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Application News

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HPLC Columns – USP Analysis

Analysis of Fingolimod Hydrochloride According to the USP Monograph 1745

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❑ Introduction

United States Pharmacopeia (USP) establishes analytical methods needed for the identity, purity, quality, strength, and potency of drug products, food ingredients, and dietary supplementary products. USP also provides reference standards that are needed for these methods. These reference standards and methods are used by manufacturer and regulatory agencies to ensure the identity, purity, quality, strength and potency of the products produced.

Here, we introduce an example of gradient analysis of Fingolimod Hydrochloride monography in compliance with General Chapter 621. Fingolimod Hydrochloride is a sphingosine-derivative and immunosuppressive agent that blocks the migration and homing of lymphocytes to the central nervous system through its action on sphingosine 1-phosphate receptors. It is marketed by Novartis under the trade name GILENYA® and used in the treatment of multiple sclerosis. Shim-pack™ GISS C18 analytical column and Prominence™-i LC-2030C Plus system were employed in this application.

Shim-pack GISS C18 is packed with ultra-high inert silica gel, provides only hydrophobic interaction to analytes and eliminate secondary interaction that leads to peak tailing. It tolerates a wide pH (1 – 10) condition and has excellent stability in 100 % aqueous mobile phases, making it suitable for analysis of many different compounds. Its rapid column equilibration property shortens the column equilibration time for analysis in gradient methods, giving reproducible elution profile, lower baseline drift and sensitivity.

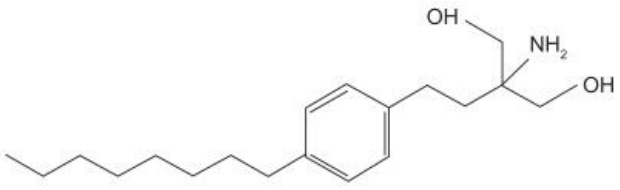

Figure 1: Structure of Fingolimod Hydrochloride

❑ Materials and Method

Analysis was performed on Prominence-i LC-2030C Plus, an integrated HPLC system. The system has touch-screen LCD display panel for easy system control and chromatogram viewing, built-in degasser, quaternary pump, autosampler with cooler module, forced-air circulation-type column oven and UV-Vis detector.

Solution A: 0.1% Phosphoric acid in water
Solution B: Acetonitrile

Mobile phase:

Time (min)	Solution A (%)	Solution B (%)
0	80	20
20	5	95
23	5	95
23.1	80	20
33	80	20

Diluent: Solution A and B (1:1)

Standard solution: 0.6 mg/mL of Fingolimod Hydrochloride in *Diluent*

Chromatographic system

Mode: LC
Detector: UV 215 nm
Column: 3-mm × 15-cm; 3-µm packing L1
Column temperature: 40ºC
Flow rate: 0.8 mL/min
Injection volume: 5 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 0.73%
Tailing factor: NMT 5

❑ Results & Discussion

The USP monograph for Fingolimod Hydrochloride calls for a 3 µm, 3.0 × 150 mm L1 column. Figure 2 shows the chromatogram for system suitability

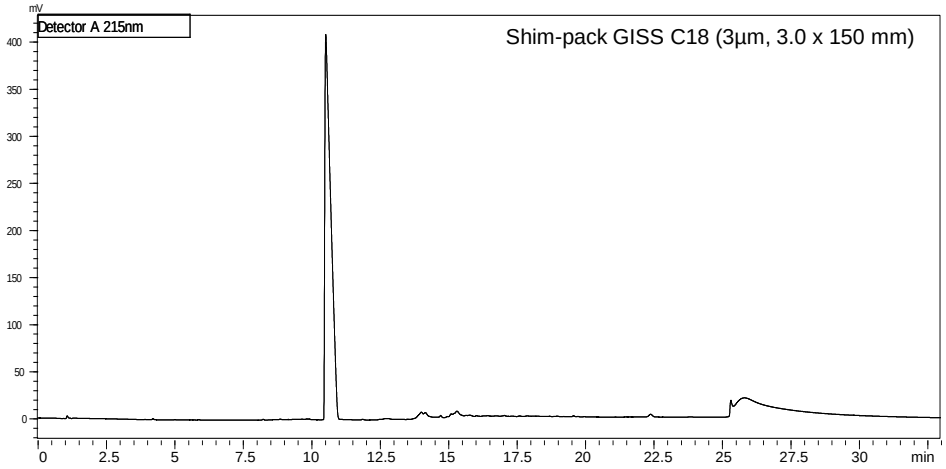

Figure 2: Chromatogram of USP analysis of Fingolimod Hydrochloride (0.6 mg/mL) with Shim-pack GISS C18 (3µm, 3.0 x 150 mm).

solution analysed on Shim-pack GISS C18 column. The retention time of Fingolimod Hydrochloride is 10.5 mins in the USP method (Figure 2, Table 1).

USP method system suitability criterion for Fingolimod Hydrochloride requires relative standard deviation NMT 0.73% and tailing factor NMT 5. The obtained RSD for retention time and area were 0.03% and 0.16%, well within the USP system suitability criteria NMT 0.73%. Also, the tailing factor acquired was 3.6, well below the acceptance criteria NMT 5.

Table 1: Analysis results of Fingolimod Hydrochloride.

System Suitability			
RSD	≤ 0.73%	Retention time	0.03
		Area	0.16
Tailing factor	≤ 5	3.6	

❑ Conclusion

This study demonstrated the ability of Prominence-i LC-2030C Plus system and Shim-pack GISS C18 column in analysis of Fingolimod Hydrochloride in conformity with the USP Monograph 1745. The obtained tailing factor for system suitability was 3.6, well below the acceptance criterion NMT 5. The relative standard deviation of retention time and area were 0.03% and 0.16%, which are well below the required criterion of NMT 0.73%.

❑ References

1.Fingolimod Hydrochloride
https://www.ncbi.nlm.nih.gov/mesh/2009891
2.USP Monograph, Fingolimod Hydrochloride, USP 40 – NF35, Second Supplement

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