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Development and validation of multi-residue method for the determination of persistent organic pollutants in soil by gas-chromatography-tandem-mass-spectrometry

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ABSTRACT

A multi-residue method capable of determining simultaneously organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in soil samples by gas-chromatograph-tandem-mass-spectrometry was developed, optimized and validated. Soil samples were extracted by two optimized extraction techniques; sonication and mechanical shaking with acetonitrile as extracting solvent. The extract was cleaned-up with solid phase extraction cartridge of amino sorbent topped with 1g anhydrous magnesium sulfate to remove matrix interferences and residual moisture, respectively. Final determination of extract which contains 1% polyethylene glycol-200 in ethyl acetate to enhance and improve separation was done by gas chromatography (GC) with mass spectrometric detection (MSD). Average recoveries by the GC"MS/MS validated method varied from 70% to 110% with relative standard deviation between 2.0% and 12.0% for amino sorbent cartridge. The method presents good linearity over the range of 0.005"1.0µg/mL, and the quantification limit for the analytes studied varied from 0.1 to 1.0 µg/kg. The method proved to be very rugged for other related soil types. The proposed method shows practical environmental and economic advantages in terms of sample processing time, simplicity, relatively safer and reduced organic solvent use and cost, and is particularly suitable for routine applications requiring a high sample throughput.

KEYWORDS

OCPs;
PCBs;
PAHs;
Soil;
Gas chromatography;
Mass spectrometry.

INTRODUCTION

Soil acts as a reservoir for many environmental contaminants. This is as a result of direct introduction (that is, pesticide application,application of sewage sludge or compost, spills, and contaminated water irrigation) or indirect by atmospheric deposition (i.e. emissions from industry, traffic or incom-

plete combustion of fossil fuels, including petroleum and coal) of contaminants into surface soil[1-2]. These contaminants may be inorganic or organic. Heavy metals and asbestos are inorganic contaminants, whilst most pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, some commercial and industrial wastes may be classified as organic contaminants. However, some of these organic contaminants such as the typical organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs), and polycyclic aromatic hydrocarbons (PAHs) are also considered persistent organic pollutants[3-5]. Persistent organic pollutants (POPs) are a group of toxic substances, which are semi-volatile, mobile in the environment, and prone to long-range transport, accumulation in abiotic matrices as well as bioaccumulation in living organisms[4]. POPs have been implicated in a broad range of adverse human health and environmental effects including reproductive failures and birth defects, immune system dysfunction, endocrine disruption and cancers[6-7]. They have the ability to remain without change in the environment for a long period of time[8-9]. They are resistant to chemical, photochemical, thermal and biochemical decomposition. Thus, the persistent nature of these organic pollutants allows their circulation and accumulation in environmental matrices such as soils, sediments and in living organisms[4].

For the determination of organic pollutants in environmental solids, it is imperative to develop extraction and clean-up techniques capable of determining simultaneously OCPs, PCBs and PAHs in the soil. Such multi-residues method must also employ the use of relatively safer organic solvents, with minimal use of solvents and also must have applicability for routine use in terms of analytical procedures and duration of their analysis.

Determination of organic residues in soil media presents with some complications, especially in extraction and clean up steps due to the complex nature of soil samples[10]. Extraction techniques such as soxhlet (SE), solid phase micro-extraction (SPME), ultra-sonication (ULS), flask-shaking (FS), microwave assisted extraction (MAE), microwave assisted soxhlet extraction (MASE), pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) have all been reported for organic contaminants analysis in soil[11-17]. Each of these extraction techniques has its own advantages and limitations[18]. Among the extraction techniques, soxhlet is the most frequently used because it has been adopted in many standardized analytical methodologies for determining organic contaminants in soils[11]. However, this technique uses drastic conditions that often destroy the structural integrity of some organic contaminants[16], and also requires long extraction hours and utilization of large volumes of hazardous organic solvent[18]. High sample throughput and relatively short extraction times are associated with MAE and ULS; however, due to the high microwave energy involved in MAE, degradation of some pesticides has been reported[16]. MASE is an upgraded version of soxhlet, incorporating microwave energy into soxhlet technique; thus same limitation of degradation of some pesticides could also be expected. According to the international organization for standardization (ISO), flask-shaking or mechanical shaking is a reference technique for the determination of PAHs in all types of soil at both low and high levels of contaminations. However, relatively large volumes of acetone and petroleum ether are simultaneously used as extracting solvents in this technique[19]. PLE and SFE are among the new techniques for environmental solid analysis; however, they cannot be used for multi-residue determination of organic pollutants. Moreover, the use of hot solvents for these methods has been shown to result in relatively very low recoveries, especially in the analysis of time-aged soils[20-21]. In the case of SPME, it is not very common technique for organic residue analysis in soil[18].

For detection and quantitation of organic contaminants in soil, gas chromatograph (GC) mass spectrometer (MS) has been used; however, GC coupled with some selective detectors such as nitrogen phosphorous detector (NPD), electron capture detector (ECD) and flame photometric detector (FPD) has also been used[12][22][23]. Liquid chromatography with ultra-violet detector and fluorescent detection has been employed for organic trace analy-

sis of less volatile and/or thermally instable and/or polar organic contaminants for GC. However, liquid chromatography coupled with tandem mass spectrometer is gaining more grounds in modern organic contaminants determinations[22-25]. The selection of an organic residue method depends on certain factors which includes the efficiency of extraction of the different contaminants from the various types of samples, the chemical properties of the contaminants such as the solubility and distribution coefficients in solvent systems of different polarity, contaminants elution patterns in chromatographic systems, the specificity and sensitivity of the method of detection and the availability of method inputs[26]. In the present work, two extraction techniques namely, ultra-sonication and continuous flask shaking were chosen using acetonitrile as extraction solvent, optimized and validated for the simultaneous determinations of OCPs, PCBs and PAHs in soil samples using gas chromatography tandem mass spectrometry.

