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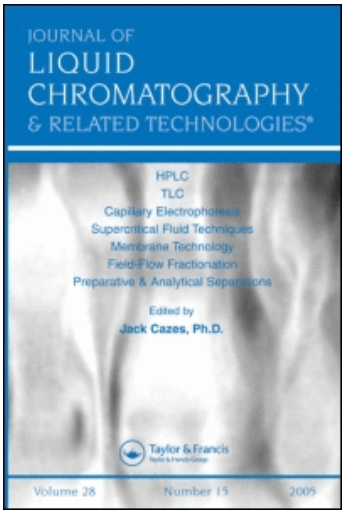

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DETERMINATION OF DIFFERENT SOLUBILITY VITAMINS IN PHARMACEUTICAL PREPARATIONS. I. HPLC COLUMN SWITCHING

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ABSTRACT

The aim of the study was to elaborate a solution enabling automation and fast quantitative determination of vitamins in pharmaceutical preparations. For this purpose, two analytical methods were combined in which different chromatographic stationary phases of different selectivity were employed. Application of the column-switching technique enabled sequential routine determination of the two different groups of vitamins. The proposed procedure was used for a quantitative determination of the vitamins: A, D_3, E, B_1, B_2, B_6, PP, and C in pharmaceutical preparations. It was proven that by means of this

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application, it is possible to perform about twelve complete analyses of vitamins within twenty-four hours.

INTRODUCTION

Simultaneous determination of all vitamins by means of one universal chromatographic method is not an easy matter. This is because not only do differences in their physicochemical properties result from different structures of parent compounds, but also because it involves many chemical individuals and physical forms of the same vitamin. Moreover, there is a broad range of concentrations in the same formulation.[1–3] Therefore, only a few methods encountered in the literature try simultaneous determination of vitamins, both fat and water-soluble. The solution presented in the study of Y. Arai and T. Hanai[4] is the application of gradient elution. The authors perform analysis on the column Inertsil ODS in gradient condition of 3% of acetonitrile in 0.1% phosphoric acid, with the addition of 5 mM pentanosulphonic acid to 97% of acetonitrile within 20 minutes. The other procedure is based on the application of a column trapping of fat-soluble vitamins and switching between two different analytical systems for each of the group vitamins.[5]

The aim of the study was to develop a procedure that enabled sequential determination of the fat- and water-soluble vitamins in pharmaceutical preparations. There are two different chromatographic systems are used: columns and mobile phase compositions. After selection of appropriate conditions for the chromatographic separation and sample preparation, the proposed analytical methods were used for determination of eight vitamins A, D_3, E_1, B_2, B_6, PP, and C. The quantitative analysis was performed in various pharmaceutical preparations including powder and tablets, both chewable and effervescent.

EXPERIMENTAL

Materials and Reagents

In the study, two different types of chromatographic columns were used: LiChrosorb RP-18 (E. Merck, Darmstad, Germany) as packing with low coverage density and Bakerbond BDC C_{18}, as dense modified surface (J.T. Baker, Deventeer, Netherlands). The characteristics of both columns are listed in Table 1.

For the preparation of standard solutions: vitamin A (retinol palmitate 250,000 I.U./g), vitamin D_3 (cholecalciferol 100,000 I.U./g), vitamin E (d, l-alpha-tocopheryl acetate 50%), vitamin B_1 (thiamine mononitrate), vitamin B_2

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Table 1. Characterisation of Columns Used in Vitamin Determinations

Parameter of Column/ Support	Code	Unit	Column I	Column II
Type of packing	–	–	LiChrosorb RP-18	Bakerbond BDC
Manufacturer	–	–	E. Merck	J.T. Baker
Column dimensions	–	mm	250×4.0	250×4.6
Particle diameter	d_p	μm	5.0	4.7
Particle shape	–	–	irregular	spherical
Pore diameter	D	nm	10	13
Pore volume	V_p	ml/g	1.0	0.65
Specific surface area	S_{BET}	m^2/g	330	170
Percent of carbon	Pc	%	16.2	11.1
Type of structure	–	–	*polymeric*	*polymeric*
Endcapped	–	–	no	yes
Surface coverage	α_{RP}	$\mu mol/m^2$	2.8	3.6

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(riboflavine), vitamin B_6 (pyridoxine hydrochloride), vitamin PP (nicotinamide), vitamin C (ascorbic acid), (Hoffmann La Roche, Basel, Switzerland) were used.

