ANALYTICAL PROCESS OF DRUGS BY ULTRAVIOLET (UV) SPECTROSCOPY – A REVIEW

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ABSTRACT

The pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs. Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes that the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer. Ultraviolet-visible spectroscopy is used to obtain the absorbance spectra of a compound in solution or as a solid.

Keywords: Ultraviolet-visible spectroscopy, Bouger-Lambert-Beer's law, Analytical chemistry.

INTRODUCTION

Ultra Violet Spectroscopy

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.

Analytical Chemistry

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity [1-3].

To be effective and efficient, analyzing samples requires expertise in:

1. The chemistry that can occur in a sample.
2. Analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade).
3. Accuracy and precision of the method.
4. Proper data analysis and record keeping.

The major stages of an analytical process are described as follows (Figure 1).

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Types of analytical chemistry

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Qualitative analysis

- ➢ Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in a sample.
- ➢ Qualitative organic analysis seeks to establish the presence of a given functional group or organic compound in a sample.

Quantitative analysis

- ➢ Quantitative analysis seeks to establish the amount of a given element (or) compound in a sample.

Methods of detecting analytes

1.   Physical
    - ✎ Mass
    - ✎ Color
    - ✎ Refractive Index
    - ✎ Thermal Conductivity
2.   Using Electromagnetic Radiation (Spectroscopy)
    - ✎ Absorption
    - ✎ Emission
    - ✎ Scattering
3.   Using Electric Charge
    - ✎ Electrochemistry
    - ✎ Mass Spectrometry

Ultraviolet Absorption Spectrophotometry

Spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The photometric methods of analysis are based on the Bouger-Lambert-Beer's law, which establishes that the absorbance of a solution is directly proportional to the concentration of the analyte. The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer. Ultraviolet-visible spectroscopy is used to obtain the absorbance spectra of a compound in solution or as a solid. What is actually being observed spectroscopically is the absorbance of light energy or electromagnetic radiation, which excites electrons from the ground state to the first singlet excited state of the compound or material. The UV-visible region of energy for the electromagnetic spectrum covers 1.5 - 6.2 eV which relates to a wavelength range of 800 - 200 nm [4,5].

The Beer-Lambert Law (Equation1) is the principle behind absorbance spectroscopy. For a single wavelength, A is absorbance (unit less, usually seen as arb. units or arbitrary units), is the molar absorptivity of the compound or molecule in solution ($M^{-1}cm^{-1}$), b is the path length of the cuvette or sample holder (usually 1 cm), and c is the concentration of the solution (M).

$$A = a\ b\ c$$

Where, A = Absorbance,
    a = absorptivity,
    b = path length,
    c = concentration.

$$C = A\ /a\ b$$

There are three types of absorbance instruments used to collect UV-Visible spectra:

1.   Single beam spectrometer.
2.   Double beam spectrometer.
3.   Simultaneous spectrometer.

All of these instruments have a light source (usually a deuterium or tungsten lamp), a sample holder and a detector, but some have a filter for selecting one wavelength at a time. The single beam instrument (Figure 2) has a filter or a monochromator between the source and the sample to analyze one wavelength at a time. The double beam instrument (Figure 3) has a single source and a monochromator and then there is a splitter and a series of mirrors to get the beam to a reference sample and the sample to be analyzed, this allows for more accurate monochromator between the sample and the source; instead, it has a diode array detector that allows the instrument to simultaneously detect the absorbance at all wavelengths. The simultaneous instrument is usually much faster and more efficient, but all of these types of spectrometers work well (Figure 4).
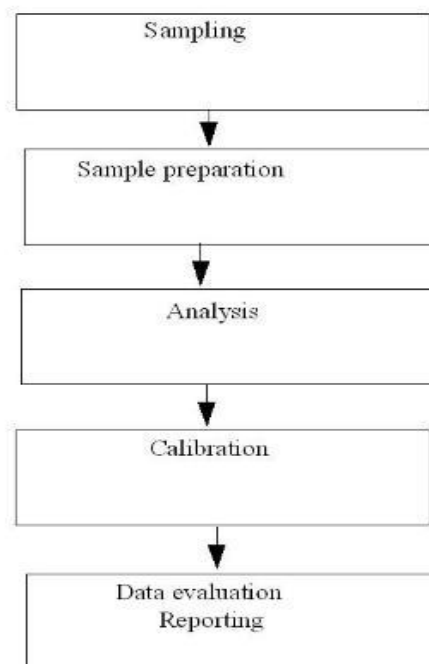
Figure 1. Steps in analytical cycle

