Analysis of nitrosamines by LC-MS/MS and HPLC, using InertSustain AQ-C18 -comparison with PFP column-

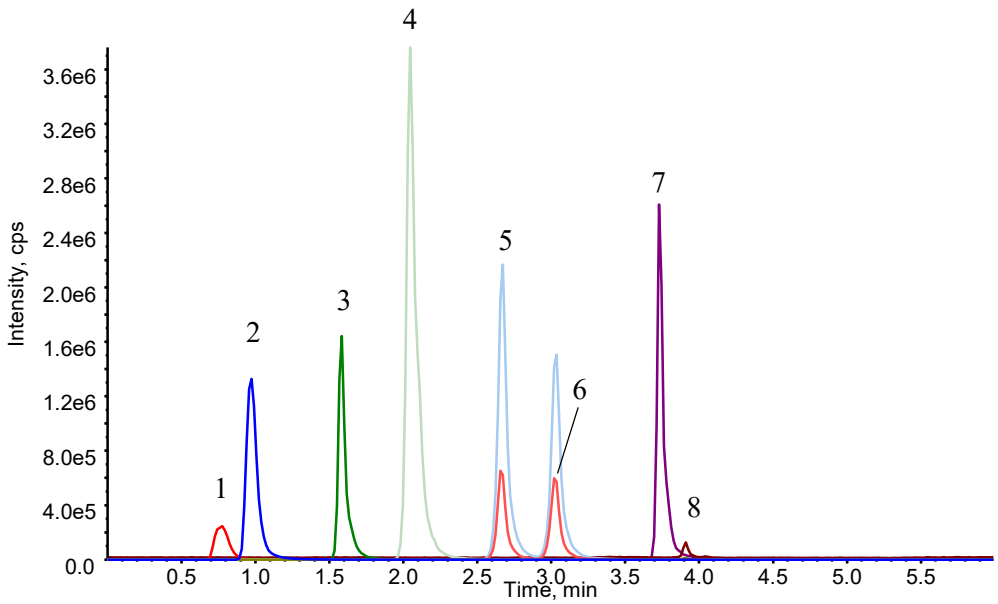
Nitrosamines are carcinogenic substances that have been detected in various products such as pharmaceuticals, chemicals, cosmetics, rubber and plastic products. The Ministry of Health, Labor and Welfare has issued a notice to self-inspect the risk of nitrosamine contamination in pharmaceuticals by April 30, 2023. Analysis examples using InertSustain PFP based on FDA (U.S. Food and Drug Administration) literature are posted on InertSearch LB691.

In this study, we examined analytical conditions for rapid measurement of eight nitrosamines by LC-MS/MS, and were able to perform analysis in 6 minutes using the InertSustain AQ-C18 HP. to introduce. In addition, an example of analysis by HPLC/DAD will also be introduced. (R.Takahashi)

Analysis by LC-MS/MS

Measurement example with InertSustain AQ-C18 HP

① InertSustain AQ-C18 HP 3µm, **50** x 2.1 mmI.D.



① Analysis conditions

system : Nexera XR UHPLC system
API 4000 Q TRAP

Eluent : A) 0.1 % HCOOH in CH_3OH
B) 0.1 % HCOOH in H_2O

Time (min)	A (vol%)	B (vol%)
0.0	10	90
0.4	35	65
1.0	40	60
2.5	50	50
2.6	73	27
3.5	78	22
3.6	90	10
4.5	90	10
4.6	10	90
6.0	10	90

Flow rate : 0.4 mL/min
Col.Temp. : 40 ℃
Detection : MS/MS
(4000Q TRAP : APCI, Positive, SRM)

CUR	CAD	NC	TEM	GS1	ihe
50	7	5	300	70	on

Inj.Vol. : 5 µL

Samples:

	Q1/Q3
1. N-Nitrosodimethylamine (NDMA)	75/58
2. N-Nitrosomorpholine (NMOR)	117/87
3. N-Nitrosodiethylamine (NDEA)	103/75
4. N-Nitrosoethylisopropylamine (NEIPA)	117/75
5. N-Nitrosodiisopropylamine (NDIPA)	131/89
6. N-Nitrosodi-n-propylamine (NDPA)	131/43
7. N-Nitrosodi-n-butylamine (NDBA)	159/103
8. N-Nitrosodiphenylamine (NDPhA)	171/171

