

Determination of gamma oryzanol in rice bran oil by HPLC with molecularly imprinted solid-phase extraction

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Article history

Received: 13 October 2015

Received in revised form:

16 December 2015

Accepted: 22 December 2015

Keywords

Gamma oryzanol
Molecularly imprinted
polymer
Photopolymerization
method
Diethylaminoethyl
methacrylate
Thai purple rice bran oil

Abstract

A molecularly imprinting technique can be used for the separation of gamma oryzanol which gives high selectivity and affinity towards template molecules. This technique was successfully applied for the molecularly imprinted polymer (MIP) synthesis via photopolymerization method by using diethylaminoethyl methacrylate (DMAEMA) as a function monomer, gamma oryzanol as template, dodecanol as porogen, 2,2'-dimethoxy-2-phenylacetophenone (DMPA) as initiator and ethylene glycol dimethacrylate (EGDMA) as cross-linker. The interaction effects (amounts of template, porogen, and cross-linker) on adsorption capacity of MIP were investigated. The strongest interaction is between the amount of porogen and the amount of cross-linker. The result of the MIP synthesis was found that 0.75 mmol of gamma oryzanol, 5 mmol of DMAEMA, 6.80 mL of dodecanol, 24.5 mmol of EGDMA and 0.18 mmol of DMPA provided the highest adsorption capacity of MIP (48 µg/mg-adsorbent). The high performance liquid chromatographic method (HPLC) was developed and validated for various chromatographic conditions for determination of gamma oryzanol in rice bran oil. The sample was separated by MIP and analyzed on chromolith® flash RP-18 column (25×4.6 mm, 5 µm), using a gradient binary phase consisting of 1.8 mM cetyl trimethylammonium bromide and methanol as mobile phase. The flow rate was kept at 1.0 mL min⁻¹. The diode array detector was set to monitor at 330 nm for gamma oryzanol. The column temperature was maintained at room temperature. Under the optimum conditions, gamma oryzanol could be determined within a concentration range of 10-50 µg mL⁻¹ which can be expressed by a correlation coefficient of 0.9960. The limit of detection and quantitation were found to be 1.90 µg mL⁻¹ and 5.70 µg mL⁻¹ respectively. The proposed method is relatively rapid and easy to perform for the separation and determination of gamma oryzanol in Thai purple rice bran oil extract.

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Introduction

Rice is a major cereal crop in the developing world. It is consumed as a staple food by over one-haft of the world's population with approximately 95% of production in Asia (Bhattacharjee *et al.*, 2002). Although widely consumed as white rice, there are many special cultivars of rice that contain color pigments such as black rice, red rice and brown. Their name refers to the kernel color (black, red or purple) which is formed by deposits of anthocyanins (Sompong *et al.*, 2011). Rice bran is one of the valuable by products of the rich milling process, particularly rich in dietary fibers and contains of starch, protein, vitamins and dietary minerals. Rice bran oil (RBO) extracted from the germ and inner husk of the rice is rich in gamma oryzanol (Patel *et al.*, 2004; Tuncel *et al.*, 2011). Gamma oryzanol, a group

of ferulic acid esters of phytosterols and triterpene alcohols, is a naturally rich source of antioxidants and unsaturated fatty acids. It is used in a wide range of applications in food, cosmetic and pharmaceutical industries (Aladedunye *et al.*, 2013; Siripairoj *et al.*, 2014). Molecular imprinting has gained popularity during the last decades as a technique of synthesizing polymer materials with chemically selective recognition sites (Alexander *et al.*, 2006; Holthoff *et al.*, 2007; Chen *et al.*, 2011). Molecular imprinting consists in the polymerization of the monomer mixture in the presence of the target molecule or template in an inert solvent. The synthesis of procedure for MIP is not expensive. The synthesis of MIP involves the radical polymerization of a mixture of functional monomers, porogen, cross-linker and a radical initiator in the presence of a template. After polymerization, the template molecule is removed

