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Routine, Ultra-Trace Analysis of Nitrosamines in Drugs using Gas-Chromatography – Orbitrap Mass Spectrometry

Dominic Roberts, Giulia Riccardino, Aaron Lamb, and Cristian I. Cojocariu

Thermo Fisher Scientific, Runcorn, UK

ABSTRACT

The Orbitrap technology was coupled with static headspace sampling to assess volatile impurities in in Valsartan and Metformin. Data was acquired in single ion monitoring (SIM) and full-scan (FS) mode allowing for both quantitative analysis of nitrosamine impurities with compliance to the FDA method requirements[1] and screening of other contaminants that can be present in the pharmaceutical products.

INTRODUCTION

Nitrosamine impurities were discovered in July 2018 when Valsartan, an angiotensin II receptor blocker, was recalled due to the presence of N-Nitrosodimethylamine (NDMA) contamination. Nitrosamines are considered a matter of concern as they are classified mutagenic carcinogens. They are formed by reaction of secondary or tertiary amines with a nitrosating agent (e.g., sodium nitrite ($NaNO_2$)) Nitrites or amines can be present as unintentional contaminants of raw materials, reagents and solvents used during the production processes and they can result in the formation of nitrosamine impurities in the final products. One of the main challenges in nitrosamines analysis is related to the high sensitivity that must be achieved as these impurities usually occur at low levels. The USFDA has published several analytical methods that may be considered when determining nitrosamine content in the API or FPP. These methods include both liquid and gas chromatography coupled with single or triple quadrupole mass analyzers to provide the sensitivity and the selectivity required to separate the analytes from chemical background by the use of single reaction monitoring (SIM) or selected reaction monitoring (SRM). High resolution accurate-mass analyzers offer the advantage of full-scan operation with a higher mass resolving power than single or triple quadrupoles but providing similar levels of selectivity and quantitative performance. Gas-chromatography is widely used in testing laboratories as it allows to achieve better chromatographic separation than liquid chromatography moreover the headspace sampling allows for the extraction of semi-volatile and volatile compounds from complex liquid and solid matrices offering the advantage of an easier and faster sample preparation compared to the liquid injection. In this study single ion monitoring (SIM) and full-scan (FS) approaches were used for quantitative analysis of NDMA, NDEA and NEIPA impurities and for screening of other contaminants that can be present in pharmaceutical products.

MATERIALS AND METHODS

Sample Preparation

EPA 521 nitrosamine mix (2000 µg/mL in dicloromethane) was purchased from Sigma Aldrich (P/N 40035-U), N-Nitrosoethylisopropylamine (25mg) was purchased from LGC (P/N DRE-C15605100) and N-Nitrosodimethylamine –d6 labeled (1000 µg/mL in dicloromethane) was purchased from Restek (P/N 3391). Standard and working solutions were diluted in GC headspace grade dimethyl-sulfoxide according to the FDA method and used to assess linearity, sensitivity, and system quantitative performance. USP <467> Class 2A residual solvent solution in DMSO was purchased from (Restek, P/N 36012). The stock solution was diluted 1:100 in headspace grade DMSO to 1/5 the concentration limits reported in the USP <467> method.[2] Valsartan and Metformin samples were prepared as described in the FDA method. Blank and samples spiked with nitrosamines and residual solvents were then prepared and used for untargeted and targeted assessment.

Test Method(s)

In all experiments, an Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system equipped with a Thermo Scientific™ Instant Connect Split/Splitless (IC-SSL) injector was coupled with a Thermo Scientific™ TriPlus 500™ HS-120 valve and loop static headspace. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-WAXMS B capillary column, 30 m × 0.25 mm × 0.5 µm (P/N 26086-2230). According to the FDA agency, high temperatures can cause the sample to generate NDMA, resulting in false positive results, therefore the incubation temperature must be limited to 120˚ C when headspace sampling is used for nitrosamine assessment.

Data Analysis

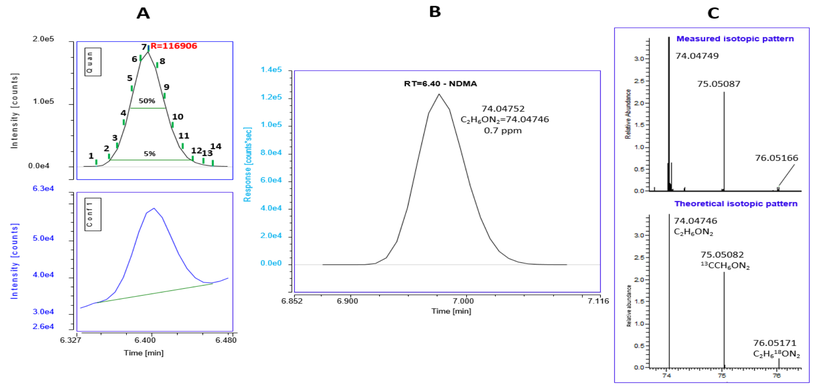
Data was acquired using the Thermo Scientific™ Xcalibur™ CDS. Freestyle™ app was used for the untargeted screening while sample quantitative analysis was carried out using the Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS), a software platform compliant with the 21 Code of Federal Regulations (CFR) part 11. Chromeleon simplified quantitative workflows delivered effective data management ensuring ease of use, sample integrity and traceability.

RESULTS

Chromatography

NDMA, NDEA and NEIPA were identified based on retention time, RT (±0.1 min window), accurate mass information (±2 ppm window) for the quantifier ion, and the characteristic fragment ions. Moreover, the elemental composition of the quantification ions was used to check the isotopic pattern fit (measured versus theoretical). An example of identification for NDMA is reported in Figure 1. An adequate number of scans/peak was achieved across the calibration curve thanks to the fast MS acquisition rate of the Orbitrap technology allowing for Gaussian peak shape, accurate peak integration and compound quantitation.

