Hindawi
Journal of Chemistry
Volume 2022, Article ID 7161318, 13 pages
https://doi.org/10.1155/2022/7161318

Research Article

Comparison of PSA to Moringa Oleifera Seed Protein as Sorbent in QuEChERS: A Response Surface Methodology Optimization for Extraction of Some Endocrine Disrupting Chemicals in Food

Thapelo Ramalepe,[1] **Heidi Richards**,[1] **Hlanganani Tutu**,[1] **Yannick Nuapia**,[2] **Eutilério Chaúque**,[3] **Imasiku Nyambe**,[4] **and Luke Chimuka**[1]

[1]*Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag X3, Johannesburg 2050, South Africa*
[2]*Pharmacy Department, School of Healthcare Sciences, University of Limpopo, Turfloop Campus, Polokwane 0727, South Africa*
[3]*Department of Chemistry, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique*
[4]*University of Zambia, School of Mines, Institute for Water Research ManageSment Centre, P.O. Box 32379, Lusaka, Zambia*

Correspondence should be addressed to Luke Chimuka; luke.chimuka@wits.ac.za

Received 20 May 2022; Accepted 29 July 2022; Published 13 September 2022

Academic Editor: Saima Q. Memon

Copyright © 2022 Thapelo Ramalepe et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This work aimed at optimizing the QuEChERS method with PSA and then comparing it with Moringa Oleifera seed protein as a clean-up sorbent for the extraction of endocrine-disrupting chemicals. The response surface methodology approach was used in the optimization. A design of experiment (DoE) was used to investigate the effect of the sample mass (0.5–3 g), centrifuge speed (3400–4000 rpm) and time (5–20 min), the mass of *NaCl* and $MgSO_4$ (1 − 3 g), and solvent extraction volume (5–10 mL). The analysis was done using GC-ECD and GC × GC TOFMS. The PSA method which was later replaced with Moringa Oleifera seed protein presented optimal values of 3 g of sample, 150 mg PSA, 4000 rpm for 6 min centrifuge conditions, including 2 g NaCl and 2 g $MgSO_4$ extracted in 10 mL methanol, respectively. Moringa Oleifera seed protein gave better selectivity, and the detection limits ranged between 0.16 and 1.77 $\mu g\,kg^{-1}$ with RSD values ≤13.32%, respectively. Moreover, recoveries were between 76.2 ± 0.85% and 105.2 ± 2.24%. Application of the developed method in food samples detected some EDCs. This study has shown that Moringa Oleifera seed protein is a promising alternative to PSA in the clean-up of food-related samples using the QuEChERS approach.

1. Introduction

The clean-up activity of extracts for chromatographic analysis is the first and far-most critical step for accurate quantification of endocrine disrupting chemicals (EDCs) in fruits, vegetables, meat, and fish [1]. Over the past 40 years, endocrine-linked diseases and complications have been significantly increasing [2]. This is associated with the presence of many man-made EDCs in the environment that eventually make their way into foodstuff [3,4]. EDCs are categorized as man-made or naturally occurring [5,6]. Man-made EDCs include some chemicals from different classes like plasticizers, pesticides, pharmaceutical compounds, and preservatives often found in detected fruits and vegetables [7]. They have been linked to interfering with the reproductive system [8,9]. For example, exposure to DDT, which is man-made, contributes to an earlier start of puberty and as females get older, the exposure has the potential to extend menstrual cycles and also speed up menopause [10].

In infants and children, the target organs for man-made EDCs are the brain and prostate glands [11]. As a result, it causes neurological and immune system defects [10,12,13]. Other man-made EDCs include bisphenol A (BPA), dioxins, polybrominated biphenyls (PBBs), and polychlorinated

biphenyls (PCBs) [14–16]. Other endocrine-related health effects include infertility, attention deficit hyperactivity disorder (ADHD), obesity and diabetes, and cancer [17, 18]. Some of these chemical pollutants can affect the hormonal system and interfere with important developmental processes in humans [19].

EDCs affect humans by mimicking the normal binding of different hormones in the body, e.g., melatonin hormone in the pineal gland, prolactin and oxytocin in the pituitary gland, insulin and glucagon in the pancreas, estrogen, and androgen in the female reproductive system, among others [8, 20]. The mimicry complicates the different natural body signals [21, 22]. This occurs by tricking the cell to accept the binding of the EDC to the hormone while causing an identical cellular response from the host as normal binding [20, 23].

There are various analytical techniques for the extraction of EDCs in food including Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), Soxhlet extraction, ultrasonic-assisted extraction (UAE), pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE) and supercritical fluid (SFE) extraction [24, 25]. A conventional Soxhlet extraction has been used for the extraction of EDCs with acceptable recoveries [24]. However, the limitation of this technique is that it is time-consuming as the reflux must be done for 24 hours, and it requires a large amount of solvent which has negative disposal effects on the environment [26–28]. UAE technique overcomes the Soxhlet extraction disadvantages of time consumption and large volume usage; however, its limitation is the repeatability of the method as the ultrasound energy that transfers from the ultrasonic bath to the sample container is dependent on the properties of the bath. As a result, a probe is needed to control the ultrasound frequency and amplitude for acceptable reproducibility [29, 30]. The elevated temperatures used in PLE may degrade unstable EDCs [31, 32]. Also, the rate of diffusion of the solvent to the sample increases as the solvent volume is increased, which is not ideal when the solvent volume is increased above the optimized amount [33]. QuEChERS extraction technique is one of the alternatives for food because it is not labor intensive and also uses a less organic solvent to produce optimal recoveries [34, 35]. This technique has been reported in the literature for various analyte applications [36–39]. The common clean-up sorbents in QuEChERS have been synthetic ones sometimes used in combination to improve matrix clean-up in samples.

Biosorbents though are receiving much attention in solid-liquid extraction in analytical chemistry [40] yet very little has been reported in the QuEChERS technique. It is anticipated that some of these biosorbents will offer as alternative commercial clean-up sorbents in the QuEChERS technique as demonstrated in this paper.

This study aimed to optimize the QuEChERS method by keeping the processes greener, cheaper, and more selective than the existing traditional QuEChERS. This was done using a response surface methodology (RSM) as the optimization tool and using Moringa seed protein as biosorbent. Furthermore, the performance of the optimized PSA approach was compared with the water-soluble Moringa Oleifera seed protein as a selective clean-up sorbent. The protein was used as a cleaning sorbent instead of the seed powder because the protein is purer thus making it more selective towards unwanted matrix. The seed protein is also not soluble in organic solvents like methanol, unlike the seed powder that has soluble components.

2. Materials and Methods

2.1. Materials and Reagents. Seven EDCs: dimethyl phthalate, 4-nonylphenol, $\beta - BHC$, diethyl phthalate, $4,4' - D\ D\ T$, $4,4' - D\ D\ D\, an\, d\, 4,4' - D\ D\ E$ with purities of over 95% were purchased from Sigma-Aldrich, Johannesburg, South Africa. Stock and standard solutions were prepared using HPLC-grade methanol from Merck (Johannesburg, South Africa). Anhydrous magnesium sulfate and sodium chloride were purchased from Sigma-Aldrich. For clean-up, primary secondary amine (PSA) bonded with silica was purchased from Sigma-Aldrich (Johannesburg, South Africa). Nitrogen gas (99.999%) was used for the evaporation of extracts to the required volume. Other chemicals used were of analytical grade. Further, certified reference materials (dimethyl phthalate, diethyl phthalate, 4-nonylphenol, 4,4'-DDT, 4,4'-DDD, and 4,4'-DDE) were bought from Dr. Ehrenstorfer GmbH of LGC chemicals (Augsburg, Germany).

2.2. GC-ECD Conditions Used in the Optimization. Gas chromatography (GC) 7890A (Agilent Technologies, DE, USA) equipped with an electron capture detector (ECD) with a WCOT fused silica capillary column (30×0.25 mm ID, 0.25 m film thickness) was used in the optimization. The software used for analysis was Chemstation version B.04.03. The GC method was programmed to get the best separation of the EDCs of interest as follows: Oven 60°C for 5 min, increased to 150°C at a rate of $^{10°C\ min\text{-}1}$, further increased to 200°C at a rate of $^{30°C\ min\text{-}1}$ then lastly to 300°C at $^{15°C\ min\text{-}1}$ for 10 min. Nitrogen gas was used for GC-ECD with a flow rate of $^{1\ mL\ min-1}$. The make-up nitrogen flow in the detector was set at $^{25\ mL\ min\text{-}1}$. The temperature of the injector operating in split less mode was held at 300°C and ECD temperature was 25°C. The volume injected was 10 μ L using manual injection.

2.3. GC × GC TOFMS Conditions Used in the Applications. A LECO GC-MS with a capacity for a GC × GC equipped with a TOFMS detector 7890B (LECO Corp., St Joseph, MI, USA) was employed for confirmation of the EDC identities. A 7683 series injector (Agilent Technologies, DE, USA) was used for the GC × GC TOFMS. ChromaTOF ® was used as the analysis software. The mass spectrometer was programmed with a transfer line temperature of 350°C; ion source temperature of 250°C and multiplier voltage of 1450 V. A programmed temperature vaporization injector operating in splitless mode was employed. The volume injected was 10 μL, split flow 50 mL min^{-1} and injection time: 0.50 min, injection flow: 100 ml min^{-1}. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Ion trap mass detection was operated in full scan mode from 50 to

500 amu. The oven temperature was from 50°C increased to 150°C at 10°C ramp rate for 1 min followed by an increase to 300°C at a rate of 5°C for 1 min.

