Journal of Chromatography, 212 (1981) 305–311
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,850

MICROEXTRACTION AND GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED PETROLEUM HYDROCARBONS IN WATER AND FISH TISSUE

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(Received February 16th, 1981)

SUMMARY

The most effective water to solvent ratio is determined for the analyses of aromatic hydrocarbons in water using hexane. The recoveries of these hydrocarbons formed in the water soluble fraction of crude oils and petroleum products are measured using a microextraction procedure. Recoveries were in the 30–40% range but are consistent for each compound. Fish muscle samples are fortified with the standards and the recoveries measured with a modified extraction procedure using dichloromethane as the primary extracting solvent. This is dispersed in water using acetone and finally extracted with hexane. Recoveries range from 90–113% with a mean value of 98%.

INTRODUCTION

Microextraction procedures for the analysis of organic contaminants in water are widely known[1–4,6–8], and are becoming more popular because of their economy of solvents, ease of extraction and speed of analysis. Since there is no need for a concentration step the problem of impurities in the solvents is reduced. Although extraction efficiencies are in the 40–60% range, consistent and reliable results are obtained.

Crude oils and petroleum products such as diesel oils and gasolines are not readily miscible with water, but when an aqueous extract is made of an oil, appreciable amounts of sparingly soluble aromatic compounds appear in the aqueous phase. The compounds selected for standardization and recovery studies are representative of those found in the water soluble fraction of crude oils and petroleum products[9–11].

A microextraction flask[3] was used to investigate the most effective water to solvent ratio at various concentration levels. A modified procedure was developed to extract these organics and measure recoveries from fish muscle samples using dichloromethane as the primary extracting solvent. The dichloromethane extract was dispersed in water with acetone. This was then extracted with 1 ml of hexane using the microextraction procedure.

0021-9673/81/0000–0000/$02.50

EXPERIMENTAL

A standard solution of ethyl benzene, 1,3,5-trimethylbenzene, 1-isopropyl 4-methyl benzene, naphthalene, 2-methyl naphthalene, 1-methyl naphthalene and 2,3-dimethyl naphthalene was made up in acetone at a concentration of 1 μg/μl of each. An internal standard of *n*-decyl benzene was made up at the same concentration in hexane.

Duplicate extractions were done with a 1-l microextraction flask in which 950 ml of water fortified with standards were extracted with 250–1000 μl of hexane. The design of the flask permitted small volumes of solvents to be conveniently recovered for direct injection into a gas chromatograph. The flask was shaken manually for 2 min to equilibrate the standards in the two phases and water was added to bring the solvent into the capillary neck of the flask for analysis.

Samples of fish muscle (5 g) were fortified at three levels of the standards (20, 10, 5 ppm) and extracted with 10 ml of dichloromethane. The slurry was passed through a coarse stainless-steel sieve and 5 ml of the liquid cleaned up on a column of dry sodium sulphate and Florisil. The compounds were eluted with dichloromethane to give 5 ml of eluate. This was transferred to the microextraction flask, 100 ml of acetone and 850 ml of water were added to produce one aqueous phase. This was then extracted with 1 ml of hexane, the internal standard was added and the solvent layer analysed by gas chromatography.

Water soluble fractions of the oils and petroleum products were prepared by shaking 50 ml of oil with 1 l of water in a separatory funnel for 5 min and allowing the layers to separate overnight. The aqueous phase was passed through two glass wool plugs in series to remove any droplets of insoluble oil. Organic materials were recovered from the aqueous phase by the microextraction procedure.

Gas chromatographic conditions

A Perkin-Elmer 900 gas chromatograph was used with an Infotronics C.R.S. 208 integrator for quantitative analysis. A 2 m × 4 mm O.D. glass column was packed with 10% Dexsil 400 coated on Chromosorb W AW, 80–100 mesh. Conditions: temperatures: flame ionization detector, 275°C; injector, 225°C; column programmed 100–250°C at 10°C/min; flow-rates: nitrogen, 25 ml/min; hydrogen, 25 ml/min; air, 200 ml/min.

