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A comparison of matrix resolution method, ratio spectra derivative spectrophotometry and HPLC method for the determination of thiamine HCl and pyridoxine HCl in pharmaceutical preparation

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Abstract

A comparison of two spectrophotometric methods and a HPLC method were described in this work for the analysis of pyridoxine hydrochloride and thiamine hydrochloride in a vitamin combination. In the first method, A_1^1 (1%, 1 cm) values of these two compounds were calculated using absorbances measured at 246.8 and 290.5 nm in zero-order spectra. The matrix was written for A_1^1 (1%, 1 cm) values and the concentration of both compounds were determined using 'Matlab' software. In the second method, the measurements in the derivative of the ratio spectra were made at 297.8 and 309.5 nm for pyridoxine hydrochloride and at 245.6 and 257.7 nm for thiamine hydrochloride. The calibration graphs were established in the range 8–40 μg/ml of both vitamins. In the HPLC method, the separation of these compounds was realized on a Nucleosil 100-5 C_{18} column with 0.1 M $(NH_4)_2C0_3$–water–methanol (5:15:80 v/v) as the mobile phase. Results of spectrophotometric and HPLC procedures were compared.

Keywords: Determination; Matrix resolution method; Ratio spectra derivative spectrophotometry; HPLC method; Pyridoxine hydrochloride; Thiamine hydrochloride

1. Introduction

The mixture of pyridoxine hydrochloride and thiamine hydrochloride as an antinevritic is widely used in vitamin combinations.

The quantitative determination of the vitamins in combinations containing pyridoxine hydrochloride or thiamine hydrochloride with other active compounds using various methods including spectrophotometry [1–7] and HPLC [8–11] have been described for several mixtures and vitamin combinations.

Salinas et al. [12] were developed a new method for the analysis of binary mixtures of compounds

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with overlapped spectra and Berzas Nevado et al. [13–15] were applied same method to determine the active compounds in different mixtures. This method was also applied by the other authors for the drugs analysis [16–19].

In this paper, the ratio spectra derivative spectroscopy, matrix resolution method and HPLC were proposed for simultaneous determination of both vitamins in a commercial vitamin preparation in Turkey. The spectrophotometric methods were compared with the HPLC method developed by us.

2. Materials and methods

2.1. Apparatus

A Shimadzu 1601 double beam spectrophotometer (the fixed slit width (2 nm)) connected to an IBM-PC computer loaded with Shimadzu UVPC Software and an HP 600 printer were used for all the absorbance measurements and treatment of data.

The HPLC equipment consisting of a Jasco PU-980 model pump, with a Jasco UV-975 model dedector connected to a computer loaded with Borwin Software and an HP 600 printer were used.

2.2. Vitamin combination

A commercial vitamin product (Benexol® film-coated tablet, Roche Pharm. Ind., Turkey, Batch no. 52), containing 250 mg pyridoxine hydrochloride (**PYD**) and 250 mg thiamine hydrochloride (**THA**) per tablet, was studied. **PYD** and **THA** were kindly donated by Roche Pharm. Ind., Turkey.

2.3. Stock solutions

Stock solutions of 100 mg/100 ml of **PYD** and **THA** were prepared in 0.1 M HCl for spectrophotometric procedures and in methanol for HPLC procedure.

2.4. Reagents

All the solvents were of analytical reagent grade. In HPLC procedure, HPLC grade methanol and double distilled water were used.

2.5. Standard solutions

For spectrophotometry: working standard solutions were prepared in 25-ml volumetric flasks containing 8–40 μg/ml of both vitamins and their synthetic mixtures were prepared by using their stock solutions. The zero-order spectra were recorded with a sampling interval of $\Delta\lambda = 0.1$ nm and a medium level of scanning speed against a reagent blank (0.1 M HCl) and stored in the computer.

For HPLC synthetic mixtures were prepared containing **PYD** and **THA** in the range 8–72 μg/ml of both vitamins, respectively with chlorpheniramine maleate (40 μg/ml) as internal standard (**IS**) in methanol. These solutions were filtered through 0.45 μm membrane filter before injection.

