Ultra-Sensitive GC-SICRIT®-HRMS Analysis of Nitrosamines in Pharmaceutical Samples featuring Hydrogen as GC Carrier Gas and Shimadzu LCMS-9030 QToF

Summary

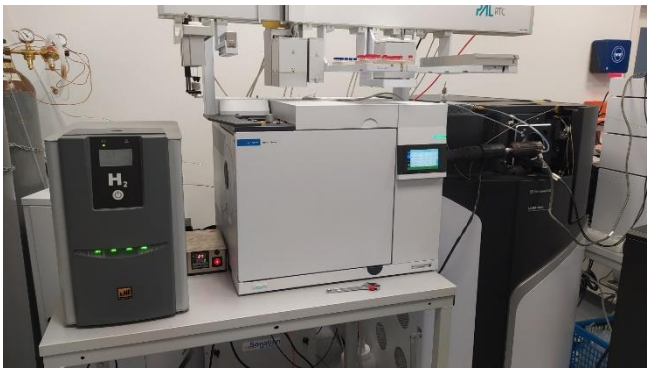
In this study we demonstrate ultra-sensitive determination of various nitrosamine compounds by GC-SICRIT®-MS using H_2 as carrier gas and full scan high resolution MS data from Shimadzu LCMS-9030. Compared to standard GC-EI-MS this combination enables a reduction in GC run times by 50% with no loss of sensitivity and the capability for parallel non target quality control. The setup was tested against real world pharmaceutical samples (dissolved tablet) spiked with nitrosamines at low pg levels.

Introduction

Cancerogenic nitrosamine impurities are of big concern in pharmaceutical quality control. In 2018, FDA and EMA initiated recall of Valsartan products of different manufacturers due to NDMA (N-nitrosodimethylamine) impurities and recommended review of manufacturing processes. Recently, both authorities prompted same actions also for Ranitidine drugs. The occurrence of nitrosamines in drugs cannot be only attributed to byproducts in raw materials, but also can be formed during the processing stage. Thus, the whole manufacturing processes must be investigated. As the recalls are associated with serious financial losses, there is a great manufacturer's need of analytical methods for tracing back nitrosamines in their quality assurance.

However, looking at the current analytical techniques, there are challenges in nitrosamine determination for pharma quality control applications. Hence pharmaceutical quality control and drug purity is mainly done by LC-MS/MS analysis, it would be obvious to include nitrosamine analysis into routine LC-MS drug purity control workflows. It has been shown that conventional LC-MS ionization techniques as ESI and APCI are limited in their ionization potential for simultaneous analysis of nitrosamines and the active drug.

In this study we show that GC coupling to (LC-)MS instruments using SICRIT® and its broad ionization range can overcome these shortcomings and presents a well-suited method for simultaneous determination of nitrosamine compounds and active ingredients at very high sensitivities.



Picture of the instrumental setup used within this study

As the cost of helium continues to increase while its availability becomes increasingly uncertain, many labs using gas chromatography are considering switching to hydrogen carrier gas. Hydrogen can be reliably produced on demand using hydrogen generators, which are more cost-effective. In addition, using hydrogen allows efficient separations to be obtained twice as fast compared to helium, which offers clear benefits to sample throughput and overall lab productivity.

Nevertheless, the change to hydrogen comes with a loss of sensitivity for most of the ionization techniques. SICRIT® however, is not affected by this loss, thus enabling faster, cheaper, and more sustainable nitrosamine analyses in pharmaceutical quality control.

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Experimental Setup

The SICRIT® technology enables flexible coupling of any GC to any (LC-)MS. Here, an Agilent 8860 GC was interfaced to a Shimadzu LCMS-9030 by SICRIT® Ion source and the SC-30 control unit. For automatic sample introduction, the GC was equipped with a PAL3 autosampler (CTC-Analytics, Zwingen). The GC was run on Hydrogen produced by a HG PRO 350 hydrogen generator (LNI Swissgas). MS detection was performed in positive scan mode (m/z 50 to 500) at a scanning frequency of 10 Hz. The data was evaluated using LabSolutions Insight Explore Software Ver. 3.8 SP3 (Shimadzu). Extracted mass traces (10 ppm window) of nitrosamines was used for quantitation.

