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

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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.

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

removal from the synthesized MIP.

For spectroscopic characterization, a Thermo Scientific (Nicolet IS5) Fourier Transform Infrared (FTIR) was employed. The surface morphology was analyzed by using scanning electron microscopy (SEM) from electron microprobe JXA 840 A (Jeol-Japan) combined with LINK analytical system.

Sample preparation

Thai purple rice used in this study was collected (Junly 2014) from Phitsanulok and Tak province, Thailand. The method was modified for the extraction of gamma oryzanol from purple rice seeds (Xu *et al*., 1999). Briefly, two grams of purple rice seeds were homogenized with 10 mL of ethanol and hexane (4:3, v/v) for 5 min at 10,000 rpm, prior to centrifugation for 20 min at 7600×g. The pellet was re-extracted twice with 10 mL of hexane and centrifuged. The supernatant were pooled and washed first with 10 mL distilled water and then with 5 mL of a 10% aqueous sodium chloride solution. The organic phase was retained and reduced to dryness under a gentle stream of nitrogen.

Preparation of gamma oryzanol imprinted and non-imprinted polymer by photopolymerization method

A prepolymerization solution consisting of 0.75 mmol of gamma oryzanol, 5 mmol function monomer (1.00 mL of DMAEMA, MAA and EMA), 24.5 mmol (4.62 mL) EGDMA, 0.18 mmol (45 mg) DMPA and 8.60 mL dodecanol were prepared in a screw-capped glass vial. The molar ratio of the template for the prepared MIPs was 1:2. The solution was sonicated for 20 min, and then purged with a stream of nitrogen for 10 min. The MIP solution was placed in UV block by photo-initiated with a UV light, which emitted light at 365 nm and irradiated for 30 min. The MIPs was then washed with acetonitrile followed by 50% of methanol in water to remove the template and residues of nonreactive species. Non-imprinted polymers (NIP) were prepared simultaneously under the same conditions without the addition of the template.

Effect of adsorption capacity of MIP and NIP for gamma oryzanol

The adsorption capacity, being the value of the amount of adsorbed substance obtained in a saturated solution (McNaught and Wilkinson, 1997) was calculated by using Equation (1):

$$\text{adsorption capacity} = \frac{\text{adsorbed gamma oryzanol (mg)}}{\text{weight of polymer added (g)}} \quad \text{Equation (1)}$$

MIP-SPE protocol

The sample was used to adsorb into the MIP which the gamma oryzanol was trapped onto the MIP. After adsorption, the surface of the MIP must be washed with acetonitrile in order to expel the target molecule prior to use as an adsorption process continued again. An UV-visible spectrophotometer was used to detect the remainder of gamma oryzanol in the acetonitrile solution to ensure the complete washing.

Various quantities of the analyzed polymers were packed into SPE glass columns equipped with porous PTFE disks at the top and at the bottom of the polymer bed. The MIP-SPE protocol accounted for the conditioning of methanol and redistilled water (3:3, mL) of the prepared MIPs cartridges before sample application. A spiked gamma oryzanol oil sample (1 mL) was then loaded. The cartridge was washed with 2 mL redistilled water. The analytes were eluted in 3 mL with ethyl acetate and hexane (20:80, v/v). The extracts were evaporated to dryness. The residue was dissolved in 200 μL mobile phase and injected into the HPLC system.

FTIR measurement

FTIR spectroscopic measurements were performed on model Thermo Scientific (Nicolet IS5) FTIR spectrometer (Thermo Nicolet Corporation, USA). The wave numbers of FITR measurement range were controlled from 500 to 4000 cm^{-1}, and collected at one data point per 2 cm^{-1}.

HPLC analysis

For HPLC analysis, 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 the 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 a 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 consisted of 1.8 mM CTAB in water (eluent A) and of methanol (eluent B). The gradient program was as follows: 90% B to 95% B (2 min), 95% B to 95% B (3 min), 95% B to 100% B (5 min) at a flow rate of 1 mL min^{-1}. The injection volume for all samples was 20 μL. The diode array detector was set to monitor at 330 nm for gamma

oryzanol. The column temperature was maintained at a temperature of 25°C. The calibration curve was constructed with the external standard.

