Optimizing the Performance of the Agilent 1290 Infinity Evaporative Light Scattering Detector

Technical Overview

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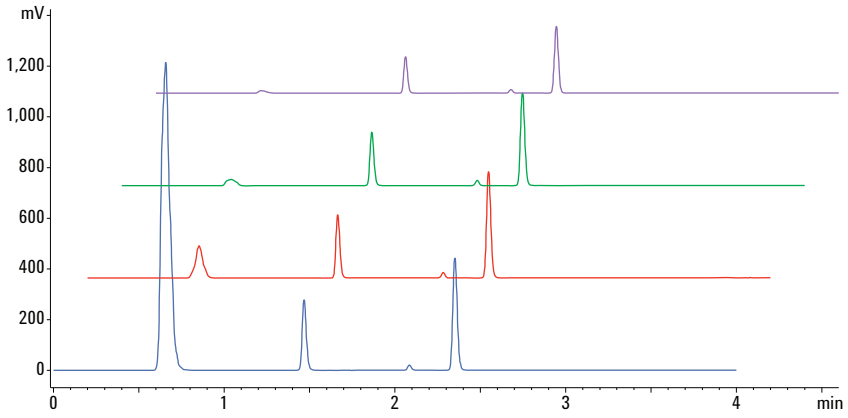
Abstract

This Technical Overview demonstrates how to achieve highest performance of the Agilent 1290 Infinity Evaporative Light Scattering Detector (ELSD). This Technical Overview shows and explains the influence of different parameter settings on sensitivity and resolution.

The primary advantage of the Agilent 1290 Infinity ELSD is the ability to adjust settings such as evaporator temperature and gas flow to remove, for example, dimethylsulfoxide (DMSO) at the low evaporator temperature of 30 °C. Additionally, this Technical Overview summarizes the influence of evaporator temperature and gas flow on the limit of detection (LOD). With the presented tips, it is easy to get the best ELSD results regarding sensitivity and resolution without further tedious method modification.



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Introduction

The advantage of the 1290 Infinity ELSD compared to detectors such as UV or MS, is the ability to analyze compounds with no chromophore, and do not ionize well. The increased laser intensity of the 1290 Infinity ELSD, coupled with a high gain photomultiplier and digital signal processing, enhances signal and reduces noise. Additionally, the ELSD handles high flow rates and rapid gradient separation and detects fast eluting semivolatile compounds.

This Technical Overview presents tips and tricks showing how to adjust different parameter settings of the 1290 Infinity ELSD to achieve best performance. One important parameter setting is the evaporator gas flow. For many applications, for example, in combinatorial chemistry or medicinal chemistry, it is necessary to remove DMSO, which is used as sample solvent, from the chromatogram. Only the 1290 Infinity ELSD has the ability to achieve DMSO transparency with two options:

- DMSO can be removed from the chromatogram with high evaporator temperature, but for some compounds, sensitivity is decreased.
- The design of the 1290 Infinity ELSD makes it possible to achieve DMSO transparency at low evaporator temperature by increasing the evaporator gas flow.

The evaporator temperature has also a huge impact on the sensitivity, especially regarding measurements around the LOD. This is demonstrated using an example of three amino acids.

The evaporator gas flow has, besides the evaporator temperature, the largest effect on the sensitivity. The unique design of the 1290 Infinity ELSD uses a patented gas flow technology in the evaporation zone to aid evaporation at low temperatures. The added stream of nitrogen, also called evaporation gas, makes it possible that less volatile solvents are easily evaporated. To efficiently remove the mobile phase, the user can regulate the evaporation gas flow and the evaporation temperature.

The smoothing factor, also called response time, is another major parameter. It has an impact on the peak width, height, resolution, and noise. This Technical Overview shows how to choose the smoothing factor according to the application.

Experimental

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

Amino acids: valine, leucine and phenylalanine as well as acetanilide, thiourea, acetaminophen, phenacetin, and dimethylsulfoxide were purchased from Sigma Aldrich, St. Louis, MO, USA with a purity of >98 %.

Software

Agilent OpenLAB CDS ChemStation version A.01.04

Table 1 System modules.

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

Results and Discussion

DMSO transparency at 30 °C evaporator temperature

A majority of drug targets in combinatorial and medicinal chemistry are stored in DMSO for stability reasons, high solvating power and low toxicity. DMSO produces a huge solvent peak at the beginning of the chromatogram and early eluting compounds underneath the DMSO peak may not be detectable by UV, especially at low wave lengths.

