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Method development and optimization for dispersive liquid–liquid microextraction factors using the response surface methodology with desirability function for the ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry determination of organic contaminants in water samples: risk and greenness assessment†

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A simple, cost effective, and efficient dispersive liquid–liquid microextraction method was developed and optimized for the determination of organic contaminants in different environmental water matrices followed by UHPLC-QTOF-MS analysis. In the preliminary experiments, the univariate optimization approach was used to select tetrachloroethylene and acetonitrile as extraction and disperser solvents, respectively. The significant factors influencing DLLME were screened using full factorial design, and the optimal values for each variable were then derived through further optimization using central composite design with desirability function. The optimal conditions were achieved with 195 µL of tetrachloroethylene as the extraction solvent, 1439 µL of acetonitrile as the disperser solvent, and a sample pH of 5.8. Under these conditions, the method provided detection limits ranging from 0.11–0.48 µg L^{-1} and recoveries ranging from 23.32–145.43% across all samples. The enrichment factors obtained ranged from 11.66–72.72. The proposed method was then successfully applied in real water samples. Only benzophenone was detected in the concentration range of 0.79–0.88 µg L^{-1} across all the water samples. The calculated risk quotient resulting from benzophenone exposure in water samples showed a low potential risk to human health and the aquatic ecosystem. The method was also evaluated for its environmental friendliness using various metrics tools such as Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE), Analytical Greenness for Sample Preparation (AGREEprep), and Sample Preparation Metric of Sustainability (SPMS). Only AES qualified the method as green while it was considered acceptable and sustainable when assessed using SPMS.

1. Introduction

Pollution of the environment by toxic organic compounds, especially those emanating from treated and untreated wastewater, has increased dramatically in recent years.[1] The wastewaters are known to harbour numerous classes of pharmaceuticals and personal care products (PPCPs) which are a diverse group of chemicals that form part of the emerging organic micropollutants. These pharmaceuticals and PPCPs are widely used in medicines and the cosmetic product industry, released in large quantities to the environment and have thus drawn the attention of global environmental researchers in recent years.[2,3] The suspected toxicity effects of these compounds (PPCPs) are well documented and hence continue to be a cause of major concern for public health prompting regulatory agencies to devise stringent guidelines for their occurrence in aquatic environments.[4,5] As a result, according to the stringency of the guidelines, highly sensitive and selective methods are required all the way from isolation/extraction and preconcentration, to the determination of the trace levels of these compounds in environmental water samples. PPCPs
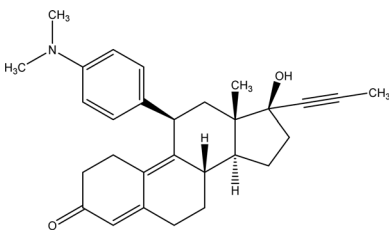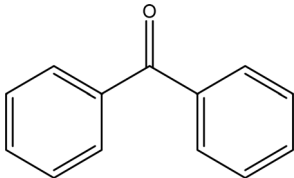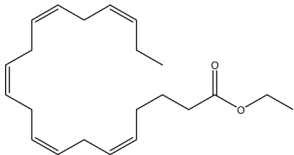
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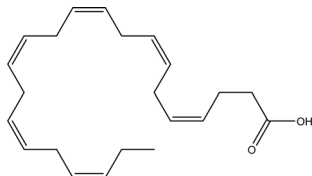*bInstitute for Nanotechnology and Water Sustainability, College of Science, Engineering and Technology, University of South Africa, Johannesburg, Florida, 1709, South Africa. E-mail: msagatam@unisa.ac.za; Tel: +27 11 670 9482*

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Table 1 Chemical structure and physicochemical properties of the analytes

Compound	Chemical structure	Therapeutic class	pK_a	$\log K_{ow}$	Reference
Mifepristone (MIF)		Pharmaceutical	5.23	5.4	27 and 28
Benzophenone (BP)		Personal care product	—	3.18	29 and 30
Ecosapentaenoic acid (EPA)		Pharmaceutical	4.82	7.85	31
Docosahexaenoic acid (DHA)		Pharmaceutical	4.89	8.62	31

cannot be directly analysed with conventional methods such as HPLC, LC-MS or GC-MS due to their low concentrations in environmental samples due to high dilution effects (ng L^{-1} up to μg L^{-1} range) and due to the complexity of the matrices such as wastewater and sediments in which they are found.[6] Thus, highly sensitive and selective extraction and preconcentration steps are required prior to the final instrumental analysis and determination. Recently, there has been a strong interest in developing sensitive, selective, fast, and low-cost sample preparation methods for organic contaminants in environmental samples. For example, in 2006 Rezaee and co-workers first introduced dispersive liquid–liquid microextraction (DLLME), a popular sample preparation technique using microliter volumes of an extraction solvent.[7] DLLME has generated a lot of interest as a green method in analytical chemistry since it was first introduced because it uses less organic extraction solvents than preconcentration techniques such as SPE and LLE, among others, and it is also less time consuming.[8] The DLLME method is based on a ternary component solvent system, in which an aqueous sample is rapidly injected with a mixture of the extraction solvent and the disperser solvent, leading to the formation of a cloudy solution. The contact area between the extraction solvent and the aqueous phase is essentially infinitely large due to the formation of the cloudy solution, and as a result the equilibrium state is rapidly reached, thus accelerating the extraction process.[7] DLLME has been used successfully to

isolate and preconcentrate a wide range of organic analytes due to its high sample throughput, rapidness, and steady sample enrichment factors.[9–12] Because sample preparation can affect important parameters including the method detection limits, precision, accuracy, linearity, and recoveries, it is thus imperative that, prior to the instrumental analysis, optimization is performed to ensure good performance in the development of analytical procedures.[13–15]

Generally, conventional techniques for optimizing a multivariable system follow the OFAT approach. However, the OFAT approach requires numerous experiments and does not represent the combined effects.[16,17] It also requires more data to determine the optimum level, which takes a long time and is unreliable.[18] Many statistical experimental design methods have recently been used in analytical chemistry to optimize process parameters.[19,20] The primary goal of the experimental design techniques is to understand the interactions among the parameters, which can aid in the optimization of experimental parameters and the generation of statistical models.[21] In this context, the multivariate design of experiments (DoE) is an important issue because it requires less time, effort, and resources than univariate procedures, and it allows for the collection of large quantities of information while reducing the number of experiments.[22] On the other hand, DoE and the response surface methodology (RSM) have been shown and proved to be useful in the development, improvement, and

optimization of processes.[23] The RSM technique, when compared to other traditional methods has an edge as it can model and optimize complex processes in analytical applications in the industrial sector, and bioprocesses, because it allows for more efficient and easier experimental setup and interpretation.[24] In addition, it is less tedious and time consuming than other procedures for extraction,[25] and it is commonly used in optimizing DLLME of several organic pollutants in environmental samples due to these benefits.[26] However, there has only been very little research done on DLLME-based statistical optimization process conditions for the trace analysis of multiclass analytes in a single extraction. Organic contaminants are expected to have different chemical properties because they belong to different chemical classes with different chemistries, heteroatoms, and functional groups. These differences may result in numerous complex interactions that preclude a direct evaluation of the extraction process.

In this study, DLLME was used to extract and preconcentrate the selected organic contaminants from water samples, which were then detected using ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). The list of analytes monitored in this study is given in Table 1. Using full factorial design (FD) and central composite design (CCD) as DoE tools, several important extraction parameters including extraction and disperser solvent type, volumes of extraction and disperser solvents, pH, and ionic strength were either statistically optimized or optimized as a single variable. Following this, the established DLLME method was then validated and applied to real-world environmental water samples. To our knowledge, this is the first application of DLLME coupled to UHPLC-QTOF-MS for the analysis of the targeted compounds in environmental water samples. In addition, the proposed method's green chemistry was assessed by using various green metrics tools such as Analytical Eco-Scale (AES), the Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE), the Analytical Greenness for Sample Preparation (AGREEprep) approach, and Sample Preparation Metric of Sustainability (SPMS). The potential risks to human health and the environment posed by the presence of the analytes in water samples were also estimated.

