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Development and Validation of New Analytical Method for Selected Anti-Cancer Drugs in Bulk and Pharmaceutical Dosage Forms

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Abstract:

The development and validation of novel analytical methods adapted for a selection of anticancer drugs, both in bulk form and within pharmaceutical dosage formulations. There is an escalating demand for precise and reliable analytical techniques in pharmaceutical research, this investigation aims to address the critical need for robust methodologies specific to anticancer medications. Through meticulous experimentation and validation processes, the proposed methods exhibit efficacy in accurately quantifying the targeted drugs, ensuring compliance with regulatory standards and facilitating quality control in manufacturing processes. By employing advanced analytical techniques, including chromatography and spectroscopy, this research advances the field's capability to assess the potency and purity of anticancer compounds in various pharmaceutical formulations. The findings not only contribute to enhancing drug development and production but also hold significant implications for clinical applications, fostering improved therapeutic outcomes and patient care in the fight against cancer.

Keywords: development, validation, anti-cancer drugs

Introduction:

A critical area of research in the pharmaceutical industry is the development and validation of new analytical techniques for the simultaneous estimate of certain medication combinations inside pharmaceutical formulations. To guarantee the safety and efficacy of the final product, it is

essential to precisely and accurately measure various medicinal components in a single formulation^[1]. Due to the existence of different drug components, excipients, and possible interferences from the sample matrix, the simultaneous estimate of several medications in pharmaceutical formulations presents a complicated problem. The exact composition of the formulation may not be correctly represented by traditional analytical methods, which sometimes require the time-consuming and laborious separation and quantification of individual pharmacological components. As such, the need for analytical methods that can estimate several medicines inside pharmaceutical formulations at the same time is growing^[2]. Creating novel analytical techniques that allow for the simultaneous estimate of certain drug combinations requires a multidisciplinary approach that includes statistical approaches, pharmacology, and analytical chemistry. To guarantee the exact and accurate quantification of medication components, researchers must carefully choose appropriate analytical techniques, sample preparation procedures, and analytical standards^[3].

The possibility of interference from other elements in the sample matrix is one of the main difficulties in creating novel analytical techniques. These interferences have the potential to cause imprecise or erroneous drug concentration readings, which might jeopardize the final product's efficacy and safety. In order to overcome this obstacle, scientists must carefully choose chromatographic settings and sample preparation methods that will reduce the likelihood of interferences. In addition to the technical challenges of developing new analytical techniques, regulatory issues also need to be taken into account. Pharma companies have certain rules and regulations that they must follow while developing and validating their analytical procedures. These legislative frameworks are designed to promote uniformity and standardization across the pharmaceutical industry while guaranteeing the safety and effectiveness of pharmaceutical goods^[4].

An important step in developing novel analytical techniques for the simultaneous estimate of targeted medication combinations is the validation of analytical methodologies. This validation procedure consists of a number of carefully planned tests and assessments intended to determine the precision, accuracy, and dependability of the analytical technique. Important evaluations usually cover elements like robustness, linearity, specificity, accuracy, and precision, among others^[5,6].

In addition, the process of validation involves the creation of suitable acceptance criteria that are specific to every parameter. These standards are customized to meet the unique requirements of the analytical technique and the intended use of the finished result. For example, a pharmaceutical

formulation intended for human consumption would require more severe acceptance criteria for an analytical technique developed for the simultaneous estimate of numerous medications than would an animal feed additive that uses the same pharmaceuticals^[7,8].

Recent research has made great progress in the development, synthesis, precise analysis, and use of pharmacological compositions for human ailment diagnosis, treatment, and even prevention^[1,2]. Low detection limit detection techniques are required for analysing tiny samples like serum, blood, and urine since some research has concentrated on examining these biological materials^[3–5].

