Analysis of citric acid and D-isoascorbic acid in beverages by High performance liquid chromatography

Rui Wang[1,2], Shiwen Hu[2,*], and Jie Chen[2]

[1]Chongqing Collaborative Innovation Center for Functional Food, Chongqing University of Education, Chongqing 400067, China
[2]College of Biological and Chemical Engineering, Chongqing University of Education, Chongqing 400067, China

Abstract. In this study, high performance liquid chromatography (HPLC) was used to analysis the content of citric acid and D-isoascorbic acid in beverages. The samples were separated by C18 chromatography column, 0.1 % phosphoric acid solution was used as the mobile phase with the flow rate of 0.5 mL/min, the column temperature was 35 °C, and the detection wavelength was 210 nm. The results showed that the content of citric acid and D-isoascorbic acid has a good linear correlation (r>0.99) within the range of 50 μg to 200 μg. The selectivity, recovery and precision of citric acid and D-isoascorbic acid were suitable. Meanwhile, this method could be used to detect the content of citric acid and D-isoascorbic acid in beverages.

1 Introduction

At present, food additives are widely used in the food industry. As we all know, food additives will not cause harm to the human body within the appropriate dosage, but if the dosage exceeds the standard, they may cause various forms of toxicity [1]. It is reported that the long-term low-dose excessive intake of colorant additives may lead to the decline of adult fertility, affect the intellectual development of children, and cause some excessive behaviors in children [2]. Therefore, strict standards have been established for the use of food additives in China [3]. As a kind of fast-consuming food, beverage has a huge market demand. In order to make the taste better and extend its shelf life, many manufacturers usually add some appropriate types of food additives to the beverage [4]. D-isoascorbic acid, also known as isovitamin C, is a common antioxidant and has many application prospects in beverages [5]. Meanwhile, citric acid is often used as a kind of sour agent in beverages. It is not only has the taste of natural fruit, but also has the effect of anti-oxidation. Moreover, it can effectively prevent browning and stickiness on the surface of foods and play a role in color protection. Currently, the detection methods of food additives in beverages mainly include the high performance liquid chromatography [6, 7], gas chromatography [8] and liquid chromatography-mass spectrometry [9], etc. However, citric acid and D-isoascorbic acid can only be detected separately by the existing methods, and there is no method for detecting them both at the same time. Therefore, this study aims to establish a high performance liquid chromatography method that can simultaneously detect citric acid and D-isoascorbic acid in beverages, and this method could be used to determine the content of related substances in commercially available beverages.

2 Materials and Methods

2.1 Chemicals and reagents

Standard D-isoascorbic acid and standard citric acid were purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China). Ultrapure water used in the experiment was of Milli-Q quality (Millipore Corp., Bedford, MA, USA). HPLC-grade Phosphoric acid was obtained from Chron Chemicals Co., Ltd (Chengdu, China). The twenty-nine kinds of beverage samples used in this experiment were randomly selected in the supermarket.

2.2 Preparation of solutions

10 mg of citric acid and 10 mg of D-isoascorbic acid standards were accurately weighed into a 10 mL volumetric flask respectively, dissolved with an appropriate amount of ultrapure water, then added the ultrapure water to make the volume to obtain a standard stock solution with a concentration of 1.0 mg/mL. 1 mL of the standard stock solutions of citric acid and D-isoascorbic acid were drawn into a 10 mL volumetric flask, ultrapure water was used to make the volume. The solution was filtered with a 0.22 μm filter membrane to obtain a mixed standard solution.

2.3 Sample preparation

1 mL of beverage sample was put into a 10 mL volumetric flask respectively. An appropriate amount of ultrapure water was mixed with the sample and made to the volume. The solution was filtered with a 0.22 μm water-based

* Corresponding author: wenfeng970211@sohu.com

filter membrane to obtain the sample solution.

2.4 Instrumentations and chromatographic conditions

Thermo Fisher Scientific UltiMate3000 HPLC System (Thermo Fisher Scientific, USA) equipped with DAD detector and an accucore C18 column (150 mm × 4.6 mm, 2.6 μm; Thermo Fisher Scientific, USA) was used in this research. The detection wavelength was 210 nm. Meanwhile, 0.1 % phosphoric acid solution was used as the mobile phase with the flow rate of 0.5 mL/min, the column temperature was 35 °C, and the injection volume was 5 μL.

