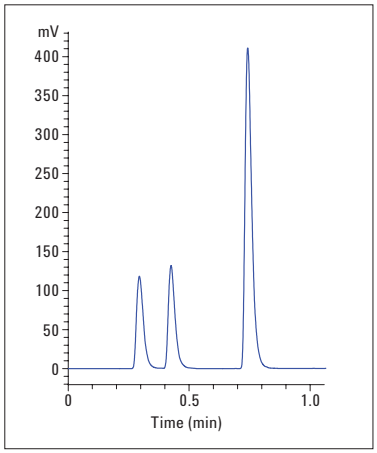
Performance Characteristics of the Agilent 1290 Infinity Evaporative Light Scattering Detector

Technical Overview



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Abstract

The Agilent 1290 Infinity Evaporative Light Scattering Detector (ELSD) provides a wide detection range for a variety of compounds with excellent sensitivity, down to low-nanogram levels, better efficiency and greater reproducibility. It is perfect for the analysis of semivolatile and nonvolatile compounds and compounds without UV chromophores such as most amino acids and sugars. The 1290 Infinity ELSD does not rely on the optical properties of a compound and is able to measure compounds without the need for derivatization and tedious sample preparation.

This Technical Overview demonstrates the improved sensitivity and performance of the 1290 Infinity ELSD coupled with the Agilent 1290 Infinity LC System. The 1290 Infinity ELSD is significantly more sensitive than any other ELSD and gives good data even in low concentrations.

Agilent Technologies

Introduction

The 1290 Infinity ELSD is an ideal detector for analytes with no chromophores. The ESLD detection is based on the universal property of the analyte and the blue laser light source delivers sensitive results in wide concentration ranges. The 1290 Infinity ELSD provides high-speed data output rates and therefore coupled with a 1290 Infinity LC the 1290 Infinity ELSD is perfect for fast LC applications. This Technical Overview shows significantly lower limits of detection than found with the Agilent 385-ESLD. The increased laser intensity coupled with a high gain photomultiplier and digital signal processing enhances signal and reduces noise.

This Technical Overview demonstrates great data reproducibility and shows an improved consistency of reliable and accurate results. Caffeine and three amino acids were separated and detected without derivatization. Detection was done using evaporative light scattering detector in-series with UV/DAD detection.

The 1290 Infinity ELSD may be used alone, or as one of several detectors on a HPLC system. The 1290 Infinity ELSD must be used as the last detector in-series if used in conjunction with other detectors because the solvent and eluent is evaporated during the analysis.

Principles of Operation

The 1290 Infinity ELSD is a highly sensitive detector for semivolatile and nonvolatile compounds in a liquid stream. The solvent stream containing the solute material is nebulized (1) and carried by a gas flow through an evaporation chamber (2). The solvent is volatilized, leaving a mist of solute particles that scatter light to a photosensitive device (3). The signal is amplified, and a voltage output provides the concentration of the solute particles passing through the optical chamber (Figure 1).

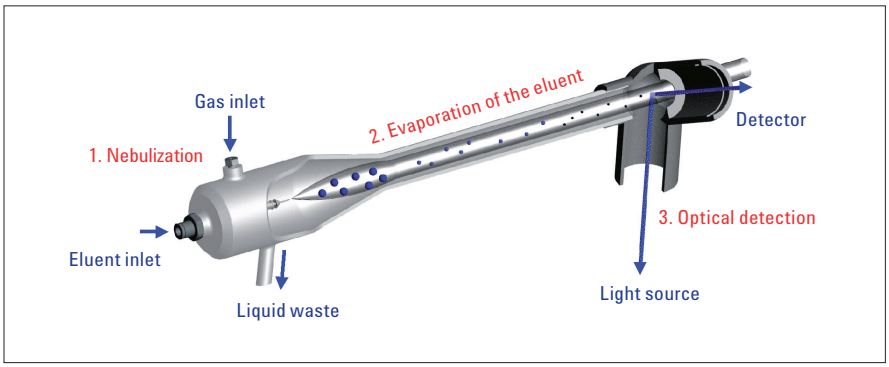

Figure 1
Schematic of the operational ELSD principles.