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from the polymer matrix, thus leaving cavities or specific binding sites in the resulting material that selectively interact with the template molecule and that give rise to molecularly imprinted polymer matrices. The high specificity and stability of MIPs as well as the possibility to synthesize polymers for practically any analyte render those attractive artificial ligands or receptors for various analytical applications. The use of the MIPs as stationary phases for liquid chromatography (Piletsky *et al.*, 2002; Turiel *et al.*, 2004), sorbents for solid-phase extraction (Yin *et al.*, 2011; Jiang *et al.*, 2012; Xu *et al.*, 2015) and ligand binding assays (Ansell *et al.*, 1996) have been reported. Although less numerous, MIPs have also been applied as recognition elements in various types of sensors, including mass spectrometric (Cao *et al.*, 2001), impedimetric biosensors (Cheng *et al.*, 2001), amperometric biosensors (Pellicer *et al.*, 2010) and potentiometric sensor (Pogorelova *et al.*, 2004; Liang *et al.*, 2009; Liang *et al.*, 2010) and chemiluminescence detection (Thongchai *et al.*, 2010).

Different analytical methods have been reported for the determination of gamma oryzanol. Apirak *et al.* (2014) described to develop and validate an image analysis method for quantitative analysis of gamma oryzanol in cold pressed rice bran oil samples. The results obtained by thin layer chromatographic method using densitometry and thin layer chromatographic image analysis methods were compared which good linearity, accuracy, reproducibility and selectivity for determination of gamma oryzanol. Amaraporn *et al.* (2012) described the determination of gamma oryzanol with MIPs from anacardic acid as a function monomer. The experimental results have shown that MIP particles prepared by the thermal polymerization method using toluene as porogen, anacardic acid as functional monomer, benzoyl peroxide as initiator and divinylbenzene as cross-linker clearly recognize property for gamma oryzanol. An analysis of variance with a 95% confidence level suggests that the interaction effect of the amount of porogen and cross-linker, the amount of template and porogen and the amount of template and cross-linker have great influence on the adsorption capacity for gamma oryzanol. Watunyu *et al.* (2014) described a molecularly imprinted polymer for adsorption of gamma oryzanol was synthesized from methacrylic acid. The experimental design and analysis of variance were used to interpret the effects of porogen, template and cross-linker on the adsorption capacity towards gamma oryzanol and regression analysis suggested a polynomial correlation predicting the adsorption capacity of MIP for the separation of

gamma oryzanol. In the preparation of the novel MIP, the following compounds were used: gamma oryzanol as a target molecule, DMAEMA as a functional monomer and EGDMA as a cross-linker. The corresponding NIPs were synthesized under the same synthesis conditions, but without gamma oryzanol.

The present study of this work is to synthesize MIPs as adsorption materials for gamma oryzanol using DMAEMA as a function monomer, dodacanol as a porogen, EGDMA as a cross-linker and DMPA as an initiator. The proposed method has been successfully applied to the separation and HPLC determination of gamma oryzanol in various Thai purple rice bran oil samples.

Materials and Methods

Chemical reagents and materials

Gamma oryzanol (98% purity) was purchased from Tokyo Chemical (Japan). Ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), ethyl methacrylate (EMA) and 2-(diethylamino) ethyl methacrylate (DMAEMA) were purchased from Merck (Germany). 2, 2'-Dimethoxy-2-phenylacetophenone (DMPA) was purchased from Sigma-Aldrich (U.S.A.). Methanol, ethanol, acetonitrile, toluene and dodecanol were purchased from Merck (Germany). Cetyl trimethylammonium bromide (CTAB) was purchased from Fluka (Buchs, Switzerland). All chemical reagents were analytical reagent grade.

Equipments

An ultraviolet-visible measurement was performed using an UV-1600 spectrophotometer (Shimadzu, Japan). The variable wavelength detector was operated at 330 nm for the determination of gamma oryzanol concentration on adsorption capacity study. High performance liquid chromatography-diode array detection analyses were performed on a Shimadzu LC-10ADVP system (Shimadzu Corporation, Kyoto, Japan), equipped with degasser (DGU-14A), solvent delivery systems (LC-10ATVP), column oven, SPD-10A UV/Visible dual detector, and LC Lab-Solution software (Shimadzu Corporation, Kyoto, Japan). All analyses were achieved on chromolith® flash RP-18 column (25×4.6 mm, 5 µm) operated at a temperature of 25°C. The mobile phase was a gradient binary phase consisting of 1.8 mM CTAB and methanol. Flow rate was kept at 1.0 mL min⁻¹ and the sample volume injected was set at 20 µL. The diode array detector was set to monitor at 330 nm for gamma oryzanol. A soxhlet apparatus was used for template