Hindawi
Journal of Food Quality
Volume 2022, Article ID 8431662, 9 pages
https://doi.org/10.1155/2022/8431662

Research Article

Simultaneous Determination of Retinols and Tocols in Egg and Milk Products Based on RP-HPLC Linked with Fluorescent and Photodiode Array

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Received 31 March 2022; Accepted 14 June 2022; Published 2 August 2022

Academic Editor: Hong Yan Zou

In this paper, we built a method of verifying the 4 retinols of vitamin A and 8 tocopherol isomers of vitamin E in the food industry based on RP-HPLC-PDA-FLR. The effect of laboratory light conditions on the target components was considered for the first time, and it was found that the loss rate of the target components was the smallest in the case of a white laboratory bench with red or LED light in the dark room. There was no finding of extraction recoveries of the target components with a significant difference under different saponification conditions. Meanwhile, it was found that using ethyl acetate/n-hexane mixed solvent as the extraction solvent could ensure the effective extraction of the target components. Finally, baseline separation of 12 components was achieved within 45 min using the C_{30} column. With the help of methodological verification, we found that the recovery rate ranged from 76.45% to 93.52%, and RSD was between 0.19% and 12.99%; the Limit of Detection minimum value was 0.01 mg/100 g and the Limit of Quantitation minimum value was 0.03 mg/100 g. The detection method was successfully applied to the distribution detection of 4 kinds of retinols and 8 kinds of vitamin E in egg and dairy products and provided technical support for the accurate nutritional evaluation of vitamin A and vitamin E.

1. Introduction

Vitamin A, also known as retinol, refers to all isoprene-like compounds with full *trans*-retinol biological activity, because it has four conjugated double bonds on the side chain; theoretically, there are 16 kinds of *cis*- and *trans*-isomers; due to the stereoteric hindrance effect, the geometric isomers that exist in nature mainly include all-*trans* retinol, 9-*cis* retinol, 13-*cis* retinol, and 11-*cis* retinol. Among the isomers, the biovalence of the all-*trans* formula is the highest (measured at 100%), followed by that of the 13-*cis* (75%), that of the 9-*cis* is 23%, and that of the 11-*cis* is 24% [1–3]. Vitamin A is one of the 13 essential vitamins in the human body; as a fat-soluble antioxidant, it can maintain vision and promote bone growth in the human body; it is mainly distributed in animal-derived foods like animal livers, egg yolk, and cream, and the main form in food is retinol and its esters [4, 5]. Vitamin E is mainly derived from various plant seeds, and it mainly has 8 kinds of forms: 4 kinds of tocopherols (α-, β-, γ-, and δ-tocopherol) and 4 kinds of tocotrienols (α-, β-, γ-, and δ-tocotrienol). Studies had shown that all forms of tocopherols had similar antioxidant activities, which was essential for the optimal functioning of reproductive, muscular, neurological, and immune systems in various animals [4, 5]. The biological activities of β-, γ-, and δ-tocopherol and α- and β-tocotrienol are 50%, 10%, 3%, 50%, and 5% of α-tocopherol [1], respectively; there is no relevant research data on the α-tocopherol activity equivalent of γ-tocotrienol and δ-tocotrienol, but studies had shown that γ- and δ-tocotrienol had a preventive effect on the prevention of colorectal cancer, gastric cancer, prostate cancer, breast cancer, and other diseases [6–9]; they also

could reduce cholesterol, alleviate cardiovascular disease, and improve nonalcoholic fatty liver and other diseases [10–12].

At present, the methods of isolating and detecting vitamin A or E in food mainly include the liquid chromatography (HPLC) method [13, 14], liquid chromatography-mass spectrometry (HPLC-MS) method [15, 16], and gas chromatography (GC) method [17, 18]. When determining vitamin E, the GC method often requires the introduction of chemical groups suitable for determining the type of detection to convert analytes through the derivatization process, and cholesterol in animal-derived foods will interfere with the determination of tocopherols. Compared with the GC method, the pretreatment process of the LC method is relatively simple and is more suitable for the simultaneous detection of retinols and tocopherols. Normal-phase liquid chromatography (NP-HPLC) method [19] and reversed-phase liquid chromatography (RP-HPLC) method [13, 15] can both be used for the analysis of vitamin E; NP-HPLC is separated by polar adsorption of compounds; it is very suitable for the separation of retinol isomers and tocopherol subtypes, but it uses n-hexane or other volatile solvents as mobile phases; the system equilibrium time is long and the reproducibility is poor. The RP-HPLC method often uses MeOH or ACN as the mobile phase, when using the traditional ODS (C_{18}) column to separate tocopherols; it is difficult to effectively separate β-tocopherol and γ-tocopherol and β-tocotrienol and γ-tocotrienol [20–22]. Separation of these two isomers requires pentafluorophenyl columns (PFP), chiral chemical columns, or long-chain alkyl-bonded silica columns (C_{30}) [23–25]. At present, most of the separation and detection of retinol *cis*- and *trans*-isomers in food focus on all-*trans* retinol and 13-*cis* retinol, and the simultaneous separation of 4 retinol *cis*- and *trans*-isomers has not been reported.

