BBCEAS-HLPC Measurements for Synthetic Antioxidants (TBHQ, BHA, and BHT) in deep-UV region below 300 nm

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Abstract: Broadband Cavity Enhanced Absorption Spectroscopy (BBCEAS) has been used for detection in an HPLC system (HPLC-BBCEAS). This technique is used as a detection system for HPLC for measurements in the deep-UV region below 300 nm. Measurements were made on synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ). The sensitivity of the measurements, as determined by the minimum detectable change in the absorption coefficient, αmin, for BHA, BHT, and TBHQ were 1.8 × 10-5 cm-1, 2.8 × 10-5 cm-1, and 1.9 × 10-5 cm-1 at 280 nm, respectively. The Limit of Detection (LOD) of the HPLC-BBCEAS setup was found to be between 4 to 30 times lower for the antioxidants when compared with a Perkin-Elmer HPLC. When compared with previous single-wavelength HPLC-CRDS studies, the sensitivity of the developed setup presents favourable results while offering simpler experimental methodology and a considerably lower-cost experimental setup.

Keywords: BBCEAS-HPLC, Deep-UV Spectroscopy, Synthetic Antioxidants

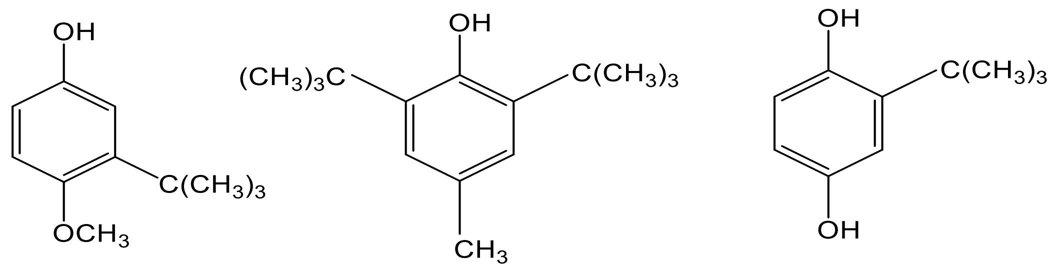
1 Introduction

Antioxidants are defined by the Food and Drug Administration as dietary supplements required to be taken in addition to normal food consumption to prevent cardiovascular, neurological or carcinogenic diseases (Yadav *et al.*, 2016). Antioxidants are compounds that slow down the oxidation rate or extend the induction period (Gulcin, 2020). They are generally added to food to prevent browning and rancidity and to maintain its freshness, thereby increasing the food's shelf life (Shahid *et al.*, 2021). Antioxidant is a general term used to describe a range of compounds that inhibit lipid oxidation through different mechanisms. These include scavenging free radicals, inactivation of peroxide and other reactive oxygen species, quenching secondary lipid oxidation products associated with oxidative rancidity, and sequestration or chelation transition metal catalysts (Santos-Sánchez *et al.*, 2019). Antioxidants used for food preservation can be classified as naturally derived and chemically synthesised. Both function as preservatives donating electron density to fats, therefore preventing their breakdown. However, they differ in their effectiveness as preservatives. Synthetic antioxidants have been reported to show higher performance levels than natural ones, because natural antioxidants show greater reluctance while donating hydrogen atoms in trying to prevent oxidation. Another advantage of synthetic antioxidants over natural ones is their affordability, being priced at approximately $10 to $20 per pound. The choice of antioxidants is also greatly dependent upon the type of food to be preserved. For example, synthetic antioxidants with higher performance levels are preferred for preserving food with high rancidity, whereas lower-performance natural antioxidants can be used for hydrogenated oils with lower rancidity (Santos-Sánchez *et al.*, 2019).

Fat, oils, and other lipid-containing food materials deteriorate via several degradation processes, which occur during heating as well as during long-time storage (Sahraee *et al.*, 2019). The major processes of deterioration include oxidation reactions and the decomposition of products of oxidation, leading to reductions in nutritional value, taste, and shelf life (Kong and Singh, 2016). The primary products of autoxidation are hydroperoxides that do not affect taste and flavour. However, their degradation products include aldehydes, ketones, acids, and polymers, which alter the physico-chemical qualities of oils (Kong and Singh, 2016). The inhibition of these oxidation processes is therefore paramount for the food industry, marketers, and direct consumers (Novais *et al.*, 2022). Oxidation may be inhibited by various methods. These include antagonizing access to oxygen, use of lower temperatures, inhibition of enzymes that catalyse oxidation reactions, lowering of oxygen tension, introduction of chelating agents, and choice of packaging material (Al-Malaika *et al.*, 2017)

A commonly applied method of protection of oils against oxidation is the introduction of additives that antagonize oxidation (Machado *et al.*, 2023). These oxidation antagonists are referred to as antioxidants. The most frequently encountered synthetic antioxidants include BHA, BHT and TBHQ (Figure 1), which stabilise free radicals and prevent the occurrence of chain reactions in oils. However, their potential risks to health have raised concerns (Atta, Mohamed and Abdelgawad, 2017). The permissible limit of these synthetic antioxidants in edible fats and oils is 20 mg/100g (Hygreeva, Pandey and Radhakrishna, 2014). The determination of the amounts of these artificial antioxidants in edible oil is therefore essential in investigating shelf life and, most importantly, safety of consumption (Nogueira *et al.*, 2019).

Although BHA, a synthetic antioxidant, has been proven to pose no stomach cancer risk at levels comparable to dietary intake, it has been shown to worsen other medical conditions like asthma as well as developmental anomalies in infants. BHA has also been demonstrated to antagonise cellular respiration by the reversal of oxidative processes associated with respiratory complexes (Surai, 2015). More significantly, it has been implicated in benign and malignant tumours in rats, male mice and hamsters (Felter, Zhang and Thompson, 2021). These health concerns have inspired legislation in different countries: for example, the European Union and the United States have a total limit of 100 mg/kg for BHT, while the limit is 200 mg/kg in Malaysia (Xu *et al.*, 2021). On the other hand, TBHQ has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at various meetings between the years 1975 and 1997; using rat, mice and dog models, the work concluded that TBHQ was not carcinogenic in those animals (Wu *et al.*, 2022). Another study demonstrated the safety of BHA in rabbits (Imbabi *et al.*, 2021).