2. Experimental

2.1 Chemicals and materials

Reference standards (benzophenone; BP, mifepristone; MIF, docosahexaenoic acid; DHA, eicosapentaenoic acid; EPA) of analytical reagent grade or higher were purchased from Merck (Johannesburg, South Africa) and used without further purification. LC-MS grade solvents such as acetonitrile (ACN), methanol (MeOH), and formic acid (FA) were purchased from Merck (Johannesburg, South Africa). Other solvents purchased form Merck included chloroform, tetrahydrofuran, methanol, acetone, acetonitrile, chloroform, tetrachloroethylene, 1,2-dichloroethane, chlorobenzene, sodium hydroxide, and sodium chloride. Surrogate/internal standards (IS): mifepristone-d3, benzophenone-d10, and lipoic acid-d5 were purchased from LGC Industrial analytical (Johannesburg, South Africa). Deuterated standards were added to the samples before or after extraction and they were also used for the quantification of the samples.

Methanol was used to prepare stock standard solutions (1 mg mL^{-1}) of each target compound. Individual working standard solutions and mixture standards were prepared by appropriate dilutions of the stock solutions and volume adjustment with methanol. The solutions were all kept at $-22\ °$C until ready for use. The procedural blank and method optimization were conducted using Milli-Q water (18.2 MΩ cm^{-1}) obtained from an Integral 10 Elix Milli-Q system (Massachusetts, USA). Validation was performed using matrix blanks prepared by diluting real samples (influent, effluent, and river water) with Milli-Q water. Glass microfibers GF/F filters (0.45 μm or 0.22 μm) were supplied by Merck South Africa. The pH was adjusted to the desired value using 0.1 M sodium hydroxide or HCl solution. The samples were prepared using a centrifuge (Thermo Electron Corporation, Massachusetts, USA) and vortex mixer (Velp, Scientifica, Italy). Nitrogen gas was purchased from Afrox South Africa.

2.2 Sample collection and preparation

Influent and effluent samples were collected from Northern Wastewater Treatment Works in Johannesburg, South Africa's most densely populated city in Gauteng province, downstream of the Jukskei River. The wastewater treatment plant discharges effluent into the Jukskei River, which is a tributary the Crocodile River. The Crocodile River flows into Hartbeespoort Dam, which serves as an irrigation and drinking water source for the Hartbeespoort area. The grab water samples were collected in glass bottles rinsed with ultrapure water and flushed several times with the samples prior to sampling. All samples were transported to the laboratory in an ice-filled cooler box. Water samples were filtered through 0.45 μm or 0.22 μm membrane filters to remove suspended particulate matter, adjusted to the appropriate pH, and stored in the dark at 4 °C until analysis.

It must be noted that DLLME factor optimization was performed in ultrapure water and applied in real water samples. The procedure for DLLME was carried out as follows: a 10 mL centrifuge capped conical bottom tube was filled with a 5 mL aliquot of the water sample (pH 5.8). Subsequently, using a 5 mL syringe, a mixture of 1439 μL of acetonitrile as a disperser solvent and 195 μL of tetrachloroethylene as an extraction solvent was rapidly injected into the sample solution, forming a cloudy solution. After this, the mixture was vortexed for 30 s and allowed to stand at room temperature for five minutes to enhance the extraction of target analytes into the tiny droplets of extraction solvent from the sample solution. Following a five minute centrifugation of the mixture at 4500 rpm, the finely dispersed tetrachloroethylene droplets sedimented at the bottom of the test tube were withdrawn using a 1000 microliter syringe and quantitatively transferred into a volume reducer (inset) in a 2 mL light-safe amber vial. A nitrogen gas stream was then used to evaporate the solvent. The residue was reconstituted in 100 μL of the mobile phase before being introduced into an UPLC-QTOF-MS instrument for analysis.

2.3 Instrumental analysis

A Dionex UHPLC system (Thermo Scientific, Bremen, Germany) equipped with a cooling autosampler, a thermostatically controlled column compartment, a binary solvent delivery system, and Analyst 1.6.2 software was used for chromatographic analysis. An Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) supplied by Waters (Ireland) was used to achieve separation, with the column temperature maintained at 35 °C. Before being used, the mobile phase, which consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was degassed for 15 minutes in an ultrasonic bath. The gradient program was as follows: 0–5 min, 2–75% B, 5–5.5 min, 75–80% B, 5.5–10 min, 80% B, followed by the equilibration of the system for an additional 3.9 min under the initial conditions. A flow rate of 0.35 mL min^{-1} was used. The autosampler was kept at 4 °C, with a 5 μL injection volume.

A Bruker Impact II Q-TOF tandem mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) was set to electrospray ionization in both positive (+) and negative modes (−). The following were the optimized operating parameters: the capillary voltage (Cap) was −4.5 kV for positive ion mode detection and +3.6 kV for negative ion mode detection. The following operating parameters were used for both positive and negative ion modes: drying gas (N$_2$) flow rate, 8.0 L min^{-1}; drying gas temperature, 220 °C; nebulizer, 1.8 bar; spray shield, −3.6 kV; collision energy, 7.0 eV. Nitrogen gas (Genius 1050) was used as the collision gas. Data were collected and analyzed using Compass 1.9 OtofControl version 4.0 (Bruker Daltonik GmbH). Mass spectra were recorded in the 50–1500 m/z range, with accurate mass measurement of all peaks. The MS instrument was calibrated using the sodium formate cluster. For each sample run, the calibrant was infused in front of the LC and was used for mass recalibration as well as calculating mass accuracy.

Quantification was performed using nwXIC (50 mDa window) of the molecular ion for each compound obtained in the TOF mode. Mifepristone and benzophenone were quantified using their deuterated surrogate/internal standards, mifepristone-d3 and benzophenone-d10, respectively, whereas lipoic acid-d5 was used as an internal standard for both docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The use of lipoic acid-d5 as the surrogate/internal standard for EPA and DHA is limited by the variability of their chemical structures and properties. However, its choice was influenced by the high cost and difficulty in obtaining deuterated DHA and EPA standards. All analytes were identified based on their mass to charge ratio (m/z), daughter ions of molecules, and retention times. Parameters of MS detection for the quantification of the target compounds are provided in the ESI (Table S1†). Because real matrix blanks were difficult to obtain for validation, water samples (influent, effluent, and river) were diluted with ultrapure water at an appropriate ratio prior to extraction and used as matrix blanks. In the prepared sample blanks, including procedural and solvent blanks, no analytes were quantitatively detected.

2.4 Analytical procedure validation

The method was validated, and the results were expressed in terms of linearity, limits of detection (LOD) and quantification (LOQ), precision and accuracy. The internal calibration approach was considered during quantification to account for potential matrix effects. Linearity was assessed for each target compound at concentration levels ranging from 1.95 to 2000 μg L^{-1} using reference standards prepared in pure solvent. The external calibration curves obtained were generated from narrow window extracted ion chromatograms (50 mDa) obtained in the TOFMS mode using linear regression analysis by plotting the relative peak area of each compound with respect to the internal standard. For each compound, the concentration range that gave a good fit ($r^2 \geq 0.9981$) was established. The corresponding deuterated compounds used as internal standards were added at a concentration of 100 μg L^{-1} to all the samples and standard solutions for the calibration curve before LC-MS analysis. Each calibration concentration level was injected three times.

