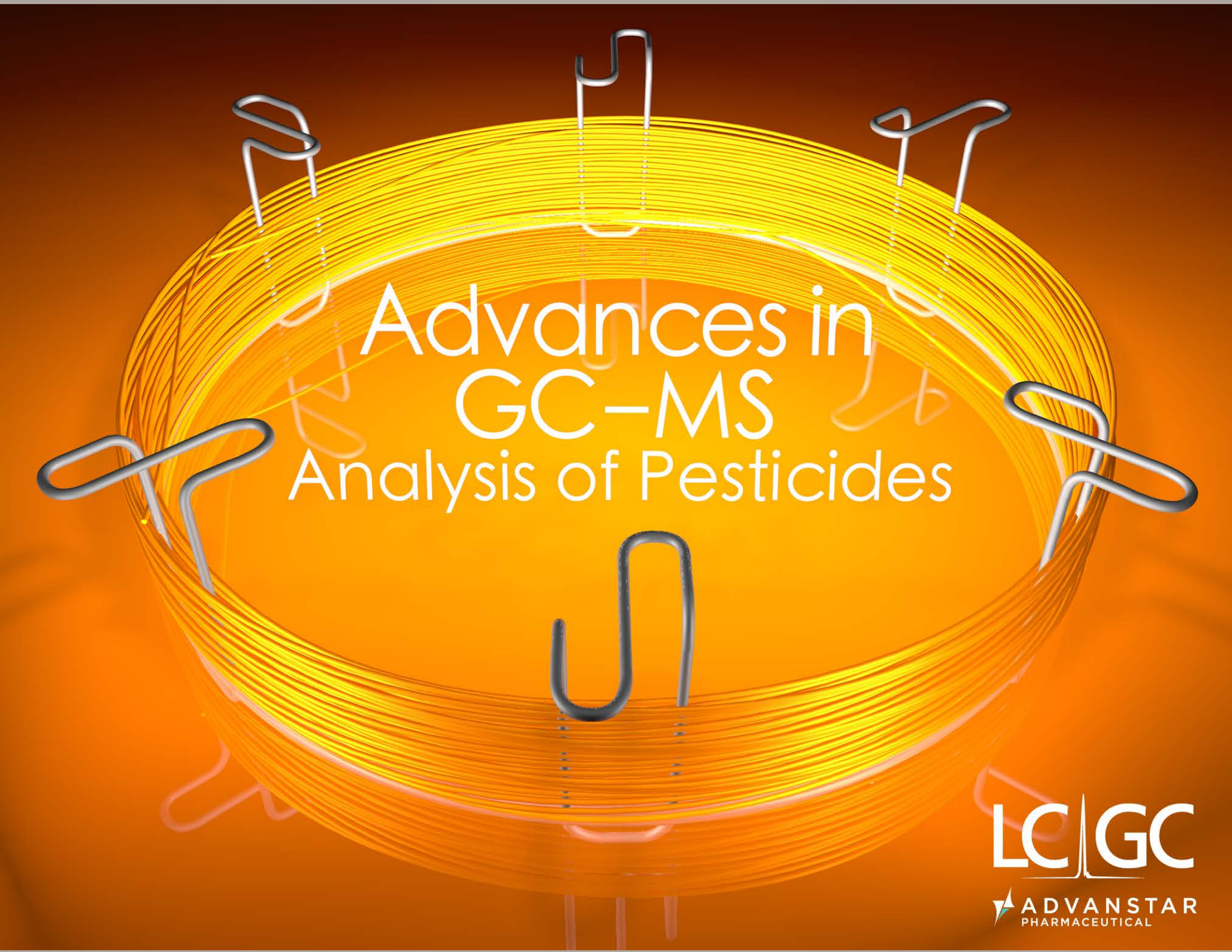
Advances in
GC–MS
Analysis of Pesticides
LC GC
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Advances in GC-MS Analysis of Pesticides

Welcome

LC GC
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Improving Sensitivity and Selectivity in Pesticide Analysis with GC–MS and GC–MS-MS

By Renata Raina-Fulton

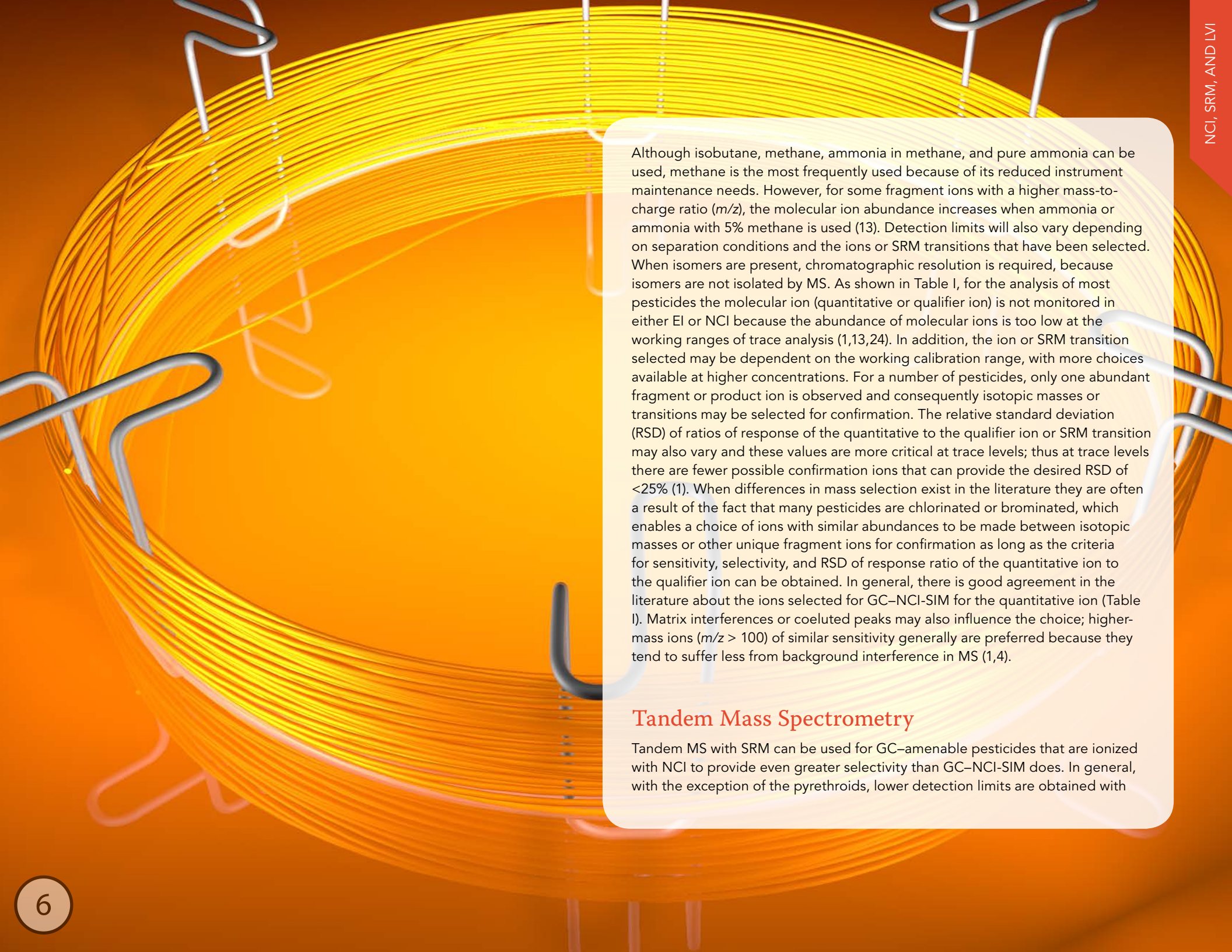
The selection of analytical methods for gas chromatography (GC)-amenable pesticides is often based on requirements for sensitivity and selectivity for regulatory needs or other monitoring requirements. Methods with both electron ionization (EI) and negative chemical ionization (NCI) are often required to cover the full range of GC–amenable pesticides at trace levels. Pesticides fragment easily in EI and CI sources such that the molecular ion is often low in abundance. NCI can provide added selectivity and sensitivity over EI methods. NCI is most commonly used in selected-ion monitoring mode. The lack of availability of parent ions for collision-induced dissociation for tandem mass spectrometry (MS) can limit the feasibility of GC–MS-MS for pesticides that significantly fragment in the ion source. Options for improving sensitivity by using of large-volume cold on-column or programmable temperature vaporizer injections are presented.

Gas chromatography (GC) continues to be a viable technique for the analysis of a range of chemical classes of pesticides including herbicides (chloroacetanilides, triazines, and selected dinitroanilines and thiocarbamates), insecticides (organochlorines, organophosphorus pesticides, and pyrethroids), and fungicides (selected triazoles, oxazoles, imidazole, dicarboximides, and phthalimides). Several approaches have been used to improve detection limits or reduce matrix interferences for GC analysis and extend the range of pesticides that can be analyzed with GC. These approaches include the use of negative chemical

ionization (NCI) rather than electron ionization (EI), tandem mass spectrometry (MS), and large-volume injections (LVI) using cold on-column (COC) injection or a programmable-temperature vaporizer (PTV). When detection limits are not the most critical factor, multiresidue pesticide analysis is frequently accomplished by GC methods with EI. This approach is cost effective, easy to set up based on the availability of an MS library, and for a few chemical classes (chloroacetanilides, triazines, and some fungicides) provides the best GC–MS sensitivity. But even though EI provides detection for the largest number of GC–amenable pesticides, it is more prone to matrix interferences and thus requires additional sample cleanup, and sensitivity may not be sufficient to meet regulatory or monitoring needs.

Negative Chemical Ionization

Chemical ionization can be used in positive- or negative-ion mode. In positive-ion chemical ionization mode, however, background and matrix interferences are observed and the analytes of interest are often coeluted with matrix compounds, as occurs with EI. As a result, positive-ion chemical ionization is not commonly used in GC–MS pesticides analysis (1–6). Pesticides that contain halogen, NO_2, or P ester groups are electron-capturing and thus have potential to exhibit enhanced response up to several orders of magnitude with NCI (1–16). The response of hydrocarbons, which are a common source of matrix interference in environmental samples, is also diminished with NCI (4). Because of the added selectivity of NCI, the coelution of other pesticides that are not electron-capturing is reduced and total run times also may be reduced. The most common pesticides that exhibit greater sensitivity with NCI include organochlorines (OCs), chlorinated cyclodiene insecticides, organophosphorus pesticides (OPs), and pyrethroids (1–16). Other selected pesticides from a variety of chemical classes that have nitro or halogen groups are also detected in NCI; these pesticides include dinitroaniline herbicides and a range of halogenated fungicides (conazole, imidazole, triazole, strobilurin, dicarboximide, and phthalimide fungicides), as shown in Table I. For many OCs and OPs the greatest sensitivity is achieved with GC–NCI in selected-ion monitoring (SIM) mode (1–4), and GC–NCI in selected-reaction monitoring (SRM) mode can provide additional confirmation ability or reduce background interferences (1,2,4,15,16). The relative abundance of the molecular and fragment ions formed in the ion source in NCI depends on the ionization conditions, with the reagent gas influencing ion abundance levels (13).



Although isobutane, methane, ammonia in methane, and pure ammonia can be used, methane is the most frequently used because of its reduced instrument maintenance needs. However, for some fragment ions with a higher mass-to-charge ratio (*m/z*), the molecular ion abundance increases when ammonia or ammonia with 5% methane is used (13). Detection limits will also vary depending on separation conditions and the ions or SRM transitions that have been selected. When isomers are present, chromatographic resolution is required, because isomers are not isolated by MS. As shown in Table I, for the analysis of most pesticides the molecular ion (quantitative or qualifier ion) is not monitored in either EI or NCI because the abundance of molecular ions is too low at the working ranges of trace analysis (1,13,24). In addition, the ion or SRM transition selected may be dependent on the working calibration range, with more choices available at higher concentrations. For a number of pesticides, only one abundant fragment or product ion is observed and consequently isotopic masses or transitions may be selected for confirmation. The relative standard deviation (RSD) of ratios of response of the quantitative to the qualifier ion or SRM transition may also vary and these values are more critical at trace levels; thus at trace levels there are fewer possible confirmation ions that can provide the desired RSD of <25% (1). When differences in mass selection exist in the literature they are often a result of the fact that many pesticides are chlorinated or brominated, which enables a choice of ions with similar abundances to be made between isotopic masses or other unique fragment ions for confirmation as long as the criteria for sensitivity, selectivity, and RSD of response ratio of the quantitative ion to the qualifier ion can be obtained. In general, there is good agreement in the literature about the ions selected for GC–NCI-SIM for the quantitative ion (Table I). Matrix interferences or coeluted peaks may also influence the choice; higher-mass ions (*m/z* > 100) of similar sensitivity generally are preferred because they tend to suffer less from background interference in MS (1,4).

Tandem Mass Spectrometry

Tandem MS with SRM can be used for GC–amenable pesticides that are ionized with NCI to provide even greater selectivity than GC–NCI-SIM does. In general, with the exception of the pyrethroids, lower detection limits are obtained with

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Organochlorine Insecticides						
α-HCH	288	71, 73	181, 111	71>35 (5), 73>37 (10)	181>145 (15), 181>109 (25)	1
β-HCH	288	71, 73	181, 111	71>35 (5), 73>37 (10)	181>145 (15), 181>109 (25)	1
γ-HCH (lindane)	288	71, 73	181, 111	71>35 (5), 73>37 (10)	181>145 (15), 181>109 (25)	1
γ-HCH (lindane)	288	255, 257, 253	181, 183, 109			2
δ-HCH	288	71, 73	181, 111	71>35 (5), 73>37 (10)	181>145 (15), 181>109 (25)	1
HCH α, β, γ, δ	88	71				15
Pentachloronitrobenzene	293	265, 249	237, 295	265>35 (35), 231>35 (25)	237>119 (15), 237>146 (25)	1
Hexachlorobenzene	285	284, 286, 282	284, 286, 282			2,4
o,p′-DDE	316	318, 316	316, 318		316>246 (15), 246>176 (25)	1
p,p′-DDE	316	318, 316	316, 318		316>246 (15), 246>176 (25)	1
o,p′-DDD	306	71, 248	235, 165		235>165 (25), 235>200 (5)	1
p,p′-DDD	306	71, 248	235, 165		235>165 (25), 235>200 (5)	1
p,p′-DDT	352	71, 73	235, 165	71>35 (5)	235>165 (25), 235>199 (15)	1

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Cyclodiene Insecticides (Chlorinated) and Their Degradation Products						
Aldrin	381	237				15
Chlordane	406	410, 408, 412	373, 375, 377			2
α-Chlordane	406	266,232,71	373, 375	71>35 (5), 266>35 (15)	375>266 (25), 375>303 (10)	1
γ-Chlordane	406	266, 71, 232	375, 373	71>35 (5), 266>35 (15)	375>266 (25), 375>303 (10)	1
Dieldrin	381	237				15
α-Endosulfan	404	372, 404, 406, 408, 410	241, 195	242>35 (15), 406>35 (10)	195>160 (5), 339>159 (15)	1
α-Endosulfan	404	372, 406, 408	241, 239, 195			2
β-Endosulfan	404	372, 404, 406, 408, 411	241, 196	242>35 (15), 406>35 (10)	195>160 (10), 339>159 (35)	1
β-Endosulfan	404	372, 406, 408	70, 239, 195			2
Endosulfan sulfate	420	386, 352	387, 272	386>97 (10), 352 >97 (15)	272>237 (25), 387>253 (10)	1
Endrin	381			380>35 (5), 346>35 (5)	263>191 (35)	6
Endrin	381	237				15
Endrin aldehyde	378	272, 270	67, 245	272>35 (20), 272>243 (15)	345>281 (10), 345>245 (10)	1
Endrin ketone	378	308, 272	317, 67	308>35 (35), 308>272 (15)	317>281 (10), 317>245 (20)	1
Heptachlor	370	266, 232	100, 274	266>35 (30), 300>35 (25)	100>65 (10), 272>237 (10)	1
Heptachlor	370	300, 266, 302	272, 100, 274			4
Heptachlor	370	300				15

