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IMPURITY PROFILING

PENKI SRAVANI

MASTER OF PHARMACY

JNTUV

Abstract

Impurities are pharmaceutical are degradable chemical or degradable product. It is defined as the any type of element or compound whose presence in a material s unintentional or unwanted. They are having organic, inorganic and residual solvents are type of Impurities is present. In the field of the pharmaceutical chemistry, these impurities are considered to be extraneous materials present in the therapeutically APC (active pharmaceutical compounds). They are expected to have unusually potent, toxic or unexpected pharmacological effects which are detrimental to human death. Many of regulatory authorities like USFDA, ICH UK-MHRA, CDSCO like emphasizing on requirements and identification of impurities in Active Pharmaceutical ingredient's (API's) as well as finished products. To characterize and isolate the impurities in pharmaceutical diverse methods are used such as gas-liquid chromatography, capillary electrophoresis, solid phase extraction method, High performance liquid chromatography, infrared spectroscopy, Ultraviolet Spectrometry, mass spectroscopy, supercritical extraction chromatography, Nuclear magnetic resonance.

Impurity profiling serves several important purposes:

1) Safety: Impurities can have harmful effects on the safety of a drug or chemical compound. They might lead to adverse reactions, toxicities, or other health risks when consumed or used.
2) Efficacy: Impurities can also affect the efficacy of drugs or the performance of chemical compounds. They might interfere with the intended effects of the substance or alter its stability.
3) Regulatory Compliance: Regulatory authorities, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have strict guidelines regarding impurity limits in pharmaceuticals and chemicals. Manufacturers must ensure that their products meet these regulatory standards to gain approval for marketing.

The process of impurity profiling typically involves the following steps:

1. Identification: Identifying the types of impurities present in the substance. These could be related to starting materials, by products, degradation products, or contaminants introduced during manufacturing.
2. Characterization: Determining the chemical structure and properties of the identified impurities. This might involve using techniques like mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy, chromatography, and other analytical methods.
3. Quantification: Measuring the amount or concentration of impurities in the substance. This helps in evaluating their potential impact on safety and efficacy.

4. Risk Assessment: Assessing the potential risks associated with each impurity based on its toxicity and concentration. This helps prioritize impurities for further action.
5. Control Strategies: Developing strategies to control and minimize impurities during the manufacturing process. This might involve process optimization, changing raw materials, or modifying manufacturing conditions.
6. Validation: Validating analytical methods used for impurity profiling to ensure accuracy and reliability of the results.
7. Documentation: Keeping comprehensive records of the impurity profiling process, including methods used, results obtained, and actions taken to address impurities.

High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for impurity profiling in pharmaceuticals and other chemical substances. It involves the separation of components in a mixture based on their interaction with a stationary phase and a mobile phase. HPLC is particularly effective for separating and quantifying impurities, degradation products, and related compounds in complex matrices. Here's how HPLC is used for impurity profiling:

1. Sample Preparation: The first step in HPLC impurity profiling involves preparing the sample. This might include dissolving the substance of interest, filtering it to remove particulates, and adjusting the sample concentration if necessary.
2. Choice of Column and Method: The choice of HPLC column and method is critical for achieving effective separation. Different columns have varying selectivity, which can impact how well impurities are resolved from the main compound. The method includes the mobile phase composition, flow rate, and gradient program if applicable.
3. Injection: The prepared sample is injected into the HPLC system. The sample is introduced into the column where the separation process begins.
4. Separation: As the sample travels through the column, components interact differently with the stationary phase, leading to their separation based on factors like polarity, size, and affinity. Impurities are typically resolved from the main compound, allowing for their individual detection and quantification.
5. Detection: Detection of separated components is crucial for impurity profiling. Various detectors can be used, including UV-visible detectors, diode array detectors (DAD), fluorescence detectors, and mass spectrometers. UV detection is common for impurity profiling as it can detect compounds with chromophores (light-absorbing functional groups).
6. Data Analysis: The output from the detector is recorded as chromatograms, which show the separation of different compounds over time. Impurity peaks are identified based on their retention times relative to the main compound and their spectral characteristics. The area under each peak is proportional to the amount of the corresponding impurity.
7. Quantification: The concentration of each impurity is determined by comparing its peak area or height to that of an appropriate standard or reference compound. Calibration curves are often used to relate peak areas to known concentrations.
8. Validation and Reporting: The HPLC method used for impurity profiling needs to be validated to ensure its reliability and accuracy. Validation involves testing aspects such as linearity, precision, accuracy, and specificity. Once validated, the results of impurity profiling are reported, often including the identities and concentrations of detected impurities.
9. Regulatory Compliance: Impurity profiling using HPLC must adhere to regulatory guidelines set by organizations such as the FDA and EMA. These guidelines define acceptable limits for impurities and degradation products to ensure the safety and quality of pharmaceutical products.

