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# A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF DECITABINE BY DIFFERENT ANALYTICAL TECHNIQUES

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**Abstract:**

*Pharmaceutical analysis is a broader term which can be defined in many ways. It is the series of processes that are used for identification, determination, separation, purification, and structure elucidation of the given compound used in the formulation of pharmaceutical products. Recent development in analytical methods has been resulted from the advancement of analytical instruments. The improvement of the analytical method development and analytical instruments like UV, HPLC, LC-MS, GC are required for reduced the time of analysis, increased precision and accuracy and reduced costs of analysis. Decitabine (i.e., 5-aza-2'-deoxycytidine) acts as a nucleic acid synthesis inhibitor.It is a medication for the treatment of myelodysplastic syndromes, a class of conditions where certain blood cells are dysfunctional, and for acute myeloid leukemia (AML). Chemically, it is a cytidine analog.*

***Key words:*** *Analytical method development, validation, Decitabine, UV, HPLC, LC-MS, GC*

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## INTRODUCTION:

**Analytical method**: Analytical methods development plays important role in drug discovery, generic product development and manufacture of pharmaceuticals. Method development is the process of identifying set of parameters which provides desired analytical performance. Below are the important parameters which needs to be considered during method development.

**Solubility profile:**

Solubility of interested compound/API in different solvents such as water, acetonitrile, methanol, isopropyl alcohol etc. is useful while selecting diluents for standard solutions and extraction solvents for test solutions. The pH solubility data of API also helps in selecting diluent during sample and standard preparation. BCS and saturation solubility data is used for selecting media for dissolution method.

**Analytical profile:**

The spectral profile is useful in understanding the absorption characteristics, which helps in selection of detector and the wavelength for analysis. Understanding the degradation profile will help in developing the method for separation and estimation of all possible impurities and degradants. Information regarding possible process related impurities and degradants shall be obtained.

**Selection and optimization of mobile phase:**

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from analyte (API) peak. The selection of mobile phase is always done in combination with selection of column (stationary phase). Following are the parameters which shall be taken into consideration while selecting and optimizing the mobile phase.

(a) Buffer, if any and its strength and pKa.

(b) pH of the buffer or pH of the mobile phase.

(c) Mobile phase composition.

**Selection of column:**

Following are the parameters of a chromatographic column which are to be considered while choosing a column for separation of interested compounds such as impurities and degradants.

(i) Length and diameter of column.

(ii) Packaging material

(iii) Shape of the particles

(iv) Size of the particles

## METHOD DEVELOPMENT

Method development consists of three main stages: feasibility—where you determine if the method will work with your sample; development—where you optimize the method; and validation—where the optimized method is validated to the relevant regulatory requirements," explains Vincent Thibon, technical development lead, RSSL. "Developing a robust method will ensure that routine testing occurs smoothly and limits the amount of testing required."

**Step one—feasibility**

The method to be suitable for drug substance (DS) initially, but maybe potentially further down the line for drug product (DP) it is crucial to collect as much background information as possible on the API to understand its characteristics or what development challenges it poses," "In order to develop an accurate, reproducible, and reliable method, there must be an understanding of the final purpose of the method. This purpose should be the driving principle behind the research and development stages," the phase of the development of the product, which impacts the amount of work required, should be assessed. Defining the phase of development early on is important, Analytes might also be unknowns belonging to broader categories of chemicals, which require a different approach compared to a targeted method for a known compound."

**Step two—development**

The next stage is about minimizing the complexity of the methods to ensure they are user-friendly for routine use, Curson continues. "[A method] will be used by different analysts and may be transferred between different labs, analysts and may be transferred between different labs," "Developers need to select an appropriate solvent system for dissolving the sample and they should also choose a suitable separation mode, such as reversed phase chromatography or hydrophilic interaction chromatography (HILIC)," states . "A detection principle should also be chosen—for example, for [ultraviolet] UV or visible light, an appropriate detection wavelength should be selected. UV detection is preferred if the analytes contain a UV chromophore due to the widespread availability of UV detectors

