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Quantitative Analysis Method of the Tea Saponin

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Abstract

In this study, the detection method of tea saponin has been studied firstly. Determining the maximum absorption wavelength is 540 nm. Standard curve equation is y = 0.0015x − 0.0885. The correlation coefficient r = 0.9983 ($p < 0.01$). The relative standard deviation is 1.13%. Reclaimable rate of adding standard sample is 89.5% - 97.7%. Comparing vanille-sulfuric acid of gravimetric determination, the maximum deviation is 3.27%, indicating that vanille-sulfuric acid method is worth of quantitative analysis of tea saponin.

Keywords

Vanillin-Sulfuric Acid Color Reaction, Saponin, Absorption Spectrum

1. Introduction

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produced when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. Tea saponin (extracted from tea seeds), a natural Non-ionic surfactant, not only has a good emulsifying, separating and dispersing capability but is also a good foaming and foam stabilizer with a great and cleaning capacity with a hydrophile-lipophilic balance value of 16 (Wu & Raven, 1998). Thus it can be used as foam stabilizer for the building concrete (Zhang et al. 1993), pesticide synergist (Lin, 1977), soil amendment (Hong et al. 2000), antioxidants (Ibrahim et al, 2006), Pesticides and surfactants (Kuo et al. 2005), so it is widely used in daily chemical industries, building materials, food industries and agriculture. Usually quantitative analyses of saponins are used by gravimetric method (General Administration of Quality Supervision,

2006). But gravimetric method detected saponins would spend more time as the complexity steps. So we explore a new colorimetric method for quantitative analysis of saponins. There are a lot of colorants that are used in colorimetric methods (Guo et al. 2011). In this paper, vanillin-sulfuric acid was selected as colorants. The principle of coloration is that saponins are dehydrogenated under the action of sulfuric acid, and then oxidized with vanillin to produce colored matter.

2. Material and Methods

2.1. Main Materials

The main materials used in this study were described in Table 1.

2.2. Main Equipments

The main instruments used in this study were described in Table 2.

2.3. The Preparation of Standard Solution (Omer et al., 2015)

Dissolve about 0.2500 g of tea saponin standard with 300 ml 85% ethyl alcohol in 500 mL dark glass volumetric flask and add alcohol up to volume, special solution 0.50 mg/ml.

2.4. Colour Reaction and Determination of Maximum Absorption Wavelength (Gu et al. 2000; Chen et al., 2012)

Add 1 ml of tea saponin standard solution with 1 ml of 8% vanillin (w/v) into 10 ml sample tube with a stopper, then put in ice-water bath, and then 8 ml of 77% sulfuric acid (v/v) was added. After shaking up, the tube was capped and placed in Oven at 60˚C for 30 min. Cool down the solution in ice-water bath for 10 min and reach solution to room temperature before UV analysis. Detecting the absorbance of the solution was used 1 cm quartz colorimetric utensil and ultraviolet-visible spectrophotometer full-band scanning. The blank solution 1:8

Table 1. Reagents.

	Grade	Manufacturer
Tea saponin standard	99%	Preparation by our lab
Crude tea saponin	≥50%	Preparation by our lab
Ethyl alcohol	Analytical pure	Guoyao chemical reagent Co. LTD
Methanol	Analytical pure	Guoyao chemical reagent Co. LTD
Sulfuric acid	Analytical pure	Guoyao chemical reagent Co. LTD
Hydrochloric acid	Analytical pure	Guoyao chemical reagent Co. LTD
Sodium hydroxide	Analytical pure	Guoyao chemical reagent Co. LTD
Acetone	Analytical pure	Guoyao chemical reagent Co. LTD
Vanillin	Analytical pure	Shanhai jingchun biochemical technology Co. LTD

Table 2. Equipments.

	Type	Manufacturer
Ultraviolet-visible spectrophotometer	UV-2550	Shimadzu corporation
Centrifuge	Avanti J-E	Beckman
Electronic balance	S-114	Sartorius
Vortex mixer	XW-80A	Haimen qilinbeier instrument manufacturing Co. LTD
Electro-thermostatic blast oven	DGG-9140	Shanghai senxin experimental instrument Co. LTD
Thermostat water bath	XMTE-8112	Shanghai jinghong experimental equipment Co. LTD
Vacuum pump	SHZ-D(Ⅲ)	Shanghai yuhua instrument equipment Co. LTD

(volume ratio) mixtures of vanillin solution and sulfuric acid were prepared as above.

