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Review

Liquid chromatography-mass spectrometry for the determination of neonicotinoid insecticides and their metabolites in biological, environmental and food commodity matrices

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ABSTRACT

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for the analysis of neonicoitinoids or their metabolites have been developed for a wide range of sample matrices including atmospheric sorbent materials, bee pollen, food commodities, soil, surface water, and urine. Neonicotinoids and their metabolites are often analyzed using a chemical class-specific approach to obtain better recoveries than with multiresidue pesticide methods. Liquid chromatography-high resolution mass spectrometry has also been used to identify new metabolites of neonicotinoid insecticides particularly where standards are not available. Electrospray ionization in positive ion mode is the most common ionization method. Reversed phase liquid chromatography with stationary phase sorbent choices including specialty-bonded C18 to silica, phenyl or biphenyl, and pentafluorophenyl bonded in a silica surface were commonly selected to provide additional selectivity for the separation to address the wide range of neonicotinoids commercially available. Gradient elution with acetonitrile as the organic modifier and 0.1% formic acid as a mobile phase additive is the most common choice, although a methanol gradient with 0.1% formic acid could also be used to obtain the required selectivity and MS sensitivity. The sample extraction and clean-up approaches varied with sample type. For liquid sample matrices the most common method of sample pre-concentration and clean-up is solid phase extraction (SPE). Neonicotinoids or their metabolites were extracted from solid sample matrices using liquid-liquid extraction, pressurized solvent extraction, QuEChERS (quick, easy, cheap, effective, rugged and safe), solid-liquid extraction, or ultrasonic extraction. When additional clean-up was required the preferred approaches included QuEChERS or modified QuEChERS or an extraction method followed by SPE. To obtain adequate recoveries and sufficient selectivity and MS sensitivity for metabolites of neonicotinoids, generally both sample preparation and liquid chromatography-mass spectrometry parameters were further optimized.

KEYWORDS: neonicotinoid insecticides, neonicotinoid metabolites, LC-ESI$^+$-MS/MS, pesticide residue analysis

1. Introduction

The first neonicotinoid insecticide (NEO) for commercial sale was introduced in 1991 and usage of NEOs has grown both in terms of the number of crops to which it is applied and the number of countries that are currently using NEOs [1-3]. Currently there are eight commercially available neonicotiniod insecticides (NEOs) that include N-nitroguanidines (clothianidin (CLO),

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dinotefuran (DIN), imidacloprid (IMI), and thiamethoxam (THX)), N-cyano-amidines (acetamiprid (ACE) and thiacloprid (THC)), nitromethylene (nitenpyram (NIT)), and a sulfoximine (sulfoxaflor (XDE-208)) [1]. Clothiandin is also a metabolite of thiamethoxam [4]. Increasing attention has been given to NEOs due to their linkage to colony collapse disorder in bees and adverse human health impacts [5-8] such that there has been an increase in the need for analytical methods capable of analyzing the full range of NEOs and their metabolites for bee and bee products, biological matrices, crops, and environmental matrices.

A variety of approaches have been used for the analysis of selected NEOs including capillary electrophoresis-mass spectrometry [9], enzyme-linked immunosorbent assay [10-15], immuno-chromatographic assays [16, 17], gas chromatography coupled with mass spectrometry or tandem mass spectrometry [18, 19], liquid chromatography with UV-visible detection [20-29] or electrochemical detection [30, 31] and supercritical fluid chromatography-mass spectrometry [32]. As shown in tables 1 and 2 the most widely used methods for the analysis of NEOs and their metabolites involve liquid chromatography coupled to mass spectrometric detection (high and low resolution) with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) in positive ion mode as these methods allow for the greatest diversity of NEOs and their metabolites to be analyzed simultaneously [4, 33-74]. Selected NEOs (most commonly acetamiprid and imidacloprid) have been analyzed in multiresidue pesticide methods utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) with inclusion of other chemical classes such as azoles, methylcarbamates, organophosphorous pesticides (OPs), phenylureas, pyrethroids, and strobilurin fungicides [43, 48, 75-90]. This review focuses on LC-MS or LC-MS/MS methods that are chemical class-specific approaches for NEOs and their metabolites. A chemical class-specific approach is often selected for the full range of NEOs or selected metabolites due to both sample preparation needs and chromatographic conditions necessary to obtain desired recoveries, adequate retention of NEOs in reversed phase systems, and optimal MS selectivity and sensitivity [4, 33-74].

2. Choice of separation conditions for LC-MS or LC-MS/MS

A wide range of reversed phase stationary phases can achieve adequate retention and resolution of the NEOs and their metabolites under proper selection of mobile phase conditions. Special attention has to be given to dinotefuran, flonicamid, and nitenpyram which are weakly retained on a number of reversed phase column choices. Ideally the column selection should include consideration for adequate retention of NEOs, resolution of all NEOs and metabolites of interest, total analysis time, and reproducibility. In pure organic solvent with 0.1% formic acid the ionization efficiency for NEOs is better with acetonitrile than methanol [41]; however under separation conditions the percentage methanol required to achieve similar retention times of NEOs (on same column choice) is higher (commonly up to 100% methanol) as compared to when acetonitrile is selected as the organic modifier, and the higher organic modifier content will improve ionization efficiency of NEOs such that adequate MS sensitivity can also be achieved using mobile phases with methanol and 0.1% formic acid. For the separation of NEOs and selected metabolites of NEOs shown in figure 1 sensitivity was significantly improved with acetonitrile as compared to methanol (up to 3 orders of magnitude) even accounting for use of up to 100% methanol as compared to 70% acetonitrile in the gradient elution. In addition, NEOs are more weakly retained than other chemical classes of pesticides including pyrethroids and strobilurin fungicides such that consideration of other pesticides present in samples or other co-eluting matrix components that require higher percentages of organic modifier in the mobile phase for elution should be made to avoid carry-over issues or excessive column flushing times between analysis [43, 48]. When methanol with 0.1% formic acid (under isocratic or gradient conditions) was used column choices that provided good resolution of NEOs included Agilent ZORBAX C18 (50 mm X 2.1 mm, 1.8 μm), Synergi Fusion RP (50 X 2 mm, 4 μm) and Kinetex biphenyl (100 mm X 4.6 mm, 2.6 μm) [33, 34, 37, 58]. Other column choices when an acetonitrile gradient was used (with 0.1% formic acid) included Acquity BEH C18 (50 X 2.1 mm,

Table 1. Liquid chromatography–mass spectrometry detection: ESI^+ SRM transitions of neonicotinoid insecticides.

Neonicotinoid insecticide	Empirical formula	Precursor ion (m/z)	Product ions (m/z) quantitative, confirmation	Reference
Acetamiprid (ACE)	$C_{10}H_{11}ClN_4$	223	126, 90	[6, 33-36]
		223	126, 56	[4, 36-42]
				[43]
		223	126, 56, 90	[44]
		223.14		[8, 45]
		223	126.00, 99.10	[46]
		223^{APCI+}	126	[47]
		223	126	[48-50]
		223, 225, 126	126, 187	[51]
		223, 126, 180		[52]
		223, 225		[53]
		223, 224, 225		
Clothianidin (CLO)	$C_6H_8ClN_5O_2S$	250	132, 169	[6, 33]
		250, 291**	169, 142, 132, 113	[54]
		250, 252	169, 132, 113, 99	[55]
		250	169, 132	[4, 7, 35, 36, 38, 39, 41, 42, 43, 48, 56]
		250^{APCI+}	169, 132	[46]
		250	169	[8, 47]
		250, 132, 169		[51]
		250, 251, 252		[53]
Dinotefuran (DIN)	$C_7H_{14}N_4O_3$	203	157, 129, 113	[43]
		203	129, 113	[33, 38, 39, 40, 42]
		203	129, 114	[6, 34]
		203, 244**	129, 157, 127, 114, 100	[54]
		203	129, 157	[32, 36, 41, 57]
		203	129, 87	[4]
		203	129	[8]
		203, 129, 113		[51]
		203, 204, 205		[53]
Flonicamid (FLO)	$C_9H_6F_3N_3O$	230	203, 148	[33]
		230	203, 174	[37, 38]
		230	203	[8]
Imidacloprid (IMI)	$C_9H_{10}ClN_5O_2$	256	209, 175	[4, 33- 37, 39- 42, 44, 59, 60]
		256	175, 209	[6, 43, 48]
		256, 297**	209, 84, 128, 175	[54, 61]
		256, 258	209, 175	[38, 55]
		256, 212,		[62]
		288^{APCI+}	209, 175, 84, 128	[59]
		$256, 258^{APCI+}$	209, 175	[46]
		256^{APCI+}		
		256	209, 239	[7]
		256	209	[8]
		256, 258, 209, 175		[49, 50]
		256, 175, 209		[51]
		256, 258		[52]
		256, 257, 258		[53]

Table 1 continued..

Neonicotinoid insecticide	Empirical formula	Precursor ion (m/z)	Product ions (m/z) quantitative, confirmation	Reference
Imidaclothiz (IMC)	$C_7H8ClN_5O_2S$	262	181, 131	[37]
Nitenpyram (NIT)	$C_{11}H_{15}ClN_4O_2$	271 271 271 271 271, 312** 271 271 271 271 271 271, 126, 225 271, 272, 273	126, 225 126, 224 99, 126 237, 224, 126 99, 225, 210, 196, 169, 126 225, 99 225, 189 189, 237 225, 56 225	[33] [34] [6] [37] [54] [4, 38] [39, 41] [36] [40] [8] [51] [53]
Paichongding (IPP)	$C_{17}H_{23}ClN_4O_3$	367.1415, 369.2381, 389.1897*	321.1314, 263.1577, 350.0735, 281.0630, 126.1909	[63]
Sulfoxaflor (XDE-208)	$C_{10}H_{10}F_3N_3OS$	278	174, 154 174, 105, 154	[4, 64] this work
Thiacloprid (THC)	$C_{10}H_9ClN_4S$	253 253 253 253^{APCI+} 253 253, 255, 126 253, 126, 186 253, 255 253, 254, 255	126, 186 126, 90 126, 99 126, 226 126	[33, 36-39, 41, 43, 48] [4, 6, 34, 42] [7, 35, 44] [46] [8] [49, 50] [51] [52] [53]
Thiamethoxam (THX)	$C_8H_{10}ClN_5O_3S$	292 292, 333** 292, 294 292 292 292^{APCI+} 292 292, 294, 244, 211 292, 211, 231 292, 294 292, 293, 294	211, 181 211, 246, 152, 132 211, 132, 99 211, 132 132, 181 129, 175 211	[4, 7, 8, 33-35, 37, 40, 42, 44, 48] [54] [55] [36, 38, 39, 43, 48] [57] [46] [8, 65] [49, 50] [51] [52] [53]
Acetamiprid-d_3	$C_{10}H_8D_3ClN_4$	226	126	[6, 34, 36]
Acetamiprid-d_6	$C_{10}H_5D_6ClN_4$	226	126	[33]
Clothianidin-d_3	$C_6H_5D_3ClN_5O_2S$	253 253 253	132, 172 172 172, 132	[33] [6, 8, 34, 36] This work
Dinotefuran-d_3	$C_7H_{11}D_3N_4O_3$	206	132	[6]

Table 1 continued..