The instrumental variation was expressed as the percentage of the relative standard deviation (% RSD) of three successive injections of a 100 μg L^{-1} standard mixture solution. The method's precision was evaluated by repeated intra-day analysis of real samples spiked at 100 μg L^{-1}, expressed as the percent RSD of the replicate measurements ($n = 3$). The instrumental detection limits (LOD) and instrumental quantification limits (LOQ) were calculated using 3SD/b and 10SD/b, respectively, where SD is the linear regression's residual standard deviation and b is the slope. Method detection limits (MDL$_{cal}$) and method quantification limits (MQL$_{cal}$) were determined according to ref. 32 as MDL$_{cal}$ = (IDL × 100)/(PE × CF), and MQL$_{cal}$ = (IQL × 100)/(PE × CF), where PE is the overall process efficiency. The concentration factor (CF) is the ratio of the initial volume of the sample (5000 μL) to the final reconstituted volume (100 μL). The enrichment factors (EFs) were determined as the ratios of target compound concentrations measured in the solvent phase to those in the standard aqueous solution.[7,33]

The percent matrix effect (% ME), the recovery (% RE) and the process efficiency (% PE) were determined according to ref. 34 at the same spike concentration level of 100 μg L^{-1}. Briefly, the determination of percentage recovery (as accuracy) involved comparing the peak area of a sample spiked before extraction to the peak area of a sample spiked after extraction. The % ME was evaluated by comparing the analyte peak area of the post-extracted sample matrices to the peak of the analyte prepared in the pure solvent. The process efficiency, also known as the apparent recovery, was calculated by comparing peak area of the standard spiked in the sample before extraction to the peak area of a standard in pure solvent. The process efficiency was used to calculate the MDL and MQL values.

2.5 Strategy for optimization, statistical tools, and analysis

The main advantage of the design of experiments (DoE) over the univariate approach is that it requires fewer experimental measurements to achieve higher precision. The optimization of the DLLME procedure was performed in two different steps in

this case. First, four factors including the kind and the volume of the extraction and the disperser solvent, pH and the ionic strength were selected as potentially influencing the extraction efficiency. As a result, for the screening study, a full factorial design was used to determine the relative influence of the factors and their interaction. Following this, only variables found to be significant were investigated further and optimized using the response surface methodology. The SAS version 1.4 statistical software package was used for the experimental design and data analysis.

3. Results and discussion

3.1 Selection and optimization of disperser and extraction solvents by a univariate approach

Prior to multivariate optimization of the DLLME method, the disperser and extraction solvents were selected and optimized using a standard one-factor-at-a-time optimization procedure. The selection of an appropriate disperser solvent is critical for an efficient extraction method. Miscibility in both the sample and the extraction solvent is a key factor in selecting the disperser solvent. In a series of experiments, tetrahydrofuran, methanol, acetone, and acetonitrile were tested in aqueous solution by rapidly injecting 750 μL of each disperser solvent containing 200 μL of each extracting solvent, chloroform, tetrachloroethylene, 1,2-dichloroethane, or chlorobenzene. When acetonitrile was used as the dispersive solvent, the extraction efficiency for most of the studied analytes, particularly benzophenone, was higher than that when other solvents were used (Fig. 1). Therefore, it was chosen as a disperser solvent for this study. Some important requirements must be met when selecting an extraction solvent, such as high density compared to water, low solubility, and volatility in water to enhance adequate separation of the analytes from the matrix, and good chromatographic behaviour.[7,35] Among the solvents with such qualifying physicochemical properties were chlorobenzene, chloroform, 1,2-dichloroethane, and tetrachloroethylene which were tested in a combination of 200 μL of each of the extraction solvents with 750 μL of each disperser solvent. The best extraction efficiency was obtained when tetrachloroethylene
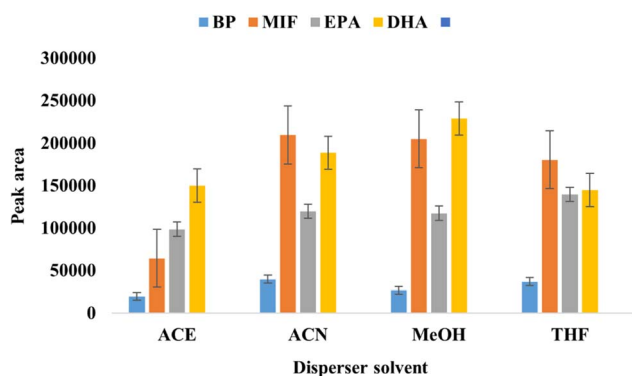

Fig. 1 The effect of different disperser solvents on the analyte's extraction efficiency ($n = 3$). Conditions for extraction: 5 mL of sample volume; 750 μL disperser solvent; 200 μL extraction solvent.
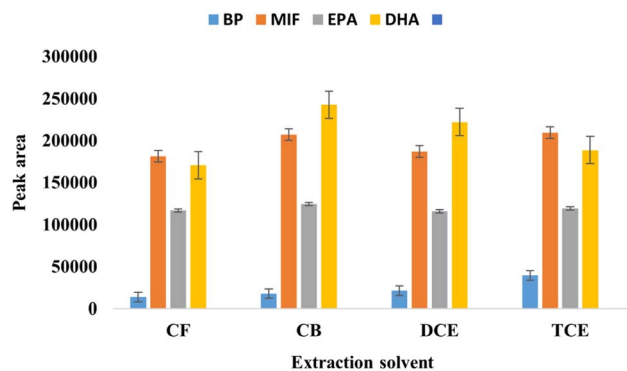

Fig. 2 The effect of extraction solvent type on analyte extraction efficiency ($n = 3$). Extraction conditions: Sample volume, 5 mL; disperser solvent, 750 μL; extraction, 200 μL.

was used as the extraction solvent (Fig. 2). Therefore, it was selected as the extraction solvent in the subsequent experiments.

3.2 Experimental screening by full factorial design

The most common first order designs are two level factorial designs, which are primarily used for screening factors, reducing the list of parameters to those that significantly influence the objective function, and modelling and refining the initial steps of an optimization process.[20,24,36,37] Following the selection of the extraction and disperser solvents, a full factorial design with 16 experiments was used for the screening study, in which the relative influence of the factors that might affect the extraction efficiency namely, volume of the disperser (X_1) and the extraction solvent (X_2), pH (X_3), and the ionic strength (X_4), and their interactions was established. The screening experiments were tested with each analyte of interest in a mixture at a spiked concentration of 10 μg L^{-1}. The order in which the experiments were performed was randomized to reduce the impact of uncontrolled variables. Table 2 lists the experimental factors, their levels, the design matrix, and the order of the experiments. The sum of peak areas obtained during LC-QTOF-MS analysis was used as the experimental response to evaluate the extraction efficiency under different experimental conditions. Analysis of variance (ANOVA) was used to assess the experiment results and determine the main effects at a 5% significance level or with 95% probability. A significant effect on the response is indicated by an F-probability ≤ 0.05, while a relative effect is indicated by $0.05 < F$-probability ≤ 0.10.[11] As shown in Table 3, the most significant factor affecting the extraction efficiency was pH (X_3) for all the analytes except for benzophenone. Other factors such as the volumes of the disperser solvent (X_1) and extraction solvents (X_2) and ionic strength had no effect on the analytes' responses. The ionic strength had no statistical impact on overall extraction efficiency, most likely due to a low salting-out effect. As a result, it was not included in any further optimization.

Due to the results obtained with the screened factors and the dependence of DLLME on the volumes of the extraction and disperser solvents, the next step involved optimising the

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Table 2 Factors, symbols, and levels corresponding to full factorial screening design

Factors	Symbol	Levels	
		Low (−)	High (+)
Volume of disperser solvent (µL)	X_1 (V_{dis})	500	1200
Volume of extraction solvent (µL)	X_2 (V_{ext})	100	250
pH value	X_3 (pH)	2	12

	X_1	X_2	X_3	X_4
1	−1(500)	−1(100)	−1(2)	−1(0)
2	+1(1200)	−1(100)	−1(2)	−1(0)
3	−1(500)	+1(250)	−1(2)	−1(0)
4	+1(1200)	+1(250)	−1(2)	−1(0)
5	−1(500)	−1(100)	+1(12)	−1(0)
6	+1(1200)	−1(100)	+1(12)	−1(0)
7	−1(500)	+1(250)	+1(12)	−1(0)
8	+1(1200)	+1(250)	+1(12)	−1(0)
9	−1(500)	−1(100)	−1(2)	+1(5)
10	+1(1200)	−1(100)	−1(2)	+1(5)
11	−1(500)	+1(250)	−1(2)	+1(5)
12	+1(1200)	+1(250)	−1(2)	+1(5)
13	−1(500)	−1(100)	+1(12)	+1(5)
14	+1(1200)	−1(100)	+1(12)	+1(5)
15	−1(500)	+1(250)	+1(12)	+1(5)
16	+1(1200)	+1(250)	+1(12)	+1(5)

