Communication to the Editor

Analysis of an Impurity, N-Nitrosodimethylamine, in Valsartan Drug Substances and Associated Products Using GC-MS

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Received January 6, 2019; accepted January 22, 2019; advance publication released online February 7, 2019

Valsartan products, commonly used to treat high blood pressure and heart failure, have been recalled in many countries due to the presence of an impurity, N-nitrosodimethylamine (NDMA), in the recalled products. We present and evaluate a GC-MS-based analytical method for the determination of NDMA levels and attempt an investigation of NDMA concentrations in valsartan drug substances and associated products. The limit of detection and limit of quantification for the method were estimated to be 0.1 and 0.5 µg/g, respectively, when testing a 0.5-g sample. A good trueness (99%) with a small relative standard deviation (1.9%) was obtained for a valsartan product spiked with NDMA at a concentration of 1.0 µg/g. Additionally, a valsartan drug substance and the associated product, which were previously determined to have NDMA contamination, were analyzed by the method. The NDMA content by our method was very close to previously determined values. Finally, six samples, including valsartan drug substances and associated, commercially available products in Japan, all of which were derived from the company implicated in the NDMA contamination, were analyzed by our method, revealing that none of these samples contained detectable concentrations of NDMA. Overall, the data indicate that the present method is reliable and useful for determination of NDMA in valsartan drug substances and associated products.

Key words N-nitrosodimethylamine; valsartan; GC-MS; drug substance; product

INTRODUCTION

Valsartan products, which are angiotensin II receptor blockers, are used to treat high blood pressure and congestive heart failure. These products contain an active tetrazole substance, valsartan. ASKA Pharmaceutical Co., Ltd., in Japan has announced a voluntary recall of several valsartan-containing products due to the presence of an impurity, N-nitrosodimethylamine (NDMA), in an active pharmaceutical ingredient supplied by Zhejiang Huahai Pharmaceutical Co., Ltd., in China.1) NDMA has been classified as a Group 2A (probably carcinogenic to humans) substance by the WHO International Agency for Research on Cancer (IARC).2) The Ministry of Health, Labour and Welfare (MHLW) in Japan released the results of NDMA analysis in valsartan drug substances and associated products that contained the active pharmaceutical ingredient supplied by not only Zhejiang Huahai Pharmaceuticals but also Zhejiang Tianyu Pharmaceutical Co., Ltd., in China,3,4) which was another company implicated in the NDMA contamination of valsartan drug substances.5) More recently, the MHLW set acceptance limits for cancer-causing substances, including NDMA, in valsartan drug substances.6) Many countries, such as European countries and the United States, have recalled valsartan products due to the contamination with NDMA.7,8)

NDMA is believed to have been introduced into the valsartan products as a result of the manufacturing process of the active pharmaceutical ingredient. The contamination is in all likelihood related to a change in the manufacturing process in Zhejiang Huahai Pharmaceuticals in 2012.9,10) Sodium azide and N,N-dimethylformamide (DMF) were used for the formation of the tetrazole ring in valsartan. Subsequently, excess sodium azide, remaining after the formation of the tetrazole ring, was quenched with sodium nitrite under acidic conditions, resulting in the formation of nitrous acid. One potential source of NDMA could be the degradation of DMF under the acidic conditions and reaction with nitrous acid.

There are a limited number of reports of analysis of NDMA in active pharmaceutical ingredients and products (as reviewed by Parr and Joseph11). More recently, the U.S. Food and Drug Administration (FDA) released GC-MS headspace12) and GC-MS/MS direct injection methods13) to determine the concentrations of cancer-causing substances, including NDMA, in valsartan drug substances and associated products. The European Directorate for the Quality of Medicines also has released analytical methods for the detection of cancer-causing substances, including NDMA, in valsartan drug substances and associated products, including methods using GC-MS headspace,14) LC-MS/MS,15) and HPLC-UV.16) Although performance of these methods should be evaluated by the user, to the best of our knowledge there are no reports on evaluation of the analytical methods for NDMA in valsartan drug substances and associated products. Here, we present and evaluate an analytical method using GC-MS for the determination of NDMA and attempt to investigate the NDMA concentrations in valsartan drug substances and the associated, commercially available products in Japan. Our analytical method using GC-MS, which has been the most widely used instrument for NDMA analysis, does not require a headspace sampler, and therefore is expected to provide high versatility in determination of NDMA in valsartan drug substances and associated products.

