Journal of Chromatography A 1756 (2025) 466093

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Optimization and validation of a QuEChERS-based method with a freezing-out clean-up for pesticide residues in commercial dry food for dogs and cats

Ana Macías-Montes [a], Octavio P. Luzardo [a,b], Annalisa Zaccaroni [c], Andrea Acosta-Dacal [a,*]

[a] Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), Universidad de Las Palmas de Gran Canaria, Paseo Blas Cabrera s/n, Las Palmas de Gran Canaria, 35016, Spain
[b] Spanish Biomedical Research Center in Physiopathology of Obesity and Nutrition (CIBERObn), Madrid, 28029, Spain
[c] Large Pelagic Vertebrate Group, Veterinary Faculty, University of Bologna, Viale Vespucci 2, Cesenatico, FC, 47042, Italy

ARTICLE INFO

Keywords:
Pet feed
LC-MS/MS
GC–MS/MS
Multi-residue method
Companion animals
Food safety

ABSTRACT

Addressing pesticide residues in feed for cats and dogs is essential to assess their potential impact on their health. In this study, we validated a QuEChERS-based multi-residue method for analyzing 211 pesticide residues in cat and dog feed by LC-MS/MS and GC–MS/MS. To overcome the challenges posed by the complex nature of pet feed, we refined the clean-up step, comparing PSA, EMR-Lipid, and freezing approaches. Freeze-out yielded the best results, with 91.9 % of analytes achieving recoveries within 70–120 % and RSDs≤20 %, and two freezing cycles proved sufficient for effective matrix removal while maintaining analyte recoveries. To the best of our knowledge, this is the first validated QuEChERS method to use freezing-out as a standalone clean-up strategy, offering a simplified and cost-effective solution for high-fat matrices. The method was validated in terms of linearity, accuracy, and precision. Matrix-matched calibration curves ($R^2 \geq 0.99$) were used for all analytes. Recoveries were within 70–120 % for all spiking levels in most compounds, with a few analytes showing recoveries between 60–130 % in the extended range allowed by SANTE guidelines. All RSDs were below 20 % by established validation guidelines. The method demonstrated high sensitivity, with most analytes achieving limits of quantification below the generic 10.0 μg/kg MRL established by EU regulations for feed. Notably, over 70 % of analytes achieved LOQs at least ten times lower. All limits of detection were equal to or below 10.0 μg/kg. The method's applicability was demonstrated by analyzing 16 commercial pet feed samples, where 112 residues of 39 pesticides were detected.

1. Introduction

The increasing popularity of pets, with over 350 million in Europe—65 % of which are dogs and cats [1] has heightened the focus on their care and nutrition, driven by growing concern among pet owners for the well-being of their companions [2,3]. Pet feed plays a key role in promoting animal health, with dry feed being one of the most popular choices due to its extended shelf life, ease of storage, and nutritional balance [4,5].

Pet feed can include a variety of components like protein sources, fats, fruits, vegetables, and grains. Its diverse and complex composition increase the risk of introducing harmful compounds such as mycotoxins [6–8], toxic elements [9–11] and pesticide residues [12]. Pesticides can enter the production chain through plant-based raw materials treated during cultivation, transportation and storage [13]. Additionally, animal protein sources can also be a route of contamination, as livestock feeds can also contain pesticide residues that accumulate in animal tissues [14,15]. Pesticides residues have been detected in the serum, hair and urine of cats and dogs, indicating potential exposure through environmental and dietary sources [16–19]. In fact, exposure to these compounds has been associated to several health issues in companion animals, including lymphoma, bladder cancer, and mammary tumors in dogs [20–23], and hyperthyroidism in cats [24], among others. Despite this, no specific regulations exist for pesticide residues in feed intended for companion animals in the European Union. In absence of specific guidelines for pet's feed, the European Union's Regulation (EC) No 396/2005 [25], which sets maximum residue levels (MRLs) for feed of plant and animal origin for production animals, is often used as a

* Corresponding author.
E-mail address: andrea.acosta@ulpgc.es (A. Acosta-Dacal).

https://doi.org/10.1016/j.chroma.2025.466093
Received 26 March 2025; Received in revised form 13 May 2025; Accepted 22 May 2025
Available online 24 May 2025

Table 1
List of Compounds with their category of use, legal status, analysis technique, and chromatographic and mass spectrometric conditions.

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
1	2-Phenylphenol	F	Approved	GC	6.30	Positive	169.0 → 115.0	30	169.0 → 141.0	15	70
2	Abamectin	I, A, AH	Approved	LC	10.99	Positive	890.5 → 567.1	10	895.5 → 751.4	45	160
3	Acetamiprid	I	Approved	LC	4.47	Positive	223.1 → 126.0	27	223.1 → 90.0	45	140
4	Aldicarb	I	Not approved	LC	5.18	Positive	116.0 → 89.1	4	208.0 → 116.0	0	100
5	Aldicarb-sulfone	Met	–	LC	3.01	Positive	240.1 → 76.0	16	223.1 → 86.1	13	75
6	Aldicarb-sulfoxide	Met	–	LC	3.06	Positive	207.1 → 131.9	0	207.1 → 89.1	10	86
7	Atrazine	H	Not approved	LC	6.80	Positive	216.0 → 68.1	55	216.0 → 103.8	30	130
8	Azinphos-methyl	I	Not approved	LC	7.31	Positive	318.0 → 132.1	8	318.0 → 261.0	0	60
9	Azoxystrobin	F	Approved	LC	7.62	Positive	404.1 → 344.1	24	404.1 → 329.1	32	110
10	Benalaxyl	F	Not approved	LC	9.00	Positive	326.2 → 148.0	20	326.2 → 208.0	12	90
11	Bendiocarb	I	Not approved	LC	5.94	Positive	224.1 → 166.9	8	224.1 → 108.9	15	100
12	Bifenthrin	I	Not approved	GC	13.85	Positive	181.2 → 165.2	25	181.0 → 115.0	60	70
13	Bitertanol	F	Not approved	LC	9.26	Positive	338.2 → 70.0	4	338.2 → 269.2	5	100
14	Boscalid	F	Approved	GC	16.53	Positive	342.0 → 140.0	15	342.0 → 112.0	45	70
15	Bromopropylate	A	Not approved	GC	13.83	Positive	341.0 → 183.0	15	341.0 → 157.0	45	70
16	Bromuconazole (two isomers)	F	Approved	LC	8,18/ 8,78	Positive	378.0 → 159.0	32	376.0 → 159.0	22	150
17	Bupirimate	F	Approved	LC	8.44	Positive	317.2 → 108.1	28	317.2 → 166.1	18	100
18	Buprofezin	I	Approved	LC	9.89	Positive	306.1 → 201.0	12	306.1 → 116.0	12	140
19	Cadusafos (ebufos)	I, AH	Not approved	LC	9.42	Positive	271.1 → 159.0	16	271.1 → 131.0	22	100
20	Carbaryl	I	Not approved	LC	6.26	Positive	202.1 → 145.1	4	202.1 → 127.1	28	90
21	Carbendazim	F	Not approved	LC	3.59	Positive	192.1 → 160.1	16	192.1 → 132.1	32	120
22	Carbofuran	I, AH	Not approved	LC	5.97	Positive	222.1 → 123.1	30	222.1 → 165.1	20	80
23	Carbofuran-3-hydroxy	Met	–	LC	4.45	Positive	238.1 → 163.1	10	238.1 → 181.1	10	110
24	Chlorantraniliprole	I	Approved	LC	7.35	Positive	483.9 → 452.9	16	483.9 → 285.9	8	105
25	Chlorfenapyr	I, A	Not approved	GC	11.98	Positive	247.0 → 227.0	15	328.0 → 247.0	20	70
26	Chlorfenvinphos	I	Not approved	LC	9.05	Positive	358.9 → 155.1	8	361.1 → 154.9	10	105
27	Chlorobenzilate	A	Not approved	GC	12.11	Positive	251.0 → 139.0	15	251.0 → 111.0	40	70
28	Chlorpropham	H	Not approved	GC	7.14	Positive	213.0 → 127.0	15	153.0 → 90.0	10	70
29	Chlorpyrifos	I	Not approved	GC	9.90	Positive	314.0 → 258.0	15	314.0 → 286.0	5	70
30	Chlorpyrifos-methyl	I	Not approved	GC	9.11	Positive	286.0 → 93.0	25	286.0 → 271.0	15	70
31	Chlorthal-dimethyl	H	Not approved	GC	10.01	Positive	300.9 → 166.9	55	300.9 → 222.9	25	70
32	Clofentezine	A	Not approved	LC	9.21	Positive	303.1 → 138.0	12	303.1 → 102.0	40	120
33	Clothianidin	I	Not approved	LC	4.16	Positive	250.0 → 169.0	8	250.0 → 131.9	8	100
34	Coumachlor	R	Not approved	LC	8.62	Positive	343.1 → 162.8	15	343.1 → 285.0	15	120
35	Coumaphos	I, A	Not approved	LC	9.02	Positive	363.0 → 227.0	30	363.0 → 306.9	15	120

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
36	Cyazofamid	F	Approved	LC	8.51	Positive	325.0 → 108.0	20	325.0 → 261.1	15	90
37	Cyflufenamid	F	Approved	GC	11.92	Positive	413.1 → 223.1	23	413.1 → 295.1	33	70
38	Cymoxanil	F	Approved	LC	4.73	Positive	199.1 → 128.0	4	199.1 → 110.9	12	90
39	Cyproconazole (two isomers)	F	Not approved	LC	8,07/ 8,27	Positive	292.2 → 70.2	18	292.2 → 125.1	24	100
40	Cyprodinil	F	Approved	GC	10.36	Positive	224.0 → 118.0	45	224.0 → 104.0	25	70
41	Demeton-S-methyl	I, A	Not approved	LC	6.02	Positive	230.9 → 88.9	5	230.9 → 61.0	30	50
42	Demeton-S-methyl-sulfone (Dioxydemeton)	I, A	Not approved	LC	3.62	Positive	263.0 → 169.0	12	263.0 → 109.0	24	120
43	Diazinon	I	Not approved	GC	8.28	Positive	304.0 → 179.0	15	137.1 → 84.0	20	70
44	Dichlorvos	I	Not approved	GC	4.71	Positive	184.9 → 93.0	10	185.0 → 109.0	15	70
45	Diethathyl (-ethyl)	H	Not approved	LC	8.75	Positive	312.2 → 238.1	15	312.2 → 162.0	30	120
46	Diethofencarb	F, MB, WP	Not approved	LC	7.61	Positive	268.2 → 226.1	5	268.2 → 152.0	20	110
47	Difenoconazole	F, MB, WP	Approved	LC	9.42	Positive	406.1 → 250.9	28	406.1 → 337.0	16	176
48	Diflubenzuron	I	Not approved	LC	8.65	Positive	311.0 → 158.0	8	311.0 → 141.0	32	90
49	Diflufenican	H	Approved	GC	13.24	Positive	395.1 → 266.0	24	395.1 → 246.0	36	150
50	Dimethenamid	H	Not approved	LC	7.74	Positive	276.1 → 244.1	10	276.1 → 168.1	20	125
51	Dimethoate	I	Not approved	LC	4.40	Positive	230.0 → 198.8	0	230.0 → 125.0	16	70
52	Dimethomorph (two isomers)	F, MB, WP	Not approved	LC	7,75/ 8,02	Positive	388.1 → 301.1	20	388.1 → 165.1	32	180
53	Diniconazole-M	F, MB, WP	Not approved	GC	12.28	Positive	326.1 → 70.0	28	328.1 → 70.0	28	110
54	Diphenylamine	PHP	Not approved	GC	6.99	Positive	170.0 → 65.0	65	170.0 → 93.0	40	200
55	Epoxiconazole	F	Not approved	GC	13.50	Positive	330.0 → 100.9	50	330.0 → 120.9	24	120
56	Ethion (diethion)	I, A	Not approved	GC	12.35	Positive	385.0 → 199.0	5	385.0 → 171.0	10	100
57	Ethirimol	F	Not approved	LC	5.18	Positive	210.2 → 140.1	20	210.2 → 98.1	28	160
58	Ethofumesate	H	Approved	GC	9.58	Positive	286.0 → 207.0	5	286.0 → 161.0	20	70
59	Ethoprophos	I, AH	Not approved	LC	8.43	Positive	243.1 → 97.0	30	243.1 → 130.9	15	90
60	Etofenprox	I, A	Approved	GC	16.71	Positive	394.0 → 359.0	10	394.0 → 135.1	40	66
61	Etoxazole	A	Approved	LC	10.36	Positive	360.1 → 304.0	16	360.1 → 113.0	58	160
62	Famoxadone	H	Not approved	LC	9.08	Positive	392.1 → 330.9	5	392.1 → 238.1	12	110
63	Fenamidone	F	Not approved	LC	7.76	Positive	312.0 → 92.2	28	312.0 → 236.1	14	100
64	Fenamiphos	I, AH	Not approved	LC	8.66	Positive	304.1 → 217.1	20	304.1 → 202.0	36	120
65	Fenamiphos-sulfone	Met	–	LC	6.27	Positive	336.1 → 308.1	12	336.1 → 188.0	31	120
66	Fenamiphos-sulfoxide	Met	–	LC	6.10	Positive	320.1 → 233.0	20	320.1 → 108.1	44	120
67	Fenarimol	F, MB, WP	Not approved	GC	15.00	Positive	139.0 → 75.0	30	139.0 → 111.0	15	70
68	Fenazaquin	A	Approved	GC	14.09	Positive	307.2 → 161.1	25	307.2 → 131.0	16	130
69	Fenbuconazole	F, V	Not approved	GC	16.16	Positive	337.1 → 70.0	40	337.1 → 125.1	33	160
70	Fenhexamid	F	Approved	LC	8.35	Positive	302.1 → 97.1	20	302.1 → 55.1	40	130
71	Fenoxycarb	I	Not approved	LC	8.72	Positive	302.1 → 88.0	20	302.1 → 116.1	10	110

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
72	Fenpropathrin	I, A	Not approved	LC	10.43	Positive	367.2 → 125.0	16	350.2 → 125.0	16	72
73	Fenpropidin	F	Approved	LC	7.27	Positive	274.3 → 147.0	30	274.3 → 86.0	25	170
74	Fenpropimorph	F	Not approved	LC	7.55	Positive	304.3 → 147.1	30	304.3 → 130.0	25	120
75	Fenpyroximate	A	Approved	LC	10.51	Positive	422.2 → 366.2	12	422.2 → 135.0	36	160
76	Fenthion	I, A	Not approved	GC	9.88	Positive	279.0 → 168.8	8	279.0 → 247.1	18	98
77	Fenthion-oxon	Met	–	LC	7.35	Positive	263.1 → 231.2	16	263.1 → 216.0	24	120
78	Fenthion-oxon-sulfone	Met	–	LC	4.67	Positive	295.0 → 217.0	15	295.0 → 104.2	24	110
79	Fenthion-oxon-sulfoxide	Met	–	LC	4.51	Positive	279.0 → 104.1	28	279.0 → 264.2	20	110
80	Fenthion-sulfone	Met	–	LC	6.42	Positive	311.0 → 125.0	22	311.0 → 109.0	28	140
81	Fenthion-sulfoxide	Met	–	LC	6.19	Positive	295.0 → 280.0	18	295.0 → 108.9	30	140
82	Fipronil	I, V	Not approved	GC	10.60	Negative	435.0 → 330.0	26	435.0 → 249.9	12	116
83	Fipronil-sulfide	Met	–	GC	10.53	Positive	351.0 → 255.0	20	420.0 → 351.0	35	70
84	Fluazinam	F	Approved	LC	10.01	Negative	462.9 → 416.0	10	462.9 → 398.0	9	140
85	Flubendiamide	I	Approved	LC	8.81	Positive	408.0 → 274.0	15	408.0 → 256.0	30	120
86	Fludioxonil	F	Approved	GC	11.55	Negative	247.0 → 180.0	62	247.0 → 125.9	32	152
87	Flufenoxuron	I, A	Not approved	LC	10.36	Positive	489.1 → 158.0	20	489.1 → 140.9	56	110
88	Fluopyram	F	Approved	LC	8.28	Positive	397.0 → 173.0	40	397.0 → 145.0	50	150
89	Fluquinconazole	F	Not approved	GC	15.79	Positive	376.0 → 307.1	56	376.0 → 108.0	24	140
90	Flusilazole	F, MB, WP	Not approved	LC	8.68	Positive	316.1 → 247.1	15	316.1 → 165.0	20	160
91	Flutolanil	F, MB, WP	Approved	LC	7.96	Positive	324.1 → 262.1	16	324.1 → 242.1	24	130
92	Flutriafol	F	Not approved	GC	11.24	Positive	302.1 → 70.1	16	302.1 → 122.9	28	90
93	Fonofos	I	Not approved	GC	8.23	Positive	246.0 → 109.0	5	246.0 → 137.0	15	70
94	Formetanate	I, A	Approved	LC	2.81	Positive	222.1 → 165.1	12	222.1 → 46.2	28	105
95	Fosthiazate	AH, V	Approved	LC	6.56	Positive	284.0 → 104.0	20	284.0 → 227.8	8	110
96	Hexaconazole	F, MB, WP	Not approved	LC	9.16	Positive	316.1 → 70.1	20	314.1 → 70.1	20	95
97	Hexaflumuron	I	Not approved	LC	9.57	Negative	459.1 → 439.0	8	459.1 → 276.1	18	100
98	Hexythiazox	A	Approved	LC	10.19	Positive	353.1 → 227.9	8	353.1 → 168.1	24	120
99	Imazalil (Enilconazole)	F	Approved	LC	6.66	Positive	297.1 → 159.0	20	296.9 → 69.1	18	140/110
100	Imidacloprid	I	Not approved	LC	4.13	Positive	256.0 → 208.9	12	256.0 → 175.0	12	110
101	Indoxacarb	I	Not approved	LC	9.51	Positive	528.1 → 293.1	10	528.1 → 202.8	48	140
102	Iprovalicarb	F	Approved	LC	8.23	Positive	321.2 → 119.0	15	321.2 → 202.9	0	108
103	Isocarbophos	I	Not approved	GC	10.35	Positive	230.0 → 155.0	25	230.0 → 198.0	10	70
104	Isofenphos-methyl	I	Not approved	LC	8.85	Positive	332.1 → 230.9	10	332.1 → 120.9	44	100
105	Isoprothiolane	F, MB, WP	Not approved	LC	7.98	Positive	291.1 → 231.1	12	291.1 → 189.0	30	100
106	Kresoxim-methyl	F	Approved	LC	8.83	Positive	314.1 → 116.0	24	314.1 → 223.0	15	98
107	Linuron	F	Not approved	LC	7.59	Positive	249.0 → 160.1	20	249.0 → 182.3	8	120

