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A review on UV validation and development method of anticancer drugs

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ABSTRACT:UV-VIS Spectroscopy is the term used for the analytical evaluation of the different types of the solvents and substances. UV- Visible spectrometers have been in general use for the last 37 years. Spectroscopy 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 a spectrum (200-400 nm). Currently it is important to develop reliable analytical methods for the determination of anticancer drugs to achieve more selectivity, sensitivity and more rapid assay method than those which are previously reported. The main objective of this work is to compare UV spectrophotometric method that has short and simple extraction procedures, consume small amounts of solvents and have a short turn-around time and gives better results at the time of validate those methods. Now as per study done it was showed that Purified or distilled water is the mainly used solvent because of its easily availability and most of drugs are easily soluble within it, it is feasible for most of method development processes and shows greater results when going to validate that results obtained. After that Methanol, absolute ethanol, acetronitrile and phosphate buffer saline etc solvents were used which showing same criteria's that are required to show better results in validation process. Next λmax also plays important role in validation of method and the maximum anticancer drugs recorded in this project shows λmax is in between 210 nm to 277 nm. So The UV methods developed in this study have the advantage of simplicity, precision, accuracy, andconvenience.

Keywords: Anti-Cancer, λmax, precision, UV-VIS Spectroscopy, validation.

I. INTRODUCTION:

Spectroscopy:Spectroscopy is the measurement and interpretation of Electro Magnetic Radiation [EMR] absorbed and emitted when the molecules or atoms or ions of a sample move from one energy states to another energy states.[1]

UV-VIS 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.[2]

PRINCIPLE OF UV-VIS SPECTROSCOPY:

Basically, spectroscopy is related to the interaction of light with matter.As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light it can absorb. There are four possible types of transitions (π–π*, n–π*, σ–σ*, and n–σ*), and they can be ordered as follows: σ–σ* > n–σ* > π–π* >n–π*

The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum which aids in the identification of the compound.

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A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the moleculesinthesample.The energy supplied by thelightwillpromoteelectronsfromtheirground state orbital to higher energy, excited state orbital or anti-bonding orbital. Potentially, three types of ground state orbitals may be involved.[3-4]
σ (Bonding)molecular
π (Bonding) molecular orbital
n (non-Bonding) atomic orbital.

In addition, two types of anti-bonding orbitals may be involved in the transition
σ* (sigma star)orbital.

π* (pi star)orbital.

There is no such thing as an n* anti-bonding orbital as the n electrons do not form bonds). Thus the following electronic transitions can occur by the absorption of ultraviolet and visible light.
σ toσ*

n to σ*

n toπ*

π toπ*

Both σ to σ* and n to σ* transitions require a great deal of energy and therefore occur in the far ultraviolet region or weakly in the region 180-240nm. Consequently, saturated groups do not exhibit strong absorption in the ordinary ultraviolet region. Transitions from then to π* and π to π* type occur in molecules with unsaturated centres, they require less energy and occur at longer wavelengths than transitions to σ* anti-bonding orbital. It will be seen presently that the wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* anti-bonding orbital which occurs in the ultraviolet region for a particular molecule may well take place in the visible region if the molecular structure is modified. Many inorganic compounds in solution also show absorption in the visible region. These include salts of elements with incomplete inner electron shells (mainly transition metals) whose ions are complexes by hydration. Such absorptions arise from a charge transfer process, where electrons are moved from one part of the system to another by the energy provided by the visible light.

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 colourless compounds in the near ultraviolet path of the spectrum (200-400nm). 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. The Beer-Lambert Law is the principle behind absorbance spectroscopy
Where,
A = Absorbance, a = absorptivity, b = path length, c = concentration.

A = a bc
C = A /a b

There are three types of absorbance instruments used to collect UV-Visible spectra:
Single beam spectrometer.
Double beam spectrometer.
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 monochromatic between the source and the sample to analyse 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 analysed, this allows

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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.

Light Source

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UVregion.

Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

MonochromatorMonochromators generally is composed of prisms and slits.

Most of the spectrophotometers are double beam spectrophotometers.

The radiation emitted from the primary source is dispersed with the help of rotating prisms.

The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose.

The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

Sample and reference cells

One of the two divided beams is passed through the sample solution and second beam is passé through the reference solution.

Both sample and reference solution are contained in the cells.