EXPERIMENTAL

Materials and standards

Certified reference chemicals (Individual OCPs; Beta-HCH [BHC], Lindane [GHC], Delta-HCH [DHC], Heptachlor [HEP], Aldrin [ALD], Gamma-chlordane [GCH], Alpha-endosulfan [AEN], Beta-endosulfan [BEN], Endosulfan sulfate [ENS], p,p'-DDD [PDD], p,p'-DDE [PDE], p,p'-DDT [PDT], Endrin END], Dieldrin [DIE] and Methoxychlor [MET]; 10ug/mL mixed polychlorinated biphenyls: PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180; and 10µg/mL mixed polycyclic aromatic hydrocarbon standards: Naphthalene [NAP], Acenaphthalene [ACA], Acenaphthene [ACE], Fluorene [FLU], Phenanthrene [PHE], Anthracene [ANT], Fluoranthene [FLT], Pyrene [PYR], Benzo(a)anthracene [BAA], Chrysene [CHR], Benzo(b)fluoranthene [BBF], Benzo(k)fluoranthene [BKF], Benzo(a)pyrene [BAP], Indeno(1,2,3-c,d)pyrene [IND], Dibenzo(a,h)anthracene [DAA] and Benzo(g,h,i)perylene [BGP]) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) with purity ranging from 94% to 100%. Reagents used in the study comprised the following: Acetonitrile, Ethyl Acetate, Acetone, Polyethylene Glycol-200, Anhydrous Sodium Sulfate and Anhydrous Magnesium Sulfate were pesticide residue grade from BDH Laboratory Supplies, England); SampliQ Amino (NH_2), 500mg/6mL and Mega BE-FL, 1000mg/6mL from Agilent Technologies, USA; Strata Si-1, Silica (55um, 70A), 1000mg/6mL was from Phenomenex, USA.

Pesticides stock solutions (1000µg/mL) of individual OCPs standards were prepared by dissolving 25mg corrected by purity of the pesticide in 25mL of ethyl acetate. Pesticide intermediate standard solution (10µg/mL) was prepared by transferring 250µL from each pesticide stock solution to a 25 mL volumetric flask and diluting to the mark with ethyl acetate. A mixed standard solution (1.0µg/mL) containing the fifteen selected OCPs, seven indicator PCBs and sixteen PAHs was prepared by transferring 1mL of each 10µg/mL mixed OCPs, PCBs and PAHs into a 10mL volumetric flask and adding ethyl acetate to make up the mark. Several standard solutions from these, with concentrations ranging from 0.005 –1.0µg/mL, were injected to obtain the linearity of detector response and the detection limits of the chemicals studied.

Apparatus

For the extraction of samples, 100mL separating funnel (Fisherbrand Glass), Horizontal mechanical shaker (Ika-Werke HS 501 Digital), 1000watts ultra-sonic bath (Grant XUB 18UK) and a centrifuge (Thermo/CR3i Multifunction) were used. A 24 pots SPE vacuum manifold (Phenomenex, USA) and a Buchi model R-210 rotavapor with circulating water chiller (Buchi, B-740) were used in extract clean-up and evaporation to just dryness of extracts, respectively.

A Varian CP-3800 gas chromatograph (Varian Associates Inc. USA) equipped with 1177 split/splitless type injector,8400 Varian autosampler and a Saturn 2200 mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 80 eV, scanning from m/z 40 to 450 at 2.0 s per scan. The optimized ion trap tem-

perature was 220°C and that of the transferline and manifold temperatures were 260°C and 80°C, respectively. The electron multiplier voltage (EM voltage) was maintained at 1650V, and a solvent delay of 3min was employed. Operating conditions were as follows: injector temperature was 270°C; helium was used as carrier gas (at 1.3mL/min constant pressure). The analytical capillary column (VF- 5ms, 30 m x 0.25 mm id., 0.25 um film thickness) temperature was maintained at 80°C for 1min and then programmed at 25°C/min to 180°C followed by a final ramp to 300°C at a rate of 5°C/min, and held for 1min. The total analysis time was 30min. The volume of sample extract injected in splitless mode was 2uL. The concentration of each compound was determined by comparing the peak areas in the sample with those found for mixtures of analytes standards of known concentration.

Analysis was performed with selected ion monitoring (SIM) mode using primary and secondary target and qualifier ions (TABLE 1). Analyzed chemicals detected were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier-to-target ion ratios. Retention times had to be within ±0.1 min of the expected time, and qualifier-to-target ratios had to be within a 10% range for positive confirmation.

Sample collection and processing for fortification

Beach soil samples were collected into zip lock plastic bags and transported to the laboratory for sample processing. For processing, soil samples were transferred into Pyrex beaker and placed in an oven overnight at 150°C. In addition, other soil type (clay, silt and loamy) were collected to check for robustness of the method. The dried soil samples were then sieved through a 2mm stainless steel sieve with the aid of a mechanical shaker, and foreign materials were removed. Fortification levels typically at the limit of determination of 10µg/kg and at levels of 100µg/kg and 50µg/kg or at levels concomitant with expected residue levels were made.

