Technical Overview

Method Transfer from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity III LC

Proof of equivalency for the transfer of conventional LC methods

Abstract

Method transfer from legacy equipment to new instruments, such as the Agilent 1260 Infinity III LC, is an important topic for all laboratories throughout different industries. Seamless method transfer ensures that the same methods can still be used, which ensures business continuity and reduced regulatory and financial risks.

This technical overview demonstrates method transfer from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity III LC for conventional LC methods covering diverse gradient conditions. Equivalent results are obtained, which proves that seamless method transfer can be expected for typical, robust, conventional LC methods. In terms of retention time precision, the 1260 Infinity III LC outperforms the 1100 LC, leading to higher trust in the data.

Introduction

Method transfer is an important topic for all laboratories throughout different industries, where HPLC methods are transferred between departments and locations as well as between different LC instruments.[1] For validated methods in the pharmaceutical industry, method transferability is compulsory.

Method transfer, or the transfer of analytical procedures from one laboratory to another in the pharmaceutical industry requires a documented process that requires comparative testing.[2] One example for instrument-to-instrument method transfer is the transfer of conventional LC methods from legacy equipment to new instruments such as a 1260 Infinity III LC.

This technical overview demonstrates method transfer from a 1100 Series Quaternary LC to a 1260 Infinity III LC for conventional LC methods. Several methods were chosen that cover diverse gradient conditions including challenging conditions such as a shallow gradient and a gradient starting at a composition extreme. This should prove that equivalent results are obtained when transferring methods from a broad application space of conventional LC methods from the 1100 Series Quaternary LC to the 1260 Infinity III LC.

Experimental

Equipment

The Agilent 1100 Series LC System comprised the following modules:

- Agilent 1100 Series quaternary pump (G1311A) with active inlet valve (AIV)
- Agilent 1100 Series degasser (G1322A)
- Agilent 1100 Series standard autosampler (G1313A)
- Agilent 1100 Series thermostatted column compartment (G1316A)
- Agilent 1100 Series diode array detector (G1315B) with standard flow cell, 10 mm, (G1315-60022)

The Agilent 1260 Infinity III LC System comprised the following modules:

- Agilent 1260 Infinity III quaternary pump (G7111B) with active inlet valve (AIV)
- Agilent 1260 Infinity III vialsampler (G7129C)
- Agilent 1260 Infinity III multicolumn thermostat (G7116A)
- Agilent 1260 Infinity III diode array detector WR (G7115A) with standard flow cell, 10 mm, (G1315-60022)

Software

Agilent OpenLab CDS, version 2.7.

Columns

- Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (part number 959993-902)
- Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm (part number 959943-902)
- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (part number 883975-902)

Chemicals

All solvents used were LC grade. InfinityLab Acetonitrile Gradient Grade for LC (part number 5191-5100)* and InfinityLab Methanol Gradient Grade for LC (part number 5191-5110)* were purchased from Agilent. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). The isocratic sample (part number 01080-68704) and the RRLC checkout (part number 5188-6529) were from Agilent. Theophylline and caffeine were purchased from Sigma-Aldrich (Steinheim, Germany). Theobromine and phosphoric acid were obtained from Alfa Aesar (Karlsruhe, Germany) and VWR (Darmstadt, Germany), respectively. Ganoderma Lucidum Fruiting Body Dry Extract (USP reference standard) was purchased from VWR (Darmstadt, Germany).

*Only available in select countries

Preparation of the purine alkaloid sample

A sample containing 100 µg/mL each of caffeine, theobromine, and theophylline in water:acetonitrile (98:2; v/v) was prepared as follows: 25 mg each of caffeine, theobromine, and theophylline were weighed into a 25 mL volumetric flask. Then, 5 mL of acetonitrile and 15 mL of water were added, and the standards were dissolved by sonication. The sample was made up to volume with water. Then, 1 mL of the resulting 1 mg/mL sample was diluted with water to 10 mL. The sample was filtered using an Agilent Captiva Premium Syringe Filter, regenerated cellulose, 0.2 µm (part number 5190-5310).

Preparation of the Ganoderma sample

A 50 mg sample of Ganoderma Lucidum Fruiting Body Dry Extract was weighed, and 5 mL of methanol was added. The mixture was sonicated for five minutes, then centrifuged at 10,000 rpm for five minutes. The supernatant was filtered using a Captiva Premium Syringe Filter, nylon, 0.2 µm (part number 5190-5088).

