Chromatography Solutions

Technical note #027

Avantor® chromatography solutions for the analysis of nitrosamines

INTRODUCTION

Nitrosamines have become an increasingly prominent concern as they are highly potent genotoxic impurities which may inadvertently be formed during the manufacture and processing of various consumer goods. The recent detection of nitrosamines in, and recall of, some pharmaceutical products has further increased concern over the presence of these compounds. Additionally, environmental contamination through release of nitrosamines, along with their formation during treatment processes (e.g. water treatment), are also areas of concern.

Nitrosamines are a class of organic compound containing a nitroso group bonded to an amine (Figure 1) and are typically formed by reaction of a nitrosating agent, such as nitrite, with various amines. Due to many nitrosamines being classified as probable human carcinogens, it is essential to establish and quantify their presence in a broad range of products and sample matrices. This technical note details several chromatographic solutions that can be applied to the determination of nitrosamines.

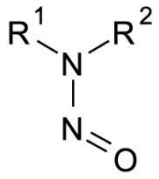


Figure 1: Generic nitrosamine structure.

CHROMATOGRAPHIC METHODS FOR NITROSAMINE DETERMINATION

Analytical methodologies for the determination of nitrosamines (including regulatory methods and guidance published by bodies such as US FDA, USP and EPA) typically utilise either liquid or gas chromatography (LC or GC). The Avantor® ACE® and Avantor® Hichrom chromatography ranges include numerous GC and LC

stationary phases which can be utilised for the analysis of nitrosamines. GC is ideally suited to the analysis of volatile nitrosamines and has therefore proved a popular choice for nitrosamine analysis, providing high resolution separations, fast analysis, and a cost effective solution. Figure 2 shows the analysis of three nitrosamines specified in EPA method 607, which can be applied to the analysis of these analytes in municipal and industrial wastewater samples. In this example, the HI-5 GC phase, a low-polarity stationary phase with a 5% phenyl, 95% methyl polysiloxane composition, provides separation of these key nitrosamines in compliance with the EPA methodology. The Avantor® Hichrom GC phase portfolio includes a wide range of standard GC phases, along with several specially developed unique phases. Columns are available in all common column formats and provide demonstrable reproducibility to meet stringent regulatory requirements. These high quality GC phases provide numerous options for new method development and for use with existing and future regulatory GC and GC-MS methods.

Liquid chromatography is also commonly used and can overcome some reported issues with GC analysis, for example allowing determination of involatile nitrosamines such as NMBA, and thermal degradation of ranitidine drug substance under GC conditions to yield NDMA.[1] LC also enables the simultaneous determination of nitrosamines alongside drug product in a single run. Figure 3 shows the separation of eight nitrosamines spiked into valsartan. This application includes key target analytes specified for monitoring by a variety of regulators including EMA and the FDA.[2,3] The separation is achieved using an Avantor® ACE® UltraCore C18 column, manufactured using ultra-high purity solid-core silica, which provides exceptional UHPLC-like separation efficiencies at low back pressure, compatible with standard HPLC systems. In this example, a high resolution separation of these key analytes from the main valsartan peak is readily achieved within 12 minutes, with excellent analyte peak shape.

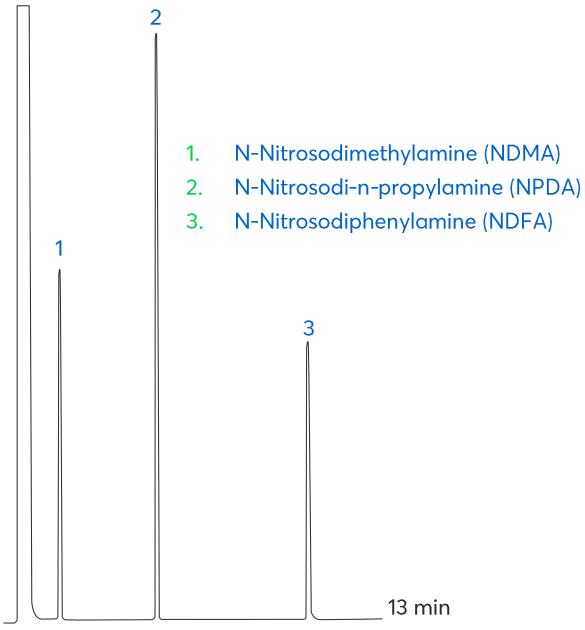


Table 1: Method conditions for Figure 2.

