Journal of Chromatography, 149 (1978) 721–733

CHROM. 10,791

CORRELATION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: A TECHNIQUE FOR IMPROVING THE DETECTION LIMIT APPLIED TO THE ANALYSIS OF PHENOLS

Tj. Th. LUB, H. C. SMIT and H. POPPE

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)

SUMMARY

Correlation chromatography was applied to the high-performance liquid chromatographic separation of phenols in aqueous medium on a reversed-phase column. For a correlation time of 2 h the detection limit was reduced by a factor of 100.

INTRODUCTION

In correlation chromatography, the chromatogram is obtained by evaluating the cross-correlation function of a random sample concentration input to the column and the resulting output. Applications to gas chromatography (GC) have been described earlier[1–7]. The most important advantage of this technique is its ability to decrease detection limits considerably without using a better detector, which is particularly important in liquid chromatography (LC), where high detection limits are a common problem. General treatments of the detection limit with respect to concentration in the sample solution were given by Huber *et al.*[8] and later by Karger *et al.*[9]. They concluded that increasing the injection volume to about the volume standard deviation of the peak will preserve the resolution to a reasonable extent and keep the dilution within the column at an acceptable level of about a factor of 3. Although even larger injection volumes can be used in order to avoid dilution, this will destroy the resolution in the chromatogram, and even then the output concentration may be too low to be detected.

The detection limit with respect to sample concentration is, for normal chromatography, therefore connected with the concentration detection limit of the measuring device used. Correlation chromatography is able to overcome this problem, owing to its signal-enhancing properties. As in all enhancement techniques, a price has to be paid with respect to the time required. This time loss, however, is very small, as will be shown in this paper.

In this work, we used phenol and 2,3-dimethylphenol as sample components. For separation, a reversed-phase system was chosen. The reasons for this choice of components and system were (1) the detection of trace amounts of phenols in water is relevant to environmental analysis and (2) reversed-phase systems do not have a good

reputation with regard to stationarity (collapsing of support, clogging of frits), and we intended to prove the usefulness of correlation techniques even for these systems.

THEORETICAL

Improvement of signal-to-noise ratio

In correlation high-performance LC (HPLC), the flow input to the column is randomly switched from pure eluent to eluent containing sample and *vice versa*. The switching function is a pseudo random binary sequence (PRBS)[6,10]. This function is to be preferred to other random inputs, for the following reasons: (1) its simplicity (the only levels are +1 and −1); (2) this function can easily be generated and reproduced; (3) its special properties offer the possibility of reducing so-called correlation noise.

The PRBS input function is cross-correlated with the detector output. The resulting cross-correlogram, $R_{xy}(\tau, T)$, is equivalent to a chromatogram as it would be

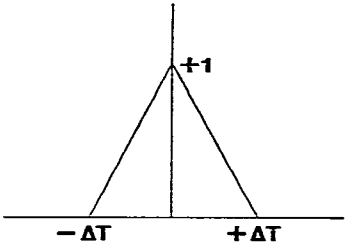

Fig. 1. Virtual injection function of chromatogram obtained by cross-correlation of PRBS and output of the system.

obtained by a two-clockperiod triangular injection (noise not considered) (Fig. 1) Treating a noise-free system, we obtain:

$$R_{xy}(\tau,T) = \frac{1}{T}\int_{-T/2}^{+T/2} x(t-\tau)y(t)\mathrm{d}t \equiv \int_{-\infty}^{+\infty} h(t')R_{xx}(\tau-t',T)\mathrm{d}t' = h(\tau) * R_{xx}(\tau,T) \quad (1)$$

where

$x(t)$ = input to column–detector system;
$y(t)$ = response of column–detector system to $x(t)$;
T = integration or correlation time;
τ = time shift;
$R_{xx}(\tau,T)$ = autocovariance function (ACVF) of $x(t)$;
$R_{xy}(\tau,T)$ = cross-correlation function of $x(t)$ and $y(t)$;
$h(\tau)$ = impulse response function of column–detector system (commonly known as a chromatogram);
$*$ represents the convolution operation.

The PRBS input $x(t)$ is a periodic function, causing $R_{xx}(\tau,T)$ to assume the same periodicity $N\Delta T$. This can be mathematically expressed by writing

$$R_{xx}(\tau,T) = \sum_{l=-\infty}^{+\infty} S(\tau-lN\Delta T,T) \quad (2)$$

If $T = kN\Delta T$, where N = sequence length of PRBS, ΔT = clockperiod of PRBS and k = positive integer, then

$$\left.\begin{aligned} S(\tau,kN\Delta T) &= 1 - \frac{|\tau|}{\Delta T}\left(\frac{N+1}{N}\right) && \text{if } |\tau| \leqslant \Delta T \\ S(\tau,kN\Delta T) &= \frac{-1}{N} && \text{if } \Delta T \leqslant \tau \leqslant (N-1)\Delta T \\ S(\tau,kN\Delta T) &= 0 && \text{if } \tau > (N-1)\Delta T \\ & && \text{or } \tau < -\Delta T \end{aligned}\right\} \quad (3)$$

For all practical purposes ($N \geqslant 127$), eqn. 3 can be replaced by the simpler expression

$$\left.\begin{aligned} S(\tau,kN\Delta T) &= 1 - \frac{|\tau|}{\Delta T} && \text{if } |\tau| \leqslant \Delta T \\ S(\tau,kN\Delta T) &= 0 && \text{if } |\tau| \geqslant \Delta T \end{aligned}\right\} \quad (4)$$

The right-hand side of eqn. 1 is a convolution of $h(\tau)$ with $R_{xx}(\tau,T)$. This corresponds to injecting with a function $R_{xx}(\tau,T)$, $R_{xy}(\tau,T)$ being the response to this (virtual) injection function.

