Analytical Techniques for the Assessment of Drug Stability

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Abstract

The expansion of the medications fetched an upheaval in the human healthcare system. These medicines would assist their role only when they do not have any contamination and being administered in the desired quantity. Stability studies are an essential part of drug development and crucial throughout all stages, with a precise timeline of analytical testing. Drug stability can be categorized into different types, including physical, chemical, microbiological, therapeutical, and toxicological stability. Different instrumental and chemical methods were devised for the analysis of drugs at regular intervals. There is always a risk of contamination in these drugs during different phases of their manufacturing, storage, and transportation, making them unsuitable for administration so they

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should be identified and quantified. Various analytical techniques and instrumentations are playing a vital role for this purpose. In this chapter, we have discussed various analytical techniques and methodologies for the assessment of the drug's quality and stability. The chapter highlights a range of analytical methods, i.e., chromatographic, electrochemical, electrophoretic, spectroscopic, and titrimetric, and their analogous techniques that have been utilized in the analysis of drugs.

Keywords

Drug stability · Chemical kinetics · Types of drug stabilities · Analytical techniques

9.1 Introduction

Drugs and pharmaceutical product's stability is an interesting subject for pharmacists, regulators, and drug manufacturers. Stability is a key quality feature, and the degree of Good Manufacturing Practice (GMP) has an essential role in the drug development process. Among all of these drug properties, this is crucial because any change occurred due to physical or chemical processes over time may affect its quality, safety, and efficacy. In most countries, stability is a regulatory prerequisite for drug registration. This is compulsory to ensure that patients have access to safe and effective products throughout the shelf life. Most of the drugs can be sensitive to environmental factors that may cause changes in the physical state or chemical structure, so it is prone to physical, chemical, and microbial degradation in different environments. This can have severe effects on its biological safety and efficacy. For manufacturers, product quality is a key element during manufacturing, shifting, and storage.

Knowledge about the stability of a drug is required to select the appropriate materials for packaging and storage conditions to evade physical and chemical alterations and interactions among drugs and excipients. The medicines contained in the pharmacopeia need to be stored under prescribed conditions to preserve quality characteristics during the shelf life. In adverse climatic conditions such as high temperature and humidity, precautions must be taken to store medicines. Stability concerns are significant in developing therapeutically effective dosage forms. Manufacturers need to perform stability studies on all final products, comprising products that are diluted or reconstituted with 5% glucose solution/saline solution before use [1].

From drug discovery and biopharmaceuticals to drug supply and pharmaceutical technology, advances in all parts of pharmaceutical science have been closely related to the development and application of analytical technologies. Without the development of HPLC and its application in plasma samples, there would be no time curve of plasma concentration, nor would the concept of bioavailability be developed. Without solid-state analysis methods (such as thermal analysis and X-ray powder diffraction), it is impossible to study the significance of the solid-state phase of a

Table 9.1 Fraction of different analytical methods approved for the assay of bulk drug materials in USP XXVII and European Pharmacopoeia, fourth edition (Ph. Eur. 4)

Analytical methods	USP 27 (%)	Ph. Eur. 4 (%)
Non-aqueous	24	36.5
Potentiometric	10	27
Indicator	14	9.5
Aqueous mixtures	5.5	21
Potentiometric	1	14.5
Indicator	4.5	6.5
Titration	40.5	69.5
GC	2.5	2
Acid base	29.5	57.5
Microbiological test, e.g., antibiotics	2.5	3
HPLC	44	15.5
UV-vis spectrophotometery	8.5	9.5
Other, i.e., argentometry, complexometry, etc.	5.5	5.5
Redox, i.e., nitritometry, lodometery, etc.	5.5	6.5
Other, e.g., NMR, IR, fluorimetry, atomic absorption, polarimetry, polarography, spectroscopy, gravimetery, etc.	2	0.5

Adapted from Ref. [2]

drug on its drug performance, let alone understand, just two examples. This list may continue infinitely. However, as the problem of drugs has been clarified, analytical techniques have also advanced. For instance, the continuous development of dissolution testing, which was initially used for quality control of dosage forms, now also has physiologically related dissolution tests to comprehend the in vivo performance of dosage forms.

In the pharmaceutical research field, analysis of major APIs, intermediates, pharmaceutical products, pharmaceutical preparations, impurities/degradation products, biological samples comprising drugs, and drug metabolites is essential. Beginning with the prescribed pharmaceutical examination, analytical methods are mentioned in the pharmacopeias and monographs aiming at describing the drug substance's quality by limiting their content of active ingredient. Recently, assay techniques in the monographs cover titration, capillary electrophoresis, chromatography, spectroscopy, and electroanalytical methods. The current state of the art is represented in the Table 9.1 which is based on the US and European pharmacopeias [2].

Analytical technology plays an essential role at all drug stages during its development from sending to market and even after its marketing because a drug's stability about its physical and chemical properties affects the design and selection of a dosage form. It also evaluates the stability of a drug product, and quantifies when applicable, and evaluates drug content and impurities in a commercial product, and also those impurities above a predetermined threshold are identified, which are necessary to assess the toxicity profile of these impurities to differentiate them from

API. The analysis of drugs and their metabolites may be qualitative or quantitative and is widely used in pharmacokinetic research. This chapter highlights the stability study, its types, and importance as well as the role of several analytical techniques and methods in drug analysis.

