

HPLC USING DIODE ARRAY DETECTION AND SPECTRODENSITOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF VITAMEN A, E AND D₃ IN MULTIVITAMIN PREPARATIONS

Abd El-Aziz El- Bayoumi

Analytical Chem. Department , Faculty of Pharmacy , Calro University, Egypt

ABSTRACT

Two selective , sensitive , reproducible methods for the rapid simultaneous determination of vitamin A palmitate , E (α - tocopherol) acetate and D₃ are described. The HPLC methods depends on a simple rapid isocratic and reserved phase C-18 for identification and quantitation of vitamin A,E and D₃ in synthetic water dispersible liquid and solid mixtures, as well as a market liquid dosage form containing specific emulsifier and additives. The HPLC determination is linear over a wide range with a lower limits of quantitation of 50 ng/ml, 10 ng/ml for vitamin , A,E and D₃ , respectively.

The spectrodensitometric method provides a rapid and precise method for the separation and quantitation of the three vitamins when present in mixtures or even in a pharmaceutical dosage form. It is linear over a range of 1-25 ng/spot with a limit of quantitation of 1 ng/spot The described methods are suitable for quality control purposes of pharmaceutical preparations containing mixtures of vitamins with each other or with additives without interferences.

INTRODUCTION

Vitamins are a large group of compounds which differ in their chemical composition, physiological action and nutritional importance.

From a nutritional point of view a sufficient intake of the fat-soluble (pro) vitamins is of great importance . This is also reflected in the legislation and labeling guidelines,

From a nutritional point of view a sufficient intake of the fat-soluble (pro) vitamins is of great importance . This is also reflected in the legislation and labelling guidelines , for vitamins A and D₃ (both dangerous in excess) and tocopherols. Therefore, accurate methods of analysis for these vitamins are needed .

Recently there has been increasing and special interest in α -tocopherol due to the possibility of its actions as a chemoprotective agent for skin cancer, since it protects dermic cells from attack by free radicals caused by ionized radiations or chemical substances.

Much work has been done to develop a satisfactory analytical method for assaying vitamins A , E and D₃ and give answers of good accuracy.

The biological methods are considered to be impractical for use in manufacturing control , because it is time consuming and expensive .

Quantitative HPLC determination of vitamin A,E and D₃ is currently used in literature utilizing different detectors , such as , coulometric detection in the redox in mode(1) , fluorescence and ultraviolet or other electrochemical detectors (2-7). Isocratic , normal and reversed-phase techniques are reported for the determination of fat-soluble vitamins by HPLC methods (8-11).

Different chromatographic methods are described for the determination of fat - soluble vitamins in foods(12-14) pharmaceutical preparations (14-16), human serum (17), biological specimens (18) and multivitamin mineral formulations(19-22). Supercritical fluid extraction (SFE) of fat-soluble vitamins is also recommended prior to chromatographic determination (23-26).

Gas chromatography without derivatisation and gas chromatography coupled with mass spectrometry is reported for the assay of fat - soluble vitamins and their principle oxidation products (27,28).

One dimensional and two-dimensional ¹H-NMR and ¹³C-NMR (29) and first-derivative spectrophotometric methods are also applied (30).

In the present work , two chromatographic methods for the simultaneous determination of vitamin A,D₃ and E in pure authentic form in water dispersible liquids are suggested. The effecte of the addition of a surfactant in such mixture of vitamins make it possible to be mixed with water to about 100 times its volume .The first method involves a very simple extraction method and the second spectrodensitometric method requires no preliminary extraction.

EXPERIMENTAL

Chemicals and Reagents:

All chemicals used were of the analytical-reagent grade and were obtained from BDH unless otherwise stated . Vitamin A (all trans-retinol) palmitate was obtained from Rhonpolank (France) and labelled to contain 1.7 MIU/g . Vitamin E (α -tocopherol) acetate was obtained from Roche-Sweizerland and labelled to contain 98% . Vitamin D₃ cholicaciferol was obtained from Solvay , Netherlands and labelled to contain 5 MIU/g.

Certificates of analysis were attached with each vitamin and the purity were determined according to the B.P. (31) or Varley et al (32) techniques.

All the vitamins were used without further purification. These substances may degrade on storage, therefore it has been protected from light, kept under nitrogen and contained a suitable preservative; vitamin E or butylated hydroxy toluene BHT.

