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# Reduction of analysis time in gas chromatography Application of low-pressure gas chromatography–tandem mass spectrometry to the determination of pesticide residues in vegetables

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**Abstract**

An alternative to conventional capillary gas chromatography (GC) is evaluated as a new approach to determine pesticide residues in vegetables. Low-pressure gas chromatography–tandem mass spectrometry (LP-GC–MS–MS) is proposed after a fast and simple extraction of the vegetable samples with dichloromethane and without clean up. The use of the above-mentioned GC technique reduced the total time required to determine 72 pesticides to less than half the present time (31 min), increasing the capability of a monitoring routine laboratory. The use of guard column and plug of carbofrit into the glass liner in combination with LP-GC was evaluated. The method was validated with limits of quantitation low enough to determine the pesticide residues at concentrations below the maximum residue levels stated by legislation. In order to assess its applicability to the analysis of real samples, 25 vegetable samples previously determined using conventional-capillary GC–MS–MS were analysed by LP-GC–MS–MS. The results obtained with the compared techniques showed differences lower than 0.01 mg $kg^{-1}$.
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*Keywords:* Gas chromatography, low pressure; Gas chromatography, fast; Vegetables; Food analysis; Pesticides

## 1. Introduction

Currently, gas chromatography mass spectrometry (GC–MS) remains the main analytical technique used for pesticide residue analysis, combining the power of separation allowed by GC, with the sensitivity, selectivity, and identification capability of MS [1–5]. In the few years, tandem MS (MS–MS) using bench top ion-trap systems has been shown to be a relevant approach in pesticide residue analysis, providing increased selectivity and sensitivity [6–12]. However, one of the main goals in the development of new GC–MS methods is an increase in analysis speed to reduce the analysis time. The relatively slow multiresidue methods currently used

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in pesticide residue laboratories restrict the number of analyses per day.

Giddings [13] showed in 1962 that the application of a vacuum at the column outlet would lead to reduced analysis times in GC. He also proposed another approach based on GC at sub-atmospheric pressure or low pressure (LP). For many years this alternative was not practical due to the lack of adequate instrumentation. However, this is now possible by connecting a wide bore capillary column (0.53 mm I.D.) to a narrow and short restriction capillary that is positioned at the injector [14–16]. On the other hand, the use of MS detectors, which also require low pressure for analysis, can provide the vacuum for LP-GC, avoiding additional instrumentation. Recently, LP-GC has enabled short analysis times with the use of a short capillary column and MS–MS detection mode [17] in pesticide residue analysis.

The main aim of this study was to demonstrate the ability of a multiresidue and LP-GC–MS–MS method for the determination of pesticides in fresh vegetables by monitoring laboratories. This method is based on a simple and fast solvent extraction of the vegetables without post-extraction clean up steps before analysis. It has been validated and applied to the analysis of real samples of vegetables (tomato, cucumber and pepper extracts) from El Ejido (Almería), which is an important agricultural area in the southeast of Spain. The results obtained by this approach were compared to those obtained using conventional capillary columns and GC–MS–MS. A comparison of the analyses of real samples allowed us to determine the feasibility of LP-GC–MS–MS for the routine analysis of pesticide residues in vegetables, processing a higher number of samples daily, which is of great interest for a routine analysis laboratory.

## 2. Experimental

### 2.1. Chemicals and reagents

Pesticide standards and the internal standard (I.S.), caffeine, were obtained from Riedel-de-Haën (Seelze-Hannover, Germany); purity was always >99%. Pesticide-quality solvents (*n*-hexane, dichloromethane, methanol and acetone) were supplied by Panreac (Barcelona, Spain). Stock standard solutions (between 75 and 550 μg $ml^{-1}$), prepared by exact weighing and dissolution in acetone, were stored in a freezer (−30 °C). Working standard solutions were prepared by appropriate dilution with cyclohexane and stored under refrigeration (4 °C). Anhydrous sodium sulfate for residue analysis was obtained from Panreac.

### 2.2. Apparatus

GC–MS analysis was performed with a Varian 3800 gas chromatograph with electronic flow control (EFC) and fitted with a Saturn 2000 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA, USA). Samples were injected into a Varian 8200 autosampler SPI/1079 split/splitless programmed-temperature injector using a 100-μl syringe operated in the large volume injection technique. The glass liner was equipped with a plug of carbofrit (Resteck, Bellefonte, PA, USA). A fused-silica untreated capillary column 2 m×0.25 mm I.D. from Supelco (Bellefonte, PA, USA) was used as a guard column connected to a Rapid-MS [wall-coated open tubular (WCOT) fused-silica CP-Sil 8 CB low bleed of 10 m×0.53 mm I.D., 0.25 μm film thickness] analytical column from Varian Instruments (Sunnyvale, CA, USA) for high speed analysis. The mass spectrometer was operated in electron impact (EI) ionization mode. The computer that controlled the system also held an EI-MS–MS library specially created for the target analytes under our experimental conditions. Other EI-MS libraries were also available. The mass spectrometer was calibrated weekly with perfluorotributylamine. Helium (99.999%) at a flow-rate of 1 ml $min^{-1}$ was used as carrier and collision gas.

A chopper (Hamilton Beach, Washington, WA, USA), a Polytron PT2100 (Kinematica, Littan/Luzern, Switzerland), and a rotary evaporator R-114 (Büchi, Flawil, Switzerland) were available for processing samples.

