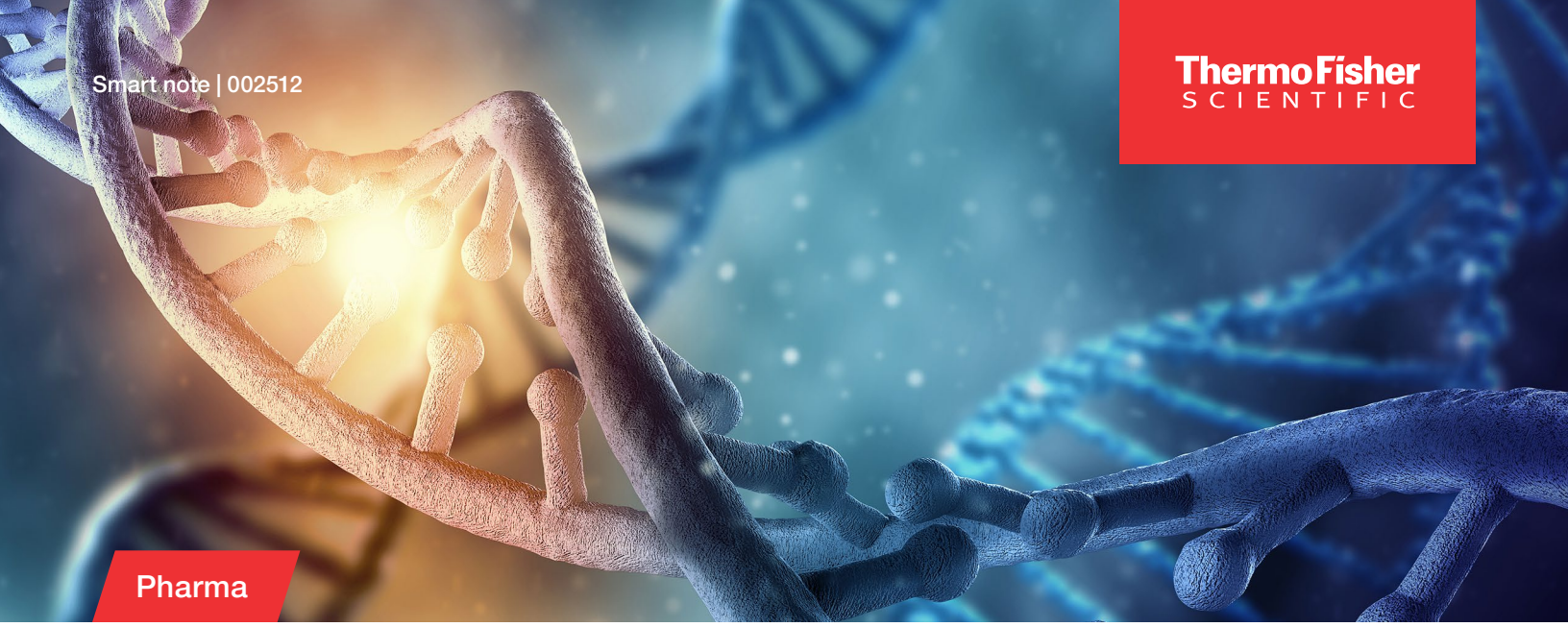


Analysis of genotoxic impurities in pharmaceuticals

Accurate, reliable, and confident quantification with high-resolution mass spectrometry

Genotoxic impurities—nitrosamines, azido, and nitroso drug substance related impurities: analytical challenges and an accurate and reliable solution to analyze them in various pharmaceuticals

Introduction

Genotoxic impurities

Genotoxic impurities (GTIs) can be broadly defined as impurities that have been demonstrated to cause deleterious changes in the genetic material, regardless of the mechanism, as per the International Council for Harmonisation (ICH) S2 (R1) Guidelines.[1] GTIs can form during the synthesis of an active pharmaceutical ingredient (API) that might require the use of reactive raw materials, which have the potential to interact with human DNA to cause mutations and cancer, even at the lowest levels. Therefore, GTIs should be avoided, or if that is not possible, reduced below a defined threshold. This may pose a challenge in manufacturing products; thus, frequent assessment of drug products for presence of GTIs is needed.

Product recalls due to GTIs

Since 2018, several products have faced recall due to greater than acceptable amounts of GTIs present, for example, general nitrosamines or nitrosamine drug substance related impurities (NDSRIs).[2] Ranitidine, metformin, valsartan, irbesartan, varenicline, quinapril, and others were recalled from the market due to GTI amounts higher than acceptable limits. The current focus, therefore, is to select the best of the techniques available today for accurate and reliable determination of GTIs in APIs and drug products.

thermo scientific

Analytical challenges

Aptly, for an accurate and versatile way of assessing GTIs, the market is greatly inclined towards LC-MS technology, which has the potential to provide a solution that is trustworthy, can detect multiple GTIs in one go, fulfills the regulatory requirements by having compliant tools, and provides complete confidence on data integrity principles. Several contemporary requirements and solutions from Thermo Fisher Scientific are addressed below.

