High-Resolution Sampling 2D-LC with the Agilent 1290 Infinity II 2D-LC Solution

Reliable Quantification of Coeluting Substances

Suitable for Agilent
1290 Infinity III LC

Technical Overview

Author

Susanne Stephan
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The Agilent 1290 Infinity II 2D-LC solution presents the ability to easily switch between comprehensive 2D-LC (LCxLC), multiple heart-cutting 2D-LC, or high-resolution sampling 2D-LC. This Technical Overview shows the benefit of high-resolution sampling 2D-LC for reliable quantification of substances coeluting in the first dimension (^{1}D). The quantification of compounds typically contained in green tea is used as an example. The compounds (+)-catechin and (–)-epicatechin were separated in the first dimension, and could be quantified based on their ^{1}D peaks using the standard workflow. The compounds (–)-epigallocatechin gallate and caffeine coelute in the first dimension, but can be separated in the second dimension. High-resolution sampling 2D-LC ensures that entire ^{1}D peaks can be transferred to the second dimension (^{2}D) column. Using this technique, a reliable quantification of the two compounds based on their second-dimension peaks was possible.

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Introduction

Quantitative analysis of compounds in a real sample can be realized for many applications with one-dimensional liquid chromatography (1D-LC). A reliable quantification, however, is only possible if there are no overlapping peaks. Using two-dimensional liquid chromatography (2D-LC) for the analysis of complex samples, compounds coeluting in the first dimension can be separated in the second dimension.

Whereas in comprehensive 2D-LC (LCxLC) the entire sample is fractionated and each fraction is transferred to the ^{2}D column[1], in heart-cutting 2D-LC, only fractions of selected peaks are sampled and further analyzed in the second dimension[2,3]. The Agilent 1290 Infinity II 2D-LC solution enables the parking of several cuts while running the ^{2}D cycle[4,5]. This Technical Overview describes high-resolution sampling as a further optional mode of 2D-LC, which can be used with the Agilent 1290 Infinity II 2D-LC solution.

Figure 1 shows that, in high-resolution sampling, target compounds can be determined by collecting several small fractions over a selected time range from the ^{1}D chromatogram. Each cut is parked in a sampling loop, and all cuts are analyzed in the second dimension consecutively. In this case, the resolution achieved in the first dimension was maintained. The technique can also be described as selective comprehensive 2D-LC[6]. In addition, it ensured that the entire amount of the selected compound was transferred to and analyzed in the second dimension. Therefore, a reliable quantification is possible after summation of all cuts belonging to one compound in the second dimension. With this setup, target compounds in a complex sample can still be quantified over their ^{1}D peaks, if their separation is sufficient. Selected coeluting compounds can be subjected to the high-resolution sampling process for a reliable quantification after 2D separation. As an example, this Technical Overview shows the quantification of (+)-catechin, (–)-epicatechin, and (–)-epigallocatechin gallate, three catechins usually found in green tea, and caffeine as a xanthine[7,8]. Produced from the tea plant *Camellia sinensis*, green tea beverages are consumed worldwide with growing attention to known healthy effects such as antioxidant, antibacterial, and antitumor actions[7,8]. Main compounds in green tea responsible for the described effects are catechins and xanthines[8,9].

Experimental

Equipment

The Agilent 1290 Infinity II 2D-LC solution was comprised of the following modules:

- Two Agilent 1290 Infinity II High Speed Pumps (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) with sample cooler (Option #100)
- Two Agilent 1290 Infinity II Multicolumn Thermostats (G7116B)
- Two Agilent 1290 Infinity II Diode Array Detectors (G7117B) with a 10-mm Max-Light cartridge cell (G4212-60008)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/4-port duo valve (2D-LC valve head, G4236A)
- Two Agilent 1290 Infinity Valve Drives (G1170A) with multiple heart-cutting valves (G4242-64000) equipped with 40 µL loops

Software

Agilent OpenLAB CDS ChemStation Edition Rev. C.01.07 SR2 [255] with Agilent 1290 Infinity II 2D-LC Acquisition Software Product Version A.01.03 [021]

Chemicals

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck, Darmstadt, Germany, and trifluoroacetic acid was from Sigma-Aldrich, Steinheim, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Standards of caffeine, (+)-catechin, (–)-epicatechin and (–)-epigallocatechin gallate were purchased from Sigma-Aldrich, Steinheim, Germany.

