

The Comparison of Silica Gel-Alumina Sorbents for Separation of PAHs and PCBs

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ABSTRACT: *The aim of this research is to develop a silica gel and alumina column chromatography for separation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) into two groups. This is achieved by optimizing the cut-off volume of eluting solvent and weight ratio of silica gel and alumina. In this process mixture of PAHs and PCBs divide in two groups and simultaneously other interferences like lipids and aliphatic compounds are removed. So the sample is prepared to be analyzed and quantified by HPLC. The main purpose of the research was to find a combination of sorbents for the column chromatography method that would permit the determination of many types of pollutants (PAHs, PCBs and aliphatic compounds) in a single run. Elution profiles for both the analytes and the interfering components were determined for several types of column chromatography sorbents (alumina, silica) and combination of them. The silica: alumina (5:5 g) column is suitable for the separation of PCBs and silica: alumina (5:10 g) is preferred for the separation of PAHs. The efficiency of the developed clean-up method was evaluated using real sediment sample.*

KEY WORDS: *Column chromatography, Sediment, Environmental analysis, PAHs, PCBs, HPLC.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are two groups of organic pollutants found ubiquitously in various environmental samples including sediments in lakes, rivers and estuaries. Determination of their concentrations involves extraction, preconcentration or volume reduction, chromatographic clean-up and instrumental analysis [1].

Currently, USEPA (1995) includes different methods for analyzing PAHs (Methods 8100, 8310) and PCBs (Method 8082). Most of the extraction procedures (Methods 3540C, 3541, 3550B) required by these methods are not specific to their analyte group [2]. Sample preparation prior to the determination of many environmental pollutants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls

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(PCBs) or organo chlorine pesticides in soil and sediments usually consists of many steps because of the complexity of the matrix. Therefore, both PAHs and PCBs can be extracted from the sample during a single extraction process. The subsequent preconcentration using vacuum evaporation is also applied to the analyses of both PAHs and PCBs. However, the clean-up procedure is very different for these two groups of pollutants. During the extraction step many interfering components are co-extracted from soil and sediment samples together with target analytes: examples include lipids, sulphur, pigments, or cholesterol and its derivatives [3,4]. The aim of the clean-up stage is to remove substances that could interfere with the final determination and quantitation of target analytes. Removal of interfering substances can be accomplished in many different ways. For example, copper compound is often used as the medium retaining sulphur. Copper was obtained from Fluka Company and activated using HCL, then rinsed sequentially with Milli-Q purified water (until PH 7), then rinsed with acetone and finally rinsed with hexane. Active copper compound can separate sulphur compound in the form of Cu_2S [5-7]. Numerous other techniques are described in the literature [8,9].

Low pressure liquid chromatographic techniques have been widely used for clean-up. Most of the clean-up procedures used for research purposes were modification of those specified in the EPA standard methods. Silica gel is the most polar sorbent available. It is useful for extract clean-up in the determination of non polar compounds. Alumina (Al_2O_3) is somewhat similar to silica because of its very polar character. The primary retention mechanisms for alumina are based on Lewis acid/base, polar and ion-exchange interactions [4].

This research has focused on the development of a consecutive elution fractionation procedure, which uses low pressure silica gel and alumina chromatographic technique to separate PCBs and PAHs into two groups. This may enable the use of a single clean-up process to prepare two subsamples for subsequent analysis by high performance liquid chromatography (HPLC) with PDA and Fluorescence detectors. By adopting this clean-up procedure, the consumption of solvent and sorbent will be reduced remarkably. This is especially meaningful when the concentration of both PAHs and PCBs are to be determined, or the sample size prohibits separate analysis for different groups of pollutants.

EXPERIMENTAL

Chemicals

The 16 PAHs analyzed were Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)Anthracene, Chrysene, Benzo(b)Fluoranthene, Benzo(k)Fluoranthene, Benzo(a)Pyrene, Dibenzo(a,h)-Anthracene, Benzo(g,h,i)-Perylene, Indeno(123-cd) Pyrene. 12 PCBs congeners with 3-8 chlorine atoms were analyzed. Their IUPAC numbers were 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180 and 194. Standard solutions of both PAHs and PCBs were purchased from Suppelco Company (Canada).

