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Application News

Shimadzu High performance liquid chromatograph The LC-2050 series

Organic Impurity Analysis of Levofloxacin Drug Material Following USP Monograph

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User Benefits

- Following USP monograph 41, impurity analysis of levofloxacin drug material was performed on the i-series LC-2050. System suitability test meets the requirements.
- Six organic impurities of trace levels were found with 4 being identified via matching the RRTs to the listed impurities in the monograph.

■ Introduction

Levofloxacin (Figure 1) is a synthetic broad spectrum antibacterial agent which is active against Gram-positive and Gram-negative bacteria. Levofloxacin is currently used in adults for the treatment of respiratory tract infections, urinary tract infections, chronic bacterial prostatitis and skin and soft tissue infections, etc.

Monitoring impurities in drug active pharmaceutical ingredient (API) and products is crucial for drug development and throughout the manufacturing process. In this application note, Shimadzu i-Series LC-2050 was used for system suitability test and organic impurities analysis of levofloxacin API following the USP monograph 41[1]. The separation was performed on a Shim-pack GIST C18 column (L1). This method with high sensitivity and selectivity can eventually be transferred and validated for QA/QC in pharmaceutical laboratories.

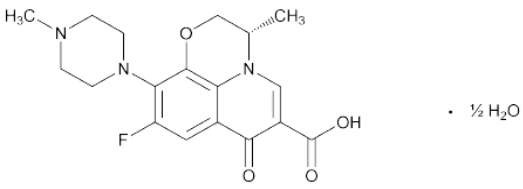

Figure 1. Chemical structure of levofloxacin

■ Experimental

Chemicals and preparation of standards

Ammonium acetate, cupric sulfate pentahydrate and L-isoleucine were obtained from Sigma Aldrich with ≥ 98 % purity. Levofloxacin standard used in this study was provided by a collaboration laboratory. The levofloxacin standard solution of 1.0 mg/mL was prepared using the mobile phase as diluent. It worked as system suitability test solution. Levofloxacin standard solution of 0.3 µg/mL was obtained by serial dilution from the stock, which is used for sensitivity test.

Analytical conditions

Shimadzu i-Series LC-2050 system was employed for analysis. The details of analytical conditions are listed in Table 1.

Table 1. Analytical conditions of levofloxacin

Column	Shim-pack GIST C18 (250 mm x 4.6 mm I.D. 5µm) packing L1
Mobile Phase	A: 8.5 g/L ammonium acetate, 1.25 g/L cupric sulfate (pentahydrate) and 1.3 g/L L-isoleucine in ultrapure water
	B: Methanol
Elution mode	Isocratic, A:B = 7:3 (Vol/Vol)
UV wavelength	360 nm
Flow Rate	0.8 mL/min
Oven Temp.	45 °C
Injection vol.	25 µL

■ Results and discussion

System suitability test

The USP method for levofloxacin was set up on the i-series LC-2050 system. Figure 2 shows the chromatogram of levofloxacin standard of 0.3 µg/mL for the system sensitivity test. The total run time is 25 minutes and levofloxacin elutes at 14.7 minutes.

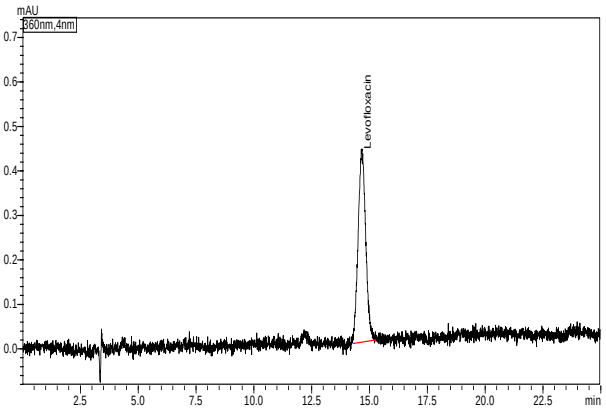

Figure 2. HPLC chromatogram of levofloxacin standard at 0.3 µg/mL

The S/N ratio of 14.4 obtained meets the criteria of NLT 10 (Table 2).

