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Influence of Chromatography and SIAM Techniques on Pharmaceutical and Medical Research and its Significance on Efficacy of Medicine

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ABSTRACT

Pharmaceutical world is huge and research in the field of Pharmaceuticals and advanced medicine involves tremendous efforts since from the beginning of discovering new chemical entities (NCEs) drugs (Drug Discovery) in clinical trials. In order to achieve safe, non-toxic, highly efficient and efficacious pharmaceutical formulations and preparations; utilization of advanced analytical tools has gained enormous importance. Chromatography, Stability indicating assay methods, developing methods and validating the same to separate active pharmaceutical ingredients from related substances and known and unknown impurities is of utmost significance. Drug discovery to advanced drug delivery several stages are involved which affect final formulation characteristics thus using high tech instruments which follow scientific approach towards developing nontoxic and high therapeutic value of medicines and Pharmaceutical formulations. Hence correlation of pharmaceutical research with analytical strategies which involve utilization of high tech instruments such as HPLC, LC, Mass spectroscopy, stability indicating assay method (SIAM), NMR, FTIR techniques for impurity assessment represents pharmaceutical research is a top-notch analysis leading to bio significance in the world of medicine.

INTRODUCTION

Since first initiated by the U. S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century" (Quality by design) has turned an essential parameter for the pharmaceutical industry that is further defined in the International Conference on Harmonization (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [1,14]. Recently, increased attention has been paid to Quality by design within the pharmaceutical industry to actively seek out quality using its underlying principles. As analytical techniques and methods are used for the quality control of pharmaceutical compounds and thereby assure patient safety and efficacy, they have become an essential part of pharmaceutical Quality by design. The topics around what is “Quality” by design for analytical methods or how to apply Quality by design to analytical method development have been discussed in the recent literature. Building-in quality as the method is developed can be achieved by defining the method objectives at the beginning of the method development effort, by using sound development tools and by applying analytical sciences knowledge to anticipate and pre-empt problems [2]. The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. This approach ensures a very high likelihood of method success during the product lifecycle. Thus, the validation, which is usually performed after method development, will serve the purpose of confirming method performance as opposed to identifying potential problem areas.

1. CHROMATOGRAPHY IN THE PHARMACEUTICAL WORLD

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all phases and steps of drug discovery, development, and production. The development of new chemical entities (NCEs) is comprised of two major activities: drug discovery and drug development. Throughout the drug discovery and drug development paradigm, rugged analytical HPLC separation methods are developed and are tailored by each development group (i.e., early drug discovery, drug metabolism, pharmacokinetics, process research, preformulation, and formulation). At each phase of development, the analyses of a myriad of samples are performed to adequately control and monitor the quality of the prospective drug candidates, excipients, and final

products. Effective and fast method development is of paramount importance throughout the drug development life cycle [3].

2. IMPLEMENTATION OF ANALYTICAL TECHNIQUES FOR QUALITY TESTING:

2.1 RELATED SUBSTANCES

Today majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. There are biologically active chemical substances generally formulated into convenient dosage forms such as tablets, capsules, suspensions, ointments and injectable. These formulations deliver the drug substances in a stable, non-toxic and acceptable form, ensuring its bioavailability and therapeutic activity. Quality, safety and efficacy of drugs Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy.

The safety of a drug is determined by its pharmacological/toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. The impurities in drugs often possess unwanted pharmacological or toxicological effects by which any benefit from their administration may be outweighed [4]. Therefore, it is obvious that the products intended for human consumption must be characterized as completely as possible. The quality and safety of a drug are generally assured by monitoring and controlling the impurities effectively. Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis.

The relationship between synthetic by-products, degradation products, and related substances is that related substances contain the sum of synthetic by-products (originating from chemical synthesis which does not change with time and conditions) and degradation products (increases with time and varies under different storage conditions). However, sometimes the synthetic by-products of the API can also be degradation products of the drug product.

2.2 ORIGIN OF IMPURITIES

Impurities in drugs are originated from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms. A sharp difference between the process-related impurities and degradation products is always not possible. However, the majority of

the impurities are characteristic of the synthetic route of the manufacturing process. Since there are several possibilities of synthesizing a drug, it is possible that the same product of different sources may give rise to different impurities [1, 5].

2.3 TYPES OF IMPURITIES

Chemical impurities are classified as organic, inorganic and residual solvents for regulatory purposes. Organic impurities can originate from impurities contained in starting materials (most often isomeric impurities), synthetic intermediates (incomplete reaction or excess reagent used) and degradation products, which may depend on alterations in reaction conditions, such as temperature, pH, or in storage conditions (hydrolysis, oxidation, ring opening, etc.). Inorganic process and are normally known and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids and charcoal etc.

