Application Note

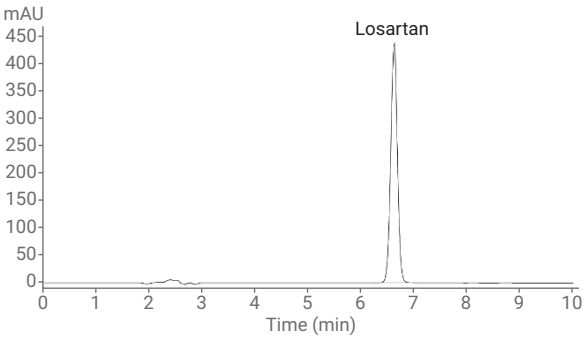
Small Molecule Pharmaceuticals

Agilent

Trusted Answers

Achieving the Required Area %RSD for the Losartan Potassium Compendial Method

Evaluation of the Performance of the Agilent 1260 Infinity II LC



Author

Kriti Tyagi
Agilent Technologies, Inc.

Abstract

This Application Note demonstrates the use of an Agilent 1260 Infinity II LC in achieving injection precision for the Losartan Potassium compendial method. As the USP is undergoing modernization of monographs, its General Chapter <621>[1] has been updated with system suitability criteria to provide high-quality standards. In USP 40–NF 35, guidelines have been introduced for achieving a %RSD of less than 0.73 %. These guidelines are applicable to all developed compendial methods for assaying drug substances. As a result, laboratories in regulated environments require reliable and robust LC systems to consistently achieve the system suitability criteria. The 1260 Infinity II LC provides the most reliable results with exceptional performance characteristics.

Introduction

The USP has updated its General Chapter <621> in terms of system suitability criteria, and introduced guidelines for %RSD of peak areas required in assays of drug substances. As per CHP<621>, the RSD must be less than 0.73 % for five consecutive standard injections in an assay method.

This Application Note demonstrates a compendial method for Losartan Potassium, which has stringent criteria, achieving less than 0.5 %RSD, and poses a unique challenge using 100 % methanol as a diluent and low injection volumes. The 1260 Infinity II HPLC is a robust and reliable system, perfectly suited to meet the system suitability requirement for achieving retention times and area %RSD within the specified limits.

Experimental

Instrumentation

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

- Agilent 1260 Infinity II Quaternary Pump
- Agilent 1260 Infinity II Vialsampler, equipped with an integrated column compartment (6-µL heat exchanger) and integrated sample cooler
- Agilent 1260 Infinity II Diode Array Detector WR

Solvent and samples

LC grade acetonitrile and methanol were sourced from J.T. Baker. Phosphoric acid was obtained from Rankem. Fresh ultrapure water was from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Software

Agilent OpenLab CDS 2.3.0 (M8413AA)

Sample preparation

Standard solution: 0.25 mg/mL of USP Losartan Potassium RS in methanol

Results and Discussion

The Losartan Potassium compendial method for assay was performed as per the chromatographic conditions given in Table 1. To obtain interday and intraday results, the analysis was performed for three consecutive days. On each day, a set of two trials were performed to analyze the repeatability of six injections. The %RSD for both peak area and retention time were calculated.

Figure 1 shows an overlay of chromatograms from six consecutive injections of Losartan Potassium standard solution. Table 2 lists the corresponding precision values. The %RSDs achieved for Losartan potassium were excellent for area and retention time, and for height as well. In addition, the %RSD was much lower than specified limits. On the first day for trial 1, the retention time RSD achieved was 0.08 % and the area was 0.14 %, and for trial 2 it was 0.00 % and 0.06 % for retention time and area, respectively.

Table 1. Chromatographic parameters for analyzing Losartan.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 250 × 4.6 mm, 5 µm
Mobile phase	0.1 % *Ortho* phosphoric acid in water (40 %):acetonitrile (60 %)
Flow rate	1.0 mL/min
Injection volume	10 µL
Column temperature	35 °C
Detection	254 nm
Diluent	Methanol
Run time	10 minutes

