thermo scientific

APPLICATION NOTE 21922

Determination of genotoxic nitrosamines in Valsartan with gas chromatography and mass spectrometry

Authors
Yanggang Zhang,
Jinshui Che, and Shen Wang
Thermo Fisher Scientific,
Shanghai, China

Keywords
GC-MS, GC-MS/MS, Genotoxic impurities, Headspace, Nitrosamines, NDMA, NDEA, Valsartan, TriPlus 500 HS, TSQ 9000, ISQ 7000

Goal

The aim of this work was to evaluate the quantitative performance of Thermo Scientific™ GC-MS solutions in combination with liquid and headspace sampling techniques for the determination of genotoxic nitrosamines in Valsartan according to the Chinese Pharmacopoeia method[1] as well as U.S. Food and Drug Administration (U.S. FDA) recommended methodology[2,3,4]. FDA methods are evolving very rapidly, so the referred methods are the one in use at the time of writing.

Introduction

Valsartan is a widely used anti-hypertensive drug known to reduce human blood pressure by dilation of blood vessels. A dominant Valsartan API supplier in China reported the detection of N-nitrosodimethylamine (NDMA) in their product in July 2018. Investigations carried out by the European Medicines Agency (EMA) and the U.S. FDA, showed that NDMA may cause cancer, and thus recall procedures of Valsartan drug were started. Moreover, the U.S. FDA found an additional unexpected genetoxic impurity, N-nitrosodiethylamine (NDEA), in three batches of recalled Valsartan drugs in September 13, 2018.

ThermoFisher SCIENTIFIC

NDMA and NDEA were classified as Class 2A carcinogens (probably carcinogenic to humans) according to the International Agency for Research on Cancer (IARC). In ICH M7 these two compounds are categorized as first class chemicals (substances with recognized genotoxicity/mutagenicity and carcinogenic), thus strict measures are in place to ensure that their levels are not higher than the acceptable limits (AL). From the datasheet released by the U.S. FDA and EMA, the acceptable limits for NDMA and NDEA in Valsartan Active Pharmaceutical Ingredient (API) were set to 0.3 ppm and 0.08 ppm, respectively, which is far below toxicological threshold (TTC) of most common genotoxic impurities (TTC of 1.5 ppm). Sensitive and reliable analytical instrumentations are therefore required for the detection and quantification of nitrosamines impurities in APIs and finished drug products.

Recently, the Chinese Food and Drug Administration (CFDA) and the U.S. FDA have released their recommended methods for the detection of nitrosamines (NDMA and NDEA) in Valsartan drug on their websites. This application note covers all the recommended GC-MS methods: liquid injection with single quadrupole GC-MS (CFDA method)[1], headspace injection with single quadrupole GC-MS[2,3] (U.S. FDA method 1), liquid injection using triple quadrupole GC-MS/MS[4] (U.S. FDA method 2). All of these methods were tested for sensitivity, robustness, and regulatory compliance.

The collection of methods tested here represents a comprehensive analytical portfolio that allows for several analytical options to be used to meet the testing requirements for existing regulations worldwide. The adoption of the triple quadrupole GC-MS/MS is the latest recommendation from the U.S. FDA, since it is offering the added advantage of improved sensitivity of detection (especially for NDEA) and excellent selectivity in matrix, representing the method of choice for nitrosamines analysis.

1. Detection of nitrosamines in Valsartan by liquid injection GC-MS

This refers to the CFDA recommended method for the detection of nitrosamine in Valsartan[1]. Methanol was used for liquid-liquid extraction of the drug (Valsartan) sample. Following centrifugation, an aliquot of the supernatant was injected and tested for nitrosamines using single quadrupole GC-MS. Using this CFDA method, only NDMA was detected in Valsartan, although both NDMA and NDEA were targeted.

1.1 Instrument method and sample preparation

A Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with Split/Splitless inlet coupled to a Thermo Scientific™ ISQ™ 7000 Single Quadrupole Mass Spectrometer was used as the analytical system. Sample injections were performed using the Thermo Scientific™ AS 1310 Autosampler. Data acquisition, processing and reporting were accomplished using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). Details of the analytical parameters used for the autosampler and GC-MS are given in Table 1.

Table 1. GC-MS conditions used for testing the Chinese Pharmacopoeia method

TRACE 1310 GC Parameters	
Injection volume (µL)	1
Liner	Splitless (P/N:453A1925)
Inlet (°C)	250
Carrier gas (mL/min)	He, 1
Injection mode	Splitless Splitless time: 1 min Split flow: 50 mL/min
Column	Thermo Scientific™ TraceGOLD™ TG-WaxMS 30 m x 0.25 mm x 0.25 µm (P/N: 26088-1420)
Oven temperature program	45 °C (hold 1 min), 15 °C/min to 180 °C, 20 °C/min to 250 °C (hold 1 min)
ISQ 7000 Parameters	
Transfer line (°C)	250
Ion source (°C)	300
Acquisition method	Timed-SIM
T-SIM parameters (compound, retention time and SIM ions)	NDMA: 6.08 min, 74, 42 *m/z* DMF: 6.35 min, 73, 44, 42 *m/z* NDEA: 6.8 min, 102, 57, 42 *m/z*

