Research Article

Determination of Propyl Gallate in Some Vegetable Oil Samples by Thin Layer Chromatography – Image Analysis Method

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ABSTRACT

A convenient and low cost thin layer chromatography (TLC) - image analysis method was developed for the determination and quantification of propyl gallate PG in some vegetable oil samples. Chromatographic separation of PG was achieved on silica gel TLC plates using benzene-ethyl acetate-methanol-glacial acetic acid as a mobile phase and Folin-Denis's reagent for spot detection . Image analysis of the TLC plates using a scanner linked to a computer was performed to quantify the amount of PG, the approach was accurate , specific, reliable for the analysis of PG in the selected oil samples, with the possibility of analyzing simultaneously 25 samples in addition to the standard curve spots. Propyl gallate calibration curve was linear up to 1 µg/spot with a detection limit 0.16 µg/spot. The results of analyzing PG using the previous approach were compared to a standard procedure employing HPLC method.

Keywords: Propyl gallate, Thin Layer Chromatography–Image Analysis, Vegetable Oils.

INTRODUCTION

Synthetic antioxidants are widely used as food additives to prevent rancidity since natural antioxidants are usually of poor stability, food manufacturers prefer to use synthetic phenolic antioxidants (SPA) such as propyl gallate (PG), tertiary butyl hydroquinone (TBHO), butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which are used in edible vegetable oil and cosmetics[1,2].

Propyl gallate, as synthetic phenolic antioxidant, display high chemical activity for suppressing chain initiation, or breaking chain propagation of peroxidation of unsaturated fatty acids.

In most countries the content of phenolic antioxidants in processed food is strictly limited.

In order to comply with safety and hygiene controls for food, it is very important to establish an effective and convenient qualitative and quantitative method.

The methods for analyzing antioxidants in food include UV – Visible spectrophotometric method, paper and thin-layer chromatographic, high performance liquid chromatography (HPLC)[3-5], gas chromatography (GC)[6,7], ion chromatography (IC)[8], capillary electrophoresis (CE)[9-11], high performance liquid chromatography electro spray mass spectrometry (HPLC-MS)[12] and gas chromatography – mass spectrometry (GC-MS)[13].

At the end of the 20th century, due to development of digital electronics, digital imaging and analyzing were applied to qualitative analysis in gel electrophoresis[14] and also to the quantitation of TLC[15]. Despite the low cost of the digital imaging and analyzing system, compared to TLC slit-densitometry, this technique was not widely used because of the existence of other competitive technologies, in addition to the immaturity of this technology itself.

Recently the performance of image analysis system using a digital camera in dark box improved greatly[16], which made the use of such a system a very good prospect for TLC quantitation. Therefore, we investigated the digital image analysis system; by replacing the digital camera with a scanner to simplify the imaging of TLC.

The scanner was coupled with a computer equipped with a convenient program (soft ware).[17]

The purpose of this study was to establish a simple, rapid, precise and low cost method for the separation and quantification of propyl gallate in some food samples using image analysis system.

MATERIALS AND METHODS

Reagents

Propyl gallate (purity ≥ 98 %) was purchased from Fluka, silica gel thin layer plates pre-coated aluminum were purchased from macherey - nagel. Folin-Denis's reagent from Fluka for spot detection (10 ml diluted to 40 ml with 2% sodium carbonate)

All reagents were of analytical reagent grade.

Apparatus

Samples solution was applied to the plates with micropipettes (Brand). The plates were developed in glass chamber (Camag). The images were acquired with a scanner (plug- N- scan 1248 UB Mustek scanner) of 600 DPI resolution, Bitmap image (bmp).

Samples preparation

Twenty test samples including five samples of soybean oil, cottonseed oil, corn oil and sunflower seed oil were purchased from local supermarkets in Damascus, Syria.

Samples for the TLC were prepared as following: 10 g of edible vegetable oil were weighed into a glass centrifuge tube and 2 ml ethanol was added. The extraction was performed by shaking the tube for 5 min, and then centrifuged for 5 min at 2000 rpm. The ethanol phase was collected and the oil phase was subsequently re-extracted twice in the same way. All the ethanol phases were combined and vaporized up to 1 ml.

