INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN: 2320-2831

IJPAR |Vol.3 | Issue 4 | Oct-Dec-2014
Journal Home page: www.ijpar.com

Research article *Open Access*

A new reverse phase HPLC method for quick quantificationof all four isomers of natural vitamin-E oil in a single analysis.

N.K.Sarathchandraprakash*, Nagaraja.P.E., Prasanna Kumar T.P., Krishan Manral

Analytical Cosmetics Division, R&D Center, The Himalaya Drug Company, Makali, Bangalore 562123, India.

***Corresponding author: N.K.Sarathchandraprakash**
E-mail id: sarathchandra@himalayawellness.com

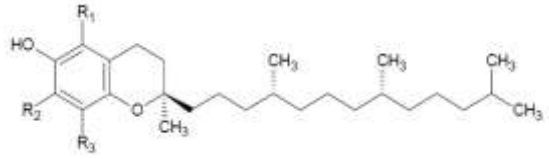
ABSTRACT

A simple and fast analytical method for quantification of natural vitamin E isomers by reverse phase High Performance Liquid Chromatography within short time. This method is developed to quantify in four natural isomers of vitamin- E, namely alpha, beta, gamma and delta tocopherol in fine formulations. The four isomers elution was carried out by using simple short column (100 X 4.6 mm) were used. And even mobile phase has used only Acetonitrile and water. The Limit of detection was achieved of isomers are very lower levels like 3 ppm. The method is capable to quantify 10 ppm to 100 % in a sample. The method is reliable robust, accurate and linear.

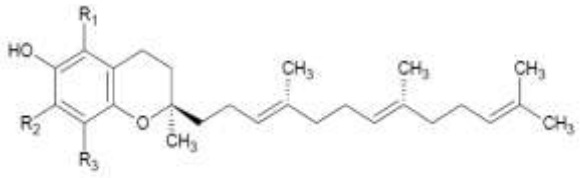
Keywords: Natural Vitamin E, Tocopherols, Alpha tocopherol, betatocopherol, gammatocopherol, delta tocopherol, tocotrienol, antioxidant.

INTRODUCTION

Vitamin E is a fat-soluble antioxidant that exists in eight major different isomeric forms – four tocopherols and four tocotrienols. Both tocopherol and tocotrienol have an alpha, beta, gamma and delta form. Each of the form has its own biological activity, measure of potency and functional use in the human body [(1)]. Alpha-tocopherol is the most active form of vitamin E in humans, and is a powerful biological antioxidant[(2)]. It is a 6-hydroxy chroman derivative (chromene) with a methyl group in positions 5, 7 and 8 and a phytyl side chain attached at carbon 2[(3)]. Tocotrienols have the same structure as tocopherols except for thethree double bonds in the side chain.Figures 1 and 2 give the schematic presentation of the molecular structuresof tocopherols and tocotrienols[(4)].



(Figure 1: Schematic presentation of the chemical structures of alpha-, beta-, gamma- and delta-tocopherol.)



(Figure2: Tocotrienols have the same basic structure as tocopherols, only with three unsaturated double bonds in the side chain.)

Compound	Formulae	R_1	R_2	R_3	Molecular weight
α-tocopherol	**C29H50O2**	**CH3**	**CH3**	**CH3**	**430**
α-tocotrienol	**C29H44O2**				**424**
β-tocopherol	**C28H48O2**	**CH3**	**H**	**CH3**	**416**
β-tocotrienol	**C29H42O2**				**410**
γ-tocopherol	**C28H48O2**	**H**	**CH3**	**CH3**	**416**
γ-tocotrienol	**C28H42O2**				**410**
δ-tocopherol	**C27H46O2**	**H**	**H**	**CH3**	**402**
δ-tocotrienol	**C27H40O2**				**396**

These commercially available mixed tocopherolsare used as antioxidants to prevent rancidity in fats and vegetable oils at a recommended dosage of 0.02-0.03% w/w of the fat content [(12)].

Commercially available vitamin E is of medium viscosity and is an oily liquid of amber to light brown incolor. It is insoluble in water but soluble in fats and vegetable oils.