Apart from $y(t)$, the detector produces noise output $n(t)$. We assume additivity of noise and signal:

$$z(t) = y(t) + n(t) \quad (5)$$

The noise will contribute to the cross-correlogram:

$$R_{xz}(\tau,T) = \frac{1}{T}\int_{-T/2}^{+T/2} x(t-\tau)z(t)\mathrm{d}t = \frac{1}{T}\int_{-T/2}^{+T/2} x(t-\tau)y(t)\mathrm{d}t + \\ + \frac{1}{T}\int_{-T/2}^{+T/2} x(t-\tau)n(t)\mathrm{d}t = R_{xy}(\tau,T) + R_{xn}(\tau,T) \quad (6)$$

As $n(t)$ is not caused by $x(t)$, no correlation with $x(t)$ exists, so $E[R_{xn}(\tau,T)] = 0$, where E represents the expected value.

The enhancement of the signal-to-noise ratio can be predicted. The power spectral density (PSD) function of R_{xz} is

$$G_{xy}(\omega) = \frac{1}{T}G_{xx}(\omega)G_{yy}(\omega) + \frac{1}{T}G_{xx}(\omega)G_{nn}(\omega) \quad (7)$$

The stochastic part of G_{xz} is $1/T\, G_{xx} \cdot G_{nn}$. Therefore, the variance of the baseline of the correlogram is:

$$\sigma^2_{b,R_{xz}} = \sigma^2_{R_{xn}} = \frac{1}{T}\int_{-\infty}^{+\infty} G_{xx}(\omega) \cdot G_{nn}(\omega)\mathrm{d}\omega \quad (8)$$

Consequently, the detection limit of the correlogram, related to peak height, can be predicted.

In general, peak height is not the best parameter and integration of a well resolved peak yields a more reliable analytical value. Baseline noise is integrated together with the analytical signal; it gives rise to an uncertainty in the integral. This uncertainty can be determined from the PSD $G_{xn}(\omega)$ or from the ACVF of the baseline of the correlogram $R_{R_{xn}R_{xn}}(\tau')$ (ref. 11):

$$\sigma_I^2 = 2\int_0^T (T-\tau)R_{xx}(\tau)\mathrm{d}\tau \tag{9}$$

where

σ_I^2 = variance of integrated baseline noise;
$R_{xx}(\tau)$ = ACVF of baseline noise;
T = integration time.

Eqn. 9 was used to determine σ_I^2, whereas $R_{RR}(\tau')$ was computed directly from the baseline noise of the cross-correlogram.

The system should be stationary. If the system is not stationary, owing to an irregular flow-rate or changes in the capacity constants, the correlogram will suffer from increased baseline noise, not caused by uncorrelated system noise $n(t)$ (ref. 4). Non-stationarity will also cause a broadening of all peaks.

Optimal conditions

When recording a correlogram, one has to pay attention to the following parameters.

(a) Optimal sequence length of the PRBS. $R_{xx}(\tau)$ is periodical function with a period $N\Delta T$, causing $R_{xy}(\tau)$ to assume the same periodicity. This implies that $N\Delta T$ has to be chosen so as to be greater than or equal to the length of the expected chromatogram (*ca.* $t_{R,\mathrm{last}} + 3\,\sigma_{\mathrm{last}}$), including all peaks, be they of analytical interest or not. If this condition is not met, peaks with high t_R will be folded back to the first part of the chromatogram.

(b) Optimal sampling rate. The highest frequency $\omega_{\mathrm{max.}}$ in the impulse response $h(t)$ is about $3/\sigma_0$, where σ_0 is the gaussian peak standard deviation of the unretarded component(s), or, if those are absent, the least retarded component. $\omega_{\mathrm{max.}}$ is the highest frequency that can pass the system, regardless of the input, because

$$G_{yy}(\omega) = G_{xx}(\omega)\cdot \mid H(\omega) \mid^2 \tag{10}$$

In order to meet the condition of Shannon's theorem, $\omega_{\mathrm{sample}} \geqslant 2\,\omega_{\mathrm{max.}}$.

(c) Optimal clockperiod of the PRBS. As the correlogram is identical with a theoretical response to a two clockperiod triangular injection, a compromise is to be reached between high resolution (= small clockperiod) and high response amplitude (= large clockperiod). The optimal value of the clockperiod will depend on the shapes and relative locations of the peaks and the theoretical plate height of the column; therefore, the clockperiod is equivalent to the injection volume in normal chromatography.

(d) Optimal filtering of the detector output. As contributions to the output

from frequencies higher than $\omega_{max.}$ are not significant [they cannot belong to $y(t)$], these can be cut off, as they could have been in a normal chromatogram recording also. The Shannon theorem is then met for noise components as well as for significant components, thus avoiding aliasing.

$f_{sample} \cdot \Delta T$ must be an integral value. If this is not so, it is impossible to take an integral number of samples during each sequence and it is not possible to choose $T = kN\Delta T$. For the same reason, sampling of the detector output and clocking of the PRBS have to be synchronized.

EXPERIMENTAL

The design of the system is shown in Fig. 2. After passing through the pump, the eluents flows through a manometer that functions as a buffer, and through a pre-column that is meant to remove possible impurities (both not shown). The flow is then split in two possible directions: it can either go through valve 1 and into the column, or into the sample coil, contents of which are passed through valve 2 and into the column as well. Either valve 1 or valve 2 is open, following the PRBS pattern. The flows are combined in a small-bore T-piece, which is located very close to the top of the column. The flow then goes through the column, which is immersed in a thermostated water-bath (25°), then through the UV detector cell, and out. The sample holding coil is filled by a separate pump, which can be connected by turning a sampling valve (not shown).

