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*Original Article*

# Analytical Approaches to Understanding and Qualifying Extraneous Peaks in Impurity Profiling of Drug Products and Active Pharmaceutical Ingredients

Kannan Jakkan[1], Narasimha Naidu Mopidevi[2]

[1]*Analytical Research & Development & Quality Control, ANI Pharmaceuticals INC, New Jersey, USA.*

[1]*Corresponding Author : kannanparform@gmail.com*

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***Abstract -*** *In chromatography, the presence of extraneous peaks refers to a serious issue, which complicates the further analytical data evaluation. It is an important problem with chromatography that extraneous peaks may appear that give unaccredited errors in analytical results. These 'overloaded' regions can be due to various factors like the presence of interfering substances in the sample matrix, the reagents, the chromatographic columns, or the formation of artifacts during the analytical process. The following review explores the potential reason that contributes to the extra peaks and identifies major points including sample preparation, system control, and quality supply products. Among others, it goes a step further in analyzing refined troubleshooting approaches as well as how to avoid the costs associated with these sudden spikes. Otherwise, by recognizing the causative factors of extra humps, the chromatographic measurements could be made more accurate and the peaks more distinctive and clearer, and therefore meet the expectations of analysts. This research work is concerned with the examination of such patterns, their detection, as well as their categorization into qualified or not. Thus, to identify the causes of extraneous peaks we systematically discuss potential sources of these peaks, including sample impurities, reagents contamination, or artificial peaks from instruments. Protein peaks under study are well characterized using Mass spectrometry and spectral analysis to quantify the values for analytical purposes. This work aims to provide, through intensive investigation and strict qualification, more credibility and fewer errors for the chromatographic analysis and, thus, the quality of the derived analytical data.*

***Keywords -*** *Chromatography, Extraneous peaks, Drug product, High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC).*

## 1. Introduction

In the pharmaceutical industry, chromatography serves widely as an analytical method, a method for purification of substances and a method for the control of the quality of the compounds and drugs produced. They include Wilhelmy violations and Alvarez whirls, which affect every phase of drug development and production. Chromatography is a versatile separation and analytical technique that could be used to identify, quantify, and sometimes isolate components within a mixture, an exponentially important phenomenon in the development of safe and potent drugs. Early to mid-stage drug discovery, chromatography helps in the identification of active pharmaceutical ingredients, or APIs, making it easier to study and find a suitable drug. In a synthesis process, chromatography provides a measure of keeping APIs and intermediates free from contaminants, which plays a vital role in formulating quality pharmaceutical products. Hanzi, Peaks that are not diluent, placebo or API of salt form are considered extraneous peaks. Since several degradants are identified at Forced degradation, peaks other than them are identified as extraneous peaks. Peaks which are present in the chromatogram in addition to all individual components are called extraneous peaks or sometimes referred to as peaks excluding residual solvents. As well, it greatly helps in stability studies where the level of degradation products is determined, which enables the assessment of the shelf life of parent products and their proper storage conditions. Besides, pharmacokinetic and metabolism studies with the use of chromatography practices of drugs are of great importance in terms of understanding their absorption, distribution, metabolism, and excretion (ADME). The high resistance to interference coupled with high efficiency makes chromatography techniques, including HPLC and GC, valuable tools in meeting regulatory mandates and protecting public health by verifying that phares and bio-products are safe and effective. In this critical procedure, extraneous peaks in chromatography present numerous issues because they

interfere with the identification as well as quantities of analytes, complicate quality control aspects, increase the overall complexity of analyses, affect compliance with regulations, and, most importantly, are a financial burden. To overcome these challenges during method development and validation, it is essential to devote considerable time and effort to the selection of the appropriate sample preparation technique, system suitability tests, and appropriate optimization parameters in chromatography.

## 2. Literature Survey

### *2.1. Historical Perspective*

The practice of impurity profiling in pharmaceutical products can be as old as chromatography, beginning in the early part of the 20th century. For many decades, analytical instrumentation grew at a rate more than required to improve impurity profiling and aid in the detection of impurities in various products.

### *2.2. Early Methods*

*2.2.1. Paper Chromatography*

The direction of one of the first unique ways to be used as a method of separating impurities. A separation technique which was first used during the 1940s, paper chromatography involves placing the sample on a strip of paper and allowing a solvent to move up through the paper via capillary action. Each of those parts of the sample has a different velocity, and there is a velocity difference which makes the sample separate. This method was, however, commonly applied for qualitative analysis because it was simple and inexpensive.

*2.2.2. Thin-Layer Chromatography (TLC)*

TLC was developed in the nineteen fifties as an evolutionary advancement over what was then known as paper chromatography, in which the material being tested is washed through a thin coating of absorbent material such as silica gel spread on a slide of glass, plastic or aluminum. TLC afford the clean separation and elegant visualization of the impurities, and it is useful for qualitative and semiquantitative analysis. The technique used in this kind of separation is simple, swift and cheap, and in producing the separation, it offers resources in the form of UV light or chemical stains for detection.

### *2.3 Modern Techniques*

*2.3.1. High-Performance Liquid Chromatography (HPLC)*

HPLC was developed in the early 1970 and, soon after that, became the most popular method for impurity profiling due to its high-resolution ability and versatility of the method as well as its capability to analyze the increased number of sample types.

By means of HPLC, the solvent is forced through a column that contains a stationary phase with the help of high-pressure pumps. Frameworks in the sample adhere to the stationary phase to qualitatively different extents, which leads to separation. Being a chromatographic technique, HPLC is not only amenable to qualitative and quantitative analysis but is also vital for the analysis of impurities present in pharmaceutical products.

*2.3.2. Gas Chromatography (GC)*

GC, which emerged in the mid-twentieth century, extended the concept of impurity profiling to Volatile and Semi-volatile impurities. In GC, the sample is first evaporated and then using a carrier gas transported though a column which has a fixed phase. Sample components are now distributed and divided depending on their solubility and response towards the stationary phase. GC is very suitable in the analysis of compounds that can undergo vaporization without undergoing chemical change since the method delivers high resolution and sensitivity.

*2.3.3. Coupled Techniques*

As for the separation itself, using additional detectors with high throughput, such as Mass Spectrometry and Nuclear Magnetic Resonance has dramatically increased the possibility of detecting and accurately measuring impurities present in the sample. These coupled techniques offer more detailed structural data of the molecule and are vital for the identification of unknown impurities. For example, the HPLC-MS and GC-MS techniques facilitate the separation of compounds in a mixture based on their polarity and mass analysis to establish the molecular weight of the compounds as well as the structure. Likewise, HPLC-NMR facilitates the identification of compounds after their separation in a manner that works synergistically with the identification offered by MS.

### *2.4 Analytical Techniques*

*2.4.1. High-Performance Liquid Chromatography (HPLC)*

Originally developed as a method utilized in the separation and determination of purity of substances. Therefore, HPLC renders high resolution and sensitivity, [1] constituting it for various pharmaceutical uses. Especially suitable for non-volatile and thermally unstable compounds to stabilize to prevent decomposition under high heat.

