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Research Article

VALIDATION OF HPLC METHOD FOR DETERMINATION OF ANTIOXIDANT VITAMIN C AND VITAMIN B_6 IN FOOD SUPPLEMENTS AND DRUGS

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ABSTRACT

Aim and methods: An isocratic HPLC method with UV – detection for identification and determination of Ascorbic acid (Vitamin C) and Pyridoxine hydrochloride (Vitamin B_6) in food supplements and drugs is developed. HPLC method is validated in respect of analytical parameters: selectivity, accuracy, precision, linearity.

Results: Analytical parameter accuracy is presented by the degree of recovery R (%) and RSD (%.) All results for R suit the relevant confidence interval: Vit. C: R[C_{200}]: 99.2 ÷ 101.82 (RSD = 0.77); R[C_{550}]: 99.9 ÷ 100.26 (RSD = 0.11); R[C_{825}]: 99.71 ÷ 100.07 (RSD = 0.11); Vit. B_6: R[B_6]: 99.33 ÷ 101.59 (RSD = 0.69). Analytical parameter precision is estimated by the uncertainty of the result, determined by SD, RSD and $\overline{X} \pm t.S\overline{X}$. All values for the obtained content of vitamins in model mixtures correspond to the respective confidence interval: Vit. C: [C_{200}]: 196.74 ÷ 205.32 (SD = 2.55, RSD = 1.27); [C_{550}]: 546.05 ÷ 554.81 (SD = 2.6, RSD = 0.47); [C_{825}]: 822.29 ÷ 827.37 (SD = 1.5, RSD = 0.18); Vit. B_6: [B_6]: 18.61 ÷ 19.65 (SD = 0.31, RSD = 1.62). The regression curves are build. The regression equations: y = a.x + b show the proportional accordance between area under the curve (AUC) and concentration (C). The obtained coefficients of regression (R^2) are higher than 0.994.

Conclusion: The validated HPLC method is appropriate for quality control of content of Vitamin C and Vitamin B_6 in food supplements and drugs.

Keywords: HPLC, validation, Vitamin C, Vitamin B_6, Food supplements.

INTRODUCTION

Antioxidants can delay the oxidation of lipids by inhibiting the initiation of oxidizing chain reactions. Antioxidant based drugs are applied for prevention of diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer 1.

Vitamin C and Vitamin B_6 are essential constituents of food and play different specific and vital functions in metabolism. Ascorbic acid protects macromolecules, including DNA from oxidative damage and plays a role in the pathogenesis prevention and therapy of cancer 2.

Vitamin C has the ability to sequester the singlet oxygen radical, stabilizes the hydroxyl radical and reduces Vitamin E back to the active state 3. Ascorbic acid is important for: a) hydroxylation reactions, that are essential for the formation of collagen; b) regulation of cellular nucleotide concentrations; c) function of immune and endocrine systems 4. Vitamin B_6 – magnesium combination can help for treatment of autism 5.

Because of relative instability of Vitamin C and Vitamin B_6 during storage, qualitative and quantitative analyses are important 6. For the simultaneous determination of pyridoxine, pyridoxal, pyridoxal – 5′ – phosphate, pyridoxamine, pyridoxamine – 5′ – phosphate and the degradation product 4 – pyridoxic acid, are described the following methods: 1) in human plasma: gradient ion – pair RP – HPLC with fluorescence detection (λexcitation = 328 nm, λemission = 393 nm), after post – column derivatization with phosphate buffer, containing 1 g/l sodium bisulfate: on C_{18} (ODS) column, mobile phase: acetonitrile (0.5 – 15 %) in a potassium phosphate buffer with 1 – octanesulfonic acid and triethylamine, pH = 2.16 for 46 min. [7]; 2) in human plasma and serum: isocratic RP – HPLC with coulometric electrochemical detection: on C_{18} column (250 mm/4.6 mm i. d./ 5 µm), mobile phase: 35 mM sodium phosphate, containing 2.5 mM heptanesulfonic acid, adjusted to pH = 3.2 with 85 % orthophosphoric acid and 12 % methanol (v/v) [8]; 3) in commercial vitaminized milk and in woman milk (with Vitamin B_2): gradient RP – HPLC with fluorescence detection, after post – column photochemical conversion: on octyl column, mobile phase: sodium pentanesulfonate in 1 % acetic acid : methanol : tetrahydrofurane 9.

