Application Note

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

Proof of equivalency and transfer to UHPLC conditions

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Abstract

Method transfer from legacy equipment to new instrumentation such as the Agilent 1260 Infinity III LC System is an important topic, especially in the pharmaceutical industry. This application note shows seamless method transfer from an Agilent 1100 Series Quaternary LC System to an Agilent 1260 Infinity III LC System for the analysis of related compounds of abacavir, according to the United States Pharmacopoeia (USP) monograph. Additionally, the method can be translated to UHPLC conditions using the 1260 Infinity III LC in combination with an Agilent InfinityLab Poroshell column, offering a significant reduction in analysis time and solvent consumption.

Introduction

The USP and other pharmacopoeias provide validated methods for the analysis of many pharmaceuticals. For validated methods in the pharmaceutical industry, method transferability is compulsory, and the transfer of analytical procedures from one laboratory to another requires comparative testing.[1] One example of instrument-to-instrument method transfer is the transfer of conventional LC methods from legacy equipment to new instruments, such as the 1260 Infinity III LC System. Each method must be verified under actual conditions of use when applied to new instruments for the first time[2], typically by determination of system suitability criteria that must be met.

This application note shows the analysis of related compounds of abacavir according to the USP monograph[3], using an 1100 Series Quaternary LC System. It has previously been shown that conventional LC methods can be transferred seamlessly from an 1100 Series Quaternary LC to a 1260 Infinity III LC.[4] Here, the seamless transfer of a USP monograph method from the 1100 Series Quaternary LC to the 1260 Infinity III LC is shown for the analysis of related compounds of abacavir.

Furthermore, the analysis of related compounds of abacavir is translated to UHPLC conditions within the permitted adjustments of chromatographic conditions in gradient elution LC, described in USP chapter 621.[5] The transfer to UHPLC conditions facilitates shorter analysis times and lower solvent consumption while maintaining separation performance, thereby reducing the cost per sample.

Experimental

Equipment

The Agilent 1100 Series LC System comprised the following modules:

- Agilent 1100 Series quaternary pump (G1311A) with active inlet valve
- 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
- 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 or later

Columns

- Agilent ZORBAX StableBond C18, 4.6 × 150 mm, 5 µm (part number 883975-902)
- Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 75 mm, 2.7 µm (part number 687975-302)

Chemicals

All solvents used were LC grade. InfinityLab methanol, HPLC gradient grade (part number 5191-5110)* and the InfinityLab LC Performance Standard Kit (part number 5191-4547) were 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). Abacavir related compounds mixture (USP reference standard) and trifluoroacetic acid (TFA; ≥ 99%; Fluka) were purchased from VWR (Darmstadt, Germany).

* Only available in select countries

System suitability solution

A 0.25 mg/mL solution of the USP abacavir related compounds mixture was prepared in water.

Results and discussion

Before starting the analysis of related compounds of abacavir, a performance check of the 1100 Series Quaternary LC and the 1260 Infinity III LC was performed by analysis of the InfinityLab LC Performance Standard. Excellent results were obtained in terms of peak shape as well as retention time and peak area precision (data not shown), demonstrating that performance of both LC systems was suitable for analysis.

The analysis of related compounds of abacavir according to the USP monograph[3] prescribes the use of a 3.9 × 150 mm, 5 µm packing L1 column and the method parameters shown in Table 1. For the assessment of system suitability, a 0.25 mg/mL solution of the USP abacavir related compounds mixture (system suitability solution) needs to be analyzed and the resolution between abacavir and *trans*-abacavir must be at least 1.5.

The USP monograph method is translated to a more common 4.6 × 150 mm, 5 µm column following the procedures described in USP chapter 621[5], which results in an adjustment of flow rate and injection volume (Table 2).

Figure 1 shows the analysis of the abacavir related compound mixture employing the 1100 Series Quaternary LC and the method parameters described in Table 2. The resolution between abacavir and *trans*-abacavir was 2.6. Therefore, the analysis fulfilled the system suitability criteria.