Standard curve Preparation

Exactly 100.0 mg of PG were dissolved in 100.0 ml volumetric flask. Appropriate dilutions of this stock solution were made by ethanol to yield 1, 0.875, 0.75, 0.5, 0.25 mg/ml of propyl gallate.

For the HPLC analysis, the standard reference method of the AOAC was used[18].

Column: Eurospher ODS 25 cm x 4.6mm, 5 µm particles.

Mobile phase: 5% Acetic Acid-water/Methanol/Acetonitrile

=6/2/2 (v/v/v)

Flow rate: 2 mL/min.

Temp: ambient

Detector: UV, 280 nm

Injection: 20 µL.

TLC procedure

1 µl of each samples were applied to the silica gel plate. The plate was developed using benzene: ethyl acetate: methanol: acetic acid (25: 5:5:1) as a mobile phase, the plate was removed when the mobile phase migrated (10) cm and then dried. For visualizing, plate was sprayed with Folin-Denis's reagent.

Digital image analyzing

The digital image of the plate was acquired using a scanner (600 DPI: resolution) and transferred to the computer. The image was properly cropped and saved in bitmap (bmp) format, and analyzed.[17]

RESULTS

Calibration curve

One µl of each standard solution were applied to the plate and it was developed and its digital image was analyzed fig (1). It was found that calibration curve was linear over 0.25 -1 µg (R^2=0.995) fig (2).

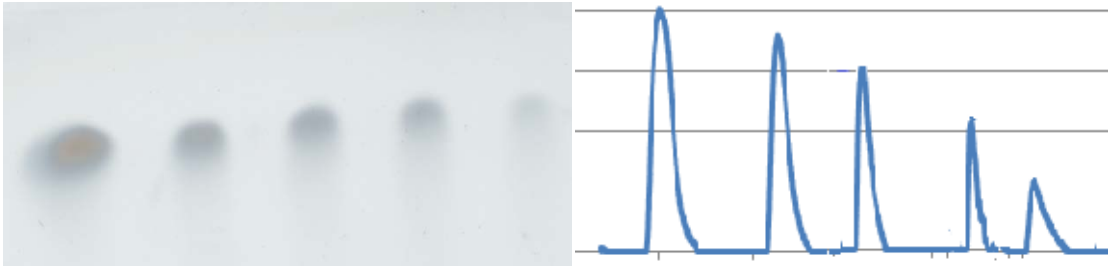

Figure 1: TLC image of standard curve spots and their corresponding peaks

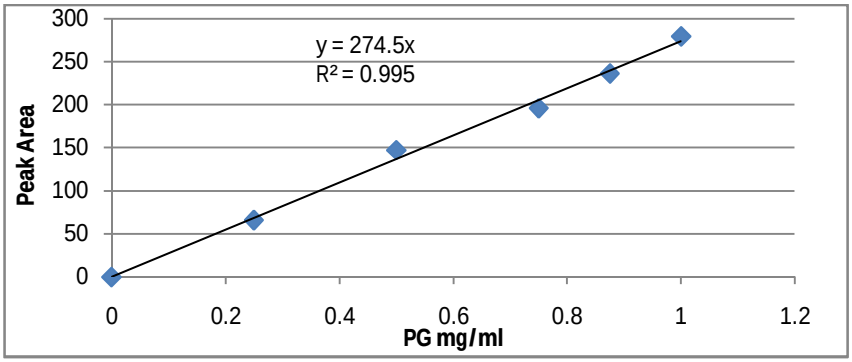

Figure 2: Calibration curve of PG

Figure 3: The image of six 0.75 µg/spot and their corresponding peaks

Detection limit

The standard solution 1mg/ml was diluted to 1:6 and 1 µl of the diluted solution was applied onto the plate, and then assayed; the limit of detection (LOD) was 0.16 µg/spot.

Repeatability

Six parallel spots of the concentration 0.75 mg/ml were applied on the plate using a 1 µl micropipette and assayed. The following values of peaks area were obtained: 194.8656-194.1276-196.2855-195.3424-197.2890-198.7632 (RSD = 1.9 %) fig (3).

