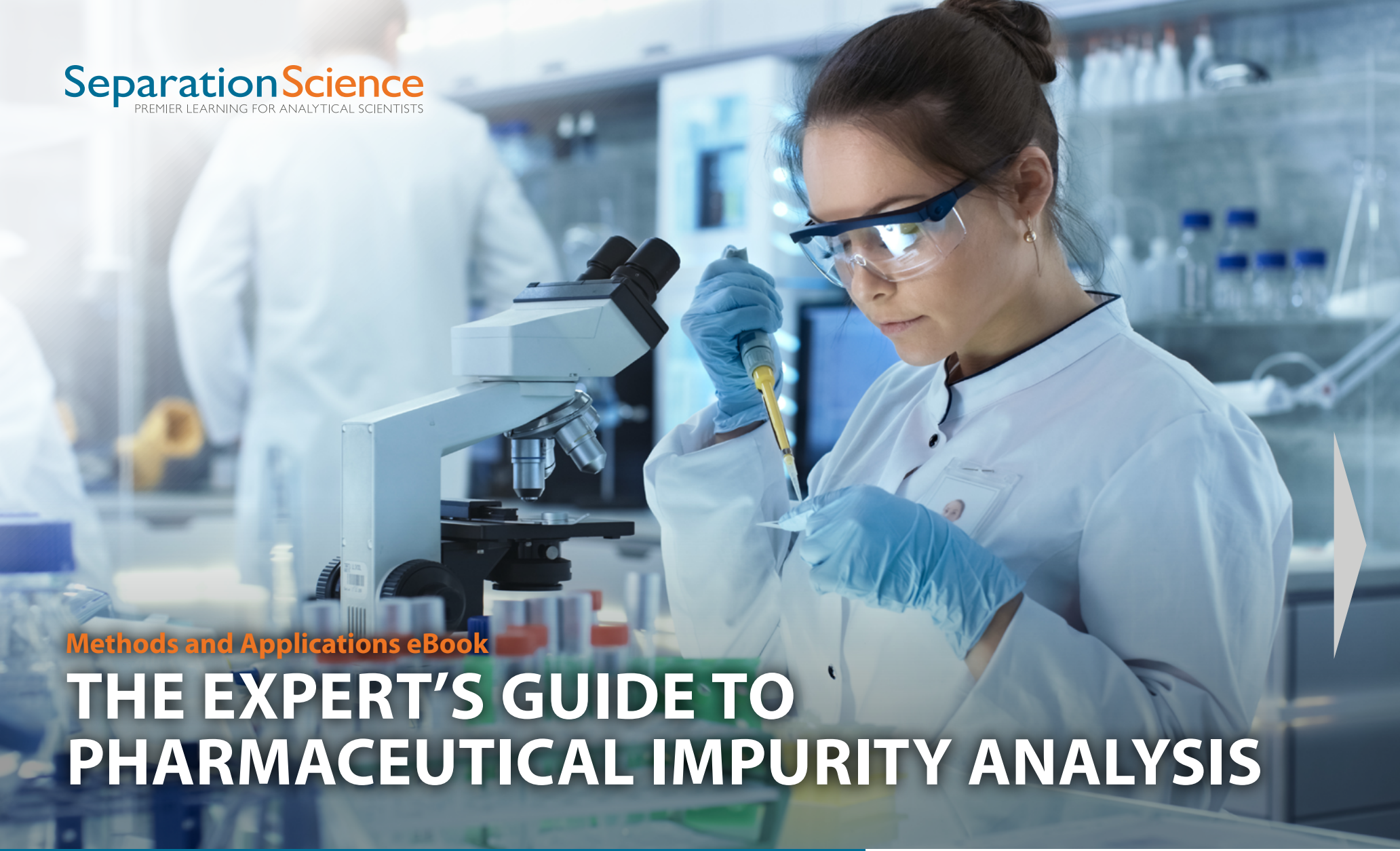


SeparationScience
PREMIER LEARNING FOR ANALYTICAL SCIENTISTS

Methods and Applications eBook

# THE EXPERT'S GUIDE TO PHARMACEUTICAL IMPURITY ANALYSIS

**Learn about:**

- Proven Workflows for Regulatory Compliance
- Enhance Drug Quality and Safety
- Simplify Analysis with Cutting-Edge Technology

In collaboration with: Agilent

# Introduction

## Proven analytical workflows to address regulatory requirements

In recent years, pharmaceutical impurities have become a growing concern and have inspired increasingly stringent regulations and controls. Drug recalls, such as an odor-related Lipitor recall in 2010 and a recall of Valsartan in 2018 due to suspected carcinogenic nitrosamine-type impurities, spurred global action. Over the past decade, regulators and standard organizations have continued to tighten regulations governing pharmaceutical impurities, emphasizing four key areas: extractables and leachables, mutagenic impurities, residual solvents, and elemental impurities.

Risk-based controls and rigorous analytical detection ensure drug quality, efficacy, and patient safety. However, tightening limits of detection and quantitation and the necessity for accuracy even with unknown or trace impurity levels can pose challenges for analytical labs, raising questions about sample preparation, method development, and compliance. Even seasoned analysts may struggle to find reliable information.

**This eBook provides:**

- Easy access to the latest regulations
- Proven end-to-end workflows, including sample preparation, analysis, detection, and reporting
- New developments in technology platforms that can simplify analysis and exceed current testing requirements

Each chapter also contains useful links to the Agilent product portfolio, application notes, and on-demand webinars. We trust you'll find this eBook engaging and valuable.

# Table of Contents

# Chapter 1: Extractable and Leachable (E&L) Impurities

## Understanding extractables and leachables

The analysis of extractables (chemicals that migrate out of test articles into model solvents under specific conditions) and leachables (a subset of extractables that end up in the final drug product) poses significant challenges. The range of packaging materials used in the pharmaceutical industry is extensive, including plastics, elastomers, glass, metals, and coatings, each with its own unique compositions and potential impurities. Polymers, in particular, can contain numerous chemical compounds, such as monomers, additives, degradation products, catalyst residues, and impurities from manufacturing processes. Moreover, the migration of compounds from these materials can be influenced by factors such as temperature, pH, storage conditions, and contact with different solvents or drug products.

