Resolving Potentially Harmful Azo-Colorant Amines Using the Distinct Selectivities of the Agilent ZORBAX Eclipse Plus Phenyl-Hexyl and StableBond Phenyl Columns

Application

Consumer Products

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Abstract

Nine aromatic amines, which can originate from azo colorants, were separated using the Agilent ZORBAX Eclipse Plus Phenyl-Hexyl column with an MS compatible mobile phase gradient. An Agilent ZORBAX StableBond Phenyl column was then substituted to provide significantly different selectivity to be used as a secondary LC method for quantification. The two phenyl phases have distinct chemical differences that made them a successful combination for separations of amines that contain phenyl groups.

Introduction

Many colorful consumer goods you use every day contain azo colorants (dyes and pigments). These are widely used in textiles, leather, inks, and plastics, and are often used in cosmetics and foodstuffs. Some azo colorants can break down to form amines that are known or suspected carcinogens. The general azo reduction reaction is:

Since consumer goods are likely to contact the skin or mouth, the azo colorants that could be reduced to toxic amines have been banned from consumer goods in many countries. Table 1 lists toxic aromatic amines that can originate from certain azo colorants; those in bold are analyzed in this work.

Table 1. Aromatic Amines That Must Not Be Found in Consumer Products According to the EU Directive 2002/61/EC [1]

Name	CAS Number
Benzidine	**92-87-5**
Biphenyl-4-amine	92-67-1 2
2-naphthylamine	**91-59-8**
4-chloro-o-toluidine	95-69-2
2, 2'-dichloro-4, 4'-methylenedianiline	101-14-4
4-chloroaniline	**106-47-8**
3, 3'-dichlorobenzidine	**91-94-1**
3, 3'-dimethoxybenzidine	**119-90-4**
3, 3'-dimethylbenzidine	**119-93-7**
4, 4'-methylenedianiline	101-77-9
4, 4'-methylenedi-o-toluidine	838-88-0
4-methyl-m-phenylenediamine	95-80-7
2-methoxyaniline	**90-04-0**
4-methoxy-m-phenylenediainine	615-05-4
6-methoxy-m-toluidine	120-71-8
4,4'-oxydianiline	101-80-4
4, 4'-thiodianiline	139-65-1
4-o-tolyazo-o-toluidine	97-56-3
o-toluidine	**95-53-4**
5-nitro-o-toluidine	99-55-8
2,4,5-trimethylaniline	137-17-7

Phenyl-type stationary phases coupled with methanolic mobile phases can have significantly different selectivity for aromatic amines than aliphatic stationary phases, such as C8 and C18 [2]. Phenyl phases can be used for analytes containing phenyl structures and, therefore, are a good first choice for method development for aro-

matic amines. The nine amines separated include aniline and eight toxic amines that can be formed from reduction of particular azo dyes. Figure 1 shows their structures and elution order on a ZORBAX Eclipse Phenyl-Hexyl column under the conditions described in the Experimental section.

Experimental

HPLC analysis was performed with the Agilent 1200 Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL with mobile phase A: 10 mM ammonium acetate in water, pH 4.7 with acetic acid; B: methanol. Flow rate was 1 mL/min. The gradient was 25% B initial composition, ramping to 90% B over nine minutes.
- G1376C automatic liquid sampler (ALS) SL. Injection volume was 2.0 µL.
- G1316B Thermally Controlled Column Compartment SL. Temperature was 25 °C.
- G1315C Diode Array Detector (DAD). Wavelength used was 220, 4 nm Ref=off, with a G1315-60024 micro flow cell (3 mm path, 2 µL volume).

ZORBAX Columns:

- ZORBAX Eclipse Plus Pheny-Hexyl 4.6 mm × 100 mm, 5 µ
- ZORBAX StableBond Phenyl 4.6 mm × 100 mm, 5 µ

Vials:	Amber screw cap (Agilent p/n 5182-0716)
Vial caps:	Blue screw cap (Agilent p/n 5282-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-1270)

Individual aromatic amines were obtained from Sigma-Aldrich and dissolved in MeOH to a concentration of about 1 mg/mL. A composite sample was then made by combining 100-µL aliquots of each individual amine solution and then diluting the mixture in water 1:10.