Hippocrates originally referred to tumors that did not create ulcers and those that did form ulcers as carcinos and carcinoma, respectively. These Greek words were then translated into the Latin word for crab, cancer, by the Roman physician Celsus. With an anticipated 9.6 million deaths from cancer in 2018, it is the second greatest cause of death globally. According to studies, cancer is caused by aberrant cells proliferating out of control and invading other organs. Through the lymphatic and circulatory systems, cancer cells can travel to other bodily areas [6]. Notably, there are more than 100 different forms of cancer. Each form of cancer is categorized into broad groups by researchers depending on the cell line or organ from whence it originated. As an example, carcinoma is a kind of cancer that develops in epithelium. Adenocarcinoma, squamous cell carcinoma, transitional cell carcinoma, and basal cell carcinoma are among the subtypes of carcinoma. Sarcoma may be a cancer of the fat, bone, muscle, cartilage, blood vessels, or supporting tissues, according to certain research. Blood-forming organs like the bone marrow are the source of leukemia, which results in aberrant blood cells. Immune system cell-derived cancers are known as lymphoma and myeloma. Central nervous system tumors are those that affect the brain and spinal cord^[8]. The pharmaceutical industry stands to gain significantly from the pursuit of creating and verifying novel analytical techniques for the simultaneous estimate of targeted medication combinations inside pharmaceutical formulations. The precise and exact measurement of several therapeutic components in a single formulation can reduce the possibility of side effects and increase the final product's effectiveness. Furthermore, the development of innovative analytical techniques can simplify and accelerate the procedures involved in medication development and production^[9].

One of the most important areas of research in the pharmaceutical industry is the development and validation of novel analytical techniques for the simultaneous assessment of specific drug combinations inside pharmaceutical formulations. Researchers may create accurate and trustworthy analytical methods that are specifically designed to satisfy the demands of the business by carefully choosing the right analytical techniques, sample preparation procedures, and

analytical standards. A fundamental component of these techniques is their validation, which entails a wide range of tests and studies meant to evaluate their precision, accuracy, and reliability. In the end, the development of novel analytical techniques has a big impact on improving the efficacy, safety, and efficiency of the procedures involved in developing and producing drugs^[10,11].

Development of Analytical Methods

Analytical chemistry encompasses methodologies for identifying, separating, and quantifying the chemical constituents of both natural and synthetic materials. The selection of an analytical method is influenced by various factors, including the chemical characteristics and concentration of the analyte³³, the nature of the sample matrix, the speed and cost of analysis, the type of measurement (quantitative or qualitative), and the volume of samples. Qualitative methods furnish information about the chemical identity of species in the sample, whereas quantitative methods provide numerical data on the relative quantities of one or more analytes present. The process of method development and validation varies depending on the type of method under consideration. Nonetheless, several common steps are typically involved in most projects:

- Defining a method development plan
- Gathering background information
- Conducting laboratory method development, which includes stages such as sample preparation, specific analytical techniques, detection, and data processing
- Generating a test procedure

An effective method should be readily validated. Its development should aim to swiftly analyze preclinical samples, formulation prototypes, and commercial samples. Various types of analytical methods exist, each with distinct validation requirements:

