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Genotoxic impurities in Famotidine according to Regulatory Perspective

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Abstract: Ensuring patient safety and therapeutic efficacy is the cornerstone of pharmaceutical development. Genotoxic impurities (GTIs) substances capable of causing DNA damage pose serious safety concerns even at trace levels in active pharmaceutical ingredients (APIs). Famotidine, a histamine H_2-receptor antagonist used for peptic ulcer and gastroesophageal reflux disease, has been under scrutiny for potential nitrosamine impurities such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA). This study investigates genotoxic impurities in Famotidine according to current international regulatory guidelines, focusing on the analytical detection, classification, and control strategies as per ICH M7 and FDA frameworks. Using LC-MS/MS and GC-MS techniques, trace-level quantification of NDMA was achieved within sub-ppm limits. The paper highlights the significance of adopting stringent analytical protocols and process optimization to minimize GTI formation and ensure pharmaceutical safety.

Keywords: Genotoxic impurities, Famotidine, NDMA, ICH M7, Regulatory guidelines, Nitrosamines, Analytical methods, LC-MS/MS

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INTRODUCTION

When it comes to patient health care, the field of pharmacy is all about making sure drugs are used efficiently and appropriately. Pharmaceuticals developed for human use can only improve health if they are completely devoid of contaminants. One important scientific field that may help improve the product's quality and safety is analytical chemistry [1], which allows

for both qualitative and quantitative examination of these contaminants. Analytical chemistry has expanded its role in the pharmaceutical industry in recent decades.

Various analytical methods, ranging from basic qualitative chemical tests to the use of very advanced devices controlled by software, may be used to conduct both quantitative and qualitative analyses. Pharmaceutical firms will be able to put more safe and effective pharmaceuticals on the market as a result of technological developments in analytical methods that allow for lower detection limits. Controlling the quality of inputs, outputs, and intermediates is essential in the pharmaceutical industry.

Active pharmaceutical ingredients (API) may include contaminants from solvents, reagents, intermediates, and degradation products, among other sources. The product's quality and safety may be jeopardised by even minute amounts of these compounds. Very low concentrations of a small number of these contaminants may have harmful effects on humans because they are carcinogens or mutagens. Analytical chemists are responsible for determining where impurities may occur during the production of API. It is important for the analytical chemist to be able to detect genotoxic impurities (GTIs) and control their levels at the early phases of synthesis.

Based on their toxicological data, all contaminants, both known and undiscovered, should be classified as either normal or genotoxic, and their safety evaluated. It is also important to try to figure out what their boundaries are so that we can identify and quantify them. Several regulatory bodies' current recommendations, including the ICH and the USFDA, call for genotoxic and possibly genotoxic impurity control to sub ppm levels [2,3]. Analytical chemists have a number of problems, including sensitivity and selectivity, when developing and routinely analysing these GTIs at sub ppm levels. In order to reduce contaminants early on in the synthetic process, it is important to determine the proper origin. Impurity control, identification, and quantification are therefore crucial steps in the drug development process. A compromised drug product may have contaminants that are more toxicologically and pharmacologically active than the active component, drug substance.

It is helpful to think about contaminants as either genotoxic or non-genotoxic so that everyone knows what to expect.

The variety and quality of the medicinal component and the raw materials employed in the procedure should be carefully considered if the safety of the end product is of utmost

importance. It is usual practice in synthetic processes to convert basic ingredients into a completed medication. It must be remembered that no reaction is totally selective and that impurities, or unwanted compounds, might be created because of the catalysts, intermediates, and starting materials that are always present. Some of these pollutants may induce genetic alterations or cancer [4]. Toxic chemicals may cause cancer, but they can also encourage chromosomal changes and rearrangements. There are a number of ways in which these chemicals might harm DNA, including alkylation and other interactions that could change genetic code. When a chemical causes changes in genes or chromosomes, it is said to be mutagenic; when it destroys DNA, it is said to be genotoxic. Consequently, genotoxic chemicals are those that alter DNAand/or its related biological components, such as spindle machinery or enzymes such as topoisomerases. No matter what causes cancer, the disease always compromises the expression or DNA integrity of the genome. Genotoxicity describes the relative likelihood of DNA damage caused by different chemical carcinogens. From a safety standpoint, things are further complicated since genotoxic chemicals may cause cancer. Substances found in drugs and other related compounds, as well as pollutants that pose structural risks, may be genotoxic [5–7]. The presence of these chemicals already puts patients and study participants at a disadvantage. Hence, genotoxic compounds and their associated contaminants provide a difficulty for regulatory bodies, particularly in the pharmaceutical industry, when attempting to characterise and control these substances.

When two possibilities emerged in the market, regulatory agencies were very vigilant. Nilfenavir, an antiviral compound manufactured by Roche and marketed under the trade name Viracept, is one example. In 2007, Roche recalled all batches of products made at their Swiss manufacturing unit because of contamination with ethyl methane sulfonate, which occurred during reactor cleaning procedures. This contamination occurred when trace amounts of methanol reacted with methane sulfonic acid, resulting in the formation of carcinogenic alkyl sulfonates. In a second instance, the European Medicines Agency rejected a medicinal molecule that had been recrystallised from acetone without anticipating the creation of misetyl oxide [8].

Keeping the risk-to-benefit and risk-to-profit ratio in producing lifesaving medications while dealing with genotoxic contaminants is a serious difficulty. Not many scientists have voiced the concern that the established boundaries may not always be realistic or have any basis in science.

Changing the synthetic pathway and starting materials may influence the formation of genotoxic impurities. The complicated and sometimes limited synthesis of API owing to the availability of chemicals and reagents makes this approach impractical in several cases. In order to control the genotoxic contaminants, purification processes are sometimes used. The development of analytical methods to reduce genotoxic contaminants will further increase the drug's time to market [9–12].

Regulatory features

The Worldwide Gathering on Harmonization (ICH) gives rules to overseeing drug substance degradants in Q3B(R) and leftover contaminations in Q3C(R). Before 2007, the presence of genotoxic synthetic substances in meds and dynamic drug fixings (APIs) was not stringently directed. Accordingly, the European Prescriptions Organization's Panel for Restorative Items (CHMP) gave its previously set of proposals in 2007 to limit genotoxic pollutants. The US Food and Medication Organization (FDA) adhered to with updates to these guidelines in 2008.

Both regulatory frameworks primarily focused on maintaining genotoxic impurity (GTI) levels below the Threshold of Toxicological Concern (TTC). For safety, the TTC recommends limiting contaminant intake to less than 1.5 µg per day. However, the acceptable impurity limits depend on the chemical’s toxicological profile. Additionally, the CHMP advocates a phased TTC approach, defining permissible daily impurity levels based on exposure duration. While the CHMP provides guidance for managing genotoxic impurities in pharmaceuticals, it does not specifically address their handling during clinical trials.