The next stage is about minimizing the complexity of the methods to ensure they are user-friendly for routine use, Curson continues. "[A method] will be used by different analysts and may be transferred between different labs,

**Step three—optimization**
Finally, the specificity and sensitivity of the method should be considered, "The analyte may be a primary component of the matrix, or it might be an impurity present at trace levels. Instrumentation and sample preparation approaches may change if trace level sensitivity is required," she reveals. "Regulatory guidelines and a knowledge of toxicology are especially important for impurity methods, as these often dictate the permissible limits. Given the trend for increasingly tight regulatory limits, such as for nitrosamines, then it might be prudent to develop a method with sensitivity beyond the minimum requirements in case regulatory authorities decide to lower limits in the future and to fully understand the risk to the consumer."

"With optimization, you want to make sure your initial method is compatible with the sample matrix," confirms Curson. "To meet the industry standard, we subject the product to harsh, acidic or basic conditions, oxidation, temperature, and heat so that we are forcing degradation products to be produced, the method must be capable of showing the degradation products and that they do not interfere with the active product potency."

**ANALYTICAL METHOD VALIDATION**
Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

**ICH Method validation parameters**
For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

- Specificity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness
- System suitability

**Specificity/Selectivity:**
Specificity is ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The terms selectivity and specificity are often used interchangeably. According to ICH the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate.

**Accuracy:**
The Accuracy of analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and value found.

Accuracy may be inferred once precision, linearity and specificity have been established. Accuracy for the area percent method should be established from 50% of the ICH reporting limit to the nominal concentration of drug substance in the sample solution.

**Precision:**
ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP XXII <1225>, 1990 incorporates the concepts described under the terms *"intermediate precision"*, *"reproducibility*" and *"repeatability*" of this guide.

**Linearity:**
Linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

**Limit of detection :**
Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not

necessarily quantitated, under the stated experimental conditions. With UV detectors, it is difficult to assure the detection precision of low level compounds due to potential gradual loss of sensitivity of detector lamps with age or noise level variation by detector manufacturer. At low levels, assurance is needed that the LOD and LOQ limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability, extraneous peak(s) could "disappear / appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for LOD from the area counts of the analyte.
The LOD may be expressed as:

$$LOD = 3.3\ \sigma / S$$

Where,

σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

**Limit of quantification:**
Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.
The LOQ may be expressed as

$$LOQ = 10\ \sigma / S$$

Where,

σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

**Robustness:**
The robustness of an analytical procedure is defined as a measure of its capacity to obtain comparable and acceptable results when perturbed by small but deliberate variations in specified experimental conditions. Robustness provides an indication of the test method's suitability and reliability during normal use. During a robustness study, conditions are intentionally varied to see if the method results are affected. Example HPLC variations are illustrated for isocratic and gradient methods, respectively.

Examples of typical variations are:

- Stability of analytical solutions
- Extraction time

**System Suitability:**
According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The purpose of the system suitability test is to ensure that the complete testing system is suitable for the intended application.

**High Performance Liquid Chromatography (HPLC) :**

HPLC is a technique in analytical chemistry used to separate, identify, and quantify specific components in mixtures. The mixtures can originate from food, chemicals, pharmaceuticals, biological, en vironmental and agriculture, etc, which have been dissolved into liquid solutions.

It relies on high pressure pumps, which deliver mixtures of various solvents, called the mobile phase, which flows through the system, collecting the sample mixture on the way, delivering it into a cylinder, called the column, filled with solid particles, made of adsorbent material, called the stationary phase.

Each component in the sample interacts differently with the adsorbent material, causing different migration rates for each component. These different rates lead to separation as the species flow out of the column into a specific detector such as UV detectors. The output of the detector is a graph, called a chromatogram. Chromatograms are graphical representations of the signal intensity versus time or volume, showing peaks, which represent components of the sample. Each sample appears in its respective time, called its retention time, having area proportional to its amount.

