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Simultaneous determination of thiamine and pyridoxine in pharmaceuticals by using a single flow-through biparameter sensor

P. Ortega Barrales, A. Domínguez Vidal, M.L. Fernández de Córdova, A. Molina Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje Las Lagunillas, E-23071 Jaén, Spain

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Abstract

For the first time, an UV-photometric flow-through sensing device has been developed for the simultaneous determination of two cationic species (thiamine and pyridoxine). The sensor is based on the retention of the analytes on a cationic ion-exchanger gel placed in the detection zone itself into a quartz flow-cell. A double discrimination is used for detecting the analytes: (a) a double and simultaneous working wavelength, performed by the use of a diode array detector; and (b) a temporary sequentiation in the arrival of the analytes to the sensing zone by on line separation using a cationic ion-exchanger (the same used in the sensing zone) placed into a minicolumn just before the flow cell. Pyridoxine is determined the first (by measuring its intrinsic absorbance at 293 nm) because it passes through the minicolumn while thiamine is strongly retained on it. Then, thiamine is conveniently eluted from the precolumn and its intrinsic UV absorbance measured at 255 nm. In both cases, transitory signals were obtained because both the carrier (in the case of the pyridoxine) and the eluting (in the case of thiamine) solutions used also eluted the respective analyte from the sensing zone. Using 1000 μl of sample, the analytical signal showed a very good linearity in the range 2–30 μg ml^{-1} for both analytes with detection limits of 0.10 and 0.084 μg ml^{-1} for thiamine and pyridoxine, respectively. The optosensor was satisfactorily applied to the determination of these two analytes in pharmaceuticals.

Keywords: Flow-through optosensor; Thiamine; Pyridoxine; Pharmaceuticals

1. Introduction

Active solid supports placed inside a flow cell in a non-destructive spectroscopic detector have been recently used in spectroscopic analytical methods [1,2]. The analyte (or a derivative com-

* Corresponding author. Tel.: +34-95-3212147; fax +34-95-3012141.
E-mail address: amolina@ujaen.es (A. Molina Díaz).

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pound from it) is retained on line on the solid support in the detection area itself and its interaction with radiation sorbed on the solid phase is continuously monitored. These continuous on-line solid phase spectroscopic systems have been called flow-through optosensors [3]. When the sensing zone is able to respond to more of one analyte, the sensor is called a multiparameter flow-through sensor [4], in opposition to those responding to only an analyte (single-parameter sensors) [5,6,8–10].

In recent years, flow-through optosensors have been applied to the analysis of active principles in pharmaceuticals using UV [1,4,7,11] or luminis-

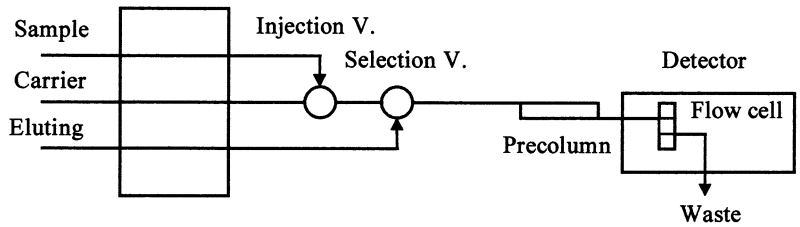

Fig. 1. Schematic diagram of the FIA system.

