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Possibilities of enhancing the sensitivity of the determination of UV-absorbing compounds in high-performance liquid chromatography

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ABSTRACT

The signal-to-noise ratio and the detection limits in high-performance liquid chromatographic (HPLC) analysis using UV detection depend on the design of the detector and on the detector settings. For the best sensitivity, the detector should be operated at a wavelength as close as possible to the absorption maximum, with a spectral band width as narrow as possible and with a frequency of the detector signal storage between 10 to 20 data per chromatographic peak. However, variations of the detector setting parameters, except for the detection wavelength, do not affect the sensitivity and the detection limits very significantly. In high-speed HPLC, computerized accumulation of several complete chromatograms from repeated runs can be used to enhance the sensitivity and to improve the detection limits. As has been verified on the example of reversed-phase chromatography of three chlorobenzenes with UV detection, this approach can be used with good accuracy to obtain detection limits below 100 ppb with an analysis time of 20–25 min. These are still considerably higher than the detection limits that can be obtained using on-line sample enrichment techniques with solid-phase extraction, but the chromatogram accumulation approach may be useful in those trace analysis problems where sample enrichment is difficult or tedious and where selective derivatization techniques or sensitive detectors are not available.

INTRODUCTION

In spite of generally poorer detection possibilities than in gas chromatography, the number of applicatons of high-performance liquid chromatography (HPLC) in the trace analysis of organic compounds is increasing steadily. On-line and off-line precolumn derivatization techniques in connection with sensitive, selective detectors are the most efficient approaches to improving the sensitivity of determination and the detection limits [1], but they are often not available or not readily applicable for some less reactice compounds or very dilute samples. Consequently, less sensitive UV detection should be still used in many applications.

Both the precision of the integrated peak areas and the limits of detection improve with increasing signal-to-noise ratio [2]. The detection limits are usually defined as the concentration of the sample solute that gives a signal-to-noise ratio of two [3] or three [4]. The signal-to-noise ratio can be improved either by enhancing the

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detector signal or by limiting the noise originating from the detector and the pumps of the instrument. The baseline noise is affected by fluctuations in the flow-rate, pressure and temperature [2] and depends on the design of the detector; signal processing may also contribute to the noise [5,6].

The useful signal, *i.e.*, the height or the integrated area of a chromatographic peak, can be enhanced by using a sensitive detector with optimized parameter settings, an efficient chromatographic column with a small dead volume, a chromatographic system allowing a low retention and simultaneously a sufficient separation selectivity and sample volumes as large as possible without deteriorating the column efficiency. The flow-rate of the mobile phase also affects the heights and areas of the peaks, which usually increase with decreasing flow-rate.

Computerized processing of the digitized detector signal offers a possibility of "bunching" and averaging several successive signal readings to be stored in the computer memory. In addition, diode-array spectrophotometric detectors make it possible to "bunch" simultaneous signals from several photodiodes in a spectral segment of a preselected width. This method of data processing can also affect the reproducibility, sensitivity and detection limits.

The signal-to-noise ratio can also be enhanced by using a filter to reduce the noise amplitude, but this approach is limited by the requirement to retain accurate peak shapes [5]. Fourier transformation of the chromatographic signal can be utilized for improving the signal-to-noise ratio by discriminating and cutting off the high-frequency noise from the useful signal [7]. Smoothing procedures have been suggested that allow one also to filter the noise with frequencies close to that of the signal [8].

High-speed HPLC employing short columns packed with a material of small particle diameter (3 μm) [9] is especially suitable for trace analysis, as the lower column dead volume means a lower dispersion of the solute band and an increased mass sensitivity in comparison with conventional columns [10,11]. High-quality separations of simpler sample mixtures can be obtained in 1–2 min using this technique [9], which suggests a possibility of using the accumulation of several chromatograms from repeated runs to enhance the signal-to-noise ratio and to reduce the limits of detection. Although this approach has been widely used in various spectroscopic techniques, its application to chromatographic analysis has not previously been reported, to our knowledge.

It was the objective of this work to investigate the practical feasibility of applying computerized accumulation of chromatograms from the repeated high-speed runs to enhance the sensitivity and to decrease the detection limits in HPLC analyses using UV detection and to compare this approach with other possibilities, such as the "bunching" and averaging of signal readings in preselected time and wavelength intervals and with the on-line sample enrichment technique using column switching, which has been widely used in the reversed-phase chromatography of aqueous samples [12–18]. For this investigation, a relatively simple reversed-phase separation of a mixture of chlorobenzenes was used.

