency) resulting in: (a) Electronic excitation caused by absorption of UV–visible radiation leading to UV–visible spectroscopy. (b) Molecular rotation by absorption of microwave radiation leading to microwave. (c) Vibrational excitation is caused by the absorption of infrared radiation leading to infrared spectroscopy.

The UV–visible spectral method involves UV–visible spectroscopy. This arises due to the absorption of ultraviolet (UV) or visible radiation with the sample resulting in an electronic transition within the molecule or ion. The relationship between the energy absorbed in an electronic transition, the frequency (ν), wavelength (λ), and wave number (ν) of radiation-producing transition is:

$$\Delta E = h\nu = h\,\frac{c}{\lambda} = h\nu c \tag{5}$$

where h is Planck's constant, c is the velocity of light. ΔE is the energy absorbed during an electronic transition in a molecule or ion from a lower-energy state (E1) (ground state) to a high-energy state (E2) (excited state). The energy absorbed is given by

$$\Delta E = E2 - E1 = h\nu \tag{6}$$

Potentially, three types of ground state orbitals may be involved: (i) σ (bonding) molecular as in C – C, (ii) π (bonding) molecular orbital as in C = C, and (iii) n (non-bonding) atomic orbital as in C – Br, C – OH. In addition, two types of antibonding orbitals may be involved in the transition, σ^* (sigma star) orbital and π^* (pi star) orbital. A transition in which a bonding σ electron is excited to an antibonding σ orbital is referred to as $\sigma - \sigma^*$ transition. In the same way, $\pi - \pi^*$ represents the transition of one electron of a lone pair (non-bonding electron pair) to an antibonding π orbital. Thus the following electronic transitions can occur by the absorption of ultraviolet and visible light: $\sigma - \sigma^*$, $n - \sigma^*$, $n - \pi^*$, $\pi - \pi^*$. The energy required for various transitions (**Figure 3**) obeys the following order: $\sigma - \sigma^* > n - \sigma^* > \pi - \pi^* > n - \pi^*$.

$\sigma - \sigma^*$ transition: This transition can occur in compounds in which all the electrons are involved in the formation of single bonds (σ-bond only) and there is no lone pair of an electron, such as saturated hydrocarbon like methane, ethane, etc. which requires radiation of high energy with short wavelength (less than 150 nm). The usual

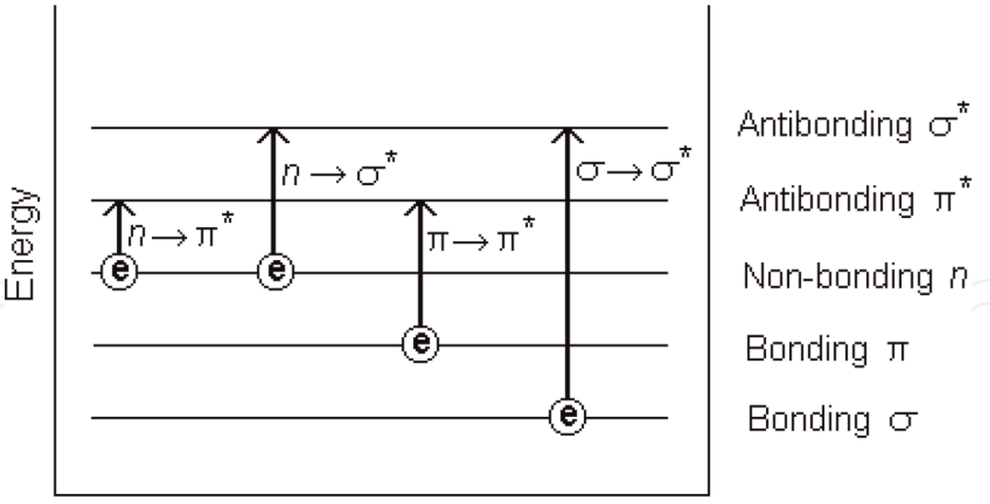

Figure 3.
The types of the transition electrons.

