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Novel applications of broadband cavity enhanced absorption spectroscopy (BBCEAS) as a detector for HPLC at ultraviolet wavelengths from 250 - 400 nm

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ABSTRACT

The first application of broadband cavity enhanced absorption spectroscopy (BBCEAS) as a detection method for high performance liquid chromatography (HPLC) in the ultraviolet (UV) spectral range of 250–400 nm is reported. A custom-designed HPLC-BBCEAS system, incorporating a laser-driven light source (LDLS) and high reflectivity cavity mirrors ($R \geq 0.99$), was employed for the separation and quantification of the polycyclic aromatic hydrocarbons (PAHs) anthracene and pyrene at near-UV wavelengths (300–400 nm). Minimum detectable absorption coefficients, $\alpha_{min(t)}$ of 9.8×10^{-6} cm^{-1} $Hz^{-½}$ and 9.6×10^{-6} cm^{-1} $Hz^{-½}$ were achieved for anthracene and pyrene at 338 nm and 333 nm, respectively, with 6.8×10^{-6} cm^{-1} $Hz^{-½}$ observed for anthracene at 356 nm. These values correspond to 25-fold and 18-fold improvements in sensitivity compared to a conventional Perkin-Elmer HPLC-UV system. In the deep-UV region (250–300 nm), the separation and detection of vitamin D_2 and D_3 were demonstrated using both the LDLS and a 280 nm LED with $R \sim 0.96$ cavity mirrors. LDLS-based measurements yielded $\alpha_{min(t)}$ values of 5.1×10^{-4} cm^{-1} $Hz^{-½}$ and 4.0×10^{-4} cm^{-1} $Hz^{-½}$ for D_2 and D_3, respectively, while the LED measurements exhibited enhanced sensitivity with values of 7.7×10^{-5} cm^{-1} $Hz^{-½}$ and 5.8×10^{-5} cm^{-1} $Hz^{-½}$. These findings demonstrate that HPLC-BBCEAS enables broadband, multiplexed, and highly sensitive detection of UV-absorbing analytes and extends the practical detection range of HPLC into the deep-UV region.

1. Introduction

1.1. Background: chromatography and HPLC detection

Chromatography is an analytical technique used for the separation, identification and quantification of components in complex mixtures [1]. A range of chromatographic methods is routinely applied across environmental and pharmaceutical laboratories. Among these, high-performance liquid chromatography (HPLC) is one of the most widely used techniques for separating and detecting compounds in the liquid phase [2]. A typical HPLC system comprises solvent reservoirs (mobile phase), a degasser, pump, injector, column, detector and a data-acquisition system. Reliable quantification depends strongly on detector choice and overall system performance.

Conventional UV–visible absorption detectors remain the most commonly employed in HPLC because they are simple, broadly applicable and relatively low cost [3]. Single-pass absorbance detectors are particularly attractive because many organic and pharmaceutical compounds possess intrinsic UV absorption bands, enabling label-free detection. However, their sensitivity is often poorer than that achieved with more complex and expensive detectors, such as fluorescence detectors and mass spectrometers. This limitation arises primarily from the short optical path length of standard HPLC flow cells, which restricts absorption sensitivity for weakly absorbing or trace-level analytes. Fluorescence detection can provide significantly improved sensitivity but is limited to fluorescent or derivatised compounds, whereas mass spectrometry instrumentation is comparatively expensive and operationally complex. Consequently, substantial effort has been directed

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towards improving the sensitivity of absorption-based HPLC detection while retaining the simplicity and broad applicability of optical absorbance measurements.

Optical cavity-based absorption spectroscopy provides a well-established route to enhanced sensitivity [4]. Compared with conventional single-pass absorption measurements, cavity-enhanced techniques can achieve substantially longer effective path lengths and correspondingly lower detection limits. Of the cavity techniques developed, cavity ring-down spectroscopy (CRDS) and cavity-enhanced absorption spectroscopy (CEAS) have been most widely adopted. Although the majority of cavity-enhanced studies have focused on gas-phase species, several studies have demonstrated liquid-phase implementations, including use as detectors for liquid chromatography.

CRDS detection in liquid chromatography using a 1 m ring-down cavity formed by two high-reflectivity mirrors (R = 0.9993 at 470 nm) and a Brewster-angle flow cell (optical path length ~300 μm; total volume 10 μL). A Nd:YAG-pumped dye laser (10 ns pulse duration) provided excitation, and a photomultiplier tube (PMT) was used for detection. Measurements were performed at a single wavelength (470 nm), yielding performance comparable to, or slightly better than, high-quality conventional UV–visible detection for the same path length [5]. Similar geometry for continuous-wave CRDS measurements at 488 nm were also reported [6].

A CRDS system using a short 2 mm cavity was demonstrated, in which the mirrors were placed in direct contact with the flowing liquid and a silicon–rubber spacer formed a 12 μL flow cell. A pulsed laser source was employed, and the transmitted light was monitored with a photodiode. This configuration enhanced absorption detection while minimising scattering losses and avoiding Brewster-angle constraints; however, the very short ring-down times complicated accurate measurements, and corrosive solvents were avoided due to direct mirror contact [7].

A later study used a similar configuration with mirror reflectivity of R ≥ 99.996% at 532 nm to separate and detect azo dyes, achieving performance comparable to earlier LC–CRDS demonstrations [8].

Further works explored LC–CRDS using a standard 1 cm flow cuvette (80 μL volume; 3 mm aperture) positioned at normal incidence within an approximately 4 cm cavity. Operation was demonstrated in the UV at 355 nm and 273 nm using a Nd:YAG laser and a frequency-doubled dye laser, respectively. Improved performance was reported at 355 nm, whereas no improvement was achieved at 273 nm because mirror reflectivity was insufficient in the deep-UV. This cuvette-based arrangement removed the need for a bespoke flow cell and reduced concerns about mirror degradation [9].

Collectively, these studies demonstrated the potential of cavity-based HPLC detectors to improve absorption sensitivity relative to conventional absorbance detection. However, previously reported LC–CRDS implementations have generally relied on pulsed laser sources, wavelength-specific operation and relatively complex optical alignment. In addition, most systems have operated at a single wavelength, limiting spectral information and multiplexing capability.

Broadband cavity-enhanced absorption spectroscopy (BBCEAS) addresses several of these limitations by combining cavity enhancement with broadband illumination and spectrometer-based detection. Unlike CRDS, BBCEAS does not require time-resolved decay measurements and can therefore employ comparatively simple continuous light sources and compact spectrometer detection systems, reducing overall system complexity and cost. The first demonstration of BBCEAS as an HPLC detector was reported using a standard flow cell at normal incidence, a low-cost broadband source (white LED), and measurements across the visible region (450–600 nm). That work demonstrated multiplexed detection of closely related dyes (rhodamine 6G and rhodamine B), while retaining a comparatively simple and low-cost experimental configuration relative to LC–CRDS [10]. The study also highlighted the advantages of broadband cavity-enhanced detection for simultaneous spectral acquisition and discrimination of analytes with closely related chromatographic behaviour.

However, many chemical, biological and pharmaceutical analytes of interest in the liquid phase exhibit strong absorption in the ultraviolet region (200–400 nm). To date, BBCEAS-based HPLC detection has not been reported across the UV range of 200–400 nm, particularly in the deep-UV region below 300 nm where optical losses increase and mirror reflectivity is typically reduced. . As a result, the applicability of BBCEAS for analytically important UV-absorbing compounds remains largely unexplored.

In this work, BBCEAS is demonstrated for the first time as a detector for HPLC at ultraviolet wavelengths across 250–400 nm, enabling broadband and multiplexed detection with enhanced sensitivity relative to conventional single-pass UV absorption detection. Two application areas are investigated. First, in the near-UV region (300–400 nm), the method is applied to the separation and quantification of the polycyclic aromatic hydrocarbons (PAHs) anthracene and pyrene. These PAHs were selected because of their environmental relevance and their closely spaced absorption features in the near-UV, making them suitable for demonstrating the multiplexing capability of broadband detection.