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Mirex	540	402, 368	272, 274	368>35 (20), 404>35 (15)	272>237 (10), 272>141 (30)	1
Mirex	540	404, 370, 439	272, 274, 270			4
Mirex	540	368, 402, 439				8
Mirex	540	366, 368, 370, 402, 404, 406				10
Herbicides						
Nitrophenyl Ether Herbicide						
Nitrofen	283	283, 138	283, 285	283>138 (10), 283>35 (35)	283>162 (20), 283>253 (10)	1
Nitrofen	283	283, 285, 284	283, 285, 202			2
Triazine Herbicide and Its Degradation Products						
Atrazine	216		200, 215		Precursor>94, 122, 132	7
DIA (desisopropyl-atrazine)	174		145, 158, 173		Precursor>68, 110	7
DEA (desethyl-atrazine)	188		172		Precursor>79, 105, 130	7
Chloroacetanilide Herbicides						
Acetochlor	270		146, 162, 174		Precursor>131	7
Alachlor	270		160, 188		Precursor>160	7
Alachlor	269		160, 188		188>160 (10), 160>130 (25)	1
Metolachlor	284		162, 238		Precursor>162	7
Propachlor	211		120, 176		176>120 (10), 176>92 (15)	1

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Phthalic Acid Herbicide						
DCPA	370	332, 330	301, 332	330>35 (10), 332>302 (5)	332>301 (5), 301>273 (15)	1
Dinitroaniline Herbicides						
Ethalfluralin	333	333,303	276, 316	333>46 (10), 333>303 (10)	276>202 (15), 316>276 (5)	1
Pendimethalin	281	281, 251, 282	252, 162, 281			4
Trifluralin	335	335, 305	306, 264	335>305 (10), 335>46 (10)	306>264 (5), 264>206 (5)	1
Trifluralin	335	335, 305, 336	306, 264, 307			2,4
Thiocarbamate Herbicide						
Triallate	303	160, 161	86, 268	160>84 (15), 160 >100(10)	268>184 (15), 268>226 (10)	1
Organophosphorus Pesticides (Insecticides)						
Acephate	183	168				16
Aspon	378		211, 253	211>35 (20)	378>210 (10), 378>115 (30)	1
Azinphos methyl	317	157, 133				13
Azinphos methyl	317	157				14,16
Azinphos ethyl	345	185				16
Bromopropylate	428	79, 81, 366, 368, 370				14
Bromophos ethyl	394	358				16
Carbofenothion	342	185, 145	157, 342	185>111 (20), 185>79 (35)	342>157 (10), 342>143 (15)	1

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Carbophenthion methyl	215	157				16
Carbophenthion	343	185, 143				13
Carbophenthion	343	185				16
Chlorfenvinphos	358	153				13,16
Chlorpyrifos ethyl	349	313, 315, 214, 212	97, 197	169>96 (15), 313>189 (10)		1
Chlorpyrifos ethyl	349	313, 314, 315, 212, 169				14
Chlorpyrifos ethyl	349	313				16
Chlorpyrifos methyl	321	214, 212	286, 125	141>126 (15), 141>96 (20)	321>268 (5), 321>208 (20)	1
Chlorpyrifos methyl	321	212, 214, 285	286, 288, 125			2,4
DEF	314	257				13,16
Diazinon	304	169, 171	137, 179	169>95 (20), 169>141 (10)	304>179 (5), 304>137 (30)	1
Diazinon	304	169, 303, 275				13
Diazinon	304	167, 168, 169	287, 302, 288			2,4
Dichlofenthion	314	278, 250	223, 97		314>223 (25), 319>81 (25)	1
Dimethoate	229	157, 159, 158	87, 93, 125			4
Dimethoate	229	157				14,16
Disulfoton	274	185				16
Dyfonate	246	169, 109	109, 137	169>107 (20), 169>141 (5)	246>137 (5), 246>109 (15)	1

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
EPN	323	323, 138, 154, 307, 201				13
Ethion	384	185, 187	231, 97	185>111 (15), 185>157 (15)	384>231 (5), 384>203 (15)	1
Ethion	384	185				13,16
Ethyl-bromophos	394	358, 257, 278, 79, 328, 169				13
Fenchlorphos	320	211, 213	125, 287	141>126 (15), 211>35 (20)	320>285 (5), 320>204 (30)	1
Fenitrothion	277	277, 168	277, 125		277>260 (5), 277>109 (20)	1
Fenitrothion	277	277, 168, 278	277, 125, 109			2, 4
Fenitrothion	277	277				16
Fenthion	278	141				16
Fonofos	246	169, 109				13,16
Isocarbophos			136, 121, 230, 289		230>155, 198, 136, 212	17
Isofenfos methyl			199, 121, 241, 231		199>121, 167, 199	17
Isofenfos	345	380, 344, 182, 153, 244, 287, 302				13
Isofenfos	345	244				16
Leptophos	410	241, 79	171, 377	241>81 (35), 257>81 (35)		1
Malathion	330	172, 157	173, 125	157>142 (15), 172>84 (5)		1
Malathion	330	157, 159, 158	173, 125, 93			2
Malathion	330	157, 172, 315				13

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Malathion	330	157				16
Omethoate	213	141				16
Methidathion	302	157				13,16
Parathion ethyl	291	291, 292, 97	97, 291	291>154 (5), 291>169 (10)		1
Parathion ethyl	291	291				16
Parathion methyl	263	263, 154, 141				13
Parathion methyl	263	154, 263				14
Parathion methyl	262	263				16
Profenophos	374	267				16
Phorate	260	222, 224	121, 75	185>111 (15), 185>157 (10)	260>75 (5), 263>231 (5)	1
Phorate	260	185				16
Phosalone	367	185				13
Phosalone	367	185, 186, 187				14
Pirimophos-ethyl	333	141				16
Profenofos	372	267, 308, 336, 79, 186, 183				13
Propetamphos	281	154				16
Ronnel	320	211, 270, 141				13
Ronnel	320	211				13
Sulfotep	322		322, 202		322>202 (10), 322>146 (25)	1

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Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Sulprofos	322	279, 247	140, 322	279>139 (20), 279>124 (35)	322>156 (10), 322>97 (20)	1
Terbufos	288	185				16
Tokuthion	344	237, 301	113, 267	269>161 (10), 237>79 (25)	344>328 (10), 344>73 (20)	1
Tributylphosphorotrithioite	314	257	169, 57	257>79 (15), 257>89 (20)	314>115 (20), 314>113 (20)	1
Trichloronate	332	213, 296	109, 297	296>108 (10), 296>79 (20)		1
Fungicides						
Phthalimide Fungicides and Their Degradation Products						
Captan	301	150, 151	79, 107, 149			3
Captan	301	150, 149, 151	79, 151, 264			4
Captan	301	150, 182				8
Captan	301	150				15
Folpet	297	146, 147, 148	260, 104, 262			2
Folpet	297	146				8
Captafol	349	150,151	79, 149, 137			3
Captafol	349	150, 217				8
Captafol	349	150				15
Phthalimide	147	147				8
Tetrahydrophthalimide	151	149				8

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Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Bridged Diphenyl Acaricide						
Dicofol	370	250, 252, 251	139, 251, 253			2
Dicofol	370	250, 262				8
Dicofol	370	250, 252, 251				12
Dicofol	370	250				15
Anilide Fungicides						
Boscalid	343			307>307 (5), 307>195.2 (5)		5
Fenhexamid	302			265>222 (5), 265>168 (5)		5
Conazole, IImidazole, Pyrazole, Triazinone, and Triazole Fungicides						
Fipronil	437	366, 384, 331	367, 369, 213			4
Fipronil	437			366>318 (5), 366>250 (5)		5
Fluquinconazole	376			348>203 (5), 348>320 (15)		5
Hexaconazole	314			257>221 (5), 257>69 (15)		5
Myclobutanil	289	288, 289, 290	179, 245, 288			2
Propiconazole	342			256>220 (5), 256>109 (20)		5
Prochloraz	377	377, 375, 379	308, 310, 266			2
Tetraconazole	372			117>97.5 (5), 117>117 (5)		5
Triazinone Herbicide						
Metribuzin	214	198, 199, 200, 184	198, 41, 57			2,4

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Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Phenylurea Herbicide						
Linuron	249	248, 250, 217	61, 248, 160			2
Dichlorophenyl Dicarboximide Fungicides						
Iprodione	330	329, 330, 331	314, 316, 187			2,4
Procymidone	284	283, 285, 284	96, 283, 285			2
Vinclozolin	286	241, 243, 245	212, 187, 124			2,4
Strobilurin Fungicides						
Azoxystrobin	403			371>356 (10), 371>371 (5)		5
Azoxystrobin	403	371, 356, 301				12
Trifloxystrobin	408			202>184 (10), 202>164 (10)		5
Pyrethroid Insecticides						
Acrinathrin	541	167, 305, 333				8
Acrinathrin	541	333, 305, 334, 167				11
Allethrin	302			167>41 (30), 167>111 (20)	123>81 (6)	6
Allethrin	302	167, 134, 168				11
β-Cyfluthrin	434			406>257 (15), 207>35 (10)	163>127 (5)	6
Bifenthrin	423			386>205 (5), 386>141 (20)		5
Bifenthrin	423	205, 241, 386				8
Bifenthrin	423	386, 241, 387, 205				11

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Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Bifenthrin	423	205, 241, 206				12
Bifenthrin	423	386, 387, 388	181, 165, 166			2,4
Cyfenothrin I-II	375	167, 198				8
Cyfluthrin I-IV	434	171, 207, 209				8
Cyfluthrin I-IV	434	207, 209, 171, 173, 211				9
Cyfluthrin I-IV	434	171, 173, 207, 209, 211				10
Cyfluthrin I-IV	434	207, 171, 209				11
Cyfluthrin I-IV	434	207				15
λ-Cyhalothrin	450			241>205 (5), 241>121 (20)		5
λ-Cyhalothrin	450	205, 241, 243				8
λ-Cyhalothrin	450	241, 205, 243				11
λ-Cyhalothrin	450	205, 241, 243				12
Cypermethrin	416	207, 209, 171	163, 165, 181			2
Cypermethrin	416			171>127 (5), 171>91 (5), 207>207 (5)		5
Cypermethrin	416	171, 173, 207, 209, 211				10
Cypermethrin	416	207, 171, 209				11
Cypermethrin	416	207, 209, 171				12
Cypermethrin	416	207				15
α-Cypermethrin	416			388>239 (15), 388>211 (25)	181>152 (30)	6

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
β-Cypermethrin	416	171, 207, 209				8
Deltamethrin	505			297>81 (15), 297>297 (15), 297>79 (15)		5
Deltamethrin	505			297>79 (15), 297>81 (15)	181>125 (25)	6
Deltamethrin	505	79,81, 297				8
Deltamethrin	505	79, 81, 137				10
Deltamethrin	505	297, 295, 505, 217				11
Deltamethrin	505	79, 81, 137, 139				12
Deltamethrin	505	79				15
Deltamethrin	505	297, 299, 79	181, 253, 251			2,4
Fenfluthrin	389	207, 209, 171				11
Fenpropathrin	349			141>141 (5), 141>97.4 (10)		5
Fenpropathrin	349	141				8
Fenpropathrin	349	141, 142, 143				11
Fenvalerate I-II	420	211, 213				8
Fenvalerate I-II	420	167, 169, 211, 213				10
Fenvalerate I-II	420	211, 213, 212				11
Fenvalerate I-II	420	211				15
Flucythrinate	451			243>199 (5), 243>133.5 (15)		5
Flucythrinate	451	243, 244, 245				11

Table I: Selected-ion monitoring (SIM) or selected-reaction monitoring (SRM) for pesticides that can be analyzed by gas chromatography–negative chemical ionization-mass spectrometry (GC–NCI-MS).

Pesticide	MW	NCI-SIM (*m/z*)	EI-SIM (*m/z*)	NCI-SRM *m/z* (Collision Energy, eV)	EI-SRM *m/z* (Collision Energy, eV)	Reference
Flucythrinate	451	243				15
Flumethrin	510			207>91 (25)		6
Fluvalinate	503	294, 296, 502				11
Fluvalinate	503	294				15
Fluvalinate-I-II	503	294, 296				8
tau-Fluvalinate	503			294>179 (10), 294>250 (10), 294>194 (15), 294>294 (5)		5
cis/trans-Permethrin	391	171, 207, 209				8
cis/trans-Permethrin	391	207, 209, 171, 173				9
cis/trans-Permethrin	391	171, 173, 207, 209, 211				10
cis/trans-Permethrin	391	207, 390, 209, 171				11
cis/trans-Permethrin	391	207, 209, 171				12
cis/trans-Permethrin	391	207				15
Phenothrin	350	167				8
Prallethrin	300	167, 132, 168				11
Tefluthrin	419	241, 382, 205, 243				11
Tetradifon	356	245, 320, 356				8
Tetradifon	356	320, 318, 245				12
Tetramethrin	331	165, 167, 331				8
Tetramethrin	331	331, 165, 332				11
Transfluthrin	371	207, 209, 171				11

GC–NCI-SIM than with GC–NCI-SRM (1,5,6). However, GC–NCI-SRM can provide additional confirmation, particularly when qualifier ions in GC–NCI-SIM are too low in abundance. Literature data are more limited for GC–NCI-SRM, however (Table I).