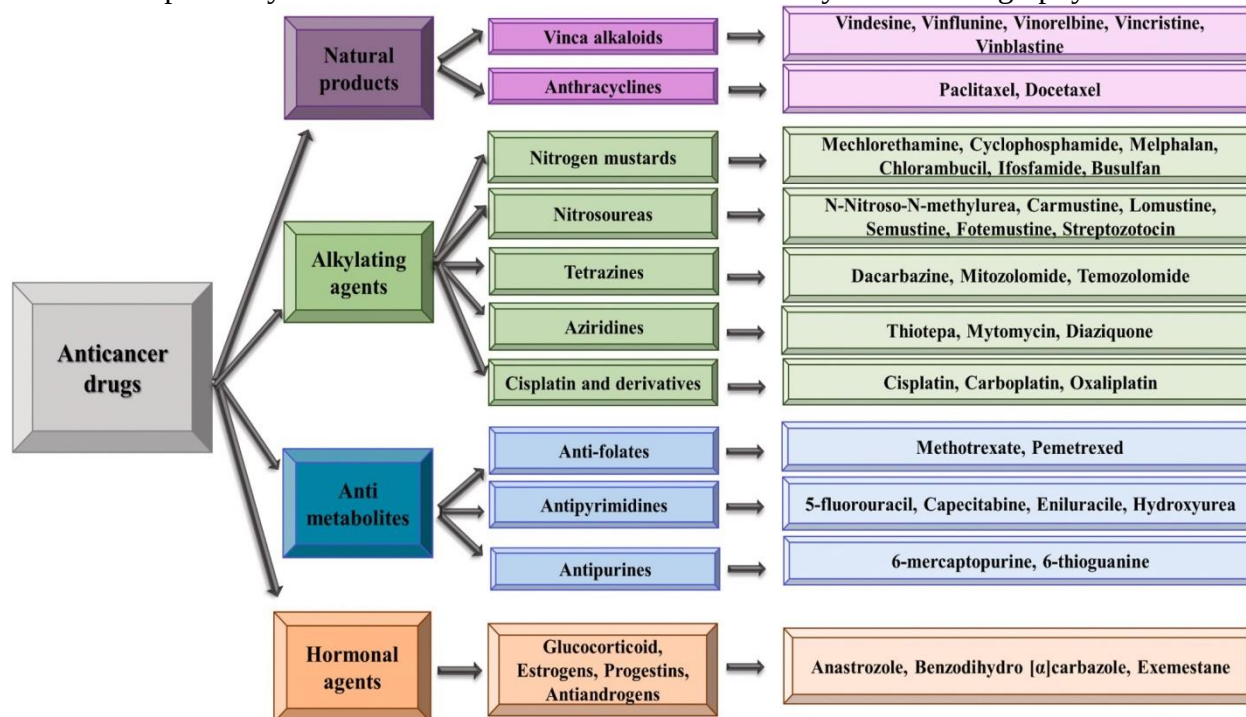
- Identification tests
- Potency assays
- Quantitative tests for impurities
- Limit tests for impurity control
- Specific tests

While the first four tests are universal, specific tests such as particle-size analysis and X-ray diffraction are tailored to control particular properties of the active pharmaceutical ingredient (API) or the drug product^[12,13].

The primary techniques utilized for the quantitative analysis of drugs and metabolites in biological samples such as blood, serum, plasma, or urine encompass Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC) ^[14,15], Thin-Layer Chromatography (TLC), and combined procedures involving GC and LC coupled with mass spectrometry (MS) such as LC-MS ^[16,17], LC-MS-MS ^[18,19], GC-MS ^[20,21], and GC-MS-MS. Moreover, techniques like Nuclear Magnetic Resonance (NMR) are employed for structural identification.

Chromatography, in its various forms, stands as the predominant analytical method for the separation of components within mixtures. The principle underlying chromatographic separation lies in the disparities in migration rates through the column, arising from different partitioning behaviors of compounds between a stationary phase (column packing) and a mobile phase flowing through the system^[21]. Chromatographic methods can be categorized based on the physical state of the mobile phase into three fundamental types: gas chromatography (GC), supercritical fluid chromatography (SFC), and liquid chromatography (LC). This technique was originally pioneered by the Russian botanist M.S. Tswett in 1903.

Today, Thin-Layer Chromatography (TLC) is increasingly adopted as a routine analytical method owing to its advantages of low operational costs, high sample throughput, and minimal sample preparation requirements. One major benefit of TLC is its ability to concurrently run several samples using a small quantity of mobile phase, unlike HPLC, thus reducing both analysis time and cost per analysis ^[23,24]. An advanced version of Thin-Layer Chromatography known as High-



Performance Thin-Layer Chromatography (HPTLC) has been developed [25,26]. Several enhancements can be implemented to the basic TLC method to automate various steps, enhance resolution, and enable more accurate quantitative measurements.

Fig.1. General Classification of anticancer drugs.

Classification of Anticancer Drugs

Recently, over 100 distinct drugs have been employed in chemotherapy, either alone or in combination with other treatments. These drugs can be categorized based on their mechanism of action into antimetabolites, natural products, hormones, and alkylating agents^[27,28]. There are also many drugs that do not fit into these categories but still demonstrate anticancer activity and are therefore used in the treatment of malignant diseases^[29]. This review scrutinizes the general classification of anticancer drugs, as illustrated.

