

Separation and Determination of Cordycepin and Adenosine in Artificial Cordyceps Sinensis Mycelium by Gradient UPLC

Feie Fu^{1,*}, Ling Chen¹ and Qiue Cao²

¹School of Yunnan National Defense Industry Vocational College, Kunming 650500, China

²School of Yunnan University, Kunming 650000, China

*Corresponding author e-mail: 280318754@qq.com

Abstract. Uracil, guanine, adenine, guanosine, adenosine, cordycepin are the most important bases in Cordyceps sinensis, but the separation and analysis of this compound is not reported. After optimizing the composition of mobile phase, program of gradient elution and flow rate, the essay used methanol-water as mobile phase, gradient elution way, flow rate 0.3mL/min, detected at 260nm, the injection volume was 3 μ L, column temperature was 40°C. The six matters could be easily determined in 10min by UPLC. This method is used to determinate the content of the bases in the artificial cordyceps, results show that the method is simple, rapid and high sensitivity, reproducible and practical value.

1. Introduction

Cordyceps sinensis is a complex of pedicel fungus parasites and larval corpses on the larvae of bat moth family Cordyceps bat moth larvae. It is a valuable medicinal material for the human body, digestive system, immune system, circulatory system, respiratory system, urinary system, endocrine system, nervous system and anti-tumor, anti-oxidation and other aspects have an important role. In order to solve the problem of natural Cordyceps sinensis, people used the liquid deep culture method to produce Cordyceps sinensis mycelium similar to the pharmacology, toxicology and composition of Cordyceps sinensis to replace the natural Cordyceps sinensis [1, 2].

The chemical components of Cordyceps sinensis mainly include amino acids, nucleosides, sterols, sugar alcohols, polysaccharides, fatty acids, esters, alkanes, vitamins, polyamines, and inorganic elements [3-5], among which nucleoside compounds it is the main active ingredient in Cordyceps militaris and its culture. In nucleoside compounds, cordycepin (a nucleoside antibiotic) has an inhibitory effect on a variety of tumors [4]. It is considered to be the most important component of Cordyceps sinensis and is an indicator component of quality control of Cordyceps sinensis. At present, there have been many reports on the separation and analysis of cordycepin and other nucleoside compounds in Cordyceps sinensis and its artificial cultures [6-8], of which chromatography is most commonly used [9-10]. However, in the existing chromatographic analysis methods, conventional high performance liquid chromatography, which is frequently used, has a long analysis time, furthermore, simultaneous isolation of uracil, guanine, adenine, guanosine, adenosine, and cordycepin in Cordyceps sinensis and its artificial cultures was not involved.



This article selected the major nucleosides of uracil, guanine, adenine, guanosine, adenosine and cordycepin contained in *Cordyceps sinensis* as the analysis objects. After optimization of the chromatographic conditions, a method for the determination of these nuclei was established. Gradient ultra-high performance liquid chromatography of glycosides, and the method was applied to the determination of the contents of various substances in artificial *Cordyceps sinensis* mycelia, it is proved that the method has the advantages of rapidity, simplicity, high sensitivity and good reproducibility, and has good practical application value.

2. Experimental section

2.1. Major Instruments and Reagents

The chromatographic analysis was performed using a Waters Acquity Ultra Performance LC system and a Waters Acquity UPLC BEH C18 column (2.1×50mm, 1.7μm) and an Empower chromatography station.

Uracil, guanine, adenine, guanosine, adenosine, cordycepin were supplied by Alfa Aesar, USA; methanol (chromatographically pure) was Fisher's product. The experimental water was Epson water UP water. All reagent solutions including water were filtered using a 0.2μm microfiltration membrane before use.

2.2. Chromatographic conditions

Column: Waters Acquity UPLC BEH C18 column (2.1×50mm, 1.7μm); mobile phase is methanol/water, the elution mode is gradient elution, the gradient program is: initial volume fraction of methanol is 0.5%, its volume The scores remain constant from 0 to 3 min, linearly increasing from 3 to 4.3 min to 10%, linearly increasing from 4.3 to 4.5 min to 15%, maintaining for 3.5 min, returning to the initial mobile phase from 8.0 to 8.5 min, balancing 1.5 Min; flow rate: 0.1mL/min; PDA detector detection wavelength: 260 nm; injection volume: 3μL; column temperature: 30°C; sampling rate: 40 points/s.

