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Journal of Economic Science Research
http://ojs.bilpublishing.com/index.php/jesr

ARTICLE

Optimization of Determination of Sucralose in Drink by HPLC

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ARTICLE INFO

Article history
Received: 4 July 2019
Accepted: 16 July 2019
Published Online: 26 July 2019

Keywords:
Drink
Sucralose
HPLC
RID

ABSTRACT

To optimize the method for determination of Sucralose in drink by high performance liquid chromatography (HPLC). Using HPLC with RID, operating conditions were C18 reversed phase chromatograph column, 40:60 = Methanol: 0.125% K_2HPO_4 as mobil phase, measured at a flow rate of 0.8 mL/min. In the range of 20 ~ 400 mg L, with the concentration of Sucralose and corresponding peak area as standard, r = 0.9999, it has good correlation, the recovery of sucralose is 94~108%.The lower limit of detection of Sucralose was 0.0024 g/kg. This method not only meets the requirements of national standards, but also fast, sensitive, and environmentally friendly, improves the detection efficiency and safety of the detection of sucralose in drink by high performance liquid chromatography.

1.Introduction

Sucralose, also known as 4,1',6'-Trichlorogalactosucrose [1], is a white or nearly white crystalline powder sucrose derivatives, its sweetness is about 600 times than sucrose. As we know that sugar is a kind of food additive with high calorie and low sweetness [2], long-term use will easily lead to health problems such as obesity and high blood fat. Therefore, low calorie and high sweetness sucralose is increasingly used in food additives. However, sucralose may also cause some health risks [3]. Therefore, there are certain limits on the addition of sucralose.

The determination methods of sucralose in food include ion chromatography [4-6], high performance liquid chromatography [7-13], gc-ms [14], HPLC-MS, UPLC — MS/MS[15] and so on, high performance liquid chromatography is the main method. Existing national food safety standard GB 22255-2014 "Determination of sucralose in food"[15] for the determination of sucralose in drinks, it is found that it has the disadvantages of long detection process, low sensitivity and large toxic and side effects through practical experiments. With the increasing demand for food, the number of test samples increases sharply. The low detection efficiency of national standards challenges the work of testers, so it is necessary to optimize and improve the existing analytical methods.

In this study, we improve the determination method of national standard, optimized the analytical conditions of instrument, and improved the detection speed, sensitivity and environmental protection of the method by comparing the separation effect of different mobile phase and flow rate.

2 Materials and Methods

2.1 Instruments and Reagents

(1) High performance liquid chromate graph (with

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DOI: https://doi.org/10.30564/jesr.v2i3.1023

RID-20A,Shimazu (China) Co., LTD);

(2) Analytical balance(MS 105DU, METTLER TOLEDO Co.,LTD);

(3) Ultrasonic cleaner(KQ-500B, Kunshan ultrasonic instrument Co. LTD);

(4) Nitrogen blowing concentrator(AutoEVA-60, Reeko Co.,LTD);

(5) Solid phase extraction column(Oasis HLB, Waters Co.,LTD), 4mL methanol and 4mL water to activate successively before use;

(6) Membrane filter(0.45μm, Agilent Technologies Co.,LTD);

(7) Sucralose(The purity of 98.8%, manhage biotechnology co., LTD);

(8) Acetonitrile, Methanol(chromatographically pure, Merck KGaA Co.,LTD);

(9) Methanol, Dipotassium phosphate(analytically pure, Guangzhou chemical reagent factory).

2.2 Experimental Method

2.2.1 Chromatographic Conditions

In this study, the experimental methods is based on the national standard method. Experimental conditions are shown in Table 1.

Table 1. Experimental method conditions

Method	Natioanal standard(GB)	My research
Column	GL Sciences Inc. 4.6 mm×150 mm, 5 μm,	
Mobile phase	Acetonitrile:Water =89:11(V:V).	Methanol:0.125% K_2HPO_4 =40:60(V:V).
Flow rate	1.0 mL/min	0.8 mL/min
Column temp.	35℃	35℃
Detector temp	35℃	35℃
Injection	20μL	20μL

2.2.2 Preparation of Standard Solution

Stock solution:weigh 0.100g sucralose in a 10-ml volumetric flask accurately, dissolve it completely with ultra-pure water, and then reach the scale with a constant volume.The concentration is about 10 mg/mL. Store at 2 to 8 ℃ for 6 months.

