Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Determination of Vitamin D3 Loaded Self-nanoemulsifying Drug Delivery Systems (SNEDDS) Based Hydrogel

Adeltrudis Adelsa DANIMAYOSTU [1,2], Endang LUKITANINGSIH [3*], Ronny MARTIEN [1], Retno DANARTI [4]

1 Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
2 Department of Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
3 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia
4 Department of Dermatology and Venereology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
* Corresponding Author. E-mail: lukitaningsih_end@ugm.ac.id (E.L.); Tel. +62-813-9280-7988.

Received: 01 December 2022 / Revised: 04 February 2023 / Accepted: 06 February 2023

ABSTRACT: High Performance Liquid Chromatography (HPLC) was used to determine vitamin D3 content from the topical dosage form. Self-nanoemulsifying drug delivery systems (SNEDDS) based hydrogel was aimed to optimize the delivery of vitamin D3 through the skin due to its high lipophilicity. Quantifying vitamin D3 was performed using the internal standard, vitamin D2, on LiChrocart C18 125-4 reverse phase column. Several characteristics of validation were referred including specificity, linearity, range, accuracy, precision, limits of detection, and quantification. The mobile phase was optimized as a mixture of acetonitrile-methanol-water for injection 94:3:3 v/v, and the flow rate was optimized at 0.8 mL/min at 25°C. Vitamin D3 detection and quantification were observed at a wavelength of 266 nm using an ultra-violet (UV) detector. The analysis method performed no excipient interference, including separating vitamin D3 from its matrix. Extraction of vitamin D3 from its matrix was observed using the dialysis membrane, with agitation (vortex) and no agitation (immersion). The drug content determination was found to be 108.22 % ± 14.77% for the immersion method and 72.04% ± 33.37% for the vortex method. Further, the high variability of the results indicated the dialysis membrane was not a suitable method for determining lipophilic drug content from the colloidal polymer matrix. The other techniques with negligible variation, such as reverse dialysis or subsequent solvent addition, are preferred.
KEYWORDS: Vitamin D3; HPLC; drug content; SNEDDS; hydrogel.

1. INTRODUCTION

Vitamin D is available from more than one source. Vitamin D2 can be obtained mainly from mushrooms, while vitamin D3 is predominantly synthesized in the skin through the stimulation of ultraviolet (UV) irradiation. Humans can get either vitamin D2 or D3 from UV exposure in the morning ambient UV exposure and food intake (fish, egg yolks, and fortified foods such as milk, margarine, and cereals) [1]. Vitamin D2 and D3 have structural differences in their side chains, particularly in their broken rings. Vitamin D2 has a double bond between C22 and C23 and a methyl group on C24 [2].

Vitamin D3 has a more diverse beneficial role in the human body that is not limited to the skin. Besides regulating calcium homeostasis, vitamin D3 positively impacts keratinocytes and hair differentiation, cancer prevention, and psoriatic and antimicrobial therapy [3]. To date, vitamin D3 is proposed to have anti-aging and photoprotective effects on the skin. Those might be contributed by anti-inflammatory actions, epidermal barrier protection, antioxidative responses, and DNA repairment [4]. Therefore, vitamin D3 for anti-aging treatment has become a prospective dosage form in the rising cosmetic market.

The solubility of vitamin D3 has been a consideration for embedding it into oil-based formulations. The higher solubility of vitamin D3 in the chosen delivery forms brings out the higher drug loading and efficacy [5]. Nanoemulsions have been rendered in cosmetics formulations due to their high solubilization capacity and physical stability. When applied to the skin, it penetrates well through the transfollicular or transepidermal route. Nanoemulsions can alter the lipid bilayer and increase the concentration gradient by

How to cite this article: Danimayostu AA, Lukitaningsih E, Martien R, Danarti R. Determination of vitamin D3 loaded self-nanoemulsifying drug delivery systems (SNEDDS) based hydrogel. J Res Pharm. 2023; 27(3): 1213-1219.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.410

working as a tiny drug reservoir. As a result, nanoemulsions enhance drug penetration, especially for lipophilic drugs [6].