Neonicotinoid insecticide	Empirical formula	Precursor ion (m/z)	Product ions (m/z) quantitative, confirmation	Reference
Imidacloprid-d_4	$C_9H_6D_4ClN_5O_2$	260 260 260	179 213 213, 179	[6] [8, 34] [35, 38, 39, 41-43]
^{13}C-Imidacloprid-d_3	$^{13}CC_8H_7ClD_3N_5O_2$	261	180, 214	[57]
Sulfoxaflor-d_3	$C_{10}H_7D_3F_3N_3OS$	281	177	[64]
Thiacloprid-d_4	$C_{10}H_5D_4ClN_4S$	296	215	[6]
Thiamethoxam-d_4	$C_8H_6D_4ClN_5O_3S$	296	215	[6]
Thiamethoxam-d_3	$C_8H_7D_3ClN_5O_3S$	295	214	[8, 34, 36]

*Na adduct; **ACN adduct.

Table 2. SRM transitions of degradation products of NEOs.

Metabolites or degradation products	NEOs	Empirical formula	Precursor ion (m/z) or SIM ions for MS	Product ions (m/z) quantitative, confirmation	Reference
6-CNA (CPM-3)	ACE, IMI, THC	$C_6H_4ClNO_2$	158, 190, 96** 158, 160 158 158 157.90 158 156^{ESI-}	122, 112 122, 78 77.90, 121. 122, 78 95 122, 78, 141, 112 112^{ESI-}	[62] [55] [45] [6] [57] [48] This work This work
AM-2	ACE	$C_9H_9ClN_4$	209.00 209 209 209	126.10 126, 90 126, 85 126, 167, 90	[45] [6, 33, 36] [7] This work
AM-2 d_3		$C_9H_6D_3ClN_4$	212	126	[6]
CPM-8	ACE	$C_8H_7ClN_2O_3$	215, 217		[45]
CM-1 (CLO-dm)	CLO	$C_5H_6ClN_5O_2S$	236, 238 236	 132, 155	[45] [56]
CM-7 (CLO-NH)	CLO	$C_6H_9ClN_4S$	205	132, 113	[56]
CM-8 (CLO-NNH_2)	CLO	$C_6H_{10}ClN_5S$	220.0456	146.97, 131.97, 113.01	[66]
	CLO	$C_6H_8ClN_5O_2$ (two identical compds different tr)	206, 208, 175, 177	132, 113	[55]
CM-11 (CL-urea)	CLO	$C_6H_8ClN_3OS$	206.0095 206	146.97, 131.97, 120.01 132, 120	[66] [56]

Table 2 continued..

Metabolites or degradation products	NEOs	Empirical formula	Precursor ion (m/z) or SIM ions for MS	Product ions (m/z) Quantitative, Confirmation	Reference
CTM-3	CLO, THM	$C_4H_2ClNO_2S$	164, 166		[45]
CTM-8	CLO, THM	$C_7H_8N_2O_3S_2$	233		[45]
CTM-11	CLO, THM	$C_2H_6N_4O_2$	119	73, 89	[56]
UF	DIN	$C_7H_{14}N_2O_2$	159	102, 67	[32, 57]
DINDN phosphate	DIN	$C_7H_{15}N_3O$	158	57, 102	[57]
TFNA-AM	FLO	$C_7H_5F_3N_3O$	191	148, 98	[33]
IM-1 (IMI, 5-hydroxy)	IMI	$C_9H_{10}ClN_5O_3$	272, 274 272, 274 272.14 272 272, 313**	 228, 228 190.99, 225.08 191, 225 225, 126, 144, 191	[45] [55] [57] [48] [61]
IMI-3 (4,5-hydroxy-IMI)	IMI	$C_9H_{10}ClN_5O_4$	288, 290 288, 329**	 207, 126, 169, 244	[45] [61]
IMI-4 (IMI-guanidine)	IMI	$C_9H_{11}ClN_4$	211.0750 211 212	84.0585, 158.0210, 126.0856 126, 84, 175, 194 127, 177	[66] [61] [36]
IMI-desnitro	IMI	$C_9H_{11}ClN_4$	211 211	126, 90 126, 90	[57] [36]
IMI-5 (IMI-hydrazone)	IMI	$C_9H_{12}ClN_5$	226.0898 226	209.05, 113.08, 96.05 126, 99, 100, 168, 190	[66] [61]
IMI-urea keto	IMI	$C_9H_{12}ClN_5$	226, 228	208, 169, 133	[55]
IMI-NH_2	IMI	$C_9H_{12}ClN_5$	226, 267**	126, 190, 168, 100, 99	[54]
IMI-6 (Nitrosoimine IMI or IMI-NO)	IMI	$C_9H_{10}ClN_5O$	240, 281** 240, 281**	209, 84, 126, 128, 175 209, 175, 128, 126, 84	[61] [54]
IM-7 (IMI-olefin)	IMI	$C_9H_8ClN_5O_2$	254, 256 254.07 254 254 254	 205.17, 171.07 236, 171 171, 205 236, 171, 205 (broad peak)	[45] [57] [36] [48] This work
IMI-9 (IMI-urea)	IMI	$C_9H_{10}ClN_30$	212, 253** 212.10 212 212	128, 99, 126, 195 128.10, 78.01 128, 99 128, 99, 126, 195, 78	[61] [57] [36] This work

Table 2 continued..

Metabolites or degradation products	NEOs	Empirical formula	Precursor ion (m/z) or SIM ions for MS	Product ions (m/z) Quantitative, Confirmation	Reference
IMI-10	IMI	$C_3H_6N_4O_2$	131.0523	86.05	[66]
IMI-11	IMI	$C_3H_4N_4O_2$	129.0479	84.05	[66]
IMI-15 (IMI-desnitro olefin)	IMI	$C_9H_9ClN_4$	209, 211 209, 250** 209 209.0521	126, 128, 99 126, 83, 173 126, 90 83.0410, 173.0832, 126.0840	[55] [61] [57] [66]
	IMI	$C_9H_6ClN_5O_2$	252	209, 84, 111, 126, 175, 194	[61]
IMI-N=CH_2	IMI	$C_8H_8ClN_4O_2$	238, 279*	209, 175, 126, 84	[54]
THC-amide	THC		271 271	126, 73 254, 228	[6] [36]
THM-urea (TM-4)	THM	$C_8H_{10}ClN_3O_2S$	248, 250, 175, 177	132, 99	[55]
THM-desmethyl	THM	$C_7H_8ClN_5O_3S$	278 278 278	132, 174 132 197, 132, 174, 167	[6] [65] This work
TM-5 (NG-A)	THM	$C_4H_8N_4O_3$	161.0675	119.0561, 147.0506, 102.0616	[66]
TM-6 (NG-B)	THM	$C_4H_9N_3O$	116.0821	102.06, 87.04	[66]
TM-8 (TM-dm-NNO)	THM	$C_7H_8ClN_5O_2S$	262.0154	197.09, 131.97	[66]
TM-13 (NG-C)	THM	$C_3H_6N_4O_3$	147.0518	87.0417, 130.0459, 102.0621	[66]
TM-14 (NG-D)	THM	$C_3H_7N_3O$	102.0678	87.04	[66]
M1	IPP	$C_{14}H_{17}ClN_4O_3$	325, 327	279, 264, 196, 153, 126, 295	[63]
M2	IPP	$C_{14}H_{19}ClN_4$	279, 281	264, 208, 153, 193, 236, 126	[63]
M3	IPP	$C_{14}H_{18}ClN_3$	264, 266	246, 236, 126, 196	[63]
M4	IPP	$C_{17}H_{23}ClN_4O_2$	351, 353	247, 333, 319, 196, 166	[63]
M5	IPP	$C_{17}H_{24}ClN_3O_2$	338, 340	278, 236, 126, 196, 169	[63]
M6	IPP	$C_{16}H_{22}ClN_3O$	308, 310	290, 264, 244, 280, 166, 126	[63]
M7	IPP	$C_{16}H_{22}ClN_3O_2$	324, 326	306, 196, 264, 137, 126, 153	[63]
X1171974	XDE-208	$C_{10}H_{12}F_3N_3O_2S$	296	174, 105	[64]

Table 2 continued..

Metabolites or degradation products	NEOs	Empirical formula	Precursor ion (m/z) or SIM ions for MS	Product ions (m/z) Quantitative, Confirmation	Reference
X11519540	XDE-208	$C_9H_{11}F_3N_2OS$	254	175, 154	[64]
X11579457	XDE-208	$C_9H_{10}F_3NO_2S$	253	174, 154	[64]
^{13}C d_3 of X11719474	XDE-208	$^{13}CC_9D_3H_9F_3N_3O_2S$	300	178	[64]

*Na adduct; **ACN adduct.

