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Article

DRUG FORMULATIONS

A Novel Ionic Liquid-Based Liquid-Liquid Microextraction Combined with High Performance Liquid Chromatography for Simultaneous Determination of Eight Vitamin E Isomers in Human Serum

Shuo Yin,[1] Yi Yang,[2] Jing Zhang,[1] Yongxin Li,[1,3] Ling Wu,[1] and Chengjun Sun[1,3]

[1]Sichuan University, West China School of Public Health and West China Fourth Hospital, Chengdu 610041, China, [2]Southwest Medical University, School of Public Health, Luzhou 646000, China, [3]Provincial Key Laboratory for Food Safety Monitoring and Risk Assessment of Sichuan, Chengdu 610041, China

Corresponding author's e-mail: sunchj@scu.edu.cn

Abstract

Background: Vitamin E deficiencies are prevalent around the world and have become one of the major public health issues. It is necessary to determine their levels in human serum for routine clinical practice.
Objective: In this study, a simple and green ionic liquid-based (IL)vortex-assisted (VA) liquid–liquid microextraction (LLME) combined with HPLC was developed for simultaneous determination of eight vitamin E isomers in human serum.
Methods: The IL, 1-octyl-methylimidazolium trifluoromethanesulfonate ([OMIM]OTf), was added into the diluted sample and vortexed to form a cloudy solution. After centrifugation, the IL phase was collected for HPLC analysis. The separation was accomplished on a Phenomenex Luna-C18 column (250 mm × 4.6 mm, 5 μm) and the column temperature was 30°C. The mobile phase was methanol/acetonitrile (80 + 20, v/v) and the flow rate was 0.7 mL/min. A fluorescence detector was used for the simultaneous detection of eight vitamin E isomers, and the detection wavelength was set at 290/327 nm. The LLME procedure can be completed within 10 min without using any organic solvent. The parameters affecting the extraction efficiencies were optimized, including the type and volume of the ILs, dispersive solvent, vortex time, and salt addition.
Results: Under the optimal conditions, limits of detection were 0.857–4.16 ng/mL. Acceptable recoveries ranging from 80.1% to 103% were achieved, with relative standard deviations less than 13.0%. The proposed method was successfully applied to the detection of eight vitamin E isomers in human serum samples.
Conclusions: This method is simple, fast, environment-friendly, cheap, and has similar linear ranges, sensitivities, accuracy, and precision as those reported chromatographic methods.
Highlights: The IL, [OMIM]OTf, was chosen as the green extractant of LLME for vitamin E extraction because of its strong adsorption property for vitamin E isomers. An IL-VA-LLME method has been developed for the analysis of 8 vitamin E isomers. The established method was successfully applied to the analysis of 8 vitamin E isomers in human serum samples.

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Vitamin E (VE), mainly classified with eight tocochromanols known as α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol, is one category of essential fat-soluble vitamins (FSVs) with various physiological functions crucial for human health (1). VE isomers are absorbed in the intestine, incorporated into chylomicrons, and then secreted into the portal vein, leading to the liver. Among different VE isomers, α-tocopherol (α-T) is the abundant and most efficient form in the human body, while γ-tocopherol (γ-T) is the most common form in the North American diet. In VE supplements, α-T, either naturally extracted from plant oils or synthetic products, is esterified, generating tocopheryl acetate. Vitamin deficiencies are prevalent around the world, especially in developing countries, and have become one of the major public health issues. The typical diseases of VE deficiency are spinocerebellar syndrome and increased oxidative cell stress (2). In addition, there is increasing evidence that VE deficiency is associated with increased risk of cancer, cardiovascular disease, diabetes, chronic inflammation, and neurological diseases (3, 4). These facts strongly increase the importance in the laboratory requests and clinical testing of VE. Each of the VE isomers has unique physiological properties (5). Thus, it is important to analyze VE isomers respectively. However, the development of simultaneous determination methods for VE isomers in clinic is still a challenge due to their chemical instability, the discrepancies between their structures, physiochemical properties, and natural concentrations in complex biological samples (6). To solve these pressing problems, establishing a fast, sensitive, robust, and cost-effective method for the simultaneous determination of VE isomers is essential in routine clinical practice (7).

Due to the chemical properties (hydrophobic, chemical instability towards light, oxygen, heat) of VE isomers, and the needs of their determination in highly polar and complex serum samples, appropriate sample preparation before detection is required (8). To date, sample preparation for the extraction of VE isomers from serum sample is mainly based on complex procedures including protein precipitation and solvent extraction using n-hexane, ethanol, acetonitrile, etc. (9, 10). These methods are tedious, time-consuming, expensive and require large amounts of toxic organic solvents, which are unsuitable for routine high-throughput analysis. In the past decades, some relatively eco-friendly sample preparation techniques have been developed for the analysis of VE and other FSVs in different matrices by miniaturing and simplifying sample preparation procedures, such as pressurized liquid extraction (10, 11), magnetic solid phase extraction (12, 13), dispersive liquid-liquid microextraction (14, 15), and hollow-fiber liquid-phase microextraction (16). However, these methods also require specific consumables, equipment, and small amounts of organic solvents. Taking account of the potential hazardous solvents used, efforts should be made in evaluating novel green solvents as extractants in extraction procedures (17).

