

Determination of Pesticide Residues in Cucumber Using GC-MS/MS With APGC™ After Extraction and Clean-up Using QuEChERS

Janitha De-Alwis, Stuart Adams, Simon Hird

Waters Corporation

Abstract

Reliable analytical methods are needed for detection, quantification, and identification of hundreds of pesticide residues in many different commodities. This application note describes the development and validation of a comprehensive method based on gas chromatography-tandem mass spectrometry (GC-MS/MS) for the determination of over 200 pesticides. Extracts of cucumber were prepared using a version of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method, including a dispersive solid-phase extraction (dSPE) step, followed by determination with GC-MS/MS. The use of GC-MS/MS utilizing atmospheric pressure gas chromatography (APGC) has been shown to offer significant improvements in performance over electron ionization (EI) for pesticide residue analysis, in terms of selectivity and specificity. The extremely high sensitivity of the APGC Xevo™ TQ-XS System was demonstrated with reliable detection for all the analytes at concentrations as low as 0.001 mg/kg, even when the injection volume was 1 µL. The method was successfully validated in cucumber using the SANTE guidelines document. The results from analysis of the spikes showed that almost all the analytes were within the required tolerance for recovery and repeatability, respectively. The

method is considered sensitive, specific, accurate, and suitable for the determination of residues of a wide range of GC-amenable pesticides in agricultural commodities, for checking compliance with maximum residue levels (MRLs) and has the potential for determination at much lower concentrations.

Benefits

- The APGC System generates extremely high sensitivity to help meet the needs of those involved with the analysis of foods for pesticide residues
- Sufficient sensitivity was achieved using splitless injection of 1 μL of acetonitrile extract
- APGC adds flexibility to the laboratory as the same tandem mass spectrometry (MS/MS) system can also operate in combination with UPLC™

Introduction

Plant protection products, more commonly known as pesticides, are used to control pests, weeds, and diseases. Pesticide residues resulting from the use of such products on crops that are used for food or feed production may pose a risk factor for public health or hinder trade. MRLs or tolerances are established in raw agricultural commodities. Some countries (e.g. EU and Japan) operate a system of "default MRL", equal to the limit of quantification (LOQ) achievable with analytical methods used for MRL enforcement, which is applicable for pesticides not explicitly mentioned in the MRL legislation. The value of this default MRL is typically 0.01 mg/kg. Compliance with MRLs is checked by the monitoring for residues in produce. Governments typically operate pesticide residue testing programs and the food industry and contract testing laboratories also carry out testing to check the levels of pesticide residues in agricultural commodities, ingredients, and finished food products.

Reliable analytical methods are needed for detection, quantification, and identification of hundreds of pesticide residues in many different commodities. One main driver for all laboratories involved with the determination of pesticide residues in food is to accurately determine the compounds of interest, at relevant concentrations, in the most cost-effective manner. Laboratories are constantly having to address issues with capacity and efficiency to address sample throughput requirements and to attain lower and lower reporting limits. The implementation of multiresidue methods, relying on generic extraction with limited clean-up (e.g. QuEChERS) and determination using both gas and liquid chromatography coupled with MS/MS has made a significant contribution to not only

extending the scope of analyses but their effective and efficient implementation.

Laboratories carrying out pesticide residue analysis are always going to need gas chromatography (GC) to complement liquid chromatography (LC) to cover the scope of analytes required. GC is a powerful technique for the determination of the more volatile, nonpolar, and low polarity pesticides. The determination of GC-amenable pesticides in food using MS/MS allows for high selectivity and sensitivity and minimizes chromatographic interferences. The most common ionization technique for GC-MS/MS is EI, as it enables the determination of a wide range of organic compounds. However, extensive fragmentation results in reduced sensitivity as the ion current is distributed over many ions with poor intensity, as well as a low selectivity due to the formation of less specific fragment ions. The use of GC-MS/MS utilizing APGC has been shown to offer significant improvements in performance over EI for pesticide residue analysis, in terms of selectivity and specificity.¹⁻⁴ Selectivity and sensitivity are enhanced if either the molecular/protonated ion or a high mass fragment ion are selected as the precursor ion for MRM transitions in MS/MS.

The objective of this study was to demonstrate the performance of a method for the determination of pesticide residues using GC-MS/MS with APGC on Xevo TQ-XS after QuEChERS. QuEChERS is a versatile, streamlined approach using rapid solvent-based extraction in a centrifuge tube, often followed by dSPE for clean-up, which is suitable for coupling with analysis of extracts by GC-MS/MS.

Experimental

Sample Preparation, Extraction, and Clean-up

Samples of cucumber were purchased from a local retail store. They were immediately homogenized in a food processor and stored frozen until required. In addition, a Quality Control Material of cucumber puree (T19290QC) was purchased from FAPAS. Samples were extracted using the CEN QuEChERS method.⁵ An overview of the details of the sample extraction and clean-up procedure used is given in Figure 1.

