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A validated stability-indicating HPLC with photodiode array detector (PDA) method for the stress tests of *Monascus purpureus*-fermented rice, red yeast rice

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Abstract

A stability-indicating reversed phase high-performance liquid chromatography (RP-HPLC) with photodiode array (PDA) detection method was developed and validated for the assay of monacolin series compounds including monacolin K, L, J and their hydroxyl acid forms as well as dehydroxymonacolin K simultaneously in *Monascus purpureus*-fermented rice, red yeast rice. Well-resolved peaks of seven main compounds of monacolin family were profiled on a C_{18} reverse-phase column using a linear gradient of 0.1% trifluoroacetic acid and acetonitrile as the mobile phase, and the detection wavelength was set at 237 nm. The method was validated with respect to specificity, chromatographic parameters, linearity, precision, accuracy, limits of detection and quantitation. The stability stress testing for fermented red yeast rice powder was carried out to show the effects of high temperature (80 °C), high humidity at room temperature (92.5% RH, 25 °C), high humidity at high temperature (75% RH, 60 °C) and light (sunlight) in solid state. The results exhibited that monacolins decreased significantly under the conditions of high humidity at high temperature (75% RH, 60 °C) and sunlight. Monacolin K and its hydroxyl acid form would be dehydrolyzed and turned to dehydromonacolin K at high temperature (80 °C) while the monacolin K, J and L would be transformed into their corresponding hydroxyl acid forms under the condition of high humidity (92.5% RH, 25 °C). The indication is that monacolins in red yeast rice powder are light-sensitive and thermal-sensitive. Therefore, it has been suggested that the preparations containing monacolins be stored in the place of cool and lightproof. The proposed degradation pathways were discussed as well. The multi-components assay for stability of botanical products could provide much more information than the normal marker-orientation method.

Keywords: Stability-indicating assay method (SIAM); Stress tests; *Monascus purpureus*; Monacolins

1. Introduction

Red yeast rice, one fermented product of rice, has been used in Chinese cuisine and medicine to promote "blood circulation" for centuries. The recent research revealed the red yeast rice forms naturally occurring hydroxymethylglutaryl-CoA reductase (HMG-CoA) inhibitors, and the medicinal properties of fermented red yeast rice have a favorable effect on lipid profiles of hypercholesterolemic patients, by decreasing low-density lipoprotein cholesterol (LDL) and elevating high-density lipoprotein cholesterol (HDL) for hypercholesterolemic adults [1,2]. Clinical trials using red yeast rice with hyperlipidemic elderly patients [3,4] as well as HIV-related dyslipidemic patients [5] have also demonstrated an improvement in lipid profiles. In recent

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reports, fermented red yeast rice preparation effectively preprandial improve and postprandial endothelial function by its potential anti-inflammatory and lipid-lowering effects [6]. This product possesses promising activity as a new hypolipidemic drug launched worldwide [7]. The HMG-CoA reductase inhibiting activity of red yeast rice comes from a family of naturally occurring substances named monacolins [8,9]. Monacolin K, also known as mevinolin or lovastatin, is the major ingredient and has been taken as a marker compound for quality control in Chinese official monograph [10], as well as the label claim of some commercial red yeast rice related products. The isolation and evaluation of seven monacolins from fermented red yeast rice were described in Ma's report [11]. In our recent research [12], totally 14 monacolin compounds including monacolin K (mevinolin), J, L, M, X, and their corresponding hydroxyl acid forms, as well as dehydromonacolin K, dihydromonacolin L, compactin, 3-hydroxy-3,5-dihydromonacolin L were identified in red yeast rice, using high-performance liquid chromatography with photodiode array detector (PDA) and tandem mass spectrometry. Meanwhile, a chemical fingerprint profiling method was established to display bioactive monacolins for the qualitative analysis of target material.

For any product used as a drug or food supplement, stability testing is imperative in order to comply with official regulations. The purpose of stability testing is to provide evidences on how the quality of a drug substance or drug product varies with time under the influence of environmental factors, such as temperature, humidity, and light, and to establish the recommended storage conditions and a shelf life for the drug product [13]. The requirement of establishing a stability-indicating assay method (SIAM) for the stability testing has become more clearly mandated in the official guidelines at the International Conference on Harmonization (ICH) [14] and United States Pharmacopeia (USP) [13]. The guidelines [13–15] also mentioned that forced decomposition studies (stress testing) under the extreme conditions as heat, oxidative and photolytic should be carried out on the drug substance so as to establish the inherent stability characteristics and degradation pathway to support the suitability of the proposed analytical procedures. More publications [16–19] reported their works of the validated stability-indicating assay methods on synthesized chemicals and Bakshi [20] presented a critical review on the comprehensive understanding of SIAM. At present, there is an increasing interest in plant ingredients and their use for drugs, teas, or food supplements, but to date, few validated stability-indicating assay method was touched upon the botanical products.

The conventional stability testing of the botanical products is to trace only the marker compound using a simple quantitative method like HPLC as well. For instance, the available publications on stability testing of red yeast rice [21–25] inclined to trace only monacolin K (mevinolin, lovastatin) by HPLC with isocratic elution. No systematic and validated stability-indicating method for the assay of the monacolins in this potential medicinal product has been reported. Furthermore, there is no available stability research either. The botanical or natural products normally contain a group of active compounds which, as a whole, contribute to their biological activities. It is more reasonable and acceptable to trace and measure the variation trend of the "group" ingredients, not only for a single one, for the quality evaluation and stability test of the analyzed products. The objective of our present study is to develop and validate a HPLC method for the stability indicating assay of multi-ingredients-containing natural products, taking monacolins in fermented red yeast rice as the analytes.