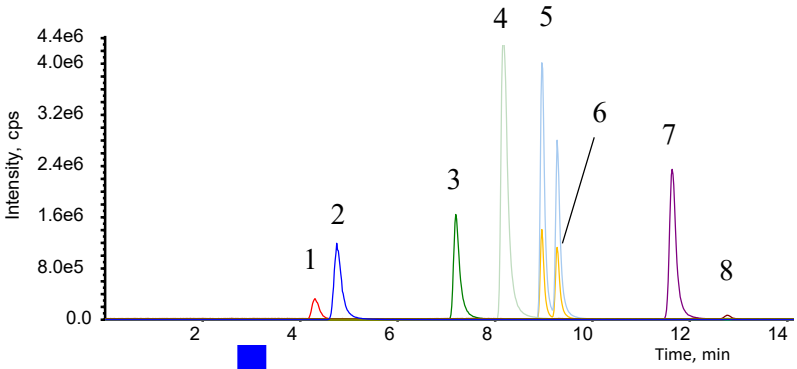
(each 1.0 mg/L in solvent)

Column selection points for analysis of nitrosamines

When high-speed analysis is performed using the PFP column adopted by the FDA, the highly polar components of nitrosamines cannot be separated well, as shown in the figure below. InertSustain AQ-C18 is an ODS column with high inertness (Inert) and high durability (Sustain), designed to retain highly polar compounds more strongly than general ODS columns. The InertSustain AQ-C18 is the best choice for high-speed analysis of nitrosamines, as the separation of highly polar components and peak shape must be carefully considered. However, for actual samples, it is necessary to select a column that considers the separation of interfering components, so it is important to use the correct column according to the situation. Please feel free to contact us if you have any problems with column selection.

High-speed analysis on InertSustain PFP HP column

② InertSustain PFP HP 3µm, 100 x **4.6** mmI.D.
(Published in Inert Search LB691)



Reduce analysis time

② Analysis conditions

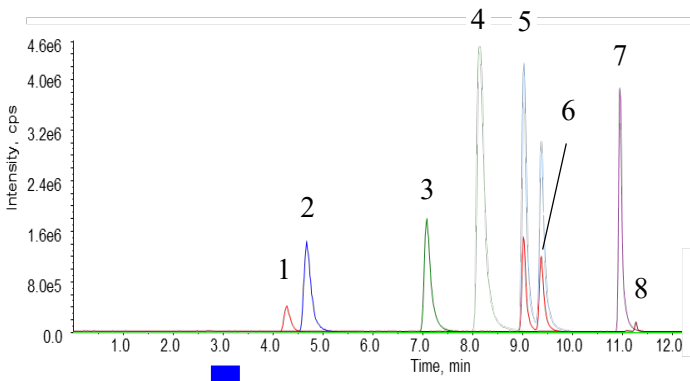
Eluent : A) 0.1 % HCOOH in CH_3OH
B) 0.1 % HCOOH in H_2O

Time (min)	A (vol%)	B (vol%)
0.0	10	90
1.5	10	90
7.0	55	45
17.0	55	45
17.1	90	10
21.0	90	10
21.1	10	90
25.0	10	90

Flow rate : 0.6 mL/min
Inj.Vol. :15 µL

* Other conditions are the same as ①.

③ InertSustain PFP HP 3µm, 100 x **4.6** mmI.D.



Change column inner diameter

③④ Analysis conditions

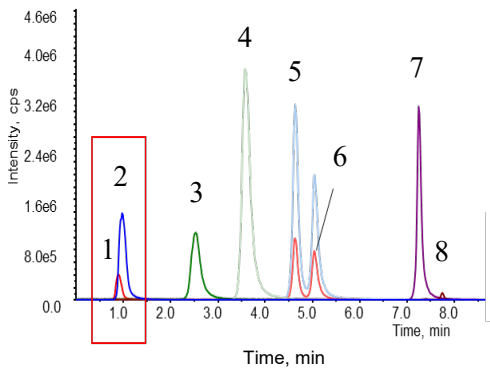
Eluent : A) 0.1 % HCOOH in CH_3OH
B) 0.1 % HCOOH in H_2O

Time (min)	A (vol%)	B (vol%)
0.0	10	90
1.0	10	90
8.0	60	40
10.5	90	10
12.0	90	10
12.1	10	90
15.0	10	90

Flow rate : 0.6 mL/min
Inj.Vol. : 15 µL

* Other conditions are the same as ①.