Ionic liquids (ILs) are a group of non-molecular compounds, with melting points below 100°C. ILs are good environment-friendly substitutes for organic extractants in extraction techniques and have received increasing attention due to their distinctive advantages, such as negligible vapor pressure, thermal stability, adjustable viscosity, density, and miscibility with water and organic phases, leading to highly selective and effective extraction and preconcentration (18, 19). There has been increasing application of ILs as extractants, especially in many sample preparation procedures as a "green" alternative for hazardous organic solvents (20). A few IL-based microextraction methods have been reported for the selective extraction of vitamin D and VE (21–23). However, there is no literature available for the simultaneous extraction of eight VE isomers in human serum using ILs.

Herein, an IL-vortex assisted (VA)-liquid–liquid microextraction (LLME) method combined with HPLC with fluorescence detection is established for simultaneous determination of eight VE isomers in human serum. In this method, the IL 1-octyl-methylimidazolium trifluoromethanesulfonate ([OMIM]OTf) was selected as an extractant for VE extraction. Various factors influencing the extraction efficiency such as the type and volume of the ILs, dispersive solvent, vortex time, and salt addition were investigated. The established method was validated and applied to the determination of eight VE isomers in human serum samples. In this work, we proceeded to explore the IL application as extractants for the extraction of VE isomers in serum, and further confirmed their vast application prospect in sample preparation procedure for biological samples.

METHOD

Samples

All the samples were collected from West China Fourth Hospital and this study got approval from Sichuan University Medical Ethics Committee, which guaranteed that the study was conducted in accordance with the principles described in the Helsinki Declaration. Forty-four human serum samples were obtained from healthy people including 6 females and 38 males aged from 22 to 55 years with a non-supplemented diet by vacuum blood collection tubes. All samples were fasting blood samples collected in the early morning. The samples were stored in a refrigerator at -18°C from the time of collection until extraction.

Apparatus

(a) *LC system*.—Dionex UltiMate 3000 HPLC system with a FLD (Thermo Fisher Scientific, MA, USA).
(b) *LC column*.—Luna-C18 column (250 mm×4.6 mm, 5 μm particle size) (Phenomenex, CA, USA).
(c) *Centrifuge*.—TDL-80-2B centrifuge (Anke, Shanghai, China).
(d) *Vortex mixer*.—ZX4 advanced IR vortex mixer (VELP, Milan, Italy).
(e) *Analytical balance*.—BSA224S analytical balance (Sartorius, Gottingen, Germany).

Reagents

(a) *Standard (purity grade)*.—α-tocopherol (α-T, 97%), β-tocopherol (β-T, 98.6%), γ-tocopherol (γ-T, 99%), δ-tocopherol (δ-T, 95.5%), α-tocotrienol (α-T3, 98.13%), β-tocotrienol (β-T3, 98.29%), γ-tocotrienol (γ-T3, 98.45%), and δ-tocotrienol (δ-T3, 98.37%) were all purchased from Sigma-Aldrich (St Louis, MO, USA).
(b) *Solvent*.—Acetonitrile (99.9%, HPLC-grade; Sigma-Aldrich), Methanol and ethanol were HPLC-grade and purchased from Kemiou (Tianjin, China).
(c) *Sodium chloride (NaCl)*.—Analytical grade (Reagent Chemical, Tianjin, China).
(d) *ILs*.—[OMIM]OTf, 1-octyl-methylimidazolium bis((trifluoromethyl)sulfonyl)imide ([OMIM]NTf_2), and 1-octyl-methylimidazolium hexafluorophosphate ([OMIM]PF_6) with purity of >98%, were purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China.

