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Application News

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HPLC Columns – USP Analysis

USP Analysis of Almotriptan Tablets and Related Substances

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❑ Introduction

Almotriptan is a triptan drug developed for the treatment of acute migraine.[1] It was reported as one of the best-responding triptans for its efficacy in pain relief and sustained pain-free rate. Almotriptan is a highly selective serotonin 5-$HT_{1B/1D}$ receptor agonist. It is believed to induce vasoconstriction and inhibition of signal transmission by acting on 5-HT_{1B} and 5-HT_{1D} receptor subtype respectively, thereby relieving a migraine attack.[2,3]

USP methods are often used as a basis for routine analysis of generically manufactured drugs. Column used in USP methods differs for each monograph. Here, we demonstrate the analysis of almotriptan tablets and related substances in accordance with the USP monograph on a Nexera™ X2 system with Shim-pack Velox™ column.[4] A C18 column with L1 designation was stated in USP monograph of almotriptan tablets. Shim-pack Velox column, a core-shell column was chosen for its efficacy, robustness and reduced backpressure. The Nexera X2 system was used for its reliable and precise performance.

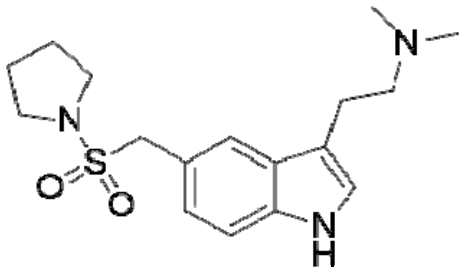

Figure 1 Structure of Almotriptan.

Permissible adjustment to HPLC parameters

The USP monograph of almotriptan tablets employs a 1.8 µm, 2.1 × 100 mm L1 column. The use of sub-2 µm column results in high backpressure and UHPLC system would be needed for the analysis. Nevertheless, drug purity analysis is often done in QC lab where there is limited number of UHPLC systems. Therefore, there is a need to transform the UHPLC analysis into a HPLC-compatible analysis.

USP monograph method can be modified according to the General Chapter 621. Adjustable parameters include particle size, flow rate, column length and diameter. Table 1 lists the allowable adjustment to HPLC parameters according to General Chapter 621. Changes to column dimension are allowed so long as the ratio of column length (L) to column particle size (dp) are within the permissible range (-25% to +50%). This is to preserve the resolution of separation.

The original USP method employs a L1 column with 2.1 mmI.D. × 100 mmL., 1.8 µm particle size (Table 2). A column of 3.0 mmI.D. × 150 mmL., 2.7 µm particle size was selected for method transfer from UHPLC to HPLC while keeping the L/dp ratio constant. Flow rates 0.55 mL/min and 0.75 mL/min were selected for the new column following the calculation as stated in USP method. Details of analytical conditions are stated under experimental section.

Table 1 Allowable Adjustment to HPLC Parameters according to General Chapter 621.

Particle size (dp)	L/dp ratio constant or theoretical plate number: -25 to +50%
Column length (L)	
Column I.D. (dc)	Any allowed if linear velocity is constant
Flow rate*	Combination of dp and dc: ± 50%
Injection vol.	Can be adjusted as consistent with precision and detection limits
Column temp.	± 10℃

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

Table 2 Selection of Columns for Analysis of Almotriptan Tablets.

USP method	Column dimension	L/dp	Ratio
Original	2.1 x 100 mm, 1.8 µm	55555	1 (100%)
HPLC	3.0 x 150 mm, 2.7 µm	55555	1 (+0%)

❑ Experimental

Analytical conditions and sample preparation

USP almotriptan Malate RS, related compound B RS, related compound C RS and related compound D RS were purchased from United States Pharmacopeia.

Standard solution: 0.5 mg/mL of USP almotriptan Malate RS in mobile phase.

System suitability stock solution: 0.1 mg/mL each of USP almotriptan Related Compound B RS, USP almotriptan Related Compound C RS and USP almotriptan Related Compound D RS in methanol.

System suitability solution: 0.001 mg/mL each of USP almotriptan Related Compound B RS, USP almotriptan Related Compound C RS and USP almotriptan Related Compound D RS prepared from the System suitability stock solution in Standard solution.

Sample solution: 0.5 mg/mL sample solution was prepared from almotriptan tablets. Eight tablets were weighed and finely powdered. The powder was transferred to a volumetric flask and 80% of the required volume of mobile phase was added. The solution was sonicated for 10 min and diluted with mobile phase to a final concentration of 0.5 mg/mL. Solution was stirred for 30 min and centrifuged at 3000 rpm for 10 min. Supernatant was filtered through a 0.45 µm Nylon syringe filter.