Table 1. Method for isocratic analysis.

Parameter	Value
Sample	Isocratic sample
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm
Solvent	A: Water B: Acetonitrile C: Acetonitrile D: Water
Gradient	Isocratic, 35% A and 65% B or Isocratic, 65% C and 35% D Stop time: 20 min or 12 min at 80 °C
Flow Rate	1.000 mL/min
Temperature	40 °C or 80 °C
Detection	254/4 nm, reference 360/100 nm 10 Hz
Injection	Injection volume: 5 µL

Table 2. Method employing a gradient starting at low %B.

Parameter	Value
Sample	Purine alkaloid sample
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm
Solvent	A: Water B: Acetonitrile
Gradient	Time (min) %B 0 1 15 15 Stop time: 20 min Post time: 10 min
Flow Rate	0.960 mL/min
Temperature	40 °C
Detection	273 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	Injection volume: 15 µL

Table 3. Method employing a fast gradient.

Parameter	Value
Sample	RRLC checkout
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 3.5 µm
Solvent	A: Water B: Acetonitrile
Gradient	Time (min) %B 0 20 6.5 90 Stop time: 8 min Post time: 4 min
Flow Rate	3.000 mL/min
Temperature	40 °C
Detection	245 nm/4 nm, reference 360 nm/100 nm 40 Hz (Infinity III LC)/20 Hz (1100 Series LC)
Injection	Injection volume: 15 µL

Table 4. Method employing a shallow gradient.

Parameter	Value
Sample	Ganoderma extract
Column	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm
Solvent	A: 0.075% Phosphoric acid in water B: Acetonitrile
Gradient	Time (min) %B 0 20 3 26.5 34 26.5 52 38.5 54 100 Stop time: 55 min Post time: 15 min
Flow rate	1.919 mL/min
Temperature	30 °C
Detection	257 nm/4 nm, reference 370 nm/60 nm 20 Hz
Injection	Injection volume: 10 µL

Results and discussion

To prove that equivalent results are obtained for the transfer of conventional LC methods from the 1100 Series Quaternary LC to the 1260 Infinity III LC, methods covering diverse gradient conditions (see Tables 1 to 4) were investigated.

For isocratic analysis, the results obtained using the 1260 Infinity III LC and the 1100 LC are shown in Figure 1. Excellent retention time precision is observed and the maximum retention time deviation between the two LC systems is 0.5%. Additionally, retention times and their reproducibility are comparable when using pump channels A and B or C and D for analysis (data not shown).

Compound		Agilent 1260 Infinity III LC		Agilent 1100 Series LC		Retention Time Deviation	
Number	Name	RT (min)	RT RSD (%)	RT (min)	RT RSD (%)	(min)	(%)
1	Dimethylphthalate	2.20	0.04	2.21	0.05	–0.01	–0.5
2	Diethylphthalate	3.02	0.06	3.03	0.06	–0.02	–0.5
3	Biphenyl	5.81	0.08	5.84	0.07	–0.02	–0.4
4	o-Terphenyl	11.72	0.11	11.77	0.08	–0.05	–0.4