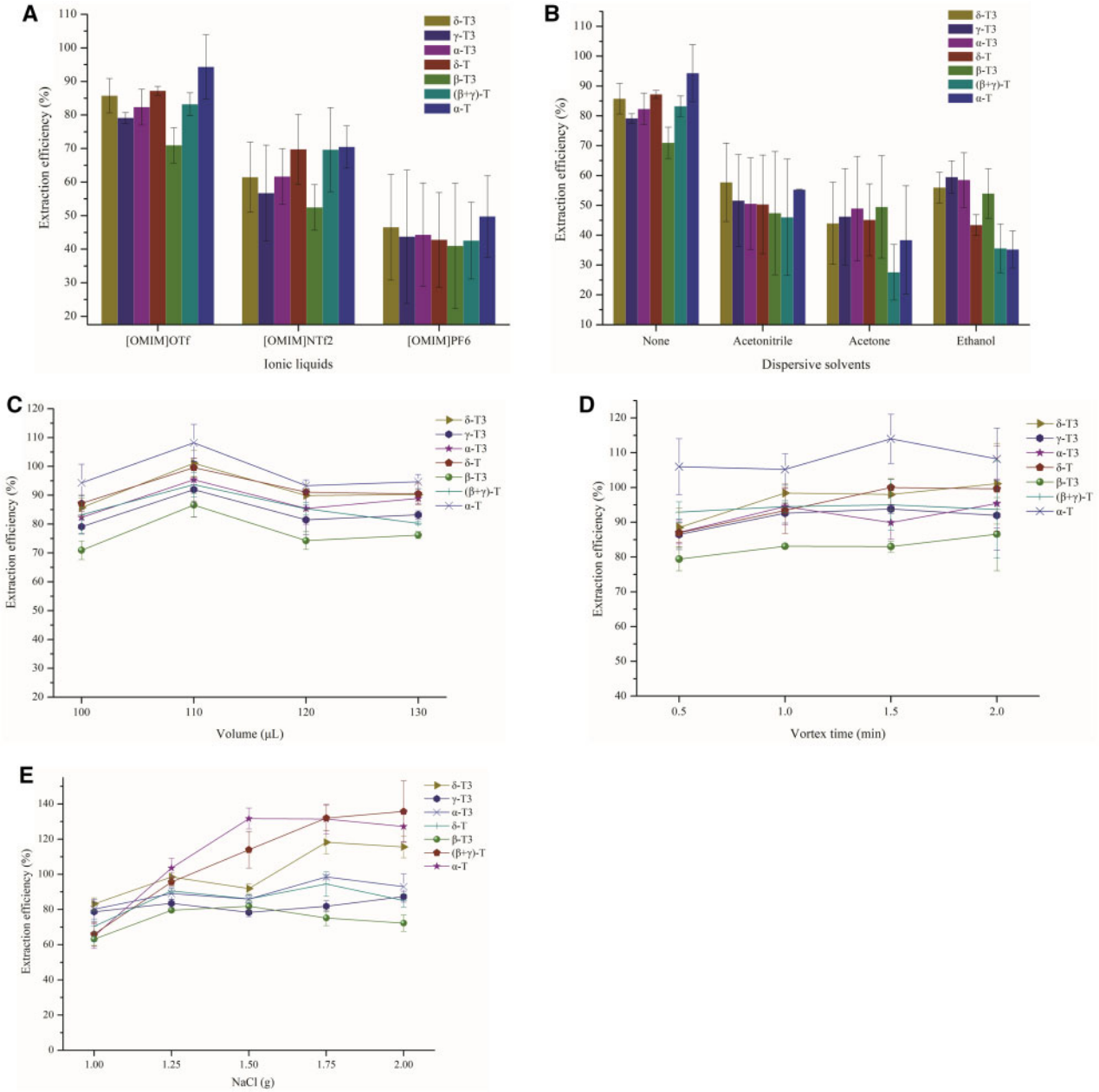

Figure 1. Effects of the operational factor on extraction efficiencies in IL-VA-LLME method. (A) Effects of different IL extractants (Conditions: 200 μL serum sample, 100 μL extractant, vortex time of 2 min, 1.25 g NaCl). (B) Effects of dispersive solvents [Conditions: 200 μL serum sample, 100 μL extractant ([OMIM]OTf), vortex time of 2 min, 1.25 g NaCl]. (C) Effects of different volumes of extractant [Conditions: 200 μL serum sample, extractant ([OMIM]OTf), vortex time of 2 min, 1.25 g NaCl]. (D) Effects of vortex time [Conditions: 200 μL serum sample, 110 μL extractant ([OMIM]OTf), 1.25 g NaCl]. (E) Effects of ionic strength [Conditions: 200 μL serum sample, 110 μL extractant ([OMIM]OTf), vortex time of 1 min].

(e) *Water*.—Ultrapure water (18.2 megohm-cm) was prepared with a Millipore Milli-Q system (Bedford, MA, USA).

Preparation of Standard Solution

The VE stock standard solutions were prepared by dissolving the standards with ethanol at 1.00 mg/mL. All stock standard solutions were sealed and stored at −18°C. The concentrations of the stock VE standard solutions were stable during the two months. As the analytes were sensitive to light and oxygen, the whole experimental procedure was carried out in a dark place and the concentrations of the VE solutions were calibrated by spectrophotometry before use (24).

Chromatographic Conditions

(a) *Mobile phase*.—Methanol/acetonitrile (80+20, v/v).
(b) *Flow rate*.—0.7 mL/min.
(c) *Column temperature*.—30°C.
(d) *Injection volume*.—20 μL.
(e) *Wavelength of detection*.—290/327 nm.

Sample Preparation

For extraction, 200 μL of the thawed serum sample was pipetted into a 15 mL centrifuge tube and diluted with 5 mL water. After adding 110 μL of IL ([OMIM]OTf) and 1.25 g sodium

chloride, the mixture was vortexed at 3000 rpm for 1 min and centrifuged at 4000 rpm for 5 min. Fifty microliter of IL phase which was on the top of aqueous phase after centrifugation was taken and injected into the HPLC system for analysis.

