Turkish Journal of Chemistry

Volume 26 | Number 3 Article 12

1-1-2002

Determination of Ternary Mixtures of Vitamins (B_{1}, B_{6}, B_{12}) by Zero-Crossing Derivative Spectrophotometry

MAHMURE ÜSTÜN ÖZGÜR

İKBAL KOYUNCU

Turk J Chem
26 (2002) , 385 – 391.

Determination of Ternary Mixtures of Vitamins (B_1, B_6, B_{12}) by Zero-Crossing Derivative Spectrophotometry

Mahmure Üstün ÖZGÜR, İkbal KOYUNCU
Yıldız Technical University, Department of Chemistry, Faculty of Science and Letters, 34210 Davutpaşa, İstanbul-TURKEY

Received 30.11.2000

A new method for determining ternary mixtures of vitamin B_1, B_6 and B_{12} using second derivative spectrophotometry is described. The procedure is accurate, nondestructive and does not require any separation step or the solving of equations. Calibration graphs were linear up to 20 $\mu g ml^{-1}$ of vitamin B_1 at 228.9 nm (r=0.9999), vitamin B_6 at 309.6 nm (r=0.9999) and vitamin B_{12} at 361.7 nm (r=0.9998). The method was successfully applied for analyzing synthetic mixtures and commercial pharmaceutical preparations.

Key Words: Derivative spectrophotometry, Vitamins, B_1, B_6, B_{12}.

Introduction

The quality control of pharmaceutical preparations of polyvitamins requires reliable and quick analytical methods. UV-visible spectrophotometry and fluorimetric methods generally involve tedious and lengthy extractions[1]. Many reversed phase high-performance liquid chromatographic (HPLC) methods have been described that use various ion-pairing reagents with preliminary automated extraction[2] and spectrophotometric or electrochemical detection[3]. In some studies ion-exchange chromatography[4] were used. Derivative spectrophotometry, the fundamental principles and applications of which have been frequently and comprehensively reviewed, has received increasing attention, in the analysis of systems of clinical and biological interest[5−9]. Derivative spectrophotometry has been applied extensively to the simultaneous analysis of binary mixtures of substances with overlapping spectra[10−13]. Berzas et al. developed a method for resolving ternary mixtures based on the use of the 1^{st} derivative of the ratio spectra of mixtures, followed by measurements at the zero crossing wavelengths of the 1^{st} derivative of ratio spectra of single components. Theoretical approach and details on the experimental procedure are found in Berzas Nevado et al.[14]. Ratio spectrophotometry has been used for determining ternary mixtures of vitamins[15] and, in the last few years, it has been used for determining quaternary mixture of vitamins[16] and hydrosoluble polyvitamins[17]. We have used derivative spectrophotometry for the simultaneous determination of a ternary mixture of food colors

(Allura Red - Sunset Yellow - Tartrazine)[18] and (Sunset Yellow - Tartrazine - Ponceau 4R)[19] in powdered drinks and ternary pharmaceutical mixtures[20].

The B-complex vitamins act favorably against inflammatory diseases and the degeneration of locomotory organs due to their particular influence on the trophism of nervous and muscular cells. Their importance in the therapeutic field and the large overlap of the absorption spectra of vitamins B_1, B_6 and B_{12} lead us to try accurate methods for a quality control of pharmaceuticals for these drugs.

The present paper describes a method that can be applied to a mixture of up to three vitamins at various concentrations. The technique was applied favorably to both synthetic mixtures and pharmaceutical dosage forms containing three hydrosoluble vitamins at different concentrations.

Experimental

Reagents

Thiamine hydrochloride (vitamin B_1), Pyridoxine hydrochloride (vitamin B_6), Cyanocobalamin (vitamin B_{12}), and Apikobal tablets (250 mg vitamin B_1, 250 mg vitamin B_6 and 1 mg vitamin B_{12}) were kindly supplied by Santa Farma İlaç Sanayi A.Ş., İstanbul. Analytical grade hydrochloric acid (E. Merck) and ionized water was used throughout the work.

Equipment

In this study, a Philips PU 8700 UV - visible spectrophotometer was used for all absorbance measurements. The derivative spectra were automatically obtained from the spectrophotometer. Suitable settings were: 2 nm band pass, 500 nm min^{-1} scan speed and very high smoothing.

