

Application of QUECHERS Extraction Coupled With GC/MS for Detection of Polycyclic Aromatic Hydrocarbons and Organochloride Pesticides in Lake Water

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Received: September 20, 2018 Accepted: November 13, 2018 Online Published: November 19, 2018

doi:10.5539/ijc.v10n4p57

URL: <https://doi.org/10.5539/ijc.v10n4p57>

Abstract

The extensive development of industries gives birth to many chemicals which brings contaminations towards environment. The present study is to apply QUECHERS extraction coupled with GC/MS for detection of polycyclic aromatic hydrocarbons (PAHs) and organochloride pesticides (OCPs) in Lake Nokoué of Benin Republic. The method was first benchmarked with standard PAHs and OCPs, and limit of detection (2-194 ppb) and limit of quantification (8-645 ppb) were obtained with recovery rate of 91-110%. The method was then applied to the detection of PAHs and OCPs in the lake water, no benzo[a]pyrene, chrysene or pyrene was detected. The main origin of PAH compounds in Nokoué Lagoon are material combustion and fuels for Polycyclic Aromatic hydrocarbons and due to remoteness pollution for organochloride pesticides compounds.

Keywords: development, analytical method, research, PAHs, OCPs, QUECHERS GC/MS

1. Introduction

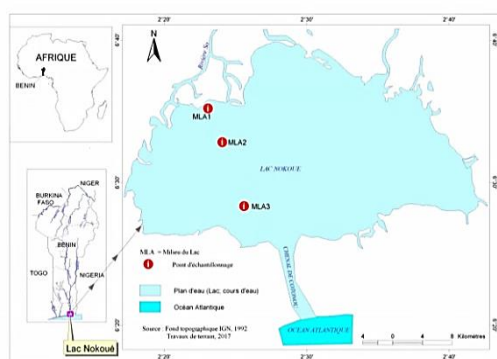
Due to diversity of pollutants released and the associated risks, analytic methods to assess the actual extent of damage are still a challenge for the analyst despite the existence of panoply of techniques. To limit exposures to very toxic organic extraction solvents and to optimize the analytical times, multi-residue pretreatment techniques have been developed and are now widely used in research of organic pollutants (Cortés-Aguado et al., 2008; Perret et al., 2004 & Bogialla et al., 2004). These techniques include assisted microwave, pressurized fluid (ASE), critical CO₂, acetonitrile extractions such as the developed and improved QUECHERS method (Anastassiades et al., 2003, Lehotay et al., 2005 & Kmeľ et al., 2010). The last method is less expensive, very reliable, effective, fast, reproducible and less toxic. This analytical approach is accompanied by the production of very powerful chromatographs equipped with high-performance sensors such as GC-MS, GC-MS / MS, LC-MS, LC-MS / MS able to separate and detect individual molecules of organic pollutants in a complex mixture resulting from a pretreatment of any environmental matrix (Chamkasem et al., 2013 & Salem et al., 2016). The choice of natural water samples for this method is based on the waters of Lake Nokoué a lake belonging to one of the most important hydrographic complexes in West Africa. This ecosystem, under a strong demographic pressure because of its location in urban areas whose consequences of mismanagement of municipal wastes, are characterized by garbage dump along its banks. It is also the receptacle of wastewater and wastes from the city of Cotonou, lacustrine villages of the town-hall of Sô-Ava, the town of Abomey-Calavi on the one hand and surface water such as the Ouémé River draining runoff that has leached cropland in large part of Benin Republic. Thus, these waters carry residues of agricultural inputs. Studies have revealed that the Cotonou Channel and Nokoué Lake ecosystems are heavily polluted by organic material of all kinds, including PAHs,

PCBs and pesticides (Soclo et al., 2008, Yehouenou et al., 2006a & b). According to the results of these authors, the concentration levels in PAH of the channel vary between 70 and 722 ng / g of dry sediments and in OCPs of a few ppb in water, sediments and living organisms. Thus, the environmental matrices of such an environment seem appropriate for the simultaneous research and monitoring of PAHs, OCPs and their derivatives by the multiresidue extraction method QUECHERS and gas chromatography coupled to mass spectrometry in an efficient manner. The objective of the study is to develop and adapt these analytical techniques to the evaluation and monitoring of contamination levels of organic pollutants in the lake.

2. Material and Methods

2.1 Stus

The surface water samples were collected in Nokoué lake, whose geographical coordinates are: 6 ° 25 '60 "N and 2 ° 27'0" E and 42 meters altitude. It covers an area of 339 ha.



Coordinates of the sampling points

Site names	X	Y
MLA1	2° 23' 52.0"	6° 27' 33.9"
MLA2	2° 24' 20.0"	6° 26' 29.1"
MLA3	2° 25' 03.1"	6° 24' 26.5"

Figure 1. Map of Nokoué Lake and coordinates of sampling points

The three sampling points are presented on the map of the figure with geographic coordinates (MLA1, MLA2, MLA3). The development, validation of analysis method and analysis of water samples were carried out at the UFR of Sciences and Techniques of the University Center of Anglet / Université Pau et des Pays de l'Adour / France.