9.2 Drug Stability

Drug stability means "a limit to which drug particle or a product can keep its integrity, within the specific time and the period through which it can be stored and further used, with the similar characteristics and features possessing at the stage of manufacturing". The type of stability is usually divided into physical, chemical, therapeutic, toxicological, and microbiological. Drug stability can also be classified as a period before the product's availability in the market. Pre-market stability approaches the clinical path where drug products can save under different strategies for safety and the ability to produce a desired or intended result. It is organized throughout the clinical examination in the drug filling duration. Commercial stability is progressive certainty on post-approval and the long-term stability examination of the drug product [3].

9.3 Importance of Drug Stability Studies

Stability studies are an essential part of drug development throughout all the stages with a precise timeline of analytical testing. The critical aim of stability testing is related to the patent's well-being, which is suffering from the disease for which the product is being designed. Aside from the degradation of non-stable products into toxic disintegration products, up to 85% activity loss that is claimed on the label may cause therapeutic failure leading to death, i.e., nitroglycerine tablets for angina and cardiac arrest. Due to this concern, it becomes a legal prerequisite to provide statistics for particular stability testing for drug regulatory authorities for new drug approval [4]. The second main concern is protecting the manufacturer's reputation by declaring the product's suitability for use, given all functionally related qualities as far as they are in the market. An additional benefit of conducting product stability studies during the development phase or on the market is the provision of a database that might be valuable in selecting appropriate formulations, excipients, and container sealing systems for the development of new products to regulate the shelf life of the product and storage conditions for developing a new product, to prepare registration documents to verify the shelf life of the required registration documents, and verifying that no alterations have been induced in the formulation/manufacturing procedure that could severely affect product stability [5].

9.4 Types of Drug Stabilities

9.4.1 Physical Stability

Drug's physical stability focuses on the physical alterations that occur in the product. These changes subject to the physical characteristics of the drug, i.e., morphology, melting point, polymorphic texture behavior, and particle size. Liquid dosage forms' physical stability is affected due to alteration in appearance, discoloration, viscosity, precipitation, drug adsorption (container surface), polymorph formation (low solubility), and microbial growth. Variations in solid dosage form's physical stability include salt or salt exchange, polymorphic transformation, solvation/desolvation, moisture adsorption, amorphization, and then recovery to crystalline form. Such alterations may cause the physical instability of the product.

9.4.2 Chemical Stability

Chemical reactions that drugs undergo in a liquid dosage form which affects product stability include oxidation (ascorbic acid, epinephrine, vitamin A), epimerization (moxalactam, tetracycline, etoposide), dehydration (glucose, prostaglandins E1 and E2, erythromycin), hydrolysis (amides, esters, imides), isomerization (cyclosporine A, cytarabine, amphotericin B), decarboxylation (etodolac, 4-aminosalicylic acid), and so on. Screening for potential toxicity of degradation products is part of the safety assessment program. Now computer-aided technology is employed to predict the toxicological performance of drug disintegration products [6].

9.4.3 Microbiological Stability

Drug's microbial stability is critical for the safety and efficiency of the product. Resistance against microbial growth or sterility must be preserved throughout the shelf life. The effectiveness of the preservatives must stay unchanged within a specified range. Multi-dose liquid formulations comprise preservatives to prevent deterioration during use. Preservatives do not affect the susceptibility of a product for its contamination (that is, the process by which microorganisms enter the product depends mainly on the design of the container). However, good design can minimize the levels of organisms entered during use and can work in concert with active preservatives to safeguard consumers. Pathogen-contaminated products can have serious concerns for consumers and manufacturers, so the product largely relies on sufficient activity of a preservative. In order to obtain approval of regulatory authorities, it is essential to demonstrate sufficient preservative activity at manufacturing time and during shelf life. Hodges discusses the biological evaluation prerequisites for preservation activity [7].

9.4.4 Therapeutic Stability

The therapeutic effect will stay unchanged.

9.4.5 Toxicological Stability

No notable increase in toxicity occurs [8, 9].

9.5 Types of Stability Studies

Stability studies are based on checking the drug product for a long duration in different temperature and atmospheric moisture. Stability studies are mainly of four types:

9.5.1 Long Term Stability

If the drug is to be divided into many geographical areas and if shipping is the demand for shipment, in these kinds of situations, long-term stability studies are of great value. These studies are performed by checking the sample at a particular duration, and conditions of external variables are changed accordingly. The primary motive of this study is to measure the shelf life of the drug substance.

9.5.2 Intermediate Stability

Studies carried out at 65% RH and 30 °C and intended to reasonably elevate the chemical degradation rate or physical alterations for a drug product/substance proposed for long-term storage at 25 °C.

9.5.3 Accelerated Testing

These studies consist of overstating storage conditions designed to study the increased rate of bodily and chemical degradation. This is part of the formal stability studies. Data from these studies is used to carry out long-term stability studies, i.e., to examine the shelf life of the drug product.

9.5.4 In-Use Stability

This kind of stability study is actually for unit-dose or multi-dose drugs. The physical stability and chemical mixture of such drugs are such that due to continual

Table 9.2 Storage conditions for stability studies of pharmaceutical products

Storage condition	Stability study method	WHO test temperature and humidity	ICH test temperature and humidity
Freezer	Long term	−20 °C ± 5 °C	−20 °C/ambient (12 months)
Refrigerated	Long term	5 ± 3 °C	5 °C/ambient [12]
	Accelerated	30 ± 2 °C/65 ± 5% RH or 25 ± 2 °C/ 60 ± 5% RH	25 ± 2 °C/ 60 ± 5% RH (6 months)
Room temperature	Accelerated	40 ± 2 °C/75 ± 5% RH (6 months)	40 ± 2 °C/ 75 ± 5% RH (6 months)
	Intermediate	30 ± 2 °C/65 ± 5% RH (6 months)	30 ± 2 °C/ 65 ± 5% RH (6 months)
	Long term	25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/ 75 ± 5% RH (12 months) 30 ± 2 °C/ 65 ± 5%RH	25 ± 2 °C/ 60 ± 5% RH (12 months)

Adapted from Ref. [10, 11]

opening and closing, it gets deteriorate due to microbial impurity. The objective of in-use stability testing is to generate where applicable an in-time limit during which a multi-dose product can be used to keep possession of quality in an accepted specification once the container is opened [9]. The storage conditions for the stability studies of the pharmaceutical products are mentioned in Table 9.2 [10, 11].