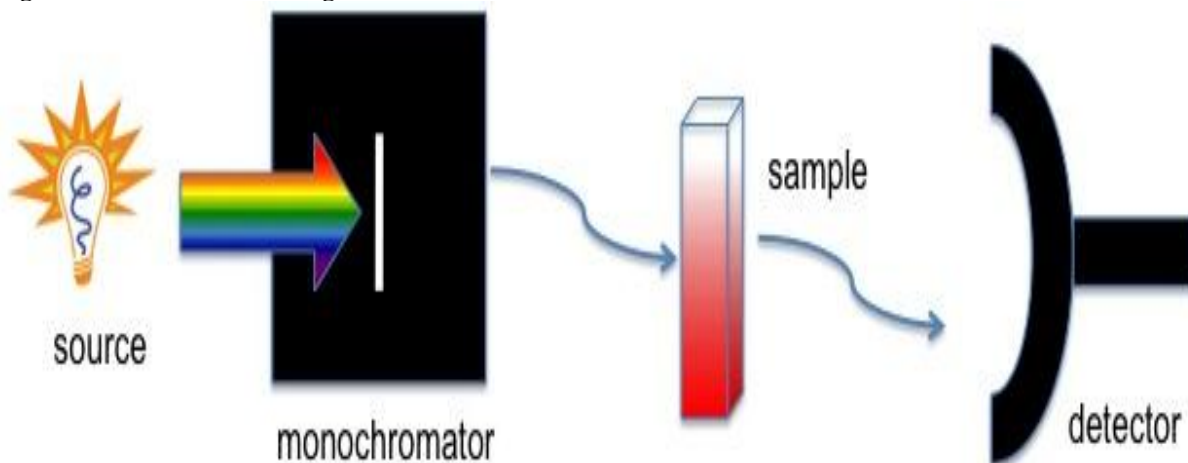**Figure 2. Illustration of a single beam UV-Visible instrument**
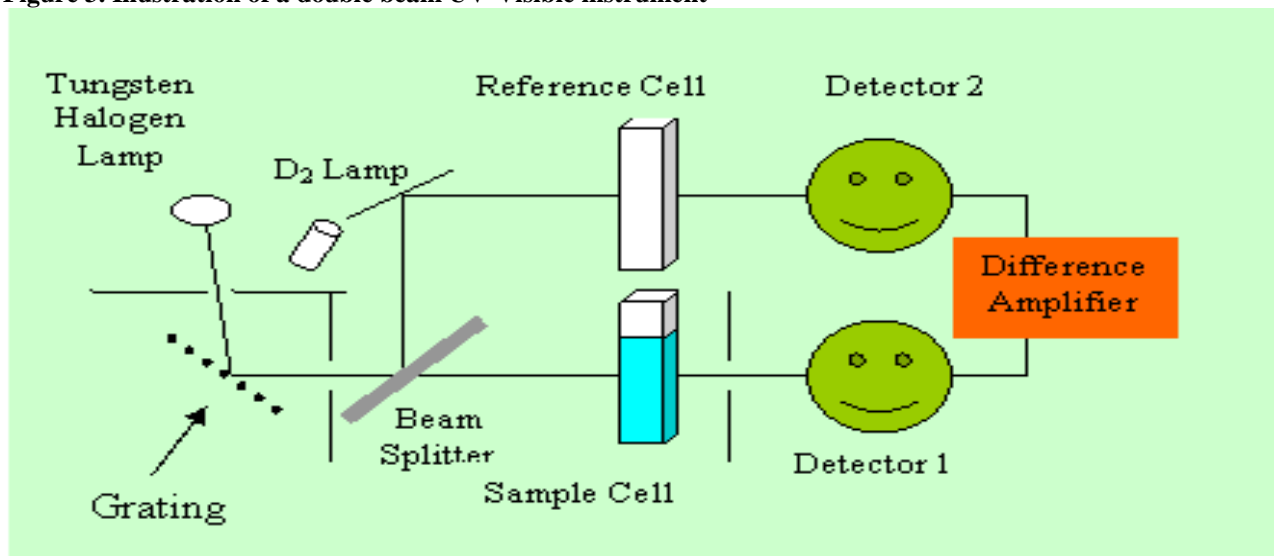

