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ACQUITY UPLC Analysis of Banned Carcinogenic Aromatic Amines

Peter J. Lee, Alice J. Di Gioia

Waters Corporation

Abstract

This application note describes the separation and identification of 19 banned aromatic

amines using the Waters ACQUITY UPLC/PDA System with Waters Empower Software and PDA library matching. The enchanced resolution, sensitivity and speed of ACQUITY UPLC allowed each of the two analyses to be completed in 10 minutes; this is up to 7.5 times faster than RP-HPLC. The ability to quickly and unambiguously analyze the carcinogenic aromatic amines can improve and accelerate the quality control of raw materials and products. The superior testing method may help manufacturers avoid product recalls and liability litigations while protecting the health of worker and public alike.

Introduction

Aromatic amines are widely used as raw materials for manufacturing chemicals such as dyestuffs, polymers, surfactants, drugs, pesticides, and corrosion inhibitors.[1-3] Although many aromatic amines are potential carcinogenic agents (1 to 19 in Figure 1),[4-6] exposure to arylamines such as benzidine (2), 2-naphthylamine (11), and 4-aminodiphenyl (16) has been shown to cause bladder cancer.[4] The suspect carcinogenic amines may be found in azo dyes[7-9] or oxidation dyes[4,10] used in consumer goods, such as, textiles, leather, foodstuffs, cosmetics, and hair dyes. The potential risks of aromatic amines to consumer health has resulted in US FDA regulations on aromatic amines in azo food dyes (21 CFR 74.705 and 21 CFR 74.706) and the European Union (EU) Directive (2002/61/EC) restricting certain azo dyes.[1,4] The EU directive bans the use of azo dyes in textile and leather articles which, on reduction, might form any of 22 listed amines.[5,6,11] The directive requires that at least two different chromatographic methods are utilized for the analysis. Although interest in the development of reliable, sensitive and rapid analytical methods for the determination of carcinogenic aromatic amines has increased, the polar nature of the arylamines makes the compounds difficult to analyze and quantify by GC. LC is a more suitable technique, and recently several RP-HPLC methods with 50- to 75-minute run times for the analysis of banned aromatic amines in textile and leather products have been reported.[1,5,9] This application note describes the separation and identification of 19 banned aromatic amines using the Waters ACQUITY UPLC/PDA System with Waters Empower Software and PDA library matching. The enchanced resolution, sensitivity and speed of ACQUITY UPLC allowed each of the two analyses to be completed in 10 minutes; this is up to 7.5 times faster than RP-HPLC. The ability to quickly and unambiguously analyze the carcinogenic aromatic amines can improve and accelerate the quality control of raw materials and products. The superior testing method may help manufacturers avoid product recalls and liability litigations while protecting the health of worker and public alike.

Experimental

Sample Preparation

Aromatic Amines: 4-methyl-m-phenylenediamine [95-80-7], 1; benzidine [92-87-5], 2; o-anisidine [90-04-0], 3; otoluidine [95-53-4], 4; 4,4’ -oxydianiline [101-80-4], 5; 4- chloroaniline [106-47-8], 6; 6-methoxy-m-toluidine [120-71- 8], 7; 4,4’ -methylenedianiline [101-77-9], 8; 3,3’ - dimethoxybenzidine [119-90-4], 9; 3,3’ -dimethylbenzidine [119-93-7], 10; 2-naphthylamine [91-59-8], 11; 4,4’ - thiodianiline [139-65-1], 12; 4-chloro-0-toluidine [95-69-2], 13; 2,4,5-trimethylaniline [137-17-7], 14; 4,4’ -methylenedio- toluidine [838-88-0], 15; 4-aminobiphenyl [92-67-1], 16; 3,3’ -dichlorobenzidine [91-94-1], 17; 2,2’ -dichloro-4,4’ -methylene-dianiline [101-14-4], 18; and o-aminoazotoluene [97-56-3], 19; were purchased from Sigma-Aldrich. The 19 aromatic amines were dissolved in MeOH to make a stock solution with 100 µg/mL of each amine. The working solution (15 µg/mL) was prepared by mixing 150 µL of the stock solution with 150 µL MeOH and 700 µL D.I. H_2O.

