Eco. Env. & Cons. 27 (November Suppl. Issue) : 2021; pp. (S267-S271)

ISSN 0971–765X

Determination of Guaiacol in presence of monovalent inorganic salt and its assessment in *Citrus sinensis* leaves and *Citrus limon* peels

N.V.S. Venugopal*[1], N. Swathi[2] and P. Padmavathi[2]

[1]Department of Chemistry, Institute of Science, GITAM University, Visakhapatnam, A.P, India
[2]Department of Chemistry, GITAM University, Visakhapatnam, A.P, India

(Received 6 March, 2021; Accepted 13 May, 2021)

ABSTRACT

Guaiacol (2-methoxy phenol) is a naturally occurring expectorant and biosynthesized by a variety of organisms. Guaiacol is also used medicinally as an expectorant, antiseptic, and local anaesthetic. In this study we reported a method for easy and rapid spectrophotometric determination of Guaiacol in presence of perbromate and periodate. The optimized method has been successfully applied for the determination of Guaiacol in orange (*Citrus sinensis*) leaves and lemon (*Citrus limon*) peels. The products obtained shows maximum absorbance at 515 nm in presence of potassium perioadate and 590 nm with potassium perbromate. Beer's law is obeyed in the linear dynamic range of 20-187.9 μg ml^{-1}. The correlation coefficient is 0.996 which indicates the linearity between the two variables. The molar absorptivity coefficient and sandal's sensitivity of the product are found to be 6.35x104L mol^{-1} cm^{-1} and 0.026 μg cm^{-2} respectively. Hence, the proposed method is fairly sensitive and reproducible.

***Key words** : Guaiacol, Perbromate, Perioadate, Citrus sinensis leaves, Citrus limon peels*

Introduction

The most common biomass materials used for energy are plants, wood, and waste. In nature we found naturally occurring chemicals such as chemicals from plants, micro-organisms, animals, the earth and the sea. Wildfires and controlled burns generate smoke particulates that could be taken up by the berries and leaves of fruit plants. Bush fires are expected to become more frequent in the coming years with global warming. In order to minimize the financial loss associated with producing smoke pollution. Guaiacol (2-methoxy phenol) (GAL) is a naturally-occurring organic compound with the formula C6H4(OH)(OCH3). Due to its aroma, it is undesirable in some natural products. It has smoky and phenolic aroma.

Although it is biosynthesized by a variety of organisms, this aromatic oil is usually derived from guaiacum or wood creosote. It is also found in es-

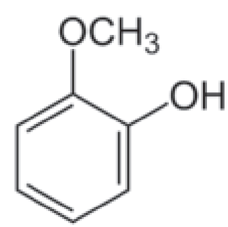

Fig. 1. Structure of Guaiacol

[2]Research Scholar

sential oils from celery seeds, tobacco leaves, orange leaves, and lemon peels. Guaiacol is also used medicinally as an expectorant, antiseptic, and local anaesthetic. Guaiacol is produced in the gut of desert locusts, *Schistocerca gregaria*, by the breakdown of plant material. This process is undertaken by the gut bacterium *Pantoea agglomerans* (*Enterobacter*). It is one of the main components of the pheromones that cause locust warming (Dillon Rod *et al*., 2000). Liquid guaiacol is colourless and it is soluble in alcohol. Guaiacol is less active antiseptic than phenol. Guaiacol can be sulphonated at 80 degree Celsius to yield a monosulphonic acid derivative which is converted to its potassium salt Almeida (Almeida *et al.,* 1999) reported the voltammetric determination of guaiacol by oxidation at a carbon paste electrode following a cathodic accumulation was first tried, with no success, because there was no accumulation of the compound. GUA are derived from phenolic compounds that are widely distributed in various plants and also be found in common foods (Kalayarasi *et al*., 2017). Reduced graphene oxide (RGO) nanosheets with high quality were chemically synthesized by hydrothermal reduction of a well-dispersed grapheme oxide (GO) suspension. The electrochemical behavior of guaiacol was studied on different carbon material surfaces (Yan Wu *et al.,* 2014). Waldemer Ternes investigated the formation of the oxidation products of guaiacol in brines and heated meat matrix: 6-nitrosoguaiacol, 4-nitroguaiacol and 6-nitroguaiacol. For this purpose we applied a newly developed HPLC-UV and LC–MS method (Sarah *et al.,* 2016). The methodology normally used in the determination of Guaiacol is gas chromatography with selective mass detection. Volta metric methods have also been proposed for the determination of phenolic compounds, based on their oxidation at the carbon paste electrode. Detection of *Alicyclobacillus acidoterrestris* was performed by determination of guaiacol in apple juice. Guaiacol produced by *A. acidoterrestris* was determined by using HPLC, UV-Vis spectrophotometer, and Minolta spectrophotometer (Bahceci *et al*., 2007).