The anhydrous forms of nanoemulsion are termed self-nanoemulsifying drug delivery systems (SNEDDS), which have a similar composition with nanoemulsions such as natural or synthetic oil, surfactants/co-surfactants while co-solvents replace aqueous media. Consequently, SNEDDS have a high portion of surfactants that co-surfactants can condense. SNEDDS provide higher drug loading and better drug partitioning between water – oil interface [7].

Vitamin D3 is a highly liposoluble vitamin, shown by its high log P 7.5 [8]. SNEDDS are suitable for lipophilic drugs having log P values greater than 5 [7]. Those data indicate SNEDDS is an appropriate dosage form for vitamin D3. A recent study demonstrated astaxanthin was successfully formulated in SNEDDS, effectively penetrating the skin to act as a UV protector, with anti-aging and anti-inflammatory effects [9].

Pharmaceutical formulations must fulfill the required drug content to ensure the quality control of the products. High Performance Liquid Chromatography (HPLC) is considered a suitable assay instrument for very tiny samples of vitamin D3 due to its sensitive and accurate equipment [10]. Validation methods of analysis are designed to represent the suitable methods for specific purposes, in this case, drug assay [11]. According to the International Conference on Harmonization (ICH) Q2(R1), the typical characteristics that need to be evaluated are specificity, linearity, range, accuracy, and precision. They will be the parameters to determine drug content based on the chosen extraction method. To the best of our knowledge, there is no published article concerning the determination method for vitamin D3 encapsulated in SNEDDS based hydrogel. This study aims to elaborate on the suitable method of vitamin D3 analysis from a sophisticated matrix in prepared SNEDDS based hydrogel.

2. RESULTS

The comparison of the chromatograms of vitamin D3 loaded SNEDDS based hydrogel, SNEDDS hydrogel base (placebo), blank, and standard vitamin D3 solution showed similar retention times (Figure 1.). There were no interfering peaks of excipients from the desired formulation.

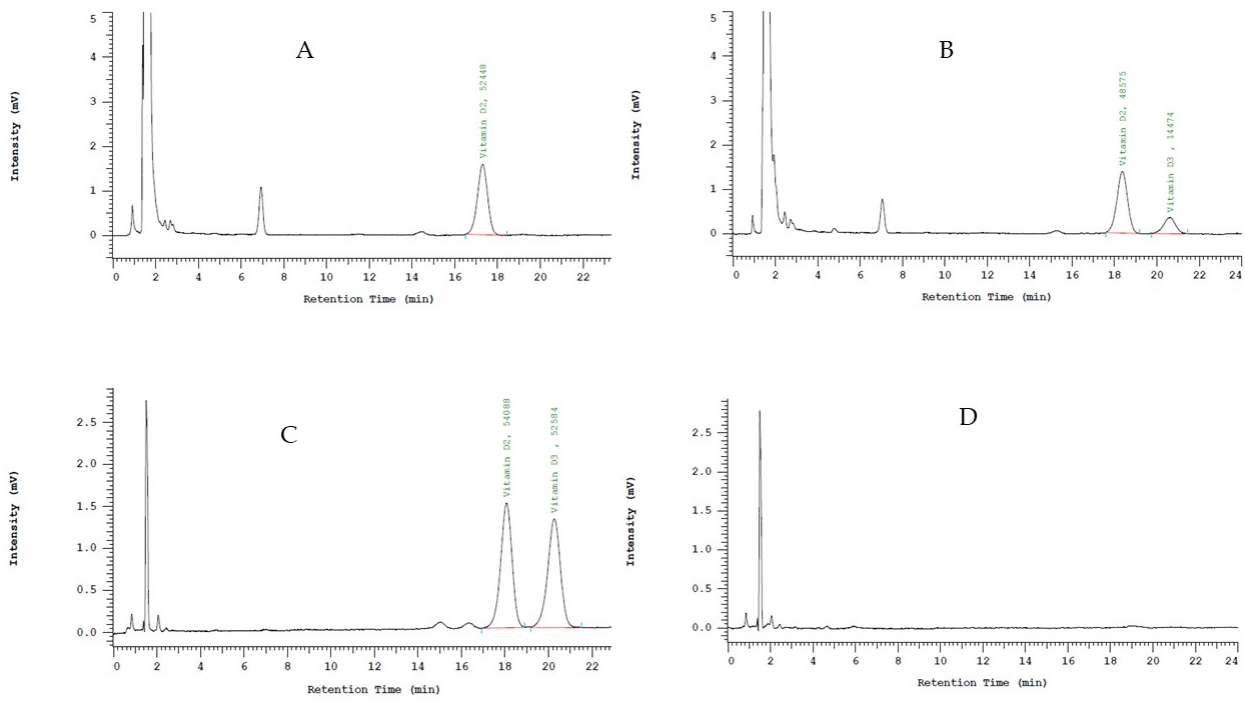

Figure 1. Chromatograms of vitamin D3 and its base (A) placebo, (B) vitamin D3 loaded SNEDDS hydrogel, (C) vitamin D3 standard solution, and (D) blank

