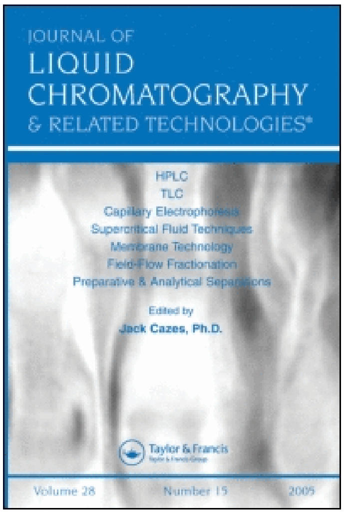

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Trace Enrichment on Pre-Columns in High Performance Liquid Chromatography. The Adsorption Capacity of a Reversed Phase Column

Ulla Lund[a]
[a] Danish Civil Defence, Analytical Chemical Laboratory, Copenhagen, Denmark

To cite this Article Lund, Ulla(1981) 'Trace Enrichment on Pre-Columns in High Performance Liquid Chromatography. The Adsorption Capacity of a Reversed Phase Column', Journal of Liquid Chromatography & Related Technologies, 4: 11, 1933 — 1945

To link to this Article: DOI: 10.1080/01483918108067553

URL: http://dx.doi.org/10.1080/01483918108067553

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(11), 1933-1945 (1981)

TRACE ENRICHMENT ON PRE-COLUMNS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. THE ADSORPTION CAPACITY OF A REVERSED PHASE COLUMN

Ulla Lund
Danish Civil Defence, Analytical Chemical Laboratory
2, Universitetsparken, DK-2100 Copenhagen
Denmark

ABSTRACT

The use of break-through experiments in investigations of the adsorption step in pre-column trace enrichment has been demonstrated.

The adsorption capacity of LiChrosorb RP-8 for phenol, 2,4-dichlorophenoxyacetic acid, ethylbenzene and pentachlorophenol has been determined as a function of the pH, ionic strength and flow-rate of the sample solution and as a function of the concentration of test substance in the sample.

The slope of the break-through curves has been found to show very little variation with the experimental conditions.

INTRODUCTION

During the last five years the trace enrichment technique has received some attention for the analysis of organic micropollutants in water. While the first reports *(e.g. 1,2)* were often limited to showing examples of the use of trace enrichment, a number of recent papers are also concerned with the efficiency of the technique. Determination of the recovery of selected test substances using one fixed sample volume has been much used (3-8). Another approach has been to compare the peak heights produced by known quantities of test substances, loaded on to the column in increasing sample volumes (8-12). Plots of peak height as a function of sample volume at fixed concentrations of test substance have also been used to determine the maximum permissible sample volume (8,13).

The present paper is concerned mainly with the adsorption step of trace enrichment. The effect of a number of variables on the adsorption capacity of a precolumn is shown using break-through experiments.

MATERIALS AND METHODS

Chemicals

2,4-Dichlorophenoxyacetic acid (Ega, Steinheim) was recrystallized from benzene.

All other chemicals and solvents were of reagent grade quality and used as received.

Apparatus

The liquid chromatograph (see Figure 1) consisted of Waters model 6000A pumps, of which one solvent selection valve was used as valve 1, a Rheodyne model 7120 injection valve (valve 2), a Waters model 440 UV-detector (254 nm) and a Waters model 660 solvent programmer. Break-through curves were recorded using an Omniscribe model 5117-2 (Houston Instruments, Texas) recorder.

Chromatography

The adsorbent used was LiChrosorb RP-8, 10 μm particles (Merck, Darmstadt). It was packed in stainless