Sensitivity: The level of GTIs to be analyzed is lower than an absolute concentration of 1 ng/mL in most cases. Low LOQ and LOD levels must be achieved, which poses a challenge in terms of sensitivity offered by the LC-MS solution in place. Therefore, more sensitive MS solutions are required to reach the desired sensitivity limits.

Selectivity: Most of the analytical methods are required to accurately measure multiple GTIs simultaneously. Therefore, sufficient mass accuracy and selectivity of the instrument are needed to accurately identify, determine the molecule of interest, and avoid any false positive data generation. Hence, the analytical instrument should give high selectivity and high throughput performance.

Compliance with regulatory requirements: Inevitably, the LC-MS instrument being used to determine GTIs should meet all regulatory requirements corresponding to data integrity principles.

As a trusted partner for pharmaceutical analysis and quality control, Thermo Fisher Scientific offers a best-in-class solution meeting the above requirements and overcoming the challenges for the analysis of GTIs.

Liquid chromatography and high-resolution mass spectrometry

Thermo Scientific™ Vanquish™ Flex UHPLC systems enable increased productivity and drive innovation without compromising quality. Like all members of the Vanquish product line, Vanquish Flex UHPLC systems offer hardware precision, detector sensitivity, and simplicity of operation.

The Vanquish UHPLC system incorporates SmartFlow™ pumping technology with automatic compensation for changes in eluent compressibility to ensure flow and gradient precision, independent of eluent composition and backpressure.

The fluidic pathway of the Vanquish Flex UHPLC system is fully biocompatible and fitted with Thermo Scientific™ Viper™ fingertight fittings, which support near-zero dead volume connections and enable sharper peaks and tool-free setup. The unique combination of active and/or passive eluent pre-heating and two column thermostatting modes—still and forced air—ensures reliable matching of the eluent and column temperature for superior UHPLC performance and allows for easy LC method transfer. The Thermo Scientific™ Vanquish™ Split Sampler FT offers outstanding speed and injection precision using Thermo Scientific SmartInject technology.

The Vanquish Flex UHPLC system integrates with the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) with compliance tools, networking capabilities, automation, and data processing. The **Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer** is designed for operational simplicity and sets a new standard in instrument productivity and ruggedness. It accelerates qualitative and quantitative confidence with consistently accurate data delivered by proven Orbitrap mass analyzer technology, whether it is for method development or everyday testing.

The next generation Thermo Scientific™ OptaMax™ source housing enables use of ESI or APCI probes, accommodating many ionization methods to broaden analytical capabilities. The robust and sensitive ion source interface, coupled to a round bore (removable) ion transfer tube and progressively spaced stacked-ring ion guide (S-lens) with a resolving injection filter, delivers high transmission and focusing of ions. Figure 1 shows the ion optics of the Orbitrap Exploris 120 mass spectrometer.

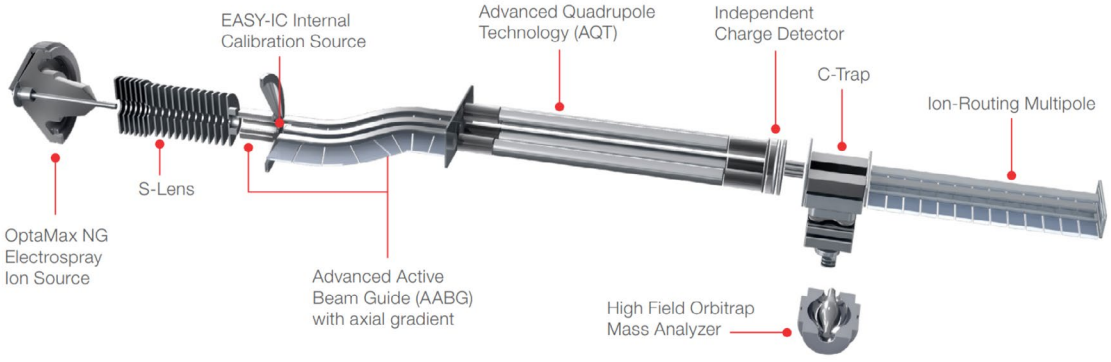


Figure 1. Schematic of the Orbitrap Exploris 120 mass spectrometer with key technology highlighted

The Orbitrap Exploris 120 mass spectrometer routinely achieves sub-3 ppm accuracy for most applications over a wide dynamic concentration range. With the standard Thermo Scientific™ EASY-IC™ (internal calibration) system, sub-ppm mass accuracies are achieved with automated introduction of an internal reference mass during sample analysis.

The segmented quadrupole mass filter allows for highly specific precursor ion selection with variable precursor isolation width starting as low as 0.4 Da for ions <*m/z* 400, while maintaining excellent ion transmission.

The ion routing multipole allows accumulation and distribution of ions within the system. It retains and fragments using higher energy collisional dissociation (HCD) or sends to the C-Trap for injection into the Orbitrap mass analyzer.

