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Nitrosamine impurity detection: unravelling the analytical puzzle

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Abstract

Because of their strong Genotoxic and Mutagenic properties, N-nitro compounds, especially N-nitrosamines, have caused concerns about worldwide safety when they are present in pharmaceutical goods.

A number of medicines were taken off the market because of the high limit of nitrosamine contamination. This paper aims to provide a succinct overview of nitrosamine impurities, including their mode of action, precise detection techniques, sample preparation steps, and regulatory limitations. Chemical structural analysis has also been done on a number of reported nitrosamine pollutants. This review paper covers nitrosamine impurity detection methods like GC-MS-HS and HPLC.

Furthermore evaluated in the study is the Decoding Nitrosation Assay: Exploring Innovative Approaches for Detection this will likely be used as a preventive measure in the future to find any potential nitrosation, N-nitro contamination, or other possibly mutagenic pollutants throughout the drug development process.

Key words : Mutagenic, Genotoxic, Nitrosamine, HPLC, GC-MS/HS, Nitrosation Assay.

"The Pharmacists Pharma Journal defines a drug as "any chemical substance that may be used on or delivered to humans to help identify, manage, cure, eliminate, or forestall disease or other abnormal conditions."[19]

Quality, safety and Efficacy of drugs :

Two key concerns in pharmacological

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therapy are the safety and effectiveness of medications. A medication's pharmacological/ toxicological profile and the unfavourable effects brought on by contaminants in dose forms and volume are what determine a drug's safety.[22]

Origin of impurities :

Drug contaminants come from a variety of sources and stages in the synthesis and dosage form manufacturing process.[33] A medication can be synthesized in a variety of ways, thus distinct contaminants could result from the same product derived from different sources For instance, impurity profiles of and orbofiban[38], fluoxetine HCl[45] that originated from various sources have been characterized in some recent works. These investigations investigated the impurity profiles of many lots of bulk medications acquired from diverse manufacturers.

Nitrosamine :

A class of chemical compounds known as nitrosamines is defined by the presence of both an amine group (NH2) and a nitro so functional group (NO). They are recognized to have the ability to cause cancer in both people and animals.[35,43]

Significance as impurities :

Carcinogenic potential :

The potential for nitrosamines to cause cancer is one of the main causes for concern. Many research investigations have connected exposure to specific nitrosamines to a higher risk of cancer. The International Agency for Research on Cancer, better known as IARC, has classified many nitrosamines as Group 1 cytotoxic to humans.[39]

Regulatory concerns :

Various regulatory authorities, notably the European Medicines Agency (EMA) and the U.S. FDA, which regulates drugs, have set acceptable levels and guidelines for nitrosamine impurities in products. The presence of nitrosamines beyond these limits can result in product recalls and regulatory actions due to the associated health risks.[5]

To sum up, this introduction to nitrosamines highlights their chemical makeup, mode of occurrence, and diversity of sources.[31]

Nitrosamines are important contaminants because of their possible carcinogenicity, regulatory risks, effects on public health, and requirement for continuous study and observation to protect consumers.[6]

Hazard :

Possibly genotoxic contaminants. Referred to in ICH M7 as a "Cohort of Concern."

Why detecting Nitrosamine impurities is crucial in various industries?

Pharmaceutical Industry :

Patient safety

Pharmaceutical impurities that contain nitrosamines have been discovered as possible carcinogens and represent a direct concern to patient safety; prolonged exposure to even

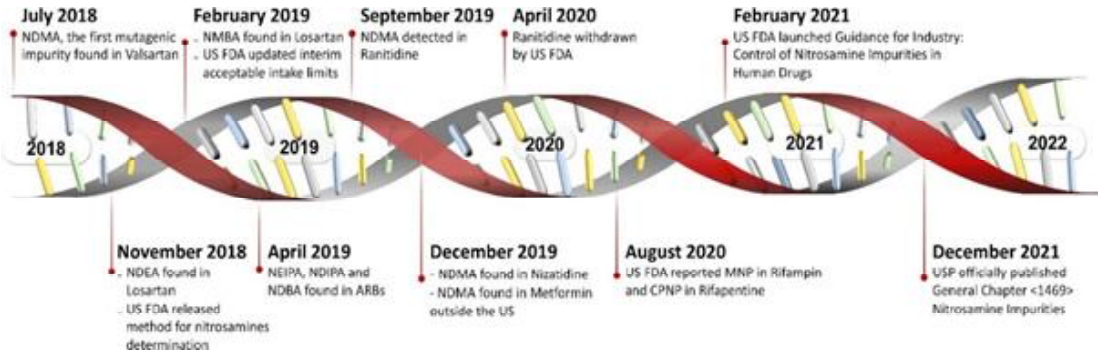

Fig. 1. Timeline of nitrosamine-contaminated drugs' evolution[15]

minute levels of these chemicals can have detrimental effects on health.[44]

