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Determination of pesticide residues in fish feed by GC-MS/MS and LC-MS/MS (WAHSPE method)

Report 33

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CONTENT:

1. Introduction

This report describes a new generic QuEChERS extraction method for the analysis of 190 pesticides in fish feed, seen the difficulties observed by the conventional QuEChERS method for the analysis of pesticide residues in fatty matrices. Fish feed are known as high fat content matrices (22% fat content). The key element of the extraction protocol is a **three-phase** extraction consisting of 5 formic acid in acetonitrile, heptane and water. Phase separation is induced by addition of ammonium formate. The water phase is assumed to retain the most polar pesticides, the acetonitrile and the heptane phase are supposed to retain the medium polar and non-polar pesticides but also fat residues. Therefore, each of the two phases (acetonitrile and heptane) are further cleaned-up using solid phase extraction, and analysed separately by GC-MS/MS and LC-MS/MS.

2. Extraction method

At least 100 g sample was milled with a mill from Retsch (Haan, Germany) using 0.5 mm mesh sieve size. 5.0 g milled sample was placed in a 50 mL plastic tube and 10.0 mL of 5 formic acid in acetonitrile, 10.0 mL of Milli-Q water and 10.0 mL of heptane were added. The mixture was shaken overhead for 1 hour. 5 g ammonium formate was added to induce phase separation, and the mixture was shaken for one minute, followed by centrifugation for 10 minutes at 5.000 x g and 20˚C. After centrifugation, the phases were separated and transferred into 15 mL plastic tubes.

The **acetonitrile** phase requires further cleaning for the removal of lipids and water. Therefore, 1.00 mL of this phase was transferred to an Eppendorf vial containing 250 mg PSA, 150 mg MgSO4 and 25 mg C18 for dSPE clean-up according to an established method [1]. The Eppendorf tube was shaken for 30 seconds and centrifuged at 10.000 X g for 10 minutes. 400 µl of the supernatant were filtered in a filter vial (0.2µm pore size). 200 µl of the filtered supernatant was transferred to a glass vial with glass insert, acidified by adding 10 µl of 1 formic acid in acetonitrile, and analysed by **GC-MS/MS and LC-MS/MS**.

The **heptane** phase requires further cleaning from the removal of water and fat residues before **GC-MS/MS** and **LC-MS/MS** analysis. Therefore, 1 mL of the heptane extract was transferred into a glass tube and 1 mL of pure acetonitrile was added. The mixture was evaporated under nitrogen at 40 ˚C until the heptane phase was evaporated leaving acetonitrile and residual lipids. This acetonitrile extract was cleaned-up using the SPE [2] column EZ-Pop NP to remove the lipids as follows: prior to clean-up, the column was conditioned with 10 mL acetone and dried for 10 minutes by vacuum. The acetonitrile extract and the residual lipids were loaded on the column. 15 mL of acetonitrile was added to elute the retained analytes into a 20 mL glass test-tube. The eluate was evaporated to 1 mL by a stream of nitrogen at 40˚C. The sample was transferred to a vial with insert and analyzed by GC-MS.

The cleaned up extracts were mixed with internal standards solution in acetonitrile in a ratio of 1:1 (V:V).

The water phase was not analyzed for pesticides residue in fish feed. However, for other applications of polar compounds (diquat and paraquat) in wheat, the water phase was analyzed by LC-MS/MS demonstrating satisfactory recoveries.

In the end, for fish feed, four injections on two different systems can be deployed to provide the coverage of the whole method: The heptane and acetonitrile phase analyzed separately via LC-MS/MS and GC-MS/MS.

3. Spiking procedure

- 190 pesticides in fish feed samples were spiked at three different spike levels in six replicates each at 5, 10 and 50 µg kg^{-1}.

Blank samples, analyzed and found free of target analytes were used as blanks and to prepare matrix matched standard curves.

