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REVIEW ARTICLE

PHARMA'S NITROSAMINE CHALLENGE: A MINIREVIEW OF A CALL FOR VIGILANCE

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Abstract

Nitrosamines are potential carcinogens (cancer-causing agents) that can form in medications under specific conditions. The European Pharmacopoeia (EP) and the United States Pharmacopeia (USP) both set strict guidelines to minimize their presence. Nitrosamines form when nitrites and amines react in acidic environments, potentially occurring in the stomach or during drug production. They are classified as probable human carcinogens, meaning long-term exposure at high levels may increase cancer risk. Fortunately, nitrosamine levels in most medications are very low and unlikely to pose significant health risks. Both EP and USP have implemented a new general chapter: Nitrosamine Impurities (EP: 2.5.42 and USP: <1469>). These chapters outline acceptable intake limits for six specific nitrosamines and a four-step risk assessment and control strategy. They also detail testing procedures and analytical methods for detecting and measuring nitrosamines in medications. There are similarities between EP and USP as both set comparable acceptable intake limits for the same six nitrosamines. Also, both employ a similar four-step risk management approach. Moreover, both provide information on testing procedures and analytical methods. Nevertheless, there are few differences between both references. There may be slight variations in the specifics of analytical procedures (e.g., detection limits, chromatographic conditions). Regulatory agencies are actively monitoring and controlling nitrosamine levels in medications, ensuring patient safety. Both EP and USP play crucial roles in setting stringent standards and ensuring their consistent implementation across the pharmaceutical industry.

Keywords: Analytical methods, carcinogenic potential, nitrosamine impurities, pharmacopoeia, regulatory guidance, risk assessment and control.

INTRODUCTION

Nitrosamines are a group of chemicals that may be formed when nitrites react with amines in the presence of acid, such as in the stomach. Some nitrosamines are **probable human carcinogens**, meaning they may increase the risk of cancer if people are exposed to them above acceptable levels and over long periods of time. Nitrosamines can damage the DNA and cause mutations that may lead to cancer. Nitrosamines have been linked to various types of cancer, such as stomach, esophageal, liver, bladder, and pancreatic cancer. However, the level of nitrosamines in most foods and medications is very low and unlikely to pose a significant health risk[1-5]. The FDA and other regulatory agencies are monitoring the levels of nitrosamines in drugs and foods and taking actions to reduce them when necessary.

How Nitrosamines can contaminate medicinal compounds

Nitrosamines are unfortunately not exclusive to processed foods; they can also be found in some medications. While the levels are usually very low and tightly regulated, understanding their potential sources is crucial for ensuring patient safety[6-10]. There are some sources from which nitrosamines might find their way into medicinal compounds:

Formation during manufacturing:

Reaction of nitrites and amines: This is the most common pathway[11]. Nitrites can be present as additives or contaminants, while amines can be inherent to the drug molecule itself or introduced during synthesis[12,13]. Specific drug reaction conditions like acidic environments or high temperatures can further promote nitrosamine formation[14,15].

Nitrosating agents: Chemicals like nitrous acid, nitrogen oxides, or nitrosyl chloride can directly nitrosate amine groups present in the drug molecule or its starting materials[16].

Nitrosamine carry-over from solvents or reagents: Trace amounts of nitrosamines might be present in solvents or reagents used during manufacturing and unintentionally contaminate the final product[17].

Post-manufacturing sources:

Storage and degradation: Over time, certain drug molecules can degrade under specific storage conditions, potentially forming nitrosamines[18]. Factors like light, heat, and pH can influence this process.

Cross-contamination: If nitrosamines are present in other drugs manufactured in the same facility, there's a risk of cross-contamination, especially in shared equipment or production lines[19].

Recycled or recovered materials: Using recycled or recovered materials in drug manufacturing can introduce nitrosamines if they were previously contaminated[16-20].

Sources specific to drug type:

Sartans: This class of blood pressure medications was heavily impacted by nitrosamine contamination due to the specific chemistry of their starting materials and the manufacturing process[19,21].

