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Note

Extra-column band spreading in high-performance liquid chromatography–mass spectrometry using a moving belt interface

Numerical evaluation of system variance

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The detrimental effects of extra-column band spreading in chromatographic separations have long been recognised and elegant theoretical treatments of the problem have appeared[1–4].

An increasing interest in microbore (and capillary) high-performance liquid chromatography (HPLC), techniques which impose great demands on chromatographic equipment, has to some extent rekindled awareness of these effects.

During our development of microbore technqiues for use with mass spectrometry (MS)[5–7] we decided to investigate the band spreading effects of the mass spectrometer when used as HPLC detector. Two important spreading effects have been identified in HPLC detectors, *viz.* those due to, for example, flow effects, including dead volumes and cross sectional area changes, and those due to electronic time constants. It has been usual practice to evaluate these effects by considering the increase in variance (or second moment of mass) of a chromatographic band. The variances have useful properties including their additivity when the contributions are independent, *i.e.* if the system time-constant is independent of the dead volume effects then the two separately calculable variances can be added to give the overall system variance. Also the commonly used measure of column efficiency, the height equivalent to a theoretical plate, is itself a measure of the increase in second moment of mass of a chromatographic band as a function of the distance travelled down the column. Although the importance of variance in extra-column band spreading has been recognised, it is unfortunately not in general practical use by chromatographers, reference being made simply to a measured or estimated dead volume in most cases.

This paper describes some results obtained using a Finnigan 4000 mass spectrometer with a moving belt liquid chromatographic (LC) interface[8], when used as a dectector for a high efficiency microbore HPLC system. In this system the column eluent is fed onto a moving belt which carries the solutes in solution under an infrared heater where the solvent is removed. Since in this technique there is no flow cell to be measured, the approach outlined here was adopted. Samples of the pesticide Lindane (γ-hexachlorocyclohexane) dissolved in methanol were injected into the LC–MS

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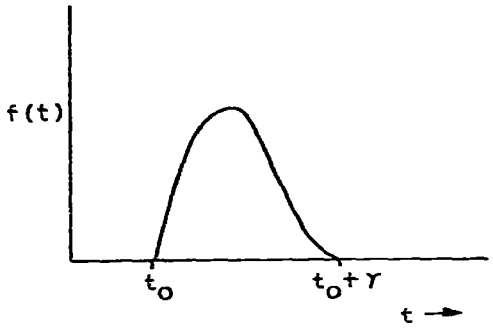

Fig. 1. Mass spectrometer output expressed as a function of time, f(t)

interface in a controlled manner using a micro-feeder. These input pulses were considered to approximate to square waves, the variance of which is given by[1]:

$$\sigma_{in}^2 = \frac{T_{in}^2}{12} \tag{1}$$

where σ_{in}^2 is the input variance, and T_{in} is the time over which the input takes place. Mass spectra were recorded using an Incos data system scanning the molecular ion region vary rapidly (0.1 sec per scan). The broadened output appeared to be a complex function together with a great deal of noise. It was not considered feasible to analyse the output algebraically and so the following calculations involving numerical integration based on Simpson's rule were used.

The ion current values produced by the mass spectrometer after subtraction of a baseline value is considered as a function of time f(t) (Fig. 1). We can then define the following:

$$A = \int_{t_o}^{t_o+\tau} f(t)\,dt = \text{area under curve} \tag{2}$$

$$\bar{t} = \int_{t_o}^{t_o+\tau} tf(t)\,dt/A \tag{3}$$

$$\bar{t}^2 = \int_{t_o}^{t_o+\tau} t^2 f(t)\,dt/A \tag{4}$$

The variance is defined as the average of the squares minus the square of the average, *i.e.*

$$\sigma^2 = \bar{t}^2 - (\bar{t})^2 \tag{5}$$

The output of the mass spectrometer is discrete, each scan being taken at a fixed time. This type of data lends itself to analysis by numerical methods, and it was decided to

