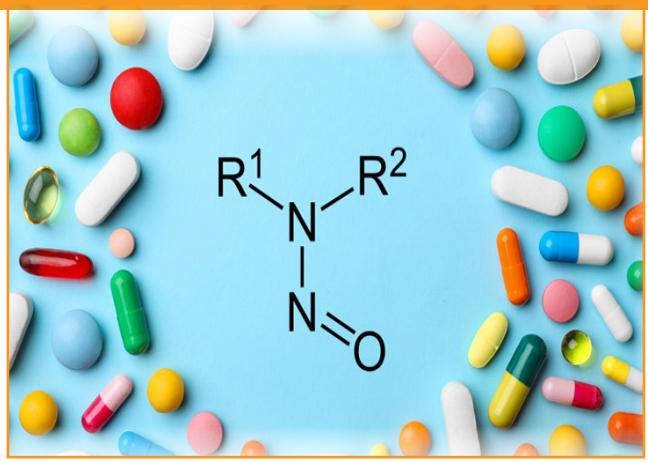
HALO®

TECHNICAL REPORT

TITLE: LC-MS/MS SCREENING OF NITROSAMINES USING HALO® COLUMNS

MARKET SEGMENT: PHARMACEUTICAL



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ABSTRACT

Seven HALO® reversed-phase stationary phases were screened with a standard mix of 12 nitrosamines in order to determine which one would provide the best combination of retention time, peak shape, and selectivity.

INTRODUCTION

Nitrosamines are a class of compounds formed from reactions between a nitrosating agent, such as nitrites or nitrates, and a nitrogenated precursor, such as a secondary or tertiary amine under acidic conditions. They can be found in food[1], medical devices, industrial products, and the environment[2] and can also be formed as drug substance related impurities in pharmaceuticals.[3] Their presence is concerning because most nitrosamines are carcinogenic. In 2018, N-nitroso-dimethylamine (NDMA) was found in valsartran, which prompted recalls of the drug. As more investigations occurred, the presence of nitrosamines was discovered in other pharmaceuticals, such as ranitidine, nizatidine, metformin, rifampicin, rifapentine, varenicline, and sitagliptin. Both gas chromatography (GC) and liquid chromatography (LC) can be used to analyze nitrosamines, however, both have their limitations. For example, GC inlet temperatures can cause some compounds to generate nitrosamines so pharmaceutical testing is mostly done by LC/MS. However, achieving adequate sensitivity of nitrosamines using ESI-LC-MS can be challenging, depending on the matrix effects and MS instrument that is used. This technical report includes LC-MS screening of seven HALO® reversed-phase stationary phases using a 12-component nitrosamine standard from LGC Standards.

EXPERIMENTAL

SCREENING LC CONDITIONS:

Column: HALO 90 Å, 2.7 µm, 2.1 x 100 mm

Phases: C18, AQ-C18, RP-Amide, PCS Phenyl-Hexyl, Biphenyl, PFP, Phenyl-Hexyl *(Phase structures shown in Figure 1.)*

Part Numbers: 92812-602, 92812-622, 92812-607, 92812-618, 92812-611, 92812-609, 92812-606

Mobile Phase A: Water/0.1% Formic Acid

Mobile Phase B: Methanol/0.1% Formic Acid

Gradient: (Time - %B) 0 min. – 5%, 1.0 min. – 5%, 3.0 min. – 20%, 7.0 min. – 100%, 9.0 min. – 100%,

Flow Rate: 0.4 mL/min.

Temperature: 30 °C

Injection volume: 1 µL of GB/T 24153-2009 Nitrosamines Mixture 137 100 µg/mL in Methanol (Part number: DRE-A50000137ME) diluted to 20 µg/mL with water. (*The compounds and their transitions are listed in Table 1.)*

HPLC Instrument: Shimadzu Nexera

Compound	*m/z Transition*
N-nitrosodibenzylamine	*227.00>91.00*
N-nitrosodiethylamine	*103.10>75.05*
N-nitrosodimethylamine	*75.10>43.25*
N-nitrosodi-n-butylamine	*159.20>57.15*
N-nitrosodiphenylamine	*199.22>169.05*
N-nitrosomethylethylamine	*89.10>61.10*
N-nitrosomorpholine	*117.10>87.10*
N-nitroso-N-ethylaniline	*151.00>121.00*
N-nitroso-N-methylaniline	*137.00>107.00*
N-nitroso-n-propylamine	*131.20>43.10*
N-nitrosopiperidine	*115.10>69.05*
N-nitrosopyrrolidine	*101.10>55.10*

Table 1. List of nitrosamines and m/z transitions.