MATERIALS AND METHODS

Materials The valsartan drug substance and its product (VALSARTAN TABLETS [AA] 80mg), which were previously determined to harbor detectable levels of NDMA, were provided by ASKA Pharmaceutical Co., Ltd., through the MHLW. The other valsartan drug substances and the commercially available valsartan products (tablets) were provided from the respective pharmaceutical companies through the MHLW. An NDMA standard (>99.0% purity) was purchased...
from Tokyo Kasei Kogyo (Tokyo, Japan), and deuterium-labeled NDMA-\(d_6\) (98% atom% D) was purchased from CDN Isotopes, Inc. (Pointe-Claire, Canada). Dichloromethane, methanol, and anhydrous sulfurous acid were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Stock solutions of NDMA and NDMA-\(d_6\) (1000\(\mu\)g/mL each) were prepared in methanol and stored at \(-20^\circ\)C. Working standard solutions, for spiking samples as well as for calibration standards, were obtained by dilution in dichloromethane. The calibration standards were prepared at 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2\(\mu\)g/mL, all with NDMA-\(d_6\) at 0.01\(\mu\)g/mL.

Sample Preparation

Valsartan tablets were milled for 10s with a 20-mm steel ball at a 30-s\(^{-1}\) frequency using a Mixer Mill (MM400; Retsch, Haan, Germany). A sample of 250–500 mg of drug substance or 500 mg of ground tablet was weighed into an eggplant-shaped flask. Forty milliliters of dichloromethane was added to the flask and the mixture was sonicated for 5 min. After the sonication, the flask was shaken for 30 min. Dichloromethane, the most widely utilized extraction solvent for NDMA analysis using GC-MS, was used in this study. Due to environmental and health concerns associated with dichloromethane, an alternative to dichloromethane is encouraged in the future. The solution was transferred to a 100-mL glass centrifuge tube and centrifuged at 1300 \(\times\) g for 5 min. The supernatant was passed through anhydrous sodium sulfate (15 g). The resulting dehydrated solution was collected in a 50-mL measuring flask and dichloromethane was added to yield a total volume of 50 mL. An aliquot (0.5–2 mL) of the resulting solution was transferred to a graduated glass centrifuge tube using a pipette and then the internal standard (NDMA-\(d_6\) 20 or 40\(n\)g) was added. Dichloromethane was added in a volume sufficient to yield a NDMA-\(d_6\) concentration of 0.01\(\mu\)g/mL, and the resulting solution was subjected to GC-MS analysis. For testing of ground tablets, the final solution was first centrifuged at 840 \(\times\) g for 10 min to remove any undissolved particulates; the resulting supernatant then was used for GC-MS analysis.

GC-MS Analysis

NDMA was analyzed by GC-MS (7890A/5975C, Agilent Technologies) operating in EI ionization (70 ev). A 1.0-\(\mu\)L sample was injected in splitless injection mode at an injector temperature of 150°C. A DB-WAX capillary column (20 m \(\times\) 0.1 mm, film thickness 0.1 \(\mu\)m; Agilent Technologies) was used with the following oven program: hold at 40°C for 2 min; ramp from 40 to 100°C at 5°C/min; ramp from 100 to 220°C at 20°C/min; ramp from 220 to 250°C at 30°C/min; hold at 250°C for 2 min. The MS was operated in selected ion monitoring (SIM) mode. For NDMA detection, \(m/z\) 42 and 74 were used for confirmation and quantification, respectively. For NDMA-\(d_6\) detection, \(m/z\) 46 and 80 were used for confirmation and quantification, respectively. The transfer line temperature was maintained at 250°C. The concentration of NDMA in a sample was obtained using data from the calibration curve prepared from calibration standards for each analytical run. The quantitation of NDMA was performed using linear least squares regression, with the ratio of peak area of NDMA vs. that of NDMA-\(d_6\) obtained using the following formula:

\[
\text{NDMA concentration (} \mu\text{g/g)} = ((A - b)\times a \times Q_i \times 50/V) / W,
\]

where \(A\) is the ratio of peak area of NDMA and NDMA-\(d_6\) in a sample (NDMA area/NDMA-\(d_6\) area), \(a\) is the slope of the calibration curve, \(b\) is the intercept of the calibration curve, \(Q_i\) is the spiking amount of NDMA-\(d_6\) (\(\mu\)g), \(V\) is the aliquot volume (mL), and \(W\) is the sample amount (g).

**Recovery Test**

The NDMA standard was added to the ground valsartan of DIOVAN® Tablets 80 mg, a form that was considered unlikely to contain NDMA because the manufacturing method differs from that used by Zhejiang Huahai Pharmaceutical Co., Ltd., in China. The sample spiked with NDMA at 1.0 \(\mu\)g/g was analyzed five separate times by the present method.

**RESULTS AND DISCUSSION**

To assess linearity, five calibration curves with six concentration points each were prepared separately. Good linearity with correlation coefficients \((r) > 0.999\) was achieved over NDMA concentrations ranging from 0.005–0.2 \(\mu\)g/mL using NDMA-\(d_6\) as an internal standard. The limit of detection (LOD) was evaluated as the concentration of NDMA with a signal-to-noise ratio (S/N) of 3. Since 0.001 \(\mu\)g/mL of NDMA corresponded to a S/N of 3, the LOD in the method was estimated to be 0.1 \(\mu\)g/g when a 0.5-g sample was tested. The limit of quantification (LOQ) was determined as the lowest concentration of NDMA on a standard curve giving a S/N > 10. The LOQ in the method was estimated to be 0.5 \(\mu\)g/g, when a 0.5-g sample was tested. Recently, the MHLW has defined an acceptable limit of NDMA impurity in valsartan drug substances. The acceptance limit of NDMA was calculated to be 0.599 \(\mu\)g/g based on the maximum daily dose of valsartan drug substance (160 mg dose/d). Therefore, the LOQ of the present method satisfied the MHLW-defined acceptable limit of NDMA in valsartan drug substances.

To evaluate the performance of the method, a recovery test of NDMA in valsartan tablets (DIOVAN® Tablets 80 mg), which were considered unlikely to be contaminated with NDMA, was carried out \((n = 5)\). The trueness of NDMA spiked at 1.0 \(\mu\)g/g was 99% with a relative standard deviation (S.D.) of 1.9% (Table 1). Figure 1 shows representative chromatograms of a sample spiked with NDMA, a non-spiked sample, and the calibration standard (0.01 \(\mu\)g/mL of NDMA). There were no interfering peaks close to the retention times of NDMA and NDMA-\(d_6\) in the samples. The overall results indicated that the present method performed well in the determination of NDMA in valsartan products without any excipient interferences.

Additional evaluation of the present method was obtained by analysis of the valsartan drug substance and the associated products, VALSARTAN TABLETS 80 mg [AA], provided by ASKA Pharmaceutical Co., Ltd., through the MHLW. These two samples were contaminated with NDMA and the content