Unfortunately, owing to the insensitivity of existing testing procedures, it is frequently not possible to ensure that medical drugs or goods are free of genotoxic pollutants. Such cases need for a risk-benefit analysis to be considered. It has been shown that a threshold mechanism allows for the passage of pollutants at low concentrations that are not expected to cause genotoxicity. Without a threshold mechanism for impurity evaluation, it is feasible to extrapolate to humans using a PDE approach. However, appropriate risk evaluations should be considered, and the impurity should be maintained to an ALARP level if there is inadequate evidence regarding its genotoxicity [13].

If there is no way to produce or acquire evidence to support ALARP, then the TTC technique is used. According to this method, the impurity might be introduced to the patient as they undergo treatment. It is worth mentioning that this technology is often used by the food

business. Investigations on more than 700 compounds in animals using the TTC method have shown that a daily dose of 1.5µg of impurity is deemed safe for an individual's lifetime [14]. Opponents of the Threshold of Toxicological Concern (TTC) approach argue that, while the concept is useful for managing genotoxic impurities at very low levels, it may not always be practical or appropriate for the production of certain high-quality medications. Critics suggest that strict adherence to TTC limits can hinder drug development, particularly when it involves compounds that are difficult to synthesize without generating trace impurities. The phased TTC method, which sets conservative exposure limits for genotoxic substances based on their chemical structure and known toxicity, may not fully account for certain real-world scenarios, leading to potential exceptions or outliers.

One limitation is that some commonly occurring contaminants, such as formaldehyde, are produced naturally through normal metabolic processes or are present in foods. In such cases, human exposure can exceed TTC-based limits without apparent adverse effects, highlighting that the TTC framework may be overly conservative for these substances. This raises concerns that applying TTC rigidly could unnecessarily restrict the use of compounds that are otherwise safe in typical dietary or physiological contexts.

Another situation where TTC application may be adjusted is in cases of severe or life-threatening conditions, such as cancer or AIDS, where therapeutic options are limited. In such circumstances, regulators and clinicians may accept higher levels of genotoxic impurities if the potential benefits of treatment outweigh the theoretical risk posed by trace contaminants. Here, the risk–benefit analysis justifies flexibility in TTC limits, recognizing that strict adherence might prevent access to potentially life-saving medications.

However, the TTC approach is less flexible for compounds with inherently high carcinogenic potential, including alkyl-azoxy, aflatoxin-like, and N-nitroso structures**.** These chemical classes are strongly associated with cancer, and even very low exposures can pose significant risks. In fact, the levels at which these substances cause genotoxic effects are often much lower than the general TTC thresholds, meaning that conventional safe intake limits are insufficient to protect human health. Consequently, these highly potent carcinogens require more stringent control measures, analytical monitoring, and, whenever possible, elimination from the drug synthesis process.

In summary, while the TTC approach provides a valuable framework for controlling low-level genotoxic impurities, it has limitations in practice. Natural exposure to certain substances, the

need for urgent therapeutic interventions, and the presence of extremely potent carcinogens create scenarios where TTC guidelines must be applied with careful consideration. Regulatory flexibility, combined with risk-based assessment and analytical vigilance, is therefore essential to balance safety, feasibility, and patient access to critical medications.

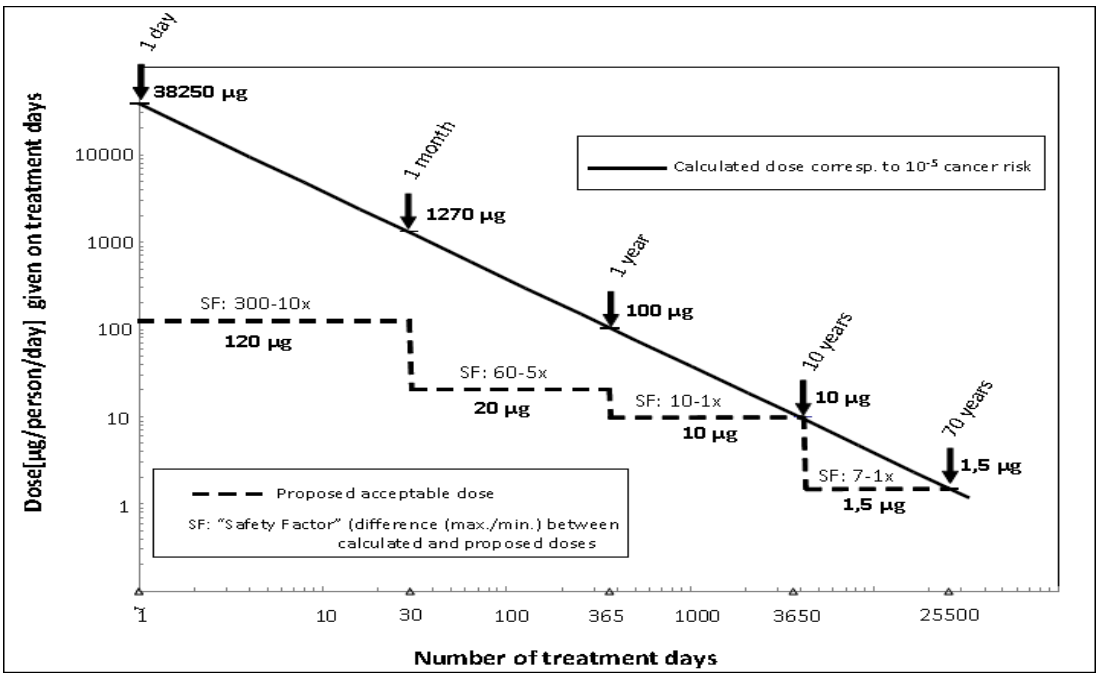

Figure 1: Staged TTC approach based on acceptable daily intake based on dose

Concerns voiced by experts include what to do in the event that a pharmaceutical moiety displays many genotoxic chemicals, as an example of an impurity [18]. Pharmaceutical preparations may include up to three contaminants, according to Bercuand colleagues (2009). In such cases, it is recommended to sum up the contaminants and handle them independently. In the beginning phases of development, when data is few, the focus is on the reagents, intermediates, and reaction products. Based on structural analysis, genotoxicity data, and the phased TTC method, acceptable criteria for impurities are determined once they have been found and categorised [Table 5] [14].

ICHM7 guidelines [15-20]

The suggestions are centred on new pharmacological substances and pharmaceutical goods, with the goal of facilitating their clinical development and future claims. This rule also applies to the newly permitted pharmacological substances in existing products.

- Changing the synthetic process can introduce new contaminants or change the

acceptability criteria for current contaminants.

- New degradation products and different acceptability criteria for old degradation products will emerge from formulation changes. Dosage regimen and indication changes will also impact carcinogenicity [15-20].
- The primary target of this recommendation is contaminants that have the potential to bind to DNA.

