Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2021, **19**(12): 954-960
doi: 10.1016/S1875-5364(22)60162-6
Chinese Journal of Natural Medicines

•Research article•

Development of an eco-friendly and fast HPLC method for quantitative analysis of four nucleosides in Cordyceps and related products

QIAN Zheng-Ming[1, 2], WU Zi[2], HUANG Qi[2], WANG Chuan-Xi[1], TAN Guo-Yin[2], LI Wen-Jia[2], SUN Wan-Yang[1], LV Gen-Pin[2*], GAO Hao[1*]

[1] *Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China;*

[2] *Ruyuan HEC Pharm Co., Ltd., Shaoguan 512700, China*

Available online 20 Dec., 2021

[ABSTRACT] An eco-friendly and fast HPLC method was developed for the determination of adenosine, inosine, guanosine and uridine in Cordyceps and related products (fermented mycelia of *Hirsutella sinensis* and *Paecilomyces hepiali*). The sample was ultrasonically extracted using 0.5% phosphoric acid solutions for 2.5 min. Sample separation was performed on a Poroshell SB-Aq column (50 mm × 4.6 mm, 2.7 μm) using eco-friendly mobile phase consisting of formic acid and ammonium formate aqueous solution at a flow rate of 1.0 mL·min^{-1}. The detection wavelength was 260 nm. The developed HPLC method showed good linearity with correlation coefficients of 1.0000 in the test range. Good precision, repeatability and stability of this method were also observed (RSD ≤ 2.81%). The recovery ranged from 91.84%−105.19% (RSD ≤ 2.59%). Compared with reported methods, the current method did not use harmful organic solvent and took only 10.5 min. It obtained a high eco-score of 91 by the "Analytical Eco-Scale" tool. The developed method is eco-friendly and fast, which is suitable for the quality evaluation of Cordyceps and related products.

[KEY WORDS] Adenosine; Inosine; Guanosine; Uridine; Cordyceps

[CLC Number] R917 **[Document code]** A **[Article ID]** 2095-6975(2021)12-0954-07

Introduction

Cordyceps (*Dong Chong Xia Cao* in Chinese) is a well-known traditional Chinese herbal medicine. It is a composite consisting of the stromata of the fungus *Cordyceps sinensis*, parasitized on the larva of some species of Hepialidae insects, and the dead caterpillar, and traditionally used to "invigorate the lung and nourish the kidneys" for thousands of years [1-3]. Cordyceps is only distributed on the Tibetan Plateau and its surrounding regions at an altitude above 3 000 m, and its resources are scarce. Thus, its related products, fermented mycelia of *Hirsutella sinensis* and *Paecilomyces hepialid*, are developed. Recently, scientific studies have proved that nucleosides are one of the important bioactive components in Cordyceps and related products [4-7]. The major nucleosides are adenosine, inosine, guanosine and uridine, which are used as the markers for quality evaluation [6].

To date, several HPLC methods for determination of these nucleosides in Cordyceps and related products have been established [8-10]. The HPLC separations are performed on common C_{18} columns (150 or 250 mm length) with organic mobile phase (methanol−water or acetonitrile−water), requiring amounts of organic solvents and long-time analysis. Recently, eco-friendly HPLC methods have attracted increasing attention, especially using rapid LC columns to reduce the consumption of organic solvents and replacing organic mobile phase with environmentally benign ones [11]. Rapid LC methods, including UPLC and core-shell LC, are developed to reduce the consumption of organic mobile phase and shorten analytical time [12-14], but harmful organic solvents (e.g. methanol) are still used as mobile phase [13, 15]. In contrast, utilizatinon of an eco-friendly solvent as mobile phase is an alternative greener method. For instance, a micellar HPLC method was developed for the analysis of nucleosides,

[Received on] 29-Apr.-2021

[Research funding] This study was supported by the National Natural Science Foundation of China (Nos. 81925037, 82073976, and 31700013), the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (No. 2017BT01Y036), Guangdong Natural Science Funds for Distinguished Young Scholar (No. 2021B1515020065), the Young Elite Scientists Sponsorship Program by the China Association of Chinese Medicine (No. QNRC2-B06), and the Dongguan Academician Workstation Project (No. DGYSZ-2018-03).