The isolation and determination of 4 retinols and 8 tocopherols in food can be better used for food nutrition evaluation, which is of great significance for the implementation of accurate nutrition assessment of residents. In this study, we established a quantitative method for the simultaneous determination of 4 retinol *cis*- and *trans*-isomers and 8 tocopherol isoforms in food by RP-HPLC. The chromatographic separation conditions and the sample pretreatment methods were optimized for better performance.

2. Materials and Methods

2.1. Chemicals and Materials. All-*trans* retinol (CAS no. 68-26-8), 9-*cis* retinol (CAS no. 68-26-8), 11-*cis* retinol (CAS no. 68-26-8), and 13-*cis* retinol (CAS no. 68-26-8) were purchased from TRC Company; α-tocopherol (CAS no. 10191-41-0), β-tocopherol (CAS no. 148-03-8), γ-tocopherol (CAS no. 54-28-4), δ-tocopherol (CAS no. 119-13-1), α-tocotrienol (CAS no. 1721-51-3), β-tocotrienol (CAS no. 490-23-3), γ-tocotrienol (CAS no. 14101-61-2), and δ-tocotrienol (CAS no. 25612-59-3) were purchased from Chromadex, USA, with purity more than 96%. Absolute ethanol (EtOH), methanol (MeOH), ethyl acetate, and n-hexane (HPLC grade) were purchased from Fisher Company of the United States; ascorbic acid, 2,6-di-tert-butyl-p-cresol (BHT), and potassium hydroxide (KOH) (analytical purity) were purchased from Aladdin company; food products were purchased from a supermarket in Hangzhou.

2.2. Instrument Conditions. High-performance liquid chromatography (Waters Company, USA) was used (equipped with PDA and FLR).

Consider the following: column: C_{30} column (150×4.6 mm, 3 μm); column temperature: 28°C; mobile phase: phase A, water; phase B, MeOH, gradient elution (see Table 1); flow rate: 0.8 mL/min; detection wavelength: PDA = 325 nm (retinol); FLR: Ex = 294 nm, Em = 328 nm (vitamin E); sample volume: 20 μL.

2.3. Experimental Methods

2.3.1. Standard Solution Preparation. After the standard stock solution is prepared by dissolving in absolute ethanol, the absorbance of each component is determined according to the wavelength of Table 2, and the corrected concentration of each standard stock solution is calculated according to the specific absorbance coefficient $E^{1\%}_{1\,cm}$. A certain amount of standard stock solution was pipetted separately to prepare the intermediate solution, of which 4 retinol concentrations were 10.0 μg/mL, and 8 tocopherol concentrations were 50 μg/mL for subsequent use.

2.3.2. Sample Preparation

Experimental Environment. The pretreatment process of this study was carried out under the white experimental table of red light (illuminance 20LUX) or white LED lamp (illuminance 70LUX) in the dark room to reduce the loss of the target compound from the influence of light factors.

Saponification. After a certain number of samples had been reduced, crushed, and homogenized as required, a homogenized sample was weighed at 0.5 g and mixed in a 50 mL centrifuge tube, 0.4 g of ascorbic acid was added, 6 mL of 0.1% BHT EtOH solution was vortexed and mixed for 30 s, and then 3 mL of KOH solution was added in it, vortexed, mixed well, and shaken at 80°C ± 2°C for 30 min (or vortexed for 16 h ± 2 h at 25°C ± 5°C).

Extraction. 6 mL of 40% ethanol aqueous solution and 5 mL of water were added to the saponification solution described above, and then 20 mL of ethyl acetate/n-hexane mixture (1/1 = v/v) was added and extracted for 10 min, and the upper layer of solution was transferred to another 50 mL centrifuge tube after high-speed centrifugation. Another 10 mL of ethyl acetate/n-hexane mixture (1/1 = v/v) was added to the original centrifuge tube and extracted for 10 min, and then the upper organic phase was merged. Water was added to 50 mL in a centrifuge tube incorporating the upper organic

Table 1: Mobile phase gradient elution conditions.

