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Determination of Vitamin B_6 in pharmaceutical formulations by flow injection-solid phase spectrophotometry

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Abstract

In this work, a new solid phase spectrophotometric method in association with flow injection analysis for Vitamin B_6 (pyridoxine) determination has been developed with direct measurement of light-absorption in C18 material. In the developed method, successive passage of the complex, previously formed in the flowing stream, and eluent through the flow cell and continuous monitoring of the process provided the analytical information needed to determine pyridoxine. Pharmaceutical samples containing Vitamin B_6 were previously dissolved in 0.1 mol l^{-1} phosphate buffer solution (pH 7.5) and a sample volume of 235 μl was injected directly into carrier stream consisting of a mixture of methanol and 0.1 mol l^{-1} phosphate buffer solution adjusted to pH 7.0 (1 + 1, v/v). The blue indophenol dye produced from the reaction between pyridoxine and *N*,*N*-diethyl-*p*-phenylenediamine after oxidation by potassium hexacyanoferrate(III) was quantitatively retained on C18 support and the spectrophotometric detection was performed simultaneously at 633 nm. The retained complex was quickly eluted from C18 material with the eluent stream consisting of a mixture of methanol and 0.01 mol l^{-1} HCl (6+4, v/v). The results showed that the proposed method is simple, rapid and the analytical response is linear in the concentration range of 0.5–10 and 0.2–4 mg l^{-1} using 235 and 860 μl of sample, respectively. The limits of detection are 0.15 and 0.060 mg l^{-1} and the R.S.D. are 3.6% (at 2 mg l^{-1} level) and 4.0% (at 1 mg l^{-1} level) using sample volume of 235 and 860 μl, respectively. The system presented an analytical throughput of 15 determinations per hour when a sample volume of 235 μl was utilized. The procedure was successfully applied to the determination of Vitamin B_6 in pharmaceutical formulations containing vitamins of B group and others active principles such as Vitamin C and minerals.

Keywords: Vitamin B_6; Solid phase spectrophotometry; Flow injection analysis

1. Introduction

Solid phase spectrophotometry coupled with continuous flow system (FI-SPS) has been employed

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to determine low concentration of several chemical species in different kinds of sample solutions [1–3]. In FI-SPS, the solid support is placed inside the flow cell in the light pathway and the detection is performed simultaneously with the analyte retention [4]. These measurements may be carried out with the help of conventional spectrophotometers, resulting in low cost and easy availability [5]. SPS offers the advantage of in situ preconcentration of the analyte due to accumulation of the analyte in a small volume. This approach permits to achieve a high sensitivity and provides a low reagent and sample consumption. By using FI-SPS an enhancement of the performance of the flow systems is observed compared to those procedures where measurement is carried out after elution or directly in solution [6].

Pyridoxine is found in the composition of analgesics, formulations containing vitamins of B group and minerals multivitamins [7]. It is essential in the diet for the metabolism of amino acids and for the maintenance of body cell [3]. Therefore, rapid, simple and highly sensitive procedures allowing the determination of pyridoxine are useful in pharmaceutical analysis.

Many spectrophotometric methods have been developed for the analysis of pyridoxine and others vitamins of B group in pharmaceuticals, included direct measurement in the UV region [3,8,9]. However, it is usual a previous separation step because of the strong spectra overlapping of other species present in the samples [7]. Solid phase spectrofluorimetry associated with flow injection analysis for determination of pyridoxine was also previously reported. This paper studied the determination of Vitamin B_6 in pharmaceutical preparation using Sephadex SP-C25 resin as solid phase [10].

In the present work, a simple, selective and sensitive one-step method has been developed for the determination of dissolved Vitamin B_6 (pyridoxine) in pharmaceutical preparations in the presence of other vitamins and mineral species by FI-SPS. The method is based on the retention of the blue indophenol dye produced in the oxidation of pyridoxine by potassium hexacyanoferrate(III) and *N*,*N*-diethyl-*p*-phenylenediamine (DPD) onto C18. The spectrophotometric detection was performed directly in the solid phase at 633 nm.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical grade reagents and high purity demineralized water obtained in a Milli-Q Water System, from Millipore (Saint Quentin Yvelines, France).