2.2.3 Establish Standard Work Curve

Remote the stock solution respectively and dilute to the following series of concentrations: 20,50,100,200,400 mg/L. Sample size is 20μL.Take the concentration (X, mg/L) as the horizontal coordinate, and the peak area (Y) as the vertical coordinate, establish standard work curve.

2.2.4 Sample Pretreatment

5g (accurate to 0.001 g) samples were accurately weighed and placed in a 25 mL plastic centrifugal tube, diluted with 5mL ultra-pure water, shaken on the vortex mixer for 30 minutes, and centrifuged for 10min at 3000r/min. All the supernatant was transferred to the pre-activated solid phase extraction column, and the liquid flow rate was controlled at 1 drop per second. When the liquid level on the column was about 2mm, 1mL ultra-pure water was added, and the liquid flow rate was kept at no more than 1 drop per second. After the liquid was completely discharged from the column, add 3mL methanol to elute, then collect methanol eluent. The eluent was blow-dried in a nitrogen blower. After the residue was dissolved in 1.00ml mobile phase, the solution was filtered through a 0.45m filter membrane. The filtrate is the sample solution prepared.

3. Results and Analysis

3.1 Optimization of Chromatographic Conditions

By adjusting the flow phase and flow rate, the peak time is advanced. In GB 22255-2014, the mobile phase is Acetonitrile: Water=89： 11(V:V), the flow rate is 1.0 mL/min, in this study, I use Methanol: 0.125% K2HPO4 =40： 60(V:V) , 0.8 mL/min instead. The detection map is shown in figure 2.

It can be seen from figure 2(a) and (b) that the peak time of the GB is relatively late and the response value is relatively low. In this study, the peak time of the target was earlier than that of the GB method, which shortened the time by more than one time, and the response value was significantly higher than that of the GB method.

There was no significant difference in sample concentration obtained by T-test($P > 0.05$). According to table 2 to table 5, the standard recoveries of both the negative samples and the samples are above 90%, showing good recoveries. My research method has a good recovery rate in the samples with quantitative limit and detection limit concentration. T- test shows that the response value (peak area) of my research method is significantly greater than that of the GB method under the same sample pretreatment conditions($P < 0.05$). According to GB, when the sampling quantity is 2.00g and the constant volume is 1.00ml, the detection limit and quantitative limit are 0.0024g/kg and 0.0075g/kg respectively. In this comparison test, it can be seen from table 1 and table 3 that the GB method does not respond to the addition of standard substances with detection limit and quantitative limit in negative samples, thus my research method has a good recovery rate. The GB method has no response until 4 times

 DOI: https://doi.org/10.30564/jesr.v2i3.1023

Table 2. Test of recovery rate of Sucralose in national standard in tea drink (negative sample) (n=4)

Standardized concentration (mg/L)	Area	Concentration (mg/L)	Average (mg/L)	Relative standard deviation (%)	Recovery (%)	Average recovery (%)	Relative standard deviation (%)
2.5	0	0	0	--	--	--	--
7.5	0	0	0	--	--	--	--
10	877	10.02	10.06	0.029	100.22	100.6	0.29
10	882	10.05			100.53		
10	887	10.08			100.85		
10	886	10.08			100.80		
20	2416	20.01	20.032	0.031	100.05	100.16	0.16
20	2417	20.02			100.10		
20	2426	20.08			100.39		
20	2417	20.02			100.09		
30	3961	30.04	30.01125	0.028	100.14	100.04	0.094
30	3950	29.97			99.91		
30	3957	30.02			100.06		
30	3956	30.01			100.04		

Note: 4 samples were taken with Standardized concentration of 2.5 mg/L and 7.5 mg/L.

