Method Transfer from an Agilent 1260 Infinity LC to an Agilent 1260 Infinity II LC

Proof of Equivalency for the Analysis of Paracetamol and its Impurities

Application Note

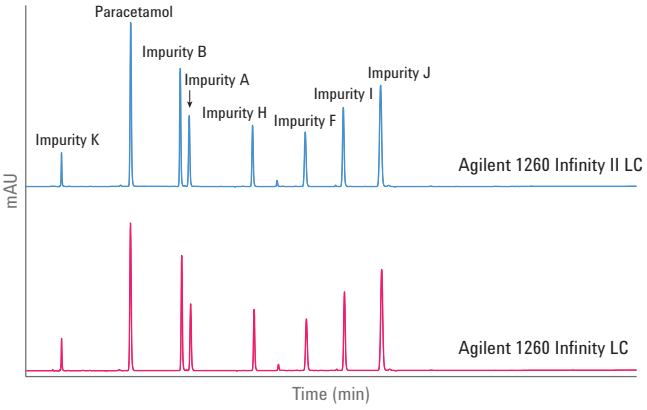
Small Molecule Pharmaceuticals

Author

Sonja Krieger
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

Instrument-to-instrument method transfer, for example to newer LC instruments such as the Agilent 1260 Infinity II LC, is an important topic in all laboratories. This Application Note shows method transfer from an Agilent 1260 Infinity LC to an Agilent 1260 Infinity II LC for the analysis of paracetamol and its impurities, and proves that equivalent results in terms of retention times and resolution are obtained.



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Introduction

Instrument-to-instrument method transfer is an important topic for laboratories throughout different industries[1]. Especially for validated methods in the pharmaceutical industry, instrument-to-instrument method transfer is compulsory, but it is also important in QA/QC in other industries. One example of instrument-to-instrument method transfer is method transfer to newer LC instruments, such as the Agilent 1260 Infinity II LC. This Application Note shows the analysis of paracetamol and its impurities using an Agilent 1260 Infinity LC, and the seamless method transfer to a 1260 Infinity II LC. In addition, decreasing the analysis time by making full use of the pressure range of the 1260 Infinity II LC will be shown.

The analysis of impurities in drugs is critical in the pharmaceutical industry. According to ICH guideline Q3A (R2), impurities at or above 0.05 % in new drug substances need to be reported, and impurities at or above 0.1 % in new drug substances need to be identified[2]. Paracetamol (4-acetamidophenol) is used as an analgesic for home medication in the treatment for relief of pain and fever[3]. It is contained in many cold and flu medications as well as prescription analgesics[3]. Unlike aspirin and ibuprofen, paracetamol does not have anti-inflammatory properties[3].

Experimental

Equipment

The Agilent 1260 Infinity II LC comprised the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1260 Infinity II Multisampler (G7167A) with sample cooler (option #100)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with a 10 mm Max-Light cartridge cell (G4212-60008)

The Agilent 1260 Infinity LC comprised the following modules:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity High Performance Autosampler (G1367E) with 1290 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with a 10 mm Max-Light cartridge cell (G4212-60008)

Software

Agilent OpenLAB CDS Version 2.1 (availability planned for September 2016)

Column

Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902T)

Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, 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). Trifluoroacetic acid was purchased from Sigma-Aldrich (Steinheim, Germany). Paracetamol, as well as impurity B (N-(4-hydroxyphenyl)-propanamide), impurity F (4-nitrophenol), impurity H (4-(acetylamino)phenylacetate), impurity I (1-(2-hydroxyphenyl)ethanone), impurity J (4-chloroacetanilide), and impurity K (4-aminophenol) were obtained from LGC Standards (Teddington, UK). Impurity A (2-acetamidophenol) was obtained from Sigma-Aldrich (Steinheim, Germany).

Sample

Mixture of paracetamol and its impurities A, B, F, H, I, J, and K. A mixture of paracetamol and its impurities A, B, F, H, I, J, and K was prepared in water/acetonitrile (95:5, v:v) with 0.1 % trifluoroacetic acid at a concentration of 50 µg/mL of each component.