2.4. Preparation of Standards and Calibration Curve. Solutions of 1000 mg.L^{-1} of each EDC were prepared in 25 mL volumetric flasks by dissolving 25 mg of each EDC in their respective volumetric flasks and filling it to the mark with methanol. The stock solutions were stored at −21°C. The 10 mg.L^{-1} each of working standard solution of the seven chemicals was prepared by withdrawing 100 μL each prepared stock solution into a 10 mL volumetric flask and diluting to the mark with methanol. The prepared mixed solutions were then stored at 4°C until analysis. From the 10 mg. L^{-1} working standard solution, calibration standard solutions of EDCs of different concentration ranging from 0.2 mg L^{-1} to 1.0 mg.L^{-1} was prepared. Five-point calibration standards were prepared.

2.5. Moringa Oleifera Seed Protein Extraction. Moringa Oleifera seed was collected from a Moringa farm in Limpopo, South Africa. The seed was manually deshelled and cleaned with distilled water to remove ground dirt. The seed was then left to dry in the oven overnight at 40°C. After drying, the seed was ground using a domestic blender and further powdered using a mortar and pestle, and sieved using a 0.20 mm sieve to obtain uniform particle size. The powder was then stored in the refrigerator at 4°C.

The water-soluble protein was extracted using a modified method from the literature [41]. Briefly, the seed powder was first mixed with hexane and petroleum ether (37 w/v%) and stirred for 30 min using a magnetic stirrer to dissolve fats, oils, or waxes constituents. The protein was then extracted using distilled water. The protein was used as a cleaning sorbent instead of the seed powder because the protein is purer and more active, thus making it more selective towards unwanted matrix. The seed powder is also not soluble in organic solvents like methanol, unlike the protein. The experimental illustration of the various Moringa Oleifera products is shown (Figure 1).

2.6. Response Surface Methodology (RSM)

2.6.1. Design of Experiments: Screening and Optimization. To get the best recovery in the application of QuEChERS to food samples, the sample mass, cleaning sorbent mass, speed and time of centrifuge, amount of NaCl and $MgSO_4$, solvent type, and solvent extraction volume was screened using L8 (3 levels with 7 factors, 6 responses, and 21 runs) linear model. The selected parameters were optimized by a central composite orthogonal (CCO) design which is composed of a full or fractional factorial design (Quadratic model, 17 runs). The statistical tool used for the design of the experiment is MODDE Pro 13.0.1 (Sartorius Stedim Biotech, Malmö, Sweden). Three center points replicates were employed for the design and for each experimental run. The screened factors are displayed in Table 1.

2.6.2. QuEChERS Extraction Method. The QuEChERS method was done using the procedure by [42], with modifications where necessary (Figure 2). The method was first performed following different screening conditions as displayed in Table 1 (a full factorial design was used as a screening tool). Each run was done following the combination shown in Table 2. Varying amounts of sample (Table 2) were weighed in a 50 mL centrifuge tube and spiked with 500 μL of 200 μg L^{-1} standard mixture to give varying concentrations in μg kg^{-1} . The spiked samples were allowed to stand for 30 min to allow the standards to integrate into the samples. Varying volumes of solvent were added according to Table 2, and the sample was vortex-shaken for 1 min. This was followed by addition of sodium chloride and anhydrous magnesium sulfate and the mixture was vortex-shaken vigorously for 1 min and then centrifuged for varying min under different speeds (Table 2). After the centrifuge, the supernatant was transferred into a second centrifuge tube for the clean-up process with anhydrous magnesium sulfate and PSA. PSA was used for screening purposes. The solution was further vortexed for 30 sec. The solution was then centrifuged for 5 min under the same conditions and filtered using first a 0.45 μm PTFE syringe filters followed by 0.22 μm PTFE syringe filters and injected into the GC-ECD for analysis.

Once all the parameters were screened and the important ones determined, the optimization process was done with reduced runs using the most important QuEChERS parameters. The optimization runs were reduced from 21 to 17 and the data is displayed in Table 3. The optimization tool employed has the advantage to determine the interaction between the independent quantitative variables, namely sample mass, speed, and time of centrifuge. It then models the system mathematically. This saved time and cost by reducing the number of trials and experiments that could have been performed when using the traditional way of optimization. The traditional way entails dealing with one parameter at a time while keeping the rest of the parameters constant. At optimal PSA amounts, the PSA was then replaced with the same amount of Moringa oleifera protein.

Appropriate quality assurance procedures were carried out to ensure reliability and reproducibility of the results such as spiking and replicate extraction of samples. Limit of detection was also calculated as a signal-to-noise ratio of three times. The CRMs were used for QuEChERS method validation. The material certificate of analysis was designed in accordance with ISO 17034 and ISO Guide 31.

3. Results and Discussion

3.1. Quality Assurance. The seven EDCs were separated within 29 min. The instrumental performance and precision for GC-ECD are given by the LOD, LOQ, and the retention times as shown in Table 4 (LOQ is 10xLOD). The linearity of this method was performed using five data points for all the compounds ranging from 0.2 mg L^{-1} to 1.0 mg L^{-1} with at least three injection repetitions.

The chromatographic representation of the individual certified reference materials (CRMs) under GC × GC

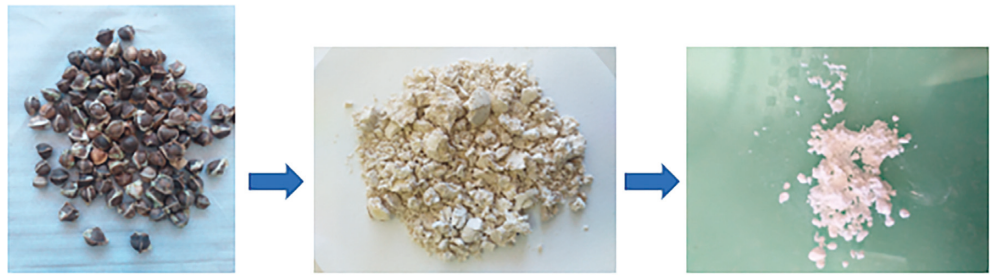


Figure 1: Schematic illustration of *Moringa Oleifera* seed protein extracted product.

Table 1: Parameters screened using L8 linear model.

QuEChERS parameter	Minimum	Maximum	Factor type
Sample mass	0.5 g	3 g	Quantitative
Centrifuge time	5 min	20 min	Quantitative
Centrifuge speed	3400 rpm	4000 rpm	Quantitative
Amount of NaCl	1 g	3 g	Quantitative
Amount of MgS O_4	1 g	3 g	Quantitative
Solvent volume	5 mL	10 mL	Quantitative
Solvent type	Methanol, acetone and acetonitrile		Qualitative

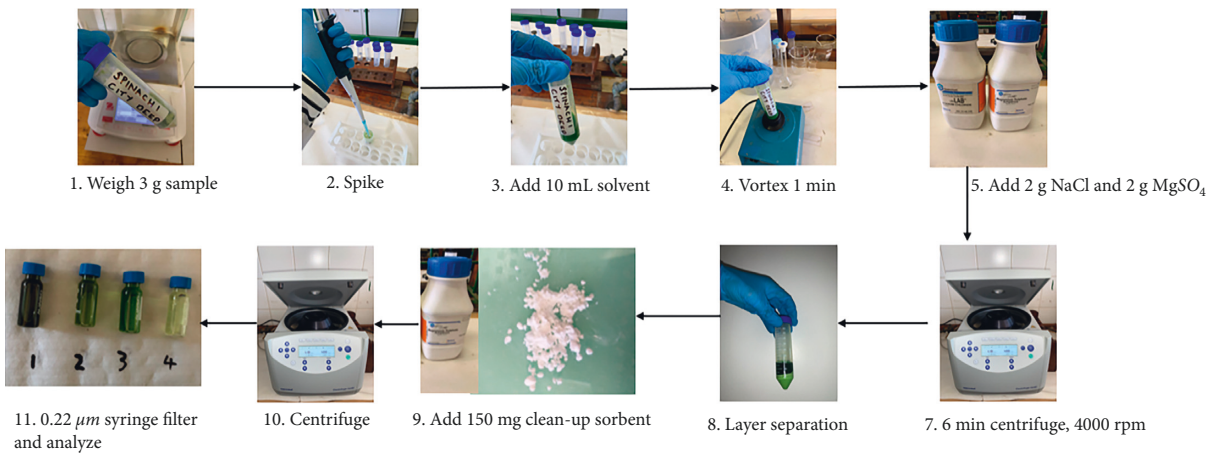


Figure 2: Schematic QuEChERS experimental procedure.

TOFMS is represented in Appendix (Figure Ia). Its recoveries after fortification are given in Table 5. The % recovery was $52.34\% \leq x \leq 114.69\%$. These recoveries are comparable to other previous studies [42–44] but low for diethyl phthalate in fish and beef samples (52–59%).

$$\%\ \text{Recovery} = \left(\frac{100^{*}\ \text{amount in extract}}{\text{amount in sample}}\right). \quad (1)$$

3.2. RSM Optimization of QuEChERS Conditions

3.2.1. Screening Experiments. The parameters which affect the efficiency of the QuEChERS method (sample mass, speed and time of centrifuge, amount of NaCl and $MgSO_4$, solvent type, and solvent extraction volume) were screened using a linear L8 model. The L8 model was then fitted using multiple linear regression (MLR).