[*]**Corresponding author]** E-mails: dyglee@163.com (LV Gen-Pin); tghao@jnu.edu.cn (GAO Hao)

These authors have no conflict of interest to declare.

in which the mobile phase simply consisted of aqueous surfactant solution [16, 17]. Furthermore, a high concentration of surfactant solution is easy to dissolve out in practical application, which may damage the column and LC instrument. However, a low concentration of buffer salt may change the elution capability of aqueous mobile phase for analytes, which is be a good choice for the analysis of nucleosides. Therefore, it may be a good strategy to develop an HPLC method for the analysis of nucleosides in Cordyceps and related products using a core-shell column and buffer salt aqueous solution as mobile phase for the purpose of green analytical chemistry.

In the present study, an eco-friendly and fast method for determination of adenosine, inosine, guanosine and uridine in Cordyceps and related products was established. Moreover, the "Analytical Eco-Scale" tool was applied to assess the greenness of the proposed HPLC method.

Methods

Chemicals and materials

Uridine (99.5%, 110887-201803), inosine (98.6%, 140669-201606), guanosine (93.6%, 111977-201501), and adenosine (99.7%, 110879-201703) were purchased from the National Institutes for Food and Drug Control (Beijing, China). Formic acid (HPLC grade), phosphoric acid (HPLC grade), and ammonium formate (HPLC grade) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Acetic acid (HPLC grade), ammonium acetate (analytical grade), and phosphoric acid (analytical grade) were purchased from Xilong Scientific Co., Ltd. (Sichuan, China). Monopotassium phosphate (analytical grade) was provided by Guangdong Guanghua Sci-Tech Co., Ltd. (Guangzhou, China). Deionized water was obtained by the Milli-Q purification system (Millipore, USA).

Cordyceps samples (s1−s11) were collected from Hubei, Qinghai, Sichuan and Xizang provinces. Related products, the fermented mycelia of *Hirsutella sinensis* and *Paecilomyces hepiali*, were also collected for comparative analysis. The fermented mycelia of *Paecilomyces hepiali* powder (FMPHP, s12 and s13) was purchased in Jiangxi Province. The fermented mycelia of *Hirsutella sinensis* powder (FMHSP, s14 and s15) was purchased in Guangdong Province. The botanical origin of materials was identified by Dr. QIAN Zheng-Ming. Voucher specimens were deposited at the Ruyuan HEC Pharma Co., Ltd., Shaoguan, China.

Preparation of reference standard solution

Reference standards stock solutions of uridine, inosine, guanosine and adenosine were prepared with 0.5% phosphoric acid solution. The resultant stock solutions were further diluted with 0.5% phosphoric acid solution to obtain a series of reference working solutions.

Preparation of sample solution

Ultrasonic extraction: The powder of samples (0.1 g) was mixed with 1.0 mL of 0.5% phosphoric acid solution and ultrasonically extracted at an ambient temperature for 2.5 min. Then the extraction solution was cooled down to room temperature and the lost weight was compensated with 0.5% phosphoric acid solution.

Reflux extraction: The powder of samples (1.0 g) was mixed with 10.0 mL of 0.5% phosphoric acid solution and reflux extracted at 100 °C for 30.0 min. Then the extraction solution was cooled down to room temperature and the lost weight was compensated with 0.5% phosphoric acid solution.

All extraction solution was filtered through a 0.2 μm filter (Agilent, California, USA) before HPLC analysis.

HPLC conditions

HPLC analysis was performed on the Agilent Series 1260 LC system (Agilent Technologies, USA) consisting of a quaternary pump, a thermostatic column compartment, an auto sampler and a diode array detector (DAD). An Agilent Poroshell 120 SB-Aq column (50 mm × 4.6 mm, 2.7 μm) was utilized for sample analysis and the column temperature was 20 °C. The mobile phase was formic acid-ammonium formate buffer solution (0.1% formic acid : 5 mmol·L^{-1} ammonium formate = 75 : 25, pH 2.87) with a flow rate of 1.0 mL·min^{-1}. The detection wavelength was 260 nm. The injection volume was 1 μL.