Table 1: ICH M7 – TTC based approach

Acceptable Daily Intakes* for an Individual Impurity, µg/day Clinical trials or marketed product								
	Single Dose	< 14 days	≤ 1 mo.	≤ 3 mo.	≤ 6 mo.	≤ 12 mo.	>1 – 10 years	>10 years to lifetime
M7	**	**	120	20	20	20	10	1.5
EMA	120	60	60	30	10	5	1.5 (marketed)	1.5

Identification and classification of potential genotoxic impurities

Guidelines For the purpose of testing for pharma genotoxicity, both ICH S2A (1995) and ICH S2B (1997) are used. Following the steps outlined in ICH S2B, genotoxic medications may be identified. This is what ICH S2B consists of:

- Mutations in the genes of bacteria.
- Chromosomal damage testing in vitro or a mouse lymphoma tk assay.
- Chromosomal damage tests in vivo in mouse haematopoietic cells.

Once the previously mentioned tests have been led and assessed in consistence with the current ideas, and the outcomes show negative, according to rules S2A and S2B, the genotoxicity wellbeing of a synthetic is laid out. In the event that a synthetic appears positive in any of those tests, it's standard methodology to see whether it could be genotoxic. Many hurtful impacts, including transformations, DNA harm, and primary chromosomal breaks, are together known as genotoxicity. As per the principles set out by the Global Meeting on

Harmonization (ICH), genotoxic substances that change DNA don't show an edge component, as opposed to genotoxic synthetics that don't change DNA [21].

The guidelines for overseeing genotoxic poisons in helpful medications will be resolved by means of a framework that is attached to limits. Without a limit component, synthetic substances that straightforwardly target DNA are harmful to a few organs, requiring cautious control at exceptionally low portions. To give only one model, it is deep rooted that such a low degree of the executives is superfluous for poisons that capability through an edge related system. The essential reasoning for the severe pollution control conventions is the potential for DNA reactivity and mutagenicity.

Dynamic practical gatherings that might respond with DNA to create mutagenicity and trigger the malignant growth process are depicted in data sets like DEREK, MultiCase, and TOPKAT [22-30]. A generally little level of DNA responsive cancer-causing agents bomb the Ames test. Since carbamates and different cancer-causing agents are not as promptly distinguished by bacterial genotoxic tests, this one could miss them. Adverse outcomes from genotoxicity tests in bacterial changes or mammalian cell tests show that the pollutant control was effective.

Classification of Genotoxic impurities

Genotoxic impurities (GTIs) can be classified in several ways based on their source, chemical structure, mechanism of genotoxicity, and regulatory categorization. Understanding these classifications is essential for identifying potential risks and developing effective control measures in pharmaceutical manufacturing.

From the perspective of their source of origin**,** GTIs are generally categorized into three main groups: process-related impurities, degradation products, and contaminants. Process-related impurities are those that arise during the synthesis of the active pharmaceutical ingredient (API). They may include unreacted starting materials, intermediates, reagents, or catalysts that remain in the final product in trace amounts. Examples include alkyl halides, sulfonate esters, and azides, which are known to possess DNA-reactive properties. Degradation products, on the other hand, are formed when the drug or excipients chemically decompose under certain conditions such as exposure to heat, moisture, or light. These by-products may develop during manufacturing, storage, or throughout the drug’s shelf life. Contaminants are impurities that unintentionally enter the product from raw materials, the environment, or equipment used during processing. Common examples include nitrosamines and metal residues.

When classified based on chemical nature**,** GTIs encompass a variety of reactive compounds capable of damaging DNA. Alkylating agents such as alkyl halides, epoxides, and sulfonate esters are among the most significant, as they can form covalent bonds with DNA bases and cause mutations. Nitroso compounds, particularly nitrosamines, are highly potent mutagens formed through the interaction of secondary amines with nitrite sources, especially under acidic or high-temperature conditions. Epoxides and aziridines, which contain strained three-membered ring structures, are also strongly reactive toward nucleophilic DNA sites. Additionally, aldehydes and peroxides contribute to genotoxicity by producing reactive oxygen species (ROS) that cause oxidative damage to nucleic acids.

In terms of mechanism of genotoxicity**,** GTIs can be divided into direct-acting and indirect-acting agents. Direct-acting genotoxic impurities are capable of interacting with DNA without requiring metabolic activation; typical examples include alkylating agents and epoxides. Indirect-acting genotoxic impurities, however, require metabolic conversion within the body to form reactive intermediates that subsequently damage DNA. Such compounds include certain aromatic amines and nitro compounds that are metabolically activated into electrophilic species.

Regulatory agencies such as the International Council for Harmonisation (ICH) provide a classification framework for genotoxic impurities under the ICH M7 (R1) guideline. This framework classifies impurities based on their mutagenic and carcinogenic potential. Class 1 impurities are known mutagenic carcinogens and should be avoided completely. Class 2 includes known mutagens with unknown carcinogenic potential that must be controlled to acceptable limits. Class 3 impurities have structural alerts but lack mutagenicity data and therefore require testing. Class 4 impurities are non-alerting compounds with sufficient evidence of non-mutagenicity, and Class 5 includes those without any structural alerts or known genotoxic risk, considered safe for use.

The categorization of genotoxic impurities (GTIs) is primarily based on their potential to cause genetic damage and their associated carcinogenic risk. Regulatory authorities, such as the International Council for Harmonisation (ICH), have developed a five-class system to help pharmaceutical scientists assess, prioritize, and control these impurities effectively. This classification system is essential for designing safer synthetic routes, optimizing purification processes, and ensuring patient safety.

Class 1 genotoxic impurities include compounds with a well-established genotoxic mechanism and a documented history of carcinogenicity in animals. These impurities are also recognized to pose a cancer risk to humans. Because of their confirmed genotoxic and carcinogenic potential, Class 1 impurities are considered unacceptable in pharmaceutical products and must be rigorously avoided during drug synthesis and production. Strict measures are implemented to eliminate these contaminants completely, and no exposure, even at trace levels, is generally tolerated.

Class 2 impurities are those that have demonstrated mutagenicity in standard genotoxic assays, such as the Ames test, but whose carcinogenic potential in humans or animals remains uncertain. While their ability to induce genetic mutations is established, there is insufficient evidence to determine whether they can cause cancer. For this reason, Class 2 impurities must be carefully controlled, and exposure levels are typically limited to well-defined thresholds based on toxicological assessments and regulatory guidance.

Class 3 impurities are identified primarily by the presence of a warning structural alert, which differs from the active pharmaceutical ingredient (API) but indicates a potential to cause genetic damage. These compounds generally contain reactive functional moieties that could interact with DNA, although they have not been specifically tested for genotoxicity. Regulatory authorities recommend evaluating Class 3 impurities on a case-by-case basis using available literature, structural analogies, and any experimental evidence. Despite the lack of direct mutagenicity data, these warning structures serve as an important tool for risk assessment and impurity control.