R^2 represents the percent of the variation of the response showing how well the model fits the data. The findings are shown in Table 6. A large R^2 (close to 1) is a necessary condition for a good model. For this model, R^2 values are in the range of 0.78–0.94.

Some factors were deemed "nonessential" from the screening. They are the type and volume of solvent and amount of salt. However, the type of solvent used for extraction needs to satisfy several requirements for maximum recovery of the EDCs. The chosen solvent needs to have high affinity and high selectivity for the endocrine-disrupting chemicals of interest. It needs to have good chromatographic

Table 2: Experimental responses of independent factors for screening.

Run order	Sample mass/g	Centrifuge speed/rpm	Centrifuge time/min	NaCl mass/g	MgSO4 mass/g	Solvent volume/mL	Solvent type	DMP	DEP	4-Nonylphenol	4,4'-DDT	4,4'-DDE	4,4'-DDD
5	0.5	3400	5	1	1	5	Methanol	44.99	123.3	68.86	23.24	123.5	132.0
8	0.5	3700	12.5	2	2	5	Acetone	76.85	132.5	88.65	37.04	96.65	96.37
18	0.5	4000	20	3	3	5	Acetonitrile	44.24	72.63	88.62	76.85	87.64	130.7
2	1.75	3400	5	2	2	5	Acetonitrile	28.88	68.85	66.96	55.67	147.9	66.61
12	1.75	3700	12.5	3	3	5	Methanol	32.65	42.25	84.56	42.55	76.68	101.9
15	1.75	4000	20	1	1	5	Acetone	67.65	142.6	136.69	71.04	119,7	145.6
10	3	3400	12.5	1	3	5	Acetone	98.89	108.1	123.25	13.66	176.64	65.55
21	3	3700	20	2	1	5	Acetonitrile	11.92	64.56	105.98	65.95	67.32	127.62
16	3	4000	5	3	2	5	Methanol	12.6	58.46	75.55	59.67	46.95	101.05
9	0.5	3400	20	3	2	10	Acetone	45.68	56,69	45.98	88.37	48.15	77.79
20	0.5	3700	5	1	3	10	Acetonitrile	47.06	64.56	32.97	74.91	119.9	68.55
1	0.5	4000	12.5	2	1	10	Methanol	16.95	45.56	45.5	75.68	38.69	140.23
3	1.75	3400	12.5	3	1	10	Acetonitrile	14.65	26.65	38.66	89.51	52.23	90.58
19	1.75	3700	20	1	2	10	Methanol	22.89	43.56	105.45	79.98	71.23	73.09
14	1.75	4000	5	2	3	10	Acetone	105.26	65.59	89.65	65.87	54.44	61.22
4	3	3400	20	2	3	10	Methanol	26.68	20.56	102.23	65.66	69.99	46.96
7	3	3700	5	3	1	10	Acetone	56.66	65.64	58.69	88.96	36.96	55.56
11	3	4000	12.5	1	2	10	Acetonitrile	42.63	32.22	71.25	108.66	56.68	75.66
6	1.75	3700	12.5	2	2	7,5	Methanol	23.36	49.68	66.69	59.69	39.64	46.96
17	1.75	3700	12.5	2	2	7,5	Methanol	21.99	33.65	65.58	55.26	29.38	86.66
13	1.75	3700	12.5	2	2	7,5	Methanol	33.1	39.65	87.88	67.85	36.66	88.89

Table 3: Experimental design for the optimization model.

Exp No	Run order	Sample mass/g	Centrifuge time/min	Centrifuge speed/rpm	Dimethyl phthalate	Diethyl phthalate	4-Nonylphenol	4,4'-DDT	4,4'-DDD	4.4'-DDE
1	4	0.5	5	3400	42.11	82.36	60.41	20.41	71.38	69.66
2	6	3	5	3400	49.81	54.11	41.91	80.41	57.85	74.21
3	12	0.5	20	3400	16.96	21.22	98.41	66.21	64.24	41.22
4	8	3	20	3400	24.58	71.11	45.27	88.22	72.64	77.71
5	3	0.5	5	4000	58.11	55.21	72.29	61.66	48.65	96.34
6	11	3	5	4000	28.96	77.41	85.69	72.77	85.21	71.02
7	15	0.5	20	4000	41.21	33.24	114.22	69.94	74.21	49.21
8	17	3	20	4000	35.91	86.96	76.96	36.66	100.05	48.76
9	16	0.1	13	3700	55.54	81.21	79.68	12.1	66.21	71.28
10	9	3,4	13	3700	58.64	101.23	71.26	39.65	88.57	56.65
11	2	1.75	2	3700	35.65	30.21	71.21	48.65	61.24	88.85
12	14	1.75	23	3700	12.71	31.24	80.21	67.41	61.21	63.33
13	1	1.75	12.5	3300	19.89	22.21	31.28	86.21	42.88	58.87
14	10	1.75	12.5	4100	32.88	54.21	88.27	52.47	64.52	71.26
15	5	1.75	12.5	3700	34.56	38.27	71.41	51.48	37.58	72.24
16	13	1.75	12.5	3700	33.24	41.27	63.41	55.21	39.21	69.71
17	7	1.75	12.5	3700	35.59	50.44	65.22	50.28	42.81	72.36

Table 4: Limits of detection (LOD - $\mu g\,kg^{-1}$), limit of quantification (LOQ $-\mu g\,kg^{-1}$) and RSD (%) for GC-ECD analytical performance (Fish).

EDC	r^2	Retention time	Detection limits ($\mu g\,kg^{-1}$)		Precision (RSD %)
			LOD	LOQ	
					$n = 3$
$\beta - BHC$	0.9318	10.871	1.03	10.3	8.55
Di methyl Phthalate	0.9252	11.643	0.61	6.1	4.39
Di ethyl phthalate	0.9479	12.829	1.18	11.8	4.88
$4 - n - Nonylohenol$	0.9887	13.214	0.89	8.9	9.86
$4,4\prime - D\,D\,D$	0.9599	17.347	0.16	1.6	13.32
$4,4\prime - D\,D\,E$	0.9567	18.214	1.12	11.2	4.44
$4,4\prime - D\,D\,T$	0.9638	20.422	1.77	17.7	2.25

Table 5: Measures of CRMs concentration ($\mu g\, kg^{-1}$) and their respective recoveries in vegetable, fish, and beef sample ($n = 3$, RSD% < 21.66).

EDC	Cabbage			Fish			Beef		
	Amount in extract (μg)	Amount in sample (μg)	Recovery %	Amount in extract (μg)	Amount in sample (μg)	Recovery %	Amount in extract (μg)	Amount in sample (μg)	Recovery %
DMP	0.146	0.1	69	0.148	0.1	67%	0.182	0.1	55
DEP	0.149	0.1	67	0.191	0.1	52%	0.168	0.1	59
4-Nonylphenol	0.087	0.1	114	0.092	0.1	108%	0.090	0.1	111
4,4-DDE	0.141	0.1	71	0.183	0.1	54%	0.156	0.1	64

Table 6: MLR statistical analysis ($n = 21$).

EDC	R^2	Q^2	RSD (%)	Model validity	Reproducibility
Dimethyl phthalate	0.92	0.64	9.80	0.68	0.95
Diethyl phthalate	0.94	0.77	11.27	0.75	0.94
4-Nonylphenol	0.88	0.58	12.18	0.88	0.78
4,4'-DDT	0.89	0.60	9.65	0.71	0.91
4,4'-DDE	0.84	0.56	20.76	0.26	0.98
4,4'-DDD	0.78	0.49	18.33	0.94	0.41

behavior and its interaction with water is also essential. The solvent needs to be immiscible with water and must be less dense than water [45].

The solvent polarity, cost, toxicity, and selectivity as well as the structure of the target analyte were taken into consideration since the chosen solvent should be able to extract all the target compounds. Figure 3 shows different peak concentrations when three solvent types are varied for the extraction of EDCs of interest. All fish samples were spiked with 106.67 $\mu g\, kg^{-1}$ of the EDCs. From Figure 3, it is essential to note the selectivity of methanol on the matrix in the presence of the target EDCs in the extraction of the hake fish sample.

The volume of the chosen solvent was also optimized. This is because extraction is based on the affinity differences between the analytes of the organic and aqueous phases. Generally, low solvent volumes are favored when considering enrichment factors. Low solvent volumes give higher enrichment factors. For this work, the final volume was reduced by nitrogen blowing. For the extraction, 10 mL of solvent was optimized to give the best extraction of the analytes while ensuring that the final volume is enough to be collected for analysis. The final volume was ± 1 mL.

The amount of NaCl and $MgSO_4$ salts were also investigated as this is the step in QuEChERS where target EDCs are extracted. The amount added may have salting-out effects, salting-in effects, or no effect at all on the extraction of endocrine-disrupting chemicals [46]. The optimal amount of salt added was determined to be 2 g of NaCl and 2 g $MgSO_4$. Increasing this amount further decreased the concentration of all seven EDCs as the solution viscosity was also increased, consequently.

