ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Validation of High-Performance Liquid Chromatography Method for Simultaneous Determination of Vitamin C and Folic Acid in Multivitamin Tablet.

Febri Annuryanti*, Tiara Vista Ramadhani, and Riesta Primaharinastiti.

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

ABSTRACT

Vitamin C and folic acid in multivitamin tablet are used to prevent and treat vitamin's deficiency. The solubility and stability differences of vitamin C and folic acid cause a problem for simultaneous determination. The objective of the research was to develop and validate a high-performance liquid chromatography method for simultaneous determination of vitamin C and folic acid in multivitamin tablet A Li-Chrospher C18 (150 x 4,6 mm; particle size 5 µm) column was used for the separation. A good selectivity of vitamin C and folic acid was obtained when 50 mM phosphate buffer pH 6.5 : methanol (90:10) used as mobile phase with flow rate of 1.5 mL/min. Detection was performed with diode array detector at 281nm. Linearity of vitamin C and folic acid was obtained with coefficient correlation (r) > 0.995. Accuracy of vitamin C and folic acid were 101.27 ± 0.15 % and 100.27 ± 0.59 %, respectively. The relative standard deviation of both analytes was ≤ 0.50.

Keywords: Vitamin C, folic acid, HPLC, simultaneous determination

Corresponding author:

ISSN: 0975-8585

INTRODUCTION

Multivitamin is one of pharmaceutical products that are used to prevent and treat vitamin's deficiency. Vitamins are essential for the nutritional balance and metabolic activity in living organism but are not naturally synthesized in the human body. Appropriate vitamin supplementation is needed to preserve the required level of vitamin [1].

Multivitamin products which contain vitamin C and folic acid are mostly in present in market. Vitamin C is used as antioxidant that can enhance other vitamins and minerals' absorption. Moreover, it is also contributed to synthesize collagen and intracellular matrices [2]. Vitamin C has characteristics as unstable in light, heat, and base solution [3]. Therefore, its preparation should be in acidic solution and protected from light and high temperature.

Folic acid, vitamin B complex's group, is insoluble in water and acidic solution but soluble in base solution [3]. It has a role on erythrocyte's formation and the essential substance on translating nitrogen base of DNA to RNA process [4]. The differences of solubility and stability of vitamin C and folic acid cause a problem for simultaneous analysis, especially in sample preparation. For the best result, folic acid is extracted in base solution, while vitamin C is more soluble and stable in acidic solution. Significant difference in amount of vitamin C and folic acid in multivitamin tablet, with ratio of 50:1, lead to homogeneity issue and make it more difficult to analyse simultaneously. Appropriate extraction procedure is important in order to get an optimum recovery [5] and it is eessential to develop selective, efficient, rapid, and reliable analytical method for simultaneous analysis of vitamin C and folic acid.

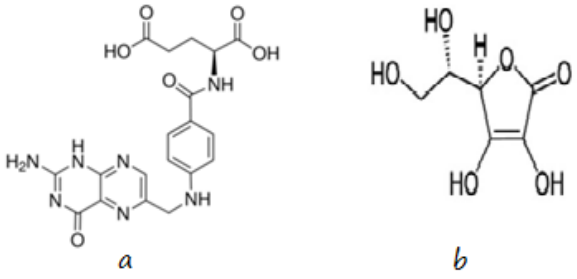

Figure 1. Structure of folic acid (a) and vitamin C (b)

Various methods including spectrophotometry, titration, high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), high performance thin layer chromatography (HPTLC), and voltametry have been used to determine vitamin C and folic acid in multivitamin products[3][5]. Reversed-phase HPLC, ion-pairing [6] and mixed-mode weak anion exchange stationary phase method [7] had been used for determination of vitamins with diode array / fluorescence / mass spectrometric detectors [3][8][9]. A HPLC is mostly used as analytical separation technique because of its ability for separating non-volatile species or thermally fragile ones. HPLC has the advantages in sensitivity and selectivity for analytical separation which is better than another separation method [10].

In this report, we develop and validate an HPLC method for simultaneous determination of vitamin C and folic acid.