4. Instrumentation

Pesticide residues in fish feed were analyzed by:

- GC-MS/MS. TSQ 8000 Evo Triple Quadrupole Mass Spectrometer (Thermo Scientific). Column: Thermo Scientific TG-5SILMS, 30m, 0.25 mm ID, 0.25µm coating, with 5m Safeguard column. Electron energy: 70eV, source temperature: 300˚C, transfer line: 280˚C, injection volume: 1µl.
- LC-MS/MS. HPLC coupled to an EVOQ Elite Bruker Triple Quadrupole instrument (Thermo Scientific). HPLC flow: 0.4ml/min, Eluent A: 0.1 formic acid and 5mM Ammoniumhydroxid in MilliQ-Water, Eluent B: Methanol, gradient: starting conditions: 98 Eluent A, 2 Eluent B; 0.1 minute: 65 Eluent A and 35 Eluent B, 7 minute: 2 Eluent A and 98 Eluent B, constant gradient 7-10 minutes 2 Eluent A and 98 Eluent B, 13 minutes: 98 Eluent A and 2 Eluent B.

5. Results

The validation was studied in accordance with the SANTE guidelines including validation acceptance criteria of recoveries between 70 and 120% and RSD < 20% [3].

Recovery results are presented in appendix 2. A total of 190 compounds were validated, 122 were analysed by GC-MS/MS and 68 were analysed by LC-MS/MS.

An LOQ of 0.005 $mg.kg^{-1}$ was achieved for 131 compounds (69% of the compounds). An LOQ of 0.01 $mg.kg^{-1}$ was achieved for 9% of the compounds (17 compounds), and a LOQ of 0.05 $mg.kg^{-1}$ was achieved for 31 compounds (16% of the compounds).

The reason for pesticides having a recovery below 70% (carbendazim and omethoate) might be because they were dissolved in the water phase to some extent, which was not measured in this experiment. Some pesticides had a recovery >120% (bixafen, coumaphos, dimoxystrobin, indoxacarb, methiocarb, procymidone). The reason for pesticides having recovery values higher than 120% might be because of interfering compounds.

Some pesticides from the organochlorine group (aldrin, dieldrin, chlordane-cis, chlordane-trans, DDD-pp, DDE-pp, DDT-op, DDT-pp, endosulfan-alpha, endosulfan-beta, endrin, HCH-alpha, HCH-beta, heptachlor, heptachlorepoxide) were found in significant percentages in both the heptane and the acetonitrile phases with combined recoveries between 82-114 % at 50 $\mu g.kg^{-1}$ (See Appendix 1), demonstrating the advantage of the water-acetonitrile-heptane followed by SPE clean-up (WAHSPE) compared to QuEChERS. The combined RSD is calculated as average of both RSDs obtained with the two phases.

The other majority of pesticides were amenable to QuEChERS, and the developed WAHSPE method showed similar recovery and LOQ values, thus no advantages. In another way, the majority of the compounds were mainly extracted by the acetonitrile phase (see Appendix 2).

Conclusions

The advantage of WAHSPE compared to QuEChERS is the inclusion of highly polar analytes in one end of the polarity scale, and non-polar analytes in fatty matrices on the other end. If only the medium polarity range of compounds are of interest, QuECHERS will generally excel as more efficient, as

WAHSPE generally will require more separate analyses to exploit its full potential, two LC analyses and two GC analyses. WAHSPE the method is therefore considered fit for the monitoring and control of pesticides residues in fatty and complex matrices such as fish feed.

References

(1) Tienstra, M.; Mol, H. G. J. Application of Gas Chromatography Coupled to Quadrupole-Orbitrap Mass Spectrometry for Pesticide Residue Analysis in Cereals and Feed Ingredients. J. AOAC Int. 2018, 101 (2), 342–351. https://doi.org/10.5740/jaoacint.17-0408.

(2) Stenerson, K. K.; Shimelis, O.; Halpenny, M. R.; Espenschied, K.; Ye, M. M. Analysis of Polynuclear Aromatic Hydrocarbons in Olive Oil after Solid-Phase Extraction Using a Dual-Layer Sorbent Cartridge Followed by High-Performance Liquid Chromatography with Fluorescence Detection. J. Agric. Food Chem. 2015, 63 (20), 4933–4939. https://doi.org/10.1021/jf506299f.

(3) Sante/11813/2017, Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues and Analysis in Food and Feed.; 2018. https://doi.org/10.13140/RG.2.2.33021.77283.

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Appendix 1. Recoveries of some organochlorine pesticides in fish feed determined in the heptane and the acetonitrile phase as well as the total recovery calculated as the sum of the recovery values of both phases. The spike level was 50 µg kg^{-1}.

