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Analytical Methods

Simultaneous determination of lovastatin and citrinin in red yeast rice supplements by micellar electrokinetic capillary chromatography

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ABSTRACT

Lovastatin is a main component of *Monascus purpureus* fermented red rice contributing to the lipid-lowering effect. Citrinin is a toxic fermentation by-product which can be found as a contaminant. An accurate, simple and rapid micellar electrokinetic capillary chromatographic method was developed for the first time for simultaneous determination of lovastatin present in lactone and hydroxy acid forms and citrinin in red rice products provided by different manufacturers and formulated in various dosage forms. Separation was achieved within only 2 min using 20 mM of phosphate buffer at pH 7.0 and 30 mM of sodium dodecyl sulphate at an applied voltage of 25 kV. Sensitivity crucial for detecting citrinin was enhanced by using an extended light path capillary. The results showed that the content of lovastatin and its acid form in dietary supplements were considerably different indicating the need for improved standardization in order to ensure efficiency and safety of these products.

1. Introduction

Red yeast rice is a traditional Chinese food and medicine known for centuries to promote blood circulation. It is produced by the fermentation of rice substrates with *Monascus purpureus*. Powdered red yeast rice and its formulated products, used worldwide as dietary supplements, have the ability of reducing blood-lipid levels in humans (Bliznakov, 2000). Several clinical trials have indicated the efficacy of red yeast rice preparations in lowering total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides in the plasma of hyperlipidaemic patients (Heber et al., 1999; Keithley et al., 2002; Wang et al., 1997). Therefore, the use of red yeast rice is appropriate in the primary and secondary prevention of heart disease and other complications of atherosclerosis. The analytical research has revealed that the main component of fermented red rice contributing to the pharmacology effect is monacolin K (Li, Zhang, Wang, & Hu, 2004). However, monacolin K present in red rice fermented with *M. purpureus* is identical to lovastatin which is a widely used statin drug.

Lovastatin was the first commercially available compound for the treatment of hypercholesterolaemia. Increased level of cholesterol is a primary risk factor for developing coronary artery disease, the leading cause of death in the world. Lovastatin decreases the amount of cholesterol by inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This is a key enzyme that catalyses the conversion of HMG-CoA to mevalonate, the rate limiting step in cholesterol biosynthesis. Lovastatin is a fermentation-derived statin drug, whereas the other statins are semi-synthetic or completely synthetic compounds. All statins share an HMG-like pharmacophore, which may be present in the inactive lactone form or in the active β-hydroxy acid form. Lovastatin is administered as lactone prodrug and subsequently enzymatically hydrolysed *in vivo* to its open-ring hydroxy acid pharmacophore.

Although statins have been available for decades, many patients seek alternative therapies and effective ways to control cholesterol level (Knox & Gaster, 2007). In the late 1990s, dietary supplement companies commercialized red yeast rice extracts able to reduce cholesterol as efficiently as statin drugs. The controversy over red yeast rice began shortly after clinical trials when its effectiveness was related to the fact that it contains a naturally-occurring form of the statin drug lovastatin discovered as the major active ingredient (Journoud & Jones, 2004). The US FDA became interested in having a tighter regulation over red yeast rice products and started monitoring lovastatin. However, in many countries the red yeast rice is still considered a food or a dietary supplement. Consequently, there is no strict quality control as there is for medicines and the content of bioactive compounds in its formulated products is still not regulated. The red yeast rice is widely available to the public as an over-the-counter dietary supplement. It is also being recommended by health care practitioners. The use of red yeast rice has augmented in recent years and is expected to increase fur-

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ther in the years ahead because high cholesterol and cardiovascular diseases are being diagnosed more frequently. Therefore, the quality control of red yeast rice products and the determination of lovastatin in these supplements are extremely important.

Lovastatin has been taken as a marker compound for quality control in the official monograph of the red yeast rice in traditional Chinese pharmacopoeia as well as the label claim of some commercial red yeast rice related products. However, the red yeast rice manufactures commonly do not disclose levels of lovastatin in their products and active ingredients are not standardized (Gordon, Cooperman, Obermeyer, & Becker, 2010). Hence, a considerable variation can be found in lovastatin content and the composition of monacolins in commercially available red yeast rice products from different suppliers and moreover discrepancies can be found between claimed and actual content of active ingredients. The food supplement manufactures have also been suspected of spiking red yeast rice preparations with purified lovastatin. Therefore, the development of simple and sensitive analytical methods for separation and determination of lovastatin in red rice products is of great importance for clinical therapy control.