Agilent 1290 Infinity ELSD – Better by Design

- **Nebulization:** Efficient nebulization using low gas flow rates is a feature of Agilent ELSDs. Independent nebulizer temperature and gas flow control provide excellent stability and reproducibility. Baseline noise is minimized by removal of any poorly nebulized eluent through a drain port.
- **Evaporation:** The nebulized stream passes through an independently temperature-controlled evaporator tube where solvent is removed at high temperature, leaving the less volatile particles behind. The 1290 Infinity ELSD features patented gas flow control technology with a short evaporator tube. This results in an extremely low swept volume for minimal peak dispersion and provides maximum resolution from the separation (especially important for work with small columns).
- **Optical detection:** The solute particles are detected as they pass through the optical chamber. The 1290 Infinity ELSD is equipped with a blue laser light source, high gain photomultiplier, and digital signal processing which gives increased signal and greatly reduced baseline noise delivering improved sensitivity. Up to tenfold increase in limit of detection can be achieved.

Experimental conditions

Solvents

Acetonitrile was LC grade and purchased from Sigma Aldrich, St. Louis, MO, USA. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher.

Samples

Valine, Leucine and Phenylalanine were purchased from Sigma Aldrich, St. Louis, MO, USA with a purity of >98%. Caffeine was obtained from Agilent Technologies, Santa Clara, USA with a purity >99% (p/n 8500-6762).

Software

Agilent OpenLAB CDS ChemStation version A.01.04

Product number	Description
G4261B	Agilent 1290 Infinity Evaporative light scattering detector (Cooled)
G4212A	Agilent 1290 Infinity Diode Array Detector (10-mm path length flow cell)
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4226A	Agilent 1290 Infinity Autosampler
G4220A	Agilent 1290 Infinity Binary Pump

Table 1
System modules.

1290 Infinity ELSD Performance

Baseline ASTM Noise and Drift

The ASTM noise and drift were measured with a restriction capillary (p/n 5022-2159) and water as mobile phase. The noise level was collected at 40 Hz data output rate and a response time (smoothing=SMTH) of 20 (2 seconds). Smoothing means the data output can be averaged to produce a smoother response.

The noise and drift for the 1290 Infinity ELSD was collected over 30 minutes of measurement. The ASTM noise was found to be at 0.05 mV for the mean of seven replicates and the drift was 0.04 mV/h. Figure 2 shows an example chromatogram for the noise and drift over 30 minutes. The baseline noise can be minimized by the removal of any poorly nebulized eluent through a drain port and therefore provides better baseline stability.

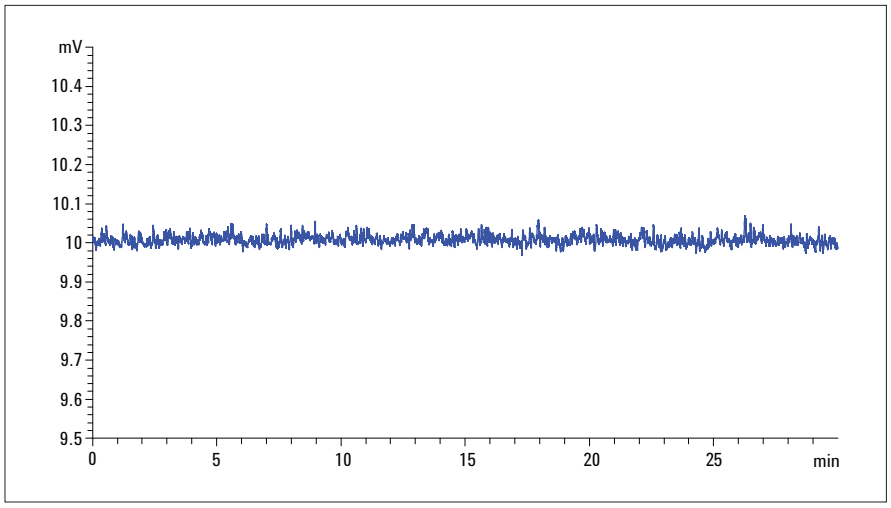

Figure 2
Chromatogram of 100% water and the ASTM noise with 0.05 mV and a drift of 0.04 mV/h over 30 minutes (n=7).

Chromatographic conditions	
Column:	Restriction capillary with 110 bar back pressure (p/n 5022-2159)
Mobile phase:	A = water
Flow rate:	1 mL/min
Stop time:	30 minutes
ELSD:	Evap. 40 °C / Neb. 40 °C / gas flow 1.2 SLM / SMTH 20 (2 s) / PMT Gain 1 / 40 Hz

Linearity and Dynamic Range

The linearity was tested using a test mixture containing an equimolar mix of valine (Val), leucine (Leu) and phenylalanine (Phe). These amino acids are considered to be nonvolatile and can be perfectly detected with the ELSD and also with the DAD, which is installed in front of the ELSD. The concentration range of the mixture is 0.05 mM to 20 mM with 10 levels. The amino acids were separated by gradient elution in approximately 3 minutes by reversed phase UHPLC.