Compound	Recovery determined in heptane phase (%) (RSD%)	Recovery determined in acetonitrile phase (%) (RSD%)	Total recovery (%)(RSD%)
Aldrin	78 (19)	11 (14)	90 (16)
Dieldrin	59 (16)	55 (13)	114 (14)
Chlordane-cis	67 (20)	37 (11)	104 (15)
Chlordane-trans	71 (19)	42 (5)	113 (12)
DDD pp	43 (8)	64 (4)	107 (6)
DDE pp	78 (29)	21 (4)	99 (10)
DDT op	72 (12)	23 (5)	95 (9)
DDT pp	59 (17)	35 (6)	95 (12)
Endosulfan-alpha	59 (23)	46 (8)	105 (16)
Endosulfan-beta	20 (48)	82 (7)	82 (7)
Endrin	52 (14)	49 (18)	101 (16)
HCH-alpha	31 (10)	72 (5)	103 (7)
HCH-beta	10 (35)	100 (4)	110 (13)
Heptachlor	75 (11)	23 (10)	98 (10)
Heptachlorepoxide	47 (9)	60 (13)	107 (11)

Appendix 2. Recoveries and LOQs of 190 pesticides in fish feed determined by GC-MS/MS and LC-MS/MS in the **heptane and the acetonitrile phase** which is the sum of the recovery values of both phases injected separately. The compounds analyzed by LC-MS/MS are highlighted in grey.

	Compound	Recovery % (RSD%) at 0.005 mg kg^{-1}	Recovery % (RSD%) at 0.01 mg kg^{-1}	Recovery % (RSD%) at 0.05 mg kg^{-1}	LOQ µg kg^{-1}
1	2-Phenylphenol	100 (7)	104 (7)	94 (5)	0.005
2	4,4´-Methoxychlor	134 (13)	128 (16)	114 (16)	0.05
3	Acetamiprid	109 (6)	114 (2)	105 (4)	0.005
4	Acrinathrin I	100 (5)	112 (16)	113 (11)	0.005
5	Aldicarb Sulfone	90 (7)	99 (4)	92 (6)	0.005
6	Aldrin	-	-	90 (16)	0.05
7	Azinphos-ethyl	95 (11)	114 (19)	118 (10)	0.005
8	Azinphos-methyl	132 (10)	123 (6)	112 (4)	0.05
9	Azoxystrobin	125 (6)	129 (9)	119 (14)	0.05
10	Bifenthrin	88 (9)	100 (12)	99 (5)	0.005
11	Bitertanol	238 (14)	136 (3)	112 (9)	0.05
12	Bixafen	126 (6)	124 (4)	122 (12)	>0.05
13	Boscalid	115 (6)	122 (3)	111 (3)	0.005
14	Bromopropylate	74 (9)	81 (2)	71 (3)	0.005
15	Bromuconazole I	97 (22)	97 (13)	96 (4)	0.005
16	Bupirimate	97 (15)	114 (7)	109 (5)	0.005
17	Buprofezin	59 (4)	62 (6)	88 (9)	0.05
18	Cadusafos	107 (11)	111 (7)	106 (6)	0.005
19	Carbaryl	111 (4)	114 (3)	104 (6)	0.005
20	Carbendazim	45 (8)	52 (6)	53 (5)	>0.05
21	Carbetamide	106 (5)	114 (4)	106 (5)	0.005
22	Carbofuran	123 (1)	127 (4)	119 (5)	0.05
23	Carbofuran, 3-hydroxy	86 (8)	98 (8)	90 (8)	0.005
24	Carbosulfan	132 (10)	123 (6)	112 (4)	0.05
25	Carboxin	109 (5)	101 (4)	92 (4)	0.005
26	Chlordane-cis	-	-	104 (15)	0.05
27	Chlordane-trans	117 (21)	130 (13)	113 (12)	0.005
28	Chlorfenapyr	92 (40)	97 (18)	98 (13)	0.005
29	Chlorfenvinphos	110 (11)	107 (7)	108 (9)	0.005
30	Chlorobenzilate	91 (4)	100 (5)	90 (3)	0.005
31	Chlorpropham	100 (14)	105 (6)	114 (14)	0.005
32	Chlorpyrifos	54 (16)	106 (17)	107 (7)	0.01
33	Chlorpyrifos-methyl	107 (15)	112 (19)	98 (10)	0.005