Several HPLC methods with diode array detection (DAD) and tandem mass spectrometry were developed for the analysis of lovastatin in red rice and its formulated products (Chairote, Chairote, Niamsup, & Lumyong, 2008; Huang, Hua, Bao, & Xie, 2006; Li et al., 2004). However, the literature review revealed that there is noticeable shortage of methods developed for the simultaneous determination of lovastatin and citrinin, a toxic fermentation by-product of *Monascus* strains. Citrinin is a known hepato-nephrotoxin, which causes functional and structural kidney damage as well as alterations in liver metabolism. It inhibits several enzymes linked to the respiratory chain of the kidney cortex and the liver mitochondria. Unfortunately, citrinin can be found as a contaminant in red fermented rice. The maximum allowed level of citrinin in red fermented rice is 200 ppb in Japan, but the European Union has the recommended limit of 100 ppb. Lovastatin and citrinin are usually determined by different analytical methods. In the literature, the HPLC method with UV detection for lovastatin analysis in red yeast rice has been published, however citrinin was determined with an indirect competitive ELISA (Chen & Hu, 2005). The HPLC method was developed for lovastatin determination in red yeast rice using DAD detector, while citrinin was quantified using another HPLC method with a mass spectrometer detector (Pattanagul, Pinthong, Phianmongkhol, & Tharatha, 2008). The HPLC method was applied to the analysis of individual and total monacolins including lovastatin in the red yeast rice, but the TLC technique was used to detect citrinin as a potential contaminant (Becker et al., 2009). Lovastatin and citrinin were separated and determined simultaneously in red yeast rice by RP-HPLC method (Lee, Wang, & Pan, 2006). The effluent from the column was passed through a photodiode array detector used for lovastatin analysis and then introduced directly into a fluorescence detector for citrinin determination. The same group of authors published another paper describing almost the same method for the simultaneous analysis of lovastatin and citrinin including only a small change in the composition of mobile phase (Wu, Kuo, Lee, Hsu, & Pan, 2011).

Although chromatographic techniques are sensitive and selective, they are usually time-consuming and require expensive equipment and solvents. Hence, simpler alternative methods for identification and quantification of lovastatin and citrinin are desirable. Capillary electrophoresis (CE) is a powerful analytical technique which is designed to separate the species based on their charge to size ratio in an electric field in a small capillary. It is a very useful tool in pharmaceutical analysis as well as the analysis of statin drugs (Nigović, Damić, Injac, Kočevar Glavač, & Štrukelj, 2009; Nigović & Vegar, 2008). Principal advantages of CE over well-established and widely used HPLC technique include

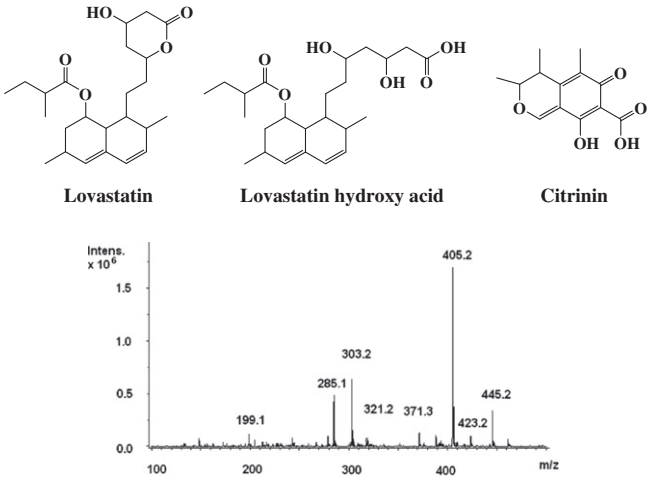

Fig. 1. Chemical structures of lovastatin, lovastatin hydroxy acid and citrinin as well as the MS spectra of lovastatin hydroxy acid.

improvements in low sample volume required, low consumption of solvents and reagents, environmental friendly, cost efficiency, simplicity, high resolution and short analysis time. Mainly employed CE modes for drug analysis are capillary zone electrophoresis (CZE) based on charge-to-mass ratio and micellar electrokinetic chromatography (MEKC) based on chromatographic partition of analytes between micelles and background electrolyte. The CZE method was developed for the determination of lovastatin in the red rice product (Li, Fan, Zhang, Sun, & Cao, 2007). In this study, high pH (10.5) was selected in order to convert lovastatin into its acidic form completely. However, earlier reported studies revealed that lovastatin and lovastatin hydroxy acid are the main active components which contribute up to 90% of the total quantity of monacolins in the red yeast rice (Li et al., 2004). Hence, the main disadvantage of the CZE method developed is that the two main components contributing to the pharmacology effect in the red yeast rice supplement were not determined individually.

Standardized manufacturing practices should be established for red rice dietary supplements in order to ensure efficiency and safety of these products, equivalence of active ingredient content in preparations being sold to the public and to limit the production of unwanted by-products of fermentation such as citrinin. Due to the shortage of analytical methods in the literature for the simultaneous determination of lovastatin and citrinin, the applicability of CE for the separation of these substances has been investigated. The objective of this study was to develop for the first time a simple MEKC method to achieve simultaneous quantification of lovastatin in the red yeast rice existing in lactone and hydroxy acid forms as well as citrinin as toxic fermentation by-product (Fig. 1). The method was validated and applied for the determination of these bioactive and toxic compounds in various commercial formulations of red yeast rice.

2. Experimental

2.1. Chemicals and reagents

Lovastatin and pravastatin sodium, used as an internal standard, were kindly donated by Pliva (Zagreb, Croatia). Citrinin was supplied from Sigma–Aldrich (Steinheim, Germany).

HPLC-grade acetonitrile, methanol and ethanol were purchased from Merck (Damstadt, Germany). Sodium dodecyl sulphate (SDS) was obtained from Poch (Gliwice, Poland). Phosphate buffer solutions were prepared from sodium dihydrogen phosphate (Fluka