Natural Products:

Plants have been identified as significant sources of natural products for drug exploration, particularly in anticancer therapies. Examples of plant-derived agents include vincristine, vinblastine, paclitaxel, etoposide, docetaxel, irinotecan, and topotecan, which are among the most effective cancer chemotherapeutics available today^[30,31]. However, these agents often exhibit poor solubility in aqueous media and considerable toxic side effects. Consequently, researchers have developed prodrugs and analogues of these agents to mitigate these issues, enhancing aqueous solubility and targeting specific tumors^[32,33].

Alkylating Agents:

Alkylating agents can directly damage DNA by adding alkyl or methyl groups to nucleotide bases, thereby preventing DNA replication and transcription. Common alkylating agents include ifosfamide, cyclophosphamide, chlorambucil, and melphalan^[34,35]. These drugs also encompass derivatives such as cisplatin and non-classical alkylating agents. Their mode of action involves blocking DNA replication and transcription, leading to DNA fragmentation and interference with cellular metabolism.

Anti-metabolites:

Antimetabolites inhibit DNA biosynthesis, affecting the synthesis of RNA, proteins, DNA, and other macromolecules. These drugs include agents that interfere with folate metabolism or are structural analogues of heterocyclic bases, which are crucial for the construction of pyrimidine and purine bases as well as nucleotides. Common antimetabolites include antipyrimidines (e.g., 5-

fluorouracil, capecitabine, eniluracil, hydroxyurea), antipurines (e.g., 6-mercaptopurine, 6-thioguanine), and antifolates (e.g., methotrexate, pemetrexed)^[36,37].

Hormones:

Hormone antagonists are used to treat tumors resulting from hormonal imbalances. In women, endogenous estrogens are steroid hormones. Research has shown that estrogen deficiency in postmenopausal women can lead to symptoms like post-menopausal symptoms, coronary heart disease, and increased risks of osteoporosis and Alzheimer's disease. Additionally, prolonged exposure to estrogen is linked to the growth of female reproductive cancers (e.g., uterus and breast cancer), often associated with hormone replacement therapy, late menopause, and early menarche. Estrogen's role in these processes depends on its binding to estrogen receptors and the activation of estrogen-responsive genes. Anticancer drugs such as anastrozole, exemestane, and benzodihydrocarbazole are used to treat breast cancer by targeting the human estrogen receptor^[38,39].

Cancer is characterized by the uncontrolled proliferation of cancer cells, leading to the loss of regulation over normal cell growth and resulting in the formation of either a solid mass known as a tumor or a liquid cancer, such as bone marrow or blood-related cancers^[24]. It can affect individuals of all ages, including fetuses, disrupting normal cellular function and differentiation throughout the body. Radiation therapy and chemotherapy represent the primary clinical treatments aimed at controlling early-stage tumor growth. The exploration of natural sources, particularly medicinal plants, has provided a rich repository for discovering and developing drugs to combat devastating diseases like cancer. Herbal remedies have demonstrated effectiveness in targeting tumor cells, with drugs derived from medicinal plants showing reduced toxicity and fewer side effects^[40]. While several chemopreventive agents show promise in treating cancer, their potential toxicity limits their widespread use.