2.6. Sample solutions

In spectrophotometric methods, 20 tablets were accurately weighed and powdered in a mortar. An amount of the powder equivalent to a tablet, was dissolved in 0.1 M HCl in 100 ml calibrated flasks. After 30 min of shaking, the solution was filtered and the residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with 0.1 M HCl (solution 1). Solution 1 was diluted 1:500 with the same solvent.

In the HPLC method, the same procedure was realized by using methanol as solvent (solution 2). Solution 2 was diluted 1:250 with methanol.

3. Application of the methods

3.1. Matrix resolution method:

This method [18–20] is based on the obtaining of equations with two unknowns using $A_1^{\%}$ values (absorbance value of the 1% solution in 1-cm cell)

calculated using absorbances measured at two suitable wavelengths for two compounds in the mixture with respect to the following equation:

$A = \alpha lC$ (pathlength (l) is equal to 1

$A_1 = \alpha_1 C_1 + \beta_1 C_2$ for λ_1

$A_2 = \alpha_2 C_1 + \beta_2 C_2$ for λ_2

where A_1 and A_2 denote the absorbances of a mixture solutions of **PYD** and **THA**. C_1 and C_2 are the concentrations of solutions of **PYD** and **THA**, whilst α and β are their respective A_1^1 (%1, 1 cm) values. The subscripts 1 and 2 refer to wavelengths of λ_1 and λ_2, respectively.

Matrix notation greatly simplifies the matters and solves system of equations with two unknowns, as shown below:

$$\begin{vmatrix} A_1 \\ A_2 \end{vmatrix} = \begin{vmatrix} \alpha_1 & \beta_1 \\ \alpha_2 & \beta_2 \end{vmatrix} \begin{vmatrix} C_1 \\ C_2 \end{vmatrix} \quad \text{or } A = EC$$

This matrix can be solved by means of the software 'Matlab' in the computer and the concentrations of each compound in the mixture were determined.

3.2. Ratio spectra first derivative spectrophotometry

The absorption spectra of the solutions at the different concentrations of **PYD** were divided by the standard spectrum of **THA** and the ratio spectra were obtained. Then, first derivatives of the ratio spectra were plotted. The amount of **PYD** was determined by measuring the signals at 297.8 and 309.5 nm corresponding to a maximum and a minimum in the spectral region 275.0–330.0 nm. In the same way, the absorption spectra of the solution at the different concentrations of **THA** were divided by the standard spectrum of **PYD**. First derivatives of the ratio spectra were plotted from the obtained ratio spectra. The concentration of **THA** was determined by measuring the signals at 245.6 and 257.7 nm corresponding to a maximum and a minimum in the first derivative of the ratio spectra in 215.0–290.0 nm region.

3.3. HPLC procedure

The chromatograms were plotted and stored in the computer. The detector responses were measured in terms of peak area. The data was evaluated using software. Separation was carried out, at ambient temperature on Nucleosil 100-5 C_{18} (250×4.6 mm I.D. 5 μm) column (Macherey–Nagel, Germany) and the mobile phase consisted of 0.1 M $(NH_4)_2CO_3$–water–methanol (5:15:80 v/v). The flow rate was 1.5 ml/min with 10 μl as injection volume. The photometric detection was performed at 254 nm. Chlorpheniramine maleate was used as internal standard.

4. Results and discussion

4.1. Matrix resolution method

In the method, the zero-order absorption spectra were plotted by a sampling interval of $\Delta\lambda = 0.1$ nm and a medium level of scanning speed in the spectrophotometer. Fig. 1 shows that the absorption zero-order spectra of the solutions of **PYD** and **THA** in 0.1 M HCl are overlapped at the region 215.0–325.0 nm. By using the matrix resolution method, the determination of the two compounds is possible for direct absorbance measurements in their zero-order spectra. In this procedure, the absorbance values were measured at 246.8 and 290.5 nm for the determination of A_1^1 values. In the method, the parameters used shown in Table 1, and the equations used have been explained in the methods section.

In this method, Beer's law was valid in the concentration range 8–40 μg/ml for both vitamins. Mean recovery and relative standard deviation of the method were found as 100.1 and 1.25% for **PYD,** and also 99.8 and 1.06% for **THA**, respectively. These results were obtained in the synthetic mixtures prepared by adding known amounts of **PYD** and **THA** (Table 2).