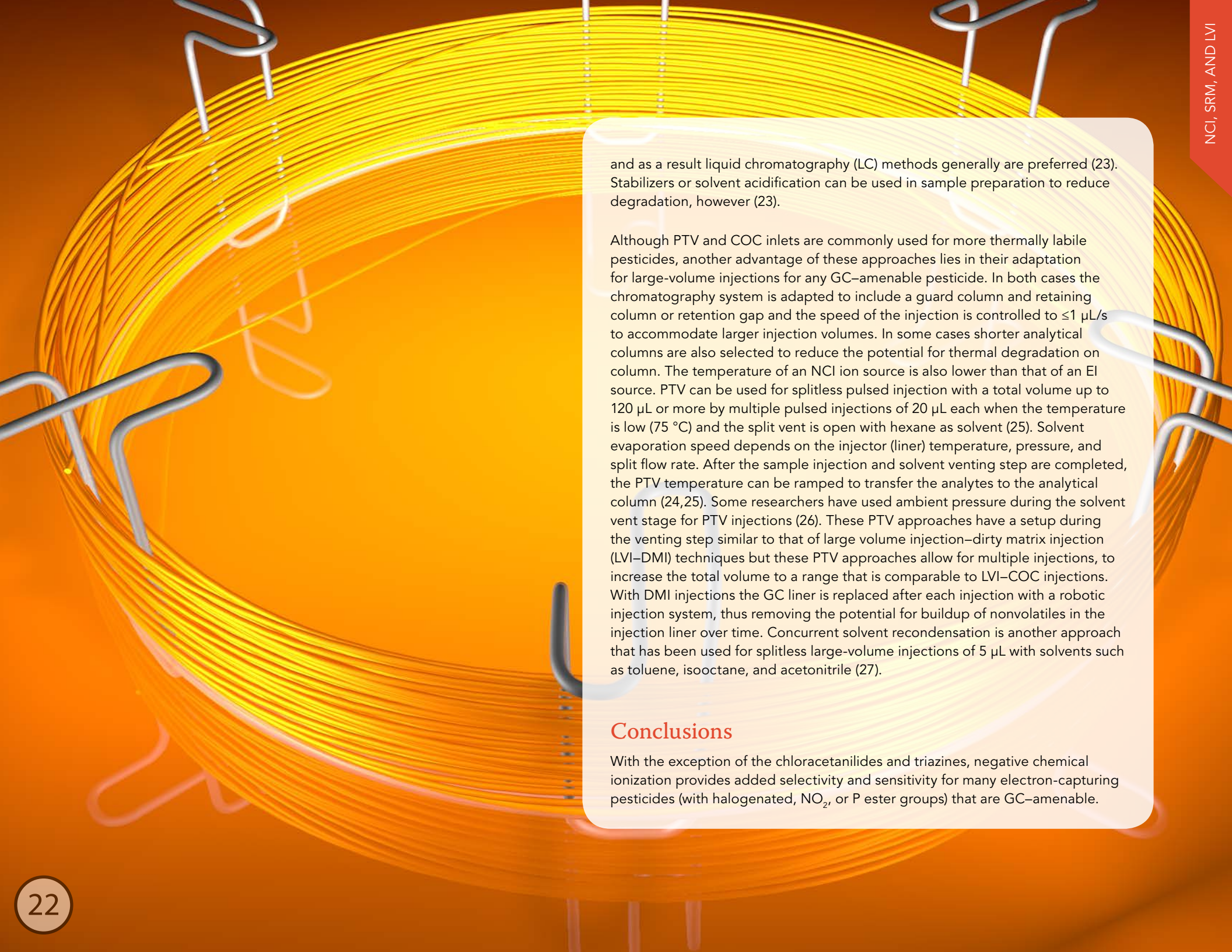
In both EI and NCI, pesticides generally fragment easily in the ion source. NCI is a softer ionization process than EI so there is a tendency to observe higher-mass ions with NCI than with EI. However, even with these higher-mass ions sometimes only one small product ion of significant abundance is formed from collision-induced dissociation (CID), such as Cl^- for a number of the OCs, chlorinated cyclodiene insecticides, and a few OPs: In Table I, see SRMs for hexachlorocyclohexanes (HCHs), dichloro-diphenyl-trichloroethane (DDT), chlordane, endosulfan, heptachlor epoxide, and mirex. For other chemical classes such as most OPs, strobilurin fungicides, and pyrethroids, higher-mass product ions from CID are generally produced. GC–NCI-SRM has been applied successfully to the quantitative and confirmation analysis of a number of pyrethroids (5,6). Pyrethroids generally have larger-mass fragment ions present in the ion source that can be selected as the parent ion for CID. The added selectivity of NCI coupled with SRM provides the opportunity for reduced analysis times because matrix interferences and the occurrence of coeluted pesticides are often reduced.

EI frequently is used for multiresidue pesticide analysis with GC–tandem MS (17–22). GC–EI-SRM is applicable to a large number of target compounds, and information is more readily available from MS libraries for parent ions formed in the EI ion source. This approach allows for the analysis of a larger range of GC–amenable pesticides together with the chloroacetanilides and triazines. GC–EI-SRM also can be used for pesticide classes that are amenable to NCI; however, in general detection limits are higher in GC–EI-SRM. Some OPs have similar sensitivity for GC–EI-SIM and GC–EI-SRM (17). GC–EI-SRM does not suffer from the background and matrix interferences observed with GC–EI-SIM and in some cases can shorten the total analysis time by reducing both sample preparation and GC run time with fewer problems of coeluted compounds (18). In our work on atmospheric samples, however, we found that GC–EI-SRM was not suitable at the low picogram-per-microliter level for OCs, cyclodiene insecticides, or most

organophosphorus pesticides (1). The response ratios of quantitative to qualifier SRM transitions at these trace levels often exceeded an RSD of 25%, thus another approach was required for confirmation (1). For other sample types, such as food commodities, where sample concentration or size can be adjusted to provide preconcentration, the use of GC–EI-SRM analysis has been sufficient to meet regulatory needs (17–22).

Large-Volume Injections Using Cold On-Column Injection or a Programmable-Temperature Vaporizer

For standard pesticide analysis, a split–splitless injector in splitless mode frequently is used with a variety of liners ranging from those containing glass wool to prepacked inlet liner material (such as CarboFrit, Restek) to improve the efficiency of vaporization. With splitless injections sample volume is limited to ~1–2 µL depending upon solvent selection. Phthalimide fungicides are prone to degradation in the injector port and must be analyzed with a different injector. Phthalimide fungicides, including both trichlorothio-phthalimides (captan and folpet) and tetrachlorothio-phthalimides (captafol), exhibit significant improvements in sensitivity with NCI and tend to form higher-mass ions in NCI (4,23). To minimize degradation in the injector port and improve detection limits, analysis can be completed with a large-volume cold on-column (COC) injection approach where a volume of up to 100 µL is injected (4). Another option is to use a PTV. The choice between using COC injection and a PTV often depends on the boiling point of the analyte and solvent used as well as the method used to clean up the extracts before analysis. Phthalamide fungicides have low boiling points and thus are less suitable to the use of a PTV inlet because certain types of PTV liners may increase sample degradation and analyte loss may occur during the solvent venting stage (23). However, large-volume (up to 20 µL) dirty matrix injection (DMI), where the sample extract is held in a microvial in a glass liner of a PTV inlet, has been shown to be successful for captan, captafol, and folpet, even with ethyl acetate as the solvent (24). Some degradation products of these fungicides have been monitored by GC–EI-SIM but sensitivity generally is too low,

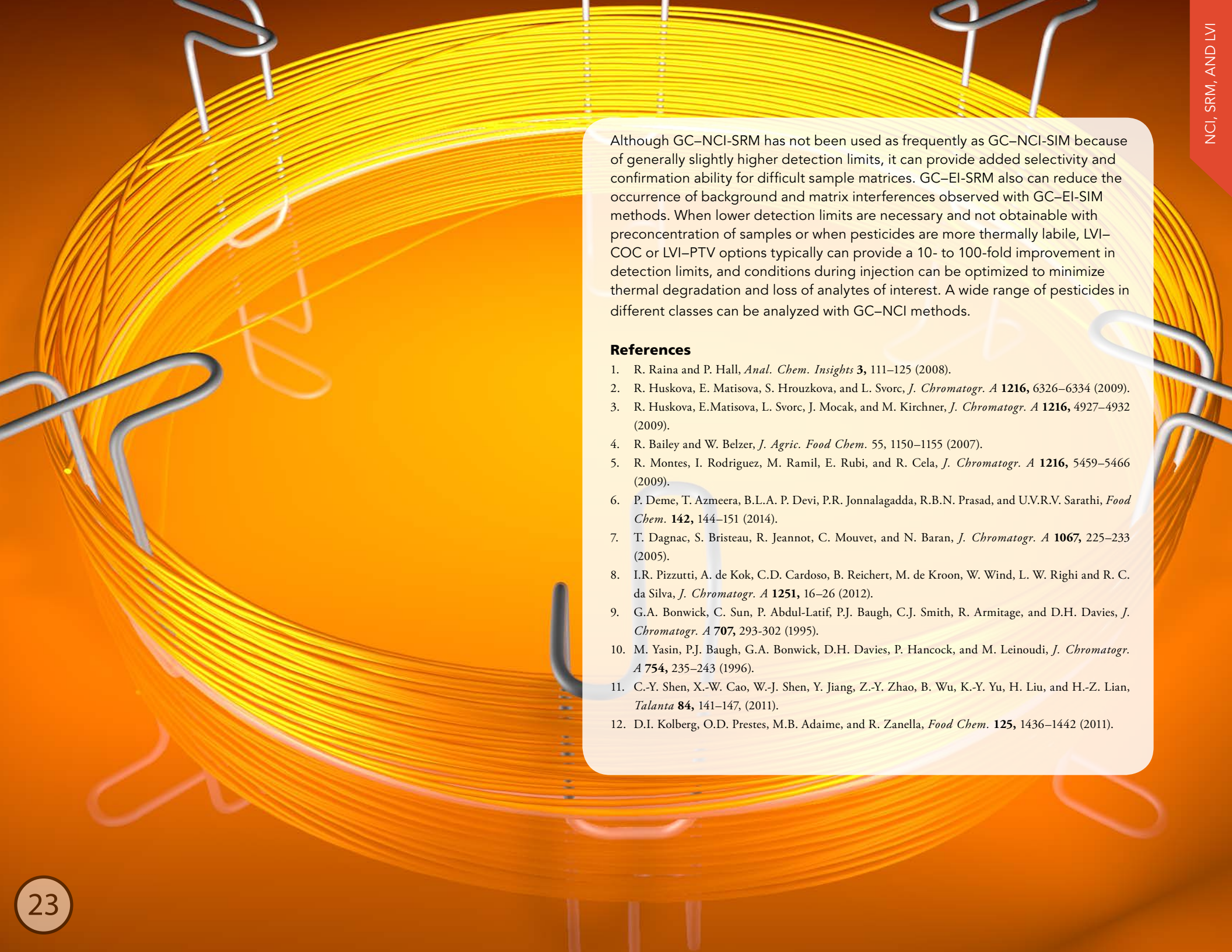


and as a result liquid chromatography (LC) methods generally are preferred (23). Stabilizers or solvent acidification can be used in sample preparation to reduce degradation, however (23).

Although PTV and COC inlets are commonly used for more thermally labile pesticides, another advantage of these approaches lies in their adaptation for large-volume injections for any GC–amenable pesticide. In both cases the chromatography system is adapted to include a guard column and retaining column or retention gap and the speed of the injection is controlled to ≤1 µL/s to accommodate larger injection volumes. In some cases shorter analytical columns are also selected to reduce the potential for thermal degradation on column. The temperature of an NCI ion source is also lower than that of an EI source. PTV can be used for splitless pulsed injection with a total volume up to 120 µL or more by multiple pulsed injections of 20 µL each when the temperature is low (75 °C) and the split vent is open with hexane as solvent (25). Solvent evaporation speed depends on the injector (liner) temperature, pressure, and split flow rate. After the sample injection and solvent venting step are completed, the PTV temperature can be ramped to transfer the analytes to the analytical column (24,25). Some researchers have used ambient pressure during the solvent vent stage for PTV injections (26). These PTV approaches have a setup during the venting step similar to that of large volume injection–dirty matrix injection (LVI–DMI) techniques but these PTV approaches allow for multiple injections, to increase the total volume to a range that is comparable to LVI–COC injections. With DMI injections the GC liner is replaced after each injection with a robotic injection system, thus removing the potential for buildup of nonvolatiles in the injection liner over time. Concurrent solvent recondensation is another approach that has been used for splitless large-volume injections of 5 µL with solvents such as toluene, isooctane, and acetonitrile (27).

Conclusions

With the exception of the chloracetanilides and triazines, negative chemical ionization provides added selectivity and sensitivity for many electron-capturing pesticides (with halogenated, NO_2, or P ester groups) that are GC–amenable.



Although GC–NCI-SRM has not been used as frequently as GC–NCI-SIM because of generally slightly higher detection limits, it can provide added selectivity and confirmation ability for difficult sample matrices. GC–EI-SRM also can reduce the occurrence of background and matrix interferences observed with GC–EI-SIM methods. When lower detection limits are necessary and not obtainable with preconcentration of samples or when pesticides are more thermally labile, LVI–COC or LVI–PTV options typically can provide a 10- to 100-fold improvement in detection limits, and conditions during injection can be optimized to minimize thermal degradation and loss of analytes of interest. A wide range of pesticides in different classes can be analyzed with GC–NCI methods.

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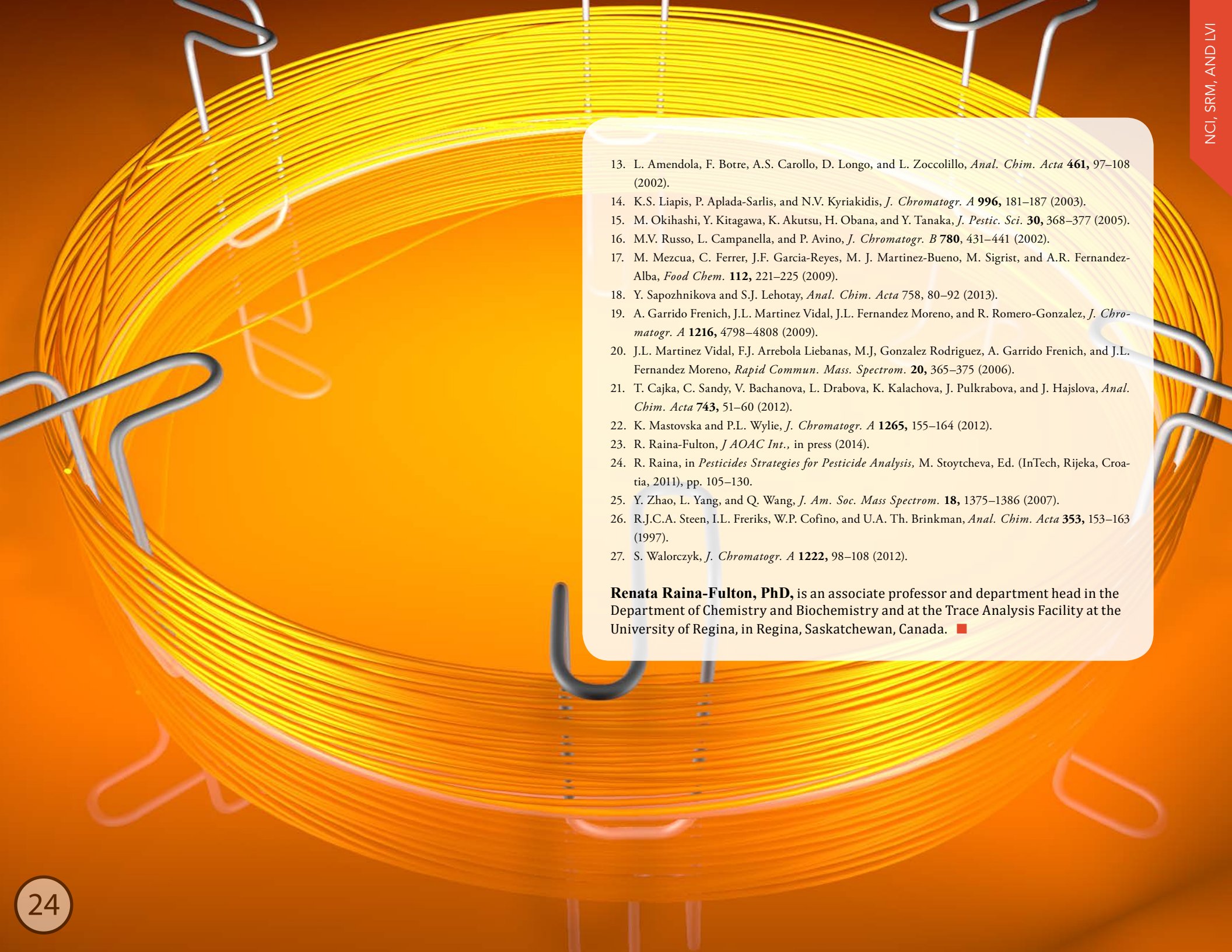

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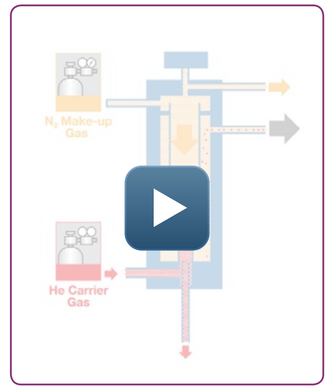

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AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

in Fruits and Vegetables by Gas Chromatography–Time-of-Flight Mass Spectrometry

By Noelia Belmonte, Samanta Uclés, Miguel Gamón, Carmen Ferrer, Milagros Mezcua, and Amadeo R. Fernández-Alba

The main objective of this study was to evaluate the capabilities of gas chromatography (GC) with time–of–flight mass spectrometry (MS) for screening pesticides in fruits and vegetables using a purpose-built accurate-mass database. Analytical performance was tested on four matrices: potato, tomato, spring onion, and orange. Two resolution modes, 7000 FWHM and 12,000 FWHM, were tested to establish the concentration range within which automatic identification was possible, considering the retention time window of 0.2 min and at least two ions with a mass error lower than 10 ppm as the identification criteria. The effects of the matrix on identification and quantification were also studied for the four selected matrices. The developed method was applied to real samples and the qualitative and quantitative results were compared with those obtained using GC with triple-quadrupole MS.

