Developing a method for the analysis of Azo Dyes using the Agilent 1290 Infinity LC Method Development System and the Agilent Method Scouting Wizard software

Application Note

Method Development Consumer Products

Author

Gerd Vanhoenacker, Frank David,
Pat Sandra
Research Institute for Chromatography
Kennedypark 26
B-8500 Kortrijk
Belgium

Abstract

The Agilent 1290 Infinity LC Method Development System was used to develop a UHPLC method for the analysis of 20 amines derived from banned azo dyes. Different columns, mobile phases, and temperatures were screened in an automated manner. The Agilent Method Scouting Wizard software proved to be a valuable tool to assist in the setup of the various methods and sequences. Initial screening analyses were performed using LC-MS, while method optimization was done with diode-array detection. The performance of the final method was evaluated.

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Introduction

Azo dyes are used extensively in cosmetics, textile, leather, food, plastics, and other consumer products. The reductive cleavage of the azo bond leads to the formation of amines of which certain have known mutagenic and/or carcinogenic properties. Therefore, the use of azo dyes that can be reduced into toxic amines is prohibited in Europe, US, and many other countries. In the European Union (EU) a directive of 2002 describes the restrictions on the marketing and use of certain azo dyes[1].

The determination of azo dyes in consumer products generally consists of the analysis of the amines after chemical reduction. LC-MS is a suitable technique to determine these amines[2]. However, routine labs often rely on UV based detectors to perform such type of analyses. This is mainly due to the high cost of purchase and use of MS detectors, and a lack of qualified personnel. The main drawback of non-MS detectors is the lack of selectivity. Consequently, when the analyses are carried out with a UV based detector, the chromatographic separation of all amines is essential. The EU directive lists a set of 22 aromatic amines and the separation of these compounds in an acceptable analysis time is not straightforward[3].

Most common method variables are column chemistry (stationary phase), mobile phase composition (pH, buffer type, organic modifier), and temperature. When screening different combinations with standard LC equipment, the intervention of the operator will be mandatory to change and condition columns or to change mobile phases. Consequently, LC method development can be a long and labor intensive process. The Agilent Method Development System consists of a combination of valves and modules to enable fully automated column and mobile phase selection. When combined with the Agilent Method Scouting Wizard software, the time consuming task of method and sequence setup can be fully automated. The software built sequence performs all analyses with the defined method variables and additionally executes all rinsing, equilibration, and column storage methods that are required when switching between methods and columns. In this way, method development can be rationalized leading to higher productivity.

This Application Note demonstrates the use of the Agilent 1290 Infinity LC Method Development System and the Agilent Method Scouting Wizard software to develop and optimize a separation of 20 amines.

Experimental

Instrumentation

An Agilent 1290 Infinity LC Method Development System equipped with the Agilent 1290 Infinity Diode Array Detector and an Agilent 6150 Single Quadrupole MS (GG6150A) were used. The Agilent Method Scouting Wizard was used to assist with the automated method development.

The Agilent 1290 Infinity LC system was configured as follows:

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220A)
- 12 position/13 port selection valve. Solvent selection binary pump channel A (G1160A)
- 12 position/13 port selection valve. Solvent selection binary pump channel B (G1160A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment with integrated 8 position/9 port valve, 1200 bar. Column selection valve, inlet from autosampler (G1316C)
- Agilent 1290 Infinity Thermostatted Column Compartment with integrated 8 position/9 port valve, 400 bar. Column selection valve, outlet to DAD and MS or waste (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light Cartridge Standard Cell (G4212-60008)

Generic method parameters

These parameters were used for the initial method screening experiments. They were adapted as necessary by the Agilent Method Scouting Wizard software.

Flow rate:	0.3 mL/min (0.272 mL/min for the 2.0 mm ID column, automatically adjusted by the software)	
Gradient:	0-1 min 1-15 min 15-15.1 min 15.1-17 min	5% B 5-90% B 90-98% B 98% B
Injection:	2 µL	
Detection DAD		
Peakwidth:	>0.025 min (10 Hz)	
Wavelength:	Signal 240/5 nm, Reference off	
Spectra acquisition:	On, 200-400 nm	
Detection MS Single Quadrupole		
Ionization mode:	JetStream Technology, Electrospray positive ionization	
Scan type:	SIM or Scan	

Standard solutions

The analyses were carried out with a standard mixture of the 20 amines shown in Table 1. The stock solution of 10 µg/mL in acetonitrile was diluted in acetonitrile or water as necessary.

