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Capillary liquid chromatographic analysis of fat-soluble vitamins and β-carotene in combination with in-tube solid-phase microextraction

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ABSTRACT

A capillary liquid chromatography (CLC) system with UV/vis detection was coupled with an in-tube solid-phase microextraction (SPME) device for the analysis of fat-soluble vitamins and β-carotene. A monolithic silica-ODS column was used as the extraction medium. An optical-fiber flow cell with a long light path in the UV/vis detector was utilized to further enhance the detection sensitivity. In the in-tube SPME/CLC system, the pre-condition of the extraction column and the effect of the injection volume were investigated. The detection limits (LOD) for the fat-soluble vitamins and β-carotene were in the range from 1.9 to 173 ng/mL based on the signal-to-noise ratio of 3 (S/N = 3). The relative standard deviations of migration time and peak area for each analyte were less than 5.0%. The method was applied to the analysis of fat-soluble vitamins and β-carotene contents in corns.

1. Introduction

Vitamins are compounds essential for the health of humans and animals. Their presence in human body is of vital importance. According to their solubility, vitamins are divided into two groups, water- and fat-soluble vitamins. The common methods for the determination of fat-soluble vitamins are normal-phase or reversed-phase liquid chromatography (LC) involved the use of UV/vis [1–4], fluorescence [5], electrochemical [6], and mass spectrometry detection [7].

Miniaturization and automation have been two important trends in LC field since 1970s. Compared with conventional LC, micro or capillary LC (CLC) offers several advantages such as significant reduction of solvent consumption, small amounts of sample required, easy coupling with other techniques. However, a minute sample injection volume and limited optical path length for on-column photometric detection in CLC result in its low detection sensitivity. In order to enhance the detection sensitivity in CLC, hyphenation with sample pre-concentration techniques is needed. One technique is on-column focusing, which is to dissolve analytes in a weaker solvent than the mobile phase and allows the injection of a large sample volume without considerable effect on band broadening [8–10]. Other sample enrichment techniques are on-line or off-line solid-phase microextraction (SPME) [11,12]. On-line in-tube SPME, introduced by Eisert and Pawliszyn [13] enables continuous extraction, concentration, desorption and injection using an autosampler, which not only shortens the total analysis time but also provides better accuracy and precision relative to manual techniques, as reviewed by Kataoka [14] and Saito and Jinno [15]. In this technique, an open tubular fused-silica capillary column was usually used as an extraction device. The ratio of the surface area of the coated layer contacted with sample solution to the volume of the capillary column is insufficient for mass transfer. Repeating draw/eject cycles of sample solution is needed to improve extraction efficiency. To overcome the problem, Shintani et al. [16] first introduced a monolithic silica-ODS column used for in-tube SPME, which demonstrated better preconcentration efficiency compared with the conventional in-tube SPME due to the merits of higher permeability and porosity possessed by a monolithic column. Jia et al. [17] developed an in-tube SPME coupled to a CLC system for analysis of cellular flavins, in which a monolithic silica-ODS column was used as an extraction device. The method proved to be beneficial to enhance the detection sensitivity. Recently, Zheng et al. [18] reported an octyl-functionalized hybrid silica monolithic column as an extraction medium for in-tube SPME coupled to a CLC system. The monolithic column allows the increment in sample injection volume without any loss in resolution, resulting in the improvement in the detection limits for several standard polycyclic aromatic hydrocarbons.

The purpose of this work was to develop a CLC method in combination with in-tube SPME for analysis of fat-soluble vitamins and β-carotene in corns, in which a monolithic silica-ODS column was used as an extraction medium. In-tube SPME as an on-column

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focusing technique and an optical-fiber flow cell with a long light path in the UV detector were combined to enhance the detection sensitivity.

2. Experimental

2.1. Chemicals and reagents

Methanol (CH_3OH) and tetrahydrofuran (THF) were of HPLC grade and purchased from SK Chemicals (Ulsan, South Korea). Phytonadione (VK_1), α-tocopherol (VE), retinyl palmitate (VA) and β-carotene were obtained from Sigma–Aldrich (USA). Stock solutions of each vitamin were prepared as 1 mg/mL in CH_3OH-THF (50/50, v/v). β-Carotene stock solution was prepared as 0.1 mg/mL in THF. The stock solutions were stored at −18 °C in brown bottles.

Water used was obtained from an Elga water purification system (ELGA, London, UK). All solutions were sonicated and filtered through 0.45 μm filters (Sartorius, Gottingen, Germany) prior to use.