T (min)	A (%)	B (%)
0.0	17	83
15.0	17	83
15.5	7	93
22.0	7	93
32.0	5	95
37.0	5	95
37.5	17	83
45.0	17	83

phase, followed by centrifugation after slight shaking, and the upper organic phase was transferred and blow-dried with nitrogen. Finally, made up to 5 mL by MeOH/Water(4/1,v/v) and passed through the microporous filter membrane, and took the filtrate to be determined.

2.3.3. Methodological Verification. The established HPLC-PDA-FLR method is verified in terms of specificity, linear range, the Limit of Detection, the Limit of Quantitation, precision, and accuracy. The linearity of each analyte is assessed by calculating the slope, intercept, and correlation coefficients of each component within a certain concentration range; the Limit of Detection and the Limit of Quantitation are determined by low concentration level standardization experiments and determined as the lowest concentrations that produce chromatographic peaks, with a signal-to-noise ratio (S/N) of 3 times and 10 times as Limit of Detection and Limit of Quantitation, respectively; and the accuracy and precision of this research method are tested by three levels of different concentration standardization experiments in infant formula. In addition, it is validated using infant/adult nutrition formulas SRM 1849a and SRM 1869 reference standards provided by the National Institute of Standards and Technology (NIST).

3. Results and Discussion

3.1. Chromatographic Conditions

3.1.1. Selection of Column and Mobile Phase. To separate and detect 4 kinds of retinols and 8 kinds of tocopherols at the same time, it is necessary to select a suitable column and optimize the mobile phase at the same time. As far as the separation of retinol is concerned, the RP-LC separation method of the *trans*- and the *cis*-isomers was less documented, and the authors had used polysaccharide derivatives to modify chiral silicone columns (OD-5H column) to separate α-tocopherol and other tocopherols and retinol isomers in infant formula [22] and obtained an ideal analysis result, but its defect was that it was impossible to separate 11-*cis* retinol and 9-*cis* retinol at the same time; what is more, the C_{18} column cannot be used for the separation of *cis*- and *trans*-retinol, β and γ-tocopherol, and β and γ-tocotrienol isomers. So we mainly considered C_{30} column and PFP column as alternative columns. Compared with the traditional column, the PFP column has a strong separation ability for easily polarizable substances such as aromatic rings and heterocyclic compounds and has a better separation effect on isomers, and it was found that it could effectively achieve separation and detection of vitamin E isomers in a short period [26], but, in this study, we cannot effectively separate 9-*cis* retinol and 13-*cis* retinol by optimizing mobile phase conditions. What is more, it was found that the C_{30} column can achieve the separation of vitamin E isomers, and, based on this study, it was found that when the proportion of the aqueous phase in the mobile phase reaches 17%, four retinol isomers can be separated. Finally, the full separation and detection of retinol and vitamin E 12 target substances can be achieved within 50 minutes by gradient elution, so the C_{30} column was selected as the conditioned column for subsequent analysis.

3.1.2. Selection of Detector. The 4 kinds of retinols have a very high UV absorption characteristic spectrum, while vitamin E has strong FLR characteristics. Therefore, we used PDA and FLD detection to detect retinol and vitamin E, respectively, and the two detectors were used in series, and the standard solution separation chromatogram of retinol and vitamin E is shown in Figure 1.

3.2. Sample Pretreatment Conditions

3.2.1. Laboratory Environmental Conditions. Because of the unstable feature of retinol and vitamin E when exposed to light, heat, and oxygen, and under the light conditions, retinol was prone to degradation or isomerization [1]. Thus, sunlight should be avoided during the experiment, the pretreatment process should be completed in a dim environment, and the solution should be stored in glassware with a low photochemical rate as much as possible. During the actual sample extraction process, it is impossible to perform in a completely light-protected environment, so this study investigated the effects of different light conditions on the target components.

The standard solution of 12 components added with BHT antioxidants was separately aliquoted in a 1 mL transparent injection flask and three experimental environments (south-facing sun laboratory, north back sun laboratory, and windowless dark room) were chosen as different light sources, and each was put for 4 hours (the storage time covered the entire time for the pretreatment process), and the standard solution before storage was synchronized with the injection control; the loss rate is shown in Table 3, retinol was sensitive to ultraviolet light, and the loss rates of the four retinols were more than 75% when stored in the south-facing sun laboratory by the window. 11-*cis* retinol was the most unstable component, whether in the south-facing sun laboratory by the window or on the north side of the back sun laboratory against the window, and the loss rate was 100%. In addition, 8 tocopherols were relatively stable under the protection of the antioxidant BHT. Ultimately, our study found that, when using a red light (illuminance 20LUX) or a white LED lamp (illuminance 70LUX) in the darkroom with a white experimental table, the loss rate of 12 components is less than 4%.

Table 2: Standard storage solution correction parameters.