9.5.5 Role of the Analytical Techniques in Drug Stability

In the field of pharmaceutical research, analysis of so many drugs, intermediates, raw materials, impure products, and biological materials that have drug products and drug metabolites is necessary. Since the initial stage of the authorized pharmaceutical assessment, assay procedures were mentioned in standard monographs to characterize the standard of a large number of drug materials by limiting the actual drug content. Recently, the assay procedures in monographs consist of spectrometry, titrimetric, chromatography, and capillary electrophoresis.

Electroanalytical techniques are also mentioned in the literature. The current stage-of-the-art is depicted by the data provided in Table 9.1 taken from US and European pharmacopeias [2]. From phases of drug growth to retail and post-retail, analytical methodology shows a significant role, considering the chemical and physical firmness of the drug, effects on the selection and dosage design, checking the firmness of the drug particles, quantization of contaminants, and recognition of those contaminants that are more than the organized threshold necessary for the assessment of toxicity profiles of these contaminants to eminent those from the API,

when validated and checked marketed drug product content. The assessment of drug and its metabolite, which could be qualitative or quantitative, is widely useful in pharmacokinetic studies [12].

9.6 Role of Chemical Kinetics in Drug Stability Studies

Chemical kinetics refers to the quantitative study of the chemical reaction rates, including factors that are affecting them. Drug stability study includes the kinetics assessment of chemical degradation reactions occurring in the drug dosage forms. It is essential to envisage the products' shelf life under distinct conditions of drug storage. The drug products can have many chemical structures and thus undergo various degradation modes having different order of reaction under different conditions. The frequently observed drugs' degradation reactions contain hydrolysis, oxidation, and photolysis. Mentioned degradation reactions mainly arise during storage, manufacturing, and usage of the drug products. The working pharmacists must be mindful of the outcomes of such reactions as a toxicity development or loss of potency in drug products to warrant the well-being of the patient. A precise evaluation of the potency loss of a product could be established through employing stability-indicating evaluation techniques which can also asses the degradants and other related compounds. Analyzed data are then passed through kinetic handling for assessing shelf life of drug products and for fixation of the expiry date. A reassessment is required in the packaging and storage parameters for any variation in dosage parameters to enhance drug stability. For this purpose, the reestablishment of the shelf life or reset phase under recommended storage conditions is required. Detailed information on the basic principles of chemical kinetics is required for the evaluation of the rates of drug degradation reaction and the estimation of shelf life and expiry date of the drug substance. These kinetic parameters can be utilized for interpretation of the degradation reactions' mechanisms enabling them to select suitable methods for drug stability. Numerous outstanding aspects of this topic with implications to the drug products' degradation kinetics are mentioned in the books [7, 13–19] monographs, [7, 20–24], and reviews [24–30].

9.7 Analytical Techniques

An analytical dimension is organized to check the limited properties of the drug material or drug outcome against traditional acceptance criteria for that property. Initial in designing a new analytical technique selection of the analytical methodology and the instrumentation that should be chosen exists on the proposed function and the capacity of the analytical technique [31]. Functions that may be assessed through the way events are care, diameters, the ceiling of observation, and limits of quantization (LOQ), range, sureness, and exactness [32].

9.7.1 Titrimetric Techniques

The procedure of titrimetric analysis originated in the eighteenth century. The term titration was originated from the invention of the volumetric method by Gay-Lussac in 1835. The assay method as being an old technique still has some advantages during recent times, such as an expansion of the non-aqueous titration technique, intensifying the area of applying these procedures to weak acids and bases and also by improving the endpoint of potentiometry to enhance the accuracy of these methods. Titrimetric methods have also gained the importance of kinetic measurements used to establish reaction rates due to the organization of functional group analysis procedures. These methodologies can be beneficial in saving time, labor, reasonable accuracy rate, and minimizing the use of reference points. In old times, titrimetric methods were used commercially for the dosage determination of albendazole [33], captopril [34], and gabapentin [2]. An antibiotic named sparfloxacin was also examined by the non-aqueous titration method [35]. These methods are mostly applicable for drug assessment but have also been used in pharmaceuticals for the assessment of the impurity of products [36].

9.7.2 Chromatography Techniques

9.7.2.1 Column Chromatography

The method of analyzing and separating chemical compounds from a mixture is known as chromatography, and the use of column chromatography can significantly purify a large number of such compounds. Column chromatography is a separation technology which is used in several fields, among which pharmacy is one of which pharmaceuticals are prepared and distributed based on this technology to warrant the effectiveness of drugs [37]. Column chromatography includes different types of columns, like flash columns, gravity columns, low- and medium-pressure columns, high-pressure columns, and vacuum columns. However, all of these columns are similar in that all columns need an absorbent, acting as a stationary phase through which samples comprising diverse compounds flow at different rates [38]. On the contrary, the adsorbent is subjected to the column in two ways, namely, a dry packing method and a slurry packing method. The slurry filling method is usually used for macro-separation, while the dry filling method is used for micro-separation. The chief advantage of column chromatography is that the stationary phase used in the method is comparatively inexpensive, and its handling is also easy. The latter stops cross-contamination and degradation of the stationary phase because of recycling. Column chromatography could be performed by gravity to move the solvent or use compressed gas to drive the solvent through the column [39].

