Analysis of a Fixed–Dose Combination Drug Using the Agilent 1290 Infinity II Evaporative Light Scattering Detector

Application Note

Small Molecule Pharmaceuticals and Generics

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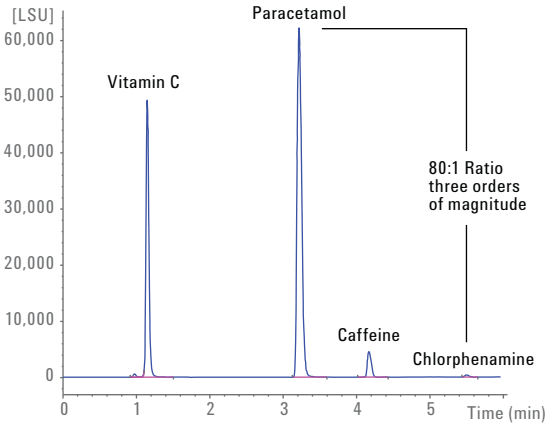
Abstract

Evaporative light scattering (ELS) is a unique and highly sensitive technique for detection of semivolatile and nonvolatile solutes in a liquid stream. As a consequence of the physics of light scattering, conventional ELS detectors exhibit nonlinear response and have a relatively small dynamic range. In contrast, the Agilent 1290 Infinity II Evaporative Light Scattering Detector (ELSD) has a more linear output and a 10-times higher dynamic range compared to conventional ELS detectors. To demonstrate the high dynamic range, a fixed-dose combination drug with low and high-dose compounds (250 to 20,000 ng/µL) was analyzed and quantified during a single run. Finally, the data was compared to the Agilent 1290 Infinity ELSD regarding area precision and sensitivity.



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Introduction

Evaporative light scattering (ELS) is ideally suited for the detection of compounds that do not have a UV chromophore. The physics of light scattering means that most ELS detectors cannot produce a voltage output greater than 1,000 mV, and that the typical dynamic range for conventional ELS detectors is two to three orders of magnitude. These characteristics make the analysis of compounds with a high concentration range difficult or even impossible. To overcome this challenge, the optical detection stage in the Agilent 1290 Infinity II ELSD uses automatic gain control to ensure an on-scale response at high concentrations, whereby at the limit of detection (LOD), the response is amplified to maximize signal-to-noise (S/N). This approach requires no user input to optimize the signal range. Hence, the 1290 Infinity II ELSD is the first ELS detector that can deliver a dynamic range of four orders of magnitude.

To demonstrate the high dynamic range of the 1290 Infinity II ELSD, this Application Note shows that a single run is adequate to reliably quantify low and high-dose compounds of a fixed-dose combination drug. All compounds were quantified within the linear range of the detector with reliable integration and quantification. These results were then compared to the results from a conventional ELS detector, and the precision of areas and sensitivity were evaluated.

Operating principle of the Agilent 1290 Infinity II ELSD

The 1290 Infinity II ELSD is based on a laser and exhibits the same sensitivity and LOD as a conventional ELS detector. The main difference is the inclusion of a high dynamic range (HDR) photomultiplier tube (PMT). The PMT extends the detector's dynamic range to four orders of magnitude.

The differences in dynamic range of the 1290 Infinity II ELSD and conventional ELS detectors can be explained by the differences in the use of the detector gains PMT. Conventional ELS detectors use the PMT with a linear response with respect to light input. As a result, the nonlinear response of the ELS detector is caused solely by the scattering effect. The 1290 Infinity II ELSD overcomes this problem by using a PMT with a nonlinear gain control with respect to light input. This nonlinear PMT has a response that is approximately inverse in shape to the natural response of the detector. As a consequence, the 1290 Infinity II ELSD continues to produce an *almost* linear response at higher concentrations beyond the 1,000-mV range. To reflect this wider dynamic range, the output signal of 1290 Infinity II ELSD is expressed in light scattering units (LSU). For a better overview, Figure 1 shows and explains the general dynamic range of high dynamic range and conventional ELS detectors.

