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"Advancement in Hyphenated Techniques: LC-MS and Beyond in Pharmaceutical Quality Control"

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Abstract A paradigm shift in human health was ushered in with the advent of drugs. Only when delivered correctly and devoid of contaminants will these medications accomplish their goals. Over time, several chemical and instrumental approaches have been developed to estimate medications, allowing them to perform their intended function. It is necessary to identify and quantify these pharmaceutical impurities since they may occur at any point in the development, transit, or storage processes, making the pharmaceutical unsafe to administer. Analytical tools and techniques are crucial for this. Analytical instruments and methodologies are discussed in this study as they pertain to medication quality assessment. This study focuses on the methodologies and techniques used in the examination of medicines, including titrimetric, chromatographic, spectroscopic, electrophoretic, and electrochemical approaches.

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1. Introduction

Pharmaceutical research has historically been crucial in the advancement of pharmaceutical development, guided by pharmacology and clinical sciences and propelled by chemistry. New pharmaceuticals are now developed via the exchange of ideas between biologists and chemists, rather than only relying on the imagination of chemists, thanks to the standard established by chemistry, pharmacology, microbiology, and biochemistry in the drug discovery process. An innovative medicinal molecule with shown therapeutic potential to fight, control, monitor, or cure illnesses is the starting point of the drug development process. Preliminary safety and therapeutic efficacy data must be generated from the synthesis and characterization of these molecules, which are known as active pharmaceutical ingredients (APIs), before drug candidates can be identified for more in-depth investigations (Valagaleti et al., 2003). An understanding of the disease's underlying cause, the information regarding the altered genes responsible for

the disease, the ways in which proteins interact with affected cells, and the changes brought about by these cells are the foundation of pre-drug discovery investigations. Using these details as a guide, a molecule is created that binds to the diseased cells and may one day become a medicine. The process of drug discovery and development, including the identification of active pharmaceutical ingredients (A.P.I.s) (http://www.phrma.org/sites/default/files/159/rd_brochure_022307.pdf).



The "compound" that will eventually become the drug molecule goes through a battery of tests to ensure it is safe to use, gets into the bloodstream, gets to where it needs to be, gets metabolized enough, and doesn't cause any harm. Animal testing for kinetics, toxicity, and carcinogenicity follows preclinical research, which includes in vitro experiments, once the substance is completed. Regulatory agencies authorize clinical studies once a product passes pre-clinical testing. The first two phases of clinical trials determine the drug's optimal dosage and schedule, while the third and fourth phases provide statistically significant data about the drug's effectiveness, safety, and overall benefit-risk association. During this phase, the drug's long-term efficacy is monitored and any possible interactions with other drugs are identified. Pharmaceuticals are made available to the public after clinical studies have shown no adverse effects. Tabulated in Table 1 are the key points from each phase of clinical studies. Chemical separation and asymmetric synthesis also paved the way for the development of single-enantiomer pharmaceuticals.



Table 1 Summary of phasewise clinical trial and motive of investigation.

Phase of clinical trail	Number and type of subjects	Investigation
Phase 1	50–200 healthy subjects (usually) or patients who are not expected to benefit from the IMP	Is the IMP safe in humans? What does the body do to the IMP? (pharmacokinetics) What does the IMP do to the body? (pharmacodynamics) Will the IMP work in patients?
Phase 2	100–400 patients with the target disease	Is the IMP* safe in patients?
Phase 3	1000–5000 patients with the target disease	Does the IMP seem to work in patients?
Phase 4	Many thousands or millions of patients with the target disease	Is the IMP really safe in patients? Does the IMP really work in patients Just how safe is the new medicine? (pharmacovigilance) How does the new medicine compare with similar medicines?

Source: guidelines in clinical trials: 2007 edition. The Association of the British Pharmaceutical Industry, 12 Whitehall London.

* IMP: investigational medicinal product i.e. the newly developed drug.

techniques. For pharmaceutical companies, the creation of single enantiomer medications has been supported by many published recommendations pertaining to chiral pharmaceuticals (FDA's, 1992; Health Canada, 2000; European Medicines Agency, 1996). The International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) established guidelines for the quality of chiral medicines (ICH Topic Q 6 A, 1999). According to the rule, applicants should treat the presence of one enantiomer as an impurity and implement identification tests that can differentiate between the two enantiomers as well as the racemic combination. In order to guarantee safe and adequate production processes, it is necessary to provide resources for effective quality systems (ICH, 2009). The products may have surface imperfections due to inadequate in-process control (Akseli et al., 2008; Varghese and Cetinkaya, 2007). Moreover, there is a possibility that the final goods will include particles of unknown alien stuff. In order to stop additional contamination, the foreign matter has to be detected and its source needs to be determined. As a result, analytical methods must be made available for the effective detection and identification of foreign materials in dosage forms (Pajander et al., 2013). Drugs on the market could come in a variety of dose formats. One way to classify formulations is by their delivery routes (Pifferi et al., 1999). The scientific basis for developing a formulation and the reasoning for an appropriate dose form are both provided by pharmaceutical development information. The standards for the data sets linked to pharmaceutical research are not fully specified in regulatory advice (U.S. Department of Health, 2003). more specific data is accessible for the evaluation of excipients' toxicological effects (US Department of Health, 2002). The bulk of solid dosage forms are excipients, which dilute the medication

component so that tablets of the right size may be formulated and which cover the tablet to prevent it from smelling or tasting bad. Physical and chemical interactions with excipients may speed up solid-state reactions in dosage forms that occur when the medication ingredient is reactive. Excipients may aid in the breakdown of medicinal substances even when they do not interact chemically (Bryn et al., 2001). Serajuddin et al. (1999) and Wirth et al. (1998) found that glycosylamines may be formed when primary and secondary amines react with lactose, glucose, and maltose.