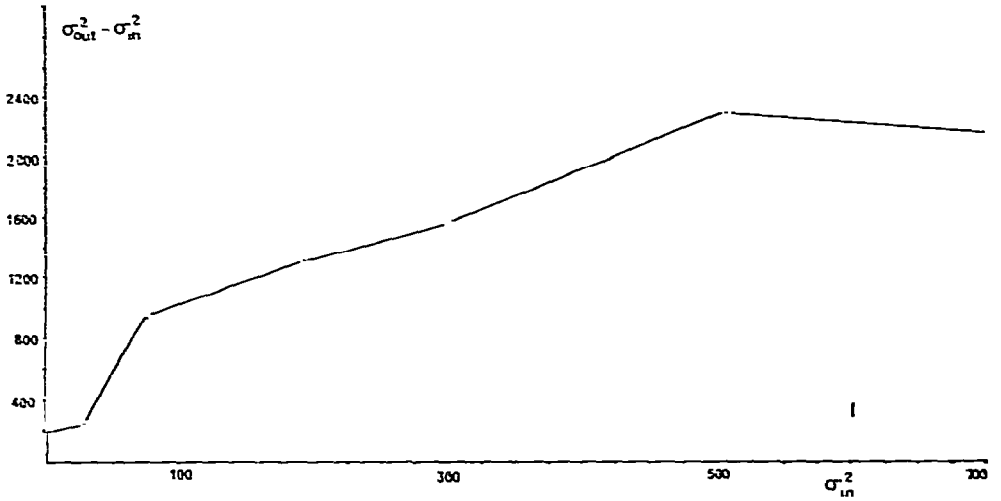

Fig. 2. Plot of output variance against input variance.

evaluate the above integrals by using Simpson's rule. Using this rule the integrals can be formulated as:

$$A = \frac{T}{3}\left\{f_1 + f_N + \sum_{n=2}^{N-1} [3 + (-1)^n]\, fn\right\} \tag{6}$$

$$\bar{t} = \frac{T}{3}\left\{tf_1 + tf_N + \sum_{n=2}^{N-1} [3 + (-1)^n]\, t^f n\right\} \tag{7}$$

$$\overline{t^2} = \frac{T}{3}\left\{t^2 f_1 + t^2 f_N + \sum_{n=2}^{N-1} [3 + (-1)^n]\, t^2\, fn\right\} \tag{8}$$

The rule requires that the peak is split into an equal number of equally spaced strips of width T (the interval between scans), which requires an odd number of data points (or scans). A Fortran computer programme was then written to evaluate the summations and to compute the output variance σ^2_{out}.

Plots of $\Delta\,\sigma^2$ ($= \sigma^2_{out} - \sigma^2_{in}$) against σ^2_{in}, and of $\Delta\,\sigma^2/\sigma^2_{in}$ against σ^2_{in} are shown in Figs. 2 and 3. Fig. 3 indicates that σ^2_{out} is proportional to σ^2_{in} at reasonable values of σ^2_{in}, but an anomaly exists at vary low values of σ^2_{in}. The volume standard deviation, σ_v, and the time standard deviation, σ_t, of a chromatographic band can be calculated using the equations:

$$\sigma_v = V_R/N^{\frac{1}{2}}$$

and

$$\sigma_t = V_R/N^{\frac{1}{2}}Q$$

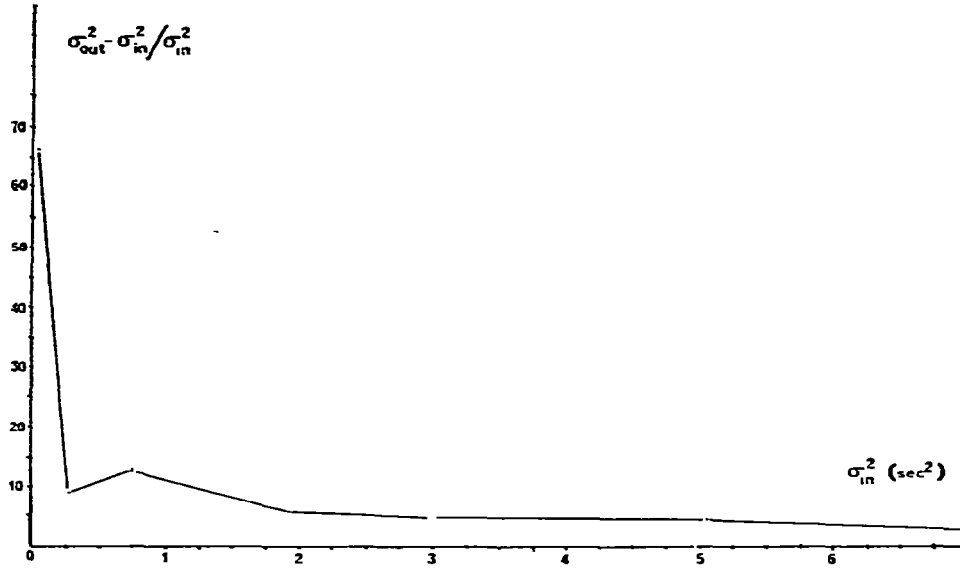

Fig. 3.

