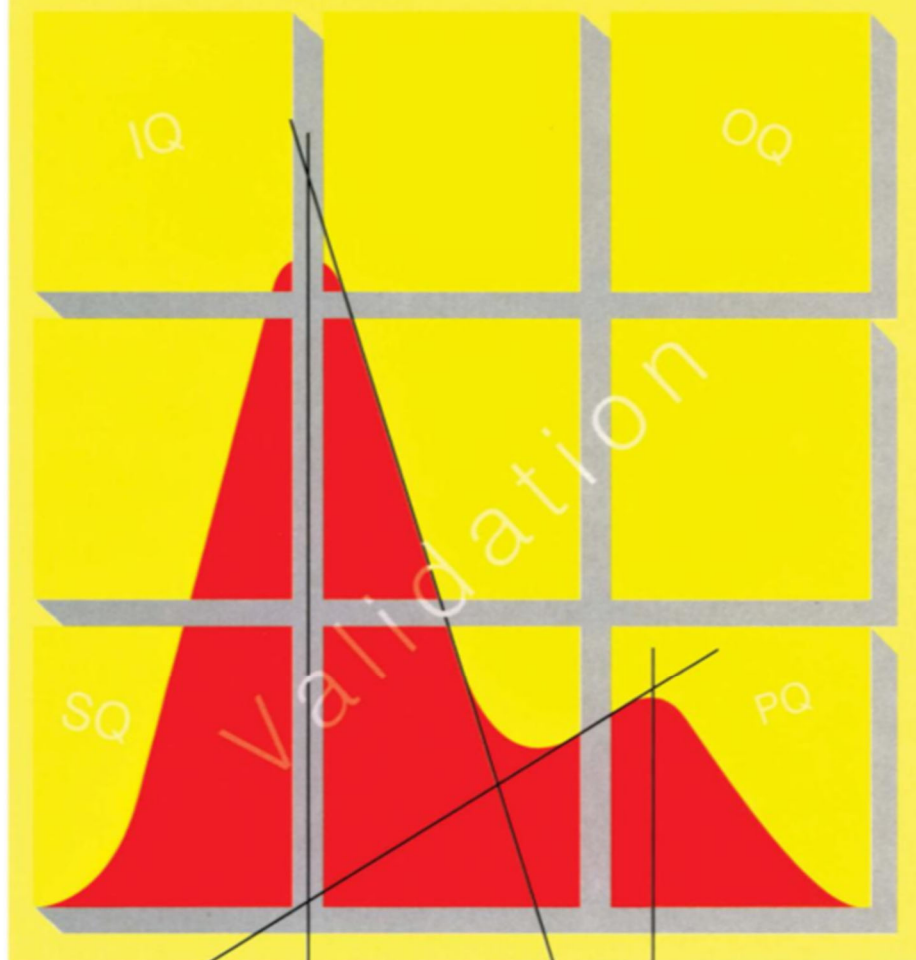


CHROMATOGRAPHIC INTEGRATION METHODS

Second Edition

by Norman Dyson



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Chromatographic Integration Methods