34	Clofentezine	116 (16)	95 (10)	97 (10)	0.005
35	Clothianidin	90 (4)	93 (4)	86 (4)	0.005
36	Coumaphos	141 (10)	141 (5)	128 (7)	>0.05
37	Cyflutrin	126 (12)	118 (16)	110 (8)	0.01
38	Cyhalothrin-lambda	107 (5)	113 (13)	112 (11)	0.005
39	Cymiazole	92 (9)	94 (5)	86 (2)	0.005
40	Cypermethrin	125 (5)	104 (4)	116 (11)	0.01
41	Cyproconazole	96 (8)	108 (4)	101 (2)	0.005
42	Cyprodinil	-	89 (18)	88 (9)	0.01
43	DDD pp	119 (16)	111 (16)	107 (6)	0.005
44	DDE pp	99 (21)	94 (20)	99 (10)	0.005
45	DDT op	79 (22)	83 (14)	95 (9)	0.005
46	DDT pp	103 (14)	93 (17)	95 (12)	0.005
47	Deltamethrin II	95 (14)	79 (11)	83 (9)	0.005
48	Demeton-S-methyl sulfone	101 (8)	116 (4)	106 (4)	0.005
49	Diazinon	101 (17)	108 (12)	106 (5)	0.005
50	Dichlorvos	106 (10)	111 (13)	88 (7)	0.005
51	Dicloran	99 (6)	111 (4)	103 (4)	0.005
52	Dicofol, o,p'-	91 (2)	100 (3)	90 (2)	0.005
53	Dicofol, p,p'-	95 (6)	105 (6)	96 (4)	0.005
54	Dieldrin	-	-	114 (14)	0.05
55	Difenoconazole I	126 (4)	125 (3)	107 (5)	0.05
56	Diflubenzuron	106 (3)	109 (4)	105 (4)	0.005
57	Dimethoate	109 (11)	106 (12)	110 (8)	0.005
58	Dimethomorph	105 (7)	115 (6)	117 (7)	0.005
59	Dimoxystrobin	121 (6)	131 (3)	121 (4)	>0.05
60	Diphenylamine	83 (19)	115 (20)	102 (8)	0.005
61	DMF	103 (2)	106 (3)	101 (4)	0.005
62	Endosulfan sulfate	140 (21)	121 (7)	105 (13)	0.01
63	Endosulfan-alpha	55 (27)	42 (23)	105 (16)	0.05
64	Endosulfan-beta	84 (26)	97 (26)	82 (27)	0.005
65	Endrin	-	-	101 (16)	0.05
66	EPN	106 (8)	122 (16)	114 (13)	0.005
67	Epoxiconazole	112 (5)	120 (4)	115 (7)	0.005
68	Ethion	84 (7)	116 (13)	114 (9)	0.005
69	Ethoprophos	92 (5)	115 (12)	109 (9)	0.005
70	Etofenprox	107 (15)	115 (12)	102 (6)	0.005
71	Famoxadone	146 (7)	139 (3)	120 (3)	0.05
72	Fenamiphos	111 (21)	113 (10)	96 (5)	0.005
73	Fenamiphos sulfone	105 (9)	109 (7)	114 (7)	0.005
74	Fenamiphos sulfoxide	35 (224)	44 (103)	83 (12)	0.05
75	Fenarimol	100 (6)	102 (7)	93 (5)	0.005
76	Fenazaquin	-	-	47 (17)	>0.05