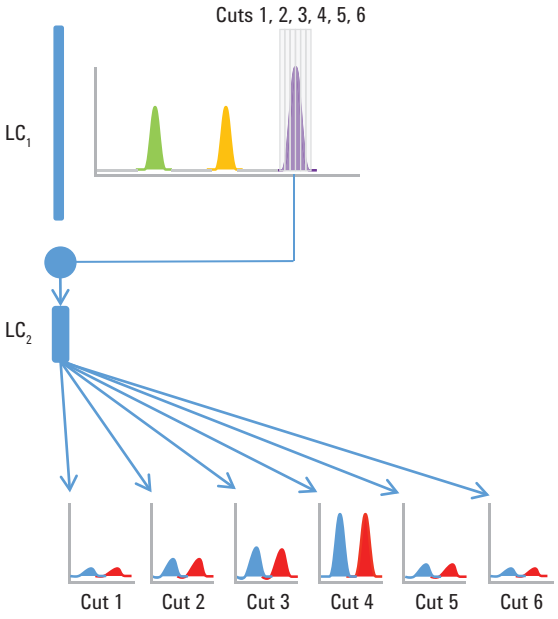

Figure 1. Illustration of high-resolution sampling 2D-LC.

Sample

Green tea (Bancha, Japan) was purchased from Ettli Kaffee GmbH, Ettlingen, Germany. An aqueous extract was prepared by adding 100 mL of boiling water to 1.75 g of dry green tea. After stirring for 5 minutes, the supernatant was filtered through a 0.45-µm pore size syringe filter (regenerated cellulose) and diluted 1:2 with water. The undiluted tea sample was injected, for quantification of (+)-catechin and (–)-epicatechin. The diluted sample was analyzed for quantification of (–)-epigallocatechin gallate and caffeine.

High-resolution sampling 2D-LC method

Parameter	Value
Columns	
First dimension	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Second dimension	Agilent ZORBAX Bonus-RP RRHD, 2.1 × 50 mm, 1.8 µm (p/n 857768-901)
^{1}D Pump	
Solvent A	Water + 0.05 % trifluoroacetic acid
Solvent B	Methanol + 0.05 % trifluoroacetic acid
Flow rate	0.2 mL/min
Gradient	0 minutes – 5 %B 10 minutes – 60 %B 11 minutes – 95 %B
^{2}D Pump	
Solvent A	Water + 0.05 % trifluoroacetic acid
Solvent B	Acetonitrile + 0.05 % trifluoroacetic acid
Flow rate	1 mL/min
Gradient	0 minutes – 5 % B 1.40 minutes – 95 % B
2D gradient stop time	1.40 minutes
2D cycle time	2.00 minutes
Stop time	15 minutes
Post time	5 minutes
High-resolution sampling	
Time based	7.07 minutes
Sampling time	2.8 seconds
Number of cuts	6
Multicolumn thermostat	
First dimension	20 °C
Second dimension	20 °C
Multisampler	
Injection volume	1 µL
Needle wash	10 seconds in methanol:water 50:50
^{1}D Diode array detector	
Wavelength	280 nm/4 nm, reference 395 nm/10 nm
Data rate	40 Hz
^{2}D Diode array detector	
Wavelength	280 nm/4 nm, reference 395 nm/10 nm
Data rate	80 Hz

Method setup for high-resolution sampling

High-resolution sampling was performed with the Agilent 1290 Infinity II 2D-LC solution, as shown in Figure 2, consisting of a 2-position/4-port-duo valve connected to two multiple heart-cutting valves holding 12 sampling loops. With this setup, up to 10 consecutive cuts can be sampled and stored until analysis. For high-resolution sampling, a maximum loop filling of 80 % is recommended to prevent any loss of sample. Figure 3 shows the method setup used for the ^{2}D pump. First, a 1D-LC separation of the sample was run, and the chromatogram was loaded as the reference signal preview window. High-resolution sampling was set up time-based, according to the peak of interest, with six cuts covering the entire peak width. Under the given ^{1}D conditions, a sampling time of 2.8 seconds equals a loop filling of 23 %.

