Matrix effects on organic pollutants analysis in marine sediment

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Abstract. Interference from the matrix sample can influence of the accurate analytical method. Accelerated Solvent Extraction and their purification methods were tried to separate the organic micropollutants respectively in marine sediment. Those matrix were as organic pollutants evaluation in marine environment. Polychlorinated Biphenyls (PCBs) and Organochlorine pesticides (OCPs) are two examples organic pollutant in environment which are carcinogenic and mutagenic. Marine sediments are important matrices of information regarding the human activities in coastal areas as well as the fate and behavior of organic pollutants, which are persistent in long-term. This research purpose to evaluate the matrice effect and the recovery from marine sediment spiking with several standar solution and deuterium of molecular target from organic pollutants in not polluted sample of sediment. Matrice samples was tested from indicate in unpolluted location. The methods were evaluated with standard calibration curve (linearity > 0.999, LOQ various ranged 0.5-1000 pg.µL−1 and LOD < LOQ). Recovery (YE) relative, Matrice Effect (ME) relative correction with deuteriated standar were evaluated the interference the matrix. Interference effect for OCPs compounds were higher than PCBs in marine sediment.

1. Introduction
Sedimentation is one of environmental problem in coastal region. Dangerous suspension or material organic from anthropogenic (such industrial and municipal sewage, agriculture, urban runoff, etc) [1] or natural (flood, erosion, volcano eruption, etc) activities can sedimented in marine sediments. Marine sediment can deposit organic matter and suspended materials from erosion effect, rock, sol and they can stored of organic compounds and also was the habitat of organismes (bivalve, shell, fish) and buried in sediment [2]. Organic pollutants could readily adsorbed into particular material, and bottom sediments act as a reservoir of hydrophobic contaminants [2,3,4] Organic pollutants persistent long term in environment commonly carcinogenic and mutagenic such PCBs which have been used as commercial industrial materials for electrical equipment for the past past 50 year (i.e. transformers, capacitors) and they are considered to be immune-toxic, liver damage, skin rashes and affect reproduction [5,6,7]. Another compounds organochlorine pesticides (OCPs) were produced commercial for pest control, crop production and their have serious short-term and long-term impacts even at low concentrations. They may also have highly non-lethal effects such as immune system and reproductive damage [8]. All components which adsorbed in sediments have complexity for analysis of organic pollutants. The “matrix” contains all components in the sample or the analytic(s) of interest [9]. Although these complex
matrices have a number of common components, not all are known and levels may vary among individuals. The matrix effect affected by the interference presence in the environmental matrices which compete for the ionization with the analyses present in low concentrations [9]. Thus, some precautions such as the use of internal standards and implementation of cleanup methods have been recommended to minimize the matrix effects in organic pollutants analyses [10,11]. For example, samples extracts which has high levels of organic matter or fats contain substances that are co-extracted and deposited on the chromatographic column, reducing the efficiency of the separation and interference of analytical method [12,13].

The evaluation of the environmental matrices requires the developing methods to validate of analytical methods. The method of standard additions (spiked) is one alternative to quantify susceptible compounds in matrix, but this approach still leads to inaccuracies and hardly feasible in multiresidue methods. The deuterium standard or isotopically labeled standard could affected of analytical procedure as native analytes for common practice in GC/MS analysis of various groups of persistent organic pollutants such as PCBs and organochlorine pesticides (OCPs). This study purpose to optimize and evaluate analytical methods with several quality control parameters (percentage of matrix effect and yield extraction) from marine sediment spiking with several standard solutions and deuterium of molecular target of organic pollutants persistent (PCBs and OCPs) in “uncontaminated” sediment samples from Jakarta Bay.

2. Methods

2.1. Chemicals

All solvents used at pro analysis, i.e. n-heptane, dichloromethane, acetone were SupraSolv grade (Merck, Darmstadt, Germany). HCl (37%) was pure grade (Fisher Scientific, Leicestershire, UK). Mixture and individual of commercial deuteriated internal standards respectively were pp’-DDT-d₄, pp’-DDE-d₄ and PCB-101-d₆ (CIL CLUZEAU, Andover, MA, USA). There were 9 individual standard solutions of OCPs used: (pp’-DDT, pp’-DDE, pp’-DDD, Hexachlorobenzen, endosulfan-1, aldrin, dieldrin [CIL CLUZEAU, Andover, MA, USA], heptachlor and heptachlor epoxide [Sigma-aldrich, St Louis, MO, USA], 7 PCBs mix standard solutions (PCB mix-3, PCB-28 (2,4,4’- Trichlorobiphynle), PCB-52 (2,2’,5,5’-Tetrachlorobiphynle); PCB-101 (2,2’,4,5,5’-Pentachlorobiphynle); PCB 118 (2,3’,4,4’,5-pentachlorobiphynle); PCB 138 (2,2’,3,4,4’,5’-Hexachlorobiphynle) PCB-153 (2,2’,4,4’,5,5’-Hexachlorobiphynle); PCB 180 (2,2’,3,4,4’,5,5’-Heptachlorobiphynle), (CIL CLUZEAU, Andover, MA, USA) were used. Standard working solutions were diluted with DCM (SGS, France). Alumina, silica gel 60 (200-300 mesh) and Cu powder (> 230 mesh) were purchased from VWR International and Merck (Darmstadt, Germany).