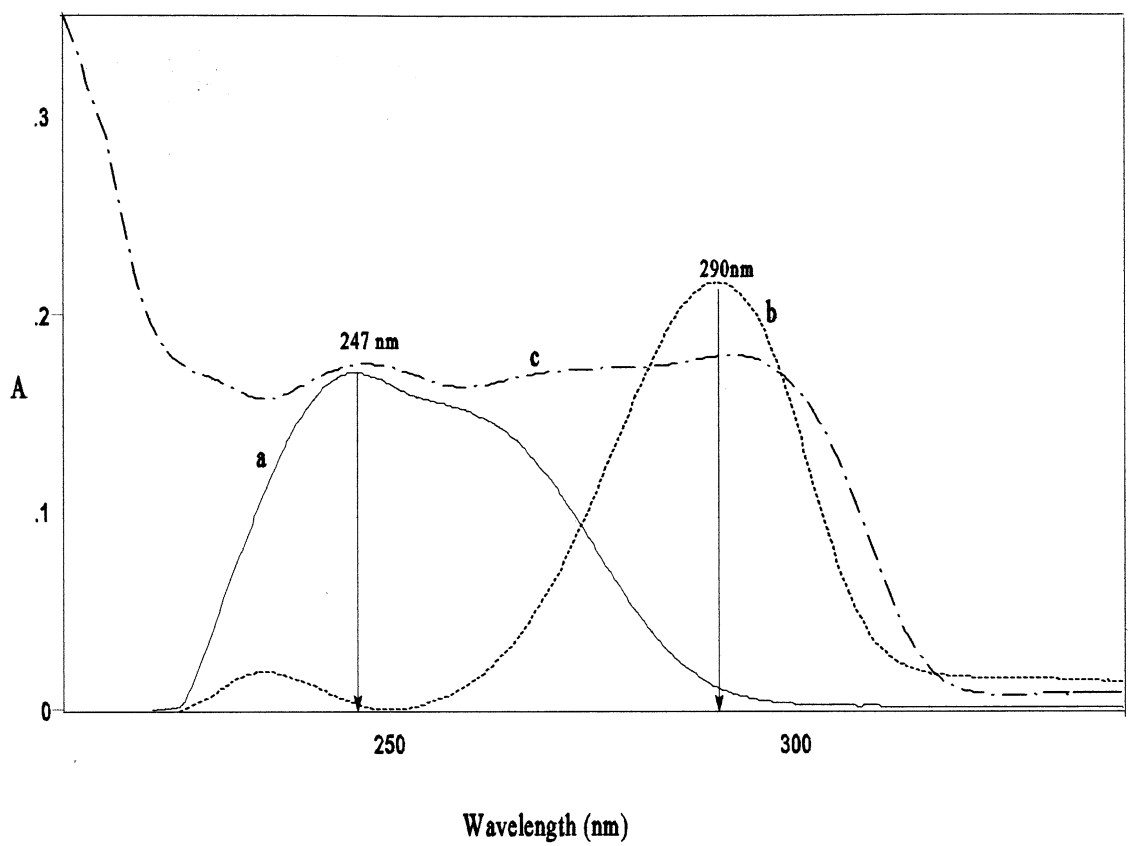

Fig. 2. Scans in solution of; (a) thiamine; (b) pyridoxine; (c) and mixture. They are made in stopped-flow with 1 mm of path length. Concentrations were in all cases 100 μg ml^{-1}.

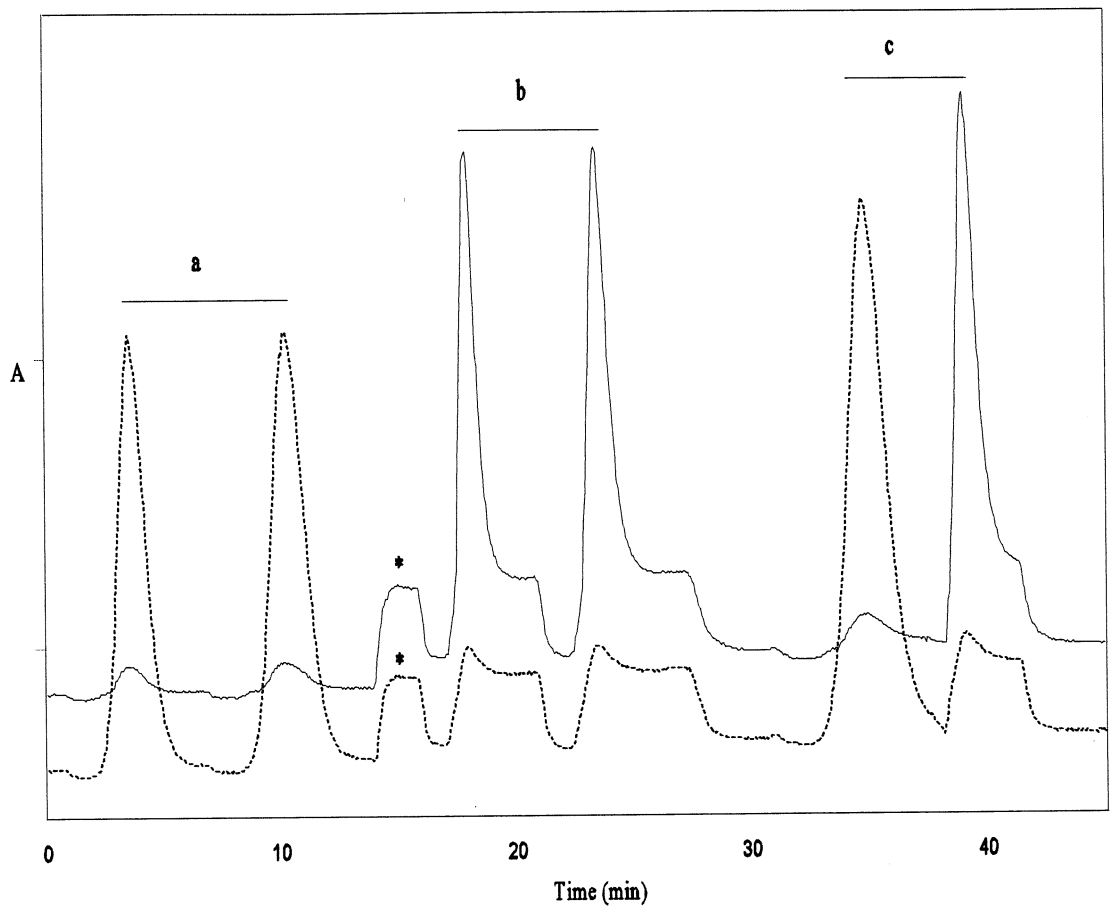

Fig. 3. Fiagram in the simultaneous determination of thiamine and pyridoxine obtained with an injection volume of 1000 μl, and concentrations of 10 μg ml^{-1} for thiamine solely (a) and pyridoxine solely (b) and 12 μg ml^{-1} for the mixture (c). *Compactation of the resin by passing 0.4 M HAc–NaAc eluting solution through the sensing zone.

