Flow-Injection Determination of Retinol and Tocopherol in Pharmaceuticals with Acidic Potassium Permanganate Chemiluminescence

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A simple and rapid flow-injection method is reported for the determination of retinol, its derivatives and α -tocopherol by its enhancement effect on a potassium permanganate–formaldehyde chemiluminescence system in an acidic medium. Linear calibration graphs were obtained between 5.0×10^{-8} to 5.0×10^{-6} mol L⁻¹ for all-*trans*-retinol, 5.0×10^{-8} – 2.5×10^{-6} mol L⁻¹ for all-*trans*-retinal, 5.0×10^{-7} – 1.0×10^{-5} mol L⁻¹ for retinoic acid, 1.0×10^{-7} – 5.0×10^{-6} mol L⁻¹ for retinyl acetate and retinyl palmitate and 1.0×10^{-8} – 5.0×10^{-6} for α -tocopherol, with relative standard deviations (RSD; $n = 4$) in the range of 0.8 – 2.8%. The limits of detection ($S/N = 3$) were in the range of 5.0×10^{-9} – 1.0×10^{-7} mol L⁻¹ with a sample throughput of 100 h⁻¹. The influence of common ions, excipients in pharmaceutical preparations and related organic compounds were studied on the determination of retinol and α -tocopherol individually. The method was applied to determine retinol and α -tocopherol in pharmaceutical preparations, and the results obtained were in reasonable agreement with the amount labelled.

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Introduction

Knowledge about the influence of oxidative stress and the role of vitamins on human health has gained much attention in the last few decades. Oxidative stress, defined as an imbalance between oxygen free-radicals formation and their scavenging by antioxidants, is recognized to play an important contributory role in the pathogenesis of numerous degenerative¹ or chronic diseases, such as arteriosclerosis, allergy or cancer.² Humans along with other aerobic organisms have evolved a variety of mechanisms to protect themselves from the potentially toxic effects of reactive molecules.¹ The so-called antioxidation complex includes enzymes, such as catalase and superoxide dismutases, repair enzymes, such as DNA glycosylases as well as water and lipid soluble vitamins, such as ascorbic acid (vitamin C), tocopherol (vitamin E), carotenoids, and retinol (vitamin A).¹ Knowledge of the level of antioxidants in human plasma allows one to estimate the state of health, and can help in treating serious diseases. Vitamins A and E are lipid-soluble vitamins essential for human health. Both groups have free-radical scavenging properties that allow them to function as physiologic antioxidants; they also play an important role in the prevention of neuromuscular deficits by maintaining a proper blood red-cell lifetime, and preventing of abnormal platelets activity.³

The major form of vitamin A is an alcohol (retinol), but it can also exist as an aldehyde (retinal) or as an acid (retinoic acid). Precursors to a vitamin (a provitamin) are present in foods of plant origin as some of the members of the carotenoid family of compounds. All forms of vitamin A have a β -ionone (4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; C₁₃H₂₀O, M_w 192.3)

ring, to which an isoprenoid (2-methylbuta-1,3-diene; C₅H₈, M_w 68.11) chain is attached. This structure is essential for vitamin activity.⁴

Natural vitamin E exists in eight different forms: four tocopherols and four tocotrienols. All feature a chromanol ring, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals and a hydrophobic side chain, which allows for penetration into biological membranes. α -Tocopherol is the most active form of vitamin E in humans. It is also a powerful biological antioxidant. Vitamin E in supplements is usually sold as α -tocopheryl acetate, a form of α -tocopherol that protects its ability to function as an antioxidant. Both tocopherols and tocotrienols occur in α , β , γ and δ forms, determined by the number of methyl groups on the chromanol ring. The chemical structures of retinol and α -tocopherol are given in Fig. 1.