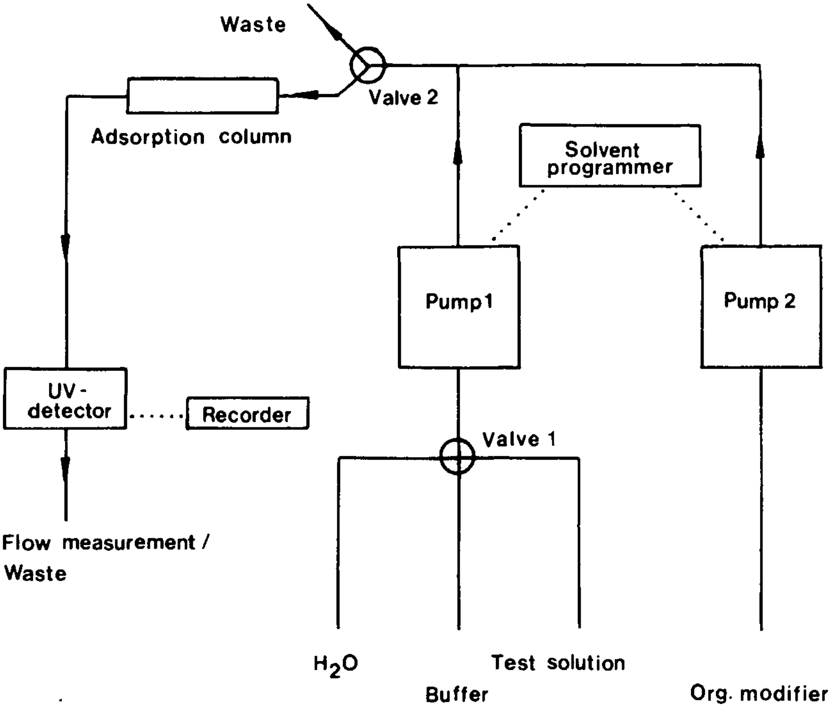

FIGURE 1

Schematic of the apparatus used for the break-through experiments.

steel columns (50 x 4.6 mm i.d.) according to a previously described procedure (14). Fittings were modified Swagelok unions. The efficiency of the columns was 950-1050 plates for phenetole (k' = 7.0) measured with methanol/water (1+1) as the mobile phase.

Procedure for Break-Through Measurements

The configuration of the liquid chromatograph used in the break-through experiments is shown in Figure 1.

Immediately before each break-through experiment the adsorption column was cleaned by running a gradient from 100 % water to 100 % methanol. After cleaning, the gradient was reversed and when 100 % water was reached valve 1 was changed to a buffer of the desired pH and ionic strength. The adsorption column was brought into equilibrium with this buffer at the flow-rate to be used in the break-through experiment after which valve 1 was switched to the test solution while valve 2 was switched to waste. Pump 1 and the tubing before valve 2 was flushed with test solution and the break-through experiment was then started by switching valve 2 back to the adsorption column.

Calculations

An example of a break-through curve is shown in Figure 2.

The area shown cross-hatched in Figure 2 corresponds to the amount of test substance adsorbed by the column, In Ref. 15 this area is integrated and the adsorption capacity calculated therefrom. If, however, the break-through curve is assumed to be symmetrical this procedure may be simplified somewhat. For a symmetrical S-curve the area under the break-through curve before the inflection point, shown by horizontal hatching in Figure 2, is identical to the area above the curve after the inflection point. This means, that the area corresponding to the amount adsorbed can be described by a rectangle, eliminating the need for integration of the S-shaped curve.

The equilibrium adsorption capacity, S (μg/g), may then be calculated from the equation:

$$S = \frac{(V_{50} - V_m)\, c_i}{W} ,$$

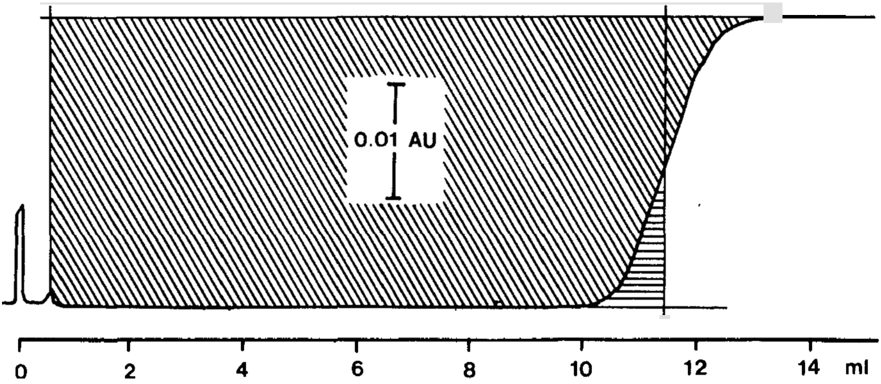

FIGURE 2

Break-through curve for phenol.
Test solution: Phenol 5.5 µg/ml in sodium phosphate buffer pH 8.0, µ 0.1. Flow-rate 5.0 ml/min.

where V_{50} (ml) is the 50 % break-through volume, V_m (ml) the void volume, c_i (µg/ml) the concentration of test substance in the test solution and W (g) the weight of adsorbent in the column.