Solvents were purchased from Merck Company (Germany). Methylene chloride, hexane, and acetone were GC grade. Silica gel (60-230 mesh), alumina (70-230 mesh) and anhydrous sodium sulphate were also purchased from Merck Company (Germany). Deionized water was produced with a Milli-Q purification system (Millipore, Milford, MA. USA). Copper was obtained from Fluka Company (Switzerland) and activated using HNO_3 , then rinsed sequentially with Milli-Q purified water (until PH 7), then rinsed with acetone and finally with hexane.

Certified reference material, IAEA-383 (sediment sample) was purchased from International Atomic Energy Agency for method validation.

Procedure

Standard samples were prepared by mixing the standard stock solutions of PAHs and PCBs. The concentrations of the 16 PAHs were 1 $\mu g/ml$ for each compound, and the concentration of 12 PCBs congeners was 500 ng/ml.

Silica gel and alumina were activated at 240 °C for 8 hours. After cooling down in a desiccator, 5 and 10 g of silica gel and alumina was weighted into a glass container, respectively and deactivated with 5 % deionized water. Each container was shaken vigorously after fitting a screw cap. The silica gel and alumina was allowed to homogenize at room temperature overnight.

The column for the fractionation is made of Pyrex glass, and was 500 mm long with 11 mm i.d. it was fitted with glass wool and a PTFE stopcock at the bottom. Activated anhydrous Na_2SO_4 (1g) was added on the top of the column (Fig. 1).

Research strategy

The first step in our investigations was the evaluation of well known and widely used sorbents, silica and alumina, for the clean-up of extracts prior to the analysis of various compounds in a single run.

In this work different column chromatography conditions for the separation of the analytes from each other was studied. 10 g silica gel column and combination of two sorbents silica: alumina (5:10 g) and silica: Aluminas (5:5 g) were chosen in this research. For the confirmation of final clean-up procedure efficiency the column chromatography with optimized conditions is used to separate interest analytes from standard sediment matrix (IAEA-383). The quantitation was performed with HPLC using PDA and Fluorescence detectors.

Elution profiles of standard solution

Basic studies with silica and alumina

A standard solution (1 ml) containing each compound of 16- PAHs (1 µg/ml) and 12-PCBs (0.5 µg/ml) was deposited on top of a pre-conditioned column chromatography. The analytes were eluted with different solvents (see table 1). Each eluted fraction was analyzed by HPLC, (Fig. 2).

Fractionation of standard sediment extract

Evaluation of the column efficiency for standard sample extract (IAEA-383) was performed. At this research stage, 5 g of sediment sample was weighted into thimble and extracted for 24 hours with 150 ml hexane-acetone (1:1 v/v) in a soxhlet extractor. Extract was transferred into glass flask by careful decantation. The residue was subsequently washed three times with 2 ml dichloromethane and these volumes were combined with the extract. Then active copper (5 g copper metal) was added to the extract for sulphur removal. The final extract was concentrated to 0.5 ml using rotary evaporator and evaporated to dryness under a gentle stream of nitrogen. Anhydrous sodium sulphate (0.5 g) was added to the top of each sorbent bed. Each fraction analyzed with HPLC, (Fig. 3).

Instrumental analysis

The HPLC system consisted of a quaternaire pump system (Waters Model, US) and a manual injector equipped with a 20 µl loop was used for the quantification

Table 1: Sorbents and elution with solvents.

Sorbents	F1 *(ml)	F2 (ml)	F3 (ml)
Silica (10 g)	40	30	20
Silica: alumina (5:5 g)	40	30	20
Silica: alumina (5:10 g)	20	30	20

* F=Fraction, F1=Hexane, F2= Hexane-Dichloromethane (9:1 v/v), F3= Hexane-Dichloromethane (8:2 v/v)

Table 2: Fluorescence setting for PAHs determination.