Due to their many similar physical properties, vitamins A and E can generally be determined using an identical sample workup procedure. Such a determination in real samples includes a sample-preparation step, which involves liquid-liquid extraction directly, or after saponification, supercritical fluid extraction and solid-phase extraction. The extract can then be used for analysis by liquid chromatography (HPLC) or other analytical methods, either directly or after additional concentration/cleanup steps, or evaporation of the solvent and re-dissolution into a solvent compatible with the polar mobile phase.^{5,6}

Several different quantification procedures have been reported in the literature for the determination of vitamins A and E in biological materials and food samples. These include colorimetry/spectrophotometry,^{7,8} spectrofluorometry,⁹ voltammetry,¹⁰ HPLC coupled with ultraviolet, fluorescence, photodiode array and electrochemical,^{11–16} and chemiluminescence¹⁷ methods that are reflected in a number of reviews.^{5,6} Table 1 gives a comparison of the analytical methods in terms of the sample matrix, linear calibration range and sensitivity for the

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determination of retinol and tocopherol.

Chemiluminescence (CL) as a technique can be used for determining trace and ultra-trace concentrations of inorganic and organic species (like drugs or pesticides) in a variety of industrial, clinical and environmental matrices. An analytical advantage of CL includes high sensitivity, very low limits of detection and a wide linear range.^{18–21}

The CL reactions of acidic potassium permanganate have been extensively studied for the determination of reductants and some enhancers.^{18,19} Among them, low-molecular weight aldehydes increase the response from many analytes. Several studies have reported that very sensitive CL emission was generated by the oxidation of hydroxyl-containing drugs, phenolics/antioxidants with KMnO_4 in an acidic medium.^{22,23} It has been reported that formaldehyde could enhance the CL emission intensity of such systems. The mechanism of enhancement by formaldehyde and related compounds has, as yet, not been fully elucidated. Various research groups have postulated that these species are oxidized to form excited-state carbon dioxide^{24,25} or singlet

oxygen;^{26,27} some have even suggested that these enhancers increase the rate of analyte oxidation.²⁸

This study reports on a flow injection chemiluminescence (FI-CL) system for the determination of retinol and tocopherol, based on their enhancement effect on the KMnO_4 -HCHO CL reaction in an acidic medium. The oxidation of tocopherol by KMnO_4 possibly results in intermediate products similar to the phenolics/antioxidants, tocoquinones/hydroquinones, which have been reported in the literature^{18,21–23} to generate CL upon a reaction with acidic potassium permanganate. A similar behavior is shown by vitamin A; however, the exact CL enhancement mechanisms of these vitamins are unclear.

Experimental

Reagents and solutions

All solutions were prepared using ultra-high purity (UHP) water (Elga, Purelab Option, UK), and all reagents were of analytical grade, unless stated otherwise. Retinyl acetate, retinal and retinoic acid from Sigma (St. Louis, USA), retinol, β -carotene and retinyl palmitate from Fluka (Buchs, Switzerland), vitamin D₃ from Acros (Geel, Belgium), DL- α -tocopherol and DL- α -tocopherol acetate from MP Biochemicals (Eschwege, Germany), potassium permanganate and formaldehyde from Merck (Darmstadt, Germany) were obtained. Individual stock (1.0×10^{-3} mol L⁻¹) solutions of retinol, retinyl acetate, retinal, retinyl palmitate, retinoic acid, β -carotene (in acetone), α -tocopherol, tocopherol acetate and vitamin D₃ were prepared by dissolving the required amounts of these compounds in absolute ethanol in brown bottles and stored at -4°C in the dark. Working standards were prepared by diluting aliquots of the stock solutions in ethanol (5%) and stored at 0°C in the dark during the day. A potassium permanganate stock (1.0×10^{-3} mol L⁻¹) solution was prepared by dissolving the required amount of the compound in UHP water. Diluted solutions of potassium permanganate and formaldehyde were prepared by mixing portions of the stock solution with the required amount of H_2SO_4 (0.05 mol L⁻¹). Stock solutions (1000 mg L⁻¹) of glycerol, sorbitol, mannitol, methyl cellulose, sucrose, glucose,

Fig. 1 Molecular structures of retinol and α -tocopherol.

