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Journal of Chromatography A, 1087 (2005) 124–130
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Optimisation of the selectivity of a pulsed flame photometric detector for unknown compound screening

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Available online 8 March 2005

Abstract

Identification of traces of chemical warfare agents (CWA) is generally performed with spectral methods such as mass spectrometry (MS) or NMR, but the use of element-specific detectors is most often required to extract interesting signals from gas chromatographic (GC) data heavily masked by natural interferents. The pulsed flame phosphorus detector (P-FPD) is able to detect phosphorus, sulphur and arsenic, and thus should be very well-suited to CWA detection.

However, first results using standard operating conditions recommended by the manufacturer of P-FPD led to false positive detection of phosphorus-containing compounds on the sulphur line. Therefore, an optimisation process of the selectivity of P-FPD for phosphorus versus sulphur or arsenic was undertaken, and allowed to identify gate delay and gate width as crucial parameters for the performance of P-FPD.

While selectivity could be significantly improved, unexpectedly, this resulted in a concurrent important loss of sensitivity (ca. 45%) for arsenic, which suggests that this detector should be carefully optimised with respect, and prior to its purported use.

Keywords: Pulsed flame photometric detection; Experimental design; Selectivity; Sensitivity; Optimisation

1. Introduction

1.1. Analysis of chemical warfare agents: context and specificities

The analysis of environmental or biological samples eventually contaminated by traces of chemical warfare agents (CWAs), especially in the context of chemical disarmament verification created by the Chemical Weapons Convention (CWC) [1], is a particularly complex analytical task. Indeed, while most analytical laboratories focus on specific matrices and/or families of chemicals, expertise in the field chemical warfare analysis requires unique capabilities, to ensure detection at minute levels of several millions of chemicals. These chemicals are not only those listed by the schedules of the CWC, but also products emerging from their degradation by various pathways. This includes very volatile to persistent agents, very polar to apolar compounds, etc., which in turn demand the use of multiple analytical methods and devices [2].

The provisions of the CWC clearly also include two important specificities, which also differs from many other analytical issues such as environmental or occupational health controls:

- qualitative analysis is sufficient for verification but false positives are completely unacceptable;
- the reference limit of detection for expert laboratories in the field is 1 ppm, which is relatively far above theoretical limits of detection of modern apparatus.

1.2. Use of gas chromatograph (GC) specific detectors for CWA analysis

Most chemical warfare agents contain characteristic heteroelements in their structure, such as phosphorus, sulphur, arsenic and/or nitrogen. Therefore, preliminary screening of organic liquid samples or extracts relies on GC hyphenated to element-specific detectors such as flame photometric detection (FPD, specific for phosphorus and sulphur) [3], nitrogen-phosphorus detector (NPD or thermoionic detector) [4], or atomic emission detector (AED) [5]. Afterwards,

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doi:10.1016/j.chroma.2005.02.045

unambiguous identification of chemical warfare agents is most often performed by means of spectrometric techniques (GC–mass spectrometry (MS), LC–MS, NMR, GC-FTIR) [6,7].

It must be stressed that information gathered by preliminary GC screening drastically reduces the time needed to search GC–MS chromatograms for relevant chemicals. Moreover, appropriate specific detection usually allows to filter much noise generated by environmental pollutants or contaminants commonly found on military fields (polymers, lubricants, fuels, explosives, . . .) [8].

As a consequence of the two specific items listed above, utmost selectivity is more important than sensitivity, since false positive detection of a heteroatom could lead to misinterpret spectral data and eventually to false positive identification of a chemical.

