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ANALYTICAL METHODS FOR VARIOUS DRUGS AND PHARMACEUTICALS: A REVIEW

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ABSTRACT

The development of pharmacotherapy and the use of drugs and pharmaceuticals is an important factor that brought about revolution in the health of human population. In order to achieve proper efficacy, the drug administered should be free from impurities and should be in proper amount. Pharmaceuticals may incorporate impurities at various stages of their development, transportation and storage which make it inefficient to be administered. Thus impurities in drugs must be detected and quantitated. Various chemical and instrumental methods were developed, in order to estimate the drugs. This review highlights the role and importance of analytical methods such as titrimetric, kinetic, electrochemical, electrophoretic, spectroscopic, chromatographic and hyphenated methods in checking the quality of the drugs.

KEYWORDS: Analytical Methods, Chromatography, Electrochemical Methods, Hyphenated Methods, Pharmaceuticals, Spectroscopy, Titrimetry

INTRODUCTION

Both qualitative and quantitative chemical analysis is used by most of the manufacturing industries to ensure the quality of raw materials and the final products. A quantitative analysis is done to establish the amount of the essential component in the raw material. The unwanted compound may interfere with the manufacturing process or may appear as a harmful impurity in the final product. The final product is checked to ensure that its essential component is present within a predetermined range of composition and the impurities do not exceed certain specified limit.

In the field of pharmaceutical research, drug analysis refers to the identification, characterization and determination of the drugs in dosage forms and biological fluids etc. The number of drugs that are being introduced into the market has been increasing day by day. These drugs may be either completely novel or may be partial structural modification of the existing drugs. So the analytical investigation of bulk drug materials, intermediates, drug products, drug formulations, impurities and degradation products, and biological samples containing the drugs and their metabolites is very important.

Starting from the stage of drug development, analytical techniques play vital roles, such as understanding the physical and chemical stability of the drug, impact on the selection and design of the dosage form, checking the stability of the drug molecules, quantification of the impurities and identification of those impurities which are above the established threshold and assessing the content of drug in the marketed products. The analysis of drug and its metabolite which may be either quantitative or qualitative is extensively applied in the pharmacokinetic studies. These methods are used in quality control laboratories to ensure the identity, purity, safety, efficacy and performance of drug products. This review highlights the role and importance of analytical methods such as titrimetric, kinetic, electrochemical, electrophoretic, spectroscopic,

chromatographic and hyphenated methods in checking the quality of the drugs.

ANALYTICAL METHODS

Electrochemical Methods

Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as large linear dynamic range, with relatively low-cost instrumentation. After developing more sensitive pulse methods, the electro analytical studies are more regularly used on industrial, environmental applications and on the drug analysis in their dosage forms. Some of the most useful electro analytical techniques are based on the concept of continuously changing the applied potentials to the electrode-solution interface and the resulting measured current [1]. Most of the chemical compounds were found to be as electrochemically active [2]. During the past years, there has been extraordinary acceleration of progress in the discovery, synthesis, sensitive electrochemical analysis [3-10].

The application of electrochemical methods in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. Various electrochemical methods in the analysis of drugs and pharmaceuticals are given in Table 1 [22]. Elelctroanalytical techniques (specially stripping analysis) are well known as excellent procedures for the determination of trace chemical species. These techniques have been developed for various cations, anions and organic molecules. Several articles that reviewed the application and the use of such voltammetric techniques in the determination of pharmaceuticals and metals in different samples have been reported. An amberlite XAD-2 and titanium dioxide nanoparticles modified glassy carbon paste was developed for the determination of imipramine, trimipramine and desipramine. The electrochemical behavior of these drugs was investigated using cyclic voltammetry, chronocoulometry, electrochemical impedence spectroscopy and adsorptive stripping differential pulse voltammetry [11].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 [12]. Electrochemical oxidation of metoclopramide hydrochloride has been reported [13], where the metoclopramide hydrochloride was determined by second-derivative adsorptive anodic stripping voltammetry with a nafion-modified glassy carbon electrode. The polarographic determination of cisapride by nitration with KNO_3 in H_2SO_4 was suggested [14]. The method is based on using Britton–Robinson buffer of pH 6.5 in presence of KNO_3/H_2SO_4 mixture as nitrating agent. Carbon paste electrode modified with poly (N-vinylimidazole) and poly (4-vinylpyridine) was used for the determination of amoxycillin in solid dosage forms without any separation step [15-17]. Voltammetric behavior of chloroquine was investigated using cyclic voltammetry and differential pulse voltammetry [18]. DNA-modified carbon paste electrode was used in this study. Captopril was subjected for different voltammetric techniques. The voltammetric behavior was studied [19, 20]. Carbon-paste electrode modified with cobalt-5-nitrolsalophen was used as a sensitive voltammetric sensor for detection of captopril [21].