HPLC is widely used for manufacturing (*e.g.*, during the production process of pharmaceutical and biological products), legal (*e.g.*, detecting performance enhancement drugs in urine), research (*e.g.*, separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (*e.g.*, detecting vitamin D levels in blood serum) purposes.

shows a basic overview of the HPLC

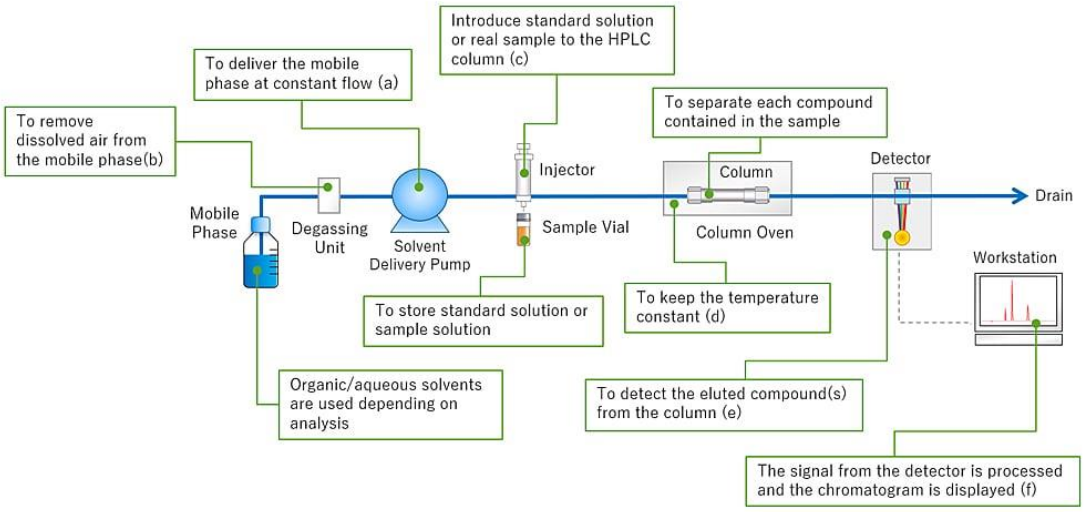


**Fig.1 HPLC FLOW DIAGRAM**

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**Liquid chromatography–Mass spectrometry (LC–MS)**

Liquid chromatography–mass spectrometry (LC–MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography – MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides spectral information that may help to identify (or confirm the suspected identity of) each separated component. MS is not only sensitive, but provides selective detection, relieving the need for complete chromatographic separation. LC–MS is also appropriate for metabolomics because of its good coverage of a wide range of chemicals. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC–MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical,
and cosmetic industries. Since the early 2000s, LC–MS (or more specifically LC–MS–MS) has also begun to be used in clinical applications.

**Gas Chromatography**

Gas chromatography (GC) is an analytical technique used to separate and detect the chemical components of a sample mixture to determine their presence or absence and/or quantities. These chemical components are usually organic molecules or gases. For GC to be successful in their analysis, these components need to be volatile, usually with a molecular weight below 1250 Da, and thermally stable so they don't degrade in the GC system. GC is a widely used technique across most industries, including for:

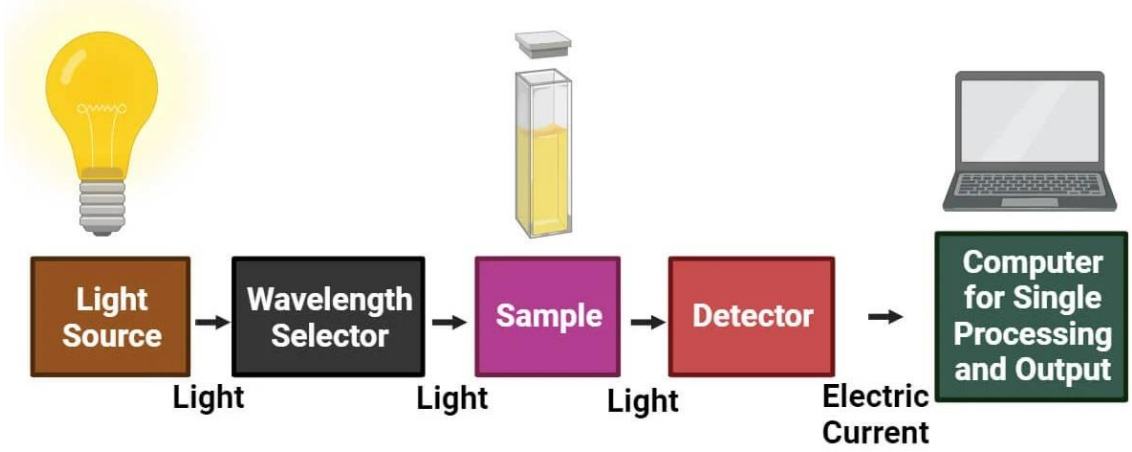
- Quality control in the manufacture of many products from cars, to chemicals and petrochemicals, to pharmaceuticals
- Research purposes from the analysis of meteorites to natural products
- Safety and monitoring from environmental samples, microplastics a nd food and wine, to forensics.