Name	Wavelength (nm)	$E^{1\%}_{1\,cm}$	Name	Wavelength (nm)	$E^{1\%}_{1\,cm}$
All-*trans* retinol	325	1830	γ-Tocopherol	298	91.4
9-*cis* retinol	323	1477	δ-Tocopherol	298	87.3
11-*cis* retinol	319	1220	α-Tocotrienol	292	86.0
13-*cis* retinol	328	1689	β-Tocotrienol	292	86.2
α-Tocopherol	292	75.8	γ-Tocotrienol	297	91.0
β-Tocopherol	296	89.4	δ-Tocotrienol	297	85.8

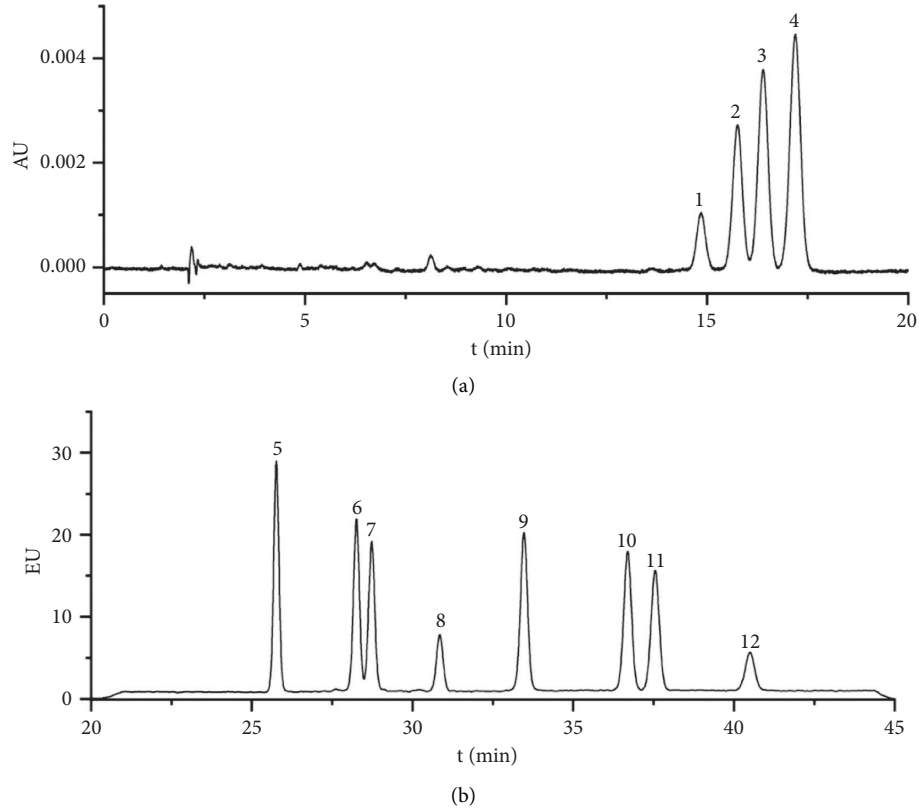

Figure 1: Standard solution chromatogram results. (a) UV = 325 nm; (b) FLR (Ex = 294 nm, Em = 328 nm) (1: 11-*cis* retinol; 2: 9-*cis* retinol; 3: 13-*cis* retinol; 4: all-*trans* retinol; 5: δ-tocotrienol; 6: γ-tocotrienol; 7: β-tocotrienol; 8: α-tocotrienol; 9: δ-tocopherol; 10: γ-tocopherol; 11: β-tocopherol; 12: α-tocopherol).

3.2.2. Saponification Conditions. Egg yolk powder is a powder obtained after a series of processes of fresh eggs as raw materials, which is a common health food and food additive on the market. Egg yolk powder is rich in various types of proteins, phospholipids, fatty acids, and so forth, with a strong emulsification function [27, 28]. Vitamins A and E are two main fat-soluble vitamins in food mainly combined with various types of lipid structures and coexist, so it is necessary to carry out a certain saponification treatment. As a common pretreatment method, the principle of saponification is to carry out an ester hydrolysis reaction under the catalytic action of alkali, to dissociate the target detection substance from the grease, which is conducive to the next step of enrichment extraction and detection. At present, the standard method for the determination of vitamins A, D, and E in food in China [29] recommends saponification conditions for 80°C constant temperature water bath oscillation for 30 min, and the saponification conditions reported in the literature were overnight cold saponification at room temperature (25°C for 15 hours in the dark room) [30, 31]. This study mainly compared the two saponification conditions above and used low background egg yolk powder as a matrix sample, through the standard recovery experiment to compare the different results of the two methods by statistical analysis, and the comparison chart is shown in Figure 2. The results showed that there was no statistical difference in the recovery rate of 12 target components under two saponification conditions in this study ($P > 0.05$), and the recovery rate of each target component was above 83.02%, basically meeting the testing requirements.