Figure 3 demonstrates the good linearity of the 1290 Infinity ELSD in a concentration range between 0.05 mM to 20 mM. The coefficient of correlation for all compounds was > 0.993. The results show good linearity for all compounds in a concentration range from 5 to 3,300 ng on column. Correlation coefficients for valine, leucine, and phenylalanine ranged from 0.9933 to 0.9972 (Table 2). With these chromatographic conditions, the lowest response values for the amino acids were obtained with 5.85 ng valine, 6.55 ng leucine, and 8.25 ng phenylalanine on column. The highest response was at 2,340 ng of valine, 2,620 ng of leucine and 3,300 ng of phenylalanine. These results show an overall dynamic range of three orders of magnitudes within just a single gain setting of 1 PMT.

The linearity was measured with identical concentrations in an equimolar mixture but the compound responses show some variation between the amino acids which demonstrates that the 1290 Infinity ELSD provides universal responses for every compound.

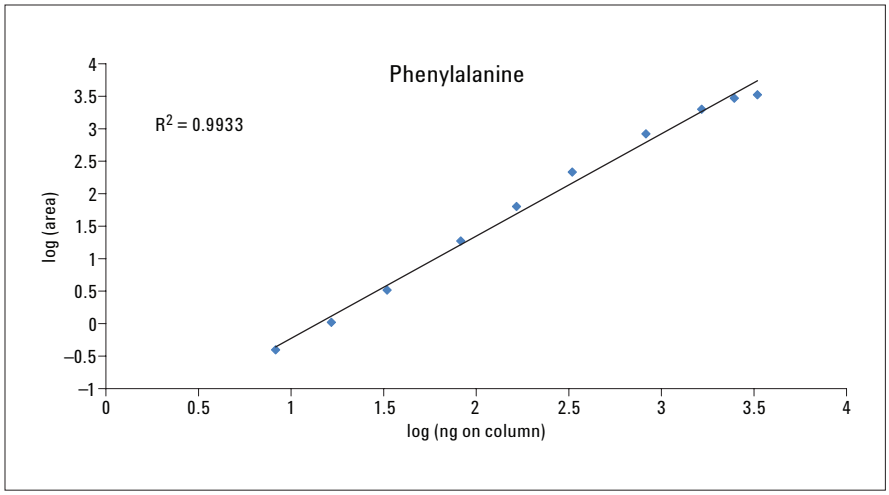

Figure 3
Dynamic range of the Agilent 1290 Infinity ELSD for all three amino acids in a concentration range between 0.05 to 20 mM.

Chromatographic conditions	
Column:	Agilent ZORBAX Eclipse Plus C18 RR HD, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase:	A: 2% acetonitrile in water
	B: acetonitrile
Flow rate:	0.6 mL/min
Gradient:	0 minutes 5 % B
	1.5 – 1.8 minutes 30% B
	1.8 – 2.8 minutes 100% B
Post time:	3 minutes
Column temperature:	25 °C
Injection volume:	1 µL
DAD:	210/4 nm, Ref off
ELSD:	Evap 50 °C/ neb 50 °C/ gas flow 1.0 SLM/ SMTH 1 (0.1 s)/ PMT Gain 1/ 40 Hz

Compound	R^2
Valine	0.9955
Leucine	0.9972
Phenylalanine	0.9933

Table 2
Coefficient of correlation for amino acids.

Sensitivity for Valine, Leucine and Phenylalanine

The sensitivity of the 1290 Infinity ELSD was determined with the limit of detection (LOD; S/N=3) and limit of quantification (LOQ; S/N=10) using a concentration of 0.1 mM for Val, Leu and Phe. The LOD and LOQ were calculated with the mean of seven replicates.

Figure 3 shows the chromatographic conditions.