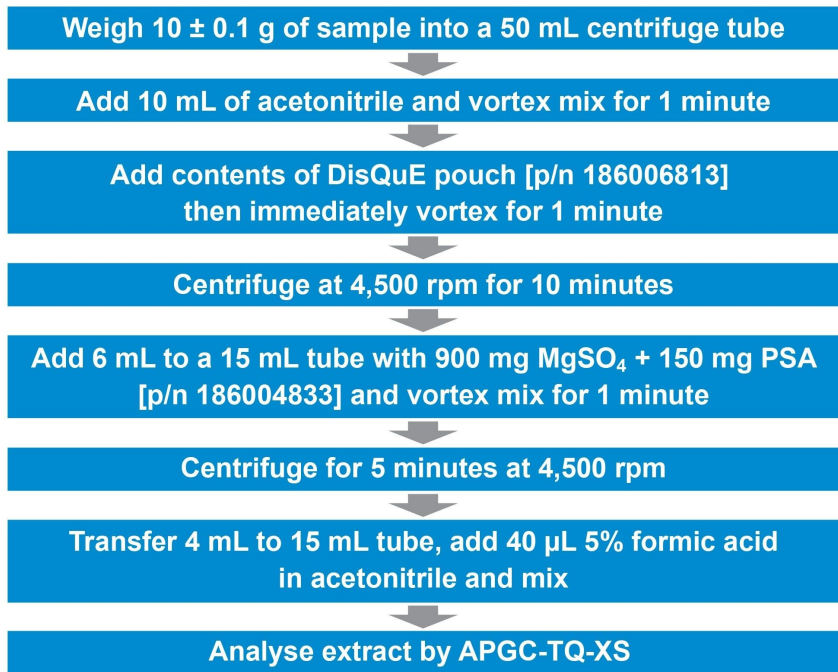


Figure 1. Overview of the details of sample preparation and clean-up for pesticide residues in cucumber.

The GC Multiresidue Pesticide Kit (Restek pn 32562) was used to prepare working solution to create matrix-matched calibration standards and for spiking the cucumber test portions. The calibration standards were prepared over the range 0.0005 to 0.10 mg/kg.

GC Conditions

GC system:	Agilent 7890A
Autosampler:	CTC CombiPal
Wash solvent(s):	Wash 1: ethyl acetate Wash 2: acetonitrile
GC column:	Restek Rxi-5Sil MS (30 m x 0.25 mm id x 0.25 µm)

film)

Carrier gas:	Helium
Oven program:	90 °C for 1 min, ramp to 330 °C at 8.5 °C/min hold for 5 min
Gas flow rate:	2 mL/min (constant flow mode)
Injection type:	Pulsed splitless
Inlet temperature:	250 °C
Pulse time:	1.2 min
Pulse pressure:	32 psi
Purge flow:	30 mL/min
Septum purge flow:	3 mL/min
Inlet liner:	Restek Topaz 4.0 mm ID Single Taper Inlet Liner w/ Wool
Injection volume:	1 µL
Makeup gas:	Nitrogen at 350 mL/min
Transfer line temperature:	280 °C

MS Conditions

Mass spectrometer:	Xevo TQ-XS
Source type:	APGC 2.0 with water as a modifier
Source temperature:	150 °C
Transfer line temperature:	280 °C
Corona current:	2.0 µA
Auxiliary gas flow:	200 L/hr
Cone gas flow:	265 L/hr

Data Management

MS acquisition software:	MassLynx™ v4.2
Quantitation software:	TargetLynx™ XS

The GC-MS/MS method for 203 pesticides and their metabolites was created using the Quanpedia™ Database, which automatically creates the MS acquisition method and processing method from a compendium of compound specific MS parameters such as transitions, and collision energy. A list of the compounds included in the method can be found in the Annex.

Method Validation

Validation was performed by replicate analysis of spiked cucumber. The following factors were assessed: selectivity, sensitivity, calibration graph characteristics, recovery, and within-laboratory repeatability (RSD_r). Recovery and repeatability were determined from the analysis of five replicates prepared at two concentrations: the EU default MRL (0.01 mg/kg) and at 10 times lower concentration (0.001 mg/kg). In addition, five replicates of the Quality Control Material (T19290QC) were prepared, analyzed and results compared to the assigned value provided by FAPAS.

Results and Discussion

Figure 2 shows the chromatography for a selection of analytes. The dwell times were automatically calculated whilst ensuring at least twelve data points for each peak for precise measurements.

