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Review Article

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Impurity Profiling for Pharmaceutical Product by using Different Analytical Techniques: An Overview

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ABSTRACT

Impurities are not acceptable in drug formulation. It is considered as unwanted chemicals or organic material which remains with active pharmaceutical ingredient (APIS). The impurity is produced either during formulation or ageing of both APIs and finished dosage form.
The existence of these undesired chemicals may influence the safety and the efficacy of the pharmaceutical finished products. In this review article we have discussed about the methodologies adopted by United States of Europe for impurity profiling given by their respective regulatory authorities. Impurity profiling, calculation, and methodologies used to represent impurities in dosage regulation related documents.

Keywords: Impurity, *Residual solvent*

INTRODUCTION

Historically the impurities in pharmaceuticals are unwanted chemicals that remain with the Active Pharmaceutical Ingredients (APIs) or develop during formulation or upon aging of both API and Formulation. The presence of these unwanted chemicals even in trace amount may influence the efficacy and safety of pharmaceutical product. The control of impurities is currently a critical issue to the pharmaceutical industry.

International Conference on Harmonization (ICH) formulated guidelines regarding the control of impurities. This review outlines the description of different types and origins of impurities and degradation routes with specific examples. In the present era, there is a tremendous upsurge of the impurity profiling of pharmaceutical products [1]. Presence of impurities in trace quantity in drug substance or drug product is inevitable. Therefore, their level should be controlled and monitored. They can reinforce or diminish the pharmacological efficacy of the active ingredients (APIs). Sometimes, the effect produced by impurities can be teratogenic, mutagenic or carcinogenic.

LITERATURE REVIEW

These can jeoparadize the human health by affecting safety, quality and efficacy (QSE) of the product. Hence, API impurity profiling (identification, isolation and characterization) is required. Their limits and threshold values should comply with the limits set and specified by official bodies and legislation (pharmacopoeia and International conference on Harmonization (ICH) guidelines). This is very important when company files investigational new

drug application (IND) or Abbreviated new drug application (ANDA). However, monitoring and controlling of impurity is different for different people. Therefore, there must be unified system to ensure that every one speak the same language when addressing "issues related to impurities".

For pharmaceutical products, impurities are defined as "substance in the product that are not the API itself or the excipients used to manufacture i.e. impurities are unwanted chemicals that remain within the formulation or API in small amounts which can influence QSE, thereby causing serious hazards. According to ICH guidelines on impurities in new drug substances and new drug products, identification of impurities below the 0.1% level is not necessary unless the potential impurities expected to be unusally potent or toxic. In all cases, impurities should be qualified. If data related to qualification of the proposed specification level of an impurity is not available then studies were required to obtain such data. According to ICH, the maximum daily dose qualification threshold is as follows [2].

CLASSIFICATION OF IMPURITIES

The three major categories of Pharmaceutical impurities:

- Organic impurities
- Inorganic impurities (elemental)
- Residual solvents

Organic Impurities in Drug Substances and Drug Products

As part of an ongoing monograph modernization initiative, the United States Pharmacopeial Convention (USP) is updating general chapter Impurities in Drug Substances and Drug Products 1086 and proposing this new chapter that addresses organic impurities testing for articles subject to applicable monographs in component of the USP, including the United States Pharmacopeia–National Formulary. This new chapter has been created to align with current scientific and regulatory approaches and to help ensure the appropriate control of organic impurities and degradation products in drug substances and drug products. The goal is to provide a science-based approach for the control of impurities in relevant monographs, and thereby to ensure the quality of the product as it relates to safety and efficacy [3].

Also excluded from this chapter are the following:

- Extraneous contaminants that should not occur in drug substances or drug products and are more appropriately addressed as good manufacturing practice issues.
- Polymorphic forms
- Impurities arising from residual solvents (see Residual Solvents 467)
- Elemental impurities (see Elemental Impurities Limits 232 Elemental Impurities Procedures 233)

Identification of impurities in drug substances and drug products: Drug substance and drug product organic impurities shall include process-related impurities that result from the manufacturing process, and degradation products observed during manufacture and stability studies. Identification of impurities shall be based on sound scientific appraisal of potential degradation pathways in the drug substance and drug product, including those impurities that arise from interactions of the drug with the environment, exipients, or the primary container closure system. Impurities observed in stability studies conducted at the recommended storage conditions shall be identified when above the identification threshold, which can be established using currently applicable regulations or other scientific means. Impurities present at a level below the identification threshold generally do not require identification.