77	Fenbuconazole	111 (7)	116 (5)	118 (5)	0.005
78	Fenitrotion	115 (7)	111 (6)	105 (3)	0.005
79	Fenoxycarb	107 (5)	118 (3)	114 (3)	0.005
80	Fenpropathrin	82 (7)	113 (15)	105 (9)	0.005
81	Fenpropimorph	81 (8)	82 (7)	86 (9)	0.005
82	Fenthion	94 (9)	101 (6)	109 (12)	0.005
83	Fenthion oxon sulfone	107 (4)	120 (7)	114 (2)	0.005
84	Fenthion oxon sulfoxide	79 (5)	93 (4)	92 (4)	0.005
85	Fenthion sulfone	130 (2)	131 (8)	119 (7)	0.05
86	Fenthion sulfoxide	98 (6)	108 (2)	113 (7)	0.005
87	Fenvalerate II	103 (6)	96 (5)	110 (11)	0.005
88	Fipronil	153 (13)	133 (7)	117 (11)	0.05
89	Fludioxonil	127 (6)	116 (7)	108 (4)	0.01
90	Flufenoxuron	-	-	72 (25)	>0.05
91	Fluquinconazole	107 (6)	114 (6)	112 (5)	0.005
92	Flusilazole	116 (12)	125 (7)	114 (4)	0.005
93	Flutriafol	119 (6)	113 (5)	104 (5)	0.005
94	Fosthiazate	126 (19)	119 (7)	106 (4)	0.01
95	HCH-alpha	84 (7)	106 (9)	103 (7)	0.005
96	HCH-beta	110 (13)	111 (4)	110 (13)	0.005
97	Heptachlor	93 (10)	98 (15)	98 (10)	0.005
98	Heptachlorepoxide	-	62 (17)	107 (11)	0.05
99	Heptenophos	113 (4)	121 (3)	115 (4)	0.005
100	Hexaconazole	107 (26)	112 (12)	86 (11)	0.005
101	Hexythiozox	117 (16)	108 (10)	106 (9)	0.005
102	Imazalil	86 (16)	81 (11)	90 (6)	0.005
103	Imidacloprid	106 (7)	108 (2)	101 (4)	0.005
104	Indoxacarb	123 (27)	132 (8)	122 (9)	>0.05
105	Iprodione	96 (14)	109 (7)	119 (12)	0.005
106	Iprovalicarb II	119 (9)	117 (9)	116 (5)	0.005
107	Isofenphos-methyl	114 (5)	113 (7)	113 (8)	0.005
108	Isoprothiolane	105 (4)	110 (5)	104 (5)	0.005
109	Isoproturon	109 (3)	112 (6)	106 (3)	0.005
110	Kresoxim-methyl	112 (19)	120 (5)	112 (2)	0.005
111	Lindane	102 (11)	101 (5)	116 (12)	0.005
112	Linuron	6 (224)	106 (23)	101 (3)	0.01
113	Malaoxon	114 (4)	118 (1)	113 (6)	0.005
114	Malathion	113 (7)	120 (5)	114 (5)	0.005
115	Mepanipyrim	91 (12)	102 (5)	113 (8)	0.005
116	Metaflumizone	111 (8)	117 (11)	114 (7)	0.005
117	Metalaxyl	119 (5)	125 (6)	117 (6)	0.005
118	Metconazole	97 (7)	116 (9)	99 (6)	0.005
119	Methacrifos	117 (21)	118 (14)	116 (13)	0.005
120	Methidathion	122 (8)	112 (5)	107 (3)	0.01

