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Original Research Article

Comprehensive Analysis of a Multi-Component Injection Solution Containing Folic Acid, Tryptophan, Niacin, and Thiamine by HPLC

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Abstract: We were tasked with testing a multicomponent aqueous solution for injection used in veterinary medicine. The composition of the solution included the following components: folic acid, tryptophan, nicotinic acid, thiamine hydrochloride, ascorbic acid, sodium acetate, and benzyl alcohol. The composition indicates that the drug is intended to stimulate the vital activity of the body. It was required to determine the activity and stability of four components: folic acid, tryptophan, nicotinic acid, and thiamine hydrochloride. The complexity of the simultaneous determination of these components was that they differ greatly in their acid-base properties and solubility in water. We managed to solve the problem using an Agilent HPLC/DAD/MS instrument and selected the parameters to determine all four components together reliably and accurately or each separately by one method.

Keywords: Folic acid, tryptophan, nicotinic acid, thiamine hydrochloride, HPLC/DAD.

Abbreviations:
DAD -Diode Array Detector
FDA - Food and drug administration
ICH- International Conference on Harmonization
LOD – limit of detection
MSD - mass selective detector
MP-mobile phase
MW- Molecular weight
N – number of theoretical plates
RP- reversed-phase
RSD – relative standard deviation
SQ - single quadrupole
T – tailing factor
UV-VIS - Ultraviolet-Visible
Sol.- solubility in water.

INTRODUCTION

Folic acid is a B vitamin. It helps the body make healthy new cells. Everyone needs folic acid (Medical Encyclopedia). Folic acid is soluble in 1 M NaOH (50 mg/mL), methanol (slightly), and alkaline solutions. The free acid is only slightly soluble in water - 0.0016 mg/mL (PubChem), 0.01g/L at 0°C (Sigma ProductInformation). K. Kida1 used HPLC with a UV detector and methanol-water mixture as the mobile phase to analyze folic acid in beverages (Kida1 et al., 2018). Folic acid in Fortified Food Products was analyzed with HPLC with the electrochemical detector (Lebiedzińska et al., 2008). Folic acid in beetroots was analyzed with HPLC/DAD, phosphate buffer -acetonitrile mixture was used as the mobile phase (Jastrebova et al, 2003). To analyze folic acid in tablets the HPLC/UV was used, and phosphate buffer – sodium perchlorate - methanol mixture was used as the mobile phase (FOLIC ACID TABLETS (USP), 2022).

Tryptophan

"Tryptophan is an amino acid needed for normal growth in infants and the production and maintenance of the body's proteins, muscles, enzymes, and neurotransmitters" (Medical Encyclopedia) Tryptophan is soluble in water: 11.4g/L 25°C, soluble in hot alcohol, and alkali hydroxides (PubChem). A review article by I. Sadok presents a list of HPLC methods for the analysis of tryptophan using C-18 RP columns and mobile phases of various compositions, most of which are buffer mixtures with methanol or acetonitrile (Sadok et al., 2017). In the works of Vitalini and Willi, tryptophan and its metabolites are analyzed by HPLC/MS/MS (Vitalini et al., 2020; Whiley et al., 2019). In this case, the mobile phase consists of volatile components. To analyze tryptophan in yogurt, Ritota and Manzi used UHPLC with a fluorescent detector (Ritota, Manzi, 2020). Niacin is a type of B vitamin. Niacin helps the digestive system, skin, and nerves to function (Medical Encyclopedia). Niacin is soluble in water (18 g/L) at 20°C, soluble in alcohol, and insoluble in most lipid solvents (PubChem). Aura Industries offers a protocol for the determination of nicotinic acid and nicotinamide by HPLC with a fluorescent detector and post-column chemical derivatization (Aura Industries). Nicotinic acid and nicotinamide adenine nucleotide were determined by HPLC with UV detector and LC/MS/MS methods (Yoshino, Imai, 2013). The joint determination of nicotinamide and thiamine in foods is described in (Anyakora et al, 2008). Nicotine amide in dietary supplements was analyzed by thin-layer chromatography followed by HPLC/MS analysis of the extract (Neamţu et al., 2020).

