Smart note | 001952

Pharmaceuticals

Genotoxic impurity analysis

Confidence in compliance. Confidence in results.

Challenges and solutions for testing/analyzing nitrosamine, azido, and NDSRI impurities in drug substances and products

Liquid chromatography and mass spectrometry solutions

Overview

Nitrosamine impurities are a group of contaminants associated with genotoxic and carcinogenic attributes. Detection of nitrosamine impurities from the drug substance or the drug product has been a subject of concern since 2018 when batches of sartans were recalled due to the presence of unacceptable levels of *N*-nitrosodimethylamine (NDMA). Since then, several other nitrosamines impurities have been identified and are being investigated by regulators in various other drug substances/products, such as ranitidine, metformin, etc.

In 2020, batches of sartans were recalled by few of the pharmaceutical companies due to the presence of unacceptable levels of another impurity called azidomethyl-biphenyl-tetrazole (AZBT). Furthermore, in 2021, there were recalls of varenicline due to the presence of *N*-nitroso-varenicline which is a type of nitroso drug substance related impurity (NDSRI).

All these events have led pharmaceutical companies to proactively investigate nitrosamines, azido, or NDSRI impurities in their drug substances/products, especially those containing secondary, tertiary, or quaternary amines. The requirements associated with the analyses of carcinogenic/mutagenic impurities presents significant challenges such as:

- **Sensitivity:** quantitation of genotoxic/carcinogenic impurities at trace level in the presence of a high concentration of active pharmaceutical ingredients (API)
- **Selectivity:** complex composition of a drug formulation that can lead to certain chemical or isobaric interferences
- **High throughput:** since the number of impurities to be monitored are increasing day by day, having a single method for the analysis of all of these impurities reduces analysis time and improves productivity.
- **Compliance:** data acquisition and processing in compliant software to ensure data integrity and security.

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 analyses of genotoxic impurities.

Liquid chromatography and mass spectrometry solutions

The Thermo Scientific™ Vanquish™ HPLC and UHPLC systems are equipped with SmartFlow™ pumping technology, which includes automatic compensation for changing eluent compressibility to ensure excellent flow and gradient precision. SmartInject technology ensures maximum sample dosage precision and improved column lifetime. Two thermostatting modes of the column compartment module ensure even temperature throughout the analyses. The active eluent pre-heater temperature can be set independently of the compartment temperature for even better control of temperature inside the column. This can be relevant when significant viscous heating occurs in the column ($T_{pre\text{-}heater} < T_{column\ compartment}$) or methods with high column temperature and elevated flow rates ($T_{pre\text{-}heater} > T_{column\ compartment}$). Tool-free Thermo Scientific™ Viper™ Fingertight Fitting systems with near-zero dead volume enable easy operation. The result of all these features is operational simplicity, unrivaled reproducibility, and robustness. The family of Thermo Scientific™ LC columns, partnered with the Vanquish HPLC or UHPLC platform to take advantage of its extended pressure capabilities and robustness, allows optimization of the separations faster and more easily.

The Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer is designed for stable, reliable performance and incorporates innovative approaches to increase robustness and reduce maintenance. The Thermo Scientific™ OptaMax™ NG ion source (APCI ready) automates all gas and voltage connections for ease-of-use. It provides optimizable spray position for ultimate performance in HESI or APCI mode. An ion beam guide with neutral blocker provides robust performance and maximum sensitivity by blocking neutrals and efficiently transmitting ions. Segmented quadrupoles enhance ion transmission and consistency to ensure excellent selectivity for Selected Reaction Monitoring (SRM) and High Resolution-SRM (H-SRM with 0.4 Da FWHM precursor filtering) acquisition and reproducible results. An enhanced dual-mode discrete-dynode electron multiplier detector extends multiplier lifetime to maintain excellent linearity and dynamic range.