The use of agrochemicals at various stages of crop cultivation has an important impact on food protection and quality preservation. For this reason, there are different regulations in each country on pesticide use but the lists of approved pesticides are not harmonized worldwide. This situation is becoming more important because the number of pesticides authorized in Europe (1) has been reduced to around 50% of the total number of compounds manufactured. The practical "target analysis" approach applied in many routine laboratories for pesticide residue testing consists of selecting a list of compounds that are amenable for analysis by gas chromatography (GC) and liquid chromatography (LC) using an established combined priority list. This means that the majority of low-frequency or misused compounds are not studied.

GC coupled to mass spectrometry (MS) is the technique of choice for the analysis of many classes of pesticides, particularly volatile, semivolatile, and low-polarity compounds. The large number of GC–MS

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

applications reported for pesticides analysis in fruits and vegetables (2–7) is the result of efficient GC separation, together with spectral information and satisfactory sensitivity provided by MS. Until recently, low-resolution single-quadrupole MS detectors working in selected-ion monitoring (SIM) mode were the instruments most commonly used because of their relatively low cost, compactness, and simplicity (8).

Single-quadrupole analyzers show limitations in analyzing complex matrices, however ion-trap detection and, more recently, triple-quadrupole analyzers allow operation in tandem MS (MS2) mode, which achieves high sensitivity and selectivity, as demonstrated in an increasing number of applications reported in various fields (9,10). Recent progress in instrumentation design (mainly optics), the use of fast recording electronics, and signal processing improvements also have led to a renaissance for time-of-flight mass spectrometry (TOF-MS) in the study of organic compounds in complex matrices, particularly in LC-based applications. TOF-MS provides greater sensitivity in full-spectrum acquisition mode compared to conventional scanning instruments, principally because of its high mass-analyzer efficiency: GC–TOF-MS can screen hundreds of compounds at high sensitivity in a single run.

In addition, GC–TOF-MS data can be acquired and reprocessed without prior knowledge of the presence of certain compounds (that is, no analyte-specific information is required). Equally important is that the presence of other compounds of interest can be investigated at any time, simply by reprocessing the data (known as *retrospective analysis*). The high mass-resolving power and mass accuracy provided by GC–TOF-MS make it feasible to obtain extracted ion chromatograms using narrow mass windows — thus excluding a large proportion of the chemical background and isobaric interferences, which significantly improves signal-to-noise ratios. Under these conditions, pesticide identification is improved in comparison to other conventional analyzers.

Over recent years, TOF-MS has been widely used for the development of screening methods for pesticide determination in fruits and vegetables, although LC-based methods are still more widely used (11,12). Nonetheless, compounds that exhibit a very low ionization yield with electrospray sources and that have

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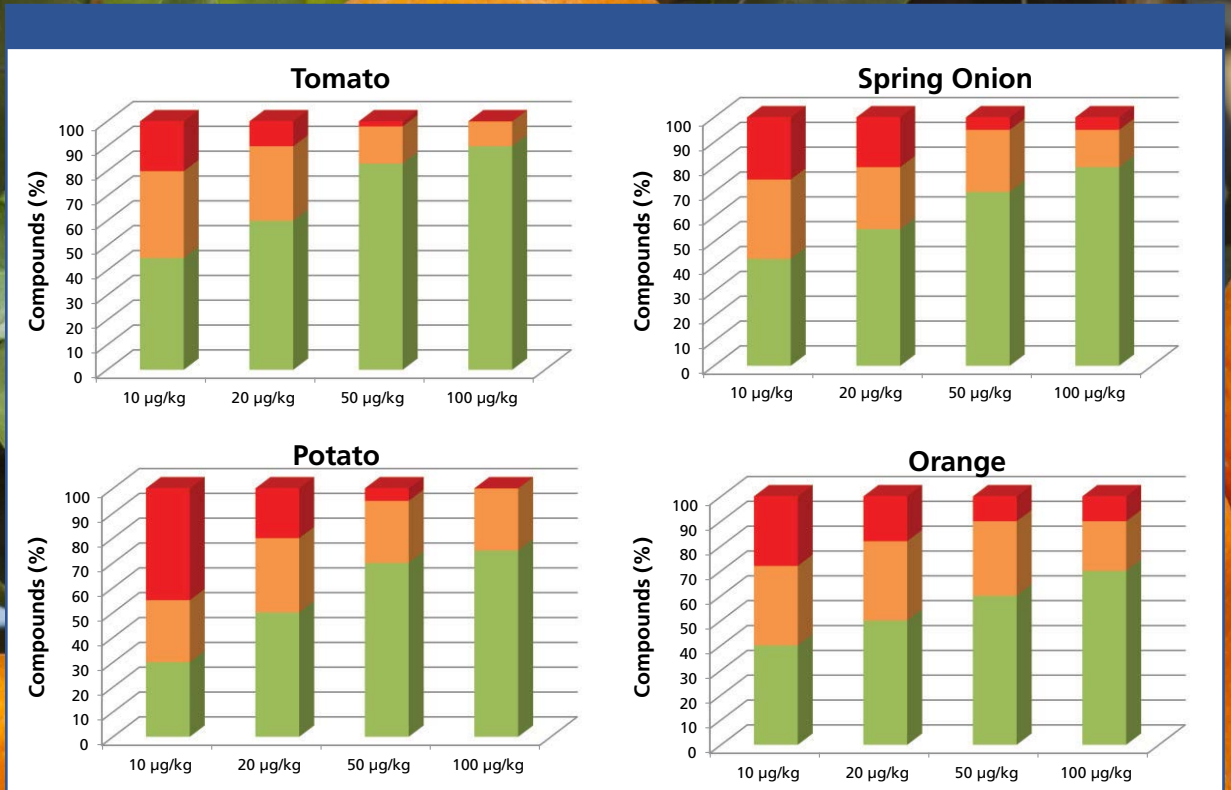

Figure 1: Percentage of compounds included in the databases identified automatically with more than two ions (green), one ion (orange), and not identified (red), working at low resolution.

low LC–MS amenability typically are outside the scope of those LC–MS screening methods.

Nowadays, GC–TOF-MS is becoming a technique of interest within the scientific community for the determination of organic compounds (13,14). The main objective of this study was to develop a purpose-built database that includes the retention time and at least two ions for a total of 110 pesticides and to use this database in the identification of these compounds in fruit and vegetable matrices at different resolving powers.

Experimental

Chemicals and Reagents

All pesticide analytical standards used in this study were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and Sigma Aldrich (Steinheim, Germany) at analytical grade (purity > 95%). A mixture standard solution contained all the pesticides studied and was prepared at 10 µg/mL in ethyl acetate and stored at –20 °C. Ethyl acetate was obtained from Fluka Analytical Pestanal (Steinheim, Germany).

Sample Treatment

The tomatoes, spring onions (called *scallions* in the United States), potatoes, and oranges used in this study were obtained from an organic farm in Almeria, Spain. The fruit and vegetable samples used for the application of the developed method were purchased from local markets.

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

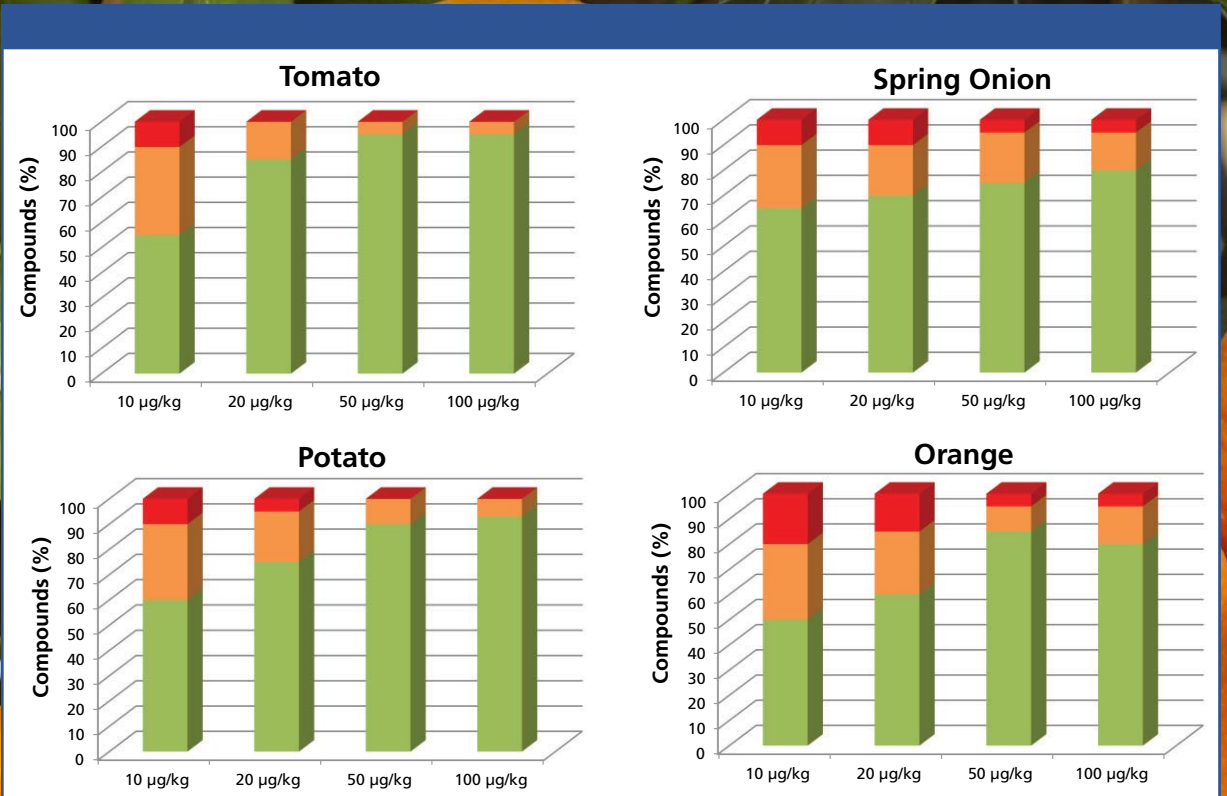

Figure 2: Percentage of compounds included in the databases identified automatically with more than two ions (green), one ion (orange), and not identified (red), working at high resolution.

The extraction method employed (15) is as follows: A representative 10-g portion of previously homogenized sample was weighed in a 200-mL PTFE centrifuge tube. Then 10 mL of ethyl acetate was added, and the tube was shaken vigorously for 3 s by hand. Next, 1.5 g of sodium chloride and 8 g of magnesium sulfate were added, and the tube was shaken in an automatic axial extractor (Agytax, Cirta Lab) for 15 min. The extract was then centrifuged at 3700 rpm for 5 min. Then the extract containing the equivalent of 1 g of sample per mL in 100% ethyl acetate was injected directly into the GC.

Gas Chromatography

The separation of the pesticides from the whole fruit or vegetable extract was carried out using an Agilent 7890 GC system with two 15 m X 0.25 mm, 0.25-µm d_f Agilent HP–5MS UI Ultra Inert GC columns connected through an auxiliary programmable control module (PCM).

The samples were injected in splitless mode through an ultra-inert liner with a glass wool frit (Agilent). The injection volume was 2 µL. The injector temperature was held at 280 °C during the entire run time. Helium (99.999% purity) was used as the carrier gas. The oven temperature program was as follows: 60 °C for 1 min, then 60–120 °C at 40 °C/min, and 120–310 °C at 5 °C/min. The analytical separation was performed under retention time locking conditions, using clorpyrifos methyl as the

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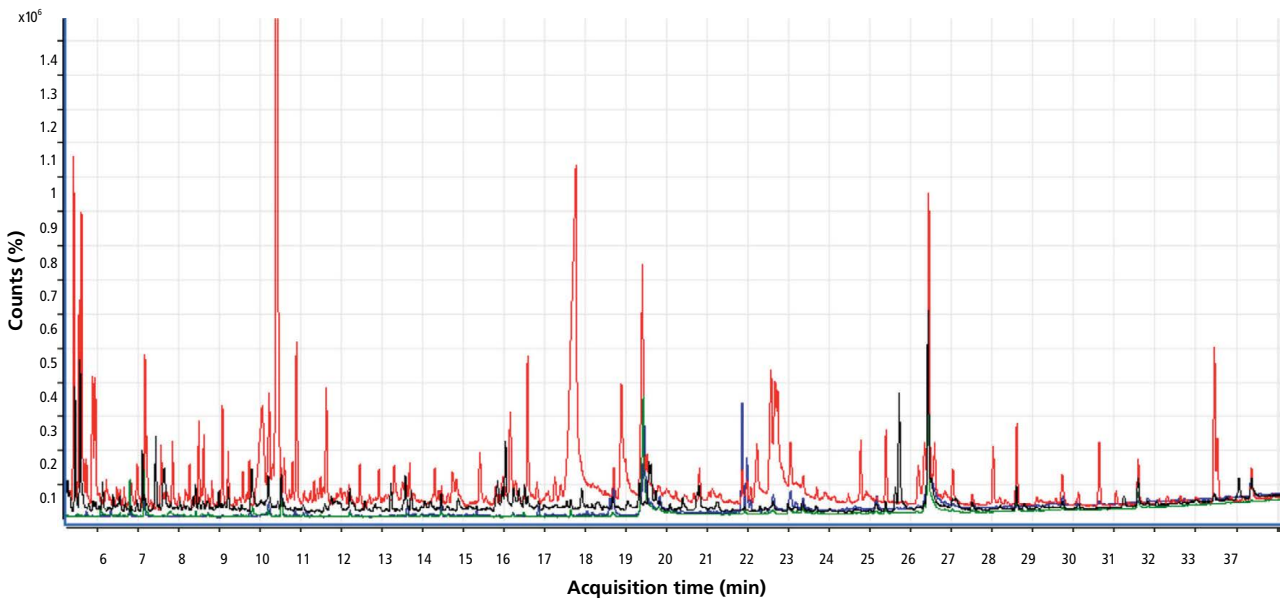

Figure 3: Total ion chromatogram for orange (red), tomato (blue), potato (green), and spring onion (black) obtained by GC–TOF-MS.

locking compound at a retention time of 18.11 min. The flow rates in column 1 and column 2 were 1.225 mL/min and 1.425 mL/min, respectively. The total run time was 40.5 min with 3 additional minutes for backflushing.

Backflushing was carried out to eliminate unwanted heavy materials from column 1, thereby shortening the analysis time, reducing system maintenance, and prolonging column life. The backflush conditions were set as follows: the oven temperature was set at 310 °C, the PCM pressure was 50 psi, and the inlet pressure was 1 psi. These conditions allow a negative flow on the column 1.

Mass Spectrometry

The GC system was connected to an Agilent Technologies model 7200 TOF-MS instrument equipped with an electron ionization source. The ion source and quadrupole analyzer temperatures were set at 280 °C and 150 °C, respectively. The TOF analyzer was operated at two different acquisition rates, 2 GHz and 4 GHz, acquiring data in the *m/z* 45–550 mass range. Perfluorotributylamine was used for daily MS calibration. The accuracy of the generated ions was controlled through an internal mass calibration performed before each injection.

