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Selective determination of pyridoxine in the presence of hydrosoluble vitamins using a continuous-flow solid phase sensing device with UV detection

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Abstract

A very simple, inexpensive and highly selective flow injection UV spectrophotometric method for the determination of vitamin B_6 is presented. The native absorbance of the analyte is continuously monitored at 290 nm when it is transiently retained on Sephadex SP C-25 cation exchanger gel beads placed in the detection area of a flow cell. The preconcentration on the active solid phase provides by itself a high increase in sensitivity compared with the same procedure carried out without a solid support. The analytical response is linear in the concentration ranges 1–10 and 2–20 μg ml^{-1} using 600 and 1250 μl of sample, respectively. The R.S.D. (%) are 0.65 (600 μl) and 0.84 (1250 μl) and the detection limits 0.08 and 0.02 μg ml^{-1}, respectively. The procedure was successfully applied to the determination of vitamin B_6 in pharmaceuticals containing (among other active principles) hydrosoluble vitamins in much higher concentrations than that tolerated by the method if performed in aqueous solution. Nevertheless they were tolerated using the proposed sensor due to the selective retention of the analyte.

Keywords: Pyridoxine; Flow injection UV spectrophotometry; UV sensing device

1. Introduction

Accurate determination of water-soluble vitamins is very important in food and pharmaceutical industries, as vitamins are essential for health. Pyridoxine hydrochloride was the first vitamin of the B_6 group to be isolated. It is essential in the diet for the metabolism of amino acids (as a cofactor in transamination and decarboxylation reactions) and for the maintenance of body cells (Kaplan and Pesce, 1989).

Several methods for the determination of pyridoxine hydrochloride have been developed including spectrofluorimetry (Bautista et al., 1996), high performance thin layer chromatography (Argekar and Kunjir, 1995), chemiluminescence (Alwarthan

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and Aly, 1998) and liquid chromatography with electrochemical detection (Hou et al., 1990). As far as spectrophotometry is concerned, pyridoxine hydrochloride has been analyzed extensively by methods based on diazo-coupling (Nirmalchander and Balasubramanian, 1988; Srividya and Balasubramanian, 1997) and other derivating reactions (Sastry et al., 1998). Spectrophotometric determination of pyridoxine in the presence of other compounds as well as resolution of mixtures of vitamins have also been performed by multicalibration techniques (Bautista et al., 1996; Yang et al., 1998).

Here we propose a very simple continuous-flow (on-line) solid-phase spectrophotometric procedure that allows the determination of pyridoxine hydrochloride in the presence of other vitamins and drugs frequently associated with it in pharmaceuticals. No derivating reaction or multicalibration is required, thus the measurement can be performed at a single wavelength very simply and quickly.

The selectivity of the measurement is achieved by means of the on-line retention of the protonated pyridoxine on the active sites of a cationic exchanger thus being isolated from the rest of the sample matrix. The intrinsic UV absorbance at the absorption maximum of pyridoxine retained on the exchanger beads is directly monitored. After the measurement, the carrier stream to which the sample was injected acted as desorbing agent achieving a fast and complete regeneration of the beads.

The use of a solid phase located in the flow-through cell of a spectrophotometer with a continuous flow system combines the important advantages of the solid phase spectrophotometric measurements: selectivity and sensitivity (Ortega-Barrales et al., 1998; Molina Díaz et al., 1999) with those of the flow systems: rapidity, low reagent consumption and minor human participation (Hase and Yoshimura, 1982; Liu et al., 1993; Ayora Cañada et al., 1998; Ruiz Medina et al., 2000).

In this case, the determination of pyridoxine in pharmaceuticals is carried out in continuous flow in the UV region and in the presence of other hydrosoluble vitamins (B_1, B_2, B_{12} and ascorbic acid) without interference from any of them. This is a consequence of the strong selective effect stated by the active solid phase in the detection area: while the species of interest (pyridoxine) is sorbed on the resin beads, the other hydrosoluble vitamins and components found along with pyridoxine in pharmaceuticals are excluded from them. In the absence of the sorbing solid phase, the analysis of vitamin B_6 in these pharmaceuticals could not be performed by direct measurement in the UV region because of the strong overlap of the spectra of these species.