System and Operation Conditions

System:	ACQUITY UPLC/PDA
Software:	Empower Software
Weak wash:	95:5 Water: CH_3CN (500 µL)
Strong wash:	50:50 Water: CH_3CN (300 µL)
Seal wash:	90:10 Water: CH_3CN (5 min)
Column temp.:	50 °C
Flow rate:	0.5 mL/min
Injection:	2.5 µL
Detection:	PDA 215 to 500 nm
Sampling rate:	10 pts/s
Filter response:	0.1 s

C_8 Column:	ACQUITY UPLC BEH C_8 2.1 x 100 mm
Mobile phase A:	[0.575g, $(NH_4)H_2PO_4$; 0.7g, Na_2HPO_4; 1000 g, H_2O; pH 6.9] + 79.1g MeOH
Mobile phase B:	MeOH
Linear Gradient:	5% B to 65% B in 10 min
C_{18} Column:	ACQUITY UPLC BEH C_{18} 2.1 x 100 mm
Mobile phase A:	10 mM NH_4OAc in 95/5 v% of H_2O/CH_3CN
Mobile phase B:	CH_3CN
Linear Gradient:	5% B to 60% B in 10 min

Results and Discussion

The EU Directive 2002/61/EC defines a limit value of 30 mg/kg of sample material since the derivation of the amines in very small amounts may lead to false positive results.5 The amines must be identified by at least two different chromatographic methods to avoid possible misinterpretation caused by an interfering substance. The goal of this study is to illustrate the rapid, sensitive separation and identification of carcinogenic aromatic amines in a complex mixture utilizing the ACQUITY UPLC System. Two different UPLC BEH reversed-phase columns (C_{18} and C_8) were used. A mixture of 19 banned aromatic amines (Figure 1) with a concentration of 15 µg/mL of each amine was prepared in 30% MeOH.[11] Figure 2 shows the PDA chromatogram extracted at 240 nm of a 10-minute separation using a ACQUITY UPLC 2.1 x 100 mm BEH C_8 Column. Despite similar chemical structures, the compounds are well-resolved by a simple linear gradient method (5%B to 65%B) where mobile phase A is ammonium phosphate and mobile phase B is MeOH. The separation is seven times faster and consumes 14 times less solvent than a similar analysis reported with conventional RP-HPLC.[5] The resolution and superior peak shape also provide increased sensitivity and more accurate quantitation. These results demonstrate the utility of a UPLC total solution for the analysis of aromatic amines.

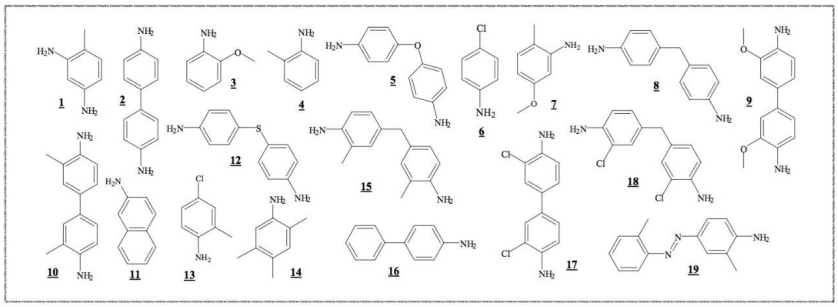

Figure 1. Chemical structure of carcinogenic aromatic amines.

