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Application News

No. L556A

High Performance Liquid Chromatography

USP-Compliant Analysis of Vitamins in Dietary Supplements
Analysis of Calcium Pantothenate by Nexera™ XR

The United States Pharmacopeia (USP) provides standards for quality control of dietary supplements and specifies testing methods and judgment standards for dietary supplements. Because many dietary supplements are distributed globally, USP compliance has become an important judgment standard for verifying the quality of supplements for consumers.

The substance that was analyzed here is pantothenic acid (pantothenate), which is a water soluble vitamin. Also called vitamin B_5, pantothenic acid is a substance in which β-alanine is bonded with pantoic acid. Many dietary supplements contain pantothenate in the form of its calcium salt.

"Oil and Water Soluble Vitamins with Mineral Tablets – Calcium Pantothenate" of USP40-NF35 describes two analysis methods using HPLC and one microorganism quantitation method. In the HPLC method in "Method 3," calcium pantothenate is detected with an ultraviolet-visible (UV-VIS) absorbance detector after separation with a reversed-phase ODS column.

This article introduces an example in which the calcium pantothenate in a dietary supplement was analyzed using a Nexera XR, which is part of the Shimadzu Nexera Series of ultra high performance liquid chromatographs. An analysis was also conducted with a Prominence™ Series HPLC, confirming that the same results can be obtained with that system, as reported here.

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System Suitability Test

A system suitability test for quantitation of calcium pantothenate was carried out. Table 1 and Table 2 show the analysis conditions and judgment standard, respectively. Fig. 1 shows the chromatogram of the calcium pantothenate standard solution, and Table 3 shows the injection repeatability result for calcium pantothenate.

The relative standard deviation %RSD of the peak area did not exceed the standard value.

Table 1 **Calcium Pantothenate Analysis Conditions**

System	:	Nexera XR
Column	:	Shim-pack™ GIS C18 USP code: L1 (300 mm × 3.9 mm I.D., 5 µm)
Mobile Phase	:	5 g/L KH_2PO_4 (pH = 3.5)*/ Methanol =9/1
Flow Rate	:	2.0 mL/min
Column Temp.	:	50 ˚C
Injection Vol.	:	25 µL
Detection	:	SPD-M40 205 nm (190 - 800 nm)

* : Adjusted to pH = 3.5 using phosphoric acid.

Table 2 **Standard Value of System Suitability Test for Calcium Pantothenate**

Relative standard deviation (%RSD) (n = 6)	≤3

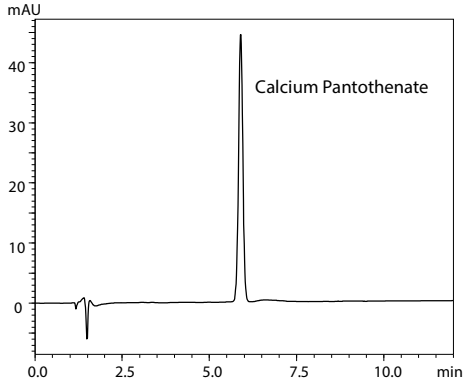

Fig. 1 **Chromatogram of Calcium Pantothenate Standard Solution (40 mg/L)**

Table 3 **Injection Repeatability of Calcium Pantothenate (n = 6)**

	%RSD	
	Retention time	Peak area
Calcium pantothenate	0.10	0.34

Analysis of Dietary Supplement

A multivitamin tablet, which is a commercially available dietary supplement, was analyzed. Fig. 2 shows the chromatogram of the multivitamin tablet, and Fig. 3 shows the pretreatment method.