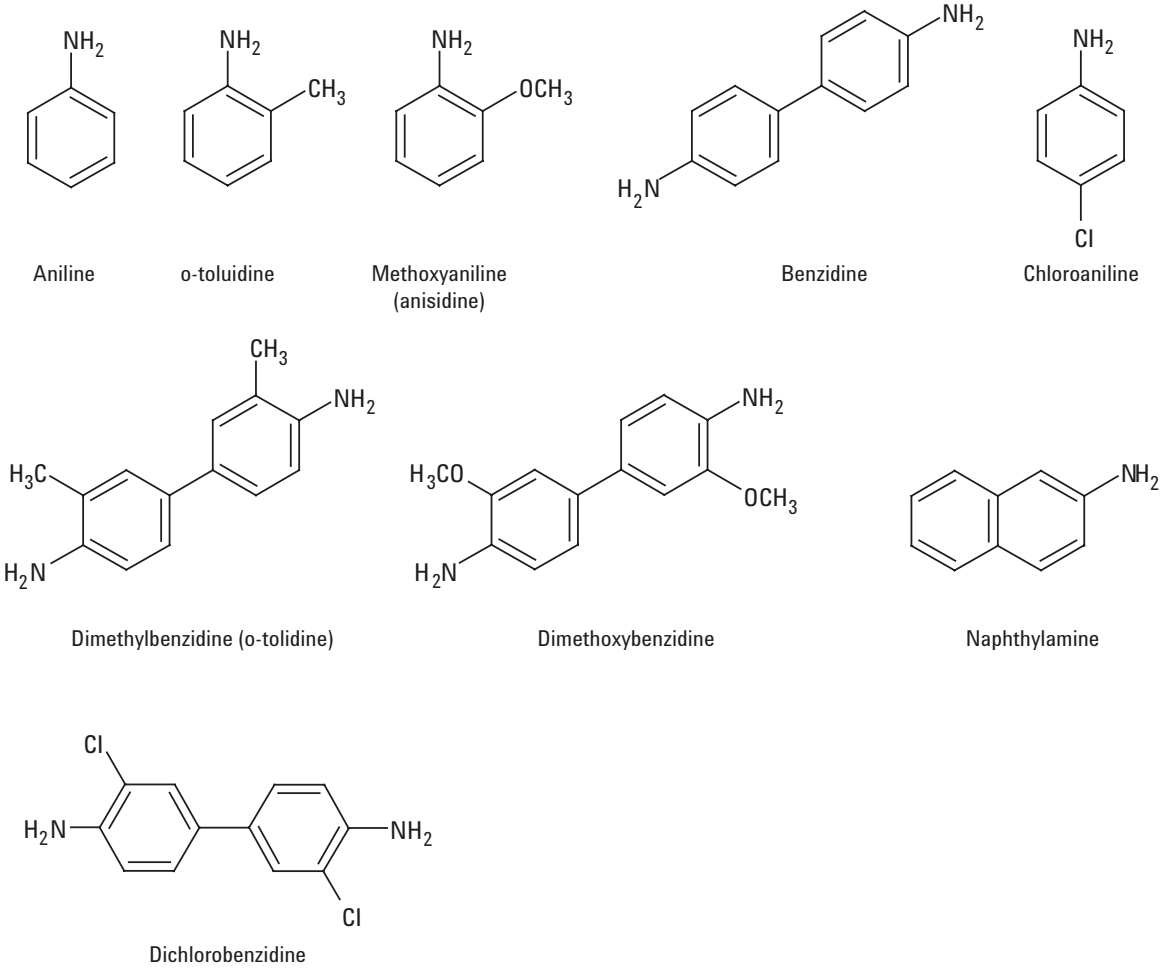


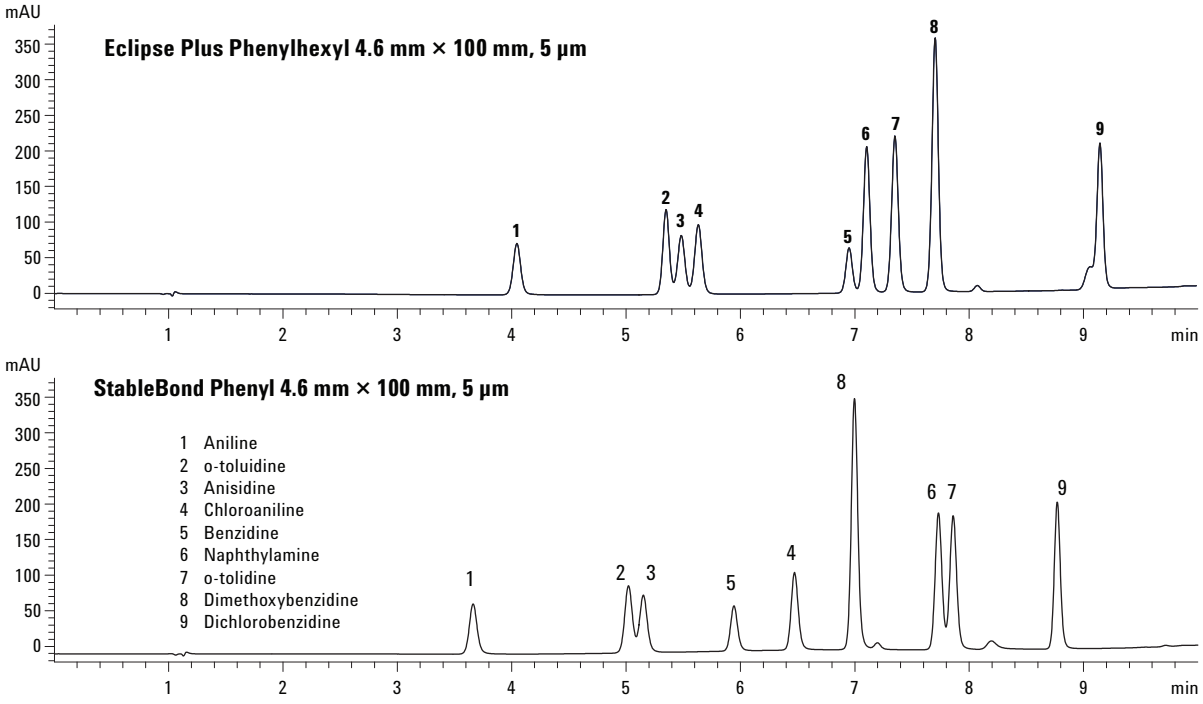
Figure 1. Structures of aromatic amines.

