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High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection

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Abstract

The method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin by high-performance liquid chromatography (HPLC) with coulometric electrochemical and UV detections is presented. The retention time of vitamins was repeatedly determined by isocratic elution using 0.05 M phosphate buffer–10% methanol and 0.018 M trimethylamine (1 ml min^{-1}, pH 3.55) as mobile phase with the Supelco LC 18 column 5 μm (25 cm × 4.6 mm). The specificity of the method was demonstrated by the retention characteristics, coulometric electrochemical and UV detection. The limits of detection of thiamine, pyridoxine and cyanocobalamin were: 9.2, 2.7 and 0.08 ng/ml, respectively. The method was characterized also by wide concentration range, high sensitivity and good accuracy (99.6–102.7%). The repeatability of the method was evaluated at different level of concentration of vitamins and the relative standard deviation was below 4.5%. The method was successfully applied for the quantification of Vitamins B_1, B_6 and B_{12} in pharmaceutical preparations and dietary supplements.
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Keywords: Vitamins B; Pharmaceutical formulations; HPLC analysis; ED; UVD

1. Introduction

Vitamins are essential for the human life. A catalytic function of vitamins in anabolic and catabolic pathways makes the human metabolism unable to synthesize these compounds. Therapeutic multivitamins are advisable for use in cases of deficiency in pathological conditions where nutritional requirements are increased, e.g. in alcoholism [1]. Therefore, multivitamins complex is recommended to be used as dietary supplementation.

The determination of water-soluble vitamins in various samples is rather difficult due to the chemical instability and complexity of the matrices in which they usually exist.

Various analytical methods are available but most of them are time-consuming or not enough accurate. These techniques base on the measurement of spectrophotometric, fluorimetric, enzymatic or microbiological properties. To the most popular chromatographic techniques belong capillary electrophoresis (CE) [1–4] and particularly high-performance liquid chromatography (HPLC). These techniques are rapid, sensitive and accurate and proven to be able for measurements of vitamin concentration in pharmaceutical formulations [4–10]. The modification of the HPLC coupled to ultraviolet-visible absorbance [4–8,10–16], fluorimetric [11,17,18] or electrochemical [19–23] detections have been presented in the literature. Critical factor for the development

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of HPLC methods is always selection of the column packing material [24–28]. Most of the published methods are based on the gradient techniques [6,10,11,29,30], as very sensitive to pH changes of the mobile phase.

The isocratic elution is usually applied for the separation of individual or combine number of vitamins [4,8,11,31]. Their determination by ion-pairing chromatography with reversed phase is the most frequent method [8,10,12,17,24,31–33]. Reversed-phase liquid chromatography, however, has become an indispensable method for the separation of analysed compounds in pharmaceutical products [25].

Determination of vitamin concentrations in pharmaceutical formulation is usually proceeded by extraction with a solvent. The technique of solid-phase extraction (SPE) has been applied for isolation of the vitamins from biological samples [13,24,31,34].

Alternative methods are based on redox reaction with electrochemical detectors such as amperometric, pulse amperometric and coulochemic instruments. The Vitamins B are easily oxidized or reduced with on-line working electrode in electrochemical cell. The high sensitivity is obtained using amperometric measurement, through the oxidation requiring a high electrical potential. In this way, many matrix components can cause increase of chromatographic interferences [19,20,22,23].

The aim of our study was to optimize the HPLC method for the simultaneous determination of concentration of thiamine, pyridoxine and cyanocobalamin in various pharmaceutical preparations and dietary supplements using coulometric electrochemical and ultraviolet detections.

2. Experimental

2.1. Apparatus

All vitamins were separated by HPLC coupled with pump P 580 (Dionex), UV diode array detector 340 S (Dionex) and Coulochem II electrochemical detector (ESA) operated by the Chromeleon Chromatography Management System.

2.2. Reagents

Thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamine were provided by Fluka (Biochemica). Sodium phosphate dibasic, heptahydrate and trimethylamine hydrochloride were obtained from Sigma–Aldrich (St. Louis, MO, USA). Other reagents were also of HPLC grade, i.e. 85% orthophosphoric acid (Riedel-de Haen), solvents: methanol and water (Baker, HPLC Analyzed).