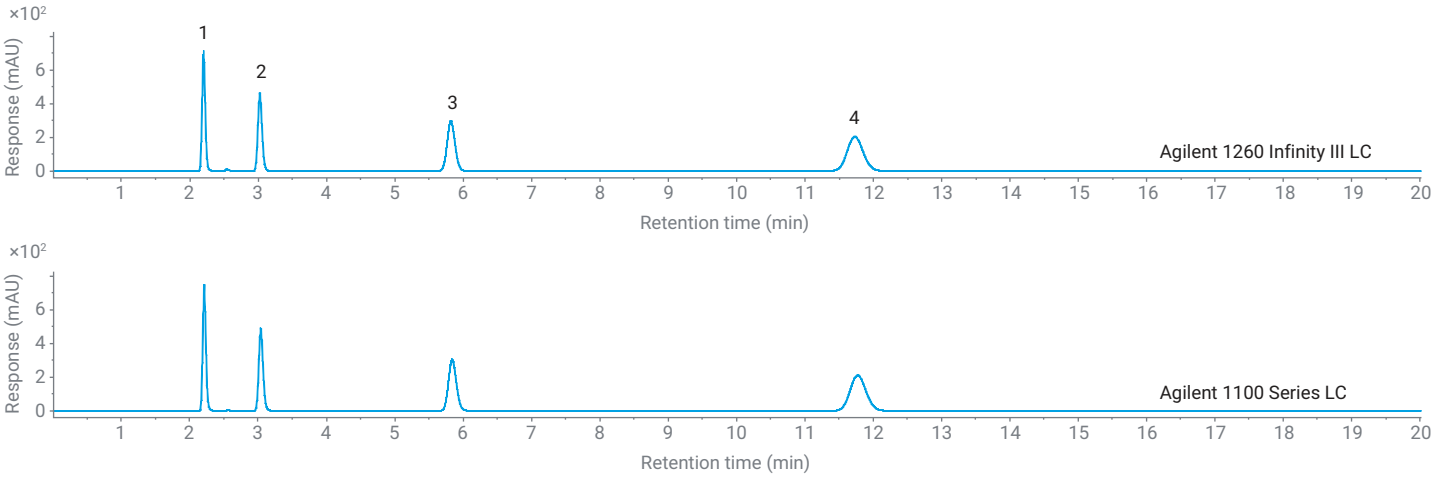

Figure 1. Analysis of the isocratic sample using the method for isocratic analysis (Table 1) at 40 °C column temperature. N = 10 for calculation of RSDs.

To investigate the influence of temperature in the column compartment, the isocratic analysis was also performed using an elevated temperature of 80 °C. Excellent retention time precision was obtained. Retention times were slightly lower when using the 1260 Infinity III LC compared to the 1100 LC, with a maximum retention time deviation of 3.2% (Figure 2). A possible explanation for this slight difference in retention time is the column compartment design and specifically that the MCT provides more ambient temperature rejection from the increased insulation of the MCT door, which may ultimately cause the analytes to elute earlier for most analytes.

Compound		Agilent 1260 Infinity III LC		Agilent 1100 Series LC		Retention Time Deviation	
Number	Name	RT (min)	RT RSD (%)	RT (min)	RT RSD (%)	(min)	(%)
1	Dimethylphthalate	1.93	0.03	1.95	0.02	–0.02	–1.2
2	Diethylphthalate	2.49	0.04	2.53	0.02	–0.04	–1.6
3	Biphenyl	4.19	0.06	4.30	0.04	–0.10	–2.4
4	o-Terphenyl	7.49	0.08	7.74	0.05	–0.25	–3.2

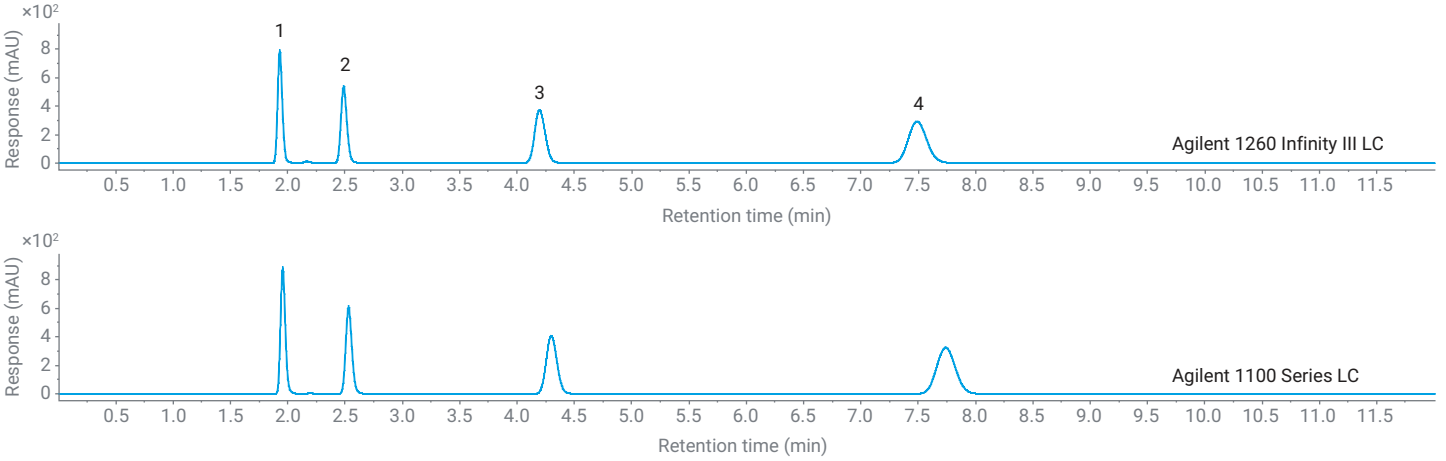

Figure 2. Analysis of the isocratic sample using the method for isocratic analysis (Table 1) at 80 °C column temperature. N = 10 for calculation of RSDs.

