Improved Sensitivity with the Agilent 1290 Infinity Evaporative Light Scattering Detector (ELSD)

Technical Overview

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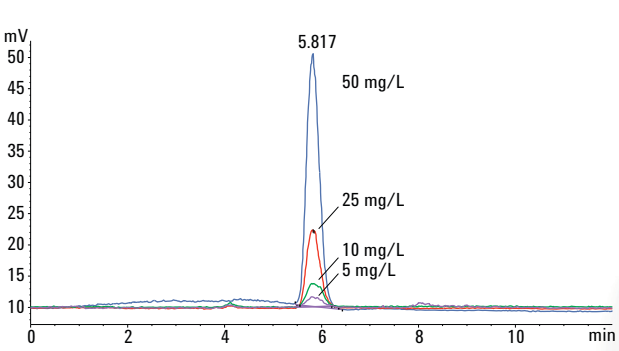
Abstract

This Technical Overview demonstrates the seamless transfer of an existing method from an Agilent 385 ELSD to an Agilent 1290 Infinity ELSD with immediate gain in sensitivity and the highest possible sensitivity performance after a slight optimization step. The measurement of lactose has been used as an example because this is an important application for the determination of residual lactose in lactose-free dairy products.



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Introduction

The food industry makes a great effort to produce lactose free dairy products because a medium percentage of the adult population (10–20% in Europe) is intolerant to lactose.

In principle, a dairy food product is declared lactose free if the content of lactose is less than 100 mg/100 g product. For quality control, detection devices must be available which are able to meet this requirement. Typically, ELSD or RI detection is used for this measurement.

The Agilent 1290 Infinity ELSD is able to keep up with this requirement[1]. Due to improvements made in the optical unit such as a blue laser light source coupled with a high gain photomultiplier and digital signal processing, the signal is enhanced and the noise is reduced. Compared to formerly used LED light sources, this results in an increase of sensitivity for the 1290 Infinity ELSD[2].

This Technical Overview demonstrates the seamless transfer of an existing method from an Agilent 385 ELSD to a 1290 Infinity ELSD with immediate gain in sensitivity and the highest possible sensitivity performance after a slight optimization step.

Experimental

Instrumentation

Description	Model number
Agilent 1220 Infinity LC	G4290B
Agilent 1290 Infinity ELSD	G4261B
Agilent 385 ELSD	(discontinued)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.03

HPLC Method

Parameter	Setting
Column	Agilent Hi-Plex Ca, USP L19, 4 × 250 mm, (p/n PL1570-5810)
Solvent	Ultrapure water
Flow rate	0.25 mL/min
Elution	Isocratic
Stop time	15 minutes
Injection volume	20 µL
Needle wash	In vial with water
Column temperature	80 °C

Agilent 1290 Infinity ELSD Method

Parameter	Setting
Nebulizer temperature	90 °C
Evaporation temperature	60 °C
Gas flow	2.2 SLM, optimized: 1.2 SLM
Detector gain	1.2 PTM
Smoothing	2 seconds
Data rate	10 Hz

Agilent 385 ELSD Method

Parameter	Setting
Nebulizer temperature	90 °C
Evaporation temperature	60 °C
Gas flow	2.2 SLM
LED intensity	100 %
Smoothing	2 seconds
Data rate	10 Hz

Chemicals

- Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

- Lactose was purchased from Sigma-Aldrich, Germany. A solution of 5 g/L was used as stock solution. For the calibration, the following dilutions were used: 5,000 mg/L – 2,500 mg/L – 1,000 mg/L – 500 mg/L – 250 mg/L – 100 mg/L – 50 mg/L.

Results and Discussion

For the determination of the limit of detection (LOD) and the limit of quantification (LOQ) of lactose for the 385 ELSD, a series of diluted concentrations was measured. The highest used concentration level was 5,000 mg/L lactose, down to the LOQ at 100 mg/L, which was found at a signal-to-noise (S/N) level of 10 (S/N = 10) (Figure 1).

