# GC-SICRIT®-MS: Fast and sensitive analysis of 6 nitrosamines by replacing helium with hydrogen as carrier gas

## Summary

A fast determination of 6 nitrosamine compounds by GC-SICRIT®-(LC)MS is demonstrated. An Agilent 8860 GC was coupled to an Agilent Ultivo TQ MS through the use of the GC SICRIT® module. Hydrogen was used as a carrier gas to increase speed by about 50%, without losing resolution and sensitivity in MS detection. Calibrating using MRMs, limits of detection (LOD) were found to be 3 ppb, with RSDs of 3.5-13%, and a linear range of 3 orders or magnitude. An example active pharmaceutical ingredient (API) was spiked with recoveries ranging from 64-135%, which are within the FDA requirements.

## Introduction

Nitrosamines have been linked to increases in cancer risk, and recently have been identified as impurities in pharmaceuticals, ultimately leading to recalls and warning notices issued by the EMA and FDA. More stringent regulations are being considered for these compounds in pharmaceuticals. EPA Method 521 utilizes gas chromatography (GC) and chemical ionization tandem mass spectrometry (MS/MS) and is conventionally used to monitor these in wastewater.

As the cost of helium continues to increase and its availability becomes more uncertain, translating GC methods to use hydrogen as carrier gas is increasing in popularity. This offers chromatographic advantages of speed and efficiency, as well as an ultimate reduction in operation cost, especially if using a hydrogen gas generator. However, there remains concerns of hydrogen as a carrier gas when using common GC mass spectrometers, particularly regarding overall sensitivity.

This sensitivity loss can be attributed to the higher optimal velocities required when using hydrogen as the carrier gas. These velocities generally relate to flows >1 mL/min, which exceed typical GC-MS instrument capabilities to achieve correct vacuum levels, ultimately leading to the loss in sensitivity [1]. The SICRIT® technology enables the connection of a GC to an LC-MS, which has greater pumping speed to handle higher gas flows.

Previously, nitrosamines were investigated using GC with helium [2] and hydrogen [3] as a carrier gas when coupled to high resolution MS through the SICRIT® source. This work demonstrates the analysis of six important nitrosamines using GC-SICRIT®-MS, with a triple quadrupole MS. Speed of separation, as well as sensitivity will be compared, using multiple reaction monitoring (MRM) and helium and hydrogen as a carrier gas.

*Figure 1: Instrumental setup used.*

## Materials and Methods

### Sample Preparation

For calibrating, a dilution series of Nitrosamine Mix ( EPA 521, 2 mg/mL in DCM (PN: 40035-U, Sigma-Aldrich)) was prepared in Methanol (MS Grade, Sigma-Aldrich) ranging from 0.3 ppb to 300 ppb. This contained six compounds: (NDMA), (NMEA), (NDEA), (NDPA), (NPIP), and (NDBA).





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Additionally, Valsartan tablets as well as an API (active pharmaceutical ingredient) were dissolved in methanol and spiked with 30 ppb of the nitrosamine mix to determine method accuracy.

## Instrumentation

An Agilent 8860 GC was interfaced to an Agilent Ultivo LC/TQ by the SICRIT® Ion source and the SC-30 control unit. For automatic sample introduction, the GC was equipped with a PAL RTC autosampler (CTC-Analytics, Zwingen) as depicted in Figure 1. When investigating hydrogen as the carrier gas, an HG PRO 350 hydrogen generator (LNI Swissgas) was utilized. MS detection was performed in positive MRM mode and all data was evaluated using MassHunter Quantitative Analysis 10.0.

## Fast GC with Hydrogen as Carrier Gas

An analysis using helium as the carrier gas was adopted from an application note on nitrosamine impurities in drug products (AD-0199, Shimadzu Corp.) [4]. With the same instrumental conditions, the RESTEK EZGC method translator was then used to optimize the analysis for speed and use hydrogen as a carrier gas [5]. The final parameters used for this fast analysis are shown in Table 1.