Standard Solutions of the Vitamins :

Accurately weighed 0.2941 g of vitamin A palmitate, 0.02 g of vitamin E (α -tocopherol) and 0.02 g of vitamin D₃ (cholicaciferol, 5 MIU/g) transferred into a mortar and mixed with an equal amount of a suitable surfactant (Tween 80 is the best one - obtained from ICI - surfactant). With the aid of minimum amount of distilled water to form stable O/W microemulsion, clear, yellow liquid was formed. Each vitamin was quantitatively transferred into a 50-ml volumetric flask, completed to volume with a mixture of equal volume of water and methanol. Ten ml of each solution was separately diluted to 25 ml with water-methanol (3:97) in volumetric flasks.

Synthetic Mixtures :

Liquid Form :

A mixture of the three vitamins to contain 100000, 20000 IU/g from vitamin A palmitate, D₃, respectively and 20 mg/g vitamin E acetate, was prepared.

The fat-soluble vitamins were treated with an equal amount of tween 80 and water to obtain stable clear yellow mixture, suitable amounts of BHT and 100 ml water was mixed to obtain a solution has a pH of about 6.5. The water used was deionized or distilled.

Solid Mixture :

A solid form containing 10000, 10000 IU/g from vitamin A palmitate and D₃ respectively was prepared, in addition to 10 mg/g of vitamin E acetate. To this mixture an equal amount of tween 80 (as surfactant), BHT (antioxidant and preservative) and a quantity sufficient of sipernat 22 were added until a homogenous solid was obtained, and completed to 100 g with lactose.

Market Preparation:

Vitamins A, E and D₃ liquid dosage form, was obtained from the Egyptian market (Adwia; Egyptian Co. for Chemicals and Pharmaceuticals) S.A.E. Egypt labelled to contain vitamin A palmitate, E acetate and D₃ in a concentration of 10000 IU/g, 20mg/g and 20000 IU/g respectively. The preparation is water dispersible mixture.

Apparatus:

- 1-Liquid chromatograph Perkin-Elmer HPLC system equipped with series 410 pump, 20 ml fixed volume injector. LC-235 variable diode array detector and Milton Roy CI-10 B integrator.
- 2-Filters, 0.45 μ m (Alltech, USA).
- 3-Ultrasonic bath, Sonorex 510s, HF frequency 35 KHz (Germany).
- 4-Beckman DU-7 Spectrophotometer.
- 5-Shimadzu, Dual wavelength flying spot scanner, CS-9000 Spectrodensitometer.

Operating Conditions :

Instrumental : Column, Nucleosil 5, RP C-18 (250 x 4.6 mm); mobile phase water-methanol (3:97) and or RP C-18 (150x4.6mm) column; flow rate, 2 ml/min. Detector, diode array UV at 254nm, 0.1 (a.u.f.s) for vitamin A and 0.06 a.u.f.s. for other vitamins. Attenuation 64, chart speed 0.5 cm/min and injection volume 10 μ l.

Spectrodensitometric Measurement :

Precoated thin layer chromatographic plates 0.2 mm thickness; Silica gel 60 HF₂₅₄ on plastic or aluminum 20 x 20 cm, E.Merck, Darmstadt.

Solvent system, chloroform-methanol (4:96) was developed in 2 hours. Plates were scanned using Shimadzu Spectrodensitometer, CS-9000 with micro-optics reflection mode, a monochromator wavelength selection at a scan speed of 20 mm/min.

Procedures :

HPLC Procedure:

The solvents were filtered through membrane filters 0.45 μ m and degassing may be carried out by sonication for 15 minutes or bubbling of helium in the solvents just before use. Aliquots ranging from 5-10 μ l of standard solutions of vitamin A palmitate, vitamin D₃ cholicaciferol and vitamin E acetate were injected into the mentioned columns.

Synthetic Prepared Mixture and Market Preparations:

Ten ml of the synthetic prepared mixture and market sample were transferred into a 25-ml volumetric flask, completed to volume with the solvent system. A volume of 5-10 μ l of this solution was injected into the HPLC column.

Spectrodensitometric Procedure:

Volumes ranging from 0.5-5 ml of each standard of the concerned compounds in chloroform-methanol (4:96) mixture were spotted into TLC plates 20 x 20 cm, 1.5 cm from the edge of the plate using

microsyringe as successive fractions. The chromatogram was developed using; chloroform-methanol (4:96) in a horizontal dark glass jar to a height of about 15 cm. Dried in a stream of cold air and scanned to investigate the optimum wavelength and the intensity of the spots was calculated for each compound separately.

For synthetic prepared mixture containing the three mentioned vitamins and market sample, were diluted to the required concentration range with a mixture of chloroform-methanol.

For both procedures the concentrations of the vitamins were calculated by reference to a preconstructed calibration graph of peak area versus concentration.

Regarding the market preparation, the standard addition technique was applied.

Calculation:

Potency of vitamin A or D₃

$$= \frac{\text{Area T} \times 100000}{\text{Area S}} = \text{IU / g.}$$

Where Area T and S correspond to area of the test and area of standard for the corresponding compound in integrated units.