### 2.3. Sample collection and storage

Fresh vegetables were sampled and transported following the 79/700/CEE directive. Pesticide free vegetables monitored by the laboratory of pesticide

Table 1
Retention time window (RTW) and GC–MS–MS conditions

| Pesticide | RTW (min) | Parent ion ($m/z$) | CID amplitude (V) | CID $R_f$ ($m/z$) | Quantification ion ($m/z$) | Range $m/z$ |
|---|---|---|---|---|---|---|
| Dichlorvos | 5.20–4.77 | 185 | 78 | 81 | 109+131 | 80–190 |
| Acephate | 5.12–5.06 | 136 | 37 | 47 | 107+119 | 80–190 |
| Heptenophos | 5.86–4.99 | 124 | 37 | 47 | 89 | 80–160 |
| Propoxur | 5.66–4.93 | 152 | 41 | 66 | 110 | 80–160 |
| Ethoprophos | 5.61–5.44 | 158 | 27 | 47 | 94+114+130 | 70–230 |
| Dimethoate | 6.75–5.52 | 125 | 55 | 60 | 79 | 70–230 |
| Lindane | 6.45–6.05 | 219 | 70 | 100 | 180 | 140–220 |
| Pyremethanil | 6.64–6.37 | 198 | 100 | 81 | 98 | 90–300 |
| Chlorthalonil | 6.81–6.37 | 266 | 90 | 85 | 133 | 90–300 |
| Disulfoton | 6.76–6.37 | 186 | 60 | 71 | 97 | 90–300 |
| Etrimphos | 6.76–6.63 | 292 | 45 | 70 | 181 | 90–300 |
| Pirimicarb | 7.04–6.75 | 166 | 49 | 53 | 83 | 80–200 |
| Caffeine | 7.07–6.81 | 194 | 56 | 60 | 120 | 80–200 |
| Formothion | 7.14–6.83 | 170 | 38 | 70 | 107 | 80–200 |
| Ethiofencarb | 7.04–6.75 | 168 | 39 | 63 | 107 | 80–200 |
| Chlorpirifos-m | 7.69–7.15 | 286 | 72 | 85 | 208 | 100–300 |
| Vinclozoline | 7.80–7.13 | 285 | 34 | 100 | 241−213 | 100–300 |
| Parathion-m | 7.68–7.04 | 263 | 48 | 80 | 136+216 | 100–300 |
| Metalaxyl | 7.61–7.44 | 206 | 54 | 75 | 132+162 | 100–300 |
| Pirimiphos-m | 7.99–7.78 | 290 | 64 | 85 | 151 | 90–300 |
| Fenitrotion | 8.04–7.73 | 260 | 65 | 71 | 122+138+170 | 90–300 |
| Malathion | 8.38–8.07 | 173 | 51 | 75 | 99 | 90–320 |
| Chlorpyrifos | 8.36–8.11 | 314 | 100 | 170 | 258 | 90–320 |
| Fenthion | 9.03–8.24 | 278 | 92 | 112 | 135 | 90–320 |
| Triadimefon | 8.73–8.40 | 208 | 62 | 75 | 144 | 90–345 |
| Tetraconazole | 9.12–8.61 | 336 | 96 | 108 | 218 | 90–345 |
| Dicofol | 9.01–8.23 | 250 | 49 | 90 | 215 | 90–345 |
| Pendimethalin | 9.26–9.01 | 252 | 62 | 95 | 208+191+162 | 90–345 |
| Penconazole | 9.72–9.20 | 248 | 77 | 89 | 192+157 | 90–300 |
| Chlozolinate | 10.01–9.32 | 331 | 88 | 145 | 259 | 90–300 |
| Isonfenphos | 9.71–9.29 | 213 | 52 | 93 | 185 | 90–300 |
| Pyrifenox | 9.89–9.11 | 263 | 90 | 100 | 192+228 | 90–300 |
| Chlorfenvinphos | 9.82–9.30 | 267 | 82 | 100 | 159 | 90–300 |
| Procymidone | 9.91–9.61 | 283 | 57 | 80 | 253:257 | 90–300 |
| Quinometionathe | 10.42–9.72 | 234 | 60 | 83 | 196 | 69–250 |
| Endosulfan α | 10.32–9.79 | 241 | 84 | 80 | 170+172 | 69–250 |
| Fenaminphos | 11.59–9.21 | 303 | 56 | 95 | 195 | 120–275 |
| Fludioxinil | 11.96–11.50 | 248 | 84 | 89 | 152+154+127 | 120–275 |
| Buprofezin | 12.14–11.22 | 249 | 50 | 80 | 191:195 | 120–275 |
| Hexaconazole | 12.43–12.03 | 231 | 100 | 100 | 159 | 120–275 |
| Bupimirate | 12.27–11.84 | 273 | 77 | 120 | 193 | 120–275 |
| Endosulfan β | 12.50–11.98 | 241 | 84 | 80 | 170+172 | 120–275 |
| Oxadixyl | 13.58–12.62 | 163 | 46 | 71 | 132 | 100–235 |
| Ethion | 13.35–13.01 | 231 | 63 | 100 | 175+203 | 100–235 |
| Benalaxyl | 14.20–13.62 | 148 | 46 | 50 | 91 | 90–345 |
| Carbofenothion | 14.47–13.36 | 342 | 64 | 131 | 199+157 | 90–345 |
| Endosulfan sulfate | 14.21–13.58 | 272 | 64 | 80 | 235+238 | 90–345 |
| Propiconazole | 15.17–14.42 | 259 | 78 | 114 | 191+173 | 100–260 |
| Nuarimol | 15.94–14.30 | 235 | 56 | 75 | 139 | 100–260 |
| Tebuconazole | 15.94–14.78 | 250 | 63 | 75 | 125 | 100–260 |
| Propargite | 16.03–15.31 | 173 | 56 | 66 | 117+145 | 100–260 |

