

COMPARISON OF APPROACHES TO DETERMINING N-NITROSODIMETHYLAMINE IMPURITY IN VALSARTAN DRUG SUBSTANCE BY GC-MS METHODS

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Methods for determining the genotoxic impurity *N*-nitrosodimethylamine (NDMA) in valsartan drug substance by gas chromatography (GC) with mass-spectrometric detection in SIM and MRM modes using direct introduction and vapor-phase analysis are compared. The obtained LOQ and DL differed insignificantly. The test results led to the conclusion that use of a method corresponding to the capabilities and equipment of the laboratory was advisable.

Keywords: quality control, GC-MS, valsartan, NDMA.

The purity of drug substances is a quality-control parameter that attracts much attention. Safety assurance demands that drug substances containing impurities must be studied and qualified and their contents normalized. Potentially genotoxic compounds that can directly or indirectly damage cellular genetic material are some of the most hazardous impurities and are rarely included in the impurity specification. Genotoxic impurities can disrupt replication and cause tumors [1]. Also, several such impurities can accumulate *in vivo* with prolonged use of “contaminated” drugs, which leads to chronic toxicity by them. Compounds containing combinations of aromatic and alkyl groups with heteroatoms [e.g., *N*-nitrosodimethylamine (NDMA)] can exhibit genotoxic properties even if introduced into the body at ultralow concentrations (ppm) [2]. Determination of ultralow concentrations of impurities has become possible with the advent of new and highly sensitive and selective methods such as HPLC-MS/MS and GC-MS/MS [3, 4].

NDMA is classified as having high genotoxic potential according to the International Council on Harmonisation of

Technical Requirements for Pharmaceuticals for Human Use (ICH). In 1987, the International Agency for Research on Cancer (IARC) classified NDMA in group 2A for probable human carcinogens. In 2018, the U. S. Food and Drug Administration (FDA) detected NDMA in valsartan drug substance [5]. Based on the aforementioned data, FDA and regulatory bodies of other countries decided to recall medications containing valsartan drug substance of Chinese manufacturers from the pharmaceutical market. As a result, patients in need of this medication found it impossible to acquire it.

A method for determining *N*-nitroso impurities (including NDMA) using various instrumental methods was developed and validated. The FDA used the results to propose an analytical method for valsartan to detect related genotoxic impurities using gas chromatography with mass-spectrometric detection (GC-MS) [6]. Two main version for the analysis have now been proposed. The first is based on vapor-phase analysis; the second, classical direct liquid introduction. In addition, a method to determine NDMA using HPLC-MS with a tandem quadrupole—time-of-flight (Q-TOF) mass detector was reported [7]. However, the method based on GC was more advantageous for determining this impurity because it is highly sensitive (limit of quantitation differs by an order of magnitude) and NDMA is a volatile compound.

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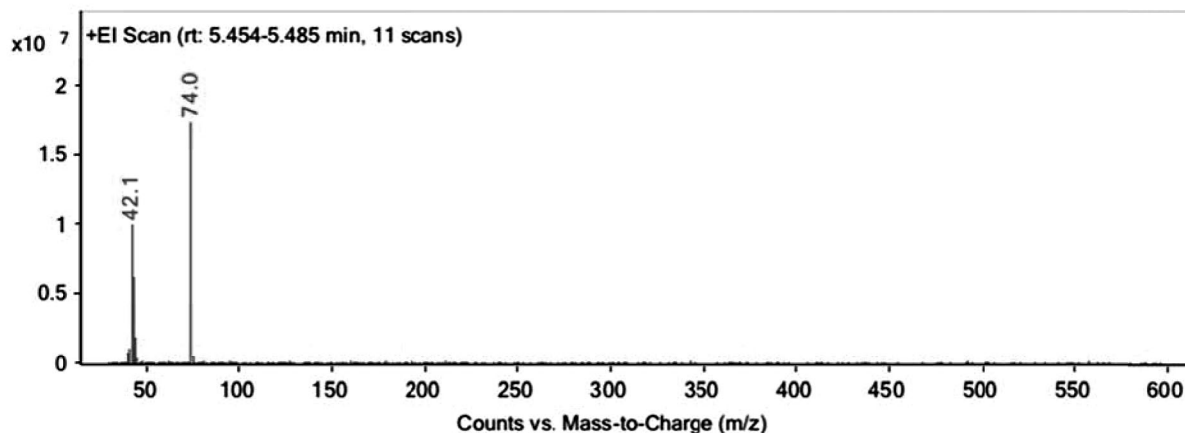


Fig. 1. Mass spectrum of NDMA at concentration 3.0 µg/mL.