Results and Discussion

Evaluation of the MIP for gamma oryzanol

The method for the preparation of the MIP was adapted from that described by Schirmer and Meisel (Schirmer *et al*., 2006). The ability of the MIP to trap the gamma oryzanol was initially evaluated in bulk using spectrophotometric detection where the DMAEMA, MAA and EMA were compared as the functional monomers. The results obtained in comparison to the polymer obtained without the incorporation of the template (NIP). It can be seen from results that much better enrichment is seen with DMAEMA. Figure 1(a) shows the scanning electron micrographs for the three different of the MIP, these show a porous surface with pore diameter distributions from 2-5 mm. During the synthesis, gamma oryzanol was used as the template molecule with DMAEMA as the function monomer, dodecanol as porogen, DMPA as initiator and EGDMA as cross-linker. The carbonyl and hydroxyl groups of gamma oryzanol can form hydrogen bonds with the functional groups (Amaraporn *et al*., 2012). EGDMA was added to the mixture as a cross-linker in order to strengthen the polymer structure. Dodecanol was also added to generate more recognition sites. The MIP structure became rigid after the 30 min of polymerization. Subsequently, the specific imprinted polymer sites were maintained after the removal of the template molecules. The gamma oryzanol MIPs did not dissolved in oil, therefore, it is possible to apply for the separation of gamma oryzanol from various source samples such as rice bran oil.

Adsorption capacity of MIP and NIP for gamma oryzanol

The adsorption of gamma oryzanol on MIP and NIP was tested for different periods of contract time up to 10 h. The binding characteristic of the MIP for gamma oryzanol was investigated by comparing the results using the MIP and the polymer without the incorporation of the template (blank). The sorption isotherms of gamma oryzanol on the MIP and NIP adsorbents were investigated. It can be seen that the sorption of gamma oryzanol by the MIP was significantly higher than that of the respective NIP controls, suggesting an imprinting effect for both MIP. The template binding for the MIP exceeded that of the NIP across the gamma oryzanol concentration of 10 mg L^{-1}. After 10 h, it was assumed that the adsorption capacity has reached the equilibrium for MIP.

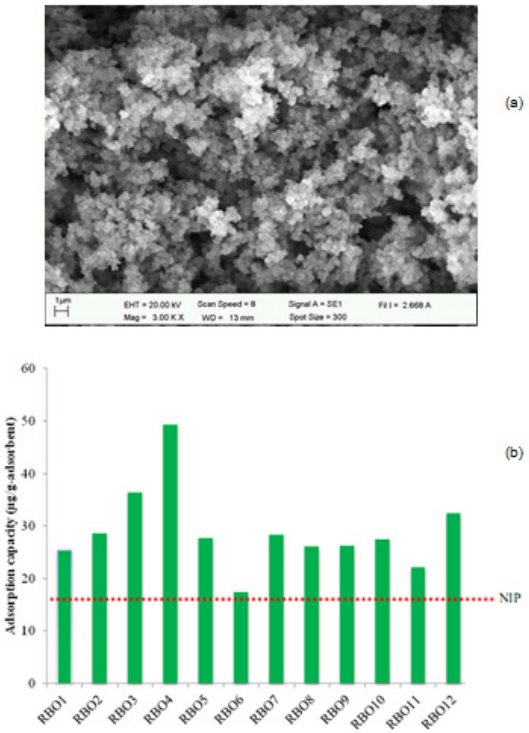

Figure 1. Scanning electron microscopy (SEM) of poly(DMAEMA-EGDMA) particles (a) and amount of gamma oryzanol bound to MIPs and NIP (b)

Effects of type and amount of monomer and porogen

The effects of the type and amount of monomer and porogen were investigated according to the rebinding test results. The adsorption capacity of all synthesized MIP can be calculated using Equation 1. The effect of the type of monomer, porogen and amount of porogen used in the preparation of MIPs and NIP are presented in Table 1. A comparison of the adsorption capacity for MIP and NIP indicated that the NIP provided the lowest capacity. The type of monomer which gave the highest adsorption capacity will be chased for further investigation. The results revealed that DMAEMA monomer gave higher adsorption capacity than MAA and EMA. In the same way, DMAEMA1 monomer was synthesized with amount of dodecanol for 8.6 mL and 4.62 mL of EGDMA gave relatively the highest adsorption capacity compared to other monomers.