EXPERIMENTAL

An HP 1090M liquid chromatograph equipped with an automatic sample injector, a column-switching valve, a 3DR solvent-delivery system, a thermostated col-

umn compartment, a Series 7994A workstation and an HP 2225 Think-Jet printer (Hewlett-Packard, Avondale, PA, USA) was used in connection with three UV spectrophotometric UV detectors. Unless stated otherwise, the chromatographic experiments were performed with a built-in standard UV diode-array detector (detector A). The other two detectors were connected to the column outlet by a 280 mm × 0.12 mm I.D. stainless-steel capillary and to the chromatographic workstation via a 760 Series analog/digital converter (Nelson Analytical): an HP 1050 wavelength programmable UV detector, equipped with a monochromator (detector B) and an HP 1050 multiple-wavelength (diode-array) UV detector (detector C), both from Hewlett-Packard. The detectors were operated at wavelengths of 223 or 230 nm.

Two conventional stainless-steel columns (250 × 4 mm I.D.) were packed with octadecylsilica Separon SGX C18, particle size (a) 5 μm and (b) 7 μm, using a high-pressure slurry technique. A high-speed column (60 × 4 mm I.D.), prepacked with Hypersil ODS, 3 μm, (c) was purchased from Hewlett-Parckard. A sample-enrichment octadecylsilica column, (40 × 2 mm I.D.) was dry-packed with Separon SGX C18, 60 μm (d). The bulk Sepharon SGX C18 materials were purchased from Tessek (Prague, Czechoslovakia).

1,4-Di-, 1,2,3,4-tetra-, 1,2,3,5-tetra- and pentachlorobenzene standards were obtained from Lachema (Bohumín, Czechoslovakia). Methanol (spectroscopic grade), purchased from Lachema (Brno, Czechoslovakia), and water, deionized and doubly distilled in glass with addition of potassium permanganate, were filtered through a 0.45-μm filter (Millipore) and used to prepare the mobile phases (90 and 80% aqueous methanol) by mixing directly in the liquid chromatograph with continuous degassing by stripping with helium. The flow-rate of the mobile phase was 1 ml/min in the experiments with the conventional columns and 3 ml/min in those with the high-speed column.

The peak areas (A) and heights (h) were measured as the arithmetic means from three repeated experiments. The detector sensitivities, S_A, S_h, were evaluated from the plots of A and h *versus* concentrations of chlorobenzenes in the range 0.5–1000 ppm (six data points) using linear regression. The baseline noise (N) was determined as the difference between the upper and lower readings of the baseline signal during a 2-min period; the minimum detectable concentrations (MDC) corresponding to the peak heights equal to twice the peak-to-peak noise were evaluated from the concentration plots of S_h for the individual solutes.

For the experiments with on-line sample enrichment using solid-phase extraction, the sorption precolumn (d) was connected to the analytical column (b) in the HP 1090M chromatograph via a standard switching valve. A U6K injector (Waters–Millipore, Milford, MA, USA) was inserted between the autosampler and the precolumn. In the sorption step, 2 ml of the aqueous sample were pushed from the sample loop through the precolumn by water pumped using channel A of the chromatographic pump. After washing the precolumn with water, the column switching valve was switched to the position directing the eluate from the precolumn to the analytical column and to the detector. Aqueous methanol (80%) as the mobile phase was then pumped using channels B and C of the chromatographic pump to elute the enriched sample from the precolumn and to accomplish the chromatographic separation on the analytical column. To test the recovery in this technique, artificial aqueous samples containing 0.01–1 ppm of each of the chlorobenzenes tested were injected in this

way and the peak areas were compared with the experiments where 20-μl samples containing equal masses of chlorobenzenes as in the enrichment experiments (concentrations 1–100 ppm) dissolved in the mobile phase were injected directly into the analytical column using the autosampler.

RESULTS AND DISCUSSION

Effects of the construction and parameters of a UV detector on the sensitivity and limits of detection

The limits of detection can be conveniently characterized as the concentration of a sample solute resulting in a detector signal, S, equal to twice the baseline noise, N, at the maximum of the chromatographic peak. The suitability of various experimental conditions to provide the best limits of detection can be tested by comparing the corresponding signal-to-noise ratios, S/N.

