ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1999) 999–1004

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Reversed-phase ion-pair HPLC determination of some water-soluble vitamins in pharmaceuticals

D. Ivanović [a,*], A. Popović [b], D. Radulović [a], M. Medenica [c]

[a] *Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, 11000, Belgrade, Yugoslavia*
[b] *Medifarm, Belgrade, Yugoslavia*
[c] *Department of Physical Chemistry, Faculty of Pharmacy, Belgrade, Yugoslavia*

Received 16 November 1997; received in revised form 26 March 1998; accepted 1 April 1998

Abstract

A reversed-phase ion-pair high-performance liquid chromatografic method (RP-IPC) was developed to assay some water-soluble vitamins in solution dosage forms. Vitamins of the B-group B_1, B_2, B_3, and B_6, including vitamin C were determined in Oligovit® coated tablets. In Beviplex® coated tablets the vitamins B_1, B_2, B_3, B_6 and *p*-aminobenzoic acid were analysed. Hexanesulphonic acid sodium salt and triethanolamine in water–methanol were used as mobile phase with adjusting pH to 2.8 with orthophosphoric acid. Phenol was used as an internal standard. For quantitative simultaneous analysis of vitamins in pharmaceutical formulations, the method of internal standard was used. All parameters for the validation of the method are given.

Keywords: Reversed-phase ion-pair high-performance liquid chromatography; Water-soluble vitamins; Pharmaceutical formulations

1. Introduction

Pharmaceutical preparations which contain vitamins are most interesting for analysing because of their complex composition. It is a special provocation for the investigators. Because of that the aim of our investigations was to develop conditions for the RP-IPC method which makes possible simultaneous determinations of some water-soluble vitamins in multi-vitamin pharmaceutical dosage forms.

It was found in the literature that vitamins from the B-group were determined individually in pharmaceutical preparations using the volumetric [1–3], spectrophotometric [4–6], spectrofluorimetric [7], and electrochemical [8,9] methods. Separation technics, such as planar [10], thin-layer [11] and high-performance liquid chromatography [12–14] were used for the qualitative and quantitative simultaneous assay of the above mentioned vitamins. Ascorbic acid was determined by volumetric [3], spectrophotometric [15,16] and electrochemical [17,18] method. RP-HPLC method was used for the investigation of ascorbic acid [19].

* Corresponding author.

0731-7085/99/$ - see front matter
PII S0731-7085(98)00109-5

The mentioned methods enable the determination vitamins individually in multi-vitamins pharmaceuticals using different methods. Applying the proposed RP-IPC technic it is possible to identify and determine simultaneously most of the vitamins in analysed pharmaceutical preparations with only one injection.

2. Experimental

2.1. Reagents

All chemicals and reagents were of an analytical reagent grade and water was distilled and filtered with a membrane filter. Methanol for HPLC (Merck, Darmstadt, Germany), Hexanesulphonic acid sodium salt (Merck) and Triethanolamine (Zorka, Šabac, Yugoslavia) were used to prepare a mobile phase and Orthophosphoric acid (Kemika, Zagreb, Croatia) for adjusting the pH values. Hydrochloric acid conc. (Zorka) was used for dissolving the samples.

2.2. Apparatus

The chromatographic LKB Pharmacia Gradient HPLC System consisted of a HPLC pump A and pump B 2150 LKB, LC controler 2152 LKB and Rapid Spectral 'diode array' UV detector 2140 LKB. LDC Analytical Consta Metric HPLC System consisted of Consta Metric pump 3000 LDC, Spectro-monitor 3100 variable wavelength detector and integrator LKB 2221. Epson LX-40 was used as a printer. Operating parameters were: attenuation 4, sensitivity 0.5 AUFS and chart speed 0.5 cm min^{-1}.

2.3. Chromatographic conditions

Separations were performed on Supelcosil column LC-8-DB 250 × 4.6 mm, with 5 μm particles sizes. Samples were introduced through a Rheodyne injection valve with a 10 μl sample loop.