The goal of the present work was to study and compare GC-MS methods in various configurations to determine genotoxic NDMA impurity in valsartan drug substance.

EXPERIMENTAL PART

Valsartan drug substance samples that could contain an NDMA impurity were selected for the study. The standard was a solution of NDMA (Sigma-Aldrich, USA, Cat. No. N7756) reference standard (RS) in MeOH at a concentration of 200 µg/mL.

The stock standard solution (SS) of NDMA was prepared via successive dilution of the RS in MeOH to reach a concentration of 3 µg/mL.

The content of genotoxic NDMA impurity was determined by preparing a series of calibrators with concentrations 0.375, 0.3, 0.225, 0.150, 0.075, 0.03, 0.0225, 0.0150, 0.01, 0.0075, and 0.005 µg/mL by successive dilutions of the stock SS. Valsartan drug substance without added SS was used as the test samples at a concentration of 100 mg/mL. The reference solution was MeOH (for HPLC).

Model solutions were prepared by dissolving valsartan drug substance (100 mg, accurate weight) in calibrators (1 mL, NDMA content 0.375 – 0.005 µg).

Chromatographic separation conditions. NDMA impurity was identified and quantified using GC-MS on an Agilent 7890A instrument (5975C MSD detector) with SIM detection and an Agilent 7890B (7010B Triple Quad MSD detector) with MRM detection. The conditions for determining NDMA impurity (column and temperature gradient) were completely identical in both versions. The column was an Agilent HP-INNOWax (30 m, 0.32 mm, 0.5 µm). Table 1 presents the chromatography parameters.

The retention time of NDMA was ~5.4 min with a total analysis time of 13.5 min.

Results were processed using Agilent MassHunter Workstation Software Qualitative Analysis Workflows ver. B.08.00.

Mass-spectrometric detection conditions. The NDMA impurity in valsartan drug substance was studied in SCAN, SIM, and MRM modes. The scan range in SCAN mode was 40 – 100 amu. The ion with 42.1 was used for qualitative determination in SIM mode; 74.0, for quantitative determination. The scan range was broad. NDMA was identified and quantified in MRM mode using the 74 → 42 transition at electron-impact energy 15 eV.

The readiness check of the chromatography system used the NDMA SS with concentration 0.03 µg/mL in SIM and MRM modes. The chromatography system was considered ready if the RSD of NDMA peak areas in repeated injections was %.

RESULTS AND DISCUSSION

Selection of mass-spectrometric detection conditions and the search for more characteristic ions used the NDMA SS with concentration 3.0 µg/mL. Figure 1 shows the mass spectrum of NDMA in SCAN mode.

The data in Fig. 1 were used to determine characteristic ions at 42.1 and 74.0, which was also confirmed by the intensities. Then, the ion at 42.1 was used for qualitative analysis because it was more specific.

The MRM transition was selected stepwise in daughter-fragments mode. The parent ion at 74.0 was the base peak. The MS2 detector operated in SCAN mode from 20 to 74.0. Mass spectra were taken with variable impact energy in the range 10 – 50 eV. The obtained fragments were strongest for impact energy 15 eV.

The methods were compared using parameters such as specificity, detection limit (DL), limit of quantitation (LOQ), analytical range, linearity, accuracy, precision, and intralaboratory precision (repeatability).