Extraction and clean-up procedure

Extraction

A 10.0g of comminuted homogenous soil sample was weighed and transferred into a 100 mL separating flask. A measured 10mL acetonitrile was added and the corked flask sonicated for 5min. After that, a further 10mL of acetonitrile was added, and the flask placed on the horizontal mechanical shaker and was shook continuously for 30min at a rate of 300mot/min. The supernatant (organic layer) was carefully transferred into 50mL centrifuge tube for centrifugation at 3000rpm for 5min. An aliquot,10 mL of the organic phase (top layer) equivalent to 5.0g soil weight was pipetted and passes over 5.0g anhydrous sodium sulphates into a 50mL round-bottom flask. Then, 5mL of acetonitrile was used to rinse the salt into the round-bottom flask. This was then evaporated to about 1mL using the rotary film evaporator set at 35°C prior to extract purification.

Extract clean-up

SampliQ Amino (NH_2) (500mg/6mL) cartridge which has 1g of anhydrous magnesium sulfate weighed on top was conditioned using 6mL acetonitrile. A 50mL pear shape flask was placed under the column in a vacuum manifold, and the extract loaded onto the cartridge. The extract was allowed to filter and the cartridge eluted with 10mL (2 x 5mL) of acetonitrile with slight intermittent vacuum use. The eluate was then concentrated just to dryness using the rotary film evaporator set at 35°C. A 20µL of 1% polyethylene glycol-200 in ethyl acetate and 980µL of ethyl acetate were added to the flask to redissolve the extract, and the dissolved extract carefully transferred into labeled 2mL GC standard opening vial prior to quantification on the GC-MS.

Quality assurance

All solvents used were analytical grade or of ultra-high purity. Prior to analysis, all glassware was rinsed with acetone. All reagents used during the analysis were exposed to same extraction procedures and solvents used were run to verify for any interfering substances within the runtime. In all batches of contaminant residues analysis; reagent blanks, procedural matrix blanks and triplicate samples were included. For the reagent blank in each extraction procedure; no or sufficiently low values were obtained for the analytes of interest (Figure

TABLE 1 : Validation results: linear range, regression coefficient, average recovery, reproducibility, limits of detection and quantification, quan ion and qualifier ions

COMPOUNDS	Linearity	R^2	%Recovery	%RSD	LoD	LOQ	Quan ion	Qual. ions
	(µg/mL)		n=18		(µg/kg)	(µg/kg)	m/z	m/z
OCPs:								
Beta-HCH	0.005 - 0.5	0.998	85	5.2	0.02	0.1	183.1	181.2/218.9
Lindane	0.005 - 0.5	0.999	92	2.0	0.03	0.1	183.1	181.2/218.9
Delta-HCH	0.005 - 0.5	0.991	87	7.1	0.03	0.1	183.1	181.2/218.9
Heptachlor	0.005 - 0.5	0.995	81	2.2	0.03	0.1	272.2	274.1/100.2
Aldrin	0.005 - 0.5	0.999	95	2.1	0.03	0.1	66.2	263.3/293.1
Gamma-chlordane	0.005 - 0.5	0.999	85	8.9	0.02	0.1	375.2	373.2/377.1
Alpha-endosulfan	0.005 - 0.5	0.998	82	2.7	0.03	0.1	241.3	269.2/243.2
P,P'-DDE	0.005 - 0.5	0.995	99	5.1	0.03	0.1	246.3	79.2/318.2
Dieldrin	0.005 - 0.5	0.997	87	3.2	0.03	0.1	79.2	81.2/279.2
Endrin	0.005 - 0.5	0.996	95	2.4	0.03	0.1	317.1	281.2/67.2
P,P'-DDD	0.005 - 0.5	0.996	92	3.0	0.03	0.1	235.3	237.2/165.3
Beta-endosulfan	0.005 - 0.5	0.998	85	7.0	0.03	0.1	195.2	243.1/269.2
P,P'-DDT	0.005 - 0.5	0.993	93	2.5	0.02	0.1	235.5	237.2/282.2
Endosulfan sulfate	0.005 - 0.5	0.999	97	5.9	0.03	0.1	272.3	274.2/387.1
Methoxychlor	0.005 - 0.5	0.999	88	2.1	0.02	0.1	227.3	240.3/228.3
PCBs:								
PCB-28	0.005 - 0.5	0.998	92	3.4	0.03	0.1	258.2	256.3/221.3
PCB-52	0.005 - 0.5	0.994	89	7.2	0.04	0.1	258.2	256.5/186.3
PCB-101	0.005 - 0.5	0.998	98	2.9	0.03	0.1	292.2	290.3/257.4
PCB-118	0.005 - 0.5	0.999	85	2.0	0.03	0.1	326.3	328.1/330.1
PCB-138	0.005 - 0.5	0.993	93	2.9	0.03	0.1	360.3	362.2/358.7
PCB-153	0.005 - 0.5	0.998	89	3.5	0.03	0.1	360.3	362.2/364.0
PCB-180	0.005 - 0.5	0.997	101	9.7	0.03	0.1	396.3	394.3/398.0
PAHs:								
Naphthalene	0.01 – 1.0	0.991	85	7.8	0.30	1.0	128.1	127.2/102.2
Acenaphthalene	0.01 – 1.0	0.996	75	3.9	0.30	1.0	152.2	151.3/153.2
Acenaphthene	0.01 – 1.0	0.992	90	5.4	0.30	1.0	153.3	154.1/76.3
Fluorene	0.01 – 1.0	0.994	105	10.8	0.30	1.0	165.2	166.2/167.1
Phenanthrene	0.01 – 1.0	0.998	71	5.1	0.30	1.0	178.2	179.1/176.3
Anthracene	0.01 – 1.0	0.999	86	7.5	0.30	1.0	178.2	179.1/176.3
Fluoranthene	0.01 – 1.0	0.991	93	3.6	0.30	1.0	202.2	201.5/200.7
Pyrene	0.01 – 1.0	0.994	90	5.3	0.30	1.0	202.2	203.2/201.6
Benzo(a)anthracene	0.01 – 1.0	0.993	82	11.7	0.30	1.0	228.3	229.2/113.3
Chrysene	0.01 – 1.0	0.992	96	9.2	0.30	1.0	228.3	229.2/114.3
Benzo(b)fluoranthene	0.01 – 1.0	0.995	102	3.7	0.30	1.0	252.5	253.2/126.4
Benzo(k)fluoranthene	0.01 – 1.0	0.991	108	4.7	0.30	1.0	253.5	252.5/126.5
Benzo(a)pyrene	0.01 – 1.0	0.992	94	2.1	0.30	1.0	252.5	253.3/126.4
Indeno(1,2,3-c,d)pyrene	0.01 – 1.0	0.994	75	10.3	0.60	2.0	276.6	277.5/138.7
Dibenzo(a,h)anthracene	0.01 – 1.0	0.991	89	11.4	0.60	2.0	278.6	281.3/276.6
Benzo(g,h,i)perylene	0.01 – 1.0	0.992	110	10.9	0.30	1.0	276.6	138.5/277.5

