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Technical Report

Multi-Residue Analysis of 210 Pesticides in Food Samples by Triple Quadrupole UHPLC-MS/MS

David R. Baker[1], Chris Titman[1], Alan J. Barnes[1], Neil J. Loftus[1], Alexander Mastoroudes[2], Simon Hird[3]

[1]Shimadzu Corporation, Manchester, UK
[2]Kings College London, London, UK
[3]Food and Environment Research Agency, York, UK

Abstract

Pesticides and their metabolites are of great concern to society as they are harmful to human health, pollute natural resources and disturb the equilibrium of the ecosystem. Consequently, stricter food safety regulations are being enforced around the world, placing pesticide analysis laboratories under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times, and all the while maintaining or reducing costs. In this study a method was successfully developed for the quantitation of 210 commonly analysed pesticides in food samples using the Nexera UHPLC and LCMS-8040. Initial validation was performed to demonstrate instrument capabilities. Limits of detection (LOD) for 90 % of compounds were less than 0.001 mg kg^{-1} (1 ppb) and all compounds were less than 0.01 mg kg^{-1} (10 ppb) for both the quantifying and qualifying transitions using only a 2 µL injection. Repeatability at the 0.01 mg kg^{-1} reporting level was typically less than 5 %RSD for compounds and correlation coefficients were typically greater than 0.997 in a variety of studied food extracts. Consequently, the LCMS-8040 is ideally suited for routine monitoring of pesticides below the 0.01 mg kg^{-1} default level set by EU and Japanese legislation.

***Keywords*: Pesticides; Multi-residue analysis; LCMS-8040; Food safety; Fruit; Vegetables**

1. Introduction

Pesticide residues in food continue to be the target of studies due to the uncertainty concerning adverse effects that those residues may have on human health after a lengthy exposure at low levels. More than 1000 active ingredients have been utilised and are formulated in thousands of different commercial products. They include a variety of compounds, mainly insecticides, herbicides and fungicides, as well as their metabolites, with very different physico-chemical characteristics and large differences in polarity, volatility and persistence.[1] Consequently, in order to ensure food safety for consumers and to facilitate international trade, regulatory bodies around the world have established maximum residue levels (MRLs) for pesticide residues in food commodities; that is, the maximum amount of pesticide residue and its toxic metabolites that might be expected on a commodity if good agricultural practice was adhered to during the use of the pesticide.[2]

In the European Union regulation 396/2005/EC was implemented in 2008 harmonising pesticide MRLS in all member states for 435 pesticide active substances in 378 commodities.[3] This EU regulation covers pesticides both currently and formerly used in agriculture in or outside the EU. For pesticide and food commodity combinations not listed in the regulation a default MRL of 0.01 mg kg^{-1} applies (Art 18(1b) of European Union Regulation No 396/2005).[3] In general, MRLs in the European Food regulation are in the range 0.01 - 10 mg kg^{-1} depending on the pesticide-commodity combination, with the lowest levels set for banned pesticides. For vegetables, fruits and cereals intended for the production of baby foods, Directive 2006/141/EC requires that baby food contains no detectable levels of pesticide residues defined as < 0.01 mg kg^{-1} and prohibits the use of certain very toxic pesticides in the production of infant foods and establishes even lower MRLs for a few other very toxic pesticides.[4] Regulatory bodies around the world, as in the EU, have produced similar guidelines. In the US, tolerances for more than 450 pesticides and other ingredients are stated in the electronic Code of Federal Regulations (US Environmental Protection Agency Office of Pesticide Programs) and are enforced by the US FDA.[5] Japan's positive list system for agricultural chemical residues in foods, introduced in 2006, contains MRLs for over 400 pesticides in various commodities.[6] China published national standard GB 2763-2005 in 2005 and more recently GB 28260-2011 which was introduced in 2012 and specifies 181 MRLS for 85 pesticides in food.[7,8]

Consequently, pesticide analysis laboratories are under increasing pressure to expand the list of targeted pesticides, detect analytes at lower levels and with greater precision, reduce analysis turnaround times and reduce usage of hazardous solvents while maintaining or reducing costs. Pesticide residues were traditionally analysed mainly by GC-based multi-residue methods often with MS detection. However, many modern

(semi)polar compounds and/or ionic compounds could not be analysed in this way due to poor thermal stability or volatility without the need for derivatisation.[9] Recent improvements in liquid chromatography - tandem mass spectrometry, combined with the discussed pitfalls of GCMS, have meant LCMSMS has become a vital technique. LC-tripe quadruple mass spectrometry enables highly selective and sensitive analysis and is well suited to the multi-class analysis of large numbers of pesticides at trace levels.

In this work, we discuss the development of a multi-residue pesticide method for 210 pesticides using the Nexera UHPLC and LCMS-8040 triple quadruple. Pesticides were matrix-matched in food matrix (lettuce, pear and dried fruit) following QuEChERS sample preparation. The method was evaluated in matrix to ensure that the necessary reporting limits were obtained according to the various regulatory guidelines around the world with acceptable precision, in addition to ensuring chromatographic resolution of pesticide isomers with identical SRM transitions.

2. Experimental

A stock of pesticides was obtained from the Food and Environment Agency, UK, at a concentration of 0.01 mg kg^{-1} (for each pesticide) in acetone:acetonitrile 1:1. Linearity was investigated over a nine-point calibration with samples ranging from 0.5 µg kg^{-1} - 0.2 mg kg^{-1} (0.5 – 200 ppb) analysed in duplicate; calibration samples were injected once in increasing order and once in decreasing order. Linearity was assessed with four calibration curves prepared by serial dilution of: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. Instrumental area repeatability was determined by replicate (n=6) injection of pear matrix at 0.01 mg kg^{-1}. LC-MS mobile phase solvents and additives were all of LC–MS quality and purchased from Sigma–Aldrich.
Food extracts were supplied by the Food and Environment Agency, UK, following established QuEChERS protocols. QuEChERS is acronym for Quick Easy Cheap Effective Rugged Safe and is a widely used sample preparation technique for the extraction of pesticides from food. Food samples included dried fruit, lettuce and pear, with the final extracts prepared in 100% acetonitrile.

LC Parameters

UHPLC:	Nexera UHPLC system		
Column:	Shim-pack XR-ODS III (150 x 2 mm, 2.2 µm particle size)		
Column temp.:	40 °C		
Mobile phase:	A = Water with 5 mM ammonium formate and 0.01 % formic acid B = Methanol with 5 mM ammonium formate and 0.01 % formic acid		
Gradient:	Time (min)	A%	B%
	0	5	95
	16	0	100
	18	0	100
	18.1	5	95
	20	5	95
Flow rate:	0.4 mL min^{-1}		
Injection volume:	32 µL (stacked injection: 2µL sample + 30µL water)		
Needle wash:	1000 µL Methanol		

MS Parameters

MS:	LCMS-8040 triple quadrupole mass spectrometer
Ionisation:	ESI - Positive and negative (15 msec. polarity switch)
SRM:	Dwell time 5 msec. Pause time 1 msec.
Desolvation line:	250 °C
Heating block:	400 °C
Drying gas:	15 L min^{-1}
Nebulising gas:	2 L min^{-1}
SRM optimisation:	1:1 water:methanol with 10mM ammonium acetate Flow rate: 0.5mL min^{-1} Flow injection analysis (No column fitted) 0.2 µL (0.01 mg kg^{-1} pesticide standard solution)
Mobile phase screening:	Carrier 1:1 water:methanol Flow rate: 0.3mL min^{-1} Flow injection analysis (No column fitted) 5µL injection (0.01 mg kg^{-1} pesticide standard solution) 1µL air gap (see text for mobile phase compositions)

Pesticide limits of detection were calculated based on the method described by the US-EPA in Title 40 Code of Federal Regulation Part 136,[10] using a standard deviation of 7 replicates in pear matrix at a concentration value that corresponds to an instrument signal to noise ratio in the range of 2.5 to 5 and a Student's t 99% confidence interval:

$$MDL = St(n-1,1-\alpha=0.99)\ x\ s.d.$$

Where, t(n-1,1-α=0.99) = Student's t value for the 99% confidence level with n-1 degrees of freedom (t = 3.14 for 7 replicates), n = number of replicates, and s.d. = standard deviation of the replicate analyses.