Methods and Experiment

Mixed tocopherols as reference were organized from Sigma-Aldrich (US) and the product number is W530066.Rest of the solvents was used HPLC grade and organizedfrom Rankem.

Standard Solution

The standard solutions of mixed tocopherols (α, β, γandδ) were prepared to a concentration of 1mg/ml in acetonitrile.Before injectingthe 5 µl solution onto the HPLC column, it was passed through a0.45-µm filter. The detection limitfor all the four isomers was 3 ppm.

Sample Solution

The natural vitamin E sample solution was prepared to a concentration of 1mg/ml in acetonitrile. Before injectingthe 5 µl solutiononto the HPLC column, it was passed through a0.45-µm filter.

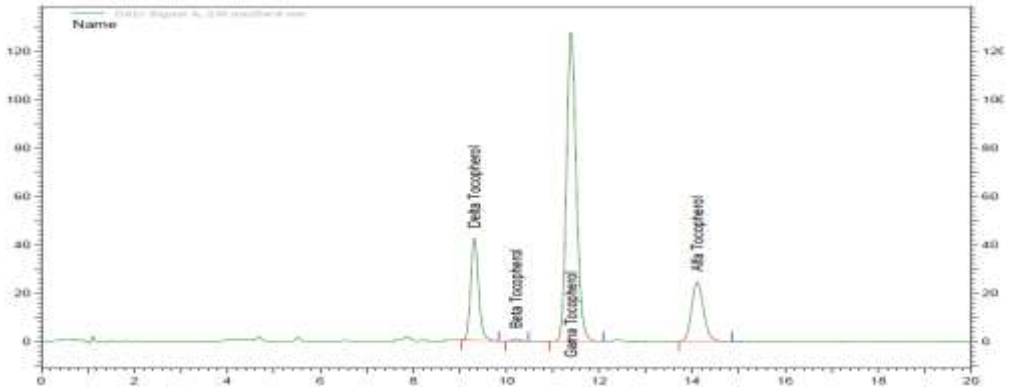
Instrumentation

Separation by HPLC was carried out using a Shimadzu. High Performance Liquid chromatography LC-20A Series, equipped with solvent Delivery Module LC-20AD, Photodiode Array UV-VIS Detector, SPD-M20A, Auto Sampler SIL-20 AHT, Column Oven CTO-10 ASvp, and Software Lab Solution LC.

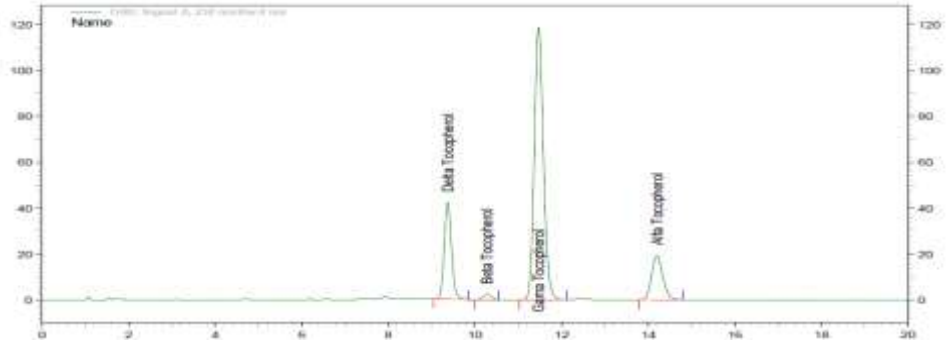
Chromatographic Conditions

The mobile phase consists of acetonitrile (95%) and water (5%) at a flow rate of 1.0 ml/min.The quantitative HPLC separation was performed on Chromolith® High Resolution RP-18 end-capped [(13)]column (100 x 4.6 mm, i.d., particle size 5 µm).The temperature of the column was 25°C andthe detector was set at a wavelength of 230 nm.

The tocopherols were identified and quantified by using the method of retention time.