Among the total 14 monacolins [12], seven main compounds, monacolin J, L, K and their corresponding hydroxyl acid forms together with dehydromonacolin K (Fig. 1) (nearly 97% of total monacolins) were chosen for assay of their concentrations in fermented red yeast rice. Other monacolins such as monacolin X, M and their hydroxyl acid forms exist in very low concentration in the analyzed product, or showed poor absorption at the detected wavelength of 237 nm and so could not be determined quantitatively. The peaks of monacolins in HPLC chromatogram (Fig. 2), were identified and traced by not only their retention time (t_R) but also their diagnostic UV spectra of mountain-like peaks acquired by using a PDA detector.

Two conditioned stability tests, accelerated testing under the condition of 40 °C and 75% relative humidity (RH) and the stress testing under high temperature (80 °C, 60 °C), high humidity (92.5% RH, 75% RH) and sunlight, of red yeast rice powder in solid state were carried out in this study.

2. Experimental

2.1. Chemicals, reagents and materials

Monacolin K (mevinolin, lovastatin) standard was purchased from Sigma Co. (St. Louis, MO, USA). The other standards of monacolin J, L and dehydromonacolin K were isolated and purified in our research lab, structurally elucidated by MS, NMR and IR evidences [11]. The red yeast rice powder was kindly provided by a healthcare company in Shanghai, China. Methanol and acetonitrile (ACN) were of HPLC grade from Merck (Darmstadt, Germany). Phosphoric acid, trifluoroacetic acid (TFA), ethyl acetate and 95% ethanol, were of analytical grade from ChromTech (Shanghai, China). De-ionized water was obtained with an in-house Milli-Q water system (Millipore Inc., Billerica, MA, USA).

2.2. Equipment

A Waters 2690 Alliance HPLC system (Waters Inc., Milford, MA, USA), equipped with an on-line degasser and an autosampler, was used for solvent management system. The raw data were detected by 996 PDA, acquired and processed by Waters Millennium^{32} chromatographic workstation loaded on a Compaq computer. Prior to each run, the HPLC–PDA system was allowed to warm up for near 30 min

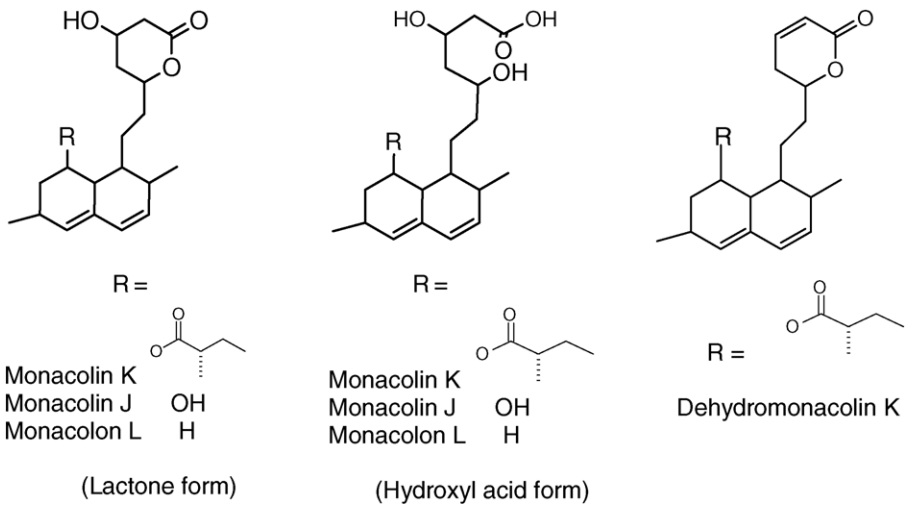

Fig. 1. Structures of monacolin compounds.

and the baseline was monitored until stable before the samples were injected. The humidity chamber for accelerated testing was Binder KBF240 (Binder GmbH, Tuttlingen, Germany), which set the storage condition of 40 °C and 75% RH.

2.3. Sample preparation

Several organic solvents including ethanol, methanol, ethyl acetate were chosen for extraction but none of them could extract the target compounds from the powder thoroughly, although the purified monacolins showed good solubility in these solvents. It was found that adding of a proper quantity of water to the solvent could benefit the dissolution of the target compounds, by helping the solvent to permeate in the rice powder and consequently promoting the extraction of monacolins from the base material. Lastly, an optimized condition, a mixture of ethanol and water (75:25) for 90 min was chosen for extraction.