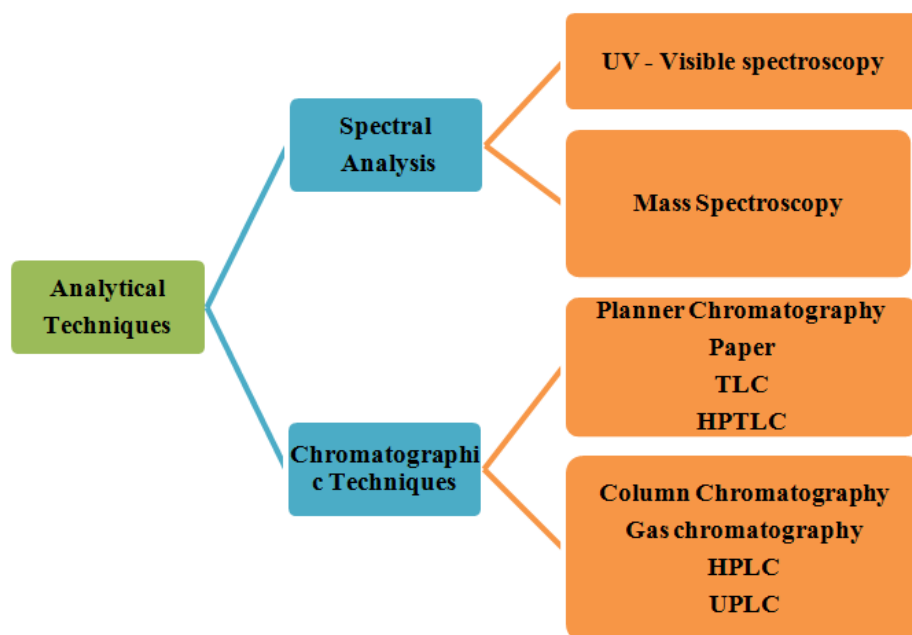


Fig.2: Schematic representation of analytical techniques

Analytical methods

HPLC:

High-performance liquid chromatography (HPLC), a specific form of column chromatography utilized in biochemistry and analysis, serves to separate, identify, and quantify active compounds. In HPLC, pumps are employed to propel pressurized liquid solvent, which carries a sample mixture, into a column packed with solid adsorbent material. While HPLC is widely used for its accuracy in quantitative and qualitative analysis of drug products, it has historically been noted for its lack of high efficiency when compared to gas chromatography and capillary electrophoresis. Nonetheless, HPLC remains one of the most potent tools in analytical chemistry. It operates on the principle of utilizing a mobile phase and a stationary phase, each with opposing polarity, and achieves separation through the interaction between them, facilitated by high-pressure pumps.

UV spectrophotometric:

To streamline the analysis of multicomponent samples, UV Spectrophotometric techniques are employed to minimize the laborious task of separating interferents. This approach allows for the determination of an increasing number of analytes, subsequently reducing analysis time and costs. Over the past 35 years, ultraviolet spectroscopy has emerged as the foremost and advanced

analytical instrument in the pharmaceutical industry. It operates by measuring the absorption of monochromatic light by colorless compounds within the near ultraviolet spectrum (200-400 nm), serving to identify, quantify, and assess the quality and purity of these substances. Analysts utilize various ultraviolet Spectrophotometric methods to swiftly analyze multicomponent formulations, biotherapeutic products, and samples with complex matrices. Among these methods, UV-Visible Spectrophotometry stands out as a preferred tool. Ultraviolet-Visible Spectrophotometers gauge the ratio or function of the ratio of the intensity of two light beams within the UV-Visible region. In qualitative analysis, this technique aids in identifying organic compounds. Its simplicity, speed, moderate specificity, and suitability for analyzing small quantities of compounds render it a versatile and widely utilized method.

LC-MS/MS:

Liquid chromatography-mass spectrometry (LC-MS/MS) is a hybrid technique that combines liquid chromatography, often referred to as HPLC, with mass spectrometry. This approach is widely utilized in laboratories for both qualitative and quantitative analyses of drug substances, drug products, and biological samples. LC-MS/MS holds a prominent position as the most commonly employed technique for such analyses. Its versatility extends to playing a crucial role in evaluating and interpreting data related to bioavailability, bioequivalence, and pharmacokinetics.

Preparation of Samples

The primary objective of sample preparation is to generate a processed sample that yields improved analytical outcomes compared to the original sample. The processed sample should constitute an aliquot that is relatively devoid of interferences, compatible with the High-Performance Liquid Chromatography (HPLC) method, and does not harm the column. The principal techniques employed in sample preparation are liquid-liquid extraction (LLE) and solid-phase extraction (SPE) ^[41,42]. These methods facilitate the separation of the analyte of interest from the sample matrix, ensuring that only a minimal number of potentially interfering species are carried through to the subsequent analytical separation stage.