KEY WORDS:

nitrosamines, HALO 90 Å Biphenyl, LC-MS

MS CONDITIONS:
MS Instrument: Shimadzu LCMS-8060NX
Nebulizing Gas: 3 L/min.
Heating Gas: 15 L/min.
Interface Temperature: 400 °C
DL Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 3 L/min.
Detection Mode: DUIS ESI + 1 kV; Corona Needle 3.5

OPTIMIZED LC CONDITIONS:
Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-611
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Methanol/0.1% Formic Acid
Gradient: (Time - %B) 0.00 min. – 5%, 8.00 min. – 100%, 9.00 min. – 100%, 9.01 min. – 5%, 12.00 min. – 5%
Temperature: 45 °C
Injection Volume: 0.6 µL of 10 µg/mL each analyte of LGC nitrosamine mix used for screening

OPTIMIZED MS CONDITIONS:
System: Shimadzu 8060NX QQQ
Detection Mode: DUIS ESI + 1 kV; Corona Needle 3.5 kV
Nebulizer Gas Flow: 3 L/min.
Interface Temperature: 300 °C
DL Temperature: 200 °C
Heat Block Temperature: 200 °C
Drying Gas Flow: 5 L/min.

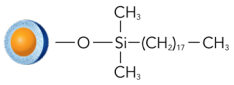
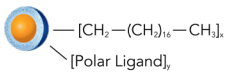
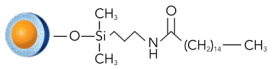
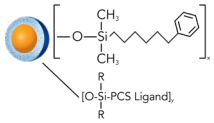
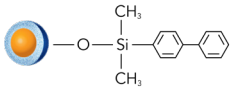
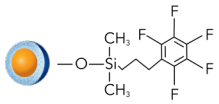
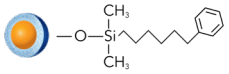
Alkyl Phases	C18 	AQ-C18 	RP-AMIDE 	
Phenyl Phases	PCS PHENYL-HEXYL 	BIPHENYL 	PFP 	PHENYL-HEXYL 

Figure 1. Structures of the HALO® phases used for nitrosamine screening. All of the phases listed here are 100% aqueous compatible except C18.

RESULTS

Results of the alkyl stationary phase screening are shown in Figure 2. A HALO® AQ-C18 column was chosen due to its 100% aqueous compatibility, allowing for increased retention for polar analytes. A HALO® RP-Amide column was also chosen, which includes a polar embedded group within the ligand, allowing for a potential difference in selectivity. Peak elution order is slightly different between HALO® AQ-C18 and HALO® C18 (peaks 7 and 8). Peaks 3 and 4 are coeluted on HALO® RP-Amide. HALO® C18 shows slightly more retention and narrower peak widths over HALO® AQ-C18 and HALO® RP-Amide.

PEAK IDENTITIES

1. N-nitrosodimethylamine
2. N-nitrosomorpholine
3. N-nitrosomethylethylamine
4. N-nitrosopyrrolidine
5. N-nitrosodiethylamine
6. N-nitrosopiperidine
7. N-nitroso-n-propylamine
8. N-nitroso-N-methylaniline
9. N-nitroso-N-ethylaniline
10. N-nitrosodi-n-butylamine
11. N-nitrosodiphenylamine
12. N-nitrosodibenzylamine

