A COMPREHENSIVE STUDY ON THE VALIDATION OF ACTIVE COMPOUNDS IN MULTI-COMPONENT DRUG FORMULATIONS

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ABSTRACT

The drug discovery and development industry has aimed at identifying single components with a clear mechanism of action as desirable candidates for potential drugs. To-date modern drug research has focused on the discovery and synthesis of single active substances. This may explain the frequently observed pleiotropic bioactivity spectra of these compounds, which may also suggest that they possess novel therapeutic opportunities. Interestingly, considerable bioactivity properties are exhibited not only by remedies that contain high doses of phytochemicals with prominent pharmaceutical efficacy, but also preparations that lack a sole active principle component. Despite that each individual substance within these multi-components has a low molar fraction, the therapeutic activity of these substances is established via a potentializing of their effects through combined and simultaneous attacks on multiple molecular targets. To overcome the difficulty in obtaining standard products, scholars have proposed achieving MCQA through the "single standard to determine multiple components (SSDMC)" approach. In the present study, a comprehensive survey on contamination profiles, occurrence, removals, temporal variation and ecological risk of multiclass multiresidue PhACs, such as antibiotics, non-steroidal anti-inflammatories, lipid regulators and phsychiatrics, was performed in wastewaters from the WWTP of Ioannina University hospital along one year period on a monthly sampling basis.

Keywords: *drug discovery, multi-components, phsychiatrics, compounds, non-steroidal anti-inflammatories,*

INTRODUCTION

These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. There is a scope; therefore, to develop newer analytical methods for such drugs Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products. Identification and quantification of impurities is a crucial task in pharmaceutical process development for quality and safety. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various

analytical methodologies are employed for the determination of related components in pharmaceuticals.

LITERATURE REVIEW

Noureddine Chaachouay [2024] thousands of years, nature has been a source of medical substances, and an astounding numeral of contemporary remedies have been identified from natural origins. Plants have long been used as folk herbal medicines to treat various disorders, and their different natural products have inspired the design, discovery, and development of new drugs. With the invention of recent molecular targets based on proteins, there is a growing need for fresh chemical diversification in screening. Natural products will play a vital part in supplying this need via the continuous exploration of global biodiversity, the majority of which remains unexplored. Even though drug discovery from medicinal plants remains an important source of novel therapeutic leads, various hurdles exist, including identifying and executing suitable high-throughput screening bioassays, scaling up the supply of bioactive molecules, and acquiring plant materials. Investigating these natural resources takes multi-disciplinary, nationwide, and global partnerships in design, synthesis, discovery, and drug development techniques.

Krishna Moorthy Manchuri [2024] This concern arises due to their potential for contamination, toxicity, carcinogenicity, and mutagenicity and their presence in many active pharmaceutical ingredients, drug products, and other matrices. N-Nitrosamine impurities in humans can lead to severe chemical toxicity effects. These include carcinogenic effects, metabolic disruptions, reproductive harm, liver diseases, obesity, DNA damage, cell death, chromosomal alterations, birth defects, and pregnancy loss. They are particularly known to cause cancer (tumors) in various organs and tissues such as the liver, lungs, nasal cavity, esophagus, pancreas, stomach, urinary bladder, colon, kidneys, and central nervous system. Similarly, drug manufacturers should be more vigilant to avoid nitrosating agents and secondary amines during the manufacturing processes. Numerous review articles have been published recently by various researchers, focusing on N-nitrosamine impurities found in previously notified products, including sartans, metformin, and ranitidine. These impurities have also been detected in a wide range of other products.

Sven Stegemann [2023] suggested physicochemical limitations for orally administered drugs, based on the analysis of chemical libraries from the early 1990s. In this review, we report on the trends in oral drug product development by analyzing products launched between 1994 and 1997 and between 2013 and 2019. Our analysis confirmed that most new oral drugs are within the Ro5 descriptors; however, the number of new drug products of drugs with molecular weight (MW) and calculated partition coefficient (clogP) beyond the Ro5 has slightly increased. Analysis revealed that there is no single scientific or technological reason for this trend, but that it likely results from incremental advances are being made in molecular biology, target diversity, drug design, medicinal chemistry, predictive modeling, drug metabolism and pharmacokinetics, and drug delivery.