1.2 Preparation of standard solution

Reference solution for NDMA and NDEA was prepared in methanol to a final concentration of 0.03 µg/mL (ppm) each. Test solution was prepared by dissolving 0.5 g of test drugs in 5 mL methanol followed by ultrasonic extraction for 15 min and centrifugation at 3000 rpm for 5 min. A volume of 2 mL of supernatant was filtered through a 0.45 µm nylon membrane. The system suitability solution containing N-dimethylformamide (DMF) as internal standard as well as NDMA and NDEA was prepared in methanol to a final concentration of 6 µg/mL (ppm). Sensitivity test solution for NDMA and NDEA was made in methanol to a concentration of 0.005 µg/mL each. The concentrations used for linearity test were: 1, 2, 5, 10, 30, 50, and 100 ng/mL (ppb).

1.3 Data processing

Thermo Scientific Chromeleon CDS version 7.2 was used for data acquisition, processing, and analysis reports. Chromeleon allows simultaneous control of instrument, method development, quantitative/qualitative analysis, and reporting. In addition, Chromeleon can be customized to display information required and also supports tags, data audit trail, and other settings easily to meet regulatory requirements for the data validity.

1.4 Results and discussion

1.4.1 System suitability test

The system suitability test requirements as described by the CFDA are:

- the resolution between NDMA and DMF must be higher than 1.5
- for the sensitivity test solution, signal-to-noise (S/N) ratio must be >10
- the reference solution must deliver a peak area relative standard deviation (RSD%) <10% for n=6 consecutive injections

The resolution of NDMA and NDEA between DMF was 2.64 and 3.17 respectively, which is greater than 1.5 and therefore meets and exceeds the CFDA resolution criteria.

As shown in Figure 1.2, the S/N of NDMA and NDEA peaks are ~85 and 61, respectively, exceeding the CFDA requirements of S/N >10.

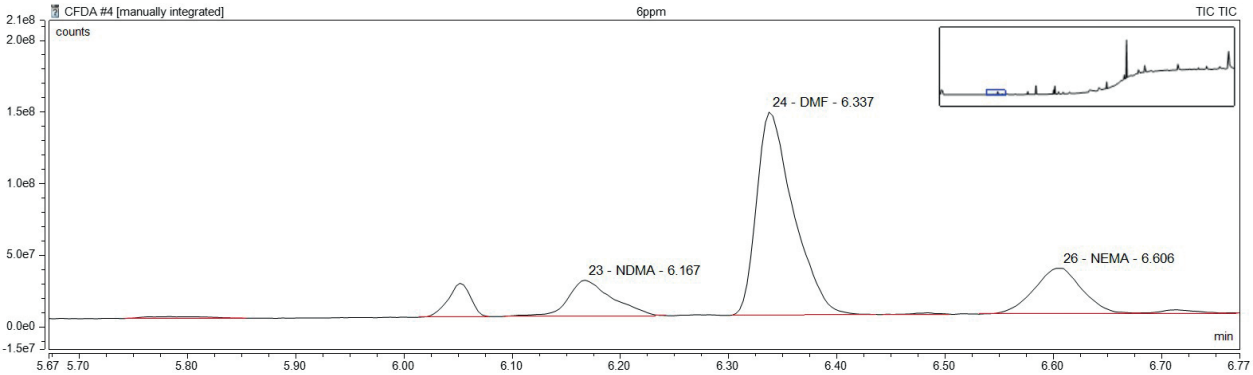

Figure 1.1. GC-MS TIC chromatogram of the system suitability test solution at 6 ppm (µg/mL) level