1.3. The pulsed flame photometric detector (P-PFD) [9]

A decade ago, Amirav and Jing [10] reported the development of a new specific detector, modified from the FPD, and designated as the "pulsed flame photometric detector". This detector allows selective detection not only for phosphorus and sulphur, but also for nitrogen, arsenic, and a variety of metals (Sn, Se, Ge, Ga, Cu, . . .) among others [11], and, as FPD, can be adapted and deployed on the field for real-time detection purposes [12]. Briefly, P-FPD exploits the time-dependence of the photon emission process during combustion of the compounds in the flame, which is not continuous but lighted on and off ca. every 300 ms; the flame pulse profile-over time is dependent on the element in the analytes that are burnt, and thus the signal may be selectively associated to an element.

The sensitivity of P-FPD confers to this detector a significantly higher power than that of FPD for trace level detection [11], especially when used in combination with mass spectrometry [13], and may account for the choice of this detector in novel method development for sulphur- [14,15] or phosphorus-containing compounds [16]. This property may be very important for applications in environmental control around chemical weapons destruction facilities, where utmost sensitivity is required [17].

Moreover, P-FPD additional capacity of selectively detecting organoarsenicals represents a substantial advantage versus older FPD for application to chemical warfare agent detection [18,19]. In the laboratory, the P-FPD (which is currently marketed by two companies) also represents a valuable alternative to the AED (whose development has been resumed by Agilent and may not represent a sustainable solution for multiple element detection) for nitrogen and especially arsenic detection.

1.4. P-FPD implementation for CWA analysis

On the basis of such promising applications in our field, and with respect to the improvement that GC–P-FPD instruments could represent towards classical GC–FPDs, it seemed important to determine the optimal way of operating the P-FPD in the context of screening of unknown chemicals, before replacing old FPDs that already offer great security in analysis.

As stated before, analysis for CWC verification purposes is performed blinded, and there is no information about the type and concentration of the chemicals that may be present in the sample. Therefore, P-FPD cannot be operated as a detector dedicated to a single element, and one has to be certain that signals reported as phosphorus (for instance) are not caused by interferences from other elements (e.g. sulphur), i.e. selectivity needs to be maximal. However, this should not be to the detriment of sensitivity of the detector for elements of interest, which would decrease the specific interest of P-FPD versus FPD. Interestingly, while much data is available on heteroatom versus carbon selectivity of P-FPD, little, if any, has been reported on inter-heteroatom selectivity.

Therefore, we report in the following an optimisation strategy using experimental design [20] which was set up to optimise the selectivity of P-FPD. Seven parameters that could influence the response of the detector were selected as variables in the experimental design, which allowed to select the parameters of greatest influence; next, optimal values for these parameters were determined with the Multisimplex algorithm. Indeed, two optimisation procedures were set up, aiming at:

a) the optimisation of P-FPD response for arsenic in order to minimise phosphorus and sulphur response;
b) the optimisation of P-FPD response for phosphorus in order to minimise arsenic and sulphur response.

During this process, we also measured the influence of the selectivity optimisation on P-FPD phosphorus, sulphur and arsenic sensitivity.

2. Experimental

2.1. Chemicals and reagents

All experiments reported in the following study were performed with a mixture of five reference chemicals. This mixture contained dimethyl methylphosphonate (DMMP, 15 $\mu g\,mL^{-1}$), tributyl phosphate (TBP, 15 $\mu g\,mL^{-1}$), malathion (15 $\mu g\,mL^{-1}$), dibenzothiophene (DBT, 15 $\mu g\,mL^{-1}$) and triphenylarsine (TPA, 15 $\mu g\,mL^{-1}$) dissolved in ethyl acetate (Pestinorm grade, Baker). All chemicals were from Sigma-Aldrich, Saint-Quentin Fallavier, France.