Vitamers pyridoxine, pyridoxal and pyridoxamine are analysed by: 1) isocratic HPLC for 12 min. – in foods [10]; 2) isocratic micellar RP – HPLC with UV – detection at λ = 290 nm – in human serum: on C_{18} column and flow – rate 1 ml/min of mobile phase: 150 mM sodium dodecyl sulphate : 2 % penthanol : dihydrogenphosphate buffer 10 mM at pH = 3 [11]; 3) fluorimetry – in serum with reagent system, containing sodium glyoxalate, potassium aluminum sulfate and manganese dioxide for quantitative conversion of pyridoxamine and it's phosphate to the corresponding aldehydes by a combination of transamination and oxidation reactions 12. HPLC is applied for quantitation of: 1) pyridoxal 5′ – phosphate and 4 – pyridoxic acid in serum, after protein precipitation, followed by centrifuge filtration [13]; 2) vitamins B_1, B_2, B_6 in foodstuffs [14] and in fruit juices [15] and B_6 and B_{12} in seafood: on C_{18} column (250 mm/4.6 mm i. d./5 µm) and flow rate 1.0 ml/min. of mobile phase: methanol : phosphate buffer = 10 : 90 and 0.018 M trimethylamine to pH = 3.55, with ultraviolet and coulometric electrochemical detections 15.

For simultaneous determination of Vitamin C and Vitamin B_6 in food products, pharmaceutical preparations and biological liquids are developed the following methods: 1) multicomponent UV – Vis spectra analysis, by terms of the algorithms: BTEM, FastICa, IPCA, MILCA, RADICAL, SIMPLISMA [16]; 2) gradient micellar RP – HPLC with UV – detection at λ = 254 nm, λ = 295 nm and λ = 361 nm, mobile phase: 16 mmol/l sodium dodecyl sulfate : 0.02 M phosphate buffer, pH = 3.6 and a gradient of 3.5 – 10 % (v/v) butanol, for 75 min. [17]; 3) isocratic hydrophilic interaction HPLC (HILIC) with UV – detection, end – capped HILIC – diol column, flow rate = 0.8 ml/min., mobile phase: acetonitrile : water = 90 : 10 v/v [18]; 4) gradient RP – HPLC with C_{18} column with: a) UV – detection: λVit. C = 265 nm; λVit. B_6 = 324 nm [19]; b) fluorescence detection [20]; c) UV – MS/MS detection: on Zorbax C_{18} column (150 mm/4.6 mm/2.5 µm/40°C), flow rate = 1.5 ml/min., gradient mobile phase: ammonium acetate 10 mM (pH = 4.5) : methanol v/v, for 35 min.; λVit. C = 260 nm, λVit. B_6 = 292 nm 6.

HPLC is the preferred technique for analysis [21] in parenteral nutrition admixtures [6], infant nutrition products [20, 22]; food [23] – tarhana [21], milk [22], almonds – on Inertsil ODS – 3 column (250 mm/4.6 mm i. d., 5.0 µm), mobile phase: 0.05 mol/l KH_2PO_4 (pH = 6.0) : methanol = 70 : 30 v/v, UV – detection at λ = 265 nm 24.

The aim of current study is to develop isocratic HPLC method with UV – detection for analysis of Vitamin C and Vitamin B_6 in food additives and drugs – tablets.

MATERIALS

I) Reference standards (RS): Vitamin C, Vitamin B_6.

II) Reagents with analytical grade quality: acetonitrile, distilled water.

METHODS

HPLC

1) Chromatographic system and conditions

a) Chromatographic system

Liquid chromatograph Shimadzu (Japan) (LC – 10 Advp), equipped with: analytical column RP – 18 ODS, column oven (CTO – 10 Asvp Shimadzu); isocratic pump (LC – 10 A); (UV – VIS – detector at fixed wavelength (SPD – 10 Avvp); 20 µl injector loop.

b) Chromatographic conditions

Stationary phase: column RP – 18 ODS (250 mm/4.6 mm i.d./5 µm); mobile phase: acetonitrile : distilled water = 60 : 40 v/v; flow rate: 1.0 ml/min.; column temperature: 40 ^oC; UV – detection at λ = 254 nm; volume for injection – 20 µl. Before using mobile phase was filtered throught membrane filter with pore size 0.45 µm.