4.2. Ratio spectra first derivative spectrophotometry

The stored spectra of the solutions prepared at

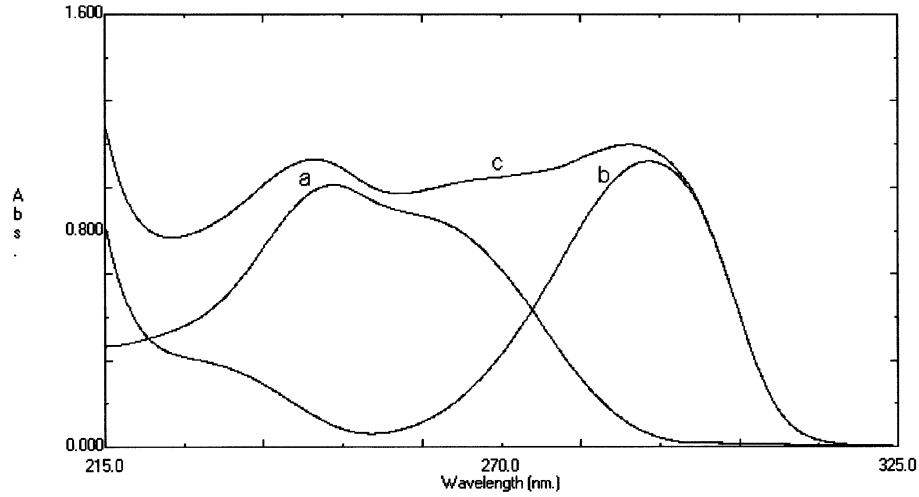

Fig. 1. Zero-order spectra of (a) 24 μg/ml thiamine hydrochloride; (b) 24 μg/ml pyridoxine hydrochloride; and (c) their mixture in 0.1 M HCl.

Table 1
Experimental parameters for matrix resolution method used for the simultaneous determination of thiamine hydrochloride and pyridoxine hydrochloride in a vitamin combination

	Thiamine HCl	Pyridoxine HCl		
λ nm	α_1	α_2	β_1	β_2
$\lambda_1 = 246.8$	408.5		32.3	
$\lambda_2 = 290.5$		20.1		434.4
Linearity range μg/ml	8–40		8–40	

Table 2
Recovery data obtained for different mixtures by using the matrix resolution method

Mixture no	Thiamine HCl			Pyridoxine HCl		
	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	24.0	24.1	100.4	8.0	8.0	100.0
2	24.0	24.0	100.0	16.0	16.1	100.6
3	24.0	23.9	99.6	24.0	24.1	100.4
4	24.0	24.1	100.4	32.0	31.5	98.4
5	24.0	23.8	99.2	40.0	39.5	98.8
6	8.0	7.9	97.5	24.0	24.2	100.8
7	16.0	16.0	100.0	24.0	24.5	102.1
8	24.0	24.3	101.3	24.0	24.0	100.0
9	32.0	31.6	98.8	24.0	23.6	98.3
10	40.0	40.2	100.5	24.0	24.3	101.3
		$\bar{x} = 99.8$				$\bar{x} = 100.1$
		RSD[a] = 1.06				RSD = 1.25

[a] RSD, Relative standard deviation.