The Vanquish HPLC and UHPLC systems integrate seamlessly with the TSQ Quantis mass spectrometer controlled by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), which enables compliance with current regulatory guidelines, such as 21 CFR Part 11. Owing to all these capabilities, the TSQ Quantis mass spectrometer coupled to Vanquish HPLC/ UHPLC systems, Thermo Scientific LC columns, and Chromeleon CDS provides the best single-vendor solution for the quantitation of nitrosamines, azido, and NDSRI impurities from various drug substances and products.

High sensitivity results

In targeted quantitation, achieving the desired levels of sensitivity and robustness of the results are key. With its segmented quadrupoles, faster rod drivers, and improved electron multipliers, the TSQ Quantis mass spectrometer delivers unprecedented robustness with best-in-class sensitivity for everyday nitrosamines, azido, and NDSRI analysis. The outstanding robustness offered by the TSQ Quantis mass spectrometer ensures maximum productivity by delivering consistent results, minimizing the downtime associated with instrument maintenance/troubleshooting and reducing investigation time for out-of-specification results. Examples listed in Table 1 showcase the capability of the TSQ Quantis mass spectrometer for quantitation of these carcinogenic/mutagenic impurities and easily meeting the regulatory requirement.

High throughput

Superior acquisition speeds on the TSQ Quantis mass spectrometer, which can acquire 600 SRMs/s, allow acquisition of sufficient data points for an expanded number of target compounds, even with ultra-high performance liquid chromatography (UHPLC) separation. Examples shown in Figure 1 demonstrate the ability of the TSQ Quantis mass spectrometer for simultaneous analysis of eight nitrosamine (NSM) impurities along with *N*-nitroso-varenicline NDSRI. A single method for the analysis of several impurities eliminates the need to analyze the same samples multiple times for different impurities. Therefore, a greater number of sample analyses can be performed within the lab operation workday and throughput can be increased. Furthermore, as new impurities are to be added to the existing method in the future, the fast scan speed of the TSQ Quantis mass spectrometer could provide the needed flexibility to include them.

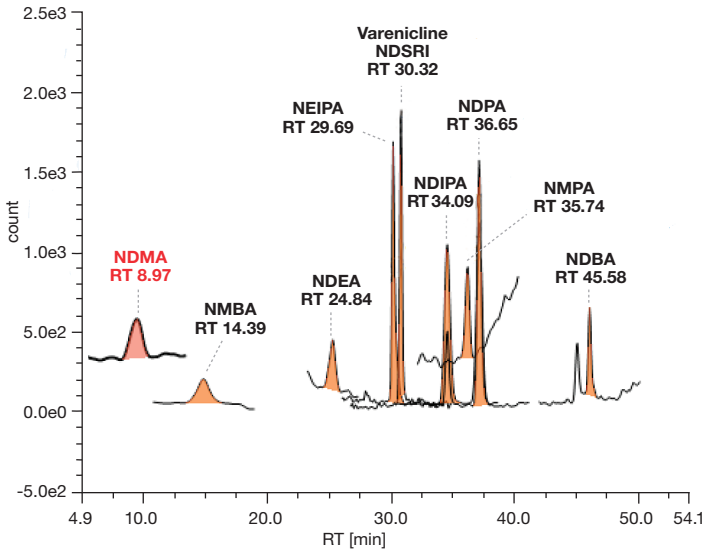

Figure 1. SRM chromatograms of eight NSM impurities and *N*-nitroso-varenicline neat standard at 1 ng/mL level