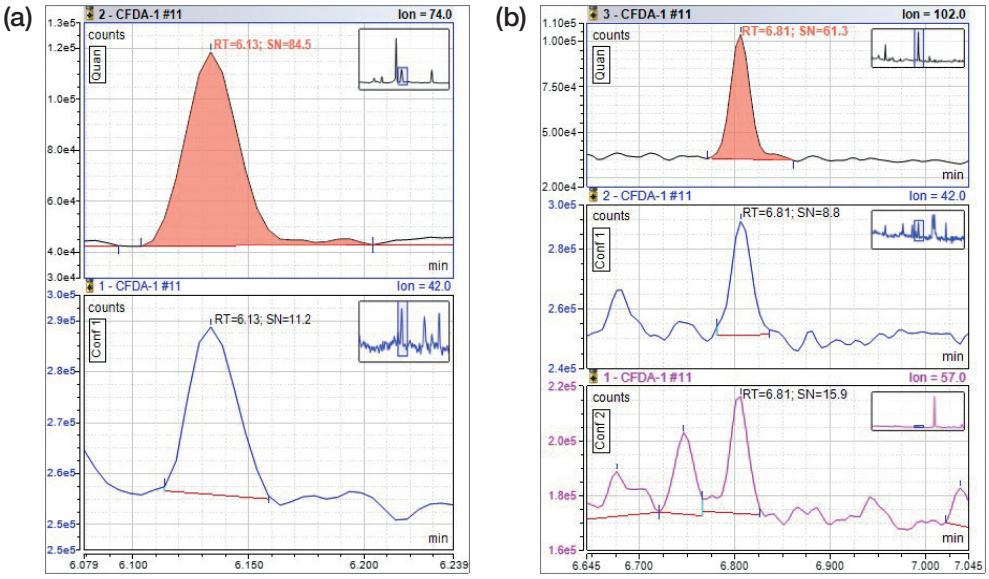

Figure 1.2. GC-MS chromatograms (SIM) of sensitivity test solution at 5 ppb (ng/mL) level for (a) NDMA; (b) NDEA

1.4.2 Method verification

The method was validated for linearity, sensitivity, repeatability, and the actual sample recoveries.

Very good linearity was obtained using external calibration for the following concentrations of NDMA and NDEA: 1, 2, 5, 10, 30, 50, and 100 ppb (ng/mL), as shown in Figure 1.3. The correlation coefficients R^2 of NDMA and NDEA were 0.9981 and 0.9979, respectively, satisfying the CFDA method requirements for linearity assessment with $R^2 > 0.995$.

Sensitivity of the method was calculated by assessing the S/N of the compounds of interest in the sensitivity test solution (5 ppb). The limit of detection (defined as LOD, S/N =3) of NDMA and NDEA was 0.18 and 0.24 ppb, respectively, whereas the limit of quantitation (LOQ, S/N = 10) was 0.6 and 0.8 ppb, respectively. The LOQ concentration corresponding to the drug (API) was 6.0 and 8.0 ppb, respectively, considering the dilution factor of 10.

The repeatability of the method was also investigated. The %RSD of the absolute peak area for consecutive n=6 injections of reference solution at 30 ppb on column was 2% and 1.3% for NDMA and NDEA, respectively (Figure 1.4).

The test sample was represented by a commercially available capsule of Valsartan. Recovery of NDMA and NDEA was investigated by spiking this sample at 5 ppb, 30 ppb, and 100 ppb level.

The calculated concentrations of NDMA and NDEA in the unspiked Valsartan capsule were 2.5 ppb and 2.7 ppb, corresponding to concentrations of 0.025 ppm and 0.027 ppm in drug, which are lower than the acceptable limits requirements of 0.3 and 0.08 ppm. Recoveries were also calculated from the spiked samples at 5 ppb, 30 ppb, 100 ppb with values of 105%, 93%, 101% for NDMA and of 104%, 92%, 102% for NDEA.

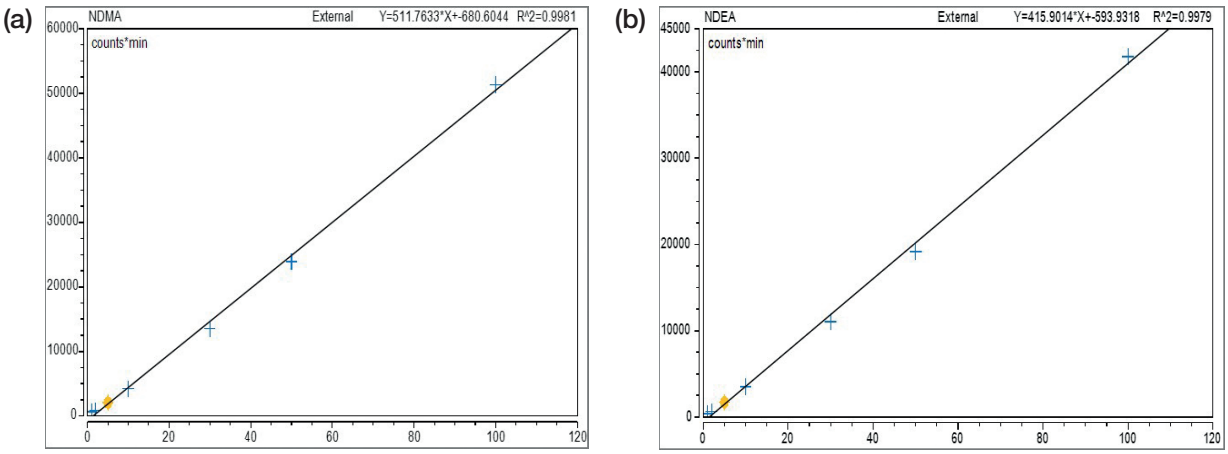

Figure 1.3. Linearity of the standard solutions over 1-100 ppb (ng/mL) for (a) NDMA; (b) NDEA