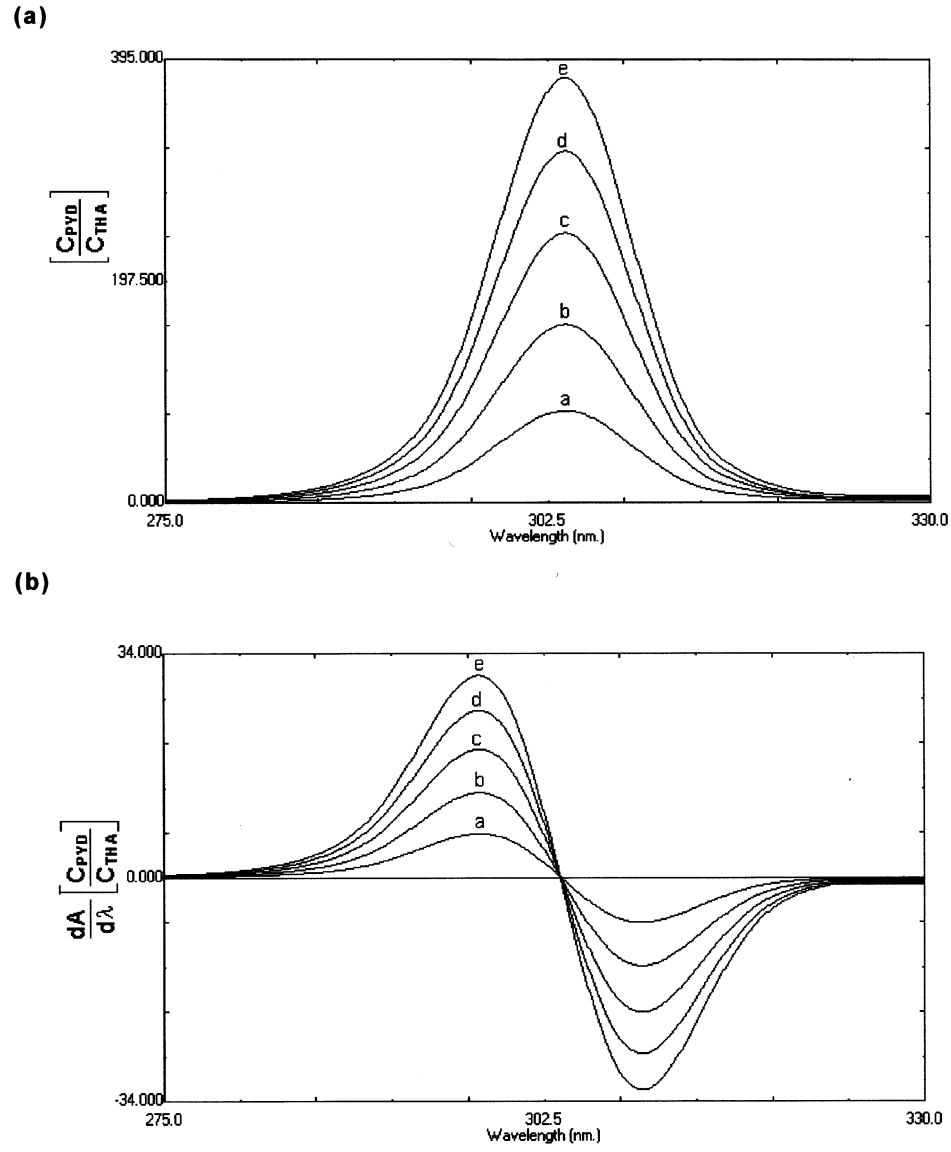

Fig. 2. Ratio spectra (a) and first derivative of the ratio spectra (b) of pyridoxine hydrochloride of (a) 8 μg/ml; (b)16 μg/ml; (c) 24 μg/ml; (d) 32 μg/ml; (e) 40 μg/ml, 24 μg/ml thiamine hydrochloride used as divisor in 0.1 M HCl ($\Delta\lambda = 8$ nm).

the increasing concentrations of PYD in 0.1 M HCl were divided the spectrum of the standard solution of 24 μg/ml THA in the same solvent. The ratio spectra were smoothened at $\Delta\lambda = 8$ nm (Fig. 2a) and their first derivative was plotted with the intervals of $\Delta\lambda = 8$ nm shown in Fig. 2b. Two calibration graphs of **PYD** were established by measuring the signals at 297.8 and 309.5 nm and were tested between 8–40 μg/ml for PYD and its binary mixtures with **THA**, as shown in Table 3. In the similar way, the absorption spectra of the solutions prepared at the increasing concentrations of **THA** in 0.1 M HCl were divided the spectrum of the standard solution of 24 μg/ml **PYD** within the same solvent. The resulting ratio spectra were smoothened at $\Delta\lambda = 8$ nm (Fig. 3a).

The first derivative of the ratio spectra was plotted with the intervals of $\Delta\lambda = 8$ nm, as shown in Fig. 3b. Two calibration graphs of **THA** were established by measuring the signals at 245.6 and 257.7 nm and were tested between 8–40 μg/ml for **THA** and its binary mixtures with **PYD** as shown in Table 3.