Class 4 impurities are linked to the API itself, sharing structural elements or functional moieties with the parent compound but undergoing modifications that may alter their biological activity. These API-related impurities are considered isolated GTIs whose precise genotoxic potential may not be fully characterized. Their control relies on an understanding of how changes in structure influence reactivity and potential DNA interaction. Analytical monitoring and risk-based assessment help ensure that these impurities remain within acceptable limits in the final product.

Class 5 impurities, in contrast, do not possess structural alerts associated with genotoxicity, nor is there sufficient evidence to suggest any genotoxic potential. Regulatory documents such as ICH Q3A, Q3B, and Q3C provide guidance for these impurities, which are generally

considered low risk. While routine monitoring may still be performed, the regulatory focus is less stringent compared to Classes 1–4, as these impurities are unlikely to pose a genetic hazard.

In summary, the classification of genotoxic impurities provides a structured approach to understanding their origin, chemical reactivity, and potential health risks. By determining whether GTIs arise from synthetic intermediates, degradation pathways, or external contamination—and evaluating their chemical and biological properties—manufacturers can implement effective analytical controls and process strategies. This systematic approach not only ensures compliance with regulatory standards but also minimizes the genotoxic risk in pharmaceutical products, thereby safeguarding patient safety and supporting the development of high-quality, reliable medications.

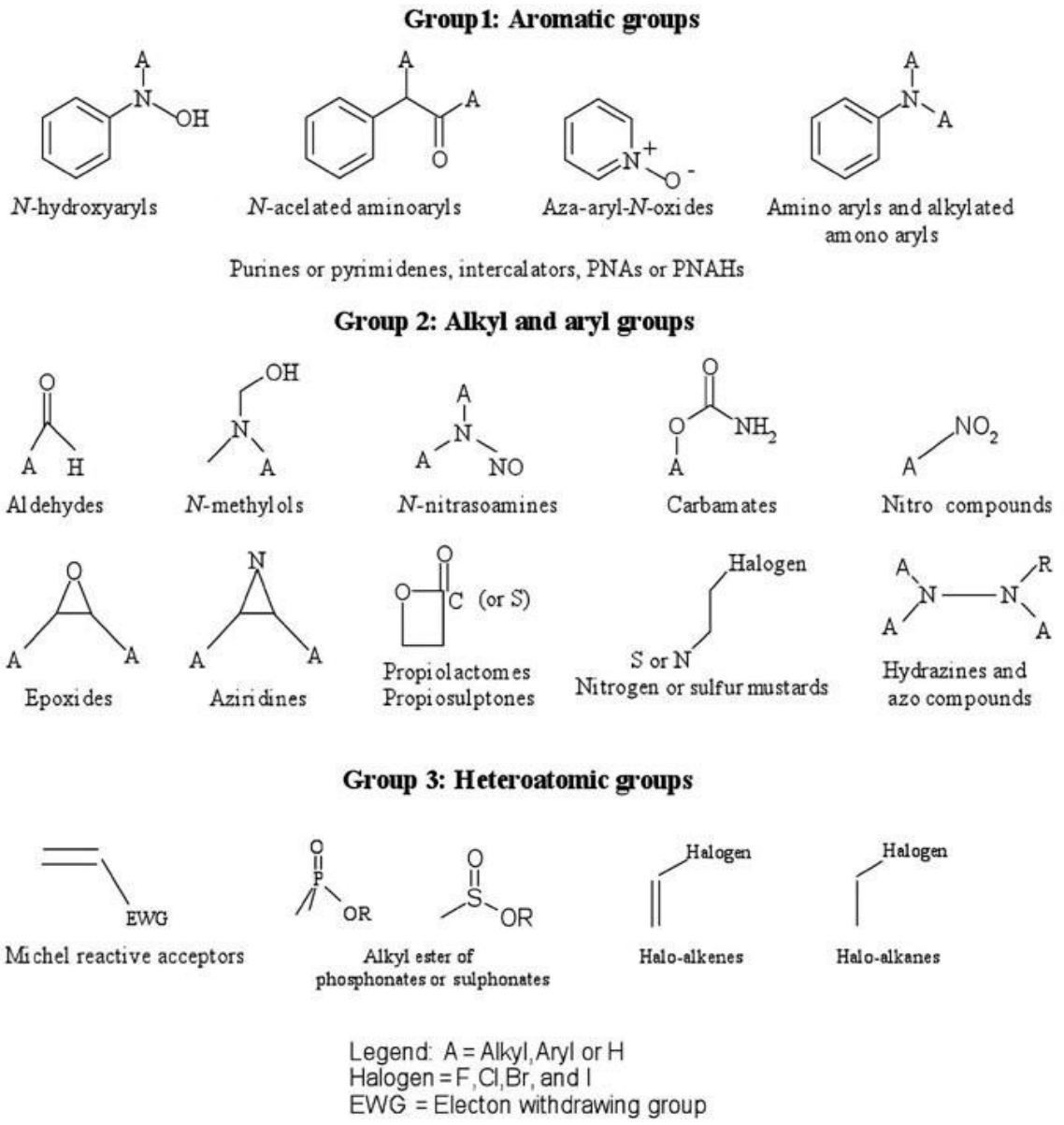

Figure 2: Structural alerts of mutagens and carcinogens

When the API isn't involved in the warning structure, knowing the synthetic process and reagents with the use of databases and QSAR allows for impurity detection. Experimental settings and environmental variables have a significant impact on drug/API impurity assessment. Before reaching any conclusions, testing should include results from other tests, including the Ames test, in addition to structural warnings, as shown in Table 6 [34-36].

Assessment and control

The next phase, after categorisation and identification, is to evaluate and regulate genotoxic contaminants.

Table 2: Control of impurities based on classification

Class	1	2	3	4	5
Definition	Known mutagenic carcinogens	Known mutagens with unknown carcinogenic data	Alerting structure unrelated to the drug substance no mutagenic data	Alerting structure related to the drug substance and are not mutagenic	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity
Proposed action of control	Control at or below compounds acceptable limit	Control at or below acceptable limit (appropriate TTC)	Control at or below acceptable limits or conduct mutagenicity assay, if non-mutagenic class 5, if mutagenic class-2	Treat as non-mutagenic impurity	Treat as non-mutagenic impurity

Impurities should be managed according to TTC and ICH criteria after classification. For known impurities of 0.15% and for unknown impurities of 0.10%, they should be considered ordinary impurities [37-39]. The target therapeutic concentration (TTC) for PGIs should be dose-dependent and maintained at 1.5µg/day. The permissible limit is 150 ppm at a 100 mg/day dosage and 0.75 ppm at a 2 g/day dose, hence the value is dose dependant.

Process and analytical scientists will face a number of obstacles, detailed below, while attempting to manage contaminants at such a low level [38, 39].

Challenges faced by Process scientist

- Reagents, starting materials, and intermediates that might be genotoxic can be difficult

to remove from synthesis.