Regulatory compliance

Pharmaceutical product nitrosamine impurities are subject to strict rules and permissible limits set by regulatory agencies. Maintaining the integrity of pharmaceutical firms and gaining market approval depend heavily on adherence to these laws.[30]

Product recalls and reputational damage High-profile :

Product recalls have resulted from the discovery of nitrosamine contaminants in medications. In addition to causing financial losses for the concerned corporations, these recalls harm their brand and erode consumer and healthcare professional trust**.**

N-nitrosodimethylamine (NDMA) contamination of valsartan-containing medications in July 2018 resulted in multiple batch recalls. This was shown to be a process impurity caused by modifications made to the active pharmaceutical, it was discovered that, in addition to the batches initially made by the Chinese company Zhejiang Zhuhai Pharmaceutical, NDMA [16] was also found in batches produced by other manufacturers (such as Zhejiang Tianyu Pharmaceutical and Hetero Labs, India). Leclerc asserts that while conducting additional tests, this discovery just so happened. Angiotensin II Component (API) such as valsartan during manufacture. It was first discovered in batches made by the Chinese company Zhejiang Huahai Pharmaceutical. In the weeks that followed, it was found in batches made by other producers, such as Zhejiang Tanya India. In the weeks that followed receptor antagonists like valsartan are prescribed to treat hypertonia, congestive heart failure, and myocardial infarction. It is incorporated with testing protocols in various pharmacopoeias. It is included in various pharmacopoeias along with procedures for assay, purity, and identity testing[12,14,34] Purity testing often concentrates on contaminants that are anticipated from synthesis and/or degradation.

Reaction :

N-nitrosamine was produced as a

result of the common NOx, $NaNO_2$, reacting with several sources of secondary and tertiary amines, as indicated below, according to the CHMP Evaluation Report of the sartans referral.[3,8] based on the two different paths of N-nitrosamine synthesis that were found, there are two primary reaction types that may be classified: 1. the secondary amines DMA and MBA are created by hydrolysing and/or heating the solvents DMF and NMP, respectively. N-nitration is the next step, which yields NDMA and NMBA.[13]

N-nitrosamine dealkylation produces NDEA, while N-nitrosative de-alkylation of the reagents TEA, DIPEA, and N, N-DMA (trialkyl amines) produces NDEA, DIPNA, EIPNA, and NMPA. The catalyst TEA HCl (quaternary ammonium salt) hydrolyses to yield the tertiary amine TEA. Both N-nitro compounds are thought to be recent instances of N-nitrosamines created after nitrosation during a manufacturing process, indicating the necessity of comprehensive nitrostatability testing of intermediates/impurities structure.[7, 10,32,37]

Sources of Nitrosamine impurities :

In essence, nitrosamine pollutants can get into drug ingredients and drug products by process production, degradation, cross-contamination, or direct introduction. Pharmacological substances are made using starting materials, intermediary compounds, chemical solvents, chemicals, and reagent this impurity may integrate and be transferred to the medicinal product if it develops or is already present during these processes.[40]

Precursors for the formation of nitrosamine impurities include primary, secondary, tertiary, and quaternary ammonium salts, as well as nitro sating agents like sodium nitrite. Nitrosamine impurities can also be formed by nitro sating carbonate, amides, and N-alkyl amides.[42] The type, structure, and concentration of the nitro sating agent are the primary determinants of the degree of nitrosamine impurity generation. It is believed that secondary amines are more reactive.

2. There is a chance that the processes recovered solvents and catalysts could lead to the creation of nitrosamines. Since residual azide in these solvents or catalysts might cause the production of nitrosamine impurities, they are treated with sodium nitrite or nitric acid to eliminate it.