Hexanesulphonic acid sodium salt and triethanolamine in water–methanol (85:15 v/v) was used for Oligovit® coated tablets. For Beviplex® coated tablets the same mobile phase was used in ratios (92:8 v/v) and (82.8:17.2 v/v). pH was adjusted to 2.8 with orthophosphoric acid. Mobile phase was filtered through a 0.2μm Millepore filter and degassed in an ultrasonic bath. Phenol was used as an internal standard. The flow rate was 2 ml min^{-1} and UV detection was performed at 280 nm, at room temperature.

2.4. Standard solutions

Concentrations of standard solutions for calibration curves were: thiamin hydrochlorid—vitamin B_1 1.48×10^{-4}–7.41×10^{-4} M, riboflavin—vitamin B_2 2.65×10^{-5}–2.65×10^{-4} M, nicotinamide—vitamin B_3 8.19×10^{-4}–8.18×10^{-3} M, pyridoxine hydrochlorid—vitamin B_6 9.72×10^{-5}–2.92×10^{-4} M, ascorbic

Table 1
The important parameters for the calibration curves

Vitamin	$y = ax + b$	r	S_a	S_b	LOD*	LOQ*
B_1	$574\,600x - 2562$	0.9978	18 572	3230	0.03	0.06
B_2	$87\,886x + 397$	0.9979	13 879	849	0.008	0.025
B_3	$36\,982x + 2262$	0.9993	2624	1611	0.0012	0.05
B_6	$495\,660x + 1012$	0.9983	37 952	1610	0.015	0.02
C	$172\,907x + 5500$	1.0003	25 807	15 845	0.0025	0.01
PABA	$180\,192x + 9806$	1.0001	96 608	39 231	0.005	0.02

[a] Experimentally determined values.
r, correlation coefficient; S_a, standard deviation of the slope; S_b, standard deviation of the intercept; LOD, limit detection; LOQ, limit quantification.

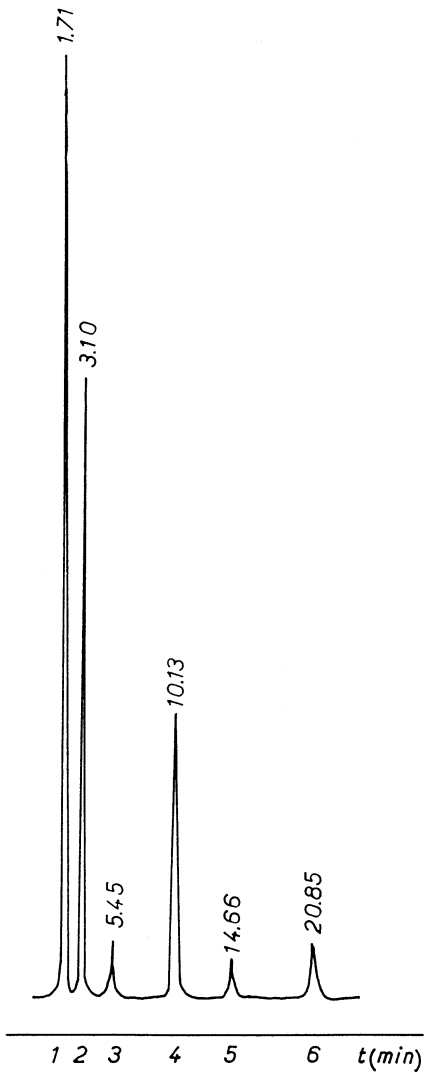

Fig. 1. The representative chromatogram of vitamins C (1), B_3 (2), B_6 (3), internal standard (4), vitamin B_1 (5) and B_2 (6) in Oligovit® coated tablets. Mobile phase: hexanesulphonic acid sodium salt and triethanolamine in water–methanol (85:15 v/v). pH was adjusted to 2.8 with orthophosphoric acid. Phenol was used as an internal standard. The flow rate was 2 ml min^{-1} and UV detection was performed at 280 nm, at room temperature.

acid—vitamin C 5.67×10^{-4}–5.67×10^{-3} M, *p*-aminobenzoic acid—PABA 2.18×10^{-3}–3.64×10^{-3} M. All substances were USP reference standards.