A 15 m × 0.1 mm I.D. capillary column was used for the analysis of the crude oils and the water soluble fractions. It was coated with SE-30 and programmed from 50–275°C at 8°C/min after a 4-min initial hold at 50°C. A splitless injection technique was used.

RESULTS

Evaporative losses typical of conventional concentration steps were measured by concentrating 5 ml of hexane to 0.5 ml on a rotary evaporator. The hexane was fortified with 100 μl of the standard solution, 100 μl of the internal standard added and the ratios of standards to *n*-decyl benzene calculated from the chromatogram. This was repeated after the concentration step and the ratios again calculated. The figures in Table I show 27–66% losses occurred during the concentration step.

TABLE I

LOSSES OF SELECTED COMPOUNDS BY CONCENTRATION WITH A ROTARY EVAPORATOR

Compound	*b.p. (°C)*	*Ratio before concentration*	*Ratio after concentration*	*Loss (%)*
Ethyl benzene	136	1.14	0.39	66
Trimethyl benzene	165	1.12	0.49	56
Isopropyl methyl benzene	177	1.08	0.50	54
Naphthalene	218	1.26	0.75	40
2-Methyl naphthalene	241	1.06	0.70	34
1-Methyl naphthalene	245	1.01	0.70	30
2,3-Dimethyl naphthalene	268	1.05	0.77	27

Using the microextraction flask, 950 ml of clean water were spiked with 50 μl of the standard solution and extracted with 250 μl of hexane. The internal standard was added and the ratios calculated from the chromatogram and expressed as a percentage recovery. This was repeated with 500, 750 and 1000 μl of hexane. The results in Table II show that the volume of hexane to extract 1000 ml of water was approaching an optimum value of 1000 μl. Larger volumes of hexane would have a diluting effect with no appreciable increase in recoveries. This optimum water to solvent ratio of 1000:1 was used to measure recoveries at four concentration levels and the results in Table III show recoveries which are consistent for each compound.

TABLE II

RECOVERIES (%) OF SELECTED COMPOUNDS AT 50 μg/l BY MICROEXTRACTION

Compound	*Volume of hexane (μl)*			
	250	*500*	*750*	*1000*
Ethyl benzene	5.5	17.2	21.4	25.4
Trimethyl benzene	9.8	30.8	33.9	35.6
Isopropyl methyl benzene	15.2	37.1	38.2	38.5
Naphthalene	5.7	19.0	24.3	27.6
2-Methyl naphthalene	10.4	33.3	35.8	37.0
1-Methyl naphthalene	9.7	32.5	37.4	39.4
2,3-Dimethyl naphthalene	16.3	39.9	40.2	40.9

Four concentration levels of the standards were added to 5 ml of dichloromethane and 100 ml of acetone used to disperse the dichloromethane into the aqueous phase in the microextraction flask. This was then extracted with 1000 μl of hexane and the recoveries calculated as before. These recoveries in Table IV were 20–30% higher using dichloromethane but were consistent for each compound within the concentration range of 10–100 ppb (10^9).

Fish muscle was spiked with three concentration levels of the standards and extracted with dichloromethane. An aliquot of the extract was run through a clean-up

TABLE III

RECOVERIES (%) OF SELECTED COMPOUNDS IN WATER WITH 1000 μl HEXANE BY MICROEXTRACTION

Compound	*Concentration (μg/l)*				*R.S.D. (%)*
	100	*50*	*25*	*10*	
Ethyl benzene	27.1	25.4	23.6	18.5	±14.6
Trimethyl benzene	36.7	35.6	34.8	31.8	± 5.8
Isopropyl methyl benzene	38.4	38.5	38.6	35.1	± 4.3
Naphthalene	28.7	27.6	27.8	24.9	± 5.8
2-Methyl naphthalene	37.7	37.0	37.4	35.2	± 2.9
1-Methyl naphthalene	40.0	39.4	35.8	32.2	± 9.4
2,3-Dimethyl naphthalene	40.5	40.9	41.8	40.7	± 2.1

