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Potentiality of Gas Chromatography−Triple Quadrupole Mass Spectrometry in Vanguard and Rearguard Methods of Pesticide Residues in Vegetables

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A new analytical strategy for the screening and confirmation/quantification of multiclass pesticide residues in vegetables has been established and validated. No complicated sample preparation was needed, but only a simple and rapid extraction using ethyl acetate and sodium sulfate, which required no cleanup. The approach is based on the use of the triple quadrupole (QqQ) mass spectrometry (MS) as detection system in gas chromatography (GC). In a first step, a GC-QqQ-MS screening method, which monitors only one MS/MS transition by compound, allows the identification of ~130 pesticides in 11.6 min. In this way, the differentiation between negative and potentially nonnegative samples is carried out. In the second step, the nonnegative samples are reanalyzed by the GC-QqQ-MS confirmation/quantification method, which monitors two or three MS/MS transitions by compound. Confirmation of pesticides was based on the comparison of intensity ratios for the main ions in samples with those obtained on the same day from the standard in a matrix containing the pesticides at a preestablished concentration level. Quantification of the identified and confirmed pesticides was based on the addition standard method, which avoids matrix effect. The proposed analytical strategy allowed a reliable identification and confirmation of the target pesticides at trace levels, reducing analysis time and increasing sample throughput in routine analytical laboratories.

Analytical chemistry can be viewed as the link between the client's problem and the chemistry metrology, selecting an appropriate analytical strategy to obtain results that will be fit for the purpose intended. Frequently, the solution of an analytical problem has to be placed on minimizing time, costs, reagents, labor, and hazards that leads to greater expeditiousness and cost-effectiveness, obtaining immediate analytical results in response to an urgent demand. This is particularly important in both the environmental and clinical fields, in which a situation of some urgency requires rapid information to make an appropriate decision. In this context, one of the trends in analytical chemistry is the development of rapid (new) methodologies that can reliably identify and quantify the analytes in complex samples at trace levels. Taking into account that, in routine analysis, a large proportion of samples may not actually contain the target compounds, rapid methodologies, such as screening methods, providing a reliable binary (yes/no, positive/negative, or presence/absence) response are of increasing interest. These methods rapidly identify whether the target compounds are present above or below a preestablished concentration level. Following this, positive samples must be analyzed by a confirmation/quantification method in order to obtain another confirmation of compound identity, as well as to quantify analyte concentrations.

Nowadays, routine laboratories of pesticide residues in foods are increasingly interested in applying methods that provide a reliable and rapid binary response in order to select those samples where the target analytes are present. In these laboratories, the control of pesticide residues in food commodities has become a requirement to enforce legislation,[1−4] for instance, for ensuring international trade or in local, regional, or national surveillance programs. Therefore, to respond to this increasing number of samples that must be analyzed with a rapid turnaround time (imported food, processed foods, pesticide regulations), screening methods are of great interest. In addition, analytical methodologies applied must be adequate to identify and accurately quantify the concentration of any pesticide residue detected, usually at very low levels, as well as being able to determine as many pesticide residues as possible from the hundreds of pesticides commonly used in modern agricultural practices.

The ideal screening method is when it can be used without a full sample treatment and also when its binary response is obtained in a direct way.[5] However, most of them need some sample

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(1) Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs.
(2) Commission Regulation (EC) No. 645/2000 of 28 March 2000 setting out detailed implementing rules necessary for the proper functioning of certain provisions of Article 7 of Council Directive 86/362/EEC and of Article 4 of Council Directive 90/642/EEC concerning the arrangements for monitoring the maximum levels of pesticide residues in and on cereals and products of plant origin, including fruit and vegetables, respectively.
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pretreatment (extraction, isolation, cleanup) or data treatment in order to convert the instrumental signal into a binary response. In the field of pesticide analysis, the overwhelming majority of direct screening methods described in the literature are related to their determination in environmental matrixes.[6−10] Nowadays, it is not possible to have a direct screening of food commodities for pesticides without a previous treatment of the samples. Screening methods that have been reported to filter the samples, i.e., giving a binary response, for the pesticide analysis in such matrixes, allow the determination of only one or two analytes using immunoassay methods, as enzyme-linked immunosorbent assay[11−14] or fluorescence polarization immunoassay.[15] Screening methods should provide information for a great number of multiclass pesticides, and not restricted to single pesticides, a few analytes, or even only an analyte family. On the other hand, a literature survey for published papers, including screening in their titles, has revealed that most of them are mainly focused on the simplification of the sample preparation steps (preconcentration/cleanup) with a minimum waste of organic solvents to get a very short sample pretreatment time. In addition to this common feature, all of them are also based in the use of a chromatographic technique.[16−20]

Presently, gas chromatography (GC) coupled with mass spectrometry (MS) seems to be the technique of choice for analysis of pesticides in food commodities. The majority of these procedures involve the simple quadrupole mass spectrometer operating in selected ion monitoring mode[16−18,20] or the ion trap mass spectrometer operating in tandem (MS/MS) mode.[21−25] The latter mode shows generally a better sensitivity and selectivity than the first one in detection of pesticides at trace levels in complex matrixes. Also, ion trap GC/MS/MS methods allow both a great confidence in the identification of peaks and an excellent quantitation of positive results. However, a major drawback of ion trap analyzers is that the product ion scan is the only available in MS/MS scan mode. This allows the determination of all the product ions generated from a selected precursor ion of a chosen mass-to-charge ratio. In practice, this MS/MS scan limits the number of overlapping compounds (no more than four or five target analytes) that can be simultaneously determined by obtaining chromatographic peaks with sufficient points.[26] Therefore, it is very important in these methods to optimize the gas chromatographic conditions in order to reach an appropriate separation between analytes while, at the same time, maintaining the total time of analysis as short as possible. Because of this limitation, ion trap GC/MS/MS methods allow analysis of up to ∼80 compounds in a single run of ∼1 h.[22] On the other hand, approaches to fast GC/MS have the characteristics of increasing speed of analysis.[27] However, few real applications[28−30] have been met in practice because other operations such as sample preparation limit overall analysis time.

GC coupled with triple quadrupole (QqQ) mass spectrometry is an alternative strategy for the rapid determination of trace-level analytes. An advantage of this technique is the possibility of operating in the four main scan modes available using MS/MS. These are product ion scans, precursor ion scans, neutral loss scans, and selected reaction monitoring (SRM). QqQ can increase the number of coeluted pesticides to be analyzed if it is operated in SRM that only monitors a few selected product ions per analyte. The absence of scanning allows the instrument to be focused on the precursor and fragment ions over longer times, increasing the sensitivity as for SRM. However, this greater sensitivity is now associated with a high increase in selectivity with respect to SRM using a single analyzer.

The aim of this paper is the development of a new analytical strategy based on the use of the QqQ as detector in GC for the determination of ∼130 pesticides in vegetable samples in a short analysis time. This new approach has two steps, the first of which is the development of a rapid screening method that allows the identification of the target pesticides in less than 12 min. This allows the differentiation between samples with pesticides above or below a preestablished concentration level achieved by monitoring for one ion of a fragmentation reaction for each compound. The second step is the confirmation and quantification of the compounds previously identified in the potentially positive samples. Confirmation is based on the monitoring of two or three product ions produced in two or three selected reactions, with quantification carried out by the addition standard method (using the quantification ion and relating peak areas to an internal standard). Both steps are based on the use of the QqQ mass spectrometer, which has less limitation in the number of analytes per segment and shorter analysis times than ion trap mass spectrometers. It could be said that in the first step the GC-QqQ-MS is used as a vanguard method and in the second one as a rearguard method.