Pharmaceutical research relies heavily on analytical investigations of biological samples containing pharmaceuticals and their metabolites, as well as on bulk pharmaceutical materials, intermediates, products, formulations, contaminants, and degradation products. With the goal of characterizing the quality of bulk medicinal materials by defining limitations of their active ingredient concentration, analytical test techniques have been included in the compendial monographs from the start of formal pharmaceutical analysis. Recent monographs have included electro analytical techniques in addition to titrimetry, spectrometry, chromatography, and capillary electrophoresis assays. Data from the most recent editions of the European (The European Pharmacopoeia and Council of Europe, 2002) and United States (United States Pharmacopoeia, 2004) pharmacopoeias are used to reproduce the current state-of-the-art in Table 2.

Analytical procedures are crucial throughout the whole medication life cycle, from discovery to commercialization and beyond. Whether it's determining the medicine's physical and chemical stability,



Table 2 Proportion of various analytical methods prescribed for the assay of bulk drug materials in Ph. Eur. 4 and USP XXVII.

Non-aqueous	36.5	24
Indicator	9.5	14
Potentiometric	27	10
Redox (Iodometry, Nitritometry, etc.)	6.5	5.5
Other (complexometry, argentometry, etc.)	5.5	5.5
UV-vis spectrophotometry	9.5	8.5

Source: S. Gorog/Journal of Pharmaceutical and Biomedical Analysis 36 (2005) 931–937.

Method	Ph. Eur. 4 (%)	USP 27 (%)
HPLC	15.5	44
GC	2	2.5
Titration	69.5	40.5
Acid–base	57.5	29.5
Aqueous mixtures	21	5.5
Indicator	6.5	4.5
Potentiometric	14.5	1
Microbiological assay (antibiotics)	3	2.5
Other (IR, NMR, polarimetry, fluorimetry, atomic absorption spectroscopy, polarography, gravimetry etc.)	0.5	2

influence the choice and development of the dosage form, evaluate the stability of the drug molecules, quantify the impurities, identify those that exceed the established threshold, assess their toxicity profiles, differentiate them from the active pharmaceutical ingredient (API) when relevant, and evaluate the drug content in the marketed products. In pharmacokinetic investigations, the medication and its metabolite are analyzed using quantitative or qualitative methods. The analytical methods and techniques used in the analysis of medicines are discussed in this overview.

2. Analytical techniques

2.1. Titrimetric techniques

The titrimetric analytical technique has its roots in the mid-eighteenth century. Titration may be traced back to the



volumetric approach, which was developed by Gay-Lussac in 1835. There have been some attempts to update the assay method, such as using non-aqueous titration methods, applying titrimetric methods to (extremely) weak acids and bases, and improving the methods' accuracy through potentiometric end point detection. Titrimetric techniques have proven useful in kinetic measurements, which are used to determine reaction rates, thanks to the introduction of functional group analysis processes. Time and labor savings, increased accuracy, and the elimination of reference standards are just a few of the many benefits linked with these technologies. Previous studies have used titrimetric techniques to determine albendazole (Basavaiah and Rahman, 2005a), captopril (Rahman et al., 2005a), and

Table 3 Chromatographic adsorbents: (approximate order is shown in the table, since it depends upon the substance being adsorbed, and the solvent used for elution).

Most strong adsorbent	Alumina	Al ₂
	Charcoal	O ₃
	Florisil	C MgO/SiO ₂ (anhydrous)
Least strong adsorbent	Silica gel	SiO ₂

Source:

<http://www.chem.wisc.edu/courses/342/Fall2004/TLC.pdf>.

Prameela, 2003) and gabapentin (Sameer and Abdulrahman Basavaiah, 2011) in commercial dosage forms. Sparfloxacin (Marona and Schapoval, 2001) was determined by the non- aqueous titration method. In addition to its application in drug estimation titrimetry has been used in the past for the estimation of degradation products of the pharmaceuticals (Matei et al., 2008).

2.2. Chromatographic techniques

2.2.1. Thin layer chromatography

Although an old technique yet it finds a lot of application in the field of pharmaceutical analysis. In thin layer chromatography, a thin coating of the solid phase, the adsorbent, is applied on a solid support, often made of glass, plastic, or aluminum. The success of this chromatographic separation method depends on a number of things. In order for there to be significant differences in the rate of elution, the adsorbent must first exhibit very high selectivity for the chemicals that are to be separated. The adsorption strengths of some adsorbents may be too high or too low to effectively separate all mixtures. Several adsorbents are listed in Table 3 according to their adsorptive ability. The unique benefits of thin layer chromatography—including its cheap cost, high sample loading capacity, flexibility in sample separation, broad range of mobile phases, and little sample clean-up—make it a preferred method for the examination of a wide variety of organic and inorganic compounds. For the purpose of screening unknown substances in bulk narcotics, TLC is an effective method (Szepesi and Nyiredy, 1996). As a result, you may be quite certain that the drug's likely constituents are isolated. Utilizing spot elution and spectrophotometric measurement, the excellent

specificity of TLC has been put to use for quantitative analytical purposes. Some steroids, such as pioglitazone, celecoxib, and nescapine, have been determined by TLC (Cimpoi et al., 2006; Gumieniczek et al., 2004; Be- bawy et al., 2002; Ashour et al., 2009). When data regarding drug substance and drug product impurities and degradation products is lacking, thin-layer chromatography (TLC) becomes an indispensable tool in the early stages of drug development (White et al., 1992; Agbaba et al., 1996).