Method validation

The current method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, repeatability and stability tests.

Linearity, LOD and LOQ

The solution of four reference components was prepared at a series of concentrations: from 1.00 to 626.35 μg·mL^{-1} for uridine, from 5.12 to 81.99 μg·mL^{-1} for inosine, from 0.98 to 615.42 μg·mL^{-1} for guanosine and from 1.07 to 668.47 μg·mL^{-1} for adenosine. The solution was analyzed for the evaluation of linearity. A standard curve was constructed by plotting the concentrations versus peak areas.

The LODs and LOQs were determined by reference component standards and recorded as the corresponding concentrations which gave signal-to-noise (S/N) ratio of approximately 3 and 10, respectively.

Precision, repeatability and stability

The intra- and inter-day assays were used to assess the precision of the established method. The intra-day precision was determined by analyzing the solution of reference components in six replicates within one day. The inter-day precision was determined by analyzing the solution of the same reference components in duplicate per day for consecutive three days. The relative standard deviation (RSD) was utilized as a measure of precision.

The repeatability of the established method was evaluated by Cordyceps sample which was extracted and the RSD of the results were tested for six times.

The stablity of sample solution was assessed by analyzing the solution of Cordyceps sample every 2 h within 24 h. Variation was evaluated by the RSD.

Recovery

A recovery test was used to evaluate the accuracy of the established method. Known amounts of four nucleosides

were added to approximate 0.05 g of Cordyceps powder (sample s2), and then extracted and analyzed by the established method. The recovery rates were calculated as 100% × (found amount – original amount)/spiked amount.

Results and Discussion

Optimization of ultrasound extraction conditions

Many methods concerning nucleoside extraction from Cordyceps were reported, such as maceration, reflux, soxhlet and ultrasonic extraction [6]. Compared with other methods, ultrasonic extraction is easy to perform and requires less energy and time. It is normally considered as a "green" and the most convenient extraction method [18, 19]. In the current study, both ultrasonic extraction and reflux extraction were compared. The results of two extraction methods were consistent, but the reflux extraction took more time (30 min). Therefore, ultrasound extraction was adopted and the parameters including extraction solvent, solid-liquid ratio and extraction time were studied.

It was reported that transformation existed during ultrasonic extraction of nucleosides from Cordyceps where water was applied as the solvent, and this process was inhibited by the addition of a certain concentration of an acid or organic solvent [20, 21]. Hence, the extraction effects were investigated, using different concentrations of phosphoric acid aqueous solutions (0%, 0.25%, 0.50%, 0.75%, and 1.00%) and different concentrations of methanol aqueous solutions (0%, 30%, 60%, and 90%). The results showed that the transformation of nucleosides was inhibited when the concentrations of phosphoric acid and methanol were not less than 0.5% and 60%, respectively. The content of the four nucleosides obtained by phosphoric acid aqueous solutions (0.50%, 0.75% and 1.00%) was equal to those obtained by methanol aqueous solution (60%). Accordingly, 0.50% phosphoric acid aqueous solution was chosen as the extraction solvent for the lowest cost and environmental protection.

Solid-liquid ratio (1 : 10, 1 : 20, 1 : 30, and 1 : 40) and extraction time (2.5, 5, 10 and 20 min) were further optimized. The results indicated that there was no obvious difference among different solid-liquid ratios and extraction times. Then, 1 : 10 solid-liquid ratio and 2.5 min of extraction time were chosen for preparation of Cordyceps samples in consideration of low consumption of solvents and time.

In sum, the optimal extraction conditions were listed as follows: extraction solvent, 0.5% phosphoric acid solution; solid-liquid ratio, 1 : 10; and extraction time, 2.5 min.