2.3. Materials

Following samples have been investigated during the experiment: Vitaminum B_1 (Polfarmex Kutno), tablet; Vitaminum B_1 (Polfa Kutno), tablet; Vitaminum B_1 0.01 (Pliva Krakow), ampules; Vitaminum B_6 (Polfarmex Kutno), tablet; Vitaminum B_6 (Pliva Krakow), ampules; Vitaminum B_{12} (WZF Polfa), ampules; Milgamma N (Wörwag Pharma), ampules; Juvit Multi (Hasco-Lek Wroclaw), drops; Multivitaminum Forte (WZF Polfa), tablet; Metabo (Metabo UP), tablet; Vibovit junior (Polfa Kutno), powder; Visolvit (GlaxoSmithKline Poznan), powder; Feminatal (Merck Darmstadt), tablet; Vita-Fem (Puritan's Pride), tablet. The dosage forms and other ingredients for pharmaceuticals are presented in Table 1.

All pharmaceutical preparations or diet supplements listed above were commercially available in pharmacy shops.

2.4. Sample preparation

Twenty Vitamin B tablets or five powder portions were weighed. The tablets were finely powdered. An accurate weight of the powder was dissolved in water and transferred into a 100 ml volumetric flask protected from light, and then diluted to the mark with water. It was mixed well and allowed to stand for 40 min. The solution was then filtered using Whatman 1 Chr filter paper. The filtrate was collected and stored in amber flask and suitable aliquots were taken for analysis. All the solutions mentioned above were stored in volumetric flasks at 4 °C.

2.5. Chromatographic conditions

The reverse phase LC 18 column 5 μm (25 cm × 4.6 mm) was produced by Supelco. Isocratic elution, with the mobile phase, consisting of 0.05 M sodium phosphate dibasic, heptahydrate, 10% methanol (v/v) and 0.018 M ion-pair reagent trimethylamine was adjusted to pH 3.55 with 85% phosphoric acid. The column was equilibrated at 22 °C at the flow rate 1 ml/min. Methanol was used in the preparation of the aqueous-organic mobile phase and for conditioning the column therefore the composition and purity of the mobile phase are critical factors for optimizing the sensitivity and selectivity of the HPLC assay with using electrochemical detection. The mobile phase was filtered through a 0.22 μm membrane filter under vacuum and degassed before use. The volume of injection was 20 μl.

The UV–vis diode array detector enables simultaneous determination of investigated compound at different wavelengths. In this study, the UV–vis detector was used only for the determination of thiamine. Pyridoxine and cyanocobalamin were detected after HPLC separation using Coulochem II electrochemical detector (model 5020A, ESA) equipped with a dual analytical cell (model 5010) and guard cell (model 5020). The guard cell was connected in-line before injection port and was used to remove oxidizable impurities in the mobile phase, to eliminate the interference with baseline stability. The hydrodynamic voltammogram (HVD) analysis was additionally performed to optimize conditions for the accurate determination of pyridoxine and cyanocobalamin in electrochemical detector.

