

Analysis of Pharmaceuticals' Impurity - Regulations and Analysis for Carcinogenic Substances -

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1. Introduction

In 2018, *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA), classified as probable human carcinogens, were detected in valsartan manufactured in China, and several drug products containing this Active Pharmaceutical Ingredients (API) have been recalled worldwide. Subsequently, detection of NDMA and NDEA in other APIs was observed one after another, and consequently API manufactures were forced to review the manufacturing process and implement stricter quality control measures.

The ICH^{*} M7 guideline "Assessment and Control of DNA-Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" provides a framework for the control of impurities with carcinogenic properties, and this concept can be applied for NDMA and NDEA as well. The ICH guidelines on impurities in pharmaceuticals are described in the Q3 category as one of the "Quality Guidelines (Quality)".

ICH Q3C and Q3D are a guidelines on residual solvents and on elemental impurities, respectively, and specific limits for each solvent and element have been established. However, there is a risk of mutation even at low concentrations in the management of substances with DNA reactivity such as carcinogens. Therefore, management at a level different from that in ICH Q3 is necessary for these substances and is independently categorized as "Guidelines on multiple areas of quality, safety, and efficacy (Multidisciplinary)." As it is necessary to analyze and control trace impurities, gas chromatography-mass spectroscopy (GC/MS) and liquid chromatography-mass spectroscopy (LC/MS) methods, which employ MS as a detector for gas and liquid chromatographs, are generally used as sensitive and selective analytical methods.

This application note provides an overview of the regulations concerning substances with carcinogenic potential as impurities in pharmaceuticals, with examples of analyses.

* International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use

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2. ICH M7 Guideline ⁽¹⁾

ICH is an international conference where pharmaceutical regulatory authorities and industry representatives work together to develop guidelines for the regulation of drugs from a chemical and technical perspective. Since its establishment in 1990, guidelines have been drawn up to harmonize international regulations to accommodate to the globalization of development, regulation, and distribution of pharmaceuticals. ICH guidelines are agreed upon through a five-step process:

Step 1: Consensus on draft Technical Document (WG ^{*1})

Step 2: Consensus on Technical Document (WG)
Adoption Draft Guideline (Regulators)

Step 3: Consultation (Regulators) and Consensus (WG)

Step 4: Adoption of an ICH Harmonized Guideline

Step 5: Implementation

The ICH M7 guideline was proposed for the purpose of providing guidance on new drug substances and new drug products during their clinical development and subsequent applications for marketing approval in 2010 (Step 1). It was agreed upon in 2014 (Step 4) and has been implemented in respective countries after an expected period of 18 months (Step 5). Substances covered under this guideline are mutagens that directly damage DNA at low levels. Therefore, classification of impurities is needed first, and if those are classified as DNA-reactive substances, acceptable values are determined in accordance with this guideline to control the manufacturing process. Table 1 shows the classification of mutagenic impurities. As a result of classification, non-mutagenic impurities that are classified in Classes 4 and 5 can be controlled according to the ICH Q3 guidelines.

Classification begins with an investigation of the carcinogenicity and mutagenicity of the target substance using literature and databases. As for those substances for which data are not available and mutagenicity is unknown (Class 3), it is allowed to carry out "Structure-activity relationship ((Q) SAR) analysis" to predict mutagenicity test results using computational modelling. Two complementary predictive models can be used, one is based on expert-rule and the other on statistics. This (Q) SAR analysis method allows us to conclude that a substance is not mutagenic if it shows that the substance does not contain warning structures of mutagenic potential. Even if a warning structure is shown, it can be concluded that the substance is not mutagenic if the Ames test is performed and is negative.

Mutagenic impurities in the drug substance or drug product are controlled by acceptable intakes. For substances classified as Class 1, a compound-specific acceptable intake can be calculated based on carcinogenic potency, for class 2 with unknown carcinogenicity, and for class 3 not tested for mutagenicity, TTC ^{*2}-based acceptable intakes can be applied. The ICH M7 guideline indicates 1.5 µg/person/day as TTC-based acceptable intake. In GMP audits of drug substance manufacturers, the following items are checked in accordance with ICH M7: whether or not they have a procedure to evaluate the potential risk of mutagenic impurities being generated as new impurities when the manufacturing process is changed; if mutagenic impurities are present, whether or not they have been verified by cleaning validation or analytical procedure validation; and whether or not they have been verified to be below TTC in quality control.

Chapter 3 introduces the concept of specific management standards for nitrosamines.

*1 Expert Working Group

*2 Thresholds of Toxicological Concern

Table 1 Classification and Control of Impurities on ICH M7

Class	Definition	Control
1	Known mutagenic carcinogen	At or Below compound-specific acceptable intake
2	Known mutagen but unknown for carcinogenic potential (positive bacterial mutagenicity results*, no-exist rodent carcinogenicity data)	At or Below acceptable limit (TTC)
3	Alerting structure unrelated to drug substances, no-exist mutagenicity data	At or Below acceptable limit (TTC) or conduct bacterial mutagenicity assay If mutagenic, class 2; if not, class 5
4	Alerting Structure related to drug substances known non-mutagen or substances related to drug substances	Non-mutagenic impurity (ICH Q3A/B)
5	No structural alert, or Alerting structure with sufficient data indicating non-mutagen or non-carcinogen	Non-mutagenic impurity (ICH Q3A/B)

* Or other data related to positive mutagenicity indicating DNA-reactive linked gene mutagenesis, for example, positive findings in *in vivo* gene mutation tests, etc

3. Analysis of nitrosamine

In 2018, probable human carcinogens, NDMA and NDEA, were detected in valsartan manufactured at a Chinese manufacturing site "Zhejiang Huahai Pharmaceutical Co., Ltd.". Subsequently, NDMA was detected and recovered from valsartan manufactured at drug substance manufacturing sites other than Zhejiang Huahai. Also, NDEA was detected and reported in irbesartan manufactured in a manufacturing facility in India, "Aurobindo Pharma Ltd.". These events triggered subsequent cases of nitrosamine detection in other drugs.

