ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 4, 2015

ISSN - 0974-2441

Research Article

SIMULTANEOUS DETERMINATION METHOD OF BUTYLHYDROXYANISOLE, BUTYL HYDROXY TOLUENE, PROPYL GALLATE, AND TERTIARY BUTYL HYDROQUINONE IN MARGARINE USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Christinawaty[1,2], Sophi Damayanti[2]*

[1]Indonesia National Agency of Drug and Food Control, Jalan Pangeran Ratu Seberang Ulu I, Palembang, Indonesia. [2]Pharmacochemistry Research Group, School of Pharmacy, Bandung Institute of Technology, Jalan Ganesa No.10, Bandung, Indonesia.
Email: sophi.damayanti@fa.itb.ac.id

Received: 23 April 2015, Revised and Accepted: 18 May 2015

ABSTRACT

Background: Antioxidants are food additives in oil and fat products usually to decrease the process of rancidity by preventing the oxidation such as in margarine. BHA, BHT, propyl gallate, and TBHQ are commonly used antioxidants. Combinations of antioxidants are generally used in the food product. Therefore, analytical method which is capable to measure the antioxidants simultaneously, is required.

Objective: This study was aimed to obtain analytical method for determination of BHA, BHT, propyl gallate, and TBHQ using HPLC in margarine.

Methods: The analysis was using Waters column Xbridge C18 (4.6 x 150 mm, 5µm) and 280 nm ultraviolet detector. The mobile phase was a mixture of 1% acetic acid in acetonitrile and 1% acetic acid (3:7). The flow rate was set at 1.7 ml/min.

Results: The calibration curve of BHA, BHT, propyl gallate and TBHQ were linear with correlation coefficient r = 0.9999, 0.9996, 0.9998 and 0.9998, respectively. Limit of detection and limit of quantitation of BHA, BHT, propyl gallate and TBHQ were 2.6217 and 7.9445 µg/ml; 4.1837 and 12.6777 µg/ml; 2.494 and 7.559 µ/ml; 2.1272 and 6.4461 µg/ml, respectively. Inter day precision of the method was expressed as RSD with result; BHA, BHT, propyl gallate, and TBHQ were 0.1553; 0.4576; 0.0237; 4.8106 %; respectively whereas recovery using standard addition method were 105.38; 107.87; 86.50; 83.64 % respectively.

Conclusions: Based on the obtained results, it can be concluded that this method has been successfully developed and validated for the determination of antioxidants BHA, BHT, propyl gallate, and TBHQ in margarine.

Keywords: Antioxidants, BHA, BHT, propyl gallate, TBHQ, HPLC, margarine.

INTRODUCTION

An antioxidant is a food additive that plays a role for protection against oxidative damage. The protection mechanism is actually able to slow down or inhibit the oxidation of a substance that is easily oxidized even in low concentrations. Antioxidants are widely used in oil and fat products because it serves to slow down the process of rancidity by preventing the oxidation of the oil and fat products [1].

Antioxidants can be used singly or in a mixture of products. For a mixture of antioxidants used must take into account the results for each food additives kind used in the mixture, then the quotient (ratio) the amount of each type of food additives with a maximum limit do not add more than one (principle 1 ratio) [2]. There are two kinds of antioxidants based on its source, namely natural and synthetic antioxidants. Natural antioxidants are usually more desirable because the level of better safety and benefits for food [3,4]. However, natural antioxidants are less stable in the process of making processed food. Natural antioxidants can be found in vegetables, fruits, and plants. Phenolic antioxidants are widely used in the food product category such as fats, oils, and oil emulsions are butylhydroxyanisole (BHA), butyl hydroxyl toluene (BHT), propyl gallate (PG), and tertiary butyl hydroquinone (TBHQ) [5].

Margarine is a food product that adds antioxidant in its formula. It is an emulsion of water in oil (W/O) with ingredient fat, salt, vitamin A, preservatives, dyes, and emulsifiers. The existence of oil in margarine urges the need of an antioxidant [6]. The simultaneous analysis of antioxidant have been developed using gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) with ultraviolet (UV), and diode array detector in several food products [7-10]. However, the determination in BHA, BHT, PG, and TBHQ in margarine has not been developed and validated. The purpose of this study was to develop and validate analytical methods simultaneous determination of levels of BHA, BHT, TBHQ, and PG in margarine products using HPLC.

