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

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Goal

The aim of the experiments described below was to evaluate the quantitative performance of the Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace (HS) Autosampler for the determination of genotoxic nitrosamines in valsartan according to Chinese Pharmacopoeia method1 as well as and US Food and Drug Administration (US-FDA) recommended methodology[2,3,4].

Introduction

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

NDMA and NDEA, were classified as class 2A carcinogens (probably carcinogenic to humans) according to 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 measured are in place to ensure that their levels are not higher than the acceptable limits (AL). From the datasheet released by FDA and EMA, the acceptable limits for NDMA and NDEA limits 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). Consequently, for the detection of such nitrosamines, the sensitivity of the analytical instrument of choice is a great challenge.

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Recently, the Chinese Food and Drug Administration (C-FDA) and the US-FDA has 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 method GC-MS (C-FDA method)[1], headspace using single quadrupole GC-MS method[2,3] (US-FDA method 1), liquid injection using triple quadrupole GC-MS/MS[4] (US-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. Liquid injection using GC-MS/MS is the latest method that US-FDA recommended, with the added advantage of improved sensitivity of detection (especially for NDEA) and excellent selectivity in matrix, therefore it is the recommended method of choice.

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

This refers to the C-FDA 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 C-FDA method, only NDMA was detected in Valsartan, although both NDMA and NDEA were targeted.

1.1 Instrument method and sample preparation

The following analytical configuration was used: Thermo Scientific™ TRACE™ 1310 Gas Chromatograph with Split/Splitless inlet coupled to Thermo Scientific™ ISQ™ 7000 Single Quadrupole Mass Spectrometer. Sample injections were performed using the Thermo Scientific AS1310 Autosampler. Data acquisition, processing and reporting was made using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). Detail 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	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)
ISQ7000 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*

Preparation of standard solution

Reference solution for NDMA and NDEA were 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 5min. A volume of 2 mL of supernatant was filtered through a 0.4 5 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 NDMA and NDEA solution were 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, 100 ng/mL (ppb).

1.2 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.

1.3 Results and discussion

1.3.1 System suitability test

The system suitability test requirements as described by the C-FDA are:

- the resolution between NDMA and DMF should higher than 1.5;
- for the sensitivity test solution, the signal-to-noise (S/N) ratio of the peak height should be >10.
- the reference solution should be injected continuously for n=6 times, and the calculated relative standard deviation (%RSD) of peak area should be < 10%.

The resolution of NDMA and NDEA between DMF were 2.64 and 3.17 respectively, all greater than 1.5 therefore meeting and exceeding the C-FDA resolution criteria.

