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Filterless, full spectral range, compound-specific detection in dual-channel photometry[1]

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Abstract

The phosphorescence of aroyl compounds in excited nitrogen has been used as a model system to demonstrate the possibility of computer-mediated, compound-specific detection by dual-channel photometry. The demonstration was carried out in the absence of any optical filter or dispersive device. It involved a sixteen-component mixture with aroyl amounts ranging over three to five orders of magnitude, all the way down to the femtomole level. Each individual compound could be successfully retrieved from one chromatographic file/separation as a single-peak Condac chromatogram. The response ratios of all sixteen compounds proved invariant from close to their detection limit to well beyond their linear range.

Keywords: Detection, GC; Dual-channel photometry; Aroyl compounds

1. Introduction

Chromatographic detectors are commonly classified as either general or selective. Of the selective ones, some are close to being specific. (The term "specific" is used here in its original analytical meaning of "infinitely selective".) The increasing quest for ever greater selectivity is driven by certain popular types of samples, e.g. of the environmental variety. The analysis of these complex mixtures is often impossible without – or is at least greatly facilitated by – a simple detector responding sensitively and selectively to some structural element or chemical property of the trace analytes.

A prime example for such a device is the common flame photometric detector [1,2]. In its dual-channel version [3] it is furthermore capable of producing computer-mediated, i.e. "virtual" or "correlation" chromatograms of much improved selectivity. So far, virtual chromatograms have been obtained by one of two principal algorithmic modes. In the "subtraction" mode, the signal from one channel is scaled and deducted from that of another [4–6]. (It should be mentioned that optical means of increasing the selectivity of this detector can be largely replaced by temporal ones. As Amirav et al. have demonstrated in their highly interesting development of a "pulsed" flame photometric detector, kinetics of emission by different elements can be used in a "dual-gate subtraction" mode to provide considerable enhancement of different types of interelemental selectivity [7–9].)

In the "Condac" (for "conditional access") mode [10,11], the algorithm allows only peaks of a given response ratio access to the chromatogram. The two

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channels monitor different wavelength ranges in accordance with analyte – and perhaps interferent – spectra. (Note that the choice of optical filter is rarely critical: many different filter combinations can serve a given element; conversely, many different elements can be accommodated by a given filter combination.)

Condac chromatograms obtained from a dual-channel flame photometric detector will display only those peaks that contain the "dialed-in" element. If Condac chromatograms were asked to show only one compound (as opposed to one element), the flame photometric detector would need to be combined with some other, response-wise orthogonal detector. Such a combination of different detectors for compound-specific response is certainly feasible [12].

But compound-specific response can sometimes be achieved even in a single detector (i.e., a single-mechanism detector offering at least two distinct, simultaneous signals). To wit, whenever the components of a sample differ in some physically measurable parameter – wavelength, mass, reaction rate, electrochemical potential, and the like – and whenever their intensity ratios in the two (optical, electronic, etc.) channels are (a) sufficiently different from one another and (b) sufficiently constant throughout the range of analytically important conditions (concentration, interference, etc.), the computer can easily generate compound-specific chromatograms.

Analytically, such an approach simplifies the chromatogram and confirms that a particular peak does indeed represent the analyte of interest. Also, if the response ratios of both the analyte and an (even perfectly) co-eluting interferent are sufficiently different (and known), algorithmically similar methodology can effectively deconvolute and hence quantitate the two peaks [13]. Most importantly, though, the demonstration of *single-compound* specificity seemed to us a primarily conceptual task: for this reason we made it the major objective of this study. An additional objective was to achieve such specificity in the absence of any optical filter or spectrometer.

Reports of open (filterless) *single*-channel flame photometry [14,15] notwithstanding, it remains a belief common to the analytical disciplines that an optical filter or, albeit at lower sensitivity, a spectrometer, are a prerequisite for achieving selectivity/specificity. The *dual*-channel flame photometric detector would seem to be in need of optical filters as a matter of course: the spectral difference between the two channels is indeed its raison d'être [3]. It became therefore rather interesting for us to investigate, in a generally applicable manner, whether this or some other dual-channel detector of the photometric type could produce Condac chromatograms in the *absence* of spectral filters or light dispersive devices. But which detector to choose?

