

# Seamless method transfer from the Agilent 1200 Series LC System to the Agilent 1260 Infinity LC System

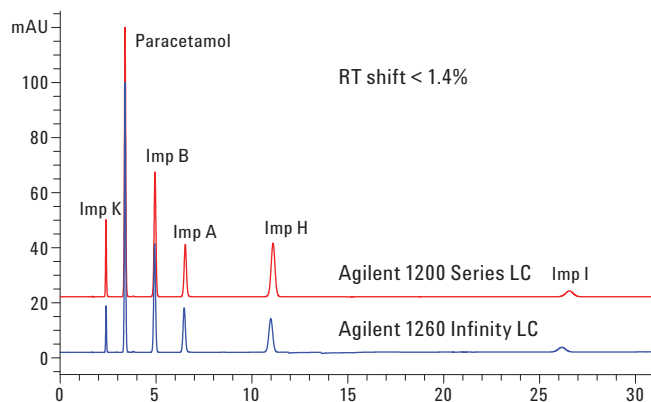
Comparison of system suitability results according EP and USP regulations for the analysis of paracetamol (acetaminophen) and its impurities

## Application Note

Pharma

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### Abstract

This Application Note shows a seamless method transfer from the Agilent 1200 Series LC System to the Agilent 1260 Infinity LC System using the analysis of paracetamol and related impurities as an example. The system suitability test, developed on an Agilent 1200 Series LC was used to test whether the Agilent 1260 Infinity LC System fulfills the acceptance criteria. The obtained results were in compliance with results typically obtained on the Agilent 1200 Series LC System. The retention time shift was < 1.4% compared to retention times obtained on the Agilent 1200 Series LC.

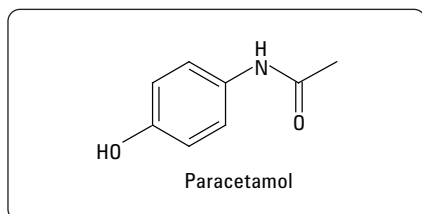


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## Introduction

Regulated environments such as the pharmaceutical industry requires that validated methods in compliance with EP or USP regulations are in use. If new instrumentation must be purchased and used, one requirement is that the validated method applied on the new instrumentation provide results inside the operating range and acceptance criteria determined for the existing instrument. This is important to avoid the need to revalidate an existing method, which is time consuming and costly.

The analysis of paracetamol and related impurities were chosen to demonstrate that seamless method transfer is possible. Paracetamol, (Figure 1) is frequently analyzed using reversed phase gradient or isocratic conditions and UV detection.<sup>1,2</sup> This study used an isocratic method with UV detection according to European Pharmacopoeia and USP regulations.<sup>3,4</sup>



**Figure 1**  
Structure of Paracetamol.

The system suitability test, developed on an Agilent 1200 Series LC was used to test whether the Agilent 1260 Infinity LC Systems fulfills the acceptance criteria. The chromatographic conditions of the system suitability test were transferred to the Agilent 1260

Infinity LC System without any changes. The obtained results were compared for compliance with results typically obtained on the Agilent 1200 Series LC System.

## Experimental

### Instrumentation

An Agilent 1200 Series LC System with an Agilent 1260 Infinity LC System with the following configurations were used (Table 1).

Agilent 1260 Infinity LC System Configuration	Agilent 1200 Series LC System Configuration
Quaternary pump with integrated vacuum degasser (G1311B)	Quaternary pump (G1354A) with external degasser vacuum degasser (G1322A)
Column compartment (G1316A)	Column compartment (G1316A)
Diode array detector (G4212B) with 10 mm path length cell	Diode array detector (G1315D) with 10 mm path length cell
Autosampler (G1367E) + sample cooling unit at 10 °C	Standard Autosampler +sample cooling unit at 10 °C
Software: ChemStation B.04.02	Software: ChemStation B.04.02

