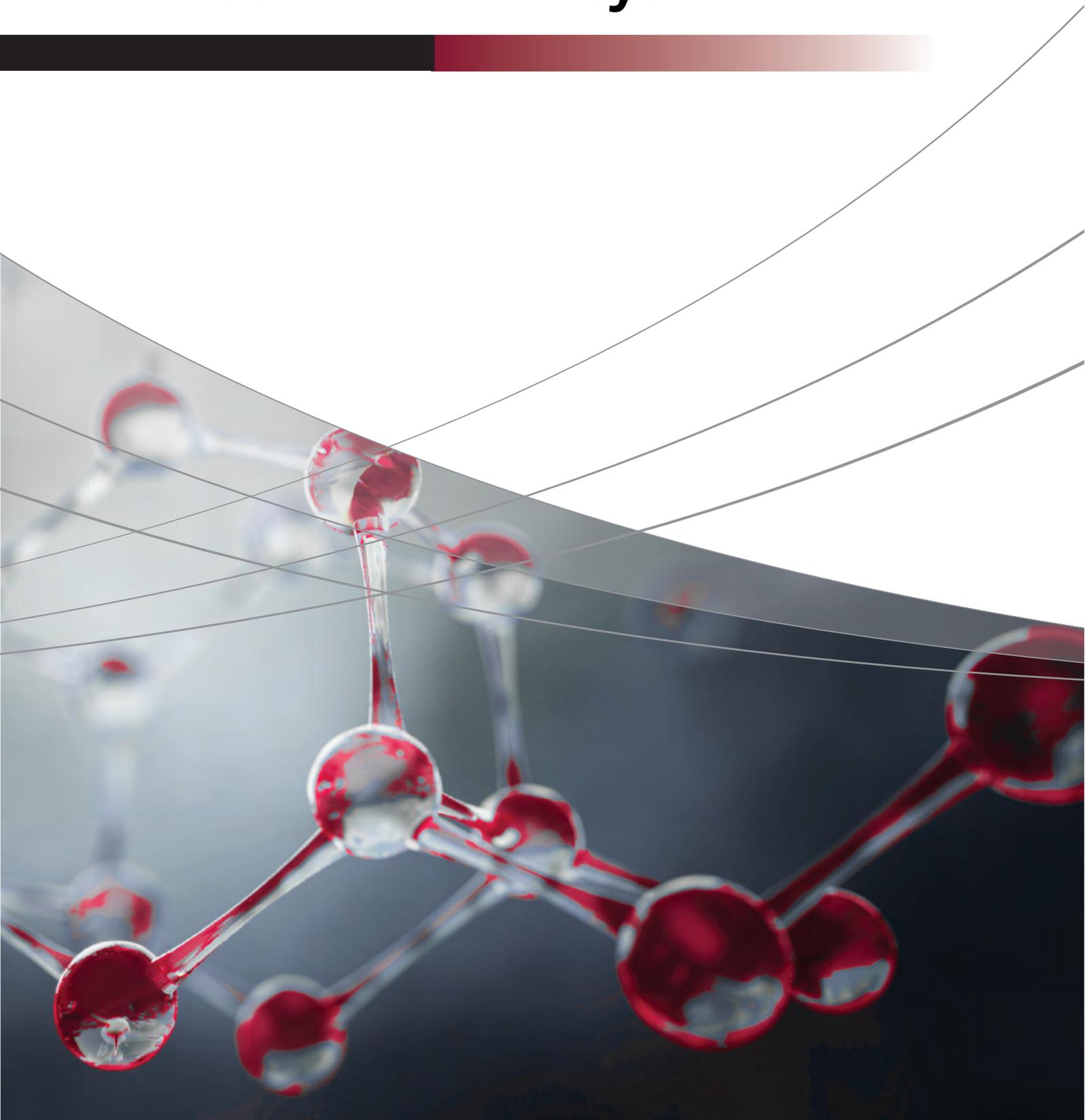


# UFMS Approach for Nitrosamine Analysis





# Nitrosamine

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Nitrosamine compounds are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). Ever since the first U.S. Food and Drug Administration (FDA) notification in June 2018 about levels of these impurities in pharmaceutical API, they have been a reason of continuous discussion globally for analytical scientists, patients and regulators. After June 2018, the number of recalls and 483's have been on a rise for not only angiotensin II receptor blockers (-ARB, also called as sartans) but also for other products like Ranitidine, Metformin etc. FDA, European Medicine Agency (EMA) and International Council for Harmonisation (ICH) have constantly set and updated the guidelines in measuring impurities in pharmaceutical products.

Holistic solution to measurement of nitrosamines need both GC-MS/MS and LC-MS/MS technologies. At Shimadzu, we have developed future proof methods for anticipated challenges like:



Triple quadrupole is gold standard for quantitation, but in such critical cases use of HRMS may also be considered. We shall share some interesting findings for both qualitative and quantitative aspects for analysis of nitrosamines using QTOF.



