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A simple colorimetric assay to evaluate the pharmaceuticals for alpha-tocopherol and its anti-oxidant potential

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Abstract: A new colorimetric method is proposed to determine α-tocopherol and its anti-oxidant activity by monitoring the ability of α-tocopherol to cause quantitative reduction of ferric ions. The method, based on Trinder reaction, includes incubating 1 µmol of ferric chloride in presence of 0 and 15 through 120 µmoles of α-tocopherol followed by detection of the iron (III) with standard mass of salicylic acid, 30 mg. Evaluation is done by monitoring decrease in absorbance at 525 nm and calculating estimated decrease in ferric ion mass as linear function of α -tocopherol over the linear test concentration range (r ± s.e. = 0.999 ± 0.001). The regression estimate, b ± s.e, obtained as 5.5 ± 0.1 nmoles iron (III) reduced μg^{-1} α-tocopherol is closer to and better than the value, 4.64, obtained with standard pharmaceutical assay for α-tocopherol based on ceric reduction. The method has been used to evaluate the effect of saponification in absence and presence of pyrogallol on the recovery of α –tocopherol, and to compare the pharmaceuticals containing α –tocopherol for their relative antioxidant potentials. The work provides an alternative simple, rapid and inexpensive colorimetric method to evaluate the pharmaceuticals for these activities.
Key words: Trinder reaction, Salicylic acid, Ferric reduction, α-tocopherol, Antioxidant.

Introduction

The Emmerie-Engel reaction is widely employed for colorimetric determination of α-tocopherol in foodstuffs, plant materials, pharmaceuticals and serum[1-4]. It has been used for detection of adulteration of dairy food products with vegetable oils[5] and for determination of anti-oxidant potential in plant materials and plasma[6-9]. The method is based on reduction of ferric ions and monitoring mass of ferrous ions by ion-specific chelators forming colored complexes. The method is widely employed for routine analytical works particularly in the laboratories where advanced and expensive technologies including those based on GC and HPLC[1, 10 -14] are not easily available. However, alternative colorimetric methods are needed to overcome the limitations of the reagents currently employed for determination of ferrous ions[1, 15-16]. Trinder reaction, based on selective interaction of ferric ions and salicylic acid, has been employed for rapid determination of salicylates with ferric reagent[17-18] and for selective determination of ferric ions by salicylic acid reagent in photometric titrimetry[19]. These observations provided rationale to optimize an alternative method based on Trinder reaction to determine free α-tocopherol and its antioxidant potential in selected pharmaceuticals by monitoring decrease in ferric ions rather than monitoring an increase in ferrous ions. Consequently, the experiments were designed to: i) optimize the Trinder reaction for spectrophotometric determination of ferric ions, ii) establish linearity parameters for quantitative relationship between increase in α-tocopherol content

and decrease in ferric ion concentration, iii) evaluate the technique by determining recovery of α-tocopherol as affected by saponification in absence and presence of pyrogallol, and (iv) evaluate test pharmaceuticals containing α-tocopherol for their relative antioxidant potentials.

Experimental

Experiments were carried out at 18 ± 1 ^{0}C ambient temperature. All chemicals used were of analytical grade. The pharmaceuticals included capsules: Bio-E 400 (Dr.Reddy's Laboratories (India) Ltd., Hyderabad), Evion 400 (Merck (India) Ltd., Goa), Tocofer 200 (Torrent Labs (India) Ltd., Ahmedabad), and Revup (Arista Pharmaceuticals (India) Ltd., Mumbai). The gross weights of Evion 400, Tocofer 200, Bio-E 400 and Revup were respectively as mean ± s.e. in mg, 674 ± 6 (n = 15), 522 ± 2 (n = 27), 573 ± 3 (n = 12), and 634 ± 6 (n =12). Bio-E contains free α-tocopherol derived from natural sources equivalent to 268.4 mg. Evion and Tocofer contain α-tocopheryl acetate (I.P.) respectively as 400 and 200 mg per capsule. Each soft gelatin capsule of Revup contains α -tocopheryl acetate 25 mg, beta-carotin 10 mg, ascorbic acid 100 mg, and minerals such as Zn 7.5 mg, Mn 1.5 mg, Cu 1 mg (all as sulfates) and Se 150 µg (as selenium dioxide). The contents of the pharmaceuticals, randomly selected from different batches, were carefully reconstituted in ethanol as 2 % (w/v) of labeled mass of active ingredient. The reconstituted samples were stored in dark at 4 ^{o}C in amber colored tightly stoppered glass containers until use.