2) Accuracy and precision (repeatability)

Three (3) equal homogenous model mixtures were prepared from all respective supplements in tablets, by adding of RS Vitamin C, equivalent to 36.5 % (200 mg), 100 % (550 mg), 150 % (825 mg) of theoretical concentration. An average weights of model mixtures were: 3.657 g (C_{200}), 4.007 g (C_{550}), 4.282 g (C_{825}). From each model mixture were prepared 3 samples, by dissolving an accurately weighed quantity, containing RS Vitamin C: 200 mg, 550 mg, 825 mg to 10.0 ml with mobile phase. Model mixture with average weight 3.48 g was prepared by adding all respective supplements in tablets and RS Vitamin B_6, equivalent to 100 % (19 mg) of theoretical concentration. From model mixture were prepared 6 samples by dissolving an accurately weighed quantity, containing 19 mg of RS Vitamin B_6 to 50.0 ml with mobile phase. Samples were filtered and 10.0 ml of each solution were diluted to 25.0 ml with mobile phase. All solutions were filtered and chromatograms were recorded.

3) Linearity

An accurately weighed quantity: 502 mg, 810 mg, 905 mg, 981 mg, 1005 mg of RS Vitamin C was dissolved in mobile phase to 10.0 ml to obtain solutions with concentration respectively: $5.02.10^{-2}$ g/ml; $8.1.10^{-2}$ g/ml; $9.05.10^{-2}$ g/ml; $1.005.10^{-1}$ g/ml. An accurately weighed content: 125 mg, 18.75 mg, 6.25 mg, 0.625 mg, 0.125 mg of RS of Vitamin B_6 was dissolved in mobile phase to 50.0 ml and 10.0 ml of each solution were diluted to 25.0 ml with mobile phase to obtain solutions with concentration correspondingly: 1.10^{-3} g/ml; $1.5.10^{-4}$ g/ml; 5.10^{-5} g/ml; 5.10^{-6} g/ml; 1.10^{-6} g/ml.

RESULTS AND DISCUSSION

Analytical parameters selectivity, accuracy, precision and linearity are studied for the development of the validation procedure for HPLC method.

Selectivity

The "placebo" solution with all labeled in tablets supplements (magnesium stearate, silicium oxide), without the active ingredients were prepared in the same manner, like the reference standard. The fact, that on chromatogram with "placebo"solution didn't exist peaks with t_R, corresponded to t_R of the respective reference standard: t_RVitamin C = 2.30 min, t_RVitamin B_6 = 7.35 min., proves the selectivity of the applied HPLC method.

Accuracy and precision (repeatability)

On Table 1 for model mixtures with Vitamin C are summarized the data for: 1) added RS Vitamin C: C_{200} (36.5 %), C_{550} (100 %), C_{825} (150 %) of labeled content in tablets; 2) weighed quantity of model mixtures: WVit.C: WC_{200}, WC_{550}, WC_{825}; 3) area under the curve: AUC C_{200}, AUC C_{550}, AUC C_{825}; 4) Chauvenet's criterion (U): U AUC C_{200}, U AUC C_{550}, U AUC C_{825}.

Table 1: Vitamin C – AUC and Chauvenet's criterion for AUC

N:	C_{200} [mg]	Weighed C_{200} [g]	C_{550} [mg]	Weighed C_{550} [g]	C_{825} [mg]	Weighed C_{825} [g]
1.	199	3.6387	548	3.9924	825	4.282
2.	200	3.657	550	4.007	826	4.2872
3.	201	3.6753	552	4.0361	826.1	4.2877
			AUC and Chauvenet's criterion for AUC (U AUC)			
N:	AUC C_{200}	U AUC C_{200}	AUC C_{550}	U AUC C_{550}	AUC C_{825}	U AUC C_{825}
1.	35842	1.031	140662	1.013	223252	1.15
2.	36682	0.065	141472	0.026	224002	0.51
3.	37372	0.966	142222	0.987	224062	0.64
$\overline{X}$	36632		141452		223772	
SD	766.22		780.19		451.33	
RSD [%]	2.09		0.55		0.2	

For model mixtures with Vitamin B_6 on Table 2 are pointed out the data for: 1) 100 % added RS Vitamin B_6; 2) weighed quantity of model mixtures: WB_6; 3) area under the curve: AUC B_6; 4) Chauvenet's criterion (U): U AUC Vit. B_6.

