Developing a UHPLC method for UV-based detection and quantification of primary aromatic amines in low concentrations

Application Note

Consumer Products

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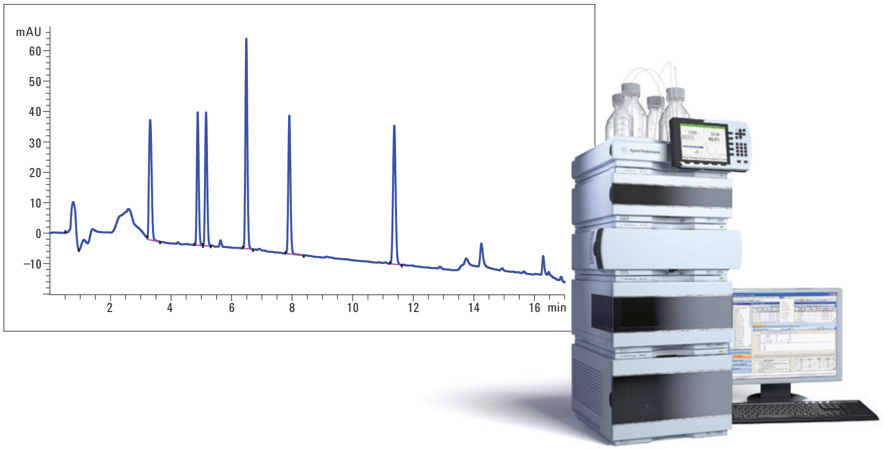

Abstract

This Application Note demonstrates that stringent sensitivity requirements for the detection of potentially harmful primary aromatic amines can be fulfilled when using an Agilent 1290 Infinity LC System equipped with the 1290 large volume injection kit. This enables the Agilent 1290 Infinity Autosampler for injection volumes up to 120 µL for sample enrichment on the column. A high sensitivity 60-mm Max-Light flow cell in the Agilent 1290 Infinity Diode Array Detector gives additional detection sensitivity.

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Introduction

Primary aromatic amines (PAAs), for example, aniline and its derivatives can originate from printing azo-dyes and azo-pigments, isocyanate based adhesives and monomers used for plastics. Since these compounds are potentially harmful and suspected to cause cancer and have other adverse effects, they have to be detected and determined, for example, in printed products, recycled paper and board material, plastic goods, and food products.

A legal requirement already exists for plastic food contact materials (FCM) within regulation 10/2011. Plastic FCM may not release PAAs in a detectable quantity. The detection limit is defined as 10 ppb (sum of all PAAs, including analytical tolerance). Similar requirements exist on the national level for paper and board materials (see BfR recommendation XXXVI on paper and board). The upcoming German regulation for printing inks used for FCM (Druckfarben V) also mentions a limit of 10 ppb for the sum of the released PAAs. Recently, problems have been reported within industry and enforcement authorities regarding the release of carcinogenic PAAs from heavily printed paper bags and napkins.

Since the legal limits apply to the sum of all PAAs, these compounds have to be detected down to a level of at least 1 ppb (1 ng/mL) for an individual PAA. One of the recommended methods for determination is HPLC with UV-based detection using a diode array detector (DAD).

This Application Note demonstrates that, in principle, this requirement can be fulfilled. Six representative primary aromatic amine compounds were tested using an Agilent 1290 Infinity LC System equipped with the 1290 large volume injection kit in the Agilent 1290 Infinity Autosampler module. This enables the Agilent 1290 Infinity Autosampler for injection volumes up to 120 µL for sample enrichment on the column. A high sensitivity 60-mm Max-Light flow cell in the Agilent 1290 Infinity Diode Array Detector gives additional detection sensitivity.

Experimental

Equipment

The Agilent 1290 Infinity LC System consisted of the following modules:

- Agilent 1290 Infinity Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A) equipped with 40-µL flex loop kit (p/n 5067-4703), 1290 large volume injection kit (G4216A) and sample cooling (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4221A)

Software

- CDS Open Lab ChemStation Edition C01.03

Column

- Agilent ZORBAX Eclipse Plus C18, RRHT, 3.0 × 100 mm, 1.8 µm

Method

- Agilent 1290 Infinity Pump

  Solvents: A: Water and 10 mM Sodium acetate, pH 5.1
  B: Methanol

  Gradient: 0 minutes–5% B,
  1 minute–5% B,
  2 minutes–20% B,
  15 minutes–70% B,
  17 minutes–90% B

