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Discussion

Discussion on "Alternatives to methanol–water elution of solid-phase extraction columns for the fractionation of high log K_{ow} organic compounds in aqueous environmental samples" by Durhan *et al.*, *J. Chromatogr.*, 629 (1993) 67–74

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With reference to the recently published article by Durhan *et al.* [1], similar problems in eluting non-polar polyaromatic hydrocarbons (PAHs) from C_{18} solid-phase extraction (SPE) columns were also observed in our laboratory. Our results confirm and expand Durhan *et al.*'s with additional PAHs and different elution solvent mixtures.

The C_{18} SPE method developed by the US Environmental Protection Agency (EPA) [2] was used to fractionate the non-polar PAHs from storm drain waters for short-term chronic toxicity tests. Our recovery study of the C_{18} SPE method used standard water solutions which contained eight commonly detected PAHs in storm drain waters (naphthalene, 2-methylnaphthalene, acenaphthene, fluorene, anthracene, pyrene, chrysene and benzo[*a*]pyrene). Low recoveries of PAHs from the C_{18} column were initially observed. The originally proposed methanol–water solvent system did not elute highly non-polar PAHs (as indicated by their high log K_{ow} values), such as chrysene and benzo[*a*]pyrene. We, like Durhan *et al.*, suspected that methanol–water mixtures and methanol were too polar to elute strongly sorbed PAHs from the C_{18} column. Therefore, a modified solvent system was developed so that compounds such as chrysene and benzo[*a*]pyrene could be fractionated for the toxicity tests.

The tolerance of the marine organisms (echinoderm fertilization test, red abalone embryo development test, and giant kelp germination/germ tube growth test, see ref. 3) in our toxicity tests limited the choice of organic solvents. Preliminary tolerance tests showed that methanol–water, methanol and methanol–methylene chloride were acceptable elution mixtures, although it was very desirable to limit the quantity of methylene chloride. Therefore, our effort was concentrated on the development of a modified

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elution system which used both methanol–water and methanol–methylene chloride mixtures.

The final elution mixture maximized recovery and separation of PAHs, although further improvements in recovery and separation are desirable. Our SPE procedures were essentially the same as those of the EPA and Durhan *et al.*, except the elution solvent volume. Our initial results showed that the overall recovery of PAHs used in the recovery study was not significantly different in using either elution solvent volumes of 2 × 1.5 ml or 2 × 1.0 ml. Therefore, 2 × 1.0 ml volumes of elution solvent were used in our recovery study as higher concentration of PAHs in the fractions could be obtained (a concentration factor of 500× as compared to 333× based on a sample volume of 1 l). Our modified elution scheme consists of six fractions: 80, 90, 100% (v/v) methanol in water and 10, 20, 50% (v/v) of methylene chloride in methanol. Unlike Durhan *et al.*, our first elution solvent is methanol–water (80:20, v/v); initial results showed that fractions with less than 80% methanol in water could not elute the most polar PAHs, such as naphthalene and 2-methylnaphthalene.

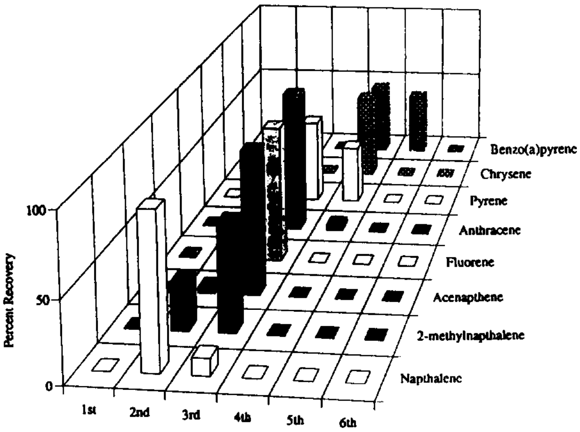

Fig. 1. An example of C_{18} SPE modified elution scheme using the modified solvent mixtures. 1st: methanol–water (80:20); 2nd: methanol–water (90:10); 3rd: 100% methanol; 4th: methylene chloride–methanol (10:90); 5th: methylene chloride–methanol (20:80); 6th: methylene chloride–methanol (50:50).

Fig. 1 is a typical elution profile of the 8 PAHs using the modified elution solvent system. Table I shows the average percent recovery and standard deviation of each PAH recovery over 8 extractions. The extraction procedures of these extractions were identical except for the concentration of PAHs. For each extraction, the concentration of all PAHs except benzo[*a*]pyrene were equal; the range of concentrations of each PAH was varied from 10 to 40 μg/l over the series of 8 extractions. The concentration of benzo[*a*]pyrene ranged from 20 to 80 μg/l. Repeatability of the extraction procedures, as

TABLE I

AVERAGE RECOVERY AND STANDARD DEVIATION OF EACH PAH FROM 8 DIFFERENT EXTRACTIONS USING THE MODIFIED ELUTION SCHEME

1st: methanol–water (80:20); 2nd: methanol–water; 3rd (90:10); 3rd: 100% methanol; 4th: methylene chloride–methanol (10:90); 5th: methylene chloride–methanol (20:80); 6th: methylene chloride–methanol (50:50).

Compound	Log K_{ow}	Average recovery (%) ± standard deviation					
		1st	2nd	3rd	4th	5th	6th
Naphthalene	3.54	0	86 ± 11	3 ± 4	0	0	0
2-methylnaphthalene	–	0	37 ± 10	43 ± 21	0	0	0
Acenaphthene	–	0	7 ± 6	76.6 ± 14	0	0	0
Fluorene	4.12	0	9 ± 9	82 ± 6	0	0	0
Anthracene	4.45	0	0.5 ± 1	84 ± 12	0.5 ± 1	0	0
Pyrene	4.88	0	0	62 ± 9	22 ± 9	0	0
Chrysene	5.61	0	0	15 ± 17	53 ± 11	3 ± 7	2 ± 5
Benzo[*a*]pyrene	6.04	0	0	0	57 ± 14	23 ± 13	0

measured by the standard deviation of the recovery, was generally within 5% for fluorene as the most repeatable, and 21% for 2-methylnaphthalene as the least repeatable.

From Table I it is observed that the methanol–water (80:20) fraction (1st fraction) eluted no PAHs. Most of the naphthalene was recovered in the 90% methanol fraction (2nd fraction). Anthracene, fluorene and acenaphthene were eluted almost entirely in the 100% methanol fraction. 2-Methylnaphthalene, pyrene, chrysene and benzo[*a*]pyrene were not well separated. Table I also shows that elution with methylene chloride (4th and 5th fractions) is required to recover those PAHs with high log K_{ow} value. Most were recovered with a maximum of 20% methylene chloride.

These results show that higher recoveries were obtained with modified elution solvents; however, several compounds were eluted in the same fraction. We worked with more varied solvent volume ratios but results were not significantly improved. Several other solvents (isopropanol, hexane and carbon tetrachloride) were also investigated but with limited success. Poor recoveries or high toxicity were observed. These results strengthen the conclusions by Durhan *et al.* and emphasize the need for further research and development.

REFERENCES

1 E. Durhan, M. Lukasewycz and S. Baker, *J. Chromatogr.*, 629 (1993) 67–74.

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