Thiamine (thiamine hydrochloride)

"Thiamine (vitamin B1) is used as a dietary supplement when the amount of thiamine in the diet is not enough" (Medical Encyclopedia). Thiamine hydrochloride is soluble in water (50 mg/ml), in ethanol (1 g/100 ml), in absolute ethanol (1 g/315 ml), insoluble in ether, benzene, hexane, and chloroform. It is stable at acidic pH but is unstable in alkaline solutions (PubChem). The thesis of Tang is devoted to the HPLC analysis of various water-soluble vitamins in pharmaceutical preparations including thiamine. The paper discusses the advantages and disadvantages of various RP columns and the composition of the mobile phase (Trang, 2013). The work of Sánchez-Machado is devoted to the simultaneous determination of thiamine and riboflavin in seaweeds by HPLC. The authors used a fluorescent detector; the mobile phase was a mixture of acetate buffer and methanol (Sánchez-Machado et al, 2004). Analysis of vitamin B1 using pre-column and post-column derivatization is described in the works of H. Ihara and M. Ofitserova, respectively (Ihara et al., 2001; Ofitserova, S. Nerkar, 2013). In both studies, an HPLC instrument with a fluorescent detector was used, and the mobile phase was based on phosphate buffer and acetonitrile. The molecular structures of the test substances and their important characteristics are shown in Figure 1.

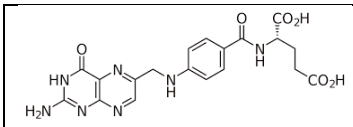	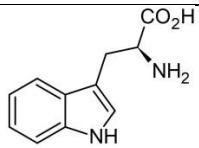	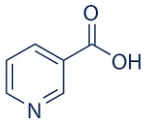	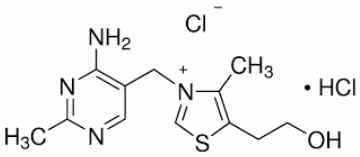
Folic acid. MW: 441.4g·mol^{-1}, pKa1=8.26 (25°C), Sol.= 0.0016 g/L at 25 °C (PubChem), 0.01g/L at 0°C (Sigma ProductInfdormation)	Tryptophan. MW: 204.2g·mol^{-1}, pKa=2.46 (25°C), Sol.= 11.4 g/L at 25 °C (PubChem)	Niacin. MW: 123.1g·mol^{-1}, pKa2=4.75 (25°C), Sol.= 18 g/L at 20 °C (PubChem)	Thiamine hydrochloride. MW: 337.3g·mol^{-1}, *pKa* = 4.8 (20°C), Sol.=1g in 1ml (PubChem)

Figure 1: Molecular structures and characteristics of the tested substances

All four tested components differ significantly in their acidic properties and solubility, which created difficulty in choosing a universal test condition. We found the universal test condition experimentally.

MATERIAL AND METHODS

Chemicals: Water HPLC grade purchased from Agilent. HPLC grade solvents were used. Reference standards of tryptophan, thiamine hydrochloride, folic acid, niacin, ascorbic acid, sodium ascorbate, benzyl alcohol, KH_2PO_4, and NaOH were from Sigma.

Mobile phase (MP): 0.01M KH_2PO_4;, Methanol 8% vol, NaOH 1N to pH=7.2.
Diluent 1: 0.5M $NaHCO_3$/Methanol 75/25 vol/vol., pH=8.5. Diluent 2: – MP.

Samples: The solution simulating a diluted injectable preparation had the following composition: 0.034 mM folic acid, 0.147mM tryptophan, 4.447mM thiamine hydrochloride, 3.249mM niacin, 0.057mM sodium ascorbate, 1.849mM benzyl alcohol. All components except folic acid were dissolved directly in 0.1M phosphate buffer containing 8% methanol

(pH=7.2). 0.15 g of folic acid was first dissolved in 34 ml of 0.1N NaOH, then the resulting solution was mixed with the total solution.
All the solutions were filtered through the 0.45µm cellulose acetate membrane filter.