Optimization of HPLC conditions

Nucleosides, a class of polar compounds, are usually separated by 100% aqueous phase [10, 20]. Ordinary reversed phase columns may be damaged in 100% aqueous phase. However, Aq column, a special reversed phase column, is often used to separate polar compounds in 100% aqueous phase with perfect retention time and chromatographic peak. For example, the nucleosides in Cordyceps was separated on a SB-Aq column (250 mm × 4.6 mm, 5 μm) within 60 min [21]. Moreover, Poroshell columns provided rapid separation for a variety of compounds with low back pressure on a conventional LC system, and were used for quality evaluation of herbs [14, 15]. Thus, the Poroshell 120 SB-Aq column may be a good choice for rapid separation of nucleosides. Previous studies generally used water-methanol or water-acetonitrile as mobile phase for analyzing nucleosides in Cordyceps and related products [6, 7, 22]. To avoid using harmful organic solvents, we tested different aqueous solutions as mobile phase to separate uridine, inosine, guanosine and adenosine in Cordyceps on the Poroshell 120 SB-Aq column.

Three different mobile phase systems (formic acid-ammonium formate, acetic acid-ammonium acetate and phosphoric acid-monopotassium phosphate) were compared as shown in Fig. S1. The retention time of adenosine was about 16.5 min in the acetic acid-ammonium acetate system, while inosine and guanosine were eluted together in the phosphoric acid-monopotassium phosphate system. The formic acid-ammonium formate system showed good resolution and short retention time for the four nucleosides. Therefore, the formic acid-ammonium formate system was used for further investigation. Nucleosides are basic compounds which can exist as ions under acid conditions. Thus, the pH value of mobile phase may have a great influence on the retention time of nucleosides. The ratio of 0.1% formic acid (solvent A) and 5 mmol·L^{-1} ammonium formate solution (solvent B) (15% B pH 2.78, 25% B pH 2.87, 35% B pH 2.97 and 45% B pH 3.07) was tested as shown in Fig. S2. The 15% B condition required the shortest time (7 min) but generated poor separation of inosine and impurities, while the 25%, 35%, and 45% B conditions showed good separation of target components. Furthermore, the 25% B condition required less time than the 35% and 45% B conditions. Thus, the 25% B condition was chosen as the optimal condition because of the good separation of inosine and impurities and the short running time (8 min) of analytes. The optimum column temperature was also investigated. As shown in Fig. S3, a higher temperature led to faster separation, with a lower resolution of analytes and impurities. The resolutions of uridine and inosine with their impurities were poor when the column temperature was above 20 °C. Thus, the column temperature was set as 20 °C.

Method validation

The developed method was validated, as demonstrated in Tables 1 and 2. Four calibration curves showed good linear regression (R = 1.000) within test ranges, the LODs were less than 0.08 μg·mL^{-1} and the LOQs were less than 0.22 μg·mL^{-1}. The RSD of precision and repeatability were less than or equal to 2.81%. The analytes in sample solution were stable within 24 h (RSD ≤ 2.78%). The recovery of four analytes ranged from 91.84%−105.19% (RSD ≤ 2.59%). Therefore, the developed HPLC method was sensitive, accurate and precise for quantitative analysis of four nucleosides in the sample.

Table 1 Linearity, LODs and LOQs of the four nucleosides

Analytes	Regression equation	*R*	Linear range (μg·mL^{-1})	LOD (μg·mL^{-1})	LOQ (μg·mL^{-1})
Uridine	$y = 5.8552x - 0.4691$	1.0000	1.00−626.35	0.03	0.08
Inosine	$y = 6.5327x + 0.0435$	1.0000	5.12–81.99	0.07	0.21
Guanosine	$y = 5.8540x - 3.8968$	1.0000	0.98–615.42	0.04	0.12
Adenosine	$y = 7.9550x - 4.5562$	1.0000	1.07–668.47	0.06	0.17

Table 2 Precision, repeatability, stability and recovery of the four nucleosides

Analytes	Precision (RSD %)		Repeatability (RSD %, $n = 6$)	Stability(RSD %, 24 h)	Recovery (RSD %, $n = 6$)
	Intra-day ($n = 6$)	Inter-day ($n = 6$)			
Uridine	2.27	2.56	2.03	1.90	91.84% (2.35)
Inosine	1.69	2.81	2.48	2.78	105.19% (2.34)
Guanosine	0.85	2.47	1.32	0.85	103.57% (2.07)
Adenosine	0.22	0.49	1.40	0.47	100.32% (2.59)