2.2.2. High performance thin layer chromatography High performance thin layer chromatography (HPTLC) has become an indispensable tool for drug analysis as the method has progressed. High-performance thin-layer chromatography (HPTLC) is adaptable enough to examine a broad range of samples swiftly. Because it is easy to use and takes little time to evaluate, this method has several benefits.

difficult or the undeveloped sample preparation. High-performance thin-layer chromatography (HPTLC) analyses the whole chromatogram with many parameters indefinitely. Results are also more reliable



since several samples and standards are developed independently on each plate at the same time. Drugs such as ethinyl estradiol and cyproterone have been quantified using high-performance thin-layer chromatography (Pavic et al., 2003; Fayed et al., 2006; Ebrahim et al., 2011).

2.2.3. Hydraulic peritoneal chromatography

High-performance liquid chromatography (HPLC) is a sophisticated method of separating molecules that helps to better understand the function of individual molecules in chemical and biological systems. According to the United States Pharmacopoeia (1980), the first procedures for the analysis of bulk medicinal materials using high-performance liquid chromatography (HPLC) were published in 1980. In USP XXVII (United States Pharmacopoeia, 2004) and, to a lesser degree but still among the most often employed ways in Ph. Eur. 4 (The European Pharmacopoeia and Council of Europe, 2002), this has emerged as the dominant approach, as seen in Table 2. It is possible to achieve enough accuracy while taking advantage of the high specificity of the HPLC approach. It must be noted, however, that comprehensive system suitability testing must be conducted prior to HPLC analysis in order to achieve the remarkable specificity, precision, and accuracy. Consequently, the cost of very specificity, precision, and accuracy must be considered.

Also, it's somewhat racy.

Among the chromatographic procedures, HPLC has been the most extensively utilized technology, according to the literature review. To ensure that all components are identified in liquid chromatography, the detection technique chosen is crucial. A UV detector, which can scan numerous wavelengths at once, is one of the most used detectors in high-performance liquid chromatography (HPLC). The presence of sufficient amounts of UV-absorbing components guarantees that they will all be recognized by the UV detector.

For spectroscopy, an integrated circuit (IC) chip containing a lined array of individual photodiodes is called a photodiode array, or PDA. It allows a spectrometer to perceive a variety of wavelengths simultaneously by being positioned at the picture plane of the instrument. To ensure that all peaks are detected when using a variable wavelength detector (VWD), a sample has to be injected many times with different wavelengths. When using PDA, a certain wavelength range may be programmed, allowing for the simultaneous identification of all chemicals that absorb within that range. By comparing spectra that fall within a peak, a PDA detector may determine the peak's purity. According to Devi Manjula and Ravi

(2012), the PDA detector is used in the pharmaceutical industry for the development of iloperidone methods.

The analytes that have little or no absorption of ultraviolet light, including polymers, sugars, carbohydrates, fatty acids, and alcohols, are best detected with the refractive index detector. Maintaining a low noise level ensures adequate trace detection capability. Although it works well at high analyte concentrations, this detector has the lowest sensitivity of any of them. For their 2010 study, Lakshmi and Rajesh analyzed the voligibose content of pharmaceutical formulations using the refractive index detector. When exposed to oxidizable or reducible compounds, the electrochemical detector will provide an electrical output as a consequence of an electron flow caused by a chemical reaction that occurs at the layer that makes up the electrode. Recent work by Spadaro et al. (2011) examined the glutathione concentration of human prostate cancer cells and lung adenocarcinoma cells using this detector.

Among LC detectors, fluorescence detectors are among the most sensitive. For highly UV-absorbing materials, its sensitivity is sometimes 10-1000 times greater than that of the UV detector, giving it a leg up when measuring certain fluorescent species in samples. Among fluorescence's many useful uses, drug estimate stands out (Ulu and Tuncel, 2012). Figure 1 shows a comparison of the many kinds of detectors used in HPLC.

Due to its superior dependability, analysis time, reproducibility, and sensitivity, the reversed-phase mode with UV absorbance detection was the method of choice for most workers for a while. Numerous pharmaceutical formulations and biological fluids have been tested for the presence of various medications using high-performance liquid chromatography (HPLC) (Siddiqui et al., 2010; Tang et al., 2012; Devika et al., 2012; Ahmed et al., 2012; Tariq et al., 2010; Sama-nidou et al., 2012; Malenovic'et al., 2012; Giorgi et al., 2012). So, high-performance liquid chromatography (HPLC) is quite useful for the pharmaceutical sector since it answers a lot of problems. On the other hand, HPLC has its drawbacks, such as the high cost of columns and solvents and the fact that the proprietary nature of column packing makes long-term repeatability impossible. One of the most groundbreaking methods developed in the latter half of the twentieth century was liquid chromatography-mass spectrometry, or LC-MS (Niessen, 1999). It was the analytical tool of choice for various phases of pharmaceutical quality control and assurance, according to Ermer (1998) and Nicolas and Scholz (1998). Hilhorst et al. (2011), D'Avolio et al. (2010), Berset et al. (2010), Ding et al.



