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Formulation evaluation development and validation of simultaneous equation method for estimation of vitamin c and vitamin D in multivitamin lipstick

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ABSTRACT

Use of vitamins in cosmetic applications has been increased exponentially during recent years. The objective of the present study involves the formulation and evaluation of multivitamin lipstick containing vitamin C and D. Evaluation parameters such as softening point, breaking load test, pay off test, rancidity, force of application, surface anomalies, skin irritation test, aging stability, solubility test were performed. All the formulations F_1,F_2, F_3 containing Vitamin C and D have better stability and showed good pay-off results and were accordingly as per daily intake of the Vitamins. A new UV simultaneous equation method was developed and validated for simultaneous estimation of Vitamin C and D according to ICH Q2 (R1) guidelines. The relationship was found to be linear in the concentration range 15-35µg/mL. Analysis was carried out at its $_{max}$ 242nm for vitamin C and at $_{max}$ 264 for vitamin D. The spectrophotometric method was optimized and validated according to ICH Q2 (R1) guidelines. The developed method was sensitive and the LOQ value was found to be 1.64µg/ mL for vitamin C and 3.65µg/ mL for vitamin D. All the parameters were within the limits.

INTRODUCTION

Cosmeceuticals are cosmetic products with bioactive ingredients purported to have medical benefits. The name is a combination of "cosmetics" and "pharmaceuticals". Cosmeceuticals affects the biological functioning of the skin (medicinal or drug like benefits).Depending upon the ingredients present in them, Cosmeceuticals increases the collagen growth in the skin and reduces the harmful effects of free radicals thus maintain the structure of keratin in good condition and making the skin healthier. There are skincare products that go beyond colouring and adorning the skin. [1, 2] Like cosmetics, cosmeceuticals are applied topically; they contain ingredients that influence the skin's biological function.Cosmeceuticals are meant to improve appearance by delivering nutrients necessary for healthy skin. Cosmeceuticals usually claim to reduce wrinkles and to improve tone, texture and radiance of the skin. [3]

Lipstick formulations are most widely used to enhance the beauty of lips and to add glamour's touch to the makeup. The present study is to treat VITAMIN D deficiency especially in women working in IT industries, as they don't get enough exposure to sunlight. In such cases, one can use medicated lipsticks for the purpose of curing deficiencies and beautification of lips. With this aim and objectives, an attempt was made to formulate and evaluate a multivitamin lipstick.

MATERIALS AND METHODS

Chemicals and Reagents:

Waxes Oils was purchased from research labs. Isopropyl myristate, Propylene glycol, Methanol, 2-propanol, Hexane was purchased from S D fine chemicals. Vitamin C and Vitamin D was received as gift sample from Hetero labs, Hyderabad.

Formulation of a multivitamin lipstick

The multivitamin lipstick was formulated as per general method of lipstick formulation. In this formulation, white bees wax, ozokerite wax, carnauba wax, lanolin, castor oil, were melted in porcelain dish on water bath with decreasing order of their melting point.Pigment lake colours, perfume and preservatives are mixed with castor oil and heated. Both phases were mixed at same temperature. Then mixture was poured into lipstick mould in excess amount and mould was kept on ice bath. After solidification surplus amount was scrapped with blade.

Lipsticks were removed from mould and flamed. Prepared lipsticks were fitted in lipstick container and used for further evaluation [6]

Evaluation of multivitamin lipstick:

***Colour*:** The formulated lipsticks were checked for colour, gloosy and smooth texture.

***Skin irritation test*:** It is carried out by applying product on skin for 10min and observed for any skin irritation.

***Force of application*:** It is test for comparative measurement of the force to be applied for application piece of coarse brown paper kept on a shadow graph balance and lipstick was applied at 45° angle to cover a 1sq. inch area until fully covered. The pressure reading is an indication of force of application.

***Perfume stability*:** The formulated multivitamin lipstick was tested for 30 days to record the fragrance.

***Surface anomalies*:** This was studied for the surface defects, such as no formation of crystals on surface, no contamination by moulds, fungi, etc.