R2 = Regression coefficient; LoD = limit of detection; LOQ = limit of quantification; RSD = relative standard deviation; n = number of observations

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4). However, any blank value was subtracted from the reading of any sample readings. All extracts were kept frozen at -18°C or lower until quantification was achieved. Recalibration curves were run with each batch of samples to check that the regression coefficient was kept around r^2=0.990. The instrument detection limits were obtained from twenty matrix blanks. The mean and the standard deviation of the blanks were calculated, and the detection limits determined by the addition of three times the standard deviation to the mean of the blank. The method used was optimized and validated using various soil types.

A fortification level of 50 µg/kg of standards mixture was chosen before analysis to evaluate the recovery of compounds in the soil samples analysed. Fortified samples were determined with good recoveries. The mean recoveries of chemical residues ranged between 70% and 110% for most of the contaminants analyzed (Figure 1). This was deemed satisfactory as far as it fell within the lower limit of 70% and the upper limit of 120%.

RESULTS AND DISCUSSION

Method optimization

For the optimization of this method, certain parameters were studied including duration of extraction, volume of organic solvent used, sorbent selection, elute concentration and MS/MS instrumentation.

Extraction

Preliminary extraction studies were made between ultra-sonication and continuous mechanical shaking. Two types of analyses were carried out respectively; one after a known amount of the standards of interest (spiked sample) were added and the other without the addition of the standards (blank sample). The analytes of interest for this method span from organochlorine pesticides, polychlorinated biphenyls to polycyclic aromatic hydrocarbons which are generally lipophilic compounds. In line with the objective of this study, acetonitrile was chosen as the extraction solvent which is considered to be safer compared to dichloromethane and other organic sol-