A standard Vitamin B_6 solution (100 mg l^{-1}) was prepared by dissolving an appropriate amount of the pyridoxine (Merck) in water. Working standard solutions (0.10–10.0 mg l^{-1}) were daily prepared by adequate dilution in 0.1 mol l^{-1} phosphate buffer (pH 7.5).

Phosphate buffer solutions (0.1 mol l^{-1}) pH range from 5.6 to 8.0 were prepared by dissolving appropriate amounts of potassium dihydrogen phosphate and potassium hydrogen phosphate.

DPD solution (0.1 g l^{-1}) was daily prepared by dissolving 0.025 g of *N*,*N*-diethyl-*p*-phenylenediamine sulfate (Merck) in 250 ml of 0.1 mol l^{-1} phosphate buffer (pH 7.0).

Potassium hexacyanoferrate(III) solution (2.0 g l^{-1}) was prepared by dissolving 0.4 g of this reagent (Merck) in 200 ml of 0.1 mol l^{-1} phosphate buffer (pH 7.0).

Carrier solution was prepared by mixing methanol (Merck) and 0.1 mol l^{-1} phosphate buffer (1 + 1, v/v) adjusting to pH 7.0 with phosphoric acid and the cleaning solution was prepared by mixing methanol (Merck) with 0.01 mol l^{-1} HCl (6 + 4, v/v).

Thirty-five milligram of C18 bonded silica (60–100 μm) obtained from Waters (Milford, MA, USA) Sep-Pak cartridges was placed inside the flow cell and was used as solid support for indophenol dye retention.

2.2. Sample preparation

The pharmaceutical preparations analyzed and the labeled contents (amount present in 1 tablet) were: *Vitamin B_6 50 mg* (GNC) containing 50 mg pyridoxine; *Essential B* (GNC) containing 10 mg pyridoxine, 10 mg thiamine, 15 mg riboflavin, 100 mg calcium pantothenate, 25 μg cyanocobalamin, 25 mg nicotinamide, 100 μg biotin, 400 μg folic acid; *Supradyn* (Roche Pharm. Ind., Rio de Janeiro) containing 10 mg pyridoxine, 20 mg thiamine, 5 mg riboflavin,

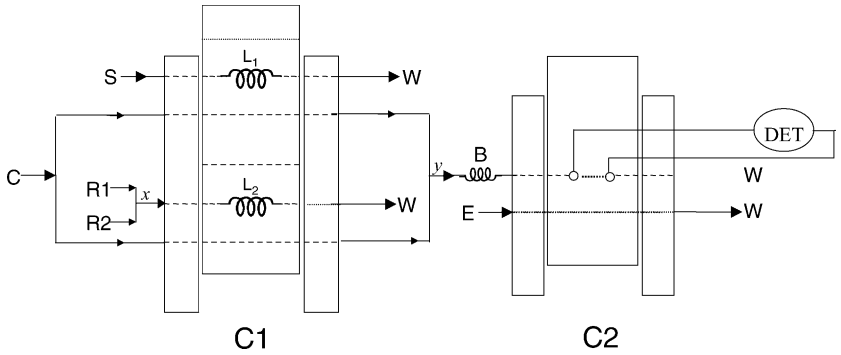

Fig. 1. Flow diagram employed for the solid-phase spectrophotometric determination of Vitamin B_6. C1 and C2: valves. S: sample (1.0 ml min^{-1}); C: carrier stream, 50% methanol in phosphate buffer pH 7.5 (1.0 ml min^{-1}); R1: reagent, DPD solution prepared in phosphate buffer (1.0 ml min^{-1}); R2: reagent, potassium hexacyanoferrate(III) solution prepared in phosphate buffer (1.0 ml min^{-1}); L1: sample loop (235 μl); L2: reagent loop (430 μl); B: 50 cm flow line; E: eluent, 60% methanol in 0.01 mol l^{-1} HCl (1.0 ml min^{-1}); w: waste; *x* and *y*: confluences; DET: spectrophotometer equipped with flow cell loaded with C18, 633 nm.