Table 1. Continued

| Pesticide | RTW (min) | Parent ion ($m/z$) | CID amplitude (V) | CID $R_f$ ($m/z$) | Quantification ion ($m/z$) | Range $m/z$ |
|---|---|---|---|---|---|---|
| Iprodione | 17.46–16.86 | 314 | 88 | 125 | 245+271 | 140–345 |
| Bromopropylate | 17.53–16.65 | 341 | 45 | 70 | 181:187 | 140–345 |
| Bifenthrin | 18.22–17.18 | 181 | 40 | 50 | 165 | 140–275 |
| Fenpropathrin | 18.26–17.66 | 265 | 72 | 95 | 210 | 140–275 |
| Tetradifon | 18.30–17.84 | 229 | 95 | 100 | 197:203 | 140–275 |
| Furathiocarb | 19.27–18.28 | 325 | 77 | 140 | 194 | 100–330 |
| Phosalone | 19.26–17.85 | 182 | 70 | 80 | 111+138 | 100–330 |
| Piriproxifen | 19.77–19.09 | 136 | 57 | 59 | 96 | 70–140 |
| Cyhalothrin | 21.38–19.65 | 181 | 90 | 80 | 152 | 120–290 |
| Amitraz | 21.22–19.66 | 162 | 50 | 71 | 132+147 | 120–290 |
| Pirazofos | 21.32–20.19 | 265 | 87 | 120 | 210 | 120–290 |
| Acrinathrin | 22.30–20.30 | 181 | 87 | 80 | 152 | 70–200 |
| Permethrin | 23.66–21.73 | 183 | 74 | 70 | 152 | 70–200 |
| Pyridaben | 22.85–20.45 | 147 | 53 | 64 | 111+105 | 70–200 |
| Cyfluthrin | 25.74–24.11 | 206 | 96 | 86 | 149:152 | 100–325 |
| Cypermethrin | 26.30–24.96 | 163 | 53 | 70 | 127 | 100–325 |
| Flucythrinate | 27.46–25.04 | 157 | 69 | 79 | 107 | 100–325 |
| Esfenvalerate | 26.85–25.96 | 225 | 51 | 70 | 119 | 100–325 |
| Difenoconazole | 29.66–28.09 | 323 | 87 | 122 | 265 | 100–325 |
| Deltramethrin | 30.79–29.05 | 253 | 57 | 90 | 172+174 | 120–350 |
| Azoxistrobina | 30.96–29.96 | 345 | 92 | 115 | 329 | 120–350 |

residues CUAM (El Ejido, Almería, Spain) were used as blank to spike samples for recovery studies and to prepare matrix matched standards for calibration. Samples were analysed in 24 h and preserved at 4 °C until the extraction time. No degradation of the pesticides was detected in the storage conditions.

### 2.4. Extraction procedure of pesticides from the vegetables

A 2-kg sample of vegetable was chopped and homogenised. An aliquot of 15 g was exactly weighted into a glass and mixed with 50 ml of dichloromethane in the Polytron for 2 min and 50 g of anhydrous sodium sulfate was added. The mixture was allowed to rest for 2 min and then filtered through a 9-cm Büchner funnel and filtered again through paper filter with anhydrous sodium sulfate to a spherical flask. Evaporation of the solvent to dryness was done in a rotary evaporator (35–40 °C). The dried residue was re-dissolved with 5 ml of cyclohexane. One millilitre of this solution was added to a 2-ml volumetric flask with 50 μl of I.S. solution of 20 mg $l^{-1}$. The final 2-ml volume was attained using cyclohexane.

### 2.5. Instrumental conditions

Sample aliquots of 10 μl were injected into the GC operating at a syringe injection flow-rate of 10 μl $s^{-1}$. The initial injector temperature of 70 °C was held for 0.5 min and then increased at 100 °C $min^{-1}$ to 310 °C, which was held for 10 min. After injection the column temperature, initially 70 °C, was held for 3.5 min, then increased at 50 °C $min^{-1}$ to 150 °C, then increased at 3 °C $min^{-1}$ to 235°C and finally raised to 300 °C at 50 °C $min^{-1}$ and held for 3 min.

The ion-trap mass spectrometer was operated in EI-MS–MS mode. The transfer line, manifold and trap temperatures were 280, 50 and 200 °C, respectively. The analysis was performed with a filament-multiplier delay of 4.75 min to prevent instrument damage. The automatic gain control (AGC) was activated with an AGC-target of 5000 counts. The emission current for the ionisation filament was set at 80 μA, generating electrons with an energy of 70 eV. The axial modulation amplitude voltage was 4.0 V.