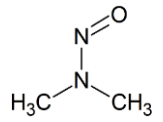
Ranitidine: This heartburn medication was withdrawn from the market due to the presence of NDMA, likely formed during the manufacturing process[22,23].

Nevertheless, it's important to note that not all drugs are susceptible to nitrosamine formation. Moreover, regulatory agencies worldwide have implemented strict limits and guidelines to minimize nitrosamine presence in medications. Pharmaceutical companies are actively investigating and implementing strategies to prevent and detect nitrosamine contamination. While the presence of nitrosamines in medications is a concern, the levels are typically very low, and the associated health risks are considered minimal for most individuals[6,7,10]. However, continued vigilance and research are crucial to ensure patient safety and prevent future contamination issues.

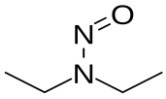
Nitrosamine Impurities in the EP

The European Pharmacopoeia (EP), which is the official collection of standards for European medicinal substances and products provided comprehensive directions about this kind of impurities. The recent EP contains a new general chapter on Nitrosamine Impurities (2.5.42), which provides guidance on the assessment and control of nitrosamine impurities in active substances and finished products. Nitrosamine impurities are probable human carcinogens that may be present in trace amounts in some pharmaceutical products[24-27]. They can originate from various sources, such as the properties of the starting materials, intermediates or active substances, the manufacturing process, the use of recovered or recycled materials, the degradation of the product, or the cross-contamination in multi-purpose facilities[28,29].

The general chapter on Nitrosamine Impurities (2.5.42) specifies the acceptable intake limits for six nitrosamine impurities: N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), N-nitrosodiisopropylamine (NDIPA), N-nitrosoethyl-isopropylamine (NEIPA), and N-nitrosodibutylamine (NDBA). These limits are based on the ICH M7(R1) guidance on the assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals. Figure 1 shows different chemical structures of the nitrosamine impurities[9,10,27,30]. The general chapter on Nitrosamine Impurities (2.5.42) also describes the risk assessment and control strategy for nitrosamine impurities, which involves four steps: identification of risk factors, evaluation of risk level, confirmation of risk control, and periodic review of risk status[31,32]. The risk assessment and control strategy should be applied to all active substances and finished products, unless otherwise justified[33]. The general chapter on Nitrosamine Impurities (2.5.42) also provides information on the testing for nitrosamines, which includes the selection of appropriate analytical methods, the validation of the methods, and the reporting of the results[30]. The British Pharmacopoeia (BP) chapter also lists some analytical procedures for the determination of nitrosamine impurities in various active substances and finished products, such as sartans, ranitidine, metformin, and pioglitazone[34-37].



N-nitrosodimethylamine (NDMA)



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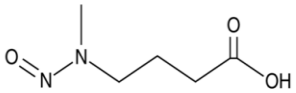
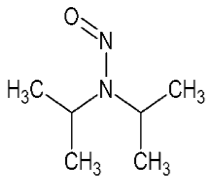
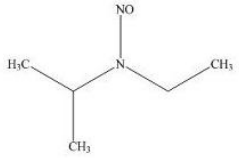


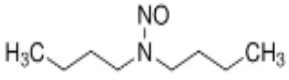
N-nitroso-N-methyl-4-aminobutyric acid (NMBA)



N-nitrosodiisopropylamine (NDIPA)



N-nitrosoethyl-isopropylamine (NEIPA)



N-nitrosodibutylamine (NDBA)

Figure 1: The six nitrosamine impurities.

Nitrosamine Impurities in the USP

The United States Pharmacopeia (USP) also addresses nitrosamine impurities through its dedicated chapter Nitrosamine Impurities.[38]Similar to the EP, this chapter serves as a guide for assessing and controlling nitrosamine levels in drug ingredients and drug products. USP establishes the same acceptable intake limits for the six identical nitrosamines mentioned previously (NDMA, NDEA, NMBA, NDIPA, NEIPA, and NDBA)[19,21,28]. These limits are derived from the same ICH M7(R1) guidance. Both USP and EP employ a four-step risk management approach, emphasizing identifying risk factors, evaluating risk levels, confirming control measures, and regularly reviewing risk status[38-40].