2.2 Sampling Campaign

Before the sampling campaign the glassware was previously depended on washing with soapy water, decontaminated with the sulfochromic mixture, cleaned with bidistilled water and acetonitrile before being packed in aluminum foil. Sampling consisted of water sample extraction from 2-liter amber glass bottles following a transect (Berryman et al., 2004a, Loyo-Rosales et al., 2007, Soclo et al., 2008, Gbaguidi et al., 2014, Bennie et al., 1997, Cortes-Aguado et al., 2008 & Gasperi et al., 2009). Before sampling, the bottles are cleaned three times with the sample of water to be taken. In order to stabilize the samples and avoid microbial degradation during transport, they undergo an addition of sulfuric acid (up to pH = 2). The samples are hermetically sealed by interposing foil between the inside of the lid and the sample. They are then labeled according to the date and place of sampling. The storage and transport of the samples to the laboratories were done in coolers equipped with cold accumulators to keep them at 40 °C ± 5 °C. The campaign was carried out during the period of the small rainy season in southern Benin where the waters of the Ouéné River drain from the north the pollutants mainly of agricultural origin in Lake Nokoué

2.3 Validation of the Analytical Method

2.3.1 Reagents

- certified solution of pesticide molecules "Stock TCL Pesticide Mix 2000 ppm in Hexane / Toluene", including: Aldrin, alpha-BHC, beta-BHC, delta-BHC, diene, alpha-endosulfan, beta-endosulfan, sulfate-endosulfan, endrin, endrin-aldehyde, endrin-ketone, gama-BHC, heptachlor, heptachlor-epoxideisomer B, methoxychlor, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT;
- internal standard (EI) of pesticide: atrazine d5;
- certified solution of PAH molecules "Stock EPA 610 PAH 100-2000 ppm in methanol" comprising 16 PAH molecules such as: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, indeno [1,2,3-cd] pyrene, benzo [g, h, i] perylene, dibenzo [a, h] anthracene. For dilutions of PAH solutions, the concentration of Acenaphthene

was used as a reference. The concentrations of the other PAH molecules were then deduced by calculation compared to their actual concentrations in the concentrated standards;

Six internal standards of PAHs namely: naphthalene-d8, acenaphthene-d10, pyrene-d10, benzo (a) pyrene-d12 and benzo [g, h, i] perylene-d12.

- analytical grade solvents such as acetonitrile, acetic acid, sulfuric acid, acetone and hexane.

All these molecules were purchased from Sigma-Aldrich in France.

2.3.2 Equipment

We use for this work:

- a gas chromatograph (GC) of the Agilent 7890A type
- a mass spectrometer (SM) with an electron impact ionisation source;
- a precision analytical balance of KERN type;
- QUECHERS Agilent Part No: 5982-5121 kits obtained from Agilent Technologies (Massy, France);
- a muffle oven.

2.3.3 Analytical Conditions

The GC-MS used is equipped with an Agilent DB5-MS UI column. The carrier gas is high purity helium (99.9995%) purchased from Air Liquide (France). The flow rate of the carrier gas is 1 mL.min⁻¹. The temperatures of the injector, the ionization source and the quadrupole are 280 °C., 230 °C. and 150 °C respectively. The pulsed mode (splitless) was adopted for the injection of the purified extracts and the injected volume is 1 µL. The oven temperature programming ramp is: initial temperature of 80 °C for 1 min, then 10 °C / min up to 160 °C and finally 3 °C / min up to 300 °C. The temperature of the interface of the mass spectrum is maintained at 300°C. Ion monitoring mode (SIM) was used for quantitative analysis, while full scan mode was used for qualitative analysis (Ben Salem et al., 2016 & Chamkasem et al., 2013).

2.3.4 Validation Criteria

The validation of the method consisted in evaluating: the retention times, the linearity through the calibration (external and internal), the repeatability and the reproducibility, the limits of detection (LOD) and of quantification (LOQ). To do this, standard certified mixed reference solutions of desired molecules are prepared in the range of 0 - 1000 ppb and injected. Using the chromatograms, the mass spectra m/z are determined as well as the retention times and the corresponding areas. From the different calculated areas and the corresponding concentrations, the external and internal calibration curves are plotted in Excel. The slopes, the coefficients of determination R² and the ordinates at the origin of the different calibration curves are calculated.

For repeatability, five (05) repetitions of the range of 0 - 500 ppb were performed in the same day. As for the reproducibility, the injection of the standard solution is made over 5 days for the range 0-500 ppb and the coefficients of variation % CV are calculated in each case. So:

$\%CV = \sigma_i / \bar{x}_i \times 100$ with σ_i the standard deviation "SD" and $\bar{x}_i$ the arithmetic mean of the analytical values obtained.