The fitted model shows cross-validated predictability of $0.49 \leq Q^2 \leq 0.77$ and total explained variance of $0.78 \leq R^2 \leq 0.94$ where Q^2 shows an estimation of the future

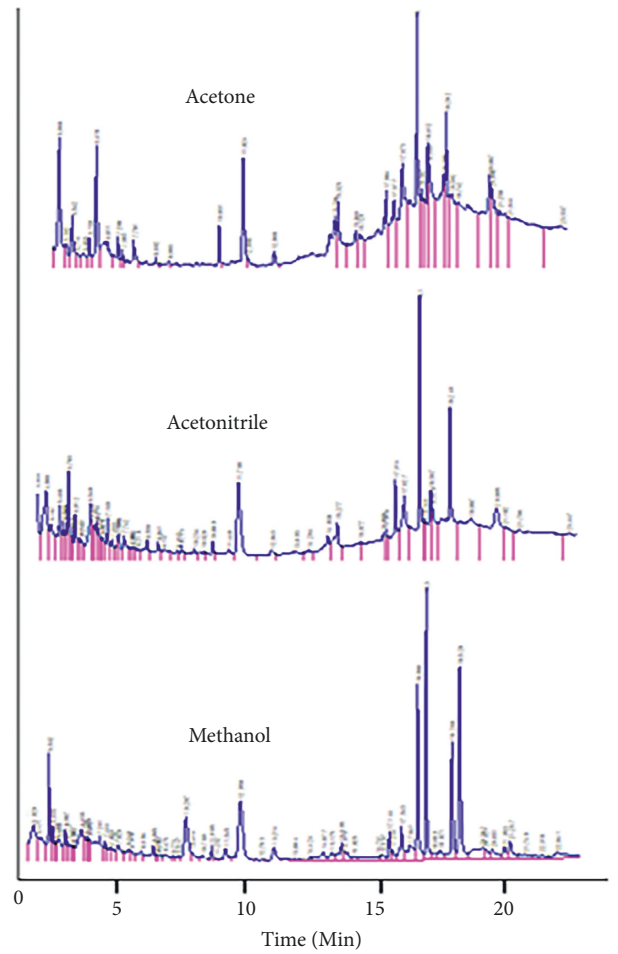


Figure 3: Effect of solvent type on QuEChERS extraction of a hake fish sample. (i) acetone (ii) Acetonitrile (iii) Methanol.

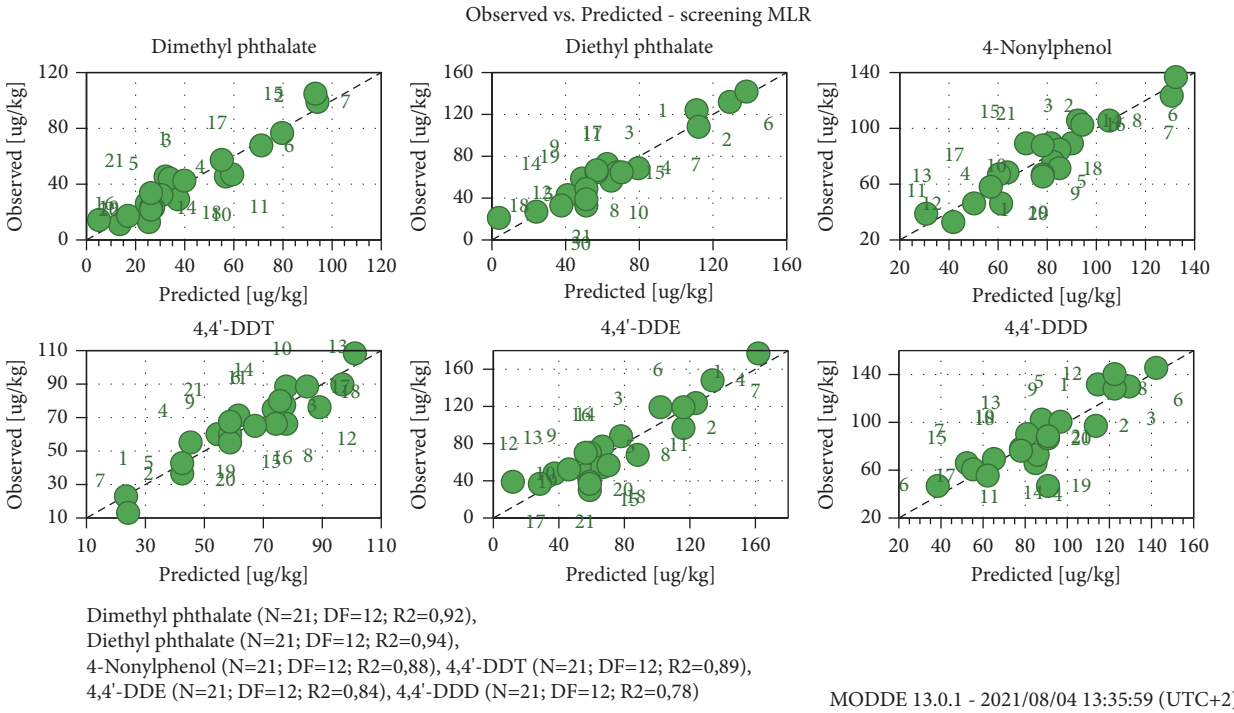


Figure 4: Observed vs Predicted concentrations of EDCs.

prediction and R^2 shows the model total variance. Figure 4 shows the linearity of the predicted versus observed values plot highlighting the validity of the model and its ability to predict the most important parameters to be optimized.

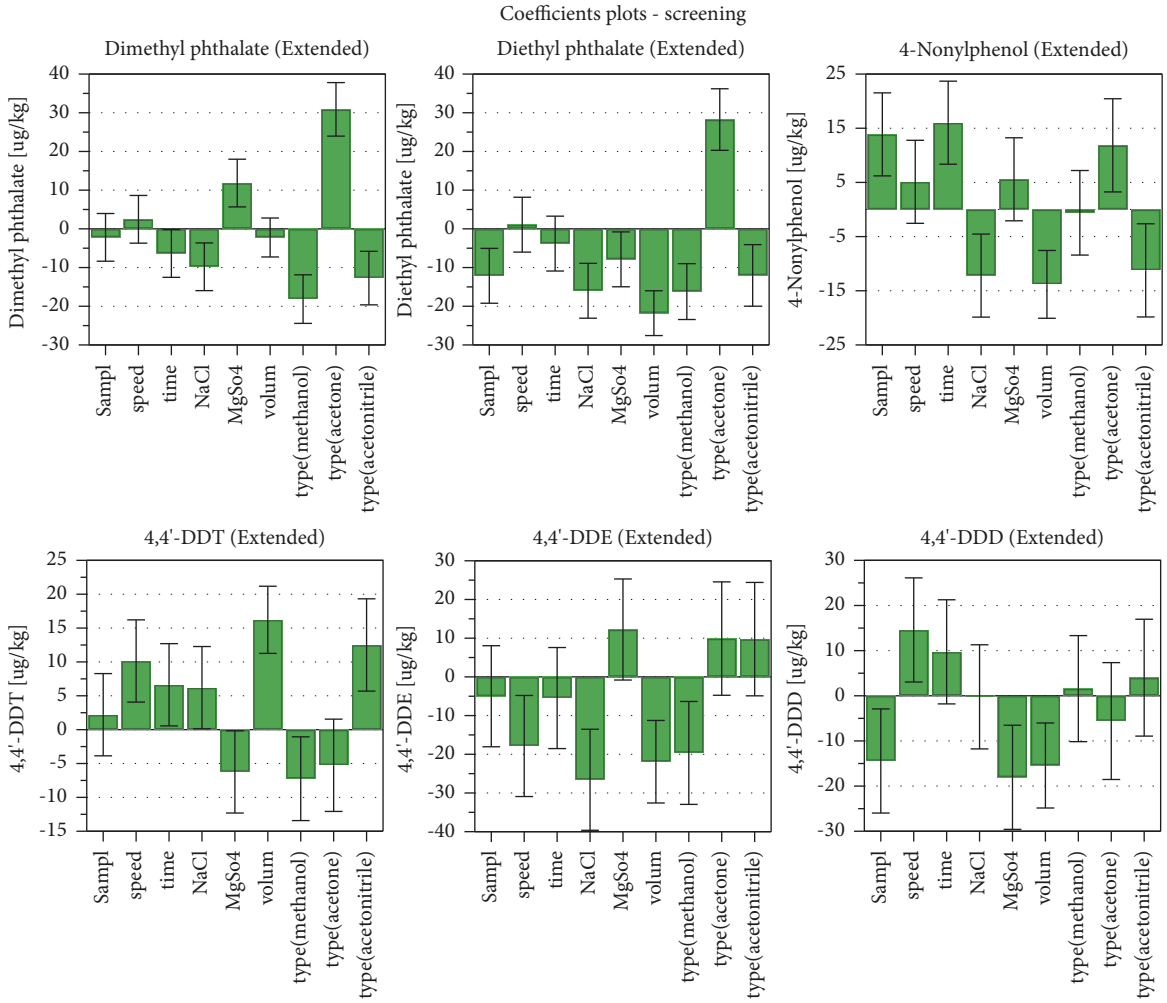
A study[47] did a similar RSM study and found that the predicted data of the response from the empirical model agrees with the observed ones in the range of the operating variables. The high value of their adjusted R2 (0.89) indicated that the model fit the observed data well. Other similar studies showed cross-validated predictability of 65% ($Q2 = 0.65$) and a total explained variance of 95% ($R2 = 0.95$). Some other studies found that verification experiments carried out for predicted and observed results show that the empirical models developed were within 6% error [48].