Detection

Following chromatographic separation, the target analyte is identified using appropriate detectors. Several commercially available detectors used in Liquid Chromatography (LC) include ultraviolet (UV) detectors ^[43], fluorescence detectors, electrochemical detectors,

refractive index (RI) detectors, and mass spectrometry (MS) detectors. The selection of the detector depends on the nature of the sample and the specific objectives of the analysis.

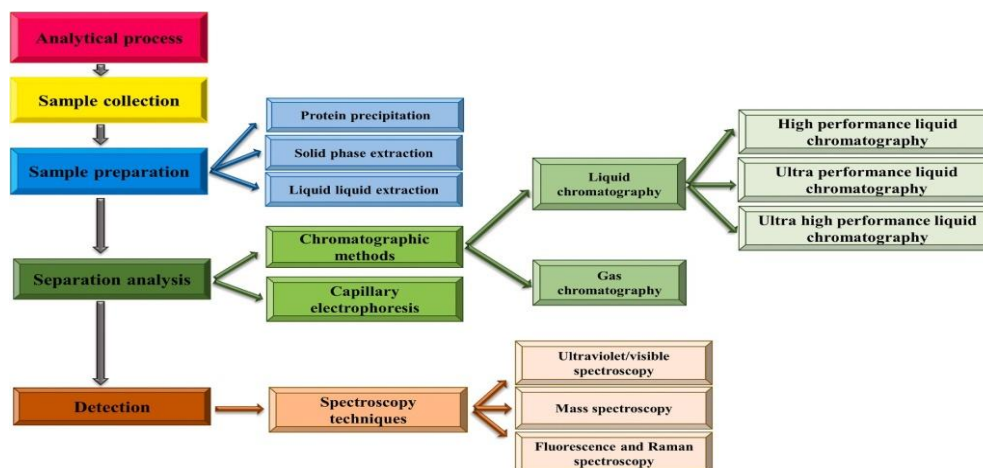


Fig. 3. Analytical analyses of anticancer drugs process

UV detectors are widely employed in HPLC due to their robustness, affordability, ease of handling, and the fact that many solutes absorb light within this frequency range^[42]. Conventional UV detectors measure absorbance at a single wavelength at a time. In contrast, a diode-array detector (DAD) can concurrently measure multiple wavelengths, eliminating mechanical errors or drift over time since no components need to be moved to alter the wavelength or perform scanning. DAD detectors have found application in various domains, including the preliminary identification of steroidal glycosides in seeds, peptide mapping, analysis of sulfamethazine in animal tissues, and identification of pesticides in human biological fluids^[44]. High-Performance Liquid Chromatography coupled with a mass spectrometer detector (LC-MS) has demonstrated superior sensitivity and selectivity compared to HPLC-UV methods.

Nuclear magnetic resonance (NMR) spectroscopy stands as a highly potent technique for elucidating the structures of compounds. This non-invasive spectroscopic analysis unveils the number of atoms, their interconnections, and consequently, the conformations of molecules. Near-infrared (NIR) spectroscopy, on the other hand, represents a rapid, non-destructive method suitable for spot analysis applications. Over the past two decades, it has witnessed growing adoption in pharmaceutical analysis.

Method Validation:

The validation of an analytical method involves laboratory studies aimed at establishing whether the method's performance characteristics meet the requirements for its intended analytical application⁸¹. The validation process adheres to the guidelines outlined by the International

Conference on Harmonization (ICH) for the validation of analytical procedures. Validation is essential for any new or modified method to ensure that it consistently yields reproducible and reliable results, regardless of the operator or laboratory employing the same equipment. The specific validation program required varies depending on the method and its proposed applications. Typical analytical parameters assessed during assay validation include:

- Accuracy
- Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range
- Robustness

Accuracy refers to the closeness between the measured and actual values, while precision denotes the agreement between measurements obtained from multiple samplings of the same homogeneous sample under prescribed conditions of repeatability^[45], intermediate precision, and reproducibility. Specificity gauges the method's ability to measure the desired analyte in the presence of expected components, such as impurities, degradants, or matrix constituents.