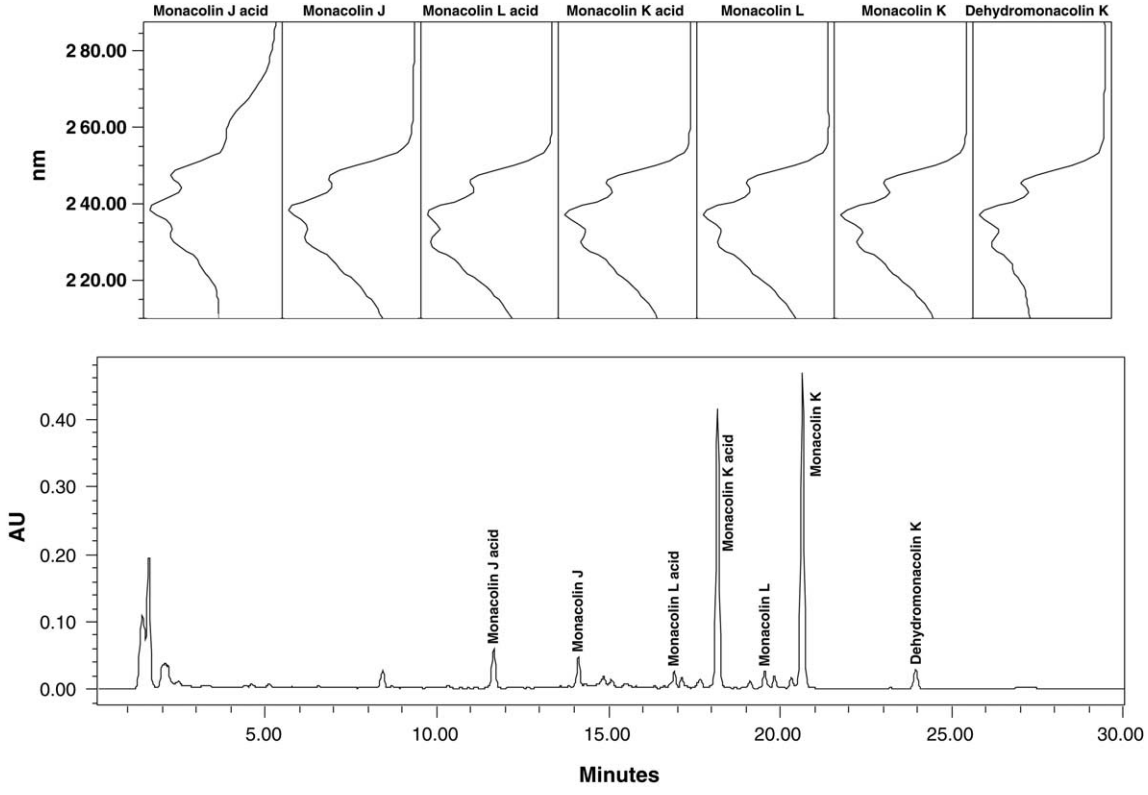

Fig. 2. HPLC chromatogram with UV spectra of the monacolins in fermented red yeast rice.

About 0.5 g of fermented red yeast rice powder as accurately weighed and transferred into a 20-ml stopper centrifuge tube. The preparations in triplicate were extracted with 8 ml of 75% ethanol for 30 min on an ultrasonic bath and subsequently centrifuged for 10 min at 3000 rpm. This extract procedure was repeated three times, and the supernatants were combined and transferred into a 25-ml volumetric flask, adding 75% ethanol to exactly 25.0 ml. The final solution was kept standing for 30 min, and then filtered through a 0.45-μm membrane before being placed in vials for HPLC analysis.

2.4. Preparation of the standard solution

Calibration curve was obtained from the purified monacolin K. The purity of the standard was found to be greater than 99.0%, evaluated by HPLC–PDA with detection at 237 nm, by recording the peak purity index via PDA, as well as by TLC chromatography.

A working standard solution at a concentration of approximately 0.1 mg per ml of monacolin K was prepared in the following manner. About 5 mg of monacolin K was accurately weighed and transferred into a 50-ml low actinic volumetric flask. Seventy-five percent ethanol was added, mixed well, then the solution was diluted to volume with 75% ethanol and used as monacolin K standard preparation.

Twenty millilitres of monacolin K standard preparation was accurately transferred into another 25-ml actinic volumetric flask and 5 ml of 0.5 N NaOH was added and the solution was allowed to stand for 30 min, and used as standard preparation of monacolin K hydroxyl acid form.

2.5. Chromatographic condition

The chromatographic condition was optimized by testing various system conditions involving stationary phase of column and mobile phase. On general reverse-phase C_{18} column, the monacolins could be well separated with a gradient elution system using acetonitrile as the organic phase and the acidic aqueous phase modulated by dilute phosphoric acid or dilute trifluoroacetic acid (TFA). Finally, a column of Waters Symmetry C_{18} (150 mm × 3.9 mm i.d., 5 μm) was chosen as the stationary phase and a gradient of acetonitrile (eluent A) and 0.1% TFA (eluent B) as the mobile phase. Linear gradient elution at 1 ml per minute from 35 to 75% A in 20 min and keeping 75% A from 20 to 28 min was applied. The total analysis time was 35 min, including column stabilization. The photo-diode array (PDA) detector was set at 210–350 nm and the chromatogram detected at 237 nm. The column temperature was set at 30 °C, and the injection volume was 20 μl.

2.6. Calibration

All the calibration of monacolins was conducted by using the relative corrected factors to the standard preparation of monacolin K, as no enough reference standards of monacolin L, J and dehydromonacolin K available. The relative corrected factors of monacolin J, L and dehydromonacolin K versus monacolin K were 1.11, 0.99 and 0.99, respectively, which were generated by injecting the corresponding minimum standards in a same HPLC system mentioned above. The hydroxyl acid forms of Monacolin L and J were calibrated versus standard preparation of the hydroxyl acid form of monacolin K based on the same relative corrected factor as their corresponding lacton forms.