Table 1
Pharmaceuticals tested during the analysis

Pharmaceuticals	Dosage form	Active substance	Other ingredients
Vitaminum B_1	Tablet	Thiamine HCl (3 mg)	Vehicle (magnesium stearate, talcum, starch, lactose, saccharose, gelatin)
Vitaminum B_1	Tablet	Thiamine HCl (25 mg)	Vehicle (magnesium stearate, talcum, starch, lactose, saccharose, gelatin)
Vitaminum B_1	Ampules	Thiamine HCl (10 mg)	Solution pro iniectione
Vitaminum B_6	Tablet	Pyridoxine HCl (50 mg)	Vehicle (magnesium stearate, talcum, starch, lactose, saccharose, gelatin)
Vitaminum B_6	Ampules	Pyridoxine HCl (50 mg)	Solution pro iniectione
Vitaminum B_{12}	Ampules	Cyanocobalamin (500 μg)	Vehicle (sodium acetate, acetic acid, natrium chloride, aqua pro iniectione)
Milgamma N	Ampules	Pyridoxine HCl (100.0 mg), thiamine HCl (100.0 mg), cyanocobalamin (1.0 mg)	Lignocaine HCl, sodium hydroxide, sodium phosphate, benzyl alkohol, potassium hexacyanoferrate(III), aqua pro iniectione
Juvit Multi	Drops	Pyridoxine HCl (4.0 mg), thiamine HCl (2.0 mg)	Vitamins: A, C, D_3, E, B_2, PP, vehicle (sodium hydroxide, sodium phosphate, citric acid, BHA, saccharin sodium, cremophore RH 40, orange flavouring, water)
Multivitaminum Forte	Tablet	Pyridoxine HCl (10.0 mg), thiamine HCl (5.0 mg)	Vitamins: A, C, D_3, E, B_2, PP, d-panthotenic Ca, vehicle
Metabo	Tablet	Pyridoxine HCl (0.3 mg), cyanocobalamin (7.5 μg)	Chromium (as chromium amino acid chelate, chromium picolinate), Proprietary blend: green tea extract (leaf), guarana seed extract (seed), oolong tea (leaf), kola nut extract (seed), bitter orange (*Citrus aurantium*) extract (fruit), cayenne (fruit), Platycodon grandiflorum extract (root), vehicle (microcrystalline cellulose, croscarmellose sodium, stearic acid, magnesium stearate, silicon dioxide, sodium carboxymethylocellulose, dextrin, dextrose, lecithin, sodium citrate)
Vibovit junior	Powder	Thiamine HCl (1.2 mg), pyridoxine HCl (1.4 mg), cyanocobalamin (2.0 μg)	Vitamins: A, D_3, E, C, PP, d-panthotenic Ca, glucose, vehicle
Visolvit	Powder sparkling	Pyridoxine HCl (1.0 mg), thiamine HCl (1.0 mg), cyanocobalamin (5.0 μg)	Vitamins: A, D_3, PP, C, d-panthotenic Ca, vehicle (tartaric acid, sodium bicarbonate, silicon dioxide, strawberry flavouring, saccharin)
Feminatal	Tablet	Pyridoxine HCl (2.2 mg), thiamine HCl (1.5 mg), cyanocobalamin (2.2 μg)	Vitamins: beta carotene, B_2, D_3, PP, C, H, d-panthotenic Ca, folic acid, minerals: Mg, Fe, Zn, Cu and I, vehicle
Vita Fem	Tablet	Pyridoxine HCl (15.0 mg), thiamine HCl (15.0 mg), cyanocobalamin (50.0 μg)	Vitamins: A, C, D_3, E, B_2, PP, H, folic acid, d-panthotenic Ca, minerals: Ca, K, I, Mg, Zn, Se, Cu, Mg, Cr, Mo, Mn, P, Cl, B, Ni, Fe; PABA, choline, inositol, betaine, L-cysteine, ginseng panax extract, vehicle (stearynic acid, silicon dioxide, methylocellulose)

2.6. *Analysis of Vitamins B_1, B_6 and cyanocobalamin in pharmaceutical formulation*

The standard stock solutions of thiamine (100 mg/l), pyridoxine (100 mg/l) and cyanocobalamin (10 mg/l) were made with the highly pure water (not less than $18\,\mathrm{M}\,\Omega\,\mathrm{cm}^{-1}$) and were stored separately in volumetric flask at the temperature of $-18\,^{\circ}\mathrm{C}$. This treatment guaranteed stability of the solution for 2 weeks. Solutions for the instrument calibration were prepared daily and further diluted with mobile phase. The detection limit of the calibration standards was defined as the minimal substrate concentration when the signal-to-noise ratio reached values greater than 3.

The method was validated with respect to accuracy and precision. The accuracy has been determined as a recovery of thiamine HCl, pyridoxine HCl and cyanocobalamin added to samples prior to extraction and calculated as the total vitamin. The standard deviation (SD) was calculated using Statistica 6.0 software-package.