Anthracene and pyrene are representative polycyclic aromatic hydrocarbons (PAHs) with closely related near-UV absorption characteristics. Anthracene is a linearly fused aromatic compound, whereas pyrene is non-linearly fused. In 95% methanol, both compounds exhibit characteristic UV absorption features between 300 and 400 nm, with strong absorption peaks occurring at 338 nm (anthracene) and 333 nm (pyrene), which were used for quantification (Fig. 1). Their closely spaced absorption features make them suitable analytes for demonstrating the multiplexing capability of broadband cavity-enhanced detection.

This study also extends BBCEAS-based HPLC detection into the deep-UV region (<300 nm) where conventional absorption-based detection is often challenged by reduced optical sensitivity and increased background losses. Vitamins D_2 ($C_{28}H_{44}O$) and D_3 ($C_{27}H_{44}O$) were selected as closely related target analytes for separation and quantification in a mixture at 282 nm (Fig. 2). Accurate determination of vitamins D_2 and D_3 is important in food and pharmaceutical manufacturing because of their nutritional significance and relative instability.

The two compounds possess very similar chemical structures and closely related chromatographic behaviour, making selective and sensitive detection challenging using conventional single-pass UV absorbance detection. Consequently, they provide a suitable test system for evaluating the performance advantages of BBCEAS-based HPLC detection in the deep-UV region. In particular, the enhanced effective optical path length provided by the optical cavity is expected to improve absorption sensitivity, while the broadband spectral acquisition capability offers additional selectivity for distinguishing closely related analytes.

2. Experimental section

2.1. Experimental setup

The home-made experimental setup for HPLC-BBCEAS approach used in this work (Fig. 3). The incident radiation was generated either a laser driven light source (LDLS;EQ-99 FC, Energetiq Technology INC, USA) or by a UV LED source with an optical output power of 30 mW (Thorlabs, UK), nominal wavelength of 280 nm, and bandwidth 12 nm. The beam was collimated and coupled into a 10 cm optical cavity. The optical cavity assembly is formed by two identical plano-concave high reflectivity mirrors. Two mirror sets have been used. One mirror set had reflectivity R ≥ 99±0.3% between 310-430±10 nm (Layertec, Germany), and was used for near-UV applications, whereas the other mirror set, had a reflectivity of R ~ 96%, between 250-400 nm (Layertec, Germany), and was employed for deep-UV applications. A commercial HPLC flow cuvette (Hellma Analytics, Germany), with an 80 μL volume, a 1 cm path length and 3 mm diameter circular aperture, was positioned in the centre of the cavity. The flow cuvette was connected to a HPLC

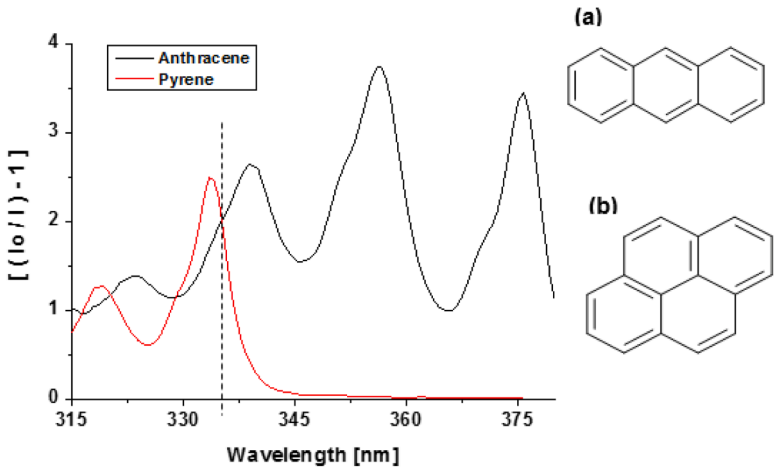

Fig. 1. The Chemical structures and the UV absorption spectra in 95% methanol solvent for analytes, (a) anthracene and (b) pyrene.

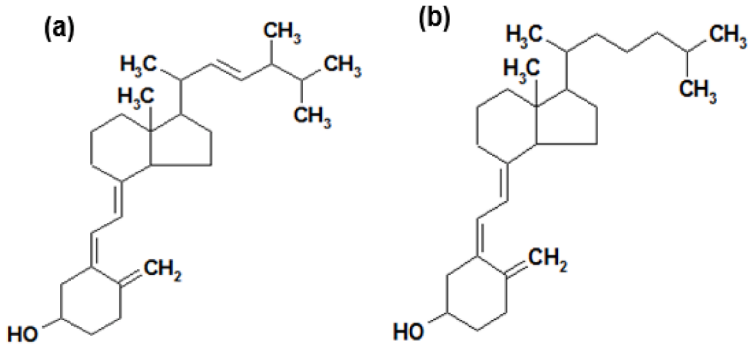

Fig. 2. Chemical structures of (a) vitamin D2 (Ergocalciferol) and (b) vitamin D3 (Cholecalciferol).

column. A Luna C18 HPLC column (Phenomenex, UK), with dimensions 150 mm length × 4.6 mm internal diameter and 100 μm particle sizes, was used for separation of the anthracene and pyrene compounds. While for separation of vitamins D2 and D3, a Discovery C18 HPLC column (Supelco, USA), with dimensions 150 mm length × 4.6 mm internal diameter and 5 μm particle sizes, was employed.

The HPLC column was coupled to a series a HPLC pump (SSI, USA) and a 6-port manual sample injector (Rheodyne, USA). The integrated light intensity outputting the cavity was focused and coupled into an AvaSpec-2048XL-USB2 UV-visible spectrometer (Avantes, The Netherlands), via a 2 m length fibre optic cable (Thorlabs U.K). The spectrometer was interfaced to a PC by means of an USB port, which allowed data transfer. The spectral data were acquired and recorded using the AvaSoft-basic software program.

Additionally, for comparison purposes, single pass measurements were carried out under the same conditions using a conventional HPLC system (Perkin-Elmer Series 200), with a UV-visible absorbance detector. The Perkin-Elmer series 200 HPLC instrument consisted of a series 200 pump, auto-sampler, the same C18 column used in HPLC-BBCEAS measurement, a 1 cm path length HPLC cuvette and a series 200 photodiode array detector. Furthermore, the PerkinElmer's TotalChrom Chromatography Data Systems software was used to control the HPLC system, while the IRIS Spectral Processing was employed to generate the spectral data.

2.2. Experimental methodology

All chemicals used in this study were of analytical grade, and all solutions were prepared using the mobile phase as a solvent. For HPLC-BBCEAS measurements in the near-UV region above 300 nm, anthracene (M.W: 178.23 g mol^{-1}) and pyrene (M.W: 202.25 g mol^{-1}), were purchased from Sigma-Aldrich, UK. Other analytical HPLC grade chemicals including methanol (CH_3OH) and HPLC water (H_2O), were obtained from Fisher Scientific, UK, and they were used in the preparation of the mobile phase, as well as the anthracene and pyrene stock and working solutions. The mobile phase was formed from 95% HPLC grade methanol and 5% HPLC water. Anthracene stock solution 5.61×10^{-3} M was prepared by dissolving 25 mg of anthracene in a final volume of 25 mL of mobile phase using a 25 mL volumetric flask. A pyrene stock solution 4.94×10^{-3} M was also prepared by the same way. Serial working solutions for both analytes were prepared directly before each measurement using the mobile phase as a diluent. Serial working solutions of anthracene in the concentration ranges of $5.61 \times 10^{-5} - 5.61 \times 10^{-4}$ M and $5.61 \times 10^{-6} - 5.61 \times 10^{-5}$ M were prepared for the single pass and multi-pass measurements, respectively. Similarly, serial working solutions of pyrene between 4.94×10^{-6} - 4.94×10^{-5} M and 4.94×10^{-7} - 4.94×10^{-6} M were also prepared. The anthracene and pyrene analytical solutions were eluted isocratically at room temperature, using the mobile phase at 1 mL min^{-1} flow rate. Then, they measured at 338 nm and 333 nm, respectively, and their mixture was measured at approximately the midpoint between their absorption peaks, 335 nm.

