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A new approach to qualitative analysis of organophosphorus pesticide residues in cucumber using a double gas chromatographic system: GC-pulsed-flame photometry and retention time locking GC–mass spectrometry

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Abstract

A qualitative method for the screening of organophosphorus pesticides (OPs) that could present in different types of vegetables has been established and validated. A typical multi-residue extraction procedure of OPs using ethyl acetate and sodium sulphate has been applied. No clean-up was required after extraction, and concentrated extracts were analysed by gas chromatography with pulsed-flame photometric detection (GC–PFPD). Confirmation of compound identities was performed by gas chromatography with mass spectrometric detection (GC–MSD) in the electron impact (EI) mode with full scan acquisition. Retention time locking (RTL) software was used in order to improve the method capability of identification and confirmation. Spiked samples at pesticide concentrations equal to the maximum residue level (MRL) were used to check chromatographic performance and for validation studies. The proposed method allows a rapid and accurate identification of the studied OPs until the ng ml^{-1} range for those whose use is forbidden, and above their MRL concentration for the rest.

Keywords: Pesticides; Screening; Qualitative analysis

1. Introduction

Organophosphorus pesticides (OPs) are applied worldwide for protection of a wide variety of crops and foodstuffs. Public concern over pesticide residues has risen notably during the last decade and, this fact has put pressure on regulatory

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agencies and private laboratories, becoming a significant food issue.

An accurate determination of pesticide residues in fruits, vegetables and related matrices is certainly of great importance in some areas of food analysis. However, in the case of routine quality control laboratories, this determination becomes unnecessary since the only information usually required is when the concentration of a particular compound is over or under a regulated limit named maximum residue levels (MRLs). In such a case, laboratories could be interested in a fast binary "yes/no" answer about the MRL avoiding the time-consuming and tedious procedure that has to be followed to perform a quantitative analysis. These are the cases when qualitative methods become special relevance [1].

Obviously, qualitative methods do not provide the same amount of information as quantitative methods but, in spite of this, some advantages make them suitable for the screening of the two groups of compounds that the analyst normally has to control in a laboratory dedicated to the pesticide residue analysis: (i) those with a regulated limit (tolerated at MRL); and (ii) those that are unauthorised ("zero tolerance") [2]. Some of these advantages are especially useful in routine laboratories, such as the avoidance of continuous recalibrations, a higher sample throughput, the achievement of a much quicker binary response to the analytical problem, the increase of sample analysis speed due to the lesser use of the GC–MSD system, the reduction of the maintenance operations in order to achieve a satisfactory instrumental response and the easier establishment of quality control programs based on control charts as long as fewer number of concentrations are under study.

This paper shows the strategy followed by the authors for the establishment of a qualitative method for the fast pesticide residue screening of 20 OPs in samples of cucumber that can be extended to other real matrices.

A previous extraction of the compounds of interest from the vegetable matrix has been performed using ethyl acetate and sodium sulphate as described by Cai et al. [3] and recommended by the Pesticide Analytical Manual [4] for the specific extraction of the OP metamidophos. The clean-up step has been eliminated for the sake of velocity due to the little benefit that can be obtained from its use when clean extracts of cucumber are under study. Besides, as pointed out by Obana et al. [5], when extraction methodologies are employed to separate compounds with different properties, the selection of an appropriate column becomes extremely hard due to the implicit risk of irreversible losing of some of the analytes of interest. In this sense, and compared with acetone or acetonitrile, ethyl acetate is a fairly suitable solvent for our purposes as long as water-soluble compounds from the matrix are hardly co-extracted with the OPs.

The screening method is based on the use of gas chromatography with pulsed-flame photometric detection (GC–PFPD) whose usefulness for these kind of analysis has been proved throughout the years and is described elsewhere [6,7]. GC–MSD in the electron impact mode (EI) with full scan acquisition in conjunction with retention time locking (RTL) software [8], provided by the supplier (RTL-GC–MSD), has been used for identification and confirmation [9] of the doubtful compounds.

A brief description of the main characteristics of both pulsed-flame photometric detector (PFPD) and RTL software will be considered below.

1.1. Pulsed-frame photometric detection

The ideal detector for qualitative pesticide analysis must be both highly selective and sensitive for the target components. Selective to avoid problems with noise, ghost peaks and overlapping peaks, and sensitive to allow a reasonable detection limit for those analytes whose use is forbidden by the authorities. The PFPD accomplishes all these premises. It is an excellent detector for the analysis of OPs due to its well-known selectivity, high sensitivity and linearity of response [10].

Operation of PFPD is described elsewhere [11]. Basically, a propagating flame, which terminates within a glass combustor, produces gas-phase reactions with the entering analytes resulting in light emissions with specific luminescence (gas chemiluminescence) spectrum and lifetimes. In

addition, the use of gated electronics permits the rejection of noise occurring outside of a specific gate window, which further improves the detectivity of the PFPD.

The dual output channel of the PFPD also allows the simultaneous collection of two signals depending on the filters used by the experimenter. In our case, phosphorous and sulphur chromatograms were obtained for each particular analysis improving notably the confirmation capability of the method due to the fact that the majority of OPs also contain sulphur.

1.2. RTL software

RT is the fundamental qualitative measurement in gas chromatography. However, the experimenter has to deal constantly with shifts in these RTs that are constantly observed in usual practice. This fact makes mandatory the re-adjustment in the values of RT for the analytes of interest every now and then. Routine maintenance procedures such as column trimming, or sometimes the establishment of inappropriate maintenance programmes, may alter RTs even when identical conditions are employed for two analyses. Similar features are observed when identical methods are used in different instruments. Even when these instruments are of the same model, RTs differ due to a high number of reasons (variables) that are often off the experimenter control.

In this sense, the RTL software appears as a very useful tool for the analytical chemist who deals with these problems. RTL is the ability to very closely match RTs when maintenance is applied or when different instruments are used. The only condition to be respected is the use of the same nominal column and operating in the constant pressure mode [12]. The RTL software determines the pressure that is necessary in the inlet to correct all the differences that may appear between identically configured GC systems.