PAHs	λ_{ex} (nm)	λ_{em} (nm)	Time (min)
Phe, Ant, Flt, Pyr	235	420	0
B [a] A, Chr	264	384	13
B [b]F,B[k]F DB[a,h]A,B[g,h,i]P	295	405	17
I[123-cd]P	300	500	20

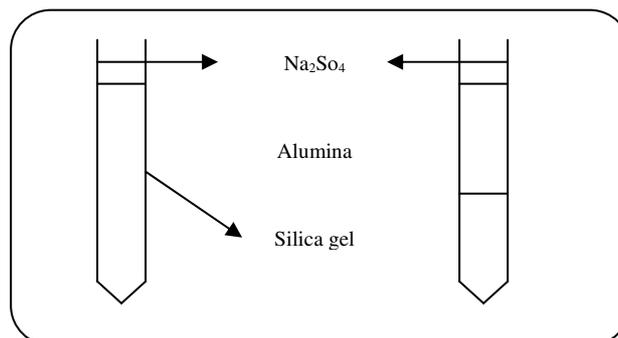


Fig. 1: Profile of column chromatography.

and determination of PCBs and PAHs. For both PAHs and PCBs, identification was based on UV spectrum of each compound.

PAHs analysis

The HPLC determination was carried out under following condition: mobile phase A consists of acetonitrile and mobile phase B consists of water. Total flowrate was 1.5 ml/min. after equilibration, 5 min at acetonitrile-water (40:60 v/v), a linear gradient from 40 to 100 % acetonitrile in 20 min is used for the elution of the PAHs. Both UV and fluorescence detection were used for analysis. UV detection was performed simultaneously at 254 nm. Fluorescence settings are shown in table 2.