By using the ratio spectra first derivative spectrophotometry for two compounds, the mean recoveries and the relative standard deviation were obtained as 99.8 and 0.87% for **PYD** and also 99.9 and 0.88% for **THA**, respectively in synthetic mixtures prepared by adding known amounts of both vitamins (Table 3).

Table 5 summarizes the regression coefficients and the linearity ranges of the calibration graphs, obtained by measuring the signals corresponding to the maximum and minimum wavelengths in the first derivative of the ratio spectra for both active compounds. For the determination of **PYD** and **THA** in their synthetic mixtures and in the tablet formulation, the calibration graphs were only used by measuring at 309.5 nm for **PYD** and 245.6 nm for **THA** in the first derivative of the ratio spectra.

The main instrumental parameter conditions were optimized to obtain the most distinct curve of first derivative of the ratio spectra. For selecting a divisor of the appropriate concentration, some divisor concentrations were tested in the determination. The standard solutions of 24 μg/ml of **PYD** and **THA** for determining **PYD** and **THA** in their binary mixtures were found suitable. The influence of the $\Delta\lambda$ and the smoothing for obtaining the first derivative was tested and a value of $\Delta\lambda = 8$ nm and a smoothing of $\Delta\lambda = 8$ nm was considered as suitable for both determinations.

4.3. HPLC method

HPLC method was developed to provide a suitable procedure for the rapid quality control analysis of **PYD** and **THA**, as comparison method for the developed spectrophotometric methods. Several mobile phase systems and different internal standards were tested for the separation and the determination of the compounds. Mobile phase of 0.1 M $(NH_4)_2CO_3$–water–methanol (5:15:80 v/v) and chlorpheniramine maleate as internal standard were found suitable for this aim. At a flow of 1.5 ml/min, retention times for **IS**, **PYD** and **THA** were defined as 1.34, 1.84 and 4.72 min, respectively (Fig. 4). The ratio of the peak areas analyte to **IS** were plotted against the concentration of **PYD** and **THA**. By using these calibration graphs, the content of

Table 3
Recovery data obtained for different mixtures by using the first derivative of the ratio spectra

Mixture no	Thiamine HCl			Pyridoxine HCl		
	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	24.0	23.9	99.6	8.0	8.1	101.3
2	24.0	24.2	100.8	16.0	16.1	100.6
3	24.0	24.0	100.0	24.0	24.0	100.0
4	24.0	24.1	100.4	32.0	31.6	98.8
5	24.0	23.8	99.2	40.0	39.8	98.5
6	8.0	8.0	100.0	24.0	24.0	100.0
7	16.0	16.0	100.0	24.0	23.9	99.6
8	24.0	24.4	101.7	24.0	24.0	100.0
9	32.0	31.6	98.8	24.0	23.7	98.8
10	40.0	39.6	99.0	24.0	24.0	100.0
			= 99.9			= 99.8
		RSD = 0.88			RSD = 0.87	