Table 3 F-probability values obtained with full factorial design with significant variables highlighted in bold

	Coded levels		F-probability			
Factors	−1	+1	BP	MIF	DHA	EPA
X_1	500	1200	0.9352	0.4004	0.1668	0.5549
X_2	100	250	0.7327	0.8878	0.7040	0.6856
X_3	2	12	0.9620	**0.0083**	**0.0007**	**0.0010**
X_4	0	5	0.9088	0.9800	0.4535	0.1793

extraction process by subjecting these two factors together with the pH of the sample to the optimization process. Thus, a central composite design was used to determine the optimum conditions using the response surface methodology. This design is suitable for exploring the quadratic response surface and building a second-order polynomial model. A CCD is composed of three types of points: (a) full factorial or fractional factorial design; (b) an additional design, often axial points in which experimental points are located at a distance α from its centre; and (c) centre points, which are often used to calculate the experimental error.[6] A full design included twenty three (23) experiments, including nine (9) replicates at the centre point with a design matrix pattern 0000 (Table 4). The sum of peak areas was chosen as the response factor for the combination of the independent variables. The most important effects and the variable interactions were assessed by analysis of variance (ANOVA) in the same way as they were in full factorial design (FFD). The volume of the extraction solvent (X_2) and pH (X_3) in

Table 4 CCD investigated experimental factors in coded and actual form, and experimental responses

Run	Independent variable (coded form)			Independent variable (actual form)			Response (peak area)
	X_1	X_2	X_3	X_1 (µL)	X_2 (µL)	X_3	
1	−1	−1	−1	500	100	3	904 547
2	−1	−1	+1	500	100	6	2 130 727
3	−1	+1	−1	500	250	3	1 645 459
4	−1	+1	+1	500	250	6	1 415 807
5	+1	−1	−1	1200	100	3	781 274
6	+1	−1	+1	1200	100	6	1 887 765
7	+1	+1	−1	1200	250	3	1 096 779
8	+1	+1	+1	1200	250	6	2 656 029
9	−α	0	0	261.37	175	4.5	2 162 859
10	+α	0	0	1438.62	175	4.5	2 339 556
11	0	−α	0	850	48.86	4.5	583 937
12	0	+α	0	850	301.13	4.5	1 695 731
13	0	0	−α	850	175	1.977	686 284
14	0	0	+α	850	175	7.022	1 935 393
15	0	0	0	850	175	4.5	2 461 594
16	0	0	0	850	175	4.5	2 445 415
17	0	0	0	850	175	4.5	2 709 101
18	0	0	0	850	175	4.5	2 185 037
19	0	0	0	850	175	4.5	3 080 568
20	0	0	0	850	175	4.5	2 693 864
21	0	0	0	850	175	4.5	3 272 168
22	0	0	0	850	175	4.5	2 522 177
23	0	0	0	850	175	4.5	3 368 711

CCD had a significant influence on the extraction procedure (Table 5). Thus, a mathematical relationship between dependent and independent variables was determined using a response surface methodology by fitting the experimental data to a second-order polynomial equation. By simply removing terms that were not statistically significant ($p > 0.05$), the empirical model in terms of the coded factors was finally obtained as follows:

$$Y \text{ (response)} = 14.79 + 0.195x_2 + 0.309x_3 - 0.340x_2{}^2 - 0.288x_3{}^2$$

The obtained model demonstrated good fitness for quadratic interactions with a coefficient of determination ($R^2 = 0.8565$) which is consistent with the adjusted R^2 value of 0.8227. The adjusted $R^2 \geq 0.8227$ is satisfactory for model adequacy, indicating a good relationship between the experimental data and the fitted model. The lack of fit p-value of 0.2105 is not significant relative to pure error and confirms the validity of the model. This shows that the model is predictive, statistically significant ($p < 0.05$), and suitable for obtaining the RSM, and can be used to describe the effect of X_2 and X_3 on the response.

The SAS software also enabled the visualisation of three-dimensional prediction response surfaces, as well as predicted values and desirability function profiles. The response surface plots obtained by CCD in Fig. 3A and B show that there is a plateau in relation to the effect of disperser volume (X_1) on the extraction volume (X_2) and pH (X_3), indicating that varying

Table 5 ANOVA for CCD, with significant variables and interactions highlighted in bold

| Source	SS	DF	MS	F-value	Prob. >F	Remarks
Model	4.7837	4	1.1959	25.3642	<0.0001	Significant
X_1	0.0006	1	0.0006	0.0147	0.9055	Not significant
X_2	**0.5193**	**1**	**0.5193**	**13.4762**	**0.0032**	Significant
X_3	**1.3020**	**1**	**1.3020**	**33.7863**	**<0.0001**	Significant
X_1X_1	0.0417	1	0.0417	1.0817	0.3188	Not significant
X_1X_2	0.0301	1	0.0301	0.7822	0.3938	Not significant
X_1X_3	0.1405	1	0.1405	3.6461	0.0804	Not significant
X_2X_2	**1.7934**	**1**	**1.7934**	**46.5367**	**<0.0001**	Significant
X_2X_3	0.1262	1	0.1262	3.2754	0.0954	Not significant
X_3X_3	**1.2878**	**1**	**1.2878**	**33.4178**	**<0.0001**	Significant
Error	0.8016	17	0.0472			
Lack of fit	0.2749	4	0.0687	1.6968	0.2105	Not significant
Pure error	0.5266	13	0.0405			
Total	5.5853	21				

its volume levels has no effect on the system under study. In fact, an appropriate volume of disperser solvent is favourable to DLLME extraction because it allows for the formation of a more homogeneous cloudy solution, and consequently, the extracting solvent is dispersed more efficiently in the aqueous solution.[11] Fig. 3C shows the effect of the extraction volume and pH on the response. With a pH value of 5.8, increasing the extraction solvent volume from 80 μL to 175 μL increases the extraction efficiency. However, when extraction volume exceeded 175 μL, the response gradually decreased, whereas pH above 5.8 had no significant effect on the extraction efficiency. The optimum conditions for the simultaneous DLLME of the analytes were predicted by the desirability function (DF) option of the SAS

software (Fig. 4). The DF was maximised to achieve high extraction efficiency of the DLLME method. Based on this criterion, the optimum conditions were obtained with 1439 μL of disperser solvent (X_1), 175 μL of extraction solvent (X_2), and 5.8 for PH (X_3) value. The desirability function value obtained was 0.94.

3.3 Analytical performance

The developed DLLME method' characteristics were tested under the optimal conditions, with linearity, limits of detection (MDL) and quantification (MQL), precision and accuracy serving as validation criteria. Tables 6 and 7 present the validation data determined for each matrix studied. All the analytes demonstrated good linearity, with the coefficient of determination (R^2) ranging from 0.9981 to 0.9998. The intra-day precision of the instrument was investigated to ensure correct quantification by analysing three successive injections of a 100 μg L^{-1} standard. The RSD values obtained from run to run experiments for instrumental variations ranged from 2.07 to 14.68%, indicating a reasonable level of instrument precision. The intra-day repeatability of the method expressed as percent RSD for three replicates ranged from 0.65–14.12% (Table S3†). The method demonstrated good variability, with intra-day variations less than 20%, confirming that the method's precision was satisfactory. The IDLs injected on the column ranged from 0.02–0.09 ng.