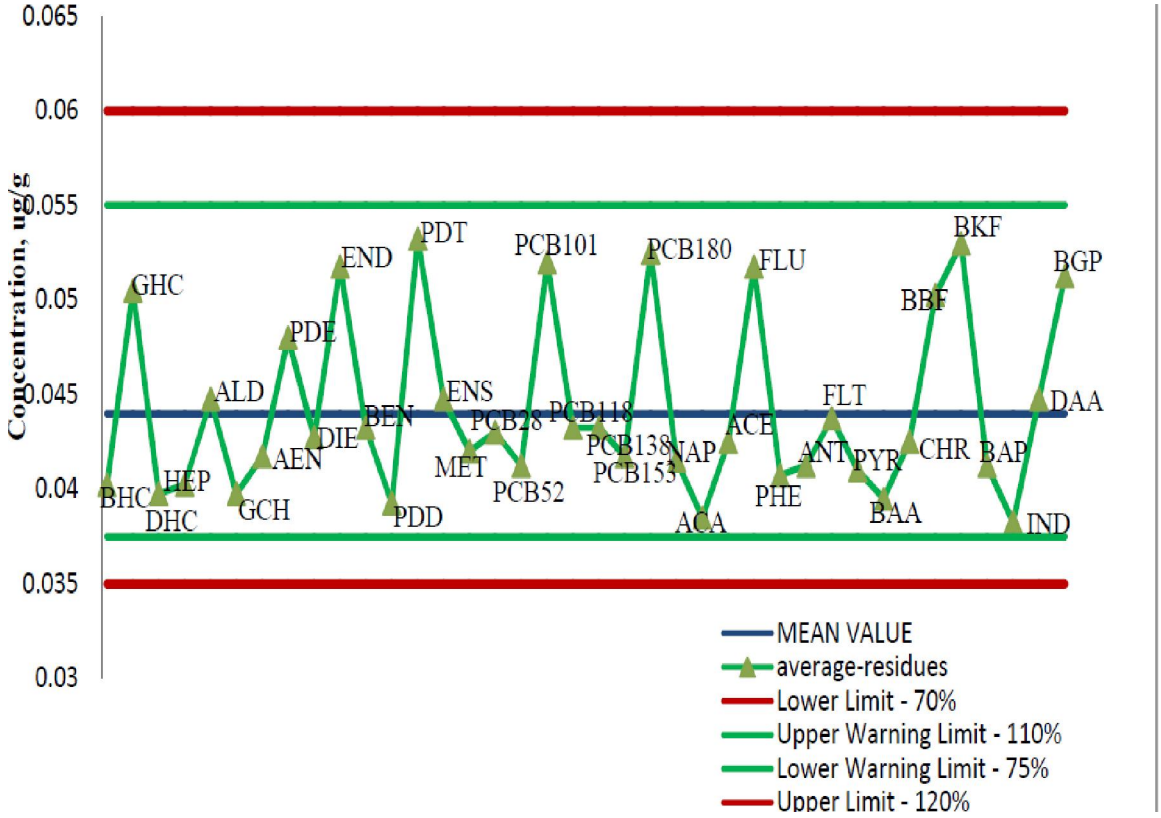

Figure 1 : Control chart for analytes of interest [Organochlorine pesticides (OCPs), Polychlorinated biphenyls (PCBs) and Polycyclic aromatic hydrocarbons (PAHs)]

vents widely used in soil analysis. For sample extraction time comparison, it appeared 60min ultrasonication extraction alone of the dried beach soil samples gave better results compared with 60min of continuous mechanical shaking extraction technique for the soil samples. And thus, combination of the two extraction techniques was considered for optimization. It was observed that there exists a gradual increment in recovery for the same level of spiked soil samples with ultra-sonication extraction durations of 1 to 5min. Similar scenario was also observed for same spiked soil samples using continuous flask shaking with extraction times of 5, 10, 20 and 30min. A comparison of 30min continuous flask shaking with 5min ultra-sonication and 60min continuous flask shaking with 5min ultra-sonication was made. The results indicated that the 30min duration of extraction procedure gave higher throughput for most of the analytes of interest than that of the 60min duration of extraction procedure (Figure 2).

For optimal organic solvent volume of extraction, it was realized that 20 mL of acetonitrile was just enough for a 10 g soil sample extraction. However, application of the method could be extended to certain dry sediment samples where 30 mL acetonitrile would be sufficient as total extraction solvent volume.

Clean-up

With the complex nature of soil samples in mind, and considering all the analytes of interest; three solid phase extraction sorbents were chosen: Amino (NH_2), Florisil (Fl) and Silica (Si). All these sorbents have been applied widely in the research of environmental pollutants, and they proved to be suitable for residue extraction from soil and other solid samples[27]. Initial extraction experiments were first conducted with all sorbents to evaluate the efficiency of each cartridge in the analysis of the target analytes. Recoveries showed that all cartridges gave good recovery percentages (higher than 70%) for most of the target analytes; however, Amino (NH_2)SPE was slightly better than silica, then florisil in that order of recovery for most of the target analytes (Figure 3). The mean recoveries from these three sorbents were subjected to a one-way analysis of variance to ascertain any statistical significance. The results proved statistical evidence of significant differences in mean recoveries of the three sorbents at 95% confidence interval with F-value of 30.301 falling out of the critical value of 3.765. Thus, though all three sorbents gave mean recoveries greater than 70% for most of the analytes of interest, statistically there exist significant differences between them in terms of their mean recoveries.

The addition of 1 g of anhydrous magnesium sulfate on top of the cartridge was very effective in mopping up residual moisture and gave clearer extracts than same quantity of anhydrous sodium sulfate. It was realized that 5 mL acetonitrile was only able to elute about 65% of the analytes from the car-

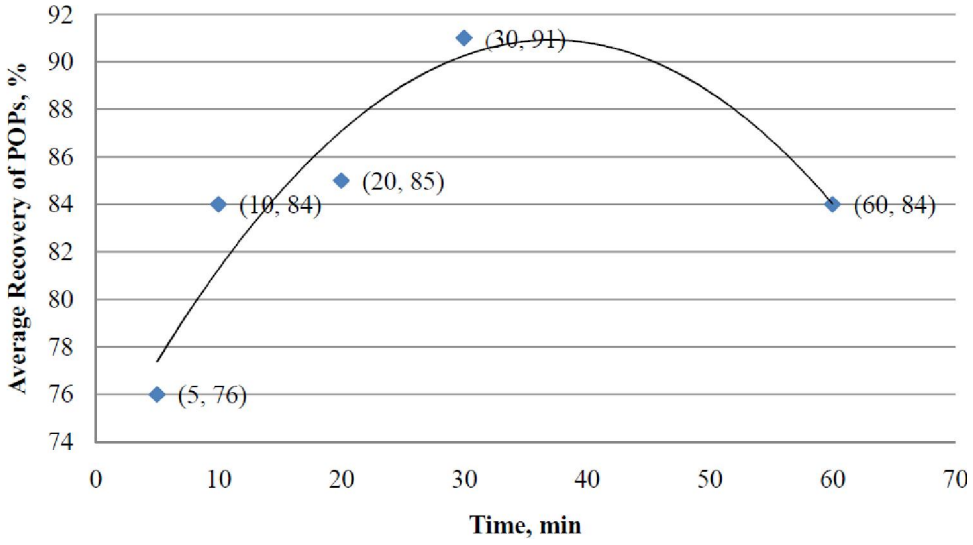

Figure 2 : Trend analysis - demonstration of effective duration for continuous flask shaking extraction