3. Results and Discussion

3.1. Selection of detection wavelength

The ultraviolet absorption spectra of uracil, guanine, adenine, guanosine, adenosine, cordycepin aqueous solution were measured. The six substances absorb at a wavelength of 260nm. Therefore, in order to simplify the measurement method and obtain better detection sensitivity, 260nm is selected as the measurement wavelength.

3.2. Optimization of Chromatographic Separation Conditions

The composition of the mobile phase, the elution method, the elution procedure, and the flow rate were optimized to obtain the optimal chromatographic conditions. Column: Waters Acquity UPLC BEH C18 column (2.1×50mm, 1.7μm); mobile phase is methanol/water, the elution mode is gradient elution, the gradient program is: initial volume fraction of methanol is 0.5%, its volume The scores remain constant from 0 to 3min, linearly increasing from 3 to 4.3min to 10%, linearly increasing from 4.3 to 4.5min to 15%, maintaining for 3.5min, returning to the initial mobile phase from 8.0 to 8.5min, balancing 1.5min; flow rate: 0.1mL/min; PDA detector detection wavelength: 260nm; injection volume: 3μL; column temperature: 30°C; sampling rate: 40points/s.

Under the above selected optimum chromatographic conditions, the chromatogram of the resulting control mixture is shown in Figure 1. It can be seen that the six components have not only been effectively separated within 10 minutes, but also have a stable baseline and an ideal peak shape.

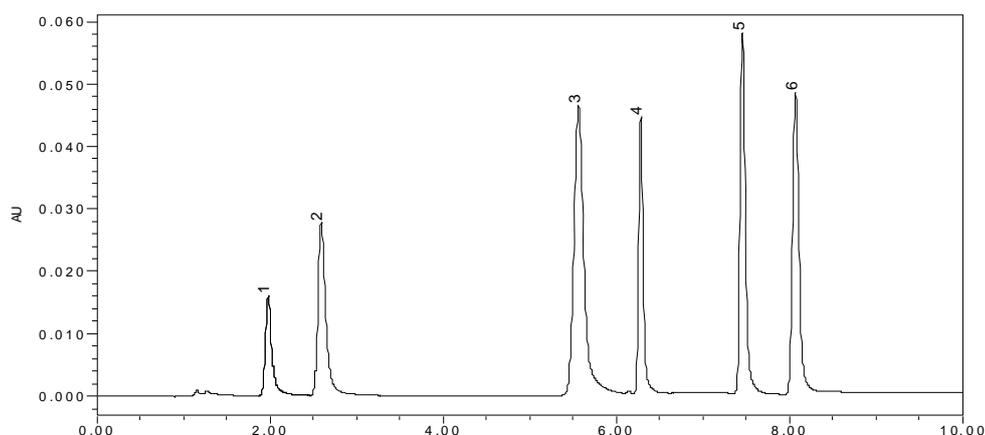


Figure 1. Chromatogram of a standard mixture under selected conditions

In the figure, peaks 1-6 are uracil, guanine, adenine, guanosine, adenosine, cordycepin.

3.3. Working curve and precision experiment

Table 1. Working curve and precision of the method

Components	Linear regression equation	<i>r</i>	Linear range (µg/mL)	<i>t</i> Mean value(n=5, min)	RSD(% n=5)	
					<i>t</i>	<i>A</i>
Uracil	$A = -4.746 \times 10^3 + 6.960 \times 10^7 C$	0.9999	9-80.0	1.99	1.35	0.39
Guanine	$A = -4.240 \times 10^3 + 4.081 \times 10^7 C$	0.9999	6-92.0	2.61	1.54	1.42
Adenine	$A = -4.299 \times 10^3 + 9.483 \times 10^7 C$	0.9996	5.0-120.0	5.56	0.67	0.31
Guanosine	$A = -7.776 \times 10^3 + 3.542 \times 10^8 C$	0.9991	6.0-110.0	6.44	0.21	0.21
Adenosine	$A = -6.279 \times 10^3 + 5.653 \times 10^7 C$	0.9998	4.0-150.0	7.47	0.09	0.25
Cordycepin	$A = -1.142 \times 10^3 + 6.091 \times 10^7 C$	0.9998	5.0-100.0	8.07	0.05	0.39

*A in the linear regression equation is the peak area; C is the concentration of each component, and the unit is µg/mL.

