Pesticides Analysis Using the Agilent 5977A Series GC/MSD

Application Note

Food Testing and Agriculture

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Abstract

A method has been developed using the Agilent 5977A Series GC/MSD that can deliver excellent linearity ($R^2 \geq 0.991$), reproducibility (RSD 11% in solvent and apple matrix), and sensitivity, most minimum detection limits (MDLs) $\leq$ 6.7 ppb of quantitation for 42 pesticides commonly analyzed in China.

Introduction

With the increasing globalization of the food industry, there is greater scrutiny on food safety, resulting in major changes in the number of pesticides that are being regulated and monitored, as well as the allowable levels of those pesticides in food. There are more than 1,000 registered pesticides worldwide, and many countries employ strict regulations on pesticide residues in food and animal feed. Currently, the lowest maximum residue level (MRL) for most pesticides in China is 0.01 mg/kg, parts per million (ppm), and can range as high as 5 mg/kg, depending on the food matrix being tested.

This application note demonstrates the ability of the 5977A Series GC/MSD to provide sensitive, accurate, and reproducible analysis for 42 commonly tested pesticides in China, with MDLs well within the required MRLs.

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Experimental

Standards and Reagents

Standards for 42 pesticides were commercially obtained (Table 1). The working calibration and matrix spiking standards were prepared in hexane using the Agilent 7696A Sample Prep WorkBench.

Table 1. 42 Pesticide Standards

No.	Classification	CAS	Name
1	Conazole	43121-43-3	Triadimefon
2	Dicarboximide	32809-16-8	Procymidone
3	Dicarboximide	50471-44-8	Vinclozolin
4	Dicarboximide	36734-19-7	Iprodione
5	Organochlorine	319-84-6	BHC-*alpha*
6	Organochlorine	319-85-7	BHC-*beta*
7	Organochlorine	58-89-9	BHC-*gamma*
8	Organochlorine	319-86-8	BHC-*delta*
9	Organochlorine	72-55-9	DDE-p,p'
10	Organochlorine	72-54-8	DDD-p,p'
11	Organochlorine	789-02-6	DDT-o,p'
12	Organochlorine	50-29-3	DDT-p,p'
13	Organochlorine	82-68-8	Pentachloronitrobenzene
14	Organophosphorus	62-73-7	Dichlorvos
15	Organophosphorus	298-02-2	Phorate
16	Organophosphorus	122-14-5	Fenitrothion
17	Organophosphorus	55-38-9	Fenthion
18	Organophosphorus	2921-88-2	Chlorpyrifos
19	Organophosphorus	56-38-2	Parathion
20	Organophosphorus	24353-61-5	Isocarbophos
21	Organophosphorus	732-11-6	Phosmet
22	Organophosphorus	2310-17-0	Phosalone
23	Organophosphorus	60-51-5	Dimethoate
24	Organophosphorus	333-41-5	Diazinon
25	Organophosphorus	298-00-0	Parathion-methyl
26	Organophosphorus	121-75-5	Malathion
27	Organophosphorus	41198-08-7	Profenofos
28	Organophosphorus	24017-47-8	Triazophos
29	Organophosphorus	99675-03-3	Isofenphos-methyl
30	Pyrazole	120068-37-3	Fipronil
31	Pyrethroid	39515-41-8	Fenpropathrin
32	Pyrethroid	91465-08-6	Cyhalothrin
33	Pyrethroid	68359-37-5	Cyfluthrin
34	Pyrethroid	70124-77-5	Flucythrinate
35	Pyrethroid	102851-06-9	Fluvalinate-*tau*
36	Pyrethroid	82657-04-3	Bifenthrin
37	Pyrethroid	51877-74-8	Permethrin
38	Pyrethroid	52315-07-8	Cypermethrin
39	Pyrethroid	51630-58-1	Fenvalerate
40	Pyrethroid	52918-63-5	Deltamethrin
41	Pyrimidine	53112-28-0	Pyrimethanil
42	Substituted Benzene	1897-45-6	Chlorothalonil

Instruments

The study was performed on an Agilent 7890B gas chromatograph equipped with a Split/Splitless Inlet and coupled to an Agilent 5977A Series GC/MSD, using SIM and Electron Ionization (EI) acquisition modes. Table 2 lists the instrument conditions.