- if $CV < 2\%$, the measurements are very homogeneous and the experiment is repeatable or reproducible;
- if $2\% < CV < 30\%$, the measurements are homogeneous and the repeatability or reproducibility of the experiment is acceptable;
- if $CV > 30\%$, the measurements are heterogeneous so the experiment is non-repeatable or non-reproducible (Gbaguidi et al., 2011, Kouzayha, 2011 & Salem et al., 2016).

The limit of detection (LOD) was determined by considering three times the standard deviation of the background noise after ten injections of the reagent blank. While the limit of quantification (LOQ) was determined by considering ten times the standard deviation of background noise after ten injections of reagent blank. The percentages of recovery of the various desired compounds are calculated using the standard solution of the calibration point 100 ppb according to the formula:

$$\%R = \frac{\text{calculated concentration} - \text{White concentration}}{\text{theoretical concentration}} \times 100.$$

2.4 Extraction of Natural Water Samples

Before analysis of the water samples by GC / MS, they underwent extraction, concentration and purification steps in

accordance with the QUECHERS methodology. This technique has allowed us to expand the range and number of individual molecules of organic pollutants and related metabolites. For the extraction, 15 mL of each water sample to be extracted were introduced into 50 mL flasks to which 15 mL of acetonitrile + 1 % of HAC (acetic acid) and 100 µL of a mixed solution were added. 1 ppm internal standard (extraction of IE) containing naphthalene d8, phenanthrene d10, perylene d12, atrazine d5 and stirred for a few seconds on vortex. To this mixture is added 6 g of MgSO₄ + 1.5 NaAC (sodium acetate) and stirred manually for 30 seconds. The flasks are centrifuged at 3000 rpm for 2 min. 8 mL of each supernatant is collected in 15 mL glass test tubes for dry evaporation using the Turbovap LV evaporator under a gentle stream of nitrogen. The dry residue was taken up with 500 µL of acetonitrile and passed through the purification in order to reach very low detection limits.

3. Results and Discussion

3.1 Determination of Retention Times and Analyte Weights

The retention times t_R , the weights M/Z of the analytes and the internal standards used are recorded in Table 1.

Table 1. Retention times t_R and weights of analytes PAHs and pesticides

PARAMETERS	t_R (min)	M/Z quantifier	M/Z qualifier
PAHs			
NAMES OF COMPOUNDS			
Acenaphthene	9.020	153	154
Acénaphylène	8.830	152	153
Anthracene	11.230	178	179
Benzo [a] Anthracene	17.670	226	228
Benzo [b] fluoranthene	20.710	252	253
Benzo [k] Fluoranthene	20.780	252	253
Benzo [g, h, i] perylene	23.890	276	277
Benzo [a] pyrene	21.430	252	253
Chrysene	17.658	228	226
Dibenzo [a, h] anth	23.480	276	277
Fluoranthene	13.820	202	203
Fluorene	9.610	166	165
Indeno [1,2,3-cd] pyrene	23.420	276	277
Naphthalene	7.230	128	129
Phenanthrene	11.150	178	179
Pyrene	14.360	202	203
INTERNAL STANDARDS OF PAHs			
Naphthalene d8	7.210	136	137
Acenaphthene d10	8.980	164	162
Pyrene d10	14.320	212	211
Benzo [a] pyrene d12	21.390	264	265
Perylene d12	21.570	264	260
Benzo [g, h, i] perylene d12	23.850	288	289
PESTICIDES			
Aldrin	12.910	263	265
Alpha BHC	10.410	183	219
BHC Beta	10.830	181	219
Delta BHC	9.150	181	219
Dieldrin	15.000	263	265
Alpha Endosulfan	14.460	241	195
Beta Endosulfan	15.670	195	241
Endosulfan Sulfate	16.580	387	272
Endrin	15.470	265	345
EndrinAldehyde	16.070	345	347
EndrinKetone	17.580	67	317
Gamma BHC	11.340	181	219
Heptachlor	12.230	100	272
Heptachlorepoxyde	13.700	353	355
Methoxychlor	17.900	227	228
4,4'-DDD	15.840	235	165
4,4'-DDE	14.940	245	248
4,4'-DDT	16.650	235	165
INTERNAL STANDARD OF PESTICIDES			
Atrazine d5	10.680	205	220