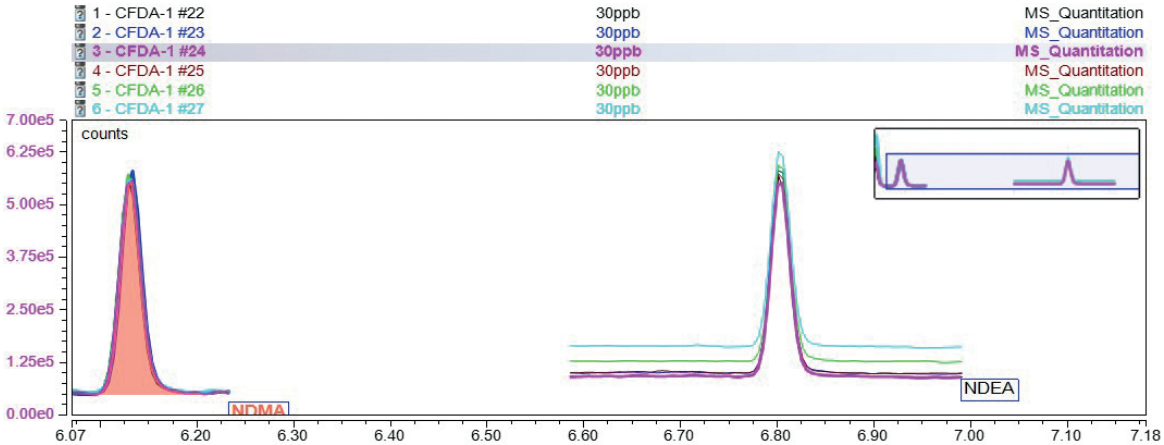

Figure 1.4. Peak area repeatability for n=6 consecutive injections of a reference solution containing NDMA and NDEA at 30 ppb on column

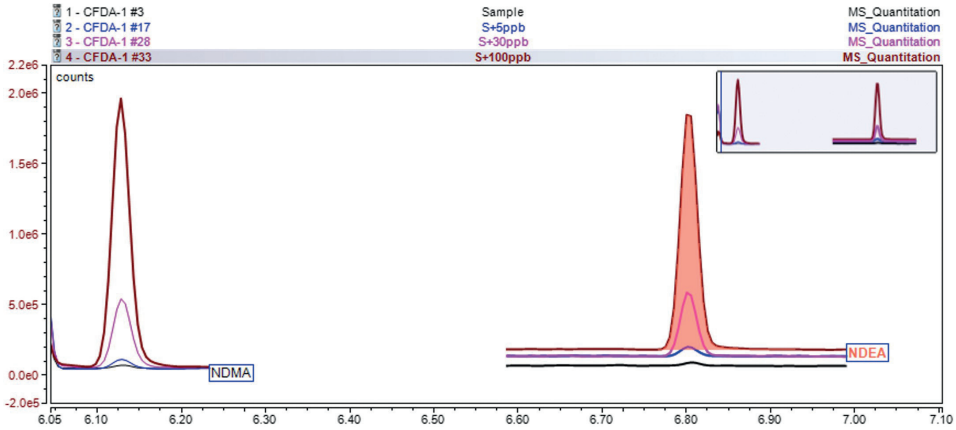

Figure 1.5. SIM chromatograms of NDMA and NDEA in an unspiked Valsartan capsule sample as well as in a Valsartan capsule sample spiked at 5, 30, and 100 ppb levels

2. Detection of nitrosamines in Valsartan by Headspace GC-MS

The static headspace sampling technique allows for a simple and efficient solventless extraction of volatile impurities from solid samples such as APIs and finished drug products. It is therefore a valid alternative to direct liquid injection, offering the advantage of eliminating time consuming sample manipulation steps.

There are two headspace methods recommend by the U.S. FDA: Method 1: "GC-MS Headspace for Detection of NDMA in Valsartan Drug Substance"[2]; Method 2: "Combined N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay By GC-MS-Headspace"[3]. Method 1 uses dimethyl sulfoxide (DMSO) to dissolve the drug and then headspace-GC-MS for the analysis of only NDMA impurity. Method 2 uses 1-methyl-2-pyrrolidone (NMP) to dissolve the drug and headspace-GC-MS for the analysis of both NDMA and NDEA impurities. The method validated in this work refers to the methods above, using dimethyl sulfoxide (DMSO) to dissolve the drugs, and headspace-GC-MS for the detection of both NDMA and NDEA.

2.1 Instrument method and sample processing

The following analytical configuration and the corresponding analytical parameters (Table 2) were used for the detection of nitrosamines in Valsartan by headspace-GC-MS:

- TRACE 1310 Gas Chromatograph
- ISQ 7000 Single Quadrupole Mass Spectrometer
- Thermo Scientific™ TriPlus™ 500 Headspace Autosampler
- Chromeleon 7.2 data processing system

The TriPlus 500 Headspace autosampler is a new platform featuring a direct column connection to assure high inertness of the sample path and full sample integrity during the injection. The capillary column is directly connected to the valve and loop manifold of the autosampler through a very short interface into the GC oven, bypassing the GC injector. The much shorter sample path makes the entire system particularly robust against possible carryover effects and assures the best recovery of target analytes.

