Application Note

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Screening of Nitrosamine Impurities in Drug Products and Drug Substances Using Agilent GC/MS/MS Instrumentation

Authors

Carole Antoine, Milan Macé, Elodie Barrau, and Emmanuel Desmartin
Eurofins Amatsi Analytics

Laurent Pascaud
Agilent Technologies, Inc.

Abstract

This application note highlights a comprehensive solution for the screening and estimation of 13 nitrosamine impurities (NDMA, NDEA, NMOR, NMEA, NPYR, NPIP, NEIPA, NDIPA, NDPA, NDBA, NMPA, NMPEA and NDPh) in drug products and drug substances in both organic or aqueous matrices at trace levels using an Agilent 8890 GC coupled to an Agilent 7010B triple quadrupole GC/MS/MS system. Briefly, product is either dispersed in methylene chloride or in water following a liquid-liquid extraction depending on its own solubility.

Calibration is made using 1 to 60 ppb calibration solutions, LOQs are obtained between 1 and 10 ppb and satisfying recovery are obtained.

The 7010B triple quadrupole GC/MS/MS is equipped with a high-efficiency source (HES) that offers excellent sensitivity, repeatability, and precision. This method allows for the performance of an initial screening of drug products (after risk evaluation) to confirm the presence or absence of nitrosamines.

Introduction

For nearly four years, the concerns regarding trace levels of N-nitrosamines in pharmaceuticals and the associated cancer risk have significantly expanded and are a major issue facing the global pharmaceutical industry. According to FDA and EMA requirements, first, a marketing authorization holder must carry out a risk-based assessment of each product. Then, a confirmatory testing should, therefore, be performed using appropriately validated and sensitive methods, and Marketing Autorisation Holders (MAHs) should inform the competent authorities immediately if tests confirm the presence of one or several nitrosamine impurities, irrespective of the amount detected.

After that, MAHs should apply for a variation in a timely manner to introduce any required changes, such as an amendment of the manufacturing process or changes to product specifications.

The screening testing can be performed either by liquid chromatography-mass spectrometry (LC/MS/MS) or by gas chromatography-tandem mass spectrometry (GC/MS/MS), using a generic sample preparation and analysis method.

These standardized procedures can be simplified according to the galenic form (powder, tabs, pills, liquid, cream, and so on), drug substance (DS), or drug product (DP) properties (polarity, solubility). The use of LC or GC technique will depend on the nitrosamines that have to be screened, the limits to be achieved, and the thermal stability of DP and excipient.

Although the screening was initially focused on N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), the list used in this method can be extended to other common or specific nitrosamines according to the risk assessment.

Our current GC/MS/MS screening allows to confirm 13 of them:

N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosomethylethylamine (NMEA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodipropylamine (NDPA), N-nitrosodibutylamine (NDBA), N-nitrosomethylphenylamine (NMPA), N-nitrosomethyl-2-phenylethylamine (NMPEA), and N-nitrosodiphenylamine (NDPh).

The screening approach is to apply this generic method to several batches of the pharmaceutical product (at least three or four batches, representative of the life cycle). The validity of the screening will be assessed by checking for each product:

- The sensitivity of the method at limit of detection (LOD) and limit of quantification (LOQ) levels
- The selectivity in the matrix
- The recovery (accuracy) of the generic method using spiked samples at two concentration levels

The approach for this limit assay can either target one nitrosamine, or screen the full list.

In case of a positive result for the screening of one or more nitrosamines, a specific quantitative method has to be developed and validated for a confirmatory testing.

This confirmatory method can either be an update of the generic method used for the screening, or a method developed specifically to the intended use (sample preparation, analysis technique, and detection).

This study performed the screening method using the 8890 GC coupled to the 7010B GC/MS/MS and found that this system provides excellent performances for the 13 nitrosamines screened. The HES, with improved ionization efficiency and 20x ion generation characteristics, delivers confident trace analysis. The 8890 GC has a touch screen interface instead of a keypad to control the GC, and offers diagnostic tests, system monitoring alerts, and mobile access.

