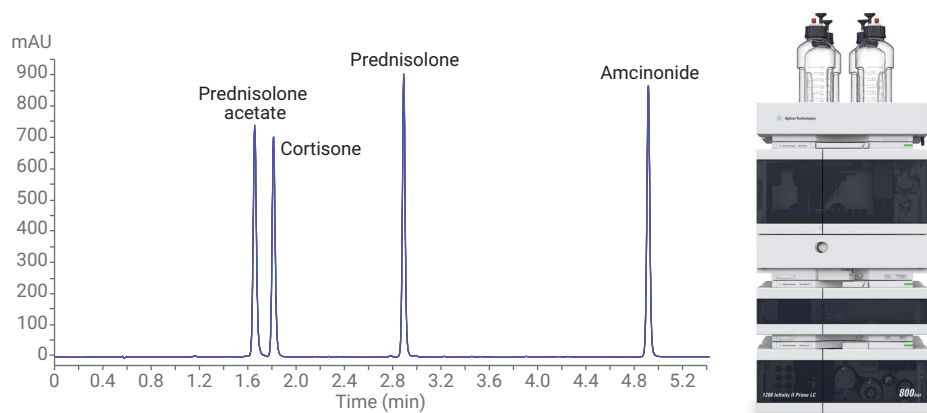


# Comparison of the 800 bar and 600 bar Agilent 1260 Infinity II Vialsampler

Demonstration of Backward Compatibility Through the Analysis of Corticosteroids



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## Abstract

Laboratories in regulated environments require reliable and efficient LC systems. The Agilent 1260 Infinity II Prime LC provides highest confidence together with excellent performance characteristics. The Agilent 1260 Infinity II Vialsampler 800 bar (G7129C) is equivalent to the previously introduced Agilent 1260 Infinity II Vialsampler 600 bar (G7129A), however it has an extended pressure range of up to 800 bar. This Technical Overview compares the two 1260 Infinity II Vialsamplers with regards to reproducibility, resolution, linearity, and sensitivity. The results clearly demonstrate an equivalent performance of the 1260 Infinity II Vialsampler 800 bar for the analysis of four corticosteroids, which allows a straight method transfer and reduced instrument revalidation.

## Introduction

The Agilent 1260 Infinity II Prime LC is a powerful, robust, and reliable system with an extended pressure range of up to 800 bar. The LC system is perfectly suited to run a wide range of applications, such as legacy methods or the analysis of small and large molecules.

The analytical method transfer from one instrument to another is an important topic for laboratories throughout different industries. In regulated pharmaceutical laboratories, method changes are restricted or even prohibited<sup>1</sup>. By transferring a method, the instrument must meet certain requirements such as equal separation, precision, or resolution to reduce the effort of method revalidation.

This Technical Overview demonstrates the backward compatibility of the Agilent 1260 Infinity II Vialsampler (800 bar) with the 600 bar 1260 Infinity II Vialsampler. Both samplers were evaluated regarding reproducibility, resolution, linearity, and sensitivity by analyzing a mix of four corticosteroids. Chlorhexidine was used to determine the carryover on both samplers.

## Experimental

### Instrumentation

The Agilent 1260 Infinity II LC System used for the experiments consisted of the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Vialsampler (G7129A and G7129C), equipped with an Integrated Column Compartment (3 µL heater; G7130-64430) and Integrated Sample Cooler (Option #100)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A)

### Solvents and Samples

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). Chlorhexidine and corticosteroids were purchased from Sigma-Aldrich Corp., St. Louis, USA.

### Software

Agilent OpenLAB CDS 2.2 (M8413AA)

**Table 1.** Chromatographic parameters for analyzing corticosteroids.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695575-302)
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.8 mL/min
Gradient	30 %B to 95 %B in 7 minutes
Stop time	8 minutes
Post time	5 minutes
Injection volume	5 µL with 3 seconds needle wash (50 % acetonitrile in water)
Column temperature	30 °C
Detection	254/10 nm, ref. wavelength 360/100 nm, 20 Hz