Gas chromatographs are frequently hyphenated to mass spectrometers (GC-MS) to enable the identification of the chemical components.

**Uv Spectroscopy**

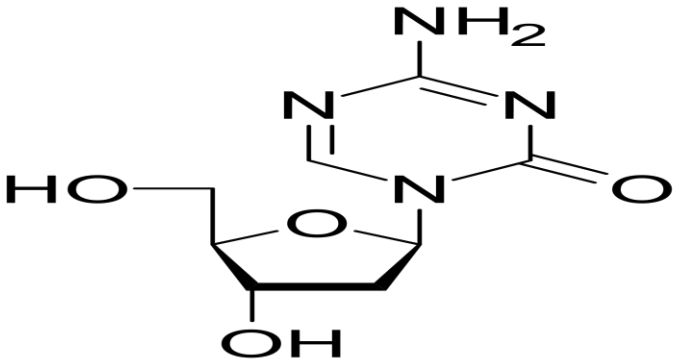
UV- spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition, potentially providing information on what is in the sample and at what concentration. Since this spectroscopy technique relies on the use of light, let's first consider the properties of light.

Light has a certain amount of energy which is inversely proportional to its wavelength. Thus, shorter wavelengths of light carry more energy and longer wavelengths carry less energy. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. Electrons in different bonding environments in a substance require a different specific amount of energy to promote the electrons to a higher energy state. This is why the absorption of light occurs for different wavelengths in different substances. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red. UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-Vis spectroscopy to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance




**DRUG PROFILE**

**Name** : Decitabine
**Description** : Decitabine is used to treat myelodysplastic syndrome Decitabine is in a class of medications called hypomethylation agents.




**IUPAC Name** : 4-Amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one

**Chemical formula** : C8H12N4O4

**Molecular mass** : 228.21g/mol

**Category** : hypomethylation agents.

**Mechanism of action** : Myelodysplastic syndromes (MDS) are a group of hematopoietic neoplasms that manifest in peripheral cytopenia's and may eventually progress to secondary acute myeloid leukaemia (sAML). Included in the over 45 genes commonly mutated in MDS patients are those involved in DNA methylation and histone modification, and it is well-established that alteration of the epigenetic landscape is a feature of myeloid leukaemia's.

Decitabine is considered a prodrug, as it requires transport into cells and subsequent phosphorylation by distinct kinases to generate the active molecule 5-aza-2'-deoxycytidine-triphosphate, which is incorporated by DNA polymerase during DNA replication. Once incorporated into DNA, decitabine is recognized as a substrate by DNA methyltransferase enzymes (DNMTs), specifically DNMT1, but due to the presence of an N5 rather than C5 atom, traps the DNMT through the irreversible formation of a covalent bond. At low concentrations, this mode of action depletes DNMTs and results in global DNA hypomethylation while at high concentrations, it additionally results in double-strand breaks and cell death.