*2.4.2. Gas Chromatography (GC)*

Applicable for working with compounds with a relatively short but measurable rate of evaporation and with compounds classified as volatile and semi-volatile. The GC is highly sensitive and yields high resolution particularly when its functionality merges with that of the MS. [4] It is frequently used on residual solvents and other volatile compounds of alcoholic preparations.

*2.4.3. Mass Spectrometry (MS)*

Present accurate molecular information, which is primary in defining unknown impurities. MS can be employed as individual technology or interactively with other chromatographic methods. It provides high sensitivity and specificity, the application of which will help diagnose very low levels of contamination.

*2.4.4. Nuclear Magnetic Resonance (NMR)*

Elucidates the structure of impurities provided. These impurities in the polymers can be studied in detail by using NMR, which offers information regarding molecular structure, dynamics of the impurities and their environment. This is especially useful when trying to reidentify unknown compounds which may have been separated from a mixture.

*2.4.5. Fourier Transform Infrared Spectroscopy (FTIR)*

More relevant to the analysis of functional groups of complex organic compounds. FTIR enables the identification of impurities by giving information on the functional groups of an object through the interaction of the object with infrared light. It is normally applied jointly with other methods to support the obtained results.

## 3. Methodology

### *3.1. Sample Preparation*

Sample preparation is the first step in defining impurity profiling. This process involves preparing the samples in a way that makes them a true reflection of all the samples and eliminating any elements that might hinder the results of the samples Figure 1.

- Selection of Sample Matrix: Use proper solvents and diluents given the nature of the drug product or API for preparation of the final formulation.
- Dilution and Filtration: Crash the sample and then filter it to get rid of particulate matter at the appropriate concentration level.
- Standard Preparation: Predict the locations of any known impurities for crosschecking and calculation.

### *3.2. Chromatographic Separation*

Some techniques that are used to cover the separation consist of High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC).

*3.2.1. High-Performance Liquid Chromatography (HPLC)*

- Column Selection: Select the proper stationary phase for the separation you are striving to accomplish.
- Mobile Phase Optimization: Adjust the solvent gradient to get more resolution or, if necessary, increase the proportion of the second solvent to get more peaks.
- Flow Rate and Temperature: All these depend on the flow rate and the temperature of the process in order to achieve a preferred level of separation.

*3.2.2. Gas Chromatography (GC)*

- Column Type: Choose good separation based on the variance and bipolarity of the compounds to be analyzed. Temperature Programming: They are uitable for the separation of complex mixtures based on the principle of temperature gradients.

### *3.3. Detection and Quantification*

A comparison of Detection Techniques of UV Detector, Mass Spectrometry, and Flame Ionization Detector, with the Sensitivity, Specificity, and Common Use are mentioned in Table 1.

- UV Detectors: Used widely in HPLC for the detection of UV active end compounds.
- Mass Spectrometry (MS): Offers good results as it targets only the entire genome with narrow specificity.
- Flame Ionization Detector (FID): Very useful in GC for the identification of many volatile compounds of significance in the flavor and aroma of foods.

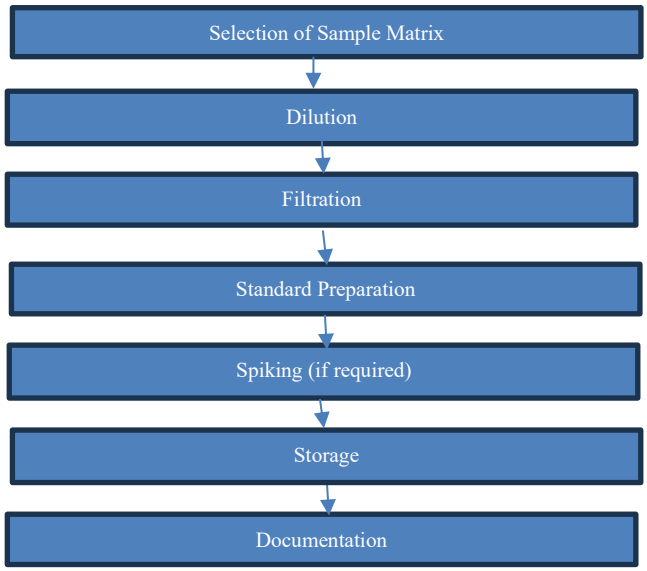


**Fig 1. Sample preparation process**

**Table 1. Comparison of detection techniques**

| Technique | Sensitivity | Specificity | Common Use |
|---|---|---|---|
| UV Detector | Moderate | Low | General impurities |
| Mass Spectrometry (MS) | High | High | Unknown impurities |
| Flame Ionization Detector (FID) | High | Low | Volatile impurities |

### *3.4. Identification*

The two techniques most useful in the identification of unknown impurities are known as Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy.

*3.4.1 Mass Spectrometry (MS)*

- Electrospray Ionization (ESI): For non-polar compounds, the separation should be reversed as indicated in the following result tables:
- Tandem MS (MS/MS): Offers structural elucidation fragmentation patterns at varying and higher resolution.

*3.4.2 Nuclear Magnetic Resonance (NMR)*

- 1D and 2D NMR: Render in-depth descriptions of the molecular structure.
- Quantitative NMR (qNMR): Crude measurement of pollutants: accuracy of impurities measurement.

### *3.5. Qualification*

Furthermore, qualification requires an understanding of the impact and safety of the mentioned impurities on the drug product or the API. Qualification Criteria for Impurities are mentioned in Table 2.

- Toxicological Assessment: Once a compound has been identified as an impurity, evaluate its potential toxicity.
- Regulatory Compliance: Therefore, concentrations of impurities should not go above the determined levels regarding ICH Q3A/B and M7.
- Stability Studies: To carry out stability studies to study the effect of time on the behavior of impurities.

### *3.6. Data Analysis*

- Accurate data analysis is critical for identifying and quantifying impurities.
- Chromatographic Peak Integration: Be very careful to obtain the correct peak area.
- MS Data Interpretation: Analyze mass spectra to identify unknowns.
- NMR Spectral Analysis: Interpret NMR spectra for structural information.

### *3.7 Sources of Extraneous Peaks*

Sources of Extraneous Peaks in Chromatography these are additional or secondary peaks which arise and reduce the ease of reading and understanding chromatographic data. Failing to do so compromises the validity of the entire analysis and thus must be addressed before continuing. Herein is a stepwise discussion of the key sources of extraneous peaks:

**Table 2. Qualification criteria for impurities**

| Impurity Type | Qualification Threshold | Regulatory Guideline |
|---|---|---|
| Genotoxic mpurities | ≤ 1.5 µg/day | ICH M7 |
| Ordinary Impurities | ≤ 0.2% | ICH Q3A/Q3B |
| Degradation Products | Case-by-case basis | ICH Q1A |

#### *3.7.1. Contaminants in Samples*

Impurities and contaminants of the sample matrix are also a source of extraneous peaks. These may include:

- By-products: Byproducts which are not the main products of a chemical reaction but are flared off along with other products.
- Degradation products: Products that were synthesized when the sample was subjected to the breaking-down process over time.
- Unintended compounds: Species that are introduced during sample collection or preparation of the sample for analysis.