Table 2: Vitamin B_6 – AUC and Chauvenet's criterion for AUC

N:	B_6 [mg]	W B_6 [g]	AUC B_6	U AUC B_6
1.	18.8	3.4434	3156831	1.25
2.	18.9	3.4617	3174431	0.896
3.	19.0	3.4800	3206431	0.252
4.	19.1	3.4983	3238431	0.392
5.	19.14	3.5056	3246431	0.553
6.	19.3	3.5349	3291231	1.454
$\overline{X}$			3218964.33	
SD			49712.56	
RSD [%]			1.54	

For all of the obtained by the applied HPLC method data for peak area of vitamins in every sample is necessary to estimate U, because when U for one value is higher than the relevant standard criterion (USt), the result must be removed as unexpected. The relations: U AUC C_{200} < 1.68; U AUC C_{550} < 1.68; U AUC C_{825} < 1.68 U (Table 1.) and AUC Vit. B_6 < 1.73 (Table 2.) show, that all experimental results for peak area of vitamins are lower, than standard requirements: Umax = 1.68 (N = 3), Umax = 1.73 (N = 6) and it isn't necessary to remove data for AUC and for the obtained content.

The content of vitamins is obtained by the method of calibration curve. For model mixtures with Vitamin C on Table 3 are indicated: N – number of the individual measurements (1 ÷ 3); [C] – obtained quantity of Vitamin C: [C_{200}], [C_{550}], [C_{825}]; UC – Chauvenet's criterion for Vitamin C: U[C_{200}], U[C_{550}], U[C_{825}]; R (%) – degree of recovery: R[C_{200}], R[C_{550}], R[C_{825}]; $\overline{X}$ – arithmetical mean; standard deviation (SD) and relative standard deviation (RSD) (%); S $\overline{X}$ – mean quadratic error; P – confidence possibility (%); t – coefficient of Student; $\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$ – confidence interval (CI); E – relative error.

Table 3: Accuracy and precision for Vitamin C – estimation by method of calibration curve

N:	[C_{200}] [mg]	R[C_{200}] [%]	U[C_{200}]	[C_{550}] [mg]	R[C_{550}] [%]	U[C_{550}]	[C_{825}] [mg]	R[C_{825}] [%]	U[C_{825}]
1.	198.4	99.7	1.03	547.8	99.96	1.01	823.1	99.77	1.15
2.	201.2	100.6	0.07	550.5	100.09	0.03	825.6	99.95	0.51
3.	203.5	101.24	0.97	553.0	100.18	0.99	825.8	99.96	0.65
$\overline{X}$ ± SD	201.03 ±2.55			550.43 ±2.6			824.83 ±1.5		
$\overline{R}$ [%] ± RSD [%]		100.51 ±0.77			100.08 ±0.11			99.89 ±0.11	
SD	2.55	0.77		2.6	0.11		1.5	0.11	
RSD [%]	1.27	0.77		0.47	0.11		0.18	0.11	
S $\overline{X}$	1.47	0.45		1.5	0.06		0.87	0.06	
P [%]	90.0	90.0		90.0	90.0		90.0	90.0	
t	2.92	2.92		2.92	2.92		2.92	2.92	
t.S $\overline{X}$	4.29	1.31		4.38	0.18		2.54	0.18	
$\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$	196.74 ÷205.32	99.2 ÷101.82		546.05 ÷ 554.81	99.9 ÷ 100.26		822.29 ÷827.37	99.71 ÷100.07	
E [%]	0.73	0.45		0.27	0.06		0.11	0.06	

For model mixtures with Vitamin B_6 on Table 4 are summarized: N – number of the individual measurements (1 ÷ 6); [C] – obtained quantity of Vitamin B_6: [B_6]; UC – Chauvenet's criterion for Vitamin B_6: U[B_6]; R (%) – degree of recovery: R[B_6]; $\overline{X}$ – arithmetical mean; standard deviation (SD) and relative standard deviation (RSD) (%); S $\overline{X}$ – mean quadratic error; P – confidence possibility (%); t – coefficient of Student; $\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$ – confidence interval (CI); E – relative error.