Table 2. Analytical parameters used for NDMA/NDEA detection and quantification in Valsartan using the US-FDA HS-GC-MS method

TriPlus 500 HS Parameters	
Incubation (°C)	150
Incubation time (min)	15
Loop/sample path (°C)	180
Injection volume (µL)	1000
Other parameters	Vial shaking: Fast Vial pressure: 130 kPa for 1 min Loop filling pressure: 70 kPa for 1 min Injection Mode: Standard for 1 min
TRACE 1310 GC Parameters	
Carrier gas (mL/min)	He, 1.0
Injection mode	Split ratio 5:1 Split flow 5 mL/min
Column	TG-WaxMS 30 m x 0.25 mm x 0.25 µm (P/N: 26088-1420)
Oven parameters	45 °C (Hold 1 min), 15 °C/min to 180 °C, 20 °C/min to 250 °C (hold 1 min)
ISQ 7000 Parameters	
Transfer line (°C)	250
Ion source (°C)	300
Acquisition mode	Timed-SIM
T-SIM parameters (compound, retention time, SIM ions)	NDMA: 6.08 min, 74, 42 *m/z* NDEA: 6.8 min, 102, 57, 42 *m/z*

2.2 Preparation of standard solution

The test solution was prepared by weighing 0.5 g of Valsartan drug and dissolving it with DMSO (or NMP) to a volume of 5 mL. Sensitivity solution was prepared by weighing appropriate amounts of the NDMA, NDEA solution which were then dissolved in DMSO to a concentration of 0.03 µg/mL. The concentrations of the standard solutions used to test the linearity were 0.03, 0.05, 0.1, 0.2, 1, 2, and 4 µg/mL.

2.3 Results and discussion

2.3.1 System suitability

System suitability of U.S. FDA method is defined as follows: coefficient of determination $R^2 > 0.995$; LOQ (defined as S/N ≥ 10) of NDMA 0.1 ppm (µg/mL), NDEA 0.05 ppm (µg/mL) in drug (API).

The correlation coefficients R^2 for NDMA and NDEA were 0.9999 and 0.9997, respectively, meeting the system suitability requirements (Figure 2.1).

As shown in Figure 2.2, the S/N ratio of NDMA and NDEA were 206.3 and 96.9 for sensitivity solution. The LODs (S/N ≥ 3) of NDMA and NDEA were 0.4 and 0.9 ppb (ng/mL), respectively; LOQ (SN ≥ 10) were 1.5 and 3.1 ppb (ng/mL). LOQ corresponding to the concentration in the drug were 0.015, 0.03 ppm (µg/mL), which is better than 0.1 and 0.05 ppm (µg/mL) required.

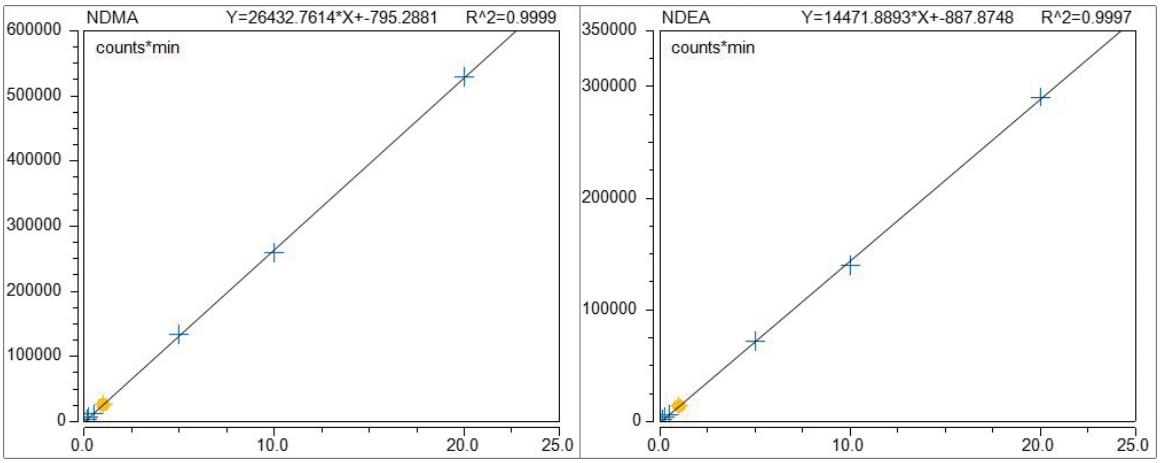

Figure 2 .1. Linearity of NDMA and NDEA (0.03 µg/mL to 20 µg/mL)