2. Experimental

2.1. Apparatus

Continuous absorption measurements were made with a Perkin Elmer Lambda 2 double beam spectrophotometer controlled by a 386 personal computer with the PECSS 4.4 software package (from Perkin Elmer). It was equipped with a Hellma 138 QS flow through cell (1 mm optical path length) housing an appropriate ion exchanger. A Rheodyne 5041 Teflon rotary valve and a Gilson Minipuls 3 peristaltic pump (four channels) were employed to set up the flow system. All tubing (0.8 mm i.d.) was made of PTFE. An ultrasonic bath (Ultrason, Selecta) and a Crison 2002 pH-meter were also used.

2.2. Reagents

All reagents were of analytical grade.

Pyridoxine stock standard solutions were prepared by dissolution of the appropriate amount of pyridoxine hydrochloride (FLUKA) in water. The solutions were stable for 2 weeks in a refrigerator at about 5°C. Working solutions were prepared daily by dilution of the stock solution with the necessary amount of doubly distilled water.

Solutions of various concentrations of NaCl, HCl, KCl and $NaNO_3$ (from PANREAC) were used.

Sephadex SPC-25 resin (ALDRICH) was used in the H^+ form without any pretreatment. The resin beads were introduced in the flow cell as an

aqueous slurry with the aid of a syringe. Other Sephadex ion exchangers tested were CM C-25, QAE A-25 and DAE A-25.

All solutions were filtered through a membrane filter (0.45-μm pore size) from Millipore.

2.3. Manifold and procedure

A single line manifold was employed. The sample solution containing 2–20 and 1–10 μg ml^{-1} of pyridoxine hydrochloride (corresponding to injections of 600 and 1250 μl, respectively) was inserted into the carrier stream (0.08 M NaCl solution adjusted at pH 2.0 with HCl flowing at 1.15 ml min^{-1}) by means of an injection valve. The analyte passing through the flow cell is retained temporarily on the cationic exchanger developing therefore the analytical signal. The measurement was continuously performed at 290 nm. The carrier itself acted as the eluent, desorbing the pyridoxine from the solid support after reaching the maximum absorbance value. In this way, the regeneration of the ion exchanger sensing microzone was performed rapidly allowing an excellent sampling frequency.

2.4. Treatment of samples

Solid samples containing vitamin B_6 as pyridoxine were ground in an agate mortar and dissolved in doubly distilled water by sonication during 10 min. Insoluble excipient was removed by filtration through a 0.45-μm Millipore membrane filter when necessary. The supernatant was then filtered, diluted to volume with water and analyzed without any pretreatment.

Liquid samples were diluted with doubly distilled water to the appropriate volume.

3. Results and discussion

3.1. Solid support selection

Different solid supports were tested. Solid phases with matrices containing aromatic rings (such as Dowex resins) were disregarded due to their incompatibility with detection in the UV region: they show a strong absorption that hinders light measurements in this spectral range. Among Sephadex resins (with polydextrane matrices), we tested cationic and anionic exchangers both in strong and weak forms, namely SP C-25, CM C-25, QAE A-25 and DEAE A-25. The retention of the analyte on these exchangers was tested in acidic, neutral and basic media. As expected, no appreciable retention of the analyte was observed neither on cationic exchanger in basic medium nor on anionic resins in acidic medium. Both anionic and cationic resins failed to retain pyridoxine at neutral pH.

