

A Review on Impurity Profile in Pharmaceutical Ingredients

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Abstract- Abstract for a drug impurity profile in pharmaceutical ingredients provides a concise summary of the impurities present in a specific pharmaceutical ingredient. It typically includes the following information
Pharmaceutical ingredient: Specifies the name and identity of the active pharmaceutical ingredient (API) or excipient being evaluated for impurities.

Impurity identification: Summarizes the impurities identified in the pharmaceutical ingredient. This can include information about impurity classes, chemical structures, and their origin (e.g., process-related impurities, degradation products).

Impurity levels: Provides an overview of the impurity levels found in the ingredient, including qualitative assessments of impurity abundance or quantitative values indicating impurity concentrations.

Analytical methods: Describes the analytical techniques used for impurity identification and quantification, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), or nuclear magnetic resonance (NMR).

Risk assessment: Includes a brief assessment of the potential risks associated with impurities detected in the pharmaceutical ingredient, considering factors like safety, toxicity.

Quality control measures: Highlights any quality control measures implemented to address impurities genotoxicity, carcinogenicity, and regulatory thresholds or limits. Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. Impurity can be of three types: Impurities closely related to the product and coming from the chemical or from the biosynthetic route itself, Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions, or the precursors which may be present in the final product as impurities.

Keywords: Impurities, HPLC, NMR, GC, MS, FDA, EMA, Humidity, Applications, Analytical Procedure.

INTRODUCTION

The impurity profile in pharmaceutical ingredients refers to the detailed analysis and characterization of impurities present in active pharmaceutical ingredients (APIs) or excipients used in drug formulations. Impurities can be defined as any component present in the pharmaceutical ingredient that is not the desired API or excipient. These impurities can originate from various sources such as raw materials, reagents, intermediates, or the manufacturing process itself. The impurity profile analysis involves the use of advanced analytical techniques such as chromatography (HPLC, GC), spectroscopy (UV-Vis, IR, NMR), mass spectrometry (MS), and other methods. These techniques allow for the separation, identification, and quantification of impurities present in pharmaceutical ingredients.

Regulatory authorities, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have established guidelines and regulations regarding impurity limits in pharmaceuticals. These regulations help ensure the quality, safety, and efficacy of drugs and require pharmaceutical companies to monitor and control impurities within acceptable limits.

By evaluating and understanding the impurity profile in pharmaceutical ingredients, manufacturers can implement appropriate measures to minimize impurities and improve product quality. These measures may include optimizing the manufacturing process, sourcing high-quality raw materials, implementing purification steps, and monitoring and controlling impurities through regular testing and analysis.

Overall, the impurity profile in pharmaceutical ingredients plays a critical role in ensuring the quality, safety, and regulatory compliance of pharmaceutical products. Thorough analysis and control of impurities contribute to the development and manufacture of effective and safe medications for patients.

Types of Impurity

- Organic Impurities
- Inorganic Impurities
- Residual Solvents
- Microbial Contaminants
- Physical Impurities
- Genotoxic Impurities

There are several types of impurities that can be present in pharmaceutical ingredients. These impurities can be categorized into different groups based on their origin, nature, and potential impact on drug quality and safety. Some common types of impurities found in pharmaceutical ingredients include:

Organic Impurities: These impurities are organic compounds that are not the intended active pharmaceutical ingredient. They can arise from various sources such as starting materials, intermediates, degradation products, or related substances formed during synthesis or manufacturing processes.

Inorganic Impurities: These impurities are typically inorganic compounds such as heavy metals, residual catalysts, or salts. They can be introduced through raw materials, solvents, or other manufacturing processes and can pose potential health hazards or cause stability issues in the drug formulation.

Residual Solvents: Residual solvents are volatile materials that can remain in the pharmaceutical ingredients after manufacturing. They are commonly used in the synthesis or purification process and can be detrimental to the safety and quality of the drug product if present above acceptable limits.