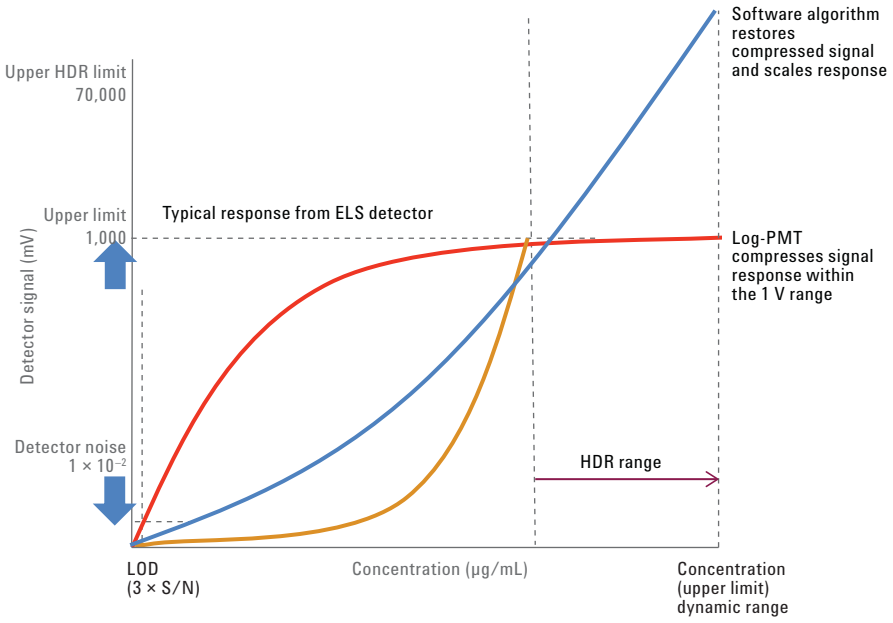

Figure 1. Schematic overview showing the different dynamic ranges of ELS detectors and the near-linear response of the high dynamic range ELS detector.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1290 Infinity II LC System comprising:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector with 10-mm flow cell (G7117B)
- Agilent 1290 Infinity II Evaporative Light Scattering Detector (G7102A)
- Agilent 1290 Infinity Evaporative Light Scattering Detector (G4261B)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS systems, revision C.01.07 [27]

Sample

A fixed-dose combination drug was used as sample with the following ingredients:

- Paracetamol and chlorphenamine at a ratio of 80:1
- Vitamin C
- Caffeine

Chromatographic conditions

Sample	Fixed dose cold medication
Injection volume	5 µL
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)
Column temperature	40 °C
Flow rate	1 mL/min
Mobile phase	A) Water + 0.1 % TFA
	B) Acetonitrile + 0.09 % TFA in water, 95/5
Gradient	5 %B at 0 minutes 5 %B at 0.5 minutes 40 %B at 6.1 minutes 95 %B at 6.5 minutes 95 %B at 8 minutes
Stop time	10 minutes
Post time	4 minutes
DAD	
Wavelength	254 nm/20 nm, reference 380/80, 10 Hz
Data rate	80 Hz
ELSD	
Evaporator temperature	55 °C
Nebulizer temperature	55 °C
Gas flow rate	1 SLM
Data rate	80 Hz
Smoothing	10

Sample preparation

1. Two capsules of cold medication were opened and dissolved in 20 mL of distilled water. The resulting solution contained:
    - 250 ng/µL chlorphenamine
    - 20,000 ng/µL paracetamol
    - 2,500 ng/µL caffeine
    - 15,000 ng/µL vitamin C
2. Extraction with ultrasonic bath for 5 minutes
3. Filtering with Agilent Premium Syringe Filter Captiva (regenerated cellulose) (p/n 5190–5109)
4. Clear liquid was filled and stored in 1.5-mL LC vials.
5. Dilution series with water (1:10, 1:20, 1:30, 1:40 and 1:50)

Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Merck, Germany. 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). Trifluoroacetic acid was purchased from Sigma Aldrich, St. Louis, USA.

Results and Discussion

The same 1290 Infinity II LC System configuration was used for all experiments except for the ELS detectors. To evaluate the performance differences between the conventional ELS detector and the 1290 Infinity II ELSD, the following experiments were performed:

- Analysis of a fixed-dose combination drug on a 1290 Infinity II LC system with a conventional ELS detector using two injections and different concentrations to determine high and low-dose drugs
- Analysis of a fixed-dose combination drug on a 1290 Infinity II LC System with the 1290 Infinity II ELSD using a single injection to determine low and high-dose drugs within the linear range

The high dynamic range of the 1290 Infinity II ELSD can be best shown by comparison to the conventional ELS detector, when the same sample is injected. Therefore, the fixed-dose combination drug was diluted 1:20, and 5 µL of the solution were injected. Figure 2 demonstrates that the 1290 Infinity II ELSD (red) can quantify the high-concentration peaks correctly without exceeding the linear range. Excellent peak shape for all compounds was obtained, while the conventional ELS detection (blue) showed saturation for the first high-concentrated (vitamin C and paracetamol) peaks. The last low-concentration peaks (caffeine and chlorphenamine) showed peak tailing and bad peak shape. To quantify all compounds in this fixed-dose combination drug with a conventional ELS detector, two injections of different dilutions would be necessary to quantify chlorphenamine, vitamin C, and paracetamol.