The high field Orbitrap mass analyzer belongs to the next generation of quadrupole-Orbitrap instruments. It provides resolution up to 120,000 (FWHM) at *m/z* 200. A wider mass range of 40 to 3,000 *m/z* and the capability of Full scan, targeted SIM, and targeted MS/MS experiments makes the Orbitrap Exploris 120 mass spectrometer a fully versatile, accurate, and reliable instrument for confident quantification of analytes of interest.

Chromeleon CDS enables compliance with current regulatory guidelines, such as 21 CFR Part 11. Owing to all these capabilities, the Orbitrap Exploris 120 mass spectrometer coupled to Vanquish HPLC/UHPLC systems, Thermo Scientific LC columns, and Chromeleon software provides the best single-vendor solution for the quantitation of nitrosamines, azido, and NDSRIs impurities from various drug substances and products.

Sensitivity, selectivity, robustness, and mass accuracy

For targeted quantitation, sensitivity, selectivity, and robustness are key. The Orbitrap Exploris 120 system makes this possible with the high field Orbitrap mass analyzer offering resolution settings up to 120,000 and high sensitivity and selectivity for the quantitation of a wide range of trace level impurities. Further it offers multiple ways of quantitative analysis, e.g., targeted SIM (tSIM) on the precursor ion, product ion scan/targeted MS/MS (tMS^2) on fragment ions, or a combination of tSIM and tMS^2 in the same method of analysis, if required, depending upon the different ionization patterns of various molecules.

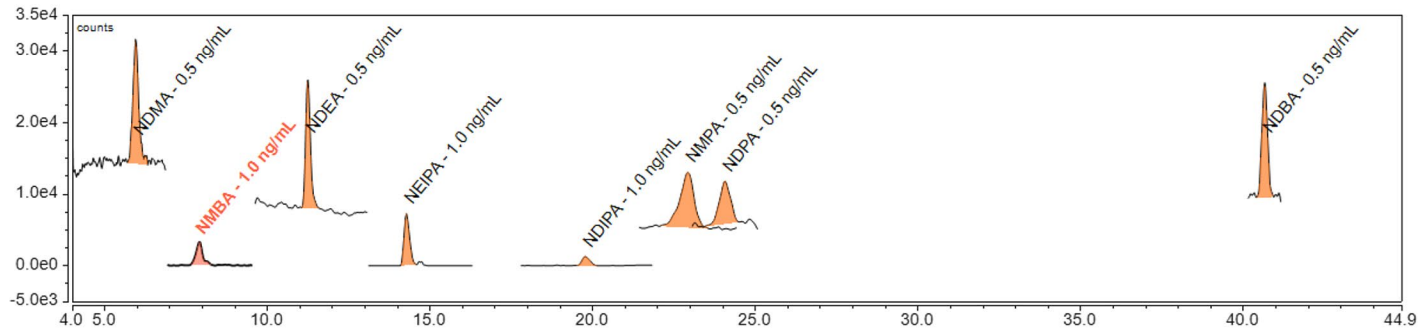
Table 1 showcases the highly sensitive and selective performance of the Orbitrap Exploris 120 mass spectrometer for quantitation of GTIs such as general nitrosamines, azido, and NDSRIs.

The outstanding robustness results, in terms of peak area response for eight nitrosamines up to 40 hours of continuous injections, as presented in Figure 2, ensures maximum productivity by delivering consistent results, and the excellent chromatographic separation between peaks of GTI was achieved by unmatched performance of the Thermo Scientific™ Acclaim™ 120 C18 column, 4.6 mm × 150 mm, 3 µm. Figure 3 represents the excellent linearity achieved for all GTIs with the high-throughput performance. Also, the easy to maintain design facilitates user-level cleaning and maintenance of ion source components that further helps avoid unwanted downtimes associated with troubleshooting and out of specification results.

Table 1. List of highly sensitive methods with 120,000 resolution

Sr. no.	Sample information	No. of impurities	Names of impurities	Scan type	LOD (ng/mL)	LOQ (ng/mL)	Spec limit (ng/mL)	Linearity range (ng/mL)
1	Ipratropium bromide + albuterol sulphate (water-based formulation)	8	NDMA, NMBA, NDEA, NEIPA, NDIPA, NDPA, NMPA, NDBA	tMS^2	0.3	0.5	1	0.5 to 1.5
2	Metformin + sitagliptin (tablets)	1	NDMA	tMS^2	0.2	0.5	3	0.5 to 30
3	Tolterodine tartrate (tablets)	8	NDMA, NDEA, NDPA, NMPA, NDBA	tMS^2	0.15	0.5	3	0.5 to 30
			NMBA, NEIPA, NDIPA		0.3	1		1 to 30
4	Lumefantrine + artemether (tablets)	1	NDBA	tMS^2	0.1	0.3	1	0.3 to 20
5	Candesartan, irbesartan, losartan, olmesartan, telmisartan and valsartan (tablets)	1	AZBT	tSIM	0.025	0.25	5	0.25 to 40
6	Bictegravir sodium (API)	1	NMBA	tSIM	0.075	0.15	0.9	0.15 to 45
7	Dydrogesterone (tablets)	7	NDMA, NMBA	tMS^2	2.054	4.107	13.691	4.107 to 41.125
			NDEA, NEIPA, NDIPA, MeNP, NDBA		0.568	1.136	3.787	1.136 to 11.375
8	Darunavir ethanolate (tablets)	1	Darunavir Impurity I (NDSRIS)	tMS^2	0.0625	0.125	1.25	0.125 to 3.75
9	Cinacalcet HCl (tablets)	1	N-Nitroso Cinacalcet (NDSRIS)	tSIM	-	1	-	1 to 20

