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Stability indicating method to determine bioactive nucleosides in crude drugs, extracts, and products from *Cordyceps sinensis* and *Cordyceps militaris*

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ABSTRACT

Objective: The aim of this study was to develop a stability indicating method to determine bioactive nucleosides including uridine, guanosine, adenosine, and cordycepin in crude drugs, extracts, and products from *Cordyceps sinensis* and *Cordyceps militaris* by reverse phase high performance liquid chromatography. **Methods:** The C_8 column (250 mm × 4.6 mm; i.d. 5 μm) was used, and the mobile phase was a mixture of water (A) and acetonitrile (B). The system was: 0-15 min, 1% B; 15-30 min, 1-15% B. The flow rate was 1 mL/min and the injection volume was 10 μL with ultraviolet detection at 254 nm. **Results:** The correlation coefficients of linearity were more than 0.9995 for uridine (0.56-11.20 μg/mL), guanosine (0.56-11.21 μg/mL), adenosine (1.13-11.30 μg/mL), and cordycepin (0.279-2.793 μg/mL). The intra- and inter-day precisions were less than 2% and 3%, respectively. The accuracy of the method was in the range of 96.65-100.64%. The studied nucleosides were stable to heat at 90°C for 12 h but were more degraded in 0.1 N H_2SO_4 and 3% H_2O_2 than 0.1 N NaOH, and sunlight. **Conclusion:** The developed method was found to be specific to uridine, guanosine, adenosine, and cordycepin in the presence of sample matrices and their degradation products and could be applied to assess the stability of crude drugs, extracts, and products from *C. sinensis* and *C. militaris*.

INTRODUCTION

Cordyceps sinensis and *Cordyceps militaris* are entomogenous fungi in class of Ascomycetes that have widely been used as an alternative medicine and dietary supplement [1,2]. *C. sinensis* has been applied in traditional Chinese medicine for hundreds of years to prolong vitality and provide treatment in cardiovascular, respiratory, and renal diseases [3,4]. Bioactive compounds from *Cordyceps* contain nucleosides, polysaccharides, sterols, and proteins [5]. Nucleosides are one of the main components in *Cordyceps* which demonstrates several therapeutic activities. Bioactive nucleosides consist of uridine, guanosine, adenosine, and cordycepin [6,7]. The structures are shown in Figure 1. Pharmacological actions of cordycepin include antineoplastic, anti-inflammatory, antiviral, antidiabetic, and antiischemic activities [8-19]. The pharmacological activities of other nucleosides include antidepressant-like activity and neurological involvement of uridine [20], anticonvulsant and anxiolytic effects of guanosine [21], and inflammatory regulator of adenosine [22,23].

The popularity of these fungi has increased and gained manufacturers' interest to produce extracts and products of

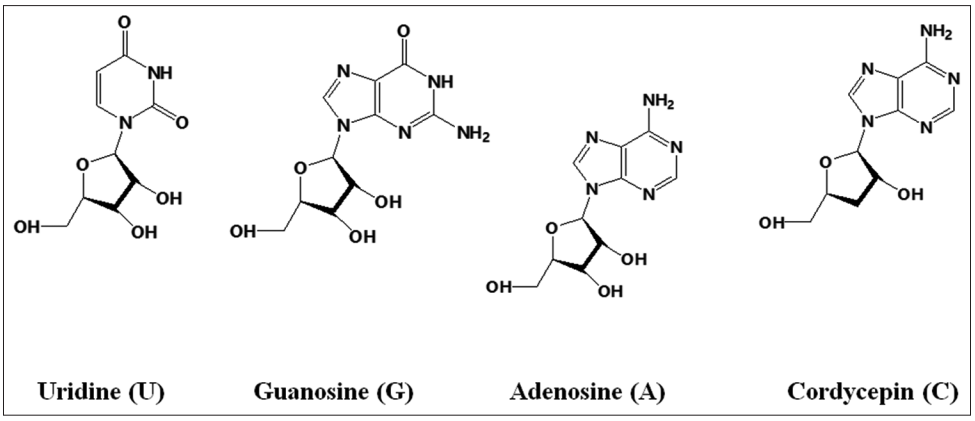

Figure 1: Chemical structures of nucleosides: Uridine (U), guanosine (G), adenosine (A), cordycepin (C)

these fungi from both natural and harvested sources [24-26]. *C. sinensis* and *C. militaris* have been marketed as a crude drug: An unprocessed and dried form, an extract: A spray-dried powder from an extracting solvent which is mostly water, a product: A dosage form that is formulated with other excipients to obtain a suitable dose [27]. The amounts of bioactive nucleosides in *Cordyceps* have been used as a chemical marker to represent therapeutic effects and quality of these medicinal fungi. The manufacturing process and storage condition could affect the stability and the amount of bioactive nucleosides in crude drugs, extracts, and products. To assure the quality and their shelf life, a stability indicating method is essential to separate and determine the amount of bioactive nucleosides.