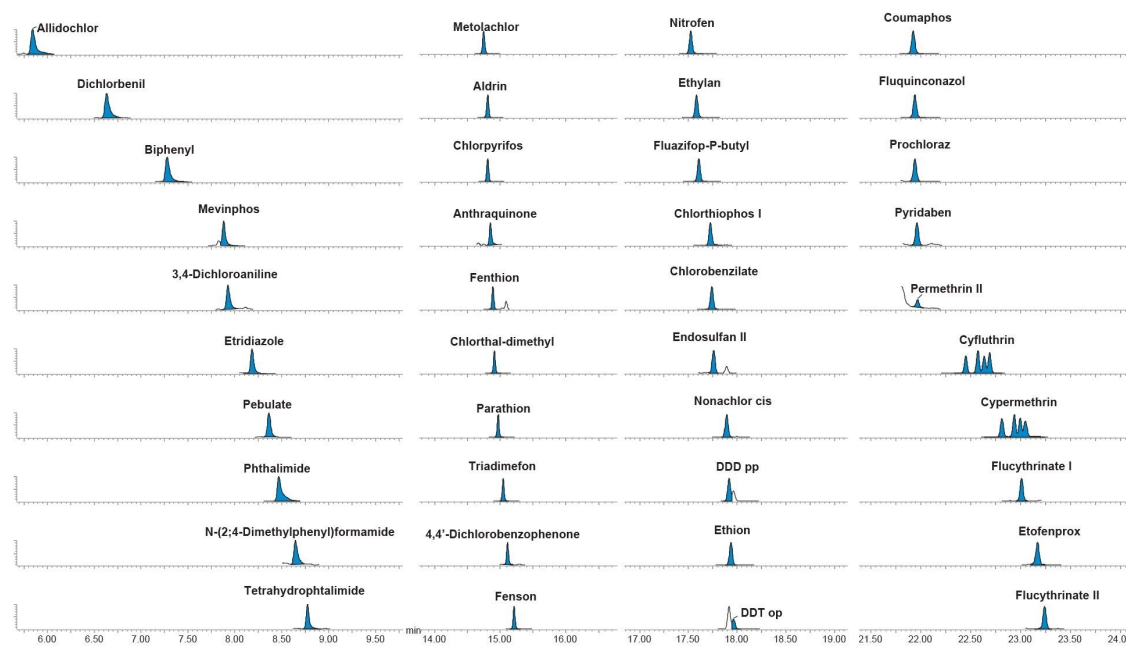


Figure 2. Chromatograms from the analysis of a selection of analytes in the cucumber matrix-matched standard at 0.001 mg/kg.

Acetonitrile has a large expansion volume, which limits the injection volume that can be used with conventional splitless injection and impacts sensitivity. Although this issue can be avoided by either using solvent exchange into another solvent such as toluene or by switching to a different design of injector, the programmable temperature vaporizer (PTV) with solvent vent, the capability to achieve sufficient sensitivity using a 1 μ L injection of acetonitrile, with an easy-to-use conventional splitless injection unit, is an attractive option.

The sensitivity of the method was evaluated by assessment of the response of the matrix-matched standard at the lowest concentration prepared (0.0005 mg/kg) and consideration of the response from the blank. Of the 203 analytes in the method, two were not detected (acequinocyl and aldrin anhydride) and from the remaining all but

one could be detected at 0.0005 mg/kg, with 85% of analytes exhibiting responses that indicated that they could be detected at much lower concentrations. Figure 3 show chromatograms from the analysis of a selection of pesticides in the cucumber matrix-matched standard at 0.001 mg/kg. This demonstrates the extreme high sensitivity of the APGC approach with reliable detection for all the analytes at very low concentrations even when the injection volume was 1 μ L.

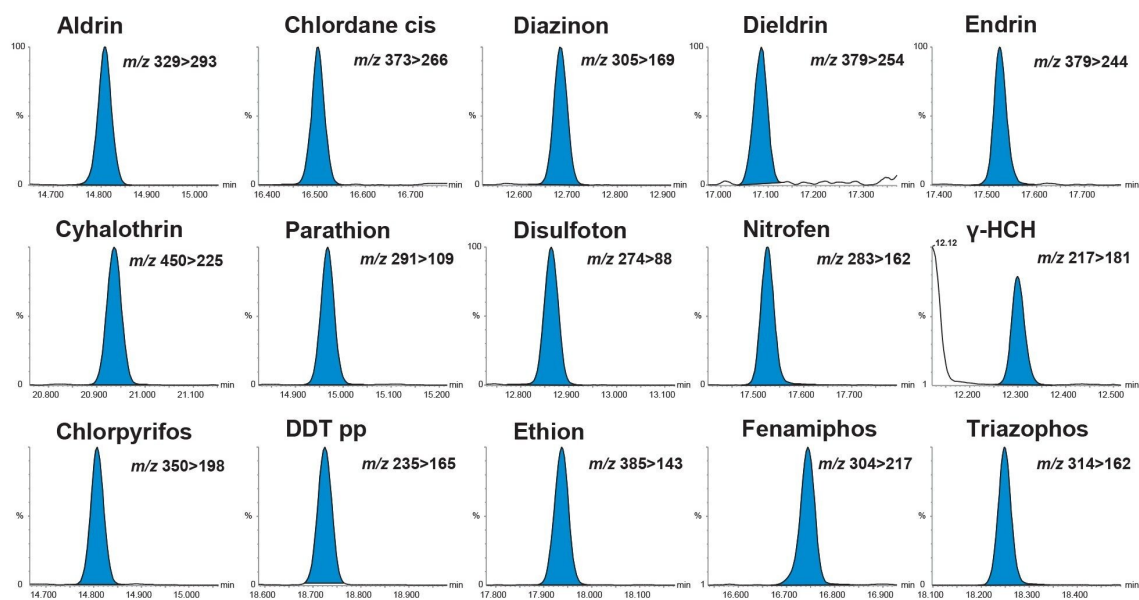


Figure 3. Chromatograms from the analysis of a selection of pesticides in the cucumber matrix-matched standard at 0.001 mg/kg.

