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Dual-channel response ratios from an integrative algorithm ☆

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Abstract

An integrative algorithm has been developed, and compared with existing differential algorithms, for automatically determining the response ratios of peaks from a dual-channel flame photometric detector. The comparison was carried out using high and low, and constant and variable concentrations of an organosulfur test compound; under different degrees of solvent quenching and at two sets of detector flow conditions; and with and without digital filtering. The new integral algorithm performed as well as —and, particularly in the presence of strong noise, significantly better than— the existing differential ones. Typically, the response ratios of good peaks varied by 2 to 3% R.S.D. for different, and by ca. 0.5% R.S.D. for similar concentrations; the former, larger variation owing to previously unnoticed spectral changes. Different algorithms, working on single, large and well-smoothed peaks, varied among themselves by typically less than 1% R.S.D. The integral response ratios were displayed on the screen in graphic form and simultaneously printed in numeric form. At the discretion of the operator, they could also be printed in the form of a scalable "response-ratio chromatogram", with or without the constituent signal traces.

1. Introduction

Response ratios (RRs) from dual- or multiple-channel detectors or sensors can serve as indicators of chemical or physical properties. RRs are commonly determined on chromatographically separated analytes. They can support such varied analytical tasks as the assessment of peak purity [1], the subtraction of matrix components [2] or interfering peaks [1], the production of element-specific chromatograms [3,4] and the determination of physicochemical constants [5]. RRs can be automatically determined and plotted as "response-ratio chromatograms" (RRCs) [1].

For studies involving the dual- or multi-channel [6] flame photometric detector (FPD), we determined RRs as *slope* ratios (i.e. by comparing the first differentials) of the twin luminescence outputs. The use of slopes served the purpose well [1,3,4,6]. Even then it was suggested, however, that determining RRs as *area* ratios (i.e. by comparing the integrals) might prove advantageous [1]. Integration is a common procedure in chromatography [7]; and it seemed likely that area ratios should be less vulnerable to shifts in phase, increases in noise, incongruities in peak shape, and —particularly in the case of sequential detection— discrepancies in retention time.

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For these reasons we decided to pursue an integrative approach to RR determination, and compare its results to those of the earlier developed differential approaches [1]. As a test system we used the dual-channel FPD that supported the prior study, and set its two channels to monitor two strong bands of the most common FPD analyte, sulfur. These two bands of the blue S_2 emission ($v = 0 \rightarrow 9$ at 394 and $0 \rightarrow 10$ at 405 nm of the system $B^3\Sigma_u^- \rightarrow X^3\Sigma_g^-$ [8]) are known to produce fairly constant RRs, even when peaks exceed the quadratic range or are quenched by co-eluting hydrocarbons [9]. To test the algorithms to their limits, the analyte was injected in variable and low, variable and high, constant and high, and constant and very high amounts; and the chromatograms, obtained from two different detector conditions, were used both with and without heavy digital filtering.

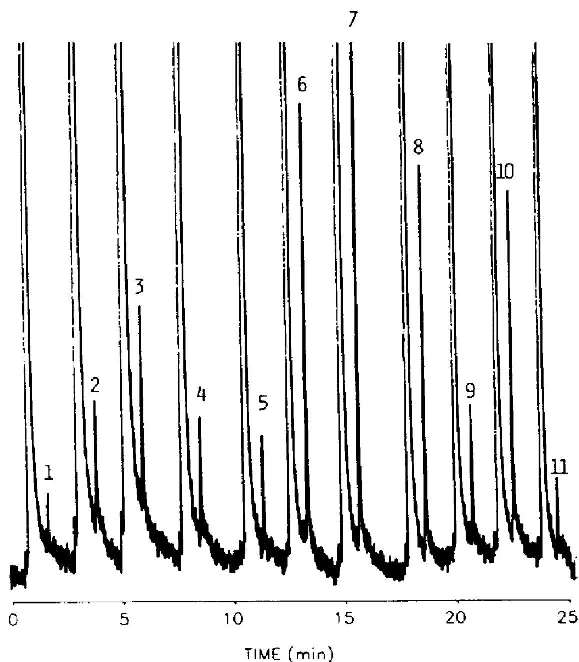


Fig. 1. Typical "unfiltered" chromatogram of "low and variable" analyte injections from ca. 0.5 to 2.0 ng of di(*tert.*-butyl) disulfide in acetone. The numbers marking the sulfur peaks are the same as those used in Tables 1 and 2. Channel 1 (405 nm); hydrogen 200 ml/min, air 50 ml/min.