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Quality of the results is not only related to the achievement of a high level of performance parameters of the confirmation/quantification method, but also it must to be related to satisfactory values of analytical features of the screening (vanguard) method. This means that both methods must be validated before they are used in the laboratory. In this paper, performance parameters of both the screening and the confirmation methods have been calculated in order to ensure the quality of the results. Finally, to assess the applicability of the new analytical strategy to the analysis of samples, spiked samples were analyzed and good results were obtained.

EXPERIMENTAL SECTION

Reagents and Chemicals. Pesticide standards and the internal standard (IS), caffeine, were purchased from Riedel-de-Haën (Seelze-Hannover, Germany); purities were always >99%. Panreac (Barcelona, Spain) supplied pesticide-quality solvents (acetone, ethyl acetate, cyclohexane). Stock standard solutions of individual compounds (with concentrations between 400 and 550 μg/mL) were prepared by exact weighing of the powder or liquid and dissolution in 100 mL of acetone, which were then stored in a freezer (−30 °C). A multicompound working standard solution (2 μg/mL concentration of each compound) was prepared by appropriate dilutions of the stock solutions with acetone and stored under refrigeration (4 °C).

Instrumentation. A GC system Varian 3800 (Varian Instruments, Sunnyvale, CA) equipped with electronic flow control was used throught the study. Samples were injected with a Combi Pal (CTC Analytics AG, Zwingen, Switzerland) into an SPI/1079 split/splitless programmed-temperature injector using a 10-μL syringe. The glass liner was equipped with a plug of Carbofrit (Resteck, Bellefonte, PA). A fused-silica untreated capillary column 2 m × 0.25 mm i.d. from Supelco (Bellefonte, PA) was used as guard column connected to a FactorFour Capillary Column VF-5ms (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Varian Instruments. Helium (99.9999%) at a flow rate of 1 mL/min was used as carrier gas. The GC was interfaced with a Varian 1200L triple quadrupole mass spectrometer operated with an electron ionization (EI, 70 eV) ion source. Positive and negative chemical ionization was also available. From the ioniation source, the ions passed through a hexapole ion guide to the mass analyzers (mass range from 10 to 1500 u). The curved collision cell presented a 180° path. Argon (99.999%) was used as collision gas. The computer controlling the system held an EI-MS-MS library created specifically for the target analytes under our experimental conditions. Other EI-MS libraries were also available. The mass spectrometer was calibrated weekly with perfluorotributylamine.

A stainless steel Polytron benchtop homogenizer (Kinematica AG, Luzern, Switzerland) was used for blending the samples at 20 000 rpm.

Chromatographic Conditions. Aliquots of sample solution (10 μL) were injected into the gas chromatographic system operating at a syringe injection flow rate of 10 μL/min. The initial injector temperature was set at 200 °C during injection, with a 2-min hold, and then increased at a rate of 200 °C/min to 300 °C, at which point it was held for 1.5 min. The injector split ratio was initially 20:1. At 0.01 min, splitless mode was switched on until minute 2. At 2 min the split ratio was 100:1, and at 4 min the split ratio was 20:1. After injection, the column temperature was set at 70 °C, with a 2-min hold, and then increased at a rate of 50 °C/min to 300 °C, which was held for 5 min.

The QqQ mass spectrometer was operated in MS/MS mode (SRM). The temperatures of the transfer line, manifold, and source of ionization were set at 280, 40, and 250 °C, respectively. The analysis was performed with a filament-multiplier delay of 3.5 min in order to prevent instrument damage. The scan time was 0.25 s for all segments. The specific MS/MS parameters used are shown in Table 1.

Sample Procedure. A 2-kg mass of vegetable sample was chopped and homogenized. An aliquot of 5 g was exactly weighed into a glass beaker and mixed with 10 mL of ethyl acetate in the Polytron for 1 min. The extract was filtered through a homemade cartridge containing a glass wool plug and ~3 g of anhydrous sodium sulfate. Following this, the filter was washed with 2 mL of ethyl acetate. The filtered extract and the rinse were collected into a test tube. Evaporation of the solvent to dryness was done with a stream of N_2. The dried residue was redissolved with 1000 μL of cyclohexane containing 0.5 μg/mL caffeine used as IS. Aliquots of this extract were used for the GC/MS/MS screening method.

Screening Method. The liquid injected into the column of the GC system consisted of 10 μL of the previous extract as well as a standard mixture of the target pesticides in the matrix at the preestablished concentration levels. Only one selected product ion was monitored for each compound. The relative instrumental response of the analyte to that of the IS, *y*, was used to identify the target pesticides by searching in its corresponding relative retention time windows (RRTWs). If none of the target compounds were identified, this sample was considered as negative and it would mean the end of the analysis. On the contrary, if some of the target pesticides were identified in its RRTW, this sample was considered as potentially nonnegative. Then, the *y* of the identified analytes was compared with the ones corresponding to the lower limit of the unreliability region. Samples with *y* values lower than the lower limit were considered as negative, while samples with values equal or higher than the lower limit were classified as nonnegative samples. These samples should be confirmed by the confirmation/quantification method.

Confirmation/Quantification Method. The 10 μL of extract of the potentially nonnegative samples was reinjected into the GC system set up for the confirmation/quantification method, monitoring only a few (two or three) selected product ions for each of the compounds previously identified. These compounds were confirmed if the tolerance of 20% for the relative intensities of the two or three selected product ions in the samples and in the standards was met. In this case, samples should be labeled as nonnegatives and should be quantified by the standard addition method. This was done using known amounts of the standard(s) of the previously confirmed pesticide(s), which were added to two test aliquots of the sample before analysis.

Internal Quality Criteria. To ensure the quality of results when the proposed methodology is applied to routine analysis, various internal quality criteria have been established. The set of samples analyzed each day was processed together with the following: (i) the analysis of a blank extract to eliminate a false positive, caused by contamination in the extraction process,