The detection limit represents the lowest amount of analyte in a sample that can be detected, though not necessarily quantitated, and can be determined visually, based on signal-to-noise ratio, or through calculation using standard deviation of the response and slope^[46-48]. The quantitation limit, on the other hand, is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. It is particularly relevant for assays targeting low levels of compounds in sample matrices, such as impurity or degradation product determination.

Linearity assesses the method's ability to generate test results directly proportional to the analyte concentration in the sample, typically evaluated through statistical methods like regression line calculation using the least squares method. The range of an analytical procedure encompasses the concentration interval of analyte in the sample over which the method has demonstrated suitable levels of precision, accuracy, and linearity.

Robustness measures the method's capacity to withstand minor deliberate variations in parameters without significant impact on its performance ^[49], offering insight into its reliability under normal conditions of use. While specificity suffices for an identification test, more complex methods, such as quantitative impurity assays, necessitate a comprehensive assessment including specificity, accuracy, linearity, range, limit of detection (LOD) ⁹¹, limit of quantitation (LOQ) ^[50], precision, and robustness testing.

Table1:Differentanalyticaltechniquesofanti-cancerdrugs

S.No.	Drug name	Analytical techniques	Description of techniques
01	Bosutinib	UPLC-MS/MS	System: Acquityultra-performanceliquidchromatography(UPLC)unit
			Column: Acquity BEHC18column(2.1mm x50mm)
			MobilePhase: ACN:0.1%Formicacid
			FlowRate: 0.40ml/min
			Run Time: 3.5min
			Linearity: 0.1-500ng/ml.
			LOQ: 0.1ng/ml
		HPLC	System: ShimadzuHPLCLC-2040C3DPluswithPDAdetector
			Column: CadenzaCX-C18column
			MobilePhase: A0.5% KH ₂ PO ₄ (pH3.5):Methanol(80:20)
			B Acetonitrile-methanol(80:20)
			InjectionVolume: 20µl
			FlowRate: 0.5ml/min
			WaveLength: 267nm
			Linearity: 10-500ng/ml
			LLOQ: 10ng/ml
		HPLC-UV	System: RP-HPLCwithUVdetector
		HPLC	MobilePhase: 0.5%Na ₂ PO ₄ H ₂ O(pH3.5)-Acetonitrile-Methanol(55:25:20v/v/v)
			Column: CapcellPAKC18MgIIreversed-phase
			FlowRate: 1.0ml/min
			UV-Detection: 250nm
			LinearityConcentrationRange: 25-1500ng/ml
			LOD: 20 ng/ml
			System: HPLCdetector
			MobilePhase: Methanol:SodiumPhosphateBuffer10mm(PH:6.5,85:15v/v)

			Column: PrimesilC18(Lenght:250nm,Diameter:4.6nm,Particle Size:5µ)
			Linearity: 10-50µg/ml
		RP-HPLC	System: JascoHPLC–PU2080PluswithPDAdetector,Software-Borwin-PDAversion-1.5
			Column: HypersilBDSC18(4.6X250mn)5µmparticlesize
			MobilePhase: Methanol:PotassiumdihydrogenPhosphatebuffer (pHadjustedwithOPA
			to 5.9)
			FlowRate: 1ml/min
			Wavelength: 266nm
			Linearity: 10-50µg/ml
			LOD: 0.2µg/ml
			LOQ: 0.90µg/ml
02	Encorafenib	RP-UPLC	System: WatersequippedwithPDAdetector
			Column: HSSC18(100X2.1mm,1.8m)
			MobilePhase: 0.01NKH ₂ PO ₄ :Acetonitrile(55:45)
			FlowRate: 1ml/min
			WaveLength: 294nm
			Linearity: 45-270µg/ml
			LOD: 0.51µg/ml
			LOQ: 1.55µg/ml
		LC-MS/MS	System: Agilent1200HPLCandanAgilent6410QqQtriplequadru poleequippedwithESI
			Column: HypersilBDSC18
			MobilePhase: 10mmolAmmoniumformatepHadjustedto3.8withFormic acid:
			Acetonitrile(38:62)
			LinearRange: 5ng/mlto500ng/ml
			FlowRate: 0.2ml/min
			InjectionVolume: 2µl
		RP-HPLC	System: WatersAlliance2695withPDA detector,Empower2Software
			Column: AgilentC18
			Mobilephase: 0.1MDiPotassiumhydrogenphosphate:Methanol(50:50v/v)
			Linearity: 7.5-22.5µg/ml
			LOD: 0.114µg/ml
			LOQ: -0.381µg/ml
03	Dabrafenib	UPLC-MS/MS	System: AcquityH-classUPLCsystem,CoupledtoaXevo TQ-SMicroTandemMass
			Spectrometer