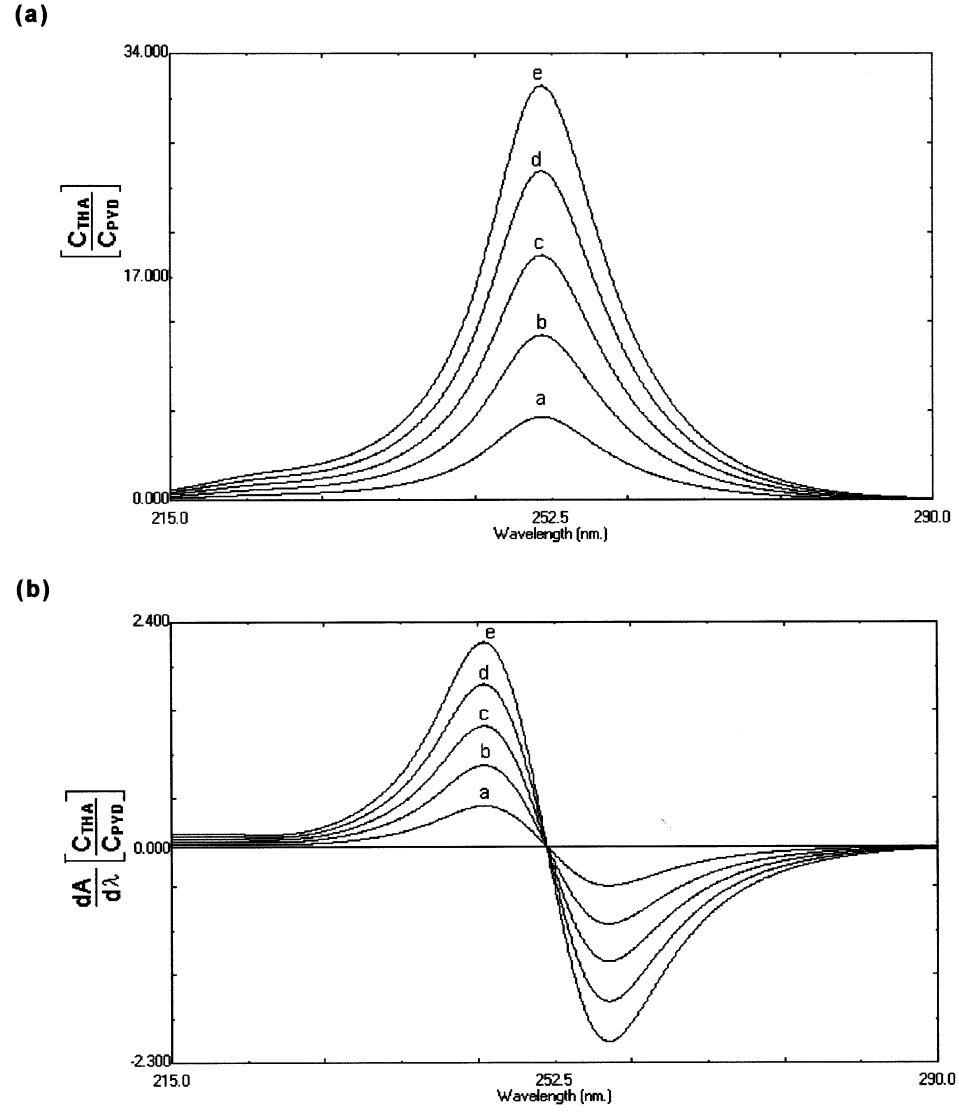

Fig. 3. Ratio spectra (a) and first derivative of the ratio spectra (b) of thiamine hydrochloride of (a) 8 μg/ml; (b) 16 μg/ml; (c) 24 μg/ml; (d) 32 μg/ml; (e) 40 μg/ml, 24 μg/ml pyridoxine hydrochloride used as divisor in 0.1 M HCl ($\Delta\lambda = 8$ nm).

PYD and **THA** are determined in the sample containing **PYD** and **THA**. Linearity range of HPLC was found as 8–72 μg/ml for both vitamins.

As seen in Table 4, in order to demonstrate the validity and applicability of HPLC method, recovery studies were performed by analyzing synthetic mixtures of **PYD** and **THA** which prepared different composition ratios. The mean recoveries and relative standard deviations of **PYD** and **THA** were found as 99.7 and 0.59% and also 100.0 and 0.53%, respectively.

Linearity ranges, regression equations and correlation coefficients were illustrated in Table 5.

A good coincidence was observed for the assay results of the tablet dosage form by the application of these three proposed in this text (Table 6). Statistical data obtained by using Student's *t*-test

and *F*-tests show no significant difference between the methods (Table 6).

5. Conclusions

In this study, by applying these methods for the analysis of synthetic binary mixtures and a pharmaceutical preparation containing **PYD** and **THA**, successful results were obtained. In spite of the two vitamins which produce a perfect overlapping spectrum in the zero-order spectra, it was observed that these two spectrophotometric methods, without requiring a separation procedure, were more very simple and very cheap than the HPLC method in this work. For example, when spectrophotometry was compared with HPLC, spectrophotometry was simple and less expensive, and require neither sophisticated instrumentation nor any prior separation procedure.