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
108	Lufenuron	I	Not approved	LC	10.06	Negative	509.0 → 339.0	5	509.0 → 326.1	15	90
109	Malaoxon	I	Not approved	LC	6.07	Positive	315.1 → 127.2	12	315.1 → 99.1	36	120
110	Malathion	I	Approved	LC	7.97	Positive	348.0 → 126.7	15	348.0 → 285.0	8	100
111	Mandipropamid	F	Approved	LC	7.92	Positive	412.1 → 328.0	10	412.1 → 356.1	4	130
112	Mepanipyrim	F, MB, WP	Not approved	LC	8.26	Positive	224.1 → 106.0	25	224.1 → 77.0	30	120
113	Metaflumizone	I	Approved	LC	9.95	Negative	505.0 → 302.0	14	541.0 → 302.0	20	110
114	Metalaxyl	F	Approved	GC	9.30	Positive	234.0 → 146.0	20	249.0 → 146.0	5	70
115	Metalaxyl-M (Mefenoxam)	F	Approved	LC	7.00	Positive	280.0 → 220.0	10	280.0 → 192.0	15	110
116	Metaldehyde	M	Approved	LC	3.95	Positive	194.1 → 61.9	5	194.1 → 106.0	5	50
117	Metconazole	F	Approved	LC	9.19	Positive	320.1 → 70.1	33	322.1 → 70.1	24	110
118	Methamidophos	I, A	Not approved	LC	1.20	Positive	142.0 → 94.0	12	142.0 → 125.0	12	85
119	Methidathion	I, A	Not approved	LC	7.16	Positive	320.1 → 144.8	8	320.1 → 85.0	30	82
120	Methiocarb	I, A, M	Not approved	LC	7.71	Positive	226.1 → 169.0	4	226.1 → 121.1	12	90
121	Methiocarb-sulfone	Met	–	LC	4.62	Positive	258.1 → 122.1	22	258.1 → 201.1	8	100
122	Methiocarb-sulfoxide	Met	–	LC	4.30	Positive	242.0 → 122.0	28	242.0 → 185.0	22	90
123	Methomyl	I, A, AH	Nor approved	LC	3.49	Positive	163.1 → 88.0	5	163.1 → 106.0	8	80
124	Methoxyfenozide	I	Approved	LC	8.03	Positive	369.2 → 149.0	10	369.2 → 313.1	0	80
125	Metrafenone	F	Approved	LC	9.29	Positive	409.1 → 209.1	8	411.2 → 209.1	12	120
126	Mevinphos (phosdrin) (two isomers)	I, A	Not approved	LC	4,44/ 4,93	Positive	225.0 → 193.1	0	225.0 → 127.0	12	65
127	Monocrotophos	I	Not approved	LC	3.77	Positive	224.1 → 126.8	12	224.1 → 98.1	15	100
128	Myclobutanil	F, MB, WP	Not approved	LC	8.12	Positive	289.1 → 70.1	16	289.1 → 125.1	32	110
129	N-2,4-Dimethylphenyl-N'-methyl-formamidine (DMPF, metabolite of amitraz)	Met [d]		LC	3.81	Positive	163.1 → 122.1	15	163.1 → 107.1	15	100
130	N,N-Dimethyl-N'-p-tolylsulphamide (DMST, metabolite of tolylfluanid)	Met	–	LC	6.11	Positive	215.1 → 106.1	10	215.1 → 151.1	4	90
131	N,N-dimethylformamidine (DMF, metabolite of amitraz)	Met [d]	–	LC	5.54	Positive	149.9 → 105.8	30	149.9 → 122.9	15	100
132	Nitenpyram	I	Not approved	LC	3.38	Positive	271.1 → 56.1	36	271.1 → 224.9	12	100
133	Nuarimol	F, MB, WP	Not approved	GC	13.21	Positive	315.0 → 252.0	30	315.0 → 81.1	28	80
134	Ofurace	F, MB, WP	Not approved	LC	6.02	Positive	282.0 → 159.9	20	282.0 → 147.9	30	100
135	Omethoate	I, A	Not approved	LC	2.55	Positive	214.1 → 124.8	22	214.1 → 183.0	5	84
136	Oxadixyl	F, MB, WP	Not approved	LC	5.48	Positive	279.1 → 219.2	5	279.1 → 132.3	32	110
137	Oxamyl	I, A, AH	Not approved	LC	3.35	Positive	237.1 → 72.0	12	237.1 → 90.0	5	70
138	Oxydemeton-methyl	I, A	Not approved	LC	3.52	Positive	247.0 → 169.0	12	247.0 → 109.0	24	100
139	Oxyfluorfen	H	Approved	GC	11.66	Positive	252.0 → 146.0	20	252.0 → 196.0	40	70
140	Paclobutrazol	H	Approved	LC	7.95	Positive	294.1 → 70.1	16	294.1 → 125.2	36	115
141	Paraoxon methyl	I	Not approved	GC	8.99	Positive	230.0 → 154.1	20	230.0 → 111.0	5	70
142	Parathion	I	Not approved	GC	9.93	Positive	290.9 → 109.0	30	138.9 → 109.0	5	70

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
143	Parathion-methyl	I	Not approved	GC	9.11	Positive	263.0 → 109.0	15	263.0 → 79.0	30	70
144	Penconazole	F, MB, WP	Approved	LC	8.89	Positive	284.1 → 70.1	15	285.1 → 195.0	30	70
145	Pencycuron	F, MB, WP	Not approved	LC	9.35	Positive	329.1 → 125.1	24	329.1 → 217.9	12	160
146	Pendimethalin	H	Approved	LC	10.21	Positive	282.2 → 212.2	10	282.2 → 194.1	17	80
147	Permethrin (two isomers)	I, A	Not approved	GC	15,56/ 15,66	Positive	183.1 → 168.1	15	183.1 → 165.1	10	70
148	Phosalone	I, A	Not approved	LC	9.22	Positive	385.1 → 182.0	20	385.1 → 110.9	55	80
149	Phosmet	I, A	Not approved	LC	7.38	Positive	318.0 → 159.9	16	318.0 → 133.0	40	90
150	Phosmet oxon	Met	–	LC	5.41	Positive	302.0 → 160.0	10	302.0 → 77.0	55	60
151	Pirimicarb	I	Approved	LC	5.34	Positive	239.1 → 72.1	20	239.1 → 182.1	12	100
152	Pirimicarb-desmethyl	Met		LC	4.03	Positive	225.1 → 72.1	20	225.1 → 168.1	8	100
153	Pirimiphos-ethyl	I, A	Not approved	GC	10.25	Positive	334.1 → 182.1	23	334.1 → 198.1	25	100
154	Pirimiphos-methyl	I, A	Approved	LC	9.18	Positive	306.1 → 108.1	32	306.1 → 164.0	20	100
155	Prochloraz	F, MB, WP	Not approved	LC	9.14	Positive	376.0 → 308.0	10	376.0 → 70.1	20	100
156	Procymidone	F, MB, WP	Not approved	GC	10.78	Positive	283.0 → 67.0	40	283.0 → 68.0	25	70
157	Profenofos	I, A	Not approved	LC	9.77	Positive	375.0 → 304.8	20	373.0 → 302.8	20	100
158	Propamocarb	F	Approved	LC	2.84	Positive	189.2 → 102.0	12	189.2 → 144.0	8	110
159	Propargite	A	Not approved	LC	10.36	Positive	368.2 → 231.1	4	368.2 → 175.0	12	88
160	Propiconazole	A	Not approved	LC	9.05	Positive	342.0 → 69.0	21	342.0 → 159.0	39	90
161	Propoxur	I	Not approved	LC	5.91	Positive	210.1 → 111.0	12	210.1 → 168.1	0	70
162	Propyzamide	H	Approved	LC	7.97	Positive	256.1 → 190.0	16	256.1 → 173.0	25	90
163	Proquinazid	F	Approved	GC	13.29	Positive	372.9 → 331.0	20	372.9 → 289.0	5	100
164	Prothioconazole-desthio	Met	–	GC	11.84	Positive	312.0 → 70.1	22	312.0 → 125.0	18	100
165	Prothiofos	F	Not approved	GC	11.43	Positive	162.0 → 63.1	5	266.9 → 221.0	20	70
166	Pymetrozine	I	Not approved	LC	2.76	Positive	218.1 → 105.0	20	218.1 → 78.0	52	120
167	Pyraclostrobin	F	Approved	LC	9.17	Positive	388.1 → 193.8	8	388.1 → 163.1	28	120
168	Pyrazophos	F, MB, WP	Not approved	LC	9.24	Positive	374.1 → 222.1	23	374.1 → 194.0	32	100
169	Pyridaben	I, A	Approved	LC	10.77	Positive	365.2 → 309.0	8	309.1 → 147.0	16	96
170	Pyridaphenthion	I, A	Not approved	LC	8.15	Positive	341.0 → 189.0	22	341.0 → 92.0	34	100
171	Pyrimethanil	F	Approved	GC	8.28	Positive	198.0 → 118.0	40	198.0 → 158.0	20	70
172	Pyriproxyfen	I	Approved	LC	10.09	Positive	322.2 → 96.0	12	322.2 → 184.9	24	80
173	Quinalphos	I, A	Not approved	LC	8.77	Positive	299.1 → 96.9	30	299.1 → 147.1	20	130
174	Quinoxyfen	F	Not approved	GC	12.85	Positive	308.0 → 197.0	32	308.0 → 161.8	55	100
175	Rotenone	I, R	Not approved	LC	8.65	Positive	395.1 → 192.1	25	395.1 → 213.1	20	150
176	Simazine	I	Not approved	GC	7.83	Positive	202.4 → 131.9	20	202.4 → 68.1	30	120
177	Spinosad A	I	Approved	LC	9.22	Positive	732.4 → 142.0	22	732.4 → 98.0	60	130
178	Spinosad D	I	Approved	LC	9.54	Positive	746.4 → 142.0	22	746.4 → 98.0	60	180

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
179	Spirodiclofen	A	Not approved	LC	10.53	Positive	411.1 → 71.2	15	411.1 → 313.0	5	110
180	Spiromesifen	I	Not approved	LC	10.29	Positive	371.0 → 273.0	15	273.0 → 187.0	15	110
181	Spirotetramat	I	Not approved	LC	8.35	Positive	374.2 → 302.2	12	374.2 → 216.1	36	150
182	Spirotetramat-enol	Met	–	LC	8.36	Positive	302.0 → 216.0	30	302.0 → 270.0	20	180
183	Spiroxamine	F	Approved	LC	7.66	Positive	100.0 → 72.0	16	100.0 → 58.0	32	120
184	tau-Fluvalinate	I, A	Approved	LC	10.86	Positive	503.0 → 208.0	10	503.0 → 181.0	26	50
185	Tebuconazole	I, A	Approved	LC	8.95	Positive	308.2 → 70.2	22	308.2 → 125.1	53	120
186	Tebufenozide	I	Approved	LC	8.69	Positive	353.1 → 132.9	22	353.1 → 297.1	0	98
187	Tebufenpyrad	A	Approved	GC	14.04	Positive	334.2 → 117.0	47	334.2 → 145.0	37	180
188	Teflubenzuron	I	Not approved	GC	5.43	Positive	197.0 → 135.0	25	197.0 → 142.0	25	70
189	Tefluthrin	I	Approved	GC	8.41	Positive	177.0 → 127.0	15	177.0 → 87.0	35	70
190	Terbufos	I, AH	Not approved	GC	8.15	Positive	231.0 → 129.0	10	231.0 → 97.0	20	70
191	Terbuthylazine	H	Approved	LC	7.76	Positive	230.0 → 174.0	16	230.0 → 96.0	28	100
192	Tetrachlorvinphos	I	Not approved	LC	8.76	Positive	367.0 → 127.0	16	364.9 → 127.0	16	110
193	Tetraconazole	F, H	Approved	LC	8.43	Positive	372.0 → 159.0	30	372.0 → 70.1	20	100
194	Tetradifon	A	Not approved	GC	14.33	Positive	158.9 → 111.0	15	229.0 → 201.0	20	70
195	Thiabendazole	F	Approved	LC	3.98	Positive	202.0 → 175.0	24	202.0 → 131.0	36	170
196	Thiacloprid	I	Not approved	LC	4.84	Positive	253.0 → 126.0	16	253.0 → 90.0	40	140
197	Thiamethoxam	I	Not approved	LC	3.63	Positive	292.0 → 211.1	8	292.0 → 132.0	22	80
198	Thiodicarb	I	Not approved	LC	6.54	Positive	355.1 → 88.1	8	355.1 → 108.1	8	60
199	Thiophanate-methyl	F	Not approved	LC	5.91	Positive	343.0 → 151.0	20	343.0 → 311.0	10	90
200	Tolclofos-methyl	F, MB, WP	Approved	GC	9.19	Positive	265.0 → 93.0	30	265.0 → 220.0	25	70
201	Triadimefon	F, MB, WP	Not approved	LC	8.07	Positive	294.1 → 69.3	20	294.1 → 197.2	15	100
202	Triadimenol	F, MB, WP	Not approved	LC	8.25	Positive	296.1 → 70.0	10	298.1 → 70.0	10	80
203	Triazophos (hostathion)	I, A	Not approved	LC	8.20	Positive	314.1 → 162.0	19	314.1 → 118.9	35	100
204	Trichlorfon	I, AH, V	Not approved	LC	4.38	Positive	256.9 → 109.0	12	258.9 → 109.0	12	170
205	Trifloxystrobin	F	Approved	LC	9.53	Positive	409.1 → 186.0	12	409.1 → 145.0	52	110
206	Triflumizole	F	Not approved	LC	9.56	Positive	346.1 → 278.0	4	346.1 → 73.0	15	85
207	Triflumuron	I	Not approved	LC	9.21	Positive	359.0 → 156.0	8	359.0 → 139.0	32	120
208	Trifluralin	H	Not approved	GC	7.27	Positive	306.0 → 264.0	5	264.0 → 160.0	15	70
209	Triticonazole	F	Approved	LC	8.42	Positive	318.1 → 70.1	16	320.1 → 70.1	33	110
210	Vinclozolin	F, MB, WP	Not approved	GC	9.08	Positive	212.0 → 145.0	45	212.0 → 109.0	40	70
211	Zoxamide	F	Approved	LC	9.05	Positive	336.0 → 187.1	25	187.1 → 88.9	40	98
1	Atrazine-d5	P–IS	–	LC	6.70	Positive	221.2 → 179.0	15	221.2 → 101.0	30	90
2	Carbendazim-d3	P–IS	–	LC	3.52	Positive	195.1 → 160.1	15	195.1 → 131.9	30	100
3	Chlorpyrifos-d10	P–IS	–	GC	9.86	Positive	324.0 → 260.0	40	324.0 → 195.0	55	70

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	Category [a]	Legal status [b]	Technique [c]	tR (min)	Polarity	Quantification		Confirmation		Frag
							MRM transition (*m/z*)	CE (eV)	MRM transition (*m/z*)	CE (eV)	
4	Cyromazine-d4	P–IS	–	LC	1.20	Positive	171.0 → 86.0	15	171.0 → 129.0	15	100
5	Diazinon-d10	P–IS	–	GC	8.25	Positive	314.0 → 183.0	15	314.0 → 199.0	5	70
6	Linuron-d3	P–IS	–	LC	7.49	Positive	255.1 → 159.8	15	255.1 → 185.0	15	100
7	Pirimicarb-d6	P–IS	–	LC	5.26	Positive	245.2 → 78.2	30	245.2 → 185.1	15	70

CE: Collision Energy; tR: Retention time.

[a] A – acaricide, MB – microbiocide, AH – anthelminthic, F – fungicide, H – herbicide, I – insecticide, R – plant growth regulator, WP – wood preservative, PHP – post-harvest preservative, M – Molluscicide, Met – metabolite, P–IS – Procedural Internal standard.

[b] The legal status reflecting the EU Pesticide Database was considered (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), which is valid for the entire EU.

[c] Gas chromatography (GC) or liquid chromatography (LC), both coupled with tandem triple quadrupole mass spectrometry.

[d] The presence of the acaricide amitraz is evaluated through theses metabolites.

reference.

Analyzing pesticide residues in pet feed poses unique analytical challenges as these feeds are complete compound feeds, formulated to provide a balanced daily ration that meets the nutritional needs of dogs and cats throughout their life stages [26,27]. Consequently, they are complex mixtures, often including both plant-based and animal-derived ingredients with varying fat and protein levels [12–29]. These components introduce a broad range of interfering substances that can compromise the detection of target analytes. This is especially critical in high-fat samples, where co-extracted lipids can significantly impact instrumental analysis by causing signal suppression or enhancement [30]. Given the need to monitor a wide variety of pesticide residues, multiresidue methods represent a practical solution for comprehensive screening. These methods must balance the extraction and detection of numerous analytes with diverse chemical properties, increasing the risk of co-extracting interfering matrix components [31].

There are relatively few studies focusing on the development of methods for pesticide extraction in animal feed, encompassing both complete or feed materials [28–34], and even fewer that include cat and dog feed among other matrices [12,13]. This limited research focus may be partly since pet feed is not intended for productive animals [8]. However, increasing awareness among pet owners about the importance of providing safe and balanced diets for their companion animals is driving the need for more robust monitoring of contaminants in pet feed. Most of available methods utilize some variation of the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique [35], which is widely used in multi-residue pesticide analysis due to its simplicity, cost-effectiveness, and adaptability to different matrices [36–41], although some of these methods employ more elaborated approaches, such as Gel Permeation Chromatography (GPC) with ethyl acetate [29–33] or a three-phase solvent partitioning with water, acetonitrile, and heptane [34]. Regardless of the extraction method employed, most existing methods for pesticide residue analysis in feed rely on LC-MS/MS or GC–MS/MS due to their ability to detect multiple compounds simultaneously with high sensitivity, selectivity, and robustness, making them well-suited for complex matrices.

The co-extraction of lipids during QuEChERS is a major challenge when analyzing pesticides in high-lipid content samples [42]. The clean-up stage aims to remove co-extracted components that may interfere with the detection of target analytes and to allow the pesticide of interest to remain in the liquid phase [30]. Dispersive solid-phase extraction (d-SPE) is commonly employed for this purpose in QuEChERS, traditionally with primary secondary amine (PSA) and anhydrous magnesium sulfate [35,43]. However, in high-fat matrices, d-SPE alone may not fully eliminate lipid interferences. Some studies have incorporated a freezing-out step in the QuEChERS protocol to precipitate lipids and other co-extractives before additional clean-up [44–47]. Although often used as a preliminary stage, freezing-out as a standalone strategy is notably rare. This approach offers a practical alternative to reduce matrix interferences without the need for additional sorbents or chemical reagents.

This study aims to develop and validate a robust and efficient method for the extraction and analysis of pesticides in dog and cat feed, with particular focus on optimizing the clean-up step to address the challenges posed by high-fat matrices. Three different clean-up strategies were tested: d-SPE with PSA, Enhanced Matrix Removal-Lipid (EMR-Lipid), and freezing-out, both alone and in combination. The validated method was further tested on commercial samples to evaluate its applicability.

2. Material and methods

2.1. Sample selection and pre-treatment

For the optimization and validation of the extraction method, we prepared a matrix to account for the variability among different pet feeds available on the market for both dogs and cats. To achieve this, we created a blend of several pet feeds, including three dog and three cat feeds with grain-based, vegetable-based, and grain-free formulations, as detailed below. Before being used to prepare the blended matrix, each of these pet food samples was individually analyzed to ensure the absence of the pesticide residues included in the scope of the method. This absence was confirmed again by separate analysis after the validation process.

To further verify the method's applicability after validation, we applied it to a selection of 16 pet feeds (eight each for dog and cats) purchased from various specialized stores and supermarkets in Gran Canaria (Canary Islands, Spain), representing grain-based, grain-free, and vegetable-based types.

Each pet feed bag was thoroughly shaken to ensure even mixing, and a representative sample was collected from various points within the bag. The feed was then ground in a food processor and stored in a zip-lock bag. The processors were thoroughly cleaned between samples to prevent cross-contamination.

2.2. Reagents, chemicals and standards

Analytical-grade acetonitrile (ACN), methanol (MeOH), and formic acid (FA, HCOOH) were obtained from Honeywell (Morristown, NJ, USA). QuEChERS salts of the AOAC method [43], PSA and EMR-lipid

Fig. 1. Workflow of the sample preparation method and optimization of clean-up strategies for pesticide extraction from pet feed.

were acquired in commercial premixes from Agilent Technologies (Palo Alto, CA, USA). Ultrapure water was generated using a Gradient A10 Milli-Q System (Millipore, Bedford, MA, USA).