measurement cannot be done below 200 nm. Thus the region of transition below 200 nm is called the vacuum ultraviolet region. Methane which contains only C – H, σ-bond can undergo σ – σ^* transition exhibiting absorption peak at 125 nm. Ethane has an absorption peak at 135 nm which also must arise from the same type of transition but here electrons of C – C bond appear to be involved. Since the strength of the C – C bond is less than that of C – H bond, less energy is required for excitation, as a result, absorption occurs at a lower wavelength. Thus organic molecules in which all the valence shell electrons are involved in the formation of σ-bonds do not show absorption in the normal ultraviolet region, that is, 180–400 nm. n – σ^* transition: This type of transition takes place in a saturated compound containing one hetero atom with unshared pair of electrons. Examples of such transitions are saturated alkyl halides, alcohols, ethers, amines, etc. which are commonly used as a solvent because they start to absorb at 260 nm. However, these solvents cannot be used when measurements are to be made in 200–260 nm. In such cases saturated hydrocarbons which only give rise to σ – σ^* transition must be used. However, the drawback is that these are poor solvating agents. π – π^* transition: This transition is available in compounds with unsaturated centers of the molecules. Examples of such transitions are alkenes, alkynes, aromatics, carbonyl compounds, etc. this transition requires lesser energy, and hence, the transition of this type occurs at a longer wavelength within the region of the UV-spectrophotometer. In unconjugated alkenes, the absorption band is around 170–190 nm. In carbonyl compounds, the band due to π – π^* transition appears at 180 nm and is more intense, that is, the value of the molar extinction coefficient is high. The introduction of the alkyl group to the olefinic linkage shifts the position of the band to a longer wavelength by 3–5 nm per alkyl group. The shift depends on the type of the alkyl group and the stereochemistry of the double bond. n – π^* transition: This type of transition occurs in unsaturated bonds containing at least one hetero atom like O, N, S, and halogen with n electron. Examples of such transitions are aldehydes and ketones, etc. Saturated aldehydes (C = O) show both types of transitions, that is, low energy n – π^* and high energy π – π^* occurring around 290 and 180 nm, respectively. In aldehydes and ketones n – π^* transition arises from the excitation of a lone pair of electrons in a 2p orbital of an oxygen atom with the anti-bonding π orbital of the carbonyl group. When hydrogen is replaced by an alkyl group as in ketone, this results in the shift of the band to a shorter wavelength. Besides the above transition,

high energy but quite intense $\pi - \pi^*$ transition also occurs in carbonyl compounds. However, the molar extinction coefficient (ε) values associated with $n - \pi^*$ transition are generally low and range from 10 to 100 while values for $\pi - \pi^*$ transition, on the other hand, normally fall in the range between 1000 and 10,000.

The quantitative analysis using UV–visible spectrophotometry is based mainly on the Beer-Lambert law, which explains the relationship between the absorbance of analyte under analysis and its concentration:

$$A = \log I_0/I = \varepsilon C x \quad (7)$$

where ε is molar absorptivity, x is the path length, and C is the concentration of analyte.

3.2 Important and application of spectrophotometric in the pharmaceutical analysis

The basis of spectrophotometric methods is the simple relationship between the absorption of radiation by a solution and the concentration of the colored species in the solution [48]. A molecule or ion exhibits absorption in the visible or UV region when the radiation (photons) causes an electronic transition in the molecule or ion containing one or more chromophoric groups (**Table 2**). The functional groups on drug molecules are targeted for quantitative analysis of pharmaceutical formulations using UV–visible spectrophotometry techniques. The quantitative analysis using UV–visible spectrophotometry is based mainly on the Beer-Lambert law, which explains the relationship between the absorbance of the analyte under analysis and its concentration:

$$A = \log I_0/I = \varepsilon C x \quad (8)$$

where ε is molar absorptivity, x is the path length, and C is the concentration of the analyte.

Methods usually are based on ion-pair, charge-transfer complex formation reactions, and redox-complexation reactions, which formed the backbone of

Chromophore	λ_{max} (nm)	ε_{max} (l mol^{-1} cm^{-1})	Transition
H2C = CH2	171	15,530	$\pi - \pi^*$
H2C = CH – CH = CH2	217	20,900	$\pi - \pi^*$
CH3 – C = O	180 290	10,000 17	$\pi - \pi^*$ $n - \pi^*$
H2C = CH – CH = O	218 320 208	18,000 30	$\pi - \pi^*$ $n - \pi^*$ $n - \pi^*$
CH3 – COH = O	208	32	$\pi - \pi^*$
H2C = CH – COOH	206 242	13,500 250	$\pi - \pi^*$

Table 2.
Examples of some common chromophoric groups.

spectrophotometric methods. The developed methods were applied to dosage forms, including tablets, injections, syrup, capsules, and also spiked human urine wherever possible [7, 14, 17, 19, 21–30, 33, 37, 44, 47]. The details of those important reactions and more examples for each application are given.