Trying to identify and correct all the variables that may be responsible for these shifts would be time-consuming and probably extremely hard. To solve this apparently tricky problem, the RTL software performs a calibration of five runs once the experimenter has selected a target compound, as follows:

1st run → nominal method pressure (P).
2nd run → $P+20\%$.
3rd run → $P+10\%$.
4th run → $P-20\%$.
5th run → $P-10\%$.

Except for the inlet pressure, the runs are made in identical chromatographic conditions. The calibration permits maintaining in the end a typical RT for the target compound that remains unchanged throughout the experiments and time. It is obvious that this fact brings about an extraordinary saving of time and effort if we just consider that 40 or 50 may be an average number of analytes in a typical environmental laboratory that uses GC methods.

2. Experimental

2.1. Chemicals

Ethyl acetate and acetone (pesticide residue analysis grade) were obtained from Scharlau Chemie (Barcelona, Spain). All pesticide standards of the highest available purity were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Each compound was dissolved in acetone to make 50 ml of stock standard solution of approximately 200 mg l^{-1}. These stock standard solutions were stored in refrigerator at a temperature below zero until use. The freshly working standard solutions were obtained by dilution with clean extracts of pesticide-free samples.

2.2. Gas chromatography equipment

A tandem of two gas chromatographs was used.

An Agilent Technologies, Inc. (Wilmington, DE) model 6890A gas chromatograph equipped with a 7673A autosampler, a split/splitless injector and a J&W Scientific DB-1701 column (30 m × 0.25 mm ID, film thickness 0.25 μm; 14% cyanopropylphenyl, 86% dimethylsiloxane), which was coupled to an OI Analytical (College Station, TX)

model 5380 pulsed-flame photometric detector. An Agilent 35900E interface is used in order to control the PFPD functioning from the Agilent Chemstation software.

An Agilent model 6890A gas chromatograph equipped with a Gerstel GmbH & Co. (Mülheim an der Ruhr, Germany) CIS-4plus injector and a J&W Sientific HP-5MS column (30 m × 0.25 mm ID, film thickness 0.25 μm; 5% diphenyl, 95% dimethylsiloxane) was coupled to an Agilent 5973N quadrupole mass spectrometer detector. The GC–MSD was equipped with the RTL pesticide library and RT database.

The GC–PFPD operating conditions were: helium carrier gas (constant flow) 1.3 ml min^{-1}; injector temperature 260 °C; detector temperature 280 °C; temperature programme 60 °C for 2 min, 6 °C min^{-1} to 120 °C, 120 °C for 3 min, 8 °C min^{-1} to 280 °C, 280 °C for 15 min; detector flows hydrogen 10.5 ml min^{-1}, air 10.5 ml min^{-1} and make-up gas 11 ml min^{-1}.

The RTL-GC–MSD operating conditions were: helium carrier gas (constant pressure) 22.04 psi (approximate as long as it depends on the results of the RTL experiment); injector temperature programme 200 °C for 0.25 min, 12 °C s^{-1} to 300 °C; transfer line heater temperature 280 °C; temperature programme 70 °C for 2 min, 25 °C min^{-1} to 150 °C, 3 °C min^{-1} to 200 °C, 8 °C min^{-1} to 280 °C, 280 °C for 10 min; MS quadrupole temperature 150 °C and MS source temperature 230 °C. The m/z ranged from 35 to 500.

2.3. Sample preparation

Samples of cucumber, obtained directly from our factory, were prepared with a conventional vegetable processing procedure for pesticide residue analysis. No clean-up step was applied.

15 g from each chopped sample was weighed and mixed with 20 g of anhydrous sodium sulphate and 50 ml of ethyl acetate for a first extraction. The mixture was homogenised with a polytron for 1 min, centrifuged for 5 min at 4500 rpm (3346 × g), and the supernatant liquid filtered through a layer of 5 g of anhydrous sodium sulphate. To the remaining solid was added another 30 ml of ethyl acetate, the process being repeated for a second extraction step. Both extracts were collected together in a round-bottomed flask and finally the solvent is removed under vacuum at 45 °C in a rotary evaporator until almost dryness. The residue was re-dissolved with 5 ml of acetone.

The clean matrix samples (free of pesticides) used for standard spiking were obtained from old samples previously analysed in our laboratory and stored frozen in our refrigerators.

2.4. GC–PFPD screening test

The screening test was based on our will of controlling the possibilities of committing type I (false-positive) and II (false-negative) errors at the same time when stating that an analysis performed to an unknown sample is negative or non-negative. To do so, an interval was developed around some replicated measurements (in our case–control solutions in which the analytes were at the concentrations of the MRLs) whose formulation was directly borrowed from the literature [17]. The interval size was directly dependent on the precision of the measurements, as well as on the values of type I and II errors that were selected as admissible for our purposes. These limits in the intervals (screening limits) also depend on the precision of the overall experiment by means of the introduction of a standard deviation in its formulation. It was evident that the screening process would have been senseless if the interval became so wide that most of the instrumental responses corresponding to unknown samples were between its limits. Hence, it was necessary to fix the values of type I and II errors in such a way that it permitted the fulfilment of a double objective: providing a fairly good control of the error and accomplishing with the screening goal stated in the method in a reasonable manner. The formulation, values of error and overall procedure that were used in our case are summarised below.