Reagents

Ferric chloride solution: The stock solution contained anhydrous ferric chloride 0.25 % (w/v) in 0.1 M HCl. equivalent to 15.4 mM iron (III). Working solution containing 1 µmole of iron (III) per mL was prepared by dilution of appropriate aliquot of stock solution in methanol at the time of use.

Salicylic acid reagent: 6 % (w/v) salicylic acid in methanol.

Ethanolic KOH: 5.6 % (w/v) KOH in absolute ethanol.

Standard α-tocopherol: Reconstituted Bio-E was used as a Reference Laboratory Standard

(RLS) for evaluation of other pharmaceuticals. The preparation was standardized against the reagent grade standard DL-α –tocopherol liquid (Hi-Media, Mumbai, India) by using the approved pharmaceutical assay based on the reduction of standard ceric sulfate as per the procedure of Indian Pharmacopoeia[20]. Ceric ammonium sulfate was standardized by iodometry against standard sodium thiosulfate with potassium dichromate as primary standard[19].

Saponfication and purification

An aliquot of 20 to 30 mL of reconstituted pharmaceutical containing α-tocopheryl acetate equivalent to 400 to 600 mg was added 10 mL ethanolic KOH per each 100 mg of the labeled active ingredient. The saponfication continued for exactly 3 minutes on thermostatically maintained hot plate at 85 ± 5 ^{0}C with flask resting on an asbestos sheet. The timing commenced from the onset of bubbling in the sample. The moistened stopper was lightly held inclined to allow the air in the flask to escape during initial warming. The stopper was removed as soon as bubbling commenced. The inner surface of the flask was constantly sprayed with a fine stream of ethanol following about one minute's onset of bubbling to avoid any thermal loss. Following saponification, the excess alkali was neutralized by drop wise addition of concentrated HCl ensuring minimal acidity in the purified samples. Each 560 mg of added KOH required about 0.9 mL of HCl for the purpose. The samples cooled to room temperature were diluted with three volumes of water, added 2 mL chloroform per 100 mg sample, shaken vigorously for a minute and allowing phases to separate. The aqueous alcoholic phase was discarded. The chloroform phase was washed thrice with 50 to 100 mL portions of distilled water till acidity of the last aqueous phase remained less than 0.01 N. Measured volume of chloroform phase was added an equal volume of ethanol, and the mixture maintained in hot water at 70 to 80 ^{0}C to remove residual chloroform and to reduce the volume to about half the volume of ethanol used. The samples were cooled to room temperature, and reconstituted to 1 % (w/v) as estimated free tocopherol in ethanol. The purified samples were maintained in dark at 4 ^{o}C until assay. The concentration of free tocopherol was calculated by multiplying the known acetate content by factor 0.91. An aliquot of RLS was subjected to the identical saponification process in absence and presence of 20 to 25 mg of pyrogallol per sample to elucidate the effects of saponification and pyrogallol on recovery of α-tocopherol, and also to enable comparative evaluation of the four test pharmaceuticals subjected to identical treatments. A parallel reagent control was simultaneously run through the whole process, and assayed along with the test preparations for any background activity. Working solutions for the assay samples were always made in methanol.

Determination of ferric ions by salicylate method

Two milliliter methanolic samples containing 0, 0.1, 0.2, 0.5, 1.0 and 2.0 µmoles ferric chloride were each added 0.5 ml salicylic acid reagent, and the volume made 4.0 ml each with methanol. The samples were mixed and allowed standing at room temperature. The

color was monitored at 525 nm twice at 25 to 30 minutes and at 50 to 60 minutes against reagent blank to optimize time factor. Each iron sample included its own reagent blank without salicylic acid reagent.

