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Pharmaceutical impurities: A review of their importance in drug safety and efficacy

Riddhi Prajapati[1], Harshil Patel[2], Dr. Vrunda Shah[3]
1.Assistant professor, Department of pharmacy, Swaminarayan university, at & po, Ahmadabad Mehsana highway, saij, kalol, Gujarat 382725
2.The university of Toledo, Toledo, USA
3. Arihant school of pharmacy and Bio research Institute, Adalaj, Gandhinagar, Gujarat
Corresponding author: Riddhi Prajapati*

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ABSTRACT:
In the field of pharmaceutical chemistry, impurities are considered as unwanted chemicals that present in the therapeutically active pharmaceutical compounds (API). They are unusually potent and expected to produce toxicity and reactivity; hence it may be shows unexpected pharmacological actions which are harmful to human health. The control of impurities is currently a critical issue to the pharmaceutical industry. The most possible source of impurities is the synthesis that involves various steps, i.e. from starting materials to finished products through the intermediate steps. Impurities in drug substances and drug products are critical issues in the generic drugs and have significant impact on the approvals of drugs hence International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) guidelines introduce the identification and qualification procedures for them, by using various analytical techniques like TLC, LC, GC, MS, LC-NMR, IR, UV, GC-MS, LC-MS, NMR etc.

KEYWORDS: API **(**active pharmaceutical compounds), Impurities, International Conference on Harmonization (ICH), Instruments.

I. INTRODUCTION:

The level of impurities present in a pharmaceutical product is the criterion to determine the quality of drug substance or drug product. Impurities with their presence, even in trace level may affect safety and efficacy of the drug. Hence formation of impurities should be eliminated or controlled during the manufacturing process and storage. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines [1-8] were developed by joint efforts of industry and regulators through scientific consensus from United States, European Union, and Japan to provide regulations and guidance to industry to manufacture high quality pharmaceutical products for human use.

In recent days, considerable emphasis is given on the improvement of the quality of life. To improve the quality of life and to increase life expectancy medicines play important role. In spite of the uses of medicines, impurities present in the drug substances pose serious threat to the human beings. Now a days the law enforcement authorities and drug regulatory agencies [9-11] across the globe are emphasizing on impurity profiling of the drug substance drug products.

Identification and characterization of unknown impurities is the most challenging aspect of developing process technology as well as a suitable analytical method to manufacture a high purity drug substance because impurities can arise during manufacturing process, degradation, and storage.

The presence of impurities in an active pharmaceutical ingredient (API) can have significant impact on the quality, safety, and efficacy of drug products. In addition, understanding of impurities in the chemical process is key to production of high-quality drug substances [12-18]. Therefore, to avoid generating the undesired impurities in the first place, it's mandatory to characterize them at early stage to understand the mechanism of their formation. ICH guidelines indicate that impurities at or above 0.05% in the drug substance require identification [3] depending on the maximum daily dosage.

The daily intake of unknown impurity by humans is deciding factor for the limit of unknown impurity to be controlled in the drug substance. Daily oral intake of 1 mg of an unknown impurity will generally meet the regulatory requirements.

Impurity profiling is the process of identification, characterization, and quantitative

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determination of known and unknown impurities (organic, inorganic and residual solvents) present in the drug substances and drug products. As a common practice, efforts should be made to identify and characterize all unknown impurities in the drug substance due to the ever-increasing demand from regulatory agencies to manufacture high purity drug substances. Thus, the analytical activities concerning the impurity profiling in drugs are among the most important issues in modern pharmaceutical analysis which is evident by the recent publications [14-17]. Characterization of unknown impurities also critical to ensure that unknown impurities are non genotoxic. Genotoxic impurities can cause chromosomal breaks, chromosomal rearrangements and gene mutations resulting in significant risk for carcinogenicity and other toxic effects [19-20]. Regulatory agencies worldwide issue multiple guidelines for the control and testing of impurities, degradation products, residual solvents, and genotoxic impurities periodically.