Column chromatography can be employed in the purification of the reaction mixture during chemical synthesis, e.g., preferred alcohol and β-ketoester were refined from traces of sulfur, purification of polychlorinated biphenyls and silver nitrate filled silica gel, and purification of phenols, pesticides, organ chlorine, and polynuclear aromatic compounds. It is also used for the purification of biomolecules,

i.e., proteins, for example, the purification of pramlintide (peptide hormone) synthesis for the treatment of type 1 and 2 diabetes. Similarly, bioactive glycolipid's purification is also done by column chromatography, which is used for the treatment of herpesvirus (HSV-1). Also, the nucleic acid purification in cultures (in vitro and in vivo) is done by employing silica gel as an absorbent as it can absorb nucleic acid [40].

9.7.2.2 Gas Chromatography

GC is usually used for the vaporous organic compounds for their detection [41]. The combination of separation and on-border detection gives an exact quantitative organization of compound mixtures, inclusive of minute quantities of amalgam (parts/trillions in specific cases) [42]. GC plays a crucial role in pharmaceutical product analysis and formation of the high-molecular mass outcome, i.e., polypeptides/melting non-stable antibiotics. The derivation is necessary for this technique due to the reason of primary restrictions which remain in the relative non-volatility of drug substances. Gas chromatography has also extended its usability for evaluation of some drugs, i.e., cocaine [43], isotretinoin [44], and used in the resolution of remaining solvents in the betamethasone valerate [45]. It is a very useful criterion for the analysis of pharmaceuticals too. Not long ago, GC by using a variety of detectors has been used for the assessment of the process-related pharmaceutical impurities [46].

9.7.2.3 High-Performance Thin-Layer Chromatography

Undertaking drug analysis seems to be much more comfortable than before due to the development of techniques like HPTLC. It consumes less time with more accuracy due to fast separation, as well as can examine a wide variety of samples. HPTLC is much easier to perform than many other techniques because it is easy to handle and less time required analyzing even the sophisticated and crude sample cleanup. HPTLC is not a time-limited technique and can evaluate the whole chromatogram with a kind of variable. Furthermore, results can be more reliable due to the nonconformist improvement of many samples and quality on each plate. HPTLC is also very useful to determine the number of drugs such as cyproterone, ethinyl estradiol [47], alfuzosin [48], tramadol, and pentazocine.

9.7.2.4 High-Performance Liquid Chromatography

HPLC is recognized as an innovative chromatographic technique mostly used in the chemical and biological structure to get a good understanding of the role of independent molecules by extracting the complex mixture of molecules [49]. HPLC methods were used for the evaluation of heavy drug constituents in 1980 for the first time [50]. HPLC method is particularly useful due to its high specificity and more precision level. The advantageous features of the HPLC method like specificity, precision, and accuracy largely depend on the condition, if widespread system fitness tests are performed before its analysis. Furthermore, the HPLC method is comparatively cost consuming than many other methods. Meanwhile, the HPLC method is still more broadly used among the chromatographic techniques. In liquid

chromatography, more expertise is needed to detect all the components used. For this purpose, a UV detector is mainly used in HPLC due to its capability to detect various wavelengths at one time, which can only be possible by the application of multiple wavelength scanning programs. For better detection of many UV-absorbing components simultaneously, an adequate quantity of UV detector is needed [51].

A photodiode arrangement (PDA) is an edge display of the detached photodiodes on a unified circuit (IC) piece for spectroscopic analysis. PDA is beneficial in spectroscopy, as it has recognized as a border arrangement of separate photodiodes on an integrated circuit (IC) chip. It can sense a wide range of wavelengths simultaneously by placing it at the resemblance strategy of a spectrometer. The application of a different wavelength detector (VWD) sample should be inserted many times with different wavelengths to ensure the detection of all possible peaks. PDA can be helpful in the identification of various compounds at one time that absorbs in its range due to its ability to develop a wavelength range. It can also examine high purity within a peak by going with spectra as well as applicable in pharmaceuticals in the method expansion of iloperidone. The refractive index detector is known to be a detector of choice because it can detect analytes like sugars, alcohols, carbohydrates, polymers, and fatty acids having low or no UV absorption. Better trace detection ability is protected via low noise. This detector is found to be having the least sensitivity as compared to other detectors but still useful for analyte with a high cluster. Lakshmi and Rajesh firstly served the refractive indication detector for voglibose content analysis in pharmaceutical products [52]. For better results of the electrochemical detector, a substance should have the oxidizable or reducible properties, and meanwhile, the chemical reaction occurring at the surface of the electrode results from an electrical output generated by triggering of electron flow. This detector has recently extended its application as being used to examine the quantity of glutathione in human lung adenocarcinoma cells and cancer [53]. Fluorescence detector is known to be the most tactful radar among the LC locater due to its very high responsiveness of about 10–1000 than UV radar for high UV absorbing materials considered advantageous for the measure of precise fluorescent species in samples. The estimation of pharmaceuticals is also considered as the backbone in fluorescence applications. For better consistency, analysis time, sensitivity, and repeatability mostly employ in UV absorbance detection use reversed-phase method. HPLC has been used for numerous drugs being assessed in biological fluids and pharmaceutical formulations, so it has resolved many problems faced in the pharmaceutical industry [54]. On the other hand, HPLC also has some limitations in use due to the high price of columns, solvents, and short-term reproduce ability pertaining to the exclusive nature of column packing.