Experimental

Sample preparation

DS and DP have to be dispersed for proper analysis. Here, two different matrices (water and methylene chloride) have been tested for further drug products and DS analysis using two different methods of preparation.

For samples soluble in organic solvents, 500 to 1,000 mg of sample (DS or DP) are directly dispersed into 5.0 mL of $MeCl_2$ and filtered through a ww-PTFE MS 0.2 µm syringe filter.

For samples soluble in water matrices, 200 to 1,000 mg of sample are dispersed into 8.0 mL of extraction mixture (NaOH 1 M solution in water). Then, a liquid-liquid extraction is made using 2.0 mL of $MeCl_2$ The organic fraction is filtered through a ww-PTFE MS 0.2 µm syringe filter and analyzed.

Standard preparations

Deuterated nitrosamines are used as internal standards: NDMA-d_6, NDEA-d_{10}, NDIPA-d_{14} and NDBA-d_{18}.

Individual internal standard stock solutions (IS stock) and individual nitrosamine stock solutions (nitro stock) were prepared by weighing each pure analytical standard in 20 mL glass tubes, then dissolving into methanol to reach 1,000 µg/mL concentration.

A mixed nitrosamine standard solution and a mixed internal standard solution at 1 µg/mL are prepared by mixing each nitro stock (or IS stock) solution with $MeCl_2$.

Mixed nitrosamine standard solution is diluted appropriately with $MeCl_2$ to obtain calibration solutions of the following concentrations: 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 ng/mL (and at 3 ng/mL of each internal standard). When samples are not soluble in organic solvents, standard solutions undergo the extraction procedure described previously using 2.0 mL of $MeCl_2$ without filtration. Calibration solutions are then transferred into a GC vial for analysis.

Particular attention

The two following technical aspects required particular attention during experimental analysis:

1. Interferences caused by the presence of trace amounts of nitrosamines in testing materials used (e.g. water, airborne sources, plastic products, rubber/elastomeric products, smokers, etc.)
2. *In situ* formation of nitrosamines

Instrumentation

Analysis was performed using Agilent 8890 GC equipped with an Agilent 7693A automatic liquid sampler coupled to an Agilent 7010B triple quadrupole GC/MS/MS. From the inlet, an Agilent J&W DB-1701 GC capillary column (14% cyanopropylphenyl/86% dimethylpolysiloxane) of dimensions 30 m × 0.25 mm, 1.0 µm was connected to the MS/MS detector.

Dwell was chosen to have sufficient data points avoiding sharp peaks.

Tables 1 and 2 display the GC and MS parameters.

Table 1. GC parameters.

Parameter	Value
MMI Injection Mode	Splitless, pressure: 7.334 psi, Total flow: 34 mL/min
Inlet Temperature	220° C
Oven Temperature Program	40 °C (0.5 min) 20 °C/min to 160 °C (0 min) 10 °C/min to 200 °C (0 min) 25 °C/min to 240 °C (2 min) 100°C/min to 280°C (10 min)
Total Run Time	24.5 min
MS Transfer Line Temperature	280 °C
Injection Volume	1 µL
Carrier Gas	Helium, 1 mL/min

Table 2. Repeatability calculated on standards.

Repeatability Std	Direct Dispersion	Aqueous Extraction
Compound	%RSD (n = 3)	%RSD (n = 3)
NDMA	2%	1%
NMEA	1%	2%
NDEA	1%	1%
NEIPA	1%	1%
NDIPA	4%	2%
NMPA	7%	4%
NDPA	1%	1%
NMOR	1%	1%
NPYR	2%	2%
NPIP	1%	1%
NDBA	5%	3%
NMPEA	4%	2%
NDPh	5%	3%

Results and discussion

The nitrosamines were separated chromatographically and detected using the advantage of a GC/MS/MS. The resolution between compounds was good enough to prevent cross-talk issues, and the target peaks were well resolved from solvent and matrix compounds. Retention times of 13 compounds are presented in Figure 1.