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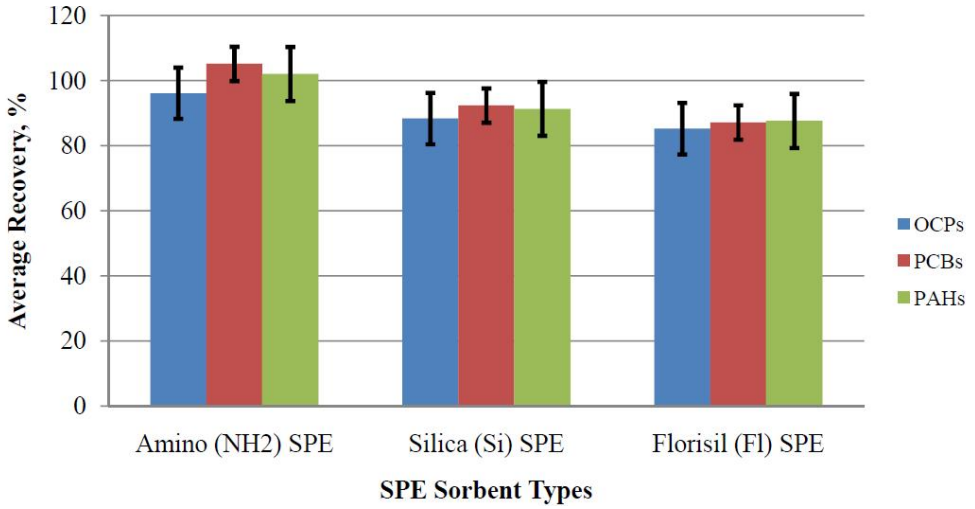

Figure 3 : SPE sorbent clean-up efficiency

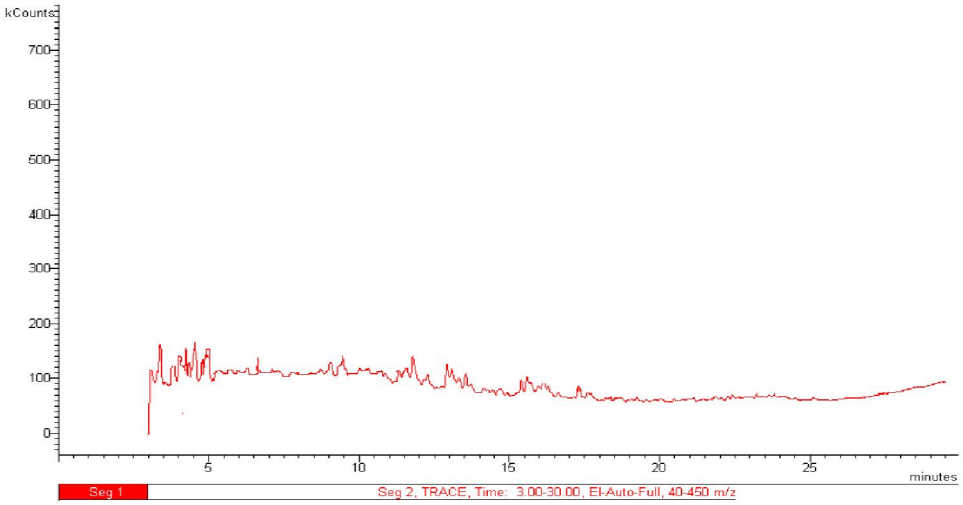

Figure 4 : Typical chromatogram of matrix blank

tridges; however, 10 mL acetonitrile in two folds (2x) 5 mL was sufficient to elute about 95% of all the analytes of interest from the cartridges.

Elute concentration with polyethylene glycol-200

Polyethylene glycol (PEG) is a polyether compound with many applications. Its application cut across; from industrial manufacturing as binders in technical ceramics preparation[28] to medicines for use as a base for a number of laxatives[29]. In this study, PEG-200 was employed to enhance the separation ability and improve the elution of analytes from the stationery phase of the analytical capillary column. Thus, PEG-200 in this case reduces the affinity of the stationery phase for the analytes of interest, and thereby increasing the sensitivity of the method.

To demonstrate this, an extract was divided into two equal halves. After rotary evaporation of the extracts to dryness, 20 µL of 1% PEG-200 in ethyl acetate and 980 µL of ethyl acetate were added to re-dissolve the extract; while in the other extract, only ethyl acetate (1000 µL) was used to re-dissolve and transferred into standard 2 mL GC vials. The results showed that the extract containing the 1% PEG-200 gave higher/better recovery concentrations of the analytes of interest than the extract without the PEG-200.

GC/MS/MS optimized parameters

Optimization of GC/MS/MS was performedto select the right and correct conditions of the Varian CP-3800 GC in tandem with the Saturn 2200 MS detector. Initially, a higher concentration of all

analytes of interest (2.0 µg/mL) was injected to run in a full-scan to obtain their retention times and toselect the optimal precursor ions for MS/MS detection. The highest and the most intense m/z ionswerechosenas precursor ions in most cases. Series of injections were then made in AMD mode (20 – 100 V) to select the best voltage to apply to achieve the right product ions from the chosen precursor ions. The most intense product ion was selected as the quantification ion, whilst the next higher ions were used for qualification purposes in the identification of the analyte. After the building of a completed compound table and MS/MS settings; the mode was converted into MS/MS and a solvent delay of 3 min was employed to run mixed standards with sufficient signal in order to obtain data points to perform accurate and reliable quantification. The optimized GC-MS/MS parameters were summarized as in subsection *apparatus* above and also in TABLE 1.