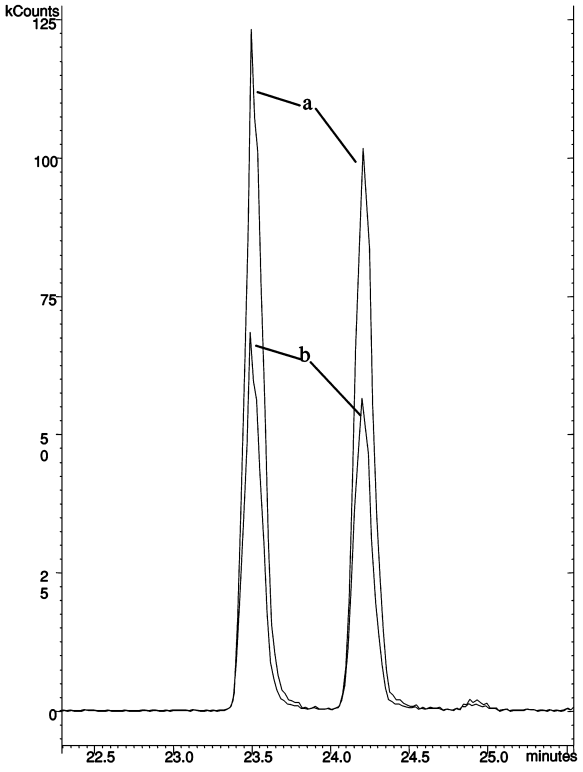


Fig. 1. LP-GC–MS–MS chromatogram obtained using (a) and without using (b) carbofrit of *cis*- and *trans*-flucythrinate pesticide.

The MS–MS process was carried out by collision-induced dissociation (CID) with a non-resonant excitation for all the compounds studied. The electron multiplier voltage was 1700 V (+200 V offset above the auto-tuning process). Scan rate and mass range scanned depended on the number of pesticides analysed simultaneously. The specific MS–MS parameters used are shown in Table 1.

## 3. Results and discussion

### *3.1. Injection step*

No special techniques for injection of samples are required with LP-GC because, despite the fact that the analytical column has to be kept under low pressure conditions, the injector works at conventional column head pressures. As a consequence, typical injection volumes can be used and the sample capacity is not limited. In this study, a large volume injection technique [11] was used in order to increase sensitivity and check the sample capacity of the analytical column proposed. The injection volume set (10 μl) allowed the determination of pesticide residues at concentrations below or equal to the maximum residue levels (MRLs) with a good peak shape. The injection of larger volumes would involve

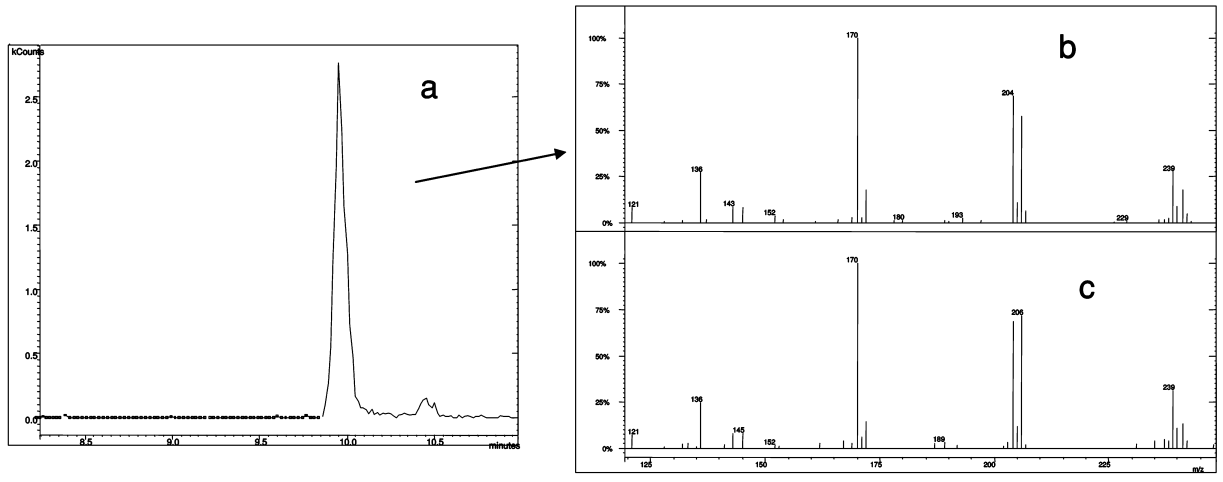


Fig. 2. (a) Endosulfan α chromatogram of a positive sample of tomato (concentration found, 0.015 mg $kg^{-1}$) and MS–MS spectra obtained for the sample (b) and library (c).

the application of a previous clean-up step to the analytical detection and an increment of the maintenance of the instrument. In addition, the injection of volumes much higher than 10 μl would not involve a significant improvement in the signal to noise ratio because of the saturation of the injector and analytical column with the components of the sample [1]. In this sense, it is important to note that the use of both a precolumn (or guard column) of conventional diameter, and a plug of carbofrit in the injection-port liner for the analysis of the complex sample extracts is favourable for eliminating matrix interferences, and consequently, it avoids the application of clean-up procedures [6,7]. On the other hand, the absence of carry over effect was tested injecting solvent after analysis of highly concentrated standards. This can be attributed to the final injector temperature (310 °C), and the further vent program. Additionally, the use of the above mentioned plug improves the deposit of the drop of sample introduced by the syringe, and the volatilisation process increases the sensitivity of the method. Fig. 1 shows the increment of sensitivity achieved when a plug of carbofrit is used.

### 3.2. Gas chromatographic separation of the pesticides

The oven temperature program applied was similar to that previously developed in our laboratory [17]. However, the use of a guard column or the use of carbofrit did not significantly affect the retention time and resolution of the pesticides, as well as the function of the restriction connected to the analytical column. All compounds were eluted in a reasonably short time (less than 31 min), as shown in Fig. 2. The use of LP-GC reduced at least to half the total time required using conventional capillary GC [6–8,10], and as a result, can double the number of samples analysed per day in a routine laboratory.

