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APPLICATION OF COMPUTERIZED ACCUMULATION OF CHROMATOGRAMS IN HPLC WITH VARIOUS DETECTION SYSTEMS AND WITH ON- AND OFF-LINE SOLID PHASE EXTRACTION SAMPLE ENRICHMENT

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Computerized accumulation of chromatograms acquired in several repeated runs may be used to enhance the signal-to-noise ratio and to decrease the detection limits in High Performance Liquid Chromatography (HPLC). The technique is feasible not only with short ("high-speed") columns, but also with conventional analytical columns, provided the separation is rapid. This approach can be applied in combination with UV, fluorescence and electrochemical detection systems and with the on- or off-line solid phase extraction (SPE) enrichment techniques. Computerized accumulation of chromatograms may further improve the detection limits achieved in HPLC with sensitive detection methods and the SPE sample enrichment.

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Introduction

Sensitivity in High Performance Liquid Chromatography (HPLC) as commonly used with UV detection is relatively low, usually in the nanogram range with respect to the sample amounts injected, even though as little as 10^{-2} ng of highly absorptive compounds can sometimes be detected. The detection is generally improved if more sensitive and selective electrochemical or fluorimetric detectors are used. Optimization of chromatographic and detection conditions is essential for good results in the trace analysis by HPLC [1]. Even with optimized separation and detection conditions, special sample pre-treatment and enrichment is often necessary to achieve low concentration limits of detection (ppb or less). Various sample enrichment techniques can be applied "off-line", prior to the chromatographic analysis, but HPLC can easily be adapted to on-line sample enrichment of aqueous samples by solid-phase extraction (SPE) on a pre-column packed with a suitable sorbent [2–4].

The limits of detection are usually defined as the concentration of the sample solute that gives a signal-to-noise ratio of two [5] or three [6]. The signal-to-noise ratio can be improved either by decreasing the noise or by enhancing the signal using a sensitive detector with optimized parameters of detection, efficient separation column (low dispersion) and chromatographic system with a low retention of sample compounds just providing sufficient resolution of sample compounds [7]. The baseline noise is influenced by fluctuations in the mobile phase flow, pressure and temperature, by the construction of the detector and by the way of the data processing [8]. High-speed HPLC using short columns is especially suitable for trace analysis, as a low column volume causes a low dispersion of the solute band, which results in increased peak height and sensitivity with respect to conventional columns [9,10].

The signal-to-noise ratio can be enhanced also by computerized processing the digitized detector signal using "bunching" and averaging several successive signal readings. With multichannel detectors such as diode-array UV detector simultaneous "bunching" of signals from several channels (photodiodes) may be useful for this purpose. A smoothing filter can be used to reduce the noise, but this process can distort peak shapes if used inappropriately [8]. 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. Smoothing procedures have been suggested that allow one also to filter the noise with frequencies close to that of the signal [11].

Computerized accumulation of spectrograms is a well-known approach in NMR, FTIR and mass spectroscopy to enhance the sensitivity and to improve the detection limits. We have applied this approach earlier to computerized accumulation of chromatograms acquired in several repeated HPLC experiments [12]. In contrast to well-known averaging of several subsequent signal readings or bunching

of signals from several detector channels (such as signals of a diode-array detector recorded at several adjacent wavelengths) during a single chromatographic run, chromatograms from several repeated runs are subsequently added to the contents of a single raw data memory register of the computer used for data processing. After this procedure, several repeated chromatograms are contained in the computer register as a new, accumulated chromatogram, which represents the sum of all repeated chromatograms. Processing of the accumulated chromatogram is as simple as that of single-run chromatograms. In the accumulated chromatogram, the signal-to-noise ratio should be increased and the detection limits are decreased proportionally to the square root of the number of accumulated chromatographic experiments with respect to the original single-run chromatograms. This procedure can be applied also to the chromatograms recorded in repeated runs used to determine the standard deviation of the results.

In the original report, a 60×4 mm I.D. "high speed" column and a HP 1090 liquid chromatograph equipped with a diode-array UV detector were used [12]. In this system, the accumulation approach could be used without problems and yielded the expected enhancement of the signal-to-noise ratio. However, the approach has some limitations and possible drawbacks.

1) The pre-requisite for successful application of the accumulation approach is outstanding reproducibility of the retention data, otherwise additional broadening of peaks occurs and the advantage of signal enhancement is lost. Although good performance was proven with high-speed analysis where the individual chromatographic runs took three minutes or less, the results may not be that good with longer separation times. One of the objectives of the present work was to verify feasibility of the accumulation approach with conventional analytical columns (15 cm long), where the separation usually takes a longer time than with short (3 – 5 cm long) columns.

