Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 1, pp. 93–99, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00
Pergamon Press plc

Short Communication

Multicomponent analysis of hydrosoluble polyvitamins by first-derivative spectrophotometry

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Keywords: *First-derivative spectrophotometry; multicomponent analysis by weighted least squares; polyvitamins.*

Introduction

The quality control of pharmaceutical preparations of polyvitamins requires reliable and quick analytical methods. UV-visible spectrophotometric and fluorimetric methods generally involve tedious and lengthy extractions [1], or thin-layer chromatography [2, 3] prior to the measurement of absorbance. Many reversed-phase high-performance liquid chromatographic (HPLC) methods have been described which use various ion-pairing reagents such as sodium hexanesulphonate [4] or sodium dioctylsulphosuccinate [5] with preliminary automated extraction [6] and spectrophotometric or electrochemical detection [7]. Other authors have used ion-exchange chromatography [8].

The application of derivative techniques in UV-visible spectrophotometry [9, 10], and the data handling capabilities of computer-based spectrophotometers have increased the range of applications of UV-visible spectrophotometry. The present paper describes a method which can be applied to a mixture of up to five vitamins at various concentrations. The technique was first assessed by using a standard mixture. Two pharmaceutical dosage forms, viz. a dried (capsules) and a liquid (for intravenous infusion) form each containing five hydrosoluble vitamins at different concentrations, were assayed by this technique.

Experimental

Instrument

A diode-array spectrophotometer HP 8451 A with disc drive 9121, plotter 7470A and 1-cm cells were used.

Reagents

Thiamine hydrochloride: Takeda USP/BP (Japan); riboflavine phosphate: Merck USP XX (RFA); pyridoxine hydrochloride: Fluka Ph. Eur. (Switzerland); nicotinamide: Sigma (USA); calcium pantothenate for biochemical use: Merck (RFA); sulphuric acid: Prolabo (France) 0.1 N solution; deionized water.

Methods

Preparation of the stock solution. Each vitamin was dissolved in 0.1 N sulphuric acid and then diluted with the same solvent in order to obtain the following final concentrations: vitamin B_1: 16 mg l^{-1}; vitamin B_2: 20 mg l^{-1}; vitamin B_6: 12.5 mg l^{-1}; vitamin PP: 13 mg l^{-1}; calcium pantothenate: 50 mg l^{-1}.

Preparation of the standard mixture and dilutions. Vitamin formulations have different concentrations of the individual vitamins. To optimize the assay parameters, the method was carried out with a standard mixture in which the concentrations of the components were

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chosen to give equal absorbance of the individual vitamins at their wavelengths of maximum absorbance (0.2 absorbance unit).

The standard mixture was prepared in 0.1 N sulphuric acid in which the concentration of each vitamin was the following: vitamin B_1: 4 mg l^{-1}; vitamin B_2: 3 mg l^{-1}; vitamin B_6: 2.5 mg l^{-1}; vitamin PP: 3.25 mg l^{-1}; calcium pantothenate: 10 mg l^{-1}. The standard solution was then diluted (1 + 99, 1 + 49, 1 + 19, 1 + 9, 1 + 4 and 1 + 3) to obtain a range of 0.01–0.25 of the standard mixture.

Preparation of the sample. Injectable solution. The sample had the following composition: vitamin B_1: 10 mg; vitamin B_2: 5.46 mg; vitamin B_6: 4 mg; vitamin PP: 40 mg; calcium pantothenate: 6 mg; water for injection to 5 ml. 0.5 ml of the infusion fluid was transferred to a 250 ml volumetric flash and diluted with 0.1 N sulphuric acid to volume.

Capsules. The stated content per capsule was: vitamin B_1: 15 mg; vitamin B_2: 15 mg; vitamin B_6: 10 mg; vitamin PP: 50 mg; calcium pantothenate: 25 mg; lactose: 23 mg. About 180 mg of a homogenous mixture of the contents of 10 capsules was accurately weighed into a 100 ml volumetric flask, dissolved in 0.1 N sulphuric acid and diluted to volume. 1 ml of this solution was diluted to 50 ml with the same solvent.