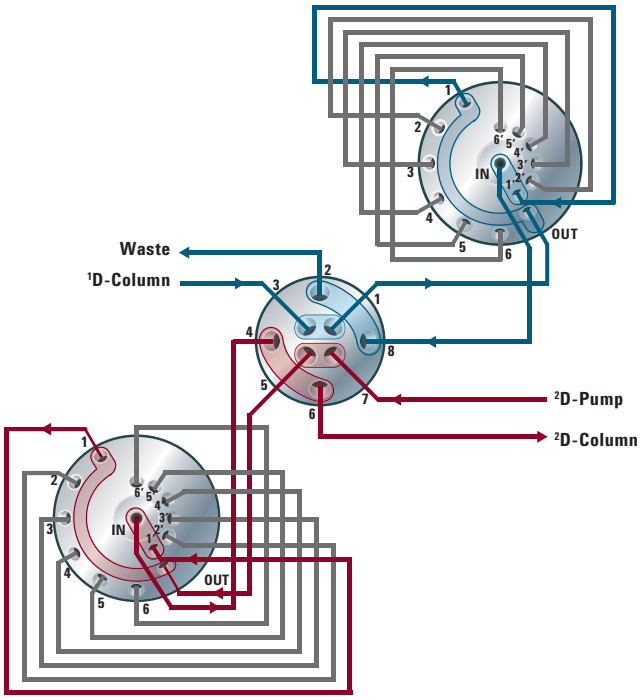

Figure 2. Setup of the Agilent 1290 Infinity II 2D-LC solution holding 12 sampling loops.