Determination of α-tocopherol

One milliliter of methanol containing 1 µmol of ferric chloride was added 1 ml methanol containing 0, 15, 30, 50, 75, 100 and 120 µg α-tocopherol, allowed standing at room temperature for about 10 minutes followed by addition of 0.5 ml salicylic acid reagent, and volume was made 4 mL with methanol. The samples were mixed, stoppered, and allowed standing for about 50 to 60 minutes. The absorbance values were monitored at 525 nm using reagent blank containing 1 µmol ferric salt in 4 ml of methanol. The concentration of ferric salt for the assay was chosen from optimization experiments. The assay was tested for linearity, and employed for evaluating the effect of saponification on the recovery of RLS. The pharmaceutical preparations following saponification were assayed in terms of RLS.

For extended time course evaluation studies, each sample of 10 µmol ferric chloride was added 0, 400, 600, 700, 900 and 1000 µg of α-tocopherol in 5 mL methanol. The samples were stoppered, and incubated for 9 days in dark. The aliquots, 0.8 mL each, from the respective samples were withdrawn at days 1, 4 and 9 for monitoring reduction of ferric salt. The assay was performed to elucidate time course of reduction by α-tocopherol.

Evaluation parameters

The parameters for observation included:

C_i = Iron (III) concentration used in the assay, i.e. 1 µmol ferric chloride per sample.

C_s = Standard α-tocopherol mass used in the assay in micrograms.

C_t = Test preparation as free α-tocopherol, estimated as per label, in micrograms.

A_i = Absorbance value obtained with C_i

A_s = Absorbance value obtained with $C_i + C_s$

A_t = Absorbance value obtained with $C_i + C_t$

These parameters have been employed for calculating three functional parameters namely FIRP, FIRA and RAOP values for evaluation of the pharmaceuticals.

FIRP is an acronym for ferric ion reducing potential. The parameter defines absolute mass of ferric ions in nmoles reduced by a given mass of test agent in microgram. It provides antioxidant potential of a test preparation.

The value is given by the equation:

FIRP value, nmol = $[C_i - (A_x / A_i)] * 1000$

where C_i = 1 and A_x stands for A_s or A_t as per the preparation.

FIRA is an acronym for ferric ion reducing activity. It defines mass of ferric ion as nmoles per sample reduced per microgram of the test material. The value is provided by either of the two methods:

(i) Regression coefficient value obtained by relating FIRP values with different masses of the test material in micrograms, and

(ii) Quotient of a FIRP value for a given mass and the mass in µg: $FIRA_x = FIRP_x / C_x$ where subscript x stands for test or standard preparation

The RAOP is an acronym for relative antioxidant potential of a test preparation in terms of a standard preparation taken as unity. The value is provided by either of the following equations depending upon the availability of the required data:

(i) $[(A_i - A_t) / (A_i - A_s)]$;

(ii) $[b_t / b_s]$;

(iii) $[FIRP_t / FIRP_s]$; and

(iv) $[FIRA_t / FIRA_s]$

where b_t and b_s stand for regression coefficients, respectively, of test and standard reparations while relating their different concentrations within the linear range versus the corresponding FIRP values.

The RAOP parameter compares two preparations for their relative antioxidant potential providing the fractional activity in the test preparation in terms of the standard preparation.

Therefore, the value when multiplied by 100 or C_s provides, respectively, per cent purity or mass of α-tocopherol in the test preparation in terms of the standard.

Results and Discussion

The popular techniques in vogue for determination of α-tocopherol are based on quantitative reduction of ferric to ferrous ions. The ferrous ions are determined by their specific interaction with chelators including dipyridy[2, 3], bathophenanthroline[1, 4], or tripyridyl triazine[6, 7]. The present work was mooted to employ the same rationale but choosing to relate decrease in standard ferric ion concentration and decrease in absorbance as parameters to determine α-tocopherol, and its antioxidant potential. The work is based on the Trinder reaction[17] that has been employed for photo-titrimetric assay of ferric ions by salicylic acid using EDTA to mark the end-point[19] and for rapid determination[17] and spot-detection[18] of salicylates in biological fluids by ferric salt.