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

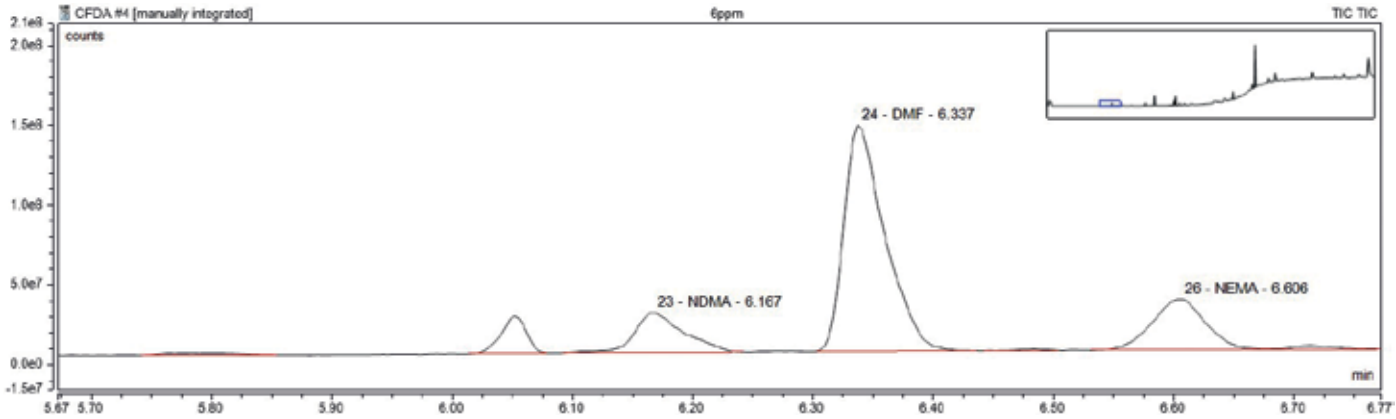

Figure 1.1. GC-MS chromatogram of the system suitability test solution at 6 ppm (µg/mL) level.

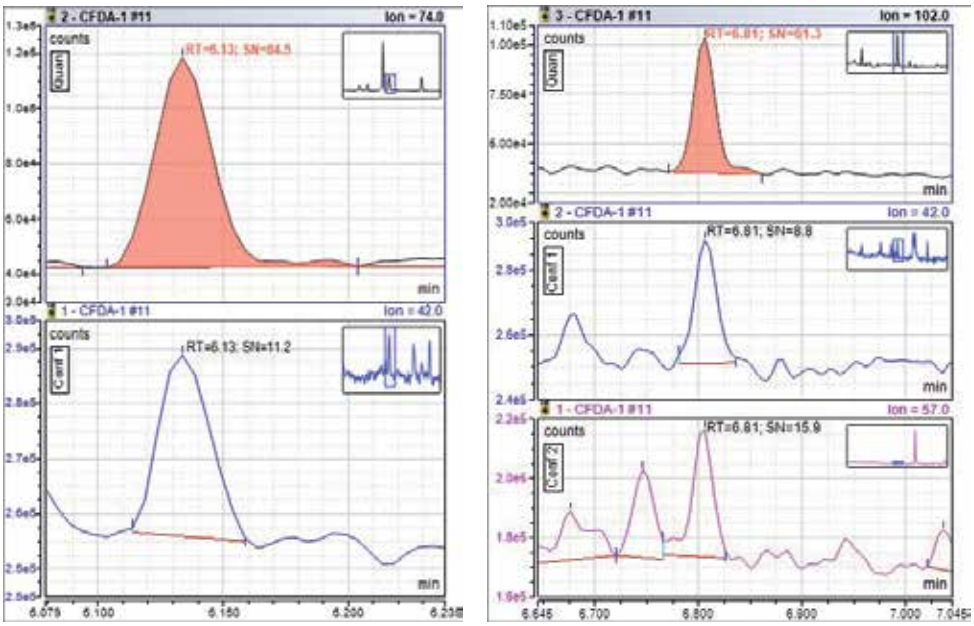

Figure 1.2. GC-MS chromatograms (SIM) of sensitivity test solution at 5 ppb (ng/mL) level.

1.3.2 Methodology verification

The following method were validated: linearity, sensitivity, repeatability, and the actual sample recoveries.

Excellent linearity was obtained using external calibration for the following concentrations of NDMA and NDEA: 1, 2, 5, 10, 30, 50, 100 ppb (ng/mL), as shown in Figure 1.3. The correlation coefficient R^2 of NDMA and NDEA is 99.81% and 99.79%, respectively, satisfying the C-FDA method requirements for linearity assessment with R^2> 99.5%.

Sensitivity of the method was calculated by the 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 were 0.18 and 0.24 ppb whereas the limit of quantitation (LOQ, S/N = 10) were 0.6 and 0.8 ppb. The LOQ concentration corresponded to the drug was 6.0 and 8.0 ppb, respectively. Method LOQs exceeded the C-FDA sensitivity requirements of 5 ppb.

The repeatability of the method was also investigated. The %RSD of the absolute peak area for continuous injection of n=6 reference solutions at 30 ppb on column were ~2% and 1.3%.