2.5. Standard Curve Determination (Zhang et al., 2009)

Series of the standard solution (0.1 ml, 0.3 ml, 0.52 ml, 0.7 ml and 0.94 ml) were taken and the mixed solutions were prepared by the method described as above for measuring absorbance values. The resulting standard curve was assessed with Shimadzu Corporation software.

2.6. Precision Experiment (Chen, 2008)

Add 0.9 ml of tea saponin standard solution to each tube (five), and the each mass was recorded to 0.45 mg, then detect the absorbance of solutions using methods of 2.4 and 2.5. Through calculating standard deviation (S) and relative standard deviation (RSD) obtained the precision of this method.

2.7. Sample Added Recovery (Zhang et al., 2009)

Add respectively 0.1500, 0.2000, 0.2500, 0.3000 and 0.3500 mg saponin standard powder into 0.4 ml of tea saponin standard solution to each tube (five), then detect the absorbance of solutions using methods of 2.4 and 2.5 and calculate sample added recovery.

2.8. Stability Experiment (Chen et al., 2012)

Absorbance changes of 0.9 ml tea saponin standard solution of within 4 hour were detected using methods of 2.4 and 2.5.

2.9. Gravimetric Determination

Refer to SNT 1852-2006 Determination of saponin content in tea saponin for export.

2.10. Data Processing Method

The resulting standard curve was assessed with Microsoft excel. The evaluation of statistical significance was determined by the one-way ANOVA test, these analyses were done with SPSS for WINDOWS, version 19.0.

3. Results and Discussion

3.1. Absorption Spectrogram of Tea Saponin

Tea saponin is maximum absorption at the wave length about 540 nm (Figure 1), so we used the characteristic curve for the determination tea saponin.

3.2. Standard Curve Determination

According to the absorbance of the standard tea saponin solution and the standard curve (Figure 2) with vanillin sulfuric acid colorimetric method, which is obtained at the maximum absorption wavelength of 540 nm at different concentration of tea saponin, the regression equation is as follows: $y = 0.0015x - 0.0885$, $R^2 = 0.9983$ ($p < 0.01$). The tea saponin concentration is positively correlated with the absorbance value; it means this equation can be used as the quantitative determination of tea saponin concentration.

3.3. Precision Experiment

Precision drawing concentration of 0.5 mg/ml reference substance solution, 0.9 ml, sample 5 consecutive times, the measured values of tea saponin, S = 0.00507, RSD = 1.13% (Table 3).

3.4. Recoveries Experiment

The recovery rate of the sample after the labeling was between 89.5% and 97.7% (Table 4). It is indicated that the veracity of the vanille-sulfuric acid method can meet the general analysis requirements.

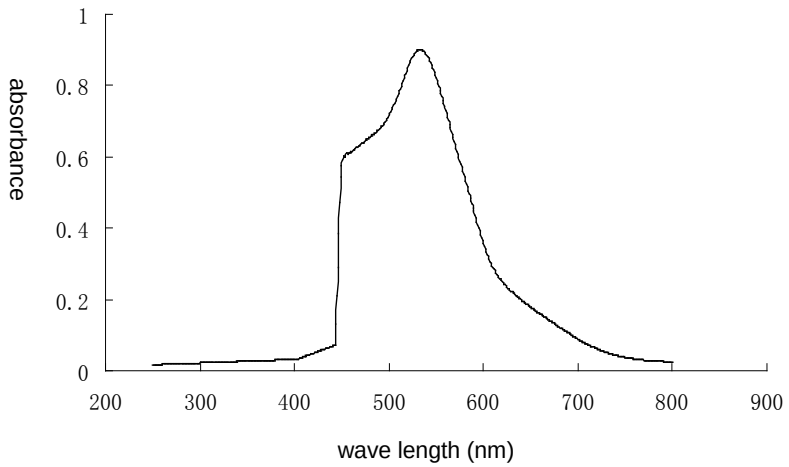

Figure 1. The absorption spectrum of tea saponin.