**Table 2.** Chromatographic conditions for carryover measurements.

Parameter	Value
Column	Agilent Pursuit XRs 3 C18, 2.0 × 50 mm (p/n A6001050X020)
Mobile phase	A) 0.1 % TFA in water B) 0.1 % TFA in acetonitrile
Flow rate	0.5 mL/min
Isocratic conditions	40 %B
Stop time	2 minutes
Injection volume	1 µL, 3 seconds needle wash (0.1 % TFA in water)
Column temperature	30 °C
Detection	254/10 nm, ref. wavelength 360/100 nm, 20 Hz

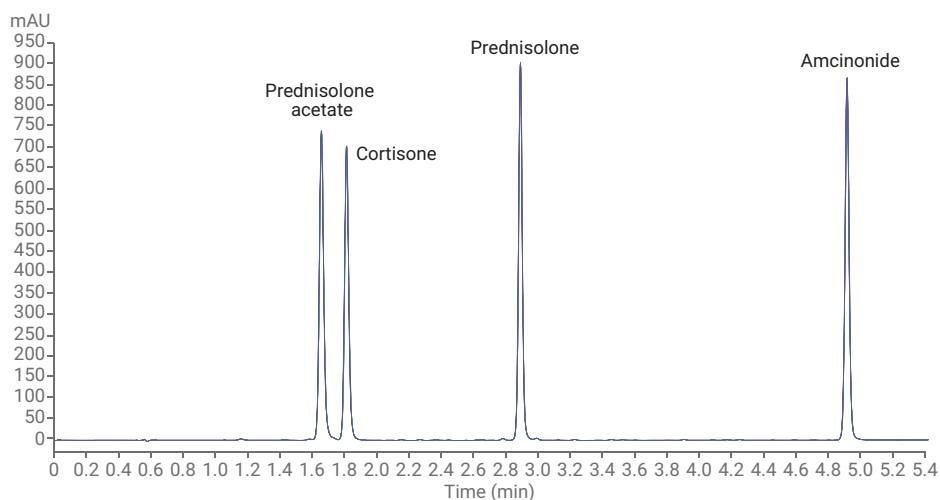
## Results and Discussion

This Technical Overview shows the analysis of four different corticosteroids: prednisolone, prednisolone acetate, cortisone, and amcinonide on a 1260 Infinity II Prime LC, equipped first with the 1260 Infinity II Vialsampler 600 bar, and next with the 800 bar 1260 Infinity II Prime LC version.

Figure 1 shows the chromatogram of the separated corticosteroids with the corresponding precision and resolution values. For this analysis, the 600 bar 1260 Infinity II Vialsampler was used.

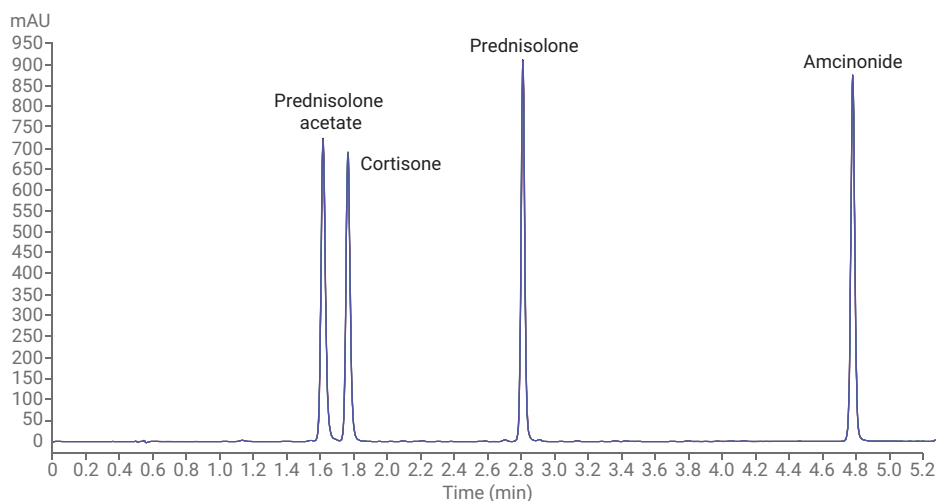
The precision values were calculated based on six consecutive runs. The retention time RSDs and area RSDs for all four compounds were clearly below the specification of 0.07 and 0.25 %, respectively.