Solvents: acetonitrile, acetic acid (Baker), ethyl acetate (Merck), n-hexane (Fisons, Loughborough, Anglia), all purity grade for HPLC, and ethanol 96%. Water for analytical purposes was prepared in laboratory using Elix 5 system (Millipore, Bedford, USA).

Other reagents: phosphoric acid 85% p.a. (POCh, Gliwice, Poland), hexane-1-sulfonic acid sodium salt and triethylamine (Merck).

Apparatus

For the sample preparation a shaker model WU-4 (Premed, Warsaw, Poland), a rotary evaporator model RE-111 (Büchi, Flawil, Switzerland), and a water bath with shaker Model 357 (Conbest, Warsaw, Poland) were used.

Chromatographic analysis was performed using a liquid chromatograph (Waters, Milford, USA) consisting of a quaternary gradient pump 60 F with controller 600, an autosampler 717 plus, and a diode array detector PDA 996. The chromatograph was equipped with a two-position motorised valve, TPMV 7750 (Rheodyne, Co., Berkeley, CA, USA). The data were recorded by a computer

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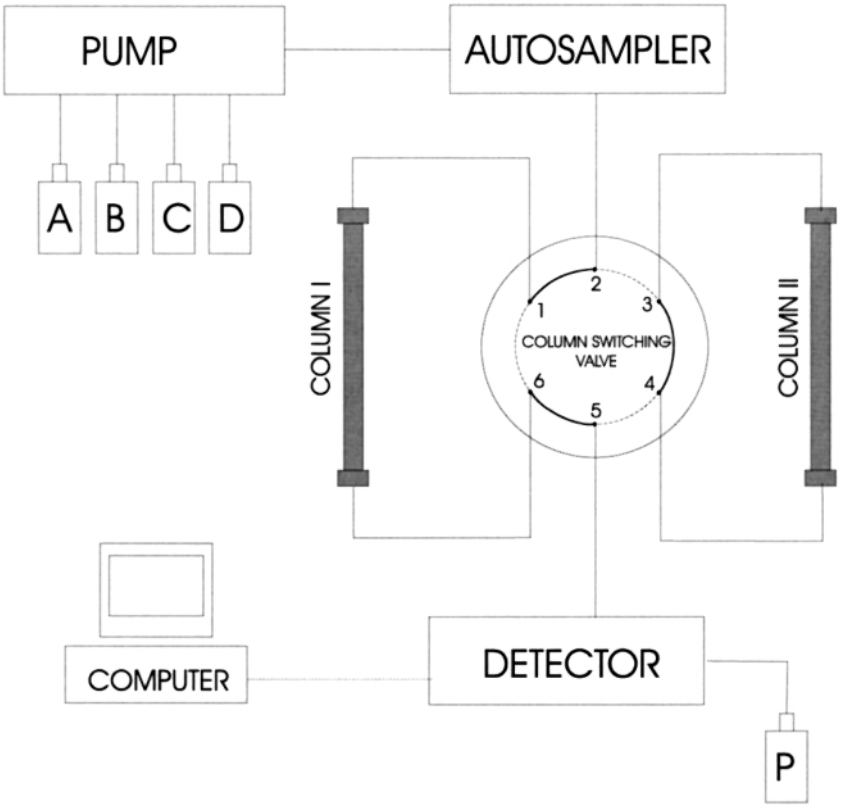

Figure 1. Schematic diagram of the apparatus. Where: A: ethyl acetate with addition 0.66 mL triethylamine for 1 L, B: mixture: water–methanol–acetic acid (730 : 270 : 10, v/v/v) with addition 1.4 g hexane-1-sulfonic acid sodium salt and 0.3 mL triethylamine for 1 L phase, C: water, D: acetonitrile, Column I: column for fat-soluble vitamins determination, Column II column for water-soluble vitamins determination. P: waste.

model 486-DX2-S 66 MHz (Optimus, Nowy Sacz, Poland) with Millennium 2.10 (Waters) software (Figure 1).

Standard Solutions

Standard solutions for the fat-soluble vitamins' determination were prepared by weighting: 20 mg vitamin A, 10 mg vitamin D_3 and 35 mg vitamin E. The weights were carried into a 300 mL Erlenmeyer flask and 25 mL 0.1 M

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solution of phosphoric acid was added, then 25 mL of ethanol and exactly 50 mL of n-hexane. The mixture was shaken within 30 minutes and was left for 5 minutes, to separate the layers. From a hexane layer, a 25 mL portion was taken and evaporated to dryness on the rotary evaporator. The residue was dissolved in 3 mL of acetonitrile–ethyl acetate mixture (80 : 20, v/v).