***Aging stability*:** The product was stored in 40° for 1 hr; various parameters such as bleeding, crystallization on surface and ease of application were observed. [7]

***Solubility test*:** The formulated multivitamin lipstick was dissolved in various solvents to observe the solubility.

***Softening point*:** Lipstick was placed with protruded salve in the flat bottom tube fixed with a thermometer through a cork in such a way that the bulb of the thermometer just touches the lipstick salve. Then inserted this arrangement into a one litre beaker filled with water to a level one centimetre above the upper tip of the lipstick salve. Slowly heat the water while stirring so that temperature rises at a rate not exceeding 2°C per minute. When the temperature reached about 45ºC, raised the temperature at the rate of 1°C per minute. Temperature was recorded when the salve starts bending and losing its shape. Softening point of lipstick should be 55°C. The same procedure is followed for evaluation of marketed lipstick.

***Rancidity*:** 5mg of lipstick and 5ml of chloroform was taken in a test tube and to it added 6ml of 3% solution of trichloroacetic acid in glacial acetic acid and 1ml of 1% solution of phloroglucinol in glacial acetic acid. The test tube was incubated at 45^{0}C for 15 minutes. After incubation, 4ml of ethanol was added and immediately measured the absorbance at 545nm. Absorbance value below 0.15 indicates no rancidity. Absorbance value greater than 0.2 indicates incipient rancidity. Absorbance value greater than 1 indicates high rancidity. The same procedure is followed for evaluation of marketed lipstick.

***Breaking load test*:** This test gives the value of maximum load a lipstick can withstand before it breaks. The lipstick container was firmly fixed with protruded salve of diameter ranging 11 to 13mm, into a screw type of chuck so that the assembly is perfectly horizontal. Above the lipstick salve, the burette was adjusted just to make a marking at a distance of 1.5cm from the base of the salve where lipstick salve sits in salve holder cup. Aluminium container along with hook was weighed and suspended it on this 1.5 cm mark to slowly release water from the burette into the aluminium container till the salve breaks. Burette reading added with the miss of the suspended container gives the breaking load of the lipstick. As per the requirement, the lipstick should withstand a breaking load of minimum 200. The same procedure is followed for evaluation of marketed lipstick.

***Payoff test*:** This test gives the idea of mass release from the lipstick salve while applying. As per the requirement, the lipstick should pass this test if pay-off is more than 0.0001gm/cm^2.The portion of lipstick salve was chopped off one centimeter from the tip using a sharp blade and made the end portion perfectly flat. Speed motor was run to determine the time required for pulling out 100 cm of paper length. Weigh the lipstick with chopped off tip. Now this lipstick was inserted in the slot arrangement so that the flattened salve portion rests on the surface of the paper strip. Started the constant speed motor and with the help of stopwatch 100 cm length of paper was allowed to run. The lipstick was re-weighed after the rub off and the length and width of the line drawn on the paper strip was measured. Pay-off value is calculated by

$$g/cm^2 = M_1\ M_2/1xb.$$

Where M_1 = mass of the lipstick before the test, M_2 = mass of the lipstick after the test, 1 = length in cm of the line drawn on paper strip, and b = breadth in cm of the line drawn on paper strip[8]. Prepared multivitamin lipsticks were compared with standard marketed formulation for all evaluation parameters mentioned above.

Determination of absorption maximum (λ_{max}):

From standard solution of vitamin C (100µg/mL) and vitamin D (100µg/mL) 10µg/ml for vitamin C and vitamin D was prepared. The scanning for solution of vitamin C and Vitamin D was carried out in the range of 200-400 nm against using methanol as a blank. The maximum absorption (λ_{max}) of vitamin C and vitamin D was found to be at 242nm and 262nm respectively.