Effects of the amount of template and cross-linker

The effects of the amount of template and cross-linker are shown in Table 1. Results showed that the optimal amount of gamma oryzanol for 0.45 g, 2.31 mL of EGDMA and 4.3 mL of dodecanol

Table 1. The effect of the type of monomer, porogen and amount of reagents used in the preparation of MIP and NIP

Polymer code	Monomer (mL)			Initiator (mg)	Cross-linker (mL)	Porogen (mL)		Gamma oryzanol (g)
	DMAEMA	MAA	EMA			Dodecanol	Toluene	
EMA_1	-	-	1.0	45	4.62	8.6	-	0.45
MAA_1	-	1.0	-	45	4.62	8.6	-	0.45
$DMAEMA_1$	1.0	-	-	45	4.62	8.6	-	0.45
EMA_2	-	-	1.0-	45	4.62	-	8.6	0.45
MAA_2	-	1.0	-	45	4.62	-	8.6	0.45
$DMAEMA_2$	1.0-	-	-	45	4.62	-	8.6	0.45
EMA_3	-	-	1.0	45	4.62	4.3	4.3	0.45
MAA_3	-	1.0	-	45	4.62	4.3	4.3	0.45
$DMAEMA_3$	1.0	-	-	45	4.62	4.3	4.3	0.45
RBO1	1.0	-	-	45	4.62	8.6	-	0.45
RBO2	1.0	-	-	45	4.62	4.3	-	0.45
RBO3	1.0	-	-	45	2.31	8.6	-	0.45
RBO4	1.0	-	-	45	2.31	4.3	-	0.45
RBO5	1.0	-	-	45	4.62	8.6	-	0.36
RBO6	1.0	-	-	45	4.62	4.3	-	0.36
RBO7	1.0	-	-	45	2.31	8.6	-	0.36
RBO8	1.0	-	-	45	2.31	4.3	-	0.36
RBO9	1.0	-	-	45	4.62	8.6	-	0.24
RBO10	1.0	-	-	45	4.62	4.3	-	0.24
RBO11	1.0	-	-	45	2.31	8.6	-	0.24
RBO12	1.0	-	-	45	2.31	4.3	-	0.24
NIP	1.0	-	-	45	4.62	8.6	-	-

respectively. Due to the role of porogen in crating increased porosity in the polymer structure for MIP, 4.3 mL of dodecanol used for the synthesis of MIP which caused higher adsorption capacity than that of 8.6 mL of dodecanol. However, for 4.62 mL of EGDMA to the excess amount of porogen lowered the adsorption capacity because of dilution of the pre-polymerization mixture (RBO2 and RBO4) (Song *et al*., 2009).

The reusability of MIP

The RBO4-MIP (Table 1) which provided the highest adsorption capacity was relatively chosen for the reusability test (Figure 1(b)). Acetonitrile was used as an eluent for the measurement of the percentage desorption of the template from the MIP and for the evaluation of reusability of the MIP. In order to test the reusability of MIP, adsorption–desorption cycle was repeated for five cycles by using the imprinted material. The adsorption capacity of the MIP repeatedly was used for five cycles. The results indicated that the RBO4 imprinted polymer can be utilized reduplicatively without a significant loss in adsorption capacity.