The characteristics of the flame photometric detector would make compound-specific detection too difficult for analytes containing the same element, i.e. those producing the same spectrum; and they would render filterless operation too easy for compounds containing different elements, i.e. those producing drastically different spectra. For these reasons, as well as for reasons of perceptual generality, simplicity and acuity, we chose to test the basic idea neither on the flame photometric detector itself nor on its combination with other detectors. Rather, we decided to use a "photometric" device singular to our laboratory, the dual-channel "aroyl luminescence detector" [13] cf. [16–19].

Why the aroyl luminescence detector? The gas-phase phosphorescence-type spectra of aroyl compounds that this detector monitors are fairly broad. They also occupy, very approximately, the same wavelength range. That notwithstanding, their features are still characteristic of individual species. This means that, as model analytes, they promise the analyst a chance of obtaining compound-specific Condac chromatograms while at the same time posing the challenge of highly similar spectra (a challenge exacerbated here by the deliberate absence of optical filters). Thus, if the aroyl luminescence detector should indeed prove capable of yielding Condac chromatograms from two filterless optical channels, it would seem reasonable to conclude that several other detectors – say, the UV-absorption detector [20] – should likewise be able of providing virtually compound-specific responses.

2. Experimental

The dual-channel aroyl luminescence detector has been described [13]. For most of the present study, it was used without optical filters. One channel was

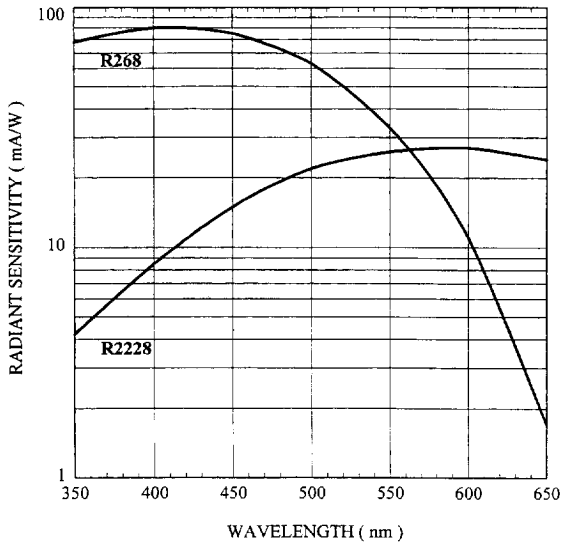

Fig. 1. Nominal radiant sensitivity of two photomultiplier tubes (from Ref. [21]).

fitted with an R-268, the other with an R-2228 Hamamatsu photomultiplier tube. Fig. 1 shows the tubes' typical radiant sensitivity in the range of interest [21].

The detector was mounted in the detector bath of an ancient Tracor Model 550 gas chromatograph. The detector temperature was usually 200°C, its operating voltage +6000 V. A borosilicate glass column of 100 cm×0.2 cm I.D., packed with Carbowax-20M bonded [22,23] onto Chromosorb W, 100–120 mesh, was used with a nitrogen flow of around 10 ml/min. (The stationary phase of this column, a surface-modified diatomaceous earth, was chosen because it typically yields short retention of polycyclic aromatics and efficient separation of positional isomers. It should be realized, however, that a good capillary column would have provided significantly larger plate numbers and signal-to-noise ratios.) The "prepurified"-grade carrier gas was further stripped of oxygen and water by passage through a Supelco Model 2-3800 cartridge [24]. Typical make-up and purge flows of nitrogen to the detector were 100 and 50 ml/min, respectively.

The determination of response ratios used the "slope ratio" method [25] for "whole" peaks. The Condac algorithm [10] and the response-ratio chromatograms have been described [25]; no changes were made to the particular computer hardware and software components that had so well served earlier studies. A 530-nm longpass filter was introduced later in this work for purpose of comparison: it was an inexpensive colored-glass disc [26].

3. Results and discussion

To test our supposition that computer-mediated compound specificity is possible – and possible even in the absence of optical filters – we prepared a few 16-component mixtures of aroyl compounds. The choice of aroyls reflected different considerations: which responded well, which represented major vs. minor structural differences, which fitted nicely and loosely into a simple demonstration chromatogram.