2.2. Equipment

All analysis were performed with a Varian 3800 gas chromatograph equipped with a pulsed flame photometric detector (P-FPD)(Varian, Les Ulis, France). The 30 m × 0.32 mm

Table 1
Variation of parameters during P/(As + S) selectivity optimisation

Trial	Gate delay (ms)	Gate width (ms)	H_2 flow (mL min^{-1})	Response function
1	4	10	13	0.9692
2	6	20	13	0.9439
3	10	5	20	0.8908
4	18.3	9.1	16.6	0.7450
6	12.5	10.4	16	0.8279
8	8.8	9.2	17	0.8976
10	9.4	11.7	15.2	0.8886
11	4.1	18.6	10.5	0.9686

i.d. capillary column was coated with 0.25 μm of CP-Sil 8CB (Varian, Les Ulis, France). Automatic injections were realised using a Varian 8200 autosampler (Varian, Les Ulis, France). The column was maintained 1 min at 50 °C, then heated to 260 °C at 10 °C/min where it was held during 8 min. Total program time was 30 min. The carrier gas was helium (99.999% purity, Air Liquide, Grigny, France).

The starting experimental conditions used for P-FPD were those recommended by Varian for arsenic detection in the user manual for the detector (Varian Analytical Instruments, USA) (see Table 2).

2.3. Design of the optimisation method

P-FPD is able to detect up to 28 elements. Unfortunately, emission spectrum of one element frequently overlaps emission spectra of the others. The first possible procedure to increase selectivity of P-FPD was described by Amirav and Jing [10]. The process utilises the dual gate subtraction method, which is included in the P-FPD operating software (Varian, Inc). The software records two chromatograms (A and B) with two different gate parameters (delay and width) theoretically corresponding to two elements. Coefficients α_1, α_2, β_1 and β_2 are empirically determined in formula $\alpha_1 A - \beta_1 B$ and $\beta_2 B - \alpha_2 A$ so that the chromatogram B does not show the element detected in the chromatogram A and conversely. Unfortunately, α and β coefficients depends on the analysed product. Therefore, with samples containing many compounds, it is often impossible to obtain a complete separation, and this method proves very difficult to set up, and may even reveal inappropriate for screening procedures.

2.4. Experimental design and Multisimplex algorithm

In this work, we preferred to optimise P-FPD parameters with a statistical method, hopefully and apparently leading to better results than the dual subtraction method. Therefore, this work was done by an experimental design procedure, completed by a statistical optimisation base on the MultiSimplex Algorithm (Version 2.1.1., Logi Labo, Paris, France), adapted from previously described work on P-FPD optimisation [22]. The response functions chosen were, 1 − ((DMMP area + TBP area + DBT area + malathion area)/TPA area) for arsenic and 1 − ((DBT area + TPA area)/(DMMP area + TBP area)) for phosphorus. The target of the algorithm was to minimise the second part of these two functions (in parenthesis), i.e. to increase selectivity towards 100% (response value equal to 1).

3. Results and discussion

3.1. Experimental design and parameters of greater influence

The use of experimental design in the course of method development and/or validation is now widely documented in scientific literature. This strategy was already used for P-FPD optimisation, for organotin [21] or organoarsenical [22] detection.

The choice of variable parameters was based on indications by P-FPD manufacturer, and on the results obtained by Jing and Amirav when developing the system [11]. Amirav reports that the composition of the flame is most important for the combustion process, and therefore for the emitting species formed in the detector. The major influence of combustion gas flow that was observed during the experimental design process shall be strongly related to this report, and was also described by others as determinant for P-FPD sensitivity towards tin element [21].

Beyond combustion gas, gate width and gate delay also came out of the process as essential parameters for selectivity and sensitivity of the P-FPD. The influence of temporal variables that appears in the optimisation process is also closely related both to former hypotheses and to the principle of the detector, where not only wavelength and intensity of emitted photons, but duration and shape of the emission profile are used for spectral interpretation and element identification.

Conversely, parameters linked to the chromatographic process, such as mobile phase flow and injector temperature did not seem to influence the detection process. Neither did detector temperature and combustor diameter, which may indicate that, in the range studied for these parameters, combustion proceeds completely and efficiently, thus giving a constantly maximal emission from burnt species.

