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Application News

No. SCA-210-058

Drug Contaminants / GC-2030 / LCMS-8050

Coupling of GC and LCMS Systems via SICRIT® Soft Ionization Source for Sensitive Detection of Nitrosamines

User Benefits

- Interface an existing LCMS system with GC inlet
- Soft-ionization of a broad rage of chemical structures and polarities
- Spectra with molecular ions and low level of fragmentation

▪ Introduction

Nitrosamine have emerged as impurities of mature concern in pharmaceutical preparations. Initially detected by the US FDA in drugs used in the treatment of hypertension and heart failure containing Sartan as active pharmaceutical ingredients. Subsequently, investigations by regulatory agencies including the European Medicines Agency (EMA) and Ministry of Health, Labour and Welfare, Japan (MHLW) led to the recall of many medicines ranging from Angiotensin II receptor antagonists, Ranitidine, Nizatidine to Metformin. This global trend reveals the importance for detection and control of nitrosamine impurities in drug manufacturing process as a critical factor to sustain business of pharmaceutical industry.

Figure 1: Coupling of GC-2030 and LCMS-8050 via SICRIT® ion source.

Plasmion SICRIT® Ion source is designed with a dedicated interface for Shimadzu LCMS Systems. It enables these mass spectrometers to use GC systems as inlet. SICRIT® (Soft Ionization by Chemical Reaction In Transfer) technology is based on flow-through cold plasma ionization. A ring-shaped cold plasma is utilized to ionize analyte molecules during their transfer from the GC column outlet to the inlet of the mass spectrometer (Figure 1).

The cold plasma ensures soft ionization of a very broad range of compounds – even of non-polar analytes as alkanes – resulting in spectra with molecular ions and a very low level of fragmentation. The efficient unbiased charge transfer qualifies the SICRIT® ion source for both targeted and non-targeted analysis.

In this study we demonstrate the application of the SICRIT® Ion source to interface a Shimadzu GC-2030 with an LCMS-8050 for the quantitative assay of nitrosamines.

Table 1: GC-MS/MS analytical conditions

[GC]	
Column Temp.	35 °C (1.5 min) → 10°C/min to 100 °C → 30 °C/min to 250 °C (0.5 min)
Split Ratio	1:10
Injector Temp.	270 °C
Injection Volume	1 µL
Carrier Gas	Helium
Carrier Gas Control	40.5 cm/sec (Constant linear velocity)
Transferline Temp.	250 °C
[MS]	
DL Temp.	250 °C
Heat Block Temp.	300 °C
Drying gas	Off
Acquisition Mode	MRM
[SICRIT®]	
Make-up Gas Flow	0.5 L/min humidified N_2
SICRIT Plasma	1.5 kV, 15 kHz

Experimental

Instruments and Analytical Conditions

Experiments were conducted using a GC-2030 coupled to an LCMS-8050 triple quadrupole mass spectrometer via the Plasmion SICRIT® Ion Source. For the experiments default settings of the SICRIT® Ion source were applied. MRM (multiple reaction monitoring) mode was used for acquisition applying publicly available MRM transition (Table 2) without further optimization. A general-purpose GC column SH-RTX-5MS (30 m length, 0.25 mm I.D., 0.25 µm film thickness, P/N 221-75861-10) was used in this study. Details of the analytical conditions are shown in Table 1.

Table 3: Information on nitrosamines*

	Target Compound	Acronym	CAS No.
1*	N-Nitrosodimethylamine	NDMA	62-75-9
2*	N-Nitrosodiethylamine	NDEA	55-18-5
3*	N-Nitrosodi-n-butylamine	NDBA	924-16-3
4	N-Nitrosomethylethylamine	NMEA	10595-95-6
5	N-Nitrosodi-n-propylamine	NDPA	621-64-7
6	N-Nitrosopyrollidine	NPYR	930-55-2
7	N-Nitrosopiperidine	NPIP	100-75-4

* 1,2, and 3 are nitrosamine impurities targeted by the US FDA[1].

Preparation of Standards

Seven nitrosamines including three listed by FDA are analyzed in this study (Table 3). From a commercially available standard solution in dichloromethane (DCM), calibration standard solutions of 0.2, 2, 20, 200, 2000, and 20000 ng/ml were prepared.