Scan to learn more

# GCMS- TQ8040 NX *with AOC-20i Plus and HS-20*

Shimadzu's GCMS-TQ8040 NX along with AOC-20i Plus liquid injector and HS-20 Headspace autosampler is a complete solution for wide variety of impurity analysis and pharmaceutical applications. Accompanied with easy sample preparation, speed of UFMS technologies and compliant platforms, GCMS-TQ8040 NX is a workhorse of any R&D or QC lab.



## Key Features

### UFMS™ Throughput

UFMS ensures no compromise in sensitivity even when you run applications at maximum speed. This allows you to perform both Targeted screening and Quantitation with the same confidence. The combination of multiple injection modes (liquid or headspace) and acquisition modes (MRM, SIM or Scan) are designed to cater to all your impurity analysis needs. This can be performed with maximum ease and flexibility.

### Minimal Maintenance

In rush of work, we need our instruments to make life easy for us. Regular tasks like maintaining the injection port or installing column are made super easy and hassle free using ClickTek™ and Easy sTop function. Active Time Management™ helps plan work schedules by giving clear estimates of autotuning, batch completion, time management during maintenance etc.

### Minimal carryover

In high throughput labs, where time is money, you don't want to spend time doing trouble-shooting and re-analysis. One of the key contributors to this trouble is the transfer line between headspace and GCMS. Shimadzu HS-20 is equipped with not only the shortest but also most inert transfer line. This means near zero carryover even for very polar compounds like triethylamine.

### Regulatory Compliance

Rest assured that data integrity and authenticity is maintained in accordance with industry regulation norms such as FDA 21 CFR Part 11. Your data is always backed up and protected from unauthorised access. We continuously provide enhanced solutions and upgrades to keep up with regulators direction.

## UFMS Measurement of 24 Nitrosamines using GCMS-TQ8040 NX

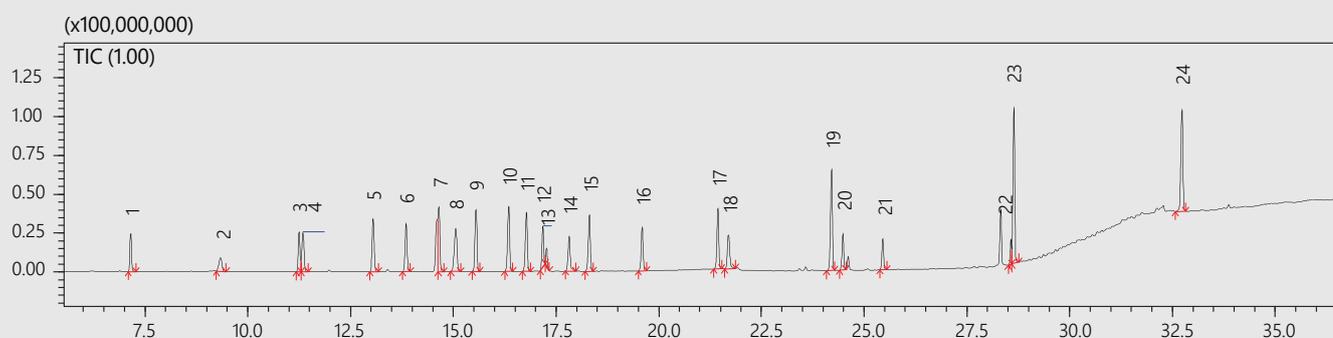


Figure 1: Chromatogram reflecting analysis of 24 Nitrosamine standards using GCMS-TQ8040 NX

- |   |                                     |                                      |
|---|-------------------------------------|--------------------------------------|
| 1. N-Nitrosodimethylamine (NDMA)        | 9. N-Nitroso-N-methylbutylamine     | 17. N-Nitrosodi-n-butylamine (NDBA)  |
| 2. N-Nitrosomethylethylamine            | 10. N-Nitrosodi-N-propylamine       | 18. N-Nitrosopiperazine Monomer      |
| 3. N-Nitrosodiethylamine (NDEA)         | 11. N-Nitroso-N-ethylbutylamine     | 19. N-Nitroso N-methyl N-phenylamine |
| 4. N-nitrosomethylisopropylamine        | 12. N-Nitroso-N-ethyl-N-phenylamine | 20. N-Nitroso-N-methylbenzylamine    |
| 5. N-Nitrosoethylisopropylamine (NEIPA) | 13. N-Nitrosomorpholine             | 21. N- Nitrosopiperazine Dimer       |
| 6. N-Nitroso ethyl-N-propylamine        | 14. N-Nitrosopyrrolidine            | 22. N-Nitrosoaminodiethanol          |
| 7. N-Nitroso-N-ethyl-tert-butylamine    | 15. N-Nitrosopiperidine             | 23. N-Nitrosodiphenylamine           |
| 8. N-Nitroso diisopropyl amine (NDIPA)  | 16. N-Nitroso-N-methylpiperazine    | 24. N-Nitrosodibenzylamine           |