*Table 1: Experimental parameters for fast $H_2$ method*

| Mass spectrometer | **Ultivo LC/TQ (Agilent)** |
|---|---|
| SICRIT® SC-30 settings | 1.5 kV, 30 kHz |
| GC/SPME Module | 280 °C; 4 psi $N_2$ |
| Gas Chromatograph | Agilent 8860 (Agilent Technologies) |
| Column | RXI-5ms, 30 m, 0.32 mm ID, 0.25 µm film thickness (Restek) |
| Inject volume | 1 µL |
| Split ratio | Splitless |
| Carrier gas | Hydrogen<br>80 cm/sec<br>Constant Pressure |
| Inlet temperature | 250 °C |
| | 38°C (0.53 min) - 160°C |
| Oven Temperature Program<br>Temperature ramp | at 23°C/min - 200°C<br>at 9.8°C/min |
| Transfer Line | 280°C |
| GC run time | 10 min |

Figure 2 shows the resulting chromatogram of the 100 ppb nitrosamine mix using helium (top) and hydrogen (bottom). A reduction in retention time from 11 min to 5.7 min for the final eluting compound, NDBA, is observed. This is approximately 50% faster than when compared to He. Additionally, this gain in speed still maintained the baseline resolution of the two critical pairs of compounds: NDMA and NMEA and NDPA and NPIP.

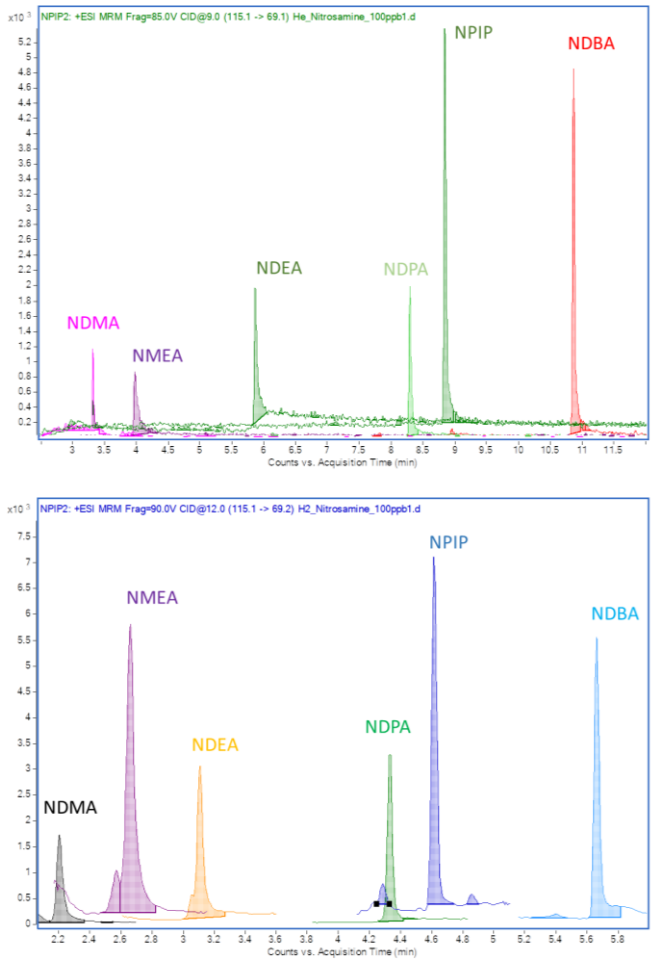


*Figure 2: Chromatographic separation of 100 ppb Nitrosamines Mix*



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*using (top) He (42.6 cm/sec) and (bottom) $H_2$ as carrier gas (80 cm/sec)*

## Results and Discussion

### *Sensitivity Comparisons Using He and $H_2$ as a Carrier Gas*

Compounds of the nitrosamine mix were ionized as $[M+H]^+$ species in this setup. For calibration, multiple reaction monitoring (MRM) mode was used with optimized transitions shown in Table 2, with their resulting chromatograms in Figure 4.