Running the analytical method again using the 1260 Infinity II Vialsampler 800 bar resulted in precision values similar to the first sampler. The retention time and area RSD values were found to be excellent, with  $\leq 0.03$  % and  $\leq 0.15$  %, respectively (Figure 2).



Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
Prednisolone	1.65	0.04	0.14	–
Cortisone	1.81	0.04	0.14	3.22
Prednisolone	2.88	0.02	0.13	24.09
Amcinonide	4.90	0.01	0.08	45.62

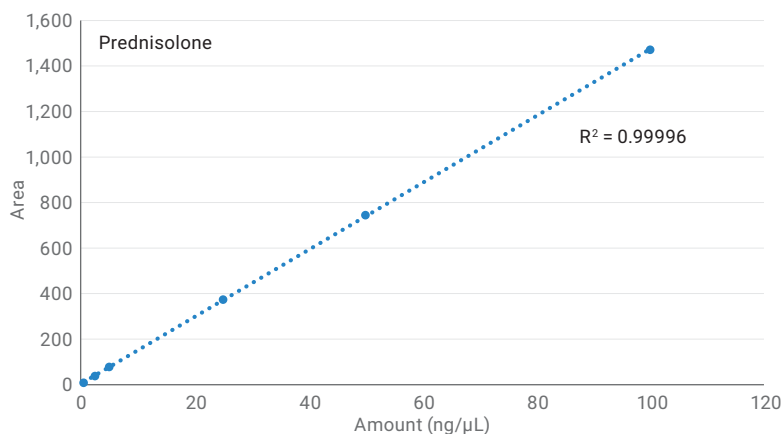
**Figure 1.** Analysis of four different corticosteroids using an Agilent 1260 Infinity II Prime LC equipped with a 600 bar Agilent 1260 Infinity II Vialsampler. The chromatogram shows an overlay of six consecutive runs.



Compound	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
Prednisolone	1.65	0.03	0.14	–
Cortisone	1.81	0.02	0.14	3.11
Prednisolone	2.87	0.02	0.15	23.98
Amcinonide	4.88	0.01	0.14	46.26

**Figure 2.** Analysis of four different corticosteroids using an Agilent 1260 Infinity II Prime LC equipped with an 800 bar Agilent 1260 Infinity II Vialsampler. The chromatogram shows an overlay of six consecutive runs.

Both systems were evaluated regarding limit of detection (LOD), limit of quantitation (LOQ), and linearity. For the calibration curve, six different concentration levels were prepared from 100 to 0.5 ng/μL for prednisolone, prednisolone acetate, and cortisone, and from 200 to 1 ng/μL for amcinonide. Figure 3 shows a representative calibration curve for prednisolone. LOD and LOQ values were calculated based on signal-to-noise ratio (S/N) of 3 and 10, respectively. Table 3 shows the evaluated LOD, LOQ, and linearity values for both system setups. Equivalent values for LOD, LOQ, and linearity were received for both Vialsamplers.



**Figure 3.** Calibration curve of prednisolone, analyzed with an Agilent 1260 Infinity II LC equipped with the Agilent Infinity II Vialsampler 800 bar.