Two pK_a values have been reported for pyridoxine, namely 5.00 and 8.96 (Perrin, 1972). Thus pyridoxine is expected to be in an anionic form in very strong basic solution ($pH > 11.0$) due to the deprotonation of the –OH group bonded to the pyridinic ring, whereas in acidic solutions ($pH < 3.0$) the pyridinic nitrogen is protonated. In neutral medium the analyte is in uncharged form that prevents its retention on ionic exchangers. Cationic resins in acidic medium provided the highest signals. Slightly lower signals were observed with anionic resins in basic solution. SP C-25 cationic resin was selected for further studies because of its large retention capability. Moreover the elution after the measurement using this resin was faster than that using the weak acid exchanger (CM C-25).

The optimum level of solid support in the measurement flow-through cell was 17 mm (measured from the bottom of the cell) that was achieved with about 20 mg of Sephadex SP C-25.

The absorption spectrum of pyridoxine shows an absorption maximum at 290 nm in aqueous solution. No changes in the position of the absorption maximum are observed when the analyte is sorbed on the anion exchanger (Sephadex SP C-25). However, the preconcentration of pyridoxine on the solid support resulted in an absorbance signal about 36 times greater than that using the same cell in homogeneous solution.

3.2. Influence of chemical variables

As mentioned above, pyridoxine retention on SP C-25 resin was found to occur only at acidic

pH values due to the retention of the protonated species on the cationic resin. In Fig. 1, the effect of the pH of the carrier solution and the sample (600 μl) is shown. The optimum retention was observed for carriers with pH values between 1.0 and 3.0. At pH values above 3.0, the analytical response decreased drastically. For pH above 4.5 the analyte was not retained.

It was necessary therefore, to use an electrolyte which enables a fast and complete baseline recovery. Several electrolytes (KCl, NaCl and $NaNO_3$) at various concentration levels were tested at pH 2.0 in all cases. NaCl provided a slightly higher analytical signal and the most complete return to the baseline.

The increase in carrier ionic strength caused a decrease in both the analytical signal and the residence time due to the competence between the analyte and the cations from the carrier for the active sites of the resin. A 0.08 M NaCl solution (pH 2.0 adjusted with HCl) was selected as a compromise between sampling rate and sensitivity.

As for the sample pH, the signal was found to be constant in a wide range from 2.0 to 11.0 (Fig. 1). This effect is due not only to the partial dispersion in the carrier solution but also to the strongly acidic medium provided by the H^+ ions retained on the solid support as counter ions. This fact notably simplified the sample preparation avoiding any pretreatment apart from dissolution.

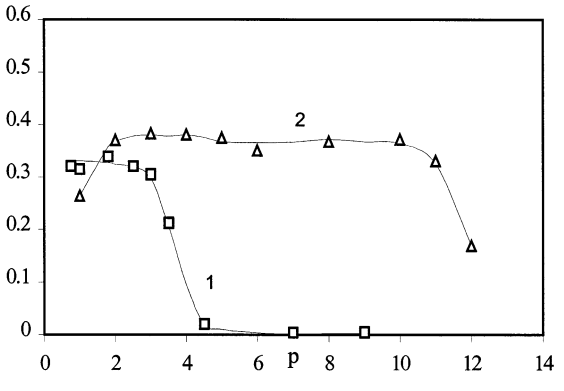

Fig. 1. Influence of pH on sensor response: (1) carrier solution, (2) sample. [Pyridoxine hydrochloride] = 4.86×10^{-5} M.

3.3. Flow system variables

The flow-rate influence was studied by changing it from 0.6 to 1.5 ml min^{-1}. Lower flow rates favored the retention process so providing greater signals. The selected flow rate (1.15 ml min^{-1}) reflects a compromise between sensitivity and time needed for the measurement, the latter being logically reduced using higher flow rates. One of the most interesting features of this sort of sensing device is the strong dependence of the response on the amount of sample flowing through the sensing microzone. The greater the volume of sample injected the higher the analytical signal as can be seen in Fig. 2 where a plot of maximum peak height versus sample volume injected is shown. So the proposed method allows pyridoxine to be determined over a wide range of concentrations (1–20 μg ml^{-1}) by simply selecting the appropriate sample volume for calibration. We chose two sample volumes (600 and 1250 μl) to construct two different calibration lines.