(2006), Wren and Tchelitcheff (2006), Zhou et al. (2011), and Breaud et al. (2009) are among the recent publications that have used HPLC-MS for drug assays. Not to mention HPLC has been used in pharmaceutical and degradation product analyses (Chitturi et al., 2011; Madireddy et al., 2011; Navaneeswari and Reddy, 2011; Thomas et al., 2012; Verbeken et al., 2011; Sabry et al., 2012; Hanysova et al., 2005; Bouchonnet et al., 2012). On its own, and in conjunction with the hyphenated technique, HPLC has been used to analyze pharmaceutical impurities.

2.2.4 Gas chromatography

Next, we have gas chromatography, which is an effective separation method for the detection of volatile organic substances and another chromatographic technique. By integrating online detection with separation, complicated mixtures may be accurately quantitatively determined, down to parts per trillions in certain instances, even incorporating minuscule amounts of chemicals. When it comes to

analyzing pharmaceutical products, gas liquid chromatography is king (Watson, 1999). This method is limited to producing polypeptides or antibiotics with a high molecular mass that are not stable enough for medical use. Because of the relative inertness of the medicinal compounds, derivatization is practically mandatory, which is its primary limitation. Isotretinoin (Lima et al., 2005), cocaine (Zuo et al., 2004), and residual solvents in betamethasone valerate (Somuramasami et al., 2011) are some of the medicines that have recently been utilized for analysis using gas chromatography. Analyzing pharmaceutical contaminants is another key use of gas chromatography. The use of gas chromatography (GC) has increased in the pharmaceutical industry in recent years for the purpose of estimating process-related impurities (Frost et al., 2003; Hiriyan and Basavaiah, 2008). GC is employed by Reddy and Reddy (2009), Hashimoto et al. (2001), Saraji et al. (2012), and Deconinck et al. (2012) to analyze residual solvents that are listed as impurities by the International Conference of Harmonization.

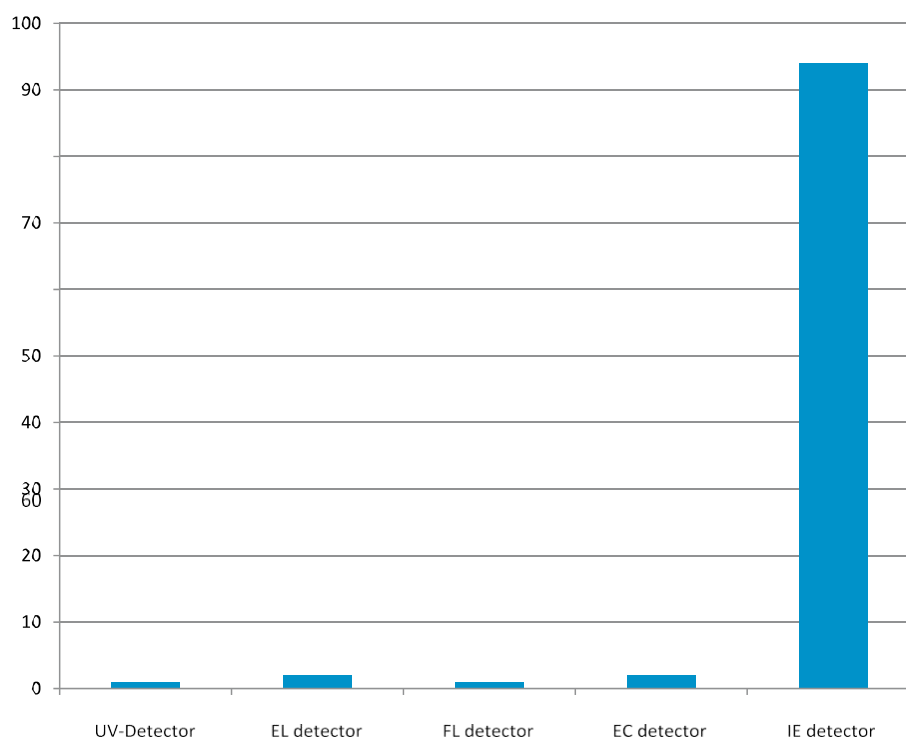


Figure 1 Usage of different detectors for HPLC analysis of drugs, Scale 0–100 represents use of the detector percentage. Source: R.N. Rao, V. Nagaraju. J. Pharm. Biomed. Anal. 2003, 33, 335–377.



2.3. Spectroscopic techniques

2.3.1. Spectrophotometry

Pharmacopoeias also make extensive use of spectrophotometric techniques, which rely on chemical processes and natural UV absorption (Gorog, 1995). The quantitative measurement of a material's reflection or transmission qualities as a function of wavelength is known as spectrophotometry. These approaches are advantageous since they require little effort and time to implement. Additionally, these procedures are quite precise. The examination of pharmacological dosage forms has seen a significant uptick in the usage of UV-Vis spectrophotometry in recent years (Tella et al., 2010; Venugopal and Sahi, 2005; Sharma et al., 2008; Ieggli et al., 2005). The following are often the cornerstones of colorimetric methods:

- Complex-formation reaction.
- Oxidation-reduction process.
- A catalytic effect.