3. A vendor's contaminated raw material or beginning material may introduce nitrosamine impurities into a drug substance or drug product.[4,27,36]

4. On the same production line, cross-contamination across different manufacturing processes and products can result in the contamination of nitrosamine impurities. Even in situations when nitro sating reagents are not utilized a process might become contaminated by the presence of nitrite in the water used in the manufacturing process.[26]

5. The breakdown of a solvent or other materials utilized in the manufacture of pharmacological substances may result in the formation of a trace amount of these contaminants. In a similar vein, contaminants known as nitrosamines may be introduced into pharmacological compounds as by-products of the drug production process. Potential NDM and NDEA contaminants can be formed by solvents like diethylacetamide (DEA), dimethylformamide (DMF), or dimethylacetamide (DMF).

Table-1. Fda's recommended limit

Impurities of Nitrosamine	FDA's approved feasible consumption (ng/day)
N-Nitrosodimethylamine (NDMA)	96
N-Nitrosodiethylamine (NDEA)	26.5
N-Nitrosoisopropylethylamine (NiPEA)	26.5
N-Nitrosodibutylamine (NDBA)*	26.5*
N-Nitrosodiisopropylamine (NDiPA)	26.5
N-Nitrosomethylphenylamine (NMPA)	26.5

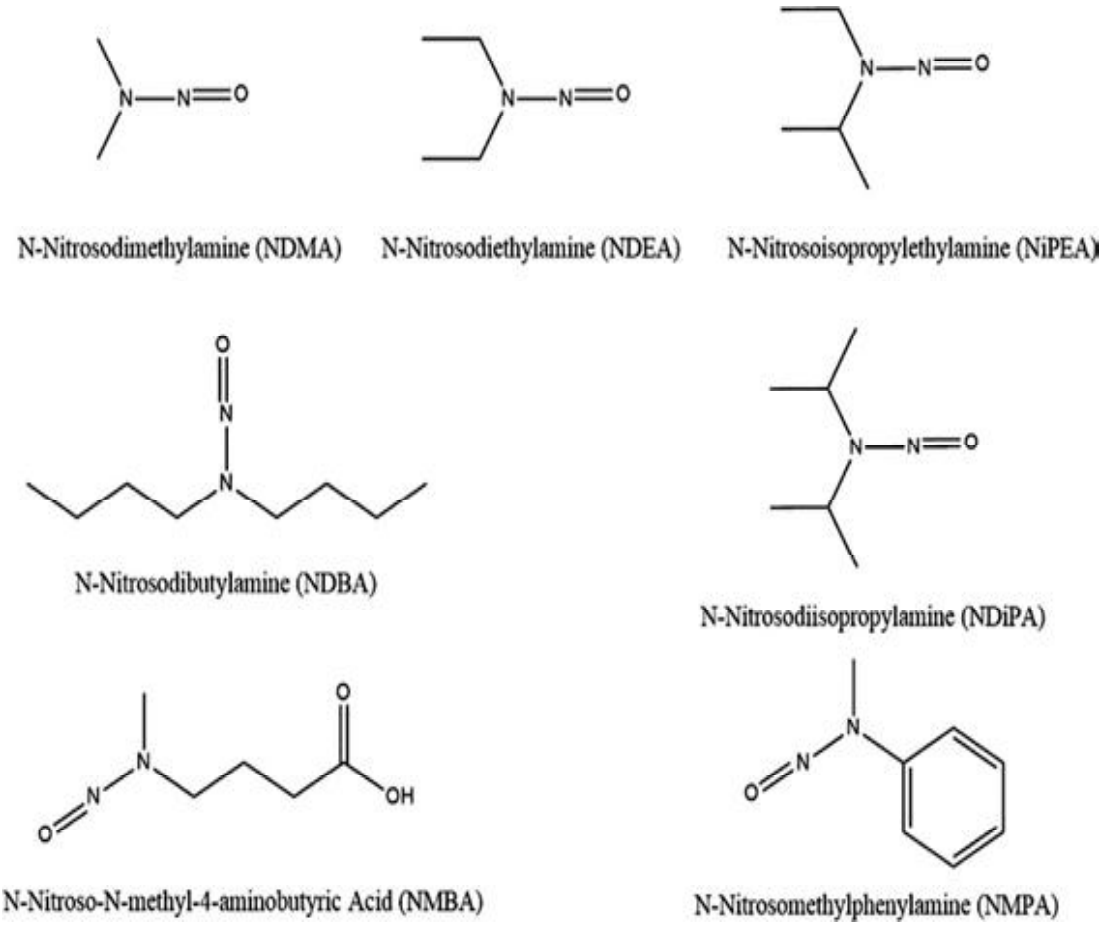

Fig. 2. Potential nitrosamine impurity structures in medication ingredients and drugs.