The 1290 Infinity ELSD has two options to remove DMSO from the chromatogram. The first option is to increase the evaporator temperature. But for some compounds, a higher temperature decreases the sensitivity.

The second option is to remove the DMSO by increasing the evaporator gas flow in combination with low temperature. In this Technical Overview, four compounds are dissolved in DMSO, with one of the early eluting compound completely coeluting with DMSO.

Chromatographic Conditions

Column:	Agilent ZORBAX SB C8 4.6 × 50 mm, 5 µm (p/n 846975-906)
Mobile phase:	A: Water B: Acetonitrile
Flow rate:	1 mL/min
Isocratic:	0 minutes 5% B 4 minutes 100% B
Column temperature:	30 °C
Injection volume:	5 µL
ELSD:	Evap. temperature 25–38 °C Neb. 25 °C Gas flow 1.6–2.5 SLM SMTH 10 (1 second) PMT gain 1/40 Hz
Sample:	Equimolar mixture of thiourea, acetaminophen, acetanilide, and phenacetin (2.3 mM)

Figure 1 shows a blank injection of DMSO. As shown, DMSO can be removed in two ways with the 1290 Infinity ELSD. Either with an evaporator temperature of 38 °C, or a gas flow rate of 2.5 SLM (standard liter per minutes) and a low temperature of 30 °C.

In a second experiment, an equimolar mixture (2.3 mM) of thiourea, acetaminophen, acetanilide, and phenacetin dissolved in DMSO was injected. Figure 2 shows that Thiourea (peak 2) has the same retention time (RT) as DMSO (peak 1, RT 0.6–0.75 minutes). For a sensitive detection of thiourea, it is necessary that all DMSO is removed from the chromatogram.

Figure 2 shows that a minimum evaporator temperature of 38 °C is necessary to remove the DMSO peak, however, acetanilide (peak 4) shows decreasing peak height with increasing evaporator temperature.

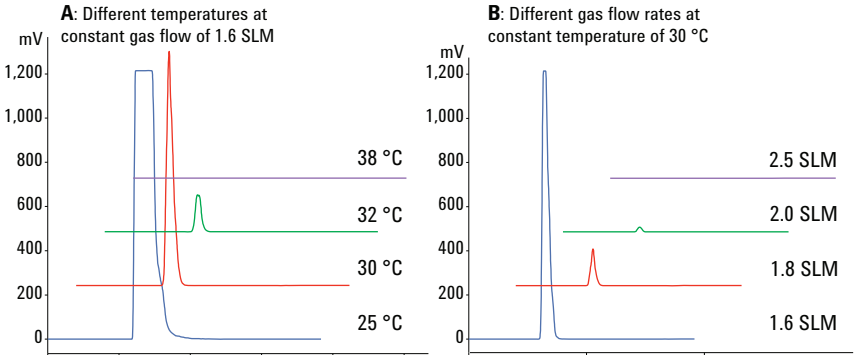


Figure 1. Pure DMSO removed with A) an evaporator temperature of 38 °C, and B) a gas flow rate of 2.5 SLM at 30 °C evaporator temperature.

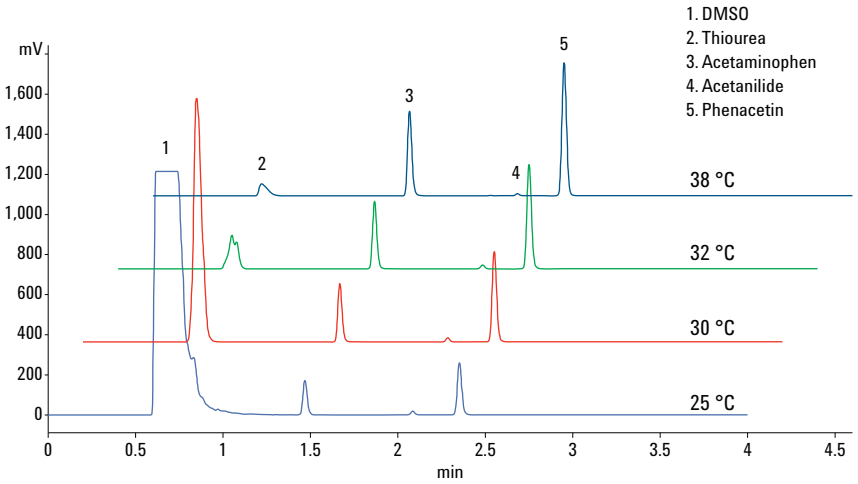


Figure 2. Chromatograms of different compounds dissolved in DMSO.