Results and discussion

A first screening phase (Phase 1A) was performed to evaluate which general conditions (column, mobile phase pH, temperature) are promising to develop further. Taking into account the conclusions from Phase 1A, a more targeted screen was carried out using more subtle mobile phase variations. A total of 240 different combinations have been tested in Phase 1 using both DAD and MS detection. Out of these experiments, several combinations proved to have potential as a starting point for method optimization. One combination was selected, further optimized, and the performance was evaluated.

Phase 1: Method Scouting

Phase 1A
Automated Non-Targeted Screening
96 methods

Columns:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm
	Agilent ZORBAX RRHD StableBond C18, 2.1 × 100 mm, 1.8 µm
	Agilent ZORBAX RRHD StableBond C8, 2.1 × 100 mm, 1.8 µm
	Acquity BEH Shield RP18, 2.1 × 100 mm, 1.7 µm (Waters)
	Acquity HSS T3, 2.1 × 100 mm, 1.8 µm (Waters)
	VisionHT C18 HL, 2.0 × 100 mm, 1.5 µm (Grace)
Mobile phase A:	0.1% Formic acid (pH 2.5)
	25 mM Ammonium formate/ formic acid (pH 4.45)
Mobile phase B:	Methanol
	Acetonitrile
Temperature:	25, 35, 45, 55 °C
Conclusions:	Ammonium formate provides better selectivity and peak spreading than formic acid.
	The selectivity with methanol is better compared to acetonitrile.
	The Acquity HSS T3 and Agilent ZORBAX Eclipse Plus C18 column give very similar selectivity.
	The Agilent ZORBAX StableBond C8 phase is significantly different from the StableBond-C18 and the other stationary phases.

Phase 1B
Automated Targeted Screening
144 methods (performed combinations from phase 1A were not re-executed)

Columns:	See Phase 1A
Mobile phase A:	25 mM Ammonium formate (pH 6.20)
	25 mM Ammonium formate/ formic acid (pH 4.85)
	25 mM Ammonium formate/ formic acid (pH 4.45)
Mobile phase B:	Methanol
	Acetonitrile
	Methanol/Acetonitrile 50/50
Temperature:	25, 35, 45, 55 °C
Conclusions:	Multiple combinations are promising. The best combination regarding peak spreading, temperature, pH was:
	Agilent ZORBAX RRHD StableBond C18, 2.1 × 100 mm, 1.8 µm
	25 mM Ammonium formate/ formic acid (pH 4.45)
	Methanol/Acetonitrile 50/50
	35 °C

Peak Code	Name	CAS no	FW
AZO 01	4-Methoxy-1,3-phenylenediamine	615-05-4	138
AZO 02	2,4-Diaminotoluene	95-80-7	122
AZO 03	4-Aminophenylether	101-80-4	200
AZO 04	4,4′-Benzidine	92-87-5	184
AZO 05	o-Toluidine	95-53-4	107
AZO 06	Bis-(4-aminophenyl)-methane	83712-44-1	198
AZO 07	4-Chloroaniline	106-47-8	127
AZO 08	2-Methoxy-5-methylaniline	120-71-8	137
AZO 09	2-Methyl-5-nitroaniline	99-55-8	152
AZO 10	3,3′-Dimethoxybenzidine	119-90-4	244
AZO 11	3,3′-Dimethylbenzidine	119-93-7	212
AZO 12	4-Aminophenylthioether	139-65-1	216
AZO 13	2-Naphthylamine	91-59-8	143
AZO 14	4-Chloro-2-methylaniline	95-69-2	141
AZO 15	2,4,5-Trimethylaniline	137-17-7	135
AZO 16	4,4′-Diamino-3,3′-dimethyldiphenyl methane	838-88-0	226
AZO 17	4-Aminobiphenyl	92-67-1	169
AZO 18	3,3′-Dichlorobenzidine	91-94-1	252
AZO 19	4,4′-Methylene-bis(2-chloroaniline)	101-14-4	266
AZO 20	4-Amino-2′,3-dimethylazobenzene	97-56-3	225

Table 1
Investigated azo dye derived amines (Azodyes-Mix 1, Dr. Ehrenstorfer, Augsburg, Germany).

