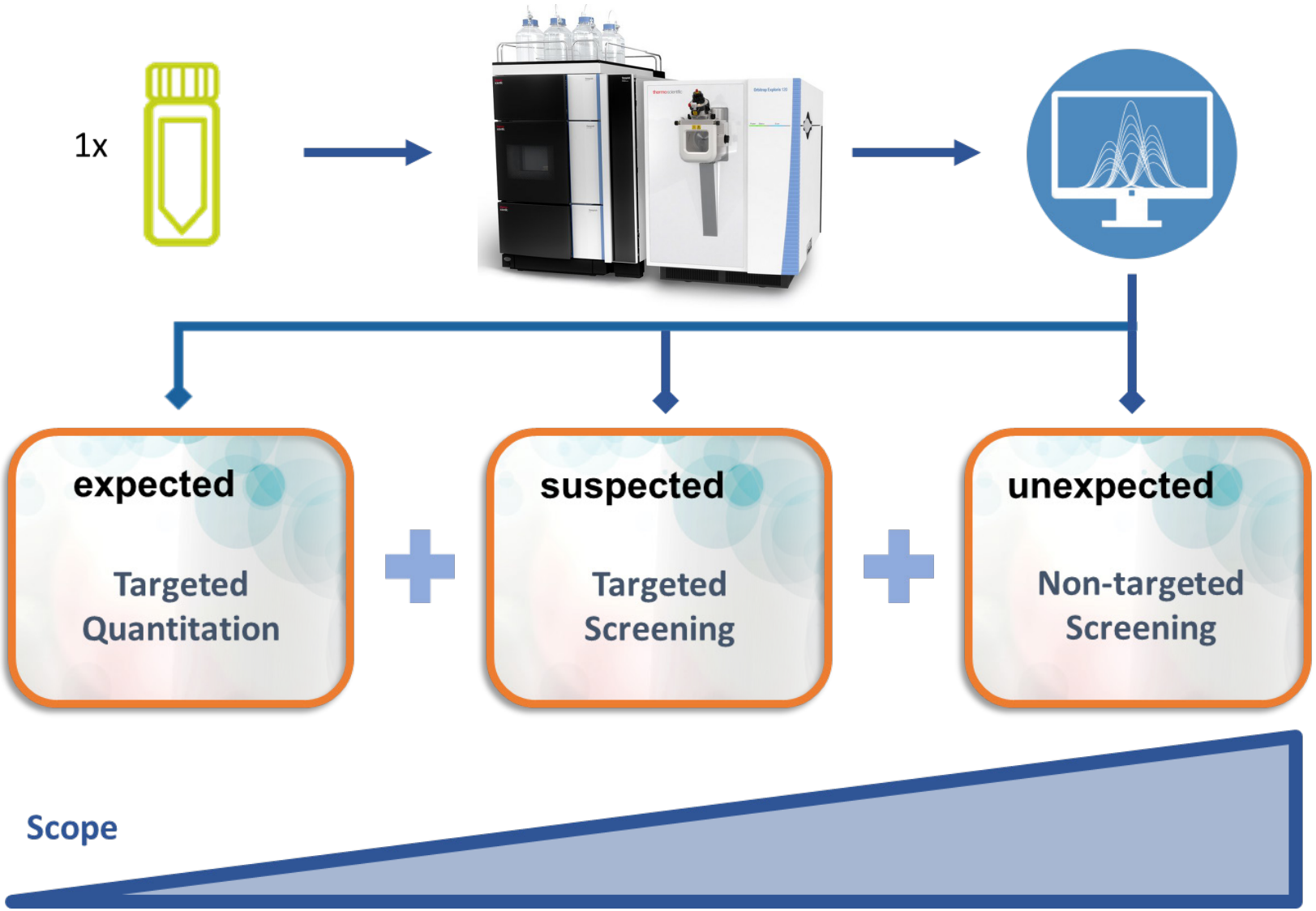
# *Comprehensive Solutions to Pharmaceutical Analysis*

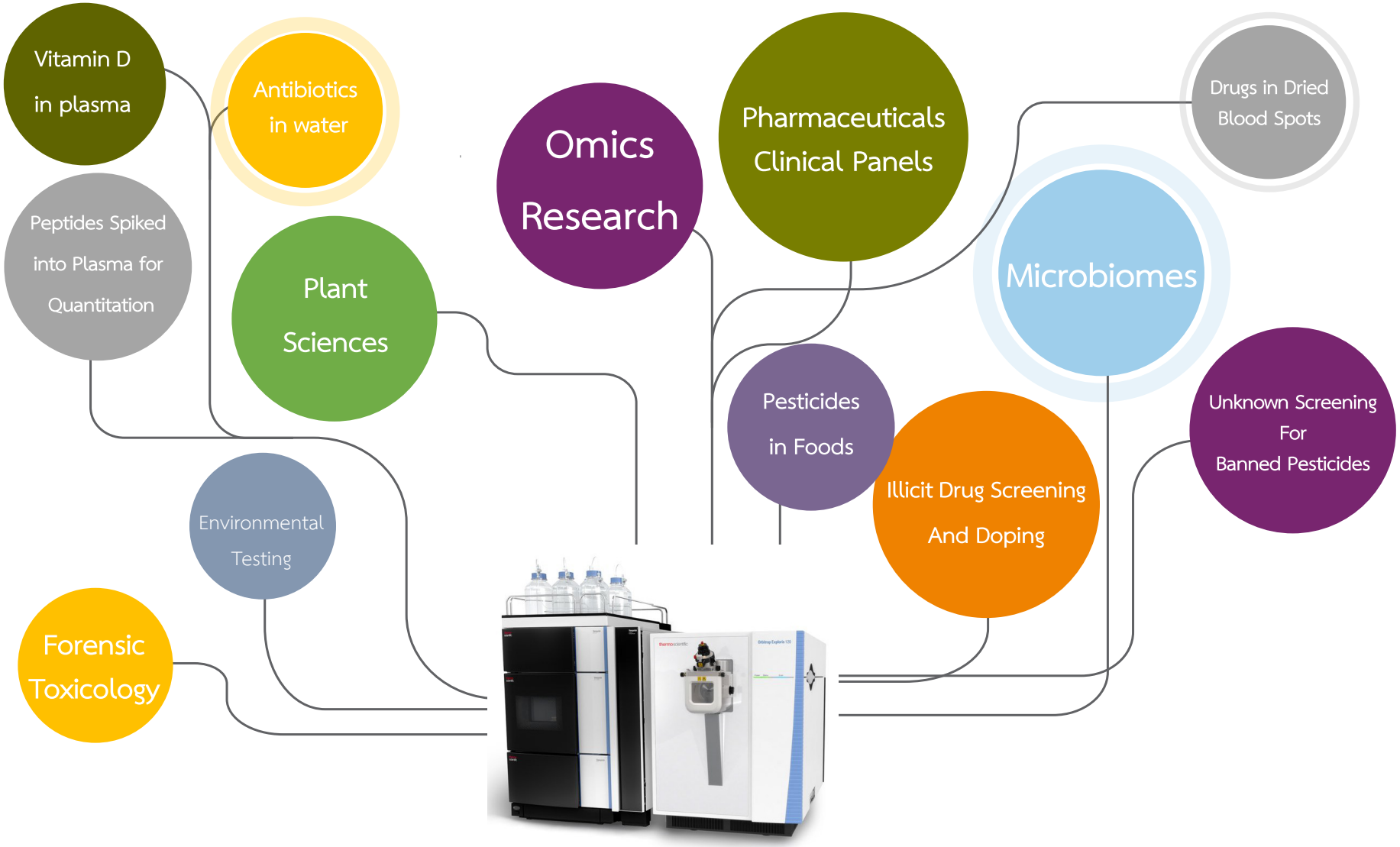
PRESENTED BY

Sci Spec Co., Ltd.