The test sample represented a commercially available capsule of Valsartan, recoveries of NDMA and NDEA were 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 a concentration of 0.025 ppm and 0. 027 ppm in drug, which is less 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 spiked samples with values for NDMA as 105%, 93%, 101% and for NDEA 104%, 92%, 102%.

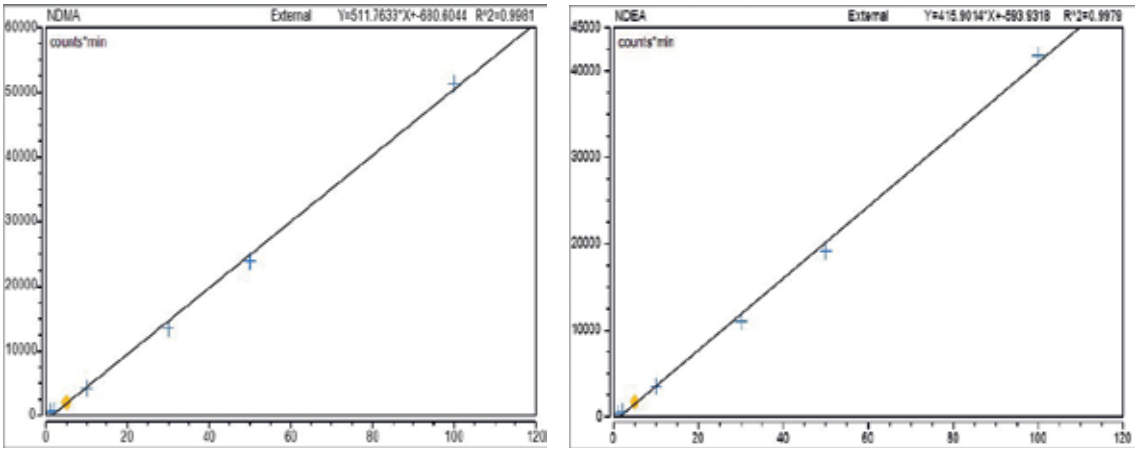

Figure 1.3. Linearity of the standard solutions over 1-100 ppb (ng/mL).

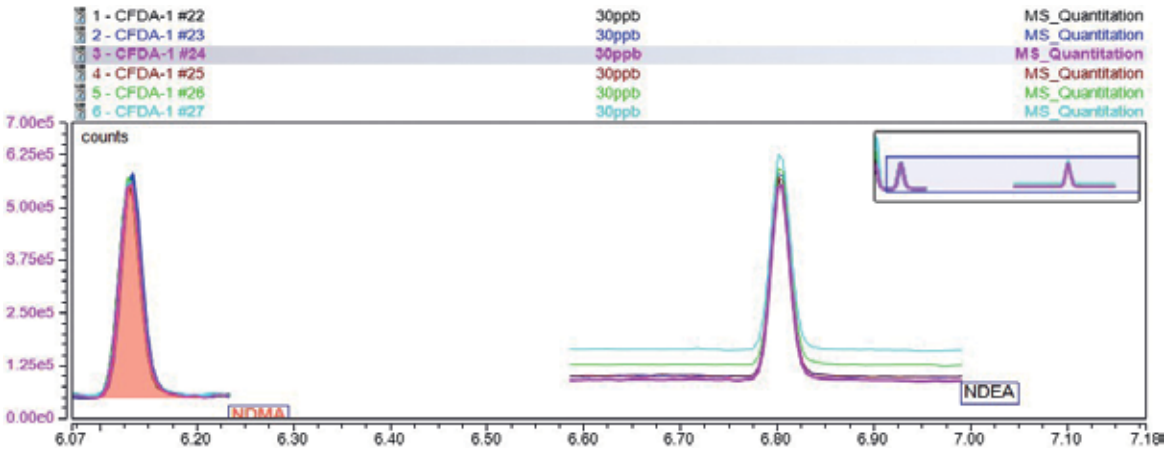

Figure 1.4. Peak area repeatability for n=6 consecutive injections of a reference solution containing NDMA and NDEA at 30 ppb on column.