#### *3.7.2. Reagent and Solvent Impurities*

Impurities and contaminants of the sample matrix are also a source of extraneous peaks. These may include:

- By-products: Byproducts which are not the main products of a chemical reaction but are flared off along with other products.
- Degradation products: Products that were synthesized when the sample was subjected to the breaking-down process over a period.
- Unintended compounds: Species that are introduced during sample collection or preparation of the sample for analysis.

#### *3.7.3. Carryover from Previous Analyses*

The DF leakage issue occurs due to the carryover of previous sample residues in the chromatography system. This is problematic if It is critical to retain the metabolites because their retention times are close to the required analysts.

#### *3.7.4. Instrumental Contamination*

Carryover can be gradual and can reach internal standards or samples, and contaminants can be trapped and retained within the chromatography system and cause extraneous peaks. Regular cleaning and maintenance are necessary to address contamination in the following ways:

- Injector: Preservatives from prior examinations or tests of the same type of samples.
- Column: Retention of substances in the stationary phase that progressively accumulates and builds up on the column's walls.
- Detector: Remaindering to which some compounds interfere with accurate detection.

#### *3.7.5. Column Degradation*

The stationary phase of the chromatography column should be stable and not disintegrate to release degradation products into the system. Proper maintenance includes:

- Regular column cleaning.
- A timely replacement of the column since, on current trends the column is approaching its maximum use capacity.

#### *3.7.6. Inadequate System Equilibration*

Insufficient equilibration of the chromatography system can result in insufficient equilibration of the chromatography system can result in:

- Baseline noise.
- Unexpected peaks. A primary requirement in deploying samples on the column is owed to its equilibration with the mobile phase before samples are run.

#### *3.7.7. Sample Preparation Artifacts*

Due to this, extraneous peaks arising from improper sample preparation are common in chromatograms. Issues may arise from:

i Inadequate filtration: A component that is not taken out or excluded from an assemblage, collection, or group.

ii Insufficient mixing: Sample component imbalances between sub-groups.

iii Introduction of contaminants: Sources due to preparation period I the type of water used for preparation The quality of water used for preparation Includes bacteria and any material that may have gotten into the water before preparation.

*3.7.8. Mobile Phase Contamination*

- Interfering substances in the mobile phase can lead to noise formation, which may result in extraneous peaks. Preventative measures include:
- The other factor that should be considered in the optimization of the separation of the said compounds is the purity of the mobile phase utilized in the separation technique.
- Correct screenings to avoid the entrance of dust, microorganisms, and chemical contaminants.

*3.7.9. Chemical Interactions*

Other chemical reactions that occur in the sample can form some chemical species that will appear as additional individual peaks. These reactions may occur between

- Solvents and sample components.
- Some of the traditional solvents are used for extraction and the stationary phase.

*3.7.10. Detector Artifacts*

- Interferences: interferences present in the detector can also cause peaks that do not represent the actual sample to be analyzed. Issues may include:
- Electronic noise: Every change in the detector related to its electronic system is reflected in the generator.
- Baseline drift: Slow deviation in the detector baseline.
- Fluctuations in sensitivity: Completing contrasts in detector response.

*3.7.11. System Hardware Issues*

Possible causes for these extraneous peaks are found inside the chromatography system; these are known to be hardware-related. Common issues include:

- Leaks in the system.
- The presence of gas bubbles in the mobile phase.
- Malfunctioning components.

*3.7.12. Environmental Contamination*

Dust or fumes from the laboratory setting can also interlope on samples and mobile phases to cause interferences or confound peaks. The analytical procedure is best done in a controlled and clean atmosphere, more so when dealing with biochemical tests.

*3.7.13. Other Sources*

Other potential sources of extraneous peaks include:

- Cross-contamination during manufacturing: Interference by the manufacturers when they give out the samples.
- Cross contamination during analysis: Interference taking place within the laboratory at the time of conducting the analysis on the samples.
- Leachable from container closure system: The content which is useful for the development of the microbial film may leach out the compounds present in the container materials.
- Excipient and container closure interaction: A specific interaction of the excipients with the container closure system in terms of chemical incompatibility.

***3.8 Investigation, Identification, and Qualification of Extraneous Peaks in Chromatography***

An important aspect of peak identification in chromatography, therefore, concerns the qualification of extraneous peaks that may compromise the results. That is why this process is subdivided into several steps and presupposes the use of different analytical methods that can help to identify the nature and source of such spikes. Below is a detailed description of the key steps and methods used for identification:

*3.8.1. Comparison with Blank Runs*

*Blank Samples*

To identify those peaks which are arising due to solvent reagents or the system itself, running blanks that consist of all constituents of the samples, but the analyte can be used. In this way, the peaks that appear in both chromatograms of the blank and the sample would indicate being due to the system or reagents used.

*Mobile Phase Blanks*

The condition of the mobile phase can be known by running only the mobile phase through the system, which can distinguish peaks originating from the impure mobile phase or contamination in the system. Any peaks obtained in the mobile phase blank are an indication that the contamination is in the mobile phase or the entire system.

*3.8.2. Use of Standard Solutions*

*Reference Standards*

The execution of standard solutions previously identified under the same conditions can assist with arbitrating between expected analyte peaks and additional noise. In this way, by comparing the peaks of the chromatogram of standard or reference as well as that of the sample, any extra peaks are characterized as impulsive.

*Spiking Experiments*

A simple approach to determining the origin of unwanted coextractives or possible degradation products is to introduce known amounts of such substances into the sample. The presence or increase in certain peaks due to changes in the chromatogram helps in identifying the contaminants themselves.

*3.8.3. Method Development and Optimization*

*Gradient Elution*

Applying gradient elution to a sample in liquid chromatography is an effective way to achieve great separation of compounds with very similar elution profiles. The gradient option allows one to make the separation of the analytes and other peaks that may be a resultant of the method distinct, making identification easier.

*Column Selection*

It is also useful when trying to separate a compound of interest from a very complex matrix or when a new column is being tested to try out different columns with different stationary phases, as this will help show peaks that may be attributed to column degeneration or interference. Thus, by performing the analyses of samples of stationary phase with different chemistries of the columns, it could be ascertained whether the extraneous peaks originated from the column.

*3.8.4. Mass Spectrometry (MS)*

*Coupling with Chromatography (LC-MS/GC-MS)*

Mass spectrometry gives deeper molecular profile analysis of peaks, which aids in categorizing extraneous peaks in synthetic and natural mixtures since tandem chromatography with MS, molecular weights and structures can be measured.

*Mass Spectral Libraries*

Analyzing extraneous peaks with the help of its mass spectra and literature spectra proved to be helpful in identifying unknown peaks. The exact nature of the unwanted compounds can subsequently be identified by comparing them with entries in a library.

