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Application News

No.L438

High Performance Liquid Chromatography

LC/MS/MS Analysis of Vitamin D_3 by the Co-Sense for Impurities System

The Co-Sense for Impurities system, as previously described in Application News No. L424, is a complete HPLC configuration that automates the sample pretreatment process in trace level analysis. The earlier article presented an example of high sensitivity detection using analyte extraction and concentration. This system offers analyte concentration and removal of impurities, and therefore comprises 3 flow lines: one for primary separation, another for concentration, and the third for secondary separation. Since a different mobile phase and column can be selected for each flow line, a combination of conditions can be used, including those that are conducive for separation at the primary separation stage and that are appropriate for detection for the secondary separation stage. Here we introduce an example in which Vitamin D_3 capsules were analyzed by LC/MS/MS using such a method.

Analysis of Standard

The Vitamin D_3 sample was injected onto the Shim-pack XR-SIL high-speed analytical column for normal phase analysis as the primary separation stage, and after being concentrated on the Shim-pack MAYI-ODS trap column, the sample was routed to the Shim-pack XR-ODS Ⅱ analytical column for reversed phase analysis as the secondary separation stage, after which it was detected by mass spectrometry. Table 1 shows the analytical conditions used for each of the processes.
The chromatogram obtained during primary separation was monitored using UV detector (A), and was verified by the peak elution position of a standard. If analysis is started with that approximate peak time set in the Co-Sense for Impurities control software, valve A switches to send that fraction to be mixed with the flow from Pump Ⅱ so it is concentrated on trap column (Ⅱ). (upper diagram of Fig. 1 and upper chromatogram of Fig. 2)
After concentration for the time specified in the software, valve B is switched so Pump Ⅲ elutes the sample from the MAYI-ODS column and delivers it to column Ⅲ for the secondary separation stage and component detection by the mass spectrometer (Detector B). (lower diagram of Fig. 1 and lower chromatogram of Fig. 2)

Table 1 Analytical Conditions

[Column Ⅰ]	: Shim-pack XR-SIL (75 mmL. × 2.0 mmI.D., 2.2 µm)
Mobile Phase	: n-Hexane/2-Propanol (100 / 1)
Flow Rate	: 0.4 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 5 µL
Detection (A)	: UV265 nm
[Column Ⅱ]	: Shim-pack MAYI-ODS (30 mmL. × 4.6 mmI.D., 50 µm)
Mobile Phase	: Water
Flow Rate	: 30 mL/min 2.55-3.2 min
[Column Ⅲ]	: Shim-pack XR-ODSⅡ (75 mmL. × 3.0 mmI.D., 2.2 µm)
Mobile Phase	: A: Methanol / Water (100 / 5), B: Ethanol
Time Program	: B Conc 0 % (0-4 min) → 100 % (4.01-5 min) ・Mixer : 20 µL
Flow Rate	: 0.4 mL/min
Detection (B)	: LCMS-8030 (APCI positive, MRM 385.00 > 259.25)