Table 3. of recovery rate of Sucralose in national standard in carbon acidic drink (positive sample)

Items	Area	Concentration (mg/L)	Average (mg/L)	Relative standard deviation (%)	Recovery (%)	Average recovery (%)	Relative standard deviation (%)
Sample	81048	432.65	433.03	0.55	--	--	--
	81015	432.48			--		
	81220	433.57			--		
	81195	433.43			--		
Standardized concentration (mg/L)							
200	119114	634.77	633.66	0.75	100.87	100.32	0.38
	118828	633.25			100.11		
	118807	633.14			100.05		
	118872	633.48			100.23		
400	152537	812.24	812.71	0.59	94.80	94.92	0.15
	152748	813.36			95.08		
	152526	812.18			94.79		
	152692	813.06			95.01		

Table 4. Test of recovery rate of Sucralose in this study in tea drink (negative sample) (n=4)

Standardized concentration (mg/L)	Area	Concentration (mg/L)	Average (mg/L)	Relative standard deviation (%)	Recovery (%)	Average recovery (%)	Relative standard deviation (%)
2.5	783	2.45	2.41	0.054	97.96	96.53	2.1
2.5	787	2.47			98.72		
2.5	769	2.38			95.16		
2.5	764	2.36			94.28		
7.5	1850	7.58	7.59	0.040	101.05	101.24	0.53
7.5	1846	7.56			100.80		
7.5	1865	7.65			102.01		
7.5	1851	7.58			101.08		
10	2356	10.01	10.04	0.034	100.09	100.37	0.34
10	2357	10.01			100.13		
10	2371	10.08			100.83		
10	2363	10.04			100.44		
20	4448	20.07	20.12	0.058	100.34	100.60	0.29
20	4451	20.08			100.41		
20	4462	20.14			100.68		
20	4475	20.20			100.98		
30	6513	29.99	30.04	0.052	99.98	100.14	0.17
30	6533	30.09			100.31		
30	6531	30.08			100.27		
30	6514	30.00			100.01		

DOI: https://doi.org/10.30564/jesr.v2i3.1023

Table 5. Test of recovery rate of Sucralose in this study in carbon acidic drink (positive sample) (n=4)

Items	Area	Concentration (mg/L)	Average (mg/L)	Relative standard deviation (%)	Recovery (%)	Average recovery (%)	Relative standard deviation (%)
Sample	85682	433.00	432.90	0.61	--	--	--
	85793	433.56			--		
	85681	433.00			--		
	85499	432.07			--		
Standardized concentration (mg/L)							
200	128137	647.22	646.83	0.29	107.16	106.96	0.14
	128036	646.71			106.90		
	128064	646.85			106.97		
	128005	646.55			106.82		
400	167026	843.45	844.31	0.62	102.64	102.85	0.16
	167188	844.27			102.84		
	167282	844.74			102.96		
	167291	844.79			102.97		

detection limit is added. Therefore, my research method can fully meet the requirements of the GB method.