Procedure. The zero-order and first-order absorption spectra were recorded in the wavelength region 190–400 nm. The first-derivative spectra were memorized in the computer, and the concentrations of the vitamins in samples were calculated by using the multicomponent program of the computer ("Concentration Method 4" of the HP8451 A calculation mode was used). This analysis is based on the absorbance characteristics of the individual components and a calculation of the combination of the individual spectra that best fits the spectrum of the mixture [11]. The statistical treatment is a weighted least squares method tailored for quantitative analysis, which uses a Savitsky–Golay smoothing algorithm. The degree of accuracy of the multicomponent calculation of concentration was assessed by three criteria.

(a) *Relative fit error.* This is the difference between the measured sample spectrum and that calculated from the spectra of the standards at the concentrations found by the multicomponent analysis.

(b) *Independence of standards.* This reflects the importance of overlapping spectra in controls and the spectral contribution of each component of the mixture.

(c) *Relative standard deviation*, defined as the ratio of the standard deviation to concentration.

Results and Discussion

The absorption spectra of five individual vitamins are shown in Fig. 1 and their corresponding first-derivative spectra are shown in Fig. 2. The zero- and first-derivative spectra of a diluted sample containing a mixture of vitamins are shown in Fig. 3.

Choice of instrumental parameters

As vitamins B_1 and B_6 are photosensitive [12] and show maximum stability in an acidic medium [13], 0.1 N sulphuric acid was selected as the solvent and solutions were analysed immediately after dilution. A 10-s recording time was selected as a compromise between imprecise measurements that would be given by a short recording time and photodecomposition that might occur if too long a recording time is used.

Absorption and derivative spectra of the standard mixture were recorded over various wavelength ranges to assess the optimal range. At low concentrations of vitamins, the accuracy of the zero-order measurements was unsatisfactory. However, the better resolution of the overlapping bands and the narrower bandwidths that are obtained in the derivative mode [9, 10] allowed concentrations as low as 1% of these in the standard mixture to be determined. The highest accuracy and precision are obtained by using the wavelength range 190–400 nm (Table 1).

Validation

The linear regression data in Table 2 obtained by assaying different dilutions of the standard mixture in the range 0.01–0.25 show that the measurements have a proportional relationship with concentration. The precision of the results for the standard mixture in terms of reproducibility and repeatability (in Table 3)

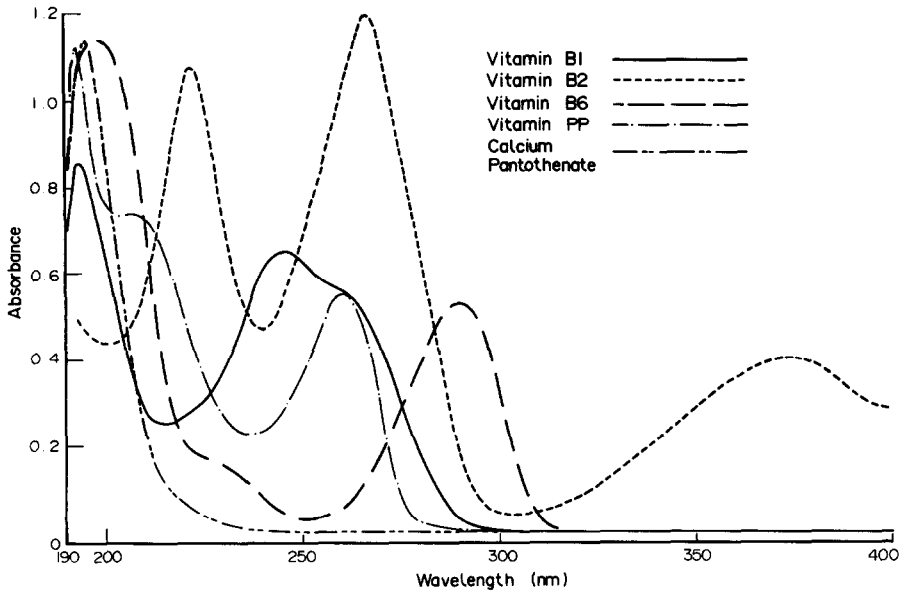

Figure 1
Absorbance spectra of the individual vitamins.