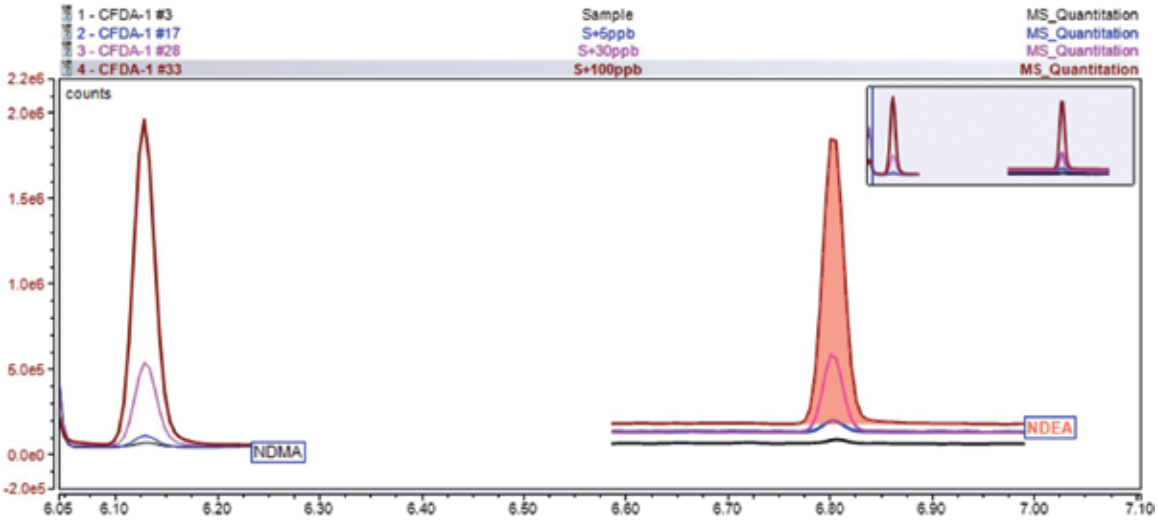

Figure 1.5. SIM chromatograms of NDMA and NDEA in an unspiked Valsartan sample as well as in a Valsartan sample spiked at 5, 30 and 100 ppb levels.

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

There are two headspace methods recommend by USFDA: "GC-MS Headspace Method 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 GCMS analyzed, only NDMA was detected. Method 2 using 1 -methyl-2- Pyrrolidone (NMP) dissolves the drug and headspace GC-MS analyzed, both NDMA and NDEA were simultaneously detected. This method refers to the above methods, using dimethyl sulfoxide (DMSO) to dissolve the drugs, and headspace GC-MS detection of both NDMA and NDEA.

2.1 Instrument method and sample processing

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

- TRACE 1310 Gas Chromatography: Split / Splitless Inlet
- ISQ 7000 Single Quadrupole Mass Spectrometer
- TriPlus500 Headspace autosampler
- Chromeleon 7.2 data processing system

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

HS500 Injector Parameters	
Incubation (°C)	150
Incubation time (min)	15
Transfer line and manifold (°C)	180
Injection volume (µL)	1000
Other parameters	Shake Mode: fast; Pressurize: 130 kPa for 1 min Loop fill: 70 kPa for 1 min Injection Mode: Standard for 1 min
TRACE 1310 GC Parameters	
Liner	headspace liner (P/N:453A1335)
Inlet (°C)	220
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)
ISQ7000 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*

Preparation of standard solution

Test solution was prepared by weighing 0.5 g of Valsartan drug sample 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 concentration of the standard solutions used to test the linearity were 0.03, 0.05, 0.1, 0.2, 1, 2, 4 µg/mL.