*3.8.5. Spectral Analysis*

- UV-Vis Spectroscopy: The information on the relationship between individual peaks in UV-Vi's spectra and the possibility of identifying compounds according to their absorbance profile. Some compounds will have a different degree of absorbance at that wavelength.
- Diode Array Detectors (DAD): Using DAD makes it possible to obtain the full UV-Vis spectra for each peak or a specific range of it. By generating the relative graphs for the obtained spectral data, it is possible to differentiate between the extraneous peaks by analyzing their absorbance profiles.

*3.8.6. Retention Time Comparison*

- Retention Index Matching: Therefore, the retention time or retention indices can be matched to the solution of other samples, and familiar substances can be excluded. This clearly means that all these compounds are present because the samples have given retention times that can be associated with the known standards.
- Retention Time Stability: Whenever there are peaks that seem to be consistent over several runs of the sample, it is possible to compare retention times and hence identify if the problems are systematic or random. When the period expenses are constant, they mean like there is a fixed problem of contamination, whereas those that are irregular imply there is abnormal contamination.

*3.8.7. Chemical Derivatization*

Derivatization Reactions: An additional step of chemical derivatization reaction helps in transforming their chromatographic characteristics, thus aiding in the identification of extraneous peaks. This is easier to achieve through adjusting the chemical composition of the compounds, making it hard to confuse with target analytes.

*3.8.8. Systematic Troubleshooting*

- Component Isolation: Separated testing of various components of the chromatography system (injector, column, detector) can be used to identify the source of contamination since making multiple changes at a time can complicate efforts to determine the reason. By analyzing every fragment differently, the main reason behind the presence of extraneous peaks can be established.
- Flow Path Examination: Checking the overall attachment to search for areas where contamination may come in through tubing, fittings and valves may also assist in eradication of extraneous peaks. Thus, from the point of view of contamination, the water can be polluted at any of the points described in the flow path, so that the assessment is detailed.

*3.8.9. Environmental Controls*

Lab Environment Monitoring: Representative common outside sources that need to be scanned and determined in the laboratory involves environmental contaminants such as airborne particles, solvent vapors, and the like that may influence extraneous peaks. Such control of contamination sources is effectively evidenced by keeping an eye on the lab arrangement.

*3.8.10. Documentation and Record Keeping*

Historical Data Comparison: To assess chromatograms of different time points, it is possible to contrast them to previous information; this situation allows us to define repeatable extraneous peaks and potential sources of the problem. Previous readings generated from excitation spectra are stored in formats such as text files effectively enabling one to look for trends in the extraneous peaks.

### *3.9. Investigation, Identification, and Qualification of Extraneous Peaks in Chromatography*

It is a significant challenge to detect and quantify extraneous peaks in chromatographic systems in most cases, and the impact of the interfering substance on the sample must be determined to obtain meaningful and accurate results [3]. These are done scientifically through several analysis steps and methods to find out the source of these usually unrecognized spikes. Below is a detailed description of the key steps and methods used for investigation, identification and qualification.Review Forced Degradation Data and Placebo Relevance to the Proposed Method and the Dosage Form Presentation

1. Forced Degradation Studies: The process of forced degradation may create extraneous peaks in the spectrum; thus, it is important to review forced degradation data to

ensure the presence and type of extraneous peaks. This requires evaluation of the stability of the analyses, its response to light, heat and the pH scale.

2. Placebo Analysis: Perform a direct comparison of chromatograms of actual products with chromatograms of placebo products; this comes with all the constituents of the actual product minus the active ingredient and helps in the identification of peaks from the excipients or other sources.

*Study of Past History*

- Stability Study: Moreover, to ensure that the peaks outside the original EXV range are genuine and not artifacts resulting from some malfunctioning in the system or a given experimental run, one can turn to the historical stability study data and look for similar extraneous peaks.
- Similar Events: Also, check the previous reports of such events – was there ANY similar issue, whether connected to troubleshooting records.

*Reinjection and Artifacts*

- Reinjection: This is to reinject the test solution in order to evaluate if the extraneous peak is due to the instruments. The repeatability of spiky signals in an equivalent reinjected sample may suggest sustained contamination.
- New Vial Injection: As you conclude the test, transfer the test solution to a new vial and inject for vial artifacts. Certain peaks, which are not present in the spiked chemical or the blank but are characteristic of the sample in the original vial, suggest contamination from the vial.
- Refiltration: Rerun the filtration process on the test solution and inject further to recognize the artefact arising from filtration. Indications of peaks that are only found prior to refiltration are indicative of contamination by the filter.
- Redilution: Use diluted format to inject to ascertain the presence of glassware-related artifacts. If peaks are no longer observed once new glassware is employed, then the source may, in fact, be tainted glassware.

### *3.10. Extended Run Time and Carryover*

*3.10.1. Extended Run Time*

They suggest letting the chromatographic run a bit longer in order to detect possible carryover from previous samples. The peaks that elute late or those that are still present in the chromatogram could be overwhelmed by residual compounds from the previous injections.

*Primary Packaging and Labels*

- Primary Packaging Injection: Puncture the solution by swab extraction made from primary pack example, Alu-Alu Foil, to help in determining if the source originates from primary packing.
- Printing Labels: Spike solutions of printing labels are prepared to assess the level of contribution of the printing process to extraneous peaks.

*Previous Product and Cross-Contamination*

- Previous Product Injection: Introduce the solution from the other product to ascertain that it comes from an area that has been cleaned to show that it has not been cleaned. Lowing peaks that signify earlier products further suggest that there were not enough cleaning processes in place.
- Parallel Product Injection: dispense solution prepared from parallel run products to cause cross-contamination whilst manufacturing.

*Solvents and Excipients*

- Solvent Injection: Introduce solvents utilized in production into the research process to recognize the cause as residual solvent remaining in the product.
- Excipient Injection: Administer single-ingredient placebos, where appropriate, if a placebo is not accessible or the quality of the placebo is doubtful. This assists when determining if excipients cause extra peaks to be encountered.

*3.10.2. Qualification of Extraneous Peaks*

To qualify an extraneous peak, one must uncover the nature of the peaks, their origins, and the influence of their interference on the analysis outcome.

Through this process, several techniques of analysis are applied to fully understand these peaks in as much as enhancing the accuracy and reliability of data in chromatography. Here are the main techniques used for the qualification of extraneous peaks [2].

*Mass Spectrometry (MS)*

- LC-MS/GC-MS: The use of the serial LC or GC together with MS means that it can easily be identified with the help of additional peaks in the molecular structure. About MS, it can provide information on molecular weight, the degradation of ions, and even the molecular structure.
- Tandem Mass Spectrometry (MS/MS): Compared to the relatively simple data obtained from the first mass spectrometer, the second source of fragmentation data in MS/MS is even better in terms of Unknown compound structure determination and qualification out spikes [7].

*Nuclear Magnetic Resonance Spectroscopy is a useful analytical tool in chemistry, especially when determining the structure of a chemical through hydrogen nuclei/Carbon*

- 1D and 2D NMR: Using NMR spectroscopy, one gets detailed information on the molecular structure of the organic compound studied. Some of the advantages that it can give include It can give more information depending on substances in the form of extra peaks, which can assist in the confirmation of their existence, taking into account their molecular form.
- NMR Comparison: The extension of the extra peak from the reference NMR spectra is another approach to the compounds' qualification.