The retention times determined were used to associate with each molecule sought the corresponding EI. Thus, it is noted that the first molecule to be released is Naphthalene d8 whose retention time t_R is 7.21 min. It is taken as internal standard for Naphthalene ($t_R = 7.23$ min). Acenaphthylene ($t_R = 8.83$ min), Acenaphthene ($t_R = 9.020$ min), Fluorene ($t_R = 9.61$ min) and Anthracene ($t_R = 11.23$ min) have for internal standard the Acenaphthene d10 with a retention time of 8.980 min. Benzo [g, h, i] perylene d12 was removed at $t_R = 23.85$ min. It is taken as the internal standard of Benzo [g, h, i] perylene. Atrazine d5 taken as internal standard of the pesticides was released at 10.68 min. Pyrene d10, $t_R = 14.32$ min was used as internal standard of the phenanthrene molecules ($t_R = 11.15$), fluoranthene ($t_R = 13.82$ min), pyrene ($t_R = 14.36$ min), of Benzo (a) Anthracene ($t_R = 17.67$ min) and Chrysene ($t_R = 17.66$ min). On the other hand, Benzo [a] pyrene d12, $t_R = 21.39$ min was used as internal standard of Benzo (b) Fluoranthene ($t_R = 20.71$ min), Benzo (k) Fluoranthene ($t_R = 20.78$ min), and Benzo (a) Pyrene ($t_R = 21.43$ min). Finally, P12, $t_R = 21.57$ min, was used as an internal standard for diBenzo [a, h] Anthracene ($t_R = 23.48$ min) and Indeno [1,2,3-cd] pyrene ($t_R = 23.42$ min). Our choices are consistent with those of Salem et al. (2016) and Miossec et al., (2018) who worked with the same analytical material as us.

3.2 External and Internal Calibration

The slopes, the coefficients of determination R^2 and the ordinates at the origin of the calibration curve differences are recorded in Table 2. The coefficients of determination obtained for the external calibration vary from 0.90 to 1 whatever the molecule considered. At the level of the PAHs sought, these coefficients vary from 0.97 to 1. For the OCPs, $0.90 \leq R^2 \leq 1$. For the internal calibration the coefficients of determination vary from 0.99 to 1 for PAHs and from 0.91 to 1 for OCPs. Whatever the external or internal calibration, the values of the coefficient of determination R^2 obtained demonstrate that the method has good linearity in the chosen calibration range. In the following, the central (average) and dispersion characteristics of the chromatogram areas obtained during the repeatability and reproducibility tests are calculated and are recorded in Tables 3 and 4.

Table 2. Slopes, coefficients of determination R^2 and ordered at the origin of the different calibration curves (external and internal calibration)

NAMES OF COMPOUNDS	Calibration externe			Calibration interne		
	Pente	R^2	ord origine	Pente	R^2	ord origine
Acenaphthene	26025.931	1	-320.999	0.338	0.99	0.155
Acénaphylène	40507.622	0.99	-1623.79	0.525	1	0.379
Anthracene	77660.874	0.97	-536.587	1.012	0.99	-0.03
Benzo [a] Anthracene	33608.963	1	-180.003	0.138	0.99	-0
Benzo [b] fluoranthene	209462.53	0.99	-536.24	1.115	1	0.033
Benzo [k] Fluoranthene	69303.2	0.99	-310.791	0.368	1	0.024
Benzo [g, h, i] perylene	84760.274	1	-552.223	0.547	1	-0.01
Benzo [a] pyrene	101138.28	0.99	-242.498	0.538	1	0.015
Chrysene	112098.39	0.99	-461.582	0.457	0.99	0.007
Dibenzo [a, h] anth	16102.971	1	-139.912	0.105	1	-0.01
Fluoranthene	132819.95	0.99	-996.356	0.539	1	0.02
Fluorene	34541.411	1	-155.078	0.45	0.99	0.027
Indeno [1,2,3-cd] pyrene	66142.127	0.98	-463.81	0.292	1	-0.02
Naphthalene	33028.887	0.99	-1140.17	0.428	1	0.074
Phenanthrene	73744.429	1	-61.104	0.299	0.99	0.019
Pyrene	139799.61	0.99	-560.202	0.568	1	0.01
Aldrin	23448.552	1	-558.931	1.485	1	-0.18
Alpha BHC	28744.851	0.99	-370.248	1.797	1	0.174
Bhc beta	29619.104	0.99	-778.547	1.867	1	-0.27
Delta BHC	8513.85	1	-66.186	0.532	0.99	0.103
Dieldrin	13525.044	1	-366.082	0.858	1	-0.14
Alpha endosulfan	9595.239	0.97	-427.169	0.611	0.98	-0.29
Beta endosulfan	12136.322	0.99	-283.795	0.764	1	-0.07
Endosulfan sulfate	11721.694	0.99	-332.445	0.744	1	-0.15
Endrin	7733.677	0.95	-403.269	0.493	0.97	-0.3
Endrinaldehyde	17354.726	0.98	-698.855	1.106	1	-0.44
Endrin ketone	24964.515	0.99	-263.432	1.559	1	0.206
Gamma BHC	29158.232	0.98	-712.455	1.828	0.99	-0.16
Heptachlor	25465.511	0.97	-1175.05	1.625	0.99	-0.82
Heptachlorepoxyde	34749.513	0.99	-979.587	2.197	1	-0.41
Methoxychlor	179834.01	0.97	-8429.74	11.476	0.98	-5.95
4,4'-DDD	142215.76	0.99	-2935.9	8.944	1	-0.41
4,4'-DDE	7522.537	0.90	-485.508	0.48	0.91	-0.38
4,4'-DDT	105413.21	0.97	-4875.54	6.728	0.99	-3.37

PAHs: Polycycliques Aromatic Hydrocarbons; OCPs: Organochlorine Pesticides.