11.6 mg calcium pantothenate, 5 μg cyanocobalamin, 50 mg nicotinamide, 150 mg ascorbic acid, 10000 U.I. Vitamin A palmitate, 10 mg tocopherol acetate, 129 mg Ca, 60 mg Mg, 50 mg Fe, 2.3 mg Zn, 2.0 mg Mn, 3.9 mg Cu, 0.25 mg Mo; *Täglich Multivitamin Minerstoffe Spurenelemente* (Pharma Aldenhoven GmbH & Co, KG) containing 1.8 mg pyridoxine, 1.4 mg thiamine, 1.7 mg riboflavin, 6 mg calcium pantothenate, 3 μg cyanocobalamin, 100 μg biotin, 75 mg ascorbic acid, 0.85 Vitamin A palmitate, 12 mg tocopherol acetate, 200 mg Ca, 150 mg Mg, 5.0 mg Fe, 5.0 mg Zn, 1.0 mg Mn, 1.0 mg Cu, 80 μg Mo, 60 μg Cr, 30 μg Se.

Ten tablets of each pharmaceutical sample were crushed to a fine powder. An amount of each powder sample in the range of 100–250 mg was dissolved in phosphate buffer solution (pH 7.5) by sonication for 15 min. Insoluble excipient was removed by filtration through a 0.45-μm membrane filter. The filtered solution was diluted to appropriate volume with phosphate buffer.

2.3. Apparatus

The set up consisted of a Cary 1E spectrophotometer (Varian, Australia) with a 78 μl home-made flow cell with 1.0 mm optical path [11,12], and a Gilson (Villiers-le-Bel, France) Minipuls 2 peristaltic pump equipped with flexible poly (vinyl chloride) tubings. The flow manifold was assembled with a homemade sliding-bar commutator [13], employing sample loops and flow lines of 0.8 mm i.d. PTFE tubes.

2.4. Flow diagram and procedure

The flow cell was filled with ca. 35 mg of C18 beads and inserted in the optical path of the spectrophotometer.

The flow diagram is shown in Fig. 1. In this situation the commutator C1 is in the position where the loops L1 (235 μl) and L2 (430 μl) are respectively being filled with sample or standards solution (S) and with DPD and hexacyanoferrate(III) solutions previously mixed in the confluence *x*, both flowing at a flow rate of 1.0 ml min^{-1}. The carrier stream, C, (methanol + buffer solution) flows through the analytical path also at 1.0 ml min^{-1}. The commutador C2 is in the position where the carrier stream flows through the flow cell.

By sliding the commutator central bar (C1), sample (or standards) and reagents aliquots are simultaneously inserted into the analytical path. So, the aliquots are mixed in the confluence *y* and transported by the carrier towards the flow cell attached in C2 commutator. Subsequently, the blue indophenol dye formed was retained in the flow cell, filled with C18, positioned in the optical pathway. Absorbance signals were continuously recorded at 633 nm.

In the last step, by displacing the sliding bar (central part) of the injector C2 to the other resting position, the cleaning process is started by passing eluent solution

(methanol + HCl) through the flow cell during 60 s at 1.0 ml min^{-1} to remove the dye from the solid phase.

3. Results and discussions

3.1. Chemical variables

The spectrum of the blue indophenol dye produced in the oxidation of pyridoxine (Vitamin B_6) by potassium hexacyanoferrate(III) and *N*,*N*-diethyl-*p*-phenylenediamine retained on C18 bonded silica was measured after baseline correction established with the solid support treated with water. On the solid phase, the dye presented an absorption maximum at 633 nm.