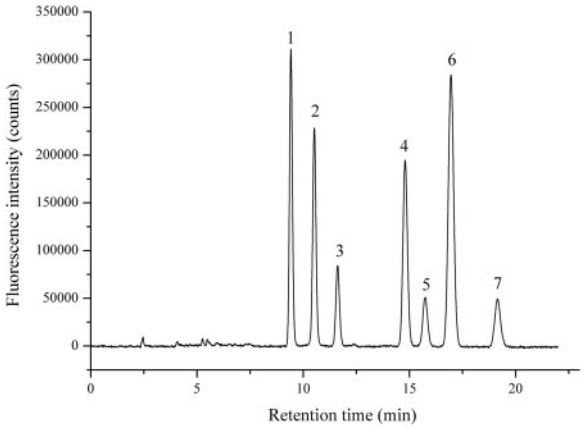

Figure 2. A typical fluorescence detection chromatogram of a 100 ng mL-1 of mixed VE isomers standards solution. (1) δ-T3, (2) γ-T3, (3) α-T3, (4) δ-T, (5) β-T3, (6) (β + γ)-T, and (7) α-T.

The sample preparation procedure can be completed within 10 min without using any organic solvent.

Results and Discussion

Optimization of Sample Preparation

Selection of the IL extractants.—

In the IL-VA-LLME process, the extractant is the most crucial factor that could affect the extraction efficiencies. The extractant should have good chromatographic performance, low water-solubility, and high extraction capability to the fat-soluble analytes. For long-chain $[CnMIM]^+$ ILs, the hydrophobic moiety of VE could be accommodated in the hydrophobic domain of the alkyl side chains so that the hydrophobic interaction between ILs and the analytes becomes stronger, and their solubility in the IL phase is also improved (23). At the same time, these ILs should be immiscible with water. Therefore, in this work, three ILs, [OMIM]PF_6, [OMIM]NTf_2, and [OMIM]OTf, were evaluated for their extraction efficiencies of eight VE isomers. The extraction efficiency, which is represented in Figure 1A, reached its highest when using [OMIM]OTf as extractant. As a result, [OMIM]OTf was selected as the extractant for the extraction of eight VE isomers.

Table 1. Linear regression equations, LODs, and LOQs of the method

Analyte	Linear ranges, ng/mL	Linear regression equations	Correlation coefficients (r)	LOD, ng/mL	LOQ, ng/mL
δ-T3	6.25-375	$y = 1.15 \times 10^2 x - 2.98 \times 10^2$	0.9993	1.16	3.85
γ-T3	6.25-375	$y = 7.91 \times 10^1 x + 1.62 \times 10^3$	0.9995	1.73	5.77
α-T3	12.5-375	$y = 5.35 \times 10^1 x + 2.78 \times 10^2$	0.9997	3.11	10.4
δ-T	6.25-375	$y = 9.00 \times 10^1 x + 6.54 \times 10^2$	0.9997	1.59	5.30
β-T3	12.5-375	$y = 2.81 \times 10^1 x + 8.51 \times 10^2$	0.9998	3.44	11.5
(β+γ)-T	$125\text{-}7.50 \times 10^3$	$y = 1.80 \times 10^2 x + 4.22 \times 10^3$	0.9996	0.857	2.86
α-T	$625\text{-}3.75 \times 10^4$	$y = 3.80 \times 10^1 x - 5.34 \times 10^3$	0.9992	4.16	13.9

Table 2. Recoveries and precisions of the method ($n = 6$)

Analyte	Background, ng/mL	Spiked, ng/mL	Found, ng/mL	Recovery, %	RSD, %	
					Intra-day	Inter-day
δ-T3	ND	50.0	51.5	103	6.301	2.24
		100	95.2	95.2	2.85	2.03
		150	148	98.7	2.74	2.71
γ-T3	17.2	50.0	64.9	95.4	8.78	13.0
		100	103.4	86.2	5.39	6.01
		150	159	94.5	2.41	1.75
α-T3	14.7	50.0	60.8	92.2	6.43	7.62
		100	94.8	80.1	5.38	9.18
		150	163	98.9	2.57	3.05
δ-T	26.1	50.0	74.5	96.8	4.69	4.02
		100	115.6	89.5	2.24	2.80
		150	173	97.9	2.97	2.90
β-T3	18.1	50.0	61.5	86.8	12.5	11.2
		100	99.3	81.2	3.61	5.77
		150	156	91.9	2.14	2.25
(β+γ)-T	892	1000	1920	103	2.05	4.31
		2000	2858	98.3	2.16	3.23
		3000	3850	98.6	1.61	1.69
α-T	10 700	5000	15 600	98.0	2.35	4.37
		10 000	20 460	97.6	2.30	2.34
		15 000	25 300	97.3	1.68	1.93

Selection of dispersive solvents.—
Adequate dispersive solvents can assist dispersion of extractant in sample solutions and increase the contact area between two phases. Three dispersants, i.e., acetonitrile, acetone, and ethanol were compared. As seen in Figure 1B, satisfactory results were obtained without using any dispersive solvents. This is possibly because these organic dispersants will dissolve parts of analytes, leading to decreased extraction efficiency. Therefore, further experiments were performed without addition of dispersive solvents.