3. Results and discussion

3.1 SRM optimisation

Target precursor and product ions were selected based on recommendations from the Food and Environment Agency, UK, and data from the EURL DataPool.[11] Typically the protonated or deprotonated molecule was used for the precursor ion. In order to try to prevent interference of SRM transitions from matrix, product ions greater than m/z 100 were selected where possible as they are typically more diagnostic.[12] Analyte specific MS parameters (Q1 pre-bias (V), Q3 pre-bias (V) and collision energy) were optimised using automated flow injection analysis. Briefly, this involves placing pesticide standards into the auto-sampler, from where they are then rapidly injected into the MS with a different parameter optimised on each injection. Each compound was optimised in only a few minutes using the automated software provided in LabSolutions. This allowed large numbers of compounds to be optimised overnight; this is in stark contrast to traditional time-consuming infusion in order to optimise parameters. The compounds studied and their associated transitions are shown in Table 1.

Table 1 *- Studied compounds and their chemical formulas, CAS numbers, SRMs, retention times, limits of detection and* R^2

Compound	Formula	CAS	Transition 1	Transition 2	Pear extract				
					RT (min.)	Transition 1 LOD (ppb)	Transition 2 LOD (ppb)	%RSD (10ppb)	R^2
Avermectin B1a	C48H72O14	71751-41-2	891 > 305	891 > 567	16.4	0.35	0.56	5.0	0.9975
Acephate	C4H10NO3PS	30560-19-1	184 > 143	184 > 49	3.0	0.17	0.31	1.0	0.9999
Acetamiprid	C10H11ClN4	135410-20-7	223 > 126	223 > 99	7.2	0.50	1.00	1.1	0.9979
Acrinathrin	C26H21F6NO5	101007-06-1	559 > 208	559 > 181	16.1	1.32	2.36	4.4	0.9990
Alachlor	C14H20ClNO2	15972-60-8	270 > 238	270 > 162	13.4	0.09	0.26	1.5	0.9995
Aldicarb	C7H14N2O2S	116-06-3	208 > 116	208 > 89	8.5	0.05	0.10	1.7	0.9998
Aldicarb sulfone	C7H14N2O4S	1646-88-4	240 > 223	240 > 86	4.3	0.17	0.13	1.8	0.9999
Aldicarb sulfoxide	C7H14N2O3S	1646-87-3	207 > 89	207 > 132	3.9	0.22	0.36	2.3	1.0000
Amidosulfuron	C9H15N5O7S2	120923-37-7	370 > 261	370 > 139	9.3	0.14	0.22	2.8	0.9984
Asulam	C8H10N2O4S	3337-71-1	231 > 156	231 > 92	3.4	0.72	2.03	3.8	0.9979
Atrazine	C8H14ClN5	1912-24-9	216 > 174	216 > 104	11.1	0.10	0.22	2.4	0.9989
Azinphos-methyl	C10H12N3O3PS2	86-50-0	318 > 132	318 > 77	11.8	0.50	0.50	2.7	0.9903
Azoxystrobin	C22H17N3O5	131860-33-8	404 > 372	404 > 344	12.1	0.03	0.30	2.1	0.9989
Bendiocarb	C11H13NO4	22781-23-3	224 > 109	224 > 167	9.8	0.10	0.09	1.5	0.9996
Benthiavalicarb-isopropyl	C18H24FN3O3S	177406-68-7	382 > 180	382 > 116	12.7	0.12	0.41	0.9	0.9997
Bispyribac sodium	C19H17N4NaO8	125401-92-5	453 > 297	453 > 179	12.1	1.41	5.43	7.4	0.9954
Boscalid	C18H12Cl2N2O	188425-85-6	343 > 307	343 > 140	12.5	0.81	1.19	4.6	0.9968
Bromoxynil*	C7H3Br2NO	1689-84-5	274 > 79	276 > 81	9.9	2.24	2.61	4.5	0.9968
Bromuconazole	C13H12BrCl2N3O	116255-48-2	376 > 159	376 > 70	13.0	0.72	1.79	2.9	0.9994
Butachlor	C17H26ClNO2	23184-66-9	312 > 238	312 > 57	15.3	0.29	0.39	1.6	0.9998
Butocarboxim	C7H14N2O2 S	34681-10-2	208 > 75	208 > 191	8.4	0.13	0.87	3.1	0.9999
Butocarboxim sulfone	C7H14N2O4S	34681-23-7	223 > 106	223 > 166	4.1	2.63	3.23	9.7	0.9949
Butocarboxim sulfoxide	C7H14N2O3S	34681-24-8	207 > 88	207 > 75	3.7	0.22	0.21	1.9	0.9999
Carbaryl	C12H11NO2	63-25-2	202 > 145	202 > 127	10.3	0.13	0.22	2.4	0.9988
Carbendazim	C9H9N3O2	10605-21-7	192 > 160	192 > 132	7.1	0.50	1.00	1.1	0.9996
Carbofuran	C12H15NO3	1563-66-2	222 > 165	222 > 123	11.1	0.12	0.18	0.7	0.9993
Carboxin	C12H13NO2S	5234-68-4	236 > 143	236 > 87	10.2	0.09	0.25	0.9	0.9991
Chlorantraniliprole*	C18H14BrCl2N5O2	500008-45-7	482 > 284	482 > 177	11.8	0.50	1.00	2.3	0.9979
Chlorfenvinfos	C12H14Cl3O4P	470-90-6	361 > 155	361 > 99	14.0	0.28	0.49	2.3	0.9966
Chloridazon	C10H8ClN3O	1698-60-8	222 > 92	222 > 104	7.2	0.20	0.18	3.2	0.9990
Chlorotoluron	C10H13ClN2O	15545-48-9	213 > 72	213 > 46	10.8	0.05	0.13	1.3	0.9967
Chromafenozide	C24H30N2O3	143807-66-3	395 > 175	395 > 91	13.0	0.05	0.60	1.0	0.9977
Clethodim	C17H26ClNO3S	99129-21-2	360 > 164	360 > 268	14.7	0.08	0.45	0.7	0.9970
Clofentezine	C14H8Cl2N4	74115-24-5	303 > 138	303 > 102	14.4	4.03	5.76	9.5	0.9967
Clothianidin	C6H8ClN5O2S	210880-92-5	250 > 132	250 > 169	6.5	0.25	0.12	1.6	0.9978
Cyazofamid	C13H13ClN4O2S	120116-88-3	325 > 108	325 > 261	13.3	0.39	3.74	2.4	0.9964
Cycloxydim	C17H27NO3S	101205-02-1	326 > 280	326 > 180	14.8	0.33	0.73	1.0	0.9989
Cyflufenamid	C20H17F5N2O2	180409-60-3	413 > 295	413 > 241	14.2	0.27	0.29	2.9	0.9982
Cymoxanil	C7H10N4O3	57966-95-7	199 > 128	199 > 111	7.7	2.99	3.52	5.5	0.9960
Cyproconazole	C15H18ClN3O	113096-99-4	292 > 70	292 > 125	12.8	0.41	0.60	3.5	0.9988
Cyprodinil	C14H15N3	121552-61-2	226 > 93	226 > 108	13.9	0.89	0.91	1.3	0.9990
Cyromazine	C6H10N6	66215-27-8	167 > 85	167 > 125	2.2	2.57	4.79	7.4	0.9994
Demeton-S-methyl sulfoxide	C6H15O4PS2	301-12-2	247 > 169	247 > 109	5.0	0.01	0.03	1.2	0.9999
Demeton-S-methyl sulfone	C6H15O5PS2	17040-19-6	263 > 169	263 > 109	5.3	0.03	0.10	3.1	0.9999
Desmedipham	C16H16N2O4	13684-56-5	318 > 182	318 > 136	11.6	0.08	0.33	0.5	0.9971
Diclobutrazol	C15H19Cl2N3O	75736-33-3	328 > 70	330 > 70	13.8	0.17	0.20	2.7	0.9988