Table 1 Comparison among several methods for the determination of retinol and tocopherol

	Matrix	Analyte	Calibration range/ $\mu\text{g mL}^{-1}$	DL/ $\mu\text{g mL}^{-1}$	R^2	Sample rate/h	Ref.
Colorimetry	Multivitamin capsules	Tocopherol acetate	0.2 – 11.0	0.012	0.9996	6	7
Spec.	Drug formulation	Retinol	2.5 – 25	NG	0.9998	NG	8
Spec. FL	Nutritional supplements	Tocopherol	0 – 5.5	NG	0.9994	NG	9
HPLC-DAD	Infant milk-based formulae	Retinol acetate	0.2 – 5	0.2	0.9999	NG	11
		Tocopherol	2 – 100	6.8	0.9993		
HPLC-FL	Beverages	Retinol	0.02 – 3.42	0.6	0.9997	NG	12
		Tocopherol	0.09 – 10.38	0.03	0.9997		
HPLC-UV	Milk based products	Retinol palmitate	0.5 – 2.5	0.33	0.9774	NG	13
		Tocopherol acetate	20 – 60	5.67	0.9850		
HPLC-FL	Human serum and plasma	Retinol	0.15 – 1.2	0.03	0.9987	NG	14
		Tocopherol	1.5 – 27	0.3	0.9999		
MLC-UV	Multivitamin syrup	Retinol palmitate	0.15 – 1.5	1.71	0.9990	NG	15
		Tocopherol acetate	2000 – 8000	4.52	0.9992		
HPLC-DAD	Food and pharmaceutical supplement	Retinol acetate	1.0 – 60	0.81	0.9998	NG	16
		Tocopherol acetate		1.12	0.9997		
FI-CL	Pharmaceuticals	Retinol	0.014 – 1.43	0.0018	0.9991	100	This method
		Tocopherol	0.004 – 2.15	0.0021	0.9991	100	

NG, not given; Spec, spectrophotometry; Spec. FL, spectrofluorometry; HPLC-DAD, higher performance liquid chromatography-diode array detector; MLC-UV, micellar liquid chromatography; FI-CL, flow-injection-chemiluminescence.

Fig. 2 Flow-injection chemiluminescence manifold for the determination retinol and α -tocopherol.

starch, gum acacia, magnesium stearate, magnesium silicate, glycerol monostearate, dextrin, cholesterol, stearic acid, oleic acid, triglycerides, Na^+ , K^+ , Ca^{2+} , Zn^{2+} , NH_4^+ , SO_4^{2-} , PO_4^{3-} , HCO_3^- , NO_3^- , and Cl^- were prepared by dissolving the required amount in ethanol or water for an interference study. All of these solutions were protected from light and stored at room temperature, and used whenever required.

Pharmaceutical formulations containing vitamin A or E were collected from the local market (Shanghai Medicines and Health Products Corporation, Shanghai-China, Zafa Pharmaceuticals Laboratories and Merck Marker Pvt. Ltd, Pakistan).

Instrumentation and procedures

The flow-injection chemiluminescence manifold used for this work is shown in Fig. 2. A peristaltic pump (Ismatec Reglo, four channels, Switzerland) was used to deliver the sample carrier and reagent solutions at a flow rate of 2.0 mL min^{-1} . All manifold tubing was Tygon (1.02 mm i.d., Ismatec, Switzerland). A rotary injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to introduce standards/samples into an aqueous ethanol (5% v/v) stream. This stream was then merged at a Y-piece with the formaldehyde and potassium permanganate reagents, and allowed to travel 1.5 cm before passing through a glass spiral flow cell (2.0 mm i.d., 25 mm diam.) positioned in front of an end-window photomultiplier tube (PMT, 9798B, Electron Tubes Ltd., Ruislip, UK). The PMT, glass coil and T-piece were enclosed in a light-tight housing. The PMT was kept constant at 900 V via a 2 kV power supply (Burle, PF1053, USA). The detector output was recorded using a chart recorder (Kipp & Zonen BD 11E, Holland).

Sample preparation

Sample solutions for vitamin A were prepared individually from each formulation by cutting 5 capsules and placed into a wide-necked glass stopper bottle containing 50 mL of ethanol. The opened capsule shell was jet rinsed with ethanol and sonicated for 10 min at room temperature to make the solutions clear.