To ensure that unknown impurity will not pose any safety risk, impurity profile (both qualitative and quantitative) is mandatory. The details of impurities, their origin, source, nature, and characterization details are required by various regulatory agencies viz. the ICH Common Technical Document 9CTD, M4Q

(R1) [21], EMEA guidelines [22], USFDA ANDA [9], EDQM [10], etc. in the dossier or drug master file applications for new drug substances and drug products. High performance liquid chromatography (HPLC) is an important tool for impurity profile analysis for identification and control the quality of active pharmaceutical ingredients for safety assessment and their manufacturing process. In recent years, liquid chromatography coupled with mass spectrometry (LC-MS/MS) has emerged as an essential tool for structural elucidation of impurities present in the drug substance [23-27]. With the advent of ionization sources such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), high performance liquid chromatography in combination with multistage mass spectrometry has become a versatile analytical tool for the determination of molecular mass of impurities, confirmation of positional isomers as well as their structural information through tandem mass spectrometry (MS/MS, MS) experiments. Evaluation of MS fragmentation data in conjunction with the process chemistry knowledge helps the scientists in proposing the potential structures of impurities.

It is evident that impurity profiling is the most challenging and critical analytical activity to ensure the quality of pharmaceutical product as well to meet the most stringent regulatory requirements. Hence it was felt necessary to develop chromatography and mass spectrometric methods for qualitative and quantitative determination of impurities associated with anti-Alzheimer, anti-epileptic and other selected drugs.

1.1 Impurities:

1.1.1 Definition of Impurity

According to ICH guidelines, an impurity is defined as “any component of the new drug substance that is not the chemical entity or any component of the drug product that is not the drug substance or an excipient in the drug product or any material that affects the purity of the material of interest [10]”.

Impurities may have different pharmacological activity or toxicity depending on chemical structure.

1.1.2 ICH Guidelines

The ICH Q3A (R2) guidelines are to provide guidance for the content and qualification of impurities in new drug substances produced by chemical synthesis. ICH guidelines classify impurities in drug substances produced by chemical synthesis into three categories (a) Organic impurities (Process and drug related), (b) Inorganic impurities, (c) Residual solvents (organic volatile impurities).

1.1.1.1 Organic Impurities

The unwanted organic chemical entities that remain with the drug substance or drug product are defined as organic impurity. Broadly the organic impurities can be classified as 1) starting materials 2) intermediates 3) process impurities 4) byproducts5) organic reagents and solvents.

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Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[2]	Qualification Threshold[3]
≤2 g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2 g/day	0.03%	0.05%	0.05%

Table 1: limit of dose(g/day)

1. Drug substance administered per day
2. Higher reporting thresholds should be scientifically defined
3. Lower thresholds should be considered for unusually toxic impurities

Starting materials and intermediates are the chemical entities which are used to synthesize a drug substance by undergoing various chemical reactions. UN-reacted starting materials involved during the short synthesis, convergent synthesis can potentially remain in the reaction mass unaltered, but synthesis involving more steps the likelihood of starting material remaining as such and as derivative are very likely. Impurities like positional isomers present in the staring material could follow the same reaction pathways as the starting material itself, and the reaction products could carry over to the final product as isomeric impurities in the final drug substances/drug products.

Intermediates generated during last steps of synthesis can potentially remain in the synthetic and purification process and are the source impurities in the final product. Side-reactions are very common during the synthesis of drug substances. By-products from the side-reactions are among the most common process impurities in drugs. By- products can be formed through a variety of side reactions, such as incomplete reaction, overreaction, isomerism, rearrangement, dimerization or unwanted reactions between starting materials or intermediates with chemical reagents. Based on the nature of the desired chemical compound and functionality present in the chemical compound molecule may undergo series of reaction during storage e.g. formation of N-oxides during storage of nitrogenous compounds. Chemical reagents, ligands, and catalysts used in the synthesis of a drug substance can be carried over to the final products as trace level impurities.

ICH guideline indicates that any impurity at a level greater than the identification threshold in the drug substance and the degradation product observed during stability study at a level greater than the identification threshold should be identified. The level of organic impurities in drug substance is controlled based on maximum daily dose and total daily intake (TDI) of the impurities.