Analytical procedures for impurities and degradation products: Manufacturers shall validate analytical procedures and demonstrate their suitability for the detection and quantitative of impurities. Manufacturers of drug substances and drug products shall refer to current applicable regulatory guidance and shall develop control strategies for establishing acceptance criteria for impurities. These acceptance criteria shall be justified with appropriate safety considerations.

These procedures shall be verified or validated and shall be suitable for their intended purpose. For impurities that are known or suspected to be highly toxic (e.g., genotoxic) or that produce undesired pharmacological effects, the quantitative/detection limit of the analytical procedures shall be commensurate with the acceptance criteria [4].

Reporting impurities and degradation products: Impurities present at a level above the reporting threshold (i.e., the disregard limit) shall be reported according to the relevant analytical methods. The reporting threshold can be established using currently applicable regulations/guidance or other acceptable scientific means. Quantitative test results shall be reported as numerical values and rounded according to conventional rules (see General Notices 7.20). Impurities at a level greater than the reporting threshold shall also be summed and reported as total impurities.

Qualification of impurities and degradation products: Establishment of acceptance criteria for impurities should focus on safety considerations. The level of any impurity or degradation product present in a drug substance or drug product that has been adequately tested in safety or clinical studies is generally considered qualified. However, highly toxic (e.g., genotoxic) impurities or degradation products shall be addressed using applicable guidance. Impurities or degradation products that are also significant metabolites are generally considered qualified. Qualification of impurities shall be based on applicable guidance, scientific rationale, or history of product use. Higher or lower thresholds for qualification of impurities may be appropriate for some products based on scientific rationale and level of concern, including drug class effects and clinical experience [4].

Inorganic Impurity

Reagents, ligands and catalysts: These create problems unless manufacturer does not take care of it.

Heavy metals: The main sources of heavy metals are the water used in the processes and the reactors.

Remedy: Using demineralized water and glass lined reactor.

Other materials: filter aids, charcoal. Regular monitoring of fibres and black particles in bulk drugs is essential to avoid these contaminations.

Solvent Residues

These are organic volatile chemicals used during the manufacturing process or generated during the production. Depending on possible risk to human health, residual solvents are divided into 3 classes [5].

Class 1 solvents: Solvents such as benzene (class.1. 2 ppm limit) and carbon tetrachloride (class 1,4 ppm limit) are to be avoided.

Class 2 solvents: Methylene chloride, Methanol, Pyridine, Toluene, Acetonitrile etc.,

Class 3 solvents: Acid, acetone, isopropyl alcohol, butanol, ethanol, ethyl acetate have permitted daily exposure of ≤ 50 mg/day.

Tests for Impurities

- Preparative Liquid Chromatography (LC)
- Liquid Chromatography And Ultraviolet Detection (LC/UV)
- Liquid Chromatography And Mass Spectroscopy (LC/MS)
- Gas Chromatography (GC)
- Capillary Electrophoresis (CE)
- Supercritical Fluid Chromatography (SFC)
- Fourier Transform Infrared Spectroscopy (FTIR)

LIQUID CHROMATOGRAPHY AND ULTRAVIOLET DETECTION (LC/UV)

Androstanolone

A sensitive, selective reverse phase method was developed for the quantitative determination of six potential impurities in Androstanolone active pharmaceutical ingredient. Efficient chromatographic separation was achieved on Zorbax Eclipse XDB C8 (250×4.6 mm, 5 µm) column with mobile phase containing a gradient mixture of solvent-A and solvent-B [6]. The elucidated compounds were monitored at 200 nm. All six potential impurities were identified by mass spectrophotometer and characterized by nuclear magnetic resonance. The developed method was validated as per ICH guidelines with respect to specificity, precision, linearity, quantitative limit, detection limit and accuracy. Regression coefficient value was greater than 0.99 for Androstanolone impurities. Detection limit of impurity-A, impurity-B, impurity-C, impurity-D, impurity-E and impurity-F were in the range of 0.0002-0.003% respectively. The quantitative limit of impurity-A, impurity-B, impurity-C, impurity-D, impurity-E and impurity-F were in the range of 0.003%-0.013% respectively with respect to sample concentration. The accuracy of the method was established based on the recovery obtained between 92.72%-106.90% for all impurities [7].

Hydrochlorothiazide and Candesartan Cilexetil

A simple, sensitive, and inexpensive high-performance liquid-chroma-tographic method has been developed for simultaneous determination of hydro-chlorothiazide and candesartan cilexetil in pharmaceutical formulations. Chro-matographic separation was achieved on a Phenyl-2 column with a 25:75:0.2 mixture of 0.02 M potassium dihydrogen phosphate, methanol, and triethyl-amine, final pH 6.0 ± 0.1, as mobile phase. Detection was at 271 nm. Response was a linear function of concentration in the range 5-45 µg mL-1 for hy-drochlorothiazide and 12–56 µg mL-1 for candesartan cilexetil; the correlation coefficients were 0.9993 and 0.9991, respectively. Total elucidate time for the two components was less than 5 min.