Microbial Contaminants: Microbial contaminants include bacteria, yeast, mold, or other microorganisms that may contaminate pharmaceutical ingredients during their production, storage, or handling. These contaminants can cause microbial growth, degrade the drug potency, or pose a risk of infection when administered.

Physical Impurities: Physical impurities are foreign particles or materials that are not supposed to be present in the pharmaceutical ingredients. They can include particles, fibers, metal fragments, or any other extraneous matter that might contaminate the formulation during manufacturing or packaging.

Genotoxic Impurities: Genotoxic impurities refer to impurities capable of damaging DNA or causing mutations. They can be potentially carcinogenic, and their presence in pharmaceutical ingredients should be limited to safe levels to minimize the risk to patients.

It is important for pharmaceutical manufacturers to identify, analyze, and control these impurities during the drug development and manufacturing process to ensure the safety, efficacy, and quality of the final pharmaceutical product. Regulatory guidelines, such as the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, provide specific limits and requirements for the identification and control of different impurity types in pharmaceutical ingredients.

Sources of Pharmaceutical Impurity

The sources of pharmaceutical impurities can vary depending on the specific ingredient and manufacturing process. Here are some common sources of impurities in pharmaceuticals:

Raw Materials: Impurities can originate from the starting materials used in the synthesis or manufacturing of pharmaceutical ingredients. Raw materials may contain impurities due to natural variations, contamination during cultivation or extraction, or inadequate purification processes.

Manufacturing Process: Impurities can be formed as by-products or degradation products during various stages of the manufacturing process, such as synthesis, purification, or formulation. Reactions, side reactions, or inadequate process controls can result in the formation of impurities.

Solvents and Reagents: Impurities can be introduced through solvents, reagents, or catalysts used in the manufacturing process. These substances may contain impurities due to inadequate purification or incomplete removal after the reaction.

Packaging and Storage: Impurities can also originate from the packaging materials used to store or transport pharmaceutical ingredients. Contamination from packaging components, such as rubber stoppers, plastic containers, or adhesives, can lead to impurity formation.

Environmental Factors: Environmental factors, such as air quality, water quality, or cross-contamination in manufacturing facilities, can contribute to impurity formation. Contaminants present in the air or water used during the manufacturing process can contaminate the pharmaceutical ingredients.

The degradation of pharmaceutical products and the formation of impurities

Can occur due to various factors, including environmental conditions, manufacturing processes, storage conditions, and even interactions with packaging materials. Here are some common sources of pharmaceutical impurities and degradation:

Hydrolysis:

This is the most common degradation pathway for pharmaceuticals. It involves the breaking of chemical bonds through water-mediated reactions. Hydrolysis can lead to the formation of various impurities, such as degradants, deamination products, or hydrolytic products.

Oxidation:

Oxidation reactions can occur due to exposure to air, light, or high temperatures. This can lead to the formation of impurities such as peroxides, hydro peroxides, or aldehydes. Oxidative degradation can result in reduced drug potency or the formation of toxic byproducts.

Photo degradation:

Some drugs are sensitive to light exposure, particularly in the ultraviolet (UV) range. Photo degradation reactions can lead to the formation of impurities, including photoproducts, isomerization products, or dimerization products.

Thermal degradation:

High temperatures during manufacturing or storage can cause thermal degradation of pharmaceuticals. This can lead to the formation of degradation products, including degradants, isomers, or impurities formed due to rearrangement of the drug molecule.

Interaction with packaging materials: Pharmaceutical products can interact with packaging materials, particularly if they contain reactive additives or leachable components. This can result in the formation of impurities, such as extractable or degradation products from packaging materials.

Reaction with excipients:

Pharmaceuticals can interact with excipients used in the formulation, leading to the formation of impurities. For example, some excipients may undergo hydrolysis or oxidation reactions, which can produce impurities or degradation products.