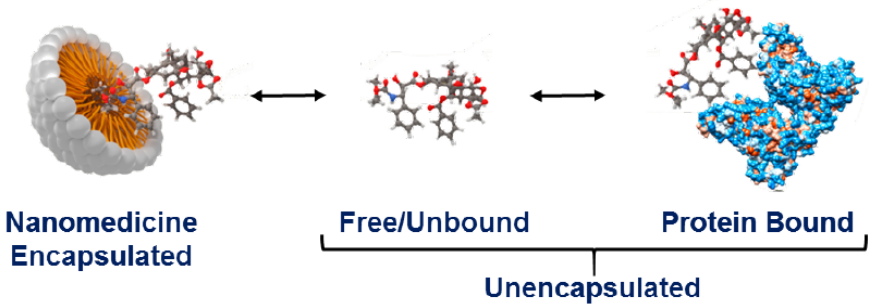
# System Main Workflows

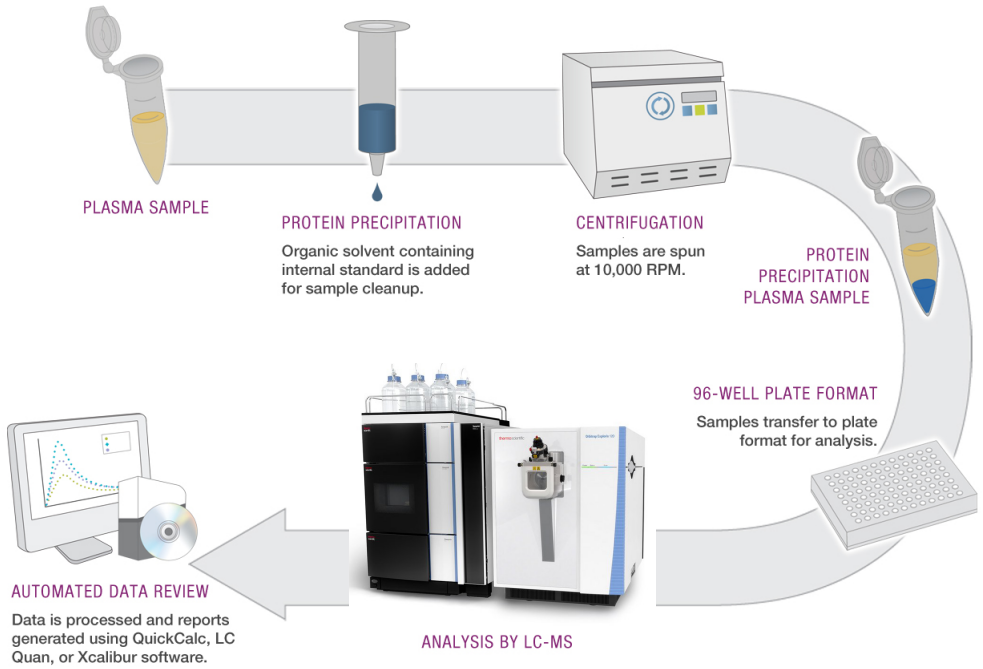


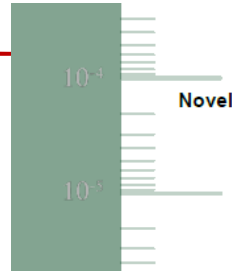
# Orbitrap Applications Universe



# Bioanalysis: Bioequivalence (BE)



**Novel Method to Determine Bioequivalence of Nanomedicines**

*summary of work conducted for*

**U.S. Food & Drug Administration**
**(Inter-Agency Award 224-16-3001S)**

NCL Nanotechnology Characterization Laboratory

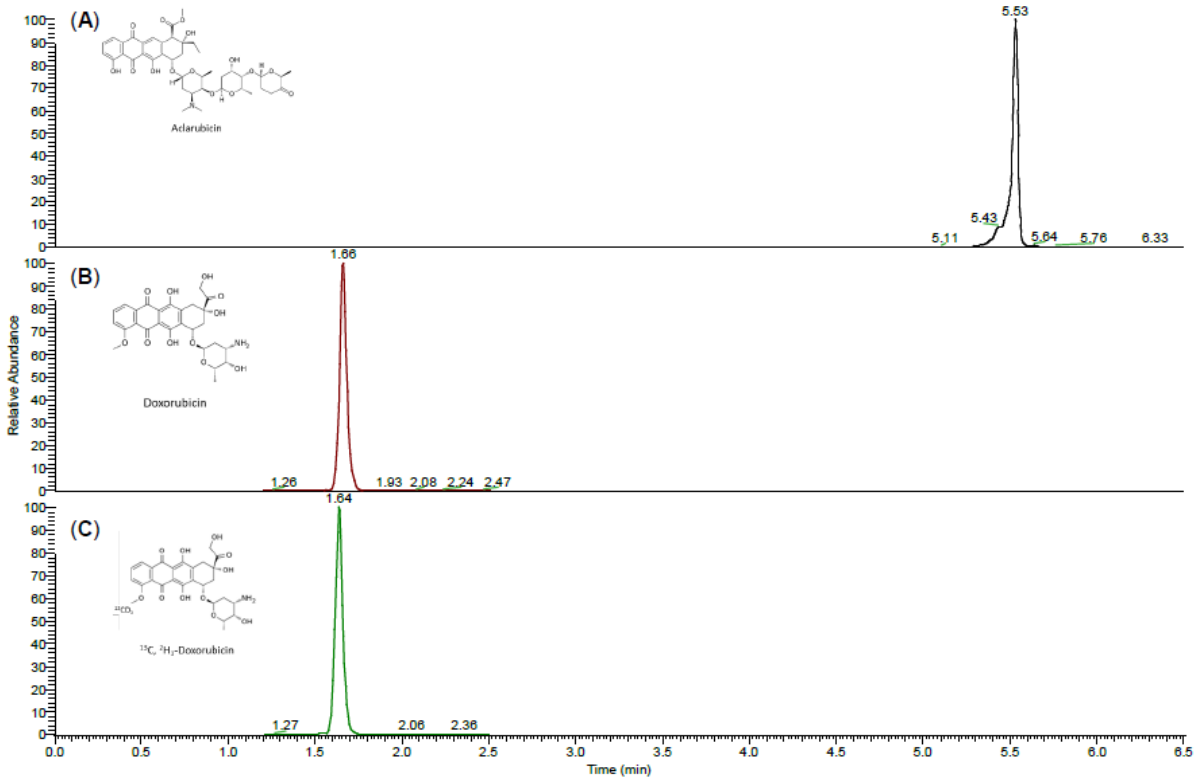
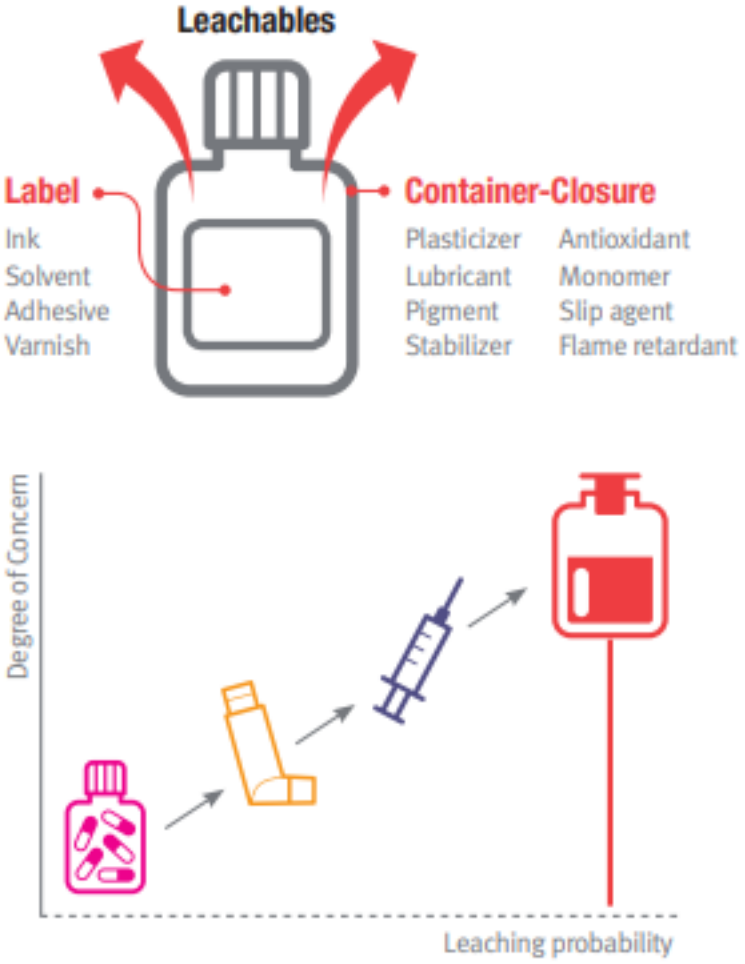
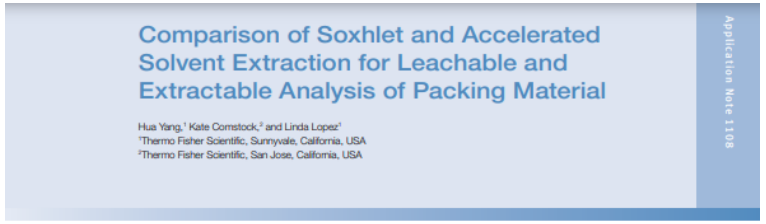



**Figure B-2. LC-Q Orbitrap Parallel Reaction Monitoring of Analytes and Internal Standard.** Displayed are chromatograms of (**A**) ISTD, (**B**) DXR and (**C**) DXR_C13. The transitions used were: DXR 544.18 → 130.08, 379.07, 397.08; DXR_C13 548.20 → 130.08, 383.09, 401.11; and Aclarubicin 812.34 → 570.23

# Extractables and Leachables





Comparison of Soxhlet and Accelerated Solvent Extraction for Leachable and Extractable Analysis of Packing Material

Application Note 1108

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[1]Thermo Fisher Scientific, Sunnyvale, California, USA
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**Key Words**
Pharmaceutical, Biopharma, U.S. FDA, Sample preparation, Packaging, Automated Extraction, HPLC, Pressurized fluid extraction

**Introduction**

According to the regulations and guidelines set by regulatory agencies, including the U.S. Food and Drug Administration (US FDA),[1] the European Medicines Agency (EMA),[2] extractable and leachable information must be included in applications for medical devices and container closure systems packaging for human drugs and biologics. The specific applications include the New Drug Application (NDA), Abbreviated New Drug Application (ANDA) and Biologics License Application (BLA).

Leachables are compounds that can migrate from components of medical devices or container closure systems into the drug product.[3] They present safety concerns and may influence the effectiveness of a drug product. Extractables are compounds that can be extracted from components and are determined through Controlled Extraction Studies.[3] The main purpose of

The accelerated solvent extraction technique is an automated technique with several advantages, including efficient extraction, reduced extraction time (<0.5 h/sample), reduced solvent use (<30 mL/sample), and flexibility in solvent selection. Using the method and sequence editor in the Dionex ASE 350 system, three