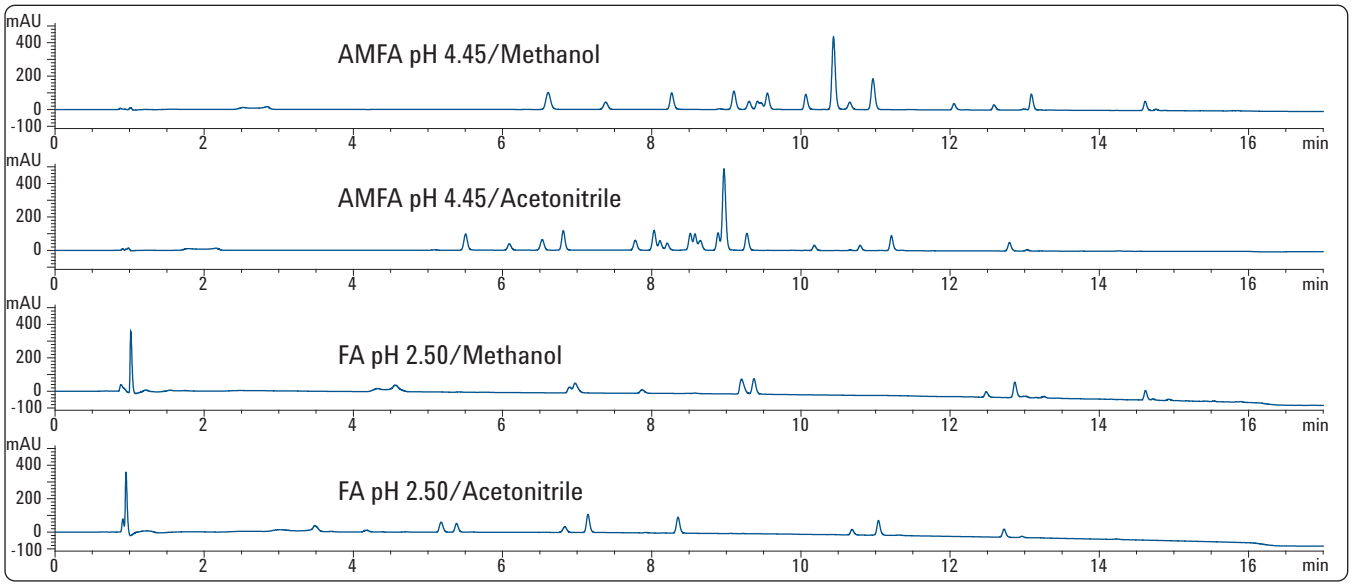

Figure 1

Phase 1A, Influence of mobile phase. Formic acid (FA) versus ammonium formate/formic acid (AMFA) and methanol versus acetonitrile. Column: Agilent ZORBAX RRHD Eclipse Plus C18, temperature: 25 °C, sample: standard solution 10 µg/mL in acetonitrile, 2 µL injection.