Methods

Table 1. Chromatographic conditions.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water + 0.1 % TFA B) Acetonitrile + 0.09 % TFA
Gradient	5 % B at 0 minutes, 5 % B at 0.67 minutes, 70 % B at 16 minutes
Stop time	16 minutes
Post time	6.7 minutes
Flow rate	1.500 mL/min
Temperature	30 °C
Detection	270 nm/4 nm Ref. 395 nm/10 nm, 20 Hz
Injection volume	10.0 µL
Sample temperature	6 °C

Table 2. Chromatographic conditions with increased flow rate.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water + 0.1 % TFA B) Acetonitrile + 0.09 % TFA
Gradient	5 % B at 0 minutes, 5 % B at 0.40 minutes 70 % B at 9.60 minutes
Stop time	9.60 minutes
Post time	4.00 minutes
Flow rate	2.500 mL/min
Temperature	30 °C
Detection	270 nm/4 nm Ref. 395 nm/10 nm, 40 Hz
Injection volume	10.0 µL
Sample temperature	6 °C

Results and Discussion

This Application Note shows the analysis of paracetamol and its impurities on a 1260 Infinity LC using the chromatographic conditions described in Table 1. This method was transferred to a 1260 Infinity II LC for proof of equivalency.

Figure 1 and Table 3 show the analysis of paracetamol and its impurities A, B, F, H, I, J, and K, as well as the corresponding retention time precision, area precision, and resolution on the 1260 Infinity LC.

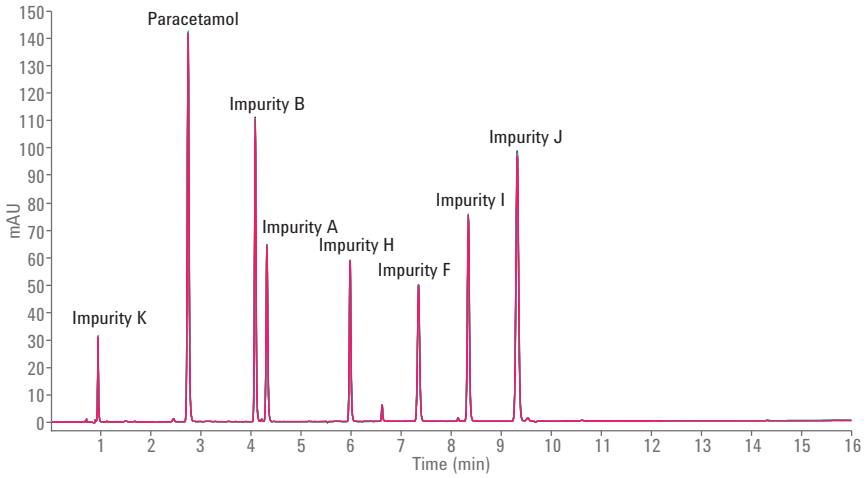

Figure 1. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity LC; overlay of 10 consecutive runs.

Table 3. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Impurity K	0.93	0.04	41.4	0.72	–
Paracetamol	2.73	0.04	360.4	0.11	37.0
Impurity B	4.08	0.02	237.8	0.17	22.0
Impurity A	4.31	0.02	156.6	0.16	3.9
Impurity H	5.98	0.02	136.2	0.24	26.9
Impurity F	7.34	0.02	157.2	0.09	19.1
Impurity I	8.34	0.02	216.0	0.26	12.6
Impurity J	9.32	0.02	372.4	0.53	11.1

The method for analysis of paracetamol and its impurities was transferred without any changes to the 1260 Infinity II LC. Figure 2 and Table 4 show the resulting separation, the corresponding retention time and area precision, as well as the resolution. In terms of area precision, the 1260 Infinity II LC outperforms the excellent 1260 Infinity LC.