**Pharmacodynamics**

Decitabine is a prodrug analogue of the natural nucleotide 2'-deoxycytidine, which, upon being phosphorylated intracellularly, is incorporated into DNA and exerts numerous effects on gene expression. The use of decitabine is associated with neutropenia and thrombocytopenia. In addition, decitabine can cause fetal harm in pregnant women; effective contraception and avoidance of pregnancy are recommended during treatment with decitabine

**Volume of distribution** : 4.59_+ 1.42 L/kg

**Protein binding** : Decitabine exhibits negligible[<1%]

**Metabolism**: It is rapidly metabolized in the liver by cytidine deaminase, which explains its short half-life of 8–30 minutes.

**Route of elimination**: Decitabine can be inactivated through its major elimination pathway involving deamination by cytidine deaminase found principally in the liver, but also in granulocytes, intestinal epithelium, and plasma. Urinary clearance of intact drug accounted for 29% of plasma clearance in mice.

**Half-life**: The terminal elimination half-life ($t_{1/2}$) of decitabine is 37–47 minutes.

**Clearance:** Decitabine has a clearance of 125 L/hr/$m^2$ (53% CV) when administered intravenously at 15 mg/$m^2$ for three hours every eight hours over three days, and a clearance of 210 L/ hr /$m^2$ (47% CV) at 20 mg/$m^2$ for one hour once daily over five days.

**Brand Name:** Dacogen, Dcitas 30mg , Mylodec ,Visvin, D-NIB, Deczuba

**Toxicity**: The most common toxicity is myelosuppression, mainly displaying as neutropenia and thrombocytopenia.

**Affected organisms** : Humans and alternative mammals.

**Table - 1 Solubility Studies of Decitabine**

| SOLVENTS | SOLUBILITY |
|---|---|
| Water | slightly Soluble |
| Propylene Glycol | Soluble |
| Ethanol | Slightly Soluble |
| Isopropyl Alcohol | Slightly Soluble |
| Methanol | Soluble |
| Toluene | Freely Soluble |
| Acetonitrile | Soluble |
| Acetone | Freely Soluble |