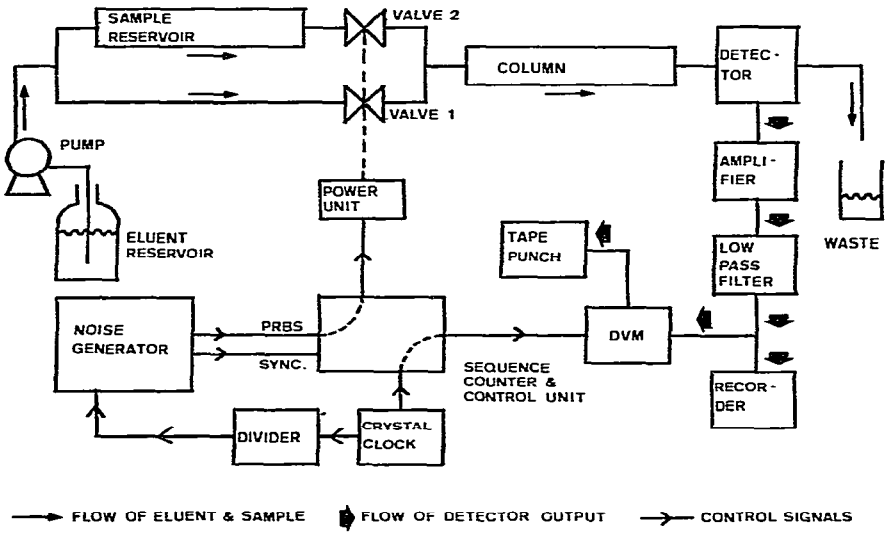

Fig. 2. System design.

The detector output is connected to an instrumental amplifier/low pass filter (24 dB per octave), then sampled by a digital voltmeter and recorded on paper-tape. It can also be monitored by a recorder.

The sampling is initiated by a crystal-stabilized pulse generator, which also

synchronizes a noise generator after division of the sample frequency by 2, which is not necessarily the optimal value. The noise generator produces the PRBS sequences controlling valves 1 and 2. These valves are powered by a system that generates a high voltage (*ca.* 40 V) for opening, a low voltage (*ca.* 15 V) for holding and a zero voltage for closing. Over-all control takes place by a counter, consisting of a series of flip-flops and triggered by pulses, generated by the noise generator at the start of each sequence. One or more sequences are used to obtain a transient-free output signal. An arbitrary integral number of sequences is then used to apply the PRBS and sampling the detector output at the same time.

Correlation is carried out off-line under control of a program developed in our laboratory. This program regenerates the PRBS using the same logic as the noise generator. The results are represented graphically on a line-printer, and can also be stored on paper-tape or on disc for further processing (Fourier transformation, construction of ACVF, off-line plotting, etc.). The program needs about 21K core memory and 110K disc storage (word length 16 bits), including disc storage needed to dump the load module. It is overlay-structured in order to save memory; the memory requirement could be further reduced by creating more overlays and/or discarding memory-consuming non-essential subroutines. The off-line correlation takes about 35 min (for 8000 samples and a correlogram length of 500 points); an on-line procedure would eliminate this extra time needed. The on-line procedure could well be realized by a microprocessor system; it could be programmed to generate the PRBS as well as to construct the cross-covariance function (CCVF). Memory requirements would be small: only the program itself, one PRBS sequence (+1 and −1 only) and the correlogram would have to reside in the memory.

Apparatus and chemicals

Column: stainless steel 316, length 15 cm, width 3 mm.
Stationary phase: Merckosorb SI 60, silanized, 10 μm.
Mobile phase: 30% ethanol–water.
Pump: Orlita MK00 reciprocating pump.
Valves: Lucifer 121 A54, stainless steel.
Detector: UV detector from DuPont 830 system.
Noise generator: Hewlett-Packard H01-3722A.
Digital voltmeter: Data Precision Model 2430.
Tape Punch: Facit 4070.
Minicomputer system: Varian V76 operating under Vortex II.

All other electronic equipment was home-made. All reagents were of laboratory grade. The column was packed with a slurry of homogeneous density. The eluent and sample were thoroughly de-gassed before use.

Experimental parameters

A flow-rate of about 32 $\mu l \cdot sec^{-1}$ was used in all experiments. The pressure at the top of the column was about 100 bar. The clockperiod of the PRBS was 1.75 sec. The cut-off frequency of the low-pass filter was 0.65 Hz.

RESULTS AND DISCUSSION

In order to express the improvement achieved in the signal-to-noise ratio numerically, we compared the detector noise $n(t)$ with its cross-correlogram $R_{xn}(t)$. The correlation time was 2 h. Fig. 3 shows a noise record and Fig. 4 a correlated noise record. Their σ_I^2–T diagrams were compared (Figs. 5 and 6). Fig. 7 shows a graph of the enhancement of the signal-to-noise ratio (related to the same injection) *versus* integration time. At low integration times (corresponding to peak height determination) the improvement is a factor of 100. The improvement is achieved in only 2 h, or 16 sequences, which corresponds to the time needed to record 16 normal chromatograms; during this time the variance σ_b^2 is reduced by a factor of 10,000. Normal signal averaging would require 10,000 runs to achieve the same enhancement, or 52 days (not even considering long-term system drift). The reason for this marked difference between correlation and signal averaging is the very high injection density used in the correlation technique, together with the intrinsic drift-suppressing property of the PRBS spectrum (see below).