- The high reactivity of certain of the starting ingredients and reagents makes them potentially genotoxic.
- Cleaning and process safety from tosylates and mesylates.
- The inevitability of PGIs means that they will remain, which drives up production costs and delays product availability.

Challenges faced by Analytical scientist

- The current technology faces a wide range of problems and issues related to low level contaminants.
- Achieving the target limit of detection may need looking outside the box at several technologies.
- Deciding on the most suitable method (GC, LCMS, NMR, UPLC).
- The impurities' stability.
- You may have to construct the analytical technique all over again if you work with new matrices (such as different formulations or reactants) every time.

Control strategy for genotoxic impurities

Figure 3 shows the proposed technique for controlling GTIs according to their entry point into the synthetic route or process.

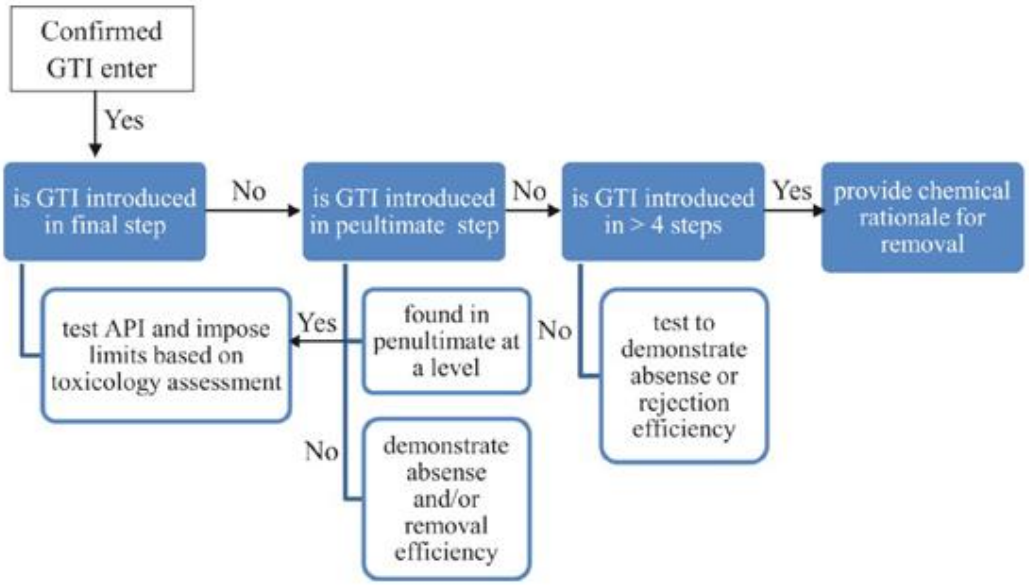

Figure 3: Control strategy of genotoxic impurities

It is often challenging for synthetic chemists to completely avoid the presence of potential genotoxic impurities (PGIs) during the synthesis of active pharmaceutical ingredients (APIs). As noted by Muller et al. (2006), many of the key reagents, intermediates, and raw materials used in chemical synthesis themselves possess genotoxic structural alerts or reactivity that can generate genotoxic by-products. Because these compounds play critical roles in facilitating specific reactions, their use cannot always be eliminated entirely. Therefore, re-evaluating and redesigning the synthetic route becomes essential to either significantly minimize or completely eliminate the formation of GTIs. This may involve selecting alternative, less reactive reagents, optimizing reaction conditions to suppress side reactions, or introducing purification steps to remove any residual impurities formed during synthesis.

The strategy for controlling genotoxic impurities largely depends on the stage at which they are introduced into the manufacturing process. When a genotoxic contaminant is introduced during the final or penultimate (semi-final) stage of synthesis, it becomes particularly crucial to incorporate strict control measures within the API specifications. This is because impurities formed or introduced in later stages have a higher likelihood of carrying over into the finished drug substance, posing a greater safety risk. For impurities that appear in earlier stages of synthesis, control measures can be based on process understanding and evidence that they are effectively removed or transformed during subsequent steps.

In cases where impurities are introduced two or three steps before the final API (often referred to as N-2 or N-3 stages), detailed and well-documented justifications must be provided for the chosen control strategy. The rationale should demonstrate that the impurity will be either chemically transformed or adequately purged in later reactions, reducing its potential to persist in the final product. Conversely, if the impurity originates four or more steps prior to the API formation, testing of the final product may not be necessary, provided that sufficient process validation confirms its removal or degradation.

If the complete elimination of genotoxic impurities is not feasible, chemists must modify their synthetic procedures to ensure that the impurity levels remain below established identification thresholds. These thresholds are typically defined by toxicological assessments and regulatory guidelines, ensuring that the residual impurity concentration poses no significant risk to human health. Analytical methods such as LC–MS and GC–MS are often employed to confirm that GTIs are below these limits. Furthermore, to substantiate the genotoxic potential of any suspected impurity, an **Ames test**—a bacterial reverse mutation assay—is required. This test determines whether the compound can induce genetic mutations in microbial DNA, thereby confirming its mutagenic nature.

In essence, effective management of genotoxic impurities requires an integrated approach that combines careful route design, analytical monitoring, and toxicological evaluation. Synthetic chemists play a central role in minimizing risk by optimizing processes, validating impurity clearance, and ensuring compliance with regulatory safety thresholds. Such efforts not only enhance product quality and patient safety but also uphold the integrity of pharmaceutical manufacturing standards.

Analytical Approach

Even when analytical and synthetic chemists possess the advanced capability to detect and quantify genotoxic impurities at levels below the specified regulatory thresholds, the overall management of these impurities throughout the development and manufacturing stages of both drug substances (DS) and drug products (DP) remains a major challenge. The complexity arises from the fact that genotoxic impurities can form at various stages of synthesis or degradation, and controlling them requires an in-depth understanding of the entire process—from raw material selection to final formulation. These challenges become particularly evident during the early phases of drug development when the synthetic route and process parameters are still being optimized. At this stage, limited information about reaction mechanisms, intermediate

stability, and degradation pathways often hinders the ability to anticipate and mitigate impurity formation effectively.

A major difficulty lies in the incomplete understanding of the efficacy and development process during the early stages of API synthesis. Without full knowledge of reaction intermediates and potential side reactions, identifying, characterizing, and controlling genotoxic contaminants becomes a complex and often uncertain task. Analytical scientists may be able to detect impurities using advanced methods such as LC–MS or GC–MS; however, establishing the chemical identity of every impurity, especially at trace levels, remains extremely challenging. Many impurities are transient or unstable, making isolation and structural elucidation difficult. Additionally, analytical methods capable of detecting impurities at such low concentrations may not always provide sufficient information about their chemical structure or mechanism of formation.