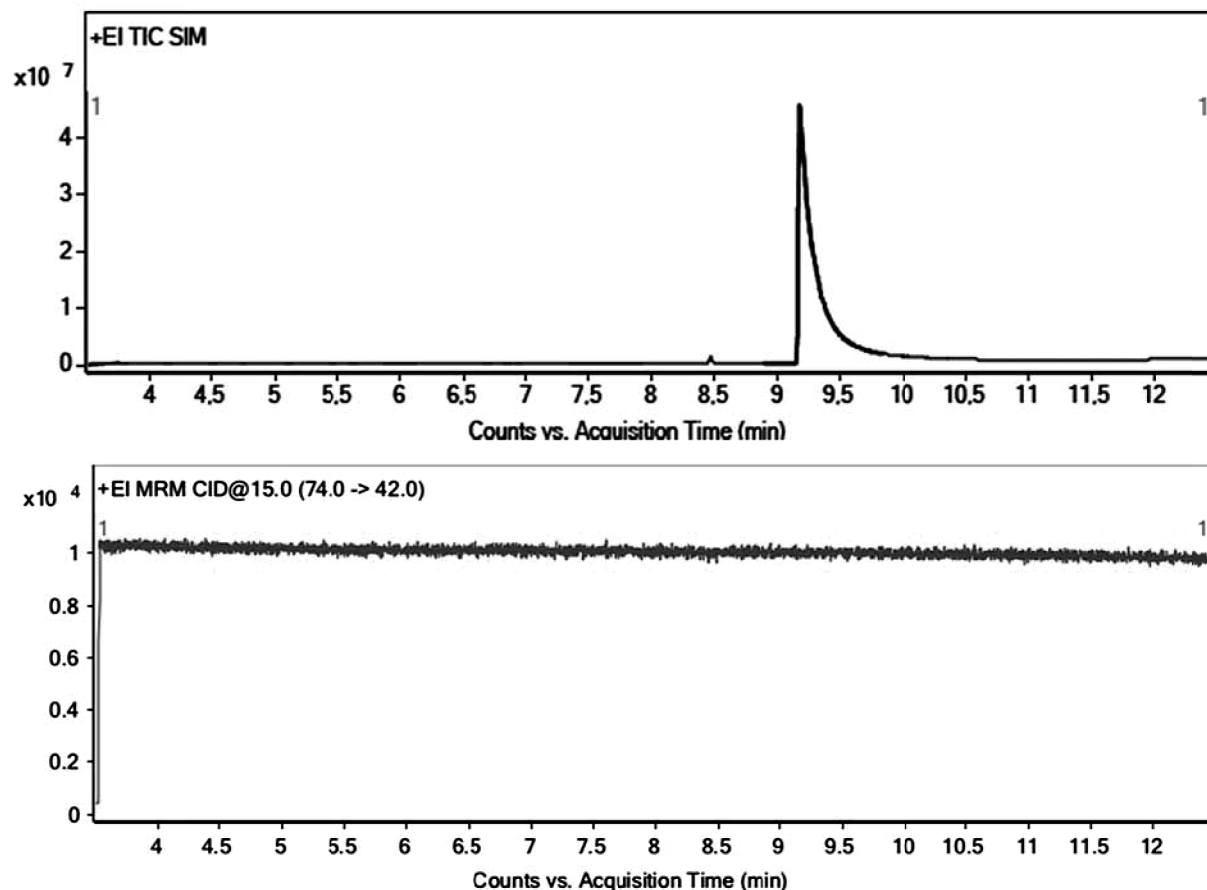


Fig. 2. Chromatograms of blank solution in SIM and MRM modes.

Specificity was demonstrated by the lack of signals in the studied region (retention time 5.4) on the chromatogram of the reference solution under the given chromatographic detection conditions. Figure 2 shows typical chromatograms of blank solutions in SIM and MRM modes.

TABLE 1. Analysis Parameters

Temperature-gradient parameters			
step	temperature, °C	rate, °C/min	time, min
1	45	–	0 – 1
2	180	15	1 – 10
3	230	20	10 – 13.5
Other analysis parameters			
Auxiliary element temperature			280° C
Ion-source temperature			230° C
Quadrupole temperature			150° C
Flow rate			1.5 mL/min
Flow division			5:1
Sample volume			1.0 µL

Detection limit (DL) was considered the concentration at which the signal-to-noise ratio (SNR) on the chromatogram was greater than 3:1 [8]. SNRs greater than 3:1 were obtained for analyses of model solutions with concentrations of 0.01 and 0.005 µg/mL and greater in SIM and MRM modes, respectively.

Limit of quantitation (LOQ) was considered the concentration at which the SNR was greater than 10:1 [9]. SNRs greater than 10:1 were obtained for analyses of model solutions with concentrations 0.03 and 0.015 µg/mL in SIM and MRM modes, respectively (Fig. 3).

Analytical range of the method should vary from the LOQ of the impurity to 120% of its allowed pharmacopoeial content because this method was a purity test [6]. Thus, linearity, precision, and accuracy of the method were determined in the concentration ranges 0.01 – 0.36 and 0.02 – 0.36 µg/mL in MRM and SIM modes, respectively.