The results of the comparison of three different variable-wavelength UV detectors under the same experimental conditions summarized in Table I suggest that the most important factors for the detector performance are the length of the detector cell and the baseline noise. At a comparable noise level, the sensitivity of detection is higher and the detection limits are lower for detector B with a longer optical path length of the cell (10 mm) in comparison with detector C (6 mm). Because of the construction differences, detector A showed a higher sensitivity (S_A *ca.* 120%, S_h *ca.* 30-50%), but a *ca.* 2.5 times higher noise level than detector C with the same optical path length, which resulted in a 1.8 times lower S/N and higher detection limits for the chlorobenzenes tested. Although these results could be expected on the basis of the Lambert-Beer law, only qualitative estimates can be made *a priori* because of other important design parameters, such as the volume and shape of the detector cell and of the connecting tubing.

TABLE I

NOISE (N), INTEGRATED PEAK AREA (S_A) AND PEAK HEIGHT (S_h) SENSITIVITIES AND MINIMUM DETECTABLE CONCENTRATIONS (*MDC*) CORRESPONDING TO PEAK HEIGHTS EQUAL TO TWICE THE BASELINE NOISE CALCULATED FROM N AND S_h FOR DETECTORS A–C

Compounds: 1 = 1,4-dichlorobenzene; 2 = 1,2,3,4-tetrachlorobenzene; 3 = 1,2,3,5-tetrachlorobenzene. Wavelength, 230 nm; band width, 4 nm.

Parameter	Compound	Detector		
		A	B	C
N (mV)		0.015	0.006	0.006
S_A (area units · $cm^3 \cdot g^{-1} \times 10^{-5}$)	1	47.60	42.97	21.38
	2	38.32	31.44	16.92
	3	38.38	33.25	17.54
S_h (mV · $cm^3 \cdot g^{-1} \times 10^{-5}$)	1	4.67	7.50	3.16
	2	2.24	3.41	1.83
	3	2.05	3.06	1.47
MDC (ppm)	1	0.064	0.016	0.038
	2	0.13	0.035	0.066
	3	0.15	0.039	0.082

It is well known that the detection wavelength should be set to the spectral absorption maximum to obtain the highest detection sensitivity. Also, the spectral band width affect S/N and the detection limits. In contrast to spectrophotometric detectors equipped with a monochromator that usually operate with a fixed width of the monochromator exit slit, diode-array detectors allow the spectral band width to be controlled by "bunching" together the signals from all the photodiodes within a preselected spectral range. We tested different spectral band width settings of detector A from 4 to 60 nm. In this range, the baseline noise was approximately constant (0.1–0.14 mV), with no systematic dependence on the spectral band width. With the detection wavelength set to the maximum of the absorption band of 1,4-dichlorobenzene, *i.e.*, 223 nm, both the area and the height of the chromatographic peak of this solute decreased with increasing spectral band width, as expected (Figs. 1 and 2). More interesting were these dependencies for the two tetrachlorobenzenes having absorption maxima below 190 nm, so that the detection wavelength could be set only at the slope of the absorption band. Here, flat maxima of the integrated peak areas were observed between spectral band widths of 30 and 50 nm. However, the increase in the integrated peak areas and in the peak heights with increasing spectral band width represents only a marginal improvement (Figs. 1 and 2), so that the selection of the detection wavelength as close as possible to the adsorption maximum and of the spectral band width as narrow as possible in agreement with common spectroscopic practice, can be recommended for obtaining the best sensitivity and detection limits, even for compounds lacking absorption maxima in the near-UV spectral region.