Table 1: Electrochemical Methods in the Analysis of Drugs and Pharmaceuticals [22]

Technique	Drugs Determined
Voltammetry	Beta blocker drugs, Rosiglitazone, Leucovorin, Secnidazole, Dopamine, Atenolol
Polarography	Nifedipine, Anti cancer drugs, Vitamin k3, Ciclopiroxolamine
Amperometry	Diclofenac, Verapamil
Potentiometry	N- acetyl- L- cysteine, Pentoxifylline

Titrimetric Methods

Titrimetric method of analysis originated in the middle of the 18th century when Gay Lussac invented the volumetric method in the year 1835. The advantages of these methods include saving time and labor, high precision and the fact that there is no need of using reference standards. Modernized titrimetric method included spreading of non-aqueous titration method, expanding the field of application of titrimetric methods to (very) weak acids and bases as well as potentiometric end point detection improving the precision of the methods. With the development of functional group analysis procedures titrimetric methods have been shown to be beneficial in kinetic measurements which are in turn applied to establish reaction rates. Titrimetric methods have been used for the determination of captopril [23], albendozole [24] and gabapentin [25] in commercial dosage forms. Nonaqueous titration method was used to determine Sparfloxacin [26]. In addition to its application in drug estimation, titrimetry has been used in the past for the estimation of degradation products of the pharmaceuticals [27].

Kinetic Methods

Kinetic method of analysis has been developing since 1950s. The growth in kinetic methods can be directed to the advancements made in principles, in automated instrumentation, in understanding the chemical and instrumentation, in data analysis methods and in the analytical application. Kinetic methods involve the measurements of concentration changes in a reactant (which may be the analyte itself) with time after the sample and reagents have been mixed manually or mechanically. Fixed time and initial rate methods are more often used for the determination of drugs in pharmaceutical formulations [28]. Popular automatic techniques are the stopped flow system [29] and the continuous addition of reagent (CAR) technique [30, 31]. In Stopped-flow systems, solutions are forced from syringes into a mixing chamber. After a very short period, flow is stopped suddenly when the observation cell is filled by an opposing piston that is linked to a sensing switch that triggers the measuring device. The schematic diagram of stopped-flow system is given in figure 1. Several drugs have been determined by using the CAR technique with photometric [32] fluorimetric detection [33].

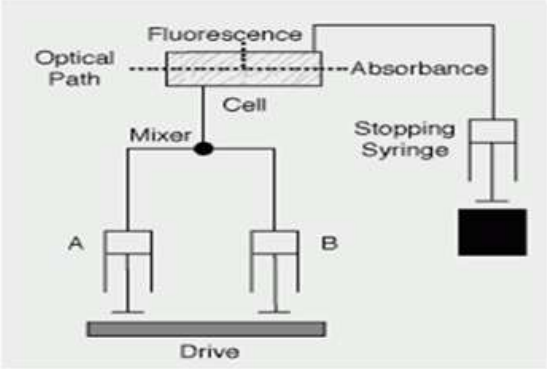

Figure 1: Schematic Diagram of Stopped-Flow System

Electrophoretic Methods

Capillary electrophoresis (CE) is the important instrument electrophoretic method used for the analysis of pharmaceuticals. CE is 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. Samples enter the tube from the right

and travel to the left to the detection system which records the chromatogram output on a computer. The advantages of CE analysis is that it is generally more effective, requires only a small amount, lesser up to Nano liter injection volumes, can be performed on a quicker time scale, and in most cases, takes place under aqueous conditions. Several reports based on the application of this technique in the routine drug analysis have been published [34, 35, 36]. Different types of capillary electrophoresis include capillary zone electrophoresis, micellar electrokinetic chromatography, isotachophoresis, capillary gel electrophoresis, isoelectric focusing and affinity capillary electrophoresis. These are applied to pharmaceutical purity testing and in bio analysis of drugs [22]. The schematic diagram of Capillary electrophoresis is shown in figure 2.

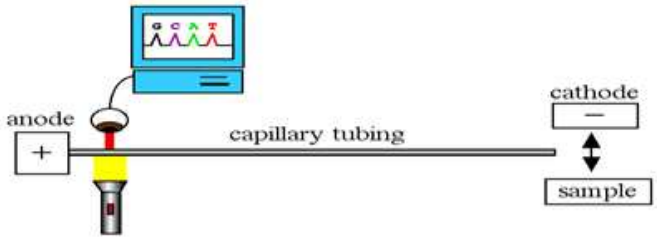

Figure 2: Capillary Electrophoresis Set Up

CHROMATOGRAPHIC TECHNIQUES

Thin Layer Chromatography (TLC)