The Chauvenet's criterion U is estimated for all of the obtained by the applied HPLC method data for the content of vitamins. From the relations: U[C_{200}] < 1.68; U[C_{550}] < 1.68; U[C_{825}] < 1.68 (Table 3.) and U[B_6] < 1.68 (Table 4.) it is obvious, that all experimental results for quantity of vitamins are lower, than standard requirements: Umax = 1.68 (N = 3), Umax = 1.73 (N = 6) and it isn't necessary to remove data for the obtained content.

For the assessment of accuracy and precision is calculated the sample standard deviation (SD), by the applying of the Bessel's correction, in which the denominator N – 1 (degrees of freedom) is used instead of N and in this case (S $\overline{X}$)^2 is an unbiased estimator for (SD)^2.

Analytical patameter accuracy is presented by the degree of recovery R (%) and RSD [25]. All results for R, suit relevant confidence inteval : 1) for Vitamin C at P = 90.0 % (t = 2.92, N = 3): R [C_{200}]: 99.2 ÷ 101.82 (RSD = 0.77); R [C_{550}]: 99.9 ÷ 100.26 (RSD = 0.11); R [C_{825}]: 99.71 ÷ 100.07 (RSD = 0.11); 2) for Vitamin B_6 at P = 99.0 % (t = 4.03, N = 6): R [B_6]: 99.33 ÷ 101.59 (RSD = 0.69). For all samples for two vitamins the values of RSD are lower than 0.77 and the relative error is lower than 0.45 %.

Analytical parameter precision is estimated by the uncertainty of the result, determined by SD, RSD and $\overline{X}$ ± t.S $\overline{X}$.

From the assessment of precision [25] it is obvious, that content of vitamins in model mixtures correspond to the relevant confidence interval: 1) for Vitamin C at P = 90.0 % (t = 2.92, N = 3): [C_{200}]: 196.74 ÷ 205.32 (SD = 2.55, RSD = 1.27); [C_{550}]: 546.05 ÷ 554.81 (SD = 2.6, RSD = 0.47); [C_{825}]: 822.29 ÷ 827.37 (SD = 1.5, RSD = 0.18); 2) for Vitamin B_6 at P = 99.0 % (t = 4.03, N = 6): R [B_6]: 18.61 ÷ 19.65 (SD = 0.31, RSD = 1.62). For all samples both for two vitamins are observed the relations: SD < 2.6; RSD < 1.62; E < 0.73 %.

Linearity

The obtained regression equations, showing the proportional accordance AUC = f (C) are: $y = 3.10^6.x - 23678$, $R^2 = 0.9945$ (Vit. C); $y = 2.10^{10}.x + 158431$, $R^2 = 0.9953$ (Vit. B_6). Calibration curves are presented on Fig. 1. (Vitamin C) and Fig. 2. (Vitamin B_6).

Table 4: Accuracy and precision for Vitamin B_6 – estimation by method of calibration curve

N:	[B_6] [mg]	R [B_6] [%]	U [B_6]
1.	18.74	99.68	1.258
2.	18.85	99.74	0.903
3.	19.05	100.26	0.258
4.	19.25	100.79	0.387
5.	19.30	100.84	0.548
6.	19.58	101.45	1.452
$\overline{X}$ ± SD	19.13 ± 0.31		
$\overline{R}$ [%] ± RSD [%]		100.46 ± 0.69	
SD	0.31	0.69	
RSD [%]	1.62	0.69	
S $\overline{X}$	0.13	0.28	
P [%]	99.0	99.0	
t	4.03	4.03	
t.S $\overline{X}$	0.52	1.13	
$\overline{X}$ – t.S $\overline{X}$ ÷ $\overline{X}$ + t.S $\overline{X}$	18.61 ÷ 19.65	99.33 ÷ 101.59	
E [%]	0.68	0.28	