The lowest calibrated level (LCL) for each analyte was established by evaluation of the calibration graph characteristics. The performance for resmethrin was considered semi-quantitative only as the calibration graph exhibited poor residuals (>20%) across the concentration range and a coefficient of determination (r^2) of 0.96. Data points at low concentrations were excluded from the calibration graphs of the following analytes due to poor residuals (>20%) and values for LCL were adjusted accordingly: captafol (0.005 mg/kg), chlorothanil (0.005 mg/kg), op DDT (0.005 mg/kg), folpet (0.001 mg/kg) and isodrin (0.001 mg/kg). After these adjustments, all analytes other than resmethrin exhibited residuals well within the $\pm 20\%$ SANTE tolerance.⁶ Other than captafol ($r^2=0.98$), the graphs for all other analytes had values for $r^2 > 0.99$. Bracketed calibration graphs from the analysis of a selection of pesticides in cucumber matrix-matched standards are given in Figure 4.

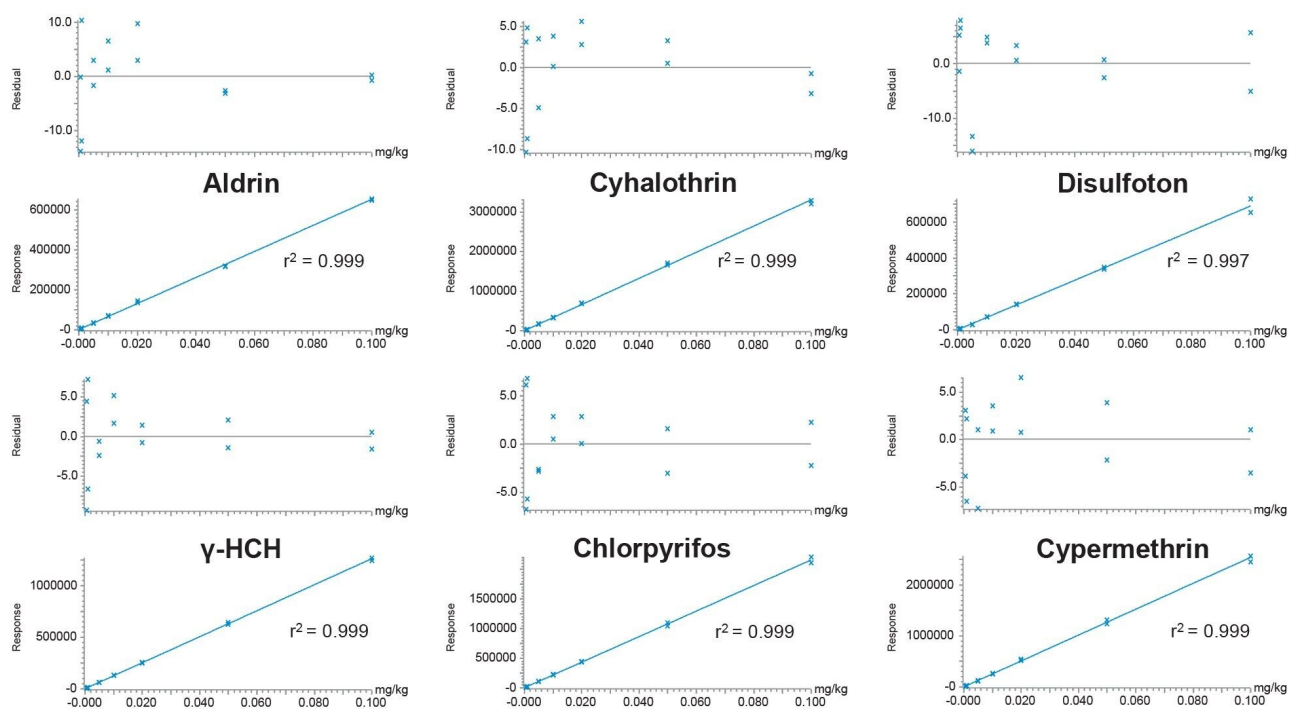


Figure 4. Calibration graphs from the analysis of a selection of pesticides in cucumber matrix-matched standards.

Identification criteria, retention times and ion ratios, were calculated and flagged using TargetLynx. The retention time and ion ratio of each analyte detected in each spiked sample should correspond to that of the calibration standard reference.⁶ The retention times of all the analytes were found to be within the tolerance of ± 0.1 minute. The ion ratios from the analysis of the samples spiked at 0.001 mg/kg were within $\pm 30\%$ of the average of calibration standards from same sequence for 97% of the analytes, the exceptions being chloroneb, cycloate, diphenylamine, 2, 3, 5, 6-tetrachloroaniline, and tetramethrin. The ion ratios from the data from analysis of the spikes at 0.01 mg/kg were within tolerance for all the analytes.