3.2.3. Extraction Reagent. China's determination standards for vitamins A, D, and E in the recommended liquid extraction extractant are petroleum ether/ether mixture [29],

Table 3: Loss rate of target components in different experimental conditions (%).

Towards	South			North			Dark room			
Placement	By the window	By the corridor countertop	By the corridor countertop	By the window	By the corridor countertop	By the corridor countertop	Red light	Red light	LED light	LED light
Illuminance (LUX)	1496–3060	565–710	565–710	1490–1800	230–310	230–310	20	20	70	70
Table color	Black	Black	White	Black	Black	White	Black	White	Black	White
9-*cis* retinol	78.48	37.47	18.90	82.43	5.89	2.11	4.97	3.48	6.28	1.78
11-*cis* retinol	100.00	52.11	34.31	100.00	18.29	4.52	15.00	3.48	15.00	2.56
13-*cis* retinol	80.04	42.73	27.65	78.02	7.99	4.91	12.40	2.16	10.14	1.06
All-*trans* retinol	76.74	28.26	18.10	73.83	4.25	1.28	3.96	2.16	3.96	1.96
α-Tocopherol	2.29	2.27	3.02	3.74	3.93	4.86	3.21	2.68	2.22	2.29
β-Tocopherol	2.71	2.06	2.31	2.48	2.26	2.48	2.36	2.61	2.68	3.12
γ-Tocopherol	2.35	2.00	2.14	2.36	2.40	2.79	3.46	2.96	2.72	3.19
δ-Tocopherol	2.62	1.85	2.50	1.92	1.63	2.81	2.09	2.63	2.28	2.21
α-Tocotrienol	2.84	2.33	3.22	3.55	1.99	4.72	3.12	3.96	3.13	3.77
β-Tocotrienol	2.18	2.46	2.52	2.59	2.36	3.10	1.66	2.81	2.41	2.79
γ-Tocotrienol	2.44	1.82	2.60	3.04	2.28	3.18	1.21	3.24	3.01	3.27
δ-Tocotrienol	2.27	2.04	2.12	2.76	2.30	2.57	4.46	2.67	2.19	2.27

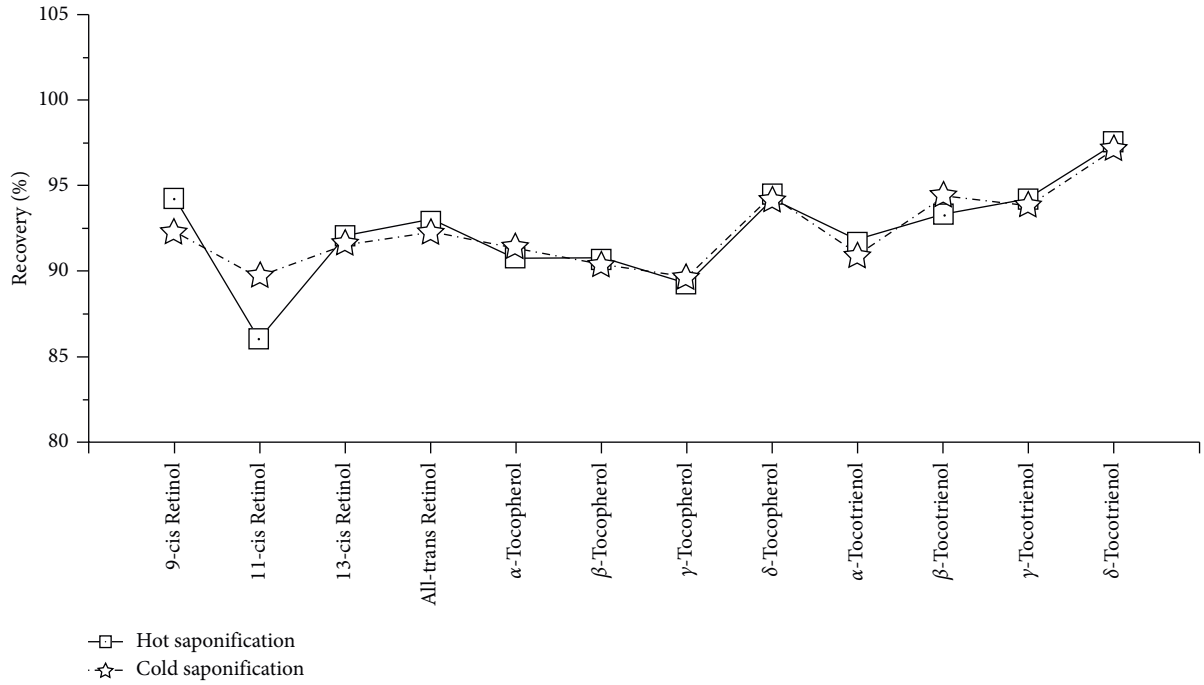

Figure 2: Recovery results for different saponification conditions.