Table 2. Agilent 7890B GC and Agilent 5977A Series GC/MSD Conditions

GC run conditions	
Analytical column	HP-5ms Ultra Inert 30 m × 250 µm, 0.25 µm (p/n 19091S-433UI)
Injection volume	1 µL
Injection mode	Splitless
Inlet temperature	280 °C
Liner	UI, splitless, single taper, glass wool (p/n 5190-2293)
Plated seal kit	Gold Seal, Ultra Inert, with washer (p/n 5190-6144)
Carrier gas	Helium, constant flow, 1 mL/min
Oven program	60 °C for 1 minute, then 40 °C/min to 170 °C, then 10 °C/min to 310 °C, then hold for 2 minutes
Transfer line temperature	280 °C

MS conditions	
Solvent delay	3.5 minutes
Acquisition mode	SIM
Tune	Etune.u
Gain factor	5.00
Source temperature	250 °C
Quadrupole temperature	150 °C
TID	On

Sample Preparation

A 20 g apple sample was homogenized with 40 mL of acetonitrile and mixed vigorously for 1 minute in a vortex mixer. A 5-g amount of NaCl was added, and the sample was mixed on the vortex mixer for another minute. The sample was then centrifuged at 4,200 rpm for 5 minutes. A 20-mL aliquot of the supernatant, equivalent to 10 g of sample, was removed and concentrated to approximately 1 mL. The extract was then cleaned up using a Bond Elut carbon/NH_2 column (500 mg/500 mg, 6 mL, p/n 12252202). The effluent was collected and evaporated to near dryness with a gentle stream of nitrogen. The residue was dissolved in hexane to a volume of 10 mL. One milliliter of the resulting blank extract, corresponding to 1 g of sample, was used to prepare a 50 ng/mL parts per billion (ppb) fortified matrix-matching standard (pesticide standards added to blank extracts).

Acquisition Parameters

Table 3 shows the ions used for acquisition.

Table 3. Retention Times and Acquisition Parameters

Target compound	RT	Q_0	Q_1	Q_2	Q_3
Dichlorvos	4.57	109	185	79	187
Phorate	7.33	75	121	260	97
BHC-*alpha*	7.47	181	219	183	217
Dimethoate	7.61	87	93	125	143
BHC-*beta*	7.84	217	181	183	219
BHC-*gamma*	7.96	217	183	219	111
Pentachloronitrobenzene	8.04	237	249	295	214
Pyrimethanil	8.10	198	199	200	77
Diazinon	8.11	179	137	152	199
BHC-*delta*	8.31	181	219	183	217
Chlorothalonil	8.39	266	264	268	270
Vinclozolin	8.91	212	285	198	187
Parathion-methyl	8.94	263	109	125	79
Fenitrothion	9.38	277	125	109	260
Malathion	9.53	173	127	125	93
Fenthion	9.71	278	125	109	169
Chlorpyrifos	9.75	197	199	314	97
Parathion	9.76	291	109	97	139
Triadimefon	9.79	57	208	85	210
Isocarbophos	9.87	136	121	120	110
Isofenphos-methyl	10.19	199	58	121	231
Fipronil	10.43	367	369	213	351
Procymidone	10.62	96	283	285	67
Profenofos	11.31	339	139	206	208
DDE-p,p'	11.39	246	318	316	248
DDD-p,p'	12.13	235	237	165	236
DDT-o,p'	12.20	235	237	165	236
Triazophos	12.41	161	162	172	77
DDT-p,p'	12.79	235	237	165	236
Iprodione	13.45	314	187	189	244
Phosmet	13.65	160	161	77	93
Bifenthrin	13.69	181	165	166	182
Fenpropathrin	13.81	181	97	125	265
Phosalone	14.33	182	121	184	367
Cyhalothrin	14.63	181	197	208	209
Permethrin	15.40	183	163	165	184
Cyfluthrin	16.12	163	206	165	227
Cypermethrin	16.24	181	163	165	77
Flucythrinate	16.44	199	157	44	207
Fenvalerate	17.14	167	125	181	152
Fluvalinate-*tau*	17.32	250	252	209	181
Deltamethrin	17.85	181	253	251	255