Regarding the presence of nitrosamines in drugs, the possibility of contaminations of by-products formed during production under certain conditions and raw materials (sodium nitrite, dimethylformamide, and triethylamine) are known. Furthermore, it is also pointed to contaminations from solvents recovered and reused⁽³⁾.

NDMA and NDEA have been classified into Group 2A (probably carcinogenic to humans) according to the International Agency for Research on Cancer (IARC) carcinogenicity classification. Applying the concept of the ICH M7 guideline to the management of carcinogenic impurities, NDMA and NDEA are classified as Class 1 in ICH M7, and should be managed at or below the permissible levels specific to the compound. As an example, Table 2 shows the guideline values (acceptable limit) for -sartan drug substances, calculated by the Japanese Ministry of Health, Labor, and Welfare based on the concept of ICH M7⁽²⁾.

In view of control of impurities, as the inspection level that can afford to detect below each limit is requested, the inspection method with lower quantitation limit (QL) and detection limit (DL) are desirable. The FDA has recommended headspace-GC/MS method⁽⁴⁾ and direct injection-GC/MS method⁽⁵⁾ for the testing of NDMA and NDEA in -sartan drugs. For reference, QL and DL requested in the FDA-released direct injection-GC/MS method are shown in Table 3. Considering the sample pretreatment, this shows that highly sensitive analysis affordable for determination of 1 ng/mL is requested.

The FDA has released a four-compound test method (Headspace-GC-MS/MS) in which *N*-nitrosoethylisopropylamine (NEIPA) and *N*-nitrosodiisopropylamine (NDIPA) are added to NDMA and NDEA, and a five-compound test method (Direct injection - GC-MS/MS) in which *N*-nitrosodibutylamine (NDBA) is added⁽⁶⁾.

In Europe, GEON (General European Official Medicines Control Laboratories Network) has developed and released a variety of methods other than GC/MS for the determination of nitrosamines in -sartan and other drugs⁽⁷⁾.

In the following sections, we introduce analysis of nitrosamines in -sartan drugs, ranitidine, and metformin using GC-MS and LC-MS/MS.

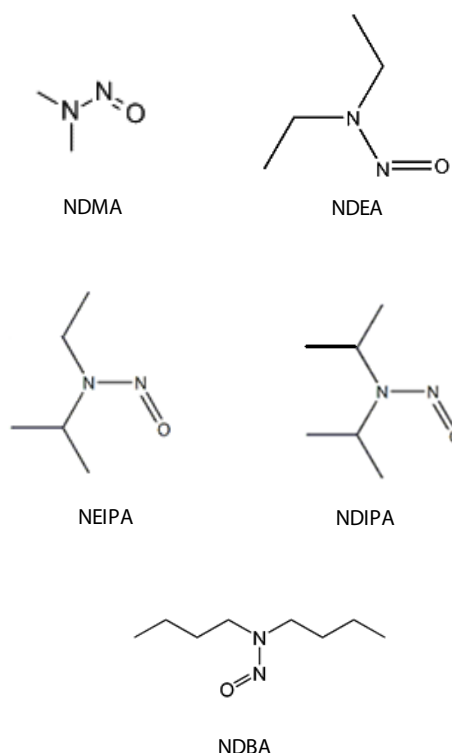


Fig. 1 Chemical Structures of Nitrosamines

Table 2 Acceptable Limit for Sartan Drug Substances

API	Maximum Daily Dosage	Acceptable Limit (NDMA)	Acceptable Limit (NDEA)
Valsartan	160 mg	0.599 ppm	0.166 ppm
Irbesartan	200 mg	0.479 ppm	0.133 ppm
Olmesartan	40 mg	2.39 ppm	0.663 ppm
Losartan	100 mg	0.959 ppm	0.265 ppm

Table 3 QL and DL in Direct Injection - GC/MS Method

	API		Pharmaceutical Formations	
	LOQ (ppm)	LOD (ppm)	LOQ (ppm)	LOD (ppm)
NDMA	0.05	0.01	0.08	0.015
NDEA	0.03	0.01	0.04	0.015

3-1. Analysis of Nitrosamine in Sartan Drugs - Headspace-GC/MS -

The following is an example of analysis of NDMA and NDEA in sartan drugs using headspace sampler HS-20 and GCMS-QP™2020 NX. The headspace method is a sample introduction method, in which volatile compounds are extracted into the gas phase by heating a vial containing sample at a constant temperature for a fixed period and injected into GC or GC-MS. As the non-volatile compounds are not introduced into GC, it has the advantage of not contaminating the sample inlet; in addition, it is also possible to directly put solid samples into vial without dissolving them and to analyze them. However, it is not suitable for substances with low vapor pressure. In this paper, the analytical conditions were optimized based on the analysis method⁽⁴⁾ released by FDA, and valsartan, losartan, and olmesartan were analyzed.



GCMS-QP™2020 NX + HS-20

The chromatogram and calibration curve of the standard solution (dimethyl sulfoxide [DMSO] solution, 2.5 - 10.0 ng/mL) are shown in Fig. 2. Excellent linearity was observed. Subsequently, the recovery test was performed. The drug products were dissolved in DMSO at a concentration of 5 % (w/v), to which the standard solutions were added at 2.5, 5.0, and 10.0 ng/mL. The results are shown in Table 5. The recovery rate was within ± 20 %.