E&L impurities can exhibit a diverse set of characteristics such as polarity, solubility, molecular weight, and concentration. These differences make it impossible to develop a universal, "one-size-fits-all" analytical method for comprehensive E&L analysis. In general, analytical tool selection in E&L is driven by impurity volatility, as demonstrated in Table 1.1. Method selection needs to ensure compatibility with safety thresholds for impurity screening.

| ANALYTE TYPE | ANALYTICAL TECHNOLOGY |
|---|---|
| Volatile Organic Compounds (VOCs) | Headspace gas chromatography coupled with mass spectrometry (HS-GC/MS) |
| Semi-VOCs | GC/MS |
| Non-VOCs | Ultra-high performance (UHPLC) and high-performance liquid chromatography coupled with ultraviolet and mass spectrometry detection (HPLC/UV/MS) |
| Elemental | Inductively coupled plasma coupled with mass spectrometry (ICP-MS) or optical emission spectrometry (ICP-OES) |

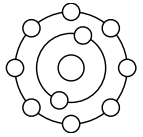
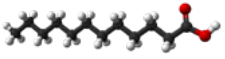
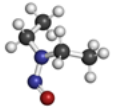
Table 1.1. Common analytical tools used for E&L analysis

### Perfecting Extractable and Leachable Testing

Learn how Nelson labs built confidence in their E&L analysis by choosing the right instruments to deliver sensitive and reliable results. Watch Video ▸

# Structures and sources of common E&L impurities

E&L impurities can arise from various sources, including manufacturing processes, packaging materials, and container closure systems. Table 1.2 lists the most prevalent types of impurities based on their chemical nature and potential sources.

| IMPURITY CLASS | SOURCE | EXTRACTABLES OR LEACHABLES | IMPURITY CLASS |
|---|---|---|---|
| Antioxidants  | Protective agents, formulation additives, raw materials, cleaning agents | Both | Non-VOC |
| Azo dyes  | Colorants in packaging, printing inks and labels, raw materials, pollution | Extractables | Non-VOC |
| Inorganics  | Foil seals and packaging, manufacturing equipment, water and process media, cleaning agents | Both | Inorganic / Elemental impurity |
| Lubricants, slip agents, fatty acids/esters  | Processing aids, packaging coatings, raw materials, cleaning agents | Both | Non-VOC |
| Nitrosamines  | Raw materials, packaging materials, process equipment, drug interactions | Both, but mostly leachables | VOC |

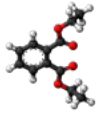
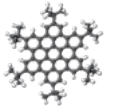
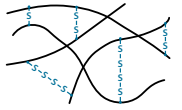
| IMPURITY CLASS | SOURCE | EXTRACTABLES OR LEACHABLES | IMPURITY CLASS |
|---|---|---|---|
| Phthalates  | Plasticizer additive | Both | Semi-VOC |
| Polyaromatic hydrocarbons (PAHs)  | Plastic impurities, printing inks and labels, contaminated equipment, environmental contamination | Extractables | VOC and semi-VOC |
| Polymer components  | Unreacted impurities, plasticizing agents, additives, environmental contamination | Both | Non-VOC |
| Silicone oils  | Machine lubricants, elastomer gaskets, container closures | Extractables | VOC |
| Vulcanizing agents  | Rubber materials, such as stoppers, seals, gaskets, and tubing | Extractables | Inorganic / Elemental impurity |

Table 1.2. Typical E&L impurities and origins

# Current E&L regulatory guidance

### U.S. Food and Drug Administration (FDA)

- Container closure systems for packaging human drugs and biologics

### European Medical Agency (EMA)

- Guideline on plastic primary packaging materials

### Product Quality Research Institute (PQRI)

- Safety thresholds and best practices for E&L in oral, inhaled, and nasal drug products
- Safety thresholds and best practices for E&L in parenteral drug products

### BioPhorum Operations Group (BPOG)

- Extractables testing of polymeric single-use components

### American Society of Mechanical Engineers (ASME)

- BPE-2016: E&L testing information for bioprocessing

### International Council for Harmonisation (ICH)

- ICH Q6A: Criteria for new drug substances
- ICH M7: Mutagenic impurities in pharmaceuticals
- ICH Q3D: Guidelines for elemental impurities

### U.S. Pharmacopeia (USP)

- Plastic biocompatibility: <661.1> , <661.2>., <665>
- 'How-to' E&L guides: <1661>, <1663> , <1664>, <1665>
- Elastomers: <381>, <382>, <1381>, <1382>
- Elemental impurities: <232>, <233>
- Biological activity: <87>, <88>

### International Organization for Standardization (ISO)

- ISO 10993-18: Characterization of medical devices within a risk management process

### American Society for Testing and Materials (ASTM)

- F1980-07: Accelerated aging of sterile barrier systems
- F619-20: Extraction of materials used in medical devices
- E3051-16: Single-use systems in biopharmaceuticals

# E&L: an evolving landscape

- The effects of new regulations are influencing E&L assessments. In October 2021, the Product Quality Research Institute (PQRI) submitted recommendations for E&Ls in parenteral drug products (PDP) to the FDA. A key part of these requirements includes the definition of basic safety and analytical thresholds.
- Improved analytical methods and accuracy ensure compatibility with testing thresholds that may evolve over time. Knowledge management tools and sample automation play increasingly important roles in E&L analysis and evaluation.
- The rising use of single-use plastics in biopharmaceutical manufacturing prompted the USP to introduce chapter <665>, scheduled to be implemented in 2026. This new chapter outlines how to test for extractables in bio-centric manufacturing environments and monitor for process equipment-related leachables (PERLs) that may escape initial screening trials. Biologics present several unique characteristics and challenges with regard to E&Ls.
- Effective project management for E&L studies requires depth of expertise; Agilent E&L applications experts can help with E&L method development and assessment. Partner with Agilent CrossLab to overcome application problems and reduce the time it takes to deploy the latest productivity and usability enhancements.

The Agilent 5977C GC/MSD is a reliable workhorse for E&L analysis of pharmaceutical impurities and features innovations such as the HydroInert source to minimize disruptions from helium shortages.

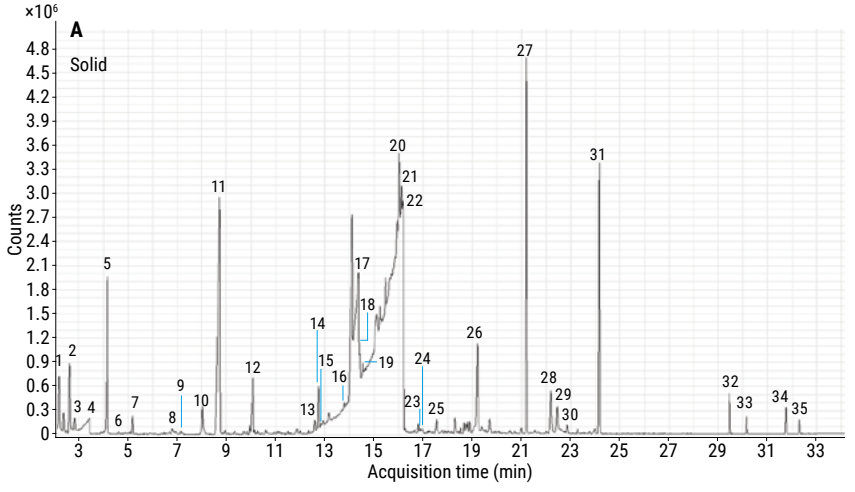
The Agilent 7250 GC/Q-TOF system delivers full-spectrum, high-resolution, accurate mass data with a wide dynamic range for identifying and quantifying GC-amenable compounds.

