MACHEREY-NAGEL
Chromatography application note

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Comparison of different aryl chemically bonded NUCLEODUR® phases for the determination of primary aromatic amines with HPLC-MS/MS

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Abstract

This application note compares different aryl chemically bonded NUCLEODUR® phases for the determination of primary aromatic amines by using HPLC-MS/MS.

Introduction

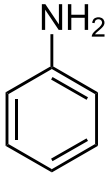
Primary aromatic amines (PAAs) are known as contaminants from azo dyes and colour pigments in commercial textile products, printed food contact materials, children´s toys and so on. Some primary aromatic amines are considered to be carcinogenic [1]. For this reason, specific regulations have been introduced in order to minimize their presence in materials used in textiles, leathers, children´s toys and printed food contact materials by the most important international authorities [2, 3]. For monitoring their occurrence leads to a demand for sensitive chromatographic methodologies.

An aromatic amine is an organic compound consisting of an aromatic ring attached to an amine. The simplest aromatic amine is aniline. It is a broad class of compounds that encompasses anilines, but also many more complex aromatic rings and many other substituents.

The separation of PAAs can be performed with different chromatographic interaction types due to their very diverse chemical structure elements. It is well known that π-π interactions with different aryl chemically bonded phases could be a useful retention mechanism for the separation of aromatic compounds like primary aromatic amines.

This application note deals with a column screening for the separation of PAAs on different aryl chemically bonded phases. In this note phenylhexyl, pentafluorophenylpropyl, biphenyl and sphinx RP, with bifunctional surface and additional π-π interactions, are compared. The influences on the retention mechanism due to the eluent system are shown for NUCLEODUR® π^2, that shows the highest π-π interaction and the chromatographic advantages are emphasized for that analyte group.

Compounds of interest



HPLC-MS / MS analysis

Chromatographic conditions

Column dimension:
EC 100/3 NUCLEODUR® X, 3 µm
(x = π^2, Phenyl-Hexyl, PFP, Sphinx RP)

Eluent A:
0.1 % formic acid in water

Eluent B:
0.1 % formic acid in methanol

Gradient:
from 5 % B to 95 % B in 10.0 min, hold for 4.0 min, back to 5 % B in 1.0 min, hold for 5.0 min

Flow rate:
0.56 mL/min

Temperature:
35 °C

Injection volume:
20 µL

MS conditions:

API 5500 (AB Sciex), ion source ESI, positive ionization mode, scan type SRM, detection window 90 s, curtain gas 35 psig, ion spray voltage 5500 V, temperature 450 °C, nebulizer gas 45 psig, turbo gas 45 psig, CAD medium

Figure 1: Simplest example for primary aromatic amines.

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Primary aromatic amines

Chromatograms

Comparison of different NUCLEODUR® aryl phases

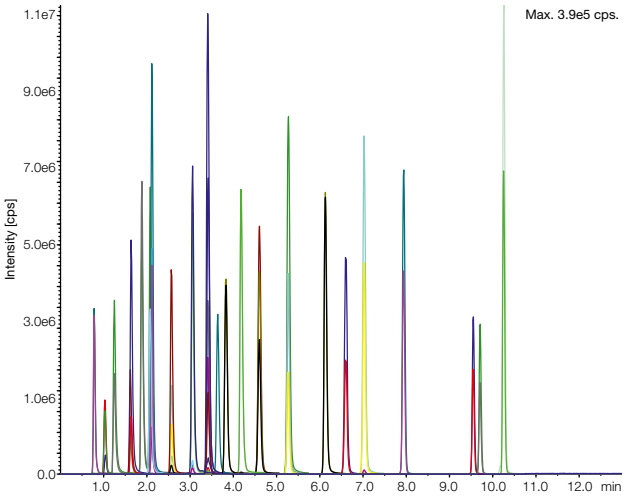

Figure 2: Separation of primary aromatic amines on NUCLEODUR® π^2 (β = 10 ng/mL in eluent A).

