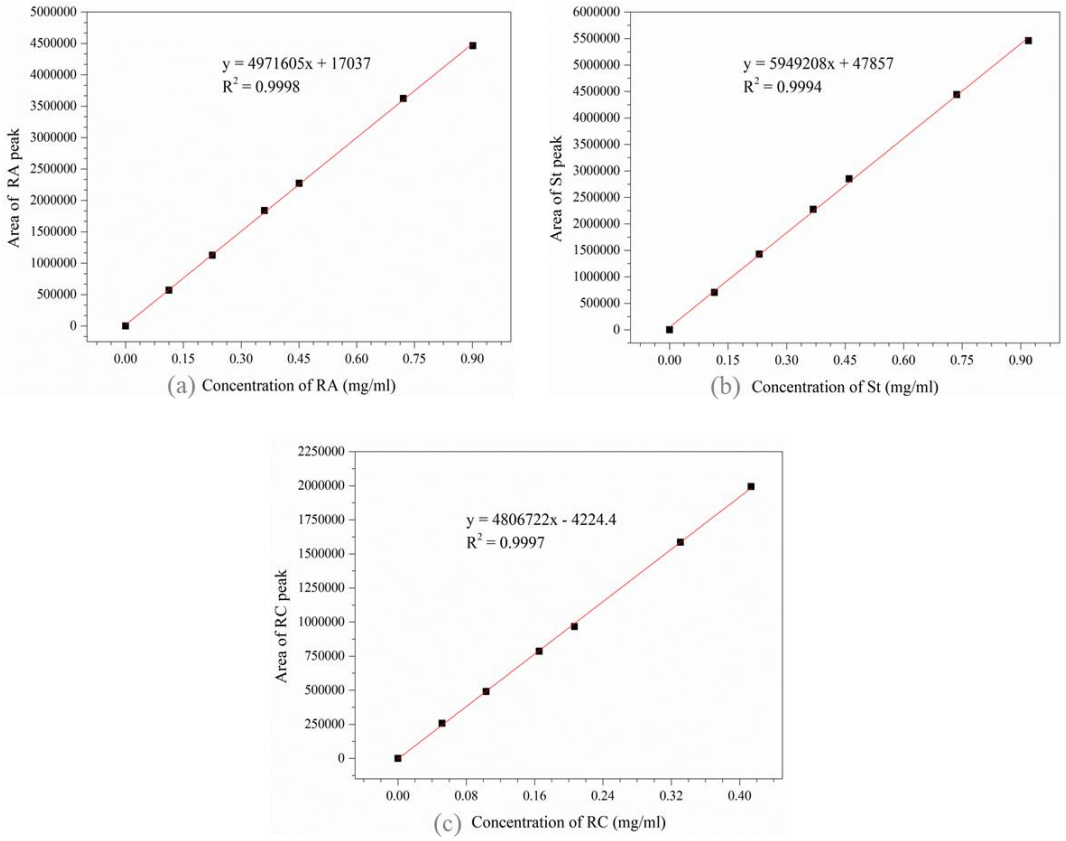

Figure S1. (a) Standard curves of RA (b) Standard curves of ST (c) Standard curves of RC

The analysis of the RSM

On the basis of single factor experiment, the concentration of crystallization solvent, solid-liquid ratio, temperature and time were selected to design the test scheme. The extraction conditions were optimized by response surface methodology to obtain the best conditions for crystallization of high purity RA from crude stevia. The influence of the interaction among the four selected factors on the experimental results was also explored.

Table S1. Test design table and test results of response surface method.

number	Crystallization time（h）	Crystallization temperature(℃)	Concentration of crystalline solvent（%）	Crystallization solid-liquid ratio	Purity of RA(%)
1	24	35	90	4	97.06
2	16	35	95	4	94.93
3	16	15	95	4	97.16
4	16	25	85	6	92.55
5	16	35	85	4	91.19
6	8	25	95	4	98.31
7	16	25	90	4	98.73

8	8	25	90	2	98.63
9	24	25	85	4	95.60
10	16	15	85	4	97.15
11	24	25	90	2	99.16
12	16	35	90	6	94.34
13	16	25	90	4	97.91
14	24	25	95	4	97.28
15	16	15	90	2	96.62
16	8	15	90	4	98.79
17	16	15	90	6	98.29
18	8	25	90	6	96.59
19	8	35	90	4	95.54
20	24	15	90	4	98.18
21	24	25	90	6	97.38
22	16	35	90	2	97.48
23	16	25	85	2	96.84
24	16	25	95	6	93.74
25	16	25	90	4	98.73
26	8	25	85	4	95.00
27	16	25	95	2	98.87

Table S2. Variance analysis of response surface test results.