Certified standards stock mix solutions of pesticides, aligning with the EU's multi-annual plan [48], were acquired from CPA Chem (Stara Zagora, Bulgaria) in 10 mixes at 100 μg mL^{-1} in ACN. Individual certified standards of additionally selected pesticides (purity 97.1 % to 99.9 %) and a selection of isotopically labeled pesticides (purity 99.3 % to 99.9 %), serving as procedural internal standards (P-IS), were sourced from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich (Saint Louis, USA). From these, individual stock standard solutions at 1000 μg mL^{-1} and mix working solutions at 1 μg mL^{-1} for P-IS and pesticides were prepared in ACN. All solutions were stored in darkness at −20 °C and checked periodically. All selected pesticides and P-IS are detailed in Table 1.

2.3. Sample preparation

Into a 50 mL centrifuge tube, 10 ± 0.05 g of pet feed and 10 mL of ACN-2.5 % FA were added and was vigorously shaken for 1 min. Next, 6 g of $MgSO_4$ and 1.5 g of CH_3COONa were incorporated, the mixture was vigorously shaken for another minute and sonicated for 15 min in an ultrasonic bath (Selecta, Barcelona, Spain). The samples were then placed in a rotary shaker (Ovan, Barcelona, Spain) for 25 min. After that, they were centrifuged for 10 min at 4200 rpm (3175.16 x g) in a 5804 R Eppendorf centrifuge (Eppendorf, Hamburg, Germany).

The supernatant was filtered through 0.20 μm Chromafil® PET filters (Macherey-Nagel, Düren, Germany) into a 5 mL tube and subjected to the different clean-up approaches to be tested:

a) Clean-up with PSA

An aliquot of 3 mL was transferred into a 15 mL tube containing 25 mg of PSA and 150 mg of $MgSO_4$. The mixture was vortexed for 1 min and centrifuged at 4200 rpm for 5 min.

a) Clean-up with EMR-Lipid

An aliquot of 3 mL of the supernatant was added to a 15 mL centrifuge tube containing 500 mg of EMR-Lipid previously activated with 1 mL of ultra-pure water. The mixture was vortexed for 1 min and centrifuged at 4200 rpm for 5 min. Then, 1 mL of the supernatant was transferred to a 2 mL tube containing 400 mg of $MgSO_4$, vortexed, and centrifuged again.

a) Clean-up by freezing-out (selected method)

An aliquot of 3 mL was transferred into a 5 mL Eppendorf tube,

frozen at –24 °C in darkness for 1 h and centrifuged at 4200 rpm for 5 min (one cycle). After that, the supernatant was carefully transferred to a 1.5 mL clean tube and refrozen one, or two times (for cycles 2 and 3, respectively).

Finally, the supernatant collected into a chromatography glass amber vial and either directly analyzed by LC-MS/MS and GC–MS/MS.

Samples for recovery experiments and Quality Controls were spiked with the volume needed to achieve the desired concentration of the standard mix solutions and 50 µl of P-IS mix solution (including blanks and samples). They were left to stand for one hour prior to extraction to ensure proper incorporation.

The sample preparation procedure, including the tested clean-up strategies (PSA, EMR-Lipid, and freezing), is summarized in Fig. 1.

2.4. Instrumental analysis

2.4.1. LC-MS/MS

LC-MS/MS analysis was conducted using a 1290 Infinity II LC System connected to a Triple Quad 6460 mass spectrometer (Agilent Technologies). Separation was achieved on a Poroshell 120 EC—C18 column (2.1 × 100 mm, 2.7 µm; Agilent Technologies) equipped with a guard pre-filter with a 0.3 µm SS frit and a pre-column (2.1 × 5 mm, 1.8 µm; Agilent Technologies) set at 50 °C. Mobile phases were 2 mM ammonium acetate and 0.1 % formic acid in water (A), and 2 mM ammonium acetate in methanol (B) in binary gradient (95 % A – 0.5 min; 95 % A – 1 min; 60 % A – 2.5 min; 15 % A – 8 min; 0 % A – 10 to 14 min; 95 % A – 14.01 min). The flow rate was 0.4 mL/min with an injection volume of 5 µL, and the total runtime was 18 min. Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) was used in both positive and negative modes in dynamic multiple reaction monitoring (dMRM). Nitrogen produced in a NGMs-1 generator (Atlas Copco, Stockholm, Sweden) was used as drying and desolvation gas at a temperature of 190 °C and a flow rate of 11 L min^{-1}. For collision gas, Nitrogen 6.0 (99.9999 % purity, Linde, Dublin, Ireland) was used. Sheath gas temperature was set at 330 °C with a flow of 12 L/min.

2.4.2. GC–MS/MS

GC–MS/MS analysis was performed in a GC System 7890B equipped with a 7693 Autosampler and Triple Quad 7010 mass spectrometer (Agilent Technologies). The chromatographic separations were achieved with two Agilent J&W HP-5MS columns (15 m, 0.25 mm i.d. and 0.25 µm of film thickness each, crosslinked 5 % phenyl-methylpolysiloxane, Agilent Technologies) connected in series by a Purged Ultimate Union (PUU; Agilent Technologies) to use the back-flushing technique (−5.8 mL min^{-1} and 315 °C for 5 min). Helium (99.999 %) was used as the carrier gas and the flow was adjusted by retention time lock (chlorpyrifos methyl tR=9.143 min). The column temperature was maintained at 80 °C for 1.8 min, increased to 170 °C at a rate of 40 °C min^{-1}, then increased to 310 °C at a rate of 10 °C min^{-1} and held for 3 min. Each analysis lasted 21 min 15 s. Injection volume was 1 µL in splitless mode using a 4 mm ultra inert liner with glass wool (Agilent Technologies) and it was set at 250 °C. MS/MS analyses were performed in electron impact (EI) ionization source in multiple reaction monitoring (MRM) mode, using 24-time segments. The EI source and the transfer line were set at 280 °C. Nitrogen 6.0 (99,9999 % purity, Linde) was used as the collision gas at a flow of 1.5 mL min^{-1} with a solvent delay of 3.7 min.

Data analysis was performed using Agilent software MassHunter Quantitative Analysis (for QQQ) version B.07.01 and MassHunter Qualitative Analysis vB.07.00 for both GC–MS/MS and LC-MS/MS.

2.5. Method validation parameters

We followed the recommendations of the European Union in the Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed SANTE 11,312/2021 v2 [49] for the validation process.

The linearity in the response was studied by injecting standards in pet food matrix extract in both instruments at ten concentration levels that ranged from 0.156 to 80 µg/kg in quintuplicate. Accuracy and precision were estimated through recovery experiments in spiked matrix samples (in quintuplicate) at 8 concentrations levels: 0.2, 0.5, 1, 2, 5, 10, 20 and 40 µg/kg. Acceptable values were considered when recoveries were between 70–120 % and relative standard deviations (RSDs) ≤20 %. The lowest concentration level that has acceptable accuracy and precision was set as the Limit of Quantification (LOQ) and the Limit of Detection (LOD) was the lowest point of the calibration curve that had a signal-to-noise ratio > 3 (Peak-to-Peak) and an accuracy between 80 and 120 %. To determine the LODs matrix-matched calibration curves were prepared in triplicate, covering a range of 0.002 to 80 µg/kg, and analyzed on each instrument.

2.6. Quantification

Analyte confirmation was performed with the acquisition of two MS/MS transitions, with a maximum ion ratio tolerance of ±30 % between the quantification and the confirmation transition. In addition, retention time maximum deviation of ± 0.1 min between the analyte in the sample and the reference standard was considered acceptable. For analytes with chiral isomers, results are reported as the sum of all isomers, in line with the residue definition. However, when the residue definition specifies a single enantiomer, each enantiomer was determined and quantified separately.

3. Results and discussion

3.1. Chromatographic and mass spectrometric parameters

MRM transitions for LC-MS/MS and GC–MS/MS were selected from an existing database established in our laboratory for environmental [50,51] and biological matrices [52–55]. To optimize method performance for this complex matrix, certain chromatographic conditions were refined.

First, all available transitions for the target analytes were tested by injecting a 20 µg/kg prepared standard containing all pesticides and P-IS in the solvent (ACN 2.5 %FA) against that of the pet feed matrix extract in both equipment. Transitions were chosen based on their selectivity and signal intensity, prioritizing those that provided the highest sensitivity for both quantification and confirmation. In LC-MS/MS, most compounds were analyzed in positive mode, with precursor ions corresponding to $[M + H]^+$, while a smaller subset of analytes showed improved response in negative mode ($[M–H]^-$). For some compounds, such as aldicarb or famoxadone, ammonium adducts ($[M+NH4]^+$) were selected due to higher signal intensity. For analytes with characteristic isotope patterns (e.g., compounds containing Cl or Br like hexaconazole or metrafenone), transitions including their isotopic ions (e.g., 35Cl/37Cl or 79Br/81Br) were included to improve selectivity.

Retention times were re-evaluated, and ion ratios for quantifier and qualifier ions were adjusted to ensure accurate identification and to minimize interferences from co-eluting matrix components.

To streamline the workflow and reduce analyte loss, acetonitrile extracts were injected directly into the LC-MS/MS and GC–MS/MS without evaporation or solvent exchange. This approach prevents the loss of volatile analytes, a known issue during solvent exchange [56]. Although acetonitrile is not the most common solvent for GC-MS/MS, it was retained due to its compatibility with our analyte panel and its satisfactory chromatographic performance. Several studies, including previous work from our laboratory [50–58], have demonstrated its suitability for this purpose. Accordingly, the GC–MS/MS conditions were adapted to accommodate this solvent; for example, the initial oven temperature was set at 80 °C, close to the boiling point of ACN, to prevent solvent condensation in the column and improve reproducible

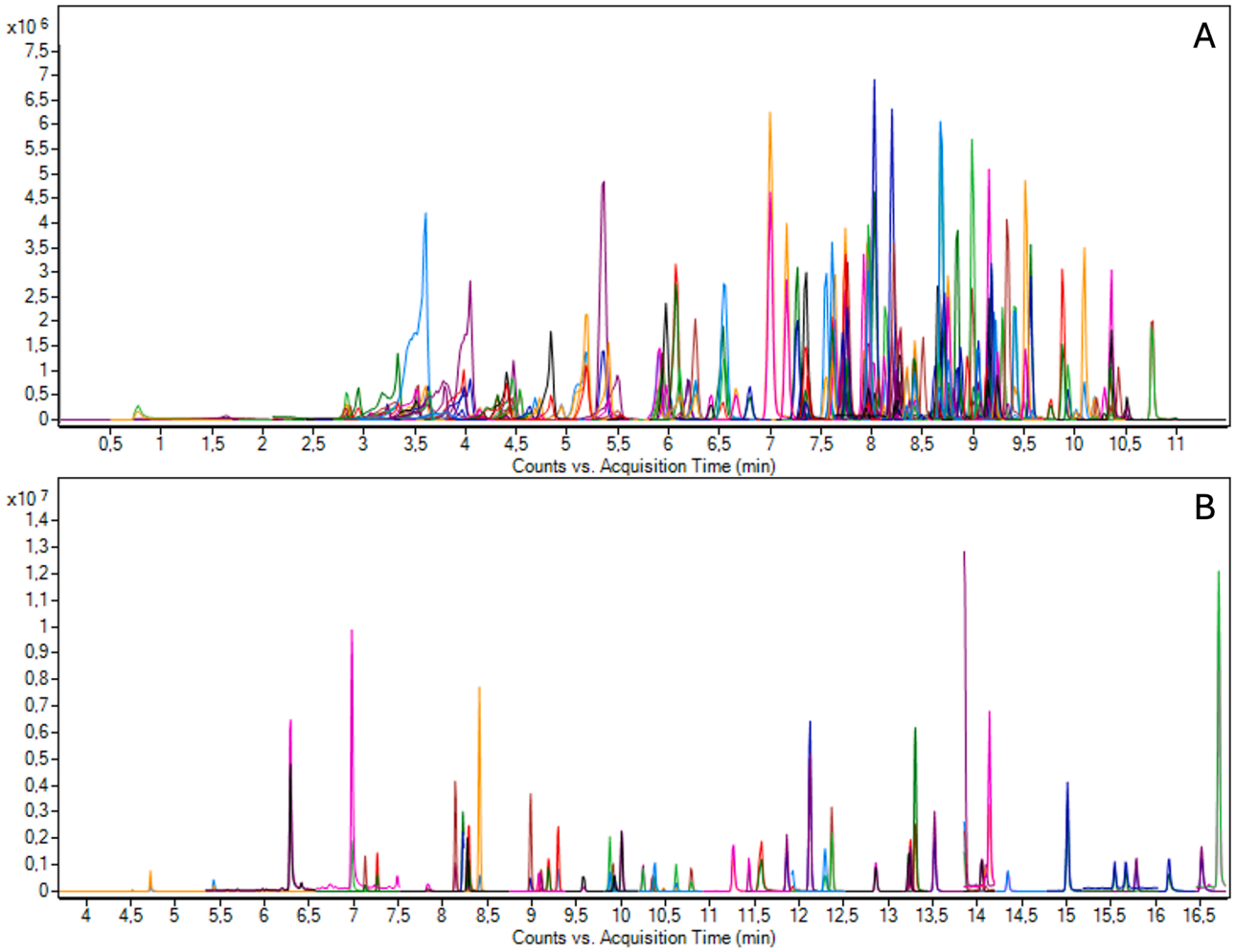

Fig. 2. Chromatograms of the analyses by LC-MS/MS (A) and GC–MS/MS (B) of pet feed matrix extract spiked with the 211 compounds and the P-IS at 20 μg/kg.

peak shapes.

The complete list of 211 analyzed compounds and P-IS, including their retention times, MRM transitions, collision energies, fragmentor values, usage categories, and legal status in the EU, is presented in Table 1. A representative chromatogram of blank pet feed matrix spiked at 20 μg/kg is shown in Fig. 2.

3.2. Optimization of clean-up step

The QuEChERS method was chosen for its rapid, economical, and straightforward approach. Specifically, we used as a starting point a single-step QuEChERS method previously optimized and validated in our laboratory for soil samples, which had demonstrated satisfactory performance in matrices with high organic matter loads [50,51]. However, due to the distinct composition of pet feed, further adaptation was required to address its particular complexity.

Given the high-fat content of pet feed samples due to the inclusion of animal-derived fats and oils as well as certain high-fat ingredients like fish meal or meat by-products, it was necessary to include a clean-up stage to ensure effective matrix interference removal. To optimize this step, we evaluated different techniques with the goal of retaining as many analytes as possible while preserving the precision and accuracy of the original method. The tested approaches included the standard QuEChERS clean-up with dispersive PSA and anhydrous magnesium sulfate ($MgSO_4$), EMR-Lipid from Agilent Technologies, and freezing-out. The QuEChERS method with dispersive PSA and $MgSO_4$ clean-up is widely recognized for its simplicity and efficiency in removing polar matrix interferences, making it a benchmark for comparison [43]. The EMR-Lipid, a commercial sorbent specifically designed for lipid-rich samples, was included due to its targeted approach to lipid removal, which is particularly relevant for high-fat matrices [42–47,43, 48–59]. Lastly, the freezing-out strategy was included as a cost-effective and sustainable alternative, avoiding the use of additional sorbents or chemicals while potentially retaining a higher number of analytes.

Before testing the different clean-up strategies, we noticed a visible phase separation in extracts that had been temporarily stored at 4 °C, suggesting the precipitation of fats and other co-extracts. This observation led us to try freezing as a potential clean-up strategy, which appeared to remove matrix interferences more effectively than simple refrigeration. Different freezing durations (30, 45, and 60 min) were tested for a single cycle, and 60 min at –24 °C was selected based on visual clarity and separation efficiency. These trials were part of the routine method setup and were not formally documented as part of the optimization study.

The clean-up experiments were conducted at a concentration of 20 μg/kg, with each experiment performed in triplicate. The clean-up was applied to the supernatant obtained after the centrifugation. Detailed procedures are provided in the Materials and Methods section, while Fig. 1 summarizes the steps followed. The complete results of the clean-up optimization study are presented in Table S1 of the supplementary material.

Fig. 3 shows the performance of each clean-up method in terms of number of compounds and recovery percentages. The compounds are categorized according to their recovery percentages for each type of

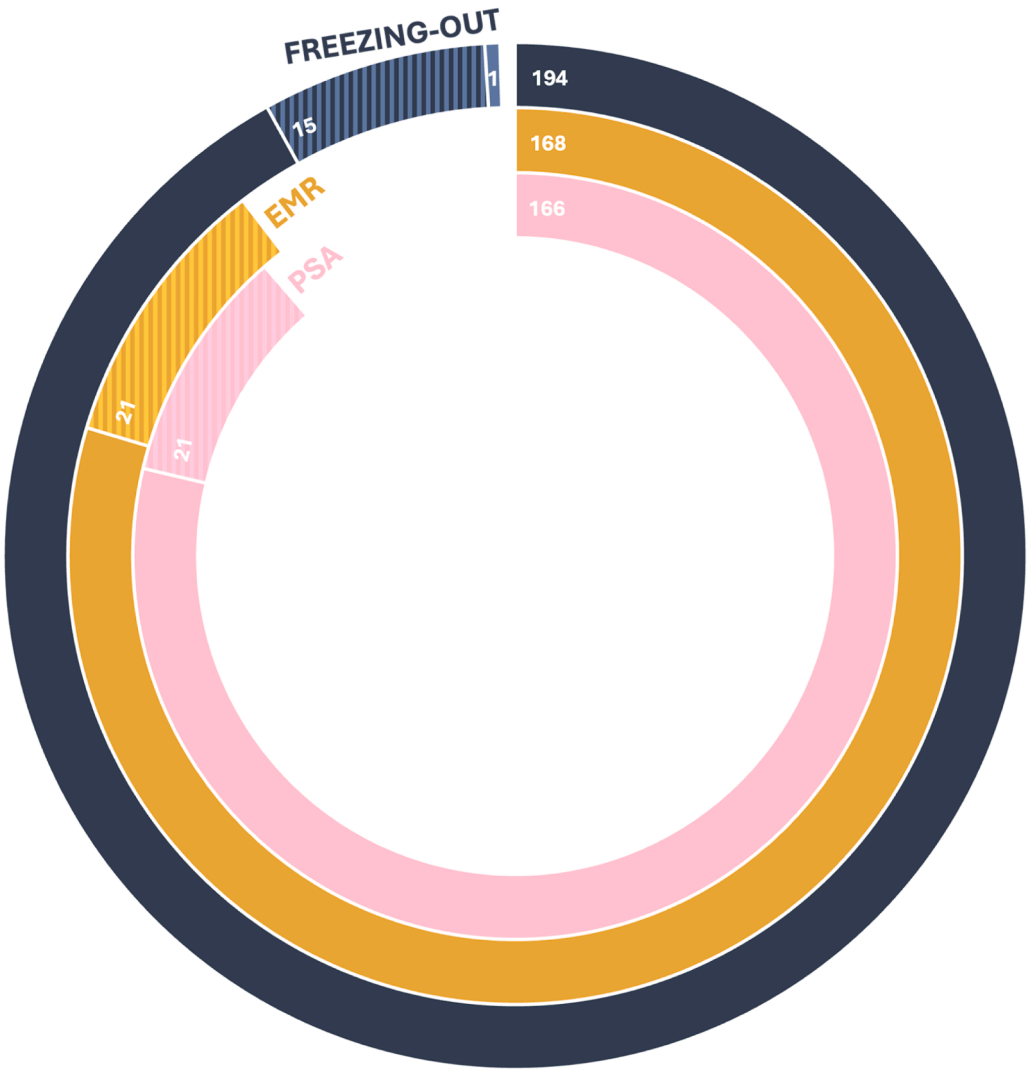

Fig. 3. Performance of clean-up tested with in terms of recovery percentages. PSA is represented in pink, EMR in yellow, and freezing-out in blue. Recovery percentages between 70 and 120 % are shown in dark solid color, between 60 and 70 % with a lined pattern, and between 120 % and 130 % in a lighter solid color. RSDs for all compounds are below 20 %.

clean-up: PSA (pink), EMR (yellow), and freezing-out (blue), and further divided as follows: between 70 and 120 % (darker solid color), between 120 and 130 % (lighter solid color), and between 60 and 70 % (lined pattern). The chosen recovery range of 70–120 % aligns with the SANTE guidelines, which designate this interval as acceptable for method validation. We also included compounds with recoveries in the 60–70 % and 120–130 % ranges as areas for potential improvement, as these values fall slightly outside the primary acceptance range but may still be considered acceptable with further optimization. All compounds included in the graph displayed acceptable RSDs below 20 %.