Table 1 *– Continued...*

Compound	Formula	CAS	Transition 1	Transition 2	Pear extract				
					RT (min.)	Transition 1 LOD (ppb)	Transition 2 LOD (ppb)	%RSD (10ppb)	R^2
Diethofencarb	C14H21NO4	87130-20-9	268 > 226	268 > 124	12.2	0.06	0.12	2.2	0.9996
Difenoconazole	C19H17Cl2N3O3	119446-68-3	406 > 251	406 > 188	14.5	0.18	0.53	2.6	0.9994
Diflubenzuron	C14H9ClF2N2O2	35367-38-5	311 > 158	311 > 141	13.5	2.21	7.48	9.2	0.9936
Dimethoate	C5H12NO3PS2	60-51-5	230 > 125	230 > 199	7.0	0.05	0.07	1.6	0.9997
Dimethomorph	C21H22ClNO4	110488-70-5	388 > 301	388 > 165	12.7	0.29	0.41	2.5	0.9991
Dimoxystrobin	C19H22N2O3	149961-52-4	327 > 205	327 > 116	13.7	0.12	0.14	0.5	0.9997
Dinotefuran	C7H14N4O3	165252-70-0	203 > 129	203 > 157	3.9	0.10	0.22	2.9	0.9994
Disulfoton sulfoxide	C8H19O3PS3	2497-07-6	291 > 213	291 > 97	10.8	0.05	0.15	2.6	0.9980
Diuron	C9H10Cl2N2O	330-54-1	233 > 72	235 > 72	11.4	0.09	0.26	0.6	0.9971
DMPF	C10H14N2	33089-74-6	163 > 107	163 > 122	4.8	1.00	2.00	2.5	0.9910
Dodine	C15H33N3O2	2439-10-3	228 > 71	228 > 60	13.5	0.30	0.54	1.7	0.9946
Epoxiconazole	C17H13ClFN3O	135319-73-2	330 > 121	330 > 101	13.3	0.12	0.37	2.5	0.9998
Ethiofencarb	C11H15NO2S	29973-13-5	226 > 107	226 > 169	10.6	0.18	0.59	0.7	0.9994
Ethiofencarb sulfone	C11H15NO2S2	53380-23-7	275 > 107	275 > 201	6.2	0.02	0.16	0.9	0.9999
Ethiofencarb sulfoxide	C11H15NO3S	53380-22-6	242 > 107	242 > 185	6.5	0.02	0.02	0.9	0.9999
Ethirimol	C11H19N3O	23947-60-6	210 > 140	210 > 98	10.8	0.14	0.24	1.8	0.9977
Etofenprox	C25H28O3	80844-07-1	394 > 177	394 > 359	16.9	0.03	0.06	3.1	0.9983
Fenamidone	C17H17N3OS	161326-34-7	312 > 92	312 > 236	12.4	0.06	0.18	1.9	0.9988
Fenamiphos	C13H22NO3PS	22224-92-6	304 > 217	304 > 202	13.5	0.05	0.28	1.9	0.9970
Fenamiphos sulfone	C13H22NO5PS	31972-44-8	336 > 266	336 > 188	10.2	0.31	0.25	4.3	0.9961
Fenamiphos sulfoxide	C13H22NO4PS	31972-43-7	320 > 108	320 > 171	10.0	0.18	0.52	3.3	0.9976
Fenbuconazole	C19H17ClN4	114369-43-6	337 > 125	337 > 70	13.4	0.23	0.40	5.0	0.9964
Fenhexamid	C14H17Cl2NO2	126833-17-8	302 > 97	302 > 55	13.1	0.75	0.95	0.9	0.9944
Fenoxycarb	C17H19NO4	79127-80-3	302 > 88	302 > 116	13.6	0.10	0.20	2.4	0.9989
Fenpropimorph	C20H33NO	67564-91-4	304 > 147	304 > 117	14.1	0.05	0.13	1.6	0.9995
Fenpyroximate	C24H27N3O4	111812-58-9	422 > 366	422 > 215	15.9	0.02	0.17	1.2	0.9997
Fenthion sulfoxide	C10H15O4PS2	3761-41-9	295 > 109	295 > 280	10.1	0.18	0.27	1.5	0.9985
Fenthion sulfone	C10H15O5PS2	3761-42-0	311 > 109	311 > 125	10.4	3.75	3.61	9.8	0.9974
Fipronil*	C12H4Cl2F6N4OS	120068-37-3	435 > 330	435 > 250	13.5	0.11	0.35	4.1	0.9998
Fluazifop acid*	C15H12F3NO4	69335-91-7	328 > 282	328 > 91	11.8	0.55	3.61	7.1	0.9983
Fluazinam*	C13H4Cl2F6N4O4	79622-59-6	463 > 416	463 > 398	15.2	0.20	0.27	2.7	0.9994
Fludioxonil*	C12H6F2N2O2	131341-86-1	247 > 126	247 > 180	12.4	1.00	1.00	4.2	0.9974
Flufenacet	C14H13F4N3O2S	142459-58-3	364 > 152	364 > 194	13.2	0.04	0.06	1.6	0.9986
Flufenoxuron	C21H11ClF6N2O3	101463-69-8	489 > 158	489 > 141	15.7	0.24	0.63	8.2	0.9989
Fluometuron	C10H11F3N2O	2164-17-2	233 > 72	233 > 46	10.6	0.12	0.14	1.3	0.9996
Fluopicolide	C14H8Cl3F3N2O	239110-15-7	383 > 173	383 > 145	12.7	0.05	0.17	2.1	0.9967
Fluoxastrobin	C21H16ClFN4O5	361377-29-9	459 > 427	459 > 188	13.1	0.19	0.22	1.7	0.9987
Fluroxypyr*	C7H5Cl2FN2O3	69377-81-7	253 > 195	255 > 197	7.8	1.13	1.75	5.7	0.9993
Flutriafol	C16H13F2N3O	76674-21-0	302 > 70	302 > 123	11.1	0.29	0.43	3.2	0.9984
Fosthiazate	C9H18NO3PS2	98886-44-3	284 > 104	284 > 228	10.7	0.05	0.12	2.7	0.9985
Furathiocarb	C18H26N2O5S	65907-30-4	383 > 195	383 > 252	15.1	0.07	0.13	1.8	1.0000
Halofenozide	C18H19ClN2O2	112226-61-6	331 > 105	331 > 275	12.3	0.05	0.05	1.7	0.9947
Halosulfuron-methyl*	C13H15ClN6O7S	100784-20-1	435 > 182	437 > 182	11.5	0.30	0.96	3.1	0.9968
Haloxyfop acid*	C15H11ClF3NO4	69806-34-4	360 > 288	362 > 290	13.3	6.20	6.86	13.4	0.9999
Heptenophos	C9H12ClO4P	23560-59-0	251 > 127	251 > 89	11.4	0.15	1.36	4.7	0.9982
Hexythiazox	C17H21ClN2O2S	78587-05-0	353 > 228	353 > 168	15.6	2.25	1.02	4.5	0.9956