It should be noted that colorimetric techniques are often used for the evaluation of bulk materials. Gorog and Szasz (1978) and Gorog (1983) both state that the blue tetrazolium test is useful for determining the formulations of corticosteroid drugs. Another use of the colorimetric approach is in the detection

Table 4 Quantitative analysis of drugs in pharmaceutical formulations by UV-visible spectrophotometric procedures.

Reagent used	Name of drug	$k_{\max}$	Reference
m-Cresol	Acetaminophen	640	Qureshi et al. (1992)
p-Chloranilic acid	Quetiapine fumarate	520	Vinay and Revenasiddappa (2012)
	Milrinone	519	Siddiqui et al. (2009)
2,3-Dichloro 5,6-dicyano-1,4-benzoquinone	Duloxetine	477	Toker and O'nal (2012)
	Amlodipine besylate	580	Rahman and Hoda (2003)
Chloranil	Dutasteride	525	Kumar et al. (2012)
	Lisinopril	520	Rahman et al. (2007)
7,7,7,8-Tetracyanoquinodimethane	Lisinopril	743	Rahman et al. (2005b)
	Alendronate sodium	840	Raza and Haq (2011)
Iodine	Flunarizine dihydrochloride	380	El Walily et al. (1995)
	Aripiprazole	400	Helmy et al. (2012)
Potassium iodide and potassium iodate	Irbesartan	352	Rahman et al. (2006a)
Ninhydrin	Pregabalin	402.6	Bali and Gaur (2011)
Ascorbic acid	Lisinopril	530	Rahman et al. (2005c)
Folin ciocalteu phenol	Oxcarbazepine	760	Gandhimathi and Ravi (2008)
	Ampicillin, amoxycillin, and carbenicillin	750, 770, 750	Ahmad et al. (2004)



European Pharmacopoeia presents the mination of cardiac glycosides. Table 4 outlines many published methodologies to spectrophotometry-based active pharmaceutical component assessment in bulk drugs and formulations.

Qualitative investigation and estimate are achieved via the use of first or higher derivatives of absorbance with respect to wavelength in derivative spectroscopy. Derivatizing spectral data was first proposed in the 1950s and shown to have several benefits. The method was underappreciated, nonetheless, since early UV-Visible spectrophotometers made derivative spectra a daunting task. With the advent of microcomputers in the late 1970s, it became mathematically feasible to produce derivative spectra in a short amount of time with little effort and good reproducibility. The utilization of the derivative approach was greatly enhanced as a result of this. In addition to ultraviolet (UV) spectrophotometry, the derivative approach has been used in infrared (McWilliams, 1969), atomic absorption (Snelleman et al., 1970; Konstantianos et al., 1994), and fluorimetry (O'Haver, 1976; John and Soutar, 1976) spectrometry. Derivative spectrometry is useful in general and may be helpful when normal spectra cannot be quantitatively studied. Derivative techniques also have their drawbacks; for example, the signal-to-noise ratio is reduced by the differential, which means that smoothing is needed in addition to differentiation (O'Haver and Begley, 1961).

Tris buffer	Diclofenac sodium	284, 305	Kramancheva et al. (1997)
Sodium metavanadate	Diltiazem HCl	750	Rahman and Azmi (2000)
Bromothymol blue	Rasagiline mesylate	414	Chennaiah et al. (2011)
Bromophenol blue	Rasagiline mesylate	414	Chennaiah et al. (2011)
Bromocresol green	Rasagiline mesylate	414	Chennaiah et al. (2011)
Potassium permanganate in alkaline medium	Isatin	60	AlOthman et al. (2013)
Brucine-sulfanilic acid in H ₂ SO ₄ medium	Nicorandil	410	Rahman et al. (2004)
3-Methyl-2-benzothiazoline	Nicorandil	560	Rahman et al. (2004)
Cu (II) & eosin	Carbinoxamine	538	Ramadan and Mandil (2006)
Potassium ferricyanide and ammonium ferric sulfate	Pantoprazole sodium	725	Rahman et al. (2006b)
Chloramin T	Zidovudine	520	Basavaiah and Anil Kumar, 2007
	Verapamil HCl	425	Rahman and Hoda (2002)

2.3.2. Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of almost any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques. The European Pharmacopoeia in chapter 2.2.40 ([The European Pharmacopoeia and Council of Europe, 2002](#)) and United States pharmacopoeias (chapter 1119 [United States Pharmacopoeia USP 26 NF 21, 2003](#)) address the suitability of NIR instrumentation for application in pharmaceutical testing. NIR spectroscopy in combination with multivariate data analysis opens many interesting perceptions in pharmaceutical analysis, both qualitatively and quantitatively. A number of publications describing quantitative NIR measurements of active ingredient in intact tablets have been reported ([Moffat et al., 2000](#); [Alvarenga et al., 2008](#); [Thosar et al., 2001](#); [Ramirez et al., 2001](#); [Blanco et al., 1996, 1999, 2000](#); [Li et al., 2003](#); [Molt et al., 1996](#); [Buchanan et al., 1996](#); [Merckle and Kovar, 1998](#); [Eustaquino et al., 1998](#); [Traford et al., 1999](#); [Corti et al., 1999](#); [Chen et al., 2001](#)). In addition to the research articles many review articles have been published citing the application of the NIRS in pharmaceutical analysis ([Luybaert et al., 2007](#); [Blanco et al., 1998](#)).