*FTIR (Fourier Transform Infrared) Spectrophotometry*

FTIR Analysis: When FTIR spectroscopy is being targeted, one can easily identify the functional groups in the compound which are responsible for the extraneous peak which is obtained. The bands' assignment in the IR spectra reveals characteristic absorption bands of the functional group as observed.

*Ultraviolet-Visible (UV-Vis) Spectroscopy*

- Spectral Analysis: UV-Vis spectroscopy is also useful when it comes to the identification of compounds in a given solution through their ''absorbance spectra.'' UV-Vis could be of help in qualifying the extraneous peaks through the spectra of the peaks in unknown samples against spectra of known standards.
- Diode Array Detection (DAD): Both peaks have DAD for corresponding peaks' full UV-Vis spectra that can be used for further evaluation, passing the qualification step.

*High-Resolution Mass Spectrometry (HRMS)*

Accurate Mass Determination: They pointed out that managing molecular weight in HRMS is much improved than in the exact mass, hence helps distinguish between structures that may be hard to tell apart merely by mass value; it also helps in evaluating the quality of interfering peaks.

*Chromatographic Techniques*

- Retention Time Comparison: Sample identification also involves matching the retention times of the extraneous peaks anew to the retention times of standards.
- Retention Index Matching: The retention indices can also assist in observing those extra peaks since, otherwise, one would compare them with the known compound. Despite this, they have not been developed perhaps to a similar level as the above e-commerce smartphone commerce types.

*Chemical Derivatization*

*1. Derivatization Reactions*

The change brought about by the derivatization process is to alter the chemical structure, especially the functional groups of compounds that take part in the process and this has the potential of changing the chromatographic property of the compounds. When these changes are monitored, they help you qualify other ill effects, such as extraneous peaks, which are normally observed in the graph.

*2. Standard Addition and Spiking Experiments*

Spiking: Merely by adding aliquots of suspected contaminants or degradation products to the sample and reconstructing the chromatogram, one can effectively qualify the observed peaks.

*3. Spectral Libraries and Databases*

- Library Matching: In relation to the identification of extraneous peaks, there is also a potential for comparison of the spectrum (for example, MS, NMR or FTIR) with the spectra existing in some spectral reference database.
- Database Searches: The unknown peaks that fit the criteria for an MLO analysis can have supplementary data collected from substances available in PubChem
- or ChemSpider, as well as additional spectral libraries available to commercial users.

*4. Systematic Troubleshooting and Isolation*

- Component Isolation: By testing the chromatography system in sequence i.e., starting with the injector, then the column, and then the detector, can assist in pinpointing the cause of the contamination.
- Environmental Monitoring: Ascertaining whether the peaks marked can be potential environmental interferents can help in judging other additional peaks by their association.

*5. Quantitative Analysis*

Quantification of Impurities: Many of the extraneous peaks may interfere with the characteristic peaks and to determine this, calibration curves and reference standards may be employed.

*Complete Flow Chart of the Process*

- Identify the structure or molecular weight of the extraneous peak (by MS/NMR).
- Literature review of the peak.
- Structure-based assessment

Step 1: Initial Screening

*Non-Mutagenic (- ve result)*

If both toxicogenical and genetic studies are negative, then based on ICH Q3A/Q3B guidelines, qualified limits are established by reference to the qualification threshold. This must not be a problem of an impurity that has the potential to harm a patient or negatively affect the outcomes of the tests Figure 2.

*Melanoma (+ ve result)*

If initial screening indicates a possible mutagenicity result, further non-clinical testing is then pursued. This includes: AMES Test: A mutagenic chemical identifying bacterial test that examines the ability of a chemical to cause reverse mutation in relevant gene sequences of bacteria.

Chromosomal Damage Study: This entails a Mouse Lymphoma Assay or other related tests to assess chromosomal mutation or injury.

Step 2: Most of the non-clinical studies described here produced satisfactory outcomes.

*Negative Result (Non-Mutagenic)*

If non-clinical studies are negative, the impurity is considered 'non-mutagenic', and the evaluation process

continues with anaerobic studies. Some restrictions are established relative to the qualification norm in accordance with ICH Q3A/Q3B.

*Positive Result (Possible Mutagenic)*

If tests with non-clinical models provide positive results, giving a hint of mutagenicity, then the real level of risk must be assessed in clinical trials. This includes: Rodent Studies: These studies aid in evaluating the mutagenic risk in vivo.

Step 3: These clinical study outcomes for this case show that suicidal ideation associated with MDD is characterized by cowardice, shame, and an absence of aggression.

*Negative Result (Non-Mutagenic)*

If the clinical study yields a negative response, then the impurity is said to be non-mutagenic and, thus, has its limits determined by the ICH guidelines Q3A/Q3B.

*Positive Result (Mutagenic)*

If the clinical study confirms mutagenicity, further assessment is needed to determine safe exposure levels: If the clinical study confirms mutagenicity, further assessment is needed to determine safe exposure levels: Evaluate LD50 (Lethal Dose 50%): This determines the hazard that the impurity presents in terms of irritant effects on the skin.Decide Limit: By proposing the LD50 and the other safety features, it is possible to propose a limit on the impurity content to protect the patient's health.

*Initial Screening*

The first tier is carried out to ascertain if an impurity of interest would probably be mutagenic. This phase employs well-known tests such as the AMES test for the quick evaluation of the mutagenic activity of substances. If the outcome of the analysis suggests that the impurity is non-mutagenic, it can be managed according to general regulation measures.

*Non-Clinical Studies*

If the first steps show a possibility of being mutagenic, further non-cliotic studies are done to gain more knowledge. The AMES test and the chromosomal damage studies are the ways to understand if the impurity causes mutations or chromosomal damage. During this phase, there are possibilities of having some undesirable impurities which must be regulated more strictly.

*Clinical Studies*

For impurities classified as potential mutagens in non-clinical studies a clinical study involving a rodent model is performed in order to determine the actual biological effect seen in a whole organism. These studies assist in establishing the extent to which impurity is a real danger to human health in terms of mutating cells within the body.

*Establishing Safe Limits*

Where clinical tests show that the chemicals have mutagenic effects, they find the maximum levels of exposure acceptable. The LD50 is used to compare the toxicity level, and with the help of these limitations, the permissible amount of the impurity is fixed within the safe limits of the final drug product.

*Batch Disposition: What is the Root Cause?*

*1. Lab Error*

If the out of specification point is deemed as a laboratory issue, there is no need for batch disposition. Correction and prevention measures for similar errors must be taken; hence, Proper Corrective and Preventive Actions (CAPA) must be undertaken, Figure 3.