# Analysis of E&L compounds from generic liquid drug formulations

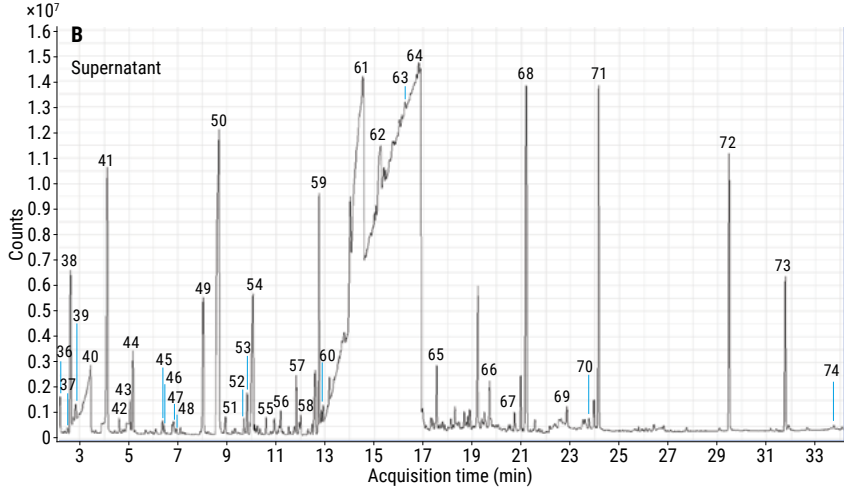
Liquid drug products have a propensity to extract substances from packaging materials, such as plastic bags, owing to their intimate contact. This application note details a controlled extraction study to determine the concentrations of additives migrating from high-density polyethylene or polypropylene packaging.

Two types of analyses were performed to identify leachable compounds in aqueous drug formulation. Components in the drug suspension were analyzed at high temperatures using an Agilent 7697A Headspace Sampler and a 7890A GC coupled with a 5977A MSD (Headspace GC/MS). Solvent extracts of drug components were analyzed using the 7693A Automatic Liquid Sampler and a 7890A GC coupled with a 5977A MSD (ALS GC/MS). The ALS GC/MS is equipped with a multimode inlet (MMI) operated in solvent vent mode. The liquid drug formulation used in this work was acquired from a generic pharmaceutical company for extractables and leachables testing.

READ THE FULL APPLICATION NOTE




Plasticizers, flavors, fragrances, pharmaceutical compounds, and their precursors were identified in the liquid drug formulation using GC/MS analysis by headspace and large-volume liquid injection.



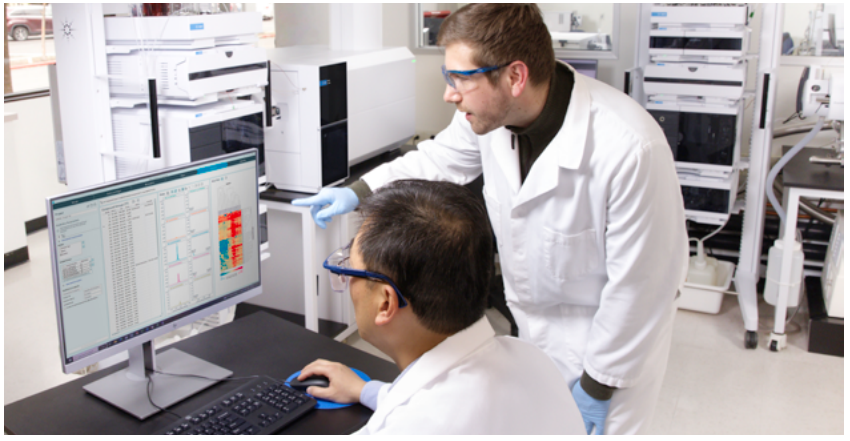
# What's next in E&L analysis?

- Advancements in data and modeling may simplify testing of well-known materials. Generation of collaborative databases with better quality information can improve modeling outcomes early in pharmaceutical development.
- Expansion of scope includes possible new E&L regulations encompassing novel therapeutics such as messenger RNA (mRNA) and cell and gene therapies.
- Standardization of extractables protocols is expected to become more prevalent, streamlining testing procedures across the industry.
- Understanding the true risk of leachables to patients or product quality remains a prime concern. Improved toxicology analysis and harmonization of safety thresholds remain subjects of consideration.

# Further information

- Find webinars, videos, application notes, and other resources on our webpage: Agilent Solutions for Extractables and Leachables Analysis
- Find additional resources on our webpage: Agilent Compliance Support and Services

The Agilent Revident LC/Q-TOF excels in routine screening, high resolution, accurate mass quantitation, identification of unknowns, and high-throughput testing of small molecules.



Agilent ChemVista software with LC/Q-TOF spectral libraries and databases integrates compound, retention time, and mass spectra from multiple sources into one location, streamlining unknowns identification workflows in MassHunter data analysis. It includes extensive, curated LC/Q-TOF libraries and databases for extractables and leachables.

# Chapter 2: Mutagenic Impurities

## Understanding mutagenic impurities

Mutagenic impurities in APIs and drug products have the potential to lead to DNA mutations and cause cancer. They pose a significant risk to patient health and safety—even in trace quantities—and are a major concern for drug makers.

Nitrosamines are a class of mutagenic impurities that is of regulatory concern. Nitrosamines can form from several processes (Figure 2.1). Table 2.1 lists several nitrosamines that have been found in recently recalled drugs.

