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Application News

No.L459

High Performance Liquid Chromatography

Simultaneous Analysis of Water-Soluble Vitamins by Newly Developed "Shim-pack MAqC-ODS Ⅰ" Column

Vitamins play vital roles in the metabolic activities of the body. However, because they cannot be synthesized in sufficient quantities in the body, if at all, they must be ingested either in our meals or by other means, such as tablets.
Vitamins can be classified into two groups, water-soluble and fat-soluble, and their analysis is generally conducted by high-performance liquid chromatography. Of the two types of vitamins, the water-soluble type consists mainly of highly polar basic components which exhibit weak retention in analysis by reversed-phase liquid chromatography. Since they exhibit weak retention, the analysis is typically conducted using ion-pair reagents.
Using ion-pair reagents can be challenging because of the longer analysis time, widening of slow eluting peaks, difficulty in obtaining sufficient sensitivity and long equilibration times. Analysis efficiency is also compromised due to additional work-flow requirements, such as mobile phase preparation and column conditioning.
Here, we introduce an example in which the newly developed "Shim-pack MAqC-ODS Ⅰ" column is used to conduct simultaneous analysis of water-soluble vitamins without the addition of ion-pair reagents to the mobile phase.

Overview of Shim-pack MAqC-ODS Ⅰ

The Shim-pack MAqC-ODS Ⅰ column is a reversed-phase column packed with a silica gel containing metal and an added octadecylsilyl (ODS) group. In addition to the hydrophobic interaction effect provided by the ODS, the metal content effectively contributes by providing cation-exchange effects. This enhances the retention of basic compounds, thereby permitting analysis using a conventional buffer instead of an ion-pair reagent such as sodium alkyl sulfonate (e.g. sodium 1-hexanesulfonate), which is required when conducting ion-pair chromatography.
It is difficult to improve throughput in ion-pair chromatography due to the time required for reequilibration following gradient elution. While on the other hand, in isocratic elution, sensitivity is compromised due to peak broadening from slow eluting substances. Thus, its application to simultaneous analysis is not always appropriate.
Use of the Shim-pack MAqC-ODS Ⅰ permits the retention of highly polar basic compounds without the use of ion-pair reagents, thereby making it possible to apply gradient elution. As a result, simultaneous analysis of highly polar basic compounds and other components can be achieved while shortening the analysis time and improving sensitivity.
These features demonstrate the powerful advantages of this column for simultaneous analysis of water-soluble vitamins, as well as impurities in pharmaceutical products that contain large quantities of basic compounds.

Analysis of Standard Solution

A standard solution was prepared following the procedure outlined in Fig. 1. As folic acid, riboflavin, and biotin are only slightly soluble in water, they were first dissolved in dilute alkali solution, and then dissolved in 10 mmol/L phosphate buffer (sodium) solution (pH 2.6). The standard solution was adjusted to obtain concentrations of 20 mg/L each.
An example of analysis using the Shim-pack MAqC-ODS Ⅰ is shown in Fig. 2, and the analytical conditions used are shown in Table 1. For comparison, analysis was conducted using a typical ODS column with mobile phase that was spiked with an ion-pair reagent. Those analysis results are shown in Fig. 3, and the analytical conditions are shown in Table 2.
The results using the Shim-pack MAqC-ODS Ⅰ demonstrate that the analysis time can be shortened due to the use of gradient analysis. Furthermore, even the late eluting substances show sharp peaks.

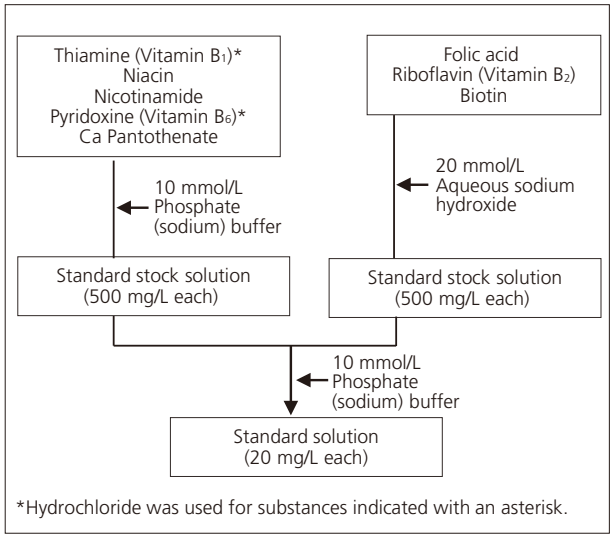
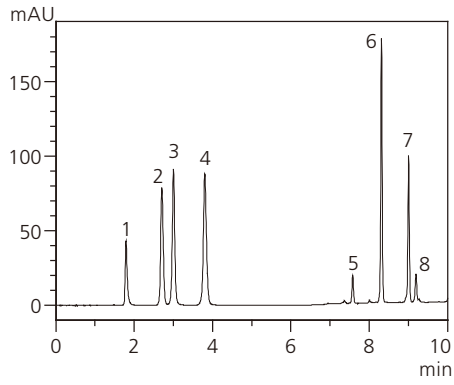