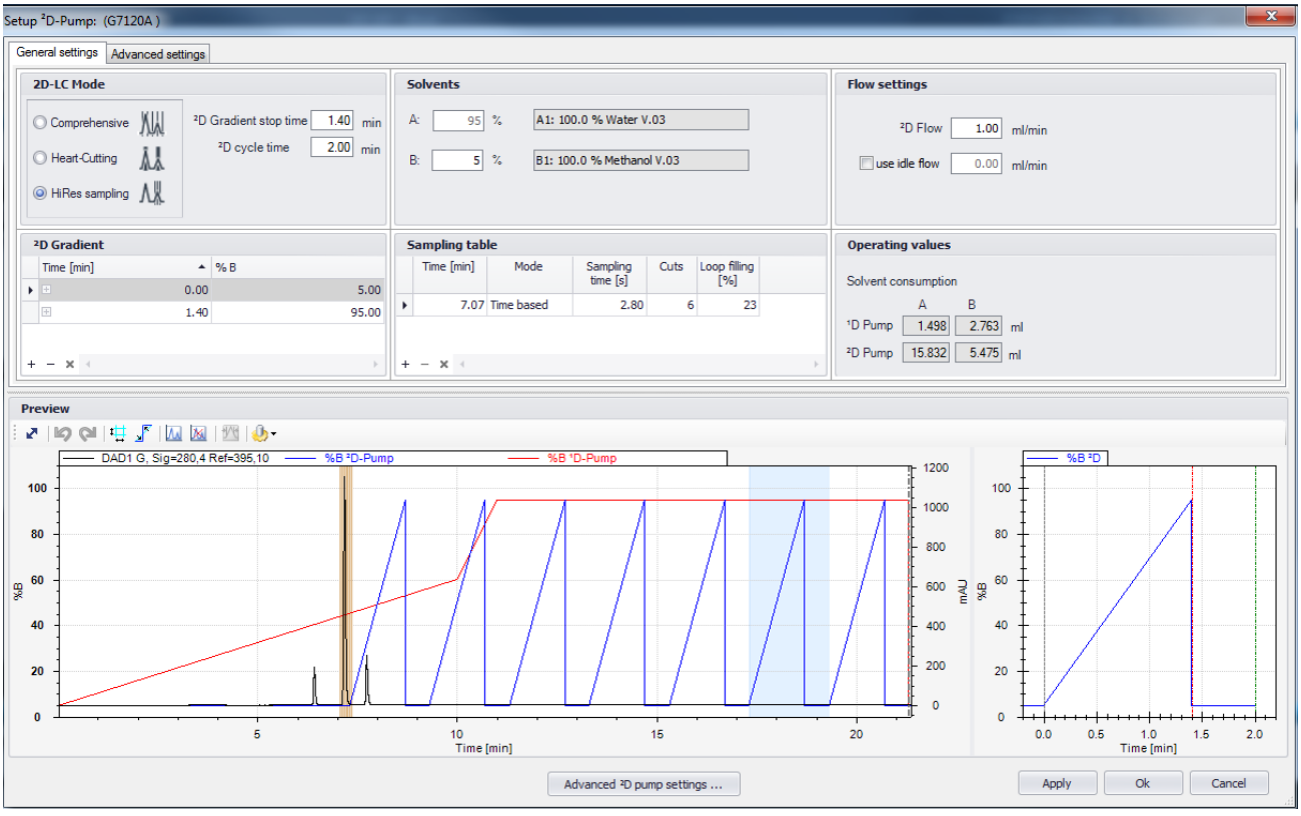

Figure 3. Method setup for the ^{2}D pump.

Results and Discussion

Quantification

An aqueous extract of green tea was analyzed with high-resolution sampling 2D-LC. Figure 4 shows a one-dimensional chromatogram of the green tea sample. (+)-catechin and (–)-epicatechin are well separated, whereas caffeine and (–)-epigallocatechin gallate coelute, and therefore cannot be quantified using the 1D-LC separation. Using high-resolution sampling, six cuts in the time range of the peak containing caffeine and (–)-epigallocatechin gallate (Figure 5A) were sampled and injected to the second dimension. A summary of the cuts is also given in the sampling table in Figure 5B. This method of sampling allows a reliable quantification, even if a slight retention time shift occurs in the first dimension, which would have a higher impact in a simple heart-cutting experiment[4]. As an additional benefit, short sampling times lead to smaller amounts of sample injected to the second dimension, resulting in higher quality chromatograms than in an experiment where the whole peak is sampled as one fraction. The ^{2}D chromatograms of all cuts, showing a good separation of caffeine and (–)-epigallocatechin gallate, are overlaid in Figure 5C. For quantification, peaks of one compound are integrated in every single ^{2}D chromatogram by the software. The peak table in Figure 5D gives the sum of peak areas for each compound.

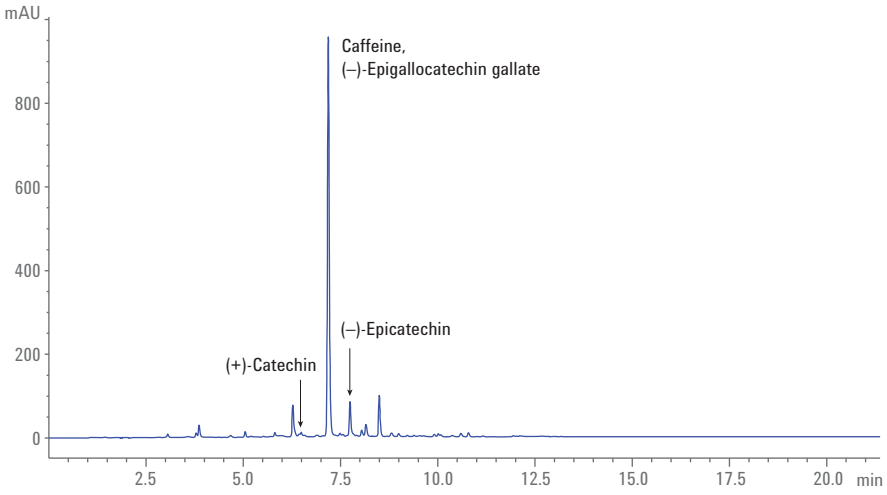

Figure 4. ^{1}D chromatogram of an aqueous green tea extract with caffeine and (–)-epigallocatechin gallate coeluting.