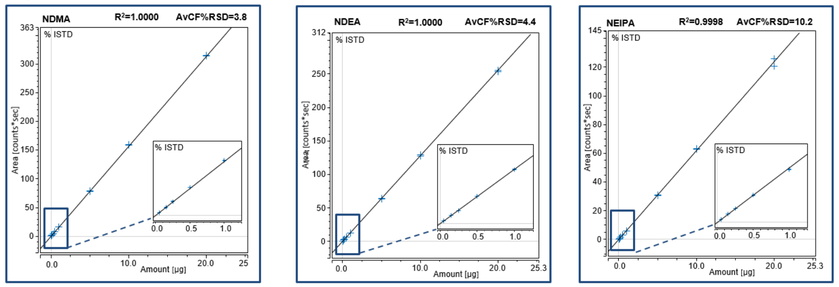
Figure 1. Identification of NDMA in solvent standard at 50 ppb showing the XIC for quantifier (*m/z* 74.04747 ± 2 ppm mass window) and confirmatory (*m/z* 42.03367) ions (A), RT (6.40 min) and mass accuracy (0.7 ppm) for quantifier ion (B), and isotopic pattern (measured vs theoretical) (C). The number of scans/peak and the exact resolution in red are annotated (A).



Linearity

A calibration curve was prepared ranging from 0.05 µg to 20 µg. Each concentration level was prepared and analysed in duplicate. The calibration curve was weighted 1/amount and plotted against the deuterated NDMA-d6 ISTD. The calculated correlation coefficients (R^2) resulted to be 1.0000 for NDMA and NDEA and 0.9998 for NEIPA with residual values (measured as % RSD of average response factors (AVCF%RSD)) <10.5%, confirming an excellent linearity as shown in Figure 2. Calculated MDL and LOQ met the FDA criteria with values of 0.01 and 0.03 ppm respectively. They can be applied after the end of the transition period (April 2021) when the limit of reporting for NDMA and NDEA will be lowered from 0.05 to 0.03 ppm.[3]

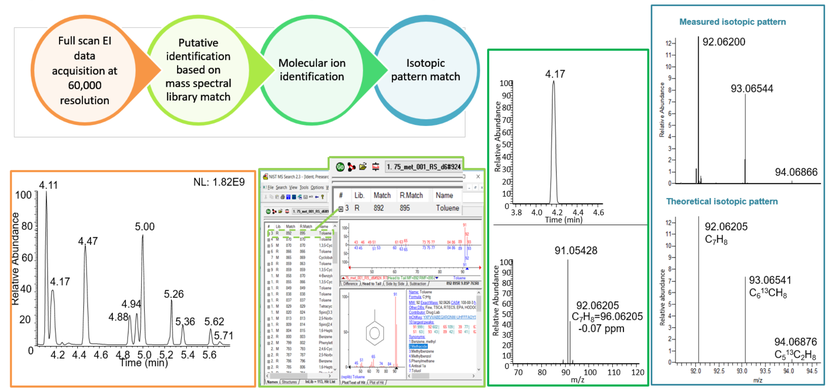
Figure 2. Calibration curves for NDMA, NDEA and NEIPA



Untargeted screening of volatile impurities

FS data was acquired at 60,000 resolution, spectral data was searched against NIST 17 nominal mass library to putatively identify the unknown compounds. The elemental composition and the mass accuracy (±1 ppm window) information were used to confirm the molecular ion. The isotopic patter match (measured vs theoretical) was used to add confidence in compound identification. As an example untargeted screening for toluene is reported in Figure 3 .

Figure 3. Applied workflow for untargeted screening of toluene



Quantification of real samples

Valsartan and Metformin samples were analysed un-spiked and spiked at three different concentrations below the LOQ, at the LOQ and above the LOQ. Low traces of NDMA could be detected in the blank samples but they resulted to be below the LOQ of 0.05 ppm. Calculated concentrations for spiked samples were ± 15% the spiked amount with % recovery within 80-120% and mass accuracy consistently below 1-ppm.

Table 1. Quantification results of real samples

Compound	Matrix	Spiked concentration (ppm)		Recovery (%)	Mass Accuracy (±2 ppm)
		Spiked	Measured		
NDMA	Valsartan	0.04	0.036	90	0.6
		0.05	0.044	88	0.4
NDEA		0.04	0.034	85	0.2
		0.05	0.052	104	0.7
NEIPA		0.02	0.022	110	0.6
		0.05	0.048	96	0.4
NDMA	Metformin	0.05	0.056	112	0.7
		1.0	1.05	105	0.5
NDEA		0.05	0.056	112	0.4
		1.0	0.99	99	0.1
NEIPA		0.05	0.054	108	0.7
		0.6	0.65	109	0.3

CONCLUSIONS

The results presented in this work demonstrate that the Exactive GC-MS system in combination with the TriPlus 500 HS autosampler delivers suitable analytical performance for the determination of NDMA, NDEA and NEIPA impurities in pharmaceuticals meeting the FDA method requirements. FS coupled with SIM acquisition allowed for both untargeted screening of volatile impurities and quantitative analysis of nitrosamines without compromising in sensitivity.

REFERENCES

1. Combined Headspace N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), and N-Nitrosodiisopropylamine (NDIPA) Impurity Assay by GC-MS/MS, 29/04/2019.
2. USP <467> Organic Volatile impurities, Chemical Tests, United States Pharmacopeia, *Interim Revision Announcement* Official November 1, 2019; Official December 1, 2020.
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TRADEMARKS/LICENSING

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