2) The most important concern may be the time of the analysis, which increases proportionally to the number of repeated runs. This limits the applicability of the accumulation approach to short analyses of relatively simple sample mixtures, preferably using short analytical columns packed with small particles (1.5 – 3 μm). In such cases, the accumulation of the data from ten repeated simple high-speed HPLC separations may take no longer than 15 – 20 min, which is comparable to the time of many single-run analyses on conventional columns and should improve the concentration detection limits three times. This point may be less important if the analyses are run unattended overnight in an automated sequence.

3) Finally, if the time of analysis is to be kept within reasonable limits, the improvement in detectability gained using the accumulation of repeated chromatograms is usually significantly lower than with other approaches such as enhancement of concentration by the sample pre-treatment, derivatization and using selective detection systems. Using the accumulation of chromatograms, only the random noise is suppressed, but the signals of interfering sample components are enhanced in the

same way as the signals of the analyzed compounds. On the other hand, the accumulation approach is simple and does not require any changes in the analytical method. From the practical point of view, other ways should be tested first to improve the sensitivity of the analysis and the accumulation approach will probably represent the "last resort" in obtaining some additional decrease of the limits of detection of an already optimized HPLC method. In the present work, we investigated further improvement of the detection limits, which can be obtained by accumulation of chromatograms combined with selective and sensitive fluorimetric and electrochemical detection methods and with SPE sample enrichment methods.

Experimental

Chemicals

Methanol and ethanol (UV spectroscopy grade), sodium tetraborate, potassium dihydrogenphosphate, sodium carbonate, 1,4-dichlorobenzene, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene were obtained from Lachema (Brno, Czech Republic), 2-phthaldialdehyde, 2-mercaptoethanol, ethylamine 70% , n-propylamine, n-butylamine, (all reagent grade) from Fluka (Buchs, Switzerland) and pentylamine from Janssen (Beerse, Belgium). All reagents were of analytical grade. Metoxuron and chlortoluron were obtained from the Agricultural Control Institute (Brno, Czech Republic).

Deionized water was doubly distilled in glass with addition of potassium permanganate. All solvents were filtered through a 0.45 μm membrane filter (Millipore, Milford, MA, USA) and used to prepare the mobile phases by mixing in required ratios directly in the liquid chromatograph.

Instrumentation

A Hewlett-Packard (Avondale, Palo Alto, USA) 1090M liquid chromatograph equipped with a diode array detector, an autosampler, a column-switching valve, a 3DR solvent-delivery system and a Series 7994A workstation was used. Programmable fluorescence detector HP 1046A (Hewlett-Packard) or three-electrode electrochemical detector HP 1049A (Hewlett-Packard) with a glassy carbon measuring electrode were connected to the workstation of the liquid chromatograph via an analog/digital convertor (Nelson Analytical). A "high speed" Hypersil ODS, 3 μm, 60×4 mm I.D. (Shandon, Runcorn, UK) or a "conventional" Separon SGX C 18, 7 μm, 150×3.3 mm I.D. (Tessek, Prague, Czech Republic) analytical columns were used.

For on-line sample enrichment using solid phase extraction, a short stainless-

steel sorption column (40×2 mm I.D.) dry packed in the laboratory with octadecyl silica Separon SGX C 18, 60 μm, (Tessek) was connected to the analytical column via a six-port automatic switching valve. A U6K sample injector (Waters, Milford, MA, U.S.A.) with variable injection volumes (1 μl to 2 ml) was inserted between the autosampler of the liquid chromatograph and the sorption column.

All columns and the switching valve were placed in the thermostatted column compartment of the chromatograph, kept at 40 °C.

Methods

Standard Preparation and Sample Pre-treatment.

Calibration standard solutions containing 0.001 to 1 ppm of each chlorinated benzene, 1 – 0.1 ppb of each metoxuron and chlortoluron and 0.02 – 0.1 ppb of each of the aliphatic amines were prepared by appropriate mixing of the basic stock solutions (0.1g of each compound was dissolved in 100 ml methanol) with freshly bi-distilled water.

For the off-line sample enrichment of samples containing phenylurea pesticides prior to the HPLC analysis, an SPE vacuum Dorcus apparatus (Tessek, Prague, Czech Republic) was used to pass large volumes of samples through a Separon SGX C18, 60 μm, SILICA-CART cartridge (Tessek). Pesticides were recovered from the cartridge with 3 ml methanol. The volume of the desorbate containing pesticides was adjusted to 5 ml and an aliquote volume was injected into the liquid chromatograph.