1.7 μm), Agilent SB-C18 (100 mm X 2.1 mm i.d., 1.8 μm), Waters Symmetry Shield™ (150 mm X 2.1 mm, 3 μm), Waters ZORBAX Eclipse XDB-C8 (100 mm X 2.1 mm i.d., 1.8 μm), Zorbax SDB C18 (100 X 2 mm, 3.5 μm), and Kinetex® C18 (150 mm X 4.6 mm i.d., 2.6 μm) [4, 35, 38-40, 49, 51]. Acetic acid (0.01%) was also used with methanol gradient with a Kinetex phenyl-hexyl (100 X 2.1 mm, 2.6 μm) column [48]. Zorbax C8 (4.6 mm X 75 mm, 3.5 μm) can also be used under isocratic conditions with acetonitrile with 0.1% formic acid [64]. Nitenpyram exhibited the largest reduction in sensitivity of the NEOs when 10 mM ammonium acetate (no formic acid) was added to the mobile phase [41]. However, ammonium acetate (5 mM or 10 mM) with 0.1% formic acid in the aqueous phase has been used with an acetonitrile gradient with a column choice of Synergi Polar-RP (50 X 2 mm, 2.5 μm), Atlantis T3 (100 X 2.1 mm, 3 μm), or ODS-AQ column (100 mm X 2.1 mm, 3 μm) [6, 8, 43]. Ammonium acetate (2 mM or 10 mM) with 0.1% formic acid in aqueous mobile phase has also been used for methanol gradient with column choice of Kinetix biphenyl (100 mm X 4.6 mm, 2.6 μm) or BDS Hypersil C-18 (250 mm X 2.1 mm, 5 μm) [34, 44, 58]. Under the same gradient conditions for separation shown in figure 1 the presence of ammonium acetate (5 mM) in the aqueous mobile phase resulted in a decrease in retention of all analytes, improvement in peak shape of early eluting analytes, and in general a small increase in sensitivity except for dinotefuran and 6-chloronicotinic acid. Nitenpyram MS response was approximately equal with or without ammonium acetate. Dikma Technolgies Inc. C18 column (250 mm X 4.6 mm, 5 μm) with an acetonitrile gradient was used for the analysis of paichongding (a new NEO not commercially available yet) [63]. Retention characteristics of NEOs on different C18 columns can vary. For example, Waters Acquity HSS T3 (100 mm X 2.1 mm i.d., 1.8 μm) provided adequate retention for nitenpyram and resolution from thiamethoxam, while under similar mobile phase conditions Waters ACQUITY BEH SHIELD RP 18 (100 mm X 2.1 mm i.d., 1.7 μm) column did not provide adequate resolution between nitenpyram and thiamethoxam [41]. Addition of 0.1 % formic acid to the mobile phase was more critical to MS sensitivity than addition of 2-10 mM ammonium acetate. Selected NEOs including ACE, CLO, IMI, THC, and THM analyzed by LC-APCI$^+$-MS/MS also used acetonitrile rather than methanol as organic modifier in the mobile phase [46, 59].

When metabolites were included with selected NEOs in the analysis column choices included Kinetex® (150 mm X 4.6 mm, 2.6 μm), Luna C18 (100 X 2 mm, 2.6 μm) and Luna PFP column (50 X 2.1 mm, 100 Å, 3 μm) with acetonitrile gradient [7, 36, 66]. AM-2 and ACE observed better separation efficiency using Phenomenex Luna PFP (50 mm X 2.1 mm, 3 μm) with acetonitrile gradient [45]. For LC-APCI$^+$-MS/MS a mobile phase containing methanol with 0.1% formic acid was used for the analysis of major metabolite 6-CNA with a Discover C18 column (25 cm X 4.6 mm, 5 μm) [62]. The LC-ESI$^+$-MS/MS analysis of 6-CNA and other IMI metabolites (as well as dinotefuran metabolites) was accomplished with a Acquity HSS T3 column (100 mm X 2.1 mm, 1.8 μm) with both

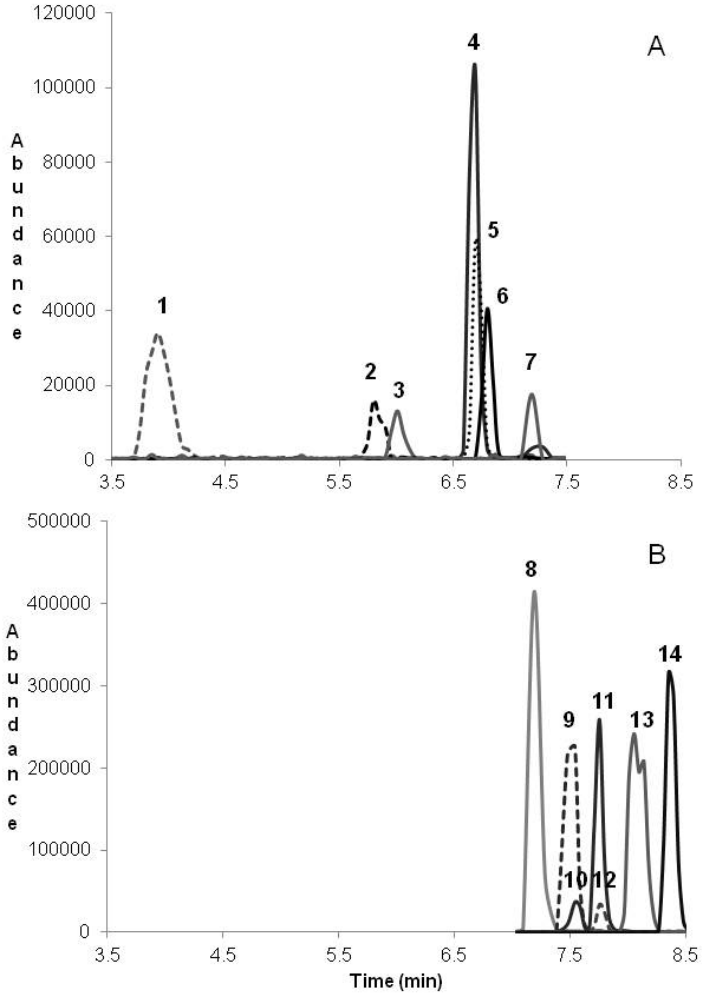

Figure 1. Selected reaction monitoring chromatogram of neonicotinoids and selected metabolites of neonicotinoids standard mixture. Analytes (quantitative SRM transition): **A**, 1. dinotefuran (203 > 157); 2. nitenpyram (271 > 99); 3. 6-chloronicotinic acid (158 > 122); 4. imidacloprid-urea (213 > 128); 5. imidacloprid-olefin (254 > 205); 6. thiamethoxam (292 > 211); 7. clothianidin (250 > 169); **B**, 8. N-desmethyl acetamiprid (209 > 126); 9. imidacloprid (256 > 175); 10. imidacloprid-d_4 (260 > 213); 11. N-desmethyl thiamethoxam (278 > 132); 12. acetamiprid (223 > 90); 13. sulfoxaflor (278 > 174); 14. thiacloprid (253 > 126). Injection of 10 µL of mixture of 50 ng/mL neonicotinoids, 100 ng/mL of metabolites of neonicotinoids, and 50 ng/mL of imidacloprid-d_4 in methanol with 0.2% formic acid. Abundance of SRM 213 > 128 and 209 > 126 full scale; abundance of all other SRM transitions expanded 10 fold for scaling of figure. Column: Synergi polar-RP column (50 mm X 2.0 mm i.d., 2 µm) with guard column (4.0 mm X 2.0 mm) C_6-phenyl Gemini). Initially the column was equilibrated with 20 v% acetonitrile with 0.1% formic acid and 80 v% aqueous phase with 0.1% formic acid and 5 mM ammonium acetate at a flow rate of 0.15 mL/min. Mobile phase gradient of acetonitrile with 0.1% formic acid was changed linearly as follows: 0 min, 20%, 3 min, 40%; 5.0-8.0 min, 60%, 9.0 min, 70%.

0.1% formic acid and 5 mM ammonium acetate as additives to methanol gradient system [57]. In another separation of IMI metabolites excluding 6-CNA, better sensitivity was found with only 0.1% formic acid as additive to the mobile phase with an acetontrile gradient on a Luna C18 column [36]. For CLO and two of its major metabolites separated on a YMC-Triart C18 (150 X 3 mm i.d., 3 µm) both 0.1% formic acid and 10 mM ammonium formate were used as additives to the aqueous phase with a gradient of acetonitrile [56]. As shown in figure 1 some metabolites

such as N-desmethyl acetamiprid and imidacloprid-urea are very sensitive relative to other target analytes in the presence of acetonitrile with 0.1% formic acid and 5 mM ammonium acetate. Methanol mobile phases have also been used for separation and identification of metabolites with LUNA C8 column with 0.1% formic acid [61, 67]. Based on this work, 6-CNA and dinotefuran have better MS sensitivity in the absence of ammonium acetate; however IMI-urea and ACE-desmethyl observed up to a 10-fold improvement in sensitivity when ammonium acetate was added. 6-CNA has an amphiprotic structure and gives similar response in positive and negative mode [38] but analysis is often completed with ESI in positive mode [6, 34, 45, 48, 55, 57, 58, 62] as most other NEOs and metabolites are more sensitive with ESI^+. In addition with ESI^+ our work shows a second sensitive selected reaction monitoring (SRM) transition 158→78 (see table 2) is available, whereas with ESI^- no sensitive confirmation SRM transition is available ($[M-H]^- \rightarrow 35$ is very weak). All NEOs and metabolites were separated with mobile phases containing 0.1% formic acid as this improve MS sensitivity and can avoid peak splitting issues. 6-CNA can exhibit peak splitting under neutral pH conditions, but peak splitting was not apparent when samples (or standards) were acidified prior to injection with 2.5 µL of formic acid [34, 58]. Table 3 shows that sample extracts are generally reconstituted after the final drying step in sample preparation procedures with methanol, acetonitrile, or in combination with an aqueous phase similar to conditions for the start of the separations. To incorporate metabolites into existing LC-ESI^+-MS/MS for NEOs it is important to consider the chromatographic resolution provided under conditions of higher aqueous content when many of the metabolites elute and additionally whether samples are analyzed for other pesticide classes that typically will have stronger retention than NEOs. Specialty reversed phases such as T3 (trifunctional C18 alkyl phase), SB C18 (sterically protected siloxane bond), biphenyl, polar RP (ether linked phenyl with polar endcapping), PFP (pentafluorophenyl bonded in silica surface) phases or other protective/endcapping choices may provide adequate selectivity and reduced peak broadening for target analytes, particularly those with shorter retention times that suffer more from peak broadening.