The recovery was evaluated using the data from the analysis of the five replicate spikes, at the two concentrations. The SANTE guidelines specifies an average recovery for each spike level tested to be between 70% and 120%.⁶ The results from analysis of the spikes at 0.001 mg/kg showed that 94% of the analytes were within that tolerance. The exceptions were azinphos methyl (58%), captafol (64%), chlorfenapyr (69%), chlorothalonil (156%), op DDT (10%), and resmethrin (no results due to poor quantification). At the higher concentration of 0.01 mg/kg, only captafol (69%) was just outside the tolerance, with results for resmethrin (74%)

being considered indicative only due to poor calibration. A summary of the recovery results is shown in Figure 5.

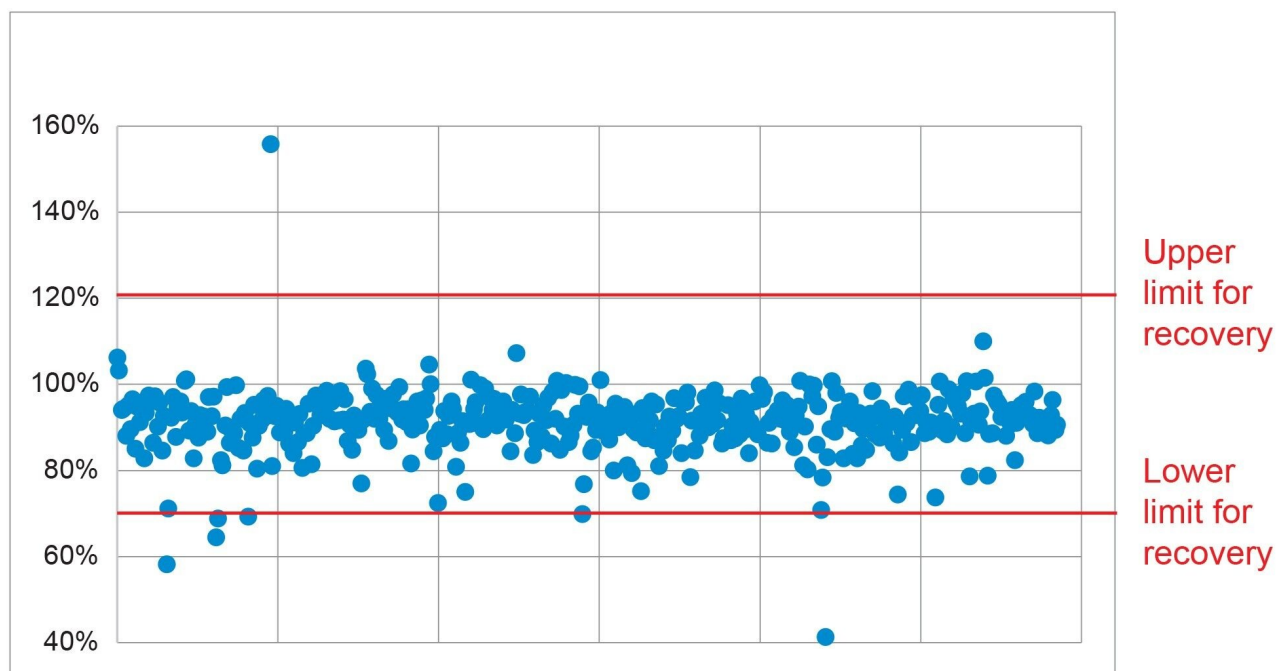


Figure 5. Summary of the recoveries (percent) from the analysis of cucumber spiked at 0.001 mg/kg and 0.01 mg/kg (extreme values for op DDT [10%] and chlorothalonil [156%] not shown)

The repeatability (RSD_r) of the method was also satisfactory. SANTE guidelines states that RSD_r for each spike level tested should be $\leq 20\%$.⁶ At 0.001 mg/kg, 99% of the analytes were within this tolerance. The exceptions were op DDT (34%) and isodrin (23%). At the higher concentration of 0.01 mg/kg, all the analytes exhibited values for $RSD_r \leq 20\%$. A summary of the repeatability results is shown in Figure 6.

