EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: **B**oscalid metabolite M510F01,
  Fenpropidin metabolite CGA289267
  Isoxaflutole metabolite RPA202248
  Isoxaflutole metabolite RPA203328
  Spiroxaminecarboxylic acid
  Fenpropimorph carboxylic acid BF-421-2
  Trifloxystrobin metabolite CGA 321113
  Dimoxystrobin metabolite 505M09
- **Commodities**: muscle, liver, kidney, milk
- **Extraction Method(s)**: QuEChERS (citrate) with C18 clean-up
- **Instrumental analysis**: LC-MS/MS

Analysis of various relevant pesticide metabolites of in products of animal origin by the QuEChERS Method using LC-MS/MS

Version 1 (last update: 25.04.2018)

Background information / Initial Observations:

Numerous pesticides are being annually re-evaluated according to Article 12 of Regulation 396/2005/EC. Where indicated, new MRLs and sometimes also new residue definitions are set. Within this framework the EURLs are involved in assessing the analytical amenability of residue definitions proposed for enforcement purposes and in elaborating possible consensus LOQs for the main commodity classes, which are considered when setting residue limits for products where no residues are expected (MRL*s). Within this context, the EURL-SRM mainly focuses on pesticides and relevant metabolites, which are known to pose problems in analysis and are thus considered “difficult” or non-amenable to multiresidue methods. It is first tested whether the analytes or residue definitions can be analyzed by introducing, preferably simple, modifications to traditional multiresidue methods (MRMs, mainly QuEChERS), and if not new methods are developed. Modifications of MRMs may entail various measures to prevent degradation or improve extractability of residues (e.g. adjustment of pH, addition of substances and adjustment of extraction temperature and time), cleavage reactions to release conjugates, special measurement conditions and other. In case of highly polar pesticides, that do not readily partition into the acetonitrile phase of the QuEChERS method, the QuPPe method, which does not involve a partitioning step, or variations thereof are tested.
The present analytical observations report deals with a number of metabolites that are relevant to products of animal origin and that, for various reasons, are expected to pose difficulties in analysis.

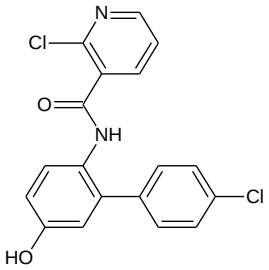
The following metabolites were selected for this study:

- Boscalid metabolite M510F01 (residue definition entails conjugates),
- Fenpropidin metabolite CGA289267 (amphoteric: acidic and basic),
- Fenpropimorph metabolite BF421-2 (amphoteric: acidic and basic),
- Spiroxamine carboxylic acid (amphoteric: acidic and basic),
- Isoxaflutole metabolite RPA 203328 (weakly acidic),
- Isoxaflutole metabolite RPA 203348 (acidic and highly polar),
- Trifloxystrobin metabolite CGA 321113 (acidic),
- Dimoxystrobin metabolite 505M09 (acidic)

In most cases these substances had to be ordered from applicants, as they were not yet available in the analytical standards market when the analyses started. Priority was given to metabolites associated with compounds being re-evaluated according to Article 12 of Reg 396/2005/EC, or that were included at some point within the scope of EU coordinated monitoring program or within the scope of the DG-SANTE Working Document, which gives guidance to the Member States in designing their own National monitoring programs.

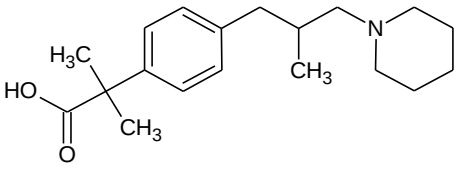
Compound details:

