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Review Paper

Extensive Review of Gas Chromatography in Pharmaceutical Applications: Emphasizing Genotoxic Impurities

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ABSTRACT

Gas chromatography (GC) is a highly versatile and precise analytical technique widely utilized in the pharmaceutical industry for identifying, quantifying, and managing genotoxic impurities. These impurities, such as nitrosamines, are of significant concern due to their potential carcinogenic effects, making their detection and control critical for public health and regulatory compliance. This review delves into the fundamental principles of GC, detailing its instrumentation, operational methodologies, and its specific applications within pharmaceutical analysis. Key topics include the development and optimization of GC methods tailored to detect trace levels of genotoxic impurities, alongside the validation procedures that ensure their reliability and reproducibility. The article also examines regulatory guidelines like International Council for Harmonisation (ICH) which set stringent impurity thresholds and explores the risk assessment and control strategies implemented to maintain impurity levels within permissible limits. Furthermore, the review highlights innovations in GC technology, such as advanced detectors and automated systems, which enhance sensitivity and efficiency. By addressing these critical aspects, this comprehensive discussion underscores GC's indispensable role in safeguarding pharmaceutical product quality, ensuring patient safety, and maintaining regulatory compliance.

INTRODUCTION

Pharmaceutical products must adhere to strict safety and quality standards to ensure patient health is not compromised. Among the various concerns in drug development and manufacturing, the presence of genotoxic impurities (GTIs) represents a significant risk. Even at trace levels, GTIs have the potential to cause genetic mutations, leading to long-term health hazards, including carcinogenesis [1]. The detection and quantification of these impurities have thus become a major focus for regulatory authorities and the pharmaceutical

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industry alike International Council for Harmonisation (ICH), European Medicines Agency (EMA) [2]. Gas chromatography (GC) is a pivotal analytical technique for GTI analysis due to its superior sensitivity, selectivity, and precision in identifying and quantifying volatile and semi-volatile impurities [3]. It enables researchers to comply with stringent regulatory guidelines while optimizing drug safety profiles [5]. This review highlights the critical role of GC in the pharmaceutical industry, particularly in the context of GTI detection, method development, and validation processes, ensuring regulatory compliance and safeguarding patient health [4]. The continual advancement of GC techniques, combined with evolving regulatory frameworks, underscores its importance in maintaining pharmaceutical quality standards.

Gas Chromatography

Principle

Gas chromatography (GC) is a powerful analytical technique used to separate and analyze volatile components in a mixture. The principle of GC is based on the differential distribution of analytes between a mobile phase, typically an inert carrier gas such as helium or nitrogen, and a stationary phase, which is a coated or packed material inside the column. When a sample is injected, it is vaporized in the injector port and carried into the column by the mobile phase. Inside the column, the analytes interact with the stationary phase based on their chemical and physical properties, such as polarity and boiling point, resulting in varying retention times. The separation efficiency depends on factors like the column's length, temperature, and stationary phase composition [7]. As each component exits the column, it is detected by a detector, such as a flame ionization detector (FID) or mass spectrometer, which provides quantitative and qualitative data [8]. GC is widely used in fields such as pharmaceuticals, food safety, and environmental monitoring due to its high resolution and sensitivity [6]. The technique's versatility makes it invaluable for analyzing complex mixtures with precision and accuracy. The Schematic diagram of the main components of a gas chromatography system shown in Figure-1.