BHA (butylated hydroxy anisole) **BHT (butylated hydroxytoluene)** **TBHQ (tert-butylhydroquinone)**

Figure 1. Chemical structures of BHA, BHT, and TBHQ

To ensure the safety and efficacy of antioxidants in food products, it is essential to accurately quantify their presence and concentration. Chromatography is an analytical technique that operates on a similar principle to extraction and is used in the quantification and division of compounds (Badawy *et al.*, 2022). In chromatography, one phase is held in place while others move past the stationary phase (Morley and Minceva, 2020). Chromatographic techniques are used in the food industry and environmental or chemical laboratories. Among the chromatographic techniques, high-performance liquid chromatography (HPLC) is the most widely used technique for the detection of analytical compounds in the liquid phase. This technique involves forcing solvents, using high pressure, through closed systems containing fine particles, which results in high-resolution separations (Kanu, 2021). However, the sensitivity of HPLC is poor in comparison to other more complex and higher-cost detection systems, such as mass spectrometry and fluorescence spectroscopy (Sammani, Clavijo and Cerdà, 2021).

Over the last few decades, efforts have been made to improve the sensitivity of absorption spectroscopy. O'Keefe and Deacon tested optical cavity-based absorption spectroscopy techniques and found them to be successful (Gianfrani, Hu and Ubachs, 2024). Further studies have identified cavity ring-down spectroscopy (CRDS) and cavity-enhanced absorption spectroscopy (CEAS) as the most used optical cavity-based absorption spectroscopy techniques. Most of these studies were carried out on gas-phase analytes; fewer liquid-phase cavity studies have been conducted and have focused on use of the HPLC detector (Dugheri *et al.*, 2021).

It is important to point out that all reported HPLC-CRDS systems have operated at a single wavelength. The use of BBCEAS as a detector has been found to provide significant improvement in the sensitivity of absorption spectroscopy through an increase in the effective path length of measurement (Thalman *et al.*, 2022). In its first demonstration, BBCEAS was used as a detector for HPLC to separate and detect rhodamine 6G and rhodamine B at visible wavelengths between 450 and 600 nm. White LEDs with a standard flow cell positioned at normal incidence geometry were used for this study, which offered a lower-cost and simpler experimental methodology in comparison to HPLC-CRDS. Furthermore, the use of HPLC-BBCEAS allows for multiplex detection of analytes across a broad range of wavelengths (Bazina *et al.*, 2022). Various foods, pharmaceutical, chemical, and biological compounds, exist in the liquid phase. At the same time, many of these compounds have strong absorbance in the deep UV region below 300 nm. This work focusses on the separation and quantification of synthetic antioxidants (BHA, BHT, and TBHQ).

It is well known that fat, oils, and other lipid-containing food materials deteriorate via several degradation processes, which occur during heating as well as during long-time storage (Sahraee *et al.*, 2019). The major processes of deterioration include oxidation reactions and the decomposition of products of oxidation, leading to reductions in nutritional value, taste, and shelf life (Kong and Singh, 2016). The primary products of autoxidation are hydroperoxides. These do not affect taste and flavour. However, their degradation products such as aldehydes, ketones, acids and polymers, alter the physico-chemical qualities of oils (Idrissi *et al.*, 2024). The inhibition of these oxidation processes is therefore paramount for the food quality. Oxidation may be inhibited by various methods. These include antagonizing access to oxygen, use of lower temperatures, inhibition of enzymes that catalyse oxidation reactions, lowering of oxygen tension, introduction of chelating agents, and choice of packaging material (Ahmed *et al.*, 2023).

A commonly applied method of protection of oils against oxidation is the introduction of additives that antagonize oxidation (Machado *et al.*, 2023). These oxidation antagonists are referred to as antioxidants. The most frequently encountered synthetic antioxidants include BHA, BHT and TBHQ (Figure 1), which stabilise free radicals and prevent the occurrence of chain reactions in oils. However, their potential risks to health have raised concerns (Hassanpour and Doroudi, 2023). The permissible limit of these synthetic antioxidants in edible fats and oils is 20 mg/100g (Hygreeva, Pandey and Radhakrishna, 2014).The determination of the amounts of these artificial antioxidants in edible oil is therefore essential in investigating shelf life and, most importantly, safety of consumption (Taghvaei and Jafari, 2015).

Although BHA, a synthetic antioxidant, has been proven to pose no stomach cancer risk at levels comparable to dietary intake, it has been shown to worsen other medical conditions like asthma as well as developmental anomalies in infants. BHA has also been demonstrated to antagonise cellular respiration by the reversal of oxidative processes associated with respiratory complexes. More significantly, it has been implicated in benign and malignant tumours in rats, male mice, and hamsters (de Oliveira Pateis *et al.*, 2018). These health concerns have inspired legislation in different countries: for example, the European Union and the United States have a total limit of 100 mg/kg for BHT, while the limit is 200 mg/kg in Malaysia (Yue *et al.*, 2019). On the other hand, TBHQ has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at various meetings between the years 1975 and 1997; using rat, mice and dog models, the work concluded that TBHQ was not carcinogenic in those animals . Another study demonstrated the safety of BHA in rabbits (Imbabi *et al.*, 2021).

2 Materials and methods

2.1 Choice of analytes.

In this study, the chemicals and solvents used were of analytical grade. Sigma-Aldrich (UK) supplied the antioxidants butylated hydroxyanisole (BHA), (M.W: 180.24 g mol-1), 2, 6-di-tert-butyl-4-methylphenol (BHT) (M.W: 220.35 g mol-1), tert-butylhydroquinone (TBHQ), (M.W: 166.22 g mol-1. In addition, HPLC grade organic solvents, such as propan-2-ol, acetic acid, methanol, and acetonitrile, were ordered from Fisher Scientific, (UK). Fresh vegetable oil was obtained from the supermarket.