In another experiment (Figure 3), DMSO was removed without changing the evaporator temperature. DMSO transparency was accomplished by increasing the evaporator gas flow rate from 1.6 to 2.5 SLM at an evaporator temperature of 30 °C (and nebulizer temperature of 25 °C).

A moderate gas flow rate of 2.5 SLM removes the DMSO completely without increasing the evaporator temperature. Peak 2, (thiourea) can be detected and the low evaporator temperature makes it possible to analyze the thermally labile compound acetanilide (peak 4) in the same run.

In contrast to Figure 2, Figure 3 shows the intensity of semivolatile compounds, such as acetanilide (peak 4), is less reduced.

Figure 4 shows a direct comparison of acetanilide with a 38 °C evaporator temperature (blue peak) and a 2.5 SLM evaporator flow rate (red peak) at 30 °C. Previous chromatograms have shown that the DMSO transparency is achieved with these settings. Table 2 lists the areas of acetanilide to show the effects of different evaporator temperatures and gas flow rate.

Table 2 demonstrates that the area of acetanilide is stable for a certain evaporator temperature and gas flow rate. With an evaporator temperature of 38 °C, the peak area is reduced to less than 50%. With a constant evaporator temperature of 30 °C and increased gas flow rate of 2.5 SLM, the loss of peak area is less than 25%.

Tip: The 1290 ELSD provides full DMSO transparency and permits detection of early eluting compounds. The system enables the removal of DMSO either by increasing the evaporator temperature or by increasing the evaporator gas flow rate.

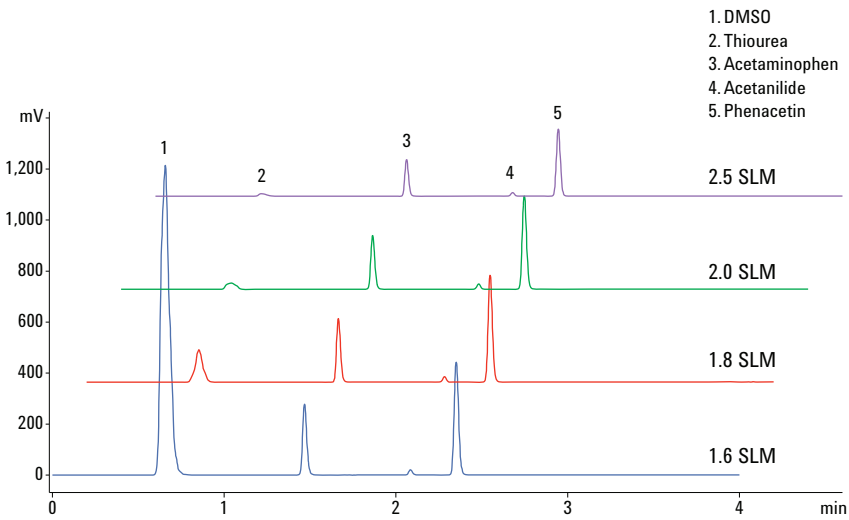
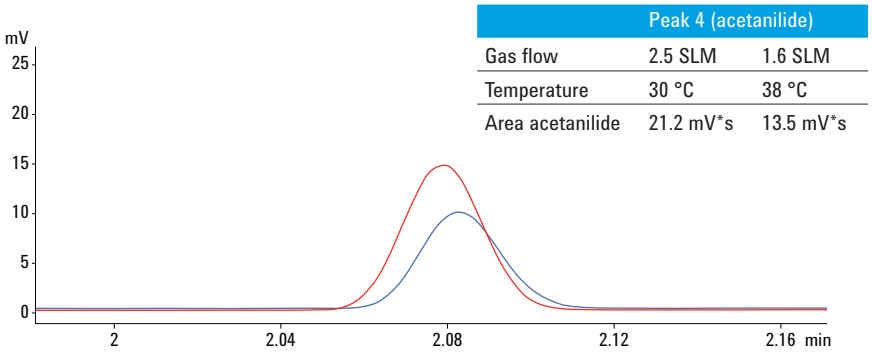


Figure 3. DMSO transparency by increasing the evaporator gas flow rate with a constant evaporator temperature at 30 °C.