Second Edition

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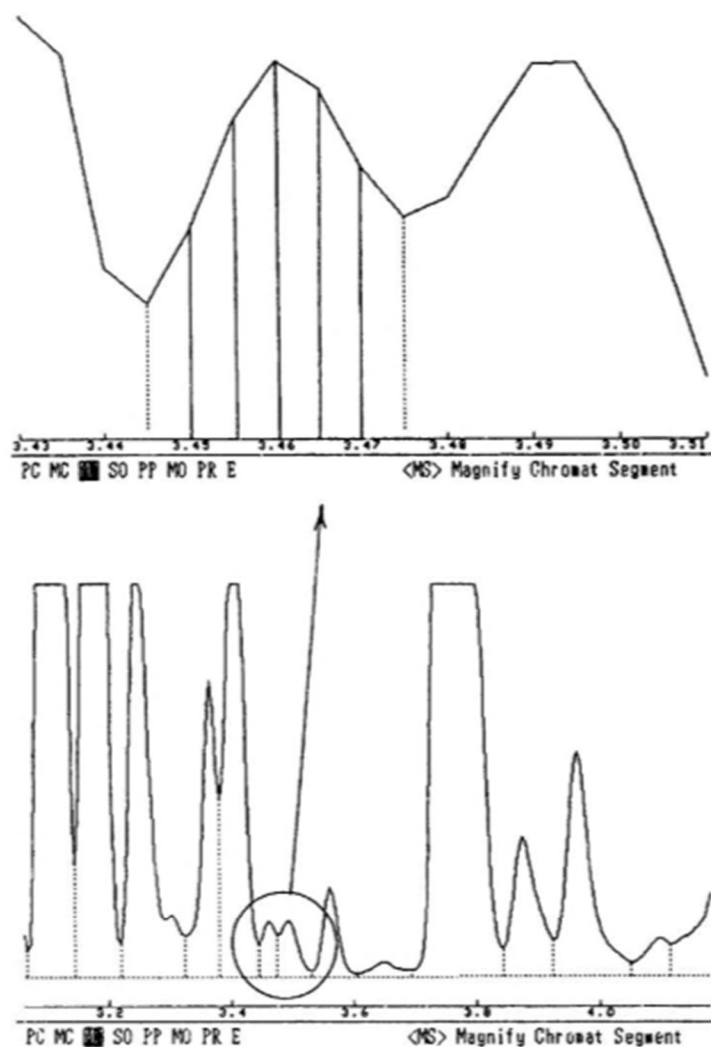
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VDU display joins up ordinates

Figure 5.15 Expansion of digitized signal shows ordinates joined to display a peak

known – applications. The shortcomings of matched filtering, like all other deconvolution techniques, lies not in the theory but in the lack of exact repeatability of chromatogram shape from repeated injections of the ‘same’ sample under ‘constant’ conditions.

3 Location and Measurement of Peaks

Accurate peak location in commercial integrators is made by coarse and fine scans where:

- (1) approximate peak location to detect every peak is obtained from Savitsky–Golay first and second derivatives;
- (2) precise location of each peak start and end point (including valleys), the limits of integration, is obtained by a local search.

Finding the Peaks

Any peak can be divided into four quadrants by lines drawn through the peak maximum and points of inflection (Figure 5.16).

Each quadrant is uniquely characterized by its first and second derivatives and this is used as a coarse peak locator and shape test (Table 5.2).

The first and second derivatives are obtained from Savitsky–Golay convolution of the stored data. Peaks are located where the first derivative departs from zero by more than a prescribed threshold, the slope sensitivity S (Figure 5.17).

The non-zero threshold distinguishes between the relatively small baseline gradients and the larger peak gradients. Slope sensitivity reduces false peak detection, but inhibits precise location of the limits of integration. Nevertheless, approximate peak location shows where the peak boundaries are to be found and later prevents the integrator making detailed searches on stretches of empty baseline, and so reduces the processing time. If the S/N ratio is large and the baseline is flat, slope sensitivity can be given a small value, and the peak limits will be located with good precision even in the 'coarse' search.