Results and Discussion

The variety of aromatic amines suggests a mobile-phase gradient would be best to separate them in a reasonable amount of time. We chose methanol as the organic part of the mobile phase to take advantage of π-π interactions between the phenyl groups of the analytes and the stationary phenyl phase. The π-π interactions are more pronounced in methanol than acetonitrile [2, 3]. Ammonium acetate buffered at pH 4.7 was used to increase retention of the amines compared to a 0.1% formic acid (pH 2) mobile phase. Reversed-phase analysis of amines commonly uses pH > 2 because the ammonium ionic form (NH_4^+) present at low pH is highly water soluble, thus poorly retained; at pH 5 the protonated NH_3 form is present and better interacts with the stationary phase. Poor peak shape from using a mobile phase near the pKa of analytes was not noticeable, possibly due to the gradient focusing the analyte bands. The buffer is also volatile, making it suitable for MS detectors.

Figure 2 is a chromatographic overlay of the azo dye amines separated on a ZORBAX Eclipse Plus Phenyl-Hexyl column and a ZORBAX StableBond Phenyl column. The methods are identical except for the different columns (stationary phases).

Comparing the chromatograms, all the analytes have different retention ($\overline{k}$) factors, thus different selectivity (α) factors and different resolution. Some analytes even elute in a different order, such as benzidine and chloroaniline, or dimethoxybenzidine and naphthylamine. The selectivity differences between the columns make ZORBAX Eclipse Plus Phenyl-Hexyl and ZORBAX StableBond Phenyl a good pair of columns to analyze banned azo-dye amines since two chromatographic methods are usually needed to confirm identification in many regulatory methods. The identical mobile phase and other method parameters allow a single automated procedure to run both methods sequentially on a single Agilent 1200 LC with a column switching valve option installed in the column compartment.



Mobile phase:	A: 10 mM NH_4 Acetate, pH 4.7	Injection volume:	2.0 µL
	B: MeOH	Temperature:	25 °C
Flow rate:	1 mL/min	Detection:	UV 220 nm
Gradient:	Time %B		
	0 25		
	9 90		

Figure 2. Aromatic amines on different Agilent ZORBAX phenyl columns show significant selectivity differences.

Table 2. Chemical Differences Between Two Agilent ZORBAX Phenyl Columns May Impart Large Selectivity Differences

	Agilent ZORBAX Eclipse Plus Phenyl-Hexyl	Agilent ZORBAX StableBond Phenyl
Endcap	Yes	No, but steric diisobutyl groups at silane bond
Phenyl linkage	Hexyl	Propyl
% carbon load	9%	6%
Silica base	Improved Type B (ZORBAX)	Type B (ZORBAX)

The different selectivity of the two phenyl phases can be explained by two factors, (1) hydrocarbon content and (2) endcapping, and is summarized in Table 2. The hexyl and propyl linkage may impart some selectivity differences between the two phases. The non-endcapping of the SB phase, coupled with its sterically bulky diisopropyl groups, would also impart a selectivity difference compared to the smaller dimethyl groups on the phenylhexyl silane and its densely endcapped silica. The net effect is significantly different columns.