2.7. Stress testing in solid state

About 0.5 g of fermented red yeast powder was accurately weighed to each glass tube, then put into the controlled storage condition at different interval times of 0, 2, 5, 10, 20 days. The conditions of high temperature were given at two ovens setting at 60 and 80 °C, separately. The condition of 75% RH was controlled in a desiccator containing saturated sodium chloride solution to hold the humidity at 75% RH, while the condition of 25 °C, 92.5% RH was maintained in a desiccator containing saturated potassium nitrate to support the humidity at 92.5% RH in an oven setting at 25 °C. For light stability test, 0.5 g of sample was exposed to summer sunlight for duration of 0, 2, 5, 10 and 20 days with an average temperature of 25 °C. The treated samples were weighed for the calculation of weight variation, and then sealed to store in a refrigerator for analysis.

2.8. Accelerated testing in solid state

Five glass tubes containing about 0.5 g of red yeast rice powder, accurately weighed, were put into a humidity chamber set at the controlled storage condition of 40 °C and 75% RH. The samples were taken out at the duration of 0, 10, 30, 60, and 90 days, weighed and then sealed to store in a refrigerator for assay.

3. Results and discussion

3.1. Method development

The monacolin compounds in red yeast rice sample preparation were traced by HPLC–PDA with the UV-index spectra, shown in Fig. 2. Seven main monacolins peaks were well separated under the optimized system condition. The peaks of monacolin K, L, J and dehydromonacolin K were identified by comparing both the retention times and the UV spectra with their corresponding standards. The hydroxyl acid form of monacolin K, J, and L were similarly identified by comparing the retention times to the corresponding standard preparations of their hydroxyl acid forms. All these monacolins showed a characteristic mountain-like UV spectrum with three maximum absorptions (λ_{max}) at 230, 237, and 246 nm, respectively [12]. The contents of monacolins K, J, L and their hydroxyl acid forms, as well

as dehydromonacolin K in fermented red yeast rice were assayed using this developed method.

3.2. Method validation

3.2.1. Specificity

The chromatogram was shown in Fig. 2 and the peak purity was assessed using the software provided in Waters Workstation Millienium[32]. The index of purity angle was calculated to demonstrate the purity of peaks. According to the theory of peak purity in Waters software, the less value of the purity angle obtained (nominal valued within the range of 0–99) indicates the high purity of the peak. The purity angle values of all the seven monacolins peaks in the chromatograms of initial and stress tests were lower than 1.5 which illustrated all the peaks were of high purity. The specificity of these monacolin peaks were also identified and evaluated by means of LC-PDA–MS in our previous report [12].

3.2.2. Chromatographic parameters

Four chromatographic parameters, i.e. tailing factor (TF), theoretical plate number (N), resolution (R_s) and relative retention time (RRT) versus monacolin K were calculated using Waters Millennium[32] Workstation and were given in Table 1. The tailing factors (TF) of all the seven peaks were near 1.00 ± 0.05 except monacolin L (TF = 1.15). The resolutions (R_s) of these seven peaks with their neighbouring peaks were all more than baseline separation of 1.5 except the hydroxyl acid form of monacolin L ($R_s = 1.15$).

3.2.3. Range of linearity

The linearity were studied for 0.02–0.5 mg/ml of both monacolin K and its hydroxyl acid form. The least-square linear regression equations of monacolin K and its hydroxyl acid were $y = 30543x + 168$ with $r = 0.9998$ and $y = 27866x + 302$ with $r = 0.9997$, respectively, where x was the concentration of standard, y was the observed response and r was the correlation coefficient. The data of the deviations of observed and predicted response were equally distributed between positive and negative values. The method was considered to be linear in the studied range.

3.2.4. Precision

The precision was investigated with respect to system precision and method precision. System precision was determined from six replicate injections of a sample preparation at the target concentration. Method precision was demonstrated from the assay of four representative samples in 1 day for within-day variation and in each of 3 days for between-days variation. The results were summarized in Table 2 and acceptable repeatability of system was observed. R.S.D.% of three ingredients (monacolin J, J acid and L acid) with lower response was found to be less than 3% while the other three with higher response (monacolin K, K acid and L) less than 1%. The obtained data revealed the established method was reproducible.

3.2.5. Accuracy/recovery

Accuracy was assessed by determination of recovery using standard addition method. Samples were prepared in triplicate by spiking with three different levels of monacolin K which covered the concentration of sample solution.

The recoveries of monacolin K and its acid form were calculated based on the ratio of adding amounts and obtained amounts. The sample preparation for recovery of hydroxyl acid of monacolin was carried out with the same procedure as standard preparation of monacolin K hydroxy acid in case of the conversion between monacolin K lacton form and acid form happened in NaOH existing [12]. The results were listed in Table 3. The data showed the good recovery of monacolin K and its acid as well as the mean recovery of 98.90% with ideal R.S.D. percentage of 1.2% for sum of both them were obtained.

3.2.6. Limit of detection (LOD) and quantitation (LOQ)

A series of diluted sample preparations were injected for 10 μl. The LOD of monacolins is 0.5 μg/ml at the peak height of three times higher than baseline noise, with reference to monacolin K. The data of six replicate injections indicated a repeatability of 6.5% (R.S.D.%).