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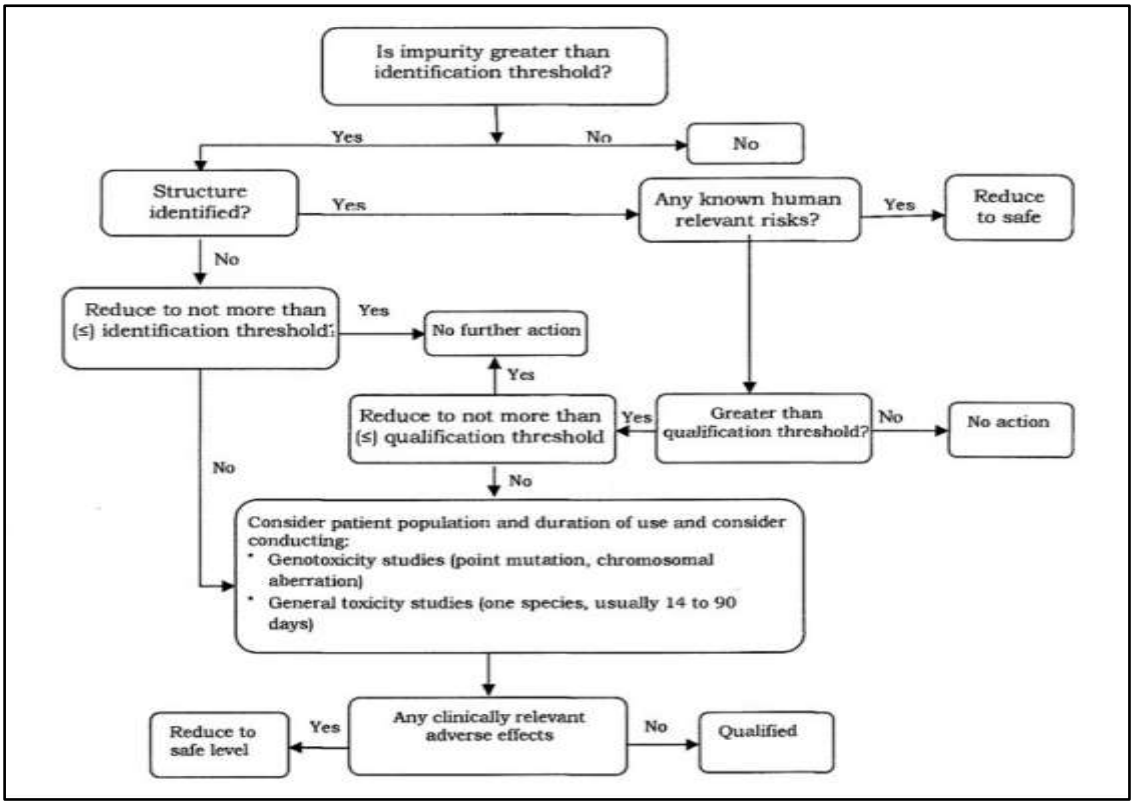

Fig. 1 ICH Decision tree for identification and qualification of impurities

ICH threshold for control of organic impurities in new drug substance is tabulated in table 1. Adequate scientific literature reference may be available to qualify an impurity, if literature reference are not available additional toxicological study is required, if the level of impurity cannot be reduced. The ICH decision tree for consideration of safety studies is given in Fig. 1. These thresholds are not applicable for genotoxic impurities. Genotoxic impurities should be controlled based on compound specific risk assessment as per threshold of toxicological concern (TTC) limits as per EMEA CHMP recommendations [19-20].

1.1.1.2 Inorganic Impurities

Inorganic impurities are carry forwarded from starting materials, introduced or generated during the manufacturing process of drug substance (e.g., catalyst, salts, metallic impurities, heavy metals). These impurities are controlled by pharmacopoeial or other specific tests [6].

1.1.1.3 Residual Solvents

Residual solvents are defined as organic volatile chemicals that are used or produced during various steps of drug substance manufacturing process (e.g., solvents for chemical reaction, extraction, washings, and crystallization) or excipients (e.g., solvents for wet granulation and coating), or in the preparation of drug products. Residual solvents are known impurities hence identification of residual solvents can be performed by either GC-HS or GC-HS-MS. Drying process removes the solvents from the drug substance, however solvents may remain with the drug substance in trace levels. As there is no therapeutic benefit from residual solvents, all residual solvents should be removed to meet product specifications. The control of residual solvents in the drug substance is described in the ICH Q3C Guideline [5]. Residual solvents are divided by a risk assessment approach into three classes.