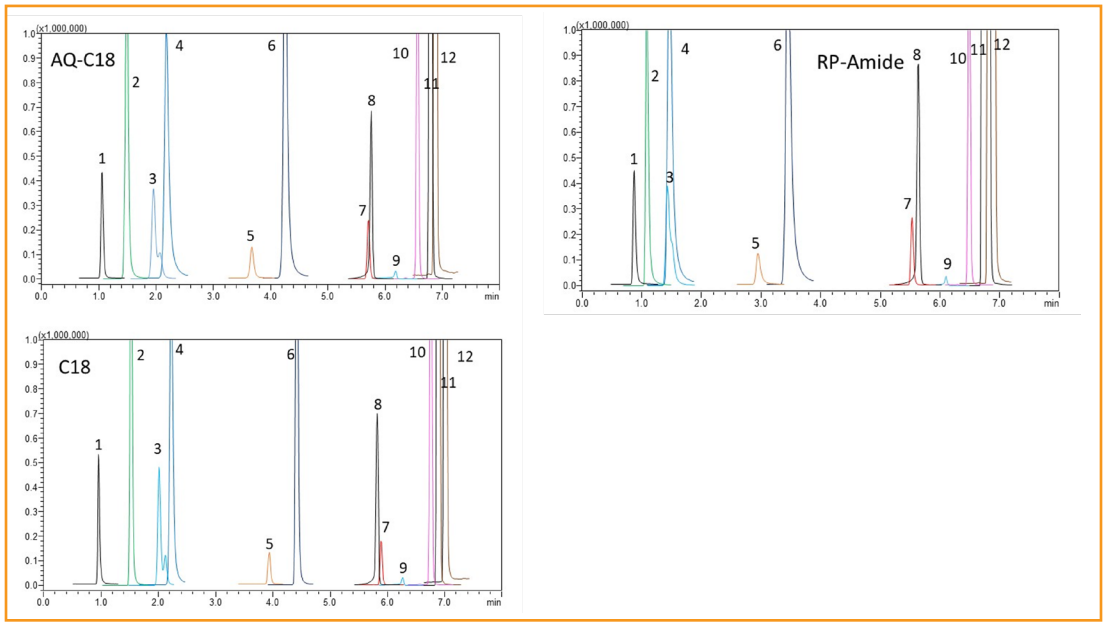


Figure 2. Screening results for AQ-C18 vs. C18 vs. RP-Amide.

Very little difference was found between HALO® PCS Phenyl-Hexyl and HALO® Phenyl-Hexyl as shown in Figure 3. The difference between these two phases is that HALO® PCS Phenyl-Hexyl contains a ligand that becomes positively charged when run at pH < 5. PCS means positively charged surface, which is designed for use with low ionic strength mobile phase and basic analytes. HALO® Phenyl-Hexyl shows increased retention over HALO® PCS Phenyl-Hexyl, most likely due to the more hydrophobic nature of HALO® Phenyl-Hexyl.

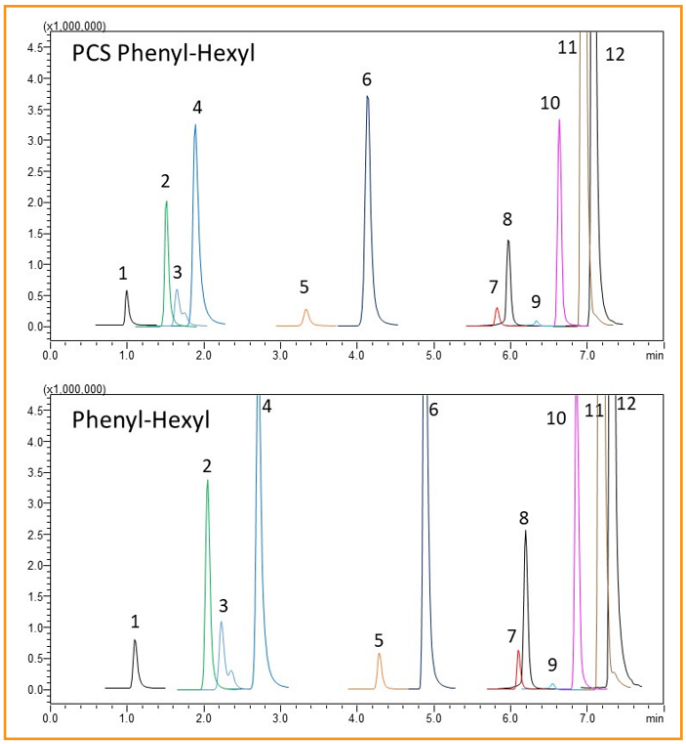


Figure 3. Screening results for PCS Phenyl-Hexyl vs. Phenyl-Hexyl. Peak identities are the same as in Figure 2.