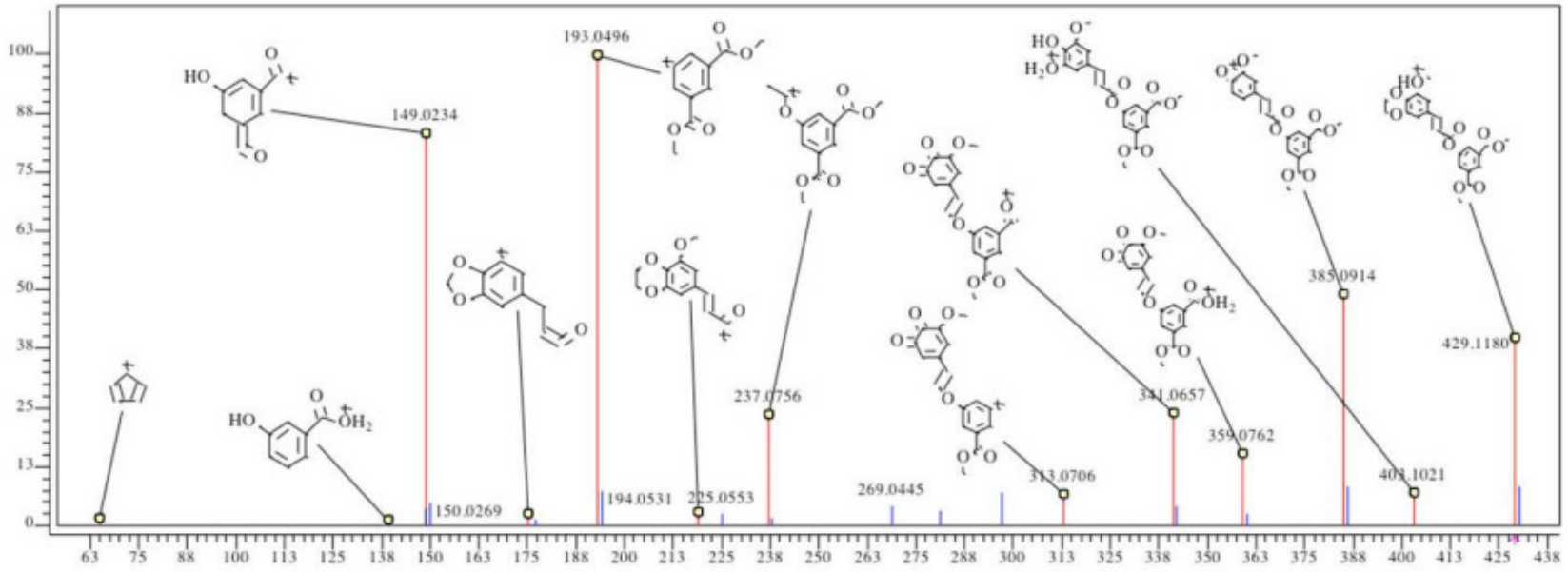
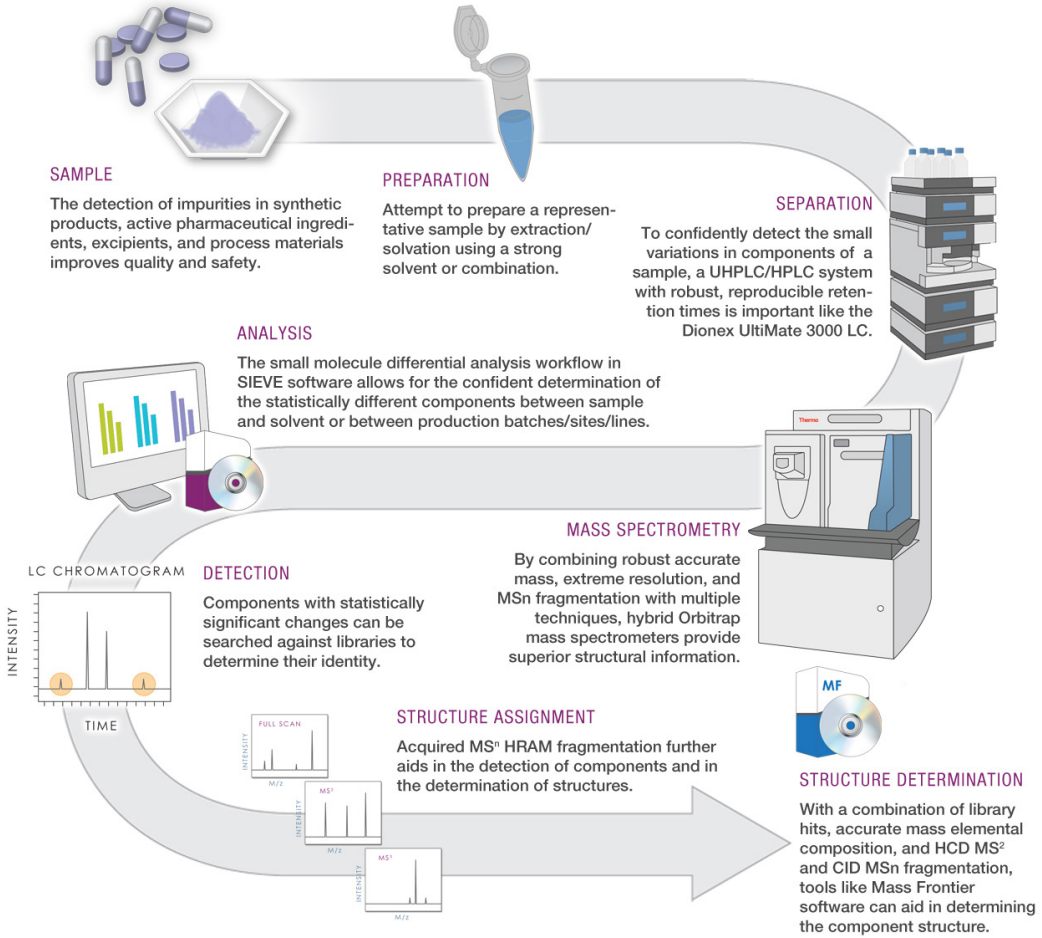
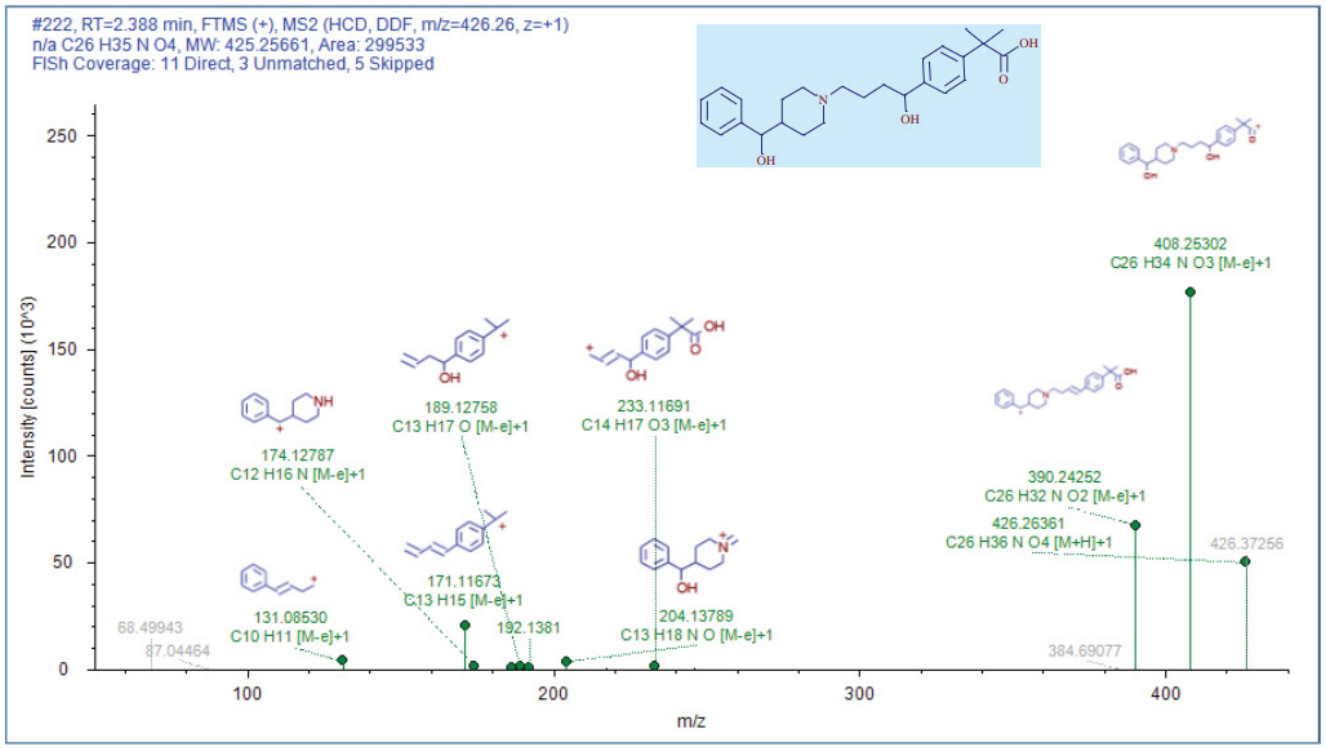




Figure 9. Auto-annotated MS/MS spectra of the proposed structure from 2-propanol extraction of the transdermal patch pouch.

# Drug Impurities Analysis



# Nitrosamine Impurities in Pharmaceutical Products

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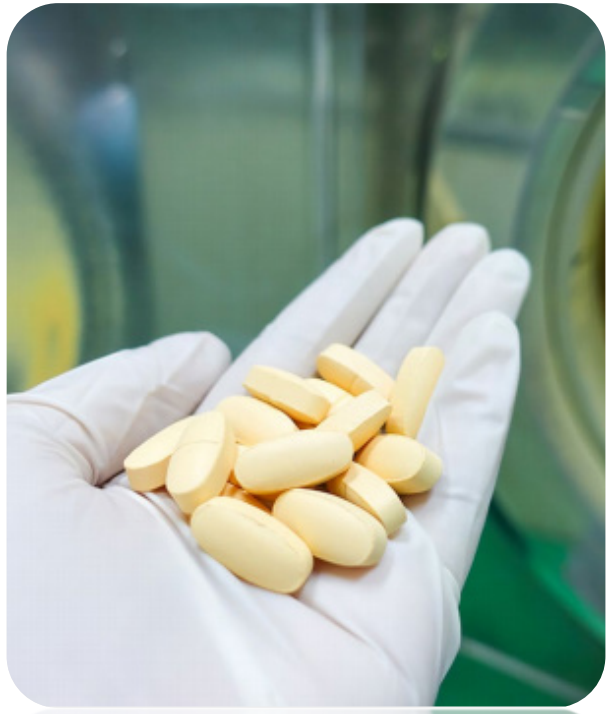
*http://pca.fda.moph.go.th/public_media_detail.php?id=2&cat=50&content_id=1722*


*https://www.khaosod.co.th/around-thailand/news_2926836*

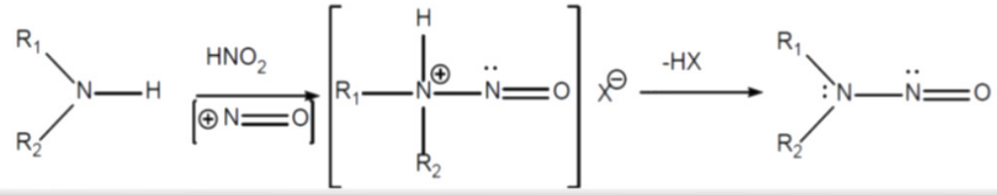
# What are Nitrosamines ?

## Nitrosamines (NAs) in APIs and drug products



- Classified as genotoxic impurities, proven as probable human carcinogen
- Detected elevated levels of NDMA and other impurities in several drug products, announced several recalls
- United States Food and Drug Administration (US FDA) published validated GC/LCMS/MS and GC/LC-HRAM methods, and interim acceptable limits for several NAs
- US FDA recently released guidance for industry to
  1) conduct risk assessments on approved or marketed drugs and pending applications,
  2) Implement control strategy to reduce and prevent formation of NAs in all API and drug

**Figure 1. Representative Reaction to Form Nitrosamines**



# Analytical Challenges


## Selectivity

- High background, formulation matrices and other impurities from drug substances and solvents
- Chemical Interferences (e.g., DMF and NDMA)
- Structural isomers (e.g., NDIPA and NDPA)

## Sensitivity

- Trace level of impurities in presence of high concentration of API
- Total nitrosamine contents should be less than 30 ppb or 26.5 ng/day based on daily maximum dosage intake of 880 mg/day


## Regulatory Expectation and Compliance

- Meet US FDA requirements in terms of sensitivity, linearity, and reproducibility
- Data collection and processing in compliant software to ensure data integrity and security

*Analytical method needs to demonstrate high selectivity, sensitivity, and meets US FDA regulatory guidelines*