Table 1: Experimental parameters

Samples	Nitrosamine Mix EPA 521, 2 mg/mL in DCM (40035-U, Sigma-Aldrich)
Solvents	Solvent MS-grade Methanol (Sigma-Aldrich)
Mass spectrometer	LCMS-9030 QTOF (Shimadzu)
SICRIT® SC30 settings	1.5 kV, 30 kHz
SPME GC/SPME Module	280 °C; 0.5 mL/min N_2 flow
GC	GC 8860 (Agilent Technologies)
Column	RXI-5ms, 30 m, 0.25 mm ID, 0.25 µm stationary phase (Restek)
Inject volume	1 µL
Split ratio	Splitless
Carrier gas	Hydrogen
Flow rate	1.75 mL/min
Injector temperature	250 °C
Start temperature	40°C, hold for 0.3 min
Temperature ramp	36.7 °C/min to 220 °C 91.5 °C/min to 325 °C
Transferline temperature	280 °C
GC run time	8.15 min

For calibration, a dilution series of Nitrosamine Mix (EPA 521) was prepared in Methanol ranging from 10 ppt to 300 ppb. For validation, a spiked real sample was prepared by dissolving a pharmaceutical tablet (400 mg/ml API) in ethyl acetate and DCM. Before analysis nitrosamine mix was added to the sample yielding a concentration of 3 ppb. The injection volume was 1 µl in splitless mode, resulting in an absolute concentration of 3 pg on the column.

Results and Discussion

All compounds of the nitrosamine mix were chromatographically separated (Figure 2) and solely ionized as $[M+H]^+$ species.

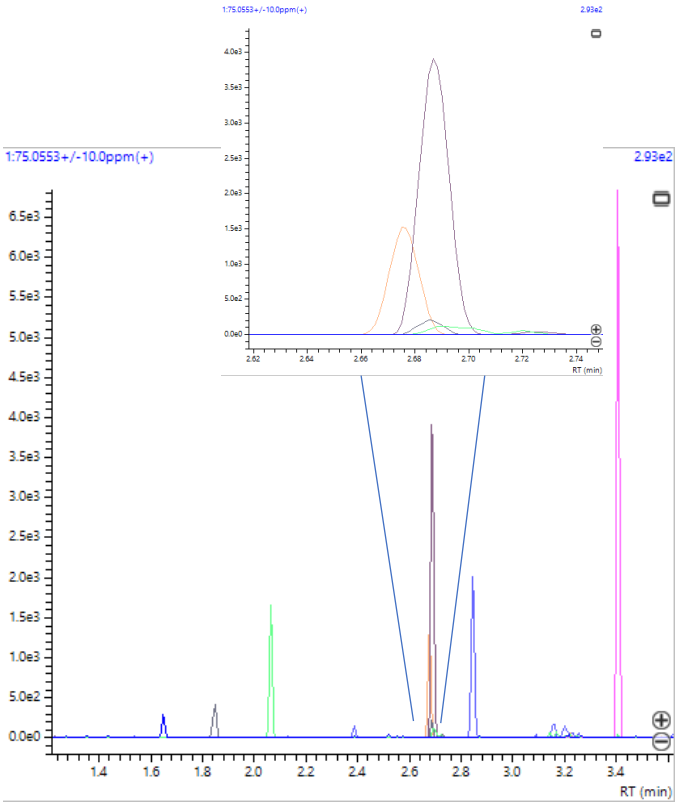

Figure 1: EICs of 300 pg/ml Nitrosamines with zoom in for NPYR and NDPA

Calibration experiments with diluted standard samples showed excellent sensitivity for all nitrosamine compounds with instrumental LODs in the low ppt range (see Figure 2 and Table 2).

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Table 2: Summary of the calibration results

Compound	Abbreviation	$[M+H]^+$ (m/z)	Retention time (min)	Exp. LOD (ng/ml)	S/N	Area (a.u)	RSD (%; n = 10; 30 ng/ml)
N-Nitrosodimethylamine	NDMA	75.0553	1.63	0.3	80.5	225	5.5
Diethylnitrosoamine	NDEA	103.0866	2.06	0.1	147.7	390	3.2
N-Nitrosodibutylamine	NDBA	159.1492	3.40	0.03	165.8	281	2.7
N-Nitrosodipropylamine	NDPA	131.1179	2.69	0.1	192.9	873	2.7
N-Nitroso-N-methylethylamine	NMEA	89.0709	1.83	0.1	inf	141	4.2
1-Nitrosopyrrolidine	NPYR	101.0709	2.67	0.1	269	422	2.8
1-Nitrosopiperidine	NPIP	115.0866	2.84	0.1	34.2	762	2.5

The lowest sensitivity of 300 pg/ml (S/N 81) was found for NDMA, whereas the highest sensitivity was found for NDBA with a S/N of 164 at 30 pg/ml. Corresponding calculated LOQs (using classic S/N>10 criteria) would result in theoretical detection limits of 37 pg/ml for NDMA and 2 pg/ml for NDBA. Linearity was preserved for most analytes up to 30 ng/ml spanning 2 orders of magnitude. To the best of our knowledge, these results represent the most sensitive analysis for nitrosamines on Shimadzu Instruments, regardless of GC-MS or LC-MS. Even the already very sensitive report for LC-APCI MS/MS analysis with the LCMS-9030[1] is outperformed by a factor of >40 with respect to the absolute amount injected. This confirms the empowerment, which can be achieved utilizing the SICRIT® ion source and GC-separation capabilities in combination with State-of-the-art high resolution mass spectrometers.