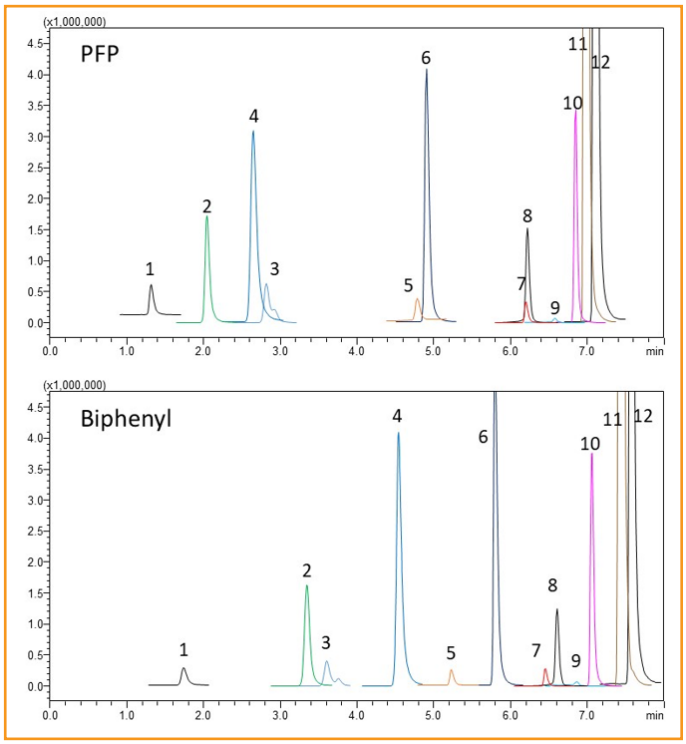


Figure 4. Screening results for PFP vs. Biphenyl. Peak identities are the same as in Figure 2.

The screening results for HALO® PFP and HALO® Biphenyl are shown in Figure 4. The peak elution order is different with peak 4 eluting before peak 3. HALO® Biphenyl shows increased retention over HALO® PFP. In fact, for the 3 earliest eluting nitrosamines, HALO® Biphenyl was the most retentive of all of the phases screened as seen in Figure 5. One explanation for the increased retention on HALO® Biphenyl could be that the lone pair electrons on the nitrogen of the nitrosamines interacts with the pi-electrons of the biphenyl rings. Since HALO® Biphenyl offered the best compromise of retention, peak shape, and selectivity, it was selected for method optimization.

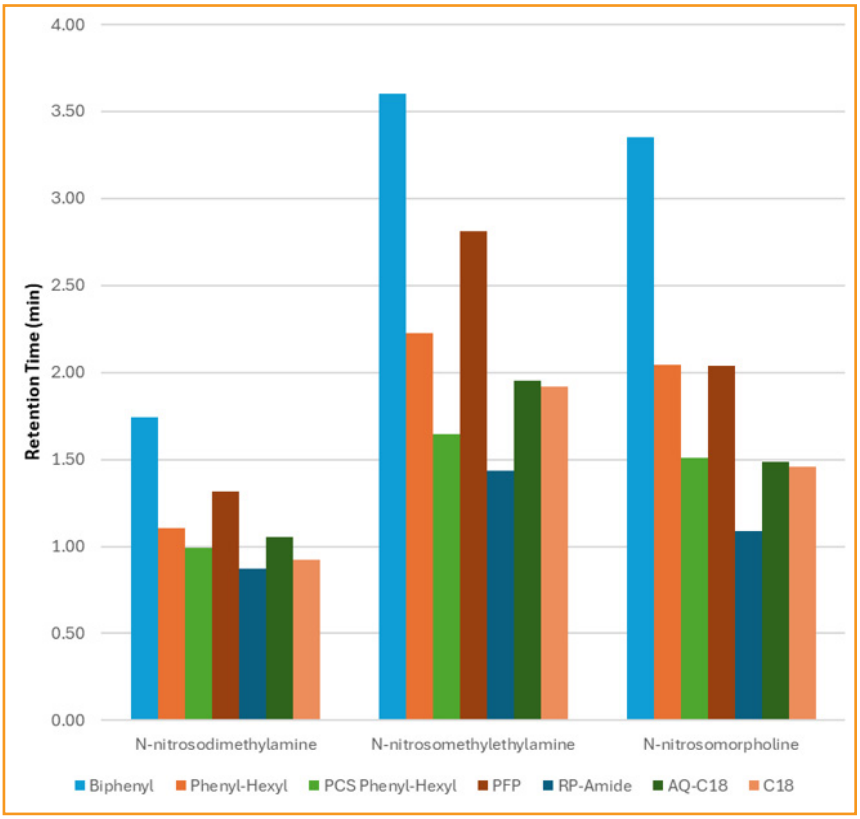


Figure 5. Retention time comparison showing HALO® Biphenyl as the most retentive phase for the 3 earliest eluting nitrosamines.