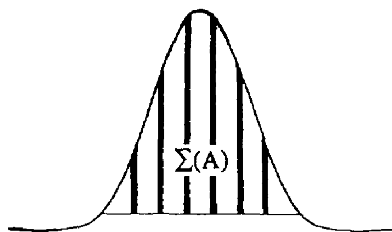
2. Experimental

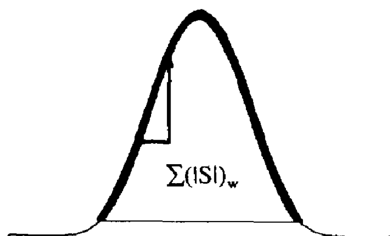
Several series of multiple di(*tert.*-butyl) disulfide injections into our ancient Shimadzu GC-4BMPF gas chromatograph with dual-channel FPD were stored in computer memory via a laboratory-developed interface and acquisition program [2]. The injections were done at variable and low (about 0.5 to 2 ng), at variable and high (about 5 to 20 ng) and, later, at constant high and very high (20 and 100 ng) levels of analyte. The variable injected amounts and volumes were selected such that the different sulfur peaks spanned the available screen range, and that the different volumes of solvent (acetone) produced tails of different prominence, i.e. of variable quenching ability. The low-analyte files deliberately included peaks that were close to the detection limit and hence encrusted by strong noise. Fig. 1 shows an example.

Both high-analyte and low-analyte files were examined "unfiltered" and "heavily filtered". The latter procedure used a finite-impulse-response (FIR) digital filter with Hamming window [10] and 32, 64 or 128 taps, whose cut-off frequency was adjusted to approach the maximum signal-to-noise ratio (an approach inevitably accompanied by a close to 50% reduction in peak height). Separate sets of individually "optimized" parameters (slope thresholds for start/stop commands, etc.) were used for differential and integral RR determinations.

The above variations were carried out at a set of general flow conditions that would typically be used for a larger number of FPD-active elements including sulfur. To guard against (only later noticed) concentration-dependent spectral changes, further data files were generated by using conditions whose gas flows were optimized for the S_2 luminescence, and by repeatedly injecting the *same* amounts of solvent and analyte.

Fig. 2 offers a simplified graphic comparison of the present integrative with the earlier differential approach: the latter averages the weighted absolute-slope ratios (the steeper the slope the heavier the weight) for the whole-peak mode; or





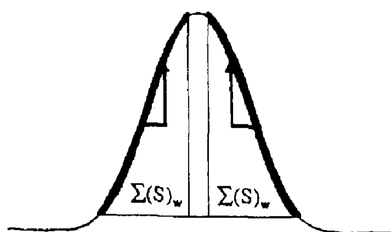


Fig. 2. Schematic representation of three different ways to evaluate chromatographic peaks (one peak only shown) for determining response ratios based on integral (area) or differential (slope) measurements. A = Amplitude; S = slope; w = weighted.

the weighted positive-slope ratios (up the peak, first "half") and negative-slope ratios (down the peak, second "half"), for the split-peak mode [1]. Note from Fig. 2 that the split-peak mode excludes a center slice containing the peak apex: slope ratios from the center, where both slopes go through zero, can under adverse conditions produce erroneously large or small numbers, particularly if the two peaks reach their maximum amplitude at a slightly different time. No such apex effect occurs in the integral mode; in fact, there the apex region represents the heaviest hence most important slice of the measurement.

Accordingly, the new integral RR algorithm[1] was written for the *whole*-peak mode only. Peak start and stop criteria were similar to those of the differential system [1]. Also as in the differential system, the objective was to cover not all but just the diagnostic parts of the peak. What mattered was not the most accurate determination of the peak areas themselves, but the most precise determination of their *ratio*. The often unreliable base of the peaks (the lowest 5–10% of peak height) was therefore routinely excluded by suitable cut-on/cut-off thresholds.