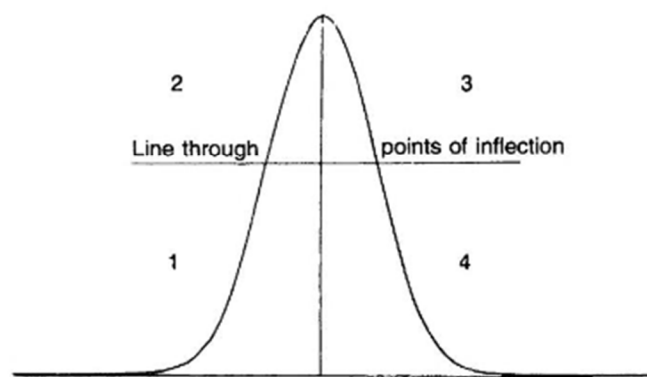


Figure 5.16 Symmetrical Gaussian peak

Table 5.2 First and second derivatives of each quadrant of a peak

Quadrant	dy/dt	d^2y/dt^2
1	> 0	> 0
2	> 0	< 0
3	< 0	> 0
4	< 0	< 0

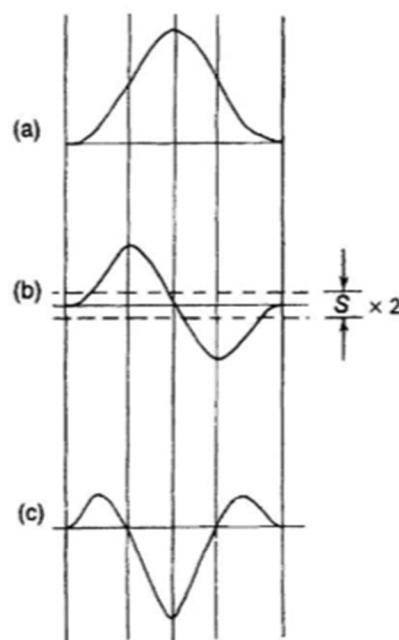


Figure 5.17 Peak, first and second derivatives

Retention Time

The peak maximum is located where the first derivative is zero between peak boundaries (Figure 5.17) and from this the retention time is measured. Without curve fitting, retention time accuracy and precision would be determined by the bunched sample interval.

Peak Shape Test

The combination of values given in Table 5.2 allows the integrator to examine the peak for shape and enables the integrator to distinguish between peaks and baseline ramps caused by solvent gradients or temperature programs. It is a measure against false peak detection.

Properties of the Smoothed Data

Savitsky-Golay processing to locate the first derivative is equivalent to the integrator comparing each smoothed datum to its M th predecessor where M is the width of the convolution window ($= 2m + 1$).

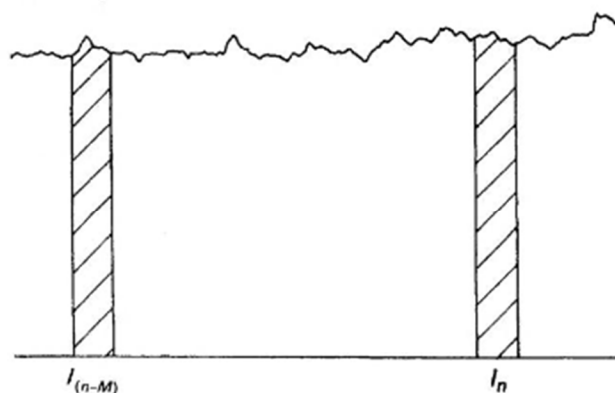


Figure 5.18 The integrator constantly computes $\Delta I_n = I_n - I_{(n-M)}$

Data Integrals

If I_n is the most recent smoothed datum or integral, the integrator computes,

$$\Delta I_n = I_n - I_{(n-M)}$$

and the value of ΔI_n is positive, negative or 'zero' (as defined by slope sensitivity) depending on whether the baseline is rising, falling or is flat. ΔI_n is therefore monitoring slope, and its value, which is a measure of the first derivative, can be used to show when a peak is emerging ($\Delta I_n > S$), or when the signal is on baseline ($-S < \Delta I_n < S$). Integrators which have slope indicators, keyboard LEDs for example, have them controlled by this quantity and they switch on and off according to its value.

The integrals are used in three ways as described below.

(1) *As an Area* Integrals are measured in units of $\mu V \text{ sec}$. When those within peak limits are summed and corrected for baseline, they will compute the peak area.

(2) *As a Height* Since the integral's value is determined for a fixed time interval against a constant reference level, -5 or -10 mV , it can only vary if the baseline rises or falls. It is therefore entirely height-dependent.

To maintain this, integrals must not change their bunch size during peak measurement, and 'time to double' instructions are only carried out after the peak end-point has been detected, when the signal is back on baseline.