Linearity of the method was determined and a calibration curve was constructed using concentrations 0.375, 0.3, 0.225, 0.150, and 0.075 µg/mL. The limiting content of NDMA impurity in valsartan drug substance, established as 0.3 µg/mL by the FDA, was considered in selecting the concentrations. The calibration curve was constructed by pro-

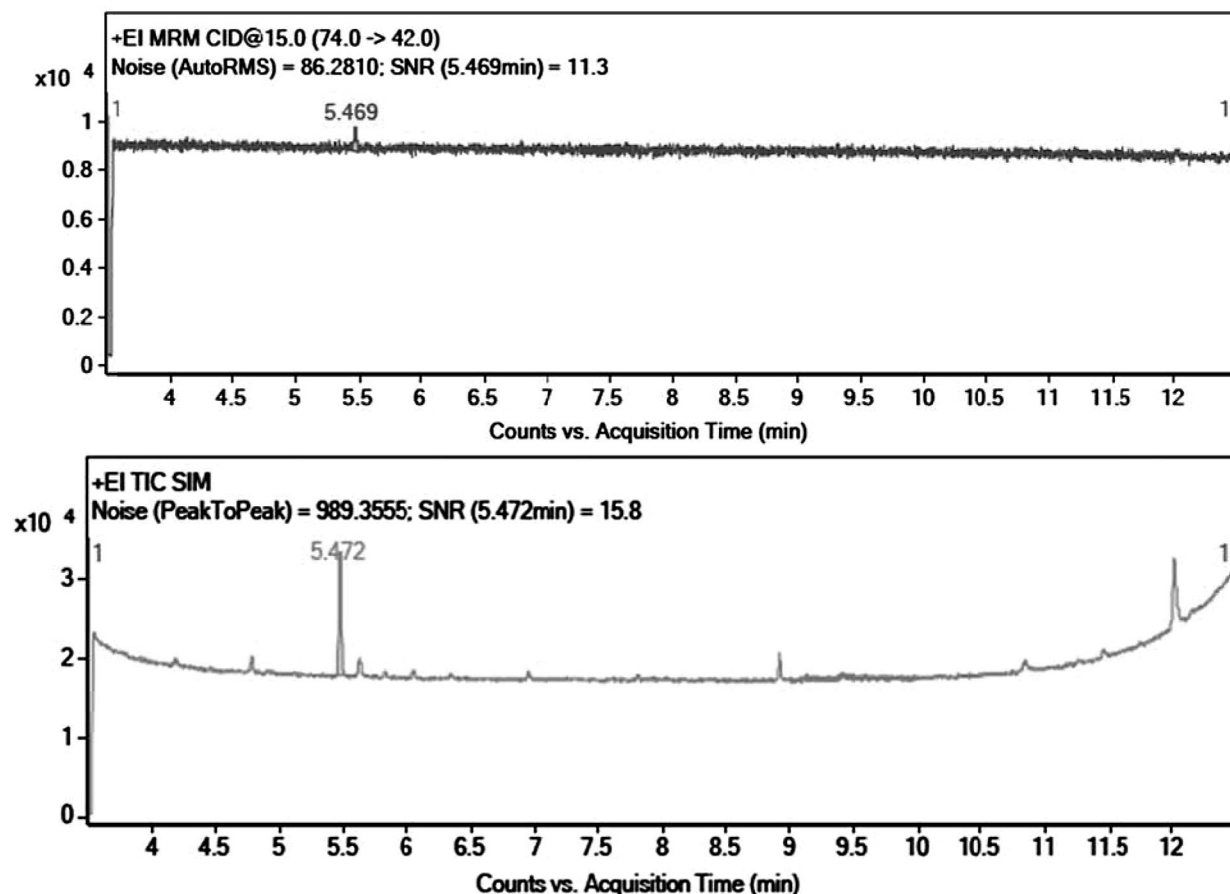


Fig. 3. Chromatograms of model solutions at concentrations 0.03 (a) and 0.015 $\mu\text{g/mL}$ (b) in SIM and MRM modes, respectively.

cessing the experimental data using a least-squares method and the linear model $y = ax + b$ (Fig. 4 shows the equations and correlation coefficients). The linearity of the method was considered satisfactory if $r^2 \geq 0.99$.

Intralaboratory precision was evaluated by determining the RSDs of calibrator peak areas with an NDMA content of 0.075 $\mu\text{g/mL}$ using SIM and MRM modes. The precision of the method was determined by different operators on different instruments simultaneously using three parallel determinations (Table 2).