Take uracil, guanine, adenine, guanosine, adenosine, cordycepin standard solution diluted with water to a series of different concentrations of mixed standard solution, determined under the selected optimal chromatographic conditions, repeated injections 3 times, taking the peak area (A) of each component as the ordinate and the corresponding concentration C(µg/mL) as the abscissa for regression analysis, the regression equation, correlation coefficient, and linear range of each component were calculated. At the same time, the concentrations of the fixed components were all 100µg/mL, and the injection was repeated 5 times. The precision of the method was verified. The working curve of the method and the relative standard deviations (RSDs) of retention time *t* and peak area *A* for each component are shown in Table 1. It can be seen that the method has the characteristics of wide linear range, good reproducibility, high sensitivity, and high analysis speed. The RSD values of the retention time and peak area of each component are determined to be below 2.0%.

3.4. Sample Determination

Using methanol as the solvent, ultrasonic extraction method was used to extract the two kinds of artificial *Cordyceps sinensis* mycelia samples, and the separation and determination were carried out under the above selected optimal chromatographic conditions. The qualitative identification of each chromatographic peak was performed by adding method. The extracts obtained by ultrasonic methanol extraction of each sample were analyzed by external standard method. The results are shown in Table 2. In order to further verify the reliability of the analysis results, the spike recovery test was performed on each sample. The results of the recovery rate are shown in Table 3.

As can be seen from Table 2 and Table 3, the recoveries of all components in each sample were between 94% and 107%, and the relative standard deviations (RSDs) of the results of the quantitative determination and the spike recovery were all 4.0%. Within, it shows that the method has good precision, and the measurement result is accurate and reliable. The results in Table 2 also show that all extracts of two artificial *Cordyceps sinensis* mycelium prepared by different techniques contained uracil, guanine, adenine, guanosine, adenosine and cordycepin.

Table 2. Results of Determination of the Components in the Sample (n=3)

sample	Uracil		Guanine		Adenine		Guanosine		Adenosine		Cordycepin	
	content(mg/g)	RS D (%)										
1	0.1246	0.19	0.07533	0.44	0.1016	0.07	0.1105	0.67	0.1802	0.20	0.09997	0.16
2	0.01360	0.52	0.01115	0.66	0.03205	0.88	0.01364	0.88	0.03727	1.28	0.0206	1.13

Samples 1 and 2 are artificial *Cordyceps sinensis* mycelium samples prepared by different processes

Table 3. Results of determination of recovery of each component in the sample (% , n=3)

sample	Uracil		Guanine		Adenine		Guanosine		Adenosine		Cordycepin	
	Recovery rate	RS D										
1	99.0	0.07	101.8	0.05	103.2	0.09	101.5	0.06	100.6	0.03	100.8	0.31
2	103.0	1.22	101.8	0.34	95.2	0.35	102.6	0.80	95.8	0.17	98.5	0.31

Sample 1 and Sample 2 are in the same table 2

4. Conclusion

After optimizing the mobile phase and gradient elution procedures, an analytical method for the determination of uracil, guanine, adenine, adenosine, guanosine, and cordycepin by gradient elution ultra-high performance liquid chromatography was established. The extraction efficiency of these six substances in two artificial *Cordyceps sinensis* mycelia by different extraction methods was studied. The results show that the ultrasonic water extraction method is conducive to the elution of the components to be measured from the sample. The established chromatographic analysis method has the advantages of simplicity, rapidness, good reproducibility, and has practical application value.

Acknowledgments

This work was financially supported by Chen Ling and Cao Qiue .Thank you for Chen Ling and Cao Qiue

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