However, the USP chapter focuses deeper into developing testing methodologies for specific nitrosamines in various drug substances and drug products[41]. It provides general analytical principles and encourages developers to create tailored methods based on their specific needs[42]. USP offers examples of established analytical procedures for certain high-risk medications like sartans, ranitidine, metformin, and pioglitazone, but encourages customization for other drugs[15,42,43].

The USP shows some key Differences with EP. Unlike the EP, USP emphasizes method development guidance over providing specific, pre-defined analytical procedures. This approach grants drug developer's greater flexibility in tailoring testing methods to their specific drug products and manufacturing processes. Although both standards share similar acceptable intake limits and risk management strategies, the USP's focus on method development guidance sets it apart from the EP's (and BP) more prescriptive approach[44,45]. Table 1 shows the overall differences briefly. The best approach depends on individual circumstances and regulatory requirements. Both EP and USP offer valuable tools for ensuring nitrosamine safety in medications, but understanding their distinct advantages and considerations is crucial for making informed decisions[17,44,45]. While the previous table (Table 1) compared the overall focus and structure of the EP and USP chapters, a more thorough overview into specific differences in detection and analysis, including Table 2 embraces key points in the testing and analysis.

Table 1: Brief comparison between the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP) on their general chapters and analytical procedures for nitrosamine impurities.

EP	USP
Nitrosamine Impurities (2.5.42) Provides guidance on the assessment and control of nitrosamine impurities in active substances and finished products	Nitrosamine Impurities <1469> Provides information on developing testing methodologies to detect and measure nitrosamine impurities in drug ingredients and drug products
Specifies the acceptable intake limits for six nitrosamine impurities: NDMA, NDEA, NMBA, NDIPA, NEIPA, and NDBA	Specifies the acceptable intake limits for six nitrosamine impurities: NDMA, NDEA, NMBA, NDIPA, NEIPA, and NDBA
Describes the risk assessment and control strategy for nitrosamine impurities, which involves four steps: identification of risk factors, evaluation of risk level, confirmation of risk control, and periodic review of risk status	Describes the risk assessment and control strategy for nitrosamine impurities, which involves four steps: identification of risk factors, evaluation of risk level, confirmation of risk control, and periodic review of risk status
Provides information on the testing for nitrosamines, which includes the selection of appropriate analytical methods, the validation of the methods, and the reporting of the results	Provides information on the testing for nitrosamines, which includes the selection of appropriate analytical methods, the validation of the methods, and the reporting of the results
Lists some analytical procedures for the determination of nitrosamine impurities in various active substances and finished products, such as sartans, ranitidine, metformin, and pioglitazone	Lists some analytical procedures for the determination of nitrosamine impurities in various active substances and finished products, such as sartans, ranitidine, metformin, and pioglitazone

The EP and USP have very similar standards and methods for nitrosamine impurities, which are based on the ICH M7(R1) guidance[1]. However, there may be some differences in the details and specifications of the analytical procedures, such as the chromatographic conditions, the detection limits, and the system suitability criteria[39]. Therefore, it is important to follow the specific procedures and requirements of the pharmacopoeia that applies to a product in the context[44,45].

Analytical Method Details:

EP: Provides specific, pre-defined procedures for certain drug classes like sartans, which can be directly applied without extensive adaptation[44,45].

USP: Offers generic principles and validation guidelines, encouraging developers to design tailored methods optimized for their specific drugs and processes.

Detection Limits:

EP and BP: May specify minimum required detection limits for listed procedures[44,45].

USP: Emphasizes achieving appropriate detection limits based on risk assessment and analytical capabilities, allowing for flexibility depending on the drug and nitrosamine of concern.