Metformin in Plasma Samples

- A LC/MS method for the analysis of the highly polar anti-diabetic drug metformin in plasma samples is compared to an ion-pair HPLC method with UV detection.
- Both methods showed good linearity in the concentration range of 50 to 2000 ng/mL, good precision and accuracy and similar sensitivity. The LC-MS method has the advantage of a simpler and faster preparation procedure, shorter analytical times and higher selectivity.

- Both methods were validated and successfully applied to bioequivalence studies of drugs containing metformin.

Cetirizine

To develop and validate stability-indicating reversed phase high performance liquid chromatographic method for simultaneous determination of ketotifen fumarate and cetirizine dihydrochloride in solid dosage forms [8].

GAS CHROMATOGRAPHY (GC)

Caprolactum

- A gas chromatographic technique has been developed for the determination of the impurities in caprolactam, using Carbowax 20M as the partition liquid and Chromosorb P as the support, treated or untreated with potassium hydroxide. The system was used on a semipreparative scale for the separation of the two main impurities of ε-caprolactam, *viz.* 6-methyl-2-piperidone and octahydrophenazine, after enrichment by continuous crystallization.
- To confirm their identity, the two impurities were synthesized and injected into the gas chromatography. Other impurities were identified by comparison of their retention times with those of known compounds [9].
- A technique was also developed to determine the degree of oxidation of caprolactam by gas chromatography.

Methanol and Chloroform from Liposomal

The use of liposomal formulations has rapidly gained popularity in pharmaceutical research and development. Their preparation often involves the use of organic solvents such as methanol and chloroform to dissolve lipophilic lipids. In the present study, gas chromatographic method for the determination of methanol and chloroform residual levels in liposomes was developed using GC 17 A Shimadzu with FID (a flame ionization detector) and the separation was carried out on BP 624 column (4% cyanopropyl phenyl and 94% dimethyl silixone, 30 m X 0.53 mm i.d. X 0.25 µm coating thickness), with nitrogen as a carrier gas in the split mode by direct injection method [10]. The method was validated according to ICH guidelines. The method described is simple, sensitive, rugged, reliable and reproducible and requires less time than other reported methods for the quantitative of methanol and chloroform levels from liposomal formulations of lamivudine and stavudine.

Nitrogen Trifluoride

Highly reactive fluorinated gaseous matrices require special equipment and techniques for the gas chromatographic analysis of trace impurities in these gases. The impurities that were analysed at the low mg/L levels included Dioxygen (O_2), Dinitrogen (N_2), Carbon dioxide (CO_2), carbon monoxide (CO), Sulfur hexafluoride (SF_6), methane

(CH_4) and nitrous oxide (N_2O). Carbon Tetrafluoride (CF_4) is also present in the product at levels of 20-400 mg/L and had to be analysed as well. This paper compares the use of a custom-built dual-channel gas chromatograph utilising single column back flush switching on one channel for the determination of O_2, N_2, CH_4 and CO with column sequence reversal on a second channel for the determination of CO_2, N_2O, SF_6 and CF_4 to a similar system using a combination of dual-column back flush and heart-cut configurations. Pulsed discharge helium ionisation detectors were used on both channels in both configurations [11].

CAPILLARY ELECTROPHORESIS (CE)

The use of Capillary Electrophoresis (CE) to determine drug-related impurities is becoming established within industrial pharmaceutical analysis laboratories. Increasingly CE is being viewed as an alternative for, and complement to, High-Performance Liquid Chromatography (HPLC). This paper comprehensively reviews the progress of CE in drug impurity determinations subdividing the reports into low pH, high pH and MECC applications. The section covering method performance and validation clearly shows that CE methods are capable of validation in this area and can often give equivalent performance to HPLC methods. Possible benefits of adopting CE for this testing include reductions in costs and improved robustness. Potential developments are covered including the use of electrolyte additives, instrumental developments and the increased implementation of electro-chromatography. It is concluded that the current status of CE is sufficiently strong to allow the analyst to view CE as a viable and attractive alternative to HPLC [12].