3. Tandem mass spectrometry fragmentation patterns

With electrospray ionization in positive ion mode NEOs and their metabolites form the protonated molecular ion $[M+H]^+$ which is always selected as the precursor ion for quantitative and confirmation analysis (see tables 1 and 2). MS^2 fragmentation patterns for selected NEOs and metabolites have been proposed [38, 41, 42, 55, 61, 66]. Loss of NO_2 and Cl radical are commonly observed in fragmentation patterns of NEOs or some metabolites [38, 41, 42, 55, 61, 66]. Some care should be taken with the proposed structures of fragment ions as some errors exist in charges and m/z calculations within these references. THM and CLO produce fragment ions at m/z of 211 and 169 corresponding to $[M+H-Cl-NO_2]^+$ [38, 42, 55]. Both imidacloprid and nitenpyram undergo fragmentation to $[M+H-NO_2]^+$ corresponding to m/z = 209 and 225, respectively [38, 41, 60]. Subsequent loss of Cl radical from the fragment ion at m/z = 209 ion produces an ion at m/z = 175, while only neutral loss of $ClC_5NH_3CH_3$ from alpha cleavage at tertiary nitrogen of the m/z = 175 ion produces m/z = 99 $[C_5H_{10}N_2]^+$. Nitenpyram also fragments to $[M+H-NO_2-Cl]^+$ resulting in an ion at m/z = 189 [41]. Dinotefuran exhibits loss of NO_2 and radical loss of NCH_2 to produce an ion at m/z = 129 with subsequent loss of NH_2 to produce an ion at m/z = 113 [38]. The fragment ion at m/z = 114 was attributed to loss of NH rather than NH_2 [68] but this is less likely and m/z = 113 is more commonly observed in the daughter ion scan (see table 1). Dinitrofen fragment ion at m/z = 157 is due to loss of NO_2 [41]. Alpha-cleavage at the tertiary nitrogen produces a common fragment ion at m/z = 132 for CLO and THM. Acetamiprid and thiacloprid undergo fragmentation to produce an ion at m/z = 126 corresponding to $ClC_5H_3NCH_2^+$ from alpha-cleavage at a tertiary nitrogen [38, 42]. Subsequent loss of HCl for this m/z = 126 fragment ion produces m/z = 90 which has been observed in the daughter ion scan of the protonated molecular ion of thiachloprid [42]. Thiacloprid also exhibits fragmentation to $[M+H-S-Cl]^+$ corresponding to an ion at m/z = 186 [38, 41]. Several MS^2 fragments

Table 3. Sample clean-up approaches for methods specific to the analysis of neonicotinoid insecticides and their metabolites.