The MDLs and MQLs of the developed method were calculated experimentally, as highlighted in Section 2.4. MDLs for river and wastewater (influent and effluent) ranged from 0.11–0.47 μg L^{-1} and from 0.10–0.48 μg L^{-1}, respectively. For both matrices, MQLs ranged from 0.34–3.64 μg L^{-1}. The results show
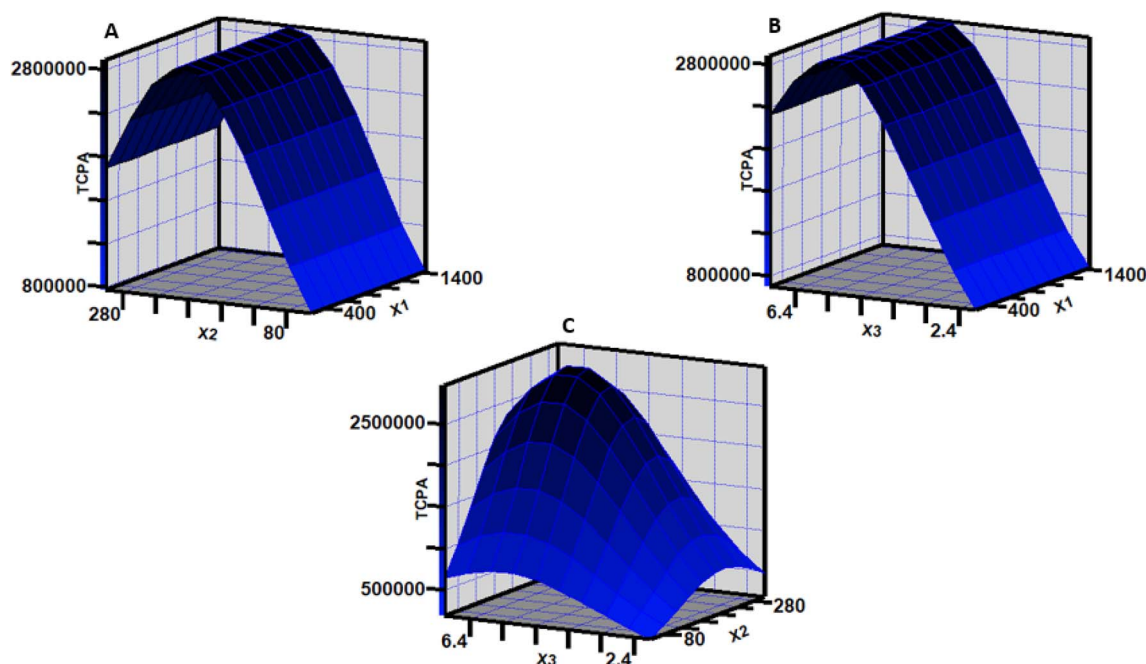
Fig. 3 Estimated response surface for simultaneous analyte optimisation using CCD by plotting (A) disperser (X_1) *vs.* extraction volume (X_2), (B) disperser volume (X_1) *vs.* pH (X_3), and (C) extraction volume (X_2) *vs.* pH (X_3).
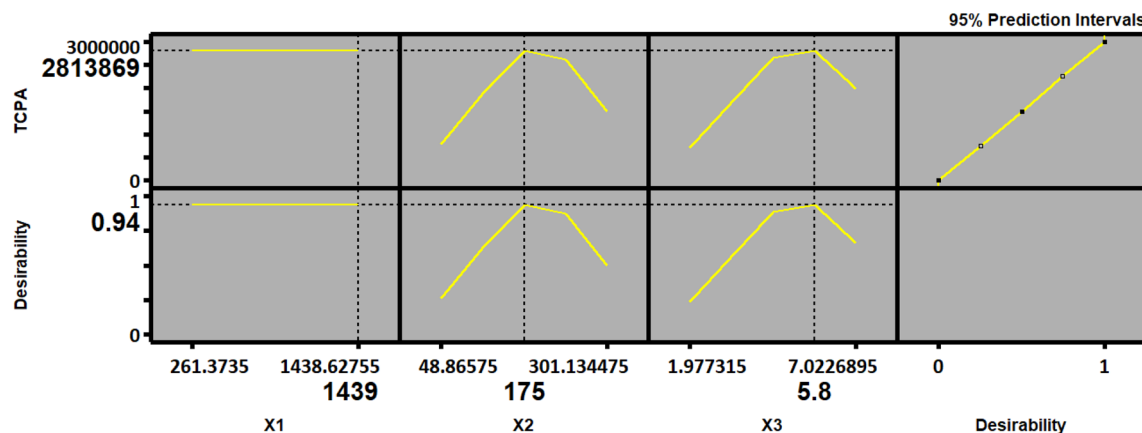
Fig. 4 Profiles for predicted values and the desirability function (DF) for total chromatography peak area (TCPA). The dashed line indicates optimized values highlighted in bold.

Table 6 Parameters and validation of the DLLME-UHPLC-MS/MS method for four organic contaminants

Compounds	Linearity (R^2)	Linearity range ($\mu g\ L^{-1}$)	IDL (ng injected)	MDL ($\mu g\ L^{-1}$) WWTP influent	WWTP effluent	River water	MQL ($\mu g\ L^{-1}$) WWTP influent	WWTP effluent	River water	Repeatability % RSD ($n = 3$)
MIF	0.9989	1.95–125	0.02	0.10	0.10	0.11	0.34	0.34	0.35	2.07
BP	0.9981	15.6–125	0.04	0.19	0.17	0.18	0.62	0.58	0.60	9.59
EPA	0.9998	31.25–500	0.05	0.19	0.48	0.22	0.62	1.60	0.73	4.83
DHA	0.9992	31.25–500	0.09	0.41	1.09	0.47	1.38	3.64	1.56	14.68

Table 7 Mean extraction recoveries for the targeted analytes

Compound	Influent ($n = 3$) % R ($\mu g\ L^{-1}$)	% RSD	Effluent ($n = 3$) % R ($\mu g\ L^{-1}$)	% RSD	River ($n = 3$) % R ($\mu g\ L^{-1}$)	% RSD
MIF	79.42	0.32	104.67	1.27	103.44	1.62
BP	145.43	6.98	119.33	10.85	94.56	5.52
DHA	122.81	13.21	23.32	19.87	66.29	7.86
EPA	64.84	12.01	31.14	9.86	85.05	6.27

that using the proposed methodology, trace amounts of the targeted analytes at parts per billion (ppb) levels could be extracted and determined in complex matrices such as influent and effluent water samples. Because no certified reference material was available, recoveries were evaluated in water samples spiked at a concentration of 100 μg L^{-1} to test the method's accuracy. The results for influent, effluent, and river samples ranged from 64.84–145.43%, 23.32–119.33%, and 66.29–103.44%, respectively. In effluent samples, DHA and EPA had reduced recovery rates of 23.32% and 31.14%, respectively. However, the low recoveries for these analytes were deemed acceptable and not considered as a barrier to their reliable determination, given that other performance data, including sensitivity and precision were satisfactory. Enrichment factors ranging from 11.66–72.72 (Table S3†) were achieved, demonstrating that the DLLME method has a considerable

concentration rate and significantly enhancing the method's detection sensitivity at the parts per billion (ppb) level.