Briefly, the integrative algorithm searched for "valid" peaks (in a procedure similar to the one used by the differential algorithms), then integrated them and determined their ratio. To be accepted as "valid", a peak had to meet *all* of the following criteria:

(1) The peak had to be located within lower and upper "clipping" levels, as defined by the operator with the help of cursors superimposed on the chromatograms. The lower clipping level allowed the operator to exclude noise or solvent-caused baseline dips; the upper clipping level allowed him to exclude peaks that exceeded the range of the original data acquisition system (and were of concern only if one or both of the chromatograms had been previously reduced in amplitude). For the present study, clipping levels were *not* needed.

(2) For sole purpose of integration, the peak was assumed to "start" when its slope exceeded a "minimum slope" threshold, set by the operator in percent of full scale (= percent of

[1] Researchers interested in this program for non-commercial purposes are invited to contact B.M. for an executable copy.

screen height) per minute. The peak was assumed to "stop" when the slope —multiplied by −1 for the descent— dropped below the same slope threshold. (*Note:* The algorithm was designed to process roughly symmetric peaks. If strongly asymmetric ones should be encountered on a regular basis, the operator may prefer to define separate and different slope thresholds for ascent and descent.) The necessary threshold values could be easily estimated on the screen by imagining tangents drawn through the desired start and stop points of some conveniently located peak.

The slope was calculated from the amplitude difference between two data points, with the distance (i.e. the time) between the data points set by the operator. For instance, a setting of "1" meant carrying out the slope determination over two adjacent data points or 0.1 s, "5" meant 0.5 s, etc. This was done to reduce the effect of short-term noise (if present). Slope values were calculated for every data point. Obtaining meaningful slope values thus depended on choosing the appropriate width of the moving window in which the slope appeared. (*Note:* If the signal had already been smoothed before the slope threshold criterion was applied, this choice mattered but little.)

(3) In order to be accepted by the algorithm as a valid peak "start", a specified, uninterrupted number of slope values had to exceed the set "minimum slope" value. For instance, if the operator answered "10" to the prompt requesting the "number of consecutive data points", at least 10 slope values in a row (1 s worth of chromatographic time) *all* had to fall above the slope threshold. The setting thus defined how long a peak-commencing upward (or a peak-terminating downward) slope had to last in order to trigger peak start and stop commands. Again, which "number of consecutive data points" to set was easily estimated on the screen from the width of the most slender peak.

With all prompts answered, i.e. all thresholds defined, the program searched for "valid" slopes throughout the chromatogram. It declared PEAKSTART and PEAKEND times if, and only if, all of the above criteria had been met for a particular peak. The algorithm to achieve this was included in a collection of in-house chromatographic routines called "CHROM-8". It analyzed the first-channel signal to define PEAKSTART and PEAKEND, then imposed these times on the second channel. This worked well if the two channels, as in this study, were fairly similar.

(Note, however, that our prime interest is analytical: it transcends the current exploratory and mainly statistical topic. In envisioned analytical methodology, the two channels could for instance originate from FPD wavelength regions spaced far apart, or they could even originate from different detectors. In addition, the sample could contain several elements or functional groups with different response characteristics. If, consequently, the two channels were to yield peaks grossly different in amplitude and perhaps even symmetry, it would be reasonable to include *both* channels in the peak diagnostics. To achieve this with minimal effort, an alternative collection of algorithms was assembled and, for practical purposes, called "CHROM-9". At its heart was a routine that took the average of the two channels, performed the diagnostics on the *averaged* peaks, and then imposed the derived PEAKSTART and PEAKEND times back onto the two constituent (original) channels —there to serve the subsequent computation of area-based response ratios. In addition, a subroutine dubbed "TD" ("time-delay") allowed one chromatogram to be temporally shifted until it best matched its twin in elution behavior. The latter feature was developed with sequential (as opposed to synchronous) detector channels in mind. For the current simultaneous signals from adjacent S_2 bands, however, the integrative CHROM-9 algorithm was unnecessary, the TD option inappropriate. Also, whenever double-checked on the data files of this study, CHROM-9 produced the same results as CHROM-8.)

The signal (amplitude, chromatographic response) at PEAKSTART time was defined as the average inside a 0.3-s window (a third of the sum of three successive data points, with the PEAKSTART datum in the middle). A similar smoothing procedure was used for the signal at

PEAKEND time. A horizontal baseline (really: a truncation line) was then defined by calculating the arithmetic mean of the smoothed PEAKSTART and PEAKEND signals. The "peak area" (for purpose of calculating area ratios) was obtained by summing all 0.1-s data point amplitudes *above that line*, from PEAKSTART to PEAKEND.