MATERIALS and METHODS

Reagents and chemicals

Dihydrogen potassium phosphate, potassium hydroxide, and ascorbic acid (vitamin C) were purchased from Merck. Folic acid was purchased from Sigma Aldrich. HPLC-grade methanol and acetonitrile were purchased from Merck and used without further purification. Spray-dried lactose, avicel PH-102, and magnesium stearate were used as placebo. All solutions were filtered through 0.22 µm nylon membran filters (Whatman, GE Healthcare, Germany) prior to use.

Instrumentation

Ultra Fast Liquid Chromatograph (UFLC) LC-20AD series with diode array detector (DAD) SPD-M20A series and degasser DGU-20A$_{SR}$ series from Shimadzu Corp. (Kyoto, Japan). Evaluation and quantification were achieved on Lab Solution software.

EXPERIMENTAL

HPLC condition

An optimum HPLC condition was obtained by doing optimization of mobile phase and flow rate. The detection wavelength was also observed since the ratio concentration of vitamin C and folic acid was 1:50. Optimum HPLC condition was as follows: Li-Chrospher C18 (150 x 4,6 mm; particle size 5 µm) was used as column. 50 mM buffer phosphate pH 6.5-methanol (90:10) with flow rate of 1.5 ml/min was used as mobile phase. The injection volume was 20 µl and analysis was done at ambient temperature. Detection wavelength was set at 281 nm.

Validation procedure

Specificity

Mixed standard solution of 2000 mg/L vitamin C and 40 mg/L folic acid was used to HPLC analysis with the optimum condition. To evaluate the separation, the resolution (Rs) value should more than 1.5 which is showed a good separation of each analyte [11].

Linearity

Eight mixed standard solutions of vitamin C and folic acid were prepared for calibration curve [12] [13]. Linear equation was established by plotting peak areas versus concentration of each vitamin. It fulfils the requirement when coefficient correlation (r) is more than 0.999. If the value of r is less than 0.999 then Vxo and Xp value should be calculated. Vxo and Xp value should be less than 5% and the smallest concentration on callibration curve respectively [11].

Accuracy

Accuracy was done by using standard addition method. Cconcentrations of 80%, 100%, and 120% from true value were used to measure recoveries value of each analyte in mixed solutions. The acceptance criteria of recovery data is 98%-102% for drug preparation [11].

Precision

Method precision (repeatability and intermediate precision) were evaluated from six independent mixed solutions of 2000 mg/L vitamin C and 40 mg/L folic acid. Repeatability was evaluated on result from the same day and intermediate precision from another day. Precision was determined by relative standard deviation (% RSD) or coefficient variance (% CV). The acceptance criteria is less than 2% [14].

RESULTS AND DISCUSSION

Detection wavelength

Both vitamin C and folic acid were observed for their maximum wavelength. The maximum wavelength of vitamin C and folic acid were 266 and 281 nm, respectively. For this experiment, the maximum wavelength of folic acid (261 nm) was choosen as wavelength detection for both vitamin C and folic acid due to ratio concentration of both analytes in samples.

ISSN: 0975-8585

Optimization of HPLC condition

Mobile phase

Various solvents were used to find optimum mobile phase. Combination of (a) 0.01% TFA-methanol and (b) 0.1 M phosphate buffer pH 7.0 – methanol with different composition were analyzed. A good peak shape was obtained when 0.1 M phosphate buffer pH 7.0 – methanol (90:10) was used as mobile phase and separated in Licrosphere C-18 column (data not shown).

Different molarity (0.05; 0.75; and 0.1 M) and pH value of phosphate buffer (6.0; 6.5; and 7.0) were further investigated. The result showed that maximum area of vitamin C and folic acid was acquired with 0.05 M phosphate buffer pH 6.5. Therefore, the experiment was done using 0.05 M phosphate buffer pH 6.5 as mobile phase.

Specificity

Figure 1 shows chromatograms of vitamin C and folic acid when analyzed at optimum HPLC condition. Retention time of vitamin C and folic acid were 1.403 and 6.085, respectively. The resolution (Rs) value of vitamin C and folic acid were 15.325.