Injection repeatability was determined with standard solution verifying RSD <20% at the 30 ppb level.

The accuracy of the method was determined by injecting three preparations of sample solution spiked at six different levels: 1, 2, 5, 10, 30, and 60 ppb, verifying RSD ≤25% and recovery within 70 and 130%.

Table 3. MS parameters.

Parameter	Value	
Mode	Electron ionization, 70 eV	
Source Temperature	230 °C	
Quadrupole Temperature	Q1 and Q2 = 150 °C	
MRM Mode Conditions		
MS1 Resolution	All compounds Unit	
MS2 Resolution	All compounds Wide	
Collision Gas Flow	Nitrogen at 1.5 mL/min,	
Quenching Gas Flow	Helium at 2.25 mL/min	
Detector Gain	2	
Quant./Qual. Transitions (FDA Method)	Time segment 1 Start time: 5.00 min	**NDMA** 74 → 44, CE 6 V 74 → 42, CE 20 V **NDMA-d_6** 80 → 46, CE 25 V **NMEA** 88 → 71, CE 5 V 88 → 56, CE 15 V
	Time segment 2 Start time: 6.75 min	**NDEA** 102-> 85 CE 4V 102 -> 56 CE 18V **NDEA-d_{10}** 112 → 62, CE 15 V **NEIPA** 116 → 99, CE 3 V 71 → 56, CE 7 V
	Time segment 3 Start time: 7.90 min	**NDIPA** 130 → 88, CE 3 V 130 → 42, CE 10 V **NDIPA-d_{14}** 144 → 96, CE 5 V **NMPA** 107 → 106, CE 14 V 106 → 51, CE 34 V **NDPA** 101 → 70, CE 3 V 130 → 43, CE 10 V
	Time segment 4 Start time: 8.95 min	**NMOR** 116 → 86, CE 5 V 116 → 56, CE 15 V **NPYR** 100 → 68, CE 10 V 100 → 41, CE 22 V **NPIP** 114 → 55, CE 23 V 114 → 42, CE 30 V
	Time segment 5 Start time: 9.90 min	**NDBA** 158 → 99, CE 7 V 84 → 56, CE 22 V **NDBA-d_{18}** 176 → 110, CE 10 V
	Time segment 6 Start time: 11.50 min	**NMPEA** 91 → 65, CE 17 V 134 → 91, CE 14 V **NDPh** 169 → 168, CE 18 V 168 → 167, CE 22 V