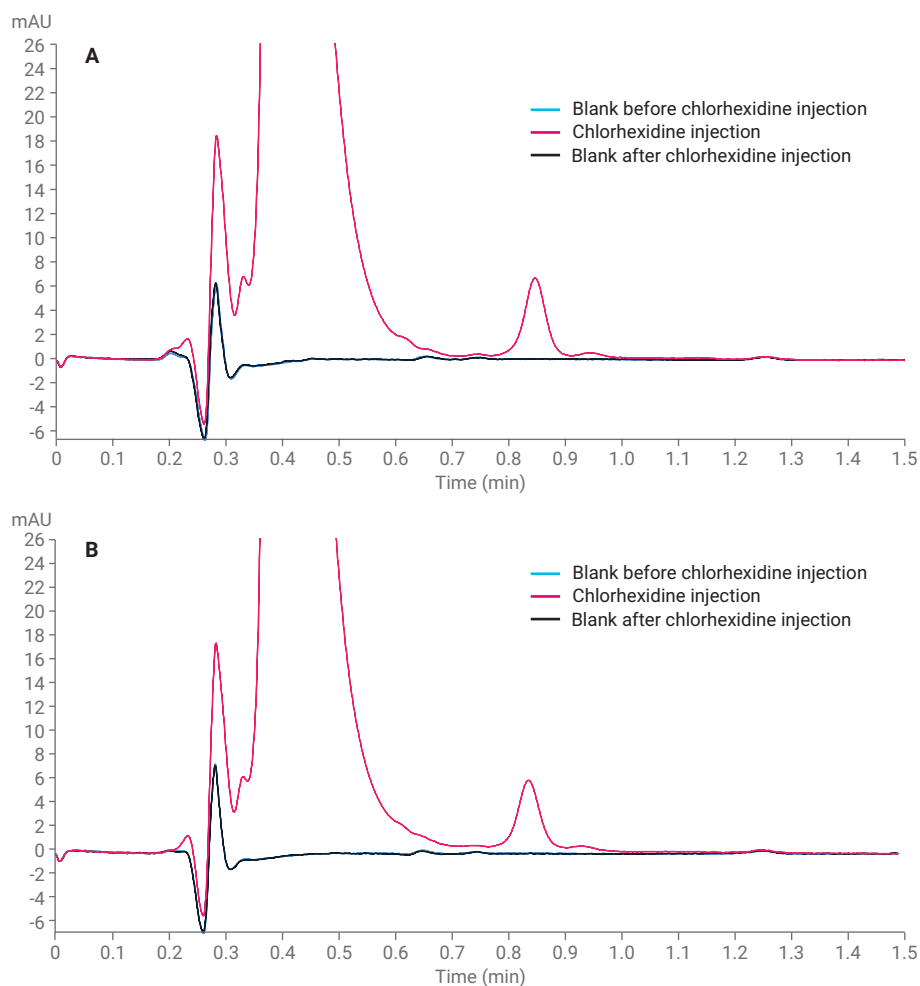
**Table 3.** Calibration data of the corticosteroids analyzed with the Agilent 1260 Infinity II Vialsampler 600 bar and Agilent 1260 Infinity II Vialsampler 800 bar. For the calibration curves, six data points in duplicate were used.

Compound	Agilent 1260 Infinity II Vialsampler 600 bar			Agilent 1260 Infinity II Vialsampler 800 bar		
	LOD (ng)	LOQ (ng)	Linearity (R <sup>2</sup> )	LOD (ng)	LOQ (ng)	Linearity (R <sup>2</sup> )
Prenisolon acetate	0.20	0.67	0.99998	0.20	0.67	0.99999
Cortisone	0.21	0.69	0.99996	0.21	0.70	0.99998
Prednisolone	0.18	0.58	0.99996	0.17	0.57	0.99998
Amcinonide	0.37	1.24	0.99997	0.37	1.22	0.99999

To determine the carryover, 1,200 ng chlorhexidine was injected with 3 seconds needle wash using 0.1 % TFA, followed by a blank injection. Figure 4 displays the results. The chromatogram overlay of the chlorhexidine injection and the blank injection clearly demonstrates that no carryover was detected, neither for the 600 bar 1260 Infinity II Vialsampler nor for the 800 bar 1260 Infinity II Vialsampler.

## Conclusion

This Technical Overview compares the Agilent 1260 Infinity II Vialsampler 600 bar with the Agilent 1260 Infinity II Vialsampler 800 bar. A standard analytical method was applied for the analysis of four different corticosteroids. Both 1260 Infinity II Vialsamplers showed great performance, with high area precision and linearity as well as comparable resolution, LODs, and LOQs. The results clearly demonstrate the equivalency of the two 1260 Infinity II Vialsamplers. Therefore, the 800 bar 1260 Infinity II Vialsampler can easily be implemented in an existing LC system.



**Figure 4.** UV chromatogram for carryover evaluation. Overlay of blank and chlorhexidine injections. For chromatogram A) the Agilent 1260 Infinity II Vialsampler 600 bar was used and for B) the Agilent 1260 Infinity II Vialsampler 800 bar.

## Reference

1. U.S. FDA, Guidance for Industry:  
Analytical Procedures and Methods  
Validation for Drugs and Biologics,  
July **2015**.

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