Boscalid metabolite M510F01	
Parameter	**Value**
Molecular Mass	359.21 g/mol
Exact mass	358.027583 Da
CAS	661463-87-2
IUPAC name	2-Chloro-N-(4′-chloro-5-hydroxybiphenyl-2-yl)nicotinamide
pKa (calculated)	ACD/Labs: pK_{a1} = 0.9; pK_{a2} = 9.4; pK_{a3} = 11.1 Chemicalize: pK_{a1} = -0.1 @ pyridine-N moiety (weakly basic) pK_{a2} = 9.3 @ phenol moiety (weakly acidic), pK_{a3} = 14.6 @ amide-N moiety (weakly acidic) Virtually non-ionized in the pH-range from 2-7
LogD (calculated)	ACD/Labs: 3.9 (pH 5.5) Chemicalize: Virtually non-ionized and with constant polarity in the pH range of 2-8 (highest logD =4.62 non-ionic form),
Residue definition EU Reg. (EU) 2016/156	Boscalid (F) (R) (A) (R) = The residue definition differs for the following combinations pesticide-code number: code 1000000 except 1040000, 1011010, 1011020, 1011050, 1012010, 1012020, 1012050, 1013010, 1013020, 1013050, 1014010, 1014020, 1014050, 1015010, 1015020, 1015050, 1016010, 1016020, 1017010, 1017020, 1017050, 1020000, 1030000: Sum of boscalid and its hydroxy metabolite 2-chloro-N-(4′-chloro-5-hydroxybiphenyl-2-yl)nicotinamide (free and conjugated) expressed as boscalid This means that the M510F01 (the hydroxyl-metabolite of boscalid) is **only relevant for liver and kidney** of swine, bovine, goat, sheep, equine, poultry and other farmed terrestrial animals as well as for other edible offal of poultry.
Boscalid is approved in…	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK
ADI / ARfD	0.04 mg/kg bw per day / Not applicable (08/44/EC)



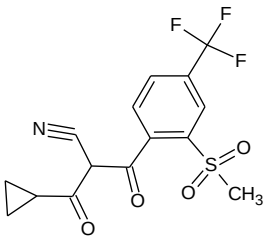
Fenpropidin metabolite CGA289267

Parameter	Value
Molecular Mass	303.4 g/mol
Exact mass	303.279829 Da
CAS	-
IUPAC name	2-methyl-2-{4-[2-(piperidin-1-ylmethyl)propyl]phenyl}propanoic acid
pKa (calculated)	ACD/Labs: pK_{a1} = 4.4 ; pK_{a2} = 9.7 Chemicalize: pK_{a1} = 4.4 @ carboxy-moiety (intermediately acidic); pK_{a2} = 10.0 @ piperidine-N (strongly basic); predominantly cationic at pH<4.5; predominantly anionic at pH >10; predominantly doubly charged at pH 4.5-10
LogD (calculated)	ACD/Labs: 1.6 (pH 5.5) Chemicalize: Lowest polarity in the range between pH 5 and 9 (highest logD = 1.87)
Residue definition EU Reg. (EU) 2014/61	Fenpropidin (sum of fenpropidin and its salts, expressed as fenpropidin) (R) (A) (R) = The residue definition differs for the following combinations pesticide-code number: fenpropidin-code 1000000 except 1040000: sum of fenpropidin, 2-methyl-2-[4-(2-methyl-3- piperidin-1-yl-propyl)-phenyl]propionic acid, and their salts, expressed as fenpropidin This means that the CGA289267 (the carboxy-metabolite of fenpropidin) is **relevant for all commodities of animal origin except apicultural products**
Fenpropidin is approved in…	AT, BE, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, SK, UK
ADI / ARfD	0.02 mg/kg bw per day / 0.02 mg/kg bw (Dir 08/66)

