Using a Triple Quadrupole GC-MS/MS System for Rapid Analysis of 303 Pesticide Residues in Green Beans

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Pesticide analysis is extremely important due to the need to ensure that foodstuff is not contaminated with pesticide residues, which can be harmful to human health. Pesticide analysis poses a number of challenges for laboratories due to the wide ranging chemistries within the contaminants. As the variety of pesticides continually expands, techniques traditionally used for pesticide residue analysis in laboratories require that one sample be analyzed using several injections with different methods in order to ensure a comprehensive screening, with no pesticides left undetected. This requirement for several injections is time consuming and significantly reduces the speed of sample analysis. This article discusses how the development of new GC-MS/MS technology significantly expands the number of pesticides that can be confirmed in a single injection, increasing laboratory productivity and protecting both the environment and human health.

Introduction

Pesticides are used to reduce or eliminate unwanted organisms or "pests" that contaminate our food and harm our environment and consequently our health. Typically, pesticides are a chemical substance, biological agent, antimicrobial or disinfectant. There are currently over 1000 recognized pesticides utilized in the production of foodstuffs worldwide, with the requirement for pesticide usage growing as consumer demand for food at a low cost and out of season increases.

Pesticide residues that can be detected in a range of agricultural products, including green beans, can be extremely harmful to human health due to their toxic nature. The consumption of pesticides such as lindane and carbendazim have been linked with long term health effects including cancer, fertility problems and hormone disruption, along with more short term ailments such as dizziness, headaches, fatigue, memory impairment, visual disorders and vomiting. They have also been known to cause skin, eye and respiratory tract irritation when consumed. As a result of these serious health effects, strict regulations governing pesticide usage have been globally implemented, with Maximum Residue Limits (MRLs) for pesticides imposed.

In 2009 the EU published Regulation (EC) No. 1107/2009 concerning the placing of 'plant protection products' on the market. This

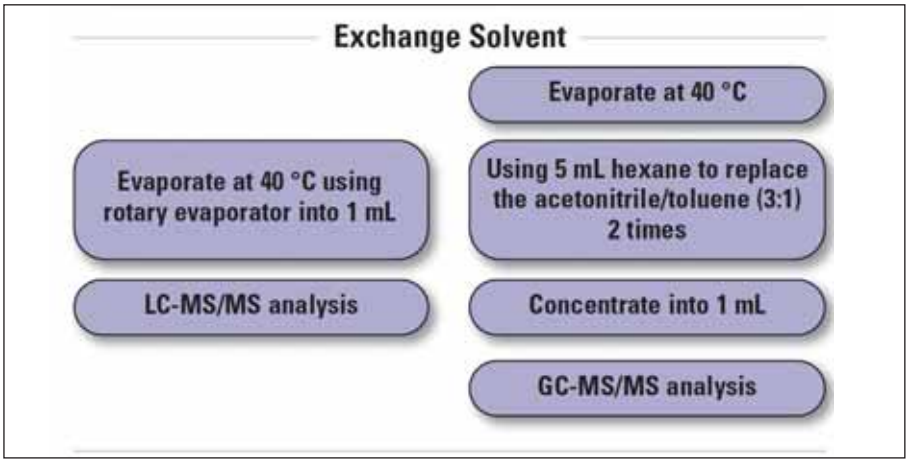


Figure 1: The final sample preparation procedure involves a process of solvent exchange.

document replaced the previously existing Directive 91/414/EEC and reflects the heightened awareness within Europe of the dangers of pesticides. The regulation emphasizes the need to 'ensure that the industry demonstrates that substances or products produced or placed on the market do not have any harmful effect on human or animal health or any unacceptable effects on the environment'.[1]

On a global level, the World Health Organization (WHO) has established the WHO Pesticide Evaluation Scheme (WHOPES)[2] to facilitate the constant evaluation of pesticides to ensure the protection of consumer health. In March 2007, WHO and The Food and Agriculture Organization of the United Nations (FAO) signed a Memorandum of Understanding to administer a cohesive global program for the management of pesticides on an international level. As a result, the International Code of Conduct on the Distribution and Use of Pesticides, which was originally established by the FAO[3] in 1985, was revised and jointly implemented in 2002. The International Code works to promote the shared responsibility of governments, industry, trade and international institutions in the management of practices that minimize health and environmental risks, including pesticide usage.