2.2 Results and discussion

2.2.1 System suitability

System suitability of US-FDA method were defined as below: coefficient of determination $R^2 > 99.5\%$; sensitivity: LOQ (defined as S/N ≥ 10) of NDMA 0.1 ppm (µg/mL), NDEA 0.05 ppm (µg/mL).

The linearity coefficient R^2 for NDMA and NDEA were 99.9 7% and 99.99% respectively, meeting the system suitability requirements.

As shown in Figure 2.2, the S/N ratio of NDMA and NDEA were 206.3 and 96.9 for sensitivity solutions. The LODs (S/N ≥ 3) of NDMA and NDEA were 0.4, 0.9, ppb (ng/mL) respectively; LOQ (SN ≥ 10) were 1.5, 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.

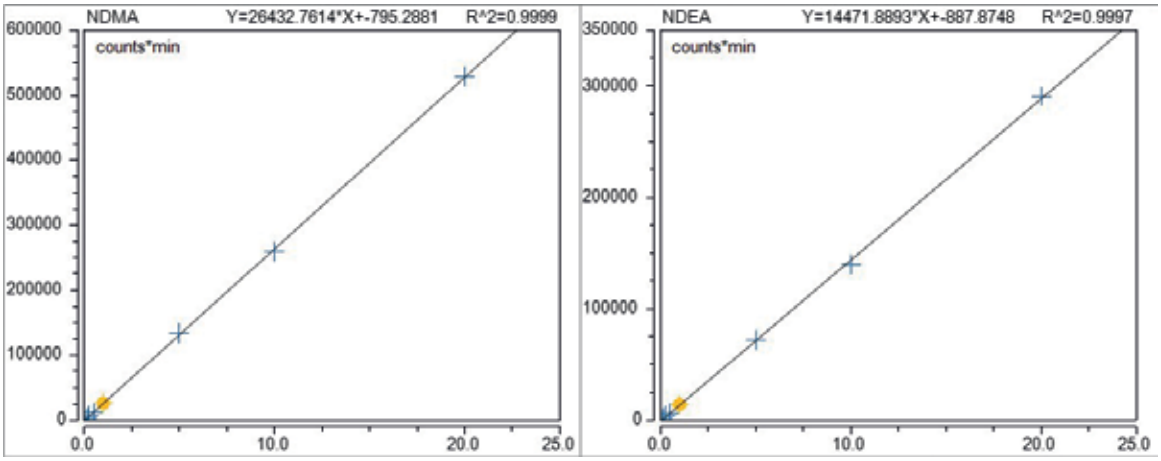

Figure 2 .1. Linearity of NDMA and NDEA (0.03 µg/mL to 20 µg/mL).