Class 1 solvents: solvents under this category are environmental hazards and carcinogens, therefore, these solvents should be avoided in the manufacturing process of drug substance and drug products. If unavoidable, the level should be strictly controlled below the acceptable limits.

Class 2 solvents: These are possible agents of neurotoxicity or teratogenicity. Class 2 solvents are controlled according to the PDEs (Permitted Daily Exposure) and Maximum Daily Dose (Option 1

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and Option 2). ICHQ3C [5] provides PDEs of all Class 2 solvents.

Class 3 solvents: These solvents are non-toxic or with low toxic potential to humans. These solvents can be controlled in the drug substance with loss on drying test with a limit of NMT 0.5% or 50 mg per day or less would be acceptable without justification.

1.2 Sources of Impurities

1.2.1 Sources of organic Impurities

Impurities can originate from several sources; such as starting materials, intermediates, penultimate intermediates, byproducts, impurities in the starting materials, isomeric impurities in starting materials, reagents, ligands and catalysts related impurities, degradation products, impurities arising during storage, stereo chemistry-related impurities, polymorphic impurities, formulation related impurities, functional group related typical degradation and impurities on ageing. Among all the above-mentioned sources, degradation impurities, stereo isomeric impurities and polymorphic impurities are more critical in safety and efficacy point of view [16-18], hence a discussion on these impurities is given below

1.2.2 Degradation impurities

Degradation of the drug substance is one of the main sources of impurities in both bulk drug and formulated product. The manufacturing conditions such as heat, humidity, solvent, pH, light, etc. may degrade the drug substance. Interactions of drug substances with other chemical entities during the formulation process can also lead to degradation of drug substance of formation of new impurity. Forced degradation studies of drug substance and the characterization of major degradants will provide useful information about degradation pathway, control of degradants and to select appropriate storage conditions [16-18].

1.2.3 Functional group-related typical degradation

Hydrolysis is the common phenomenon for ester type of drugs, especially in liquid dosage. Oxidative degradation of drugs, hydroxyl group directly bonded to an aromatic ring, conjugated dienes, heterocyclic aromatic rings, nitroso and nitrite derivatives, and Aldehydes are all susceptible to oxidative degradation. Photolytic cleavage includes example of pharmaceutical products that are exposed to light while being manufactured as solid or solution, packaged, or when being stored. Photochemical energy creates free radical intermediates, which can perpetuate chain reactions. Most compounds will degrade as solutions when exposed to high-energy UV exposures.

1.2.4 Stereo isomeric Impurity

The three-dimensional orientation of pharmacophoric group in biological environment greatly affects pharmacological properties. Organic molecules possess four different substitutions in one carbon is known as chiral carbon but now a days the word chiral has been replaced by asymmetric carbon. The term asymmetric is more appropriate because some of the Spiro and biphenolic compound does not possess the chiral centre but still exists in stereo isomeric forms because of the presence of asymmetry in the molecule. The stereo isomeric impurity can be enantiomeric impurity or diastereomeric impurity or epimoric impurity. Determinations of impurities in this category often require chiral chromatographic techniques.

1.2.5 Polymorphic impurities

The phenomenon of drug substances to exist in multiple crystalline and solid forms is known as polymorphism. Polymorphism is the term used to indicate crystal systems where substances can exist in different crystal packing arrangements, all of which have the same elemental composition, whereas in solvent molecules are being incorporated in the crystal packing arrangements, withdifferent elemental composition. When water is the solvent within the crystal lattice such compounds are known as hydrates (also known as pseudo polymorphs). Amorphous materials do not have three-dimensional long-range order and transforms into liquid, upon heating through glass transition process. Although polymorphs are not impurities, an understanding of the crystalline forms, hydration or solvation states, or amorphous nature is critical to the overall characterization of the drug substance [28]. 80-90% of organic compounds can exist in polymorphic forms, physicochemical properties of polymorphic compounds can have significant differences in solubility, density, melting point, colour, stability, hygroscopic nature, and can have an impact on bio-availability and bioequivalence. As per regulatory guidelines drug substance manufacturer has to demonstrate that polymorphic form active pharmaceutical ingredient manufactured

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consistently has the same purity and remains stable throughout the shelf life.

