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A novel approach to the analysis of multivitamin by online supercritical fluid extraction/supercritical fluid chromatography

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Introduction

Vitamin is a series of basic trace substances which could maintain normal life forms of the animal body. Due to the chemical structure, fat-soluble vitamins such as vitamin A, vitamin E, etc. have strong hydrophobicity, low solubility in polar organic solvents. The analytical methods of those compounds are various, such as vitamin A by reversed phase liquid chromatography (RP-HPLC), vitamin D by normal phase liquid chromatography (NP-HPLC), and vitamin E normally used gas chromatography. Because of original method diversity, it is difficult to develop a new method of simultaneous analysis for fat-soluble vitamins. Supercritical Fluid Chromatography (SFC) is an unconventional chromatographic separation technology by using supercritical fluid and a small amount of modifier as mobile phase. Supercritical CO_2 ($scCO_2$) with its character of safe, inexpensive, non-toxic, facile, chemical inertness and other factors become the main mobile phase of SFC. Supercritical fluid ($scCO_2$) with low viscosity, high diffusivity and solubility characteristics is used in a wide variety of fields. Nexera UC Online SFE - SFC system is the latest products of supercritical fluid chromatograph in Shimadzu, which realized SFE (supercritical flow extraction) and SFC online combination, and simplify and unify the pretreatment method with high automation, extraction efficiency, and repeatability. In this study, a simultaneous analytical method for fat-solvable vitamins in drug and health care food was developed by using Nexera UC. It provides effective analysis and detection means for a variety of fat-soluble vitamins, and can be the reference for the quantitative study of this kind of material.

Methods and Materials

Sample Preparation

The analytical method for 5 kinds of fat-soluble vitamins was established in this study. Take the five standard include vitamin A acetate (VAA), vitamin A palmitate (VAP), vitamin E acetate (VEA), vitamin D2 (VD2), and vitamin D3 (VD3) and dissolves with n-hexane, diluted to a series of mixture concentration samples with ethanol. Then, dropped them to extraction tank and analyzed for standard curve. For commercially available vitamin A, vitamin E gelatin pearl, capsule and tablets, take out the contents into extraction tank to analyzed.

Experimental condition

Instrument

Nexera UC Online SFE-SFC system

configuration:

SFE-30A (SFE module), LC-30ADSF (CO_2 deliver pump), LC-20ADXR (modifier deliver pump), DGU-20A5 (degasser), CTO-20AC (column oven), SFC-30A×2 (back pressure adjustment module), SPD-20A (UV detector), CBM-20A (system controller), LabSolutions Ver5.8 (workstation).

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SFE condition	
Extraction agent	: $scCO_2$
modifier	: MeOH(5%)
flow rate	: 5 mL/min
static extraction	: 3 min
dynamic extraction	: 3 min
SFE temperate	: 50 °C
back pressure	: A-14.8 MPa, B-15 MPa
SFC condition	
Column	: GL Science ODS-P 4.6 mm I.D.×250 mm L., 5 µm
Mobile phase A	: $scCO_2$
Mobile phase B	: MeOH
Gradient program	: 0%B (6 min)-2%B (9 min)-10%B (16 min)-50%B(16.1-17 min)
Flow rate	: 3 mL/min
Oven temperature	: 40 °C
back pressure	: 10 MPa
detector wavelength	: 325 nm; 284 nm

Results and Discussion

Supercritical fluid extraction

Samples were loaded to the extraction vessel, and then set in a supercritical fluid extraction module for extraction. Liquid CO_2 and modifier of methanol (98/2, v/v) were delivered through the pumps into the extraction vessel (Figure 1), and changed to supercritical fluid under the setting of temperature and pressure. Methanol, as modifier, is to adjust the polarity, solubility and other properties of supercritical fluid to improve the extraction efficiency. Kept the vessel filled with supercritical fluid in 3 min at a stable temperature and pressure for static extraction.

