Bulletin of Health,
Science and Technology
BHST
ISSN 0858-7531
Volume 13, Number 1, 2015 : 50-55

DEVELOPMENT OF A NEW LC-MS METHOD FOR QUANTITATIVE ANALYSIS OF COLD PRESSED RICE BRAN OIL

Thatree Phadungcharoen[1,*], Tossaton Charoonratana[1], Tanate Panrat [2] and Nijsiri Ruangrungsi[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand, 12000.
[2]Prince of Songkla University International College, Prince of Songkla University, Hatyai, Songkhla, Thailand, 90110.
[3] Public Health Sciences Programme, College of Public Health Sciences, Bangkok 10330, Thailand ; Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand, 12000

[*]Corresponding author: thatree.p@chula.ac.th

Abstract: A new developed LC-MS method for analysis of cold pressed rice bran oil (RBO) must be capable to detect and quantify several kinds of valuable constituents. RBO was purchased from the market, in which was claimed to be manufactured by compression method. All samples were subjected into LC-ESI-MS with a quadrupole-ion trap so as to analyze the active pharmaceutical compounds. The method validation was also conducted to observe the parameters according to ICH guideline. Analyses were performed in isocratic mode using acetonitrile and methanol (60:40). The C-18 column was maintained at 30^{o}C with a flow rate of 0.18 ml/min and the injection volume was 10 µl. The chromatographic condition was feasible. Short time for analysis and simple method were achieved. Eight metabolites including linolenic acid, linoleic acid, oleic acid, stearic acid, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate were identified by comparison the retention times and mass spectrum of RBO sample with reference standards. All parameters were validated to guarantee the suitable of this method for analysis the metabolites in RBO.

Keywords: cold pressed rice bran oil, LC-MS, fatty acids, γ-oryzanol

---

บทคัดย่อ: งานวิจัยนี้มีเป้าหมายเพื่อพัฒนาระบบวิธีการวิเคราะห์สารสำคัญในน้ำมันรำข้าวชนิดบีบเย็นที่วางขายตามท้องตลาด โดยใช้วิธีการวิเคราะห์เป็น LC-ESI-MS ที่ต่อกับ quadrupole-ion trap รายละเอียดของวิธีการทดลองคือใช้เฟสเคลื่อนที่เป็น acetonitrile และ methanol ในอัตราส่วน 60:40 และใช้เฟสอยู่กับที่เป็น C-18 โครมาโทกราฟี ใช้อุณหภูมิ 30 องศาเซลเซียส และอัตราเร็วของเฟสเคลื่อนที่เป็น 0.18 มิลลิลิตรต่อนาที ระบบการวิเคราะห์เสร็จสิ้นภายใน 25 นาที ซึ่งสามารถตรวจวัดสารสำคัญได้ 8 ชนิดคือ linolenic acid, linoleic acid, oleic acid, stearic acid, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate และ β-sitosteryl ferulate โดยดูเทียบกับค่าเวลาที่สารถูกชะออกมากับค่ามวลของสารมาตรฐาน นอกจากนั้นงานวิจัยนี้ยังทำการตรวจรับรองวิธีการวิเคราะห์ที่ได้ตามเกณฑ์มาตรฐานของ ICH อีกด้วยเช่นกัน

คำสำคัญ: cold pressed rice bran oil, LC-MS, fatty acids, γ-oryzanol

---

INTRODUCTION

There has been global interest in rice bran oil (RBO) in recent times. It is rapidly growing as an important application in the nutritional industry. This is because of RBO providing benefits to health in its natural state, as it is full of valuable constituents. RBO was reported to possess sterols, phytosterols, tocopherols, tocotrienols, fatty acids, and γ-oryzanol (Godber and Wells, 1994; Patel and Naik, 2004; Ghosh, 2007). Tocopherols, tocotrienols, and γ-oryzanol exhibit antioxidant and hypocholesterolemic effects (Chung and Lee, 2003; Nam et al, 2003; Ausman et al, 2005; Berger et al, 2005). Moreover, they were reported to prevent the diseases in cardiovascular system (Simopoulos, 2008). In addition, RBO has high levels of fatty acids, such as linolenic acid, linoleic acid, oleic acid. All of them show the benefit to reduce the risk of cancer, cardiovascular disease, inflammation, and etc (Sales-Campos et al, 2013).

In Thailand, cold pressed RBO has been developed using the screw compression method. It was used in small factories, which has allowed local communities to produce commercial RBO. The benefit of cold pressed RBO is low cost and its simple method of preparation. In this article, LC-ESI-MS was used as a tool for investigation of cold pressed RBO. LC-ESI-MS is known as a superior technique which can be used to analyze wide range of molecules. This method was validated according to ICH guideline (ICH, 2005) and can be used in a quality control process of cold pressed RBO.