Table 1 *– Continued...*

Compound	Formula	CAS	Transition 1	Transition 2	Pear extract				
					RT (min.)	Transition 1 LOD (ppb)	Transition 2 LOD (ppb)	%RSD (10ppb)	R^2
Imazalil	C14H14Cl2N2O	35554-44-0	297 > 159	297 > 69	11.8	0.30	0.48	3.5	0.9988
Imidacloprid	C9H10ClN5O2	138261-41-3	256 > 209	256 > 175	6.4	0.50	0.50	1.9	0.9966
Indoxacarb	C22H17ClF3N3O7	144171-61-9	528 > 203	528 > 150	14.5	0.40	0.37	3.9	0.9964
Ioxynil*	C7H3I2NO	1689-83-4	370 > 127	370 > 215	11.0	0.12	1.00	3.6	0.9961
Iprovalicarb	C18H28N2O3	140923-17-7	321 > 119	321 > 203	13.1	0.06	0.23	2.5	0.9981
Isazofos	C9H17ClN3O3PS	42509-80-8	314 > 120	314 > 162	12.9	0.04	0.13	2.2	0.9994
Isocarbofos	C11H16NO4PS	24353-61-5	307 > 231	307 > 121	11.4	0.07	0.12	2.7	0.9991
Isofenphos	C15H24NO4PS	25311-71-1	346 > 245	346 > 217	14.3	0.17	0.13	1.7	0.9991
Isofenphos-methyl	C14H22NO4PS	99675-03-3	332 > 231	332 > 273	13.8	0.03	0.13	1.2	0.9996
Isoprocarb	C11H15NO2	2631-40-5	194 > 95	194 > 137	11.1	0.20	0.49	1.9	0.9990
Isoprothiolane	C12H18O4S2	50512-35-1	291 > 189	291 > 231	12.6	0.10	0.09	0.9	0.9994
Isoproturon	C12H18N2O	34123-59-6	207 > 72	207 > 46	11.3	0.10	0.11	1.7	0.9996
Isoxaben	C18H24N2O4	82558-50-7	333 > 165	333 > 150	12.6	0.02	0.06	0.9	0.9989
Kresoxim-methyl	C18H19NO4	143390-89-0	314 > 116	314 > 206	13.8	0.15	0.18	3.3	0.9991
Lenacil	C13H18N2O2	2164-08-1	235 > 153	235 > 136	11.2	0.18	0.64	2.2	0.9987
Linuron	C9H10Cl2N2O2	330-55-2	249 > 160	249 > 182	12.2	3.15	3.20	3.7	0.9979
Lufenuron*	C17H8Cl2F8N2O3	103055-07-8	509 > 339	509 > 175	15.2	0.35	2.39	3.8	0.9918
Malathion	C10H19O6PS2	121-75-5	348 > 127	348 > 331.2	12.6	0.04	0.31	1.0	0.9989
Mandipropamid	C23H22ClNO4	374726-62-2	412 > 328	412 > 356	12.5	0.11	0.45	4.2	0.9991
Mecarbam	C10H20NO5PS2	2595-54-2	330 > 227	330 > 97	13.2	0.15	0.30	2.0	0.9992
Mepanipyrim	C14H13N3	110235-47-7	224 > 106	224 > 77	13.1	0.19	0.39	3.6	0.9993
Mepronil	C17H19NO2	55814-41-0	270 > 119	270 > 91	12.7	0.05	0.07	1.1	0.9972
Mesosulfuron-methyl	C17H21N5O9S2	208465-21-8	504 > 182	504 > 83	10.9	0.27	0.96	3.4	0.9996
Metaflumizone	C24H16F6N4O2	139968-49-3	507 > 178	507 > 287	15.1	2.63	3.42	6.6	0.9986
Metalaxyl	C15H21NO4	57837-19-1	280 > 220	280 > 192	11.3	0.04	0.06	1.9	0.9998
Metamitron	C10H10N4O	41394-05-2	203 > 175	203 > 104	7.0	0.21	0.44	2.3	0.9990
Metconazole	C17H22ClN3O	125116-23-6	320 > 70	322 > 125	14.2	0.10	0.30	3.6	0.9976
Methabenzthiazuron	C10H11N3OS	18691-97-9	222 > 165	222 > 150	11.1	0.11	0.19	0.9	0.9989
Methamidophos	C2H8NO2PS	10265-92-6	142 > 94	142 > 125	2.3	0.06	0.69	1.3	0.9991
Methiocarb	C11H15NO2S	2032-65-7	226 > 121	226 > 169	12.3	0.10	0.28	2.9	0.9948
Methiocarb sulfoxide	C11H15NO3S	2635-10-1	242 > 122	242 > 170	6.9	0.04	0.15	1.5	0.9996
Methomyl	C5H10N2O2S	16752-77-5	163 > 88	163 > 106	5.0	0.10	0.10	0.8	0.9996
Methoxyfenozide	C22H28N2O3	161050-58-4	369 > 149	369 > 313	12.7	0.50	1.00	1.7	0.9980
Metobromuron	C9H11BrN2O2	3060-89-7	259 > 148	259 > 91	10.9	0.35	0.63	3.2	0.9987
Metolachlor	C15H22ClNO2	51218-45-2	284 > 252	284 > 176	13.4	0.06	0.31	1.5	0.9962
Metolcarb	C9H11NO2	1129-41-5	166 > 109	166 > 94	9.1	0.12	0.29	2.4	0.9996
Metosulam	C14H13Cl2N5O4S	139528-85-1	418 > 175	418 > 140	10.1	0.24	0.23	2.2	0.9968
Metoxuron	C10H13ClN2O2	19937-59-8	229 > 72	229 > 156	8.7	0.04	0.30	1.4	0.9997
Metrafenone	C19H21BrO5	220899-03-6	409 > 209	409 > 227	14.4	0.09	0.10	1.3	0.9993
Metsulfuron-methyl	C14H15N5O6S	74223-64-6	382 > 167	382 > 77	9.2	0.19	0.97	1.2	0.9982
Mevinphos	C7H13O6P	7786-34-7	225 > 127	225 > 193	7.1	0.05	0.16	2.5	0.9998
Molinate	C9H17NOS	2212-67-1	188 > 126	188 > 55	12.9	2.08	1.25	3.1	0.9956
Monocrotophos	C7H14NO5P	6923-22-4	224 > 193	224 > 127	5.6	0.72	1.35	4.8	0.9991
Monuron	C9H11ClN2O	150-68-5	199 > 72	199 > 46	9.4	0.13	0.21	1.6	0.9995
Myclobutanil	C15H17ClN4	88671-89-0	289 > 70	289 > 125	12.8	0.23	0.44	2.6	0.9990
Neoquassin	C22H30O6	76-77-7	391 > 373	391 > 207	10.2	0.29	1.63	2.3	0.9970