(A)



(B)

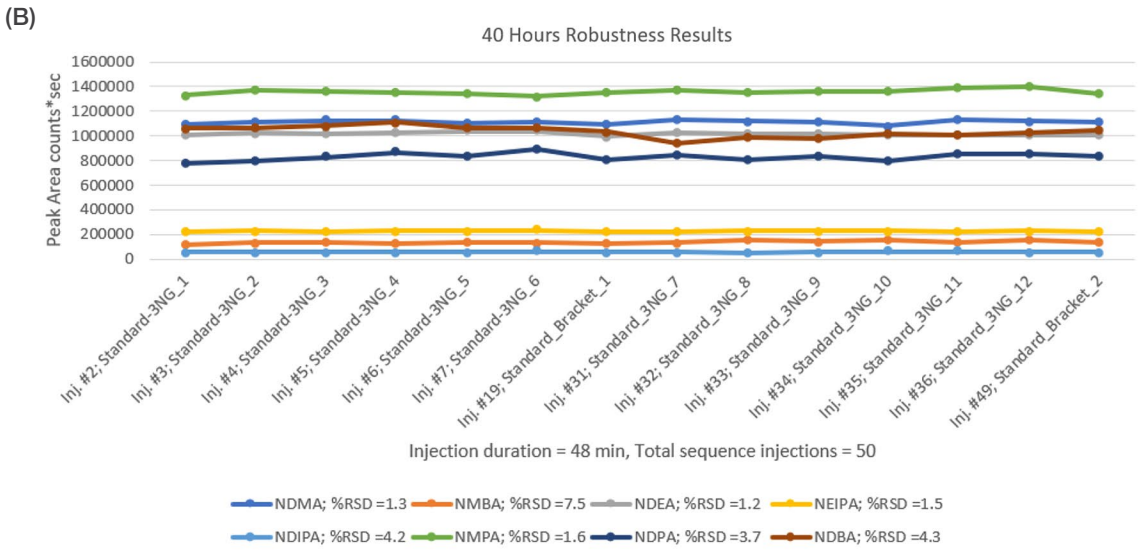


Figure 2. (A) LOQ level chromatogram – Eight nitrosamines in tolterodine tartrate tablets, (B) robustness chart