A comparison of results obtained in the present work with those from the GC-MS/headspace method published by the FDA showed that the LOQ and DL of the direct-introduction methods were much less than the claimed characteristics of the headspace method. The LOQ and DL for the headspace methods were 0.3 and 0.05, respectively, according to data on the FDA website [9]. The LOQ and DL obtained by us were 0.03 and 0.01 for SIM mode and 0.015 and 0.005 for MRM mode, respectively. Later, FDA published data for the detection of NDMA impurity in valsartan drug substance and preparation using a GC-MS/MS headspace method [10]. According to these data, the LOQ and DL of valsartan drug substance were 0.05 and 0.01, respectively.

These parameters were several times worse than those from the direct-introduction method according to the data given above and were also confirmed by our studies.

The present work studied determination of NDMA impurity in valsartan drug substance using direct-introduction GC-MS with detection in SIM and MRM modes, as proposed by the FDA. The methods were studied for specificity, DL, LOQ, analytical range, linearity, accuracy, precision, and intralaboratory precision.

The method with detection in MRM mode had better linearity and repeatability. This was confirmed by the correla-

TABLE 2. Determination of Method Precision

Operator	GC-MS method			GC-MS/MS method		
	measure- ment 1	measure- ment 2	measure- ment 3	measure- ment 1	measure- ment 2	measure- ment 3
1	94215	94212	94221	7643	7652	7649
2	94212	94210	94221	7658	7654	7653
3	94213	94197	94198	7654	7645	7642
RSD %	6.22			5.56		

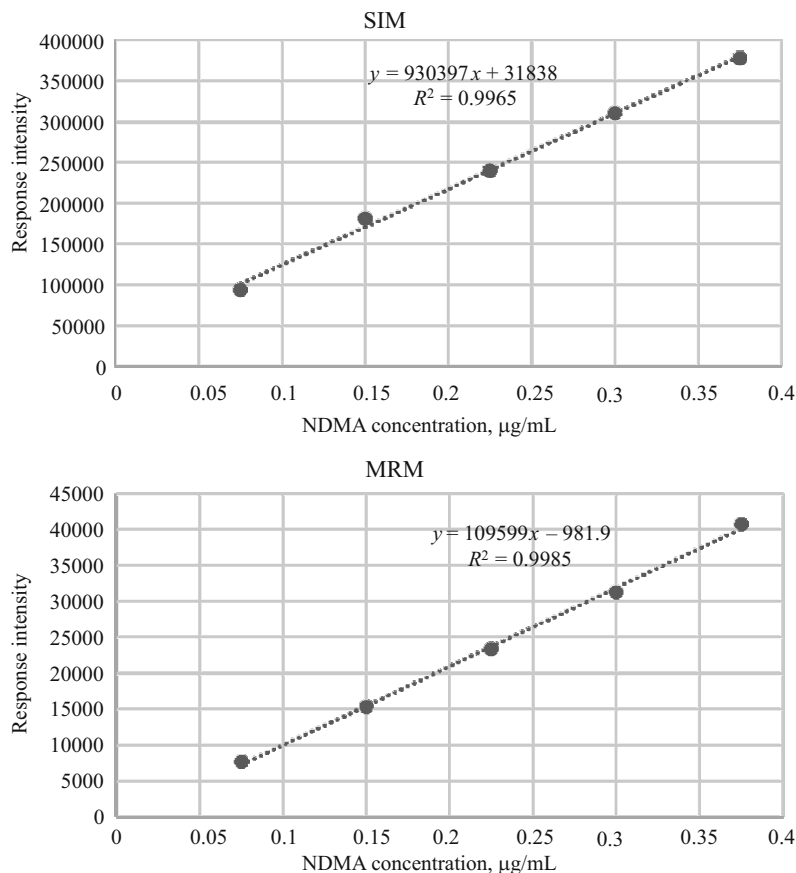


Fig. 4. Typical calibration curves where y is the mean response; x , NDMA concentration; slope (a) and intercept of the function linear section (b).

tion coefficients and RSDs given above. The accuracy and sensitivity of the method were confirmed by the measured LOQ and DL. The relative signal intensity could be increased by using a triple-quadrupole detector because the noise was suppressed. Nevertheless, differences in method parameters were considered insignificant because the limiting allowed concentration of NDMA impurity in the preparation was situated in a much higher range.

Thus, both detection modes were capable of detecting NDMA in valsartan drug substance at lower levels than the MAC. The studies also found that the direct-introduction SIM and MRM methods were comparable to SIM and MRM methods using vapor-phase analysis. This was confirmed by comparing results with those published on the FDA website [9, 10].

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