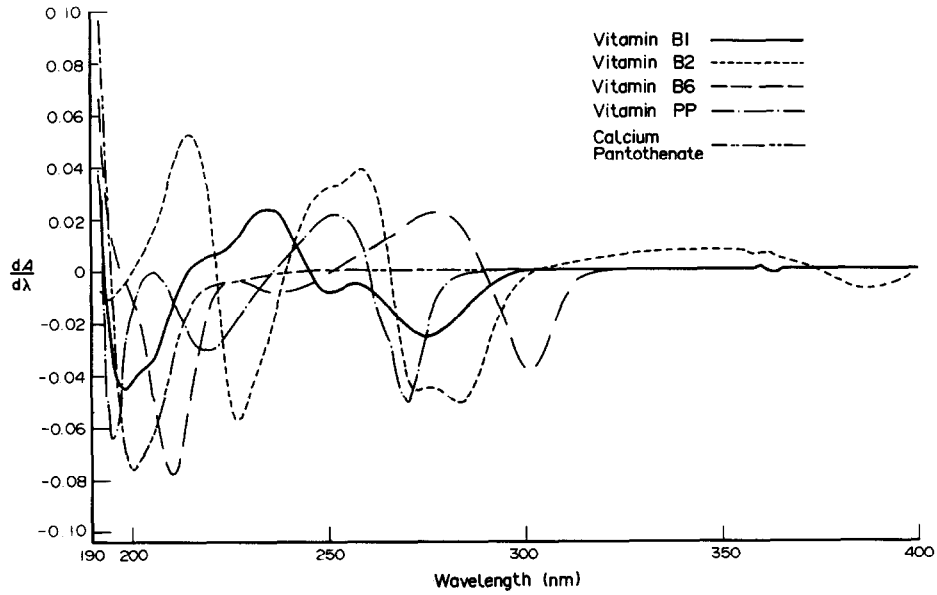

Figure 2
First-derivative spectra of the individual vitamins.

shows that the method has satisfactory precision. To assess the limit of quantification, two standard mixtures at concentrations, considerably lower than those in the sample solutions after dilution as described in the Experimental section, were assayed. The results in Table 4 shows that even at these low concentrations, reasonably satisfactory recoveries were obtained.

Application to pharmaceutical formulations

An injectable solution and a batch of capsules containing the five vitamins were assayed by the proposed method. The results, and the precision in terms of repeatability and reproducibility, are shown in Table 5. The results for vitamins B_1, B_2, B_6 and PP in both formulations and for calcium pantothenate in the capsule formulation were close to the stated

Table 1
Effect of wavelength range on the accuracy and precision of the results for the standard mixture using the first-derivative mode

	Results for the standard mixture (n = 10)								
	190–400 nm		190–350 nm		190–320 nm		190–280 nm		
	Average mg l^{-1}	Standard deviation	Average mg l^{-1}	Standard deviation	Average mg l^{-1}	Standard deviation	Average mg l^{-1}	Standard deviation	Theory mg l^{-1}
VIT B1	3.938	0.017	3.938	0.018	3.930	0.019	3.91	0.036	3.965
VIT B2	3.058	0.019	3.054	0.021	3.054	0.020	3.042	0.037	3.0525
VIT B6	2.495	0.017	2.489	0.017	2.480	0.021	2.487	0.044	2.518
VIT PP	3.288	0.011	3.287	0.011	3.288	0.011	3.3068	0.015	3.255
Calcium pantothenate	10.66	0.089	10.72	0.106	10.76	0.119	10.67	0.212	10.42

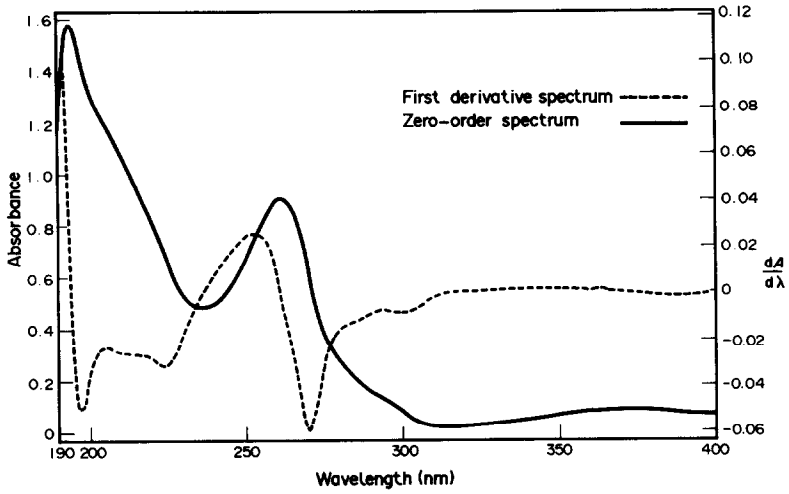

Figure 3
Absorption and first-derivative spectra of the polyvitamin mixture.