Selection of analytical concentration ranges (5-25µg/mL) and Construction of Calibration Curve to check Linearity:

Various concentrations were prepared from the working standard stock solution of vitamin C and Vitamin D (100µg/ml), appropriate aliquots like 1.5, 2.0, 2.5, 3.0, 3.5ml solutions were pipetted in 10 mL graduated tubes. The solution in each tube was made up with methanol to obtain working standard concentration ranging from 15-35µg/ mL. The absorbance of these solutions was measured at 242nm & 262nm by taking vitamin C & vitamin D prepared concentrations in sample cell and methanol in reference cell. Absorbance measured and calibration curve was plotted.

Simultaneous Equation Method: Absorbance of the resulting solution was measured at 242nm for determination of Vitamin C, and at 264 nm for determination of Vitamin D. The amounts of the Vitamin C & Vitamin D present in the sample solutions were calculated by formula as

$$C_x = \frac{A_2ay_1\ A_1ay_2}{ax_2ay_1\ .ax_1ay_2} \text{ ------- equation no 1.}$$

$$C_y = \frac{A_1ax_2\ A_2ax_1}{ax_2ay_1.\ ax_1ay_2} \text{ ------- equation no 2.}$$

$$At\ \lambda_1\ A_1 = ax_1bcx + ay_1bcy$$

$$\lambda_2\ A_2 = ax2bcx + ay_2bcy$$

DILUTED SAMPLES (FORMULATION → A1& A2)

Where A_1 and A_2 are the absorbances of mixture at λ_1 and λ_2

respectively, λx_1 and ax_2 are absorptivities of VITAMIN C at $\lambda_1$and λ_2 respectively. λy_1 and ay_2 are absorptivities of VITAMIN D at λ_1 and $\lambda_2$respectively[9].

Analytical method validation

***Linearity*:** From the mixed standard stock solution II, aliquots ranging from 1.5-3.5mL of Vitamin C & Vitamin D were transferred into a series of 10mL volumetric flasks to provide final concentration range of 15-35 µg/mL.The absorbance of the solutions was measured at 242nm and 264 nm against solvent blank and the absorbance were recorded and coefficient of correlation was calculated.

***Limit of detection and limit of quantification*:** The sensitivity of proposed method for measurement of Vitamin C & Vitamin D were estimated in terms of LOD & LOQ. Limit of detection (LOD) is the lowest amount of an analyte that can be detected but not necessarily as an exact value. Limit of quantification (LOQ) is the lowestamount of an analyte in a sample that can be quantitativelydetermined with suitable precision and accuracy.The LOD and the LOQ were determined according to the International Conference of Harmonization (ICH) guidelines for the validation of analytical procedure.Standard deviation of the response and the slope were calculated by using following formula

$$LOD = 3.3\,\sigma / S, LOQ = 10\,\sigma / S.$$

Where, σ = standard deviation of the y intercept of regression lines and S = slope of the calibration curve. The mean of the slope and standard deviation of response were obtained after plotting three calibration curves.

Precision

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings of homogeneous sample. It provides an indication of random errors in results and expressed as relative standard deviation (%RSD). Precision of the method is reported as repeatability, intraday and inter day precision.

Repeatability

Repeatability assessment of an analytical method is performed by analyzing six replicates of single concentration that is 25µg/mL of Vitamin C and 25µg/mL of Vitamin D. Absorbance of samples were recorded at 242nm and 264nm for Vitamin C and Vitamin D respectively.

Intraday and Interday precision

Variations of results within the same day (intraday) and variation of results between days (inter day) were analyzed.The Intra-assay precision of the proposed method was determined on samples of drug solutions at varying concentration levels (20µg/mL, 25µg/mL and 30µg/mL for Vitamin C & Vitamin D) by analyzing three replicates of each sample as a batch in a single assay run at 242nm, for Vitamin C and at 264nm for Vitamin D respectively. The Inter-assay precision was determined by analyzing the same samples (20µg/mL, 25µg/mL and 30µg/mL for Vitamin C & Vitamin D) in three consecutive days at 242nm, for Vitamin C and at 264nm for Vitamin D respectively.