Instrumentation

GC instrumentation consists of several key components:

1. **Carrier Gas**
2. **Flow Controllers**
3. **Injection Devices**
4. **Columns**
5. **Detectors**

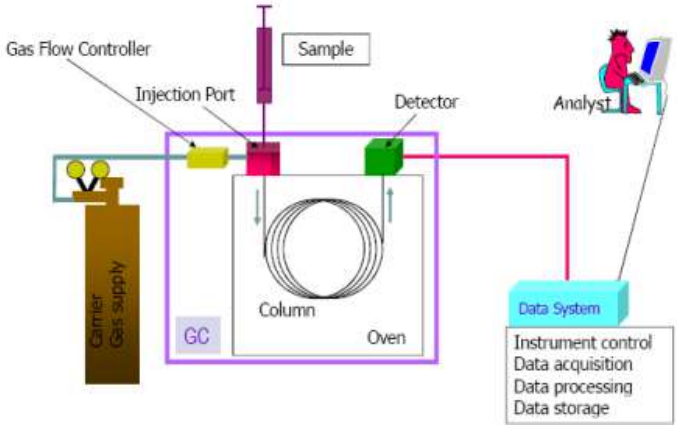

Figure 1. Schematic diagram of the main components of a gas chromatography system[13]

Carrier Gas

The carrier gas in gas chromatography (GC) acts as the mobile phase, transporting analytes through the column. Commonly used carrier gases include helium, nitrogen, and hydrogen, chosen for their inertness and suitability to the analysis

requirements. The carrier gas must maintain consistent flow rates to ensure reliable and reproducible results. Helium is preferred for its excellent inertness and compatibility with most detectors, while hydrogen offers faster analysis times and improved resolution in some applications [7]. Selection of carrier gas also depends on factors like sensitivity and operational costs [15].

Flow Controllers

Flow controllers in GC are essential for regulating the carrier gas flow rate, ensuring consistent pressure and optimal separation efficiency. Traditional systems use manual controllers, but modern setups often employ electronic flow controllers (EFCs) for enhanced precision and stability. EFCs allow for automated adjustments, improving reproducibility and accommodating method development [8]. Accurate flow control minimizes peak broadening, enhances resolution, and ensures consistent retention times, which are critical for reliable analytical results [9].

Injection Devices

Injection devices play a crucial role in GC by introducing samples into the system. Common injection techniques include split injection for concentrated samples, splitless injection for trace analysis, and on-column injection for thermally labile compounds. The choice of injection method depends on the analyte's volatility and sample matrix [10]. Precision in sample introduction is essential to maintain peak shape and avoid contamination. Innovations in autosamplers have enhanced reproducibility and throughput, further improving the injection process [8].

Columns

GC columns are critical for separation efficiency and resolution. They are classified into packed and capillary (open tubular) types. Capillary columns, featuring a thin stationary phase coating on the inner wall, provide superior resolution and sensitivity, making them widely used in modern GC [15]. The stationary phase's polarity is selected based on analyte characteristics, such as functional groups and polarity, to maximize separation [7]. Column temperature programming further optimizes analyte separation by accommodating differences in volatility.

Detectors

Detectors in GC translate separated analytes into measurable signals, enabling identification and quantification. Popular detectors include the Flame Ionization Detector (FID) for hydrocarbons, the Electron Capture Detector (ECD) for halogenated compounds, and Gas Chromatography-Mass Spectrometry (GC-MS) for precise structural analysis. Detector selection depends on the analytes' chemical properties and sensitivity requirements [9]. GC-MS is particularly valuable for its ability to provide both qualitative and quantitative data, making it indispensable in fields like environmental analysis and pharmaceuticals [10].

Procedure (Stepwise)

1. **Sample Preparation**: The sample is extracted and purified to eliminate interfering substances, ensuring accurate results [7].
2. **System Setup**: The carrier gas, column, and detector are configured for optimal performance, depending on the analysis requirements [15].
3. **Injection**: The sample is injected using the appropriate device, such as splitless or on-column injection, based on analyte characteristics [10].
4. **Separation**: Analytes are separated by the column based on their interaction with the stationary phase, influenced by polarity and boiling point [7].
5. **Detection**: The detector measures the analytes as they exit the column, generating a signal corresponding to their concentration [9].

6. **Data Analysis**: Chromatograms are processed to quantify analytes and identify their chemical composition [10].