Figure 3. Illustration of a double beam UV-Visible instrument

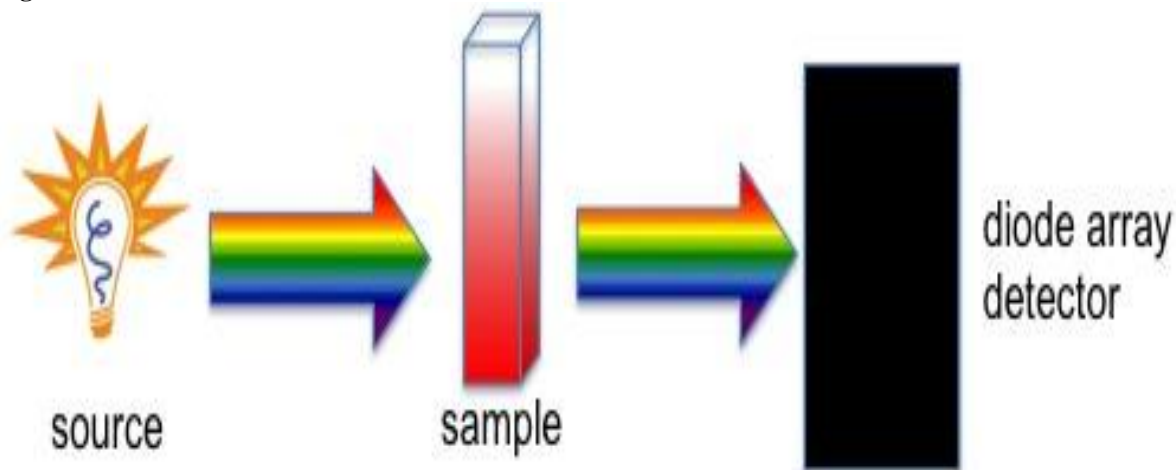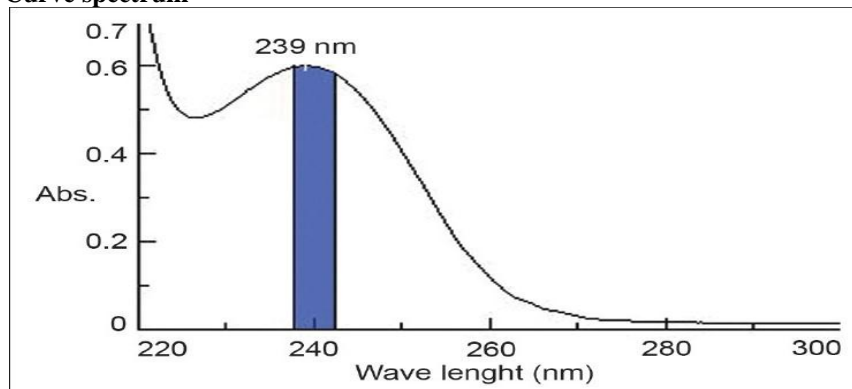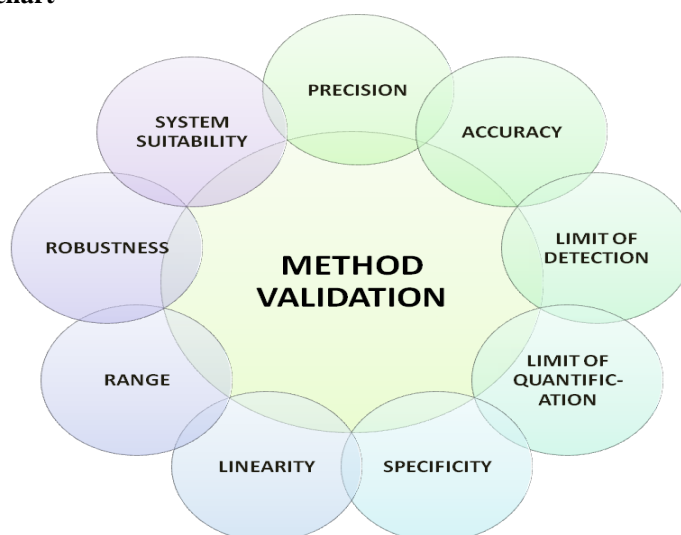**Figure 4. Illustration of a simultaneous UV-Visible instrument**

Figure 5. Area Under Curve spectrum

Figure 6. Method validation chart

UV-Visible spectra

UV-Visible spectroscopic data can give qualitative and quantitative information of a given compound or molecule. Irrespective of whether quantitative or qualitative information is required it is important to use a reference cell to zero the instrument for the solvent the compound is in. For quantitative information on the compound, calibrating the instrument using known concentrations of the compound in question in a solution with the same solvent as the unknown sample would be required. If the information needed is just proof that a compound is in the sample being analyzed, a calibration curve will not be necessary; however, if a degradation study or reaction is being performed, and concentration of the compound in solution is required, thus a calibration curve is needed.