Stability indicating method is defined as an analytical procedure that accurately and precisely measures active ingredients without interferences from impurities, excipients, and degradation products [28]. Various analytical methods have been developed to analyze nucleosides such as high performance liquid chromatography (HPLC) with ultraviolet (UV) detector, liquid chromatography with mass spectrometry, and capillary electrophoresis with mass spectrometry [29-36]. Most studies focused on qualitative and quantitative determination of nucleosides in crude drugs from different *Cordyceps* species [29,30,34,35,37,38]. To develop a stability indicating method, forced conditions for nucleoside degradation is required to test specificity of the developed method. Forced degradation is composed of acidic and alkaline hydrolysis, oxidation, photodecomposition, and thermal decomposition. A reverse phase HPLC method was selected in this study because it could provide both high sensitivity and selectivity to achieve the nucleoside separation in the presence of sample matrices and their degradation products. Moreover, mobile phase in reverse phase is considered to be low toxicity and convenient in waste management. Because adenosine and cordycepin are much more hydrophobic than uridine and guanosine, a gradient elution would be operated to overcome a fast analysis.

The objectives of this study were to develop and validate a reverse phase HPLC system with gradient elution as a stability indicating method to determine 4 bioactive nucleosides: Uridine, guanosine, adenosine, and cordycepin according to AOAC guidelines for single-laboratory validation of chemical methods for dietary supplement and botanicals [39].

METHODS AND MATERIALS

Chemicals

Reference standards of uridine, inosine, guanosine, adenosine, cordycepin (Sigma®, Germany) contained the purity of 100.0, 100.0, 99.2, 98.0, 99.7%, respectively. Uridine, guanosine, adenosine, and cordycepin were used as markers and inosine was used as a resolution solution to check system suitability. Ultrapure water was obtained freshly from the water purifier (Maxima®, England). Acetonitrile (Labscan®, Ireland) and methanol (Merck, Germany) were of HPLC grade. *C. sinensis* (Tek Sheng Hing. Co. Ltd., Thailand) was from a natural source, and *C. militaris* (Freshville Farm, Thailand) was grown from a laboratory. *Cordyceps*' tablets (Seven Star Pharmaceutical, Thailand) and capsules (Kress Pharma, Thailand) were manufactured in Thailand. Two *Cordyceps* extracts were purchased from Changsha Organic Herb in China and Kress Pharma in Thailand.

Instrumentation and HPLC Conditions

The analysis was performed on an HPLC system consisting of HPLC binary pump (YL clarity® YL 9111, Korea), YL clarity® autosampler (YL 9100, Korea), and UV detector (YL clarity® YL 9120, Korea). The column was ACE® Generix 5 C_8 column (250 mm × 4.6 mm; i.d. 5 μm). The flow rate of the mobile phase was set at 1 mL/min. The injection volume was 10 μL. The UV wavelength was 254 nm. The mobile phase included acetonitrile and water which was 1% acetonitrile for 15 min, and the percentage of acetonitrile was increased to 15% over the following 15 min. The total run time was 30 min. After each injection, the column was cleaned with 20% acetonitrile for 10 min and equilibrated with 1% acetonitrile for 10 min. The software of YL clarity® was used to record and interpret all HPLC chromatograms.

Standard Preparation

The reference standards of uridine, inosine, guanosine, and adenosine were accurately weighed and diluted with ultrapure water to produce stock solutions at the concentration of 100 μg/mL, but the stock solution of cordycepin was prepared at the concentration of 30 μg/mL. Then, the stock standard solutions were pipetted and diluted in ultrapure water to produce the range of 0.5-12 μg/mL for uridine and

guanosine, 1-12 μg/mL for adenosine, and 0.28-2.8 μg/mL for cordycepin.

Sample Preparation

There were three kinds of samples: Crude drugs, extracts, products; including *C. sinensis*, *C. militaris*, 2 *Cordyceps* extracts, tablets, and capsules. *C. sinensis*, *C. militaris*, and *Cordyceps* tablets were crushed and powdered before analysis. The samples were accurately weighed 0.1 g for *C. sinensis*, 2 extracts, tablets, and capsules and 0.3 g for *C. militaris* into 10 mL plastic tubes and added with 5 mL of ultrapure water. The samples were sonicated for 10 min and centrifuged at 10000 g for 10 min. The supernatants were collected in new plastic tubes. The extraction was repeated 3 times for *C. sinensis*, 2 extracts, and 2 products and 6 times for *C. militaris*. The supernatants were filtered and injected into the HPLC system.

System Suitability

The suitability of the HPLC system was assessed from 6 injections of the standard solution at 10 μg/mL for uridine, guanosine, adenosine and at 3 μg/mL for cordycepin. Inosine as a resolution solution was added in the standard solution at the concentration of 10 μg/mL. The parameters used in the system suitability testing included capacity factor (k'), number of theoretical plate (N), tailing factor (T), resolution (R), and relative standard deviation of peak area (%RSD).