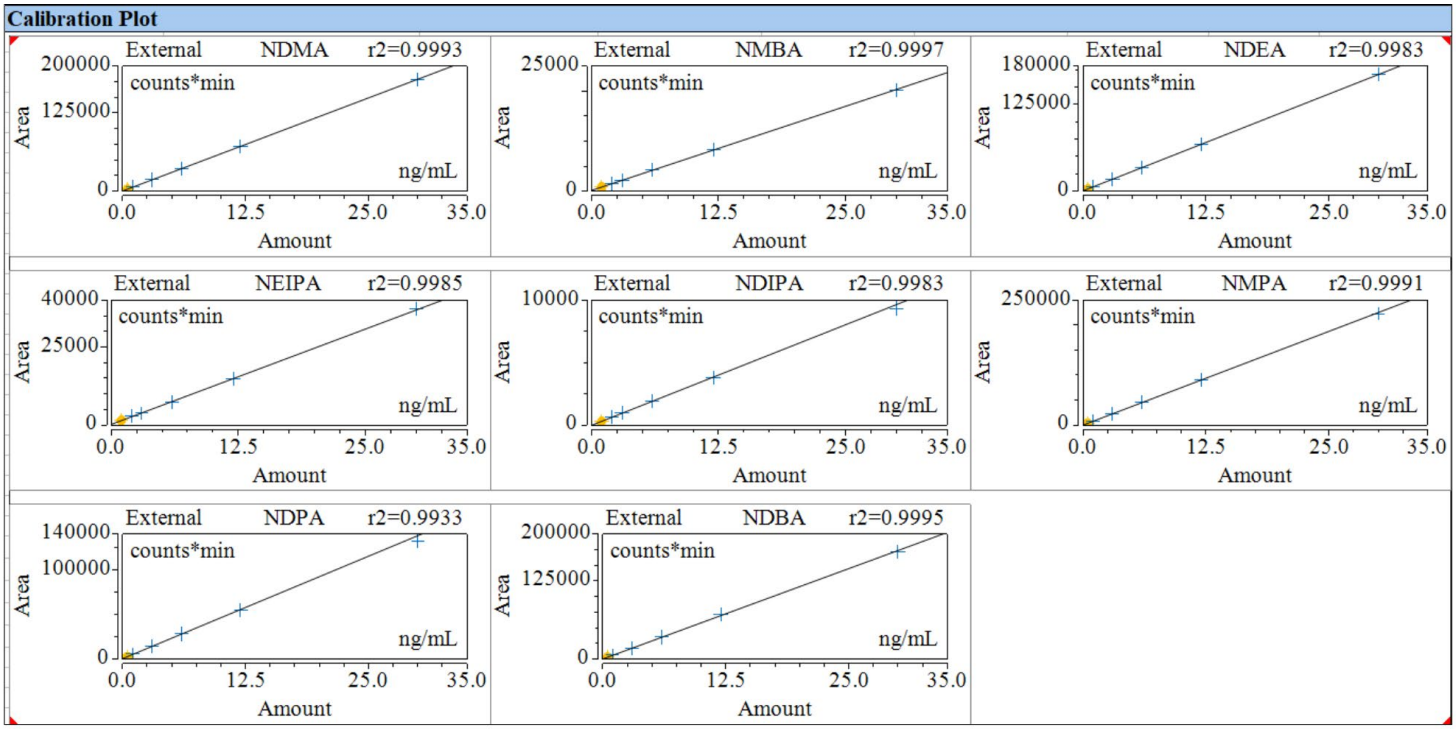


Figure 3. Linearity – Eight nitrosamines in the method of tolterodine tartrate

With respect to mass accuracy in the Orbitrap Exploris 120 mass spectrometer, external calibration achieves <3 ppm RMS drift over 24 hours; internal lock mass calibration achieves <1 ppm RMS drift over 24 hours; EASY-IC achieves <1 ppm RMS drift for at least 5 days. In addition, the One-Point (Self) Mass Calibration achieves <3 ppm RMS drift over at least 4 weeks. This exceptional mass accuracy ensures optimal confidence in the analytical results.

Compliance

Compliance with regulatory requirements is critical for many laboratories and needs to be implemented and maintained without compromising the productivity of the lab. Chromeleon CDS is built to satisfy these needs for efficiency and data integrity. It provides secure data management capabilities with comprehensive audit trails and electronic signatures to ensure the traceability of data and comply with 21 CFR Part 11/Annex 11 regulations, as well as FDA, EMA, MHRA, and PIC/S requirements. Figure 4 shows the comprehensive audit trail feature including extensive audit trail areas of Instrument, Administration, and Data.

With one central implementation for both chromatography and MS workflows, Chromeleon CDS simplifies repetitive tasks, reduces errors, and offers advanced reporting capabilities, enabling users to easily create and share reports with stakeholders, further streamlining laboratory operations.

The combination of the Vanquish Flex UHPLC, Orbitrap Exploris 120 mass spectrometer, and Chromeleon CDS provides a complete and compliance-ready solution for laboratory workflows, offering productivity, efficiency, and data security while meeting regulatory requirements.

Chromatography and consumables

To address the best performance of an LC-MS instrument, two vital factors are the quality of chemicals, solvents, or other consumables used and the appropriate utilization of LC-MS with due precautions in place.

Fisher Scientific™ Optima™ LC-MS grade highly pure chemicals and solvents play a pertinent role in sample/mobile phase preparations as well as in achieving low chemical noise and good signal-to-noise ratios with GTIs. In addition, a divert valve installed with the LC-MS helps to protect the mass spectrometer from very high concentrations of the drug sample, which is unwanted and may pose serious contamination issues that further calls for instrument downtime. The proper utilization of a divert valve can be seen in Figure 5 where the impurity to be analyzed is sent to the mass spectrometer and the remaining portion goes into UV/waste. In this case, the magnificent chromatographic separation between the azido impurity and six sartan drug substances was achieved with the Thermo Scientific™ Accucore™ Biphenyl column, 2.1 mm × 100 mm, 2.6 µm, that resulted in creation of a robust method of analysis.

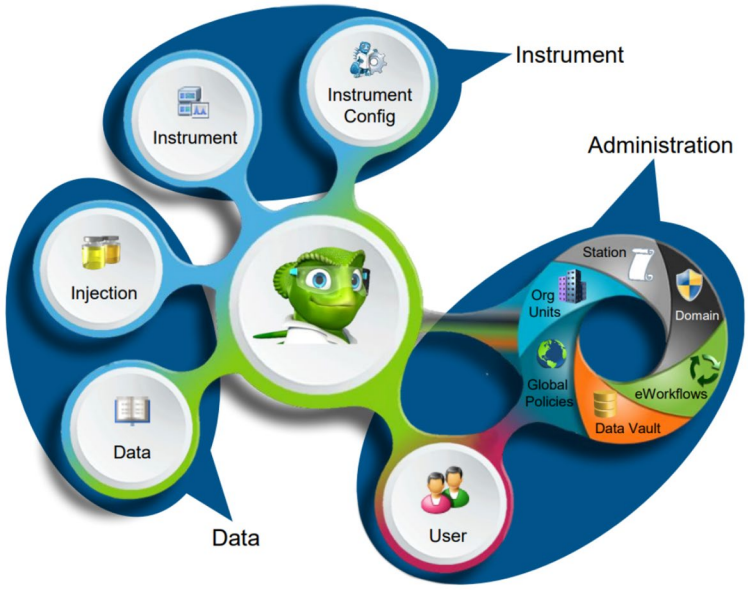


Figure 4. Comprehensive audit trail features of Chromeleon CDS including eleven audit trail areas and three main audit trails (Instrument, Administration, and Data)