Instrument: Agilent HPLC/DAD/MS instrument consists of the following components: Diode Array Detector (DAD). The following wavelengths have been established: 280 nm (folic acid), 210 nm (niacin and tryptophan), and 232 nm (thiamine); Reversed-phase (RP) Column Poroshell 120 EC-C18 250x4.6mm with particles size 2.7 µm, and guard precolumn; Quaternary pump with the flow: 0.7 ml/min, and high-pressure limit of 600 bar. For this analysis was chosen the isocratic elution was. MSD was not in use because the chosen mobile phase has non-volatile components (Agilent Single Quadrupole LC/MS instrument, 2019).

Qualitative analysis of the components was carried out using UV spectra specific for each of the components (Fig 3). Based on these spectra, 3 working wavelengths were chosen, namely 210, 232, and 280 nm.

Quantitative analysis was done using a calibration curve built for each of the components.

The system's suitability has been validated according to the Center for Drug Evaluation and Research (CDER, 1994) and the System Suitability Assessment Guidelines (Evaluating System Suitability CE, GC, LC, and A/D ChemStation, 2019). Parameters were peak area, retention time, number of theoretical plates (N), and tailing factor (T).

Calibration curve and coefficient of correlation: The concentration range of the calibration curve was chosen so that the expected concentration of the component was near the middle. In this range, the calibration curve should be strictly linear ($r \geq 0.999$).

The precision/accuracy of the method was determined by the RSD value from the analysis of five samples of the same concentration under the same experimental conditions. The intraday and interday analysis was compared by RSD and recovery.

Limits of detection (LOD): LOD characterizes the sensitivity of a method; it is the minimum amount of a substance that can be measured by a given method, whereas the LOQ is the lowest concentration with acceptable linearity, accuracy, and precision. If the equation of the calibration curve is an equation of the first degree (straight line) then LOD is calculated by formula (1):

$$\mathbf{LOD = 3.3* \sigma/a} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where the (σ) is the residual standard deviation of the regression line, and (a) is the slope of the line (European Medicines Agency. ICH, 2006). LOQ is 3 times LOD.

A measure of repeatability is the RSD of the mean of five independent tests of the samples of the same concentration.

To prove the specificity of the method, the peak areas of the component in the drug sample and the standard solution of the same concentration were compared. At the same time, the retention time of the component in both chromatograms was almost the same (RSD<1.2%). A minor discrepancy in the magnitude of the peak area indicated the specificity of the method for this component.

To demonstrate the robustness of the method flow rate, column temperature, and mobile phase composition were varied. The tailing factor (T) and a number of theoretical plates (N) were calculated. The results were compared with the acceptable limits.

Statistical analysis included calculating mean, standard deviation (S.D.), relative standard deviation (RSD), and correlation coefficient (r). Results $p < 0.05$ were considered statistically significant. The Least-squares regression analysis was used (FDA Guidance, 2015). In most cases, the calculation was performed automatically by the OpenLAB CDS program.

RESULTS AND DISCUSSION

System suitability (Table 1) The standard solution of each of the components was tested five times. The results were averaged, and the RSD was calculated automatically using the OpenLAB CDS software. The acceptable limit is in line with the recommendations (Dr. Deepak, 2013; Bose, 2014).

The chromatograms are presented in Figure 2. The extracted UV- spectra are presented in Figure 3.