ACCURACY

Accuracy is the closeness of test results obtained by the method to the true value. Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80%-120% (20µg/mL, 25µg/mL, and 30µg/mL for Vitamin C & Vitamin D) by analyzing three replicates of each sample as a batch in a single assay.Recovery studies for drug product were carried out by adding known amount of standard drug (25µg/mL) to the sample solution (20µg/mL, 25µg/mL, and 30µg/mL).From the extracted sample, 1000µL of sample is transferred in to 10 mL volumetric flask. The volume was adjusted to the mark with the methanol to obtain concentration of 25µg/mL. This solution is used for the estimation of Vitamin C & Vitamin D in formulated lipstick.

RESULTS

Formulations of Lipsticks

Formulation of Lipsticks with Vitamin C & Vitamin D were shown in the Table No. 1 as picture wise. These formulations were

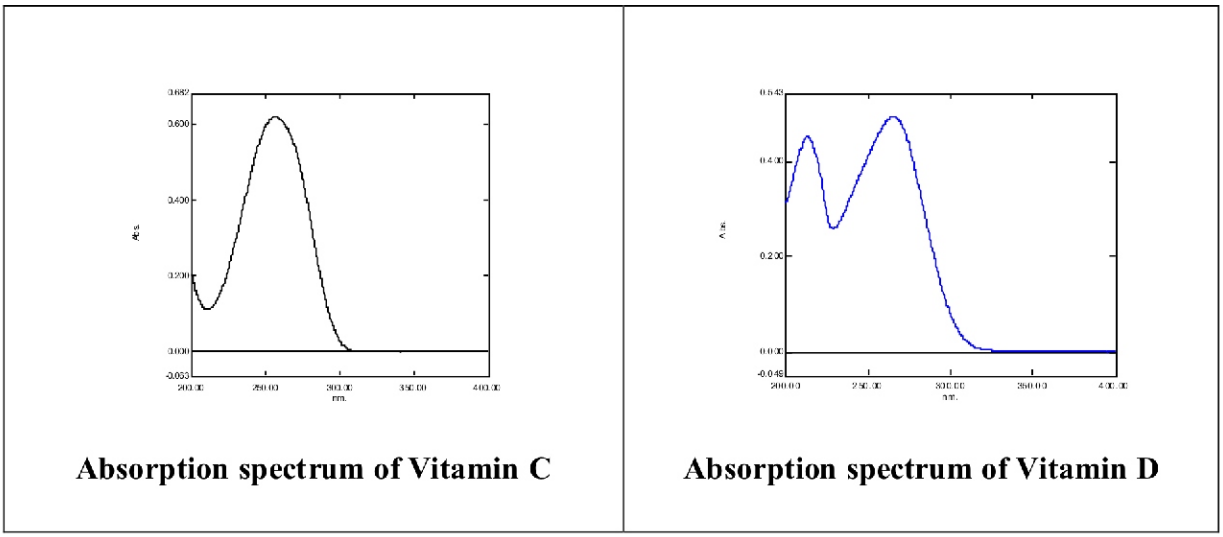


Fig. 1 : Determination of Absorption Maximum of Vitamin C &Vitamin D

Absorption maximum of vitamin c and vitamin d at 242nm and 264nm.

Table 1 : Compositions and images of different formulations of Lipsticks with vitamin C and vitamin D are shown.

FORMULATIONS			
INGREDIENTS	**Formulation 1(10 Lipsticks)**	**Formulation 2(10 Lipsticks)**	**Formulation 3(10 Lipsticks)**
VITAMIN D	250mg	250mg	250mg
VITAMIN C	250mg	250mg	250mg
BEES WAX	9g	9g	9g
OZOKERITE WAX	5g	5g	5g
CARNAUBA WAX	1.5g	1.5g	1.5g
CETYL ALCOHOL	1g	1g	1g
LANOLIN	2.5g	2.5g	2.5g
CASTOR OIL	10mL	10mL	10mL
ISOPROPYL MYRISTATE	5ml	5ml	5ml
PROPYLENE GLYCOL	5ml	5ml	5ml
LAKE COLORS	0.25mg	0.25mg	0.25mg
PERFUMES, PRESERVATIVES	Q.S	Q.S	Q.S
Image of Lipstick			

further used for evaluation tests and validation process.