Column	Avantor® Hichrom HI-5
Dimensions	0.53 mm, 1.50 µm, 15 m
Oven Program	40 °C (4 min), 20 °C/min, 240 °C
Carrier Gas:	Hydrogen, 10 mL/min
Injector	On-column, 1 µL
Detector	FID, 280 °C
Sample	20 µg/mL each in Methanol

Figure 2: Nitrosamine analysis using the HI-5 Phase (EPA Method 607, Application note C-13033).[4]

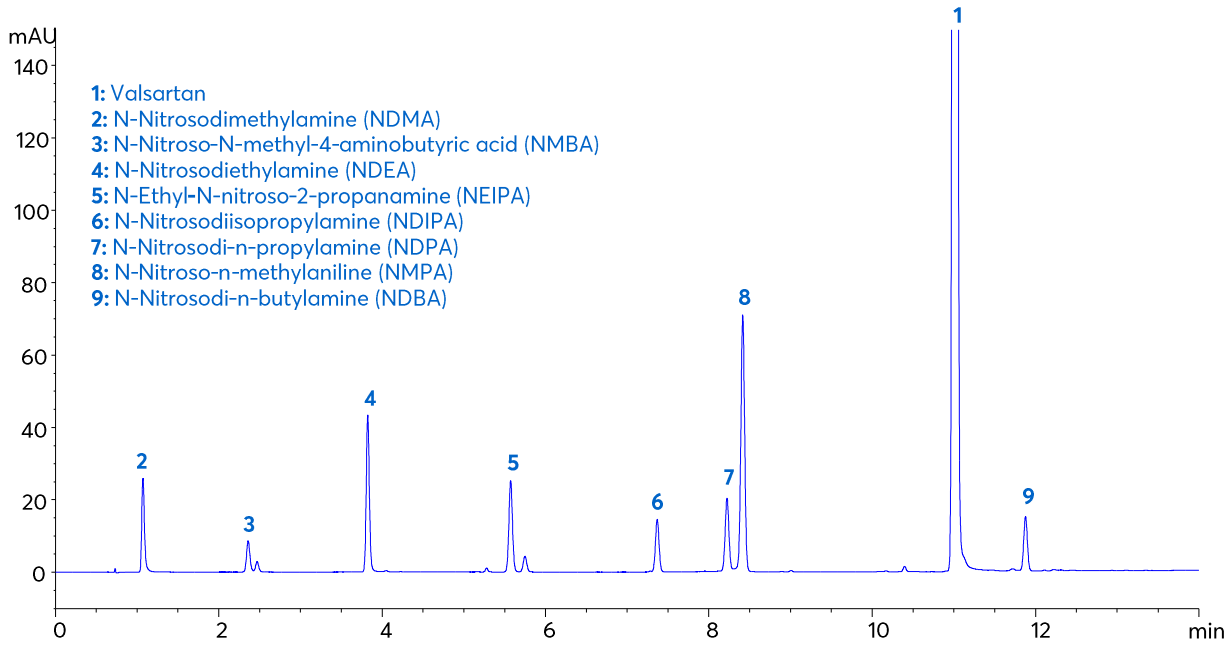


Figure 3: Separation of nitrosamine contaminants in valsartan API (Application note AN7250).[4]

Table 2: Method conditions for Figure 3.

Column	Avantor® ACE® UltraCore C18
Dimensions	100 x 3.0 mm, 3.5 µm (95 Å pore size)
Mobile phases	A: 20 mM KH_2PO_4 pH 2.7 in H_2O B: 20 mM KH_2PO_4 pH 2.7 in ACN/H_2O 7:3 v/v
Flow Rate	0.6 mL/min
Injection	1 µL
Temperature	20 °C
Detection	UV, 254 nm

Table 3: Gradient table for Figure 3.

Time (mins)	%B
0	4
1	4
15	95
17	95
17.5	4
25	4

Due to the low-level detection limits required for genotoxic impurities, the use of mass spectrometry detection is often desired. Figure 4 demonstrates an alternative solution for the LC-MS/MS separation of nitrosamines according to the European Union EN 71-12 safety of toys standard. In this example, a simple acidic mobile phase is used to separate a wide range of nitrosamines on an Avantor® ACE® Excel 2 C18-PFP column. Often the alternative selectivity offered by non-C18 phases such as pentafluorophenyl (PFP) can be highly advantageous, enabling the separation of analytes that cannot be achieved using a C18 phase.[5,6] The alternative selectivity offered by this phase when compared to a standard C18 phase is clearly demonstrated in Figure 5A and 5B. However, shorter, more chemically labile ligands typically utilised for such phases can potentially show high levels of phase bleed, resulting in reduced or variable MS signal sensitivity. Figure 5A, C and D clearly demonstrates the high levels of bleed that can potentially arise when using a propyl PFP phase compared to a standard C18 phase. The ACE C18-PFP overcomes this issue (Figure 5B) by combining the alternative selectivity of a PFP phase with the longer chain, rugged and stable C18 ligand to substantially reduce stationary phase bleed (Figure 5E), providing superior LC/MS compatibility.