RESULTS AND DISCUSSION

The described methods introduced a surfactant to reduce the consumption of solvents and provide mild extraction conditions for the analysis of the labile vitamins.

From the experimental point of view, Tween 80 is the best surfactant which gives good recoveries and reliable results without any interference.

Isocratic, reversed phase HPLC Nucleosil 5 C-18 column, size 250 x 4.6 mm or RP-18 (150 x 4.6mm) column were used for the separation of the three fat-soluble vitamins individually or in authentic mixture using methanol; water (97:3) as a mobile phase with diode array detection.

Figure (1) shows the HPLC chromatogram of vitamins D₃ (R_t 4.44), vitamin E (R_t 6.27) and vitamin A (R_t 15.85) using the long column and methanol-water (97:3) as a mobile phase.

Figure (2) shows the HPLC chromatogram of the three fat-soluble vitamins using a short column (150 x 4.6 mm), in which vitamin D₃ appears at R_t 3.16, vitamin E appears at R_t 5.38 and vitamin A appears at R_t 9.49 min.

This simple isocratic HPLC system was described to perform a rapid separation, identification, and quantitation of the three vitamins. These conditions allowed best base line separation of the fat-soluble vitamins in reasonably short analysis time, which was ranged from about 10-16 minutes (according to the column used). Good resolution was obtained from the long column, in a maximum analysis time of about 16 minutes, especially for vitamin A palmitate.

The linearity test for quantitation was carried out over the range 50-100 ng/ml, 10-20 ng/ml and 5-15 ng/ml for vitamin A, E and D₃, respectively.

The detection limits for each vitamin expressed as amount injected was found to be 0.9 ng, 0.7ng and 0.5 ng for vitamin A, E and D₃, respectively.

The equations for the calibration graphs (n=9) for the selected vitamins were found to be:

$$Y = 0.0171 x + 0.0005 \text{ For vitamin A}$$

$$Y = 0.0142 x + 0.0013 \text{ For vitamin E}$$

$$Y = 0.0153 x + 0.0012 \text{ For vitamin D}_3$$

Where :

$$r = 0.9936, 1.0000 \text{ and } 0.9942 \text{ for vitamin A, E and D}_3, \text{ respectively.}$$

The Proposed HPLC method was applied for the determination of the three vitamins in authentic oily form after treatment with tween 80 as a surfactant.

The results obtained was presented in table (1). The mean percentage recoveries were found to be 100.18 (SD, 0.040), 100.51 (SD, 0.204) and (SD, 0.283) for vitamin A, E and D₃, respectively.

The results illustrated in table (1) provided evidence for the quantitative, recovery of the three vitamins and good reproducibility (low standard deviation) of the HPLC method upon using Tween 80 as a surfactant.

The HPLC method was applied to synthetic liquid, solid mixtures and market liquid dosage forms containing the three vitamins, surfactant, antioxidant (Butylated hydroxy toluene), lactose and water. The solid mixture assumed to contain an additive substance (Sipernat 22) to produce a stable solid form and did not contain water.

In order to assess the performance of the proposed method for the determination of the three fat-soluble vitamins in the synthetic mixtures and market dosage forms, three standard additions were added from each vitamin and the recoveries from four replicates were calculated and presented in table (2).

The mean percentage recoveries of the added vitamins to synthetic liquid, solid mixtures and market