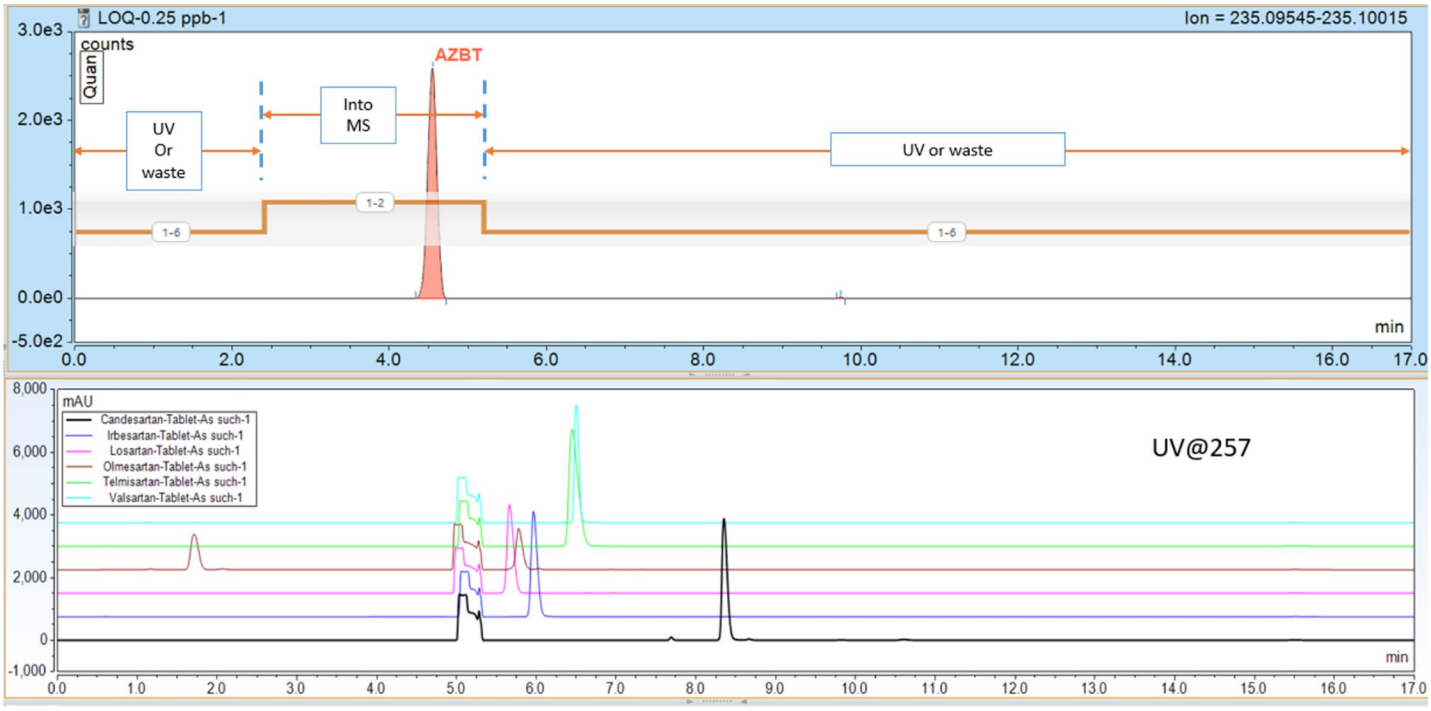


Figure 5. AZBT impurity in six sartans; MS and UV chromatograms with divert valve setup to protect the mass spectrometer

To explain further, HPLC/UHPLC columns play an unmatched role, providing proper peak shapes to impurities and separating them out from the peak of a drug sample. Although, to achieve this, method development is essential. Figure 6 shows seven nitrosamines are properly resolved from the peak of drug sample with an Acclaim 120 C18 column, 4.6 mm × 150 mm, 3 µm. This also helps in appropriate setup of the divert valve program where the desired portion of the sample flow (containing only impurities) goes into the mass spectrometer and the portion containing API goes either to the UV or waste, thereby protecting the system from being contaminated by the high concentration of the sample.

Besides the above, for the current combination of the Vanquish Flex UHPLC and Orbitrap Exploris 120 mass spectrometer, we deployed various column chemistries to achieve desired results with various methods. These HPLC columns with specified dimensions have been observed to show a high level of reproducibility and robustness and that is how these are the preferred choices for GTIs, azido, and NDSRIs analysis. All the columns as well as the other consumables that are used in sample preparation are also listed here.