**Table 1**  
Instrument configurations.

### Chromatographic Conditions According to USP Method<sup>5</sup>

Column	Agilent ZORBAX StableBond-C8, 4.6 mm × 250 mm, 5 μm
Mobile phase	Mix together 375 mL of a 17.9 g/L solution of disodium hydrogen phosphate, 375 mL of a 7.8 g/L solution of sodium dihydrogen phosphate, and 250 mL of methanol containing 6 mL of a 400 g/L (40%) solution of tetrabutylammonium hydroxide in methanol
Pump settings	No gradient (in accordance with EP regulations)
Stop time	45 min
Flow rate	1.5 mL/min, isocratic
Injection volume	20 μL
Column temp	35 °C
Detector	Agilent 1200 Series LC System with 10 mm path length Agilent 1260 Infinity LC System with 10 mm path length Peak width 0.1 min (5 Hz) Signal 245 nm

For all experiments the same column, same batch of prepared sample and the same batch of mobile phase was used.

## Preparation of Samples

The reference solution was prepared as follows, in accordance with EP regulations. Five milligrams of paracetamol and 5 mg of each impurity were dissolved in methanol and diluted to 20 mL with the same solvent. One milliliter of the solution was diluted to 25 mL with the mobile phase. One milliliter of the 25 mL was diluted 1:10 with the mobile phase. The substances to be checked were paracetamol and impurities K, A, B, H, I and J.

Impurity K = 4-aminophenol

Impurity A = 2-acetamidophenol

Impurity B =

N-(4-hydroxyphenyl)propanamide

Impurity H = 4-(acetylamino)phenyl

Acetate (N,O-Diacetyl-4-aminophenol)

Impurity I =

1-(2-hydroxyphenyl)ethanone

Impurity J = 4-chloroacetanilide

The following solutions were prepared. The last 1:10 diluted concentration was used for injection (Table 2).

## Results and Discussion

The operating range for paracetamol was defined and an appropriate chromatographic method was developed and validated on the Agilent 1200 Series LC System.

In addition, the EP regulations require system suitability testing with a reference solution (see Preparation of Samples). The USP Chromatography General Chapter states:

"System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such."<sup>3</sup>

System suitability testing has to be

done to verify that the LC system fulfills the acceptance criteria for the validated method. It must be performed before and throughout all regulated assays. The system suitability test, developed on the Agilent 1200 Series Quaternary LC System for paracetamol and related impurities included the following acceptance criteria:

- Precision of areas must be < 2% RSD
- Precision of retention times must be < 0.5% RSD
- Resolution must be > 4 for impurity K and paracetamol
- Signal-to-noise ratio must be > 50 for impurity J
- Plate number for impurity H > 2000 plates
- Tailing factor < 2
- Peak symmetry 0.8 and 1.1

Evaluation of column performance parameters such as plate number, symmetry and tailing factors help determine the influence of different instruments on these parameters.