Once the screening limits (upper and lower) were established, the instrumental responses corresponding to unknown samples were directly compared with them. The comparison offers three possibilities as follows:

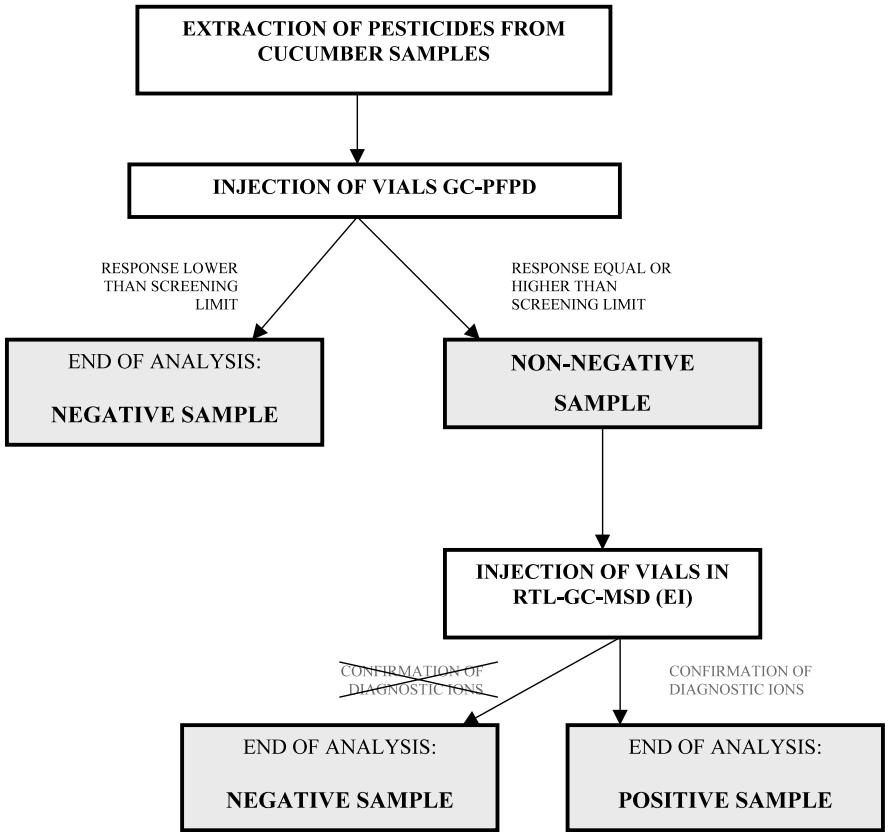


Fig. 1. Method flow chart.

a) the instrumental response of the unknown sample may be lower than the lower screening limit,
b) the instrumental response of the unknown sample may be between the values of (or equal to) the lower and upper screening limits, and
c) the instrumental response of the unknown sample may be higher than the higher screening limit.

Case (a) would mean the end of the analysis. In such a case, the unknown sample would be labelled as negative because the peak area found for the suspect compound is off the interval established to control the concentrations established in the law (MRLs). Contrarily, cases (b) and (c) would label the samples as non-negatives and direct them to confirmation by RTL-GC–MSD (EI).

Fig. 1 shows the flow chart that summarises the whole process of extraction, screening and further confirmation.

An internal quality control (IQC) program was implemented in order to monitor both chromatographic features (injection volume, carrier gas flow rate, column performance and detector performance) and analytical parameters (peak-time retention, peak area and precision). IQC procedures are based on the use of control samples and calibration standards, which are included in the analytical batch and treated in the same way as the test samples. Because no studies are available on

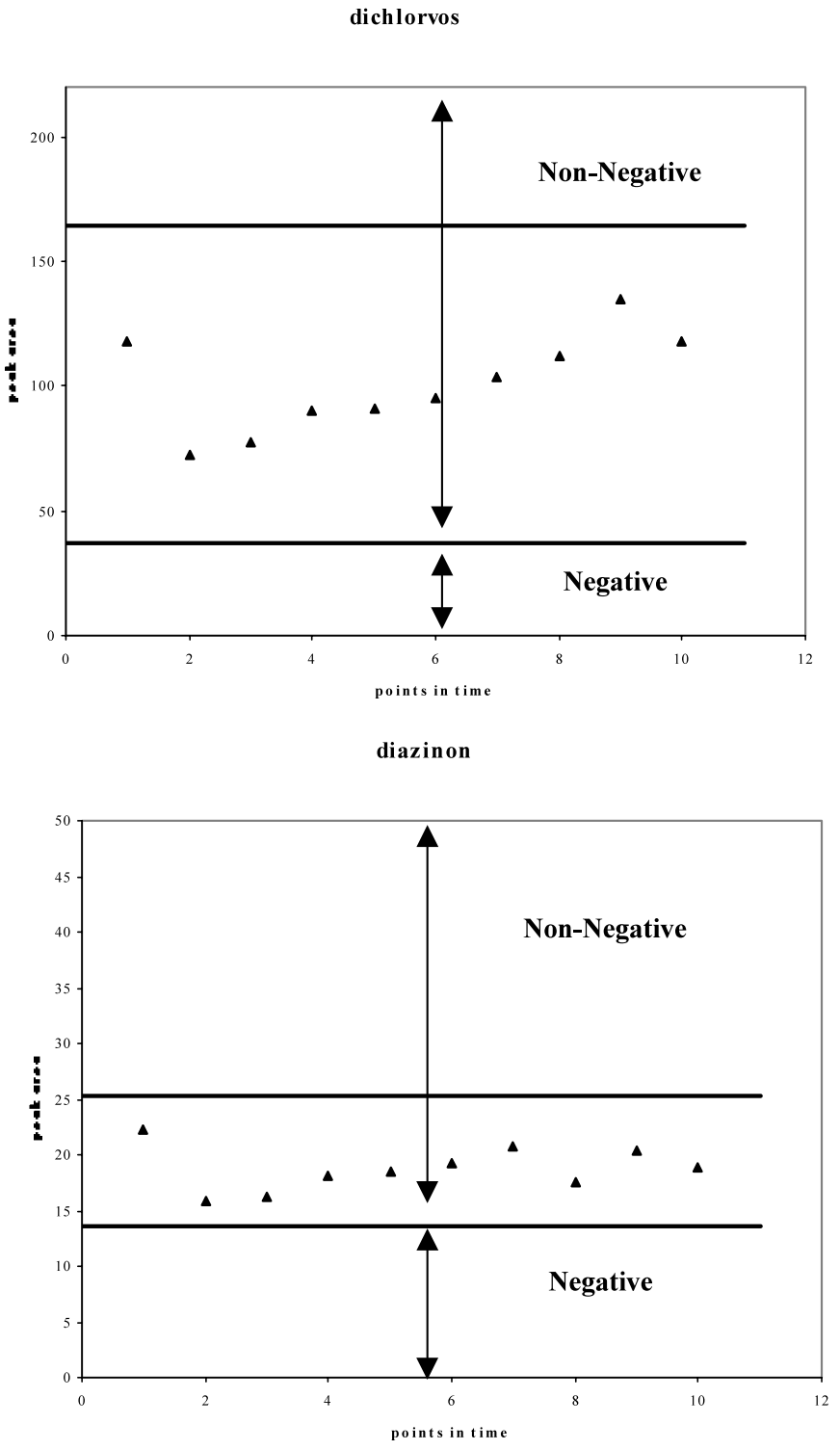
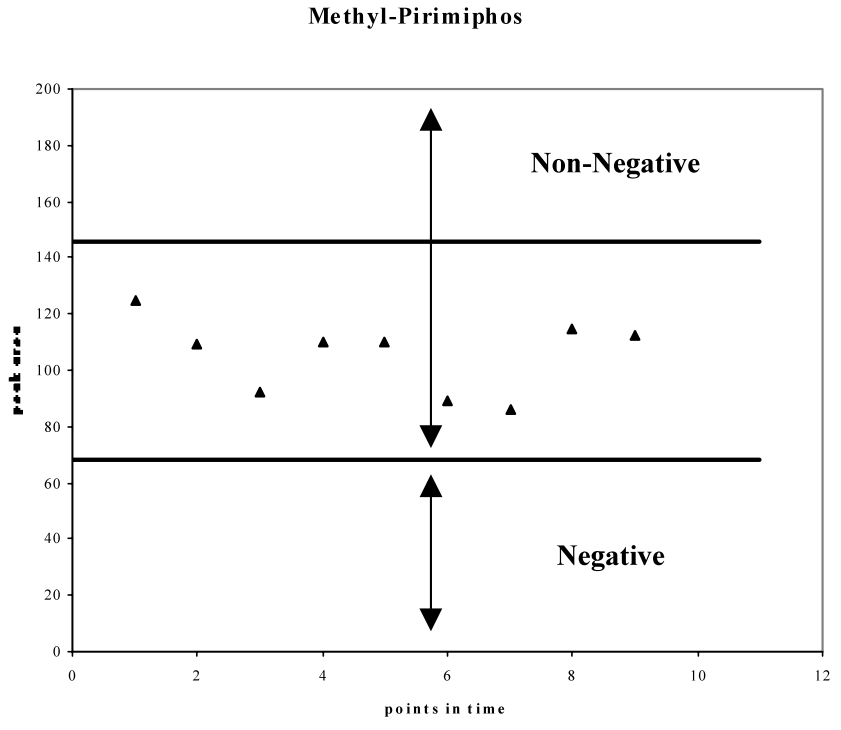