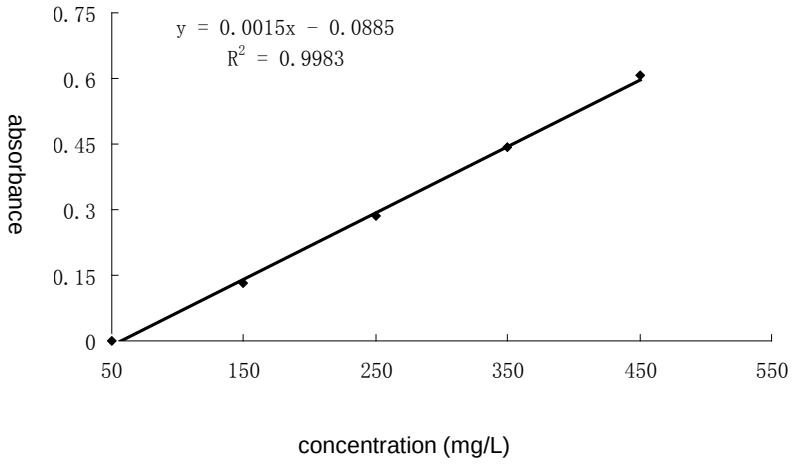

Figure 2. The standard curve of tea saponin.

Table 3. The results of precision experiment.

No.	Weight of tea saponin /mg	Determination weight of tea saponin /mg	S/mg	RSD/%
1	0.450	0.432	0.00507	1.13
2	0.450	0.424		
3	0.450	0.431		
4	0.450	0.438		
5	0.450	0.429		

Table 4. The results of recoveries experiment.

No.	Weight of tea saponin in first/mg	Additive amount/mg	Determination weight of tea saponin /mg	Recovery rate/%
1	0.200	0.153	0.338	90.2
2	0.200	0.205	0.397	96.1
3	0.200	0.252	0.441	95.6
4	0.200	0.294	0.463	89.5
5	0.200	0.345	0.537	97.7

3.5. Stability Experiment

The storage time is positively correlated with the absorbance value (**Figure 3**), the regression equation is as follows: $y = 0.0003x - 0.782$, $R^2 = 0.9983$ ($p < 0.01$). Precision drawing the first absorbance 0.7891 for the sample solution, each interval of sometime detected, a continuous inspection 4 h, the results of tea saponin absorbance RSD values of 1.62% (50 min) and 7.6% (4 h), indicating that the basic stability within 1 h.

3.6. Comparison of Two Detection Methods

Exactly the same conditions and samples, comparing vanille-sulfuric acid of gravimetric determination, the maximum deviation is 3.27% (**Table 5**), indicating

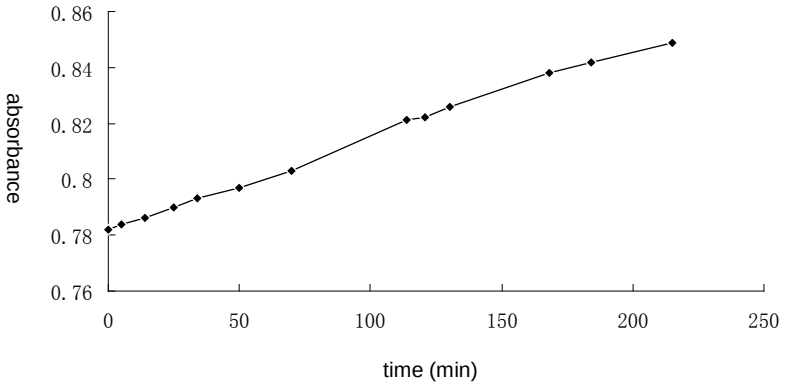

Figure 3. The result of stability test.

Table 5. The comparison of two detection methods.

Method	Weight of saponin/%			
	1	2	3	mean value
Gravimetric determination	56.97	57.12	56.63	56.91 ± 0.25
Vanille-sulfuric acid	59.05	58.59	59.31	58.98 ± 0.36

that vanille-sulfuric acid method is worth of quantitative analysis of tea saponin.

4. Conclusion

Quantitative analysis method of the tea saponin vanillin-sulfuric acid color reaction has been analyzed. Determining the maximum absorption wavelength is 540 nm. Standard curve equation is y = 0.0015x − 0.0885. The correlation coefficient r = 0.9991. The relative standard deviation is 1.13%. Reclaimable rate of adding standard sample is 89.5% - 97.7%. Comparing vanille-sulfuric acid of gravimetric determination, the maximum deviation is 3.27%, indicating that vanille-sulfuric acid method is worth of quantitative analysis of tea saponin.

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