Freezing-out provided the best overall performance, with 91.9 % of the compounds falling within the 70–120 % recoveries and 7.5 % in the established extended range, covering nearly 100 % of compounds. Only formetanate fell outside the acceptable range, with a recovery of 59 %. In comparison, EMR-Lipid and PSA clean-up techniques achieved <80% each, with a complete loss of 22 and 24 compounds, respectively.

To further evaluate its potential, we tested the integration of a freezing step prior to the d-SPE clean-ups using PSA and EMR-Lipid to determine its impact on analyte recoveries. For EMR-Lipid, the addition of a freezing step slightly improved its performance, with 176 compounds achieving recoveries within the 70–120 % range and 11 within the 60–70 % range, but still with a complete loss of 24 compounds. However, for PSA, the inclusion of a freezing step did not lead to overall improvement and even resulted in the loss of some compounds compared to using PSA clean-up alone. Only 166 compounds fell within the 70–120 % range, while 14 were in the extended 60–70 % range.

Based on these findings, freezing-out was selected as the unique clean-up method to proceed with further optimization. To visualize the impact of the different clean-up strategies on chromatographic performance, the peak shapes of four selected compounds (clothianidin, mepanipyrim, boscalid, and cyprodinil) are compared in Fig. 4 for each type of tested clean-up strategy: PSA, EMR-Lipid, and freezing-out (1, 2, and 3 cycles). Broader or slightly distorted peaks are observed for the PSA and EMR-Lipid clean-up methods, particularly for mepanipyrim and cyprodinil. In contrast, freezing-out clean-up methods produce sharper and more symmetrical peaks, as observed with clothianidin and boscalid, demonstrating better performance in reducing matrix interference.

Next, once we had selected the freezing-out as the clean-up method, we further investigated the effect of increasing the number of freezing cycles to determine the optimal conditions for the clean-up step. We evaluated 1, 2, or 3 freezing cycles, with each cycle consisting of freezing the samples for one hour at –24 °C in darkness, followed by centrifugation. The results of this experiment are presented in Fig. 5, where compounds are numbered according to Table 1 and recovery values are shown for each freezing cycle. Shades of blue (from light to dark)

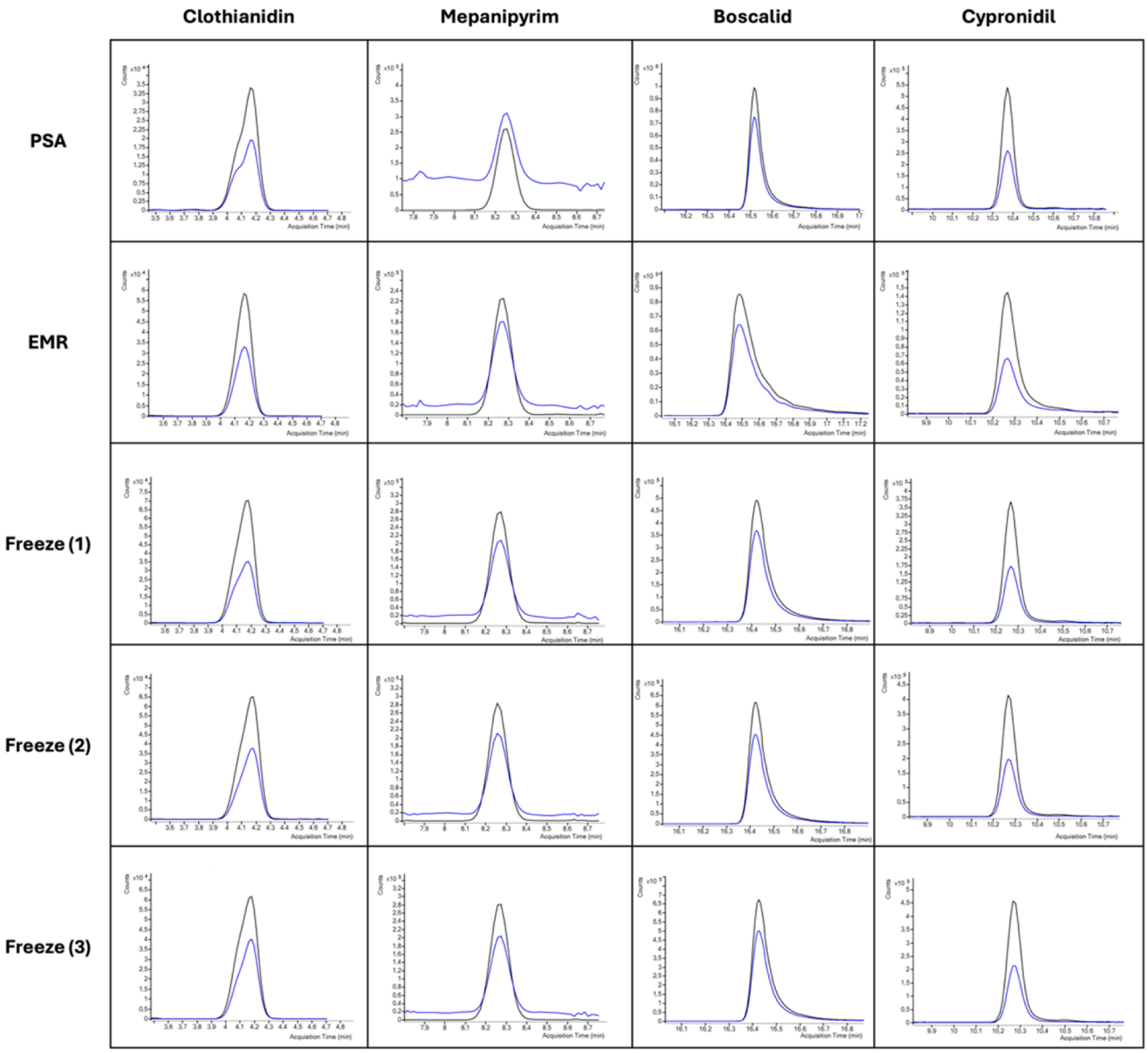

Fig. 4. Chromatographic peak shapes of four selected compounds: clothianidin, mepanipyrim, boscalid and cyprodinil (two analyzed by LC-MS/MS and two by GC–MS/MS) shown on the horizontal axis, under different clean-up procedures tested: PSA, EMR-Lipid, and freezing-out with 1, 2, and 3 cycles, indicated on the vertical axis.

represent the increasing number of cycles. Bold dotted lines and a gray shaded area highlight the primary recovery limits (70–120 %) according to SANTE guidelines, while gray dotted lines indicate a broader range (60–130 %) to assess borderline recoveries.

According to our results, adding a second freezing cycle improved the recoveries of eight compounds, bringing them into the desirable range of 70–120 %. Additionally, formetanate, previously outside the acceptable range, moved into the extended range of 60–70 % with a mean recovery of 61.1 %, resulting in a total of nine compounds that benefited from the second cycle. While the improvement of adding a second freezing cycle over a single cycle is evident, moving to three cycles does not provide additional benefits. Although fenazaquin and pymetrozine showed slight enhancements, recoveries for ethirimol, methamidophos, and permethrin were reduced. These results are consistent with the findings shown in Fig. 4: while there is little difference in peak shape and sensitivity between two and three cycles, the improvement over a single cycle is noticeable in both peak quality and sensitivity.

Considering these results, two freezing cycles were selected as the optimal number for the freezing-out clean-up step, balancing efficiency and performance. The final procedure, incorporating this optimized clean-up process, is detailed in Section 2.3 (Sample Preparation) of the Materials and Methods.

A comparative summary of the clean-up strategies, including estimated reagent and consumable costs and hands-on time per sample, is provided in Table S2 (Supplementary Material). Freezing-out is by far the most cost-effective option among the three, although it requires more processing time due to the two freezing cycles. Nevertheless, this is offset by the substantial savings in consumables and, more importantly, by its superior analytical performance, achieving the highest number of compounds within the acceptable recovery range, as shown above.

3.3. *Matrix effect study*

Cat and dog feed are highly complex and diverse matrices, with substantial variation among products due to the range of compositions available and the specific nutritional requirements for each species. This variability can lead to signal suppression or enhancement in analytical

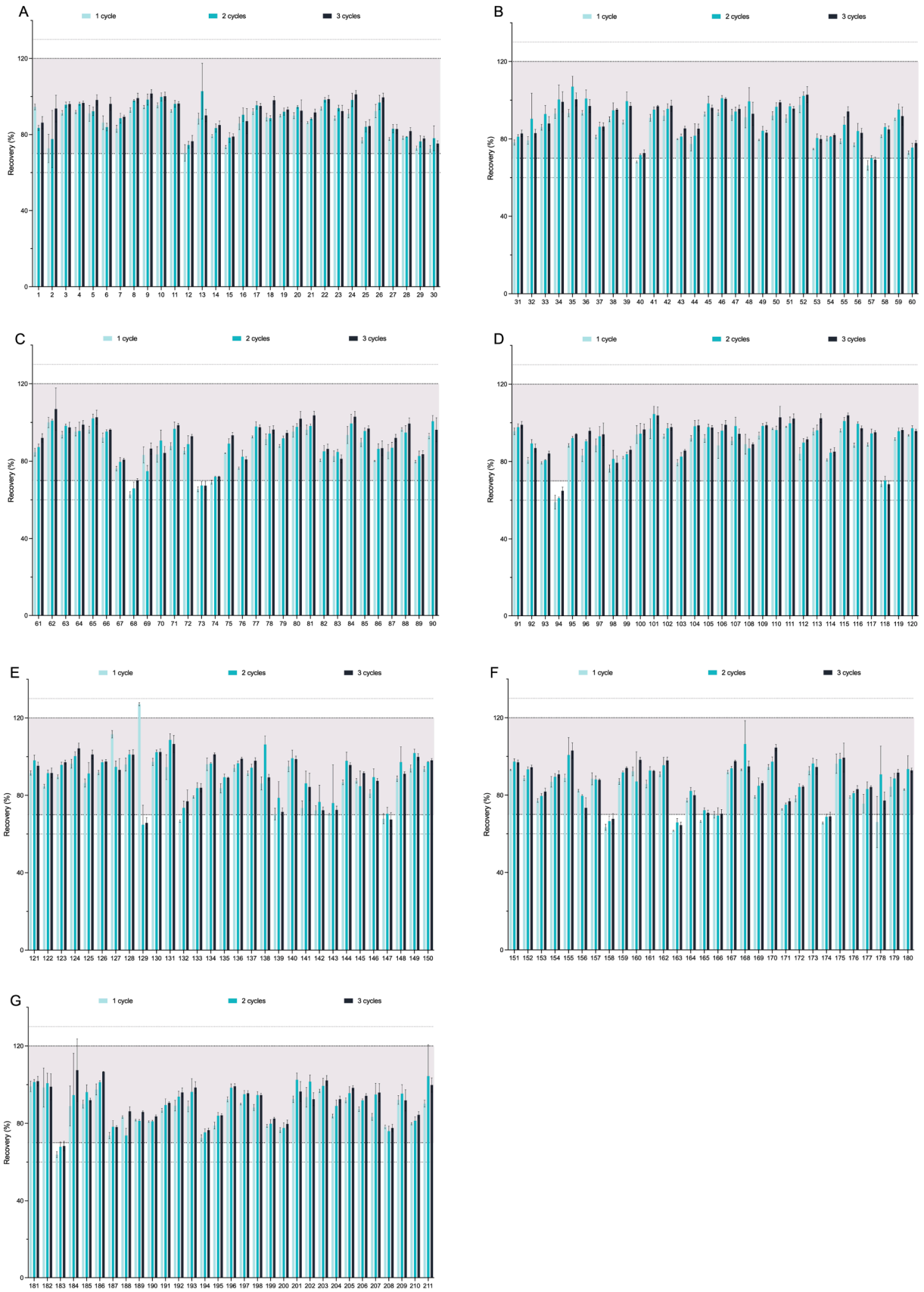

Fig. 5. Optimization of freezing cycles for the clean-up step. Each compound (numbered as in Table 1) is shown with results across three freezing cycles, represented in shades of blue from light to dark as the number of cycles increases. Compounds are numbered as in Table 1. Bold dotted lines and a gray shaded area mark the 70 % to 120 % recovery range (SANTE guidelines). Gray dotted lines indicate an extended range (60 %–130 %). The graph is divided into 7 panels for clarity.

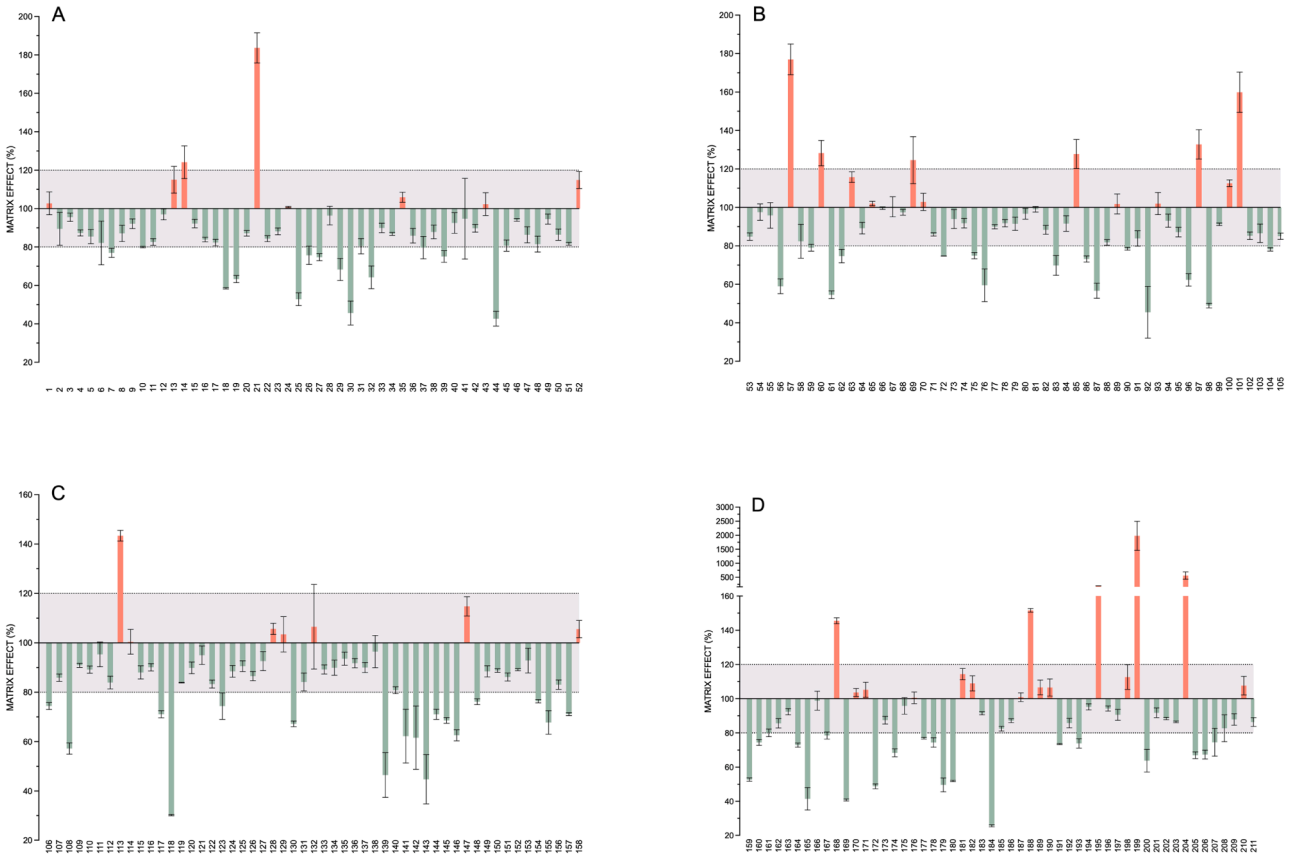

Fig. 6. Matrix Effect Study. The bars depict the average matrix effect percentage (*ME* %) for each analyte, and the whiskers indicate the standard deviation. The space between the dotted lines denotes the acceptable tolerance range, within which the matrix effects are considered negligible for the analyte. The compounds are presented in order with the number assigned in Table 1 and have been divided into four panels for clarity.

instruments, which poses challenges in quantitative analyses [31–47,43, 48–61]. To address these issues, the optimized freezing-out clean-up step described in the previous section was designed to minimize matrix interferences by removing a significant portion of interfering components. However, despite this improvement, a matrix effect study remains crucial to assess any residual influences and to determine the most appropriate calibration approach accordingly.

The matrix effect (*ME*) was assessed by comparing the slopes of the calibration curves (*ME* (%) = (S_M/S_s) ×100) prepared in solvent (S_s) (ACN 2.5 % FA) and pet feed matrix extracted with the selected method (S_M), covering the range of 1.25 to 40 μg/kg in triplicate. All curves were adjusted to a linear regression curve. Thus, the effect of the matrix components on the signal is qualified as the percentage of suppression or enhancement if the *ME* of each compound is below or above 100 %, respectively. A tolerance range where a compound had no significant matrix effects was established between 80 % and 120 % [49] and it is represented in a gray area in Fig. 6. The ME ranges for each compound and replicate are provided in Supplementary Table S3.

As can be seen, most compounds (133) do not exhibit any matrix effect. However, among those that do, the most marked tendency is the suppression in the signal (64 compounds), with 21 compounds under 60 % of ME, while only 14 show signal enhancement. Of those, 9 are above 140 % with trichlorphon and thiophanate-methyl reaching 1213.6 % and 1977.0 %, respectively. This trend has been reported in other studies, potentially due to co-eluting matrix components competing with analytes during the ionization process, thereby reducing the efficiency of ion generation for the target compounds [12–47,43, 48–63].

A small number of compounds with matrix effects was detected in GC–MS/MS, only 22, representing 40 % of the compounds analyzed in this equipment. In comparison, 56 out of 156 compounds analyzed by LC-MS/MS exhibited matrix effects. This indicates similar percentage of compounds with matrix effects in both techniques.

To compensate the observed variability, we opted for using matrix-matched calibration to ensure accurate quantification of the target analytes.

3.4. Method validation

The optimized method was validated under the terms stated in the "Method Validation Parameters" section.

Linearity in the response was satisfactory for both techniques in the studied range (0.156–80 μg/kg) in the pet feed matrix extract, with R^2 values exceeding 0.99 for all the analytes.

The method demonstrated acceptable accuracy (recoveries within the range of 70–120 %) and precision (RSD<20 %) for all tested concentrations (0.2, 0.5, 1.0, 2, 5, 10, 20 and 40 μg/kg) from the LOQ of each analyte to the highest level. However, some of the analytes showed at least one recovery value outside the desirable range. These compounds were included due to their relevance in pesticide monitoring, with the following criteria: recoveries between 60–130 % and RSD<20 %. While the SANTE guideline allows the validation of compounds with recoveries between 30–140 % if they exhibit high reproducibility (RSD<20 %) in routine analyses [49], we decided to include only those within a narrower range to balance inclusivity of key analytes with a higher level of reliability in the method's performance.