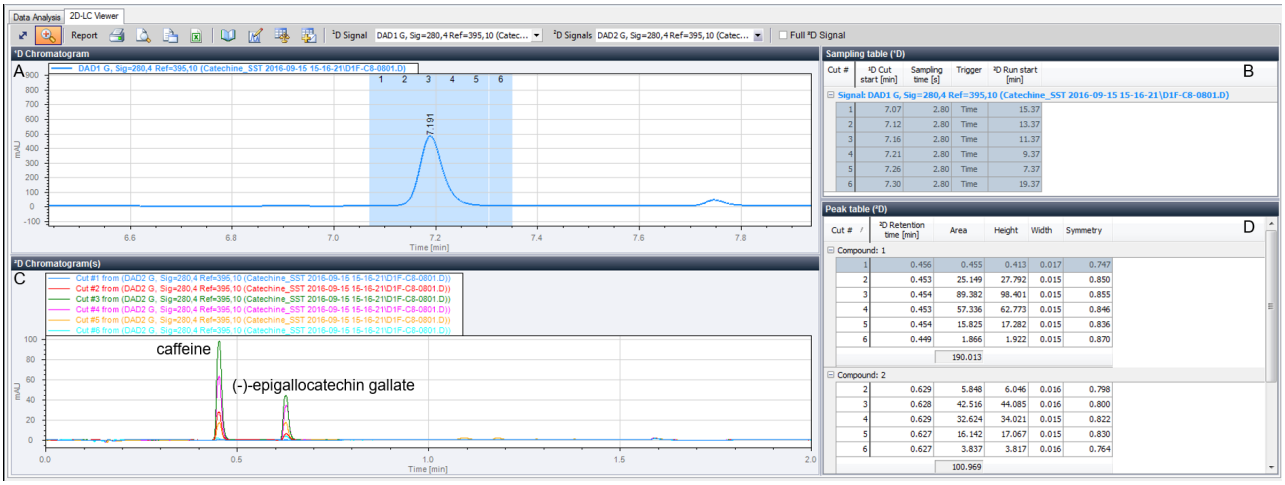

Figure 5. 2D-LC Viewer. A) ^{1}D chromatogram with six consecutive cuts over the entire width of the peak containing caffeine and (–)-epigallocatechin gallate. B) Sampling table with six cuts taken from the first dimension. C) Overlay of all ^{2}D chromatograms of six cuts. D) Peak table of the second-dimension chromatograms with peak areas in each cut and sum of peak areas for two compounds.

Standard solutions of (+)-catechin, (–)-epicatechin, (–)-epigallocatechin gallate and caffeine in a concentration range from 5 to 220 µg/mL were analyzed twice with the described method. For the calibration of (+)-catechin and (–)-epicatechin, first-dimension peaks were evaluated using the standard integration and calibration functionalities of the ChemStation software. Figures 6A and 6B show the calibration curves for both compounds, with good linearity ($R^2 > 0.999$). Calibration of (–)-epigallocatechin gallate and caffeine was performed using the sum of ^{2}D peak areas of each compound. Figure 7 shows that this sum of peak areas, calculated in the 2D-LC Viewer tab in ChemStation, can be added as a new level to the calibration table in the Data Analysis tab. For both compounds, a good linearity across the entire calibration range is given, with R^2 values greater than 0.999, as shown in Figures 6C and 6D.

The target compounds in the green tea sample were quantified using the ^{1}D peak for (+)-catechin and (–)-epicatechin, as well as the sum of second-dimension peaks for (–)-epigallocatechin gallate and caffeine, as described above. The amounts (average of two analyses) found in the determined tea beverage were 5.2 µg/mL (+)-catechin, 68.9 µg/mL (–)-epicatechin, 198.8 µg/mL (–)-epigallocatechin gallate, and 167.7 µg/mL caffeine.