Capillary Electrophoresis/Mass Spectrometry using Various Ionization Techniques

- Capillary Electrophoresis/mass Spectrometry (CE/MS) is predominantly carried out using Electrospray ionization (ESI). Recently, Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photoionization (APPI) have become available for CE/MS. With the VUV lamp turned off, the APPI source may also be used for CE/MS by Thermospray Ionization (TSI).
- In the present study the suitability of ESI, APCI, APPI and TSI for drug impurity profiling by CE/MS in the positive ion mode is evaluated. The drugs carbachol, lidocaine and proguanil and their potential impurities were used as test compounds, representing different molecular polarities. A background electrolyte of 100 mM acetic acid (pH 4.5) provided baseline separation of nearly all impurities from the respective drugs. APPI yielded both even and odd-electron ions, whereas the other ionization techniques produced even-electron ions only. In-source fragmentation was more pronounced with APCI and APPI than with ESI and TSI, which was most obvious for proguanil and its impurities [13]. In general, ESI and TSI appeared the most efficient ionization techniques for impurities that are charged in solution achieving detection limits of 100 ng/Ml (full-scan mode). APPI and APCI showed a lower efficiency, but allowed ionization of low and high polarity analytes, although quaternary ammonium compounds (e.g. carbachol) could not be detected. Largely neutral compounds, such as the lidocaine impurity 2,6-dimethylaniline, could not be detected by TSI, and yielded similar detection limits (500 ng/mL) for ESI, APPI and APCI.

- In many cases, impurity detection at the 0.1% (w/w) level was possible when 1 mg/mL of parent drug was injected with at least one of the CE/MS systems. Overall, the tested CE/MS systems provide complementary information as illustrated by the detection and identification of an unknown impurity in carbachol [14].

Anti-Cancer Drug Impurities

- Due to the low therapeutic index of anti-cancer drugs, they should be closely monitored for evidence of potential contamination that may be of high toxicity and not to have the desired therapeutic effect. Therefore, analytical methods to detect drugs related substances at low concentrations are necessary.
- Capillary electrophoresis allows for fast and clear separation of drug derivatives. A multitude of submethods make selection of suitable environment for various types of chemicals possible. Publications concerning separation of drugs such as cisplatin, carboplatin, lobaplatin, methotrexate, tamoxifen, paclitaxel from their derivatives, which are their potential contaminations, show that capillary electrophoresis provides the appropriate tools to analyze the impurities of these anti-cancer drugs and is able to partially displace such technique as thin layer chromatography and high performance liquid chromatography, which still play a major role in this field [15].

Ciprofloxacin

- Capillary zone electrophoresis (CZE) has been elaborated for separation, identification and determination of ciprofloxacin and its impurities.
- The separation, phosphate buffer pH 6.0 was supplemented with 0.075 M pentane-1-sulfonic acid sodium salt.
- The elaborated method was validated. The selectivity, linearity, Limits Of Detection (LOD) and Quantification (LOQ), precision, and accuracy of capillary zone electrophoresis were evaluated.
- The results obtained by CZE were also compared with those obtained by liquid chromatography. Regarding the validation results the CE method fulfils the current European Pharmacopoeia (Eur. Ph.) requirements. The evaluated CE method could be applicable to the analysis of different medicinal products containing ciprofloxacin [16].

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

Parallel Factor Analysis for Column Testing in a Wide Range of Operational Conditions

- Retention mechanisms involved in Supercritical Fluid Chromatography (SFC) are influenced by interdependent parameters (temperature, pressure, chemistry of the mobile phase, and nature of the stationary phase), a complexity which makes the selection of a proper stationary phase for a given separation a challenging step. For the first time in SFC studies, Parallel Factor Analysis (PARAFAC) was

employed to evaluate the chromatographic behavior of eight different stationary phases in a wide range of chromatographic conditions (temperature, pressure, and gradient elution composition).

- Design of Experiment was used to optimize experiments involving 14 pharmaceutical compounds present in biological and/or environmental samples and with dissimilar physicochemical properties. The results showed the superiority of PARAFAC for the analysis of the three-way (column × drug × condition) data array over unfolding the multiway array to matrices and performing several classical principal component analyses [17].
- Thanks to the PARAFAC components, similarity in columns' function, chromatographic trend of drugs, and correlation between separation conditions could be simply depicted: columns were grouped according to their H-bonding forces, while gradient composition was dominating for condition classification. Also, the number of drugs could be efficiently reduced for columns classification as some of them exhibited a similar behavior, as shown by hierarchical clustering based on PARAFAC components.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Anti-parasitics