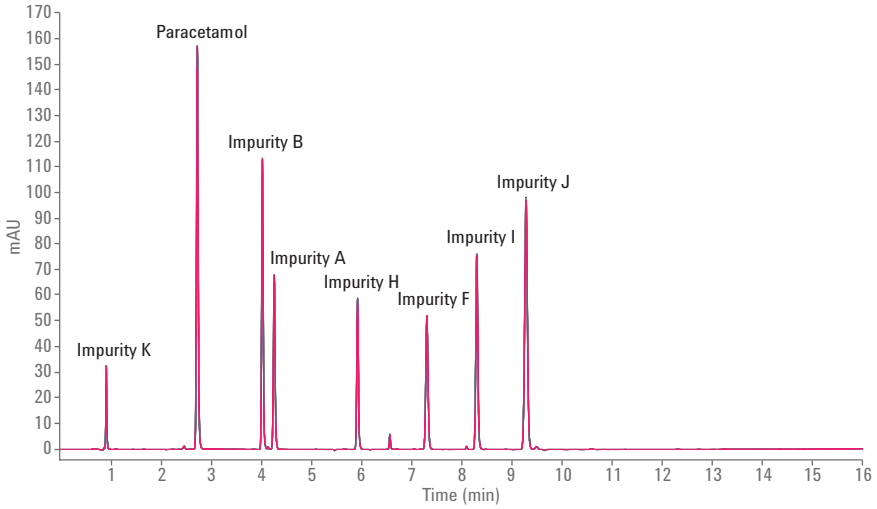

Figure 2. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 4. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity II LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Impurity K	0.93	0.04	43.3	0.50	–
Paracetamol	2.74	0.05	373.4	0.11	38.7
Impurity B	4.04	0.02	243.4	0.09	22.1
Impurity A	4.27	0.02	163.3	0.10	4.0
Impurity H	5.93	0.02	138.2	0.08	26.8
Impurity F	7.32	0.01	165.6	0.08	19.1
Impurity I	8.31	0.02	220.6	0.07	12.5
Impurity J	9.29	0.01	375.3	0.38	11.0

Figure 3 and Table 5 compare the retention times of paracetamol and its impurities analyzed on the 1260 Infinity LC and the 1260 Infinity II LC. With a maximum retention time deviation of less than 1 %, excellent agreement of retention times between the 1260 Infinity LC and the 1260 Infinity II LC was observed. The same peak resolution achieved using the 1260 Infinity LC was also obtained using the 1260 Infinity II LC. This proves the possibility for seamless method transfer from the 1260 Infinity LC to the 1260 Infinity II LC for the analysis of paracetamol and its impurities.

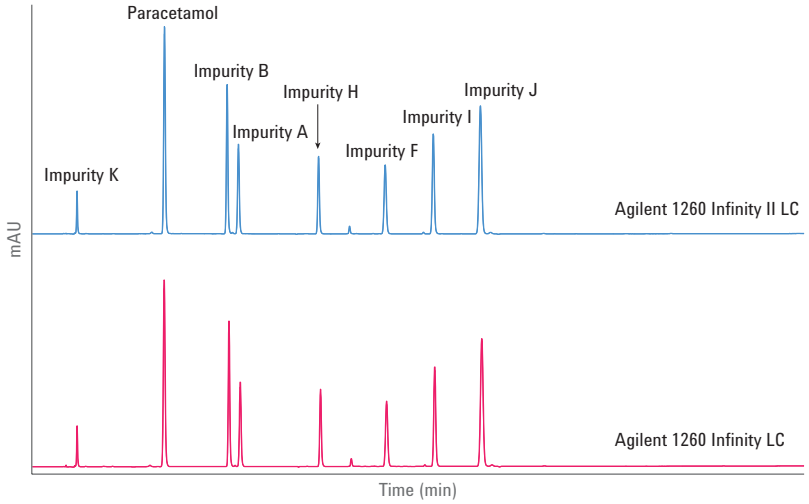

Figure 3. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity LC and an Agilent 1260 Infinity II LC.

Table 5. Analysis of paracetamol and its impurities on an Agilent 1260 Infinity LC and an Agilent 1260 Infinity II LC; comparison of retention times.

Compound	RT Deviation (min)	RT Deviation (%)
Impurity K	0.00	-0.2
Paracetamol	0.01	0.3
Impurity B	-0.04	-0.9
Impurity A	-0.04	-0.9
Impurity H	-0.04	-0.7
Impurity F	-0.03	-0.4
Impurity I	-0.03	-0.4
Impurity J	-0.03	-0.3

For this Application Note, instrument control of the 1260 Infinity LC and the 1260 Infinity II LC as well as data analysis was performed using Agilent OpenLAB CDS Version 2.1. OpenLAB CDS Version 2.1 offers a single software for liquid chromatography, gas chromatography, and mass spectrometry. It provides a flat user interface, and customized, interactive reporting with drag and drop template creation. Figure 4 shows the data analysis in OpenLAB CDS Version 2.1. The layout of the data analysis is user-configurable and enables, for example, the simultaneous display of selected chromatograms, the peak explorer, injection results, and peak details for a selected peak.