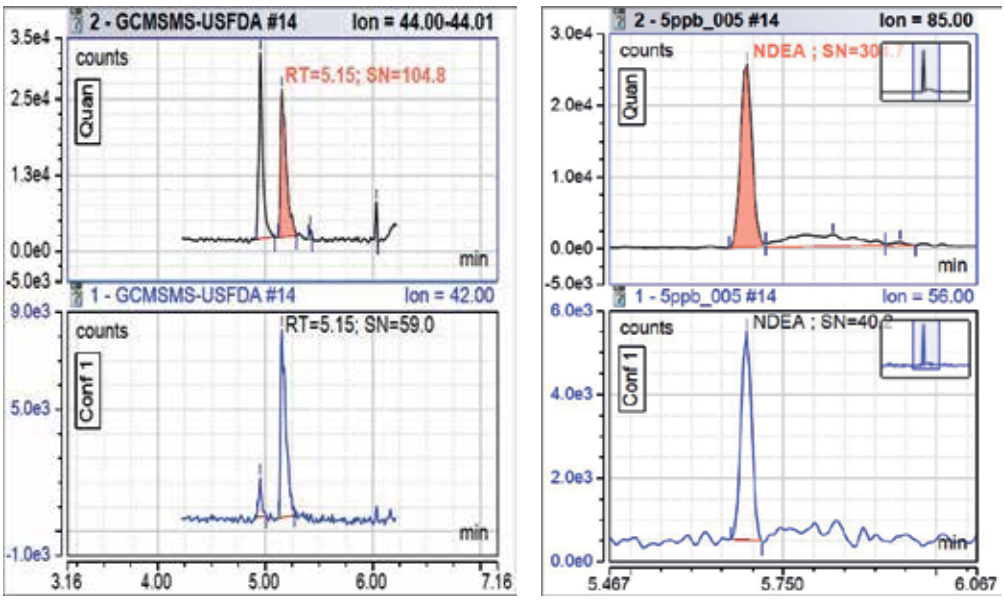

Figure 2.2. GC-MS chromatograms (SIM) of NDMA (right) and NDEA (left) at 0.03 ppb (ng/mL) on column (sensitivity test solution).

2.2.2 Method validation

Besides of above-described linearity and sensitivity tests, repeatability and recoveries were also examined.

As shown in Figure 2.3, the precision of peak area repeatability calculated as %RSDs derived from n=6 consecutive injection 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 US-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 were 3.1 ppb whilst 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, NDMA 103%, 84%; NDEA were 92%, 81%.

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

Until recently the latest method for the detection of nitrosamines in pharmaceuticals recommended by the US-FDA was "Combined Direct Injection N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine. (NDEA) Impurity Assayby GC-MS" [4]. This method used deuterated and C^{13} double-labelled 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, the method was slightly modified by employing the use of external standards. One can also use deuterated NDMA as internal standard if internal standard method was required.

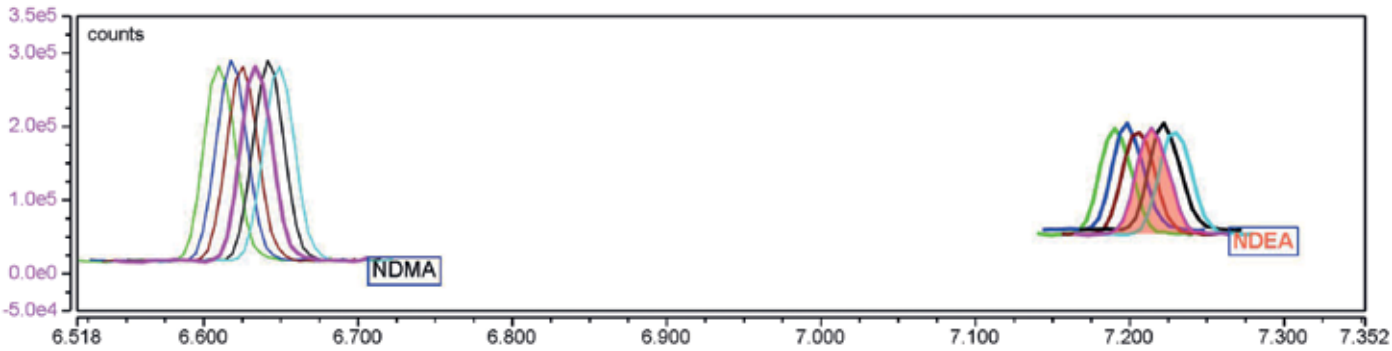

Figure 2.3. Repeatability of peak area assessed from n=6 consecutive injection of a standard containing NDMA and NDEA at 50 ppb (ng/mL) level.

