Multiresidue Analysis of 100 Pesticides in Food Samples by LC / Triple Quadrupole Mass Spectrometry

Application

Food Safety

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Abstract

An analytical methodology for confirming the presence of a group of 100 pesticides in vegetable and fruit samples was developed using the Agilent G6410AA Triple Quadrupole Mass Spectrometer (QQQ). One transition per parent compound was monitored in a single chromatographic run containing two time segments. The sensitivity obtained meets the maximum residue levels (MRLs) established by the European Union regulation for food monitoring programs. The analytical performance of the method was evaluated for different types of fruit and vegetables — orange, tomato, and green pepper — showing little or no matrix effects. Linearity of response over two orders of magnitude was demonstrated (r > 0.99). This study is a valuable indicator of the potential of the QQQ for routine quantitative multiresidue analysis of pesticides in vegetables and fruits.

Introduction

In recent years, the established regulations regarding the maximum residue limits (MRLs) in commodities have become more and more stringent. The European Union (EU) has set new directives for pesticides at low levels in vegetables in order to meet health concerns. For fruits and vegetables intended for production of baby food, an MRL of 10 µg/kg is applicable for all pesticides, and compounds without a stated regulation also have the lowest MRLs at 10 µg/kg. The low MRLs have encouraged the development of more sensitive analytical methods to meet the requirements in complex samples. In this sense, liquid-chromatography tandem-mass spectrometry (LC-MS-MS) with triple quadrupole in multiple reaction monitoring (MRM) mode has become, so far, the most widely used technique for the monitoring and quantitation of pesticides in food, as reported extensively in the literature. On the other hand, high-resolving power mass spectrometric techniques, such as time-of-flight mass spectrometry (TOF-MS), have been applied recently for screening purposes as well. Nevertheless, the simplicity of methodologies using triple quadrupole as a detection technique, together with the low limits of detection achieved and the MS/MS capability make this technique a valuable tool for routine

monitoring programs established in regulatory official laboratories. The easiness of use is sometimes an essential for these types of regulatory agencies, which lack the high-skilled personnel required for more sophisticated techniques such as TOF-MS. Triple quadrupole technology is not new in the sense that it needs to be validated for monitoring purposes and its basis is already well-established for routine analysis.

Our study in this report is one of the first of its kind to examine the new Agilent Triple Quad for the analysis of pesticides in fruit and vegetables. This topic was chosen because of the relevance of these compounds and their significant use on food commodities. The sensitivity of the QQQ easily meets the levels required by the regulations on pesticides in food.

Experimental

Sample preparation

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany). Individual pesticide stock solutions (around 1,000 µg/mL) were prepared in pure acetonitrile or methanol, depending on the solubility of each individual compound, and stored at –18 °C. From these mother solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Vegetable samples were obtained from the local markets. "Blank" vegetable and fruit extracts were used to prepare the matrix-matched standards for validation purposes. In this way, two types of vegetables and one fruit (green peppers, tomatoes, and oranges) were extracted using the QuEChERS method already described in a previous application [1]. The vegetable extracts were spiked with the mix of standards at different concentrations (ranging from 2 to 100 µg/kg) and subsequently analyzed by LC/MS/MS.

LC / MS / MS Instrumentation

LC Conditions

Column:	Agilent ZORBAX Eclipse® XDB C-8, 4.6 mm × 150 mm, 5 µm, (p/n 993967-906).
Column temperature:	25 °C
Mobile phase:	A = 0.1% formic acid in water B= Acetonitrile
Flow-rate:	0.6 mL/min
Gradient:	10% B at 0 min 10% B at 5 min 100% B at 30 min
Injection volumes:	1-5 µL

MS Conditions

Mode:	Positive ESI using the Agilent G6410AA Triple Quadrupole Mass Spectrometer
Nebulizer:	40 psig
Drying gas flow:	9 L/min
V capillary:	4000 V
Drying gas temperature:	350 °C
Q1 resolution:	Unit
Q2 resolution:	Unit
Fragmentor voltage:	70 V
Collision energy:	5–25 V
MRM:	1 transition for every compound as shown in Table 1
Dwell time:	15 msec

Results and Discussion

Optimization of LC / MS / MS conditions

A preliminary study of the optimal MRM transitions for every compound was carried out by injecting groups of analytes (around 10 analytes in one chromatographic run) at a concentration level of 10 µg/mL. Various collision energies (5, 10, 15, 20, and 25 V) were applied to the compounds under study. The optimum energies were those that gave the best sensitivity for the main fragment ion and, as a general rule, left about 10% of parent compound in the spectra, and they were selected as optimum ones. Only one fragment ion was chosen as the most abundant product ion for every target compound. Results are shown in Table 1.

