Asian Journal of Chemistry Vol. 22, No. 6 (2010), 4939-4944

Determination of Fat Soluble Vitamins E, D_2, A, K in Syrian Pharmaceutical Preparations by High Performance Liquid Chromatography with UV-DAD Detection

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HPLC method was developed and applied to the determination of fat soluble vitamins FSV (E, A, K, D_2) in Syrian pharmaceutical preparations in three different columns. High performance liquid chromatography with UV-DAD detection was employed. The developed chromatographic method employed an ODS, CN and C_8 columns with dimension for each (250 mm × 4.6 mm; 5 μm). Solvent systems were employed depending on the expected column type in the studied samples. With first column the mobile phase was (methanol: dichloromethane). For the second column the mobile phase was (*n*-hexane:isopropanol). finally with third column it was (water:methanol:acetonitrile). This new method has offered a determination of fat soluble vitamins (E, A, K, D_2) directly with a high accuracy and authenticity for the results without extraction need.

Key Words: Separation, Vitamins E, D_2, A, K, Comparison chromatographic columns.

INTRODUCTION

Vitamins are organic compounds which are required in diet in small amounts and are essential substances for normal health and growth. The sufficient amounts should be supplied by food as its deficiency may cause different diseases. Thus multivitamins preparation should be taken in order to prevent vitamin deficiency. The content of vitamins in pharmaceutical preparations needs to be checked in order to ensure the correct intake the label statements[1] so that pharmaceutical products' should be analyzed. Fat soluble vitamins has been determined by various methods such as fluorescence[2,3] which can determine a small concentration of vitamins but it determines only fluorescent vitamins and it can't determinate vitamins mixture in one step so it cost a lot of time and power. Some of the vitamins have been separated and determinated by gas chromatography[4] but it can't be applied for all vitamins because some vitamins destroyed by heat. Vitamin A has been determine by spectrophotometric methods but this method also can't determinate vitamins mixture[5], Also vitamins have been separated and estimated by high performance liquid chromatography with several detections such as electrochemical detection[6], mass spectroscopy[7], fluorescence[8,9] and UV, visible detection[10,11] HPLC give ability

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to determine mixture of vitamins in one step and with high accuracy and good sensitivity for separated vitamins and that help us to save time, solvents and money.

Fat soluble vitamins (E, D_2, A, K) have been separated by many columns as C_{18} column[12-15], vitamin E has been determined on NH_2[16], vitamin D has been determined on CN[17], C_8[18] and phenyl C_6H_5[19] columns.

We tried in this study to separate the fat soluble vitamins (E, D_2, A, K) at the same time and to compare the separation results in three different column such as ODS, CN and C_8 columns and profiting from HPLC-DAD detection to increase the method sensibility.

HPLC-DAD detection can give us high absorption for every vitamin peak, so we have the ability to determine each vitamins in specific wavelength where we have a small amount of each vitamin in different products correctly and in one step.

EXPERIMENTAL

The chromatograms were obtained by using Hitachi liquid chromatograph equipped with a diode array detector (DAD) Hitachi L-2200, pump Hitachi L-2130, column oven Hitachi L-2350 and autosampler Hitachi L -2200. All columns ODS, CN and C_8 with dimension for each (250 mm × 4.6 mm; 5 μm) were from MN company. Altrasonic 405 from Hwashin Technology (Korea), Micropipette IsoLap (Germany).

Standard vitamins (E, A, K, D_2) were purchased from Dr. Ehrenstorer (Germany), dichloromethane, HPLC grade acetonitrile, *n*-hexane and 2-propanol, water were purchased from Merck (Germany), methanol HPLC was purchased from ACROS (USA).

Stock standard solutions: Standard stock solutions of vitamins (E, D_2, A, K) were prepared in concentration of (0.4 mg/mL for each vitamin (D_2, A, K) and 3 mg/mL for vitamin E) by dissolving required amount of vitamins (E, D_2, A, K) in diluted solutions and adjusting the volume by diluting solution.

Standard solutions: The standard solutions were prepared in the range (27-300 μg/mL). For vitamin K, (12-250 μg/mL) for vitamin A, (10-400 μg/mL) for vitamin D_2 and (75-100 μg/mL) for vitamin E by diluting a required amount from the standard stock solution and adjusting the volume by diluting solution.

Diluted solution: The diluted solvents are presented in Table-1.