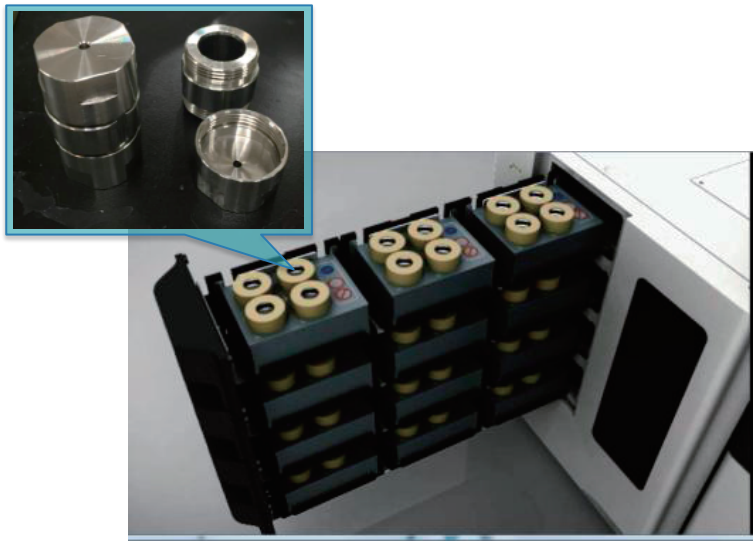

Figure 1 Extraction vessel and SFE

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Then, through SFE unit flow switch valve, supercritical fluid flow through the extraction vessel and extracted components from sample in 3 min by dynamic extraction. In the process of dynamic extraction, extract was directly introduced into subsequent SFC system. SFC separation and analysis was start after the completion of the extraction. The whole process of online SFE - SFC is shown in figure 2.

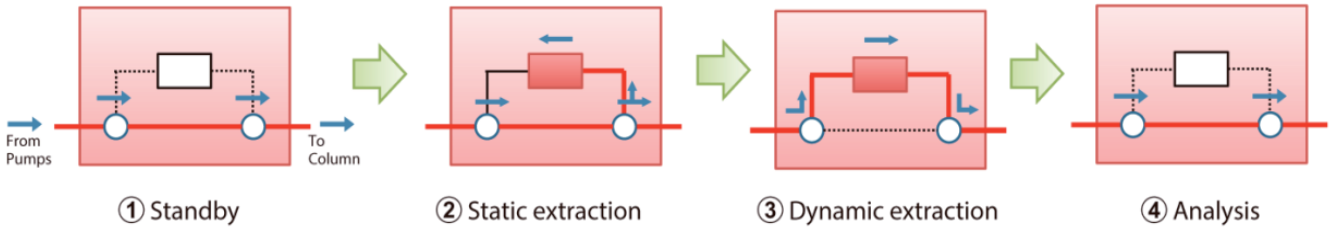

Figure 2 Pretreatment processing of SFE

For estimating the extraction efficiency of 5 compounds under the condition of setting, repeated extraction and analysis for the same vessel was performed. Peak area of every compound was calculated and peak area ratio of first extraction to total three times was recorded in table 2 to show the extraction yield of every compounds. The results showed that the SFE extraction yield of 5 vitamins were above 85% under the condition of settings.

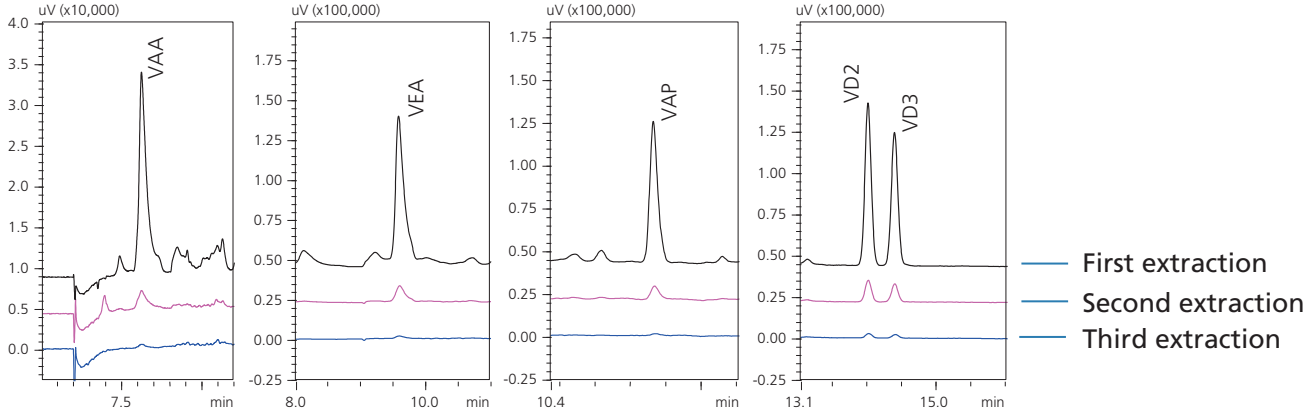

Figure 3 Chromatographs of three extractions for 5 vitamins

Table 1 Yield of three extractions for 5 vitamins

	VAA	VEA	VAP	VD2	VD3
1st extraction	94.2	88.6	89.3	86.2	86.0
2nd extraction	5.8	10.1	9.7	11.6	12.0
3rd extraction	0	1.3	1.0	2.2	2.0