DryLab® was chosen as the method optimization software to accelerate method development. This software can be used for 2 or 3 parameter optimizations of the following: gradient time, temperature, pH, and mobile phase composition/additive. Two gradient times (10 and 30 minutes) and two temperature (30 and 50 °C) were chosen for the training runs for DryLab®. Figure 6 shows the comparison between the experimental and predicted mass spectra. Only 11 peaks are included with the predicted result since there was no need to model peak 1 (NDMA) as it had no closely eluting peaks near it. The two results match very well for retention time. There were some problems with the response variability of the MS so this explains why some of the peak areas do not match exactly.

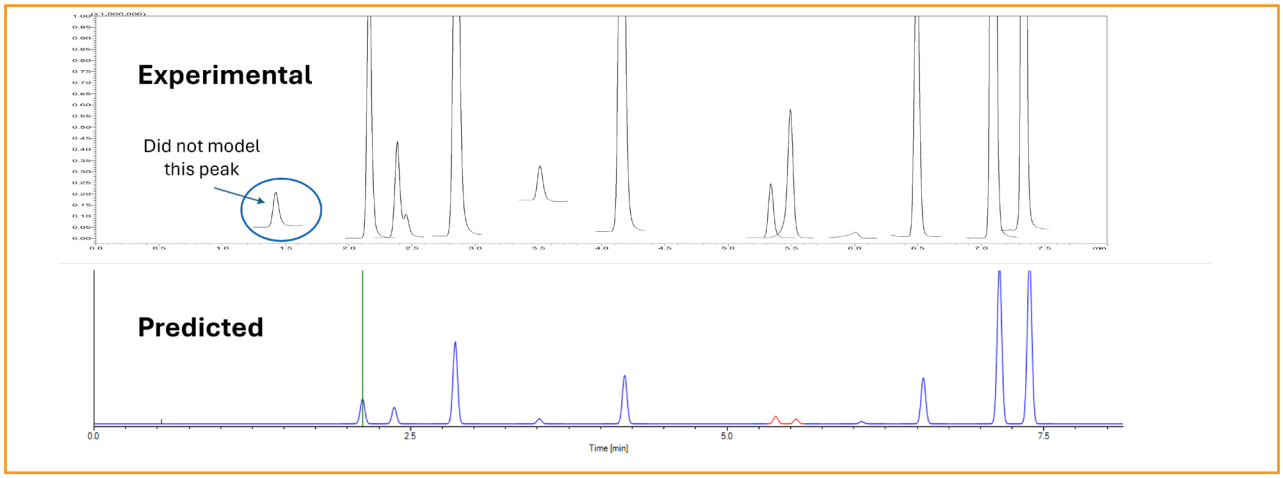


Figure 6. DryLab® Optimization: Experimental results compared to predicted results.

The optimized method using the HALO® Biphenyl column is shown in Figure 7. Compared to the original screening method, this method shows increased resolution between peaks 11 and 12. Table 2. lists the compounds and retention times.

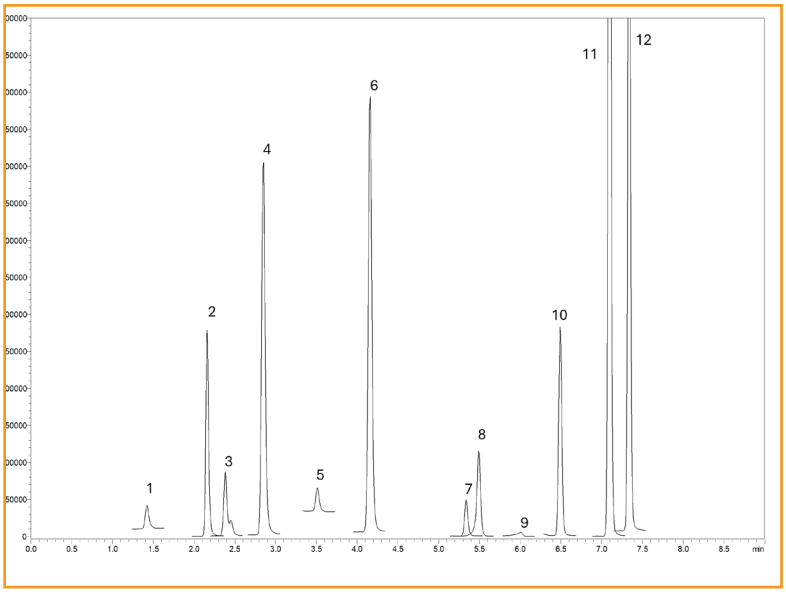


Figure 7. Optimized nitrosamines method using a HALO® Biphenyl column.