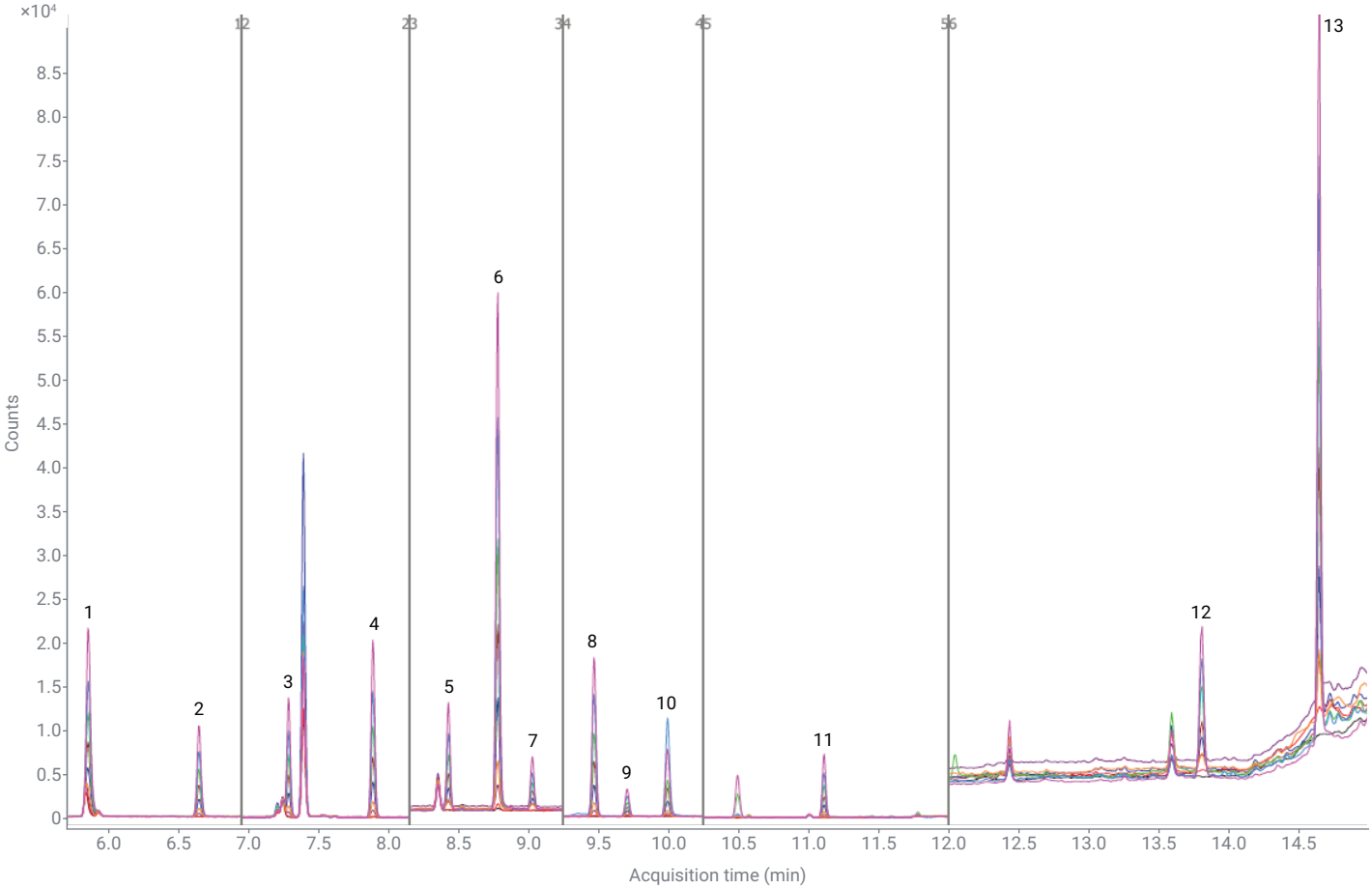

Figure 1. MRM TIC chromatogram overlay of nine calibration levels for the 13 impurities in dichloromethane (Agilent 8890 GC). (1) NDMA, (2) NMEA, (3) NDEA, (4) NEIPA, (5) NDIPA, (6) NMPA, (7) NDPA, (8) NMOR, (9) NPYR, (10) NPIP, (11) NDBA, (12) NMPEA, (13) NDPh.

For both sample preparation methods (with water and methylene chloride), excellent recoveries within 70 to 130% were observed for each of the nitrosamines with recovery % RSD less than 20% at 30 ppb. The findings are reported in Tables 4 and 5.

The response linearity for nitrosamines was evaluated over the range studied.

A mixed nitrosamine standard solution and a mixed internal standard solution at 1 µg/mL are prepared with $MeCl_2$.

Table 4. Repeatability calculated on samples using the direct dispersion in $MeCl_2$ procedure.