TLC is a technique used for fast screening of samples. It is used to identify herbal products and to differentiate between herbal species [39, 40]. TLC plays a crucial role in the early stage of drug development when information about the impurities and degradation products in drug substance and drug product is inadequate. Various impurities of pharmaceuticals have been identified and determined using TLC [37, 38]. One of the main advantages of TLC is that it enables optimization of operational parameters, such as the sample application, plate development and derivatization. The main disadvantages of TLC are its low reproducibility and resolution [40-42], need for high compound concentration for detection [40] and the semi-quantitative nature of the technique, in the analysis and quality control of herbal drugs. The main factors that are hard to control in TLC and affect its precision include sample spotting, saturation of the developing chamber and the instability of colour appearance when coloring reagents are used for detection [40]. The introduction of digital scanning and documentation software has improved the applicability of TLC for the comprehensive identification and assessment of herbal drugs. In addition, high performance thin layer chromatography (HPTLC) that make use of a smaller particle size range (5–20 mm) and automation of different steps has increased the producibility and resolution [39, 40, 41, 43]. Micro-emulsion TLC (ME-TLC) development lead to increased separation efficiency and signal enhancement and resulted in an improved sensitivity [40].

Gas Chromatography

Gas chromatography is a well established analytical technique commonly used for the characterization, quantification and identification of volatile compounds [44]. Combining separation and on-line detection allows accurate quantitative determination of complex mixtures, including traces of compounds down to parts per trillion in some specific cases. Gas liquid chromatography plays a substantial role in the analysis of pharmaceutical product [45]. The scope of Gas chromatography technique includes the creation of high-molecular mass products such as polypeptides, or thermally unstable antibiotics. Gas chromatography has been used for assay of drugs such as isotretinion [46], cocaine [47] and employed in the determination of residual solvents in betamethasone valerate [48]. Gas chromatography is also an

important tool for analysis of impurities of pharmaceuticals [49, 50]. The residual solvents listed as impurity by the International Conference of Harmonization are analyzed by the GC using a variety of detectors [51, 52, 53, 54].

High-Performance Liquid Chromatography (HPLC)

HPLC is an advanced form of liquid chromatography, which was appeared for the first time for the assay of bulk drug materials in the year 1980 [55]. It is used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to recognize better the role of individual molecules. This has become the principal method in USP XXVII [56] and to a lesser extent but one of the most widely used methods also in Ph. Eur. 4 [57]. The HPLC method has excellent specificity and simultaneously sufficient precision is also attainable.

Literature survey explains that it HPLC has been the most widely used system among the chromatographic techniques. In liquid chromatography the choice of detection approach is crucial for guaranteeing detection of all the components. UV detector is one of the widely used detectors in HPLC which is capable of monitoring several wavelengths concurrently by applying a multiple wavelength scanning program. If present in adequate quantity, UV detector assures detection of all the UV-absorbing components [22].

Several drugs have been assayed in pharmaceutical formulations [58, 59, 60, 61] and in biological fluids [62, 63, 64] using HPLC. Thus, HPLC plays a major role in answering many questions of the pharmaceutical industry. However, the disadvantages of HPLC include the requirement of expensive machinery, large volumes of environmentally unfriendly liquids, the undetected co-elution of compounds, the susceptibility of conventional (silica-based) columns to relatively basic (pH>9) or acidic (pH<2) mobile phases and high temperatures, price of columns, solvents and a lack of long term reproducibility due to the proprietary nature of column packing.

SPECTROSCOPIC TECHNIQUES

Spectrophotometry

Spectrophotometric methods are based on natural UV absorption and chemical reactions [65]. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantages of these methods are low time and labour consumption. The method also has excellent precision. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years [66, 67, 68]. The colorimetric methods are usually based on complex-formation reaction, oxidation-reduction process and a catalytic effect. Colorimetric methods are regularly used for the assay of bulk materials. For example, the blue tetrazolium assay is used for the determination of corticosteroid drug formulations [69, 70]. The colorimetric method is also exploited for the determination of cardiac glycosides and is presented in European Pharmacopoeia.

Several approaches using spectrophotometry for determination of active pharmaceutical ingredients in bulk drug and formulations have been reported. New sensitive and rapid spectrophotometric methods for the determination of four analgesic drugs namely, nalbuphine (NALB), naltrexone (NALT), morphine (MORF) and tramadol (TRAM) in pharmaceutical formulations were developed and optimized [71]. Two simple, rapid, sensitive, low-cost, and accurate methods (A and B) for the micro determination of amantadine HCl (AMD) in pure form and in pharmaceutical formulations are developed. Method A is based on the formation of tris (o-phenanthroline)-iron (II) complex (ferroin) upon reaction of amantadine HCl with an iron (III)-o-phenanthroline mixture in sodium acetate acetic acid buffer media.