System suitability samples: Standard solution and system suitability solution.

Table 3 Analytical Conditions

Column	: 1. Shim-pack Velox C18, 1.8 µm, 2.1 x 100 mm (P/N: 227-32007-03) 2. Shim-pack Velox C18, 2.7 µm, 3.0 x 150 mm (P/N: 227-32010-04)
Mobile phase	: Acetonitrile/ phosphate buffer※ = 10/90 (v/v)
Flow rate of mobile phase	: Column 1: 0.55 mL/min Column 2: 0.55 mL/min, 0.75 mL/min
Column temp.	: 40 °C
Injection vol.	: 3 µL
Detection	: 210 nm

※1L of 0.01 mol/L phosphoric acid was added with 10 mL of triethylamine. pH was adjusted with phosphoric acid to a pH of 6.0.

Table 4 Other consumables

Item
1.5ml Screw-thread amber vial with write on spot, caps with PTFE/white silicone septa
1L Solvent bottle
Solvent safety caps kit
LC solvent waste kit
Ultra-High pressure fitting
PEEK fitting
0.45 µm Nylon syringe filter

Please contact Shimadzu for information on consumables.

❑ Results & Discussion

The assay for almotriptan tablets and organic impurities were performed on Nexera X2 system. The USP monograph calls for a 1.8 µm, 2.1 × 100 mm L1 column in both analysis. Figure 2 and 3 show the chromatograms for standard solution and system suitability solution analysed on Shim-pack Velox C18 column.

The original USP method with 1.8 µm, 2.1 × 100 mm L1 column had a backpressure of 55.3 MPa which constrains the analysis to be conducted on a UHPLC system. In view of this, method transfer to a HPLC system has been performed. Method transfer onto 2.7 µm, 3.0 × 150 mm Shim-pack Velox C18 column displayed a system backpressure of lower than 40 MPa , indicating a feasible HPLC method.

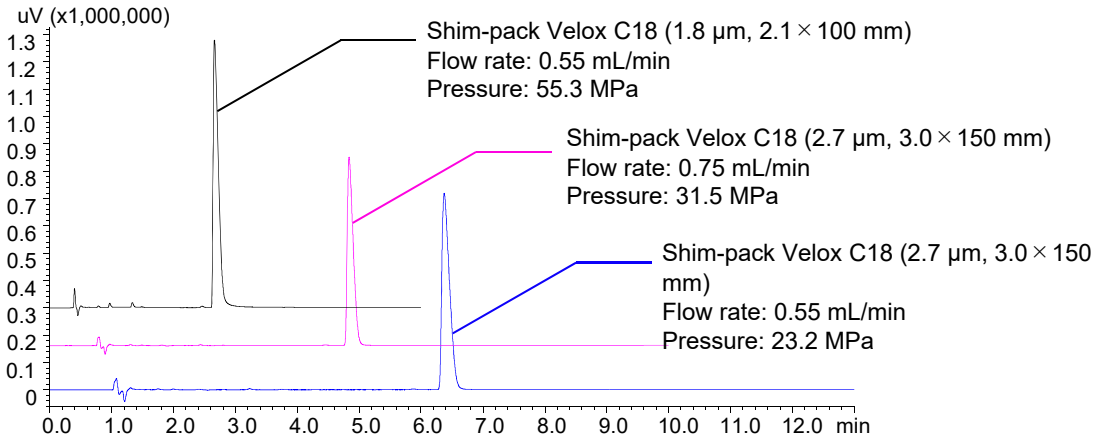

Figure 2 Chromatogram of Standard Solution

Table 5 Results on Analysis of Almotriptan Standard Solution and System Suitability Solution.