Analysis of samples

Totally, 11 batches of Cordyceps (s1−s11) and 4 batches of related product powder (s12−s15) samples were analyzed according to the developed method. The representative chromatograms of reference standards and Cordyceps samples were illustrated in Fig. 1. The amounts of the four nucleosides in Cordyceps and related product samples were listed in Table 3. The content of the four nucleosides in Cordyceps samples (s1−s11) ranged from 0.22−0.66 mg·g^{-1} (uridine), 0.20−0.46 mg·g^{-1} (inosine), 0.08−0.27 mg·g^{-1} (guanosine) and 0.21−0.48 mg·g^{-1} (adenosine), respectively. The total content ranged from 0.94−1.70 mg·g^{-1}, which was consistent with previous reports [9, 23]. Notably, the total content of the four nucleosides in FMHRP and FMPHP (s12−s15) was much higher than that in Cordyceps. This difference can be used to distinguish Cordyceps from the fermented mycelia powder as previously reported [24, 25].

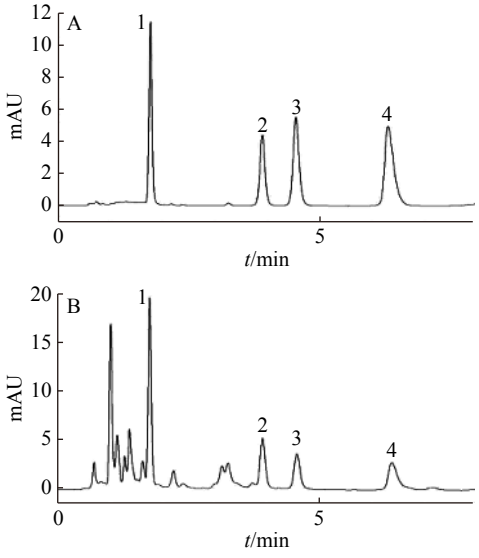

Fig. 1 HPLC chromatograms of reference standards (A) and Cordyceps sample s2 (B) 1-Uridine; 2-Inosine; 3-Guanosine; 4-Adenosine

Comparison of the current and reported methods

Several HPLC and UHPLC methods (Methods I, II and III) for analyzing the four nucleosides in Cordyceps was reported before the present study [13, 15, 26]. The "Analytical Eco-Scale" tool was used to evaluate the greenness of the proposed method and reported ones [27]. The "Analytical Eco-Scale" tool was regarded as an excellent quantitative tool for the greenness assessment [28]. In this evaluation system, a higher score for an analytical method means the method is more eco-friendly. The result of the "Analytical Eco-Scale" evaluation was shown in Table 4. Method I using a long C_{18} column (250 mm) got a low score of 78, which employed a lot of poisonous solvents and produced large amounts of waste [26]. Method II received the lowest score of 77 because more harmful organic solvent was consumed and more waste was produced during sample preparation [13]. Method III combining HPLC with Poroshell column scored a high point of 87, which reduced the consumption of organic solvent during sample extraction and HPLC separation [15]. The proposed HPLC method got the highest Eco-Scale score of 91, indicating that this method is the greenest among the methods. In the current HPLC method, harmful organic solvent was replaced by aqueous solution. Thus, less waste was produced. These results suggested that the current method is an eco-friendly HPLC method for the analysis of four nucleosides in Cordyceps and related products.