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Supercritical Fluid Chromatography

The online SFE-SFC analytical results of 5 fat-soluble vitamins were showed in Fig 4. Vitamin A acetate and other four compounds were isolated obviously. The standard curves of absolute amount of compound added in extraction vessel to the detector response shown in Figure 5, it indicates 5 compounds with good linearity in their respective concentration, and regression coefficient of R2 in 0.997-0.999.

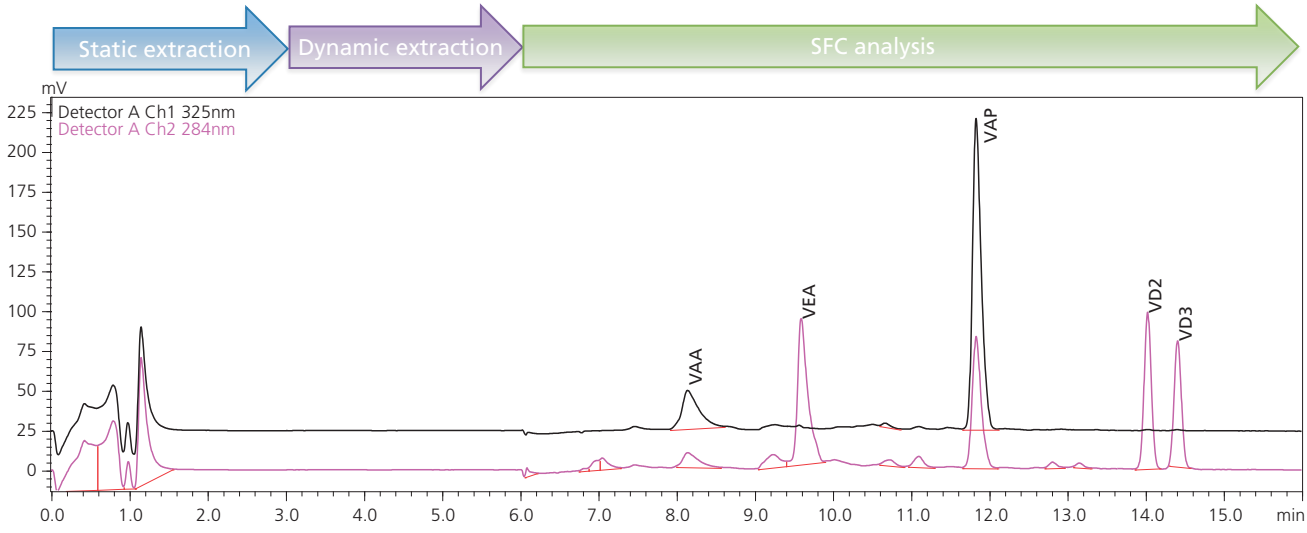

Figure 4 Chromatogram of simultaneous analysis for 5 vitamins

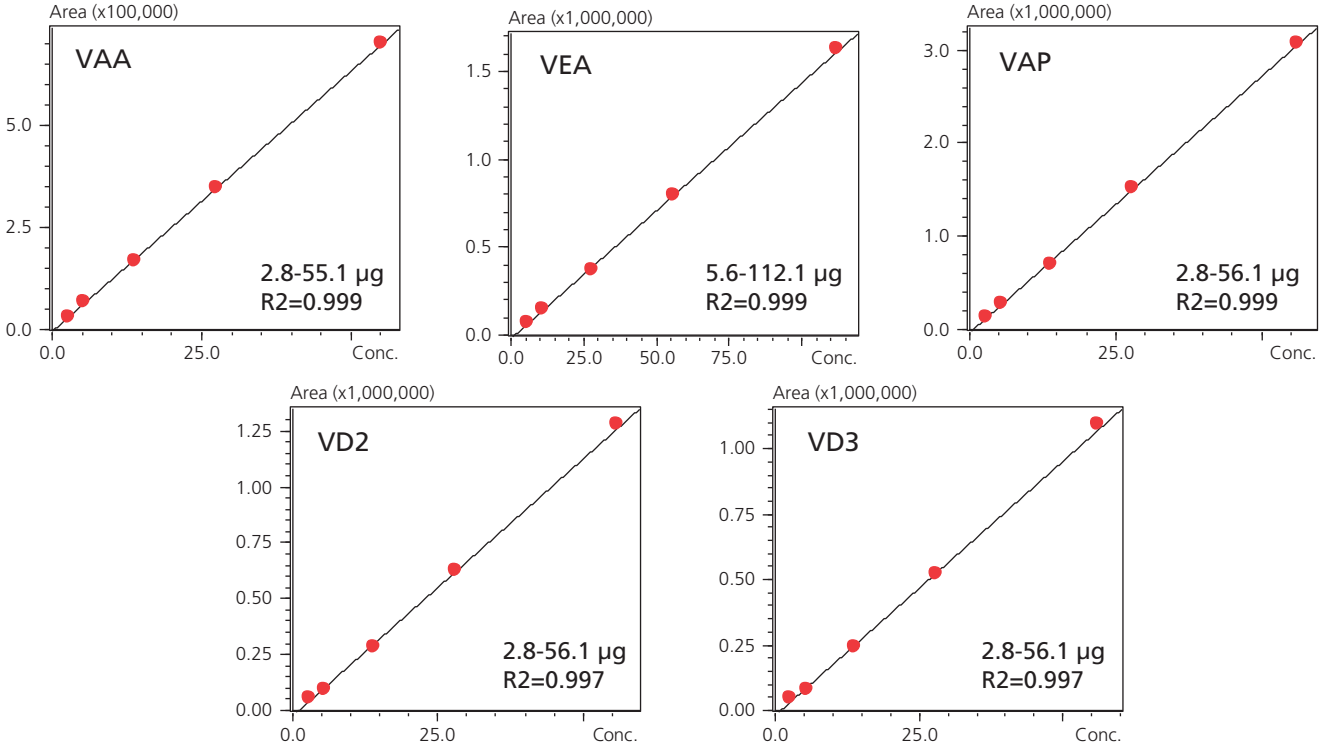

Figure 5 Calibration curves of 5 vitamins

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Repeatability and recovery