Despite these challenges, scientists can still perform risk classification and assessment if the presence of an impurity exceeds the established identification threshold. In such cases, toxicological evaluation and structure–activity relationship (SAR) analyses can help determine whether the impurity poses a potential genotoxic risk. However, if the impurity level remains below the threshold of toxicological concern (TTC), regulatory guidelines generally do not require extensive assessment or control, since the risk is considered negligible. This approach helps focus analytical and manufacturing efforts on impurities that may pose a genuine health hazard. Nonetheless, the limitation of this approach is that certain impurities, though below the threshold, may remain undetected or uncharacterized unless a process failure or unexpected reaction occurs.

Determining the exact chemical structure of every impurity is often one of the most complex aspects of impurity management. Some impurities may exist in minute concentrations or have overlapping chromatographic properties, making it difficult to isolate and identify them unambiguously. Moreover, structural elucidation requires sophisticated analytical tools and techniques such as high-resolution mass spectrometry (HRMS) or nuclear magnetic resonance (NMR), which may not always provide clear results for low-level, unstable, or reactive compounds [40].

In summary, while modern analytical technologies have significantly improved the ability to detect and quantify genotoxic impurities, comprehensive control remains an ongoing challenge. Effective impurity management requires not only sensitive analytical tools but also

a thorough understanding of chemical synthesis, reaction mechanisms, and degradation behavior. Continuous process knowledge, risk-based assessment, and adherence to regulatory guidelines are essential to ensure that potential genotoxic contaminants are kept well below harmful levels throughout the entire drug development and production lifecycle.

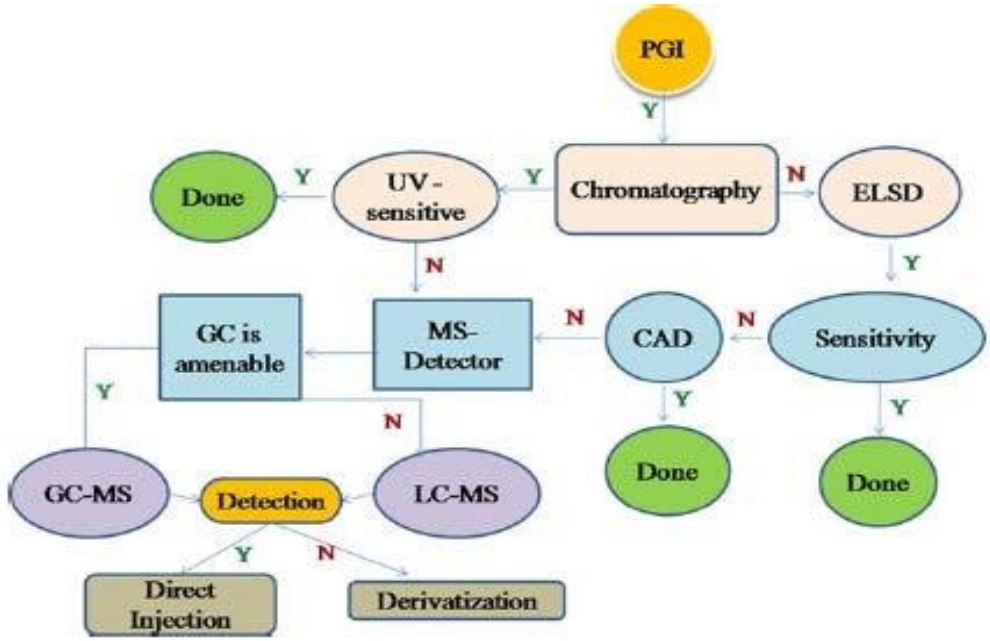

Figure 4: Analytical flow chart for testing GTI'S

METHODOLOGY

Selection of drugs

Drugs were selected based on the basis of exhaustive literature survey and market research. Online data, journals, analytical papers were comprehensive explored to screen out the drug candidates whose impurities were difficult to analyse using available methods and techniques. Furthermore, ease of availability of these drugs was also ascertained before initiating present studies.

In-silico methods for toxicity assessment

After the impurities were finalised, they were subjected to toxicity testing. This testing made use of several computational approaches, such as DEREK, MCase, and TOPKAT, to determine if the impurities in the compounds were mutagenic or carcinogenic. The rationale behind this is that these computational approaches lessen the need for costly and inefficient preclinical testing, which in turn saves money.

Procurement of analytical standards and impurities

Sigma Aldrich of India supplied the ranitidine sulfonate impurities while Neuland Laboratories of Hyderabad provided the acenocoumerol. The source of the Famotidine and Famotidine standard impurities was Sigma Aldrich in India, whereas the source of the quetiapine impurities was the same source.

In vitro assessment of toxicity

After in-silico prediction of impurities, they were further submitted to Ames test for mutagenicity and carcinogenicity assessment.

Selection of analytical techniques

Indeed, even at incredibly low focuses, PGIs should be distinguished and evaluated. In this review, GC-MS and LC-MS were chosen to handle this trouble. As indicated by the Van Deemter condition, GC-MS and LC-MS use sections with a molecule size of under 2 µm, which improves speed, responsiveness, and goal. As well as expanding awareness, GC-MS and LC-MS diminish additional segment impacts. The recognition limits expected to measure genotoxic foreign substances can't be met by usually utilized indicators like PDA, UV-VIS, ELSD, and refractive file. Because of its awareness and selectivity, GC-MS was picked over elective LC-MS identifiers. Charged species isolated by mass-to-zoom proportions are the focal point of fluid chromatography-mass spectrometry. To work on the insightful strategy's selectivity and responsiveness for genotoxic pollutant recognition and measurement, a few tests were led, including as full sweep MS, SIM, and MRM. For LC-MS to work, it could deal with a solitary particle at a time, and then split it up in the impact cell to make section particles that were more designated to the analyte particle. Since less foundation particles had the option to arrive at the locator, the sign to-commotion proportion improved.Even at incredibly low focuses, PGIs should be recognized and measured. In this review, GC-MS and LC-MS were chosen to handle this trouble. As per the Van Deemter condition, GC-MS and LC-MS use segments with a molecule size of under 2 µm, which upgrades speed, responsiveness, and goal. As well as expanding awareness, GC-MS and LC-MS decrease additional section impacts. The identification limits expected to evaluate genotoxic pollutants can't be met by generally utilized indicators like PDA, UV-VIS, ELSD, and refractive file. Because of its awareness and selectivity, GC-MS was picked over elective LC-MS identifiers. Charged species isolated by mass-to-zoom proportions are the focal point of fluid chromatography-mass spectrometry. To

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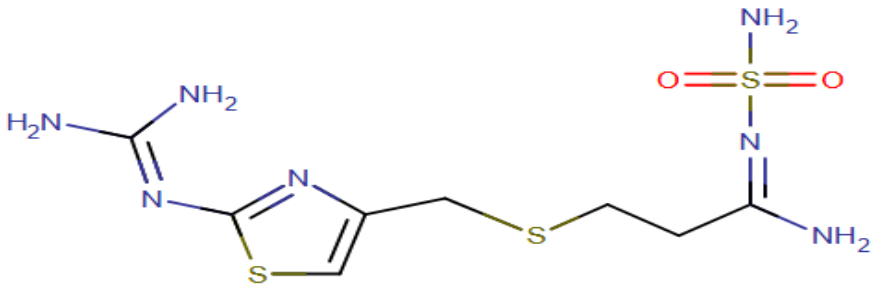
Method development and validation

The genotoxic impurities were distinguished and measured utilizing a straightforward, specific, delicate, and quick methodology. Following its turn of events, the logical procedure went through approval as per the ICH rules. Various variables were assessed, including linearity, accuracy, power, exactness, and cutoff points of discovery and measurement.