Another variable detection parameter is the frequency with which the detector signal is digitized and stored in the computer memory during the elution of a chromatographic peak. Several subsequent "readings" of the detector signal may be

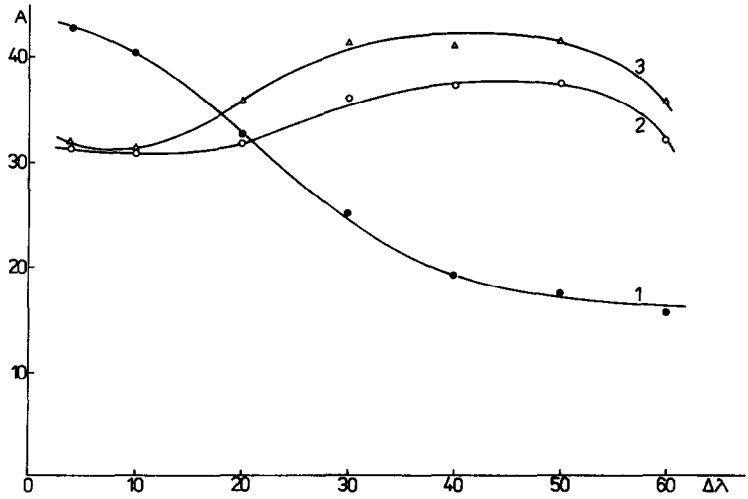

Fig. 1. Dependence of the peak areas A (in integrator units) on the spectral band width $\Delta\lambda$ (in nm) for (1) 1,4-dichlorobenzene, (2) 1,2,3,4-tetrachlorobenzene and (3) 1,2,3,5-tetrachlorobenzene. Column, Hypersil ODS, 3 μm, 60 × 4 mm I.D.; mobile phase, 80% methanol in water, 3 ml/min: sample volume, 5 μl; detector A, λ = 223 nm, 0.32 s data storage period.

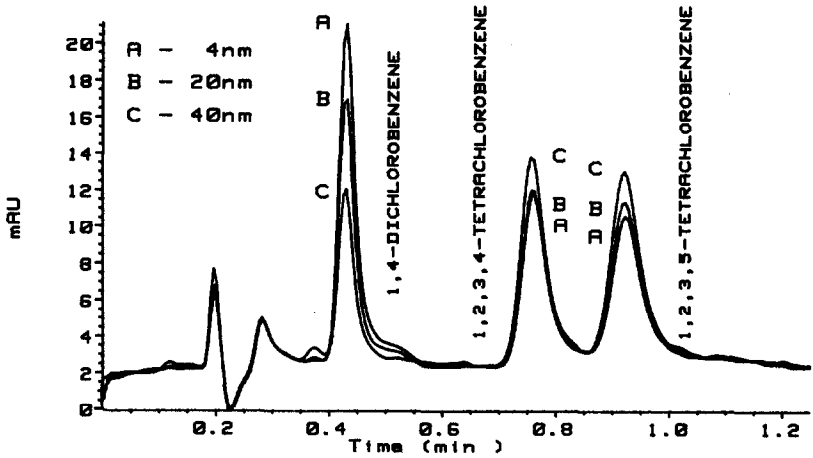

Fig. 2. Overlayed chromatograms for a mixture of three chlorobenzenes at different spectral band widths. Conditions as in Fig. 1.

"bunched" and averaged within the signal storage period, which results in reduced baseline noise. For example, the experimental noise of detector A at 223–230 nm, with 90% methanol as the mobile phase, was reduced from 0.11 mV at a signal storage period of 0.32 s to 0.025 mV at a signal storage period of 1.28 s. However, the signal storage frequency should not fall below the minimum required to give 10–20 signals stored per chromatographic peak, otherwise the peak shape is not reconstructed accurately and poor reproducibility of quantitation may result. To achieve the best S/N, this minimum signal storage frequency should not be exceeded.

Computer-assisted accumulation of chromatograms

The computerized accumulation of spectrograms is a well known approach to increase the sensitivity of various spectroscopic methods, such as NMR, Fourier transform IR and mass spectrometry, but to our knowledge this technique has not previously been applied to the improvement of sensitivity and detection limits in chromatography. Unlike the signal "bunching" and averaging during a single chromatographic run discussed earlier, several chromatograms from the subsequent repeated runs are added to the contents of a single raw data memory register so that the register eventually contains the sum of all the chromatograms from the repeated runs. In the final accumulated chromatograms reconstructed from the contents of the register, the chromatographic signal is amplified proportionally to the number of individual repeated chromatographic runs, but because of the random character of the baseline noise, the final noise after the accumulaton is expected to increase proportionally to the square root of the number of accumulations, yielding an increase in S/N and a decrease in the detection limits proportional to the square root of the number of accumulations, as in the accumulated spectrograms.