(Chromatogram-1: MixedTocopherol (α, β, γand δ) standard)

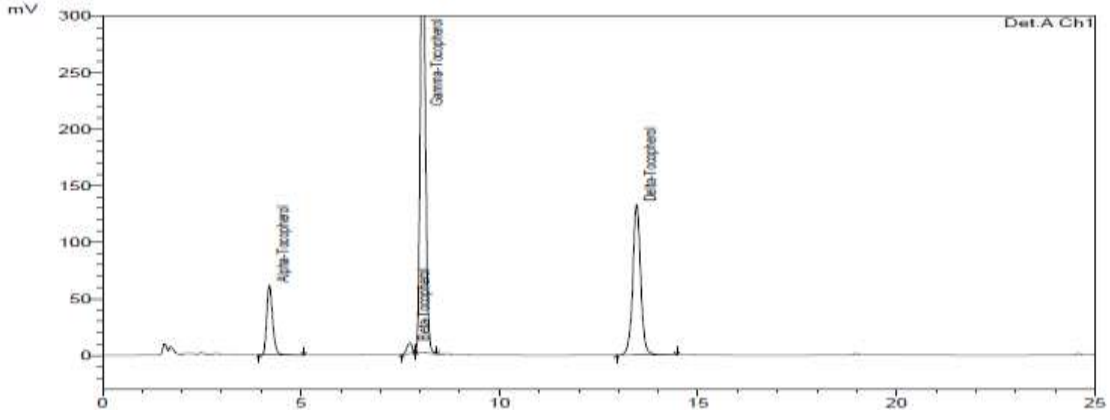


(Chromatogram-2: Commercially obtained natural vitamin E)

Table-1

Sl.No.	Tocopherols	Results(in mg)
1	α–tocopherol	103.7
2	β-tocopherol	30.2
3	γ-tocopherol	472.8
4	δ-tocopherol	148.8

(Table 1 shows the quantification of individual tocopherols in commercially available natural vitamin E)



Chromatogram 3: Mixedtocopherol (α, β, γandδ)standards bynormal phase chromatography

Normal phase HPLC, the most widely used technique to analyze tocopherol, separates all the isomers of tocopherol (α, β, γ and δ) [14] [15] [16] [17]. Some reverse phase HPLC methods can also be used to separate the

isomers of tocopherol, but all isomers cannot be separated (β, γ); they can only separateisomers likeα, β+γ and δ [18] [19].Here, we have developed a reverse phaseHPLC method that can separate all isomers of tocopherols (α, β, γ and δ) within a short run time.

Validation of the Optimized Method

The method was validated as per the ICH guidelines for the validation of analytical procedures, [22] and parameters for accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness were determined.

DEMONSTRATION OF LINEARITY

Standard Linearity: Linearity was checked by preparing five concentrations of the substance ranging from 50% to 150%. A concentration of 100 ppm was proposed in the procedure. Hence, the test substance was prepared at concentrations of 0.5mg/ml, 0.75mg/ml, 1mg/ml, 1.25mg/ml and 1.50mg/ml for determininglinearity. Estimations were carried out as per the procedure mentioned. Observations were recorded and a linearity curve was prepared using regression analysis.