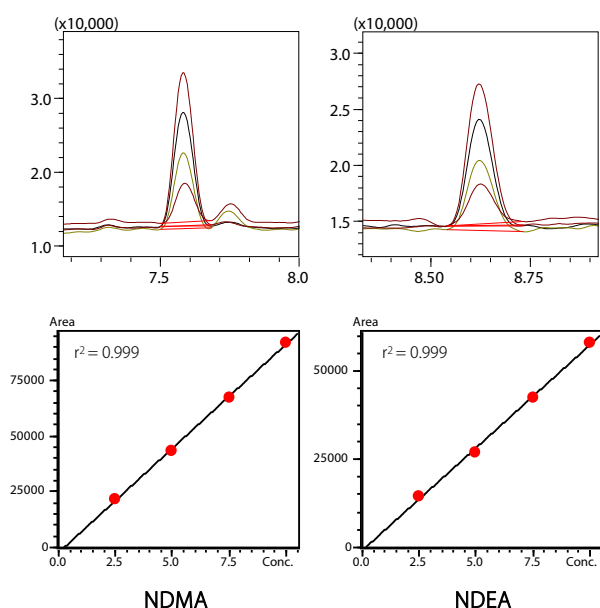


Fig. 2 Chromatogram and Calibration Curve of NDMA, NDEA Standard Solution

Table 4 Analytical Conditions

[HeadSpace Sampler]	
Instrument	: HS-20
Mode	: Loop (1 mL)
Pressure	: 103 kPa
Oven Temp.	: 120.0 °C
Warming Time	: 15 min
[GC-MS]	
Instrument	: GCMS-QP2020 NX
Column	: SH-StabilWax™, 30 m × 0.32 mm I.D., 0.25 μm
Oven Program	: 40 °C (2 min)_10 °C/min_120 °C_ 25 °C/min_230 °C (5.6 min)
Flow Control Mode	: Constant linear velocity
Linear Velocity	: 45.6 cm/sec.
Injection Mode	: Splitless
Ion source Temp	: 200 °C
Ionization	: EI (70 V)
Mode	: SIM (NDMA: m/z 74.0, NDEA m/z 102.0)

Table 5 Summary of Recovery Test Result (%)

API	Valsartan		Losartan		Olmesartan	
	NDMA (N.D.)	NDEA (130.4 ng/g)	NDMA (N.D.)	NDEA (74.1 ng/g)	NDMA (N.D.)	NDEA (130.4 ng/g)
2.5 μg/L	108.6	104.6	95.0	113.4	103.4	114.0
5.0 μg/L	99.9	96.5	96.1	107.9	89.8	88.7
10.0 μg/L	100.5	114.2	99.8	107.7	98.3	102.7

3-2. Analysis of Nitrosamine in Sartan Drug - Direct Injection-GC-MS/MS -

When the GC/MS method is employed, the direct injection method is more suitable than the headspace method for simultaneous analysis of samples containing less volatile substances. This section presents an example of simultaneous analysis of seven nitrosamines using the GC-MS/MS, including NDMA, NDEA, and NDBA, which are contained in the FDA released method. Four other nitrosamines are *N*-nitrosmethylethylamine (NMEA), *N*-nitrosdipropylamine (NDPA), *N*-nitrospyrrolidine (NPYR), and *N*-nitros piperidine (NPIP), which are also believed to be probable human carcinogens. The stable isotope NDMA-D6 was added as the internal standard. Four samples of -sartan drug were dissolved and extracted with dichloromethane, subsequently centrifuged, filtered, and directly injected into GC-MS/MS.



GCMS-TQ™8050 NX

Figs 3 and 4 show chromatograms of 50 ng/mL mixed standard solutions of seven nitrosamines and 5 ng/mL of NDMA, NDEA, and NDBA, respectively. The linearity at 2.5 - 100 ng/mL, repeatability of the standard solution (5 ng/mL), and Signal to Noise were satisfactory, showing an excellent performance (Table 6). The recovery of olmesartan extract by addition of standard solution (5 ng/mL) was 105 - 144 %.

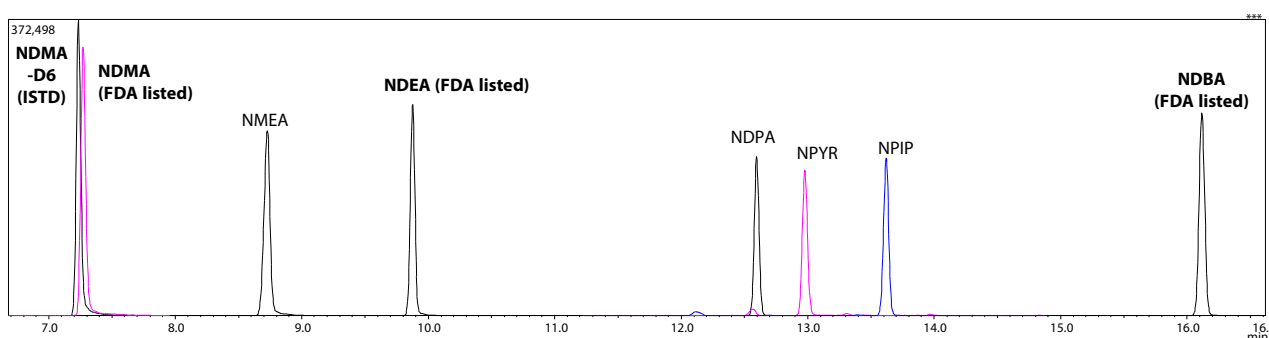


Fig. 3 MRM Chromatogram of Seven Nitrosamines in Standard Solution (50 ng/mL)

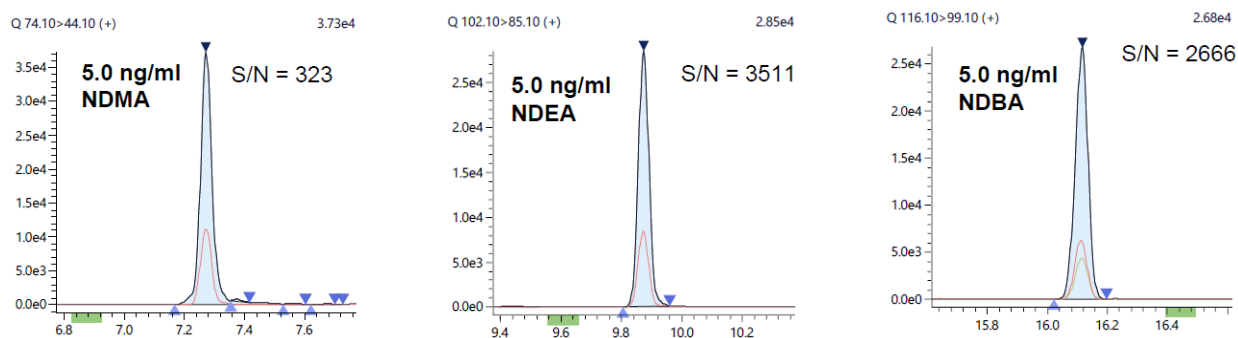


Fig. 4 MRM Chromatograms and S/N of NDMA, NDEA, NDBA in Standard Solutions (5 ng/mL)

