CHIMIA 51 (1997) Nr. 10 (Oktober)

Chimia 51 (1997) 751–752
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ISSN 0009–4293

Error Tracking Down: A Peculiar Playground?

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Abstract. Errors in an analytical method are often not recognized as such or are underrated. Therefore, it is useful to compile and publish them. Error tracking down can even be an exciting field of research. Two different errors which can occur in chromatography are presented: The area of poorly resolved peaks cannot be integrated accurately by the usual integrators or data systems due to geometrical reasons. Second, injected air can give rise to a retained peak in HPLC. Air injection can happen during a malfunction of the autosampler but the extra peak is unwanted because it can overlap with the analyte peaks.

Sometimes a scientific question or field of research seems to act like a person, it is going to look after you and enters your life; you did not intend to meet it but now it is here. Some years ago I was busy doing a lot of quantitative gas chromatographic analyses, and because the integrating software sometimes made inacceptable decisions, it was necessary to re-integrate the chromatograms manually. Little by little some questions concerning the quality of the analysis arose. Two problems are presented in *Fig. 1*. Where are the accurate peak delimiters (peak start, peak end, baseline) if the signal-to-noise ratio is low? And what about the true area of overlapping peaks? How true are our analytical results which will be hopefully published in a journal of excellent reputation? (They were published, of course [1].)

1. Overlapping Peaks

It turned out that the problem of poorly resolved peaks had not found much attention so far. It seems to be more interesting to develop new separation techniques or applications than to look for errors [2]. Surprisingly enough, the small number of papers found in the literature did not clarify the situation. With regard to a pair of *Gaussian* peaks such as shown in *Fig. 2*, contradictory statements were presented. 'The area of the peak to the right is always larger than the true area' [3]; 'The area of the small peak is too small' [4]; 'Dropping a perpendicular favours the taller peak but short-changes the smaller one' [5]; 'Assuming peaks of equal width it makes sense that the smaller peak is overestimated' [6]; 'Using the perpendicular to integrate peaks, I do not see why the area of a small *Gaussian* peak would be underestimated' [7]. Who is right?

It is not too difficult to find the correct answer. Synthetic peaks of any shape (*Gaussian* or tailed) and of any resolution can be generated with the appropriate software [8] and analyzed by any integrator or data system. In a pair of overlapping *Gaussian* peaks, the area of the small peak is too small whereas the area of the large one is too large, irrespective of the elution order, if the peaks are separated by a perpendicular drop. In the case of tailed peaks (which are usually observed in real chromatograms) the area of the second peak is too large and the first peak is integrated with too small an area, irrespective of the size ratio [9]. In both cases the reason is purely geometrical in nature; it is easily understood with tailed peaks but not obvious at all with *Gaussian* bands.

These results make plain that baseline resolution is a prerequisite for accurate area integration. The chromatographic resolution R, expressed in units of the four-sigma peak width, needs to be increased drastically if extreme peak size ratios (*e.g.* 100:1) occur in the chromatogram [10]. Too often analysts sin against this principle, keenly looking for precision and forgetting about accuracy.

The topic of overlapping peaks has another aspect as well. The separating power or peak capacity of chromatographic columns is limited, less so in gas chromatography, more pronounciated in liquid chromatography. With complex mixtures (whereas 'complexity' begins with *ca.* 10 peaks in HPLC or *ca.* 20 peaks in GC) the analyst is confronted with statistical peak overlap [11], and the probability of obtaining a complete separation of all compounds without the use of extra selectivity effects (such as tailor-made gradients or temperature programs) is low.

It can be assumed that the problem of overlapping peaks has not yet been recog-

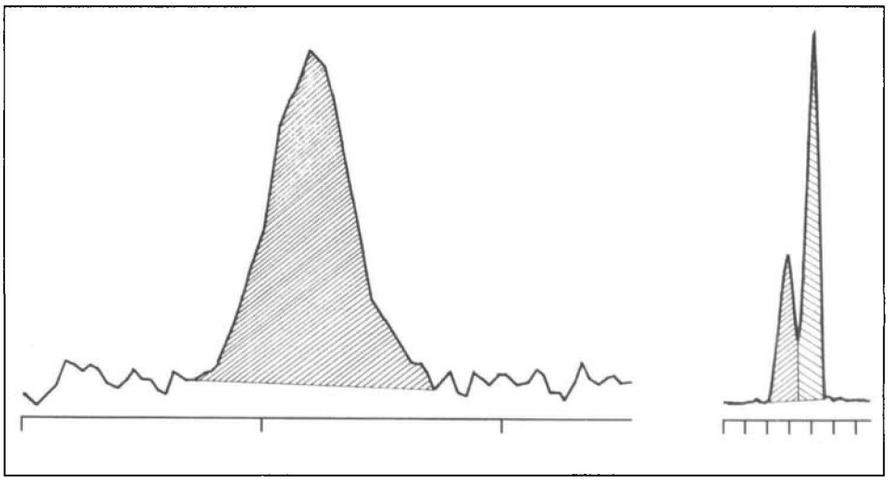

Fig. 1. Left: *A peak of* D-*allo-isoleucine at a signal-to-noise ratio of* ca. *9; one does not know if the integration is performed accurately.* Right: *A peak pair of* D/L-*proline with severe overlap; the integrator calculates a* D/L *area ratio of 0.450 but this value may be wrong.* The amino acids are in the form of *N*-trifluoroacetyl isopropyl ester derivatives. GC Separation on *Chirasil-Val.* The *x*-axis marks indicate time intervals of 10 s.