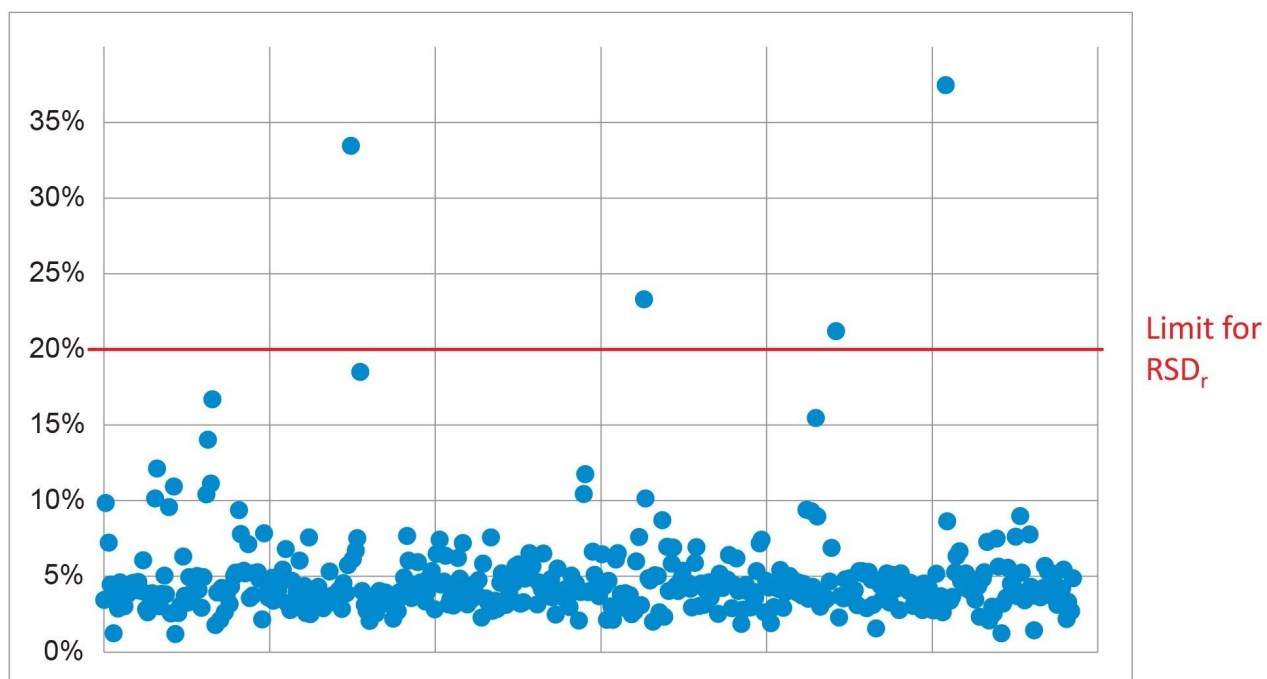


Figure 6. Summary of the repeatability (%RSD_r) from the analysis of cucumber spiked at 0.001 and 0.01 mg/kg.

A cucumber puree reference material (T19290QC) was analyzed to further evaluate the performance of the method. The measured values agreed well with the assigned values for five of the analytes, with good repeatability (Table 1). The mean value for tolylfluanid (0.049 mg/kg) was much lower than the assigned value of (0.081 mg/kg) but inside the concentration range within the limits of ± 2 z-scores (0.040–0.103 mg/kg). These measurements exhibited good repeatability (RSD 5.8%). Tolyfluanid is associated with stability issues and has been shown to degrade rapidly during sample preparation, if samples are allowed to defrost and during dSPE clean-up when using the PSA sorbent.^{7,8}

Analyte	Mean measured value (mg/kg)	RSD _r (%)	Assigned value (mg/kg)	Range for z ≤ 2
Cyhalothrin lambda	0.200	4.6	0.153	0.088–0.218
Dicloran	0.058	4.1	0.067	0.037–0.096
Dieldrin	0.042	1.9	0.042	0.023–0.060
Oxadiazon	0.060	3.1	0.057	0.032–0.082
Phosmet	0.090	9.0	0.083	0.045–0.116
Tolyfluanid	0.049	5.8	0.081	0.045–0.116

Table 1. Comparison of the measured and assigned values for pesticides from the analysis of the cucumber puree reference material.

Conclusion

This application note describes a sensitive and accurate multiresidue method for the determination of pesticide residues using GC-MS/MS (Xevo TQ-XS fitted with APGC). The method allowed for reliable quantitation down to concentrations well below typical MRLs and was successfully validated according the SANTE guidelines, presenting satisfactory results for 200 pesticides in cucumber. The method exhibited very high sensitivity (LODs typically <0.0005 mg/kg) without the need for solvent exchange, PTV or large volume injection. The results from analysis of the spikes showed almost all the analytes were within the required tolerance for recovery and repeatability, respectively. For example, at 0.001 mg/kg, 94% were within the tolerance for recovery and 99% for repeatability. The procedure can be applied to the analysis of other commodities after suitable validation. This method has been demonstrated as suitable for checking compliance with MRLs and has the potential for determination at much lower concentrations.