The first step of this experiment was to ensure that the analysis method was suitable for the intended characteristics of the drug and the purposes of analysis. The system suitability test was evaluated by the resolution, N plate, retention time (Rt), and area of vitamin D3 and its internal standard, vitamin D2. The test

was assessed for six replicate injections of vitamin D3 reference standard solution with 0.8 mg/L. The chromatographic separation times of vitamin D3 and D2 were 19.60 ± 0.24 min and 17.60 ± 0.21 min, respectively (Table). It leads to an analysis time of 22 min per sample injection. USP 32 NF 27 stated that the relative standard deviation (RSD) for the vitamin D3 peak response must be less than or equal to 2.0%.

Table 1. System suitability parameters

Parameters	Result		%RSD	
	Vitamin D3	Vitamin D2	Vitamin D3	Vitamin D2
Retention factor (min)	19.60 ± 0.24	17.60 ± 0.21	1.21	1.17
N plate	6728 ± 44.76	6605 ± 52,68	0,67	0,80
Asymmetric factor	0,96 ± 0,01	0,97 ± 0,01	0,85	0,57
Resolution	2.20 ± 0.01		0.48	
System precision (n=6)				
	Theoretical Concentration		Calculated Concentration	
Concentration (mg/L)	0.8000	1.000	0.8032	1.004

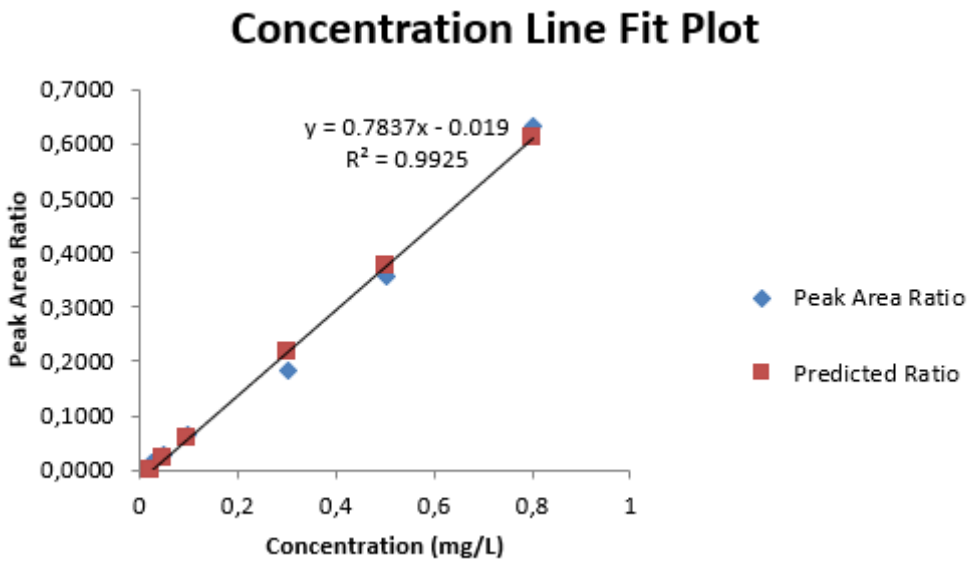

Figure 2. Vitamin D3 linearity plot –peak area vs. concentration ratio (mg/L) aligned with the predicted ratio

The vitamin D3 linearity calibration curve was plotted to correlate the relationship of concentration range to the peak area. The linear equation Y= 0.7869x-0.019 was proved by R^2 = 0.9925. The linearity plot was close enough to the predicted one. The LOD and LOQ were calculated theoretically using the following equations:

(Eq. 1) Limit of detection (LOD) = $\gamma_B + 3S_B$
(Eq. 2) Limit of quantification (LOQ) = $\gamma_B + 10S_B$

Where γ_B is a blank signal while S_B is the standard deviation of the blank, those two terms can be found by linear regression. It is the calculated intercept, *a*, which is used as γ_B. The estimated value of the standar deviation S_B value is obtained from S_y/x. The values of LOD and LOQ were subsequently 0.1433 mg/L and 0.43415 mg/L, respectively [12]. The recovery percentage of vitamin D3 standard solution shown 92.57-102.52% and %RSD 2.45-4.70%, while the %recovery of vitamin D3 loaded SNEDSS based hydrogel was 72.04-99.63% and %RSD 8.29-12.58%. AOAC official method analysis stated the expected value for one ppm analyte concentration mean recovery as 80-110% and a minimum of 11% for RSD. Standard solutions of vitamin D3 have qualified within the required range. On the contrary, the vitamin D3 loaded SNEDSS based hydrogel results were out of range, while the %RSD depicted high variability.

The developed RP-HPLC method aims to determine drug content from vitamin D3 loaded SNEDDS based hydrogel formulations. Vitamin D3 must be extracted from its carrier (SNEDDS and hydrogel). Two

alternatives were compared to choose the extraction method of vitamin D3 from SNEDDS based hydrogel. The drug content determination was found to be 108.22% ± 14.77% for the immersion method and 72.04% ± 33.37% for the vortex method. The vortex instrument used was not hands-free. The inconsistency due to manual tube holding was causing high variability of the results. The immersion method for several hours seems more effective than the vortex method, but they both have high variability. It can be deducted by a high %RSD, more than the acceptability criteria, 11% [13].

3. DISCUSSION

The mean recovery and, mainly, the RSD results of SNEDDS based hydrogel above indicate two causes of problems in the analysis: the methods cannot extract the intended analyte from its matrix and the imprecision between independent tests under stipulated conditions. The imprecision increases as the analyte concentration decreases [13]. A problem was detected regarding determining drug content using the dialysis membrane method. High variability of the measured drug concentration indicates that the "bottleneck" of the problem could be the diffusion or release process.

Experiments of lipophilic drug content determination from a polymer matrix often result in high recovery percentage variability [14]. Several methods are applied to determine a matrix's lipophilic or nanoparticle drug content. Ultrafiltration and centrifugation are used to separate the nanoparticulate released drug from its carrier matrix. They do not always render complete drug extraction, probably due to high shear stress and long high-speed circulation times, which might induce premature drug release at the separation time. Continuous flow methods cause fluctuations in flow rates, delayed response time, and filter clogging, thus affecting the drug release profile. Membrane diffusion techniques such as dialysis membranes are also mentioned as an alternative method for drug release, but they have many reports of obstacles and result errors [15]. The dialysis method is considered not suitable due to its high variability results. Those also happened in this experiment, possibly caused by insufficient agitation inside the dialysis sac. The immersion method was chosen in this experiment due to the lack of concentration used. Another reason is high viscosity of hydrogel could become a barrier retarding the diffusion process [16].

The type of dosage forms affects the diffusion process and, consequently, the measured drug. A study of lipophilic drug (diazepam) release denoted incomplete and slower release rates in emulsion than in hydroalcoholic solution. It indicated that oil as one of the emulsion components caused a significant decrease of lipophilic substance in the aqueous phase. The low concentration of lipophilic substance The lowering of gradient concentration in the dialysis membrane affects permeation to become the critical point of the process. Those experiments also suggest that the dialysis bag method is inappropriate for colloidal carrier release testing [17]. Vitamin D3 is a lipophilic drug dissolved in SNEDDS droplet entrapped in hydrophilic carbopol based gel. To be transferred to the acceptor compartment, vitamin D3 should pass the aqueous phase. A significant decrease in vitamin D3 concentration could impact the measured drug in the acceptor compartment.

By diffusion, drugs need to be dissolved in the solvent to penetrate the dialysis membranes and follow Fick's first law of diffusion. The sink condition is an absolute environment for the kinetic system. The consistency of the sink solution is needed to maintain the gradient concentration. The volume of the donor compartment is expected to be much less than the acceptor compartment to reach the sink condition. Levy & Benita,1990 even stated that dialysis bag volume is about 10-15 mL compared with 500 mL of sink solution, indicating a 1:50 ratio of the donor and acceptor compartment. In this experiment, we used 100 µL of donor compartment volume and 5 mL sink solution, which has a proportion of 1:50 with the reference mentioned above [17].