Sample Repeatability Obtained Doing a Direct Dispersion in $MeCl_2$							
	Mean Recovery						%RSD
Level (ppb)	1	2	5	10	30	60	30
NDMA	93	97	98	96	98	98	1.0
NMEA	101	102	103	101	102	106	2.0
NDEA	109	110	99	98	95	96	1.3
NEIPA	108	104	103	102	104	109	2.3
NDIPA	121	110	100	98	96	96	3.4
NMPA	122	108	95	85	117	NA(2)	6.5
NDPA	104	103	99	95	99	99	2.6
NMOR	116	107	97	94	97	96	2.9
NPYR	NA(1)	128	109	103	105	101	1.8
NPIP	120	117	117	104	95	NA(3)	2.5
NDBA	112	108	97	99	103	NA(2)	2.2
NMPEA	NA(1)	76	119	107	102	106	7.5
NDPh	123	108	87	80	89	87	2.5

(1) Not applicable because S/N <10 for 1 ppb.
(2) Not applicable because accuracy performed with a calibration range from 1 to 40 ppb following out-of-acceptance criteria obtained for accuracy levels 1 and 2 ppb with a calibration range from 1 to 60 ppb.
(3) Not applicable because accuracy performed with a calibration range from 1 to 40 ppb following out-of-acceptance criteria obtained for accuracy level 1 ppb with a calibration range from 1 to 60 ppb.

Table 5. Repeatability calculated on samples using the aqueous extraction procedure.

Sample Repeatability Obtained Doing an Aqueous Extraction						
	Mean Recovery					%RSD
Level (ppb)	1	2	5	10	30	30
NDMA	115	99	98	95	95	2.5
NMEA	111	104	104	103	103	3.0
NDEA	95	95	97	96	98	3.3
NEIPA	107	107	102	102	104	3.9
NDIPA	126	106	102	97	98	4.5
NMPA	125	96	82	79	75	7.3
NDPA	104	108	106	103	99	3.7
NMOR	101	97	97	98	96	3.2
NPYR	NA(1)	134	106	100	97	4.1
NPIP	107	101	105	101	98	4.0
NDBA	81	92	99	109	104	7.5
NDPh	92	91	93	92	87	5.9

(1) Not applicable because S/N <10 for this level.

Linear curves were generated using a linear fit. Correlation coefficient is equal to 1.00 for all nitrosamines on the studied range, which complies with acceptance criteria (r ≥0.98), establishing excellent linear response through the range (Tables 6 and 7).

To assess the sensitivity, LODs were calculated based on the signal-to-noise (S/N) values for diluted standards to determine which LODs could be achieved, enabling very trace level detection. The LODs were in the range of 0.05 to 2 ppb and LOQs based on accuracy results were in the range of 1 to 10 ppb. This result allows a good screening sensitivity, which should be checked on each DS or DP matrix, by spiking, in the run during the day of analysis.

Calibration curves, correlation coefficient, and LOD are presented in Tables 6 and 7.

Table 6. Calibration curve obtained and validated range on the 13 nitrosamines screened with associated LODs using the direct dispersion in $MeCl_2$ procedure.

Compound in $MeCl_2$	Range Studied	Equation	r	Working LOD and Associated Concentration
NDMA	1 to 60 ppb	y = 0.073036x + 0.003881	1.00	0.1 ppb (S/N = 3)
NMEA	1 to 60 ppb	y = 0.054100x – 0.001531	1.00	0.05 ppb (S/N = 4)
NDEA	1 to 60 ppb	y = 0.065633x – 0.002706	1.00	0.05 ppb (S/N = 4)
NEIPA	1 to 60 ppb	y = 0.112296x – 0.011425	1.00	0.05 ppb (S/N = 6)
NDIPA	1 to 60 ppb	y = 0.033903x – 0.010897	1.00	0.1 ppb (S/N = 3)
NMPA	1 to 40 ppb(1)	y = 0.113250x – 0.022100	1.00	0.1 ppb (S/N = 3)
NDPA	1 to 60 ppb	y = 0.014889x – 0.002761	1.00	0.05 ppb (S/N = 3)
NMOR	1 to 60 ppb	y = 0.090466x – 0.020014	1.00	0.05 ppb (S/N = 6)
NPYR	2 to 60 ppb(2)	y = 0.007464x – 0.005057	1.00	0.5 ppb (S/N = 3)
NPIP	1 to 40 ppb(3)	y = 0.020842x + 0.004194	1.00	0.5 ppb (S/N = 7)
NDBA	1 to 40 ppb(1)	y = 0.061255x + 4.087211E-004	1.00	0.5 ppb (S/N = 12)
NMPEA	2 to 60 ppb(2)	y = 1.188428x + 0.061221	1.00	1 ppb (S/N = 7)
NDPh	1 to 60 ppb	y = 2.326033x – 0.108300	1.00	0.05 ppb (S/N = 6)