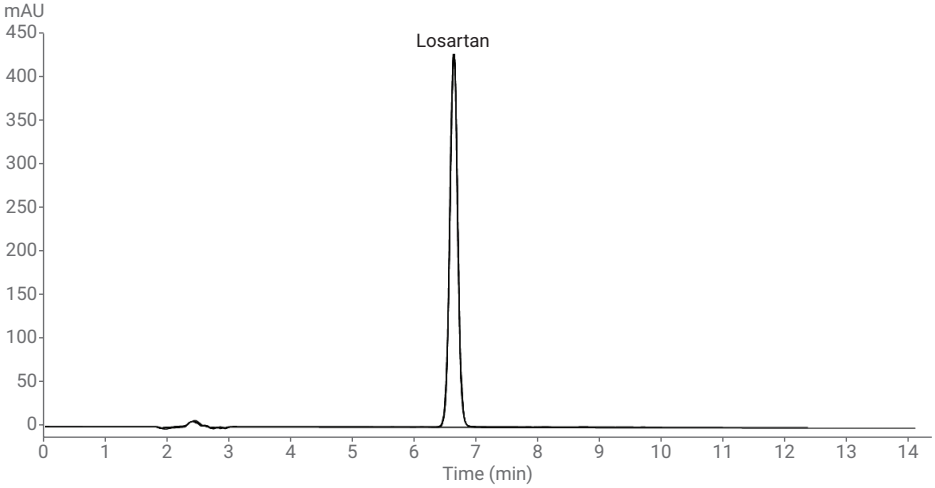


Figure 1. Six-replicate overlay of Losartan standard on day 1 using a 1260 Infinity II LC.

Similar tests were performed on the second and third day, and excellent repeatability was achieved for both trials. Figure 2 shows an overlay of chromatograms for six consecutive injections of Losartan Potassium standard solution on day 2. Table 3 lists the corresponding precision values. A %RSD of lower than 0.1 % was achieved on both days.

On the third day, a sequence of 50 consecutive injections of Losartan standard were also performed to check the repeatability and robustness of the method on a 1260 Infinity II LC. Exceptional precision below 0.2 % was observed. The calculated value of the area RSD was 0.16 %, and retention time RSD was 0.14 % for 50 consecutive standard injections of Losartan standard. Figure 3 displays the chromatographic overlay of 50 standard injections of Losartan.

Table 2. Losartan Potassium analysis performed on day 1.

		Trial 1			Trial 2		
	Injection number	RT (min)	Area	Height	RT (min)	Area	Height
Day 1	1	6.64	3,814	425	6.63	3,810	435
	2	6.64	3,803	426	6.63	3,815	433
	3	6.64	3,814	427	6.63	3,816	435
	4	6.64	3,810	428	6.63	3,813	433
	5	6.63	3,817	428	6.63	3,816	430
	6	6.63	3,816	429	6.63	3,814	429
	%RSD	**0.08**	**0.14**	**0.34**	**0.00**	**0.06**	**0.58**

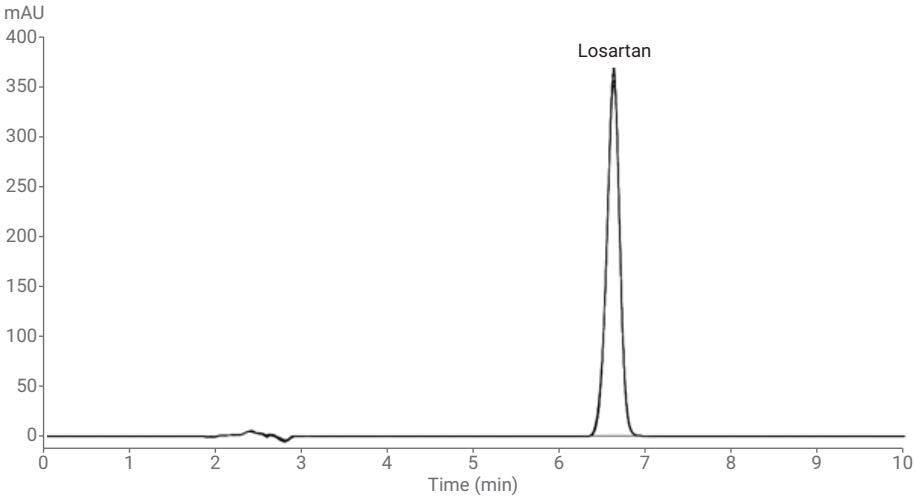


Figure 2. Six-replicate overlay of Losartan standard performed on day 2 using a 1260 Infinity II LC.

Table 3. Losartan Potassium analysis performed on days 2 and 3.