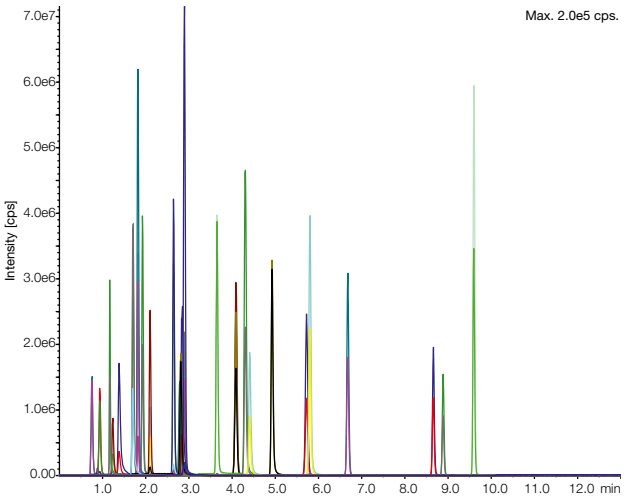

Figure 3: Separation of primary aromatic amines on NUCLEODUR® Phenyl-Hexyl (β = 10 ng/mL in eluent A).

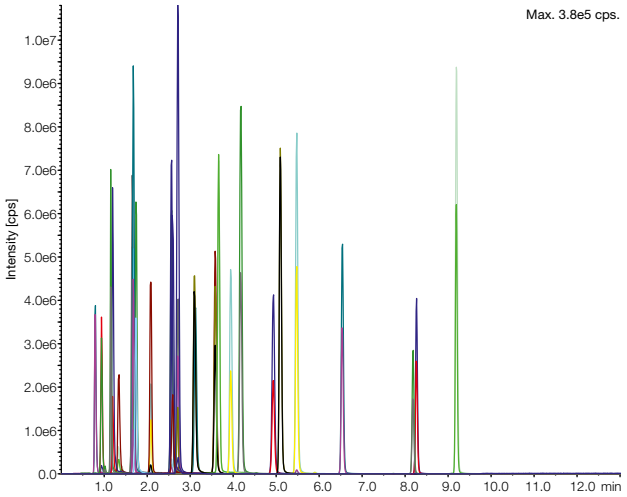

Figure 4: Separation of primary aromatic amines on NUCLEODUR® PFP (β = 10 ng/mL in eluent A).

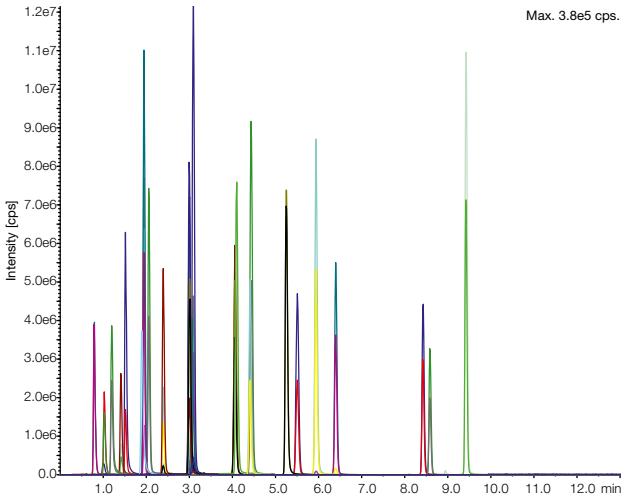

Figure 5: Separation of primary aromatic amines on NUCLEODUR® Sphinx RP (β = 10 ng/mL in eluent A).