Agilent InfinityLab columns and supplies work together perfectly with the 1260 Infinity II LC for maximum performance and efficiency of LC workflows. The Agilent InfinityLab Quick Connect (p/n 5067-6166, Quick Connect fitting with a 0.17 × 105 mm capillary) and Quick Turn Fittings (p/n 5067-5966) enable a tool-free, fast and easy column installation, ensuring a perfect column connection independent of the user. The setup of the 1260 Infinity II LC on the Agilent InfinityLab Flex Bench rack (p/n 5043-1252) enables efficient use of lab space, and an ergonomic approach with easy access to the instrument.

Agilent InfinityLab Poroshell columns, in combination with the pressure range of up to 600 bar of the 1260 Infinity II LC, enable UHPLC analyses, offering time and solvent savings while maintaining or increasing peak resolution. When ordering a 1260 Infinity II LC, the customer has the choice between different InfinityLab Poroshell columns that can be delivered with the system, for example the InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column (p/n 695975-902T).

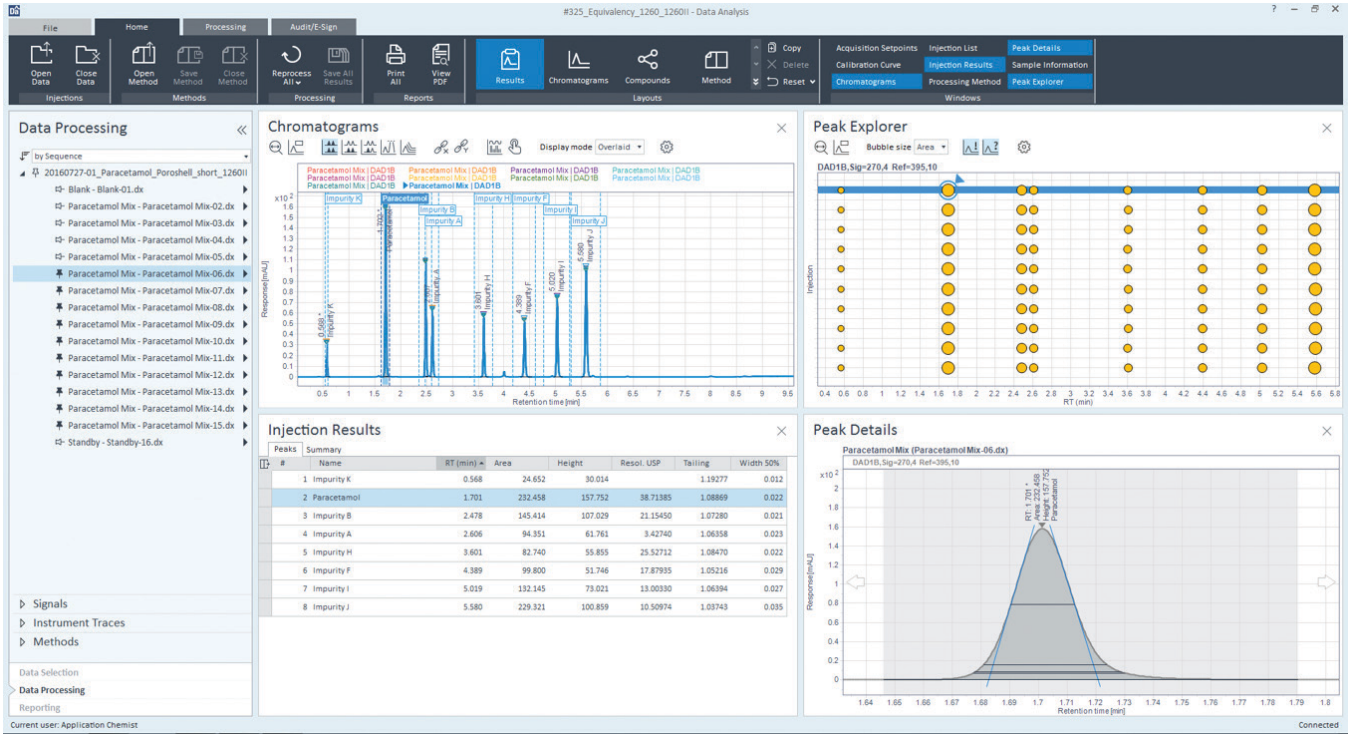

Figure 4. Impression of the data analysis in Agilent OpenLAB CDS Version 2.1.