The standard stock solutions of water-soluble vitamins were prepared as follows: 30 mg of vitamin B_1 (thiamine mononitrate), 15 mg of vitamin B_2 (riboflavine), 30 mg vitamin B_6 (pyridoxine hydrochloride), and 250 mg of vitamin PP (nicotinamide) weighted accurately were transformed into 100 mL flasks. About 60 mL of the mixture: water–acetonitrile–acetic acid (94 : 5 : 1, v/v/v) was added and it was stirred until the vitamins were completely dissolved. The contents of the flask with vitamin B_2 were heated within about 15 minutes in a temperature of 70°C in a water bath, and then cooled to the room temperature. Solutions in the flasks were supplemented to 100 mL with the same mixture and mixed. For standard solution preparation, 1 mL of vitamin B_1, B_6, and PP; 2 mL of vitamin B_2 standard stock solutions; and 10 mg of vitamin C were placed in 10 mL flasks, filled up to volume with a mixture of water–acetonitrile–acetic acid (94 : 5 : 1, v/v/v), and mixed. Concentrations of vitamins in the prepared solution were, respectively, B_1 0.03 mg/mL, B_2 0.03 mg/mL, B_6 0.03 mg/mL, PP 0.25 mg/mL, and C 1 mg/mL.

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Sample Preparation

Preparations in the form of powder were analysed after mixing the contents of several packages. Tablets were powdered in a mortar and then the procedure was in accordance with the description below.

For the determination of fat-soluble vitamins, a suitable sample of the tested preparation was weighted and transferred into a 300 mL Erlenmeyer flask, then 25 mL 0.1 M of phosphoric acid solution, 25 mL of ethanol, and exactly 50 mL of hexane were added. The mixture was shaken within 30 minutes and then was left for 5 minutes so that layers might become distinct. From the hexane layer, a 25 mL portion was collected, then it was evaporated till complete dryness on a rotary evaporator, and the residue was dissolved in 2.5 mL of the mixture: acetonitrile–ethyl acetate (80 : 20, v/v), and volume was filled to 3 mL.

For determination of water-soluble vitamins tested, adequate samples were weighted into a 100 mL flask and about 60 mL of the water–acetonitrile–acetic acid (94 : 5 : 1, v/v/v) mixture was added. Samples were heated within 15 minutes in a temperature of 70°C in a water bath. The solutions were cooled to room temperature and filled up with the same mixture to 100 mL.

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Chromatographic Analysis

For determination of fat-soluble vitamins, a LiChrosorb RP-18 5 μm, 250 × 4 mm column was used, mobile phase was an acetonitrile–ethyl acetate (85 : 15, v/v) mixture with the addition of 0.1 mL of triethylamine to 1 L, flow rate 1.5 mL/min, injection 50 μL, detection UV-Vis, $\lambda = 254$ nm.

Determination of water-soluble vitamins was performed using Bakerbond chromatographic column BDC C_{18}, 5 μm 250 × 4.6 mm, mobile phase water–methanol–acetic acid (730 : 270 : 10, v/v/v) with the addition of 1.4 g hexane-1-sulfonic acid sodium salt and 0.3 mL triethylamine to 1 L, flow rate 1.0 mL/min, injection 20 μL, detection UV-Vis, $\lambda = 280$ nm.

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RESULTS AND DISCUSSION

System Description

Taking into consideration some significant differences between properties of the tested compounds, the chromatographic separations were made in two distinct chromatographic systems, using the columns that differ in properties of stationary phase and column-switching technique that enabled automation of the study and with one chromatograph used to perform analyses. The application of an electrically controlled two-position valve let the determinations be performed without stopping work, changing columns, mobile phases, etc. The valve that changes the column was equipped with a stopper motor and a system for remote switching by means of electrical impulses. The work of the valve was steered by a chromatograph pump in accordance with a sequence of commands realised by the software.

The analysis was started with the determination of a vitamin from the first group, then a column was washed out, mobile phase flow was stopped, and subsequently, the column was exchanged and vitamins from the second group were analysed (Table 2). The work was initiated by 30 minutes stabilisation of the column used for the determination of fat-soluble vitamins.