3.2. Optimisation of sensitivity for phosphorus and loss in P/S selectivity

Starting parameters were the standard ones indicated by the manufacturer of the system for combustion gas flow, gate delay and gate width, completed by empirical values, as required by the algorithm in the initial step. As expected, sensitivity could be significantly increased from the standard value, but it is likely that the final optimised parameters are dependent from individual instruments, e.g. linked to unique modifications due to the manufacturing process. Unfortunately, the use of these parameters for screening for P-, S- and As-containing chemicals among unknown chemicals re-

vealed inconsistencies with the results yielded by a Varian 3400 FPD used in parallel with a phosphorus filter. These inconsistencies finally revealed that optimisation has resulted into a severe loss of P/S selectivity of P-FPD, thus leading to detection of sulphur-containing chemicals on the phosphorus line. Such a result can reasonably be considered a false positive detection, which is, as stated before, especially penalising in the context of CWC verification.

Examination of the literature on P-FPD reveals that inter-heteroatom selectivity has already been identified as a potential pitfall of this detector, which is due to partially overlapping emission profiles of several elements detected by the P-FPD [10,11]. An illustration of this phenomenon is given by Bravo et al. [23], who observed interference between emission of Sn- and S-containing species, and hypothesise that tin could also be concerned by insufficient selectivity, in P-FPD detection, towards phosphorus.

Indeed, the use of specific combinations of filters, recommended by the P-FPD designers [11] may partly solve this problem, but this is not compatible with efficient and timely screening of several elements in complex mixtures, which is for instance a strength of the concurrent atomic emission detector.

Killilea and Aldstadt [22], when studying organoarsenical speciation, also report on potential confusion between As- on the one hand, and S-, OH- and C-emitting species on the other hand; these authors however describe a suitable method for overcoming these problems by a convenient combination of gate parameters for signal processing. Therefore, the optimisation procedure was restarted, but the optimised parameter was changed to P/(As + S) and As/(P + S) selectivity instead of single atom sensitivity.

3.3. Optimisation of inter-heteroatom selectivity

3.3.1. General

The detailed optimisation results are presented on Figs. 1 and 2. On both figures, the curve at the top describes the progression of the optimised parameter towards a maximum value of 1, while superimposed chromatograms show significant evolutions of detection during the process. Selectivity for one element (e.g. As) was quantified as the ratio between peak areas corresponding to analytes containing this element and peak areas of other analytes. Malathion, which contains both P and S, was not taken into account for P/(As + S) optimisation. This results in the following formulae:

$$\frac{\mathrm{P}}{\mathrm{As}+\mathrm{S}} = 1 - \left(\frac{(\mathrm{DBT\ area} + \mathrm{TPA\ area})}{(\mathrm{DMMP\ area} + \mathrm{TBP\ area})} \right)$$

$$\frac{\mathrm{As}}{\mathrm{P}+\mathrm{S}} = 1 - \left(\frac{\mathrm{DMMP\ area} + \mathrm{TBP\ area} + \mathrm{DBT\ area} + \mathrm{malathion\ area}}{\mathrm{TPA\ area}} \right)$$

Table 2
Variation of parameters during As/(P + S) selectivity optimisation

Trial	Gate delay (ms)	Gate width (ms)	H_2 flow (mL min^{-1})	Response function
1	6	20	13	−2.5117
2	10	5	20	−1.2264
3	18.3	9.1	17.0	0.7087
4	16	3	24	0.077
6	10.4	12.9	16.7	−0.3164
7	19.8	11.7	18.5	0.7596
10	14.2	10.4	18.3	0.4754
11	18.9	17.8	11.9	0.8487

3.3.2. Phosphorus versus sulphur and arsenic

In a first series of experiments, selectivity for phosphorus versus sulphur and arsenic was optimised. Table 1 presents the starting and final parameters associated with this process.