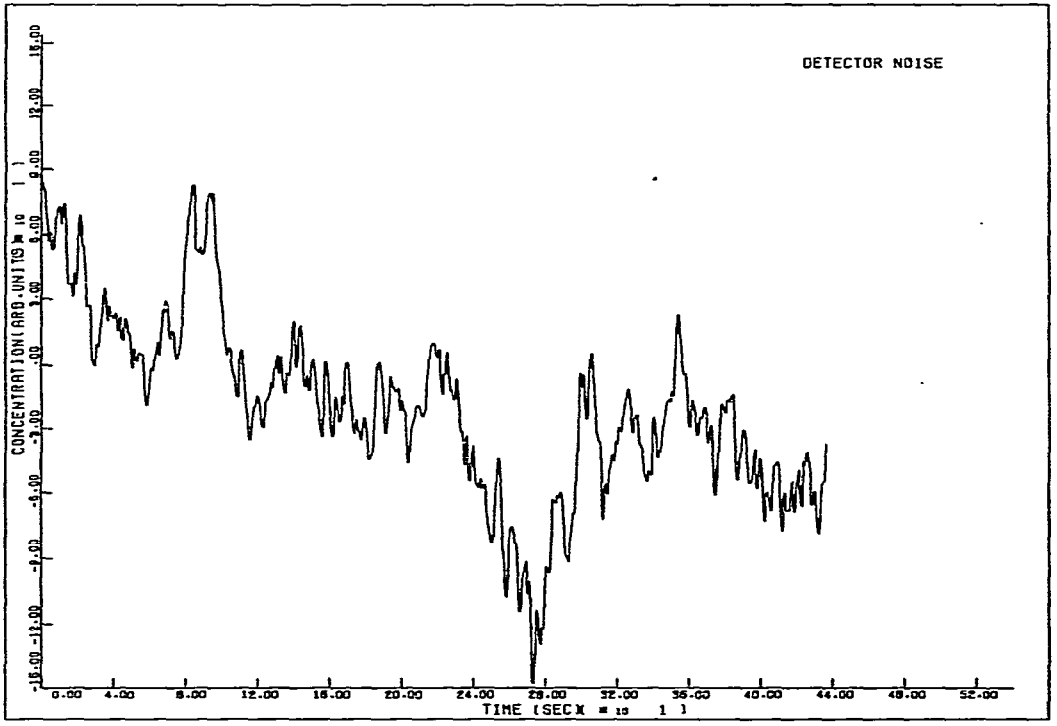

Fig. 3. Part of noise record of detector. 1 arbitrary unit = $5 \cdot 10^{-6}$ absorption units in all plots.

There is a marked improvement in the enhancement as T becomes larger. This is explained by the spectra (Figs. 8 and 9); the correlated noise spectrum contains less low frequencies, apart from an overall reduction of power. This is caused by the PRBS, which contains no lower frequencies than $1/N\Delta T$ (except a d.c. term). The

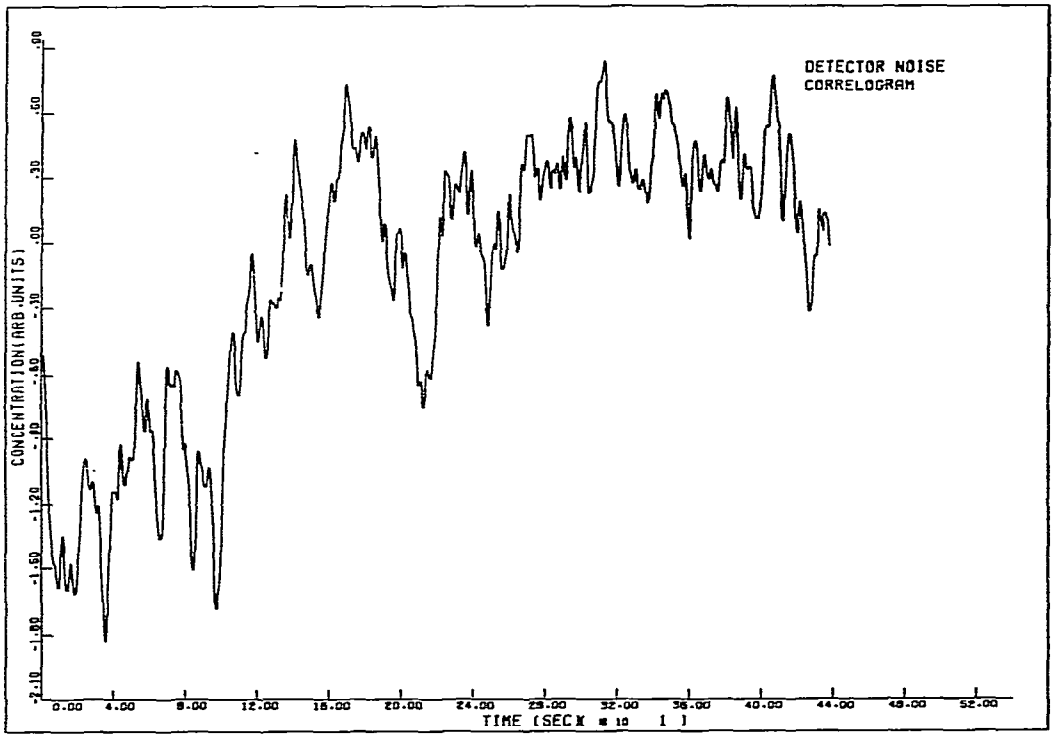

Fig. 4. Detector noise (Fig. 3) cross-correlated with PRBS. $N = 511$, $\Delta T = 1.75$ sec, $k = 8$.

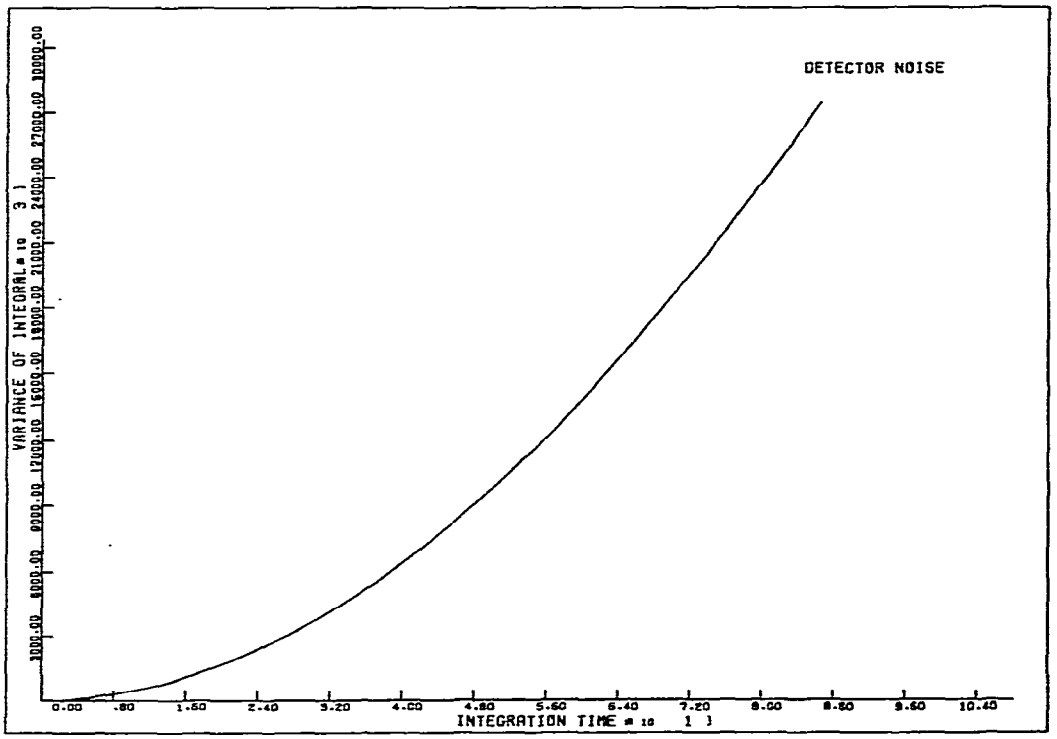

Fig. 5. Variance of integrated detector noise (Fig. 3) *versus* integration time. Eqn. 9 was used.