Source	Sum of Squares	d f	Mean Square	F Value	p-value Prob > F	
Model	95.02	14	6.79	6.15	0.0016	significant
A-temperature	20.37	1	20.37	18.47	0.0010	
B-time	0.27	1	0.27	0.24	0.6306	
C-concentration of solvent	11.92	1	11.92	10.80	0.0065	
D- solid-liquid ratio	18.03	1	18.03	16.35	0.0016	
AB	1.15	1	1.15	1.04	0.3280	1.15
AC	3.47	1	3.47	3.15	0.1013	3.47
AD	5.78	1	5.78	5.24	0.0410	5.78
BC	0.66	1	0.66	0.60	0.4535	0.66
BD	0.016	1	0.016	0.014	0.9065	0.016
CD	0.17	1	0.17	0.16	0.6999	0.17

A^2	7.26	1	7.26	6.58	0.0248	7.26
B^2	0.18	1	0.18	0.16	0.6947	0.18
C^2	25.34	1	25.34	22.97	0.0004	25.34
D^2	2.59	1	2.59	2.35	0.1516	2.59
Residual	13.24	12	1.10			
Lack of Fit	12.79	10	1.28	5.72	0.1579	not significant
Pure Error	0.45	2	0.22			
Cor Total	108.26	26				

The results of variance analysis and credibility analysis of this regression model were shown in Table S1 and Table S2 respectively. Among them, F value and P value were used to judge the significance of the model and various variables to the response results. According to Fischer's theory, when the p value<0.05, it was significant in statistical analysis.

In this study, the quadratic regression model was significant (F=6.15，p=0.0016), and the loss of fit term was not significant (F=5.72，p=0.1579), indicating that the model was sically significant and no lack of fit existed.

The software was used to analyze the RSM experiment results in Table S2, and the quadratic regression model between purity and the four selected factors could be obtained.

$$S=98.46-1.30A+0.15B+1C-1.23D+0.54AB+0.93AC-1.20AD-0.41BC+0.063BD-0.21CD-1.17A^2+0.18B^2-2.18C^2-0.70D^2$$

Where S, A, B, C and D respectively represented RA purity, crystallization temperature, crystallization time, concentration of crystalline solvent crystallization solid-liquid ratio respectively.

The fitting equations of the process were analyzed by the experimental results, and the fitting equations at all levels were listed in Table S3. According to the data analysis in Table S3, the experiment conforms to the quadratic equation model, and the analysis of variance was shown in Table S2.

Table S3. Calculation of fitted equations

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-squared	
Linear	0.0060	0.0749	0.3705	0.2027	
2FI	0.6925	0.0655	0.3033	-0.2419	
Quadratic	0.0028	0.1579	0.7351	0.3102	Suggested

Cubic	0.0167	0.7928	0.9661	0.8352	Aliased

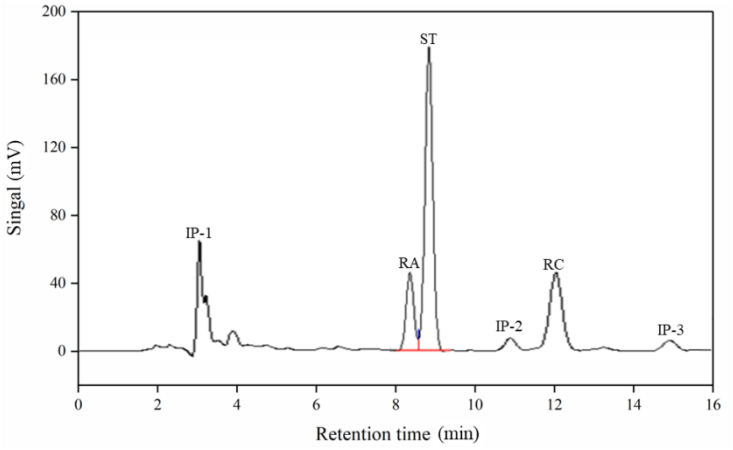

Figure S2. Chromatogram of the solid phase after second crystallization