(3) *As a Gradient* ΔI_n is not only a difference in baseline height, it is a difference in height measured over the fixed time interval of M data slices, and so ΔI_n is also a gradient (Figure 5.19). This gradient has a discontinuity when the bunching is changed on baseline, but the integrator is programmed to account for it.

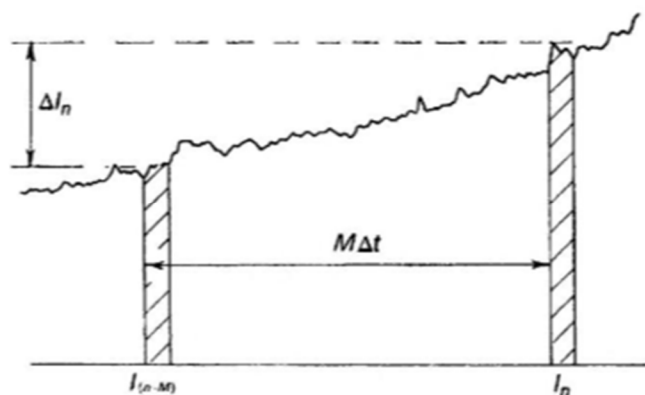


Figure 5.19 ΔI_n is measured over a fixed time interval of M samples

Baseline Fluctuations and Slope Sensitivity

Slope sensitivity is determined by giving ΔI_n a threshold value. It is the smallest shift in baseline position which the integrator sees as a positive or negative slope.

Programming Slope Sensitivity

Judging what value to give to slope sensitivity is not intuitive. Integrators therefore provide a test facility to monitor the baseline for a while, measure the average value of signal fluctuations, and self-program a value. The length of the test is linked to the peak width setting and lasts for approximately 100 bunched integrals.

Daily use of the test should make the analyst familiar with the empirical range of values to be expected; different columns, detectors and sample types all contribute to the range. With experience, the analyst may even choose to over-rule the integrator and enter an alternative value for slope sensitivity, for example, to allow more micro-peaks to be seen if these are important.

Slope Sensitivity and Representative Baseline

Automatic tests to determine baseline noise levels are performed when the signal is on 'representative' baseline.

If an integrator is asked to measure baseline noise following a thorough reconditioning of the column, it will measure a different value to that produced after samples have been analysed and the baseline is littered with chromatographic rubble. If this latter baseline is the normal one, it will be more appropriate to determine a slope sensitivity value which includes the baseline rubble and considers it to be 'zero slope'.

If programming errors are to be made, it is better to make slope sensitivity too sensitive. The integrator will see more noise and measure too many small peaks, but these can be removed from the final report by setting a minimum area threshold.

Updating Peak Width and Slope Sensitivity during Analyses

As analyses progress peaks become broader and flatter. The initial values for peak width and slope sensitivity require revision and updating. Integrators do this automatically by monitoring peak widths and comparing them with the programmed value. If there is a significant difference, the peak width parameter is increased (*i.e.* the sample bunching is increased).

Alternatively, the parameters may be programmed to increase on a timed basis via a time program.

When bunching is changed it changes the values of both the peak width parameter and the slope sensitivity (Figure 5.20).

If the bunching is doubled the effective width of each data integral is doubled, and ΔI_n is measured over twice the time interval while still recognizing the same shift in baseline level as the slope threshold. Expressed as a gradient, slope sensitivity is halved and becomes twice as sensitive.