3. Results and discussion

3.1. *Chromatography*

The mobile phase used in the system proposed offers a suitable environment for the separation of thiamine as well as electrochemical activation and separation of pyridoxine and cyanocobalamin. Optimization procedure includes studies the composition of the mobile phase: buffer type (phosphoric or acetic buffer), pH of the mobile phase (from 2.5 to 4.0), the ion-pair reagent (sodium dodecyl sulphate or trimethylamine), organic modifiers (methanol or acetonitrile) and flow rate and temperature (from 20 to 40 °C). The ion-pair reagent trimethylamine was proven at the concentration 0.018 M to be good for the separation of vitamins and significantly prolonged the retention times in the presence of 10% methanol. Optimum characteristic of peak resolution was achieved at pH 3.55 in room temperature in a C 18 column in isocratic mode. However, the RP-HPLC in the isocratic mode with

acetic buffer (at various pH of the mobile phase), sodium dodecyl sulphate or trimethylamine and methanol or acetonitrile could not be used for the simultaneous separation Vitamins B_1, B_6 and B_{12} because the chromatographic peaks are not well resolved.

The hydrodynamic voltammogram (HVD) was developed by injecting solution (20 μl of 500 ng/ml of standard pyridoxine and 25 ng/ml of standard cyanocobalamin) and measuring the current produced by vitamins at the electrodes. Electrical potentials varied in the range from 0.05 to 0.95 V depending on the examined substance. Fig. 1 shows the HVD as function of the oxidation potential used for pyridoxine and cyanocobalamin detection. The lowest potential required for the electrodes to produce maximum current response by oxidizing pyridoxine and cyanocobalamin was determined. The optimal potential level for oxidative Vitamin B_6 detection was found to be 0.85 V. Corresponding level for cyanocobalamin was 0.70 V. For improving selectivity of pyridoxine, the voltage at E2 was set equal to the established oxidation potentials of the vitamins. In the case of cyanocobalamin, the E2 voltage has value exceeding slightly the established oxidation potentials of the vitamins. Finally, for the detection of pyridoxine and cyanocobalamin, electrode potentials were set as: 0.90, 0.35 and 0.85 V, for the guard cell, E1 and E2, respectively. A high enough electrical potential at the electrodes has to be given in order to achieve high sensitivity and baseline stability. It is essential that the water must be of top purity and that the mobile phase should be continually degassed.

In our method, the eluent flows trough a porous graphite electrode on the coulometric detector and next through the ultraviolet detector, in single run. Compared to the coulometric detection, the UV detection is less sensitive and more susceptible to interfering substances. Fig. 2 presents the UV diode array spectra of aqueous solution of thiamine. The ultraviolet chromatogram at the limit of quantification for standard of thiamine is presented in Fig. 3A. In this experiment, the spectra were recorded in the range of 280–288 nm for selective detection of thiamine. The ultraviolet detector has lower sensitivity for the determination of pyridoxine, than electrochemical coulochemic detector. Fig. 3B shows

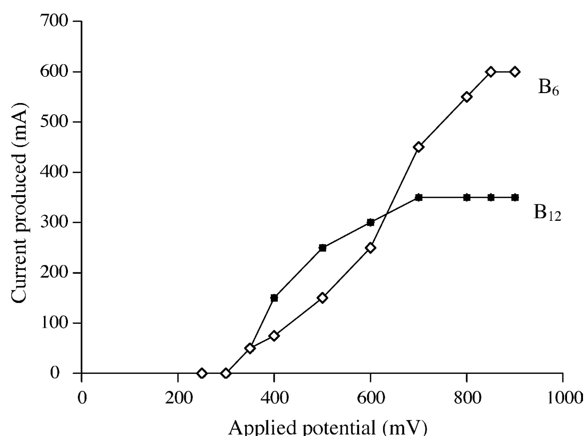

Fig. 1. The hydrodynamic voltammogram (HDV) of pyridoxine and cyanocobalamin. The HVD was developed by plotting the current produced by 10 ng pyridoxine and 0.5 ng cyanocobalamin at various oxidation potentials.