but the extract has a special pungent odor and strong toxicity, which is not conducive to the experimenter's own protection. In recent years, some studies used n-hexane and other reagents instead of petroleum ether, ether, and other highly toxic reagents [13]. In this study, the extraction effects of six common extraction reagents (petroleum ether, ether, petroleum ether/ether, ethyl acetate, n-hexane, and ethyl acetate/n-hexane) were compared with the extraction effects of retinol and vitamin E, 6 samples were determined in parallel with each reagent, and the target components were extracted twice in turn to achieve a better enrichment effect, and the final experimental results are shown in Figure 3. The results showed that when ethyl acetate/n-hexane was used as the extraction reagent, the recovery rate of the target components was comparable to that of petroleum ether/ether mixture, and the experimental error was even smaller, so ethyl acetate/n-hexane was selected as the extractant for this study.

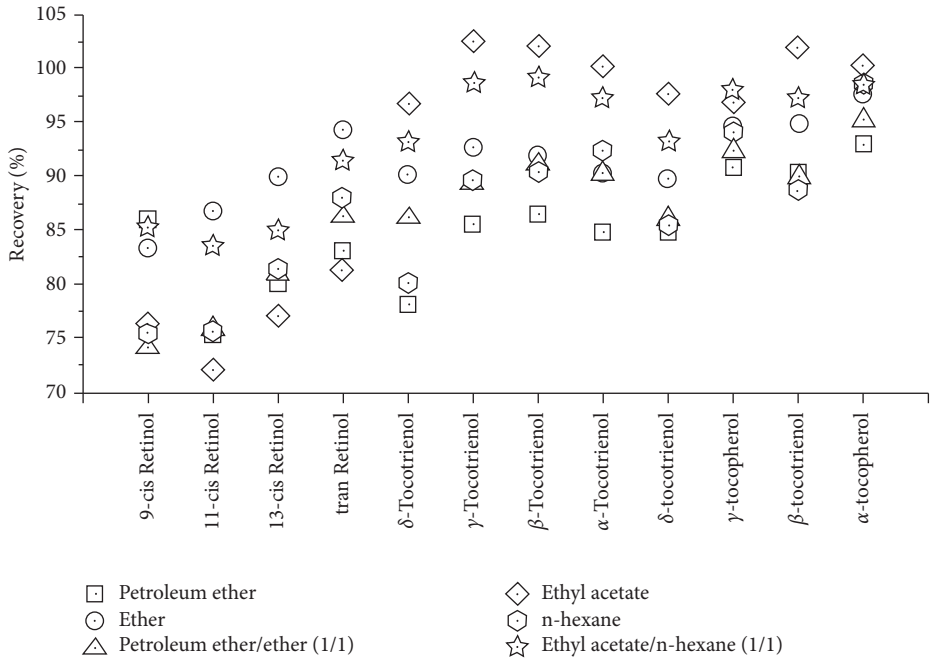

Figure 3: Results for different extraction reagents comparison.

3.3. Method Validation

3.3.1. Standard Curve Linear. The linearity calculation was based on the six increasing concentrations of each isomer. Ranged from 0.02 μg/mL to 0.50 μg/mL for retinols, and ranged from 0.10 μg/mL to 2.50 μg/mL for tocols. The standard curve was drawn with the concentration as the abscissa and the peak area as the ordinate, and the calibration curve and related coefficients are shown in Table 4.

3.3.2. Method Limit of Detection and Limit of Quantitation. Skimmed milk powder was selected as a blank background sample according to the method of sample preparation in 2.3.5, according to the signal-to-noise ratio (S/N) evaluation Limit of Detection and Limit of Quantitation, 3 times of the S/N is the method Limit of Detection value, and 10 times of the S/N is the method Limit of Quantitation value. The results showed that when the solid sample was weighed at 0.50 g and the volume was fixed to 5.00 mL; the Limits of Detection of 9-*cis* retinol, 11-*cis* retinol, 13-*cis* retinol, and all-*trans* retinol were 10 μg/100 g, and the Limits of Quantitation were 30 μg/100 g; and the Limits of Detection for the eight tocopherols were 0.05 mg/100 g and the Limits of Quantitation were 0.15 mg/100 g, respectively.