where V_R = retention volume, N = number of theoretical plates, and Q = volume flow-rate. For a typical microbore case using a 250 × 0.5 mm I.D. column we may have:

$$N = 10{,}000; \qquad V_0 = 30\ \mu l; \qquad Q = 10\ \mu l\ min^{-1}$$

V_R is replaced by V_0, the column void volume, as this represents at the most difficult

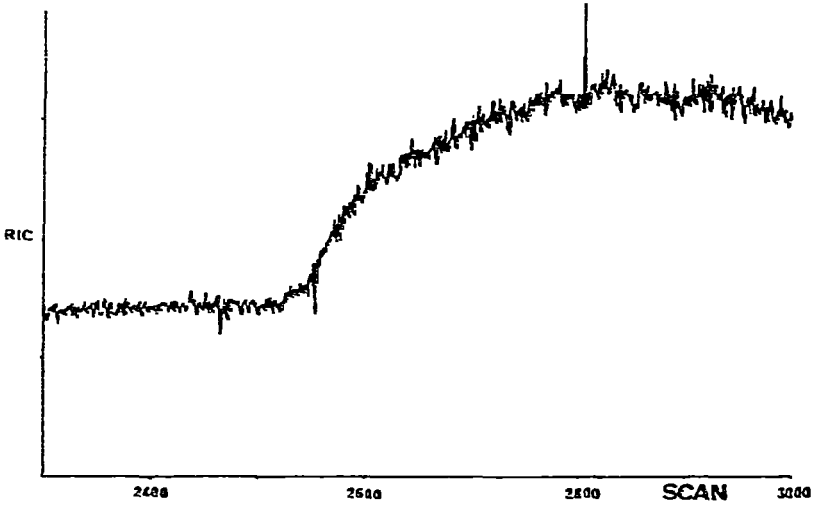

Fig. 4. A typical response curve for a belt transport LC–MS interface[8].

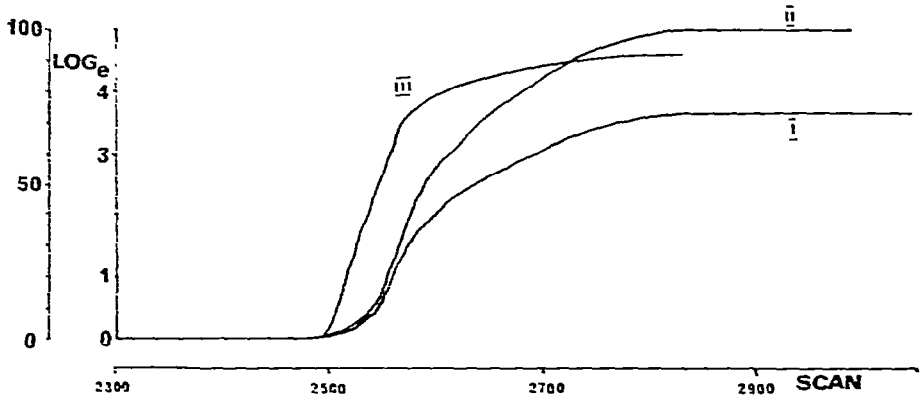

Fig. 5. Curve I: Smoothed response curve taken from Fig. 4; curve II: normalised response curve; curve III: logarithm curve, the slope of the straight line portion giving the instrument time constant.

peak to handle. The time based standard deviation and variance then compute to:

$$\sigma_t = 1.8 \text{ sec and } \sigma_t^2 = 3.24 \text{ sec}^2$$

By reference to Fig. 3 we see that this takes us well into the flat area of the plot. From this and a comparison of the mass spectrometer detector with a micro flow cell UV detector of dead volume 0.3 μl, we conclude that the mass spectrometer is a suitable low effective dead volume detector for microbore HPLC.

We have also measured the time constant of the mass spectrometer detector. A single ion was monitored at maximum scan rate and a flow of sample was injected into the interface. A typical response is shown in Fig. 4. This response curve shows a high level of noise which makes further manipulation difficult. We decided to simply average the noise by drawing a line through the centre points of the curve to produce Fig. 5 (line I). After normalisation (line II) the logarithm was plotted (line III). The slope of the straight line portion of the logarithm curve gives the instrument time constant. An average of three values gave a commendably low 0.08 sec.

In conclusion this work shows the mass spectrometer to be a low time constant, low effective dead volume detector suitable for microbore HPLC. We also hope that this approach to the estimation of extra-column band broadening by consideration of system variance will promote further discussion of the problem among chromatographers.

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