2.2. Instrumentation

The CLC system was comprised of a microflow pump (MP711, GL Sciences, Tokyo, Japan), an UV/vis detector with an optical-fiber flow cell (MP701, GL Sciences, Tokyo, Japan), and a manual injection valve (Valco VICI, Switzerland). A monolithic silica-ODS column (27 cm × 100 μm I.D.) was prepared according to the method previously described by Tanaka research group [19]. Isocratic elution was performed with CH_3OH as the mobile phase at a flow rate of 1.0 μL/min. The detection wavelength was set at 290 nm for VE, 250 nm for VK_1, 325 nm for VA, and 452 nm for β-carotene. The switch of the detection wavelength was controlled by a time program.

2.3. In-tube SPME

The in-tube SPME system consisted of a syringe pump (PHD 2000 series, Harvard, USA) and a monolithic silica-ODS column (10 cm × 200 μm I.D.), which was mounted in the position of the sample loop of the six-port valve. The syringe pump was used to control the injection volume and flow rate of the sample solution. The monolithic column was washed and conditioned first by CH_3OH 50 μL, then 50% CH_3OH 50 μL at a flow rate of 5 μL/min prior to extraction. The extraction of the sample into the monolithic column was performed by the injection of the sample at a flow rate of 5 μL/min with the six-port valve in the LOAD position. After the valve was switched to the INJECT position, the extracted sample was directly desorbed from the monolithic column by mobile phase flow, and transported to the CLC column, then detected by UV/vis detector.

2.4. Sample preparation

Corn sample was purchased from market. The sample preparation procedure was similar to the method described previously [20]. Briefly, weigh 120 mg corn sample accurately (Sartorius CP224S analytical balance, Goettingen, Germany) and put it into a 15 mL plastic tube. Cold CH_3OH (−20 °C, 4 mL) was added to the tube containing the corn sample. The CH_3OH solution was mixed thoroughly for about 30 s and then placed at −20 °C for 30 min. The solution was centrifuged at 4 °C and 5000 rpm for 30 min. The upper layer was withdrawn and centrifugally filtered through a Millipore 5 kDa-cutoff filter to remove proteins and other debris. The filtrate was evaporated using a micro centrifugal vacuum concentrator Christ RVC 2–18 (Osterode am Harz, Germany) at 30 °C. Prior to analysis, the dried sample was reconstituted in 0.5 mL of 50% CH_3OH.

3. Results and discussion

3.1. Optimization of CLC conditions

In the optimization experiments of CLC conditions, the volume of sample loop was 100 nL. The standard mixture was used as the test sample to optimize the CLC conditions, where the concentration of each fat-soluble vitamin was 10 μg/mL and β-carotene 2 μg/mL. The detection wavelength was set at 290 nm for VE, 250 nm for VK_1, 325 nm for VA, and 452 nm for β-carotene.

The mobile phase composition and flow rate were optimized. When CH_3OH was used as the mobile phase while keeping the flow rate at 1.0 μL/min, the separation of the fat-soluble vitamins and β-carotene was finished in 11 min, as shown in Fig. 1A. Based on the experimental results, isocratic elution with CH_3OH as the mobile phase at a flow rate of 1.0 μL/min was utilized for subsequent experiments.