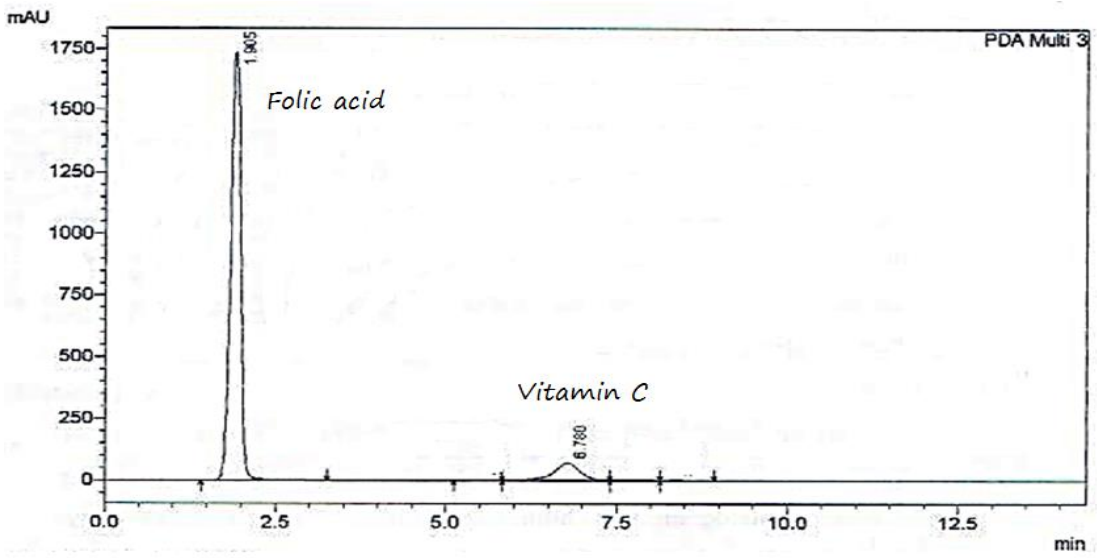

Figure 2. Chromatograms of folic acid and vitamin C analyzed using mobile phase of 50 mM buffer phosphate pH 6.5-methanol (90:10) with flow rate of 1.5 ml/min

Linearity

The results of linearity are shown in Figure 3 and Figure 4. A good linear regression was shown by both of vitamin C and folic acid. The correlation coefficient (*r*) of vitamin C and folic acid were 0.9952 and 0.9999 with Vxo value less than 5%.

ISSN: 0975-8585

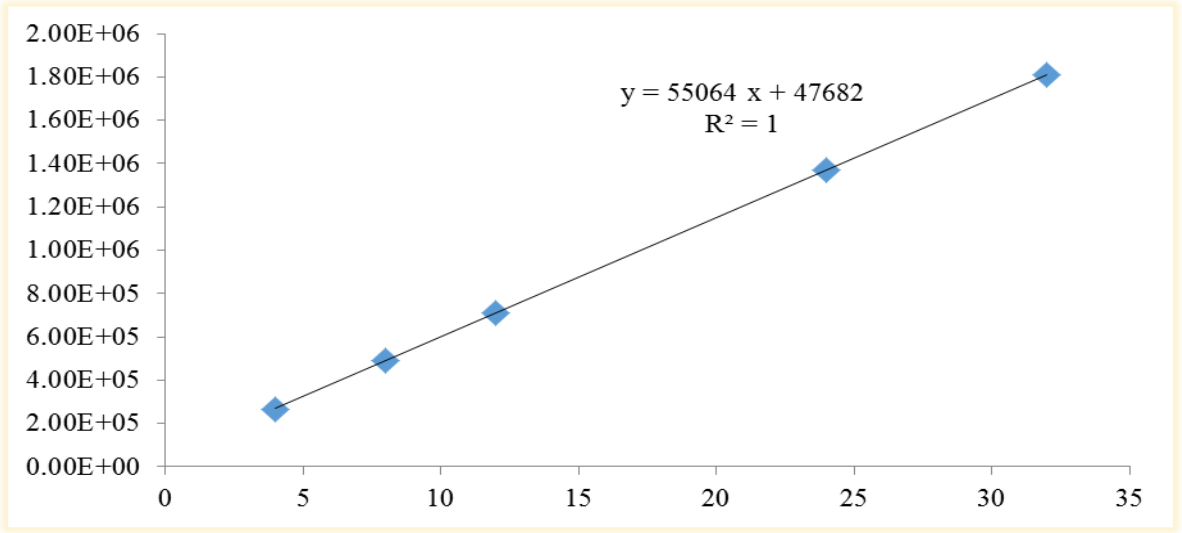

Figure 3. Linearity of folic acid shows good linear regression from concentration 4 mg/L to 32 mg/L