Linearity graph

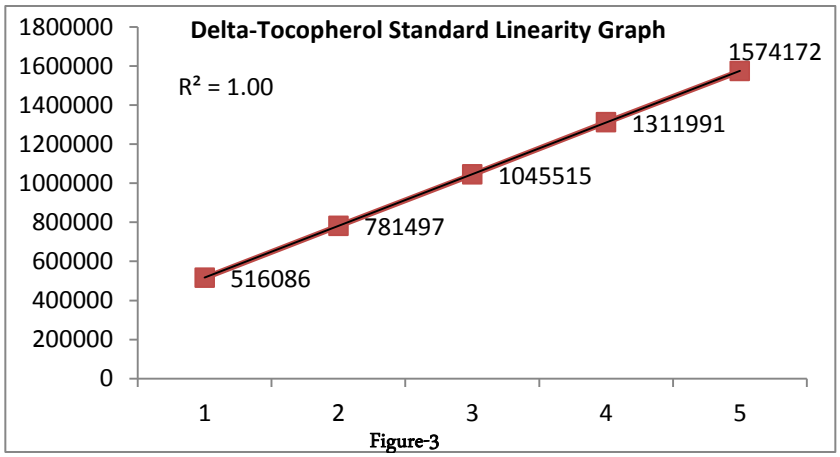


Figure-3

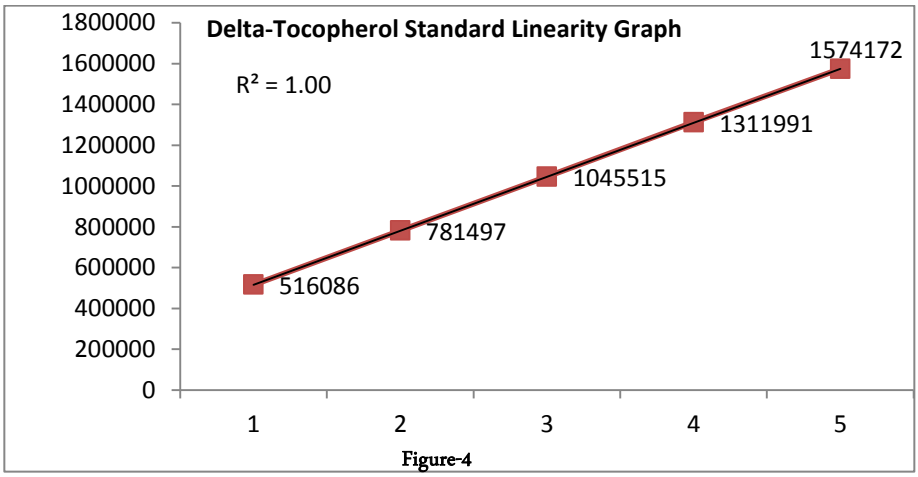


Figure-4

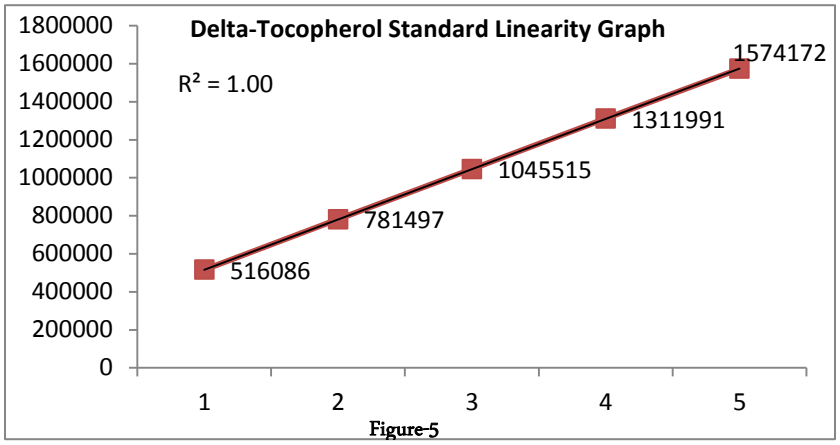


Figure-5

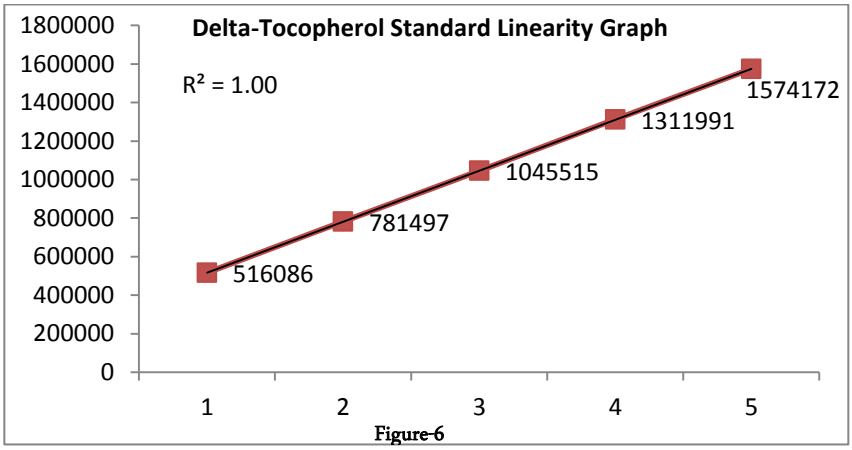


Figure-6

Sample Linearity

Linearity was checked by preparing at different concentrations of 0.5mg/ml, 0.75mg/ml, 1mg/ml, 1.25mg/ml and 1.50mg/ml for determining linearity. Estimations were carried out as per the procedure mentioned. Observations were recorded and a linearity curve was prepared using regression analysis.