Due to the complicated components, analysis of Chinese herbal medicine is usually time-consuming, especially during the process of sample preparation and LC analysis [29, 30]. Most of the currently available methods for analyzing four nucleosides in Cordyceps required more than 60 min. For example, a HPLC method developed by Xiao [26]required 55

Table 3 The contents of analytes in samples

No.	Name	Collection place	Uridine (mg·g^{-1})	Inosine (mg·g^{-1})	Guanosine (mg·g^{-1})	Adenosine (mg·g^{-1})	Total nucleosides (mg·g^{-1})
s1	Cordyceps	Hubei	0.22 ± 0.002	0.20 ± 0.032	0.08±0.001	0.48±0.001	0.98
s2			0.46 ± 0.009	0.31 ± 0.001	0.21±0.003	0.34±0.003	1.32
s3			0.53 ± 0.002	0.46 ± 0.009	0.22±0.001	0.21±0.000	1.42
s4			0.66 ± 0.002	0.40 ± 0.006	0.27±0.002	0.37±0.001	1.70
s5			0.39 ± 0.002	0.39 ± 0.005	0.17±0.001	0.24±0.002	1.19
s6			0.35 ± 0.004	0.33 ± 0.007	0.26±0.006	0.41±0.003	1.35
s7			0.29 ± 0.002	0.35 ± 0.003	0.20 ± 0.012	0.28 ± 0.012	1.12
s8			0.23 ± 0.001	0.24 ± 0.002	0.25 ± 0.020	0.30 ± 0.001	1.02
s9		Qinghai	0.52 ± 0.001	0.10 ± 0.000	0.18 ± 0.025	0.22 ± 0.001	1.02
s10		Sichuan	0.65 ± 0.008	0.10 ± 0.000	0.29 ± 0.011	0.32 ± 0.000	1.36
s11		Xizang	0.46 ± 0.004	0.11 ± 0.001	0.08 ± 0.000	0.29 ± 0.002	0.94
s12	FMPHP	Jiangxi	3.13 ± 0.021	0.03 ± 0.002	2.50 ± 0.019	2.26 ± 0.008	7.92
s13			3.76 ± 0.185	0.07 ± 0.004	2.89 ± 0.129	2.54 ± 0.122	9.26
s14	FMHSP	Guangdong	1.27 ± 0.013	N\A	1.03 ± 0.001	1.36 ± 0.014	3.66
s15			2.54 ± 0.015	N\A	2.14 ± 0.041	2.37 ± 0.031	7.05

*Data are expressed as mean ± standard deviation (n = 2)

Table 4 Comparison of the proposed method and reported methods

Method	Analytical Eco-Scale		Penalty points	References
Proposed Method	Reagents	Formic acid	2	N\A
		Phosphoric acid	2	
		Ammonium formate	0	
	Instruments	Sonicator	1	
		LC	1	
		Occupational hazards	0	
		Waste	3	
	Total penalty points		9	
	Eco-Scale		91	
Method Ⅰ	Reagents	Methanol	6	[26]
		Acetic acid	4	
	Instruments	Sonicator	1	
		Centrifuge	1	
		LC	1	
		Occupational hazards	0	
		Waste	5	
	Total penalty points		18	
	Eco-Scale		78	

Continued

Method	Analytical Eco-Scale		Penalty points	References
Method Ⅱ	Reagents	Methanol	6	[13]
		Acetic acid	4	
		Acetonitrile	4	
	Instruments	ASE	2	
		UHPLC	0	
		Rotary evaporator	2	
		Occupational hazards	0	
		Waste	5	
	Total penalty points		23	
	Eco-Scale		77	
Method Ⅲ	Reagents	Formic acid	2	[15]
		Acetonitrile	4	
	Instruments	Sonicator	1	
		LC	1	
		Occupational hazards	0	
		Waste	5	
	Total penalty points		13	
	Eco-Scale		87	

min in sample extraction and 35 min in LC separation with a total of 90 min. An UPLC method developed by Yang [13] consumed only 15 min including sample extraction on an ASE system and LC separation on a UPLC system. The instruments used were expensive and unsuitable for wide application. In the current method, extraction was operated on an ultrasonic apparatus, and separation was performed on a normal HPLC system. The whole process took only 10.5 min, including 2.5 min of sample extraction and 8 min of HPLC separation. It was faster and more convenient compared with reported methods.

The current method was able to analyze the four nucleosides in Cordyceps and related product samples within 10.5 min, without the use of any harmful organic solvent, which is an eco-friendly and rapid method.

