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Quantitation of vitamin C content in herbal juice using direct titration

Leena Suntornsuk [a,*], Wandee Gritsanapun [b], Suchada Nilkamhank [b], Anocha Paochom [b]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Rajathevee, Bangkok 10400, Thailand*

[b] *Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayudhaya Road, Rajathevee, Bangkok 10400, Thailand*

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Abstract

Vitamin C content in fresh and freeze-dried herbal juice, such as guava (*Psidium guajava Linn.*) emblic myrobolan (*Phyllanthus embica Linn.*), lemon (*Citrus aurantifollia Swing*), sweet pepper (*Capsicum annuum Linn.*) *Garcinia schomburgkiana Pierre* and passion fruit (*Passiflora laurifoia Linn.*) was determined by direct titration with iodine. The method showed excellent linearity ($r^2 > 0.99$) over the concentration ranges tested (100–500% of the amount found in the juice samples), good precision (R.S.D. < 1.5%) and recovery (> 97%). The limit of detection and limit of quantitation were 2.2 and 7.3 mg, respectively. The amount of vitamin C found were 80.1 mg/100 g for guava, 226.0 mg/100 g for emblic myrobolan, 52.8 mg/100 g for sweet pepper, 39.1 mg/100 g for passion fruit, 10.5 mg/100 g for lemon and 4.6 mg/100 g for *G. schomburgkiana*. The stability of vitamin C during the first 4 weeks was remarkably improved after freeze-dried process. The percent reductions of vitamin C after freeze-dried process were 41.4 and 20.4% for guava and emblic myrobolan, respectively. After 8 weeks, the freeze-dried samples contained only traces amount of vitamin C tested by thin layer chromatography.

Keywords: Direct titration; Vitamin C; Ascorbic acid; Guava; Emblic myrobolan; Lemon; Sweet pepper; *Garcinia schomburgkiana Pierre*; Passion fruit

1. Introduction

Vitamin C or ascorbic acid is water-soluble vitamin, which is present in fresh fruit, especially citrus fruit and vegetable. It involves in wound healing, tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, synthesis of lipids and protein, iron metabolism, resistance to infections and cellular respiration [1]. In addition, vitamin C shows antioxidative effects and under certain conditions can protect against oxidativlely induced DNA damage [2]. Determination of vitamin C can be performed by various methods. For example, first derivative ultra-violet

* Corresponding author. Tel.: +66-2-644-8695; fax: +66-2-247-4696.

E-mail address: lleena65@hotmail.com (L. Suntornsuk).

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(UV) spectrophotometry with zero-crossing technique was employed for analysis of mixture of ascorbic acid, pyridoxine hydrochloride and tyrosine [3]. Aburjai [4] reported the used of second derivative UV spectrophotometry and high performance thin layer chromatography (HPTLC) for the simultaneous determination of vitamin C and dipyrone in pure form and in pharmaceutical dosage forms. The Association of Official Analytical Chemists (AOAC) [5] recommends the volumetric titration using 2,6 dichloroindophenol as a titrant for the determination of vitamin C in vitamin preparations and juices and reduced ascorbic acid content in ready-to-feed milk-based infant formula. Whereas, microfluorometry and semi-automated fluorometry is suggested for quantitation of total vitamin C in vitamin preparations and food, respectively [5]. Botre and coworkers [6] measured vitamin C in 11 different exotic fruits by high performance liquid chromatography (HPLC). Vitamin C in fruit juices was also determined by enzymatic method using ascorbate oxidase from starfruit (*Averrhoa carambola*) [7]. Recently, flow injection analysis coupling with different detectors has been employed for the analysis of vitamin C [8–11].

The aims of this work are to validate a direct titration method for the determination of vitamin C content in fresh and freeze-dried herbal juice and to study the stability of vitamin C in selected fresh juice and freeze-dried samples. Unlike other methods, the direct titration method using iodine as a titrant is inexpensive, simple, reliable and rugged. No extraction or sample pre-treatment is required prior to titration, thus the analysis time is less than 5 min per sample allowing, as many as, 12 titrations per hour.

2. Experimental

2.1. Instrumentation

The titrations were carried out using a Schott Gerate Titrator apparatus (Schotte Gerate GmbH, Postfach, Germany). This instrument is equipped with a titrator TR 156, an automatic buret 10 ml TA 10 and a stirrer TM 120.

2.2. Materials and reagents

Guava (*Psidium guajava Linn.*), emblic myrobolan (*Phyllanthus embica Linn.*), lemon (*Citrus aurantifollia Swing*), sweet pepper (*Capsicum annuum Linn.*) *Garcinia schomburgkiana Pierre* and passion fruit (*Passiflora laurifoia Linn.*) were purchased from local markets. Ascorbic acid and arsenic trioxide were obtained from Sigma Chemical (St. Louis, MO). All other chemicals were analytical grade and also obtained from Sigma. Deionized water was used for all preparations.