### 3.3. Extraction of the pesticides

The extraction procedure prior to the instrumental determination is an important factor. The extract obtained should not jeopardise the injector, column or detector systems. However, the low concentrations of the pesticides in foodstuffs require concentrating the extracts with the corresponding increment of the target analyte and interference signals. In this sense, expensive and time-consuming clean-up steps are recommended [18–22]. For MS-based methods there is a tendency to omit the clean-up step, especially when MS–MS is used [6,7]. However, the use of previous clean-up steps increases the time between instrumental maintenance. A simple method with dichloromethane is proposed as an extraction procedure based on the capability of this solvent to extract substances with a wide range of polarities. It obviously also includes more matrix interferences but they can be minimised using a plug of carbofrit into the glass liner and a guard column. In this way, the tedious clean-up step can be avoided. The method was partially miniaturised in order to reduce the amount of dichloromethane used (environmental impact of chlorinated solvents) [6,7]. Despite the absence of clean-up, additional maintenance of the LP-GC column was not necessary, demonstrating the reliability of the proposed chromatographic technique.

### 3.4. Optimisation of the MS–MS parameters

The MS–MS process involves two fundamental steps between the formation and detection of ions. In the first step the precursor ion or an entire cluster of parent ions is isolated in the trap, and in the second stage the dissociation of the precursor ion or ions is performed by collisional activation with an inert gas. Usually, the most intense parent ions are selected, but when those ions have low mass it may be better to select a slightly less intense ion at a higher mass. After this choice it is necessary to set the excitation storage level [12] before optimising the CID step.

For the last task the instrument software has a procedure known as automated method development (AMD) that allows us to perform this work with a few injections. The main parameters involved in this process are the excitation amplitude (or resonance excitation voltage) and the excitation storage level. The final values used in this study are summarised in Table 1. The excitation time was set constant at 20 ms.

Once the MS–MS conditions were optimised, the quantitation ions were selected. The MS–MS spectra

Table 2
Accuracy and precision at two concentration levels of the LP-GC–MS–MS method