Muhammad Amjad [2023] The analytical method development in Pharmaceuticals is most important because it is the regulatory requirements. The determination of Active Ingredients of Pharmaceuticals (API) and Excipients is only possible when the testing

method will be validated as per ICH, WHO guidelines or GMP requirements. The development will be reliable when the correct and precise strategy will be followed. Here, the systematic method development technique and validation parameters have been discussed precisely. This comprehensive review will be very helpful for analysts to develop analysis method of new molecules present in any pharmaceutical formulation. The validation concept and various techniques of validation have also been argued to create interest for the researchers.

Hartmut Beck [2022] Bayer Chemical Research Laboratory in Wuppertal, Germany. A significant number of prominent small-molecule drugs, from Aspirin to Xarelto, have emerged from this research site. In this review, we shed light on historic cornerstones of small-molecule drug research, discussing current and future trends in drug discovery as well as providing a personal outlook on the future of drug research with a focus on small molecules.

Development of Generic Drug Products

Developing a generic drug product in pharmaceutical industry is a scientific and technical approach which is totally different from developing an innovator product that takes several years and stages to be registered as an innovator product. Generic products are sometimes termed as "Super Generic" or "Therapeutic hybrid". In terms of active ingredient(s), strength, dosage form, method of administration, quality, safety, performance characteristics, and therapeutic indication, bio-equivalence means that a generic drug product is virtually comparable to the originator product. Generic drug products are cost-effective than their respective brand products. Pharmaceutical industries try to formulate and develop a generic product which is therapeutically equivalent to the innovator product. Though development of generic product does not require that much time and cost compared to the innovator company, but it is very difficult to develop a product containing the same therapeutic efficacy compared to innovator and to meet all the regulatory requirements as well.

Innovator and Generic Drug Products

Innovator products are also called "Reference Listed Drug" or "Patent Drug". Innovator Company or organization develop the medicinal product by trial and error and must have to conduct human trial or bio-availability study. As mentioned earlier, the generic drug product has to be bioequivalent to the innovator product and ensure the same biological effect with proper safety and efficacy. For example, "ZentivaPharma UK Limited" invented an active, named "Dicycloverine Hydrochloride" and the marketed products was "Dicycloverine Hydrochloride 20 mg and 10 mg Tablets". Another pharmaceutical industry "Teva UK Limited" developed bio-equivalent product by conducting bioequivalence study against the innovator product. So, the product of Zentiva is innovator and the product of Teva is generic product.

Source Selection on Generic Drug Products

Drug products are formulated with API and excipients. Drug product has to enter the market in any country by adapting either of the two processes viz. new drug product approval process or generic drug product approval process. Generic drug products are similar to the new drug products in safety, efficacy and quality. Has published the manual for API supplier selection "USP pharmaceutical ingredient supplier qualification program". Generic drug

product approval process was introduced with the intent of marketing the drug products at a lower cost than the innovative drug product so as to provide monetary benefit to the patients. Since, all the approved generic drug products are similar to the innovative drug product; cost of the generic drug product plays a critical role in helping the generic drug product manufacturer acquire a significant market share in the competitive and crowded generic pharmaceutical market. The major share of the generic drug product price is mainly driven by cost of the API. Also, API attributes such as material purity, physical and chemical properties are the discerning factors that decide the generic drug product quality. So, the generic drug manufacturers are enforced to select the suitable API material with required attributes and an appropriate API supplier for the drug product development. The decision to choose an API supplier is an important decision in generic drug product development since most of the generic companies do not have their own API development and manufacturing unit. In the highly competitive generic business it is important for the drug product manufacturers to maintain an entrusted long term strategic relationship with the API suppliers to get an early access to high quality active pharmaceutical ingredients as well as to overcome the pricing burdens.

Drug Products with Complex Mixtures as the Active Ingredients

Certain drug products may contain complex drug substances (i.e., active moieties or active ingredients that are mixtures of multiple synthetic and/or natural source components). Some or all of the components of these complex drug substances cannot be characterized with regard to chemical structure and/or biological activity. Quantification of all active or potentially active components in pharmacokinetic studies to document BA and BE is neither encouraged nor desirable. Rather, we recommend that BA and BE studies be based on a small number of markers of rate and extent of absorption. Although a case-by-case determination, criteria for marker selection include amount of the moiety in the dosage form, plasma or blood levels of the moiety, and biological activity of the moiety relative to other moieties in the complex mixture. Where pharmacokinetic approaches are infeasible to assess rate and extent of absorption of a drug substance from a drug product, in vitro approaches may be preferred.