In-Depth Investigation into Nitrosamine Analysis: EP vs. USP comparison

Both the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP) play crucial roles in ensuring medication safety, including regulating nitrosamine impurities. While both share the same goal, their approaches to nitrosamine analysis differ in several key aspects. Unfortunately, directly comparing

specific chromatographic steps like column, detector, and mobile phase across EP and USP for nitrosamine analysis isn't straightforward[47-50]. While both advocate for analytical method validation and performance evaluation, the focus on pre-defined procedures in EP versus customizable methods in USP leads to significant differences:

Pre-defined vs. customizable approaches:

EP: Provides specific procedures for certain drug classes, outlining extraction techniques, column types (e.g., C18), mobile phase compositions, detector types (e.g., LC-MS/MS), and data analysis parameters. Developers can directly apply these procedures with limited adaptation[44,45, 49-53].

USP: Focuses on general principles and validation guidelines. Developers design and validate their own methods based on their specific drug and manufacturing process, leading to potentially varied chromatographic steps optimized for each case.

Table 2: In-depth holistic comparison of nitrosamine detection and analysis: EP vs. USP.

Feature	European Pharmacopoeia (EP)	United States Pharmacopeia (USP)
Regulatory Acceptance	Methods listed in the chapter are generally accepted by regulatory authorities in the Europe and other regions following European Pharmacopoeia standards	Developer-created methods need to be submitted for regulatory review and approval based on their validation data and adherence to USP guidelines
Knowledge Sharing	May have limited opportunities for sharing and adapting methods internally or externally due to their specific and pre-defined nature	Encourages method development knowledge sharing between developers and regulatory agencies, potentially leading to advancements and improvements in nitrosamine detection techniques
Analytical procedures	Specific procedures listed for some active substances and finished products	General principles provided, encouraging developers to create tailored methods
Method development guidance	Limited	Extensive, emphasizing flexibility and drug-specific adaptation
Validation requirements	Yes, for the listed procedures. Validates the listed procedures in-house, ensuring they meet performance criteria	Yes, for developer-created methods. Requires developers to validate their custom methods, placing responsibility for demonstrating suitability on the manufacturer
Reporting requirements	Reporting results and method details	Reporting results, method details, and justification for method selection
Available Resources	Offers specific, readily available procedures for some drugs, potentially reducing method development time and resources	Requires more upfront effort from developers but allows for potentially more efficient and targeted analysis methods specific to their needs
Flexibility for developers	Lower	Higher, allowing for adaptation to specific drugs and processes
Overall approach	More prescriptive, providing specific procedures	More guidance-oriented, empowering developers to design suitable methods

Additional notes: Both EP and USP share the same acceptable intake limits for the six key nitrosamines. Both employ a similar four-step risk management strategy for nitrosamines. The choice of EP or USP methods depends on the specific context and regulatory requirements for the drug product in question. Further considerations: This table provides a general overview. Specific details and nuances may vary depending on the nitrosamine of interest and the drug product being analyzed. It's crucial to consult the latest versions of both EP and USP chapters for the most up-to-date information and guidance.

Challenges in direct comparison:

Drug-specificity: The optimal column, detector, and mobile phase depend heavily on the individual drug and nitrosamine of interest.

Evolving Techniques: Analytical methods are constantly evolving with new technologies and advancements. Specific examples from EP and USP chapters might become outdated by the time of comparison.

Regulatory Focus: Both EP and USP prioritize method performance and validation over specific steps[39,40,51,53,54]. Comparing individual steps without considering the complete validated method and its suitability for the target drug wouldn't be relevant for regulatory purposes.

Highlights in Nitrosamine analysis: General principles and key differences

General principles of nitrosamine analysis:

1. **Sample Preparation:**
   - Extraction techniques often involve liquid-liquid extraction with organic solvents or solid-phase extraction cartridges.
   - Considerations include maximizing nitrosamine recovery, minimizing interferences, and ensuring compatibility with subsequent chromatography[39,40,45].
2. **Chromatography:**
   - **Liquid chromatography (LC):** The most widely used technique, employing reversed-phase columns (e.g., C18) and various mobile phase compositions depending on the nitrosamine and matrix[51,53,54].
   - **Gas chromatography (GC):** Can be used for volatile nitrosamines, requiring appropriate derivatization techniques.

