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Development of HPLC analysis for the determination of retinol and alpha tocopherol in corn oil nanoemulsion lotion

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Abstract

In this study was to extract and analyses composition of corn oil and develop cosmetics from corn oil extract. A simple high performance liquid chromatographic procedure for the determination of retinol and alpha tocopherol from corn oil nanoemulsions was proposed. The preparation condition for nanoemulsions of corn oil by high pressure homogenization was investigated. It appeared that the appropriate number of homogenization cycle was 8 cycles under the pressure of 1,000 bars at 25ºC for the formula which contained 40% of corn oil, 15% of the mixed surfactant between Tween 80 and Span 80. The droplet sizes of the corn oil nanoemulsions were found to be 115.5±0.6 nm. The HPLC method was developed and validated for various chromatographic conditions for simultaneous determination of retinol and alpha tocopherol in corn oil nanoemulsion. The sample was analyzed on an Inersil C8-3 (4.6 mm×150 mm), using methanol and water as mobile phase with gradient elution system and detected at 292 and 325 nm for alpha tocopherol and retinol, respectively by spectrophotometer. The flow rate was used at 1 mL min^{-1} to complete separation of both analyzes. Under the optimum conditions, retinol and alpha tocopherol could be determined within a concentration range of 5-40 µg mL^{-1} which can be expressed by the regression equation y = 57507x+136421 (r^2 = 0.9990) and y = 10434x+26394 (r^2 = 0.9980), respectively. The limit of detection and quantitation were found to be 0.063 and 0.192 µg mL^{-1} for retinol and 0.067 and 0.203 µg mL^{-1} for alpha tocopherol, respectively. The proposed method was applied to the determination of retinol and alpha tocopherol contents in corn oil nanoemulsions.

Introduction

Although various indigenous plant oils are discovered in Thailand, only some of them were used in industry such as palm and soybean oils, because they can be produced in an industrial scale. However, plant oils can obtain higher value in some other applications such as in cosmetic products if their efficacy is scientifically proven. Because the major composition of plant oil is triglycerides, they can prevent water loss from skin by acting as a temporary barrier (Boyce and Williams, 1993; Siddappa, 2003). Moreover, certain oils contain a high amount of bioactive compounds such as tocopherols and retinol which have significant antioxidant activity. Their antioxidant function is mainly inhibition of lipid peroxidation and scavenging reactive oxygen species. Singlet oxygen can generate the entire oxygen free radical cascade with oxidation of nucleic acids, proteins, and lipids, resulting in skin cancer and that a stable aqueous solution of 15% ascorbic acid and 1% tocopherols when applied topically to skin can provide 4 fold photoprotection for skin (Lin *et al*., 2003; Kruk *et al*., 2005). Corn oil is a popular vegetable oil in the United States and in many other countries. Because of its pleasant nutty flavor, its good stability, and its popularity for making margarines, corn oil has long been considered a premium vegetable oil. Among all of the vegetable oils, corn oil ranks tenth in terms of annual production, and it represents about 2% of the vegetable oil produced worldwide. The chemical composition of corn oil is distinguished by its high levels of polyunsaturated fatty acids (an average value of 60–75% linoleic acid), and among the commodity vegetable oils corn oil has the highest levels of unsaponifiables (>2%), the highest levels of phytosterols (>1%), and the highest levels of tocopherols (about 0.10%) (Boruff and Miller, 1937; Mendis *et al*., 1989; Robert *et al.*, 1996: Jonathan and Karlene, 2002). Nanoemulsions have recently become increasingly important as potential vehicles for the controlled delivery of cosmetic and

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for the optimized dispersion of active ingredients in particular skin layers. Due to their lipophillic interior, nanoemulsions are more suitable for the transport of lipophillic compounds than liposomes. Similar to liposomes, they support the skin penetration of active ingredients and thus increase their concentration in the skin. Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation, or coalescence that is observed with macroemulsions. The incorporation of potentially irritating surfactants can often be avoided by using high-energy equipment during manufacturing (Izquierdo *et al.*, 2002).