References

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Annex

Name	RT	CV(s)	MRMs (CE, eV)	Name	RT	CV(s)	MRMs (CE, eV)
2,3,5,8-Tetrachloroaniline	10.6	30	232>158 (40)	Fonofos	12.5	30	247>109 (20)
2,4-Methoxychlor	19.0	10	227>141 (30)	HCH α	11.5	10,30	181>146 (15)
2-Phenylphenol	9.1	10	171>153 (30)	HCH β	12.1	10,31	181>146 (15)
3,4-Dichloroaniline	7.9	30	162>127 (30)	HCH γ	12.3	10,32	181>146 (15)
4,4'-Dichlorobenzophenone	15.1	30	253>141 (20)	HCH δ	13.0	10,33	181>146 (15)
4,4-Methoxychlor olefin	16.5	30	309>166 (50)	Heptachlor	14.0	40	335>264 (20)
Acetochlor	13.7	30	223>146 (14)	Heptachlor epoxide	15.7	20	351>217 (20)
Acinethathrin	21.2	10	428>260 (20)	Hexachlorobenzene	11.6	30	284>214 (35)
Alachlor	13.9	30	238>162 (20)	Hexazinone	18.8	30	253>71 (30)
Aldrin	14.8	20	367>293 (15)	Iodofenphos	16.8	30,60	412>377 (5)
Allioquinolone	5.9	5	174>81 (15)	Iprodione	19.6	30	330>245 (10)
Antraquinone	14.9	20	208>152 (20)	Isazofos	12.9	30	314>162 (15)
Atrazine	12.2	10	216>104 (30)	Isodrin	15.5	30	363>159 (20)
Azinphos ethyl	21.2	5	132>77 (20)	Isopropalin	15.4	5	310>206 (15)
Azinphos methyl	20.5	5	261>125 (20)	Lanauil	18.9	30	235>153 (30)
Benfluralin	11.2	10	335>202 (10)	Leptaphos	20.5	5	413>317 (20)
Bifenthrin	19.8	10	181>165 (20)	Linuron	14.6	30	249>182 (15)
Bioallethrin	15.9	30	303>151 (10)	Malathion	14.7	20	331>127 (5)
Biphenyl	7.3	30	154>152 (25)	Metakyl	14.1	30	280>160 (20)
Bromfenvalphos	16.7	30	405>99 (30)	Metazachlor	15.6	20	278>210 (10)
Bromfenvalphos methyl	15.8	30	377>127 (20)	Methacrifos	8.9	30	241>125 (20)
Bromophos ethyl	16.2	10	393>337 (20)	Methoxychlor	19.9	10	227>196 (20)
Bromophos methyl	15.3	30	363>210 (20)	Methidathion	14.7	20	284>178 (20)
Bromopropylate	19.8	50	341>183 (20)	Mevinphos	7.9	30	225>127 (10)
Bupirimate	17.2	30	317>108 (30)	MGK 264 1*	15.3	30	276>210 (20)
Captafol	19.1	5	348>312 (10)	MGK 264 2*	15.6	30	276>210 (20)
Captan	15.9	5	264>106 (10)	Mirex	20.9	10	272>237 (15)
Carbofenthothion	18.5	5	343>157 (20)	Myclobutanil	17.1	30	288>179 (10)
Carfentazone ethyl	18.5	10	412>346 (20)	N-(2,4-Dimethylphenyl)formamide	8.6	5	149>106 (15)
Chlorobutadiene	16.2	20	268>125 (15)	Nitralin	19.3	30	345>300 (15)
Chlordane cis	16.5	30	373>296 (25)	Nitrofen	17.5	10	283>253 (15)
Chlordane trans	16.2	25	373>296 (25)	Nonachlor cis	17.9	30	407>300 (25)
Chlorfenvinphos	17.4	20	409>271 (20)	Nonachlor trans	16.6	30	407>300 (25)
Chlorfenson	16.7	5	303>159 (10)	Norfurazon	18.5	20	303>145 (20)
Chlorfenvinphos	15.8	30	359>170 (30)	Oxadiazon	17.1	5	345>177 (30)
Chlorobenzilate	17.7	30	307>251 (20)	Oxyfluorfen	17.2	30	361>300 (15)
Chloroneb	9.0	60	191>113 (20)	Paclobutrazol	16.4	30	294>70 (30)
Chlorothalonil	12.7	30	265>133 (30)	Parathion	15.0	30	291>109 (15)
Chlorpyrifos	14.8	40	350>322 (10)	Permethrin methyl	13.8	30	264>125 (20)
Chlorpyrifos methyl	13.7	40	322>290 (20)	Pebulate	8.4	30	204>72 (20)
Chlorthol dimethyl	14.9	30	332>301 (15)	Penconazole	15.7	5	284>159 (25)
Chlorthiophos 1*	17.7	30	360>269 (20)	Pendimethalin	15.6	20	264>201 (20)
Chlorthiophos 2*	18.0	30	360>269 (20)	Pentachloroaniline	13.3	15	266>230 (20)
Chlormezinone	15.7	30	331>188 (20)	Pentachloroisole	11.7	30	280>237 (25)
Cimazone	12.2	30	240>126 (30)	Pentachlorobenzene	9.1	40	248>178 (30)
Coumaphos	21.9	30	363>207 (20)	Pentachlorobenzonitrile	12.3	20	276>241 (20)
Cyfluthrin (sum)	22.6	10	434>127 (30)	Pentachloroethioanisole	14.5	30	297>250 (40)
Cyhalothrin	20.9	10	450>141 (20)	Permethrin 1*	21.8	10	391>255 (15)