Methods of Analysis for Nitrosamine Impurity Detection :

HPLC (high-pressure liquid chromatography):

Liquid chromatography is one important separation method that significantly affects the pharmaceutical sector. According to earlier research published by the National Agency for Pharmaceuticals and Health Products Safety and the French Drugs Control Laboratories (OMCL), NDMA contaminants in Valsartan were successfully found using a UV detector set at 228 nm. Moreover, consistent nitrosamine detection by HPLC could be achieved with the use of post-column photolysis, which occurs and chemiluminescence detectors (LC-PR-CLD).[17] Li *et al*. first employed diode array detectors (DAD) in the HPLC to locate NDMA pollutants in the wavelength range of 230–233 nm.[33]

A technique for detecting N-nitrosodi-ethanolamine (NDELA) in cosmetic goods was developed by Ho young *et al*. Following their separation from cosmetics, the NDELA fractions were subjected to HPLC analysis in conjunction with a thermal energy analyser (TEA). TEA indicated that the LOD of NDELA was 2-3 ng, which is almost twice as high as the 20-30 ppb found in the cosmetic product. The NDELA detection was performed on the hair grooming gel and emulsion cream.[24] An HPLC approach for the quantitative measurement of NDMA contaminants in Valsartan was developed by Masada *et al*. The LOD and LOQ that were reported were 0.0085 µg/mL and 0.0285 µg/mL, respectively. The technique may achieve the necessary linearity, precision, and accuracy to analyse the NDMA impurity in Valsartan. The process quickly screened and and detected NDMA contamination in Valsartan.[24]

Current developments in HPLC technique development for process impurity monitoring in bulk pharmaceuticals .HPLC is currently used for the great majority of drug-related impurity determinations. It provided great automation with the required sensitivity for trace level measurements. The use of HPLC to all drug classes is made possible by the large range of stationary phases and operation modes. The majority of the time, standard UV detectors can be used to routinely meet the 0.1% or lower detection limits for drug-related contaminants using HPLC. HPLC was widely employed during the review period to assess and monitor the purity of chemical components used in the production of bulk medicines. Numerous methods have been proposed to determine the impurity profiles of synthesized medicines.[46]

There are various obstacles and factors to take into account when preparing a sample for HPLC analysis.

Matrix interference :

Nitrosamines are frequently extracted from liquid samples using SPE. The material is run through a solid-phase extraction cartridge in this procedure. Complicated sample matrices, like food extracts or biological fluids, may include substances that obstruct the HPLC analysis. Matrix interference may have an impact on the precision and accuracy of the findings. Several techniques for sample preparation are used to guarantee sensitive and accurate nitrosamine detection. The following are some standard methods for nitrosamine

assay sample preparation[20] where the analytes are retained, and impurities are washed away. Elution with a suitable solvent then recovers the nitrosamines for subsequent analysis.[16]

Liquid-liquid extraction (LLE) :

Nitrosamines must be divided into two immiscible liquid phases in LLE. The analytes are separated from the contaminants by mixing the sample with an appropriate organic solvent and nitrosamines. The nitrosamines are then recovered for further investigation by elution using an appropriate solvent.[9] Split up into the phase of organic matter. The organic phase is collected for further analysis upon separation.

Solid-phase Micro extraction (SPME) :

Using a coated fibre that adsorbs the analytes selectively, SPME extracts nitrosamines. After that, the fiber is desorbed for examination in the injection port of a liquid or gas chromatograph (GC or LC).[23]

Derivatization :

Derivatization can make GC or LC analysis easier. Frequently used derivatization in conjunction with LC- or GC-MS. In order to improve nitrosamines' chromatographic performance or detectability, derivatization entails changing their chemical structure. Derivatization can make GC or LC analysis easier. Pentafluoro benzyl bromide and dansyl chloride are common derivatization agents.[5]

Protein precipitation :

Protein precipitation is often used for biological samples. Proteins are precipitated through the inclusion of a precipitating agent (such as acetonitrile or alcohol) and then concentrating the mixture. The supernatant can then be analysed directly or subjected to further sample clean-up.[29]

The sample matrix, the kind of nitrosamines present, and the analytical method employed for detection all influence the sample preparation procedure selection. Every method has benefits and drawbacks, and in order to get the greatest outcomes for a certain application, optimization is frequently required.[29]

There are a number of difficulties with nitrosamine detection by HPLC, and resolving these difficulties is essential to producing accurate and trustworthy results. One of the biggest problems facing analytical scientists is the need for highly sensitive and specific analytical procedures with limit of quantifications (LOQs) in the ppb and sub-ppb range. Here are some of the current difficulties with nitrosamine detection utilizing HPLC.[36]

Artifactual nitrosamine synthesis during sample preparation and injection that results in an overestimation of nitrosamines has also drawn a lot of attention.