Methods

Preparation of the stock solution

Each vitamin was dissolved in 0.1N hydrochloric acid and then diluted with the same solvent in order to obtain 200 μgml^{-1} final concentrations. Working solutions had a concentration of 20 μgml^{-1}.

Preparation of the standard solutions and synthetic mixtures

Standard solutions: Standard solutions were prepared in 10 ml volumetric flasks containing 4-20 μgml^{-1} of vitamin B_1, B_6, B_{12} and diluted to volume by 0.1N hydrochloric acid and several synthetic ternary mixtures of these vitamins in different concentrations (8-20 μgml^{-1}).

Preparation of the sample: The stated content per tablet was vitamin B_1: 250 mg, vitamin B_6: 250 mg and vitamin B_{12}: 1 mg. About 354 mg of a homogeneous mixture of the contents of 10 tablets was accurately weighed into a 50 ml volumetric flask, dissolved in 0.1N hydrochloric acid and diluted to volume. 0.5 ml of this solution was diluted to 100 ml with the same solvent.

Procedure

The absorbance and second order absorbance spectra were recorded in the wavelength region 200-400 nm. First, the suitable derivative orders with appropriate $\Delta\lambda$ and wavelength, where each vitamin could be

analyzed in the presence of the other, were determined. Then, measuring the signal and using an appropriate calibration graph at the selected derivative order and wavelength, their concentrations were calculated. These calibrations were prepared by varying the concentrations of the vitamin, in the absence of the other. In order to test the validity of the proposed method, several synthetic ternary mixtures of vitamin B_1, B_6 and B_{12} in different proportions were prepared and resolved by the method described.

Results and Discussion

As vitamins B_1 and B_6 are photosensitive[1] and show maximum stability in an acidic medium[17], 0.1N hydrochloric acid was selected as the solvent and the solutions were analyzed immediately after dilution.

The optimum value of $\Delta\lambda$ should be determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Some values of $\Delta\lambda$ were tested. By second order derivative, 2 nm was selected as the optimum in order to give a satisfactory signal to noise ratio.

In Figures 1 and 2, the absorbance and second derivative spectra of vitamin B_1, B_6 and B_{12} and their mixture are presented. It can be seen from Figure 2 that vitamin B_1 can be determined in the presence of B_6 and B_{12} at 228.9 nm. On the other hand, vitamin B_6 and B_{12} can also be determined in the presence of the others at 309.6 and 361.7 nm, respectively.

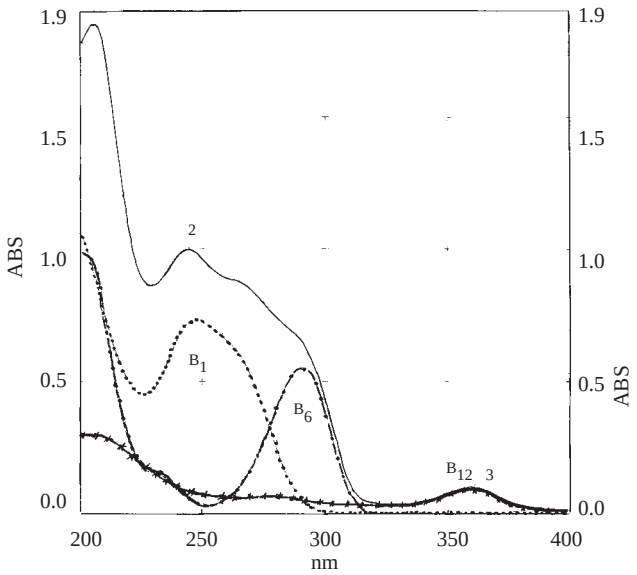

Figure 1. Absorption spectra of vitamin B_1 (20 μmgl^{-1}), B_6 (20 μmgl^{-1}) and B_{12} (20 μmgl^{-1}) and their mixture (——).