3.3. Stress testing of red yeast rice powder

Five stress testing conditions at different sampling intervals were conducted to indicate the changes of monacolins in red yeast rice powder. The data of weight variation of the powder (Table 4) reflected that fermented red yeast rice could absorb much water (up to 21.9%) in the circumstance of high humidity (92.5% RH), and lose its weight quickly under the condition of high temperature (80 °C, 60 °C).

Table 1
Chromatographic parameters

Ingredient	Tailing factor (TF)	Theoretical plate number (N)	Resolution factor (R_s)	Relative retention times (RRT)
Monacolin J acid	1.01	76020	1.88	0.56
Monacolin J	0.95	105908	1.72	0.68
Mona L acid	1.08	85235	1.15	0.82
Monacolin L	1.15	159550	1.89	0.95
Monacolin K acid	1.00	145568	1.59	0.88
Monacolin K	0.98	180360	1.51	1.00
Dehydromonacolin K	1.00	170126	1.67	1.16

Table 2
Precision

Ingredient	System		Within-day		Between-days	
	Mean (%)	R.S.D.%	Mean (%)	R.S.D.%	Mean (%)	R.S.D.%
Monacolin J acid	0.0114	2.81	0.0118	1.85	0.0115	3.06
			0.0115	2.10		
			0.0111	2.29		
Monacolin J	0.0125	2.75	0.0127	2.31	0.0124	3.05
			0.0126	2.12		
			0.0120	2.56		
Monacolin L acid	0.0197	2.95	0.0193	3.80	0.0199	4.10
			0.0208	2.58		
			0.0195	2.68		
Monacolin L	0.0268	0.95	0.0262	2.53	0.0263	2.32
			0.0270	2.15		
			0.0258	2.05		
Monacolin K acid	0.1230	0.13	0.1237	1.01	0.1229	0.65
			0.1221	1.35		
			0.1230	2.01		
Monacolin K	0.2780	0.20	0.2770	0.71	0.2788	0.76
			0.2811	1.03		
			0.2783	0.80		
Dehydromonacolin K	0.0430	0.88	0.0433	2.23	0.0428	1.64
			0.0431	2.05		
			0.0420	2.12		
Total	0.5141	0.32	0.5140	1.56	0.5146	0.65
			0.5182	1.08		
			0.5116	1.87		

Table 3
Accuracy/recovery for monacolin K and monacolin K hydroxyl acid form

Ingredient	Level (%)	Adding (mg)	Intra-adding level		Inter-adding level	
			Mean (%)	R.S.D.%	Mean (%)	R.S.D. (%)
Monacolin K	80	1.31	98.82	1.12		1.04
	100	1.65	99.18	1.04	98.88	
	120	1.97	98.65	0.98		
Monacolin K acid	80	0.42	98.43	2.15		1.92
	100	0.54	99.07	1.82	98.92	
	120	0.66	99.25	1.78		
Sum of monacolin K and its acid	80	1.73	98.63	1.07	98.90	1.18
	100	2.19	99.12	0.84		
	120	2.63	98.95	1.65		

The effects of high temperature on red yeast rice powder was conducted at a high temperature of 80 °C, in dryness condition, with different storage intervals of 0, 2, 5, 10 and 20 days. The contents of the seven monacolins were assayed using the above-developed method and the results were drawn in Fig. 3. The variation of monacolins in red yeast rice powder under the condition of high humidity (92.5% RH, 25 °C) for the duration times at 0, 2, 5, 10 and 20 days were plotted in

Table 4
Weight variation

Days	80 °C (%)	60 °C (%)	60 °C, 75% RH (%)	25 °C, 92.5% RH (%)	Light, RM (%)
2	−4.1	−2.5	8.9	9.9	3.1
5	−4.2	−2.7	8.6	13.8	3.1
10	−4.5	−3.1	8.4	18.6	3.2
20	−4.9	−3.4	7.5	21.9	3.5

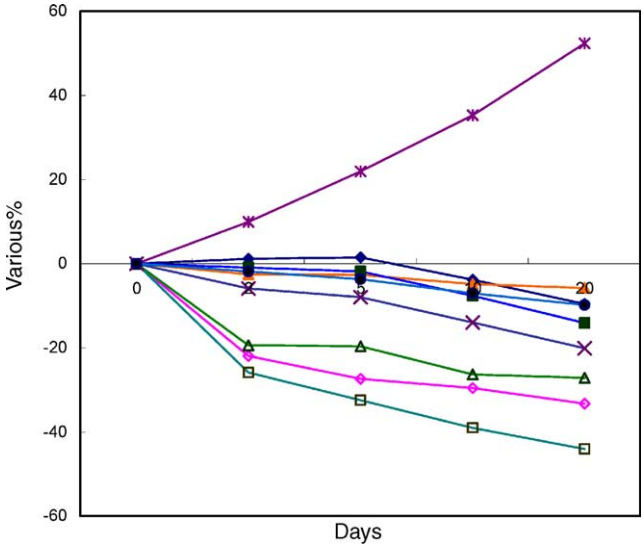

Fig. 3. Monacolins in red yeast rice at high temperature (80 °C, dryness) ((●) monacolin L; (◊) monacolin L hydroxyl acid form; (▲) monacolin J; (△) monacolin J hydroxyl acid form; (✕) dehydromonacolin K; (□) mevinolin K hydroxyl acid form; (■) monacolin K (mevinolin); (✕) sum of monacolin K and its acid; (|) sum of the total monacolins).