*2. Product Related*

Possible application of other wavelengths for product-specific identification In order to execute proper measurements for a product-related extraneous peak, additional investigation is required. This can be due to:

*a) Previous Product Cross Contamination*

Check if the observed value is less than the Maximum Allowable Carryover (MACO) of the previous product:

- Yes: Launch the batch with CAPA to release the batch so that such an occurrence is not repeated.
- No: It is necessary to dispose of the batch with CAPA and review the cleaning validation procedures. Non-Mutagenic Impurity, Mutagenic Impurity and Nitrosamine Impurity

*b) Develop and Validate method*

- For the mentioned impurities, the sort of analytical methods need to be developed and effectively validated.
- Dose at which a contaminant is present in the food below QT/AI nivel.

**Table 3. Summary of analytical tests for impurity profiling**

| Test Type | Description | Purpose |
|---|---|---|
| AMES Test | Bacterial reverse mutation assay | Initial mutagenicity screening |
| Chromosomal Damage Study | Assays like Mouse Lymphoma | Detect chromosomal mutations/damage |
| Rodent Studies | In vivo studies in rodents | Confirm mutagenic risk in the whole organism |
| LD50 Evaluation | Determination of lethal dose for 50% of the population | Assess acute toxicity |

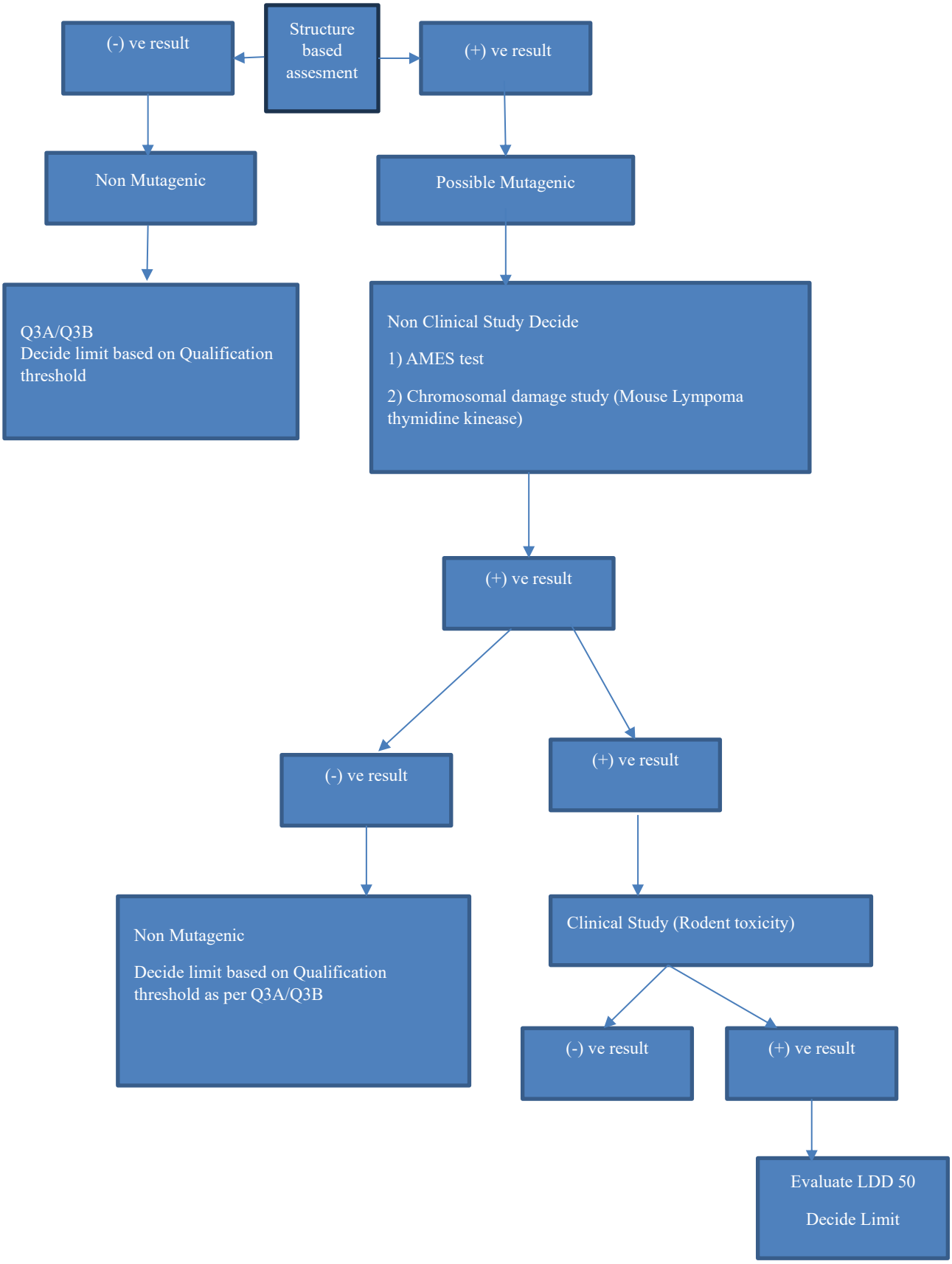


**Fig. 2 Extraneous peak process**

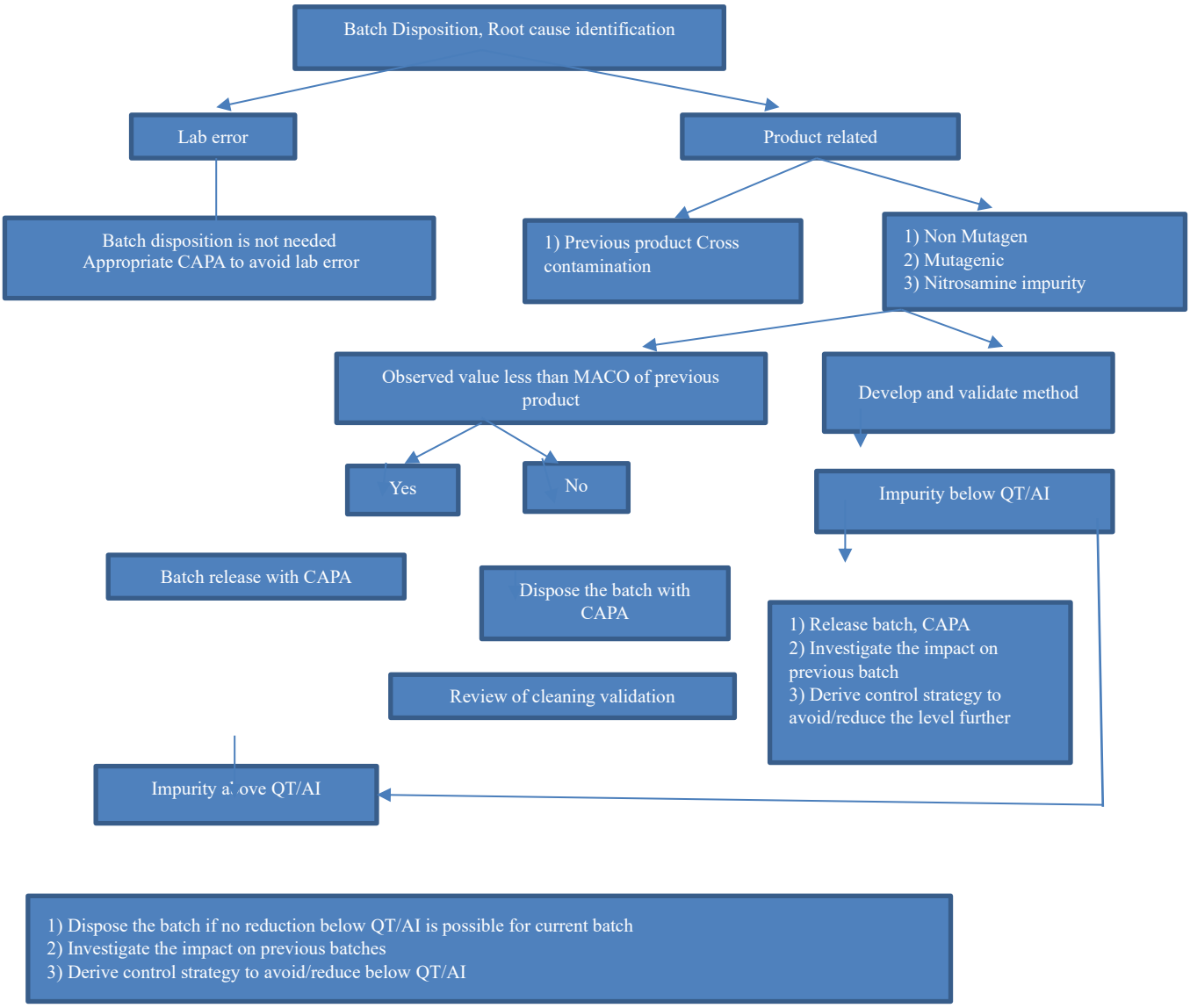


**Fig. 3 Batch disposition**

*c) If the impurity is below QT/AI levels, perform the following steps:*

- The current recommended option for their release should be to release the batch with CAPA.
- Examine the effect on previous batches that were processed with the new system.
- The following are some of the specific actions, which should be taken to avoid or to reduce the extent of the impurities in the materials.

*d) Impurity Above QT/AI*

If the impurity is above QT/AI levels, consider the following:

- Discard the batch if refining is nQT practicable to bring impurities levels below traditional OT/AI for the current batch.
- Explore the effects on the former groups.
- Come up with control measures that would minimize or eradicate incidences of such impurity levels reaching QT/AI.

*Explanation of the Diagram*