Low concentrations and sensitivity :

Challenge: Nitrosamines are frequently found in very small amounts, particularly in medications and some food items. It is difficult to attain the sensitivity needed for precise quantification, and improving detection limits is still a priority.[47]

Complex sample matrices :

Challenge: Food items, environmental

samples, and pharmaceutical formulations are examples of complex sample matrices that contain nitrosamines. The HPLC method's accuracy and selectivity may be impacted by interference from matrix elements.[29]

Structural diversity of Nitrosamines :

Challenge: The structural diversity of nitrosamines is evident, and their chemical characteristics and polarity might differ amongst them. It is difficult to create a universal technique that can efficiently separate and identify the full spectrum of nitrosamines.

Derivatization requirements :

Challenge : Certain nitrosamines might not have enough UV absorbance or fluorescence, so they would need to be derivatized in order to be more detectable. Steps in the derivatization process have the potential to increase technique complexity and unpredictability.[36]

Method Robustness :

Challenge : It is vital for routine analysis that HPLC methods achieve resilience. The reliability of nitrosamine detection can be affected by variations in parameters like temperature, mobile phase composition, and column performance.

Regulatory Scrutiny and Stringency :

Challenge **:** The pharmaceutical and other industries have stricter regulations for nitrosamine contaminants. The strain on analytical laboratories to meet changing regulatory standards and guidance for detection limits is increased.[18]

Emerging Nitrosamines :

Challenge: Analytical techniques must be continuously modified in order to account for the identification of novel nitrosamines or variants of recognized nitrosamines. A problem for technique development and validation is making sure that current methods can identify newly emergent nitrosamines.[3]

High-Throughput requirements :

Challenge : High-throughput techniques are needed in sectors such as pharmaceuticals in order to examine big volumes of samples. Finding a compromise between the need for quick throughput and sensitive, precise detection is challenging.[11]

Standardization and Harmonization :

Challenge : It can be difficult to harmonize with international regulatory requirements and standardize HPLC procedures amongst various laboratories. Method differences may be caused by variations in staff competence, reagents, and equipment.[28]

Evolving analytical technologies :

Challenge: It can be difficult to stay up to date with new developments in analytical technology and to choose the best detection methods. In order to preserve cutting-edge capabilities, laboratories might need to make an investment in current equipment.[28]

Below is a summary of the methods that are currently being used in non-European states and within the OMCL network. Further details are available on the EDQM website. For the relevant drug compound (DS) or drug product (DP), most labs use a direct extraction

Table-2. Summary of the Methods That Are Currently Being Used In Non-European States and Within the Omcl Network[11]

Analytical technique	GC-MS/MS (DI)	GC-MS (HS)	LC-MS/MS	HPLC-UV	High throughput Rapid-fire®-MS
Analytes(s)	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA	NDMA, NDEA
Sample amounts (DS and/ or DP)	250-500 mg DS or DP containing 250 mg of DS	50-500 mg DS or 50-250 mg DP; 'one tablet'	50-100 mg DS or DP containing 50- 100 mg of DS	62-320 mg DS	DS (unknown)
Workup procedure	DE with Me OH or DCM; LLE with NaOH and DCM	Direct H Sanalysis after dissolution in NMP or DMSO	DE with MeOH	DE with Me OH/, H_2O (35:65 V/V)	DE with MeOH
DS	valsartan irbesartan losartan candesartan olmesartan	valsartan irbesartan losartan candesartan olmesartan	valsartan irbesartan losartan candesartan olmesartan	valsartan irbesartan losartan candesartan olmesartan	Losartan
NDMA – LOD	0.002-0.01 ppm (DS)	0.005-0.04 ppm (DS)	0.010-0.15 ppm (DS)	0.02-0.10 ppm	10 ppm
NDMA – LOQ	0.005-0.05 ppm (DS)	0.1 ppm (DS)	0.08-0.5 ppm (DS)	0.04-0.25 ppm	25 ppm
NDEA – LOD	0.002-0.01 ppm (DS)	0.02 ppm (DS)	0.006-0.02 ppm (DS)	0.04-0.10 ppm	25 ppm
NDEA – LOQ	0.002-0.01 ppm (DS)	0.05-0.08 ppm (DS)	0.02-0.15 ppm (DS)	0.08 – 0.50 ppm	25 ppm