For HPLC-BBCEAS measurements in the deep-UV region below 300 nm, vitamin D2 (Ergocalciferol, M.W: 396.66 g mol-1) and vitamin D3 (cholecalciferol, M.W: 384.65 g mol-1), were ordered from Sigma-Aldrich, UK. Moreover, HPLC grade organic solvents including acetonitrile and methanol were obtained from Fisher Scientific, UK. The acetonitrile solvent was used as a mobile phase, whereas the methanol solvent was employed in preparation of vitamins D2 and D3 stock and working solutions. A stock solution of 100 mg/L for each vitamin was prepared. In addition, serial working solutions in the concentration

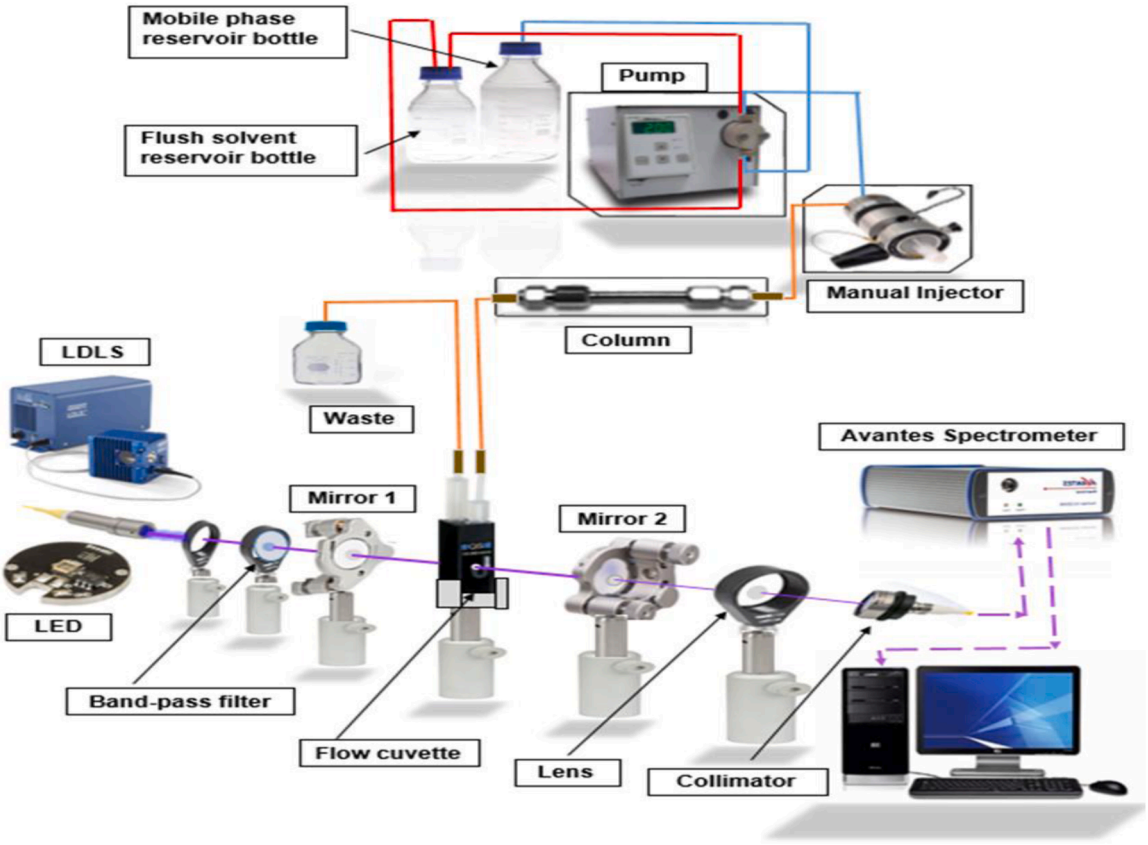

Fig. 3. A schematic diagram showing the home-made experimental setup for the HPLC-BBCEAS technique used for measurements at UV wavelengths from 260 - 400 nm.

ranges of 10 – 100 mg/L and 1 - 10 mg/L were prepared for single-pass and multi-pass measurements, for each vitamin, respectively. These working solutions for vitamins D2 and D3 were individually measured at 282 nm, and additionally their mixture was also measured at the same wavelength. Single pass measurements for both analytes were carried out using a commercial Perkin-Elmer HPLC instrument, and by employing the HPLC-BBCEAS system after removing one of the cavity mirrors. Furthermore, multi-pass HPLC-BBCEAS measurements were conducted twice for each analyte. In the first set of experiments, the HPLC-BBCEAS setup was used with the LDLS as the light source and a Semrock bright-line band-pass UV filter (Laser 2000, UK) to reduce the wavelength output to the range 260-340 nm, whereas in the second set of experiments, the HPLC-BBCEAS setup was used with a high power 280 nm LED (Thorlabs, UK), as the light source. The LED had a typical emitted power of 30 mW and a nominal peak wavelength of 280 nm with full width at half maximum (FWHM) of 12 nm. For all the vitamin D2 and vitamin D3 experiments, the analytes were eluted isocratically at room temperature. The mobile phase (acetonitrile) was chromatographed at a 1 mL min-1 flow rate.

In this work, the results of HPLC-BBCEAS measurements were represented using the main Fig.s of merits include the cavity enhanced factor (CEF), the normalised baseline noise ($\Delta ABS_{min}(t)$), the sensitivity ($\alpha_{min}(t)$) and the limit of detection (LOD). These Fig.s of merits were calculated using the equations described Table 1). The CEF value was calculated using the multi-pass absorption extinction coefficient value (the gradient of the regression curve in the cavity) divided by 2.303 times the single pass absorption extinction coefficient (ε) value at the same wavelength. The Δ $ABS_{min}(t)$ was obtained from the mean of 10 standard deviation values for blank spectra of the eluent, which was measured at a 10 nm bandwidth around the wavelength and multiplied by the square root of the acquisition time used. The $\alpha_{min}(t)$ was

Table 1
Equations used for the calculation of Fig.s of merit in the HPLC-BBCEAS measurements.

Fig. of merit	Equation
CEF	$CEF(\lambda) = \frac{\left[\left(\frac{Io}{I}\right)-1\right]_{(\lambda)}}{2.303\varepsilon_{(\lambda)}Cl}$
$\Delta ABS_{min}(t)$	$\Delta ABS_{min}(t) = STDEV\left[\left(\frac{Io}{I}\right)-1\right]\sqrt{Total\ acquisition\ time\ (s)}$
$\alpha_{min}(t)$	$\alpha_{min}(t) = \frac{3\Delta ABS_{min}(t)}{l_{eff}}$
LOD_{LRM}	$LOD_{LRM} = \frac{3\ STDEV\ of\ the\ intercept}{Slope}$
LOD_{SM}	$LOD_{SM} = \frac{3\ \alpha_{min}(t)}{2.303\ \varepsilon_{(\lambda)}}$

calculated from 3 times the Δ $ABS_{min}(t)$ and divided by the effective path length (L_{eff}), where (L_{eff} = the absorption path length in cm × CEF). The LOD for each analyte was calculated by two methods: the linear regression method and the spectral method. The LOD value from the linear regression method (LOD_{LRM}) was obtained from 3 times the standard deviation of the intercept divided by the slope of the calibration curve [18]. While the LOD value from the spectral method (LOD_{SM}) was calculated from 3 times the $\alpha_{min}(t)$ divided by 2.303 times the (ε). For comparison, the baseline noise (ΔABS), the sensitivity and the limit of detection for the conventional HPLC experiments were calculated by the same way. Conversely, the chromatogram of the analyte's mixture was obtained by plotting the retention time versus the absorption [(Io/I) –1] of analytes in the cavity. In addition, the calibration curves and the representative absorption spectra were also obtained by plotting the concentration range and the wavelength range, respectively, against the

absorption values.