  Stop time: 17 minutes

  Post time: 3 minutes

  Flow rate: 0.75 mL/min

- Agilent 1290 Infinity Autosampler

  Injection volume: 120 µL

  Needle wash: 6 seconds in MeOH

  Sample temperature: 10 °C

- Agilent 1290 Infinity Thermostatted Column Compartment

  Column temperature: 40 °C

- Agilent 1290 Infinity Diode Array Detector

  Wavelength: 240/8 nm

  Ref. wavelength: 360/100 nm

  Slit: 8 nm

  Data rate: 20 Hz

  Cell: 60-mm Max-Light high sensitivity cell

Samples

Stock solutions of: aniline, o-anisidine, o-toluidine, 2-methyl-5-nitroaniline, 2,4-dimethylaniline, 2,4-dichloraniline at 10 mg/100 mL in acetonitrile each. Diluted to a mixed stock solution at 1,000 ng/mL each in water /methanol 95/5 (v/v). Dilution series: 100, 50, 20, 10, 5, 2, 1, 0.5 ng/mL in water/methanol 95/5 (v/v).

Preparation of blank matrix from colorized napkins for spiking experiments:

A cold water extract of pretested napkins was prepared according to CEN standard 645. The napkin was cut into small pieces, 10 g of which were put in a flask and filled with 200 mL of distilled water. The extract was allowed to stand at room temperature for 24 hours, then the paper was separated by decantation and the water adjusted to a final volume of 250 mL.

Results and discussion

To meet the requirement of highly sensitive UV based detection of PAAs down to an individual level of 1 ppb, two modifications were made to the standard configuration of the Agilent 1290 Infinity LC System.

First, the standard 10-mm Max-Light flow cell in the 1290 Infinity Diode Array Detector was replaced with a 60-mm Max-Light high sensitivity flow cell. Second, the standard 20-µL loop in the 1290 Infinity Autsampler was replaced with a 40-µL loop by including the 1290 large volume injection kit to inject volumes up to 120 µL[1].

The method that was finally applied was developed by using a mixture of the six PAAs at a concentration of 100 ng/mL each (Figure 1). The method starts with an enrichment step for the first minute followed by a steep increase to the starting conditions at 20% methanol. The gradient separation was done in the following 13 minutes up to 70% methanol.

With the final method, a calibration was done for all PAAs from 100 ng/mL down to a level of 2 ng/mL (2 ppb). All linearity correlation coefficients were above 0.99983 (Figure 2). The concentration level at 2 ng/mL was defined as the limit of quantification (LOQ) because the compounds' signal-to-noise ratio was around 10 at this point. The limit of detection (LOD) was 0.5 ng/mL at a signal-to-noise ratio around 3 for all compounds.

A statistical evaluation was done on the 10 ng/mL level as an example for the lower level concentrations. The relative standard deviation (RSD) of the retention times was between 0.039 and 0.057%, the area RSDs were between 0.5 and 2.7%. The precision of the measured concentrations was in the same range (Table 1). Relative standard deviation of the compound response factors were between 0.004 and 0.017%.

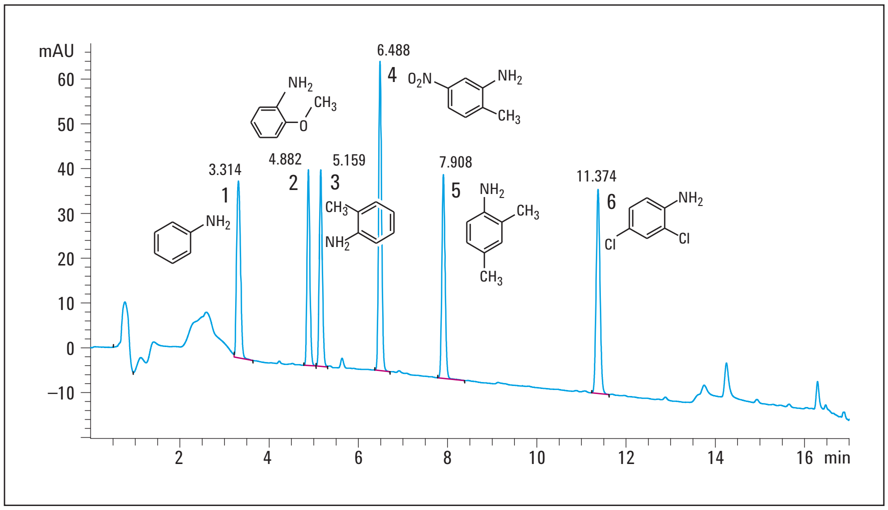


Figure 1
Primary aromatic amines at 100 ng/mL, 1) aniline: 3.314 minutes, 2) o-anisidine: 4.882 minutes, 3) o-toluidine: 5.159 minutes, 4) 2-methyl-5-nitroanilin: 6.488 minutes, 5) 2,4-dimethylanilin: 7.908 minutes, 6) 2,4-dichloranilin: 11.373 minutes.