2.5. Laboratory mixtures

Laboratory mixtures which corresponded to Oligovit® coated tablets were prepared of vitamin B_1 (1.50×10^{-4} M), B_2 (1.40×10^{-4} M), B_3 (3.86×10^{-3} M), B_6 (1.08×10^{-4} M) and vitamin C (5.64×10^{-3} M) in mobile phase. For the chro-

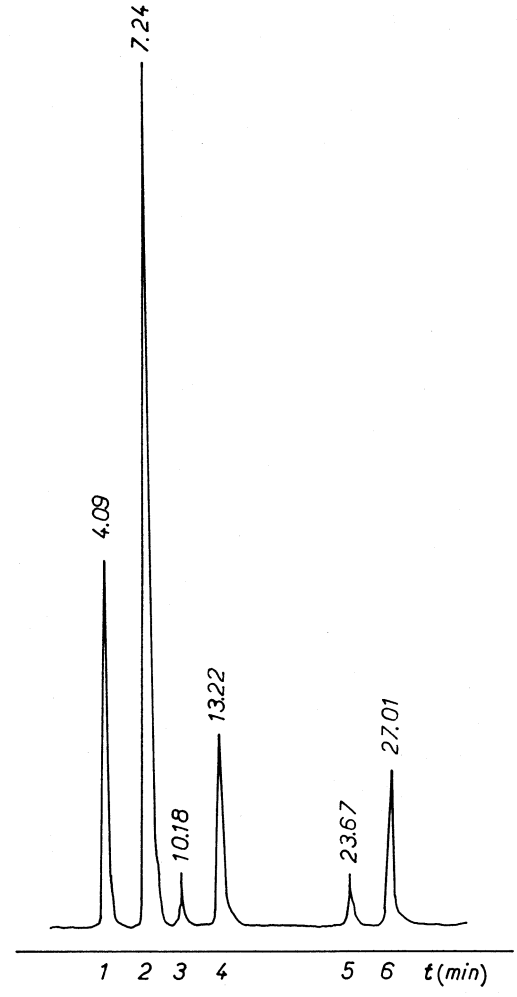

Fig. 2. The chromatogram of vitamin B_3 (1), PABA (2), vitamin B_6 (3), internal standard (4), vitamins B_1 (5) and B_2 (6) in Beviplex® coated tablets. Mobile phase: hexanesulphonic acid sodium salt and triethanolamine in water–methanol (92:8 v/v) for the first ten minutes and (82.8:17.2 v/v) to the end of the separation. pH was adjusted to 2.8 with orthophosphoric acid. Phenol was used as an internal standard. The flow rate was 2 ml min^{-1} and UV detection was performed at 280 nm, at room temperature.

Table 2
Determination of vitamins in Oligovit® coated tablets

Vitamin	Amount inj. (mg ml^{-1})	Found (mg ml^{-1})	S.D. (mg ml^{-1})	Recovery (%)	t_n
B_1	0.05	0.0488	0.0008	99.83	1.50
B_2	0.05	0.0489	0.001	97.70	1.10
B_3	0.50	0.5070	0.003	105.40	2.33
B_6	0.025	0.0241	0.0007	99.15	1.28
C	1.00	1.0012	0.0007	100.02	1.71

* $n = 10$.

matographic separation, phenol was added as an internal standard (1.08×10^{-2} M).

Laboratory mixtures which corresponded to Beviplex® coated tablets were prepared of vitamin B_1 (2.46×10^{-4} M), B_2 (2.90×10^{-4} M), B_3 (4.26×10^{-3} M), B_6 (2.60×10^{-4} M) and PABA (3.07×10^{-3} M) in mobile phase. For the chromatographic separation phenol was added as an internal standard (1.08×10^{-2} M).