Use of Gas Chromatography in Pharmaceutical Industry

Gas chromatography (GC) plays an essential role in the pharmaceutical industry by ensuring the safety and quality of drug products. One of its significant applications is the detection of residual solvents and impurities. After pharmaceutical synthesis, trace amounts of solvents may remain, which could compromise the safety of the final product. GC is highly sensitive and provides the necessary precision to detect these residual solvents, thereby helping pharmaceutical companies comply with stringent regulatory standards [11]. Additionally, GC is utilized to quantify volatile organic compounds (VOCs) in formulations, as VOCs can impact the stability and safety of a drug product [10]. The ability to monitor and measure VOCs ensures that products meet safety requirements for both active pharmaceutical ingredients and excipients [7].

GC is also crucial for the analysis of genotoxic impurities, such as nitrosamines, which are harmful even at low concentrations. As part of regulatory guidelines, GC allows for the detection of these impurities in drugs, mitigating potential health risks to patients [11]. Moreover, GC supports stability studies and formulation development by analyzing how drug compounds degrade over time under various conditions. These insights are vital for ensuring the long-term safety and efficacy of pharmaceutical products.

Types and Sources of Impurities

Pharmaceutical impurities can arise from several sources during the development, manufacturing, and storage of drug products.

Process-related Impurities include byproducts, reagents, or intermediates that are formed during the synthesis of active pharmaceutical ingredients (APIs). These impurities are typically present as a result of incomplete reactions or side reactions in the manufacturing process [8].

Degradation Products occur when the drug substances break down under certain conditions, such as exposure to light, heat, or moisture. These products can compromise drug stability and efficacy over time, making it essential to assess their formation during stability studies [10].

Contaminants, on the other hand, are unwanted substances introduced during manufacturing, storage, or handling processes. They may include microorganisms, foreign particles, or even packaging material residues that can adversely affect the safety and quality of the pharmaceutical product [15]. Proper quality control is crucial in minimizing the presence of these impurities to ensure patient safety.

Pharmaceutical impurities can arise from various sources:

- **Process-Related Impurities:** Byproducts, reagents, or intermediates.
- **Degradation Products:** Resulting from instability under specific conditions.
- **Contaminants:** Introduced during manufacturing or storage.

Classification Based on ICH Guidelines

The **International Council for Harmonisation (ICH)** has provided guidelines to classify pharmaceutical impurities into three main categories: **organic impurities**, **inorganic impurities**, and **residual solvents**.

Organic impurities encompass process-related impurities and degradation products that can arise during the synthesis or storage of the pharmaceutical product [2].

Inorganic impurities typically include residual reagents, catalysts, and metal traces from manufacturing equipment or the synthesis process [7].

Residual solvents are volatile chemicals that are often used in the manufacturing process but must be removed to ensure that the final drug product

complies with regulatory standards [1,2]. These impurities can vary widely depending on the drug's formulation and the processes involved in its production.

Method Development Regulations

Method development for gas chromatography (GC) is a meticulous process essential to ensure reliable and efficient analytical outcomes, especially in regulated environments like the pharmaceutical industry. A key starting point is selecting appropriate stationary and mobile phases. The stationary phase must provide optimal interaction with the analyte, affecting the separation efficiency and retention times, while the mobile phase should facilitate the movement of compounds through the column [8]. These choices directly influence the chromatographic resolution and the ability to detect trace impurities. Additionally, the optimization of temperature programs is a critical aspect of GC method development. Temperature control enhances the separation process by affecting the volatility of analytes, which is especially crucial when analyzing complex mixtures or volatile substances [10]. For a method to be both effective and reliable, ensuring reproducibility and robustness is vital. The method must consistently provide accurate results under varied conditions to ensure its applicability across different laboratories or analytical sessions [15]. Regulatory bodies like the FDA and EMA stress the importance of method development that meets rigorous scientific and quality standards. These agencies require that any GC method developed for pharmaceutical applications undergo thorough validation to confirm its suitability for the intended purpose and its compliance with regulatory requirements [1,2]. The method development details in some drug substances is presented in Table 1.