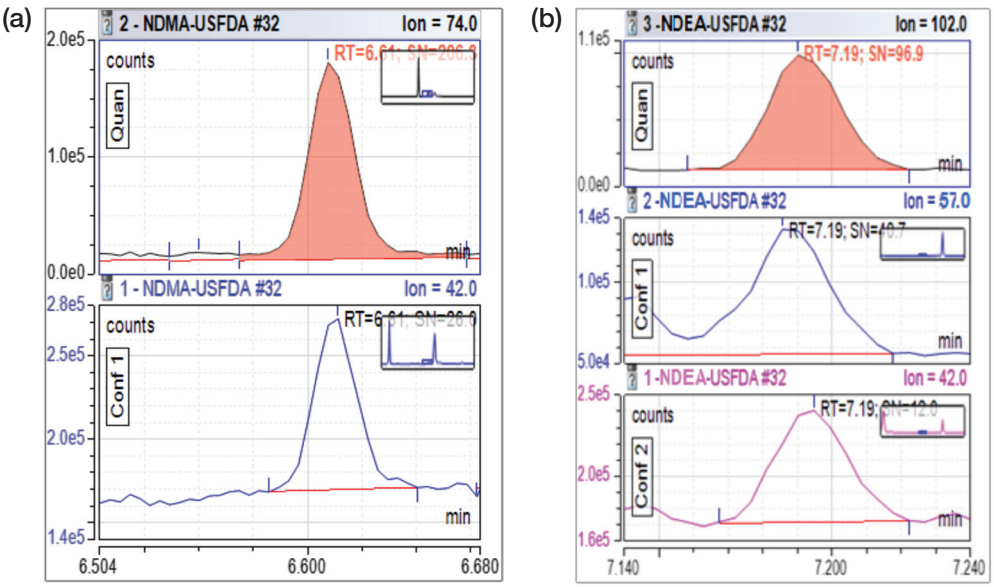

Figure 2.2. GC-MS chromatograms (SIM) of NDMA (a) and NDEA (b) at 0.03 ppb (ng/mL) on column (sensitivity test solution)

2.3.2 Method validation

Besides the linearity and sensitivity tests, repeatability and recoveries were also examined.

As shown in Figure 2.3, the precision of peak area repeatability calculated as %RSD of n=6 consecutive injections of a 50 ppb (ng/mL) standard, was 2.4% for NDMA and 2.1% for NDEA, exceeding the acceptable threshold 5% required in the U.S. FDA method. Recoveries of NDMA and NDEA were assessed by spiking a test sample (representing a commercially available capsule of Valsartan) at 5 ppb, 30 ppb, and 100 ppb level.

The detected value of NDMA in the unspiked Valsartan capsule was 3.1 ppb while NDEA was not detected N.D. ppb, corresponding to a concentration of 0.031 ppm NDMA in drug, which is less than the requirement (0.3 and 0.08 ppm). Recoveries were also calculated, for 0.25 ppb, 5 ppb spiked samples, as 103%, 84% for NDMA and 92%, 81% for NDEA.

3. Detection of nitrosamines in Valsartan by GC-MS/MS

Until recently the method for the detection of nitrosamines in pharmaceuticals recommended by the U.S. FDA was "Combined Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC-MS"[4]. This method used deuterated and C^{13} double-labeled NDMA as an internal standard, and triple quadrupole GC-MS/MS to detect and quantify NDMA and NDEA. This approach combines the advantages of the first and the second method, with the highest sensitivity and selectivity.

Since deuterated and C^{13} double-labeled NDMA are difficult to obtain, for this work the method was slightly modified by employing the use of external standards.

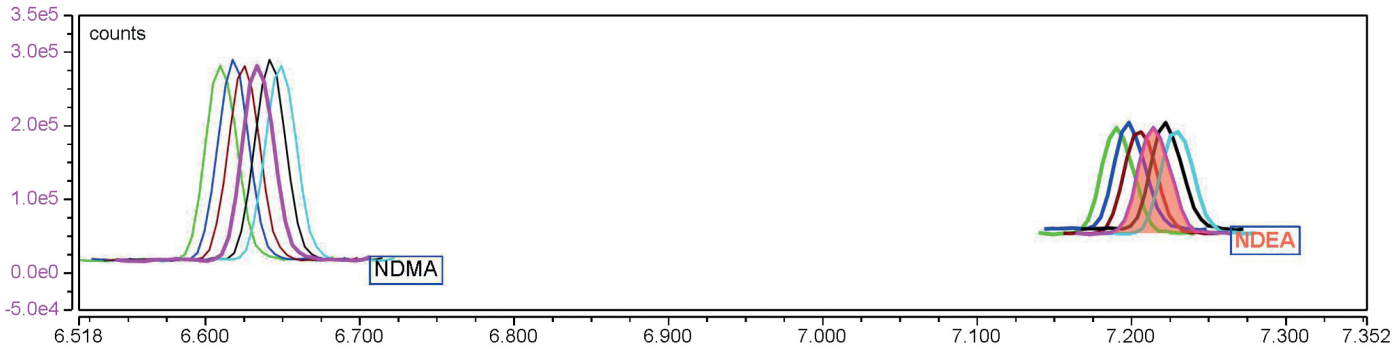

Figure 2.3. Repeatability of peak area assessed from n=6 consecutive injection of a standard containing NDMA and NDEA at 50 ppb (ng/mL) level

3.1 Analytical configuration and sample preparation

The following analytical configuration was used: a Thermo Scientific TRACE 1310 Gas Chromatograph configured with a split/splitless inlet was coupled to a Thermo Scientific™ TSQ™ 9000 triple quadrupole mass spectrometer equipped with a Thermo Scientific™ ExtractaBrite ion source. Sample injection was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler and data was acquired and processed with Chromeleon 7.2 CDS. Instrument parameters are shown in Table 3.