Fig. 4. Fig. 5 showed the effect of sunlight on the contents of these monacolin compounds for the interval periods of 0, 2, 5, 10 and 20 days at the average temperature of 25 °C. The changes of monacolin K, monacolin K hydroxyl acid, sum of monacolin K and its acid, as well as the dehydromonacolin K under five different conditions, 60 °C, 80 °C, 60 °C with 75% RH, 92.5% RH at 25 °C, and sunlight, were summarized in Fig. 6(a)–(d).

From Figs. 3–6, the results suggested (i) the contents of both Monacolin K, J and L and their hydroxyl acid forms

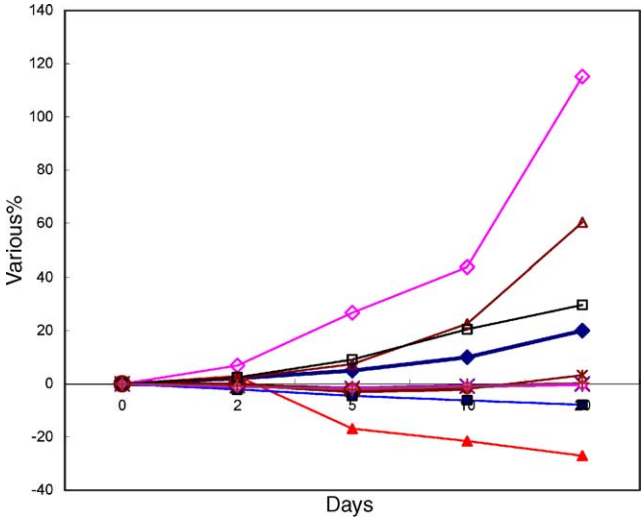

Fig. 4. Monacolins in red yeast rice in high humidity (92.5% RH, 25 °C) ((●) monacolin L; (◊) monacolin L hydroxyl acid form; (▲) monacolin J; (△) monacolin J hydroxyl acid form; (✕) dehydromonacolin K; (□) mevinolin K hydroxyl acid form; (■) monacolin K mevinolin; (✕) sum of monacolin K and its acid; (|) sum of the total of monacolins).

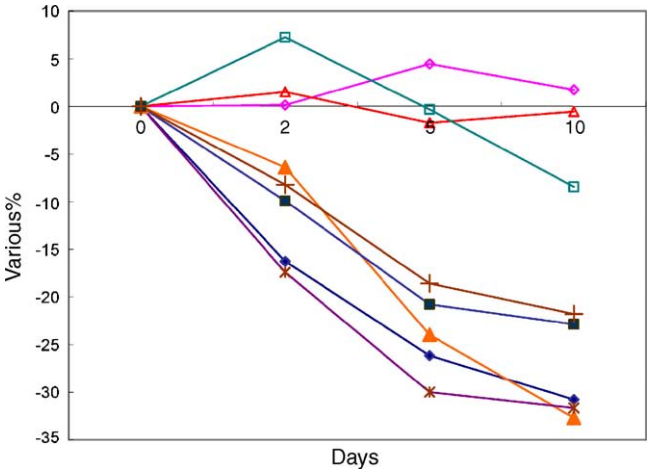

Fig. 5. Sunlight effects on monacolins in red yeast rice powder ((●) monacolin L; (◊) monacolin L hydroxyl acid form; (▲) monacolin J; (△) monacolin J hydroxyl acid form; (✕) dehydromonacolin K; (□) mevinolin K hydroxyl acid form; (■) monacolin K (mevinolin); (✕) sum of monacolin K and its acid).

decreased, while dehydromonacolin K increased under the condition of high temperature (80 °C, dryness) (Fig. 3). It indicated that the monacolins and their hydroxyl acid form could be dehydrated and turned into their dehydromonacolins, as seen in the transformation of dehydromonacolin K from monacolin K and its acid form under the high temperature (Fig. 6). (ii) Under the condition of high humidity (92.5% RH, 25 °C), the contents of monacolin K, J and L descended while their corresponding hydroxyl acid forms ascended, and the total amounts of both the lactone and the hydroxyl acid forms keep less changes (Figs. 4–6). The results revealed that it was notable for the transformation of monacolin lacton forms into their acid forms in the red yeast powder in high humidity. (iii) Almost all individual monacolins and the total amounts of monacolins were decreased in the sunlight (Figs. 5 and 6), which illustrated the light-sensitivity of monacolins in red yeast rice powder. (iv) The degradation of monacolin K was relative quick under the conditions of high temperature with high humidity (60 °C, 75% RH) and sunlight (Fig. 6(a)). Besides, the color of red yeast rice powder also changed from reddish to a whitish on exposing to sunlight. The light- and thermal-sensitivity of these researched material could be concluded and a special light-proof packaging, cool and dryness storing condition were proposed.