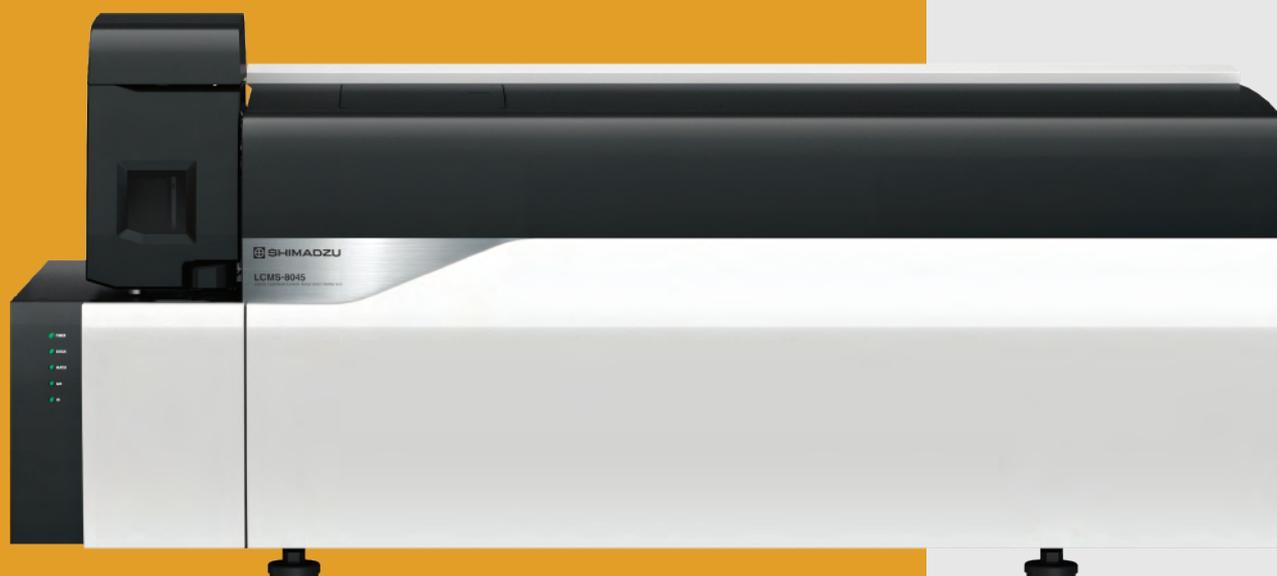
Scan to learn more

# LCMS-8045

*(Field Upgradable to  
LCMS-8050 & LCMS-8060)*

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LCMS-8045 provides optimum sensitivity and ease of use for demanding and high throughput impurity applications. A true Ultra Fast Mass Spectrometer (UFMS), LCMS-8045 is an ideal answer to demanding impurity analysis. This system is packed with key design features for quick and easy use of LCMS technology for your lab at both R&D and remote manufacturing QC sites.



## Key Features

### UFscanning™ & UF-MRM™ with High Sensitivity

Equipped with a heated ESI probe, the LCMS-8045 has the highest sensitivity in its class. It is capable of providing accurate and stable data over long periods of time. The inclusion of Shimadzu's ultra-high-speed high-voltage power supply enables the world's fastest scan speed (30,000 u/s) and polarity switching time (5 ms). High-speed acquisition benefits the laboratory by reducing run times for increased throughput, and also shortens method development time.

### Easy Workflow

LabSolutions LCMS features an intuitive user interface and offers the latest features designed to enhance laboratory productivity and streamline workflows with complete compliance

### Superior Robustness

The LCMS-8045 is designed to be robust. The heated ESI probe, high-temperature heating block, heated desolvation line, drying gas, and focusing optics all act to maximize sensitivity and minimize contamination. This means long periods of continuous operation in the laboratory with reliable data collection.

### Regulatory Compliance

Simple and easy to use workflows are inherently designed to deliver compliance. Your data is always backed up and protected from unauthorized access. We continuously provide enhanced solutions and upgrades and keep up with regulators direction.