3. **Detection:**
   - **Mass spectrometry (MS):** The preferred detector due to its high sensitivity and selectivity.
   - Triple quadrupole MS/MS is commonly used for specific and quantitative detection of individual nitrosamines.
4. **Data Analysis:**
   - Quantification based on calibration curves with reference standards.
   - Calculation of limits of detection, quantification, and linearity.
   - Evaluation of matrix effects and specificity.

Key considerations for method validation:

- **Selectivity:** Ensure the method differentiates the target nitrosamines from potential interferences in the sample matrix.
- **Sensitivity:** Achieve detection limits low enough to meet regulatory requirements and quantify relevant nitrosamine levels.
- **Accuracy and Precision:** Demonstrate consistent and reliable measurements.
- **Robustness:** Ensure the method performs consistently under varied experimental conditions.

EP vs. USP approach:

- **EP:**
  - Provides specific, pre-defined procedures for analyzing nitrosamines in certain drug classes.
  - Offers limited flexibility in adapting these procedures.
  - Requires validation of the listed procedures but not developer-created adaptations.
  - Regulatory acceptance in regions following European Pharmacopoeia standards.
- **USP:**
  - Offers general principles and validation guidelines for developing analytical methods.
  - Requires developers to design and validate their own methods based on their specific drug and process.
  - Greater flexibility to optimize analysis for individual needs.
  - Developer-created methods need regulatory review and approval.

Investigation and exploration of risk assessment & control: EP vs. USP nitrosamine strategies

While both EP and USP outline a four-step risk assessment and control strategy for nitrosamine impurities (Figure 2), the specifics within each step reveal intriguing differences. These differences could be outlined as the following[30,39,40]:

1. Identification of risk factors:

- **EP:** Provides a comprehensive list of potential risk factors, including starting materials, reaction conditions, manufacturing processes and product storage.
- **USP:** Offers a more general framework, encouraging developers to consider all relevant factors specific to their drug and manufacturing process.

2. Evaluation of risk level:

- **EP:** Employs a tiered approach based on pre-defined criteria like the potency of nitrosamine and the amount of drug substance administered.
- **USP:** Adopts a more flexible, qualitative risk assessment, considering factors like potential exposure, toxicity data, and manufacturing controls.

3. Confirmation of risk control:

- **EP:** Specifies established control measures based on the identified risk factors, such as process modifications or raw material selection.
- **USP:** Encourages developers to implement appropriate risk mitigation strategies based on their risk assessment, allowing for customized solutions.

4. Periodic review of risk status:

- **EP:** Requires periodic reviews at defined intervals to ensure continued effectiveness of control measures.
- **USP:** Emphasizes the importance of ongoing monitoring and risk reassessment based on new information or changes in the manufacturing process.

Additional key differences:

- **EP:** Offers a more prescriptive approach with defined risk factors, evaluation criteria, and control measures. This provides clear guidance but may limit flexibility.
- **USP:** Focuses on principles and encourages developers to tailor the risk assessment and control strategy to their specific context. This offers more flexibility but requires greater expertise and effort.

Choosing the right approach:

- **EP:** Suitable for manufacturers who prefer a clear, step-by-step approach with pre-defined criteria.
- **USP:** Preferred by those seeking flexibility and the ability to customize the risk assessment and control strategy to their specific needs.

Ultimately, the optimal approach depends on factors like regulatory requirements, the complexity of the drug product, and the manufacturer's risk management expertise. However, beyond this comparison, it's crucial to consult the latest versions of both EP and USP chapters for comprehensive details and any regulatory updates, in addition to Seeking additional guidance from regulatory agencies or experts if needed, especially for complex cases or novel drug formulations. Importantly, it should be remembered that ensuring drug safety through effective nitrosamine risk management is a shared responsibility. Understanding the nuances of EP and USP approaches empowers manufacturers to choose the strategy that best suits their context and contributes to patient safety. The diagram in Figure 2 is titled "Deep Dive into Risk