2.2 Standard HPLC Measurements.

The PerkinElmer series 200 HPLC instrument consisted of a series 200 pump, an auto-sampler, the same C18 column used for the HPLC-BBCEAS measurements, a 1 cm path-length HPLC cuvette, and a series 200 photodiode array detector. IRIS Spectral Processing software was employed to generate the spectral data, while PerkinElmer's TotalChrom Chromatography Data Systems software was used to control the HPLC system.

2.3 Experimental Set up

Figure 2 presents the homemade experimental setup for the HPLC-BBCEAS approach used in the present work. A high-power (45 mW) UV LED (Thorlabs, UK) generated the collimated incident radiation beam with a nominal wavelength of 285 nm and bandwidth of 12 nm and was collimated and coupled into a 10 cm length optical cavity. Two identical plano-concave high-reflectivity mirrors formed the optical cavity assembly. Two mirror sets were used, one with a reflectivity of R ~ 0.96, between 250 and 400 nm (Layertec, Germany), employed for the deep-UV applications. At the centre of the cavity, a commercial HPLC flow cuvette (Hellma Analytics, Germany) with 1 cm path length, 3 mm diameter circular aperture and 80 µL volume was positioned. An HPLC column connected to the flow cuvette. A Luna C18 HPLC column, of 150 mm length, 4.6 mm internal diameter and 100 µm particle size (Phenomenex, UK), was used for the separation of TBHQ, BHA and BHT in edible oils. An HPLC pump (SSI Series 2, USA) and a six-port manual sample injector (Rheodyne, USA) were coupled to the HPLC column. A two-metre-length fibre-optic cable (Thorlabs, UK) was used to focus and couple the light intensity outputting from the cavity into an AvaSpec-2048XL-USB2 UV-visible spectrometer (Avantes, The Netherlands). For ease of data transfer, the spectrometer was interfaced to a PC by means of a USB port. The AvaSoft Basic software program was used to acquire and record spectral data.

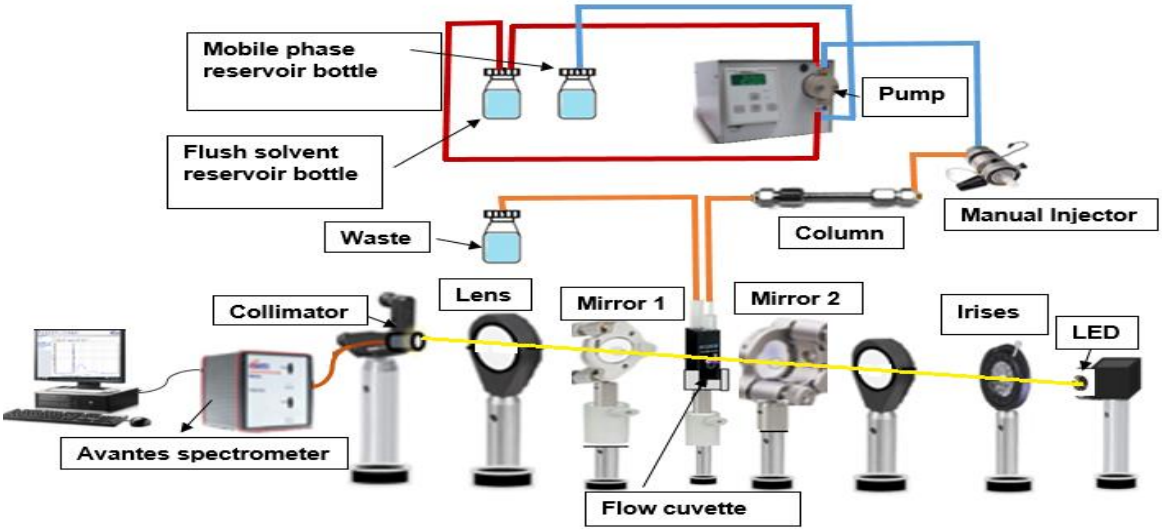


Figure 2. Schematic diagram showing the home-made experimental setup for the HPLC-BBCEAS technique used for measurements at UV wavelengths from 250 to 300 nm.

2.4 Experimental Methodology

For the separation and quantitative determination of Antioxidants TBHQ, BHA, and BHT, the mobile phase consisted of acetonitrile, acetic acid, and water in the ratio of [70:1:30 (v/v)]. The antioxidants, stock and working solution used were prepared using water acetic acid, solvent, and acetonitrile. Furthermore, the antioxidants and oil were spiked with propan-2-ol using a 50 mL volumetric flask; a stock solution of 1 mg/mL of the preferred antioxidants was obtained by dissolving 50 mg of each antioxidant in 50 mL of mobile phase. For single-pass and multi-pass measurements, serial solutions of 10 to 100 mg/L and 1 to 10 mg/L concentration ranges respectively were prepared for every antioxidant.

Fresh vegetable oil spiked with 20 mg of each of the three antioxidants (TBHQ, BHA and BHT) was blended properly with an ultrasonic bath for 15 minutes. Thereafter, 10 mL of the spiked stock solution was mixed with 10 mL of propan-2-ol/acetonitrile and shaken for two minutes in a mini shaker. Then, the mixture was centrifuged at a rotation speed of 3,500 rpm for about 10 minutes. This was followed by taking a portion of the top layer of the spiked solution of all the antioxidants used in the study (TBHQ, BHA and BHT) and inserting them into the PerkinElmer HPLC. A mixture of the antioxidants was also injected into the PerkinElmer HPLC, and the same procedure was conducted for the HPLC-BBCEAS technique.