For the off-line enrichment of the samples of aliphatic amines, the cartridges packed in the laboratory with Spheron C 1000, 25 – 40 μm, spherical polymeric weak cation exchanger (Lachema, Brno) were employed in the Dorcus apparatus. Adsorbed amines were recovered with 3 ml 1M perchloric acid. The desorbate from the Dorcus apparatus containing amines was neutralized with sodium hydroxide and mixed with 3 ml of derivatization agent prepared by mixing 1.5 ml ethanolic 2-phthaldialdehyde (10 mg ml^{-1}), 1.5 ml ethanolic 2-mercaptoethanol (4.5 mg ml^{-1}) and 90 ml borate buffer, pH 10.5 (1.7 g sodium tetraborate and 1.6 g sodium carbonate in 200 ml of bi-distilled water). The reaction mixture was left for three minutes at the ambient temperature, the volume was adjusted to 10 ml with methanol, and an aliquote volume was used for injection [13].

Chromatography

A) A Hypersil ODS column, mobile phase consisting of 80% methanol in water at the flow rate 3 ml min^{-1} and UV detection at 223 nm were used for the

separation of the individual chlorobenzenes after on-line SPE sample enrichment on a Separon SGX C18 sorption column. In the sorption step, the sorption column was equilibrated with water and a 2 ml sample was injected by means of the U 6K injector. The outlet from the sorption column was directed to the waste container. The sorption column was washed with 1 ml water, then the six-port valve was switched over to the second position and the mobile phase was directed to the sorption column, from where the enriched sample compounds were desorbed and led to the analytical column to be separated from each other in the mobile phase.

B) A Separon SGX C18 column, mobile phase consisting of 0.02 mol l^{-1} KH_2PO_4 in 60% methanol at the flow rate of 1 ml min^{-1} and electrochemical detection at the potential of +1.3 V were used for the separation of phenylurea pesticides.

C) A Hypersil ODS column, mobile phase consisting of 65% methanol in water at the flowrate of 1 ml min^{-1} and fluorimetric detection with the excitation wavelength set at 223 nm and the emission wavelength at 435 nm were used for the separation of derivatized aliphatic amines.

Data Acquisition and Processing

In the accumulation procedure, several chromatograms from the subsequent runs were added to the contents of a single raw data memory register, so that the register eventually contained the sum of all the chromatograms from the repeated runs, allowing to reconstruct the accumulated chromatogram.

The mean values of the peak areas and heights of the peaks obtained either from the single-run or from the accumulated chromatograms for the calibration standard solutions were used to construct the calibration curves. Minimum detectable concentrations corresponding to the peak heights twice the peak-to-peak noise were evaluated from the calibration plots.

Results and Discussion

Accumulation of Chromatograms with UV Detection and On-line Enrichment of the Samples of Chlorinated Benzenes.

Using on-line enrichment of a 2 ml sample containing 1,4-dichlorobenzene, 1,2,3,4-tetrachlorobenzene and 1,2,3,5-tetrachlorobenzene in water by solid phase extraction technique, the sensitivity is increased 80 times with respect to direct injection of a 25 µl sample. A single-run chromatogram (Fig. 1A) of a sample containing 10 ppb of each sample component obtained on a "high-speed" analyti-