This equation is similar to equations derived from statistical (16) and physico-chemical (17) considerations.

Another quantity of interest is the slope of the break-through curve. This may be found from mathematical descriptions of break-through curves (*e.g.* 17) but in the present investigation a simplified measure of the slope is used:

$$V_{10,rel} = \frac{V_{10}}{V_{50}} ,$$

that is, the 10 % break-through volume is measured relative to the 50 % break-through volume.

RESULTS AND DISCUSSION

Break-through experiments were conducted on LiChrosorb RP-8 for some selected test substances, *viz.* phenol, pentachlorophenol, ethylbenzene and 2,4-dichlorophenoxyacetic acid, at three concentration levels. The effects of flow-rate, pH and ionic strength of the test solution were examined.

Slope of Break-Through Curve

The influence of flow-rate (1-10 ml/min) on the relative 10 % break-through volume for phenol was examined and proved to be non-significant. Also the type of test substance had little effect on the slope, although the relative 10 % break-through volume for phenol (mean: 0.92) was slightly smaller compared to the other compounds (mean: 0.94). Phenol has the smallest absolute break-through volume and the difference observed is therefore not surprising, using evidence from elution liquid chromatography. Here it is known that the efficiency of the system increases with increasing capacity ratio in the beginning of a chromatogram, while the effect levels off at greater capacity ratios.

The age of the adsorption column, defined as the number of experiments performed on the column, proved to have the largest effect on the relative 10 % break-through volume, as shown in Figure 3. Experimental conditions other than the age of the adsorption column have not been kept constant in the figure. This procedure is justified by the fact, that these conditions have very little influence on the slope. But even the effect of adsorption column age is small, and when the column is used for a limited number of experiments, *e.g.* fifteen, the effect is negligible.

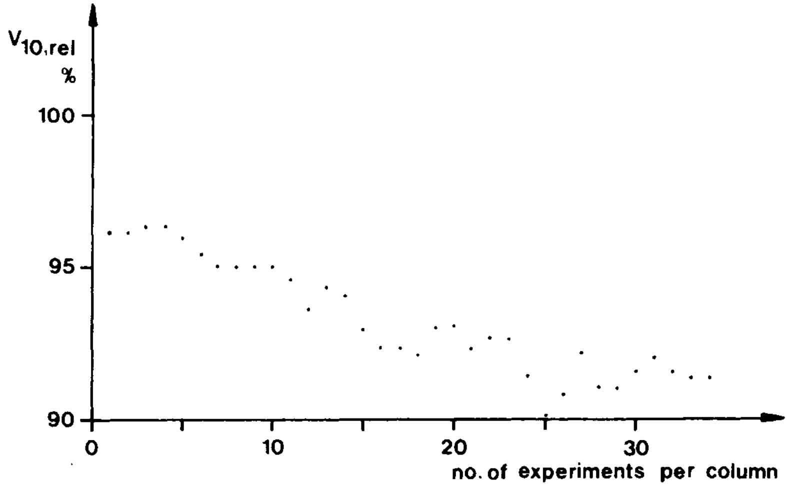

FIGURE 3

Influence of the age of the adsorption column, defined as the number of experiments performed per column, on the relative 10 % break-through volume. Points shown are mean values for seven columns.

The conclusion is, that the slope of the break-through curve under the conditions tested is nearly constant as long as the adsorption columns are not used more than fifteen times.

Adsorption Capacity

The adsorption isotherms for the four test substances at pH 8 are shown in Figure 4.