At a wavelength of 280 nm, each working solution of antioxidants, as well as their mixtures, were measured separately using the PerkinElmer HPLC, with single-pass measurements conducted of both analytes. Accordingly, upon the removal of the lone cavity mirror, the HPLC-BBCEAS technique measurements were also conducted on the analytes. Additionally, HPLC-BBCEAS multi-pass measurements were measured, in triplicate, for all the analytes. An LED light source of 280 nm (Thiorlabs, UK) was used for the initial set of tests using the HPLC-BBCEAS technique.

The LED was equipped with an emission power of 45 mW with a 280 nm nominal peak wavelength and 12 nm full width at half peak (FWHM). It was also equipped with a Semrock bright-line UV filter (Laser 2000, UK) to restrict the wavelength output to the range of 260 to 340 nm.

The experimental setup consisted of two identical mirrors of R~0.96 over a bandwidth of 250 to 400 nm and a C18 HPLC column (Supelco, USA) with internal diameter of 4.6 nm, length of 150 nm, and particle size of 5 µm. At room temperature, each of the antioxidants was diluted isocratically and the mobile phase consisting of water, acetic acid and acetonitrile was chromatographed at 1mL min^{-1} flow rate.

Although the acquisition times varied, the HPLC-BBCEAS technique was deployed in the study. Experimental data were obtained at a total dilution time of 18 minutes per sample run at the rate of one sample point every two seconds. The HPLC-BBCEAS technique was deployed using a 280 nm LED, and measurements were conducted at a total acquisition time of 0.4 seconds (4 ms and 100 averages) at a similar wavelength. The CEF, ∆ABSmin (t) αmin (t) and LOD values were obtained using HPLC-BBCEAS and conventional HPLC techniques. Also, calibration plots, chromatograms and representative absorption spectra were obtained.

2.5 Statistical analysis

Results from BBCEAS measurements are presented using several figures of merit, and representative absorption spectra for the analytes. The figures of merit include the cavity enhancement factor (CEF) or the number of passes, normalised base-line noise

(ΔABSmin(t)), the sensitivity (αmin(t)) and the limit of detection (LOD) of the analytes, which were calculated using the equations presented in Table 1 (Bazina *et al.*, 2022).

Table 1: Equations used for the calculation of figures of merit in the liquid-phase BBCEAS measurements in this work.

Figure of merit	Equation
$\boldsymbol{CEF}$	$CEF(\lambda) = \frac{[\left(\frac{Io}{I}\right) - 1]_{(\lambda)}}{2.303\varepsilon_{(\lambda)}Cl}$
$\Delta\boldsymbol{ABS_{min}(t)}$	$\Delta ABS_{min}(t) = STDEV\ [\log_{10}\left(\frac{Io}{I}\right)]\sqrt{Total\ acquisition\ time\ (s)}$
$\boldsymbol{\alpha_{min}(t)}$	$\alpha_{min}(t) = \frac{2.303\Delta ABS_{min}(t)}{l_{eff}}$
$\boldsymbol{LOD}$	$LOD = \frac{3\ \alpha_{min}(t)}{2.303\ \varepsilon_{(\lambda)}}$

3 Results and Discussion

3.1 Standard HPLC Measurements for Antioxidants.

The measurement of antioxidants (TBHQ, BHA and BHT) was carried out using a commercial PerkinElmer HPLC system in a 1 cm HPLC cuvette at a wavelength of 280 nm. Table 2 presents the results of these measurements. Figure 3 to *Figure **5*** to present linear calibration plots and representative absorbance spectra for the antioxidants. For the same concentrations, a separate set of PerkinElmer HPLC measurements were conducted on 50% (v/v) mixtures of antioxidants, shown in Figure *6*.

Table 2: Results of the individual PerkinElmer HPLC measurements for antioxidants TBHQ, BHA and BHT at 280 nm

Analyte	Δ ABS	α_{min} [cm^{-1}]	LOD [mg/L]
TBHQ $(\lambda)_{280}$ nm	6.4×10^{-5}	1.5×10^{-4}	0.1
BHA $(\lambda)_{280}$ nm	8.6×10^{-5}	1.9×10^{-4}	0.3
BHT $(\lambda)_{280}$ nm	1.1×10^{-4}	2.6×10^{-4}	1.7

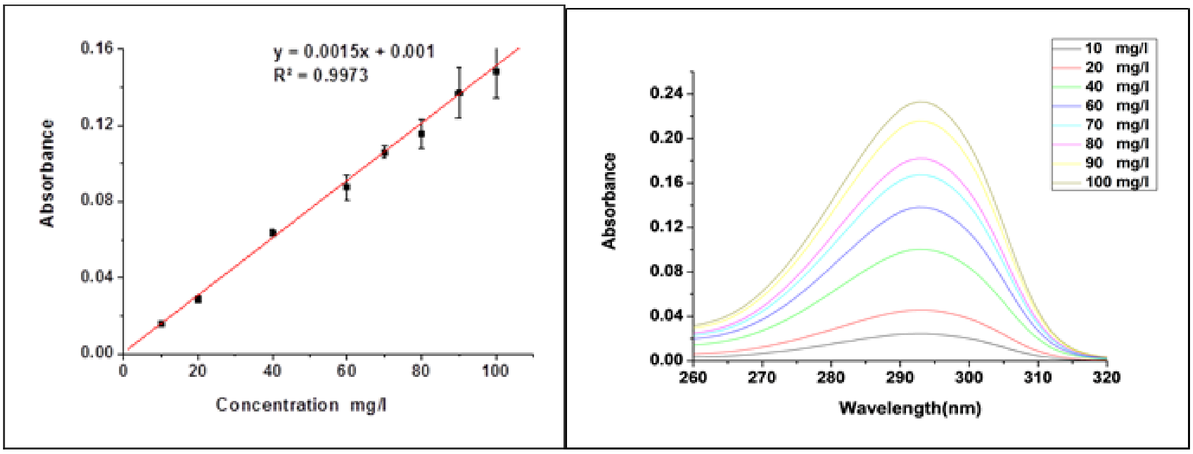


Figure 3: Representative absorbance spectra and calibration plot for antioxidant TBHQ recorded using the conventional HPLC PerkinElmer instrument.