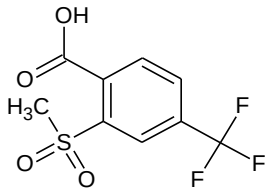


Isoxaflutole metabolite RPA202248

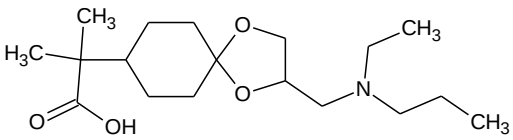
Parameter	Value
Molecular Mass	359.3 g/mol
Exact mass	359.043912 Da
CAS	143701-75-1
IUPAC name	3-cyclopropyl-2-[2-methanesulfonyl-4-(trifluoromethyl)benzoyl]-3-oxopropanenitrile
pKa (calculated)	ACD/Labs: pK_{a1} = 1.1 Chemicalize: pK_{a1} = 5.4 @ CH moiety in alpha position to CO, CO and CN (weakly acidic)
LogD (calculated)	ACD/Labs: logD -0.3 (pH 7) Chemicalize: Lowest polarity when pH <4 (highest logP= 2.03), highest polarity when pH >9 (lowest logP= 0.11); logP 1.92 (pH 5); 1.5 (pH 6)
Residue definition EU Reg. (EU) 2015/845	Isoxaflutole (sum of isoxaflutole and its diketonitrile-metabolite, expressed as isoxaflutole) Footnote: RPA 202248 is 2-cyano-3-cyclopropyl-1-(2-methylsulfonyl-4-trifluoromethylphenyl) propane-1,3-dione. RPA 203328 is 2-methanesulfonyl-4-trifluoromethylbenzoic acid. This means that the RPA202248 (the diketonitrile-metabolite of isoxaflutole) is **relevant for all commodities of plant and animal origin.** The mentioning of RPA 203328 in the footnote must be an error.
Isoxaflutole is approved in…	AT, BE, BG, CZ, DE, EL, ES, FR, HR, HU, IE, IT, LU, NL, PL, PT, RO, SI, SK, UK
ADI / ARfD	0.02 mg/kg bw per day / ARfD not applicable



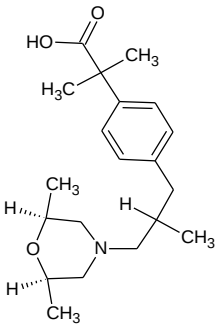
Isoxaflutole metabolite RPA203328	
Parameter	**Value**
Molecular Mass	268.21 g/mol
Exact mass	268.001713 Da
CAS	142994-06-7
IUPAC name	2-methanesulfonyl-4-(trifluoromethyl)benzoic acid
pKa (calculated)	ACD/Labs: pK_{a1} = 2.0; No base pK_a Chemicalize: pK_{a1} = 2.3 (strongly acidic, carboxy moiety)
LogD (calculated)	ACD/Labs: -1.2 (pH 7) Chemicalize: logD 1.16 (pH2); 0.54 (pH3), -0.38 (pH 4), -1.3 (pH 5), -2.0 (pH 6).
Residue definition EU Reg. (EU) 2015/845	Not regulated. **The mentioning of RPA 203328 in the footnote must be an error (see above)**
Isoxaflutole is approved in…	AT, BE, BG, CZ, DE, EL, ES, FR, HR, HU, IE, IT, LU, NL, PL, PT, RO, SI, SK, UK
ADI / ARfD	0.02 mg/kg bw per day / ARfD not applicable



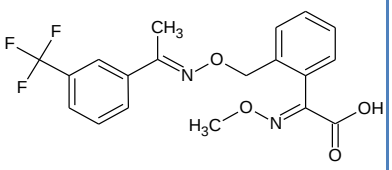
Spiroxamine carboxylic acid	
Parameter	**Value**
Molecular Mass	327.46 g/mol
Exact mass	327.240959 Da
CAS	156042-38-5
IUPAC name	2-(2-{[ethyl(propyl)amino]methyl}-1,4-dioxaspiro[4.5]decan-8-yl)-2-methylpropanoic acid
pKa (calculated)	ACD/Labs: pK_{a1} = 4.8; pK_{a2} = 8.8 Chemicalize: pK_{a1} = 4.1 @ carboxylic acid moiety (intermediately acidic); pK_{a2} = 9.3 @quarternary amine (intermediately basic); predominantly cationic at pH<4; predominantly anionic at pH >9; predominantly doubly charged at pH 4-9
LogD (calculated)	ACD/Labs: 0.7 (pH 7) Chemicalize: Lowest polarity in the range between pH 5 and 8.5 (highest logD = 0.84)
Residue definition EU Reg. (EU) 2016/452	Spiroxamine (sum of isomers) (A) (R) (A) = The EU reference labs identified the reference standard for spiroxamine carboxylic acid metabolite M06 as commercially not available. When re-viewing the MRL, the Commission will take into account the commercial availability of the reference standard referred to in the first sentence by 30 March 2017, or, if that reference standard is not commercially available by that date, the unavailability of it. (R) = The residue definition differs for the following combinations pesticide-code number: Spiroxamine — code 1000000 except 1040000: Spiroxamine carboxylic acid metabolite M06, expressed as spiroxamine (sum of isomers) This means that the spiroxamine carboxylic acid is **relevant for all commodities of animal origin except apicultural products**
Spiroxamine is approved in…	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FR, HR, HU, IE, IT, LT, LU, LV, PL, PT, RO, SI, SK, UK
ADI / ARfD	0.025 mg/kg bw per day / 0.1 mg/kg bw