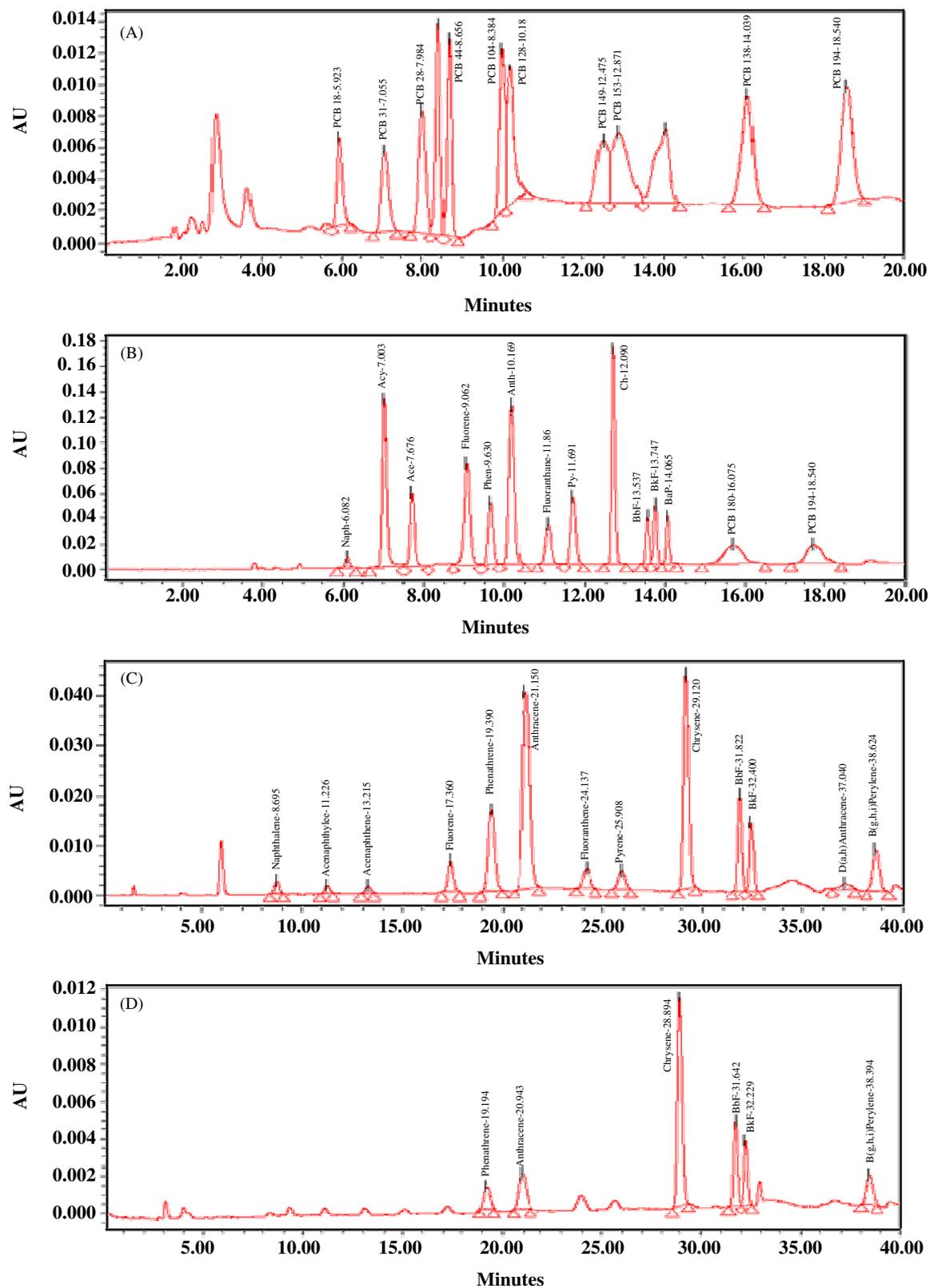


Fig. 2: (A) Chromatograms of PCBs standard, (B) chromatograms of PAHs standard, (C) chromatograms of PAHs with silica gel (D) chromatograms of PAHs with silica gel- alumina.

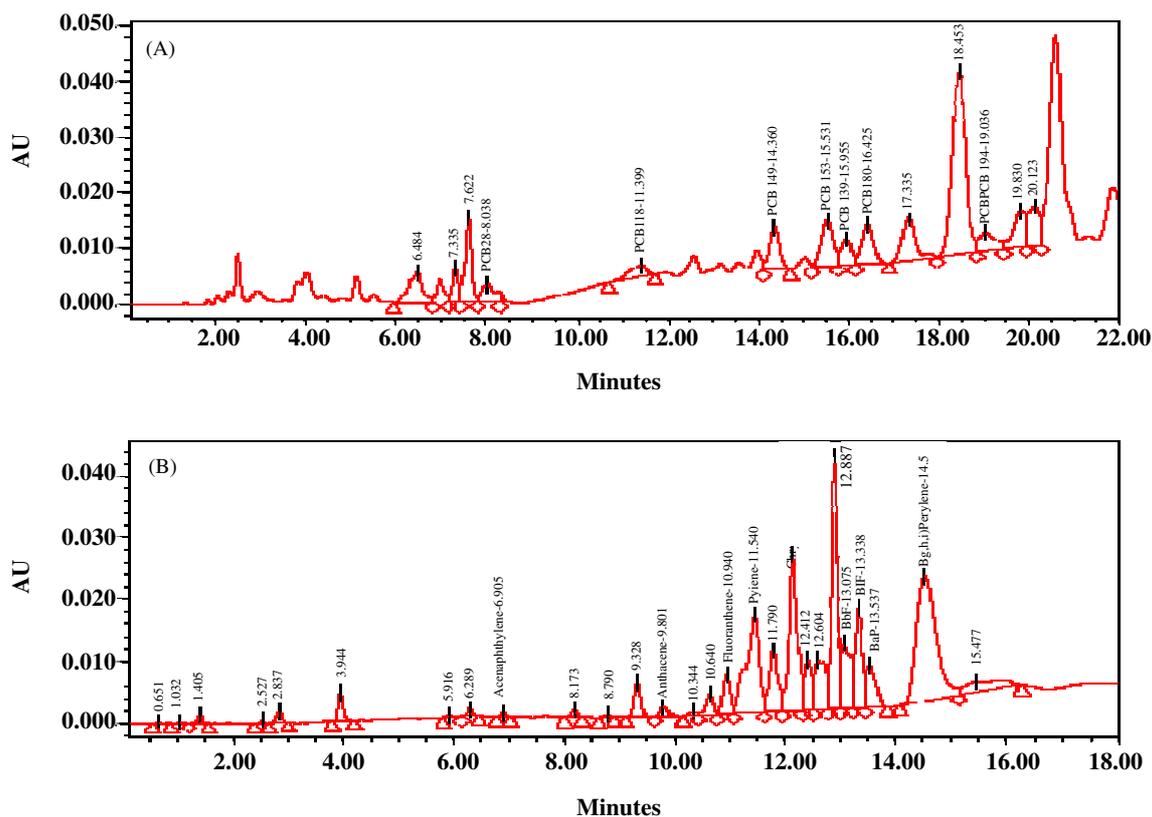


Fig. 3: (A) Chromatograms of PCBs in IAEA sample, (B) chromatograms of PAHs in IAEA sample.