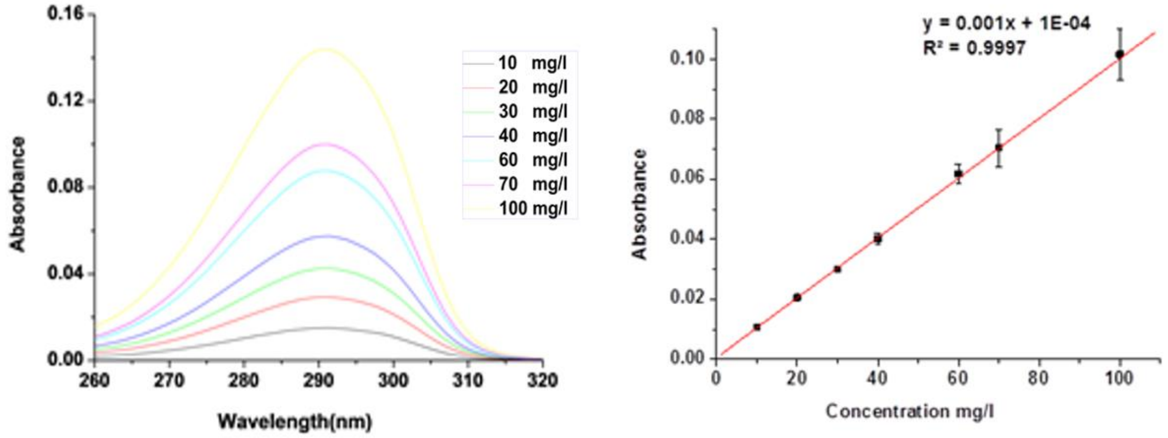


Figure 4. Representative absorbance spectra and calibration plot for antioxidant BHA achieved using the conventional HPLC PerkinElmer instrument.

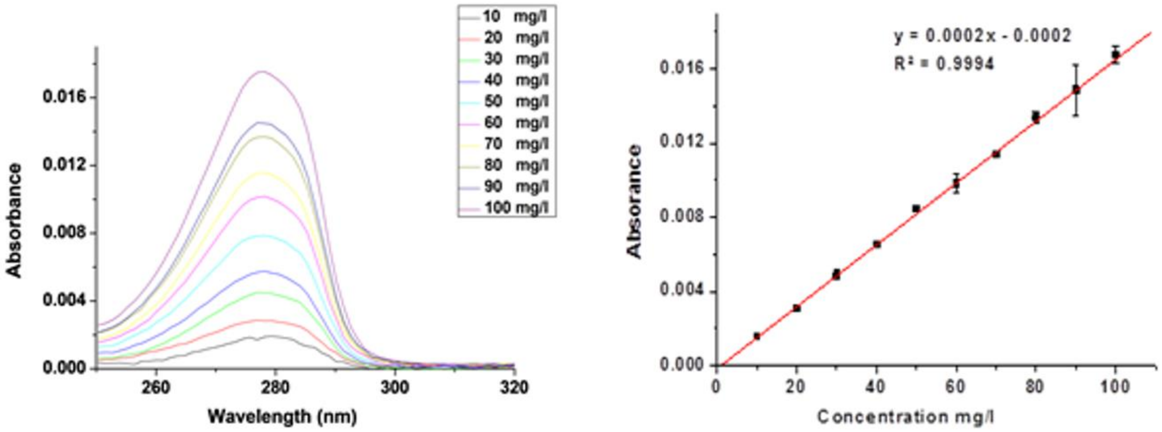


Figure 5. Representative absorbance spectra and calibration plot for antioxidant BHT achieved using the conventional HPLC PerkinElmer instrument.

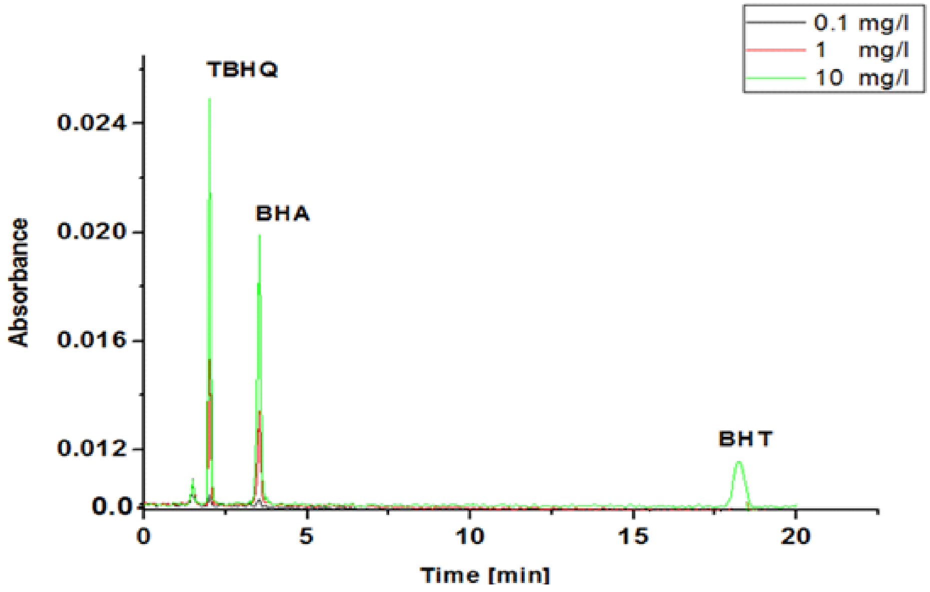


Figure 6. Chromatogram obtained using the conventional PerkinElmer HPLC system for a 50% (v/v) mixture of antioxidants TBHQ, BHA and BHT at different concentrations (0.1, 1 and 10 mg/L).