MATERIALS AND METHODS

1) *Materials*:

RBO was purchased from the market. Linoleic acid (C 18:3), linolenic acid (C 18:2), oleic acid (C 18:1), and stearic acid (C 18:0) were purchased from Sigma Aldrich (USA). Standard γ-oryzanol was purchased from Tokyo Chemical Industry (Japan). HPLC grade acetonitrile and methanol were purchased from B&J (Korea).

Individual stock solutions (1.0 mg/ml) of all standards were prepared in isopropanol and filtered through 0.45 µm membrane filter. Working standards, linoleic acid (0.1-10 µg/ml), oleic acid (0.1-10 µg/ml), linolenic acid (0.05-5 µg/ml), stearic acid (0.05-5 µg/ml), and γ-oryzanol (1.0625-17 µg/ml) were prepared by diluting the consequent stock solution with isopropanol.

2) *Methods*:

LC-MS analysis

In this study, Dionex Ultimate TM 3000 HPLC coupling with Bruker Amazon SL mass spectrometer were used as the instrument. A stationary phase was Poroshell 120 EC-C18 column (2.7 µm, 2.1 mm x 150 mm). Analyses were performed in isocratic mode using acetonitrile and methanol (60:40). The column was maintained at 30^{o}C with a flow rate of 0.18 ml/min and the injection volume was 10 µl. The mass spectrometer was equipped with an ESI ion source and a quadrupole-ion trap. LC-MS evaluation was performed using full scan in negative mode recorded on a mass range of *m/z* 100-800. Capillary voltage was set at 4,500 V and drying gas temperature was 220^{o}C with a flow rate of 7.0 l/min. Nebulizer pressure was set at 2 bars. The constituent amount in cold pressed RBO was quantified mostly from the calibration curve. For cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate, the relative amount was calculated based on the proportion of its being in γ-oryzanol. Data were processed by Compass 1.3 SR2 program.

Method validation

The method was validated in accordance with the ICH guidelines Q2(R1) (ICH, 2005).

Linearity

Linearity test solutions of the method were prepared at five concentrations of each reference standards. The calibration curves should show a coefficient of correlation (R^2) ≥ 0.999.

Limit of quantification

The limit of quantification (LOQ) was determined by means of a serial dilution based on signal-to-noise ratios of 10:1. The LOQ concentration was evaluated by precision of six replicate injections.

Precision

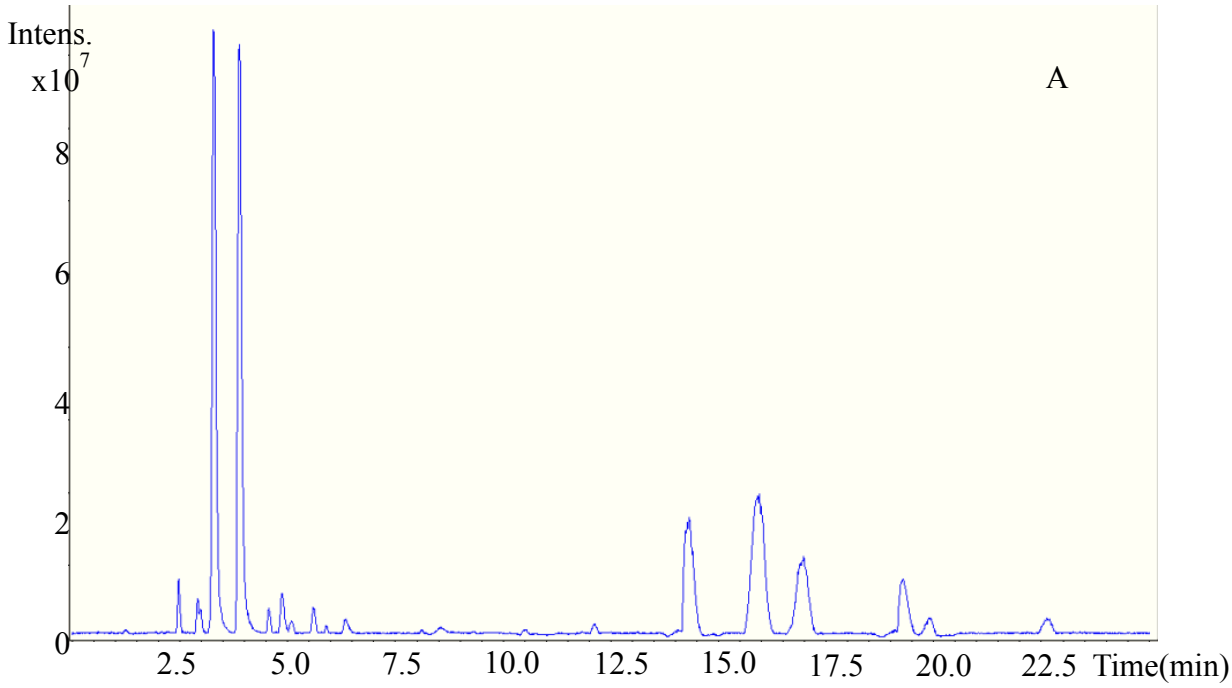
The intra-day precision was optimized by six replicate analyses and was calculated as the relative standard deviation (RSD) of results from the same day. The inter-day precision was optimized by comparing the RSD of analyze samples on three consecutive days.