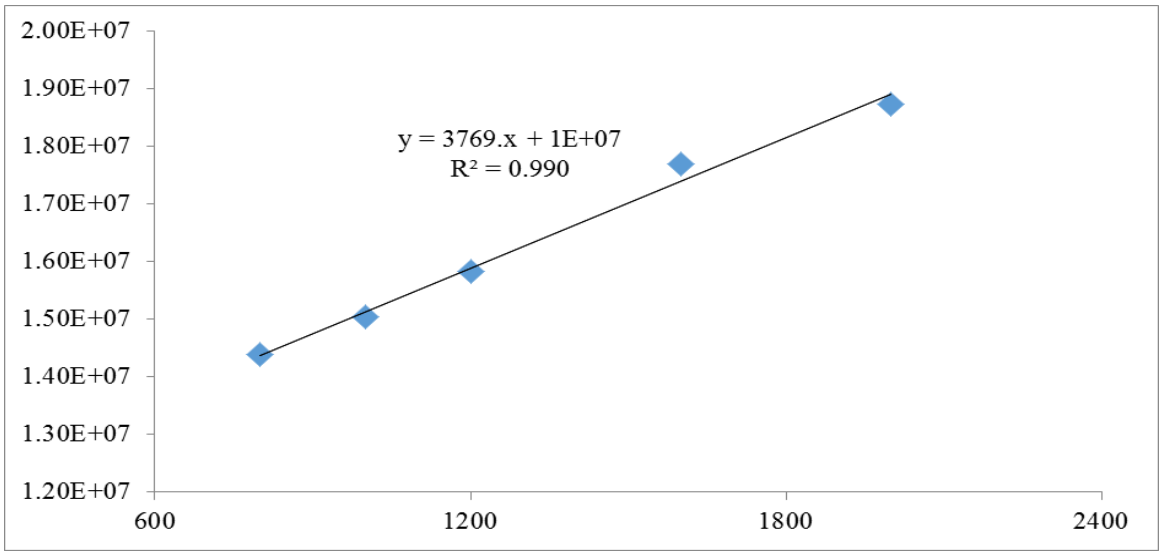

Figure 4. Linearity of vitamin C shows good linear regression from concentration 800 mg/L to 2000 mg/L

Accuracy

Accuracy was done using three concentration in triplicate. The result is shown on Table 2 and were in range of 98%-102% of acceptance criteria.

Table 2. The result of accuracy for vitamin C and folic acid

	Added amount (mg/L)	Obtained amount (mg/L)	% recovery
		Vitamin C	
80%	826.67	838.00	101,40 ± 0.17
100%	1027.33	1040.67	101,15 ± 0.49
120%	1223.33	1236.33	101,06 ± 0.45
		Folic acid	
80%	17.03	17.11	100.46 ± 0.41
100%	20.80	20.94	100.73 ± 0.35
120%	24.80	24.69	99.56 ± 0.50

ISSN: 0975-8585

Precision

Table 3 and 4 shows the precision of vitamin C and folic acid. Both of repeatability and intermediate precision were less than 2% of CV acceptance criteria.

Table 3. The *repeatability* and *intermediate precision* of vitamin C

Repl.	Day 1			Day 2		
	Real conc. (ppm)	Obtained conc. (ppm)	% *rec*	Real conc. (ppm)	Obtained conc. (ppm)	% *rec*
I	1020	1028	100,8	1036	1033	99,7
II	1040	1056	101,6	1040	1037	99,8
III	1022	1038	101,5	1042	1038	99,6
IV	1030	1035	100,5	1036	1037	100,1
V	1036	1055	101,8	1046	1043	99,7
VI	1050	1064	101,3	1050	1056	100,6
	Mean		101,3	Mean		99,91
	SD		0,51	SD		0,38
	CV (%)		0,50	CV (%)		0,38