Other methods have been observed to solve the problem of dialysis. Therefore, reverse dialysis, which has significantly lower variation results, is recommended [16]. A study by Williams et al. 2001 successfully used subsequent solvent addition to stipulate alprazolam content from a hydroxymethyl cellulose (HPMC) matrix-based tablet. Hot water was added to stimulate polymer swelling, followed by cold water to dissolve the polymer. Strong solvent acetonitrile (ACN) was added to extract alprazolam from the matrix using its solubility in ACN. This method has shown the minor variable result of recovery percentage [14].

Vitamin D2 has been chosen as this experiment's internal standard for quantifying vitamin D3. Both of them have very similar chemical structures. Vitamin D3 and D2 are generally in the same sample, either food, drinks, tissues, or blood. Those two substances were separated using another internal standard, dihydrotachysterol [18–20]. The specificity of two substances closest to each other can be interpreted from the resolution (Rs) value [11]. The resolution (Rs) value was even less than 2. Rs ≥2 is an accepted value for excellent and adequate separation between two peaks [10,20].

Our chromatogram results are remarkably similar to the findings of the Mattila et al. study. They used vitamin D2 as an internal standard for vitamin D3 analysis and vice versa [21,22]. Those peaks were separated well with the analysis's run time above 20 min. Vitamin D2 was eluted first, followed by vitamin D3, both eluting in 18-22 min. They used methanol: water as the mobile phase in analytical HPLC and n-hexane: isopropanol for semipreparative HPLC [22–24]. Otherwise, another study mentioned that the run time of the analysis reached almost 40 min [21].

4. CONCLUSION

The RP-HPLC vitamin D3 loaded SNEDDS based hydrogel method was found to be selective. The resulting peaks have a good resolution but quite a lengthy analysis duration. The chosen method for determination of vitamin D3 content, dialysis membrane, rendered a low precision. It indicated the dialysis membrane is not a suitable method for determining lipophilic drug content from the colloidal polymer matrix. We prefer other ways with the slightest variation, such as reverse dialysis or subsequent solvent addition.

5. MATERIALS AND METHODS

5.1. Materials

Vitamin D3 (Xi'an Lyphar Biotech Co., Ltd, China), Carbopol 940 (Sumitomo Seika Chemicals Co., LTD., Japan), Mygliol 812 N (IOI Oleochemical, Germany), Polysorbate 80 (Kao Chemicals Global, Japan), Polyethylene Glycol 400/PEG 400 (Kao Chemicals Global, Japan)

5.2. Reagents and chemicals

HPLC grade acetonitrile (SmartLab, Indonesia), HPLC grade methanol (JT Baker, United States), Water for injection (PT Ikhapharmindo Putramas Pharmaceutical Laboratories, Indonesia), Cholecalciferol (Sigma-Aldrich Pte Ltd, Singapore), Ergocalciferol (Sigma-Aldrich Pte Ltd, Singapore)

5.3. Methods

5.3.1. Preparation of SNEDDS based hydrogel

Vitamin D3 was encapsulated in self nanoemulsifying drug delivery systems (SNEDDS) to improve its solubility and acceptability. SNEDDS is composed of Mygliol 812 N, Tween 80, and PEG 400, while the hydrogel base consists of Carbopol 940, Triethanolamine (TEA), phenoxyethanol, and aquadest. Vitamin D3 loaded SNEDDS was optimized by water titration method as the other general SNEDDS preparation. Vitamin D3 was dissolved in Miglyol 812 N before being thoroughly mixed with the surfactant mixture (Smix) of Tween 80 and PEG 400 using a magnetic stirrer in moderate agitation. SNEDDS was incorporated in hydrogel to obtain a 10 µg/g vitamin D3.