| Pesticide | 1st Cal. level | | | 2nd Cal. level | | | LOD ($\mu g\ kg^{-1}$) | LOQ ($\mu g\ kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | Conc. ($mg\ kg^{-1}$) | Recovery (%) | RSD (%) | Conc. ($mg\ kg^{-1}$) | Recovery (%) | RSD (%) | | |
| Dichlorvos | 0.050 | 71.2 | 25.5 | 0.250 | 87.3 | 9.0 | 1.0 | 3.5 |
| Acephate | 0.020 | 73.8 | 7.9 | 0.100 | 80.5 | 7.3 | 4.0 | 13.0 |
| Heptenophos | 0.010 | 85.3 | 8.9 | 0.050 | 103.3 | 14.6 | 0.1 | 0.5 |
| Propoxur | 0.050 | 83.5 | 16.9 | 0.250 | 110.7 | 15.7 | 1.1 | 3.6 |
| Ethoprophos | 0.010 | 70.3 | 13.4 | 0.050 | 115.3 | 12.7 | 0.2 | 0.8 |
| Dimethoate | 0.020 | 89.7 | 16.5 | 0.100 | 114.3 | 12.4 | 2.7 | 8.9 |
| Lindane | 0.100 | 71.8 | 13.5 | 0.500 | 82.5 | 11.4 | 0.3 | 1.0 |
| Pyremethanil | 0.020 | 115.6 | 12.2 | 0.100 | 112.3 | 11.2 | 0.6 | 1.9 |
| Chlorthalonil | 0.100 | 87.2 | 14.6 | 0.500 | 83.6 | 14.0 | 1.9 | 6.4 |
| Disulfoton | 0.020 | 78.2 | 13.1 | 0.100 | 77.3 | 14.5 | 10.0 | 22.0 |
| Etrimphos | 0.010 | 76.7 | 14.0 | 0.050 | 111.3 | 10.5 | 0.1 | 0.2 |
| Pirimicarb | 0.020 | 76.7 | 14.1 | 0.100 | 78.5 | 8.0 | 0.5 | 1.7 |
| Caffeine | – | – | – | – | – | – | – | – |
| Formothion | 0.050 | 74.6 | 14.3 | 0.250 | 112.7 | 8.1 | 3.8 | 12.8 |
| Ethiofencarb | 0.020 | 118.2 | 10.3 | 0.100 | 73.3 | 12.8 | 2.2 | 7.4 |
| Chlorpirifos methyl | 0.020 | 95.0 | 14.7 | 0.100 | 88.3 | 13.1 | 0.1 | 0.8 |
| Vinclozoline | 0.050 | 73.0 | 13.2 | 0.250 | 70.4 | 14.5 | 0.1 | 0.2 |
| Parathion methyl | 0.100 | 97.9 | 14.3 | 0.500 | 120.0 | 12.3 | 2.6 | 8.7 |
| Metalaxyl | 0.050 | 74.4 | 4.7 | 0.250 | 111.6 | 9.3 | 0.2 | 0.8 |
| Pirimiphos met | 0.010 | 73.3 | 5.0 | 0.050 | 108.0 | 8.6 | 0.1 | 0.2 |
| Fenitrotion | 0.100 | 100.8 | 9.6 | 0.500 | 102.0 | 14.0 | 0.3 | 1.0 |
| Malathion | 0.100 | 112.8 | 9.4 | 0.500 | 70.6 | 7.8 | 0.7 | 2.3 |
| Chlorpyrifos | 0.020 | 82.4 | 5.6 | 0.100 | 118.3 | 7.7 | 0.1 | 0.5 |
| Fenthion | 0.020 | 109.0 | 10.8 | 0.100 | 112.0 | 7.8 | 0.2 | 0.6 |
| Triadimefon | 0.050 | 115.6 | 7.5 | 0.250 | 116.0 | 10.0 | 0.8 | 2.7 |
| Tetraconazole | 0.010 | 71.2 | 9.5 | 0.050 | 85.0 | 9.7 | 0.1 | 0.2 |
| Dicofol | 0.020 | 119.8 | 9.8 | 0.100 | 73.3 | 8.2 | 0.2 | 0.8 |
| Pendimethalin | 0.020 | 78.2 | 9.2 | 0.100 | 106.7 | 9.2 | 0.1 | 0.2 |
| Penconazole | 0.010 | 97.4 | 15.3 | 0.050 | 77.3 | 12.4 | 0.1 | 0.2 |
| Chlozolinate | 0.020 | 87.4 | 12.5 | 0.100 | 94.3 | 11.3 | 0.3 | 0.8 |
| Isonfenphos | 0.010 | 100.8 | 9.5 | 0.050 | 100.0 | 9.2 | 0.2 | 0.6 |
| Pyrifenox | 0.050 | 106.7 | 8.3 | 0.250 | 90.9 | 13.4 | 0.2 | 0.7 |
| Chlorfenvinphos | 0.050 | 81.5 | 9.2 | 0.250 | 108.7 | 9.3 | 0.1 | 0.4 |
| Procymidone | 0.100 | 117.5 | 11.4 | 0.500 | 116.0 | 10.8 | 0.5 | 1.7 |
| Quinometionathe | 0.020 | 109.0 | 10.6 | 0.100 | 96.7 | 9.9 | 0.1 | 0.5 |
| Endosulfan $\alpha$ | 0.025 | 90.3 | 6.6 | 0.125 | 104.5 | 10.1 | 0.2 | 0.7 |
| Fenaminphos | 0.020 | 78.0 | 13.6 | 0.100 | 89.5 | 9.5 | 0.1 | 0.2 |
| Fludioxinil | 0.020 | 105.4 | 14.7 | 0.100 | 104.7 | 8.8 | 0.2 | 0.5 |
| Buprofezin | 0.010 | 85.8 | 7.0 | 0.050 | 110.0 | 9.4 | 0.1 | 0.4 |
| Hexaconazole | 0.010 | 71.8 | 11.3 | 0.050 | 104.0 | 10.4 | 0.1 | 0.3 |
| Bupimirate | 0.010 | 115.2 | 12.5 | 0.050 | 106.0 | 9.2 | 0.1 | 0.2 |
| Endosulfan $\beta$ | 0.025 | 76.7 | 13.2 | 0.125 | 114.1 | 5.7 | 0.5 | 2.7 |
| Oxadixyl | 0.050 | 72.5 | 15.3 | 0.250 | 111.1 | 7.2 | 0.1 | 0.3 |
| Ethion | 0.020 | 74.4 | 13.1 | 0.100 | 109.7 | 8.9 | 0.1 | 0.2 |
| Benalaxyl | 0.050 | 82.1 | 8.8 | 0.250 | 87.3 | 7.6 | 0.2 | 0.8 |
| Carbofenothion | 0.010 | 97.4 | 12.7 | 0.050 | 113.2 | 10.2 | 0.1 | 0.3 |
| Endosulfan sulfate | 0.025 | 79.6 | 11.1 | 0.125 | 92.3 | 14.0 | 0.3 | 1.0 |
| Propiconazole | 0.020 | 126.9 | 11.6 | 0.100 | 115.3 | 9.6 | 0.1 | 0.4 |