Calibration curve: To construct the calibration curve, five replications of (25 μL) for each standard solution were injected immediately after preparation into ODS, CN and C_8 columns and peak areas of chromatograms were measured at a specific wavelength for each vitamin, as it is shown in Fig. 1.

Sample preparation: 20 tablets or capsules containing fat soluble vitamins were weighed and finely powdered and the average amount of one tablet or capsule have been taken to 25 mL volumetric flask and the volume was adjusted by diluted solution. After the flask sonication by ultrasonic bath for 20 min, the solution has been filtrated and 1 mL of solution transfer to 10 mL volumetric flask and adjusted

TABLE-1
CHROMATOGRAPHIC CONDITIONS TO SEPARATE
AND DETERMINATE FSV BY STUDIED COLUMNS

Column conditions	C_{18} (250 × 4.6)	C_8 (250 × 4.6)	CN (250 × 4.6)
Mobile phase	Methanol:dichloromethane (77:23) %	Water:methnol:acetonitrile (87:4:9) %	*n*-Hexane 100 %
Flow rate: mL/min	1.0	1.2	0.8
Wavelength	Multi wavelength	Multi wavelength	Multi wavelength
Diluted solution	Ethanol	Ethanol	2-Propanol
Injection volume	25 µL	25 µL	25 µL

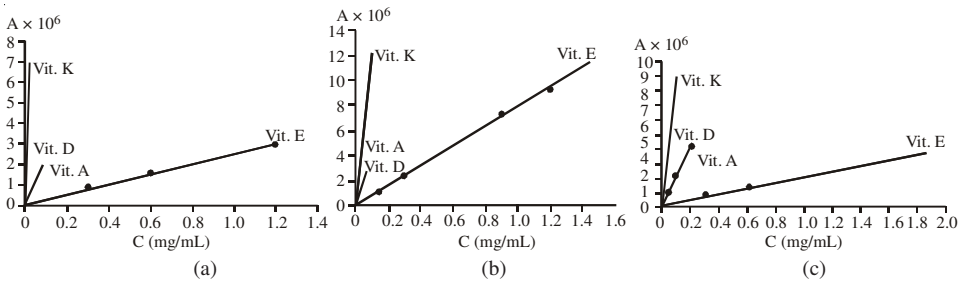

Fig. 1. Calibration curves of fat soluble vitamins on C_8 column, CN column and C_{18} column

to volume by diluted solution. The obtained solution was filtrated through 0.45 µm filter. At the case of ampoules, five ampoules have been taken to Becher and the volume of one ampoule has been taken to 25 mL volumetric flask and the volume was adjusted by diluted solution, after putting the flask to ultrasonic for 20 min, the solution has been filtrated through 0.45 µm filter and 1 mL of filtered solution transferred to 10 mL volumetric flask and adjusted to volume by diluted solution. The same preparation procedure was adopted for all samples. Finally 25 µL of each diluted sample was injected into the ODS, CN and C_8 columns and data were recorded. Fat soluble vitamins concentrations in the samples were then calculated by using peak data and standard curves.

The chromatographic conditions obtained as an experimental results for each ODS, CN and C_8 columns are presented in Table-1.

RESULTS AND DISCUSSION

We used a new rapid chromatographic conditions which has offered a determination of fat soluble vitamins (E, A, K, D_2) directly with a high accuracy and authenticity for the results without extraction need, by using three different columns represented by C_8 column shown in Fig. 2, CN column in Fig. 3 and C_{18} column in Fig. 4. The PDA detection permit us to realize the fat soluble vitamins (E, A, K, D_2) determination by using malty wavelength, so it was difficult to determine them especially with UV detection alone where we have a very dosage variation for each one.

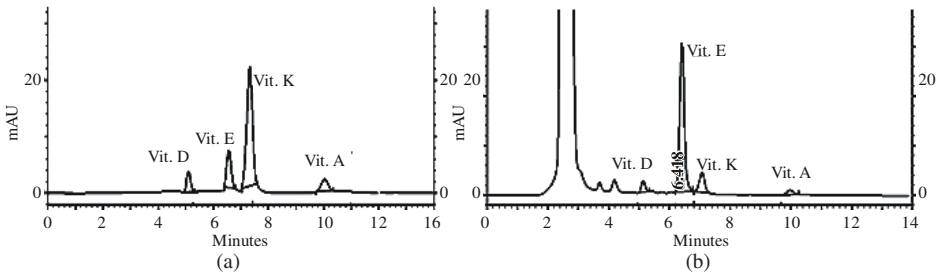

Fig. 2. Typical chromatogram of fat soluble vitamins standard solution and (cenvite) product in the mobile phase on C_8 column