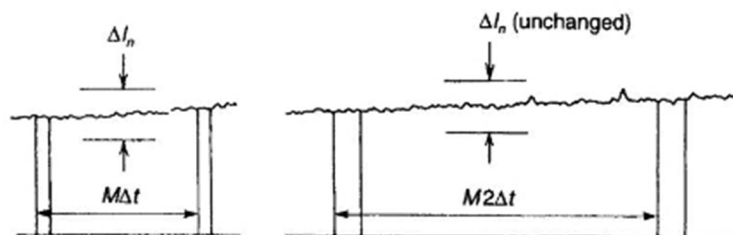


Figure 5.20 If peak width parameter is doubled ΔI_n is measured over twice the time interval and slope sensitivity is halved

Location of the Limits of Integration

Figure 5.21 shows a single peak on a flat baseline, sliced into bunched integrals as determined by the peak width parameter, and a reference level above which the detector signal is measured. The reference level is the lower limit of the integrator's operating range (-5 or -10 mV). If the detector signal drifts lower, or is inadvertently sent lower by careless use of detector back-off control, it will create a dead band error and only that part of the detector signal which projects above the reference level will be measured.

The approximate peak start is located where the detector signal first exceeded the programmed slope sensitivity value. The integrator proceeds to conduct a fine search for a more accurate location among the preceding data samples. The range of its search is limited by the time interval between first peak location S , and the peak maximum. This is no more than 2σ , where σ is the peak standard deviation; the integrator looks back over the range 4σ from the peak retention time. The 'correct' peak start is located at the lowest baseline level in this region.

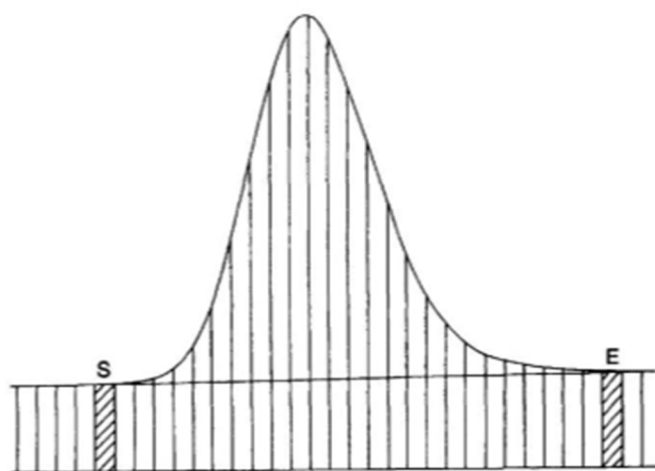


Figure 5.21 Peak start, S, and end, E

As the peak emerges, the integrals increase in value compared with their predecessors (Figure 5.22).

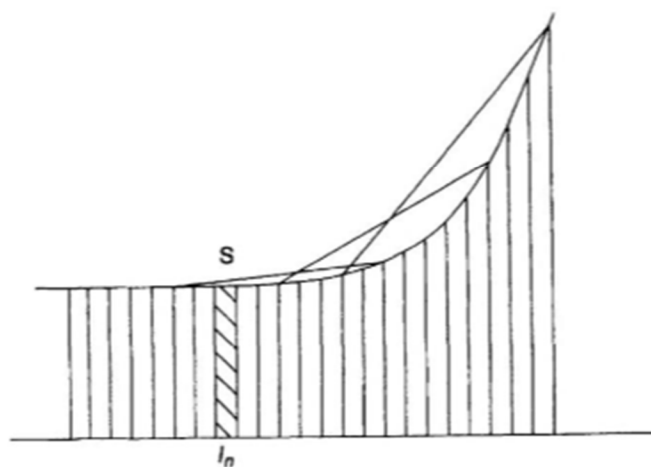


Figure 5.22 The peak starts at S when $\Delta I_n > \text{slope sensitivity}$

To be recognized as the start of a peak and to trigger integration, ΔI_n must be positive and increasing in value for P consecutive integrals, where P has a value which depends on the moving window size (and manufacturer) but ranges from about 5 to 10. If it is too large the integrator will be slow to make decisions. If it is too small the integrator will measure residual noise peaks.