3.2.1 Ion-pair complex

Ion-pair formation results from electrostatic according to Coulomb attraction law without the formation of a covalent bond [49]. The formation of an ion-pair complex between the drug and the choice dye followed by its extraction into an organic solvent for absorbance measurement is a widely used reaction as the basis of spectrophotometric assays for pharmaceutical formulations. These are the simplest of the spectrophotometric methods ever developed since they involve mere mixing of drug and dye solutions in an organic solvent before measuring the absorbance of the colored species. However, these methods require the drug to be present in the base or acid forms for complexation. For example, typical, some methods based on extractive spectrophotometric are used for the determination of some formulations after selecting the optimum conditions by preliminary experiments, such as pH, buffer, and solvent; at the wavelength of maximum absorption; (a) bromocresol purple used for terbinafine [21, 24], nifedipine [50], sulfadimidine, sulfaguanidine, sulfametrole, sulfaquinoxaline, and sulfamethoxazole [51], hydroxyzine dihydrochloride [52], lercanidipine [53], oxomemazine hydrochloride [54], and atorvastatin [55].

The widely used spectrophotometric methods in trace analysis are based on the possibility of converting the constituent to be determined into a substance whose solution is strongly colored. Such a solution shows differential absorption to light of different wavelengths (**Table 3**).

3.2.2 Redox-complexation (oxidation:reduction reactions)

These reactions involve a transfer of electrons between two species and a change in the oxidation number of species by gaining or losing an electron, generating a colored species. Many scientific published papers indicated oxidation-reduction reactions, that include; valganciclovir hydrochloride with iron (III) and $KMnO_4$ [30], amoxycillin, ampicillin, and cloxacillin with iodate [17], Olanzapine with N-Bromosuccinimide and Cerium (IV) sulfate [56], captopril with a mixture of $KBrO_3$ and KBr [57], to name a few.

Ultraviolet	<400 nm
Violet	400–450 nm
Blue	450–500 nm
Green	500–570 nm
Yellow	570–590 nm
Orange	590–620 nm
Red	620–800 nm
Infrared	>800 nm

Table 3.
The wavelengths of the visible and ultraviolet regions.

3.2.3 Charge-transfer complexation

The charge-transfer complex is formed from a combination of two molecules, one of which acts as an electron donor and the other as an electron acceptor. Based on the charge-transfer complexation, albendazole with picric acid [58] (**Figure 4**), chloranilic with tyrosine [59], carvedilol with iodine [60] to name a few, were determinates using spectrophotometric techniques.

4. The general methodology for the development of spectrophotometric methods

To develop a quantitative method for an unknown concentration substance using spectroscopic methods, the first step is by chooses the appropriate wavelength that corresponds to the highest absorbance. We can also choose the appropriate wavelength from the literature.

4.1 Step 1: method development

4.1.1 Optimization conditions

Several experimental variables such as the pH of the buffer system, choice of organic solvent, the volume of dye, and shaking time for the extraction of the ion-pair complex were tested with respect to their effect on complex formation.

For the development of spectroscopic methods, oxidation and reduction reactions, and complex formation are considered the backbone of these methods. These types of reactions produce colored products whose absorbance is measured. The sensitivity of the method and the degree of color stability are studied through several variables, the most important of which is the acid concentration/pH reagent concentration, nature of solvent, temperature, etc. until we reach the optimum conditions for the method. Before all that, the wavelengths of the colored products are scanned until the maximum wavelength (λ_{max}) is obtained. The range at which the attainment of maximum color and stability occurs in the color species formation is termed as an optimum condition of each parameter.

4.2 Study of the composition of the complex (stoichiometry)

Job's method of continuous variation [61] was followed for finding out the composition of the ion-association complex formed between the studied drugs and selected dyes.