Recovery

Oil samples extracts of known propyl gallate concentration were spiked with propyl gallate standard. The spiked samples were analyzed in the duplicated. Analysis of the samples gives recoveries in the range 92-101.3% as shown in table 1.

Table 1: Analytical recoveries of standard glutamate additions to oil extract

PG added (mg/ml)	PG found (mg/ml)	% Recovery
0	0	-
0.25	0.23	92
0.33	0.31	93.9
0.5	0.48	96
0.75	0.76	101.3
1	0.98	98

Table 2: Comparison of PG level in oil samples obtained from local supermarkets by using TLC-image analysis method and HPLC method (n = 3).

Name of Sample	PG (mg/ml)	
	TLC-image analysis	HPLC
soybean oil (1)	n.d.	n.d.
soybean oil (2)	n.d.	n.d.
soybean oil (3)	n.d.	n.d.
soybean oil(4)	n.d.	n.d.
soybean oil(5)	n.d.	n.d.
cottonseed oil(1)	n.d.	n.d.
cottonseed oil(2)	n.d.	n.d.
cottonseed oil(3)	n.d.	n.d.
cottonseed oil(4)	n.d.	n.d.
cottonseed oil(5)	n.d.	n.d.
corn oil(1)	n.d.	n.d.
corn oil(2)	n.d.	n.d.
corn oil(3)	n.d.	n.d.
corn oil(4)	n.d.	n.d.
corn oil(5)	n.d.	n.d.
sunflower seed oil(1)	n.d.	n.d.
sunflower seed oil(2)	n.d.	n.d.
sunflower seed oil(3)	n.d.	n.d.
sunflower seed oil(4)	n.d.	n.d.
sunflower seed oil(5)	n.d.	n.d.

n.d.: not detected

Analysis of food Samples

In order to evaluate the effectiveness of the proposed method, a variety of edible vegetable oils from different manufactories were selected for analysis table 2.

DISCUSSION

To have an optimal extraction, we took in consideration the solubility of PG in several solvents (methanol-acetonitrile-ethanol), we choose ethanol because it is less toxic from other solvents (methanol- acetonitrile) when evaporated. The extracts were concentrated by vaporization up to 1 ml to increase the sensitivity of the method.

The recovery of extraction was increased from 75% to achieve 98% in the third extraction.

In the literature, silica gel plates with several mobile phases system were used in TLC assay of propyl gallate present in oil samples, and $FeCl_3$ was widely used for spot detection.[19]

When experimenting the system chloroform- acetic acid (17:3), which was used to separate octyl gallate, dodecyl gallate and propyl gallate in food samples.[20] It was found that it could not develop propyl gallate and separate it from the others in oil samples.

A number of other mobile phases such as aceton-water 5:3, benzene-ethyl acetate-methanol 25:5:5 were also experimented. Our observations revealed that the later could develop propyl gallate, but, unfortunately, with sever tailing, therefore, the addition of acetic acid reduced the tailing.

A satisfactory separation was obtained by using a mobile phase:benzene-ethyl acetate-methanol-acetic acid (25:5:5:1).

Using the proposed solution of Folin reagent for spot detection gave less colored background of the plate than other reagents ($FeCl_3$-$AgNO_3$) with a convenient sensitivity.

Once spraying the plate with Folin reagent, the whole plate was colored, which led to an interference of the resulting background with the color of the spot.

To eliminate the interference of the reagent's color, the reading of an area PG free and equal to that of a spot was subtracted.

The same results were obtained by subtraction the reading of blank spot obtained from oil sample PG free examined by HPLC.

The calibration curve cannot start from zero, if we do not subtract the mentioned blank value from the reading of the calibration curve spots, and it started from zero when this subtraction was realized.

Pure substances were used in the quantification studies of TLC - image analysis, with validation depending only on a calibration plot[21]. The validation in the proposed

method was performed with many additional items according to the Analytical Procedures of International Conference on Harmonization (ICH).[22]

The obtained linearity (R^2=0.995), repeatability (RDS 1.6 %), detection limit (LOD = 0.16 µg/spot), and accuracy were all satisfactory.