Gas Chromatography (GC) is another powerful analytical technique used for impurity profiling in pharmaceuticals and chemical substances. While GC is primarily suited for analyzing volatile and semi-volatile compounds, it can be highly effective for separating and quantifying impurities that can be vaporized without undergoing significant decomposition. Here's how GC is utilized for impurity profiling

1. Sample Preparation: Similar to other analytical techniques, sample preparation is a critical step. The sample needs to be prepared for injection into the GC system. This can involve dissolving the sample in a suitable solvent, derivatizing certain compounds to improve their volatility, and ensuring the sample is free from particulates.
2. Injection: The prepared sample is introduced into the GC system through a syringe injector. The sample is volatilized and injected into the chromatographic column.
3. Choice of Column and Method: The choice of GC column and method depends on the nature of the impurities and the sample matrix. The column's stationary phase determines the separation based on factors like polarity and volatility. The method includes parameters like column temperature, carrier gas flow rate, and gradient programming if applicable.
4. Separation: As the vaporized sample components move through the column, they interact with the stationary phase and separate based on their volatility. Less volatile components, including impurities, may have longer retention times in the column, leading to their separation from more volatile compounds.
5. Detection: Various detectors can be used in GC, including Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), and Mass Spectrometer (MS). FID and TCD are commonly used for impurity profiling in GC. FID is sensitive to organic compounds that can be ionized and burned, while TCD is sensitive to changes in thermal conductivity.
6. Data Analysis: The detector output generates chromatograms that show the separation of components over time. Impurity peaks are identified based on their retention times relative to the main compound and their detector response characteristics.
7. Quantification: The concentration of each impurity is determined by comparing its peak area or height to that of a known standard or reference compound. Calibration curves are established to relate peak areas to known concentrations.
8. Validation and Reporting: The GC method for impurity profiling must be validated to ensure its reliability and accuracy. Validation involves testing aspects such as linearity, precision, accuracy, and specificity. The results are reported, including the identities and concentrations of detected impurities.
9. Regulatory Compliance: GC-based impurity profiling must adhere to regulatory guidelines, ensuring the limits set by organizations like the FDA and EMA are met to guarantee product safety and quality.
10. GC is particularly useful for analyzing volatile organic impurities, residual solvents, and other compounds that can be vaporized without decomposition. It's commonly used in pharmaceuticals, petrochemicals, environmental analysis, and food safety testing.

Mass Spectrometry (MS): Is an advanced analytical technique used extensively in impurity profiling for pharmaceuticals and other chemical substances. MS provides valuable information about the molecular weight, structure, and composition of compounds, making it a powerful tool for identifying and quantifying impurities. Here's how Mass Spectrometry is utilized for impurity profiling:

1. Ionization: The sample is introduced into the mass spectrometer, where it is ionized. Various ionization techniques can be used, including Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), and Electron Impact (EI) for gas-phase samples. The ionization process imparts a charge to the molecules, generating ions.