## UFMS Measurement of 15 Nitrosamines using LCMS-8045

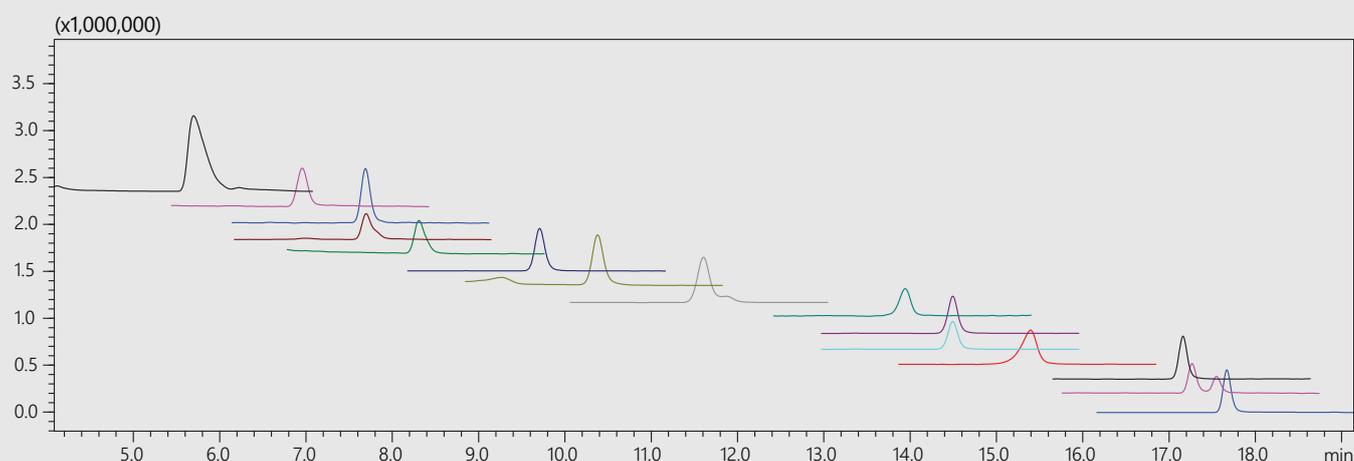
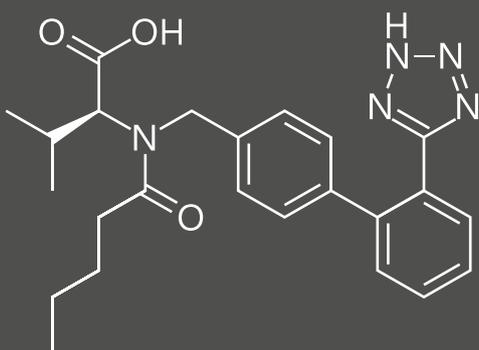


Figure 2: Chromatogram reflecting analysis of 15 Nitrosamine standards using LCMS-8045

- |  |                                     |                                       |
|--|-------------------------------------|---------------------------------------|
| 1. N-Nitrosodimethylamine                        | 6. N- Nitrosodibutylamine           | 11. N-nitrosomorpholine               |
| 2. N-Nitrosodiethylamine                         | 7. N-Nitrosopiperidine              | 12. N-nitroso di n propyl amine       |
| 3. N-Nitrosoisopropylethylamine                  | 8. N-Nitroso-n- ethyl n-propylamine | 13. N-nitroso methylethylamine        |
| 4. N-Nitroso-N-methyl-4-aminobutyric Acid (NMBA) | 9. N-nitrosopyrrolidine             | 14. N-nitroso diphenylamine           |
| 5. N-Nitrosodiisopropylamine                     | 10. N-nitrosodibenzylamine          | 15. N-nitroso-n-methyl n-phenyl amine |



# Nitrosamine Analysis in Valsartan

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Sartans or Angiotensin II receptor blockers (ARBs), are a group of pharmaceuticals that modulate the renin–angiotensin system, hence used for treatment of hypertension, congestive heart failure etc. Some commonly used sartans include valsartan, telmisartan, losartan, irbesartan, olmesartan etc. Recently they have captured limelight due to large number of recalls caused by detection of nitrosamine impurities which are potentially carcinogenic.

Valsartan was the first API which was noticed positive for nitrosamine. Since then different regulatory agencies have issued guidance on determination level, which is based on maximum daily dose. Table 1 describes the interim limits for some nitrosamines in Valsartan (these values are applicable to other sartans as well)

# Limits and Range

Table 1: LOD, LOQ and calibration range values obtained on Shimadzu GCMS-TQ8040 NX and LCMS-8045

	GCMS-TQ8040 NX (in ppm)			LCMS-8045 (in ppm)		
	LOD	LOQ	Range	LOD	LOQ	Range
NDMA	0.0010	0.0025	0.0025 - 0.2000	0.0025	0.0050	0.0050 - 0.1000
NDEA	0.0010	0.0025	0.0025 - 0.2000	0.0010	0.0025	0.0025 - 0.1000
NEIPA	0.0010	0.0025	0.0025 - 0.2000	0.0005	0.0010	0.0010 - 0.1000
NDIPA	0.0010	0.0025	0.0025 - 0.2000	0.0010	0.0025	0.0025 - 0.1000
NDBA	0.0010	0.0025	0.0025 - 0.2000	0.0025	0.0050	0.0050 - 0.1000
NMBA	N.A	N.A	N.A	0.0025	0.0050	0.0050 - 0.1000

## Analysis of 5 Nitrosamines in Valsartan using GCMS-TQ8050 NX

All required nitrosamine impurities were checked in valsartan API using GCMS-TQ8050 NX. The LOD, LOQ and calibration range (wrt sample) are mentioned in table 1. Method was tested for linearity and  $R^2 > 0.999$  was obtained for all the impurities. Importantly, excellent repeatability was obtained using developed method on GCMS-TQ8050 NX. %RSD of LOQ was found to be less than 12% (for n=6) and %RSD for working standard solution for all impurities was less than 1.8% (for n=6)