Method Validation

Specificity

The specificity of the stability indicating method was evaluated from the ability to separate 4 nucleosides in the presence of sample matrices and their degradation products. The mixed standard solution containing uridine, guanosine, adenosine at the concentrations of 10 μg/mL and cordycepin at the concentration of 3 μg/mL was injected into the HPLC system. Then, the sample solutions including *C. sinensis*, *C. militaris*, two extracts, tablets, and capsules were injected into the HPLC system.

The forced degradation of 4 nucleosides was induced under the conditions of 0.1 N H_2SO_4, 0.1 N NaOH, 3% H_2O_2, sunlight, and heat at 90°C. The solutions used in the forced degradation were prepared at the concentrations of 4 μg/mL for uridine, guanosine, adenosine and the concentration of 1 μg/mL for cordycepin. The forced degradation solutions were kept in amber tubes at room temperature for 3 days in 0.1 N H_2SO_4 and 0.1 N NaOH and for 6 h in 3% H_2O_2. The forced solutions under sunlight were kept in transparent tubes at room temperature for 28 days. The forced solutions at 90°C were kept in amber tubes and heated in a water bath for 12 h. All solutions were filtered with 0.45 μm nylon membrane filter and injected into the HPLC system.

The forced degradation of 6 sample solutions including *C. sinensis*, *C. militaris*, 2 extracts, and 2 products was induced under the conditions of 0.1 N H_2SO_4 and 3% H_2O_2. The forced degradation solutions were kept in amber tubes at room temperature for 3 days in 0.1 N H_2SO_4 and for 6 h in 3% H_2O_2. All solutions were filtered with 0.45 μm nylon membrane filter and injected into the HPLC system.

The specificity of the method was assessed from the resolutions of 4 bioactive nucleosides. They should also be separated from interferences in both sample matrices and their degradation products.

Linearity and range

The mixed standard solutions of uridine, guanosine, adenosine, and cordycepin were diluted from the stock standard solutions by pipeting 50-1000 μL of the uridine and guanosine stock standard solutions, 100-2000 μL of the adenosine stock standard solution and 100-1000 μL of the cordycepin stock standard solution into 10-mL volumetric flasks and diluted with ultrapure water. The concentrations of uridine and guanosine were in the range of 0.5-10.0 μg/mL, and the concentrations of adenosine were in the range of 1.0-20.0 μg/mL and the concentrations of cordycepin were in the range of 0.3-3.0 μg/mL. The standard solutions were filtered through 0.45 μm nylon membrane filter, and triplicately injected into the HPLC system. When the chromatograms were obtained, the peak areas and the concentrations of each standard were plotted as $y = mx + c$; y = peak area, x = concentration in μg/mL, m = slope, and c = y-intercept to obtain the regression equation. The correlation coefficient (R) was calculated to determine the linearity of the calibration curve.

Accuracy

The solutions at the concentration of 0.5, 4.0, 10.0 μg/mL were prepared for uridine and guanosine; 1.0, 4.0, 20.0 μg/mL for adenosine; and 0.3, 1.0, 3.0 μg/mL for cordycepin. Each concentration was triplicately prepared. Then the solutions were filtered through 0.45 μm Nylon membrane filter and injected into the HPLC system. The peak areas of each nucleoside obtained from the chromatograms were converted into the found amounts. The accuracy was expressed in % recovery which was found amount × 100/ added amount. The percentage of recovery should be in the range of 92-105% [39].

Precisions

The intraday precisions were assessed by preparing the solutions at the concentrations of 0.5, 4.0, 10.0 μg/mL for uridine and guanosine; 1.0, 4.0, 20.0 μg/mL for adenosine; 0.3, 1.0, 3.0 μg/mL for cordycepin. Each concentration was triplicately prepared. Then, the solutions were filtered through 0.45 μm nylon membrane filter and injected into the HPLC system. Intraday precisions were tested at 3 concentrations on the same day, while interday precisions were tested at 3 concentrations on 3 different days. The RSDs were calculated from 3 determinations within the same day for intraday precisions and 9 determinations from 3 different days for interday precisions. The RSDs should be less than 2% for intraday precisions and less than 4% for interday precisions [39].

Sensitivity

Limits of detection (LOD) of the method to detect each standard were tested by diluting the standard solution at the lowest concentrations of the calibration curves to create the signal-to-noise ratios at 3:1. The diluted solutions were then injected into the HPLC system to obtain HPLC chromatograms. The peak heights of signal and noise were measured to calculate the signal-to-noise ratios. Limits of quantitation

(LOQ) to accurately and precisely determine each standard were assessed from the accuracy and precisions of the lowest concentrations of the calibration curves.