Table 1. Analytical Conditions and Limits of Detection (LOD) for Each of the Compounds Tested

Compound name	Retention time (min)	Protonated molecule $[M+H]^+$	Product ion (*m/z*)	Collision energy	LOD (pg)
Segment 1					
Cyromazine	2.7	167	125	20	10
Thiosultap	2.7	312	232	10	90
Cartap	3	150	105	15	10
Thiocyclam	4.5	182	137	10	8
Aldicarb sulfoxide	6.4	207	89	5	9
Carbendazim	6.6	192	160	15	5
Thiabendazole	7.9	202	175	25	10
Aldicarb sulfone	10.8	223	148	5	50
Nitenpyram	11	271	225	10	7
Hydroxyatrazine	11.2	198	156	15	3
Methomyl	11.5	163	88	5	4
Deisopropylatrazine	11.9	174	132	15	18
Imazapyr	12.5	262	234	15	8
Metamitron	13.9	203	175	15	8
Fenuron	14.5	165	72	15	2
Deethylatrazine	14.8	188	146	15	4
Imidacloprid	14.8	256	209	10	7
Dimethoate	15.4	230	199	5	7
Acetamiprid	15.5	223	126	15	6
Prometon	15.7	226	184	20	4
Irgarol metabolite	16	214	158	15	0.8
Methiocarb sulfone	16.4	258	122	5	6
Nicosulfuron	16.9	411	182	15	6
Thiacloprid	17	253	126	15	3
Imazalil	17.2	297	159	15	7
Mebendazole	17.2	296	264	20	2
Aldicarb	17.5	213	89	10	10
Imazaquin	17.8	312	284	20	15
Oxadixyl	17.9	279	219	10	10
Fluroxypyr	17.9	255	209	10	120
Simazine	18	202	132	15	5
Monuron	18	199	72	10	2
Lenacil	18.4	235	153	10	20
Cyanazine	18.5	241	214	10	70
Metolcarb	18.5	166	109	5	2
Spiroxamine	18.6	298	144	15	10
Dichlorvos	18.7	221	109	15	10
Metribuzin	18.9	215	187	15	5
Chlorotoluron	19.4	213	72	15	3
Prometryn	19.5	242	200	20	2
Terbutryn	19.5	242	186	15	1
Carbofuran	19.6	222	165	10	2
Bendiocarb	19.7	224	167	5	2
Segment 2					
Spinosad A	20	732	142	5	12
Carbaryl	20.1	202	145	5	2
Irgarol 1051	20.3	254	198	15	0.1
Atrazine	20.3	216	174	15	0.3
Metalaxyl	20.4	280	248	10	5
Difenoxuron	20.4	287	123	15	5
Isoproturon	20.4	207	72	15	1
Bensultap	20.5	432	290	15	6

Table 1. Analytical Conditions and Limits of Detection (LOD) for Each of the Compounds Tested (Continued)

Compound name	Retention time (min)	Protonated molecule $[M+H]^+$	Product ion (m/z)	Collision energy	LOD (pg)
Diuron	20.5	233	72	15	5
Spinosad D	20.7	746	558	5	100
Ethiofencarb	20.7	226	107	5	5
Dimethomorph isomer 1	21.3	388	301	20	11
Propachlor	21.6	212	170	10	1
Dimethomorph isomer 2	21.7	388	301	20	8
Prochloraz	21.9	376	308	10	6
Propanil	22.2	218	162	15	10
Cyproconazole	22.5	292	70	10	6
Methiocarb	22.6	226	169	5	15
Terbutylazine	22.7	230	174	15	0.3
Bromuconazole isomer 1	22.8	376	159	20	6
Fenamiphos	23	304	217	15	0.7
Methidathion	23	303	145	5	5
Azoxystrobin	23.2	404	372	10	0.4
Phosmet	23.2	318	160	5	2
Captan	23.2	300	264	10	50
Dimethenamide	23.3	276	244	10	1
Promecarb	23.3	208	151	10	5
Bromuconazole isomer 2	23.7	376	159	20	6
Molinate	23.7	188	126	10	5
Diflubenzuron	24.1	311	158	10	9
Iprodione	24.6	330	245	10	8
Propiconazole isomer 1	24.7	342	159	20	5
Malathion	24.8	331	127	5	5
Propiconazole isomer 2	24.9	342	159	20	5
Metolachlor	24.9	284	252	10	2
Triflumizole	24.9	346	278	10	7
Alachlor	25	270	238	10	8
Acetochlor	25.1	270	224	10	8
Flufenacet	25.2	364	194	5	5
Difenoconazole isomer 1	25.3	406	251	20	4
Difenoconazole isomer 2	25.4	406	251	20	4
Chlorfenvinphos	25.5	359	155	10	8
Benalaxyl	25.8	326	294	5	5
Parathion ethyl	26.2	292	236	10	9
Triclocarban	26.4	315	162	15	8
Hexaflumuron	26.5	461	158	10	7
Buprofezin	26.7	306	201	10	1
Diazinon	26.8	305	169	15	1
Teflubenzuron	26.9	381	158	15	22
Chlorpyrifos methyl	27.1	322	212	15	15
Profenofos	27.6	373	303	10	7
Lufenuron	27.9	511	158	10	10
Prosulfocarb	28	252	91	15	2
Flufenoxuron	28.5	489	158	10	6
Butylate	28.7	218	57	10	2
Pendimethalin	29.2	282	212	5	5
Trifluralin	29.7	336	236	15	30