The ferroin complex is spectrophotometrically measured at kmax 509 nm against reagent blank. Method B is based on the reduction of Fe (III) by the drug which forms colored complex with 2, 20-bipyridyl [72]. In the literature about 22 methods were reported for the estimation of pioglitazone using spectrophotometry, of which 8 methods are for pioglitazone determining alone, while the others are for quantifying pioglitazone in combination with other drug substances [73].

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. Its major advantages over other analytical techniques are 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 [22]. The major pharmacopoeias have generally adopted NIR techniques. The European Pharmacopoeia in chapter 2.2.40 [57] and United States pharmacopoeias in 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. In addition to the research articles many review articles have been published citing the application of the NIRS in pharmaceutical analysis [74].

Nuclear Magnetic Resonance Spectroscopy (NMR)

The first report describing the use of NMR spectroscopy to screen for the drug molecules appeared in 1996 [75]. NMR has application in quantitative analysis in order to determine the impurity of the drug [76], characterization of the composition of the drug products and in quantization of drugs in pharmaceutical formulations and biological fluids [77].

Hyphenated Techniques

A hyphenated technique is developed by coupling of a separation technique and on-line separation technique. The last two decades saw a remarkable advancement in the hyphenated techniques and its application in pharmaceutical industry. A variety of hyphenated techniques such as GC-MS, LC-MS, CE-ICP-MS, CE-MS and LC-NMR have been applied in the analysis of pharmaceuticals. HPLC together with various types of detection such as ultraviolet, fluorescence, and mass spectrometry has become the method of choice for bioanalytical method development [78, 79].

The Lab-on-a-Chip concept aims at miniaturizing laboratory processes to enable automation and/or parallelization via microfluidic chips that are capable of handling minute sample volumes. One of the first hyphenated LOC-MS systems was reported by Xue et al [80]. Comprehensive reviews on LOC-MS have recently been published by Gao et al [81]. Feng et al. [82] and Oedit et al [83].Two highly sensitive methods for the determination of genotoxic alkyl methane sulfonates (AMSs) and alkyl paratoluene sulfonates (APTSs) in lamivudine using hyphenated techniques have been presented. AMSs were determined by GC–MS method using GSBP- INOWAX (30 m X 0.25 mm X 0.25 μm) column. APTSs were determined by LC-MS using Zorbax, Rx C8, 250 mm X 4.6 mm, 5 μm column as stationary phase. 0.01 M ammonium acetate is used as buffer. The mixture of buffer and methanol in 75:25 (v/v) ratio was used as mobile phase A and mixture of buffer and methanol in 5:95 (v/v) ratio was used as mobile phase B [84]. A new stability-indicating RP-HPLC assay method was developed and validated for quantitative determination of Levocabastine HCl in bulk drugs

and in ophthalmic suspensions in the presence of degradation products generated from forced degradation studies [85]. A dissolution method with robust high performance liquid chromatographic (HPLC) analysis for immediate release tablet formulation was developed and validated to meet the requirement as per International Conference on Harmonization (ICH) and United States Food and Drug Administration (USFDA) guidelines. Two- way analysis of variance (ANOVA) was applied for evaluating the statistical difference between the assay results obtained via both NASSAM and RP–HPLC methods and ultimately no significant difference was found between both the methods [86]. A method has been developed for the separation of moxifloxacin HCl and ketorolac tromethamine using reverse phase high-performance liquid chromatography (RP-HPLC) on C18 column (250 X 4.6 mm, 5 μm) with UV detection at 308 nm [87].

Tandem MS/MS instruments coupled with HPLC or UPLC are capable of analyzing multiple analytes simultaneously as well as reaching sensitivity limits corresponding to those achieved by GC–MS/MS [88]. Experiments were performed to identify the presence of human pharmaceuticals in the tropical aquatic environment of Malaysia. Water samples collected at different sites along the Langat River and effluents from five sewage treatment plants were extracted by solid phase extraction and analyzed using liquid chromatography coupled with tandem mass spectrometry [89]. This study confirmed the presence of mefenamic acid, salicylic acid and glibenclamide in all river water samples [22]. A HPLC-MS/MS method has been reported for the determination of six kinds of parabens in food [90]. The method was successfully applied to the determination of methyl, ethyl, propyl, butyl, isopropyl and isobutyl esters of 4-hydroxybenzoic acid.

CONCLUSIONS

The main aim of the pharmaceutical drugs is to save humans from diseases and to prevent illness. In order to achieve proper efficacy, the drug administered should be free from impurities and should be in proper amount. This review is aimed at focusing the role of various analytical techniques in the assay of pharmaceuticals. The review also highlights the advances made in the field of analytical techniques from the older titrimetric method to the advanced hyphenated techniques.

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