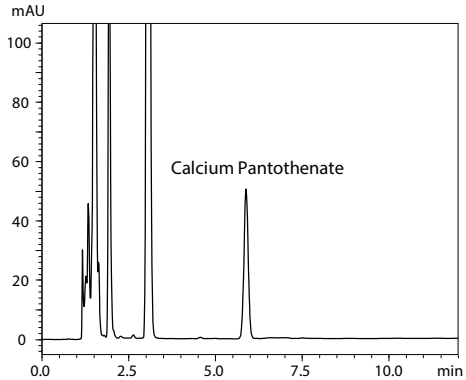

Fig. 2 **Chromatogram of the Multivitamin Tablet**

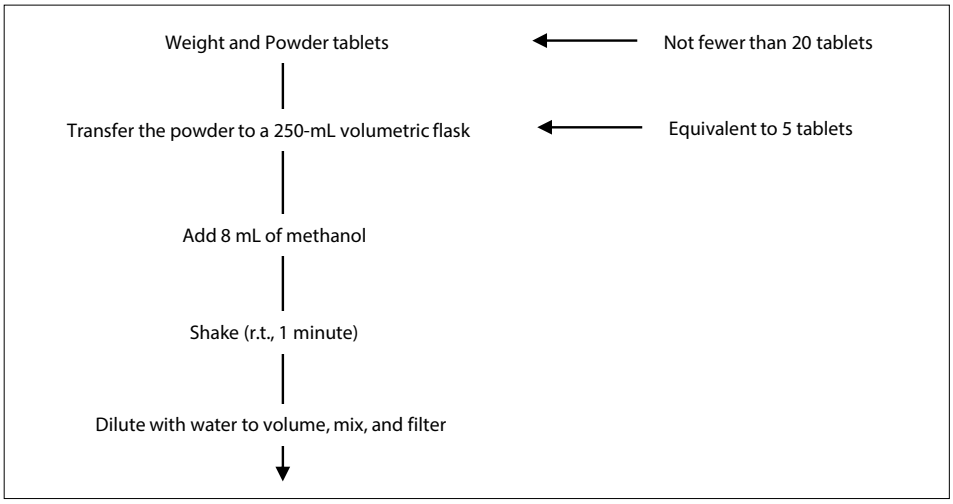

Fig. 3 **Pretreatment Procedure in Analysis of Calcium Pantothenate**

Compatibility with Prominence Series

The analysis carried out with a Prominence Series HPLC was also conducted in the same manner with the Nexera XR, as described above. The analysis conditions and pretreatment method were as shown in Table 1 and Fig. 3.

Fig. 4 shows the chromatogram of the standard solution of calcium pantothenate, Fig. 5 shows the chromatogram of the multivitamin tablet, and Table 4 shows the measurements of calcium pantothenate in the multivitamin tablet.

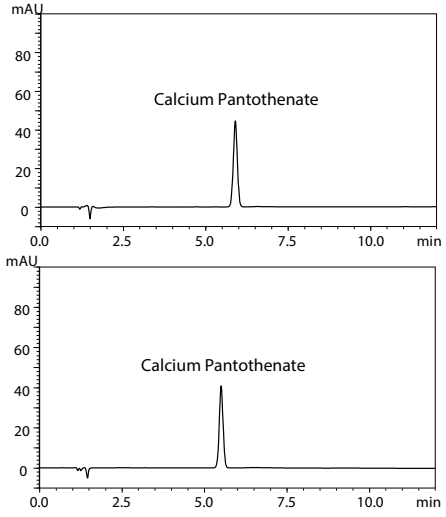

Fig. 4 **Chromatograms of Calcium Pantothenate Standard Solution (40 mg/L) (Top: Nexera XR, Bottom: Prominence)**

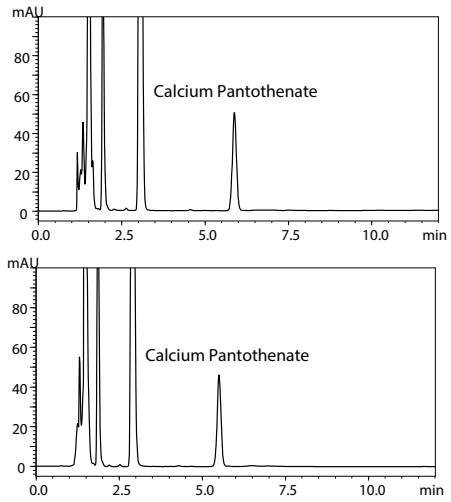

Fig. 5 **Chromatograms of the Multivitamin Tablet (Top: Nexera XR, Bottom: Prominence)**

Table 4 **Measurements of Calcium Pantothenate in the Multivitamin Tablet**

Calculated amount/tablet	Nexera XR	1.7 mg
	Prominence	1.7 mg
Labeled amount/tablet		1.5 mg

* : Standard specified in USP40-NF35: 90% to 150% of labeled amount.

Conclusion

The calcium pantothenate in a commercially available dietary supplement was analyzed under analysis conditions conforming to USP40-NF35 using a Nexera XR, which is a new product Nexera series, and a Prominence Series. This experiment confirmed that the same results as with the Nexera XR can also be obtained with the Prominence.

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