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Application News

High Performance Liquid Chromatography

High Speed Analysis of Cephalexin in accordance with chapter 621 in USP 39

No. SCA_190_035

High throughput analysis has been advanced dramatically in recent years with the increasing necessity to improve productivity and operational efficiency. Especially HPLC has also been in the spotlight thanks to significant advances in ultra-high-speed analysis technology, in particular ultra-high performance LC and micro-particle column packing material. The recently revised General Chapter 621 of the United States Pharmacopoeia (USP 621) now permits a degree of adjustment of HPLC and GC parameters, specifically aimed to satisfy the requirements of system suitability. Taken account of USP 621, this Application News introduces an example of isocratic analysis of Cephalexin monography in accordance with USP and still fulfilling the allowable adjustment criteria. Cephalexin is an antibiotic. It is used to treat different bacterial infections. Additionally, an example of analysis that can be completed in a significantly shorter time than that described in the USP General Chapter 621 Chromatography is presented here.

■ Allowable adjustments to HPLC parameters

Table 1 shows the parameters which may be changed according to USP 621. The analysis was performed under isocratic conditions. Additionally, the actual permissible ranges within these LC parameters are shown.

■ Speed enhancement for USP method

The permissible ranges within the analytical conditions may be modified and are specified in the USP General Chapters: <621> Chromatography. Changing these analytical conditions within the range makes it possible to shorten the analysis time. For details regarding changes that can be used to allow fast USP-compliant analysis, please refer to Application News L464. Shortening analysis time can be accomplished in three ways, 1) by shortening the column, 2) by lowering the inner diameter and 3) by increasing the flowrate while maintaining the linear velocity. To preserve the resolution of the separation, the column length and particle size may be modified as long as the ratio of L (column length) to dp (column particle size) remains in the specified range (permissible range: -25 % to +50 %). For the original USP method, a column with the dimensions 250 mmL. x 4.6 mm I.D., and 5 µm particle size was used. We selected a column size of 100 mmL. x 2.1 mm I.D., and 2 µm particle size with constant L/dp ratio (Table 2). For further details, please see Table 3. The flowrate, proportional to the column cross-sectional area, and inversely proportional to the particle diameter (see text for permissible limits), was determined as 0.5 mL/min. Table 4 shows the analytical conditions.

Table 1: Allowable adjustments to HPLC parameters according to USP 621

Particle size(dp)	L/dp ratio constant or Theoretical plate number: -25 to + 50%
Column length(L)	
Column ID(dc)	Any allowed if linear velocity is constant
Flowrate	Combination* of dp and dc : ±50%
Injection Vol.	Can be adjusted as consistent with precision and detection limits
Column Temp.	±10 °C

* $F_2 = F_1 \times [(dc_2^{\,2} \times dp_1)/(dc_1^{\,2} \times dp_2)]$
F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters; and dp_1 and dp_2 are the particle sizes.

Table 2: Selection of column for speed enhancement

	Column size	L/dp	Ratio
USP Original Method	250 x 4.6 mm 5 µm	50000	1 (100%)
USP Fast Method	100 x 2.1 mm 2 µm	50000	1 (+0%)

Table 3: Column selection for speed enhancement in case of fixing the particle size and column I.D.

	USP method of Ibuprofen	Allowable range	Modified method
Particle size(dp)	5 µm	2 µm	2 µm
Column ID(dc)	4.6 mm	2.1 mm	2.1 mm
Column length(L)	250 mm	75 - 150 mm	100 mm
Flowrate	1.5 mL/min	0.39 - 1.17 mL/min	0.5 mL/min
Injection Vol.	20 µL	Variable	2 µL
Column Temp.	Unspecified	Variable	40 °C

Table 4: Analytical conditions

System	LC-2040C 3D
Column	(1) Shim-pack GIST C18 (100 x 2.1 mm, 2 µm) (2) Shim-pack GIST C18 (250 x 4.6 mm, 5 µm)
Mobile phase	0.985 g /L of sodium 1-pentanesulfonate in a mixture of Acetonitrile, Methanol, Triethylamine and water (20/10/3/170), adjusted with phosphoric acid to a pH of 3.0
Flowrate	(1) 0.5 mL/min; (2) 1.5 mL/min
Column Temp.	40 °C
Injection Vol.	(1) 2 µL; (2) 20 µL
Detection	LC-2030/2040 PDA; D2 at 190-350 nm

■ Results

The results are shown in Figure 1 and 2 and in Table 5. The speed enhancement is shown in Figure 1. Here, the retention time is much shorter with 5.3 minutes for Cephalexin compared to the retention time in Figure 2 (7.6 minutes).

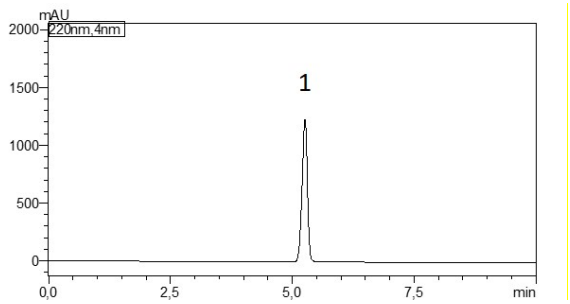

Figure 1: Chromatogram of USP fast method for Cephalexin (Peak 1) (0.44 mg/mL) with column (1) Shim-pack GIST C18 (100 x 2.1 mm; 2 µm)

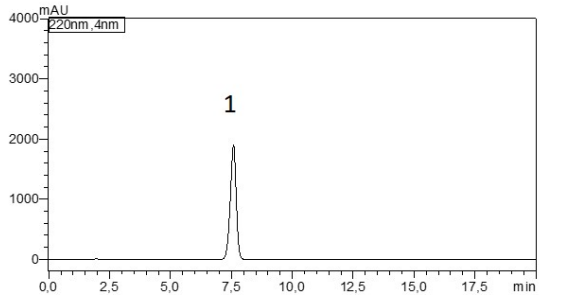

Figure 2: Chromatogram of USP original method for Cephalexin (Peak 1) (0.44 mg/mL) with column (2) Shim-pack GIST C18 (250 x 4.6 mm; 5 µm)

Table 5: Results of system suitability test using USP Method (original method and fast method).

System Suitability		Ref. Value	USP original Value		Fast method Value	
RSD%	Cephalexin	≦2.0%	0.28	Pass	0.26	Pass

■ Conclusion

With the fast USP method, the original USP method, according to the reference value, was improved because the analysis time is shorter and solvent consumption is reduced. Ongoing with this, the cost per analysis is reduced significantly. Additionally, both column conditions are better than the USP reference.