The steps of the procedure are given in Table 2. The reason for washing with solutions given in the table was the statement that after a few days of the analyses retention times of water-soluble vitamins started to increase. It was caused by residue of hydrophobic compounds, among others fat-soluble vitamins, which remained on the chromatographic column. As a result, the hydrophobic properties of the column increased, and consequently, retention times of the analytes were prolonged. One should pay attention to usefulness of the proposed

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Table 2. Conditions for Analysis, Columns Stabilisation and Cleaning

Mobile Phase Composition	Flow Rate, mL/min.	Time, min	Description
COLUMN I			
15% **A** 85% **D**	1.5	30	Stabilisation column for fat-soluble vitamins determination
15% **A** 85% **D**	1.5	25	Analysis standards and samples of fat-soluble vitamins
30% **A** 70% **D**	1.5	30	Column washing
100% **D**	1.0	15	System flushing with pure acetonitrile
	0	2	Stop flow
Column Switching			
COLUMN II			
100% **D**	od 0 do 1	2	Gentle flow start from 0 to 1 ml/min
100% **D** do 73% **C** 27% **D**	1	10	Mobile phase changing
73% **C** 27% **D** do 100% **B**	1	10	Mobile phase changing
100% **B**	1	30	Stabilisation column for water-soluble vitamins determination
100% **B**	1	25	Analysis standards and samples of water-soluble vitamins
100% **B** do 100% **C**	1	10	Mobile phase changing
100% **C**	1	30	Flushing column with water
100% **C** do 100% **D**	1	20	Mobile phase changing
100% **D** do 30% **A** 70% **D**	1	5	Mobile phase changing
30% **A** 70% **D**	1	20	Column washing
30% **A** 70% **D** do 100% **D**	1	10	Mobile phase changing
100% **D**	1	10	System flushing with pure acetonitrile

A: Ethyl acetate with addition of 0.66 mL triethylamine for 1 L, B: Mixture: water–methanol–acetic acid (730 : 270 : 10, v/v/v) with addition of 1.4 g hexane-1-sulfonic acid sodium salt and 0.3 mL triethylamine for 1 L phase, C: Water, D: Acetonitrile, Column I: Column for fat-soluble vitamin determination, Column II: Column for water-soluble vitamin determination.

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procedure because it is possible to analyse, by means of the used arrangement, the contents of vitamins in approximately twelve samples within 24 hours.

Chromatographic Separation

Most suitable for the determination of fat-soluble vitamins, were methods that use non aqueous reverse phase (NARP) in an arrangement which was proposed in the literature, consisting of acetonitrile and more nonpolar organic solvents.[6] Because of the fact that in solvents such as chloroform or methylene chloride isomerization of vitamin A may appear,[7] mobile phase composition and the solution for samples used for determination of the fat-soluble vitamins was changed through replacing proposed methylene chloride with ethyl acetate. It was observed that when solvents with HPLC purity were used in the described conditions, unrepeatable areas of peak of vitamin A with tendency to increase respectively with successive injections were observed. For the purpose of obtaining a proper repetitiveness of vitamin A peak, addition of triethylamine to the mobile phase appeared to be necessary. One may assume that the reason for such an occurrence was the remaining free silanol groups presented on the surface of stationary phase. After application of the mentioned addition to the mobile phase, relative standard deviation of the peak surface for vitamins A and E did not exceed 1% and with an exception for vitamin D_3 when it was exceeded 1.5% (Table 3).

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Table 3. Properties of Chromatographic System (Fat-Soluble Vitamins, $t_o = 1.974$, Water-Soluble Vitamins, $t_o = 3.184$)

Vitamin	Retention Time, t_r, min	Capacity Factor, k	Peak Area Repeatability, RSD %, (n = 6)	Asymmetry Factor, f_{as}
E (d,l-alpha-tocopheryl acetate)	6.43	2.26	1.00	2.11
D_3 (cholecalciferol)	8.21	3.16	1.78	1.16
A (retinal palmitate)	13.25	5.71	0.99	1.11
C (ascorbic acid)	2.99	0.06	0.22	0.94
PP (nicotinamide)	4.05	0.27	0.18	1.03
B_6 (pyridoxine hydrochloride)	6.33	0.99	0.17	1.09
B_2 (riboflavine)	8.05	1.53	0.17	1.07
B_1 (thiamine mononitrate)	15.28	3.79	0.20	1.10

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Because there are water-soluble vitamin compounds containing polar functional groups, the Bakerbond BDC C_{18}, with a modified stationary phase surface, was used which minimizes the possibility of contact between solute molecules and the residual silanol groups. Such fillings are especially suitable for analyses of compounds with basic properties,[8,9] the example from the tested compounds is vitamin B_1. By means of the used stationary phase, a good peak symmetry was obtained; asymmetry factor did not exceed $f_{as} \leq 1.2$ for any of the analysed vitamins. There was good repetitiveness of peak area, relative standard deviations below 0.25% were also obtained (Table 3).