Evaluation parameters

Various parameters were evaluated for lipsticks formulation and the results were given in the Table 2.

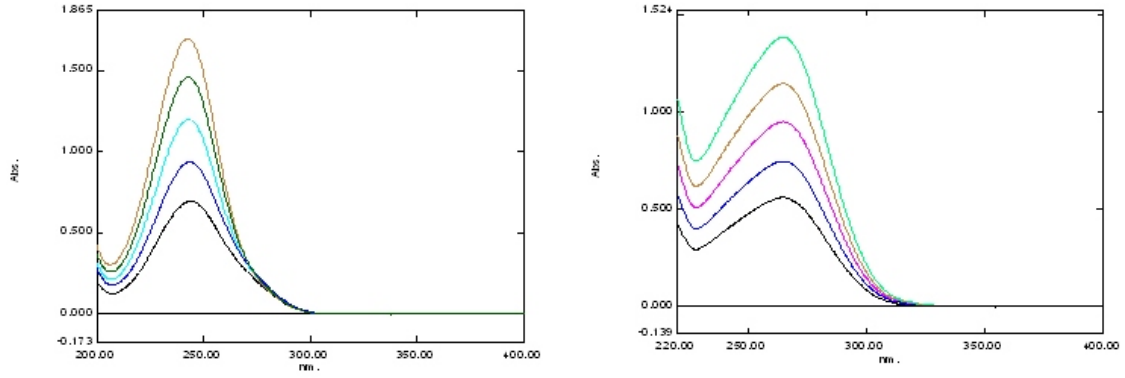
Determination of absorption maximum (λmax):

From standard solution of vitamin C (100µg/ml) and vitamin D (100µg/ml) 10µg/ml for vitamin C and vitamin D was prepared. The scanning for solution of vitamin C and Vitamin D was carried out in the range of 200-400 nm against using methanol as a blank. The maximum absorption (λmax) of vitamin C and vitamin D was found to be at 242 nm and 262 nm. The spectrum is shown in figure 1.

Table 2 : Evaluation parameters

S.No.	Evaluation Parameter	Inference			Marketed lipstick
		F1	F2	F3	
1	Colour	Nude	Orange	Blue	Red
2	Skin irritation test	No	No	No	No
3	Force of application	Good	Good	Good	Good
4	Perfume stability	+++	+++	+++	+++
5	Surface Anomalies	No Defect	No Defect	No Defect	No Defect
6	Aging stability	Smooth	Smooth	Smooth	Smooth
7	Solubility test	Chloroform	Chloroform	Chloroform	Chloroform
8	Softening point	50^0C	50^0C	55^0C	55^0C
9	Rancidity	2.793	1.623	2	2.793
10	Breaking Load Test	160	210	200	210
11	Pay-off Test	0.034g/cm^2	0.029g/cm^2	0.022g/cm^2	0.038g/cm^2

The results obtained for evaluation parameters such as softening point, rancidity, breaking load test and pay-off test are shown in the table above.



Vitamin C Vitamin D

Fig. 2 : Vitamin C & D overlay spectrum linearity

Simultaneous equation method

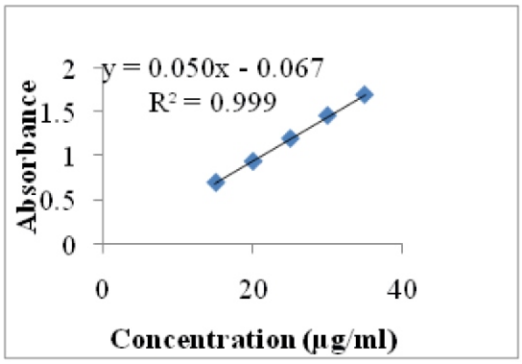
Absorbance values of extract form and pure form of Vitamin C and Vitamin D was performed by simultaneous equation method and values were given in the Table No. 3. The concentration value of Vitamin C and Vitamin D were checked and found to be 24.45µg/ml and 24.25µg/ml respectively.