Effect of Forced Conditions on Nucleoside Degradation

The degradation of uridine, guanosine, adenosine, and cordycepin under the forced degradation conditions was assessed from the remaining percentage of each standard in the acidic, basic, oxidative, sunlight conditions at 0, 3, 7, and 28 days and in the heat condition at 90°C at 0, 1, 2, 3, 5, 10, and 12 h. The remaining percentage was calculated as shown below. Peak $area_0$ was the peak area of compounds of interest at the initial day or hour, and peak $area_n$ was the peak area of compounds of interest at any days or hours.

$$\% \text{ remaining} = \frac{\text{Peak area}_n}{\text{Peak area}_0} \times 100$$

RESULTS

System Suitability

The developed method to determine 4 bioactive nucleosides including uridine, guanosine, adenosine, and cordycepin in this study required the analysis time of 30 min. The retention times of uridine, guanosine, adenosine, cordycepin were 7.6, 14.6, 25.9, and 27.6 min, respectively. The HPLC system required an isocratic elution of 1% acetonitrile in the first 15 min and a gradient elution from 1% to 15% acetonitrile during the following 15 min. The ratio of acetonitrile should be as low as 1% so that the nucleosides can retain in the C_8 column and this system maximizes the resolution between inosine and guanosine with the resolution of 2.3. Inosine is a component in *Cordyceps* that previous methods showed incomplete separation between guanosine and inosine. Therefore, in this study inosine was used as a resolution solution to assess the system suitability of the HPLC system. After uridine, inosine, and guanosine have been separated at 7.6, 13.6, 14.6 min, the ratio of acetonitrile should be increased from 1% to 15% over the following 15 min to accelerate the elution of adenosine and cordycepin at 25.9 and 27.6 min. The developed method has been shown to be suitable since the resolutions between nucleosides were more than 2, all peaks were symmetrical because tailing factors were 1.1. The column contained high numbers of theoretical plates not less than 16300. All peaks could be retained long enough in the column because the capacity factors were in the range of 1.5-8.0. The RSDs of peak areas were less than 1% as shown in Table 1.

Method Validation

Specificity

The chromatograms of crude drugs, 2 extracts, and 2 dosage forms showed that all 4 nucleosides: uridine, guanosine, adenosine, and cordycepin were successfully separated as shown in Figure 2. Therefore, the developed method was specific without interferences from sample matrices. The specificity of 4 nucleosides in 5 forced conditions was resulted as following: in the condition of 0.1 N H_2SO_4 for 3 days; the peaks of degradation products from all 4 nucleosides were eluted at 2.7 min while the peak of a degradation product from guanosine was shown at 6.1 min and the peak of a degradation product from cordycepin was shown at 13.9 min. In the condition of 0.1 N NaOH for 3 days, small peaks of degradation products from all nucleosides were found at about 2.5 min. In the condition of 3% H_2O_2 for 6 h, all 4 nucleosides displayed the interfering peaks at 2.9 min. In the condition of sunlight for 28 days, a degradation peak at 22.3 min was generated from cordycepin. In the condition of heat at 90°C for 12 h, no peak of degradation product was noticeable. The forced conditions on nucleoside degradation revealed that the peaks of uridine, guanosine, adenosine, and cordycepin could be separated from the degradation products as shown in Figure 3.

To ensure that the developed method could separate the nucleosides in sample degradation, the specificity of the method was tested with the forced condition by 0.1 N H_2SO_4 for 3 days and 3% H_2O_2 for 6 h in six sample solutions from 2 crude drugs, 2 extracts, and 2 products. The chromatograms from both forced conditions exhibited the successful separation of uridine, guanosine, adenosine, and cordycepin. The chromatograms of sample degradation in 0.1 N H_2SO_4 for 3 days were shown in Figure 4.

Linearity and range

The linearity of uridine, guanosine, adenosine, and cordycepin was assessed from correlation coefficients of the calibration curves. The concentration ranges were 0.56-11.20 μg/mL for uridine, 0.56-11.21 μg/mL for guanosine, 1.13-11.30 μg/mL for adenosine, 0.279-2.793 μg/mL for cordycepin. The correlation coefficients of the calibration curves of 4 nucleosides were found to be 0.9999 as shown in Table 2. The developed method was found to be linear at the studied ranges.

Table 1: System suitability testing (n=6) for the developed HPLC conditions: Concentrations of uridine, inosine, guanosine, adenosine were 10 μg/mL and the concentration of cordycepin was 3 μg/mL; inosine was added at 10 μg/mL in the standard solution as a resolution solution

Standard	Retention time (min)	Resolution (R)	Tailing factor (T)	Number of theoretical plate (N)	Capacity factor (k')	%RSD of peak area
Uridine	7.6	-	1.1	17300	1.5	0.20
Inosine	13.6	18.3	1.1	16500	3.5	0.41
Guanosine	14.6	2.3	1.1	16300	3.8	0.32
Adenosine	25.9	36.4	1.1	399000	7.5	0.44
Cordycepin	27.6	10.1	1.1	451000	8.0	0.51