2.3. Sample preparations

Fresh guava, emblic myrobolan, lemon, sweet pepper *G. schomburgkiana Pierre* and passion fruit were cleaned, chopped into small pieces and passed through a juice extractor (Moulinex®, France). The juice was filtered through a Whatman paper no. 1 prior analysis. Freeze-dried sample of guava and emblic myrobolan was prepared by pouring 200 ml of guava and emblic myrobolan juice into stainless trays, froze and put into the freeze drier (Labconco®, USA).

2.4. Preparation and standardization of 0.1 N iodine

Iodine (14 g) was dissolved in potassium iodide solution (100 ml), acidified with hydrochloric acid (1 N), diluted with water to 1000 ml and standardized with primary standard arsenic trioxide prior use. The primary standard arsenic trioxide (150 mg) was dissolved in 1 N sodium hydroxide (20 ml) and diluted with water to 40 ml. The solution was acidified with diluted hydrochloric acid using methyl red as an indicator. Sodium bicarbonate (2 g), water (50 ml) and starch TS (3 ml) was added into the adicified solution prior titration with iodine solution. Each ml of 0.1 N iodine is equivalent to 4.946 mg arsenic trioxide.

2.5. Determination of vitamin C in herbal fresh juice

The vitamin C content in herbal juice was determined by direct titration with iodine. Each

25 ml of the herbal fresh juice was transferred into a 250 ml Erlenmeyer flask. Twenty-five milliliter of 2 N sulfuric acid was added, mixed, diluted with 50 ml of water and 3 ml of starch T.S. was added as an indicator. The solution was directly titrated with 0.1 N iodine previously standardized with primary standard arsenic trioxide. A blank titration was performed prior titration of each sample ($n = 5$). Each ml of 0.1 N iodine is equivalent to 8.806 mg ascorbic acid.

2.6. Linearity

The linearity of the method was determined by adding standard ascorbic acid 100, 200, 300, 400 and 500% of the amount found in the juice samples into the sample. Triplicate titrations were made for each standard added solution. The linear regression line was plotted between the amount of standard ascorbic acid found and the amount of standard ascorbic acid added using Microsoft EXCEL®. The regression equation and the regression coefficient (r^2) values were obtained.

2.7. Precision and accuracy

Intra- and inter-day precision was studied by determining vitamin C content in guava and emblic myrobolan juice from the same source by the same analyst on the same and different days. Five titrations were made for each sample. Precision was expressed as % relative standard deviation (%R.S.D.). The accuracy calculated from percentages of bias and recovery was determined using standard addition method. Standard ascorbic acid 100, 200, 300, 400 and 500% of the amount found in the guava and emblic myrobolan juice was added into the juice samples. Vitamin C in the added juice sample was determined by direct titration method as described in the previous section. Triplicates titrations were made for each standard added solution. The percentage bias and recoveries were calculated using the expressions:

$$\%\ \text{Bias} = \frac{\text{Measured amount} - \text{True amount}}{\text{True amount}} \times 100$$

$$\%\ \text{Recovery} = \frac{\text{Amount of standard recovered}}{\text{Amount of standard added}} \times 100$$

2.8. Ruggedness

Ruggedness of the method was performed by analyzing the vitamin C content in guava and emblic myrobolan juice from the same source by different analysts. Five replicates were made for each sample.

2.9. Limit of detection and quantitation

The limit of detection (LOD) was determined by decreasing the concentration of standard ascorbic acid 10-fold each time. The amount of standard ascorbic acid, which could be detected by observation of end-point, was considered to be limit of detection. The lowest amount of standard ascorbic acid that could be quantified with reasonable precision and accuracy was considered the limit of quantitation (LOQ). The LOQ was calculated by multiplying the LOD by a factor of 3.3.

2.10. Stability of vitamin C in herbal fresh juice and freeze-dried samples

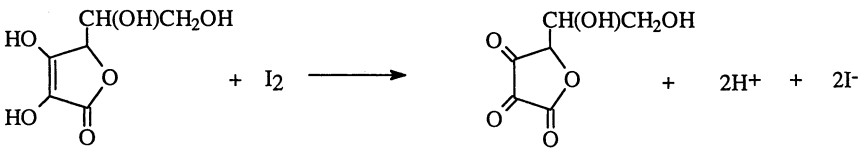
Stability of vitamin C content in guava and emblic myrobolan juice was determined weekly for 4 weeks after storage in a refrigerator maintained at 4 °C. All samples were kept in light protected containers. Freeze-dried samples were kept from light in a dessicator and were tested for the stability of vitamin C content after 4 and 8 weeks. Vitamin C content in herbal fresh juice and freeze-dried sample after 4-week storage was determined using direct titration method. Freeze-dried samples after 8 week were analyzed by thin layer chromatography. Silica gel 60 F_{254} was used as stationary phase and *n*-butanol–glacial acetic acid–water (16:4:1, v/v/v) was employed as the solvent system. TLC plates were dried at 100 °C for 5 min and sprayed with 10% H_2SO_4 in ethanol. A loss exceeding 10% of the initial content is considered to indicate instability of vitamin C.