Reference: 0.1N hydrochloric acid

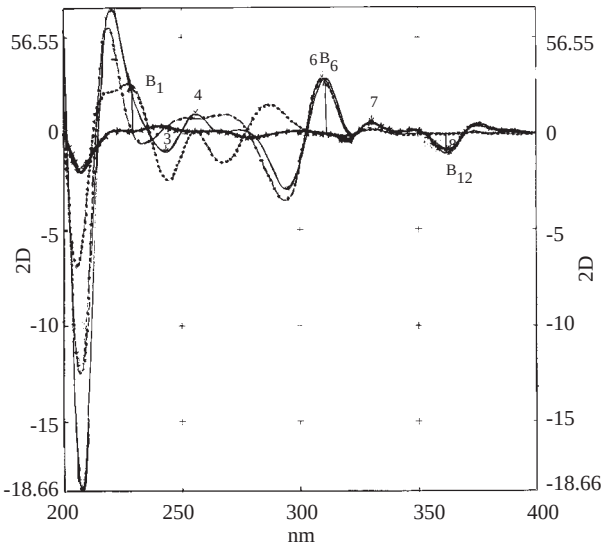

Figure 2. Second derivative spectra of vitamin B_1 (. . . . .), vitamin B_6 (-.-.-.-.), vitamin B_{12} (-*-*-*-), and mixtures of vitamins B_1, B_2 and B_{12} (——).
Reference: 0.1N hydrochloric acid

The calibration graphs were obtained by using the range of 4-20 μgml^{-1} concentrations of vitamin B_1, B_6 and B_{12} (Figures 3,4). The statistical data obtained from calibration graphs are summarized in Tables 1 and 3 and the results obtained from the resolution of the synthetic ternary mixtures are summarized in Table 2. These results indicate that the second derivative spectrophotometric method is suitable for the determination of vitamins B_1, B_6 and B_{12} in different proportions of synthetic ternary mixtures. The precision of the results for the synthetic mixture in terms of repeatability shows that the method has satisfactory precision.

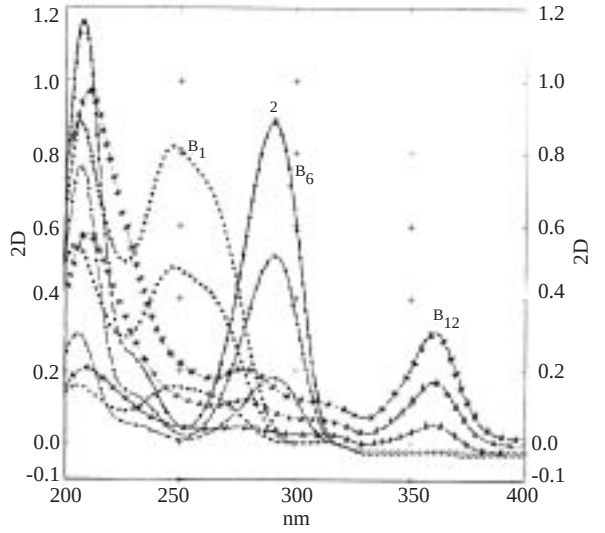

Figure 3. Absorption spectra of standard solutions of vitamin B_1 (. . . . .), vitamin B_6 (-.-.-.-.), and vitamin B_{12} (-*-*-*-).
Reference: 0.1N hydrochloric acid

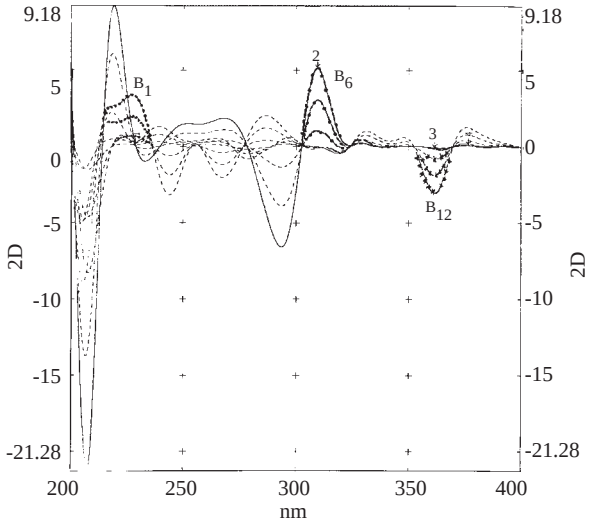

Figure 4. Second derivative spectra of standard solutions of vitamin B_1 (. . . . .), vitamin B_6 (-.-.-.-.), and vitamin B_{12} (-*-*-*-).
Reference: 0.1N hydrochloric acid

Application

The utility of second derivative method was tested on commercial tablets (Apikobal). The absorption and second derivative spectra of tablet sample solution and the diluted sample solution containing a mixture of vitamins are shown in Figure 5. The results of the determination of vitamins B_1, B_6 and B_{12} in tablets at the selected wavelengths are shown in Table 3.