Simple gas chromatography-tandem mass spectrometry (GC-MS/MS) method for quantification of guaiccol reported (Zhiqian Liu *et al*., 2020). GC-MS has been the predominant analytical technique in combination with various sample preparation protocols, including liquid-liquid extraction (LLE) from berry homogenate and wine (Wilkinson *et al.,* 2011), headspace solid-phase microextraction (HP-SPME) from beer (Pizarro *et al*., 2010), polymer stir bar sportive extraction from beer and wine (Zhou *et al*., 2015), and trimethylsilyl (TMS)-based derivatization following purification by solid-phase extraction (SPE) of berry homogenate and wine (Allen *et al*., 2013). The reported methods shows that guaiacol is determined through different methods whrein reagents and solvents are of less familiar and methods involving complicated procedures. The author reported the determination of Guaiacol with potassium ioadate and with bromate using aminobenzoic acid (ABA) as reagent

Materials and Methods

Preparation of reagents and solutions

All chemicals used were of analytical grade and were used as such. All laboratory reagents were freshly Prepared. 0.01 percent Guaiacol solution is prepared in methanol. 0.2 percent Aminobenzoic acid (ABA) solution is prepared in double distilled water 0.01M Potassium periodate and potassium per bromated solutions are prepared in distilled water.

Buffer solutions: Hydrochloric acid (1.0M) and sodium acetate (1.0M) are mixed to get the required pH (1.0-3.5), 0.2M sodium acetate and 0.2M acetic acid are mixed to get the required pH (4.0-6.0) and 0.01M Potassium dihydrogen phosphate and 0.01M disodium hydrogen phosphate are mixed to get the required pH (pH 7.0). The pH of the above buffer solutions are measured by a pH meter and finally adjusted suitably.

Apparatus

All spectrophotometric measurements were carried out using a UV-1800 Shidmazu and 752w UV - Vis grating with a silica glass cell of l cm thickness. All glassware are washed with a mixture of concentrated sulfuric acid and nitric acid (1:1) before use.

Procedures

With potassium periodate: 5.0 ml Guaiacol solution is taken in a volume of 15 ml buffer solution and it is mixed with 2 ml of potassium periodate and 1mL of ABA solution. The values of optical densities of the pink colored product obtained are measured at different wavelengths.

With potassium perbromate: 5.0 ml Guaiacol solution is taken in a volume of 15mL buffer solution and it is mixed with 2 ml of potassium perbromate and 1mL of ABA solution. The value of optical densities of the yellow colored product obtained is measured at different wavelengths.

Results and Discussion

The present study was formulated to assess the Guaiacol content in the leaves of *Citrus sinensis* and *Citrus limon*. This aromatic oil is usually extracted from guaiacum and found in celery seeds, tobacco leaves, orange leaves, and lemon peels. The oxidation of Guaiacol in presence of strong oxidising agents like perbromate and periodate was studied as shown in Fig. 2.