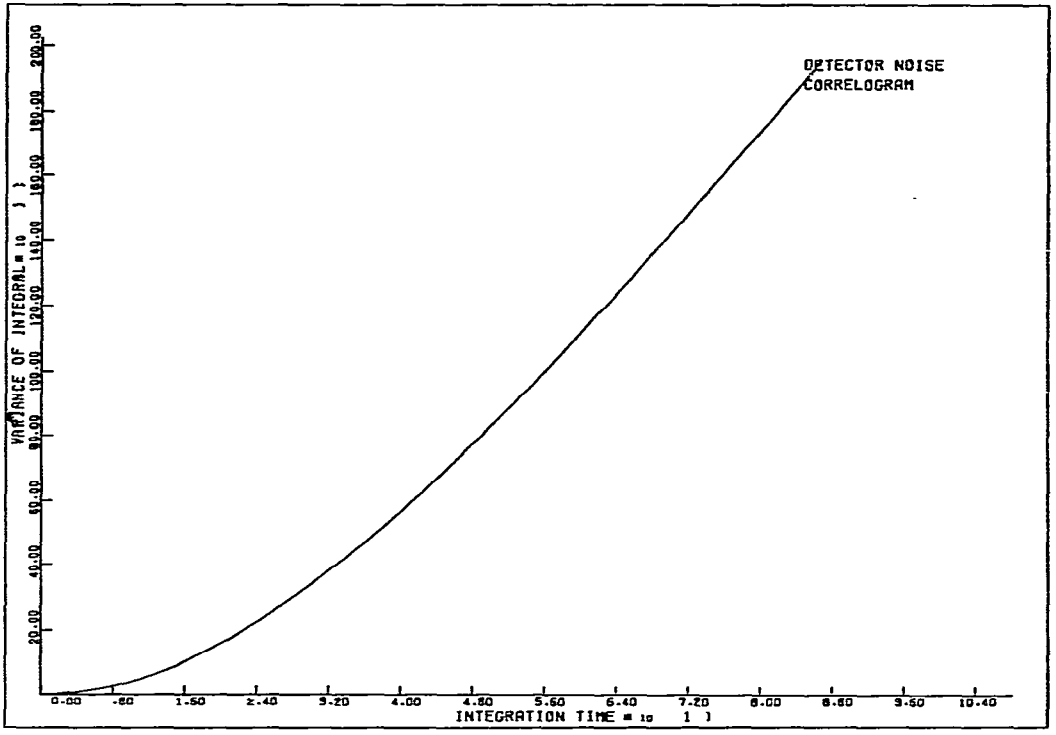

Fig. 6. Variance of integrated baseline noise of correlogram (Fig. 4) *versus* integration time.

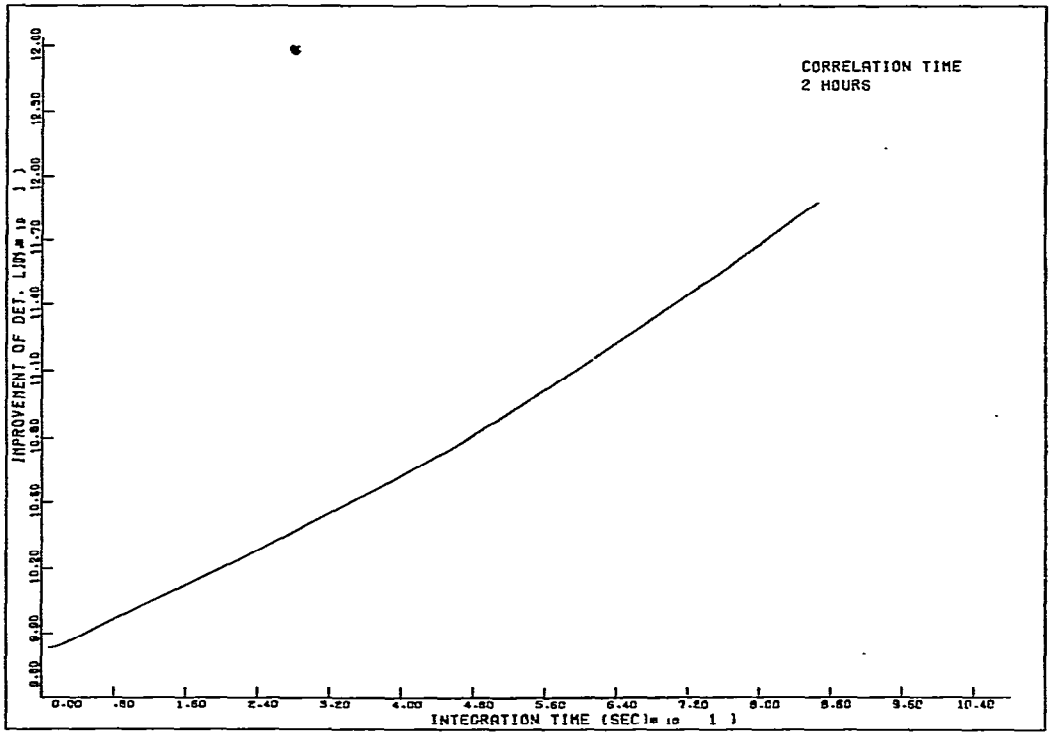

Fig. 7. Plot of (σ_I, detector noise/σ_I, correlogram) *versus* integration time. Detector noise record in Fig. 3, correlogram in Fig. 4.

spectra were constructed under control of a program developed in our laboratory[12].