2.2. Sampling and preparation

The sediment samples were taken from one station from Jakarta Bay which supeicious low concentration as the the matrix interference test adding with standard solution. This station was described from our previously study [14]. Sediment samples were stored in cool box (-4°C) before preparation in laboratory. Samples were freeze-dried at -40°C at pressure 0.080 bar for 48h, then dry samples were weighed and grinded with mortar then sieved < 200µmesh and stored before analytical treatment.

2.3. Extraction and purification of sediment

Extraction and Purification of PCBs and OCPs were carried at same time at one method which has been modified from previously studies [15, 16]. Those compounds were extracted with ASE (Accelerated Solvent Extraction) using 33mL thimble cell with filter fibre containing 3 g alumine at the base of cells, 10 g sediment and 3 g of Cu powder which has been previously washed with HCl (1:4, v:v) and cells were completed with adding fontaine bleu sand. The HEPT:ACETONE (1:1) was choosed as extract solvent for following conditions conducted for 2 cycle at 100°C and 1500Psi then evaporated at turbovap...
(Lab commerce, Inc, San Jose, CA) under nitrogen stream until 0.5mL. Analysis of PCBs and OCPs were carried out by gas chromatography-triple quadrupole mass spectrometry (GC-QQQ/MS) system.

2.4. Analytical procedure
Analysis method conducted by GC-QQQ/MS (Gas Chromatography triple quadrupole Mass Spectrometry; Agilent 7000, USA). The analysis of the parameters was carried with the aid of QQQ-Quantitative and QQQ-Qualitative analysis agilent technology software. The modification for analytical method [16],[17]. The chromatographic conditions were: splitless injection (30s), DB5MS column (30m x 0.25mm i.d. x 0.25μm film thickness), and the GC oven was temperature-programmed from 80°C (held two minutes) to 300°C at 12°C.minutes⁻¹, then held for five minutes until run time 25.33minuates. The flow rate of the carrier gas was constant at 3mL.minutes⁻¹ and the PSS injector was programmed from 80°C (0.1minutes) to 250°C(200°C.minutes⁻¹), held for ten minutes, and then reduce to 60°C(200°C.minutes⁻¹) to minimize the solvent peak.

Mass spectrometry is a highly selective analytical technique that can be used to monitor the masses of specific ions generated from the analytes of interest [18]. The mass spectrometer was operated in positive electron impact ionization (EI) mode (70eV) and simultaneously scanned in both full scan (FS) for all molecular targets compounds.

PCBs and OCPs analyses have been optimal when scanned in product ion mode (PIM) in order to select the ion product at several energy collision, the highest intensity was chosen to used the energy collision then scanned in Multiple Resonance Magnetic (MRM) methods[17]. Quadrupoles can be used in scanning or filtering mode. Triple quadrupoles give more advantage to a higher obtained selectivity and reproducibility, especially at low concentrations, selectivity, accuracy, resolution, reproducibility especially at low concentration, better signal/noise (S/N) and wider linear range allowing quantitation with lower limit quantification.

2.5. Quality control
Calibration of PCBs and OCPs with standard solution and individual standard deuterium were monitored several parameters. The linearity areas for PCB and OC analysis were determined from eight points covering a concentration range from 5 to 1000pg.μL⁻¹ alues below this confidence interval were rejected. The LOD (Limit of Detection) below then 5-50 pg.μL⁻¹. There are three replicates performed for analysis. Replicates were extracted, purified and analyzed in the same sequences and have the same operating conditions. The samples was divided into three control samples (see fig.1).The standard solutions were prepared by diluting the solutions to 10 mg.L⁻¹ in 1mL DCM and then doping them with 20μL of each deuterated internal standard solution 100pg.μL⁻¹. Quality Control (QC) solutions (pure solvent in a standard range concentration) were inserted into the sequence to ensure that there was no drift of the analytical signal. The quantifications were made by plotting the area ratios (target compound / deuterated compound) as a function of concentration ratios. Calculations of errors might be below 20% (80-120% accuracy) and linearity > 0.9990.