Determination of ferric ions by salicylate method

Salicylic acid interacts selectively and quantitatively with ferric ions over 0.1 through 2 µmoles in a 4 mL sample forming linear color intensities (Fig.1). Ferrous ions, from ferrous sulfate or ferrous ammonium

Table 1 Effect of saponification on recovery of standard α-tocopherol and parametric analysis

Parameter	Mass, µg	Standard	Saponification	
			Pyrogallol (-)	Pyrogallol (+)
Absorbance	40	0.291 ± 0.001	0.317 ± 0.001	0.309 ± 0.002
	60	0.258 ± 0.001	0.288 ± 0.002	0.258 ± 0.001
	100	0.161 ± 0.001	0.207 ± 0.001	0.163 ± 0.003
FIRP values, nmoles	40	263.3 ± 1.4	197.3 ±1.4	216.0 ± 3.1
	60	347.7 ± 3.0	270.0 ± 4.9	346.0 ± 2.1
	100	591.7 ± 3.3	475.7 ± 2.3	588.3 ± 7.4
r ± s.e	-	0.996±0.003	0.996±0.003	0.999±0.001
b ± s.e	-	5.56±0.17[a]	4.71±0.14[b]	6.18±0.09[c]
FIRA values $nmol\mu g^{-1}$	40	6.57±0.03	4.94±0.03	5.77 ± 0.04
	60	5.80±0.05	4.50±0.08	5.77 ± 0.04
	100	5.92±0.03	4.76±0.02	5.88 ± 0.07
Mean FIRA, $nmol\mu g^{-1}$ (RSD)	-	6.10±0.12[a] (5.9)	4.73±0.07[b] (4.4)	5.81±0.03[c] (1.5)
RAOP[d]	40	1.000	0.751±0.005	0.878±0.005
	60	1.000	0.773±0.014	0.994±0.006
	100	1.000	0.804±0.001	0.994±0.012
Recovery, µg (Per cent)	40	(100)	30.0 ± 0.2 (75.1 ± 0.5)	35.1 ± 0.2 (87.8 ± 0.5)
	60	(100)	46.5 ± 0.8 (77.3 ± 1.4)	59.7 ± 0.4 (99.4 ± 0.6)
	100	(100)	80.4 ± 0.1 (80.4 ± 0.1)	99.4 ± 1.2 (99.4 ± 1.2)
Overall per cent recovery (RSD, %)	40 - 100	-	77.7±0.9 (3.5)	95.6±2.0 (6.3)
	60 - 100	-	79.0±0.9 (2.8)	99.4±0.6 (1.5)

The values at individual concentrations are mean ± s.e of three observations. The values with identical superscripts are not different (P>0.1, n=9 each). [d] RAOP values for treatments are calculated with standard FIRA mean of corresponding concentration. RSD is relative standard deviation. Mean absorbance value standard iron (III), 0.395 ± 0.003 (n=3).

sulfate, over the equivalent concentrations fail to show any visible coloration with the salicylic acid. The absorbance values and regression coefficient observed at about 60 minutes (Fig.1) do not differ from the corresponding values recorded at about 30 minutes (P > 0.1, n = 25 each).

Optimum pH conditions

The Trinder reaction is conducted at acidic pH[17, 18]. The optimal pH ensured by using acetate -HCl buffer for photometric titration of ferric salicylate method is 1.7 to 2.3[19]. The mean pH during the present assay, without use of any buffer, has remained 1.82 ± 0.07 (n =54) with range from 1.55 ± 0.01 (n = 27) to 2.10 ± 0.11 (n=27) respectively in presence and absence of free tocopherol. Acidic pH is largely contributed by salicylic acid reagent followed by acidified ferric chloride solution. The pH values beyond these limits are not favorable for the assay. For instance, in presence of potassium acetate, 20 through 400 µmoles in methanol per sample, the ferric salicylate exhibits a uniform discoloration and opalescence showing increase in pH from 4.12 ± 0.04 through 6.5 ± 0.02 (n = 4 each). Discoloration is lesser, and opalescence fainter with 10 µmoles of acetate, and the normal purplish color returns on addition of 0.1 through 0.3 mL of 0.05 M HCl in methanol with pH returning to 1.87 ± 0.33 (n = 6). Further acidification with addition of the acid from 0.5 through 2 mL causes linear fall in color intensity from mean absorbance of 0.32 down to 0.09 with concomitant drop in mean pH from 0.50 to 0.05 (r = 0.94). Similar observations on decolorization of ferric salicylate have been observed with increase in addition of HCl[17]. Addition of acetic acid, 0.1 to 0.3 mL of 1M in methanol does not alter mean absorbance values 0.422 ± 0.007 or mean pH 2.01 ± 0.13 (n= 6 each) compared to the respective values of 0.420 ±0.015 and 1.93 ± 0.21 of standard (n= 3 each) in