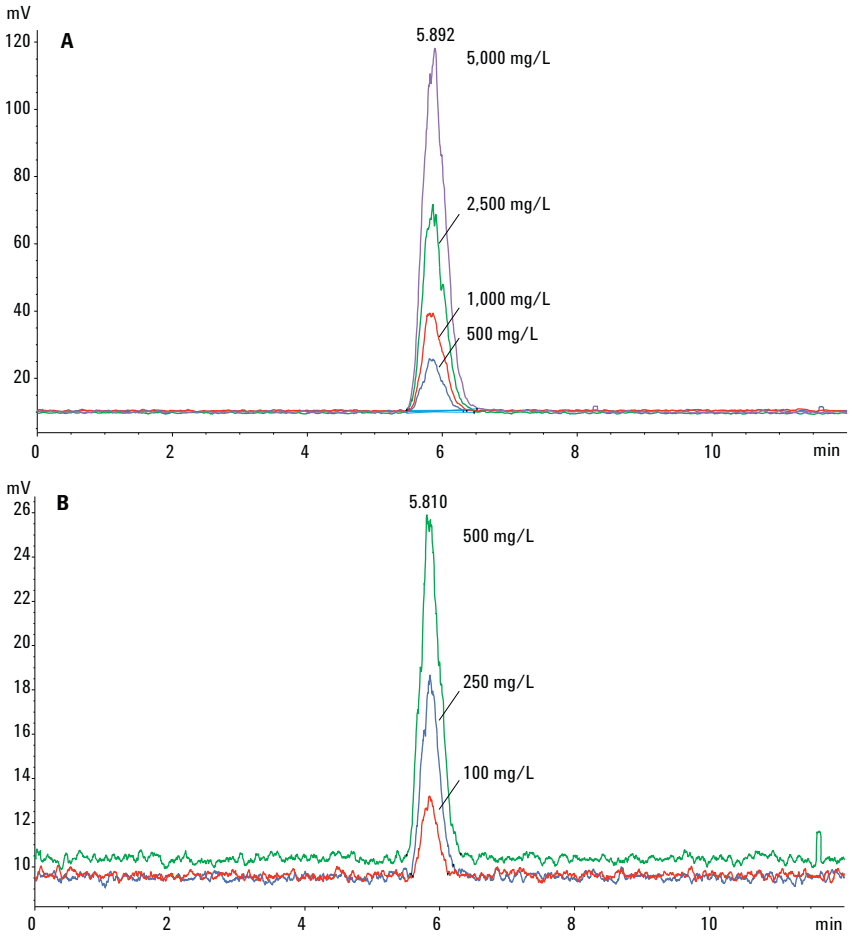

Figure 1. Injection of a dilution series of Lactose for calibration and determination of the LOQ and LOD on an Agilent 385 ELSD. **A**) 5,000–500 mg/L, **B**) 500–100 mg/L, LOQ 100 mg/L at S/N > 10, LOD at 50 mg/L at S/N < 3.

The measured dilution series was used to create a calibration curve to demonstrate the linearity of the 385 ELSD in this range. In the double logarithmic calibration plot, the linearity coefficient was 0.9987 (Figure 2). The LOD for lactose measured on the Agilent 385 ELSD was found at a concentration level of 50 mg/L, at an S/N = 2.5 (Figure 3). The relative standard deviation (RSD (%)) for lactose was determined from replicate measurement of the 500 mg/L concentration level. The retention time RSD was determined to be 0.29% and the peak area RSD 3.68% (Table 1).

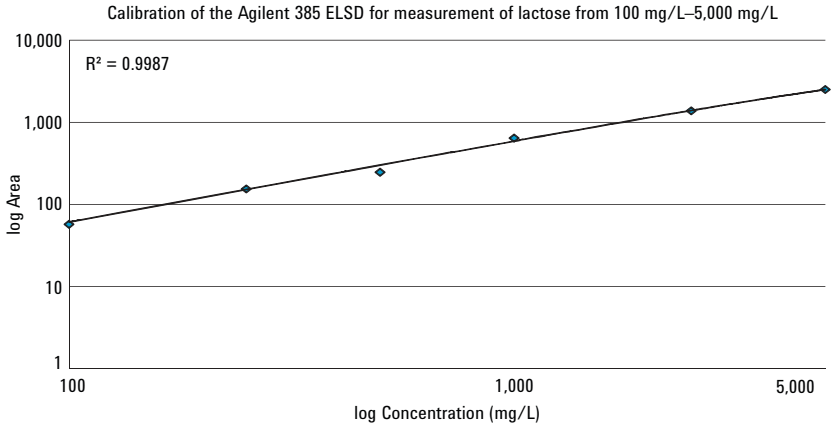

Figure 2. Calibration of the Agilent 385 ELSD for measurement of lactose from 100 mg/L–5,000 mg/L, LOQ 100 mg/L at S/N > 10.