The algorithm then calculated the area ratio of corresponding peaks in the two channels. (Any magnification factors previously applied to one or both chromatograms were automatically factored into this response-ratio calculation.) The numerical RRs, together with the PEAKSTART and PEAKEND times (down to a tenth of a second), were arranged in a table —similar to the one shown in Ref. [1]— and forwarded to a matrix printer.

Meanwhile, the RRs appeared on the screen in the form of a (logarithmic) RRC [1], superimposed on the two constituent chromatograms. With the help of an auxiliary program, all three could be individually magnified or reduced in amplitude. The screen images could also be vertically offset and, if so desired, forwarded singly or in combination to a printer. Fig. 3 shows the example of a coarse "screen dump", as routinely obtained from an inexpensive matrix printer and typically destined for a laboratory notebook.

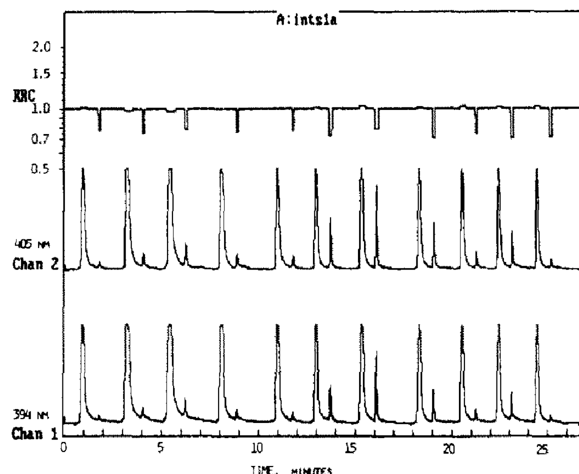


Fig. 3. Response-ratio chromatogram with its constituent (405 and 394 nm) chromatograms. Scaled for easier viewing; numbers and axis captions manually inserted. Screen-dumped on 8-point matrix printer (hence the stepped appearance). The caption shown on top, "A:intsla", is the automatically printed file title.

3. Results and discussion

There is little doubt that dual-channel RR algorithms perform well on strong peaks towering above straight and smooth baselines; just as conventional integrator algorithms [7] do. The obvious question, however, is how well they cope with the more difficult types of terrain in which smaller peaks —overlapping one another, perhaps— rise from an undulating baseline overgrown with noise. In such cases, conventional integrators have been shown to produce errors (in estimating single-peak areas) of up to 40% [11].

The test protocol offered therefore chromatograms of considerable challenge: by including non-filtered data {i.e. data filtered only by the resistor–capacitor (RC) time constant of the electrometer (cf. Ref. [12]) }; by approaching the detection limit; by letting peaks ride on strong or weak solvent tails (and thus be strongly or weakly quenched); and by injecting variable amounts of both analyte and solvent.

Table 1 shows an example of such a worst-case scenario: it lists the RRs for the sulfur peaks numbered in the Fig. 1 chromatogram. Table 1 lists in addition the means, together with the relative standard deviation (± R.S.D.) values, both for a *single peak* determined by all four algorithms (row), and for all peaks determined by a *single algorithm* (column). As expected for a worst-case scenario, the "precision" of the measurements is unacceptably large —with the important exception, however, of the 7.2% R.S.D. value for the *integral* (area-ratio) mode. In contrast, the differential (slope-ratio) mode produces not only very large relative deviations; it also produces highly inaccurate means.

This is as expected, since summed *differential* ratios should be much more susceptible to severe variations in slope, i.e. to noise obscuring the algorithmically important parts of the peak. The