absence of acetic acid (P>0.1). At higher concentrations of acetic acid, 0.25 through 2.35 M, the pH remains fairly stable, 1.72 ± 0.10 (n=12), while mean absorbance falls from about 0.34 through 0.03 with increase in acid concentration (r = 0.95). The decolorizing effect of higher concentration of acetic acid is presumably due to formation of acetylsalicylic acid that renders free phenolic group of salicylic acid inaccessible for the required activity. Ferric chloride in presence of alcohol imparts slight palish-green tinge to the solution. The color does not absorb at 525 nm but necessitates running of corresponding blanks for each iron (III) concentration.

Table 2 Comparative evaluation of the pharmaceuticals containing α-tocopherol

Product [a]	Absorbance[b]		FIRA	RAOP	Purity %	RSD %
	A_i	A_x				
Standard(3)	0.395 ± 0.003	0.161 ± 0.001	5.92 ± 0.03	1.000	100	0.9
Bio-E(3)	0.395 ± 0.003	0.207 ± 0.001	4.76 ± 0.02	0.804 ± 0.001	80.4 ± 1.0	0.7
Evion(6)	0.402 ± 0.001	0.201 ± 0.002	5.01 ± 0.05	0.847 ± 0.009	84.7 ± 0.9	2.4
Tocofer(6)	0.402 ± 0.001	0.230 ± 0.002	4.29 ± 0.06	0.724 ± 0.009	72.4 ± 0.9	3.4
Revup(4)	0.402 ±0.003	0.239 ± 0.002	4.05 ± 0.06	0.684 ± 0.011	68.4 ± 1.1	3.0

[a] The content equivalent to 100 µg α-tocopherol is subjected to saponification in absence of pyrogallol. [b]Absorbance values with standard iron (III) 1 µmol per 4 mL sample in absence, A_i, and presence of the pharmaceutical, A_x where subscript x stands for standard or test preparation. FIRA is ferric ion reducing activity, nmoles ferric ion reduced per µg α-tocopherol base. RAOP is relative antioxidant potential.RSD is relative standard deviation calculated on FIRA. The values within parentheses are number of observations.

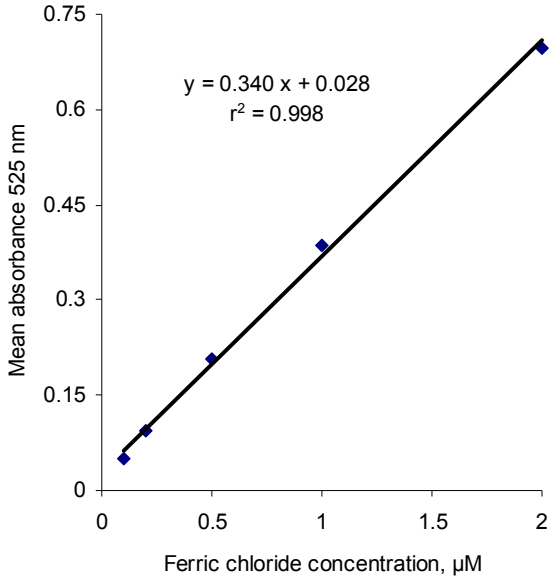

Fig. 1

Fig.1 Absorbance values as a function of iron (III) mass by Trinder reaction

Determination of α-tocopherol by ferric salicylate method

One µmol of iron (III) was chosen as optimal mass for the assays as it afforded better magnitude of standard response. For instance, incubating 60 µg of α-tocopherol with 0.5, 1.0 and 2.0 µmole ferric ions per sample provide FIRA values respectively as 2.76 ± 0.09, 5.29 ± 0.09 and 5.12 ± 0.11 nmoles iron(III) reduced μg^{-1} (n=3 each). The magnitude of response at 1 µmol ferric ion per sample is significantly better than the values obtained at other concentrations ($P<0.01$). At standard ferric iron concentration, α-tocopherol 15 to120 µg causes decrease in absorbance, and reduction in ferric iron in direct proportion to increase in mass of α-tocopherol (r ± s.e. = 999 ± 0.001; b ± s.e. = 0.00214 ± 0.00004 and 5.49± 0.10, respectively for decrease in absorbance and increase in FIRP values) (Figs 2a and 2b).