Because of the high cost of a slit - scanning densitometry, which is not much less than that of the HPLC[23], the image analysis using a digital camera was proposed, the later exhibits a major limitation concerning the homogeneity lighting of the spot, causing unstable baseline, and the existence of lens in the camera decreases the reflected light which affects the quality of the image, in addition to the necessity of using a dark cabinet which render it more expensive.[24]

In this study we used a scanner to acquire the image of TLC plates, which

Provides a homogenous lighting which increases the accuracy by improving the quality of the image and decreasing the background noise without using a dark cabinet, and it offers the possibility of simultaneous analysis of at least 25 samples (six slides 10 × 10 cm) regarding the surface of the scanner.

This method is not solvents consumption in comparison to HPLC and GC.

The simplicity of the materials which are available in most laboratories where HPLC and slit- densitometry is very expensive and not available in all laboratories.

The proposed method offers another alternative for the qualitative determination of PG in oils and it gave good results and can be carried out easily with facilities existing in most analytical laboratories.

To evaluate the effectiveness of the proposed method, it was applied to the analysis of a total of twenty samples of edible vegetable oils from different manufactories.

PG was not found in any sample, the addition of PG to the oil is inappropriate for frying due to its poor stability at high temperatures, it decomposes at its melting point of 148ºC.[25]

The results of analyzing PG using the previous approach were compared to a standard procedure employing HPLC method which gave the similar results.

PG is permitted for food use by the FDA and the USDA at 0.02% and 0.01%, respectively. Linearity of the calibration curve covers limited range of concentration between (0.25 -1 µg). The calibration curve does not cover the permitted of value (0.01-0.02%).

In case of sample with high concentration, it is recommended to reduce the quantity of the sample, thus the proposed method will be able to determine any concentration of food samples.

The obtained results by the proposed method for analyzing some edible vegetable oils were similar to those obtained by Ni. Y et al, 2000.[26]

CONCLUSION

TLC- image analyzing method by using a scanner for the determination of propyl gallate in some oil samples has some advantages over several current methods, such as simplicity in application where no complicated instruments are needed and, rapidity where 25 samples could simultaneously be analyzed in relatively short time.

Low cost method in comparison with other methods especially HPLC and slit - scanning densitometry. The use of a scanner has the advantage of realizing homogeneity of lighting and without the need of dark cabinet as in the case of a digital camera. The method was validated and found to be accurate, reliable and convenient for the analysis of propyl gallate in oil samples.

REFERENCES

1. Guan, Y. Q., Chu, Q. C., Fu, L., & Ye, J. N. Determination of antioxidants in cosmetics by micellar electrokinetic capillary chromatography with electrochemical detection. Journal of Chromatography A, 1074(2), 2005, 201–204.

2. Guo, L., Xie, M. Y., Yan, A. P., Wan, Y. Q., & Wu, Y. M. Simultaneous determination of five synthetic antioxidants in edible vegetable oil by GC–MS. Analytical and Bioanalytical Chemistry, 386(6), 2006, 1881–1887.

3. Borremans, M., Van, L. J., Roos, P., & Goeyens, L. Validation of HPLC analysis of 2-Phenoxyethanol, 1-phenoxypropan-2-ol, Methyl, Ethyl,Propyl, Butyl and Benzyl 4-Hydroxybenzoate (parabens) in cosmetic products, with emphasis on decision limit and detection capability. Chromatographia, 59(1–2), 2004, 47–51.

4. Christian, P., & Liliane, M. Quantification of synthetic phenolic antioxidants in dry foods by reversed-phase HPLC with photodiode array detection. Food Chemistry, 77(1), 2002, 93–100.

5. Lozano, P. P., Montoya, E. G., Orriols, A., Minarro, M., Tico, J. R., & Snegre, J. M. A new validated method for the simultaneous determination of benzocaine, propyl paraben and benzyl alcohol in a bioadhesive gel by HPLC. Journal of Pharmaceutical and Biomedical Analysis, 39(5), 2005, 920–927.

6. Mu, T. N., Xu, H., & Li, W. Gel perme chromatography with GC–FID to simultaneous determination of BHA and BHT in the edible oil. Science and Technology of Food Industry, 1, 2007, 228–230 (in Chinese).

