

A New HPLC Method for Analysis of Natural Monacolin K in Red Yeast Rice Pharmaceutical Preparations

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Abstract

Monacolin K (lovastatin or mevinolin) is a naturally occurring hypocholesterolemic statin used to prevent cardiovascular diseases for those persons suffering from hypercholesterolemia, a condition characterized by very high levels of cholesterol in the blood. It is originally derived from the fungus *Monascus ruber* and *Monascus purpureus* family Monascaceae. Monacolin K is a lactone polyketide derived compound converted in the body to the active form, B-hydroxy acid of monacolin K. It acts by inhibiting the hepatic HMG-CoA Reductase enzyme. In this paper, we report a selective precise and accurate new HPLC method for analysis of both lactone form and acid form of monacolin k in red rice pharmaceutical preparations.

Keywords: Red yeast rice; *Monascus purpureus*; Monacolin K; Lovastatin; HPLC; Diode array detector (DAD)

Introduction

Red yeast rice is a fermented food product produced by inoculating *Monascus purpureus* into steamed rice [1]. Extracts from red yeast rice are well known for their content of starch, sterols, isoflavones, mono-unsaturated fatty acids and monacolins. The functions of red yeast rice products include reducing blood serum cholesterol [2], anti-fatigue [3], fighting Alzheimer [4], preventing obesity development [5], and prevention of diabetes development [6]. Monacolins are polyketide derived compounds that has the ability of lowering the blood lipid levels. Increased levels of cholesterol and triglycerides are known to be risk factors for developing coronary artery diseases. Lipid-lowering agents that inhibit HMG coenzyme-A reductase are now prominent among the drugs of choice for treating hypercholesterolemia. Monacolin k (Figure 1) is a potent inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis and has been demonstrated to be effective in reducing both cholesterol and triglyceride. Fourteen naturally occurring monacolin compounds were identified in red yeast rice (Figure 2), those are monacolin K, monacolin J, monacolin L, monacolin M, monacolin X, and their hydroxy acid form, as well as dehydromonacolin K, dihydromonacolin L, compactin, 3 α hydroxy-3,5-dihydromonacolin L [7]. Monacolins are a group of statins existing in both lactone forms and hydroxyl acid forms [8]. Monacolin K is an active pharmaceutical ingredient in the *Monascus* capsules. Its role is to reduce serum total cholesterol, triglyceride, and LDL cholesterol, increase HDL cholesterol, treat for hyperlipidemia and cardio-cerebrovascular, diseases caused by hyperlipidemia and atherosclerosis. Statins are a novel class of drugs widely used for the treatment of hypercholesterolemia and atherosclerosis commonly known to act as 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors (Figure 3) [9,10]. High-performance liquid chromatography (HPLC) shows many advantages in the chemical analysis of herbal medicines and their preparations, such as high sensitivity, reproducibility, good resolution, linearity, the ability to analyze multiple constituents and the ease of automation. Because of the acidity of statin compounds, acidic modifiers are usually used to suppress their ionization and to achieve optimal separation. The modifier used here is di-hydrogen phosphate potassium salt [11]. In this research paper we are using the usefulness of instability of the Monacolin K lactone

compound [8-[2-(4-hydroxy-6-oxo-oxan-2-yl) ethyl] -3,7-dimethyl-1,2,3,7,8,8a-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2-methylbutanoate which can be caused by hydrolysis in aqueous media to determine both the lactone and acid form (Figure 4) [12]. The determination of Monacolin K in the capsule of *Monascus* has great significance for the control of clinical therapy. So, a simple, rapid, and sensitive analytical method for separation and determination of Monacolin K in the capsule of *Monascus* is of great interest. HPLC has been applied for the qualitative and quantitative analysis of Monacolin K in the capsule [11,13]. The objective of this paper was to quantify the amount of monacolin k in commercial pharmaceutical preparations containing *M. purpureus* existing in both lactone form and hydroxy acid form using technology of hyphenated HPLC/DAD. The content of monacolin K in red yeast rice commercial preparation was estimated referenced to standard commercially available monacolin K lactone form (MKL).

Experimental

Materials and chemicals

Capsules of red yeast (Biochol BIO PHARMA EGYPT S.A.E.) were purchased from the Egyptian Drugstores. Reference standard Monacolin K and KH_2PO_4 analysis reagent grade were purchased from sigma. Acetonitrile HPLC grade and Methanol HPLC grade were purchased from Merck.