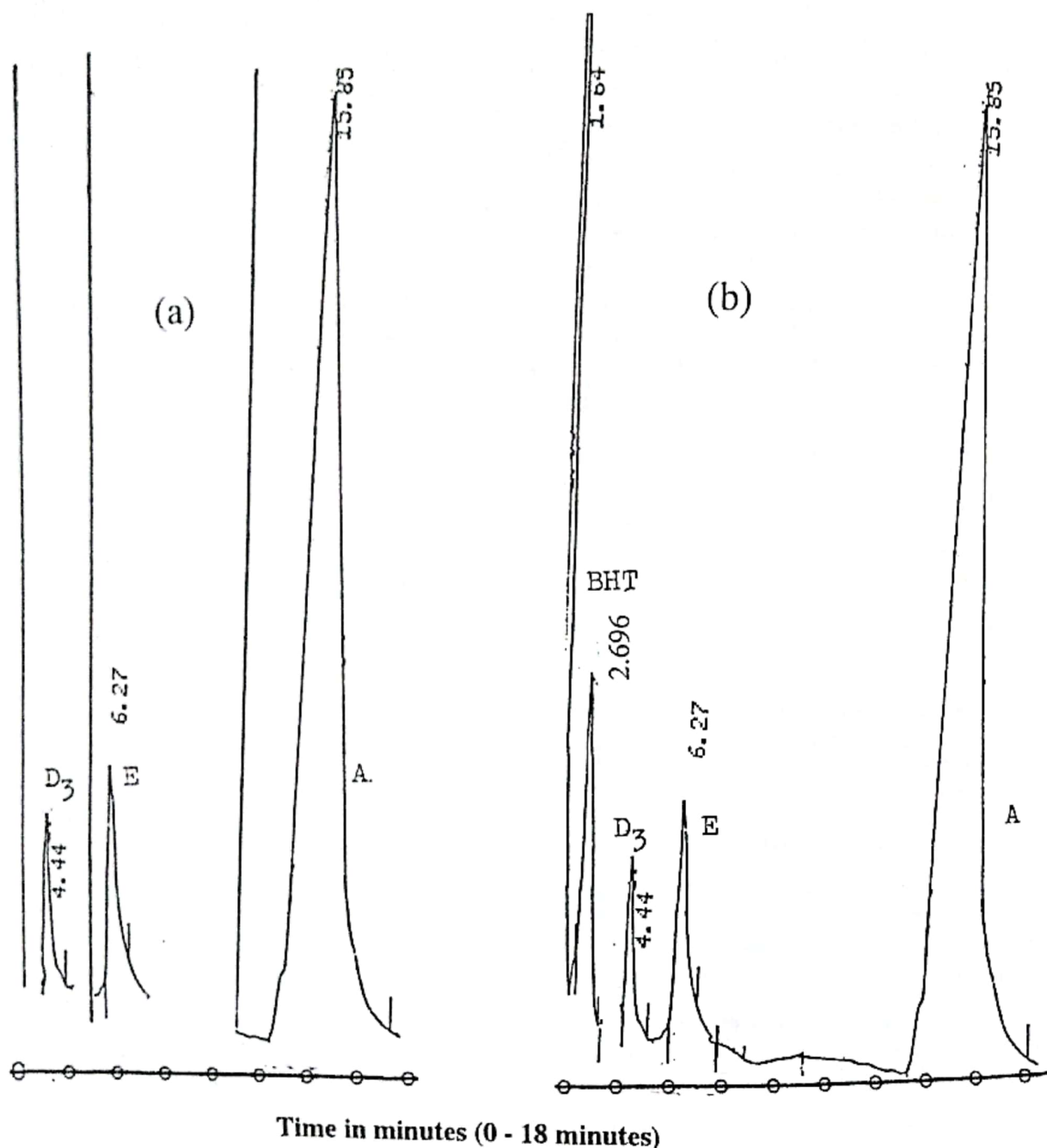


Fig. (1 a and b): Representative chromatograms obtained by injection of standard vitamin A, E and D₃ into a Nucleosil 5 RP C-18, HPLC column, size 250 x 4.6 mm. (1a) while (1b) shows an HPLC chromatogram obtained by injection of a synthetic liquid mixture containing the concerned vitamins, a preservative (BHT) and treated with tween 80. Mobile phase: Methanol-water (97:3).

Detector : Diode array
Chart speed : 0.5

Attenuation : 64
injection volume: 5-10 μ l.

preparation were ranged from 97.78-98.55 for vitamin A, 96.91-98.67 for vitamin E and 94.95-96.67 for vitamin D₃.

Regarding the second proposed spectrodensitometric method, the three vitamins were separated using a precoated silica gel HF₂₅₄ aluminum plate (20 x 20cm) and a mixture of chloroform methanol as a

mobile phase. The R_f values were found to be ; 0.86 , 0.806 and 0.953 for vitamin A, E and D₃ , respectively.

A Typical TLC separation of the three vitamins was presented in Figure (3) , in which different concentrations were spotted and compared with external standards . The spectrodensitometric measurement of the intensity of the spots was carried out at 265 nm.

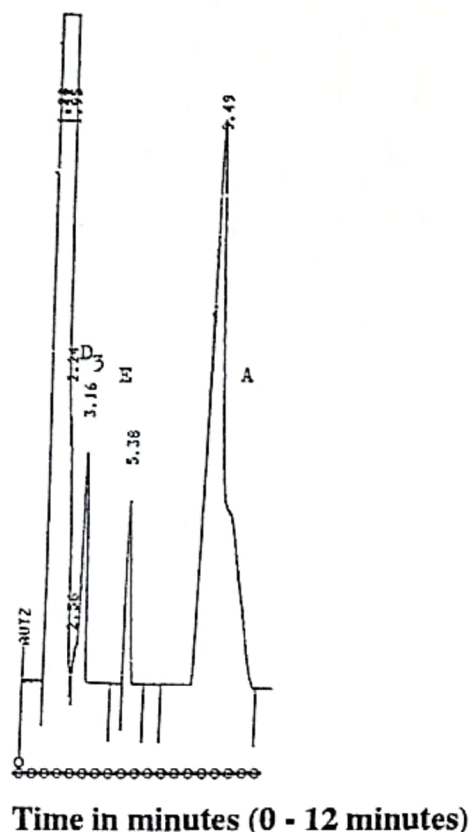


Fig (2) : Representative HPLC chromatogram of a market preparation containing vitamin A,E and D₃ separated on a relatively short RP C-18 HPLC column, size 150 x 4.6 mm.