Alpha-tocopherol up to 675 µg fails to show any visible direct interaction with salicylic acid. The method works with any of the test salicylates viz, sodium salicylate, methyl salicylate and salicylic acid. Salicylic acid is chosen for its better solubility in alcohol. Methyl salicylate (10% v/v) in methanol is an alternative substitute to salicylic acid. In fact methyl salicylate 2 to 20 mg is assayable with 5 µmol ferric iron in 5 mL methanol (r ± s.e. = 0.994 ± 0.005, b ± s.e. = 0.027±0.001). The assay performs better with ferric chloride than with ferric ammonium sulfate. Time optimization studies revealed a faster reduction process. The mean absorbance value, 0.259±0.002, at standard ferric ion mass with 60 µg of α-tocopherol following 2-3 minute incubation does not differ from the value, 0.259±0.003, observed following about 20 minute incubation ($P>0.1$, n=3 each). A 10-minute incubation period has been employed during the study for practical convenience. The evaluation of extended time course response over 9 days incubation has provided a consistent response with no difference in FIRA values of 5.71 ± 0.08 (n = 5), 5.51±0.09 (n = 6) and 5.49±0.09 (n = 8) observed on days 1, 4 and 9 respectively ($P >0.1$). The assay works well in methanol and ethanol. Extracts in benzene or in chloroform are assayable. Salicylic acid method in vogue for determination of ferric ions by photometric titration employs acetone[19]. Acetone up to 0.5 mL in 4 mL volume does not interfere, but higher concentrations seriously impair color development. Hexane and heptane also impair the development of color presumably by segregating the reacting moieties into different organic phases owing to different extents of solubility. These observations would suggest appropriate choice of organic solvent for conduct of the assay. Addition of α-tocopherol to the preformed ferric salicylate complex is weakly active compared to the effect of its presence before the formation of the complex.

Saponification and purification of extracts

Alpha-tocopherol acetate derived from the test pharmaceuticals fails to elicit any response when added up to 600 µg while free tocopherol as little as 15 µg produces discernible reduction in ferric salicylate formation. The mean absorbance value with 100 µg acetate form, 0.361 ± 0.002, does not differ significantly from the value, 0.354 ± 0.001, provided by standard iron (III) in absence of treatment even with prolonged incubation up to 2 hours ($P>0.1$, n=5 each). Alpha tocopherol is quite stable to heat, acid and alkali while the air is detrimental particularly in presence of alkali[1]. Keeping the flask stoppered during saponification tends to decrease recovery of α-tocopherol possibly due to mixing of air in the flask with the material under process. Recoveries improve when the moistened stopper is held slightly inclined to allow the air present in the flask to escape during initial heating, and filling the available space with ethanolic vapor. This provides an optimal reducing environment. Prolonged saponification beyond 3-minute period is detrimental to the assay while 1-minute incubation is insufficient. The mean FIRA value obtained with 3-min incubation has been 5.13 compared to the values 3.52 and 2.06 observed, respectively, following 1-min and 5-min incubation. Twenty minute incubation causes nearly complete destruction of free tocopherol such that the absorbance value, 0.416±0.003, with 100 µg equivalent free α-tocopherol with standard iron mass does not differ from the value, 0.423±0.002, observed without α-tocopherol ($P>0.1$, n= 3 each). Saponification losses are minimized by using pyrogallol which provides reducing environment in conjunction to ethanol. Overall, recovery of α-tocopherol in presence of pyrogallol 20 -25 mg per sample, providing about 0.1 % concentration during saponification, increases to nearly 100 % from about 80 % obtained in absence of pyrogallol (Table1). Use of pyrogallol during saponification has been recommended to minimize saponification related losses in α-tocopherol[10, 12, 21]. It may not be required if the intent is to compare relative antioxidant potential of the pharmaceuticals. The criterion has been applied for comparing the relative potentials of the test pharmaceuticals. Saponification control sample fails to produce any effect on ferric reduction implying that the process and the reagents do not contribute in any way to the observed effect.