Volume of IL extractant.—
Extraction efficiencies for VE extraction with different volumes of IL (80–130 μL) were compared. The experimental results showed that, IL volume <100 μL made it difficult to collect IL phase for HPLC analysis. But the highest extraction efficiencies could be obtained when the volume of IL was 110 μL (Figure 1C). Therefore, 110 μL [OMIM]OTf was used in the following study.

Vortex extraction time.—
The influence of extraction time (0.5, 1.0, 1.5, 2.0 min) on the extraction efficiencies was investigated. As shown in Figure 1D, the extraction efficiencies of [OMIM]OTf on VE improved when the vortex time increased from 0.5 to 1 min. As the vortex time continued to increase, the extraction efficiencies didn't increase any more. Thus, the optimized vortex extraction time was set at 1 min.

Salt addition.—
Addition of salt can potentially decrease the solubility of the analytes in the aqueous phase and enhance their partitioning into the IL phase. What's more, addition of salt can increase the density of the sample solution and make the IL drop float on the top of aqueous phase, facilitating its collection. The protein in serum sample can also be precipitated and form a dense membrane when adding an appropriate amount of salt. On the other hand, excessive salt may reduce the mass transfer process and enhance the solubility of ILs in the aqueous phase and decrease the extraction efficiency. The effect of NaCl addition on the recoveries of VE isomers was studied in the range of 0–2 g NaCl. The results showed that the IL phase could hardly be collected when the NaCl amount was less than 0.75 g. The extraction efficiencies of [OMIM]OTf on VE isomers increased when the NaCl amount increased from 1 to 1.25 g and the best results were achieved when the NaCl amount was 1.25 g (Figure 1E). Therefore, 1.25 g NaCl was added to the diluted serum samples for further study.

Optimization of the HPLC conditions

The maximum excitation and emission wavelengths of VE isomers were obtained by FLD scanning. Among the 8 VE isomers, α-T3, β-T3, and α-T show relatively lower fluorescence signal at the same concentration. Thus, the detection for VE isomers was operated at these three isomers' maximum excitation wavelength (290 nm) and emission wavelength (327 nm).

For better chromatographic separation of VE isomers, the composition of the mobile phase was investigated. B- and γ-T are positional isomers and could not be separated on conventional reversed-phase-HPLC columns. Therefore, these two VE isomers appeared as one peak in this work. When the mobile phase was methanol, (β + γ)-T and β-T3 could not be separated. Addition of acetonitrile could improve the separation of the VE isomers. The resolution of (β + γ)-T and β-T3 was significantly

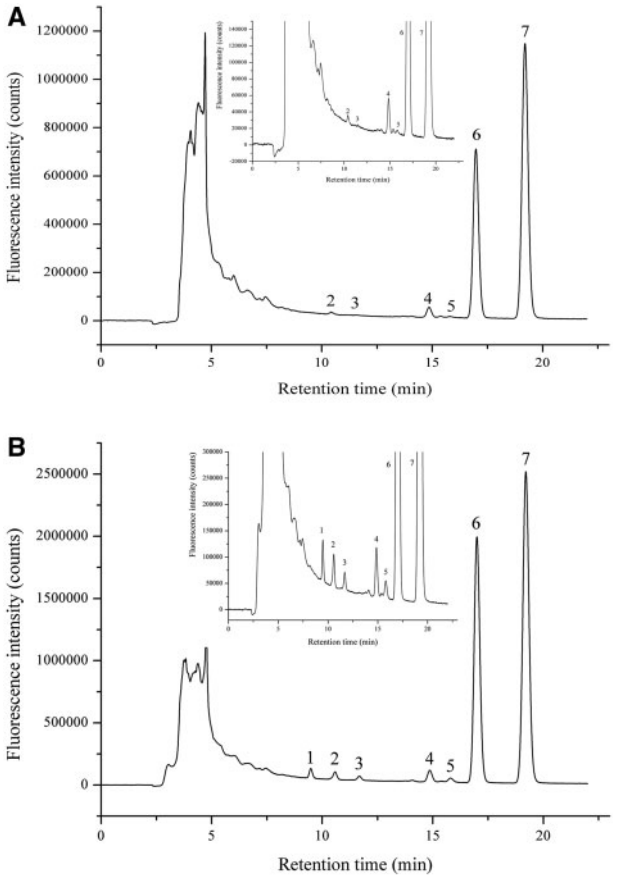

Figure 3. Chromatograms of a serum sample (A) and the sample spiked with middle level of standard solution (B). (1) δ-T3, (2) γ-T3, (3) α-T3, (4) δ-T, (5) β-T3, (6) (β + γ)-T, and (7) α-T.

improved when increasing the proportion of acetonitrile, whereas the resolution of δ-T and β-T3 became worse. When the proportion of acetonitrile was 20%, peak resolutions among (β + γ)-T, β-T3, and δ-T were >1.5. Therefore, the mobile phase was set as methanol and acetonitrile (80+20, v/v).