# Objective

*Thermo Scientific™*

***Vanquish™***

*UHPLC System*

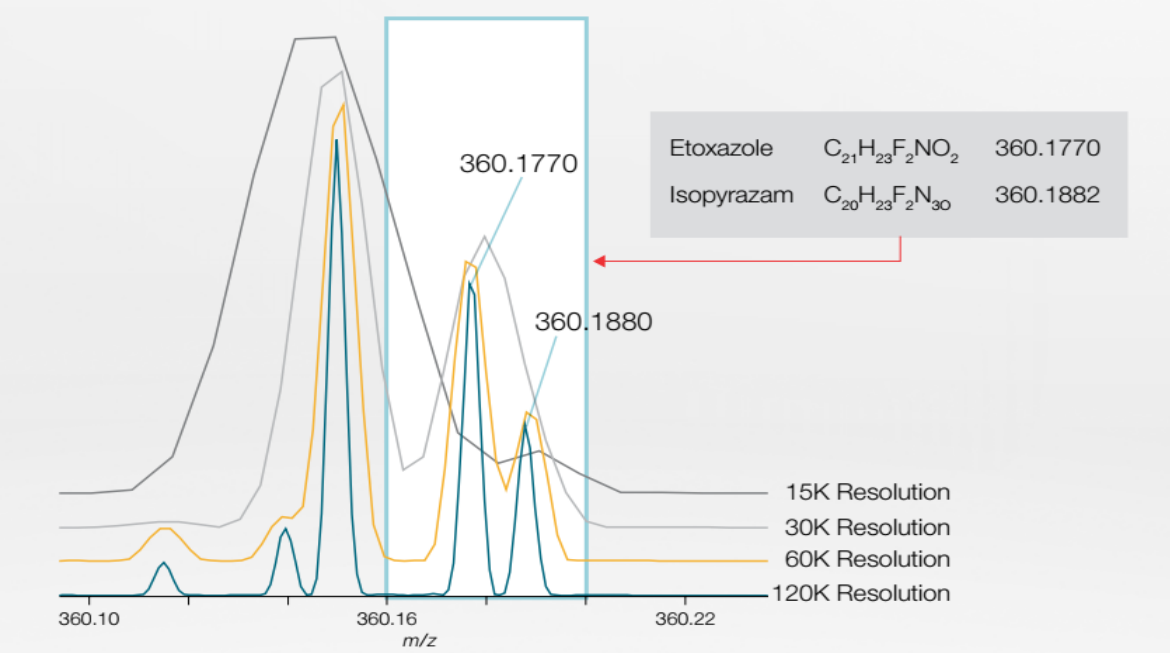
*Thermo Scientific™*

***Exploris™ 120***

*HRAM-MS System*

*A rapid, highly selective and sensitive LC-HRAM-MS method was developed for detection and quantitation of 9 nitrosamines in commercial ranitidine drug product*

# Product Specification

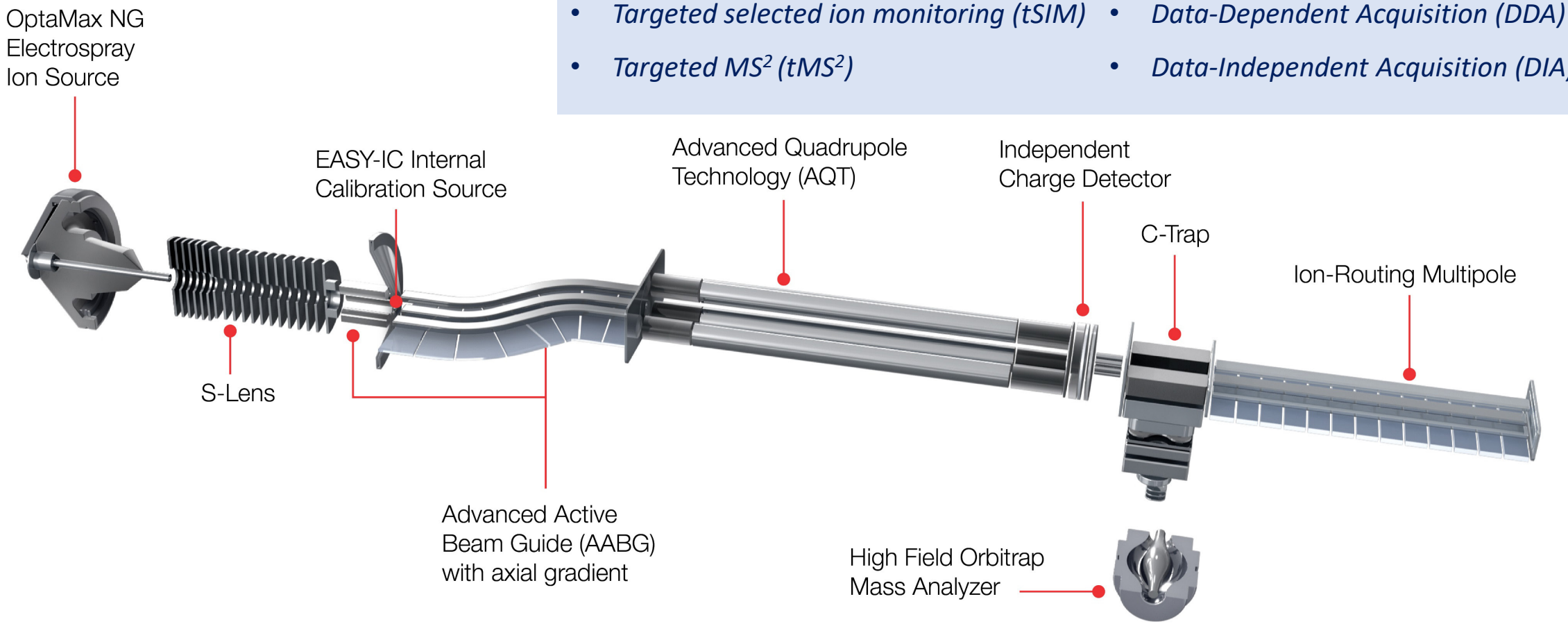



| | Orbitrap Exploris 120 |
|---|---|
| Resolution @ *m/z* 200 | up to 120,000 FWHM |
| Mass Range | 40 – 3,000 |
| Scan Rate | up to 22 Hz |
| Sensitivity | MS/MS: 200 fg reserpine on column S/N 100:1<br>tSIM: 200 fg reserpine on column S/N 250:1 |

# Orbitrap™ Technology

## *Data Acquisition Mode*

- *Full MS scan*
- *Targeted selected ion monitoring (tSIM)*
- *Targeted MS² (tMS²)*
- *All Ion Fragmentation (AIF)*
- *Data-Dependent Acquisition (DDA)*
- *Data-Independent Acquisition (DIA)*



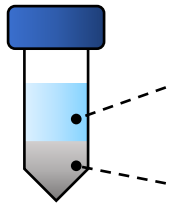
# Target compounds

| Analyte | Internal standard (ISTD) |
|---|---|
| N-Nitrosodiethylamine (NDEA) | NDEA-$D_{10}$ |
| N-Nitrosodimethylamine (NDMA) | NDMA-$D_6$ |
| N-Nitrosodi-n-butylamine (NDBA) | NDBA-$D_{18}$ |
| N-Nitroso-di-n-propylamine (NDPA) | NDPA-$D_{14}$ |
| N-Nitrosomethylethylamine (NMEA) | NMEA-$D_3$ |
| N-Nitrosopiperidine (NPIP) | NPIP-$D_{10}$ |
| N-Nitrosopyrrolidine (NPYR) | NPYR-$D_8$ |
| N-Ethyl-N-nitroso-2-propanamine (NEIPA) | NEIPA-$D_5$ |
| N-Nitroso-di-isopropylamine (NDIPA) | NDIPA-$D_{14}$ |

# Sample Preparation

## *Ranitidine substance*

**Dissolution**

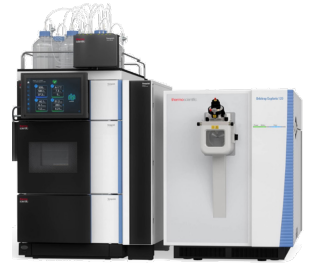


1 ml MeOH
+ 10 µl 500 ppb ISTD
30 mg ranitidine hydrochloride

*Total concentration = 30 mg/mL API*

Filter 750 µl solution using 0.2 µm PVDF syringe filter

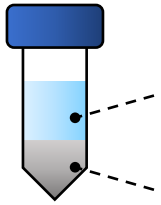
Inject 5 µl to LC-HRMS



## *Drug Product*

Ranitidine tablet

**Extraction**



10 ml MeOH
+ 100 µl 500 ppb ISTD
300 mg API, ground ranitidine tablet

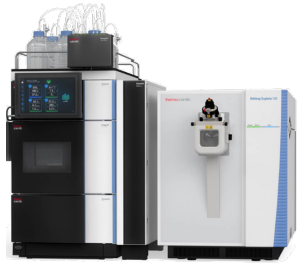
*Total concentration = 30 mg/mL API*

Shake for 40 min

Centrifuge for 15 min at 4000 RPM

Filter 750 µl supernatant using 0.2 µm PVDF syringe filter

Inject 5 µl to LC-HRMS



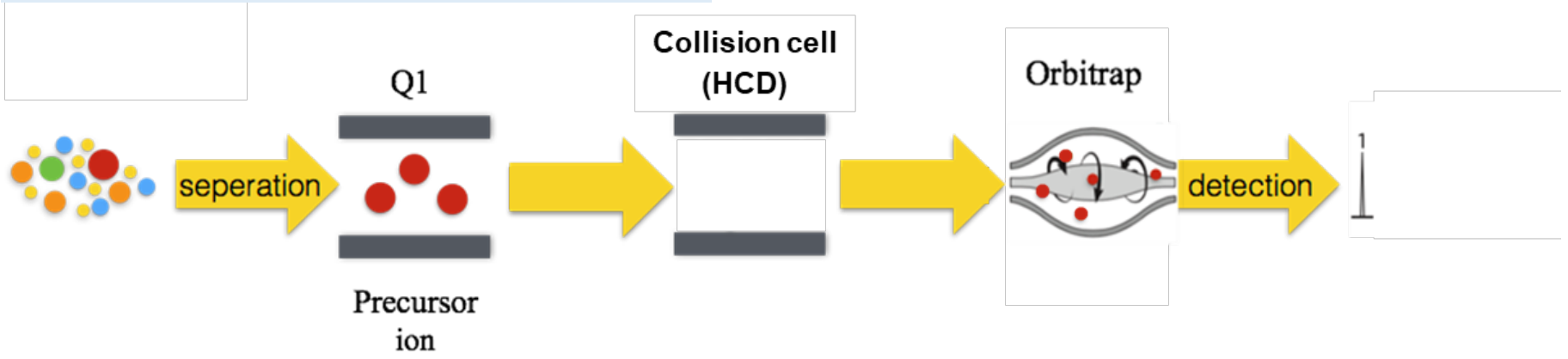
# LC-MS Condition

| Parameters | Value | | |
|---|---|---|---|
| HPLC column | Acclaim Polar Advantage II, 100 × 2.1 mm, 2.2 µm | | |
| Column temperature | 40 °C | | |
| Flow rate | 0.5 mL/min | | |
| Mobile phase A | Water + 0.1% formic acid | | |
| Mobile phase B | Methanol + 0.1% formic acid | | |
| Gradient | Time (min) | %Mobile phase A | %Mobile phase B |
| | 0.0 | 95 | 5 |
| | 0.5 | 95 | 5 |
| | 8.0 | 5 | 95 |
| | 9.0 | 5 | 95 |
| | 9.1 | 95 | 5 |
| | 12.0 | 95 | 5 |
| Injection volume | 5 µL | | |