Table 6 Linearity and Repeatability

Compounds	MRM Transition	Correlation Coefficient	%RSD 5 ng/mL, (2.5 - 100 ng/mL) n=6
NDMA	74.10 > 44.10	0.9995	4.37
NDEA	102.10 > 85.10	0.9998	3.42
NDBA	116.10 > 99.10	0.9999	3.96
NMEA	88.10 > 71.10	0.9997	1.79
NDPA	130.10 > 113.10	0.9993	5.61
NPYR	100.10 > 55.10	0.9991	2.68
NPIP	114.10 > 84.10	0.9997	5.27
NDMA-D6 (ISTD)	80.10 > 50.10		

Table 7 Analytical Conditions

Instrument	: GCMS-TQ8050 NX
Column	: SH-Rxi™-624Sil MS, 30 m × 0.25 mm I.D., 1.4 μm
Oven Program	: 38 °C (1.0 min)_12 °C/min_160 °C_5°C/min_200 °C (1.0 min)
Flow Control Mode	: Constant linear velocity
Linear Velocity	: 42.6 cm/sec
Injection Mode	: Splitless (250 kPa, 2 min)
Ion source Temp	: 230 °C
Ionization	: EI (70 V)
Mode	: MRM

3-3. Analysis of Nitrosamine in Sartan Drug - LC-MS/MS -

In addition to the GC/MS method, the FDA and OMCL have also adopted the LC/MS/MS method for the analysis of nitrosamines. An example of the analysis of six nitrosamines (NDMA, NDEA, NDIPA, NDBA, NEIPA, and *N*-nitrosomethylbutylamine (NMBA)) in olmesartan using the triple quadrupole liquid chromatograph mass spectrometer LCMS™-8060 is shown below. Olmesartan was dissolved in 300 µL of dichloromethane and methanol containing 10 % acetic acid was added to make 1 mL. Glycerol was then added, evaporated to dryness, and the residue was dissolved in 1 mL of water/methanol (7/3) solution for analysis.



The chromatogram of the 0.1 ng/mL standard solution, 1/10 of the limit of quantification (as the concentration of solution) requested by the FDA, is shown in Fig. 5. The linearity at 0.1 - 10 ng/mL was over 0.99, and the accuracy in this concentration range was within 80 - 120 %, showing excellent results.

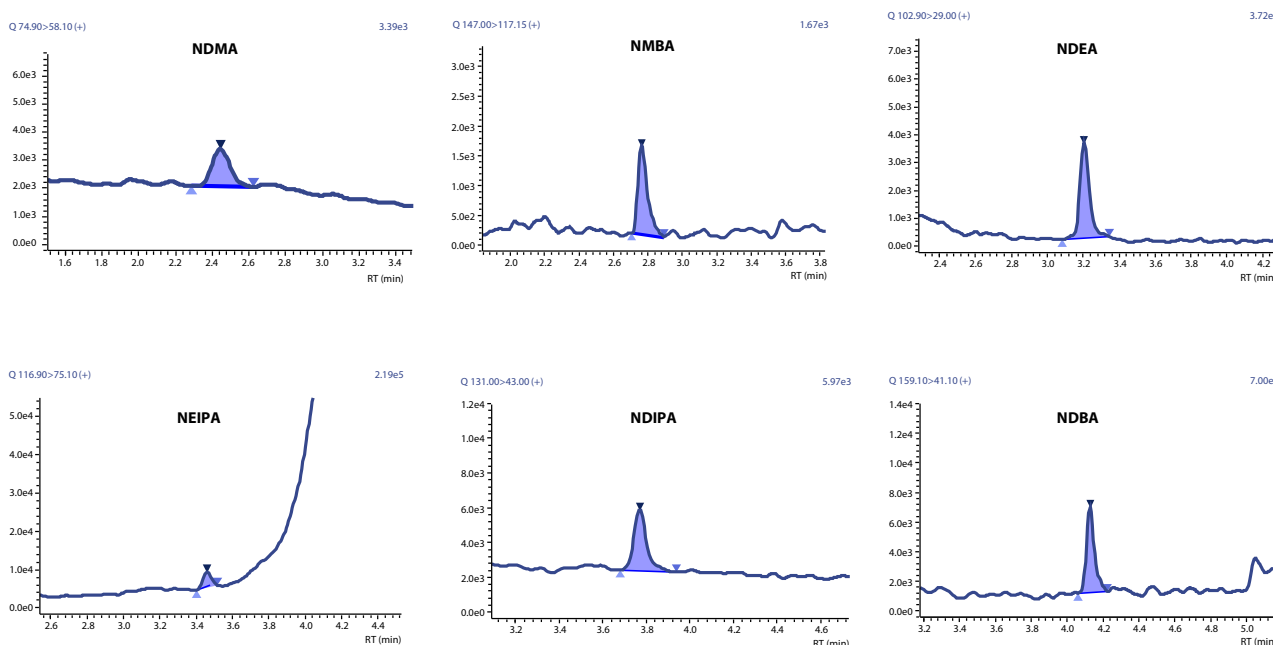


Fig. 5 Chromatogram of Nitrosamines in 0.1 ng/mL Standard Solution

Table 8 Analytical Conditions

Instrument	: LCMS-8060
[LC]	
Column	: Shim-pack Arata™ C18, 150 mm × 3.0 mm I.D., 2.2 µm
Mobile Phase	: A 0.05 % formic acid Water B 0.05 % formic acid in Methanol Gradient
Flow Rate	: 0.50 mL/min
Column Temp.	: 50 °C
Injection Volume	: 40 µL
[MS]	
Ionization	: APCI (Positive)
Mode	: MRM
	NDMA : 74.90>58.10
	NMBA : 147.00>117.15
	NDEA : 102.90>29.00
	NEIPA : 116.90>75.10
	NDIPA : 131.00>43.00
	NDBA : 159.10>41.10

3-4. Analysis of Nitrosamine in Ranitidine Drug

In September 2019, EMA and FDA announced that trace amounts of NDMA were detected in API and pharmaceutical formations containing ranitidine hydrochloride, a histamine H₂ receptor antagonist. Ranitidine contains dimethylaminomethyl structurally and is known to have the possibility to form NDMA in the presence of nitrites during the manufacturing process. The FDA also recommends LC/MS method because NDMA is formed when ranitidine and nizatidine are analyzed by the headspace-GC/MS method under high temperature condition. Here, we present an example of the analysis of NDMA in ranitidine by quadrupole time-of-flight (QTOF) LC/MS. Nizatidine, which has dimethylaminomethyl group in its structure, must be tested for NDMA, similar to Ranitidine.