2.6. Pharmaceutical preparations

Beviplex® dragee (ICN Yugoslavia): one dragee contains: 4 mg of vitamin B_1 in form thiamine chloride, 5 mg of vitamin B_2 in form Lactoflavine, 2 mg of vitamin B_6 in form pyridoxine hydrochloride, 1 mg of vitamin B_{12} in form cyanocobalamin, 5 mg of vitamin B_5 in form calcium panthotenate, 25 mg of vitamin B_3 in form nicotinamide and 20 mg of PABA in form *p*-aminobenzoic acid.

Oligovit® dragee (ICN Yugoslavia): One dragee contains: 5 mg of vitamin B_1 in form thiamine chloride, 5 mg of vitamin B_2 in form lactoflavine, 50 mg of vitamin B_3 in form nicotinamide, 10 mg of vitamin B_5 in form Calcium Panthotenate, 2.5 mg of vitamin B_6 in form pyridoxine hydrochloride, 0.0025 mg of vitamin B_{12} in form cyanocobalamin, and 100 mg of vitamin C in form ascorbic acid.

2.7. Sample solutions

An amount of ground dragees equivalent to average fill was weighted from the composite of 10 Oligovit® or Beviplex® dragees (ICN Yugoslavia). This procedure was used to compensate any variation of weight in individual dragees. Each sample was transferred with 0.01 M hydrochloric acid to a 50 ml volumetric flask to make the final concentration of the solution, approximately the same as the stock standard solution. The flask was placed in an ultrasonic bath for 10 min and was centrifuged for 10 min. 1 ml of supernatant was mixed with 1 ml of an internal standard solution. Resulting solution was injected into the column.

3. Results and discussion

For the separation and determination of water-soluble vitamins in analysed pharmaceutical preparations Oligovit® and Beviplex® coated tablets, the best results were obtained with the following mobile phase. Hexanesulphonic acid sodium salt and triethanolamine in water–methanol (85:15 v/v) was used for Oligovit® coated tablets. For Beviplex® coated tablets the same mobile phase was used in ratio (92:8 v/v) for the first 10 min (eluent A) and after that to the end of the separation, in the ratio (82.8:17.2 v/v). This ratio was obtained by the combination of two pumps with different eluent, using 90% of the mobile phase (eluent A) and 10% of methanol (eluent B).

The robustness of a method describes the effect of minor changes in the analytical parameters, such as pH value, eluent composition, temperature, flow rate, etc. In our investigations, it can be concluded that RP-IPC method is robust, because slight variations in some experimental parameters have little or no effect on the results. The composition of Hexanesulphonic acid sodium salt and

Table 3
Determination of vitamins in Beviplex® coated tablets

Vitamin	Amount inj. (mg ml^{-1})	Found (mg ml^{-1})	S.D. (mg ml^{-1})	Recovery (%)	t_n
B_1	0.08	0.0812	0.0004	104.02	3.00
B_2	0.10	0.0975	0.0009	97.40	2.77
B_3	0.50	0.4925	0.002	99.66	3.75
B_6	0.04	0.0408	0.0003	100.09	2.66
PABA	0.40	0.4090	0.002	106.90	4.50

* $n = 10$.

triethanolamine was changed $\pm 5\%$, pH was changed in the interval from 2.5 to 3.0 and the ambient temperature from 18 to 25°C. There were no remarkable changes of the retention time except that the increase of temperature decreased the retention time and the shape of the peak was finer. These little changes didn't interfere with the quantitative results.

A linear relationship of peak area over the mentioned concentration range for vitamins B_1, B_2, B_3, B_6, C and PABA were obtained, respectively. The important parameters of calibration curves, such as slope (a), intercept (b), correlation coefficient (r), standard deviation of the slope (S_a) and standard deviation of the intercept (S_b), are presented in Table 1. Detection limit (LOD) and quantification limit (LOQ) were experimentally determined and they are presented in Table 1.

Fig. 1. shows a representative chromatogram of analysed Oligovit® coated tablets with good separation of vitamins C, B_3, B_6, internal standard, vitamins B_1 and B_2.

Fig. 2. represents the chromatogram of vitamins B_3, PABA, vitamin B_6, internal standard, vitamins B_1 and B_2 in Beviplex® coated tablets.