The resolution, retention time, and peak area of the analytes were also affected by the flow rate and column temperature. The flow rate of the mobile phase was optimized and the results indicated that a flow rate of 0.7 mL/min was appropriate for the separation of eight VE isomers within 20 min. The column temperature was also investigated at 20–40°C, and 30°C was optimal for all the analytes' separation. Figure 2 shows a typical chromatogram of a 100 ng mL^{-1} of mixed VE isomers standard solution under the optimal HPLC conditions.

The method performance

In the IL-VA-LLME procedure, although the IL is almost immiscible with water, a small proportion of diluted sample solution will be dissolved in the IL phase, which makes it hard to work out the accurate volume of extractant. Therefore, the working curves were used for the calibration to eliminate the error. As shown in Table 1, the correlation coefficients (*r*) were between 0.9992 and 0.9998. The limits of detection (LODs; S/N = 3) were in the ranges of 0.857–4.16 ng/mL. Serum samples were spiked with mixed standard solutions at three levels before sample preparation for evaluating the accuracy of the proposed method. The precision was obtained from the spiked samples at

Table 3. Comparison of the reported methods with the proposed method

Analytes	Sample	Sample preparation methods	Devices required in extraction procedure	Cost of per extraction procedure (RMB)	Detection techniques	Organic solvent consumed in sample preparation, μL	LOD, ng/mL	Linear range, ng/mL	Recoveries	Ref.
VA, 25(OH)D_3, α-T	100 μL plasma	PP: ethanol; SPE: HLB μElution plate	Centrifuge, nitrogen evaporator, vortex mixer, ultrasonic cleaner, SPE device	6.8	LC-MS/MS	1520	10.03 (VA), 1.20 (25(OH)D_3), 0.04 (α-T)	140-14 320 (VA), 1.80-180.29 (25(OH)D_3), 6.03-602.99 (α-T)	80-120%	[26]
25(OH)D_2, 25(OH)D_3, 3-epi-25(OH)D_3, VA, α-T	100 μL serum	PP: methanol; LLE: hexane	Centrifuge, nitrogen evaporator, vortex mixer, ultrasonic cleaner	1.9	LC-MS/MS	1950	0.660 (25(OH)D_2), 0.761 (3-epi-25(OH)D_3), 0.681 (25(OH)D_3), 28.6 (VA), 431 (α-T)	1.65-66 (25(OH)D_2), 1.60-64 (3-epi-25(OH)D_3), 1.60-80 (25(OH)D_3), 28.6-1144 (VA), 1724-30 170 (α-T)	87-112.9%	[6]
VA, α-T, β-T, γ-T, δ-T, α-T3, β-T3, γ-T3, δ-T3	100 μL serum	PP: ethanol; LLE: hexane	Centrifuge, nitrogen evaporator, vortex mixer, ultrasonic cleaner	1.7	HPLC-DAD	1600	1-2	25-50 000 (α-T); 25-10 000 ((β+γ)-T); 10-5000 (VA); 25-2500 (δ-T, α-T3, β-T3, γ-T3, δ-T3)	86.3-110 %	[9]
α-T, β-T, γ-T, δ-T, α-T3, δ-T3, γ-T3, 25(OH)D_2, 25(OH)D_3, VK_1, lutein, zeaxanthin, β-cryptoxanthin, retinal	200 μL plasma	PP: isopropanol; SLE: heptane	Centrifuge, vortex mixer, Extrahera robot (SLE automatic device)	18.7	UHPSFC-MS/MS	5600	–	20-1400 (lutein, zeaxanthin, α-T3, γ-T3, δ-T3, β-T, γ-T,δ-T, 25(OH)D_2, 25(OH)D_3); 0.2 – 14 (VK_1), 1600-11 2000 (α-T)	85-115%	[27]
α-T, β-T, γ-T, δ-T, α-T3, β-T3, γ-T3, δ-T3	200 μL serum	IL-LLME: [omim]OTf	Centrifuge, vortex mixer	1.1	HPLC-FLD	0	0.857- 4.16	625-37 500 (α-T); 125-7500 ((β+γ)-T); 6.25-375 (δ-T, β-T3, γ-T3, δ-T3), 12.5-375 (α-T3)	80.1-103%	This work

three levels within a day (intra-day RSD) and on 6 successive days (inter-day RSD). As seen in Table 2, the mean recoveries ranged from 80.1–103%. The intra- and inter-day RSDs for eight VE isomers in serum samples were 1.61–12.5% and 1.69–13.0%, respectively. Figure 3 presents the typical chromatograms of a sample and the sample spiked with mixed standard solutions.