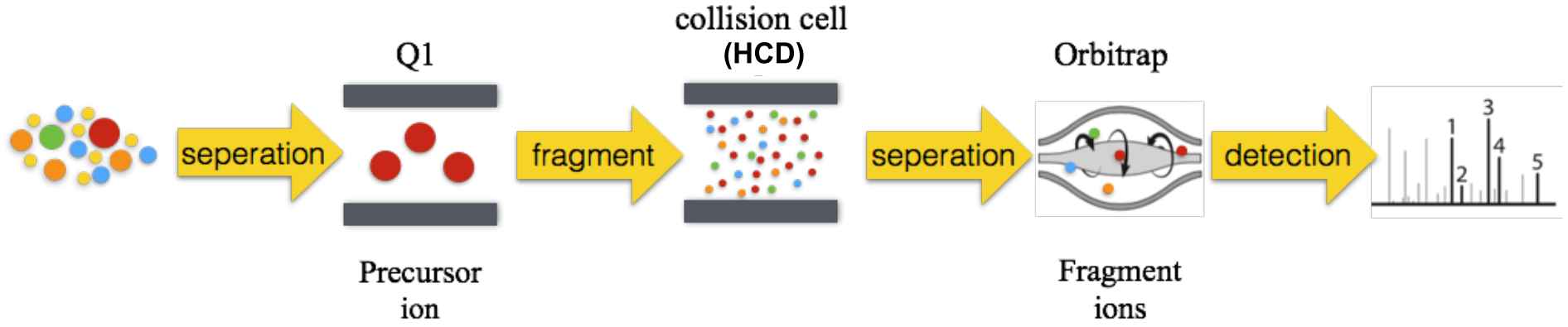
| Parameters | Value | |
|---|---|---|
| Ionization | APCI | |
| Polarity | Positive | |
| Spray current | 2 µA | |
| Sheath gas | 45 | |
| Auxiliary gas | 10 | |
| Sweep gas | 0.5 | |
| Ion transfer tube temp. | 200 °C | |
| Vaporizer temp. | 300 °C | |
| Scan mode | tSIM | $tMS^2$ |
| Polarity | Positive | Positive |
| Resolution | 120,000 | 120,000 |
| AGC target | 1e5 | 1e5 |
| Maximum IT | Auto | Auto |
| Isolation window | m/z 2.0 | m/z 2.0 |

# Acquisition Mode (Target Quantitation)

*Targeted selected ion monitoring (tSIM)*




*Targeted $MS^2$ ($tMS^2$)*



# Optimized MS Condition for Target Nitrosamines

| | Scan type | Scan Start – End (min) | Polarity | *m/z* of Quan. ion | *m/z* of Qual. Ion | Normalized CE (%) |
|---|---|---|---|---|---|---|
| NDMA | $tMS^2$ | 0.25–1.75 | Positive | 75.0552 | 43.0290 | 60 |
| NDMA-$D_6$ | $tMS^2$ | 0.25–1.75 | Positive | 81.0928 | 46.0480 | 60 |
| NMEA | $tMS^2$ | 0.75–2.25 | Positive | 61.0397 | 89.0708 | 15 |
| NMEA-$D_3$ | $tMS^2$ | 0.75–2.25 | Positive | 64.0585 | 92.0898 | 30 |
| NPYR | $tMS^2$ | 0.75–2.25 | Positive | 101.0709 | 55.0540 | 60 |
| NPYR-$D_8$ | $tMS^2$ | 0.75–2.25 | Positive | 109.1212 | 62.0980 | 30 |
| NDEA | $tMS^2$ | 1.95–3.45 | Positive | 103.0866 | 75.0550 | 45 |
| NDEA-$D_{10}$ | $tMS^2$ | 1.95–3.45 | Positive | 113.1493 | 81.0930 | 45 |
| NPIP | $tMS^2$ | 2.35–3.85 | Positive | 115.0866 | 69.0699 | 60 |
| NPIP-$D_{10}$ | $tMS^2$ | 2.35–3.85 | Positive | 125.1494 | 78.1260 | 60 |
| NEIPA | $tMS^2$ | 2.85–4.35 | Positive | 75.0553 | 117.1022 | 15 |
| NEIPA-$D_5$ | $tMS^2$ | 2.85–4.35 | Positive | 80.0866 | 122.1336 | 15 |
| NDIPA | tSIM | 3.75–5.25 | Positive | 131.1179 | - | - |
| NDIPA-$D_{14}$ | tSIM | 3.75–5.25 | Positive | 145.2058 | - | - |
| NDPA | tSIM | 4.25–5.75 | Positive | 131.1179 | - | - |
| NDPA-$D_{14}$ | tSIM | 4.25–5.75 | Positive | 145.2058 | - | - |
| NDBA | $tMS^2$ | 5.75–7.25 | Positive | 159.1492 | 103.0866 | 15 |
| NDBA-$D_{18}$ | $tMS^2$ | 5.75–7.25 | Positive | 177.2622 | 66.1264 | 15 |

# Thermo Scientific™ Chromeleon™ CDS

## …control all your instrumentation

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- Comprehensive control for more than 300 instruments from other manufacturers
- Customizable ePanels provide a consistent look and feel for all instruments

## …get the most out of your data

- Deliver accurate and reliable results with built-in reporting tools
- Create any report you need using the spreadsheet-based report designer
- Find, collate and report results across multiple sequences using powerful data mining tools

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- Network existing Chromeleon CDS stations to create a compliant laboratory-wide solution with no additional licenses
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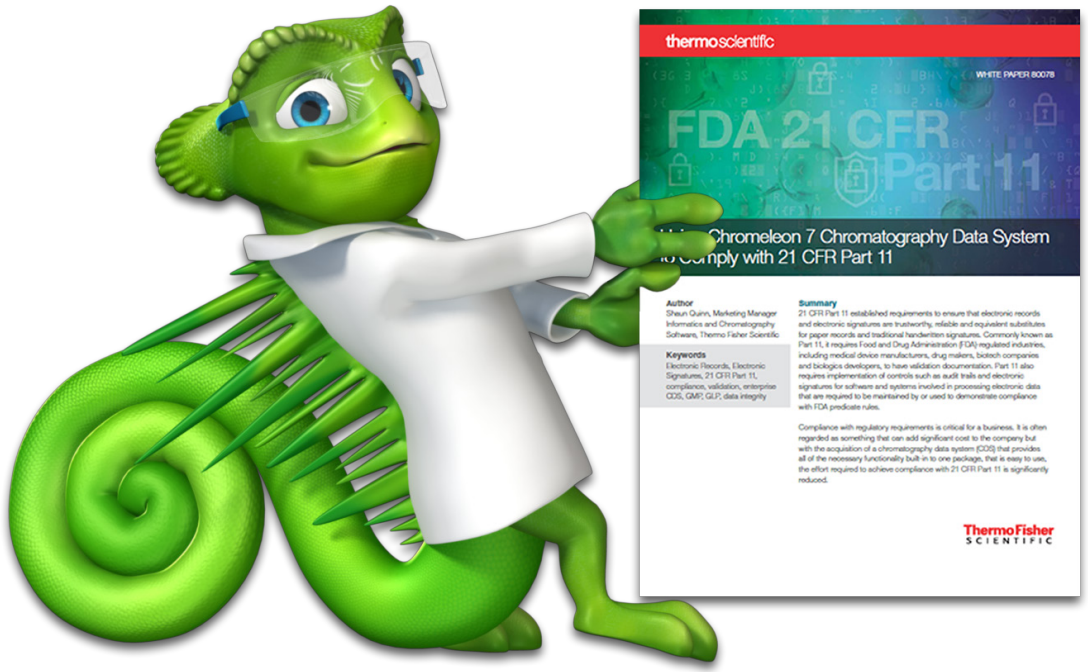
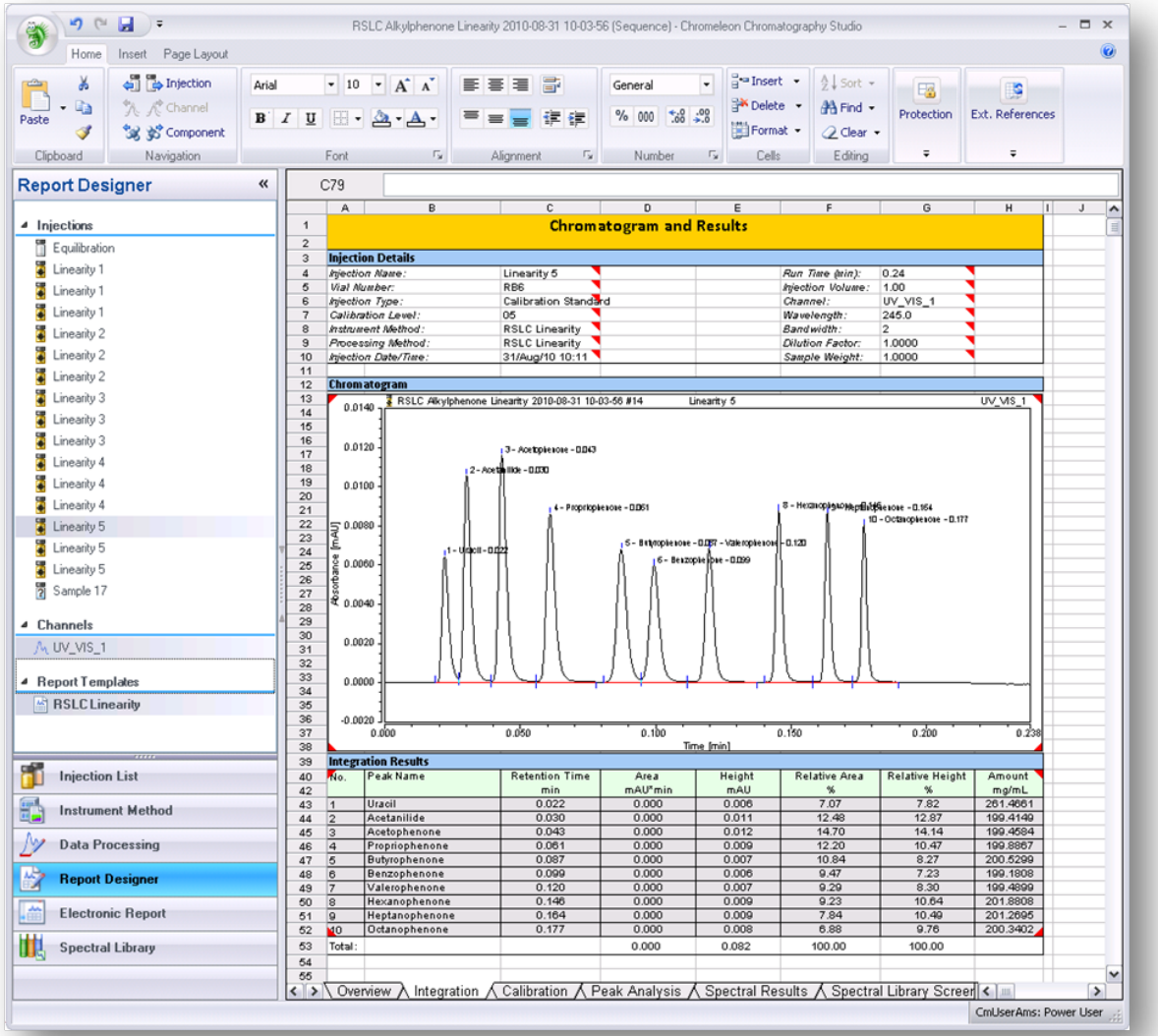
# Thermo Scientific™ Chromeleon™ CDS