Table 1. Conditions MS/MS

pesticide	seg	precursor ion (*m/z*)	product ion,[a] *m/z* (V)
methamidophos	1	141	94* (−10), 79 (−30)
1,2-dichlorobenzene	1	146	111* (−30), 75 (−40)
1,2,4-trichlorobenzene	1	180	145* (−30), 109(−50)
trichlorfon	1	185	93* (−20), 108 (−30)
dichlorvos	1	185	109* (−50), 145 (−30)
acephate	2	136	94* (−20), 70−140(−20)
propoxur	2	152	110* (−10), 90−160(−10)
omethoate	2	156	110* (−10), 79 (−30)
molinate	2	187	126* (−10), 83 (−30), 98 (−30)
mevinphos	2	192	164* (−10), 127 (−30)
etoprophos	2	199	129* (−20), 97 (−40)
heptenophos	2	215	109* (−20), 89 (−30)
thionazin	2	248	140* (−10), 106 (−20)
pentachlorobenzeno	2	250	180* (−40), 145 (−50),215 (−20)
phorate	2	260	75* (−10), 129 (−50)
sulfotepp	2	322	202* (−20), 146 (−30), 174 (−20)
pirimicarb	3	166	96* (−10). 80−170(−10)
pyrimethanil	3	198	183* (−20), 118 (−50)
simazine	3	201	173* (−10), 158 (−20)
dicloran	3	206	176* (−40), 124 (−20)
terbuthylazine	3	214	104* (−30), 83 (−30), 132 (−50)
atrazine	3	217	202* (−10), 160 (−20), 174 (−20)
lindane	3	219	183* (−10), 147 (−40), 109 (−50)
carbofurane	3	221	164* (−10), 149 (−50), 122 (−20)
dimethoate	3	125	79* (−30), 93 (−20)
fosfamidone	3	264	109* (−50), 95 (−50)
chlorthalonil	3	266	133* (−50), 160 (−30), 231 (−40)
disulfoton	3	274	88* (−10), 97 (−50)
hexachlorobenzene	3	284	249* (−20), 177 (−50), 214 (−40)
etrimphos	3	292	281* (−10), 153 (−30), 125 (−50)
quintocene	3	297	267* (−10), 239 (−20)
diazinon	3	304	179* (−20), 137 (−50)
malathion	4	173	99* (−20), 127 (−10), 145 (−10)
cyromazine	4	151	109* (−20), 82 (−30)
caffeine	4	194	109* (−20), 120 (−50), 137 (−30)
metalaxyl	4	206	132* (−20), 104 (−40), 117 (−50)
triadimefon	4	209	182* (−10), 112 (−40), 127 (−20)
formothion	4	224	155 (−10), 109 (−50), 125 (−20)
ethiofencarb	4	225	168* (−10), 107 (−20)
endosulfan ether	4	241	170* (−40), 207(−50)
terbutryn	4	241	185* (−10), 111 (−50), 170 (−20)
hepthachlor	4	272	237* (−20), 165 (−50), 141 (−50)
fenitrothion	4	277	260* (−10), 109 (−20), 125 (−20)
fenthion	4	278	109* (−20), 125 (−40), 169 (−40)
vinclozoline	4	285	212* (−10), 145 (−40), 198 (−30)
chlorpyriphos methyl	4	286	208* (−10), 112 (−40), 127 (−20)
parathion ethyl	4	291	137* (−10), 81 (−30), 109 (−10)
pirimiphos methyl	4	305	290* (−20), 125 (−50), 180 (−10)
chlorpyriphos ethyl	4	314	258* (−20), 286 (−20)
dichlofluanide	4	332	123* (−30), 167 (−10)
tetraconazole	4	336	155* (−10), 204 (−50), 217 (−40)
triadimenol	5	168	168* (−10), 81 (−20)
isofenphos	5	213	185* (−5), 121 (−10)
anilazina	5	239	178* (−20), 142 (−50), 116 (−50)
penconazole	5	248	192* (−20), 157 (−50), 206 (−20)
dicofol	5	251	216* (−10), 111 (−40), 139 (−20)
pendimethalin	5	252	162* (−20), 118 (−50), 206 (−50)
chlozolinate	5	259	188* (−20), 145 (−50), 153 (−40)
pyrifenox	5	263	228* (−20), 116 (−40), 201 (−30)
parathion methyl	5	263	246* (−1), 109 (−10), 153 (−1)
procymidone	5	283	185* (−50), 145 (−50), 96 (−20)
triflumizol	5	287	218* (−10), 193 (−50)
aldrin	5	291	256* (−20), 185 (−50), 221 (−20)
endosufan lactone	5	321	267* (−10), 159 (−50), 296 (−10)
chlorfenvinphos	5	324	267* (−20), 159 (−50), 296 (−10)
heptachlor epoxide	5	353	253* (−30), 289 (−10), 217 (−40)
fipronil	5	367	213* (−30), 255 (−30)
quinalphos	6	146	118 (−20), 90 (−40)
buprofezin	6	172	131* (−10), 115 (−20)
tolilfluanide	6	181	137* (−30), 91 (−50)
kresoxin methyl	6	206	131* (−20), 89 (−40)
michlobuthanil	6	206	179* (−10), 150 (−40)
quinomethionate	6	234	206* (−10), 116 (−40), 148 (−40)
endosulfan α	6	241	241* (−20), 133 (−40), 170 (−40)
endosufan *β*	6	241	241* (−20), 133 (−40), 170 (−40)
fludioxinil	6	248	183* (−20), 127 (−50), 154 (−20)
captan	6	263	107* (−10), 148 (−20)
bupimirate	6	273	193* (−10), 108 (−20), 150 (−10)
methidathion	6	302	145* (−10), 85 (−30)
fenamimphos	6	303	195* (−10), 260 (−20), 153 (−40)
p,p-DDE	6	318	247* (−20), 177 (−50)
propenofox	6	338	267* (−20), 249 (−40)
oxyfluorfen	6	361	300* (−20), 252 (−40), 317 (−10)
benalaxyl	7	148	118* (−30), 105 (−30)
fosmet	7	160	133* (−10), 104 (−20)
oxadixyl	7	163	132* (−20), 105 (−40), 117 (−40)
tetramethrin	7	164	135* (−10), 107 (−30), 93 (−10)
acrinathrin	7	181	152* (−40), 126 (−50)
cihalothrin	7	181	152* (−30), 126 (−50)
bifenthrin	7	181	165* (−40), 141 (−20), 153 (−20)
fenoxycarb	7	186	157* (−20), 109 (−30), 77 (−40)
iprodione	7	187	124* (−40), 159 (−30)
furatiocarb	7	194	161* (−10), 151 (−30), 105 (−40)
hexaconazole	7	215	159* (−30), 145 (−50), 124 (−50)
famfur	7	218	109* (−30), 93 (−10)
ciproconazole	7	222	125* (−30), 82 (−10)
methoxichlor	7	227	169* (−40), 140 (−50), 115 (−50)
ethion	7	231	175* (−20), 129 (−30), 185 (−10)
p,p′-DDD	7	235	165* (−30), 199 (−20)
p,p′-DDT	7	235	165* (−40), 199 (−20)
o,p′-DDT	7	236	165* (−35), 201 (−10)
tebuconazole	7	250	125* (−30), 153 (−10), 163 (−10)
propiconazole	7	259	173* (−20), 145 (−50), 191 (−20)
fenpropathrin	7	265	210* (−20), 89 (−40), 181 (−50)
endosulfan sulfate	7	272	237* (−10), 165 (−50)
dieldrin	7	277	206* (−20), 170 (−50), 241 (−30)
endrin	7	281	211* (−30), 245 (−20), 208 (−40)
norflurazon	7	303	145* (320), 173 (−10)
butoxide of piperonil	7	338	176* (−10), 117 (−40), 131 (−40)
bromopropylate	7	341	183* (−30), 155 (−50)
carbofenothion	7	342	157* (−10), 97 (−50), 143 (−50)
endrin aldehyde	7	345	245*(−40), 281(−30),101(−20)
piriproxifen	8	136	96* (−10), 70−140 (−10)
azinphos methyl	8	160	132* (−10), 105 (−20)
cipermethrin	8	163	127* (−10), 91 (−20)
fosalone	8	182	102* (−30), 111 (−20), 138 (−10)
permethrin	8	183	153* (−30), 127 (−40), 115 (−50)
flucythrinate	8	199	157* (−10), 107 (−40)
cifluthrin	8	206	150* (−50), 176 (−40)
pyrazophos	8	221	193* (−20), 148 (−30), 138 (−30)
esfenvalerate	8	225	119* (−30), 147 (−30)
tetradifon	8	227	199* (−20), 143 (−40), 164 (−30)
deltramethrin	8	253	172* (−10), 118 (−40), 198 (−30)
mirex	8	272	237* (−20), 140 (−40), 167 (−40)
tebufenocide	8	278	193* (−10), 133 (−40)
pyridaben	8	309	147* (−20), 117 (−50), 132 (−50)
difeconazole	8	323	265* (−20), 201 (−50)
feranimol	8	330	139* (−20), 111 (−50)
azoxistrobine	8	344	329* (−30), 172 (−50), 156 (−40)