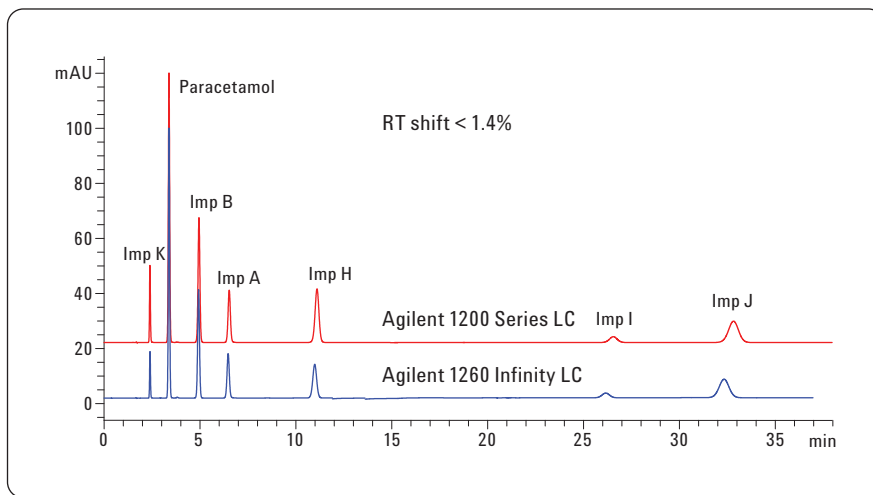
	Stock solution in methanol (µg/100 µL)	1:25 diluted in mobile phase (µg/1 mL)	1:10 diluted in mobile phase (ng/20 µL)
Impurity K = 4-aminophenol	220	8.8	17.6
Impurity H = 4-(acetylamino)phenyl acetate (N,O-diacetyl-4-aminophenol)	330	13.2	26.4
Impurity F = nitrophenol	360	14.4	28.8
Impurity A = 2-acetamidophenol	320	12.8	25.6
Impurity B = N-(4-hydroxyphenyl)propanamide	305	12.2	24.4
Impurity J = 4-chloroacetanilide	215l	8.6	17.2
Impurity I = 1-(2-hydroxyphenyl)ethanone	315	12.6	25.2
Paracetamol	410	16.4	32.8

**Table 2**  
**Sample concentrations.**

An overlay of the chromatograms obtained on the two LC systems is shown in Figure 2.

The retention times do not differ more than 1.4% from the reference chromatogram of the Agilent 1200 Series LC System. The system suitability test was applied to the new instrument and the results compared to results obtained on the Agilent 1200 Series LC. The results are combined in Tables 3–5. Data for retention times, symmetry, USP tailing, plates and resolution can be obtained from the *Extended Performance* report. This report is based on calibrated peaks. Signal-to-noise data are included in the *Performance and Noise* report. Statistical data are included in the *Sequence Summary* report. All report styles are available from the *Specify Report* menu in the ChemStation.<sup>6</sup>

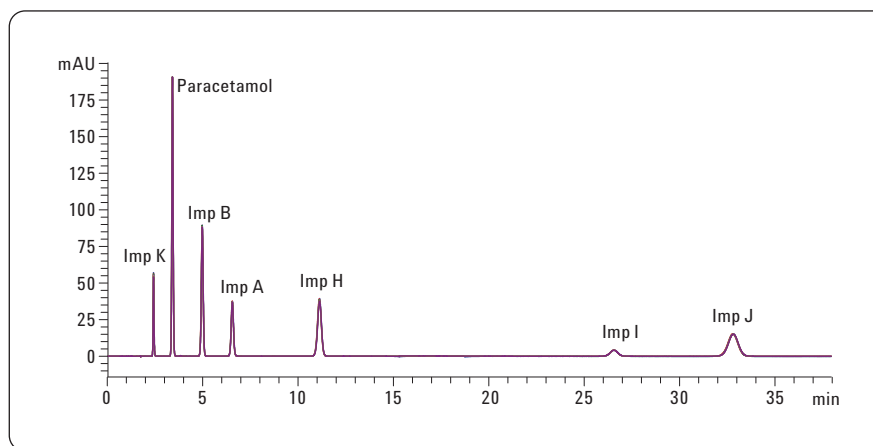
An overlay of six consecutive runs performed on the Agilent 1260 Infinity LC System is shown as example for precision evaluation of retention times and areas (Figure 3).