3. Results and Discussion
The quantification of PCBs and OCPs in sediment samples performed by several steps, such as sampling, extraction, purification, and instrumental analysis. Among those steps, the extraction step have more effect to remove the compounds from the matrix to the solvent [19, 20]. Extraction technique using Accelerated Solvent Extraction (ASE) have more effective extraction with less organic solvent. Soxhlet and sonication [20]. Modification of ASE technique by adding the alumina for purification method at the same time can reduce separation time. The organic solvent using HEPT:ACETONE (1:1) as a suitable solvent extraction that used to extracts of PCBs and OCPs because it can reduces the amount of interferences and improves signal to noise (S/N) [21]. The matrix effect is also used to confirm the recovery of spiked samples compared with the recovery of an analyte from blanks to evaluate their interfering compounds at the same retention time as the analytes [13]. The identification of PCBs and OCPs was carried out by comparison of the retention times and mass spectra of commercial standards.
solution. Based on the utilization of internal standards, they have various criteria:

- They should be absent from the considered samples.
- Their retention time should be close to the compounds but not match the target compounds.
- It is necessary that their response factor is similar to the studied molecules.

Gas chromatographic with triple quadrupole is effective method for analysis multiresidues molecules targets. There were three types of quadrupoles with different functions. The first quadrupole filters a specific precursor ion of interest. Ions generated from an ion source with a different m/z can not pass in Quadrupole 1. The collision cell is optimized to produce a characteristic product ion by the collision of the precursor ion with a neutral collision gas such as nitrogen. This process is called Collision Induced Dissociation (CID). Generated product ions then transferred into the third quadrupole, where only a specific m/z is allowed to pass. The MRM mode works like a double mass filter that drastically reduces noise and increases selectivity [18].

There are 3 replicates simulation for measuring percentage of Matrix Effect (%ME) and Yield Extraction (%YE) relative at Figure 1.

![Diagram of simulation](image)

**Figure 1.** The 3 replicates simulation for measuring percentage of Matrix Effect (%ME) and Yield Extraction (%YE) relative

The yield of extraction (YE) relative determined by comparing the areas of the chromatographic peaks of C2 (AC2) and C1 (AC1) for each of the target compounds. The analytical signal resulting from the contamination of all the blank matrices was removed by subtracting the measured area of the C1 control (AC1), finally, yields calculated using (1).

\[
%YE = \frac{AC3 - AC1}{AC2 - AC1} \times 100
\]  

(1)

Matrix effects (ME) relative determined by comparing the responses obtained in the control sample C2 with a standard solution prepared in pure solvent at the same concentration. There were 7 PCBs that use 1 internal standard deuteriated (PCB-110) and 7 OCPs use 2 internal standards deterium (pp'-DDT-d4 and pp'-DDE-d4). Matrix effects calculated using (2).

\[
%ME = \frac{AC2 - AC1}{A_{std}} \times 100
\]  

(2)
A_{\text{STD}}$ represents the area of the chromatographic peak measured for the standard solution. The determination of matrix effects can evaluate the quality of purification during the development of sample preparation and protocols to eliminate maximum interfering (minimizing of matrix effects).

All standard solutions were separated with same method corrected with adding their deuteriated internal standar. 17 compounds from mixing standard solutions and their deuteriated internal standard have been separated from 12 minute to 19 minute at same method see figure 2.

**Figure 2.** Capillary Column gas chromatograms of mixture of 7 PCBs and 9 OCPs of standard solution and their deuteriated internal standar

Based from Figure 2, the average of Limit of Quantification (LOQ) range from 5 to 1000 pg.µL$^{-1}$ or 0.5–100 µg.kg$^{-1}$ in 10g dry weight sample. The degree of chlorination determines the molecular weight of PCBs, and also being responsible for the properties of each PCB congener. The degree of chlorination, just like the molecular weight of PCBs, is related to the number of chlorine atoms in the molecules [20]. The 7 PCBs congener can be represent to analysis other congener of PCBs. Based from chromatograms, PCB-28 and PCB-52 which have range from 2 to 4 chlorine atoms or low chlorinated PCBs shows the large intensity as the result. The high chlorinated PCBs (contain from 5 to 9 chlorine atoms) such as PCB-153 and PCB-180 have less intensity because they are more co-elution than low chlorinated PCBs in chromatograms.