Linearity graph

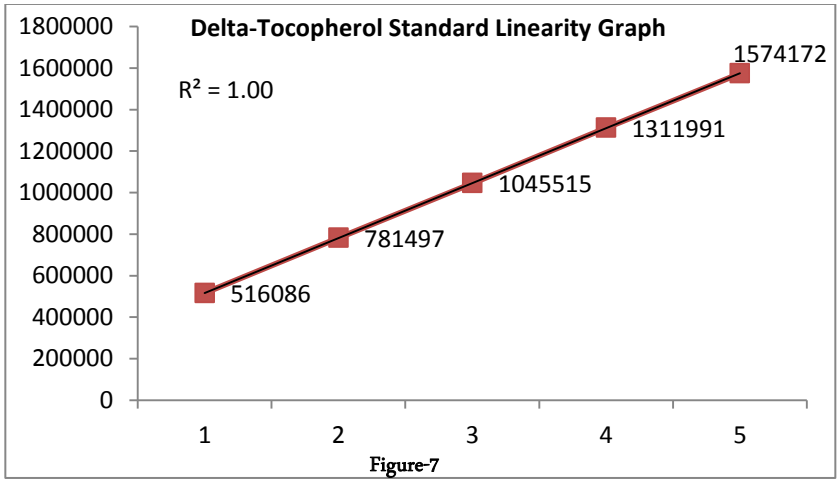


Figure-7

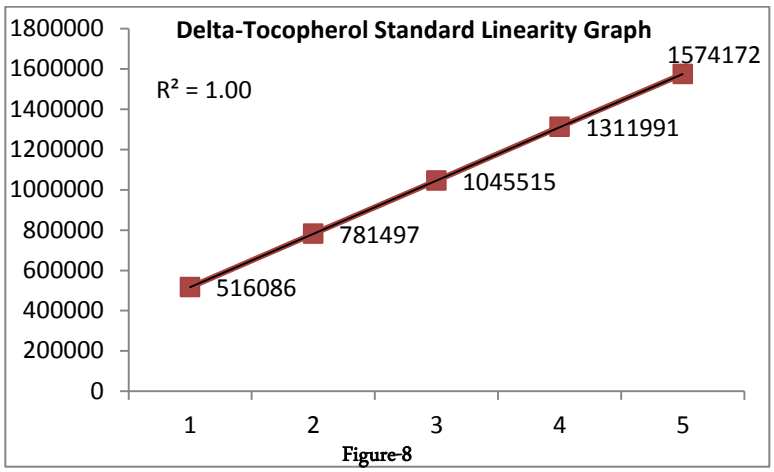


Figure-8

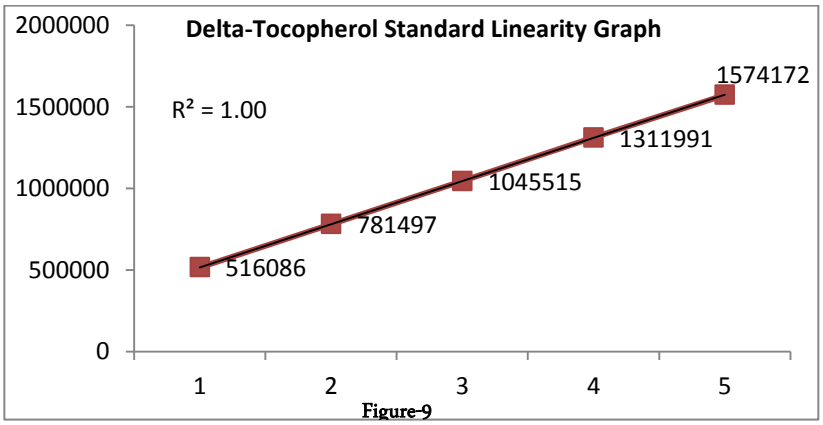


Figure-9

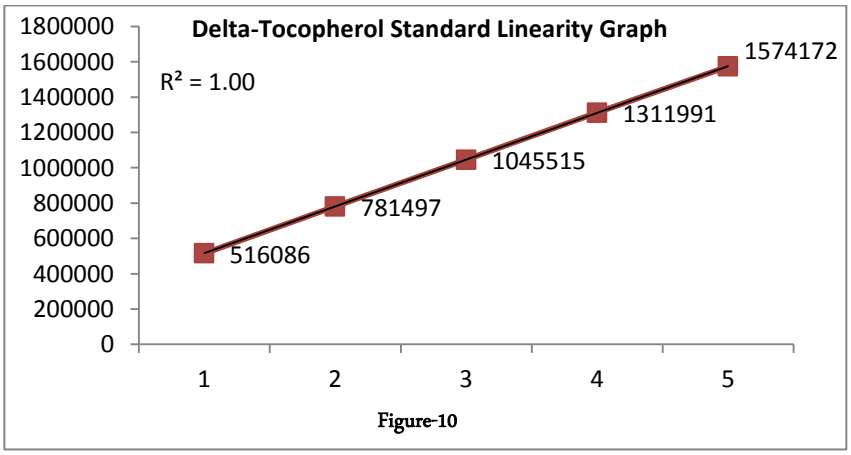


Figure-10

ROBUSTNESS

During the robustness study, a slight change in the column flow was observed butnone of the instruments showed any variation during the process. The change in flow rate did not haveany impact on the percentage of RSD, which was found to be 0.48%, 0.51%, 0.43% and 0.74% for delta-tocopherol, beta-tocopherol, gamma-tocopherol and alpha-tocopherol, respectively. With the help different systems, the % RSD was found to be 0.90%, 1.00%, 0.86% and 1.20% for Delta Tocopherol, Beta Tocopherol, Gama Tocopherol and Alpha Tocopherol respectively.

DEMONSTRATION OF ACCURACY

Accuracy was determined by spiking the standard mixed tocopherols at three different levels of 0.75mg/ml, 1mg/ml and 1.25mg/ml.