RT – retention time in minutes

Q_0 – quantifier ion

Q_1, Q_2, Q_3 – qualifier ions

Results and Discussion

Linearity

Calibration curves were constructed from 20 to 200 ng/mL (ppb) in hexane for most target compounds. All 42 compounds had calibration coefficient values ≥ 0.991 (Table 4).

Table 4. Calibration Coefficients (R^2) for the 42 Pesticides

Target compound	R^2	Target compound	R^2
Dichlorvos	0.998	Fipronil	0.997
Phorate	0.998	Procymidone	0.998
BHC-*alpha*	0.998	Profenofos	0.997
Dimethoate	0.995	DDE-p,p'	0.998
BHC-*beta*	0.995	DDD-P,P'	0.998
BHC-*gamma*	0.998	DDT-o,p'	0.998
Pentachloronitrobenzene	0.997	Triazophos	0.995
Pyrimethanil	0.998	DDT-p, p'	0.996
Diazinon	0.998	Iprodione	0.995
BHC-*delta*	0.998	Phosmet	0.995
Chlorothalonil	0.999	Bifenthrin	0.996
Vinclozolin	0.999	Fenpropathrin	0.996
Parathion-methyl	0.993	Phosalone	0.995
Fenitrothion	0.991	Cyhalothrin	0.995
Malathion	0.998	Permethrin	0.992
Fenthion	0.998	Cyfluthrin	0.995
Chlorpyrifos	0.998	Cypermethrin	0.995
Parathion	0.989	Flucythrinate	0.994
Triadimefon	0.994	Fenvalerate	0.996
Isocarbophos	0.991	Fluvalinate-*tau*	0.995
Isofenphos-methyl	0.998	Deltamethrin	0.992

Reproducibility and Minimum Detection Limits in Solvent

Table 5 illustrates the good reproducibility obtainable with the 5977A Series GC/MSD in solvent. The majority of the relative standard deviation values (RSDs) across all 42 pesticide compounds were ≤ 5%, with none exceeding 11%, across eight injections.

The corresponding MDLs, calculated as the product of the RSD multiplied by the Student t test value, ranged from 3 to 14.3 ppb, with the majority being ≤ 6.7 ppb.

*Table 5. Reproducibility RSDs and Calculated MDLs for a 50 ppb Standards Mix Sample in Solvent**

Compound	RSD (%)	MDL (ppb)	Target compound	RSD (%)	MDL (ppb)
Dichlorvos	5	6.4	Fipronil	6	8.3
Phorate	5	6.3	Procymidone	4	4.7
BHC-*alpha*	3	4.3	Profenofos	10	12.0
Dimethoate	7	8.3	DDE-p,p'	3	4.5
BHC-*beta*	3	3.6	DDD-p,p'	5	6.7
BHC-*gamma*	2	3.0	DDT-o,p'	3	3.6
Pentachloronitrobenzene	3	3.8	Triazophos	8	9.9
Pyrimethanil	4	5.0	DDT-p,p'	3	4.0
Diazinon	4	4.7	Iprodione	10	12.2
BHC-*delta*	3	3.5	Phosmet	8	9.7
Chlorothalonil	3	4.1	Bifenthrin	6	8.2
Vinclozolin	3	4.2	Fenpropathrin	6	8.6
Parathion-methyl	5	5.9	Phosalone	7	9.1
Fenitrothion	5	6.6	Cyhalothrin	8	10.6
Malathion	5	6.2	Permethrin	8	9.6
Fenthion	5	5.9	Cyfluthrin	9	11.3
Chlorpyrifos	4	5.7	Cypermethrin	9	11.0
Parathion	5	5.6	Flucythrinate	11	13.1
Triadimefon	4	5.5	Fenvalerate	9	12.0
Isocarbophos	8	9.1	Fluvalinate-*tau*	11	14.3
Isofenphos-methyl	5	6.7	Deltamethrin	9	11.2