Fig. 10 shows a normal chromatogram obtained with a sample loop valve. Fig. 11 shows a correlogram of a much lower concentration (200 ppb of phenol, 400 ppb of 2,3-dimethylphenol). The retention times are different because of slightly different flow-rates due to column aging. Fig. 12 shows the response to a 186-μl injection of the 200 + 400 ppb mixture; the expected response is 9.2 arbitrary units (first peak). Only noise appears, which emphasizes the fact that this concentration can be made visible only by correlation.

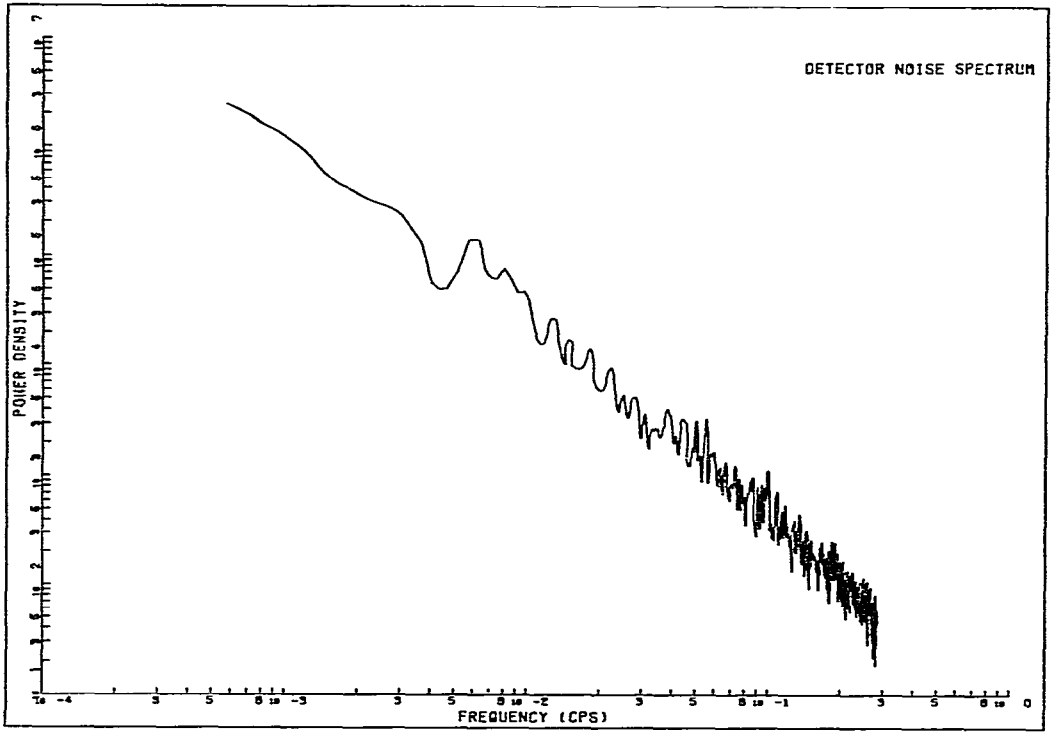

Fig. 8. Power spectral density of detector noise (Fig. 3). Normalized bandwidth = 0.0008 Hz.

This technique can theoretically lead to an unlimited improvement in signal-to-noise ratio. In practice, the improvement will be limited, for example, by non-stationarity of the system and/or a limited (available) amount of sample. Peaks will suffer more from non-stationarity if there are other, much larger peaks. The greatest asset of the correlation technique, especially in HPLC, is its ability to obtain information from very dilute samples without the necessity for pre-concentration. If an aqueous eluent can be used, and the sample is also aqueous, the only requirement is to match the sample and eluent with respect to moderator content, absorbance and pH, and, of course, the sample must be freed from solid particles. Environmental analysis is, at first sight, a possible field of application of correlation chromatography.

If the sample can be re-concentrated to the concentration that existed before

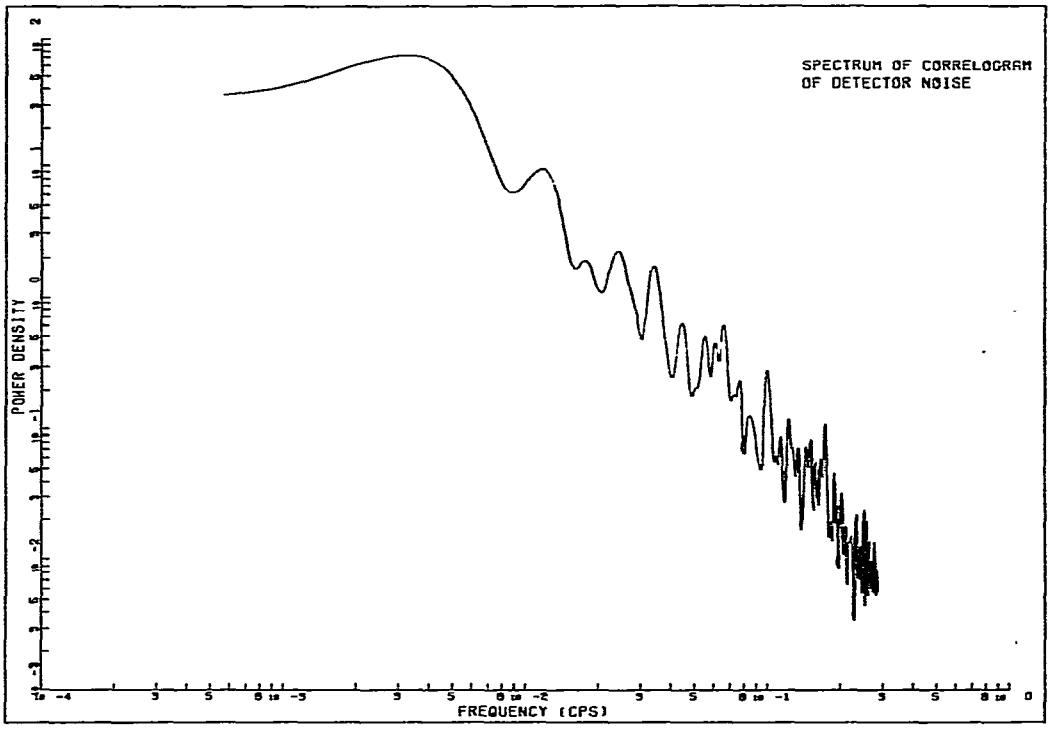

Fig. 9. Power spectral density of baseline noise of correlogram (Fig. 4). Normalized bandwidth = 0.0023 Hz.