Characterization of polymorphs are performed using, [25-29]

1) Powder x-ray diffraction (PXRD),
2) Infrared spectroscopy (IR),
3) Differential scanning calorimetry (DSC) / modulated differential scanning calorimetry (MDSC), hyper differential scanning calorimetry (HDSC),
4) Microscopy,
5) Raman spectroscopy,
6) Solid state nuclear magnetic resonance spectroscopy
7) Thermo gravimetric analysis (TGA).

1.3. Identification and Characterization of Impurities

Identification and characterization of impurities is the most critical and challenging activity to ensure the quality of drug substances and drug products. Structural elucidation of unknown impurities will eventually help scientists in understanding the pathway of impurity formation, to propose control mechanism and to prevent their formation. Generally structural elucidation of unknown impurities is complex and tedious due to the complexity of organic reactions. A general methodology usually followed by the scientists for impurity profiling is showed in Fig. 2. Characterization of impurities is a multistage process, involving separation, detection, identification, isolation, synthesis and structural elucidation, by combination of liquid chromatography, LC-MS/MS, UV, IR, NMR and elemental analysis [14, 16-18]. Techniques such as single crystal-XRD can also be used for ultimate characterization of unknown compounds.

1.3.1 Separation methods

The primary and most important activity of characterization of impurity is the separation of impurities by chromatographic techniques such as high performance liquid chromatography (HPIC), ultra performance liquid chromatography (UPLC) gas liquid chromatography (GLC), gas liquid chromatography with head-space sampler (GC-HS), liquid chromatography with head-space sampler coupled with mass detector (GC-HS-MS), thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC) and capillary electrophoresis (CE).