Mobile phase: Methanol-water (97:3).
 Detector : Diode array Attenuation:64
 Chart speed :0.5 injection volume: 5-10 µl..

This wavelength gave the maximum absorbance with minimum noise.

The linearity range for quantitation was calculated to be ; 1-25 ng/spot , with detection limits of 0.5 , 0.3 and 0.1 ng/spot for vitamin A, E and D₃, respectively.

The regression equations and correlation coefficients were presented in table (3) which indicated the applicability of the proposed method for the rapid determination of the three vitamins in authentic form with sensitivity of 1 ng/spot , (n=9).

Quantitative determination of the three fat-soluble vitamins in pure form utilizing the spectrodensitometric method was presented in table (4).

The mean percentage recoveries were ranged from 102.15-98.71 for vitamin A, E and D₃, respectively. The standard deviations varied from 1.1349 , 0.2234 and 0.167 , which provide evidence for

Table (1) : HPLC Determination of Vitamin A, E and D₃ in Pure Forms.

Vitamin	Added ng ml ⁻¹	Found ng ml ⁻¹	% Recovery
A	50	50.10	100.20
	60	60.15	100.25
	70	70.12	100.17
	80	80.14	100.17
	90	90.13	100.14
	100	100.15	100.15
	Mean SD		
E	10	10.05	100.50
	12	12.10	100.83
	14	14.05	100.35
	16	16.10	100.62
	18	18.10	100.55
	20	20.05	100.25
	Mean SD		
D ₃	5	5.05	101.00
	8	8.05	100.62
	10	10.08	100.80
	12	12.02	100.16
	14	14.10	100.71
	15	15.08	100.53
	Mean SD		

good quantitative recovery of vitamins and good reproducibility.

The results shown in table (5) in which the proposed spectrodensitometric method was applied to synthetic liquid , solid mixtures and market liquid dosage forms. To standardize the method , three standard solutions from each vitamin were added . The spots were applied to the silica gel precoated plates without preliminary extraction, applying the mobile phase chloroform-methanol (4:96), since all the preparations contain Tween 80 as a surfactant.

The thin layer chromatographic method separated the three vitamins from each other and from other additives and excipients.

In conclusion, the proposed HPLC and spectrodensitometric methods were very useful tool to evaluate (in a single chromatographic run , without preliminary harmful solvent extraction) vitamins A, E and D₃ . The results also confirmed that the methods did not give rise to artifacts , and in particular , there was no interconversion or side reactions especially with the selected surfactant which did not retained the vitamins as indicated by the higher percentage recovery in both methods.

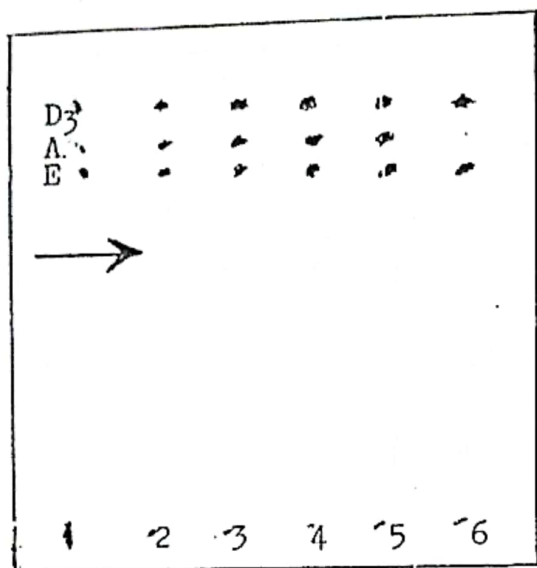


Fig (3 a) : TLC chromatogram
vitamin A,E,D₃.
Mobile phase: Chloroform-
methanol (4:96).
Adsorbent: Silica gel HF₂₅₄