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

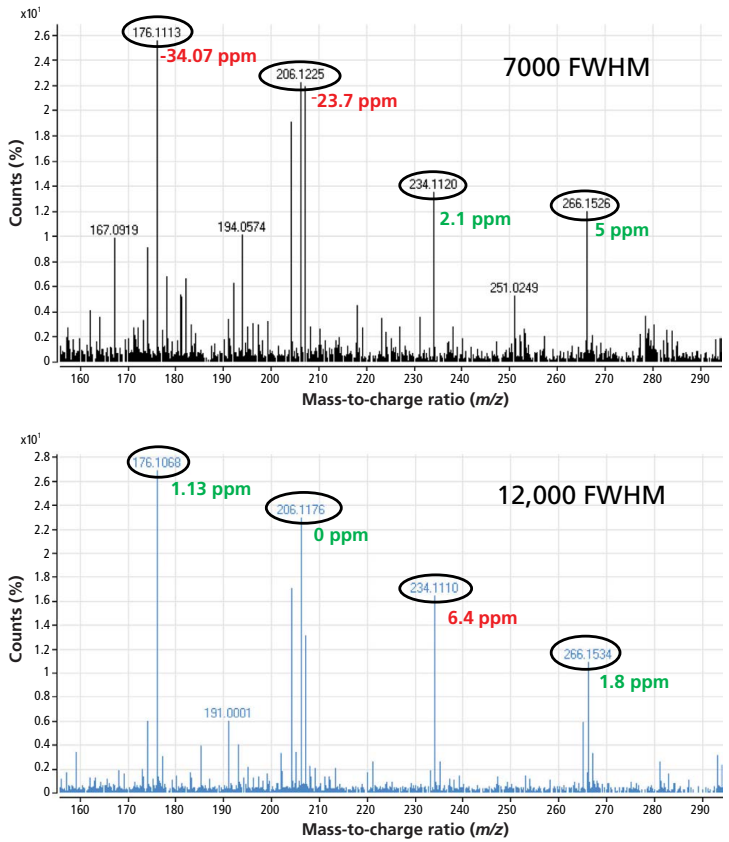

Figure 4: Mass spectra for benalaxyl in tomato at 20 µg/kg obtained at low and high resolution using GC–TOF-MS.

Creation of the Database

The experimental conditions described above were applied to create an accurate-mass database of 110 pesticides. The selected pesticides were run in a tomato matrix at a concentration of 100 µg/kg. The full-scan spectrum was studied and at least two characteristic ions (with a relative abundance higher than 30% with respect to the base peak) were selected. In most cases, the molecular ion of the pesticides was not present in the mass spectrum or its relative abundance was too low to be considered. After the ions were selected, a molecular formula was assigned, and their exact masses were calculated and used to create the database together with their retention times. All the information was collected in an Excel file, which was converted into a CSV format to be used as the library and linked to Mass Hunter data analysis software (Agilent Technologies).

Automatic Identification Using the Pesticide Database

The selected matrices were spiked with all the pesticides at four concentration levels, 10, 20, 50, and 100 µg/kg. Automatic identification was performed at two acquisition rates, 2 GHz and 4 GHz, which allowed respective mass resolutions of 7000 and 12,000 full width at half maximum (FWHM). The search parameters were 0.2 min for the retention time window and 10 ppm for the mass error tolerance.

Results and Discussion

The information obtained from this study has been discussed considering two different goals. The first was to perform screening intended to produce a yes-or-no answer while considering only two identification criteria: the retention time and a single ion with a mass accuracy of less than 10 ppm mass error. The second goal was to obtain confirmation of results and semiquantification considering two ions with a mass accuracy of less than 10 ppm mass error and the correct retention time.

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

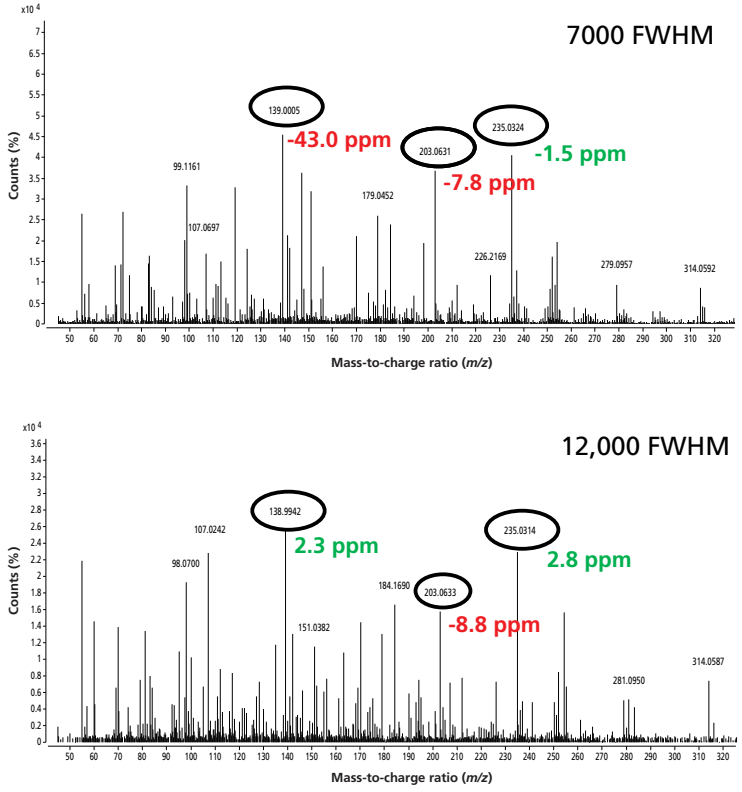

Figure 5: Mass spectra for nuarimol in orange at 20 µg/kg obtained at low and high resolution using GC–TOF-MS.

Goal 1: Screening and Automatic Identification

The four selected extracts spiked at 10, 20, 50, and 100 µg/kg were analyzed at low resolution (7000 FWHM) and at high resolution (12,000 FWHM). The analysis was processed with data analysis software using the purpose-built database as the library.

The pesticides, identified automatically with the correct retention time and one ion with a mass accuracy of less than 10 ppm mass error, were counted and compared with the total number of pesticides included in the database to calculate the percentage of pesticides identified.

The results obtained operating at 7000 FWHM are shown in Figure 1. The green columns for each concentration level are the pesticide percentages with at least two ions having an error lower than 10 ppm and a retention time difference of less than 0.2 min. The orange columns are the pesticide percentages with at least one ion with a mass error lower than 10 ppm and a retention time error of less than 0.2 min. The columns in red are the compound percentages not detected or detected with a mass error higher than 10 ppm.

At the 10-µg/kg level, the percentages of pesticides identified with at least one fragment and the correct retention time

Table I: Real samples identified and quantified with the proposed method and compared to GC–triple-quadrupole MS. Values listed in parentheses are the concentrations that were found in the samples.

Sample	GC–TOF-MS	GC–Triple-Quadrupole MS	Comments
Pepper	Boscalid (0.25 mg/kg)	Boscalid (0.23 mg/kg)	
Tomato 1	Iprodione (0.20 mg/kg)	Iprodione (0.27 mg/kg)	
	Pyriproxifen (0.054 mg/kg)	Pyriproxifen (0.062 mg/kg)	
Tomato 2	Bifenthrin 1F (0.12 mg/kg)	Bifenthrin (0.13 mg/kg)	Ion 166.099 error > 10 ppm
Melon 1	Bupirimate (0.15 mg/kg)	Bupirimate (0.13 mg/kg)	
Tomato 3	Buprofezin (0.49 mg/kg)	Buprofezin (0.16 mg/kg)	
	Metalaxyl (0.060 mg/kg)	Metalaxyl (0.068 mg/kg)	
Zucchini	Pirimicarb (0.33 mg/kg)	Pirimicarb (0.24 mg/kg)	
	Bupirimate (0.11 mg/kg)	Bupirimate (0.048 mg/kg)	
	Penconazole (0.091 mg/kg)	Penconazole (0.074 mg/kg)	
Brussels sprouts	Boscalid (0.21 mg/kg)	Boscalid (0.12 mg/kg)	
Tomato 4	Bifenthrin (0.10 mg/kg)	Bifenthrin (0.125 mg/kg)	Ion 166.099 error > 10 ppm
Bean 1*	Iprodione 1F (0.830 mg/kg)	Iprodione (1.02 mg/kg)	After dilution
Bean 2	Azoxystrobin ND	Azoxystrobin (0.337 mg/kg)	Coelution
	Iprodione (0.36 mg/kg)	Iprodione (0.25 mg/kg)	
Bean 3	Azoxystrobin ND	Azoxystrobin (0.11 mg/kg)	Coelution
	Iprodione (0.19 mg/kg)	Iprodione (0.078 mg/kg)	
Melon 2	Azoxystrobin ND	Azoxystrobin (0.16 mg/kg)	Coelution
	Bupirimate (0.11 mg/kg)	Bupirimate (0.08 mg/kg)	
Bean 4*	Iprodione (1.82 mg/kg)	Iprodione (1.70 mg/kg)	After dilution
Bean 5*	Iprodione (1.27 mg/kg)	Iprodione (1.70 mg/kg)	After dilution
Grape	Iprodione (0.15 mg/kg)	Iprodione (0.11 mg/kg)	
	Myclobutanil (0.041 mg/kg)	Myclobutanil (0.041 mg/kg)	

*** These samples were injected after being diluted 1:2 to achieve correct identification and quantification.**
1F = one fragment detected
ND = not detected

were as follows: 80% in tomato, 50% in potato, 76% in spring onion, and 70% in orange. As expected, at higher concentration levels, the percentage of pesticides found was higher.

Figure 2 shows the results of the studied matrix at the different concentration levels obtained when operating the system at high resolution (12,000 FWHM). At this acquisition rate, the percentage of positives at all concentration levels, and in all matrices, is higher than when operating at low resolution. At 10 µg/kg, the percentages of pesticides identified with at least one fragment and the correct retention time were as follows: 88% in tomato, 91% in potato, 88% in spring onion, and 82% in orange. At 20 µg/kg, 100% of the pesticides included in the database for tomato were identified with at least two ions and a mass error < 10 ppm along with a retention time < 0.2 min. The pesticide identification rates for the spring onion, potato, and orange were 95%, 97%, and 85%, respectively. Operating at high resolution, the number of pesticides found with a mass error below the level established as the mass tolerance was higher than at low resolution.

An important influence on mass assignments is the ability of a mass spectrometer to resolve two peaks on the *m/z* scale when they are close together.

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES

When peaks are not (fully) resolved, the resulting measured mass profile will be the sum of the two individual mass profiles, and the maximum of the combined profile will lie somewhere between the exact masses of the two individual peaks. As a consequence, the mass assignment, which is based on a centroiding algorithm of the detected profile, will result in an incorrect analyte mass. As sample complexity becomes greater (a larger number of matrix peaks at high levels relative to the analytes), the mass resolution (defined as an MS instrument's ability to distinguish between two ions with similar *m/z* values) of a mass spectrometer can become a key parameter in the correct assignment of analyte masses.

Figure 3 shows the full-scan chromatogram of the four matrices selected for this study. The total ion chromatogram in red corresponds to the orange matrix; the chromatogram in black corresponds to spring onion; green corresponds to potato; and blue corresponds to tomato. Orange shows the largest number of matrix interferences.

Figure 4 is an example showing the analysis of benalxyl at 20 ppb in tomato analyzed at both low and high resolution. If we set the mass tolerance to 10 ppm, only two ions are identified when operating at 7000 FWHM, whereas all the ions are identified at 12,000 FWHM.

Figure 5 is an example of an analysis in the orange matrix. In this case the mass spectrum of the pesticide nuarimol is shown at low and high resolution. At low resolution only one ion is correctly identified, whereas at high resolution two ions were identified with a mass error lower than that established.

Goal 2: Confirmation and Semiquantification

With the purpose of using the developed method for confirmation and semiquantification, we considered the identification criteria to be the identification of at least two ions with a mass error lower than 10 ppm and a retention time error lower than 0.2 min. The pesticide percentages that meet these criteria are as follows: Operating at high resolution, more than 70% of the pesticides were detected at the 20 µg/kg level in tomato, potato, and spring

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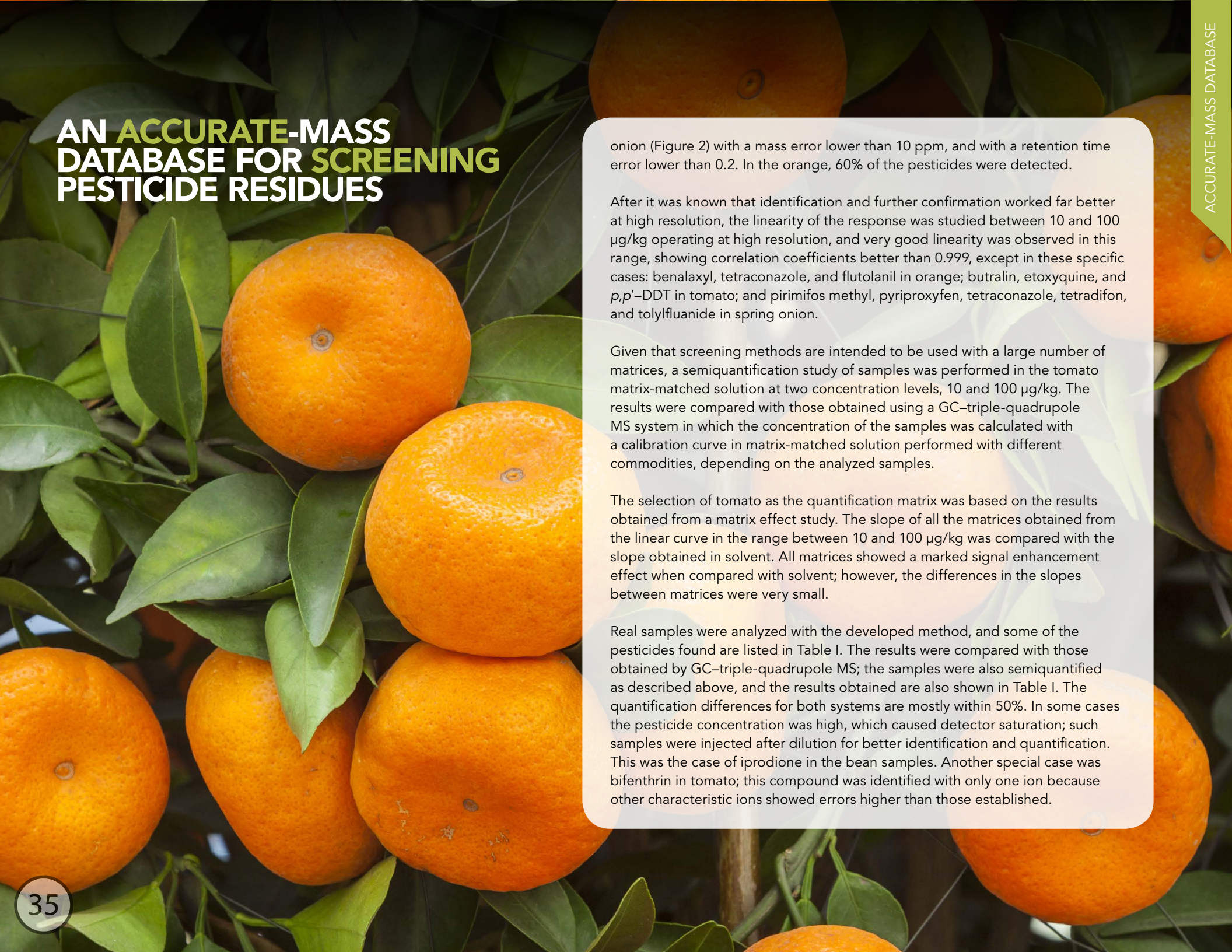


onion (Figure 2) with a mass error lower than 10 ppm, and with a retention time error lower than 0.2. In the orange, 60% of the pesticides were detected.