The aim of this study was to developed indigenous corn oil nanoemulsion lotions which have good antioxidant activity and high performance liquid chromatographic (HPLC) analysis of alpha tocopherol and retinol in nanoemulsion lotion was investigated. The HPLC was developed for simple, rapid and reliable HPLC method with spectrophotometric detection for the quantitation of alpha tocopherol and retinol in corn oil nanoemulsion lotions. This method has been applied to the development of the HPLC method using C-8 silica column and their applications to the quantitation of alpha tocopherol and retinol in corn oil nanoemulsion lotions.

Material and Methods

Apparatus and instruments

The chromatographic system for the separation and analysis of alpha tocopherol and retinol in nanoemulsion lotion were carried out with Shimadzu model SCL-10A liquid chromatography, thermostatic column compartment, online degasser and an UV-visible detector model SPD-10A. An analytical column used was an Inertsil C8-3 (4.6 mm×150 mm, 5 µm, Canadian Life Science Inc.). Mobile phase was a mixture containing varying ratios of methanol and DI water with gradient elution system. The flow rate was adjusted to 1 mL min^{-1}. The injection volume was adjusted to 20 µL and the absorptions of alpha tocopherol and retinol were made at 290 and 325 nm respectively. The sample solution was prepared and vacuum-filtered through 0.45 µm nylon membrane (Whatman® filters, USA) before use. The following instruments were also used; simultaneous spectrophotometer (UV mini-1240, Shimadzu, Japan) was used to scan the spectra of alpha tocopherol and retinol, pH-meter (Model pH 900, Precisa, Switzerland), water bath and shaker (Model SB-200-10, Thailand), Ultrasonicator (Model 889, Cole Parmer, USA) and a rotary evaporator (EYELA N-1200B series, USA, polytron (PT-MR 3000), Kinematica AG, Switzerland) and high pressure homogenizer (EmulsiFlex® Model-C3, Canada).

Chemical and reagents

All chemicals used were of analytical reagent grade. Distilled water was used throughout the experiment (Milli-Q water purification system, Millipour Co., USA). Standard of alpha tocopherol and retinol were purchased from Sigma-Aldrich (Saint Louis, USA). Methanol, ethanol and dimethyl sulfoxide (DMSO) were purchased from Fluka (Buchs, Switzerland). Acetonitrile, n-hexane, Tween 80, Span 80 and petroleum ether were obtained from Carlo-Erba (Italy).

Standard solutions

The stock standard solutions of alpha tocopherol and retinol were prepared in DMSO to provide a concentration of 1 mg mL^{-1}. These stock solutions were freshly prepared each time and stored below 4°C and protected from light. The solutions were diluted with DMSO and methanol (50:50, v/v) to the desired concentration levels just before performing the analysis.

Sample and sample pre-treatments

Corn oil preparation

All corn samples were bought (September 2013) from commercial sources in Phitsanulok province, Thailand and analyzed in the same year of purchase. The corn was collected in fresh and dried in hot air oven at 50°C for 36 hours. The dried material was ground to a fine powder and kept in an air-tight container at 4°C until further use. The powder corn sample (1.0 kg) was extracted with n-hexane and petroleum ether by soxhlet extraction process for 3 hour. The organic solution was evaporated to dryness at 60°C by mean of a rotary evaporator (Buchi, Switzerland). The 0.5 g of corn oil was transferred into a 5 mL volumetric flask and made up to volume with DMSO. An aliquot of this solution was filtered through a 0.45 µm nylon membrane. Then 20 µL of this solution was injected into HPLC system for analysis of retinol and alpha tocopherol.

Nanoemulsion preparation

Aqueous (Tween 80 and water) and oil (Span 80 and corn oil) phases were prepared separately. The water phase was poured into the oil phase. The nanoemulsion was continuously stirred for 1 hour and pre-homogenized with a polytron (PT-MR 3000), Kinematica AG, Switzerland) at 8,000 rpm for 20 minutes. Then the nanoemulsion was more dispersed

using a high pressure homogenizer (EmulsiFlex® Model-C3, Canada) at 1,000 bars for 8 cycles. The final corn oil and surfactant concentration in the formula were mixed of 40% and 15% respectively.