cent detection [12–14]. Most of them are monoparameter optosensors: the sensing microzone responds to the active principle and it is appropriately regenerated after developing the signal, so remaining ready for the next sample. Usually, the solution carrier elutes the analyte from the sensing microzone [5,7–10] but, sometimes, an additional eluting solution have to be used for this purpose [1,4,9,10,14]. In the first case, a simple monochannel manifold is used with a buffer solution as carrier/self-eluting agent. In the second case, an additional channel have to be used for the eluting agent. As an intrinsic property is usually measured (absorbance or fluorescence) it should be emphasized that these simple optosensors do not use derivatizing reagents, so the cost per analysis is very low and the sampling frequency high; moreover, they show very good analytical features in terms of sensitivity, selectivity, repeatability and linearity. They exhibit two main advantages versus the corresponding conventional spectrophotometric or fluorimetric methods as a consequence of the selective separation (sorption) and, simultaneously, pre-concentration of the analyte(s) on line in the detection area itself. In addition, the costs are similar and the instrumentation the same than that used in conventional spectroscopic methods.

Nevertheless, two previous conditions have been performed in order to use a flow-through

optosensor: (1) the analyte have to be retained on the solid sensing support; and (2) then eluted from it. These two requirements can be satisfied for a lot of active principles if an appropriate couple of solid support and carrier/self-eluting agent are chosen. Thus, mixtures of two or even three active principles have been resolved using UV optosensors [1,4,11,13].

Flow-through optosensors can also compete very favourally against chromatographic methods in therms of: (a) cost, as a much less sofisticate instrumentation is used; and (b) time spent in the analysis when one or up to three principles are going to be analysed [1].

When a higher number of analytes have to be simultaneously determined, chromatographic methods offer a higher potentiality although they usually do not show a sensitivity as high as the respective optosensors.

Hiterto, a few biparameter flow-through optosensors have been described [11,13,15] . All of them operates sequentially (two separate injections are needed) by using two alternate carriers and an only sensing zone.

Sometimes [13,15] the sensors responds only to one of the analytes in the first injection and in the second one, the signal corresponds to the sum of both, so one of them is determined by difference. Therefore, the signals have to be additive (this

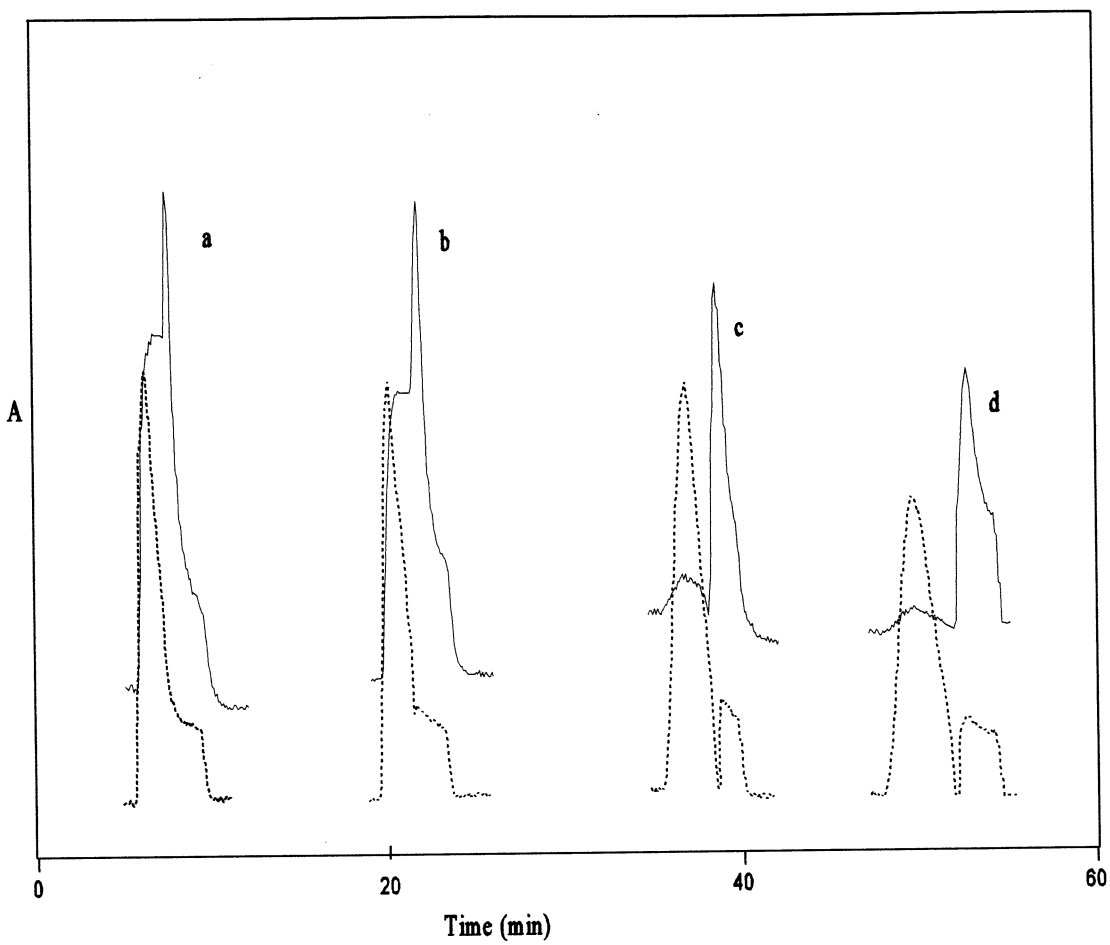

Fig. 4. Fiagram showing the influence of the pre-column length in the separation: (a) Without pre-column; (b) pre-column of 7 mm, (c) pre-column of 15 mm (chosen); and (d) pre-column of 25 mm.