Fenpropimorph carboxylic acid (BF-421-2)	
Parameter	**Value**
Molecular Mass	333.47 g/mol
Exact mass	333.230394 Da
CAS	121098-45-1
IUPAC name	2-(4-{3-[(2R,6S)-2,6-dimethylmorpholin-4-yl]-2-methylpropyl}phenyl)-2-methylpropanoic acid
pKa (calculated)	ACD/Labs: pK_{a1} = 4.4; pK_{a2} = 7.3 Chemicalize: pK_{a1} = 4.4 @ carboxylic acid moiety (intermediately acidic); pK_{a2} = 8.5@morpholine-N (intermediately basic); predominantly cationic at pH<4.5; predominantly anionic at pH >8.5; predominantly doubly charged at pH 4.5-8.5
LogD (calculated)	ACD/Labs:1.3 (pH 7) Chemicalize: Lowest polarity in the range between pH 5 and 8 (highest logD = 1.65)
Residue definition EU Reg. (EU) 2017/170	Fenpropimorph (sum of isomers) (F) (R) R) = The residue definition differs for the following combinations pesticide-code number: Fenpropimorph - code 1000000: Fenpropimorph carboxylic acid (BF 421-2) expressed as fenpropimorph This means that fenpropimorph carboxylic acid is **relevant for all commodities of animal origin including apicultural products**
Fenpropimorph is approved in…	AT, BE, BG, CZ, DE, EE, EL, ES, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, RO, SE, SI, SK, UK
ADI / ARfD	0.003 mg/kg bw per day / 0.03 mg/kg bw

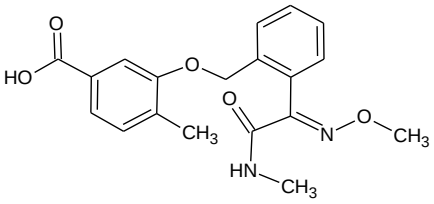


Trifloxystrobin metabolite CGA 321113	
Parameter	**Value**
Molecular Mass	394.34 g/mol
Exact mass	394.114042 Da
CAS	252913-85-2
IUPAC name	(2E)-2-(methoxyimino)-2-[2-({[(E)-{1-[3-trifluoromethyl) phenyl]ethylidene}amino]oxy}methyl)phenyl]acetic acid
pKa (calculated)	ACD/Labs: pK_{a1} = 2.8; No base pK_a Chemicalize: pK_{a1} = 3.2@carboxylic acid moiety (strongly acidic); pK_{a2} = 2.25 @imino-moiety (weakly basic); pK_{a3} = -0.45 @methoxy-imino-moiety (very weakly basic);
LogD (calculated)	ACD/Labs: 0.6 (pH 7) Chemicalize: lowest polarity (highest logD = 4.2) at pH 2.8; pH4 logD=3.5; pH5 logD=2.6; pH5 logD=1.7
Residue definition EU Reg. (EU) 2017/626	Trifloxystrobin (A) (F) (R) (A) = The EU reference labs identified the reference standard for CGA321113 as commercially not available. When re-viewing the MRL, the Commission will take into account the commercial availability of the reference standard referred to in the first sentence by 23 July 2016, or, if that reference standard is not commercially available by that date, the unavailability of it. (F) = Fat soluble (R) = The residue definition differs for the following combinations pesticide-code number: Trifloxystrobin- code 1000000 except 1040000: the sum of trifloxystrobin and its metabolite (E, E)-methoxyimino- {2-[1-(3-trifluoromethyl-phenyl)-ethylideneamino-oxymethyl]-phenyl}-acetic acid (CGA 321113)
Tritosulfuron is approved in…	AT, BE, BG, CY, CZ, DE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, MT, NL, PL, PT, RO, SE, SI, SK, UK
ADI / ARfD	0.1 mg/kg bw per day / ARfD not applicable