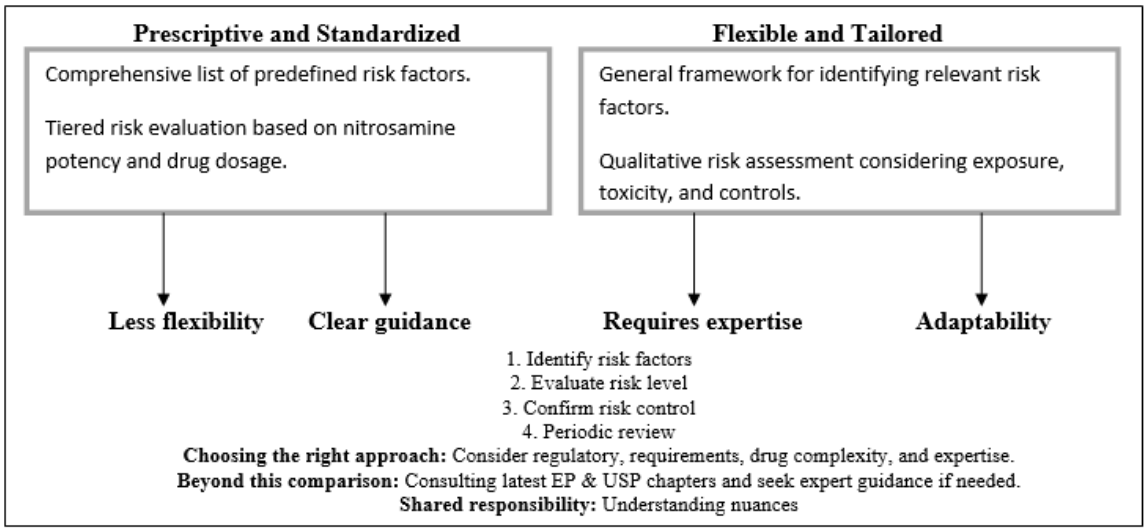


Figure 2: Deep dive into risk assessment & control: EP vs. USP Nitrosamine strategies.

Assessment & Control: EP vs. USP Nitrosamine Strategies[4,5,6].

It visually represents the differences in how the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP) approach the four-step risk assessment and control process for nitrosamine impurities in medications.

Figure 2 visually emphasizes the concept of risk assessment and control for nitrosamine strategies employed by EP and USP. A central core is the "Risk Assessment & Control Process" outlines the four key steps: identification of risk factors, evaluation of risk level, confirmation of risk control, and periodic review of risk status. Two separate approaches flank the central one, representing the EP and USP concepts. The EP rectangle, labeled "Prescriptive and Standardized", details a comprehensive list of predefined risk factors, tiered risk evaluation based on potency and dosage, established control measures, and defined intervals for review. Benefits are mentioned: clear guidance and efficiency for routine cases. However, limitations include reduced flexibility and potential difficulties adapting to unique situations.

On the other hand, the USP rectangle, labeled "Flexible and Tailored", outlines a general framework for identifying risk factors, a qualitative assessment considering exposure, toxicity, and controls, with encouragement for customized mitigation strategies. Ongoing monitoring and reassessment based on new information are emphasized. The benefits of this approach are adaptability to specific needs and the potential for more effective control. However, limitations include the need for expertise and increased effort for initial implementation. Connection exists depicting the relationships between the components.

The bottom section provides additional information and guidelines for selection. Text box discusses choosing the right approach by considering regulatory requirements, drug complexity, and available expertise. It emphasizes consulting the latest EP and USP chapters and seeking expert guidance when necessary. Finally, the text underscores the shared responsibility: understanding the nuances empowers manufacturers to ensure drug safety.

Reported incidents of nitrosamine toxicity in consumables: A closer look

While nitrosamines have raised concerns due to their potential carcinogenicity, direct evidence of human toxicity from consuming nitrosamines in food or medication is limited. Some cases concerning the exposure to Nitrosamine compounds could be illuminated as the following:

Acute toxicity: No confirmed cases of acute nitrosamine poisoning have been reported from consuming food or medication. Acute toxicity typically involves high-dose exposure and rapid onset of symptoms like nausea, vomiting, dizziness, or liver damage.58Studies in animals suggest high doses of nitrosamines can cause acute toxicity, but these doses far exceed levels typically found in consumable products[55-57].