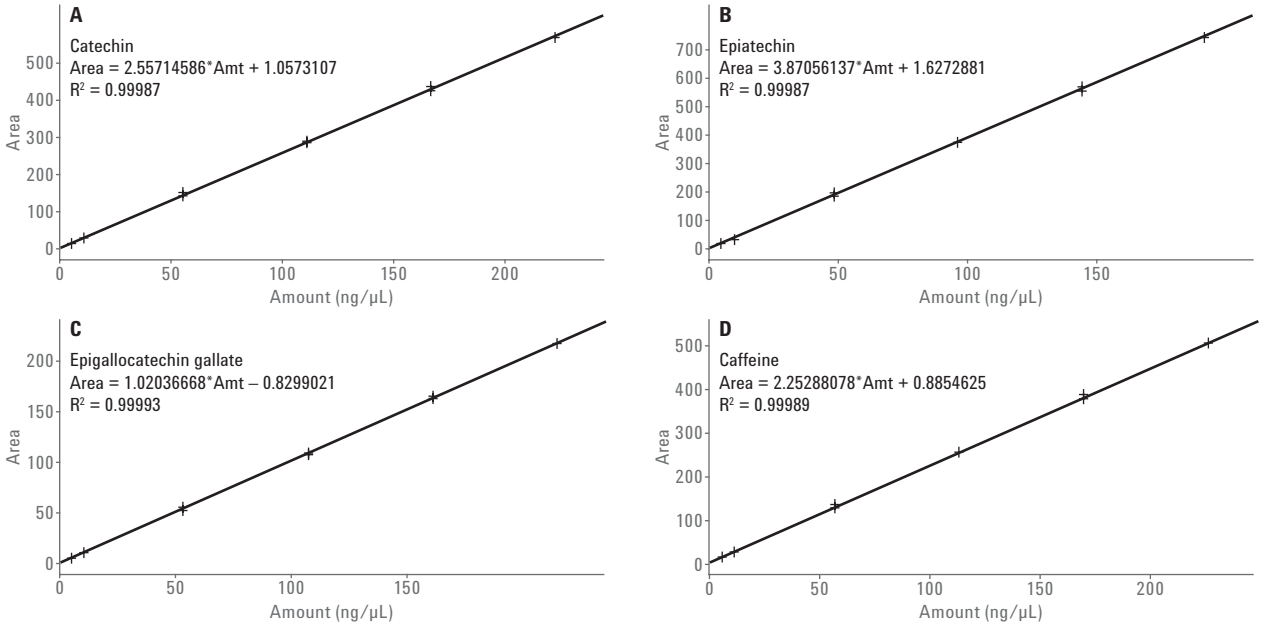

Figure 6. Calibration curves between 5 and 220 µg/mL for the four target compounds, generated from the ^{1}D peak for (+)-catechin (A) and (–)-epicatechin (B), and from the sum of ^{2}D peaks for (–)-epigallocatechin gallate (C) and caffeine (D).