Application of the method

Under optimized conditions, the established method was applied to the determination of eight VE isomers in 44 human serum samples. The ranges of each analyte were 5260–33 500 ng/mL for α-T, 420–3460 ng/mL for (β + γ)-T, 8.53–459 ng/mL for δ-T, not detected–73.7 ng/mL for α-T3, not detected–117 ng/mL for β-T3, not detected–78.2 ng/mL for γ-T3, and not detected–7.21 ng/mL for δ-T3. The concentrations of the analytes detected by use of the method were within the range of reported results in other published HPLC methods (9, 25).

Comparison of reported methods with the proposed method

Table 3 shows a comparison of the proposed method with some reported chromatographic methods for the analysis of VE and other FSVs in human serum or plasma samples. Albahrani et al. (6) reported a simultaneous method for the determination of vitamins A, D, and E in human serum using liquid chromatography (LC) tandem mass spectrometry (MS/MS). The sample preparation includes protein precipitation and LLE procedure, which is commonly used for FSVs extraction. The LODs were 0.660–431 ng/mL and the recoveries were 87–112.9%. Our group (9) developed a HPLC-diode-array detection (DAD) method for simultaneous determination of vitamin A and 8 VE isomers in human serum in 2015. Ethanol and *n*-hexane were used for precipitating proteins and extracting targeted analytes from serum samples, respectively. The extractant was then evaporated under nitrogen flow and the residue was dissolved with methanol for HPLC analysis. Mean recoveries of the method were 86.3–110%, with RSDs less than 14.9%. Zhang et al. (26) established a LC-MS/MS method for the simultaneous determination of vitamin A, 25(OH)D_3, and α-T in plasma samples. Samples were precipitated by ethanol and then extracted with the HLB μElution SPE plate. The recoveries for all analytes ranged from 80–120%, and the RSDs were less than 10.0%. Petruzziello et al. (27) developed a quantitative method for FSVs in human plasma using a supercritical fluid chromatography-mass spectrometry (SFC-MS). Automated supported liquid extraction was used and optimized in the sample preparation procedure. Recoveries of the developed method were from 85–115%, and the RSDs were 2.7–13.8%, respectively. Compared with these reported chromatographic methods, our method has the merits of being simple, fast, environment-friendly, and cheap without using any organic solvents and specific devices in sample preparation. The LLME procedure can be completed within 10 min, and the IL extractant is cheap for routine analysis. The enrichment factor of this IL-VA-LLME method was 1.82, which has the same magnitude as LLE traditional methods reported in the literature (4.5 and 0.875 when using *n*-hexane as solvent) (9, 25).

Moreover, HPLC-FLD was chosen for the detection of VE targets. The cost of HPLC-FLD is significantly lower than LC-MS/MS and SFC-MS, making it suitable for routine analysis in ordinary laboratories. Also, our method has the similar linear ranges, sensitivities, recoveries, and precisions with reported methods.

Conclusions

A novel and green IL-VA-LLME technique combined with HPLC method was proposed for the analysis of eight VE isomers. In this method, the IL was directly injected into the diluted serum samples without protein precipitation. No hazardous organic solvent was used in the whole sample preparation process. Under the optimal conditions, this IL-VA-LLME method is simple, rapid, environment-friendly, and exhibits good linearity, sensitivity, recoveries, and precisions for the simultaneous determination of eight VE isomers in human serum samples. However, there is still work to be done to improve this method in the future. For example, the enrichment factor should be improved for the accurate detection of some VE isomers at trace levels, such as δ-T3 and β-T3. What's more, the performances of the proposed method in other biological fluids and food samples should be investigated.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