HPLC: High performance liquid chromatography, RSD: Relative standard deviation

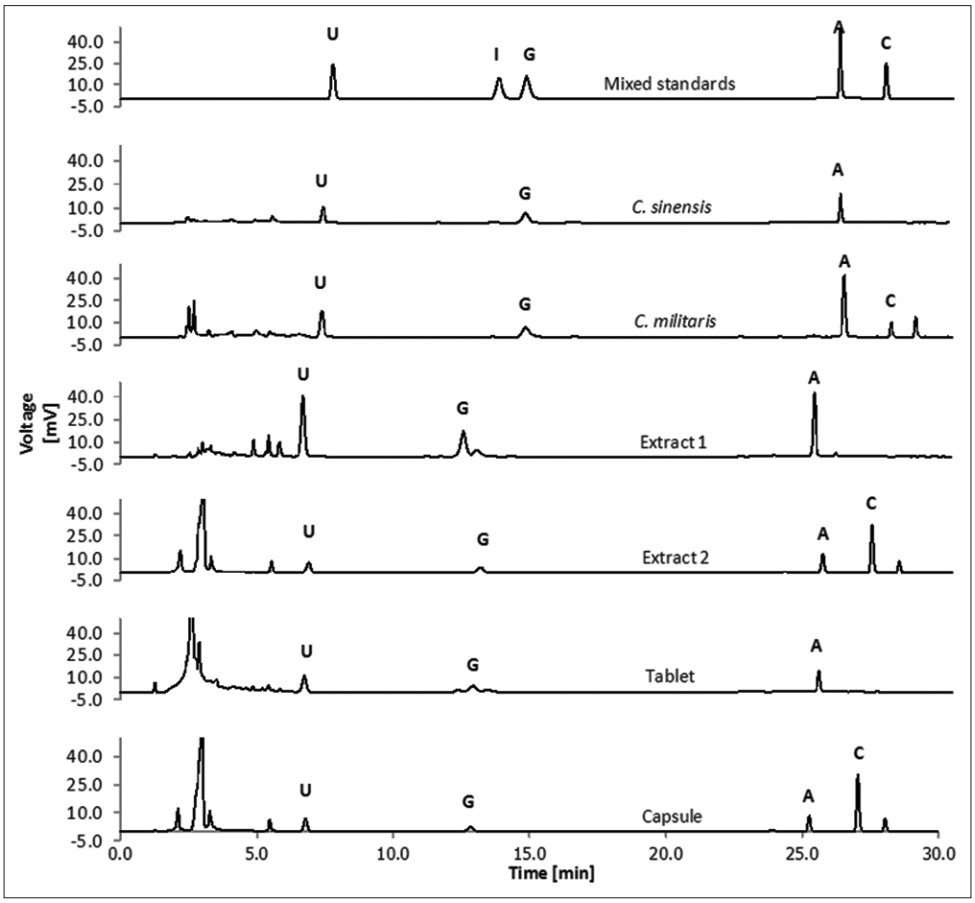

Figure 2: High performance liquid chromatography chromatograms of 4 nucleosides including uridine (U), guanosine (G), adenosine (A), cordycepin (C) in the standard solution, and the sample solutions including 2 crude drugs: *Cordyceps sinensis*, *Cordyceps militaris*, 2 extracts, and 2 dosage forms. The concentrations of standard nucleosides were 10 μg/mL for uridine, guanosine, adenosine and 3 μg/mL for cordycepin. Inosine (I) at 10 μg/mL was used as a resolution solution in the mixed standard solution

Table 2: Method validation parameters for range, linearity, accuracy, precision, LOD, and LOQ

Standard	Range (µg/mL)	Linear regression equation (n=3)	R (n=3)	% recovery	Intraday precision (% RSD)	Interday precision (% RSD)	LOD (µg/mL)	LOQ (µg/mL)
Uridine	0.56-11.20	y=19.689x+0.173	0.9999	97.31-100.39	0.18-1.33	0.58-1.27	0.02	0.56
Guanosine	0.56-11.21	y=24.894x−1.2417	0.9999	99.87-100.64	0.27-1.63	1.04-2.25	0.02	0.56
Adenosine	1.13-11.30	y=28.084x+1.9577	0.9999	98.75-100.14	0.22-1.40	0.52-2.02	0.01	1.13
Cordycepin	0.279-2.793	y=55.97x−0.5141	0.9999	96.65-99.97	0.21-1.28	0.70-2.76	0.01	0.279

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation, R: Correlation coefficient

Accuracy

The accuracy of uridine and cordycepin were found to be in the range of 97.31-100.39% and 96.65-99.97%. The accuracy of guanosine and adenosine were found to be in the range of 99.87-100.64 and 98.75-100.14%. The accuracy of the developed method was shown as the percentages of recovery to be 96.65-100.64 for all four nucleosides which were in the acceptable range of 92-105% as shown in Table 2.

Precision

Intraday precisions of uridine, guanosine, adenosine, and cordycepin were found to be not more than 1.63%, and interday precisions were found to be not more than 2.76% as shown in Table 2. The precisions of all four nucleosides were shown as the RSDs of intra- and inter-day precisions which were acceptable less than 2 and 4%.