<table>
<thead>
<tr>
<th>Table 1. Recovery of NDMA from DIOVAN® Tablets 80 mg</th>
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<tbody>
<tr>
<td><strong>Sample</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>DIOVAN® Tablets</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

a) Non-spiked samples \((n = 2)\) and spiked samples \((n = 5)\) were analyzed using the present method. b) Repeatability c) According to the medication package insert, tablets consist of 38% of valsartan drug substance.
was previously determined based on the GC-MS analysis according to the document released by ASKA Pharmaceutical Co., Ltd.\(^3\) Using the method described here, we determined the NDMA content in the two samples with three replicates. As shown in Table 2, we detected NDMA in the two samples, which exhibited mean NDMA content of 59 \(\mu g/g\) in the valsartan drug substance and 4.1 \(\mu g/tablet\) in the product; these values were obtained with very small S.D.s. Again, there were no interfering peaks close to the retention times of NDMA and NDMA-\(d_6\) in the chromatograms of the samples (data not shown). The determined NDMA content was close to previously reported values, suggesting that the present method

### Table 2. Comparison with the Values Reported from Analysis of Valsartan Drug Substance and Associated Product (Tablets) Contaminated with NDMA

<table>
<thead>
<tr>
<th>Sample</th>
<th>NDMA content</th>
<th>Mean (\pm) S.D.</th>
<th>Reported NDMA value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((\mu g/g))</td>
<td>((\mu g/tablet))</td>
<td>((\mu g/g) or (\mu g/tablet))</td>
</tr>
<tr>
<td>Substance</td>
<td>60</td>
<td>—</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VALSARTAN TABLETS 80 mg [AA]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablets(^c)</td>
<td>16</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>4.2</td>
<td>4.1 (\pm) 0.1</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>4.0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\(^a\) Contained amount of NDMA in a tablet; calculated by multiplying the analytical concentration and the weight of the tablet. \(^b\) Previously determined values by GC-MS analysis.\(^3\) \(^c\) According to the medication package insert, tablets consist of about 32\% of valsartan drug substance.
is reliable for the determination of NDMA in valsartan drug substances and associated products.

We then investigated, using the present method, the concentrations of NDMA in two valsartan products (tablets) that are commercially available in Japan and four valsartan drug substances. Zhejiang Tianyu Pharmaceutical Co., Ltd., another company implicated in the NDMA contaminations of valsartan, supplied the drug substances for use in the analysis. The two tablets tested in the analysis also contained a drug substance supplied by Zhejiang Tianyu Pharmaceutical Co., Ltd. For the valsartan drug substances, a sample of 250 mg of substances was tested due to the limited sample availability, resulting in two times higher LOD in the substances compared to that in the tablets. No peak for NDMA was detected in any of the tested samples, indicating that the concentrations of NDMA in these samples were below the LOD (<0.1 µg/g for tablets and <0.2 µg/g for substances), as shown in Table 3. The NDMA concentrations of the four valsartan drug substances were within the acceptance limit for NDMA (0.599 µg/g). The European Medicines Agency has reported that the concentrations of NDMA detected in batches of valsartan from Zhejiang Tianyu Pharmaceutical Co., Ltd., were considerably lower than those found in the valsartan drug substance from Zhejiang Huahai Pharmaceutical Co., Ltd. Also, the manufacturing method of the valsartan drug substances in the tested samples might have been different from that used in other countries to generate the NDMA-containing valsartan. These differences may explain why NDMA was not detectable in the samples tested in the present work.

The overall results indicated that the present method is reliable and useful for determination of NDMA in valsartan drug substances and associated products. The present method is also simple, allowing us to determine NDMA without any purification steps. We observed no significant decrease in sensitivity of NDMA in the GC-MS analysis for at least two weeks, in spite of the presence of large amounts of valsartan and excipients. The described method provides an additional option for regulatory-purpose analysis for NDMA in valsartan drug substances. In order to use the method as a specification test for NDMA impurity, the method should be fully evaluated for its trueness, repeatability and intermediate precision at the acceptable limit of NDMA in valsartan substances.

Acknowledgments This study was supported by a Grant from the Ministry of Health, Labour, and Welfare of Japan. The authors gratefully acknowledge Mr. Satoshi Isagawa and Mr. Shuhei Fueki (Japan Food Research Laboratories, Tama Laboratory, Tokyo, Japan) for their helpful discussions.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