Table 3. Analytical parameters used for NDMA/NDEA detection and quantification in Valsartan using the US-FDA GC-MS/MS method.

TRACE 1310 GC Parameters	
Injection volume (µL)	1.0
Liner	Splitless (P/N:453A1925)
Inlet (°C)	250
Carrier gas (mL/min)	He, 1.0
Injection mode	Splitless Splitless time 1 min Split flow 50 mL/min
Column	TG-WaxMS 30 m x 0.25 mm x 0.25 µm PN: 26088-1420
Oven parameters	40 °C (hold 0.5 min), 20 °C/min to 200 °C, 60 °C/min to 250 °C (hold 3 min)
TSQ 9000 Parameters	
Transfer line (°C)	250
Ion source (°C)	300
Acquisition mode	Timed-SRM
t-SRM transitions	NDMA: 5.16 min 74>44 (CE 5V); 74>42 (CE 15V) NDEA: 5.65 min 102>85 (CE 5V);102 >56 (CE 18V)

Standard solutions were prepared by weighing appropriate amounts of NDMA and NDEA and diluting to the corresponding concentration with DCM. Test solution was prepared by accurately weighing 0.5 g of Valsartan drug and dissolving it in DCM to a final volume of 5 mL. This was followed by ultrasonic extraction for 15 min and centrifugation at 3000 rpm for 5 min. A volume of 2 mL was filtered through a 0.45 µm nylon membrane and used for GC-MS/MS injection. Sensitivity solution was prepared in DCM to a final concentration of NDMA and NDEA of 5 ng/mL. Concentrations of standard calibration levels were: 1, 2.5, 5, 10, 25, 50, 80, and 100 ng/mL (ppb).

3.2 Results and discussion

3.2.1 System suitability criteria and method performance

The system suitability criteria required by the U.S. FDA for this method are as follows: the correlation coefficient of the method must be R^2>0.998 and the NDMA and NDEA sensitivity in the test solution (5 ng/mL) must give a S/N ratio > 10 for both target compounds.

As demonstrated in Figure 3.1, the correlation coefficients R^2 of NDMA and NDEA over the concentration range of 1-100 ng/mL were 0.9982 and 0.9993, respectively, meeting the method requirements.

As shown in Figure 3.2, S/N values for NDMA and NDEA were 105 and 305, respectively, exceeding the minimum acceptable values. Based on these S/N values, the calculated LOD (3x S/N) were 0.15 ppb for NDMA and 0.05 ppb for NDEA; whereas the LOQ (10x S/N) were 0.5 ppb for NDMA and 0.16 ppb for NDEA.

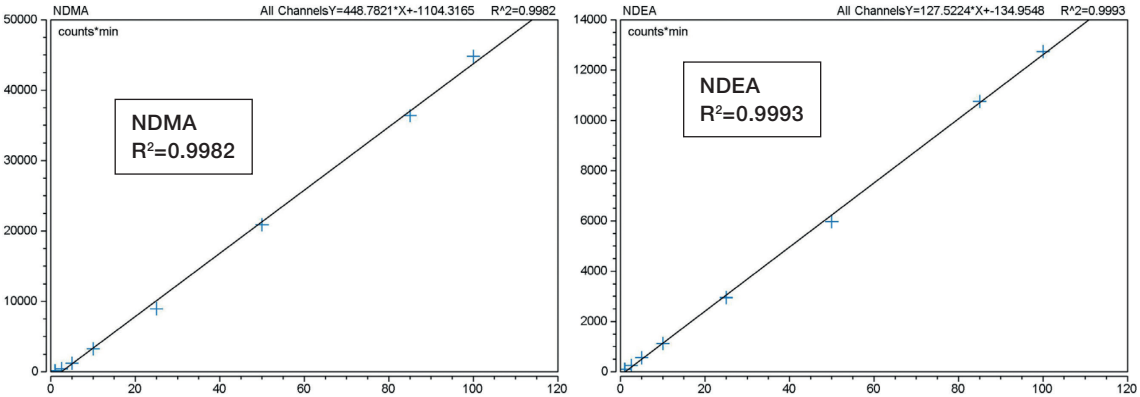

Figure 3.1. Linearity of NDMA and NDEA assessed in solvent standards over a concentration range of 1-100 ng/mL (ppb)