*Using **Chromeleon 7** Chromatography Data System to Comply with **21 CFR Part 11***

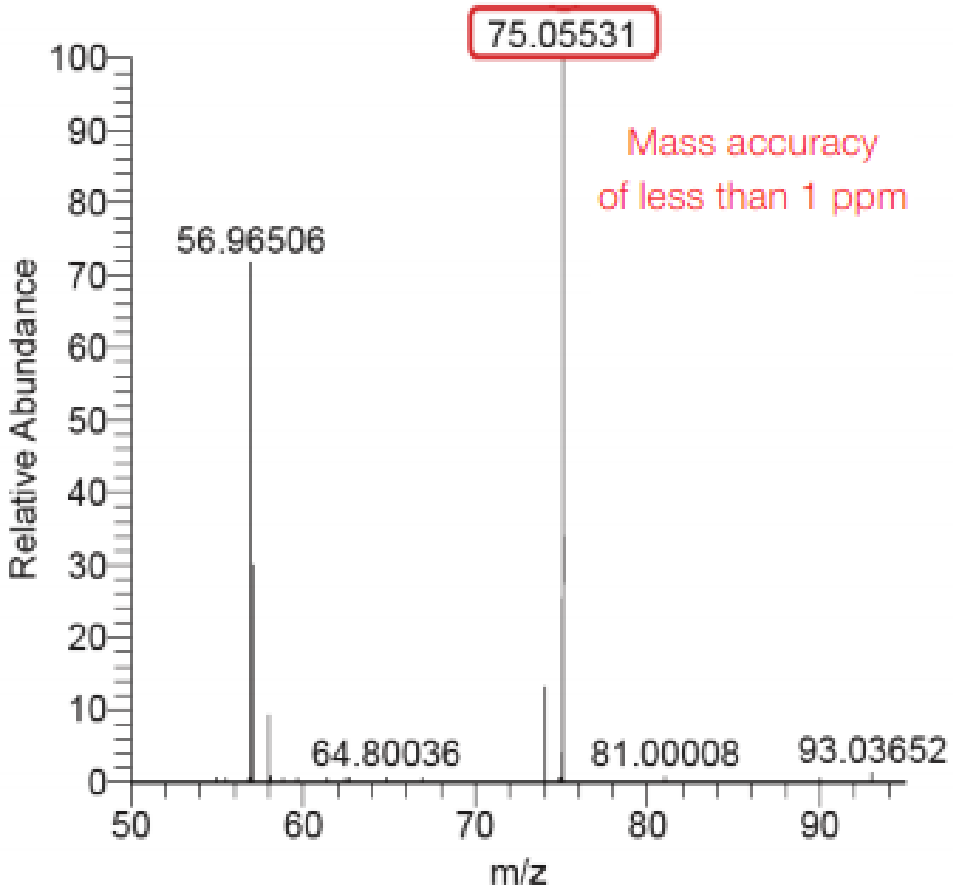


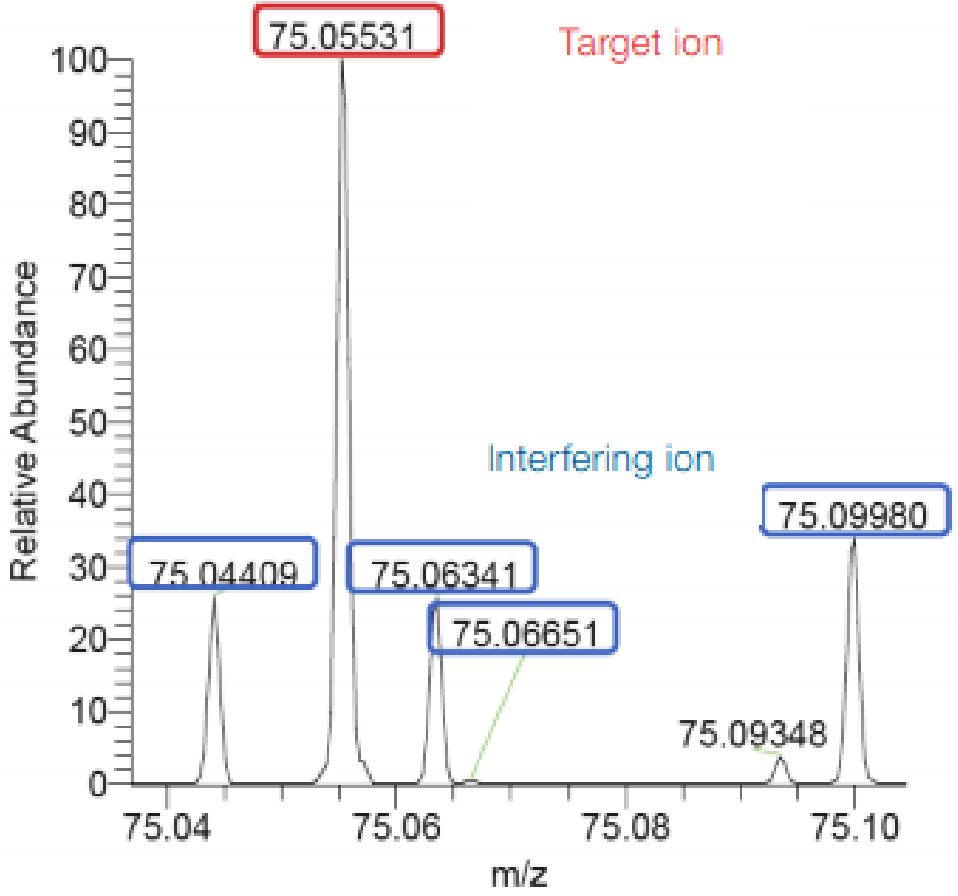



# Selective Determination of Nitrosamine Impurities

*Mass spectrum of 1 ng/ml NDMA standard solution*

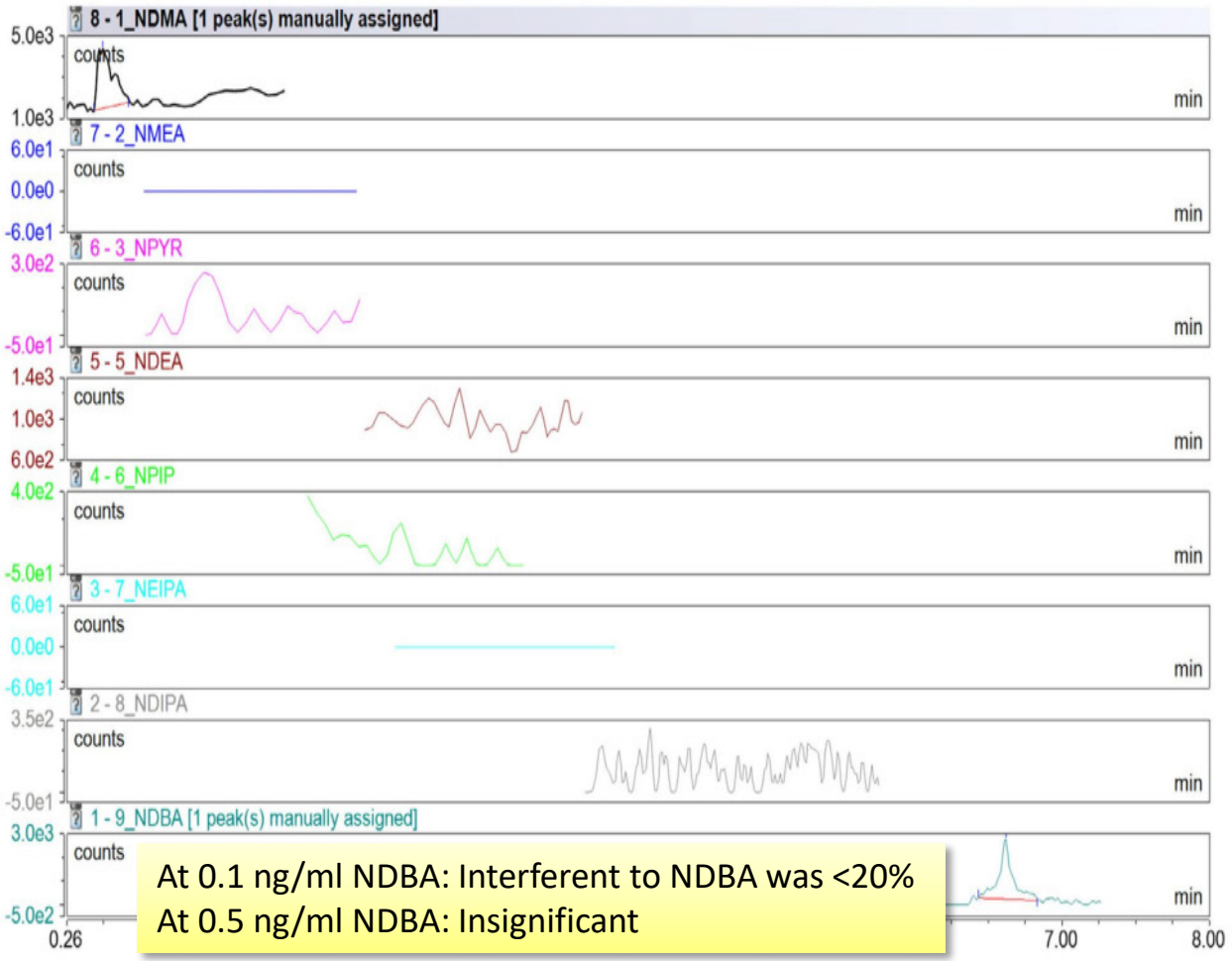
*Target monoisotopic mass of NDMA = 75.0552*