	Peak 4 (acetanilide)	
Gas flow	2.5 SLM	1.6 SLM
Temperature	30 °C	38 °C
Area acetanilide	21.2 mV*s	13.5 mV*s

Figure 4. Overlay of acetanilide (peak 4) at evaporator temperatures of 38 °C (blue) and at 30 °C with a flow rate of 2.5 SLM (red).

Table 2. Different evaporator settings and the effects on acetanilide peak area.

Area of acetanilide with different evaporator temperatures		Area of acetanilide with different evaporator gas flow rates		
25 °C	29.0 mV*s	1.6 SLM	29.9 mV*s	
30 °C	29.7 mV*s	1.8 SLM	30.6 mV*s	
32 °C	27.8 mV*s	2.0 SLM	28.8 mV*s	
38 °C	13.5 mV*s	2.5 SLM	21.2 mV*s	← DMSO removal

DMSO removal maximizes the sensitivity of compounds that typically coelute with the DMSO peak. Gas flow of 1.8 SLM is normally enough to remove or significantly reduce the DMSO peak at 30 °C. If a flow rate of 1.8 SLM is not enough, increase the gas flow until the DMSO is removed.[2,3]

Evaporator Temperature Effect on LOD

The evaporator temperature has a huge effect on the sensitivity of different compounds. To show the temperature effect in more detail, three amino acids were measured around their LOD level.

Chromatographic Conditions

Column:	Agilent ZORBAX Eclipse Plus C18 RRHD, 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 40–70 °C Neb 50 °C Gas flow 1.0 SLM SMTH 1 (0.1 seconds) PMT gain 1/40 Hz
Sample:	Valine, leucine, phenylalanine (0.1 mM)

An equimolar mixture of: valine (RT 0.29 minutes), leucine (RT 0.42 minutes), and phenylalanine (RT 0.73 minutes) was measured at an evaporator temperature of 40 °C, 50 °C, 60 °C and 70 °C.

Figure 5 shows an overlay of all chromatograms generated with different temperatures.

Figure 5 and Table 3 show that the evaporator temperature has a significant effect on the sensitivity. If the temperature is too low, for example, 40 °C, the evaporation tube is not able to remove the solvent completely, which leads to a low sensitivity. The optimum for these amino acids is reached at 50 °C. With higher temperatures, such as 60 °C and 70 °C, the sensitivity decreases. Table 3 indicates that with nonoptimized temperature settings, the signal, and signal-to-noise (S/N) are reduced.

Tip: To give a general idea how to choose the right temperature, set the temperature at approximately 80 °C to 90 °C for nonvolatile compounds. For semivolatile compounds and compounds with a low molecular weight, set the temperature at approximately 20 °C to 30 °C. To optimize an ELSD method, choose a midrange temperature and adjust it to see if there is any significant intensity effect.[2,3]

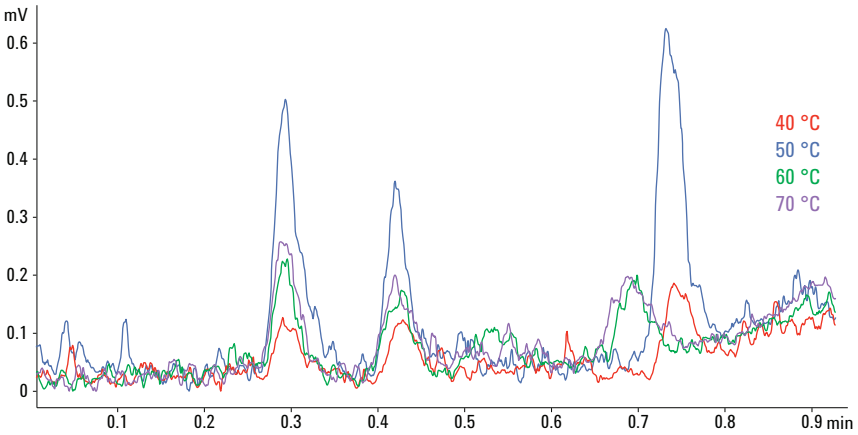


Figure 5. Overlay chromatograms of amino acids (0.1 mM) with different evaporator temperatures.

Table 3. Amino acids with different temperatures and their signal, noise, and S/N.