[a] Asterisk indicates screening transition.

instrument, or chemicals used; (ii) the analysis of a blank sample spiked immediately prior to the instrumental determination with standards of the pesticides at the preestablished concentration level of each compound to state the response to this level; and (iii) the analysis of a blank vegetable spiked with the standards of the pesticides at the preestablished concentration level and then extracted to check the recovery of the extraction step. The efficiency of the extraction procedure is checked by comparison with the responses of the standard. Analysis of samples within the sequence was performed if recoveries were between 60 and

Table 2. Relative Retention Time Windows (RRTWs), Recovery, and Intraday Precision at 10 mg/kg and Detection Limits (LOD) for the Target Pesticides

pesticide	RRTW (min)	% R^a	(% RSD)[a]	LOD[b] (μg/kg)	pesticide	RRTW (min)	% R^a	(% RSD)[a]	LOD[b] (μg/kg)
caffeine[c]	1.00−1.03				heptachlor epoxide	1.07−1.09	91.8	11.1	0.19
methamidophos	0.76−0.78	90.8	7.3	0.17	fipronil	1.08−1.09	76.4	10.1	0.19
1,2-dichlorobenzene	0.60−0.62	85.6	14.6	0.01	quinalphos	1.12−1.13	109.2	12.4	0.13
1,2,4-trichlorobenzene	0.71−0.73	75.3	16.9	0.01	buprofezin	1.13−1.14	100.0	10.6	0.05
trichlorfon	0.74−0.75	104.1	12.7	0.01	tolilfluanide	1.13−1.15	81.1	11.8	0.16
dichlorvos	0.74−0.75	101.2	12.4	0.06	kresoxin methyl	1.11−1.13	93.4	10.6	0.02
acephate	0.84−0.86	92.9	11.4	0.11	michlobuthanil	1.12−1.14	89.0	12.5	0.10
propoxur	0.82−0.84	85.3	11.2	0.07	quinomethionate	1.11−1.12	109.1	7.0	0.01
omethoate	0.90−0.92	101.1	14.0	0.14	endosulfan α	1.12−1.13	109.5	11.3	0.39
molinate	0.90−0.92	97.0	14.5	0.01	endosufan β	1.12−1.13	104.3	12.5	0.35
mevinphos	0.88−0.90	103.2	10.5	0.01	fludioxinil	1.12−1.14	79.8	13.2	0.09
etoprofos	0.91−0.93	90.8	8.0	0.14	captan	1.12−1.13	74.3	15.3	0.17
heptenophos	0.89−0.90	81.1	11.2	0.14	bupimirate	1.12−1.13	107.7	13.1	0.01
thionazin	0.90−0.92	99.2	8.1	0.26	methidathion	1.11−1.12	72.5	8.8	0.28
pentachlorobenzene	0.88−0.89	92.9	12.8	0.03	fenamimphos	1.10−1.12	75.8	12.7	0.12
phorate	0.94−0.95	80.9	13.1	0.64	*p,p*-DDE	1.12−1.13	70.6	11.1	0.05
sulfotepp	0.92−0.94	73.0	14.5	0.02	propenofox	1.12−1.13	107.7	11.6	0.81
cyromazine	0.96−1.01	94.7	12.3	0.21	oxyfluorfen	1.11−1.12	97.3	10.7	0.08
pirimicarb	0.99−1.00	107.1	9.3	0.34	benalaxyl	1.18−1.19	106.3	18.9	0.28
pyrimethanil	0.98−0.99	70.9	8.6	0.13	fosmet	1.25−1.27	101.5	11.5	0.12
simazine	0.96−0.97	85.3	14.0	0.12	oxadixyl	1.16−1.17	105.1	7.9	0.57
dicloran	0.97−0.98	79.8	7.8	0.04	tetramethrin	1.23−1.24	101.2	5.9	0.16
terbuthylazine	0.97−0.98	97.6	7.7	0.04	acrinathrin	1.24−1.25	102.8	8.1	0.32
atrazine	0.96−0.97	99.3	7.8	0.03	cihalothrin	1.24−1.25	105.7	11.6	0.27
lindane	0.98−0.99	102.3	10.0	0.65	bifenthrin	1.22−1.24	109.3	12.2	0.02
carbofurane	0.95−0.97	70.1	9.7	0.02	fenoxycarb	1.25−1.26	101.3	13.9	0.10
dimethoate	0.97−0.98	82.9	18.5	0.29	iprodione	1.20−1.22	104.1	7.4	0.20
fosfamidone	0.99−1.00	99.3	9.2	0.15	furatiocarb	1.26−1.27	104.0	11.8	0.11
chlorthalonil	0.99−1.00	97.1	12.4	0.27	hexaconazole	1.07−1.08	106.8	7.9	0.38
disulfoton	0.98−1.00	106.9	11.3	0.02	famfur	1.18−1.19	109.2	14.0	0.09
hexachlorobenzene	0.96−0.98	70.2	9.2	0.01	ciproconazole	1.14−1.18	105.1	9.1	0.05
etrimphos	0.98−0.99	78.1	13.4	0.01	methoxichlor	1.20−1.21	72.9	15.0	0.22
quintocene	0.98−0.99	74.7	9.3	0.15	ethion	1.14−1.19	105.6	7.7	0.03
diazinon	0.97−0.98	67.7	10.8	0.01	*p,p′*-DDD	1.16−1.17	100.6	9.9	0.09
malathion	1.03−1.04	94.8	9.9	0.01	*p,p′*-DDT	1.16−1.17	104.0	9.7	0.09
metalaxyl	1.02−1.03	86.1	10.1	0.00	*o,p′*-DDT	1.16−1.17	84.5	10.6	0.10
triadimefon	1.03−1.05	105.2	9.5	0.10	tebuconazole	1.22−1.23	102.6	13.5	0.40
formothion	0.98−1.05	106.9	8.8	0.13	propiconazole	1.18−1.20	104.3	11.1	0.05
ethiofencarb	0.99−1.05	99.3	9.4	0.12	fenpropathrin	1.24−1.26	108.6	9.0	0.08
endosulfan ether	1.03−1.05	72.1	10.4	0.04	endosulfan sulfate	1.15−1.17	81.6	9.2	0.10
terbutryn	1.01−1.02	109.7	9.2	0.12	dieldrin	1.15−1.20	109.3	7.2	0.10
hepthachlor	1.03−1.04	83.8	10.9	0.09	endrin	1.15−1.16	101.3	14.0	0.13
fenitrothion	1.00−1.04	77.5	7.2	0.02	norflurazon	1.19−1.20	70.4	7.8	0.55
fenthion	1.05−1.06	74.5	8.9	0.23	butoxide of piperonil	1.20−1.21	71.1	7.7	0.14
vinclozoline	1.01−1.02	77.0	7.6	0.05	bromopropylate	1.25−1.26	97.1	7.8	0.07
chlorpyriphos methyl	1.01−1.02	95.8	10.2	0.09	carbofenothion	1.19−1.20	87.6	10.0	0.08
parathion ethyl	1.05−1.06	67.6	14.0	0.02	endrin aldehyde	1.18−1.21	104.3	9.7	0.09
pirimiphos methyl	1.02−1.03	70.4	9.6	0.04	piriproxifen	1.29−1.30	84.9	18.5	0.03
chlorpyriphos ethyl	1.04−1.05	81.3	6.5	0.02	azinphos methyl	1.31−1.33	71.1	9.2	0.23
dichlofluanide	1.06−1.07	77.9	8.3	0.13	cipermethrin	1.37−1.38	97.2	12.4	0.13
tetraconazole	1.04−1.06	93.0	14.6	0.12	fosalone	1.29−1.31	104.5	11.3	0.03
triadimenol	1.09−1.11	70.8	13.5	0.13	permethrin	1.37−1.38	69.0	9.2	0.23
isofenphos	1.07−1.08	91.3	5.4	0.05	flucythrinate	1.49−1.50	80.4	13.4	1.15
anilazina	1.07−1.08	87.1	8.4	0.13	cifluthrin	1.43−1.43	71.1	9.3	2.35
penconazole	1.07−1.09	108.8	7.9	0.02	pyrazophos	1.32−1.33	108.2	10.8	1.62
dicofol	1.06−1.07	105.1	10.0	0.01	esfenvalerate	1.49−1.49	73.6	9.9	2.12
pendimethalin	1.05−1.12	103.1	7.1	0.03	tetradifon	1.29−1.30	85.1	10.1	0.58
chlozolinate	1.05−1.12	108.9	11.3	0.15	deltramethrin	1.71−1.72	108.1	9.5	2.65
pyrifenox	1.08−1.10	88.4	19.7	0.15	mirex	1.36−1.37	81.6	14.6	2.06
parathion methyl	1.08−1.10	70.8	7.7	0.01	tebufenocide	1.33−1.34	85.1	16.9	3.21
procymidone	1.08−1.10	77.9	10.2	0.06	pyridaben	1.40−1.41	108.9	12.7	0.95
triflumizol	1.08−1.09	83.2	5.1	0.34	difeconazole	1.68−1.69	85.1	12.4	2.95
aldrin	1.06−1.11	87.1	12.3	0.12	feranimol	1.35−1.36	109.4	11.4	1.36
endosulfan lactone	1.09−1.10	76.4	10.3	0.30	azoxistrobine	1.73−1.79	97.1	9.2	2.51
chlorfenvinphos	1.06−1.10	105.1	7.2	0.13					