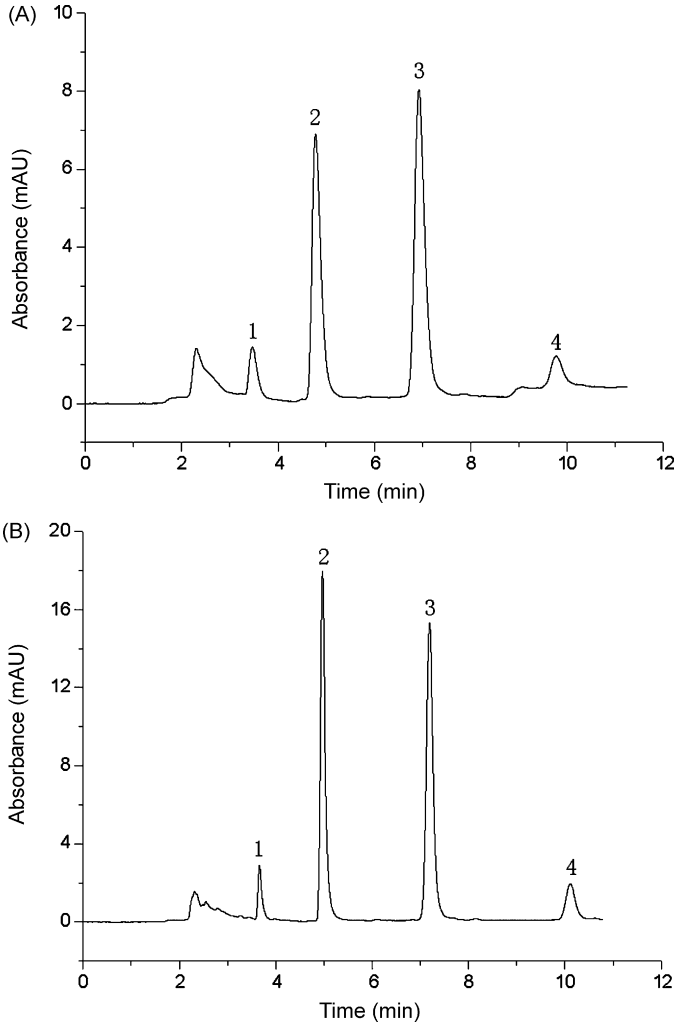

Fig. 1. Effect of sample matrix. (A) CH_3OH; (B) 50% CH_3OH. CLC conditions: Isocratic elution was performed with CH_3OH as mobile phase at a flow rate of 1.0 μL/min. Injection volume was 100 nL. The concentration of each fat-soluble vitamin was 10 μg/mL and β-carotene 2 μg/mL. Analytes: (1) VE; (2) VK_1; (3) VA; (4) β-carotene.

3.2. Effect of sample matrix

In the CLC system, an optical-fiber flow cell with a long light path of 3 mm was utilized to improve the detection sensitivity. Moreover, solvent gradient zone sharpening effect as an on-column focusing technique was also used to enhance the detection sensitivity. When the sample dissolved in a weaker solvent than the mobile phase, the sample zone is sharpened during its injection, resulting in the improvement of the detection sensitivity of analytes. Hence, the effect of the sample matrix was investigated. When the concentration of CH_3OH in the sample matrix was in the range of 50–100%, the peak width at half height of analytes decreased with the decrease of the concentration of CH_3OH in the sample matrix due to the existence of the injection zone sharpening effect, resulting in the increase in the peak height of analytes. With less than 50% CH_3OH in the sample matrix, the further decrease in the concentration of CH_3OH had little effect on the peak width at half height of analytes. As shown in Fig. 1, with 50% CH_3OH as the sample matrix, there were 2.2-, 2.6-, 1.9- and 2.2-fold sensitivity enhancement in terms of peak height for VE, VK_1, VA and β-carotene, respectively, compared with that using CH_3OH as the sample matrix. Thus, 50% CH_3OH was chosen as the sample matrix for subsequent experiments.

3.3. Investigation of in-tube SPME

Under the existence of the solvent gradient zone sharpening effect, the detection sensitivity can be improved by enabling the injection of large volume of sample without compromising the resolution since the sample dissolved in a weak solvent undergoes on-column concentration at the column inlet during its injection. In our work, an in-tube SPME device was on-line coupled to CLC to enhance the detection sensitivity.

In the in-tube SPME/CLC method, the standard mixture, in which the concentration of VE was 1 μg/mL and other analyte 0.2 μg/mL, was used as the test sample. CH_3OH solution (50%) was used for pre-conditioning the extraction column. The effects of different injection volumes on the separation were investigated. With the injection volume in the range from 5 to 100 μL, the peak heights of the analytes had linear relationship with the injection volume. Fig. 2 shows the chromatograms of the fat-soluble vitamins and β-carotene by in-tube SPME/CLC system at injection volume of 10 and 100 μL. In comparison with the conventional injection (100 nL), with the injection volume at 100 μL in in-tube SPME, the sensitivity enhancement factors for the analytes (defined as the following, the ratio of the peak height of analytes with in-tube SPME to that with conventional injection (100 nL) × dilution factor) were 13 (VE), 86 (VK_1), 224 (VA), and 724 (β-carotene), respectively. However, when the injection volume was larger than 50 μL, the analytes of larger retention factors, e.g., VA and β-carotene, showed significantly fronting shapes due to the sample overloading. Hence, the injection volume of 10 μL was used for calibration experiments.

3.4. Calibration

A series of standard solutions with the concentration ranging from 1 to 10 μg/mL for VE and 0.2 to 2 μg/mL for other analytes were used to determine the calibration parameters of the analytes. The standard mixture, in which the concentration of each analyte was 0.2 μg/mL except for VE 1 μg/mL, was utilized for the determination of the reproducibility of the method with three consecutive runs. The limits of detection (LODs) for the fat-soluble vitamins and β-carotene were calculated at signal-to-noise ratio equal to 3 (S/N = 3). As shown in Table 1, the linearity of the calibration functions for the analytes was satisfactory with the correlation coefficients above 0.9963. The LODs for the analytes were 173 ng/mL (VE), 16.4 ng/mL (VK1), 3.6 ng/mL (VA), and 1.9 ng/mL (β-carotene), respectively. The relative standard deviations (RSDs) for the migration time and peak area of each analyte were less than 5.0%.