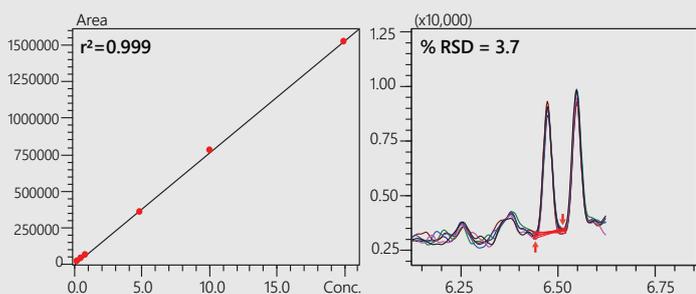


Figure 3a: Calibration and overlay of 6 LOQ of NDMA with %RSD < 3.7%

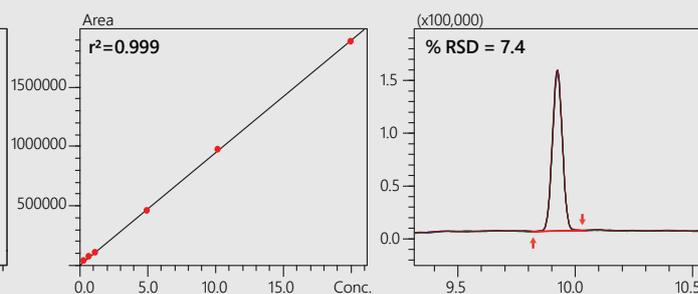


Figure 3b: Calibration and overlay of 6 LOQ of NDBA with %RSD < 7.4%

## Analysis of 6 Nitrosamines in Valsartan using LCMS-8045

A robust chromatographic method was developed using Shim-pack Velox PFPP column (P/N: 227- 32023-03) enabling clear chromatographic separation between API and all nitrosamine impurities along with sharp peak shapes. This eliminates any ion suppression effect which may occur due to multi fold difference in concentration of API and impurities. Limit of detection, quantitation and calibration range are mentioned in table above with  $R^2$  of each calibration being > 0.99

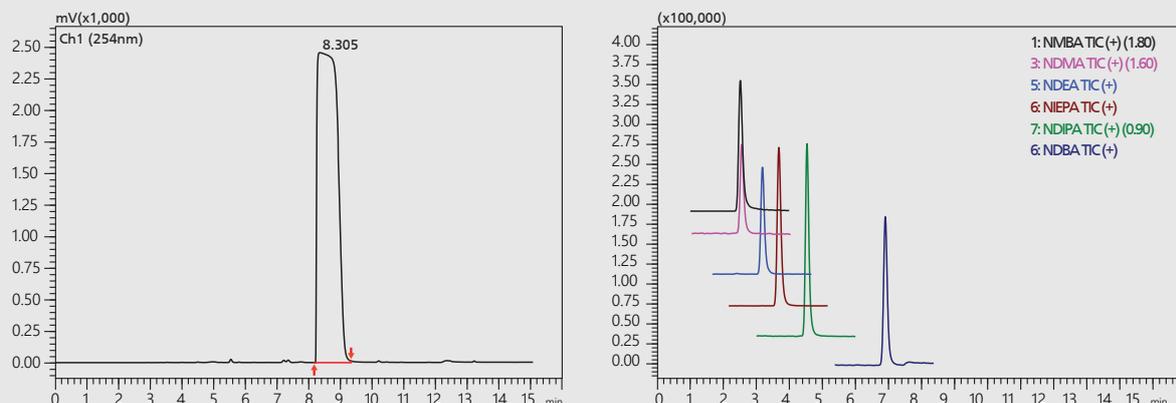
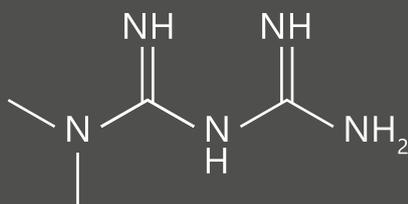


Figure 4: UV Chromatogram of Valsartan (left) and MS chromatograms of nitrosamines (right) show complete chromatographic separation of API and 6 impurities.



# Nitrosamine Analysis in Metformin

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Metformin is a prescription drug used to control high blood sugar in patients with type 2 diabetes. Following the trend of sartans, Health Sciences Authority, Singapore issued notification for recall of 3 out of 46 metformin medicines. Though U.S. FDA statement released on 3rd Feb, 2020 does not mention of any positively tested batch, it is clear that Metformin is placed under an active scrutiny.