TABLE IV

RECOVERIES (%) OF SELECTED COMPOUNDS FROM DICHLOROMETHANE WITH 1000 μl HEXANE BY MICROEXTRACTION

Compound	*Concentration (μg/l)*				*R.S.D. (%)*
	100	*50*	*25*	*10*	
Ethyl benzene	33.6	32.3	32.5	26.0	±10.5
Trimethyl benzene	46.2	46.2	47.2	41.6	± 5.2
Isopropyl methyl benzene	53.5	56.0	56.2	50.6	± 4.5
Naphthalene	33.9	32.4	32.1	30.0	± 4.7
2-Methyl naphthalene	48.0	48.3	48.9	44.5	± 3.9
1-Methyl naphthalene	50.7	46.4	44.8	39.9	± 9.1
2,3-Dimethyl naphthalene	56.3	59.6	60.5	57.1	± 3.2

TABLE V

RECOVERIES (%) OF SELECTED COMPOUNDS FROM FORTIFIED FISH MUSCLE BY MICROEXTRACTION

Compound	*Concentration (ppm)*			*R.S.D. (%)*
	20	*10*	*5*	
Ethyl benzene	98.7	98.1	102.0	±5.8
Trimethyl benzene	97.7	95.7	94.2	±7.1
Isopropyl methyl benzene	97.4	93.8	89.9	±5.3
Naphthalene	98.3	100.6	113.2	±3.8
2-Methyl naphthalene	96.1	91.7	104.4	±4.0
1-Methyl naphthalene	102.6	94.3	99.0	±4.0
2,3-Dimethyl naphthalene	98.0	91.9	99.6	±4.4

column, transferred to the aqueous phase in the microextraction flask with 100 ml of acetone and re-extracted with 1 ml of hexane. The ratios were again calculated from the chromatograms and using the mean recovery figure for each compound from Table IV, the results were expressed as a percentage recovery. These recoveries from fish muscle in Table V range from 90–113% with a mean value of 98%. The relative standard deviation (R.S.D.) of results in Tables IV–V was less than 9% with the exception of ethyl benzene at the lowest concentration, possibly because it was most volatile.

DISCUSSION

It has been demonstrated that many types of organic compounds show losses during concentration steps such as rotary evaporation, use of micro Snyder column or blowing nitrogen over a solution[3,5]. The microextraction procedure overcame this disadvantage in that it extracted and concentrated in one step with minimum of loss due to handling and transfers.

Recoveries of selected compounds approached a maximum when 1 ml of hexane was used to extract 1 l of water containing 10–100 μg of contaminants. Recoveries from dichloromethane using the modified procedure were improved by 20–30% and were consistent over the concentration range investigated. When fish muscle was fortified with the standards in the 5–20 ppm range the corrected recoveries were close to 100%.

The advantage of using dichloromethane as a primary extracting solvent for fish samples was in the concentrating effect; the compounds extracted in 5 ml of dichloromethane were finally extracted into 1 ml of hexane with no evaporative step in the process. There were no detectable hydrocarbon impurities in the dichloromethane and the clean-up step using sodium sulphate and Florisil produced a clear eluate suitable for microextraction. Other solvent systems no doubt exist which would permit the transfer of organic compounds through a solvent–water–solvent phase system to achieve concentration in the final solvent.

The effect of adding inorganic salts to the aqueous layer before extraction was not investigated since consistent recoveries were achieved without this step.

Recoveries for ethyl benzene and naphthalene have been reported in the 90% range[1,2] using water to solvent ratios of 100:1 and 20:1. These high recoveries are due to the choice of pentane (b.p. 36°C) as a solvent. The use of lower water to solvent ratios partially negated the concentrating effect in that less organics were available for extraction. This was offset to some extent by higher extraction efficiencies, but the overall recovery was improved, four to five fold, when a large volume of water was extracted with a small volume of solvent. In our experience, hexane (b.p. 69°C) was found to be more suitable than pentane because it was less volatile and less soluble in water. Extracts could be stored more easily without evaporative losses and more hexane was recovered from the microextraction.