3.3 Repeatability

In the repeatability tests (Table 3) we noticed that at 10 ppb, 58.54 % of the tests have undetermined % CV (ND) compared to 32.5 % whose % CV have values between 7 and 30 %, have. At the 50 ppb concentration level, 70% of the tests have acceptable repeatability ($5 \leq \% CV \leq 30$). On the other hand 80 % of the tests have a % CV between 0.35 and 30 at the concentration of 100 ppb. More than 92% of the tests have a % CV between 4 and 30 at the concentration of 500 ppb. According to Salem et al., (2016), these high proportions of ND in low concentrations would be related to a loss of low molecular weight analyte by evaporation during dry extracts resulting in concentration levels under the limit of detection (Miossec et al., 2018). If this hypothesis is true in their case, in our tests the evaporation did not concern that the molecules of low molecular weight. It has affected all molecular weight ranges. To overcome this difficulty, the natural samples to be analyzed are concentrated more than 30 times to get out of the background noise.

Table 3. Repeatability test results

TESTS	10 ppb			50 ppb			100 ppb			500 ppb		
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
PAHs												
Names of Compounds												
acenaphthene	2141.8	619	29	7824.8	477	6	18343.4	1824	9.94	87866.4	10688	12
Acenaphthylene	ND	ND	ND	649.25	185	29	1552.6	220	14.17	6884.8	1415	21
anthracene	ND	ND	ND	160.33	92	57	494.25	143	28.97	3813.8	453	12
Benzo [a] Anthracene	230.0	33	14	4188.8	1072	26	6577.25	2132	32.41	39537.4	3839	10
Benzo [b] fluoranthene	ND	ND	ND	4409.33	789	18	8194	347	4.24	38568.4	3862	10
Benzo [k] Fluoranthene	ND	ND	ND	1927	226	12	3761.4	363	9.66	18833	1721	9
Benzo [g, h, i] perylene	364.0	121	33	1134.6	164	14	2454.2	517	21.05	11225.2	682	6
Benzo [a] pyrene	ND	ND	ND	1289	147	11	2320.4	263	11.34	12411.8	1743	14
Chrysene	ND	ND	ND	634.67	427	67	2181.33	465	21.31	11408.4	1272	11
Dibenzo [a, h] anth	397.33	121	30	2436.2	381	16	5756.4	900	15.64	27364.2	3398	12
Fluoranthene	ND	ND	ND	683.2	137	20	1631	210	12.88	7949	1305	16
Fluorene	ND	ND	ND	710	158	22	2103.5	442	21.02	10757	2395	22
Indeno [1,2,3-cd] pyrene	1055.7	264	25	3699.8	597	16	7704.2	913	11.85	33456.4	4715	14
Naphthalene	ND	ND	ND	882.2	292	33	1754.8	232	13.24	8837.8	1978	22
Phenanthrene	253.3	81	32	1347	256	19	2881	492	17.09	14483.6	1837	13
INTERNAL STANDARDS OF PAHs												
Naphthalene d8	6950.4	963	14	6739.8	858	13	7449	1452	19.49	7371.6	930	13
Acenaphthene d10	ND	ND	ND	552.5	138	25	606.5	2	0.35	668	136	20
Pyrene d10	20776.6	3434	17	22444.4	3718	17	22592.2	4499	19.91	21604.8	3881	18
Benzo [a] pyrene d12	9111.2	603	7	8988.75	840	9	8342	239	2.86	9422.4	410	4
Perylene d12	8085.5	735	9	8353.67	645	8	8423.25	373	4.42	8182.6	863	11
Benzo [g, h, i] perylene d12	7809.75	750	10	7550.67	364	5	7768.5	673	8.66	8142.2	802	10
PESTICIDES												
Aldrin	ND	ND	ND	686.67	237	35	1784.5	518	29.04	11339.8	2300	20
Alpha BHC	ND	ND	ND	1272.8	291	23	3101.5	201	6.49	13112.4	4081	31
BHC Beta	114.0	21	19	986.4	195	20	2566.2	818	31.87	12382.4	3127	25
Delta BHC	ND	ND	ND	786.5	368	47	2093	1038	49.59	5078.66667	3348	66
Dieldrin	ND	ND	ND	297.5	18	6	1061.67	30	2.83	6573	377	6
Alpha Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	ND	3860.33333	305	8
Beta Endosulfan	ND	ND	ND	346.33	146	42	1051.5	189	17.94	5365.2	442	8
Endosulfan Sulfate	23.6	53	-	398.67	143	36	758.75	203	26.72	5444.8	893	16
Endrin	ND	ND	ND	ND	ND	ND	283	130	45.83	2610	688	26
EndrinAldehyde	ND	ND	ND	236.5	175	74	834	366	43.89	7035.6	816	12
EndrinKetone	340.0	191	56	1410.25	205	15	2694	248	9.20	11384	997	9
gamma BHC	117.5	9	8	594.33	117	20	2248.4	618	27.49	11626.2	2207	19
heptachlor	ND	ND	ND	247.67	57	23	1114.33	534	47.91	9652.5	2823	29
HeptachlorEpoxide	ND	ND	ND	1166	340	29	2302.5	694	30.15	15016.2	2041	14
Methoxychlor	ND	ND	ND	2050.67	256	12	5193.75	1126	21.68	62609.2	9638	15
4,4'-DDD	961.8	274	29	5940.25	360	6	11816.5	1061	8.98	64102.8	5202	8
4,4'-DDE	ND	ND	ND	ND	ND	ND	117.5	21	17.45	3086.5	728	24
4,4'-DDT	ND	ND	ND	1300	255	20	3445.75	566	16.41	40566.4	4826	12
INTERNAL STANDARD OF PESTICIDES												
Atrazine d5	509.33	99	19	1352.5	451	33	1630.4	183	11.23	1728.6	715	41
% Repeatability	32.5			70.0			80.0			92.5		