It is crucial for pharmaceutical manufacturers to understand and control these potential sources of impurities and degradation. Proper storage conditions, packaging selection, and process optimization can minimize the formation of impurities and ensure the stability and quality of pharmaceutical products..

Reagents, ligands, and catalysts-

These chemicals are less commonly found in APIs; however, in some cases they may pose a problem as impurities.

In general, an individual API may contain all of the above-mentioned types of organic impurities at levels varying from negligible to significant. A detailed investigation of impurities in semi-synthetic penicillin was performed both by the manufacturers and the different research groups. A review paper on penicillin's and cephalosporin's describes methods of isolation, detection, and quantification of degradation products, and antigenic polymeric by-products. Studies show the presence of traces of ampicillin polymers and hydrolyzed products in the API [7]. It has also been found that the presence of certain chemicals such as triethylamine has a degradative effect on the product. Ampicillin trihydrate samples having triethylamine content of 2000 ppm to 4000 ppm (determined by visual color method developed by Gist- Brocades, Delft, Holland)(7) were found to be stable under accelerated stability testing. However, the product showed appreciable degradation when triethylamine content became 7000 ppm. Recent pharmacopoeia [8] included the limit tests for the traces of impurities present in ampicillin and amoxicillin bulk raw materials. The residual solvents associated with these APIs have also been determined [7]. As the organic impurities are the most common product- as well as process-related impurities, it is the responsibility of both the manufacturers of APIs and the users (ie, formulators) to take care of these impurities according to ICH guidelines or compendia. In addition, for an optically active single isomer drug there could be enantiomeric impurities present in the API.

Enantiomeric impurities:

The single enantiomeric form of a chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index with a more favorable adverse reaction profile.9 However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard [9]. In any case, cost benefits as well as the patient's compliance need to be considered in selecting drugs. For the manufacturers of single enantiomeric drug (eutomer), the undesirable stereoisomers in drug control are considered in the same manner as other organic impurities. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), levalbuterol (R-albuterol), esomeprazole (S-omeprazole). Inorganic impurities may also derive from the manufacturing processes used for bulk drugs. They are normally known and identified and include the following

• Heavy metals-

The main sources of heavy metals are the water used in the processes and the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place. These impurities of heavy metals can easily be avoided using demineralized water and glass-lined reactors.

• Other materials (eg: filter aids, charcoal)-

The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants, and, in many cases, activated carbon is also used. The regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminations

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. It is very difficult to remove these solvents completely by the work-up process; however, efforts should be taken to the extent possible to meet the safety data. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into 3 classes. Solvents such as benzene (Class I, 2 ppm limit) and carbon tetrachloride (Class I, 4 ppm limit) are to be avoided. On the other hand, the most commonly used solvents such as methylene chloride (600 ppm), methanol (3000 ppm), pyridine (200ppm), toluene (890 ppm), N,N-dimethylformamide (880 ppm), and acetonitrile (410 ppm) are of Class II. Class III solvents (acetic acid, acetone, isopropyl alcohol, butanol, ethanol, and ethyl acetate) have permitted daily exposures of 50 mg or less per day. In this regard, ICH guidelines [10] for limits should be strictly followed. Apart from bulk drug-related impurities, the formulated form of API may contain impurities that form in various ways.

Method related A known impurity,

1-(2,6-dichlorophenyl)indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium if it is terminally sterilized by autoclave [11]. It was the condition of the autoclave method (ie, 123 + 2°C) that enforced the intramolecular cyclic reaction of diclofenac sodium forming the indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on the initial pH of the formulation. The concentration of the impurity in the resultant product in the ampoule exceeds the limit of the raw material in the BP..

Humidity:

For hygroscopic products, humidity is considered detrimental to both bulk powder and formulated solid dosage forms. Aspirin and ranitidine are classical examples.