Fig. 2. Construction of the screening intervals for the pesticides dichlorvos, diazinon, methyl-pirimiphos and quinalphos. Negative and non-negative peak area intervals are highlighted.



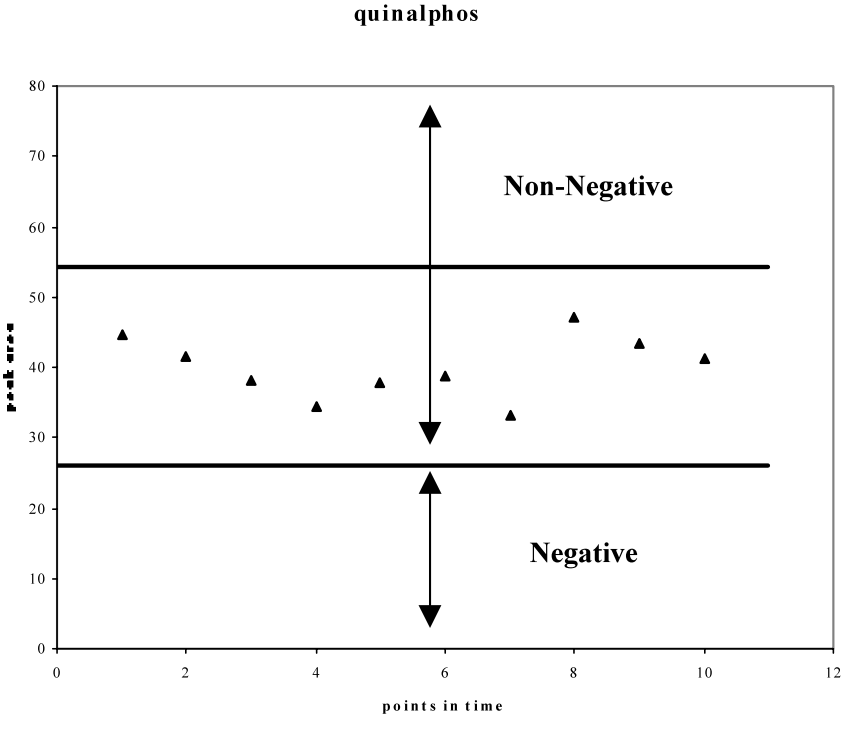


Fig. 2. (*Continued*)

IQC procedures for pesticide qualitative assays, some very recent published recommendations for anti-doping control laboratory [13] were followed.

Additionally, strict maintenance procedures have been followed (septa and liners replacement, column trimming) to avoid the influence of the instrumental drift.

2.5. GC–MSD confirmation test

RTL-GC–MSD (EI) with full scan monitoring was used to change the conclusion of the analysis from non-negative to negative/positive. The confirmation had to be done in all instances for each pesticide involved in the process. To assure the presence of a compound, all the fragment ions with abundances higher than 10% of the base peak should be distinguished in the GC–MSD spectrum at the RTs established by means of the RTL software. Confirmation is done only if the tolerances usually found in the literature for the relative intensities diagnostic ions/base peak ion are met [14–17]. The use of a single ion monitoring (SIM) approach was found unnecessary.