Table 1. Targeted screening results

Sr. no.	Name of the API	No. of impurities analyzed simultaneously	Name of the impurity analyzed	LOD (ng/mL) neat standard	LOQ (ng/mL) neat standard
1	Dapagliflozin and Metformin	Eight	NDMA, NMBA,NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA	NA	1 ng/mL
2	Lercarnidipine	Seven	NDMA, NMBA,NDEA, NEIPA, NDIPA, NDPA, NDBA	NA	1 ng/mL
3	Metformin	Ten	NDMA, NMOR, NMBA, NDEA, NPIP, NEIPA, NDIPA, NMPA, NDPA, NDBA	0.2 ng/mL (for NPIP, NEIPA) 0.4 ng/mL (for others)	0.5 ng/mL (for NPIP, NEIPA) 1 ng/mL (for others)
4	Valsartan and Losartan	Ten	NDMA, NMOR,NMBA, NDEA, NPIP, NEIPA, NDIPA,NMPA, NDPA, NDBA	0.4 ng/mL	1 ng/mL
5	Varenicline	Nine	NDMA, NMBA, NDEA,NEIPA, NDIPA, NMPA,NDPA,NDBA, *N*-nitroso-varenicline	0.5 ng/mL	1 ng/mL
6	Ciprofloxacin	Ten	NMBA, NDMA, NMOR, NDEA, NPIP, NEIPA, NDPA, NDIPA, NMPA, NDBA	NA	2.5 ng/mL (for NDMA, NMBA) 0.68 ng/mL (for others)
7	Candesartan, Irbesartan, Losartan, Omesartan, Telmisartan, and Valsartan	One	AZBT	0.025 ng/mL	0.5 ng/mL
8	Rifampicin	One	*N*-nitroso-methyl-piperazine	0.16 ng/mL	0.32 ng/mL

Selectivity

The TSQ Quantis mass spectrometer delivers excellent quantitative performance in both SRM and H-SRM for fit-for-purpose selectivity. The enhanced active Q2 collision cell improves product ion transmission, especially for low-mass product ions, which can increase detection efficiency. H-SRM mode with a Q1 resolution of 0.4 Da FWHM helps in **minimizing the baseline noise**, which in turn **enhances the detection limit** for certain nitrosamines. An example of simultaneous quantitation of ten NSM impurities spiked at 3 ng/mL level from metformin drug product with 0.4 Da FWHM for Q1 resolution highlights the H-SRM capability of TSQ Quantis mass spectrometer (Figure 2).

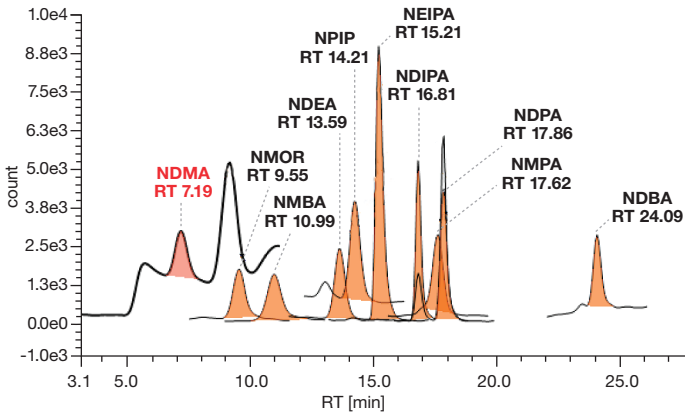

Figure 2. H-SRM chromatogram (with Q1 resolution of 0.4 Da FWHM) of 10 NSM impurities spiked at 3 ng/mL level in a metformin tablet sample

Compliance

Compliance with regulatory requirements is critical for many laboratories. It needs to be implemented and maintained without compromising the productivity of the lab. Chromeleon 7 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.

With one central implementation for both chromatography and MS workflows, Chromeleon software 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 TSQ Quantis mass spectrometer and Chromeleon CDS provides a complete and compliant solution for laboratory workflows, offering productivity, efficiency, and data security while meeting regulatory requirements.

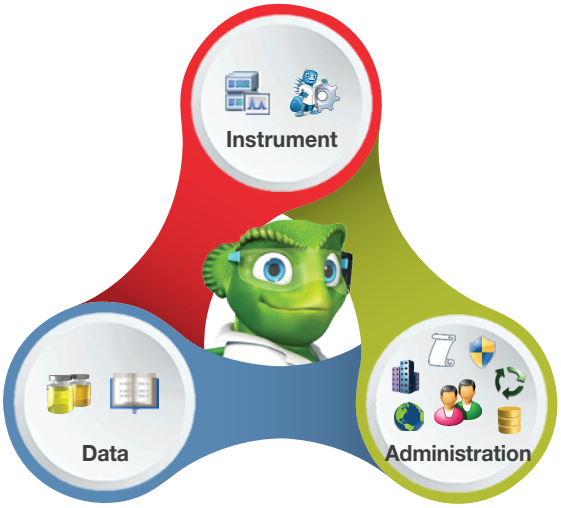

Figure 3. Chromeleon CDS provides comprehensive audits trails in all functional areas for both chromatography and MS workflows