Dosage form factors related

Although the pharmaceutical companies perform pre-formulation studies, including a stability study, before marketing the products, sometimes the dosage form factors that influence drug stability force the company to recall the product. Fluocinonide Topical Solution USP, 0.05%, (Teva Pharmaceuticals USA, Inc., Sellersville, Pennsylvania) in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub-potency [15]. In general, liquid dosage forms are very much susceptible to both degradation and microbiological contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are critical factors.

APPLICATIONS

Numerous applications have been sought in the areas of drug designing and in monitoring Quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, Extracted from natural products or produced by recombinant methods. The applications include Alkaloids, amines, amino acids, analgesics, antibacterial, anticonvulsants, antidepressant, Tranquilizers, antineoplastic agents, local anesthetics, macromolecules, steroids, miscellaneous Impurities in pharmaceutical ingredients play a crucial role in the development and safety of drugs. Here are some key applications of impurities in pharmaceutical ingredients:

Identification and characterization: Impurities help in identifying and characterizing the active pharmaceutical ingredient (API) and excipients used in drug formulations. By detecting and analyzing impurities, scientists can determine the composition and purity of the ingredients, ensuring the quality and efficacy of the drug.

Quality control: Impurities act as critical quality parameters for pharmaceutical ingredients. Regulatory authorities such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have established guidelines and limits for impurity levels in pharmaceuticals. These impurity limits help manufacturers maintain the quality and safety of drugs, ensuring that they meet regulatory requirements.

Safety evaluation: Impurities in pharmaceutical ingredients need to be evaluated for potential health risks. Some impurities can be toxic or have adverse effects on human health. Therefore, rigorous testing and risk assessment of impurities are conducted to ensure patient safety. Regulatory agencies require manufacturers to assess and control impurities, especially those with known or suspected genotoxic or carcinogenic properties.

Stability evaluation: Impurities can also influence the stability of pharmaceutical formulations. They may cause degradation or chemical changes in the drug, affecting its shelf-life, potency, and efficacy. Monitoring and controlling impurities during the formulation and storage of pharmaceutical ingredients are crucial to maintain stability and prolong the shelf-life of drugs.

Process optimization: Impurities in pharmaceutical ingredients can arise from various sources, including raw materials, manufacturing processes, or storage conditions. Analyzing impurities helps in optimizing manufacturing processes to minimize their formation. Through process optimization, manufacturers aim to reduce impurities, enhance product quality, and improve manufacturing efficiency.

Overall, the application of impurities in pharmaceutical ingredients is an essential aspect of drug development, quality control, and patient safety. By analyzing, monitoring, and controlling impurities, manufacturers can ensure the efficacy, safety, and stability of pharmaceutical products.

General Scheme for Drug Impurity Profiling

The general scheme for drug impurity profiling involves several steps. Here is an overview of the typical process:

Impurity identification: The first step is to identify the impurities present in a drug substance or formulation. Various analytical techniques such as liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy are used to identify impurities. Comparison with reference standards or databases helps in characterizing the impurities.

Impurity isolation: Once the impurities are identified, the next step is to isolate them. This can involve techniques such as preparative chromatography or extraction methods. Isolating impurities allows for further analysis and characterization, as well as determination of their quantities.

Impurity quantification: Quantifying impurities is crucial for assessing their levels and compliance with regulatory limits. Analytical techniques like LC or GC coupled with suitable detection methods (UV, MS) are used to quantify impurities. Calibration curves with reference standards are used to determine the impurity levels accurately.

Impurity profiling: Impurity profiling involves a comprehensive analysis of all impurities present in the drug substance or formulation. This includes the identification, quantification, and evaluation of impurities based on their origin, chemical class, structure, and potential risks. The impurity profile provides insights into the quality, safety, and stability of the drug.

Impurity control and mitigation: Based on the impurity profile, steps are taken to control and mitigate impurities. This may involve optimizing manufacturing processes, sourcing higher quality raw materials, or implementing adequate storage and transportation conditions. The objective is to reduce impurity levels and ensure the final drug product meets regulatory requirements.