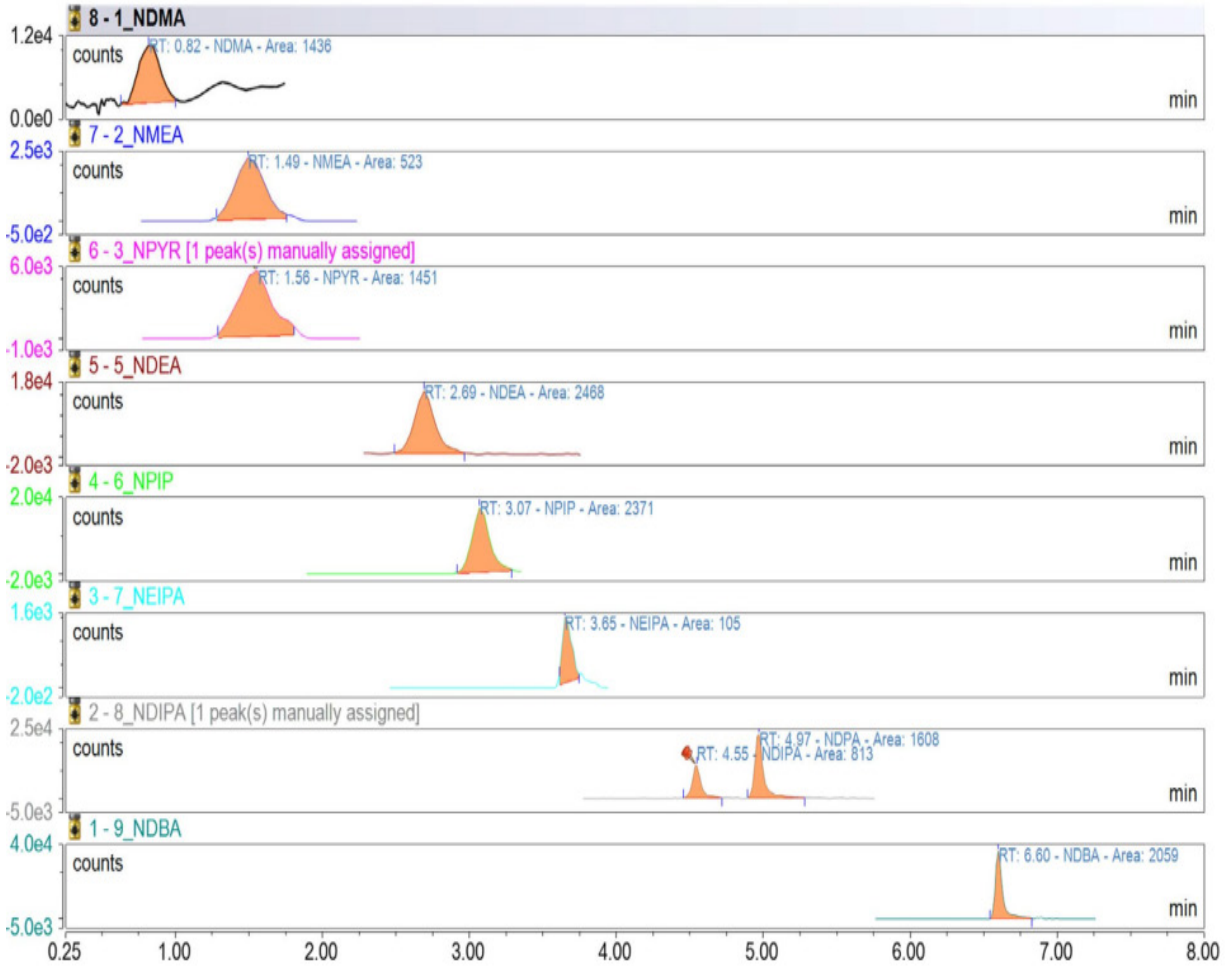
# Selective Determination of Nitrosamine Impurities

## *Blank (Excipient)*




At 0.1 ng/ml NDBA: Interferent to NDBA was <20%
At 0.5 ng/ml NDBA: Insignificant

## *Standard at 0.5 ng/ml or 17 ppb (ng/g API)*



# Calibration Curves for All Compounds in Excipient

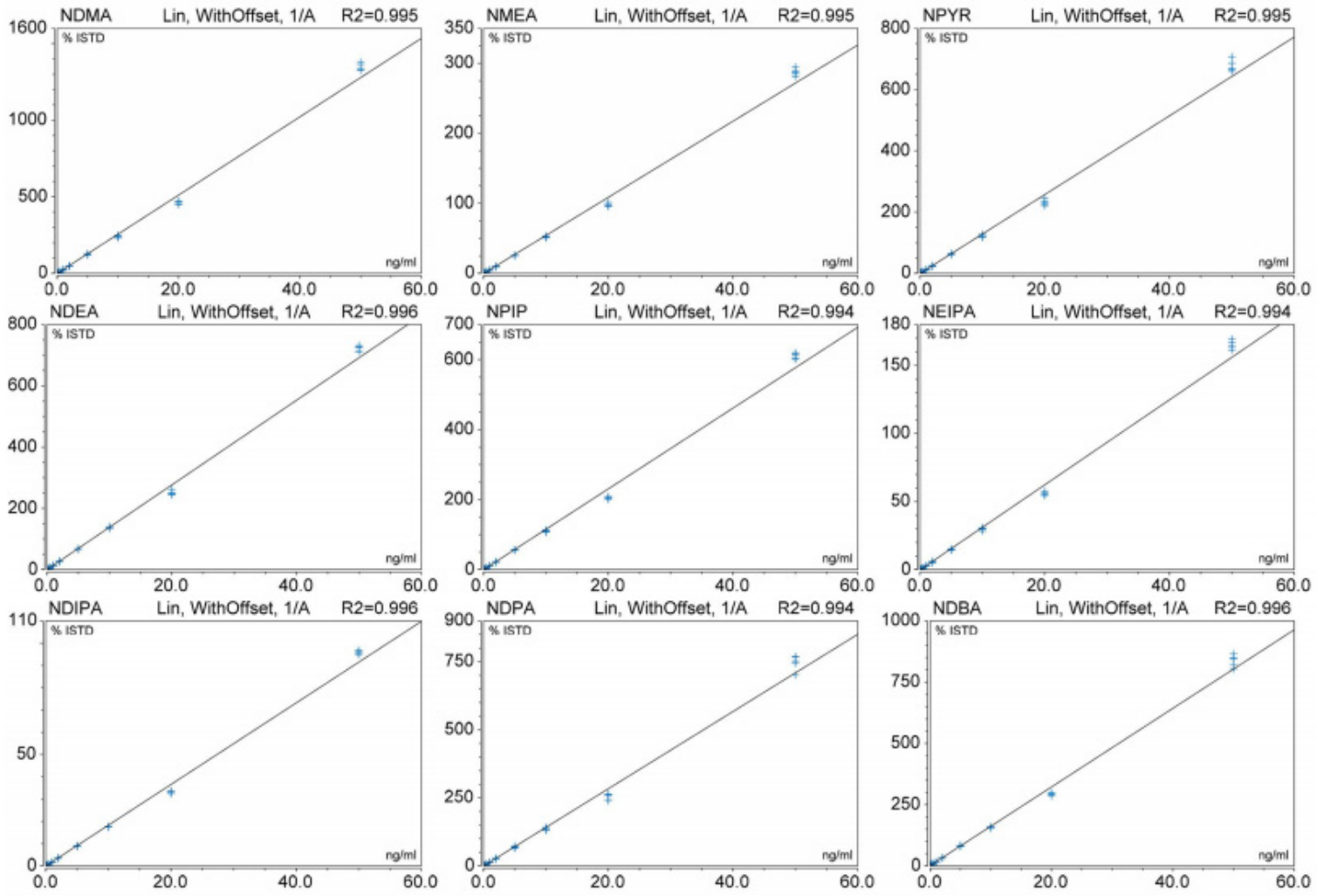



Figure 2. Calibration curves for all compounds in excipient

The newly recommended detection limits from the US FDA : < 30 ppb for a total of 7 nitrosamines

| | LOD | | LLOQ | | |
|---|---|---|---|---|---|
| | ng/mL | PPB | ng/mL | PPB | Linearity |
| NDMA | 0.2 | 6.8 | 0.2 | 6.8 | |
| NMEA | 0.2 | 6.8 | 0.2 | 6.8 | |
| NPYR | 0.2 | 6.8 | 0.2 | 6.8 | |
| NDEA | 0.1 | 3.4 | 0.1 | 3.4 | |
| NPIP | 0.2 | 6.8 | 0.2 | 6.8 | LLOQ – 50 |
| NEIPA | 0.5 | 17.0 | 0.5 | 17.0 | |
| NDIPA | 0.1 | 3.4 | 0.1 | 3.4 | |
| NDPA | 0.1 | 3.4 | 0.1 | 3.4 | |
| NDBA | 0.1 | 3.4 | 0.5 | 3.4 | |

LOD defined as within 20% accuracy, and 15% RSD.

LOQ defined as within 15% accuracy, and 15% RSD.

PPB is calculated based on 30 mg/mL of drug substance and product extract.

# Recovery, Accuracy, and Precision

## *Sample recovery and reproducibility*

*Spiked 2 and 5 ng/mL standard in blank excipient matrix before and after the extraction process (n=5)*

| | 2 ng/mL | | 5 ng/mL | |
|---|---|---|---|---|
| | % Recovery | % RSD | % Recovery | % RSD |
| NDMA | 95 | 5.5 | 99 | 3.2 |
| NMEA | 96 | 6.3 | 98 | 1.2 |
| NPYR | 99 | 7.4 | 100 | 3.9 |
| NDEA | 97 | 2.4 | 98 | 3.2 |
| NPIP | 96 | 2.6 | 99 | 5.5 |
| NEIPA | 99 | 8.4 | 98 | 2.2 |
| NDIPA | 99 | 2.8 | 98 | 2.2 |
| NDPA | 95 | 4.4 | 96 | 8.4 |
| NDBA | 99 | 2.9 | 101 | 1.5 |

The recovery for all nitrosamines during the extraction process was between 95 and 101%, and the reproducibility of the replicate injections was within 10% RSD

## *Accuracy and precision*

*0.5 ng/mL (17 ppb) check standard in excipient (n=5)*

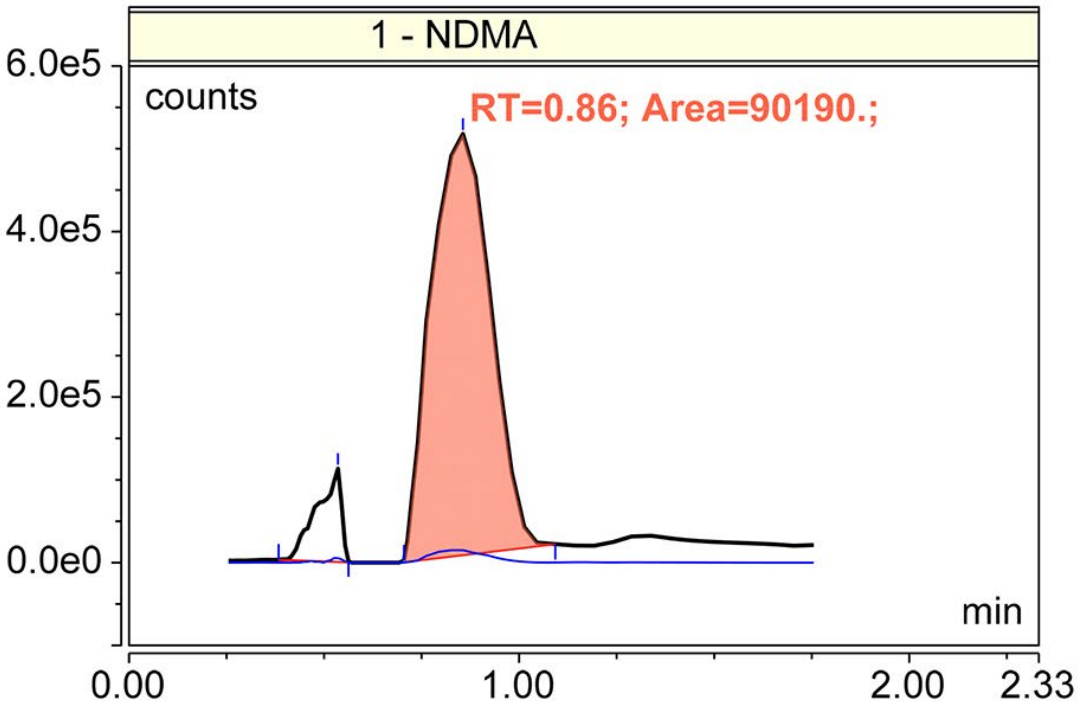
| | %Accuracy | %RSD |
|---|---|---|
| NDMA | 92 | 10.9 |
| NMEA | 95 | 4.2 |
| NPYR | 92 | 2.7 |
| NDEA | 95 | 3.4 |
| NPIP | 98 | 5.1 |
| NEIPA | 93 | 7.8 |
| NDIPA | 97 | 2.9 |
| NDPA | 96 | 3.0 |
| NDBA | 97 | 4.1 |