3. Results and discussion

An interval was developed around the average peak area ($\bar{A}$) obtained from 10 injection replicates of standard solutions at the MRL concentrations (MRLs in the Spanish law) made-up with clean extracts of cucumber obtained in identical way as the real samples [18]. The intervals allowed us not only to control the possibilities of committing type I (false-positive) and type II (false-negative) errors at the same time, but also obtaining a great deal of information that was used with a triple goal: first of all for method validation as long as some of the validation experiments refer directly to the value of $\bar{A}$; secondly, to verify good chromatographic performance by means of control solutions; and finally for method discrimination, since the peak areas obtained from unknown samples are compared with the intervals and classified as negatives/non-negatives. The expression for its calculation is: $\bar{A} \pm \Delta(\alpha, \beta, \nu) s_{\mathrm{MRL}}$, where $\Delta(\alpha,\beta,\nu)$ is the non-central parameter of a non-central t-distribution with ν degrees of freedom (the number of experiments -1), α and β are the accepted error probabilities of committing type I and II errors, respectively, and S_{MRL} is the standard deviation of the replicated peak area values obtained for every pesticide under study according to the recommendations of Pulido et al. [18].

Due to our commitment to strictly comply with the Spanish legal framework, our laboratory decided to control the probability of releasing false-negative results. Therefore, it was decided to reduce type II error to 5% leaving type I to 10%. This decision brought about a considerable enlargement of the intervals, but we thought it necessary to develop an adequate control.

Fig. 2 shows the intervals and the points used for their development for the pesticides methylpirimiphos, quinalphos, dichlorvos and diazinon as well as the negative and non-negative area intervals for all compounds.

The intervals allowed easy discrimination between negative and non-negative samples in a very simple manner. The peak area of a suspect peak in a chromatogram of a real sample (appearing at the same RT as a controlled pesticide) is directly compared with the intervals and classified as negative or non-negative. Non-negative samples were later confirmed by RTL-GC–MSD and labelled either positive or negative.

3.1. Method validation

One of the most important issues to be controlled when dealing with qualitative methods is to ensure the quality of the results that are about to be obtained. Thus, method validation represents an objective demonstration that some particular requirements are fulfilled by an analytical methodology.

Some information to this respect can be found in the literature although it is only referred to minimum requirements for the validation of qualitative methods [19]. The lack of a detailed standard procedures for validation in the area of qualitative analysis of pesticides, made us use the proposals found in the literature dedicated to anti-doping control laboratories [20] due to the similar approach that can be made in some instances.

Their guidelines have been slightly modified and adapted to our particular circumstances as pointed out in the text.

3.1.1. Selectivity/specificity

The complete process of extraction was performed with several samples of cucumber that were free of pesticides. The additions of fixed volumes of standard solutions of pesticides at the MRL levels were carried out just before the redissolution of the extracts. The same process was done with a single clean sample of cucumber. No addition was performed onto it as long as it was named the matrix blank. All the samples were injected five times in the GC.

The methodology used herein for the detection of pesticides over their MRL in cucumber with GC–PFPD was regarded as selective enough only if the signal-to-noise (S/N) ratios for each one of them are ≥ 2 (S/N ratios calculated with the chromatograms of the spiked and non-spiked cucumber samples at the same RTs). The results are shown in Table 1. Neither false-positive nor false-negative results were observed in the experiment.

3.1.2. Limit of detection

The limit of detection (LOD) was obtained from the same experiments referred to in the previous section. The lack of calibration curves is one of the benefits of qualitative methods in terms of rapidity. However, a great deal of information is lost due to the drastic reduction in the number of experiments. In this sense, the LODs could only be considered estimations when obtained through this particular approach.

The concentration of pesticide corresponding to the LOD is commonly estimated by simple correlation making use of the S/N ratio obtained in the previous experiment using the MRL concentration (signal) and the non-spiked clean extracted samples (noise) and finding out the concentration that

Table 1
Validation parameters

Pesticide	MRL (mg kg^{-1})	S/N ratio (at MRL concentration)	Selectivity[a]	LOD[b]	Precision (%RSD)	Percent of recovery[c]
Carbophenothion	0.02	244	Acceptable	< MRL	13.22	63.5
Chlorpyriphos	0.05	21168	Acceptable	< MRL	12.66	66.7
Diazinon	0.02	13640	Acceptable	< MRL	11.63	68.9
Dichlorvos	0.10	24774	Acceptable	< MRL	17.97	74.4
Ethion	0.10	214139	Acceptable	< MRL	13.05	65.9
Etrimfos	0.05	35210	Acceptable	< MRL	10.95	69.4
Fenamiphos	0.05	106087	Acceptable	< MRL	13.28	77.5
Fenitrothion	0.50	263056	Acceptable	< MRL	13.56	70.2
Fenthion	0.05	278	Acceptable	< MRL	11.58	61.9
Heptenophos	0.10	97195	Acceptable	< MRL	11.55	80.5
Methyl-chlorpyriphos	0.05	33645	Acceptable	< MRL	19.08	63.7
Methyl-pirimiphos	0.10	118447	Acceptable	< MRL	10.33	67.9
Mevinphos	0.10	36890	Acceptable	< MRL	12.89	78.9
Omethoate	0.20	89295	Acceptable	< MRL	21.94	88.0
Parathion	0.50	577526	Acceptable	< MRL	11.52	65.9
Methyl-parathion	0.20	64207	Acceptable	< MRL	13.03	74.8
Pyrazophos	0.50	496935	Acceptable	< MRL	14.23	69.7
Pyridaphenthion	0.05	37326	Acceptable	< MRL	13.61	74.4
Quinalphos	0.05	39460	Acceptable	< MRL	10.97	70.7
Triazophos	0.02	2300	Acceptable	< MRL	16.65	62.9

[a] Selectivity acceptable whenever S/N > 2.
[b] LOD < MRL whenever S/N > 3.
[c] Calculated by comparison with $\bar{A}$ for each pesticide.

would provide an S/N of 3. The results obtained by this approach became somehow exaggerated and probably useless for comparison purposes, even when very low LODs have been reported elsewhere when using a flame photometric detector to determine OPs that are extracted from water [21].