Impurity monitoring: Continuous monitoring of impurities is essential throughout the drug development and manufacturing process. Stability studies are conducted to assess the degradation kinetics of impurities over time. Batch-to-batch monitoring ensures consistent impurity levels, and long-term stability studies assess impurity formation during the drug's shelf-life.

Regulatory compliance: Finally, the impurity profile is evaluated for regulatory compliance. Regulatory agencies, such as the FDA or EMA, have defined guidelines and limits for impurities in drugs. The impurity profile data are submitted as part of the regulatory documentation for drug approval, demonstrating compliance with quality and safety standards.

Method development: Develop analytical methods for the detection and quantification of impurities. This may involve techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR). Validated methods should be capable of separating and analyzing impurities with sufficient sensitivity and selectivity.

Impurity identification: Perform impurity identification to determine the chemical structure of individual impurities. This step involves isolating impurities using preparative chromatography or other separation techniques. Various spectroscopic techniques like NMR, MS, and infrared (IR) spectroscopy are employed to characterize the impurity structure. Reference standards and databases are used for comparison and identification.

Impurity classification: Categorize impurities based on their origin and chemical structure. Common impurity classifications include organic impurities, inorganic impurities, process-related impurities, degradation products, and residual solvents. This step helps in understanding the source of impurity and determining potential risks associated with each impurity class.

Quantification: Perform quantitative analysis of impurities to determine their levels or concentrations in the drug substance or drug product. Validation of the analytical method for impurity quantification is necessary to ensure accuracy, precision, and linearity. Quantification can be achieved by using calibration curves, internal standards, or other appropriate techniques.

Risk assessment: Evaluate the potential risks associated with each impurity. Determine impurity limits considering factors like safety, toxicity, genotoxicity, carcinogenicity, and regulatory guidelines. Risk assessment helps in setting appropriate quality specifications for impurities to ensure patient safety.

Process optimization: Identify potential sources of impurities during the drug manufacturing process. Optimize synthetic or manufacturing processes to minimize the formation of impurities. This may involve modifying reaction conditions, purification methods, or raw material selection to reduce impurity levels.

Stability studies: Conduct stability studies to evaluate the degradation pattern and formation of impurities over time. This is done under different storage conditions to simulate the expected shelf-life of the drug product. Stability studies help assess the impact of impurities on drug stability and provide valuable information for the formulation and packaging of pharmaceutical products.

Regulatory compliance: Ensure compliance with regulatory guidelines set by authorities such as the FDA, EMA, or other local regulatory bodies. Follow requirements for impurity identification and control throughout the drug development process and during commercialization.

By following this general scheme for drug impurity profiling, pharmaceutical companies can ensure the quality, safety, and efficacy of their drug products, minimizing the risks associated with impurities.

Acceptance Criteria for Impurities

Acceptance criteria for pharmaceutical impurities in quality control (QC) are established to ensure that the levels of impurities present in the pharmaceutical product are within acceptable limits. These criteria are determined based on regulatory guidelines, pharmacopoeial standards, and specific product requirements. Here are some common considerations for setting acceptance criteria for pharmaceutical impurities in QC:

Regulatory Guidelines: Pharmaceutical regulatory agencies, such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA), provide guidelines on the maximum allowable levels of impurities for different drug products. These guidelines help ensure the safety and efficacy of pharmaceuticals.

Analytical Procedures

- **Method Development Method development**
- **Spectroscopy**
- **Chromatography**
- **Radiation Techniques**

There are several analytical techniques commonly used for the detection and quantification of impurities in pharmaceuticals. The choice of technique depends on the nature of the impurity, its concentration level, and the specific requirements of the pharmaceutical product. Here are some common analytical techniques for finding impurities in pharmaceuticals:

High-Performance Liquid Chromatography (HPLC): HPLC is one of the most widely used techniques for impurity analysis. It separates and quantifies impurities based on their different retention times in a chromatographic column. HPLC can be coupled with various detectors such as UV-Vis, fluorescence, or mass spectrometry for identification and quantification.