Fig. 2 shows the two single-channel chromatograms of a typical mixture, topped by a whole-peak response-ratio chromatogram [25]. It is obvious that, as expected, the different radiant sensitivity profiles

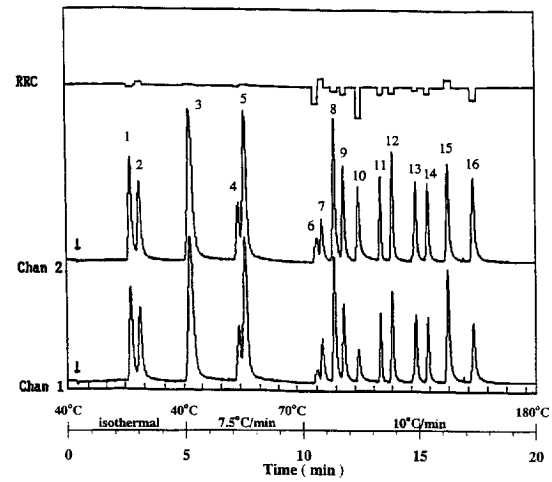

Fig. 2. Screen-dump of individual channels and the slope-based, whole-peak response-ratio chromatogram [25] of a mixture of aroyl compounds; time/temperature scale added. Channel 1: PMT-268 (no filter, −600 V); Channel 2: PMT-2228 (no filter, −600 V). Compounds in order of elution: 0.5 μl hexane (solvent), 3 ng benzaldehyde (1), 3 ng 4-fluorobenzaldehyde (2), 18 ng acetophenone (3), 3 ng 4-ethylbenzaldehyde (4), 12 ng 3-methylacetophenone (5), 60 ng 2,6-dichlorobenzaldehyde (6), 60 ng α-tetralone (7), 3 ng isophthalaldehyde (8), 3 ng methyl-4-formylbenzoate (9), 30 ng 1,4-naphthoquinone (10), 3.2 ng 3,3'-bis(trifluoromethyl)benzophenone (11), 2.4 ng benzophenone (12), 1.8 ng 4-methylbenzophenone (13), 2.4 ng 4-chlorobenzophenone (14), 0.3 ng xanthone (15) and 0.42 ng anthraquinone (16).

of the two photomultiplier tubes have indeed generated differences in response ratio for the sixteen components. These differences are not large. The question, however, is whether they are large enough to permit the Condac algorithm to succeed.

As the experiment clearly demonstrates, they are. Somewhat to our own surprise, however, it was even possible to obtain (from a single file, i.e. a single injection) the maximum possible complement of sixteen individual Condac chromatograms. Seven of these chromatograms are shown stacked up in the middle of Fig. 3.

It may be well to recall that, in the process of asking the computer to produce a Condac chromatogram [10], it is given a numerical, "true" response ratio together with the values for three quality parameters (thresholds). The first parameter defines the fraction by which the determined response (really: slope) ratio is allowed to differ from the "true" one; the second defines what percentage of

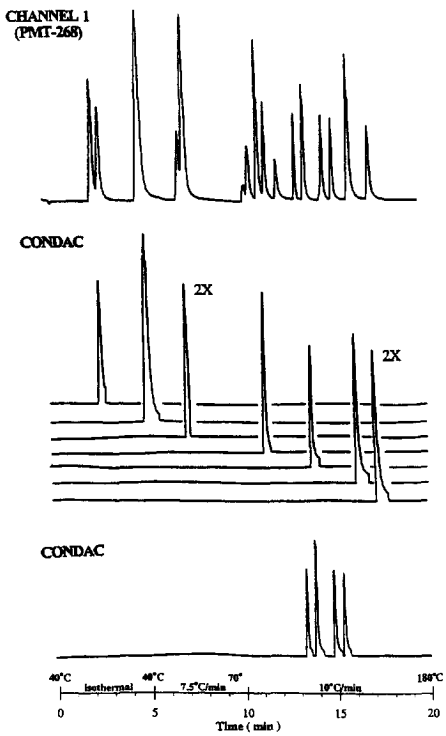

Fig. 3. Selected single-channel (top), single-Condac (middle), and group-Condac (bottom) chromatograms from one chromatographic separation. Conditions and compounds as in Fig. 2.

the 10-Hz data points within a Condac peak has to be accepted by the above criterion; and the third defines the minimum of time over which the other two conditions have to be met. The numerical values typically used to set these thresholds were 0.001, 90%, and 10 s, respectively.