[a] $n = 6$. [b] The lowest concentration injected that yielded a signal-to-noise (S/N) ratio of 3 when the quantification ion was monitored. [c] Retention time of caffeine (IS), 6.41 min.

110% for the spiked matrix, and interferences were not observed at the RRTWs of the target pesticides. Furthermore, in the case of pesticide confirmation, the correlation coefficient of the standard addition curve must be greater than or equal to 0.99.

RESULTS AND DISCUSSION

GC-QqQ-MS Parameters. The gas chromatographic separation of the target analytes in QqQ-based methods is not very critical because this MS/MS detection mode is able determine

Table 3. Number of Positives (*P*) and Negatives (*N*) Detected at Different Concentrations by the Screening Method, Relative Area at the Cutoff Value of Each Compound and Unreliability Interval for Each Compound[a]

compound	concn, μg/kg (*n* = 10) 5 *P*	5 *N*	8 *P*	8 *N*	10 *P*	10 *N*	rel area	unreliability interval (μg/kg) low value	unreliability interval (μg/kg) upper value
caffeine									
methamidophos[b]	0	10	0	10	8	2	0.005	5.6	14.4
1,2-dichlorobenzene[c]	0	10	2	8	6	4	0.001	7.8	22.2
1,2,4-trichlorobenzene[b]	4	6	6	4	9	1	0.025	4.4	14.4
trichlorfon	0	10	0	10	10	0	0.004	5.3	14.7
dichlorvos	0	10	2	8	10	0	0.007	6.8	13.2
acephate	0	10	0	10	10	0	0.013	6.0	14.0
propoxur	0	10	2	8	10	0	0.404	5.7	14.3
omethoate	0	10	0	10	10	0	0.007	6.4	13.6
molinate	0	10	0	10	10	0	0.024	5.7	14.3
mevinphos	0	10	0	10	10	0	0.021	4.9	15.1
etoprofos	0	10	0	10	10	0	0.002	6.4	13.6
heptenophos	0	10	4	6	10	0	0.001	3.7	16.3
thionazin	0	10	0	10	10	0	0.002	5.1	14.9
pentachlorobenzene	0	10	0	10	10	0	0.003	8.1	11.9
phorate	0	10	0	10	10	0	0.001	5.2	14.8
sulfotepp	4	6	6	4	10	0	0.008	7.2	12.8
cyromazine	0	10	0	10	10	0	0.003	4.2	15.8
pirimicarb	0	10	0	10	10	0	0.026	8.0	12.0
pyrimethanil	0	10	0	10	10	0	0.014	6.8	13.2
simazine	0	10	0	10	10	0	0.013	8.3	15.1
dicloran	0	10	2	8	10	0	0.001	6.2	13.8
terbuthylazine	0	10	0	10	10	0	0.003	7.3	12.7
atrazine	0	10	4	6	10	0	0.003	7.4	12.6
lindane	0	10	0	10	10	0	0.002	7.1	12.9
carbofurane	0	10	5	5	10	0	0.006	6.7	13.3
dimethoate	0	10	0	10	10	0	0.036	7.9	12.1
fosfamidone	0	10	0	10	10	0	0.013	4.9	15.1
chlorthalonil	0	10	0	10	10	0	0.002	6.2	13.8
disulfoton	2	8	5	5	10	0	0.004	6.6	13.4
hexachlorobenzene	2	8	5	5	10	0	0.006	7.8	12.2
etrimphos	0	10	0	10	10	0	0.017	6.8	13.2
quintocene	2	8	4	6	10	0	0.001	6.8	13.2
diazinon	0	10	4	6	10	0	0.014	6.8	13.2
malathion	2	8	0	10	10	0	0.019	6.8	13.2
metalaxyl	2	8	6	4	10	0	0.005	7.3	12.7
triadimefon	0	10	4	6	10	0	0.005	7.6	12.4
formothion	0	10	0	10	10	0	0.006	6.5	13.5
ethiofencarb	0	10	0	10	10	0	0.003	7.8	12.2
endosulfan ether	2	8	0	10	10	0	0.010	7.2	12.8
terbutryn	6	4	5	5	10	0	0.011	5.9	14.1
hepthachlor	2	8	0	10	10	0	0.006	7.2	12.8
fenitrothion	0	10	5	5	10	0	0.006	7.2	12.8
fenthion	0	10	5	5	10	0	0.017	7.2	12.8
vinclozoline	0	10	5	5	10	0	0.002	6.2	13.8
chlorpyriphos methyl	4	6	4	6	10	0	0.002	6.1	13.9
parathion ethyl	0	10	2	8	10	0	0.004	5.3	14.7
pirimiphos methyl	4	6	4	6	10	0	0.004	5.9	14.1
chlorpyriphos ethyl	0	10	5	5	10	0	0.005	7.4	12.6
dichlofluanide	0	10	0	10	10	0	0.007	8.1	11.9
tetraconazole	0	10	0	10	10	0	0.003	5.1	14.9
triadimenol	4	6	6	4	10	0	0.019	8.5	11.5
isofenphos	0	10	0	10	10	0	0.015	6.9	13.1
anilazina	0	10	0	10	10	0	0.007	8.1	11.9
penconazole	0	10	0	10	10	0	0.004	7.9	12.1
dicofol	4	6	0	10	10	0	0.015	5.9	14.1
pendimethalin	0	10	0	10	10	0	0.002	5.7	14.3
chlozolinate	0	10	0	10	10	0	0.001	7.2	12.8
pyrifenox	0	10	0	10	1	0	0.006	7.2	12.8
parathion methyl	0	10	0	10	10	0	0.004	5.3	14.7
procymidone	2	8	2	8	10	0	0.002	8.1	11.9
triflumizol	2	8	0	10	10	0	0.006	7.0	13.0
aldrin	0	10	0	10	10	0	0.006	7.2	12.8
endosufan lactone	0	10	2	8	10	0	0.003	5.5	14.5
chlorfenvinphos	0	10	0	10	10	0	0.003	6.5	13.5
heptachlor epoxide	2	8	8	2	10	0	0.006	6.2	13.8
fipronil	2	8	0	10	10	0	0.011	4.4	15.5
quinalphos	0	10	4	6	10	0	0.021	4.6	15.4