Method validation

The performance of the optimized extraction procedure, amino(NH_2) sorbent SPE clean-up and GC-MS/MS analysis method was validated evaluating the linearity, recoveries, reproducibility, limits of detection (LoDs) and quantification (LOQs) and robustness. These were done to ascertain if the proposed multi-residue method was fit for the intended purpose. The results are as listed in TABLE 1.

Linearity was determined for the instrumental response. The range of concentration studied was 0.005 – 1.0µg/mL analyzing reference standard solution at seven concentration levels (0.005, 0.01, 0.02, 0.05, 0.1, 0.5 and 1.0 µg/mL). The linear calibration curves were obtained by plotting the peak area for each analyte versus its concentration. Each compound showed good linearity for the GC-MS/MS analysis in the studied working range, with regression co-efficient (R^2) greater than 0.990 (TABLE 1).

The recovery and reproducibility (RSD) of the developed analytical method were evaluated on spiked soil samples. Three levels of fortification were chosen. These levels were carefully chosen to represent first of all, contaminations at regulation levels, contamination below and then contamination above the regulation levels. The analysis was performed in replicates of six (n = 6) at each level in order to evaluate the reproducibility of the method. Recoveries were calculated with the analytes of interest (OCPs, PCBs and PAHs) and the values ranged between 70% - 110% for most of the analytes using the amino (NH_2) sorbents SPE cartridge. The overall method reproducibility for all analytes of inter-

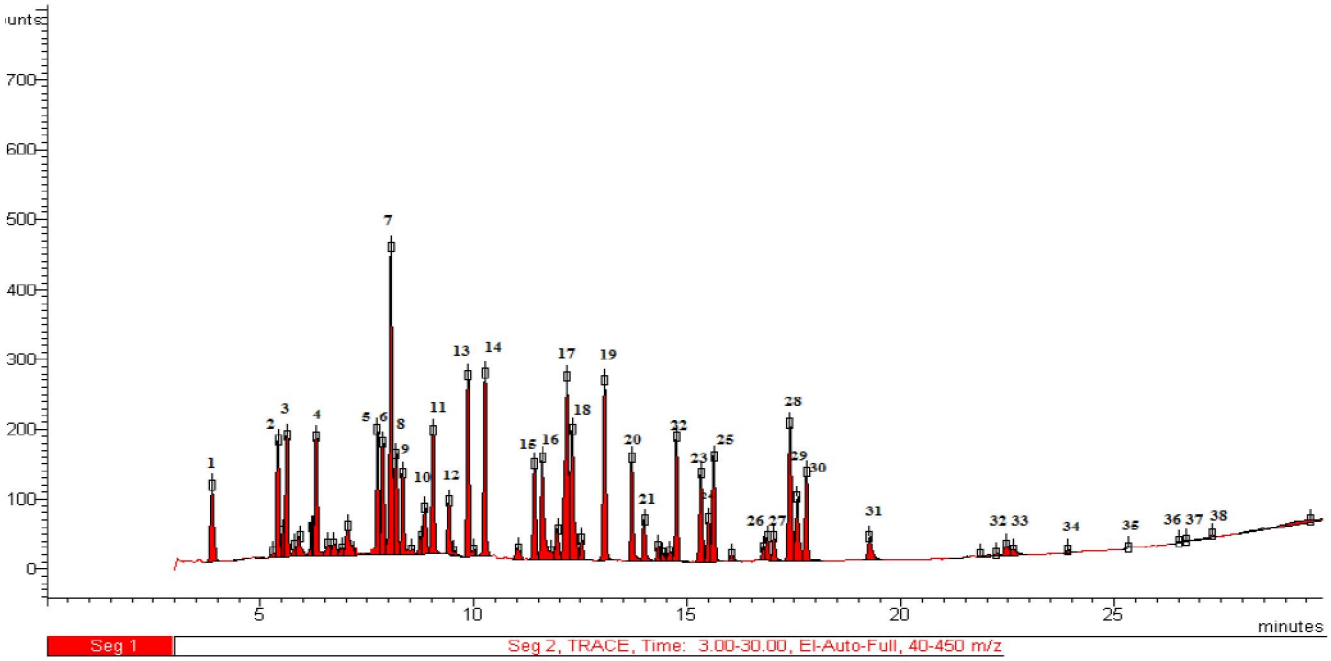

Figure 5 : Typical chromatogram of 10 µg/kg spiked soil sample with OCPs, PCBs and PAHs