Gas Chromatography (GC): GC is mainly used for volatile and semi-volatile impurities. It separates impurities based on their boiling points and vapor pressures. GC is commonly coupled with detectors like flame ionization detector (FID), mass spectrometry (GC-MS), or electron capture detector (ECD) for identification and quantification.

Thin-Layer Chromatography (TLC): TLC is a simple and cost-effective technique used for qualitative analysis of impurities. It involves the separation of impurities based on their differential migration on a thin layer of stationary phase. The separated spots can be visualized by various staining or visualization methods.

Capillary Electrophoresis (CE): CE is a powerful technique for separating charged impurities based on their electrophoretic mobilities in a capillary filled with an electrolyte. CE is commonly used for the analysis of charged impurities or related substances.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy is used to identify and characterize organic impurities based on their unique infrared absorption spectra. It can be performed in solid, liquid, or gas phases for impurity analysis.

Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR spectroscopy is used to identify and quantify impurities based on their unique resonance signals in the NMR spectrum. It provides valuable structural information about the impurities and is particularly useful for complex organic molecules.

Mass Spectrometry (MS): Mass spectrometry is a powerful technique for identification and quantification of impurities. It can provide information about the molecular weight, fragmentation pattern, and elemental composition of the impurities. Common MS techniques used include GC-MS, liquid chromatography-mass spectrometry (LC-MS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).

These are just a few examples of the analytical techniques used for finding impurities in pharmaceuticals. Depending on the specific impurity and its characteristics, other techniques like X-ray diffraction (XRD), elemental analysis, or specific spectroscopic methods may also be employed.

CONCLUSION

In conclusion, the profiling and analysis of impurities in pharmaceuticals is of utmost importance to ensure the safety, efficacy, and quality of the drugs. Various analytical techniques are employed to detect and quantify impurities in pharmaceutical products.

High-performance liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography (TLC), capillary electrophoresis (CE), Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) are among the commonly employed techniques.

HPLC and GC are particularly useful for separating and quantifying impurities based on their different properties such as retention time or boiling point. TLC is a cost-effective method for qualitative analysis, while CE is suitable for charged impurities. FTIR and NMR spectroscopy provide valuable information about the structure of impurities, while MS allows for their identification and quantification at a molecular level.

REFERENCES:

1. International Conference on Harmonization (2000) Draft Revised Guidance on Impurities in New Drug Substances. Federal Register Q3A(R) 65 (140): 45085.
2. International Conference on Harmonization (2000) Draft Revised Guidance On Impurities in New Drug Products. Federal Register Q3B(R) 65 (139): 44791.
3. International Conference on Harmonization (1997) Impurities, Q3C- Guidelines for Residual Solvents, Q3C. Federal Register 62(247): 67377.
4. International Conference on Harmonization (1999) Specifications, Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical substances 65 (146):67488.
5. Parimoo P., et al; „A Text Book of Pharmaceutical Analysis“, CBS publishers and distributors, New Delhi, 1998, 14.
6. Van Krimpen, PC, Van Bennekom WP, and Bult A. Penicillins and cephalosporins: Physicochemical properties and analysis in pharmaceutical and biological matrices. Pharm Weekbl [Sci].1987; 9:1-23.
7. J, Roy Mohammad G, and Banu A. Pharmaceutical analysis and stability of locally manufactured ampicillin trihydrate. Indian Drugs. 1993; 5(30)5:211-218.
8. British Pharmacopoeia. The British Pharmacopoeia Commission, Vol 1. The Stationary Office, London, UK; 2001:123,128.
9. Riley TN. Steric aspects of drug action. Pharmacist. 1998;23(3):40-51.
10. International Conferences on Harmonization, Impurities- Guidelines for residual Residual solvents. Q3C. Federal Register. 1997; 62(247):67377.