These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UVregion.

Detector

Generally two photocells serve the purpose of detector in UVspectroscopy.

One of the photocell receives the beam from sample cell and second detector receives the beam from the reference.

The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.

Amplifier

The alternating current generated in the photocells is transferred to the amplifier.

The amplifier is coupled to a smallservometer.

Generally current generated in the photocells is of very low intensity, the main purpose of amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording devices

Most of the time amplifier is coupled to a pen recorder which is connected to the computer.

Computer stores all the data generated and produces the spectrum of the desired compound.

APPLICATIONS OF UV SPECTROSCOPY

Detection of Impurities: It is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

Structure elucidation of organic compounds: It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of heteroatoms.

UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UVradiation.

UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation thus used in qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.

This technique is used to detect the presence or absence of functional group in the compound. Absence of a band at particular wavelength regarded as an evidence for absence of particular group.

Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.

Many drugs are either in the form of raw material or in the form of formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at specific wavelength.

Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.

UV spectrophotometer may be used as a detector forHPLC.

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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.

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[9].

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 analysing synthetic mixtures spiked with known quantities of components. For the quantitation of impurities, accuracy is determined by analysing 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 termspecificity, 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. 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

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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 Quantification (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 itsintended 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 concentrationsthat 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 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.

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.

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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 absorbance's 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

- **Different Uv Validation And Development Methods Of Anticancer Drugs**

A simple, rapid, cost effective and extractive UV spectrophotometric method was developed for the determination of Gemcitabine HCl (GMCT) in bulk drug and pharmaceutical formulation. It was based on UV spectrophotometric measurements in which the drug reacts with gold nanoparticles (AuNP) and changes the original colour of AuNP and forms a dark blue coloured solution which exhibits absorption maximum at 688 nm. The apparent molar absorptivity and Sandell's sensitivity coefficient were found to be 3.95×10−5 l mol−1 cm−1 and 0.060 g cm−2 respectively. Beer's law was obeyed in the concentration range of 2.0–40 g ml−1. This method was tested and validated for various parameters according to ICH guidelines. The proposed method was successfully applied for the determination of GMCT in pharmaceutical formulation (parental formulation). The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%). As it is simple, cheap and less time consuming, it can be suitably applied for the estimation of GMCT in dosage formsLetrozole (4-[(4-cyanophenyl)-(1,2,4-triazol-yl)methyl] benzonitrile, is an oral non-steroidal aromatase inhibitor for the treatment of hormonally-responsive breast cancer after surgery. Currently it is important to develop reliable analytical methods for the determination of Letrozole to achieve more selectivity, sensitivity and more rapid assay method than those which are previously reported. The main objective of this work is to develop a RP-UFLC and UV spectrophotometric method that has short and simple extraction procedures, consume small amounts of solvents and have a short turn-around time. During the study acetonitrile: phosphate buffer (pH6.8) (50:50 v/v) was used as mobile phase for RP- UFLC and methanol for UV spectrophotometric method. The samples were analyzed by using UFLC consisting reversed phase C-18 column, PDA detector set at 241 nm. The mobile phase consisting a mixture of PBS (pH 7): acetonitrile (50:50 v/v) which was pumped at a flow rate of 1.0 mLmin-1 showed best separation of drug and internal standard (Letrozole- d4).[12]

The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Chlorambucil, an anti-cancer drug, in bulk and pharmaceutical dosage form. The solvent used was acetonitrile and the lmax or the absorption maxima of the drug was found to be 258 nm. A linear response was observed in the range of 2-10µg/ml with a regression coefficient of 0.9994. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Chlorambucil in quality control of formulation without interference of the excipients.[13]

A simple, sensitive, sectrophotometric method in UV region has been developed for the determination of Letrozole is bulk and tablet dosage form. Standard solution of Letrozole shows maximum absorbance at 240 nm with apparent molar absorptive of 3.3016 × 104 l/mol/cm. Beers law was obeyed in the concentration range of 1 -10 µg /m.l with regression, slope and intercept 0.9998,-0.016, 0.1164 respectively. Result of the analysis was validated statically and by recovery studies. The results percentage recovery is 100.63 ± 0.4215 which shows that the method is free from interference of additives and impurities during the estimation drug in formulation. This shows the adoptability of the method for the routine quality control analysis of the drug in bulk and in formulation.[14]