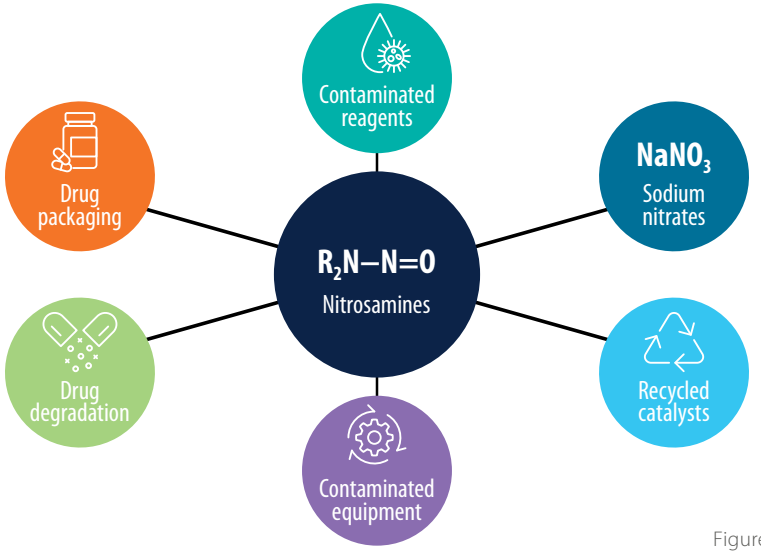



Figure 2.1

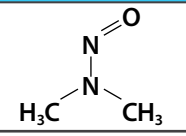
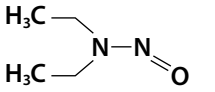
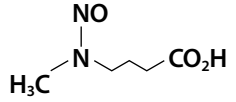
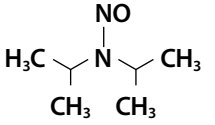
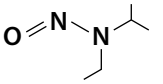
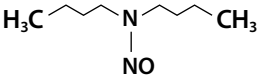
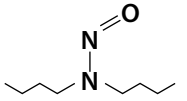
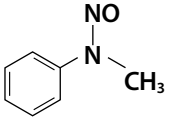
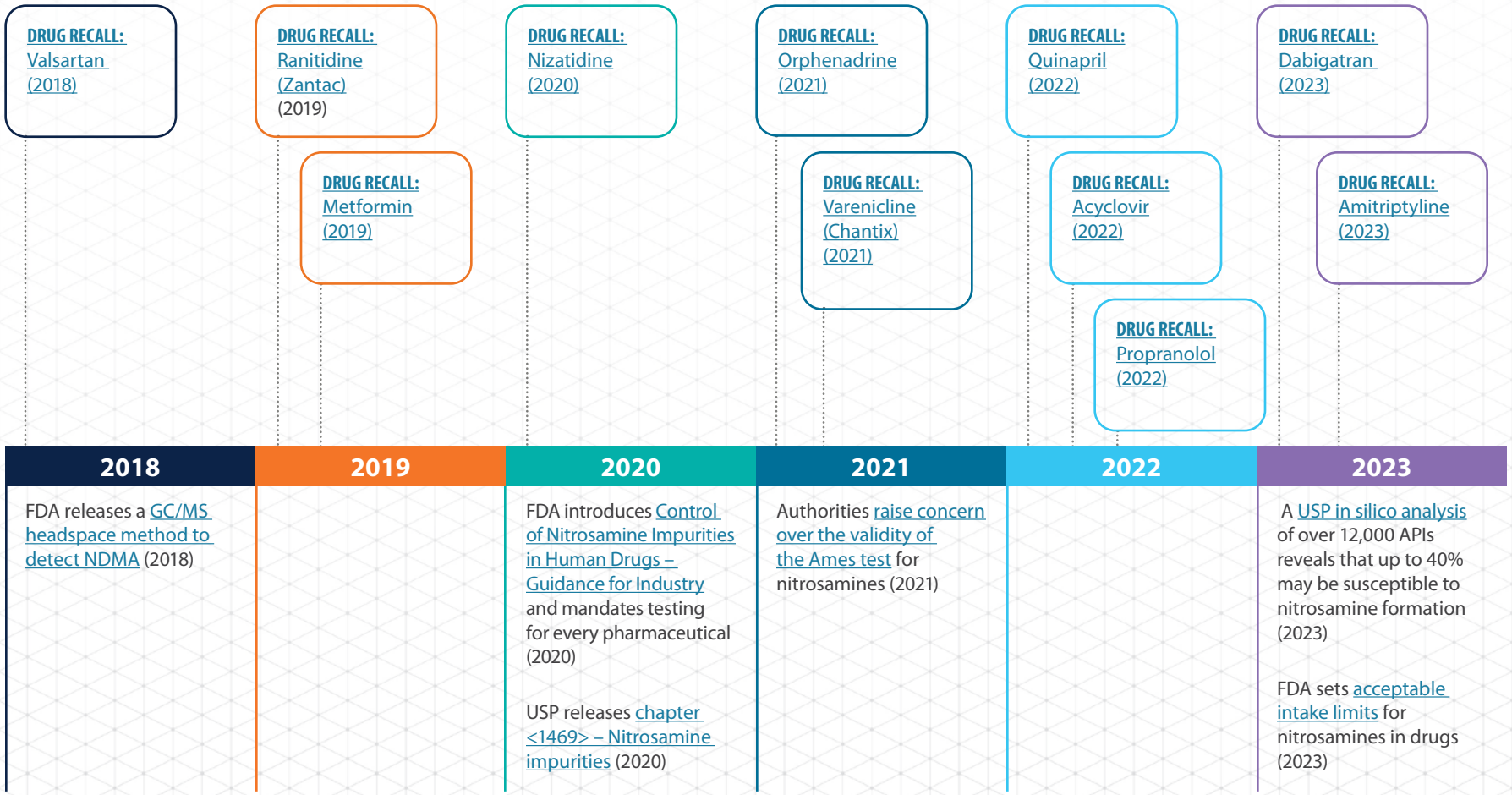
| ABBREVIATION | CHEMICAL NAME | CHEMICAL STRUCTURE |
|---|---|---|
| NDMA | N-nitrosodimethylamine |  |
| NDEA | N-Nitrosodiethylamine |  |
| NMBA | N-nitroso-N-methyl-4-aminobutyric acid |  |
| NDIPA | N-nitroso diisopropylamine |  |
| NEIPA | N-nitroso ethylisopropylamine |  |
| NDBA | N-nitro sodibutylamine |  |
| NDPA | N-nitro sodi-n-propylamine |  |
| NMPA | N-nitroso methylphenylamine |  |

Table 2.1. Chemical structures of common nitrosamine contaminants

# Ascending threats: A timeline of nitrosamine regulatory actions

The pharmaceutical industry has undertaken a major safety audit to identify nitrosamine impurities. While initially associated with specific medicines, the hunt for nitrosamines has expanded to various drug classes, posing a risk to historic and new medicines.

The high number of nitrosamine-based recalls underscores the effectiveness of analytical tools in detecting and eliminating hidden sources of contamination.



# Mutagenic impurity analysis, an evolving landscape

- Increased regulatory harmonization is becoming possible, as the FDA released guidance on acceptable intake limits for nitrosamine impurities in drug substances in August 2023, aligning with the ICH's adoption of M7(R2) guidelines in April. This framework helps assess mutagenic and carcinogenic risks in drug products. The FDA provides specific information on its website, including suggested acceptable intake limits, testing methods, and safety assessments.
- The main challenge in measuring mutagenic impurities lies in the requirement for exceptionally low detection limits. Newly released guidelines demand highly sensitive analytical techniques capable of detecting and measuring these impurities at very low levels, often in parts per million (ppm) to parts per billion (ppb) ranges.
- Advanced technologies, such as triple quadrupole mass spectrometry systems, can be critical tools for reaching ultra-low detection limits. These instruments provide:
    - Lower baselines for better signal-to-noise (S/N) ratios, and hence better method LOQs
    - Lower LOQs that promise a forward thinking system (safety limits may be further reduced)
- Multiple reaction monitoring (MRM) can reduce interferences, increase method selectivity and specificity, and help confirm the presence/absence of impurities.