Table 1 *– Continued...*

Compound	Formula	CAS	Transition 1	Transition 2	Pear extract				
					RT (min.)	Transition 1 LOD (ppb)	Transition 2 LOD (ppb)	%RSD (10ppb)	R^2
Nitenpyram	C11H15ClN4O2	120738-89-8	271 > 126	271 > 225	4.7	0.15	0.29	2.6	1.0000
Nuarimol	C17H12ClFN2O	63284-71-9	315 > 252	315 > 81	12.2	0.75	2.66	2.8	0.9990
Omethoate	C5H12NO4PS	1113-02-6	214 > 125	214 > 183	3.6	0.16	0.18	1.6	0.9998
Oxadixyl	C14H18N2O4	77732-09-3	296 > 279	296 > 219	9.0	0.25	0.26	1.7	0.9999
Oxamyl	C7H13N3O3S	23135-22-0	237 > 72	237 > 90	4.6	0.03	0.10	1.5	0.9999
Paclobutrazol	C30H40Cl2N6O2	76738-62-0	294 > 70	294 > 125	12.6	0.18	2.74	2.4	0.9982
Penconazole	C13H15Cl2N3	66246-88-6	284 > 70	284 > 159	13.9	0.17	0.20	2.6	0.9992
Pencycuron	C19H21ClN2O	66063-05-6	329 > 125	329 > 218	14.4	0.03	0.39	1.5	0.9992
Phenmedipham	C16H16N2O4	13684-63-4	318 > 168	318 > 136	11.8	0.36	0.32	1.0	0.9949
Phenthoate	C12H17O4PS2	2597-03-7	321 > 79	321 > 247	13.7	0.32	0.55	2.3	0.9993
Phorate sulfone	C7H17O5PS2	2588-04-7	293 > 171	293 > 97	11.0	0.51	0.26	3.4	0.9964
Phorate sulfoxide	C7H17O4PS2	2588-05-8	277 > 97	277 > 199	10.8	0.26	0.13	0.9	0.9979
Phosphamidon	C10H19ClNO5P	297-99-4	300 > 174	300 > 127	9.3	0.10	0.19	1.0	0.9998
Phoxim	C12H15N2O3PS	14816-18-3	299 > 77	299 > 129	14.1	0.25	0.30	2.0	0.9992
Picolinafen	C19H12F4N2O2	137641-05-5	377 > 238	377 > 145	15.2	0.26	1.38	5.4	0.9999
Picoxystrobin	C18H16F3NO4	117428-22-5	368 > 145	368 > 205	13.5	0.12	0.17	1.3	0.9994
Pirimicarb	C11H18N4O2	23103-98-2	239 > 72	239 > 182	10.8	0.05	0.10	2.1	0.9996
Pirimicarb-desmethyl	C10H16N4O2	152-16-9	225 > 72	225 > 168	8.5	0.04	0.04	1.7	0.9996
Prochloraz	C15H16Cl3N3O2	67747-09-5	376 > 308	376 > 70	14.3	0.10	0.19	2.8	0.9987
Profenofos	C11H15BrClO3PS	41198-08-7	375 > 305	375 > 347	15.0	0.30	0.38	2.6	0.9997
Promecarb	C12H17NO2	2631-37-0	208 > 109	208 > 151	12.5	0.44	0.42	3.1	0.9993
Prometryn	C10H19N5S	7287-19-6	242 > 158	242 > 200	13.1	0.07	0.08	1.6	0.9998
Propamocarb free base	C9H20N2O2	24579-73-5	189 > 102	189 > 74	3.1	0.23	0.22	1.4	0.9984
Propaquizafop	C22H22ClN3O5	111479-05-1	444 > 100	44 > 371	15.2	0.15	0.85	1.2	0.9990
Propiconazole	C15H17Cl2N3O2	60207-90-1	342 > 159	342 > 69	14.0	0.23	0.60	3.6	0.9998
Propoxur	C11H15NO3	114-26-1	210 > 111	210 > 168	9.7	0.07	0.08	2.6	0.9998
Propyzamide	C12H11Cl2NO	23950-58-5	256 > 190	258 > 192	12.7	1.83	1.94	6.0	0.9915
Prosulfuron	C15H16F3N5O4S	94125-34-5	420 > 141	420 > 167	11.7	0.43	0.82	2.0	0.9940
Prothioconazole	C14H15Cl2N3OS	178928-70-6	312 > 70	314 > 70	13.4	0.16	0.50	2.3	0.9952
Pymetrozine	C10H11N5O	123312-89-0	218 > 105	218 > 79	5.0	0.05	0.39	2.9	0.9994
Pyraclostrobin	C19H18ClN3O4	175013-18-0	388 > 194	388 > 163	14.2	0.50	1.00	1.9	0.9996
Pyrethrin I	C21H28O3	121-21-1	329 > 161	329 > 105	15.9	0.25	1.20	2.3	0.9998
Pyrethrin II	C22H28O5	121-29-9	373 > 161	373 > 133	14.6	0.70	2.27	4.2	0.9992
Pyrimethanil	C12H13N3	53112-28-0	200 > 107	200 > 82	12.3	0.10	0.50	0.9	0.9999
Pyriproxyfen	C20H19NO3	95737-68-1	322 > 96	322 > 185	15.5	0.07	0.10	0.6	0.9999
Quassia	C22H28O6	76-78-8	389 > 223	389 > 163	9.1	0.57	0.80	2.7	0.9968
Quinmerac	C11H8ClNO2	90717-03-6	222 > 204	222 > 141	6.8	0.09	0.45	1.8	0.9966
Quinoxyfen	C15H8Cl2FNO	124495-18-7	308 > 197	308 > 162	15.6	0.18	0.23	3.2	0.9998
Rimsulfuron	C14H17N5O7S2	122931-48-0	432 > 182	432 > 325	10.0	0.31	0.64	2.8	0.9989
Rotenone	C23H22O6	83-79-4	395 > 213	395 > 192	13.5	0.44	0.52	3.5	0.9976
Spinosyn A	C41H65NO10	131929-60-7	733 > 142	733 > 98	14.1	0.03	0.19	1.6	0.9997
Spinosyn D	C42H67NO10	131929-63-0	747 > 142	747 > 98	14.6	0.20	0.97	3.3	1.0000
Spiromesifen	C23H30O4	283594-90-1	388 > 273	388 > 371	15.6	0.05	0.34	2.3	0.9998
Spiroxamine	C18H35NO2	118134-30-8	298 > 144	298 > 100	11.7	0.08	0.18	2.1	0.9999
Sulcotrione	C14H13ClO5S	99105-77-8	329 > 139	329 > 69	7.5	0.70	5.00	4.3	0.9969
Tebuconazole	C16H22ClN3O	107534-96-3	308 > 70	310 > 70	13.9	0.10	0.34	2.1	0.9993