C18 support was very stable in acidic and methanolic solutions, allowing its use for more than 60 measurements without affecting the retention of indophenol dye. The mixture of methanol and hydrochloric acid employed as eluent solution allowed proper dye elution without change the retention characteristics of the solid phase. Different mixtures of methanol and 0.01 mol l^{-1} hydrochloric acid were tested and it was found that with a mixture of methanol and HCl (60 + 40, v/v), the retained complex was quickly and completely eluted from C18 material.

The influence of the pH on the color development of blue dye and on its retention onto C18 support was investigated within the range of pH 5.5–8.0. No significant variation in the retention efficiency was observed for the 7.0–7.6-pH range. Thus, in further experiments, sample, reagents and carrier solutions were previously buffered at pH 7.5 with phosphate buffer solution.

The effect of the DPD concentration was studied in the range between 0.05 and 0.3 g l^{-1}, maintaining the potassium hexacyanoferrate(III) solution with a constant concentration. Better results were verified when DPD had a concentration of 0.1 g l^{-1} for a pyridoxine concentration of 4.0 mg l^{-1}.

The ratio between hexacyanoferrate(III) and DPD could also be an important chemical factor that can influence the reaction rate. Therefore, several ratios were tested maintaining the DPD solution with a fixed concentration at 0.1 g l^{-1}. It was observed that potassium hexacyanoferrate(III) solution must be used in the system in a minimum concentration of 0.5 g l^{-1} using DPD concentration of 0.1 g l^{-1}. No effect was observed for quantitative retention of pyridoxine and on the absorbance signal when potassium hexacyanoferrate(III) solution was used in the range from 0.5 to 5.0 g l^{-1}. Thus, a concentration of 2.0 g l^{-1} was chosen for the procedure.

3.2. FI variables

Total flow rate of the system was maintained at 1.0 ml min^{-1} because at higher values fluid leakage was observed due to the increase of the pressure inside the system. Using flow rates <1.0 ml min^{-1}, the absorbance signal was unaffected, indicating that the process of reaction/retention is fast. So, it was selected a flow rate of 1.0 ml min^{-1} to increased the productivity of the method.

In order to improve the reaction condition, the volume of the coil B located after mixing the samples and reagents was varied between 50 and 150 cm. It was observed that the analytical signals were maximized reducing the volume of the reaction coil. This fact suggest that the reaction is rapid and indophenol dye produced from the reaction between pyridoxine and *N*,*N*-diethyl-*p*-phenylenediamine after oxidation by potassium hexacyanoferrate(III) was not very stable. For this reason, the flow line B (Fig. 1) was maintained as short as possible (ca. 50 cm).

An important advantage of solid phase spectrophotometry is the potentiality of improving sensitivity by increasing the sample volume from which the analyte is concentrated in the solid support. This fact is important since it can provide different alternatives for varying the sensitivity of the procedure as a function of the pyridoxine concentration in the samples. In this experiment, a linear relation ($r = 0.996$) was observed between the absorbance signal obtained at 633 nm and the sample volume injected, in the volume range 100–450 μl. This relation can be described by the equation

$$A = 8.0 \times 10^{-4} V + 0.0153$$

where A is the absorbance and V the injected sample volume (μl) of a 4 mg l^{-1} solution of pyridoxine. On the other hand, by increasing the sample volume, an inherent decrease in the sampling rate was also observed. Thus, a 235 μl sample volume was chosen as a compromise between sensitivity and sample throughput.