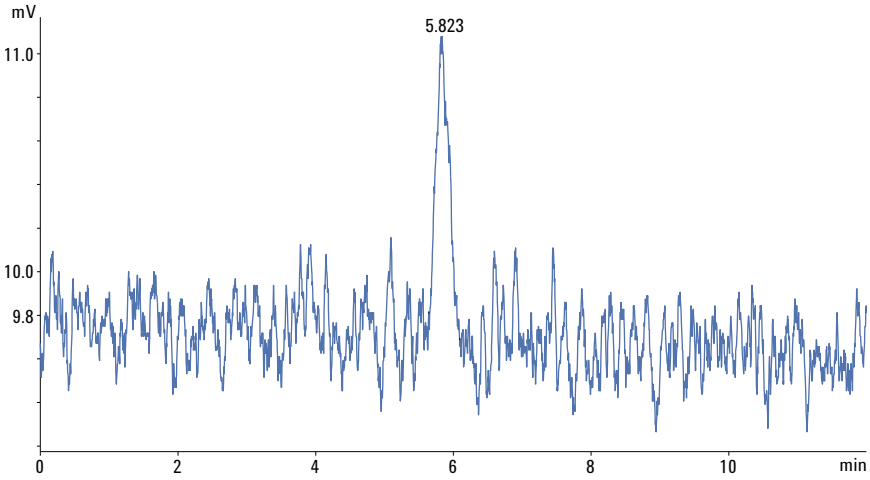

Figure 3. Lactose measured with the Agilent 385 ELSD at LOD, 50 mg/L, S/N = 2.5.

Table 1. Retention time and area RSD (%), lactose at 500 mg/L, n = 10.

	Retention time (min)	Area
Average	5.816	17.49
s.d.	0.016721	0.643843
RSD (%)	0.29	3.68

Transfer of the method for measurement of lactose from a 385 ELSD to the 1290 Infinity ELSD with the same settings gave an improvement in sensitivity of a factor of approximately 5–10. After optimization of the gas flow settings, the 1290 Infinity ELSD delivered an improvement in sensitivity of a factor of 20. The injection of a dilution series of lactose on a 1290 Infinity ELSD under optimized gas flow setting from 50 mg/L down to 5 mg/L (2.5 mg/L) showed the LOQ at 5 mg/L with S/N > 10 and the LOD at 2.5 mg/L with S/N < 3 (Figure 4)[1].

Transferring this method from the 385 ELSD to the 1290 Infinity ELSD under fully optimized conditions showed an improvement in sensitivity by a factor of 20. Table 2 summarizes all the achieved results for the 385 ELSD and the 1290 Infinity ESLD.

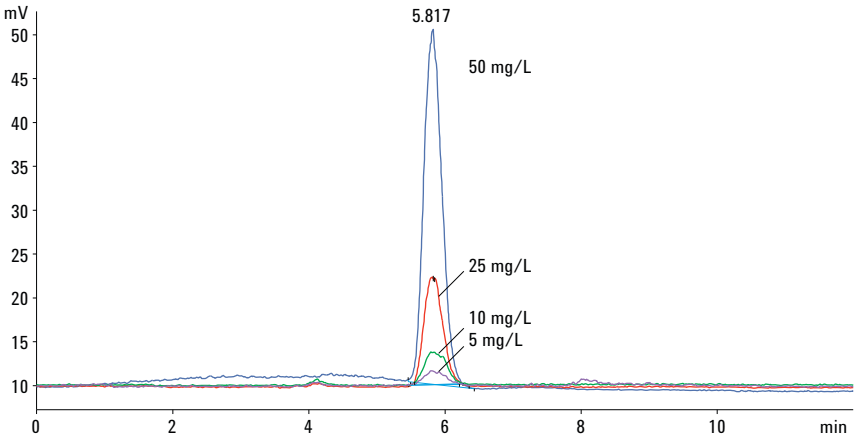

Figure 4. Injection of Lactose on an Agilent 1290 Infinity ELSD under optimized gas flow setting 50–5 mg/L, LOQ 5 mg/L at S/N > 10, LOD at 2.5 mg/L at S/N < 3.

Table 2. Comparison of LOD, LOQ and statistical parameters for the Agilent 385 ELSD and an Agilent 1290 Infinity ELSD.

	Agilent 385 ELSD	Agilent 1290 Infinity ELSD
LOD (S/N) = 3	50 mg/L	2.5 mg/L
LOQ (S/N) = 10	100 mg/L	5 mg/L
r.t. RSD (%)	0.29	0.11
Area RSD (%)	3.68	3.51

Conclusion

This Technical Overview demonstrates the ability to transfer methods from the Agilent 385 ELSD directly to the Agilent 1290 Infinity ESLD with immediate gain in sensitivity, and further sensitivity improvement by minor efforts in the adjustment of the method parameters.

References

1. Agilent Application Note, Publication number 5991-2252EN.

2. Agilent Technical Overview, Publication number 5991-2097EN.

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