The result of the yield and matrix effect relative obtained various values (see Table 1). The mean yield extraction, the yield extraction (YE) and matrix effect (ME) relative have given mostly good performance with accuracy 80-120%. However, interferences were still found in several compounds that obtained higher or lower than accuracy especially in several organochlorine pesticides (OCPs). The percentage of yields and matrix effects in sediments showed the presence of multi-component mixtures of molecular target which are soluble at same organic solvent (see Table 1)

The analysis of the blank matrix sample as “uncontaminant” sediment sample from Jakarta Bay originally have two PCBs (PCB-28 and PCB-52) and pp’-DDE with concentration level below 3 µg.kg$^{-1}$ dry weight. It means, on the evaluate process of the matrix, the concentration of those compounds are larger than another compounds, because they have been presence in blank matrix sample. From the result, most of PCBs intensity produce small concentration at their time retention. Although those concentrations could not determined but they were still detected in chromatograms. Those values have
still lower than LOD (Limit of Detection). The percentage of matrix effect (ME) relative and yield extraction (YE) relative with their deuteriated internal standard normally range from 80% to 120%. The result show that %YE relative of PCBs range from 79% to 137%. The %YE relative of PCB-101 are the biggest value that reach 137% because its probably co-elution of massive chlorine between their deuteriated internal standard and standard solution at same retention time. Several OCPs were observed the % YE relative ranged from 87 to 117% and % ME relative obtained from 82 to 138%. %ME more than 120% (such Aldrin, Heptachlore epoxy, Endosulfan-1) but only Aldrin has abundance interference for standar solution refer to its matrix. Although there was not found aldrin in blank samples, it was probably from their co-elution of chlorine to other molecular targets close the retention time of aldrin. The percentage indicated the interference of the peak corresponding with their deuteriated internal standard due to closely range of retention time at each other and the co-elution of chlorine to certain molecules target could make possibility increasing the presence of interference effect at ionitation process so the peak of molecular target could reduce or abundance intensity [13, 19, 21].

The percentage of matrix effect relative and yield extraction relative were observed in sediments conclude that the recovery of standard solution refer to sediment samples to deuteriated internal standard and was better in PCBs than OCPs.

### Table 1. Percentage Yield Extraction (YE) and Matrix effect (ME) relative with internal standard deuterium and concentration of PCBs and OCPs in blank samples (n=3)

| Compounds          | YE relative (%) | ME relative (%) | Concentration (µg.kg⁻¹) |
|--------------------|-----------------|-----------------|-------------------------|
| PCB-28             | 100             | 109             | 1.63±0.24               |
| PCB-52             | 79              | 81              | 2.23±0.24               |
| PCB-101            | 137             | 87              | < LOD                   |
| PCB-118            | 91              | 114             | < LOD                   |
| PCB-153            | 111             | 96              | < LOD                   |
| PCB-138            | 87              | 110             | < LOD                   |
| PCB-180            | 90              | 111             | < LOD                   |
| Hexachlorobenzen   | 104             | 80              | < LOD                   |
| Heptachlor         | 87              | 138             | -                       |
| Aldrin             | 155             | 127             | -                       |
| Heptachlore epoxy  | 89              | 139             | -                       |
| Dieldrin           | 97              | 109             | -                       |
| Endosulfan-1       | 117             | 126             | -                       |
| pp'-DDE            | 85              | 82              | 2.97±0.34               |
| pp'-DDT+pp'-DDD    |                 |                 |                         |

Most of OCPs were not presence in blank matrix except the pp'-DDE at its retention time. The yield extraction evaluate the interferences from extraction until analysis process and Matrix Effect related the interferences from analysis process which compare the signal between the blank sample and the standard solution. Further studies will need to evaluate the interfering peaks also comparing with other methods that they have been generally used at sediment sample and their organic matter contains in sediment. In future studies, this result can be a reference to develop analysis method using other validation parameter or Certified Reference Materials (CRMs) of sediment to evaluate the matrix effect.

### 4. Conclusion

The parameters method tested such Matrix effect (ME) and Yield Extraction (YE) relative refer to deuteriated internal standard and standard solution into blank matrix samples can be applied to sediment samples for quality control procedures used for PCBs and OCPs analysis but it must still apply by CRM.
(Certified Reference Materials) or several validation parameters to developing the validation method. Using GC-QQQ/MS analysis, the percentage of matrix effect resulted a few OCPs and almost without any PCBs in sediments. Further studies need to evaluate the interfering peaks of massive chlorine atoms and scanning organic matters properties of sediment sample which can interact with molecular targets.

The development method based on quality control and the evaluation of matrix effects will improve the accuracy of analytical results, helping future environmental studies to determine the real impact of PCBs and OCPs in coastal region also their impact to human.

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