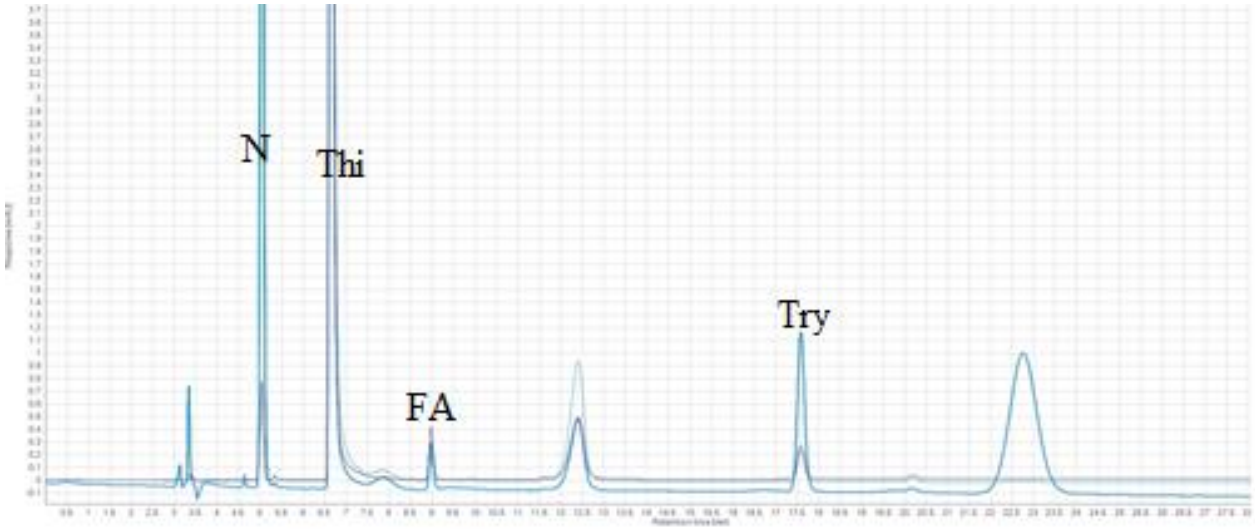

Figure 2: Representative chromatogram. Folic acid (FA), Tryptophan (Try), Niacin (N), Thiamine (Thi)

As can be seen in Figure 2, all four tested components are well separated. The slowest of the four compounds tested, tryptophan has a retention time of 17.4 minutes (Table 1), but for the column to be completely purified, we set a run time of 30 minutes. UV spectra of all four components are represented in Figure 3. Each of the spectra has its specific maximums and may be used for qualitative analysis. Folic acid has maximums of 194 and 280 nm, tryptophan has a maximum of 218 nm, niacin has maximums of 210 and 262 nm, and thiamine has maximums of 232 and 266 nm.