METHOD VALIDATION

Linearity

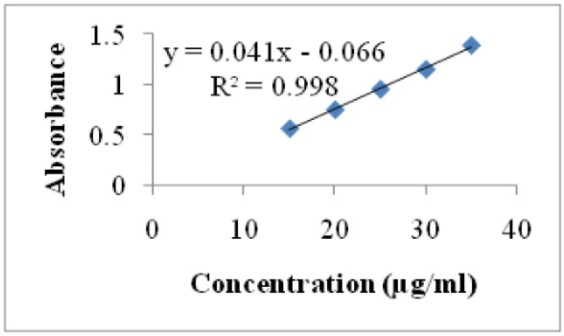
Linear relationship was found in the concentration range of 15-35µg/mL for Vitamin C and Vitamin D and results are shown in Table No.4 and the calibration curve is shown in graph no 1 & 2.

Graph 1: Calibration curve of Vitamin C



The standard graph was plotted between absorbance and concentration and is shown as graph.

Graph 2: Calibration curve of Vitamin D



The standard graph was plotted between absorbance and concentration and is shown as graph

Table 1 : Examples of NSAIDs, conjugates and their activity.

S.NO	DRUG		Abs at 242nm	Abs at 264nm
1	Extract form	Vitamin C	1.018	000
		Vitamin D	000	0.795
2	Pure form	Vitamin C	1.173	0.882
		Vitamin D	0.488	0.609

Absorbance values of extract form and pure form of vitamin C and vitamin D. The concentration value of vitamin C and vitamin D was checked and found to be 24.45µg/ml and 24.25µg/ml.

Table 4 :

Method	Simultaneous Equation Method	
Solvent (diluent)	Methanol	
Wavelength range	200-400nm	
Drug	Vitamin C	Vitamin D
Wavelength for quantitation	242nm	264nm
Linearity range (µg/ml)	15-35 µg/ml	15-35 µg/ml
Coefficient of correlation (r^2)	0.999	0.998
Slope	0.050x	0.041x
Limit of Detection (µg/ml)	0.54	1.20
Limit of Quantification (µg/ml)	1.64	3.65
Precision (% RSD) Intraday precision Interday precision	 0.14 1.26	 0.15 0.28
Accuracy	1.27	1.01
Recovery studies	96.9	98.5

Optical conditions and statistical data of linearity data and values of LOD, LOQ, precision, accuracy and recovery studies data are mentioned in the above table.

Limit of detection and limit of quantification

The values for limit of detection and limit of quantitation by both methods are mentioned in the Table No.4

Precision

The results of intra-day and inter-day precision were expressed as % RSD and it was found to be NMT 2. The results of intra and inter day precision are shown inTable No.4

Accuracy

Accuracy for drug substance was determined on samples of drug solutions for Vitamin C & Vitamin D). The %RSD was calculated and reported in Table No.4it was found that the%RSD values are within the acceptance limits.Recovery studies for drug product were carried out by adding known amount of standard drug (25µg/mL) to the sample solution (20µg/mL, 25µg/mL, and 30µg/mL). The % recovery were calculated for Vitamin C & Vitamin D and recorded in the Table No.4 respectively.

DISCUSSION

Cosmeceuticals is a rapid growing area in personal care sectors, which extends from facial products to skin and body products[10,11,12].A forecasted report suggested that by 2025, the global market for cosmeceuticals will reach ~45 billion dollars and lead to a great demand in near future[13].Cosmetics include lotions, powders, lipsticks, deodorants, baby products, bath oil, bubble bath products, bath salts and are in great demand in both developing and developed countries[14]. Lipstick is a lipcolouringformulation that has its earliest use dating back to the prehistoric age. In the present day, the use of lipsticks has increased and are available in variety ofcolorshades, textures and lusters[15].