The technique of computerized accumulation of chromatograms is practically feasible only in connection with high-speed separations, to avoid excessive analysis times. It can be speculated that good reproducibility of the retention times is also

required for this approach, otherwise both the leading and the trailing edges of the small peaks of trace compounds in some of the subsequent individual chromatograms could be shifted outside the integration area of the accumulated peak, resulting in a negative error in the integrated peak area in the final reconstructed chromatogram.

The main objective of this work was to investigate the practical feasibility of applying computerized accumulation of chromatograms in high-speed HPLC. The same three chlorobenzenes were used as sample compounds as in the previous section, with a high-speed Hypersil ODS column. In 80% methanol as the mobile phase, the separation of the three solutes could be accomplished in 1.2 min at a flow-rate of 3 ml/min. This means that twenty individual chromatograms could be accumulated in *ca.*. 24 min, which is the analysis time commonly accepted for many separations on conventional analytical columns. For this investigation, detectors B and C were no longer available to us, so that detector A had to be used. On the basis of the results reported in the previous section, we adjusted the detection parameters to the optimum settings: a detection wavelength of 223 nm and a spectral band width of 4 nm were the optimum values for 1,4-dichlorobenzene (Figs. 1 and 2) and a signal storage period of 0.32 s provided 13–18 data stored during the elution of a peak for the three sample compounds, which is in the aforementioned optimum signal storage frequency range.

Fig. 3 shows a non-accumulated chromatogram from one of the subsequent repeated runs with the sample containing 0.1 ppm of each of the three chlorobenzenes. According to Table I, this concentrations is sligthly above the detection limit for 1,4-dichlorobenzene and below the detection limits for the two tetrachlorobenzenes, whose peaks could be only tentatively attributed in the baseline noise on the basis of the retention times of the more concentrated standards. The peak heights and areas for the 0.1 ppm sample are necessarily subject to significant errors (relative standard deviations 35–60%, Table II).

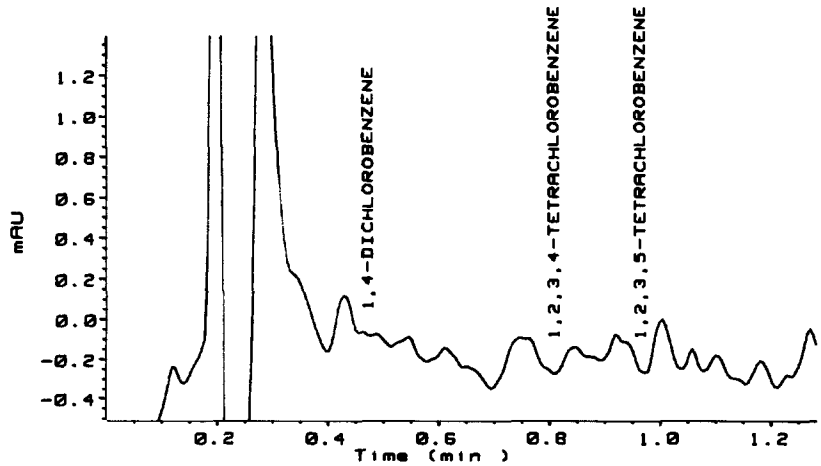

Fig. 3. Single chromatogram for 5 μl of 0.1 ppm methanolic solution of chlorobenzenes. Conditions as in Fig. 1; spectral band width 4 nm.

TABLE II

EFFECT OF THE COMPUTERIZED ACCUMULATION OF CHROMATOGRAMS ON SENSITIVITY AND DETECTION LIMITS

Column, Hypersil ODS, 3 μm, 60 × 4 mm I.D.; mobile phase, 80% methanol in water, 3 ml/min; 5 μl of methanolic solutions of 1,4-dichlorobenzene (1), 1,2,3,4-tetrachlorobenzene (2) and 1,2,3,5-tetrachlorobenzene (3); detector A, 223 nm, spectral band width 4 nm, data storage period 0.32 s. A_i, A_a, h_i and h_a, integrated peak areas (in integrator area units) and peak heights (in mAU) for an unaccumulated chromatogram (*i*) and for the chromatogram reconstructed after 20 acumulations (*a*). *MDC* = minimum detectable concentration (ppm).