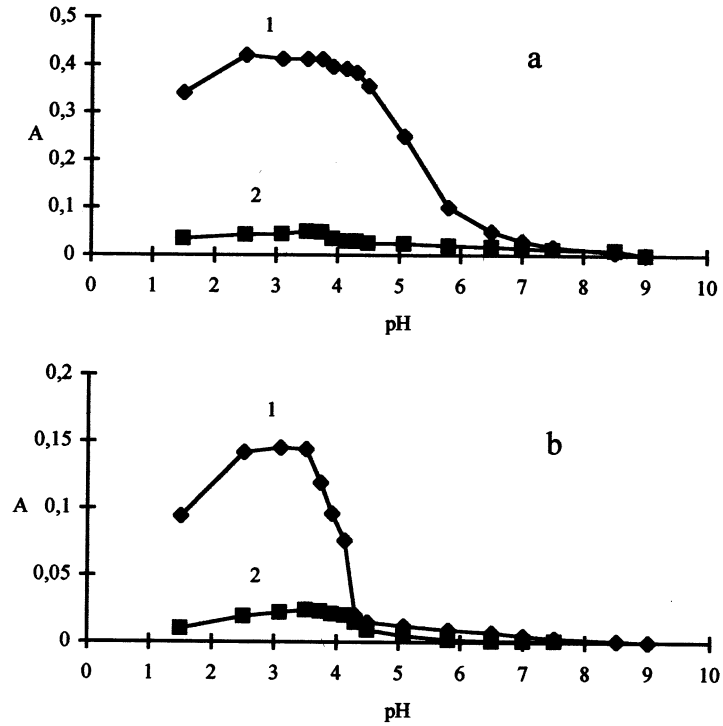

Fig. 5. Influence of pH (40 μl was used as injection volume in all cases): (a) for thiamine (50 μg ml^{-1}); peak heights at: (1) 255 nm (working wavelength); and (2) 293 nm (appropriate wavelength for pyridoxine). (b) for pyridoxine (30 μg ml^{-1}); peak heights at: (1) 293 nm (working wavelength); and (2) 255 nm (appropriate wavelength for thiamine).

Table 1
Analytical features of the method

Parameter	Thiamine		Pyridoxine	
Injected volume/μl	40	1000	40	1000
Intercept	4.7×10^{-3}	1.8×10^{-3}	1.0×10^{-3}	6.0×10^{-3}
Slope/ml μg^{-1}	7.4×10^{-3}	3.53×10^{-2}	9.1×10^{-3}	3.39×10^{-2}
Linear dynamic range/μg ml^{-1}	7–130	2–30	5–110	2–30
Correlation coefficient (r)	0.9999	0.9999	0.9995	0.9998
RSD/% ($n=10$)	2.1	2.4	2.7	3.0
Detection limit/μg ml^{-1}	0.40	0.10	0.21	0.084
Quantification limit/μg ml^{-1}	1.38	0.34	0.69	0.28

requirement is not usually found when spectral measurements are performed on a solid phase). In other cases, the sensor responds alternately to only one of the analytes each time. Here, the required conditions are that the two chosen carriers have to allow each the selective retention of only one of the analytes in the presence of the another one [11].

In this paper we describe, for the first time, a biparameter sensor using an only sensing zone (Sephadex SP-C25 cation exchanger gel) combined with an on-line ion-exchange separative previous process to determine simultaneously (that is, using only one sample injection) two analytes: thiamine and pirydoxine.

These authors have previously developed two separate monoparameter optosensors for the individual determination of each of these vitamins. Nevertheless, because of the simultaneous retentions and strong spectral overlap without additive signal behaviour of the system the only discrimination provided by the use of a diode array detector tuned at two working wavelengths does not allow resolving the mixture.

The biparameter sensor here developed is based on the strong retention of thiamine on an ion-exchanger column placed on line just before the cell, while pyridoxine passes through it developing the analytical signal in the solid phase transductor. Then, thiamine is conveniently eluted from the column and carried also to the cell in the detector. In both cases, the intrinsic UV absorbance of the analyte at the respective maximum wavelength is used as analytical signal which is acquired with a diode array detector. So, the solid phase acts as a dual sensing zone responding successively to the two analytes, whose arrival to the detector area is, in this way, temporarily discriminated. It was successfully applied to the determination of these vitamins in pharmaceuticals.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical reagent-grade chemicals by using doubly distilled water.