Table 1 shows directly the S/N ratio obtained at the MRL for all the pesticides in the method presented which is in all cases much bigger than 3 due to the little background noise that is observed in the PFPD.

3.1.3. Extraction recovery

15 g of clean chopped cucumber was spiked with solutions in acetone containing the pesticides under study. The spiking was done in order to obtain samples with pesticides at the MRL levels. These samples were homogenised, extracted and the extracts were re-dissolved in the same way explained before.

The experiment was repeated twice by two different analysts and each injection was replicated for five times. Finally, we obtained 10 peak areas corresponding to each one of the pesticides under study. The upper and lower results were eliminated to prevent the presence of outliers and the average of the remaining eight points compared with the mean of the peak areas obtained when the intervals were obtained (100% recovery). All recoveries expressed as percentages are collected in Table 1.

Although we recognise that extraction recoveries must be as close to 100% as possible, no acceptance criterion was defined for this issue as long as adequate detection was achieved for most of the compounds under study [22].

3.1.4. Repeatability (intra-assay precision)

The agreement between different results obtained was evaluated by this parameter. The simple relative standard deviation (%RSD) obtained with the peak areas corresponding to the 10 experiments performed for the intervals constructions plus the five results experiments done in the validation part corresponding to clean samples of cucumber spiked with pesticides before the redissolution of the extracts were used with this purpose. RSD lower than 25% was considered acceptable. The results are shown in Table 1.

3.2. Application

Two clean samples of cucumber were fortified with a mixture of methyl-pirimiphos, quinalphos, dichlorvos and diazinon at concentrations equal to the MRL for dichlorvos and diazinon (100 and 20 μg kg^{-1}, respectively) and to MRL/2 for methyl-pirimiphos and quinalphos (50 and 25 μg kg^{-1}, respectively). The injection is replicated twice being the average of the peak areas obtained for each pesticide shown in Table 2. A typical GC–PFPD chromatogram obtained for the experiment is shown in Fig. 3.

The peak area results obtained for each pesticide are compared with those in the screening intervals and the method validation. The comparison between the upper and lower thresholds obtained in the interval construction and those obtained in the application show fair agreement as long as non-negative conclusions are got for dichlorvos and diazinon, whereas a negative conclusion is obtained for both methyl-pirimiphos and quinalphos.

Finally, the sample extracts were injected for confirmation in the RTL-GC–MSD. The spectra obtained for dichlorvos and diazinon confirmed

Table 2
Method of application: GC–PFPD screening

Pesticide	Peak area	Upper threshold[a]	Lower threshold[a]	Conclusion
Dichlorvos	115.0	164.3	37.5	Non-negative
Diazinon	15.8	25.2	13.5	Non-negative
Methyl-pirimiphos	59.6	156.4	72.3	Negative
Quinalphos	16.1	60.1	26.9	Negative

[a] Values obtained in the construction of the screening intervals.

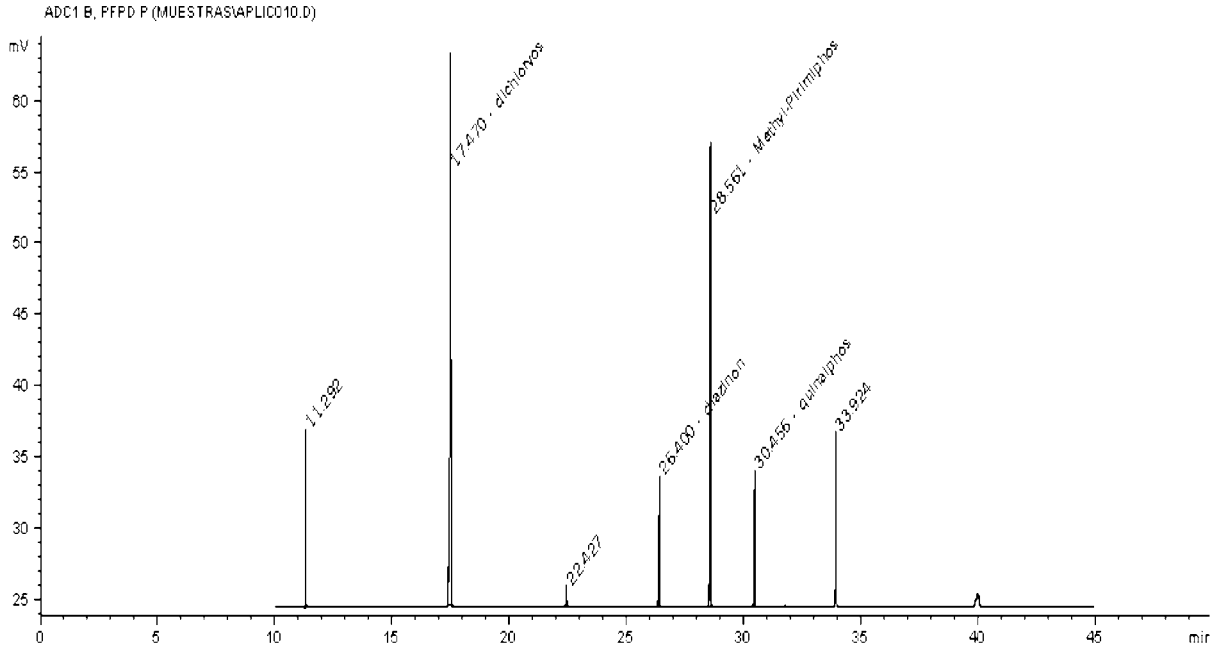


Fig. 3. GC–PFPD chromatogram corresponding to the method application: a clean (free of pesticide residues) sample of cucumber spiked with the pesticides dichlorvos, diazinon at the MRL concentration level, methyl-pirimiphos and quinalphos at 10% of the MRL concentration.