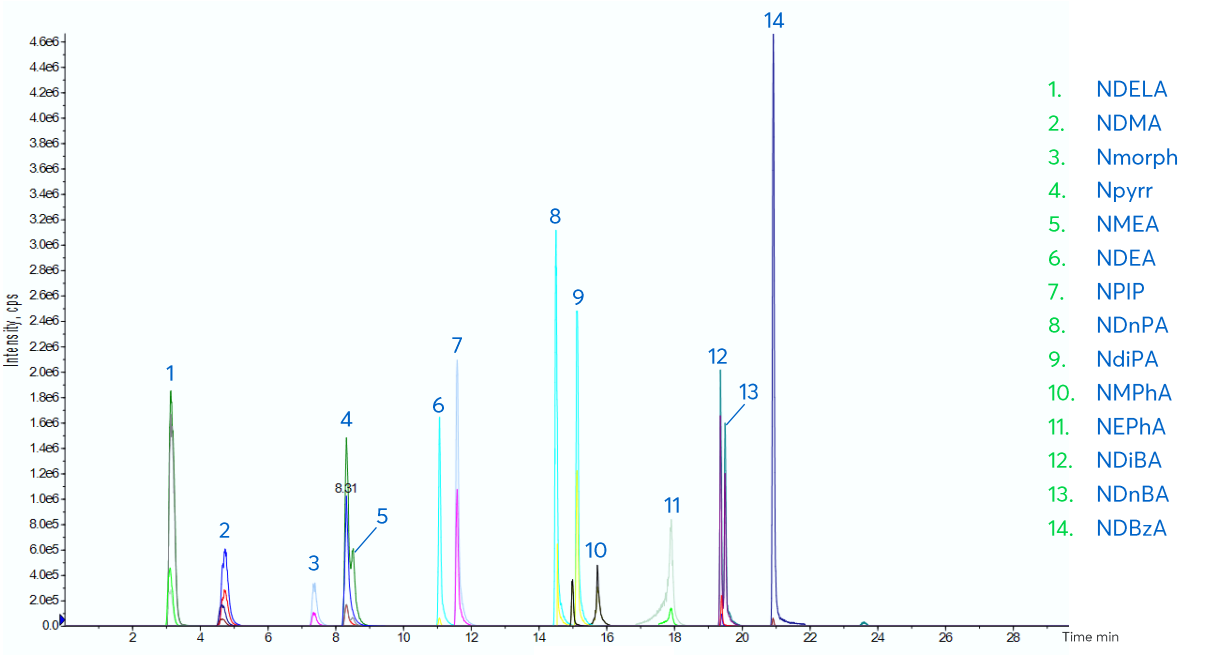


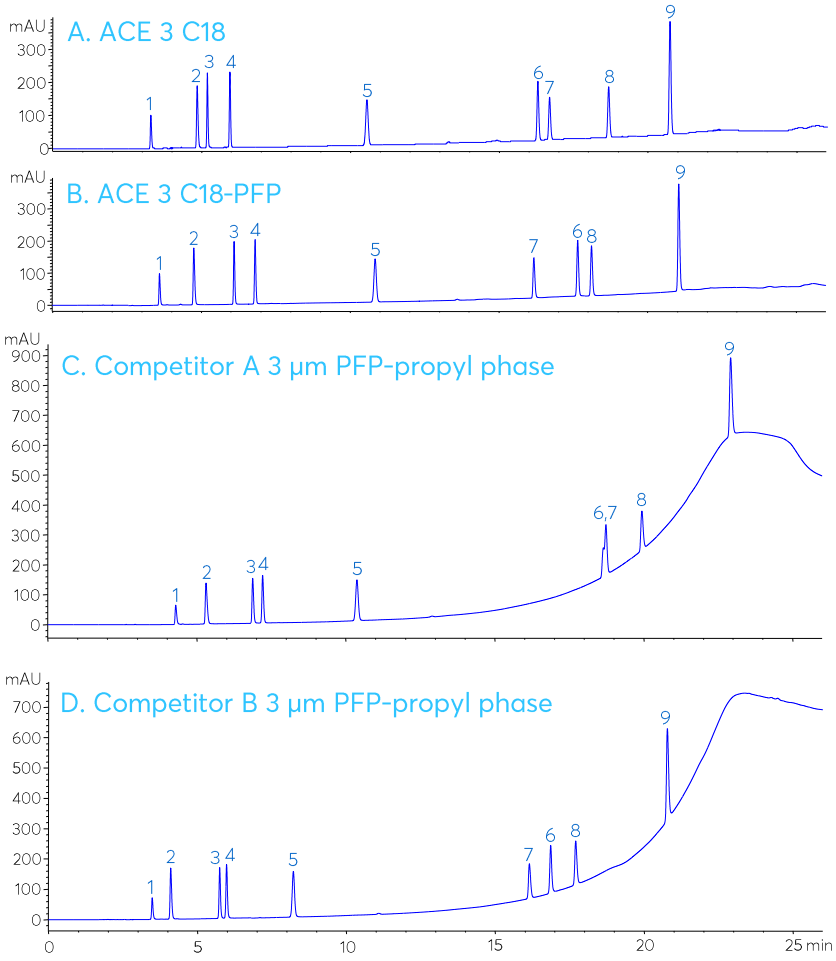
Figure 4: Separation of nitrosamines according to the European safety of toys standard method by LC-MS/MS (Please refer to Application Note AN1110 for MRM transitions[4]). Reproduced with permission of LGC Limited, UK.