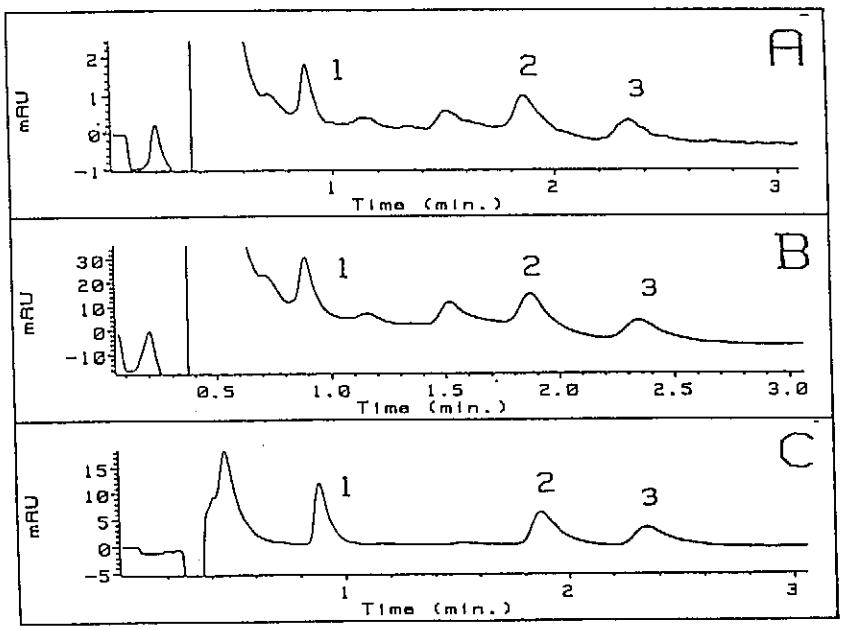

Fig. 1 Chromatograms of 2 ml aqueous samples containing 1,4-dichlorobenzene (1), 1,2,3,4-tetrachlorobenzene (2) and 1,2,3,5-tetrachlorobenzene (3) using on-line sample enrichment: A – single-run chromatogram, 10 ppb of each sample component; B – accumulated chromatogram after 20 runs, 10 ppb of each sample component; C – single-run chromatogram, 200 ppb of each sample component. Sorption column, Separon SGX C18, 60 μm, 40 × 2 mm i.d., "high-speed" analytical column, Hypersil ODS, 3 μm, 60 × 3 mm i.d., mobile phase, 80% methanol in water (v/v), flow rate 3 ml min^{-1}, UV detection at 230 nm.

cal column is compared with the chromatogram obtained after 20 accumulations of repeated chromatograms (Fig. 1B), in which similar peak areas are apparent as in a single-run chromatogram of 2 ml sample containing 200 ppb of each sample component (Fig. 1C).

The recovery of the individual chlorinated benzenes in the SPE enrichment step was 75 – 90% in the range of 20 – 1000 ppb. The accuracy of the accumulation process was tested by comparing peak heights h_a and areas A_a in the chromatogram accumulated after 20 runs with the sum of the peak heights $\sum h_i$ or areas $\sum A_i$ recorded in 20 individual single-run chromatograms (Table I). The differences between the sums of the single-run data and the data from the accumulated chromatograms was 4% or less for the peak areas and 10% or less for the peak heights at 10 ppb concentration levels.

The minimum detectable concentration corresponding to twice the baseline noise is 1.26 – 3.29 ppb for single-run chromatograms and improves approximately

Tab. I Integrated peak areas (A_i, A_a, in arbitrary units) and peak heights (h_i and h_a, in milliabsorbance units) in a single-run chromatogram (i) and in the chromatogram after 20 accumulations (a).

	ppb	A_i	$\sum A_i$	A_a	$\sum h_i$	h_a	MDC_i	MDC_a
1	10	4.42	88.43	88.24	24.38	22.56	1.26	0.29
2	10	6.09	121.87	127.67	18.80	16.07	1.63	0.41
	1	1.48	29.6	10.72	4.24	1.75		
3	10	4.15	83.04	80.93	9.31	7.86	3.29	0.84
	1	1.67	33.32	10.83	4.20	1.61		

$\sum A_i$, $\sum h_i$ are the sums from 20 single chromatograms.On-line sample enrichment and separation of a 2 ml sample of 10^{-8} g l^{-1} (10 ppb) and 10^{-9} g l^{-1} (1 ppb) 1,4-dichlorobenzene (1), 1,2,3,4-tetrachlorobenzene (2), 1,2,3,5-tetrachlorobenzene (3) on a "high-speed" analytical column with UV detection. MDC = minimum detectable concentrations (in ppb units).