A total of 120 mg of a ranitidine preparation was dissolved in 4 mL of methanol and analyzed. The linearity at 1.14 -146 ng/mL of the standard solution was >0.999, and the accuracy at each calibration curve point was 80 - 120 % (Fig. 6). The S/N at 1.0 ng/mL (Equivalent to 0.033 ppm concentration in the drug product) of the standard solution was 13, and the relative standard deviation of the peak area values at 2.0 ng/mL was 8.4 % (Table 10). NDMA in the drug preparation was 0.11 ppm. The average recovery of the drug substance (3 samples) added with 0.1 ppm of the standard solution was 88.4 % (Fig. 7).



Table 9 Analytical Conditions

Instrument	: LCMS-9030
[LC]	
Column	: Shim-pack™ GIST C18, 150 mm × 4.6 mm I.D., 5.0 μm
Mobile Phase	: A 0.1 % formic acid I Water B Methanol Gradient
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Volume	: 5 μL
[MS]	
Ionization	: APCI (Positive)
Mode	: Scan (m/z 50-95)

Table 10 Repeatability of NDMA in Standard Solution (2.0 ng/mL)

	Peak Area	Retention Time (min)
1	1892	4.107
2	1953	4.133
3	1595	4.118
4	1820	4.123
5	2040	4.142
6	1776	4.127
Average	1846	4.122
%RSD	8.4	0.29

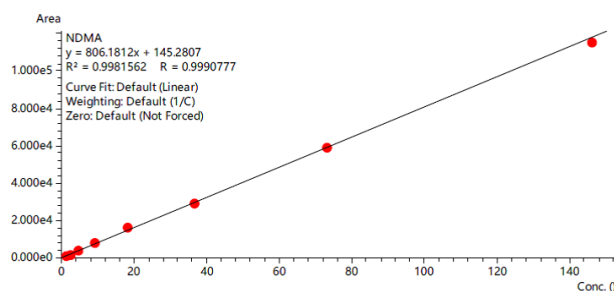


Fig. 6 Calibration Curve

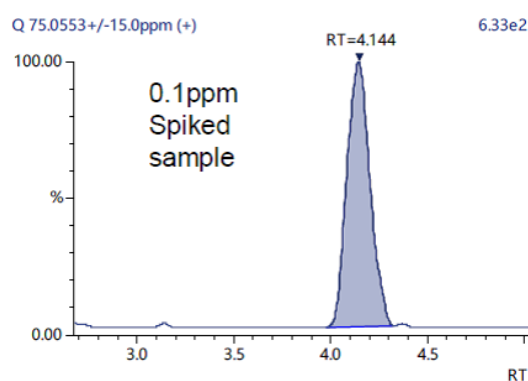
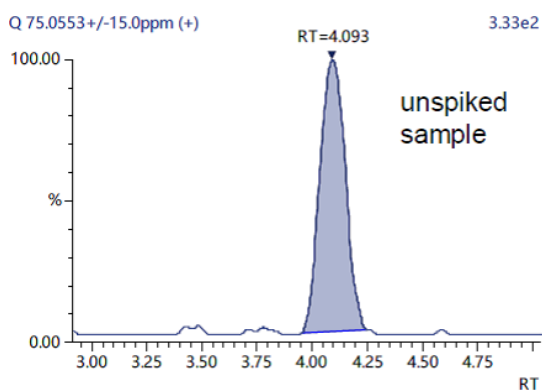


Fig. 7 Chromatograms of NDMA in Drug Substance and in Standard Addition

3-5. Analysis of Nitrosamine in Metformin

In 2019, following the detection of NDMA in preparations containing metformin hydrochloride, a biguanide hypoglycemic agent, Singapore's Health Sciences Authority announced that some business operators in Singapore have initiated voluntary recall of such preparations. GC/MS method with a high-resolution mass spectrometry has been employed in Singapore to test the presence of NDMA in metformin preparations⁽⁸⁾, but here, an example of analysis using the triple quadrupole gas chromatograph GCMS-TQ™ 8050 NX is shown.

After pulverizing the sample, an amount equivalent to 0.5 g of metformin was weighed, dissolved in 10 mL of 1 mol/L hydrochloric acid, extracted using 10 mL of dichloromethane, and analyzed.



GCMS-TQ™8050 NX

Figs 8 and 9 and Table 12 show the results of sensitivity using the standard solution (0.25 ng/mL) and the recovery following the addition of the standard solution. These results indicate that the triple-quadrupole gas chromatograph mass spectrometer GCMS-TQ 8050 NX can be effectively employed for the analysis of NDMA in metformin.