Vitamin D is a fat-soluble vitamin generally referred as Vitamin D3 (cholecalciferol) comes under the group of calsiferols[16]. 90% of Vitamin D is synthesized endogenously in the skin under sun exposure[17].It is used tomaintain normal blood

levels of calcium and phosphate that are required for normal mineralization of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. It is also important for immune function, for inflammation, cell proliferation, and differentiation[17,18].

Vitamin D deficiency is quite rampant in India. Increased indoor lifestyle, (prevent adequate exposure to sunlight) modernization, pollution (hamper the synthesis of Vitamin D in the skin by UV rays)[19], changing food habits (like low dietary calcium and Vitamin D intake), increased skin pigmentation and application of sunscreens, cultural practices such as the burqa and purdah system[20] are various reasons for vitamin deficiency in the urban population.

Vitamin C (ascorbic acid) plays a major role in the metabolism ranging from the synthesis of collagen, carnitine and norepinephrine to a large number of antioxidant activities[21]. Human body cannot synthesize vitamin C and is dependent on dietary sources, mainly citrus fruits and vegetables.It is needed for the health and repair of various tissues in body, including skin, bone, teeth and cartilage. Vitamin C deficiency in the diet can lead to a condition called scurvy and symptoms include bruising, bleeding of gums, joint and muscle pains.Life style changes like western dietary intake is the main reason for the deficiency of vitamin C in the urban women.

From the extensive literature survey, our study initiated to formulate multivitamin lipstick which is a common cosmetic used by urban women in day-to-day life and can be a simple solution to prevent vitamindeficiency.

The aim of this study is to formulate and evaluate vitamin D and C in multivitamin lipstick. This study is also aimed to develop and validate the simultaneous equation method for estimation of vitamin D and C in multivitamin lipstick.

Multivitamin lipstick is formulated by incorporating vitamin D and C and evaluated for organoleptic parameters like colour, skin irritation. The results were found to be in line with standards and marketed lipstick as shown in Table 1. The linearity range for vitamin D and C was found to be in the range of 15-35µg/mL at respective selected wavelengths (Gragh 1 &2). The coefficient of correlation for vitamin D at 264 nm and for vitamin C at 242 nm is 0.998 and 0.999, respectively. Both drugs showed good regression values at their respective wavelengths, and the results of a recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method.

The validity and reliability of proposed method was assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non-interference of formulation additives in estimation (Table 4). Precision was determined by studying the repeatability and intermediate precision. Repeatability indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. The % RSD was calculated for vitamin D and vitamin C. The results are mentioned in Table 4. A precision study expresses within a laboratory variation in different days. In intra- and inter-day precision studies for the proposed method % RSD are not more than 2.0% indicates good repeatability and intermediate precision [table 4]. The LOD and LOQ values for vitamin D and vitamin C are 1.20, 0.54µg/ml, and 3.65, 1.64 respectively. Low values of LOD and LOQ indicates good sensitivity of proposed methods shown in Table 4.

CONCLUSION

The present work was aimed to formulate a lipstick using vitamin C and D to prevent the vitamin's deficiency in women. The prepared formulations (F_1, F2, and F3) were evaluated for organoleptic properties such as spreading, hardness, surface anomalies, aging, softening point and found to be satisfactory. A new, simple and sensitive UV simultaneous equation method was developed and validated for the simultaneous estimation of vitamin Cand vitamin D.From the results, it can be concluded that the formulated and developed method of lipstick is simple, accurate, precise, sensitive and cost effective and can be used for simultaneous estimation of vitamin C and vitamin D from the formulated lipstick. This type of formulations can become cost effective treatments for vitamin deficiencies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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