3. Results

The HPLC–BBCEAS detection system was first evaluated at near-UV wavelengths between 300 and 400 nm using the PAHs anthracene and pyrene as model analytes. Initial conventional HPLC measurements were performed separately for anthracene and pyrene using a Perkin–Elmer HPLC instrument equipped with a standard 1 cm path-length flow cuvette. Detection wavelengths of 338 nm and 333 nm were used for anthracene and pyrene, respectively. Table 2) summarises the results of these measurements in terms of the Fig.s of merit. Furthermore, Figs. 4 and 5 show representative absorbance spectra and the calibration plots for both analytes. Likewise, the second series of HPLC multi-pass measurements of the PAHs anthracene and pyrene were conducted using the HPLC-BBCEAS technique. Table 3) summarises the results of these measurements, in addition, linear calibration plots and their representative absorption spectra for both analytes are shown in Fig. 6) and Fig. 7). Moreover, the chromatograms for the mixture at 335 nm obtained using the HPLC-BBCEAS technique are shown in Fig. 8).

The second application of HPLC-BBCEAS detection system for measurements at deep-UV wavelengths between 250-300 nm. A commercial Perkin-Elmer HPLC system was first used for measurements of vitamin D2 and vitamin D3, separately, in a 1 cm HPLC cuvette at the same wavelength of 282 nm. The results of these measurements are summarised as Fig.s of merit in Table 4).

Likewise, multi-pass measurements for vitamins D2 and D3 were conducted using the HPLC-BBCEAS technique. Table 5) summarises the results of these measurements at the two different light sources used. Figure 9)Figure 10)Figure 11)Figure 12) show linear calibration plots and representative absorption spectra for both analytes at the two different light sources used. Moreover, the chromatograms for the vitamin D2 and vitamin D3 mixture obtained using the HPLC-BBCEAS setup with the two light sources, at 282 nm, are presented in Figure 13) and Figure 14).

4. DISCUSSION

This study reports the first application of HPLC-BBCEAS detection system for measurements at UV wavelengths. HPLC-BBCEAS measurements at near-UV wavelengths between 300-400 nm for anthracene and pyrene were firstly carried out individually on the analytes at 338 nm and 333 nm. The molar absorption coefficients for anthracene and pyrene were obtained by single pass measurement using a double beam (JASCO V-630) spectrophotometer and produced values of 6.2×10^3 M^{-1} cm^{-1} and 5×10^4 M^{-1} cm^{-1} at 338 nm and 333 nm respectively. However, these values decreased by 9 and 7 folds respectively in the HPLC measurements due to chromatographic broadening by the column.

The results showed approximately similar CEF values of 29.5±0.13 and 30.1±0.12 respectively. These CEF values were calculated using the HPLC based molar absorption coefficients for both analytes obtained from their calibration curves recorded by using the HPLC-BBCEAS experimental setup after the removal of one of the cavity mirror. The resulting CEF values are slightly lower than the CEF values of 40 and 45 for rhodamine 6G and rhodamine B using their HPLC-BBCEAS system at visible wavelengths between 450-600 nm [10]. The decrease in CEF values in this work could be due to comparatively higher scattering and absorption optical losses in the UV light by the cuvette surfaces and the solvents used, as well as lower mirror reflectivity at the wavelengths studied. Moreover, the absorption measurements were not conducted at the maximum absorption peaks for both analytes. Thus, the CEF value for anthracene was also calculated for the larger absorption peak at 356 nm. The results showed an increase in the CEF value for anthracene of 42.3±0.15 which is close to the CEF value [10]. This was possibly due to greater mirror reflectivity at 356 nm than at 338 nm. Further improvements could be made using fibre optic coupling of the light source instead of the current free space coupling to improve alignment.

Furthermore, this HPLC-BBCEAS study produced sensitivity $\alpha_{min}(t)$ values of 9.8×10^{-6} cm^{-1} $Hz^{-½}$ and 9.6×10^{-6} cm^{-1} $Hz^{-½}$ for anthracene and pyrene at 338 nm and 333 nm, respectively, and 6.8×10^{-6} cm^{-1} $Hz^{-½}$ for anthracene at 356 nm. These $\alpha_{min}(t)$ values are found to be 25 and 18 times smaller rather than the α_{min} values obtained using the conventional Perkin-Elmer HPLC system, respectively, (Tables 2 and 3) These results indicate that the use of BBCEAS as a detection system can improve the sensitivity of the conventional HPLC instruments up to 25 times for near-UV measurements. The baseline noise was also quantitatively compared with that of the conventional absorbance-based HPLC detector. For the near-UV measurements, the HPLC-BBCEAS baseline noise was 1.2×10^{-4}, which is comparable to the conventional Perkin-Elmer values of 1.1×10^{-4} for anthracene and 7.5×10^{-5} for pyrene. Therefore, the improved sensitivity mainly arises from the increased effective optical path length provided by the cavity rather than from lower baseline noise. Compared with previous CRDS and CEAS HPLC studies, this study shows nearly 3 times better sensitivity compared to these studies (Table 6.)

In addition, this study recorded the LOD values for both anthracene and pyrene by the linear regression method, as well as, by the spectral method. The results showed large differences between the LOD values obtained by the two methods as described in Tables 2) and3). The LOD_{SM} values achieved by the spectral method were more than 10 times lower than the comparative LOD_{LRM} values obtained by the linear regression method. These differences in LOD values between the two methods were also found in the first HPLC-BBCEAS study which stated that the LOD_{LRM} values are substantially worse [10]. Nevertheless, the LOD_{LRM} values are often the preferred method in conventional analytical absorption spectroscopy measurements. This is because the calculation is based on the standard deviation of the instrument response (y-residuals or y-intercepts) and three replicates and thus they represent all points on the regression line; while the LOD_{SM} values depend on the sensitivity (α_{min}) of the measurement calculated from the standard deviation of baseline noise on a blank spectrum. Cavity enhanced studies have tended to calculate LODs based on the spectral method rather than the linear regression method. We compared both methods in this study by carrying out measurements for both analytes at concentration ranges close to the LOD_{LRM} concentrations. The results exhibited linear standard curves with approximately the same previous CEF, sensitivity as the LOD_{SM} results, and seem to indicate that the LOD_{SM} values are a more accurate representation of the true LOD of the HPLC-BBCEAS system. Furthermore, the HPLC-BBCEAS measurements for anthracene and pyrene displayed LOD_{SM} values lower approximately 43 and 48 times than LOD_{SM} values obtained using the conventional Perkin-Elmer HPLC system. HPLC-BBCEAS measurements were also carried out on mixtures containing equal volumes of anthracene and pyrene at 335 nm and consisting of different concentrations. These results demonstrated the ability of the HPLC-BBCEAS system to separate and detect both analytes at concentration levels down to 0.01 mg/L (Fig. 8). Conversely, this sensitivity was not possible with the conventional Perkin-Elmer HPLC instrument. Even at 0.1 mg/L concentration level, the

Table 2
Summary of results of the individual Perkin-Elmer HPLC measurements for anthracene and pyrene.

Analyte	(λ) [nm]	ΔABS	α_{min} [cm^{-1}]	LOD_{LRM} [M]	LOD_{SM} [M]	ε_{eff} [M^{-1} cm^{-1}]
Anthracene	338	1.1×10^{-4}	2.5×10^{-4}	3.5×10^{-5}	7.8×10^{-7}	4.2×10^{2}
Pyrene	333	7.5×10^{-5}	1.7×10^{-4}	7.1×10^{-7}	8.2×10^{-8}	2.7×10^{3}