Liquid chromatography (LC), together with the mass spectrometry (MS), has produced excellent results during the previous decay of the twentieth century. It has got significant importance in the pharmaceutical industry and is a method to choose for analytical support during different phases of quality assurance and quality control. HPLC-MS has also been employed for the evaluation of drugs. The use of HPLC in analyzing the drugs alone can also be applied in combination with the hyphenated technique to determine the impurities of the pharmaceuticals and degradation products [55].

9.7.2.5 Thin-Layer Chromatography

It is known to be an old technique but still got so much importance in various drug product analyses. In thin-layer chromatographic technique, an adsorbent, a solid phase is generally spread on aluminum, plastic, or glass (reliable support) as a thin layer. The effectiveness of chromatographic separation depends on several factors. In this chromatographic technique, the selectivity of adsorbent is significant as it should be highly selective toward the elements being separated as the differences in the elution rate be high. Adsorbent selection is also crucial in the separation of any kind of mixture as they can be a strong or weak adsorbing agent.

Thin-layer chromatography is being used widely for the checking of numerous inorganic and the organic materials due to the following advantages: minimal sample cleanup, huge sample loading range, multiple options of mobile phases, the pliability in sample difference, and less cost [56, 57]. It is also very useful and gained the primary importance for the screening of unidentified materials in bulk drugs as well as shows high degree certainty for the separation of required components of the drug. These properties of the TLC technique, along with its high specificity, extended its use to do quantitative analytical tests following spectrophotometric measurement. Thin-layer chromatography has also been used for the identification of few steroids [58], noscapine [59], pioglitazone [60], and celecoxib [61]. Usually, during the beginning process of drug growth, there is a lack of evidence about the degradation of products and impurities in drug products; TLC plays an essential role during that stage. TLC can also identify many different kinds of degradation products in pharmaceuticals [62, 63].

9.7.3 Spectroscopic Techniques

9.7.3.1 Spectrophotometry

On the bases of natural UV absorption and chemical reactions, spectrophotometric methods are groups of methodologies having a significant part in pharmacopeias. Generally, spectrophotometry can be stated as the measurement (quantitative) of transmission or reflection characteristics of a substance with varying wavelengths. These techniques are considered to be better than many others due to their property of less time and labor consumption, along with a high precision rate. A combination of UV-vis spectrophotometry has played an essential role in the examination of pharmaceutical dosage form during the past few years [64, 65].

Usually, colorimetric methods consisted of the following three parameters:

(i) A catalyze reaction
(ii) Compound formation reaction
(iii) Oxidation-reduction reaction

Colorimetric procedures have been employed permanently for the assessment of large materials, such as drug formulations (corticosteroids), which are usually determined by the blue tetrazolium assay [66]. This technique is also used for the

assessment of cardiac glycosides mentioned in the European Pharmacopeia. For qualitative analysis and assessment, the derivative of spectroscopy that uses the upper or first derivatives of absorbance following wavelength is being employed. Derivatizing the spectral data concept came out in the 1950s, offering many advantages. On the other hand, this technique could not get high consideration, especially due to the difficulty of creating derivative spectra using primary UV-visible spectrophotometers. In the late 1970s, the introduction of microcomputers made it comparatively easy to use mathematical methods for the generation of derivative spectra more rapidly and reproducibly, which increase the use of this technique. The derivative method has extended its uses beyond UV spectrophotometry to infrared, atomic absorption, fluorimetry, and fluorescence spectrometry. This method is not limited in its use but can also help solve problems in the quantitative study of standard spectra. Derivative methods have also shown some disadvantages, such as the discrepancy breaks down the signal-to-noise ratio, so more smoothing is needed in combination with discrepancy [67].

9.7.3.2 Near-Infrared Spectroscopy (NIRS)

NIRS is fast as well as a nondestructive technique that encompasses the testing of multiple constituents of almost any matrix. In the current era, NIRS has obtained a broader appreciation in pharmaceutical manufacturing industries for process monitoring, testing of raw materials, and product quality control. The increased pharmaceutical attention toward NIRS is perhaps due to its main advantages over other analytical procedures, like expectancy of physicochemical parameters of the sample from the single spectrum, and involves easy preparation of sample and likelihood of the separating sample measurement site through the usage of fiber optic probes. The main pharmacopeias have opted for NIR procedures. USP, as well as EP in chapter 2.2.40, mentions the appropriateness of the NIR technique for testing pharmaceutical samples. NIRS, together with multivariate data examination, unlocks numerous stimulating insights in medicinal testing, both quantitatively and qualitatively. Many research papers covering NIR quantitative analysis of active pharmaceutical contents in whole tablets have been published [68, 69]. Additionally, numerous review literatures have been issued, quoting the uses of NIRS in the testing of pharmaceuticals [70]. NIR has been extensively useful for traditional Chinese medicines (TCM) testing for both polar and nonpolar constituents. NIRS for TCM constituents, which are correlated to distinct complexes like saponins, carbohydrates, lipids, essential oils, phenolics, and alkaloids, is used [71]. For quantitative testing of an active ingredient in diverse manufacturing stages of solid dosage forms, the content of the drug is measured at two phases, i.e., after granulation as well as after tablet coating in the pharma industry [72]. NIRS is consuming a portable instrument (microNIR) connected with chemometrics mockups, hierarchical cluster analysis (HCA), and principal component analysis (PCA), and partial least squares (PLS) regression has been applied to determine cocaine as well as to segregate synthetic drugs through functional chemical configuration in 23 medicine samples,19 ecstasy tablets, and 22 seals of designer drugs [73]. NIR process was established, which allows determining olanzapine precisely as

well as accurately in commercial drug products with slight pretreatment of the sample [74].