MATERIALS AND METHODS

Materials

Reference standards BHA (PPOMN, Jakarta), BHT (PPOMN, Jakarta), PG (PPOMN, Jakarta), TBHQ (PT Indofood, Palembang), acetonitrile pro HPLC (Merck), acetic acid (Merck), methanol pro HPLC (Merck), demineralized water, and some margarine products.

Methods

Standards of BHA, BHT, PG, and TBHQ each carefully weighed approximately 25 mg inserted into 25 mL volumetric flask, diluted

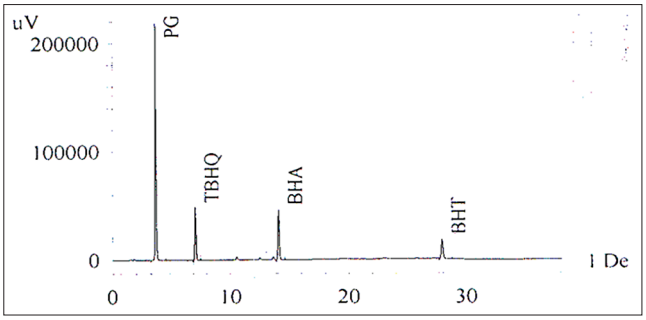

Fig. 1: Chromatogram of standard solution mix

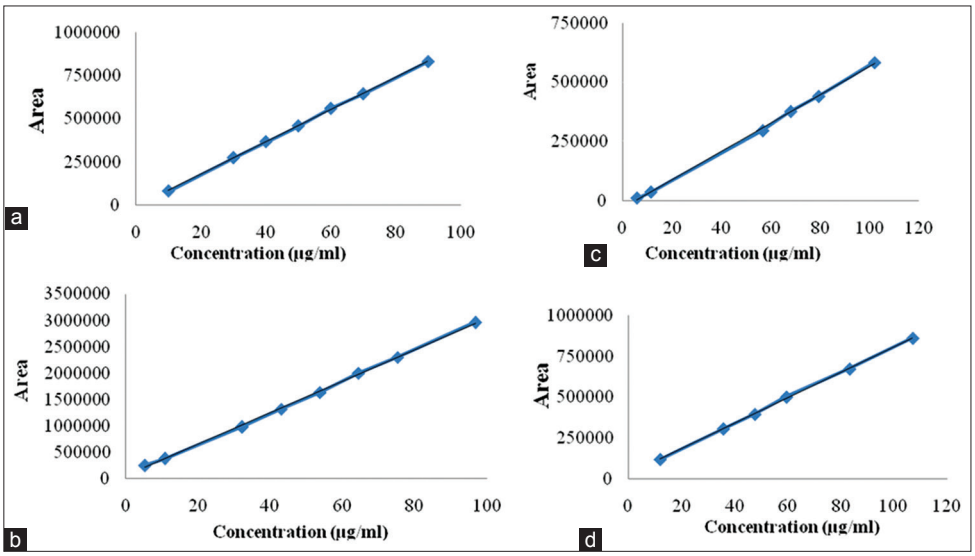

Fig. 2: Calibration curve of BHA, y=9385.2x–10263 (a), BHT, y=5970.1x–31248 (b), Propyl Gallate, y=29851x+50616 (c), and TBHQ, y=7793.1x+27100 (d)

Table 1: Determination system suitability test of BHA, BHT, PG, and TBHQ

Standard	Theoretically plate number (N)	Resolution	Symmetry factor	Capacity factor	CV of time retention
BHA	476605.4320	35.5028	1.2417	2.8157	0.2371
BHT	1162496.5385	57.8187	1.2595	6.5565	0.1430
PG	51986.9362	-	1.2368		0.2654
TBHQ	154358.3047	19.0668	1.2767	0.911	0.2774