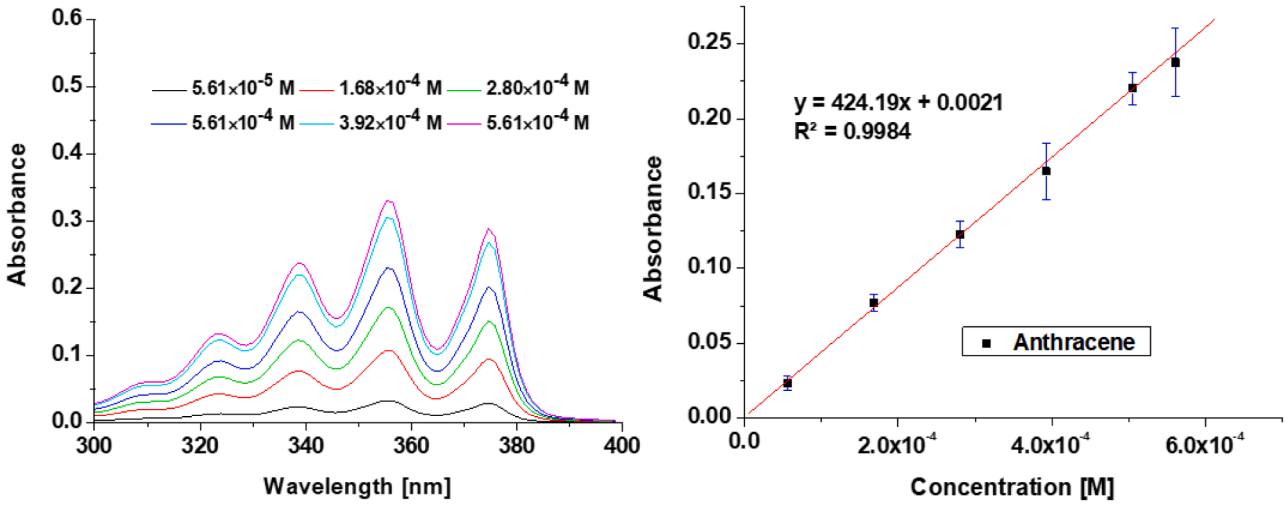

Fig. 4. Representative absorbance spectra and calibration plot for anthracene obtained using the conventional Perkin-Elmer HPLC instrument.

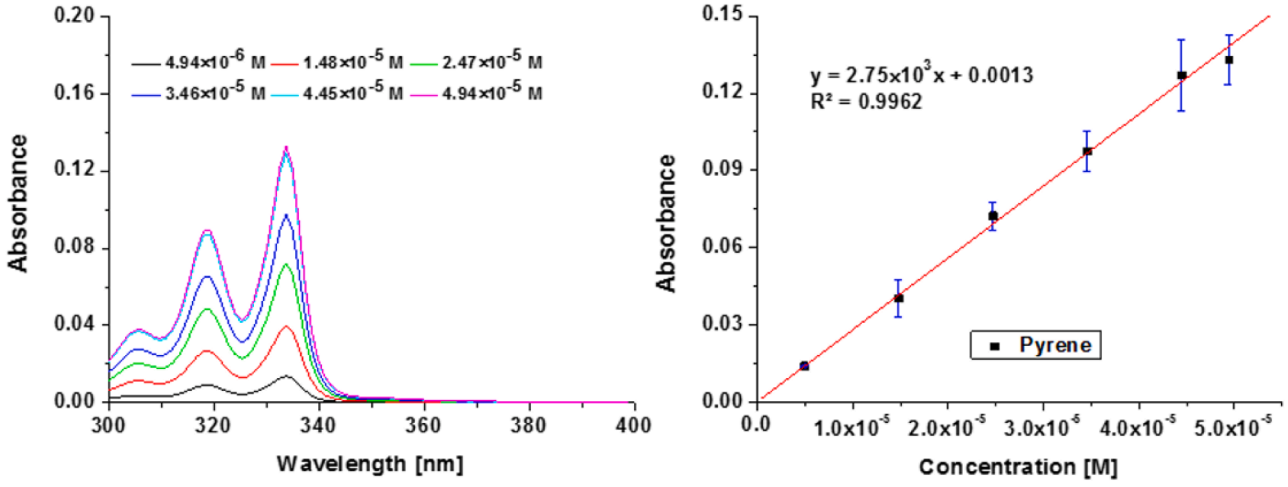

Fig. 5. Representative absorbance spectra and calibration plot for pyrene obtained using the conventional Perkin-Elmer HPLC instrument.

Table 3
Summary of results of the individual HPLC-BBCEAS measurements at 338 and 356 nm for anthracene and at 333 nm for pyrene with R ≥ 0.99 mirror set.

Analyte	CEF	$\Delta ABS_{min}(t)$	$\alpha_{min(t)}$ [cm^{-1} $Hz^{-½}$]	LOD_{LRM} [M]	LOD_{SM} [M]	ε_{eff} [M^{-1} cm^{-1}]
Anthracene λ_{338} nm	29.5 ±0.1	1.2×10^{-4}	9.8×10^{-6}	4.0×10^{-7}	1.8×10^{-8}	4.6×10^{4}
Anthracene λ_{356} nm	42.3 ±0.1	1.2×10^{-4}	6.8×10^{-6}	3.3×10^{-7}	1.3×10^{-8}	6.7×10^{4}
Pyrene λ_{333} nm	30 ±0.1	1.2×10^{-4}	9.6×10^{-6}	4.5×10^{-8}	1.7×10^{-9}	5×10^{5}

instrument displayed the statement "no peak can be defined". These outcomes suggest that BBCEAS can be used as a highly sensitive, simple and low-cost detector for HPLC applications in the near-UV region between 300 and 400 nm.

In this study we also demonstrate the first application of the BBCEAS approach as a detection system for HPLC measurements at deep-UV wavelengths of less than 300 nm. HPLC-BBCEAS measurements in a 1 cm HPLC flow cuvette for vitamin D2 and vitamin D3 dissolved in methanol have been carried out individually and then in a partial mixture at 282 nm. This discussion considers some of the Fig.s of merit obtained from this work and compares them with the results achieved using the conventional HPLC system, and with the previous HPLC-CRDS and HPLC-BBCEAS studies.

Vitamin D2 and vitamin D3 were determined to have effective absorption coefficients of 1.0 L g^{-1} cm^{-1} and 0.83 L g^{-1} cm^{-1} at 282 nm. These absorption coefficient values were used in calculation of the CEF values for both vitamins. Table 5) lists CEF values of 11.5±0.3 and 14.6 ±0.7 for vitamin D2 and vitamin D3, respectively, with the LDLS light source; in addition to CEF values of 7.6±0.3 and 10.0±0.3 for the analytes with the LED light source. The CEF values are much lower than the near-UV measurements as the reflectivity of the mirror set used (~0.96) is much lower than the ~0.99 reflectivity near-UV mirrors. In addition, there is greater scattering and absorption of light by the cuvette and solvent in the deep-UV region. The LED light source is more difficult to collimate and align through the HPLC flow cell than the LDLS which probably explains why fewer passes are obtained compared to the LDLS.

The sensitivity ($\alpha_{min(t)}$) of all measurements in this study are shown in Table 5). The LDLS measurements produced values of 5.1 × 10^{-4} cm^{-1} and 4.0 × 10^{-4} cm^{-1} for vitamin D2 and vitamin D3, respectively, whilst the LED measurements produced values of 7.7 × 10^{-5} cm^{-1} $Hz^{-½}$ and 5.8

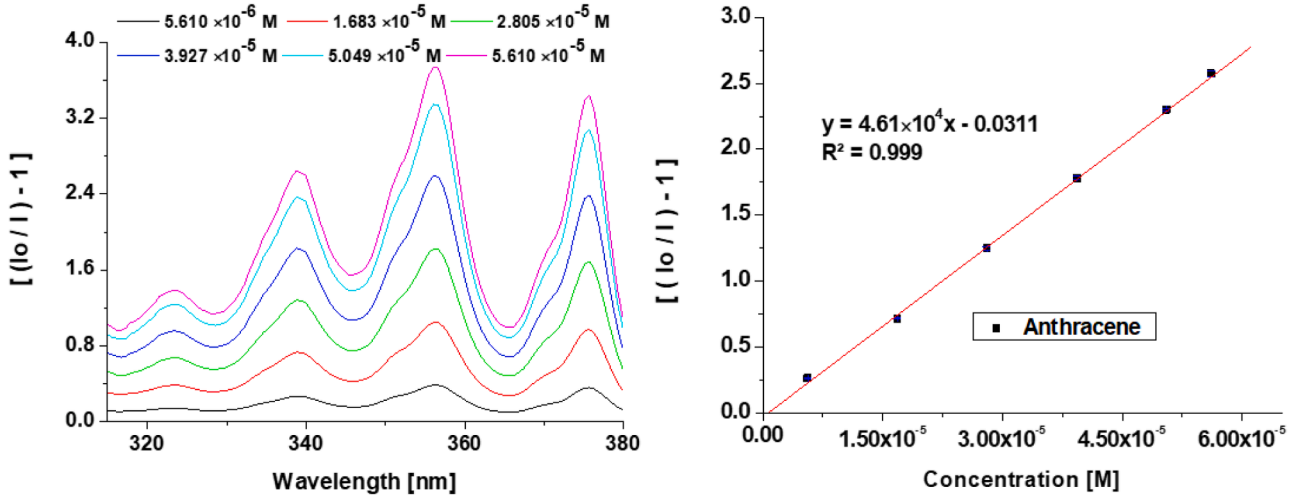

Fig. 6. Representative absorption spectra and calibration plot for anthracene at 338 nm obtained using the HPLC-BBCEAS system at a concentration range 5.6 – 56 μM with the LDLS light source, 1 cm HPLC flow cuvette, R ≥ 0.99 mirror set and the Avantes detector.