3.2 Standard HPLC-BBCEAS Measurements for Antioxidants.

The HPLC-BBCEAS technique was used for multi-pass measurements for antioxidants TBHQ, BHA and BHT. The results of these measurements are summarised in Table 4.4 as figures of merit for both analytes. The linear calibration plots and representative absorption spectra for both analytes obtained using the HPCL-BBCEAS setup coupled with the LED light source are presented in Figures 4.11 and 4.12. Figures 4.13 and 4.14 present the chromatograms for the antioxidant mixture of TBHQ, BHA and BHT obtained using the HPLC-BBCEAS setup with the LED light source at 280 nm.

Table 3: Summary of results of the individual HPLC-BBCEAS measurements for antioxidants with TBHQ, BHA and BHT, with the R ~ 0.96 mirror set at 280 to 289 nm using LED light source.

Analyte	CEF	$\Delta ABS_{min}(t)$	$\alpha_{min}(t)$ [cm^{-1} $Hz^{-½}$]	LOD [mg/L]
TBHQ	8.7 ± 0.3	7.4×10^{-5}	1.9×10^{-5}	0.01
BHA	8.7 ± 0.7	7.3×10^{-5}	1.8×10^{-5}	0.01
BHT	9.6 ± 0.3	1.2×10^{-4}	2.8×10^{-5}	0.4

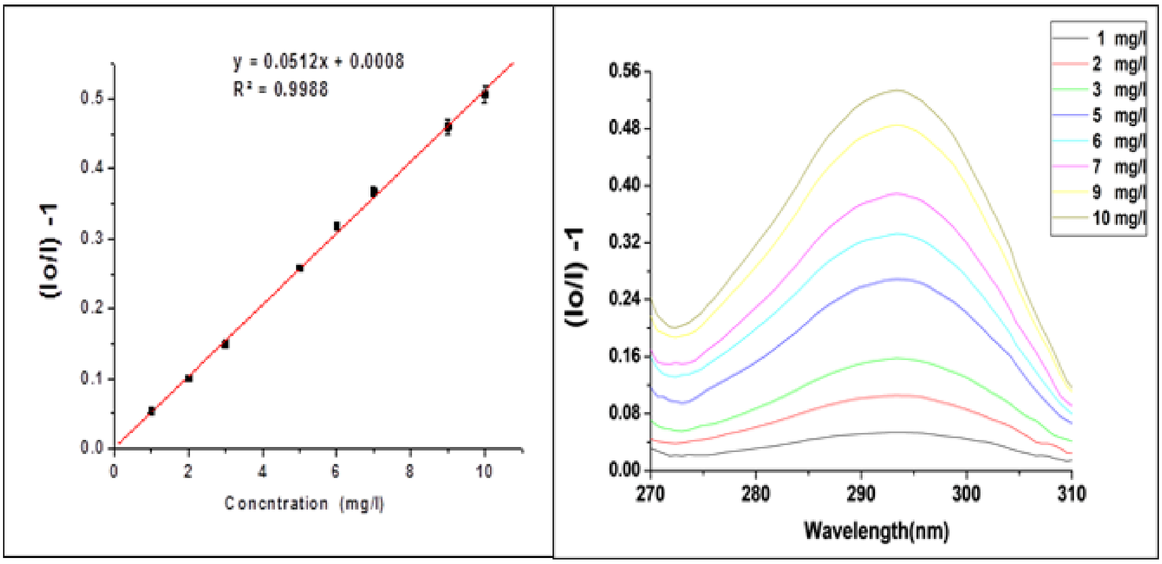


Figure 7: Representative absorption spectra and linear calibration plot for antioxidant TBHQ recorded using the HPLC-BBCEAS technique coupled with the LED light source at 289 nm.

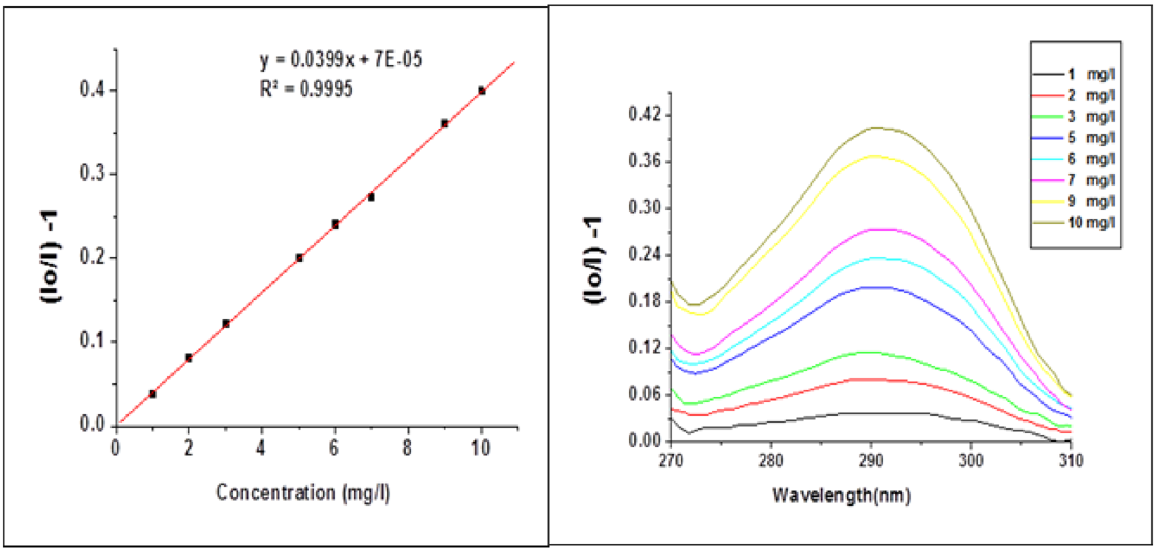


Figure 8: Representative absorption spectra and linear calibration plot for antioxidant BHA recorded using the HPLC-BBCEAS technique coupled with the LED light source at 289 nm.