Sensitivity

The sensitivity of the method was evaluated from LOD and LOQ. LOD of all 4 nucleosides was considered from signal-to-noise ratio of the standard solutions to be 3:1. The LOD were found to be 0.02 μg/mL for uridine and guanosine, and 0.01 μg/mL for adenosine and cordycepin. The LOQ should demonstrate both accuracy and precision of the analysis. In this study, the lowest concentrations of the calibration curves were limits of quantitation. The LOQ of uridine, guanosine,

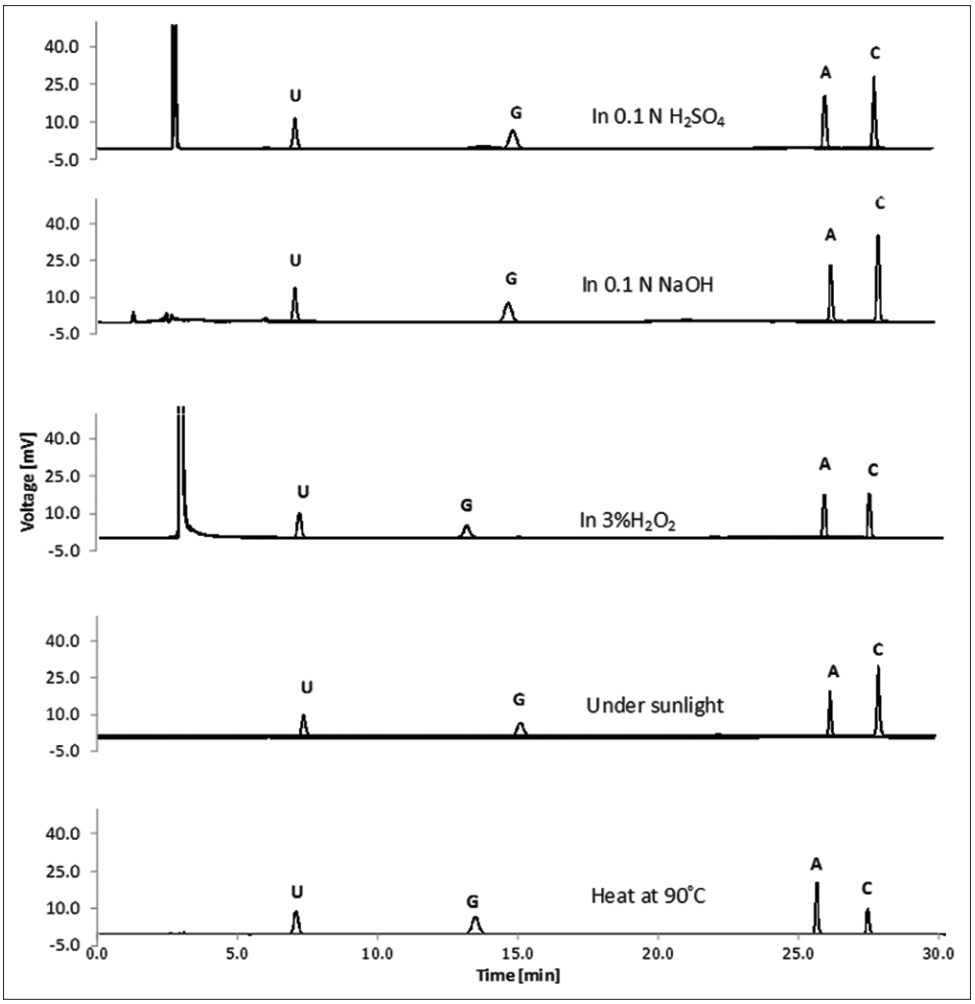

Figure 3: Overlaid high performance liquid chromatography chromatograms of 4 nucleosides including uridine (U), guanosine (G), adenosine (A), and cordycepin (C) in forced degradation conditions as the following: In 0.1 N H_2SO_4, 0.1 N NaOH for 3 days, under sunlight for 28 days, in the heat at 90°C for 12 h, and in 3% H_2O_2 for 6 h. The initial concentrations were 4 μg/mL for uridine, guanosine, adenosine and 1 μg/mL for cordycepin

adenosine, and cordycepin were found to be 0.56, 0.56, 1.13, 0.279 μg/mL as shown in Table 2.