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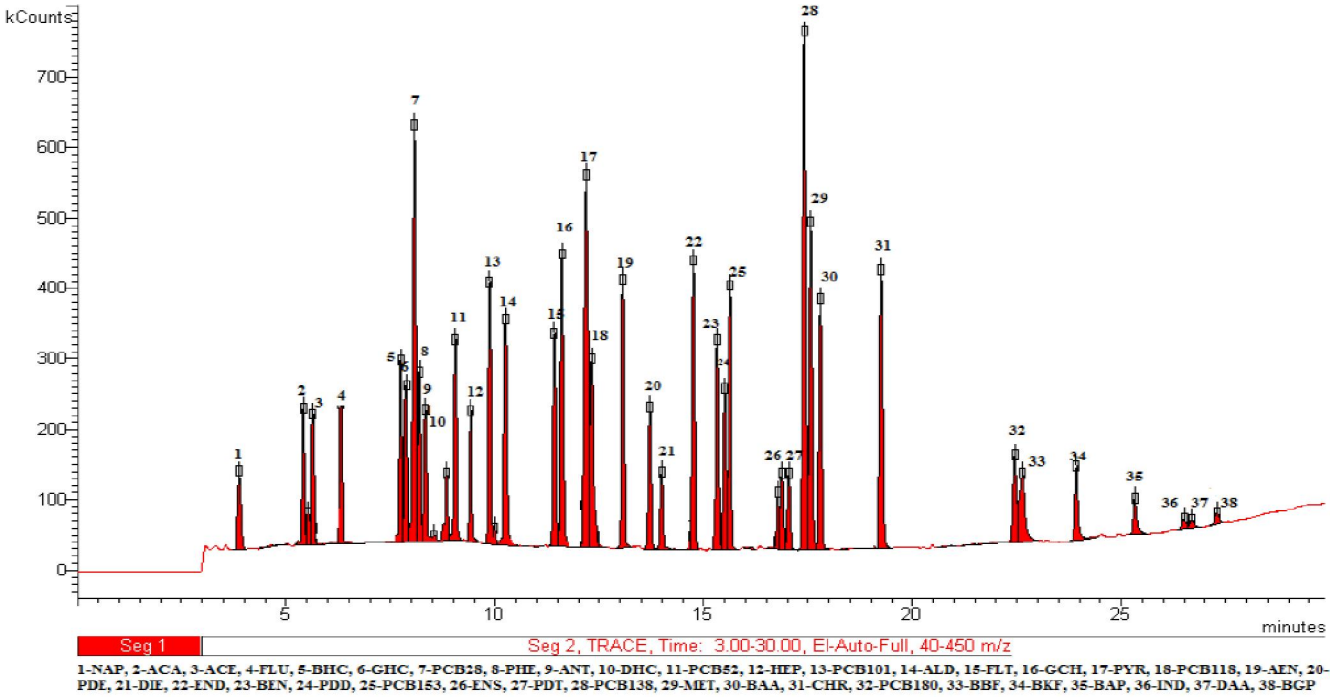

Figure 6 : Typical chromatogram of 0.05 µg/mL mixed reference standards of OCPs, PCBs and PAHs

est expressed as RSDs was satisfactory and was in the range of 2.0% - 11.7%. TABLE 1 shows the average recoveries and RSD values for the selected OCPs, PCBs and the sixteen PAHs. Figure 4-6 shows typical chromatograms of the selected analytes of interest.

The limits of detection and quantification were evaluated by preparing twenty individual method blanks and analyzing each one. The mean and corresponding standard deviations were calculated, and the limit of detection determined from the equation:

$Y_{ld} = Y_{blank} + 3s$

Where Y_{ld} is the detection limit, Y_{blank} is the mean value of the twenty blanks and s is the standard deviation. The limit of quantification was obtained by multiplication of the standard deviation by a factor of 10. And these were used to estimate the statistical significance of differences between low level analytes responses and the combined uncertainties in both the analyte and the background measurement. TABLE 1 shows the various limits of detection and quantification.

To determine the robustness of the method, other soil types were taken through the extraction and clean-up steps. Clay, silt, loamy types of soil and sediment were investigated. Recovery test of the analytes of interest in these soil types and sediments (65% - 109%) proved very successful. All the recovery results showed that the method is capable of analyzing OCPs, PCBs and PAHs in soil and soil-related matrices. As a result, this analytical method can be applied in routine for the trace analysis of organochlorine pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in any soil type; and as such it had been adopted for the analysis of trace organic contaminants in soil and soil-related matrices in the Pesticide Residue Laboratory, Testing Division of the Ghana Standards Authority, under the code GSA-SM-T24.

CONCLUSION

Rapid, simple and easy multi-residue method for the simultaneous determination of organic pollutants in soil has been developed. This study presents two combined optimized extraction techniques and an SPE cartridge clean-up method using acetonitrile for the extraction of fifteen organochlorine pesticides, seven indicator polychlorinated biphenyls and sixteen polycyclic aromatic hydrocarbons in soil samples. Optimized conditions include 5min sonication, 30min mechanical shaking and 10mL elution

on an amino sorbent SPE cartridges followed by an addition of 20µL of 1% polyethylene glycol-200 in ethyl acetate and 980µL ethyl acetate after a concentration stage. Under the optimized conditions, the method showed good recoveries higher than 70% for most of the analyzed compounds using all three selected sorbents (amino, silica and florisil) SPE cartridges. Linearity and reproducibility were evaluated, obtaining satisfactory results for all analytes tested(0.005 – 1.0 µg/mL) and (2% - 11.7%), respectively. Limits of quantification were between 0.1 µg/kg and 1.0 µg/kg across the selected analytes. The developed method proved to be very rugged for other related soil types. The method as validated and adopted shows practical environmental and economical advantages in terms of sample processing time, simplicity, relatively safer and reduced organic solvent use and cost, and is particularly suitable for routine applications requiring a high sample throughput.

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