## REVIEW OF LITERATURE:

1. **Ankit Awasthi, et al., [2020] :** Objectives: In the proposed study, a basic, novel, protected economic, delicate and cost-effective UV-Spectrophotometric technique for the analysis of Decitabine which is antineoplastic drug. Methods: The created method was approved according to ICH rules. The decitabine indicated maximum absorption at 221 nm. This strategy can successfully apply for estimation of Decitabine in active pharmaceutical ingredient (API) form for routine examination with UV identification at 221 nm. A Shimadzu UV-Visible spectrophotometer 1800 with 1 cm quartz cells and Acetonitrile (ACN) as diluent was utilized in this technique. Results: The Developed technique obeyed Beer's-Lambert's law in the conc. of 5.0-15.0 ug/ml, having R2 of 0.9993 and 99.82%±0.75 recovery of decitabine was found. Different approval parameters like linearity, accuracy, precision and robustness were performed and were found to be within the limits as per the guidelines of ICH.
2. **Suresh Reddy Yelampalli, et al., [2019]:** Decitabine is an anti-cancer chemotherapy drug. This article describes method development and method validation of related substances of Decitabine (α- decitabine, α-isomer, methyl 4-chlorobenzoate, β-isomer, O-Acetyl and unknown impurities) in decitabine drug substance and finished dosage forms by using a high Performance liquid chromatography. In this high performance liquid chromatography, the resolution was achieved on Inertsil ODS 3V, 250×4.6 mm, 5µm column with a gradient elution at a flow rate of 1.2 mL/min using a mobile phase A as Ammonium acetate buffer solution and mobile phase B as water: acetonitrile (10:90% v/v) at wavelength 254 nm by an UV detector. The method was validated in the concentration range of 0.7 ppm to 7.1 ppm, 1.2 ppm to 11.0 ppm, 1.2 ppm to 10.9 ppm, 1.2 ppm to 11.0 ppm, 1.2 ppm to 10.9 ppm and 0.8 ppm to 7.7 ppm for related substances of α- decitabine, α-isomer, methyl 4-chlorobenzoate, β-isomer, O-Acetyl and Decitabine respectively. The obtained recovery was in between 90.0 % to 110.0 % and the % RSD was not more than 10.0.
3. **Byasabhusan Das, et al., [2017] :** Development of a new rapid, efficient and reproducible reverse phase-HPLC method for the analysis of Decitabine in bulk drug and tablet dosage form. This separation was achieved by using Develosil ODS HG-5 RP column 150mm x 4.6mm, 5µm (particle size) i.d. in isocratic mode, with mobile phase containing ACN : Acetate buffer (55:45), adjusted to pH 3.9 using ortho phosphoric acid. The flow rate was maintained at 1.0 ml/min and analyte were monitored at 254 nm. The retention time of Decitabine was found to be 2.97 min. The linearity for Decitabine were in the range of 0-60 µg/ml. The recoveries of Decitabine were found in the range of 100.4933%, 101.4733% and 100.0467%. The developed method was validated as per ICH guidelines and successfully applied to the further analysis of Decitabine in bulk and tablet dosage form
4. **Y.Neupane, et al., [2014**]: The aim of our present work was to develop and validate a reverse phase high-performance liquid chromatography (RP-HPLC) method for the determination of Decitabine (DCB). The developed method was further applied to observe the degradation of DCB under various stress conditions. Methods: Chromatographic separation was achieved on C18, 250 × 4.6 mm, particle size 5 µm, Agilent column, using ammonium acetate (0.01M) as mobile phase with flow rate of 1mL/min and injection volume was 20 µL. Quantification was carried out with UV detector at 230 nm with a linear calibration curve in the concentration range of 10–100 µg/mL based on peak area. Thus, developed method was validated for linearity, accuracy, precision, and robustness. Results: Linearity was found to be in the range between 10–100 µg/mL with a significantly higher value of correlation coefficient r = 0.9994. The limits of detection (LOD) and the limits of quantification (LOQ) were found to be 1.92µg/mL and 5.82 µg/mL respectively.
5. **Sunil kumar , Adupa , et al., [2014] :** A new, precise, rapid, accurate RP- HPLC method was developed for the estimation of Decitabine in pharmaceutical dosage form. After optimization the good chromatographic separation was achieved by Isocratic mode with a mixture of Ammonium Acetate buffer of pH 4.5: Acetonitrile (985:15) v/v as the mobile phase with Develosil Rp Aqueous-AR-5 (150 x 4.6 mm, 5 µm), column as stationary phase at flow rate of 1.5 mL/min and detection wavelength of 244 nm. The Retention time of decitabine was found to be 3.796 min. The linearity of this method was found in the concentration range of 50-150 µg/mL. The correlation coefficient R 2 value is found to be 0.998. The LOD for this method was found to be 0.0003 µg/mL. The LOQ for this method was found to be 0.0009 µg/mL. This method was found to be good percentage recovery about 99.77 indicates that the proposed method is highly accurate.

6. **Karuna Reddy,et al;[2011]:**simple, sensitive, precise and stability indicating Reverse phase high performance liquid chromatographic method has been developed for the quantitative analysis of Decitabine drug present in tablet formulation and bulk drug. The HPLC separation was achieved on Zorbax bonus C18 Column (250mm x 4.6 mm, i.d, 5 µm particle size) with the mobile phase and detection at 254nm.The proposed method provided linear responses within the concentration range 400-1200 µg mL−1 for Decitabine and its related compounds. LOD and LOQ values for the active substance were 0.26 and 0.8 µg mL−1 , respectively. Correlation coefficients (r) of the regression equations for the impurities were greater than 0.999 in all cases. The precision of the method was demonstrated using intra- day assay RSD% values which were less than 1% in all instances.

## CONCLUSION:

A survey of literature reveals that smart analytical strategies don't seem to be offered for the drug Decitabine. Despite the fact that only a few strategies of estimation of on top of medicine square measure offered, several of them suffer from one disadvantage or the opposite, like low sensitivity, lack of property and ease etc. the present chemical science strategies square measure inadequate to fulfil the requirements; hence its planned to enhance the present strategies and to develop new strategies for the assay of Decitabine in pharmaceutical dose forms adapting totally different offered analytical techniques like Uv spectrophotometry, HPLC, GC and LC-MS

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## CONSENT AND ETHICAL APPROVAL

It is not applicable

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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