2.5 Method validation

2.5.1 Selectivity

The mixed standard solution of D-isoascorbic acid and citric acid was determined according to the HPLC method. The retention time and the chromatographic peaks of two additives were obtained to study the selectivity of the detection method.

2.5.2 Linear range

The appropriate amount of D-isoascorbic acid standard solution and citric acid standard solution was precisely measured and diluted with mobile phase to the concentration of 20, 40, 60, 80, 100 μg/mL, then analyzed by the established detection method. After that, the concentrations of standard substance were set as abscissa and the peak area were set as ordinate to draw the standard curves.

2.5.3 Precision

The appropriate amount of D-isoascorbic acid standard solution and citric acid standard solution was taken. It was detected six times to calculate the intra-day precision. Meanwhile, it was detected once a day to calculate the inter-day precision in a five consecutive day period.

2.5.4 Recovery

50 μg/mL, 100 μg/mL and 150 μg/mL of D-isoascorbic acid standard solution and citric acid standard solution were added respectively to the 100 μg/mL standard mixed solution, and perform five times detection according to the established HPLC method. Then the recoveries of D-isoascorbic acid and citric acid were calculated respectively.

2.6 Determination of D-isoascorbic acid and citric acid in beverages

Twenty-nine kinds of beverages were randomly selected from the market. The content of D-isoascorbic acid and citric acid in each beverage was detected by the established detection method.

3 Results and Discussions

3.1 Selectivity

The retention time of D-isoascorbic acid and citric acid was 2.5 min and 2.8 min respectively as shown in Figure 1, and the separation of the two additives was obvious. These results showed that the selectivity of the HPLC method meets the experimental requirements.

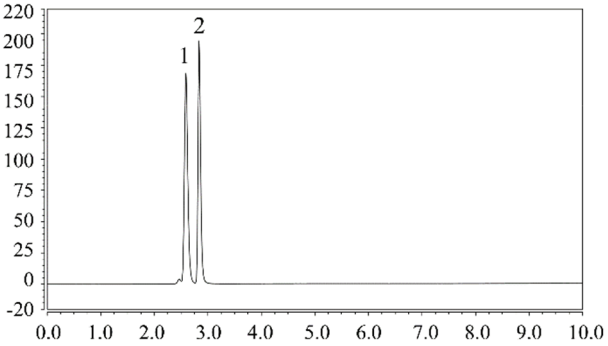

Fig. 1. HPLC chromatogram of standard. (1) D-isoascorbic acid; (2) citric acid.

3.2 Linear range

As shown in Table 1, the correlation coefficient (r) of D-isoascorbic acid and citric acid was 0.998 and 0.996 respectively. The results demonstrated that the content of D-isoascorbic acid and citric acid has a good linear correlation (r>0.99) within the range of 50 μg to 200 μg.

Table 1. Standard curve and linear range of two additives.

additive	standard curve	correlation coefficient (r)	linear range (μg/mL)
D-isoascorbic acid	Y=0.01X + 0.0438	0.998	50-200
citric acid	Y=0.1098 X + 0.602	0.996	50-200

3.3 precision

As shown in Table 2, the intra-day precision of D-isoascorbic acid and citric acid was 0.602 % and 1.366 % respectively. Meanwhile, the inter-day precision of D-isoascorbic acid and citric acid was 0.815 % and 1.601 % respectively. These results indicated that the HPLC method has a good precision.

Table 2. Intra-day and inter-day precision.

additive	RSD (%)	
	Intra-day	Inter-day
D-isoascorbic acid	0.602	0.815
citric acid	1.366	1.601

3.4 Recovery

The recoveries of D-isoascorbic acid were ranged in 86 % to 112 %, and the recoveries of citric acid were ranged in 87 % to 97 %, respectively (Table 3). It can be seen from the results that the recovery of the HPLC method was good.

Table 3. Recoveries of two additives.