Of the 211 compounds included in the validation, 71.1 % achieved a LOQ of 1 μg/kg or lower, with nearly 25 % reaching the minimum LOQ of 0.2 μg/kg. This is at least ten times below the generic MRL of 10 μg/kg established by Regulation (EC) No 396/2005 for food and feed of plant and animal origin intended for production animals [25]. Although the

Table 2
Method validation results: LOD, LOD, Linearity, recoveries (REC, %) and RSD (%). Concentrations are expressed in μg/kg.

N°	Compound	Technique[a]	LOD[b]	LOQ[c]	Linearity	0.2		0.5		1		2		5		10		20		40	
						REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD
1	2-Phenylphenol	GC	0.625	1.0	0.9988	–	–	–	–	72.0	3.8	70.9	7.4	68.6	4.2	76.5	5.3	76.8	1.8	77.1	4.3
2	Abamectin	LC	10.000	20.0	0.9957	–	–	–	–	–	–	–	–	–	–	–	–	114.0	8.6	89.7	5.6
3	Acetamiprid	LC	0.625	1.0	0.9989	–	–	–	–	108.0	8.4	101.9	4.7	92.0	3.2	97.9	3.4	98.5	1.6	95.9	4.4
4	Aldicarb	LC	0.039	0.2	0.9981	90.6	6.0	84.2	5.3	84.1	3.2	91.0	4.0	91.6	1.3	96.8	3.3	97.2	1.5	96.6	4.2
5	Aldicarb-sulfone	LC	1.250	2.0	0.9972	–	–	–	–	–	–	116.9	6.8	92.7	4.9	96.0	5.0	95.7	1.9	93.0	2.7
6	Aldicarb-sulfoxide	LC	5.000	5.0	0.9966	–	–	–	–	–	–	–	–	106.1	8.0	99.1	7.2	87.3	8.0	82.5	5.9
7	Atrazine	LC	0.156	0.5	0.9980	–	–	92.6	3.8	80.4	7.8	81.1	6.5	80.9	4.2	88.0	3.3	89.0	1.8	87.2	2.8
8	Azinphos-methyl	LC	0.313	0.5	0.9987	–	–	119.8	14.5	93.2	2.4	95.0	10.4	92.1	5.8	100.3	2.8	98.8	2.9	99.4	3.8
9	Azoxystrobin	LC	0.039	0.5	0.9986	–	–	72.8	12.7	85.8	5.5	92.7	4.0	96.4	6.3	104.3	3.0	102.8	1.6	101.3	5.1
10	Benalaxyl	LC	0.078	0.2	0.9984	95.4	19.2	87.0	7.2	89.4	8.1	91.8	2.4	94.0	3.1	98.7	2.8	100.3	2.0	98.1	3.5
11	Bendiocarb	LC	0.313	0.5	0.9988	–	–	114.5	5.9	96.4	8.2	96.2	5.3	90.8	4.5	98.7	3.8	99.2	1.7	97.1	3.0
12	Bifenthrin	GC	1.250	2.0	0.9991	–	–	–	–	–	–	95.2	5.5	83.0	5.3	75.8	6.4	70.4	8.5	69.8	4.6
13	Bitertanol	LC	0.625	1.0	0.9952	–	–	–	–	125.4	6.5	102.5	11.0	96.2	2.8	94.2	7.6	97.9	7.7	104.1	2.9
14	Boscalid	GC	0.078	0.2	0.9996	71.7	16.6	79.9	7.1	79.2	2.5	80.2	5.0	74.7	3.5	74.9	3.8	72.0	1.4	70.0	5.5
15	Bromopropylate	GC	0.156	0.2	0.9998	85.2	8.4	79.9	2.8	79.2	1.9	81.6	6.6	76.5	2.2	75.7	2.6	73.7	1.3	72.6	4.8
16	Bromuconazole (two isomers)	LC	5.000	5.0	0.9980	–	–	–	–	–	–	–	–	98.2	3.8	88.4	12.6	89.6	7.4	90.5	6.7
17	Bupirimate	LC	0.313	0.5	0.9993	–	–	113.9	8.0	90.9	6.7	91.9	6.0	91.0	4.2	95.3	6.9	96.7	2.5	93.1	3.7
18	Buprofezin	LC	0.156	0.5	0.9978	–	–	89.6	4.9	79.7	8.0	82.3	9.0	78.6	2.1	83.0	2.6	82.8	1.2	82.7	2.5
19	Cadusafos (ebufos)	LC	0.156	0.5	0.9980	–	–	100.1	9.5	82.7	7.1	83.5	8.7	81.3	5.7	85.6	3.5	85.4	1.4	84.4	3.9
20	Carbaryl	LC	0.156	0.2	0.9989	118.2	5.1	92.7	4.8	87.1	2.8	90.1	8.2	91.3	2.6	95.3	2.4	95.0	1.8	93.8	3.8
21	Carbendazim	LC	0.156	0.5	0.9982	–	–	101.7	3.2	88.4	5.9	92.9	5.6	89.4	1.6	86.4	22.9	90.8	6.9	87.7	6.2
22	Carbofuran	LC	0.156	0.5	0.9989	–	–	106.7	6.3	94.0	3.2	98.4	5.1	98.4	1.4	103.0	3.4	102.8	1.7	102.2	3.3
23	Carbofuran-3-hydroxy	LC	0.313	0.5	0.9983	–	–	108.7	8.8	89.5	5.9	91.3	6.5	89.3	1.7	96.7	6.5	96.9	2.5	94.8	4.6
24	Chlorantraniliprole	LC	0.625	1.0	0.9979	–	–	–	–	116.0	10.3	103.5	8.1	93.9	4.4	100.1	4.9	99.1	4.5	101.0	3.8
25	Chlorfenapyr	GC	1.250	2.0	0.9991	–	–	–	–	–	–	93.1	10.6	87.1	1.6	82.0	5.3	79.4	2.1	75.9	5.6
26	Chlorfenvinphos	LC	0.625	1.0	0.9995	–	–	–	–	98.8	12.4	102.2	8.6	97.9	5.1	100.9	4.9	96.9	1.8	91.6	4.0
27	Chlorobenzilate	GC	0.078	0.2	0.9997	94.5	12.2	92.5	3.9	88.3	1.9	88.5	4.6	84.8	3.1	82.4	4.4	81.0	1.9	78.4	4.7
28	Chlorpropham	GC	0.313	0.5	0.9995	–	–	79.1	13.6	83.7	7.6	81.6	4.3	76.6	2.7	78.8	4.3	76.3	0.9	76.4	4.5
29	Chlorpyrifos	GC	0.156	0.2	0.9993	78.2	19.0	75.5	10.3	74.3	2.8	78.2	5.1	73.9	4.0	72.8	3.4	70.6	1.5	68.9	5.5
30	Chlorpyrifos-methyl	GC	0.313	0.5	0.9993	–	–	76.4	8.1	79.4	4.0	81.5	2.2	73.5	2.3	73.3	3.1	69.9	2.0	66.0	5.1
31	Chlorthal-dimethyl	GC	0.078	0.2	0.9994	93.6	3.9	88.9	4.4	85.0	1.5	85.6	4.6	81.7	3.3	81.1	3.7	79.0	1.5	76.6	5.5
32	Clofentezine	LC	0.313	0.5	0.9985	–	–	77.2	9.7	78.1	10.2	75.9	10.9	84.2	5.9	86.9	2.8	84.4	5.6	81.4	2.3
33	Clothianidin	LC	0.625	1.0	0.9953	–	–	–	–	111.6	8.8	102.9	10.0	87.1	5.6	89.1	4.1	89.6	2.4	87.2	4.1
34	Coumachlor	LC	0.625	1.0	0.9982	–	–	–	–	103.2	9.0	97.1	12.0	91.2	12.6	104.7	10.0	95.5	6.7	98.3	4.4
35	Coumaphos	LC	0.156	1.0	0.9974	–	–	–	–	85.8	13.9	92.6	12.3	93.0	3.3	102.3	3.8	102.3	4.1	97.2	4.1
36	Cyazofamid	LC	1.250	2.0	0.9981	–	–	–	–	–	–	107.9	8.7	99.6	4.4	103.0	2.6	101.6	3.0	99.9	3.8
37	Cyflufenamid	GC	0.156	0.2	0.9994	102.9	7.2	95.9	7.4	89.3	5.4	92.4	5.6	83.7	4.5	85.7	3.6	82.7	0.9	81.8	4.8
38	Cymoxanil	LC	5.000	5.0	0.9991	–	–	–	–	–	–	–	–	106.8	1.6	102.3	3.5	96.6	2.0	93.2	1.7
39	Cyproconazole (two isomers)	LC	0.625	1.0	0.9988	–	–	–	–	112.3	3.1	100.3	7.0	96.6	4.3	99.5	5.2	99.2	2.0	95.3	2.4
40	Cyprodinil	GC	0.313	0.5	0.9994	–	–	83.4	11.5	79.0	4.7	76.5	4.0	71.9	4.8	71.3	4.8	70.2	2.0	68.8	4.5
41	Demeton-S-methyl	LC	10.000	10.0	0.9999	–	–	–	–	–	–	–	–	–	–	117.5	10.0	94.7	16.0	103.5	13.8
42	Demeton-S-methyl-sulfone (Dioxydemeton)	LC	0.625	1.0	0.9983	–	–	–	–	110.2	4.6	101.3	4.0	90.9	3.9	95.9	3.7	95.1	2.2	91.6	3.9
43	Diazinon	GC	0.156	0.2	0.9997	101.8	3.6	88.9	4.1	85.8	2.7	85.0	5.8	79.2	4.4	80.2	3.2	78.3	1.3	78.3	4.8
44	Dichlorvos	GC	0.625	1.0	0.9973	–	–	–	–	119.5	4.8	102.9	5.0	91.3	5.7	84.4	9.4	78.7	6.9	73.4	6.4
45	Diethathyl (-ethyl)	LC	0.078	0.2	0.9991	96.9	12.4	81.5	6.2	89.2	5.5	101.9	8.4	97.8	1.6	101.5	4.2	102.3	2.4	100.4	3.7
46	Diethofencarb	LC	0.156	0.5	0.9994	–	–	102.2	12.3	92.7	6.6	97.5	5.0	99.8	2.3	106.4	3.1	104.9	2.0	103.8	3.9
47	Difenoconazole	LC	0.625	2.0	0.9930	–	–	–	–	–	–	76.8	7.2	81.0	5.5	87.8	2.4	92.7	1.9	93.0	4.3
48	Diflubenzuron	LC	2.500	5.0	0.9934	–	–	–	–	–	–	–	–	85.7	8.1	87.3	14.3	90.5	8.2	94.0	5.3
49	Diflufenican	GC	0.156	0.2	0.9996	88.6	9.1	87.9	4.8	85.8	2.0	87.5	6.0	84.4	1.9	85.3	3.0	83.4	1.6	81.8	5.0
50	Dimethenamid	LC	0.156	0.5	0.9992	–	–	102.0	3.4	93.9	3.7	101.8	8.4	98.7	4.1	104.8	2.9	102.1	1.8	99.5	3.0
51	Dimethoate	LC	0.313	0.5	0.9979	–	–	114.2	7.2	88.9	5.4	91.7	5.8	88.7	2.0	91.8	3.5	93.6	1.8	90.4	2.8

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	Technique[a]	LOD[b]	LOQ[c]	Linearity	0.2		0.5		1		2		5		10		20		40	
						REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD
52	Dimethomorph (two isomers)	LC	0.156	0.2	0.9989	118.7	15.3	97.1	7.4	102.6	9.4	100.7	9.6	105.5	3.9	114.6	3.3	113.9	1.8	113.4	5.1
53	Diniconazole-M	GC	0.156	0.2	0.9995	111.3	6.3	93.5	3.5	84.9	2.9	87.0	6.2	82.6	3.3	80.5	3.3	79.8	1.2	77.9	5.6
54	Diphenylamine	GC	0.625	1.0	0.9989	–	–	–	–	117.0	5.6	94.6	10.2	83.3	7.2	83.4	4.9	79.4	1.6	78.2	4.3
55	Epoxiconazole	GC	0.156	0.2	0.9996	83.8	8.0	87.9	6.5	88.2	3.5	88.3	6.1	82.2	2.7	83.0	3.0	81.2	0.7	79.0	5.2
56	Ethion (diethion)	GC	0.313	0.5	0.9994	–	–	97.7	6.3	86.2	8.6	91.3	4.9	80.3	5.2	78.2	3.4	76.5	2.0	72.4	6.0
57	Ethirimol	LC	0.625	1.0	0.9982	–	–	–	–	116.6	5.6	93.4	2.0	78.3	1.8	79.6	2.3	77.8	2.1	74.7	1.9
58	Ethofumesate	GC	0.078	0.2	0.9992	108.1	4.4	97.8	2.7	91.7	1.6	89.3	4.0	87.4	2.5	86.6	6.4	85.3	2.9	81.6	6.8
59	Ethoprophos	LC	0.313	0.5	0.9990	–	–	112.7	8.8	96.9	8.9	98.1	5.6	93.5	5.4	96.3	6.4	94.7	1.9	91.7	3.4
60	Etofenprox	GC	0.625	1.0	0.9996	–	–	–	–	84.8	5.9	79.7	6.0	74.8	3.2	73.4	3.6	70.3	1.4	67.3	5.6
61	Etoxazole	LC	0.625	1.0	0.9972	–	–	–	–	103.8	4.1	88.4	9.7	83.2	2.9	84.2	4.1	82.0	1.6	79.9	2.9
62	Famoxadone	LC	1.250	5.0	0.9977	–	–	–	–	–	–	–	–	85.6	13.3	84.4	9.1	90.8	1.7	89.7	6.4
63	Fenamidone	LC	0.156	0.2	0.9979	107.4	13.9	101.0	6.0	97.0	4.8	104.5	7.2	105.9	6.3	113.9	1.8	108.5	1.7	106.0	4.5
64	Fenamiphos	LC	0.078	0.2	0.9986	88.8	7.0	93.6	10.9	88.4	5.1	97.5	4.9	95.7	6.9	103.3	2.7	101.5	2.2	99.7	4.3
65	Fenamiphos-sulfone	LC	0.078	0.2	0.9967	102.5	11.7	100.1	18.0	85.6	10.3	91.0	7.2	92.3	1.5	106.9	4.0	107.6	2.2	109.0	4.0
66	Fenamiphos-sulfoxide	LC	0.625	1.0	0.9988	–	–	–	–	114.9	5.1	103.7	7.7	96.3	2.4	103.4	3.3	103.5	2.3	103.3	3.5
67	Fenarimol	GC	0.313	0.5	0.9996	–	–	110.1	1.2	92.7	1.2	86.3	5.8	78.2	3.7	76.5	3.2	72.9	1.6	70.4	5.0
68	Fenazaquin	GC	2.500	10.0	0.9996	–	–	–	–	–	–	–	–	–	–	60.1	7.1	61.4	1.8	61.1	5.5
69	Fenbuconazole	GC	0.625	1.0	0.9995	–	–	–	–	96.0	12.3	92.2	6.1	79.0	2.4	76.1	3.2	73.6	1.1	70.8	6.1
70	Fenhexamid	LC	5.000	5.0	0.9975	–	–	–	–	–	–	–	–	98.2	9.8	102.7	7.3	89.7	6.2	90.3	4.1
71	Fenoxycarb	LC	0.156	0.5	0.9992	–	–	109.2	7.4	98.8	12.7	100.0	5.4	99.7	3.1	98.5	2.9	98.4	2.3	96.8	6.0
72	Fenpropathrin	LC	5.000	5.0	0.9968	–	–	–	–	–	–	–	–	91.8	3.4	85.8	6.3	80.9	3.9	77.4	3.1
73	Fenpropidin	LC	0.156	1.0	0.9977	–	–	–	–	70.6	6.4	83.4	9.8	81.1	3.7	87.2	2.2	88.2	2.9	89.4	4.5
74	Fenpropimorph	LC	0.156	0.2	0.9987	105.5	7.4	80.1	10.8	72.4	2.0	74.5	6.5	72.3	3.9	76.2	2.1	77.3	2.5	77.8	3.3
75	Fenpyroximate	LC	0.625	1.0	0.9989	–	–	–	–	113.2	5.3	98.3	3.4	84.7	2.9	84.1	2.8	80.8	0.7	80.2	2.6
76	Fenthion	GC	0.313	0.5	0.9997	–	–	79.3	8.3	78.4	2.0	83.4	3.1	77.7	2.2	75.6	4.8	72.9	1.4	69.6	5.6
77	Fenthion-oxon	LC	0.039	0.2	0.9987	89.7	8.4	89.4	7.0	86.5	5.9	95.8	6.2	94.3	2.7	100.6	2.8	100.9	1.6	98.7	4.1
78	Fenthion-oxon-sulfone	LC	0.313	1.0	0.9956	–	–	–	–	101.8	6.9	92.9	4.3	88.4	5.7	96.6	3.6	97.9	2.7	99.3	4.9
79	Fenthion-oxon-sulfoxide	LC	1.250	2.0	0.9991	–	–	–	–	–	–	107.5	3.7	93.2	2.3	96.3	2.9	96.6	1.9	94.2	3.9
80	Fenthion-sulfone	LC	0.156	0.5	0.9973	–	–	90.7	11.7	87.8	13.6	90.3	7.7	89.6	5.6	97.2	3.4	99.6	1.9	102.6	4.0
81	Fenthion-sulfoxide	LC	0.625	1.0	0.9986	–	–	–	–	107.3	3.0	101.5	9.5	98.4	3.7	105.2	4.0	104.9	1.2	104.9	3.8
82	Fipronil	GC	0.078	0.2	0.9984	99.3	4.2	94.2	5.1	86.2	4.5	87.3	3.5	82.3	2.5	82.8	2.5	80.0	1.6	78.3	4.6
83	Fipronil-sulfide	GC	0.313	1.0	0.9980	–	–	–	–	101.1	10.0	99.8	5.3	94.6	3.5	89.6	8.0	91.3	1.7	86.7	5.9
84	Fluazinam	LC	0.625	2.0	0.9948	–	–	–	–	–	–	95.5	14.8	92.1	11.0	101.2	4.4	99.5	2.7	101.6	6.1
85	Flubendiamide	LC	1.250	2.0	0.9981	–	–	–	–	–	–	103.7	14.9	101.5	12.2	104.6	8.2	109.2	0.9	108.4	4.5
86	Fludioxonil	GC	0.156	0.2	0.9988	92.6	9.4	91.3	1.5	88.2	3.1	88.8	3.9	87.4	4.1	84.4	4.6	83.9	1.5	81.2	6.4
87	Flufenoxuron	LC	0.625	1.0	0.9939	–	–	–	–	99.2	5.8	89.7	6.7	75.8	3.5	75.5	6.3	77.1	1.4	78.2	3.3
88	Fluopyram	LC	0.156	0.5	0.9970	–	–	75.8	19.2	78.1	10.1	94.4	10.0	95.6	3.2	99.6	4.8	98.0	3.3	97.5	5.7
89	Fluquinconazole	GC	0.078	0.2	0.9995	89.2	9.2	81.7	1.6	78.9	1.9	79.5	7.0	73.0	3.0	72.6	1.3	70.8	1.7	68.6	4.0
90	Flusilazole	LC	0.625	1.0	0.9984	–	–	–	–	116.5	11.0	108.2	15.9	101.3	5.1	101.3	3.0	99.1	1.2	96.7	2.3
91	Flutolanil	LC	0.156	0.5	0.9980	–	–	94.2	6.3	92.5	3.7	101.4	10.3	104.5	1.8	108.5	3.9	107.7	1.8	102.8	2.7
92	Flutriafol	GC	0.625	1.0	0.9960	–	–	–	–	122.4	9.3	106.8	6.6	99.0	4.6	90.8	8.1	89.2	4.7	84.1	7.8
93	Fonofos	GC	0.078	0.2	0.9996	92.4	7.1	87.6	4.0	85.9	3.4	83.4	6.2	79.8	2.2	82.4	3.4	80.8	1.0	80.4	4.0
94	Formetanate	LC	0.625	1.0	0.9963	–	–	–	–	94.5	8.6	92.7	5.5	73.1	6.3	85.4	5.4	77.0	4.4	70.6	4.2
95	Fosthiazate	LC	0.156	0.2	0.9981	111.2	2.9	92.8	5.0	87.7	4.1	93.2	5.3	90.9	2.5	97.4	2.5	97.3	2.2	95.4	3.7
96	Hexaconazole	LC	0.313	1.0	0.9973	–	–	–	–	78.8	11.4	78.5	16.0	83.8	7.4	90.3	8.0	88.6	8.5	90.9	4.1
97	Hexaflumuron	LC	2.500	5.0	0.9902	–	–	–	–	–	–	–	–	104.6	12.9	111.2	5.5	107.6	2.8	100.4	8.8
98	Hexythiazox	LC	0.625	1.0	0.9905	–	–	–	–	105.4	4.1	81.3	6.9	68.0	4.5	67.9	3.6	69.8	3.0	66.4	2.5
99	Imazalil (Enilconazole)	LC	0.156	1.0	0.9971	–	–	–	–	71.8	14.6	84.6	6.7	83.9	5.2	92.2	6.3	93.7	2.6	94.5	3.6
100	Imidacloprid	LC	0.625	2.0	0.9978	–	–	–	–	–	–	107.3	11.6	96.3	12.0	98.0	4.9	98.3	3.8	96.6	1.5
101	Indoxacarb	LC	0.625	1.0	0.9996	–	–	–	–	67.2	15.0	80.6	15.9	82.8	4.1	88.6	11.0	89.7	3.3	87.6	3.9
102	Iprovalicarb	LC	0.078	0.2	0.9991	100.2	9.3	93.3	6.9	91.5	2.8	96.7	6.3	97.7	3.2	102.7	2.1	100.3	2.5	100.3	3.7
103	Isocarbophos	GC	0.625	1.0	0.9995	–	–	–	–	89.8	5.6	85.1	6.6	82.6	2.8	81.8	4.2	80.5	1.5	78.4	5.0
104	Isofenphos-methyl	LC	0.313	0.5	0.9993	–	–	101.6	12.3	101.5	2.5	98.3	4.7	93.2	2.8	96.5	3.5	96.7	4.7	96.0	3.6
105	Isoprothiolane	LC	0.156	0.2	0.9990	97.9	10.1	94.0	9.9	94.7	5.1	102.1	5.3	97.2	1.8	103.3	3.0	99.6	1.5	97.9	2.0