BHA: Butylhydroxyanisole, BHT: Butyl hydroxyl toluene, PG: Propyl gallate, TBHQ: Tertiary butyl hydroquinone

Table 2: Value of r, Vx0, Limit of Detection (µg/ml) and Limit of Quantitation (µg/ml) of BHA, BHT, PROPYL GALLATE and TBHQ

Compound	r	V_x0 (%)	LOD (µg/mL)	LOQ (µg/mL)
BHA	0.9999	0.885	2.622	7.945
BHT	0.9996	2.355	4.184	12.678
PROPYL GALLATE	0.9998	1.583	2.494	7.559
TBHQ	0.9998	1.230	2.127	6.446

BHA: Butylhydroxyanisole, BHT: Butyl hydroxyl toluene, PG: Propyl gallate, TBHQ: Tertiary butyl hydroquinone, LOD: Limit of detection, LOQ: Limit of quantitation

Table 3: Value of precision (CV)% and recovery (%) of BHA, BHT, PG, and TBHQ

Compound	Precision (CV) %			Recovery (%)
	Day 1	Day 2	Day 3	
BHA	4.2527	4.0355	4.2298	105.3843
BHT	2.9349	2.6599	2.5451	107.8665
PG	2.1634	1.4825	1.5001	86.5000
TBHQ	3.1514	4.1161	5.7873	83.6394

BHA: Butylhydroxyanisole, BHT: Butyl hydroxyl toluene, PG: Propyl gallate, TBHQ: Tertiary butyl hydroquinone

with methanol up to the mark. Then the standard solution was made between the standard solution and the standard solution was diluted with aquademineralisasi work up to the mark with a concentration of 1-90 µg/mL. The solution was filtered using a membrane filter of 0.45 µm. The mobile phase was a mixture of 1% acetic acid in acetonitrile and 1% acetic acid in the ratio of 30:70. About 2 g of margarine sample was accurately weighed that have been homogenized, put in a 10 mL centrifuge tube and add 4 mL of methanol. Supernatant obtained was filtered with a membrane filter of 0.45 µm, HPLC analysis with UV detector at a wavelength of 280 nm. Determination of sample performed in three repetitions. Validation of the HPLC method includes linearity, LOD and quantitation limits, accuracy and precision [11-13].

RESULTS

Result of system suitability is shown in Table 1 whereas result of validation of the HPLC method includes linearity, limit of detection and quantitation limits, accuracy and precision are shown in Figure 2 and Table 2. Moreover determination of sample is 3 repetition is shown in Table 3.

DISCUSSION

Chromatography is a separation technique one or more components of a sample that was taken by the mobile phase passes through the stationary phase (can be solid or liquid). HPLC is a modern liquid chromatography column. The equipment consists of HPLC column, high-pressure pump, and detector sensitive [14]. Factors that support a well separation is an appropriate stationary phase, the detector that is corresponding to the wavelength of the component to be measured, the comparison of mobile phase, pH and temperature appropriate and columns [15].

Antioxidants are used in margarine products are often found to be more than one kind. Therefore, simultaneous methods are needed.

Optimization was done by the selecting ratio of mobile phase composition and flow rate. The optimum conditions obtained the composition of the mobile phase of 1% acetic acid in acetonitrile and 1% acetic acid in the ratio of 30:70 and a flow rate of 1.7 mL/min. Optimization results obtained in this study have a resolution separation of qualified acceptance, as well as the stages of sample preparation is simpler compared with Saad, *et al*. (2007) using HPLC with UV detector.

Chromatographic system was using Xbridge Waters C_{18} column with a mobile phase of 1% acetic acid in acetonitrile and 1% acetic acid in the ratio of 30:70, a flow rate of 1.7 mL/min; and using the program time. Optimization showed good results of separated peaks (Fig. 1).

The standard peak value for the resolution of standard TBHQ, BHA, and BHT in a mixed standard solution was 19.576; 36.854; and 60.771. Thus, it is qualified for resolution value, which is more than 1.5 [16].