Quantitative Analysis

In the proposed conditions, the complete separation of the analysed compounds from the remaining components of the tested preparations was obtained. The time of the chromatographic run of both groups of vitamins did not exceed 25 minutes (Table 3). There were coeluting peaks, except vaniline, a component added to some of the tested preparations, that appeared on chromatograms of water-soluble vitamins (near peak of vitamin B_2) (Figure 2).

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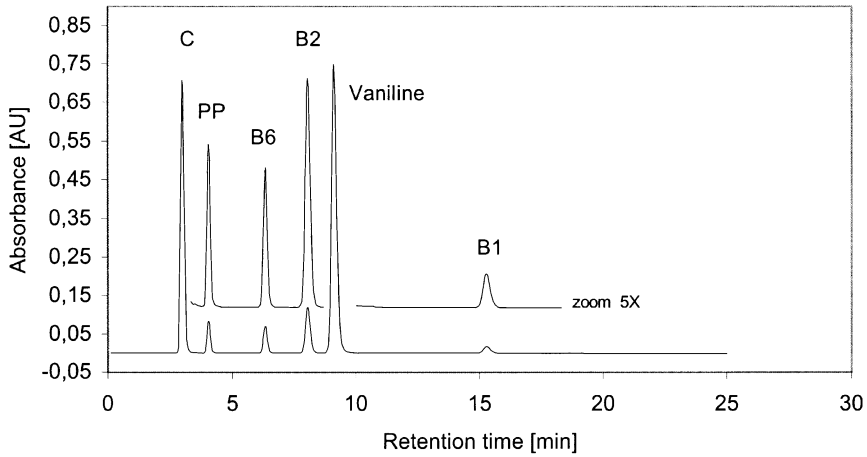

Figure 2. Separation of the water-soluble vitamins. Conditions: column Bakerbond BDC C_{18}, 250 × 4.6 mm, mobile phase water–methanol–acetic acid (730 : 270 : 10, v/v/v) with addition 1.4 g hexane-1-sulfonic acid sodium salt and 0.3 mL triethylamine for 1 L, flow rate 1.0 mL/min, detector $\lambda = 280$ nm, injection 20 µL.

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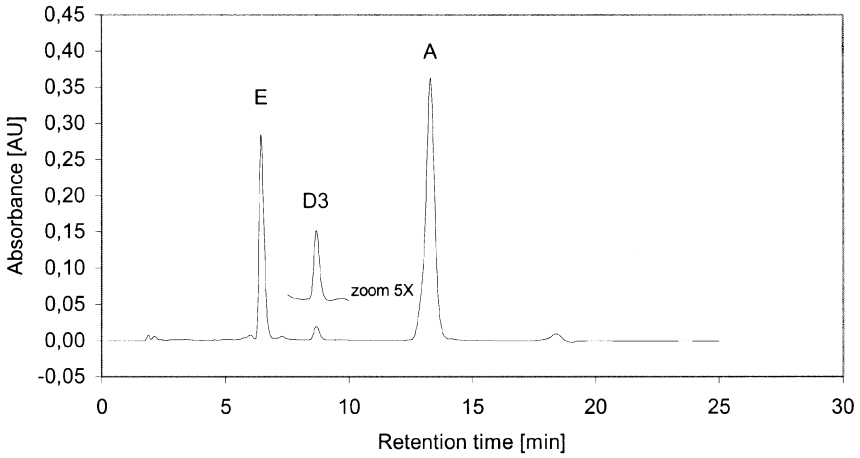

Figure 3. Separation of the fat-soluble vitamins. Conditions: column LiChrosorb RP-18 (5 μm), 250 × 4 mm, mobile phase acetonitrile–ethyl acetate (85 : 15, v/v) with addition of 0.1 mL triethylamine for 1 L, flow rate 1.5 mL/min, detector $\lambda = 254$ nm, injection 50 μL.

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The appearance on chromatograms of samples containing fat-soluble vitamins of additional peaks was derived from raw materials of the marked vitamins, mainly from raw material of vitamin A (Figure 3).

The results of determinations of vitamins in the tested preparations were in agreement with the declared data. The differences did not exceed a few percent (Table 4).