40 °C	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	1.83	0.06	3.14
	Leucine	1.83	0.06	3.24
	Phenylalanine	2.50	0.06	4.45
50 °C	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	9.25	0.04	15.14
	Leucine	4.75	0.04	8.52
	Phenylalanine	11.01	0.04	17.91
60 °C	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	4.75	0.04	8.77
	Leucine	4.01	0.04	7.35
	Phenylalanine	3.25	0.04	6.20
70 °C	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	6.01	0.04	10.69
	Leucine	4.50	0.04	7.68
	Phenylalanine	3.75	0.04	6.57

Evaporator Gas Flow Effect on LOD

Another very important parameter is the evaporator gas flow which has, beside the evaporator temperature, the largest effect on the sensitivity. To increase sensitivity, the gas flow has to be optimized in addition to the temperature. In this experiment, the gas flow was varied between 1.0, 1.3, and 1.6 SLM with a constant temperature of 50 °C to show the huge impact of the sensitivity.

Figure 6 shows an overlay of three chromatograms for valine, leucine, and phenylalanine with a concentration of 0.1 mM. Figure 6 and Table 4 show that decreasing the evaporator gas flow from 1.6 SLM to 1.0 SLM doubles the signal intensity. As demonstrated by the previous experiment, a midrange evaporator temperature of 50 °C and a low evaporator gas flow of 1.0 SLM is the best choice for these compounds.

The adjustable evaporator gas flow of the 1290 Infinity ELSD is primarily dependent on the mobile phase, while the temperature is set according to the compound of interest. This is very important, and differentiates the 1290 Infinity ELSD from other ELSDs. The user should be aware of the evaporator gas and temperature parameters which belong closely together.

Tip: The evaporator gas flow is responsible for the ELSD´s evaporation process control. Set the gas value according to the mobile phase. An aqueous eluent needs a higher gas flow rate than an organic solvent. Generally, the higher the evaporator temperature, the lower the gas flow setting is required, regardless of mobile phase composition. Conversely, if the evaporator temperature is reduced, the gas flow needs to be increased for compensation.[1]

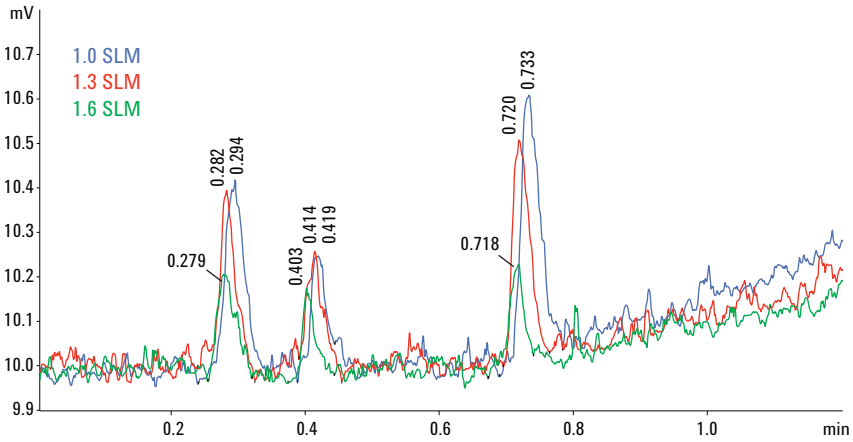


Figure 6. Overlay of three consecutive runs of three amino acids with different gas flow rates and their effects on sensitivity.

Table 4. Amino acids with different evaporator gas flow rates and the influence on the signal, noise, and S/N.

1.6 SLM	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	5.4	0.05	9.7
	Leucine	2.6	0.05	4.6
	Phenylalanine	5.2	0.05	9.5
1.3 SLM	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	6.3	0.06	12.1
	Leucine	4.6	0.06	8.5
	Phenylalanine	8.5	0.06	15.1
1.0 SLM	Compound	Signal (mV)	Noise (mV)	S/N
	Valine	9.25	0.04	15.14
	Leucine	4.75	0.04	8.52
	Phenylalanine	11.01	0.04	17.91

Smoothing Factor Effects

The functionality of the smoothing factor is to mathematically average the output data to achieve smoother response and to reduce noise. The smoothing factor is set to the number of data points over which the data is averaged and can be regarded as a digital time constant.[1]

In this experiment, an equimolar mixture of 1 mM valine, leucine, and phenylalanine was analyzed with four different smoothing rates of 1 (0.1 second), 10 (1 second), 20 (2 seconds), and 30 (3 seconds) and with a fixed data rate of 40 Hz.