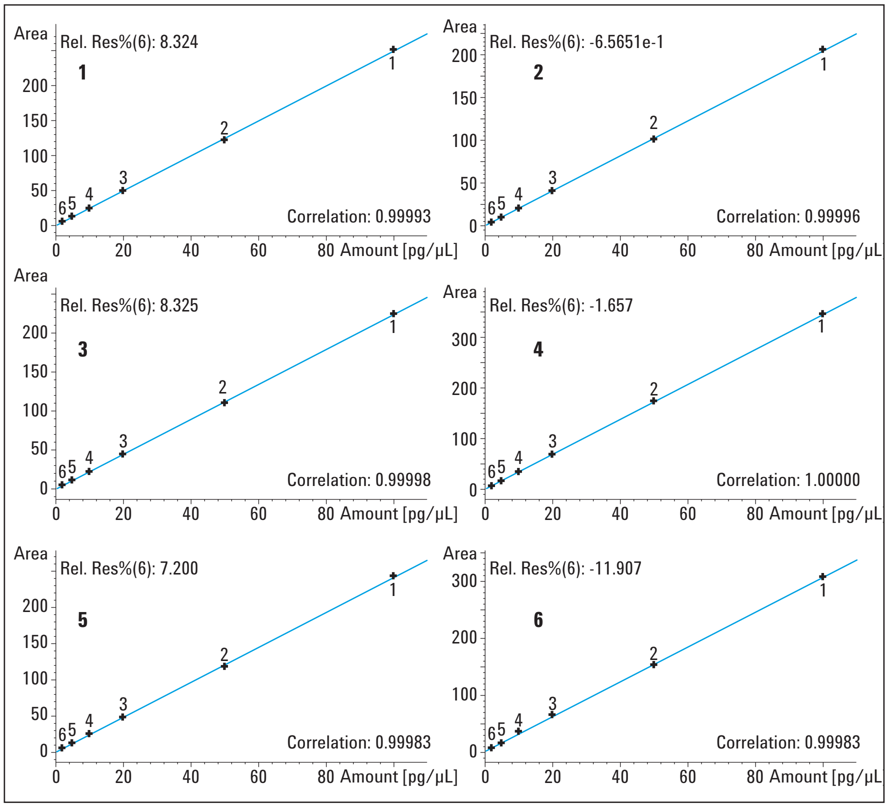


Figure 2
Calibration curves 2–100 ng/mL of the used paAs, 1) aniline: Correlation: 0.99993, 2) o-anisidine: Correlation: 0.99996, 3) o-toluidine: Correlation: 0.99998, 4) 2-methyl-5-nitroanilin: Correlation: 1.00000, 5) 2,4-dimethylanilin: Correlation: 0.99983, 6) 2,4-dichloranilin: Correlation: 0.99983

To test the method, a blank matrix sample produced from a heavily colored napkin was spiked with the six PAAs at various concentrations (Figure 3). All six PAAs could be well separated from the matrix compounds and identified and quantified with the developed method and calibration. However, for some PAAs, a sample cleaning step using SPE prior to the chromatographic analysis might be necessary as indicated by a recovery that was too high, for example, of o-toluidine. This may have been caused by matrix interferences and has to be considered in further method development activities.