Interestingly, when using standard gate and combustion gas values given by the manufacturer of our instrument, artefacts due to sulphur interference were observed on the phosphorus line at the retention time of dibenzothiophene. Conversely, triphenylarsine gave no detectable signal. which confirms that our observations are not due to carbon interference, since triphenylarsine, despite carbon-rich (18 atoms per molecule) does not give rise to artefacts. It also suggests that standard parameters confer satisfactory P/As selectivity: actually, total P/(As + S) selectivity is close to 0.97 at the start of the optimisation. Fig. 1 evidences that the optimisation algorithm does not afford any significant progression, with a total selectivity hardly reaching the original value, and dibenzothiophene artefact being reduced almost by the same factor than true phosphorus response.

3.3.3. Arsenic versus sulphur and phosphorus

A second experiment was dedicated to selectivity for arsenic versus sulphur and phosphorus. Astonishingly, standard operating parameters gave good sensitivity, but almost no selectivity for arsenic (As/(P + S) selectivity as determined in our model was negative!). This time, the optimisation process revealed extremely efficient, and rewarded an much better selectivity (As/(P + S) = 0.85), keeping in mind that arsenic and sulphur signals are described by Jing and Amirav [11] to be particularly difficult to isolate, which may explain the difficulty to overcome this threshold. Unfortunately, this entailed a decrease in the triphenylarsine peak area, illustrating sensitivity for arsenic, of ca. 45% (Fig. 2).

The examination of the gate parameters obtained at the end of the optimisation process may bring out an explanation to this result. Indeed, the emission of arsenic is maximal after a delay of ca. 15 ms, and is prolonged for several ms. The optimised gate delay is 18.9 ms: this is necessary to suppress all overlap with phosphorus and/or sulphur emission, but also means that most of the signal corresponding to arsenic emis-