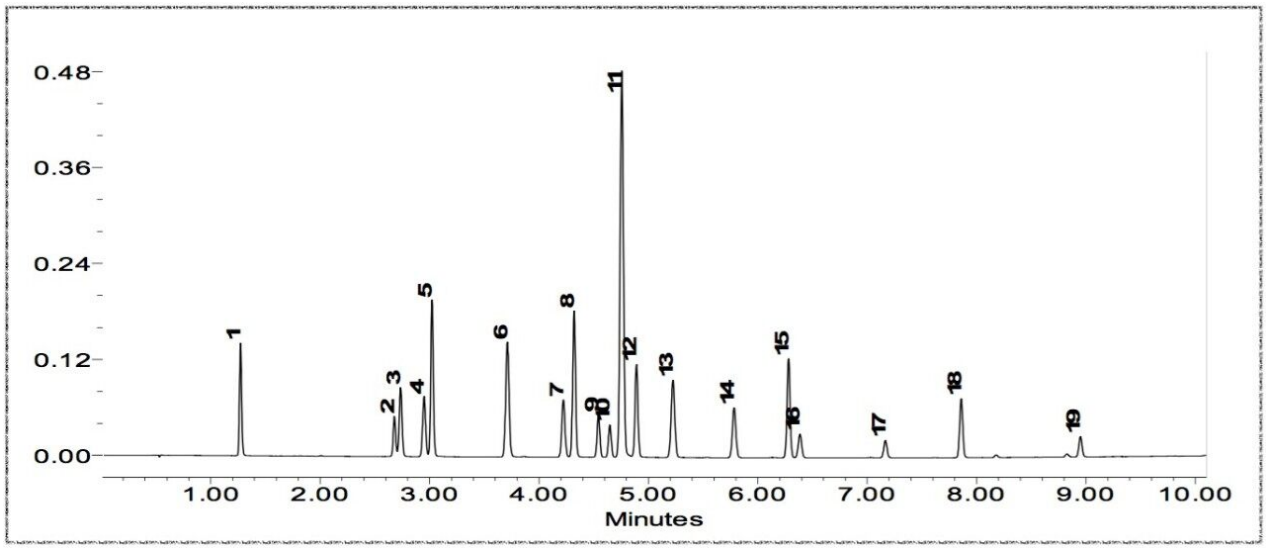

Figure 2. PDA extracted chromatogram (240 nm) of 19 aromatic amines by UPLC BEH C_8 2.1 x 100 mm Column.

Photodiode array (PDA) is a powerful detector for chromatographic analyses[12,13] and can provide UV spectra of analytes for component identification. Empower Software allows plotting PDA timed wavelength chromatograms using the λmax of each analyte for increasing the detection limit and improving quantification when the analyte concentration is low. UV libraries for library matching and peak purity for compound confirmation can be readily created. Figure 3 shows the UV spectra of several amines extracted from the PDA data. The difference in UV spectra of the 19 aromatic amines is sufficient for compound identification.