It should also be noted that, in general, the narrow leeway established by the above threshold values must be broadened for real-life tasks. There, sample numbers and complexity, and chromatographic noise and overlap, are bound to increase. This and the required precise reproducibility of response ratios – required notwithstanding the passing of time and dirty samples – may impose a limit on how many single-compound Condac chromatograms can be obtained from one separation. Our tour-de-force of wrenching single-compound Condac chromatograms from a single-separation computer file for each and every one of the sixteen peaks may not have been achievable had the same mixture been dissolved in a complex sample matrix, had it been injected repeatedly, and had it been analyzed with pre-established, fixed values for the three algorithmic thresholds – all this in a filterless detector offering only a limited spread of response ratios (i.e. under conditions that could conceivably prevail in some over-ambitious analytical service project).

It is equally obvious, however, that if not most so at least many compound-specific chromatograms can indeed be obtained from a single file/separation with relative ease. (For purpose of analyte confirmation, the appearance in the Condac chromatogram of two or more compounds of similar response ratio but clearly different retention would hardly reduce the chromatogram's information content.) The measured response ratios of the 16-component mixture, at the particular setting of photomultiplier voltages used, varied from 0.430 for 1,4-naphthoquinone to 1.165 for xanthone. This is a fairly large range, particularly since the two photomultiplier cathodes provide the only venue of spectral discrimination in this filterless device. Many more compounds could easily fit into, and be safely differentiated within, this range.

Nevertheless, compounds of a very similar structure are more likely to possess very similar response ratios. An example is benzophenone and its 3,3′-bis(trifluoromethyl), 4-methyl, and 4-chloro derivatives. When the criterion of response ratio bandwidth

is suitably relaxed, the four benzophenones (with response ratios, at the prevailing supply voltages, of 0.840, 0.835, 0.845, and 0.816, respectively) appear together on the Condac chromatogram. This is illustrated in the bottom section of Fig. 3.

Similar "group Condacs" may be used to good avail: they indicate the probable structural similarity of peaks produced by an "unknown" sample. Given a two-dimensional data base of relative retention times and response-ratio windows, the identity of a peak may often be conveniently surmised (though rarely positively proven). The important point here is that such however scant information can be obtained in the picogram to nanogram range, whereas procuring the (more informative) whole spectrum requires far more sample and far more time.

The spread of response ratios obtained by two different, filterless photomultiplier tubes (though impressive in its own right) is nevertheless significantly narrower than the range accessible through even a single, simple filter. The example given in Fig. 4 shows chromatograms analogous to those of Fig. 2, but now derived from (nominally) identical photomultipliers (both R-268 tubes): one in an open (filterless) channel, the other in a channel fitted with a 530 nm longpass colored-glass filter [26]. Note that the open channel still preserves full spectral access. Yet it is obvious from the comparison of ordinates in Figs. 2 and 4 that the range of response ratios has significantly widened: Condac chromatograms are therefore much easier to obtain.

Further spectral optimization could – indeed, should – be carried out for analytical applications. Such optimization may, for instance, involve the use of two filters, possibly of the interference (as opposed to the colored-glass) type. These filters could yield a much more extended spread of response ratios and hence a much more rugged specificity for single (or spectrally related groups of) compounds. By narrowing spectral range they could, however, also diminish the number and sensitivity of potential analytes.