3. REGULATORY ASPECTS

Control is more important today than ever. Until the beginning of the 20th century, drug products were produced and sold having no imposed control. Thereupon the Food, Drug and Cosmetic act were revised requiring advance proof of safety and various other controls for new drugs. The impurities to be considered for new drugs are listed in regulatory documents of the Food and Drug Administration (FDA), International Conference on the Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the United States Pharmacopoeia (USP) [1, 6, 7]. Nevertheless, there are many drugs in existence, which have not been studied in such detail. The USP and National Formulary (NF) are the recognized standards for potency and purity of new drugs. The most critical aspect of the elaboration of the guidelines was the definition of the levels of impurities for identification and qualification

Table No. 1: ICH guidelines for identification and qualification of impurities in bulk drugs and formulations [1, 3, 14]

Dose	Threshold for	
	Identification (%)	Qualification (%)
<1mg	1	1
1-10mg	0.5	1
10-100mg	0.2	0.5
100mg-2g	0.1	0.2
>2g	0.1	0.1

4. ROLE OF CHROMATOGRAPHIC TECHNIQUES IN DETERMINATION OF IMPURITIES IN DRUGS

TLC is a powerful tool for screening unknown materials in bulk drugs. Its application to bulk drugs is limited owing to the problems involved in detection systems. At present, HPLC is the most widely used technique for the analysis of bulk drugs and their formulations. Derivatization of the drugs prior to analysis is normally not required. Gradient elution, temperature, and wavelength programming techniques provide valuable information regarding the undetected components of a given drug. Gradient elution, although extensively used in pharmaceutical research, is not popular because many of the above advantages are lost.

Instead, screening for potential impurities is often performed by a combination of isocratic HPLC methods. The choice of proper detection mode is crucial to ensure that all the components are detected. With UV detection, this problem could be overcome by using a multiple wavelength scanning programs which are capable of monitoring several wavelengths simultaneously. It provides assurance that all the UV-absorbing components are detected if present in sufficient quantity. Photodiode-array detectors are used to record spectro-chromatograms simultaneously. Fluorescence, electrochemical, refractive-index, and conductivity detectors are appropriate for specific applications. Chiral detectors are useful in determining the purity of enantiomeric drugs by HPLC [8,9].

5. STABILITY INDICATING ASSAY METHOD (SIAM)

Stability Indicating Assay Method can be defined as validated quantitative analytical method that can detect the changes with time in the chemical, physical or microbiological properties of the drug substance and drug product, and that are specific so that the contents of active ingredients, degradation products and other components of interest can be accurately measured. The stability-indicating assay is a method that is employed for the analysis of stability samples in pharmaceutical industry [10]. With the ICH guidelines, the requirement of the establishment of stability indicating assay method has become more clearly mandated. The guidelines conditions, like pH, light, oxidation, dry heat, etc. and separation of drug products.

5.1 DEVELOPMENT OF STABILITY INDICATING ASSAY METHOD (SIAM)

5.1.1 Critical Study of drug structure

This should be the first step in the development of SIAM. Much information on degradation pathway of a drug can simply be gained from the structure of the drug, by the study of the functional groups. There are definite functional group categories, like amides, esters, lactams, lactones, etc. that undergo hydrolysis, others like thiols, thioethers, etc. undergo oxidation and compounds like olefins, aryl halo derivatives, aryl acetic acids and those with aromatic nitro groups, N-oxides undergo photo de-composition.

5.1.2 Collection of Information on Physicochemical Properties

Before method development is taken up, it is generally important to know various physicochemical parameters like pKa, log P, solubility, absorptivity and wavelength maximum (λ_{max}) of the drug in question. The knowledge of pKa is important as most of the pH-related changes in retention occur at pH values within 1.5 units of the pKa value. The ionization value also helps in selecting the pH of the buffer to be used in the mobile phase. The knowledge of logP for the drug and the identified degradation products provides well insight into the separation behavior likely to be obtained on a particular stationary phase. The analysis of the drug or degradation products requires that they are soluble in HPLC compatible solvents in the first place [11,12].

The availability of the solubility data in aqueous, organic and commonly used HPLC solvents and their combinations can thus prove to be very useful in the selection of the sample solvent and the mobile phase. As the HPLC analysis, employing a UV detector is usually carried out at the wavelength maximum or at a wavelength where all components show good absorbance, therefore, the necessity to know the wavelength maxima and extinction of the drug and degradation products in different solvents and at different pH becomes an absolute requirement.