Table 1
Response ratios from a non-filtered, low and variable level chromatogram

Peak	Area ratios	Slope ratios			Average (% R.S.D.)
		Whole peak	Split peak		
			First half[a]	Second half[a]	
1	0.851	2.305	1.319	2.984	1.865 (51.5)
2	0.716	1.639	1.141	2.096	1.398 (42.8)
3	0.786	1.689	1.129	2.910	1.629 (57.2)
4	0.731	1.416	1.350	1.425	1.231 (27.2)
5	0.762	1.448	1.095	1.413	1.180 (27.2)
6	0.734	1.344	1.260	1.833	1.293 (34.8)
7[b]	0.779	0.809	0.736	1.500	0.956 (38,1)
8	0.639	0.798	0.880	0.965	0.821 (16.9)
9	0.729	1.613	1.622	1.103	1.267 (34.2)
10	0.721	1.171	1.376	0.901	1.042 (27.8)
11	0.786	1.984	1.763	1.159	1.423 (38.6)
Average (% R.S.D.)	0.749 (7.21)	1.474 (30.8)	1.243 (23.9)	1.663 (43.8)	

Conditions: hydrogen 200 ml/min, air 50 ml/min. RC time constant of electrometer 0.22 s. Injections vary from 0.5 to 2.0 ng di(*tert.*-butyl) disulfide in acetone.
[a] The "half" is actually less than 50% since the center slice that contains the peak apex is excised by the split-peak algorithm (see Fig. 2).
[b] Off-scale peak.

Table 2
Response ratios from a filtered, low and variable level chromatogram

Peak	Area ratios	Slope ratios			Average (% R.S.D.)
		Whole peak	Split peak		
			First half	Second half	
1[a]	0.808	0.904	0.885	0.873	0.868 (4.8)
2	0.756	0.760	0.770	0.739	0.756 (1.7)
3	0.787	0.784	0.771	0.796	0.785 (1.3)
4	0.767	0.786	0.761	0.796	0.778 (2.1)
5	0.789	0.788	0.770	0.803	0.788 (1.7)
6	0.730	0.723	0.714	0.703	0.724 (1.0)
7[b]	0.799	0.837	0.831	0.833	0.825 (2.1)
8	0.712	0.715	0.708	0.720	0.714 (0.71)
9	0.748	0.776	0.770	0.760	0.764 (1.6)
10	0.716	0.722	0.722	0.721	0.720 (0.40)
11[a]	0.764	0.810	0.786	0.795	0.789 (2.4)
Average (% R.S.D.)	0.761 (4.3)	0.782 (7.1)	0.772 (6.7)	0.766 (6.8)	

Conditions as in Table 1, except FIR cut-off frequency of 0.2 Hz.
[a] Values obtained in zoom mode to avoid screen resolution problems.
[b] Off-scale peak in non-filtered chromatogram.

numbers in Table 1, measured under deliberately marginal conditions, clearly indicate that integral measurements (of truncated peaks) provide preciser —and as we shall see later, also more accurate— data than differential measurements.

Again as expected, the situation is much improved by heavily filtering the chromatograms before measuring the RRs. Table 2 shows the results. All R.S.D. values are now acceptable. (On a poor data file such as this, we consider an R.S.D. of less than ± 10% to represent a reasonable criterion of acceptability). The means are very close to one another, and are essentially identical to the "integral" mean of Table 1. It is interesting to note that the four algorithms now differ less among each other when evaluating the *same peak* (most values are in the 1–2% R.S.D. range, with the median at 1.7%), than peaks differ among each other when evaluated by the *same algorithm* (these values all fall into the 4–7% R.S.D. range).

The precision improves, as expected, when larger amounts of analyte are used. Fig. 4 shows the "non-filtered" first-channel chromatogram of this series. It should be mentioned that the better RRs are not due to a diminution of quenching: the sulfur peaks are still quenched by the same percentage as in Fig. 1. This is not immediately obvious from the picture —the solvent appears to tail very little at the higher attenuation— but percent quenching depends only on the concentration of the quencher, not on the concentration of the analyte [13]. The quencher, i.e. the solvent and/or its fragmentation products, has not reached the limit of its quenching power —it has reached the limit of its own luminescence. The fact that the linear range of (probably CH and CC) luminescence has indeed been exceeded, can be appreciated from the very similar peak heights resulting from very different volumes of solvent.

Table 3 contains the RRs for the chromatographic data file shown in Fig. 4, i.e. for the typical example of a "non-filtered", high-analyte level run. The individual integral and differential algorithms now produce essentially the same R.S.D. (close to 3%) for *all* peaks combined. When the action of the four algorithms is compared for the *same* peak, the R.S.D. values are generally lower (mostly between 0.5 and 3%, median 1.1% R.S.D.).