The coefficient plots (Figure 5) show the parameters that have a significant influence on the EDC extraction when the QuEChERS method is employed. It shows that centrifuge conditions and sample mass have significant influence on the extraction of DDT, DDD, DDE, and 4-nonylphenol. Acetone as the solvent is not essential as it is not the optimum solvent even though it influences the phthalates group significantly (Figure 5). The reasoning behind the significances is given in Section 3.2.2. Figure 5 further shows that acetonitrile as a solvent is very relevant to the extraction of 4-Nonylphenol. [49] found that acetonitrile solvent greatly impacts polyphenols extraction in their study. No other study has reported the effect of partition salts (NaCl and M_gSO_4) on QuEChERS extraction using chemometrics.

3.2.2. Optimization (RSM) Using CCO Quadratic Design. The optimum values from the optimization are given in Table 7 with their factor contributions. From the design employed, it is essential to note that the sample mass, centrifuge time, and centrifuge speed are the factors with the highest contributions. This is due to their large influence on the efficiency of QuEChERS extraction of EDCs over the other screened parameters.

Centrifuge speed and time are essential parameters in QuEChERS extraction to get the target compound in the final extract from the solid. The centrifuge is used in two phases: first, to separate the supernatant that contains the extracted target analytes from the solid sample after addition of partition salts (NaCl and M_gSO_4); second, to separate the final extract from the sample matrix in the extract. In this work, Figure 6 shows the optimum extraction conditions obtained by using graphical and numerical analysis based on the response surface plots. The contour plot shows that at the optimal centrifuge speed of 4000 rpm, the optimal centrifuge time is 6 min. E.g., For 4,4'-DDE, increasing the centrifuge time from 6 to 17 min decreases the 4,4'-DDE concentration from 90 to 60 $\mu g\,kg^{-1}$, corresponding to the coefficient plot at a 95% confidence interval (Figure 5).

3.2.3. Optimization of PSA as Cleaning Sorbent Using Single Factor Experiments. The optimization of the amount of PSA was investigated using single-factor experiments. PSA amount was varied from 25 to 75 mg (25 mg increments) while keeping the other parameters constant at 1.5 g of



Dimethyl phthalate (N=21; DF=12; R2=0,92),
Diethyl phthalate (N=21; DF=12; R2=0,94),
4-Nonylphenol (N=21; DF=12; R2=0,88), 4,4'-DDT (N=21; DF=12; R2=0,89),
4,4'-DDE (N=21; DF=12; R2=0,84), 4,4'-DDD (N=21; DF=12; R2=0,78),
Confidence=0,95

Figure 5: Coefficient plots: Effect of different screening factors on the concentration of EDCs.

Table 7: RSM- Optimised values.

Factor	Role	Value	Factor contribution
Sample mass	Free	3	52,8265
Centrifuge time	Free	6	24,3663
Centrifuge speed	Free	4000	22,8072

sample, 4000 rpm for 6 min centrifuge, 1 g of each salt and 5 mL of methanol. With the increase of PSA mass from 25 to 75 mg, the extraction efficiency of most analytes gradually increased and then remained the same above 75 mg for most analytes (data not shown). Therefore, 75 mg of PSA was selected as the optimal clean-up amount. Further increasing the PSA mass past 75 mg did not have any effect on the quantification of EDCs or on the cleanliness of the chromatogram (Figure Ib appendix).

After the PSA optimization, each factor was scaled up by a factor of 2, consequently giving 150 mg Moringa Oleifera protein as the optimum cleaning sorbent mass to obtain an acceptable recovery which correlates to 3 g samples mass, 2 g NaCl mass, 2 g M_gSO_4 mass, and 10 mL solvent volume.

3.3. Comparison of PSA and Moringa Oleifera Seed Protein

3.3.1. Selectivity. Figure 7(a) shows the comparison of selectivity between PSA and Moringa Oleifera seed protein as clean-up sorbents in QuEChERS in the extraction of beef samples. In the same way, Figure 7(b) shows comparison of the same but for extraction of the fish sample. In both cases, chromatograms using Moringa Oleifera seed protein as clean-up show much selectivity compared to PSA.

3.3.2. Comparison of the Recovery between PSA and Moringa Oleifera Seed Protein. Besides selectivity comparison between PSA and Moringa Oleifera seed protein in QuEChERS as clean-up sorbents, recovery was also compared. The

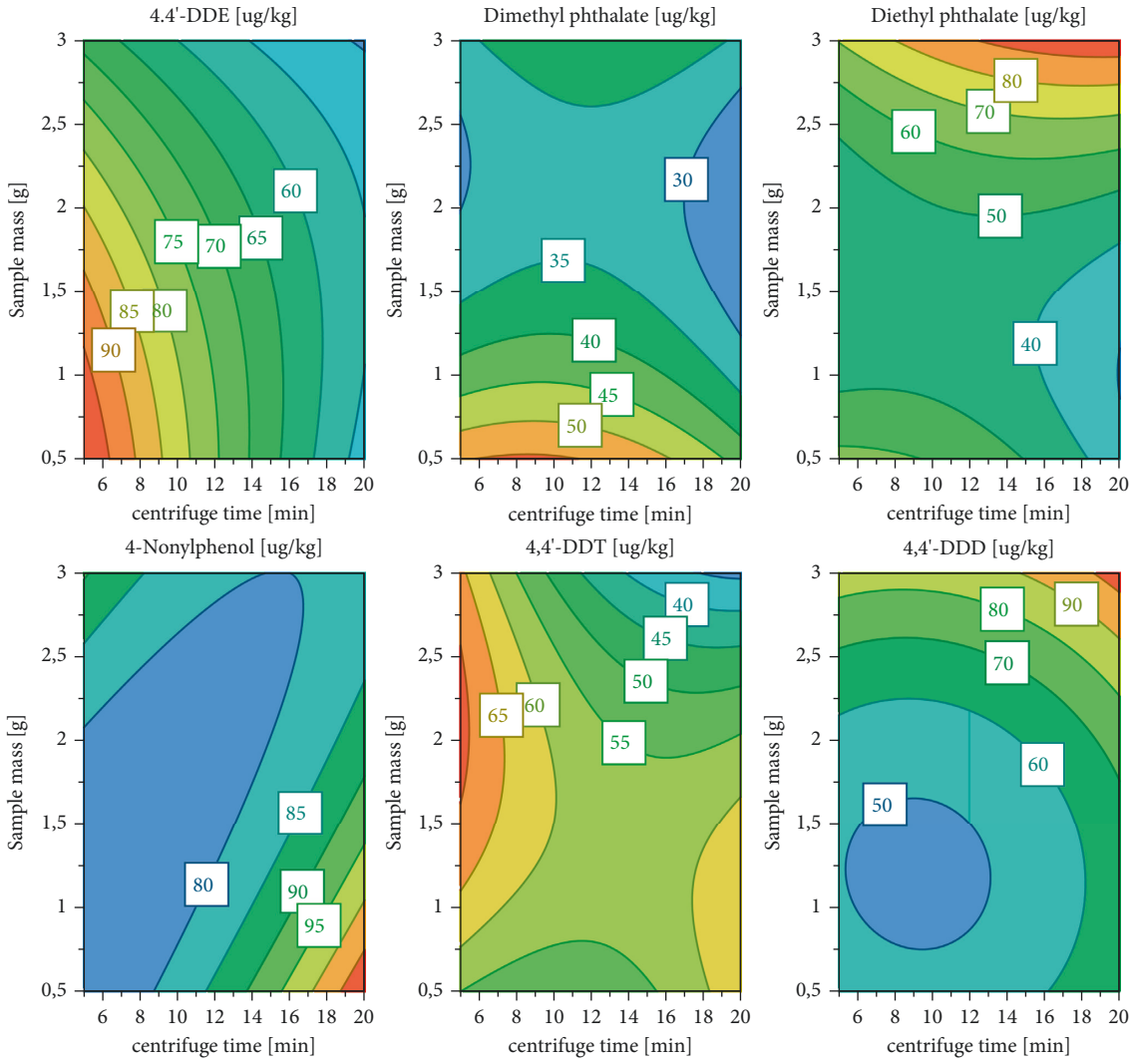


Figure 6: Contour plots of the interaction of the selected parameters on the concentrations of target endocrine disrupting chemicals.

results are given in Tables 8 and 9. Overall, recoveries using Moringa Oleifera seed protein as clean-up sorbent were slightly higher than those from PSA. These recoveries in QuEChERS are comparable to those reported by [50].