3.4 Matrix effects

The limitations of quantitative LC-MS analysis are related to matrix effects caused by co-eluting residual matrix components, which impairs ionization efficiency, resulting in inaccurate quantification of the target analytes.[38] As a result, it was critical to investigate the matrix effect when developing and validating the method. The % ME was calculated by comparing the analyte peak area of the post-extracted sample matrices to the analyte prepared in the pure solvent at the same concentration levels.[34] A value of 100% indicates that there is no matrix effect, *i.e.*, the response in the pure solvent and in the extract is the same. A value of >100% indicates signal enhancement, while a value of <100% indicates signal suppression. Table 8 shows the results

Table 8 Matrix effects of the analytes in different water samples

Compound	% ME (influent)	% ME (effluent)	% ME (river)
Mifepristone	126.45 ± 0.05	98.14 ± 0.02	95.21 ± 0.04
Benzophenone	64.54 ± 0.04	84.89 ± 0.15	102.35 ± 1.03
Docosahexaenoic acid	78.14 ± 0.13	155.90 ± 0.13	128.44 ± 0.10
Eicosapentaenoic acid	155.20 ± 0.25	125.96 ± 0.12	101.01 ± 0.10

of MEs in extracts spiked at 100 μg L^{-1} for the compounds studied. The observed signal enhancement was the most noticeable for hydrophobic analytes such as DHA and EPA, which showed the highest signal enhancement in effluent (155.90%) and influent (155.20) samples, respectively. In contrast, lower but still significant signal suppression values (64.54–78.14%) were observed in influent samples for BP and DHA. EPA was the only analyte that showed signal enhancement across all the samples. MIF and BP showed moderate matrix effects (close to 100%) in both effluent and river samples, as did EPA in river samples. In general, water samples, particularly effluent and river water, are less loaded with concomitant matrix components than influent samples, and thus large matrix effects from these matrices are not expected. However, different chemical compounds in water may interfere with the analytes' quantification signals, resulting in varying enhancement and suppression matrix effects. Polar chemical compounds in the positive ionization mode are widely known to be more susceptible to signal suppression; however, signal enhancement can be caused by matrix components that act as a dopant, increasing the ionization efficiency of analytes with high ionization energy.[39] Even though the chromatographic signal was enhanced and suppressed by the matrix, even with the use of internal standards observed for all analytes in both water matrices, the results indicate that the current proposed method could be used for the determination of the targeted analytes in water samples. However, in addition to the current approach in this study, there are other approaches that can also be investigated to reduce the matrix effects. Matrix-matched calibration curves, for example, could be used for determination. The main disadvantage of this approach is the difficulty in obtaining an analyte-free representative matrix.[40] In contrast, the standard addition method (SAM) has been consistently used for accurate determination. Unfortunately, the SAM adds time and analysis cost.[38] The analytical method proposed in this study reduces analysis time and cost while maintaining acceptable analytical data performance. This study's protocol could be applied for the analytes under investigation, justifying the use of external calibration with internal standards for quantification. Therefore, for accurate determination of the analytes in different matrices, a classical approach based on external calibration with internal standards is justified.[41]

3.5 Application in real environmental water samples

The validated method was used to determine the concentration levels of the targeted organic contaminants in the wastewater (influent and effluent) and river water samples (Table 9). The peak areas of DHA, EPA, and MIF in any of the samples analyzed

were below the detection limit (b.d.l.) and could not be integrated to be quantified. Benzophenone was quantified using peak areas of the precursor ion molecules based on accurate mass measurement at a prescribed retention time. Fig. 5 depicts a typical UHPLC-QTOF-MS chromatogram and MS spectra obtained for BP and BP-d10 influent water extract. Benzophenone was present at mean concentration levels of 0.79 ± 0.03 μg L^{-1}, 0.84 ± 0.02 μg L^{-1}, and 0.88 ± 0.01 μg L^{-1} in influent, effluent, and river water, respectively. The findings indicate that the wastewater treatment plant was ineffective at removing these pollutants before discharging effluents into the environment. The efficiency of the studied WWTPs was calculated using the formula removal efficiency = $100 - (C_{eff}/C_{inf}) \times (100)$, where C_{eff} represents concentration in effluent and C_{inf} represents concentration in influents of the WWTPs, in μg L^{-1}. The results showed a low to moderate negative removal efficiency of −6.34%. Compared to the influents, the concentration levels of benzophenone in the river samples were 5.49 higher. Several factors considerably influence the behaviour of organic compounds in water, which may help to explain the increased concentration of benzophenone in the effluent. According to Shigei and co-workers,[42] these factors include partitioning to organic matter or bioaccumulation during biological treatment in the WWTPs, as well as the presence of conjugates or metabolites that may cleave back to the parent compound. Benzophenone has a log K_{ow} of 3.18 and is potentially hydrophobic.[29] Palma and co-workers found that compounds with log K_{ow} greater than 3.0 exhibit hydrophobic behaviour and have a high potential for bioaccumulation.[43] Similar observations of concentration enhancement due to possible compound accumulation in some compartments during the wastewater treatment process have been reported elsewhere in the literature.[44] The increased concentration of benzophenone detected in the river downstream compared to the concentration found in the influent indicates that effluent discharge into the river has a direct impact. The increased concentrations could also be attributed to potential human activities upstream of the WWTP,

Table 9 The mean concentration ($n = 3$) of analytes from the Northern Johannesburg Wastewater Treatment Plant and the Jukskei River

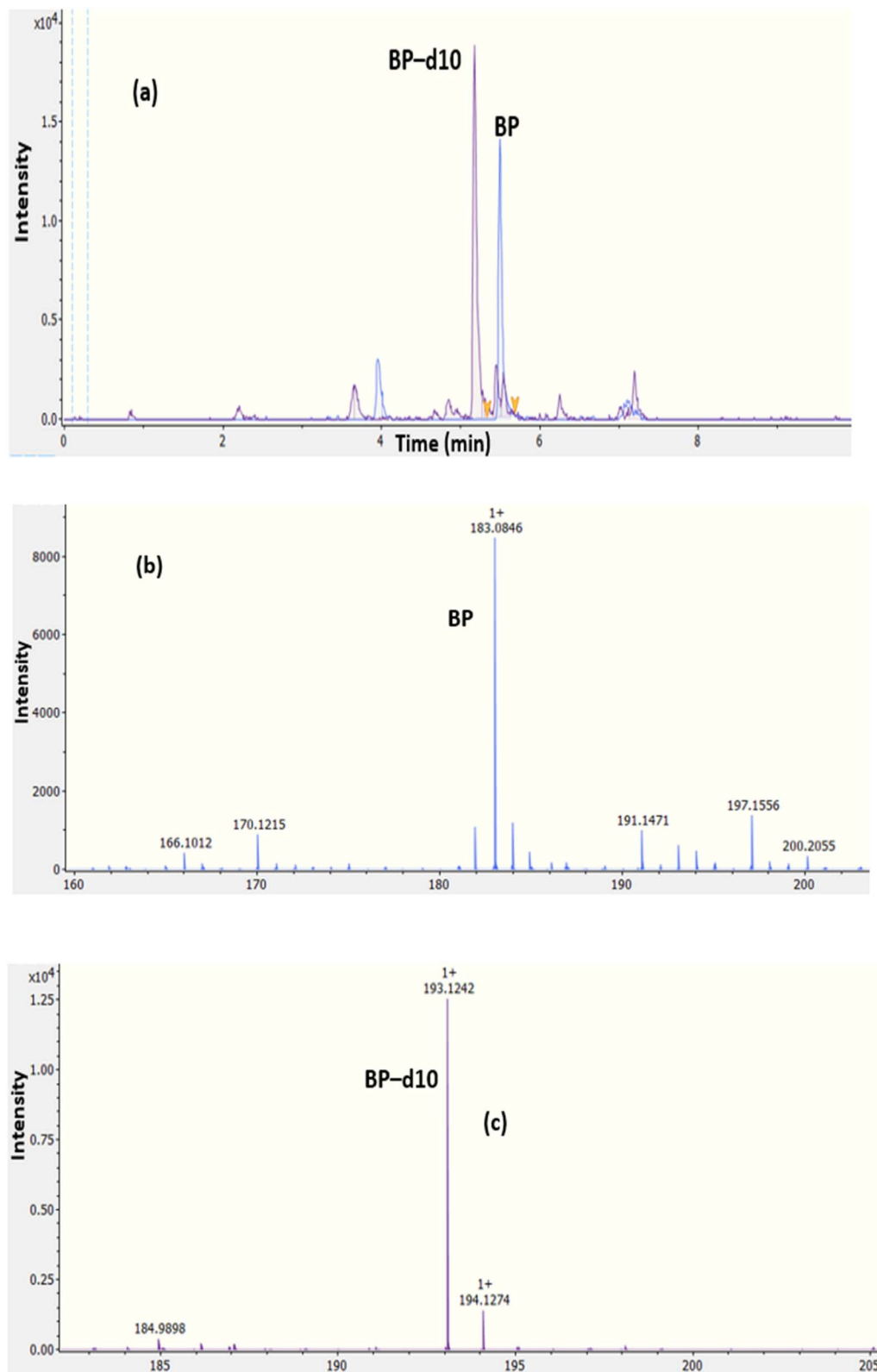| Analyte	Influent (μg L^{-1})	Effluent (μg L^{-1})	River downstream (μg L^{-1})
MIF	b.d.l.	b.d.l.	b.d.l.
BP	0.79 ± 0.03	0.84 ± 0.02	0.88 ± 0.01
EPA	b.d.l.	b.d.l.	b.d.l.
DHA	b.d.l.	b.d.l.	b.d.l.