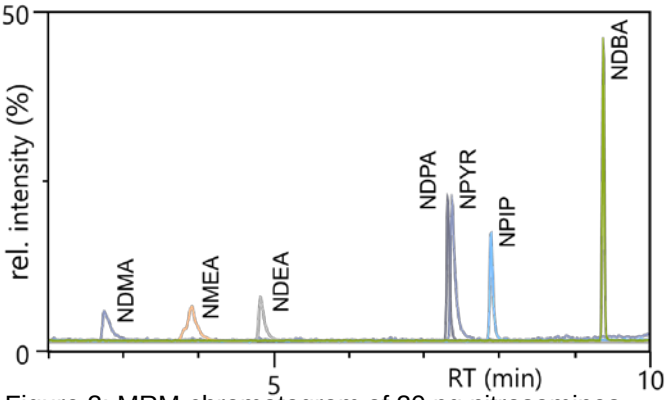


Figure 2: MRM chromatogram of 20 pg nitrosamines

Results

The chromatographic separation of the seven nitrosamines is displayed in Figure 2. All the analytes were monitored via one quantitative and one or two qualitative MRM transitions for confirmation. The details of MRM transitions and quantitation parameters are shown in Table 2.

The peak shape of the early eluting compound is compromised by the general-purpose column used in this study. Significant optimization is achieved by a more advanced column selection (compare [2]).

For peak identification, a deviation of absolute retention time of less than ±0.15 min and relative ion ratios within ±30% of the set ion ratio were used as criteria. The areas of the nitrosamine peaks were plotted against their amounts on column. All linear calibration curves without the use of internal standards displayed correlation coefficients, $R^2 \geq 0.998$, which fulfils the FDA requirement. Calibration curves are displayed including a representative MRM peak in Figure 3.

Table 2: MRM transitions and quantitation parameters

Compound	MRM transition	CE (V)	Ion Ratio (%)	RT (min)	Cal Range (pg)	R^2	LOQ (pg)
NDMA	75.1 > 43.1	-22	100	2.62	2-2000	0.995	2.0
	75.1 > 58.0	-14	24				
NDEA	103.0 > 75.3	-16	100	4.79	2-2000	0.997	1.0
	103.0 > 47.0	-21	34				
NDBA	159.3 > 57.2	-15	100	9.37	0.2-200	0.999	0.2
	159.3 > 41.1	-22	60				
	159.3 > 103.1	-14	55				
NMEA	89.0 > 61.1	-15	100	3.77	2-2000	0.999	1.8
	89.0 > 43.0	-22	14				
NDPA	131.1 > 89.1	-14	100	7.32	0.2-200	0.999	0.2
	131.1 > 43.0	-15	98				
NPYR	101.0 > 55.0	-20	100	7.4	2-200	0.999	1.9
	101.0 > 41.0	-30	15				
NPIP	115.2 > 69.0	-15	100	7.88	2-200	0.999	1.9
	115.2 > 41.6	-26	7				