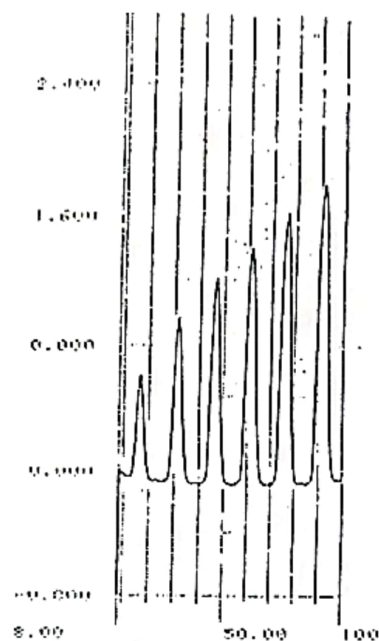


Fig (3b)

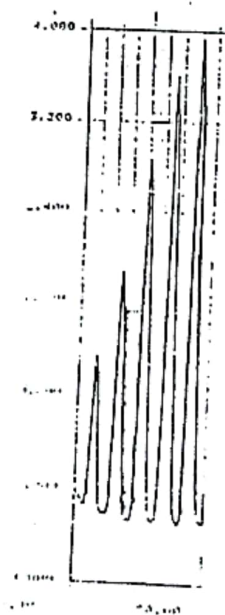


Fig.3c

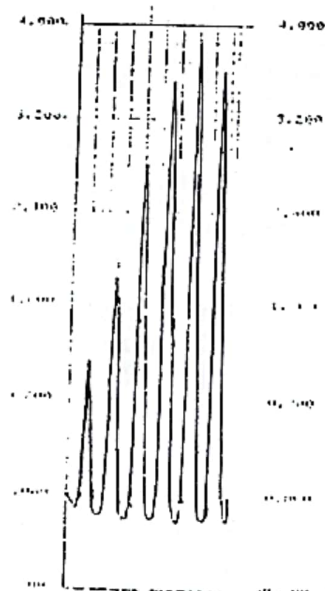


Fig.3d

Fig (3): Thin layer chromatographic separation of standard vitamin A, E and D₃ in different ratios (fig.3a).while figures (3b, c and d) show the spectrodensitometric measurements of standard vitamin D₃,A and E in different concentrations , respectively.

Table (2) : Analytical Recoveries of Fat- Soluble Vitamins in Synthetic Mixtures and Market Preparation Containing the Three Vitamins Using HPLC Method.

Vitamin Preparation	A		E		D ₃	
	Found ng ml ⁻¹	% Recovery	Found ng ml ⁻¹	% Recovery	Found ng ml ⁻¹	% Recovery
Liquid Mixture	49.2	98.40	9.82	98.20	4.80	96.00
	58.3	97.16	11.79	98.21	6.70	95.70
	68.5	97.80	14.65	97.66	8.58	95.33
Mean (SD)	97.78 (0.62)		98.03 (0.327)		95.67(0.335)	
RSD	0.634		0.333		0.35	
Synthetic Solid Mixture	49.10	98.20	9.70	97.00	4.75	95.00
	58.05	96.75	11.65	97.08	6.58	94.00
	68.25	97.50	14.50	96.66	8.59	95.40
Mean (SD)	97.48(0.725)		96.91(0.223)		94.8(0.721)	
RSD	0.743		0.23		0.760	
Market Multivitamin Liquid prep.	49.70	99.40	9.90	99.00	4.80	96.00
	58.80	98.00	11.82	98.50	6.70	95.71
	68.78	98.25	14.78	98.53	8.65	96.11
Mean (SD)	98.55(0.746)		98.67(0.28)		95.94(0.206)	
RSD	0.756		0.283		0.214	

Table (3) : Regression Equations and Correlation Coefficients of Calibration Curves Relating the Mean Area to Concentration (Four Replicates) of Fat-Soluble Vitamins Determined by the Spectrodensitometric Method.

Vitamin	Regression Equation	Correlation Coefficients (r)
A	$Y = 0.0505 + 0.0048X$	0.999
E	$Y = 0.0613 + 0.0045X$	0.998
D ₃	$Y = 0.0570 + 0.0174X$	0.995

The two methods retained their accuracy and precision when the standard addition technique was applied to synthetic mixtures or pharmaceutical dosage forms.

Therefore the two suggested methods were considered to be simple , rapid reproducible , sensitive , selective and suitable for quality control analysis of pharmaceutical preparations containing fat-soluble vitamins.

Table (4) : Quantitative Determination of Pure Fat- Soluble Vitamins Applying the Spectrodensitometric Method.