3.4. Application of the Developed Method to Food Samples. The optimized Moringa Oleifera method was applied to real food samples collected from open markets in Johannesburg and Pretoria, South Africa. All EDCs monitored in this study were detected in Johannesburg food items in the order: DMP (8 samples) > 4-Nonylphenol (4 samples) > DEP (3 samples) > DDT (3 samples) > DDE (2 samples) (Table 10). The lowest detected mean concentration in Johannesburg samples was 4,4'-DDT in cabbage samples (5.2 ± 6.82 $\mu g\,kg^{-1}$), and the highest detected concentration was also 4,4'-DDT in *Tilapia* fish (188.9 ± 8.89 $\mu g\,kg^{-1}$) and beef muscle (143.7 ±11.87 $\mu g\,kg^{-1}$) followed by DMP in banana (169.5 ±12.28 $\mu g\,kg^{-1}$). The high concentration of 4,4'-DDT in fish can be attributed to the extent of bioaccumulation of the pesticides in fish' fat or lipid membranes. This is due to extensive pesticide use in farms in the past years since DDT was banned in the 1990s. Since 4,4'-DDT is highly persistent in nature, it eventually leaches into water bodies and aquatic environment in rainy seasons. Half the DDT in soil will only break down in 2–15 years [51]. The 4,4'-DDT detected in this study in fish and beef samples was generally comparable to the results reported by [52], who reported 4,4'-DDT in beef as 167.89 ± 1.50 $\mu g\,kg^{-1}$ and 134.57 ± 5.20 $\mu g\,kg^{-1}$, respectively, in Johannesburg open markets. The levels of 4,4'-DDT detected in this study were higher than the limits recommended by the FAO/WHO (2012). No target EDCs were detected in tomato samples.

The mean concentration of 4-nonylphenol varied from 34.7 $\mu g\,kg^{-1}$ to 141.5 $\mu g\,kg^{-1}$, the highest being in the chicken liver. The closest published work working on nonylphenols in South Africa is reported by [53]. They analyzed a chicken liver sausage sample, targeting 4-nonylphenol. They reported its concentration at 13 $\mu g\,kg^{-1}$. Their highest concentration is 18.5 $\mu g\,kg^{-1}$ in potato samples. For this study, 4-nonylphenol was not detected in any potato samples. Nonylphenols accumulate in fat

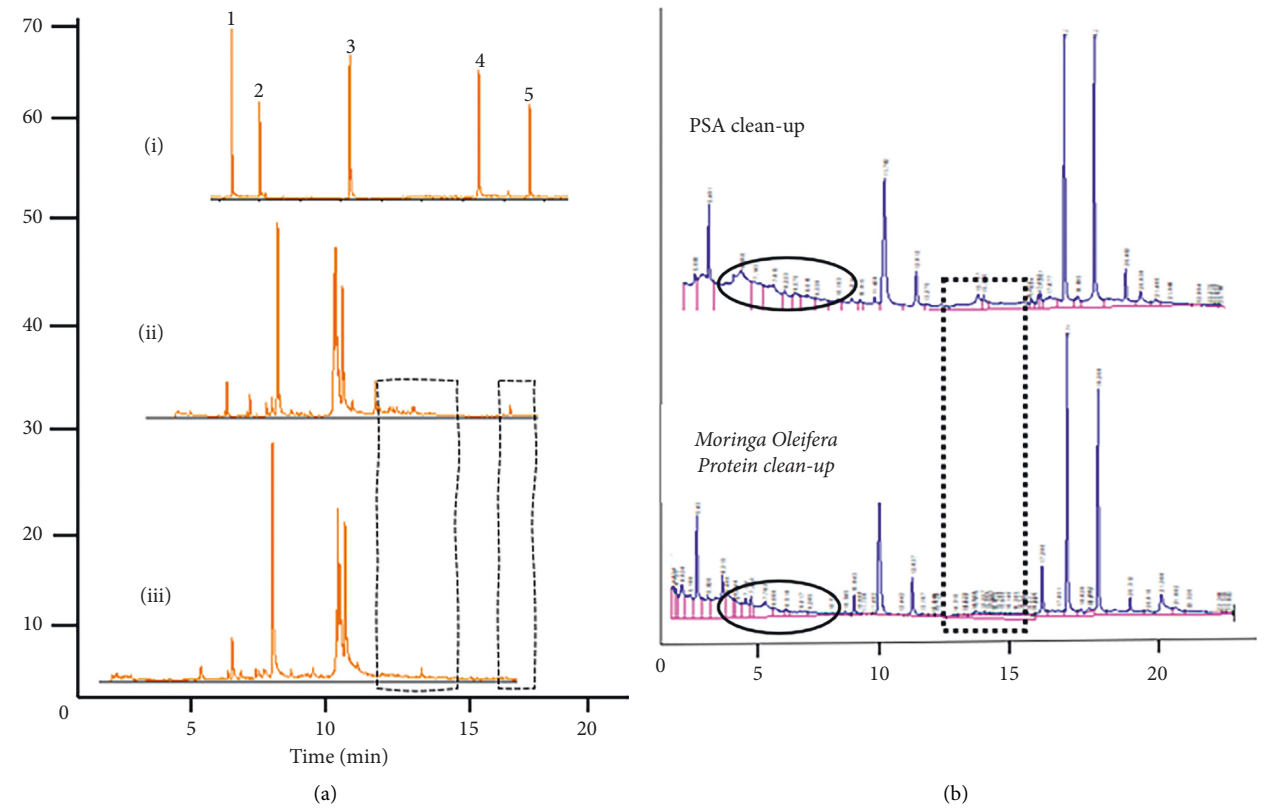


Figure 7: (a) GC × GC MSTOF chromatograms of (i) 1 mg L^{-1} standard mixture of five EDCs standards, (ii) PSA-Cleaned beef sample spiked with 142.86 $\mu g\,kg^{-1}$ standard mixture, (iii) Moringa Oleifera seed protein cleaned beef sample spiked with 142.86 $\mu g\,kg^{-1}$ standard mixture (Peak markers: 1. dimethyl phthalate. 2: diethyl phthalate 3: 4-nonylphenol 4:4,4'-DDE, 5:4,4'-DDT). (b) GC-ECD chromatogram of fish samples extracted using QuEChERS and (i) PSA and (ii) Moringa Oleifera seed protein clean-up sorbent.

Table 8: Analytical spike recovery and standard deviation (SD) of five endocrine disrupting chemicals for different food items sold in Johannesburg open markets at 142.86 $\mu g\,kg^{-1}$ and 714.29 $\mu g\,kg^{-1}$ fortification levels.

EDC	Banana		Potato		Onion		Cabbage	
	142.86 $\mu g\,kg^{-1}$	714.29 $\mu g\,kg^{-1}$	142.86 $\mu g\,kg^{-1}$	714.29 $\mu g\,kg^{-1}$	142.86 $\mu g\,kg^{-1}$	714.29 $\mu g\,kg^{-1}$	142.86 $\mu g\,kg^{-1}$	714.29 $\mu g\,kg^{-1}$
DMP	87.8 ± 0.41	89.0 ± 0.52	85.9 ± 1.36	81.3 ± 1.69	93.9 ± 2.20	99.3 ± 1.39	76.3 ± 2.23	74.2 ± 1.12
DEP	88.9 ± 0.33	86.3 ± 0.38	101.2 ± 1.21	104,1 ± 0.96	80.6 ± 1.02	78.2 ± 2.89	86.3 ± 2.56	86.3 ± 2.23
4-n-OH	83.9 ± 0.17	85.9 ± 0.23	94.3 ± 1.23	86.6 ± 0.96	87.4 ± 1.27	84.4 ± 0.78	87.4 ± 1.04	86.8 ± 1.01
4,4'-DDE	89.2 ± 0.88	86.9 ± 1.20	74. 3 ± 1.08	74.1 ± 1.26	78.0 ± 0.85	80.1 ± 1.05	86.1 ± 0.76	87.2 ± 0.87
4,4'-DDT	102.3 ± 0.29	94.3 ± 0.09	100.1 ± 0.26	77.5 ± 0.25	78.8 ± 1.55	75.1 ± 0.96	98.9 ± 1.23	100.5 ± 0.22

DMP: Dimethyl phthalate, DEP: Diethyl phthalate, SD: standard deviation.

environments since they are hydrophilic, which correlates to the highest levels in this study being detected in beef and liver samples. However, some 4-nonylphenol was detected in onion and cabbage with minimal fat.

The optimized method was also applied to Pretoria samples and the results are displayed in Table 11. When comparing Johannesburg and Pretoria samples, Johannesburg samples generally report the highest detected endocrine-disrupting chemicals in fruits and vegetables. There could be too many possible sources of the EDCs around Johannesburg compared to Pretoria. Poorly monitored municipal wastewater treatment plants around Johannesburg could be a source of some of these chemicals in surface water used for farming purposes. Further studies are needed to link possible sources of these EDCs in surface water.

4. Concluding Remarks

Response surface methodology was found to be an easy way of QuEChERS optimization. Moringa Oleifera seed protein was found to be an alternative biosorbent for food sample clean-up in QuEChERS. The recovery was comparable with

Table 9: Choice of clean-up sorbent: Recovery comparison between PSA and Moringa Oleifera-extracted Protein on selected food from Johannesburg open markets at 142.86 $\mu g\,kg^{-1}$ and 714.29 $\mu g\,kg^{-1}$ fortification levels.