Narrow Therapeutic Range Drugs

This guidance defines narrow therapeutic range10 drug products as containing certain drug substances subject to therapeutic drug concentration or pharma co dynamic monitoring, and/or where product labeling indicates a narrow therapeutic range designation. Examples include digoxin, lithium, phenytoin, theophylline, and warfarin. Because not all drugs subject to therapeutic drug concentration or pharmacodynamics monitoring are narrow therapeutic range drugs, sponsors and/or applicants can contact the appropriate review division at CDER to determine whether a drug can or cannot be considered to have a narrow therapeutic range. This guidance recommends that sponsors consider additional testing and/or controls to ensure the quality of drug products containing narrow therapeutic range drugs. The approach is designed to provide increased assurance of interchangeability for drug products containing specified narrow therapeutic range drugs. It is not designed to influence the practice of medicine or pharmacy.

Drugs during Drug Product Development

Safety and efficacy are key aspects of drug research and development; therefore, formulations must be designed in a way that ensures appropriate bioavailability of a drug and its physico-chemical stability over the determined shelf-life. The chemical stability of a drug is an intrinsic property that is determined by its chemical structure. The dosage form can lead to drug instability because of the presence of other compounds (e.g., excipients). Also, the drug manufacturing process, packaging, and storage must be monitored for drug stability. In this context, the problem of drug product stability is an very important area in drug research and development, not only for new drugs, but also for generic drugs.

A drug product can undergo physical and chemical changes. The first affects the form of the chemical substance but not its chemical composition, which means that no chemical bonds are broken or formed. The physical instability of a drug manifests as changes in its appearance, the release of the drug, polymorphic changes, adsorption, and many others. On the other hand, chemical changes refer to changes in the chemical structure that arise from degradation of the drug substance and the reactions between the drug and the excipients in the formulation.

RESEARCH METHODOLOGY

Determining the correct concentration of each active ingredient to achieve the desired therapeutic effect, considering the interactions between the components. Develop and optimize the logical system to achieve separation, discovery, and quantification of each active emulsion. Optimize parameters similar as column temperature, mobile phase composition, inflow rate, injection volume, and discovery wavelength. Each active compound in the formulation must be selected based on its therapeutic properties, compatibility with other ingredients, and its pharmacokinetic profile. Choose a suitable logical fashion similar as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), or Mass Spectrometry (MS) and grounded on the parcels of the active composites and the expression matrix. The entire research work was focused on using readily available, lowest price chemicals and HPLC columns. Develop a suitable sample medication system to prize and insulate the active composites from the expression matrix. The drug formulation is exposed to elevated temperature and humidity conditions to predict its shelf life. This testing helps evaluate the degradation of active compounds over time and under varying conditions. Quantify each active emulsion and calculate the chance of each element. dissect the data using statistical software and calculate mean, standard divagation, and RSD. Interpret the results and compare with the marker claim and acceptance criteria. Transfer the validated logical system to other laboratories or instruments. corroborate the system transfer by assaying replicate samples and comparing results.

RESULTS AND DISCUSSIONS

The benzyl group is often abbreviated "Bn", thus benzyl alcohol is denoted as BnOH. Benzyl alcohol is a colorless liquid with a mild, pleasant aromatic odor. It is a useful solvent due to its polarity, low toxicity, and low vapor pressure. Benzyl alcohol has a moderate water solubility and is miscible with alcohol and diethyl ether. The anion produced by deprotonation of the alcohol group is known as benzylate or benzyloxide .Benzyl alcohol is an aromatic alcohol used

in a wide variety of cosmetic formulations as a fragrance component, preservative, solvent, and viscosity-decreasing agent. Benzyl alcohol is used as a bacteriostatic preservative at a low concentration in intravenous medications, cosmetics, and topical drugs.

Instrument and Chemical Details

The current research work was done at the analytical research and development lab of Aspiro Pharma Limited, hence the below mentioned instruments, chemicals, and standards (Table 1 & 2) were used, which are already available in the lab.