Table 2
Linearity of the method: standard (0–0.01–0.02–0.05–0.1–0.2–0.25)

	VIT B1	VIT B2	VIT B6	VIT PP	Calcium pantothenate
Correlation coefficient	0.99996	0.99997	0.99995	0.99997	0.9981
Slope	1.002	0.986	0.986	0.995	1.064
Intercept ($\times 10^{-3}$)	0.93	8.89	11.71	−0.52	−2.68

Table 3
Repeatability and reproducibility of the standard mixture

	Repeatability $n = 10$		Reproducibility $n = 10$	
	% Found	RSD %	% Found	RSD %
VIT B1	99.3	0.42	98.9	0.82
VIT B2	100.1	0.63	101.0	0.99
VIT B6	99.1	0.7	98.6	1.1
VIT PP	101.0	0.35	100.9	0.56
Calcium pantothenate	102.3	1.04	103.5	1.7

Reproducibility was measured on different days by the same analyst using the same spectrophotometer. Repeatability was measured by replicate analysis on the same day.

quantities. However, the results for calcium pantothenate in the injection solution showed considerable variation and the concentrations found differed significantly from the stated concentrations. This is probably due to the weak absorbance of calcium pantothenate. Even at its λ_{max} (194 nm, Fig. 1). The low concentration in the diluted injection (0.4 mg l^{-1}) results in an absorbance of only 0.054. In contrast to the other vitamins, the contribution of calcium pantothenate to the total spectrum is small and consequently the accuracy and precision of the assay of this vitamin is less than those of the other vitamins.

As the spectra of the vitamins and their degradation products were different, the method could not be used for degraded mixtures. However the statistical data generated allowed the presence of degradation products in the dosage forms to be detected. The levels of degradation products tolerated by the method have been empirically evaluated at 2% for each vitamin. Lactose, the only additive in the capsules, had no significative absorbance in

Table 4
Limits of quantification

	Mixture 1		Mixture 2		Theoretical concentration of vitamins in the diluted injection	Theoretical concentration of vitamins in the sample solution of the capsule contents
	Concentration mg l^{-1}	% found	Concentration mg l^{-1}	% found	(mg l^{-1})	(mg l^{-1})
VIT B1	0.2	102.4	0.1	69.4	4.0	3.9
VIT B2	0.075	97.0	0.03	62.7	2.184	3.9
VIT B6	0.125	100.9	0.062	112.1	1.6	2.6
VIT PP	0.08	107.5	0.03	243.7	16	13
Calcium pantothenate	3	92.3	2	85.2	2.4	6.5

Table 5
Repeatability and reproducibility of the assay in pharmaceutical formulations

	Injectable solution						Capsules			
		Repeatability $n = 10$		Reproducibility $n = 10$			Repeatability ($n = 10$)		Reproducibility ($n = 10$)	
	Stated conc. mg 5 ml^{-1}	Found mg amp^{-1}	Standard deviation	Found mg amp^{-1}	Standard deviation	Stated content mg $capsule^{-1}$	Found mg $capsule^{-1}$	Standard deviation	Found mg $capsule^{-1}$	Standard deviation
Thiamine hydrochloride (B1)	10	9.94	0.01	10.11	0.05	15	14.99	0.03	14.91	0.032
Riboflavin phosphate (B2)	5.46	5.50	0.018	5.4	0.08	15	15.09	0.07	14.97	0.09
Pyridoxine hydrochloride (B6)	4	3.92	0.011	3.91	0.046	10	9.92	0.08	9.89	0.068
Nicotinamide PP	40	39.59	0.028	39.78	0.27	50	50.10	0.07	50.08	0.08
Calcium pantothenate	6	5.16	0.52	6.95	0.93	25	24.54	0.24	24.87	0.31

Conditions for measuring reproducibility and repeatability are the same as Table 3.

the 190–400 nm wavelength range in the derivative mode and consequently did not interfere with the determination.

Conclusion

The advantage of a diode array spectrophotometer, which records entire spectra very rapidly and of the data handling and processing capabilities of microcomputers, are utilised in the proposed procedure for the assay of five vitamins in injectable and capsule formulations. The speed of the assay and its ability to detect (but not quantify) decomposition products permit its use for in-process control of the final products. It is possible that the method can be used with combinations of other vitamins also.

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[Received for review 7 December 1988; revised manuscript received 24 April 1989]