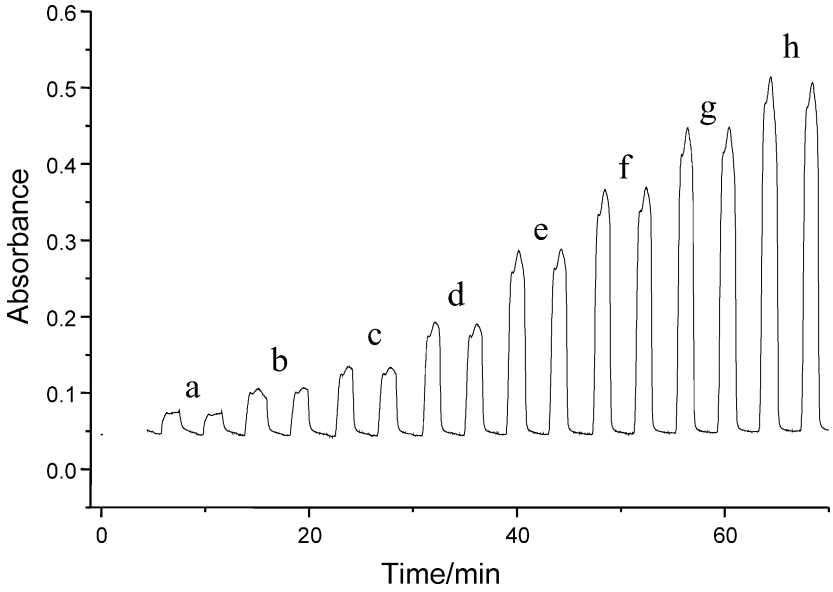

Fig. 2. FIA recording obtained for the direct injection of pyridoxine standards. (a) 0.0; (b) 0.5; (c) 1.0; (d) 2.0; (e) 4.0; (f) 6.0; (g) 8.0; (h) 10.0 mg l^{-1} of pyridoxine.

The effect of the flow rate on the desorption of indophenol dye previously retained on C18 was investigated using the mixture of methanol and hydrochloric acid as cleaning solution. A change in flow rate from 0.5 to 1.0 ml mim^{-1} did not affect the indophenol desorption. Flow rates >1.0 ml mim^{-1} caused fluid leakage in the joints due the increase in back pressure. Therefore, a flow-rate value of 1.0 ml mim^{-1} was selected as a compromise between sample throughput and system stability.

3.3. Effect of foreign species

A systematic study of the effect of potentially interfering species on the pyridoxine determination in pharmaceutical preparations was undertaken. In order to evaluate the potential effect of foreign species, a interference study was carried out involving those compounds that are more frequently found along with pyridoxine in pharmaceuticals, including other vitamins of the B group. This study was carried out by

Table 1
Comparison of the analytical characteristics of the proposed procedure[a] and the previously work [3]

Analytical characteristic	Proposed method (sample volume)		Previously method (sample volume) [3]	
	235 μl	860 μl	600 μl	1250 μl
Linear dynamic range (mg l^{-1})	0.5–10	0.2–4	2–20	1–10
Correlation coefficient (r)	0.9979	0.9960	0.9997	0.9999
Variation coefficient (% R.S.D.), $n = 10$[b]	3.6	4.0	0.65	0.84
Detection limit (mg l^{-1}), 3σ	0.15	0.060	0.08	0.02
Apparent molar absorptivity, ε (l mol^{-1} cm^{-1})	8.89×10^4	1.89×10^5	–	–
Sample throughput (h^{-1})	15	11	44	32

[a] Using 1.0 mm cell.
[b] Calculated by sequential repetition of retention, measuring and elution operations.

Table 2
Reagent consumption during 1 h of operation

Reagent	Consume
DPD	0.003 g
$K_3[Fe(CN)_6]$	0.06 g
Methanol P.A.	66 ml
Na_2HPO_4	0.26 g
$NaH_2PO_4{\cdot}H_2O$	0.16 g
Concentrated HCl	0.032 ml

adding a known amount of foreign species to a pyridoxine solution of 2 mg l^{-1}. The tolerance limit was taken as ±5% change in absorbance. The following species, when present in the amounts shown in brackets (in mg l^{-1}) do not interfere: Fe [18], Mg [150], Mn [20], Mo [0.10], Zn [6.0], Cu [1.0], Cr [10], Se [2.0], thiamine [200], riboflavin [20], cyanocobalamin [0.1], ascorbic acid [120], vitamin A palmitate [20], tocopherol acetate [13.2], lactose [200], folic acid [2.0]. When calcium was present in sample, it was previously separated by filtration after its precipitation at sample dissolution step.