*Table 2: Optimized MRM transitions for Nitrosamines (RT window 1 min)*

| | Precursor Ion (m/z) | Product Ion (m/z | Fragmentor (V) | CE (V) | Dwell (ms) |
|---|---|---|---|---|---|
| **NDMA** | 75.1 | 58.2 | 80 | 10 | 178 |
| | 75.1 | 43.1 | 80 | 15 | |
| **NDEA** | 103.1 | 75.2 | 70 | 8 | 177 |
| | 103.1 | 47.1 | 70 | 14 | |
| **NDBA** | 159.1 | 41.1 | 80 | 25 | 250 |
| | 159.1 | 57.2 | 80 | 10 | |
| **NDPA** | 131.3 | 89.3 | 90 | 7 | 96 |
| | 131.3 | 43.1 | 90 | 10 | |
| **NMEA** | 89.1 | 61.2 | 70 | 10 | 121 |
| | 89.1 | 43.1 | 70 | 10 | |
| **NPIP** | 115.1 | 41.2 | 90 | 20 | 131 |
| | 115.1 | 69.2 | 90 | 12 | |

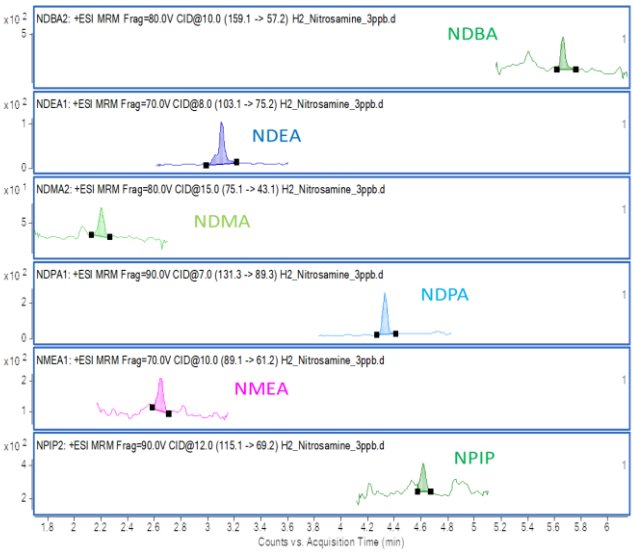


*Figure 4: MRMs of 3 ppb of Nitrosamines using $H_2$ as a carrier gas at a velocity of 80 cm/sec.*

Calibration curves of all transitions show good linearity over the calibration range from 3 - 300 ppb. Correlation coefficients ($r^2$) > 0.99 for all compounds can be seen in Figure 5 using hydrogen. However, it should be noted that using helium, two compounds, NDMA and NMEA had higher limits of detection (LOD), as shown in Table 3. Here, the use of hydrogen does not hinder sensitivity. These compounds were the first two eluting compounds in the mixture and can be prone to adsorption onto active sites within the GC system, such as the liner and column. Therefore, with faster elution times, the potential for adsorption is removed, which could result in the improved sensitivity.

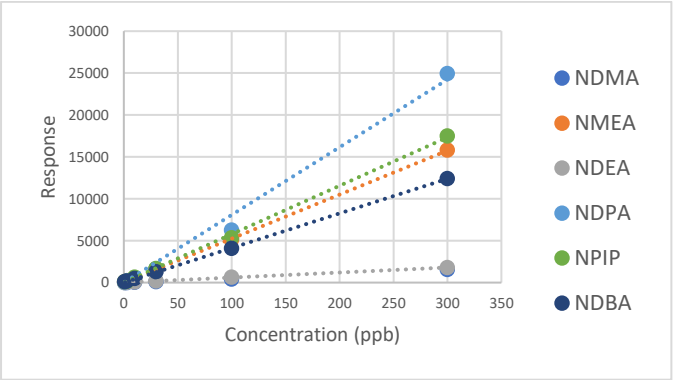


*Figure 5: Calibration curve for nitrosamines using hydrogen as the carrier gas over the range of 3 – 300 ppb.*

*Table 3: Figures of merit using He and $H_2$ as carrier gas*

| Carrier Gas | He | | | $H_2$ | | |
|---|---|---|---|---|---|---|
| | RT (min) | LOD (ppb) | RSD (n=5) (%) | RT (min) | LOD (ppb) | RSD (n=5) (%) |
| **NDMA** | 3.4 | 10 | 15.8 | 2.2 | 3 | 7.9 |
| **NDEA** | 5.9 | 3 | 6.5 | 3.1 | 3 | 3.5 |
| **NDBA** | 10.9 | 3 | 1.9 | 5.7 | 3 | 5.5 |
| **NDPA** | 8.3 | 3 | 5.6 | 4.3 | 3 | 8 |
| **NMEA** | 4.0 | 30 | 13 | 2.7 | 3 | 12.8 |
| **NPIP** | 8.8 | 3 | 3.3 | 4.6 | 3 | 7.2 |