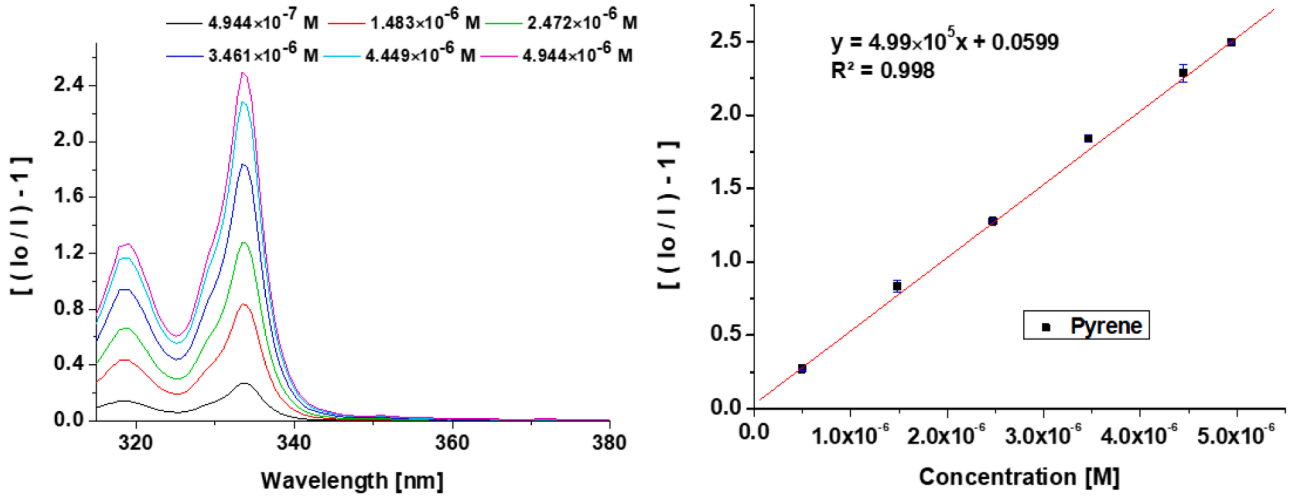

Fig. 7. Representative absorption spectra and calibration plot for pyrene at 333 nm obtained using the HPLC-BBCEAS system at a concentration range 0.49 – 4.9 μM with the LDLS light source, 1 cm HPLC flow cuvette, R ≥ 0.99 mirror set and the Avantes detector.

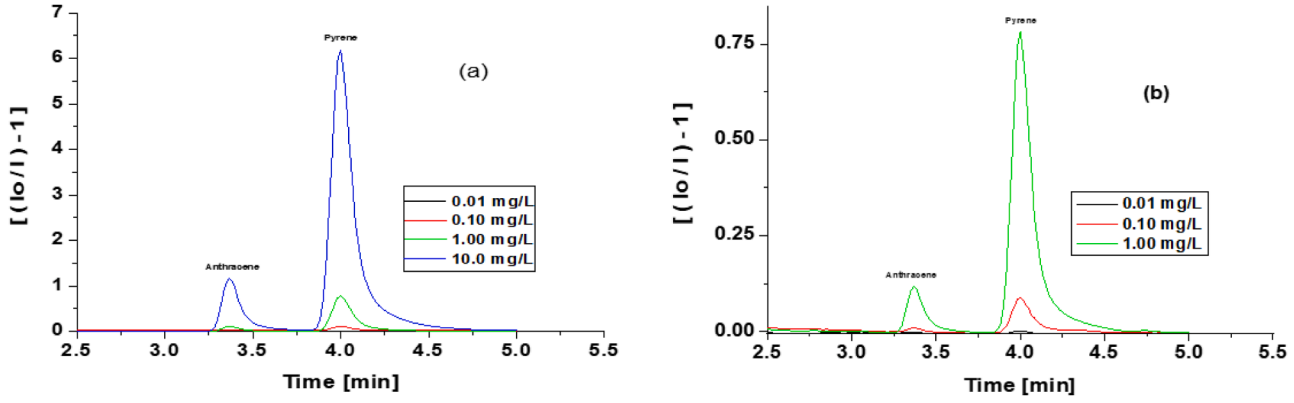

Fig. 8. Chromatograms obtained at 335 nm using the HPLC-BBCEAS system for a mixture of anthracene and pyrene at different concentrations; (a) (0.01, 0.1, 1 and 10 mg/L) and (b) (0.01, 0.1 and 1 mg/L).

$\times 10^{-5}$ cm^{-1} $Hz^{-½}$ respectively. The difference in the values obtained with the two light sources is mainly due to the poor photon flux after the cavity at ~280 nm of the LDLS compared to the LED. This resulted in a much longer integration time being required for the LDLS measurements (250 ms against 4 ms for the LED) and thus the time normalised baseline noise $\Delta ABS_{min}(t)$ was approximately 10-fold worse. The CEF values

Table 4
Summary of results of the individual Perkin-Elmer HPLC measurements for vitamins D2 and D3 at 281.7 nm.

Analyte	Δ ABS	α_{min} [cm^{-1}]	LOD_{LRM} [mg/L]	LOD_{SM} [mg/L]	$E_{ff}(\varepsilon)$ [L g^{-1} cm^{-1}]
Vitamin D2 $(\lambda)_{282}$ nm	7.9×10^{-5}	1.8×10^{-4}	3.7	0.27	8.6×10^{-7}
Vitamin D3 λ_{282} nm	6.5×10^{-5}	1.5×10^{-4}	3.3	0.26	7.4×10^{-7}

Table 5
Summary of results of the individual HPLC-BBCEAS measurements for vitamin D2 and vitamin D3 with the R ~ 0.96 mirror set at 282 nm using different light sources.

Analyte	CEF	$\Delta ABS_{min}(t)$	$\alpha_{min}(t)$ [cm^{-1} $Hz^{-½}$]	LOD_{LRM} [mg/L]	LOD_{SM} [mg/L]	$E_{ff}(\varepsilon)$ [L g^{-1} cm^{-1}]
Vitamin D2 (LDLS)	11.5 ±0.3	2.5×10^{-3}	5.1×10^{-4}	0.70	0.64	2.7×10^{-5}
Vitamin D3 (LDLS)	14.6 ±0.7	2.5×10^{-3}	4.0×10^{-4}	0.85	0.63	2.8×10^{-5}
Vitamin D2 (LED)	7.6 ±0.3	2.5×10^{-4}	7.7×10^{-5}	0.64	0.09	1.7×10^{-5}
Vitamin D3 (LED)	10.0 ±0.3	2.5×10^{-4}	5.8×10^{-5}	0.47	0.09	1.9×10^{-5}

were worse for LED measurements, and these slightly reduced the gap between the sensitivity of the LDLS and LED measurements. (Table 6)