Table 3
Method application: GC–MS (EI) confirmation

Pesticide	Diagnostic ions recognised	Expected RI[a] (diagnostic ion/BP[b])	Actual RI[a] (diagnostic ion/BP[b])	Matching probability RTL[c] software (%)	Conclusion
Dichlorvos	109 (BP)			97%	Positive
	185	25%	25%		
	79	18%	21%		
	187	10%	8%		
Diazinon	179 (BP)				Positive
	137	121%	178%	89%	
	152	85%	72%		
	199	67%	79%		
Methyl-pirimiphos	Not applied	Not applied	Not applied	Not applied	Not applied
Quinalphos	Not applied	Not applied	Not applied	Not applied	Not applied

[a] Relative intensity.
[b] Base peak.
[c] Retention time locking.

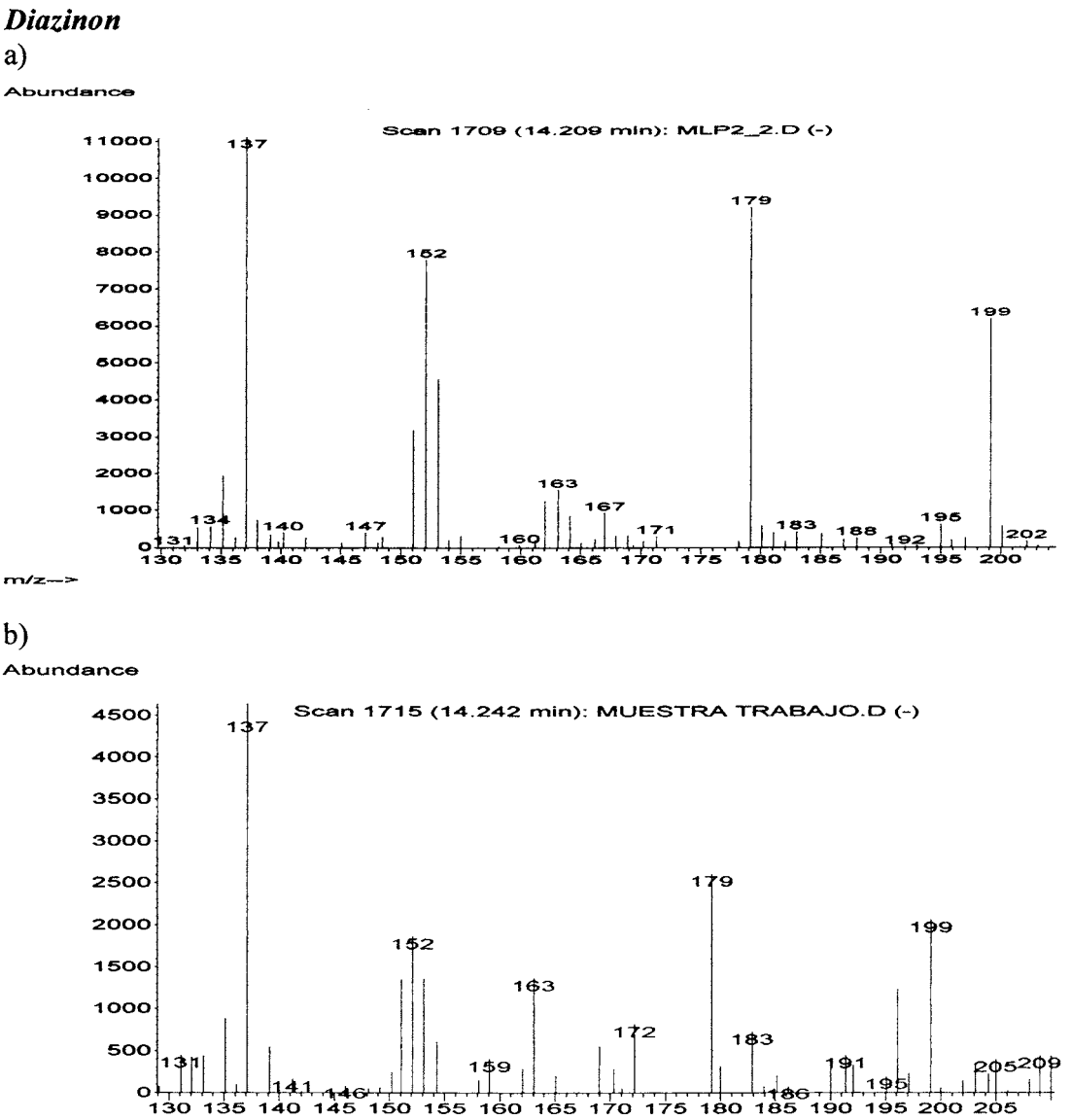


Fig. 4. MS spectra of diazinon and dichlorvos corresponding to: (a) the standard spectra in solvent and (b) the method application obtained by GC–MS (EI) obtained in the full scan mode without sample clean-up.

the positive conclusion if we take into account the presence of, at least, four diagnostic ions allowing a 20% margin on ion abundance ratios [14,15]. Some influence of the background noise is noticed in the relative intensities that should be expected for the diagnostic ions (especially in the diazinon MS spectrum where 137 is considerably higher than expected). One possible explanation to this fact relies on the nature of the comparison (the standard spectrum was obtained in solvent), but the low concentration to be confirmed for diazinon probably points out the need of using either