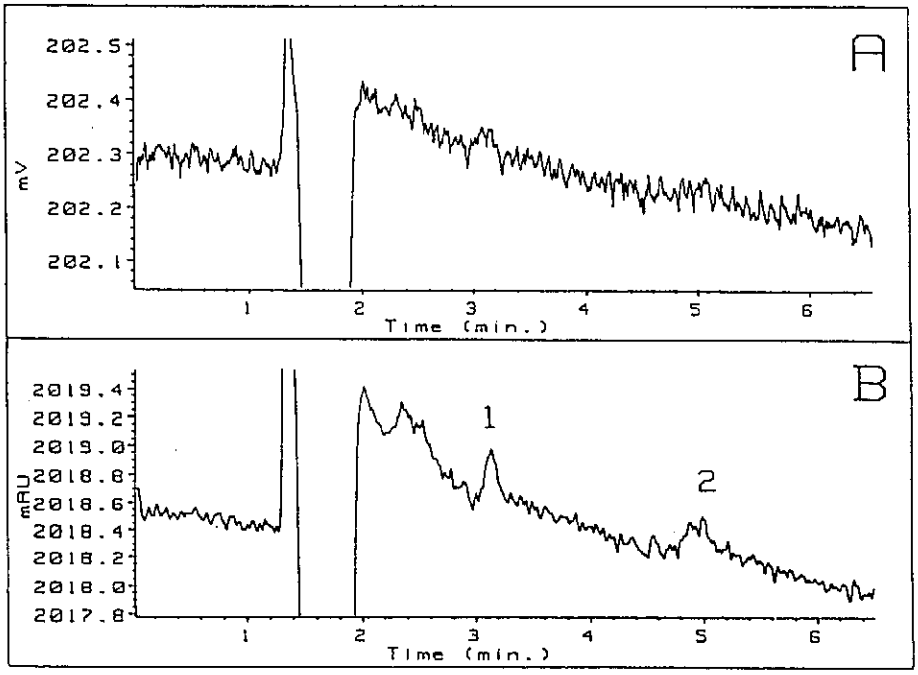

Fig. 2 Chromatogram of 2 ml aqueous samples containing 1,2,3,4-tetrachlorobenzene (1) and 1,2,3,5-tetrachlorobenzene (2) using on-line sample enrichment: A – single-run chromatogram, 1 ppb of each tetrachlorobenzene; B – accumulated chromatogram after 20 runs, 1 ppb of each tetrachlorobenzene. Conditions as in Fig. 1

four times after twenty accumulations, i.e. to 0.29 – 0.84 ppb, as expected. The concentration detection limits (MDC) in a 2 ml sample correspond to approximately 1.5 ppb for the two tetrachlorobenzene isomers, as it is demonstrated in Fig. 2. The peaks of chlorinated benzenes cannot be seen in the baseline noise in a single-run chromatogram of the aqueous sample containing 1 ppb of each tetrachlorobenzene isomer after the on-line sample enrichment step (Fig. 2A). In the chromatogram (Fig. 2B) reconstructed after twenty accumulations of the repeated experiments the peaks of isomeric tetrachlorobenzenes can be distinguished from the baseline noise.

Accumulation of Chromatograms with Electrochemical Detection after Off-line Enrichment of the Samples of Phenylurea Pesticides

Electrochemical detection is generally more sensitive and more selective than UV detection, so that the interferences caused by impurities are suppressed and the detection limits are improved. The effect of the accumulation of chromatograms of the phenylurea pesticides metoxuron and chlortoluron on a conventional analytical column after off-line sample enrichment on a Separon SGX C18 SILICA-CART cartridge with the electrochemical detection is illustrated by Fig. 3 and Table II. The recovery of the SPE step was 70% for metoxuron and 90% for chlortoluron, respectively, the calibration curves being linear in the range of 0.5 – 10 ppb. The peak areas and heights a in single-run chromatogram agree with the data in the chromatogram obtained after ten accumulations of the run with the sample diluted ten times.

Figure 3 shows a single-run chromatogram of 25 μl of an aqueous sample containing 10 ppb (Fig. 3A) and 1 ppb (Fig. 3B) of each metoxuron and chlortoluron after off-line SPE with the enrichment factor 100. The chromatogram 3C obtained with the same sample as in Fig. 3B after ten accumulations demonstrates considerable improvement in the signal to noise ratio. The sample containing 0.1 ppb of each pesticide yielded a single-run chromatogram with no detectable peaks (Fig. 4A). After the accumulation of ten chromatograms, it was possible to clearly distinguish the peak of metoxuron from the baseline noise and the peak of chlortoluron was just at the detection limit (Fig. 4B). The minimum detectable concentrations for metoxuron and chlortoluron are 0.36 and 0.50 ppb in non-accumulated chromatograms and 0.13 and 0.20 ppb in chromatograms re-constructed after ten accumulations, which is in agreement with the expected three fold decrease in detection limits.

This example shows that the reproducibility of the experimental data with modern instrumentation is sufficient for the accumulation approach even with conventional analytical columns and longer analysis times (the time of analysis was six minutes, i.e., approximately one hour with ten accumulations).