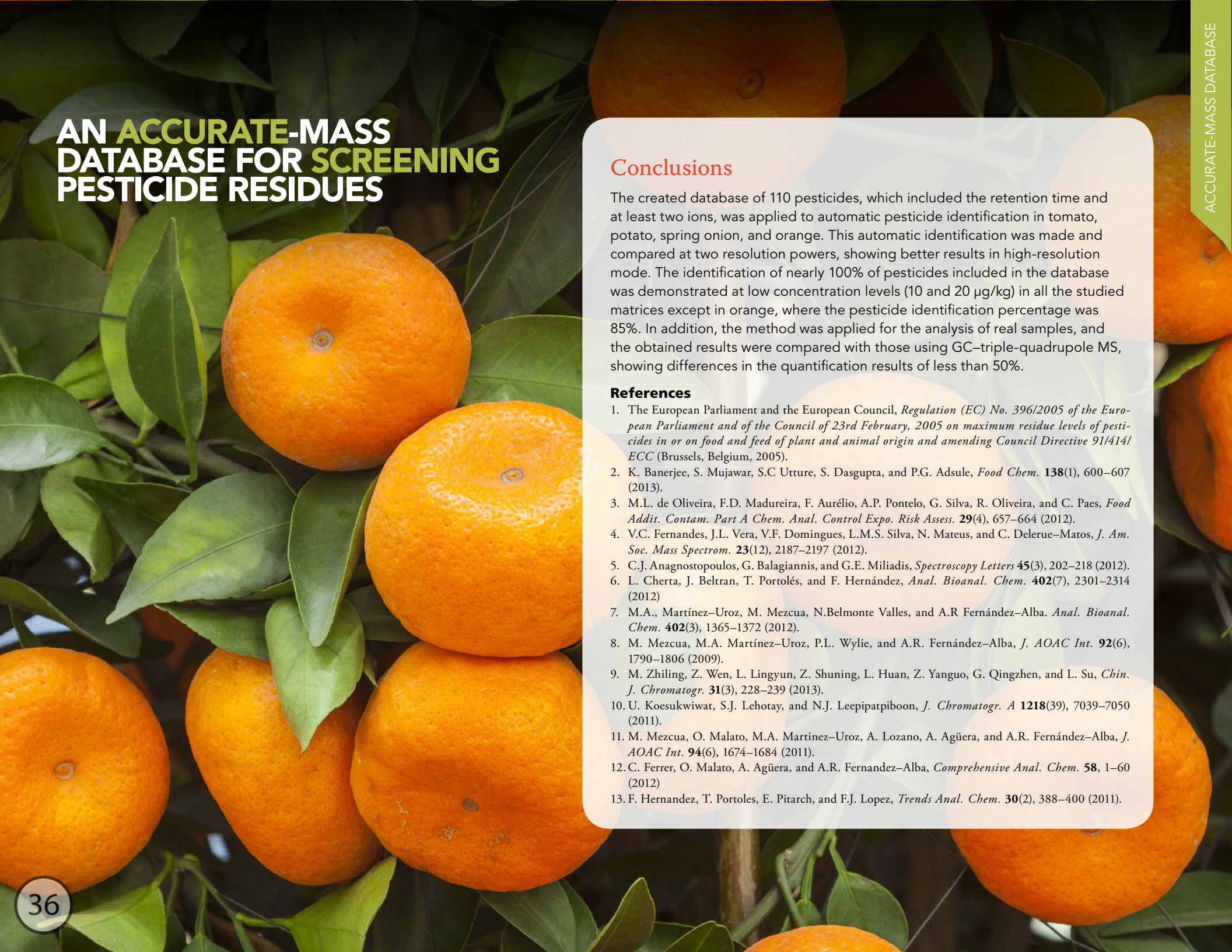
After it was known that identification and further confirmation worked far better at high resolution, the linearity of the response was studied between 10 and 100 µg/kg operating at high resolution, and very good linearity was observed in this range, showing correlation coefficients better than 0.999, except in these specific cases: benalaxyl, tetraconazole, and flutolanil in orange; butralin, etoxyquine, and *p,p'*–DDT in tomato; and pirimifos methyl, pyriproxyfen, tetraconazole, tetradifon, and tolylfluanide in spring onion.

Given that screening methods are intended to be used with a large number of matrices, a semiquantification study of samples was performed in the tomato matrix-matched solution at two concentration levels, 10 and 100 µg/kg. The results were compared with those obtained using a GC–triple-quadrupole MS system in which the concentration of the samples was calculated with a calibration curve in matrix-matched solution performed with different commodities, depending on the analyzed samples.

The selection of tomato as the quantification matrix was based on the results obtained from a matrix effect study. The slope of all the matrices obtained from the linear curve in the range between 10 and 100 µg/kg was compared with the slope obtained in solvent. All matrices showed a marked signal enhancement effect when compared with solvent; however, the differences in the slopes between matrices were very small.

Real samples were analyzed with the developed method, and some of the pesticides found are listed in Table I. The results were compared with those obtained by GC–triple-quadrupole MS; the samples were also semiquantified as described above, and the results obtained are also shown in Table I. The quantification differences for both systems are mostly within 50%. In some cases the pesticide concentration was high, which caused detector saturation; such samples were injected after dilution for better identification and quantification. This was the case of iprodione in the bean samples. Another special case was bifenthrin in tomato; this compound was identified with only one ion because other characteristic ions showed errors higher than those established.

AN ACCURATE-MASS DATABASE FOR SCREENING PESTICIDE RESIDUES



Conclusions

The created database of 110 pesticides, which included the retention time and at least two ions, was applied to automatic pesticide identification in tomato, potato, spring onion, and orange. This automatic identification was made and compared at two resolution powers, showing better results in high-resolution mode. The identification of nearly 100% of pesticides included in the database was demonstrated at low concentration levels (10 and 20 µg/kg) in all the studied matrices except in orange, where the pesticide identification percentage was 85%. In addition, the method was applied for the analysis of real samples, and the obtained results were compared with those using GC–triple-quadrupole MS, showing differences in the quantification results of less than 50%.

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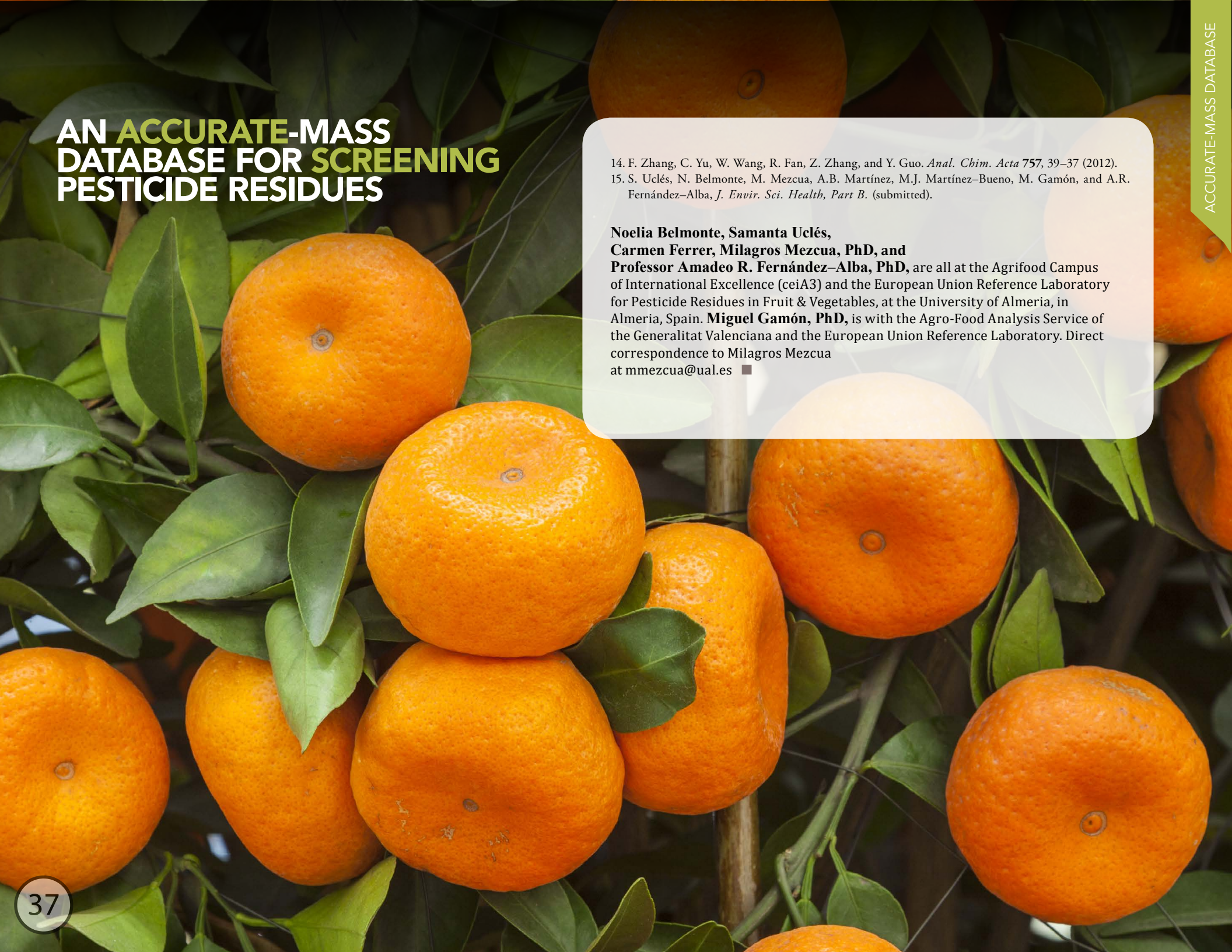

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RAPID SAMPLE PREPARATION AND FAST GC–MS-MS

for the Analysis of Pesticides and Environmental Contaminants in Fish

By Yelena Sapozhnikova

A rapid, high-throughput analytical method was developed and evaluated for the simultaneous determination of pesticides and environmental contaminants in fish. The compounds included polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and flame retardants. The method was based on a QuEChERS (quick, easy, cheap, effective, rugged, and safe) technique with acetonitrile extraction, and a dispersive solid-phase extraction (dSPE) cleanup. Three sorbent combinations were compared for cleanup efficiency and recoveries of the contaminants: C18+PSA, traditionally used for lipid removal in dSPE, and two novel sorbents, based on silica coated with zirconium dioxide (ZrO_2) and ZrO_2/C18, designed for phospholipid removal. The dSPE cleanup with ZrO_2 sorbent provided the highest efficiency with the lowest baseline, as well as satisfactory recoveries (70–120% calculated based on isotope-labeled internal standards) for the most analytes. The method allows for quick sample preparation of fish samples for the analysis of almost 200 targeted contaminants using fast, low-pressure gas chromatography with tandem mass spectrometry (GC–MS-MS), thus providing a wide scope of analysis.

Advances in pesticide residue analysis in recent years led to development of methods for the analysis of multiple pesticides of different classes with a single sample preparation technique and preferably one chromatographic run. Other classes of contaminants, which were previously analyzed by separate methods, requiring either a different sample preparation technique or an additional chromatographic run, are now integrated into multiclass, multiresidue methods, allowing for faster, less expensive, high-throughput analysis. Thus, we recently reported a method for the simultaneous determination of >140 pesticides (1) and 50 environmental contaminants (2) in fish using fast sample preparation based on QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction and dispersive solid-phase extraction (dSPE) and low-pressure vacuum-outlet gas chromatography with tandem mass spectrometry (low-pressure GC–MS-MS). The reported method

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covers a wide range of pesticides along with legacy and novel environmental contaminants: polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and flame retardants, providing wide analytical scope. The method allows for rapid and simple sample preparation (10 homogenized fish samples can be prepared in 1 h), and low-pressure GC–MS-MS affords a fast (9-min) separation of more than 200 analytes, achieving high throughput. The method can easily be extended to include additional GC-amenable emerging contaminants as the need arises.

During the extraction of complex matrices, multiple unwanted components from the matrix are extracted along with the compounds of interest. These coextractive matrix compounds may fill the active sites of the GC inlet and column causing matrix-induced ion enhancement (3), resulting in overestimating of the calculated concentrations. After continuous accumulation of matrix compounds on the inlet liner and the front part of the GC column, opposite effects can be observed — a "matrix-induced diminishing effect" (4), or a reduced ion signal, leading to underestimating of the calculated concentrations. Matrix compounds may also cause retention time shifts and analyte peak distortion, thus compromising quality of the analysis.

To remove interfering matrix compounds from extracts, various cleanup procedures such as gel permeation chromatography, solid-phase extraction, column chromatography, and others are commonly used. These techniques are laborious and time consuming, and require large amounts of organic solvents. Sample cleanup by dSPE is fast, inexpensive, and removes coextractive interfering matrix components while requiring no solvents for conditioning sorbents or eluting compounds of interest. Sorbents such as C18 and primary secondary amine (PSA) are traditionally used for dSPE cleanup of fish extracts. Recently, two novel zirconium dioxide–based (ZrO_2) sorbents, specifically designed to remove phospholipids, were introduced. A sorbent based on silica coated with ZrO_2 is recommended for samples with <15% fat, and a sorbent based on silica coated with ZrO_2 and incorporated C18 (ZrO_2/C18) is recommended for samples with >15% fat.

In this article, we aim to evaluate and compare the C18+PSA, ZrO_2, and ZrO_2/C18

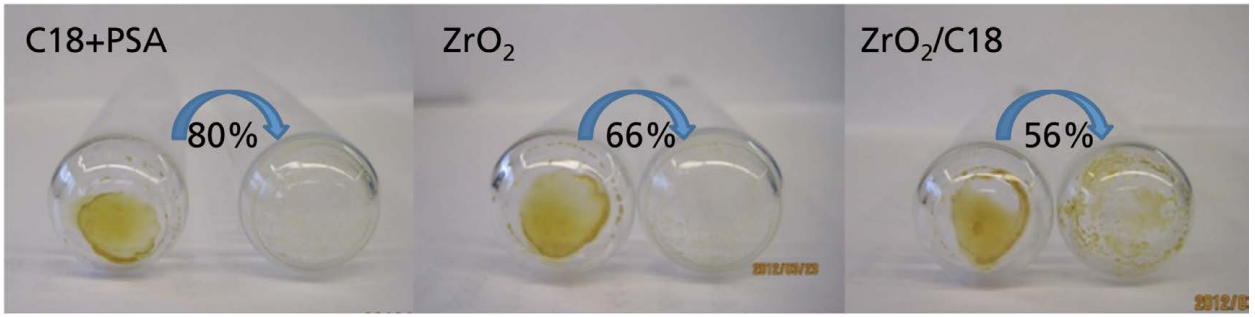

Figure 1: Coextractive materials removal efficiency.

sorbents for dSPE in terms of coextractive removal efficiencies and target analyte and internal standard recoveries, evaluate matrix effects and identify matrix compounds causing them, and investigate how these matrix compounds alter the chromatographic behavior of coeluted analytes. Lastly, we sought to examine the relationship of analyte recoveries on different sorbents and their log K_{ow} values.