Table 1: Details of Instruments

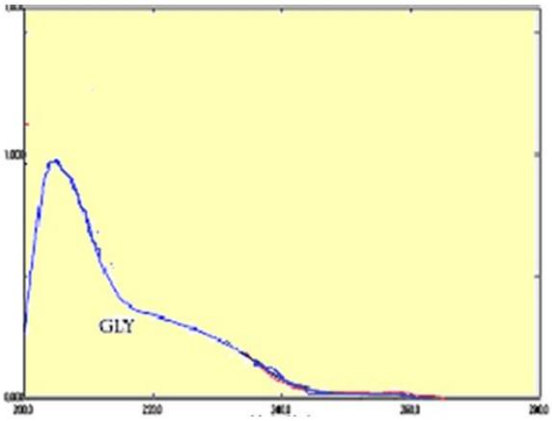
S. No	Name	Make	Model
01	HPLC system	Waters	2489 / 2998
02	Detector (UV/ PDA)	Sartorius	MSA225S-100-DA
03	Analytical balance	Thermo scientific	Orion Star A211
04	pH meter	PCI analytics	USB 3.5L 100
05	Ultrasonic bath	Waters	2489 / 2998

According to the literature, glycopyrrolate is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, and benzyl alcohol is moderately soluble in water and miscible in all alcohols.

Table 2: Details of Chemicals

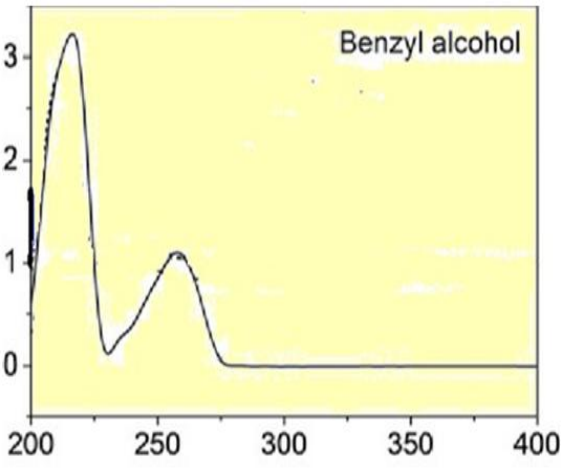
S. No	Name of the Chemical/ Reagent	Grade	Make	Catalogue no / Part No.
01	Potassium dihydrogen phosphate	Emparta	Merck	1.93205.0521
02	Anhydrous sodium sulfate	Emparta	Merck	1.9261.0521
03	Pentane-1-sulphonic acid sodium salt	Emparta	Merck	QG5Q651977
04	Sulfuric acid	Emparta	Merck	S0520
05	Orthophosphoric acid (88%)	Emparta	Merck	1.93003.0521
06	Methanol	HPLC	Merck	M0275
07	Acetonitrile	HPLC	Merck	A0754
08	Water	Milli-Q	NA	NA
09	Benzyl alcohol	HPLC	Merck	305197
10	Glycopyrrolate API	Available material at Aspiro AR&D lab		
11	Glycopyrrolate Injection	Available material at Aspiro AR&D lab		

Based on the knowledge which was gained through literature search and, as mentioned above, further development experiments were conducted and those experiments are mentioned below.



Graph 1: Glycopyrrolate UV spectrum

Based on the solubility data of glycopyrrolate and benzyl alcohol, the reverse phase HPLC chromatography mode with C18 stationary phase was chosen for separation and estimation assay of glycopyrrolate active moiety and benzyl alcohol preservative in glycopyrrolate liquid injection of 0.2 mg/mL. Since both glycopyrrolate and benzyl alcohol are having chromophores, based on available literature, the UV detection mode was chosen [graph 1-2] for estimation of glycopyrrolate and benzyl alcohol in glycopyrrolate liquid injections of 0.2 mg/mL.



Graph 2: Benzyl alcohol UV spectrum

CONCLUSIONS

Consistent, high-quality results from the analytical methods mentioned above are crucial for the approval and market release of the drug formulation. Standardization and quality control measures, including advanced analytical techniques like HPLC, LC-MS, and NMR, provide reliable data to confirm the presence and stability of the active compounds throughout the shelf life of the drug. Every therapeutic intervention, either a mono- or multicomponent drug, results in changes of intra- and intercellular signalling events, and finally leads to pleiotropic effects that affect an organisms' homeostasis. Consequently, more detailed dose-effect relationships can be further analysed via assays that focus on particular activities, and aid investigators in extracting the necessary parameters for assessing large-scale quantitative data. Optimize sample medication parameters similar as solvent composition, birth time, and temperature. dissect the multi-component medicine phrasings using the validated logical system. The integration of such datasets with comprehensive knowledge bases containing direct and indirect molecular interactions aids in deciphering the most prominent modulated pathways. Furthermore, the validation process ensures that multi-component formulations maintain therapeutic consistency and do not pose unintended risks to patients. As the pharmaceutical industry continues to innovate with complex formulations, robust validation practices remain critical to safeguarding public health and meeting regulatory requirements.

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