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Drinking water	ACE, IMI, THC, THM	100 mL or 1000 mL	SPE LiChrolut EN 200 mg, eluted 3 mL EA/MeOH 50:50 v/v%; dried, re-dissolved 0.01% acetic acid in 40/60 MeOH/H_2O.	LC-ESI^+-MS	[50]
Surface water	ACE, CLO, DIN, FLO, IMI, NIT, THM Metabolite: 6-CNA	100 µL	Direct aqueous injection.	LC-ESI^+-MS/MS	[34]
Water	XDE-208 (sulfoxaflor) and metabolites	10 mL	Add 100 µL 1.0 N HCl and mix. Oasis HLB (60 mg, 3 mL), wash with 1 mL 0.01 N HCl, elute with two 500 µL ACN/H_2O (80:20) with 0.1% HCOOH, dilute to 2 mL with H_2O/ACN (95:5) with 0.1% HCOOH.	LC-ESI^+-MS/MS	[64]
Water	CLO, IMI, THM Metabolites: hydroxyl-IMI, urea-keto-IMI, desnitro olefin-IMI 6-CNA, THM-urea and 2 unnamed	100 mg/L NEOs with immobilized TiO_2 as photocatalyst	Sample preparation only includes irradiated samples.	LC-ESI^+-MS	[55]
Wine	ACE, CLO, IMI, THC, THM	10 mL or 2 mL wine, diluted with equivalent volume H_2O	SPE Oasis HLB (200 mg or 60 mg), eluted with 2 mL ACN. Florisil (total elution volume 5 mL ACN), dried and reconstituted with 1 mL ACN, diluted 1:1 for analysis.	LC-ESI^+-MS/MS	[35]
Serum and Urine	ACE, CLO, DIN, FLO, IMI, NIT, THC, THM Metabolites: AM-2, 5-AMAM-2-CP, 5-AAM-2-CP	1 mL sample diluted with 2 mL H_2O	SPE Extrelut® NT3, elute with 10 portions of 3 mL chloroform/2-propanol (3:1 v/v), dried, reconstituted in 100 µL MeOH and 900 µL of 4 mM NH_4Ac-0.1% HCOOH buffer (pH 3).	LC-ESI^+-MS/MS	[72]
Urine	ACE, CLO, IMI, NIT, THC, THM Metabolite; AM-2	50 mL collected frozen and thawed prior to analysis of 1 mL	WAX SPE (225 mg, 60 µm, 2 mL volume), wash step with 2 mL 0.2 M NaOH, 2 mL 0.1 M sodium phosphate buffer (pH 7.4) and 2 mL H_2O. Elution with 2 mL ACN, dried and reconstituted with 100 µL H_2O/ACN (97/3 v/v).	LC-ESI^+-MS/MS	[7]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Urine	ACE, CLO, DIN, IMI, THC, THM in Fraction A NIT fraction B	1 mL urine, 1 mL 2% phosphate solution and IS	Incubate diluted sample at 37 °C for 10 min, SPE Bond Elut PCX, 30 mg, load 2 mL diluted sample and wash with 0.5 mL 2% HCOOH, elution of fraction A with 0.5 mL MeOH; fraction B eluted with 0.5 mL MeOH/ACN 1:1 v/v with 5% NH_3, dried, reconstituted with 170 µL ACN. Sonicate and centrifuge prior to analysis.	LC-ESI^+-MS/MS	[73]
Urine (24 hr pooled)	ACE, CLO, DIN, IMI, NIT, THC, THM Metabolites: Desmethyl-ACE, desmethyl-THM, THC amide	1 mL urine	Column clean-up: diatomite column (InertSep K-solute, 2 mL) eluted with 25 mL CCl_2H_2, dried to 1 mL. Supelclean ENVI-Carb-II/PSA SPE, 500 mg, eluted with 10 mL 20% v/v CCl_2H_2/ACN (20/80 v/v), dried, reconsituted in 30/70 MeOH/ H_2O v/v.	LC-ESI^+-MS/MS	[6]
Urine from humans and mice	ACE, CLO, IMI Metabolites: AM-2, IMI-1; IMI-3, IMI-7, CM-1, CPM-3, CPM-8, CTM-3, CTM-8 and others	20 µL of diluted urine	Centrifuged at 15000 rpm for 10 min and filtered. Human urine diluted 2-fold and mouse urine diluted 5-fold with 5% ACN/H_2O, followed by adjusting pH to have aliquots that are acidic, basic and neutral. SPE (Plexa, 30 mg, 40 µm). Neutral SPE 20 µL with 480 µL H_2O for loading, wash with 1 mL H_2O and elute with 500 µL ACN; dried and reconstituted with 5% ACN/H_2O with 0.1% HCOOH; acidic aliquot: for SPE added 1.25 µL and 2.50 µL of HCOOH during loading of mice and human urine samples; basic aliquot: for SPE added 10 and 20 µL of 25% NH_4OH to mice and human urine samples during loading.	LC-TOFMS (+ and – ionization)	[45]
Urine	6-CNA derivatized with HFIP	3 mL urine	Frozen and thawed, added H_2SO_4 (5 M) and heated at 90 °C for 1 h and cooled. Clean-up with Amberlite XAD-4, wash with 2 mL H_2O (pH 1.3) and 2 mL hexane followed by elution with 7 mL diethyl ether and passed through Na_2SO_4. Dried and reconstituted in 1 mL hexane. Derivatization with 10 µL HFIP (1,1,1,3,3,3-hexafluoropropan-2-ol) followed by 15 µL of DIC (diisopropyl-carbodiimide). Neutralized excess derivatization agent with 5% K_2CO_3 (aq). Organic layer analyzed.	GC-EI MS/MS	[69]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Urine	6-CNA, 2CTCA, 3FA	2 mL urine	Added 50 µL H_2SO_4, heated to 86 °C for 2 h followed by centrifugation for 10 min. Clean-up of supernatant by SPE, wash with 500 µL 2% HCOOH and elution with 500 µL MeOH. Eluant divided into two fractions and dried, and reconstituted with 250 µL ACN for 2CTCA and toluene for 6-CNA and 3FA followed by derivatization with 40 µL BSTFA-TMCS (or HFIP or trimethylsilyldiazomethane).	GC-EI MS/MS	[70]
Air sorbent materials for gas phase collection Coverall PPE Urine	ACE	Chromosorb 102, 125 mg Coverall pieces 3 mL	Sonication with EA for 20 min, 3 times repeated, dried extract, reconstituted in 10 mL MeOH/H_2O 50:50 v/v%. Extracted using shaker, 30 min at 30 rpm, with 250-350 mL MeOH/H_2O 50:50 v/v%. SPE C18 500 mg, 2 X 4mL H_2O for clean-up followed by elution of desired fraction with 3 mL MeOH, dried to 1.5 mL and 1.5 mL H_2O added.	LC-ESI^+-MS/MS	[47]
Particulate matter air sample	ACE, CLO, DIN, IMI, NIT, THC, THM	Glass fiber filters	Pressurized solvent extraction, cell size 30 mL, EA 1.5 times cell volume, dried, reconstituted with MeOH and IS for clean-up. SPE C18 1000 mg, 250 µL sample diluted with 750 µL H_2O for SPE loading step, and wash with 1 mL 20/80 MeOH/H_2O, elution with 5 mL MeOH, dried to ~1 mL.	LC-ESI^+-MS/MS	[43]
Soil or dust from Planter Exhaust	CLO and THM	10 g diluted with H_2O 200 X	Sifted for size of particles, diluted with water. 20 mL aliquot extracted with 20 mL ACN, then addition of $MgSO_4$, 1 g NaCl, 1 g Na_3citrate tribasic, 0.5 g NaHcitrate sesquihrate. 4 mL of ACN phase then dried and reconstituted with 1 mL of 1:1 MeOH:H_2O (v/v) with 5 mM HCOOH.	LC-ESI^+-MS/MS	[71]
Soil	IMI Metabolites: 6-CNA, IMI-olefin, IMI-nitrosimine, IMI-urea, IMI-5-hydroxy, nitroguanidine	15 g	QuEChERS method: Add 30 mL ACN and homogenize, centrifuge at 15000 rpm, add 10 g NaCl and rotospin for 5 min, centrifuge at 2500 rpm for 3 min to separate. Organic layer removed and 10 g activated Na_2SO_4 added followed by rotospin for 2-3 min. Transfer of 6 mL organic phase for clean-up. 0.15 g PSA, 0.9 g activated anhydrous $MgSO_4$ and 0.05 g GCB added, vortexed for 30 s followed by centrifugation for 1 min at 2500 rpm, top 4 mL transferred and dried to 2 mL.	LC-PDA	[20]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Soil cocoa plantation		5 g	QuEChERS method: to sample add 5 mL H_2O and mix followed by addition of 10 mL ACN with 1% CH_3COOH, shake for 1 min. Additives for QuEChERS methods: 1) 4 g $MgSO_4$, 1.5 g NaOAc; 2) 4 g $MgSO_4$ and 1 g NaCl; 3) 4 g $MgSO_4$, 1 g NaCl, 1 g SCTD and 0.5 g SCDS. Shake for 1 min and centrifuge. Clean-up dSPE additives: 1) 25 mg PSA + 150 mg $MgSO_4$; 2) 25 mg of PSA and C-18 and 150 mg $MgSO_4$; 3) 25 mg PSA + 7.5 mg GCB (+150 mg $MgSO_4$). Shake and centrifuge, followed by dilution of organic layer 1:1 with H_2O.	LC-ESI^+-MS/MS	[44]
Soil	IPP and degradation products	1 g	Extracted with 50 mL 0.01 M $CaCl_2$ and then pH adjusted to 3 and extracted with equivalent volume of ACN/CCl_2H_2 (1:1 v/v) 3 times. The ACN/dichloromethane phase merged, with other extractions, with ACN/H_2O, MeOH, and CCl_2H_2 phase. Dried and reconstituted in 1 mL ACN.	LC-ESI^+-MS/MS	[63]
Soil, plants, and pollen		20 g soil 10 g plants and flowers 10 g pollen	Soil: mixed with MeOH/NH_4OH 0.05% (3:1 v/v). Add 2 g celite and then vacuum filter with 2 g wetted celite on a glass frit; dried and rinsed with 15 mL CCl_2H_2, dried, reconstituted in ACN/H_2O 50/50 and centrifuged. Plants: Grinding with 100 mL MeOH/H_2SO_4 0.04% (4:1 v/v), 2 g celite and vacuum filtered as above, dried to 5-10 mL, water diluted to 15 mL with H_2O and centrifuged; C18 SPE clean-up, elute with CCl_2H_2, partially dried and diluted to 1 mL with ACN/H_2O and centrifuged. Pollen: 20 mL of EtOH/H_2O (3:1) mixed in Ultra-Turax blender for 1 min. centrifuged, rinsed with EtOH/H_2O, combined extract dried to 5 mL with 10 µL buffer added (pH 7 Na_2CO_3/CH_3COOH), repeat washing twice and combine washes. Extraction of combined washes with 20 mL CCl_2H_2. Organic layer removed and dried, reconstituted in 2 mL hexane and diluted with 1 mL ACN/H_2O followed by centrifugation.	LC-$APCI^+$-MS/MS	[59]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Bovine tissue	ACE, CLO, DIN, IMI, NIT, THC, THM	2.5 g bovine tissue with 2 g diatomaceous earth	Pressurized solvent extraction, 11 mL cell, sample extracted with H_2O, 80 °C, 5 min, 2 static cycles, volume solvent 22 mL. Extract cooled at -20 °C for 15 min then centrifuge at 3800 rpm for 5 min. Oasis HLB SPE, 500 mg, wash with 5 mL MeOH/H_2O 20/80 v/v%, elute with 3 mL MeOH.	LC-ESI+-MS/MS	[39]
Eels	ACE, CLO, DIN, IMI, NIT, THC, THM	2.5 g 2.5 g 2.5 g	Add 2.0 g diatomaceous earth to sample. Pressurized solvent extraction, 11 mL cell, extracted with H_2O, 120 °C, 5 min, 2 static cycles, volume extract 22 mL. Ultrasonic extraction, extraction solvent was ACN (15 mL). Sonicate for 30 min. followed by centrifugation at 8000 rpm for 5min. Sample re-extracted and extracts combined. Cool extract to -20 °C for 2 h, centrifuge at 4 °C, dry and re-dissolved in 5 mL 20/80 v/v% MeOH/H_2O. Extraction with 2 X 15 mL ACN with shaking at 3000 rpm for 30 min, centrifuge at 8000 rpm for 5 min at 4 °C, supernatant cooled to -20°C for 2 h, centrifuge, dry and reconstitute in 5 mL 20/80 v/v% MeOH/H_2O. All types of extracts undergo SPE using Oasis HLB 200 mg, wash with 5 mL water, 5 mL MeOH-H_2O, and elute with 3 mL MeOH, dried, reconstituted in 5 mL 20/80 v/v% MeOH/H_2O.	LC-ESI^+-MS/MS	[38]
Bee Pollen	ACE, CLO, DIN, IMI, NIT, THC, THM	2 g	Solid-liquid extraction with 10 mL CH_2Cl_2, vortex for 10 min, centrifuge for 10 min. Extract dried, re-dissolved in 1 mL 50/50 v/v ACN/H_2O.	LC-ESI^+-MS	[51]
Honey and bee pollen	IMI IMI metabolites: olefin, 5-hydroxy, urea, desnitro olefin, desnitro HCl, 6-CNA	15 g mixed with 12 mL H_2O	Modified QuEChERS: Mixed with 15 mL triethylamine in ACN with tissuemizer for 3 min. Salting-Out: Add 6 g $MgSO_4$ and 1.5 g NaOAc, shaken for 2 min and centrifuged. Organic phase removed, 0.5 g $MgSO_4$ added and shaken. 12 mL extract for C-18 SPE clean-up (1 g), eluted with 2% TEA/ACN, dried and re-dissolved in 1 mL ACN/H_2O 3:1.	LC-ESI^+-MS/MS	[57]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Honey and bee pollen	DIN DIN metabolite: UF	2 g diluted with 5 mL H_2O	QuEChERS: addition of 5 mL ACN/CH_3COOH (99/1 v/v), vortex for 5 min, followed by addition of 4 g $MgSO_4$ and 1.5 g NaOAc and vortex for 1 min, centrifuge for 5 min. Supernatant clean-up with 50 mg PSA, 50 mg C18, 10 mg GCB and 150 mg $MgSO_4$ for pollen and 50 mg PSA, 50 mg C18 and 150 mg $MgSO_4$ for honey, followed by vortexing, centrifugation and filtration of organic phase.	SFC-MS/MS Enantiosepartion with post-column addition of 0.1% HCOOH in MeOH	[32]
Honey Liqueur		5 mL 15 mL	DLLME using 0.5 mL ACN as dispersive solvent and 2.0 mL CCl_2H_2 as extraction solvent. Vortex for 1 min, sonicate for 10 min, dried, re-dissolved with 0.5 mL MP: 10% ACN, 90% 0.1% HCOOH in H_2O. QuEChERS: ACN salt out with the buffer salts added (4000 mg $MgSO_4$, 1000 mg NaCl, 500 mg citrate dibasic sesquihydrate, and 1000 mg sodium citrate tribasic dehydrate) followed by vortexing for 10 min and subsequent centrifugation at 3000 rpm. Aliquot (1.5 mL) of ACN layer transferred and dried, reconstituted in 0.15 mL mobile phase.	LC-ESI^+-MS/MS	[40]
Honey Pollen	CLO, IMI, THM Metabolites: TM-5, TM-6, TM-8, TM-13, TM-14, CM-8, CM-11, IMI-4, IMI-5, IMI-10, IMI-11	5 g honey dissolved in 10 mL H_2O 2 g pollen	Honey: SPE Clean-up with Strata X 33 µm, 250 mg. Wash with 2 mL MeOH:H_2O 7:3 v/v, elution with 2 mL ACN:H_2O 45:55 v/v. Pollen: Add 19 mL CCl_2H_2, shake for 10 min, then centrifuge. Organic phase removed and filtered, dried and reconstituted with 1 mL ACN/H_2O 50:50 v/v and filtered.	LC-ESI^+-QTOF	[66]
Honey	ACE, IMI, THC, THM	5 g honey mixed with 20 mL H_2O	SPE with Extrelut-NT 20 (20 mL, diatomaceous earth) of high pore volume. Elute with five 20 mL portions of CCl_2H_2, dry and re-dissolve in 1 mL MeOH.	LC-ESI^+-MS	[52]
Honey	ACE, CLO, DIN, FLO, IMI, NIT, THC, THM Metabolites: IM-2-1, TFNA-AM	1 g honey diluted with 10 mL water	Add 20 mL MeOH, homogenize for 1 min and filter. Wash filter with 20 mL 75:25 MeOH/H_2O. Dried to remove organic solvent such that aqueous portion remains. Subsequent clean-up on ChemElut 1010 column. Target analytes eluted with 80 mL cyclohexane/ethyl acetate (50:50). Dried and reconstituted in 1 mL MeOH/H_2O (20:80).	LC-ESI^+-MS/MS	[33]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
		5 g honey diluted with 10 mL H_2O	QuEChERS: Add 10 mL CAN, homogenize. Add 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3citrate dehydrate, 0.5 g Na_2Hcitrate, shake for 1 min, centrifuge for 5 min. 6 mL aliquot of organic phase cleanup with dSPE using 900 mg $MgSO_4$ and 150 mg PSA. 2 mL supernatant dried and reconstituted in 1 mL MeOH/H_2O (20:80).		
Honey	ACE, CLO, IMI, THC, THM	2 g mixed with 10 mL	SPE using Discovery DSC-18Lt, 500 mg. Elute with 1.5 mL ACN. DLLME procedure: 1.5 mL extract added to mixture of 10% w/v NaCl and 100 µL $CHCl_3$. After extraction centrifuge and collect organic phase. Organic phase dried and reconstituted in ACN 50 µL.	LC-APCI$^+$-MS/MS	[46]
Honey bee honey	ACE, CLO, DIN, IMI, THC, THM Metabolites: AM-2, IMI-guanidine, IMI-urea, IMI-olefin, IMI-desnitro, THC-amid	2 g honey bee 2 g honey	Honey bee frozen with liquid nitrogen for 10 min followed by incubation in dark at 4 °C for 30 min. 8 mL ACN/EA (8:2 v/v) added and homogenized with vortex mixer 1 min and ultrasonic bath 15 min followed by centrifuging mixture. Filtration of supernatant with Sep-Pak Alumina N Plus without preconditioning, followed by drying and reconstitution with 300 µL H_2O. Sample filtered prior to analysis. Honey: incubated as above and then 10 mL ACN/EA (8:2 v/v) added and 200 µL of 20% v/v TEA/ACN. Homogenized and sonicated followed by centrifugation as above. Cleanup of supernatant with Strata X-CW with water wash and elution with 2 times 3 mL ACN/EA (8:2) followed by eluant drying and reconstitution in 250 µL water and filtering.	LC-ESI$^+$-MS/MS	[36]
Beebread	ACE, CLO, IMI THC, THM Metabolites: 5-hydroxy-IMI, IMI olefin, 6-CNA	2 g	Diluted with 5 g water and 5 mL hexane. Modified QuEChERS: 10 mL ACN with 2% TEA, shaken, added $MgSO_4$ and acetate buffer shaken, centrifuged. ACN layer incubated for 15 h at -18 °C, 6 mL portion transferred for dSPE with 150 mg PSA and 900 mg $MgSO_4$. Supernatant dried and reconstituted in MeOH and final dilution of 40 µL sample/160 µL water.	LC-ESI$^+$-MS/MS	[48]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Beeswax	ACE, CLO, DIN, IMI, NIT, THC, THM	2 g homogenized	Dissolved with 15 mL n-hexane/isopropanol mixture 8:2 v/v and heated 3 min at 50 °C, then add 10 mL H_2O and centrifuge at 700 rpm, 50 °C for 5 min. Aqueous phase (10 mL) loaded onto Isolute® HM-N diatomaceous earth cartridge and eluted with 20 mL acetone, dried, re-dissolved in 1 mL ACN/H_2O 50/50 v/v% and filtered.	LC-ESI$^+$-MS	[53]
Beeswax	ACE, CLO, DIN, IMI, NIT, THC, THM	5 g spiked with NEOs diluted with 25 mL of 0.25% oxalic acid (aq)	2 g homogenized beeswax and 15 mL hexane/isopropanol (8:2 v/v) heated to 50 °C for 3 min. Aqueous phase clean-up with diatomaceous earth cartridge eluted with 20 mL acetone. Dried, reconstituted with 1 mL ACN/H_2O (50:50 v/v).	CE-MS/MS	[9]
Rabbit hair Rabbit urine Human hair	IMI, 6-CNA	50 mg hair 0.5 mL urine	Add IS and 2 mL MeOH, sonicate for 3 hrs, repeat, filter, dry, reconstitute in 100 µL MeOH. Add IS and dilute with 1.5 mL H_2O and 2 mL CCl_2H_2, shake for 10 min, centrifuge at 4000 rpm for 5 min, remove CCl_2H_2, add 10 µL 6 M HCl to aqueous phase, repeat extraction with CCl_2H_2. Combine CCl_2H_2 fractions, dry, re-dissolve in 100 µL MeOH.	LC-APCI$^+$-MS	[62]
Water and protein	CLO, DIN, IMI, NIT, THM Metabolites: IMI-NO, IMI-NH_2, IMI-N=CH_2, NIT-NO, NIT-CN, dm-THM, THM-N-CH_2, dmTHM-NO, dmTHM-NH_2, CLO-NO, CLO-NH_2, DIN-NO, DIN-NH_2	Kinetic measurements 50 µM neonics in aqueous solution with protein at 2 and 5 mg/mL	Liquid/liquid extraction with EA, dried, reconstituted in ACN/0.1% trifluoroacetic acid (25:75) and filtered.	LC-ESI$^+$-MS/MS	[54]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Human liver microsomes	IMI and IMI metabolites: Olefin, hydrazone, guanidine, triazolone, nitrosoimine, 4,5-dihydroxyl, urea, 5-hydroxyl	2.5 mg of protein	Sample incubated with [3H]IMI (395 ng) and NAPPH (0 or 1.5 mM) in pH 7.4 100 mM phosphate buffer (155 µL) for 120 min at 37 °C. 1. Deproteination by addition of ACN (310 µL) followed by concentration and addition of CH_3COOH for 3:7 ACN/H_2O with 0.1% CH_3COOH. 2. Treat with NaOH and HCl with extraction with CCl_2H_2 and chloroform/ACN (5:2).	LC-ESI^+-MS/MS	[61]
Mouse and liver microsome	IMI, THM metabolites: 5-hydroxylIMI 4,5-dihydroxylIMI IMI-NH 4-hydroxylTHM		Homogenate prepared by sonic dismembrator followed by vortex, ACN extraction, dried, reconstituted in 75:25:0.1 ACN/H_2O/trifluoroacetic acid (300 µL).	LC-ESI^+-MS	[67]
Larvae of tobacco budworm, *Heliothis virescens*, and fall armyworm, *S. frugiperda* (Lepidoptera: Noctuidae), and green peach aphid, *Myzus persicae* (Hemiptera: Aphididae) Cotton plants	CLO, IMI, THM Metabolites: N-methyl IMI, N-desmethyl THM	Haemolymph of 3 larvae combined and mixed with equal volume of MeCN Cotton leaves	Larvae: centrifuge for10 min, direct injection of supernatant. Cotton Leaves: cut and pulverized with liquid N_2 and mortar and pestle. Added 4 mL ACN for 1 g leaves, centrifuged for 5 min. Direct injection of supernatant.	LC-ESI^+-MS/MS	[65]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Crops: crown daisy, sedum, amuranth	CLO CLO metabolites: MNG, THM, TZMU, TZNG	10 g	QuEChERS: 10 g homogenized sample with 6 g $MgSO_4$ and 1.5 g NaCl. Add 20 mL ACN, shake, centrifuge. To 15 mL supernatant add 1.5 g $MgSO_4$ and 0.5 g PSA, shake, centrifuge. ACN layer dried and reconstituted in 2 mL MeOH/H_2O with 1% CH_3COOH and filtered.	LC-ESI^+-MS/MS	[56]
Cotton leaves	DIN, NIT, THM	10 g	Leaves chopped with blender and mixed with 50 mL ACN and shaken for 90 min. Addition of 5 g NaCl and 8 g $MgSO_4$, shaken for 1 min and then centrifuged for 5 min. 25 mL supernatant dried and reconstituted with 2 mL ACN. Clean-up using a dual layer GCB (500 mg)/PSA (500 mg), eluted with 3 times 6 mL ACN. Combined eluant dried and reconstituted in 2 mL ACN followed by filtering.	LC-ESI^+-MS/MS	[4]
Spinach, cucumber, apple, pomelo	ACE, IMC, IMI, NIT, THC, THM	10 g homogenized sample	Addition of 10 mL ACN, shaken for 30 min, added 4 g $MgSO_4$ and 1 g NaCl, vortexed for 1 min, centrifuged. Organic phase filtered with direct injection.	LC-ESI^+-MS/MS	[37]
Chicken, egg, milk, pork, rice, tea, apple, potato, cabbage	ACE, CLO, DIN, IMI, NIT, THC, THM	1 g homogenized 10 g homogenized	To sample added 20 mL ACN and then shaken or sonicated for 30 s, centrifuged for 10 min. Supernatant removed and repeated extraction 2 times. Supernatants were combined and dried and redissolved in 5 mL H_2O. Oasis HLB SPE was used to cleanup a 3 mL portion of this sample. SPE tube washed with 3 mL H_2O and elution with 3 mL MeOH. Eluant dried and reconstituted with 1 mL MeOH/H_2O (1:1 v/v) and filtered.	LC-ESI^+-MS/MS	[41]
Rice	IMI	0.5 g	MIP-MSPD extraction. Rice and 1 g MIPs homogenized and loaded in cartridge. Wash with 5 mL 20/80 MeOH/H_2O and eluted with 8 mL MeOH. Eluent dried and reconstituted with 1 mL 75:25 0.1% CH_3COOH/ACN.	LC-ESI^+-MS/MS	[60]
Olive, spinach, and others Honey Pollen	ACE, CLO, DIN, FLO, IMI, NIT, THC, THM	10 g homogenized 5 g diluted 10 mL H_2O 2 g diluted 8 mL H_2O	Add 10 mL ACN and 3 mL hexane for pollen and 5 mL hexane for olive (others 0 mL hexane), shake for 30 s. QuEChERS citrate salt extraction with the use of a ceramic homogenizer followed by shaking for 40 s, centrifuge. Supernatant (1 mL) cleanup with d-SPE with 50 mg PSA+50 mg C18+150 mg $MgSO_4$ for honey, olive, and pollen; 50 mg PSA+50 mg GCB+150 mg $MgSO_4$ for spinach; and 25 mg PSA+7.5 mg GCB+150 mg $MgSO_4$ for other fruits and vegetables. 600 µL of supernatant dried and reconstituted with 200 µL of 15/85 ACN/H_2O and filtered.	LC-ESI^+-MS/MS	[8]