Table 1. Statistical data for calibration graphs

	Vitamin B_1 $^2D_{228.9}$	Vitamin B_6 $^2D_{309.6}$	Vitamin B_{12} $^2D_{361.7}$
Correlation coefficient (n=5)	0.9999	0.9999	0.9998
Slope	0.1111	0.2474	0.1482
Intercept	0.0449	0.0401	0.2136

Table 2. Determination of vitamins in synthetic mixture by second derivative spectra

Theoretical μgml^{-1}			Recovery % (n=3)		
Vitamin B_1	Vitamin B_6	Vitamin B_{12}	Vitamin B_1	Vitamin B_6	Vitamin B_{12}
8	20	8	101.5	95.3	99.9
12	16	12	101.3	99.4	101.4
16	12	16	97.3	99.6	99.1
20	8	20	98.6	101.0	100.4
			99.7∓2.0*	98.8∓2.40*	100.2∓0.96*

*Standard deviation

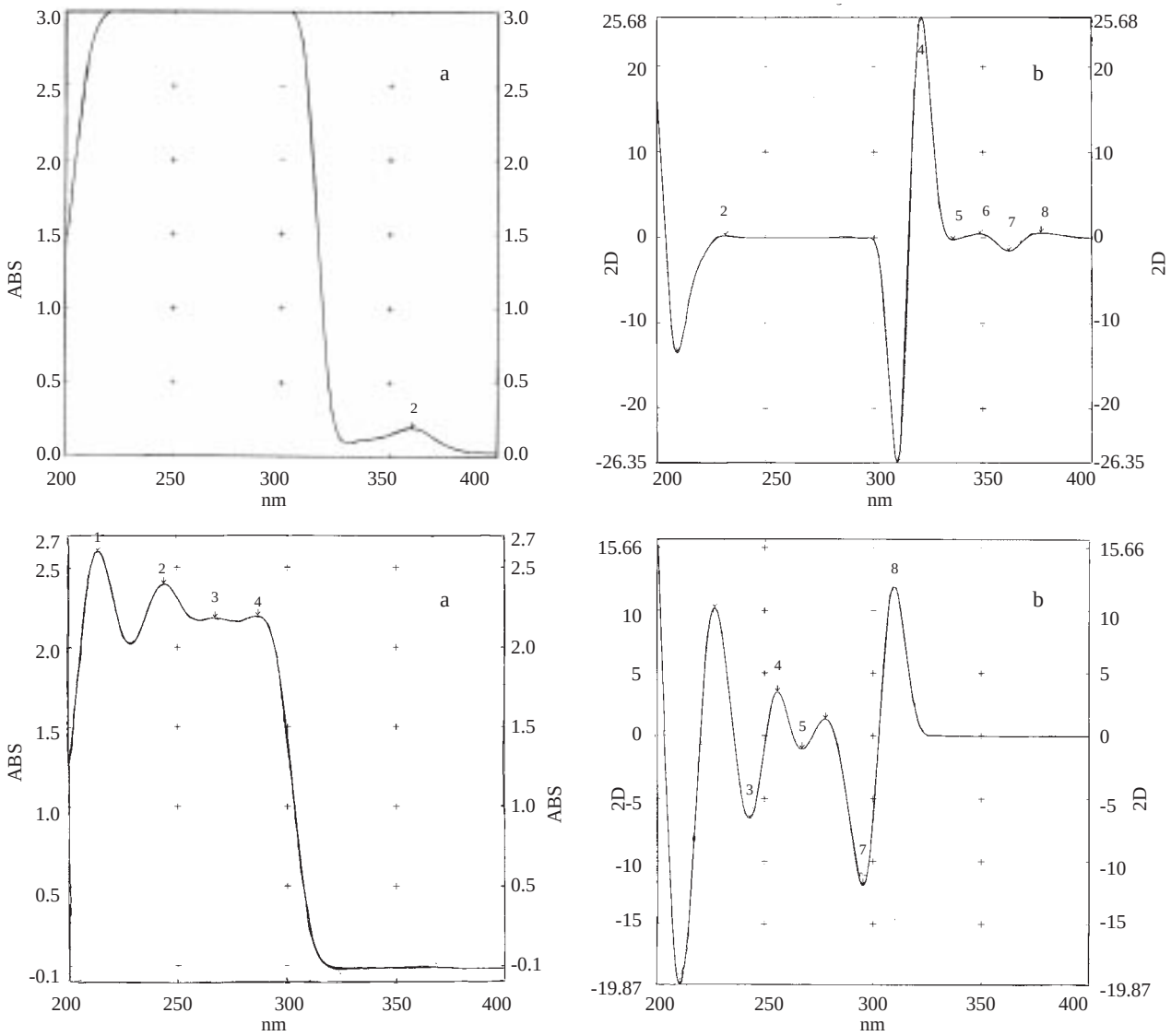

Figure 5. Absorption (a) and second derivative spectra (b) of Apikobal tablet.

Table 3. Repeatability of the assay in tablets

Repeatability n=6	Stated Conc. mg/tablet	Found mg/tablet	Relative Standard deviation
Vitamin B_1	250	236.2	1.13
Vitamin B_6	250	242.2	1.81
Vitamin B_{12}	1	1.03	3.58

References

1. F. Saccani and C. Neri, **Boll. Chim. Farm, 109,** 275-277 (1970).
2. M. Amim and J. Reusch, **J. Chromatogr. 390,** 448-453 (1987).
3. E. Wang and W. Hou, **J. Chromatogr. 447,** 256-262 (1988).
4. R.C. Williams, D.R. Baker and J.A. Schmitt., **J. Chromatogr. Sci. 11,** 618-624 (1973).
5. A.M. Wahbi, F.A. El Yazbi, M.H. Berary, S.M. Sabri, **Analyst, 117**, 785-793 (1992).