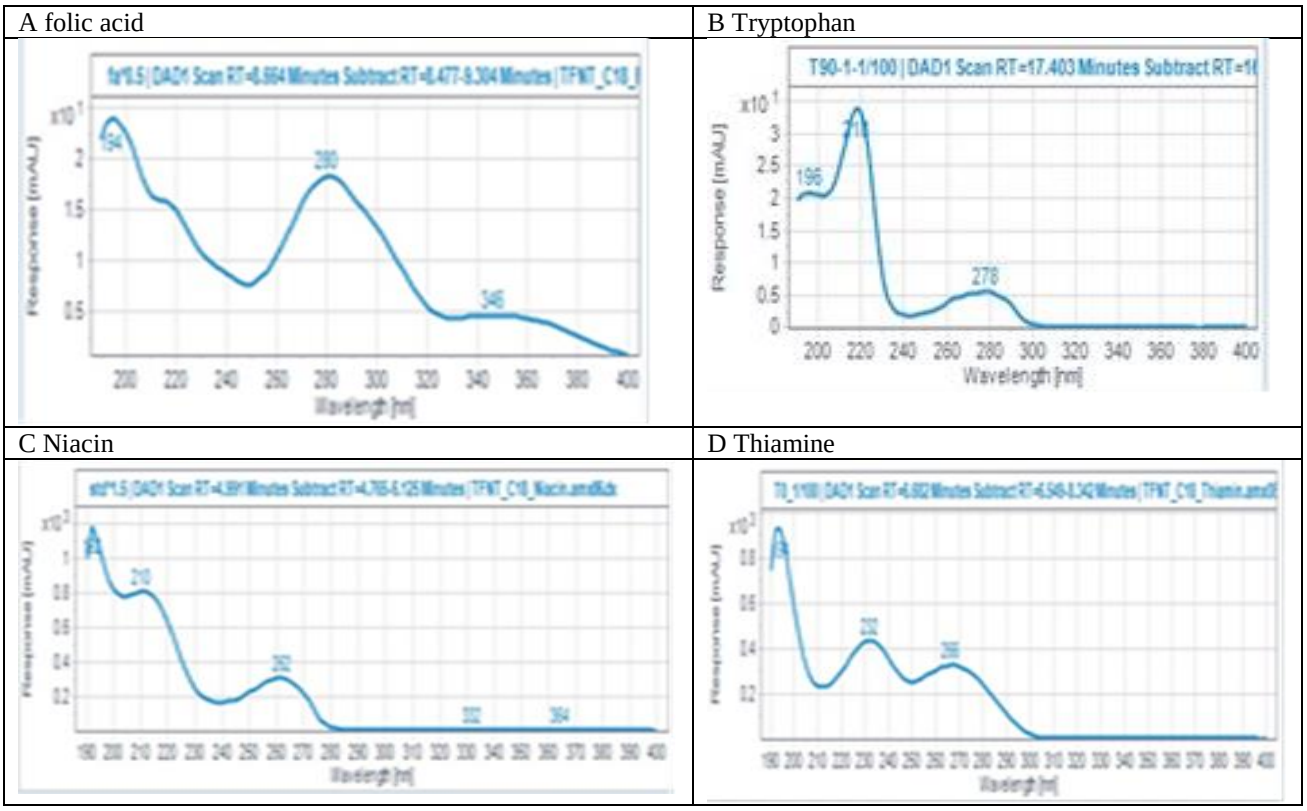

Figure 3: A, B, C, D. UV-Spectra. A Folic acid, B Tryptophan, C Niacin, D Thiamine

Linearity, Range, and Limit of Detection

The working range for each of the four components is set, it corresponds to the range of the linear section of the calibration curve (Table 2). The linearity is more than satisfactory, the correlation coefficient is almost equal to one. The limit of detection is more than satisfactory for testing pharmaceutical products (Table 2). We state the LOD in micrograms, which is complete information on the sensitivity of the method, as opposed to indicating the minimum

concentration, as some authors do, since indicating the concentration without specifying the injection volume creates uncertainty. The typical injection volume of our device is between 0.1 and 20 microliters.

Table 2: Linearity, Range, LOD. "Y"- the peak area; "Y calc." – the calculated peak area; "ΔY" – the residues; "a"- the slope of the regression line; "b"- the intercept; "r" – the correlation coefficient; "S.D. ΔY" – the residual standard deviation of the regression line (σ), (p <0.05)

Folic acid (μg)	Mean Y (n=5)	Y calc.	ΔY	Trypto phan (μg)	Mean Y (n=5)	Y calc.	ΔY	Niacin (μg)	Mean Y (n=5)	Y calc.	ΔY	Thiamine (μg)	Mean Y (n=5)	Y calc.	ΔY
0.005	21	21	0.05	**0.010**	101	102	-0.63	**0.107**	565	570	-4.67	**0.263**	661	666	-4.50
0.01	38	39	-0.93	**0.015**	152	151	1.50	**0.161**	827	829	-1.84	**0.525**	1324	1322	2.94
0.015	58	57	0.87	**0.035**	345	345	-0.10	**0.375**	1862	1864	-2.19	**1.050**	2629	2633	-3.44
0.025	93	93	0.18	**0.040**	392	393	-1.31	**0.428**	2137	2123	14.35	**1.575**	3952	3944	7.95
0.05	183	183	-0.17	**0.076**	734	733	0.52	**0.803**	3929	3935	-5.64	**3.150**	7874	7877	-2.94
a	**3607.36**			**a**	**9613.56**			**a**	**4838.14**			**a**	**2497.41**		
b	**3.03**			**b**	**4.66**			**b**	**52.13**			**b**	**10.40**		
r	**0.9999**			r	**1.0000**			r	**1.0000**			r	**1.0000**		
S.D. ΔY	0.65			S.D. ΔY	1.08			S.D. ΔY	8.18			S.D. ΔY	5.31		
LOD (μg)	**0.00059**			**LOD**	**0.00037**			**LOD**	**0.00558**			**LOD**	**0.00701**		