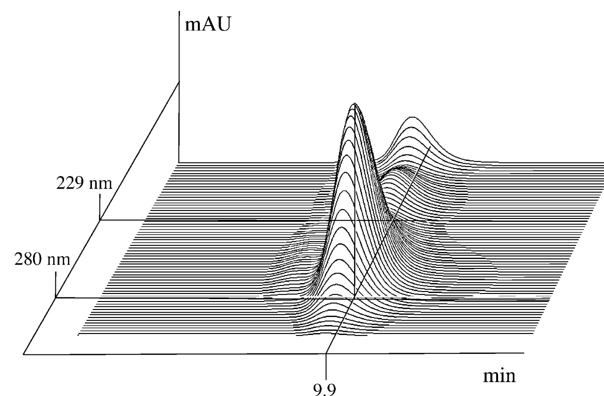

Fig. 2. The UV spectra of thiamine in aqueous solution. LC 18 column 5 μm (4.6 mm × 25 cm) Supelco Inc., Mobile phase: 0.05 M phosphate buffer (pH 3.55) with 10% methanol containing 0.018 M trimethylamine, flow rate 1.0 ml min^{-1}.

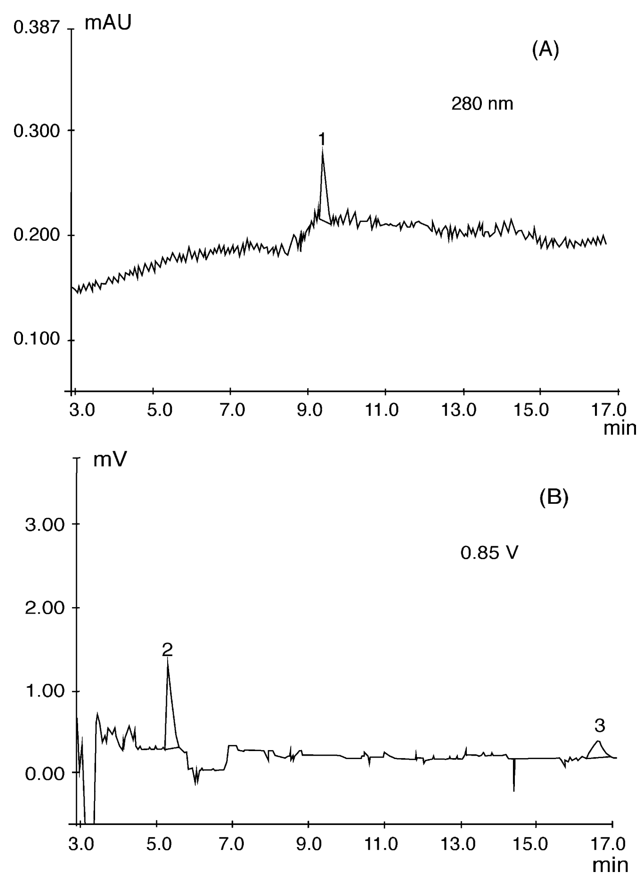

Fig. 3. Chromatograms of standard mixture at the LOQ: (A) 1, thiamine (t_R 9.9 min, 29.0 ng/ml); (B) 2, pyridoxine (t_R 5.5 min, 9.3 ng/ml); 3, cyanocobalamin (t_R 16.2 min, 0.28 ng/ml). LC 18 column 5 μm (4.6 mm × 25 cm) Supelco. Mobile phase: 0.05 M phosphate buffer (pH 3.55) with 10% methanol containing 0.018 M trimethylamine, flow rate 1.0 ml min^{-1}.

coulochemic chromatogram at the limit of quantification of pyridoxine and cyanocobalamin standards at working electrode E2. The potentials at the electrode provide peak area response with a minimum background and are the basis for quantification. Standard deviation values of t_R for thiamine and pyridoxine at $\lambda = 280$ nm were 9.9 ± 0.04 and 5.8 ± 0.02 min, respectively, while these values for pyridoxine and cyanocobalamin at potential E2 amounting to 0.85 V were 5.5 ± 0.07 and 16.2 ± 0.06 min, respectively. Figs. 4 and 5 illustrate chromatograms of the vitamins studied in samples of Vibovit and Visolvit, respectively.

Many scientific papers on the electrochemistry of cyanocobalamin and pyridoxine including polarographic studies and cyclic voltammetry have been published in the recent years [16,19,20,22,35–38]. Some results provide evidence that Vitamin B_{12} has a redox relation to the cobalt atom. Vitamin B_{12} containing Co(III) can be reduced to Vitamin B_{12} with Co(II), and subsequently to Vitamin B_{12}, that contains Co(I), all in aqueous media. The reduced product can be observed by the spectra change in the UV range. Zagal et al. [39] claimed that the spectrogram peak related to reversible transition Co(II) ↔ Co(III) is very sensitive to the electrolyte pH. Zheng and Lu [35] reported that the cyclic voltammograms (CVs) of cyanocobalamin are different in the solutions with pH higher than 3.