Accuracy

The accuracy was carried out by adding sample with the known quantities of the standard solutions. Prior to fortification, the background levels of linoleic acid, linolenic acid, oleic acid, stearic acid, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate in the sample were determined so as to calculate actual recoveries. The amounts of each standard were determined in triplicate and the percentage recoveries were calculated.

RESULTS AND DISCUSSION

Different composition of acetonitrile and methanol were tried to figure out the appropriate condition. The most appropriate peaks were showed when an isocratic mode using acetonitrile and methanol (60:40). The elution order was linolenic acid (tR = 3.0 min), linoleic acid (tR = 3.5 min), oleic acid (tR = 4.1), stearic acid (tR = 5.0), cycloartenyl ferulate (tR = 14.4), 24-methylenecycloartanyl ferulate (tR = 16.1), campesteryl ferulate (tR = 16.9), and β-sitosteryl ferulate (tR =19.4), the chromatograms were shown in Figure 1.



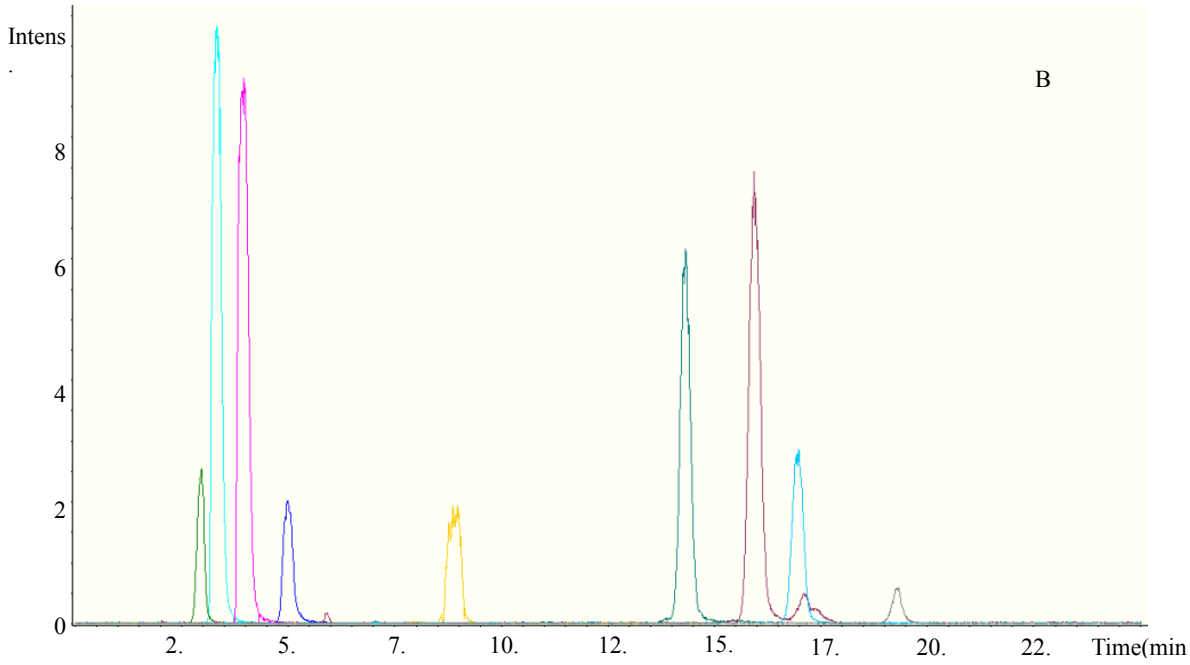


Figure 1. (A) LC-MS chromatograms of cold pressed RBO; (B) LC-MS chromatograms of standard linolenic acid (tR = 3.0 min), linoleic acid (tR = 3.5 min), oleic acid (tR = 4.1 min), stearic acid (tR = 5.0 min), α-tocopherol (tR = 8.9 min), cycloartenyl ferulate (tR = 14.4), 24-methylenecycloartanyl ferulate (tR = 16.1 min), campesteryl ferulate (tR = 16.9 min), and β-sitosteryl ferulate (tR =19.4 min)

Method validation

Linearity

The linearity was determined by means of linear regression analysis. All calibration curves were linear over their concentration ranges with the coefficient of correlation (R^2) ≥ 0.999. The concentration ranges of each standard are linoleic acid (0.1-10 µg/ml), oleic acid (0.1-10 µg/ml), linolenic acid (0.05-5 µg/ml), stearic acid (0.05-5 µg/ml), and γ-oryzanol (1.0625-17 µg/ml).