6. J.J. Berza Nevado, J. Lemus Gallego, G.S.M. Castane da Penalvo, **J. Pharm. Biomed. Anal. 11,** 607-614 (1993).
7. G. Carlucci, P. Mazzeo, T. Fantozzi, **Anal. Lett. 26,** 2193-2205 (1993).
8. C. Altesor, P. Corbi, M. Don, I. Knochen, **Analyst, 118,** 1549-1561 (1993).
9. J.E. Perkin, **J. Pharm. Biom. Anal. 11,** 609-626 (1993).
10. M.Ü. Özgür, S. Sungur, **Pharmazie, 47,** 459-460 (1992).
11. M.Ü. Özgür, S. Sungur, **Chimica Acta Turcica, 23,** 119-123 (1995).
12. M.Ü. Özgür, S. Sungur, L. Ersoy, **Pharmazie, 47,** 558-559 (1992).
13. A. Bozdoğan, M. Özgür, İ. Koyuncu, **Anal. Lett. 33,** 2975-2982 (2000).
14. J.J. Berzas Nevado, C. Guibertau Cabanillas and F. Salinas, **Talanta, 39,** 547-563 (1992).
15. B. Morelli, **Anal. Lett. 27,** 2751-2768 (1994).
16. B. Morelli, **J. Pharm. Sci. 84,** 34-37 (1995).
17. J. Petiot, P. Prognon, E. Postaire, M. Larue, F. Laurencon-Courteille and D. Pradeau, **J. Pharm. Biomed. Anal. 8,** 93-99 (1990).
18. İ. Koyuncu, M.Ü. Özgür, **37^{th} IUPAC Congress,** 14-19 August 1999, Berlin, Germany, APP-2-126, 743.
19. M.Ü. Özgür, İ. Koyuncu, **37^{th} IUPAC Congress,** 14-19 August 1999, Berling, Germany, APP-2-127, 744.
20. M.Ü. Özgür, G. Alpdoğan and B. Aşçı, **Monatshefte,** in press (2001).