Thiamine and pyridoxine stock solutions, 1000 mg l^{-1} (as hydrochloride) were prepared from thiamine hydrochloride (Fluka) and pyridoxine hydrochloride (Fluka). Solutions were stable for at least 4 weeks at 4–5°C. Work solutions were prepared fresh daily by appropriate dilution with doubly distilled water.

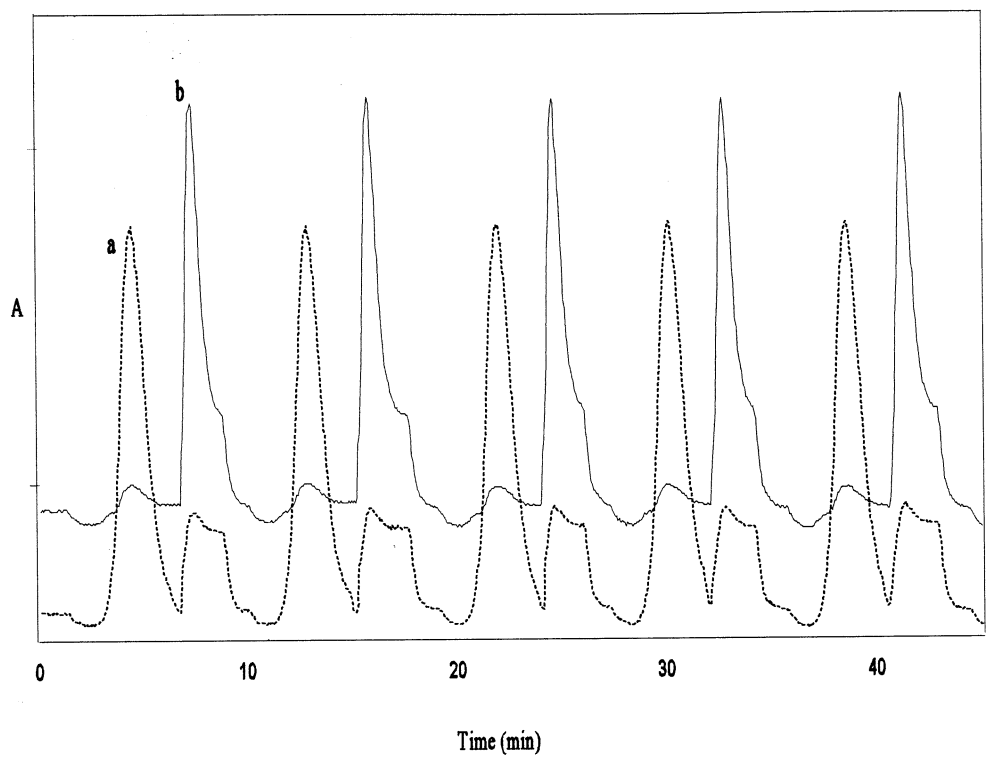

Fig. 6. Reproducibility fiagram study for 10 μg ml^{-1} with 1000 μl as injection volume for (a) pyridoxine and (b) thiamine.

Table 2
Effect of foreign species

Foreign species	Tolerance limit (μg ml^{-1} interfering species/μg ml^{-1} analyte)	
	Thiamine	Pyridoxine
Lactose, glucose, saccharose, L-carnitine	>100[a]	>100[a]
Ca Pantothenate	90	50
Nicotinamide	25	20
Pyridoxine	>20[a]	–
Thiamine	–	>20[a]
Cyanocobalamine	10	10
Saccharin	7.5	7.5
Folic acid, Sodium Diclophenac, Biotin	5	5
Riboflavin	5	2.5
Ascorbic acid	10	20
Ascorbic acid (with retention in Sephadex QAE-A25)	100	100

[a] Maximum ratio tested.

Sodium citrate–citric acid buffer solution, 0.1 M at pH 3.0, was used as carrier solution.

Sodium acetate–acetic acid (Hac–NaAc) buffer solution, 0.4 M at pH 4.80, was used as eluting solution.

Sephadex SP-C25 (Aldrich) ion exchanger gel (40–120 μm; in H^+ form without any preliminary treatment), packed both in a precolumn of 15 mm length and 2 mm i.d. and in a Hellma 138-QS flow-through cell, was used for measurement of solid phase UV light absorption in the sensing zone.

2.2. Instrumentation

A Milton Roy 3000 Array Spectrophotometer, equiped with a Hellma 138-QS flow cell (1-mm optical path length and 50 μl inner volume), was used for absorbance measurements. It was controlled by a microprocessor fitted with the software package MILTON ROY Rapid Scan 2.01. Exposition and cycle times were fixed in 2 s.

A four-channel Gilson Minipuls-3 peristaltic pump with rate selector, teflon tubing of 0.8 mm i.d. and two Rheodyne Model 5041 injection valves were also used. One was the injection valve and another was connected as selection valve.