9.7.3.3 Nuclear Magnetic Resonance Spectroscopy (NMRS)

Arena of NMR-based screening has progressed rapidly after the appearance of the first report in 1996, revealing the application of NMR spectroscopy in order to screen drug molecules [75]. Over the last few years, many advanced methods have been introduced and, also, extensive use of this technique in academic as well as pharmaceutical research [76, 77]. NMR spectroscopy has made its position in analytical testing in order to characterize the composition of the pharmaceutical products, analysis of impurities, and quantification of active ingredients present in biological fluids as well as in pharmaceutical formulations [78–80]. NMR has an essential part in drug discovery development, enabling it to develop as well as evolve its role constantly. NMR is likely to uphold this evolution over the upcoming 10 years with advancements in-cell imaging techniques, automation, speed of structure calculation, as well as the expansion of NMR amenable goals. NMR being adaptable is effective in elucidating high-affinity ligands for biological macromolecules, recognizing small molecules with wide-ranging binding affinity, elucidating ligand-binding sites, as well as it has a major role in structure-based drug designing, and therefore evidencing to be appreciated. Such NMR screening procedures have been built on target observation as well as ligand resonances presenting as detection tool for the identification of weak binding composites and also helping their progression into potent inhibitors (drug-like) for employing in drug discovery as a lead compound. NMR spectroscopy has developed into a significant method in the provision of structure-based drug designing. NMR plays a vital role in the stability analysis of cyclotide cysteine, ethylene-vinyl acetate (EVA) copolymer, β-galactosidase [81]. It is also useful in the determination of drug impurities and also for the quantitative analysis of drugs in biological fluids and pharmaceutical products [75, 78].

9.7.3.4 Phosphorimetry and Fluorimetry

The manufacturers of pharma manufacturing units always show interest in sensitive testing procedures consuming small sample size. Fluorescence spectrometry is a procedure that gives high sensitivity without losing precision as well as specificity. In the recent past, an increased amount of literature covering fluorimetry [82, 83] as well as phosphorimetry [84, 85] usage during quantitative measurements of several active pharmaceutical contents in drug products as well as in biological solutions, have been seen.

9.7.4 Electrochemical Methods

Over the last few years, electrochemical procedures are being used rapidly in the testing of pharmaceuticals. The attention toward electrochemical techniques can be accredited to have more sophistication in instrumentation and easily

comprehensible. Furthermore, numerous electroanalytical techniques are being presented for the pharmaceutical product's quantitative analysis. In order to quantify desipramine-imipramine, trimipramine, titanium, ambulate XAD-2, and dioxide nanoparticles, altered glossy carbon paste was established [86]. Adsorptive stripping differential pulse voltammetry, chronocoulometry, electrochemical impedance spectroscopy, and cyclic voltammetry were used to analyze the electrochemical response of these drugs. Capsaicin adapted carbon nanotube-altered basal plane pyrolytic graphite electrode, and p-chloranil-altered carbon paste electrodes have been introduced for quantification of lidocaine and benzocaine [87]. In order to determine norepinephrine, levodopa, adrenaline, and dopamine, copper (II) complex, as well as silver nanoparticle-altered glassy carbon paste electrode, was used [88]. The electrochemical response of such medications was considered employing adsorptive stripping square wave voltammetry, electrochemical impedance spectroscopy, and cyclic voltammetry and chronocoulometry techniques [89]. Electrochemical response by clioquinol having widespread clinical applications was analyzed by square wave voltammetry and cyclic differential pulse voltammetry at a wide range of pH employing a glassy carbon electrode. The adsorptive stripping differential pulse voltammetry technique was established for the estimation of venlafaxine as well as desvenlafaxine using a Nafion-carbon nanotube compound glossy carbon electrode. Carbon nanotube paste electrode altered at a precise place by Triton X100 was established for separation as well as the concurrent identification of caffeine, aspirin, and acetaminophen. In order to quantify the sub-nanomolar concentration of bismuth, an electrochemical procedure based on potentiometric stripping testing using cryptand as well as carbon nanotube-altered paste electrode has been proposed. An innovative technique, capillary electrophoresis (CE) along with a perimetric detector, has been developed for quick as well as the practical determination of benserazide and levodopa along with its impurity in co-beneldopa pharmaceutical preparations [90]. Electrical methods have been used for quantitative measurement of desipramine imipramine, trimipramine, lidocaine, and benzocaine, levodopa, norepinephrine, epinephrine, and dopamine as well as for effective determination of benserazide and levodopa and its contamination (R, S)-2-amino-3-hydroxy propanohydrazide in co-beneldopa therapeutic preparations [87–90].

9.7.5 Kinetic Method of Analysis (KMA)

This procedure of testing has been emerging since the 1950s; additionally, until now, this is winning the main revival inactivity. The monotonous attention in these procedures can be attributed to the developments made in principles, in comprehending the chemical as well as instrumentation, in investigational data approaches, and in the testing application and automated instrumentation. It is evident from the literature that the kinetic method of analysis is somewhat general, having many benefits over the conventional equilibrium method. Fundamentally, kinetic procedures involve a determination of amount changes (noticed by

alterations in signals) in a reactant with time after mixing of reagents and samples mechanically or manually [91].