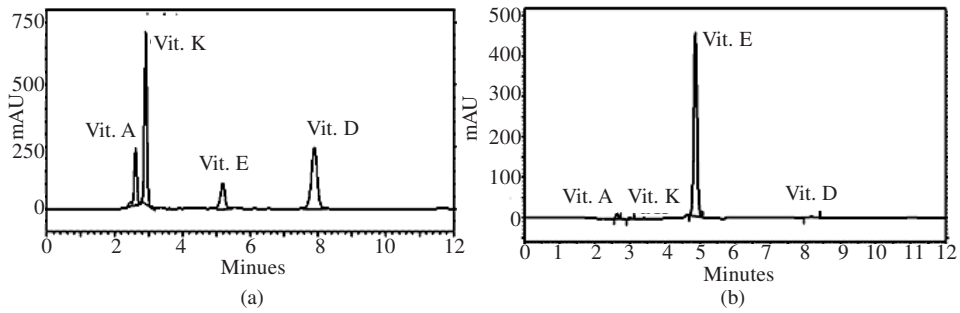

Fig. 3. Typical chromatogram of fat soluble vitamins standard solution and (centaraz) in the mobile phase on CN column

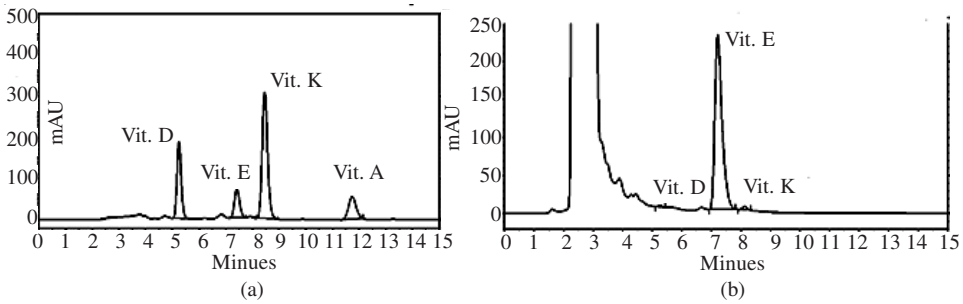

Fig. 4. Typical chromatogram of fat soluble vitamins standard solution and (cenvite) in the mobile phase on C_{18} column

We present the results obtained of fat soluble vitamins (FSV) in some Syrian pharmaceutical products on C_8 column in Table-2, on CN column in Table-3 and on C_{18} column as it is shown in the Table-3.

Conclusion

In this work, we developed and realized some new chromatographic methods and found a new condition of both stationary and mobile phase as previous presentation which allowed us to determine fat soluble vitamins (E, A. K. D_2) in some Syrian pharmaceutical.

TABLE-2
AMOUNT OF FAT SOLUBLE VITAMINS IN SOME SYRIAN PHARMACEUTICAL PRODUCTS ON C_8 COLUMN

Products Trade mark	Compan	Vit. E mg/dose		RSD (%)	Vit. K mg/dose		RSD (%)	Vit. D_2 mg/dose		RSD (%)	Vit. A mg/dose		RSD (%)
		T	E		T	E		T	E		T	E	
Adavit	Adamco	50	52	1.0	–	–	–	–	–	–	0.27	0.26	2.1
Centaraz	Razi	30	33	1.0	0.025	0.024	3.1	0.010	0.009	3.5	0.27	0.26	2.4
Supradyn	MBI	10	10	1.1	0.030	0.028	3.0	0.005	0.005	3.2	0.80	0.81	2.0
Cenvite	Pharmasyr	30	31	1.0	0.025	0.023	2.8	0.010	0.009	3.2	0.27	0.25	1.9
Damvita-M	Ultra medica	50	52	0.9	–	–	–	–	–	–	0.27	0.26	2.0
Viteax	Alpha	70	68	0.8	–	–	–	–	–	–	1.60	1.60	1.9
K-VIT	IBN	–	–	–	10	11	1.4	–	–	–	–	–	–
Calciferol	Amrit	–	–	–	–	–	–	10	11	1.2	–	–	–
Pharmavit	Pharmasyr	70	72	0.4	–	–	–	–	–	–	1.60	1.40	1.9
Sterogyl 15	Oubari	–	–	–	–	–	–	10	10.2	1.4	–	–	–