ND = Not detected, SD = Standard Deviation, %CV = coefficient of variation

3.4 Reproducibility

Analysis of the reproducibility data in Table 4 shows that the percentages of validated reproducibility tests vary from 67.7 to 97.5 %. The low percentages are found at the concentration of 10 ppb. Overall, the reproducibility percentages of the assays are acceptable and are considered satisfactory for validating the method and for use in real-world analyzes.

Table 4. Reproducibility test results

TESTS	10 ppb			50 ppb			100 ppb			500 ppb		
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
NAMES OF COMPOUNDS												
OF						PAHs						
Acenaphthene	722	48.477	6.714	3295.104	116.339	3.531	6615.2	4.324	0.065	29819.92	11.573	0.039
Acenaphthylene	2154.84	53.364	2.476	7864.936	89.092	1.133	15037.8	7381.269	49.085	87893.76	59.412	0.068
Anthracene	20.74	1.8105	8.730	646.572	9.661	1.494	1553.72	5.186	0.334	6885.48	1.411	0.020
Benzo [a] Anthracene	3.2	3.962	123.823	161.416	2.517	1.560	496.844	4.845	0.975	3813.08	1.724	0.045
Benzo [b] Fluoranthene	234.26	27.857	11.892	4196.78	19.225	0.458	6568.6	7.403	0.113	39536.84	2.930	0.007
Benzo [k] Fluoranthene	77.92	4.912	6.304	4413.55	22.774	0.516	8182.8	92.815	1.134	38570.96	5.111	0.013
Benzo [g, h, i] Perylene	27.36	1.585	5.794	1927.2	5.933	0.308	3763	12.042	0.320	18836.6	12.602	0.067
Benzo [a] pyrene	354.18	16.812	4.747	1134.796	3.618	0.319	2448.8	9.654	0.394	11240.08	33.682	0.300
Chrysene	2	2.549	127.475	1403.6	272.381	19.406	2317.8	5.630	0.243	12425	39.779	0.320
Dibenzo [a, h] anth	2.6	3.435	132.120	635.952	2.690	0.423	2185	8.689	0.398	11430.36	39.235	0.343
Fluoranthene	392.904	8.750	2.227	2434.24	4.625	0.190	5763.4	21.973	0.381	27361.04	14.294	0.052
Fluorene	57.714	2.631	4.560	685.84	12.224	1.782	1630.2	3.271	0.201	7946.8	9.680	0.122
Indeno [1,2,3-Cd] Pyrene	36.696	4.911	13.383	707.4	7.127	1.007	2100	9.165	0.436	10760.8	22.830	0.212
Naphthalene	1066.78	59.936	5.618	3699.96	9.111	0.246	7721.8	44.059	0.571	33472.48	31.544	0.094
Phenanthrene	46.3	4.872	10.524	885.8	9.445	1.066	1744	31.313	1.796	8843.8	11.498	0.130
Pyrene	252.306	5.784	2.293	1346	3.808	0.283	2874.6	17.473	0.608	14471.4	17.170	0.119
INTERNAL STANDARDS OF PAHs												
Naphthalene d8	6965.858	49.045	0.704	6741.8	4.817	0.071	7455.4	28.763	0.386	7365.12	39.062	0.530
Acenaphthene d10	151.552	1.701	1.122	553.05	4.705	0.851	594.6	22.645	3.808	672.6	18.379	2.733
Pyrene d10	17090.86	8390.001	49.091	22435.378	42.803	0.191	59211.4	93653.195	158.167	21718.6	175.005	0.806
Benzo [a] pyrene d12	9012.206	95.297	1.057	8973.2	55.585	0.619	8338.2	26.167	0.314	9423.08	4.672	0.050
Perylene d12	8038.76	105.221	1.309	8361.372	22.474	0.269	6896.4	3414.184	49.507	8206.92	53.771	0.655
Benzo [g, h, i] Perylene d12	7892.992	124.115	1.572	7563.754	21.947	0.290	7844	216.669	2.762	8129	38.588	0.475
PESTICIDES												
Aldrin	12	5.788	48.233	680.2	12.677	1.864	1843	100.628	5.460	11381.2	89.452	0.786
Alpha BHC	31.344	1.171	3.735	1271.2	6.906	0.543	3107.56	22.651	0.729	13116.2	8.843	0.067
Beta BHC	110.2	7.629	6.923	985.32	3.670	0.372	2550.2	32.438	1.272	12409	54.397	0.438
Delta BHC	3.8	3.564	93.782	776.6	10.899	1.403	2060	117.847	5.721	5078.93467	0.595	0.012
Dieldrin	2.8	1.923	68.698	298.298	5.711	1.914	1074.774	82.856	7.709	6599.6	57.134	0.866
Alpha endosulfan	12	5.788	48.233	2	2.549	1.275	13.2	4.970	37.651	3867.6	20.744	0.536
Beta endosulfan	3.4	3.781	111.222	348.2	7.497	2.153	1068.2	32.011	3.00	5367.6	21.478	0.400
Endosulfan sulfate	22.98	1.988	8.651	397.178	12.036	3.030	752.6	10.164	1.350	5445.2	8.585	0.158
Endrin	4	3.391	84.779	112.4	5.413	4.816	273.4	23.818	8.712	2617.4	16.994	0.649
Endrin aldehyde	12	5.788	48.233	825.2	14.601	1.769	834	0	0	7047.4	30.171	0.428
Endrin ketone	2.8	2.049	73.192	1423.2	43.234	3.038	2677.4	46.377	1.732	11376.8	84.221	0.740
Gamma BHC	115.7	3.493	3.019	596.6	8.649	1.450	2245	11.180	0.498	11709.8	167.512	1.430
Heptachlor	2	1.581	79.057	244.8	6.457	2.638	1106.2	15.254	1.379	9641.4	31.604	0.328
Heptachlor epoxide	78.554	1.224	1.558	1158.6	42.253	3.647	2300.6	7.537	0.328	15022	13.038	0.087
Methoxychlor	155.472	2.794	1.797	2037.8	27.344	1.342	5133	144.477	2.815	51346.8	25179.692	49.038
4,4'-DDD	950.622	34.186	3.596	4875	2389.817	49.022	11704.4	464.696	3.970	64080	88.031	0.137
4,4'-DDE	14.8	5.541	37.437	70.8	2.775	3.919	117.32	4.314	3.677	3095.2	71.733	2.318
Aldrin	112.686	5.339	4.738	1298.4	11.929	0.919	3400.2	227.912	6.703	40565.8	6.340	0.016
INTERNAL STANDARD OF PESTICIDES												
Atrazine d5	509.616	1.954	0.383	1351.2	6.760	0.500	1636.6	12.361	0.755	1728.2	7.6941	0.445
% Reproducibility	67.5			97.5			92.5			97.5		