Figure 7 and Tables 5 and 6 demonstrate the effects of the smoothing factor on peak width, resolution, peak height, peak area, and noise. For fast applications, such as in this experiment, a small smoothing factor is recommended to give a good and accurate peak shape and area. The resolution between the peaks valine and leucine is better with a small smoothing factor.

A higher smoothing factor has the advantage of decreased noise.

Tip: For most applications, a smoothing factor of 10 to 30 (1 to 3 seconds) can be used. However, to gain highest sensitivity, decrease the smoothing rate to enhance the signal height. This recommendation is valid for fast separations where peak widths are very small (< 3 seconds) and the smoothing factor also has an impact on the resolution.[1] For applications requiring highest sensitivity, the smoothing factor has to be optimized for maximum peak height and minimum noise.

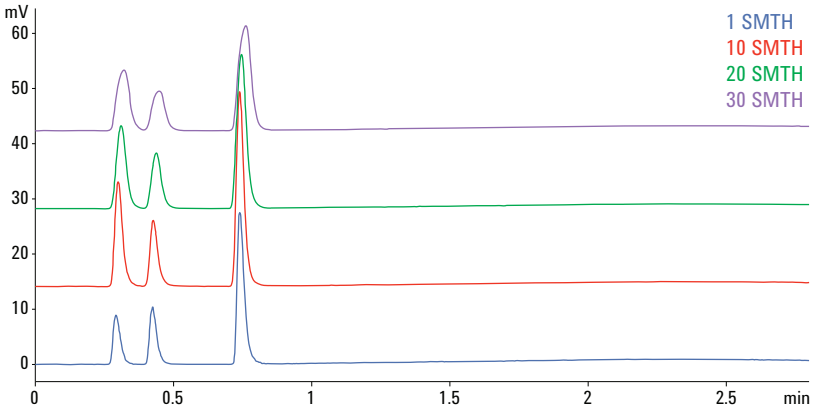


Figure 7. Overlay of four runs and the effect of different smoothing factors.

Table 5. Effects of the smoothing factor on the peak width and the resolution for valine and leucine and phenylalanine.

Peak width (5*sigma)				
Smooting rate	1	10	20	30
Valine	0.067	0.073	0.086	0.098
Leucine	0.066	0.077	0.087	0.099
Phenylalanine	0.070	0.071	0.085	0.098
Resolution				
Smoothing rate	1	10	20	30
Valine/leucine	2.72	2.29	1.91	1.44

Table 6. Comparison of peak height and area of three amino acids and different smoothing factors.

Peak height (mV)				
Smooting rate	1	10	20	30
Valine	17.8	17.6	14.2	10.7
Leucine	11.1	11.3	10	6.9
Phenylalanine	33.1	32.1	27.1	19.1
Noise	0.04	0.01	0.01	0.007
Peak area (mV*s)				
Smooting rate	1	10	20	30
Valine	33.5	35.8	36.5	34.1
Leucine	22.1	23.5	23.9	22.4
Phenylalanine	61.7	63.1	65.7	61.2

Conclusion

This Technical Overview facilitates method development to achieve the best performance with the Agilent 1290 Infinity ELSD.

With the unique design of the 1290 Infinity ELSD, the DMSO transparency can be achieved by enhancing the evaporator gas flow instead of increasing the evaporator temperature. Since high temperatures can result in a decrease in sensitivity for some compounds, DMSO can be totally removed at 30 °C evaporator temperature and a gas flow rate of 2.5 SLM.

In general, for method development, optimize the evaporator temperature first, because this is one of the most important parameters to gain the best sensitivity. Secondly, adjust the evaporator gas flow according to the temperature. Usually a high temperature needs a low gas flow rate to achieve the best sensitivity. This Technical Overview shows on three amino acids that an evaporator temperature of 50 °C and a very slow evaporator gas flow rate of 1.0 SLM gave the highest sensitivity.

Finally, the right smoothing factor has to be chosen. A smoothing rate of 20 (2 seconds) is satisfactory for the most applications. For fast applications, when good resolution or high sensitivity is required, decrease the smoothing factor.

References

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2. Agilent 1200 Infinity Series ELSD Service Manual, Part Number G4260-90001, **2012**
3. S. Ball, "Analysis of Pharmaceuticals using HPLC with Evaporative Light Scattering Detection", Agilent Technologies Application Compendium, Publication Number 5990-9455EN, **2012**

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