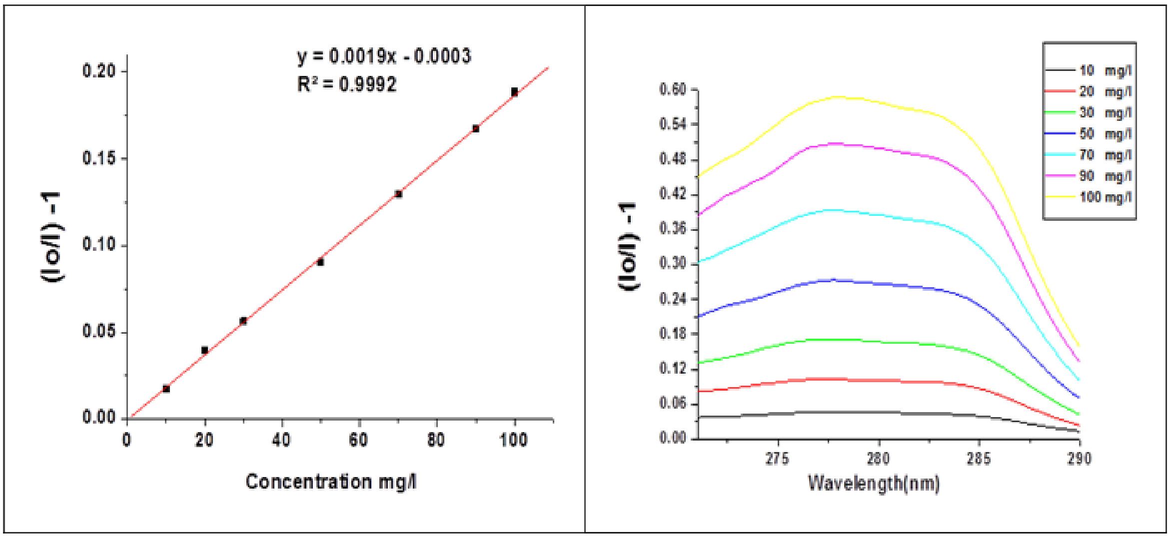


Figure 9: Representative absorption spectra and linear calibration plot for antioxidant BHT recorded using the HPLC-BBCEAS technique coupled with the LED light source at 280 nm.

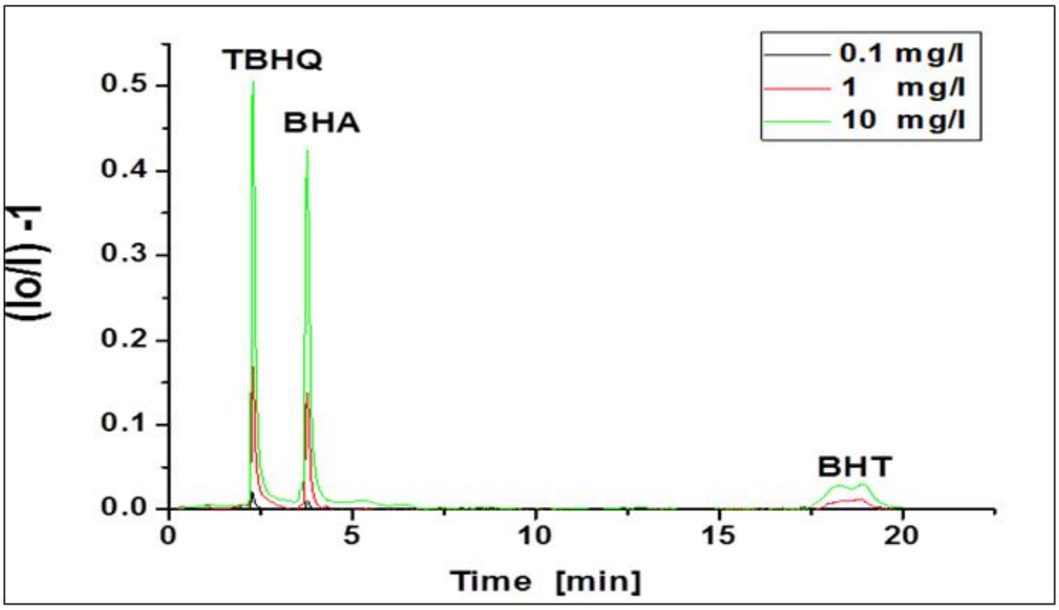


Figure 10: The chromatogram obtained using the HPLC-BBCEAS technique coupled with the LED light source for a 50% (v/v) mixture of antioxidants TBHQ, BHA and BHT at different concentrations (0.1, 1 and 10 mg/L).

3.3 Antioxidants Extraction in fresh vegetable oil samples using HPLC.

For the determination of antioxidants in fresh oil using commercial PerkinElmer HPLC, an average extraction yield of 88.8, 70.3 and 85.2% with relative standard deviations (RSD) of 19.56, 5.15 and 5.52% were obtained for the antioxidants for the triplicate samples each. This is presented in Table 4.

Table 4: Summarises the extraction of antioxidants in fresh oil samples using Perkin –Elmer HPLC.

Analyte	Concentration (mg/L)	Extraction Yield (%)	RSD (%)
BHA	196.71	88.8	19.56
BHT	152.8	70.3	5.15
TBHQ	190.10	85.2	5.52

3.4 Antioxidants Extraction in fresh vegetable oil samples using BBCEAS-HPLC.

As stated earlier, the three antioxidants were added to the fresh oil samples separately. An average yield for the triplicate samples was 99.8%, 76.3 and 99.1% were obtained for the BHA, BHT and TBQH antioxidants. Relative standard deviations (RSD) of 2.9, 7.2 and 1.26% were obtained using BBCEAS-HPLC.

Table 5 presents the extraction of antioxidants in the fresh oil samples using BBCEAS-HPLC. An increase in 12, 8.5 and 16% improvement in the extraction yield was obtained for BHA, BHT and TBHQ using this method compared to the commercial PerkinElmer HPLC.