buprofezin	2	8	0	10	10	0	0.021	6.9	13.1
tolilfluanide	0	10	0	10	10	0	0.063	6.0	14.9
kresoxin methyl	0	10	0	10	10	0	0.026	7.0	13.0
michlobuthanil	1	9	0	10	10	0	0.004	5.9	14.1
quinomethionate	1	9	1	9	10	0	0.037	6.8	13.2
endosulfan α	0	10	0	10	10	0	0.003	6.0	14.0
endosufan β	0	10	0	10	10	0	0.003	6.0	14.0
fludioxinil	0	10	0	10	10	0	0.007	6.2	14.8
captan	0	10	2	8	10	0	0.044	5.8	14.2
bupimirate	0	10	0	10	10	0	0.055	6.9	13.1
methidathion	1	9	0	10	10	0	0.005	7.6	12.4
fenamimphos	0	10	0	10	10	0	0.071	7.7	12.3
p,p-DDE	2	8	0	10	10	0	0.007	5.6	14.4
propenofox	0	10	3	7	10	0	0.002	6.2	13.8
oxyfluorfen	0	10	1	9	10	0	0.006	8.2	11.8
benalaxyl	0	10	0	10	10	0	0.011	6.9	13.1
fosmet	1	9	0	10	10	0	0.014	7.7	12.3
oxadixyl	1	9	0	10	10	0	0.024	4.6	15.4
tetramethrin	0	10	0	10	10	0	0.025	4.3	15.7
acrinathrin	0	10	0	10	10	0	0.028	5.8	14.2
cihalothrin	0	10	0	10	10	0	0.006	5.9	14.1
bifenthrin	0	10	1	9	10	0	0.223	6.4	13.6
fenoxycarb	0	10	0	10	10	0	0.003	7.0	13.0
iprodione	0	10	0	10	10	0	0.006	5.6	14.4
furatiocarb	0	10	1	9	10	0	0.006	5.6	14.4
hexaconazole	2	8	1	9	10	0	0.002	6.9	13.1
famfur	0	10	1	9	10	0	0.004	5.8	14.0
ciproconazole	0	10	2	8	10	0	0.018	6.0	14.0
methoxichlor	0	10	1	9	10	0	0.003	6.3	13.7
ethion	0	10	2	8	10	0	0.030	4.4	15.6
p,p′-DDD	0	10	2	8	10	0	0.021	4.5	15.5
p,p′-DDT	0	10	2	8	10	0	0.021	4.4	15.6
o,p′-DDT	0	10	2	8	10	0	0.020	6.5	13.5
tebuconazole	1	9	1	9	10	0	0.011	5.5	14.5
propiconazole	0	10	0	10	10	0	0.014	7.3	12.7
fenpropathrin	0	10	0	10	10	0	0.007	6.8	13.2
endosulfan sulfate	0	10	3	7	10	0	0.003	6.0	14.0
dieldrin	0	10	0	10	10	0	0.002	6.6	13.4
endrin	0	10	0	10	10	0	0.028	4.5	15.5
norflurazon	0	10	2	8	10	0	0.009	7.4	12.6
butoxide of piperonil	0	10	2	8	10	0	0.006	6.4	13.6
bromopropylate	0	10	3	7	10	0	0.016	6.0	14.0
carbofenothion	0	10	3	7	10	0	0.003	7.2	12.8
endrin aldehyde	0	10	2	8	10	0	0.037	6.9	13.1
piriproxifen	0	10	5	5	10	0	0.005	7.1	12.9
azinphos methyl	0	10	2	8	10	0	0.024	7.4	12.6
cipermethrin	0	10	4	6	10	0	0.063	6.4	13.6
fosalone	1	9	2	8	10	0	0.006	7.8	12.2
permethrin	0	10	2	8	10	0	0.020	6.8	13.2
flucythrinate	0	10	2	8	10	0	0.051	6.4	13.6
cifluthrin	0	10	2	8	10	0	0.002	5.8	14.2
pyrazophos	1	9	3	7	10	0	0.008	6.5	13.5
esfenvalerate	0	10	0	10	10	0	0.006	5.1	14.9
tetradifon	0	10	0	10	10	0	0.011	6.0	14.0
deltramethrin	0	10	0	10	10	0	0.002	6.9	13.1
mirex	0	10	4	6	10	0	0.005	6.7	13.3
tebufenocide	0	10	3	7	10	0	0.156	6.9	13.1
pyridaben	0	10	2	8	10	0	0.010	5.9	14.1
difeconazole[b]	0	10	4	6	9	1	0.002	6.3	13.7
feranimol	0	10	4	6	10	0	0.002	6.7	13.3
azoxistrobine	0	10	0	10	10	0	0.002	6.6	14.4

[a] Caffeine area: 6.272×10^7. [b] Cutoff value: 12 μg/kg. [c] Cutoff value: 15 μg/kg.

between 25 and 30 compounds at the same time in the selected experimental conditions. So, to obtain a minimum analysis time, a temperature gradient of 50 °C/min was applied. This temperature program allowed development of a rapid GC-QqQ-MS method for the monitoring of ~130 pesticides in only 11.6 min, which means a 5-fold gain in analysis time saved, compared to GC/MS methods using an ion trap. On the other hand, to program the isolation of precursor ions for every compound along the chromatographic run, the overall analysis time was split into nine segments. Within each time segment, precursor ions were isolated in the first quadrupole, and then fragmented by collision-induced decomposition in the second quadrupole with the goal of generating spectra at different voltages, and in the third quadrupole, the product ions were separated before detection. To optimize the resources of the instrument for a rapid analysis, only the MS/MS transition that gave the highest response for each compound was used for its determination in the screening method. However, for confirmation of the identity of a previously detected compound, the acquisition method contained a total of two or three MS/MS transitions depending on the pesticide (Table 1).