The analysis of related compounds of abacavir using the method parameters described in Table 2 were transferred to the 1260 Infinity III LC (Figure 2). Excellent results were obtained in terms of retention time precision, leading to high trust in the data. The resolution between abacavir and *trans*-abacavir was 2.6, which fulfilled the system suitability criteria.

Table 1. USP compendial method for analysis of related compounds of abacavir sulfate.

Parameter	Value
Column	Packing L1, 3.9 × 150 mm, 5 µm
Solvent	A) TFA and water (0.05:99.95) B) Methanol and water (17:3)
Gradient	Time (min) %B 0.0 5 20.0 30 35.0 90 35.1 5 50.0 5 Stop time: 50 min
Flow Rate	1.0 mL/min
Temperature	30 °C
Detection	UV 254 nm
Injection Volume	20 µL

Table 2. USP compendial method for analysis of related compounds of abacavir sulfate translated to a 4.6 mm id column.

Parameter	Value
Column	Agilent ZORBAX StableBond C18, 4.6 × 150 mm, 5 µm
Solvent	A) TFA and water (0.05:99.95) B) Methanol and water (17:3)
Gradient	Time (min) %B 0.0 5 20.0 30 35.0 90 35.1 5 50.0 5 Stop time: 50 min
Flow Rate	1.39 mL/min
Temperature	30 °C
Detection	254 nm/4 nm 10 Hz
Injection Volume	27.82 µL

Table 3. USP compendial method for the analysis of related compounds of abacavir sulfate translated to a 3.0 × 75 mm, 2.7 µm column.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 75 mm, 2.7 µm
Solvent	A) TFA and water (0.05:99.95) B) Methanol and water (17:3)
Gradient	Time (min) %B 0.00 5 5.40 30 9.45 90 9.48 5 13.50 5 Stop time: 13.50 min
Flow Rate	1.10 mL/min
Temperature	30 °C
Detection	254 nm/4 nm 20 Hz
Injection Volume	5.92 µL

Compound		Agilent 1100 Series LC			
Number	Name	RT (min)	RT RSD (%)	Area	Area RSD (%)
1	Descyclopropyl abacavir	13.13	0.02	49.78	2.57
2	Abacavir	19.84	0.02	6,282.26	0.03
3	*trans*-Abacavir	20.83	0.01	48.20	0.20
4	O-Pyrimidine derivative abacavir	25.09	0.01	23.30	1.62
5	*t*-Butyl derivative abacavir	30.95	0.01	15.62	1.60