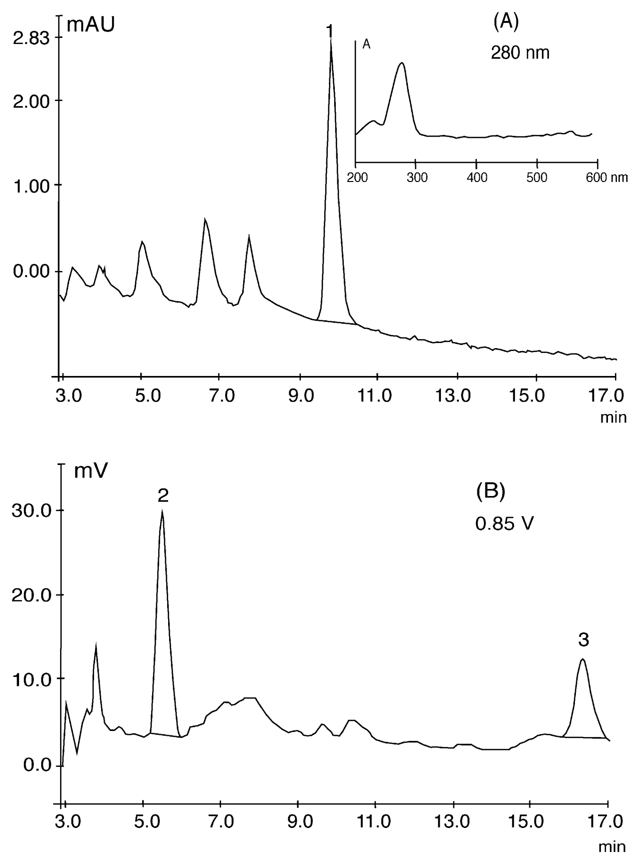

Fig. 4. Chromatograms of the pharmaceuticals (Vibovit powder): (A) 1, thiamine (t_R 9.9 min, 2.95 μg/ml); (B) 2, pyridoxine (t_R 5.5 min, 1.2 μg/ml); 3, cyanocobalamin (t_R 16.2 min, 0.01 μg/ml). LC 18 column 5 μm (4.6 mm × 25 cm) Supelco. Mobile phase: 0.05 M phosphate buffer (pH 3.55) with 10% methanol containing 0.018 M trimethylamine, flow rate 1.0 ml min^{-1}.