- Thermo Scientific™ Acclaim™ 120 C18 column, 4.6 mm × 150 mm, 3 µm (P/N 059133)
- Thermo Scientific™ Accucore™ C18 column, 4.6 mm × 150 mm, 2.6 µm (P/N 17126-154630)
- Thermo Scientific™ Accucore™ Biphenyl column, 2.1 mm × 100 mm, 2.6 µm (P/N 17826-102130)
- Thermo Scientific™ Acclaim™ Mixed Mode WCX-1 column, 3 mm × 150 mm, 3 µm (P/N 070092)
- Thermo Scientific™ Hypersil GOLD™ C18 selectivity columns, 4.6 mm × 250 mm, 5 µm (P/N 25005-254630)
- Formic acid, Optima™ LC/MS grade, Fisher Chemical™ (Fisher Scientific P/N A117-50 or equivalent)
- Methanol, Optima™ LC/MS grade, Fisher Chemical™ (Fisher Scientific P/N A456-4 or equivalent)
- Water, Optima™ LC/MS grade, Fisher Chemical™ (Fisher Scientific P/N AAB-W6-4 or equivalent)
- Invitrogen™ 2 mL microcentrifuge tubes (P/N AM12475)
- Thermo Scientific™ Titan3™ 0.2 µm PVDF syringe filters (P/N 42213-PV)
- Thermo Scientific™ SureSTART™ 2 mL GOLD-grade glass screw top vials (P/N 6PSV9-1PG)
- Thermo Scientific™ SureSTART™ 9 mm screw caps (P/N 6PSC9TST)
- Thermo Scientific™ Nunc™ 15 mL extraction/conical sterile polypropylene centrifuge tubes (P/N 339650)

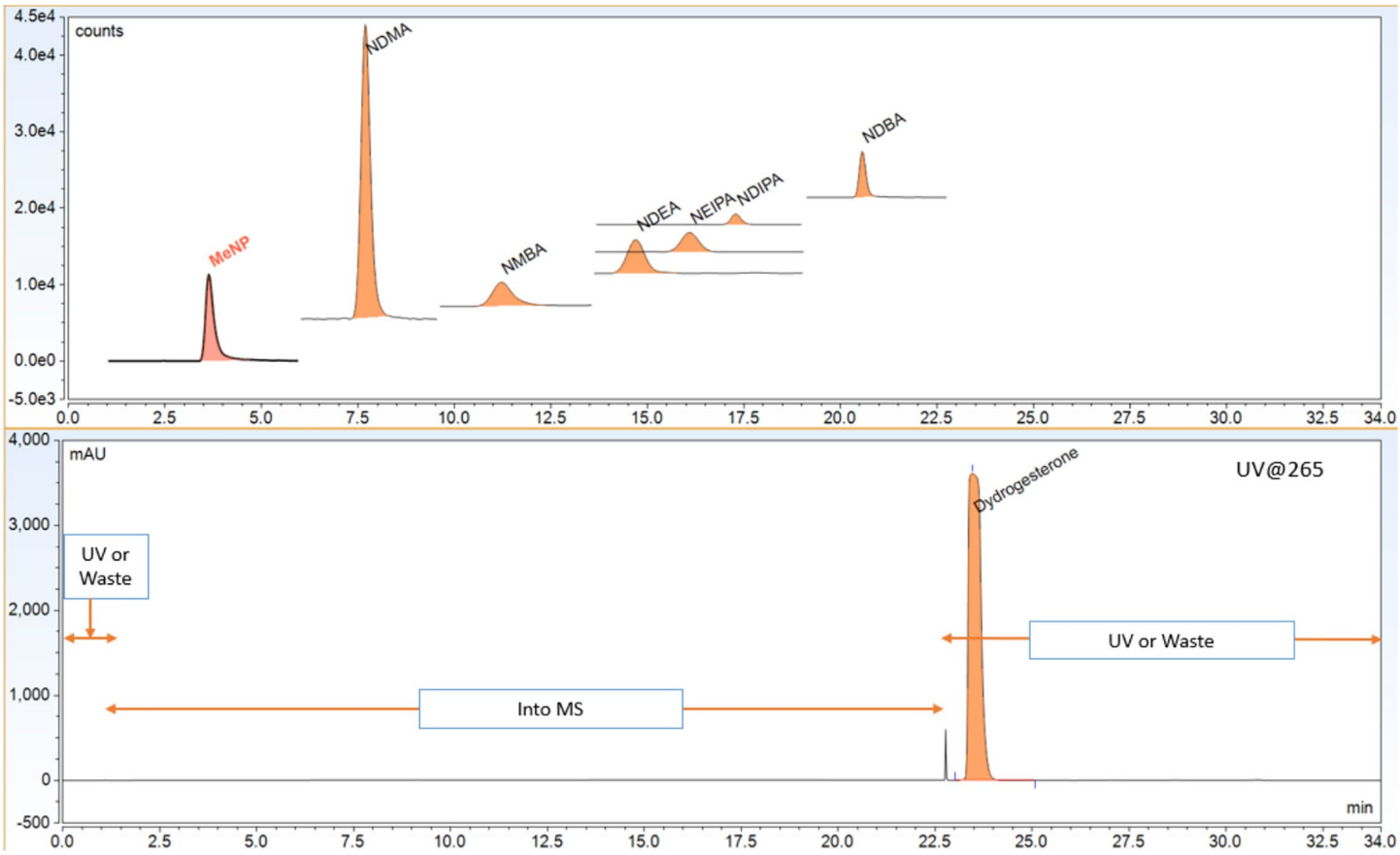


Figure 6. Chromatographic separation between seven nitrosamines and dydrogesterone drug substance