121	Methiocarb	16 (105)	84 (32)	125 (15)	>0.05
122	Methiocarb sulfone	77 (11)	77 (7)	82 (10)	0.05
123	Methiocarb sulfoxide	83 (3)	85 (3)	81 (2)	0.005
124	Methomyl	90 (43)	112 (21)	95 (12)	0.005
125	Metribuzin	109 (5)	114 (4)	109 (5)	0.005
126	Mevinphos	101 (4)	105 (6)	109 (4)	0.005
127	Monocrotophos	93 (8)	104 (3)	107 (6)	0.005
128	Myclobutanil	126 (6)	126 (7)	113 (5)	0.05
129	Omethoate	38 (16)	47 (10)	42 (12)	>0.05
130	Oxadixyl	128 (3)	131 (3)	114 (2)	0.05
131	Oxamyl	93 (6)	92 (6)	86 (6)	0.005
132	Oxychlordane	28 (63)	123 (19)	106 (9)	0.05
133	Paclobutrazol	95 (12)	104 (9)	100 (4)	0.005
134	Paraoxon-methyl	255 (81)	112 (56)	119 (10)	0.01
135	Parathion	107 (11)	102 (11)	99 (4)	0.005
136	Parathion-ethyl	82 (19)	80 (23)	106 (7)	0.005
137	Parathion-methyl	115 (7)	118 (10)	107 (3)	0.005
138	Penconazole	83 (4)	96 (4)	94 (6)	0.005
139	Pencycuron	95 (5)	105 (4)	103 (6)	0.005
140	Pendimethalin	112 (22)	100 (15)	106 (7)	0.005
141	Perrmethrin I+II	165 (19)	129 (24)	104 (7)	0.05
142	Phenthoate	104 (10)	98 (11)	94 (7)	0.005
143	Phosmet	144 (8)	126 (7)	111 (5)	0.05
144	Phosphamidon	106 (14)	108 (13)	107 (5)	0.005
145	Phoxim	105 (5)	112 (5)	108 (3)	0.005
146	Pirimicarb	99 (4)	103 (3)	95 (4)	0.005
147	Pirimicarb desmetyl	85 (9)	96 (7)	94 (4)	0.005
148	Pirimiphos methyl	42 (19)	62 (22)	106 (10)	0.05
149	Pirimiphos-ethyl	61 (17)	122 (16)	116 (7)	0.05
150	Pirimiphos-methyl	71 (14)	78 (8)	105 (8)	0.05
151	Prochloraz	104 (5)	111 (6)	101 (8)	0.005
152	Procymidone	258 (25)	193 (11)	125 (4)	>0.05
153	Profenofos	78 (19)	77 (11)	87 (18)	0.05
154	Propargite	108 (25)	109 (25)	88 (9)	0.005
155	Propiconazole	84 (9)	97 (4)	103 (6)	0.005
156	Propyzamide	85 (8)	103 (5)	108 (4)	0.005
157	Prothioconazole-desthio	75 (8)	90 (5)	92 (3)	0.01
158	Prothiofos	-	80 (21)	98 (7)	0.01
159	Pyraclostrobin	111 (6)	120 (7)	112 (5)	0.005
160	Pyrazophos	113 (2)	118 (2)	112 (5)	0.005
161	Pyridaben	123 (16)	114 (11)	105 (7)	0.01
162	Pyrimethanil	96 (22)	103 (17)	92 (5)	0.005
163	Pyriproxyfen	113 (14)	112 (14)	109 (8)	0.005
164	Quinoxyfen	102 (15)	101 (15)	95 (8)	0.005

165	Resmethrin	-	91 (24)	72 (21)	0.01
166	Simazine	-	-	98 (2)	0.05
167	Tebuconazole	94 (13)	110 (3)	99 (3)	0.005
168	Tebufenpyrad	70 (8)	109 (32)	104 (13)	0.01
169	Teflubenzuron	101 (9)	102 (10)	101 (6)	0.005
170	Tefluthrin	98 (9)	108 (10)	104 (6)	0.005
171	Terbuthylazine	66 (15)	84 (8)	101 (4)	0.01
172	Tetraconazole	117 (16)	126 (7)	114 (7)	0.005
173	Tetradifon	79 (11)	77 (4)	111 (14)	0.05
174	Tetramethrin1	86 (10)	93 (6)	96 (4)	0.005
175	Thiacloprid	111 (3)	113 (3)	107 (4)	0.005
176	Thiamethoxam	95 (12)	96 (13)	91 (5)	0.005
177	Thiodicarb	108 (4)	116 (6)	102 (4)	0.005
178	Thiometon	98 (11)	98 (8)	99 (5)	0.005
179	Tolclofos-methyl	112 (18)	111 (18)	108 (9)	0.005
180	Triadimefon	97 (6)	108 (5)	114 (6)	0.005
181	Triadimenol	109 (9)	112 (9)	105 (4)	0.005
182	Triallate	52 (11)	60 (5)	87 (9)	0.05
183	Triazophos	112 (3)	119 (3)	113 (7)	0.005
184	Tricyclazole	75 (4)	78 (6)	76 (4)	>0.05
185	Trifloxystrobin	104 (14)	118 (7)	116 (4)	0.005
186	Triflumuron	97 (6)	104 (4)	103 (3)	0.005
187	Trifluralin	102 (18)	108 (16)	108 (7)	0.005
188	Triticonazole	73 (10)	94 (5)	99 (6)	0.01
189	Vinclozolin	118 (8)	118 (10)	107 (6)	0.005
190	Zoxamide	109 (8)	116 (2)	115 (5)	0.005