3.4. Analytical figures of merit

The calibration graphs (Fig. 2 inset) are linear for 2–20 and 1–10 μg ml^{-1} for 600 and 1250 μl of sample, respectively. The analytical parameters are summarized in Table 1. The reproducibility was established for 10 independent analyses of solutions containing 15 and 8 μg ml^{-1} (for 600 and 1250 μl, respectively), R.S.D. being 0.65 and 0.84%. The detection limit calculated using the IUPAC definition was obtained from the S.D. of the blank signal, and detection limits as low as 0.08 (for 600 μl) and 0.02 μg ml^{-1} (for 1250 μl) were obtained. The use of a carrier solution that also acted as a desorbing agent allowed very high sampling frequency: 44 and 32 h^{-1} for 600 and 1250 μl, respectively.

3.5. Study of interferences

In order to determine the potential effect of foreign species, a tolerance study was carried out involving those compounds that are more frequently found along with pyridoxine in pharmaceuticals, including other vitamins of the B group.

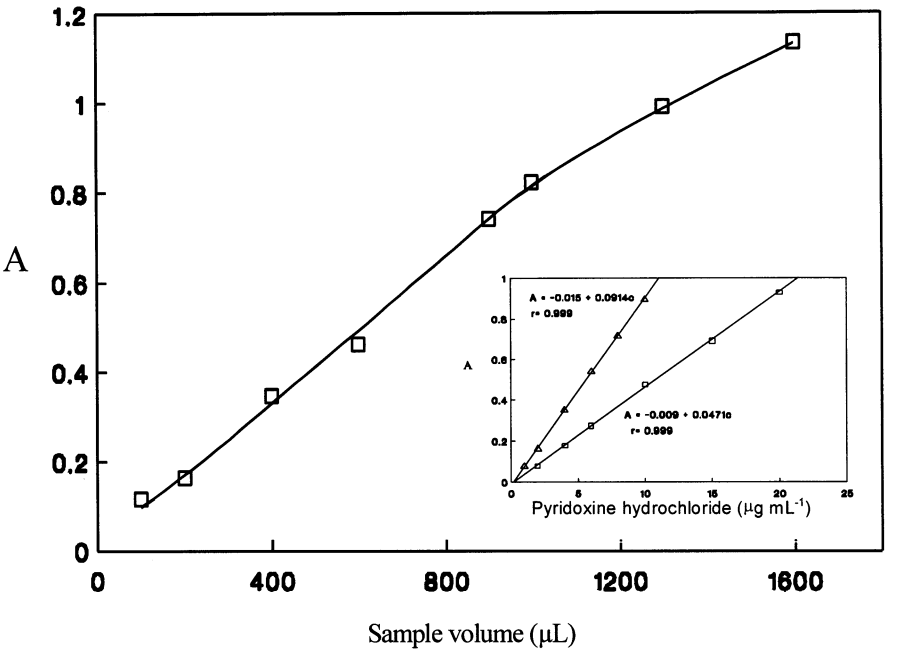

Fig. 2. Effect of sample volume injected on the analytical signal and slope of calibration lines (inset) for 600 μl (□) and 1250 μl (△).

The tolerance limit was taken as the maximum amount of foreign species causing less than 5% error in the determination of pyridoxine. The maximum w/w ratio tested was 100. The results from this study on 18 different foreign species are summarized in Table 2.

The proposed procedure shows a high level of tolerance to most of the species tested. Only sodium diclofenac interferes, when it is found at the same level of concentration as pyridoxine, although this fact did not hinder the determination of the analyte in pharmaceuticals, where diclofenac was in a lower concentration than B_6 vitamin. We must also emphasize that the sensor shows much higher tolerance levels than the conventional spectrophotometric method in solution as can be seen in Table 3 for species such as caffeine, hydrosoluble vitamins, etc. Tolerance levels are notably increased using the proposed sensor (up to 10 000 times for ascorbic acid) due to the selective retention of cationic pyridoxine on the solid phase and its consequent separation from the matrix.