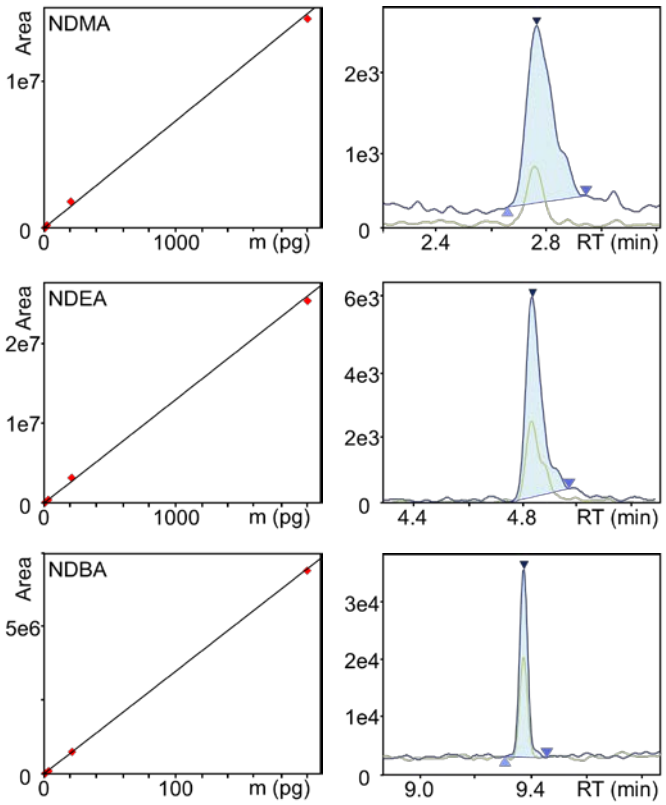


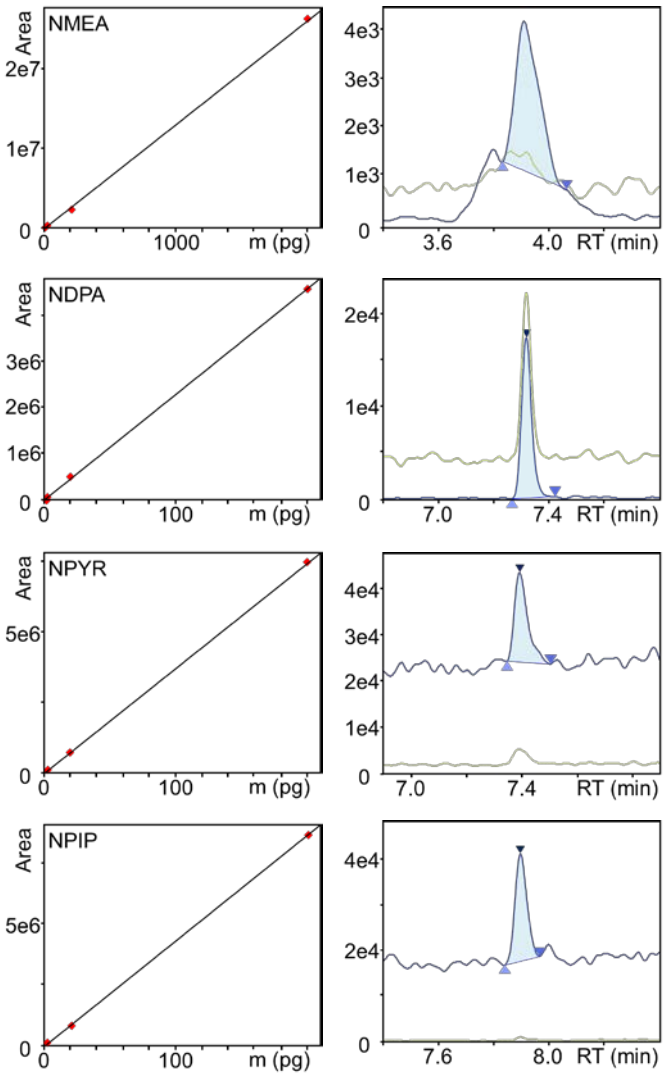
Figure 3: Calibration curves and MRM chromatograms at 2 pg nitrosamine on column.

▪ Conclusion

The Plasmion Sicrit® Ion Source is well suited for the interface between GC and LCMS systems. It provides a soft ionization for a wide range of compounds with different structures and polarities. Here we display the utilization of the system to analyze nitrosamines. Using the default parameter set for the MS and without significant optimization of GC and MRM conditions, we reach LOQs comparable to dedicated GCMS systems.

▪ References

1. FDA U.S. Food & Drug Administration, Combined Direct Injection N-Nitrosodimethyl-amine (NDMA) and N-Nitrosodiethylamine (NDEA) Impurity Assay by GC/MS
2. Shimadzu Application News AD-0199, Determination of Nitrosamine Impurities in Sartan Drug Products by GC-MS/MS Method



▪ The Package

- ❑ *Main Unit*
  LCMS-8050, TQ mass spectrometer
  GC-2030, gas chromatograph
- ❑ *Consumables*
  SH-Rtx-5MS (P/N 221-75861-10)
- ❑ *Software and Libraries*
  LabSolutions LCMS
- ❑ *Plasmion*
  SICRIT MSInterface SZ2
  SC-30 Control Unit
  GC TransferLine

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