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Application News

High Performance Liquid Chromatography

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

No. SCA_190_041

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 Indometacin monograph in accordance with USP and still fulfilling the allowable adjustment criteria. Indometacin is a nonsteroidal anti-inflammatory drug. It is an analgesic and antipyretic. An example of analysis that can be completed in a significantly shorter time than that described in the USP General Chapter 621 is presented here.

▪ Allowable adjustments to HPLC parameters

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

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
Flow rate (F)	Combination* of dp and dc : ±50%
Injection Vol.	Can be adjusted as consistent with precision and detection limits
Column Temp.	±10 ºC

* $F2 = F1 \times [(dc2\ 2 \times dp1)/(dc12 \times dp2)]$
F1 and F2 are the flow rates for the original and modified conditions, respectively; dc1 and dc2 are the respective column diameters; and dp1 and dp2 are the particle sizes.

▪ Speed enhancement for USP method

The allowable ranges within the analytical conditions may be modified and are specified in the USP General Chapter: <621>. 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 flow rate 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 (allowable range: -25% to +50%).

For the original USP method, a column with the dimensions of 250 mmL. x 4.6 mm I.D., and 5 µm particle size was used. We selected a column size of 150 mmL. x 3.0 mm I.D., and 3 µm particle size with constant L/dp ratio (Table 2). For further details, please see Table 3. The flow rate, proportional to the column cross-sectional area, and inversely proportional to the particle diameter (see text for allowable limits), was determined as 1.2 mL/min. Table 4 shows the analytical conditions.

Table 2: Selection of column for speed enhancement

	Column size	L/dp	Ratio
USP Original Method	250 mmL x 4.6 mm ID; 5 µm	50000	1 (100%)
USP Fast Method	150 mmL X 3.0 mm ID; 3 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 Indometacin	Allowable range	Modified method
Particle size(dp)	5 µm (2)	3 µm	3 µm (1)
Column ID(dc)	4.6 mm (2)	3.0 mm	3.0 mm (1)
Column length(L)	250 mm (2)	112 - 225 mm	150 mm (1)
Flow rate	1.5 mL/min	0.53 – 1.59 mL/min	1.2 mL/min
Injection Vol.	10 µL	Variable	5 µL
Column Temp.	30 ºC	Variable	30 ºC

Table 4: Analytical conditions

System	LC-2040C 3D
Column	(1) Shim-pack™* GIST C18-AQ (150 mmL x 3.0 mm ID, 3 µm) (2) Shim-pack GIST C18-AQ (250 mmL x 4.6 mm ID, 5 µm)
Mobile phase	A) 0.1 % Formic acid B) Acetonitrile; A/B= 55/45 (v/v)
Flow rate	(1) 1.2 mL/min; (2) 1.5 mL/min
Column Temp.	30 ºC
Injection Vol.	(1) 5 µL; (2) 10 µL
Detection	LC-2030/2040 PDA; D2 at 190-800 nm

▪ Results

The results are shown in Figure 1 and 2 and in Table 5. The speed enhancement is shown in Figure 1. The retention times were much shorter with 9.5 minutes for Indometacin, 1.3 minutes for 2-(5-Methoxy-2-methyl-1 H-indol-3-yl (Compound A) and 2.2 minutes for 4-Chloro-benzoic acid (Compound B) compared to that in Figure 2 (25.5 minutes for Indometacin, 3.8 minutes for Compound A and 6.1 minutes for Compound B).

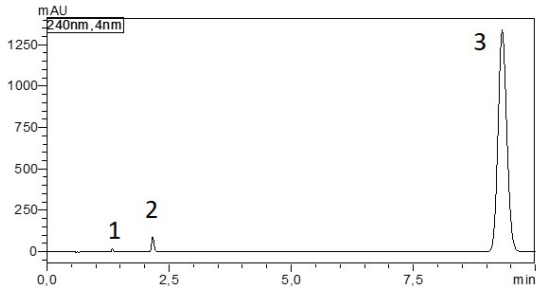

Figure 1: Chromatogram of USP fast method for Indometacin (Peak 3) (2000 mg/mL) with column (1) Shim-pack GIST C18-AQ (150 x 3.0 mm; 3 µm). Peak 1: Compound A (2 mg/L) Peak 2: Compound B (10 mg/L)

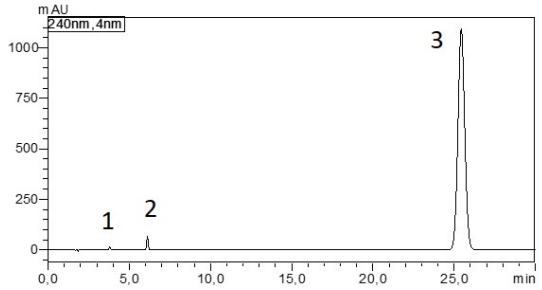

Figure 2: Chromatogram of USP original method for Indometacin (Peak 1) (2000 mg/L) with column (2) Shim-pack GIST C18-AQ (250 x 4.6 mm; 5 µm). Peak 2: Compound A (2 mg/L) Peak 2: Compound B (10 mg/L)

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	
Resolution (Comp.A and B)		≧2.0	12.3	Pass	8.8	Pass
Tailing Factor	Indometacin	≦2.0	1.1	Pass	1.1	Pass
	Compound A	≦2.0	1.1	Pass	1.2	Pass
	Compound B	≦2.0	1.0	Pass	1.1	Pass

▪ Conclusion

With the fast USP method, the original USP method, according to the reference value, was improved because the analysis time is shorter (- 63%) and solvent consumption is reduced about 20%. Ongoing with this, the cost per analysis is lowered significantly. Additionally, obtained results with both columns were better than the USP reference values.

* Shim-pack is a trademark of Shimadzu Corporation in Japan and/or other countries.

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