The MRM transitions were included in the method with a dwell time of 15 msec, and two different time segments were recorded in the chromatographic run (each one of them containing about half of the pesticides studied). Figure 1 shows the chromatogram corresponding to 100 pg on column for all the compounds studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective protonated molecule and product ion MRM transition.

Linearity and Limits of Detection

Linearity was evaluated by analyzing the standards solutions at five different concentration levels in the range 2 to 100 pg on column. As an example, the calibration curve generated for atrazine is shown in Figure 2. As it can be observed in this figure, the linearity of the analytical response across the studied range is excellent, with a correlation coefficient of 0.998. Similar results were obtained for the rest of the compounds analyzed.

The limits of detection (LOD) were estimated from the injection of standard solutions at concentration levels corresponding to a signal-to-noise ratio of about 3. The results obtained are included in Table 1 as well. The best limits of detection were obtained for the triazines (from 100 fg to 2 pg on column) and the highest limits of detection were for fluoroxypyr and spinosad D (above 100 pg).

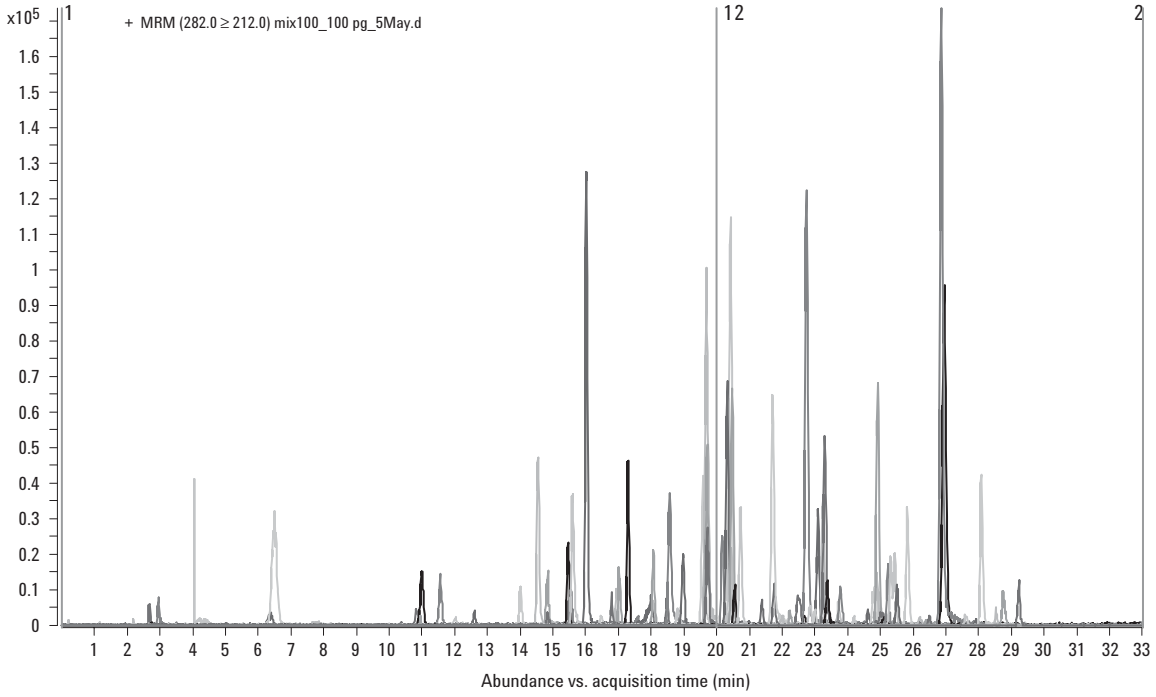

Figure 1. Product ion chromatograms of a mix of 100 pesticides (concentration: 100 pg on column).