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	Technique[a]	LOD[b]	LOQ[c]	Linearity	0.2		0.5		1		2		5		10		20		40	
						REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD
106	Kresoxim-methyl	LC	1.250	2.0	0.9998	–	–	–	–	–	–	103.3	8.1	89.1	3.2	92.7	8.3	91.3	5.1	89.1	2.3
107	Linuron	LC	0.156	0.5	0.9981	–	–	93.7	10.9	84.6	13.8	88.8	8.2	88.7	3.7	92.9	4.1	93.4	3.4	88.8	5.2
108	Lufenuron	LC	0.625	1.0	0.9902	–	–	–	–	123.5	9.8	89.4	12.4	79.7	6.9	84.5	6.1	88.6	3.8	92.0	2.9
109	Malaoxon	LC	0.039	0.2	0.9985	90.3	7.9	90.2	1.8	85.7	2.4	95.6	3.8	93.9	2.0	101.0	3.6	101.2	1.5	100.4	4.6
110	Malathion	LC	0.156	0.5	0.9986	–	–	99.7	11.2	95.2	6.6	99.4	5.1	100.9	4.3	106.5	3.3	104.5	3.6	100.5	3.7
111	Mandipropamid	LC	0.156	0.5	0.9981	–	–	81.0	12.3	95.1	4.9	104.6	5.9	102.1	1.5	108.0	4.9	107.1	1.9	106.3	3.0
112	Mepanipyrim	LC	5.000	10.0	0.9981	–	–	–	–	–	–	–	–	–	–	87.3	4.6	88.2	3.2	85.0	2.9
113	Metaflumizone	LC	0.313	0.5	0.9963	–	–	81.6	11.7	86.6	4.2	96.4	11.4	105.5	3.6	115.8	4.4	111.4	3.1	108.8	3.5
114	Metalaxyl	GC	0.078	0.2	0.9996	89.8	3.8	90.2	4.4	88.3	1.4	88.8	5.0	84.6	3.0	85.2	3.7	83.5	0.9	82.0	5.0
115	Metalaxyl-M (Mefenoxam)	LC	0.078	0.2	0.9967	104.2	0.9	97.8	1.7	95.3	3.0	103.6	5.3	103.3	1.4	108.0	3.2	107.2	1.7	103.8	3.3
116	Metaldehyde	LC	10.000	10.0	0.9988	–	–	–	–	–	–	–	–	–	–	113.7	2.0	104.5	1.1	101.7	4.4
117	Metconazole	LC	0.313	0.5	0.9991	–	–	108.9	18.7	93.3	11.2	96.9	15.0	87.4	4.9	98.6	1.6	98.8	3.6	94.5	4.2
118	Methamidophos	LC	2.500	5.0	0.9942	–	–	–	–	–	–	–	–	81.0	2.8	77.8	3.0	71.0	2.9	63.2	3.1
119	Methidathion	LC	0.156	0.2	0.9993	118.2	8.9	97.2	5.0	91.5	2.6	92.4	6.3	91.1	2.5	96.5	2.3	96.6	1.5	95.3	3.7
120	Methiocarb	LC	1.250	2.0	0.9995	–	–	–	–	–	–	103.7	6.1	95.4	3.8	98.5	4.0	95.3	2.6	93.4	4.2
121	Methiocarb-sulfone	LC	0.313	1.0	0.9959	–	–	–	–	100.4	6.7	96.3	6.2	85.4	3.1	89.6	5.8	92.7	1.9	92.6	1.1
122	Methiocarb-sulfoxide	LC	1.250	2.0	0.9981	–	–	–	–	–	–	121.7	4.8	93.1	1.4	94.1	4.5	94.0	2.7	87.9	3.4
123	Methomyl	LC	0.313	0.5	0.9976	–	–	118.9	5.2	91.9	3.3	90.7	7.3	83.8	3.4	91.2	4.1	92.1	1.3	90.0	3.0
124	Methoxyfenozide	LC	0.039	0.2	0.9972	87.9	9.8	96.2	7.3	99.3	2.7	101.4	3.4	101.3	2.0	109.8	3.6	107.9	1.2	104.8	3.6
125	Metrafenone	LC	0.156	1.0	0.9979	–	–	–	–	72.0	14.8	81.6	1.4	84.3	3.9	87.4	3.7	89.6	3.2	88.2	2.7
126	Mevinphos (phosdrin) (two isomers)	LC	2.500	5.0	0.9977	–	–	–	–	–	–	–	–	99.2	5.4	101.4	3.4	95.5	1.6	95.1	2.9
127	Monocrotophos	LC	1.250	2.0	0.9980	–	–	–	–	–	–	112.7	8.0	92.8	4.4	95.8	4.6	92.2	2.6	87.0	3.9
128	Myclobutanil	LC	0.313	0.5	0.9986	–	–	101.7	11.2	100.7	9.0	97.9	9.2	103.5	2.1	109.5	4.7	109.5	1.6	109.7	7.0
129	N-2,4-Dimethylphenyl-N'-methyl-formamidine (DMPF, metabolite of amitraz)	LC	2.500	5.0	0.9989	–	–	–	–	–	–	–	–	96.2	4.4	86.7	1.3	83.9	1.9	79.5	3.6
130	N,N-Dimethyl-N'-p-tolylsulphamide (DMST,metabolite of tolylfluanid)	LC	0.625	1.0	0.9990	–	–	–	–	109.4	4.5	101.1	7.6	102.3	4.0	108.7	3.2	110.8	2.3	111.2	4.7
131	N,N-dimethylformamidine (DMF, metabolite of amitraz)	LC	2.500	5.0	0.9949	–	–	–	–	–	–	–	–	90.8	6.0	84.9	4.7	82.8	4.5	79.7	3.3
132	Nitenpyram	LC	2.500	10.0	0.9982	–	–	–	–	–	–	–	–	–	–	103.0	7.2	86.5	4.1	84.9	7.7
133	Nuarimol	GC	0.156	0.2	0.9996	96.8	11.6	90.1	4.8	86.4	2.1	87.5	4.3	83.3	3.1	83.3	3.7	82.2	1.4	79.7	4.8
134	Ofurace	LC	0.313	0.5	0.9972	–	–	118.8	3.5	99.7	4.8	99.3	4.0	90.1	3.5	99.3	3.4	102.9	1.4	101.4	3.7
135	Omethoate	LC	2.500	5.0	0.9976	–	–	–	–	–	–	–	–	97.3	3.9	95.1	5.2	88.8	3.3	84.9	4.6
136	Oxadixyl	LC	0.625	1.0	0.9982	–	–	–	–	112.2	2.5	101.1	7.7	94.2	2.2	99.3	2.9	99.0	1.6	99.1	4.6
137	Oxamyl	LC	0.313	0.5	0.9985	–	–	112.6	7.0	89.2	4.5	90.5	3.8	88.4	1.3	94.9	3.5	95.0	0.9	90.8	3.3
138	Oxydemeton-methyl	LC	0.313	1.0	0.9953	–	–	–	–	97.6	6.9	94.7	7.4	91.1	4.0	99.7	6.4	101.6	2.1	94.5	3.0
139	Oxyfluorfen	GC	1.250	2.0	0.9984	–	–	–	–	–	–	101.0	8.4	74.6	6.4	76.6	3.5	73.3	2.3	70.7	6.9
140	Paclobutrazol	LC	1.250	2.0	0.9993	–	–	–	–	–	–	101.6	6.2	103.0	5.3	106.4	5.2	102.5	1.4	97.4	4.7
141	Paraoxon methyl	GC	10.000	20.0	0.9965	–	–	–	–	–	–	–	–	–	–	–	–	108.7	8.3	87.1	7.6
142	Parathion	GC	5.000	5.0	0.9994	–	–	–	–	–	–	–	–	75.1	3.5	73.0	3.3	67.2	2.7	67.5	6.4
143	Parathion-methyl	GC	2.500	5.0	0.9991	–	–	–	–	–	–	–	–	74.8	2.7	72.4	3.1	66.3	2.3	63.4	6.2
144	Penconazole	LC	0.313	1.0	0.9989	–	–	–	–	97.5	12.7	98.5	12.2	89.8	2.4	97.5	5.1	92.6	2.9	90.5	2.3
145	Pencycuron	LC	1.250	2.0	0.9984	–	–	–	–	–	–	109.9	10.8	96.4	7.0	95.3	3.8	88.1	2.1	86.1	3.3
146	Pendimethalin	LC	1.250	2.0	0.9928	–	–	–	–	–	–	100.4	6.1	77.2	7.5	69.2	3.2	71.2	2.5	68.1	4.6
147	Permethrin (two isomers)	GC	5.000	10.0	0.9995	–	–	–	–	–	–	–	–	–	–	62.3	4.4	63.9	1.3	64.6	5.9
148	Phosalone	LC	0.313	1.0	0.9995	–	–	–	–	94.7	5.9	96.7	11.0	88.0	5.2	94.8	3.8	91.2	3.2	87.6	5.1
149	Phosmet	LC	0.625	1.0	0.9998	–	–	–	–	110.8	7.4	102.1	5.3	97.9	2.6	100.6	3.9	100.1	2.5	97.1	3.7
150	Phosmet oxon	LC	1.250	2.0	0.9997	–	–	–	–	–	–	109.0	6.7	99.1	2.9	103.6	2.5	101.7	1.1	98.2	3.5
151	Pirimicarb	LC	0.078	0.2	0.9981	100.2	5.2	93.9	3.9	89.9	3.9	95.9	5.4	93.3	1.7	97.7	2.9	98.5	1.1	97.1	3.8
152	Pirimicarb-desmethyl	LC	0.313	0.5	0.9981	–	–	106.2	7.7	94.5	4.4	96.5	3.6	91.0	0.7	94.2	3.6	94.9	1.3	93.4	4.0
153	Pirimiphos-ethyl	GC	0.078	0.2	0.9991	94.6	10.3	86.7	9.0	81.6	4.8	82.0	5.1	78.2	5.1	80.0	4.3	78.3	1.5	77.4	4.7
154	Pirimiphos-methyl	LC	1.250	2.0	0.9980	–	–	–	–	–	–	97.1	14.7	95.4	8.2	94.0	4.5	93.1	1.1	91.0	2.9
155	Prochloraz	LC	0.625	1.0	0.9986	–	–	–	–	118.6	9.9	115.5	10.6	96.1	4.3	99.1	9.6	97.7	3.8	94.5	3.0

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	Technique[a]	LOD[b]	LOQ[c]	Linearity	0.2		0.5		1		2		5		10		20		40	
						REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD
156	Procymidone	GC	2.500	5.0	0.9989	–	–	–	–	–	–	–	–	88.2	9.3	86.6	3.4	83.1	1.5	79.4	5.4
157	Profenofos	LC	0.313	1.0	0.9929	–	–	–	–	97.5	13.7	80.2	11.7	79.1	7.3	82.5	1.3	84.5	2.9	84.0	4.6
158	Propamocarb	LC	10.000	20.0	0.9996	–	–	–	–	–	–	–	–	–	–	–	–	86.3	2.0	78.7	3.1
159	Propargite	LC	1.250	2.0	0.9992	–	–	–	–	–	–	93.6	8.0	87.1	3.0	83.3	3.0	79.6	2.2	76.6	2.9
160	Propiconazole	LC	1.250	2.0	0.9981	–	–	–	–	–	–	107.8	11.7	95.5	9.8	91.9	7.1	95.1	5.2	94.6	2.6
161	Propoxur	LC	0.313	0.5	0.9984	–	–	120.1	6.5	98.9	6.8	96.8	5.5	91.4	1.7	94.6	2.3	93.7	1.7	92.4	3.2
162	Propyzamide	LC	0.156	0.2	0.9979	109.7	12.8	109.3	19.1	90.9	6.6	94.1	11.0	91.0	3.0	101.1	7.6	97.6	3.1	94.1	3.7
163	Proquinazid	GC	0.078	0.2	0.9997	72.0	7.9	68.4	4.3	65.8	2.8	66.2	5.1	63.7	2.4	63.7	2.7	62.2	1.3	60.6	3.9
164	Prothioconazole-desthio	GC	0.313	0.5	0.9988	–	–	84.2	9.8	76.3	4.7	81.8	4.7	79.2	3.4	77.4	4.3	77.0	1.6	74.9	5.9
165	Prothiofos	GC	1.250	2.0	0.9993	–	–	–	–	–	–	69.7	5.9	67.5	5.2	65.2	4.6	64.9	2.5	62.4	4.0
166	Pymetrozine	LC	1.250	2.0	0.9958	–	–	–	–	–	–	117.5	7.9	78.1	3.3	87.1	6.0	80.5	6.1	73.7	1.9
167	Pyraclostrobin	LC	0.156	0.5	0.9987	–	–	85.1	13.3	91.8	6.4	96.1	8.7	94.6	2.0	98.5	1.9	97.7	1.6	97.4	4.8
168	Pyrazophos	LC	0.313	0.5	0.9997	–	–	119.4	11.6	98.2	4.9	97.9	6.2	93.0	3.4	101.2	4.0	99.7	1.8	98.2	4.1
169	Pyridaben	LC	0.625	1.0	0.9972	–	–	–	–	99.6	8.2	86.9	4.4	77.4	3.0	74.3	3.4	74.0	1.2	72.8	2.9
170	Pyridaphenthion	LC	0.156	0.5	0.9996	–	–	94.8	7.7	93.6	8.8	87.6	7.5	99.2	5.4	105.5	3.5	105.2	3.8	103.3	4.5
171	Pyrimethanil	GC	0.078	0.2	0.9995	86.5	7.3	81.2	5.7	77.6	3.0	77.6	7.0	73.6	3.4	74.8	3.4	73.1	1.1	72.7	4.4
172	Pyriproxyfen	LC	0.313	0.5	0.9982	–	–	97.6	6.3	80.9	6.3	78.6	6.2	72.1	3.6	73.1	2.2	71.9	1.4	71.1	3.4
173	Quinalphos	LC	1.250	2.0	0.9965	–	–	–	–	–	–	95.8	10.1	90.4	6.4	99.7	3.0	100.5	3.0	97.7	2.9
174	Quinoxyfen	GC	0.078	0.2	0.9992	103.6	5.6	81.0	2.8	72.6	5.4	70.5	7.8	64.7	2.4	64.4	2.6	63.3	1.3	62.4	3.7
175	Rotenone	LC	1.250	2.0	0.9975	–	–	–	–	–	–	115.7	12.2	100.7	5.3	106.7	5.6	104.5	8.4	106.5	6.2
176	Simazine	GC	0.313	0.5	0.9994	–	–	104.7	5.5	95.1	4.0	88.0	5.6	81.2	3.0	82.6	4.1	80.3	1.6	78.8	4.7
177	Spinosad A	LC	0.156	0.2	0.9990	106.4	12.9	88.0	7.2	87.8	4.9	88.4	4.6	85.9	2.8	94.7	3.3	92.4	1.5	90.6	2.7
178	Spinosad D	LC	1.250	2.0	0.9970	–	–	–	–	–	–	110.7	9.1	88.1	8.9	88.9	4.9	83.6	2.3	84.0	3.2
179	Spirodiclofen	LC	1.250	2.0	0.9947	–	–	–	–	–	–	91.5	5.5	81.5	5.7	76.9	4.1	76.2	3.6	74.4	4.7
180	Spiromesifen	LC	1.250	2.0	0.9968	–	–	–	–	–	–	113.5	4.5	90.4	4.0	84.9	2.6	82.8	1.7	81.3	3.8
181	Spirotetramat	LC	0.313	1.0	0.9946	–	–	–	–	107.5	9.4	102.8	9.7	100.4	6.9	111.3	7.4	108.7	4.8	114.9	7.4
182	Spirotetramat-enol	LC	2.500	5.0	0.9903	–	–	–	–	–	–	–	–	114.9	3.3	118.3	11.9	108.6	6.9	115.7	3.0
183	Spiroxamine	LC	0.078	0.2	0.9990	91.9	4.3	76.7	2.2	76.8	2.7	86.0	7.2	80.4	3.1	88.5	2.5	87.6	2.3	87.1	3.9
184	tau-Fluvalinate	LC	10.000	10.0	0.9907	–	–	–	–	–	–	–	–	–	–	88.1	12.3	76.8	10.2	68.6	10.8
185	Tebuconazole	LC	0.156	1.0	0.9976	–	–	–	–	97.5	14.8	87.8	9.9	85.7	6.1	93.4	8.4	91.7	3.0	88.8	2.9
186	Tebufenozide	LC	0.156	0.5	0.9917	–	–	96.9	8.7	94.3	6.2	102.4	7.3	105.4	2.7	109.2	4.2	109.9	1.2	103.9	2.5
187	Tebufenpyrad	GC	0.156	0.2	0.9993	89.1	4.6	82.3	4.6	82.3	3.0	81.7	5.2	77.4	2.9	76.8	3.5	75.2	1.3	73.6	5.3
188	Teflubenzuron	GC	0.313	0.5	0.9996	–	–	100.1	5.9	87.9	7.5	83.9	8.4	73.4	6.4	76.6	3.2	74.6	3.0	75.7	5.0
189	Tefluthrin	GC	0.156	0.2	0.9998	84.1	5.0	85.7	4.8	86.0	3.0	85.0	4.8	81.4	3.5	82.2	3.1	80.1	0.7	80.0	4.2
190	Terbufos	GC	0.078	0.2	0.9997	93.6	5.0	86.7	2.2	84.6	3.9	83.7	7.3	78.9	2.3	81.1	3.2	79.6	0.9	78.7	4.6
191	Terbuthylazine	LC	0.078	0.2	0.9974	97.0	9.4	87.8	5.4	86.5	8.7	92.8	6.6	91.3	1.3	94.0	3.3	93.2	2.2	86.8	1.6
192	Tetrachlorvinphos	LC	0.625	1.0	0.9968	–	–	–	–	92.8	16.3	94.3	13.8	92.2	11.0	103.1	4.0	100.6	4.7	96.9	1.1
193	Tetraconazole	LC	0.625	5.0	0.9971	–	–	–	–	–	–	–	–	93.0	6.2	99.3	8.3	107.0	2.8	100.2	7.9
194	Tetradifon	GC	0.625	1.0	0.9992	–	–	–	–	109.9	2.2	86.8	2.9	75.9	3.8	71.9	2.5	69.1	1.0	66.7	5.4
195	Thiabendazole	LC	0.625	1.0	0.9963	–	–	–	–	99.9	4.0	91.0	5.8	80.2	2.4	82.7	4.9	83.7	1.0	79.3	4.9
196	Thiacloprid	LC	0.078	0.2	0.9976	112.4	7.1	90.0	7.6	84.0	5.9	91.2	4.4	91.8	2.1	99.9	3.5	99.6	1.4	98.6	3.8
197	Thiamethoxam	LC	0.625	1.0	0.9989	–	–	–	–	106.0	6.0	98.3	3.3	88.0	2.7	97.0	3.2	95.5	1.9	89.9	2.6
198	Thiodicarb	LC	0.156	0.5	0.9988	–	–	100.8	4.6	89.1	2.3	93.3	4.9	89.8	3.3	99.6	3.1	99.5	2.0	93.2	3.5
199	Thiophanate-methyl	LC	0.313	0.5	0.9994	–	–	99.7	4.9	79.6	4.1	71.0	5.7	69.1	2.3	68.7	5.1	70.2	2.9	68.5	1.5
200	Tolclofos-methyl	GC	0.625	1.0	0.9994	–	–	–	–	121.1	3.9	99.5	4.0	77.9	3.0	72.6	3.1	68.6	3.4	63.2	4.1
201	Triadimefon	LC	1.250	2.0	0.9985	–	–	–	–	–	–	123.2	9.9	112.2	8.2	94.1	6.4	96.7	5.2	99.4	4.4
202	Triadimenol	LC	1.250	2.0	0.9993	–	–	–	–	–	–	110.3	6.1	90.8	3.6	95.4	4.3	98.2	5.3	100.2	6.1
203	Triazophos (hostathion)	LC	0.156	0.2	0.9993	118.3	8.6	103.6	5.0	96.3	6.7	100.2	5.4	93.7	2.0	99.8	1.9	98.9	2.7	94.6	3.8
204	Trichlorfon	LC	1.250	2.0	0.9977	–	–	–	–	–	–	111.6	8.2	90.5	6.4	91.9	4.3	94.5	4.3	89.2	6.2
205	Trifloxystrobin	LC	0.156	0.2	0.9981	76.2	17.1	87.5	6.5	80.4	6.8	89.9	7.1	89.8	3.3	93.7	1.8	95.2	1.3	93.3	1.8
206	Triflumizole	LC	0.156	0.5	0.9983	–	–	92.0	4.7	88.9	4.2	89.5	5.0	89.3	4.4	93.1	3.7	91.5	2.3	92.4	3.3
207	Triflumuron	LC	0.625	2.0	0.9975	–	–	–	–	–	–	98.0	13.4	92.1	8.4	98.7	7.4	97.1	5.6	91.3	4.0
208	Trifluralin	GC	0.078	0.2	0.9984	90.8	4.7	83.5	4.1	81.8	3.7	81.8	4.5	75.3	2.8	76.0	4.7	73.3	2.5	73.9	5.7
209	Triticonazole	LC	1.250	2.0	0.9991	–	–	–	–	–	–	106.2	8.9	106.2	4.5	108.7	6.0	103.5	3.6	102.4	3.5