PCBs analysis

The HPLC determination was carried out under following condition: mobile phase A consists of methanol and mobile phase B consists of water. Total flowrate was 0.7 ml/min. after equilibration, 5 min at methanol-water (90:10 v/v), a linear gradient from 90 to 100 % methanol in 20 min is used for the elution of the PCBs. For both PAHs and PCBs, identification was based on matching retention times and PDA-UV library. Concentrations were determined from calibration curves, which were established for each compound by analyzing at least three external standards. Average R^2 of the calibration curves was greater than 0.99 for PAH and PCB analyses. Concentrations of analytes in all injected samples were within the concentration range of their respective calibration curves.

RESULTS AND DISCUSSION

Separation of PAHs and PCBs

In general, almost all PCBs were eluted prior to the elution of PAHs [11]. The earlier elution of PCBs than

PAHs may be explained by the stronger interactions between PAHs and silica gel sorbent due to the higher polarizability of most PAHs compared to PCBs. Within the groups of PAHs or PCBs, the orders of elution depended on the molecular weight of the compounds, but in different patterns. For PCBs, higher molecular weight congeners like hepta and hexachlorobiphenyl were eluted more quickly than lower weight congeners. These heavier congeners were completely eluted out of the column with 40 ml of the eluting solvent, (Fig. 2 A,B).

In contrast to PCBs, lower molecular weight PAHs eluted earlier than the heavier ones. Therefore, in order to separate PCBs and PAHs into two groups during a consecutive fractionation/clean-up process, the lower molecular weight compounds in both groups are of most concern.

Fractionation of standard solution using sorbents and combination of sorbents

Two sorbent (silica gel and alumina) and two combination sorbents (see table 1) were examined using

Table 3: Mean Recoveries \pm SD^a of PAHs and PCBs for three column chromatography.

Analytes	Mean Recovery \pm SD		
	Silica (10 g)	Silica : alumina (5:5)	Silica : alumina (5:10)
PCB 18	14.2 \pm 1.1	80.6 \pm 2.2	41.9 \pm 0.5
PCB 31	20.4 \pm 0.8	87 \pm 0.7	40.9 \pm 0.8
PCB 28	24.25 \pm 0.4	83.1 \pm 1.5	49.4 \pm 1.2
PCB 52	32.1 \pm 1.27	93.2 \pm 1.2	46.1 \pm 1.2
PCB 44	36.35 \pm 2.3	94.5 \pm 0.7	54.4 \pm 1.9
PCB 101	29.15 \pm 1.6	94.2 \pm 1.1	79.4 \pm 1.5
PCB 118	29.2 \pm 1.6	92.2 \pm 1.7	62.3 \pm 1.4
PCB 149	30.5 \pm 2.1	95.2 \pm 2.5	79.5 \pm 1.1
PCB 153	44.3 \pm 1.4	96.9 \pm 0.7	75.1 \pm 1.1
PCB 138	52.2 \pm 2.8	94.1 \pm 1.2	67.3 \pm 1.2
PCB 180	43.1 \pm 1.4	98.1 \pm 1.4	69.8 \pm 0.4
PCB 194	62.5 \pm 3.5	97.4 \pm 0.9	82.4 \pm 1.4
Naphthalene	18.6 \pm 0.5	23.15 \pm 1.6	41.1 \pm 1.2
Acenaphthylene	41.5 \pm 3.5	88.6 \pm 0.8	76.7 \pm 2.2
Acenaphthene	49.8 \pm 3.1	77.3 \pm 1.8	80.5 \pm 1.4
Fluorene	71.1 \pm 1.4	87.4 \pm 1.4	83.8 \pm 0.9
Phenanthrene	66.4 \pm 2.2	55.1 \pm 1.6	67.5 \pm 0.7
Anthracene	47.3 \pm 2.8	74.6 \pm 1.6	80.2 \pm 1.7
Fluoranthene	24.4 \pm 1.4	83.2 \pm 1.7	84.2 \pm 1.9
Pyrene	65.6 \pm 2.3	70.8 \pm 2.1	75.2 \pm 1.3
Chrysene	34.2 \pm 1.7	44.5 \pm 0.7	61.5 \pm 1.9
Benzo [b] fluoranthene	19.2 \pm 0.49	43.9 \pm 1.7	55 \pm 1.4
Benzo [k] fluoranthene	54.2 \pm 2.8	70.6 \pm 2.2	77.5 \pm 1.6
Benzo [a] Pyrene	29.7 \pm 1.7	78.7 \pm 1.1	83.9 \pm 0.7
Dibenzo (a, h) anthracene	20.2 \pm 1.6	57.1 \pm 1.6	69.9 \pm 0.8
Benzo (g, h, I) Perylene	51.7 \pm 2.5	67.1 \pm 1.5	67.5 \pm 1.9
In (1,2,3-c, d) Pyrene	36.5 \pm 0.7	81.1 \pm 2.5	75.4 \pm 1.2