2.3. Procedure

Using the flow diagram shown in Fig. 1, 40 μl (or 1000 μl) of an aqueous solution containing both thiamine (7–130 μg ml^{-1} or 2–30 μg ml^{-1}, respectively) and pyridoxine (5–110 μg ml^{-1} or 2–30 μg ml^{-1}, respectively) were inserted into the carrier solution (sodium citrate–citric acid, 0.1 M, pH 3.0) and pumped at a flow rate of 0.95 ml min^{-1}. Thiamine was retained on the cation exchanger placed in the precolumn, while pyridoxine, because of its faster retention–elution kinetic process, passed through the pre-column, was carried to the flow cell and retained in. Pyridoxine retention signal was monitored at 293 nm.

When pyridoxine was totally eluted by the carrier itself, by turning the selection valve, HAc–NaAc buffer solution (0.4 M, pH 4.8) was used as eluting solution for thiamine retained in the precolumn, carrying it, in turn, to the flow cell. The retention signal had its maximum height at 255 nm. Then, by turning again the selection valve, the baseline was restored and it was possible another sample injection.

3. Results and discussion

3.1. Preliminary study

The spectral features of both analytes in homogeneous solution were previously established and they are shown in Fig. 2. Maximum absorbance wavelengths were 247 nm for thiamine and 290 nm for pyridoxine. The scans of the analytes were overlapped, so it was impossible its simultaneous determination without significant errors.

Due to its cationic nature, thiamine was quickly and strongly sorbed on Sephadex SP C-25 cation exchanger gel. At an appropriate acidic pH value, pyridoxine was also sorbed on Sephadex SP C-25 resin due to its protonation in the N atom.

The spectral characteristics of both analytes on the solid phase suffered a bathochromic effect, which moved the absorbance maximum up to 255

and 293 nm for thiamine and pyridoxine, respectively. However, the spectral overlapping also appeared when the two analytes were simultaneous retained on the solid support.

Because of this problem, a pre-column, placed before the flow cell and filled with the same support in the flow cell (Sephadex SP C-25), was used to hold thiamine while pyridoxine was detected. This was possible because pyridoxine had a faster retention–elution process through the active solid support, and it was measured first. Then, with an appropriate eluting solution, thiamine was carried to the cell and measured.

The chosen wavelengths for simultaneous determination of the analytes, were 255 (thiamine) and 293 nm (pyridoxine), the peak height being used as analytical signal.

3.2. Optimization of variables

3.2.1. Level of the packed flow cell

The level of the support in the flow cell is an

Table 3
Determination of thiamine and pyridoxine in pharmaceutical preparations

Pharmaceuticals	Thiamine			Pyridoxine		
	Labelled (mg)	Found[l] (mg ± RSD)		Labelled (mg)	Found[l] (mg ± RSD)	
		Methods			Methods	
		Proposed	Reference		Proposed	Reference
Benerva Roche[a]	300	297.2 ± 0.5	298.3 ± 0.7	–	–	–
Benadon Roche[b]	–	–	–	300	298.1 ± 0.7	297.4 ± 0.6
Agudil[c]	–	–	–	10	10.1 ± 0.1	10.2 ± 0.3
Antomiotopic[d]	–	–	–	25	26.8 ± 0.2	25.1 ± 0.5
Nervobión[e]	100	99.1 ± 0.1	103.2 ± 0.6	100	98.5 ± 0.1	97.6 ± 0.5
Dolo-Nervobión[f]	50	51.9 ± 0.1	49.8 ± 0.4	200	199.5 ± 0.3	200 ± 1
Antineurina[g]	125	118.4 ± 0.6	127.1 ± 0.8	125	131.1 ± 0.1	124.1 ± 0.5
Neurodavur Plus[h]	50	50.1 ± 0.6	50.6 ± 0.9	50	50.0 ± 0.4	51.3 ± 0.7
Actilevol Orex[i]	50	51.3 ± 0.4	50.8 ± 0.7	50	52.0 ± 0.2	51.1 ± 0.9
Trimetabol [j]	30	30.2 ± 0.1	28.5 ± 0.5	30	29.6 ± 0.2	28.4 ± 0.4
Calcinatal[k]	5	4.9 ± 0.5	4.7 ± 0.9	10	9.9 ± 0.1	9.8 ± 0.8

[a] Tablets (Roche) containing thiamine 300 mg.

[b] Tablets (Roche) containing pyridoxine 300 mg.

[c] Tablets (Sigma-Tau) containing L-asparagine 25 mg; D,L-fosforilserine 25 mg; L-glutamine 25 mg; pyridoxine.HCl 10 mg.

[d] Tablets (Cibavision) containing dry extract of Vaccinium Myrtillus 50 mg; retinol acetate 2500 u.i.; α-tocopherol 25 mg; L-cytruline 10 mg; pyridoxine HCl 25 mg; L-acetil aspartic acid 10 mg.

[e] Capsules (Merk) containing thiamine nitrate 100 mg; pyridoxine HCl 100 mg; cyanocobalamine 1 mg.

[f] Capsules (Merk) containing sodium diclophenac 50 mg; $B_1 \cdot HCl$ 50 mg; $B_6 \cdot HCl$ 200 mg; methylcobalamine 1 mg; saccharose 63.74 mg.

[g] Injectable vials (Tedec-Meiji Farma) containing thiamine · HCl 125 mg; pyridoxine · HCl 125 mg; lidocaine · HCl 3 mg; B_{12} 5000 gammas.