3.3.3. Method Precision and Accuracy. Blank background skimmed milk powder was selected as the standardized matrix; the standard recovery rate test was carried out at the three concentration levels of the lowest, intermediate, and highest points of the standard curve, respectively. Each concentration level was tested for 6 times, and the results were located in Table 5. The average recovery rate of low-level standardization was between 76.45% and 87.10%, RSD was between 3.06% and 12.99%, the intermediate concentration recovery was between 80.72% and 92.50%, RSD was between 0.19% and 8.44%, and the high concentration standard recovery was between 78.52% and 93.52%, and RSD was between 0.44% and 2.75%; and the results indicated that the method established had great reproducibility and precision.

3.3.4. Detection of Certificated reference Materials. This study also used the certificated reference materials SRM 1869 and SRM 1849a provided by the National Institute of Standards and Technology (NIST) to verify the method; each reference standard underwent three parallel replicates to ensure the accuracy of the results, and the specific results are detailed in Table 6. From the experimental results, it can be seen that 11-*cis* retinol, β-tocotrienol, and δ-tocotrienol were not detected in the two certificated reference materials; the all-*trans* retinol of the two certificated reference materials was low compared to the nominal retinol values but was comparable to the declared values when the other isomers were counted; α-tocopherol's measurements were within the reference range of the indicated values; the detection values of β-tocopherol, γ-tocopherol, and δ-tocopherol in SRM 1869 were basically the same as those indicated. The detection values of the two certificated reference materials indicated that the test results of this method were true and reliable, and the method was suitable for the separation and detection of retinol and vitamin E isomers in real samples.

3.4. Actual Sample Results. This study also analyzed and detected the retinol and vitamin E isomer content in 15 kinds of formula milk powders and 6 kinds of egg yolk

Table 4: Linear range for retinol and tocopherol isomers.

Name	Range (μg/mL)	Calibration curve	R^2
9-*cis* retinol	0.02~0.50	$Y = 432.3 + 211783.7X$	0.9999
11-*cis* retinol	0.02~0.50	$Y = 488.7 + 82274.3X$	1.0000
13-*cis* retinol	0.02~0.50	$Y = 157.8 + 285759.4X$	0.9999
All-*trans* retinol	0.02~0.50	$Y = 119.1 + 389663.3X$	1.0000
α-Tocopherol	0.10~2.50	$Y = -69853.9 + 1116543.5X$	0.9993
β-Tocopherol	0.10~2.50	$Y = -42411.4 + 2535083.8X$	0.9998
γ-Tocopherol	0.10~2.50	$Y = -60077.5 + 2782667.2X$	0.9997
δ-Tocopherol	0.10~2.50	$Y = 663.2 + 3344578.7X$	1.0000
α-Tocotrienol	0.10~2.50	$Y = -91398.5 + 1004661.7X$	0.9992
β-Tocotrienol	0.10~2.50	$Y = -46799 + 2424243.3X$	0.9996
γ-Tocotrienol	0.10~2.50	$Y = -65585.5 + 2760020.3X$	0.9995
δ-Tocotrienol	0.10~2.50	$Y = -4499.3 + 2834712.8X$	0.9999

Table 5: Method precision and accuracy of experimental results.

Name	Addition level 1		Addition level 2		Addition level 3	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
9-*cis* retinol	76.79	4.52	85.92	1.66	86.41	2.75
11-*cis* retinol	79.85	7.94	80.72	8.44	78.52	1.02
13-*cis* retinol	76.45	3.06	89.08	2.08	91.52	2.24
All-*trans* retinol	87.10	7.41	91.28	1.56	93.52	0.98
α-Tocopherol	76.56	2.62	88.46	3.31	90.07	0.44
β-Tocopherol	76.63	12.99	90.82	4.35	90.46	1.44
γ-Tocopherol	76.53	11.54	92.50	2.85	92.85	1.49
δ-Tocopherol	78.12	3.76	89.34	2.54	90.40	0.80
α-Tocotrienol	79.93	8.50	84.79	2.21	83.71	2.02
β-Tocotrienol	79.28	4.09	82.44	1.97	80.78	2.06
γ-Tocotrienol	80.73	4.22	84.53	1.92	83.68	1.99
δ-Tocotrienol	79.61	4.23	81.91	0.19	82.73	1.32

Table 6: Certificated reference materials test results.