Table 1 *– Continued...*

Compound	Formula	CAS	Transition 1	Transition 2	Pear extract				
					RT (min.)	Transition 1 LOD (ppb)	Transition 2 LOD (ppb)	%RSD (10ppb)	R^2
Tebufenozide	C22H28N2O2	112410-23-8	353 > 133	353 > 297	13.5	0.04	0.10	1.5	0.9980
Tebufenpyrad	C18H24ClN3O	119168-77-3	334 > 117	334 > 147	15.2	0.30	0.28	0.9	0.9998
Teflubenzuron*	C14H6Cl2F4N2O2	83121-18-0	379 > 339	379 > 359	15.3	0.29	0.40	3.6	0.9973
Terbufos sulfone	C9H21O4PS3	56070-16-7	321 > 97	321 > 171	12.1	0.55	0.52	3.8	0.9956
Terbufos sulfoxide	C9H21O3PS3	10548-10-4	305 > 187	305 > 97	12.1	0.09	0.09	1.3	0.9989
Tetraconazole	C13H11Cl2F4N3O	112281-77-3	372 > 159	372 > 70	13.2	0.29	0.55	2.6	0.9950
Thiabendazole	C10H7N3S	148-79-8	202 > 175	202 > 131	8.2	2.50	2.50	1.5	0.9987
Thiacloprid	C10H9ClN4S	111988-49-9	253 > 126	253 > 90	7.9	0.10	0.50	1.0	0.9991
Thiamethoxam	C8H10ClN5O3S	153719-23-4	292 > 211	292 > 181	5.3	0.04	0.08	2.4	0.9995
Thiodicarb	C10H18N4O4S3	59669-26-0	355 > 88	355 > 108	10.6	0.08	0.18	1.1	0.9991
Thiophanate-methyl	C12H14N4O4S2	23564-05-8	343 > 151	343 > 311	9.7	0.25	0.62	1.1	0.9967
Tolfenpyrad	C21H22ClN3O2	129558-76-5	384 > 197	384 > 91	15.3	0.28	0.73	3.0	0.9983
Triadimefon	C14H16ClN3O2	43121-43-3	294 > 69	294 > 197	12.8	0.24	0.31	2.6	0.9985
Triadimenol	C14H18ClN3O2	55219-65-3	296 > 70	298 > 70	13.1	0.24	0.54	3.7	0.9982
Triasulfuron	C14H16ClN5O5S	82097-50-5	402 > 141	402 > 167	9.6	0.42	0.36	1.5	0.9993
Triazamate acid*	C11H18N4O3S	112143-82-5	287 > 198	287 > 170	10.1	0.09	0.26	4.4	0.9996
Triazophos	C12H16N3O3PS	24017-47-8	314 > 162	314 > 119	12.9	0.02	0.12	1.5	0.9992
Triclopyr*	C7H4Cl3NO3	55336-06-3	256 > 198	254 > 196	11.1	1.95	1.81	8.9	0.9969
Tricyclazole	C9H7N3S	41814-78-2	190 > 136	190 > 163	8.3	0.10	0.20	2.3	0.9993
Trifloxystrobin	C20H19F3N2O4	141517-21-7	409 > 186	409 > 145	14.6	0.02	0.05	1.2	0.9994
Triflumizole	C15H15ClF3N3O	68694-11-1	346 > 278	346 > 43	14.8	0.09	0.09	1.3	0.9996
Triflumuron*	C15H10ClF3N2O3	64628-44-0	357 > 154	357 > 176	14.2	1.76	3.12	4.6	0.9991
Triforine	C10H14Cl6N4O2	26644-46-2	435 > 390	437 > 392	11.7	0.92	3.53	4.8	0.9963
Triticonazole	C17H20ClN3O	131983-72-7	318 > 70	320 > 70	13.2	0.40	0.41	1.9	0.9993
Zoxamide	C14H16Cl3NO2	156052-68-5	336 > 187	336 > 159	14.0	0.09	0.29	1.3	0.9951
2,4-D*	C8H6Cl2O3	94-75-7	219 > 161	219 > 125	10.3	1.09	5.00	9.7	0.9980

* Negative electrospray ionisation

3.2 Rapid screening of different mobile phase compositions on signal response

The signal intensity in LCMS can be strongly influenced by the mobile phase composition. In order to optimise the signal intensity, pesticides were added into vials containing different mobile phase compositions and injected into the interface with no column installed. The Nexera auto-sampler was setup to inject an air gap both before and after the injected sample in order to prevent the sample mixing with carrier mobile phase. This approach enables a large number of potential mobile phase compositions to be screened in a short automated period of time and without the need to manually change mobile phases. Ten different mobile phase compositions were tested, including: ammonium acetate, ammonium formate, formic acid, acetic acid, and ammonium formate with formic acid in water:methanol or acetonitrile 1:1. A total of 23 different pesticides were assessed, selected to include a range of different polarities and both positively and negatively ionised compounds. The different mobile phases tested and their peak area response, relative to the highest peak area response obtained for that compound, are shown in Table 2.

As expected with multi residue methods, there was not one optimum mobile phase for all pesticides. Overall, the lowest signal was achieved for mobile phases containing water:methanol only, and the mobile phase containing water:acetonitrile 10 mM ammonium acetate. Negatively ionised compounds (fludioxinil and ioxynil) provided superior responses in water:methanol 10mM ammonium acetate, while the addition of either formic acid or acetic acid decreased response. The highest signals were typically found in 10 mM ammonium formate, 10mM ammonium acetate, and 10 mM ammonium formate with 0.1 % formic acid. The effect of methanol and acetonitrile in the mobile phase was also investigated. Comparison of 10mM ammonium formate in methanol and acetonitrile showed that intensities were typically lower with the use of acetonitrile. Similarly the use of ammonium acetate in methanol and acetonitrile presented the same trend. The same observation with regards to methanol and acetonitrile for pesticide analysis have been reported by others.[13]

Table 2 *- Results of rapid mobile phase screening using flow injection analysis for 23 pesticides. All peaks areas were normalised against the maximum peak area achieved for that compound. Accordingly, 100 % indicates the highest peak area achieved and is highlighted.*

Compound	H2O:MeOH	H2O:MeOH 0.05% Formic acid	H2O:MeOH 0.1% Formic acid	H2O:MeOH 0.2% Formic acid	H2O:MeOH 5mM Ammonium acetate	H2O:MeOH 10mM Ammonium acetate	H2O:MeOH 20mM Ammonium acetate	H2O:MeOH 50mM Ammonium acetate	H2O:MeCN 10mM Ammonium acetate	H2O:MeOH 10mM Ammonium formate	H2O:MeCN 10mM Ammonium formate	H2O:MeOH 0.1% Acetic acid	H2O:MeOH 0.1% Formic acid 50mM ammonium formate
Atrazine	52	**100**	99	88	52	71	66	62	48	80	50	52	87
Azinphos-methyl	14	32	32	27	75	98	87	59	26	**100**	26	30	96
Azoxystrobin	27	30	29	25	69	87	77	58	65	**100**	82	29	99
Carbendazim	66	**100**	91	92	37	42	38	32	26	71	36	64	81
Chlorantraniliprole	**100**	46	52	41	69	81	92	69	27	91	60	94	56
Cyprodinil	66	94	88	86	55	63	57	41	51	**100**	82	67	78
Difenoconazole	27	85	90	72	70	**100**	92	73	59	99	62	61	90
Fludioxinil	69	42	38	37	74	**100**	95	84	60	94	81	55	76
Imazalil	85	69	62	63	66	78	73	62	51	**100**	68	58	74
Ioxynil	**100**	47	41	43	41	60	60	51	34	62	53	55	53
Isoproturon	28	34	34	30	74	93	84	75	78	**100**	90	30	98
Metalaxyl	30	31	31	25	68	92	81	76	79	**100**	87	31	92
Myclobutanil	15	71	75	57	65	**100**	91	73	23	86	25	58	84
Pirimicarb	82	85	76	78	66	90	80	68	68	**100**	80	66	78
Pirimicarb-desmethyl	72	90	81	83	64	85	74	67	64	**100**	82	70	86
Prochloraz	38	**100**	94	89	47	65	56	45	45	61	46	64	64
Pyraclostrobin	33	32	30	27	62	78	70	55	61	**100**	82	26	93
Pyrimethanil	54	**100**	92	91	54	65	54	31	48	92	74	62	76
Tebufenozide	28	40	40	36	70	88	78	65	73	96	84	33	**100**
Thiabendazole	96	**100**	91	89	58	69	61	48	37	99	60	67	84
Thiacloprid	16	28	28	25	53	59	45	32	34	86	49	18	**100**
Thiophanate methyl	24	21	24	17	62	77	62	44	34	98	43	31	**100**
Triadimenol	17	96	**100**	81	56	88	86	74	44	79	46	66	74
Minimum	14	21	24	17	37	42	38	31	23	61	25	18	53
Maximum	100	100	100	92	75	100	95	84	79	100	90	94	100
Average	**50**	**64**	**62**	**57**	**61**	**80**	**72**	**58**	**49**	**91**	**63**	**52**	**83**