The FTIR characterization of MIP and NIP molecularly imprinted polymers and non imprinted polymers were prepared via photo polymerization method using a non-covalent approach. The interaction between functional monomer and template molecule provides the affinity and high selectivity of MIP. In this study FTIR in Figure 2 (a) was used to confirm peaks after co-polymerization had been done. The FTIR results show a broad OH stretching vibration peak at 3,600 cm^{-1} for MIP. These peaks can be associated with carboxylic group (COOH). The $-CH_2$ stretching peak was also observed at 2,949 cm^{-1} can be attributed for MIP and this can be due to the presence of methylene group in both DMAEMA and EGDMA. The carbonyl group (C=O) of the ester group stretching peak was observed in MIP at 1,721 cm^{-1} and this might have originated from DMAEMA and EGDMA in MIP. Weak combination bands from 1,451-957 cm^{-1} and sharp bands C-N at 1,145 cm^{-1} specifically on MIPs spectra indicate the presence of tertiary amine of the DMAEMA. For NIP shown in Figure 2 (b), the characteristic signals are

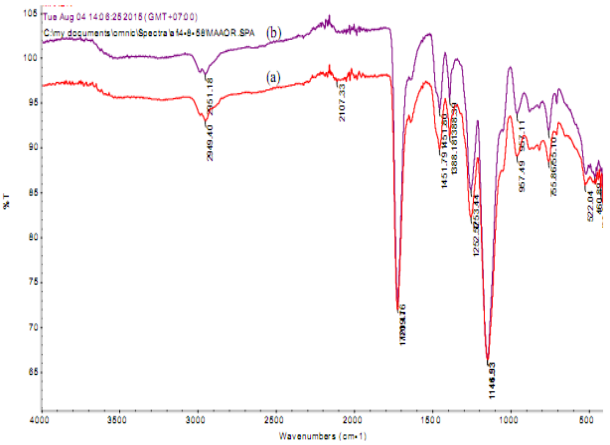

Figure 2. Comparative FT-IR spectra of gamma oryzanol molecularly imprinted (a) and non-molecularly imprinted (b) polymers by photopolymerization method

similar to gamma oryzanol-MIP. However, there are two different points: first, the intensity of the -CH2 (2,951 cm^{-1}) stretching vibration peak is lower than that of gamma oryzanol-MIP; second, the strength of C-N (1,146 cm^{-1}) and C=O (1,720 cm^{-1}) stretching vibration peaks are higher than that of gamma oryzanol-MIP. There are probably two reasons: the one is the polymerization of NIP being devoid of gamma oryzanol, leading DMAEMA remained and taking on strong peak of C-N (1,146 cm^{-1}); the other is that the gamma oryzanol, assembled with DMAEMA by hydrogen bonded interaction, which is essential to the DMAEMA and EGDMA co-polymerization. Consequently, stable imprinting cavities were formed by ordered distribution of functional groups containing C=O and C-N. This result confirmed that the gamma oryzanol-MIP possessed cavities creating abundance functional groups, behaving selective capabilities.

Validation of the method

To test the suitability of the proposed HPLC system and to validate its performance characteristics such as linearity, limit of detection, limit of quantitation, precision and accuracy were performed using under the selected conditions.

Linearity of calibration graph

The linearity of responses to gamma oryzanol was determined. Calibration curve was obtained by using the least-square linear regression analysis of the studied gamma oryzanol peak area (y) versus analyte concentration (x). Each concentration was tested in triplicate. Linear calibration curve was obtained over the concentration range of 10-50 µg mL^{-1} of gamma oryzanol. The standard solution was injected into the HPLC system. Linear calibration graph over the concentration range 10-50 µg mL^{-1} of gamma oryzanol was obtained with a correlation coefficient of 0.9960.

Detection limit and quantification limit

The detection limit of the method was investigated by injecting standard solution of gamma oryzanol into the HPLC column. The detection limit (LOD) was found to be 1.90 µg mL^{-1}. It is calculated as three times of the standard deviation. The quantification limit (LOQ) value was found to be 5.70 µg mL^{-1}. This value was calculated directly from the calibration graph. It is defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision, LOQ may be expressed as: LOQ = 10SD/S where S is the slope of calibration graph and SD is standard deviation (Miller *et al*., 1993).