2.3.3. Nuclear magnetic resonance spectroscopy (NMR)

Since the first report appeared in 1996 ([Shuker et al., 1996](#)) describing the use of NMR spectroscopy to screen for the drug molecules, the field of NMR based screening has proceeded promptly. Over the last few years, a variety of state-of-the-art approaches have been presented and found a widespread application in both pharmaceutical and academic research. Recently NMR finds its application in quantitative analysis in order to determine the impurity of the drug



(Mistry et al., 1999), characterization of the composition of the drug prod-

ucts and in quantitation of drugs in pharmaceutical formula- tions and biological fluids (Salem et al., 2006; Reinscheid, 2006), Many reviews on the application of NMR in pharma- ceuticals have been published (Holzgrabe et al., 2005; Malet- Martino and Holzgrabe, 2011).

2.3.4. Fluorimetry and phosphorimetry

The pharmaceutical industries continuously look for the sensi- tive analytical techniques using the micro samples. Fluores- cence spectrometry is one of the techniques that serve the purpose of high sensitivity without the loss of specificity or precision. A gradual increase in the number of articles on the application of fluorimetry (Rahman et al., 2012, 2009) and phosphorimetry (De Souza et al., 2013; Chuan et al., 2000) in quantitative analysis of various drugs in dosage forms and biological fluids has been noticed in the recent past.

2.4. Electrochemical methods

The application of electrochemical techniques in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. The renewed interest in electrochemical tech- niques can be attributed in part to more sophisticated instru- mentation and to increase the understanding of the technique themselves. Here the application of various electro- chemical modes in the analysis of drugs and pharmaceuticals is presented in Table 5.

Moreover, a large number of electroanalytical methods are available for quantification of pharmaceuticals. An amberlite XAD-2 and titanium dioxide nanoparticles modified glassy car- bon paste was developed for the determination of imipramine, trimipramine and desipramine. The electrochemical behavior of these drugs was investigated using cyclic voltammetry, chro- nocoulometry, electrochemical impedance spectroscopy and adsorptive stripping differential pulse voltammetry (Sanghavi and Srivastava, 2013).The capsaicin modified carbon nanotube modified basal-plane pyrolytic graphite electrode or p-chloranil modified carbon paste electrodes have been developed for the determination of benzocaine and lidocaine. The electrochemi- cally initiated formation of capsaicin- benzocaine adduct causes a linear decrease in the voltammetric signal corresponding to capsaicin which correlates to the added concentration of

Table 5 Determination of drug by various electrochemical techniques

Technique	Drugs determined	Remark	Reference
Voltammetr y	b-blocker drugs	Nafion-coated glassy carbon electrode	Nigovic'et al. (2011)
	Rosiglitazone	Square wave adsorptive stripping voltammetry	Al-Ghamdi and Hefnawy (2012)
	Leucovorin	Silver solid amalgam electrode	S' eles'ovska'et al. (2012)
	Secnidazole	Cathodic adsorptive stripping voltammetry	El-Sayed et al. (2010)
	Acetaminophen and tramadol	At glassy carbon paste electrode	Sanghavi and Srivastava (2011a)
	Dopamine	Differential pulse stripping voltammetry	Abdoljavadi and Masrournia (2011)
Polarograph v	Atenolol	Using nanogold modified indium tin oxide electrode	Goyal et al. (2005)
	Nifedipine		Jeyaseelan et al. (2011)
	Anti cancer drug, Vitamin K3		TasA^ et al. (2011)
	Ciclopirox olamine		Ibrahim and El-Enany (2003)
Amperometr y	Diclofenac		Gimenes et al. (2011)
	Verapamil		Ortuno et al. (2005)
Potentiometr y	N-acetyl-L-cysteine		Prkic'et al. (2011)
	Pentoxifylline		Alarfaj and El-Tohamy (2011)

benzocaine (Kachoosangi et al., 2008). A copper (II) complex and silver nanoparticles modified glassy

carbon paste electrode was constructed and used for the determination of dopamine, levodopa, epinephrine and norepinephrine. The electrochemical behavior of



these drugs was studied using cyclic voltammetry, electrochemical impedance spectroscopy, chronocoulometry and adsorptive stripping square-wave voltammetry techniques (Sanghavi et al., 2013). The electrochemical behavior of clonidine, a molecule with a large spectrum of clinical applications, was studied by cyclic, differential pulse and square-wave voltammetry over a wide pH range using a glassy carbon electrode (Ghalkhani et al., 2011). Adsorptive stripping differential pulse voltammetric method has been developed for the determination of venlafaxine and desvenlafaxine using Nafion-carbon nanotube composite glassy carbon electrode (Sanghavi and Srivastava, 2011b). A carbon nanotube paste electrode modified in situ with Triton X 100 was developed for the individual and simultaneous determination of acetaminophen, aspirin and caffeine (Sanghavi and Srivastava, 2010). An electrochemical method based on potentiometric stripping analysis employing cryptand and carbon nanotube modified paste electrode has been proposed for the subnanomolar determination of bismuth (Gadhari et al., 2010). A novel method, capillary electrophoresis with amperometric detection, has been established for rapid and effective measurement of levodopa and benserazide and its impurity (R,S)-2-amino-3-hydroxy propanohydrazide in combination with levodopa pharmaceutical formulations (Wang et al., 2005). Potentiometric stripping analysis of antimony based on carbon paste electrode modified with hexathia crown ether and rice husk has also been reported (Gadhari et al., 2011).