When the integrator observes P positive values of ΔI_n in succession, such that,

$$\Delta I_n > S \quad (15)$$

and
$$\Delta I_{n+1} > \Delta I_n \quad (16)$$

and
$$\Delta I_{n+2} > \Delta I_{n+1} \quad (17)$$

$$\vdots$$

and
$$\Delta I_{n+P} > \Delta I_{n+P-1} \quad (18)$$

and it satisfies the conditions of the peak shape test that dy/dt and d^2y/dt^2 are both positive. The integrator triggers integrate mode which initiates several operations:

- (1) data samples are accumulated from the peak start;
- (2) a number of baseline integrals immediately before the peak start are averaged and stored;
- (3) screen or keyboard indicators display when a peak is confirmed;
- (4) a peak event mark (if available) will be added to the chromatogram.

When integrators and strip chart recorders were separate instruments, the event mark added by the integrator was drawn late by the recorder, at the point where the peak start was confirmed, not where peak onset was first noted. Just how late was determined by the peak width value. Now that intelligent plotters are part of the integrator, printing of the chromatogram is delayed slightly so that the event mark can be added to the correct place, but this has sacrificed a useful indicator of whether the peak width parameter has been programmed correctly.

Small Peak Filtering

Unless small peaks show positive slope for the correct length of time, $P \times \Delta t$, they will not trigger integration and will not be measured. Varying the bunch width (Δt) varies the qualifying time, allowing selective filtration of small peaks. This defines the fastest measurable peak.

Location of Peak End

The integrator continues to accumulate peak integrals until it locates the baseline again (Figure 5.23). The approximate location of peak end is determined when the

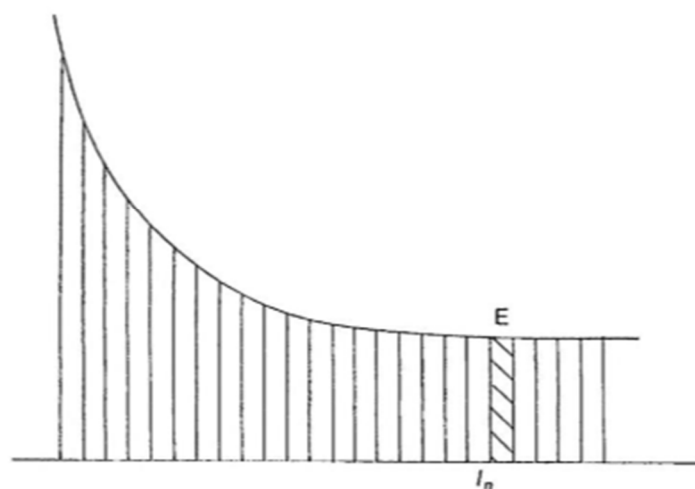


Figure 5.23 Location of peak end

detector signal gradient ΔI_n , becomes zero for a qualifying time of Z consecutive samples. Z is larger than P because of peak tailing which has to be monitored longer*. The integrator conducts a fine search among the successive data samples to find a more accurate location. The search range is determined by the time interval from the peak maximum to the approximate end, which will be large if there is tailing. The correct end point of the peak is taken to be the lowest baseline point in the search region.

When baseline is confirmed, more operations come into play:

- (1) Integrate mode is terminated and accumulation of peak integrals stops at the peak end. The integrator now holds in its memory the sum of the data points from peak start to end (Figure 5.24).
- (2) Several of the new baseline integrals immediately after the peak are averaged and stored.
- (3) A peak event mark is recorded on the chromatogram, and keyboard peak indicators switch off.

Measurement of Peak Area

Peak area measurement is completed by subtracting the 'baseline area' from the accumulated integral count (Figure 5.25). The baseline area is calculated from the average of the stored baseline integrals before and after the peak, and the peak width (from the number of integrals accumulated within the peak limits). The area is calculated as a trapezium, and so the method is commonly called 'trapezoidal baseline correction'. There are three points to note:

*On at least one integrator $Z = 2P$



This popular series is designed to provide a collection of thorough and informative bench-top guides for the practising chromatographer.

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Norman Dyson, after 7 years of study at Newcastle University, spent time in research and marketing with multinational companies before founding Dyson Instruments in 1974. As well as a wealth of experience with chromatographic techniques, Dr Dyson has lectured extensively to researchers and industrialists.

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