With y = peak area ratio and x = concentration (ppb)

(1) Range studied from 1 to 40 ppb due to the out-of-acceptance criteria obtained for accuracy levels 1 and 2 ppb with a range from 1 to 60 ppb.

(2) Range studied from 2 to 60 ppb due to the S/N <10 obtained for standard solution at 1 ppb.

(3) Range studied from 1 to 40 ppb due to the out-of-acceptance criteria obtained for accuracy level 1 ppb with a range from 1 to 60 ppb.

Table 7. Calibration curve obtained and validated range on the 13 nitrosamines screened with associated LODs using the aqueous extraction procedure.

Compound in Water	Range Studied	Equation	r	Working LOD and Associated Concentration (ppb)
NDMA	1 to 30 ppb	y = 0.089432x + 0.011075	1.00	0.05 (S/N = 4)
NMEA	1 to 30 ppb	y = 0.079262x + 0.015512	1.00	0.05 (S/N = 6)
NDEA	1 to 30 ppb	y = 0.067200x + 0.009409	1.00	0.1 (S/N = 3)
NEIPA	1 to 30 ppb	y = 0.118563x + 0.021747	1.00	0.05 (S/N = 3)
NDIPA	1 to 30 ppb	y = 0.035273x – 0.005707	1.00	0.05 (S/N = 5)
NMPA	1 to 30 ppb	y = 0.178605x – 0.055821	1.00	0.5 (S/N = 5)
NDPA	1 to 30 ppb	y = 0.016135x – 9.566144E-004	1.00	0.05 (S/N = 6)
NMOR	1 to 30 ppb	y = 0.078884x + 0.001414	1.00	0.1 (S/N = 3)
NPYR	2 to 30 ppb(1)	y = 0.007386x – 0.004633	1.00	0.05 (S/N = 4)
NPIP	1 to 30 ppb	y = 0.024298x + 0.006008	1.00	0.1 (S/N = 3)
NDBA	1 to 30 ppb	y = 0.059460x + 0.008600	1.00	0.1 (S/N = 2)
NMPEA	10 to 30 ppb(2)	y = 1.082170x – 1.109961	1.00	2 (S/N = 4)
NDPh	1 to 30 ppb	y = 2.278087x + 0.482510	1.00	0.05 (S/N = 3)

With y = peak area ratio and x = concentration (ppb)

(1) Range studied from 2 to 30 ppb because LOQ reached at 2 ppb for NPYR (S/N <10 obtained for standard solution at 1 ppb).

(2) Range studied from 10 to 30 ppb because LOQ reached at 10 ppb for NMPEA (S/N <10 obtained for standard solutions at 1, 2, and 5 ppb).

Conclusion

The results of these experiments demonstrate:

- A good resolution between the 13 compounds
- Accurate and precise recovery with spiking concentrations within 70 to 130% recovery and % RSD <20% at 30 ppb level
- Excellent linearity was observed in the range
- Low detection limits were achieved

These features enabled reliable screening of all 13 residues. Each day of analysis, the reliability of the method is verified by spiking the analyzed matrices with each required nitrosamine. This allows estimation of the % recovery of spiked samples.

This method highlights the capabilities of the 8890-7010B GC/MS/MS system for the detection and screening of 13 nitrosamine drug impurities, at trace level, in drug substances and drug products.

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