Commercially available formulations containing vitamin E exist as DL- α -tocopherol acetate, which is quite stable to oxidation, does not shown any significant chemiluminescence, and require conversion into tocopherol by transesterification. Tocopherol acetate was converted into tocopherol by a previously reported procedure.⁷ For each sample: one or two capsules were individually cut opened and placed into a wide-necked glass stopper bottle containing 200 mL of ethanol. The opened capsule shell was jet rinsed with ethanol, and solution was sonicated for 10 min at room temperature. Standards were prepared by taking 50 – 250 μL from each sample and stock solutions of tocopherol acetate in a 20 mL of a brown glass bottle, and 25 μL of concentrated sulfuric acid (as a catalyst)

and 10 mL of methanol were added to each bottle. The bottles were covered with aluminium foil by leaving a space for evaporation; the bottles were then heated at $70 - 80^\circ\text{C}$ in a water bath for 80 – 120 min by intermittent purging of nitrogen gas; during this period the contents of the bottles were evaporated to almost dryness. The end product of transesterification was dissolved in 10 mL of methanol and analyzed accordingly.

Results and Discussion

Selection of flow-injection manifold parameters

In order to establish the optimum conditions for the determination of retinol and α -tocopherol, various experimental parameters were investigated using a univariate approach. The key parameters optimized were sulfuric acid, potassium permanganate, and formaldehyde concentrations, the sample volume, the flow rate of the sample carrier and the reagents streams. All of these studies were performed with a $1.0 \times 10^{-7} \text{ mol L}^{-1}$ retinol and α -tocopherol standard solution (60 μL).

Since vitamins A and E are lipid soluble vitamins, ethanol was used to dissolve the compound from the stock solution. It is known that organic solvents generally influence the CL behavior; hence, the effect of ethanol was examined. When the ethanol concentration was increased up to 20% (v/v), high blank values were observed with non-reproducible CL signals. Higher ethanol concentrations also caused a decrease in CL intensities; therefore, 5% (v/v) ethanol was selected as a suitable concentration for the present work as a sample carrier stream and for dilution of the standards and samples.

The concentration of H_2SO_4 is an important parameter for the CL reaction involving KMnO_4 . This study was carried out in the range of $0.01 - 0.25 \text{ mol L}^{-1}$, and the maximum CL intensity was obtained at $0.05 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$, which indicates that under this concentration condition, the amounts of the reactive species of the oxidants in the solution was maximum as shown in Fig. 3a. Hence, $0.05 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ was subsequently used for preparing both potassium permanganate and formaldehyde solutions.

The concentration effect of KMnO_4 on the CL light intensity was examined over the range of $1.0 - 10 \times 10^{-5} \text{ mol L}^{-1}$ in the optimized sulfuric acid concentration. The results are shown in Fig. 3b. The maximum emission intensity was observed at $2.5 \times 10^{-5} \text{ mol L}^{-1} \text{ KMnO}_4$, above which the CL response decreased. Hence, $2.5 \times 10^{-5} \text{ mol L}^{-1} \text{ KMnO}_4$ was selected and used in all subsequent experiments. The concentration influence of formaldehyde on the CL intensity was studied over the range of $0.1 - 1.0 \text{ mol L}^{-1}$, as shown in Fig. 3c. An increase in the CL intensity was observed up to 0.5 mol L^{-1} , and a further increase in the formaldehyde concentration resulted in a higher background and a decrease in the CL response of the analytes. Hence, a solution of 0.5 mol L^{-1} formaldehyde was selected for subsequent studies.

The effect of the flow rate and the sample volume on the CL light intensity was investigated in terms of the sensitivity, reagent consumption and sample throughput. The flow rates for each of the three channels were simultaneously investigated over the range of $0.5 - 3.0 \text{ mL min}^{-1}$, and a flow rate of 2.0 mL min^{-1} gave the maximum CL response with a steady baseline and reproducible ($<3.0\%$ RSD) peak height, and was subsequently used for all three channels. The effect of the sample volume on the sensitivity was studied in the $60 - 240 \mu\text{L}$ range, with a maximum CL light intensity for volumes of $120 \mu\text{L}$ and it was subsequently used for economy of sample consumption.

Analytical figures of merit

Under the selected experimental conditions mentioned above, the calibration graphs of the relative CL intensity *versus* the concentration of retinol, retinal, retinoic acid, retinyl acetate, retinyl palmitate and α -tocopherol were measured with relative standard deviations ($n = 4$) in the range of 0.8 – 2.8%. The figures of merit are summarized in Table 2, with a sample throughput of 100 h^{-1} . The method was applied to determine vitamins A and E in pharmaceutical preparations. The obtained results are in reasonable agreement with the amount labelled. A representative chart showing the recorder outputs for the determination of various concentrations of retinol is shown in Fig. 4.