It is confirmed from the literature that initial rate and fixed-time methods have been applied frequently for the determination of drugs in dosage forms. Automatic approaches for KMA are usually based on open systems. Stopped flow systems, as well as the continuous addition of reagent (CAR) techniques, are among the standard techniques [92]. Many drugs have been measured with the help of the CAR technique, having photometric as well as the fluorometric detection system. In order to make analytical reactions quicker, catalysts can be used as they are feasible with both equilibrium estimations and reaction rate. Now the application of micellar media in the KMA is appreciated in order to augment the rate of reaction via micellar catalysis. Besides, it may enhance the selectivity as well as sensitivity, which in turn decreases the time for analysis of the desired active ingredient.

Multicomponent determinations by a kinetic method of analysis, most often stated as differential rate procedures, are getting full attention in the pharmaceutical exploration area. In order to deal with the component's overlapping spectra in binary mixtures, two novel methods, i.e., H-point standard addition method as well as kinetic wavelength pair method, have been recommended [48].

9.7.6 Electrophoretic Procedures

Capillary electrophoresis is another significant instrument vital for the testing of pharmaceutical formulations. CE is a comparatively innovative procedure having the basis of segregation of analytes carrying charge by little capillary underneath the influence of the electric field. In a procedure, solute particles are considered as peaks as these analytes pass through the detector, while the specific peak area is directly proportional to their amounts that permit quantification. Electrophoretic procedures are also used for the testing of biopolymer as well as inorganic ions. Capillary electrophoresis testing is usually more efficient, takes less time for analysis, lesser up to nanoliter injection volumes, involves only a small quantity, and, in many instances, happens in aqueous mediums [93]. These four features of this technique have confirmed to be valuable to many pharmaceutical uses. Numerous reports have seemed on the usage of this procedure in the testing of the drugs. Many approaches of capillary electrophoresis such as micellar electrokinetic chromatography electrophoresis, capillary gel, isoelectric focusing, affinity capillary electrophoresis, isotachophoresis, and capillary zone electrophoresis were established and used for pharmaceutical purity analysis as well as in bio testing of pharmaceuticals [94, 95].

9.7.7 Flow Injection Analysis/Sequential Injection Analysis (FIA and SIA)

In the second half of the 20th era, laboratory automation was presented. Ruzicka and Hansen in Denmark, as well as Steward in the USA, made the FIA method for

automation of the chemical process [96, 97]. This procedure emerged to alter the beginning of automation in the chemical testing by allowing instrumental quantification in the absenteeism of chemical as well as physical equilibrium. FIA method's principle is the inoculation of a fluid sample into a non-segmented, flowing, and continuous carrier stream of an appropriate fluid. The inoculated sample makes a zone moving to a detector, which analyses fluctuations in the electrode potential, absorbance, and additional physical restriction occurring bypassing analyte into the flow cell [98, 99]. Flow injection testing steps can be seen in Fig. 9.1.

After the extensive use of computers in the lab, the second-generation flow testing was presented by [100], who named it SIA. Although it is the same as flow injection analysis, being a non-segmented continuous flow process founded on the similar basic concept of controlled dispersion as well as reproducible operation of FIA discernment, its working method includes the basis of programmable flow theory.

FIA method has hired a noteworthy input for the automation development in pharma testing, and its benefits are fully recognized in a specialized monograph [101] and numerous review articles [102]. The SIA technique has made the scientific community to take an interest in automation in the pharmaceutical testing domain. Several articles are published regarding pharmaceutical testing, comprising two review articles using sequential injection testing techniques to extensive diversity of matrices, like solid matrices, i.e., liquids (solution, suspensions, emulsions) and pastes (creams, ointments), as well as encompassing several entities having different healing properties. By profiting from the benefits in the elevated sampling rates as well as the economy of reagents, the major uses are devoted to the quantification of API for quality control in medicinal preparations [103].

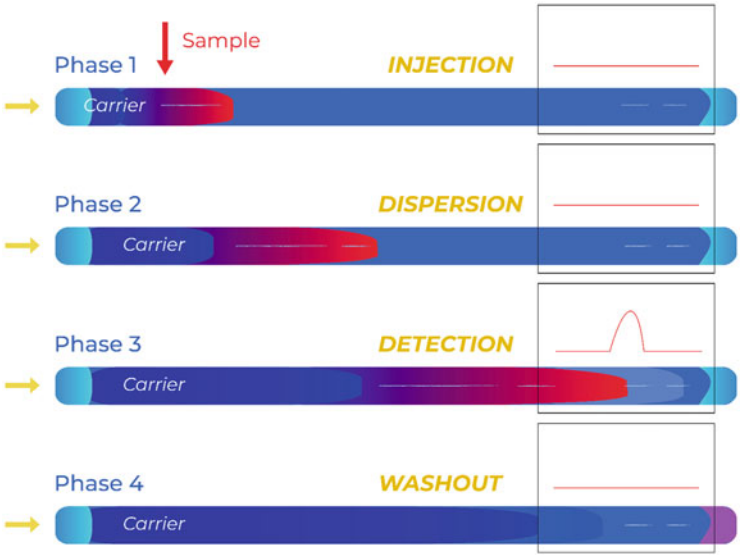

Fig. 9.1 Different steps of flow injection analysis. (Adapted from Refs. [98, 99])