Validation of the Methodology. In most of the cases, an accurate determination of pesticide residues in food commodities becomes unnecessary in routine quality control laboratories since the only information required is whether the concentration of a specific compound in a particular matrix is under or over a regulated value, named maximum residue level (MRL). Only accurate quantification is needed when the detected quantity is expected to exceed the MRL. MRL values are both compound and matrix dependent, and in addition, these levels can differ from one country to another. In general, MRLs are established at trace levels, with 10 μg/kg being the lowest value stated. This paper shows the validation of the approach developed at this concentration level in cucumber matrix. Obviously, this target concentration level can be selected in function of the MRL in each food commodity.

Screening Method. Performance characteristics of any analytical method applied to monitor samples should be assessed. There is a lack of guidance dealing with screening method validation in the area of pesticide residues. This was the reason for selecting quality parameters based on the Eurachem Guide,[31] such as, selectivity, percent false positives and negatives, cutoff, detection limit, and unreliability region. On the other hand, due to the particular characteristics of our chromatographic method, RRTWs, trueness, and precision (repeatability conditions) were also considered.

The identification of the pesticides was carried out by searching for them in the appropriate RRTWs, defined as the average relative retention time (the ratio of the chromatographic retention time of the analyte to that of the IS) ± 3 standard deviations of the relative retention times obtained when 10 blank cucumber samples spiked at 10 μg/kg of each compound were analyzed (Table 2). Recoveries between 67 and 110%, with relative standard deviation (RSD) lower than 20%, were obtained for all compounds in the target matrix (Table 2). Despite international guidelines[28] indicating that for the validation of quantitative methods mean recovery should be within the range 70−110%, we considered that the values obtained were fit for a purpose in a screening method. Selectivity (specificity) was evaluated by analysis of uncontaminated cucumber samples (n = 10) and checking the absence of concomitant substances in the RRTWs of the pesticides and the IS.

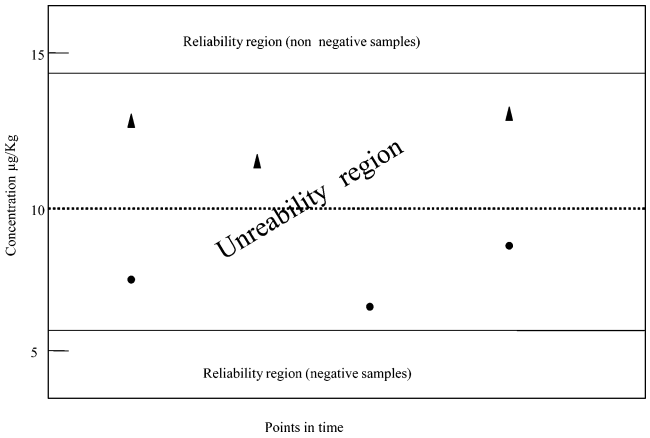

Figure 1. Representation of the reliabilty and unreliability region for propoxur; false positive (▲) and false negative (●).

The absence of any chromatographic signal (with a S/N ≥ 3) at the same retention time of the target pesticides and IS suggested that no chemical interferences were occurring. Limit of detection (LOD) values ranging from 0.01 to 3.21 μg/kg were obtained (Table 2). The percentage of false positives and negatives expressed for qualitative methods was considered as

$$\%\ \text{false positives} = \frac{\text{false positives}}{\text{total known negatives}} \times 100$$

$$\%\ \text{false negatives} = \frac{\text{false negatives}}{\text{total known positives}} \times 100$$

These values were calculated using unspiked blank samples and blank samples spiked at 5 and 8 (below the MRL), 10 (MRL), and 12 and 15 μg/kg (above the MRL) with the target pesticides. Ten replicates were measured at each concentration level. Table 3 shows the number of positives and negatives at 5, 8, and 10 μg/kg. It can be seen in Table 3 that positive identification of the target analytes is 100% reliable from 10 μg/kg, except for methamidophos, 1,2,4-trichlorobenzene, difeconazole, and for 1,2-dichlorobenzene (8 positives, 2 negatives at 12 μg/kg). This means that just this four compounds would show false negatives at a concentration level equal or higher than 10 μg/kg. In consequence 10, 12, or 15 μg/kg, is the reliable cutoff concentration for the target compounds. It can also be noticed that no false positives were found when unspiked blank samples were monitored, although this is not the case for several pesticides at a concentration level of 5 and 8 μg/kg. Also, from these results it can be concluded that sensitivity values of 100% at 10 μg/kg were obtained for all compounds, except for the ones previously indicated; sensitivity being calculated as

$$\text{sensitivity} = \frac{\text{number of found positive samples}}{\text{total number of known positives}} \times 100$$

The unreliability region is the analyte concentration range, around the cutoff value, in which false positive or false negative

(31) CITAC/EURACHEM GUIDE. *Guide to Quality in Analytical Chemistry: An Aid to Accreditation*; 2002.

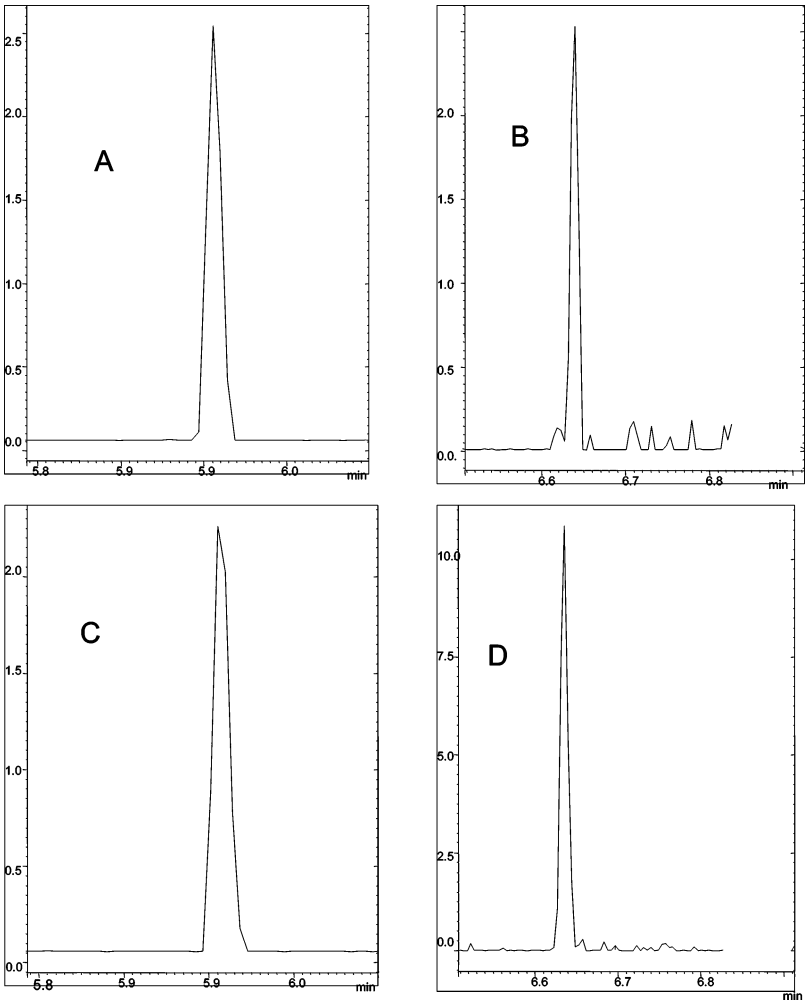

Figure 2. Chromatographs of (A) sulfotepp (12 μg/kg) and (B) malathion (5 μg/kg) in sample 1, and of (C) sulfotepp (10 μg/kg) and (D) malathion (10 μg/kg) in a standard sample.

responses are produced. The lower and upper limits that define this range depend on the statistical probability, which is assumed by the analyst. To do this, a probability of 5% error was fixed to obtain a false positive or a false negative. Table 3 shows the values obtained. Once these limits were established, the concentration (C) values obtained from unknown samples would be in two different regions: (a) region of reliability, if the C obtained was lower (or higher) than the lower (upper) limit, that means negative (or positive) sample, and (b) region of unreliability, if the C obtained was lower (or higher) than the cutoff value corresponding to the region of false positive (or false negative) sample. Figure 1 shows these regions for the propoxur pesticide. All samples with C values equal to or higher than the lower limit shall be confirmed.