		Trial 1		Trial 2			Trial 1	
	Injection number	RT (min)	Area	RT (min)	Area		RT (min)	Area
Day 2	1	6.62	3,815	6.62	3,819	Day 3	6.51	3818
	2	6.62	3,835	6.63	3,820		6.51	3816
	3	6.62	3,816	6.64	3,823		6.51	3821
	4	6.62	3,826	6.64	3,819		6.51	3819
	5	6.62	3,821	6.64	3,826		6.5	3822
	6	6.62	3,818	6.64	3,816		6.51	3823
	%RSD	**0.00**	**0.20**	**0.13**	**0.09**		**0.06**	**0.07**

Figure 4 displays Interday and intraday comparisons, showing the overall day-to-day peak area %RSD compared to the USP required levels of less than 0.5 % for assay.

Conclusion

The 1260 Infinity II Vialsampler is designed for the highest precision and accuracy, and meets the current requirement of system suitability criteria of the USP. It also performs precise injections at low volumes, where 100 % organic solvent is used as diluent. This Application Note demonstrates the exceptional performance of a 1260 Infinity II LC for achieving the lowest possible %RSD of area and retention time.

Reference

1. United States Pharmacopoeia USP NF 40, USP<621> General chapter of Chromatography.

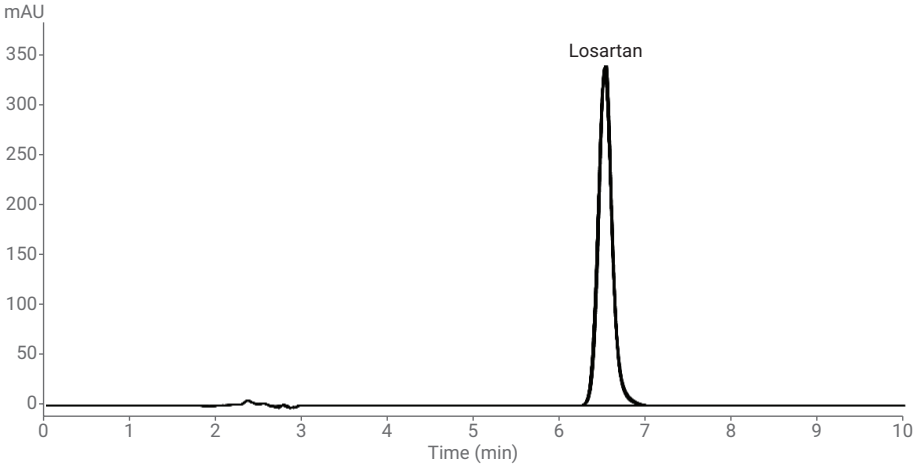


Figure 3. 50-Replicate injection overlay of Losartan standard performed on day 3 using a 1260 Infinity II LC.

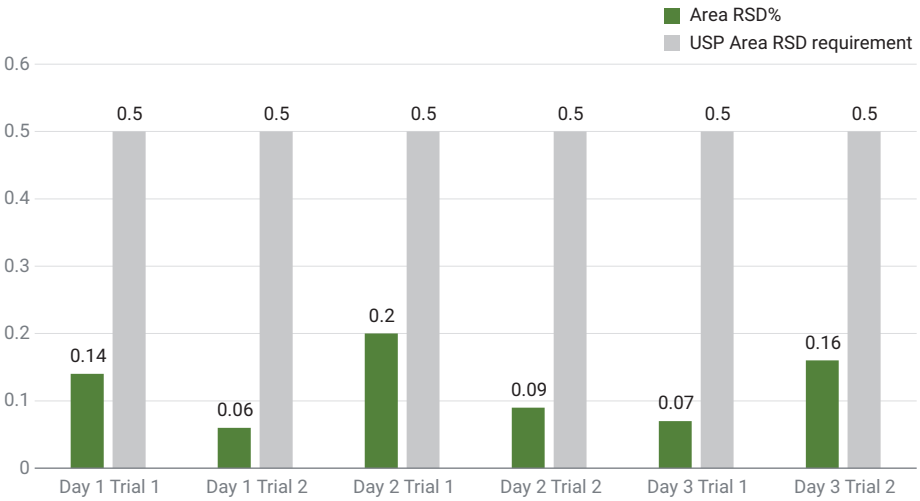


Figure 4. Comparison of interday and intraday trials performed on a 1260 Infinity II LC for Losartan standard solution.

www.agilent.com/chem

Printed in the USA, November 16, 2018
5994-0495EN

Agilent
Trusted Answers