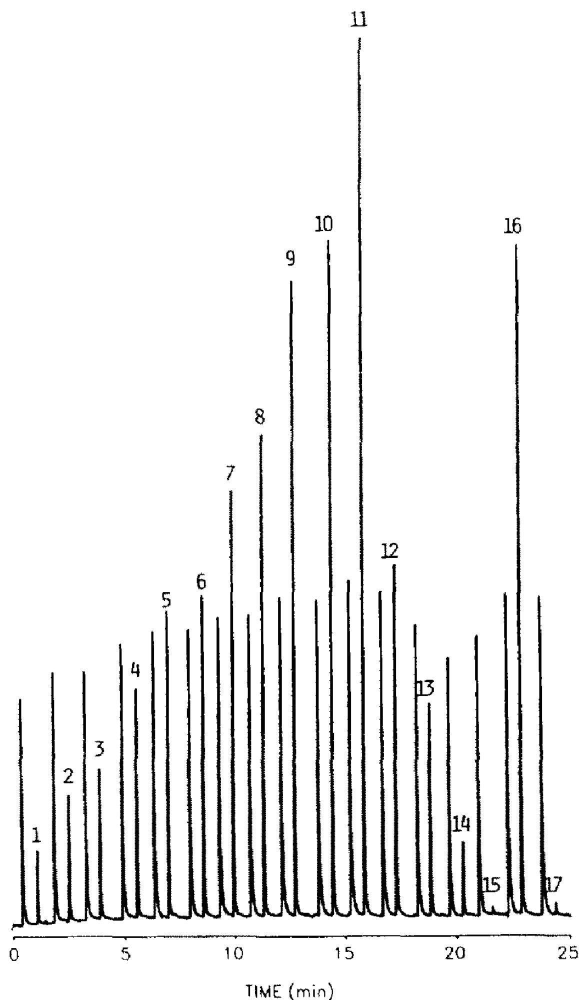


Fig. 4. Typical "unfiltered" chromatogram of "high and variable" analyte injections from ca. 5 to 20 ng of di(*tert.*-butyl) disulfide in acetone. The numbers marking the sulfur peaks are the same as those in Table 3. Channel 1 (405 nm); hydrogen 200 ml/min, air 50 ml/min.

The same chromatogram as given in Fig. 4, but now heavily filtered, produces data hardly different from those of Table 3, at least as far as the mean response ratios are concerned. Also, the R.S.D. values for each of the four algorithms, working separately on *all* peaks, are again similar (close to 2.7%). However, the

Table 3
Response ratios from a non-filtered, high and variable level chromatogram

Peak	Area ratios	Slope ratios			Average (% R.S.D.)
		Whole peak	Split peak		
			First half	Second half	
1	0.795	0.801	0.801	0.828	0.806 (1.8)
2	0.795	0.819	0.809	0.806	0.807 (1.2)
3	0.801	0.797	0.809	0.797	0.801 (0.71)
4	0.803	0.805	0.811	0.816	0.809 (0.73)
5	0.811	0.806	0.808	0.825	0.813 (1.1)
6	0.816	0.819	0.826	0.823	0.821 (0.54)
7	0.822	0.825	0.827	0.836	0.828 (0.73)
8	0.821	0.821	0.821	0.837	0.825 (0.97)
9	0.835	0.833	0.830	0.851	0.837 (1.1)
10	0.834	0.856	0.874	0.851	0.854 (1.9)
11[a]	0.888	0.855	0.833	0.894	0.868 (3.3)
12	0.839	0.834	0.833	0.852	0.840 (1.0)
13	0.834	0.832	0.838	0.841	0.836 (0.48)
14	0.828	0.851	0.836	0.840	0.839 (1.1)
15[b]	0.779	0.880	0.890	0.844	0.848 (5.9)
16	0.843	0.846	0.847	0.862	0.850 (1.0)
17[b]	0.843	0.872	0.870	0.872	0.864 (1.6)
Average (% R.S.D.)	0.823 (3.1)	0.832 (3.0)	0.833 (3.0)	0.840 (2.8)	

Conditions as in Table 1 except injections from 5 to 20 ng di(*tert.*-butyl) disulfide in acetone.
[a] Off-scale peak.
[b] Values obtained in zoom mode to avoid screen resolution problems.