Table 3 continued..

Matrix	NEOs and metabolites	Sample size	Sample preparation and addition clean-up steps	Analysis method	Reference
Apricot, celery, courgette, peach, pear	ACE, IMI, THC, THM	25 g homogenized sample	Add 60 mL acetone and shaken 2 min, filtered, and washed to 100 mL acetone. 20 mL aliquot for Extrelut-NT20 cleanup with elution with five 20 mL portions of CCl_2H_2. Dried and reconstituted with 1 mL MeOH.	LC-ESI^+-MS	[49]
Chestnut, shallot, ginger, tea	ACE, CLO, DIN, IMI, THC, THM	1 g (0.5 g tea) homogenized powder diluted with 1 mL H_2O	Add 10 mL ACN, shake for 30 s, vortex for 1 min and organic phase removed with process repeated 3 times. Combined extracts dried with Na_2SO_4 for 30 min and dried to 4 mL. Extract cleaned with activated carbon SPE with elution with 5 mL ACN followed by drying and reconstitution with 10 mL H_2O; followed by Oasis HLB SPE with elution with 2 X 5 mL MeOH, dried and reconstituted with mobile phase of ACN/0.1% HCOOH.	LC-ESI^+-MS/MS	[42]
Plant	CLO	NA	Plant materials placed in universal adapter with no further sample preparation.	Sheath-Flow Probe ESI MS (TOF)	[73]
Cucumber, green beans, pepper, tomato	IMI (analyzed as hydrolysis product 1-(6-chloro-3-pyridylmethyl)imidazolin-2-one)	20 g homogenized, diluted with 100 mL H_2O	Filtered and rinsed with H_2O to 250 mL, hydrolyzed by addition of 0.4 g NaOH, heated and then neutralized with 1:1 HCl, followed by liquid-liquid extraction (10:1 ratio) with chloroform. Organic phase dried with Na_2SO_4 and then dried to 200 µL.	GC-EI MS	[19]

from metabolites of CLO, IMI, and THM are also formed from loss of NO_2, and Cl radical or triazole moiety [66]. The structures of the IMI guanindine, hydrazone and triazolone have also been proposed [61]. Loss of nitroso and methyl loss has been observed for piachongding and its metabolites [50]. Sulfoxaflor is a newly registered NEO in North America and has not been included in many separation methods of NEOs. Sulfoxaflor fragments to produce ions at m/z =74 and 154 [4, 64] which are proposed to be from cleavage at the tertiary C to produce $[M+H\text{-}SONCH_3CN]^+$ with neutral loss of HF. The fragment ion at m/z = 105 was also observed in the daughter ion scan which is the alternative result of cleavage at the tertiary C from $[M+H\text{-}CF_3C_5NH_3CCH_3]^+$ and was stronger in abundance than the fragment ion of m/z = 154, although all 3 SRM transitions are of similar magnitude. Table 1 shows the major precursor ions of the NEOs match with these proposed fragmentation patterns.