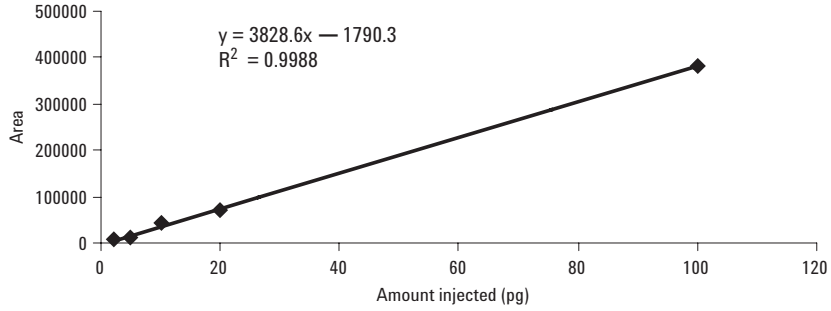

Figure 2. Calibration curve for atrazine using a linear fit with no weighting and no origin treatment.

Application to Vegetable Matrices

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were analyzed in three different matrices — green pepper, tomato, and orange — at two different concentration levels (10 and 100 µg/kg). Figure 3 shows the analysis of a green pepper spiked with the pesticide mix at 10 µg/kg (10 pg on column). As it can be observed in two of the MS/MS extracted product ion chromatograms, for dimethoate and azoxystrobin, compounds can be easily identified in these complex matrices due to the selectivity of the MRM transitions, thus fulfilling the regulation limits imposed by the EU directives. In general, the LOD obtained meet the requirements regarding the MRLs imposed by the existing European regulations.

Reference

1. Imma Ferrer and E. Michael Thurman, "Determination of Fungicides in Fruits and Vegetables by Time-of-Flight and Ion Trap LC/MS" (2005) Agilent Technologies, publication 5989-2209EN www.agilent.com/chem.

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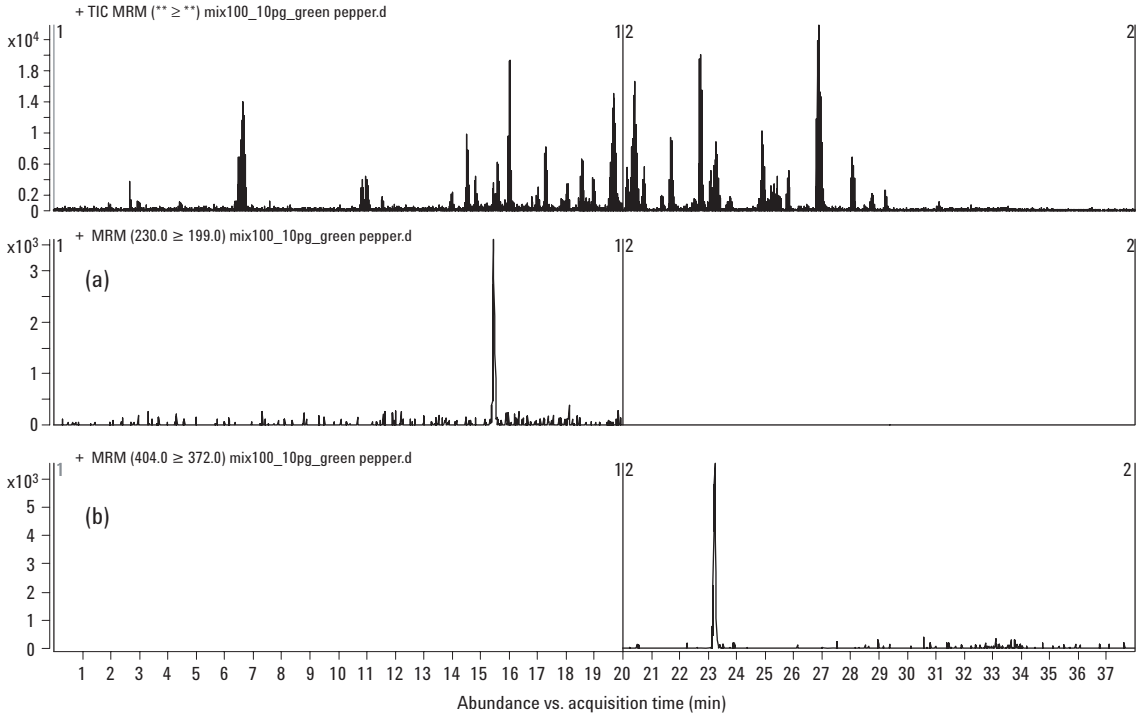

Figure 3. MRM chromatogram of a spiked green pepper sample at 10 µg / kg. Product ion chromatograms for (a) dimethoate and (b) azoxystrobin.

Printed in the USA
August 9, 2006
5989-5469EN

Agilent Technologies