Isotherms for phenol and ethylbenzene are linear in this concentration range, the isotherm for 2,4-dichlorophenoxyacetic acid is curved, while the isotherm for pentachlorophenol shows a marked curvature. Pentachlorophenol and 2,4-dichlorophenoxyacetic acid are most pro-

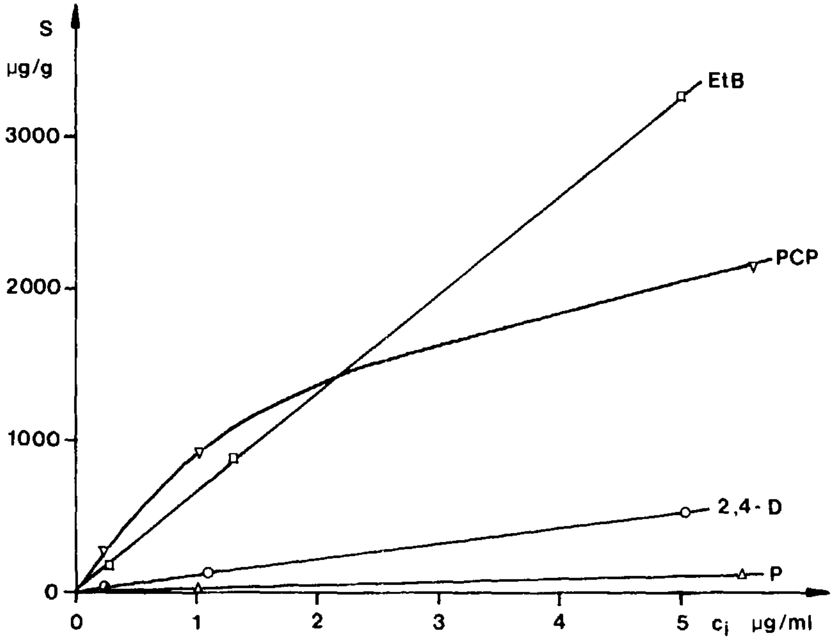

FIGURE 4

Adsorption isotherms at pH 8, µ 0.1.
Substances: Ethylbenzene (EtB), Pentachlorophenol (PCP), 2,4-Dichlorophenoxyacetic acid (2,4-D), Phenol (P).

bably retained as ion-pairs with sodium from the buffer as the counter ion, since they are both fully ionized at this pH. The linear isotherms mean, that the 50 % break-through volumes for phenol and ethylbenzene are independent of the concentration of test substance, while the 50 % break-through volumes for the two substances with convex adsorption isotherms give decreasing 50 % break-through volumes with increasing concentration of test substance.

The flow-rate of the test solution had no effect on the adsorption capacity for phenol in the range of flow-rates examined (1 - 10 ml/min). Assuming the same to be the case for the more retained test substances the rest

TABLE 1

50 % Break-through Volumes (ml) of Phenol (P), 2,4-Dichlorophenoxyacetic acid (2,4-D), Ethylbenzene (ETB) and Pentachlorophenol (PCP) at Concentrations of 5 +/- 0.5 μg/ml. Numbers Given in Parentheses are Standard deviations (n = 4).

		P	2,4-D	ETB	PCP
pH 8	μ 0.01	11 (0.1)	20 (0.3)	310 (10)	120 (4)
	μ 0.1	11 (0.3)	50 (0.3)	330 (6)	190 (11)
pH 6	μ 0.01		30 (1)		380 (48)
	μ 0.1		60 (2)		570 (60)
pH 4	μ 0.01	11 (0.5)	60 (1)	300 (2)	>5000
	μ 0.1	12 (0.3)	80 (3)	330 (8)	

of the experiments were conducted at a fixed flow-rate of 5 ml/min.

The effects of pH and buffer concentration of the test solution are seen in Tabel 1.

It is evident, that for phenol which is unionized at the pH-values in question and for ethylbenzene which is non-ionic, no measurable effect of pH on the break-through volumes is found. The buffer concentration has a small influence, which is only measurable for ethylbenzene, in that "salting out" is observed as the buffer concentration increases.