The best resolution was obtained using phenol as an internal standard. Chromatograms obtained for the laboratory mixtures and for the pharmaceutical samples show a good selectivity and specificity of procedure. The influence of the excipient did not interfere to the selectivity of the method.

In neither of multi-vitamin pharmaceutical preparations (Oligovit® and Beviplex® coated tablets) it was possible to detect vitamin B_5 because it didn't absorb at 280 nm. Vitamin B_{12} is present only in the trace in comparison with the amount of other vitamins in the mentioned preparations.

The concentrations of vitamins were calculated by the internal standard method.

In the Tables 2 and 3 are given the results of the reversed-phase ion-pair HPLC determination of the vitamins in Oligovit® and Beviplex® coated tablets. The important statistical value for the accuracy validation of the method (t_n coefficient of confidence) is given in Table 2 an Table 3 with the other results which show a good recoveries and a small standard deviations. The precision (reproducibility) of the results is minor than 4% and the repeatability is minor than 3% for the analysed vitamins.

The results show that the described HPLC method is reproducible and can be used for the determination of water-soluble vitamins. The proposed method is rapid, accurate, sensitive and the results are reproducible. The authors propose this method for the simultaneous determination of water-soluble vitamins in multicomponent pharmaceutical formulations.

4. Conclusion

This RP-IPC method is rapid, accurate and the results are reproductive. Therefore the authors propose this method for the separation, identification and simultaneous quantitative assays of some water-soluble in multi-vitamin pharmaceutical dosage forms.

References

[1] S.N. Borisevich, G.I. Savel'eva, Zh. Anal. Khim. 39 (1984) 171–174.
[2] I.P. Koka, P.S. Koltun, Farm. Zh. (Kiev) 4 (1986) 64–65.
[3] K. Verma, A. Jain, R. Rawat, J. Assoc. Off. Anal. Chem. 67 (1984) 262–265.
[4] T. Sane, V.J. Doshi, S. Jukar, S.K. Joshi, S.V. Sawant, U.R. Pandit, J. Assoc. Off. Anal. Chem. 68 (1985) 83–85.
[5] C.S.P. Sastry, N.R. Singh, M.N. Reddy, Analysis 14 (1986) 355–356.
[6] R.T. Sane, V.J. Doshi, S.K. Joshi, J. Assoc. Off. Anal. Chem. 66 (1983) 158–160.
[7] M. Barary, M. Abdel-Hamid, E. Hassan, M. Elsayed, Pharmazie 41 (1986) 483–485.
[8] C. Wang, Y. Guo, Yaoxue Tongbao 21 (1986) 143–144.
[9] Z. Dai, Yaoxue Tongbao 21 (1986) 77–79.
[10] S. Sriewoelan, S. Igna, R.H.P. Sirait, Acta Pharm. Indones. 9 (1984) 143–151.
[11] S. Baczyk, M. Lewandowska, T. Szwajca, Mikrochim. Acta 1 (1983) 437–442.
[12] N. Sadlej-Sosnowska, D. Blitek, I. Wilczynska-Wojtulewicz, J. Chromatogr. 357 (1986) 227–232.
[13] G.R. Ramana, S.S.N. Murty, K.R. Mohan, Indian Drugs 19 (1982) 451–455.
[14] T. Canella, G. Bichi, Boll. Chim. Farm. (1983) 122.
[15] W. Baker, T. Lowe, Analyst 110 (1985) 1189–1191.
[16] D. Ivanović, M. Medenica, E. Nivaud-Guernet, M. Guernet, Spectrosc. Lett. 28 (1995) 557–571.
[17] V.G. Belikov, S.V. Volokitin, Farmatsiya (Moscow) 34 (1985) 43–46.
[18] A.C. Calokerinos, T.P. Hadjiioannou, Microchem. J. 26 (1983) 464–469.
[19] D. Ivanović, M. Medenica, E. Nivaud-Guernet, M. Guernet, Chromatographia 40 (1995) 652–656.