3. Results and discussion

The method based on the oxidation of ascorbic acid to deydroascorbic acid by iodine (Scheme 1) was used for the determination of vitamin C content in fresh and freeze-dried herbal juice.

3.1. Method validation

Direct titration with iodine was employed for determining the vitamin C content in herbal juice. The method was validated for linearity, precision, accuracy, ruggedness, limit of detection and limit of quantitation using guava and emblic myrobolan juice as samples. A linear relationship of the amount of standard ascorbic acid found and the amount of standard ascorbic acid added over concentration of 100–500% of the amount found in the guava and emblic myrobolan juice was obtained. The validating parameter of each calibration curve, such as slope (a), intercept (b), r-squared (r^2), relative standard deviation of the slope (R.S.D.) and intercept ($R.S.D_b$) are present in Table 1. The method was precise and the %R.S.Ds for intra-day assay was within 1.3% in all cases as shown in Table 2. The %R.S.Ds for



Scheme 1.

Table 1
Calibration curve parameters and statistics: vitamin C in guava juice and emblic myrobolan juice

Curve	Guava juice			Emblic myrobolan juice		
	Slope	y-intercept	r^2	Slope	y-intercept	r^2
1	0.9982	0.0013	0.9995	0.9780	0.0017	0.9999
2	0.9998	0.0011	0.9990	0.9814	0.0006	0.9999
3	0.9812	0.0020	0.9985	0.9798	0.0015	1.0000
Mean ($n = 3$)	0.9931	0.0015		0.9797	0.0013	
S.D.	0.0103	0.0005		0.0017	0.0006	
R.S.D. (%)	1.0	32.2		0.1736	46.3	

Table 2
Precision determination of vitamin C content in guava and emblic myrobolan juice

Number of sample	Vitamin C in guava juice (mg)			Vitamin C in emblic myrobolan juice (mg)		
	Freshly prepared	7 days	14 days	Freshly prepared	7 days	14 days
1	25.69	25.10	24.36	59.25	49.99	41.65
2	25.84	24.92	24.52	58.17	50.17	42.92
3	26.31	25.15	24.49	58.25	50.17	42.91
4	26.54	24.99	24.23	58.27	49.29	42.94
5	26.12	25.15	24.68	58.44	49.38	42.71
Mean ($n = 5$)	26.10	25.06	24.45	58.58	49.80	42.63
S.D.	0.343	0.103	0.171	0.415	0.432	0.557
R.S.D. (%)	1.3	0.4	0.7	0.7	0.9	1.3

Table 3
Recovery of vitamin C from guava juice

Strength (%)	Amount added (g)	Amount found (g)	Recovery (%)	Bias (%)
100	0.0215	0.0219	101.86	1.86
	0.0220	0.0228	103.64	3.64
	0.0222	0.0228	102.70	2.70
200	0.0435	0.0455	104.57	4.57
	0.0440	0.0458	104.09	4.09
	0.0442	0.0459	103.85	3.85
300	0.0670	0.0689	102.84	2.84
	0.0652	0.0670	102.76	2.76
	0.0665	0.0680	102.26	2.26
400	0.0878	0.0873	99.43	0.57
	0.0870	0.0870	100.00	0.00
	0.0888	0.0909	102.36	2.36
500	0.1097	0.1113	101.46	1.46
	0.1087	0.1102	101.38	1.38
	0.1101	0.1085	98.55	1.45

Table 4
Recovery of vitamin C from emblic myrobolan juice

Strength (%)	Amount added (g)	Amount found (g)	Recovery (%)	Bias (%)
100	0.0820	0.0811	98.90	1.10
	0.0847	0.0836	98.70	1.30
	0.0829	0.0816	98.43	1.57
200	0.1823	0.1787	98.03	1.97
	0.1749	0.1723	98.51	1.49
	0.1686	0.1666	98.81	1.19
300	0.2330	0.2301	98.76	1.24
	0.2375	0.2345	98.74	1.26
	0.2318	0.2290	98.79	1.21
400	0.3594	0.3513	97.75	2.25
	0.3470	0.3394	97.81	2.19
	0.3462	0.3405	98.35	1.65
500	0.4328	0.4265	98.54	1.46
	0.4214	0.4151	98.50	1.50
	0.4312	0.4240	98.33	1.67