# Proven GC/TQ method to detect NDMA and other nitrosamines

The Agilent 8890 GC system equipped with an Agilent 7693A automatic liquid sampler coupled to an Agilent 7010 series triple quadrupole GC/MS is a recommended solution for analyzing NDMA and mutagenic impurities. The system demonstrates excellent performance for determination of nine nitrosamine drug impurities in sartan drug products and substances, with reliable quantification of all nine impurities up to 3 ppb.

DOWNLOAD THE NITROSAMINES ANALYSIS BY GC/TQ CONSUMABLES ORDERING GUIDE

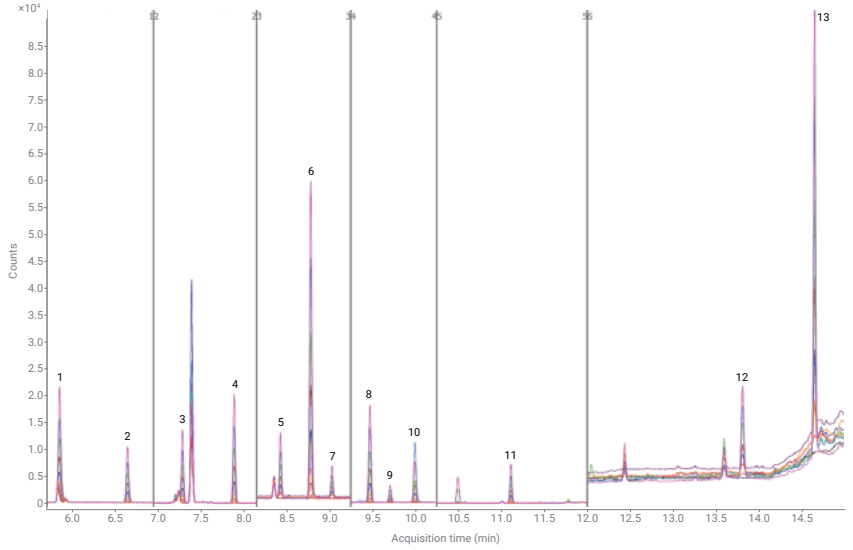
# Screening of nitrosamine impurities in drug products and drug substances using Agilent GC/MS/MS instrumentation

Regulatory bodies such as the FDA and EMA require marketing authorization holders to conduct risk assessments and confirmatory testing using validated methods. While GC/MS/MS methods initially focused on N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), the screening now extends to other nitrosamines based on risk assessment.

This application note describes a comprehensive solution for the screening and estimation of 13 nitrosamine impurities (NDMA, NDEA, NMOR, NMEA, NPYR, NPIP, NEIPA, NDIPA, NDPA, NDBA, NMPA, NMPEA and NDPh) in drug products and drug substances in both organic or aqueous matrices at trace levels using an Agilent 8890 GC coupled to an Agilent 7010 series triple quadrupole GC/MS/MS system.

In case of a positive result, a specific quantitative method is developed and validated for confirmatory testing. This study demonstrates the effectiveness of the 8890 GC coupled to the 7010 series GC/MS/MS system, offering excellent performance and enhanced ionization efficiency for confident trace analysis.

READ THE FULL APPLICATION NOTE



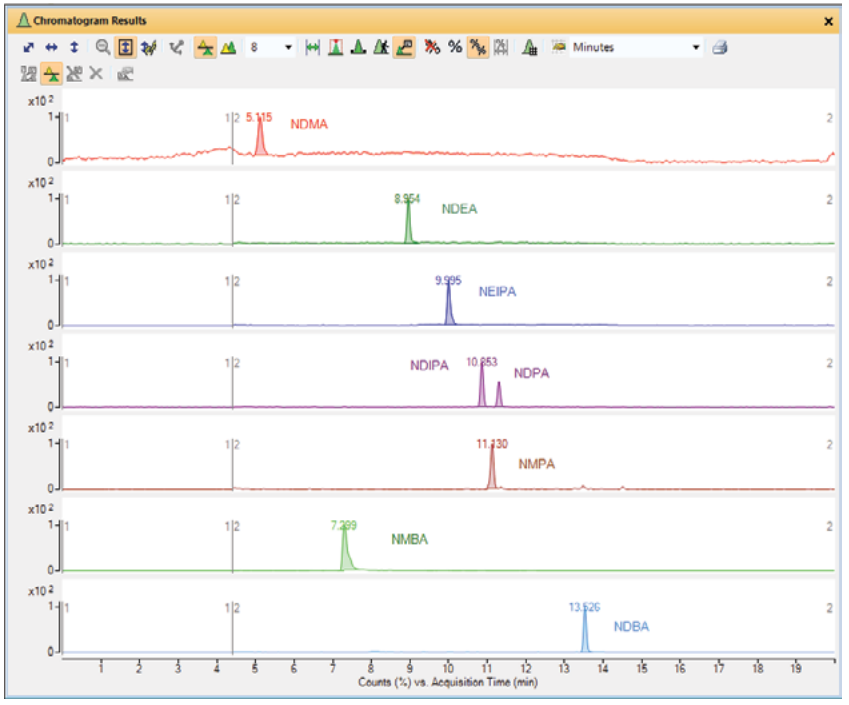

A Multiple Reaction Monitoring / Total Ion Chromatogram overlay showing the retention times of 13 nitrosamine impurities.

# Simultaneous determination of eight nitrosamine impurities in metformin

Recent recalls of metformin by regulatory authorities such as the FDA, EDQM, and Health Sciences Authority due to the presence of NDMA have spotlighted the need for comprehensive testing across various APIs and drug products, extending beyond angiotensin II receptor blocker (ARB) drugs. However, the challenge lies in achieving the necessary sensitivity levels for robust analytical methods.

This application note presents a highly sensitive LC/MS/MS method, built upon the Agilent 6470 triple quadrupole technology, designed for the simultaneous determination of eight nitrosamine impurities in metformin drug substance: NDMA, NDEA, NMBA, NEIPA, NDIPA, NMPA, NDPA, and NDBA.

The Agilent MassHunter acquisition software was used to analyze and acquire data, while the MassHunter acquisition optimizer software determined MRM parameters including precursor and product ions, fragmentor voltages, and collision energies.




A representative Multiple Reaction Monitoring chromatogram of the nitrosamine impurities (0.6 ng/mL) in a standard solution.