Conclusions

An eco-friendly and rapid method has been established, using Poroshell column with green mobile phase, for the determination of adenosine, inosine, guanosine and uridine in Cordyceps and related product samples. The newly developed method is proved to be accurate, sensitive and reliable. It can be used as an efficient alternative method for the determination of nucleosides, so as to improve the quality evaluation of Cordyceps and related products.

References

[1] Shashidhar MG, Giridhar P, Udaya Sankarb K, *et al*. Bioactive principles from *Cordyceps sinensis*: a potent food supplement–a review [J]. *J Funct Foods*, 2013, **5**(3): 1013-1030.

[2] Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People's Repulic of China*. Vol 4 [M]. Beijing, China Medical Science Press, 2020: 119.

[3] Zhan XT, Lai GP, Zhu DX. Study progress in pharmacological effects of *Cordyceps sinensis* and its ferment mycelia [J]. *Med Recapitulate*, 2012, **18**(10): 1566-1569.

[4] Yao CL, Qian ZM, Tian WS, *et al*. Profiling and identification of aqueous extract of *Cordyceps sinensis* by ultra-high performance liquid chromatography tandem quadrupole-orbitrap mass spectrometry [J]. *Chin J Nat Med*, 2019, **17**(8): 631-640.

[5] Li SP, Yang FQ, Tsim KWK. Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine [J]. *J Pharmaceut Biomed*, 2006, **41**(5): 1571-1584.

[6] Ai Z, Qian ZM, Li WJ, *et al*. Recent advances in the analysis of nucleosides in Chinese cordyceps [J]. *Mycosystema*, 2016, **35**(4): 388-403.

[7] Xiao JH, Qi Y, Xiong Q. Nucleosides, a valuable chemical marker for quality control in traditional Chinese medicine *Cordyceps* [J]. *Recent Pat Biotechnol*, 2013, **7**(2): 153-166.

[8] Fan H, Li SP, Xiang JJ, *et al*. Qualitative and quantitative determination of nucleosides, bases and their analogues in natural and cultured *Cordyceps* by pressurized liquid extraction and high performance liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) [J]. *Anal Chim Acta*, 2006, **567**(2): 218-228.

[9] Yang FQ, Li SP. Effects of sample preparation methods on the quantification of nucleosides in natural and cultured *Cordyceps* [J]. *J Pharmaceut Biomed*, 2008, **48**(1): 231-235.

[10] Yuan JP, Zhao S, Wang Y, *et al*. Distribution of nucleosides and nucleobases in edible fungi [J]. *J Agr Food Chem*, 2008,