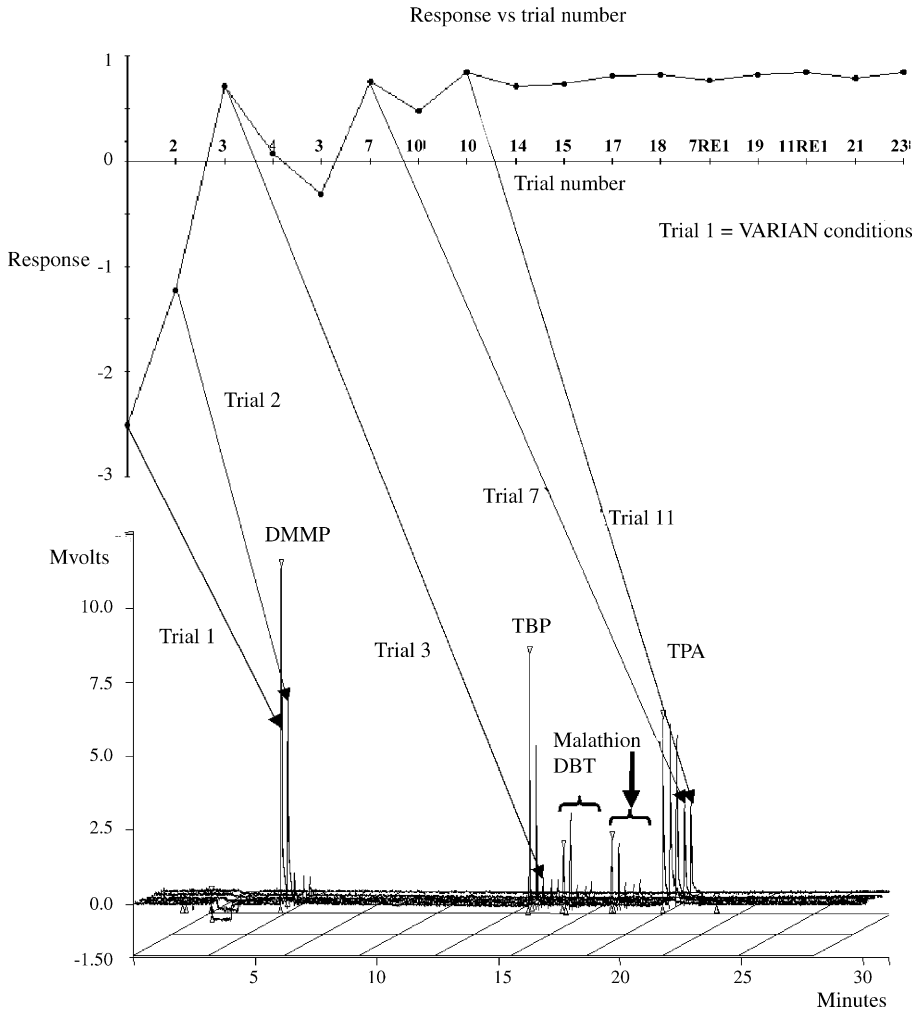

Fig. 1. Evolution of P/(S + As) selectivity (see text for details) during the optimisation steps, and corresponding P-FPD profile of a test mixture containing P-, S- and As-containing chemicals (M = malathion. Circles indicate the five iterations of the algorithm illustrated by a chromatogram).

sion in the flame is not recorded. This is probably responsible for the concomitant drop in sensitivity observed at the end of the optimisation process.

It may be worth mentioning that the final parameters for maximal As selectivity were used in our laboratory for various samples. In all cases no interfering signal was detected on arsenic chromatograms, which suggests the robustness of these parameters towards nature of sample and chromatographic conditions.

3.4. Additional comments and discussion

Very little is described in the literature on detector parameters, which are never (to our knowledge) detailed in Section 2. Therefore, it is difficult to discuss our observations on manufacturer parameters at the light of others' experience, all the more since it is doubtless that best parameters depend on single instruments. However, it should be valuable to report P-FPD operating parameters in further studies.

Moreover, in most P-FPD development reports such as [10,11], selectivity is presented mainly as a requisite for accuracy in quantification (insufficient selectivity results in addition or quenching of signals from different elements for a single analyte), but is not really discussed as a problem when dealing with completely unknown analytes; therefore, pertinent information relative to the issues raised here is quite limited.

These observations, as well as other points noticed during our first-hand experiments on the P-FPD, such as large discrepancies in sensitivity according to the analyte (sensitivity for phosphorus in DMMP and TEP is nearly three times that for phosphorus in malathion), require further experiments to ascertain eventual limitations to the use of P-FPD for un-