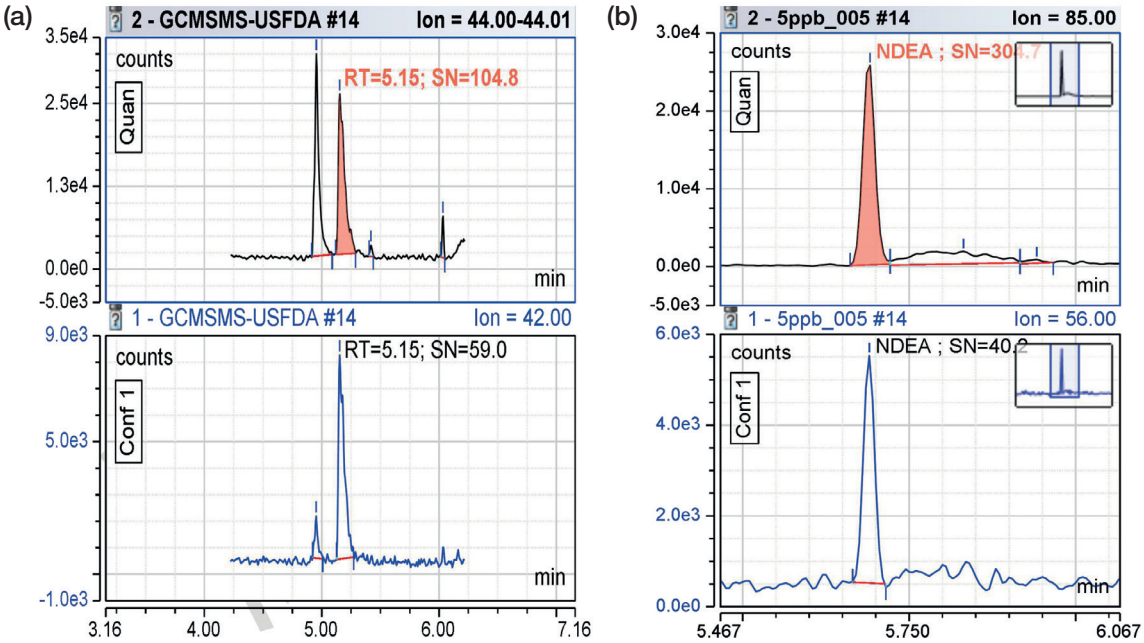

Figure 3.2. SRM chromatograms for NMDA (a) and NDEA (b) of the sensitivity test solution (5 ng/mL)

3.2.2 Method verification

Besides the above-described method performance tests, repeatability of the results and recoveries were also examined.

The %RSD values calculated from n=6 consecutive injections of a 25 ng/mL standard sample were 2.7% and 2.8% for NDMA and NDEA, respectively, well lower than the acceptable maximum threshold of 5%.

The test sample (commercially available Valsartan capsule) was used to assess compound recoveries at 5 ppb, 25 ppb, and 100 ppb spiking levels. Recovery values for these levels were: 103%, 107%, and 108% for NDMA and 96%, 97%, and 84% for NDEA.

The detected concentrations of NDMA and NDEA in the unspiked Valsartan capsule were 2.6 ppb and 1.5 ppb, respectively, corresponding to a concentration of 0.026 ppm NDMA and 0.015 ppm NDEA in the drug, which is lower than the allowable limits (0.3 and 0.08 ppm).

The GC-MS/MS approach resulted in higher sensitivity for the determination of nitrosamines impurities in drugs. Particularly for NDEA, the high selectivity of the SRM acquisition allows to unravel matrix interferences and reach much lower values of LOD and LOQ for more accurate quantitative results.

Conclusions

The results presented in this work clearly demonstrate that the Thermo Scientific GC-MS platforms can be used to produce results that are compliant with the CFDA and U.S. FDA standard methods for nitrosamines detection and quantification in Valsartan, providing excellent flexibility and analytical performance for routine laboratory use.

Three recommended instrumental approaches (GC-MS, HS-GC-MS, GC-MS/MS) were covered. The proved analytical performance of the three configurations met the regulation requirements in terms of sensitivity and repeatability, exceeding the expected requirements of the control limits.

The static headspace injection technique, compared to the direct liquid injection, offers a simplified workflow for sample handling, not requiring additional steps of sample preparation, but still providing high recovery and suitable sensitivity in compliance with U.S. FDA limits of detection.

All the methods are consistent for the determination of NDMA, while for NDEA the use of GC-MS/MS offers higher sensitivity and more accurate quantitative results, resulting in the method of choice for the quantification of trace level of nitrosamines in drugs.

Whether using single quadrupole or triple quadrupole GC-MS, the unique design of the vacuum probe interlock (VPI) featured by the Thermo Scientific ISQ 7000 GC-MS and the TSQ 9000 triple quadrupole GC-MS/MS systems allows for quick maintenance such as source cleaning or analytical column replacement without the need of venting the MS system. These GC-MS solutions allow for higher sample throughput with almost no downtime, making these analytical systems particularly suitable for the routine detection of trace genotoxic impurities in complex drug matrix.

References

1. http://www.chp.org.cn/view/ff80808164eede7e016545903eea6d2f?a=BZHXYP
2. https://www.fda.gov/downloads/Drugs/DrugSafety/UCM618053.pdf
3. https://www.fda.gov/media/117843/download
4. https://www.fda.gov/media/117807/download

Find out more at **thermofisher.com**