Pesticide analysis poses a number of challenges for laboratories due to the wide ranging chemistries within the contaminants. Gas chromatography (GC) with element-selective detectors or single quadrupole gas

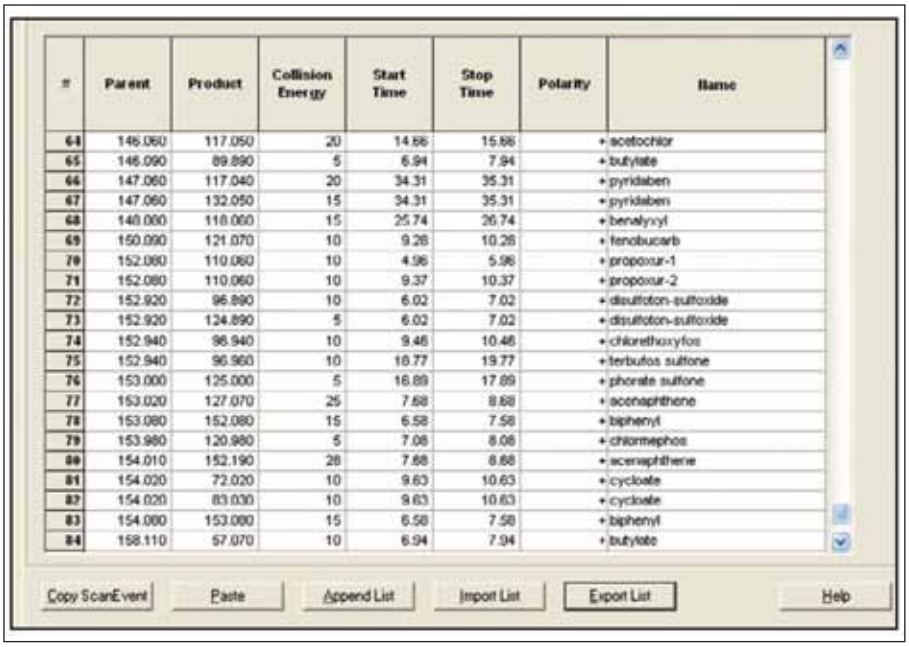
chromatography/mass spectrometry (GC/MS) are common techniques used for GC-amenable pesticide residue analysis in labs. However, as the variety of pesticides continually expands, these techniques require that one sample be analyzed using several injections with different methods. This is to ensure a comprehensive screening, with no pesticides left undetected. The requirement for several injections is time consuming and significantly reduces the speed of sample analysis.

This article will discuss how new GC-MS/MS technology significantly expands the number of pesticides that can be confirmed in a single injection, increasing productivity and safeguarding human health. This was demonstrated through the analysis of 303 pesticides in green beans by using an acetonitrile extract, followed by solid phase extraction (SPE) clean-up steps, and analysis by the use of a GC-MS/MS system using timed-SRM mode (Thermo Scientific TSQ Quantum, Thermo Fisher Scientific).

Sample Preparation

303 pesticides, including various classes such as organochlorine, organophosphorus, carbamates, and pyrethroids, were analyzed in green beans by GC-MS/MS in 40 minutes. The sample preparation procedure included a process of extraction, two clean-up stages and a solvent exchange.

10g of chopped sample was added to an 80mL centrifuge tube, followed by 20mL acetonitrile. This was then homogenized and extracted for one minute at 1500 r/min, before adding 5g of sodium chloride. The sample was then extracted for an additional one minute before being placed back in the centrifuge tube at 3000 r/min. Following this, the 10 mL (top layer MeCN) was transferred for SPE clean-up. For the first clean-up stage, C18 SOE columns were conditioned with acetonitrile. 10mL extract (from the extraction step) and 15mL acetonitrile was then added to the SPE column. All the eluting liquid was collected and then evaporated at 40°C using a rotary evaporator into 1mL. For the second clean-up step, a graphitized carbon SPE was connected to the top of the aminopropyl SPE column. The column was conditioned with 4mL acetonitrile/toluene (3:1) and 1mL of extract was added to the SPE column. The sample bottle was washed with 2mL acetonitrile/toluene (3:1) three times and washing liquid was added to the SPE column. Following this, 25mL acetonitrile/toluene (3:1) was added to the SPE column, with all the eluting liquid then collected. The process of solvent exchange was then performed as illustrated in Figure 1.