3.1. Quantitative and confirmation analysis using liquid chromatography-tandem mass spectrometry

Most recent low resolution LC-MS/MS analysis of NEOs and their metabolites include two selected reaction monitoring (SRM) transitions to achieve unambiguous identification of NEOs with precursor selected as $[M+H]^+$ and two unique fragment ions selected (as shown in tables 1 and 2). This satisfies the criteria for four identification points introduced in European Union guidelines (93/256/ECC) with 1 identification point for the precursor ion and 1.5 identification points for each of the two fragment ions [91]. The most intense SRM is selected for quantification and the second most intense SRM is selected for confirmation. In some cases both transitions have similar magnitude such as for CLO 250→132 and 250→169, DIN 203→129, 203→157, sulfoxaflor as noted above (Table 1) and the selection of the quantitative and confirmation transition may consequently vary. In addition, generally criteria for confirmation of target analyte in the sample includes using ratios of peak areas obtained from $SRM_{quantitative}/SRM_{confirmation}$ for unknown samples matching standards to within 10% or relative standard deviation % determined from standards run on day of analysis to minimize issues with matrix interferences [43, 58, 91]. The NEO metabolites have less agreement on reported SRMs in the literature and fewer options for the confirmation SRM. One major challenge in the analysis of metabolites of NEOs is the availability of commercially available analytical standards and relatively few studies completed to date. Figure 1 shows the chromatogram of several commercially available NEO metabolites (6-chloronicotinic acid (6-CNA), N-desmethyl acetamiprid (AM-2), imidacloprid olefin (IM-7), imidacloprid urea (IM-9), and N-desmethyl-thiamethoxam (dm-THC)). In general the quantitative SRM of metabolites of NEOs have good agreement between the published studies and our work presented herein. As new commercial analytical standards of metabolites of NEOs become available it should be feasible to incorporate these metabolites into existing LC-MS/MS methods thus allowing for their simultaneous analysis which is critical as often sample size or extract volumes available for analyses are limited.

3.2. High-resolution mass spectrometry (HRMS) and low resolution mass spectrometry for the identification of metabolites of NEOs

As most metabolites are not commercially available as standards LC-QTOF (MS) has been used to identify potential new metabolites of NEOs often from plant materials, honey, pollen, soil or bee products [45, 63, 66]. Liquid chromatography coupled with high-resolution mass spectrometry has had wide use for the identification of chemical contaminants such as those in food commodities applying both targeted and non-targeted screening approaches. LC-high-resolution MS methods have the ability to be used for targeted analysis with accurate mass comparison to calculated molecular weights. A number of the metabolites of NEOs were identified using at least one of the following approaches: accurate mass analysis of the precursor ion of a chlorinated metabolite where ^{35}Cl:^{37}Cl ratios could be used for confirmation; identification of new peaks in chromatograms of treated versus untreated or blank samples; and agreement with molecular weights of proposed urinary or plant metabolites or hydrolysis products from literature studies [45, 66]. In cases where the NEOs or the metabolites are chlorinated the ^{37}Cl isotope of $[M+H]^+$ may be used for confirmation of identity in any LC-MS approach

although typically not used for quantitative LC-MS/MS analysis [39, 49, 52, 53, 60, 66]. In addition, when LC-MS is used the sodium or acetonitrile adduct may be used for additional confirmation [50, 54]. Low resolution tandem-mass spectrometry methods for NEO metabolites are also highlighted in table 2 and require commercially available standards or other sources of standards to be available for completion of quantitative and confirmation analysis. The analysis of the largest number of metabolites of an individual NEO has been completed for IMI metabolites partly due to the availability of standards from various sources [36, 45, 48, 54, 55, 57, 61, 66].

Awareness of the metabolism of NEOs is important in identifying potential metabolites to include in exact mass calculations that are not commercially available as standards and often metabolites can be grouped by the chemical structure similarities. ACE, IMI, NIT, and THC have a chloropyridinyl moiety and undergo metabolism to 6-chloronicotinic acid (6-CNA) which is among the most widely analyzed metabolites of NEOs [45, 70, 71]. However, it is important to note that other NEOs including CLO, DIN, THM, and sulfoxaflor do not yield 6-CNA through metabolism. IMI can also produce imidacloprid-guanidine in mammals [69]. CLO, DIN, IMI, and THM contain the nitroguanidine moiety and aldehyde oxidase will reduce nitroguanidines to nitroso and aminoguanidines, and nitromethylenes also form from metabolism of NIT [54]. NEO metabolism can also occur with CYP450, and phase II enzymes in addition to aldehyde oxidase and schemes for precursor-product relationships in metabolism have been developed for CLO, IMI, THC, and THM with a number of metabolite products in brain and liver tissues identified by LC-MS of which the desmethyl and hydroxyl metabolites have been of greatest interest [67]. Metabolites of IMI have been the most widely studied and measured with more detailed metabolic pathways available with the inclusion of 6-CNA, hydroxyl, nitroso, olefin, urea, and triazinone metabolites [57, 62]. N-desmethyl-acetamiprid (AM-2) is the major urinary metabolite in animals and humans [45]. A scheme of formation of potential common and unique metabolites of ACE, IMI, CLO, and THM is available [45]. THM and CLO which is also a degradation product of THM have in common the chlorothiazole structure and are metabolized to 2-chloro-1,3-thiazole-5-carboxylic acid (2CTCA) after conjugation with glycine or glucuronic acid. Dinotefuran is metabolized to 3-furoic acid (3FA) and dinotefuran DN phosphate [57, 70]. Some studies have included databases of nominal weights of molecular formula of up to 57 known metabolites [45]. This notation (listed in table 2) includes AM-1-12 which are unique to ACE, IM-1-11 which are unique to IMI, CM-1-10 which are unique to CLO, CPM-1-10 which are common to chlorpyridinyl NEOs, and CTM-1-10 which are common to CLO and THM. Those listed in table 2 were identified in samples using LC-MS (high resolution) [45].

3.3. Sample preparation and matrix interferences

A wide variety of sample matrices have been analyzed for NEOs and their metabolites as shown in table 3. In general sample preparation is required for removal of matrix components that can co-elute with target analytes in the separation even with optimization of column and mobile phase conditions. Potential matrix interferences can lead to significant matrix enhancement or suppression in MS detection particularly when concentrations of NEOs or their metabolites are in the μg/L or lower range. Target analytes that elute earlier are often more prone to matrix effects. In addition NEOs need to be extracted from solid matrices with exchange of solvent to a compatible solvent for LC-MS or LC-MS/MS analysis such that there is often a drying or evaporation step prior to reconstitution of the sample in the solvent compatible for analysis. Nitenpyram and sulfoxaflor have considerably higher volatility than the other NEOs and hence require more care during drying (evaporation of solvent). The approach taken in sample preparation is selected to deal with the complexity of the sample matrix and removal of sufficient interferences while providing adequate recoveries of NEOs (and their metabolites).

3.4. Liquid matrices: Water, urine and wine

For liquid sample matrices solid phase extraction is typically used to pre-concentrate and clean-up the targeted NEOs and metabolites prior to LC-MS/MS analysis with the most common SPE sorbents including LiChrolut EN and Oasis HLB [35, 50, 64]. Table 3 shows that a wider variety of

SPE sorbents have been used to pre-concentrate NEOs or for additional clean-up of urine sample extracts including Extrelut® NT3, WAX, Bond Elut PCX, Amberlite XAD-4, or a diatomaceous earth [6, 7, 50, 70, 72, 73]. NEOs and metabolites are soluble in organic solvents including ethyl acetate, methanol, and acetonitrile. These solvents range in polarity and are often selected to elute the NEOs from the SPE sorbents. The solvents are also compatible with the need to evaporate the solvent prior to reconstitution in a mobile phase solvent. With all SPE sorbents the elution solvents selected should be evaluated for recoveries of targeted NEOs and their metabolites. If analysis is by GC-EI-MS/MS such as for 6-CNA, 2CTCA, and 3FA then a derivatization step is required [69, 70].