Table 1: Details of method development for different drug substances.

S. No	Product Name	Type of Injection	Type of Column	Type of Detector	Name of the Solvent	Citation
1	Paracetamol	Split	Capillary (DB-5)	Flame Ionization	Methanol	[16]
2	Ibuprofen	Split/Splitless	Non-polar (SPB-1)	Flame Ionization	Acetonitrile	[15]
3	Aspirin	Split	Polar (HP-FFAP)	Flame Ionization	Acetonitrile	[1]
4	Diazepam	Split/Splitless	Polar (Carbowax 20M)	Flame Ionization	Methanol	[2]
5	Caffeine	Split	Capillary (DB-1)	Flame Ionization	Acetonitrile	[16]
6	Omeprazole	Split/Splitless	Non-polar (ZB-1)	Flame Ionization	Acetonitrile	[15]
7	Metformin	Split/Splitless	Non-polar (Rtx-5MS)	Flame Ionization	Acetonitrile	[1]
8	Ketorolac	Split	Polar (DB-FFAP)	Flame Ionization	Acetonitrile	[2]
9	Codeine	Split/Splitless	Capillary (DB-17)	Flame Ionization	Ethanol	[16]
10	Lorazepam	Split	Polar (HP-5)	Flame Ionization	Acetonitrile	[15]

Method Validation Guidelines

Method validation is a critical step to ensure that analytical methods provide reliable and accurate results, especially in routine analysis. Specificity refers to the method's ability to distinguish the analyte of interest from any potential interferences, ensuring that only the target compound is measured. Accuracy and precision assess the method's consistency in producing results close to true values and its ability to produce reproducible measurements under the same conditions. Linearity ensures that the response from the detector is proportional across a wide range of concentrations, making the method suitable for quantifying varying amounts of the analyte. Additionally, the limit of detection (LOD) and limit of quantification (LOQ) establish the sensitivity of the method, determining the smallest concentration that can be reliably detected and quantified. These parameters, guided by regulatory standards, ensure that the method is robust, reliable, and suitable for its intended purpose [12,14]. The method validations related parameters according to international regulatory authority and its acceptance criteria is presented in Table 2.

Table 2: Validation parameters with respect to different parameters and acceptance criteria.

Validation parameter	International Council for Harmonisation-Q2(R2) [14]	Acceptance Criteria
Specificity	Ability to distinguish analytes from interferences	Must show no interference from other substances
Accuracy	Comparison with a known reference or standard	Typically, 80-120% of known value or standard
Precision	Repeatability and intermediate precision	Relative standard deviation (RSD) ≤ 15% for repeatability, ≤ 15-20% for intermediate precision
Linearity	Response proportional to concentration across the range	Correlation coefficient (R^2) ≥ 0.999 for calibration curve
Limit of Detection (LOD)	Lowest analyte concentration that can be reliably detected	Typically ≥3:1 signal-to-noise ratio for LOD
Limit of Quantification (LOQ)	Minimum analyte concentration that can be reliably quantified	Typically ≥10:1 signal-to-noise ratio for LOQ

Risk Assessments

Risk assessments are critical for identifying potential sources of genotoxic impurities (GTIs) in pharmaceutical manufacturing and evaluating their potential impact on drug safety. These assessments aim to quantify the risk posed by GTIs and ensure that their presence in drug products is minimized. Failure Mode and Effects Analysis (FMEA) is one technique used to systematically evaluate potential failure modes in manufacturing processes and their consequences, helping to identify where GTIs could be introduced [10]. Additionally, control threshold determinations are employed to define acceptable levels of GTIs in drug products, based on toxicological data and regulatory limits [1,2]. Risk assessments allow for the prioritization of mitigation efforts, such as improving manufacturing controls and refining purification processes, to ensure compliance with safety standards set by regulatory agencies like the Food and drug administration (FDA) and EMA [1,2,12]. By identifying and managing potential risks early in the drug development process, pharmaceutical companies can reduce the