Interferences

In order to assess the selectivity of the proposed CL method, the influences of common foreign species were tested under the optimized conditions containing a standard solution of 1.0×10^{-7}

mol L^{-1} retinol and tocopherol individually. The tolerance of each foreign species was taken as the largest concentration yielding less than $\pm 5\%$ of the error of the adoptive concentration of retinol and tocopherol. No clear interference could be found with 1000-fold for glycerol, Na^+ , K^+ , and SO_4^{2-} , and 500-fold for tocopherol acetate, methyl cellulose, Ca^{2+} , Mg^{2+} , NH_4^+ , vitamin D₃, cholesterol, and stearic acid, 200-fold for sorbitol, mannitol, sucrose, glucose, starch, gum acacia, magnesium stearate, dextrin, Zn^{2+} , PO_4^{3-} , NO_3^- , HCO_3^- , and Cl^- , 100-fold for oleic acid, triolein, EPA, and DHA, and 50-fold for polyvinyl alcohol.

Analytical applications

The proposed FI-CL method was applied for the determination of vitamin A in commercially available cod liver oil capsules containing vitamins A and D₃. Pharmaceuticals containing only vitamin E (DL- α -tocopherol acetate) were used after transesterification. Appropriate dilution of each solution was made with aqueous ethanol (5% v/v) and injected into the proposed FI-CL manifold. The obtained results are given in Table 3. It can be seen from Table 3 that there were no significant differences between the labeled contents in the pharmaceutical formulation samples and those obtained by the proposed CL method. Furthermore, the standard-addition method was also adapted by adding a variable amount of pure vitamins to the previously analyzed fraction of capsules. The results given in Table 3 confirm that the proposed method is not liable to interferences. They seem to be accurate, and the method was successfully applied to the determination of vitamins A and E in the presence of common excipients.

Fig. 3 Variation of the CL intensity with (a) H_2SO_4 , (b) KMnO_4 and (c) HCHO concentrations. Conditions: retinol and tocopherol, $1.0 \times 10^{-7} \text{ mol L}^{-1}$ (60 μL), (a) KMnO_4 , $5.0 \times 10^{-5} \text{ mol L}^{-1}$; HCHO , 0.25 mol L^{-1} (in H_2SO_4), (b) HCHO , 0.25 mol L^{-1} , (c) KMnO_4 , $2.5 \times 10^{-5} \text{ mol L}^{-1}$.

Fig. 4 Representative recorder outputs for a series of retinol standards under the proposed conditions by FI-CL system.

Table 2 Analytical characteristics of vitamins

Figures of merit	Linear range/ mol L^{-1}	R^2 ($n = 8$)	Calibration equation	RSD range, % ($n = 4$)	DL/ mol L^{-1} ($S/N = 3$)
Retinol	$5.0 \times 10^{-8} - 5.0 \times 10^{-6}$	0.9991	$I = 4.0579C + 1.002$	1.0 – 1.6	6.5×10^{-9}
Retinal	$5.0 \times 10^{-8} - 2.5 \times 10^{-6}$	0.9989	$I = 4.1044C + 1.731$	1.3 – 2.2	6.5×10^{-9}
Retinyl acetate	$1.0 \times 10^{-7} - 5.0 \times 10^{-6}$	0.9985	$I = 0.7345C + 2.319$	1.2 – 1.9	2.5×10^{-8}
Retinyl palmitate	$1.0 \times 10^{-7} - 5.0 \times 10^{-6}$	0.9989	$I = 0.4531C + 1.825$	1.1 – 2.8	2.5×10^{-8}
Retinoic acid	$5.0 \times 10^{-7} - 1.0 \times 10^{-5}$	0.9992	$I = 0.0756C + 2.102$	1.4 – 2.1	1.0×10^{-7}
α -Tocopherol	$1.0 \times 10^{-8} - 5.0 \times 10^{-6}$	0.9991	$I = 7.2181C + 1.170$	0.8 – 1.9	5.0×10^{-9}