Table 2. Continued

| Pesticide | 1st Cal. level | | | 2nd Cal. level | | | LOD (μg kg$^{-1}$) | LOQ (μg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | Conc. (mg kg$^{-1}$) | Recovery (%) | RSD (%) | Conc. (mg kg$^{-1}$) | Recovery (%) | RSD (%) | | |
| Nuarimol | 0.010 | 92.8 | 10.7 | 0.050 | 112.7 | 6.5 | 0.2 | 0.7 |
| Tebuconazole | 0.020 | 79.5 | 16.4 | 0.100 | 110.0 | 8.3 | 0.5 | 1.5 |
| Propargite | 0.050 | 128.5 | 11.5 | 0.250 | 122.0 | 14.6 | 1.8 | 7.7 |
| Iprodione | 0.100 | 80.4 | 7.9 | 0.500 | 118.7 | 13.5 | 0.1 | 0.2 |
| Bromopropylate | 0.100 | 66.7 | 5.9 | 0.500 | 113.3 | 5.4 | 0.1 | 0.2 |
| Bifenthrin | 0.010 | 107.7 | 8.1 | 0.050 | 117.3 | 8.4 | 0.1 | 0.2 |
| Fenpropathrin | 0.020 | 107.7 | 11.6 | 0.100 | 90.3 | 7.9 | 0.1 | 0.2 |
| Tetradifon | 0.050 | 102.6 | 12.2 | 0.250 | 86.7 | 10.0 | 0.1 | 0.3 |
| Furathiocarb | 0.050 | 72.3 | 13.9 | 0.250 | 111.2 | 7.1 | 0.1 | 0.2 |
| Phosalone | 0.100 | 105.1 | 7.4 | 0.500 | 101.3 | 11.3 | 0.1 | 0.3 |
| Piriproxifen | 0.010 | 110.3 | 11.8 | 0.050 | 105.3 | 19.7 | 0.1 | 0.2 |
| Cyhalothrin | 0.050 | 92.3 | 7.9 | 0.250 | 106.7 | 7.7 | 0.1 | 0.3 |
| Amitraz | 0.020 | 128.2 | 14.0 | 0.100 | 87.6 | 10.2 | 0.1 | 0.4 |
| Pirazofos | 0.010 | 91.9 | 9.1 | 0.050 | 104.0 | 5.1 | 0.1 | 0.2 |
| Acrinathrin | 0.010 | 95.4 | 15.0 | 0.050 | 71.3 | 12.3 | 0.2 | 0.8 |
| Permethrin | 0.050 | 89.7 | 7.7 | 0.250 | 119.3 | 10.3 | 0.6 | 2.0 |
| Pyridaben | 0.010 | 71.8 | 9.9 | 0.050 | 119.3 | 7.2 | 0.1 | 0.3 |
| Cyfluthrin | 0.020 | 89.7 | 9.7 | 0.100 | 110.0 | 11.1 | 0.6 | 2.2 |
| Cypermethrin | 0.100 | 83.3 | 10.6 | 0.500 | 113.3 | 10.1 | 0.7 | 2.5 |
| Flucythrinate | 0.020 | 96.9 | 13.5 | 0.100 | 114.1 | 12.4 | 0.3 | 0.9 |
| Esfenvalerate | 0.020 | 88.5 | 11.1 | 0.100 | 123.3 | 10.6 | 0.3 | 1.1 |
| Difenoconazole | 0.010 | 82.1 | 9.0 | 0.050 | 92.2 | 11.8 | 0.1 | 0.2 |
| Deltramethrin | 0.050 | 105.1 | 9.2 | 0.250 | 118.7 | 10.6 | 0.1 | 1.2 |
| Azoxistrobina | 0.050 | 76.9 | 7.2 | 0.250 | 70.7 | 12.5 | 0.1 | 0.4 |

obtained in the final experimental conditions were stored in our own-made MS–MS library.

### 3.5. Validation of the method

In order to check the feasibility of the LP-GC method for the analysis of pesticide residues in fresh vegetable sample extracts, it was validated using cucumber extracts. Cucumber was selected as a representative commodity for the validation of the method for the determination of pesticide residues in matrices of high water content, like the ones studied here, according to the SANCO guide [23].

#### 3.5.1. Identification and confirmation of target analytes

The identification of the pesticides was based on the retention time windows (RTW) that are defined as the retention time average±3 S.D.s of the retention time when 10 blank samples spiked at the second calibration level of each compound were analysed. The confirmation of a previously identified compound was done by comparing the MS–MS spectra obtained in the sample with another stored as reference spectrum in the same experimental conditions. The reference spectra were obtained daily by injecting a blank cucumber sample spiked at the concentration of the second calibration point.

#### 3.5.2. Quantitation of target analytes

##### 3.5.2.1. Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ values were calculated through the definition based on the standard deviation of the signal of the blank (in our study, blank cucumber extracts) injections following IUPAC recommendations [24]. LOD values ranging from 0.02 to 4 μg kg$^{-1}$ and LOQ ranging from 0.06 to 13 μg kg$^{-1}$ were obtained (Table 3). The exception was disulfoton, which showed higher LOD and LOQ values (10 and 22 μg kg$^{-1}$, respectively)

*3.5.2.2. Linearity*

The linearity of the method was determined by injecting 10 μl of spiked blank matrix extracts. Linear calibration graphs were constructed by least-squares regression of concentration versus peak area and height ratio (analyte/I.S.) of the calibration standards. Slightly better results were achieved using relative areas for all compounds. Table 2 summarises the slopes, intercepts and correlation coefficient values for the validation study. Good linearity was found in the concentration range studied, with correlation coefficients between 0.97 and 0.99. For all compounds, the first point of the calibration curve was set at a concentration between the LOQ and the smallest MRL found for the vegetables studied and the different EU legislation. In cases like acephate, when the LOQ was not much lower than minimum MRL, the first calibration point was the MRL. On the other hand, for all pesticides, the highest calibration concentration was set to 15 times the first calibration concentration.

*3.5.2.3. Accuracy and precision*

Recovery efficiency data were obtained by analysing uncontaminated cucumber extracts ($n=10$) spiked at two different concentration levels (Table 3). Recoveries higher than 70.0% were obtained for all pesticides. We assumed, as a criterion for validation of the compounds, recoveries between 70 and 130%. These values indicated acceptable recovery for the assay procedure. The precision (repeatability, $n=10$) of the overall method was also evaluated at two concentration levels, and expressed as relative standard deviation (RSD). Table 3 shows the results with RSD values lower than 17% for all pesticides, except for dichlorvos (25.5%) at the lower spiking level.