3.3 Performance Optimising Injection Sequence (POISe)

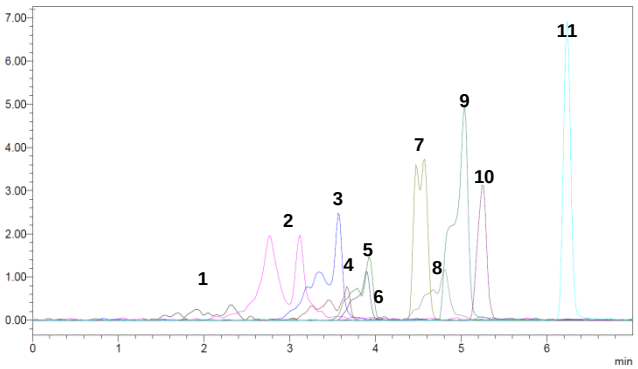
In reversed phase UHPLC, early eluting compounds typically display the greatest peak distortion. Peak distortion is a particular problem is pesticide analysis as samples are typically extracted by QuEChERS, with samples diluted in 100% acetonitrile (a strong eluting solvent). To solve this issue, laboratories may decide to dilute the acetonitrile extracts in water before LCMS injection. However, doing so adds an additional sample preparation step and dilution in water can also negatively affect the stability of some analytes.[14]

To minimise peak dispersion with the injection of acetonitrile extracts, one potential solution is the use of a band compression technique.[15] Band compression is achieved by injecting a band of weak eluting solvent onto the column after the analytes. As the analyte and the weak eluting solvent bands travel towards the column, minute mixing occurs. Therefore, the analytes are dissolved in a weak eluting solvent when they reach the column leading to isocratic band compression.

The performance optimising injection sequence (POISe) was evaluated by injecting between 5 – 40 µL of water following a 3 µL injection of pear extract in 100% acetonitrile. This was achieved using the Nexera auto-sampler (SIL-30AC) pre-treatment program to perform this function.

Figure 1 shows the injection of pear extract *with* and *without* the performance optimising injection sequence. Using POISe, band dispersion was minimised considerably for early eluting pesticides, with peak widths reduced by 5-69%. The optimum amount of water to inject following the sample was found to be 30 µL. Increasing this volume to 40 µL did not provide any significant improvements. Early eluting compounds are affected by the injection of a weak eluting solvent band to a much larger extent in comparison to analytes with higher retention factors. This improvement is due to the reduction in the sample solvent elution strength, which has a large impact on the early eluting compounds. Whereas, analytes with higher retention factors will experience some degree of band compression in the mobile phase already. Table 3 lists the peak width for 11 early eluting compounds. Compounds are arranged in retention time order to show the improvement using the POISe on early eluting analytes**.**

(A) 3 µL pear extract injection *without* the POISe



(B) 3 µL pear extract injection *with* the POISe (30 µL water)

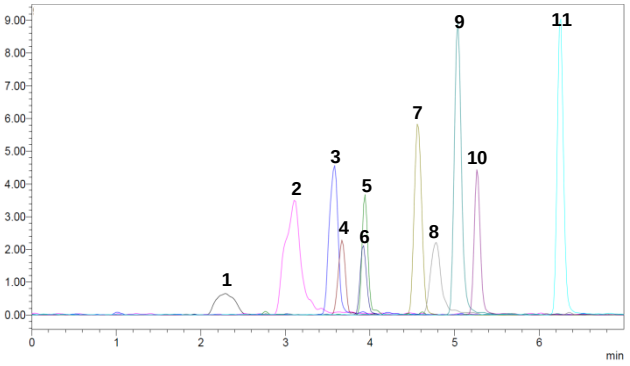


Figure 1 *– Pear extract (0.050 mg kg^{-1}) injected without (A) and with (B) the performance optimising injection sequence*

Table 3 *– Peak widths obtained with and without the performance optimising injection sequence*

No.	Compound	*Peak width (min.)*		Peak width change (%)
		Without POISe	*With POISe*	
1	Methamidophos	1.193	0.466	**-60.9**
2	Propamocarb	0.937	0.473	**-49.5**
3	Omethoate	0.773	0.247	**-68.0**
4	Butocarboxim sulfoxide	0.664	0.205	**-69.1**
5	Aldicarb sulfoxide	0.545	0.195	**-64.2**
6	Dinotefuran	0.460	0.247	**-46.3**
7	Oxamyl	0.317	0.248	**-21.8**
8	DMPF	0.309	0.254	**-17.8**
9	Demeton-S-methyl sulfoxide	0.418	0.271	**-35.2**
10	Demeton-S-methyl sulphone	0.277	0.248	**-10.5**
11	Ethiofencarb sulphone	0.233	0.220	**-5.6**

3.4 UHPLC gradient optimisation

Based on the results of the mobile phase screening investigation (section 3.2) the three superior compositions were tested: 1) 10 mM ammonium formate, 2) 10 mM ammonium acetate and 3) 10 mM ammonium formate with 0.1 % formic acid. Separation was achieved using a Shim-Pack XR-ODS III, 2.0 x 150 mm, 2.2 µm particle size. Ammonium formate was found to be the most effective compromise for all 210 compounds in terms of signal to noise ratios and peak shapes.

However two problems with ammonium formate were observed; early elution of asulum and poor peak shape of propamocarb. Consequently, 0.01 % formic acid was tested and found to increase the retention of asulum, and improve the peak shape of propamocarb. The addition of acid was found to shorten the retention time of cyromazine (RT 2.2 min.), yet this retention time was still in excess of 2 column volumes as required in quality control procedures for pesticide residues analysis in food and feed.[13]

A number of pesticide isomers have identical transitions and consequently must be separated chromatographically. Employing a 16 minute gradient resulted in resolution greater than 1 between all necessary pesticides including: butocarboxim sulphoxide / aldicarb sulphoxide, ethiofencarb sulphone / methiocarb sulphone, diuron / fluometronsulam and desmedipham / phenmedipham. Figure 2 highlights the excellent peak shapes achieved on the Nexera UHPLC.

3.5 Final method performance

In order to assess the performance of the LCMS-8040 for real samples, limits of detection, linearity and repeatability were determined in food extracts. Linearity was assessed from 0.5 – 200 ppb in four types of sample: (1) acetonitrile, (2) dried fruit extract, (3) lettuce extract and, (4) pear extract. All 210 pesticides achieved excellent correlation coefficients greater than 0.99 in all four types of matrix with typical values greater than 0.997. Correlation coefficients are listed in Table 1 for all pesticides in pear extract, and the calibration curves of eight selected pesticides shown in Figure 3.

Pesticide limits of detection were calculated based on the method described by the US-EPA (see experimental section). Limits of detection were assessed for both the quantifying transition and the qualifying transition and are listed in Table 1. All of the studied pesticides presented LODs less than the 0.01 mg kg^{-1} reporting level for both transition 1 and 2.

A limit of detection less than 0.001 mg kg^{-1} (1ppb) was achieved for the quantifying transition and less than 0.002 mg kg^{-1} (2 ppb) for the qualifying transition for 90 % of compounds: thereby highlighting the excellent sensitivity of the LCMS-8040 for pesticide analysis. Furthermore, these limits of detection were achieved with an injection volume of only 2 µL. Therefore, detection limits could be reduced even further with larger injection volumes. An injection volume of 2 µL was used in the study to allow the injection of 100 % acetonitrile extracts without detriment to early eluting peak shapes.

Repeatability was assessed at the 0.01 mg kg^{-1} reporting level as peak area %RSD for six replicate injections in pear extracts. Repeatability less than 5 %RSD was achieved for 92 % of the 210 pesticides studied. All of the studied compounds presented repeatability less than 10 %RSD, with exception of haloxyfop acid (13.4 %).