References

1. Fanali, C., D'Orazio, G., Fanali, S., & Gentili, A. (2017) *Trac-Trends Anal. Chem.* **87**, 82–97. doi:10.1016/j.trac.2016.12.001
2. Albahrani, A.A., & Greaves, R.F. (2016) *Clin. Biochem. Rev.* **37**, 27–47
3. Goncalves, A., & Amiot, M. (2017) *Curr. Opin. Clin. Nutr.* **20**, 492–497. doi:10.1097/MCO.0000000000000412
4. Wade, L., Nadeem, N., Young, I.S., Woodside, J.V., McGinty, A., McMaster, C., & McEneny, J. (2013) *Atherosclerosis* **226**, 392–397. doi:10.1016/j.atherosclerosis.2012.11.032
5. Lu, D., Yang, Y., Li, Y., & Sun, C. (2014) *CPA* **11**, 66–78. doi:10.2174/1573412910666140630170055
6. Albahrani, A.A., Rotarou, V., Roche, P.J., & Greaves, R.F. (2016) *J. Steroid Biochem. Mol. Biol.* **159**, 41–53. doi:10.1016/j.jsbmb.2016.02.019
7. Mata-Granados, J.M., Luque De Castro, M.D., & Quesada Gomez, J.M. (2008) *Clin. Biochem.* **41**, 676–680. doi:10.1016/j.clinbiochem.2008.02.003
8. Lazzarino, G., Longo, S., Amorini, A.M., Di Pietro, V., D., Urso, S., Lazzarino, G., Belli, A., & Tavazzi, B. (2017) *J. Chromatogr. A* **1527**, 43–52. doi:10.1016/j.chroma.2017.10.053
9. Yang, Y., Lu, D., Zhang, J., Li, Y., Zheng, B., & Sun, C. (2015) *Chromatographia* **78**, 1359–1366. doi:10.1007/s10337-015-2951-6
10. Milagros, D.M., Bustamante-Rangel, M., Sierra-Manzano, S., Verdugo-Jara, M., & Carabias-Martinez, R. (2009) *J. Sep. Sci.* **32**, 1430–1436. doi:10.1002/jssc.200800707
11. Bustamante-Rangel, M., Delgado-Zamarreño, M.M., Sánchez-Pérez, A., & Carabias-Martínez, R. (2007) *Anal. Chim. Acta* **587**, 216–221. doi:10.1016/j.aca.2007.01.049
12. Jiao, Z., Jiao, S., Guo, Z., Chen, H., Zhang, N., & Huang, W. (2017) *Food Anal. Methods* **10**, 820–826. doi:10.1007/s12161-016-0633-0
13. Momenbeik, F., & Yazdani, E. (2015) *J. AOAC Int.* **98**, 336–344. doi:10.5740/jaoacint.13-403

14. Goudarzi, N., Farsimadan, S., Arab, C.M., & Bagherian, G.A. (2015) *J. Sep. Sci.* **38**, 3254–3261. doi:10.1002/jssc.201500522
15. Viñas, P., Bravo-Bravo, M., López-García, I., & Hernández-Córdoba, M. (2013) *Talanta* **103**, 166–171. doi:10.1016/j.talanta.2012.10.027
16. Saber-Tehrani, M., Aberoomand-Azar, P., & Raziee, M. (2014) *J. Liq. Chromatogr. R. T* **37**, 404–419. doi:10.1080/10826076.2012.745144
17. Armenta, S., Garrigues, S., & de la Guardia, M. (2015) *Trac-Trends Anal. Chem.* **71**, 2–8. doi:10.1016/j.trac.2014.12.011
18. Martinis, E.M., Berton, P., & Wuilloud, R.G. (2014) *Trac-Trends Anal. Chem.* **60**, 54–70. doi:10.1016/j.trac.2014.04.012
19. Ventura, S.P.M., e Silva, F.A., Quental, M.V., Mondal, D., Freire, M.G., & Coutinho, J.A.P. (2017) *Chem. Rev.* **117**, 6984–7052. doi:10.1021/acs.chemrev.6b00550
20. Płotka-Wasylka, J., Rutkowska, M., Owczarek, K., Tobiszewski, M., & Namieśnik, J. (2017) *Trac-Trends Anal. Chem.* **91**, 12–25. doi:10.1016/j.trac.2017.03.006
21. Qin, L., Zhang, J., Cheng, H., Chen, L., Qi, Z., & Yuan, W. (2015) *ACS Sustainable Chem. Eng.* **4**, 583–590. doi:10.1021/acssuschemeng.5b01330
22. Liang, R., Bao, Z., Su, B., Xing, H., Yang, Q., Yang, Y., & Ren, Q. (2013) *J. Agric. Food Chem.* **61**, 3479–3487. doi:10.1021/jf305558b
23. Yang, Q., Xing, H., Cao, Y., Su, B., Yang, Y., & Ren, Q. (2009) *Ind. Eng. Chem. Res.* **48**, 6417–6422. doi:10.1021/ie801847e
24. Franke, A.A., Murphy, S.P., Lacey, R., & Custer, L.J. (2007) *J. Agric. Food Chem.* **55**, 769–778. doi:10.1021/jf0623844
25. Siluk, D., Oliveira, R.V., Esther-Rodriguez-Rosas, M., Ling, S., Bos, A., Ferrucci, L., & Wainer, I.W. (2007) *J. Pharm. Biomed. Anal.* **44**, 1001–1007. doi:10.1016/j.jpba.2007.03.033
26. Zhang, H., Quan, L., Pei, P., Lin, Y., Feng, C., Guan, H., Wang, F., Zhang, T., Wu, J., & Huo, J. (2018) *J. Chromatogr. B* **1079**, 1–8. doi:10.1016/j.jchromb.2017.12.017
27. Petruzziello, F., Grand-Guillaume Perrenoud, A., Thorimbert, A., Fogwill, M., & Rezzi, S. (2017) *Anal. Chem.* **89**, 7615–7622. doi:10.1021/acs.analchem.7b01476