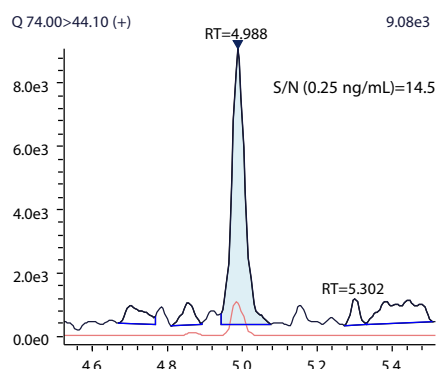


Fig. 8 Chromatogram of NDMA in Standard Solution (0.25 ng/mL)

Table 11 Analytical Conditions

Instrument	: GCMS-TQ8050 NX
Column	: SH-Rtx™-Wax, 30 m × 0.25 mm I.D., 0.5 μm
Oven Program	: 60 °C (0.5 min)_15 °C/min_150 °C_20 °C/min_240 °C (2 min)
Flow Control Mode	: Constant linear velocity
Linear Velocity	: 51.6 cm/sec
Injection Mode	: Splitless
Ion source Temp	: 230 °C
Ionization	: EI (70 V)
Mode	: MRM (Target: 74.00 > 44.10, Reference: 74.00 > 42.10)

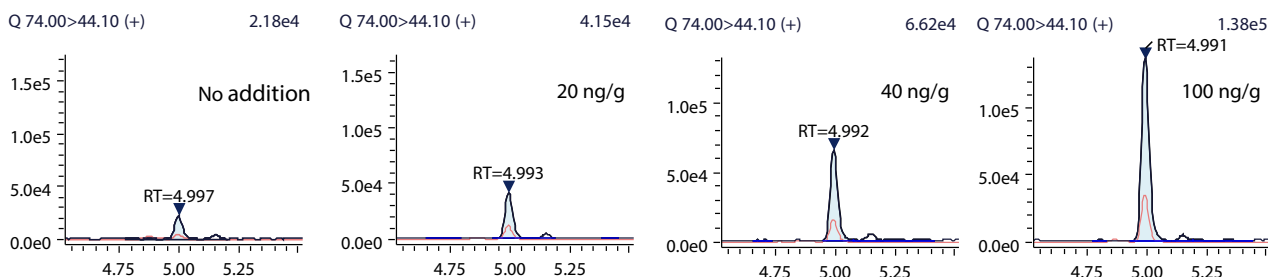


Fig. 9 Chromatograms of NDMA in Metformin Drug Substance and in Standard addition

Table 12 Result of Recovery

Spiked Conc. (ng/g)	Background Conc. (ng/g)	Detection Conc. (ng/g)						Average Conc. (ng/g)	RSD%	Recovery (%)
		1	2	3	4	5	6			
20	13.6	28.6	31.0	29.6	32.0	31.2	30.8	30.6	3.94	85.0
40	13.6	45.8	47.0	49.8	47.0	50.6	50.4	48.4	4.39	87.0
100	13.6	101.2	98.8	101.4	99.4	99.0	100.8	100.2	1.20	86.6

4. Analysis of Methanesulfonate

Methanesulfonic acid is widely used in the manufacturing of bulk drug substances as a counter ion in salt formation, and it is known that harmful alkyl esters are formed as by-products. IARC has classified methyl methanesulfonate (MMS) in Group 2A (probably carcinogenic to humans) and ethyl methanesulfonate (EMS) in Group 2B (possibly carcinogenic to humans). In accordance with the concept of ICH M7 guideline, these compounds correspond to Class 1 on ICH M7, similar to NDMA and NDEA, and it is requested to be controlled below the permissible levels specific to the compound. The following is an analysis example of MMS in methanesulfonic acid, and MMS, EMS, and isopropyl methanesulfonate (IMS) in the drug substances by optimized method with reference to the EP method ⁽⁹⁾.



GCMS-QP™2020 NX

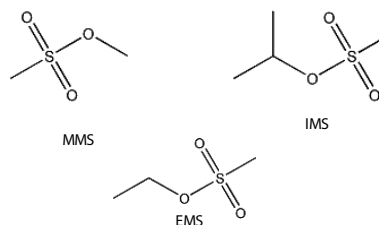


Fig. 10 Chemical Structures of Mesylate Esters

4-1. Analysis by GC/MS

The analysis was performed by the internal standard (IS) method using butyl methanesulfonate (BMS) as IS. The chromatogram is shown in Fig. 11. The detection limit (S/N = 3) calculated from chromatogram of 1.0 ng/mL was 0.3 ng/mL. The chromatogram of the standard solution and calibration curve (1.0 - 10,000 ng/mL) are shown in Fig. 12. When methanesulfonic acid was diluted with water and extracted into dichloromethane, 10.9 µg/g of MMS and 0.03 µg/g each of EMS and IMS were detected.

Table 13 Analytical Conditions

Instrument	: GCMS-QP2020 NX
Column	: non porous, 15 m × 0.25 mm I.D., 1.0 µm
Oven Program	: 55 °C (4.0 min)_8 °C/min_95 °C (4.0 min)_ 40 °C/min_295 °C (1 min)
Flow Control Mode	: Constant Flow
Linear Velocity	: 1.0 mL/min
Injection mode	: Splitless
Ion source Temp	: 230 °C
Ionization	: EI (70 V)
Mode	: SIM

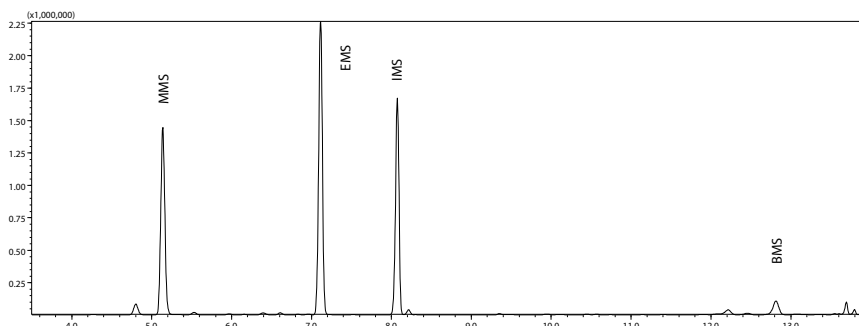


Fig. 11 Chromatogram of MMS, EMS, IMS in Standard Solution (1000 ng/mL)