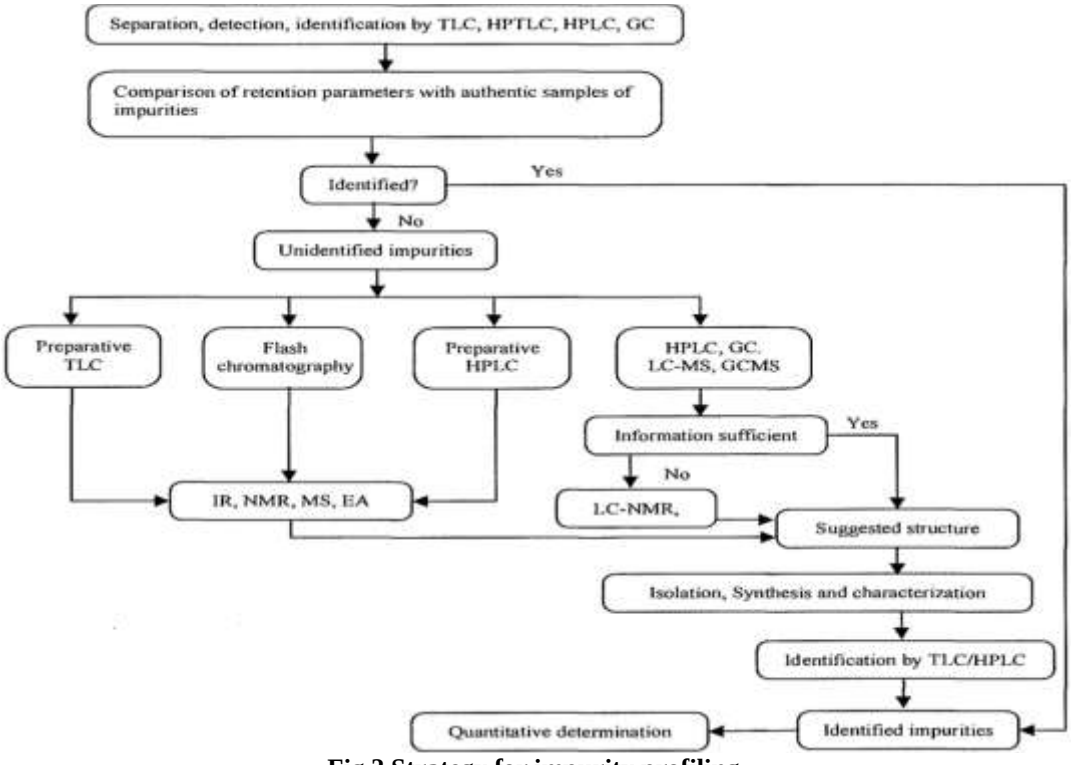

Fig.2 Strategy for impurity profiling

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Reliable and meaningful analytical data is needed to manufacture a new drug in various stages of development (24-26). 1. For the analytical method development sample set selection is required. 2. Screening of chromatographic conditions and phase, typically using the linear solvent strength model of gradient elution. 3. Optimization of the method to fine-time parameters related to ruggedness and robustness. Assuring the safety of a new pharmaceutical product or drug substance demands that the new drug substance should meet the established standards for purity and safety as a chemical entity or when

admixed with animal feeds for toxicity studies or when formulated with or without pharmaceutical excipients for human use. Furthermore, it should exhibit excellent stability throughout its shelf life. These requirements mandate that the analytical method (s) employed for this purpose should be sufficiently sensitive to measure low levels of impurities. This has resulted in development of analytical techniques that are appropriate for measurement of trace/ultra-trace levels, i.e., submicrogram quantities of a variety of chemical entities.

The role of reference standards Reference standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standards are needed not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates and excipients. Analytical techniques: · Spectroscopic methods · Chromatography methods or Separation methods Spectroscopic methods The following spectroscopic measurement techniques have been used for characterizing impurities; most of these are very useful as detectors for chromatographic methods: 1. Ultraviolet (UV). 2. Infrared (IR). 3. Raman spectroscopy. 4. Mass spectrometry (MS). 5. Nuclear magnetic resonance (NMR). Separation methods The following methods can be used for separation of impurities and degradation products: 1. Capillary electrophoresis (CE). 2. Chiral separations. 3. Gas chromatography (GC). 4. High-pressure liquid chromatography (HPLC). 5. Supercritical fluid chromatography (SFC). 6. High performance thin-layer chromatography (HPTLC). REGULATORY PERSPECTIVE The International Conference on Harmonization (ICH)

II. CONCLUSION:

This review provides a perspective on impurities in drug substance and drug product. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more attention from the public. Also provides the valuable information about the impurities, their types and classification, various analytical techniques for the determination, qualification of impurities and critical factors to be considered while preparation of the bulk drugs. Now a day, it is mandatory requirement in various pharmacopoeias to know the impurities present in API's. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biologicalsafety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

REFERENCES:

[1]. ICH, Q1A(R2), Stability Testing of New Drug Substances and Products (2006)
[2]. ICH Guidelines, Q2 (RI), Validation of analytical procedures: test and methodology. November 2005.
[3]. ICH, Impurities in new drug substances Q3A (R2), in: International Conference on Harmonization, IFPMA, Geneva (Switzerland), 2006.I
[4]. ICH, Impurities in new drug products Q3B (R2), in: International Conference on Harmonization, IFPMA, Geneva (Switzerland), 2006.
[5]. ICH, Impurities: Guideline for residual solvents Q3C (R5), in: International conference on Harmonization, IFPMA, Geneva (Switzerland), 2011.
[6]. ICH, Impurities: Guideline for metal impurities Q3D, in: International Conference on Harmonization, IFPMA, Geneva (Switzerland), 2009.
[7]. ICH, Q6A, Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (2006)
[8]. ICH, Q7, Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients. (2006)
[9]. U.S. Food and Drug Administration. Center for Drug Evaluation and Research, www.fda.gov/CDER/GUIDANCE
[10]. European Directorate for the Quality of Medicines and HealthCare, http:// www.edqm.eu/site/Homepage-628.html