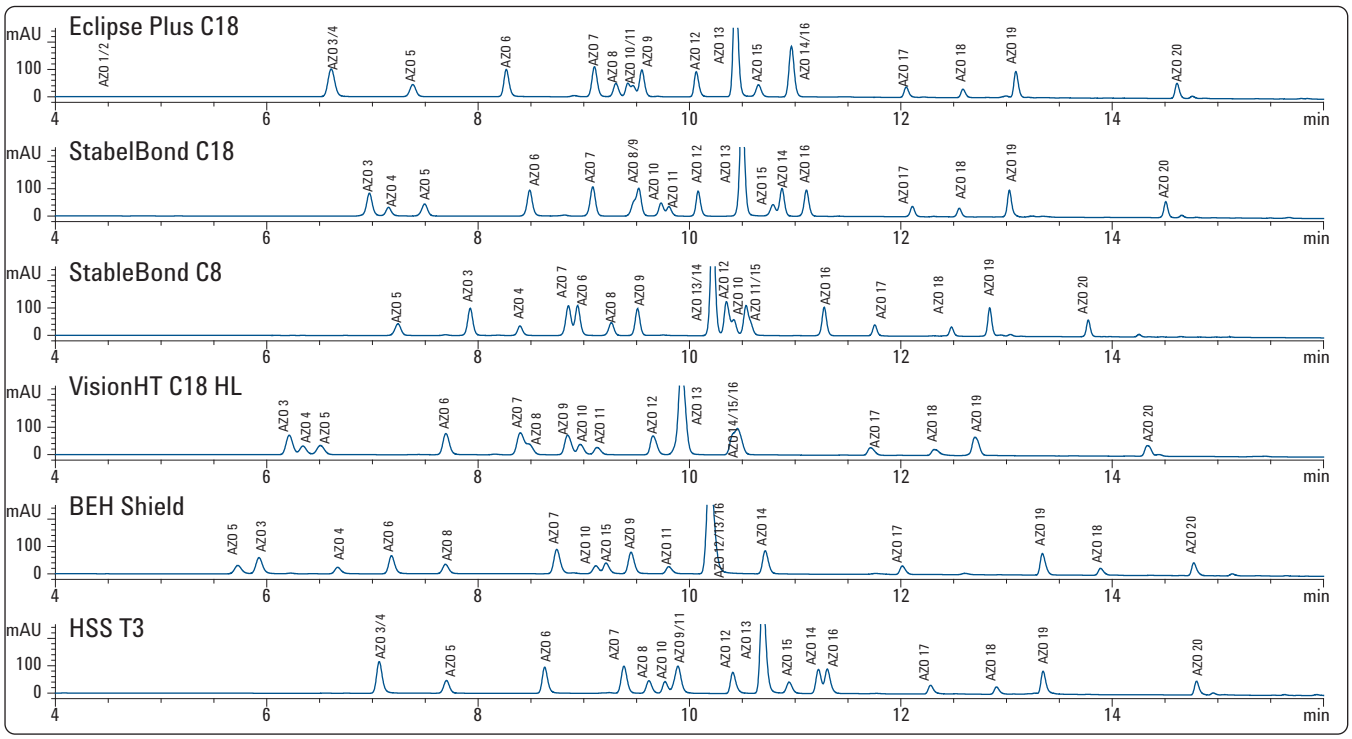

Figure 2

Phase 1A, Influence of stationary phase. Mobile phase: ammonium formate/formic acid and methanol, temperature: 25 °C, sample: standard solution 10 µg/mL in acetonitrile, 2 µL injection.