Repeatability test was conducted with the system suitability solution of 1 mg/mL. The %RSD (n = 6) of peak area and retention time of levofloxacin are shown in Table 2, which meet the system suitability requirement of %RSD NMT 1.0 % following USP requirement. The result for tailing factor was within the criteria as listed in Table 2.

Table 2. System suitability test parameters of levofloxacin

Test factors	Solutions (µg/mL)	Acceptance Criteria	Values (n=6)	Results
Area %RSD	1000	NMT 1.0%	0.07%	Pass
R.T. % RSD	1000	NMT 1.0%	0.12%	Pass
Signal-to-noise ratio	0.3	NLT 10	14.4	Pass
Tailing factor	1000	0.5-1.5	0.61	Pass

Identification of organic impurities in levofloxacin

The 1 mg/mL of levofloxacin solution was used for impurity profiling analysis. The HPLC chromatogram is shown in Figure 3.

Trace level impurity peaks of levofloxacin from same solution could be seen on the zoomed chromatogram as shown in Figure 3 (bottom). Six impurities were detected as listed in Table 3. Four of the impurities were identified by matching to the relative retention time (RRT) listed in the Table 1 of USP monograph 41. Two unknown impurities with RRT of 0.55 and 0.67 were found. The total impurity was 0.29%.

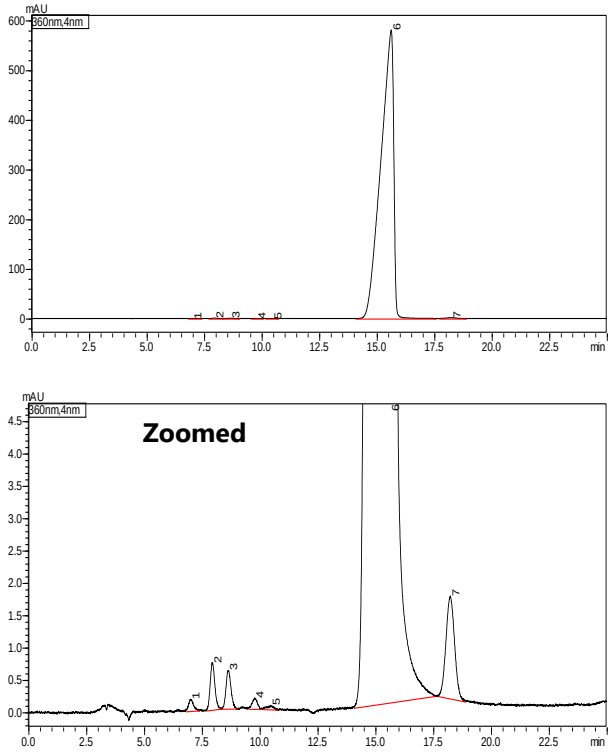

Figure 3. Chromatogram of levofloxacin of 1 mg/mL (top). The zoomed chromatogram (bottom) shows the smaller impurity peaks.

Table 3. Impurities identified in levofloxacin solution of 1.0 mg/mL

Peak#	RT (min)	Area (%)	RRT	Name
1	6.987	0.011	0.447	N-Desmethyl levofloxacin
2	7.941	0.045	0.508	Diamine derivative
3	8.624	0.039	0.552	unknown impurity
4	9.771	0.011	0.625	Levofloxacin N-oxide
5	10.464	0.005	0.670	unknown impurity
6	15.627	99.708	1.000	Levofloxacin
7	18.235	0.181	1.167	D-isomer
Total Impurities		0.292		

■ Conclusion

The USP method for impurity analysis of levofloxacin was tested on the i-series LC-2050 system with Shim-Pack GIST C18 column. The results of system suitability test are sufficient as per the requirements in the USP 41 monograph. The impurities were identified by matching to the relative retention time listed in Table 1 of USP monograph 41. Four out of five impurities listed in USP monograph were identified in the levofloxacin standard solution at 1 mg/mL.

■ Reference

1. USP (USP 41, May 1, 2018) official monograph / Levofloxacin 2395

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