Name		SRM 1869		SRM 1849a	
		Measured value (mg/kg)	Certified value (mg/kg)	Measured value (mg/kg)	Certified value (mg/kg)
Retinol	9-*cis* retinol	0.45	19.27 ± 0.32 (for retinol)	0.60	7.68 ± 0.23 (for retinol)
	11-*cis* retinol	ND		ND	
	13-*cis* retinol	1.42		1.39	
	All-*trans* retinol	18.66		6.60	
Vitamin E	α-Tocopherol	214.7	217.2 ± 6.2 (for total α-tocopherol)	204.70	219 ± 16 (for total α-tocopherol)
	β-Tocopherol	3.79	4.22 ± 0.69	6.30	—
	γ-Tocopherol	99.83	99.4 ± 5.1	144.1	—
	δ-Tocopherol	33.21	32.5 ± 2.9	82.3	—
	α-Tocotrienol	1.20	—	1.83	—
	β-Tocotrienol	ND	—	ND	—
	γ-Tocotrienol	0.41	—	1.20	—
	δ-Tocotrienol	0.05	—	ND	—

ND: not found; —: not referred.

powder commonly used in the market. Retinol examination results showed that three retinol isomers (9-*cis* retinol, 13-*cis* retinol, and all-*trans* retinol) could be detected in formula milk powder, and a small amount of 11-*cis* retinol can be detected; this may be due to the effect of steric resistance in space, making the presence of 11-*cis* retinol unstable in nature, making it impossible to obtain effective separation detection. In the egg yolk powder sample, only one of the samples detected retinol isomers, and none of the remaining samples detected retinol; it may be that some processes in the production and processing destroy the retinol in it. Except for the four detectable *cis*- and *trans*-isomers of retinol, there was a high unknown peak between 11-*cis* retinol and 9-*cis* retinol during the detection of some actual samples, and if the content of the component was calculated by all-*trans* retinol, it accounted for 2.53% to 8.54% of the total all-*trans*

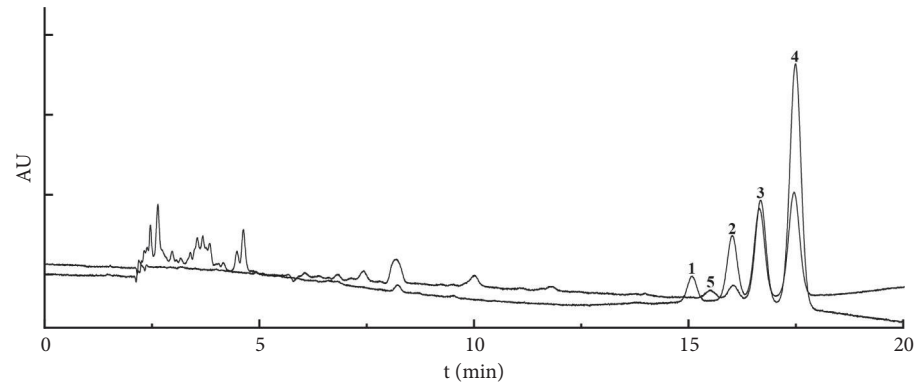

Figure 4: Sample and standard contrast chromatogram (1: 11-*cis* retinol; 2: 9-*cis* retinol; 3: 13-*cis* retinol; 4: all-*trans* retinol; 5: unknown component).

retinol, so the confirmation of the component needs to be further studied to confirm whether it is a retinol isomer or an impurity component. The detailed sample and the standard were compared to detect the chromatogram as shown in Figure 4, and the number 5 chromatographic peak in the figure was the unknown component chromatographic peak.

The test results of the vitamin E isomer showed that the presence of four tocopherols could be found in almost all samples, with the content being α-tocopherol > γ-tocopherol > δ-tocopherol > β-tocopherol; however, tocotrienols were detected less often, and the presence of various types of tocotrienols can be detected in some formula milk powders (such as four samples nos. 5, 6, 8, and 11). According to the biological activity equivalent of other forms of tocopherols known to the study, after conversion, it was found that the activity equivalent of other isomers detected in the sample accounted for 0.11% to 59.18%. Therefore, it is not perfect to evaluate the nutritional value of food only in line with α-tocopherol, and it is necessary to isolate and test other forms of tocopherol and include them in the calculation according to their active equivalent and improve the evaluation criteria further.

4. Conclusion

In this study, the influence of experimental light conditions on the degree of loss of the target components was considered for the first time, and, finally, the study found that, in the case of open red light (illuminance 20LUX) or white LED lamp (illuminance 70LUX) in the dark room, the loss rates of 12 target components were less than 4%. On this basis, the saponification conditions and liquid-liquid extraction solvents were studied, and it was found that there was no significant difference in the influence of hot saponification and cold saponification conditions on the target components of this study. The selection of ethyl acetate/n-hexane mixed solvent as the extraction solvent could meet the requirements of the extraction and detection of the target components while ensuring the safety of the experimenter. The optimized research method had the advantages of simple operation, high accuracy, and good repeatability, which can meet the simultaneous separation and detection of retinol isomers and vitamin E homologues and can be effectively applied in dairy products.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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