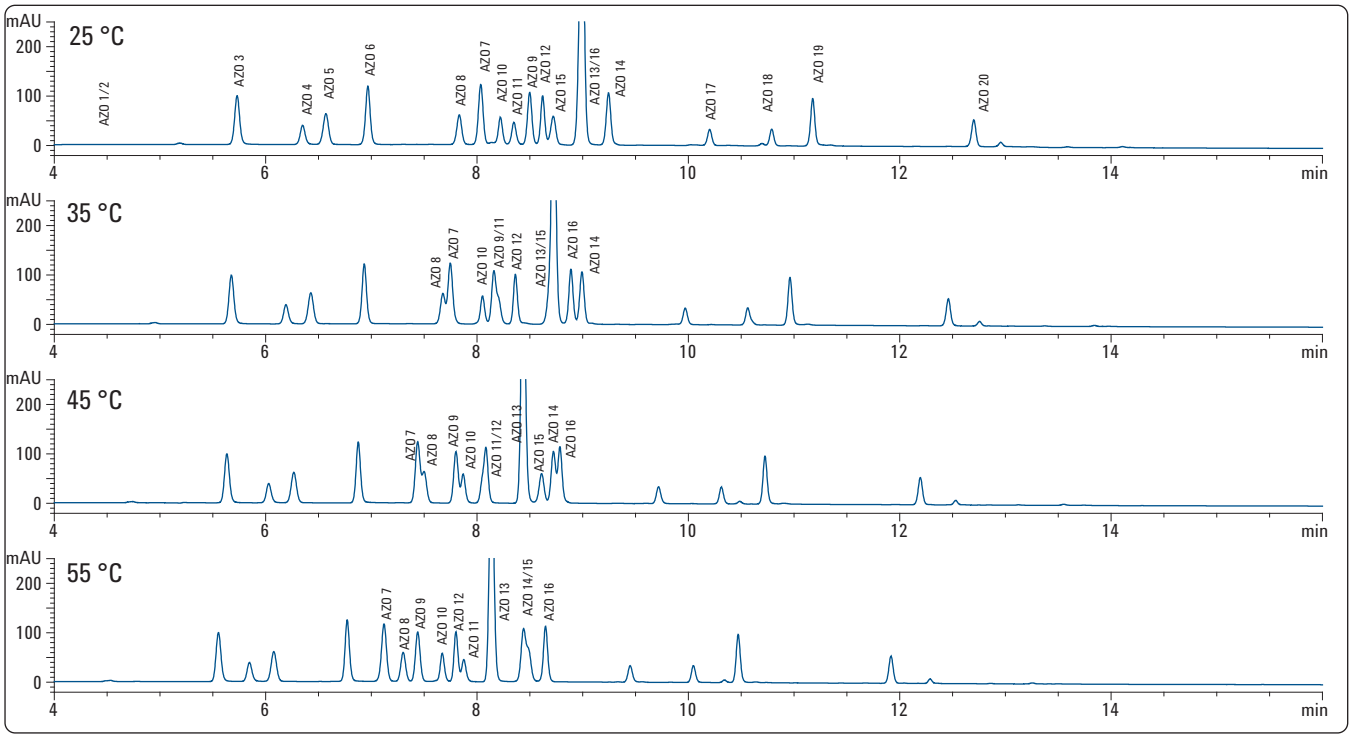

Figure 3

Phase 1A, Influence of column temperature. Column: Agilent ZORBAX RRHD StableBond-C18, mobile phase: ammonium formate/formic acid and acetonitrile, sample: standard solution 10 µg/mL in acetonitrile, 2 µL injection.

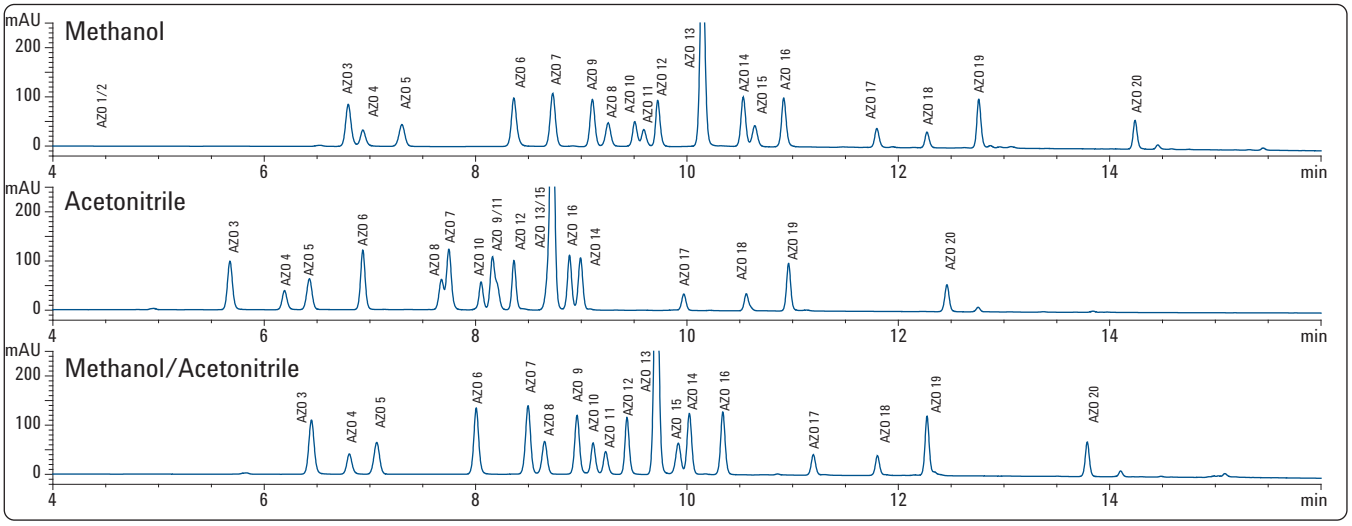

Figure 4

Phase 1B, Influence of organic modifier. Column: Agilent ZORBAX RRHD StableBond-C18, mobile phase A: ammonium formate/formic, temperature: 35 °C, sample: standard solution 10 µg/mL in acetonitrile, 2 µL injection.