Summary

Thermo Fisher Scientific provides a complete and comprehensive solution to the accurate and reliable quantification of genotoxic impurities above and beyond general nitrosamines and NDSRIs. This was achieved using an unmatched configuration of the Vanquish Flex UHPLC system coupled with the Orbitrap Exploris 120 mass spectrometer with Orbitrap high-resolution accurate-mass technology. In terms of chromatographic capabilities, the Thermo Scientific HPLC columns with exact suitable column chemistry and proven performance in terms of retentivity and selectivity drive the faster development of liquid chromatography-mass spectrometry based methods for the determination of genotoxic impurities in drug substances and drug formulation products.

References

1. https://www.europeanpharmaceuticalreview.com/article/108031/genotoxic-impurities-in-pharmaceutical-products/
2. https://www.pharmaceuticalprocessingworld.com/10-prominent-drugs-recalled-over-nitrosamines/

Useful links to different solutions provided by Thermo Fisher Scientific for nitrosamines analysis

- Thermo Fisher Scientific's solution to the interference induced by DMF (dimethyl formamide) leading to false positive estimation of NDMA in drug products:
  - HRAM LC-MS method for the determination of nitrosamine impurities in drugs
  - Reliable quantitation of 11 nitrosamine impurities in metformin drug products using Orbitrap Exploris 120 mass spectrometry
- Nitrosamine impurities analysis solution guide
- Columns and chromatography solutions for nitrosamine impurity analysis
- Dedicated blog on nitrosamine analysis solution – Analyte Guru
- A validated LC-HRAM-MS method for the rapid and confident determination of azido (AZBT) impurity in sartan drug products

For more information, please contact:

Varun Khali, Thermo Fisher Scientific, India
varun.khali@thermofisher.com

Manoj M. Kushwaha, Thermo Fisher Scientific, India
manoj.m.kushwaha@thermofisher.com

Sachin Pandey, Thermo Fisher Scientific, India
sachin.pandey@thermofisher.com

Deepti Bhandarkar, Thermo Fisher Scientific, India
deepti.bhandarkar@thermofisher.com

Biswajayee A. Patra, Thermo Fisher Scientific, India
biswajayee.patra@thermofisher.com

Devendra Kamat, Thermo Fisher Scientific, India
devendra.kamat@thermofisher.com

Aaron Lamb, Thermo Fisher Scientific, UK
aaron.lamb@thermofisher.com

Acknowledgments

Varun Khali, Thermo Fisher Scientific, India

Sachin Pandey, Thermo Fisher Scientific, India

Manoj Kushwaha, Thermo Fisher Scientific, India

Deepti Bhandarkar, Thermo Fisher Scientific, India

Saravanan Kumar, Thermo Fisher Scientific, India

Biswajayee Patra, Thermo Fisher Scientific, India

Sunil T. Kumar, Thermo Fisher Scientific, India

Sarthak Gupta, Thermo Fisher Scientific, India

Devendra Kamat, Thermo Fisher Scientific, India

Aaron Lamb, Thermo Fisher Scientific, UK

Alan Atkins, Thermo Fisher Scientific, UK

Jon Bardsley, Thermo Fisher Scientific, UK

Darren Barrington-Light, Thermo Fisher Scientific, UK

Kelly Broster, Thermo Fisher Scientific, UK

Sukhdev Bangar, Thermo Fisher Scientific, UK

Scott Peterman, Thermo Fisher Scientific, USA

Min Du, Thermo Fisher Scientific, USA

Sven Hackbusch, Thermo Fisher Scientific, USA

Melissa Nemenz, Thermo Fisher Scientific, USA

Maci Wang, Thermo Fisher Scientific, USA

Hao Yang, Thermo Fisher Scientific, USA

Frank Steiner, Thermo Fisher Scientific, Germany

Mauro De Pra, Thermo Fisher Scientific, Germany

Susanne Fabel, Thermo Fisher Scientific, Germany

Kai Scheffler, Thermo Fisher Scientific, Germany

Sebastien Morin, Thermo Fisher Scientific, Canada

Kaja Gleerup, Thermo Fisher Scientific, Denmark

Lizzie Gallagher, Thermo Fisher Scientific, Ireland

Maciej Bromirski, Thermo Fisher Scientific, Poland

Learn more at **thermofisher.com/nitrosamines**