Added ng/spot	A		E		D ₃	
	Found ng/spot	% Recovery	Found ng/spot	% Recovery	Found ng/spot	% Recovery
5	5.10	102.00	5.02	100.40	4.95	99.00
8	8.05	100.62	8.01	100.12	7.90	98.75
10	10.10	101.00	10.05	100.50	9.85	98.50
15	15.50	103.33	15.10	100.66	14.80	98.66
20	20.60	103.00	20.05	100.25	19.75	98.75
22	22.65	102.95	22.15	100.68	21.70	98.63
Mean SD	102.15		100.43		98.71	
	1.1349		0.2234		0.167	
Average of four replicats						

Table (5): Spectrodensitometric Determination of Fat-Soluble Vitamins In Synthetic Mixtures and Market Preparation Applying Standard Addition Technique .

Vitamin	Synthetic liquid mixture		Synthetic Solid mixture		Market Liquid Preparation	
	Found ng/spot	% Recovery	Found ng/spot	% Recovery	Found ng/spot	% Recovery
A	9.80	98.00	9.75	97.50	9.70	97.00
	14.60	97.33	14.50	96.66	14.45	96.33
	19.40	97.00	19.42	97.10	19.35	96.75
Mean	97.44		97.08		96.69	
(SD)	0.416		0.343		0.276	
RSD	0.4269		0.3539		0.285	
E	9.75	97.50	9.72	96.50	9.60	96.00
	14.55	97.00	14.60	96.00	14.30	95.33
	19.35	96.75	19.40	96.25	19.15	95.75
Mean	97.08		97.17		96.78	
(SD)	0.311		0.1350		0.299	
RSD	0.3211		0.1396		0.3080	
D ₃	9.70	97.00	9.65	96.50	9.60	96.00
	14.50	96.66	14.40	96.00	14.30	95.33
	19.30	96.50	19.25	96.25	19.15	95.75
Mean	96.72		96.25		95.69	
(SD)	0.208		0.204		0.276	
RSD	0.2150		0.2119		0.2888	

Three standards were added from each vitamin . (n = 4).

REFERENCES

- 1-H.Takeda, T. Shibuya, K. Yanagawa, H. Kanoch and M. Takasaki; *J. Chromatogr. A*, 722 (1-2), 287-94, (1996).
- 2-B. Dimitrova, M. Poyre, G. Guiso, A. Badiali and S. Caccia; *J. Chromatogr. Biomed Appl.* 681 (1), 153-60 (1996).
- 3-G. N. Nul *J. Chromatogr. A*, 721(2), 247-59, (1996).
- 4-M.M.D. Zamarrero, A.S. Perez, C.G. Perez and J. H. Hernandez; *J. Chromatogr.* 623(1), 69-94, (1992).
- 5-G. Panfili, P. Manzi and L. Pizzoferrato; *Analyst*; 119(6), 1161-5, (1994).
- 6-S.K. Jensen; *J. Dairy Res.*, 61(2), 233-40, (1994).
- 7-T. Meda Ichikawa and O.I. Garash; *J. Nutr. Sci. Vitaminol-Tokyo*, 39 (3), 207-19, (1993).
- 8-P.C. Hollman, J.H. Slangen, P.J. Wagstaffe, U. Faure, D. A. Southgate and P. M. Figas; *Analyst*, 118(5), 475-80, (1994).
- 9-E. Brinkman, I. Mehlitz, H.B. Oei, R. Tiebach and W. Baltes; *Z. Lebensm. Unters. Forsch.*, 199 (3), 206-9, (1994).
- 10-G. M. Landers and J. A. Olson; *J. Chromatogr.* 438 (2), 383-92, (1988).
- 11-V.K. Agarwal; *J. Assoc. Off. Anal. Chem.* 72 (6), 1007-9, (1989).
- 12-H. E. Indyk; *Analyst*, 115 (12), 1525-30, (1990).
- 13-Y. Satomura, M. Kimura and Y. Itokawa; *J. Chromatogr.* 625 (2) 372-6 (1992).
- 14-A. Baruffini, E. De Lorenzi, C. Gandini, M. Kitsos and G. Massolini; *J. Chromatogr.*, 593 (1-2), 95-7, (1992).
- 15-K. E. Savolainen, K. M. Pynnonen, S. P. Lapinjoki and M.T. Vidgren; *J. Pharm. Sci.*, 77 (9), 802-803, (1988).
- 16-V. K. Agarwal; *J. Assoc. Off. Anal. Chem.*, 71 (1), 19-22, (1988).