Equipment (Apparatus and chromatographic conditions)

HPLC was performed with a Dionex ultimate 3000 isocratic chromatograph equipped with Ultimate 3000 pump, Ultimate 3000

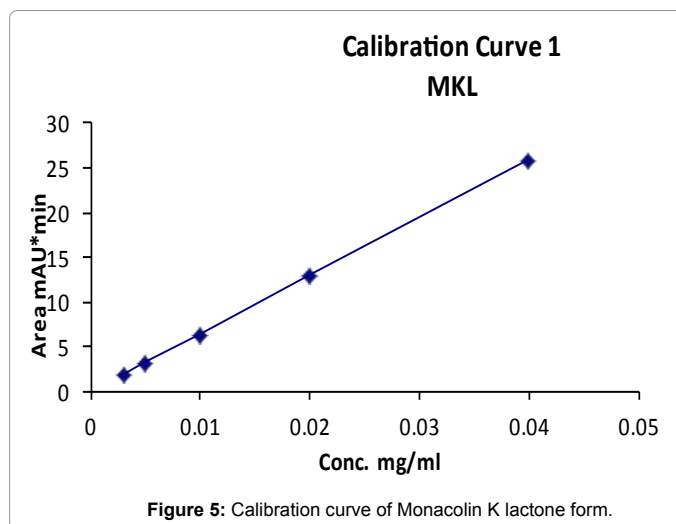
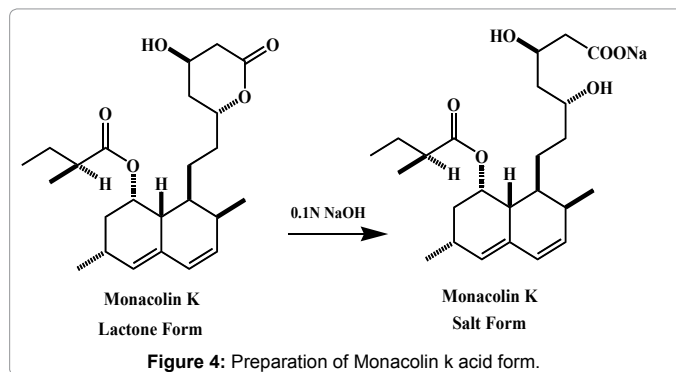
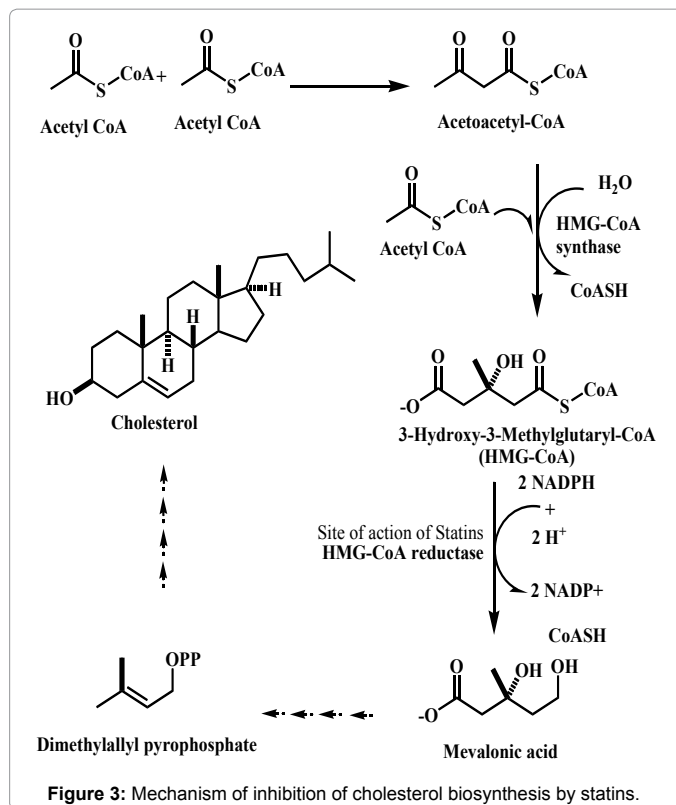
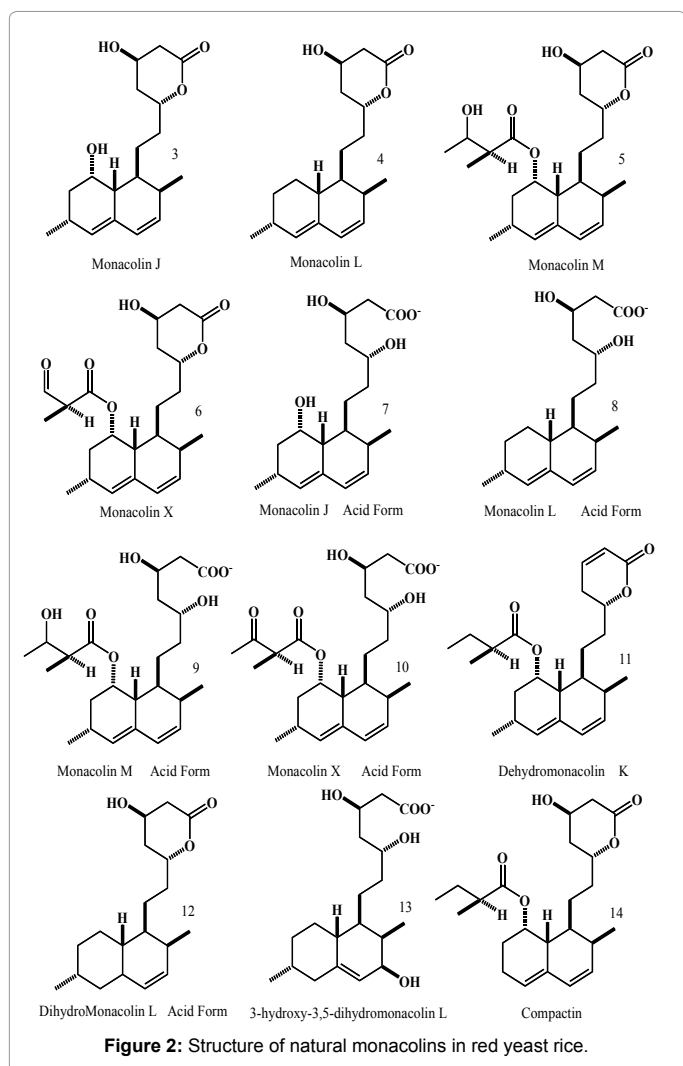
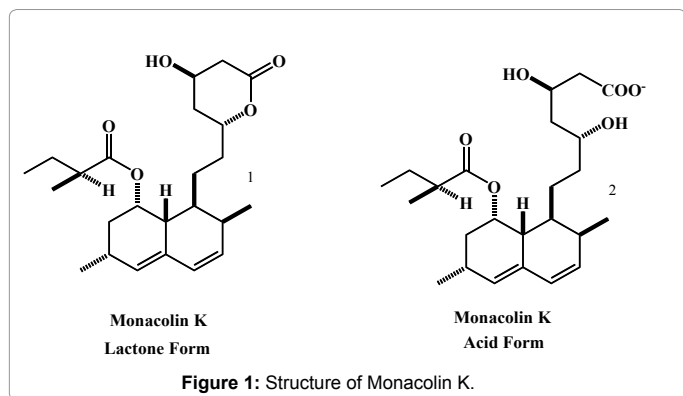
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column compartment, controlled by Ultimate 3000 autosampler and Ultimate 3000 photodiode array detector (PAD) was used for peak purity test and analysis of compounds. Analysis was performed on a Dionex octadecyl silyl silica gel column (250 mm x 4.6 mm I.D., 5 μm particle size). The mobile phase was Isocratic acetonitrile (A) and 50mM KH₂PO₄ pH 3.5 (B) 60:35. The injection volume was 20 μl and the samples were monitored at 237 nm. A constant flow-rate of 1.5 ml/min was used during analysis. HPLC grade solvents and bi-distilled