EDC	Fish: Tilapia				Beef			
	142.86 μg kg^{-1}		714.29 μg kg^{-1}		142.86 μg kg^{-1}		714.29 μg kg^{-1}	
	PSA	Protein	PSA	Protein	PSA	Protein	PSA	Protein
DMP	89.7 ± 1.04	88.2 ± 0.85	81.0 ± 1.20	89.3 ± 1.26	90.7 ± 0.53	93.6 ± 0.21	104.5 ± 0.21	105.1 ± 2.24
DEP	93.3 ± 1.36	86.5 ± 0.56	107.4 ± 0.88	98.5 ± 0.85	79.8 ±1.35	76.2 ± 0.85	80.8 ±2.32	98.6 ± 0.45
4-Nonylphenol	86.0 ± 0.89	91.1 ± 1.11	88.1 ± 1.03	91.2 ± 0.63	82.7 ±1.22	90.9 ± 2.23	85.2 ± 1.01	97.7 ± 1.24
4,4'-DDE	90.8 ± 1.01	103.2 ± 0.68	79.9 ±0.96	101.6 ± 0.52	88.4 ± 0.53	88.8 ± 0.52	80.7 ± 0.52	92.1 ± 0.28
4,4'-DDT	103.1 ± 0.96	94.3 ± 0.89	96.5 ± 1.01	92.6 ± 0.75	86.7 ± 0.21	96.9 ± 1.18	82.2 ± 0.32	96.0 ± 0.96

Table 10: Concentration of EDCs in food items ($\mu g\,kg^{-1}$) in Johannesburg Market.

	DMP	DEP	4-Nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Banana	169.5 ±12.28	88.3 ± 9.96	Nd	nd	nd
Potato	155.9 ± 4.56	Nd	Nd	nd	nd
Carrot	45.6 ± 2.28	Nd	nd	nd	nd
Onion	37.2 ± 8.86	Nd	77.2 ± 7.23	nd	nd
Cabbage	8.1 ± 5.69	Nd	34.7 ± 6.39	nd	5.2 ±6.8
Fish	17.2 ± 10.11	72.6 ± 5.52		86.3 ± 8.56	188.9 ±8.89
Beef muscle	21.2 ± 5.56	Nd	93.8 ± 4.56	62.8 ± 8.86	143.7 ±11.87
Liver	23.7 ± 6.69	76.1 ± 6.63	141.5 ± 7.68	nd	nd

nd: Not detected.

PSA and in some cases gave cleaner extracts. Application of

Table 11: Concentration of EDCs in food items ($\mu g\,kg^{-1}$) in Pretoria Market.

	DMP	DEP	4-Nonylphenol	4,4'-DDE	4,4'-DDT
Sample					
Banana	210.6 ± 5.65	73.2 ± 5.21	nd	nd	nd
Potato	23.2 ± 3.69	Nd	nd	nd	nd
Carrot	46.7 ± 8.96	95.8 ± 6.69	nd	nd	nd
Onion	74.1 ± 1.10	Nd	67.2 ± 2.36	nd	nd
Cabbage	22.4 ± 6.69	Nd	28.8 ± 3.69	nd	nd
Tomato	34.6 ± 8.85	75.8 ± 7.63	nd	nd	nd

nd: Not detected.

the optimized method to various food samples found in Johannesburg and Pretoria, South Africa revealed the presence of some EDCs like dimethyl phthalate, diethyl phthalate, 4-nonylphenol, 4,4'-DDT, 4,4'-DDD, and 4,4'-DDE.

Abbreviations

CRM: Certified Reference Material
EDC: Endocrine disrupting chemical
PSA: Primary secondary amine
RSM: Response surface methodology.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This was funded by the National Research Foundation of South Africa (Grant No: 118473).

Supplementary Materials

Appendix I Figure Ia: GC × GCTOFMS chromatogram of 1 mg L^{-1} certified reference materials. Figure Ib: Chromatograms of QuEChERS fish sample extracted using QuEChERS and PSA as a clean-up sorbent at quantities: (i) 25 mg, (ii) 50 mg, (iii) 75 mg. (*Supplementary Materials*)

References

[1] R. A. Trenholm, B. J. Vanderford, J. C. Holady, D. J. Rexing, and S. A. Snyder, "Broad range analysis of endocrine disruptors and pharmaceuticals using gas chromatography and liquid chromatography tandem mass spectrometry," *Chemosphere*, vol. 65, no. 11, pp. 1990–1998, 2006.

[2] A. Bergman, United Nations Environment Programme, and World Health Organization, *State of the Science of Endocrine Disrupting Chemicals - 2012 an Assessment of the State of the Science of Endocrine Disruptors*, WHO, Geneva, Switzerland, 2013.

[3] E. Nilsen, K. L. Smalling, L. Ahrens et al., "Critical review: grand challenges in assessing the adverse effects of contaminants of emerging concern on aquatic food webs," *Environmental Toxicology and Chemistry*, vol. 38, no. 1, pp. 46–60, 2019.

[4] G. Crawford, P. Hurrel, K. Paroschy, and C. Pereira, *Pharmaceuticals and Other Endocrine Disrupting Compounds in Natural Water Systems*, Muskoka Watershed Counc, Bracebridge, Canada, 2017.

[5] M. Čelić, B. D. Škrbić, S. Insa, J. Živančev, M. Gros, and M. Petrović, "Occurrence and assessment of environmental risks of endocrine disrupting compounds in drinking, surface and wastewaters in Serbia," *Environmental Pollution*, vol. 262, Article ID 114344, 2020.

[6] M. Petrovic, E. Eljarrat, M. J. Lopez De Alda, and D. Barceló, "Endocrine disrupting compounds and other emerging contaminants in the environment: a survey on new monitoring strategies and occurrence data," *Analytical and Bioanalytical Chemistry*, vol. 378, no. 3, pp. 549–562, 2004.

[7] E. Vlassi, E. Bempelou, K. Liapis, and G. Arapis, "Consumer safety evaluation after monitoring of endocrine disruptor pesticide residues: a case study of Thessaly, Central Greece," *Toxicological and Environmental Chemistry*, vol. 102, no. 1–4, pp. 105–123, 2020.

[8] Y. Combarnous and T. M. D. Nguyen, "Comparative overview of the mechanisms of action of hormones and endocrine disruptor compounds," *Toxics*, vol. 7, no. 1, pp. 5–11, 2019.

[9] A. Guarino, A. Aruta, P. Ebrahimi et al., *Organochlorine Pesticides in the Soils of the Acerra Plain: Concentration and Distribution of DDT Isomers and Metabolites*, EGU General Assembly, Vienna, Austria, 2021.

[10] A. Matsushima, "A novel action of endocrine-disrupting chemicals on wildlife; ddt and its derivatives have remained in the environment," *International Journal of Molecular Sciences*, vol. 19, no. 5, p. 1377, 2018.

[11] T. C. Bleak and G. M. Calaf, "Breast and prostate glands affected by environmental substances (Review)," *Oncology Reports*, vol. 45, no. 4, pp. 20–18, 2021.

[12] N. U. Benson, O. H. Fred-Ahmadu, S. I. Ekett et al., "Occurrence, depth distribution and risk assessment of PAHs and PCBs in sediment cores of Lagos lagoon, Nigeria," *Regional. Studies. Marine. Science.*vol. 37, Article ID 101335, 2020.

[13] H. C. Wu, B. A. Cohn, P. M. Cirillo, R. M. Santella, and M. B. Terry, "DDT exposure during pregnancy and DNA methylation alterations in female offspring in the Child Health and Development Study," *Reproductive Toxicology*, vol. 92, pp. 138–147, 2020.

[14] A. R. Zota, R. J. Geller, L. E. Romano et al., "Association between persistent endocrine-disrupting chemicals (PBDEs, OH-PBDEs, PCBs, and PFASs) and biomarkers of inflammation and cellular aging during pregnancy and postpartum," *Environment International*, vol. 115pp. 9–20, January, 2018.

[15] L. K. Akinola, A. Uzairu, G. A. Shallangwa, and S. E. Abechi, "A computational insight into endocrine disruption by polychlorinated biphenyls via non-covalent interactions with human nuclear receptors," *Ecotoxicology and Environmental Safety*, vol. 214, Article ID 112086, 2021.

[16] E. Diamanti-Kandarakis, E. Palioura, S. A. Kandarakis, and M. Koutsilieris, "The impact of endocrine disruptors on endocrine targets," *Hormone and Metabolic Research*, vol. 42, no. 08, pp. 543–552, 2010.

[17] M. Street, S. Angelini, S. Bernasconi et al., "Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: highlights from a national italian meeting," *International Journal of Molecular Sciences*, vol. 19, no. 6, p. 1647, 2018.

[18] T. T. Schug, A. F. Johnson, L. S. Birnbaum et al., "Minireview: endocrine disruptors: past lessons and future directions," *Molecular Endocrinology*, vol. 30, no. 8, pp. 833–847, 2016.

[19] T. K. Kasonga, M. A. A. Coetzee, I. Kamika, V. M. Ngole-Jeme, and M. N. Benteke Momba, "Endocrine-disruptive chemicals as contaminants of emerging concern in wastewater and surface water: a review," *Journal of Environmental Management*, vol. 277, Article ID 111485, 2021.

[20] M. V. Maffini, B. S. Rubin, C. Sonnenschein, and A. M. Soto, "Endocrine disruptors and reproductive health: the case of bisphenol-A," *Molecular and Cellular Endocrinology*, vol. 254-255, no. 255, pp. 179–186, 2006.