From the data obtained on the exacting test system of gas-phase aroyl luminescence, similar *compound*-specific behavior could be expected from, say, the UV–Vis absorption detector [20]. Likewise, *element*-specific behavior could be expected from, say, the flame photometric detector [10]. Furthermore, both

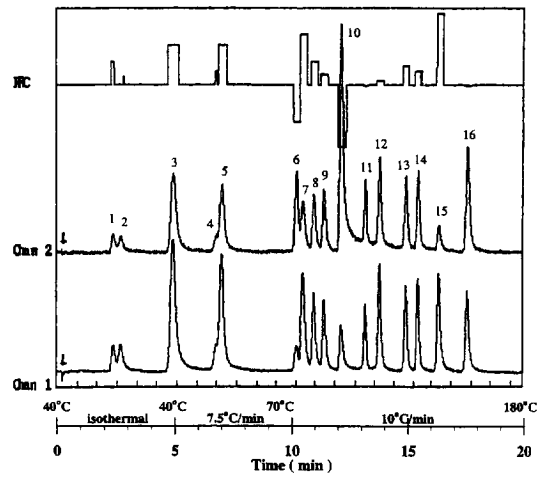

Fig. 4. Individual channels and slope-based, whole-peak response-ratio chromatogram of a mixture of aroyl compounds, comparable to Fig. 2. Channel 1: PMT-268 (no filter, −600 V, attenuation: 8000 ×); Channel 2: PMT-268 (530 nm longpass filter, −600 V, attenuation: 200 ×). Compounds in order of elution: 0.5 μl hexane (solvent), 1 ng benzaldehyde (1), 1.2 ng 4-fluorobenzaldehyde (2), 25 ng acetophenone (3), 4 ng 4-ethylbenzaldehyde (4), 16 ng 3-methylacetophenone (5), 80 ng 2,6-dichlorobenzaldehyde (6), 80 ng α-tetralone (7), 4 ng isophthalaldehyde (8), 4 ng methyl-4-formylbenzoate (9), 40 ng 1,4-naphthoquinone (10), 3.2 ng 3,3′-bis(trifluoromethyl)benzophenone (11), 3.2 ng 4-chlorobenzophenone (12), 2.4 ng 4-methylbenzophenone (13), 3.2 ng 4-chlorobenzophenone (14), 0.4 ng xanthone (15) and 0.56 ng anthraquinone (16).

types of specificity could be obtained in the absence of optical filters or dispersion devices. We therefore consider the titular supposition of this study to be sufficiently established. However, for reasons of analytical applicability we would like to buttress this basic methodology with some additional experimental evidence.

This evidence addresses the integrity of dual-channel response ratios. Clearly, the analytical practicality of using response ratios hinges on their constancy in the face of changing circumstances. In an earlier study, optical conditions had to be selected with great care so that the elemental response ratios in the dual-channel flame photometric detector would remain constant over an elemental concentration range that spanned several orders of magnitude – and that they would do so in the presence of hydrocarbons that severely quenched most analyte peaks [27].

Proving themselves even more rugged, the aroyl-luminescence response ratios of all sixteen test compounds remained invariant throughout and beyond their conventional calibration curves, in accordance with expectations based on the singularity of their excited states. The constancy of aroyl response ratios was indeed never compromised, despite the large number of probed concentrations. Typically these concentrations ranged from close to the $S/N_{p-p}=2$ detection limit (as marked by a star in Figs. 5 and 6) to well beyond the end of the linear range (as marked by an arrow). Fig. 5 documents this picture-perfect behavior for two *different* photomultipliers and no filter; Fig. 6 does the same for two *similar* photomultipliers, one of which resides behind a colored-glass window. In Figs. 5 and 6, straight and strictly horizontal lines have been drawn for some of the compounds. In order to avoid obscuring the graph, however, other compounds – which, though closely approaching each other, remained similarly on the straight and narrow – are represented here merely by the numbers that identify them.

The relative standard deviation of the response ratio for any given compound over the *whole* tested concentration range – i.e. over 3 to 5 orders of magnitude depending on compound sensitivity – is, on average, ±2.3% (lowest value 1.1%, highest value 5.6%). That puts it well within practical limits of Condac chromatography. Figs. 5 and 6 also offer a good pictorial representation of the ample response space still available to new compounds, even in the filterless system. As has been mentioned before, gas-phase phosphorescence spectra occupy a restricted, mostly common region on the wavelength scale. In this regard they differ from, say, typical flame-energized band spectra. The latter cover a much wider wavelength range with much less spectral overlap: they would hence be expected to expand even farther the response-ratio domain.