Accuracy/recovery and precision (Table 3)

Samples containing three different concentrations of the component of interest were measured five times, and the mean value and the relative standard deviation were calculated. The recovery was determined based on the calibration curve. The data in Table 3 confirm the accuracy, reproducibility, and precision of the method. Interday analysis (check the next day) shows no significant degradation.

Table 3: Accuracy, Recovery, Repeatability

FA (μg)	Mean recovery (μg)	±S.D.	RSD (%)	Recovery (%)
0.01	0.010	0.000	1.00	100
0.015	0.015	0.000	0.00	103
0.025	0.025	0.000	0.00	100
0.015	0.0152	0.000	0.99	101
Tryptophan (μg)				
0.0152	0.015	0.000	1.00	100
0.0354	0.035	0.000	1.00	100
0.0404	0.040	0.000	1.00	100
0.0354	0.0353	0.000	1.00	100
Niacin (μg)				
0.3745	0.375	0.004	1.00	100
0.428	0.428	0.004	0.00	100
0.8025	0.802	0.008	0.00	100
0.428	0.428	0.004	1.00	100
Thiamine (μg)				
1.05	1.05	0.011	1.00	100
1.575	1.575	0.016	0.00	100
3.15	3.15	0.032	0.00	100
1.575	1.550	0.016	1.02	98
Recovery data presents an average value of five independent determinations (n=5). The highlighted line corresponds to the inter-day analysis.				

Selectivity (Specificity) assay

The results of the analysis of the standard solution and the test solution with the same concentration of the test component were compared. The presence of other ingredients does not affect the recovery of the tested component. The relative standard deviation of the compared peak areas does not exceed 1.5% (Table 4). Thus, the method is specific to each of the tested components.

Table 4: Specificity

Active component	µg per injection	Mean peak area. (Standard) (n=5)	Mean peak area (Drug) (n=5)	RSD (%)
Folic acid	0.0228	69	71	1.5≤2
Tryptophan	0.033	321	320	0.24≤2
Niacin	0.43	2108	2121	0.43≤2
Thiamine	1.44	35024	35724	1.4≤2
Two peaks are compared, one for the standard solution and the other for the diluted injection solution. The concentration of the test component in both solutions is the same.				

Robustness (Table 5)

As part of establishing the robustness of the method, the chromatographic parameters (T and N) of each of the four components were determined with a change in flow rate, column temperature, and composition of the mobile phase. These parameters changed insignificantly and were within acceptable limits. Thus, the method is robust.

Table 5: Robustness

Parameter	Folic acid (0.015µg)				Tryptophan (0.0354µg)				Niacin (0.428 µg)				Thiamine (0.525 µg)			
	T	RSD	N	RSD	T	RSD	N	RSD	T	RSD	N	RSD	T	RSD	N	RSD
Flow rate 0.70 mL/min	1.14	0.5	9E+05	1.8	1.097	0.5	1.E+06	1	1.383	0.5	6E+05	1.1	1.7	0.5	1.E+05	1.8
Flow rate 0.75 mL/min	1.13	1	9E+05	1.7	1.119	1	1.E+06	1	1.13	1	5E+05	1.3	1.7	0.9	1.E+05	1.8
Temperature 38°C	1.11	0.8	9E+05	1.5	1.075	2	9.E+05	1	1.11	0.8	7E+05	1.4	1.8	0.8	1.E+05	1.5
Temperature 40°C	1.14	0.5	9E+05	1.8	1.097	0.5	1.E+06	1	1.383	0.5	6E+05	1.8	1.7	0.5	1.E+05	1.6
Mobile phase composition																
Formic acid 0.1%	1.14	0.5	9E+05	1.8	1.097	0.5	1.E+06	1	1.383	0.5	6E+05	1.6	1.7	0.7	1.E+05	1.7
Formic acid 0.13%	1.15	0.7	9E+05	1.6	1.119	0.7	1.E+06	1	1.15	0.7	5E+05	1.6	1.7	0.7	1.E+05	1.6
T= Tailing factor (mean); N= number of theoretical plates (mean); RSD - relative standard deviation (%); n=5.																