To illustrate this extraordinary increase in sensitivity, the spectra of binary mixtures all containing 10 μg ml^{-1} of pyridoxine and different species

Table 1
Analytical figures of merit

Analytical parameter	Sample volume	
	600 μl	1250 μl
Linear range (μg ml^{-1})	2–20	1–10
Slope (ml μg^{-1})	4.71×10^{-2}	9.14×10^{-2}
Intercept	−0.0009	−0.015
Correlation coefficient (r)	0.9997	0.9999
Relative standard deviation (% R.S.D.) ($n = 10$)	0.65 (15)*	0.84 (8)*
Limit of detection (μg ml^{-1})**	0.08	0.02
Limit of quantification (μg ml^{-1})***	0.26	0.07
Sampling frequency (h^{-1})	44	32

* Concentration (μg ml^{-1}) at which R.S.D. was established.
** 3σ criterion (IUPAC, 1976).
*** 10σ criterion (Guidelines for Data Acquisition, 1980).

Table 2
Study of interferences

Foreign species	Tolerance (μg ml^{-1} interferent) (μg ml^{-1} B_6)
Lactose, glutamic acid, L-carnitine, L-lysine, sucrose, L-methionine, saccharin, ascorbic acid	>100
Calcium pantothenate	75
Biotine, vitamin B_{12}	50
Caffeine, lidocaine	10
Nicotinamide, salicylamide	7.5
Vitamin B_2	5
Vitamin B_1	2.5
Diclofenac sodium	1

in aqueous solution (vitamins of the B group, vitamin C, caffeine and salicylamide) were measured and shown in Fig. 3. In all cases, the determination of pyridoxine is impossible due to the strong spectral overlap. Nevertheless with the use of the proposed sensor the spectral contributions of these species are eliminated completely due to the selective retention of pyridoxine on the resin. This can be appreciated in Fig. 3b in which the spectra in the solid phase (obtained in stopped-flow conditions at the maximum of the transitory peak) are shown for a solution containing 10 μg ml^{-1} of pyridoxine alone and a mixture containing 10 μg ml^{-1} of each component.

Table 3
Comparison of tolerance in solution determination and with the proposed sensor

Interfering species	Tolerance (μg ml^{-1} interferent)/(μg ml^{-1} B_6)	
	Solution	Proposed sensor
Caffeine	0.1	10
Salicylamide	<0.1	7.5
Ascorbic acid	0.1	100
Diclofenac	<0.1	1
Vitamin B_{12}	<0.1	50
Vitamin B_2	<0.1	5
Vitamin B_1	0.2	2.5

3.6. Applications

The flow-through sensing device developed was applied to the determination of pyridoxine in 11 commercial pharmaceutical preparations with different composition and presentation (tablets, capsules, sachets, ampoules and solutions). They included other vitamins of the B group (B_1, B_2 and B_{12}) and ascorbic acid among other active principles that make the analysis of pyridoxine impossible by direct UV measurement in solution.

In all cases the standard calibration graph method was used with 600 μl of sample. Results are shown in Table 4. Moreover, in order to check the accuracy of the proposed method, a recovery