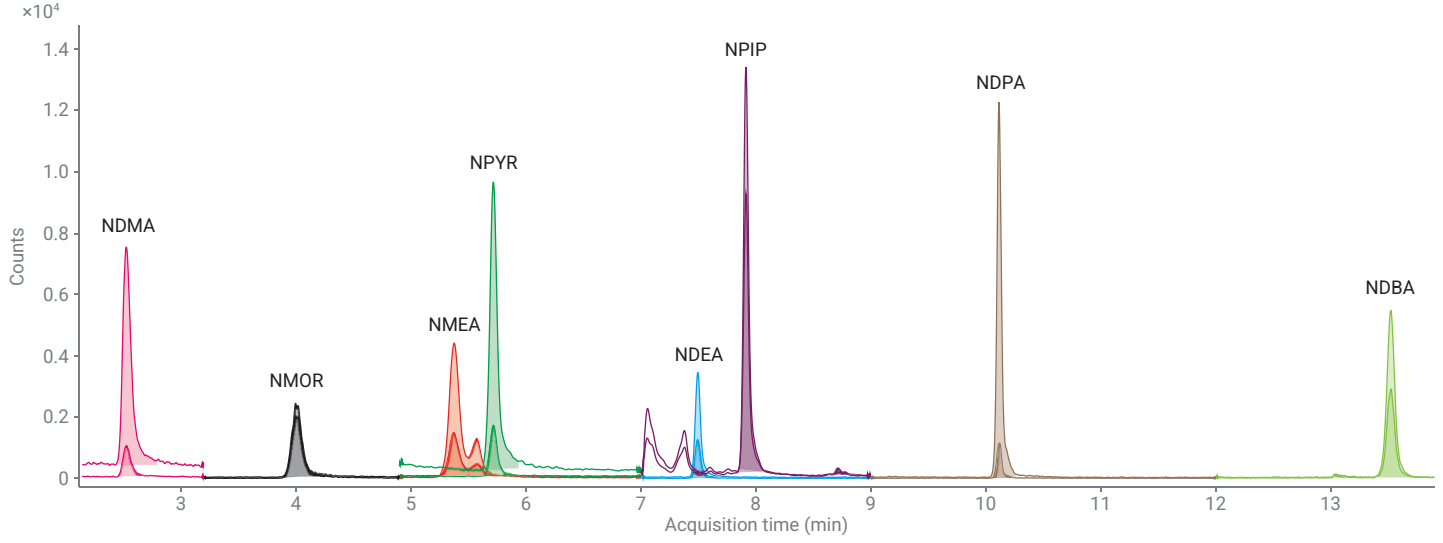
**DOWNLOAD THE NITROSAMINES ANALYSIS USING LC/MS CONSUMABLES WORKFLOW ORDERING GUIDE**

**READ THE FULL APPLICATION NOTE**

# Determination of nitrosamine impurities using the Agilent 6475 triple quadrupole LC/MS system

The Agilent 6475 triple quadrupole LC/MS system can confidently quantify the nitrosamine impurities NDMA, NMOR, NMEA, NPYR, NDEA, NPIP, NDPA, and NDBA at the low concentration levels specified by regulatory requirements. This method can be used to quantify these impurities in different ARB drug products, with some changes in chromatographic conditions based on the elution pattern of the drug product.

Key findings from the application note include the achievement of excellent chromatographic separation and peak shapes for all analytes, as illustrated in the figure. The study also demonstrated outstanding precision and accuracy across all tested levels.




Multiple Reaction Monitoring chromatogram of eight nitrosamine impurities (1 ng/mL) in a standard solution.

READ THE FULL APPLICATION NOTE

# What's next in mutagenic impurity analysis?

- Regulatory guidance and acceptable intake limits may become even more specific, with new FDA guidance requiring pinpointing certain nitrosamine impurities by examining data or comparing them to similar compounds as a reference.
- Predictive methodologies for carcinogenic risk are set to become increasingly important. The latest FDA guidance aligns with ICH M7(R2) principles when assigning risk levels to different API categories. Impurity profiling can expedite the analysis process.
- Traditional carcinogenicity assessments, such as rodent bioassays, may give way to computational tools that predict the presence of nitrosamines. While in-silico tools for mutagenicity prediction of pharmaceutical impurities are accepted by regulatory agencies, similar approaches for nitrosamines as contaminants are not yet widely reported.

# Further information

- Find webinars, videos, application notes, and other resources on our webpage: Agilent Solutions for Mutagenic Impurities Analysis
- Find additional resources on our webpage: Agilent Compliance Support and Services

The Agilent 1290 Infinity II LC, coupled with the Agilent 6475 LC/TQ makes a powerful analytical system providing high performance, sensitivity, and increased sample throughput day in and day out.

The Agilent 6495 LC/TQ is an ultrahigh-performance system built for testing large batches of samples. Equipped with the innovative iFunnel technology, this LC/MS/MS achieves trace detection limits for the most challenging samples.

# Chapter 3: Residual Solvents

## Understanding residual solvent characterization

Residual solvents are unwanted chemicals that persist in APIs or drug product formulations. Residual solvents can arise from various sources:

- Synthesis or reaction byproduct
- Inherent instability exhibited by some drug substances
- Excipients and water used in the manufacturing process
- Interactions with manufacturing equipment and packaging materials, including container closure systems (CCSs).

The primary guiding regulations for residual solvents include USP <467>, which aligns closely with ICH Q3C(R8) guidelines. The document classifies residual solvents into three categories:

- Class 1: Solvents to be avoided, including known human carcinogens, strongly suspected human carcinogens, and solvents posing environmental hazards.
- Class 2: Solvents to be limited, comprising nongenotoxic animal carcinogens or agents causing other irreversible toxicities such as neurotoxicity or teratogenicity. This class also includes solvents suspected of causing significant but reversible toxicities.
- Class 3: Solvents with low toxic potential, having no health-based exposure limit.

The Agilent 8697 Headspace Sampler with the 8890 GC provides fast, accurate, and reproducible analysis of Class 1 and Class 2A/B solvent content in pharmaceutical products.

# Residual solvent testing methodology

USP <467> provides a comprehensive framework for testing methodologies and decision-making regarding residual solvents. When this chapter was first introduced, some labs faced challenges demonstrating enough sensitivity to meet the signal-to-noise requirements for low-level Class 1 reference standards. It soon became apparent that part of the problem arose from the instructions for multi-level aqueous dilution of the Class 1 standards.

Now, manufacturers have two design options when it comes to compliance with residual solvent analysis. They can either perform direct analysis of the final drug product or a cumulative analysis of solvents throughout the manufacturing chain. Testing the official product is acceptable in all cases. When no solvents are used during the manufacturing of the official product, a cumulative approach is employed. This approach calculates the solvent levels in the API and the excipients or dietary ingredients to determine the overall solvent content in the official product.

If solvents are used during the manufacturing of the official product, the cumulative approach from step one can still be applied. For each solvent used, determine the content in the finished product or after the manufacturing step.