(*continued on next page*)

Table 2 (*continued*)

N°	Compound	Technique[a]	LOD[b]	LOQ[c]	Linearity	0.2		0.5		1		2		5		10		20		40	
						REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD	REC	RSD
210	Vinclozolin	GC	0.156	0.2	0.9996	111.8	13.2	93.9	7.3	89.0	4.9	87.5	5.4	82.3	2.6	82.9	3.7	81.2	0.6	80.0	5.3
211	Zoxamide	LC	0.313	0.5	0.9994	–	–	107.0	8.7	96.6	8.2	98.7	8.0	94.2	6.2	98.7	3.3	96.8	2.6	92.4	4.2

[a] Technique used for the analysis of each compound: LC – Liquid Chromatography or GC – Gas Chromatography, both coupled with tandem triple quadrupole mass spectrometry.
[b] LOD – Limit of Detection.
[c] LOQ – Limit of Quantification.

regulation is primarily aimed at production animal feed, it allows its application to feed for non-food-producing animals to ensure consistent safety standards and facilitate controls. In fact, all compounds, except for abamectin, paraoxon-methyl, and propamocarb, achieved LOQs within the generic MRL of 10 μg/kg or lower. Regarding the Limits of Detection (LODs), 73.5 % of the compounds achieved values of 0.625 μg/kg or lower, while only six compounds exhibited a LOD equal to 10 μg/kg.

Most existing methods aim to achieve LOQs equivalent to the generic MRL for feed. The method developed and validated here achieves lower LOQs than those reported in most multi-residue pesticide methods for complete feed and feed materials [12–32]. Eyring et al. [34]. reported LOQs of 5 μg/kg (half of the generic MRL value) for 139 of their target pesticides, whereas 95.3 % of analytes in our method achieved LOQs at this concentration or lower. Van der Lee et al. [29]. developed a multi-residue method for 106 compounds in GCxGC-TOF_MS and reported 35 of the pesticides achieving LOQs of 10 μg/kg or lower. However, it is important to note that their LOQs were calculated using the US Food and Drug Administration (FDA) approach (defined as 10 times the intercept's standard deviation divided by the slope using matrix-matched standards) whereas our method follows the more stringent criteria set by the SANTE guidelines. Several of these methods use QuEChERS with d-SPE clean-up, and some, like Kumar et al. [13] or Walorczyk and Drozdzyński [28], incorporate an additional freezing step. In contrast, our method relies solely on a two-cycle freezing-out clean-up, simplifying the process while reducing materials, cost, environmental contamination and time.

After validation, the method was shown to be both accurate and reliable for analyzing the selected pesticides in cat and dog feeds. The results of the validation process, including linearity, LODs, LOQs, and recovery experiments, are summarized in Table 2.

3.5. Application to real samples

The validated method was tested on 16 commercial pet feed samples to evaluate its performance in detecting pesticide residues. These samples included eight dog feeds and eight cat feeds and were categorized by composition: four vegetable-based, two grain-based, and two combining vegetables and grains for each type of feed. The results are presented in Table 3.

A total of 112 residues from 39 different pesticides were detected, representing 18.5 % of the compounds of the validated method. Among the pesticides detected, their primary uses were fungicides and insecticides, with 21 and 14 compounds, followed by two acaricides, an herbicide, and a post-harvest pesticide. Notably, 12 of the pesticides detected are currently not approved for use in the European Union [64] and account for 24 % of the residues detected.

All samples analyzed contained at least one pesticide residue. The highest number of residues detected in a single sample was 21, observed in a vegetable-based dog feed. Two additional vegetable-based samples had 18 residues each, another one for dog and one for cat feed. As shown in Fig. 7, most fungicide residues originate from compounds approved for use in the EU, with only 12 % stemming from non-approved compounds. In contrast, nearly half of the insecticide residues are attributed unauthorized active substances.

Tebuconazole was the most prevalent pesticide, found in 9 samples—6 from dog feed and, 3 from cat feed—followed by the not-approved insecticide chlorpyrifos, detected in half of the samples. Most compounds (31) were identified in 4 or fewer samples.

Regarding concentration levels, the highest levels were recorded for pirimiphos methyl, an approved insecticide, with concentrations of 184.28 μg/kg and 27.7 μg/kg in two samples. Both samples belong to cat feed, vegetable-grained, and vegetable, respectively. In dog feed, pirimiphos methyl also exhibited the highest concentration at 21.44 μg/kg in a grained sample, followed by fludioxonil, an approved fungicide, at 16.56 μg/kg in vegetable-grained feed. Notably, several detected

Table 3
Pesticides detected in 16 samples of cat and dog dry feed. Concentrations are expressed in μg/kg.

Compound	1 DG	2 DG	3 DV	4 DV	5 DV	6 DV	7 DVG	8 DVG	9 CG	10 CG	11 CV	12 CV	13 CV	14 CV	15 CVG	16 CVG	Frequency (%)
2-Phenylphenol	–	–	–	–	2.89	–	–	16.56	3.15	–	–	–	–	–	–	–	18.8
Acetamiprid	–	–	–	–	–	–	–	–	–	–	–	–	0.91	–	–	–	6.3
Azoxystrobin	–	–	0.34	–	0.30	–	–	–	–	–	–	0.23	0.32	–	–	–	25.0
Boscalid	–	–	7.83	0.76	4.05	–	–	–	–	–	1.08	–	8.94	–	–	–	31.3
Carbendazim	–	–	–	–	0.48	–	–	–	–	–	–	6.32	–	–	–	–	12.5
Chlorantraniliprole	–	–	–	–	1.04	–	–	–	–	–	–	–	1.65	–	–	–	12.5
Chlorpropham	–	–	7.40	–	–	–	–	–	–	–	0.40	–	–	–	–	–	12.5
Chlorpyrifos	–	1.13	1.01	–	0.17	–	–	–	0.70	0.44	–	0.49	–	–	0.70	1.83	50.0
Chlorpyrifos methyl	0.53	0.32	–	–	–	–	2.90	0.62	–	–	–	–	–	–	0.36	5.62	37.5
Cyflufenamid	–	–	–	–	–	–	–	–	–	–	–	–	0.23	–	–	–	6.3
Cyprodinil	–	–	0.77	–	0.40	–	–	–	–	–	–	–	2.19	–	–	–	18.8
Difenoconazole	0.94	–	1.84	0.78	1.52	–	–	–	–	–	–	–	3.47	–	–	–	31.3
Diphenylamine	–	–	–	–	–	–	–	–	–	–	–	1.17	–	–	–	–	6.3
Epoxiconazol	0.21	0.23	–	–	–	–	0.27	–	–	–	–	–	–	–	–	–	18.8
Fenazaquin	–	–	–	–	–	–	–	–	–	–	–	–	–	27.70	–	–	6.3
Fenoxycarb	–	–	0.36	–	–	–	–	–	–	–	–	–	–	–	–	–	6.3
Fenpropidin	0.17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6.3
Fludioxonil	–	–	8.60	0.73	7.59	–	–	–	–	–	0.75	–	10.06	–	–	–	31.3
Fluopyram	–	–	4.47	0.49	1.00	–	–	–	–	–	–	0.18	2.33	–	–	–	31.3
Flutolanil	–	–	–	–	0.28	–	–	–	–	–	–	0.16	–	–	–	–	12.5
Hexythiazox	–	–	0.75	–	–	–	–	–	–	–	–	–	–	–	–	–	6.3
Imazalil (Enilconazole)	–	–	0.69	0.71	1.21	–	–	–	–	–	–	–	18.06	–	–	–	25.0
Isoprothiolane	–	–	–	–	0.16	–	–	–	–	–	–	–	0.80	–	–	–	12.5
Methoxyfenozide	–	–	–	–	0.06	–	–	–	–	–	–	–	–	–	–	–	6.3
Metrafenone	–	–	–	–	–	–	–	–	–	–	0.46	–	–	–	–	–	6.3
Phosmet	–	–	–	–	1.06	–	–	–	–	–	–	–	–	–	–	–	6.3
Pirimicarb	–	–	0.10	0.08	0.22	–	–	–	–	–	–	–	0.46	–	–	–	25.0
Pirimiphos methyl	21.44	–	–	–	–	3.38	–	–	–	–	–	–	–	–	6.63	184.28	25.0
Pyraclostrobin	–	–	6.05	0.81	4.62	–	–	–	–	–	1.64	0.23	12.09	–	–	–	37.5
Pyrimethanil	–	–	2.04	0.59	3.76	–	–	–	–	–	1.50	–	10.25	–	–	–	31.3
Pyriproxifen	–	–	–	–	–	–	–	–	–	–	–	–	1.01	–	–	–	6.3
Spiroxamine	–	0.14	–	–	–	–	0.65	–	–	–	–	–	–	–	–	–	12.5
Tebuconazole	–	0.41	3.54	0.52	1.97	–	0.32	0.30	–	0.48	0.36	–	7.39	–	–	–	56.3
Tebufenocide	–	–	0.83	–	–	–	–	–	–	–	–	–	–	–	–	–	6.3
Thiabendazole	–	–	–	–	0.70	–	–	–	–	–	–	–	0.85	–	–	–	12.5
Thiacloprid	–	–	0.31	–	–	–	–	–	–	–	–	–	–	–	–	–	6.3
Thiophanate-methyl	–	–	–	–	–	–	–	–	–	–	–	0.41	–	–	–	–	6.3
Triazophos (hostathion)	–	–	–	–	–	–	–	–	–	–	–	0.19	–	–	–	–	6.3
Trifloxystrobin	–	–	0.54	–	0.37	–	–	–	–	–	0.20	–	0.60	–	–	–	25.0
Number of residues	5	5	18	9	21	1	4	3	2	2	8	9	18	1	3	3	–

DG – Dog Food with Animal Protein and Grains.
DV – Dog Food with Animal Protein and Vegetables.
DVG – Dog Food with Animal Protein, Vegetables, and Grains.
CG – Cat Food with Animal Protein and Grains.
CV – Cat Food with Animal Protein and Vegetables.
CVG – Cat Food with Animal Protein, Vegetables, and Grains.

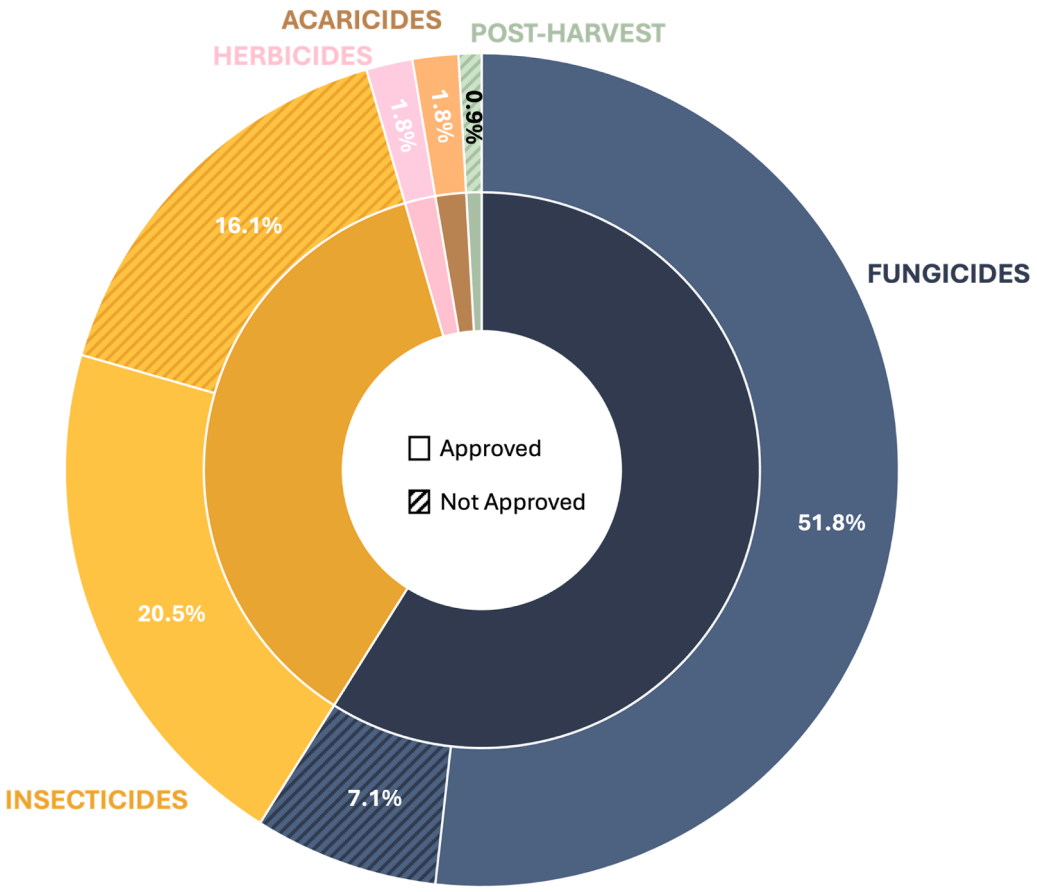

Fig. 7. Distribution of pesticide residues detected in the analyzed cat and dog feed samples by their application usage and legal status in the European Union.

concentrations exceed the generic MRL for feed.

This analysis demonstrates the applicability of the validated method for detecting a wide range of pesticide residues in diverse pet feed samples. However, given the limited number of samples analyzed, it is not possible to draw definitive conclusions regarding whether certain feed types or compositions are more prone to pesticide contamination. Similarly, it remains unclear whether the presence of specific pesticides is linked to their typical applications or the feed's composition. Further studies with larger and more diverse sample sets are required to confirm these initial observations and provide a more comprehensive assessment of pesticide residues in pet feed.

4. Conclusions

In this study, we successfully validated a QuEChERS-based method for the extraction and analysis of 211 pesticides in cat and dog feed using LC-MS/MS and GC–MS/MS. Our approach employs a simplified clean-up strategy based on a two-cycle freezing-out, avoiding the need for additional sorbents or chemicals while offering a more efficient and cost-effective alternative to handle the higher levels of fat and animal protein in complete dog and cat feed.

The validated method demonstrated high sensitivity with all but three compounds presenting LOQs equal to or below the generic MRL and achieving LOQs below 1 µg/kg for over 70 % of the compounds. Its applicability was tested on 16 commercial pet feed samples, where 112 residues from 39 pesticides were detected, including compounds not approved for use in the EU. These findings highlight the need for continuous monitoring of pesticide residues in pet feed to ensure regulatory compliance and consumer safety.

To our knowledge, this is the first validated QuEChERS approach employing freezing-out as a standalone clean-up step. Its versatility, simplicity, and high sensitivity make it a practical tool for routine monitoring of pesticide residues in pet feed, offering a cost-effective solution for both regulatory authorities and industry stakeholders. Additionally, its potential applicability to other high-fat matrices could further expand its utility in pesticide residue analysis.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve the readability and language of the manuscript, as English is not their first language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Ana Macías-Montes: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Octavio P. Luzardo:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization. **Annalisa Zaccaroni:** Writing – review & editing, Investigation. **Andrea Acosta-Dacal:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was partially funded by the Catalina Ruiz Research Staff Training Program of the Canary Islands Government's Ministry of Universities, Science, Innovation, and Culture, and the European Social Fund, through a postdoctoral grant awarded to Andrea Acosta-Dacal (APCR2022010003) at the University of Las Palmas de Gran Canaria.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2025.466093.

Data availability

Data will be made available on request.