For pentachlorophenol and 2,4-dichlorophenoxyacetic acid, which have pK_a-values of 5.3 and 2.9, respectively, the break-through volumes increase as the degree of ionization decreases. For these compounds the buffer concentration has a larger influence on the break-through volumes, supporting the theory that the compounds in the ionized state are adsorbed as ion-pairs with the buffer cations. This is further supported by the fact that for 2,4-dichlorophenoxyacetic acid the influence of the buffer concentration decreases with decreasing pH-value.

Figure 4 shows, that the adsorption isotherms for pentachlorophenol and 2,4-dichlorophenoxyacetic acid are more or less curved at pH 8. Decreasing the pH-value improved the linearity of the adsorption isotherms for these two compounds. As an example the isotherms for 2,4-dichlorophenoxyacetic acid at pH 4, 6, and 8 are shown in Figure 5.

The isotherms are slightly convex at pH 6 and 8, but at pH 4, where the substance is no longer fully ionized, the isotherm is linear. Pentachlorophenol

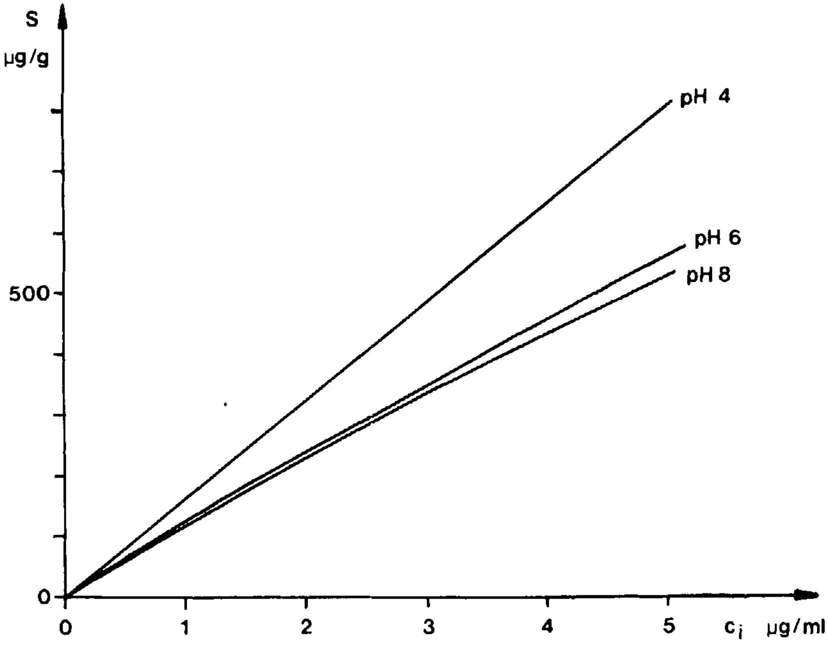

FIGURE 5

Adsorption isotherms for 2,4-Dichlorophenoxyacetic acid at three pH-values.

gave isotherms that at pH 6 were still curved, but less so than at pH 8. These findings agree well with experience from bonded-phase chromatography where many compounds have the best chromatographic properties at pH-values where the compounds are unionized (18).

CONCLUSION

The results found show, that the adsorption isotherms for the unionized and non-ionic test substances are linear and that the linearity of the isotherms for the ionized, anionic substances improves as the degree of ionization decreases. Trace enrichment of solutes in the unionized state is to be preferred, because the adsorption capacity of LiChrosorb RP-8 is largest for unionized solutes and because the degree of loading of the adsorption column is more easily controlled when the concentration of test substance has little influence on the break-through volume. In the range of buffer concentrations used very little "salting out effect" is observed.

The slope of the break-through curves show very little variation under the conditions tested. Loading of the adsorption columns up to 80-90 % of their adsorption capacities can therefore be used with very little risk of loss of the test substance in the effluent.

ACKNOWLEDGEMENT

The financial support of the Danish Council for Scientific and Technical Research (grant no. 16-9180) is gratefully acknowledged.

REFERENCES

1. Little, J.N. & Fallick, G.J., New Considerations in Detector-Application Relationships, J. Chromatogr., 112, 389, 1975.