While methodologies are specific to water-soluble and water-insoluble drug products, testing decision trees follow three procedures:

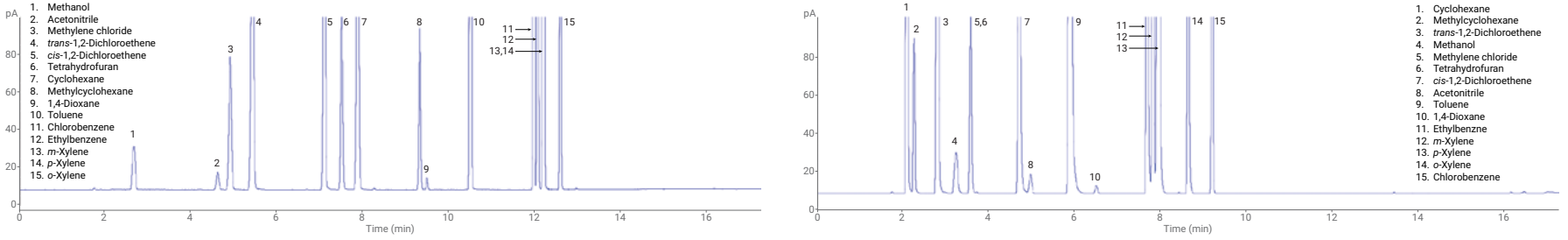
- Procedure A for identification and limit test
- Procedure B for confirmatory test
- Procedure C for quantitative test


The Agilent Intuvo 9000 GC system, when configured as a dual-column, dual FID setup with the Agilent 8697 headspace sampler, reduces analysis time and simplifies the process of detecting residual solvents. The Intuvo Residual Solvent Analyzer is pre-configured and factory tested to provide fast, accurate, and reproducible residual solvent analysis of Class 1 and Class 2A/B solvents in pharmaceutical products.

# Improve throughput of residual solvent testing

USP <467> sets the standard for analyzing residual solvents in pharmaceuticals, addressing the critical role of solvent choice during manufacturing. USP <467> prescribes a dual-column analysis with confirmation, traditionally requiring two separate runs on conventional gas chromatographic systems. However, the Agilent Intuvo 9000 GC system, equipped with an inlet splitter for dual flame ionization detection (FID), revolutionizes the process, enabling both analyses in a single run, reducing time and boosting efficiency.

In chromatographic analysis of Class 2A solvents, this method provides exceptional peak symmetry. Elution order variations arise between the Agilent DB-624 Select UI and Agilent DB-WAX UI columns due to distinct stationary phases. While the DB-624 Select UI column excels in resolving critical analytes like carbon tetrachloride within Class 1 solvents, the DB-WAX UI column offers superior resolution of xylene isomers within Class 2A. Running both columns concurrently proves advantageous, ensuring optimal chromatography for both Class 1 and Class 2A solvents. This streamlined approach allows for enhanced performance while simplifying the analysis process.




Analysis of Class 2A solvents on an Agilent DB-624 Select UI column (left image) and an Agilent DB-WAX UI column (right image)

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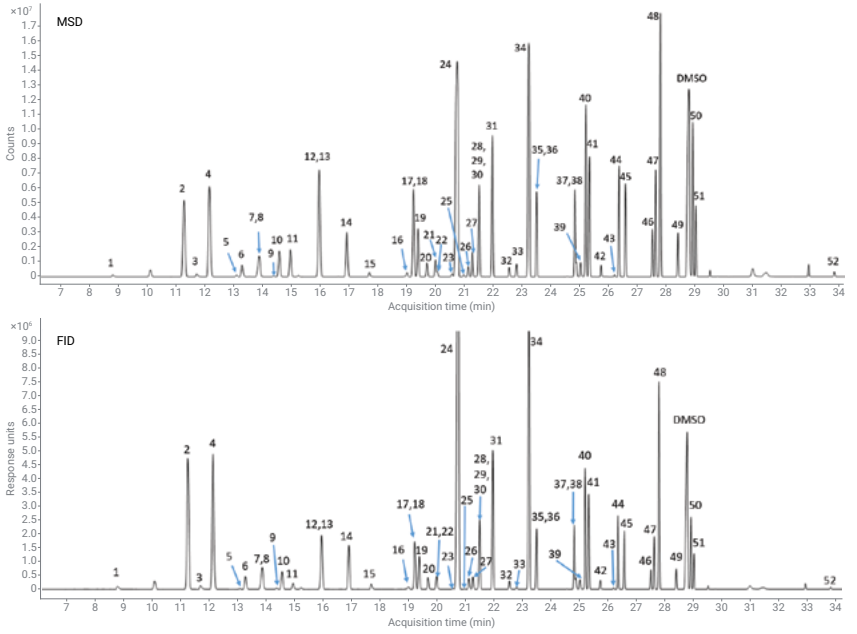
# Analysis of USP <467> residual solvents of Class 1, Class 2, and Class 3

Mass spectrometry detection (MSD) is a good choice for identification of volatile organic solvents, especially for unknown solvents. Usually, GC and GC/MSD are two separate systems, and it may take a long time for users to transfer methods between the two systems that may use different carrier gas or columns. This application note used a single 8890 GC system uniquely configured with both a Flame Ionization Detection (FID) and MSD for the three classes of residual solvents analysis. Samples introduced by the headspace sampler or the automatic liquid sampler were split 1:1. FID or MSD can be used as the tools for quantitative analysis, while MSD also can be used for qualitative analysis of unknown components.

Chromatographic analysis shows good peak shapes for various compounds in both GC/MS/SCAN and FID detectors. However, some pairs of compounds, such as tert-butylmethyl ether and trans-1,2-dichloroethene, co-elute on the DB-624 column, making quantification challenging with FID. In such cases, unique ions for each compound can be extracted and processed separately for accurate measurement. Isopropanol and ethyl formate also co-elute and were quantified together in this study, but selecting different columns with distinct properties can improve compound separation for precise quantification.




GC/MSD (upper image) and FID chromatograms (lower image) of 52 compounds on an Agilent DB-624 column.

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# What's new in residual solvent analysis?

- In May 2021, ICH announced it had reached Step 4 in the revision of its Q3C(R8) guideline, which establishes new permitted daily exposures (PDEs) for three residual solvents: 2-methyl tetrahydrofuran (2-MTHF), cyclopentyl methyl ether (CPME), and tertiary butyl alcohol (TBA). This guideline, which aims to provide recommendations for less toxic solvents in drug manufacturing, has been in place since 1997, and the recent revision is part of an ongoing process to update PDE levels based on new toxicological data for solvents.

# Further information

- Find webinars, videos, application notes and other resources on our webpage: Agilent Solutions for Residual Solvent Analysis
- Find resources on our webpage: Agilent Compliance Support and Services

It may be risky to rely on aging equipment to meet stringent regulations and productivity requirements. Learn about the Agilent technology refresh program.