3.4. Accelerated testing of red yeast rice powder

The accelerated tests in the storage conditions of 40 °C and 75% RH were conducted for 0, 10, 30, 60 and 90 days. All samples were assayed with the developed method and the results were shown in Table 5. The data revealed that the contents of monacolins as K, L, J in lactone forms descended while their hydroxyl acids ascended, and the total of them

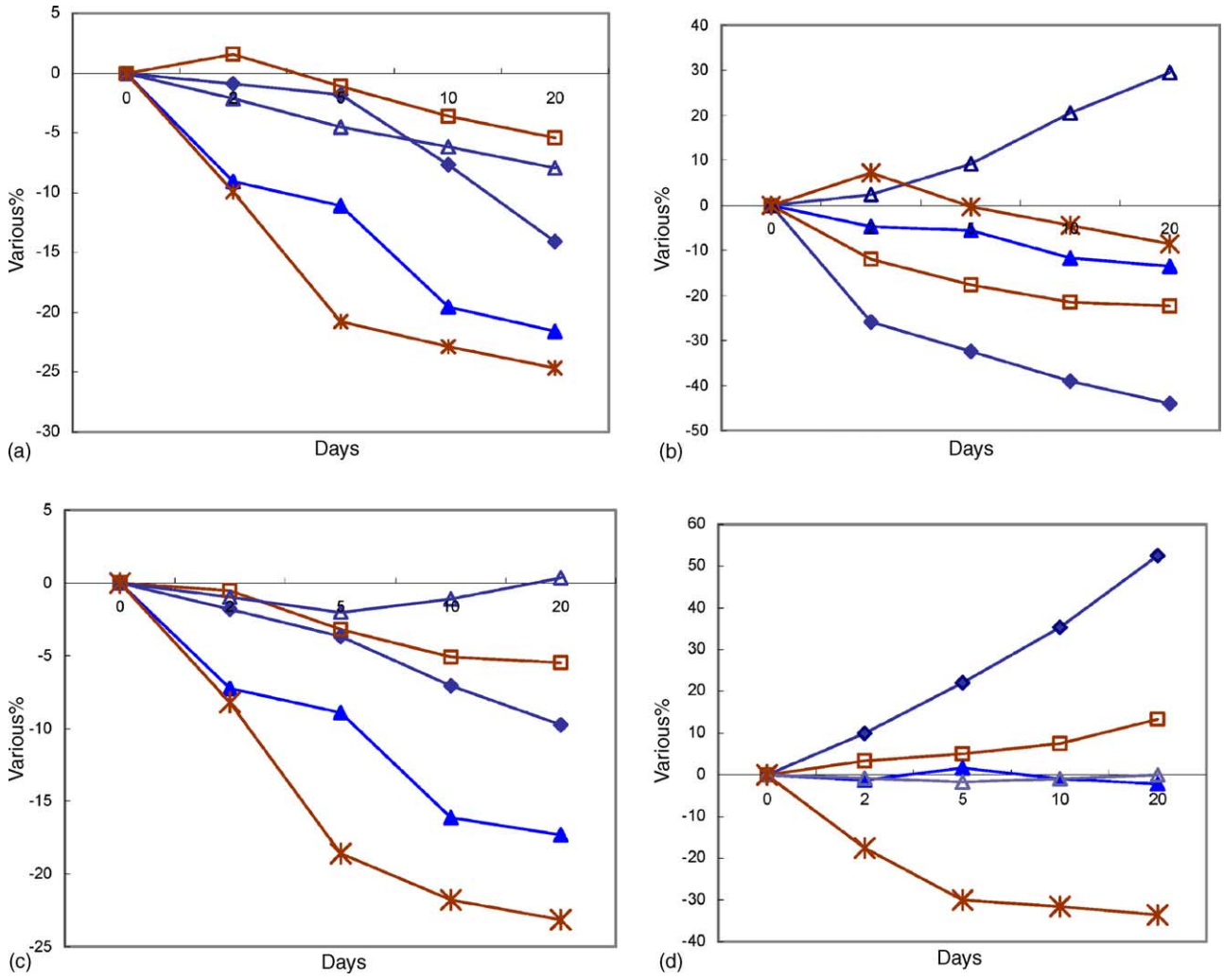

Fig. 6. Variation of monacolin K and related compounds in five stress testing condition (a) monacolin K; (b) monacolin K hydroxyl acid; (c) sum of monacolin K and its acid; (d) dehydromonacolin K ((■) 80 °C, (□) 60 °C, (▲) 60 °C, 75% RH, (△) 25 °C, 92.5% RH, (✱) Sunlight).

with about 6% loss at the end of three 3 months in terms of accelerated test condition.

From summary of above stress testing and accelerated testing data, two basic degradation pathways were proposed: The first pathway was that the lactone-ring of monocolins hydrolyzed and transformed into their hydroxyl acid forms even in solid phase, especially in high humidity condition; the second pathway was that both the lactone-forms and acid-forms of monocolins were dehydrated into dehydromonacolins. The pathway of degradation, photolysis and

Table 5
Variation of monacolins in fermented red rice for the accelerated testing (40 °C, 75% RH)