Conclusion

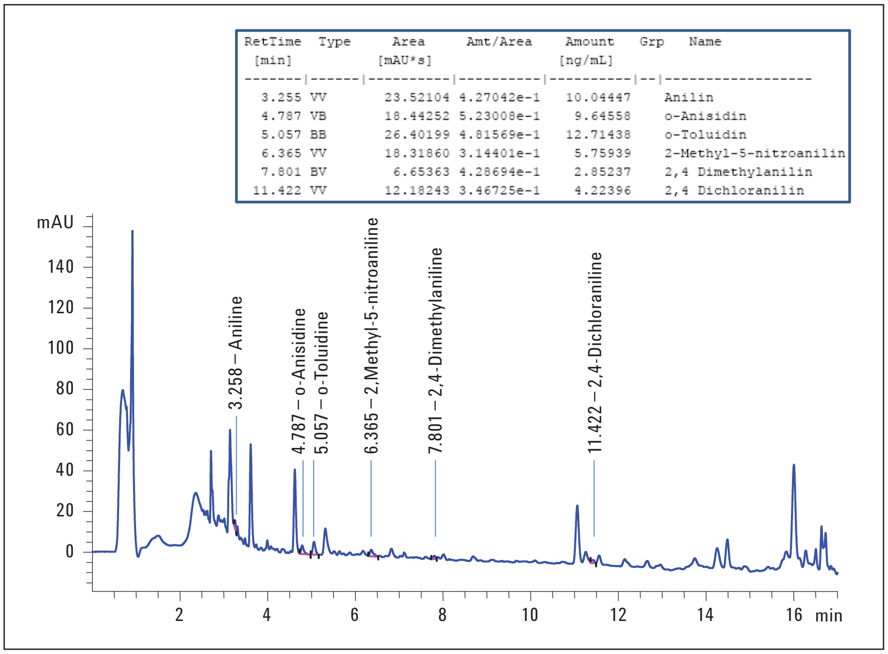
This Application Note shows that the Agilent 1290 Infinity LC System can detect low amounts of primary aromatic amines by using the 60-mm Max-Light high sensitivity cell in the diode array detector, and the 1290 large volume injection kit in the autosampler for an enrichment step. Data are shown which prove an excellent linearity of the calibration down to the LOQ at 2 ppb. The LOD was found at 0.5 ppb. The lower level calibration points show good relative standard deviations for the retention time and the area as well as good precision for the measured concentrations. The PAAs could be detected and quantified in a sample with heavy matrix load at lowest levels.

Reference

1.
Enhancing the maximum injection volume of the Agilent 1290 Infinity Autosampler, Agilent Technical Overview, Publication Number 5990-9533EN, **2011**.

Compound	**Aniline**				**o-Anisidine**				**o-Toluidine**			
	RT	**Area**	**Amount**	**Compound response factor**	**RT**	**Area**	**Amount**	**Compound response factor**	**RT**	**Area**	**Amount**	**Compound response factor**
Average	3.273	22.474	9.610	0.427	4.815	19.131	9.988	0.522	5.084	20.848	10.014	0.480
Standard deviation	0.001	0.117	0.048	7.049E-05	0.002	0.094	0.048	2.870E-05	0.002	0.567	0.271	5.390E-05
RSD (%)	0.039	0.521	0.505	0.016	0.047	0.494	0.489	0.005	0.047	2.722	2.715	0.011
Compound	**2-Methyl-5-nitroaniline**				**2,4-Dimethylaniline**				**2,4-Dichloraniline**			
	RT	**Area**	**Amount**	**Compound response factor**	**RT**	**Area**	**Amount**	**Compound response factor**	**RT**	**Area**	**Amount**	**Compound response factor**
Average	6.400	31.729	9.913	0.312	7.827	22.871	9.925	0.433	11.367	28.067	10.050	0.358
Standard deviation	0.004	0.279	0.087	1.298E-05	0.004	0.198	0.086	2.950E-05	0.006	0.321	0.116	6.251E-05
RSD (%)	0.057	0.880	0.880	0.004	0.050	0.869	0.875	0.006	0.049	1.146	1.163	0.017

Table 1
Retention time precision, area precision, concentration precision and compound response factor precision for the six used standard paAs at 10 ng/mL.



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RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [mAU*s]                 [ng/mL]
-------|------|----------|----------|----------|--|------------------
  3.255 VV       23.52104 4.27042e-1   10.04447     Anilin
  4.787 VB       18.44252 5.23008e-1    9.64558     o-Anisidin
  5.057 BB       26.40199 4.81569e-1   12.71438     o-Toluidin
  6.365 VV       18.31860 3.14401e-1    5.75939     2-Methyl-5-nitroanilin
  7.801 BV        6.65363 4.28694e-1    2.85237     2,4 Dimethylanilin
 11.422 VV       12.18243 3.46725e-1    4.22396     2,4 Dichloranilin
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Figure 3
Matrix sample from a heavily colored napkin spiked with the six paAs.

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