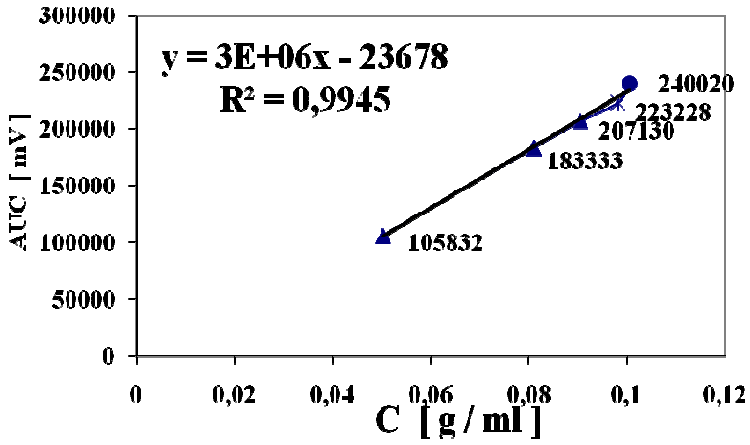

Fig. 1: Linearity for Vitamin C

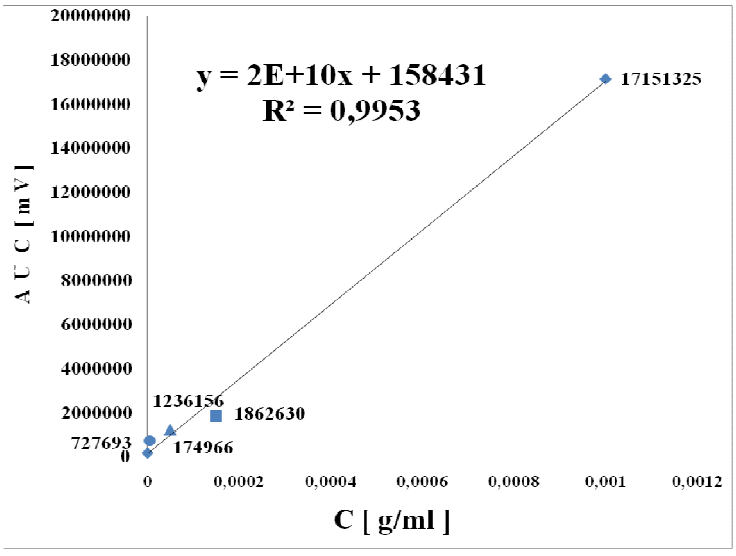

Fig. 2: Linearity for Vitamin B_6

CONCLUSION

The validated HPLC method is appropriate for quality control of content of Vitamin C and Vitamin B_6 in food supplements and drugs.

All data for $\overline{R}$ [%] ± RSD (%) suit standard requirements: Vitamin C: C_{200}: 100.51 ± 0.77; C_{550}: 100.08 ± 0.11; C_{825}: 99.98 ± 0.22; Vitamin B_6: 100.46 ± 0.69. The obtained quantities correspond to the relevant confidence interval. The validated HPLC method is appropriate for quality control of content of Vitamin C and Vitamin B_6 in food supplements and drugs.