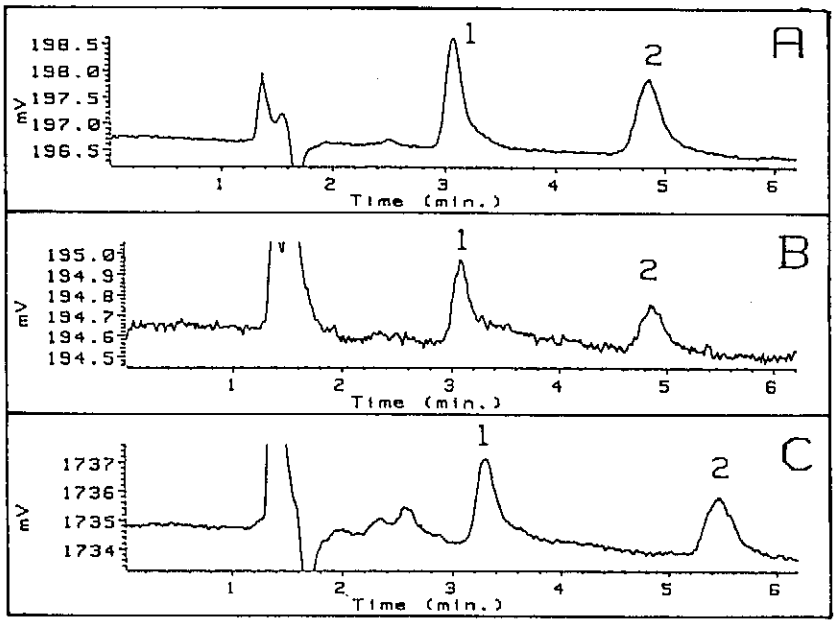

Fig. 3 Chromatogram of 2 ml aqueous sample containing phenylurea herbicides metoxuron (1) and chlortoluron (2) using off-line sample enrichment: A – single-run chromatogram, 10 ppb of each herbicide; B – single-run chromatogram, 1 ppb of each herbicide; C – accumulated chromatogram after 10 runs, 1 ppb of each herbicide. Sorption cartridge, SILICA-CART Separon SGX C18, 60 μm, "conventional" analytical column, Separon SGX C18, 7 μm, 150 × 3 mm i.d., mobile phase, 0.02 M KH_2PO_4 in 60% methanol, 0.5 ml min^{-1}, electrochemical detection at +1.3 V

Accumulation of Chromatograms with Fluorimetric Detection and Off-line Sample Enrichment Combined with Derivatization of the Samples of Aliphatic Amines

Fluorimetric detection is connected with similar gain in selectivity and sensitivity with respect to the UV detection as the electrochemical detection. The chromatograms of four n-alkylamines (ethyl- to n-pentyl) on a "high-speed" column after off-line SPE enrichment on a SILICA-CART cartridge packed with Spheron C1000 (enrichment factor 50) and derivatization with 2-phthaldialdehyde and 2-mercaptoethanol are shown in Fig. 5. In a single-run chromatogram of 25 μl of enriched and derivatized sample containing 0.02 ppb of each amine (Fig. 5A), it is possible to distinguish the two first peaks (ethylamine and propylamine), but not the peaks of butyl- and pentylamine. From the chromatogram of this sample after twenty accumulations (5B), quantitative evaluation of the peaks of ethylamine and n-propylamine is possible and the peak of n-butylamine can be detected. This chroma-

Tab. II Integrated peak areas (A_i, A_a, in arbitrary units) and peak heights (h_i and h_a, in milliabsorbance units) in a single-run chromatogram (i) and in the chromatogram after 10 accumulations (a).

	ppb	A_i	$\sum A_i$	A_a	$\sum h_i$	h_a	MDC_i	MDC_a
1	1	3.48	34.77	32.33	2.94	2.54	0.36	0.13
	0.5	1.66	16.58	17.55	1.98	1.84		
	0.1			3.89		0.41		
2	1	3.32	33.19	32.31	2.15	1.82	0.50	0.20
	0.5	1.56	15.57	16.45	1.18	1.14		
	0.1			3.04		0.26		

$\sum A_i$, $\sum h_i$ are the sums from 10 single chromatograms. Separation of 2 ml aqueous sample of 10^{-9} g l^{-1} (1 ppb), 5×10^{-10} g l^{-1} (0.5 ppb) and 10^{-10} g l^{-1} (0.1 ppb) metoxuron (1) and chlortoluron (2) on a Separon SGX C18, 7 μm, 150×3 mm i.d. conventional analytical column with electrochemical detection at +1.3 V, after off-line SPE enrichment on a SILICA-CART sorption cartridge packed with Separon SGX C18, 60 μm. MDC = minimum detectable concentrations (in ppb units).

Regression equations for the concentration dependencies of integrated accumulated peak areas (concentration c in ppb; R = correlation coefficient):

1. $A_a = 1.11 + 31.52c$; $R = 0.9991$
2. $A_a = -0.06 + 32.49c$; $R = 0.9999$

togram is very similar to that obtained after 20 accumulations of chromatograms with the direct injection of 25 μl sample of 1 ppb of the amine derivatives (5C). The peak of pentylamine is still burried in the baseline noise.