9.7.8 Hyphenated Techniques

Hyphenated techniques develop from the linkage of separation technique with online separation technique. Previous 20 years observed a notable development in the hyphenated methods and their use in medicinal testing. A wide range of hyphenated techniques like GC-MS, CE-ICP-MS, LC-NMR, CE-MS, and LC-MS [104] has been used for testing of pharmaceutical products [105]. The measurement of medicinal entities in biological fluids is a significant phase in drug discovery as well as drug development. High-performance liquid chromatography with different types of detectors like PDA mass spectrometry and fluorescence has become the method of choice for testing pharmaceuticals products. A review of high-performance liquid chromatography with PDA or MS/MS detector is reported for testing meloxicam in biological samples as well as medicinal products. For qualitative as well as quantitative testing of metabolites of *Zuojinwan Rhizome* and *copies* formulation; after oral intake in the rat urine, a liquid chromatography along electrospray ionization mass spectrometry has been established. The same testing method was used for the instantaneous quantification of acetylsalicylic acid as well as L-ascorbic acid in aspirin C effervescent tablets [106]. C_{18} column is used for the separation of urine samples with the help of a mobile phase containing water and acetonitrile having formic acid (0.1%) in the ratio of 70:30. Abuse of recreational drugs is a rising problem, and innovative materials are commonly noticed in forensic and clinical samples. Diphenyl-2-pyrrolidinemethanol is one of such items. So, this and its biologically degraded products in rat urine have been identified with the help of GC-MS and LCMS. Trials were made to detect the existence of humanly used medicinal entities in the aquatic areas of Malaysia territory. Required aquatic testing samples are assembled from diverse locations along Langat River as well as effluents from 05 sewerage treatment plants after solid-phase extraction and tested with the help of LC having a tandem mass spectrometry detection system [107]. This analytical investigation has proved the existence of glibenclamide, salicylic acid, and mefenamic acid in all the river water samples. Drug-drug interaction of clopidogrel, as well as rabeprazole in a healthy Chinese population, has been investigated. After oral intake, the amount of rabeprazole, as well as clopidogrel blood, has been analyzed by using liquid chromatography linked with mass spectrophotometry. A new liquid chromatography linked with the mass spectrophotometry-MS technique was established for the identification of bacterial isolates' carbapenemase response. Liquid chromatography-tandem mass spectrometry was used to evaluate the distinctive pharmacokinetic parameters of the individual analyte like caffeine, midazolam, warfarin, omeprazole, and metoprolol [108].

9.7.9 Thermal Analytical Techniques

The existing arena of thermal exploration is both diverse and dynamic. Theoretically, entire thermal analytical methods evaluate the alterations of a particular attribute of material with a varying temperature that leads scientists to have

Table 9.3 Thermal analytical methods and measured properties

Sr.#	Analytical technique	Measured property
1	Dynamic mechanical analysis (DMA)	Deformation
2	Evolved gas analysis	Gaseous decomposition
3	Dielectric thermal analysis (DEA)	Deformation
4	Thermogravimetric analysis (TGA)	Mass
5	Differential scanning calorimetry (DSC)	Enthalpy
6	Differential thermal analysis (DTA)	Temperature difference
7	Thermo-optical analysis (TOA)	Optical properties

information regarding macroscopic concepts of matter like irreversible kinetics and thermodynamics as well as equilibrium. Merely a few practices are commonly used in the pharmaceutical sciences. These involve differential scanning calorimetry (DSC, differential thermal analysis (DTA), dynamic mechanical analysis (DMA), and thermogravimetric analysis (TGA). Table 9.3 describes the properties measured by several thermal analytical techniques [109, 110].

9.7.9.1 Differential Scanning Calorimetry (DSC)

The conception of DSC was formerly originated from earlier DTA apparatuses. The primary difference between DSC and DTA is that theoretically, DSC measures enthalpy change, while DTA determines a difference in temperature of reference as well as a sample [111, 112]. The International Confederation for Thermal Analysis and Calorimetry (ICTAC) explains that DSC is an analytical technique for measuring heat flow rate change between reference material the sample. Commercially two major categories of DSC apparatuses exist, i.e., power compensation DSC (pc-DSC) and heat flux DSC (hf-DSC), originally denoted to as quantitative DTA. At this time, there is another significant difference that no common method is available for presenting DSC data. Based on the apparatus being employed, the default settings could show exothermic measures in a downward direction while endothermic measures in an upward direction [113].

9.7.9.2 Thermogravimetric Analysis (TGA)

TGA is a procedure in which a difference in mass is detected and determines the physicochemical processes that happen upon heating the test sample. Simplified TGA apparatuses design consists of an accurate analytical balance attached with a sample pan, suspended within a heater, which in turn controlled by computer. TGA results like DSC may differ prominently based on test material as well as experimental [114, 115]. Thermogravimetric analysis is precious for studying numerous kinetic procedures of solids/liquids as far as the methods encompass mass loss. This can be attained by the use of accurate balances, also including accurate control of rates of heating and cooling and atmospheric circumstances. Additional common uses in pharmaceutical sciences involve hydrate characterization comprising an evaluation of decomposition, sublimation temperatures, or vaporization and desolvation procedure. Though most thermal analytical techniques involve the

characterization of samples as solids, semi-solids, or liquids, common applications encompass:

- Characterization of the physicochemical attributes of crystalline solids.
- Identification as well as detection of polymorphism.
- Due to increased utilization of solid dispersions as well as other polymeric formulations, thermal analytical techniques are employed more frequently for development and characterization.
- To study the effects of lyophilization as well as to develop optimal lyophilization dosage forms and cycles [116].

9.8 Conclusion

The key purpose of the pharmaceuticals is to assist the human so that they can avoid possible ailment or can prevent the disease. For achieving this anticipated purpose of medicines, they must be free from all types of impurities and other factors that may cause any harm to humans. In this chapter, our main target was to discuss drug stability and which type of different analytical techniques could be utilized for the assessment of stability and quality of drug substance. Various analytical techniques for the assay of drugs are discussed with detailed instrumentation involved. This chapter also highlighted the applications of these analytical techniques and their development from the old trimetric method to the latest hyphenated technique.

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