To demonstrate the wide concentration range of the 1290 Infinity II ELSD, a calibration curve is shown in Figure 3 for paracetamol, the highest peak in the chromatogram. Due to exponential response of the detector, the type of calibration curve *power* was chosen. The calibration curve showed an exceptional correlation factor of 0.9996 from 400 to 2,000 ng/µL.

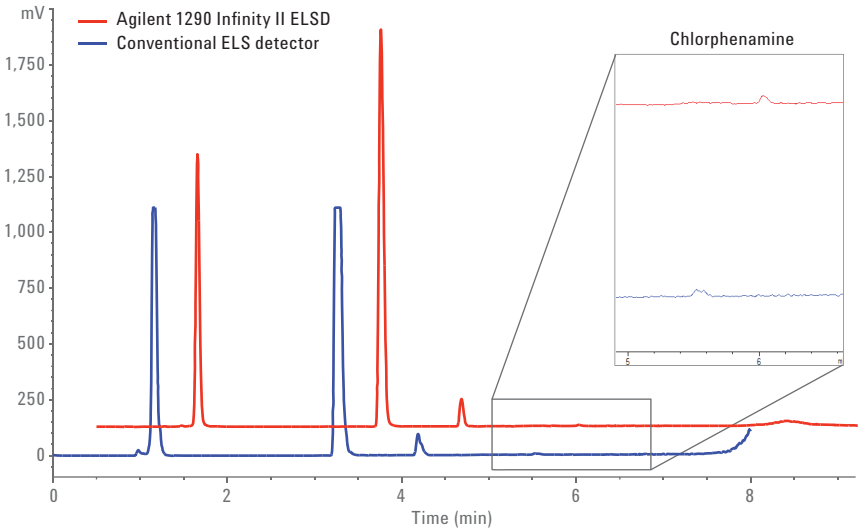

Figure 2. Fixed-dose combination drug was injected with the Agilent 1290 Infinity II ELSD (red) and the conventional ELSD (blue). The conventional ELSD is saturated with the high concentration of the first two peaks while the last two peaks show peak tailing and poor quantification properties.

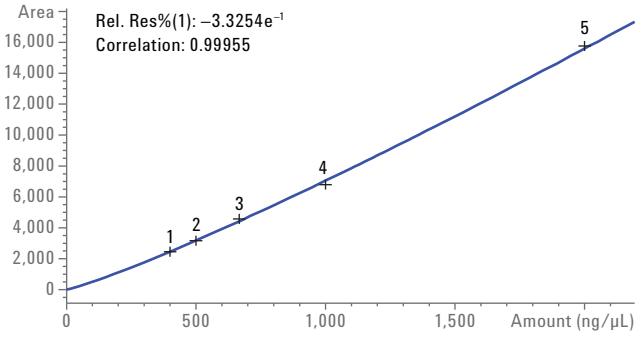

Figure 3. Agilent 1290 Infinity II ELSD: The wide concentration range (400 to 2,000 ng/µL) is shown by the example of paracetamol and its calibration curve with a correlation factor of 0.9996.

Even the smallest peak, chlorphenamine, with a total ratio of 1:80 relative to paracetamol, showed good quantification properties and had a good correlation factor of 0.9969 from 5 to 25 ng/µL, see Figure 4.

Because of the high dynamic range of the 1290 Infinity II ELSD, even the undiluted fixed-dose combination drug can be analyzed (see Figure 5), with three orders of magnitude between the highest and the lowest peak in the chromatogram without exceeding the saturation point of the detector.

As shown in Figure 2, the dynamic range of the conventional ELS detector was too small to determine the dilution 1:20 of the fixed-dose combination drug, so a further dilution up to 1:50 was necessary.