Ingredient	Days [mg/g, (variation %)]				
	0	10	30	60	90
Monacolin J acid	0.055	0.06 (9.1)	0.062 (12.7)	0.067 (21.8)	0.068 (23.6)
Monacolin J	0.112	0.109 (−2.7)	0.094 (−16.1)	0.085 (−24.1)	0.082 (−26.8)
Monacolin L acid	0.078	0.086 (10.3)	0.111 (42.3)	0.121 (55.1)	0.125 (60.3)
Monacolin L	0.199	0.185 (−7.0)	0.176 (−11.6)	0.169 (−15.1)	0.167 (−16.1)
Monacolin K acid	1.048	1.055 (0.7)	1.064 (1.5)	1.081 (3.1)	1.092 (4.2)
Monacolin K	3.351	3.223 (−3.8)	3.128 (−6.7)	3.072 (−8.3)	3.006 (−10.3)
Dehydromonacolin K	0.493	0.494 (0.2)	0.49 (−0.6)	0.486 (−1.4)	0.481 (−2.4)
Total	5.336	5.212 (−2.3)	5.125 (−4.0)	5.081 (−4.8)	5.021 (−5.9)

oxidation were under further research, and the possible biological activities differences deduced from the variations of structures are worth of well elucidated for the consideration of insuring the safety and efficacy of these kind of products when used in medical or food supplement preparations.

4. Conclusion

In the preceding method for determination of monocolins, a gradient elution HPLC system with diode array detection was applied successfully for good resolution of these seven manocolin family compounds with similar and characteristic UV spectra. The assay method of monacolins in red yeast rice was validated, with a high degree of accuracy and precision. With the well-separation of monacolins from each other as well as the degradation and transformation of monacolins in stability samples traced, a method developed which proved to be a stability-indicating assay method, applicable and reliable for stability testing of red yeast rice and other related products.

Forced degradation studies could provide the evidence about relevant storage conditions while the accelerated testing could give the expectation of shelf life of given products. Compared to the traditional approach of the single marker-oriented method for stability testing, simultaneously assaying multiple ingredients like monacolin K, J, L and their hydroxyl acid forms in this research could explore more information and lead to deeper understanding of chemical variation in botanical products.

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References

[1] J.X. Wang, Zh.L. Lu, J.M. Chi, Curr. Ther. Res. 58 (1997) 964–978.
[2] Ch.L. Li, Y. Zhu, Y.Y. Wang, S.Sh. Zhu, J. Chang, Nutr. Res. 18 (1998) 71–81.
[3] D. Heber, I. Yip, J.M. Ashley, D.A. Elashoff, Am. J. Clin. Nutr. 69 (1999) 231–236.
[4] J.X. Wang, M.Zh. Su, Z.L. Lu, W.R. Kou, Chin. J. Exp. Trad. Med. Formula 1 (1995) 51–60.
[5] J.K. Keithley, B. Swanson, B.E. Sha, et al., Nutrition 18 (2002) 201–204.
[6] S.P. Zhao, L. Liu, Y.C. Cheng, M.H. Shishehbor, M.H. Liu, D.Q. Peng, Y.L. Li, Circulation 110 (2004) 915–920.
[7] M. Journoud, P.J.H. Jones, Life Sci. 74 (2004) 2675–2683.
[8] P. Juzlova, L. Martinkova, V. Kren, J. Ind. Microbiol. 16 (1996) 163–170.
[9] A. Endo, J. Antibiot. 32 (1979) 852–854.
[10] Ministry of Public Health of China, WS-193 (X-183)-95, Monograph.
[11] J.Y. Ma, Y.G. Li, Q. Ye, J. Li, Y.J. Hua, D.J. Ju, J. Agric. Food Chem. 48 (2000) 5220–5225.
[12] Y.G. Li, F. Zhang, Zh.T. Wang, Zh.B. Hu, J. Pharma. Biomed. Anal. 35 (2004) 1101–1112.
[13] The United States Pharmacopeia (USP), 24th revision, United States Pharmacopeial Convention, Inc., Rochville, MD, 2000.
[14] International Conference on Harmonisation (ICH), Stability Testing of New Drug Substance and Products, ICH Harmonised Tripartite Guideline, Q1A(R), 2002.
[15] Food and Drug Adminstration (FDA), Guidance for Industry, Stability Testing of Drug Substance and Drug Products, Rockville, MD, 1998.
[16] M.L. Storms, J.T. Stewart, J. Pharm. Biomed. Anal. 30 (2002) 49–58.
[17] N.A. E1 Ragehy, A.M. Badawey, S.Z. E1 Khateeb, J. Pharm. Biomed. Anal. 28 (2002) 1041–1053.
[18] M. Chorny, D. Levy, I. Schumacher, C. Lichaa, B. Gruzman, O. Livshits, Y. Lomnicky, J. Pharm. Biomed. Anal. 32 (2003) 189–196.
[19] Y. Shao, R. Alluri, M. Mummert, U. Koetter, S. Lech, J. Pharm. Biomed. Anal. 35 (2004) 625–631.
[20] M. Bakshi, S. Singh, J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.
[21] R.J. Stubbs, M. Schwartz, W.F. Bayne, J. Chromatogr. 383 (1986) 438–443.
[22] G. Morovjan, G. Szakacs, J. Fekete, J. Chromagr. A 763 (1997) 165–172.
[23] J. Wen, P. Chang, X.L. Gu, Z.L. Ju, Food Sci. 21 (2000) 100–102.
[24] X.Q. Zhang, F.R. Zhou, J.M. Shi, J. Chin. Trad. Med. 22 (1997) 222–224.
[25] Ch.L. Wang, J. Beijing Agric. Coll. 15 (2002) 48–51.