Hovever, linearity range of the HPLC method was more larger than the matrix resolution and

Table 4
Recovery data obtained for different mixtures by using HPLC

Mixture no	Thiamine HCl			Pyridoxine HCl		
	Added (μg)	Found (μg)	Recovery (%)	Added (μg)	Found (μg)	Recovery (%)
1	40.0	40.2	100.5	8.0	8.0	100.0
2	40.0	39.9	99.8	24.0	23.9	99.6
3	40.0	40.1	100.3	40.0	40.0	100.0
4	40.0	39.9	100.6	56.0	55.8	99.6
5	40.0	40.0	100.0	72.0	72.0	100.0
6	8.0	8.0	100.0	40.0	39.8	99.5
7	24.0	23.9	99.6	40.0	39.7	99.3
8	40.0	39.9	99.8	40.0	40.1	100.3
9	56.0	56.0	100.0	40.0	40.0	100.0
10	72.0	72.3	100.4	40.0	39.5	98.8
			= 100			= 99.7
		RSD = 0.53			RSD = 0.59	

Table 5
Calibration data in the determination of pyridoxine hydrochloride and thiamine hydrochloride[a]

Methods	λ (nm)	Linearity range (μg/ml)	Equation	Regression coefficient (*r*)
Ratio spectra first derivative spectrophotometry	297.08	8–40	$Y = 5.5 \times 10^{-1} C_{\mathbf{PYD}} + 8.0 \times 10^{-2}$	0.9992
	309.5	8–40	$Y = 8.0 \times 10^{-1} C_{\mathbf{PYD}} + 7.3 \times 10^{-2}$	0.9990
	245.6	8–40	$Y = 5.4 \times 10^{-2} C_{\mathbf{THA}} - 5.4 \times 10^{-3}$	0.9999
	257.7	8–40	$Y = 5.2 \times 10^{-2} C_{\mathbf{THA}} + 5.0 \times 10^{-3}$	0.9999
HPLC method	254.0	8–72	$Y = 1.4 \times 10^{-1} C_{\mathbf{PYD}} - 5.0 \times 10^{-2}$	0.9990
	254.0	8–72	$Y = 2.1 \times 10^{-1} C_{\mathbf{THA}} - 2.2 \times 10^{-2}$	0.9994

[a] $C_{\mathbf{PYD}}$, μg/ml of pyridoxine hydrochloride HCl; $C_{\mathbf{THA}}$, μg/ml of thiamine hydrochloride; Y, absorbance values.

Table 6
The results obtained in commercial vitamin combination (mg)[a]

	Matrix resolution method	Ratio spectra derivative spectrophotometry	HPLC
Thiamine HCl			
Mean ± SD	249.8 ± 0.8	249.5 ± 0.8	250.0 ± 0.9
$t_{calculated}$	0.530	0.101 $t_{theoretical}$	2.26 (P = 0.05)
$F_{calculated}$	0.742	0.836 $F_{theoretical}$	3.18
Pyridoxine HCl			
Mean ± SD	250.1 ± 0.9	249.6 ± 0.8	249.8 ± 0.9
$t_{calculated}$	0.256	0.961 $t_{theoretical}$	2.26 (P = 0.05)
$F_{calculated}$	0.989	0.873 $F_{theoretical}$	3.18

[a] Results obtained are the average of ten experiments. SD, standard deviation.

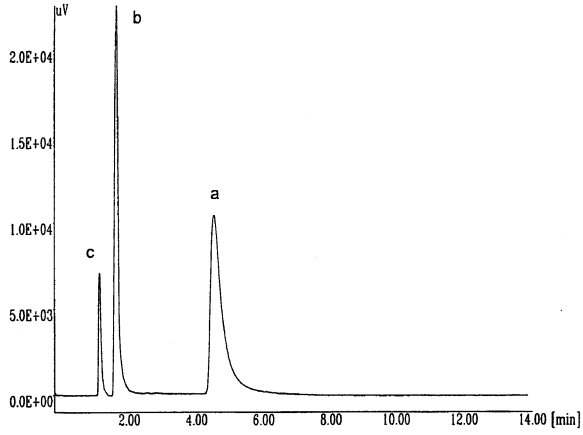

Fig. 4. Typical chromatograms of (a) thiamine hydrochloride and (b) pyridoxine hydrochloride; (c) chlorpheniramine maleate as internal standard (IS).

ratio spectra derivative methods for the determination of **PYD** and **THA**.

These three methods proposed were found to be suitable for the determination of both vitamins in their binary mixtures. Recovery data on added drugs were excellent and the procedures were shown applicable to commercial formulations containing these compounds.

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