Table 5: Summarises the extraction of Antioxidants in fresh oil samples using BBCEAS-HPLC

Analyte	Concentration (mg/L)	Extraction Yield (%)	RSD (%)
BHA	220.99	99.8	2.81
BHT	165.88	76.3	7.22
TBHQ	221.12	99.1	1.31

3.5 Discussion

The antioxidants showed effective absorption coefficients of 4.01×10^{-2} L mg^{-1} cm^{-1} for BHA, 1.9×10^{-3} L mg^{-1} cm^{-1} for BHT, and 5.01×10^{-2} L mg^{-1} cm^{-1} for TBHQ, after modifying the HPLC-BBCEAS setup. These coefficients were crucial for calculating the cavity enhancement factors (CEF), resulting in values of 8.7±0.7 for BHA, 9.6±0.3 for BHT, and 8.7±0.3 for TBHQ using the LED light source.

The deep-UV region posed challenges due to higher light absorption and scattering by the cuvette and solvent. CEF values from UV-visible measurements exceeded those in the deep-UV due to the difficulties in light collimation and alignment through the HPLC flow cell's 3 mm aperture. Sensitivity values, αmin (t), were recorded as 1.8×10^{-5} cm^{-1} for BHA, 2.8×10^{-5} cm^{-1} for BHT, and 1.9×10^{-5} cm^{-1} for TBHQ with LED, demonstrating improvements of 11, 9, and 8 times, respectively, over conventional HPLC. The LED light source reduced sensitivity slightly due to worse CEF values but allowed a shorter integration time of 4 seconds, enhancing αmin (t) values about eightfold compared to the PerkinElmer HPLC system.

Comparison with previous cavity-enhanced HPLC studies, specifically HPLC-CRDS by (Van der Sneppen *et al.*, 2006) highlighted that the LED measurements were up to 48 times more sensitive at a deep UV wavelength of 273 nm, using a more straightforward and cost-effective setup. The linear regression and spectral methods determined the limits of detection (LOD) for the antioxidants, with HPLC-BBCEAS demonstrating superior performance over the PerkinElmer HPLC instrument. BHA showed a thirty-fold improvement in LOD, BHT a fourfold improvement, and TBHQ a tenfold improvement.

The HPLC-BBCEAS system, tested with concentrations of 0.1, 1, and 10 mg/L, successfully separated antioxidants in sample mixtures down to 1 mg/L. This method exhibited high sensitivity and reduced noise compared to conventional HPLC. The extraction yields for the antioxidants were significantly higher with BBCEAS-HPLC, achieving 99.7% for BHA, 76.4% for BHT, and 96% for TBHQ, which were 12%, 8.5%, and 16% higher, respectively, than those obtained with the PerkinElmer HPLC technique.

4 Conclusion

In recent years, the BBCEAS technique has been employed as a detector for HPLC systems at visible wavelengths. To date, no studies that uses the HPLC-BBCEAS approach for separation and quantification at deep UV wavelengths have been published.

This study presents the first demonstration of the HPLC-BBCEAS technique for the separation and quantification of synthetic antioxidants in the deep-UV region. The BBCEAS-HPLC method showed effective absorption coefficients of 4.01×10^{-2} L mg^{-1} cm^{-1} for BHA, 1.9×10^{-3} L mg^{-1} cm^{-1} for BHT, and 5.01×10^{-2} L mg^{-1} cm^{-1} for TBHQ, resulting in cavity enhancement factors (CEF) of 8.7±0.7, 9.6±0.3, and 8.7±0.3, respectively. Sensitivity values, αmin (t), were significantly improved, recording 1.8×10^{-5} cm^{-1} for BHA, 2.8×10^{-5} cm^{-1} for BHT, and 1.9×10^{-5} cm^{-1} for TBHQ, demonstrating enhancements of 11, 9, and 8 times over conventional HPLC.

Compared to the PerkinElmer HPLC system, the HPLC-BBCEAS setup achieved a thirty-fold improvement in the limit of detection (LOD) for BHA, a four-fold improvement for BHT, and a ten-fold improvement for TBHQ. The method also successfully separated antioxidants in sample mixtures down to 1 mg/L with high sensitivity and reduced noise. The extraction yields were significantly higher using the BBCEAS-HPLC, achieving 99.7% for BHA, 76.4% for BHT, and 96% for TBHQ, representing improvements of 12%, 8.5%, and 16%, respectively, over the PerkinElmer HPLC technique.

These results demonstrate that the HPLC-BBCEAS technique offers superior sensitivity, efficiency, and reliability for detecting and quantifying synthetic antioxidants in the deep-UV region. This method's high performance and cost-effectiveness make it a promising tool for food quality control and safety assessment. Moreover, the versatility of the BBCEAS-HPLC approach suggests its potential applicability for the separation and analysis of various other liquid-phase compounds.

5 Recommendations

To further advance the BBCEAS technique, future research should explore using more powerful deep-UV light sources with higher photon flux and narrower bandwidths, along with higher reflectivity mirrors ($R \geq 0.99$) to increase the number of passes and effective path length. Integrating more sensitive spectrometers, such as those with advanced detectors like CCDs or PDAs, will enhance measurement accuracy and reliability. Expanding the application of the BBCEAS-HPLC method to a broader range of synthetic and natural antioxidants will demonstrate its versatility. Optimising experimental conditions, including flow rates, solvent compositions, and column characteristics, will enhance separation efficiency and sensitivity for various antioxidants. Applying this technique to real-world food and oil samples will provide valuable insights into its efficacy and safety in commercial products. Comparative studies with other advanced detection techniques, such as mass spectrometry or fluorescence spectroscopy, will further highlight the BBCEAS-HPLC method's strengths.

Declaration of Competing Interest

The authors state that they have no competing financial interests or personal relationships that could have influenced the research presented in this paper.

Data Availability

Data will be made available on request.

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