The recovery of the amines after the SPE enrichment was between 90 and 100% in the range of 1 – 10 ppb. The peak heights and areas in the accumulated chromatograms correspond to approximately 90 – 95% of the sums of the data from the individual single-run chromatograms. The minimum detectable concentrations decreased from 0.021 – 0.043 ppb with non-accumulated chromatograms to 0.008 – 0.015 ppb with the chromatogram reconstructed after twenty accumulations, which is slightly less than expected (Table III). The calibration curves for the accumulated peak heights and areas of ethyl and n-propylamine show similar slopes and correlation coefficients as the calibration curves for the sum of single-run chromatograms, which proves that no significant peak shape deformation occurs due to the accumulation approach.

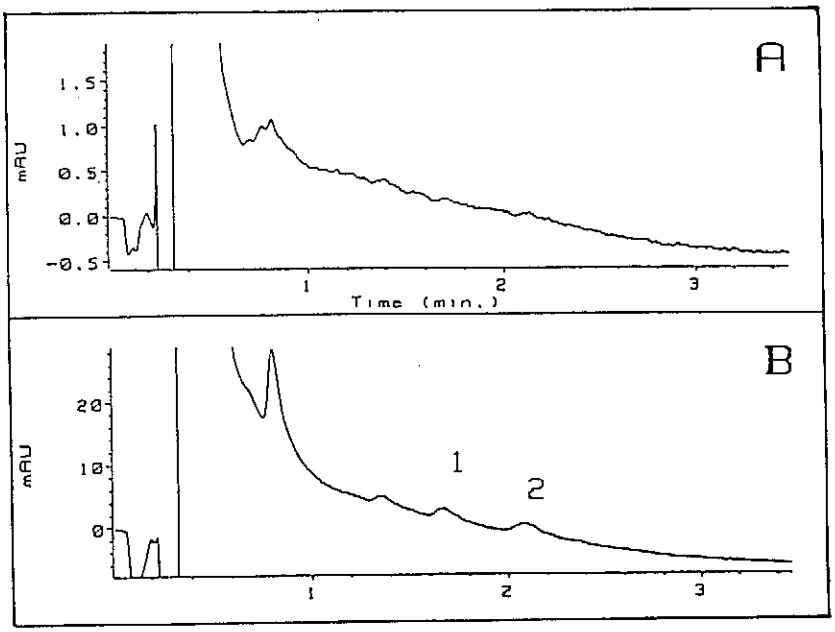

Fig. 4 Chromatogram of 2 ml aqueous sample containing phenylurea herbicides metoxuron (1) and chlortoluron (2) using off-line sample enrichment: A – a single-run chromatogram, 0.1 ppb of each herbicide; B – the accumulated chromatogram after 10 runs, 0.1 ppb of each herbicide. Conditions as in Fig. 3

Conclusion

Computerized accumulation of subsequently acquired chromatograms from repeated runs can be applied to obtain additional improvement of detection limits in HPLC, where the sensitivity has been optimized using other methods, such as combination with both on-line and off-line sample enrichment techiques, derivatization and using sensitive fluorescence and electrochemical HPLC detection, suppressing of band broadening in the column by using short and efficient columns and as low a retention of sample compounds as allowed by the resolution required. In this way, it is possible to obtain three or four times decreased concentration limits with respect to non-accumulated runs, even lower than 0.1 ppb. Long-term stability of the modern instrumentation allows one to apply this approach in connection with conventional analytical columns for simple separations of early eluted compounds, but short "high-speed" chromatographic columns are to be preferred, because of low peak dispersion and short time required to process great number of repeated single runs.

Tab. III Integrated peak areas (A_i, A_a, in arbitrary units) and peak heights (h_i and h_a, in milliabsorbance units) in single-run chromatogram (i) and in chromatogram after 20 accumulations (a).

	ppb	A_i	$\sum A_i$	A_a	$\sum h_i$	h_a	MDC_i	MDC_a
1	0.1	2.10	21.03	19.91	13.54	11.89	0.021	0.008
	0.05	1.11	11.13	10.32	7.03	6.17		
	0.02	0.49	4.87	4.15	3.42	2.60		
2	0.1	1.89	18.92	16.14	12.72	9.64	0.018	0.007
	0.05	0.95	9.54	8.94	6.43	4.56		
	0.02	0.48	4.78	4.04	3.59	2.03		
	0.1	1.21	12.13	11.92	6.29	5.88	0.038	0.011
	0.05	0.77	7.68	7.06	3.55	3.08		
	0.02			2.72		1.25		
	0.1	0.90	8.97	8.80	5.14	4.21	0.043	0.015
	0.05	0.55	5.54	4.63	2.84	1.77		
	0.02			2.02		0.56		