As can be seen (Table 5 and Figure 2), the thiamine tail is within the acceptable limit but somewhat larger than that of the other components. Reducing the tail by diluting the sample more poorly fits this situation because the concentration of folic acid is 100 times lower, and at higher dilution, it is too low for good analysis. Decreasing the pH of the mobile phase from 7.2 to 4.0 reduces the tailing factor from 1.7 to 1.2, but the separation of the components deteriorated. So, we didn't change anything. In the future, we plan to reduce the analysis time using the available UHPLC instrument. For this purpose, we ordered a 1200 - 1300 psi RP column.

CONCLUSION

An HPLC/DAD method for the analysis of folic acid, tryptophan, niacin, and thiamine in a multi-component solution for injection has been developed and validated. The method makes it possible to analyze all four components simultaneously without derivation and special pre-treatment of the sample. The analysis was carried out at 3 wavelengths 210, 232, and 280nm. The method has high sensitivity, selectivity, specificity, and robustness. The method can be recommended for the analysis of all four components, any combination of them, or each of the components separately.

REFERENCES

1. Medical Encyclopedia. https://medlineplus.gov/ency/article/002332.htm.
2. PubChem https://pubchem.ncbi.nlm.nih.gov/.
3. Sigma ProductInformation. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/102/092/f8758pis.pdf.
4. Kida, K., Tomotake, M., Sasako, H., Matsuda, Y., Sasaki, N., & Yamamoto, N. (2018). Small amounts of ethanol attenuate folic acid stability in acidic beverages during storage, *Food Sci Nutr*, 6, 214–219. https://onlinelibrary.wiley.com/doi/pdf/10.1002/fsn3.549.