TABLE-3
AMOUNT OF FAT SOLUBLE VITAMINS IN SOME SYRIAN PHARMACEUTICAL PRODUCTS ON CN

Products Trade mark	Company	Vit. E mg/dose		RSD (%)	Vit. K mg/dose		RSD (%)	Vit. D_2 mg/dose		RSD (%)	Vit. A mg/dose		RSD (%)
		T	E		T	E		T	E		T	E	
Adavit	Adamco	50	51	0.3	–	–	–	–	–	–	0.27	0.25	1.5
Centaraz	Razi	30	31	0.2	0.025	0.024	1.5	0.010	0.0090	2.3	0.27	0.26	1.8
Supradyn	MBI	10	11	0.5	0.030	0.028	1.1	0.005	0.0049	3.1	0.80	0.81	1.2
Cenvite	Pharmasyr	30	32	0.2	0.025	0.023	1.4	0.010	0.0090	2.8	0.27	0.25	1.2
Damvita-M	Ultra medica	50	52	0.1	–	–	–	–	–	–	0.27	0.26	1.6
Viteax	ALPHA	70	72	0.1	–	–	–	–	–	–	1.60	1.60	1.2
K-VIT	IBN	–	–	–	10	11	0.9	–	–	–	–	–	–
Calciferol	Amrit	–	–	–	–	–	–	10	11	1.1	–	–	–
Pharmavit	Pharmasyr	70	71	0.2	–	–	–	–	–	–	1.6	1.4	1.4
Sterogyl 15	Oubari	–	–	–	–	–	–	10	10.2	0.9	–	–	–

TABLE-4
AMOUNT OF FAT SOLUBLE VITAMINS (FSV) IN SOME SYRIAN PHARMACEUTICAL PRODUCTS ON C18 COLUMN

Products Trade mark	Company	Vit. E mg/dose		RSD (%)	Vit. K mg/dose		RSD (%)	Vit. D_2 mg/dose		RSD (%)	Vit. A mg/dose		RSD (%)
		T	E		T	E		T	E		T	E	
Adavit	Adamco	50	53	0.6	–	–	–	–	–	–	0.27	0.24	1.9
Centaraz	Razi	30	32	0.8	0.025	0.025	2.1	0.010	0.0089	2.4	0.27	0.26	1.3
Supradyn	MBI	10	9.8	1.1	0.030	0.029	2.2	0.005	0.0049	2.2	0.80	0.81	2.2
Cenvite	Pharmasyr	30	32	0.8	0.025	0.023	1.9	0.010	0.0096	3.1	0.27	0.26	2.1
Damvita-M	Ultra medica	50	51	0.6	–	–	–	–	–	–	0.27	0.25	2.9
Viteax	ALPHA	70	72	0.3	–	–	–	–	–	–	1.60	1.70	1.3
K-VIT	IBN	–	–	–	10	9.9	0.8	–	–	–	–	–	–
Calciferol	Amrit	–	–	–	–	–	–	10	11	0.8	–	–	–
Pharmavit	Pharmasyr	70	71	0.2	–	–	–	–	–	–	1.6	1.58	1.7
Sterogyl 15	Oubari	–	–	–	–	–	–	10	11	1.0	–	–	–

T: The theory amount, E: the experimental amount.

Under the suggested chromatographic condition, we give to the chemist to make a choice on which column will be used to separate the fat soluble vitamins as the available ODS, CN and C_{18} columns, analysis time, kind of analysis or the product.

By comparison with the results it is noticed that the shortest time of analysis was on CN column about 9 min then C_{18} and finally C_8 about 13 min.

Under the recent study, we could have a good determination, separation and fine peaks of fat soluble vitamins (E, A, K, D_2) in a short time. So it can save a lot of solvents and chemical regents as well as time.

ACKNOWLEDGEMENTS

This work was financially and technically supported by the Ministry of High Education through the University of Aleppo, Faculty of Science, Department of Chemistry in cooperation with Faculty Pharmacy, Food and Analytical Department in University of Aleppo, Aleppo, Syria.

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(*Received*: 11 January 2010; *Accepted*: 2 March 2010) AJC-8495