Add 2 times of LLOQ for each compound into extraction vessel to test repeatability and recovery. Results were shown in table 2.

Table 2 Repeatability and recovery of 5 vitamins (n=6)

	VAA	VEA	VAP	VD2	VD3
Rt (RSD%)	0.24	0.15	0.10	0.04	0.03
Area (RSD%)	13.0	5.2	4.1	4.5	5.9
Recovery (%)	94.4	101.1	100.0	90.5	90.5

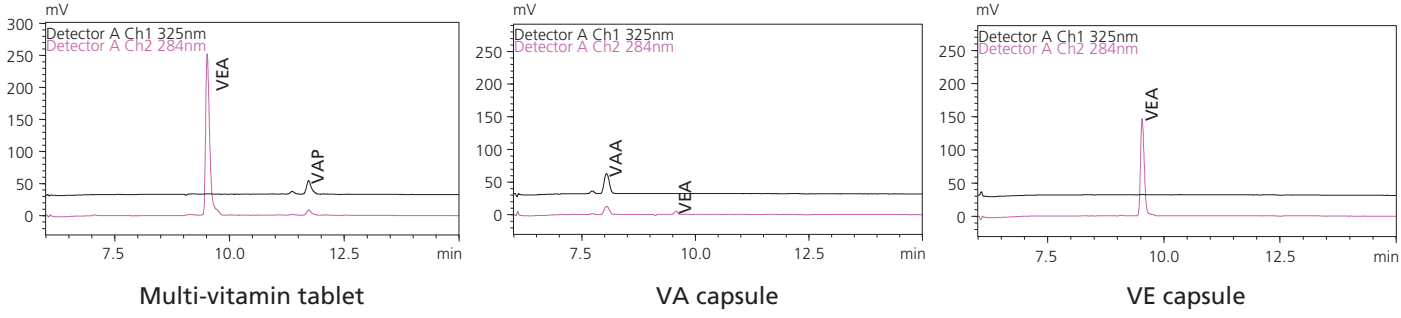

Figure 6 Chromatogram of three real samples which were analysed by using Nexera UC

Conclusions

An Online SFE-SFC method has been developed for quantitative analysis of 5 fat-soluble vitamins in drugs and health care food sample. It provided a new way for simultaneous analysis for 5 vitamins which combined the processing of pretreatment and analysis together. The results showed that this method is rapid and reliable.

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