**Figure 2**  
Overlay of chromatograms.

Compound	Agilent 1200 Series LC System			Agilent 1260 Infinity LC System		
	Retention time (min)	Resolution	Signal-to-noise	Retention time (min)	Resolution	Signal-to-noise
Impurity K	2.398		3169.5	2.402		2804.3
Paracetamol	3.393	<b>9.53</b>		3.393	<b>9.68</b>	
Impurity B	4.957	10.34	5148.3	4.939	10.27	6161.4
Impurity A	6.536	7.85	2163.9	6.482	7.74	2522.3
Impurity H	11.117	15.17	2199.6	11.005	15.11	2002.1
Impurity I	26.583	27.53	242.3	26.195	27.11	270.8
Impurity J	32.857	7.28	<b>884.1</b>	32.369	7.18	<b>1042.5</b>

**Table 3**  
Comparison of retention times, signal-to-noise and retention data.



**Figure 3**  
Overlay of six consecutive runs using the Agilent 1200 Series LC System; evaluation of precision data.

The results for the Agilent 1260 Infinity LC System shows that it is fully compliant with the system suitability acceptance criteria stated for the Agilent 1200 Series.

The retention time shift is < 1.4% for all peaks on the new instrument. The signal-to-noise ratio is typically improved for the Agilent 1260 Infinity LC System. The precision of retention times is improved by a factor 1.5–2 on the Agilent 1260 Infinity LC System. The precision of areas is comparable. The plate number at half height, peak symmetry and the USP tailing factors are comparable.

Compound	Agilent 1200 Series LC System Precision		Agilent 1260 Infinity LC System Precision	
	RSD RT(%)	RSD area (%)	RSD RT(%)	RSD area (%)
Impurity K	0.050	1.4330	0.038	1.1679
Paracetamol	0.056	0.6078	0.032	0.2772
Impurity B	0.055	0.0683	0.022	0.0586
Impurity A	0.053	0.0796	0.027	0.0878
Impurity H	0.048	1.4295	0.026	1.1386
Impurity I	0.032	0.3905	0.024	0.4442
Impurity J	0.043	0.1589	0.029	0.0945

**Table 4**  
Comparison of precision data.

Compound	Agilent 1200 Series LC System			Agilent 1260 Infinity LC System		
	Plate number (HH)	Peak symmetry	Peak tailing	Plate number	Peak symmetry	Peak tailing
Impurity K	13642	0.886	1.095	14000	0.927	1.058
Paracetamol	11861	0.947	1.038	12132	0.969	1.019
Impurity B	12348	0.991	1.003	12388	0.989	1.005
Impurity A	14005	0.973	1.014	13775	0.960	1.031
Impurity H	13728	1.011	0.984	13654	0.998	0.995
Impurity I	19332	1.007	0.994	19936	0.937	1.021
Impurity J	17576	1.018	0.979	17659	0.981	0.993

**Table 5**  
Comparison of plate numbers, peak symmetry and peak tailing.

## Conclusion

This study demonstrated that seamless method transfer from an Agilent 1200 Series LC System to an Agilent 1260 Infinity LC System is possible, using an isocratic EP method. Analysis of acetaminophen (paracetamol) in pain relievers was used as an example in this study. The system suitability test, developed on an Agilent 1200 Series LC System for this method, was applied on the new Agilent 1260 and the Agilent 1220 Infinity LC System. The obtained results were compared with results typically obtained on the Agilent 1200 Series LC System (Table 6).

Both new instruments fulfilled the acceptance criteria of the system suitability test. The retention time shift for all peaks was < 1.4% using the same column and the same batch of mobile phase. Better results have been obtained for the resolution on the Agilent 1260 Infinity LC, for the signal-to-noise ratios and for the precision of retention times.

Parameter and acceptance criteria	Agilent 1200 Series LC System	Agilent 1260 Infinity LC System
RSD RT < 0.5% RSD	< 0.057% RSD	< 0.039% RSD
RSD area < 2% RSD	< 1.44% RSD	< 1.17% RSD
Rs Paracetamol > 4	9.53	9.68
S/N Imp J > 50	884.1	1042.5
Plate number Imp H > 2000	13728	13654
Tailing factors < 2	0.98 – 1.10	0.99 – 1.06
Symmetry factors 0.8 – 1.1	0.880 – 1.019	0.927 – 0.999

**Table 6**  
**Comparison of acceptance criteria.**

## **References**

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