Peak #	Compound	Retention Time (min)
1	N-nitrosodimethylamine	1.42
2	N-nitrosomorpholine	2.16
3	N-nitrosomethylethylamine	2.38
4	N-nitrosopyrrolidine	2.85
5	N-nitrosodiethylamine	3.51
6	N-nitrosopiperidine	4.16
7	N-nitroso-n-propylamine	5.34
8	N-nitroso-N-methylaniline	5.49
9	N-nitroso-N-ethylaniline	6.01
10	N-nitrosodi-n-butylamine	6.49
11	N-nitrosodiphenylamine	7.10
12	N-nitrosodibenzylamine	7.34

Table 2. List of nitrosamines and retention times on the HALO® Biphenyl column.

In order to both increase the sensitivity and reduce the mobile phase consumption for the method, it was transferred to a 1.5 x 100 mm HALO® Biphenyl column. For more details on moving methods to 1.5 mm ID columns, please see the white paper Increasing Sensitivity while Reducing Solvent Consumption with HALO® 1.5 mm UHPLC Columns. The same method was run on both a competitor SPP Biphenyl 2.6 µm, 2.1 x 100 mm and a HALO® Biphenyl 2.7 µm, 1.5 x 100 mm column except the flow rate was adjusted to 0.2 mL/min. for the 1.5 mm ID to maintain the same linear velocity. MarvelXACT™ connectors were used to reduce extracolumn volume. A 75 µm x 350 mm connector was used from column outlet to ground while a 75 µm x 150 mm connector was used from ground to source. The comparative MS results are shown in Figure 8. On average, 34% larger peak heights were observed with the 1.5 mm ID HALO® Biphenyl column. On average, 14% narrower peak widths were observed with the 1.5 mm ID HALO® Biphenyl column. Half the solvent that would typically be needed for the 2.1 mm ID column was consumed by the 1.5 mm ID HALO® Biphenyl column.

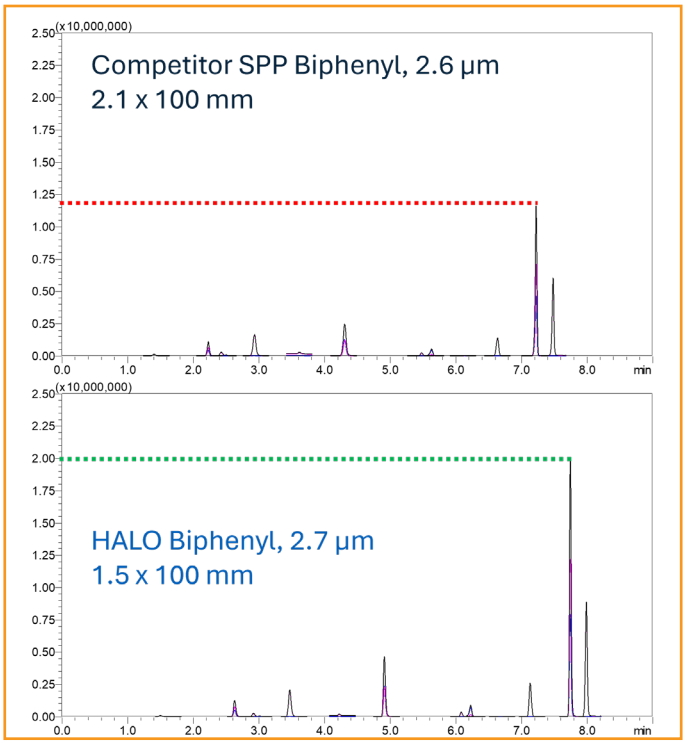


Figure 8. HALO® Biphenyl 1.5 mm ID column compared to a competitor 2.1 mm ID Biphenyl column.

CONCLUSION

Of the 7 HALO® stationary phases screened, the HALO® Biphenyl gave the best combination of retention time, peak shape, and resolution for the mix of 12 nitrosamines. The use of DryLab® enabled linear gradient conditions to be used without the need for a step gradient while improving the resolution of peaks 11 and 12. HALO® Biphenyl in 1.5 mm ID gives sharper peaks and increased peak height over 2.1 mm ID columns provided that the extra column volume has been minimized by reducing the post column volume by going to smaller ID, shorter length tubing. 1.5 mm ID columns offer 50% solvent savings over 2.1 mm ID columns for greener, more sustainable methods.

REFERENCES

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