[h] Ampoules (Belmac) containing hydroxicobalamine (acetate) 5 mg; thiamine · HCl 50 mg; pyridoxine · HCl 50 mg; Dexametasone (sodium phosphate) 1.6 mg; lidocaine · HCl 12.5 mg.

[i] Packets (Wassermann) containing carnosine 100 mg; hematoporfirine · HCl 3 mg; ascorbic acid 250 mg; pyridoxine · HCl 50 mg; thiamine 50 mg; B_{12} 0.25 mg.

[j] Syrup (Uriach) containing per 5 ml: L-carnitine 375 mg; cyproheptadine 1.75 mg; thiamine · HCl 30 mg; pyridoxine · HCl 30 mg; lysine · HCl 250 mg; cyanocobalamine 1 mg.

[k] Tablets containing Vit. A 1250 ui; Vit D_3 400 ui; Vit B_1 5 mg; Vit B_2 2 mg; Vit B_6 10 mg; Vit PP 15 mg; Vit B_{12} 5 mcg; Vit C 50 mg; calcium pantothenate 3 mg; Ca 100 mg; P 30 mg; Fe 1.83 mg; Cu 0.19 mg; Mn 0.02 mg; Sr 0.14 mg; Zn 0.02 mg; I 0.21 mg; F 0.12 mg; Mg 4.52 mg.

[l] Data are average of three determinations.

Table 4
Recovery study of thiamine and pyridoxine in pharmaceuticals

	Thiamine		Pyridoxine	
Pharmaceuticals	Added (mg/unit)	Recovery ± RSD (%)	Added (mg/unit)	Recovery ± RSD (%)
Benerva	50	100.3 ± 0.6		
	75	97 ± 1		
	100	99 ± 1		
Antomiotopic			15	99.6 ± 0.3
			30	99 ± 1
			75	100 ± 2
Nervobión	25	98.9 ± 0.5	25	99.8 ± 0.4
	50	99 ± 1	50	98 ± 1
	75	100 ± 1	75	101 ± 2
Dolo-Nervobión	12	101 ± 3	50	101.2 ± 0.6
	25	99 ± 2	100	102 ± 1
	75	99 ± 1	150	100 ± 1

interesting variable. If it falls below the bottom of the light beam, the measurement of the absorbance of the analyte is carried out only in solution and absorbance remains constant with a low value. If the level is over the light beam, signal height is not affected but the peaks became wider so decreasing sampling frequency.

As the light beam section of the spectrophotometer was circular, and it was projected to the top of the cell, it became necessary filling up the flow cell. However, due to the different chemical nature and concentration of the carrier (0.1 M) and the eluting (0.4 M) solutions, the resin suffered alternately swelling (with the carrier) and compactation (due to unswelling when the eluting solution passed through it) (see Fig. 3), so altering the level of the resin in the cell. For preventing compactations lowering the resin level below the light beam, the flow cell was just filled up passing through it the eluting solution.

3.2.2. Amount of the resin in the packed pre-column

As indicated above, with only the resin in the flow cell, the signals of the analytes at the respective wavelengths were overlapped. We tried to separate them before they reached the detection zone by using the different kinetic of the retention–elution process of the analytes in Sephadex SP C-25 (faster for pyridoxine). Therefore, we used a pre-column of Sephadex SP-C25 just before the cell in order to retain thiamine in it, while pyridoxine was carried to the flow cell.

The pre-column length (and consequently, the resin amount) was studied from 7 to 25 mm, using an i.d. of 2 mm. The separation of the analytes was completed from 15 mm. (Fig. 3 and Fig. 4). We chose a pre-column length of 15 mm because it gave a satisfactory and complete separation in the minimum possible time.

3.2.3. Influence of pH, nature and concentration of carrier and eluting solutions

The optimum pH value of the carrier solution for the retention of both analytes in the resin was studied at the two working wavelengths by injecting each one of them solely. The single monochannel manifold in Fig. 1 was used, but without the pre-column. The carrier solutions consisted of NaCl solutions (0.04 M for pyridoxine and 0.08 M for thiamine) at different pH values adjusted with HCl and NaOH solutions. After reaching the maximum signal, the analytes were eluted by the respective carrier. From the results obtained (Fig. 5), pH 3.0 was selected as the optimum for the determination of pyridoxine.

In order to make the analytes not to reach the sensing detection zone at the same time, but sequentially, a prior separation had to be performed. Based in the faster-elution kinetic process for pyridoxine in Sephadex SP C-25, the separation would be easily performed using a pre-column with Sephadex SP-C25. A citric acid–sodium citrate solution 0.1 M at pH 3.0 was used as carrier solution. Thiamine was strongly retained in the pre-column while pyridoxine passed through it, reached the detection zone, developed its analytical signal at the optimum pH value and was eluted by the carrier itself. Then thiamine was eluted from the pre-column by rotating the selection valve and passing a HAc–NaAc solution (0.4 M, pH 4.8) through it. When thiamine reached the detection zone, its analytical signal was developed and the analyte eluted by the HAc–NaAc solution itself. Due to the higher ionic strength of this solution with respect to that used for pyridoxine, the compactation of the resin increased so altering the baseline (see * in Fig. 3). Then, the carrier solution (citric acid–sodium citrate solution) was passed through the system by rotating again the selection valve and the initial baseline was restaured, so remaining the system ready for a next injection.