Procedure

All analyses were performed at room temperature. The chromatographic conditions were carried out in the gradient elution mode using a mixture of methanol and water as mobile phase. The flow rate was set at 1.0 mL min^{-1}. The analytical column was an Inertsil C8-3 (4.6 mm×150 mm, 5 µm). Each sample and standard aliquots of 20 µL was injected onto the analytical column. The effluent from the analytical column was monitored by UV detection at 292 and 325 nm for alpha tocopherol and retinol, respectively. The quantitation was achieved based on peak area of retinol and alpha tocopherol. Calibration curve of analyst was constructed by plotting peak areas versus various concentrations of retinol and alpha tocopherol.

Results and Discussion

Nanoemulsion characterization

Droplet size

The mean droplet size and the size distribution were determined by photon correlation spectroscopy with a Malvern Zetasizer® Nano Series (Malvern Instruments, UK) at 25°C by diluting 10 µL of the nanoemulsion with 10 mL of DI water. The size distribution was showed by polydispersity index (PI) values. The effect of number of homogenization cycle on droplet size of corn oil nanoemulsions, upon increasing the number of cycles from 2 to 8, a decreased in droplet size and PI value was investigated. From these results showed that, corn oil formulations which passed through the homogenizer for 8 cycles showed the lowest droplet size and PI values. The average droplet sizes and the PI values of the formulation were found to be 115.5±0.6 nm and 0.095 respectively. The PI values lower than 0.25 indicated a close size distribution providing good stability of nanoemulsion.

Surface charge

The surface charge was determined using a Malvern Zetasizer® Nano Series. The nomoemulsion sample was done by diluting 10 µL of the nanoemulsion with 10 mL of DI water and measuring the zeta potential (ZP). The ZP values of corn oil nanoemulsion were below -32 mV. The ZP characterizes of particles and thus it gives information about repulsive forces between particles and droplets, to obtain stable nanoemulsion by preventing flocculation and coalescence of the nanodroplets (ZP value above±30 mV).

Table 1. Gradient elution program for the chromatographic separation of retinol and alpha tocopherol in corn oil nanoemulsion sample

Step	Time (min)	Mobile phase composition (%)		Flow rate (mL min^{-1})	Wavelength (nm)
		methanol (A)	water (B)		
1	0.01	85	15	1	325
2	5.00	85	15	1	325
3	5.01	90	10	1	325
4	7.01	90	10	1	292
5	15.00	95	5	1	292

Method optimization

A preliminary experiment was carried to investigate the spectral characteristics of retinol and alpha tocopherol. The absorption spectrum was studied by batch wise spectrophotometry. The absorption spectrum was obtained by scanning the wavelength over the range of 200-400 nm. The UV spectrum of retinol and alpha tocopherol standard solutions showed the absorption maxima at 325 and 292 nm respectively. Secondly, the optimization work mainly addressed the program of the chromatographic elution in order to maximize both resolution and sensitivity. An aqueous solution of retinol and alpha tocopherol, methanol (A), and water (B) were still adopted as components of the mobile phase, whereas the optimized elution program for corn oil sample is reported in Table 1. The best sensitivity compromise for all analytes was reached using two different operative wavelengths (325 nm for retinol and 292 nm for alpha tocopherol). The chromatographic separation was performed at a flow rate of 1.0 mL min^{-1}. The method proposed is rapid: all analytes were completely eluted within 5 min and the whole chromatographic run was completed in 15 min. Figure 1 shows the chromatograms obtained by analyzing a mixture of standards (Figure 1(A)), and an corn oil sample (Figure 1(B)).