inter-day assay was 0.83% for guava juice and 7.99% for emblic myrobolan juice. Accuracy data is present in Tables 3 and 4 and the percent biases were 0–4.57% for guava juice and 1.10–2.25% for emblic myrobolan juice. Recoveries of standard ascorbic acid were within 98.55–104.57% and 97.75–98.90% for guava and emblic myrobolan, respectively. Titration results from two analysts ($n = 5$) on determination of vitamin C content in guava and emblic myrobolan juice were in close agreement as demonstrated in Table 5. The LOD and LOQ of vitamin C content were 2.20 and 7.26 mg, respectively.

3.2. Determination of vitamin C content in herbal fresh juice and freeze-dried samples

The validated method was employed for determinations of vitamin C content in herbal juice. The herbal juice in this study contained varied amount of vitamin C. The average ($n = 5$) vitamin C content was 80.1 mg/100 g for guava, 226.0

mg/100 g for emblic myrobolan, 52.8 mg/100 g for sweet pepper, 39.1 mg/100 g for passion fruit, 10.5 mg/100 g for lemon and 4.6 mg/100 g for *G. schomburgkiana*. Among these plants, guava and emblic myrobolan contained the highest vitamin C content, then they were freeze-dried and used for further study. The freeze-dried guava appeared as viscous green thin film with the yield of 4.97%, whereas that of emblic myrobolan was yellowish green powder with the yield of 11.42%. The vitamin C content in freeze-dried samples ($n = 5$) was 46.9 mg/100 g for guava and 179.8 mg/100 g for emblic myrobolan. Results show that vitamin C degraded after freeze-dried process and the percents of reduction were 41.4 and 20.4% for guava and emblic myrobolan, respectively.

3.3. Stability of vitamin C in herbal fresh juice and freeze-dried samples

Guava and emblic myrobolan juice were stored in a refrigerator maintained at 4 °C and re-analyzed for the vitamin C content on a weekly basis for 4 weeks. The percents of vitamin C reduction in guava after 1, 2, 3 and 4 weeks were 9.2, 17.1, 27.6 and 38.6%, respectively. For emblic myrobolan, the percents of vitamin C reduction were 56.5, 63.0, 68.4 and 73.6%, respectively. Stability of vitamin C in freeze-dried samples was studied after 4 and 8 week storage. After 4 weeks, vitamin C content reduced from 46.9 to 33.8 mg/100 g (27.9%) for freeze-dried guava and from 179.8 to 172.7 mg/100 g (3.9%) for freeze-dried emblic myrobolan. After 8 weeks, the amount of vitamin C was too small to be quantitatively analyzed by direct titration method. TLC data shows that only trace amount of vitamin C remained in these samples. The data indicate vitamin C content in guava fresh juice was unstable after 1 week, while in emblic myrobalan it was degraded during the first week. However, the stability of vitamin C in both juices remarkably improved after they were freeze-dried.

4. Conclusions

Direct titration with iodine was utilized for determination of vitamin C in fresh and freeze-dried herbal juice. The method was validated and showed good linearity, precision, accuracy, recovery, sensitivity and ruggedness. With this method analysis of vitamin C in herbal juice can be performed without extraction or derivatization. Among the tested samples, guava and emblic myrobolan juice contained the highest vitamin C content. The vitamin C content in fresh juice was greater than that in freeze-dried samples indicating degradation of vitamin C during the freeze-dried process. The stability of vitamin C in freeze-dried samples, however, was higher than in fresh juice after 4-week storage. But after 8 weeks, the content was dramatically reduced and made it unable to be analyzed by direct titration. Comparing with other methods the proposed procedure is

Table 5
Ruggedness data of vitamin C content in guava and emblic myrobolan juice

Number of sample	Vitamin C in guava juice (mg)		Vitamin C in emblic myrobolanjuice (mg)	
	Analyst I	Analyst II	Analyst I	Analyst II
1	29.70	28.91	87.16	87.36
2	28.92	29.75	86.11	86.12
3	29.71	28.92	87.15	86.12
4	29.32	29.71	87.59	87.11
5	28.95	29.35	87.45	86.61
Mean ($n = 5$)	29.32	29.33	87.09	86.67
S.D.	0.389	0.407	0.582	0.563
R.S.D. (%)	1.3	1.4	0.7	0.6

simple and less time consuming since sample manipulation prior the titration is not required. In addition, the method is reliable and cost-effective in term of the instrumentation and reagents. The proposed method is successfully applied for determination of vitamin C content in fresh and freeze-dried juice and can be used as a stability-indicating method of vitamin C in both samples.

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