$\sum A_i$, $\sum h_i$ are the sums from 10 single chromatograms. Separation of 2 ml aqueous samples of 10^{-10} g l^{-1} (0.1 ppb), 5×10^{-11} g l^{-1} (0.05 ppb) and 2×10^{-11} g l^{-1} (0.02 ppb) of ethylamine (1), propylamine (2), butylamine (3) and pentylamine (4) after off-line SPE enrichment on a SILICA-CART sorption cartridge, packed with Spheron C1000, 25 – 40 μm and derivatization, on a "high-speed" analytical column, Hypersil ODS, 3 μm, 60×4 mm i.d. with fluorimetric detection, excitation wavelength 223 nm, emission wavelength 435 nm.

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

1. $A_a = 0.33 + 196.47c$; $R = 0.9998$
2. $A_a = 1.18 + 150.51c$; $R = 0.9994$
3. $A_a = 0.82 + 113.18c$; $R = 0.9939$
4. $A_a = 0.35 + 84.61c$; $R = 0.9999$

1. $\sum A_i = 0.83 + 202.60c$; $R = 0.9998$
2. $\sum A_i = 1.00 + 177.86c$; $R = 0.9991$

1. $h_a = 0.32 + 115.95c$; $R = 0.9999$
2. $h_a = -0.02 + 95.79c$; $R = 0.9989$
3. $h_a = 0.13 + 57.68c$; $R = 0.9997$
4. $h_a = -0.42 + 45.95c$; $R = 0.9988$

1. $\sum h_i = 0.81 + 126.88c$; $R = 0.9998$
2. $\sum h_i = 1.04 + 115.32c$; $R = 0.9974$

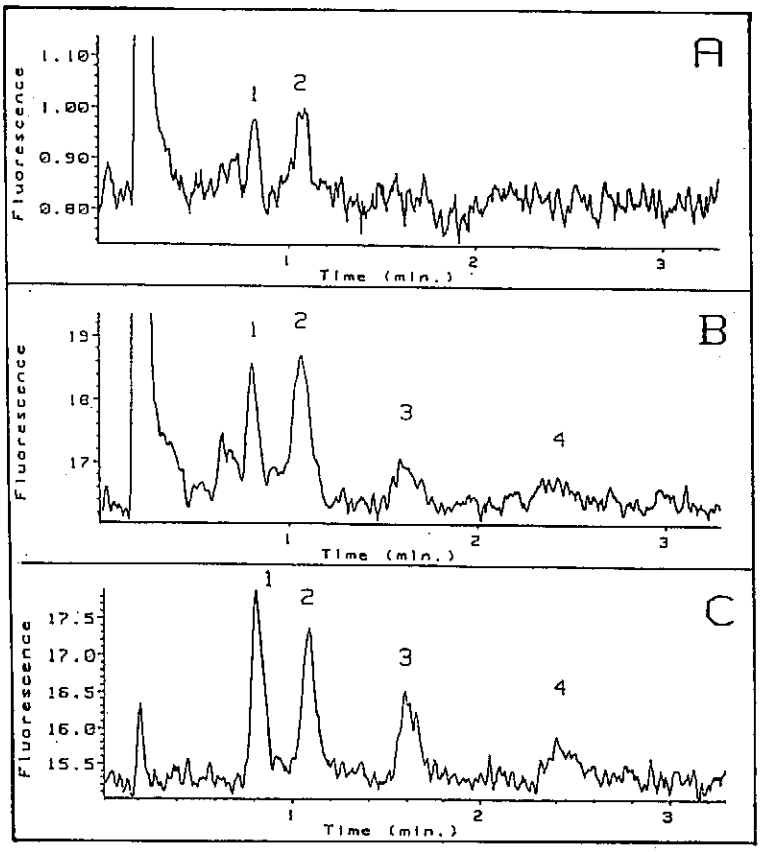

Fig. 5 Chromatogram of a 2 ml aqueous sample containing ethylamine (1), n-propylamine (2), n-butylamine (3) and n-pentylamine (4) using off-line sample enrichment: A – a single-run chromatogram, 0.02 ppb of each amine; B – the accumulated chromatogram after 20 runs, 0.02 ppb of each amine C – direct injection of 25 µl aqueous sample without enrichment, 1 ppb of each amine Sorption cartridge, SILICA-CART packed with Spheron C1000, 25 – 40 µm, "high-speed" analytical column, Hypersil ODS, 3 µm, 60 × 4 mm i.d., mobile phase, 65% methanol in water, 2 ml min^{-1}, fluorimetric detection, excitation wavelength 223 nm, emission wavelength 435 nm

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