This – plus the additional analytical dimension provided by a chromatographic, i.e. largely resolved sample input – suggest that the filterless approach developed in this study can be used not just on the aroyl luminescence detector but on a variety of other "photometric" instruments and samples as well. Sometimes (e.g. in a destructive source such as a flame or plasma) the computer-mediated "specificity" will refer to single elements; sometimes (e.g. in a non-destructive source such as an absorption or, as

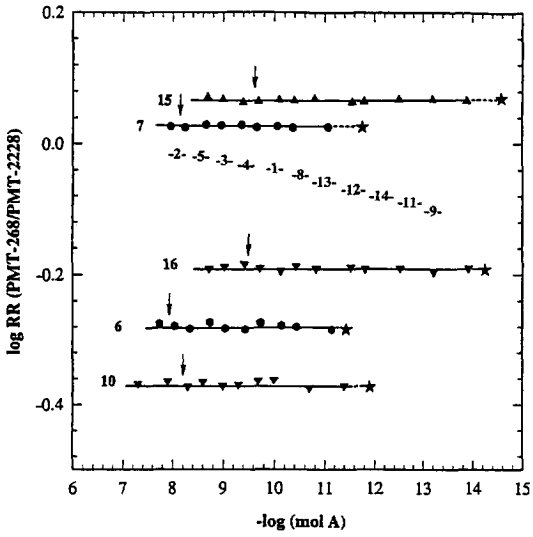

Fig. 5. Whole-peak response ratio vs. injected molar amount of 16 aroyl test compounds. First channel: PMT-268 (no filter, −850 V), second channel: PMT-2228 (no filter, −1000 V). Peak Nos. as in Fig. 2. Data points for compounds closely approaching each other are omitted for clarity. Arrow: upper end of linear range (−10% deviation); Star: minimum detectable amount ($S/N_{p-p}=2$).

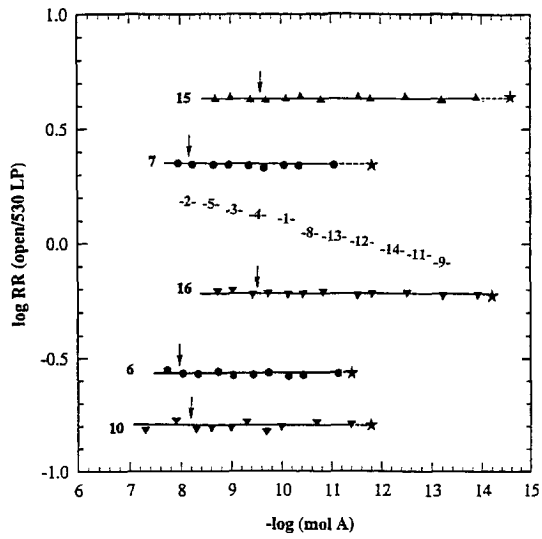

Fig. 6. Whole-peak response ratio vs. injected molar amount of 16 aroyl test compounds. First channel: PMT-268 (no filter, −850 V), second channel: PMT-268 (530 nm longpass filter, −850 V). Comparable to Fig. 5.

here, emission system) it will even refer to single compounds.

4. Conclusions

Simple dual-channel detector responses that relate to analyte structure, e.g. those based on compound-specific spectra, can be used to obtain virtual chromatograms that display only a single, selected compound from among a multitude of separated ones. Many single-peak (or structurally defined multiple-peak) Condac chromatograms can thus be obtained from a one-time separation stored in computer memory. While this study was carried out on the aroyl luminescence detector, other types of "photometric" detectors – and, even beyond these, detectors of other than a spectral nature – should be capable of similar single-compound (or single-group) specificity.

This study has also demonstrated that it is possible to obtain spectrally based, virtual separations from a detector of full wavelength range that employs neither optical filters nor dispersive devices. Obviously, however, the purpose of this demonstration was not to question the use of filters (which are some of the most helpful and inexpensive items in the chromatographic arsenal) but to draw attention to how small a spectral difference can be turned into how large an analytical advantage.

Acknowledgments

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