Table 4. The *repeatability* and *intermediate precision* of folic acid

Repl.	Day 1			Day 2		
	Real conc. (ppm)	Obtained conc. (ppm)	% *rec*	Real conc. (ppm)	Obtained conc. (ppm)	% *rec*
I	20,700	20,728	100,1	21,900	22,102	100,9
II	21,000	21,032	100,2	21,960	22,194	101,1
III	21,260	21,358	100,5	21,900	22,115	101,0
IV	20,600	20,745	100,7	21,920	22,127	100,9
V	20,800	21,032	101,1	21,960	22,361	101,8
VI	20,980	21,063	100,4	21,980	22,357	101,7
	Mean		100,5	Mean		101,2
	SD		0,37	SD		0,41
	CV (%)		0,37	CV (%)		0,41

CONCLUSION

A rapid, specific, accurate, and precise reversed phase HPLC method was optimized and validated for simultaneous analysis of vitamin C and folic acid. Ionic strength, pH, and quantity of buffer in mobile phase become the critical point of simultaneous analysis of vitamin C and folic acid.

ACKNOWLEDGEMENTS

Authors thank Dean of Faculty of Pharmacy, Airlangga University for the financial support. Authors also thank M. Kusaeri, Kustiawan, and Dasuki for their assisstance during research.

REFERENCES

[1] Kucukkolbasi, S., Bilber, O., Ayyildiz, H.F., and Kara, H. Quim. Simultaneous and accurate determination of water- and fat-soluble vitamins in multivitamin tablets by using an RP-HPLC method. Nova 2013; 36 (7) : 1044-1051

[2] Chebrolu, K.K., Jayaprakasha, G.K., Yoo, K.S., Jifon, J.L., and Patil B.S. An improved sample preparation method for quantification of ascorbic acid and dehydroascorbic acid by HPLC. LWT - Food Science and Technology 2012; 47 : 443-449

[3] Jin, P., Xia, L., Li, Z., Che, N., Zou, D., and Hu, X. Rapid determination of thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine, folic acid and ascorbic acid in Vitamins with Minerals Tablets by high-performance liquid chromatography with diode array detector. J. Pharm and Biomed Anal 2012; 70 : 151-157

[4] Badan Pengawas Obat and Makanan (BPOM). Info POM : Manfaat Suplementasi Folat. 2009; 10 (2) : 1 - 4
[5] Patil, S.S., Srivastava, A.K. Development and Validation of Rapid Ion-Pair RPLC Method for Simultaneous Determination of Certain B-Complex Vitamins Along with Vitamin C. J AOAC Int 2012; 95 (1) : 74 - 83
[6] Amin, M. and Reusch, Simultaneous Determination of Vitamins B_1, B_2, B_6, B_{l2} and C, Nicotinamide and Folic Acid in Capsule Preparations by Ion-pair Reversed-phase High-performance Liquid Chromatography. J. Analyst 1987; 112 : 989 - 991
[7] Dabre, R., Azad, N., Schwämmle, A., Lämmerhofer, and M., Lindner, W. Simultaneous separation and analysis of water- and fat-soluble vitamins on multimodal reversed-phase weak anion exchange material by HPLC-UV. J Sep Sci 2011; 34 : 761–772
[8] Chen, P., Atkinson, R., and Wolf, W.R. Single-Laboratory Validation of a High-Performance Liquid Chromatographic-Diode Array Detector-Fluorescence Detector / Mass Spectrometric Method for Simultaneous Determination of Water-Soluble Vitamins in Multivitamin Dietary Tablets. J AOAC Int 2009; 92 (2) : 680 – 687
[9] Li, H.B. and Chen, F. Simultaneous Determination of Twelve Water- and Fat-Soluble Vitamins by High-Performance Liquid Chromatography with Diode Array Detection. Chromatographia 2001; 54 : 270 - 273
[10] Skoog, D.A., Holler, F.J., Crouch, S.R. Principles of Instrumental Analysis 2007; 6: 367-374, 816-851
[11] Yuwono, M., Indrayanto, G. Validation of Chromatographic Methods of Analysis. Profiles Of Drug Substances, Excipients, and Related Methodology 2005; 32 : 243 - 258
[12] International Conference on Harmonisation (ICH). ICH Harmonised Tripartite Guideline Validation of Analytical Procedures : Text and Methodology Q2(R1) Current Step 4 version. 2005; 6 - 13
[13] United Nations Office on Drugs and Crime (UNODC). Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens. 2009; 10 - 13
[14] Snyder, L.R., Kirkland, J.J., Glajch, J.L. Practical HPLC Method Development 1997; 690 - 691