Method Validation

Range and linearity

Under the selected chromatographic conditions, the linear range of the signal response for retinol and alpha tocopherol were studied over the concentration

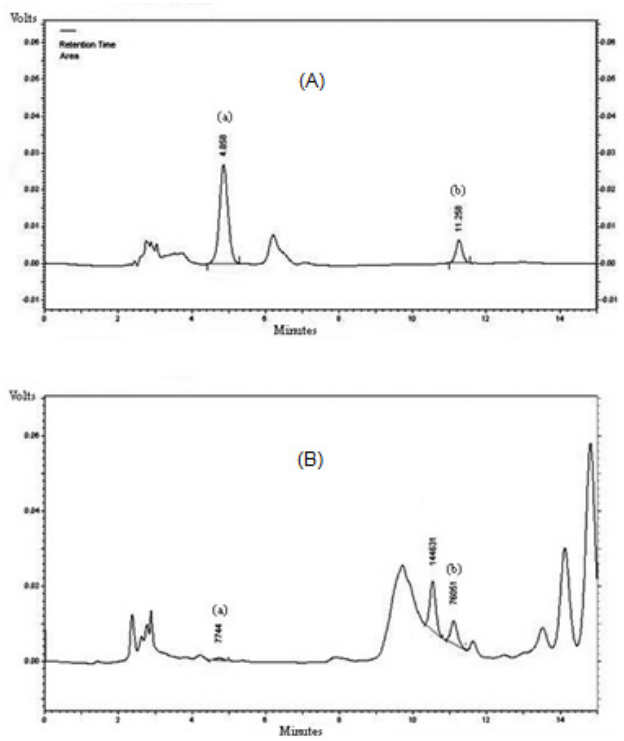

Figure 1. (A) chromatogram of a standard mixture of retinol (a) and alpha tocopherol (b), each at a concentration of 10 mg L^{-1}; (B) chromatogram of corn oil nonoemulsion sample

range of 1.0-100.0 µg mL^{-1}. The linearity of calibration graph was determined using the optimal experimental parameters. Eight standard solutions ranging from 5.0-40.0 µg mL^{-1} in concentration, in five replicates each, were injected into the HPLC system. The calibration graph was obtained by plotting the absorbance of the solutions against the standard concentrations. Linear calibration graph over the concentration range of 5.0-40.0 µg mL^{-1} of retinol and alpha tocopherol were obtained with the regression equation; $y = 57507x + 136421$ ($r^2 = 0.9990$) and $y = 10434x + 26394$ ($r^2 = 0.9980$), respectively.

Detection limit and quantification limit

Limit of detection (LOD) and limit of quantitation (LOQ) of retinol and tocopherol were estimated from the calibration curve using the expression, LOD = 3.3SD/S where SD is standard deviation of the blank (or the intercept of the calibration curve) and S is the slope of calibration curve and LOQ = 10SD/S (Miller and Miller, 1993). The limit of detection and limit of quantitation values were found to be 0.063 and 0.192 µg mL^{-1} for retinol and 0.067 and 0.203 µg mL^{-1} for alpha tocopherol, respectively.

Precision

The precision of the method was determined by measuring the repeatability (intraday precision) and the intermediate precision (interday precision), both expressed as relative standard deviation (R.S.D). The precision was evaluated by assaying seven replicate injections of 5, 10, and 20 µg mL^{-1} of retinol and alpha tocopherol standard solutions, respectively. The repeatability was evaluated each sample on the same day under the same experimental conditions. The intermediate precision was evaluated by assaying each sample on three different days. The results of repeatability and intermediate precisions were showed in Table 2.

Table 2. Repeatability and intermediate precision for the studied retinol and alpha tocopherol standard solutions (n=7)

Compounds	concentration (µg mL^{-1})	Intra-day precision %RSD	Inter-day precision %RSD
Retinol	5	2.34	2.02
	10	1.81	1.00
	20	1.48	1.01
Alpha tocopherol	5	2.16	2.05
	10	1.36	1.21
	20	1.94	1.33

Accuracy

Accuracy of the method was assessed with recovery using the addition of four known concentration levels of 5, 10, 20 and 30 µg mL^{-1}. All samples were injected in three replicates for each concentration. The concentration found was calculated against the concentration added (Table 3). Additives and excipients did not interfere in the determination of those active ingredients since the samples used to evaluate recovery were prepared with those additives and excipients present.