All target nitrosamines at 17 ppb (0.5 ng/mL) could be detected and quantified with high accuracy

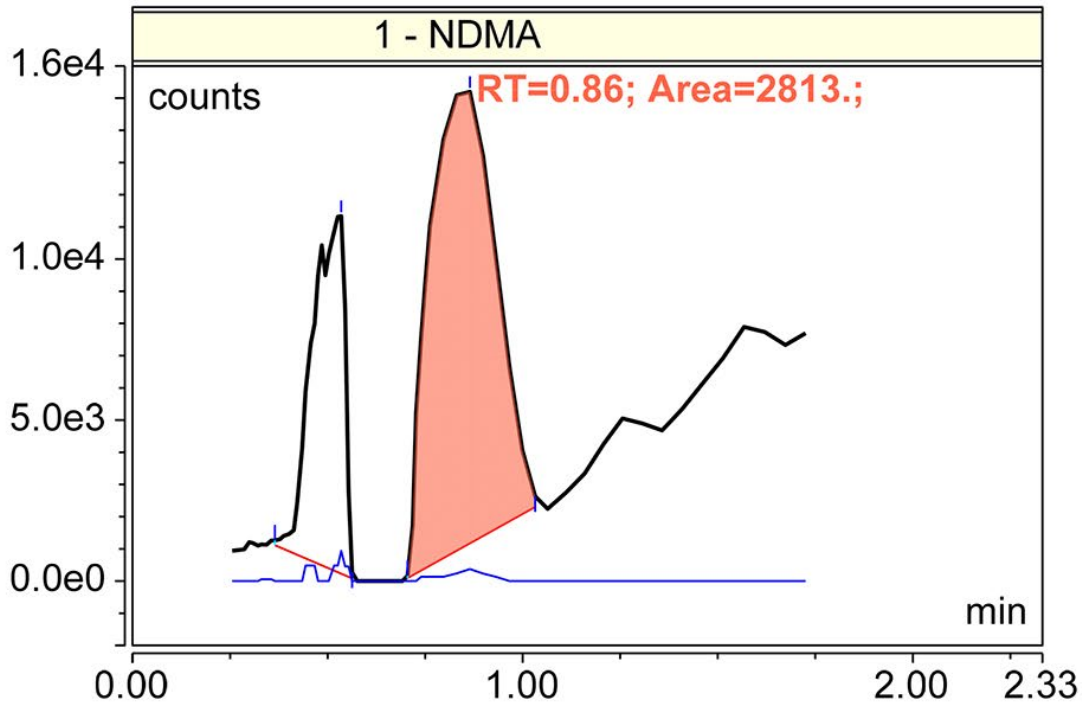
# Nitrosamine Impurity Levels in Ranitidine Drug Product

*NDMA in ranitidine drug substance and tablet. Data processed with a mass tolerance setting of 3 ppm.*

a) Ranitidine drug substance




b) Ranitidine drug tablet




The measured amount of NDMA in 30 mg/mL ranitidine drug substance exceeded the upper limit of calibration and was estimated to be more than 7 ppm

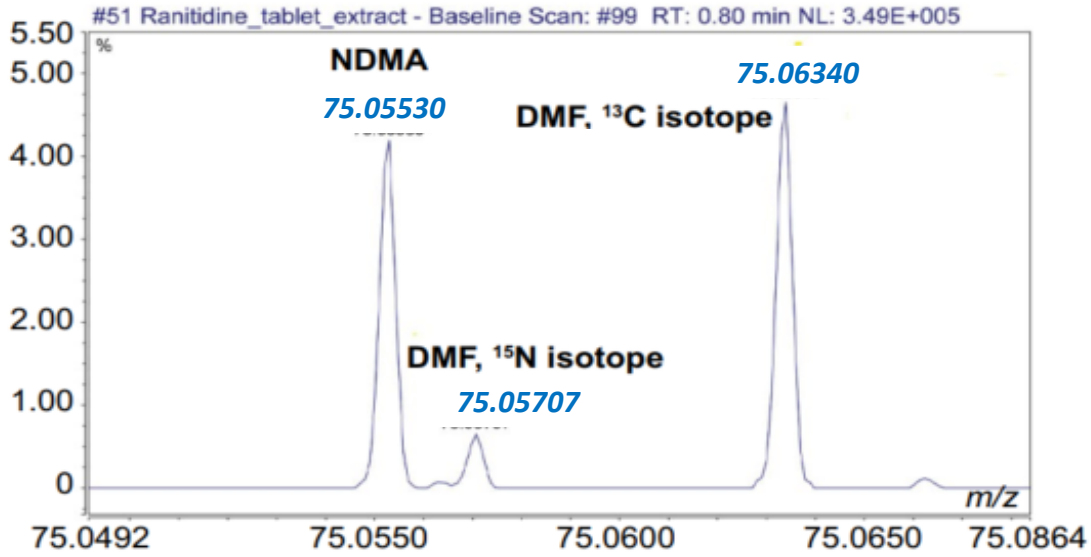
the measured amount of NDMA in 300 mg ranitidine tablet was 82 ppb

**Both exceeded the acceptable regulatory limit !**

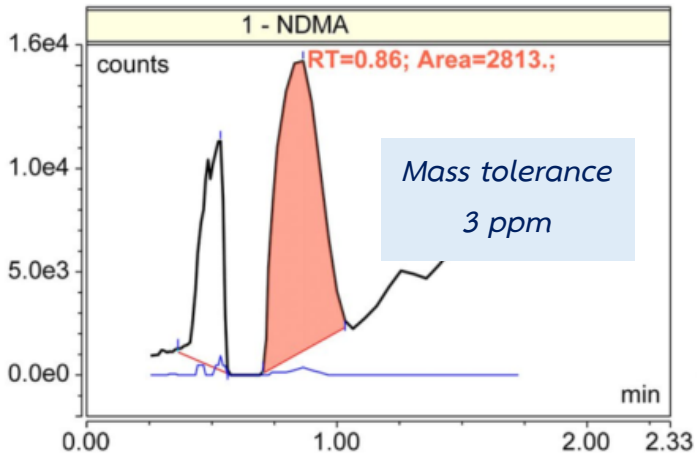
# Nitrosamine Impurity Levels in Ranitidine Drug Product

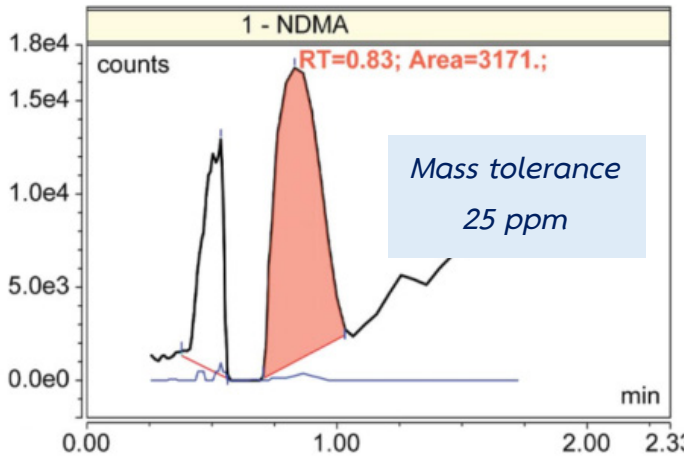
- Mass spectrum of ranitidine drug tablet showing co-elution of NDMA and N,N-dimethylformamide (DMF), which could cause overestimation of NDMA when inadequate mass tolerance setting was used for data processing
- The mass difference between NDMA and DMF $^{15}N$ isotope is only 21 ppm
- DMF in pharmaceuticals is allowed to be up to 880 ppm as per the ICH Q3C(R6) guideline.
- A minimum resolution setting of 45,000 and a maximum mass tolerance of 15 ppm are required to prevent overestimation of NDMA when quantifying NDMA using the monoisotopic ion.

*Spectrum of ranitidine drug tablet sample containing NDMA and DMF with a resolution setting of 120,000*




Overestimation of NDMA when processing the data with mass tolerance set at 25 ppm; the resultant quantitation is 13% higher as compared with results obtained at 3 ppm



# Conclusions

- A rapid, highly selective, and sensitive method was developed using the Acclaim Polar Advantage II column, Vanquish Horizon UHPLC system and Orbitrap Exploris 120 mass spectrometer for detection and quantitation of nine nitrosamines in commercially available ranitidine drug products.
- By combining the robust and reproducible chromatography with the 120,000 mass resolving power, fast scanning speed, and sub-ppm mass accuracy of the Orbitrap Exploris 120 system, the resultant method can provide reliable and confident quantitation of nine nitrosamine impurities to meet the September 2020 US FDA regulatory acceptance limits.

# References

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APPLICATION NOTE 73814

## HRAM LC-MS method for the determination of nitrosamine impurities in drugs

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Keywords: Nitrosamines, NDMA, APCI, high resolution accurate mass, mass spectrometry, Orbitrap Exploris 120, Chromatography Data System, compliance-ready, generic drugs, impurities, genotoxic impurities, ranitidine, excipient, tSIM, tMS²

**Application benefits**

- Detection and quantification of nine nitrosamines with a single liquid chromatography-high resolution accurate mass (HRAM) mass spectrometry method
- Quantitation of nitrosamine impurities in ranitidine drug substance and product below the daily acceptable intake level, that meets the requirements of FDA regulatory guidelines

- Use of Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for both data collection and processing in a 21 CFR 11 compliant environment with full data integrity and security capabilities for cGMP facilities

**Goal**

To demonstrate fast, highly sensitive quantitation of nine nitrosamines with a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer, and the use of the LC-MS method to measure nitrosamine impurities in commercially available ranitidine drug substances and products

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