Compound	Concentration (ppm)	A_i[a]	ΣA_i[b,*]	A_a*	h_i	h_a*	MDC_i	MDC_a
1	10	43.85 ±1.0	876.9	869.2	14.2	300	0.3	0.06
	1	3.34 ±0.83	66.8	69.9		36		
	0.1	0.39 ±0.14	7.8	6.4	0.2	4.1		
2	10	31.36 ±1.05	627.2	619.3	7.2	150	0.6	0.12
	1	3.61 ±1.11	72.1	59.0		18		
	0.1	0.36 ±0.15	7.2	4.6	0.1	2.2		
3	10	34.42 ±1.14	688.3	688.0	6.2	125	0.7	0.15
	1	3.20 ±1.01	64.1	60.0		15		
	0.1	0.37 ±0.22	7.4	5.1	0.1	2.2		

[a] Arithmetic means from twenty repeated experiments ± standard deviaton.

[b] Sum of the areas evaluated from the individual non-accumulated chromatograms.

* Regression equations for the concentration dependences (concentrations c in ppm; R = correlation coefficient):

Solute: 1 $A_a = -9.803 + 87.830c$; $R = 0.9999$
2 $A_a = -2.341 + 62.153c$; $R = 0.9999$
3 $A_a = -5.349 + 69.303c$; $R = 0.9999$
1 $\Sigma A_i = -0.550 + 88.692c$; $R = 0.9998$
2 $\Sigma A_i = 0.262 + 62.244c$; $R = 0.9999$
3 $\Sigma A_i = -0.104 + 69.014c$; $R = 0.9999$
1 $h_a = 3.611 + 29.664c$; $R = 0.9987$
2 $h_a = 1.889 + 14.823c$; $R = 0.9999$
3 $h_a = 1.778 + 12.330c$; $R = 0.9999$.

Fig. 4 shows a reconstructed accumulated chromatogram after the summation of twenty individual chromatograms such as that shown in Fig. 3. Comparison of Figs. 3 and 4 shows that the peaks of the chlorobenzenes are clearly distinguished from the baseline noise in the accumulated chromatogram, with the peak heights amplified *ca.* 20-fold. Should there be other trace impurities in the sample, their peaks would be amplified in the same ratio as the peaks of the chlorobenzenes. However, the amplitudes of all the other "peaks" in Fig. 4 were 0.9 mAU or lower, in comparison

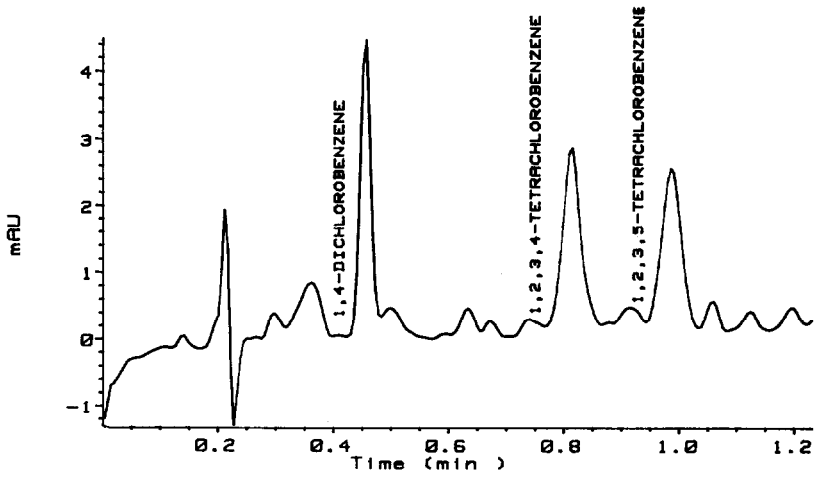

Fig. 4. Chromatogram for 5 μl of 0.1 ppm methanolic solution of chlorobenzenes after twenty accumulations of repeated experiments. Conditions as in Figs. 1 and 4.

with the maximum amplitude of the baseline noise in the non-accumulated chromatogram of 0.22 mAU (Fig. 3). This is only a 4.1-fold increase after the acculumation, corresponding well with the expected noise increase of $\sqrt{20}$ = 4.5-fold, which allows us to conclude that no impurities were present in the sample in concentrations comparable to those of the sample solutions and to attribute all the small "peaks" in Fig. 4 to the baseline noise.