Limit of quantification

The lowest reliable amount for quantification of each metabolite was determined. The LOQ values of linolenic acid and stearic acid were 1.5 ng/ml, while linoleic acid and oleic acid were 1.0 ng/ml. For the ferulate esters, while the LOQ of campesteryl ferulate and β-sitosteryl ferulate were 21.0 ng/ml, the LOQ of cycloartenyl ferulate and 24-methylenecycloartanyl ferulate were 4.2 ng/ml as shown in table 1.

Precision

The precision of the method was evaluated by determining of intra-day and inter-day analysis. The method was shown to be reliable with both intra-day and inter-day precision values, the RSD were found to be 0.34-0.50% and 1.22-1.94%, respectively (Table 1).

Accuracy

The spiking procedure was performed by adding the standards to RBO. Before spiking, the background levels of all standards in RBO were analyzed so as to calculate actual recoveries. Mean recoveries in the range of 101-103% were observed for all standards (Table 1).

The method presented in this study is more suitable than the other method published previously for RBO determination since its capability to determine wider range of compounds found in RBO in one single run (Lu, 2014).

Table 1: Validation data of the developed LC-MS method

Markers	LOQ (ng/ml)	RSD (%)		%Recovery
		Intra-day	Inter-day	
Linolenic acid	1.5	0.50	1.94	102.47±1.10
Linoleic acid	1.0	0.39	1.55	101.14±0.55
Oleic acid	1.0	0.38	1.22	101.38±0.99
Stearic acid	1.5	0.45	1.47	102.64±0.98
Cycloartenyl ferulate	4.25	0.34	1.30	102.29±0.83
24-methylenecycloartanyl ferulate	4.25	0.41	1.73	101.88±0.92
Campesteryl ferulate	21.0	0.47	1.49	101.22±1.23
β-sitosteryl ferulate	21.0	0.34	1.34	102.70±0.75

CONCLUSION

Fatty acids and γ-oryzanol which found in cold pressed RBO possess significant dietary value and a range of pharmacological effects, thus new method which can be used to detect these compounds must be built up. It was found that the presented LC-MS method suited for the quality control process of cold pressed RBO manufacturing. This new validated method proposes the development of a fast, simple, sensitive, and reliable LC-MS method for the determination of several compounds in cold pressed RBO.

ACKNOWLEDGMENTS

The authors wish to thank all staffs in Sino-Thai Traditional Medicine Research Center, Rangsit University, Pathum Thani, Thailand for all instrumental equipments. The grant number 51/2557 from the Research Institute of Rangsit University was also acknowledged.

REFERENCES

Ausman LM, Rong N, Nicolosi RJ. 2005. Hypocholesterolemic effect of physically refined rice bran oil: studies of cholesterol metabolism and early atherosclerosis in hypercholesterolemic hamsters. J Nutr Biochem ; 16:521-529.

Berger A, Rein D, Schafer A, Monnard I, Gremaud G, Lambelet P, *et al.* 2005. Similar cholesterol-lowering properties of rice bran oil, with varied γ-oryzanol, in mildly hypercholesterolemic men. Eur J Nutr ; 44:163-173.

Chung YA, Lee JK. 2003. Antioxidative properties of phenolic compounds extracted from black rice. Korean Soc Food Sci Nutr ; 32(6):948-951.

Ghosh M. 2007. Review on recent trends in rice bran oil processing. J Am Oil Chem Soc ; 84:315-324.

Godber JS, Wells JH. 1994. Rice bran: as a viable source of high value chemicals. Louisiana Agriculture ; 37(2):13-17.

ICH. 2005. Validation of analytical procedures: text and methodology Q2(R1), [cited 2015 April 16]; Available from:http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures- text-and-methodology.html.

Lu W, Niu Y, Yang H, Sheng Y, Shi H, Yu LL. 2014. Simultaneous HPLC quantification of five major triterpene alcohol and sterol ferulates in rice bran oil using single reference standard. Food Chem ; 148:329-334.

Nam SH, Jang SM, Kang MY. 2003. Varietal difference in antioxidative activity of ethanolic extracts from colored rice bran. J Korean Soc Appl Biol Chem ; 46(1):16-22.

Patel M, Naik SN. 2004. Gamma-oryzanol from rice bran oil. J Sci Ind Res ; 63:569-578.

Simopoulos AP. 2008. The importance of the omega-6/omega-3 fatty acid ration in cardiovascular disease and other chronic diseases. Exp Biol Med ; 233(6):674-688.

Sales-Campos H, Souza PR, Peqhini BC, da Silva JS, Cardoso CR. 2013. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev Med Chem ; 13(2):201-210.