Application of developed analytical method

Strategies that have been created consider the productive arrival of Programming interface clumps for detailing by recognizing and measuring PGI's in arbitrarily picked clusters of restorative fixings. Routine QC testing research facilities for the previously mentioned drug intensifies furthermore utilized the proposed strategies.

Drug Profile [Famotidine]



Chemical Name	**3-(((2-guanidino-4-thiazolyl)methyl)thio)-N-(aminosulfonyl)propanamide**
Molecular Weight	337.4 g/mol
Molecular Formula	C8H15N7O2S3
Description	White or off-white crystalline powder
Solubility	Slightly soluble in water; freely soluble in dimethylformamide
Melting Point	~163–164°C
Category	Histamine H_2-receptor antagonist
pKa	~6.8
Mechanism of Action	Competitively inhibits H_2-receptors on gastric parietal cells, reducing acid secretion.
Related Impurities	Impurities listed in pharmacopoeias (e.g., USP, BP)

Genotoxic Impurities (*)

Estimation of N- Nitrosodimethylamine (NDMA) content (By LC-MS/MS)

Mobile Phase A (0.1% formic acid in water)

Accurately transfer 1 mL of Formic acid in to the 1000 mL of Milli-Q water.

Mobile Phase B (0.1% formic acid in methanol)

Measure out 1000 millilitres of methanol and carefully add 1 millilitre of formic acid.

Diluent

Dilute the solution with water.

Chromatographic parameters

The liquid chromatograph has a data processor, injector, and mass detector.

Table 3: Chromatographic parameters

Instrument details	:	Agilent 6470 LC/TQ LC/MS-MS system with APIC source or Equivalent
Column	:	ACE Excel C18-AR (50 mm x 4 6 mm), 3 µm
Columnoven temperature	:	30°C
Flow rate	:	0.6 ml/minute
Injection volume	:	10 µl
Run time	:	14 minutes
Autosampler temperature	:	4-8 °C
Retention time	:	About 2.20 minutes

Table 4: Gradient programme

Time (minutes)	% Of Mobile Phase A	% Of Mobile Phase B
0.0	95	5
1.0	95	5
3.0	80	20
7.0	0	100
9.0	0	100
9.1	95	5
14.0	95	5

Table 5: Source parameters

Ion sources	APCI
Gas temperature	325'C
APCI heater	400
Gas flow (L/minutes)	6
Nebulizer (psi)	45
Capillary (V)	4000
APCI needle positive	5

Table 6: Scan Parameters

Polarity: Positive ion

Scan type: MRM

Scan time: 1-3.0 minute; Delta EMV (+): 400

	Precursorion	**MS1 Res**	**roduct io**	**MS2 Res**	**Dwell**	**Fragmentor**	**Collision Energy**	**Cell Accelerator voltage**
Quantifier	75.1	Unit	43.1	Unit	200	90	15	5
Quantifier	75.1	Unit	58.1	Unit	200	90	10	5

Table 7: Time programme

Index	Start time (minute)	Scan type	Ion made	Div valve	Delta EMV (+)	Store
1	0	MRM	APCI	To MS	400	Yes
2	2.65	MRM	APCI	To Waste	0	Yes
3	12.5	MRM	APCI	To MS	400	Yes

Preparation of NDMA impurity stock solution: (100 ng/mL)

Place 10.0 milligrams of N-Nitrosodimethylamine (NDMA) into a 100 milliliter volumetric flagon after exact gauging. Add around 70 milliliters of diluent, sonicate until broke down, and afterward weaken to volume with diluent. Mix well. In a 100.0 mL volumetric flagon, add 1.0 mL of the arrangement, then, at that point, add diluent to bring it up to volume, blending great. Add 10.0 mL of volumetric flagon to which you have proactively added 1.0 mL of standard stock arrangement, and mix well.

Standard preparation (1.0 ng/mL):

Add 1.0 mL of pollutant standard stock answer for a 100.0 mL volumetric cup and mix well. (Centralization of NDMA 1.0 ng/mL) OR

Preparation of NDMA impurity stock solution (200 ppm)

Utilize 200 ppm NDMA standard arrangement make: Sigma Aldrich (Item no. 48670)

Before use, weaken 0.1 mL of 200 ppm NDMA arranged arrangement with 10 mL of diluent, blending great. Utilize 100 mL of diluent to weaken 1 mL of this arrangement, and mix well. Twenty sections for each billion of NDMA

Standard preparation: (1.0 mg/mL)

Reduce the volume of the impurity standard stock solution by 5 millilitres and add it to a 100.0 millilitre volumetric flask. (Concentration of NDMA 1.0 ng/mL)

Sample preparation

After removing the tops from five ampoules, pour the contents into a clean beaker or test tube and stir to combine. Here is the pooled sample: this well mixed solution. (Concentration of Ranitidine 25 mg/mL)

Procedure

Infuse single infusion of diluent, six reproduce infusions of standard arrangement and single infusion of test arrangement and record the chromatogram.

Ignore any top because of clear.

System suitability

The recurrent infusion of weakened standard readiness shouldn't surpass 10.00 as far as the overall standard deviation of pinnacle region inferable from NDMA.

Calculation

Utilise the following formula to determine the sample's NDMA impurity percentage.

$$\text{Impurity (in ppb)} = \frac{\text{NDMA}_{Au}}{\text{As}} \times 0.2 \times \frac{0.1}{10} \times \frac{1}{100} \times \frac{5}{100} \times \frac{1}{\text{LC}} \times P \times 10000 \times 1000$$

OR

$$\text{Impurity (in ppb)} = \frac{\text{NDMA}_{Au}}{\text{As}} \times \frac{Ws}{100} \times \frac{1}{100} \times \frac{1}{10} \times \frac{1}{100} \times \frac{1}{\text{LC}} \times \frac{X}{P} \times 10000 \times 1000$$

Where:

Au = Area of peak of NDMA obtained from single injection of the sample preparation.

As = Average area of peak of NDMA obtained from six replicate injections of standard preparation.

Ws = Weight of NDMA Impurity use to prepare standard solution, in mg.

LC = Label claim of injection, in mg/mL.

P = Potency of NDMA impurity standard.