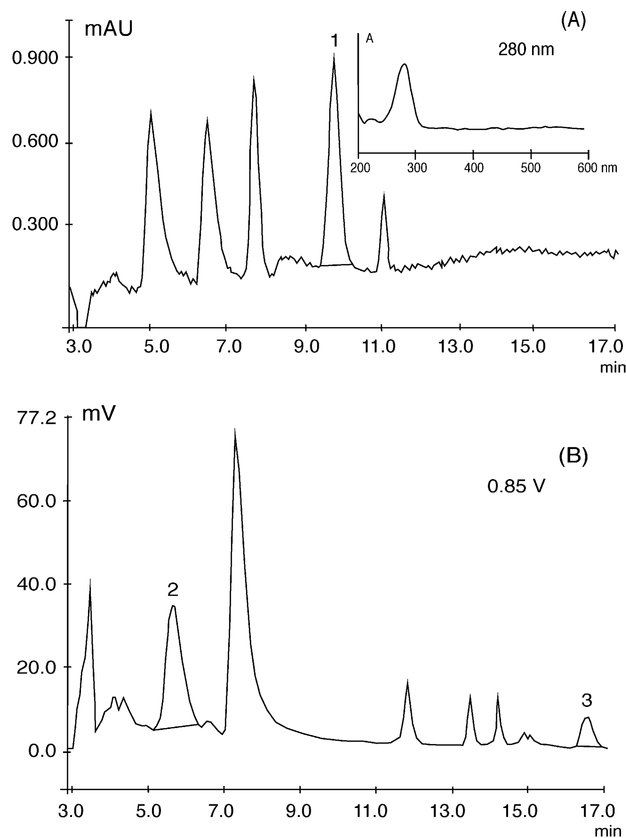

Fig. 5. Chromatograms of the pharmaceuticals (Visolvit): (A) 1, thiamine (t_R 9.9 min, 1.43 μg/ml); (B) 2, pyridoxine (t_R 5.5 min, 0.95 μg/ml); 3, cyanocobalamin (t_R 16.2 min, 0.01 μg/ml). LC 18 column 5 μm (4.6 mm × 25 cm) Supelco. Mobile phase: 0.05 M phosphate buffer (pH 3.55) with 10% methanol containing 0.018 M trimethylamine, flow rate 1.0 ml min^{-1}.

A wide variety of compounds is capable of being monitored with a coulochemic detector. In general, electroactivity is dependent on the presence of functional group of molecules [40]. The oxidized pyridoxine and cyanocobalamin contain the hydroxy- or amine groups with a lone pair of electrons. These effects are difficult to interpret, since it is unknown how the Vitamin B_{12} is adsorbed on the graphite electrode at positive potential. Qu et al. [20] describe electrochemical behaviour of pyridoxine, using a chemically-modified glassy carbon electrode. They observed sensitive oxidation peak appeared at 0.80 V. In the coulometric detector all coeluting compounds which have the oxidation potential less than that for the compound of interest will be oxidized at the screen electrode. This phenomenon makes the use of the screen mode as a powerful tool to enhance selectivity [41,42]. The parameters such as minimal potential of electrode oxidation, longer electrode high-life and good baseline stability are advantages of the coulometric detector.

Table 2
Validation of the method regarding linearity and the limits of detection and quantification

Analytical parameter	Thiamine	Pyridoxine	Cyanocobalamin
Concentration range (μg ml^{-1})	1.4–10.8	0.5–3.5	0.005–0.0275
Intercept	−0.0881	−0.0170	0.0065
Slope ± SD[a] (ml μg^{-1})	10.2 ± 1.8	314.4 ± 7.4	15.8 ± 2.5
Coefficient of correlation (r)	0.9997	0.9999	0.9998
Limit of detection (ng ml^{-1})	9.2	2.7	0.08
Limit of quantification (ng ml^{-1})	29.0	9.3	0.28

[a] Represents the standard deviation of the slope calibration curves obtained by plotting in triplicate six different concentrations of each vitamin by UV and coulometric response.

Table 3
Inter and intra-day repeatability for thiamine, pyridoxine and cyanocobalamin

Vitamin	Concentration (μg ml^{-1})	SD (%)			
		Day 1[a]	Day 2[a]	Day 3[a]	Overall[b]
Thiamine	2.6	0.9	1.6	3.5	3.4
	7.8	1.1	0.7	2.1	0.8
Pyridoxine	1.5	4.4	0.4	1.6	4.3
	2.6	0.7	2.0	0.6	2.8
Cyanocobalamin	0.0095	0.3	1.3	1.7	0.8
	0.0155	0.5	0.7	1.5	2.5

[a] $n = 5$.
[b] $n = 3$.

The peak current at the positive potential was proportional to the concentration of pyridoxine and cyanocobalamin showing that the response should be caused by these electroactive vitamins. In these conditions, the simultaneous determination of pyridoxine and cyanocobalamin was achieved.

3.2. Validation

Calibration graphs were constructed by triplicate injection of six standard solutions of the vitamins. Table 2 summarizes the parameters of the calibration curves: slope, intercept, coefficient of correlation (r), limit of detection and limit of quantification. The limit of detection was calculated using 3 s criterion that corresponds to a signal equal to three times the standard deviation of the background noise. The limit of detection for thiamine hydrochloride amounted to 9.2 ng/ml. Subsequently, the limits for pyridoxine and cyanocobalamin were 2.7 and 0.08 ng/ml, respectively. The present method is characterized by higher sensitivity than ultraviolet [4,6], capillary electrophoresis [2] and fluorescence [11,43] techniques. The limit of pyridoxine detection achieved in our study (2.7 ng/ml) is comparable with that reported for voltammetric determination [22]. A calibration plot was linear for wide concentration ranges with correlation coefficient $r \geq 0.999$ (Table 2).