5.3.2. HPLC analysis

Identification of vitamin D3 was carried out on Hitachi high-performance liquid chromatograph, LaChrom elite D2000 L-Series, Japan, equipped with a UV Vis detector L-2420, autosampler L-2200 and Pump L-2130. The reversed-phase column LiChrosphere® 100 RP-18 (5µm), LiChrocart C18 125-4 used acetonitrile-methanol-water (94:3:3) as a mobile phase at a flow rate 0.8 mL/min was performed in isocratic elution mode at 25°C. The injection volume was set at 20 µL, and the detection wavelength was accomplished at 266 nm.

5.3.3. Specificity

Specificity measures the chance of any interferences of formulation excipients to the intended drug. Samples from SNEDDS hydrogel base, blank, standard vitamin D3 solution, and vitamin D3 loaded SNEDDS based hydrogel were injected, respectively. Evaluation of the chromatograms aims to observe the presence of any interfering peaks from excipients.

5.3.4. System suitability test

The standard solutions were prepared by dissolving weighed 10.25 mg of cholecalciferol and 10.57 mg of ergocalciferol, as an internal standard (IS), into 50.0 mL methanol in separate volumetric flasks. Appropriate volumes of each standard solution were pipetted into one 10.0 mL volumetric flask, resulting in a 2 mg/L concentration. The mixture of cholecalciferol and its internal standard was injected six times to calculate the RSD on retention time, area, N value, and resolution.

5.3.5. Linearity analysis method

The stock standard was prepared by accurately weighing 10.0 mg of respectively cholecalciferol and ergocalciferol into a 25.0 mL volumetric flask (c = 400 mg/L). Appropriate volumes of cholecalciferol stock solution were pipetted into one 10.0 mL volumetric flask, resulting in the following concentration: 0.025; 0.05; 0.1; 0.3; 0.5; and 0.8 mg/L. Each of those working standards contains 1.0 mg/L internal standard, ergocalciferol.

5.3.6. Drug content determination of vitamin D3

Determination of drug content was observed using the chosen extraction method, the dialysis bag technique. This method is usually used to study in vitro drug release from nanoparticles in oral dosage forms. There have been no documented drug content studies for SNEDDS based hydrogel. We optimize the best procedure to obtain vitamin D3 concentration detected on HPLC. The SNEDDS of vitamin D3 based on hydrogel was weighed 500.0 mg and was inserted into a dialysis bag, along with methanol 75 µL and an internal standard 25 µL of 400 mg/L. Both ends of the dialysis bag were tightly tied, preventing the leakage risk. The dialysis bag was immersed in 5 mL methanol. This stage was divided into 2 treatments:

A. The dialysis bag was immersed in 5 mL methanol for 9 hours and moved to another 5 mL methanol for 9 hours
B. The dialysis bag in 5 mL methanol was vortexed for 3 minutes and moved to another 5 mL methanol to repeat the exact handling

The total volume of methanol obtained from the extraction procedure was 10 mL. It became the sample to be injected directly into HPLC.

Acknowledgments: -.

Author contributions: Concept – A.A.D., E.L., R.M., R.D..; Design – A.A.D., E.L., R.M., R.D.; Supervision – E.L., R.M., R.D..; Resources – R.M.; Materials – R.M.; Data Collection and/or Processing – A.A.D.; Analysis and/or Interpretation – A.A.D., E.L., R.M., R.D.; Literature Search – A.A.D.; Writing – A.A.D.; Critical Reviews – A.A.D., E.L., R.M., R.D.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, Chope G, Elina H, Berry J, Vieth R, Lanham-New S. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;95(6):1357-1364. https://doi.org/10.3945/ajcn.111.031070

[2] Sirajudeen S, Shah I, Al Menhali A. A narrative role of vitamin D and its receptor: With current evidence on the gastric tissues. Int J Mol Sci. 2019;20(15):3832. https://doi.org/10.3390/ijms20153832

[3] Piotrowska A, Wierzbicka J, Zmijewski MA. Vitamin D in the skin physiology and pathology. Acta Biochim Pol. 2016;63(1):17-29.https://doi.org/10.18388/abp.2015_1104

[4] Bocheva G, Slominski RM, Slominski AT. The impact of vitamin d on skin aging. Int J Mol Sci. 2021;22(16):9097. https://doi.org/10.3390/ijms22169097

[5] Bi Y, Xia H, Li L, Lee RJ, Xie J, Liu Z, Qiu Z, Teng L. Liposomal vitamin D3 as an anti-aging agent for the skin. Pharmaceutics. 2019;11(7):311. https://doi.org/10.3390/pharmaceutics11070311