Chronic toxicity: The primary concern surrounding nitrosamines lies in their potential to contribute to cancer risk over time. This risk stems from their ability to damage DNA, potentially leading to mutations and cancer development. Epidemiological studies investigating links between nitrosamine intake and cancer risk have yielded mixed results. Some studies suggest a potential association, while others haven't found conclusive evidence[58-60]. Current evidence suggests the cancer risk from consuming nitrosamines in food or medication is likely very low, especially considering the typically low levels present and the body's natural detoxification mechanisms.

Important Caveats: Individual susceptibility and vulnerability to nitrosamine-induced carcinogenicity might vary based on factors like genetics, pre-existing health conditions, and overall dietary patterns[28,60,61]. Research on nitrosamine toxicity is ongoing, and new findings could shed further light on potential risks and individual variations.

Specific cases: In 2019, several medications were recalled due to the presence of NDMA, a nitrosamine, above acceptable limits. However, no adverse health effects were reported in consumers who took these

medications. Outbreaks of liver disease in some parts of the world have been linked to high nitrosamine exposure from contaminated drinking water[62,64]. These cases highlight the potential dangers of long-term exposure to high nitrosamine levels.

It could be extrapolated that while the presence of nitrosamines in consumables warrants continued monitoring and regulatory efforts to minimize exposure, direct evidence of human toxicity from typical consumption levels remains limited. Ongoing research is crucial to refine risk assessments and personalize approaches to managing potential risks associated with nitrosamines.

Nitrosamines effect on human health and how they are generated

Nitrosamines are a group of chemicals that may be formed when nitrites react with amines in the presence of acid, such as in the stomach. Some nitrosamines are probable human carcinogens, meaning they may increase the risk of cancer if people are exposed to them above acceptable levels and over long periods of time. Nitrosamines can damage the DNA and cause mutations that may lead to cancer. Nitrosamines have been linked to various types of cancer, such as stomach, esophageal, liver, bladder, and pancreatic cancer. However, the level of nitrosamines in most foods and medications is very low and unlikely to pose a significant health risk. The FDA and other regulatory agencies are monitoring the levels of nitrosamines in drugs and foods and taking actions to reduce them when necessary. Nitrosamines form via various reactions, most prominently involving the interaction between secondary or tertiary amines with nitrite (NO_2^-)[65-68]. There few examples that could be highlighted:

1. Reaction with nitrosating agents:

$$N\text{-}R1R2 + NO_2^- \text{----> } R\text{-}N\text{-}NO + R2^-$$

Where N-R1R2 represents a secondary amine and R-N-NO represents the nitrosamine product. Other nitrosating agents like N-nitrosodimethylamine (NDMA) can also participate.

2. Diazotization-coupling:

A primary amine reacts with sodium nitrite and hydrochloric acid to form a diazonium salt, which then couples with secondary amine to form a nitrosamine.

3. Nitrosation by nitrogen oxides:

NO_2 reacts with amines under acidic conditions to form nitrosamines.

4. Nitrosation by nitroso compounds:

N-nitroso compounds like NDMA can directly nitrosate other amines.

Protein Modifications:

S-nitrosylation: Nitrosamines can nitrosylate protein cysteine residues, altering their function and impacting various cellular processes.

Formation of nitrosothiol species: These reactive intermediates can further modify proteins and other biomolecules, contributing to potential cellular damage.

Drug Interactions of Nitrosamines

Nitrosamines can interact with various drugs and alter their metabolism, efficacy, or toxicity. The specific interactions depend on the individual drug and the nitrosamine involved. Here are some general categories of interactions:

1. Enzyme inhibition: Nitrosamines can inhibit cytochrome P450 enzymes, responsible for drug metabolism, potentially leading to increased drug levels and toxicity.

2. DNA alkylation: Nitrosamines can alkylate DNA, causing mutations and potentially increasing cancer risk. This interaction is particularly concerning due to the potentially carcinogenic nature of nitrosamines themselves.