Precision and accuracy

The precision of the method was determined by measuring the repeatability (intraday precision) and the intermediate precision (interday precision), both expressed as relative standard deviation (RSD). The precision was evaluated by assaying six replicate injections of 10, 20 and 30 µg mL^{-1} of gamma oryzanol. The repeatability was evaluated each sample on the same day under the same experimental conditions, 2.4%, 2.0% and 1.8% respectively. The intermediate precision was evaluated by assaying each sample on three different days, 2.0%, 2.8% and 1.0% respectively.

The recoveries were determined by using standard addition method. Gamma oryzanol standard concentration were added and mixed with known aliquots of sample solutions, the sample was extracted and analyzed using the proposed method. The percentage recoveries of the spiked gamma oryzanol in sample solution were found to be 101.22-118.45%. Results are presented in Table 3.

The contents of gamma oryzanol

The proposed reversed-phase high performance liquid chromatography-diode array detection was applied to the determination of gamma oryzanol in rice berry and purple rice of a Thai rice variety collected from Phitsanulok and Tak province, Thailand. The sample was prepared according to the sample preparation as described in experimental section and the content of gamma oryzanol in each sample solution was determined using the optimum conditions. The samples gave well-defined peaks. There is no interference peak present in either sample (Figure 3). The average contents of gamma oryzanol of rice berry and purple rice were found to be 0.019,

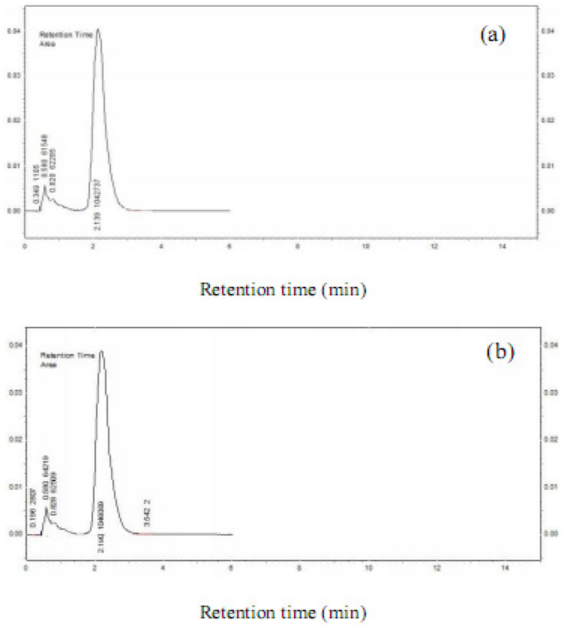

Figure 3. Chromatograms of (a) gamma oryzanol standard and (b) gamma oryzanol in Thai purple rice bran oil sample

0.032, 0.015 and 0.024 µg mg^{-1} respectively (Table 2).

Table 2. The gamma oryzanol contents of rice berry and purple rice bran oil

Sites	Thai rice bran oil	Gamma oryzanol content (µg mg^{-1})	% Recovery
1	Rice berry	0.019±0.11	107.64
	Purple rice	0.032±0.05	118.45
2	Rice berry	0.015±0.03	101.22
	Purple rice	0.024±0.09	112.52

Conclusion

The new monomer of DMAEMA for gamma oryzanol was synthesized by photopolymerization method at 365 nm and irradiated for 30 min using gamma oryzanol as a template molecule, dodecanol as porogen, DMPA as an initiator and EGDMA as cross-linker. The highest adsorption capacity was observed for RBO4-MIP which was synthesized from 0.75 mmol of gamma oryzanol, 6.80 mL of dodecanol, 4.62 mL (24.5 mmol) of EGDMA and 45 mg (0.18 mmol) of DMPA provided the highest adsorption capacity of MIP. The reusability of RBO4-MIP was demonstrated for more than five times without significant loss of performance. The proposed method is relatively rapid and easy to perform for the separation and HPLC determination of gamma oryzanol in Thai purple rice bran oil extract.

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