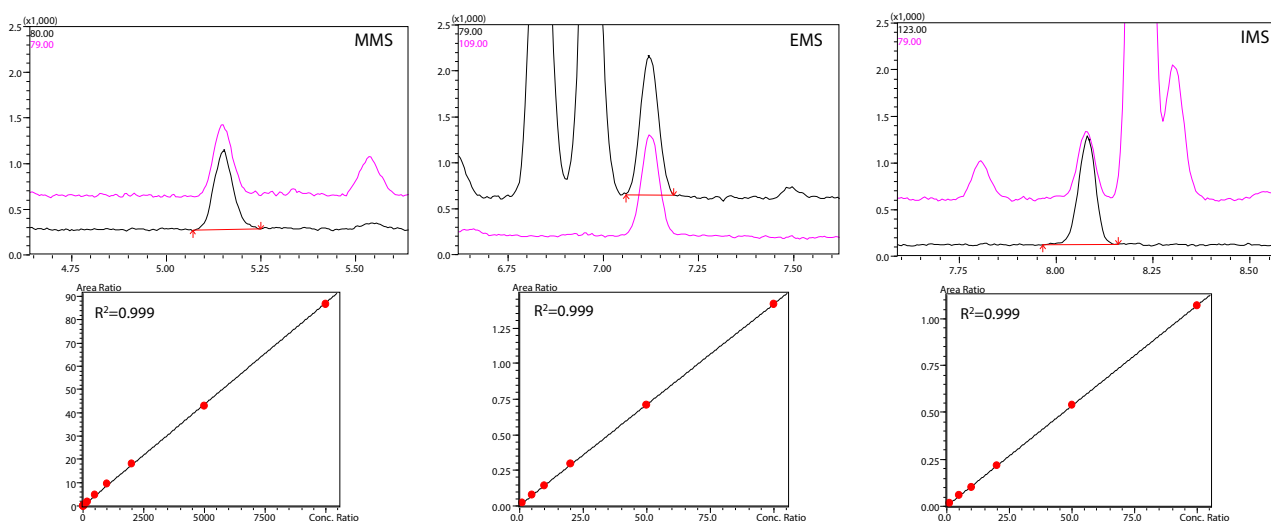


Fig. 12 Chromatograms and Calibration Curves of MMS, EMS, IMS in Standard Solution (1.0 ng/mL)

4-2. Analysis by Headspace-GC/MS

This section presents an example of headspace-GC/MS analysis of MMS, EMS, and IMS in betahistine mesilate, which is used to relieve symptoms, such as dizziness caused by Meniere's disease. Methanesulfonate has high boiling point above 200 °C and is difficult to be extracted into gas phase as it is. Therefore, measurement was carried out after it was derivatized to methyl iodide, ethyl iodide, and propyl iodide in a headspace vial. The internal standard method using BMS was used in a similar manner as in the GC-MS method. The derivatization solution was prepared by dissolving 30 mg of anhydrous sodium thiosulfate and 60 mg of sodium iodide in 50 mL water. The derivatization solution (0.5 mL) was added into a headspace vial along with 25 mg of sample and dissolved the sample before analysis. The mixed standard solutions of MMS, EMS, and IMS were derivatized in the headspace vial in the same way and measured. The calibration standards were prepared in the range of 1.5 - 250 ng/mL (3.0 - 250 ng/mL for IMS). Figs 13 and 14 show chromatograms of standard solutions (250 ng/mL and 3 ng/mL). As MMS, EMS, and IMS were not detected in the samples, recovery tests using the addition of standard solutions were conducted, and excellent recovery was demonstrated (Table 15).



GCMS-QP™2020 NX + HS-20

Table 14 Analytical Conditions

[HeadSpace Sampler]	
Instrument	: HS-20
Mode	: Loop (1 mL)
Oven Temp.	: 60.0 °C
Warming Time	: 30 min
[GC-MS]	
Instrument	: GCMS-QP2020 NX
Column	: Polar (PEG), 30 m×0.25 mm I.D., 1.0 μm
Oven Program	: 40 °C (1 min)_10 °C/min_130 °C_40 °C/min_240 °C (7 min)
Flow Control Mode	: Constant Flow
Linear Velocity	: 0.5 mL/min
Injection Mode	: Splitless
Ion source Temp	: 240 °C
Ionization	: EI (70 V)
Mode	: SIM

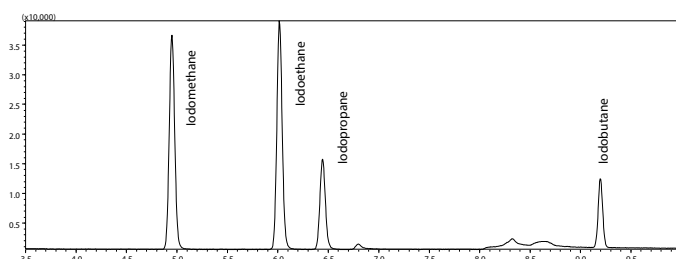


Fig. 13 Chromatogram of MMS, EMS, IMS in Standard Solution (250 ng/mL)

Table 15 Result of Recovery

Compounds	Concentration (μg/g)	Quantities of Addition (ng)	Recovery (Ave.(n=3), %)
MMS	N.D.	40	102.3
EMS	N.D.		107.7
IMS	N.D.		110.9