3. Formation of reactive nitrogen species: Nitrosamines can generate reactive nitrogen species like nitric oxide (NO) which can further interact with drugs and biological molecules.

4. Competition for binding sites: Nitrosamines may compete with drugs for binding sites on proteins or receptors, altering their effects. This can be relevant for drugs with similar structures or binding mechanisms to nitrosamines.

Specific reactions

The specific interactions and reactions depend heavily on the individual nitrosamine and drug involved their concentrations, and the cellular context.

•Advanced glycation end product (AGE) formation: Nitrosamines can react with sugars to form AGEs, implicated in aging and age-related diseases.

•Metal-catalyzed reactions: Under specific conditions, metal ions can facilitate nitrosamine decomposition or formation of reactive intermediates with enhanced toxicity.

Final thoughts, insights and lessons learned

The challenges and risks in pharmaceutical industry are diverse and each has its unique assessment and evaluation methodologies[69-77]. This short review offers a detailed approach in the analysis of nitrosamines in medications, highlighting their potential risks, sources of contamination, and strategies for control. There are some key points to consider and novel perspectives. **Regulatory Landscape:** Both the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP) play a crucial role in setting standards and guidance for nitrosamine control in medications. They share the same acceptable intake limits for six key nitrosamines but differ in their analytical and risk management approaches. Analytical Methods: EP offers pre-defined procedures for specific drug classes, while USP emphasizes creating customized methods tailored to individual drugs and processes. This highlights a trade-off between ease of use and flexibility.

Risk Assessment and Control: EP utilizes a prescriptive approach with defined risk factors, evaluation criteria, and control measures. USP offers a more flexible framework that requires manufacturers to tailor the strategy to their specific needs. This creates a decision point for manufacturers based on their expertise and the complexity of the drug. Shared **Responsibility:** Ultimately, ensuring drug safety through effective nitrosamine risk management is a shared responsibility between manufacturers, regulatory agencies, and healthcare professionals.

For these points novel perspectives could be explored in multipronged approach. Beyond current regulations: The focus has primarily been on minimizing nitrosamine formation during manufacturing. A future perspective could involve exploring strategies to minimize nitrosamine generation during storage and degradation of medications. Individualized Risk **Assessment:** Current approaches focus on population-wide risk assessment. Future research could explore incorporating individual factors like genetics and dietary patterns into nitrosamine risk assessment for high-risk patients.

Balancing Detectability vs. Efficiency: While pre-defined methods offer ease of use, they might not be as efficient or sensitive for certain drugs. Future advancements could involve creating standardized, yet adaptable analytical methods for broader applicability. Finally, nitrosamines in medications are a concern, but the levels are typically very low, and the associated health risks are likely minimal for most individuals. Continued vigilance and research are crucial to ensure patient safety and minimize future contamination issues. The contrasting approaches of EP and USP highlight the importance of finding the right balance between clear guidance, flexibility, and scientific expertise for effective nitrosamine control.

CONCLUSIONS

This review underscores the critical issue of nitrosamine contamination in medications. It details methods for analyzing nitrosamine levels, explores potential health risks, and proposes control strategies. Interestingly, the analysis highlights contrasting approaches by the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP). The EP offers pre-defined, standardized methods, while the USP prioritizes customizable approaches. This creates a choice for manufacturers: ease of use versus adaptability. Similarly, risk assessment reflects different philosophies. The EP lays out defined criteria, whereas the USP encourages a more flexible framework based on individual drug needs. This empowers manufacturers but demands greater expertise. Looking beyond current regulations, future efforts could address nitrosamine formation during storage and degradation. Additionally, personalized risk assessment, considering factors like genetics and diet, could benefit high-risk patients. Finally, while nitrosamine levels are typically low, continued vigilance is crucial. The EP and USP approaches highlight the importance of striking a balance between clear guidance, flexibility, and scientific knowledge for optimal nitrosamine control in medications.

AUTHOR'S CONTRIBUTION

Eissa ME: Writing original draft, review, methodology, data curation, literature survey, editing.

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DATA AVILIABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

None to declare.

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