- This study shows that Fourier Transform Infrared (FTIR) spectroscopy, Thermo Gravimetric Analysis (TGA), and Scanning Electron Microscopy (SEM) can be used as supporting tools for the evaluation of the quality of antiparasitics [18]. In addition, an analytical methodology was developed and validated to quantify simultaneously Thiabendazole (TB), Febantel (FB), Toltrazuril (TZ), and Fluazuron (FZ) in bulk and in their veterinary pharmaceutical formulations using Reverse Phase High Performance Liquid Chromatography (RP-HPLC).
- In order to investigate stability, pharmaceuticals were submitted to degradation processes under different conditions, such as recommended by the International Conference on Harmonization. The chromatographic conditions were optimized and the validation parameters, such as selectivity, linearity, detection limit, quantification limit, precision, accuracy, and robustness showed results within acceptable standards [19].
- All analytes were stable in the stability assays in acid and basic media and thermal conditions, except in the oxidation process, which presented two degradation peaks. Physicochemical characterization by TGA, FTIR, and SEM of raw materials of TB, FB, TZ, and FZ provided information about the authenticity of the analytes, proving the wide applicability of the instrumental techniques.
- The RP-HPLC proposed method was found to be accurate, precise, and reproducible and can in addition be used for routine quality control analysis.

Simvastatin Drug

In the present study a reversed phase high performance liquid chromatography (RP-HPLC) method with diode array detector (DAD) at room temperature was used for obtaining impurity profiles of 20 drug products containing

simvastatin as an active substance. Fourier-transform infrared spectroscopy (FT-IR) was carried out to obtain absorption spectra of samples [20].

The Partial Least Squares (PLS) model was built to predict the relative content of lovastatin, the main impurity of simvastatin, and sum of statin-like impurities. In order to build the PLS model, peak areas obtained from HPLC chromatograms were related to FT-IR spectra of drugs. The PLS model based on signal normal variate and orthogonal signal correction (SNV+OSC) transformed FT-IR a spectrum was able to predict the content of drug impurities in real samples with a good prediction ability (R2>0.95).

Rosuvastatin

The objective of the present study was to develop Floating Drug Delivery System (FDDS) of rosuvastatin calcium is one method to accomplish tedious gastric residence times, provide convenience for both local and systemic drug action. Thus, gastro retention could help to provide higher availability of new products and subsequently improved therapeutic activity and considerable benefits to patients. In this article aims at summarizing the floating drug delivery system along with types, access for designing the floating dosage form, advantages and disadvantages of FDDS. In this article, aims to maintain increasing floating retention time at the gastric site to reinforce the bioavailability and release rate of drug. The floating dosage form was processed by direct compression method adopting sodium bicarbonate as gas generating agent [21]. The release rate was sustained up to 20 hrs with 1:1.5 ratio of HPMC K4M and Xanthan gum, but the Floating Lag time was construct to be more with the combination. Evaluations of granules like physical parameters, weight variation, drug content uniformity, bulk density, tapped density, buoyancy studies, Swelling Index, angle of repose was done. Similarly, the aggregate between HPMC K4M and Guar gum also controlled the release more than 20 hrs was detected. The aggregate between HPMC K100M and Carbopol 934P with the ratio of 2:1 was found to be acceptable with release profile. Hence the Formulation F10 was optimized by for further studies. The formulation (F10) also gratify the Swelling Index, Buoyancy time controlled the drug release up to 24 hrs. The mechanism of drug release pursued the Zero order kinetics with the co-efficient (R2) value 0.996 [22].

Phenytoin Sodium

Phenytoin Sodium (PS) has a tendency to convert to its base form Phenytoin base (PHT) during manufacturing, packaging, shelf life and in-use conditions that can influence its clinical performance. The objective of the present work was to develop a non-destructive, quick and easy analytical method for quantification of PHT in the drug product. A formulation was prepared to contain the excipients of commercial capsule formulation of PS. The formulation containing either 100% PHT or PS was prepared and these formulations were mixed in different proportion to achieve 0%-100% PHT matrices [23]. FTIR, NIR and Raman spectra of samples were collected. Data were truncated and mathematically pretreated before development of Partial Least Squares (PLS) and principal component analysis (PCA) regressions model. The models were assessed by slope, intercept, R, R2, Root Mean Square Error (RMSE) and Standard Error (SEP).

DISCUSSION AND CONCLUSION

The models exhibited good linearity over the selected range of PHT in the formulations with low error as indicated by slope that was close to one and small values of intercept, RMSE and SE. The models of NIR based data were more accurate and precise than Roman data based models as indicated by the low values of RMSE and SE. Prediction accuracy of independent samples containing 25% PHT using NIR models were similar to Roman models. On the other hand, the prediction was more precise for the independent sample containing 5% PHT using NIR data based models compared to Roman data based models as indicated by standard deviation. In conclusion, chemometric models based on NIR and Roman spectroscopies provides a fast and easy way to monitor the disproportionation of PS in the drug products.

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