3.5. Solid matrices: Biological animal tissues, honey, pollen, plants, soil and sorbent materials used in gas or particle phase atmospheric sample collection of NEOs

NEOs from solid matrices can be extracted with a variety of approaches (see table 3). Pressurized solvent extraction and ultrasonic extraction (sonication) are used for extraction of NEOs from sorbent materials and filters used in air sampling (gas or particle phase), as well as bovine tissues, eels, hair, and honey [36, 38, 39, 43, 47, 62]. Liquid-liquid extractions have been used to extract NEOs [9, 38, 41, 47, 53, 54, 61, 63]. Solid-liquid extraction has also been used for pollen and homogenized food commodities [4, 37, 38, 41, 49, 51, 59, 60, 65, 66]. A variety of organic solvents of varying polarity have been used. The most common solvents are methylene chloride, ethyl acetate, acetone, methanol, acetonitrile, water or combinations of these solvents (see table 3). Better recoveries were reported for solid-liquid extraction of NEOs including dinotefuran and nitenpyram in bee pollen when dichloromethane was selected as the extraction solvent rather than acetone, ethyl acetate, acetonitrile, or 50/50 v/v% ethyl acetate/dichloromethane [51]. As these extraction procedures are not selective many of these extraction steps are followed by clean-up of the extract primarily by SPE with common SPE sorbents including C18, Oasis HLB, Strata X-CW (weak cation mixed mode), and graphitized carbon black (GCB)/primary secondary amine (PSA) [4, 36, 38, 39, 41, 42, 47, 65]. Bonded phases with C18 bonded to silica (reversed phase) and Oasis HLB (polymeric reversed phase with mixed hydrophilic/lipophilic monomers for greater retention of polar analytes) are the most popular. With Oasis HLB the wash step was performed with water, while with C18 20/80 v/v% methanol/water could be used without loss of NEOs thus potentially allowing matrix of lower polarity to be removed [41, 43]. Both SPE methods used methanol to elute NEOs from the SPE sorbent [41, 43]. Strata X-CW gave the best recoveries of NEOs and some metabolites with acetonitrile/ethyl acetate 8:2 v/v and did not require the use of other additives such as triethylamine [36]. Under these conditions recoveries for IMI metabolites were better with Strata X-CW than Oasis HLB or C18 [36]. Some diluted samples with water including honey or homogenized food samples undergo direct SPE with Strata X, Extrelut-NT-20, ChemElut 1010, Discovery DSC18Lt, and Isolute® HM-N [33, 49, 52, 53, 66]. Florisil was generally not selected for NEOs with the exception of wine samples where Florisil or PSA reduced matrix effects [35, 53]. Molecularly imprinted-matrix solid-phase dispersion has also been designed for the analysis of IMI [60].

The quick, easy, cheap, effective, rugged and safe (QuEChERS) method or modified forms of QuEChERS are one of the most popular sample preparation methods for pesticide residue analysis including that of NEOs in a wide range of fruits, honey, pollen, soil, and vegetable samples [8, 20, 32, 33, 40, 44, 57, 92]. To obtain a representative food commodity sample for extraction the solid food sample is typically homogenized. If the water content of the sample is low then a wetting step is also included prior to extraction. When feasible, initial sample preparation steps are often done at lower temperatures than room temperature to minimize degradation of target pesticides. Often an internal standard is utilized to correct for commodity-to-commodity differences in water content. QuEChERS has been used to recover NEOs and metabolites of NEOs with recoveries >80% for LC methods with MS detection as well as UV-visible detection. The process involves an acetonitrile salt-out extraction with anhydrous $MgSO_4$ in combination with sodium salts of citrate, chloride, or acetate (in increasing pH order) which causes phase separation of water and acetonitrile

layers and "salting-out" of the target analytes into the acetonitrile layer. If the sample has a low water content such as some samples of honey, pollen, and soil then a wetting step is added prior to or in combination with the acetonitrile salt-out extraction [8, 32, 33, 44, 56, 57]. Acetate or citrate-buffered QuEChERS is a good choice to improve recoveries for pH-sensitive pesticides [8, 32, 33, 40]. Metabolites of NEOs are generally more sensitive to the type of salt added in the salt-out extraction step with generally sodium acetate providing better recoveries of metabolites of IMI with the exception of imidacloprid desnitro HCl which still had recoveries of only 49% [57]. Recoveries of some metabolites (IMI olefin, 5-hydroxy-IMI, IMI-urea) and IMI and CLO were not impacted by the type of salt added. In soil matrices citrate in combination with $MgSO_4$ is often used in the salt-out procedure. At higher levels of NEOs in soil samples the salt type (NaCl versus Na_3 citrate) did not influence significantly the recoveries of NEOs; but at low levels of NEOs, recoveries were reduced in the presence of citrate which was attributed to greater influence of matrix such that NaCl was selected over Na_3 citrate [44]. NaCl was also selected in the QuEChERS method for CLO metabolites [56].

The dispersive SPE (dSPE) clean-up of the acetontrile layer follows with typically primary-secondary amine (PSA), graphitized carbon black (GCB) or C18. Anhydrous $MgSO_4$ may be added to remove residual water molecules in the acetonitrile layer prior to the dSPE clean-up. C18 is mainly used to remove fats and non-polar matrix components; GCB is used to remove pigments so often selected for plant extracts, and PSA is used to remove organic acids, fatty acids and sugars from sample extracts. 6-CNA may bind strongly to PSA as organic acids bind to this sorbent [57]. PSA alone had better recoveries of NEOs for soil samples than with C18 or GCB [44], while acceptable recoveries with PSA and GCB for IMI metabolites including 6-CNA in soil were found [20]. PSA was found to provide acceptable recoveries (72-111%) for clothianidin and its metabolites in crop samples [56]. Metabolites can have poor recoveries such as for desnitro imidacloprid olefin, desnitro imidacloprid HCl, and 6-chloronicotinic acid with the standard QuEChERS method [57]. Modified QuEChERS utilizing C18 SPE eluted with 2% triethylamine in acetonitrile (alternatively 1-5% acetic acid) has been used with addition of 2% triethylamine to elute more strongly bound metabolites from the SPE cartridges [57]. The method was further improved by not having the drying step of the acetonitrile layer with anhydrous $MgSO_4$ prior to SPE. Some NEOs such as nitenpyram and dinotefuran are strongly bound to SPE sorbents such as ChemElut 1010 (diatomaceous earth) and not eluted with cyclohexane/ethyl acetate, and QuEChERS with addition of Na_3citrate and Na_2H citrate during salt-out (with $MgSO_4$ and NaCl) provides better recoveries (70-120%) for NEOs, N-desmethyl acetamiprid and flonicamid metabolite (TFNA-AM), than observed with SPE clean-up [33]. Dispersive liquid-liquid microextraction (DLLME) with dichloromethane as the extraction solvent also gave lower recoveries for nitenpyram and dinotefuran than QuEChERS (acetonitrile salt-out extraction) due to the higher polarities of nitenpyram and dinotefuran such that extraction efficiency was better in the more polar extraction solvent used in the QuEChERS method [40]. DLLME and QuEChERS both provided good performance for other NEOs. Particularly, for more polar analytes such as nitenpyram and dinotefuran the polarity of the solvent selected in all sample preparation procedures should be considered. In addition metabolites of NEOs may need consideration of pH of the solvents selected to improve recoveries.

3.6. Matrix interferences and LC-MS/MS calibration approaches

Table 3 shows that most methods required sufficient clean-up procedures during sample preparation to minimize matrix interferences for LC-MS/MS. In most cases matrix-matched standards can be prepared such that evaluation of matrix effects can be completed by comparison of standards prepared in solvents (calibration curve commonly using internal standard approach) [50]. Table 1 shows a number of options available for deuterated internal standards with imidicloprid-d_4 most commonly used due to its good MS sensitivity. In the majority of sample types listed in table 3 matrix-matched standards were used. Matrix effects for nitenpyram, dinotefuran, and thiamethoxam were

greater than for other NEOs with QuEChERS as compared to DLLME as expected from the use of acetonitrile in QuEChERS which will co-extract high concentrations of polar matrix components [51]. Beeswax samples showed 61 to 78% matrix effect [53]. Matrix effects also varied for CLO and its metabolites in different crop samples [56]. Differences in relative response for NEOs have also been observed for different fruit matrices [49].

4. Conclusion

Neonicotinoids and their metabolites can be analyzed simultaneously by LC-ESI$^+$-MS/MS for a wide range of sample matrices. A variety of reversed phase columns can be used but consideration for sufficient retention of dinotefuran, nitenpyram and metabolites should be made. In general an acetontrile gradient with 0.1% formic acid will provide the best selectivity and sensitivity. Addition of ammonium acetate will provide shorter analysis times, potential improvement in peak shapes, and small improvements in sensitivity for a large number of target analytes. Gradients with methanol (or acetonitrile) and formic acid without ammonium acetate should be considered when MS sensitivity for 6-chloronicotinic acid and dintoefuran is the highest priority. Although more than 2 SRM transitions are often provided for metabolites of neonicotinoids (see tables 1 and 2) in general, sensitivity is not sufficient for trace analysis for more than one confirmation SRM. Sample preparation approach depends upon sample matrix with SPE preferred for liquid samples, QuEChERS or modified QuEChERS methods for fruit, bee products, pollen, or vegetation samples. For other sample matrices pressurized solvent extraction, ultrasonic extraction, liquid-liquid extraction, and solid-liquid extractions are selected. SPE is used to further clean-up extracts from these less selective extraction procedures.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest to declare.

ABBREVIATIONS

ACE	:	Acetamiprid
ACN	:	Acetonitrile
AM-2	:	N-Desmethyl acetamiprid
APCI	:	Atmospheric pressure chemical ionization
BSTFA	:	N,O-Bis (trimethylsilyl) trifluoroacetamide
CLO	:	Clothianidin
CM-1	:	N-Desmethyl-clothianidin
6-CNA (CPM-3)	:	6-Chloronicotinic acid
CPM-8	:	N-N-(6-Chloronicotinoyl) -glycine
2CTCA	:	2-Chloro-1,3,-thiazole-5-carboxylic acid
CTM-3	:	2-Chlorothiazole-5-carboxylic acid
CTM-8	:	N-(2-(methylsulfanyl) thiazole-5-carboxyl)-glycine
DIN	:	Dinotefuran
DLLME	:	dispersive liquid-liquid microextraction
dSPE	:	Dispersive solid phase extraction
EA	:	Ethyl acetate
ESI	:	Electrospray ionization
3FA	:	3-Furoic acid
GCB	:	Graphitized carbon black
GC-EI-MS/MS	:	Gas chromatography-electron impact-tandem mass spectrometry
IMC	:	Imidaclothiz
IMI	:	Imidacloprid
IMI-1	:	5-Hydroxy imidacloprid
IMI-3	:	4,5-Dihydroxy-imidacloprid
IMI-7	:	Imidacloprid olefin
IMI-9	:	Imidacloprid urea
IS	:	Internal standard
LC-QTOF	:	Liquid chromatography-quadrupole time of flight
LC-MS/MS	:	Liquid chromatography-tandem mass spectrometry
MeOH	:	Methanol
MP	:	Mobile phase
NEO	:	Neonicotinoid
NIT	:	Nitenpyram
PSA	:	Primary secondary amine

QuEChERS	:	quick, easy, cheap, effective, rugged, safe
TFMA-AN	:	4-trifluoromethyl-nicotinamide
THC	:	Thiacloprid
THM	:	Thiamethoxam
TMCS	:	Trimethylchlorosilane
XDE-208	:	Sulfoxaflor

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