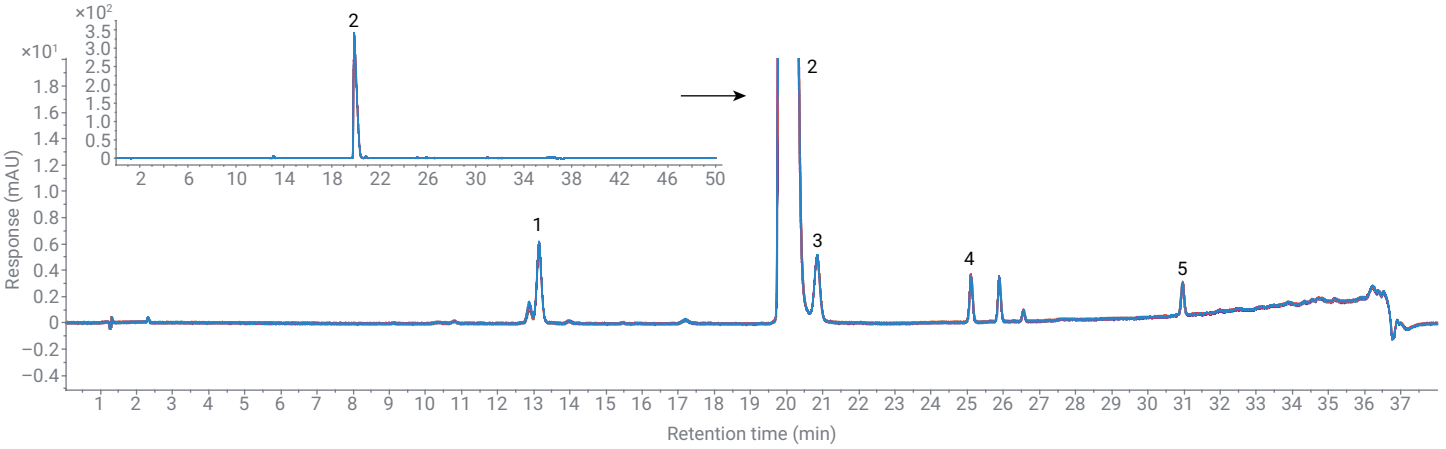

Figure 1. Analysis of the abacavir related compounds mixture using the USP compendial method on an Agilent ZORBAX StableBond C18, 4.6 × 150 mm, 5 µm column using an Agilent 1100 Series LC System. The chromatogram shows a zoom, and the inset shows an overview. Overlay of six consecutive analyses. N = 6 for calculation of RSDs.

Compound		Agilent 1260 Infinity III LC			
Number	Name	RT (min)	RT RSD (%)	Area	Area RSD (%)
1	Descyclopropyl abacavir	13.03	0.04	53.02	5.12
2	Abacavir	19.76	0.03	6,299.74	0.10
3	*trans*-Abacavir	20.76	0.02	48.87	0.55
4	O-Pyrimidine derivative abacavir	24.99	0.01	21.63	2.50
5	*t*-Butyl derivative abacavir	30.84	0.01	14.57	1.79

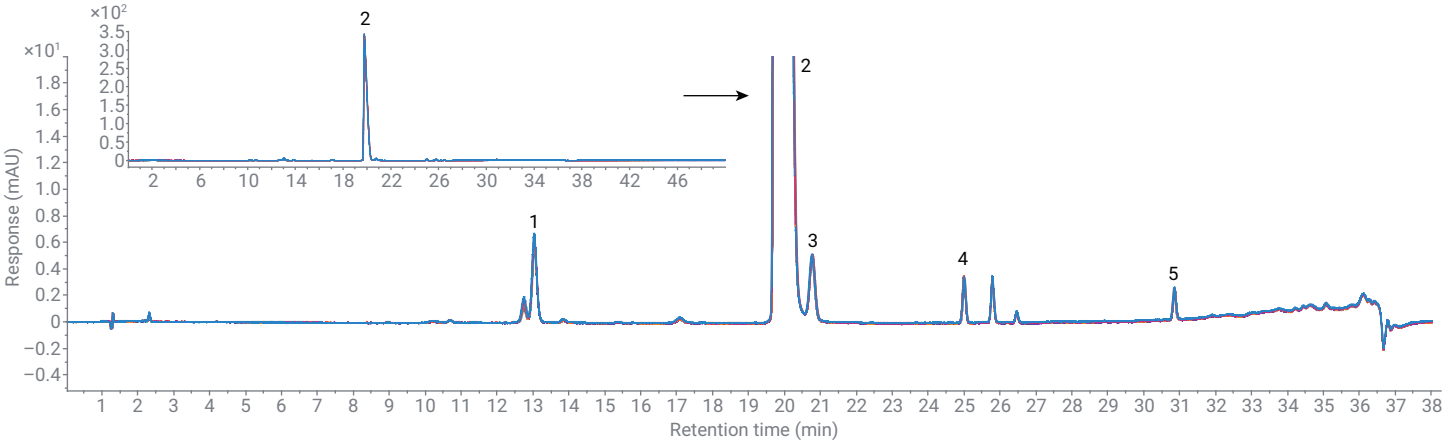

Figure 2. Analysis of the abacavir related compounds mixture using the USP compendial method on an Agilent ZORBAX StableBond C18, 4.6 × 150 mm, 5 µm column using an Agilent 1260 Infinity III LC System. The chromatogram shows a zoom, and the inset shows an overview. Overlay of six consecutive analyses. N = 6 for calculation of RSDs.

Figure 3 shows the comparison of the analysis of the abacavir related compounds mixture performed using the 1260 Infinity III LC and the 1100 Series Quaternary LC. Excellent agreement of retention times was observed, with a maximum deviation of 0.8%. The possibility for seamless transfer of the analysis of related compounds of abacavir from an 1100 Series Quaternary LC to a 1260 Infinity III LC saves time and money, as the same validated method can still be used.