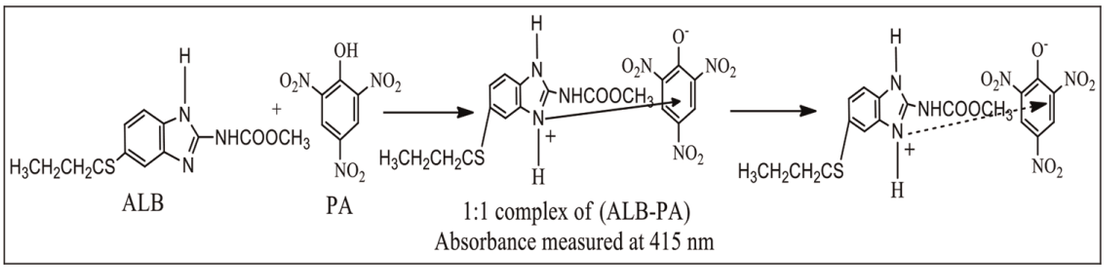

Figure 4.
Reaction pathway for the formation of electron donor-acceptor complex due to Albendazole and picric acid interaction [58].

4.3 Chemistry of the colored species formed

The chemistry of the colored species formed in each method is ascertained either through probability with the existing experimental evidence or through analogy with the literature methods.

5. Method validation (validation of analytical results)

After optimizing the experimental variables for maximum complex formation and extraction, some analytical performance characteristics such as linearity, limits of detection (LOD) and quantification (LOQ), accuracy and precision, robustness and ruggedness, and selectivity were investigated using standard solutions of drugs under study.

5.1 A calibration curve (linearity)

In quantitative analyzes using spectroscopic methods, the standard curve is always needed. Where the active substance of the pure drug is subjected to the same optimal conditions for the samples under study and the absorbance was measured at the maximum length. This is followed by plotting the absorbance measurements against the concentrations of the samples. A straight line passing through the origin is obtained if Beer's law is obeyed. This curve may then be used in the subsequent determination of the constituent under the same conditions.

5.2 Sensitivity of the method (LOD and LOQ)

Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing the sensitivity. For more sensitive spectrophotometric methods, ε is $> 1 \times 10^4$ L. mol^{-1}. cm^{-1} and values of $\varepsilon < 1 \times 10^3$ l. mol^{-1}. cm^{-1} correspond to less sensitive methods. Sandell's sensitivity [62] refers to the number of μg of the constituent determined, converted to the colored product, which is a column solution of cross section 1 cm^2, shows an absorbance of 0.001 (expressed as μg/cm^2). Limits of detection LOD and LOQ are the smallest amount of an analyte that can be determined and quantified by a particular method. The LOD and LOQ values were calculated using the formulae:

$$\text{LOD} = \frac{3.3S}{m} \text{ and } \text{LOQ} = \frac{10S}{m} \tag{9}$$

where S is the standard deviation of replicate (n = 7) absorbance of blanks and m is the slope of the calibration curve.

5.3 Precision and accuracy

The purpose of carrying out a determination is to obtain a valid estimate of a true value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging the results generated by the analytical procedure.

To evaluate the precision and accuracy of the methods, standard drug solution at three concentration levels was subjected to analysis on the same day (intra-day) in seven replicates and on five consecutive day (inter-day) by preparing all solutions afresh each day. Mean (×) and standard deviations (SD) were obtained by back-calculated drug concentration at each level. Accuracy and precision were evaluated in terms of relative error (RE) and relative standard deviation (RSD), respectively.

5.4 Robustness and ruggedness

Robustness is the measure of its capacity to remain unaffected by small, but deliberate, variations in parameters of the method and indicates its reliability during normal usage, while ruggedness represents the degree of reproducibility of examined results, found by analyzing the same samples under condition variables. The assay procedure was repeated after making a small incremental variation in the optimized condition such as the pH of buffer and reagent volume, and the effect of these variations was investigated to assess the robustness of the method. To evaluate ruggedness, the determination was performed by a single analyst using three instruments in the same laboratory and also by three analysts using a single instrument. Each study was performed on three levels of analyte.