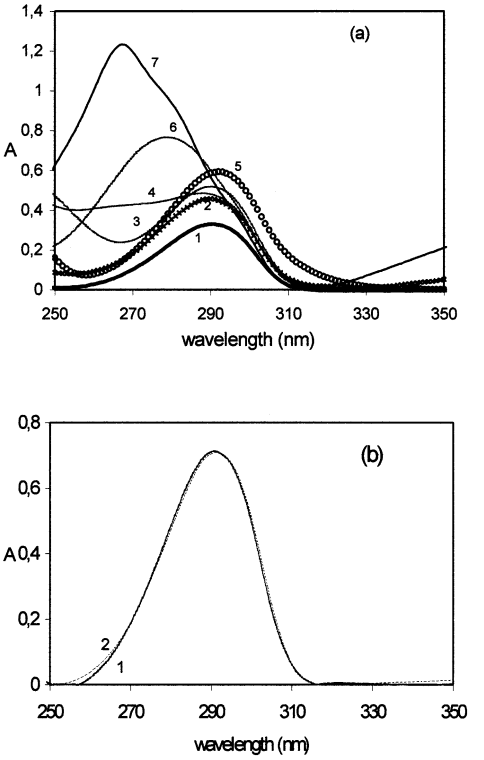

Fig. 3. (a) Spectra in aqueous solution of vitamin B_6 alone (1) and binary mixture solutions of vitamin B_6 with: (2) vitamin B_{12}, (3) vitamin C, (4) vitamin B_1, (5) salicylamide, (6) caffeine and (7) vitamin B_2. Concentration of all species: 10 μg ml^{-1}. Light path length: 10 mm. (b) Spectra in the solid sensing zone of: (1) vitamin B_6 and (2) a mixture of all the above species. Concentration of all components: 10 μg ml^{-1}. Sample volume injected: 600 μl. Light path length: 1 mm.

Table 4
Analytical applications

Sample	Other active principles	Pyridoxine hydrochloride labeled (mg)	Pyridoxine hydrochloride found (mg)
Antomiopic[a]	Vitamins (A, E), L-citrulline, *N*-acetyl-L-aspartic acid	25	25.1 ± 0.5
Neurodavur Plus[b]	Vitamins (B_1, B_{12}), lidocaine, dexamethasone phosphate disodium salt	50	51 ± 2
Trofi-milina[c]	Phospholipids	150	151 ± 2
Vertigum[a]	Dixirazine, inositol nicotinate	100	100 ± 2
Agudil[a]	L-Asparagine, *o*-phospho-DL-serine, L-glutamine	10	10.1 ± 0.3
Dolo-Nervobión[c]	Diclofenac sodium, vitamins (B_1, B_{12})	200	200 ± 2
Audione Oral[a]	Vitamins (A, E, PP, B_1)	50	50.8 ± 0.8
Actilevol Orex[d]	Vitamins (C, B_1, B_{12}), carnosine, hematopophyrin, ciproheptadine	50	51.1 ± 0.9
Astenolit[e]	Vitamins (B_1, B_{12}), inositol, carnitine, potassium and sodium aspartate, acetylglutamine	50	49.8 ± 0.8
Salvacolon[d]	Vitamins (B_1, B_2, B_{12}, PP), calcium pantothenate, lysine	8	7.98 ± 0.09
Trimetabol[f]	Vitamins (B_1, B_{12}), carnitine, lysine, ciproheptadine acephylinate	6	6.08 ± 0.05

[a] Tablets.
[b] Injectable vials.
[c] Capsules.
[d] Sachets.
[e] Ampoules.
[f] Solution.

study was carried out for each pharmaceutical by means of addition of standard pyridoxine hydrochloride to the sample. Three different concentration levels (between 5 and 200 mg per unit depending on the pyridoxine content) were assayed. Results of recovery obtained by comparison of the amount recovered with that added were between 95 and 106% in all cases.

4. Conclusions

The proposed method for direct determination of pyridoxine in the presence of other hydrosoluble vitamins (e.g. 10-fold of ascorbic acid) and drugs frequently associated with it in pharmaceuticals by continuous flow UV solid phase spectrophotometry is highly selective and sensitive in spite of its UV spectrophotometric nature. Rapidity, low cost and reagent consumption (no derivative reactions are needed) and simplicity are found among other interesting features of the procedure which make it applicable to drug analysis and quality control of pharmaceuticals.

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