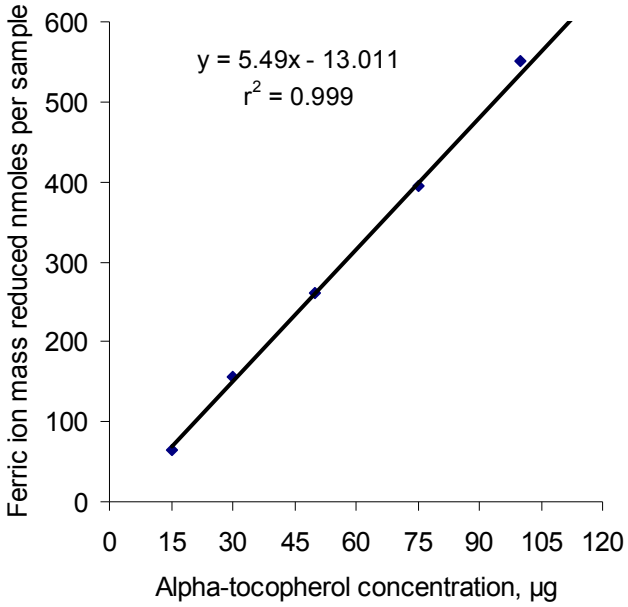

Fig. 2b

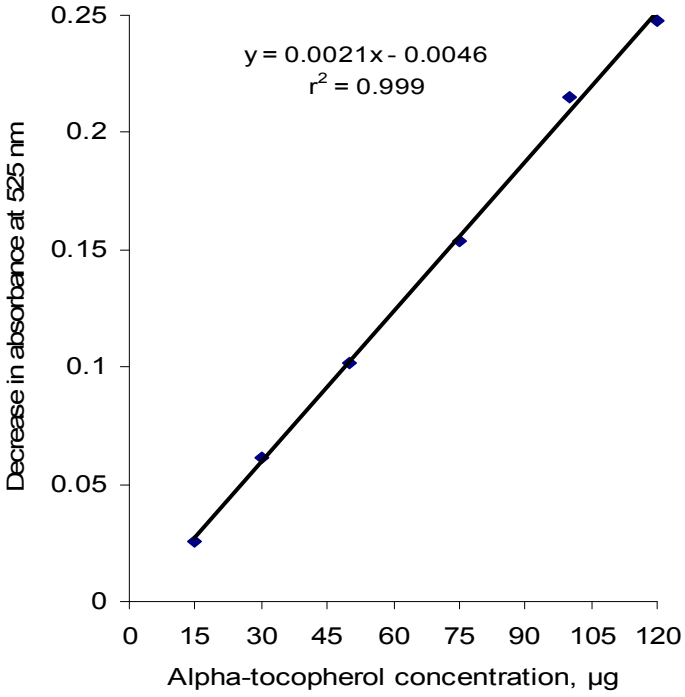

Fig. 2a

Fig.2 Linear relation between varying mass of α-tocopherol and decrease in absorbance at 525 nm (2a) or in ferric ion mass, nmoles per sample (2b)

Alpha tocopherol acetate is the only active ingredient in Evion and Tocofer, while the Revup additionally contains beta-carotene, ascorbic acid and mineral salts of Se, Mn, Cu and Zn. The extraction procedure ensured removal of all undesirable constituents. Thorough washing of chloroform phase with water completely eliminates the possible interference from residual ascorbic acid that otherwise produces the same effect as produced by free tocopherol. The final purified ethanolic extract from Revup retains a faint tinge of beta-carotene. The content of beta-carotene in assayable aliquots is estimated to be less than 1 µg per ml as measured by monitoring concentrated samples against calibration curve obtained with standard β -carotene at 460 nm in chloroform solvent. However, β-carotene 0.4 through 4 µg remains undetected while higher concentrations cause physical discoloration of the ferric-salicylate complex making measurements difficult to assess. Bio-E preparation was chosen as RLS as it contains free α-tocopherol from natural sources, and so does not require any saponification. The titrimetric pharmaceutical assay revealed per cent purity of reconstituted bulk sample as 99.6 ± 0.7 (n = 6) compared to 98.4 ± 1.1 (n = 6) obtained with reagent grade α-tocopherol having labeled minimum per cent purity as 98.