2. Mass Separation: The ions generated from the sample are then subjected to an electric or magnetic field, which separates them based on their mass-to-charge ratio (m/z). This separation allows the different ions to be analyzed individually.
3. Mass Analysis: The separated ions are detected by their response to the mass analyzer. Different types of mass analyzers, such as Quadrupole, Time-of-Flight (TOF), Ion Trap, and Orbitrap, offer varying levels of resolution, accuracy, and sensitivity.
4. Data Acquisition: The mass spectrometer generates spectra that provide information about the mass of the ions detected. These spectra are used to identify the molecular weight and fragmentation patterns of compounds present in the sample.
5. Identification: The mass spectra are compared to reference spectra or databases to identify the compounds present, including impurities. The fragmentation pattern can provide insight into the structural characteristics of the impurities.
6. Quantification: MS can be used for quantifying impurities by measuring the intensity of the ion signals corresponding to the impurities and the main compound. Calibration curves are established to relate ion intensities to known concentrations.
7. High-Resolution MS: High-resolution mass spectrometers can provide detailed information about the elemental composition of ions, aiding in the identification of impurities with high precision.
8. Tandem Mass Spectrometry (MS/MS or MSn): MS/MS involves performing multiple stages of mass analysis. The precursor ion is selected in the first stage and fragmented, and the resulting fragments are then further analyzed in subsequent stages. This technique enhances structural information and can be useful for confirming impurity identities.
9. Isotope Labeling and Stable Isotope Dilution: Isotope labeling involves introducing isotopically labeled internal standards to the sample, which allows for more accurate quantification. Stable isotope dilution involves adding a known amount of an isotopically labeled compound to the sample before analysis.
10. Data Analysis and Reporting: MS data are processed and analyzed using specialized software. The results are reported, including the identities and concentrations of detected impurities.

Nuclear Magnetic Resonance (NMR) spectroscopy is a sophisticated analytical technique that provides detailed information about the structure of molecules, making it a valuable tool for impurity profiling in pharmaceuticals and other chemical substances. NMR spectroscopy allows researchers to analyze the connectivity of atoms within a molecule and can be highly effective in identifying and characterizing impurities. Here's how NMR spectroscopy is used for impurity profiling:

1. Sample Preparation: The sample needs to be prepared for NMR analysis. This often involves dissolving the sample in a suitable solvent and ensuring it is properly purified and concentrated.
2. Instrument Setup: The NMR instrument is calibrated and the sample is loaded into a specialized NMR tube. The tube is placed within the NMR spectrometer.
3. NMR Acquisition: The NMR spectrometer generates a magnetic field, which causes the nuclei (usually protons, carbon-13, nitrogen-15, etc.) in the sample to absorb energy from radiofrequency radiation. When the nuclei return to their equilibrium state, they emit radiofrequency signals that are recorded as NMR spectra.
4. Spectral Interpretation: NMR spectra consist of peaks that correspond to different types of atoms within the molecule. The chemical shifts (expressed in parts per million, ppm) of these peaks provide information about the local electronic environment of the atoms. The multiplicity and coupling patterns of peaks provide information about neighboring atoms and functional groups.
5. Identification: Impurities can be identified based on their unique chemical shifts, coupling patterns, and integrals (peak areas). Comparison of impurity spectra to reference spectra or databases aids in identification.

6. Quantification: The intensity or area of NMR peaks is proportional to the number of nuclei generating the signal. By comparing the peak areas of impurities to those of known standards, impurity concentrations can be determined.
7. Two-Dimensional (2D) NMR: 2D NMR techniques such as COSY (COrrelation SpectroscopY), HSQC (Heteronuclear Single Quantum Coherence), and HMBC (Heteronuclear Multiple Bond Correlation) provide enhanced structural information by revealing correlations between nuclei that are connected within a molecule.
8. NOESY (Nuclear Overhauser Effect Spectroscopy): NOESY can provide information about the spatial arrangement of atoms in a molecule, aiding in the determination of the overall structure.
9. High-Resolution NMR: High-resolution NMR instruments offer greater detail and resolution in spectra, allowing for better differentiation of impurities from the main compound.
10. Solid-State NMR: Solid-state NMR is used for samples that are not in solution, such as crystalline solids or certain polymers. It provides information about the local environment and interactions of atoms within the solid matrix.

Fourier Transform Infrared (FTIR) Spectroscopy: Fourier Transform Infrared (FTIR) spectroscopy is a widely used analytical technique for impurity profiling and structural characterization of pharmaceuticals and chemical substances. FTIR spectroscopy provides information about the functional groups present in a molecule based on their absorption of infrared radiation. Here's how FTIR spectroscopy is used for impurity profiling:

1. Sample Preparation: The sample needs to be prepared for FTIR analysis. Typically, the sample is ground into a fine powder and mixed with a suitable matrix (often KBr) to create a thin pellet. This pellet is placed in the FTIR instrument for analysis.
2. Instrument Setup: The FTIR instrument generates a broad-spectrum infrared beam that is passed through the sample. The beam interacts with the sample, and the resulting absorption spectrum is collected.
3. Spectral Acquisition: The absorption spectrum is obtained by measuring how much infrared radiation is absorbed by the sample at different wavelengths. This absorption is due to the vibrations of different chemical bonds present in the molecules.
4. Spectral Interpretation: The FTIR spectrum is represented as a plot of intensity (absorbance) against wavenumber (1/wavelength). Peaks in the spectrum correspond to specific vibrational modes of functional groups within the molecules.
5. Identification: Impurities can be identified by comparing the FTIR spectrum of the sample to reference spectra or databases. Each functional group has characteristic absorption bands, which aids in identifying the types of impurities present.
6. Quantification: The intensity of absorption peaks can provide semi-quantitative information about the relative concentrations of impurities. However, quantitative analysis using FTIR can be challenging due to potential overlapping peaks and matrix effects.
7. ATR (Attenuated Total Reflectance) FTIR: ATR is a sampling technique that allows for direct analysis of solid and liquid samples without extensive sample preparation. ATR crystals are used to couple the sample to the FTIR beam, allowing for rapid analysis.
8. 2D FTIR Spectroscopy: Similar to 2D NMR, 2D FTIR techniques provide more complex information about molecular interactions and structural features by correlating vibrations.
9. FTIR Microscopy: FTIR microscopy allows for spatially-resolved analysis, which can be valuable for studying the distribution of impurities within a sample.
10. Validation and Data Interpretation: FTIR methods used for impurity profiling need to be validated to ensure reliability and accuracy. Interpretation of FTIR spectra requires expertise in identifying characteristic peaks and understanding the relationships between spectral features and molecular structure.

Liquid Chromatography-Mass Spectrometry (LC-MS): Liquid Chromatography-Mass Spectrometry (LC-MS) is used for impurity profiling, specifically focusing on the instrumentation aspects:

1. Sample Introduction: The sample is prepared by dissolving the substance of interest in a compatible solvent. It is then injected into the LC-MS system using an autosampler. The autosampler can handle multiple samples and ensures consistent and accurate injection volumes.

2. Liquid Chromatography (LC): The sample is introduced into the LC system, which typically consists of a pump, an injector, and a chromatographic column. The LC separates the components of the sample based on their interactions with the stationary phase (inside the column) and the mobile phase (solvent). The LC separation can be tailored to separate impurities from the main compound and from each other.

3. Ionization: After separation by LC, the eluted compounds are directed into the mass spectrometer for ionization. The most common ionization techniques in LC-MS are Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). ESI is particularly useful for polar and ionic compounds, while APCI is more suitable for less polar compounds.

4. Mass Spectrometry (MS): The ionized compounds are introduced into the mass spectrometer for analysis. The MS consists of several components:

Mass Analyzer: The mass analyzer separates ions based on their mass-to-charge ratio (m/z). Different types of mass analyzers, such as Quadrupole, Time-of-Flight (TOF), and Orbitrap, are used in LC-MS instruments.

Detector: The detector records the ions' intensities at different m/z values, generating mass spectra.

5. Data Acquisition and Mass Spectra: The mass spectrometer generates mass spectra that show the m/z ratios of the ions detected. The intensities of the peaks correspond to the abundance of different ions. Each peak in the mass spectrum represents a specific ionized compound.

6. Tandem Mass Spectrometry (MS/MS): For more detailed structural information, MS/MS can be employed. In MS/MS, specific precursor ions are selected from the first mass analyzer, fragmented, and then the resulting fragments are analyzed in the second mass analyzer. This provides information about the fragmentation patterns and connectivity of atoms within the molecules, aiding in impurity identification.

7. High-Resolution Mass Spectrometry: High-resolution mass spectrometers provide greater accuracy in determining the m/z values of ions. This is particularly useful when distinguishing between closely related compounds with similar masses.

8. Data Analysis and Identification: The mass spectra are analyzed using specialized software. Impurities are identified based on their m/z ratios and fragmentation patterns, which can be compared to reference spectra or databases.

9. Quantification: The intensity of ion signals in the mass spectra can be used for semi-quantitative analysis of impurities. Isotope-labeled internal standards can be used for more accurate quantification.

10. Reporting: The results of the LC-MS analysis are reported, including the identities and concentrations of detected impurities. The information is used for quality control, process optimization, and regulatory compliance.