### *3.6. Application to the analysis of real samples*

In order to test the feasibility of the LP-GC–MS–MS approach for routine analysis of pesticide residues in real samples and to compare it with conven-

Table 3
Results obtained (mg $kg^{-1}$) in the analysis of tomato (T) samples by LP-GC and conventional GC (values in parenthesis)

| Pesticide | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Endosulfan α | 0.015 (0.014) | | | | | | | | | | |
| Chlorothalonil | 0.058 (0.105) | 0.033 (0.075) | 0.113 (0.173) | | 0.090 (0.090) | | | | | 0.141 (0.127) | 0.878 (0.878) |
| Iprodione | 0.129 (0.154) | 0.095 (0.068) | | | | 0.176 (0.150) | | | | | |
| Buprofezin | 0.001 (0.029) | | | | | 0.009 (0.016) | | | | | |
| Procimidone | | | | 0.318 (0.317) | | | | | | | 0.454 (0.434) |
| Oxadixyl | | | | | 0.012 (0.011) | | | 0.050 (0.048) | | 0.024 (0.027) | 0.014 (0.018) |
| Pirimiphos-m | | | | | | 0.051 (0.042) | 0.050 (0.048) | | | | |
| Tebuconazole | | | | | | | | 0.093 (0.079) | | | 0.018 (0.017) |
| Piriproxifen | | | | | | | | | | | 0.019 (0.014) |
| Fludioxinil | | | | | | | | | 0.035 (0.034) | | |
| Bifenthrin | | | | | | | | | 0.017 (0.011) | | |
| Metalaxyl | | | | | | | | | | 0.037 (0.038) | |

Table 4
Results obtained (mg $kg^{-1}$) in the analysis of cucumber (C) and pepper (P) samples by LP-GC and conventional GC (values in parenthesis)

| Pesticide | C1 | C2 | C3 | C4 | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endosulfan α | 0.049 (0.041) | 0.015 (0.013) | | 0.009 (0.001) | 0.064 (0.051) | 0.003 (0.015) | | 0.012 (0.020) | 0.015 (0.013) | | | 0.022 (0.022) | | |
| Endosulfan β | | | | 0.019 (0.007) | | | | 0.032 (0.025) | 0.018 (0.019) | | | 0.031 (0.024) | | |
| Endosulfan sulfate | 0.027 (0.036) | | | | | | | 0.026 (0.026) | | | | | | |
| Acrinathrin | | | 0.032 (0.023) | | | 0.006 (0.006) | 0.006 (0.010) | | | | | | | |
| Permethrin | | | 0.318 (0.317) | | | | | | | | | | | |
| Chlorothalonil | | | 0.033 (0.033) | 0.012 (0.043) | | | | | | | | | | |
| Iprodione | | | | | | | 0.077 (0.125) | | | 0.109 (0.106) | 0.109 (0.106) | | | |
| Procymidone | | | | | | | | | | | 0.086 (0.098) | 0.076 (0.090) | | 0.136 (0.118) |
| Buprofezin | | | | | | 0.002 (0.002) | | | 0.003 (0.005) | | | | | |
| Piridaben | | | | | | | 0.016 (0.013) | 0.011 (0.012) | | | 0.001 (0.005) | | | |
| Pirimiphos-m | | | | | | 0.010 (0.027) | | 0.107 (0.110) | | | | 0.007 (0.011) | | |
| Tebuconazole | | | | | | 0.032 (0.031) | | | | | | | | 0.007 (0.015) |
| Fludioxinil | | | | | | 0.007 (0.012) | | | | 0.002 (0.002) | | 0.027 (0.033) | | |
| Bifenthrin | | | | | | | | 0.007 (0.012) | | 0.011 (0.012) | | | | |
| Fenpropatrin | | | | | | | | | | | | 0.025 (0.025) | | |
| Cypermethrin | | | | | | | | | | | | 0.028 (0.064) | | |
| Pyremethanil | | | | | | | | | | | | 0.005 (0.017) | | |
| Nuarimol | | | | | | | | | | | | | 0.037 (0.033) | |

tional GC–MS–MS, 25 samples of vegetables (cucumber, tomato and pepper) were analysed for the target compounds. All samples came from CUAM laboratory located in Almería, where they were previously analysed by GC–MS–MS with positive residues (a total of 70 positive residues). Tables 3 and 4 summarise the results obtained by both approaches. The same positive pesticide residues were detected in the samples by LP-GC. Endosulfan α (36%), chlorothalonil (32%), iprodione (26%) and procymidone (20%) occurred more frequently followed by endosulfan β, buprofezin, oxadixil, bifentrin and tebuconazol (16%), pirimifos methyl, acrinathrin and fluodixil (12%), and finally, endosulfan sulfate, metalaxyl, nuarimol, fenpropathrin, pyriproxifen, permethrin and cypermethrin (4%). The rest of the target pesticides were not found in any of the analysed samples. Only one of the positive residues exceeded the EU regulation, and specifically for the acrinathrin pesticide, by the two methods.

In general, differences lower than 0.01 mg $kg^{-1}$ were obtained. A higher disagreement was obtained for the chlorothalonil pesticide, which always showed lower values by LP-GC. However, in any case the chlorothalonil residues detected exceeded the MRLs.

## 4. Conclusions

A multiresidue method by LP-GC–MS–MS has been validated for the determination of pesticides in fresh vegetable samples. The introduction of a precolumn and carbofrit in the chromatographic system did not show any problem in LP-GC, and in addition avoided the application of previous clean-up steps to the complex extracts. The effectiveness of this approach for routine analysis was evaluated by its application to real samples that had been previously analysed by GC–MS–MS. The excellent agreement between the results demonstrates the applicability of the LP-GC–MS–MS to routine analysis.

In future, the application of LP-GC–MS–MS methods, as described in this paper, will change conventional capillary GC–MS–MS methods in monitoring laboratories. Analysis that in the past needed more than 1 h might now be performed in half that time with this approach.

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