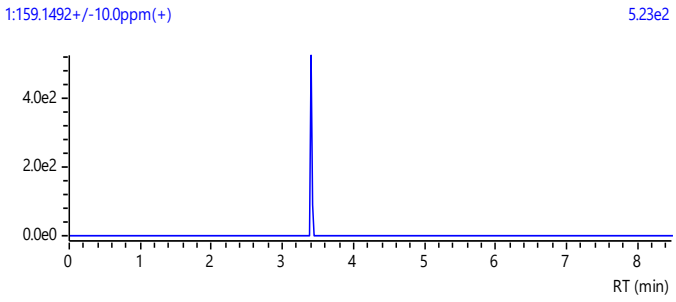

Figure 2: EIC of 100 pg/ml NDBA

The presented data also confirms the potential of the SICRIT® GC-(LC)MS approach using H_2 as carrier gas instead of He, without any compromise of sensitivity. This results in a potential speed up of routine GC analysis by more than 50%, since even higher flowrates (>20 ml/min) are possible with the SICRIT solution than any classical GC-MS could handle.

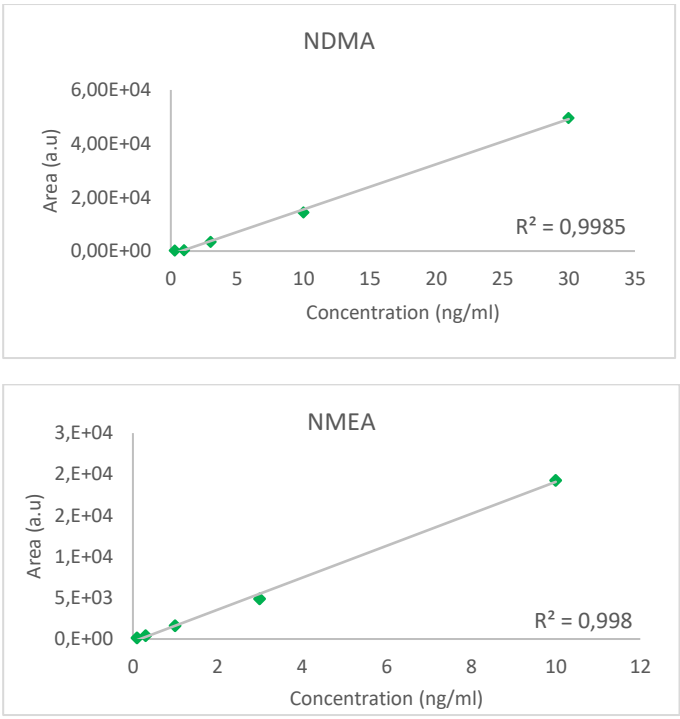

Figure 3: Calibration curves for NDMA and NMEA

In the validation experiment using a spiked sample, the nitrosamines and the active ingredient were detected (Figure 4) in parallel. Recoveries of the spiked amount

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ranged between 87% to 140%. NMEA was not quantified in this MS approach, due to an isobaric interference by ethyl acetate. For routine application this can be overcome by applying a MS/MS method, described for the same instrument. [1]

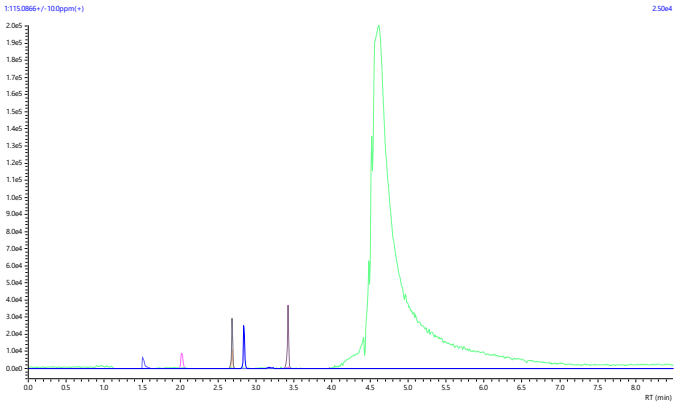

Figure 4: EICs of 30 ng/ml Nitrosamines and in the spiked real sample. The green EIC shows the mass interference with ethyl acetate and NMEA

Table 1: Recovery of spiked nitrosamines (3 ppb) in %

recovery of nitrosamines in real sample in %	
NDMA	88.8
NDEA	140.1
NDBA	95.1
NDPA	123.9
NMEA	n/a
NPYR	87.2
NPIP	138.7

Conclusion

The presented data show sensitive detection of nitrosamine compounds by GC-SICRIT®-MS using H_2 as carrier gas. Furthermore, GC run time was shorten by 50 % without any loss of sensitivity. In combination with the high resolution of the QTOF no need of MRM measurements was given, offering high flexibility of scan run. This setup also works well in real samples.

Acknowledgements

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References

[1] Shimadzu application news 04-AD-0240-EN

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