#	Parent	Product	Collision Energy	Start Time	Stop Time	Polarity	Name
64	146.060	117.050	20	14.66	15.66	+	acetochlor
65	146.090	89.090	5	6.94	7.94	+	butylate
66	147.060	117.040	20	34.31	35.31	+	pyridaben
67	147.060	132.050	15	34.31	35.31	+	pyridaben
68	148.000	118.060	15	25.74	26.74	+	benalyxyl
69	150.090	121.070	10	9.26	10.26	+	fenobucarb
70	152.080	110.060	10	4.96	5.96	+	propoxur-1
71	152.080	110.060	10	9.37	10.37	+	propoxur-2
72	152.920	96.890	10	6.02	7.02	+	disulfoton-sulfoxide
73	152.920	124.890	5	6.02	7.02	+	disulfoton-sulfoxide
74	152.940	96.940	10	9.46	10.46	+	chlorethoxyfos
75	152.940	96.960	10	18.77	19.77	+	terbufos sulfone
76	153.000	125.000	5	16.89	17.89	+	phorate sulfone
77	153.020	127.070	25	7.68	8.68	+	acenaphthene
78	153.080	152.080	15	6.58	7.58	+	biphenyl
79	153.980	120.980	5	7.08	8.08	+	chlormephos
80	154.010	152.190	28	7.68	8.68	+	acenaphthene
81	154.020	72.020	10	9.63	10.63	+	cycloate
82	154.020	83.030	10	9.63	10.63	+	cycloate
83	154.080	153.080	15	6.58	7.58	+	biphenyl
84	158.110	57.070	10	6.94	7.94	+	butylate

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Figure 2: A portion of the timed-SRM instrument method automatically generated from importing transition information from a spreadsheet.

TriPlus™ Liquid Autosampler	
Injection Volume:	1 µL
Injection Mode:	hot needle
Pre & Post Injection Dwell Mode:	3 s and 2 s
TSQ Quantum GC Mass Spectrometer	
Ion Source Temp:	280
Emission Current:	25 µA
Ionization Mode:	EI
Ion Volume:	Closed EI
Analytical Mode:	timed-SRM (Selected Reaction Monitoring)
Scan Width:	0.002 *m/z*
Cycle Time:	0.4 s
Peak Width:	Q1 0.7 Da; Q3 0.7 Da
Collision Gas Pressure:	1.5 mTorr (Ar)
Chrom Filter Peak Width:	10 s
TRACE GC Ultra™ Gas Chromatograph	
Injection Mode:	splitless with surge
Surge Pressure:	250 kPa (1 min)
Injection Temp:	250 °C
Oven Program Temp:	50 °C for 1 min, 20 °C min to 150 °C 3 °C/min to 230 °C 10 °C/min to 300 °C, hold for 10 mins
Flow Rate:	1.5 mL/min
Transferline Temp:	280 °C

Table 1: Selected instrumental conditions for the autosampler, GC, and mass spectrometer

Concentration (mg/kg)	Clodinafop-propargyl 349-238/349-266	Diallete-1 234-192/234-150	Molinate 187-158/187-126
0.0040	78.74	84.58	33.54
0.0100	73.00	86.60	26.44
0.0200	75.71	84.47	29.68
0.0400	70.75	85.33	33.26
average	74.55	85.25	30.73
RSD%	3.45	0.98	3.36

Table 2: The ion ratios of two SRM transitions for four selected pesticides

No.	Compounds	RT (min)	R^2	0.010 mg/kg Mean	CV (%)
1	dichlorvos	5.99	0.990	92.7	14.7
2	allidochlor	6.29	0.997	82.3	13.5
3	disulfoton-sulfoxide	6.58	0.995	109.9	5.2
4	dichlorbenil	6.82	0.993	85.3	9.2
5	EPTC	6.82	0.995	79.0	14.6
6	dichlormid	6.84	0.993	85.7	9.4
7	biphenyl	7.09	0.986	85.6	12.2
8	butylate	7.50	0.991	78.5	16.1
9	methacrifos	7.55	0.996	99.2	5.8
10	mevinphos	7.55	0.997	98.7	8.3
11	chlormephos	7.62	0.996	84.7	9.3
12	vernolate	7.67	0.994	84.2	12.3
13	propham	7.80	0.996	95.4	9.6
14	pebulate	7.81	0.994	79.1	11.1
15	acenaphthene	8.21	0.994	84.1	8.8
16	chloroneb	8.42	0.992	91.7	12.5
17	2-phenylphenol	8.56	0.997	76.4	17.4
18	crimidine	8.58	0.999	87.5	14.2
19	molinate	8.75	0.998	92.3	16.1
20	isoprocarb	8.79	0.999	98.5	8.0
21	heptanophos	9.32	0.999	97.6	7.7
22	chlorfenprop-methyl	9.51	0.998	88.0	9.5
23	thiomazin	9.74	0.993	90.4	9.9
24	tecnazene	9.74	0.995	105.2	18.6
25	fenobucarb	9.77	0.998	99.5	10.6

Table 3: Summary of linearity, average recovery and precision for 303 pesticides

Experimental

For this experiment, matrix-matched calibration standards were prepared by spiking a mix of 303 pesticide standards into blank green bean samples, followed by extraction for GC-MS/MS analysis. The final concentrations of standards in the matrix were 0.0040, 0.0100, 0.0200 and 0.0400 mg/kg. Five replicate quality control (QC) samples at 0.010 mg/kg were also prepared under the same sample preparation procedure to evaluate average recovery and precision.