R.S.D. values for the four algorithms working on the *same* peak now fall mostly into the 0.1 to 0.3% range (median 0.23%): a significant improvement when compared to the "non-filtered" data. (The full data set is not shown here to save space.)

It thus appears that all tested integral and differential algorithms concur within relatively narrow margins of error (certainly below 1% R.S.D.) when measuring the same, good-quality peak. As supported by this concurrence —and the low probability of all four algorithms having the same bias— we consider all four algorithms to be essentially accurate. The variability of the RRs themselves thus seems to be due not to algorithmic but to chromatographic, i.e. spectral differences. This should come as no surprise in light of the differently sized and differently quenched peaks. Although the S_2 bands predominate, smaller contributions of other excited sulfur species [9,14] may still be present —not to mention carbon-based emissions and/or background luminescences. Chromatographic response ratios, in addition to their many other roles in analysis, can thus serve as exquisitely sensitive probes of spectrochemical change.

Although we originally did not plan to do so, the relatively strong RR variation of differently sized and quenched peaks demanded that we either confirm or deny the algorithms' potential involvement in the comparatively large ± 3% R.S.D. spread. To obtain data files that excluded, as far as convenient, any spectral variation, the detector conditions were optimized for the S_2 emission (earlier conditions had been those that afforded good overall response to a rather wide variety of elements, including sulfur). Also, the same analyte amount and the

same injection volume were used throughout each experimental run.

Table 4 shows the results from one set of heavily filtered chromatograms, as obtained from very high, constant levels of analyte (just bordering the upper end of the linear range, in fact). The obvious difference to the earlier, variable-analyte runs is the much lower R.S.D. value for the *same* algorithm working on *all* 16 peaks: it is of similar magnitude as the R.S.D. value for all four algorithms working on the same peak. Somewhat lower (but still constant) analyte levels gave the same result, i.e. the R.S.D. values for heavily filtered chromatograms were close to 0.5% for the integral and the two split-peak differential modes.

The values for the *whole*-peak differential mode were, however, clearly worse (in the 1–1.5% range). Speculatively we believe this to be a consequence of working close to the upper linear-range limit. The likely relevant effect to consider here is the behavior of the peak apex. It is the peak apex that approaches or breaches the linear range; and it is the peak apex where, as argued earlier on grounds of principle, *slope* ratio measurements are likely to show the greatest error. Accordingly, the apex-free *split*-peak values of the differential approach agree well with the (*whole*-peak) values of the algorithmically quite different integral approach; but they disagree with the values of the otherwise similar *whole*-peak differential approach —both in mean and in % R.S.D. (0.734, 0.736 and 0.737 vs. 0.746; and 0.42, 0.41 and 0.38 vs. 1.2; respectively). Since the apex-burdened whole-peak differential mode is here the odd algorithm out, its forced inclusion in the group of four brings about an R.S.D. value higher than expected.

In other words: if the spectral conditions are fairly constant, three algorithms will produce essentially the same response ratio —with both

Table 4
Response ratios from a filtered, very high and constant level chromatogram

Peak	Area ratios	Slope ratios			Average (% R.S.D.)
		Whole peak	Split peak		
			First half	Second half	
1	0.739	0.745	0.736	0.737	0.739 (0.546)
2	0.733	0.739	0.733	0.734	0.735 (0.391)
3	0.733	0.753	0.733	0.734	0.738 (1.334)
4	0.733	0.746	0.734	0.736	0.737 (0.809)
5	0.731	0.739	0.731	0.732	0.733 (0.527)
6	0.731	0.745	0.732	0.736	0.736 (0.866)
7	0.733	0.744	0.734	0.737	0.737 (0.674)
8	0.741	0.748	0.743	0.742	0.744 (0.418)
9	0.738	0.749	0.739	0.742	0.742 (0.669)
10	0.737	0.743	0.738	0.739	0.739 (0.356)
11	0.737	0.777	0.738	0.740	0.748 (2.590)
12	0.735	0.743	0.736	0.737	0.738 (0.487)
13	0.733	0.740	0.736	0.738	0.737 (0.405)
14	0.731	0.742	0.734	0.737	0.736 (0.637)
15	0.735	0.746	0.736	0.739	0.739 (0.672)
16	0.732	0.741	0.736	0.735	0.736 (0.508)
Average (% R.S.D.)	0.734 (0.419)	0.746 (1.209)	0.736 (0.406)	0.737 (0.377)	

Conditions: hydrogen 50 ml/min, air 40 ml/min. FIR cut-off frequency 0.2 Hz. Injections of 100 ng di(*tert.*-butyl) disulfide in acetone.

types of R.S.D. values hovering around 0.5%. In contrast, the fourth (the whole-peak differential mode) is a bit off and its R.S.D. a bit worse (in the 1–1.5% range, typically). To keep matters in perspective, however, even this poorer precision should still amply satisfy most analytical requirements.