Fig. 1 Standard Solution Preparation

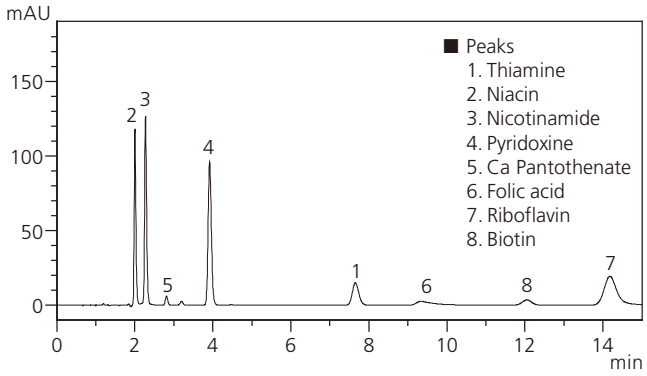


■ Peaks
1. Thiamine
2. Niacin
3. Nicotinamide
4. Pyridoxine
5. Ca Pantothenate
6. Folic acid
7. Riboflavin
8. Biotin

Table 1 Analytical Conditions: Shim-pack MAqC-ODS Ⅰ

Column	: Shim-pack MAqC-ODS Ⅰ (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A) 10 mmol/L phosphate (sodium) buffer (pH 2.6) B) Acetonitrile
Time Program	: B Conc. 1 % (0 min) → 1 % (2.5 min) → 50 % (10 min) → 1 % (10.01 min → 15 min)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 ˚C
Detection	: SPD-M20A at 210 nm
Injection Vol.	: 10 µL

Fig. 2 Chromatogram of Standard Mixture of 8 Water-Soluble Vitamins (Shim-pack MAqC-ODS Ⅰ)



■ Peaks
1. Thiamine
2. Niacin
3. Nicotinamide
4. Pyridoxine
5. Ca Pantothenate
6. Folic acid
7. Riboflavin
8. Biotin

Table 2 Analytical Conditions: Shim-pack VP-ODS

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A) 100 mol/L phosphate (sodium) buffer (pH 2.1) Containing 0.8 mmol/L sodium 1-octanesulfonate B) Acetonitrile A/B = 10/1 (v/v)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 ˚C
Detection	: SPD-M20A at 210 nm
Injection Vol.	: 10 µL

Fig. 3 Chromatogram of Standard Mixture of 8 Water-Soluble Vitamins (Shim-pack VP-ODS)

■ Analysis of Multivitamin Tablet

Fig. 5 shows a chromatogram of a multivitamin tablet that was analyzed using the Shim-pack MAqC-ODS Ⅰ column. The analytical conditions were the same as those shown in Table 1. Sample preparation was conducted according to the procedure outlined in Fig. 4.

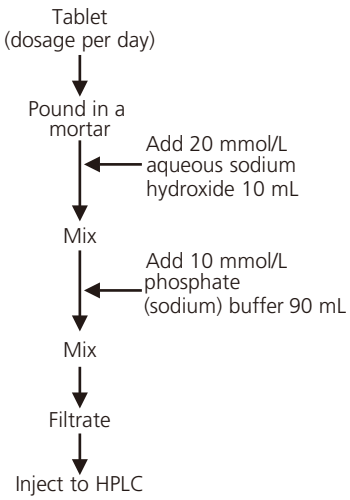


Fig. 4 Sample Preparation Procedure

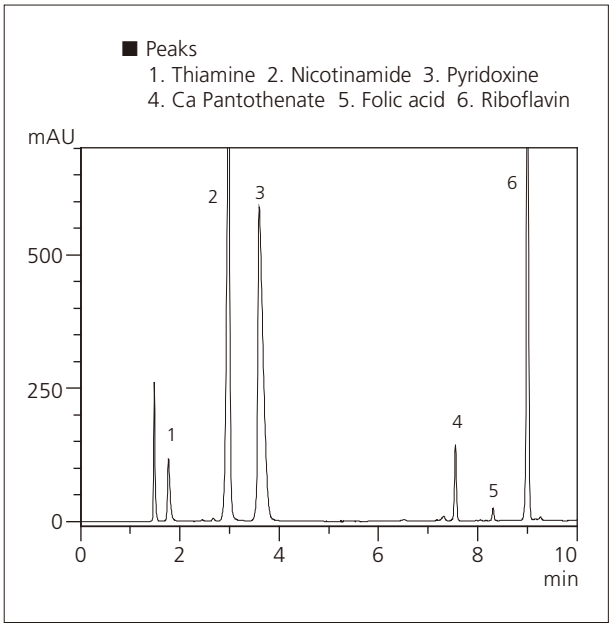


Fig. 5 Chromatogram of Multivitamin Tablet

The Shim-pack MAqC-ODS Ⅰ was developed jointly with Eisai Co., Ltd.

First Edition: Aug. 2014

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