3.1 Analytical configuration and sample preparation

The following analytical configuration was used:

Thermo Scientific TRACE 1310 Gas Chromatography configured with a split/splitless inlet and couled to a Thermo Scientific™ TSQ™ 9000 triple quadrupole mass spectrometer equipped with 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. Addition 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)	1
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)
TSQ9000 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 >56CE 18V)

Standard solutions were prepared by weighinh appropriate amounts of NDMA and NDEA and diluting to the corresponding concentration with DCM. Test solution were prepared by accurately weighing 0.5 g of Valsartan 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 were prepared in DCM to a final concentration of NDMA and NDEA of 5 ng/mL. Concentrations of standard curves were: 1, 2.5, 5, 10, 25, 50, 80, 100 ng/mL (ppb).

3.2 Results and Discussion

3.2.1 System suitability criteria and method performance

The system suitability criteria required by US-FDA for this method were: the linearity coefficient of the method should be R2>0.998 and the NDMA and NDEA sensitivity in the test solution (5 ng/mL) should 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 a concentration range of 1-100 ng/mL, were >998 and >999, respectively, meeting the method requirements.

As shown in figure 3.2, S/N values for NDMA and NDEA were 105 and 305, 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.

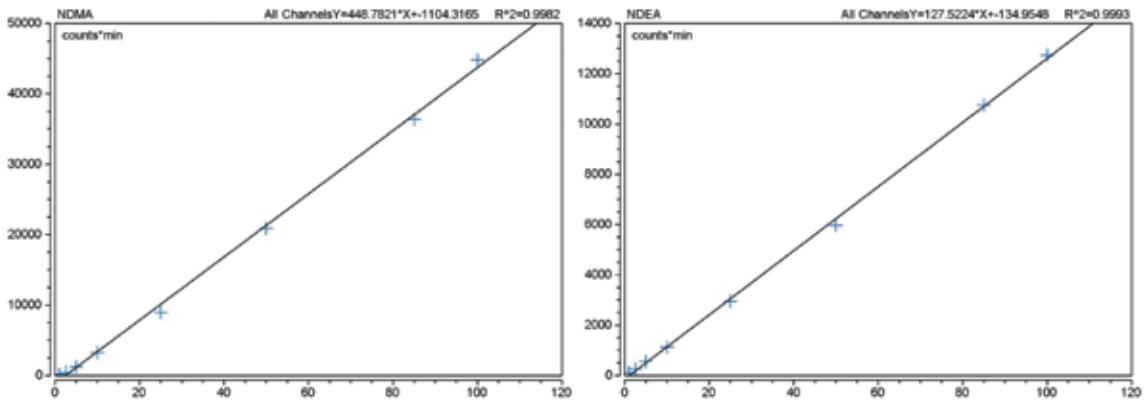

Figure 3.1. Linearity of NDMA and NDEA assessed in solvent standards over a concentration range of 1-100 ng/mL (ppb).

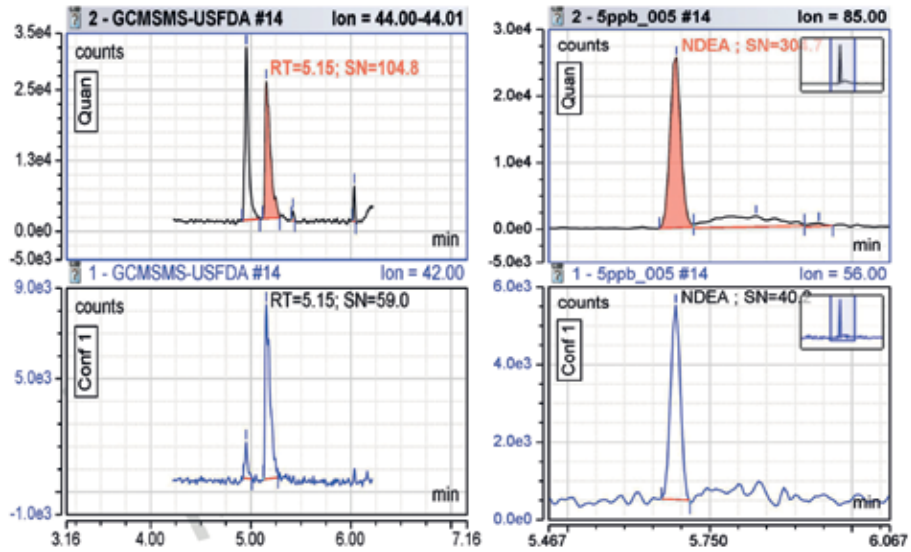

Figure 3.2. SRM chromatograms for NMDA (left) and NDEA (right) of the sensitivity test solution (5 ng/mL).