3.2.4. Optimization of FIA variables

FIA variables are injection volume and flow rate.

One of the main advantages of the sensor is the potential increase in the sensitivity as the sample volume taken for analysis is increased. Injecting in the flow system different volumes of a solution containing both analytes, thiamine (10 μg ml^{-1}) and pyridoxine (12 μg ml^{-1}) could be assessed the effect of this variable on the analytical signal. Absorbance increased linearly up to 1500 μl (thiamine: $A = 0.0582 + 3.17 \cdot 10^{-4}\ v$, $r = 0.9929$; pyridoxine: $A = 0.0929 + 5.17 \cdot 10^{-4}\ v$, $r = 0.9979$), with increasing injection volume (v, μl). Higher volumes did not increase the signal significantly. 40 and 1000 μl were chosen to calibrate the sensor.

Flow rate is an interesting variable in this sensor; the higher the rate the higher the compactation in the pre-column (and in the flow cell). This effect decreased the real rate through the cell and made excessive pressure in the system. So, the flow rate could be only studied in the range 0.15–0.95 ml min^{-1} as beyond 0.95 ml min^{-1} pressure problems occurred. The signal increased as the flow rate increased and so 0.95 ml min^{-1} was chosen as working flow rate.

3.3. Analytical features of the proposed method

Calibration graphs were obtained simultaneously for both analytes by following the proposed method. The analytical figures of merit for two different sample volumes are given in Table 1. Data were fitted by standard least-squares treatment. Very good linearity was found in the concentration range 7–130 and 5–110 μg ml^{-1} for thiamine and pyridoxine, respectively, using 40 μl of sample volume, and 2–30 μg ml^{-1} for both analytes, using 1000 μl. Detection limits were estimated as the concentration of analyte which produced an analytical signal equal to three times the standard deviation of the background absorbance. Reproducibility was established for ten analyses of solutions containing 10 μg ml^{-1} of both thiamine and pyridoxine. Sampling frequency was 12 and 9 h^{-1} with 40 and 1000 μl of sample volume, respectively. Five of these determinations are shown in Fig. 6.

3.4. Effect of foreign species

In order to determine the effect of foreign species, a tolerance study was carried out with those compounds that are usually found along with thiamine and pyridoxine in pharmaceuticals.

The study was carried out with 10 μg ml^{-1} of both thiamine and pyridoxine using 1000 μl as sample volume. Foreign species were added to the samples at concentrations higher than those usually found in pharmaceutical preparations.

The tolerance limit (Table 2) was established as the maximum concentration of foreign species that caused a relative error of $\pm$ 3% in the analytical signal. As can be seen, the tolerance to the presence of foreign species (including other vitamins B) is very much higher than the amount in which these compounds are usually found to-

gether with the analytes in pharmaceuticals. In addition, the tolerance limits are very much higher than those ones corresponding to the determination of the analytes by direct UV measurements in solution-only methods. This is due to the selectivity conditions stated by the active solid support which excludes from it, and consequently, from the detection zone, all those species that can not be retained on it in the working conditions.

The tolerance to ascorbic acid was drastically increased by means its retention 'in line' on an anion exchanger resin (Sephadex QAE A-25) placed just before the injection valve. The regeneration of this resin, after several determinations, was carried out with the same buffer solution used as eluting solution (HAc–NaAc, 0.4 M, pH 4.8).

3.5. Application of the method

The proposed sensor was applied to the determination of thiamine and pyridoxine in pharmaceuticals using the standard calibration graph method for an injection volume of 1000 μl. In order to check the accuracy of the proposed procedure, the AOAC reference fluorimetric method [16] (based on the oxidation of thiamine to thiochrome by potassium ferricianide), and a reverse-phase HPLC method [17] using a C_{18} nucleosil column and water at pH = 2.0 as mobile phase, with UV detection at 285 nm were used for thiamine and pyridoxine determination, respectively. Table 3 shows satisfactory results.

In addition, a recovery study was also performed using the proposed procedure by adding different known amounts of the analytes to four pharmaceuticals. Percentage of recovery is shown in Table 4.

4. Conclusions

The use of an on line pre-column in a continuous flow solid phase UV spectrophotometric system allowed the simultaneous determination of two cationic species: vitamins thiamine and pyridoxine. The cation exchanger resin used in the flow cell worked as a dual sensing zone responding sequentially to each analyte, the diode array detection making possible to work in the maximum UV signal for both analytes. Their arrival to the detector/sensing zone was time discriminated owing to the strong retention of thiamine in the on-line pre-column. This is the first photometric flow-through biparameter sensor that allows the simultaneous determination of two analytes with the same ionic nature. Therefore, this is a very simple, rapid and cheap sensor, which contributes to the desired progress [18] in the area of the flow-through spectroscopic sensors applicable to the analysis of real samples. It shows very good analytical features, does not use any derivative reaction and it has been demonstrated its suitability for routine analysis of pharmaceuticals, the dissolution of the sample being the only required pre-treatment.

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