water were used in the chromatographic studies. All chromatographic experiments were performed at constant column temperature 40°C.

Procedure

Sample preparation

150 mg of the red yeast capsule (Biochol Capsule) content were accurately weighed and transferred to 100 ml volumetric flask. 80 ml of mobile phase {acetonitrile-50mM KH_2PO_4 pH 3.5 (60:35)} were added. Mixture was sonicated for 10 minutes to be dissolved. Volume was completed to 100 ml with the mobile phase. Sample was filtered through a 0.45 μm syringe membrane filter prior to injection (20 μL) into HPLC.

Preparation of standard solutions

Preparation of standard Monacolin K lactone form (MKL): An accurately weighed amount of lovastatin (Monacolin K) 25 mg (99.5% purity) was transferred to 100 ml volumetric flask. 70 ml of mobile phase {acetonitrile - 50mM KH_2PO_4 pH 3.5 (60:35)} was added. Mixtures were sonicated for 10 minutes to be dissolved. Dilution with mobile phase to 100 ml was carried out to form the parent standard solution.

Standard Solution 5 (0.0398 mg/ml): 4 ml of the parent standard solution was transferred to a 25 ml volumetric flask; volume was completed and adjusted to 25 ml with the mobile phase acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35).

Standard Solution 4 (0.0199 mg/ml): 2 ml of the parent standard solution was transferred to a 25 ml volumetric flask; volume was completed and adjusted to 25 ml with the mobile phase acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35).

Standard Solution 3 (0.00995 mg/ml): 1ml of the parent standard solution was transferred to a 25 ml volumetric flask; volume was completed and adjusted to 25 ml with the mobile phase acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35).

Standard Solution 2 (0.00497 mg/ml): 5 ml of the Standard Solution 3 was transferred to a 10ml volumetric flask; volume was completed and adjusted to 10 ml with the mobile phase acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35).

Standard Solution 1 (0.00298 mg/ml): 3 ml of the Standard Solution 2 was transferred to a 5 ml volumetric flask; volume was completed and adjusted to 5 ml with the mobile phase acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35).

Preparation of standard Monacolin K acid form (MKA): Standard Solution 5b (0.0398 mg/ml): 2 ml of the Standard Solution 5 were mixed with 2 ml 0.1 N NaOH and kept for 2 h at 30°C. The mixture was dried under vacuum, and then dissolved in 2 ml acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35). The solution was filtered through a 0.45 μm membrane syringe filter prior to analysis using HPLC.

Standard Solution 4b (0.0199 mg/ml): 2 mls of the Standard Solution 4 were mixed with 2 ml 0.1 N NaOH and kept for 2 h at 30°C. The mixture was dried under vacuum, and then dissolved in 2 ml acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35). The solution was filtered through a 0.45 μm membrane syringe filter prior to analysis using HPLC.

Standard Solution 3b (0.00995 mg/ml): 2 mls of the Standard Solution 3 were mixed with 2 ml 0.1 N NaOH and kept for 2 h at 30°C.

The mixture was dried under vacuum, and then dissolved in 2 ml acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35). The solution was filtered through a 0.45 μm membrane syringe filter prior to analysis using HPLC.

Standard Solution 2b (0.00497 mg/ml): 2 mls of the Standard Solution 2 were mixed with 2 ml 0.1 N NaOH and kept for 2 h at 30°C. The mixture was dried under vacuum, and then dissolved in 2 ml acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35). The solution was filtered through a 0.45 μm membrane syringe filter prior to analysis using HPLC.

Standard Solution 1b (0.00298 mg/ml): 2 mls of the Standard Solution 1 were mixed with 2 ml 0.1 N NaOH and kept for 2 h at 30°C. The mixture was dried under vacuum, and then dissolved in 2 ml acetonitrile-50 mM KH_2PO_4 pH 3.5 (60:35). The solution was filtered through a 0.45 μm membrane syringe filter prior to analysis using HPLC.

Calibration curves

The calibration curve for Monacolin K lactone form (Figure 5) was obtained by plotting the peak areas of Monacolin K lactone form and Monacolin K acid form versus concentration of Monacolin K lactone form over the range of 0.002985-0.039800 mg/ml. The calibration curve for Monacolin K acid form (Figure 6) was obtained by plotting the peak areas of Monacolin K acid form versus concentration of Monacolin K lactone form (MKL) over the range of 0.002985-0.039800 mg/ml. The data of analysis of the calibration curve are shown in (Table 1 and 2). The recoveries of Monacolin K lactone form (MKL) and Monacolin K acid form (MKA) were found to be in the range of 99-101%. The proposed HPLC method was applied to the determination of equivalent amount of Monacolin K lactone form (MKL) in the Biochol capsule dosage form.

Method development

The procedure was conducted on the liquid chromatography with photodiode array detector was optimized by testing various system conditions. General reverse-phase C18 and ODS columns were used, and several different elution systems were trialed. The resolution and symmetry factors of peaks as monacolin K with its neighboring peaks were satisfied by using acetonitrile-water system. The symmetry and resolution was increased by lowering the pH value of elution (pH 3.5). Several aqueous solutions such as the phosphoric buffer, acetate acid as well as dilute phosphoric acid and dilute trifluoroacetic acid (TFA) together with an organic phase of methanol or acetonitrile etc. were used for the condition optimization. The results suggested a system composed of acetonitrile - 50 mM KH_2PO_4 pH 3.5 (60:35) as an ideal system for the separation of the monacolin compounds. The photodiode array detector was set at 210-350 nm and the chromatogram detected at 237 nm. The column temperature was set at 40°C, and the injection volume was 20 μL . The accuracy of the method was determined by calculating recoveries of Monacolin K by method of standard additions. Selection of mobile phase was performed based on resolution and theoretical plates obtained for both monacolin K lactone form and acid form. The mobile phase acetonitrile and 50 mM KH_2PO_4 (60:35) was found to be satisfactory and gave two well-resolved peaks for Monacolin K lactone form and Monacolin K acid form. The retention time for Monacolin K acid form and Monacolin K lactone form were 5.621 min and 9.07 min, respectively (Figure 7) UV spectra of both the monacolin K lactone form and monacolin K acid form showed that both the drugs absorbs appreciably at 237 nm so, 237 nm was selected as the detection wavelength in HPLC.