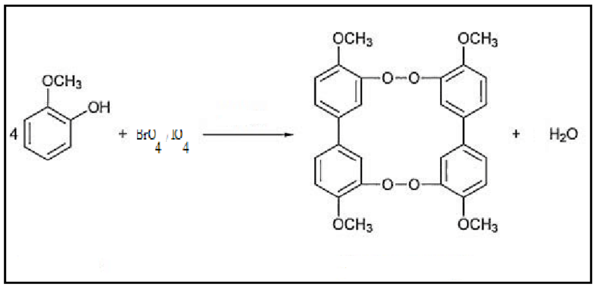

Fig. 2. Oxidation of Guaiacol

Absorption spectra

The absorption spectrum of the products obtained by the reaction between guaiacol and ABA in the presence of potassium perioate and potassium perbromate is shown in Figure 3. The reagent blank exhibited eligible absorbance despite having a wavelength in the same region from the absorption spectra, it is clear that the products obtained shows maximum absorbance at 515 nm in presence of potassium perioadate and 590 nm with potassium perbromate.

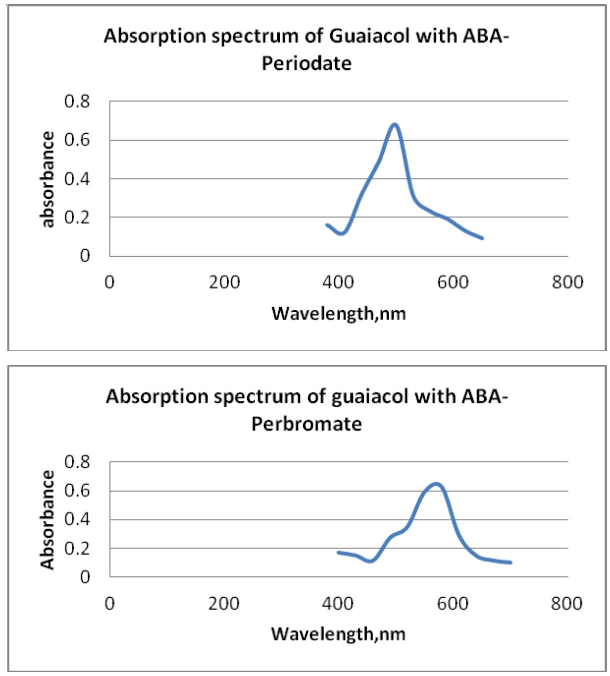

Fig. 3. Absorption spectra of GAL

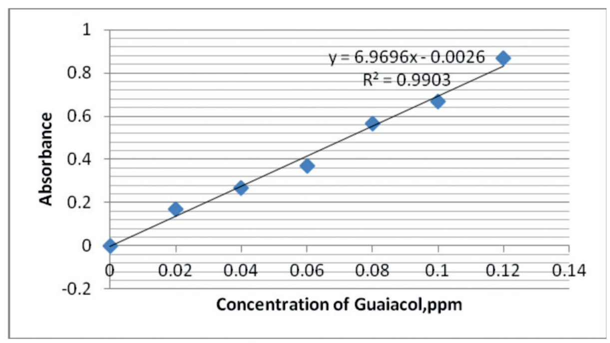

Fig. 4. Calibration graph of Guaiacol with ABA-periodate

Applicability of Beer's law

Aliquots of 10ml solutions, each containing constant volumes of 3 ml of buffer (pH 5.0) 1.0 ml of ABA and periodate or perbromate and 1.0 mL of Guaiacol in the range from 0.02-10 ìg mL-1 are prepared. Absorbance measurements of these solutions are measured at 515 nm in presence of potassium perioadate and 590 nm with potassium perbromate. A graph plotted between the concentration of Guaiacol and its absorbance in presence of perbromate and periodate were given in Figs. 4 and 5. From the graph, it is observed that a linear plot passing