Phase 2: Method Optimization

Now that the column, pH range and temperature range are selected, further optimization can be performed. This work was done without MS detection. Since the final method will be carried out with UV based detection, the volatile formate buffer was replaced with a UV transparent phosphate buffer of the same pH and with similar ionic strength (to maximize sensitivity). The chromatographic separation was maintained as can be seen from the result shown in Figure 5.

After some further adjustment of the buffer preparation (and consequently pH), the Agilent Method Scouting Wizard was used to perform an automated temperature and gradient optimization. The final method was then optimized for speed by doubling the flow rate and gradient slope. This adjustment gave rise to some minor selectivity changes caused by the frictional heat generated by the high mobile phase velocity in the column. This is a known phenomenon which can be overcome by lowering the set temperature of the thermostatted column compartment[4], in this case from 37 to 36 °C. A final method was obtained with an analysis time of 8.5 min and an operating pressure of 760 bar (Figure 6).

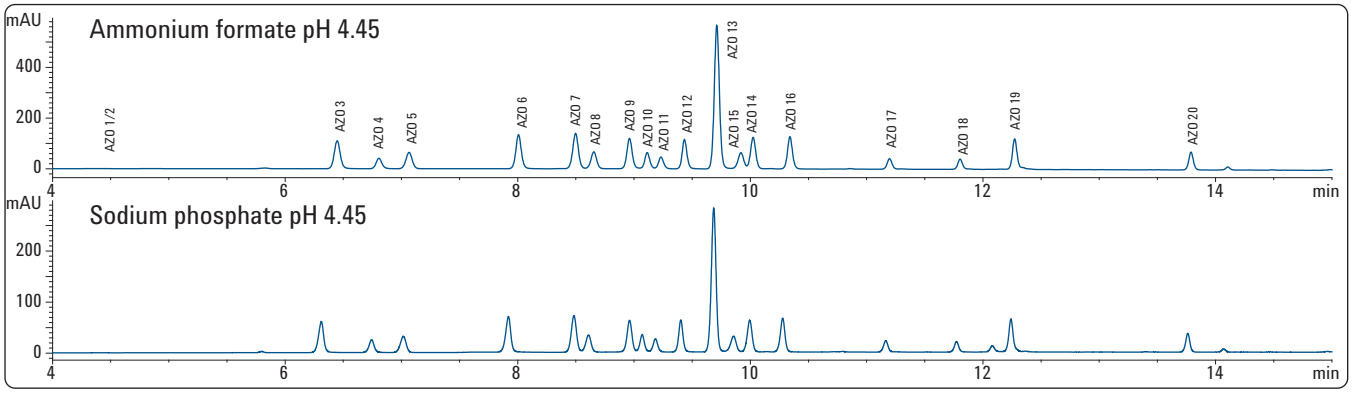

Figure 5
Phase 2, Transfer of the mobile phase buffer. Column: Agilent ZORBAX RRHD StableBond-C18, organic modifier: methanol/acetonitrile, temperature: 35 °C, sample: standard solution 10 µg/mL in acetonitrile, 2 µL injection.