Confirmation/Quantification Method. Positive samples in the screening method were injected again for confirmation in the acquisition GC-QqQ-MS method with two or three transitions by compound previously identified.

To demonstrate that the method is suitable for the intended use, quantification (LOQs) limits, trueness, and precision at a medium concentration level (100 μg/kg) were calculated. LOQs were determined as the lowest pesticide concentration injected that yielded a signal-to-noise (S/N) ratio of 10 (when the quantification ion was monitored). LOQ values ranging 0.05−10 μg/kg were obtained. Recoveries higher than 75% were obtained for all compounds, while the intraday precision ($n = 6$) of the overall method, expressed as relative standard deviation (RSD) was lower than 15% for all pesticides.

Once the confirmation of identity was checked, samples were quantified by the standard addition method. Known amounts of the standard(s) of the previously confirmed pesticide(s) were added to two aliquots of the test sample before analysis. In this way, three aliquots were analyzed per sample; the first portion analyzed unchanged and the other two portions with the aliquots added. We checked that good results were obtained if the amount of the standard pesticide added was between 2 and 5 times the estimated amount of the analyte in the sample. Analyses ($n = 5$) carried out with samples spiked at 25 and 100 μg/kg quantified by the addition of 50 and 125 μg/kg and 200 and 500 μg/kg, respectively, using the addition standard method, showed predicted values between 23 and 27 μg/kg and 95 and 102 μg/kg, respectively. The selected calibration method presents the advantages of elimination of (i) the variable matrix effect exhibited when analyzing different commodities[32] and (ii) the necessity of obtaining several blank matrixes in the laboratory for every sample type.

(32) SANCO: Quality control procedures for pesticide residue analysis. Document SANCO/10476/2003, February 2004.

Table 4. Application of the GC-QqQ-MS Screening Method

	(area pesticide/area IS)			
pesticides	sample 1	sample 2	low value of unreliability region	screening result
sulfotepp	0.0196		0.0110	nonnegative
malathion	0.0226		0.0306	negative
piridaben	-	0.0022	0.0026	negative
etrimphos	-	0.0210	0.0285	negative

Application to the Analysis of Vegetable Samples. To test the feasibility of the proposed analytical strategy for the analysis of pesticide residues, two spiked samples of cucumber were analyzed. One of the spiked samples contained sulfotepp at 12 μg/kg and malathion at 5 μg/kg (the cutoff/2 level), while the other sample was spiked with piridaben and etrimphos at concentrations equal to the cutoff/2 level (5 μg/kg for each one). Three replicates were performed for each sample. First, all the pesticides were searched in the appropriate RRTWs. Only the relative retention time of sulfotepp and malathion in the first sample, and piridaben and etrimphos in the second sample, matched that of the standard within its specified RRTW. The average of the peak areas obtained for the identified pesticides in both samples, relative to that of the IS, as well as the ones at corresponding to the lower concentration level of the unreliability region, are shown in Table 4. Their corresponding comparisons show fair agreement, as long as a nonnegative conclusion is achieved for sulfotepp. However, negative conclusions are obtained for the other pesticides despite the fact that they were positively identified. A typical GC-QqQ-MS chromatogram obtained by the screening method for the first sample is shown in Figure 2.

Following screening, the extract of the first sample was reinjected by the confirmation/quantification method. The spectra obtained for sulfotepp (Figure 3) confirmed the positive conclusion, taking into account the presence of the three ions with a variability of less than ±20%. Finally, the sample was quantified by the standard addition method. For that, 20 and 50 μg/kg of the sulfotepp pesticide, respectively, were added to two aliquots of the test sample. The estimated concentration was 10.7 μg/kg, the correlation coefficient of the standard addition curve, 0.99. Moreover, in this case, the quantity of pesticide in the sample extract was also calculated by single calibration with the standard of 10 μg/kg, because this is the concentration that is closest to the estimated concentration. Now, a concentration of 10.5 μg/kg was estimated being in agreement with the spiked concentration of 12 μg/kg.

CONCLUSION

This paper has demonstrated the potentiality of the triple quadrupole mass spectrometer coupled to GC as a valuable tool for the rapid identification and confirmation of ~130 multiclass pesticide residues in vegetables. The new analytical strategy developed can be summarized as follows: (a) simple and fast extraction of the compounds from the vegetables; (b) analysis by the GC-QqQ-MS screening method that allows labeling the samples as negatives or potentially nonnegatives; (c) analysis of the potential nonnegative samples by the GC-QqQ-MS confirmation method, which allows determining the true nonnegative samples, and (d) quantification of these last samples by the standard addition method. The approach has been validated, obtaining good results for every parameter. Also, it was applied to spiked blank cucumber samples obtaining satisfactory results.

This analytical strategy tries to be a new alternative to the conventional confirmation/quantification methods usually applied in most pesticide residue control laboratories. This methodology allows a considerable gain in total analysis time and, therefore, an increase in sample throughput, which is one of the most important aspects in choosing an analytical method for routine applications.

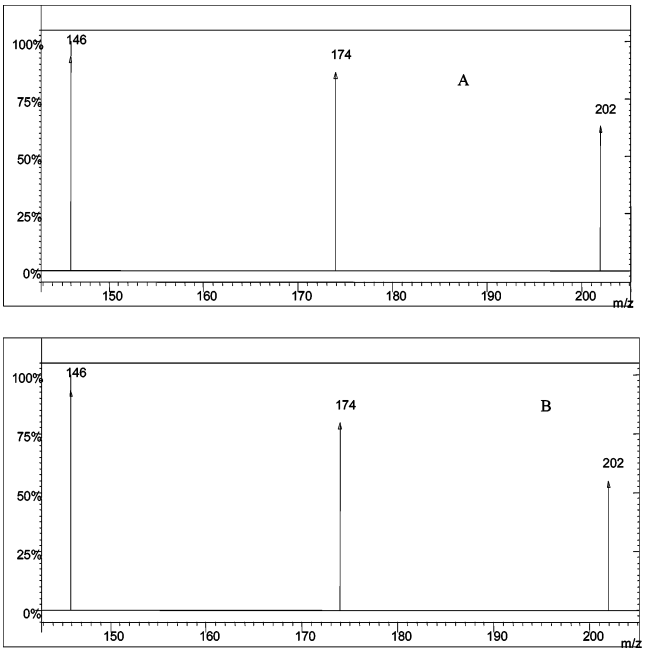

Figure 3. MS/MS spectrum of sulfotepp in (A) standard at 10 μg/kg and (B) sample 1.

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