likelihood of harmful levels of GTIs reaching patients [7]. An in-silico evaluation of the genotoxicity of drug substances and their raw materials must be conducted using appropriate tools like Derek Nexus, Sarah Nexus and Lhasa. This evaluation is performed in accordance with the ICH M7 guideline, which focuses on the "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk." Impurities with potential mutagenic effects must be assessed and classified according to the principles outlined in the ICH M7 guideline to determine their mutagenic potential. This process involves reviewing toxicological literature and conducting database searches, followed by computational assessments using quantitative structure-activity relationships ((Q)SAR) and knowledge-based approaches. The classification of mutagenic and carcinogenic impurities according to the ICH M7 guideline is presented in Table 3.

Table 3: Impurity Classification with Respect to Mutagenic and Carcinogenic potential proposed in the ICH M7 Guidance.

Class	Definition	Proposed action for control
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit
2	Known mutagens with unknown carcinogenic potential (Bacterial mutagenicity positive), no rodent carcinogenicity data)	Control at or below acceptable limits (Appropriate TTC)
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (Appropriate TTC) or conduct bacterial mutagenicity assay. If non-mutagenic = Class 5 If mutagenic = Class 2
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity

Limit Calculation:

Formula:

$$\text{Calculation (ppm)} = \frac{1.5\ \mu g}{\text{Maximum daily dosage of drug substance in grams}}$$

Control Strategies

Control strategies are essential to ensure that genotoxic impurities (GTIs) in pharmaceutical products remain within acceptable limits, complying with regulatory standards. Analytical controls involve regular testing and monitoring throughout the manufacturing process to detect and quantify impurities, ensuring they are below predetermined thresholds [10]. This can include the use of techniques like gas chromatography (GC) and high-performance liquid chromatography (HPLC). Process controls focus on optimizing manufacturing processes to minimize the formation of impurities. This may involve refining synthesis conditions, improving purification steps, or implementing more efficient separation technologies [7]. Regulatory compliance is critical to ensuring that the developed strategies meet the required safety standards, such as those outlined in the ICH M7 guidelines for assessing and

controlling mutagenic impurities in pharmaceutical products. By implementing robust control strategies, pharmaceutical companies can mitigate risks and ensure that products are safe for patient use. The control of mutagenic and carcinogenic impurities according to the ICH M7 guideline is presented in Table 4.

Table 3: The control of mutagenic and carcinogenic impurities according to the ICH M7 Guidance.

Product Name	Impurity Name	ICH Classification	Impurity Limit (ppm)	LOQ (ppm)	LOD (ppm)	Citation
Paracetamol	4-Nitroaniline	Class 2A	1	0.05	0.01	[16]
Ibuprofen	2-Nitropropane	Class 2B	0.5	0.03	0.005	[15]
Aspirin	Benzene	Class 1	0.1	0.02	0.005	[1]
Diazepam	Ethyl acrylate	Class 2A	0.5	0.05	0.01	[2]
Caffeine	Acetamide	Class 2B	1	0.1	0.02	[16]
Omeprazole	Methacrylate	Class 1	0.2	0.05	0.01	[15]
Metformin	Formaldehyde	Class 1	0.5	0.1	0.02	[1]
Ketorolac	Methyl iodide	Class 2A	0.1	0.02	0.005	[2]
Codeine	Dimethyl sulfate	Class 1	0.05	0.01	0.005	[16]
Lorazepam	Chloromethyl methyl ether	Class 1	0.05	0.01	0.005	[15]

CONCLUSION:

Gas chromatography remains a cornerstone analytical technique for addressing the critical challenge of genotoxic impurities in pharmaceuticals. Advances in instrumentation and regulatory frameworks continue to enhance its effectiveness and reliability. Future innovations will likely focus on improving sensitivity, automation, and environmental sustainability, ensuring safer pharmaceutical products for global health.

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