Experimental

Sample Preparation

Sample preparation was as follows: First, 10 g of homogenized fish sample was placed into a 50-mL polypropylene centrifuge tube, and internal standards and analytes were added for recovery experiments. After 15 min, 10 mL of acetonitrile was added and the tube was vigorously shaken by hand for 30 s. Then the entire extract, including the tissue, was poured into another 50-mL centrifuge tube containing 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride and was vigorously shaken by hand for 1 min. The tube was then centrifuged for 2 min at 3250 rcf. Then, 1 mL of the extract was placed into a 2-mL centrifuge tube for dSPE cleanup with 150 mg of anhydrous magnesium sulfate and either 50 mg of C18 plus 50 mg of PSA; 50 mg of ZrO_2; or 50 mg of ZrO_2/C18. The

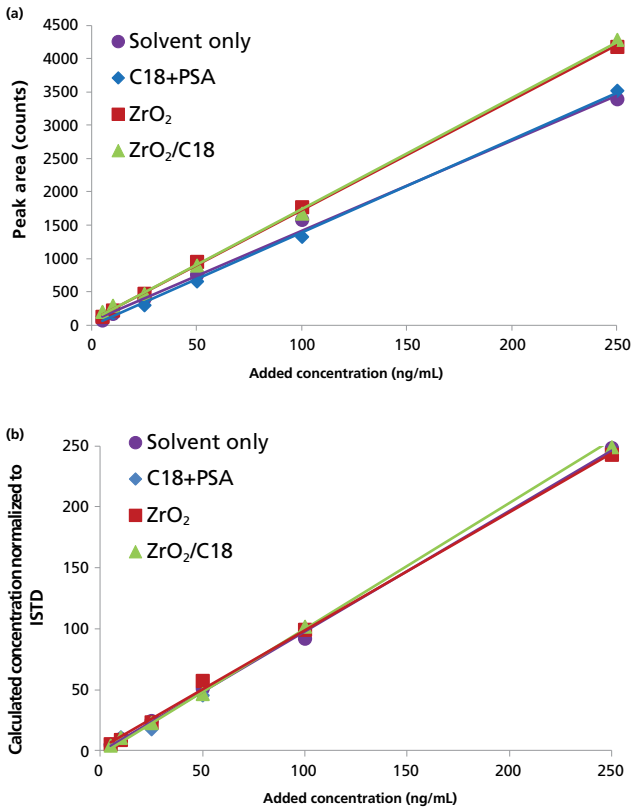

Figure 2: Solvent-only and matrix-matched calibration curves for triphenyl phosphate prepared using dSPE with C18+PSA, ZrO_2, and ZrO_2/C18 based on (a) external calibration and (b) normalized to an internal standard.

tubes were then shaken vigorously for 30 s and centrifuged for 2 min at 3250 rcf. Lastly, 0.5 mL of the treated extract was transferred into an autosampler vial and subjected to low-pressure GC–MS-MS analysis.

Sorbents for dSPE

J.T.Baker Bakerbond C18 sorbent (40 µm) was purchased from Thomas Scientific (Swedesboro, New Jersey). Primary secondary amine (PSA) was purchased from UCT (Bristol, Pennsylvania), and zirconium dioxide-based Supel QuE Z-Sep (ZrO_2) and QuE Z-Sep Plus (ZrO_2/C18) sorbents were purchased from Supelco (Bellefonte, Pennsylvania).

Low-Pressure GC–MS-MS

An Agilent Technologies 7000B triple-quadrupole mass spectrometer with electron ionization (EI) interfaced to an Agilent 7890A gas chromatograph was used. The GC instrument had a 220-V fast oven heating upgrade, which allowed for fast heating rates. A 15 m × 0.53 mm, 1-µm d_f Rti-5ms Restek GC column was used, and it was connected to a 5 m × 0.18 mm non-coated guard column (Restek) at the injector, and to a 17 cm × 0.53 mm phenyl-methyl deactivated guard column (Restek) at the transfer line with Ultimate union column connectors (Agilent) as previously described (2). The carrier gas was ultrahigh purity helium at 2 mL/min constant flow rate. The GC oven temperature program was as follows: 70 °C for 1.5 min, 70–180 °C at 80 °C/min, 180–250 °C at 40 °C/min, 250–320 °C at 70 °C/min, and then 320 °C until a total run time of 9 min was reached. The transfer line and ion source were set at 250 °C and 320 °C, respectively. The triple-quadrupole collision gas was nitrogen at 1.5 mL/min and the quench gas was helium at 2.25 mL/min. The EI energy was –70 eV, the quadrupole temperatures were set at 150 °C, and the solvent delay was at 2 min. A multimode inlet (MMI, Agilent) was operated as a programmable temperature vaporizer (PTV) with solvent vent mode of He flow at 50 mL/min, and the injection volume was 5 µL. The inlet temperature program was as follows: 80 °C for 0.31 min (at which point the vent was closed), 80–420 °C at 320 °C/min, and 420 °C for the rest of the GC run.

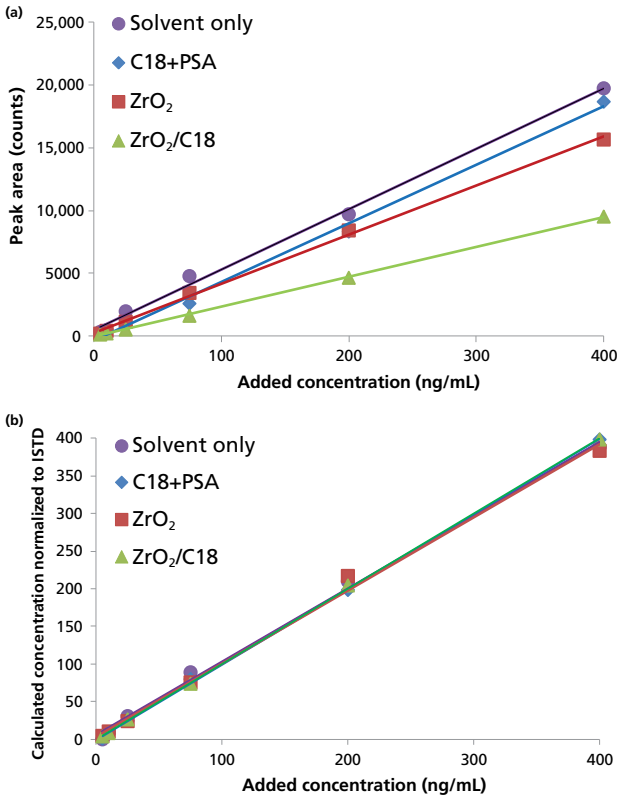

Figure 3: Solvent-only and matrix-matched calibration curves for *o,p´*-DDE prepared using dSPE with C18+PSA, ZrO_2, and ZrO_2/C18 based on (a) external calibration and (b) normalized to an internal standard.

Coextractives Determination

A sample of 10 g of catfish was extracted with 10 mL of acetonitrile by shaking for 1 min, then the entire extract, including the tissue, was poured into another 50-mL centrifuge tube with 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride, vigorously shaken for 1 min, and centrifuged as described above. An aliquot of the extract (2.5 mL) was placed into a previously weighed glass tube, evaporated to dryness with nitrogen, and dried in the oven at 100 °C for 20 min; at that point the tube with extractable residue was weighed again. To compare the coextractive removal efficiency, portions of 5 mL of the extract were placed into three previously weighed glass tubes and were treated with C18+PSA, ZrO_2, and ZrO_2/C18, 250 mg in each case, all in combination with 750 mg of anhydrous magnesium sulfate. After the tubes were centrifuged, 2.5 mL of the treated extracts was evaporated to dryness and dried in the oven at 100 °C for 20 min, and the tube was weighed again. The experiment was conducted in triplicate.

Three sets of matrix-matched calibration curves were prepared in catfish extracts after each dSPE cleanup (C18+PSA, ZrO_2, and ZrO_2/C18) as described above. Analyte and internal standard solutions were added to the treated extracts to yield needed concentrations for six calibration points. The solvent-only calibration curve was prepared in acetonitrile.

Results and Discussion

Coextractive Compounds and Matrix Effects

Coextractive matrix compounds may alter chromatographic behavior of analytes and cause matrix effects, leading to inaccurate quantification. Therefore, it is important to develop a sample preparation procedure that yields as few coextractive components as possible. In this method, we selected acetonitrile as an extraction solvent because of its ability to extract compounds with a wide range of polarities without coextracting a significant amount of fat or lipids compared to nonpolar solvents (such as hexane or ethyl acetate). For example, the amount of total lipid and fat in catfish is approximately 6% (5), and using acetonitrile for extraction, the total measured amount of coextractives was 8 mg/g, or 0.8%.

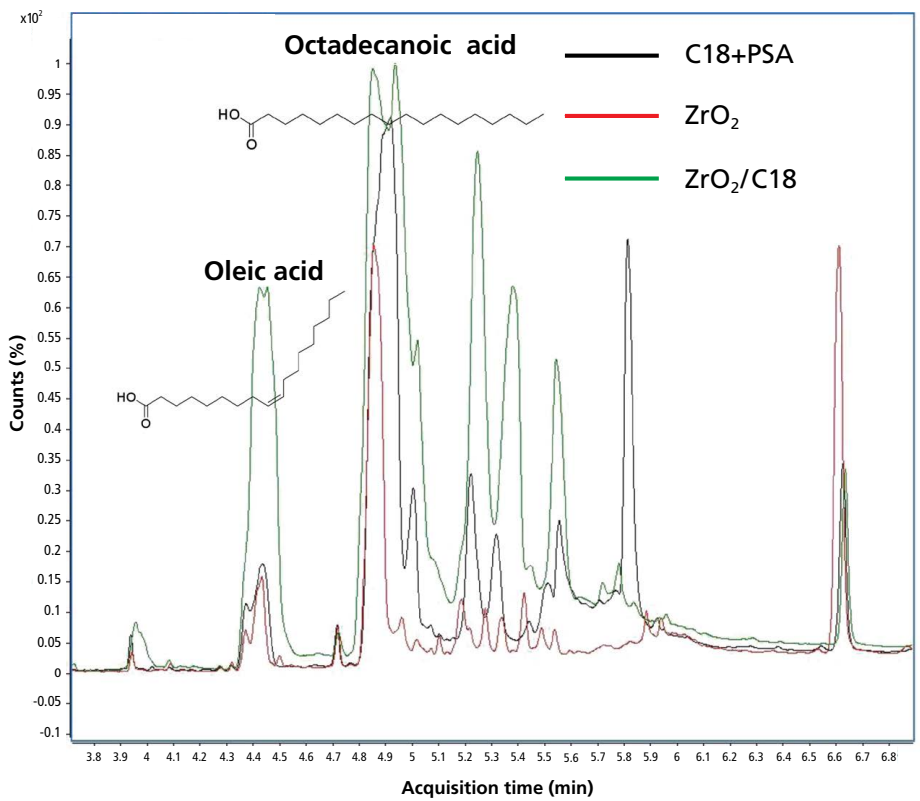

Figure 4: Overlaid total ion chromatograms for C18+PSA, ZrO_2, and ZrO_2/C18 dSPE treatments.

Dispersive SPE is commonly used to remove coextractive materials that interfere with the analysis of target analytes. In this work, three different sets of sorbents in dSPE were compared: C18+PSA, ZrO_2, and ZrO_2/C18, each in the amount of 50 mg per 1 mL of extract, all in combination with 150 mg of anhydrous magnesium sulfate.

Coextractive material removal efficiency was estimated gravimetrically by measuring the difference in weight before and after each dSPE treatment and calculated using the following formula:

$$removal\ efficiency\ \% = \left(\frac{\text{coextractive weight before dSPE} - \text{coextractive weight after dSPE}}{\text{coextractive weight before dSPE}}\right) \text{X } 100\% \text{ [1]}$$

Figure 1 shows the glass tubes from our experiments with coextractive materials before and after dSPE with C18+PSA, ZrO_2, and ZrO_2/C18. As it can be seen, the tube after dSPE with ZrO_2/C18 still had an appreciable amount of coextractive material that was not removed. The calculated removal efficiency was 80% for C18+PSA, 66% for ZrO_2, and 56% for ZrO_2/C18 dSPE. This means that 1 mL of extract after dSPE cleanup contained 1.6 mg (C18+PSA), 2.72 mg (ZrO_2), or 3.52 mg (ZrO_2/C18) of coextractive materials, and with an injection volume of 5 µL, 8 µg (C18+PSA), 13.2 µg (ZrO_2), or 17.6 µg (ZrO_2/C18) of coextractives were introduced in the GC injector with each injection. Although the amounts of matrix compounds left in the extract seem very small, they still may affect chromatographic behavior of coeluted analytes and cause matrix effects. Matrix-matched calibrations were used to account for matrix effects in each dSPE treatment. Difference in adsorption of analytes on active sites in neat solvent and sample extract containing matrix compounds can be seen as a difference between slopes of

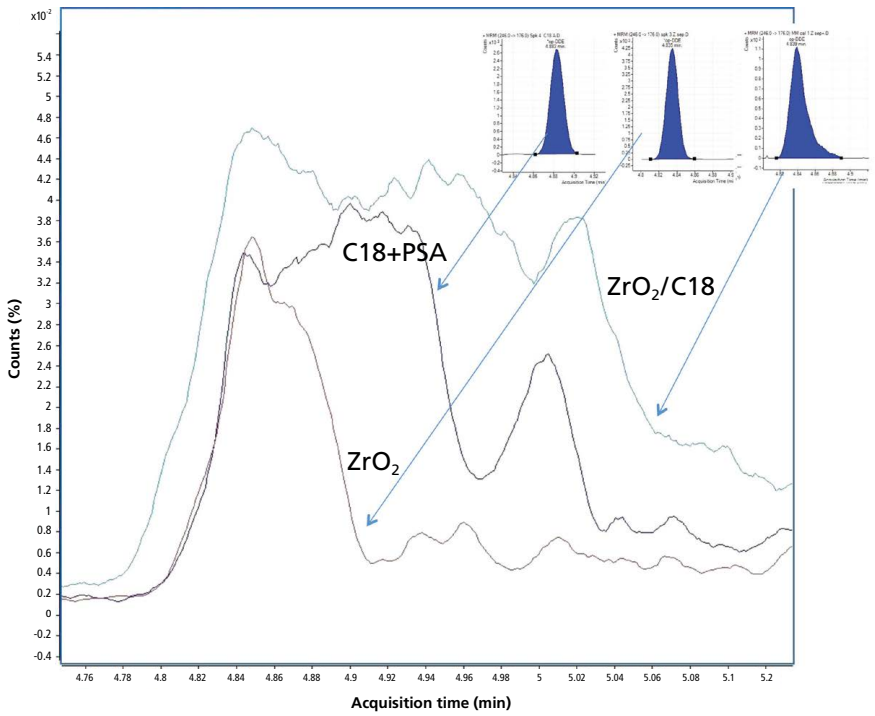

Figure 5: Extracted ion chromatogram for ion 176 representing *o,p´*-DDE at t_R = 4.854 min for extracts treated with C18+PSA, ZrO_2, and ZrO_2/C18.

solvent-only and matrix-matched calibration curves. For example, Figure 2a shows solvent-only and matrix-matched calibration curves for triphenyl phosphate, a flame retardant. Matrix effects (ME in equation 2) were calculated as a difference between the slope of the matrix-matched (MM) calibration curve for each dSPE treatment and solvent-only (SO) calibration curve.

$$ME, \% = \left(\frac{\text{slope of MM calibration curve - slope of SO calibration}}{\text{curve slope of SO calibration curve}} \right) \text{X } 100\% \text{ [2]}$$

Calculated matrix effects for triphenyl phosphate were 4% (C18+PSA), 24% (ZrO_2), and 25% (ZrO_2/C18) (Figure 2a), based on peak areas (not normalized to an internal standard), illustrating signal enhancements in matrix-matched calibration by up to 25%. However, when normalized to the internal standard, $13C_{18}$-triphenyl phosphate, calculated matrix effects were 4% (C18+PSA), –2% (ZrO_2), and 1% (ZrO_2/C18) (Figure 2b).