④ InertSustain PFP HP 3µm, 100 x **2.1** mmI.D.



Samples:

	Q1/Q3
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2. N-Nitrosomorpholine (NMOR)	117/87
3. N-Nitrosodiethylamine (NDEA)	103/75
4. N-Nitrosoethylisopropylamine (NEIPA)	117/75
5. N-Nitrosodiisopropylamine (NDIPA)	131/89
6. N-Nitrosodi-n-propylamine (NDPA)	131/43
↓-Nitrosodi-n-butylamine (NDBA)	159/103
↓-Nitrosodiphenylamine (NDPhA)	171/171

(each 1.0 mg/L in solvent)

High-speed analysis reduces resolution on PFP columns.

Analysis example by HPLC/DAD

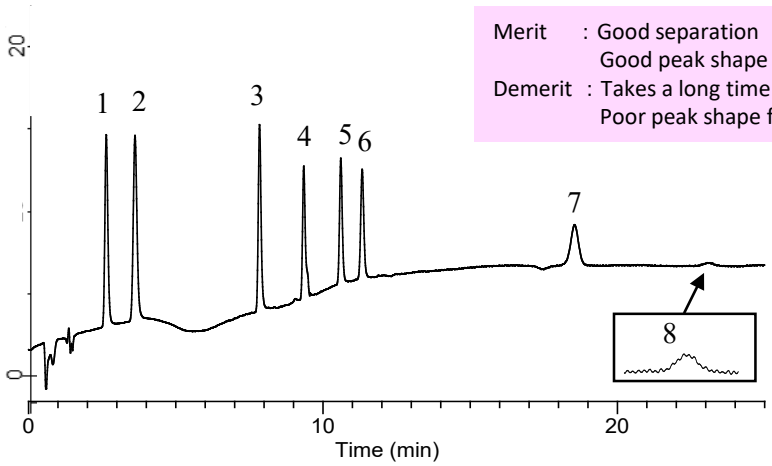
InertSustain PFP HP and InertSustain AQ-C18 HP with the same column size (3 µm, 100 x 4.6 mm I.D.) were analyzed by HPLC/DAD, and the measurement results at UV 240 nm and the characteristics when comparing the two columns are described. Also, the UV absorption spectrum from 220 to 400 nm obtained with the DAD detector is shown on the next page. As with LC/MS/MS, please select a column according to the analytical components and contaminant components.

Measurement example when analyzed with the same method

Example of InertSustain AQ-C18 HP

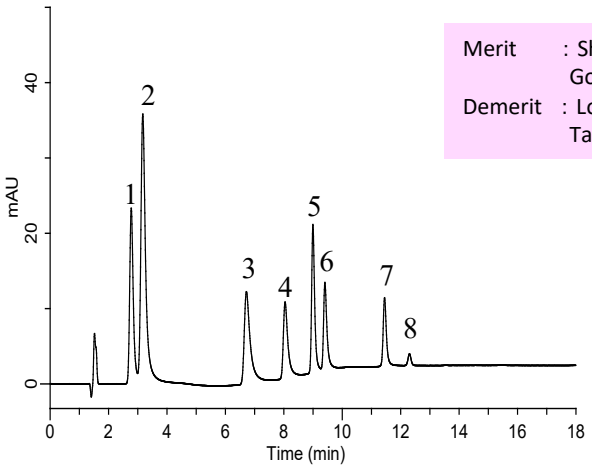
Comparing the two columns...

Merit : Good separation
Good peak shape for weakly retained components
Demerit : Takes a long time to measure
Poor peak shape for components with strong retention



Example of InertSustain PFP HP

Merit : Short measurement time
Good peak shape even for components with strong retention
Demerit : Low resolution
Tailin g is likely to occur depending on the ingredients



Analysis conditions

System : Primaide HPLC system
Flow rate : 1.0 mL/min
Col.Temp. : 40 ℃
Detection : UV 240 nm
(1430 Diode Array Detector)
Inj.Vol. : 10 µL

Eluent : A) CH_3OH
B) H_2O

Time (min)	A (vol%)	B (vol%)
0.0	10	90
1.5	10	90
7.0	55	45
30.0	55	45
30.1	90	10
35.0	90	10
35.1	10	90
40.0	10	90