The 1:50 dilution was used to show and compare the performance of the two detectors in Table 1. Because of the dilution of 1:50, the conventional ELS detector can quantify paracetamol and vitamin C without exceeding the dynamic range, but, in return, the smallest peak in the chromatogram, chlorphenamine, could not be detected anymore. Also, caffeine shows a high area RSD at 15.25 %.

In comparison, with the high dynamic range 1290 Infinity II ELSD all compounds showed a good area RSD of less than 2 %, except chlorphenamine with 17.81 %. But chlorphenamine can still be quantified with a S/N of 16.

In summary, these results demonstrate that the conventional ELS detector cannot analyze a fix-dose combination drug with a wide concentration range reliably in a single run. In contrast, the high dynamic range 1290 Infinity II ELSD achieved excellent results for all compounds. With a 10-times wider dynamic range, one injection was enough to quantify low and high-dose compounds of a fixed-dose combination drug with excellent results.

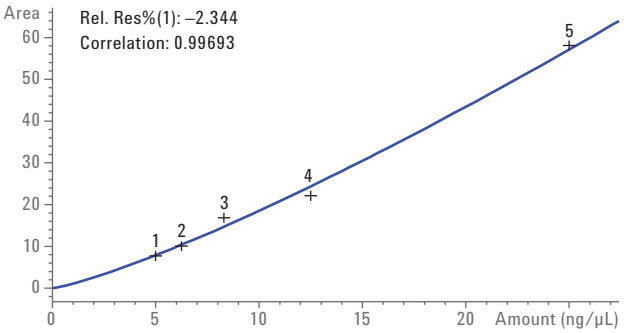

Figure 4. Agilent 1290 Infinity II ELSD: Chlorphenamine shows a good correlation factor of 0.9969 with a concentration range from 5–25 ng/µL.

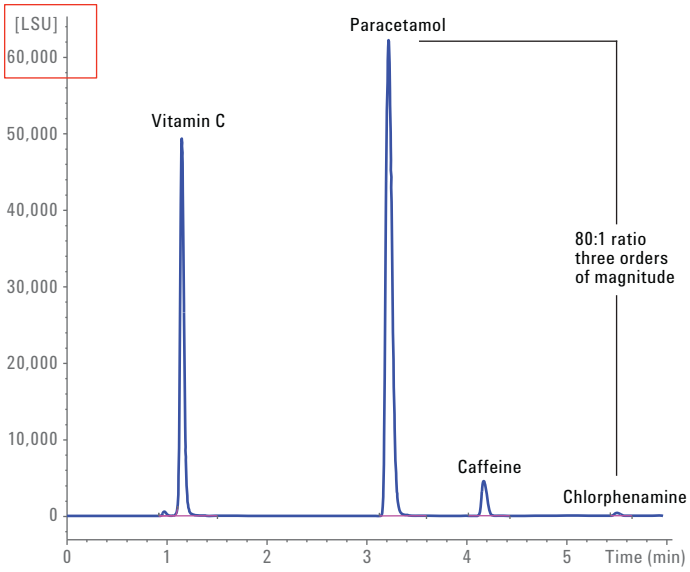

Figure 5. Even the undiluted fixed-dose combination drug can be injected without reaching the saturation point of the Agilent 1290 Infinity II ELSD. Three orders of magnitude are between the highest and lowest peak with a ratio of 1:80

Table 1. Comparison of the Agilent 1290 Infinity II ELSD to a conventional ELSD (n = 5).

Compound	Agilent 1290 Infinity II ELSD area RSD (%)	Conventional ELSD area RSD (%)
Vitamin C	2.73	4.23
Paracetamol	1.38	1.33
Caffeine	2.06	15.27
Chlorphenamine	17.81	–

Conclusion

This Application Note shows the performance and efficiency gain in sample throughput of the Agilent 1290 Infinity II Evaporative Light Scattering Detector coupled to an Agilent 1290 Infinity II LC System. Due to the 10-times higher dynamic range, a fixed-dose combination drug with low and high-dose compounds can be analyzed and quantified with just one injection. The dynamic range and area RSDs are compared to a conventional ELS detector. While the conventional ELS detector is not able to detect the smallest and highest peak with one sample injection, good results are found for the 1290 Infinity II ELSD, even for the smallest peak in the chromatogram. The extended dynamic range of the 1290 Infinity II ELSD enables detection and quantification of compounds in a fixed-dose combination drug, which have a concentration ratio of more than 80:1.

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This information is subject to change without notice.

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