- 17-N.Bealulieu,N.M.Curran, C. Gane, M. Gravelle and E.G.Lovering; *J. Assoc. Off. Anal. Chem.*, 72(2), 247-54,(1989).
- 18-K.S.Epher,R.G.Zeigler and N.E.Craft; *J. Chromatogr.*, 619 (1),37-48,(1993).
- 19-J. P. Hart and P. H. Jordan; *Analyst*, 114, 1633-35 , (1989).
- 20-D. K. Feeter, M.F.Jacobs and H. W. Rawlings; *J. Pharm. Sci.*, 60 (6), 913 -16 , (1971).
- 21-J.W.Strong ; *J. Pharm. Sci.*, 65 (7) , 969 -74 , (1976).
- 22-S.Tomiyama,T. Nitta and S.Yamada; *Steroids* , 59(9),559-63,(1994).
- 23-A.Ortiz and T.A.Trauel; *Biochem. Int.*, 25 (3) ,429-435,(1991).
- 24-S.Scallia,A.Renda,Guberto and F.Bonina ; *J. Pharm.Sci.*, 84(4),433-6,(1995).
- 25- S.Scallia,A.Renda,Guberto,F.Roning E .Menegatti; *J. Pharm. Biomed. Anal.* 13 (3), 273-7, (1995).
- 26-D.Watson , K. D.Stchell and R. Ross ; *J. Chromatogr. Biomed. Appl.*, 5 (4),153-60, (1991).
- 27-E.Ballesteros , M.Gallego and M.Valcarcel; *J. Chromatogr.* A.719 (1),221-7,(1996).
- 28-D.C.Leibler, J.A.Burr,L.Philip and A. J. Ham; *Anal. Biochem.*, 236 (1), 27-34 , (1996).
- 29-J.K.Baker and C.W. Mayer; *Pharm. Res.*, 8 (6),763-70,(1993).
- 30-A. A. Wahbi, M.A.Abounassif and H. M. Alkhatani; *J.Pharm.Biomed.Anal*, 7(1),39 - 43, (1989).
- 31-The British Pharmacopoeia ; Her Majisty Stationary Office, London, (1993).
- 32-H.Varley, A.H. Gowenlock and M.Bell; "Practical Clinical Biochemistry,Hormones , Vitamins, Drugs and Poisons". Vol. 2 , W. Heinemann Medical Books Ltd, London, (1976).

Received : July 5 , 1998

Accepted : Sept. 12, 1998

كروماتوجرافيا السائل ذات الضغط العالي باستخدام كاشف الديود اراي وقياس كثافة البقع علي شرائح من الطبقة الرقيقة للتقدير المتزامن لكل من فيتامين أ ، هـ وفيتامين د₃ في مستحضرات متعددة الفيتامينات

عبدالعزیز البيومي عبدالعزیز

قسم الكيمياء التحليلية - كلية الصيدلة - جامعة القاهرة - جمهورية مصر العربية

تم في هذا البحث اجراء طريقتان تميزان باختبارية ، حساسية وقابلية للتطبيق في تقدير فيتامين أ بالميتات ، فيتامين هـ (الفا توكوفيرول) اسيتات وفيتامين د₃ .

وتعتمد طريقة الكروماتوجرافيا السائلة ذات الضغط العالي على فصل سهل باستخدام نفس النسبة من المذيبات وفصلها بعمود معكوس C-18 نيكلوسيل ٥ مع كاشف الديود اراي لتحقيق فصل سريع ، و التعرف على الفيتامينات المذكورة وتقديرها كيميا في صورتها النقية. ولقد تم تطوير الطريقة المذكورة لتقدير الفيتامينات التي لا تذوب في الماء كي تكون مختلطة بالماء في صورتها السائلة والصلية وكذلك المستحضرات الصيدلانية باستخدام المواد الفعالة التي تعمل على السطح وفي وجود الإضافات المختلفة .

وتتميز طريقة الكروماتوجرافيا السائلة ذات الضغط العالي بأنها تعطى علاقة مستقيمة في مدى كبير مع امكانية التقدير الكمي لحدود صغيرة تتراوح بين ٥٠ نانوجرام لكل مليلتر ، ١٠ نانوجرام لكل مل ، ٥ نانوجرام / مل لكل من فيتامين أ ، هـ ود₃ على التوالي . أما الطريقة الثانية وهي قياس كثافة البقع على شريحة من الطبقة الرقيقة فتتميز بأنها سريعة ودقيقة لفصل وتقدير الفيتامينات الثلاثة المذكورة سواء وجودها في مخاليط أو مستحضرات صيدلانية.

والطريقة المذكورة تعطى علاقة خط مستقيم في مدى من ١ - ١٥ - نانوجرام / بقعة مع أقل حد ممكن لتقديرها كيميا هو ١ نانوجرام / بقعة. والطريقتان المذكورتان تناسبان أغراض الرقابة النوعية على المستحضرات الصيدلانية التي تحتوى على مخاليط من الفيتامينات مع بعضها أو في وجود الإضافات الأخرى دون تداخل .