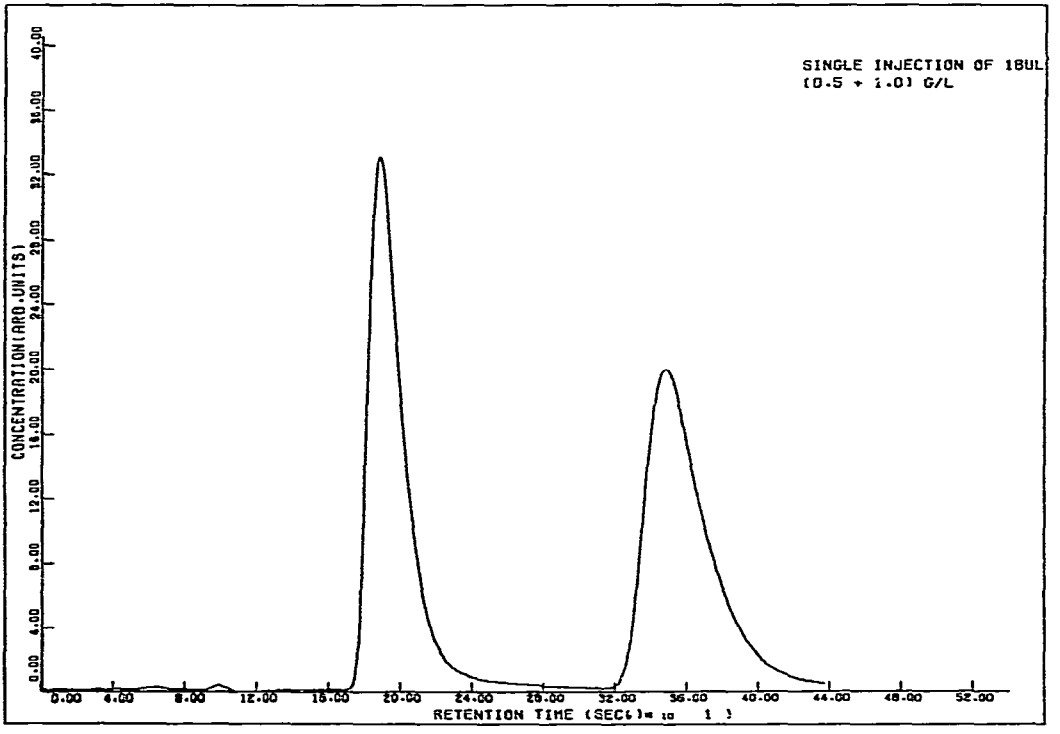

Fig. 10. Normal chromatogram made with sample loop. Phenol 0.5 $g \cdot l^{-1}$, 2,3-dimethylphenol 1.0 $g \cdot l^{-1}$. Injection volume 18.2 μl.

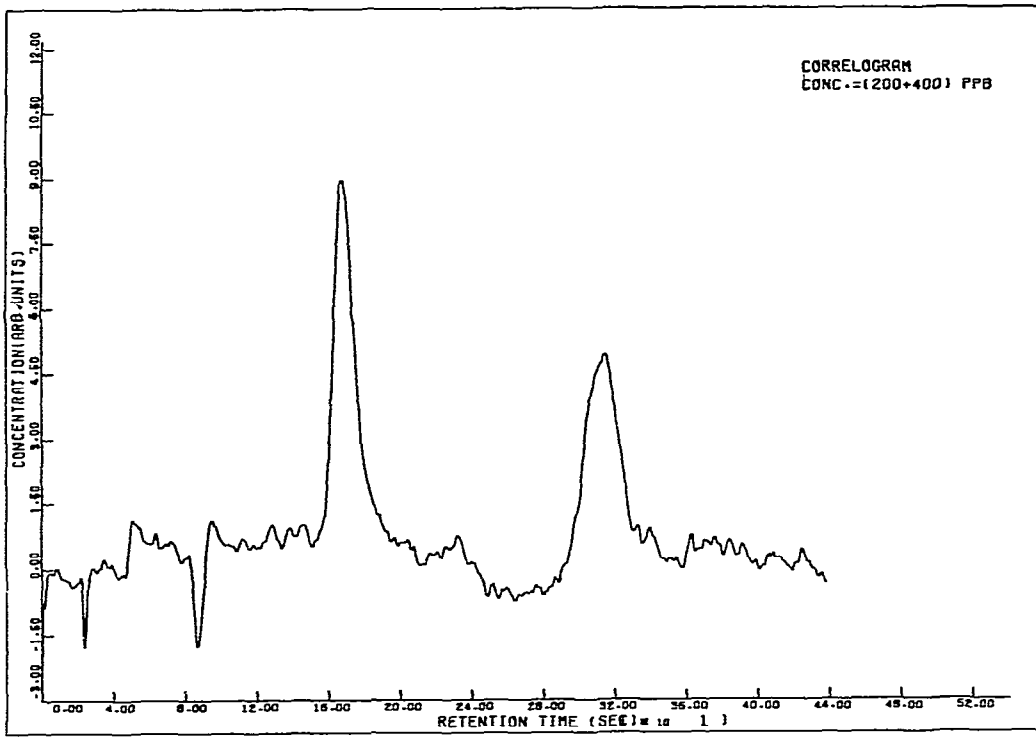

Fig. 11. Correlation chromatogram. Phenol 200 ppb, 2,3-dimethylphenol 400 ppb. $N = 255$, $\Delta T = 1.75$ sec, $k = 16$. Virtual injection volume 56 μl.