References

[1] FEDIAF, Annual report 2024, 2024. https://morth.nic.in/sites/default/files/AR-MoRTH_Annual Report_2023-24_English.pdf.
[2] M. Schleicher, S.B. Cash, L.M. Freeman, Determinants of pet food purchasing decisions, Can. Vet. J. 60 (2019) 644–650.
[3] P.A.M. Overgaauw, C.M. Vinke, M.A.E. van Hagen, L.J.A. Lipman, A one health perspective on the human-companion animal relationship with emphasis on zoonotic aspects, Int. J. Environ. Res. Public Health 17 (2020) 1–29, https://doi.org/10.3390/ijerph17113789.
[4] P. Roudebush, Pet food additives, J. Am. Vet. Med. Assoc. 203 (1993) 1667–1670.
[5] M. Hu, Chapter 9 - oxidative stability and shelf life of low-moisture foods (Eds.), in: M. Hu, C. Jacobsen (Eds.), Oxidative Stability and Shelf Life of Foods Containing Oils and Fats, AOCS Press, 2016, pp. 313–371, https://doi.org/10.1016/B978-1-63067-056-6.00009-4.
[6] L.Y. Bissoqui, M.S. Frehse, R.L. Freire, M.A. Ono, J.G. Bordini, M.T. Hirozawa, A. J. de Oliveira, E.Y. Ono, Exposure assessment of dogs to mycotoxins through consumption of dry feed, J. Sci. Food Agric. 96 (2016) 4135–4142, https://doi.org/10.1002/jsfa.7615.
[7] S.D. Singh, S. Baijnath, A.A. Chuturgoon, A comparison of mycotoxin contamination of premium and grocery brands of pelleted cat food in South Africa, J. S. Afr. Vet. Assoc. 88 (2017) 1–4, https://doi.org/10.4102/jsava.v88i0.1480.
[8] A. Macías-Montes, C. Rial-Berriel, A. Acosta-Dacal, L.A. Henríquez-Hernández, M. Almeida-González, Á. Rodríguez-Hernández, M. Zumbado, L.D. Boada, A. Zaccaroni, O.P. Luzardo, Risk assessment of the exposure to mycotoxins in dogs and cats through the consumption of commercial dry food, Sci. Total Environ. 708 (2020) 134592, https://doi.org/10.1016/j.scitotenv.2019.134592.
[9] R.V.A. Zafalon, R.S. Pedreira, T.H.A. Vendramini, M.F. Rentas, V. Pedrinelli, R.B. A. Rodrigues, L.W. Risolia, M.P. Perini, A.R. Amaral, J.C. de Carvalho Balieiro, C.F. F. Pontieri, M.A. Brunetto, Toxic element levels in ingredients and commercial pet foods, Sci. Rep. 11 (2021) 1–19, https://doi.org/10.1038/s41598-021-00467-4.
[10] A.C.C. Paulelli, A.C. Martins, E.S. de Paula, J.M.O. Souza, M.F.H. Carneiro, F. B. Júnior, B.L. Batista, Risk assessment of 22 chemical elements in dry and canned pet foods, J. Verbraucherschutz Leb. 13 (2018) 359–365, https://doi.org/10.1007/s00003-018-1178-5.
[11] A. Macías-Montes, M. Zumbado, O.P. Luzardo, Á. Rodríguez-Hernández, A. Acosta-Dacal, C. Rial-Berriel, L.D. Boada, L.A. Henríquez-Hernández, Nutritional evaluation and risk assessment of the exposure to essential and toxic elements in dogs and cats through the consumption of pelleted dry food: how important is the quality of the feed? Toxics 9 (2021) 133, https://doi.org/10.3390/toxics9060133.
[12] T.W. Na, H.J. Seo, S.N. Jang, H. Kim, H. Yun, H. Kim, J. Ahn, H. Cho, S.H. Hong, H. J. Kim, S.H. Lee, Multi-residue analytical method for detecting pesticides, veterinary drugs, and mycotoxins in feed using liquid- and gas chromatography coupled with mass spectrometry, J. Chromatogr. A 1676 (2022) 463257, https://doi.org/10.1016/j.chroma.2022.463257.
[13] A. Kumar, A. Bhattacharyya, R. Shinde, M. Dhanshetty, C.T. Elliott, K. Banerjee, Development and validation of a multiresidue method for pesticides and selected veterinary drugs in animal feed using liquid- and gas chromatography with tandem mass spectrometry, J. Chromatogr. A (2020) 1627, https://doi.org/10.1016/j.chroma.2020.461416.
[14] H.W. Cho, K. Seo, J.Y. Jeong, J.L. Chun, K.H. Kim, Evaluating sulfoxaflor residues in pig tissues using animal modeling, J. Anim. Sci. Technol. 64 (2022) 911–921, https://doi.org/10.5187/jast.2022.e67.
[15] D.J. MacLachlan, R. Bhula, Transfer of lipid-soluble pesticides from contaminated feed to livestock, and residue management, Anim. Feed. Sci. Technol. 149 (2009) 307–321, https://doi.org/10.1016/j.anifeedsci.2008.06.007.
[16] J. Norrgran Engdahl, A. Bignert, B. Jones, I. Athanassiadis, Bergman, J.M. Weiss, Cats' Internal exposure to selected brominated flame retardants and organochlorines correlated to house dust and cat food, Environ. Sci. Technol. 51 (2017) 3012–3020, https://doi.org/10.1021/acs.est.6b05025.
[17] N. Ali, R.N. Malik, T. Mehdi, S.A.M.A.S. Eqani, A. Javeed, H. Neels, A. Covaci, Organohalogenated contaminants (OHCs) in the serum and hair of pet cats and dogs: biosentinels of indoor pollution, Sci. Total Environ. 449 (2013) 29–36, https://doi.org/10.1016/j.scitotenv.2013.01.037.
[18] Z.M. Li, M. Robinson, K. Kannan, An assessment of exposure to several classes of pesticides in pet dogs and cats from New York, United States, Environ. Int. 169 (2022) 107526, https://doi.org/10.1016/j.envint.2022.107526.
[19] G.M. Forster, D.G. Brown, G.P. Dooley, R.L. Page, E.P. Ryan, Multiresidue analysis of pesticides in urine of healthy adult companion dogs, Environ. Sci. Technol. 48 (2014) 14677–14685, https://doi.org/10.1021/es503764s.
[20] S. Gautam, N.K. Sood, K. Gupta, C. Joshi, K.K. Gill, R. Kaur, I. Chauhan, Bioaccumulation of pesticide contaminants in tissue matrices of dogs suffering from malignant canine mammary tumors in Punjab, India, Heliyon 6 (2020) e05274, https://doi.org/10.1016/j.heliyon.2020.e05274.
[21] C.F. Wise, S.C. Hammel, N.J. Herkert, M. Ospina, A.M. Calafat, M. Breen, H. M. Stapleton, Comparative assessment of pesticide exposures in domestic dogs and their owners using silicone passive samplers and biomonitoring, Environ. Sci. Technol. 56 (2022) 1149–1161, https://doi.org/10.1021/acs.est.1c06819.
[22] B.B. Takashima-Uebelhoer, L.G. Barber, S.E. Zagarins, E. Procter-Gray, A. L. Gollenberg, A.S. Moore, E.R. Bertone-Johnson, Household chemical exposures and the risk of canine malignant lymphoma, a model for human non-Hodgkin's lymphoma, Environ. Res. 112 (2012) 171–176, https://doi.org/10.1016/j.envres.2011.12.003.
[23] K.R. Luethcke, J. Ekena, R. Chun, L.A. Trepanier, Glutathione S-transferase theta genotypes and environmental exposures in the risk of canine transitional cell carcinoma, J. Vet. Intern. Med. 33 (2019) 1414–1422, https://doi.org/10.1111/jvim.15504.
[24] M.E. Peterson, C.R. Ward, Etiopathologic findings of hyperthyroidism in cats, Vet. Clin. North Am. Small Anim. Pract. 37 (2007) 633–645, https://doi.org/10.1016/j.cvsm.2007.05.001.
[25] European Commission, Regulation (EC) No 396/2005 of the European parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending council directive 91/414/EEC, (2005). https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881, 20100701:EN:PDF.
[26] European Parliament and Council, Regulation (EC) No 767/2009 of the European parliament and of the council of 13 July 2009 on the placing on the market and use of feed, Off. J. Eur. Union 229 (2009) 1–28. L/.
[27] FEDIAF, Nutritional guidelines: for complete and complementary pet food for cats and dogs, 2024. 10.1016/S0140-6736(84)91417-X.
[28] S. Walorczyk, D. Drozdzyński, Improvement and extension to new analytes of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry revisited, J. Chromatogr. A 1251 (2012) 219–231, https://doi.org/10.1016/j.chroma.2012.06.055.
[29] M.K. van der Lee, G. van der Weg, W.A. Traag, H.G.J. Mol, Qualitative screening and quantitative determination of pesticides and contaminants in animal feed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry, J. Chromatogr. A 1186 (2008) 325–339, https://doi.org/10.1016/j.chroma.2007.11.043.
[30] H. Musarurwa, L. Chimuka, V.E. Pakade, N.T. Tavengwa, Recent developments and applications of QuEChERS based techniques on food samples during pesticide analysis, J. Food Compos. Anal. 84 (2019) 103314, https://doi.org/10.1016/j.jfca.2019.103314.
[31] D. Steiner, R. Krska, A. Malachová, I. Taschl, M. Sulyok, Evaluation of matrix effects and extraction efficiencies of LC-MS/MS methods as the essential part for proper validation of multiclass contaminants in complex feed, J. Agric. Food Chem. 68 (2020) 3868–3880, https://doi.org/10.1021/acs.jafc.9b07706.
[32] H.G.J. Mol, P. Plaza-Bolaños, P. Zomer, T.C. De Rijk, A.A.M. Stolker, P.P.J. Mulder, Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes, Anal. Chem. 80 (2008) 9450–9459, https://doi.org/10.1021/ac801557f.
[33] V. Sharma, B.K. Wadhwa, H.J. Stan, Multiresidue analysis of pesticides in animal feed concentrate, Bull. Environ. Contam. Toxicol. 74 (2005) 342–349, https://doi.org/10.1007/s00128-004-0590-y.
[34] P. Eyring, M. Tienstra, H. Mol, S.S. Herrmann, P.H. Rasmussen, H.L. Frandsen, M. E. Poulsen, Development of a new generic extraction method for the analysis of pesticides, mycotoxins, and polycyclic aromatic hydrocarbons in representative animal feed and food samples, Food Chem. 356 (2021) 129653, https://doi.org/10.1016/j.foodchem.2021.129653.
[35] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.
[36] O. Golge, A. Koluman, B. Kabak, Validation of a modified QuEChERS method for the determination of 167 pesticides in milk and milk products by LC-MS/MS, Food Anal. Methods 11 (2018) 1122–1148, https://doi.org/10.1007/s12161-017-1066-0.
[37] Y. Ni, H. Yang, H. Zhang, Q. He, S. Huang, M. Qin, S. Chai, H. Gao, Y. Ma, Analysis of four sulfonylurea herbicides in cereals using modified Quick, easy, cheap, effective, rugged, and safe sample preparation method coupled with liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1537 (2018) 27–34, https://doi.org/10.1016/j.chroma.2018.01.017.
[38] W. Zheng, J.A. Park, D. Zhang, A.M. Abd El-Aty, S.K. Kim, S.H. Cho, J.M. Choi, J. H. Shim, B.J. Chang, J.S. Kim, H.C. Shin, Determination of fenobucarb residues in animal and aquatic food products using liquid chromatography-tandem mass spectrometry coupled with a QuEChERS extraction method, J. Chromatogr. B

Analyt. Technol. Biomed. Life Sci. 1058 (2017) 1–7, https://doi.org/10.1016/j.jchromb.2017.05.008.
[39] X. Xu, X. Zhang, E. Duhoranimana, Y. Zhang, P. Shu, Determination of methenamine residues in edible animal tissues by HPLC-MS/MS using a modified QuEChERS method: validation and pilot survey in actual samples, Food Control 61 (2016) 99–104, https://doi.org/10.1016/j.foodcont.2015.09.030.
[40] Á. Grande-Martínez, F.J. Arrebola-Liébanas, J.L. Martínez-Vidal, M.E. Hernández-Torres, A. Garrido-Frenich, Optimization and validation of a multiresidue pesticide method in rice and wheat flour by modified QuECHERS and GS-MS/MS, Food Anal. Methods 9 (2015) 548–563, https://doi.org/10.1007/s12161-015-0214-7.
[41] A.B. Bordin, L. Minetto, I. do Nascimento Filho, L.L. Beal, S. Moura, Determination of pesticide residues in whole wheat flour using modified QuEChERS and LC–MS/MS, Food Anal. Methods 10 (2017) 1–9, https://doi.org/10.1007/s12161-016-0542-2.
[42] L. Han, J. Matarrita, Y. Sapozhnikova, S.J. Lehotay, Evaluation of a recent product to remove lipids and other matrix co-extractives in the analysis of pesticide residues and environmental contaminants in foods, J. Chromatogr. A 1449 (2016) 17–29, https://doi.org/10.1016/j.chroma.2016.04.052.
[43] S.J. Lehotay, Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study, J. AOAC Int. 90 (2007) 485–520, https://doi.org/10.1093/jaoac/90.2.485.
[44] P. Parrilla Vázquez, E. Hakme, S. Uclés, V. Cutillas, M. Martínez Galera, A. R. Mughari, A.R. Fernández-Alba, Large multiresidue analysis of pesticides in edible vegetable oils by using efficient solid-phase extraction sorbents based on quick, easy, cheap, effective, rugged and safe methodology followed by gas chromatography–tandem mass spectrometry, J. Chromatogr. A 1463 (2016) 20–31, https://doi.org/10.1016/j.chroma.2016.08.008.
[45] G. Bernardi, M. Kemmerich, L.C. Ribeiro, M.B. Adaime, R. Zanella, O.D. Prestes, An effective method for pesticide residues determination in tobacco by GC-MS/MS and UHPLC-MS/MS employing acetonitrile extraction with low-temperature precipitation and d-SPE clean-up, Talanta 161 (2016) 40–47, https://doi.org/10.1016/j.talanta.2016.08.015.
[46] J.A. Ferreira, J.M.S. Ferreira, V. Talamini, J. de F. Facco, T.M. Rizzetti, O. D. Prestes, M.B. Adaime, R. Zanella, C.B.G. Bottoli, Determination of pesticides in coconut (Cocos nucifera Linn.) water and pulp using modified QuEChERS and LC–MS/MS, Food Chem. 213 (2016) 616–624, https://doi.org/10.1016/j.foodchem.2016.06.114.
[47] S. Schanzer, E. Kröner, G. Wibbelt, M. Koch, A. Kiefer, F. Bracher, C. Müller, Miniaturized multiresidue method for the analysis of pesticides and persistent organic pollutants in non-target wildlife animal liver tissues using GC-MS/MS, Chemosphere 279 (2021), https://doi.org/10.1016/j.chemosphere.2021.130434.
[48] European Commission, Commission Implementing Regulation (EU) 2024/989 of 2 April 2024 concerning a coordinated multiannual control programme of the Union for 2025, 2026 and 2027 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure, (2024) 1–15.
[49] European Comission, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE 11312/2021, European Commission, Health & Consumer Protection Directorate-General (2021) 1–57. https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp, ?CntID=727.
[50] A. Acosta-Dacal, C. Rial-Berriel, R. Díaz-Díaz, M. del M. Bernal-Suárez, O. P. Luzardo, Optimization and validation of a QuEChERS-based method for the simultaneous environmental monitoring of 218 pesticide residues in clay loam soil, Sci. Total Environ. 753 (2021) 142015, https://doi.org/10.1016/j.scitotenv.2020.142015.
[51] A. Acosta-Dacal, C. Rial-Berriel, R. Díaz-Díaz, M. del M. Bernal Suárez, M. Zumbado, L.A. Henríquez-Hernández, O.P. Luzardo, Supporting dataset on the optimization and validation of a QuEChERS-based method for the determination of 218 pesticide residues in clay loam soil, Data Brief. 33 (2020) 106393, https://doi.org/10.1016/j.dib.2020.106393.
[52] C. Rial-Berriel, A. Acosta-Dacal, M. Zumbado, O.P. Luzardo, Micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife, Sci. Total Environ. 736 (2020), https://doi.org/10.1016/j.scitotenv.2020.139444.
[53] C. Rial-Berriel, A. Acosta-Dacal, F. González, N. Pastor-Tiburón, M. Zumbado, O. P. Luzardo, Supporting dataset on the validation and verification of the analytical method for the biomonitoring of 360 toxicologically relevant pollutants in whole blood, Data Brief. 31 (2020) 105878, https://doi.org/10.1016/j.dib.2020.105878.
[54] C. Rial-Berriel, A. Acosta-Dacal, M. Zumbado, L.A. Henríquez-Hernández, Á. Rodríguez-Hernández, A. Macías-Montes, L.D. Boada, M.D.M. Travieso-Aja, B. M. Cruz, O.P. Luzardo, A method scope extension for the simultaneous analysis of pops, current-use and banned pesticides, rodenticides, and pharmaceuticals in liver. Application to food safety and biomonitoring, Toxics 9 (2021), https://doi.org/10.3390/toxics9100238.
[55] C. Rial-Berriel, Á. Ramos-Luzardo, A. Acosta-Dacal, A. Macías-Montes, P. Fernández-Valerón, L. Alberto Henríquez-Hernández, M. Zumbado, L.D. Boada, O.P. Luzardo, Validation of a method scope extension for simple biomonitoring of 353 pollutants in serum samples, Toxics 11 (2023) 498, https://doi.org/10.3390/TOXICS11060498, 2023Page 498 11.
[56] Ł. Dabrowski, Evaluation of solvents used as keepers in the determination of organic pollutants by GC/MS, Molecules 25 (2020), https://doi.org/10.3390/molecules25194419.
[57] A. Acosta-Dacal, C. Rial-Berriel, R. Díaz-Díaz, M.D.M. Bernal-Suárez, M. Zumbado, L.A. Henríquez-Hernández, P. Alonso-González, E. Parga-Dans, O.P. Luzardo, Validation of a method scope extension for the analysis of pops in soil and verification in organic and conventional farms of the canary islands, Toxics 9 (2021) 101, https://doi.org/10.3390/toxics9050101.
[58] K. Maštovská, S.J. Lehotay, Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues, J. Chromatogr. A 1040 (2004) 259–272, https://doi.org/10.1016/j.chroma.2004.04.017.
[59] K. Iosif, I. Konstantinou, Determination of pesticide residues in olive oil using QuEChERS extraction and liquid chromatography–Orbitrap high-resolution mass spectrometry: comparison of different clean-up sorbents and validation study, Sustainability 15 (2023), https://doi.org/10.3390/su15118714.
[60] D. Remane, M.R. Meyer, D.K. Wissenbach, H.H. Maurer, Ion suppression and enhancement effects of co-eluting analytes in multi-analyte approaches: systematic investigation using ultra-high-performance liquid chromatography/mass spectrometry with atmospheric-pressure chemical ionization or electrospray ionizat, Rapid Commun. Mass Spectr. 24 (2010) 3103–3108, https://doi.org/10.1002/rcm.4736.
[61] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, An overview of matrix effects in liquid chromatography–mass spectrometry, Mass Spectrom. Rev. 30 (2011) 491–509, https://doi.org/10.1002/mas.20298.
[62] S. Walorczyk, Development of a multi-residue screening method for the determination of pesticides in cereals and dry animal feed using gas chromatography-triple quadrupole tandem mass spectrometry, J. Chromatogr. A 1165 (2007) 200–212, https://doi.org/10.1016/j.chroma.2007.07.071.
[63] S. Walorczyk, Development of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry. II. Improvement and extension to new analytes, J. Chromatogr. A 1208 (2008) 202–214, https://doi.org/10.1016/j.chroma.2008.08.068.
[64] European Commission, EU pesticides database, (2024). https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en.