Effect of Forced Conditions on Nucleoside Degradation

Uridine, guanosine, adenosine, cordycepin were tested in the forced conditions of 0.1 N H_2SO_4, 0.1 N NaOH, 3% H_2O_2, sunlight, and heat at 90°C. The degradation data of all 4 nucleosides were shown as the remaining percentage. All nucleosides were susceptible to 3% H_2O_2 as the remaining percentages of the nucleosides were rapidly reduced during the first 3 days. The remaining percentages of all 4 nucleosides at 3, 7, 28 days were in the range of 0-43.6%, 0-7.1%, and 0% as shown in Figure 5b. Moreover, the nucleosides were also mainly degraded in 0.1 N H_2SO_4. The remaining percentages of the nucleosides at 3, 7, 28 days were in the ranges of 85.8-98.0%, 57.8-92.6%, and 8.6-90.2% as shown in Figure 5a. The nucleosides were less degraded in 0.1 N NaOH and under sunlight than in 0.1 N H_2SO_4 and 3% H_2O_2 as shown in Figure 5c and 5d. They were stable to heat at 90°C for 12 h as the remaining percentages of the nucleosides at 12 h were found to be not less than 97.9% as shown in Figure 5e. The degradation data suggested that 4 nucleosides were sensitive to acidic and oxidative conditions.

DISCUSSION AND CONCLUSIONS

Stability indicating method should be able to distinguish bioactive compounds from sample matrices, excipients, and degradation products. Stability indicating methods in herbal quality control have been shown for glycyrrhetic acid in *Glycyrrhiza glabra* crude drug and extract [40]; and 6-gingerol in polyherbal formulations containing *Zingiber officinale* [41]. Requirement to control the quality of herbal medicine has been raised to ensure efficacy and safety. Nucleosides are essential markers to control the quality of *Cordyceps* because they are bioactive components with various therapeutic effects. In this study, we presented that bioactive nucleosides including uridine, guanosine, adenosine, and cordycepin could be decomposed in acidic and oxidative environments. They were found to be more susceptible to 0.1 N H_2SO_4 and 3% H_2O_2 than 0.1 NaOH and sunlight so acid resistant preparations or antioxidants should be recommended for *Cordyceps*

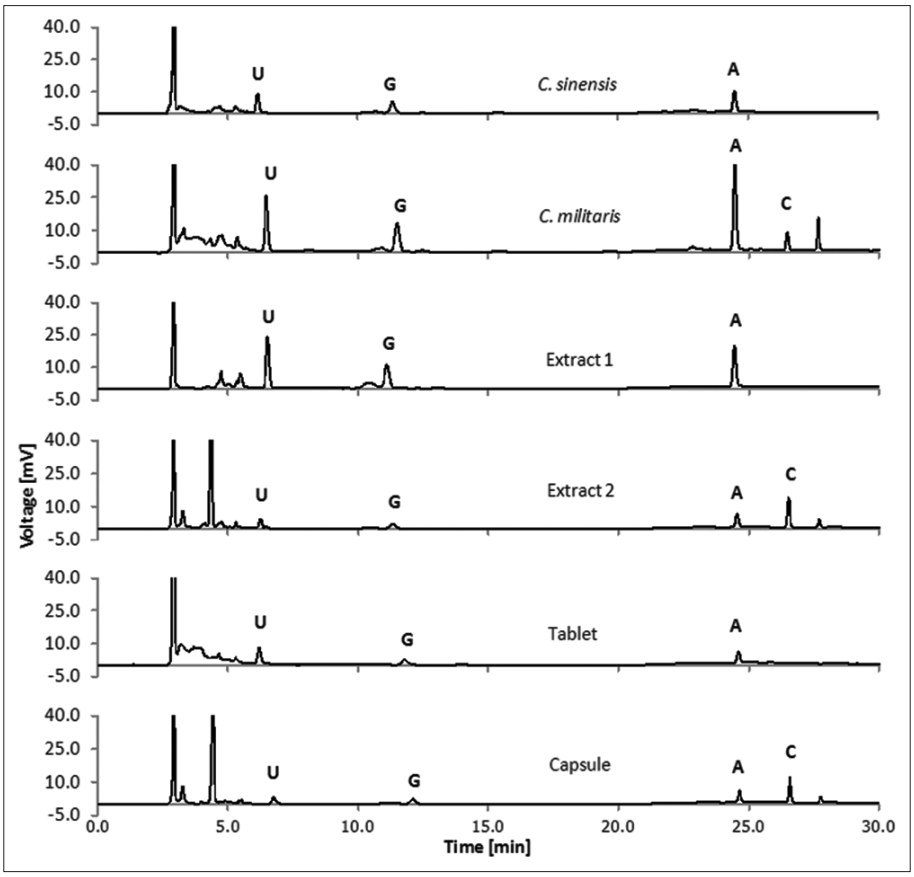

Figure 4: High performance liquid chromatography chromatograms of 6 *Cordyceps* samples including *Cordyceps sinensis, Cordyceps militaris*, 2 extracts, and 2 products in the forced acidic degradation by 0.1 N H_2SO_4 for 3 days. Bioactive nucleosides included uridine (U), guanosine (G), adenosine (A), and cordycepin (C)