The importance of using clean water and solvents must be emphasized. Distilled water was obtained directly from a commercial still without passage through plastic pipes since these contaminated water with phthalate esters[3]. Each batch of solvents was checked for interfering contaminants before use but four small peaks persisted in the water blank chromatogram and have been attributed to impurities in the water.

The chromatograms in Fig. 1 illustrate the complex nature of the organic compounds found in crude oils and their water soluble fractions. Tentative identification have been made of some of the major components from retention time data of known compounds. The peaks numbered in the chromatogram of Alberta Crude are the *n*-alkanes present in all crude oils in varying proportions. Pristane and phytane are seen next to C_{17} and C_{18} respectively and serve as helpful points of identification. In the water soluble fraction chromatograms, the peaks numbered 1–7 are those selected compounds which appear in all water soluble fractions, again in varying proportions. Although nominally insoluble, these compounds are soluble in the low ppb range of these extractions.

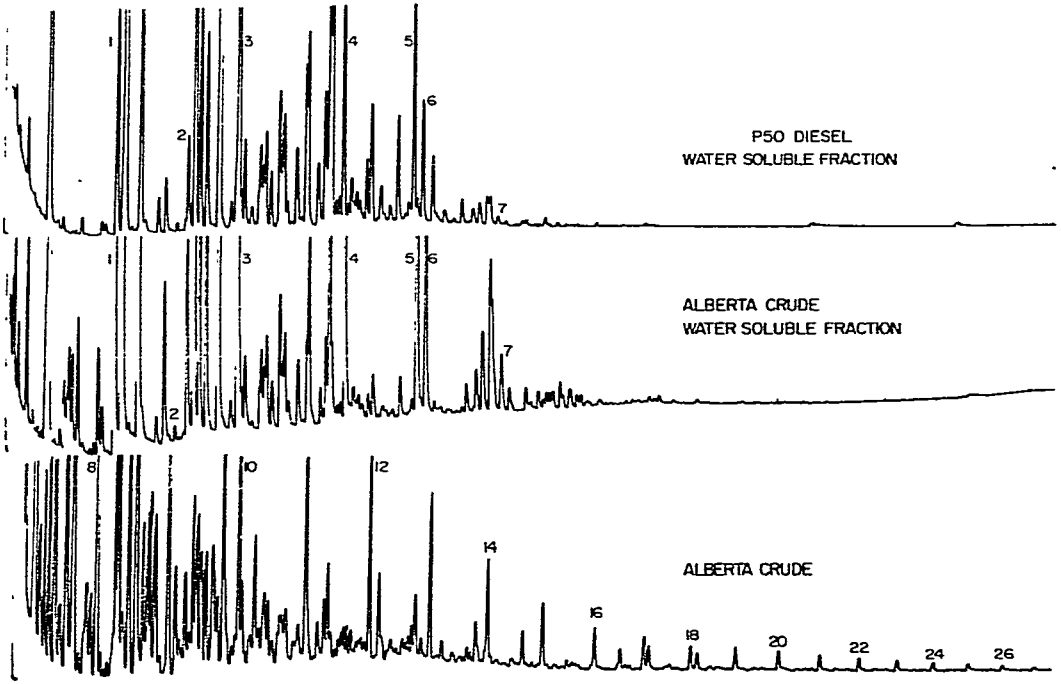

Fig. 1. Gas chromatograms of microextractions of the water soluble fractions of diesel and crude oil compared to the crude oil using a 15-m wall-coated open tubular SE-30 column and a splitless injection.

It is of interest that crude oils and petroleum products all produced similar water soluble fractions, showing the same pattern of major peaks. Further work on the sub-lethal toxic effects of these compounds on fish, invertebrates and other organisms is presently underway using the described techniques.

ACKNOWLEDGEMENTS

We thank B. G. E. de March for statistical advice and Dr. D. C. G. Muir for assistance in preparing this manuscript.

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