5.1.3 Stress (Forced Decomposition) Studies

The next step in the development of SIAM is the conduct of forced decomposition studies to generate degradation products of the drug. The ICH guideline Q1A suggests the following conditions to be employed 10ºC increments temperatures above the

increase in a linear manner, moisture content and residual water, hydrolysis across broad dimensions of pH values also oxidation and photolytic degradability. However, the guideline provides no details on how hydrolytic, photolytic and oxidative studies have to be actually and accurately performed.

Subsequent to preliminary chromatographic studies, the t_R and relative retention times (RRT) of all products formed should be tabulated for each reaction condition. PDA spectra or LC-MS profile of such components are obtained and critically evaluated to ascertain whether the products are same or different. In the final, a mixture of the reaction solutions is prepared and subjected again to resolution behavior study. While making this mixture, it is not always necessary to add all reaction solutions withdrawn at the different time for all conditions, as that would make the situation too complex. Rather, only those solutions are mixed where different products are formed in sufficient quantity [3, 13, 15]. Resolution in the mixture is studied closely, to see whether the resolution is similar to that obtained in individual samples. To separate the close or close eluting peaks, the method is optimized by changing the mobile phase ratio, pH, flow rate, solvent type and column and its type.

5.1.4 Identification and Characterization of Degradation Products, Preparation of Standards

Before moving to the validation of a SIAM, it is necessary to identify the drug degradation products and arrange for their standards. These are required to establish specificity/selectivity of the method. The work on this aspect can even be initiated once an idea on the nature and number of degradation products formed under different degradation conditions is obtained from preliminary separation studies. To identify the resolved products, a conventional way is to isolate them and determine the structure through spectral (MS, NMR, IR, etc.) and elemental analysis. However, this approach is tedious and time-consuming when multiple degradation products are formed. Against it, the modern approach is to use hyphenated LC techniques coupled with mass spectrometry.

This strategy integrates into a single instrument approach, analytical HPLC, UV detection, full scan mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS-MS) and provides a fair idea on the identity of resolving components. These days a

further integrated approach is becoming popular wherein LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information, and the further detailed structural information is obtained through LC-NMR analysis.

5.6 Validation of Stability Indicating Assay Method

There are two stages in the validation of a SIAM. The first stage is early in the development cycle when drug substance is subjected to forced decomposition studies and the SIAM is established based on the knowledge of drug degradation behavior. The focus of validation at this stage is on the establishment of specificity/selectivity, followed by other parameters like accuracy, precision, linearity, range, robustness, etc. This validated method finds application in the analysis of stability samples of the bulk drug for determination of its retest or expiry period. In the second stage, when the SIAM so developed is extended to formulations or other matrices, the emphasis gets limited to just prove the pertinence of the established validation parameters in the presence of excipients or other formulation constituents. Here only parameters of critical importance like specificity/selectivity, accuracy and precision are revalidated. If the SIAM is being developed directly for a formulation, without involving the bulk drug route, then all validation parameters are necessary to be established [5, 16].

The specificity/selectivity of a SIAM can be established very simply if degradation chemistry of the drug is known and the standards of the products are available. The only effort involved than is the development of a method that separates components from a physical mixture of drug and the degradation products. At this stage, only peak purity becomes crucial. The most commonly used technique is the PDA analysis, the principle of which is the comparison of the spectra of the analyte peak, taken upslope, at the apex and on the downslope. If these spectra do not match then the peak is non-homogeneous [17].

CONCLUSIONS

The complexity of multi-component pharmaceutical solid, liquid dosage forms involves multiple entities and excipients that poses a considerable challenge to the pharmaceutical research scientists during the development of assay procedure. In the early period of this century, colorimetric and spectrophotometric methods were used for drug analysis due to reasons of economy and easy availability. These methods, however, are used to a lesser extent today because they lack specificity, sensitivity, and accuracy. For the simultaneous

estimation of the drugs, present in multicomponent solid or liquid dosage forms, Nano formulations, Injectable etc. HPLC method is considered most suitable as it gave reproducible results and it affirms precision of the analytical strategies reducing nonspecificity of the technique. Several HPLC, RP HPLC, HPTLC techniques have been reported by researchers in pharmaceutical science as method development and validation for novel drugs and drug delivery systems, marketed formulations, as well as estimation of impurities with stress degradation analysis of drug products.

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