Name	Accuracy Level	Amount added	Amount recovered	Recovery (%)	Acceptance Criteria
Delta-tocopherol	0.75 mg/ml	0.75 mg/ml	0.77 mg/ml	103.21	Between 80 and 120%
	1.00 mg/ml	1.00 mg/ml	1.06 mg/ml	105.53	
	1.25 mg/ml	1.25 mg/ml	1.31 mg/ml	105.06	
Beta-tocopherol	0.75 mg/ml	0.75 mg/ml	0.77 mg/ml	103.24	
	1.00 mg/ml	1.00 mg/ml	0.99 mg/ml	99.34	
	1.25 mg/ml	1.25 mg/ml	1.28 mg/ml	102.00	
Gamma-tocopherol	0.75 mg/ml	0.75 mg/ml	0.80 mg/ml	107.04	
	1.00 mg/ml	1.00 mg/ml	1.09 mg/ml	109.65	
	1.25 mg/ml	1.25 mg/ml	1.34 mg/ml	107.60	
Alpha-tocopherol	0.75 mg/ml	0.75 mg/ml	0.75 mg/ml	99.57	
	1.00 mg/ml	1.00 mg/ml	1.07 mg/ml	102.65	
	1.25 mg/ml	1.25 mg/ml	1.25 mg/ml	100.11	

(Table 2: Recovery (%) of mixed tocopherol standard)

DEMONSTRATON OF PRECISION

Standard solution was analyzed as per procedure by analyst 1 in 6 replicates (Repeatability). Intermediate precession was performed with different analyst (Analyst 2).