2.5. Kinetic method of analysis

Kinetic method of analysis has been developing since 1950s and yet in modern days it is taking a major resurgence in activity. The repetitive interest in the kinetic methods can be credited to the advancements made in principles, in automated instrumentation, in understanding the chemical and instrumentation, in data analysis methods and in the analytical application.

From the literature it is evident that the kinetic approach to analytical chemistry is rather general with several advantages over traditional equilibrium approach (Pardue, 1989; Mottola, 1988; Perez-Bendito and Silva, 1988). Essentially, kinetic methods trust the measurements of concentration changes (detected via signal changes) in a reactant (which may be the analyte itself) with time after the sample and reagents have been mixed manually or mechanically.

Going through the literature it can be evident that fixed-time and initial rate methods have been used more often for the determination of drugs in pharmaceutical formulations (Darwish et al., 2010;

Rahman and Kashif, 2010). Automatic techniques for the kinetic methods are generally based on open systems, among the popular techniques are the stopped flow system (Andrade et al., 2010) and the continuous addition of reagent (CAR) technique (Jimenez-Prieto and Silva, 1998, 1999). Several drugs have been determined by using the CAR technique with photometric (Marquez et al., 1990) and fluorimetric detection (Marquez et al., 1989). The usage of catalysts to accelerate analytical reactions is feasible with both reaction rate and equilibrium estimations. The use of micellar media in kinetic method is recently encouraged to enhance the rate of reaction, through micellar catalysis and may additionally improve the sensitivity and the selectivity which in turn lessen the analysis time for the analyte (Monferrer-Pons et al., 1999; Pérez-Bendito et al., 1999; Georgiou et al., 1991).

Multicomponent kinetic estimations, most often referred to as differential rate methods, are also receiving wide acceptance in the field of pharmaceutical research (Sultan and Walmsley, 1997). Two new approaches i.e. kinetic wavelength pair method (Pena et al., 1991) and H-point standard addition method (Givianrad et al., 2011) have been proposed for dealing with overlapping spectra of components in the binary mixtures.

2.6. Electrophoretic methods

Another important instrument essential for the analysis of pharmaceuticals is capillary electrophoresis (CE). CE is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the impact of an electric field. In this technique solutes are perceived as peaks as they pass through the detector and the area of individual peak is proportional to their concentration, which allows quantitative estimations. In addition to pharmaceutical studies it finds an application in the analysis of biopolymer analysis and inorganic ions. CE analysis is generally more effective, can be performed on a quicker time scale, requires only a small amount, lesser up to Nano liter injection volumes, and in most cases, takes place under aqueous conditions. These four characteristics of CE have proven to be beneficial to many pharmaceutical applications. Several reports have appeared on the application of this technique in the routine drug analysis (Nehme et al., 2010; Zhang et al., 2009; Calcara et al., 2005). Different modes of capillary electrophoresis such as capillary zone electrophoresis (Hamoudora and Pospisilova, 2006; Nevado et al., 2006; Hauze et al., 2005; Neth et al., 2011; Amin et al., 2012), micellar electrokinetic chromatography (Hamoudova et al.,



2006; Al Azzam et al., 2011; Theurillat et al., 2010), isotachopheresis (Pospisilova et al., 2005; Kuba-cak et al., 2005), capillary gel electrophoresis (Liu et al., 1995; Srivatsa et al., 1994), isoelectric focusing (Lasdun et al., 2001; Liu et al., 1996) and affinity capillary electrophoresis (Li et al., 2011; Martinez-Pla et al., 2004) have been developed and applied to pharmaceutical purity testing and in bio analysis of drugs.

2.7. Flow injection and sequential injection analysis

Laboratory automation was introduced in the second half of the XX century. Steward in the U.S. as well as Ruzicka and Hansen in Denmark, created the flow injection analysis (FIA) technique for the automation of chemical procedure (Stewart et al., 1976; Ruzicka and Hansen, 1975). The introduction of this technique approached to transform the conception of automation in chemical analysis by permitting instrumental measurement to be carried out in the absence of physical and chemical equilibria (Ruzicka and Hansen, 1988; Valcarcel and Luque de Castro, 1987; Karlberg and Pacey, 1989). The basis of Flow injection analysis (FIA) is injection of a liquid sample into a moving, non-segmented uninterrupted carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that uninterruptedly records the changes in absorbance, electrode potential, or other physical

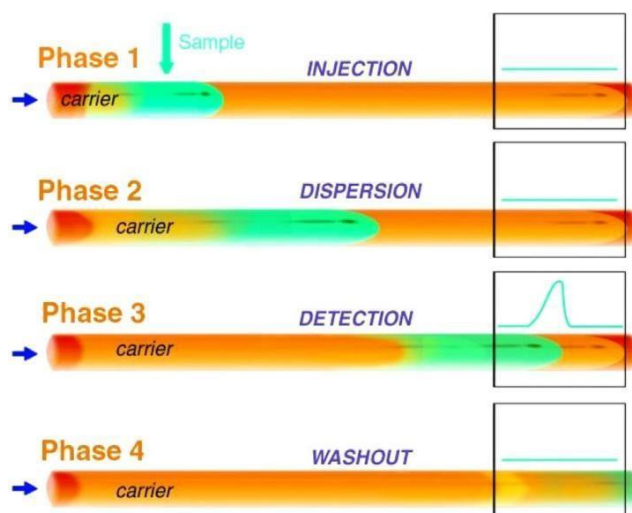


Figure 2 Stages of flow injection analysis. Source: <http://ww2.chemistry.gatech.edu/class/analyt/fia.pdf>. variable that is produced as the substance being sampled moves through the flow cell. As shown in Figure 2, the flow injection analysis consists of many steps. In 1990, Ruzicka and Marshall introduced a new kind of flow analysis they called sequential injection analysis (SIA). This was in response to the widespread use of computers in everyday laboratory procedures. This is a continuous flow