To make a calibration curve, at least three concentrations of the compound will be needed, but five concentrations would be most ideal for a more accurate curve. The concentrations should start at just above the estimated concentration of the unknown sample and should go down to about an order of magnitude lower than the highest concentration. The calibration solutions should be spaced relatively equally apart, and they should be made as accurately as possible using digital pipettes and volumetric flasks instead of graduated cylinders and beakers [6,7].

Calibration and reference

A Blank reference will be needed at the very beginning of the analysis of the solvent to be used (water, hexanes, etc), and if concentration analysis needs to be performed, calibration solutions need to be made accurately. If the solutions are not made accurately enough, the actual concentration of the sample in question will not be accurately determined.

Choice of solvent or container

Every solvent has a UV-visible absorbance cutoff wavelength. The solvent cutoff is the wavelength below which the solvent itself absorbs all of the light. So when choosing a solvent is aware of its absorbance cut off and where the compound under investigation is thought to absorb. If they are close, chose a different solvent. Table 1 provides an example of solvent cut offs [8,9].

Table 1. UV absorbance cutoffs of various common solvents

Solvent	UV absorbance cutoff (nm)
Acetone	329
Benzene	278
Dimethyl formamide (DMF)	267
Methanol	205
Ethanol	205
Toluene	285
O.1N HCL	210

The material the cuvette (the sample holder) is made from will also have a UV-visible absorbance cutoff (table 2).

Table 2 UV absorbance range of different sample cells

Material	Wavelength range (nm)
Glass	380 – 780
Plastic	380 – 780
Fused quartz	below 380

Glass will absorb all of the light higher in energy starting at about 300 nm, so if the sample absorbs in the UV, a quartz cuvette will be more practical as the absorbance cutoff is around 160 nm for quartz.
The different types of cuvettes commonly used, with different usable wavelengths.
The important applications are
  ➢ Identification and Quantitative determination of many types of organic,
     inorganic molecules and ions.
  ➢ Quantitative determination of mixtures of analytes.
  ➢ Monitoring and identification of chromatographic of effluents.
  ➢ Determination of stoichiometry and chemical reactions.
  ➢ Monitoring of environmental and industrial process.
  ➢ Typical analysis times range from 2 to 30 minutes for sample.

Concentration of solution

To obtain reliable data, the peak of absorbance of a given compound needs to be at least three times higher in intensity than the background noise of the instrument. Obviously using higher concentrations of the compound in solution can combat this. Also, if the sample is very small and diluting it would not give an acceptable signal, there are cuvettes that hold smaller sample sizes than the 2.5 mL of standard cuvettes. Some cuvettes are made to hold only 100 µl, which would allow for a small sample to be analyzed without having to dilute it to a larger volume, lowering the signal to noise ratio.

AREA UNDER CURVE STUDIES

The principle for Area under curve method is "the area under two points on the mixture spectra is directly proportional to the concentration of the compound of interest".AUC is particularly suitable for the compounds where there is no sharp peak or broad spectra are obtained, which is shown in the figure 5.AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. Area calculation processing item caliculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

ANALYTICAL PARAMETERS

Validation may be defined as a process involving confirmation or establishing by laboratory studies that a method/ system/ analyst give accurate and reproducible result for intended analytical application in a proven and established range.

Method Validation

Method validation is completed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range that an analyte will be analyzed. Method validation provides an assurance of reliability during normal use, and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do" and parameters are shown in figure 5.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. According to the ICH, precision should be performed at three different levels: repeatability, intermediate precision, and reproducibility. Repeatability is the results of the method operating over a short time interval under the same conditions (inter-assay precision).

It should be determined from a minimum of nine determinations covering the specified range of the procedure (for example, three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration. Intermediate precision is the results from within lab variations due to random events such as different days, analysts, equipment, etc. In determining intermediate precision, experimental design should be employed so that the effects (if any) of the individual variables can be monitored. Reproducibility refers to the results of collaborative studies between laboratories. Documentation in support of precision studies

should include the standard deviation, relative standard deviation, coefficient of variation, and the confidence interval [10].