56(3): 809-815.
[11] Assassi AL, Roy CE, Perovitch P, *et al*. Green analytical method development for statin analysis [J]. *J Chromatogr A*, 2015, **1380**: 104-111.
[12] Welch CJ, Wu N, Biba M, *et al*. Greening analytical chromatography [J]. *Trend Anal Chem*, 2010, **29**(7): 667-680.
[13] Yang FQ, Guan J, Li SP. Fast simultaneous determination of 14 nucleosides and nucleobases in cultured *Cordyceps* using ultra-performance liquid chromatography [J]. *Talanta*, 2007, **73**(2): 269-273.
[14] Tanaka N. Core−Shell, ultrasmall particles, monoliths, and other support materials in high-performance liquid chromatography [J]. *Anal Chem*, 2016, **88**(1): 279-298.
[15] Li WQ, Li WJ, Qian ZM, *et al*. Rapid analysis of polar components in *Ophiocordyceps sinensis* by conventional liquid chromatography system [J]. *Chin Herbal Med*, 2014, **6**(3): 217-221.
[16] Alwera V, Sehlangia S, Alwera S. A sensitive micellar liquid chromatographic method for the rectification of enantiomers of esmolol, and determination of absolute configuration and elution order [J]. *J Liq Chromatogr Related Technol*, 2020, **43**(17−18): 742-749.
[17] Kim YN, Brown PR. Micellar liquid chromatography for the analysis of nucleosides and bases [J]. *J Chromatogr*, 1987, **384**: 209-220.
[18] Iftikhar M, Zhang H, Iftikhar A, *et al*. Study on optimization of ultrasonic assisted extraction of phenolic compounds from rye bran [J]. *LWT-Food Sci Technol*, 2020, **134**: 110243.
[19] Vinatoru M, Mason TJ, Calinescu I. Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials [J]. *Trend Anal Chem*, 2017, **97**: 159-178.
[20] Qian ZM, Zhen DM, Li WQ, *et al*. Adenosine transformation pathway during water extraction of Chinese cordyceps [J]. *World Chin Med*, 2016, **11**(5): 758-762.
[21] Yang FQ, Li DQ, Feng K, *et al*. Determination of nucleotides, nucleosides and their transformation products in *Cordyceps* by ion-pairing reversed-phase liquid chromatography–mass spectrometry [J]. *J Chromatogr A*, 2010, **1217**(34): 5501-5510.
[22] Zhao J, Xie J, Wang LY, *et al*. Advanced development in chemical analysis of *Cordyceps* [J]. *J Pharmaceut Biomed*, 2014, **87**: 271-289.
[23] Zong SY, Han H, Wang B, *et al*. Fast simultaneous determination of 13 nucleosides and nucleobases in *Cordyceps sinensis* by UHPLC–ESI–MS/MS [J]. *Molecules*, 2015, **20 (12)**: 21816-21825.
[24] Zong SY, Han H, Duan R, *et al*. Quantitative analysis and evaluation of 13 nucleosides in cordyceps health care products by UHPLC-ESI-MS/MS [J]. *Chin Med Mat*, 2016, **39**(10): 2264-2267.
[25] Zhang WW, Gong T, Han DH, *et al*. Comparative research on components of cultured *Cordyceps militarise* and *Cordyceps sinensis* [J]. *Beijing J Tradit Chin Med*, 2016, **35**(1): 87-91.
[26] Xiao YC, Hu FZ, Dong Q, *et al*. Comparative study of fifteen kinds of nucleosides in *Cordyceps sinensis* from different origin of Yushu prefeuture, Qinghai province [J]. *Chin Pharm J*, 2014, **49**(22): 1983-1988.
[27] Gałuszka A, Konieczka P, Migaszewski ZM, *et al*. Analytical eco-scale for assessing the greenness of analytical procedures [J]. *Trend Anal Chem*, 2012, **37**: 61-72.
[28] Saleh SS, Lotfy HM, Tiris G, *et al*. Analytical tools for greenness assessment of chromatographic approaches: application to pharmaceutical combinations of indapamide, perindopril and amlodipine [J]. *Microchem J*, 2020, **159**: 105557.
[29] Li D, Xiao JQ, L WY, *et al*. Vitellaria paradoxa nutshells from seven sub-saharan countries as potential herbal medicines for treating diabetes based on chemical compositions, HPLC fingerprints and bioactivity evaluation [J]. *Chin J Nat Med*, 2019, **17**(6): 446-460.
[30] Ding PL, He CM, Cheng ZH, *et al*. Flavonoids rather than alkaloids as the diagnostic constituents to distinguish *Sophorae flavescentis* radix from *Sophorae tonkinensis* radix et rhizoma: an HPLC fingerprint study [J]. *Chin J Nat Med*, 2018, **16**(12): 951-960.

Cite this article as: QIAN Zheng-Ming, WU Zi, HUANG Qi, WANG Chuan-Xi, TAN Guo-Yin, LI Wen-Jia, SUN Wan-Yang, LV Gen-Pin, GAO Hao. Development of an eco-friendly and fast HPLC method for quantitative analysis of four nucleosides in Cordyceps and related products [J]. *Chin J Nat Med*, 2021, **19**(12): 954-960.

Dr. GAO Hao is the head of Institute of Traditional Chinese Medicine & Natural Products, Jinan University and the dean of Department of Chinese Materia Medica, College of Pharmacy from 2016. His main research interests focus on the effective substances of traditional Chinese medicines and bioactive natural products. He has authored 100+ scientific publications including Nat. Commun., Chem.Sci., Org. Lett., Chem.Commun., J. Nat. Prod. et al., and got 10+ licensed patents.