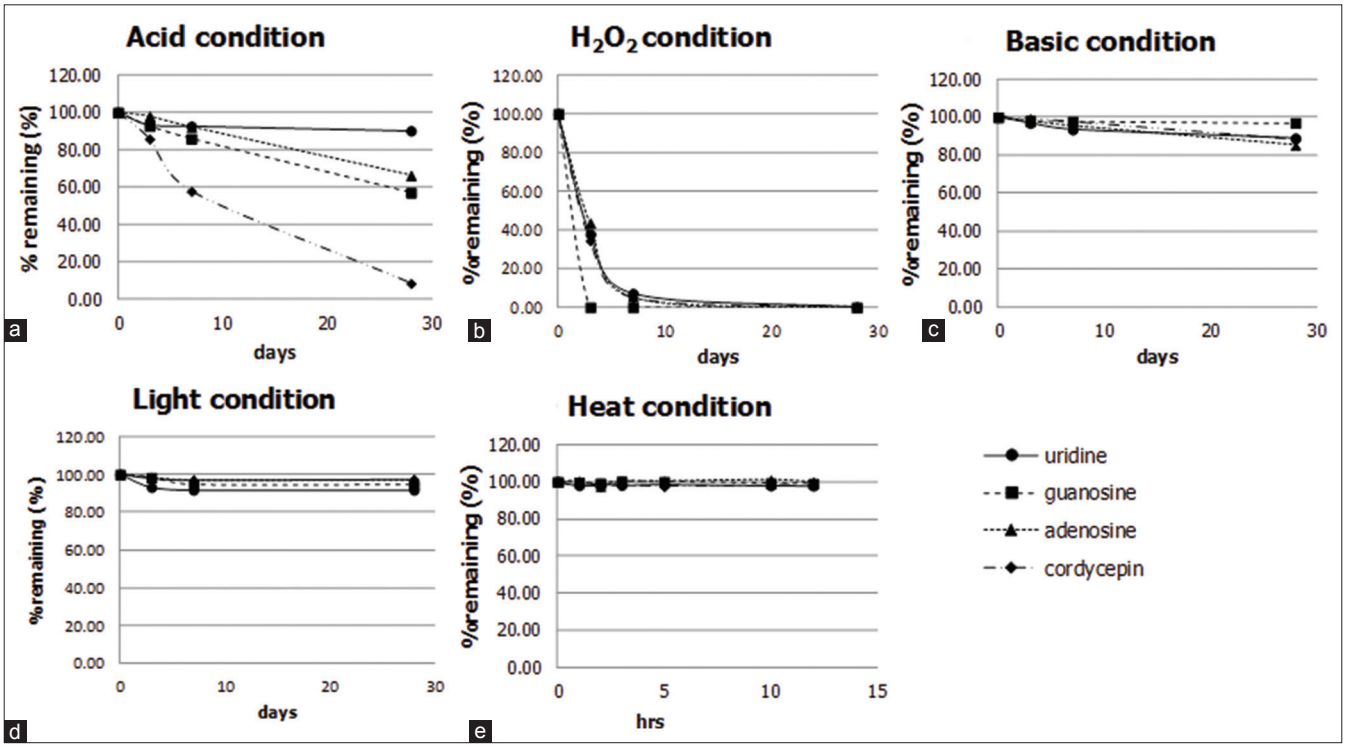

Figure 5: (a-e) Degradation data of 4 nucleosides including uridine, guanosine, adenosine, and cordycepin in forced degradation conditions: 0.1 N H_2SO_4, 0.1 N NaOH, 3% H_2O_2, sunlight, and heat at 90°C

products. Nucleosides could undergo acidic hydrolysis by breakage of nucleobase and sugar or oxidative decomposition [42]. Therefore, stability indicating method for bioactive nucleosides would be necessary to determine quality and shelf life of *Cordyceps* for efficacy and safety.

The stability indicating method was developed by reverse phase HPLC to determine 4 bioactive nucleosides in 2 crude drugs from *C. sinensis* and *C. militaris*, 2 extracts, and 2 dosage forms. The HPLC system required an isocratic elution of 1% acetonitrile in water for 15 min and gradient elution of 1-15% acetonitrile in water over the next 15 min. The system suitability parameters showed a suitable HPLC system, and the analysis time was within 30 min. Specificity results demonstrated that the HPLC method was specific to all 4 bioactive nucleosides in the presence of sample matrices, excipients, and forced degradation products. The advantages of the developed method include high specificity to bioactive nucleosides particularly to polar nucleosides such as uridine and guanosine without interferences from sample matrices and degradation products and application flexibility to various samples: Crude drugs, extracts, and products.

In conclusion, the developed method has been validated and found to be specific, accurate, precise, and sensitive. It has successfully separated uridine, guanosine, adenosine, cordycepin in *C. sinensis*, *C. militaris*, extracts, tablets, capsules, and distinguished all 4 nucleosides in the presence of the their degradation products from 0.1 N H_2SO_4, 0.1 N NaOH, 3% H_2O_2, and sunlight. The method could be applied as a stability indicating method to determine shelf life of crude drugs, extracts, and products from *C. sinensis* and *C. militaris*.

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