Sample Name	Conc. mg/ml	Ret. Time	Area mAU*min	Height mAU
Std 1	0.00299	9.05	2.0008	8.25
Std 2	0.00498	9.04	3.2806	13.33
Std 3	0.00995	9.06	6.4449	26.47
Std 4	0.0199	9.05	13.1608	54.56
Std 5	0.0398	9.06	25.8249	107.81

Table 1: Concentrations (mg/ml) and peak areas of standard Monacolin K lactone form.

Sample Name	Conc. mg/ml	Ret. Time	Area mAU*min	Height mAU
Std 1 _o	0.00299	5.621	2.6677	11
Std 2 _o	0.00498	5.62	4.3741	17.77
Std 3 _o	0.00995	5.621	8.5932	35.29
Std 4 _o	0.0199	5.621	17.5477	72.75
Std 5 _o	0.0398	5.62	34.4332	142.76

Table 2: Concentrations (mg/ml) and peak areas of standard Monacolin K acid form.

Peak No.	Compound	Ret. Time	Conc. mg/ml
1	Monacolin K acid form	5.621	0.0027
2	Monacolin K lactone form	9.07	0.0035

Table 3: Concentration of monacolin K in the sample prepared.

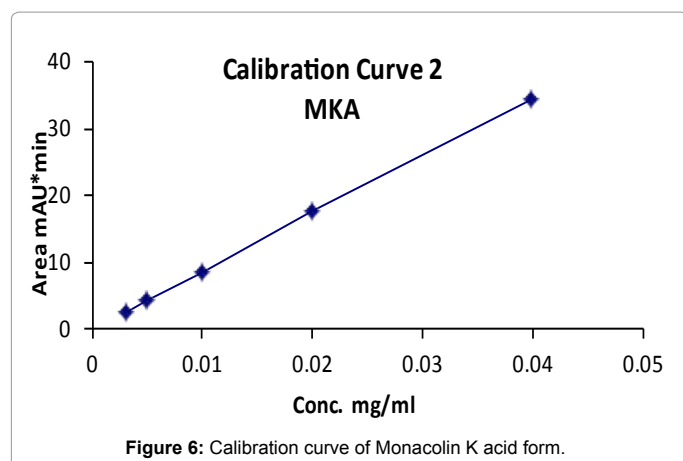


Figure 6: Calibration curve of Monacolin K acid form.

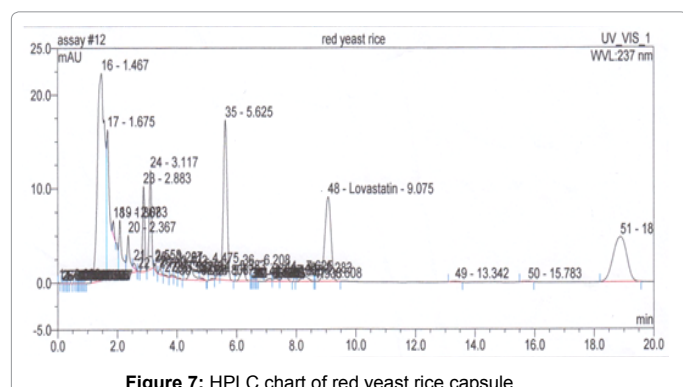


Figure 7: HPLC chart of red yeast rice capsule.

Results

From the constructed standard calibration curve: Concentrations of Monacolin K lactone form and Monacolin K acid form were found to be 0.0035 mg/ml and 0.0027 mg/ml. The total concentration of Monacolin K was found to be 0.0062 mg/ml. Monacolin K in 100 ml sample solution (150 mg powdered red yeast) is 0.62 mg. Concentration of monacolin K in 600 mg red yeast capsule (Biochol) is 2.48 mg/capsule (103.3% of the corresponding labeled amount) (Table 3).

Discussion

The results of the experiment demonstrate that the proposed HPLC method is suitable for the determination of Monacolin K content in the commercial Red yeast rice capsule. In addition, the method is reliable, simple and sensitive.

Conclusion

This work is the first work that can show how to determine both Monacolin K lactone form (MKL) and monacolin k acid form (MKA) in the complex extract of the Red Yeast Rice Pharmaceutical Preparations by the powerful HPLC technique.

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