Performance of the 80-nL UV flow cell

Technical Overview

Karlheinz Blum
Mark Stahl
Angelika Gratzfeld-Huesgen

Abstract

In this Technical Overview the performance of the new 80-nL cell designed for micro bore and capillary LC UV detection is evaluated. Using a standard chromatographic separation allows to compare analytical parameters of the 500-nL cell to those of the new 80-nL cell. This clearly demonstrates a great improvement in chromatographic separation.

Introduction

Especially, but by far not limited to bioanalysis, the sample amount available for analysis is becoming smaller and smaller. Therefore, lower column diameters in combination with lower flow rates are being used. This results in higher analyte concentration at the detector since the sample is eluted in less solvent. Some years ago capillary LC was still highly sophisticated, however, in the last years it has become a more or less routine technique. Although capillary LC is often used in combination with mass spectrometry, there are also numerous applications using UV detection. This created a need for an appropriate UV flow cell which does not create too much band broadening in the detector, thus reducing the chromatographic performance. The first step into this direction was the introduction of the 500-nL flow cell, but as separation dimensions have been minimized further a need for an even lower UV detection cell arose. Therefore, Agilent developed a 80-nL cell resulting in lower peak dispersion and thus improving chromatographic separation at low flow rates.s used.

This application has been verified using an Agilent 1200 Series LC system, and showed comparable or even better performance.

Materials and methods

An Agilent 1100 Series capillary LC system with a diode-array detector equipped with either a 500-nL or an 80-nL flow cell was used for the analysis. Detection was done at 254 nm with a reference wavelength of 360 nm. For the isocratic separation 1 µL of the Agilent isocratic standard sample (Dimethylphtalat, Diethylphtalat, Biphenyl, O-Terphenyl) with the addition of thiourea was injected. The analyses were done on ZORBAX XDB C18 0.5 or 0.3 x 150-mm columns with a particle size of 3.5 mm at 60 °C. For the isocratic separation a water/acetonitril (both solvents with 0.1 % TFA) mixture (20/80 v/v) was used with a flow rate of 4 µl/min for the 0.3-mm column and 10-µL/min for the 0.5-mm column.

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Results and discussion

From the chromatogram and the chromatographic parameters presented in figure 1 and table 1 it becomes obvious that compared to the 500-nL flow cell the 80-nL cell improves resolution without compromising too much sensitivity. Even though the shorter path length of the smaller flow cell decreases sensitivity, some is gained back due to narrower peaks resulting in increased peak height. Due to the lower internal cell volume peak dispersion is reduced leading to better chromatographic separations shown by peak widths, chromatographic plates and resolutions.

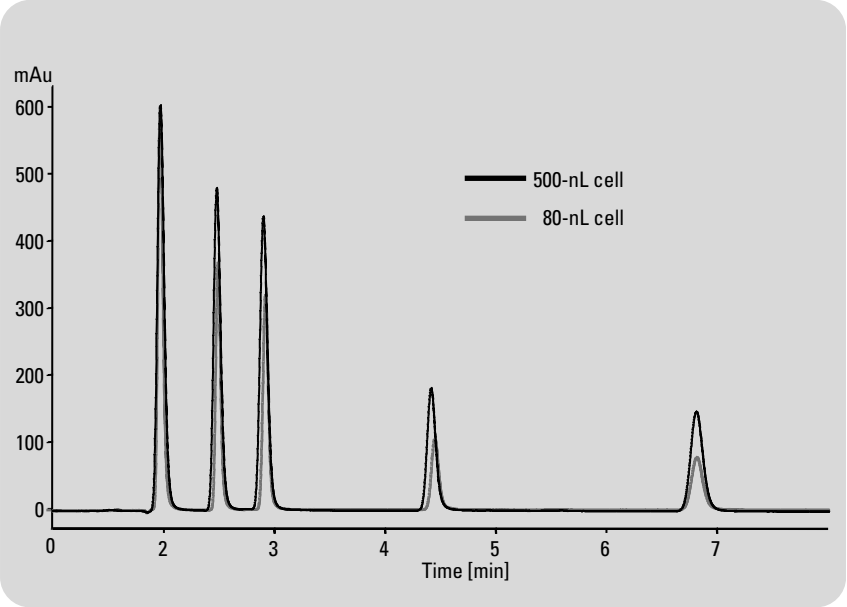

Figure 1
Chromatogram of the separation of the Agilent isocratic standard sample plus thiourea on a ZORBAX XDB C_{18} 0.5 x 150 mm, 3.5-µm column with a 500-nL and a 80-nL flow cell for DAD detection

Conclusion

The Agilent 80-nL flow cell for UV detection improves chromatographic separation. Due to the low internal cell volume, peak widths, resolutions and chromatographic plates improve compared to the 500-nL cell. As the path length of such a low volume cell is shorter, sensitivity typically decreases slightly, however this is absorbed to some extent by the narrower peak widths resulting in larger peak heights. Thus, the 80-nL flow cell is well suited for UV detection at low flow rates.

Mark Stahl and Angelika Gratzfeld-Huesgen are Application Chemists and Karlheinz Blum is Technical Specialist for Capillary and Nano Columns at Agilent Technologies GmbH, Waldbronn, Germany.

		Peak area (mAu/s)	Peak height (mAu)	Peak width (min)	Resolution	Theor. plates	Signal/ Noise	Noise (mAu)
Peak 1	**80-nL cell**	1936	378	0.075	-	5872	565	0.3609
	500-nL cell	2689	537	0.075	-	4506	725	0.2175
Peak 2	**80-nL cell**	1685	345	0.071	4.2	9693	648	0.3609
	500-nL cell	2079	411	0.077	4.1	6690	503	0.2175
Peak 3	**80-nL cell**	1637	313	0.075	3.5	11337	303	0.3609
	500-nL cell	2012	373	0.082	3.2	7945	457	0.2175
Peak 4	**80-nL cell**	701	112	0.089	10.8	16770	201	0.3609
	500-nL cell	1014	167	0.092	10.4	14118	625	0.2175
Peak 5	**80-nL cell**	855	96	0.131	12.6	17089	217	0.3609
	500-nL cell	1192	140	0.130	12.7	16340	504	0.2175

Table 1
Chromatographic parameters of the separations shown in figure 1

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