5.4.1 Selectivity

It can be defined as the degree to which a method can quantity the analyte accurately in the presence of interferes. The selectivity of the developed methods was examined using placebo blank and synthetic mixture analyses. To a certain amount (mg) of the placebo blank (talc, starch, sucrose, lactose, and other compounds) prepared, accurately known amount (mg) of pure drug was added, mixed thoroughly and the mixture extract was prepared as usual; and then steps described under the procedure for dosage forms were followed. The % recovery of pure drug in the mixture was computed, which is taken as a measure of selectivity.

5.5 Accuracy by recovery experiments (standard-addition method)

Accuracy by recovery experiments: To ascertain the accuracy of the proposed methods, recovery experiments were performed *via* the standard addition technique. If the % of recovery calculated using the formula given below is satisfactory, confidence in the accuracy of the procedure is enhanced.

$$\%recovery = \frac{\sum XY - \sum X \sum Y}{\sum X^2 - (\sum X)^2} \tag{10}$$

where X = amount of the constituent added in μg (spectrophotometry) or mg (titrimetry), Y = amount of the constituent found, μg or mg.

5.6 Evaluation of accuracy and precision by comparison of two methods

To evaluate the accuracy and precision of the method, one often compares the method being developed or the "test method" with an existing method called the reference, standard or official method [63]. Student's t-test (comparison of two

means); suppose that a sample is analyzed by two different methods, each repeated several times and that the mean values obtained are different, student's t-test will tell, with a given probability, whether it is worthwhile to seek an assignable cause for the difference between the two means. The test gives a yes or no answer to the correctness of the null hypothesis with a certain confidence, such as 95% or 99%. The procedure is as follows: suppose that sample has been analyzed by two different methods (test and reference methods) yielding means X_1 and X_2 and standard deviations S_1 and S_2, n_1 and n_2 is the number of individual results obtained by two methods, t is calculated using the following formula:

$$t = \frac{X_1 - X_2}{S}\sqrt{\frac{n_1 n_2}{n_1 + n_2}} \tag{11}$$

Here, it is presupposed that S_1 and S_2 are the same. If S_1 and S_2 are different, S is calculated using the following formula:

$$S = \sqrt{\frac{\Sigma(X_1 - X_1)^2 + \Sigma(X_2 - X_2)^2}{n_1 + n_2 - 2}} \tag{12}$$

F-test (comparison of two standard deviations); using the formula:

$F = S^2_T/S^2_R$.

where S^2_T is the variance of the test method, S^2_R is the variance of the reference method.

F-test uses for the calculation of F-ratio (larger variance/smaller variance). If the calculated F-value is in the table [64, 65], one can conclude that the methods are not significantly different in precision at a given confidence level.

6. Conclusion

Realizing the importance and usefulness of these two techniques; titrimetry and spectrophotometry and valuing their unique features, the author has attempted to explain of applications these simple and inexpensive techniques for the determination of different pharmaceutical formulations. The advantages and superior performances of these two techniques; titrimetry and spectrophotometry compared with the existing techniques are rapidly, simplicity, sensitivity, and use of inexpensive reagents and chemicals.

Modern methods of analysis (LCMS, GCMS, NMR, and Mass) involve sophisticated and costly equipment and pose problems of maintenance. Hence, they may not be within the reach of most of the laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations. Among various techniques, titrimetry and spectrophotometry, still enjoy a significant role in the assay of several classes of drugs at macro, semi-micro (titrimetry), micro, or nanogram (spectrophotometry) levels. They are simple, economically viable, and easy to carry out. Visible spectrophotometry is the simplest of the spectrophotometric techniques and it is in wide use in the quantitative analysis of active substances. The spectrophotometric procedure is also recommended in Pharmacopoeial monographs such as Indian Pharmacopeia, British Pharmacopeia, USP, EP, etc. Hence, spectrophotometry is generally preferred in small-scale industries and most laboratories for routine quality assurance

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because of its overwhelming advantages, such as speed, simplicity, cost-effectiveness, specificity/selectivity, and sensitivity. Titration is also a simple technique giving accurate and precise results. The non-aqueous titration with visual or potentiometric end point detection has maintained its importance in pharmaceutical analysis and has been accepted by a majority of modern pharmacopeias as an official analytical method.

Conflicts of interest

There are no conflicts of interest to declare.

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