	System suitability	Shim-pack Velox C18		
Particle size (µm)	-	1.8	2.7	
Column I.D. (mm)	-	2.1	3.0	
Column length (mm)	-	100	100	
Flow rate (mL/min)	-	0.55	0.55	0.75
System backpressure (MPa)	-	55.3	23.2	31.5
Retention time of almotriptan (min)	-	2.66	6.38	4.84
Theoretical plate number (almotriptan)	-	4309	11519 (+167 %)	11138 (+ 158%)
Resolution (almotriptan and related compound C)	≥ 1.5	1.68	2.57	2.53
Tailing factor (almotriptan)	≤ 3.0	2.32	1.92	1.84
%RSD retention time (almotriptan)	≤ 2.0 %	0.06	0.01	0.01
%RSD area (almotriptan)	≤ 2.0 %	0.36	0.06	0.09
Elution time of the last peak (min)	-	3.89	8.99	7.12

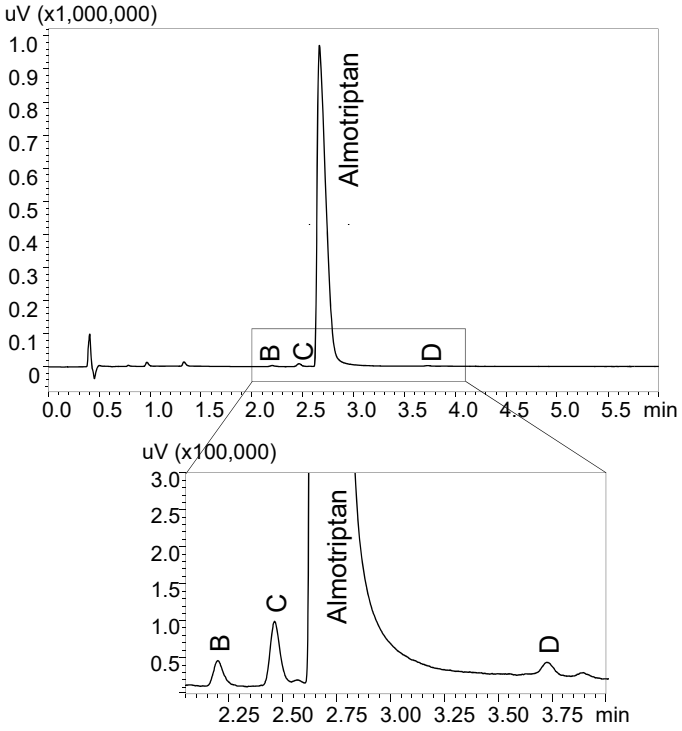

Figure 3 Chromatogram of system suitability solution

Table 5 summarizes the results obtained after six replicate injections on each column. The resolution between almotriptan related compound C and almotriptan obtained with the original USP method was 1.68, meeting the acceptance criteria NLT 1.5. Besides that, the tailing factor of almotriptan was 2.32, falling within the acceptance criteria NMT 3.0. The obtained %RSD for almotriptan was 0.06% for retention time and 0.36% for area, well below the acceptance criteria NMT 2.0 %. The low RSD value indicates excellent repeatability of Shim-pack Velox C18 column and Nexera X2 system.

For method transfer onto HPLC with new column under two flow rates, resolution value and %RSD obtained met the acceptance criteria. Results in Table 5 denote the success in method transfer. Number of theoretical plates increased by 167% and 158% at flow rate of 0.55 mL/min and 0.75 mL/min respectively. Nevertheless, analysis time increased from 6 min to 10 min (0.75 mL/min) and 13 min (0.55 mL/min) when method is transferred from UHPLC to HPLC.

Table 6 Relative Retention Time of Almotriptan and Organic Impurities.

Name	Relative retention time			
	USP monograph	Shim-pack Velox C18		
		1 (0.55 mL/min)	2 (0.55 mL/min)	2 (0.75 mL/min)
Almotriptan related compound B	0.82	0.83	0.83	0.82
Almotriptan related compound C	0.93	0.92	0.92	0.92
Almotriptan	1.0	1.0	1.0	1.0
Almotriptan related compound D	1.39	1.40	1.37	1.37

Almotriptan and the related compounds were well separated as seen in Figure 3. Relative retention time of almotriptan and the related compounds are stated in Table 6. Results show that the relative retention time are consistent with values stated in the USP monograph. This again demonstrates the suitability and applicability of Shim-pack Velox C18 column for analysis of almotriptan tablets in accordance with USP monograph.

❑ Conclusion

This study has evaluated the ability of Nexera X2 UHPLC system and Shim-pack Velox C18 column in analysis of almotriptan tablets in conformity with the USP Monograph USP41 – NF36, 2659. The Shim-pack Velox C18 column demonstrated excellent, robust and reliable performance as seen from the system suitability test results. A change in the column dimension lowered the system backpressure and allowed method transfer onto a HPLC system.

❑ References

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4. USP Monograph, Almotriptan Tablets, USP41 – NF36, 2659. The United States Pharmacopeia Convention, official from Feb 1, 2017.

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