Inter and intra-day repeatability were studied at two different concentrations. The results obtained are shown in Table 3. It can be seen that the relative standard deviations were below 4.5%.

3.3. Quantification of the vitamins in pharmaceutical formulations

As is shown in Table 4, satisfying recoveries ranging from 99.6 to 100.8% were obtained for added to pharmaceuticals known amounts of all the vitamins studied. The analytical results for eight pharmaceutical preparations and dietary supplements, containing the studied vitamins are summarized in Table 5. The assayed samples indicate retention time below 17 min. The obtained results agree with the labelled values for Vitamins B in the studied pharmaceutical preparations and dietary supplements. No interference was found during analysis of the pharmaceutical preparations. Although in the case

Table 4
Recovery study of thiamine, pyridoxine and cyanocobalamin in pharmaceuticals, $n = 5$

	Added (mg/ampules)	Recovery (%)	SD (%)
Pharmaceutical 1[a]	10	100.7	1.8
	5	100.3	1.9
	2	99.6	0.9
Pharmaceutical 2[b]	42.3	102.7	2.9
	28.2	100.1	0.9
	14.1	100.2	0.7
Pharmaceutical 3[c]	0.4	100.1	0.2
	0.24	100.8	1.4
	0.16	99.9	0.8

[a] Containing 10 mg thiamine.
[b] Containing 50 mg pyridoxine.
[c] Containing 0.5 mg cyanocobalamin.

Table 5
The concentration of thiamine, pyridoxine and cyanocobalamin in pharmaceutical and dietary supplements

Pharmaceuticals	Thiamine HCl			Pyridoxine HCl			Cyanocobalamin		
	Labelled (mg)	Found[a] (mg)	SD (%)	Labelled (mg)	Found[a] (mg)	SD (%)	Labelled (μg)	Found[a] (μg)	SD (%)
Vitaminum B_1	3	2.94	3.2	–	–	–	–	–	–
Vitaminum B_1	25	24.7	2.6	–	–	–	–	–	–
Vitaminum B_1	10	10.5	0.8	–	–	–	–	–	–
Vitaminum B_6	–	–	–	50	49.9	0.9	–	–	–
Vitaminum B_6	–	–	–	50	50.2	0.2	–	–	–
Vitaminum B_{12}	–	–	–	–	–	–	500	501.2	0.3
Milgamma N	50	49.7	0.6	50	50.3	0.2	500	500.4	0.7
Juvit Multi	2.0	1.98	0.6	4.0	3.9	0.1	–	–	–
Multivitaminum forte	5.0	5.1	0.3	10.0	10.1	0.2	–	–	–
Metabo	–	–	–	0.3	0.29	0.6	7.5	7.49	0.3
Vibovit junior	1.2	1.20	0.5	1.4	1.39	0.3	2.0	2.02	1.7
Visolvit	1.0	1.1	0.4	1.0	1.02	0.3	5.0	4.9	0.8
Feminatal	1.5	1.4	0.2	2.2	2.3	0.7	2.2	2.1	0.6
Vita Fem	15.0	14.8	0.9	15.0	15.1	0.9	50.0	49.8	1.5

[a] Data are average of five determinations.

of dietary supplement Metabo UP were observed additional peaks, they do not interfere with well-distinguished vitamin peaks. Results of the tests performed demonstrate the ability of the proposed method for successful quantitative evaluation of the vitamins studied in pharmaceutical preparations: tablets, powders, ampules and dietary supplements. The high sensitivity and selectivity of HPLC–electrochemical detection method upgraded by coulometric and UV detectors with isocratic elution can be applied to accurate determination of Vitamins B_1, B_6 and B_{12} in a single run.

4. Conclusion

A method for the simultaneous determination of Vitamins B_1, B_6 and B_{12} by combined HPLC with coulochemic and UV detections was described. Such detections of the three vitamins have not yet been reported previously. The method has been proven to have good sensitivity, linearity, accuracy and enlarged range of B_1, B_6 and B_{12} quantification in a single run. The proposed separation and detection procedures were successfully applied for evaluation of the Vitamins B in pharmaceutical preparations and dietary supplements.

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