RESULTS

Famotidine

There are significant safety concerns about the presence of genotoxic impurities (GTIs) that might cause cancer in famotidine, an H2-receptor antagonist that is routinely used for acid reflux and ulcers. The most prevalent nitrosamine contaminants in pharmaceuticals are N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA), however there are many more. Research into the potential formation of nitrosamine impurities during manufacture or storage of famotidine continues, despite the fact that its structure differs from that of ranitidine, which was terminated due to high levels of NDMA. Regulatory agencies such as the FDA and the EMA have set strict limits for these pollutants, typically below 96 ng/day for NDMA, to ensure patient safety. Additionally, genotoxicity may be caused by famotidine's sulfonamide-related impurities. Chemicals with thioether groups in their molecular structures may undergo oxidation to form sulfone or sulfoxide derivatives, which in turn can generate reactive species that have the potential to cause genotoxicity. In addition, genotoxic problems might arise from process-related pollutants that are not adequately removed during manufacture, including residual solvents like dimethylformamide (DMF) and methylene chloride. The use of alkyl halides in synthesis may lead to the introduction of alkylating agents, which can harm DNA. Furthermore, famotidine's breakdown products might provide a genotoxic risk if they are produced in very humid settings or if the medicine is not maintained correctly. Sulfone or nitroso derivatives may be produced via degradation processes; their levels should be carefully monitored to ensure they do not exceed acceptable limits. The risks associated with famotidine's genotoxic impurities may be managed and reduced by following regulatory requirements such as ICH M7 (R1). Researchers employ state-of-the-art analytical techniques including high-performance liquid chromatography

(HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) to detect and quantify these pollutants. As part of risk mitigation measures, it is important to utilise appropriate synthesis methods to reduce impurity formation, perform thorough purification processes to eliminate genotoxic intermediates, and maintain storage conditions to prevent degradation. Consistent testing and monitoring per regulatory standards is necessary to maintain the efficacy and safety of famotidine formulations. Pharma firms may ensure the continued safety of famotidine and reduce the risk of genotoxic contamination by adhering to these guidelines. An established active component, famotidine is described in the European Pharmacopoeia (Ph.Eur.). Crystalline powder with a white or yellowish-white hue that is very insoluble in water is the active component. Fatotidine crystallises in two different forms: stable polymorph A and metastable polymorph B. A drug's efficacy is independent of its polymorphism, according to the MAH. Acharyal agents include acutidine. Through the use of the CEP technique, the active component is produced. A certificate of appropriateness may be requested by manufacturers or distributors of pharmaceutical substances via the Council of Europe's approved Certification Procedures of the EDQM. The chemical purity and microbiological quality of the material may be controlled according to the applicable specialised monograph, or the risk of Transmissible Spongiform Encephalopathy (TSE) can be evaluated according to the general monograph, or both can be attested to by this certificate. Assuring that chemicals are of sufficient quality and conform to Ph.Eur standards is the goal of this procedure.

Table 8: Peak Results

Sample ID	RT (min)	NDMA Area	NDMA (ng/g)	ICH Limit	Status
Famotidine-01	3.45	1523	18.5	96 ng/g	Pass
Famotidine-02	3.46	1430	17.2	96 ng/g	Pass
Blank	-	-	ND	-	Pass
Standard (20ng/g)	3.45	1600	20.0	-	-

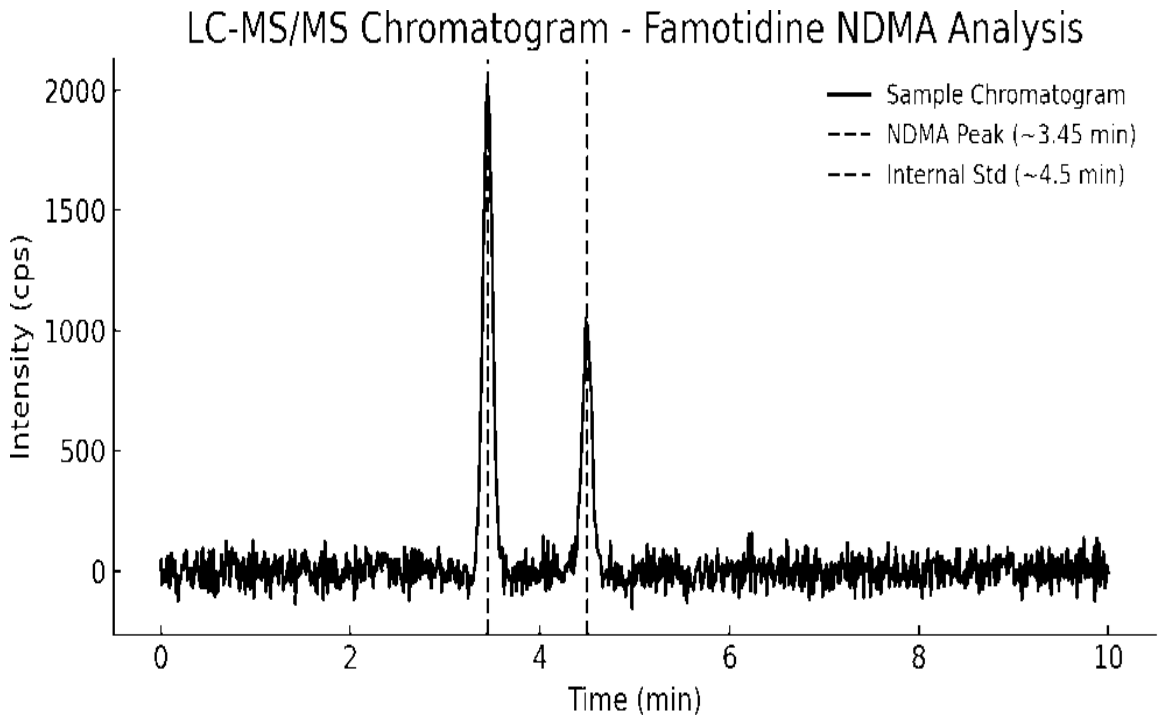


Figure 5: LC-MS chromatogram for NDMA (Famotidine)

CONCLUSION

The study underscores the critical need for stringent monitoring of genotoxic impurities in pharmaceutical substances such as Famotidine. Despite its established therapeutic efficacy, the presence of even trace quantities of nitrosamine impurities like NDMA poses a significant carcinogenic risk. Regulatory frameworks such as ICH M7 and FDA guidance emphasize maintaining GTIs below the TTC limit of 1.5 µg/day. Advanced analytical techniques including LC-MS/MS and GC-MS have proven indispensable in achieving sub-ppm detection and ensuring compliance with regulatory expectations.Effective control strategies ranging from synthetic route modification, purification optimization, and analytical surveillance must be integrated early in the drug development process. Future work should focus on predictive modeling and in-silico genotoxic risk assessment to proactively identify potential impurities. Ensuring patient safety requires a robust synergy between synthetic chemistry, analytical science, and regulatory compliance.

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