Table 3 lists the LODs, LOQs, and signal-to-noise (S/N) values for Val, Leu, and Phe. Figure 4 shows a chromatogram for the amino acids at a 100 µM equimolar concentration and different gain settings. All compounds could be detected with high sensitivity. To increase the signal, adjust the detector gain (PMT) setting, especially for low concentrations. The gain sets the factor by which the detector output signal is amplified. When setting the gain, both the signal and noise are simply amplified by the value set, so S/N values are rarely affected. The raw signal output displayed on the parameter screen reflects this increase or decrease in signal amplification. The gain can be adjusted from 1 to 10. Table 4 shows three different gain settings and how they affect the LODs (S/N=3) for low concentrated samples (100 µM).

Especially for low concentrations, it is useful to increase the gain setting if manual integration is required. The change in LOD for different gain settings is more of a data acquisition effect rather than an ELSD effect.

In general, the droplet size determines the sensitivity of an ELSD. The larger the droplet, the larger the final particle will be at the optical stage, and the greater the scattering. To achieve the ideal droplet size, the user can control the nebulization gas and temperature.

Compound	LOD (ng/µL) (S/N=3)	LOQ (ng/µL) (S/N=10)
Valine	4.22	14.07
Leucine	8.09	26.97
Phenylalanine	5.02	16.72

Table 3
Shows the LOD, LOQ, and S/N values for valine, leucine, and phenylalanine (100 µM; n=7) with a gain setting of 1.

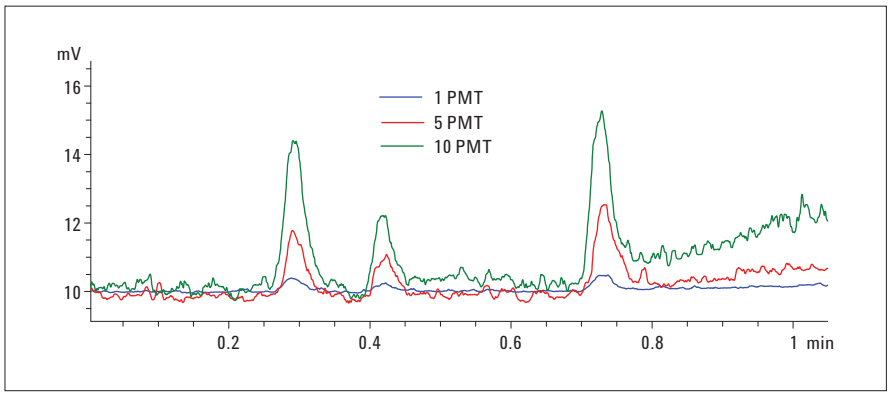

Figure 4
Chromatogram with 0.1 mM of valine (RT 0.290), leucine (RT 0.418), and phenylalanine (RT 0.731) at a gain setting of 1 (blue), 5 (red), and 10 (green). With a higher gain setting just the visibility of the compounds is better.

	LOD (ng/µL)		
Compound	Gain 1	Gain 5	Gain 10
Valine	4.22	3.74	2.75
Leucine	8.09	7.23	5.78
Phenylalanine	5.02	4.77	3.47

Table 4
LODs are rarely changed with a higher gain setting.

Leucine	1 PMT	5 PMT	10 PMT
Area	0.52	2.6	4.76
Height	0.23	1.23	2.24
S/N	6.04	5.43	6.79

Table 5
Different PMT gain settings will just change the visibility of the peaks in a chromatogram. Figure 5 shows leucine as an example.

Comparison DAD and ELSD - Detect Compounds with no UV Chromophores

The ELSD is especially useful for compounds, which have no chromophore and are not detectable with the DAD. Even in mid-range concentration (1 mM), valine and leucine are barely detectable with the DAD compared to the ELSD (Figure 5). To measure the difference in sensitivity between the DAD signal and the ELSD signal, the DAD and ELSD are connected in series on-line.