Retention Time (minutes): The largest value is passed along the x-axis as time, with units in minutes representing the retention time. Intensity (mAU): So in this particular diagram, on the vertical axis, there is a scale marked up to the milli absorbance unit abbreviated as ‘mAU’, which represents the level of intensity. Chromatographic Separation: When two distinct phases or components of the sample are located across the blue line, it is possible to partition them according to this curve. Impurity Peaks: On the peaks representing impurities in the above barcode, red crosses are appended to indicate the following: These peaks are named to make it easy for one to follow the trend of the graph if any), organizational.

**Table 4. Chromatographic Separation Data**

| Retention Time (minutes) | Intensity (mAU) |
|---|---|
| 0 | 0 |
| 1 | 10 |
| 2 | 30 |
| 3 | 50 |
| 4 | 20 |
| 5 | 10 |
| 6 | 5 |
| 7 | 3 |
| 8 | 2 |
| 9 | 1 |
| 10 | 0 |

*Lab Error Investigation*

Even if one is faced with an extraneous peak that originates from a laboratory error, it is vital to localize the error. The common cause may be unsuitable preparation of the sample to be tested, improper functioning of the analyzing instrument, or mistakes made by an operator. It is about time that CAPA re-direct its attention to improving lab procedures, training staff and equipment maintenance in order to avoid reoccurrence.

*Product-Related Investigations*

If the extraneous peak is product-related, then method development investigation is necessary to diagnose if the peak is coming from cross-contamination or is an ability of the method.

When present in the product, it can be confirmed that it stems from cross-contamination by evaluating the mac values of the impurities found with MACO values from the previous products.

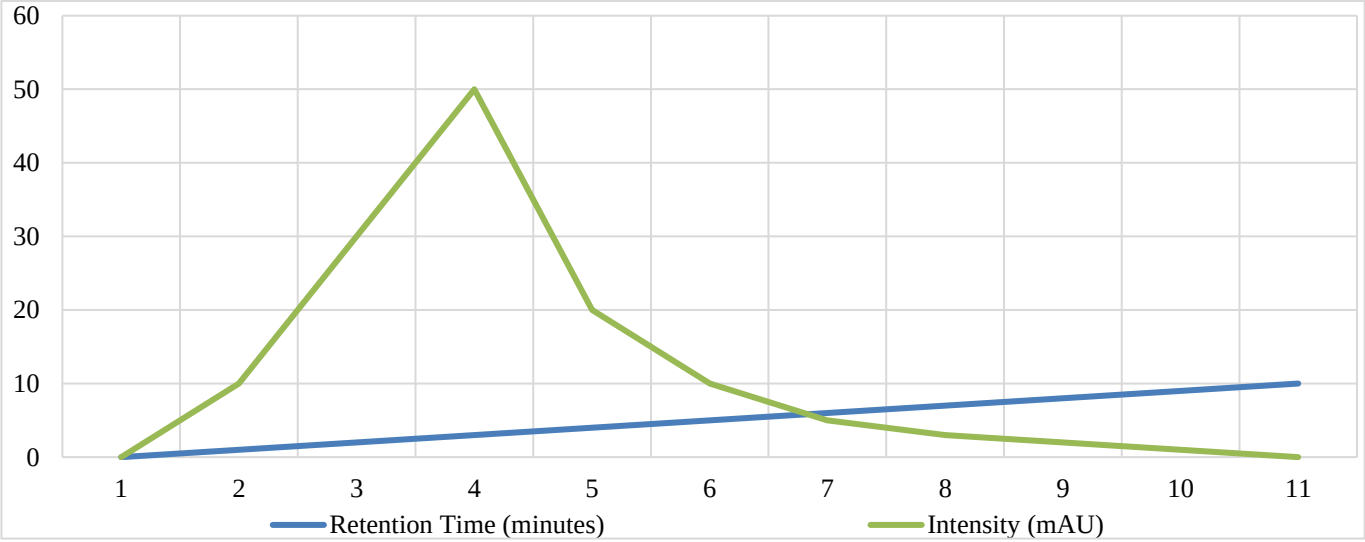


**Fig. 4 Retention time and intensity**

For other types of impurities, like mutagenic or nitrosamine impurities, separate methods need to be identified and applied, and their analytical procedures need to be fixed and approved. AMDDV The process of designing and establishing suitable methods for analytically measuring a substance of interest in a matrix of a given complexity is known as Analytical Method Development and Validation (AMDDV). The fact of the matter is that the identification and quantification of impurities remains a significant challenge today: Advanced analytical methods are of paramount importance when it comes to the methodology of impurity profiling. This entails the choice of the right type of chromatographic technique and detection method for analysis, tuning of different parameters, and method documentation in compliance with the regulatory requirements.