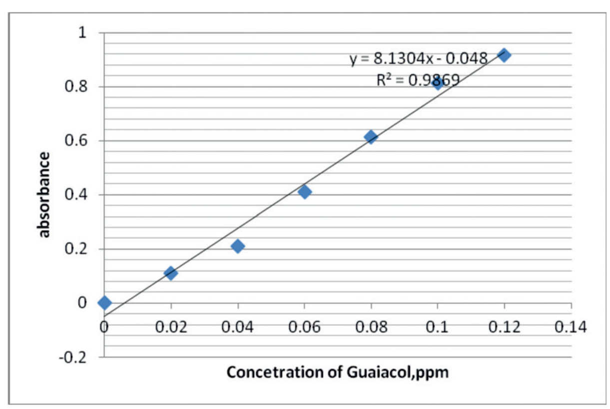

Fig. 5. Calibration of GAL with ABA-perbromate

through the origin obeys Beer's law in the range from 20-187.9 micro grams of Guaiacol. The correlation coefficient is 0.996 which indicates the linearity between the two variables. The molar absorptivity coefficient and sandal's sensitivity of the product are found to be 6.35 x 104L mol^{-1} cm^{-1} and 0.026 µg cm^{-2} respectively. The selected analytical paprameters in presence of periodate and perbromate are given in Table 1 and 2.

Suitable volume of aliquot was analyzed according to the proposed in presence of periodate and perbromate. The results are tabulated in Table 3.

Determination of Guaiacol in orange and lemon leaves

The *Citrus sinensi* leaves, *Citrus limon* peels used in this study were harvested from dierent regions of Visakhapatnam district, Andhra Pradesh, India. They were cleaned with running water, freed of dust and then shadow dried in the shade for 7 days and then transferred to an oven at forty degress celcius, *until* a stable weight. Twenty g of dried plant material each powder factory were extracted by absolute methanol at the ambient temperature (25 °C) for 24 h (200 ml, w/v: 1/10). The extracts were evaporated in the drying oven at 40 °C until the complete evaporation of methanol to obtain the crude extracts.

The prepared solutions are analyzed according to the optimized procedure. The obtained results are shown in the Table 4.

Table 3. Quantitative determination of Guaiacol

	Amount of Guaiacol, µg Found	
Taken	Periodate	Perbromate
22.00	21.75	21.81
54.00	53.58	52.89
126.00	125.6	125.8
188.00	185.6	187.9

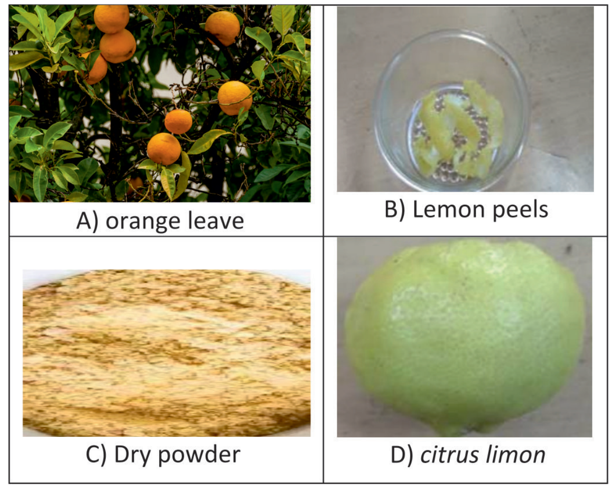

Fig. 6. Guaiacol in orangeleaves and lemon peels

Conclusion

The determination of Guaiacol in presence of

Table 1. Selected analytical parameters obtained in presence of periodate

Parameter	Examined range	Selected value
Wavelength	380-750	515
pH	3.0-5.0	5.0
Temperature °C	-	25
Molar absorptivity L mol–1 cm–1 -	-	6.35 × 104
Sandal's sensitivity, µg cm-2	-	0.026
Detection limit/µg l–1 -	-	0.5
Regression coefficient	-	0.996

Table 2. Selected analytical parameters obtained in presence of perbromate

Parameter	Examined range	Selected value
Wavelength, nm	380-750	590
pH	3.0-5.0	5.0
Temperature °C	-	25
Molar absorptivity L mol–1 cm–1 -	-	6.85 × 104
Sandal's sensitivity, µg cm^{-2}	-	0.036
Detection limit /µg l–1 -	-	0.5
Regression coefficient	-	0.99