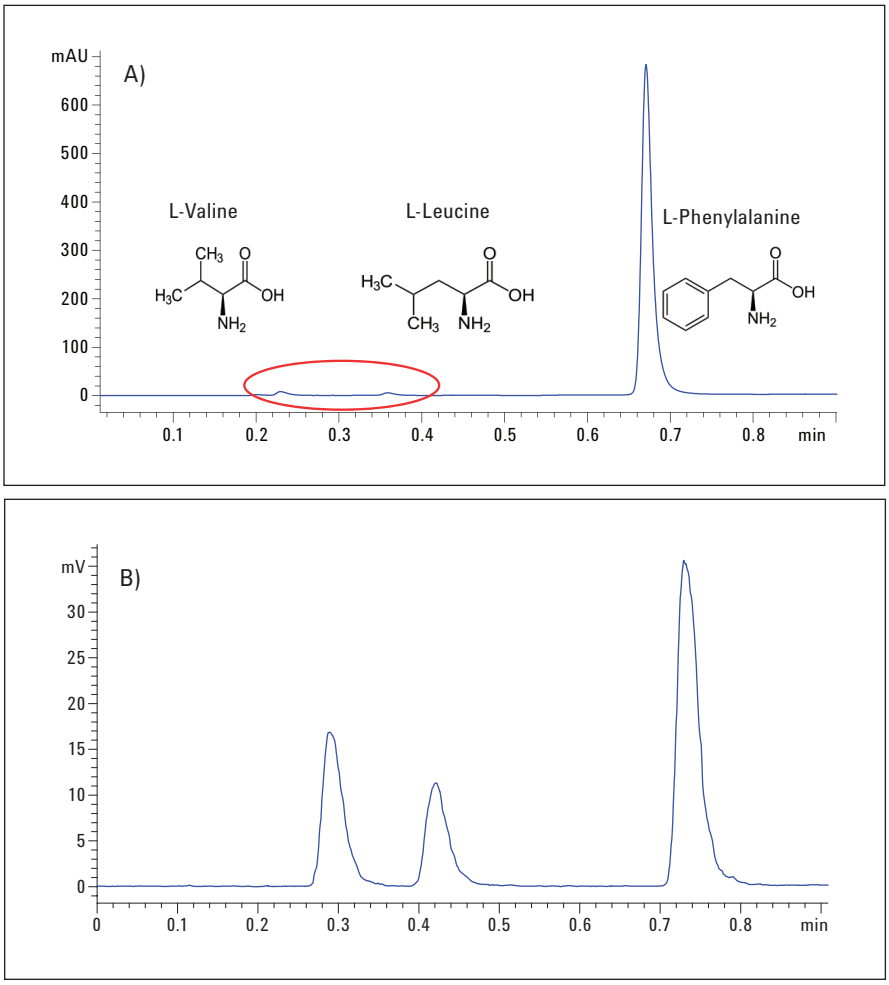

Figure 5
A) UV chromatogram of valine, leucine and phenylalanine. The first two compounds are almost not visible with UV detection. B) ELSD chromatogram for the same compounds.

Sensitivity for Caffeine

A good sensitivity was also shown for caffeine, a semivolatile compound. A 40 ng amount of caffeine on column was injected.

The S/N ratio for 40 ng caffeine on column was 29.2 and showed the good sensitivity of the 1290 Infinity ELSD, as depicted in Figure 6. The LOD for caffeine is 4.1 ng on column.

In summary, for amino acids and caffeine, two different chemical substance classes, very low sensitivities can be demonstrate. LODs for all compounds are below 10 ng on column and great S/N ratio was achieved for all tested compounds. The 1290 Infinity ELSD with cooling is ideal to detect even semivolatile compounds.

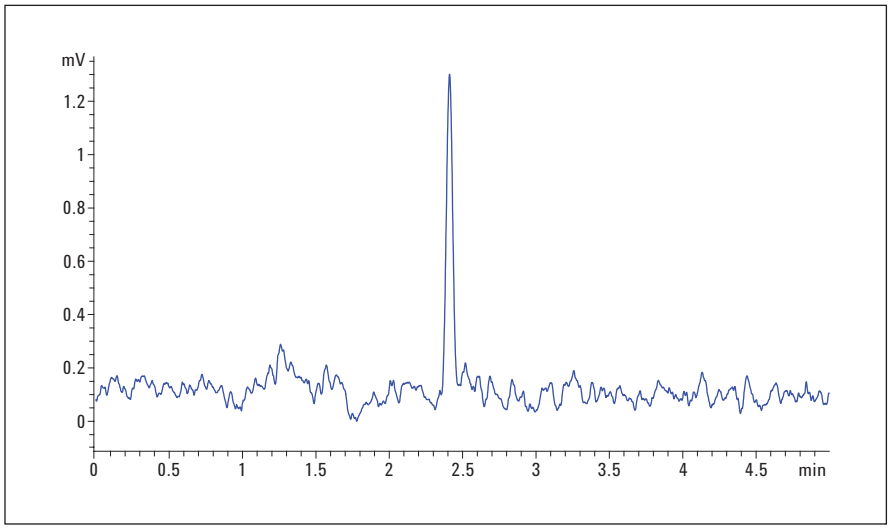

Figure 6
Injection of 40 ng caffeine on column with S/N ratio of 29.2.