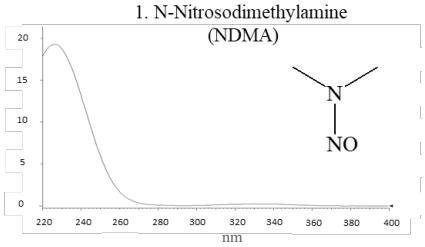
Samples:

1. N-Nitrosodimethylamine (NDMA)
2. N-Nitrosomorpholine (NMOR)
3. N-Nitrosodiethylamine (NDEA)
4. N-Nitrosoethylisopropylamine (NEIPA)
5. N-Nitrosodiisopropylamine (NDIPA)
6. N-Nitrosodi-n-propylamine (NDPA)
7. N-Nitrosodi-n-butylamine (NDBA)
8. N-Nitrosodiphenylamine (NDPhA)

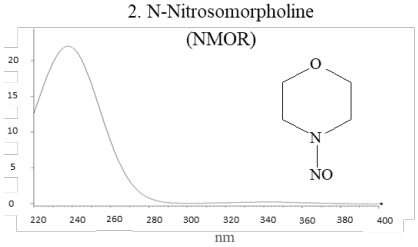
(each 5 mg/L in solvent)

UV spectrum

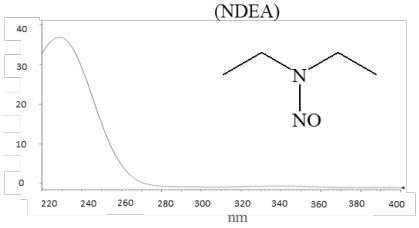
1. N-Nitrosodimethylamine (NDMA)



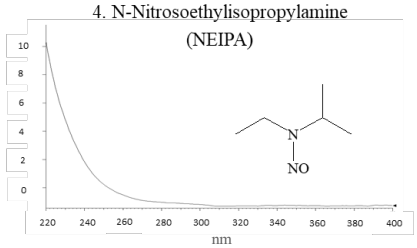
2. N-Nitrosomorpholine (NMOR)



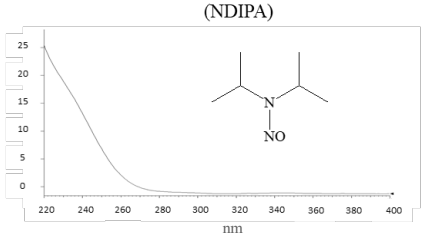
3. N-Nitrosodiethylamine (NDEA)



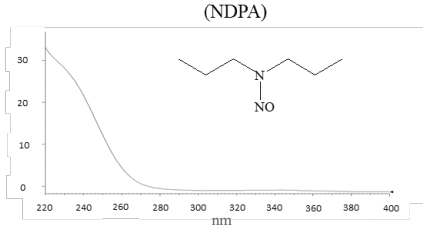
4. N-Nitrosoethylisopropylamine (NEIPA)



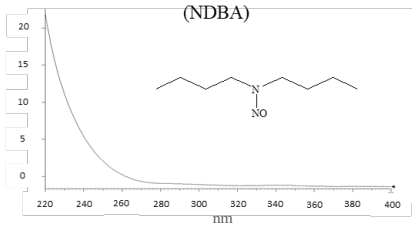
5. N-Nitrosodiisopropylamine (NDIPA)



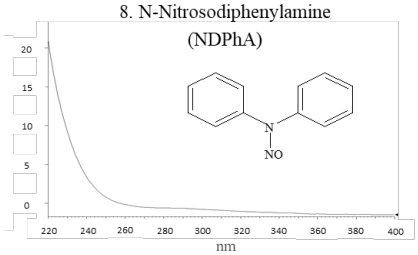
6. N-Nitrosodi-n-propylamine (NDPA)



7. N-Nitrosodi-n-butylamine (NDBA)



8. N-Nitrosodiphenylamine (NDPhA)



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

Product used

Column	Size	Cat. No.
InerttSustain AQ-C18 HP	3µm, 100 x 2.1 mmI.D.	5020-89923
InerttSustain AQ-C18 HP	3µm, 50 x 2.1 mmI.D.	5020-89921
InerttSustain AQ-C18 HP	3 µm, 100 x 4.6 mmI.D.	5020-89935
InerttSustain PFP HP	3 µm, 100 x 4.6 mmI.D.	5020-87932
InerttSustain PFP HP	3µm, 100 x 2.1 mmI.D.	5020-87920

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