Working at composition extremes poses a challenge to quaternary pumps. To investigate transfer of a method employing extreme composition conditions, a sample of purine alkoids was analyzed using a gradient starting at 1% B. Excellent retention time precision was observed, with the 1260 Infinity III LC clearly outperforming the 1100 LC (see Figure 3). Retention times on both LC systems were comparable, with a maximum deviation of 2.4%.

To assess the transfer of a fast gradient method, the RRLC checkout was analyzed using a gradient ranging from 20 to 95% B within 6.5 minutes at a high flow rate of 3.0 mL/min. The results are shown in Figure 4. Excellent retention time precision was obtained on both LC systems, and excellent agreement of retention times was observed with a maximum retention time deviation of 0.04 minutes.

Compound		Agilent 1260 Infinity III LC		Agilent 1100 Series LC		Retention Time Deviation	
Number	Name	RT (min)	RT RSD (%)	RT (min)	RT RSD (%)	(min)	(%)
1	Theobromine	4.38	0.20	4.49	1.09	–0.11	–2.4
2	Theophylline	5.74	0.18	5.82	0.90	–0.08	–1.4
3	Caffeine	8.27	0.15	8.45	0.72	–0.18	–2.2

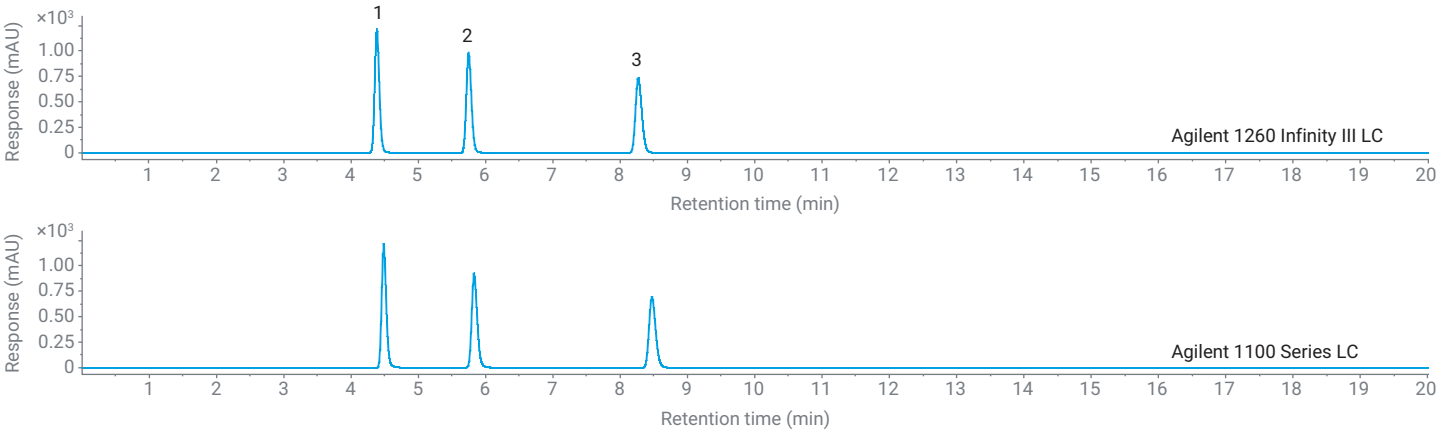

Figure 3. Analysis of the purine alkaloid sample using a gradient method that starts with a low %B (Table 2). N = 10 for calculation of RSDs.