3.4. Features of the method

In the proposed procedure, the Beer's law was strictly obeyed from 0.5 to 10.0 mg l^{-1} when a sample volume of 235 μl was employed, according to the equation: $A = 0.0791C + 0.0922$, with $r = 0.9979$. In this equation, A represents the absorbance signals measured as peak height and C the concentration of pyridoxine in mg l^{-1}. As can be seen in Fig. 2, the blank solution has absorbance signal due reagents absorption at 633 nm.

The variation coefficient for 10 independent measurements was 4.0% and the detection limit, calculated as three times the standard deviation of the blank solution, was 0.15 mg l^{-1}. The apparent molar absorptivity was estimated as 8.89×10^4 l mol^{-1} cm^{-1} which is more than 10 times higher than that obtained in the analogous flow injection spectrophotometric method, in which larger sample volume (ca. 500 μl) and a cell with a larger optical path (10 mm) were employed [8].

A comparison of the analytical characteristics of the proposed procedure using sample injected volumes of 235 and 860 μl is summarized in Table 1. FIA recordings obtained by duplicate injections of pyridoxine standards are shown in Fig. 2. From these recordings a sample throughput of 15 h^{-1} was calculated and good stability in the baseline and precision could be observed. The method has a low reagent and sample consumption with a waste generation of 242 ml h^{-1}. In Table 2 is indicated the amounts of reagents required for 1 h of operation.

A previously work [3] used direct UV measurement carried out in the solid phase for determination of pyridoxine in pharmaceuticals in the presence of hydrosoluble vitamins. As the proposed method present in this paper, the previously work is selectivity and sensitivity. A comparison of the analytical characteristics of the proposed procedure and the previously work is summarized in Table 1.

3.5. Determination of pyridoxine in pharmaceutical preparations

The proposed method has been applied to the determination of pyridoxine in pharmaceutical preparations. Results are showed in the Table 3. Each result was obtained as the average of two replicates of three independent solutions of the same pharmaceutical preparation. The supplier labels and the result obtained by direct measurement in the UV region at 290 nm [14] or HPLC method using Nucleosil 100-5 C18 column with 0.1 mol l^{-1} $(NH_4)_2CO_3$–water–methanol

Table 3
Determination of pyridoxine in pharmaceutical preparations (mg per tablet)

Sample	Labeled contents	Comparative method	Proposed method
Vitamin B_6 50 mg	50.0	61.0 ± 0.5[a]	63.2 ± 3.3
Essential B	5.0	5.3 ± 0.3[b]	4.8 ± 0.2
Supradyn	10.0	10.6 ± 0.9[b]	10.5 ± 0.8
Täglish	1.8	2.1 ± 0.1[b]	2.3 ± 0.1

[a] Direct measurement in the UV region.
[b] HPLC method.

(5 + 15 + 80, v/v) as mobile phase and detection at 280 nm [15] were used for comparision. The application of the paired *t*-test (95% confidence level) did not show significant differences between the values obtained.

4. Conclusions

The developed procedure provides a highly sensitive and simple approach for the determination of pyridoxine by the retention of the blue indophenol dye produced in the oxidation of pyridoxine by hexacyanoferrate(III) and DPD onto C18 without on-line or off-line preconcentration or separation steps.

The FI-SPS proposed method is rapid and it has low cost since the measurements may be carried out with the help of conventional spectrophotometers. Small amounts of sample, sorbent and reagent are required. High enrichment factors can be attained by employing large sample volumes making possible to work at different concentration levels.

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