[11]. Australian government, Department of Health and Ageing, Therapeutic goods administration, www.tga.gov.au
[12]. E.M. Sheldon, J.B. Downar, Development and validation of a single robust HPLC method for the characterization of a pharmaceutical starting material and
[13]. omed. Anal. 23 (2000) 561-572.
[14]. E. M. Sheldon, Development of a LC-MS complete heart-cut approach for the characterization of a pharmaceutical compounds using standard instrumentation, J. Pharm. Biomed. Anal. 31 (2003) 1153-1166.
[15]. D. Bartos, S. Gorog, Recent advances in the impurity profiling of drugs, Curr. Pharm. Anal. 4 (2008) 215-230.
[16]. R.N. Rao, V. Nagaraju, An overview of the recent trends in development of HPLC methods for the determination of impurities in drugs, J. Pharm. Biomed. Anal. 33 (2003) 335-377.
[17]. S. Gorog (Ed.), Determination of impurities in drugs, Elsevier sciences, Amsterdam, 1999.
[18]. S. Ahuja, Impurities evaluation in pharmaceuticals, Marcel Dekker, New York, 1998.
[19]. S. Hussein, R.N. Rao, Monitoring of process impurities in drugs, in: Z. Deyl, I. Miksik, F. Taglirao, E. Tesarova (Eds.), Advanced chromatographic and electromigration methods in bio-sciences, Elsevier Science, Amsterdam, Netherland, 1998, pp. 834-888.
[20]. L. Müller, R.J. Mauthe, C.M. Riley, M.M. Andino, D.D. Antonis, C. Beels, J. DeGeorge, A.G.M.D. Knaep, D. Ellison, J.A. Fagerland, R. Frank, B. Fritschel,
[21]. S. Galloway, E. Harpur, C.D.N. Humfrey, A.S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D.K. Ness, M.R. O'Donovan, M.D. Smith, G. Vudathala, L. Yotti, A rationale for determining, testing, and controlling specific impurities in pharmaceuticals that possess potential for genotoxicity, Regul. Toxicol. Pharmacol. 44 (2006) 198-211.
[22]. European Medicines Agency Evaluation of Medicines for Human Use, Committee for medicinal products, Guidelines on the limit of genotoxic impurity CPMP/ICH/174/95, 1998.
[23]. ICH, The common technical document for the registration of pharmaceuticals for human use: Quality - M4Q (R1), in: International Conference on Harmonization, IFPMA, Geneva (Switzerland), 2002.
[24]. CPMP, Chemistry of new active substances (CPMP/QWP/130/96), Committee for Proprietary Medicinal Products, EMEA, London (UK), 2004.
[25]. S. Görög (Ed.), Identification and determination of impurities in drugs, Elsevier Science, Amsterdam, Netherland, 2000.
[26]. J. Fiori, R. Gotti, C. Bertucci, V. Cavrini, Investigation on the photochemical stability of lercanidipine and its determination in tablets by HPLCUV and LC-ESI-MS/MS, J. Pharm. Biomed. Anal. 41 (2006) 176-181.
[27]. J. Wang, V. Krishnamoorthi, E. Wang, C. Yang, D. Baptista, X. Wu, M. Liu, M. Gardner, P. Elkins, J. Hines, P. Liu, LC/MS characterization of impurities and degradation products of a potent anti-tumor peptidic dimer, CU201, J. Pharm. Biomed. Anal. 51 (2010) 824-833.
[28]. A. Schneider, L.A. Wessjohann, Comparison of impurity profiles of orlistat pharmaceutical products using HPIC tandem mass spectrometry, J. Pharm. Biomed. Anal. 53 (2010) 767-772.
[29]. I. Pasáková, P. Kovarikova, R. Kucera, J. Klimes, J. Sochor, A. Hrabálek, Development and validation of an LC-ESI-MS ion-trap method for analysis of impurities in transkarbam 12, a novel transdermal accelerant, Chromatographia 69 (2009) 977-983.
[30]. N. Ching, T. Rades, J. Aaltonen, An overview of recent studies on the analysis of pharmaceutical polymorphs, J. Pharm. Biomed. Anal. 55 (2011) 618-644.