A simple, rapid, cost-effective and green high-performance liquid chromatographic assay for determination of capecitabine in human plasma using a C18 reversed-phase analytical column was developed and validated. The separation was conducted by means ofamobile phase composed of formic acid solution (pH=3): ethanol (55:45) running at a flow- rate of 1.0mLmin–1 with UV detection at 310 nm. The column temperature was set at 50 °C. Sample preparation involved protein precipitation by zinc sulfate–ethanol solution. This method is consistent with a high recovery of capecitabine in human plasma ranging from 95.98 to102.50 %.The calibration curves were linear over concentration range of 0.05–10.00 µg mL–1 (r2 > 0.9999). Between- and within-day

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variability was less than 15 % and the bias was within ±15 %. This validated method was successfully applied to a pharmacokinetic study enrolling seven Iranian cancer patients after administration of a morning oral dose of 1500 mg.[15]

Anastrozole is a nitrile and triazole derivative, act as a selective non-steroidal aromatase inhibitor. It used in the treatment of estrogen nuclear receptor breast cancer in postmenopausal women. A new spectrophotometric method was developed in the UV region for determination of anastrozole in dissolution samples. In water, its solubility is very poor, (0.5 mg/mL at 25 deg C) and solubility is dependent of pH in the physiological range. Absorption characteristics of pure drug was checked insolvents like water, ethanol, phosphate buffer pH 6.8 and pH 7.4 phosphate buffer saline and in pH 7.4 phosphate buffer salineit has good absorption characteristics. Absorption maximum was found at 210 nm and the method was optimized and validatedusing this wavelength. All validation parameters were found to be in the acceptance range. The developed UVspectrophotometric method was successfully applied for the In Vitro studies of anastrozole in its invasomal formulation. [16]

Development and validation of a simple UV- Spectroscopy method was done for the quantitative analysis of Ellagic Acid (EA). The stock solution of 50µg/ml was prepared and scanned, for which absorption maxima was found to be 277nm. Further dilutions to different concentrations (1-5µg/ml) were prepared and analyzed at 277nm. The method so developed was validated as per ICH guidelines for: linearity, robustness, precision, accuracy, limit of detection and quantification. The Lambert- Beer's law is followed in the range (1-5µg/ml) with correlation coefficient value 0.9994. It was observed that the method is precise and accurate for EA analysis with good recovery percent of 94.47% to 106.83%. The method developed was further employed for determining the entrapment efficiency of ellagic acid and its release from its nanoparticle dosage form. The method may be utilized for determining the concentration of EA when present as formulation and in combination with other drugs.

The objective of this study is a cost effective, precise, accurate, simple stability indicating UV-Spectrophotometric method was developed for the estimation of Sorafenib in tablet dosage form. Instruments used ELICO Double beam SL 210 Ultra violet-Visible spectrophotometer consisting two matched quartz cells with one cm light path was utilized for measuring of absorbance of Sorafenib. Sorafenib shows highest λmax at 265.5 nm. Beer's law was found over a concentration range of 2-10 µg/ml with superior correlation coefficient (r2 = 0.9998). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were found to be 0.3741 µg/ml and 1.1337 µg/ml respectively. The results of the Sorafenib recovery analysis were found to be 99.9580 ± 0.02095 to 99.9787 ± 0.0106. Percentage assay of Sorafenib tablets (Sorafenat) got more than 99.88 %. Sorafenib was subjected to alkali, acidic, oxidation, thermal, UV light degradation. Sorafenib is more unstable in acidic, oxidation, thermal and stable in alkaline and ultra violet (UV) light irradiation. The Proposed spectrophotometric method was validated as per the ICH Q1A (R2) guidelines. While estimating the Sorafenib in tablet formulation there was no interference of additives & excipients. Hence this method can safely be employed for the routine quality control analysis of Sorafenib in bulk and tablet formulations.[18]

The present work deals with development of two rapid, precise and accurate spectrophotometric methods for the estimation of Imatinib Mesylate in bulk and solid dosage form. Method A is first order derivative spectroscopy where derivative amplitudes were calculated by considering minima and maxima of the curve. Method B is area under the curve in which wavelength range 237-277nm was selected for estimation of Imatinib Mesylate. Linearity was observed in the concentration range 5-30µg/ml for both the methods (r2=0.9992 for method A and r2=0.9996 for method B). All the methods were found to be simple, precise and accurate and can be employed for routine quality control analysis of Imatinib Mesylate in bulk as well as in its solid dosage form.[19]