Standards	Preparation	Results	%RSD	Acceptance Criteria
Delta-tocopherol	100% concentration	1 mg/ml	0.29	Less than 10%
Beta-tocopherol			1.84	
Gamma-tocopherol			0.56	
Alpha-tocopherol			0.55	

Table 3: Repeatability study (Analyst 1)

Standards	Preparation	Results	%RSD	Acceptance Criteria
Delta-tocopherol	100% concentration	1 mg/ml	0.24	Less than 10%
Beta-tocopherol			1.79	
Gamma-tocopherol			0.09	
Alpha-tocopherol			0.30	

Table4: Intermediate precision study (Analyst 2) Sample (natural vitamin E) solution was analyzed as per the procedure by analyst 1 in 6 replicates (Repeatability)

Standards	Preparation	Results	%RSD	Acceptance Criteria
Delta-tocopherol	**100% concentration**	**1 mg/ml**	**0.29**	**Less than 10%**
Beta-tocopherol			**1.84**	
Gamma-tocopherol			**0.56**	
Alpha-tocopherol			**0.55**	

Table 5: Sample repeatability (Analyst 1)

Limit of Detection and Quantification

Beta-tocopherolhas the lowest response compared to other isomers like delta-, alpha- and gamma-tocopherol. Hence,beta-tocopherol is taken into account for LOD and LOQ tests. For beta-tocopherol, the limit of detection is 3 ppm and limit of quantification is 10 ppm.

RESULTS AND DISCUSSION

The new developed reverse phase HPLC method was validated as per ICH guidelines. The method shows very good, simple, linear, accurate and repeatable.

REFERENCE

[1] Barrie Tan, Ronald Ross Watson, VictorR.Preedy., "TOCOTRIENOLS: vitamin e beyond tocopherols", page no.23, Second Edition-2012.
[2] Jack Challen, "User's Guide to Nutritional Supplements", page no.49,2003.
[3] W P Edwards,"the Science of Bakery Products", page no-103, 2007.
[4] Ronald Eitenmiller,JunsooLee, "Vitamin E-Food Chemistry,Composition and Analysis", Page no.03, 2004.
[5] The EFSA Journal 640, page no.06, 2008.
[6] Maria Laura Colombo,Molecules, ISSN 1420-3049, page no.2107, 2010.
[7] V.R.Preedy and R.R.Watson, "The Encyclopedia of Vitamin E", .page no.05, 2007.
[8] Cheryl M.Burgess, "Cosmetic Dermatology",page no.20, 2005.
[9] Lester Packer,Jurgen Fuchs, "Vitamin E in Health and Disease",page no.03, 2003.
[10] AroleS. Ramke, "How to stop Colds. Allergies and more", page no.132, 2012.
[11] www.adm.com/en-US/products/Documents/ADM-Natural-vs-Synthetic-Vitamin-E.
[12] Richard D.O, Brien, "Fats and Oils", page no.216, 2009.
[13] www.merckmillipore.com/showBrochure/200803.023.ProNet.pdf,page no.181,
[14] Leo M.L.Nollet, FidelToldra., "Food analysis by HPLC" , thirdedition, page no.293, 2012.
[15] Journal of Chromatography A, 881, 217–227,page no.221, 2000.
[16] Journal of Liquid Chromatography&RelatedTechnologies, page no.790, 2005.
[17] Anna maijalampi,Cereal Chem. 85(2),page no.248–251.
[18] Not Bot HortiAgrobo, 41(1), page no.94, 2013.
[19] Journal of Chromatography A, 881, page no.253, 2000.
[20] Lloyd R. Snyder, Joseph J. Kirkland,John W. Dolan" Introduction to modern Liquid Chroma - tography",Thirdedition., Wiley, 2011,
[21] Maya Shankar Singh., "Reactive Intermediates in Organic Chemistry", page no.115, 2014.
[22] ICH Guidelines (ICH Q2 R_1) , 2005.