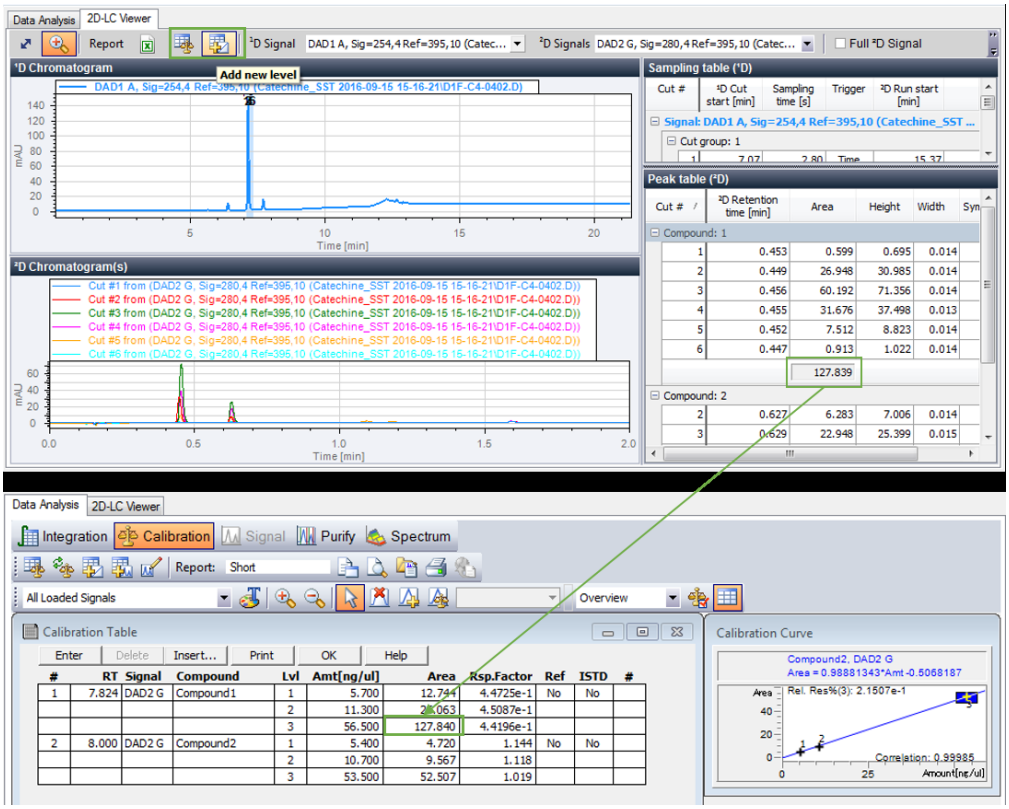

Figure 7. Performing a calibration in Agilent OpenLab CDS ChemStation with Agilent 1290 Infinity II 2D-LC Acquisition software after high-resolution sampling 2D-LC analysis. The sum of ^{2}D peak areas of each compound as shown in the 2D-LC Viewer tab (marked in green) is transferred to the Calibration Table in the Data Analysis tab by clicking **Add new level**.

Repeatability

To determine the repeatability of the high-resolution sampling 2D-LC method, six consecutive analyses of a standard mix containing 100 µg/mL of caffeine and the three catechins were performed. Figure 8A shows the overlay of all ^{1}D chromatograms. In Figure 8B, total ^{2}D chromatograms are overlaid. Total peak areas of caffeine and (–)-epigallocatechin gallate were determined from the ^{2}D chromatograms, as described above. Relative standard deviations (RSDs) were 1.2 % for caffeine and 1.1 % for (–)-epigallocatechin gallate, respectively.

Conclusions

This Technical Overview shows the high-resolution sampling functionality of the Agilent 1290 Infinity II 2D-LC Solution. Compounds coeluting in the first dimension were sampled in several fractions, and could be separated consecutively in the second dimension. Exemplified through a green tea sample, it shows that compounds already separated in the first dimension can be quantified over their ^{1}D peak, whereas compounds coeluting in the first dimension can be analyzed and reliably quantified over high-resolution sampling 2D-LC within the same run.

References

1. Marriott, P. J.; Wu, Z.; Schoenmakers, P. Nomenclature and Conventions in Comprehensive Multidimensional Chromatography–an Update. *LCGC Eur.* **2012**, *25*(5), 266–275.

2. Lee, C.; *et al.* Application of Heart-Cutting 2D-LC for the Determination of Peak Purity for a Chiral Pharmaceutical Compound by HPLC. *Chromatographia* **2013**, *76(1–2)*, 5–11.

3. Naegele, E. Detection of Impurities by Heart Cutting Using the Agilent 1290 Infinity 2D-LC Solution, *Agilent Technologies Application Note*, publication number 5991-0834EN, **2012**.

4. Buckenmaier, S. Agilent 1290 Infinity 2D-LC Solution for Multiple Heart-Cutting, *Agilent Technologies Technical Overview*, publication number 5991-5349EN, **2015**.

5. Krieger, S. Application of Multiple Heart-Cutting 2D-LC in Method Development for Impurity Analysis, *Agilent Technologies Application Note*, publication number 5991-5349EN, **2015**.