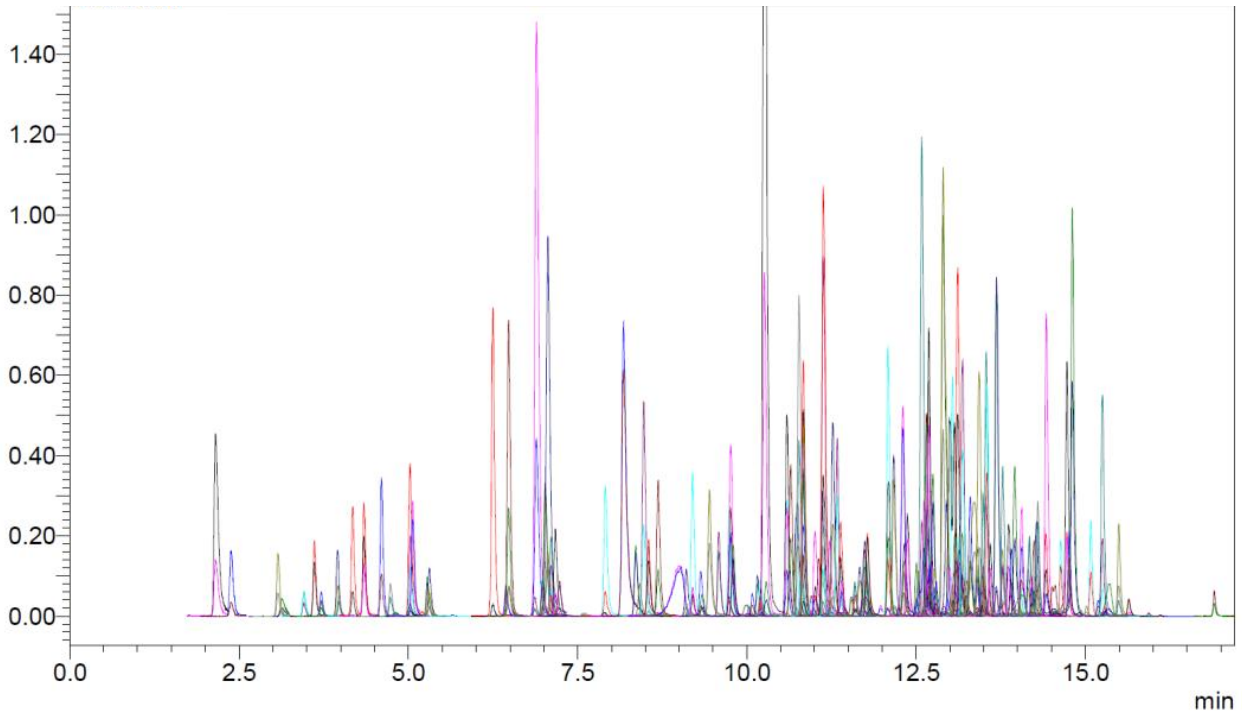


Figure 2 *– Extracted ion chromatogram of 210 pesticides using the Shimadzu Nexera UHPLC and the Shimadzu LCMS-8040; 2 µL injection of a* 0.05 mg kg^{-1} *standard solution.*

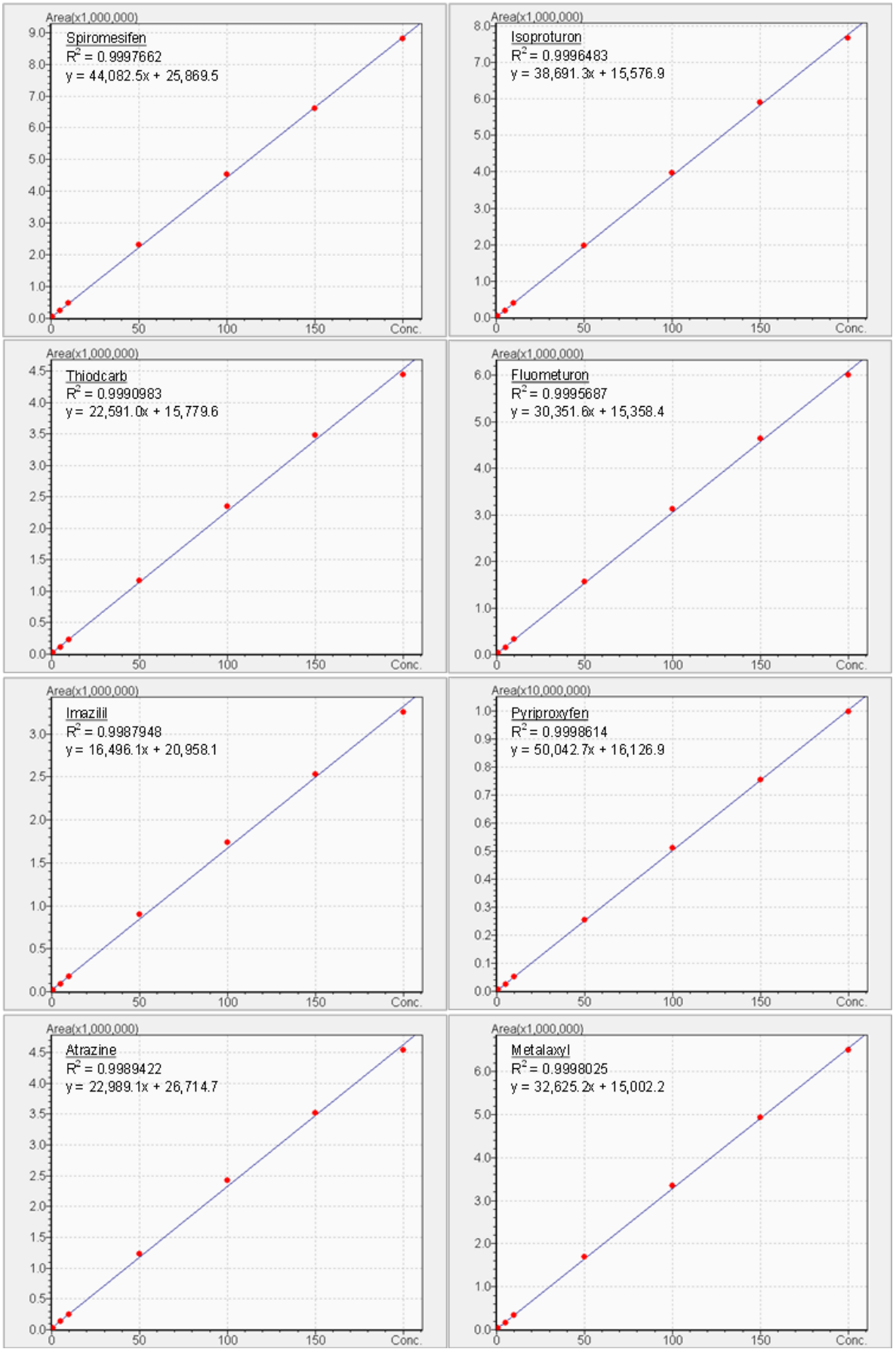


Figure 3 – *Calibration curves,* 0.5 µg kg^{-1} - 0.2 mg kg^{-1} (0.5 – 200 ppb), *of eight pesticides in pear matrix*

4. Conclusion

The results of the developed methodology show that the Shimadzu LCMS-8040 triple quadrupole can achieve excellent sensitivity, linearity and repeatability in food extracts for over 200 commonly analysed pesticides. Limits of detection were less than 0.01 mg kg^{-1} (10 ppb) for both the quantifying and qualifying transitions for all compounds studied, while for 90% of compounds was less than 0.001 mg kg^{-1} (1ppb) (quantifying transition) and 0.002 mg kg^{-1} (2 ppb) (qualifying transition); therefore providing excellent response, especially given that the injection volume was only 2µL. The sensitivity of the LCMS-8040 was able to meet the 0.01 mg kg^{-1} (10 ppb) requirements of regulatory guidelines such as those established by the EU and Japan. Repeatability at the 0.01 mg kg^{-1} reporting level was less than 5% for nearly all compounds and correlation coefficients greater than 0.99 for all compounds in a variety of food samples. Consequently the LCMS-8040 is ideally suited for routine monitoring of pesticides in regulatory laboratories.

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