Accuracy

Accuracy is the measure of exactness of an analytical method, or 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. It is measured as the percent of analyte recovered by assay, by spiking samples in a blind study. For the assay of the drug substance, accuracy measurements are obtained by comparison of the results with the analysis of a standard reference material, or by comparison to a second, well-characterized method. For the assay of the drug product, accuracy is evaluated by analyzing synthetic mixtures spiked with known quantities of components. For the quantitation of impurities, accuracy is determined by analyzing samples (drug substance or drug product) spiked with known amounts of impurities. (If impurities are not available, see specificity.)

Specificity

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only. That no co-elution exists. Specificity is measured and documented in a separation by the resolution, plate -count (efficiency), and tailing factor. Specificity can also be evaluated with modern photodiode array detectors that compare spectra collected across a peak mathematically as an indication of peak homogeneity. ICH also uses the term specificity, and divides it into two separate categories: identification, and assay/impurity tests.

Limit of Detection

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value. It is expressed as a concentration at a specified signal-to-noise ratio, usually two- or three-to-one. The ICH has recognized the signal-to-noise ratio convention, but also lists two other options to determine LOD: visual non-instrumental methods and a means of calculating the LOD. that 0.211µg. The maximum limit of LOD value must be not more than 2 µg, from the standard references.

Linearity and Range

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range reported as the variance

Visual non-instrumental methods may include LOD's determined by techniques such as thin layer chromatography (TLC) or titrations. LOD's may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula:

$$LOD = 3.3(SD/S)$$

The standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines. The method used to determine LOD should be documented and supported, and an appropriate number of samples should be analyzed at the limit to validate the level. LOD values are always specific for a particular set of experimental conditions. Anything that changes the sensitivity of a method, including instrument, sample preparation etc. will change detection limits.

Limit of Quantification

The Limit of Quantificaion (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Like LOD, LOQ is expressed as a concentration, with the precision and accuracy of the measurement also reported. Sometimes a signal-to-noise ratio of ten-to-one is used to determine LOQ. This signal-to-noise ratio is a good rule of thumb, but it should be remembered that the determination of LOQ is a compromise between the concentration and the required precision and accuracy. That is, as the LOQ concentration level decreases, the precision increases. If better precision is required, a higher concentration must be reported for LOQ. This compromise is dictated by the analytical method and its intended use. The ICH has recognized the ten-to-one signal-to-noise ratio as typical, and also, like LOD, lists the same two additional options that can be used to determine LOQ, visual non-instrumental methods and a means of calculating the LOQ. The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula:

$$LOQ = 10(SD/S)$$

Again, the standard deviation of the response can be determined based on the standard deviation of the blank, on the residual standard deviation of the regression line, or the standard deviation of y-intercepts of regression lines. The value of LOQ is almost 10 times higher than that of the blank. The LOQ was found with in limit concentrations of the slope of the regression line. Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results obtained by the method. The ICH guidelines specify a minimum of five concentration levels,

along with certain minimum specified ranges. For assay, the minimum specified range is from 80-120% of the target concentration. For an impurity test, the minimum range is from the reporting level of each impurity, to120% of the specification. (For toxic or more potent impurities, the range should be commensurate with the controlled level.)

Ruggedness

Ruggedness, according to the USP, is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. These conditions include different laboratories, analysts, instruments, reagents, days, etc. In the guideline on definitions and terminology, the ICH did not address ruggedness specifically. This apparent omission is really a matter of semantics, however, as ICH chose instead to cover the topic of ruggedness as part of precision, as discussed previously.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic, pH, ionic strength, temperature, etc., and determining the effect (if any) on the results of the method. As documented in the ICH guidelines, robustness should be considered early in the development of a method. In addition, if the results of a method or other measurements are susceptible to variations in method parameters, these parameters should be adequately controlled and a precautionary statement included in the method documentation.

Recovery studies

Recovery studies were carried out by using spiking method. In this method the test sample having the concentration of 10μg/ml .To this standard drug is spiked by adding into the test solution. A concentration of 8, 10 and 12μg/ml are added to the sample solutions and the absorbances of the three spiked concentrations were taken. From this absorbance we can determine the amount of drug that can be recovered by the proposed method [9-13].

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