# Limits and Range

Table 2: LOD, LOQ and calibration range values obtained on Shimadzu GCMS-TQ8040 NX and LCMS-8045

	GCMS-TQ8040 NX (in ppm)			LCMS-8045 (in ppm)		
	LOD	LOQ	Range	LOD	LOQ	Range
NDMA	0.0010	0.0025	0.0025 - 0.1000	0.0025	0.0050	0.0050 - 1.0000
NDEA	0.0010	0.0025	0.0025 - 0.1000	0.0010	0.0025	0.0025 - 1.0000
NEIPA	–	–	–	0.0010	0.0025	0.0025 - 1.0000
NDIPA	–	–	–	0.0010	0.0025	0.0025 - 1.0000
NDBA	–	–	–	0.0010	0.0025	0.0025 - 1.0000
NMBA	–	–	–	0.0010	0.0025	0.0025 - 1.0000

## Analysis of NDEA & NDMA in Metformin using GCMS-TQ8040 NX

NDEA and NDMA were checked in metformin API using GCMS-TQ8040 NX. The LOD, LOQ and calibration range (wrt sample) are mentioned in table 2. Method was tested for linearity and  $R^2 > 0.999$  was obtained for both the impurities. Importantly, excellent repeatability was obtained using developed method on GCMS-TQ8040 NX. %RSD of LOQ was found to be less than 15% (for n=6) and %RSD for working standard solution for all impurities was less than 3.4% (for n=6). Recovery study was performed by spiking different levels from 0.25 ppb to 10 ppb in the metformin sample and it was found to be well within the criteria.

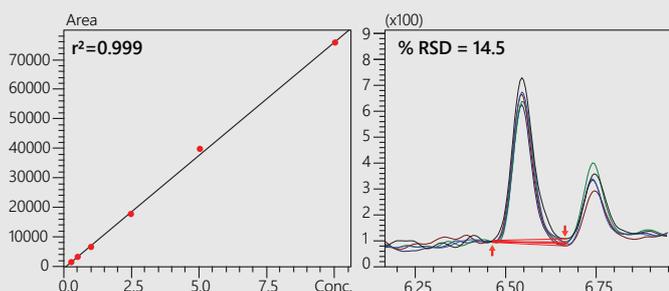


Figure 5a: Calibration and overlay of 6 LOQ of NDMA with %RSD <14.5

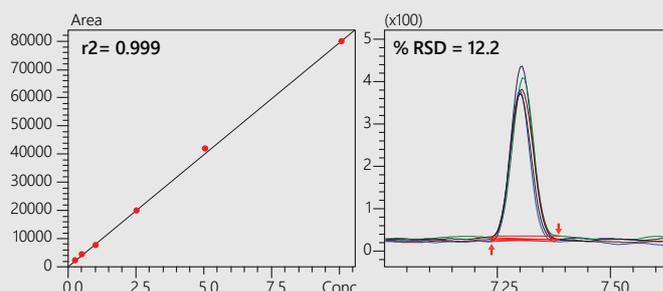


Figure 5b: Calibration and overlay of 6 LOQ of NDEA with %RSD <12.2

## Analysis of 6 Nitrosamines in Metformin using LCMS-8045

Chromatographic method was developed using Shim-pack GIST column (P/N: 227-30017-07) with clear separation between early eluting peak of metformin and later eluting peak of nitrosamines. Method was developed using 2 internal standards: NDMA-D6 and NDEA-D10. LOQ, LOQ and calibration range are mentioned in table 2. Coefficient of correlation  $R^2$  was found to be  $> 0.999$  for all the impurities. Recovery study was performed by spiking 0.005 ppm in the metformin sample and it was found to be well within the criteria.

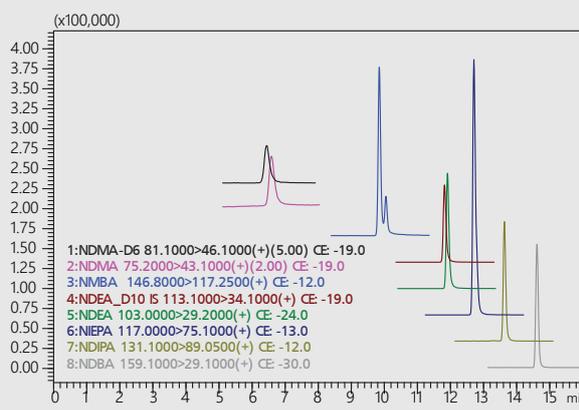
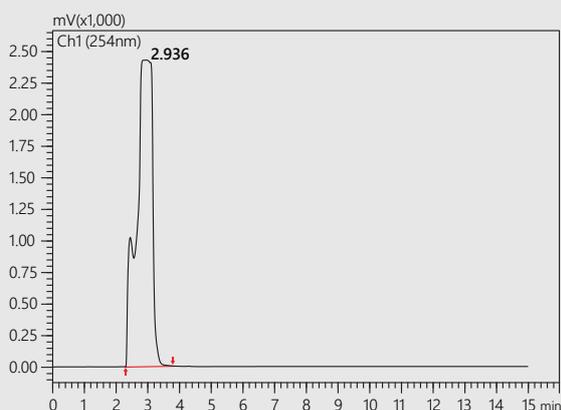
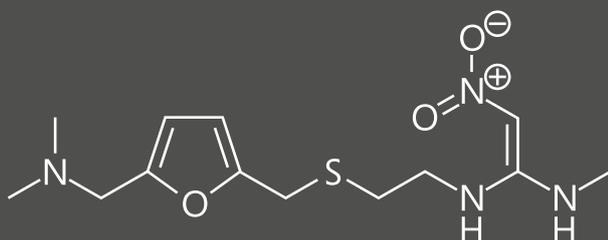


Figure 6: UV Chromatogram of Metformin (left) and MS chromatograms of nitrosamines (right) show complete chromatographic separation of API and 6 impurities.