Method application

The proposed HPLC method was applied to the determination of retinol and alpha tocopherol in corn oil nonoemulsion. Corn oil sample was prepared according to sample preparation and the contents of retinol and alpha tocopherol in each sample solution were determined using the optimum conditions. The samples gave well-defined peaks. There is no interference peak present in each sample. The average contents of retinol and alpha tocopherol from corn oil nanoemulsion samples were found to be (corn oil (A) and corn oil (B)) were found to be 0.28, 12.80 µg g^{-1} and 0.34, 10.07 µg g^{-1} respectively (Table 4).

Table 3. Accuracy of the proposed HPLC method (n=5)

Retinol	Concentration (mg L^{-1})		%Recovery (average ± SD)	Alpha tocopherol	Concentration (mg L^{-1})		%Recovery (average ± SD)
	Added	Found			Added	Found	
	5	5.50 ± 0.13	109.97 ± 2.52		5	5.31 ± 0.13	106.16 ± 2.52
	10	10.97 ± 0.35	109.69 ± 3.47		10	8.82 ± 0.35	88.22 ± 3.47
	20	22.15 ± 0.26	110.73 ± 1.73		20	19.83 ± 0.26	99.16 ± 1.73
	30	34.30 ± 0.23	114.3 ± 1.15		30	30.14 ± 0.23	100.45 ± 1.15

Table 4. Determination of retinol and alpha tocopherol in nanoemulsion samples using the proposed HPLC method

Sample	Content	
	Retinol (μg g^{-1})	Alpha tocopherol (μg g^{-1})
Corn oil A	0.28	12.80
Corn oil B	0.34	10.07

Conclusion

In conclusion, the proposed HPLC procedure can be used for the determination of retinol and alpha tocopherol in corn oil nanoemulsion samples. The detection limit of this method was reasonable accepted. Sample pretreatment is not necessary. This method is simple, fast, relatively inexpensive, precise, accurate and sensitive. Then, the speed of analysis and the precision make this method suitable for quality control of retinol and alpha tocopherol in corn oil nanoemulsion samples. It is therefore suitable for quality control in cosmetic and pharmaceutical formulations containing corn oil.

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References

Boruff, C.S. and Miller, D. 1937. Solvent extraction of corn oil from distillers grains. Oil and Soap 14: 312-313.

Boyce, S.T. and Williams, M.L. 1993. Lipid supplemented medium induces lamellar bodies and precursors of barrier lipids in cultured analogues of human skin. Journal of Investigative Dermatology 101(2): 180-184.

Izquierdo, P., Esquena, J., Tadros, T.F., Dederen, C, Garcia, M.J., Azemar, N. and Solans, C. 2002. Formation and stability of nano-emulsions prepared using the phase inversion temperature method. Langmuir 18: 26-30.

Jonathan, W.D.V. and Karlene, R.S. 2002. Determination of vitamins A (retinol) and E (alpha tocopherol) in foods by liquid chromatography: collaborative study. Journal of AOAC International 85(2): 424-434.

Kruk, J., Hollander-Czytko, H., Oettmeier, W. and Trebst, A. 2005. Tocopherol as singlet oxygen scavenger in photosystem II. Journal of Plant Physiology 162: 749-757.

Lin, J.Y., Selim, M.A., Shea, C.R., Grichnik, J.M., Omar, M.M., Monteiro-Riviere, N.A. and Pinnell, S.R. 2003. UV photoprotection by combination topical antioxidants vitamin C and vitamin E. Journal of the American Academy of Dermatology 48: 866–874.

Mendis, S., Wissler, R.W., Bridenstine, R.T. and Podbielski, F.J. 1989. The effect of replacing coconut oil with corn oil on human serum lipid profiles and platelets derived factors active in atherogenesis. Nutrition Reports International 40(4): 773-782.

Miller, J.C. and Miller, J.N. 1993. Statistics for analytical chemistry, Ellis Horwood PRT Prentice-Hall: New York; 3rd Edition.

Robert, A.M., Michael, J.P. and Kevin, B.H. 1996. Extraction and quantitative analysis of oil from commercial corn fiber. Journal of Agricultural and Food Chemistry 44(8): 2149-2154.

Siddappa, K. 2003. Dry skin conditions, eczema and emollients in their management. Indian Journal of Dermatology, Venereology and Leprology 69: 69-75.