In another example, matrix effects calculated for *o,p´*-DDE based on peak areas were –3% (C18+PSA), –19% (ZrO_2), and –51% (ZrO_2/C18) (Figure 3a), revealing a less common example of signal diminishing effect (4) by up to 51%. However, when using the internal standard fenthion-d_6, calculated matrix effects were (–3%) for C18+PSA, (0%) for ZrO_2, and (–3%) for ZrO_2/C18 (Figure 3b). These examples demonstrate the value of internal standards to compensate for matrix effects and eliminate the need for matrix-matched calibration in some cases. Similar findings were reported for matrix effects of multiple pesticides in various food crops (6). Indeed, it is difficult, if not impossible, to have "clean," contaminant-free materials to prepare matrix-matched calibrations for many matrices. In case of "megamethods" with multiple analytes of different types, it is rare to find such an "analyte-free" matrix.

Interestingly, calculated matrix effect values for triphenyl phosphate and *o,p´*-DDE gave Pearson correlation coefficients of 0.93 and –0.96, respectively, when linked to the amounts of coextractive materials for each dSPE treatment.

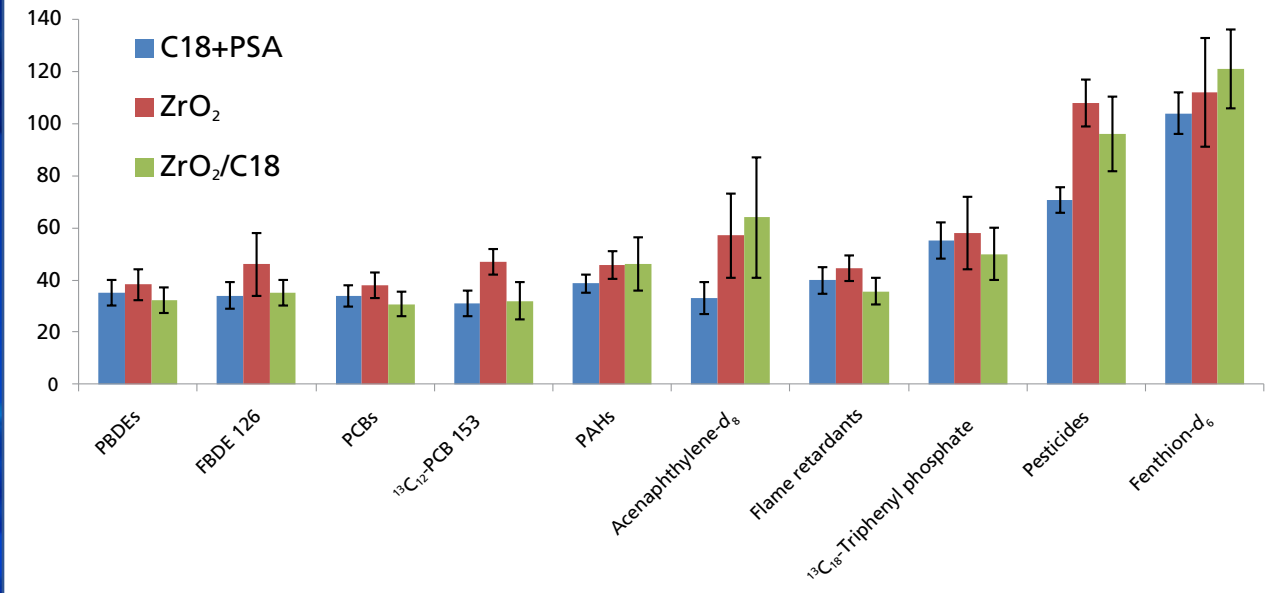

Figure 6: Average recoveries (%) of PBDEs, PCBs, PAHs, flame retardants, and pesticides and representative internal standards. Error bars represent standard deviations (%).

Furthermore, we looked into what particular coextractive compounds may cause matrix effects. Extracts treated with the three dSPE sorbents were run in scan mode, and total ion chromatograms (TICs) for these extracts are shown in Figure 4. Deconvolution software (Agilent Mass Hunter) and the NIST 2011 library identified 24 compounds in the range of 3–7 min on the TIC after dSPE with C18+PSA, 29 compounds in extracts treated with ZrO_2 dSPE, and 17 compounds in extracts treated with ZrO_2/C18. Most of these compounds were lipids, fatty acids, and their derivatives. Although fewer coextracted compounds were identified in the ZrO_2/C18 chromatogram, their intensities were greater than those treated with C18+PSA or ZrO_2 (Figure 4). Two of the largest peaks, identified on the TICs, were oleic acid (t_R = 4.4 min) and octadecanoic acid (t_R = 4.9 min), fatty acids from animal and vegetable fat and oil. Oleic acid amounts, calculated from peak areas, which remained in the extracts after dSPE cleanup with C18+PSA and ZrO_2, were 20% and 13%, respectively, of the amount of oleic acid in the extract after dSPE with ZrO_2/C18 (100%), suggesting that ZrO_2/C18 was not effective in removing this fatty acid. Likewise, octadecanoic acid amounts were 13% (C18+PSA) and 2% (ZrO_2) of the amount of this acid in ZrO_2/C18 (100%). Pearson correlation

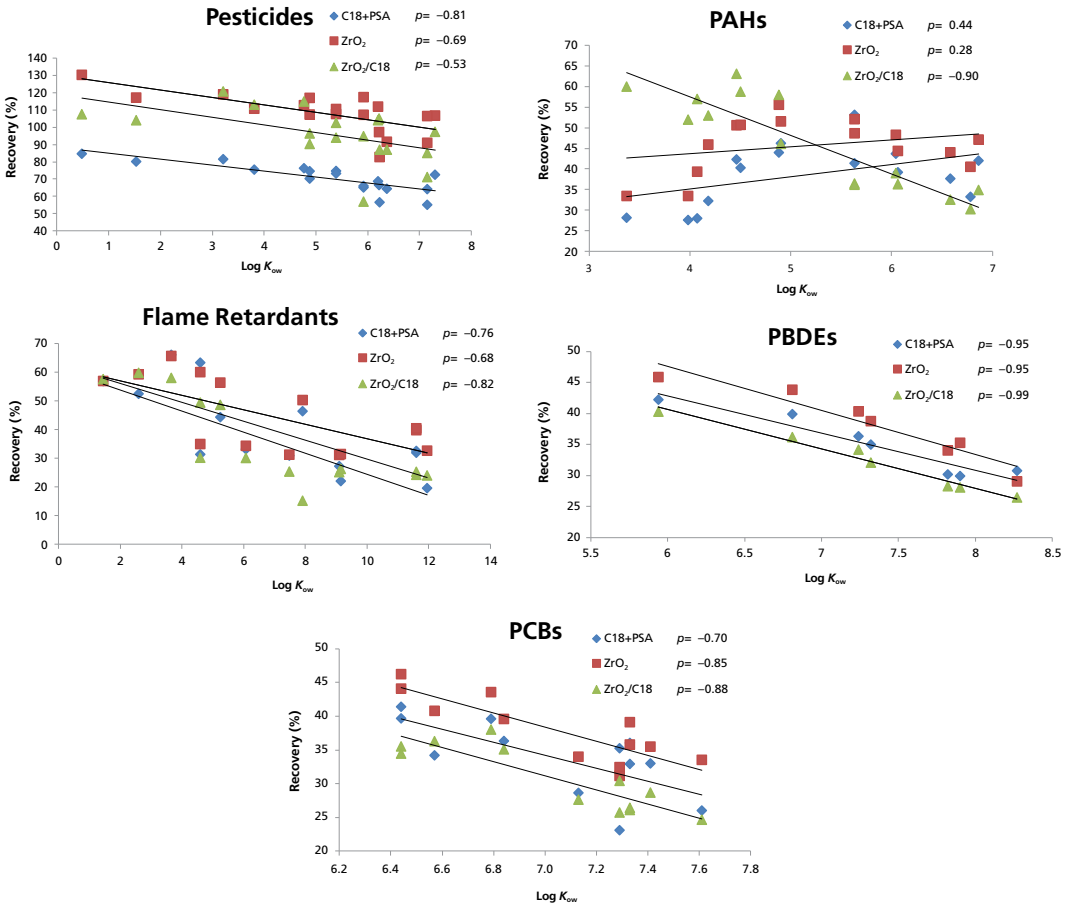

Figure 7: Correlation between recoveries (%) and log K_{ow} values for pesticides, PAHs, flame retardants, PBDEs, and PCBs.

coefficients for peak areas of oleic and octadecanoic acids and amounts of coextractive materials in these extracts were 0.89 and 0.88, respectively, proposing their significant contributions to the total amount of coextractive materials. The compound *o,p´*-DDE is coeluted with octadecanoic acid, and therefore may be affected by this coextractive interference. In fact, a good correlation was observed for matrix effects calculated for *o,p´*-DDE and the peak areas of octadecanoic acid for the three dSPE treatments, with r^2 = 0.97.

Figure 5 shows an extracted ion chromatogram for ion 176 representing *o,p´*-DDE at t_R = 4.854 min for the three dSPE treatments and corresponding *o,p´*-DDE multiple reaction monitoring (MRM) chromatograms of the same extracts. MRM peak widths for *o,p´*-DDE were 2.98 ± 0.19 s for C18+PSA treatment, 2.97 ± 0.33 s for ZrO_2, and 3.90 ± 0.39 s for ZrO_2/C18 for *n* = 10 injections. The peak on the ZrO_2/C18 MRM chromatogram was wide and asymmetrical with visible tailing, perhaps showing an example of interference by coeluted matrix compounds.

ZrO_2 treatment removed the highest amounts of both oleic and octadecanoic acids in our experiment, and thus provided the "cleanest" chromatograms with lowest background levels. Several publications to date have described successful applications of ZrO_2 in dSPE for cleanup of matrices with high amounts of lipids, and agreed that compared to C18+PSA and ZrO_2/C18, ZrO_2 provided the cleanest extracts, lowest background noise on the chromatograms, lowest amounts of coextractive matrix compounds (7), and highest amounts of analyzed contaminants showing acceptable recoveries and lowest standard deviations.

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Recovery Study

As the need for multiresidue, multiclass analysis of contaminants in foods and environmental samples continues to increase, megamethods covering multiple classes of contaminants in one uncomplicated technique are being introduced. As we have demonstrated, the use of internal standards helps to account for matrix effects and enables quite accurate quantification. Because it is impractical to match every single analyte with the isotope-labeled internal standard, efforts were made in our method development to use one representative internal standard labeled with an isotope or an alternate label for each class of contaminants. Thus, all organophosphate flame retardants were quantified with $13C_{18}$-triphenyl phosphate, all PCB congeners with $13C_{12}$-PCB 153, all PBDE congeners and brominated flame retardants with fluoro-BDE 126 (FBDE 126), and all pesticides with fenthion-d_6. PAHs were quantified with various isotope-labeled PAHs because surrogate PAH cocktails are readily available and relatively inexpensive.

The recoveries of nearly all analytes were within the acceptable range of 70–120% while using calculations normalized to the internal standards (1,2). In the present study, we attempted to estimate "real" recoveries of the analytes without internal standard correction and recoveries of representative internal standards. This information gives a valuable insight into what quantity of analytes and internal standards were lost during the sample extraction and cleanup process. Recoveries were calculated as an average for all representative compounds of one class: PCBs, PBDEs, PAHs, flame retardants, and pesticides. For the three dSPE treatments, the average recoveries of representative compounds in a class and corresponding internal standards were as follows: PBDEs: 32–38%, internal standard FBDE 126: 34–46%; PCBs: 31–38%, internal standard $13C_{12}$-PCB 153: 31–38%; PAHs: 39–46%, internal standard acenaphthylene-d_8: 33–64%; flame retardants: 36–45%, internal standard $13C_{18}$-triphenyl phosphate 50–58%; and pesticides: 71–108%, internal standard fenthion-d_6: 104–121% (Figure 6). For all classes of contaminants, except for pesticides, the recoveries were 2–3 times lower when not normalized to an internal standard, reflecting incomplete recovery during the sample preparation process. For pesticides, however, the recoveries were comparable with and without normalizing to an internal standard.

For all classes, except for PAHs, the recoveries using dSPE with ZrO_2 were greater compared to the use of dSPE with C18+PSA or ZrO_2/C18 (Figure 6). Also, standard deviations for dSPE with ZrO_2/C18 were greatest compared to the other two treatments, compromising the repeatability of the method. Some of the matrix effects and coextractive compounds discussed above may be responsible for higher standard deviations in extracts treated with ZrO_2/C18.

Correlation Between Recoveries and Log K_{ow} Values

As is well known, the octanol–water partition coefficient, log K_{ow}, is one of the factors affecting sorption of organic compounds. During a dSPE step, organic compounds in the extract undergo a sorption–desorption process on the sorbent, with their recoveries representing their retention on the sorbent. We explored a correlation between recoveries of targeted analytes from various classes and their log K_{ow} values. For all classes of contaminants, except for PAHs, Pearson correlation coefficients were negative, indicating that increasing log K_{ow} values lead to decreased recoveries. Chemicals with higher log K_{ow} values are more lipophilic, and therefore tend to have a higher affinity for lipids, adsorbed by the sorbent during the SPE cleanup process. Similar to our study, Rajski and colleagues (7) examined the relationship of 92 pesticide recoveries from fatty matrices and log K_{ow}, and concluded that the majority of pesticides with higher log K_{ow} values exhibited lower recoveries.

The correlations were more pronounced for PBDE and PCB congeners, which are homologues with similar structures, unlike other classes of chemicals that include compounds with rather diverse chemical structures. Thus, for PBDE congeners, the calculated correlation coefficients were as follows: –0.95 for C18+PSA, –0.99 for ZrO_2, and –0.99 for ZrO_2/C18 (Figure 7). For PCB congeners, the correlation coefficients were –0.70 for C18+PSA, –0.85 for ZrO_2, and –0.88 for ZrO_2/C18 (Figure 7). In contrast to the negative correlation observed for all pesticides, PCBs, PBDEs and flame retardants on all sorbents, the correlation coefficient for PAHs was negative only for ZrO_2/C18 treatment (–0.90), and weakly positive for C18+PSA (0.44) and for ZrO_2 (0.28).

Another interesting relationship was observed between t_R and log K_{ow}. Pearson correlation coefficients were 0.99 for PAHs, 0.95 for PBDEs, 0.98 for PCBs, 0.92 for flame retardants, and 0.55 for pesticides.

Conclusions

In this study we compared the efficiency of the three sorbents for dSPE cleanup of fish extracts for the analysis of pesticides and environmental contaminants: C18+PSA, ZrO_2, and ZrO_2/C18. C18+PSA removed the highest amounts of coextractive materials by weight (80%), followed by ZrO_2 (66%) and ZrO_2/C18 (55%). In terms of target analyte recovery, ZrO_2 provided the highest recoveries for most contaminants, and ZrO_2/C18 showed the highest standard deviations, possibly caused by matrix interferences. Recoveries of analytes not normalized to internal standards were 2–3 times lower for all classes, except for pesticides, reflecting losses during the sample preparation.

Two major coextractive compounds identified in the extracts that contributed to matrix effects were oleic and octadecanoic acid, and dSPE with ZrO_2 removed these compounds most effectively. Peak shapes and widths were altered by coeluted matrix interferences as we observed in the case of *o,p´*-DDE.

Calculated matrix effects for triphenyl phosphate and *o,p´*-DDE based on peak areas were lowest for dSPE with C18+PSA, followed by ZrO_2 and ZrO_2/C18; however, they were ≤4% for all the treatments when normalized to internal standards, indicating the need to use an internal standard to compensate for matrix effects. Finally, Pearson correlations were reported for compound recoveries and t_R and log K_{ow} values.

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