3.2.2 Method verification

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

As shown in Figure 3.3, the %RSDs values calculated from n=6 consecutive injection of a 25 ng/mL standard sample, were 2.7% and 2.8% for NDMA and NDEA respectively, meeting and exceeding the acceptable threshold of 5%.

The test sample (commercially available capsule Valsartan) was used to assess compound recoveries at 5 ppb, 25ppb, and 100ppb spiking levels. Recoveries 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, corresponding to a concentration of 0.026 ppm NDMA and 0.015 ppm NDEA in drug, which is lower than the allowable limits (0.3 and 0.08 ppm).

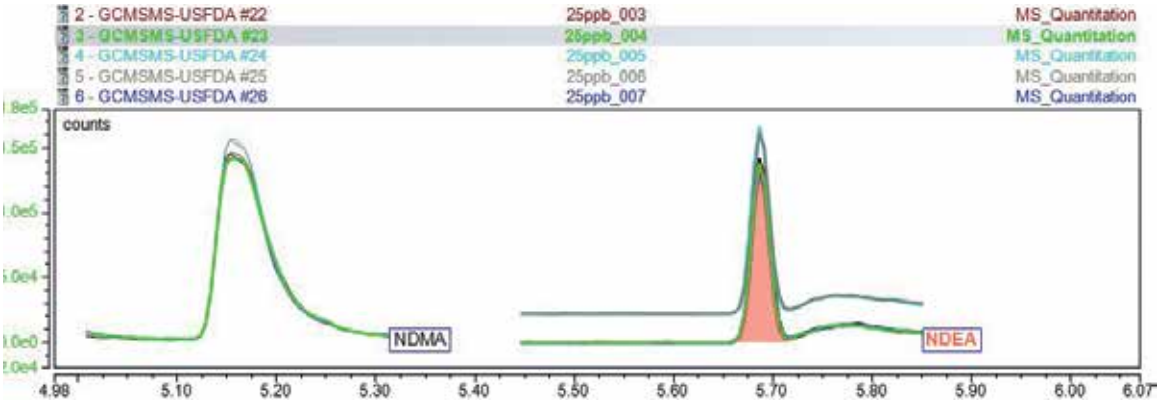

Figure 3.3. Peak area repeatability (SRM chromatograms) of NMDA and NDEA from n=6 consecutive injection of a 25 ng/mL standard solution.

Conclusions

This application note demonstrates the flexibility and suitability of Thermo Fisher Scientific GC-MS analytical configurations for the assessment of nitrosamines in Valsartan according to the current methods of detecting genotoxic impurities.

Three recommended methods (GC-MS, HS-GC-MS, GC-MS/MS) were covered. These methods met all the requirements of current regulations, sensitivity and repeatability exceeded the expected requirements of the control limits.

Moreover, with the unique design of the vacuum interlock (VPI) the Thermo Scientific™ ISQ™ 7000 GC-MS and the TSQ 9000 triple quadrupole GC-MS/MS systems allow for quick maintenance such as source cleaning or analytical column replacement without the need of venting the MS system. Consequently deliver consistent results longer and achieve higher sample throughput with almost no downtime, making these analytical systems especially suitable for the detection of trace genotoxic impurities in complex drug matrix.

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 C-FDA and US-FDA standard methods for nitrosamines detection and quantification in Valsartan providing excellent flexibility and analytical performance for routine laboratory use.

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