Fig. 5   Representative chromatograms of BP and BP-d10 (a), and precursor ion scans at m/z 183.0846 (b) and m/z 193.1242 (c) in ESI positive mode in the influent sample.

in addition to the effluent discharge from WWTPs, or other unidentified sources of contamination in the river. However, no samples from upstream river were analysed. The results are limited to allow a detailed discussion of the distribution and behavioural patterns of the analytes, and additional research will require considering seasonal variation in data collection,

including analysis of upstream river samples. Nevertheless, studies assessing the risks to human health and the environment are warranted due to the high enough measured concentrations of BP in the water samples.

3.6 Preliminary human health and ecological risk assessment

Considering the concentrations of benzophenone detected, as well as the negative removal efficiencies of the wastewater treatment plant under study, the ecotoxicity risk quotient (RQ) for the detected benzophenone can be used to evaluate the potential toxic effects of this compound in water samples. The RQs were determined using the equation RQ = MEC/PNEC, where RQ represents the risk quotient, MEC represents the maximum environmental concentration, and PNEC represents the predicted non effect concentration.[45] Based on the predicted values, the analytes were classified as high (RQ ≥ 1), medium (0.1 ≤ RQ < 1), and low risk (0.01 ≤ RQ < 0.1).[46] Fig. 6 depicts the RQ values derived based on PNECs for ecological risk due to the concentrations of benzophenone detected in influent, effluent, and river samples. The risk assessment of benzophenone showed that the RQ values were categorized into the low risk category of RQ < 0.1, indicating that benzophenone poses little risk to organisms at various trophic levels, except in river water, where daphnids were the main organisms exposed to these risks.

In addition, as a worst-case scenario for human exposure, estimates of age specific RQs based on the maximum detected benzophenone concentrations in water samples were investigated. The drinking water equivalent level (DWEL) values for the detected benzophenone ranged from 242.6 µg L^{-1} (1–2 years) to 1131.3 µg L^{-1} (16–21 years), with RQs ranging from 0.000787 (16–21 years age group) to 0.00367 (1–2 years age group). Fig. 7 shows that among various age groups, children (1–11 years age group) had higher RQs than teenagers (11–21 years age group) and adults (>21 years age group). In general, RQs for the detected benzophenone were less than 0.2, indicating that the detected concentration of benzophenone posed no significant issues and is unlikely to endanger human health through drinking water consumption.
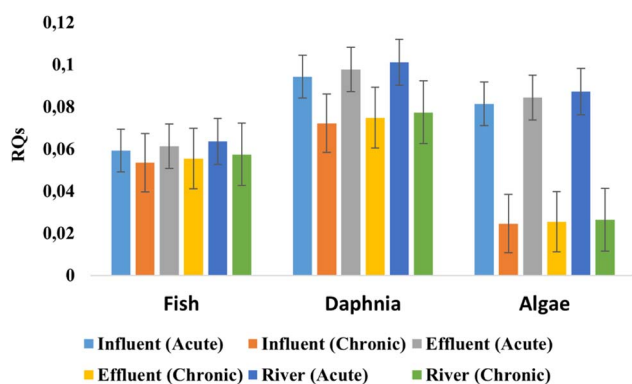

Fig. 6 RQ profile of benzophenone in water samples under acute and chronic exposure.
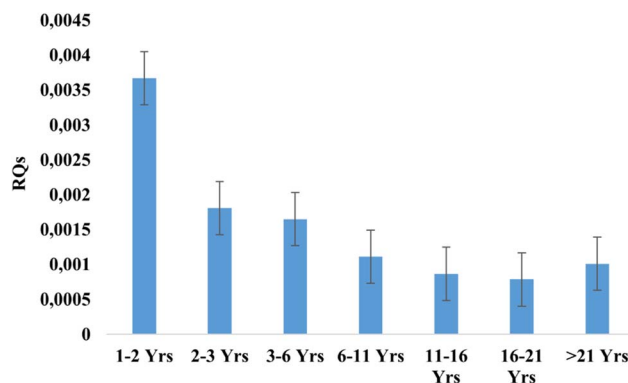

Fig. 7 Human health life-stage RQs for benzophenone in river water.

3.7 Greenness of method profile assessment

The Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI), Analytical Greenness Calculator (AGREE), Analytical Greenness for Sample Preparation (AGREEprep), and Sample Preparation Metric of Sustainability (SPMS) were used to assess the proposed method's greenness. Briefly, the AES is a numerical metric with no pictograms. It is calculated by subtracting the penalty points (PPs) allocated to reagents/solvents, instruments, and equipment based on their energy consumption, occupational hazards, waste generation, and disposal from a total of 100 points.[47] The AES defines a score of more than 75 as excellent green analysis. A score of more than 50 suggests acceptable green analysis, whereas a score of less than 50 indicates insufficient green analysis. The GAPI is a semiquantitative tool that involves various steps of the analytical procedure such as sampling and sample preparation, chemicals and their toxicity, instrument energy consumption, and waste generation and disposal.[48] In addition, the GAPI tool considers additional variables such as health and safety. It assesses and quantifies the environmental impact of each stage of an analytical method using five pentagrams, with green, yellow, and red representing low, medium, and high environmental impact, respectively.[48] The central pentagram specifies the method's general type, whether it was used for qualification or for both qualification and quantitation. AGREE, on the other hand, is a recent automated software metric tool developed in 2020 by Pererira and co-workers.[49] The AGREE pictogram has twelve sections, which correspond to the twelve GAC principles. Each section and the core area of the AGREE symbol can be coloured from red to green, depending on how green the method is. The method's total score, which varies from zero to one depending on how green it is, is automatically calculated with the score displayed in the centre of the pictogram.[49] Very recently, Pena-Pereira and co-workers[50] introduced AGREEprep as an effective metric for thorough sustainability evaluation focusing on sample preparation. The foundation of this metric tool is ten effect categories, which are then converted into a 0–1 scale sub-score. Each part of the pictogram is represented by an array of colours, ranging from red to green. The assessment technique generates an overall greenness score for the approach as a pictogram with a score in the middle. SPMS is the most

Table 10 Parameters for the metrics used in this study's proposed methodology

Eco-scale		GAPI	AGREE	AGREEprep	SPMS
Reagents	**PPs**				
Acetonitrile	4				
Water	0				
Formic acid	6				
Tetrachloroethylene	3				
NaOH	2				
HCl	4				
Instruments					
UHPLC-MS	2				
Centrifuge	1				
Occupational hazard	0				
Waste	3				
Total PPs	**25**				
Eco-scale	**75**				
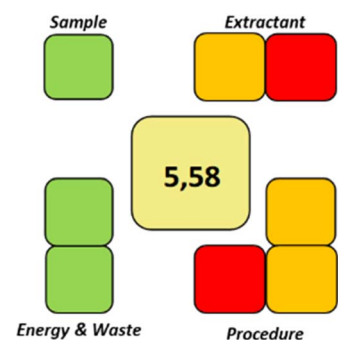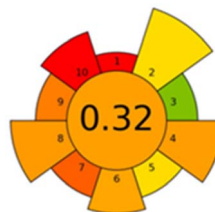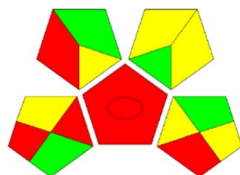
recent green metric tool available for evaluating the environmental impact of sample preparation steps by assessing factors such as sample and extractant quantity, extractant nature, procedure steps, extraction time, additional post-extraction steps, sample throughput, energy consumption, waste production, and extractant reusability.[51] Each step in a clock diagram is represented by a coloured square, with the colours green and red denoting good and not good, respectively. The overall sample preparation procedure score, which varies from 0.0 to 10.0, is displayed in the central large square.[51] The score is displayed as a gradient of green to red based on the result. The tool can effectively distinguish between comparable sample preparation techniques in terms of sustainability using both visual and numerical evaluations.[51,52]