Concentration of added standard (μg/mL)	D-isoascorbic acid		citric acid	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
50	95	0.9	87	1.3
100	112	1.2	97	1.6
150	86	1.6	94	0.9

3.5 Determinations of D-isoascorbic acid and citric acid in beverages

As shown in Table 4, the content of D-isoascorbic acid and citric acid in beverages were analyzed by HPLC. The results of the study showed that citric acid was added to all of the 29 kinds of beverages, while D-ascorbic acid was not added to 8 kinds of beverages. The content of D-isoascorbic acid in 29 kinds of beverages was mostly in the range of 100 to 200 μg/mL, and there were 4 samples with added D-isoascorbic acid below 100 μg/mL, while there were 6 samples with added D-isoascorbic acid above 200 μg/mL. Moreover, the content of citric acid was mostly in the range of 10 to 30 μg/mL, 14 samples with concentration below 10 μg/mL, and 4 samples above 30 μg/mL. Therefore, the established high performance liquid chromatography method in this study could detect the content of D-isoascorbic acid and citric acid in beverages simultaneously and accurately.

Table 4. Determination of D-isoascorbic acid and citric acid in beverages.

Sample	D-isoascorbic acid (μg/mL)	citric acid (μg/mL)
1	74.02	4.32
2	605.02	23.62
3	not detected	8.39
4	153.42	5.17
5	not detected	12.67
6	253.62	7.67
7	206.72	14.54
8	40.72	4.50
9	47.12	2.63
10	138.12	10.75
11	not detected	20.40
12	598.62	28.12
13	181.82	18.42
14	183.92	8.55
15	not detected	39.67
16	142.42	11.58
17	116.92	14.64
18	200.82	3.96
19	236.22	41.32
20	not detected	11.90
21	not detected	1.84
22	106.42	31.70
23	146.42	1.13
24	not detected	6.28
25	not detected	1.34
26	136.92	4.19
27	51.42	13.94
28	190.92	69.12
29	120.22	4.35

4 Conclusion

This study was optimized on the basis of the existing detection methods of D-isoascorbic acid and citric acid to establish a high performance liquid chromatography method that can simultaneously determine the content of D-isoascorbic acid and citric acid in beverages. The samples were separated by C18 chromatography column, 0.1 % phosphoric acid solution was used as the mobile phase with the flow rate of 0.5 mL/min, the column

temperature was 35 °C, and the detection wavelength was 210 nm. The results showed that the retention time of D-isoascorbic acid and citric acid was 2.5 min and 2.8 min respectively. The content of D-isoascorbic acid and citric acid has a good linear correlation (r>0.99) within the range of 50 μg to 200 μg. Meanwhile, the recoveries of D-isoascorbic acid were ranged in 86 % to 112 %, and the recoveries of citric acid were ranged in 87 % to 97 %, respectively. The precision of citric acid and D-isoascorbic acid were suitable. Furthermore, the content of D-isoascorbic acid in 29 kinds of beverages was mostly in the range of 100 to 200 μg/mL, while the content of citric acid was mostly in the range of 10 to 30 μg/mL. In summary, this established HPLC method could analyze the content of D-isoascorbic acid and citric acid in beverages quickly. Therefore, this HPLC method has certain application value.

Acknowledgments

This research was funded by Teaching Reform Project of Chongqing University of Education (JG201730).

References

1. H. F. Liu, X. Zhou. Automotive Quality, **4**, 42 (2006)
2. Q. Liu, X. Li, X. X. Wu, L. L. Liu, P. Liu. Physical Testing and Chemical Analysis (Part B: Chemical Analysis), **53**, 693 (2017)
3. J. Wei, Z. M. Guo, F. F. Zhang, X. M. Liang. Journal of Food Safety & Quality, **4**, 395 (2013)
4. X. L. Fang. The Food Industry, **39**, 314 (2018)
5. Y. T. Dai, J. Q. Yang, S. L. Ge, S. Y. Yu. Food and Fermentation Sciences & Technology, **55**, 81 (2019)
6. M. X. Liu, Q. Zhou, W. P. Chen. Science and Technology of Food Industry, **34**, 376 (2013)
7. Y. Zhang, H. M. Wu, W. Wang, J.Q. Wang, L. P. Bai. Journal of Food Safety & Quality, **27**, 18 (2010)
8. Y. Luan, J. Zhao, W.Y. Quan, H. Z. Li. Chinese Journal of Public Health **20**, 487 (2004)
9. R.H. Ke, L. J. Wang, H.M. An, J.J. Yin, Q. H. Song. Beverage Industry, **16**, 35 (2013)