Compound		Agilent 1260 Infinity III LC		Agilent 1100 Series LC		Retention Time Deviation	
Number	Name	RT (min)	RT RSD (%)	RT (min)	RT RSD (%)	(min)	(%)
1	Acetanilide	0.60	0.04	0.59	0.07	0.01	1.0
2	Acetophenone	1.31	0.03	1.32	0.03	–0.01	–0.8
3	Propiophenone	2.07	0.02	2.10	0.02	–0.02	–1.1
4	Butyrophenone	2.76	0.02	2.79	0.02	–0.03	–1.2
5	Benzophenone	3.06	0.01	3.10	0.01	–0.03	–1.1
6	Valerophenone	3.40	0.01	3.43	0.01	–0.04	–1.0
7	Hexanophenone	3.98	0.01	4.01	0.01	–0.03	–0.8
8	Heptanophenone	4.51	0.02	4.54	0.01	–0.03	–0.7
9	Octanophenone	5.01	0.02	5.03	0.01	–0.03	–0.5

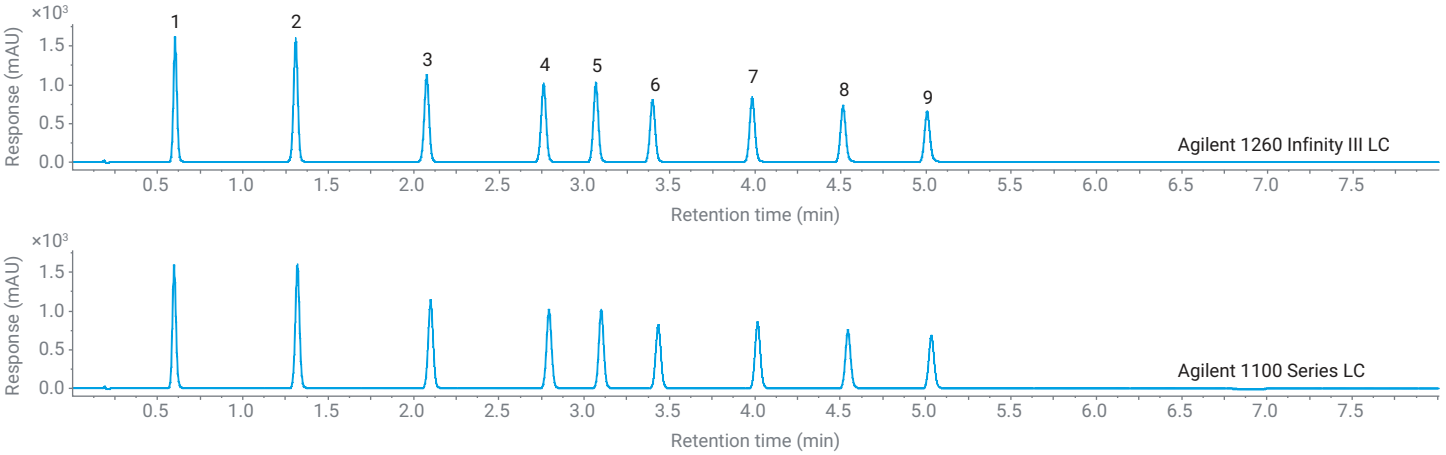

Figure 4. Analysis of the RRLC checkout using the fast gradient method (Table 3). N = 10 for calculation of RSDs.

Next to composition extremes, shallow gradients also pose challenges to quaternary pumps. The method for analysis of a Ganoderma extract (Table 4) based on a USP compendial method[3] employs a shallow gradient. The results obtained transferring this method from the 1100 LC to the 1260 Infinity III LC are depicted in Figure 5. Excellent retention time precision is obtained with the 1260 Infinity III LC outperforming the 1100 LC. Retention times are equivalent between the two LC systems, with a maximum retention time deviation of 1.0%.

The transfer of a selection of conventional LC methods from a broad application space from the 1100 Series Quaternary LC to the 1260 Infinity III LC shows that equivalent results are obtained on both LC systems, with the 1260 Infinity III LC outperforming the 1100 LC in terms of retention time precision. This proves that equivalent results can be expected for the transfer of typical, robust, conventional LC methods from the 1100 Series Quaternary LC to the 1260 Infinity III LC. A higher likelihood of differences between the results obtained using the 1100 Series Quaternary LC and the 1260 Infinity III LC might however exist for certain conditions, such as low flow rates, challenging gradients, temperature sensitive analytes, and combinations thereof.