Making full use of the pressure range of the 1260 Infinity II LC, the InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm column can be operated at a relatively high flow rate of 2.5 mL/min (chromatographic conditions described in Table 2). Figure 5 and Table 6 show the resulting analysis of paracetamol and its impurities, the corresponding retention time and area precision, as well as resolution. The increased flow rate decreases the analysis time by 40 %, with only marginal loss in resolution.

Conclusions

Method transfer for the analysis of paracetamol and its impurities from the Agilent 1260 Infinity LC to the Agilent 1260 Infinity II LC showed a maximum retention time deviation of less than 1 % while achieving the same peak resolution. This proves the equivalency of the 1260 Infinity II LC compared to the 1260 Infinity LC for the analysis of paracetamol and its impurities, and shows the possibility of seamless method transfer. In addition, by increasing the flow rate and making full use of the pressure range of the 1260 Infinity II LC the analysis time could be decreased by 40 %.

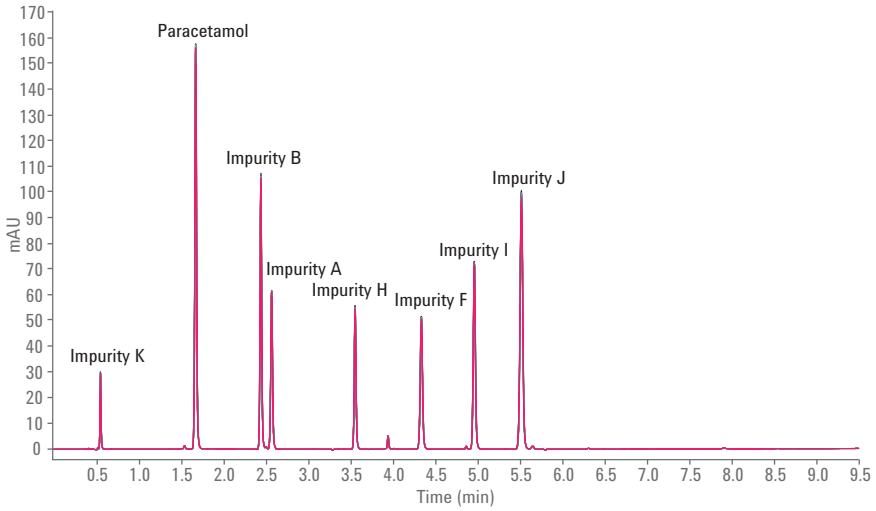

Figure 5. Analysis of paracetamol and its impurities with increased flow rate on an Agilent 1260 Infinity II LC; overlay of 10 consecutive runs.

Table 6. Analysis of paracetamol and its impurities with increased flow rate on an Agilent 1260 Infinity II LC; retention time and area precision determined from 10 consecutive runs.

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Impurity K	0.57	0.03	24.6	0.29	–
Paracetamol	1.70	0.03	232.6	0.07	38.5
Impurity B	2.48	0.01	145.5	0.08	21.0
Impurity A	2.61	0.02	94.4	0.07	3.4
Impurity H	3.60	0.01	82.8	0.09	25.3
Impurity F	4.39	0.02	99.8	0.09	17.7
Impurity I	5.02	0.02	132.2	0.07	12.9
Impurity J	5.58	0.02	228.3	0.34	10.4

References

1. Agilent 1290 Infinity with ISET, *Agilent Technologies User Manual*, part number G4220-90314, **2015**.

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3. Bosch, *et al.* Determination of paracetamol: Historical evolution, *J. Pharm. Biomed. Anal.* **2006**, *42*, 291–321.

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