procedure, which is followed by a dilution and filtration step. After extraction, the supernatants are transferred to equipment for direct injection (DI) measurements, such as GC- or LC-MS (often LC-UV). Another widely used method uses GC headspace (HS)-MS to dissolve the sample directly in dimethyl sulfoxide (DMSO) or N-methylpyrrolidine (NMP). These rapid workup methods were chosen because NDMA and NDEA are volatile compounds, which could result in losses.

The Nitrosation assay method as a strategy for Risk analysis:

The biggest obstacle to risk assessment is learning enough about the chemistry of the by-products to be an adequate advisor. Regulatory agencies, industry experts, and researchers all disagree with well-established impurity norms like ICH Q3A (R2) and ICH Q3B (R2). It's important to be aware about unique chemical reactions that result in trace

amounts of unknown contaminants at ppm and especially ppb levels because these reactions are typically hazy and difficult to understand. As such, it is unlikely that risk assessments will cover all potential danger. The quality control of hazardous materials is very crucial.[28]

The scientific community is working to find solutions to these problems through method development, optimization, and collaboration. Staying up to date on the newest developments and taking part in collaborative activities are crucial to solving the problems with nitrosamine detection by HPLC.

Insights into potential future developments or improvements in HPLC methods for Nitrosamine analysis :

Further advancements and improvements in HPLC methods for nitrosamine measurement are expected as analytical techniques and technology progress. These could provide some useful information about upcoming trends and areas for development.

Improved Sensitivity and Selectivity :

Upcoming advancements might concentrate on improving HPLC techniques' sensitivity and selectivity for nitrosamine detection even more. This could entail developments in stationary phase design, detector sensitivity, and column technology.

Detector integration :

It might become more common to integrate increasingly sophisticated detectors, like new fluorescence detectors or high-resolution mass spectrometry (HRMS). These detectors can provide improved accuracy in identifying and quantifying nitrosamines, as well as greater selectivity and specificity.

High-throughput screening and automation:

Further developments in automation may result in HPLC techniques that are more efficient and high-throughput. More advanced automated procedures for data processing, injection, and sample preparation may be developed in the future, increasing productivity and lowering the need for human intervention.

Subsequent investigations could examine novel derivatization methods that improve nitrosamine detectability without complicating the process. This could increase sensitivity while streamlining sample preparation.

Multi-mode Chromatography:

The advancement of multi-mode chromatography systems, which combine several separation modes onto a single platform, may provide increased adaptability for the analysis of nitrosamine. In complicated matrices, this might improve the separation of structurally different nitrosamines.

Chemo metrics and data processing :

Using sophisticated data processing methods, such as chemo metrics and machine learning, more often could make handling large, complicated data sets easier. This could help identify patterns, find outliers, and increase the general dependability of the outcomes.

Global standardization :

Attempts are being made to standardize nitrosamine detection techniques globally. Analytical method harmonization may make nitrosamine testing and reporting more consistent.

Better calibration procedures :

In the future, efforts may be directed toward improving calibration procedures, particularly when complicated matrices are involved the use of matrix-matched standards and isotopically marked internal norms may increase measurement accuracy.

Green analytical chemistry:

The development of HPLC techniques for nitrosamine analysis may be impacted by the adoption of green analytical chemistry concepts, such as utilizing environmentally friendly chemicals and minimizing solvent use.[1,44,47]

Nitrosamines are environmentally hazardous and genotoxic pollutants that are extremely hazardous. Regulatory organizations including the US-FDA, EMA, and CDSCO are attempting to quantify the presence of nitrosamine contaminants in different products. These contaminants' high hydrophilicity and low molecular weight provide a problem. For the accurate identification and measurement of nitrosamines in pharmaceutical APIs, new analytical instruments and procedures are being developed. Regulating bodies have set permissible limitations, therefore in order to guarantee the safety of items, scientists and industrialists must keep coming up with new ideas in this field.

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