Chromatographic conditions	
Column:	Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 µm (p/n 993967-902)
Mobile phase:	A: 80/20 (water/acetonitrile)
Flow rate:	1 mL/min
Gradient:	isocratic 5 minutes
Column temperature:	25 °C
Injection volume:	10 µL of 21 µM caffeine standard (p/n 8500-6762)
DAD:	273/4 nm, Ref off
ELSD:	Evap 40 °C/ neb 40 °C/ gas flow 1.2 SLM/ SMTH 20 (2 s)/ PMT Gain 5/ 40 Hz

Area and Retention Time Precision for Caffeine

Retention time and area precision was tested with 250 ng caffeine on column. The relative standard deviation (RSD) of retention times was 0.22% RSD and area precision was 1.10% RSD for the mean of seven replicates. Figure 7 shows the area and retention time precision.

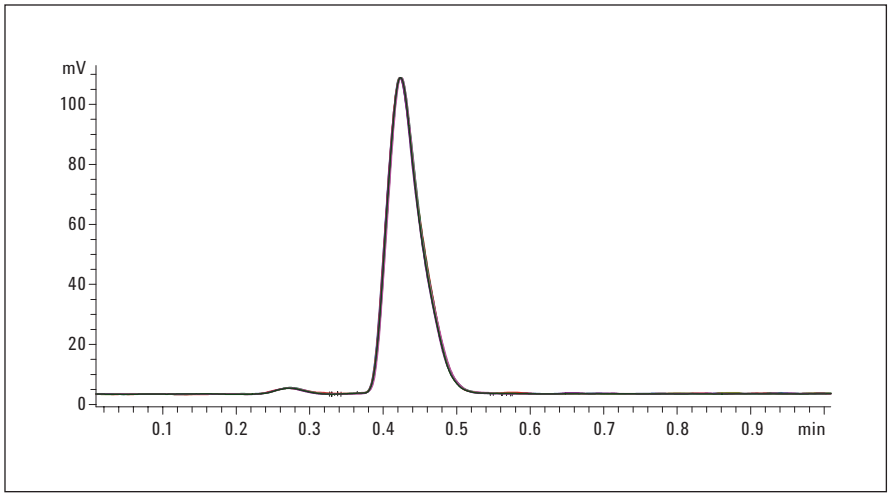

Figure 7
Overlay of seven replicates with 10 µL caffeine standard.

Chromatographic conditions	
Column:	Agilent ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 µm (p/n 993967-902)
Mobile phase:	A: 80/20 (water/acetonitrile)
Flow rate:	1 mL/min
Gradient:	isocratic 5 minutes
Column temperature:	25 °C
Injection volume:	10 µL of 25 µM caffeine standard (p/n 8500-6762)
DAD:	273/4 nm, Ref off
ELSD:	Evap 40 °C/ neb 40 °C/ gas flow 1.2 SLM/ SMTH 20 (2 s)/ PMT Gain 5/ 40 Hz

Conclusion

This Technical Overview demonstrates the high sensitivity of the Agilent 1290 Infinity ELSD coupled with an Agilent 1290 Infinity LC System. High quality data is presented where the ASTM noise was found at 0.05 mV and the drift was 0.04 mV/h. Excellent S/N values were found, for example, a S/N ratio of 29.2 was found for 40 ng caffeine on-column. The LODs for all tested compounds were < 10 ng on-column. The typical LOD was between 1 to 25 ng on-column for the most tested compounds using the 1290 Infinity ELSD. This high sensitivity was achieved because the 1290 Infinity ELSD uses a blue (405 nm) laser instead of a LED to maximize the scattering from small particles and digital signal processing to minimize baseline noise

The dynamic range is approximately three orders of magnitude for the analyzed amino acids and typically, for most compounds, between two and three orders of magnitude.

A great RSD value for retention time and area precision could be achieved. Precision of retention times was typically 0.22% RSD and the precision for areas was typically 1.10% RSD.

The laser-based 1290 Infinity ELSD provides significantly higher sensitivity, better efficiency, and greater reproducibility. The 1290 Infinity ELSD is ideally suited for the detection of semivolatile and nonvolatile solutes in pharmaceutical, drug discovery, food quality testing, and chemical analysis. Also, low dispersion and high speed data output rates (80 Hz) are ideal for fast LC applications, especially for applications with small columns.

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