ND = Not Detected, SD = Standard Deviation, CV = Coefficient of Variation, LOD= Limits of Detection, LOQ= Quantification

Table 5 shows the values of the detection and quantification limits and the percentages of recovery

Table 5. Limits of Detection (LOD), Limits Of Quantification (LOQ), and Recovery percentages (% R)

NAMES OF COMPOUND	Detection Limits (LOD)	Quantization Limits (LOQ)	Recovery Percentage
	« $\mu\text{g/g}$ »	« $\mu\text{g/g}$ »	(%R)
Acenaphthene	0.037	0.123	99
Acénaphylène	0.120	0.401	100
Anthracene	0.021	0.069	99
Benzo [a] Anthracene	0.016	0.053	98
Benzo [b] fluoranthene	0.012	0.039	110
Benzo [k] Fluoranthene	0.013	0.045	100
Benzo [g, h, i] perylene	0.019	0.065	99
Benzo [a] pyrene	0.017	0.058	110
Chrysene	0.012	0.041	99
Dibenzo [a, h] anth	0.026	0.087	108
Fluoranthene	0.022	0.075	108
Fluorene	0.013	0.045	99
Indeno [1,2,3-Cd] Pyrene	0.021	0.070	107
Naphthalene	0.104	0.345	109
Phenanthrene	0.002	0.008	98
Pyrene	0.012	0.040	100
Aldrin	0.071	0.238	100
Alpha BHC	0.039	0.129	109
BHC Beta	0.079	0.263	100
Delta BHC	0.023	0.078	107
Dieldrin	0.081	0.271	108
Alpha Endosulfan	0.133	0.445	98
Beta Endosulfan	0.070	0.234	100
Endosulfan Sulfate	0.085	0.284	100
Endrin	0.156	0.521	97
EndrinAldehyde	0.121	0.403	106
EndrinKetone	0.034	0.115	100
Gamma BHC	0.073	0.244	99
Heptachlor	0.138	0.461	98
HeptachlorEpoxide	0.086	0.282	100
Methoxychlor	0.141	0.468	98
4,4'-DDD	0.062	0.206	100
4,4'-DDE	0.194	0.645	91
4,4'-DDT	0.139	0.462	99