Table 3 Application to pharmaceutical formulations

Capsule	Composition labeled/capsule	Found ^a Proposed method ^b	Taken ^a	Added ^a	Found ^a
Vitamins A and D ₃	Retinyl palmitate 3000 IU and vitamin D ₃ 300 IU	2910.59	25	0	24.4 ± 0.8
				25	51.2 ± 1.5
				50	75.1 ± 1.8
				100	123.8 ± 2.0
Vitamins A and D ₃	Retinyl palmitate 4000 IU and vitamin D ₃ 400 IU	3852.89	50	0	50.2 ± 1.7
				50	102.3 ± 2.5
				100	148.9 ± 2.9
				200	251.0 ± 6.0
Vitamin E	DL- α -Tocopheryl acetate 200 mg	196.82	2	0	1.97 ± 0.11
				2	4.05 ± 0.15
				3	4.95 ± 0.21
				4	6.01 ± 0.25
Vitamin E	DL- α -Tocopheryl acetate 400 mg	401.51	3	0	3.02 ± 0.12
				2	4.95 ± 0.21
				4	7.09 ± 0.25
				6	8.91 ± 0.3
Vitamin E	DL- α -Tocopheryl acetate 600 mg	595.9	6	0	5.89 ± 0.25
				2	8.05 ± 0.15
				4	10.11 ± 0.19
				6	12.20 ± 0.21

a. Average of five. b. International Unit for first two samples and milligrams for the rest of samples.

Possible CL mechanism

It is well documented that molecules containing phenol or amine moieties efficiently generate CL upon oxidation with acidic potassium permanganate.^{18,19} Tsaplev *et al.*²⁹ proposed a mechanism to explain the CL generation with acidic potassium permanganate; it involves the reduction of Mn(III) ions to electronically excited Mn(II) ions, which subsequently emit light to return to the ground state. Mn(III) can be obtained either by the oxidation of Mn(II) or the reduction of Mn(VII). In a number of articles^{30–32} dealing with the oxidation of various compounds with acidic potassium permanganate it is proposed that the CL emitter in these reactions is the excited Mn(II) species. In a recent communication, Adcock *et al.*³³ show a comparison of the laser-induced photoluminescence of Mn(II) with chemiluminescence from the reaction of acidic potassium permanganate and sodium borohydride to confirm that the characteristic red emission from acidic potassium permanganate emanates from an electronically excited Mn(II) species. In an earlier investigation, Barnett *et al.*³⁴ negated the possibility of generating CL from potassium permanganate oxidations *via* singlet oxygen, sulfur dioxide, molecular nitrogen or nitric oxide species. It has also been postulated that there is no appreciable dependence on the nature of the reducing agent oxidation with potassium permanganate, the CL emitter being the same excited Mn(II) species.

Retinol and α -tocopherol are antioxidants. Tocopherol contains the phenol moiety (6-chromanol) which upon oxidation forms quinones and hydroquinones.³⁵ It is feasible that Mn(II) may be complexed *via* the phenolic oxygen, and it is known³⁶ that the Mn(II) complexes can be oxidized to the Mn(III) species using acidic potassium permanganate. It was postulated³¹ that Mn(III) complexes can be reduced to an electronically excited Mn(II) complex, which then emits a photon. It is well known³⁷ that dilute permanganate solution rapidly oxidizes olefins to diols *via* the transfer of two oxygen atoms from potassium permanganate to olefins by complex formation. It can therefore be assumed that retinol makes a complex with permanganate during which the reduction of permanganate occurs, and for

generation of CL *via* an electronically excited Mn(II) complex is possible. The role of formaldehyde possibly in these reactions is to enhance the reduction of potassium permanganate to a lower excited-state Mn(II) species, which in turn gives CL, as background CL, which was observed when potassium permanganate reacted with formaldehyde alone.

Conclusion

The CL reaction of acidic potassium permanganate with formaldehyde can be significantly enhanced in the presence of retinol and tocopherol. Based on this sensitizing effect, the proposed FI-CL method for the determination of retinol and tocopherol is simple and rapid (100 h⁻¹ sample throughput) with a limit of detections in the nanomolar range. This method is better in terms of the limit of detection, and the linear range, and is subject to less interference from the methods reported in Table 1. The method was applied to pharmaceutical preparations. The results were in reasonable agreement with the quoted value. The method can also be applied to determine retinol and tocopherol in blood serum after separation. Further work is underway for the separation and simultaneous determination of retinol and tocopherol in blood serum.

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