Columns, solvents, and consumables

Besides the performance of a mass spectrometer, a few other factors are crucial for the carcinogenic/mutagenic impurity analyses from drug substance/product, such as the chromatographic performance. It is imperative to separate the drug from the impurities to divert high concentration of drug into waste and prevent the contamination of the mass spectrometer. Selection of the right column chemistry with the right dimensions plays a vital role for developing such methods. Moreover, selection of a correct column chemistry can sometimes allow using a single method for quantification of genotoxic impurities from different drug substances and products. The example shown in Figure 4 is of AZBT impurity analysis from six different sartan drug substances/products and increasing throughput.

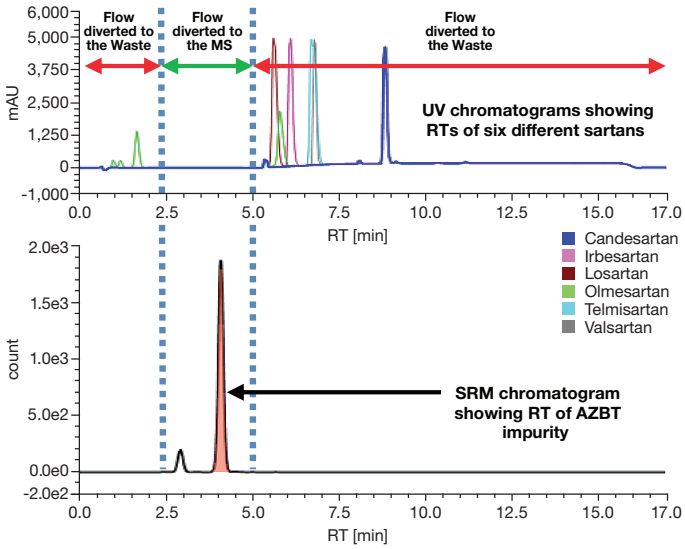

Figure 4. UV and SRM chromatograms showing separation of AZBT impurity from six different sartan drugs

Another important factor is the quality of the solvents used for mobile phase or sample preparation in case of such trace level quantitation of genotoxic impurities. Poor quality of solvents can lead to higher baseline noise, ion suppression, chemical interferences, and so on, which can negatively impact the analyses. Hence, using high quality LC/MS grade solvents is of the utmost importance for such analyses. Different column chemistries useful for method development and LC/MS grade solvents that ensure high quality of data, along with a few other consumables useful for such analyses are listed here:

- Thermo Scientific™ Acclaim™ 120 C18 column, 4.6 × 150 mm, 3 µm (P/N 059133)
- Thermo Scientific™ Accucore™ C18 column, 4.6 × 150 mm, 2.6 µm (P/N 17126-154630)
- Thermo Scientific™ Accucore™ Biphenyl column, 2.1 × 100 mm, 2.6 µm (P/N 17826-102130)
- Thermo Scientific™ Acclaim™ Mixed Mode WCX-1 column, 3 × 150 mm, 3 µm (P/N 070092)
- Thermo Scientific™ Hypersil GOLD™ C18 selectivity columns, 4.6 × 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™ Nunc™ 15 mL extraction/conical sterile polypropylene centrifuge tubes

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

- Highly sensitive and robust LC-MS/MS solution for quantitation of nitrosamine impurities in metformin drug products
- LC-MS/MS method for the quantification of 10 nitrosamine impurities in metformin
- Nitrosamine impurities analysis solution guide
- Columns and chromatography solutions for nitrosamine impurity analysis
- Dedicated blog on Nitrosamine analysis solution – Analyte Guru
- Sensitive and robust determination of genotoxic AZBT impurity in six sartan drug products

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