2. Hertz, H.S., May W.E., Chesler, S.N.& Gump, B.H., Petroleum Analysis: Methodology for Quantitative and Qualitative Assessment of Oil Spill, Environ. Sci. Technol., 10, 900, 1976.

3. Oyler, A.R., Bodenner, D.L., Welch, K.J., Liukkonen, R.J., Carlson, R.M., Kopperman, H.L. & Caple, R., Determination of Aqueous Chlorination Reaction Products of Polynuclear Aromatic Hydrocarbons by Reversed Phase High Performance Liquid Chromatography - Gas Chromatography, Anal. Chem., 50, 837, 1978.

4. Otsuki, A. & Shiraishi, H., Determination of Poly-(oxyethylene)alkylphenyl Ether Nonionic Surfactants in Water at Trace Levels by Reversed Phase Adsorption Liquid Chromatography and Field Desorption Mass Spectrometry, Anal. Chem., 51, 2329, 1979.

5. Ishii, D., Hibi, K., Asai, K. & M. Nagaya, Studies of Micro High-Performance Liquid Chromatography, III. Development of a "Micro-Pre-Column Method" for Pretreatment of Samples, J. Chromatogr., 152, 341, 1978.

6. Kummert, R., Molnar-Kubica, E. & Giger, W., Trace Determination of Tetrachloroethylene in Natural Waters by Direct Aqueous Injection High-Pressure Liquid Chromatography, Anal. Chem., 50, 1637, 1978.

7. May, W.E., Chesler, S.N., Cram, S.P., Gump, B.H., Hertz, H.S., Enagonio, D.P. & Dyszel, S.M., Chromatographic Analysis of Hydrocarbons in Marine Sediments and Seawater, J. Chromatogr. Sci., 13, 535, 1975.

8. van Vliet, H.P.M., Bootsman Th.C., Frei, R.W. & Brinkman, U.A.Th., On-Line Trace Enrichment in High-Performance Liquid Chromatography Using a Pre-Column, J. Chromatogr. 185, 483, 1979.

9. Frei, R.W., Trace Enrichment and Chemical Derivatization in Liquid Chromatography; Problems and Potential in Environmental Analysis, Int. J. Environ. Anal. Chem., 5, 143, 1978.

10. Eisenbeiss, F., Hein, H., Joester, R. & G. Naundorf, The Separation by LC and Determination of Polycyclic Aromatic Hydrocarbons in Water Using an Integrated Enrichment Step, Chromatogr. Newsl. 6, 8, 1978.

11. Ogan, K., Katz, E. & Slavin, W., Concentration and Determination of Trace Amounts of Several Polycyclic Aromatic Hydrocarbons in Aqueous Samples, J. Chromatogr. Sci. 16, 517, 1978.

12. Schauwecker, P., Frei, R.W. & Erni, F., Trace Enrichment Techniques in Reversed-Phase High-Performance Liquid Chromatography, J. Chromatogr., 136, 63, 1977.

13. Saner, W.A., Jadamec, J.R., Sager, R.W. & Killeen, T.J., Trace Enrichment with Hand-Packed CO: PELL ODS Guard Columns and Sep-Pak C-18 Cartridges, Anal. Chem., 51, 2180, 1979.

14. Lund, U & Hansen, S.H., Simultaneous Determination of Atropine and Its Acidic and Basic Degradation Products by Mixed-Column High-Performance Liquid Chromatography, J. Chromatogr., 161, 371, 1978.

15. Huber, J.F.K. & Gerritse, R.G., Evaluation of Dynamic Chromatographic Methods for the Determination of Adsorption and Solution Isotherms, J. Chromatogr., 58, 137, 1971.

16. Grubner, O. & Burgess, W.A., Simplified Description of Adsorption Break-Through Curves in Air Cleaning and Sampling Devices, Am. Ind. Hyg. Assoc. J., 40, 169, 1979.

17. Bergström, G., Ekedahl, E. & Sillén, L.G., Leaking Curves for Activated Carbons with Physically Sorbed Gases at Low Leaking Concentrations, FOA Reports, 5, 1, 1971.

18. Snyder, L.R. & Kirkland, J.J., Introduction to Modern Liquid Chromatography, 2. ed., Wiley, New York, 1979.