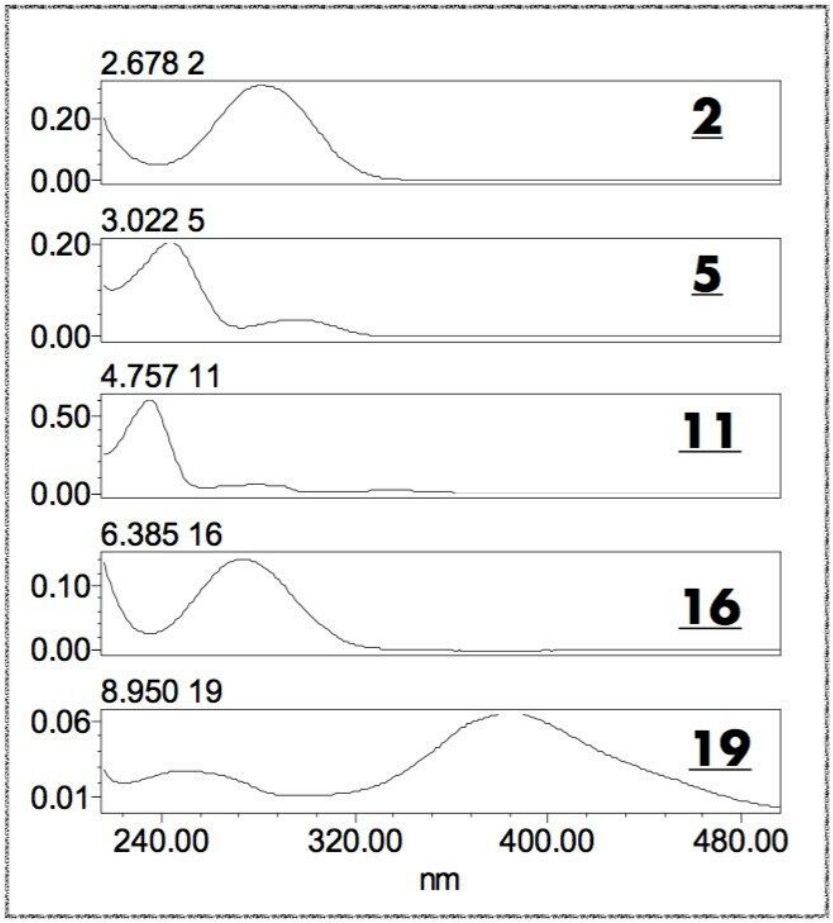

Figure 3. UV spectra of 2, 5, 11, 16, and 19 extracted from PDA data.

Table 1 lists the PDA library matching results of the separation of the mixture of 19 amine standards with the C_8 column. The values of parameters (match angle and threshold, purity angle and threshold) indicate that the amines 1–19 were well-separated and matched with PDA library of banned aromatic amines.[14] The similarity of the UV spectra for compounds 2 and 16 (Figure 3) did not impede the positive matching results in Table 1. With Empower, a PDA library of banned amines with UV spectra and retention times can be easily built for operator unattended, automatic PDA matching and rapid product screening.

	Name	RT	Match1 Spect. Name	Match1 Angle	Match1 Threshold	Purity 1 Angle	Purity 1 Threshold
1	1	1.271	4-methyl-m-phenylenediamine	0.112	1.195	0.202	0.501
2	2	2.678	Benzidine	0.099	1.185	0.257	0.434
3	3	2.735	o-anisidine	0.452	1.626	4.027	0.787
4	4	2.950	o-toluidine	0.247	1.567	0.395	0.748
5	5	3.022	4,4-oxydianiline	0.148	1.306	0.329	0.543
6	6	3.711	4-chloroaniline	0.225	1.568	0.408	0.749
7	7	4.223	6-methoxy-m-toluidine	0.292	1.667	0.528	0.889
8	8	4.321	4,4-methylenedianiline	0.170	1.364	0.285	0.593
9	9	4.544	3,3-dimethoxybenzidine	0.136	1.280	0.207	0.486
10	10	4.648	3,3-dimethylbenzidine	0.160	1.331	0.233	0.504
11	11	4.757	2-naphthylamine	0.085	1.132	0.143	0.366
12	12	4.892	4,4-thiodianiline	0.183	1.334	0.330	0.598
13	13	5.225	4-chloro-o-toluidine	0.270	1.706	0.577	0.975
14	14	5.783	2,4,5-trimethylaniline	0.337	1.844	0.555	0.965
15	15	6.281	4,4-methylenedi-o-toluidine	0.226	1.513	0.425	0.780
16	16	6.385	4-amionbiphenyl	0.178	1.440	0.415	0.730
17	17	7.166	3,3-dichlorobenzidine	0.201	1.333	0.422	0.668
18	18	7.859	2,2-diCl-4,4-methylene-dianili	0.290	1.580	0.597	1.004
19	19	8.950	o-aminoazotoluene	0.209	1.426	0.385	0.612

Table 1. PDA library matching results of C_8 column separations.

To demonstrate the simplicity and flexibility of the UPLC system for aromatic amine analysis, a 2.1 x 100 mm C_{18} Column and a solvent system (mobile phase A, 10 mM NH_4OAc in 5% CH_3CN; mobile phase B, CH_3CN) more suitable for mass spectrometry were used. Figure 4 shows the 10-minute separation chromatogram using a simple linear gradient method (5% B to 60% B). Again, the mixture of carcinogenic aromatic amines was well separated.