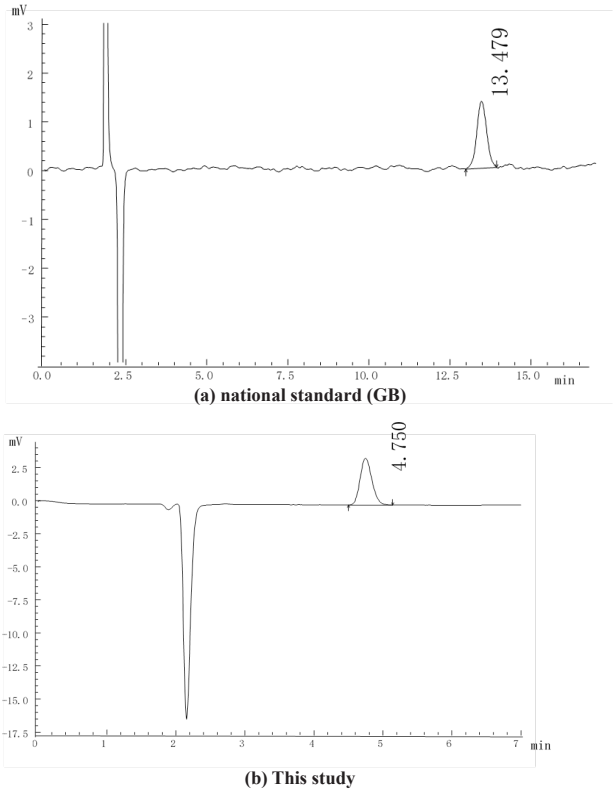

Figure 2. Chromatogram of Sucralose standard

3.2 Detection limit and quantitative limit

When the concentration of sucralose standard solution was 20 mg/L, the signal to noise ratio was 49.61.The detection limit of the method in this study is 0.0024 g/kg based on the calculation of 3-fold signal-to-noise ratio. It is basically consistent with the detection limit of 0.0025g /kg in the GB method. The minimum detection concentration of the sample is 0.0025g /kg, which can meet the needs of determination. The quantitative limit is 0.0081 g/kg.

3.3 Precision test

The same content sample was determined 6 times with standard deviation RSD of 3.6%, shown in table 6.

As shown in figure 3, the concentration of sucralose has a good linear relationship with the peak area, the concentration (X, mg/L) is the horizontal coordinate, the peak area (Y) is the vertical coordinate, the regression equation is Y=207.99X+274.1, and the correlation coefficient is 0.9999.The linear range is 20~400 mg/L.

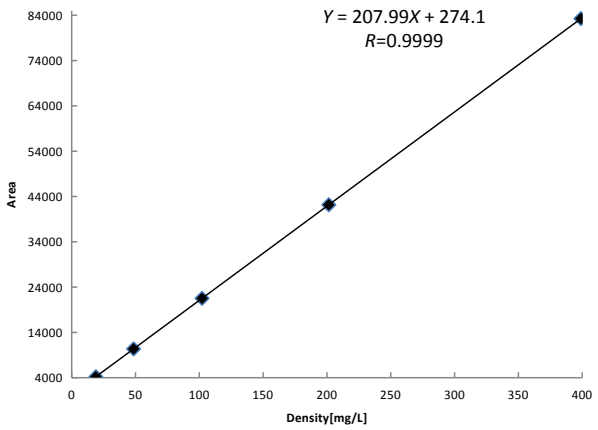

Figure 3. Calibration curve of sucralose

Table 6. Precision of the method for sucralose content in this study (n=6)

Standardized concentration (mg/L)	Area	Concentration (mg/L)	Average (mg/L)	Relative standard deviation (%)
200	41917	200.22	200.25	3.6
	41925	200.25		
	41917	200.21		
	41921	200.23		
	41934	200.30		
	41932	200.29		

 DOI: https://doi.org/10.30564/jesr.v2i3.1023

4. Conclusion

In this study, the optimized national standard method was used to detect sucralose in drink, which was stable in operation and could fully meet the needs of daily analysis in terms of precision, accuracy and detection limit, as well as compared with the national testing standards[16] and the results of the study of some scholars [11, 17-18], the analysis speed is accelerated, the sensitivity is improved, the toxicity and cost are reduced by replacing Acetonitrile with Methano, the stability is improved by adding phosphate , the balance of the detector is accelerated, the use of mobile phase is saved, the detection efficiency is improved, and the results of this study are consistent and suitable for daily testing of a large number of drink samples, which is consistent with those of Gan Bingbing [9] . And compared with the previous studies of Chen Xiaoxia[19], the column temperature and detection temperature are reduced, the loss of the column is reduced, and the service life of the column is extended. The author has also tried to use with the same water-based chromatographic of Wang Guihua [10] column in the study, although ultra-pure water is used as the flow is easier to prepare, but the peak time is relatively late, resulting in the waste of reagent, and the water-based chromatographic column on the pretreatment method has relatively strict requirements, improper treatment, will cause damage to the water-based chromatographic column, reduce the service life of the column, should not be promoted. In the future, we can further investigate whether this research method can be applied to the detection of other types of samples, and continue to optimize the pre-treatment method to reduce the time needed for the whole experimental cycle and improve the detection efficiency.

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DOI: https://doi.org/10.30564/jesr.v2i3.1023