To test the accuracy of the accumulation approach, the peak areas and heights before and after the accumulation were compared for three different concentrations of the solutes tested (Table II). The areas of the peak in the accumulated chromatograms, A_a, differ from the sum of the peak areas in the original twenty chromatograms, A_i, by *ca.* 1% for 10 ppm samples and 10–20% for 1 ppm samples. The differences between A_a and A_i relative to A_a were less than the relative standard deviation of a single experiment. Also, the dependences of both A_a and A_i on the concentration of the sample solutes, c, showed good linearities, with the slopes differring by less than 1%. The increased absolute values of the intercepts of the A_a *versus* c plots can possibly be attributed to the accumulation of the noise.

The heights of peaks in the accumulated chromatograms are slightly greater than the sums of the peak heights in the individual non-accumulated chromatograms (*ca.* 1–5% for 10 ppm samples and 3–10% for 0.1 ppm samples). The plots of the peak heights in the accumulated chromatograms *versus* the concentrations of chlorobenzenes in the samples analysed were linear, like the plots of the peak areas *versus* c. The miminum detectable concentrations corresponding to the peak heights twice the baseline noise were 0.3–0.7 ppm for non-accumulated chromatograms and decreased to 0.06–0.15 ppm after twenty accumulations, *i.e.*, *ca.* 5-fold, in agreement with the theoretical prediction ($\sqrt{20}$ = 4.5).

On the basis of the present results, it can be concluded that the technique of computerized accumulation of chromatograms in high-speed HPLC can be perform-

TABLE III

LINEARITY AND RECOVERY TESTS OF THE ON-LINE ENRICHMENT TECHNIQUE FOR AQUEOUS SAMPLES OF CHLOROBENZENES

Analytical column, Separon SGX C18, 7 μm, 250 × 4 mm I.D.; enrichment precolumn, Separon SGX C18, 60 μm, 40 × 2 mm I.D.; mobile phase, 80% methanol in water, 1 ml/min; sample volume, 2 ml; solutes, as in Table I; detector A, 230 nm. A = Integrated areas in integrator units; c = concentrations of sample solutes; R = correlation coefficient; recovery as % of areas determined in the experiments with direct injection of 20-μl samples containing equal amounts of sample solutes as in the enrichment experiments.

Regression equations for the concentration dependences

Solute	Equation	R
1	$A = -6.41 + 3357\,c$	0.998
2	$A = 12.1 + 3639\,c$	0.999
3	$A = 26.7 + 3423\,c$	0.999

Concentration dependences of recoveries

c (ppm)	Recovery (%)		
	Solute 1	Solute 2	Solute 3
1	83.0	84.7	80.8
0.5	86.5	78.0	78.2
0.1	82.8	75.7	80.0
0.05	86.8	65.4	76.2
0.02	58.5	76.3	87.1
0.01	70.7	67.2	94.8

ed with adequate accuracy. It allowed the detection limits of the chlorobenzenes tested to be decreased about 5-fold to the 100 ppb level in a tolerable time of analysis of 24 min.

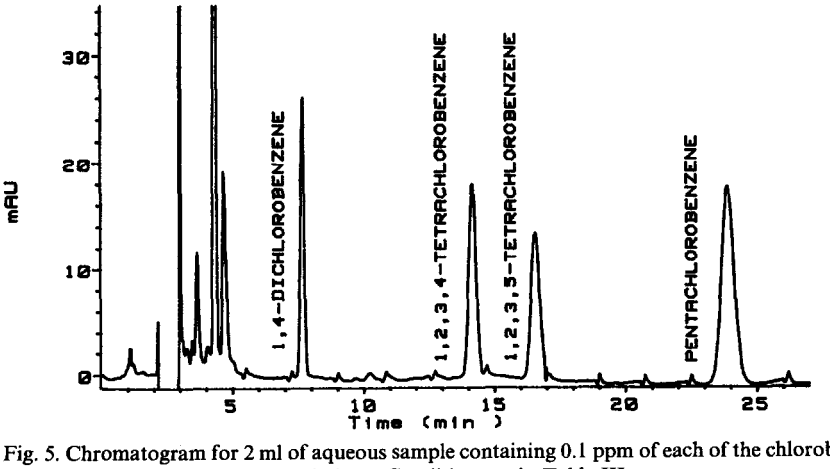

Fig. 5. Chromatogram for 2 ml of aqueous sample containing 0.1 ppm of each of the chlorobenzenes using the on-line sample enrichment technique. Conditions as in Table III.