Conclusions

The ZORBAX Eclipse Phenyl-Hexyl and ZORBAX StableBond Phenyl columns are good choices to separate analytes containing phenyl-type structures such as aromatic amines. ZORBAX Eclipse Plus Phenyl-Hexyl's specially designed column characteristics, such as the hexyl linkage and exhaustive silanol endcapping process, result in a column with different selectivity than ZORBAX StableBond-Phenyl columns. These unique stationary phases, coupled with methanolic mobile phase selectivity, enhance the chemical interactions of analyte phenyl groups with the stationary phase.

References

1. Opinion on: Report (Final Draft) on "Assessment of the risks to human health posed by azo colorants in toys, writing inks and paper products, and analysis of the advantages and drawbacks of restrictions on their marketing and use." Opinion expressed at the 24th CSTEE plenary meeting, Brussels, 12 June 2001 (http://ec.europa.eu/health/ph_risk/committees/sct/docshtml/sct_out109_en.htm)
2. M. Yang, et al, "Impact of Methanol and Acetonitrile on Separations Based on π-π Interactions with Reversed Pphase Phenyl Column," *J. of Chromatography*, 1097, 124–129, 2005
3. W. Long and J. Henderson, "Unique Selectivity and High Throughput Applications of SB-Phenyl RRHT," Agilent Technologies publication 5989-6067EN, 2007

Appendix

Available Agilent ZORBAX Phenyl-Hexyl Columns

959990-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 4.6 mm × 250 mm
959993-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 4.6 mm × 150 mm
959963-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 4.6 mm × 150 mm
959996-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 4.6 × 100 mm
959961-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 4.6 mm × 100 mm
959964-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 4.6 mm × 100 mm
959933-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 4.6 mm × 75 mm
959946-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 4.6 mm × 50 mm
959943-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 4.6 mm × 50 mm
959941-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 4.6 mm × 50 mm
959936-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 4.6 mm × 30 mm
959931-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 4.6 mm × 30 mm
959993-312	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 3.0 × 150 mm
959963-312	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 3.0 mm × 150 mm
959961-312	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 3.0 mm × 100 mm
959964-312	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 3.0 mm × 100 mm
959941-312	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 3.0 mm × 50 mm
959701-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 2.1 mm × 150 mm
959763-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 2.1 mm × 150 mm
959793-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 2.1 mm × 100 mm
959764-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 2.1 mm × 100 mm
959746-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm, 2.1 mm × 50 mm
959743-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 2.1 mm × 50 mm
959741-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 2.1 mm × 50 mm
959733-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 3.5 µm, 2.1 mm × 30 mm
959731-912	ZORBAX Eclipse Plus Phenyl-Hexyl, 1.8 µm, 2.1 mm × 30 mm
820950-938	ZORBAX Eclipse Plus Phenyl-Hexyl Guard, 5 µm, 4.6 mm × 12.5 mm (4 pk)
821125-938	ZORBAX Eclipse Plus Phenyl-Hexyl Guard, 5 µm, 2.1 mm x 12. 5 mm (4 pk)

Available Agilent ZORBAX SB-Phenyl Columns

877250-112	ZORBAX PrepHT SB-Phenyl, 21.2 mm × 250 mm, 7 µ cartridge
861954-312	ZORBAX SB-Phenyl 3.5 µm, 3.0 mm × 100 mm
863954-312	ZORBAX SB-Phenyl 3.5 µm, 3.0 mm × 150 mm
835975-912	ZORBAX SB-Phenyl 3.5 µm, 4.6 mm × 50 mm
866953-912	ZORBAX SB-Phenyl 3.5 µm, 4.6 mm × 75 mm
863953-912	ZORBAX SB-Phenyl 3.5 µm, 4.6 mm × 150 mm
860975-912	ZORBAX SB-Phenyl 5 µm, 2.1 mm × 50 mm
883700-912	ZORBAX SB-Phenyl 5 µm, 2.1 mm × 150 mm
883975-312	ZORBAX SB-Phenyl 5 µm, 3.0 mm × 150 mm
880975-312	ZORBAX SB-Phenyl 5 µm, 3.0 mm × 250 mm
820975-912	ZORBAX SB-Phenyl 5 µm, 4.0 mm × 80 mm (ZGC)
883975-912	ZORBAX SB-Phenyl 5 µm, 4.6 mm × 150 mm
880975-912	ZORBAX SB-Phenyl 5 µm, 4.6 mm × 250 mm
880975-212	ZORBAX SB-Phenyl 5 µm, 9.4 mm × 250 mm
820950-917	ZORBAX SB-Phenyl Guard 5 µm, 4.6 mm × 12.5 mm 4/pk (ZGC)
861753-912	ZORBAX SB-Phenyl Narrow Bore RR 3.5 µm, 2.1 mm × 100 mm
861953-912	ZORBAX SB-Phenyl RR, 4.6 mm × 100 mm, 3.5 µm

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