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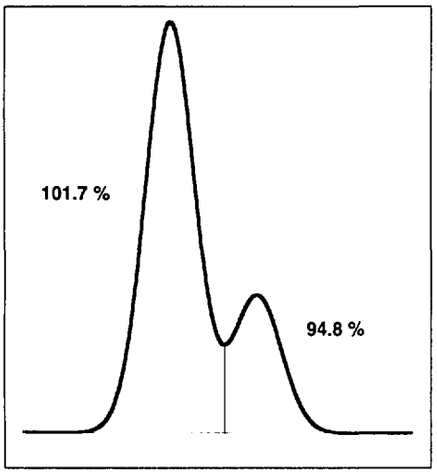

Fig. 2. *A* Gaussian *peak pair with incomplete resolution.* In the case shown here, the chromatographic resolution is 0.9 and the size ratio is 3:1. This results in a + 1.7% area error for the large peak and a – 5.2% error for the small one.

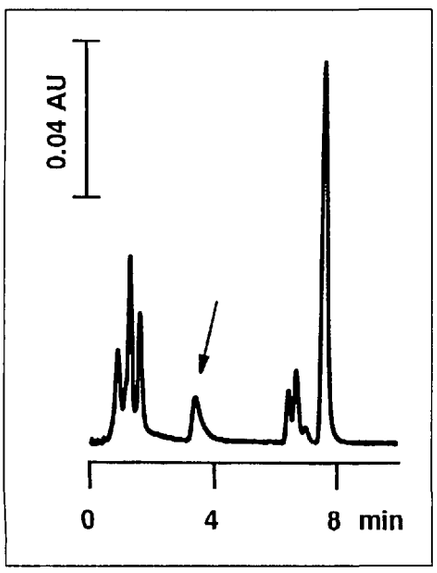

Fig. 3. *Air peak in HPLC.* Conditions: sample, 5 µl of octogen solution (the last peak is octogen) and 5 µl of air; column, 2 mm × 15 cm; stationary phase: *YMC 120 ODS-AQ*, 3 µm; mobile phase, water/acetonitrile 67:33, 0.3 ml/min; detector: UV 210 nm.

nized by many laboratories, including even such ones involved in routine analysis. Three different stategies can be followed: To create programmed separations (with temperature programs in GC and with gradients in HPLC) which isolate the peaks to be quantitated from neighbouring peaks; to use hyphenated techniques such as GC-MS, HPLC-UV or HPLC-MS even in routine analysis [12]; or to analyze the peak shapes and deconvolute the individual peaks by appropriate software packages [13][14]. It is a matter of course that all three approaches need thorough validation.

2. Other Errors in Chromatography

Once infected with the hunting fever for sources of error, a field is open which deserves compilation and research (in this order, yes). The chase is interesting and pleasant although one feels a consanguinity with *Jean Paul*'s Dr. *Katzenberger* whose passion was the collection of human and animal monstrosities [15]. It can be assumed that both major fields of instrumental chromatography, GC and HPLC, are equally interesting hunting grounds. Personally I limited my activities to liquid chromatography. Errors or unexpected incidents can occur at every step of an HPLC anlysis, from sample preparation to data evaluation. Their influence on the analytical result can be minor or severe. Laboratory work is not excluded from *Murphy*'s laws [16]. A wrong solvent may be used to elute the analyte from the solid-phase cartridge; the mobile phase may be prepared by a different procedure than usually; the analyte may decompose in the autosampler rack or during the separation process itself; a leak within the HPLC instrument is not detected by the operator [17]; the detection wavelength is no longer the one which is requested by the standard operating procedure; chromatographic resolution may be lost due to column ageing which leads to increased tailing and decreased separation performance. It was a pleasure to document all these and many more incidents and to publish them as a book [18].

The most recent example of an unwanted event in HPLC analysis is the fact that injected air can give rise to a retained peak. This was demonstrated in a chromatography journal [19] and could be easily confirmed with a different separation system. *Fig. 3* shows the peak which is obtained by injection of 5 µl of air into a reversed-phase column. Although nobody is injecting air intentionally, this can happen by careless manual injection, by malfunction of the autosampler or by incomplete filling of the vials. The extra peak which is observed at low detection wavelengths is unwanted because it could overlap with other peaks.

3. Not Peculiar but Necessary

Error tracking down may be a passion, however, a beneficial one. Today industrial and governemental laboratories make great efforts for validation and certification/accreditation (an aspect of analytical chemistry which is neglected by universities although the majority of chemists will be inevitably persecuted by these matters during their professional carreer). It is detrimental if qualified personnel produces inaccurate results by using expensive instruments only because the one or other source of error was overlooked. Nobody is perfect, but once an error is tracked down the best one can do is to discuss it with the colleagues and even to publish it if it is a new or unexpected one.

Received: June 26, 1997

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