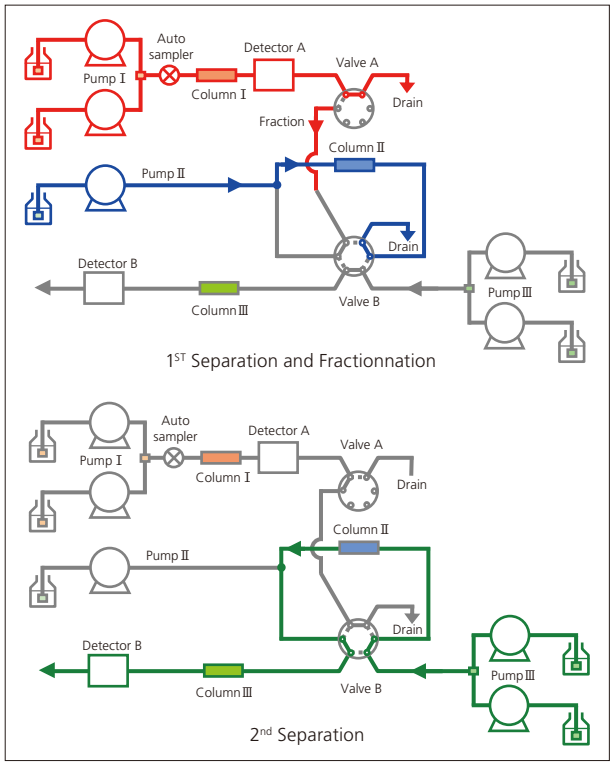

Fig. 1 Flow Diagrams

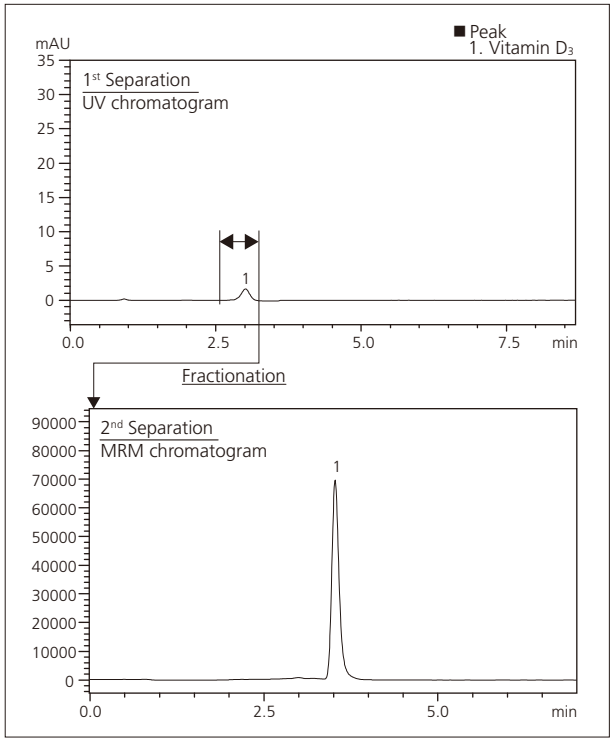

Fig. 2 Chromatograms of Vitamin D_3

The entire process described above took approximately 9 minutes for a complete analysis cycle.
Although LC/MS is not suitable for detection during the normal phase primary separation, it provided good detection in the secondary separation on the reversed phase column. Excellent calibration curve linearity from 0.005 – 0.5 µg/mL was obtained, with a correlation coefficient R > 0.999.
Furthermore, the peak area repeatability obtained using MRM chromatograms generated from 6 replicates of a standard solution (0.5 µg/mL) was 1.55 %RSD. The chromatograms are shown in Fig. 3.

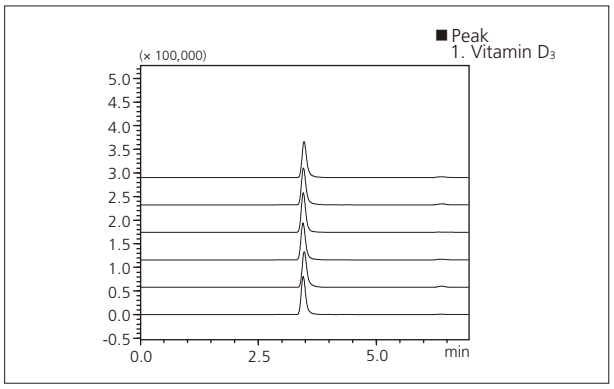

Fig. 3 Repeatability of MRM Chromatograms

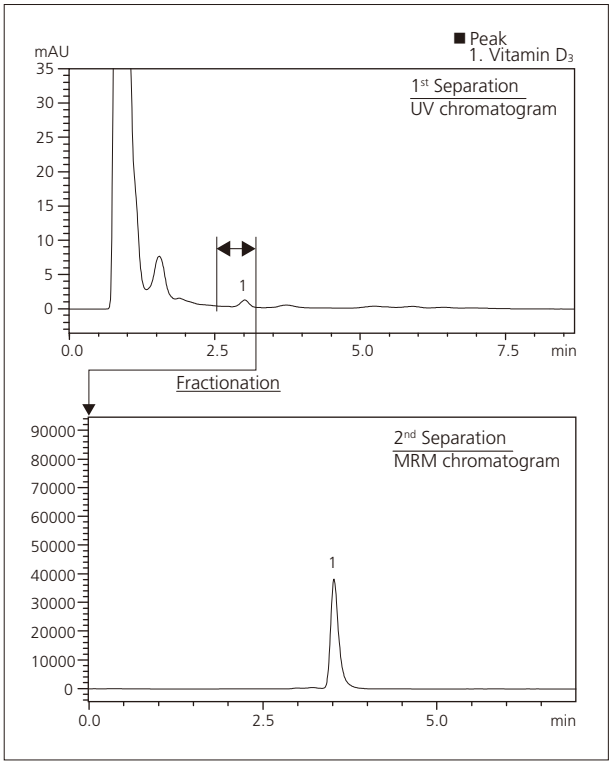

Fig. 4 Chromatograms of Oil-Soluble Vitamin Mixture Capsule

Analysis of Vitamin D_3 in Vitamin Capsules

We analyzed two commercially available capsules using these conditions.
The liquid in the capsule that contained oil-soluble vitamins was dissolved in hexane.
The capsule content of a commercially available multivitamin supplement was dissolved in a mixture of DMSO/hexane, and after heating, hexane extraction was conducted.
These hexane solutions were injected into a Co-Sense for Impurities system, and the results are shown in Fig. 4 and Fig. 5. In both cases, the large amount of impurities present in the hexane solution, as shown in the upper tier chromatograms, are quickly eliminated during the primary separation, and only the Vitamin D_3 and a small amount of impurities included in the normal phase fraction were selectively introduced into the secondary separation flow line.

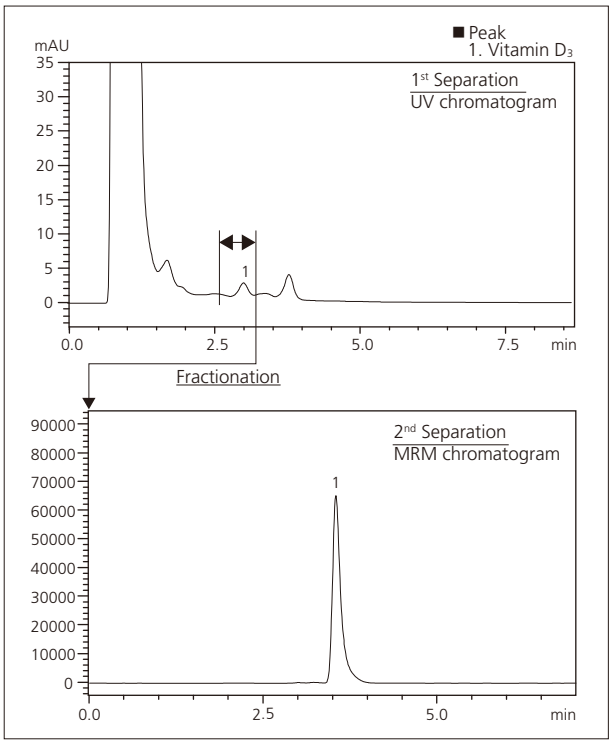

Fig. 5 Chromatograms of Oil- and Water-Soluble Vitamin Mixture Capsule

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