The proposed method obtained a score of 75 on the Eco-scale, suggesting that it is an excellent green method, as shown in Table 10. The GAPI demonstrated that the proposed method is not environmentally sustainable, because only four fields were shaded green, six yellow and five red. In contrast, the developed method obtained an overall AGREE score of 0.42. Even though the proposed methodology only had three red colours in the AGREE pictogram, it is not regarded as green and hence has a detrimental ecological impact on the environment. According to the literature, the method must have an AGREE score of at least 0.6 to be considered green.[53,54] The method can be enhanced by emphasizing *in situ* measurement, minimizing energy usage, reducing waste generation, analysing numerous analytes, and utilizing less toxic solvents. The results of the AGREEprep metric tool are also shown in Table 10. The proposed method for determining the targeted analytes in water samples was evaluated using DLLME. *Ex situ* extraction was carried out using tetrachloroethylene and acetonitrile, both of which are not considered sustainable or renewable. Due to their contact with the sample, the solvents that were used were considered to be waste. The sample also contained toxic and corrosive NaOH and HCl used for pH adjustment in addition to formic acid, which was added to the mobile phase for extract reconstitution before LC-MS analysis. Three steps were involved in the manual sample preparation. With a sample throughput

of 4 per hour, the energy demand was estimated to be 152 W h. The waste quantity included pure solvents and mixtures, and vial insets. The analytical technique was LC-MS, and more than four hazards were identified because of the toxicity of the solvents used. With a final score of 0.32, the AGREEprep metric demonstrated that the applied method did not satisfy the conditions for the method to be regarded as green. This method, as assessed by AGREEprep, can be made green by minimizing or replacing toxic solvents and reagents with safer greener ones, automating the system, preparing the sample *in situ*, and assuring the operator's safety. The DLLME method was also assessed using SPMS and found to be both acceptable and sustainable, with a score of 5.58. In general, the SPMS score for a DLLME method differs from those obtained using other metrics. For the evaluated method, for instance, the numerical score from the SPMS metric is higher than those derived from comparable quantitative metrics such as AGREE and AGREEprep. The advantage of SPMS is in the fact that it solely concentrates on the sample preparation procedure, which typically may result in higher sustainability ratings than other metrics. In conclusion, the proposed method was found to be ecologically unsustainable as assessed by the selected metrics tools, except for AES and SPMS. It is distinguished by a range of different green chemistry principles that draw attention to detrimental environmental problems, such as those pertaining to health and safety. With only the AES qualifying the proposed method as green and SPMS as acceptable and sustainable, it is evident that the disagreement between the metric tools is due to the enormous differences in the criteria employed to evaluate the proposed method. Nevertheless, the information collated using these metric tools can assist researchers in making informed decisions on how to reduce the environmental impact of an analytical procedure.

3.8 Method comparison

The method proposed in this study is compared to previously reported methods for determining organic contaminants in water samples as shown in Table 11. The reported method in this study demonstrated a competitive performance in terms of

Table 11 Comparisons of the performance of the method reported in this study with extraction methods for determining organic contaminants in different water matrices

Extraction	Analytes	Matrix	Sample volume	Extraction/solvents	Analysis	MDLs	% Recoveries	Reference
DLLME	Benzophenone and bisphenols	Wastewater and surface water	5 mL	Acetonitrile and dichloromethane (2.153 mL)	LC-MS/MS	2–20 ng L^{-1}	60–121	55
SPE	Mifepristone and other compounds	Wastewater	100 mL	Methanol (6 mL)	MEKC	1.2–7.7 µg mL^{-1}	72.5–86.1	56
LLE	EPA and DHA	River and sediment	400 mL	Chloroform–methanol (5 mL)	GC-MS	—	—	57
DLLME-SFO	Benzophenone compounds	Tap, lake, and wastewater	8 mL	Alpha-terpineol (80 µL)	HPLC-DAD	0.12–0.53 µg L^{-1}	80.2–108.4	30
SPE	Mifepristone and other compounds	Wastewater	1000 mL	Ethyl acetate (12 mL)	LC-MS	—	—	58
DLLME	BP, MIF, DHA, and EPA,	Wastewater and river water	5 mL	Acetonitrile and tetrachloroethylene (1.634 mL)	LC-MS/MS	0.10–0.48 µg L^{-1}	23.32–145.43	This work

MDLs. While some analytes have calculated recoveries that are lower than those reported in previous work, the proposed method offers a simple cost-effective procedure with significantly lower solvent consumption. Additionally, DLLME extraction time is relatively rapid, does not necessitate a special approach and instruments in the pre-treatment step, and provides advantages in terms of reusability of extraction materials such as centrifuge tubes. In comparison to other miniaturized techniques such as DLLME-SFO,[30] our method demonstrated its efficiency for simultaneous extraction of analytes with different physicochemical properties in a single extraction. The main advantage of this method compared to other methods mentioned in the literature is that it has never been applied to simultaneously analyse the targeted analytes in water samples. In addition, the method has been evaluated for its environmental friendliness as well as human and ecological risk assessment. This method, however, had some limitations. Using lipoic acid-d5 as a surrogate/internal standard for EPA and DHA limited the applicability of the proposed method, while other analytes, such as MIF and BP, were quantified in relation to their corresponding standards. Other limitations include the method's reproducibility not being tested through inter-day analysis and its accuracy being tested using only one spiking level concentration. Nevertheless, this work demonstrated the proposed method's quantitative capabilities in the analysis of the targeted organic contaminants in complex matrices such as wastewater treatment plant influent and effluent water samples.

4. Conclusions

In this study, a DLLME procedure was developed and used to simultaneously determine BP, MIF, DHA, and EPA in water samples using UHPLC-QTOF-MS. The proposed method is simple, fast, and cost-effective. The OFAT experimental approach was used to optimize the selection of the extraction and disperser solvents, and the parameters influencing the extraction efficiency were statistically optimized using the DoE approach and CCD with DF. The method was validated under optimum conditions and demonstrated good linear ranges and sensitivity, satisfactory accuracy, and acceptable recoveries, indicating its applicability and feasibility in real water samples. Assessments of the health and ecological risks were also performed to evaluate the potential risk of exposure to organic contaminants such as benzophenone in different water samples with the results showing no risk. However, further risk assessment analysis would require considering seasonal variation in data collection to gain better insights into contaminant distribution and exploit the gaps that appear to exist in sampling, analysis, and monitoring. The developed method was also evaluated for its environmental friendliness, and it represented a non-sustainable green one as measured by various metric tools, with only the AES classifying the method as green and SPMS as acceptable and sustainable.

The current study is the first to report on the determination of the targeted analytes using DLLME and LC-QTOF-MS analysis. More broadly, the results indicate that the proposed

method, while not environmentally friendly when evaluated with green metrics tools such as the GAPI, AGREE, and AGREEprep, can be a valuable approach for determining the selected compounds in different water samples. Additionally, the compounds under investigation, specifically poly-unsaturated fatty acids (PUFAs), docosahexaenoic acid, and eicosapentaenoic acid, may represent novel sources of pollution. It may be argued that the sensitivity of the method could be compromised if all aspects of GAC were fully incorporated during method development. However, the method can be made more environmentally friendly by reducing the amount of hazardous materials used, or by replacing them with eco-friendly alternatives, while still balancing the method's sensitivity and environmental friendliness.

Data availability

Data will be made available upon request.

Author contributions

Tlou Auguston Makwakwa: conceptualization, data curation, methodology, project administration, investigation, validation, formal analysis, visualization, resources, software, writing – original draft. Elsie Dineo Moema: resources, formal analysis, validation, writing – review & editing. Titus Alfred Makudali Msagati: funding acquisition, project administration, supervision, validation, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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