			Column: CORTECSUPLC18Column(2.1×50nm,1.6µmParticle size, Waters)
			MobilePhase: 0.1%formicacidandwater:CAN
			Linearity: 10-4000µg/ml
			FlowRate: 0.8ml/min
			LLOQ: 10.0µg/ml
			HLOQ: 3999.2µg/ml
		RP-HPLC	System: WaterAlliancee2695HPLCdevicewith2998PDAdetector
			Column: Symmetry ODSC18(4.6mm×150mm)5µmParticlelength
			MobilePhase: Methanol:Zero.1%Orthophosphoricacid(64:36% v/v)
			WaveLength: 224nm
			Linearity: 20-100µg/ml
			LOD: 0.97µg/ml
			LOQ: 2.91µg/ml
		RP-UPLC	System: WaterAcquityH- classUPLCSystemcoupledwithQSM,SampleManagerand
			Photodiodearray(PDA)detector,EmpowerPRO2.0software
			MobilePhase: 0.05%Ortho-phosphoricacidinwaterandmethanol
			Column: AcquityBEHC18(100nm×2.1nm,1.8µm)
			FlowRate: 0.3mlmin ⁻¹
			InjectionVolume: 5µl
			WaveLength: 225nm
			Linearity: 12.5to125ngml ⁻¹
			LOD: 12.5ngml ⁻¹
			LOQ: 25ngml ⁻¹
		LC/MS/MS	Massspectrometricdetection: Mode- Multiplereactionmode(MRM)andAPI5500(TQ-
			MS)Triple- quadrupolemassspectrometrypositiveionmode,softwareversion 1.5.2(Sciex)
			MobilePhase: 10nmAmmoniumbicarbonateInwater:methanol
			Cloumn: GeminiC18Column(5.0µm50×2.0mm)
			FlowRate: 0.250ml/min
			Linearity: 2.0-200ng/ml
			InjectionVolume: 2µl
			LLOQ: 5; 2ng/ml

Table2:Brandnamesforanticancerdrugs

S.No.	Brandname	API	Manufacturing company
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01	Bosutris	Bosutinib	Mylanpharmaceuticals
02	Bosulif	Bosutinib	PfizerInc
03	Braftovi	Encorafenib	PfizerInc
04	Tafinlar	Dabrafenib	NovartisEuropharmLimited
05	Rafinlar	Dabrafenib	NovartisEuropharmLimited

Conclusion:

The development and validation of analytical methods is an essential step in the development of medicinal products. This review focuses on cancer drugs and shows that the drugs in table 1 are subjected to HPLC, UV and LC-MS analyzes for identification, purification and measurement. In addition, table 2 shows the brand names of the drugs. The primary goal of diagnostic development is to isolate and identify impurities and degradation products. Validation studies confirmed the reliability and specificity of the proposed method, which is suitable for routine analysis in quality control laboratories. Moreover, this method can be extended to other matrices, such as biological samples, for pharmacokinetic investigations. Future research endeavors may explore the application of the proposed method for analyzing other drug combinations and devising alternative techniques to expedite analysis and enhance sensitivity. Recent advancements in the pharmaceutical and biotechnological domains have intensified the demand for analytical methods. Swift and accurate quantification of substrates and drug products holds paramount importance in process development. Advancements in analytical instrumentation have led to the emergence of new techniques such as isocratic and gradient RP-HPLC, which have become primary methods for analyzing nonvolatile APIs and impurities. These analytical methods constitute critical components of pharmaceutical development, underscoring the significance of devising efficient and meticulously validated analytical methods to ensure the development of safe and efficacious drugs.

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