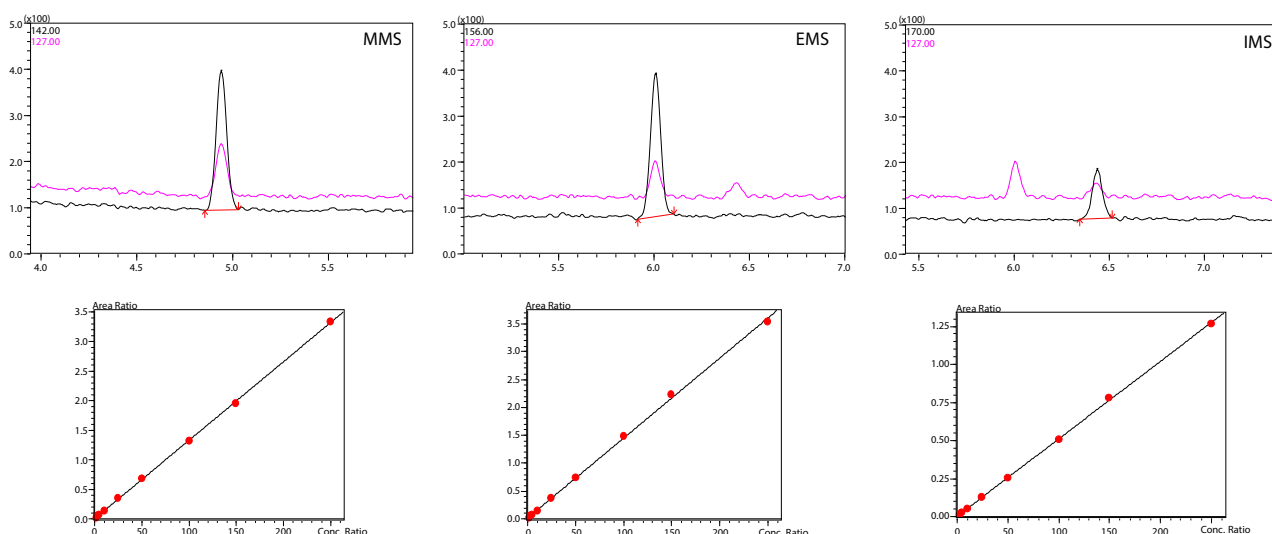


Fig. 14 Chromatograms and Calibration Curves of MMS, EMS, IMS in Standard Solution (3.0 ng/mL)

5. Analysis of Benzenesulfonate, *p*-Toluenesulfonate

The following are analytical examples of benzenesulfonic acid and toluenesulfonic acid, which are also used as counter ions in pharmaceuticals. Methyl benzenesulfonate (MBS), ethyl (EBS), and isopropyl (IBS) in amlodipine besylate, an antihypertensive agent, and methyl *p*-toluenesulfonate (MTS), ethyl (ETS), and isopropyl (ITS) in sultamicillin tosylate, a penicillin antibiotic, were analyzed using headspace-GC/MS. Similar to the analysis of methanesulfonate in betahistine mesilate, headspace GC/MS with derivatization method was employed. Refer to 4-2 for preprocessing operations and analysis conditions. Calibration curves were prepared in the range of 1.5 - 250 ng/mL (3.0 - 250 ng/mL for IMS), and correlation coefficient was >0.999 for all compounds. The recovery test of the addition of standard solution also showed excellent results (Tables 16 and 17).



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Chromatograms of standard solution (3.0 ng/mL) are shown in Fig. 15. In addition, the detection limit (S/N = 3) was calculated from the results of the minimum concentration of the calibration curve. Quantification limits of Benzenesulfonate and *p*-toluenesulfonate were almost similar to methanesulfonate, indicating that there was no significant difference in the yields of derivatization.

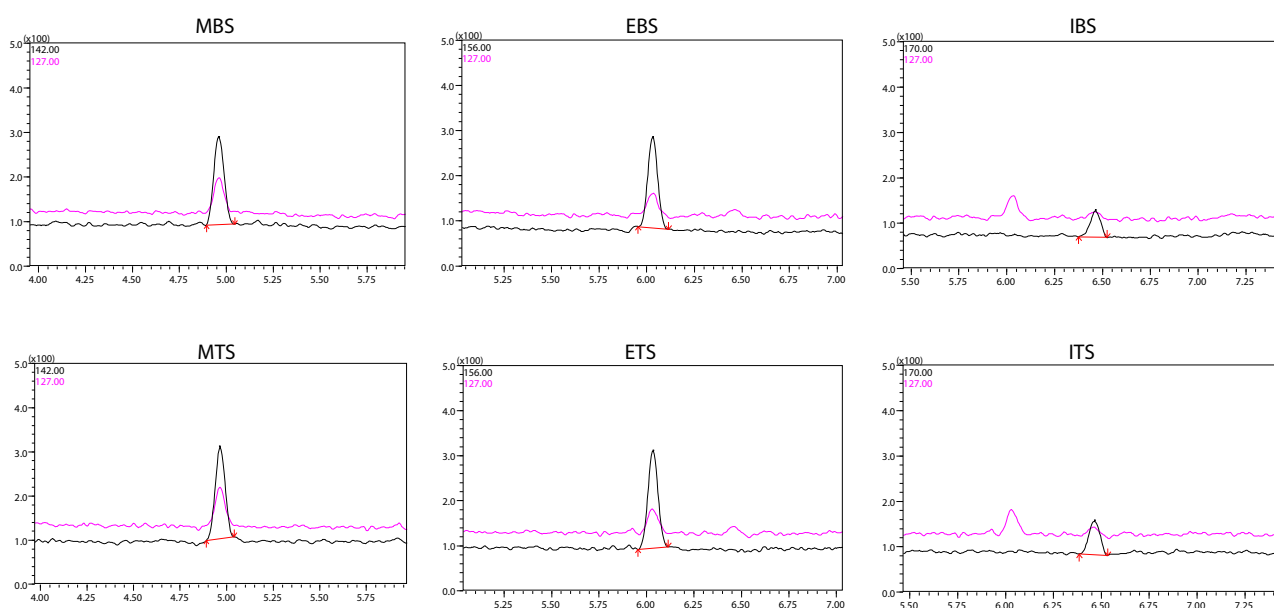


Fig. 15 Chromatograms of Besylate and Tosylate in Standard Solution (3.0 ng/mL)

Table 16 Result of Recovery (Besylate)

Compounds	Concentration (μg/g)	Quantities of addition (ng)	Recovery (Ave.(n=3),%)
MBS	0.17	2.5	102.1
EBS	0.04		95.9
IBS	N.D.		86.7

Table 17 Result of Recovery (Tosylate)

Compounds	Concentration (μg/g)	Quantities of addition (ng)	Recovery (Ave.(n=3),%)
MTS	0.81	10	106.6
ETS	0.05		105.4
ITS	N.D.		91.8

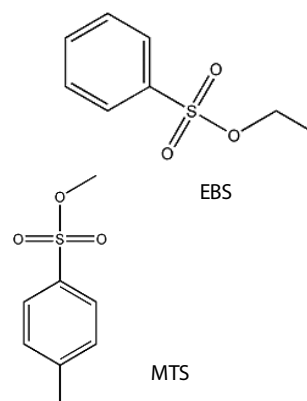


Fig. 16 Chemical Structures of Ethyl Benzenesulfonate and Methyl Tosylate

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