A simple, sensitive, precise, reproducible and validated UV spectrophotometric methods have been developed for the determination of vincristine (VCR) in the pure and dosage forms. The method was founded on the simple solubility of VCR in purified water, and their characteristic maximum absorption λ (max) at 295 nm for VCR in the UV regions. The nature of obedience, to the Bouguer-Lambert-Beer's law by the VCR in the range of concentration 5-50 µg/ml was employed to this method. Accuracy and reproducibility of the

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proposed method were statistically validated by recovery studies. The accuracy of the method for the VCR was ~ 100.4 % with good reproducibility. The analytical curves were linear over
a wide concentration range (5-50 µg/ml), with a correlation coefficient (r)-0.9998, and 0.9999 for VCR in that order. The method was showed sufficient precision, with a relative standard deviation (RSD) less than 1%. The method was validated in accordance with Russian general pharmacopoeia article (RGPA) 42-0113-09 and ICH guidelines. Validated method can easily apply for fast, precise and reliable rapid assessment of drug forms and pure substances in the laboratory. [20]

The objective of this work was to develop and validate a UV method for the quantitative analysis of melphalan, an anticancer drug from lyophilized nanosuspension. The melphalan was detected and quantitated using a UV detector at a wavelength of 254 nm. The method was shown to be specific and linear in the range of 10-50 µg.mL-1 with correlation coefficient of 0.9979 and was precise at the intra-day level as reflected by relative standard deviation, accurate at recovery rate 99.75±0.08 and robust to change mobile phase and column brand. The detection and quantitation limits were 0.2956 µg. mL-1 and0.5874 µg.mL-1, respectively. The proposed method could be advantageous in estimation of melphalan quantitation in lyophilized nanosuspension form in the presence of excipients. Conclusion: The method was found to be simple, specific, rapid, precise, accurate and reproducible. The method was successfully applied for determination of the entrapment efficiency of melphalan from lyophilized nanosuspension and was found to 93.56 ± 4.32%. [21]

It is a simple, precise and economical UV-spectrophotometric method has been developed for the estimation of Vandetanib from bulk. Two methods was developed First method (A) applied was area under curve (AUC) in this method area was integrated in wavelength from 323.59-333.36nm. Second method (B) was first order derivative spectrometric method. In this method absorbance at λmin=311.27nm, λmax=340.54nm and zero cross=328.37nm was measured. Both the method linearity was observed in the concentration range of 5-30µg/ml at the λmax=328.44nm. Accuracy and precision studies were carried out and result was satisfactory obtained. The drug at each of the 80 %, 100 % and 120 % levels showed good recoveries that is in the range of 97.00 to 99.00% for both methods, hence it could be said that the method was accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were determined for the method. The method was validated by the International Conference on Harmonization. All validation parameters were within the acceptable limit. The developed method was successfully applied to estimate the amount of vandetanib in pharmaceutical formulation.

II. RESULT AND DISCUSSION

- Method of Gemcitabine HCl: Transfer aliquots equivalent to 20–400 g GMCT into a series of 10 ml volumetric flask. Add 1 ml of AuNP (1 mM) in each flask with constant stirring. Allow to stand them for 2 min, and then make the volume up to the mark with water. The UV–vis spectrum of the GMCT–AuNP solution shows absorption maximum at 688nm.
- Method of Letrozole: About 10 mg of Letrozole-d4 was weighed accurately, and transferred to a 10 mL clean glass volumetric flask. It was dissolved in methanol and the volume is made up with the same to produce a solution of 1mg/mL of Letrozole-d4. The UV–vis spectrum of the Letrozole solution shows absorption maximum at 241 nm.
- Method of Chlorambucil: 1ml was pippetted into a 10ml volumetric flask and the volume was made up to the mark with acetronitrile to prepare a concentration of 10µg/ml. Then 4µg/ml sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using acetronitrile as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be258nm.
- Method of Letrozole: Twenty tablets were weighed and powdered well from that powder equivalent to 100mg of Letrozole was transferred to a 100ml standard volumetric flask. Add small amount of absolute ethanol and make up to mark with ethanol to get stock solution (1mg/ml) from this stock 6 µg/ml was prepared for the estimation. From standard stock solution, aliquots of solution were taken and diluted to get different concentrations like 2,4,6,8 and 10 µg/ml. The standard solutions were scanned between 200-400nm, they showed λ-max at 240nm.
- Method of Capecitabine: Standard stock solution (1.0 mg mL–1) of capecitabine was prepared in methanol and stored in refrigerator

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at 4 ± 2 °C. The standard solutions were scanned between 200-400nm, they showed λ-max at 266nm.