When compared to the measurements using the conventional Perkin-Elmer HPLC system (Table 4), the $\alpha_{min}(t)$ values obtained using the LDLS were approximately 3 times worse even though a CEF of between 11.5 ±0.3 and 14.6±0.7 was obtained. This again can be attributed to the low photon flux after the cavity at ~280 nm of the LDLS leading to a ~40-fold poorer $\Delta ABS_{min}(t)$ compared to the conventional Perkin-Elmer HPLC system. So that even a CEF of ~11 - 15 is not sufficient to improve the sensitivity over the conventional system. By contrast, the measurements with the LED which had a much higher photon flux at ~280 nm allowed a much shorter integration time of 4 ms to be used leading to a baseline noise which was much closer to that of the conventional HPLC system and consequently $\alpha_{min}(t)$ values which were ~3 times more sensitive. Quantitatively, the LDLS-based BBCEAS baseline noise was 2.5×10^{-3}, compared with 7.9×10^{-5} and 6.5×10^{-5} for the conventional detector, whereas the LED-based BBCEAS baseline noise was 2.5×10^{-4}, approximately 3–4 times higher than the conventional detector but still sufficient to provide improved αmin values because of cavity enhancement. When compared with previous cavity enhanced HPLC studies only the HPLC-CRDS study reported by van der Sneppen et al. [12] was made at a deep-UV wavelength of 273 nm. They obtained an α_{min} value of 1.0×10^{-3} cm^{-1}. By comparison the LDLS measurements in this study are ~2 times more sensitive whilst the LED measurements are up to 17 times more sensitive whilst using a simpler and cheaper experimental setup.

The LOD values for both vitamins were calculated by the linear regression method and by the spectral method. The results obtained using the HPLC-BBCEAS technique with the LDLS light source surprisingly showed approximately similar LOD_{SM} values for both analytes compared to the corresponding LOD_{LRM} values probably because the baseline noise was high for the LDLS measurements. The LDLS LODs were also higher than those obtained with the conventional HPLC system. By contrast, the LOD_{SM} values for vitamin D2 and vitamin D3 achieved using the HPLC-BBCEAS setup with the LED light source were 7 and 5 times, respectively, smaller than their corresponding LOD_{LRM} values obtained by the linear regression method. Moreover, they were also ~ 3 times lower than the LOD_{SM} values for both vitamins obtained using the conventional Perkin-Elmer HPLC instrument. HPLC-BBCEAS measurements were also carried out on mixtures containing equal volumes of vitamin D2 and vitamin D3 at 282 nm and using three different concentrations: 1, 10 and 100 mg/L. The results showed the ability of the HPLC-BBCEAS system to separate both vitamins in sample mixtures down to 1 mg/L concentration levels as shown in Figs. 13) and 14). This separation was also possible to conduct using the conventional Perkin-Elmer HPLC instrument. The results also showed that the chromatogram obtained using the HPLC-BBCEAS setup with the LDLS, was much noisier than the corresponding chromatogram achieved using the HPLC-BBCEAS setup with the LED light source due to poorer sensitivity.

5. Conclusion and future work

This study has demonstrated the first application of HPLC-BBCEAS detection system for measurements at UV wavelengths. Its reports the application of the HPLC-BBCEAS technique at near-UV wavelengths between 300 – 400 nm for the first time, using a simple and low-cost setup with a LDLS as the light source. The results showed improved sensitivity compared to the previous CRDS and BBCEAS studies, and a significant improvement in sensitivity compared to measurements with a conventional Perkin-Elmer HPLC instrument. In addition, this study

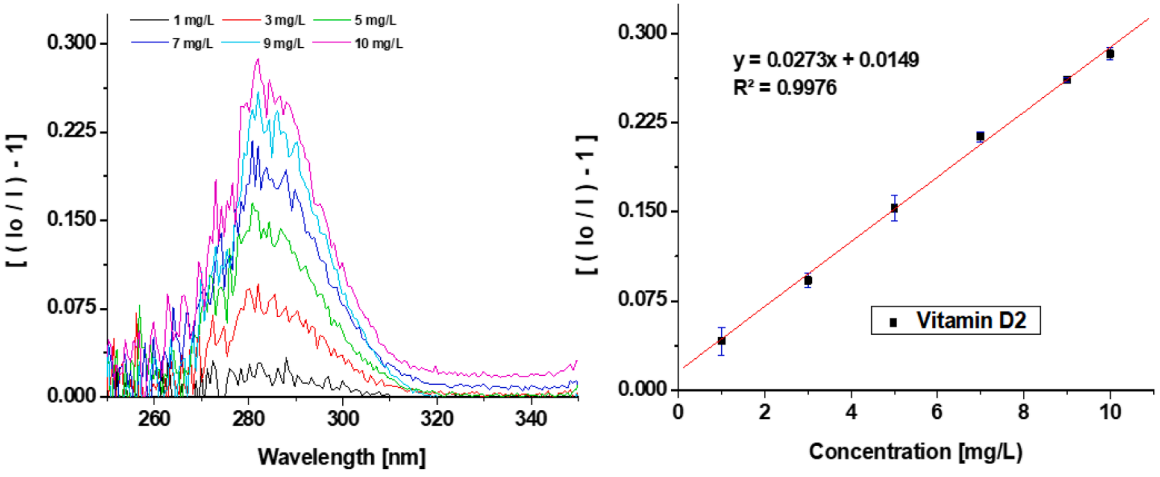

Fig. 9. Representative absorption spectra and linear calibration plot for vitamin D2 recorded using the HPLC-BBCEAS technique coupled with the LDLS light source at 282 nm.

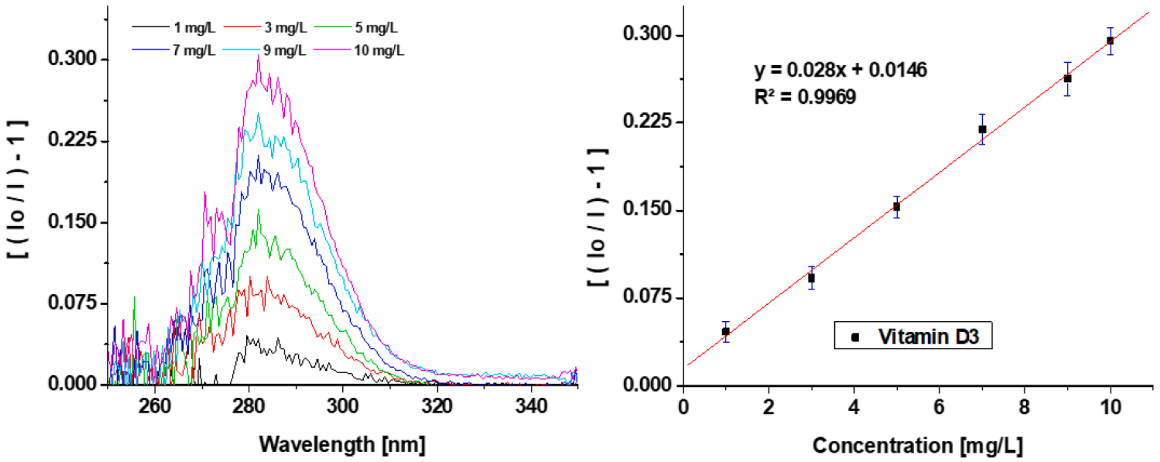

Fig. 10. Representative absorption spectra and linear calibration plot for vitamin D3 recorded using the HPLC-BBCEAS technique coupled with the LDLS light source at 282 nm.