4. Conclusions

To summarize —and at the same time to include summaries of some directly comparable data sets not reproduced in full here for reasons of space— Table 5 lists the R.S.D. roster for three typical chromatographic files being exposed to all four algorithmic treatments. (Other files run for this study follow the same trends.) A variety of conclusions and expectations can be drawn from these files:

(1) The algorithms do perform satisfactorily over the whole concentration range —even peaks close to the detection limit (see Fig. 1) can be successfully evaluated.

(2) In the particular case of S_2 —and likely of some other FPD emitters as well (cf. Ref. [9])— care has to be taken that the spectral characteristics of the emitter do indeed remain constant throughout the concentration/quenching ranges of analysis. Under reasonable circumstances, the algorithms are sensitive enough to pick up changes in spectrum smaller than 1% —a change that would, for example, not be normally recognized in conventional single-channel spectrophotometry. (Hence, such algorithms might find use in spectrochemically motivated work.)

(3) In this study, heavy filtering invariably improved the precision of RR data. Although the dependence of R.S.D. values on the time constant of the filter has not been investigated in any detail, it is obvious that dual-channel chromatograms should be routinely filtered before the response ratios of their peaks are determined.

(4) In general, the *split*-peak values —although having less than half of the peak to operate on— are as precise, or preciser than, the whole-peak values in the *differential* mode. We speculatively attribute this to the —slope-ratio-wise— less precise apex section being excised by the split-peak algorithm.

(5) On noisy chromatograms, the *integral*

Table 5
Summary of relative standard deviations (%) of dual-channel FPD response ratios as determined by different algorithms on chromatograms varying in detector conditions, analyte levels and time-constant settings

No. of peaks	Analyte injection level[a]	Chromatogram filtered?[b]	Area ratios	Slope ratios			Median of Single peak R.S.D.s[c]
				Whole peak	Split peak		
					First half	Second half	
11	Low, variable	No	7.2	31	24	44	35
		Yes	4.3	7.1	6.7	6.8	1.7
17	High, variable	No	3.1	3.0	3.0	2.8	1.1
		Yes	2.6	2.7	2.6	2.7	0.23
16	Very high, constant	No	0.42	3.0	1.3	1.1	2.4
		Yes	0.42	1.2	0.41	0.38	0.59

[a] Typical levels of di(*tert.*-butyl) disulfide in acetone: low and variable: 0.5 to 2.0 ng; high and variable: 5 to 20 ng, both at hydrogen 200 and air 50 ml/min; very high and constant: 100 ng, hydrogen 50, air 40 ml/min.

[b] "No" represents an RC time constant of 0.22 s; "Yes" represents an FIR filter with filter taps and cut-off frequency set to give peak height reduction in the 50 to 60% range (close to maximum S/N).

[c] Median of same-peak R.S.D. values as determined by the four algorithms (see utmost right columns in Tables 1–4).

(area ratio) approach performed better than the *differential* (slope ratio) approach.

(6) The *integral* algorithm is at present available in *whole*-peak mode only. However, the addition of a split-peak integral mode —if desired for checks of peak purity, peak overlap, etc.— should require only the minor software adjustment of bisecting the peak at the apex. Similarly, operator-selected (i.e. cursor-defined) peak slices could be evaluated by the integrative (as opposed to the already existing differential) approach as well.

(7) General principles suggest that —in comparison with RRs determined by the differential (slope) method— RRs determined by the integral (area) method should be less susceptible to those detrimental effects of shifts in retention time and/or concentration profile that arise from monitoring two *sequential* detectors. They should also be less susceptible to the insidious effects of electronic phase shifts that can occur even when two channels of the *same* detector are monitored.

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