# Nitrosamine Analysis in Ranitidine

---

Ranitidine HCl is a prescription and over the counter medication used to treat acid reflux. The drug is a histamine-2 receptor antagonist (acid inhibitor or H<sub>2</sub> blocker). Being an OTC drug, it is consumed by mass population and thus, it is necessary to ensure the safety of this drug. At the same time, it is also necessary to avoid false positive results for presence of carcinogenic impurities.

# Analysis of NDMA in Ranitidine using LCMS-8045

FDA has advised companies to recall their ranitidine if testing shows levels of NDMA above the acceptable daily intake (96 nano-grams per day). Hence, quantitation of NDMA from ranitidine at trace levels has become the necessity. Since GC based methods had been observed to elevate NDMA levels in tested materials, an alternative method which prevents the degradation of ranitidine and the subsequent formation of NDMA was therefore needed. Liquid chromatograph coupled with triple quadrupole mass spectrometer is considered as a gold standard for trace level quantitation. Here, LC-MS/MS method for the determination of NDMA in ranitidine drug substance is described. Required LOD of 0.01 ppm and LOQ of 0.033 ppm were achieved.

MRM method is developed for the NDMA where 75 > 43 is used as a quantifier and 75>58 is used as a qualifier. Internal standard method is used for the quantification of NDMA in ranitidine drug substance. Shim-pack GIST C18 AQ (P/N: 227-30724-05) column is used for the separation of N-nitroso-di-methylamine (NDMA) impurity from ranitidine . It is important to separate NDMA peak from drug substance in order to avoid ion suppression and contamination of mass spectrometer. As observed in Figure 7 NDMA peak is well separated from ranitidine drug substance also without any blank interference.

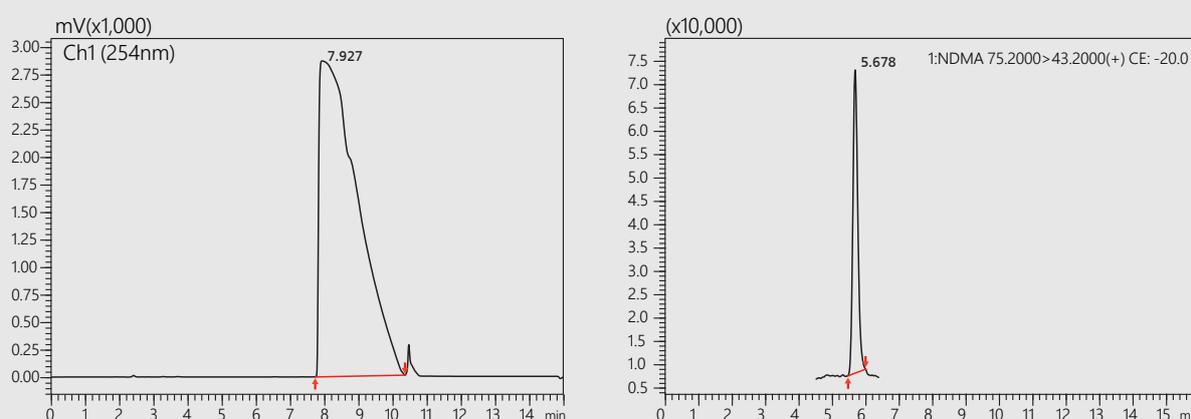


Figure 7: UV Chromatogram of Ranitidine (left) and MS chromatograms of NDMA (right) show complete chromatographic separation of API and impurities.

Calibration curve was plotted by using internal standard method with coefficient of correlation of 0.999 over a range of 1.0 to 100.0 ng/mL (refer Figure 8). Recovery study was performed by spiking LOQ level in the ranitidine sample and it was found to be well within the criteria.