- Method of Anastrozole: Standard stock solutions of anastrozole (1000 µg/mL) were prepared separately in pH 7.4 phosphate buffer saline. Working standard solutions of the drug (100 µg/mL) was obtained by dilution of the respective stock solutions in PBS. Absorption maximum was found at 210 nm and the method was optimized and validatedusing this wavelength.
- Method of Ellagic Acid: From the stock solution, the samples with 1-5µg/ml concentrations range were prepared in triplicate and analyzed in the spectrophotometer at 277nm against 10% PEG200 solution in water asblank.
- Method of Sorafenib: Sorafenib 10 µg/ml standard stock solution was prepared by transferring accurately weighed 10 mg of standard Sorafenib to 10 ml volumetric flask and dissolved in MEOH and ACN. The volume was made upto the mark with MEOH and ACN. To estimate the maximum λmax, Sorafenib 10 µg/ml of working standard solution was prepared and scanned in UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 265.5 nm which was chosen as the detection wavelength for the determination ofSorafenib.
- Method of Imatinib Mesylate: The standard stock solution of Imatinib Mesylate was prepared by transferring, accurately weighed 100mg of Imatinb Mesylate to100ml of volumetric flask. The drug was dissolved with sonication in 50ml of distilled water and volume was made up to the mark by using distilled water. Wavelength range was selected around wavelength maxima257nm.
- Method of Vincristine: VCR solution A (stock solution)–vincristine-Richter lyophilisate, a dosage of 1.0 mg five ampules, were accurately transferred into 50 ml volumetric flask separately and diluted with purified water to nominal volume, at the final solution concentration was 100 µg/ml. The characteristic maximum absorption λ (max) obtained at 295 nm.
- Method of Melphalan: Standard stock solutions of Melphalan (1 mg mL−1) were daily prepared by dissolving the appropriate amounts of the drug in methanol. The melphalan was detected and quantitated using a UV detector at a wavelength of 254nm.
- Method of Vandetanib: Standard solution of Vandetanib was prepared by transferring accurately weighed 10 mg of drug into a 100ml volumetric flask and the volume was made up to 100ml using methanol as a solvent to get the concentration of 100µg/ml. The maximum absorbance of solution was measured at the wavelength328.44nm.
- So as per this results it was discussed that Purified or distilled water is the mainly used solvent because of its easily availability and most of drugs are easily soluble within it, it is feasible for most of method development processes and shows greater results when going to validate that results obtained. After that Methanol, absolute ethanol, acetronitrile and phosphate buffer saline etc solvents were used which showing same criteria's that are required to show better results in validation process. Next λmax also plays important role in validation of method and the maximum anticancer drugs recorded in this project shows λmax is in between 210 nm to 277nm.

III. CONCLUSION

- As per result & discussion it was concluded that most of method developments and validation are successfully done on anticancer drugs. In which it starts with solvent selection according to particular drugs solubility within it. Because when method development starts at first stage searching for various solvents as per solubility parameter then its easy availability and showing of the intense absorption peaks. Then go for the proposed analytical method is simple, sensitive, easy and cost effective. The proposed method is accurate and precise. UV spectrophotometric method provides suitably quick results and has an important role to play in method development and validation. Now as per the results were obtained it is concluded that Purified or distilled water is the mainly used solvent because of its easily availability and most of drugs are easily soluble within it, it is feasible for most of method development processes and shows greater results when going to validate that results obtained. After that Methanol, absolute ethanol, acetronitrile and phosphate buffer saline etc solvents were used which showing same criteria's that are

required to show better results in validation process. Next λmax also plays important role in validation of method and the maximum anticancer drugs recorded in this project shows λmax is in between 210 nm to 277 nm. So The UV methods developed in this study have the advantage of simplicity, precision, accuracy, and convenience.

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