An analysis of the data shows that LODs range from 0.002 $\mu\text{g/g}$ to 0.194 $\mu\text{g/g}$. LOQs range from 0.008 to 0.645 $\mu\text{g/g}$. The lowest LOD is obtained for phenanthrene while the highest value is found in 4,4'-DDE. The percentages of recovery vary between 91 and 110 for respectively 4,4'-DDE and Benzo [a] pyrene. Salem et al., (2016) under conditions similar to ours for a multiresidue assay of 16 PAHs, 12 PCBs and 9 OCPs in the sediments, obtained R^2 coefficient of determination greater than 0.95 for all the compounds analyzed. The quantification limits (LOQ) found by these authors vary between 0.02 and 9.64 ppb. These differences would be related to the state of the columns and detectors used. The column in our tests is relatively old which would be the cause of the limits of detection and quantification too high. Despite these high limits of detection and quantification the method has good linearity, good repeatability and reproducibility for concentrations greater than 10 ppb and can therefore validly be used in real-world for investigations. As such, the internal calibration has been used.

3.5 Application of the Method to the Analysis of Lake Nokoué Water Samples

The application of the validated method allowed us to search and to dose these two (02) families of contaminants sought in the waters of Lake Nokoué. The analysis results are shown in Table 6.

Table 6. Lake Nokou éwater analysis results

Names of compounds	[MLA1] in ppb	[MLA2] in ppb	[MLA3] in ppb
Acenaphtene	2.492	2.894	2.503
Ac énaphtyl ène	ND	1.679	0.294
Anthracene	0.046	0.115	0.131
Benzo [a] Anthracene	219.31	0.009	0.017
Benzo [b] fluoranthene	0.0001	ND	ND
Benzo [k] Fluoranthene	0.086	ND	ND
Benzo [g, h, i] perylene	0.008	0.0188	0.025
Benzo [a] pyrene	ND	ND	ND
Chrysene	ND	ND	ND
Dibenzo [a, h] anth	0.068	0.159	0.173
Fluoranthene	1207.332	ND	ND
Fluorene	0.677	0.865	1.23
Indeno [1,2,3-cd] pyrene	0.037	0.041	0.037
Naphthalene	10.225	5.796	10.516
Phenanthrene	2.073	0.821	1.309
Pyrene	ND	ND	ND
Aldrin	0.119	0.119	0.120
Alpha BHC	10.209	ND	ND
BHC Beta	11.371	0.15	0.149
Delta BHC	109.280	ND	ND
Dieldrin	12.269	0.167	0.17
Alpha Endosulfan	37.976	0.493	0.49
Beta Endosulfan	0.093	0.119	0.111
Endosulfan Sulfate	0.206	0.206	0.206
Endrin	20.137	0.615	0.606
EndrinAldehyde	0.399	0.399	0.399
EndrinKetone	45.784	ND	ND
Gamma BHC	13.291	0.099	0.094
Heptachlor	42.940	0.533	0.518
HeptachlorEpoxide	0.184	0.184	0.184
Methoxychlor	0.518	0.521	0.5189
4,4'-DDD	4.209	0.0463	0.046
4,4'-DDE	19.324	0.809	0.813
4,4'-DDT	38.829	0.522	0.554

ND: Not Detected

From the analysis of the data in Table 5, we note at the level of PAHs that, out of 16 molecules sought in the lake, three (03) were not detected. Nine (09) out of sixteen PAH molecules were detected at all sites. Contamination levels vary from not determined to 1207.33 ppb on the MLA1 site.

At the level of the OCPs, if on the MLA1 site all the eighteen (18) searched molecules are found, at the MLA sites 2 and 3, three (03) molecules were not detected. These are Delta BHC, EndrineKetone and Alpha BHC. So a total of 15 molecules out of 18 OCPs were detected and quantified at all sites. That is a percentage of 83% of OCPs. The origin of PAH compounds in Nokou éLagoon are material combustion and fuels for polycyclic aromatic hydrocarbons and due to remoteness pollution for organochloride pesticide compounds (Soclo, 2008; Yehouenou, 2006a & b).

4. Conclusion

Whatever the external or internal calibration, the values of the coefficient of determination R^2 obtained are between 0.98 and 1. These values demonstrate that the method has good linearity in the chosen calibration range. For the repeatability the validation percentages vary from 32.5 to 92 %. The low percentages are the repeatability tests for the 10 ppb concentration. This state of affairs would be related to a loss of analyte during the dry extracts. Overall, the reproducibility percentages of the assays are acceptable and are considered satisfactory for validating the method and for use in real-world analyses. For all the molecules sought, the percentage of recovery varies between 91 and 110 % corresponding respectively to Benzo [b] fluoranthene and Benzo [a] pyrene.

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