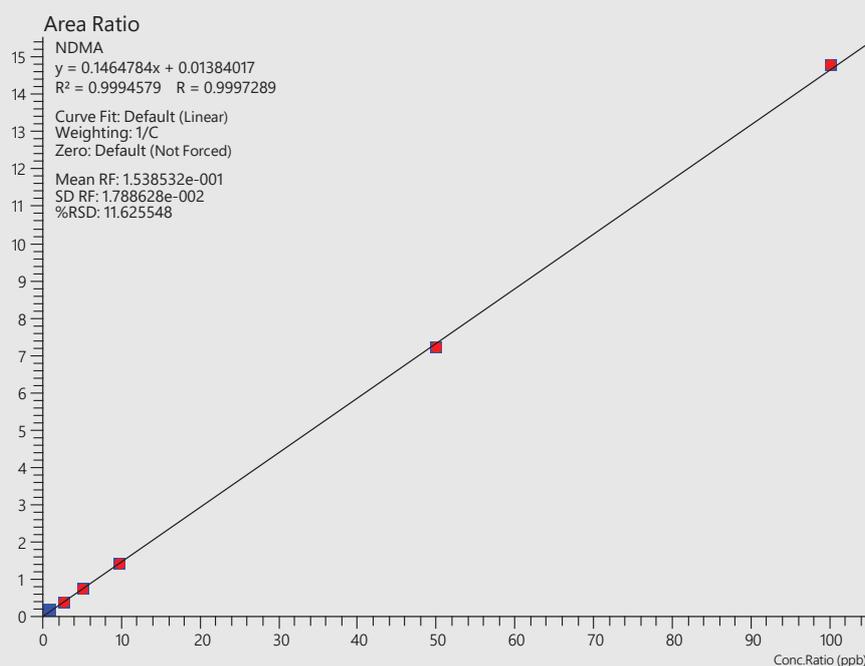


Figure 8: Calibration curve of NDMA



Scan to learn more

# LCMS-9030

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LCMS-9030 Quadrupole Time Of Flight Mass Spectrometer enhances the most important features of Q-TOF instrumentation - mass accuracy with sensitivity, speed and stability to address qualitative and quantitative challenges with genuine confidence and ease.



## Key Features

### Mass Accuracy

The Mass Accuracy of the LCMS-9030 is exceptional over a wide mass range to allow high-confidence identification of unknowns. But even more exceptional is the stability of this mass accuracy. Intelligent flight tube temperature controls enable the LCMS-9030 to reproduce accurate results regardless of fluctuation in laboratory temperatures.

### Sensitivity

Because of its high sensitivity, the LCMS-9030 requires only a small sample size for identification of unknowns. With less contamination of the instrument, it offers robust function for a longer time. High sensitivity also enables users to perform quantitation down to extremely low levels. This means the LCMS-9030 can address both qualitative and quantitative challenges.

### Stability of Mass Accuracy

The mass accuracy stability of the LCMS-9030 ensures that users can achieve the same identification results time after time. Rock stable mass accuracy also means that less frequent calibration is required, making the routine work easy to manage.

### Speed

Users achieve the quantitation results they need: 100 scans per second for high-sensitivity quantitation of targeted compounds. 100 scans per second also applies to MS/MS acquisition for a comprehensive screening of unknowns. The LCMS-9030 not only addresses qualitative and quantitative challenges — it can address both simultaneously.

## HRMS quantitation of NDMA in Ranitidine

A common LC methodology was implemented for LCMS-8045 and LCMS-9030 to quantitatively determine NDMA concentration in ranitidine API. Analysis was performed at 15 ppm accuracy window. For long continuous analysis, linearity > 0.999 was achieved without use of internal calibration. A comparable LOD and LOQ like triple quadrupole was observed along with similar calibration range proves capability of LCMS-9030 for both qualitative and quantitative workflows.

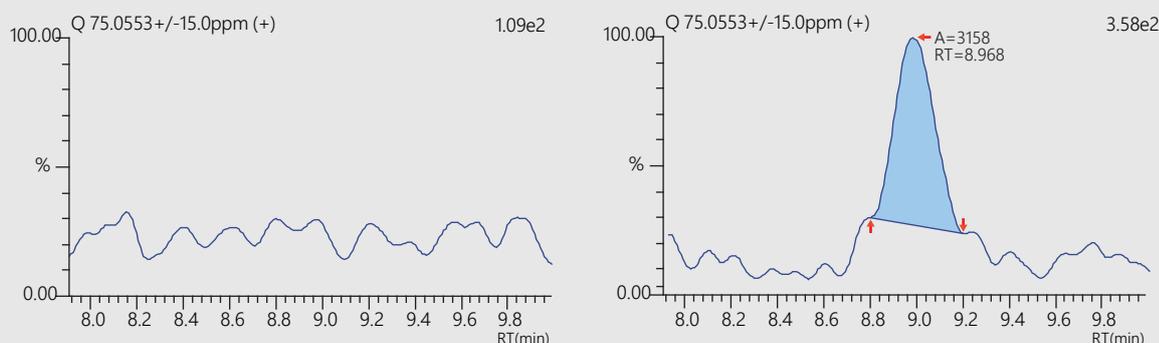


Figure 9a: Blank and NDMA (1ng/mL) chromatogram

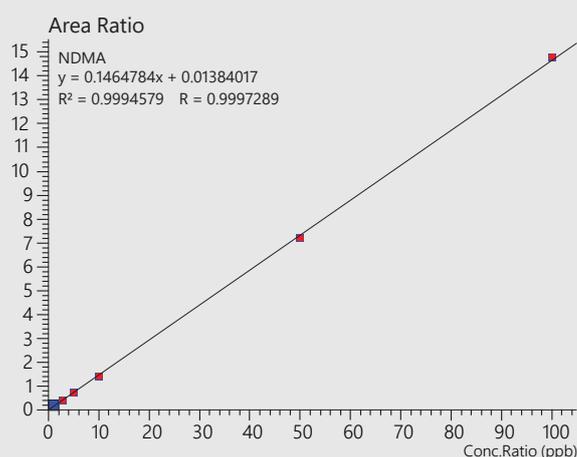


Figure 9b: Calibration curve for NDMA 1-100 ng/mL

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