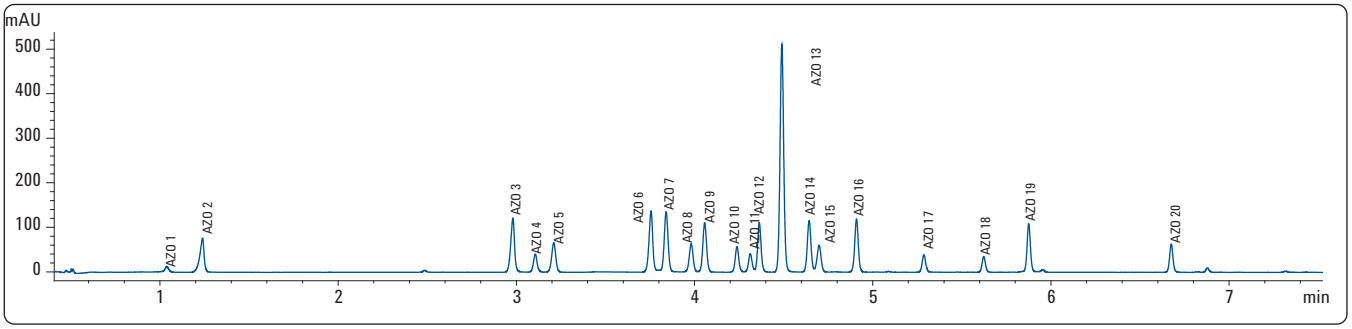

Figure 6
Final result, 1 ppm solution.

After optimization of the sample solvent (water/acetonitrile 9/1), injection volume (20 µL), and detection wavelength the performance of the method was evaluated. The results are summarized in Table 2.

The final method parameters are:

Column:	Agilent ZORBAX RRHD StableBond-C18, 2.1 × 100 mm, 1.8 µm	
Mobile phase:	A=20 mM NaH_2PO_4, pH 4.60	
	B=methanol/acetonitrile 50/50	
Flow rate:	0.6 mL/min	
Gradient:	1-7.5 min	5-90% B
	7.5-7.6 min	90-98% B
	7.6-8.5 min	98% B
	8.5-9.3 min	5% B (post-time)
Temperature:	36 °C	
Injection:	20 µL, needle wash (4 s, flushport, mobile phase B)	
Detection DAD		
Peak width:	>0.0063 min (40 Hz)	
Wavelength:		
A=Time programmed:	0-2.5 min Signal 210/5 nm, Reference off	
	2.5-6.2 min Signal 262/10 nm, Reference off	
	6.2-8.5 min Signal 386/15 nm, Reference off	
B=Signal 235/20 nm, Reference off		
C=Signal 245/10 nm, Reference off		
D=Signal 285/30 nm, Reference off		
Spectra acquisition:	On, 200-400 nm	

		RSD% (n=6)			
Compound	Signal	Ret Time	Area, 10 ng/mL	Area, 100 ng/mL	Area, 1000 ng/mL
AZO 01	210/5 nm	0.05	2.16	1.40	0.58
AZO 02	210/5 nm	0.05	1.76	0.55	0.13
AZO 03	245/10 nm	0.09	1.39	0.22	0.04
AZO 04	285/30 nm	0.09	0.81	0.15	0.06
AZO 05	235/20 nm	0.08	1.00	0.10	0.07
AZO 06	245/10 nm	0.08	3.26	0.77	0.65
AZO 07	245/10 nm	0.07	6.37	1.61	0.66
AZO 08	235/20 nm	0.07	2.75	0.85	0.27
AZO 09	235/20 nm	0.07	0.80	0.31	0.20
AZO 10	285/30 nm	0.08	2.07	0.18	0.03
AZO 11	285/30 nm	0.07	1.45	0.13	0.02
AZO 12	262/10 nm	0.08	0.71	0.50	0.52
AZO 13	235/20 nm	0.07	0.89	0.17	0.07
AZO 14	245/10 nm	0.07	0.82	0.18	0.03
AZO 15	235/20 nm	0.07	4.37	0.92	0.64
AZO 16	245/10 nm	0.07	3.45	0.10	0.21
AZO 17	285/30 nm	0.04	1.35	0.48	0.08
AZO 18	285/30 nm	0.03	0.48	0.19	0.07
AZO 19	245/10 nm	0.02	0.32	0.35	0.95
AZO 20	386/15 nm	0.01	0.57	0.11	0.08

Table 2
Repeatability of the final method.

Conclusion

The Agilent 1290 Infinity LC Method Development System is a flexible and user-friendly instrument for automated method screening and development. The combination of solvent and column selection valves with the Agilent Method Scouting Wizard software results in a powerful tool to assist the setup of large method development sequences. The system greatly reduces the amount of man hours involved with exchanging, equilibrating, and rinsing columns and flushing solvent lines. All components (columns and mobile phases) can be installed on the system and combined the software.

A method for the analysis of 20 banned azo dye derived amines was developed and optimized for resolution and speed.

References

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