REFERENCES

1. Acharya S, Sahu AR, Satyajit, Mohanta SR. Free radical scavenging activity of Thalamus of Nymphacea stallata willd. International Journal of Pharmacy and Pharmaceutical Sciences 2010;2(4):61-63.
2. Madhuri K, Vani K, Rabbani S, Alshatwi AA. Role of Ascorbic acid as an antioxidant in gastric cancer patients in South indian population. International Journal of Pharmacy and Pharmaceutical Sciences 2011;3(4):179-81.
3. Kaminski M, Boal R. An effect of ascorbic acid on delayed - onset muscle soreness. Pain 1992;50:317-21.
4. Groff JL, Gropper SS, Hunt SM. Advanced Nutrition and Human Metabolism. Minneapolis, West Publishing Company 1995;222-37.
5. Angley M, Semple S, Hewton C, Paterson F, McKinnon R. Children and autism - part 2 - management with complementary medicines and dietary interventions. Aust Fam Physician 2007;36(10):827-30.
6. Vazquez R, Le Hoang M - D, Martin J, Yahia YA, Graffard H, Guyon F et al. Simultaneous quantification of water-soluble and fat-soluble vitamins in parenteral nutrition admixtures by HPLC-UV-MS/MS. European Journal of Hospital Pharmacy Science 2009; 15(2):28-35.
7. Bisp MR, Bor MV, Heinsvig E - M, Kall MA, Nexø E. Determination of Vitamin B_6 Vitamers and Pyridoxic Acid in Plasma: Development and Evaluation of a High- Performance Liquid Chromatographic Assay. Analytical Biochemistry 2002;305(1):82-9.
8. Marszałł ML, Lebiedzińska A, Czarnowski W, Makarowski R, Kłos M, Szefer P. Application of the high - performance liquid chromatography method with coulometric detection for determination of vitamin B_6 in human plasma and serum. Journal of Chromatography B 2009;877(27):3151-58.
9. Gatti R, Gioia MG. Liquid chromatographic determination with fluorescence detection of B_6 vitamers and riboflavin in milk and pharmaceuticals. Analytica Chimica Acta 2005;538(1-2):135-41.
10. Kall MA. Determination of total vitamin B_6 in foods by isocratic HPLC: a comparison with microbiological analysis. Food Chemistry 2003;82(2):315-27.
11. Esteve - Romero J, Capella - Peiró M - E, Monferrer - Pons L, Gil – Agustí M. Micellar liquid chromatography in clinical chemistry: application to the monitorization of B_6 vitamins. Clinica Chimica Acta 2004;348(1-2):69-77.
12. Chauhan MS, Dakshinamurti K. Fluorometric assay of B_6 vitamers in biological material. Clinica Chimica Acta 1981;109(2):159-67.
13. Rybak ME, Pfeiffer CM. A simplified protein precipitation and filtration procedure for determining serum vitamin B_6 by high - performance liquid chromatography. Analytical Biochemistry 2009;388(1):175-77.
14. Ndaw S, Bergaentzlé M, Aoudé - Werner D, Hasselmann C. Extraction procedures for the liquid chromatographic determination of thiamin, riboflavin and vitamin B_6 in foodstuffs. Food Chemistry 2000;71(1):129-38.
15. Lebiedzińska A, Marszałł ML, Kuta J, Szefer P. Reversed - phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B_1 (thiamine), B_6 (pyridoxamine, pyridoxal and pyridoxine) and B_{12} in animal and plant foods. Journal of Chromatography A 2007;1173(1-2):71-80.
16. Monakhova YB, Mushtakova SP, Kolesnikova SS. Determination of Vitamins in Mixtures of Various Composition by Spectrophotometry with Self - Modeling Curve Resolution. Journal of Analytical Chemistry 2010;65(6):588-95.
17. Ghorbani AR, Momenbeik F, Khorasanian JH, Amini MK. Simultaneous micellar liquid chromatographic analysis of seven water-soluble vitamins: optimization using super-modified simplex. Anal Bioanal Chem 2004;379(3):439-44.
18. Karatapanis AE, Fiamegos YC, Stalikas CD. HILIC separation and quantitation of water-soluble vitamins using diol column. Journal of Separation Science 2009;32(7):909-17.
19. Li HB, Chen F. Simultaneous determination of twelve water- and fat - soluble vitamins by high-performance liquid chromatography with diode array detection. Chromatographia 2001;54:270-73.
20. Zafra - Gómes A, Garballo A, Morales JC, García - Ayuso LE. Simultaneous Determination of Eight Water – Soluble Vitamins in Supplemented Foods by Liquid Chromatography. J Agric Food Chem 2006;54(13):4531-36.
21. Ekinci R, Kadakal Ç. Determination of seven water - soluble vitamins in tarhana, a traditional turkish cereal food, by high-performance liquid chromatography. Acta Chtomatographica 2005;15:289-297.
22. Konings EJ. Water - soluble vitamins. JAOAC Int 2006;89(1):285-88.
23. Engel LA, Stefanovits - Bányai É, Fodor P. Simultaneous determination of water soluble vitamins in fortified food products. Acta Alimentaria 2010;39(1):48-58.
24. Shen Y, Zhang P, Kong X, Guo C, Wang J. Simultaneous determination of water - soluble vitamins C, B_1, B_2 and B_6 in almonds by high performance liquid chromatography. Se Pu 2005;23(5):538-41.
25. Dimov NP. Method validation of a chromatographic method. Industrial Pharmacy – science and technology series. Volume 1: Chromatography in Pharmaceutical Analysis. First edition Chemical Pharmaceutical Research Institute (NIHFI), Sofia, Bulgaria, 1999;205-29.