SRM transitions

Analyte	CAS number	$[M+H]^+$	Q_1 (Quantifier)	Q_2 (Qualifier)
2,4-Diaminotoluene	95-80-7	123.1	106.0	77.1
4-Chloroaniline	106-47-8	128.1	93.0	111.0
4,4'-Diaminodiphenylmethane	101-77-9	199.1	106.1	77.1
4-Chloro-2-methylaniline	95-69-2	142.0	107.1	106.1
4,4'-Methylene-bis (2-methylaniline)	838-88-0	227.1	120.1	77.0
4,4'-Oxydianiline	101-80-4	201.1	108.1	80.1
Benzidine	92-87-5	184.0	156.0	167.1
4-Aminobiphenyl	92-67-1	170.1	152.1	153.0
o-Anisidine	90-04-0	124.1	109.1	80.1
4-Aminoazobenzene	60-09-3	198.1	77.1	93.1
4,4'-Thiodianiline	139-65-1	217.1	124.1	80.0
o-Toluidine	95-53-4	108.1	91.1	93.1
o-Aminoazotoluene	97-56-3	226.1	91.1	121.1
o-Tolidine	119-93-7	213.1	198.0	181.0
2-Naphthylamine	91-59-8	144.1	127.1	77.0
4,4'-Methylene-bis (2-chloroaniline)	101-14-4	267.0	230.0	140.1
5-Nitro-*o*-toluidine	99-55-8	153.0	107.0	90.0
2,4-Dimethylaniline	95-68-1	122.1	107.1	77.0
o-Dianisidine	119-90-4	245.1	230.0	186.0
2-Methoxy-5-methylaniline	120-71-8	138.1	123.1	106.0
2,6-Dimethylaniline	87-62-7	122.1	107.1	77.0
3,3'-Dichlorobenzidine	91-94-1	254.0	218.0	121.0
2,4-Diaminoanisole	615-05-4	139.1	124.1	107.1
2,4,5-Trimethylaniline	137-17-7	136.2	121.2	91.0
1,4-Phenylenediamine	106-50-3	109.1	92.1	65.1
1,3-Phenylenediamine	108-45-2	109.1	92.1	65.1
4-Aminophenol	123-30-8	110.1	65.0	93.1
2,5-Diaminotoluene	95-70-5	123.1	106.0	77.1
2-Methoxy-4-nitroaniline	97-52-9	169.1	152.1	122.1
2-Amino-6-ethoxynaphthalene	293733-21-8	188.1	156.0	167.1
Aniline	62-53-3	94.1	77.0	51.0

Table 1: SRM transitions for primary aromatic amines.