[6] Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, Chourasia MK. Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release. 2017;252:28-49. https://doi.org/10.1016/j.jconrel.2017.03.008

[7] Devireddy SK, Jonnalagadda LP. A literature review on self nanoemulsifying drug delivery system (SNEDDS). Int J Pharm Sci Rev Res. 2021;70(1):85-94. https://doi.org/10.47583/ijpsrr.2021.v70i01.011

[8] Cholecalciferol. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Cholecalciferol. (accessed July 4, 2022)

[9] Ponto T, Latter G, Luna G, Leite-Silva VR, Wright A, Benson HAE. Novel self-nano-emulsifying drug delivery

systems containing astaxanthin for topical skin delivery. Pharmaceutics. 2021;13(5):649. https://doi.org/10.3390/pharmaceutics13050649
[10] Ravisankar P, Naga Navya C, Pravallika D, Sri DN. A review on step-by-step analytical method validation. IOSR J Pharm. 2015;5(10):2250-3013.
[11] European Medicines Agency. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. London: European Medicines Agency; 2006.
[12] Miller JN, Miller JC. Statistics and chemometrics for analytical chemistry. Sixth. London: Pearson Education Limited; 2010. 119-127.
[13] AOAC International. Appendix F: Guidelines for Standard Method Performance Requirements. AOAC Int Off Method Anal. 2016;1-18.
[14] Williams RO, Sykora MA, Mahaguna V. Method to recover a lipophilic drug from hydroxypropyl methylcellulose matrix tablets. AAPS PharmSciTech. 2001;2(2):E8. https://doi.org/10.1208/pt020208
[15] Knoke S, Bunjes H. Transfer of lipophilic drugs from nanoemulsions into lipid-containing alginate microspheres. Pharmaceutics. 2021;13(2):173. https://doi.org/10.3390/pharmaceutics13020173
[16] Xu X, Khan MA, Burgess DJ. A two-stage reverse dialysis in vitro dissolution testing method for passive targeted liposomes. Int J Pharm. 2012;426(1-2):211-218. https://doi.org/10.1016/j.ijpharm.2012.01.030
[17] Levy MY, Benita S. Drug release from submicronized o/w emulsion: a new in vitro kinetic evaluation model. Int J Pharm. 1990;66(1-3):29-37. https://doi.org/10.1016/0378-5173(90)90381-D
[18] Dimartino G. Simultaneous determination of cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) in foods by selected reaction monitoring. J AOAC Int. 2009;92(2):511-517. https://doi.org/10.1093/jaoac/92.2.511
[19] Shah I, Petroczi A, Naughton DP. Method for simultaneous analysis of eight analogues of vitamin D using liquid chromatography tandem mass spectrometry. Chem Cent J. 2012;6:112. https://doi.org/10.1186/1752-153X-6-112
[20] Lipkie TE, Janasch A, Cooper BR, Hohman EE, Weaver CM, Ferruzzi MG. Quantification of vitamin D and 25-hydroxyvitamin D in soft tissues by liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2013;932:6-11. https://doi.org/10.1016/j.jchromb.2013.05.029
[21] Mattila PH, Piironen VI, Koivistoinen PE, Uusi-Rauva EJ. Contents of cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites in milk products and raw meat and liver as determined by HPLC. J Agric Food Chem. 1995;43(9):2394-2399. https://doi.org/10.1021/jf00057a015
[22] Mattila PH, Piironen VI, Uusi-Rauva EJ, Koivistoinen PE. New analytical aspects of vitamin D in foods. Food Chem. 1996;57(1):95-99. https://doi.org/10.1016/0308-8146(96)00144-6
[23] Mattila PH, Piironen VI, Uusi-Rauva EJ, Koivistoinen PE. Vitamin D contents in edible mushrooms. J Agric Food Chem. 1994;42:2449-2453. https://doi.org/10.1021/jf00047a016
[24] Mattila PH, Piironen VI, Uusi-Rauva EJ, Koivistoinen PE. Cholecalciferol and 25-hydroxycholecalciferol contents in fish and fish products. J Food Compos Anal. 1995 (8). 232-243. https://doi.org/10.1006/jfca.1995.1017