Capillary Electrophoresis (CE) is an analytical technique that separates charged molecules in a capillary tube under the influence of an electric field. CE is particularly useful for analyzing ionic and charged compounds, making it a valuable tool for impurity profiling in pharmaceuticals and other chemical substances. Here's how Capillary Electrophoresis is used for impurity profiling:

1. Sample Preparation: The sample is prepared by dissolving the substance of interest in a suitable buffer solution. The buffer solution provides the necessary ions for electrical conductivity and can affect the separation and resolution of charged compounds.

2. Capillary Tube: The sample is injected into a narrow capillary tube that is typically made of fused silica. The capillary is filled with the buffer solution, and both ends are immersed in buffer reservoirs. The capillary tube acts as the separation column.

3. Ionization: CE separates charged molecules, so the sample must contain ionic or charged compounds. If the compounds of interest are not inherently charged, they might need to be derivatized or ionized prior to analysis.

4. Electrophoresis: When an electric field is applied across the capillary, the charged molecules in the sample migrate towards the electrode with the opposite charge. The migration rate is influenced by the size and charge of the molecules, as well as the viscosity of the buffer.

5. Separation: As the charged compounds migrate through the capillary, they separate based on their charge-to-mass ratio. Smaller and more highly charged molecules migrate faster than larger and less charged molecules.

6. Detection: Detection in CE can be accomplished using various methods, such as UV-absorption, fluorescence, conductivity, and mass spectrometry. The detector is positioned at the end of the capillary where the separated compounds pass through, generating signals as they elute.

7. Data Acquisition: The detector generates signals that correspond to the different compounds as they elute from the capillary. These signals are recorded as electropherograms, which show the intensity of the signals over time.

8. Data Analysis: Electropherograms are analyzed to identify and quantify the compounds present. Peaks in the electropherograms correspond to the separated compounds. Comparisons to reference standards or databases aid in impurity identification.

9. Quantification: The peak areas or heights in the electropherograms can be used for semi-quantitative analysis. For accurate quantification, internal standards of known concentrations can be added to the sample.

10. Reporting: The results of the CE analysis are reported, including the identities and concentrations of detected impurities. The information is used for quality control, process optimization, and regulatory compliance.

Thin-Layer Chromatography (TLC): Thin-Layer Chromatography (TLC) is an analytical technique that separates compounds based on their differential migration on a thin layer of adsorbent material, typically coated onto a glass or plastic plate. While not as advanced as some other techniques like HPLC or GC, TLC is a simple and cost-effective method that can be used for preliminary impurity profiling and qualitative analysis. Here's how Thin-Layer Chromatography is used for impurity profiling:

1. Sample Application: A small amount of the sample is spotted onto a designated starting line on the TLC plate. Multiple samples can be run on the same plate for comparison.

2. Developing the Plate: The TLC plate is placed in a developing chamber with a solvent (mobile phase) that travels up the plate through capillary action. The compounds in the sample are carried by the solvent, and their migration is influenced by their affinity for the stationary phase (adsorbent) and the solvent.

3. Separation: As the solvent travels up the plate, the compounds in the sample move at different rates based on their polarity, size, and interactions with the adsorbent. The less polar compounds generally migrate faster, while more polar compounds remain closer to the starting line.

4. Visualization: After development, the TLC plate is removed from the chamber and the solvent front (highest point reached by the solvent) is marked. The plate is then dried and visualized using various detection methods:

UV Absorption: If the compounds have chromophores (light-absorbing functional groups), UV light can be used to visualize the spots.

Staining Reagents: Specific reagents can be applied to the plate to develop colored spots, which can aid in compound identification.

Iodine Vapor: Iodine vapors can be used to visualize compounds as brown spots on the plate.

Fluorescence: If the compounds are fluorescent, the plate can be visualized under UV light.

5. Rf Value Calculation: The Retention Factor (Rf) value is calculated for each compound. It's the ratio of the distance traveled by a compound to the distance traveled by the solvent front. Rf values are unique to each compound and can be used to compare migration rates.

6. Qualitative Analysis: The TLC plate provides a qualitative profile of the sample. The separation pattern and Rf values can give insights into the number and polarity of compounds present. Comparison to known standards or reference compounds can aid in identification.

7. Limitations: TLC has limitations when it comes to quantification and precision. It's mainly used for qualitative analysis and preliminary impurity profiling. Quantitative information is limited, and compound identification might require additional techniques for confirmation