A TriPlus liquid autosampler (Thermo Fisher Scientific) was used to inject a 1 µL aliquot of the final extract, using a hot needle injection. The sample was then separated using a 5% diphenyl/95% dimethyl polysiloxane, 30m x 0.25mm i.d., 0.25 µm film thickness column. The determination of the target 303 pesticides was carried out by the GC triple quadrupole mass spectrometer operated in timed-SRM mode. At least two SRM transitions for each pesticide and their collision energies were selected from the Thermo Scientific Pesticide Analyzer Reference and after a simple modification in an Excel® file, the transitions were imported directly to the instrument method (Figure 2). Detailed GC-MS/MS conditions are given in Table 1.

Results and Discussions

According to the European Council Directive 96/23/EC[4], at least two transitions per pesticide are required to ensure its confirmation. To adhere with this Directive, a total of 652 SRM transitions were acquired in one analytical run using the TSQ Quantum in timed-SRM mode. The timed-SRM function allowed all 652 transitions to be set in multiple small overlapping windows, based on the retention times of each pesticide. The dwell time of each transition was automatically maximized for each compound to give the best sensitivity for all pesticides in one run.

The responses of SRM transitions were used for quantification analysis and the ratio of two SRM transitions for each compound was used for confirmation at the same time. Two SRM transitions for Clodinafop-propargyl, Diallete-1 and Molinate show the stability of ion ratio crossing the concentration range from 0.0040 mg/kg- 0.0400mg/kg with the relative standard deviation less than 5% (see Table 2).

Calibration curves from 0.004 to 0.040 mg/kg were created using matrix-matched standard calibration solutions. A summary of the linearity of calibration standards, average recovery and precision data from five replicated QC samples at 0.010 mg/kg are given in Table 3 for all 303 pesticides in green

beans. The correlation coefficient (R^2) for most pesticides was greater than 0.99. The signal-to-noise ratios for all 303 pesticides at the lowest calibrated level were easily more than 10:1. The average recoveries for most pesticides at 0.010mg/kg in matrix were within the range of 73% to 110% with average precision of 10.3% CV.

Conclusion

In order to protect consumer health, environmental regulations regarding levels of pesticides that can exist within agricultural products are becoming increasingly stringent. As a result, the requirement for a rapid and cost-effective method of pesticide analysis is growing. In this application, 303 pesticides in green beans were analyzed using triple quadrupole GC-MS/MS, with 652 SRM transitions taking place under timed-SRM mode. The analysis was conducted with standard sample preparation including SPE as a clean-up procedure. Evaluation of the analysis demonstrates that sensitivity can easily reach up to 0.004 mg/kg for all pesticides in green beans, which is in line with strict industry regulations. In addition, linearity and recoveries were within industry requirements at 0.010 mg/kg. Confirmations are demonstrated through the use of two SRM transitions for each pesticide, with good stability of their ion ratios at different concentrations.

As demonstrated by the analysis, timed-SRM mode is a highly effective method for screening and determining large amounts of pesticides at low levels in the sample matrix, which is extremely important to safeguarding human health. Furthermore, the instrument set up for hundred of pesticide SRM transitions was simplified by using conditions given in the Pesticide Analyzer Reference, which minimized complex SRM method development work. By adopting this method, food safety laboratories can benefit from significantly increased sample throughput while ensuring that a high level of accuracy in matrix is maintained

References

1. *Health and Safety Executive, 'Regulation (EC) no 1107/2009 of the European Parliament and of the Council', Official Journal of the European Union, (http://www.pesticides.gov.uk/environment.asp?id=1980)*
2. *World Health Organization, 'WHO Pesticide Evaluation Scheme (WHOPES)', (http://www.who.int/whopes/en/).*
3. *Food and Agriculture Organization of the United Nations, International Code of Conduct on the Distribution and Use of Pesticides', (http://www.fao.org/docrep/005/Y4544E/y4544e00.htm).*
4. *European Council Directive 96/23/EC http://ec.europa.eu/food/food/chemicalssafety_htm*