USP chapter 621[5] describes permitted adjustments of chromatographic conditions in gradient elution LC, and allows method transfer to superficially porous particle (SPP) columns. The 1260 Infinity III LC offers a pressure range of 600 bar, thereby enabling UHPLC analyses using 2.7 µm SPP columns. Table 3 shows the method parameters for the analysis of related compounds of abacavir translated to an InfinityLab Poroshell 120 SB-C18, 3.0 × 75 mm, 2.7 µm column, following the procedures described in USP chapter 621.[5] The results of the analysis of related compounds of abacavir using the 1260 Infinity III LC and the method parameters shown in Table 3 are presented in Figure 4.

Excellent retention time precision was observed during the analysis of the abacavir related compounds mixture on the InfinityLab Poroshell 120 SB-C18, 3.0 × 75 mm, 2.7 µm column employing the 1260 Infinity III LC. Resolution between abacavir and *trans*-abacavir was increased to 3.1, fulfilling system suitability requirements. Method transfer of the analysis of the abacavir related compounds mixture to a 3.0 × 75 mm, 2.7 µm column resulted in a 70% reduction in analysis time and 78.6% less solvent consumption per injection, offering the possibility to significantly reduce the cost per sample.

Compound		Agilent 1100 LC RT (min)	Agilent 1260 Infinity III LC RT (min)	RT Difference (min)	RT Difference (%)
Number	Name				
1	Descyclopropyl abacavir	13.13	13.03	−0.10	−0.78
2	Abacavir	19.84	19.76	−0.08	−0.39
3	*trans*-Abacavir	20.83	20.76	−0.07	−0.34
4	O-Pyrimidine derivative abacavir	25.09	24.99	−0.10	−0.41
5	*t*-Butyl derivative abacavir	30.95	30.84	−0.11	−0.34

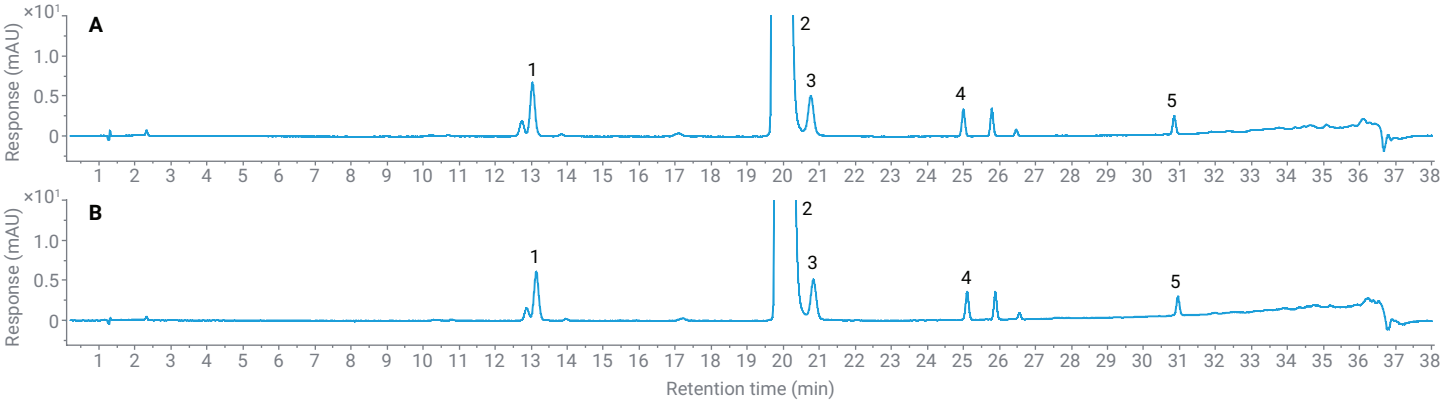

Figure 3. Comparison of the analysis of the abacavir related compounds mixture, performed using an Agilent 1260 Infinity III LC System (A) and an Agilent 1100 Series LC System (B).