Cypermethrin (sum)	22.9	20	416>127 (30)	Permethrin 2*	22.0	10	391>255 (15)
Cyprodinil	15.6	40	226>133 (30)	Phenothrin	20.4	20	351>193 (40)
DDD op	17.2	30	235>199 (20)	Phorate	11.4	10,50	260>75 (5)
DDD pp	17.9	30	235>199 (20)	Phosalone	20.5	10	368>111 (40)
DDE op	16.3	30	316>246 (25)	Phosmet	19.7	20	318>160 (10)
DDT pp	17.0	30	316>246 (25)	Piperonyl butoxide	19.2	30,70	338>176 (5)
DDT op	18.0	30	235>199 (20)	Primingphos methyl	14.4	5	306>125 (30)
DDT pp	18.7	30	235>199 (20)	Pirimiphos-ethyl	15.3	10	334>198 (23)
Deltamethrin	24.7	5	504>279 (10)	Pretlachlor	16.9	20	312>252 (15)
Di-allate cis	11.4	30	270>86 (13)	Prochloraz	21.9	30	378>309 (40)
Di-allate trans	11.7	30	270>86 (13)	Procymidone	16.0	30	284>228 (20)
Diazinon	12.7	40	305>153 (30)	Prodiamine	14.5	5	333>288 (20)
Dichlorofenidol	14.5	20	334>226 (20)	Profenofos	16.9	5	375>305 (25)
Dichlorobenzil	6.6	30	172>136 (15)	Profluralin	12.5	30,20	348>331 (10)
Dicloran	11.8	30	206>176 (10)	Propachlor	10.3	30	212>94 (30)
Dieldrin	17.1	20	379>325 (10)	Propargite	19.1	30	350>201 (10)
Dimethachlor	13.6	30	256>224 (15)	Propisochlor	14.0	30	284>148 (20)
Diphenamid	15.3	30	240>134 (20)	Propyzamide	12.6	30	256>173 (20)
Diphenylamine	10.6	10	170>152 (20)	Prothiofos	16.8	5	345>241 (15)
Disulfoton	12.9	20	274>88 (5)	Pyraclifos	21.4	5	361>211 (20)
Edifenphos	16.5	30	310>171 (15)	Pyrazophos	21.1	33	374>194 (32)
Endosulfan ether	13.3	30	341>170 (30)	Pyridaben	22.0	10,50	365>147 (25)
Endosulfan I	16.5	5	406.8>170 (40)	Pyridaphenthrin	19.6	30	341>189 (20)
Endosulfan II	17.8	5	406.8>170 (40)	Pyrimethanil	12.7	30	200>82 (25)
Endosulfan sulfate	18.6	10	323>217 (30)	Pyriproxyfen	20.7	30	322>96 (15)
Endrin	17.5	30	379>343 (10)	Quinalphos	15.9	30	299>271 (10)
Endrin ketone	19.6	30	381>281 (10)	Quintozene	12.2	5	296>279 (20)
EPN	19.8	30	324>296 (15)	Resmethrin	19.3	30	339>143 (20)
Esfenvalerate/Fenvalerate 1* (sum)	23.8	30	410>222 (10)	Sulfotep	11.2	30	323>171 (20)
Ethion	17.9	5	385>125 (20)	Sulphos	18.3	10	323>139 (20)
Ethofenprox	23.2	10,20	376>163 (10)	Tau-fluvalinate	24.0	40	503>208 (10)
Etridiazole	8.2	30	210>140 (20)	Tebuconazole	19.0	30	308>70 (30)
Fenamiphos	16.7	40	304>217 (20)	Tebufenpyrad	20.1	30	333>171 (15)
Fenarimol	21.1	40	331>139 (30)	Tecnazene	10.1	10	259>201 (15)
Fenitrothion	14.4	40	278>200 (20)	Telluthrin	13.0	5	419>325 (10)
Fenpropathrin	20.0	25	350>125 (10)	Terbacil	12.9	20	161>118 (20)
Fenvalerate 2*	24.1	30	419>225 (10)	Terbufos	12.5	5	289>103 (15)
Figronil	15.7	30	437>315 (30)	Tetradifon	20.3	10	354>227 (15)
Fluxifop-P-butyl	17.6	30	384>328 (15)	Tetrahydrophtalimide	8.8	5	151>80 (10)
Fluchloralin	12.8	30	304>161 (15)	Tetramethrin 1*	19.7	50	332>164 (13)
Flucythrinate 1*	23.0	5	412>219 (30)	Tetramethrin 2*	19.9	50	332>164 (13)
Flucythrinate 2*	23.2	5	412>219 (30)	Tolclofos-methyl	13.9	5	301>128 (20)
Fludioxinil	16.8	30	248>154 (20)	Tolyfluamid	16.7	5	346>137 (30)
Fluquinconazole	21.9	30,45	375>340 (7)	Tranfluthrin	14.0	35	371>83 (10)
Fluridone	23.4	30	330>310 (30)	Triadimefon	15.0	40	294>197 (10)
Flusilazole	17.2	30	316>247 (20)	Triadimenol	16.0	30	296>70 (10)
Flutolanil	16.8	30	323>145 (50)	Tri-allate	13.1	5	268>184 (15)
Flutriafol	16.8	30	310>200 (20)	Triazophos	18.3	30	314>182 (20)
Folpet	16.0	5	260>130 (10)	Tricyclazole	16.9	5	190>183 (20)
				Triflumizole	16.1	10	346>266 (20)
				Trifluralin	11.2	30	336>232 (20)
				Vinclozolin	13.8	5	286>242 (10)
							286>172 (20)

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