5. Lebiedzińska, M., Dbrowska, P., Szefer, P., & Marszałł, M. (2008). High-Performance Liquid Chromatography Method for the Determination of Folic Acid in Fortified Food Products, *Toxicology Mechanisms, and Methods*, 18(6), 463-467. https://www.tandfonline.com/doi/full/10.1080/15376510701623870.
6. Jastrebova, J., Witthoft, C., Grahn, A., Svensson, U., & Jagerstad, M. (2003). HPLC determination of folates in raw and processed beetroots, *Food Chemistry*, 80, 579–588 https://ucanr.edu/datastoreFiles/608-314.pdf.
7. FOLIC ACID TABLETS (USP). (2022). https://www.sigmaaldrich.com/technical-documents/articles/analytical-applications/hplc/dissolution-testing-folic-acid-tablets.html.
8. Sadok, I., Gamian, A., & Staniszewska, M. (2017). Chromatographic analysis of tryptophan metabolites. *J Sep Sci*, 40, 3020–3045. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jssc.201700184.
9. Vitalini, S., Dei Cas, M., Rubino, F., Vigentini, I., Foschino, R., Iriti, M., & Paroni, R. (2020). LC-MS/MS-Based Profiling of Tryptophan-Related Metabolites in Healthy Plant Foods, *Molecules*, 25, 311. Whiley, L., Nye, L., Grant, I., Andreas, N., Chappell, K., Sarafian, M., Misra, R., Plumb, R., Lewis, M., Nicholson, J., Holmes, E., Swann, J., & Wilson, I. (2019). Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry with Electrospray Ionization Quantification of Tryptophan Metabolites and Markers of Gut Health in Serum and Plasma—Application to Clinical and Epidemiology Cohorts, *Anal. Chem.* 91(8), 5207–5216. https://pubs.acs.org/doi/10.1021/acs.analchem.8b05884#.
10. Ritota, M., & Manzi, P. (2020). Rapid Determination of Total Tryptophan in Yoghurt by Ultra-High-Performance Liquid Chromatography with Fluorescence Detection Molecules, 25, 5025. https://www.mdpi.com/1420-3049/25/21/5025/pdf.
11. Aura Industries Inc., http://www.aura-inc.com/Aura%20Industries%20Niacin%20Application-1.pdf
12. Yoshino, J., & Imai, S. (2013). Accurate measurement of nicotinamide adenine dinucleotide (NAD^+) with high-performance liquid chromatography. *Methods Mol Biol*, 1077, 203–215. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3935825/.
13. Anyakora, C, Afolami, I., Ehianeta, T., & Onwumere, F. (2008). HPLC analysis of nicotinamide, pyridoxine, riboflavin, and thiamin in some selected food products in Nigeria. *African Journal of Pharmacy and Pharmacology*, 2(2), 029-036. https://www.researchgate.net/publication/228861153_HPLC_analysis_of_nicotinamide_pyridoxine_riboflavin_and_thiamin_in_some_selected_food_products_in_Nigeria.
14. Neamţu, T., Biţă, A., Scorei, I., Rău, G., Bejenaru, L., Bejenaru, C., Rogoveanu, O., Oancea, C., Radu, A., Pisoschi, C., Neamţu, J., & Mogoşanu, G. (2020). Simultaneous Quantitation of Nicotinamide Riboside and Nicotinamide in Dietary Supplements via HPTLC–UV with Confirmation by Online HPTLC–ESI–MS. *Acta Chromatographica*, 32(2), 128–133. https://akjournals.com/view/journals/1326/32/2/article-p128.xml.
15. Trang, H. (2013). Development of HPLC methods for the determination of water-soluble vitamins in pharmaceuticals and fortified food products. Theses, 8, Clemson University. https://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2745&context=all_theses.
16. Sánchez-Machado, I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada, P. (2004). Simultaneous Determination of Thiamine and Riboflavin in Edible Marine Seaweeds by High-Performance Liquid Chromatography, *Journal of Chromatographic Science*, 42, 117-120. https://academic.oup.com/chromsci/article-pdf/42/3/117/793866/42-3-117.pdf.
17. Ihara, H., Hashizume, N., Hirano, A., & Okada, M. (2001). A simplified HPLC method for thiamine and its phosphate esters in whole blood. *Journal of Analytical Bio-Science*, 24(3), 235-238. http://plaza.umin.ac.jp/j-jabs/24/24.235.pdf.
18. Ofitserova, M., & Nerkar, S. (2013). Analysis of Vitamin B1 in Foods and Dietary Supplements by HPLC with Post-Column Derivatization and Fluorescence Detection, *Pickering Laboratories Inc.* https://www.chromatographyonline.com/view/analysis-vitamin-b1-foods-and-dietary-supplements-hplc-post-column-derivatization-and-fluorescence-d.
19. Agilent Single Quadrupole LC/MS instrument. (2019). https://www.agilent.com/en/products/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/single-quadrupole-lc-ms/lc-msd.
20. CDER. (1994). Center for Drug Evaluation and Research, FDA. Reviewer Guidance, Validation of Chromatographic Methods; FDA, Rockville. https://www.fda.gov/media/75643/download.
21. Evaluating System Suitability CE, GC, LC and A/D ChemStation. (2019). Revisions: A.03.0x-A.08.0x https://www.agilent.com/cs/library/Support/Documents/a10424.pdf.
22. European Medicines Agency. ICH (2006) Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf.
23. FDA Guidance for Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry (2015). https://www.fda.gov/media/87801/download.
24. Deepak. (2013). How to calculate System Suitability in Chromatography https://lab-training.com/2013/02/27/how-to-calculate-system-suitability-in-chromatography/.
25. Bose, A. (2014). HPLC Calibration Process Parameters in Terms of System Suitability Test. https://pdfs.semanticscholar.org/c378/3cd6c88c294be0aecee3b77cfd1eb35789fc.pdf.