The performance of RLS over the years has remained quite stable. For instance, the regression coefficient, 5.49±0.10 (n=30), obtained over 15 through 120 µg RLS during May 2007 is comparable to the value, 5.44 ±0.19 (n=8), obtained with 20, 60 and 100 µg RLS during March 2009 (r ± s.e. = 0.999±0.001 each, P>0.1).

Evaluation parameters

The two parameters viz., decrease in absorbance, and increase in FIRP values while incubating with standard iron mass, permit evaluation of the pharmaceuticals for their α-tocopherol contents and for determining their relative antioxidant potentials. Ordinarily the test can be performed with a single concentration of each test and standard preparation using comparable concentrations within the linear range. However, multiple concentrations can be compared using either their regression coefficients for comparative evaluation, or actually calculating the parameters (FIRP, FIRA) for individual concentrations. The parametric analysis is demonstrated through the data obtained with saponification study (Table 1). The regression coefficient obtained over the linear range 15 to120 µg α-tocopherol, 5.49±0.10 (n=30, Table 1), and with standard over 40 to 100 µg during the saponification study, 5.56±0.17 (n=9, Table 1), do not differ significantly from the value, 6.12 ±0.12 (n=9, Table 1), calculated from individual observations concentration wise (P>0.1). A similar feature is observed when regression coefficients obtained with saponification, in absence and presence of pyrogallol, are compared with corresponding average FIRA values (P>0.1, Table 1). This implies that either procedure is applicable for determination of FIRP and FIRA values. Furthermore, choosing the concentration range 60 to 100 µg for comparative analysis provide better recovery and improved precision than when lower concentration of 40 µg is included in the analysis (Table 1). Multiplication of each FIRA value by the corresponding concentration gives mean content of α--tocopherol. The ratio of FIRA values of any two preparations gives their relative contents and their relative antioxidant potentials. The parameters enable comparative evaluation of test pharmaceuticals. The four pharmaceuticals, subjected to uniform treatment of saponification, reveal their relative potency order (content and antioxidant wise) (Table2):

Evion ≥ Bio-E > Tocofer > Revup.

Per cent purity of test pharmaceuticals, excluding saponification loss, ranges from ca. 68 to ca. 84. The saponification loss accounts for about 20 per cent in absence of pyrogallol (Table 1). The results would suggest that the pharmaceutical quality of Evion is slightly better than that of Bio-E, or may reflect that the Bio-E as free tocopherol is more vulnerable to saponification losses than an acetate form. Decreased recovery with Revup and Tocofer may reflect either a decrease in the available content in the test capsules, or an increased mass in Bio-E and Evion (for compensating losses incurred on storage) as against their labeled claims. Methods based on reduction of ferric ion for determination of α-tocopherol are non-specific. The selectivity for a given chemical moiety would be ensured by proper selection of extraction and purification procedures.

Conclusions

Salicylic acid method based on Trinder reaction is proposed as a simple, and an inexpensive alternative colorimetric method for determination, and evaluation of α-tocopherol in the pharmaceuticals. The evaluation parameters include determination of FIRP and FIRA values to determine relative antioxidant activity, estimated content and per cent purity of test preparations in terms of the standard. The study reveals that each µg of α-tocopherol is capable of reducing 5.5 to 6 nmoles of ferric ions under standard conditions. The value is closer to and better than the stoichiometric value provided by standard titrimetric pharmaceutical assay wherein each µg of α-tocopherol causes reduction of 4.64 nmoles of ceric ions (GOI, 1996). The relative potency order of the test pharmaceuticals has been found to be: Evion ≥ Bio-E > Tocofer >Revup, with recovery in the range of about

68 to 84 per cent excluding about 20 % loss in recovery on account of saponification without pyrogallol. The proposed method would be also useful for screening of test materials for antioxidant activities, and for selective determination of ferric ions over the concentration range of 0.1 to 2 µmoles, equivalent to about 5 through 100 µg per sample. The author is routinely employing the method for screening the indigenous plant extractives for their antioxidant potential.

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