7. Yang, M. H., Lin, H. J., & Choong, Y. M. A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. Food Research International, 35(7), (2002), 627–633.

8. Lian, H. Z., Li, D. L., Wu, X. X., & Tian, L. C. Determination of phenolic preservatives in gelatin and vacant capsules for medicine use by preservatives in gelatin and vacant capsules for medicine use by ionsuppress reversed-phase high performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis, 37, 2005, 369–372.

9. Jaworska, M., Szulinska, Z., & Wilk, M. Application of a capillary electrophoresis method for simultaneous determination of preservatives in pharmaceutical formulations. Journal of Separation Science, 28(2), 2005, 137–142.

10. Memon, N., Bhanger, M. I., & Khuhawer, M. Y. Determination of preservatives in cosmetics and food samples by micellar liquid chromatography. Journal of Separation Science, 28(7), 2005, 635–641.

11. Xiang, Q., Gao, Y., & Xu, Y. H. Capillary electrophoresis–amperometric determination of antioxidant propyl gallate and butylated hydroxyanisole in foods. Analytical Science, 23(6), 2007, 713–717.

12. Lee, M. R., Lin, C. Y., Li, Z. G., & Tsai, T. F. Simultaneous analysis of antioxidants and preservatives in cosmetics by supercritical fluid extraction combined with liquid chromatography mass spectrometry. Journal of Chromatography A, 1120(1–2), 2006, 244–251.

13. Guo, L., Xie, M. Y., Yan, A. P., & Wan, Y. Q. Simultaneous determination of three antioxidants in edible vegetable oil by GC–MS. Journal of Analytical Science, 23(2), 2007, 169–172. in Chinese.

14. Patton, W.F. Biologist's perspective on analytical imaging systems as applied to protein gel electrophoresis. J. Chromatogr. A, 698, 1995, 55–87.

15. Poole, C.F. Thin-layer chromatography: challenges and opportunities. J. Chromatogr. A 1000, 2003, 963–84.

16. Hemmateenejad, B., Mobaraki, N., Shakerizadeh-Shirazi, F., & Miri, R. Multivariate image analysis-thin layer chromatography (MIA-TLC) for simultaneous determination of co-eluting components. Analyst, 135(7), 2010, 1747-58.

17. Safadi, F., Al ataie, M., and Rajab., M. Un systeme polyvalent pour les measures photometriques spectrophotometriques par l' analyses d'image. (2001), Bulletin officiel de le proprieté industrielle Paris, France, No 01139, No de puplication 2806796.

18. Official Methods of Analysis (16th ed.). Method 983.15 Association of Official Analytical Chemists, 1995, Arlington, VA USA.

19. Sharma, Sh., Saxena, R.K. Evaluation of the versatility of the tannases produced from Aspergillus niger and Penicillium variable with respect to gallic acid production, gallate ester synthesis, animal feed improvement, tannery effluent degradation and tannin stain removal. Research in Biotechnology, 3(5), 2012, 09–20.

20. Crompton, T. R. Additive Migration from Plastics into Food; A Guide for Analytical Chemists: A Guide for the Analytical Chemist, 298.

21. Zhang, L., Lin, X.G. Quantitative evaluation of thin-layer chromatography with image background estimation based on chargecoupled device imaging. J. Chromatogr, A 1109, 2006, 273–78.

22. ICH. Validation on analytical procedures. Commission of the European Communities. Proceedings of the International Conference on Harmonization (ICH) [C]. 1996.

23. Xiu-Qin, L., Chao, J., Yan-Yan, S., Min-Li, Y., Xiao-Gang, Ch. Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS, Food Chemistry, 113, 2009, 692–700.

24. Tie-xin, T., Hong, W. An Image Analysis System for Thin-Layer Chromatography Quantification and Its Validation. Journal of Chromatographic Science, 46, 2008, 560-564.

25. Fereidoon, Sh., Ying, Zh. Bailey's Industrial Oil and Fat Products., 6, 2005, 498.

26. Ni, Y., Wang, L., Kokot, S. Voltammetric determination of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tert-butylhydroquinone by use of chemometric approaches. Analytica Chimica Acta, 412, 2000, 185–193.

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