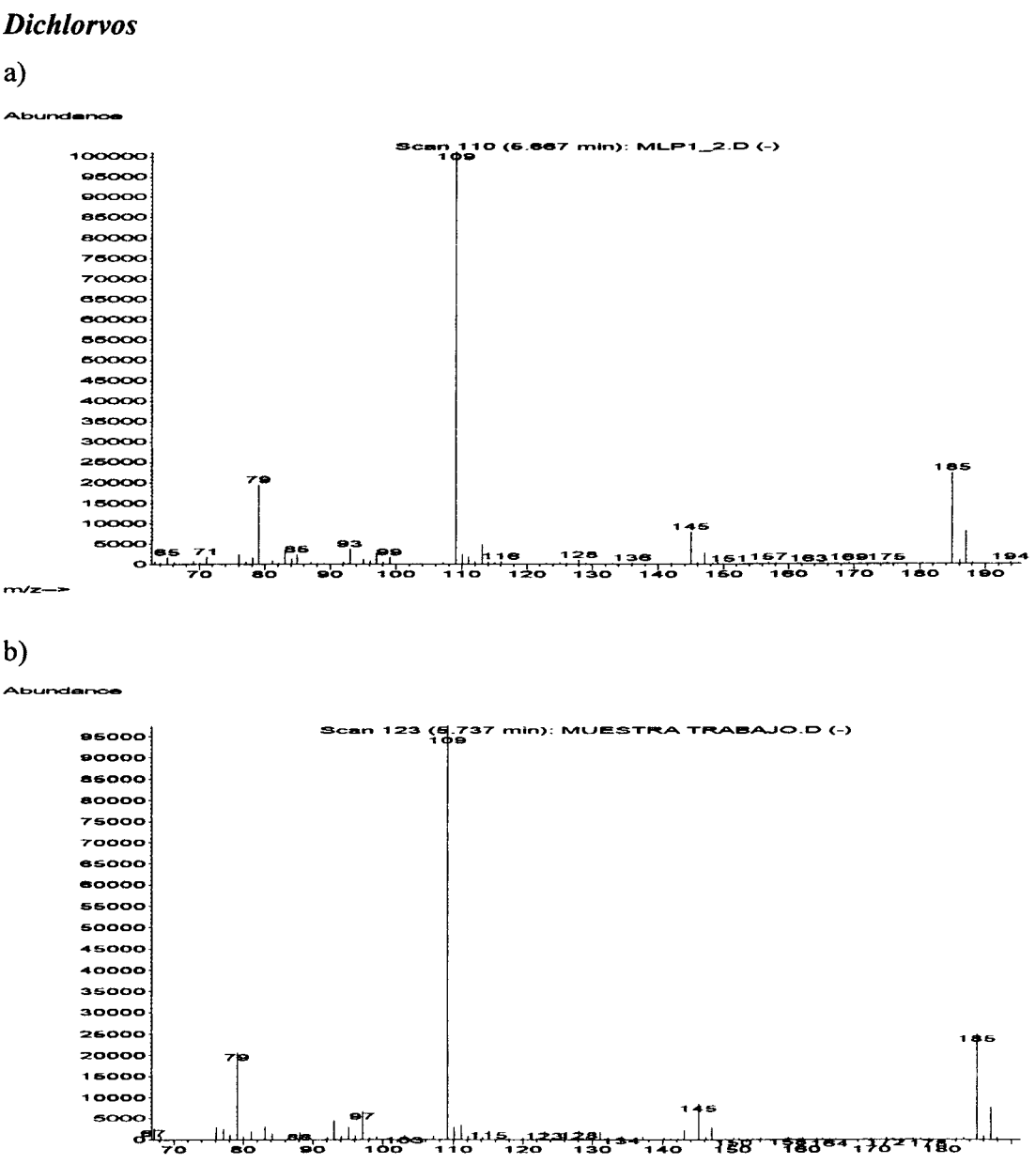


Fig. 4. (*Continued*)

SIM or clean-up approaches if we want to work in this concentration range for this particular pesticide.

Both spectra and their respective standards in solvent are shown in Fig. 4. We confirmed the presence of both pesticides. There are no doubts for dichlorvos whichever be the criteria applied. Unfortunately, diazinon presents what can be considered drawbacks for confirmation. However, if one attends to the similarity found in the spectra, the presence of four diagnostic ions, the matching probability provided by the RTL software and the

fair agreement in the relative intensities (base peak/diagnostic ion) obtained for 152 and 199 ions, the positive conclusion should be clear. In spite of our conclusion, little controversy about the possibility of false-positives could be accepted if some more strict criteria found in the literature are attended [16,17].

The RTL software was optimised with a 1 mg l^{-1} solution of methyl-chlorpyriphos (target compound) in acetone and was used to get a rapid and accurate detection of the compounds of interest in the chromatograms.

Table 3 shows the diagnostic ions recognised in the spectra, the actual and expected relative intensities for the diagnostic ions, the percentage results of the comparisons done by the RTL software and the conclusions of the analysis in each particular case.

4. Conclusions

A qualitative method devoted to the detection of OPs in cucumber has been presented. The method pays special attention to the control of the MRLs established in the Spanish law achieving a thorough knowledge of the detector response at those concentration levels for the 20 OPs studied. This method can be summarised as follows:

1) extraction of the pesticides from the sample matrices,
2) screening of the samples using GC–PFPD and naming the samples as negatives or non-negatives,
3) confirmation of the non-negative samples using RTL-GC–MSD, and
4) conclusion labelling of the samples as positives or negatives.

The method has been validated obtaining good results for every issue and applied, as an example, to a spiked clean sample of cucumber. The results that have been obtained in the application are completely satisfactory. Hence, this qualitative method tries to offer a new chance to the widespread quantitative determinations usually employed in most pesticide laboratories throughout the world. The method is fast, simple and reliable, and it focuses their information search in those concentration levels that are under strict control by the authorities. The development of an interval using replicated detector responses for calibration samples at those MRLs permits a fast classifying of the samples between negatives and non-negatives which is a huge advantage when dealing with large daily sample inputs. Only non-negatives are confirmed.

For validation purposes, we have made use of the anti-doping literature as long as the patterns of our method are very similar. There is no need to go into the time-consuming procedure of 20 calibrations and, therefore, effort and time are saved obtaining similar information to prove the quality of the method. In this sense, the results obtained for selectivity, LOD, recovery and precision estimation show clearly the applicability of the method because of the high amount of information that is accumulated for those concentration levels that are controlled (that are needed to control).

All these reasons make the qualitative approach very attractive for routine laboratories no matter the technique used. Obviously, some repeatability in the detector response is needed but, even in low repeatability situations, the usual re-building of the intervals may allow its application. In the case of pesticide laboratories making use of conventional detectors like FPD, ECD or FID, the approach is definitely and without the shadow of a doubt an interesting approach if providing quantitative results is not a must.

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