Primary aromatic amines

Influence on the retention mechanism for NUCLEODUR® π²

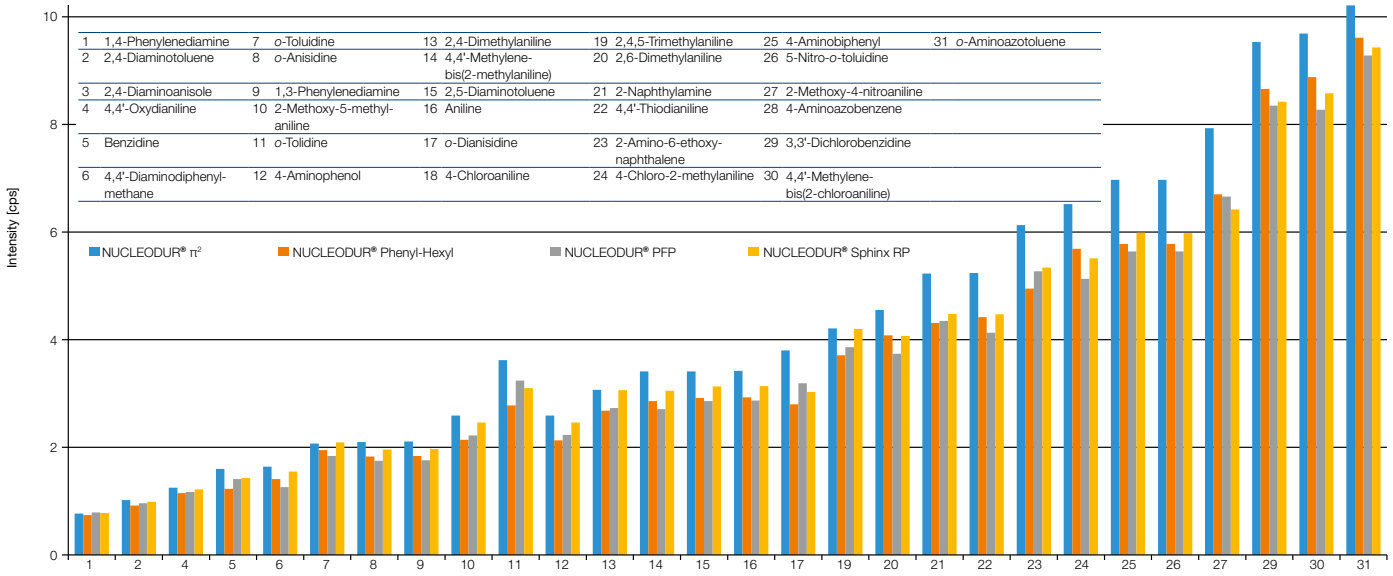

Figure 6: Comparison of retention times for tested aryl phases.

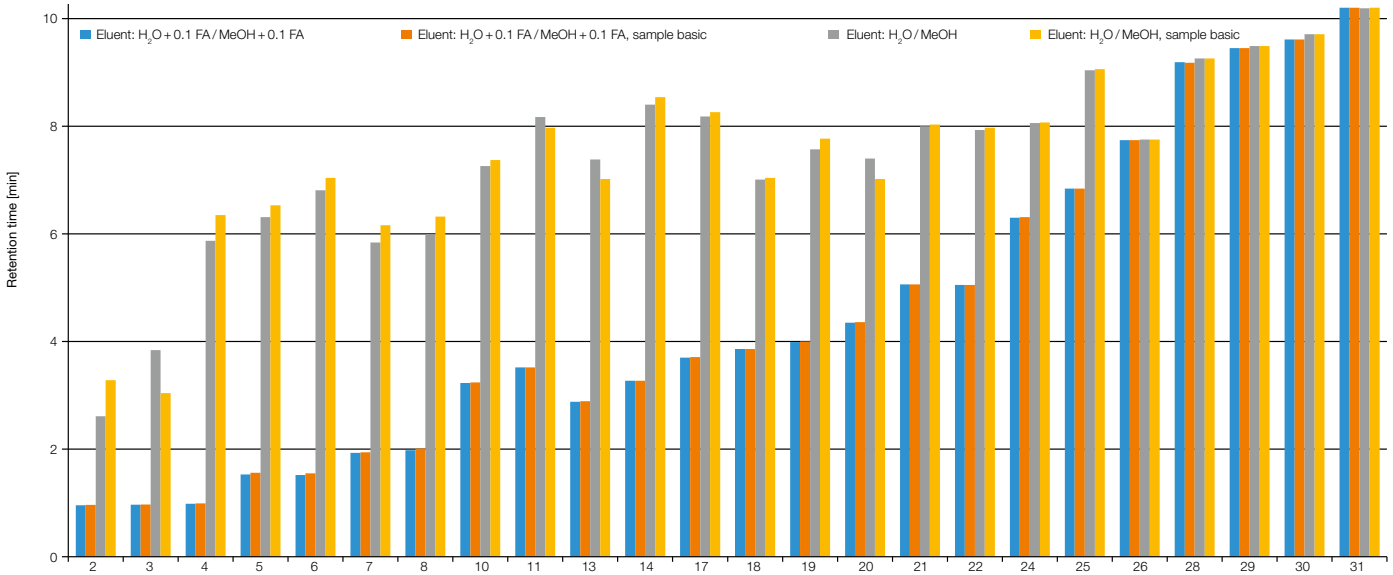

Figure 7: Influence on retention time due to methanol as eluent, eluent additive and pH of sample solution for NUCLEODUR® π².