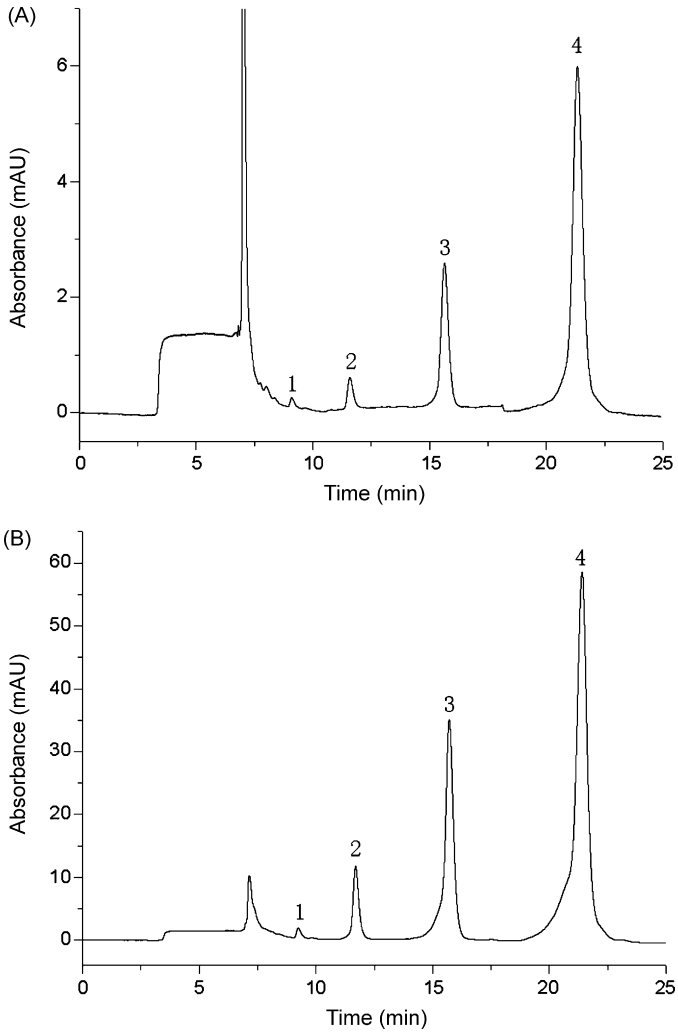

Fig. 2. Effect of injection volume. (A) 10 μL; (B) 100 μL. In-tube SPME/CLC conditions: Isocratic elution was performed with CH_3OH as mobile phase at a flow rate of 1.0 μL/min. Sample matrix was 50% CH_3OH. The concentration of each analyte was 0.2 μg/mL except for VE (1 μg/mL). Analyte peak numbering is the same as in Fig. 1.

3.5. Analysis of corn samples

When the developed in-tube SPME/CLC method was applied to the analysis of corns, only VE was detected in the corn samples,

Table 1
Reproducibility, linearity and sensitivity for the fat-soluble vitamins and β-carotene.

Analyte	Calibration line	r^2	LOD (ng/mL)	RSD (%, $n = 3$)	
				Migration time	Peak area
VE	$y = 4.58x - 0.96$	0.9977	173	1.3	3.1
VK_1	$y = 25.09x - 4.87$	0.9965	16.4	1.0	4.2
VA	$y = 62.62x - 5.94$	0.9997	3.6	1.0	5.0
β-Carotene	$y = 121.37x + 4.08$	0.9963	1.9	0.3	2.9

In-tube SPME/CLC conditions: Isocratic elution was performed with CH_3OH as mobile phase at a flow rate of 1.0 μL/min. Detection wavelength was set at 290 nm. Sample matrix was 50% CH_3OH. Injection volume was 10 μL. y: peak area; x: analyte concentration (μg/mL); r: correlation coefficient.