Table 4: Method conditions for Figure 4.

Column	Avantor® ACE® Excel 2 C18-PFP
Dimensions	150 x 3.0 mm
Mobile phases	A: 0.1 % formic acid in H_2O B: 0.1 % formic acid in MeOH
Flow Rate	0.35 mL/min
Injection	20 µL
Temperature	40 °C
Sample temperature	4 °C
Detection	Applied Biosystems 4000 Q-Trap MS Source: APCI (positive mode) Collision energy: 10-30 V Source temperature: 300 °C
Method	European Union EN 71-12 safety of toys: N-Nitrosamines and N-nitrosatable substances e.g. Analysis of nitrosamines in balloon extracts

Table 5: Gradient table for Figure 4.

Time (mins)	%B
0.00	5
1.00	5
10.00	65
14.00	65
15.00	90
20.00	90
21.00	5
30.00	5



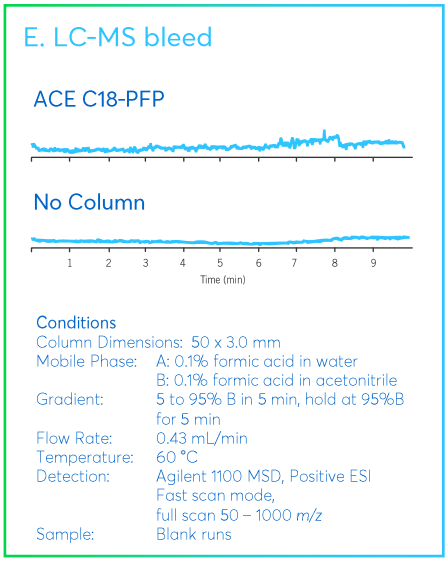


Figure 5: LC-UV analysis of a set of basic compounds showing varying level of observed bleed on a C18 phase (A), C18-PFP phase (B) and two competitor pentaflurophenyl-propyl stationary phases (C & D). Figure 5E demonstrates the low level of LC-MS bleed observed on the ACE C18-PFP.

Conditions for A-D: Column dimensions: 150 x 4.6 mm, Mobile phase: A: 20 mM KH_2PO_4 pH 2.7 (aq) B: 20 mM KH_2PO_4 pH 2.7 in MeOH/H_2O 65:35 v/v, Gradient: 5-100 %B in 20 minutes, hold for 5 mins, Flow rate: 1 mL/min, Injection volume: 5 µL, Temperature: 60 °C, Detection: UV, 210 nm.

Sample: 1) Nicotine 2) Benzylamine 3) Procainamide 4) Terbutaline 5) Phenol 6) Proprietary base 1 7) Proprietary base 2 8) Remacemide 9) Nortriptyline.

Comparative data may not be representative of all applications. Phenomenex columns were not used in the above comparison.

CONCLUSION

As concern over the presence of nitrosamines in various consumer products, pharmaceutical products and the environment grow, industry regulators are demanding increasingly sensitive methods for their determination. Liquid and gas chromatography, especially when coupled to mass spectrometry, provide highly selective and sensitive analytical approaches to this challenge. This Technical Note has provided examples of how the Avantor® ACE® and Avantor® Hichrom ranges of LC and GC columns provide numerous options for the high resolution separation of nitrosamines. The high quality and reproducibility of all Avantor® chromatography products ensures that customers have confidence that methods will meet stringent regulatory performance requirements and offer consistent and reliable long-term performance.

REFERENCES

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