[21] A. Ghassabian and L. Trasande, "Disruption in thyroid signaling pathway: a mechanism for the effect of endocrine-disrupting chemicals on child neurodevelopment," *Frontiers in Endocrinology*, vol. 9, 2018.

[22] J. González-Sálamo, "New extraction protocols for the determination of endocrine disrupting compunds in samples of environmental and agri-food interest," 2018, https://www.proquest.com/openview/8ed0998b66517bcdd0648022ca5e3806/1?pq-origsite=gscholar&cbl=2026366&diss=y.

[23] T. T. Schug, A. Janesick, B. Blumberg, and J. J. Heindel, "Endocrine disrupting chemicals and disease susceptibility," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 127, no. 3–5, pp. 204–215, 2011.

[24] P. L. Cloutier, F. Fortin, P. E. Groleau, P. Brousseau, M. Fournier, and M. Desrosiers, "QuEChERS extraction for multi-residue analysis of PCBs, PAHs, PBDEs and PCDD/Fs in biological samples," *Talanta*, vol. 165, pp. 332–338, 2017.

[25] M. G. Rasul, "Conventional extraction methods use in medicinal plants, their advantages and disadvantages," *International Journal Basic Sciences and. Applied. Computing*, vol. 6, pp. 10–14, 2018.

[26] M. Hirondart, N. Rombaut, A. S. Fabiano-Tixier, A. Bily, and F. Chemat, "Comparison between pressurized liquid extraction and conventional Soxhlet extraction for rosemary antioxidants, yield, composition, and environmental footprint," *Foods*, vol. 9, no. 5, p. 584, 2020.

[27] F. Al Juhaimi, M. M. Özcan, K. Ghafoor, E. E. Babiker, and S. Hussain, "Comparison of cold-pressing and soxhlet extraction systems for bioactive compounds, antioxidant properties, polyphenols, fatty acids and tocopherols in eight nut oils," *Journal of Food Science & Technology*, vol. 55, no. 8, pp. 3163–3173, 2018.

[28] Q. W. Zhang, L. G. Lin, and W. C. Ye, "Techniques for extraction and isolation of natural products: a comprehensive review," *Chinese Medicine*, vol. 13, no. 1, pp. 20–26, 2018.

[29] R. Nuerxiati, A. Abuduwaili, P. Mutailifu et al., "Optimization of ultrasonic-assisted extraction, characterization and

biological activities of polysaccharides from Orchis chusua D. Don (Salep)," *International Journal of Biological Macromolecules*, vol. 141, pp. 431–443, 2019.

[30] L. Wu, L. Li, S. Chen, L. Wang, and X. Lin, "Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from Moringa oleifera L. leaves: optimization, comparison and antioxidant activity," *Separation and Purification Technology*, vol. 247, no. May, Article ID 117014, 2020.

[31] B. Yang, Q. Wu, Y. Luo, Q. Yang, X. Wei, and J. Kan, "High-pressure ultrasonic-assisted extraction of polysaccharides from Hovenia dulcis: extraction, structure, antioxidant activity and hypoglycemic," *International Journal of Biological Macromolecules*, vol. 137, pp. 676–687, 2019.

[32] N. S. Marcon, R. Colet, D. Bibilio, A. M. Graboski, C. Steffens, and C. D. Rosa, "Production of ethyl esters by direct transesterification of microalga biomass using propane as pressurized fluid," *Applied Biochemistry and Biotechnology*, vol. 187, no. 4, pp. 1285–1299, 2019.

[33] R. Nuerxiati, A. Abuduwaili, P. Mutailifu et al., "Optimization of lutein extraction from scenedesmus almeriensis using pressurized liquid extraction," *Chemical. Engineering. Transactions.*vol. 87, pp. 475–480, 2021.

[34] N. Montemurro, J. Joedicke, and S. Pérez, "Development and application of a QuEChERS method with liquid chromatography-quadrupole time of flight-mass spectrometry for the determination of 50 wastewater-borne pollutants in earthworms exposed through treated wastewater," *Chemosphere*, vol. 263, Article ID 128222, 2021.

[35] J. d. M. Campêlo, T. B. Rodrigues, J. L. Costa, and J. M. Santos, "Optimization of QuEChERS extraction for detection and quantification of 20 antidepressants in postmortem blood samples by LC-MS/MS," *Forensic Science International*, vol. 319, Article ID 110660, 2021.

[36] O. Golge, A. Koluman, and B. Kabak, "Validation of a modified QuEChERS method for the determination of 167 pesticides in milk and milk products by LC-MS/MS," *Food Analytical Methods*, vol. 11, no. 4, pp. 1122–1148, 2018.

[37] S. J. Lehotay, K. A. Son, H. Kwon et al., "Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables," *Journal of Chromatography A*, vol. 1217, no. 16, pp. 2548–2560, 2010.

[38] Á. Santana-Mayor, B. Socas-Rodríguez, A. V. Herrera-Herrera, and M. Á. Rodríguez-Delgado, "Current trends in QuEChERS method. A versatile procedure for food, environmental and biological analysis," *TrAC, Trends in Analytical Chemistry*, vol. 116, pp. 214–235, 2019.

[39] M. Anastassiades, E. Scherbaum, and D. Bertsch, "Validation of a Simple and Rapid Multiresidue Method and its Implementation in Routine Pesticide Analysis," *Filtration*, vol. 7, 2003.

[40] K. M. Billiard, A. R. Dershem, and E. Gionfriddo, "Implementing Green Analytical Methodologies Using Solid-Phase Microextraction: A Review," *Molecules*, vol. 25, p. 22, 2020.

[41] T. G. Kebede, S. Dube, and M. M. Nindi, "Removal of non-steroidal anti-inflammatory drugs (NSAIDs) and carbamazepine from wastewater using water-soluble protein extracted from Moringa stenopetala seeds," *Journal of Environmental Chemical Engineering*, vol. 6, no. 2, pp. 3095–3103, 2018.

[42] M. João Ramalhosa, P. Paíga, S. Morais, C. Delerue-Matos, and M. B. Prior Pinto Oliveira, "Analysis of polycyclic aromatic hydrocarbons in fish: evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method," *Journal of Separation Science*, vol. 32, no. 20, pp. 3529–3538, 2009.

[43] H. R. Norli, A. Christiansen, and E. Deribe, "Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry," *Journal of Chromatography A*, vol. 1218, no. 41, pp. 7234–7241, 2011.

[44] P. Tölgyessy, Z. Miháliková, and M. Matulová, "Determination of selected chlorinated priority substances in fish using QuEChERS method with dual dSPE clean-up and gas chromatography," *Chromatographia*, vol. 79, no. 21–22, pp. 1561–1568, 2016.

[45] S. Berenji Ardestani, M. A. Sahari, and M. Barzegar, "Effect of extraction and processing conditions on anthocyanins of barberry," *Journal of Food Processing and Preservation*, vol. 40, no. 6, pp. 1407–1420, 2016.

[46] L. Geis-Asteggiante, S. J. Lehotay, and H. Heinzen, "Effects of temperature and purity of magnesium sulfate during extraction of pesticide residues using the QuEChERS method," *Journal of AOAC International*, vol. 95, no. 5, pp. 1311–1318, 2012.

[47] H. Ceylan, S. Kubilay, N. Aktas, and N. Sahiner, "An approach for prediction of optimum reaction conditions for laccase-catalyzed bio-transformation of 1-naphthol by response surface methodology (RSM)," *Bioresource Technology*, vol. 99, no. 6, pp. 2025–2031, 2008.

[48] I. S. Jeong, B. M. Kwak, J. H. Ahn, and S. H. Jeong, "Determination of pesticide residues in milk using a QuEChERS-based method developed by response surface methodology," *Food Chemistry*, vol. 133, no. 2, pp. 473–481, 2012.

[49] O. Galarce-Bustos, L. Novoa, J. Pavon-Perez, K. Henriquez-Aedo, and M. Aranda, "Chemometric optimization of QuEChERS extraction method for polyphenol determination in beers by liquid chromatography with ultraviolet detection," *Food Analytical Methods*, vol. 12, no. 2, pp. 448–457, 2019.

[50] E. Hakme and M. E. Poulsen, "Evaluation of the automated micro-solid phase extraction clean-up system for the analysis of pesticide residues in cereals by gas chromatography-Orbitrap mass spectrometry," *Journal of Chromatography A*, vol. 1652, Article ID 462384, 2021.

[51] A. K. Chopra, M. K. Sharma, and S. Chamoli, "Bioaccumulation of organochlorine pesticides in aquatic system-an overview," *Environmental Monitoring and Assessment*, vol. 173, no. 1–4, pp. 905–916, 2011.

[52] Y. Nuapia, L. Chimuka, and E. Cukrowska, "Assessment of organochlorine pesticide residues in raw food samples from open markets in two African cities," *Chemosphere*, vol. 164, pp. 480–487, Dec. 2016.

[53] K. Günther and J. Sumpter, "Environmental M Ne," 2002, https://www.google.com/search?q=%22Environmental+M+Ne%22&rlz=1C1GCEJ_enIN1019IN1019&oq=%22Environmental+M+Ne%22&aqs=chrome.69i57j0i7i8i10i30j0i13i30j0i5i13i30l7.1857j0j7&sourceid=chrome&ie=UTF-8.