6. Stoll, D. R.; *et al.* Direct Identification of Rituximab Main Isoforms and Subunit Analysis by Online Selective Comprehensive Two-Dimensional Liquid Chromatography-Mass Spectrometry, *Anal. Chem.* **2015**, *87(16)*, 8307–8315.

7. Jankana, B.; Wandee, Y. Catechins and Caffeine Contents of Green Tea Commercialized in Thailand. *J. Pharm. Biomed. Sci.* **2012**, *22(22)*, 1–7.

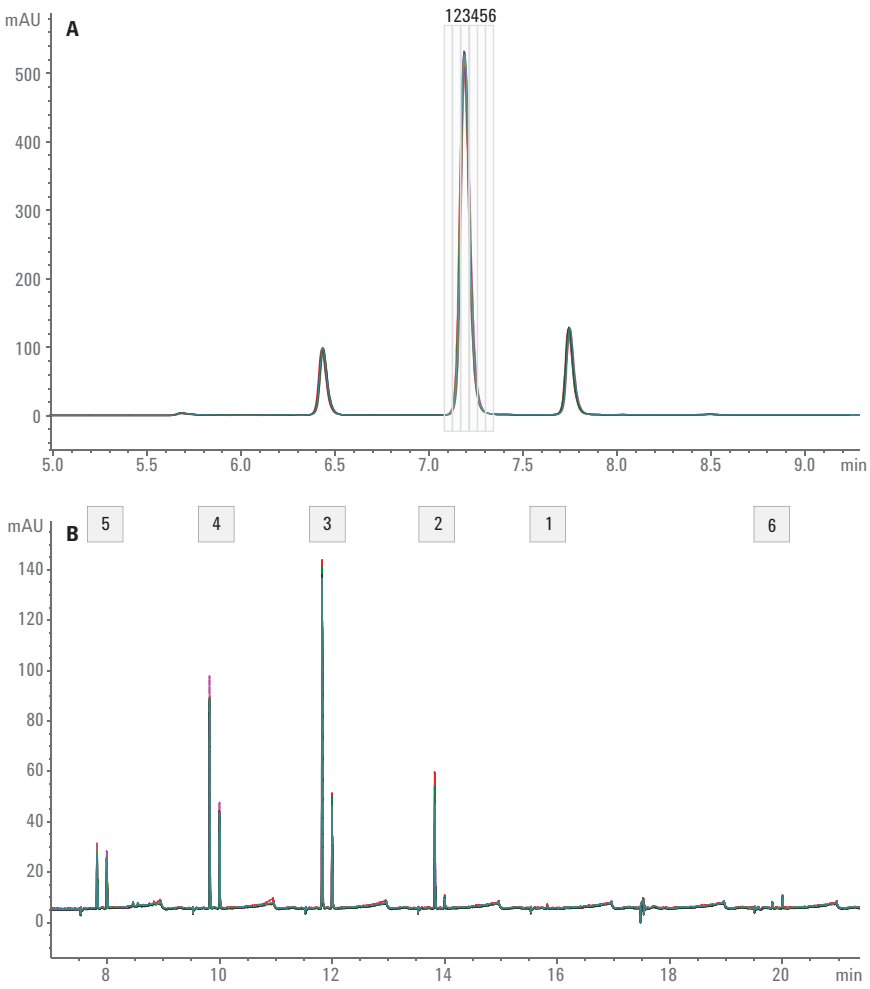

Figure 8. Overlay of eight consecutive injections of a standardmix containing caffeine, (–)-epigallocatechin gallate, (+)-catechin, and (–)-epicatechin. A) ^{1}D chromatogram with six fractions sampled time-based, beginning at 7.07 minutes (sampling time 2.8 seconds). B) Full ^{2}D signal of the six fractions.

8. Dufresne, C. J.; Farnworth, E. R. A Review of Latest Research Findings on the Health Promotion Properties of Tea. *J. Nutr. Biochem.* **2001**, *12(7)*, 404–421.

9. Rahim, A. A.; Nofrizal, S.; Saad, B. Rapid Tea Catechins and Caffeine Determination by HPLC Using Microwave-Assisted Extraction and Silica Monolithic Column. *Food Chem.* **2014**, *147*, 262–268.

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