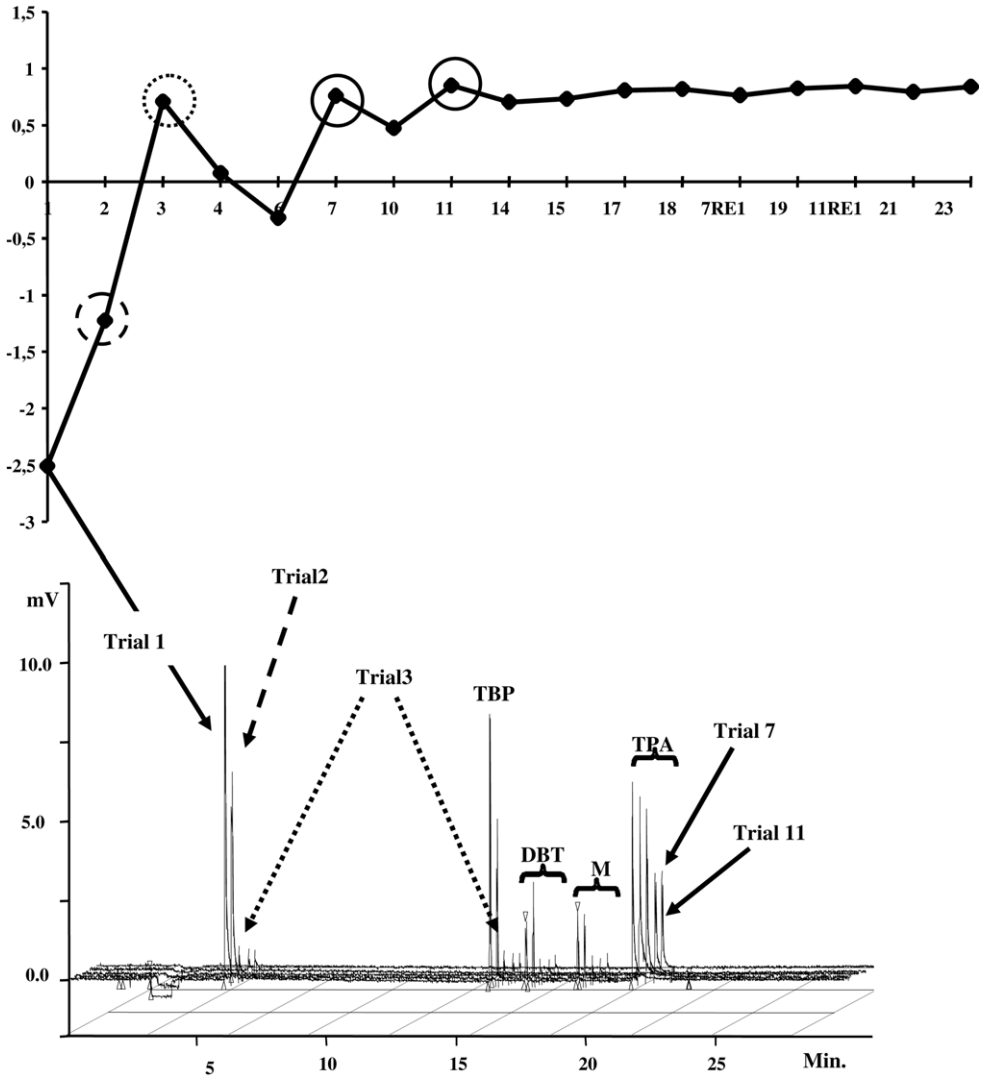

Fig. 2. Evolution of As/(S + P) selectivity (see text for details) during the optimisation steps, and corresponding P-FPD profile of a test mixture containing P-, S- and As-containing chemicals (M = malathion. Circles indicate the five iterations of the algorithm illustrated by a chromatogram).

known sample screening, where confidence towards the detector needs to be unquestionable.

4. Conclusion

The results presented here both confirm that the pulsed flame photometric detector is highly sensitive and versatile, and that it requires great care when optimising its capabilities, especially for qualitative analyses of unknown chemicals.

Indeed, it seems necessary to determine which parameter is most important to the purported P-FPD analysis before starting an optimisation process such as the ones described here. In particular, careless maximisation of the sensitivity towards a single element may result in false positive detections, due to selectivity decrease; conversely, selectivity optimisation appears as detrimental to sensitivity. It is important to stress that, as suggested previously by others, subtle variations in parameters of the detector such as gate delay and gate width strongly influence signal emission, acquisition and processing.

Finding suitable values (which are likely to be unique for each instrument) for these parameters is required to avoid tedious and time consuming use of multiple filters or photomultipliers, which would decrease the specific advantages of P-FPD versus other commercial GC detectors. Therefore, ongoing experiments combining use of different filters and detector parameters aim now at finding an optimal equilibrium between number of runs, sensitivity, and indisputable inter-heteroatom selectivity, to use with a greatest benefit the potential of P-FPD for the screening of complex mixtures eventually containing traces of chemicals related to the Chemical Weapons Convention.

Acknowledgements

We would like to acknowledge Mr. Aviv Amirav for answers to our numerous questions concerning PFPD and Mrs. Claudette Morand for excellent technical assistance during this study.

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