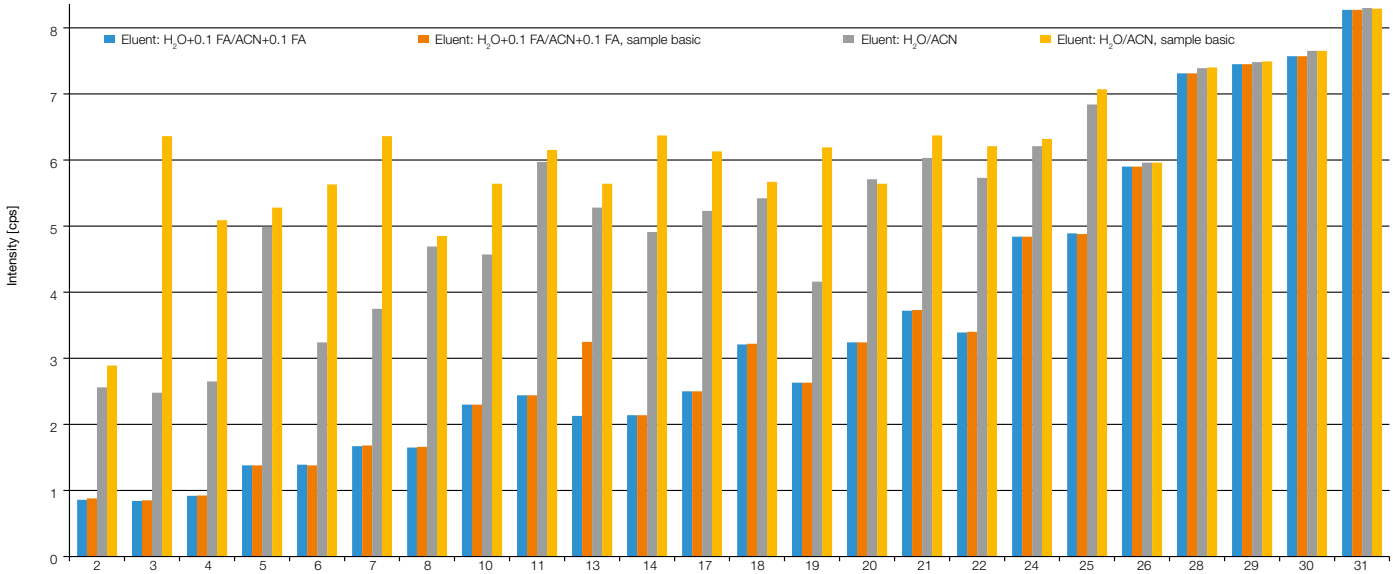

Figure 8: Influence on retention time due to acetonitrile as eluent, eluent additive and pH of sample solution for NUCLEODUR® π².

Primary aromatic amines

Conclusion

The results show that the determination of PAAs could be carried out successfully with all the tested aryl phases. Figure 6 shows that the NUCLEODUR® π^2 shows the highest retention for PAAs due to strong π-π interaction. This retention mechanism can be reinforced by chromatographic conditions.

By using methanol and water for eluents in a binary gradient system the maximum of interaction could be achieved with polar analytes like 2,4-Diaminotoluene and 2,4-Diaminoanisole. Working without typical additive formic acid to enhance electrospray ionization in positive ion mode leads to improved retention behavior. If acetonitrile is preferred as eluent it is useful to add base to the sample solution. A high pH value in the sample solution has a significant influence on π-π interactions.

In summary the presented application describes a chromatographic separation for the determination of primary aromatic amines on different aryl phases with mass spectrometry detection.

References

[1] Romualdo Benigni (2002), Carcinogenicity of the aromatic amines: from structure–activity relationships to mechanisms of action and risk assessment. Mutation Research 511 (2002) 191–206.

[2] COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

[3] Updated BfR Opinion No. 041/2012, 6 July 2012.

Additional information

The following applications regarding “Comparison of different aryl chemically bonded NUCLEODUR® phases for the determination of primary aromatic amines with HPLC-MS/MS” and further applications can be found on our online application database at *www.mn-net.com/apps*

HPLC:	MN Appl. No. 128700
	MN Appl. No. 128710

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760636.30, EC 100/3 NUCLEODUR® π^2, 3 µm

REF 760576.30, EC 100/3 NUCLEODUR® Phenyl-Hexyl, 3 µm

REF 760446.30, EC 100/3 NUCLEODUR® PFP, 3 µm

REF 760812.30, EC 100/3 NUCLEODUR® Sphinx RP, 3 µm