USP uses three analytical procedures for identification and quantification of residual solvents. Find proven methods and access the consumables you need with Agilent's USP and ICH Q3C (R5) Consumable Ordering Guide.

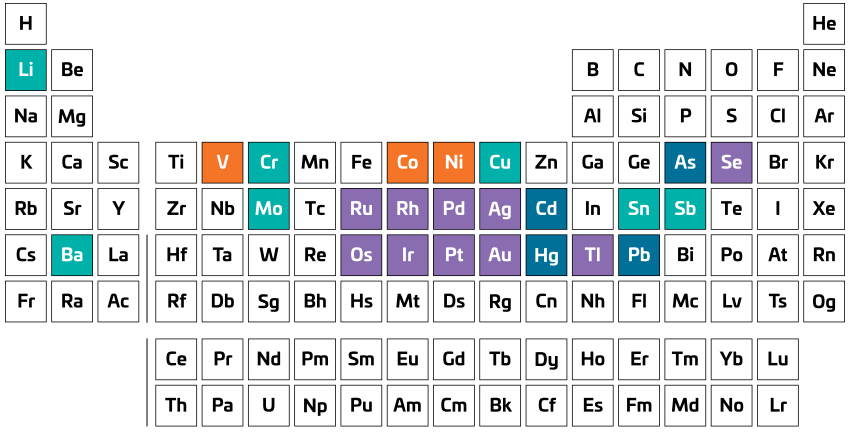
# Chapter 4: Elemental Impurities

## Understanding elemental impurities

Elemental impurity analysis plays a crucial role in pharmaceutical development and quality control (QC) in manufacturing. Elemental impurities (Figure 4.1) can be toxic when ingested, even in trace amounts. Therefore, their presence in pharmaceutical products can pose serious health risks.

Manufacturing processes can introduce elemental impurities into pharmaceutical products through contact with equipment, containers, or raw materials. Routine QC testing ensures that these impurities are within acceptable limits. Elemental impurity analysis is used to verify the batch-to-batch consistency of drug products and can provide critical data on assessing and mitigating risk.

Advances in analytical instrumentation and techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES), can be used to develop accurate and specific methods for elemental impurity analysis. These modern techniques provide enhanced sensitivity over traditional colorimetric methods and offer the ability to detect a wider range of elements at trace-level concentrations.




**Class 1:** Recognized as human toxicants and generally not utilized in the production of pharmaceuticals.

**Class 2:** Level of toxicity is influenced by the method of administration.

*Class 2A:* Have a higher likelihood of being found in drug products.

*Class 2B:* Have a lower likelihood of appearing in drug products.

**Class 3:** Exhibit relatively mild toxic effects (with high PDEs) when administered orally. However, their risk profile may need to be re-evaluated for inhalation and parenteral administration.

Figure 4.1. Elemental Impurities with established Permitted Daily Exposures (PDEs). Note that other elements, including Al, B, Ca, Fe, K, Mg, Mn, Na, W, and Zn, do not have defined established PDEs but might still require consideration. *Source: ICH Q3D guidelines*

# Regulatory landscape for elemental impurities

For many years, USP chapter <231> was the standard method for testing for heavy metals in pharmaceuticals. However, it had several limitations, including relying on colorimetric tests, which lacked specificity and accuracy.

In 2014, the ICH Q3D guideline on elemental impurities was adopted to provide a harmonized approach to evaluating and controlling elemental impurities in drug products.

In 2018, the USP further revised its standards for elemental impurity testing. This led to the replacement of USP chapter <231> with USP chapter <232>, which introduced a more modern and scientifically rigorous approach to elemental impurity testing. USP chapter <233>, titled "Elemental Impurities—Procedures," complements chapter <232>.

In 2022, Pharmacopeial Forum introduced significant changes to chapter <232>, adding PDEs for cutaneous and transcutaneous routes and correcting calculation errors for nickel, gold, and silver PDEs. This aligns the pharmacopeial requirements with ICH guideline Q3D (R2) in effect since September 2022, ensuring improved safety and compliance in pharmaceuticals.

# Key changes in USP chapter <232>/<233> include:

- Adoption of ICP-MS and ICP-OES instrumentation as the primary analytical techniques for elemental impurity analysis due to their superior accuracy and sensitivity
- Expansion of the list to 24 elements to be tested, covering a broader range of possible impurities
- Establishment of specific permissible daily exposure (PDE) limits for individual elements based on toxicological data
- Introduction of criteria for risk assessment and control strategies for elemental impurity levels in pharmaceuticals.

# Analyzing artificial tear eye drops for elemental impurities

This application note details an approach using the Agilent 7900 ICP-MS to accurately measure elemental impurities in Sterile Artificial Tear Eye Drops (SATED). The importance of this analysis stems from the fact that ophthalmic solutions do not have established PDEs for elemental contaminants as per USP and ICH. By leveraging parenteral PDEs and a daily dose assumption of 5 g/day, concentration limits (J values) for 24 elements were derived.

SATED samples underwent spiking at levels of 0.5, 1.0, and 1.5 J. The criteria for acceptance at each spike level involve recoveries falling within the range of 70% to 150% after subtracting the amount present in the unspiked sample. The figure below illustrates that spike recoveries satisfy this requirement within a 10% margin for all 24 elements at each level.


Accuracy results for SATED samples spiked at 0.5, 1.0, and 1.5 J obtained with the Agilent 7900 ICP-MS.

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# What's next in elemental impurity analysis?

- Innovation in analytical techniques is set to continue. Advancements in high-resolution ICP-MS and AI-driven methods are set to further simplify the analysis of routine samples.
- Enhanced data collaboration through the establishment of robust databases promises to elevate early-stage elemental impurity identification and research.
- A broadened regulatory horizon is anticipated, potentially encompassing emerging drug modalities such as nanoparticles and biologics.
- Unified testing protocols will become more streamlined. As the field matures, more standardized elemental testing procedures will likely emerge, ensuring consistency across the pharmaceutical landscape.

# Further information

- Elemental Analysis Product Selector Tool
- Agilent ICP-MS Series
- Find webinars, videos, application notes, and other resources on our webpage: Agilent Solutions for Elemental Impurity Analysis
- Find additional resources on our webpage: Agilent Compliance Support and Services

The Agilent 7900 ICP-MS is a flexible single quadrupole ICP mass spectrometry instrument well-suited for routine pharmaceutical QA/QC analysis. The system provides the industry's best matrix tolerance, most effective helium collision mode, lowest detection limits, and widest dynamic range. ICP-MS measures each sample in around 3 minutes, making it particularly beneficial in high-throughput laboratories. ICP-MS can quantify nearly every element (and measure isotopic composition) at parts per trillion to percent-level concentrations. It can also be connected to a chromatographic system for speciation analysis.

For more information visit Agilent Solutions for Pharmaceutical Small Molecule Development

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