Table 4. Concentration of Guaiacol in presence of perioadate and perbromate in the leaves of *Citrus sinensis & Citrus limon*

Sample	*Citrus sinensis* (ppm)		*Citrus limon* (ppm)	
	Perioadate	Perbromate	Perioadate	Perbromate
1	0.610	0.592	0.037	0.029
2	0.307	0.309	0.043	0.036
3	0.160	0.154	0.0017	0.0011
4	0.093	0.085	0.224	0.116
5	0.110	0.107	0.0014	0.0009

perbromate and perioadate in orange and lemon leaves has been developed by using a sensitive and selective analytical reagent. The reported method offers advantages like sensitivity and quick colour development. The developed method found to be quantitative compared to other methods and applied to orange and lemon leaves.

Acknowledgements

We gratefully acknowledge the management of GITAM University and Department of Chemistry, GITAM institute of science for providing facilities and encouragement.

References

Allen, D., Bui, A.D., Cain, N., Rose, G. and Downey, M. 2013 Analysis of free and bound phenolics in wine and grapes by GC-MS after automated SPE. *Anal. Bioanal. Chem*. 405 : 9869- 9877. DOI: 10.1007/s00216-013-7405-0.

Bahçeci, K.S. and Acar, J. 2007 Determination of guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice by using HPLC and spectrophotometric methods, and mathematical modeling of guaiacol production. *Eur Food Res Technol*. 225 : 873–878. https://doi.org/10.1007/s00217-006-0495-6

Kalaiyarasi, J., Meenakshi, S., Pandian, K., Subash, C. and Gopinath, B. 2017. Simultaneous voltammetric determination of vanillin and guaiacol in food products on defect free graphene nanoflakes modified glassy carbon electrode. *Microchim Acta*. 184 : 2131–2140, DOI 10.1007/s00604-017-2161-z

Pizarro, C., Pérez-del-Notario, N. and González-Sáiz, J.M. 2010. Optimisation of a simple and reliable method based on headspace solid-phase microextraction for the determination of volatile phenols in beer. *J. Chromatogr.* A 1217 : 6013–6021. DOI: 10.1016/j.chroma.2010.07.021.

Sarah-Maria Bölicke and Waldemar Ternes, 2016 Isolation and identification of oxidation products of guaiacol from brines and heated meat matrix. *Meat Science.* 117 : 18-26. https://doi.org/10.1016/j.meatsci.2016.02.031

Wilkinson, K.L., Ristic, R., Pinchbeck, K.A., Fudge, A.L., Singh, D.P., Pitt, K.M., Downey, M.O., Baldock, G.A., Hayasaka, Y. and Parker, M. 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. *Aust. J. Grape Wine Res*. 17 : S22–S28. https://doi.org/10.1111/j.1755-0238.2011.00147.x

Yan,Wu, Meng Huang, Nannan Song and Weibing Hu, 2014. Electrochemical detection of guaiacol in bamboo juice based on the enhancement effect of RGO nanosheets. *Anal. Methods.* 6 : 2729-2735. https://doi.org/10.1039/C4AY00195H

Zhiqian Liu, Vilnis Ezernieks, Priyanka Reddy, Aaron Elkins, Christian Krill, Kieran Murphy, Simone Rochfort and German Spangenberg, 2020. A Simple GC-MS/MS Method for Determination of Smoke Taint-Related Volatile Phenols in Grapes. *Metabolites*. 10 : 294; doi:10.3390/metabo10070294

Zhou, Q., Qian, Y. and Qian, M.C. 2015. Analysis of volatile phenols in alcoholic beverage by ethyleneglycol-polydimethylsiloxane based stir bar sorptive extraction and gas chromatography–mass spectrometry. *J. Chromatogr.* A 1390 : 22–27. DOI: 10.1016/j.chroma.2015.02.064.