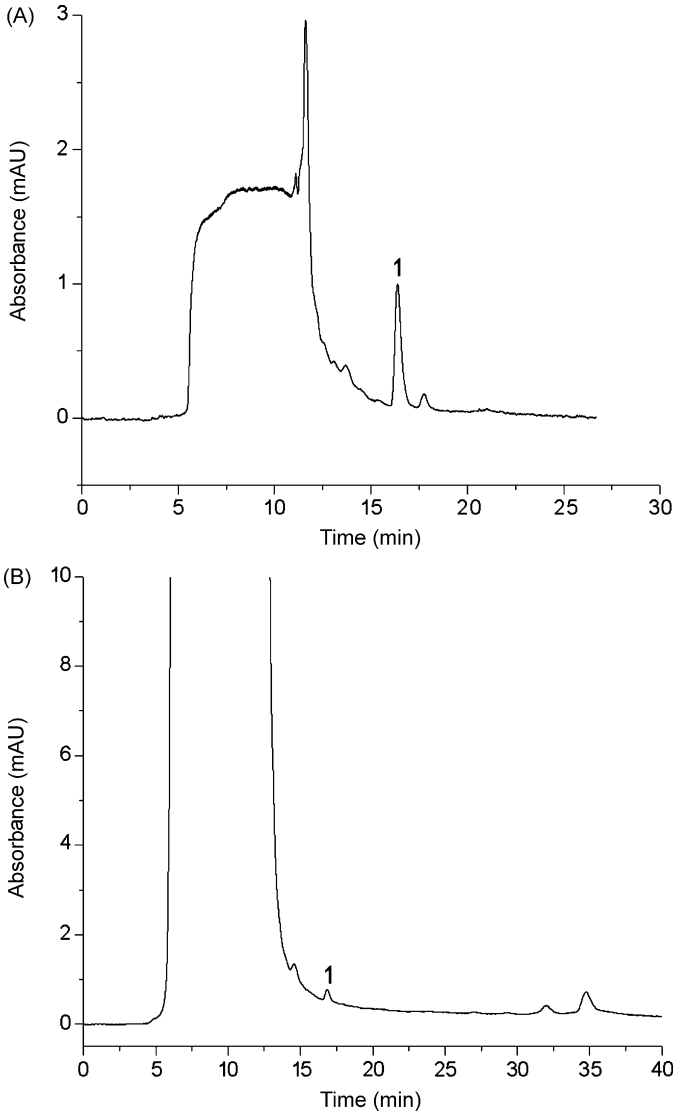

Fig. 3. Chromatograms of standard sample and corn samples by in-tube SPME/CLC. (A) Standard VE sample; (B) Corn sample. In-tube SPME/CLC conditions: Isocratic elution was performed with CH_3OH as mobile phase at a flow rate of 0.6 μL/min. Detection wavelength was set at 290 nm. Sample matrix was 50% CH_3OH. Injection volume was 10 μL. Analyte peak numbering is the same as in Fig. 1.

Table 2
Analysis results of corn samples ($n = 3$).

Analyte	Estimated concentration (μg/g)	Recovery (%)
VE	6.04 ± 0.12	92.7 ± 6.0

other fat-soluble vitamins and β-carotene were not detected. However, VE could not be separated sufficiently from the sample matrix due to the interference from other substances. In order to determine the content of VE accurately, the flow rate was decreased to separate VE from other substances in corn baseline. The experimental results showed that with the flow rate at 0.6 μL/min, VE obtained better separation from other substances. As β-carotene and the fat-soluble vitamins except for VE were not detected in the corn sample using the developed method, only VE was quantified at a flow rate of 0.6 μL/min. A series of VE with different concentrations were used to determine the calibration parameters. Satisfactory linearity was obtained ($y = 6.44x - 0.50$, where y is peak area and x concentration of VE in μg/mL, $r^2 = 0.9997$). The sample with the concentration of VE at 5 μg/mL was utilized for the determination of the reproducibility of the method with three consecutive runs. The RSD for the migration time was 0.5%, for peak area 3.7%. Fig. 3 shows the chromatograms of standard VE and corn samples analyzed by the in-tube SPME/CLC method. The analytes in the sample were identified by two methods: (1) comparing their migration times with those of standards; (2) spiking the standards to the sample. As shown in Table 2, the content of VE in the corn was evaluated to be 6.04 ± 0.12 μg/g by triplicate analysis of the corn sample. In order to investigate the measured accuracy of VE in a complex corn sample using the developed in-tube SPME/CLC method, the recovery experiments were carried out based on 1.50 μg/mL of standard VE added to the prepared corn sample. The recovery for VE was determined to be 92.7% by triplicate analysis, with the RSD 6.0%.

4. Conclusion

A CLC method in combination with in-tube SPME with an UV/vis detector was developed for simultaneous analysis of fat-soluble vitamins and β-carotene. In-tube SPME as an on-line sample concentration method and an optical-fiber flow cell with a long light path of 3 mm were combined to enhance the detection sensitivities of the analytes. The developed method was applied to evaluate VE content in corns.

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