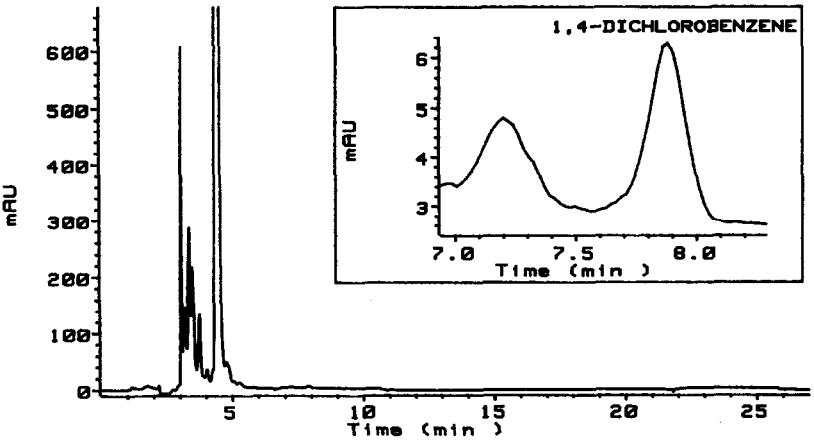

Fig. 6. Chromatogram for 2 ml of a sample of waste water from an industrial plant, containing 14 ppb of 1,4-dichlorobenzene, using the on-line sample enrichment technique. Conditions as in Table III.

On-line sample enrichment using solid-phase extraction

The tests of the on-line sample enrichment technique in reversed-phase HPLC of the three chlorobenzenes, summarized in Table III, showed good linearity of the calibration graphs in the range 50–1000 ppb with average recoveries of 80%. Fig. 5 shows the chromatogram for 2 ml of an artificial sample containing 0.1 ppm of each of the chlorobenzenes in water. The chromatogram of a practical sample of waste water from an industrial plant, containing 14 ppb of 1,4-dichlorobenzene (identified on the basis of both the retention time and the UV spectrum), is shown in Fig. 6. The detection limits for the 2-ml samples are in the range 2–5 ppb, *i.e.*, approximately 30 times lower than those achieved with 5-μl samples using high-speed HPLC with twenty accumulations of the repeated experiments. The amount of 1,4-dichlorobenzene in the sample in Fig. 6 is below the detection limits of the chromatogram accumulation technique.

A combination of computerized accumulation of chromatograms with the on-line sample enrichment technique could possibly further decrease the limits of detection. However, (1) it would require a low inner-volume switching valve suitable for work with high-speed columns (such a valve was not available to us), (2) longer analysis times should be tolerated and (3) the detection limits in on-line sample enrichment techniques are likely to be controlled by the "chromatographic" noise originating from trace impurities in the sample or in the components of the mobile phase rather than by the baseline noise; this "chromatographic" noise, of course, cannot be reduced by the technique of computerized accumulation of chromatograms. On the other hand, the chromatogram accumulation approach may offer a means of increasing the sensitivity and decreasing the limits of determination for analyses where the application of sample enrichment techniques would be tedious of where there is no adequate sorbent for solid-phase extraction.

CONCLUSIONS

The computerized accumulation of subsequent repeated chromatograms in high-speed HPLC makes it possible to increase the signal-to-noise ratio and to decrease the minimum detectable concentrations approximately in proportion to the square root of the number of accumulations with good accuracy of quantification. A compromise between the time of analysis and the detection limits should be chosen; however, the accumulation of the data from twenty repeated simple high-speed HPLC separations at 100 ppb levels may take no longer than 20–30 min, which is a time comparable to those for many single-run analyse on conventional columns.

To obtain low detection limits using the accumulation approach it is important to use a detector designed to yield a high signal-to-noise ratio. The UV detector set to the wavelength of the absorption maximum should use a narrow spectral band width setting and a signal acquisition frequency of 10–20 signal readings during the elution of a chromatographic peak.

On-line sample enrichment techniques based on solid-phase extraction result in detection limits at least one order of magnitude lower than those achievable using computerized accumulations of repeated chromatograms, but the latter approach may be a useful option for analyses where no adequate sorbent is available or where the sample enrichment techniques are too tedious.

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