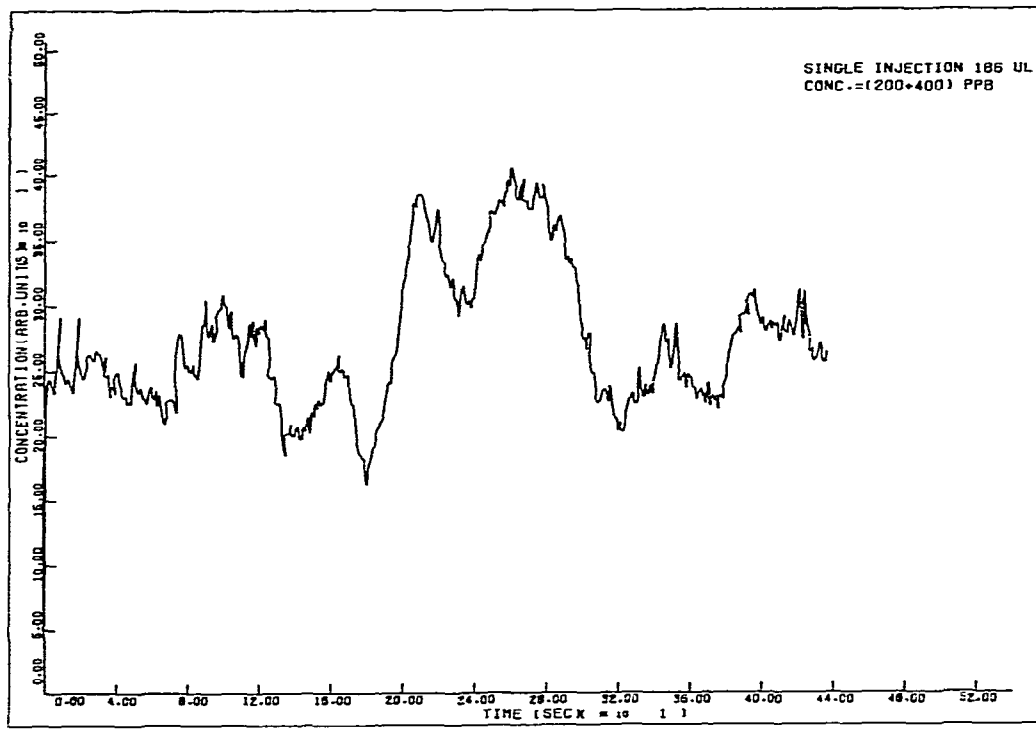

Fig. 12. Response to an injection of 186 μl. Phenol 200 ppb, 2,3-dimethylphenol 400 ppb.

it entered the system (which means concentrating by a factor of 2), not only small concentrations but also small amounts of sample can be handled.

LIST OF SYMBOLS

$x(t)$	input function
$y(t)$	response of system to $x(t)$
$h(t)$	response of system to unit impulse
$n(t)$	system noise
$z(t)$	system noise plus response of system to $x(t)$
$R_{xx}(\tau,T)$	autocovariance function of $x(t)$ over a correlation time T
$R_{xy}(\tau,T)$	cross-correlation function of $x(t)$ and $y(t)$ over a correlation time T
$R_{RR}(\tau,T)$	autocovariance function of a cross-correlation function
τ	time shift
N	sequence length of PRBS
ΔT	clockperiod of PRBS: minimum time lapse during which the PRBS function remains in the same state
k	number of PRBS sequences used to construct a correlation function
T	integration or correlation time
$G_{xx}(\omega)$	spectral power density function of $x(t)$
$G_{nn}(\omega)$	spectral power density function of $n(t)$
$G_{xz}(\omega)$	spectral cross-power density function of $x(t)$ and $z(t)$
$H(\omega)$	transfer function of system
σ_b	standard deviation of baseline noise
σ_I	standard deviation of integrated baseline noise
$t_{R\text{last}}$	retention time of most retarded component
σ_{last}	gaussian standard deviation of most retarded peak
$\omega_{\text{max.}}$	maximum angular frequency contained by $y(t)$
$f_{\text{max.}}$	maximum frequency contained by $y(t)$
ω_{sample}	angular sampling frequency used to digitize the output $z(t)$
f_{sample}	sampling frequency
ω	$2\pi f$
1 ppb	10^{-9} g·l^{-1}

REFERENCES

1 H. C. Smit, *Chromatographia*, 3 (1970) 515.
2 C. Largeau and B. Espiau, *J. Chim. Phys.*, 71 (1971) 1143.
3 G. C. Moss, P. J. Kipping and K. R. Godfrey, in S. G. Perry (Editor), *Proceedings of the 9th International Symposium on Gas Chromatography, Montreux, October 9–13, 1972*, Applied Science Publ., Barking, 1973, p. 187.
4 R. Annino and L. E. Bullock, in S. G. Perry (Editor), *Proceedings of the 9th International Symposium on Gas Chromatography, Montreux, October 9–13, 1972*, Applied Science Publ., Barking, 1973, p. 171; *Anal. Chem.*, 45 (1973) 1221.
5 C. Largeau and F. Barras, *Chromatographia*, 8 (1975) 373.
6 R. Annino, *J. Chromatogr. Sci.*, 14 (1976) 265.
7 J. B. Philips and M. F. Burke, *J. Chromatogr. Sci.*, 14 (1976) 495.
8 J. F. K. Huber, J. A. R. J. Hulsman and C. A. M. Meijers, *J. Chromatogr.*, 62 (1971) 79.
9 S. L. Karger, M. Martin and G. Guiochon, *Anal. Chem.*, 46 (1974) 1640.
10 G. A. Korn, *Random Process Simulation and Measurements*, McGraw-Hill, New York, 1966, p. 83.
11 H. C. Smit and H. L. Walg, *Chromatographia*, 8 (1975) 311.
12 H. L. Walg and H. C. Smit, *Anal. Chim. Acta*, 103 (1978) in press.