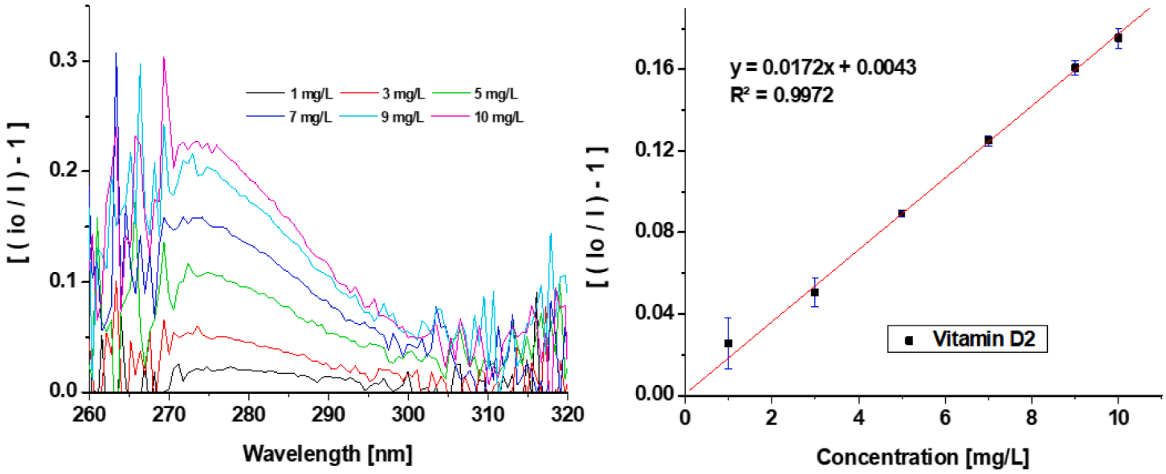

Fig. 11. The representative absorption spectra and the linear calibration plot for vitamin D2 recorded using the HPLC-BBCEAS technique coupled with the LED light source at 282 nm.

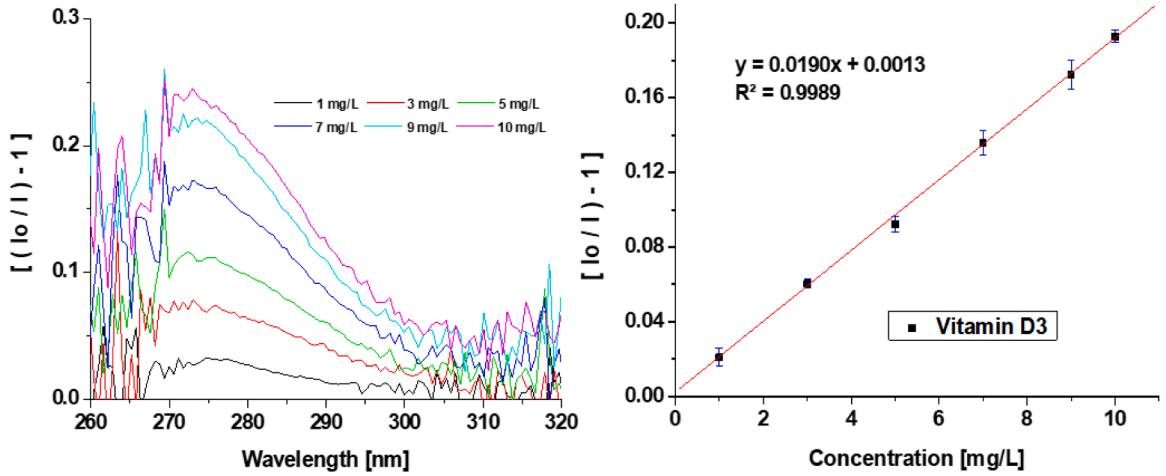

Fig. 12. The representative absorption spectra and the linear calibration plot for vitamin D3 recorded using the HPLC-BBCEAS technique coupled with the LED light source at 282 nm.

also shows that it is possible to use BBCEAS as a detection system for HPLC measurements in the deep-UV region with high sensitivity, simplicity and low-cost. For future work, improvements could be obtained by fibre optic coupling of the light source to minimise alignment and collimation losses for near-UV measurements and carry out the measurements at the maximum reflectivity of the mirrors. For the deep-

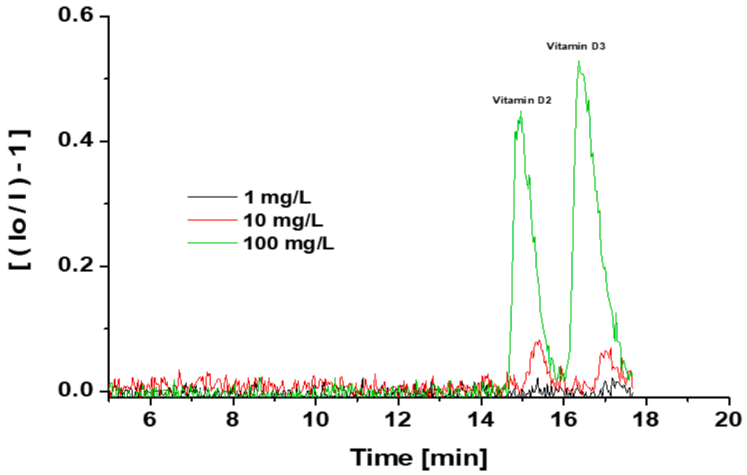

Fig. 13. The chromatogram obtained using the HPLC-BBCEAS technique coupled with the LDLS light source for a mixture of vitamin D2 and vitamin D3 at different concentrations (1, 10 and 100 mg/L).

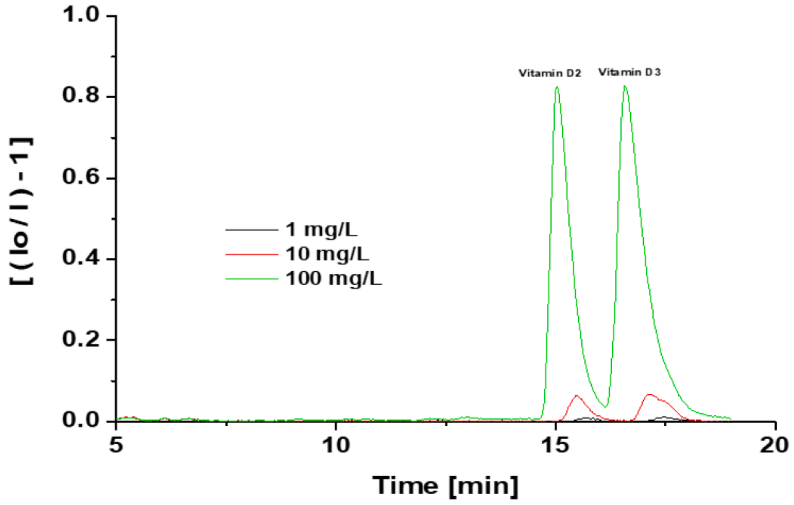

Fig. 14. The chromatogram obtained using the HPLC-BBCEAS technique coupled with the LED light source for a mixture of vitamin D2 and vitamin D3 at different concentrations (1, 10 and 100 mg/L.

Table 6
Comparison between this study and some of previous HPLC studies used optical cavity-based techniques as a detector.

The study	Technique	R^2	λ [nm]	α_{min} [cm^{-1}]	Fold Reduction in LOD Compared with Conventional Detection (×)
This study	BBCEAS	0.99	300-400 Below 300	6.8×10^{-6}	Anthracene (43) pyrene (48) Vit D2 (7) Vit D3 (5)
Seetohul et al. [43]	BBCEAS	0.99	450-600	1.9×10^{-5}	Rhodamine 6G (54) Rhodamine B (77
van der Sneppen et al. [81]	CRDS	0.9995 0.9991	355 273	3.0×10^{-5} 1.0×10^{-3}	dyes and nitro-polyaromatic hydrocarbons (nitro-PAHs), (30)

LOD values for anthracene and pyrene from this work were converted from mol L^{-1} to mg L^{-1} using molecular weights of 178.23 g mol^{-1} for anthracene and 202.25 g mol^{-1} for pyrene. LRM = linear regression method; SM = spectral method; LDLS = laser-driven light source; LED = light-emitting diode; N.R. = not reported.

UV region, further improvements in sensitivity could be obtained by coupling more powerful deep-UV light sources with higher reflectivity (R ≥ 0.99%) deep-UV mirrors and a more sensitive spectrometer.

CRediT authorship contribution statement

Mostafa Almdaaf: Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Naser Bazina:** Writing – review & editing, Visualization, Validation, Methodology. **Tariq Ahmed:** Writing – review & editing, Software. **Mosharraf Sarker:** Supervision, Software, Resources, Methodology, Investigation. **Meez Islam:** Visualization, Validation, Supervision, Software, Resources, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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