Linearity test of each compound was carried out by determining the area under the curve of series of standard solutions (Fig. 2 and Table 2). Parameters value of r, Vx0, LOD (µg/mL), and LOQ (µg/mL) of each compound were fulfilled the requirements [11,12,17].

Interday precision of the method was expressed as RSD with result; BHA, BHT, PG, and TBHQ were 0.1553; 0.4576; 0.0237; 4.8106%; respectively whereas recovery using standard addition method were 105.38; 107.87; 86.50; 83.64%, respectively (Table 3).

Perrin*, et al.* (2002) using HPLC PDA with extraction methods carried out a two-stage recovery yield for PG 85-106%, BHA 95-104%, and octyl gallate 83-85%; while Ding, *et al*. (2012) using GC-MS method.

CONCLUSION

The optimum conditions obtained the composition of the mobile phase of 1% acetic acid in acetonitrile and 1% acetic acid in the ratio of 30:70 and a flow rate of 1.7 mL/min. Interday precision of the method was expressed as RSD with result; BHA, BHT, PG, and TBHQ were 0.1553; 0.4576; 0.0237; 4.8106%; respectively whereas recovery using standard addition method were 105.38; 107.87; 86.50; 83.64%, respectively. Based on the obtained results, it can be concluded that this method has been successfully developed and validated for the determination of antioxidants BHA, BHT, PG, and TBHQ in margarine.

ACKNOWLEDGMENTS

This research was funded by Master Program Scholarship Indonesia National Agency of Drug and Food Control.

REFERENCES

1. Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food Antioxidants. New York: Marcell Dekker Inc.; 1996.
2. Mahindru SN. Food Safety: A Techno Legal Analysis. New Delhi: MDH Ltd.; 2000.
3. Potter NN, Hotchkiss H. Food Science. 5th ed. New York: Chapman & Hall; 1995.
4. Race S. Antioxidants the truth about BHA, BHT, TBHQ and other antioxidants used as food additives. London: Tigmor Books; 2009.
5. Carocho M, Isabel CF. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 2013;51:15-25.
6. Hasenhuettl GL, Hartel RW. Food Emulsifier and Their Applications. New York: Chapman & Hall, International Thompson Publishing; 1997.
7. Perrin C, Meyer L. Quantification of synthetic phenolic antioxidants in dry foods by reversed-phase HPLC with photodiode array detection. Food Chem 2002;77(1):93-100.
8. Ding M, Zou J. Rapid micropreparation procedure for the gas chromatographic–Mass spectrometric determination of BHT, BHA and TBHQ in edible oils. J Food Chem 2012;131:1051-5.
9. Saad B, Sing YY, Asri MN, Hashim N, Salhin AM, Idris MS, *et al*. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. J Food Chem 2007;105:389-94.
10. Sin WM, Wong YC, Mak CY, Sze ST, Yao WY. Determination of five phenolic antioxidants in edible oils method validation and estimation od measurement uncertainty. J Food Compost Anal 2006;19:784-91.
11. ICH Harmonized Tripartit Guideline. Text on Validation of Analytical Procedure; 1994.
12. Ahuja SM, Dong W. Handbook of Pharmaceutical Analysis by HPLC Separation Science and Technology. London, New York: Elsevier Academic Press; 2005.
13. Ermer J, Miller JH, editor. Performance Parameters, Calculations and Tests, in Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. Weinheim, Wiley-VCH Verlag GmbH & Co.; 2005.
14. Kromidas S. HPLC Made to Measure: A Practical Handbook For Optimization. Weinheim: Wiley-VCH Verlag GmbH & Co.; 2006.
15. Kazakevich Y, LoBrutto R. HPLC for Pharmaceutical Scientists. New Jersey: John Wiley and Sons, Inc.; 2007.
16. Adamovics JA. Chromatographic Analysis of Pharmaceuticals. 2nd ed. New Jersey: Marcel Dekker Inc.; 1997.
17. Ibrahim S. Berbagai pendekatan pengujian kelinieran kurva baku pada metode analisis instrumental. Acta Pharm Ind 2005;30(1):1-5.