Table 4: Mean Recoveries \pm SD^a for PAHs and PCBs from (IAEA-383).

PAHs compounds	Concentration (ng/g)	Mean recovery \pm SD	RSD (%)
Acenaphthylene	47	69.2 \pm 0.6	0.9
Anthracene	30	15.7 \pm 9.3	6.2
Fluoranthene	290	196.6 \pm 7.76	8
Pyrene	280	186.5 \pm 7.6	8.5
Chrysene	170	78.5 \pm 5.1	6.4
B[K]F	73	56.4 \pm 3.2	5.6
B[a]P	120	53 \pm 4.3	8.2
B(g,h,i)P	110	109 \pm 9.6	6.4
PCB 28	100	83.5 \pm 0.4	0.5
PCB 118	100	95.2 \pm 1.2	1.3
PCB 149	100	75.5 \pm 1.2	1.6
PCB 153	100	111.5 \pm 1.2	1.1
PCB 138	100	114.3 \pm 1.7	1.5
PCB 180	100	67.5 \pm 0.8	1.1
PCB 194	100	70.1 \pm 1.4	2.1

combined samples (Fig.2 C,D). Elution profiles of selected PAHs and PCBs were determined for silica and alumina column chromatography using hexane, dichloromethane and their mixture for analyte elution. For most of the sorbents and sorbent combination, recoveries between 55 and 109 % were achieved. In the case of silica gel (10 g) PCBs eluted in first fraction with 40 ml hexane and PAHs eluted in the second and third fractions with 30 ml Hexane-Dichloromethane (H:DCM) (9:1 v/v) and 20 ml Hexane-Dichloromethane (8:2 v/v), respectively. Whereas in the case of silica gel: alumina (5:5 g) aliphatic compounds eluted at first fraction with 20 ml hexane and PCBs eluted with 20 ml hexane. The second and third fraction contains PAHs. At the end with silica gel: alumina (5:10 g), aliphatic compounds eluted with 20 ml hexane and PAHs eluted with 30 ml H-DCM (9:1). The result of recoveries for different columns is represented in table 3. The low recoveries for low molecular mass PCBs and high recoveries for high molecular mass PCBs obtained from three columns. The reason is that PCBs with lower molecular weight coelute with PAHs and distribute between fraction of PAHs and PCBs.

The table 4 illustrates the recoveries of individual compounds obtained from the run of reference sediment sample extract with silica-alumina columns (5:5 g) and (5:10 g) for PCBs and PAHs, respectively.

CONCLUSIONS

This research evaluated the fractionation of standard solution samples and sediment standard using silica and alumina sorbents for extract clean-up prior to the final determination of analytes ranging widely in polarity. Neither silica nor alumina should be used for extract clean-up in a single step when analyzing compounds of various polarities. After evaluating different column chromatography conditions for the separation of the analytes from each other, the combination of two sorbents silica, alumina (5:10 g) for separation of PAHs and silica, alumina (5:5 g) for separation of PCBs was chosen as the best of tested. The matrix effect on the separation is insignificant for the sediment samples used in this study. This procedure tends to have higher reliability for PCBs and PAHs of higher molecular weight.

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