Compound		Agilent 1260 Infinity III LC			
Number	Name	RT (min)	RT RSD (%)	Area	Area RSD (%)
1	Descyclopropyl abacavir	3.85	0.02	17.02	3.03
2	Abacavir	5.67	0.05	1,752.03	0.12
3	*trans*-Abacavir	5.97	0.05	13.65	0.46
4	O-Pyrimidine derivative abacavir	7.36	0.02	6.03	1.98
5	*t*-Butyl derivative abacavir	8.91	0.01	3.67	1.35

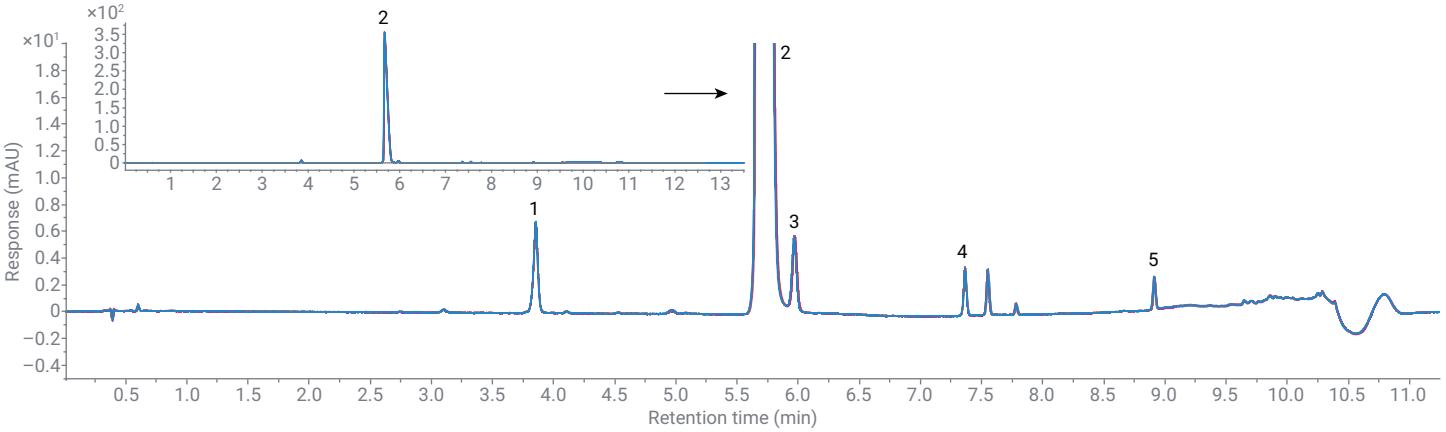

Figure 4. Analysis of the abacavir related compounds mixture on an Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 75 mm, 2.7 µm column using an Agilent 1260 Infinity III LC. The chromatogram shows a zoom, and the inset shows an overview. Overlay of six consecutive analyses. N = 6 for calculation of RSDs.

Conclusion

The analysis of related compounds of abacavir according to the USP monograph can seamlessly be transferred from an Agilent 1100 Series Quaternary LC to an Agilent 1260 Infinity III LC. Employing the 1260 Infinity III LC, the method can also be translated to UHPLC conditions using an Agilent InfinityLab Poroshell 120 column, enabling significant reductions in analysis time and solvent consumption, thereby reducing the cost per sample.

References

1. 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).
2. USP general chapter <1226> Verification of Compendial Procedures, official as of 01-Dec-2019. https://online.uspnf.com/uspnf/document/1_GUID-18A6E56B-8EA7-4B37-AB7D-82352B73A309_3_en-US?source=Activity (accessed 14 November 2024).
3. USP Monograph on Abacavir Sulfate, official as of 01-May-2020. https://online.uspnf.com/uspnf/document/1_GUID-0F0CC075-551C-4DC0-BBDD-D317593E2324_3_en-US?source=Activity (accessed 10 January 2025).
4. 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. *Agilent Technologies technical overview*, publication number 5994-8031EN, **2025**.
5. USP general chapter <621> Chromatography, official as of 01-Dec-2024. https://online.uspnf.com/uspnf/document/1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_9_en-US?source=TOC (accessed 10 January 2025).

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