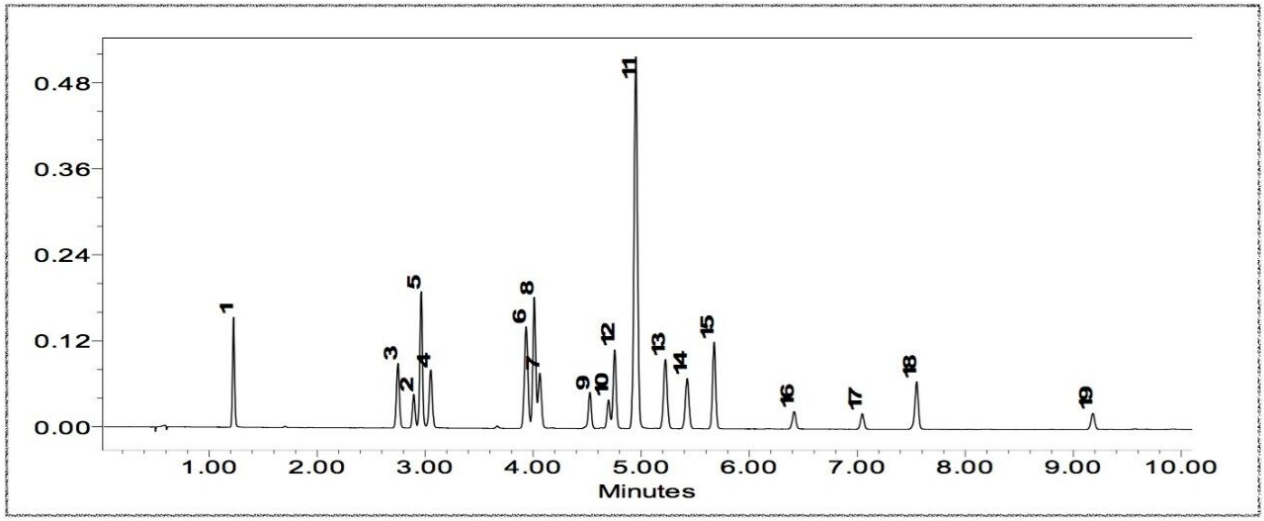

Figure 4. PDA extracted chromatogram (240 nm) of 19 aromatic amines by ACQUITY UPLC BEH C_{18} 2.1 x 100 mm Column.

The data also illustrates that the retentivity and selectivity of aromatic amines is noticeably different between C_8 and C_{18} columns. The availability of two column chemistries is extremely useful in LC method development. The separation using the mass spectrometer friendly solvent (NH_4OAc/ CH_3CN) suggests the extension of this work to UPLC with a single or tandem quad MS detector should provide fast and unambiguous identification of banned aromatic amines in complex mixtures.

Conclusion

The ACQUITY UPLC provides a sensitive, baseline resolved separation of 19 banned aromatic amines in 10 minutes. This is seven times faster and consumes 14 times less solvent than HPLC systems. Empower PDA features further reduce analysis time by automatically identifying the amines. Potential applications of this method include the identification and quantification of aromatic amines in azo dyes, textiles, leather, polymer additives, food dyes, cosmetics and hair dyes.

References

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11. Amines 1 to 19 and the following three amines are in the EU list of 22 banned aromatic amines: 5-nitro-o-toluidine [99-55-8],20; 4-aminoazobenzene [60-09-3], 21; and 4-methoxy-m-phenylenediamine [615-05-4], 22. Under the sample preparation condition of EU methods (EN 14362-1 & 2), amines 19 and 20 are further reduced to 1 and 4; and 21 is reduced to aniline and 1,4-phenylenediamine. Amine 22 was not in the test mixture because it was not available from several major vendors for common chemical reagents in USA.

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13. Peak Identification with Waters 996 Photodiode Detector (PDA): Library Matching, Waters Corporation, Waters Performance PerSPECtives, WPP12, Library No. 960405.

14. Empower\Help\Interpreting PDA Peak Purity Results.

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