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*Spike and Recovery with an Active Pharmaceutical Ingredient (API):*

Recoveries of 30 ppb nitrosamine mix in the Valsartan tablet and an API are shown in Table 4. The first two eluting compounds, NDMA and NMEA, show under recovery. As seen in the matrix spiked chromatogram (Figure 5), the matrix has an effect on the second transition of NDMA resulting in this under recovery. These are within acceptable limits, according to the FDA guidelines of 70 - 140% [5], with the exception of NDMA.

*Table 4: Recovery of 30 ppb nitrosamine mix spiked in a tablet and API matrix.*

| | Tablet | | API | |
|---|---|---|---|---|
| | Measured concentration in spike (ppb) | recovery (%) | Measured concentration in spike (ppb) | recovery % |
| **NDMA** | 24 | 80 | 19 | 64 |
| **NDEA** | 35 | 115 | 35 | 115 |
| **NDBA** | 40 | 135 | 30 | 100 |
| **NDPA** | 30 | 100 | 36 | 119 |
| **NMEA** | 25 | 83 | 22 | 75 |
| **NPIP** | 31 | 102 | 35 | 116 |

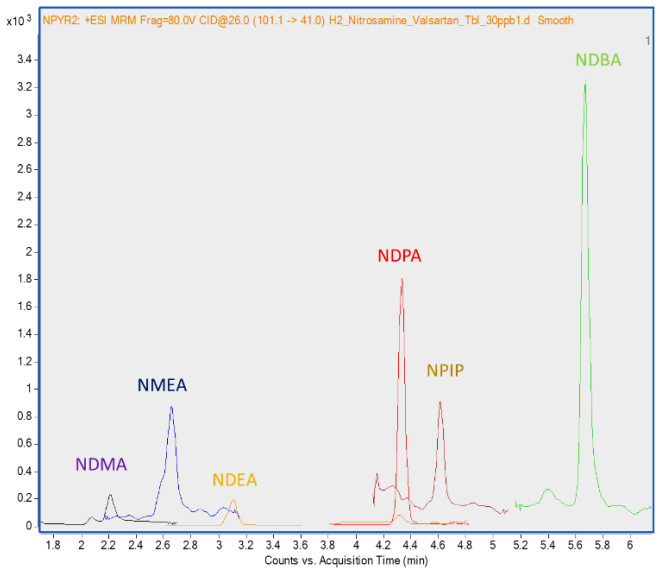


*Figure 5: MRMs of 30 ppb nitrosamines mix spiked into the tablet extract*

## Conclusion

The SICRIT® GC-(LC)MS setup using $H_2$ as carrier gas can be applied to the analysis of nitrosamines. This allows for a fast chromatographic runtime, while maintaining the very high MS sensitivity. The SICRIT® ionization source enables the connection of a GC with an LCMS, which has a greater pumping capability allowing it to maintain good vacuum. With that, sensitivity is also maintained with hydrogen as a carrier gas at high flow rates.

Hydrogen with higher flow rates allows for faster chromatographic run times, permitting resolution between critical pairs are maintained. Here, a reduction in elution time of the last eluting compound is reduced by 50%. Further the sensitivity of the MS is maintained, allowing for detection limits of 3 ppb of all nitrosamines tested.

## References

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[2] Sensitive Detection of Nitrosamines for Drug Quality Control using SICRIT® Soft Ionization-MS (www.plasmion.de)

[3] Ultra-Sensitive GC-SICRIT®-HRMS Analysis of Nitrosamines in Pharmaceutical Samples featuring Hydrogen as GC Carrier Gas and Shimadzu LCMS-9030 QToF (www.plasmion.de)

[4] AD-0199 : Determination of Nitrosamine Impurities in Sartan Drug Products by GC-MS/MS Method (shimadzu.com)

[5] EZGC Method Translator and Flow Calculator (restek.com)

[6] US FDA-2020-D-1530, Control of Nitrosamine Impurities in Human Drugs, Guidance for Industry. https://www.fda.gov/media/141720/download



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