*CAPA Implementation*

By analyzing the source of those impurities, CAPA helps in putting in place corrective actions that should be taken. This entails revisiting some of the key cleaning practices, refining the production methods, and meeting the required safety codes.

*Regulatory Compliance*

It is very important to control and ensure that those impurities get to an acceptable level in compliance with the law. Taking some time to ensure that the set analytical methods are checked and verified makes it possible to maintain compliance with the set forth guidelines on the production of pharmaceutical products.

**Table 4. Common sources of impurities**

| Source | Description |
|---|---|
| Raw Materials | Impurities originating from raw ingredients |
| Intermediates | By-products formed during synthesis |
| Reagents | Residual chemicals used in the process |
| Solvents | Residual solvents used during formulation |
| Degradation | Products formed due to chemical instability |

**Table 5. Regulatory guidelines for impurity levels**

| Guideline | Description |
|---|---|
| ICH Q3A (R2) | Impurities in New Drug Substances |
| ICH Q3B (R2) | Impurities in New Drug Products |
| FDA Guidance | Impurities in Drug Substances and Products |

## 4. Results and Discussion

### *4.1. Fingerprinting Analysis of Impurities by Chromatographic Techniques*

In other words, conforming to the results of the HPLC analysis, several additional peaks of impurities in the drug product and APIs were detected. These parameters were noted, and subsequently, the quantification of these impurities and identification of the peaks was achieved. The following table briefly outlines retention times and the relative peak areas of the observed impurities:

**Table 6. Chromatographic data of detected impurities**

| Peak No. | Retention Time (min) | Peak Area (mV*min) | Identification |
|---|---|---|---|
| 1 | 2.5 | 15.4 | Impurity A |
| 2 | 4.8 | 8.3 | Impurity B |
| 3 | 6.2 | 12.7 | Impurity C |
| 4 | 7.5 | 5.1 | Impurity D |

### *4.2. Mutagenicity Assessment*

Preliminary antagonist/AME test revealed that AM AS test using impurities A and B had no mutagenic effect. However, they identified one impurity, C and D, which needed further sourcing. The chromosomal damage assays of impurity C and impurity D results further demonstrated that impurity C was non-mutagenic, but impurity D might be mutagenic and require clinical trials Table 7.

**Table 7. Summary of mutagenicity testing**

| Impurity | AMES Test Result | Chromosomal Damage Study | Clinical Study Result |
|---|---|---|---|
| Impurity A | Negative | Not required | Not required |
| Impurity B | Negative | Not required | Not required |
| Impurity C | Negative | Negative | Not required |
| Impurity D | Positive | Positive | Positive |

### *4.3. Discussion*

*4.3.1. Chromatographic Separation Efficiency*

A high degree of efficiency in the separation of the impurities was observed by utilizing the chromatographic method used in this study from the peaks eluted. Since it is important to identify and quantify as many components of the samples as possible, the decision to separate impurities was vital here.

*4.3.2. Mutagenicity Evaluation and Regulatory Implications*

The outcomes of the mutagenicity evaluation indicated that a more thorough analysis should be performed to evaluate the presence of GPI. The AMES test offered a first step, which offered a way to assess non-mutagenic contaminants, which were revealed by having A and B codes accordingly. In other cases, if they identified other impurities possibly causing mutagenicity (impurity D), then it was a requirement to conduct more non-clinical and clinical tests to establish the risk. Generally, the positive results that have emerged from the clinical study of impurity D require extra caution, hence the need for extra measures to be put in place. By ICH guidelines Q3A/Q3B, all such impurities should be controlled within predefined limits to protect patients or consumers. These thresholds have been set based on toxicologically well-studied values such as LD50 values.

*4.3.3. Control Strategies for Impurities*

For impurities considered non-mutagenic the ICH Q3A/Q3B guidelines outline a qualification threshold that gives an idea of the acceptable amount for these to be present in a drug product. Mobile studies would suggest that for mutagenic impurities, the findings show there is a need for tighter control measures. This ranges from the optimization of the chemical synthesis procedures to reduce the generation of such substances to the high-level cleaning and validation procedures required to avoid the contamination of products by dirty equipment.

### *4.4. Future Directions and Recommendations*

The subsequent studies must focus on enhancing the analytical techniques involved in the detection of this biomarker with reference to both the sensitivity as well as the specificity of the methods involved. Moreover, a pivotal awareness of a variety of other unknown or documented insufficient impurity profiles of various DP and APIs will enable a quicker detection and assessment of risks associated with such impurities. Impurity profiling is also expected to be improved by the development of additional chromatographic techniques and detection methods since the improvement is very much a continuous one here.

## 5. Conclusion

Thus, the identification and qualification of unknown impurities that can be detected by chromatography separation techniques play a significant role in guaranteeing the safety, efficacy and quality of pharmaceutical products and other chemical products. Thus, various techniques including molecular analysis by LC-MS, GC-MS, and NMR that are used for qualitative and quantitative analyses of impurities, allow obtaining the exact information even at the trace level. This process is also useful not only in satisfying the required amount of the material necessary for regulations but also in the development of new products because synthesis pathways and degradation mechanisms are taken into consideration. Furthermore, as chromatography technologies are still under development, new findings and advancements in chromatographic techniques are still being established and provide enhancements of the capability for profiling and identifying the impurities; it is consistent with the ongoing

development of high standards of analysis and has broader applications in several industries. It is important to note that with the future progress of these capacities, the collaboration between academic institutions, companies, and regulatory authorities will be instrumental in the future advancement of these technologies to foster a purer and more efficient analysis when it comes to developing safer products for consumers around the world.

## References


[1] What is HPLC (High Performance Liquid Chromatography)?, Shimadzu. [Online]. Available: https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/basic/what_is_hplc.html

[2] Investigation of Extraneous Peaks in Chromatography, Pharma Beginers. [Online]. Available: https://pharmabeginers.com/investigation-of-extraneous-peak-in-chromatography/

[3] Assad J. Kazeminy, ICH Q3C Impurities: Residual Solvents Contract Laboratories Perspectives, USP-NF, pp. 1-41. [Online]. Available: https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/presentationKazeminy.pdf

[4] What is Gas Chromatography?, Shimadzu. [Online]. Available: https://www.ssi.shimadzu.com/service-support/faq/gas-chromatography/what-is-gas-chromatography/index.html

[5] How an FTIR Spectrometer Operates, LibreTexts Chemistry. [Online]. Available: . https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Vibrational_Spectroscopy/Infrared_Spectroscopy/How_an_FTIR_Spectrometer_Operates

[6] Eshita Garg, and Muhammad Zubair, "Mass Spectrometer," *StatPearls*, 2023. [Google Scholar] [Publisher Link]

[7] Impurities in New Drug Substances Q3A(R2), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, pp. 1-15, 2002. [Google Scholar] [Publisher Link]

[8] Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7(R2), *International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*, pp. 1-32, 2023. [Google Scholar] [Publisher Link]