*Eight consecutive injections were used to calculate the RSDs.

Reproducibility and Minimum Detection Limits in Apple Matrix

Reproducibility was quite good in apple matrix, with many of the RSDs being lower than those calculated for analysis in solvent (Table 6 versus Table 5). All but two of the MDLs were ≤ 7.5 ppb, none was higher than 10.1 ppb, and all were well below the Chinese MRL requirements (Table 6).

*Table 6. Reproducibility RSDs and Calculated MDLs for a 50 ppb Standards Mix Sample Spiked into Apple**

Target compound	RSD (%)	Calculated MDL (ppb)	Required MRL† (ppb)
Dichlorvos	2	2.6	200
Phorate	3	4.2	10
BHC-*alpha*	3	4.1	50**
Dimethoate	3	3.7	1,000
BHC-*beta*	4	5.9	50**
BHC-*gamma*	3	5.2	50**
Pentachloronitrobenzene	3	4.0	20 (watermelon)
Pyrimethanil	3	3.9	1,000 (pear)
Diazinon	3	4.1	100 (cereal)
BHC-*delta*	3	4.3	50**
Chlorothalonil	2	2.9	1,000
Vinclozolin	3	4.0	1,000 (cucumber)
Parathion-methyl	3	4.1	10
Fenitrothion	2	3.2	500
Malathion	3	3.7	2,000
Fenthion	3	5.0	50
Chlorpyrifos	3	4.5	1,000
Parathion	3	4.5	10
Triadimefon	3	3.7	1,000
Isocarbophos	3	6.0	10
Isofenphos-methyl	3	3.7	10
Fipronil	2	3.4	20 (cereal)
Procymidone	3	4.5	5,000 (grape)
Profenofos	4	5.8	50
DDE-p,p'	3	3.5	50***
DDD-p,p'	1	1.8	50***
DDT-o,p'	2	2.4	50***
Triazophos	2	3.7	200
DDT-p,p'	2	2.9	50***
Iprodione	3	5.3	5,000
Phosmet	3	4.1	5,000 (orange)
Bifenthrin	3	5.0	500
Fenpropathrin	3	3.8	5,000
Phosalone	2	2.6	1,000 (spinach)
Cyhalothrin	4	5.1	200
Permethrin	4	5.5	2000
Cyfluthrin	7	10.1	500
Cypermethrin	5	7.3	2,000
Flucythrinate	5	7.5	500
Fenvalerate	4	5.7	1,000
Fluvalinate-*tau*	3	4.1	500 (spinach)
Deltamethrin	2	3.1	100

* Eight consecutive injections were used to calculate the RSDs.
† Chinese regulation GB 2763-2012, MRL: Maximum Residue Level
** Four isomers in total
*** Four DDT, DDE, DDD in total

Conclusion

The Agilent 5977A Series GC/MSD can deliver sensitive, accurate and reproducible results for the analysis for 42 commonly tested pesticides in China, including MDLs in some food matrices that can be well within the required limits of Chinese regulation GB 2763-2012. The use of automation such as the Agilent 7696A Sample Prep WorkBench to prepare standards also results in more linear calibration curves.

For More Information

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Printed in the USA
April 12, 2013
5991-2212EN

Agilent Technologies