arrangement that does not use segments, similar to the FIA, and it operates according to the notion of programmable flow; nevertheless, it does not share the FIA's concept of controlled dispersion and repeatable manipulation. Several review articles (Kar-licek et al., 1994; Calatayud et al., 1990; Calatayud and Garcia Mateo, 1992a,b; Evagen'ev et al., 2001; Fletcher et al., 2001) and a specialized monograph (Calatayud, 1996) have well-documented the advantages of the FIA technique, which has contributed significantly to the advancement of automation in pharmaceutical analysis.

The scientific community's interest in pharmaceutical automation has been reawakened with the advent of SIA (Christian, 1992). Subsequent injection analysis has been applied to numerous matrices, including solid matrices, pastes (ointments, creams), and liquids (emulsions, suspensions, solutions), in numerous pharmaceutical articles (e.g., Liu and Fang, 2000; Solich et al., 2004). These matrices encompass a wide range of active ingredients with diverse healing activities. Many applications are devoted to determining active components for pharmaceutical formulation quality control, taking use of the benefits in reagent economy and enhanced sample rates.

2.8. Methods with hyphens

Hybrid techniques are the result of combining traditional separation methods with online separation methods. The hyphenated methods and their use in drug analysis have come a long way in the last twenty years. Several hyphenated methods, including LC-MS (Qian et al., 2012; Wang et al., 2012; Nandakumar et al., 2012), GC-MS (De Lima Gomes et al., 2011; Wollein and Sch-Studies on pharmaceuticals have made use of LC-NMR (Lindon et al., 2000), CE-ICP-MS (Timerbaev et al., 2012), and CE-MS (Blasco et al., 2009), among others. An essential part of the process of finding and developing new medications is determining their presence in biological materials. An important part of the process of discovering and developing new medications is the determination of pharmaceuticals in biological materials. According to Nova'kova et al. (2008), the development of bioanalytical methods now primarily involves the use of high-performance liquid chromatography (HPLC) in conjunction with many detection techniques, including ultraviolet, fluorescence, and mass spectrometry. For the purpose of analyzing meloxicam in pharmaceutical formulations and biological materials, a recent review (Brezovska et al., 2013) presents HPLC with UV or MS/MS detection. The method for the qualitative and quantitative determination of metabolites in rat urine after oral administration of Rhizome coptidis and Zuojinwan preparation has been developed using a liquid chromatography-electrospray ionization-mass spectrometry (Rui et al., 2012). In a related study, Walaidur et al. (2013) found that aspirin C effervescent tablets simultaneously contain L-ascorbic acid and acetyl salicylic acid. The urine samples were separated on a C18 column with a mobile phase consisting of a



combination of water and acetonitrile in a proportion of 30:70 v/v. The mobile phase contained 0.1% formic acid. The prevalence of recreational drug addiction is on the rise, and forensic and clinical samples often reveal the presence of novel compounds. Among these compounds, diphenyl-2-pyrrolidinemethanol and its metabolites have been identified in rat urine by the use of gas chromatography-mass spectrometry and liquid chromatography-high resolution-mas spectrometry (Meyer et al., 2013). The presence of human medications in Malaysia's tropical aquatic environment was determined via the use of experiments. Al-Odaini et al. (2013) used liquid chromatography in conjunction with tandem mass spectrometry to evaluate water samples that had been collected at various locations along the Langat River as well as effluents from five sewage treatment facilities. The samples were extracted using solid phase extraction. All river water samples tested positive for mefenamic acid, salicylic acid, and glibenclamide, according to this research. Research on the drug-drug interaction between clopidogrel and rabeprazole has been conducted in healthy Chinese volunteers. At various time intervals after delivery, the LC-MS/MS analysis of rabeprazole and clopidogrel plasma concentrations was conducted (Wu et al., 2013). Peaper et al. (2013) developed a unique LC-MS/MS approach for the identification of carbapenemase activity from bacterial isolates. According to Cao et al. (2013), six different types of parabens may be identified in food using an HPLC-MS/MS technique. The following 4-hydroxybenzoic acid esters were successfully determined using the method: methyl, ethyl, propyl, butyl, isopropyl, and isobutyl. Caffeine, warfarin, omeprazole, metoprolol, and midazolam were given orally or intravenously to mini pigs in order to study the pharmacokinetics of human cytochrome P450s' specific substrates. Liquid chromatography-tandem mass spectrometry was used to determine each analyte's usual pharmacokinetic characteristics from plasma samples taken up to 24 hours after dose (Mogi et al., 2012).

3. Conclusion

The fundamental goal of pharmaceutical medications is to help people by eradicating or preventing diseases. In order for the drug to work as intended, it has to be free of any impurity or interference that might hurt people. A comprehensive literature assessment of the instruments used in pharmaceutical analysis is the goal of this study, which also aims to highlight the function of these instruments in the assay of pharmaceuticals. Starting with the more archaic titrimetric approach and progressing to the more modern hyphenated technique phases, the review also emphasizes the methods' development.

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