Compound		Agilent 1260 Infinity III LC		Agilent 1100 Series LC		Retention Time Deviation	
Number	Name	RT (min)	RT RSD (%)	RT (min)	RT RSD (%)	(min)	(%)
1	Ganodermic acid C	9.04	0.10	9.12	0.10	−0.08	−0.9
2	Ganodermic acid C	10.20	0.10	10.30	0.09	−0.10	−1.0
3	Ganodermic acid G	13.94	0.08	14.00	0.17	−0.05	−0.4
4	Ganodermic acid B	14.60	0.10	14.65	0.17	−0.04	−0.3
5	Ganodermic acid B	15.92	0.10	15.98	0.16	−0.06	−0.4
6	Ganodermic acid A	23.41	0.12	23.42	0.14	−0.01	0.0
7	Ganodermic acid D	32.16	0.08	31.91	0.23	0.26	0.8
8	Ganodermic acid D	36.75	0.08	36.52	0.21	0.23	0.6
9	Ganodermic acid F	46.01	0.02	45.86	0.09	0.15	0.3

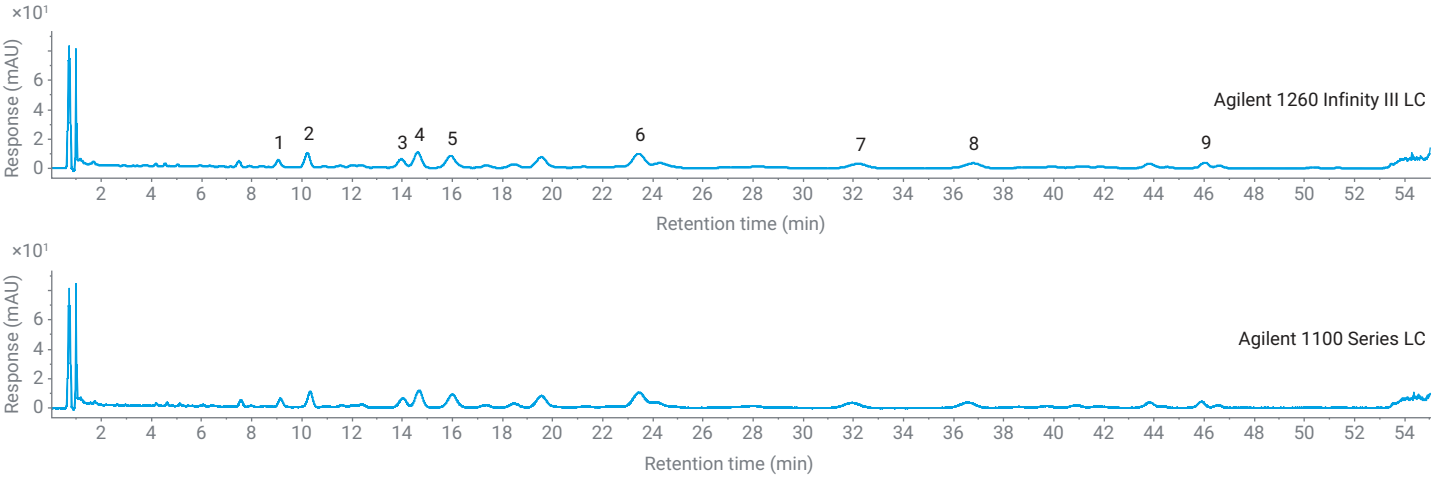

Figure 5. Analysis of the Ganoderma extract using the shallow gradient method (Table 4). N = 6 for calculation of RSDs.

Conclusion

The transfer of conventional LC methods covering diverse gradient conditions from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity III LC leads to equivalent results, thereby proving that seamless method transfer can be expected for typical, robust, conventional LC methods. In terms of retention time precision, the 1260 Infinity III LC outperforms the 1100 LC, leading to a higher trust in the data.

References

1. Agilent InfinityLab LC with ISET. *Agilent Technologies user manual*, publication number G7120-90310 Rev. B, **2022**.
2. USP general chapter <1224> Transfer of Analytical Procedures, official prior to 2013, https://online.uspnf.com/uspnf/document/1_GUID-41AB3326-5D3F-44DA-B8A0-2CF631D49095_4_en-US?source=TOC (accessed 14 November 2024).
3. USP Monograph on Ganoderma Lucidum Fruiting Body, official as of 1-Dec-2015, https://online.uspnf.com/uspnf/document/1_GUID-799BE3E2-2A56-41AA-8ACA-163F14B04BA6_2_en-US?source=Search%20Results&highlight=Ganoderma (accessed 15 November 2024).

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