The methods were successfully used in many preparations such as multivitamin tablets, effervescent tablets containing calcium and vitamin D_3, chewable tablets, and multivitamin tablets with microelements. Acceptable results for concentrates used as fodder additives were also obtained. In the case of some preparations, it was necessary to heat the samples during dissolving to a temperature of about 40°C to dissolve the coatings and release the fat-soluble vitamins. The determination of water-soluble vitamins by means of this method failed for multivitamin tablets with microelements. It would be necessary in this case to make a modification of the sample preparation, e.g., by application of the SPE technique. These proposed methods failed to mark the rest of water-soluble vitamins added to the tested preparations, vitamin B_{12} and calcium panthotenate, because of too small quantities of B_{12} and lack of sufficient UV absorption pantotenate in the conditions of the analysis. For the determination of these

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Table 4. Results of the Quantitative Analysis of the Two Different Multivitamin Preparations

	Preparation 1—Determined Content of the Vitamins in 2 g							
Theoretical Content of the Vitamins in 2 g	A I.U. 2400	D_3 I.U. 500	E mg 8.4	B_1 mg 1.32	B_2mg 1.44	B_6 mg 1.54	PP mg 12.6	C mg 77
Sample 1	2448	511	8.31	1.23	1.28	1.41	11.32	71.92
2	2520	520	7.86	1.19	1.28	1.42	11.40	70.38
3	2320	458	7.59	1.28	1.33	1.51	12.50	73.81
4	2195	503	7.67	1.35	1.35	1.39	11.91	69.40
5	2468	465	7.86	1.28	1.32	1.61	11.74	71.18
6	2518	457	8.15	1.29	1.47	1.40	12.10	79.5
	Preparation 2—Determined Content of the Vitamins in 2 g							
Theoretical Content of the Vitamins in 2 g	A I.U. 1800	D_3 I.U. 500	E mg 5.25	B_1 mg 0.77	B_2 mg 0.96	B_6 mg 0.88	PP mg 8.4	C mg 55
Sample 1	1650	486	4.81	0.69	0.89	0.81	8.17	50.88
2	1781	505	5.18	0.71	0.93	0.85	8.58	54.25
3	1850	523	5.15	0.69	0.89	0.85	8.31	55.65
4	1812	465	4.89	0.77	1.00	0.92	8.71	55.33
5	1700	514	4.79	0.70	0.87	0.90	8.22	51.02
6	1852	468	5.05	0.69	0.88	0.86	8.09	51.88

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vitamins, either modification of sample preparation or other methods of detection should be performed.

CONCLUSIONS

The proposed approach allows the determination to be considerably hastened, although it does not solve the problem of a chromatographic system for simultaneous determination both fat- and water-soluble vitamins. The system enables the complete analyses to be performed in approximately twelve samples of vitamin preparations within twenty four hours, by means of one chromatograph equipped with an autosampler.

Both methods used for chromatographic determination obtain complete separation of tested vitamins within the time shorter than 25 minutes and meet the demands necessary for the methods used in control laboratories.

The advantage of the chromatographic methods, in relation to traditional methods of determination of the contents of vitamins, is the possibility of determining contents of several different vitamins. It allows decreasing the time needed for performing analyses and decreasing the expenses as well.

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REFERENCES

1. Stancher, B.; Zonta, F.J. Chromatogr. **1983**, *256*, 93.
2. Hollman, P.C.H.; Slangen, H.; Wagstaffe, P.J.; Faure, U.; Southgate, D.A.T.; Finglas, P.M. Analyst **1993**, *118*, 475.
3. Kountourellis, J.E.; Raptouli, A.; Botsoglou, N.A.; Georgarakis, M. Pharmazie **1985**, *40*, 193.
4. Arai, Y.; Hanai, T.J. Liquid Chromatogr. **1988**, *11*, 2049.
5. Mikami, A. Jpn. Kokai Tokyo Koho. **1989**, *111*, 6215f.
6. Barnett, S.A.; Frick, L.W.; Baine, H.M. Anal. Chem. **1980**, *52*, 610.
7. Mulry, M.C.; Schmidt, R.H.; Kirk, J.R. J. Assoc. Off. Anal. Chem. **1983**, *66*, 746.

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8. Buszewski, B.; Suprynowicz, Z.; Staszczuk, P.; Albert, K.; Pfleiderer, B.; Bayer, E.J. Chromatogr. **1990**, *499*, 305.
9. Buszewski, B.; Jezierska, M.; Welniak, M.; Berek, B. J. High Resol. Chromatogr. **1998**, *21*, 267.

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