Dimoxystrobin metabolite 505M09

Parameter	Value
Molecular Mass	356.37 g/mol
Exact mass	356.137222 Da
CAS	1418095-11-0
IUPAC name	3-({2-[(E)-(methoxyimino)(methylcarbamoyl) methyl]phenyl}methoxy)-4-methylbenzoic acid
pKa (calculated)	ACD/Labs: pK_{a1} = -1.7; pK_{a2} = 4.2; pK_{a3} = 11.3 Chemicalize: pK_{a1} = 4.06@carboxylic acid moiety (intermediately acidic); pK_{a2} = 14.66 @methylamide moiety (very weakly basic); pK_{a3} = 0.62 @methoxy-imino-moiety (very weakly basic);
LogD	ACD/Labs: 1.3 (pH 7) Chemicalize: lowest polarity (highest logD ~3) at pH 1.5-3 logD 2.8 (pH 4), 2.0 (pH 5), 1.1 (pH 6)
Residue definition EU Reg. (EU) 2015/1040	Dimoxystrobin (R) (A) (A) = The EU reference labs identified the reference standard for 505M09 as commercially not available. When re-viewing the MRL, the Commission will take into account the commercial availability of the reference standard referred to in the first sentence by 1 July 2016, or, if that reference standard is not commercially available by that date, the unavailability of it. (R) = The residue definition differs for the following combinations pesticide-code number: Dimoxystrobin — code 1000000 except 1040000: 505M09, expressed as dimoxystrobin. Metabolite 505M09 = 3-({2-[(1E)-N-methoxy-2-(methylamino)-2-oxoethanimidoyl]benzyl}oxy)-4-methylbenzoic acid
Dimoxystrobin is approved in…	AT, BE, BG, CZ, DE, EE, FR, HR, HU, LT, LU, LV, PL, RO, SK, UK
ADI / ARfD	0.004 mg/kg bw per day / 0.004 mg/kg bw



Consumables:

Analytical Standards:

Table 1: Sources of analytical standards (exemplary)

Substance	Purity	CAS	Source	Order number
Boscalid met. M510F01	95.7%	661463-87-2	BASF (friendly donation)[1]	-
	on request		HPC Standards GmbH	681008
	>=95		Sigma-Aldrich Chemie GmbH	28001-10MG
Fenpropidin met. CGA289267	98%	?	Syngenta (friendly donation)	-
Isoxaflutole met. RPA202248	99.5%	143701-75-1	Bayer CropScience (friendly donation)	-
	on request		HPC Standards GmbH	679125
	on request		Toronto Research Chemicals	R700915
Isoxaflutole met. RPA203328	95%	142994-06-7	Fluoro Chem Ltd.	-
	on request		HPC Standards GmbH	679380
	on request		Toronto Research Chemicals	M328135
	on request		Sigma-Aldrich Chemie GmbH	MAT370172678-1G/074529-1G
Spiroxamine carboxylic acid	71.1%	156042-38-5	Bayer CropScience (friendly donation)	-
Fenpropimorph carboxylic acid (BF-421-2)	99.8%	121098-45-1	BASF	-
	on request		HPC Standards GmbH	681497
	on request		Toronto Research Chemicals	F249510
	on request		CHEMOS GmbH & Co. KG	-
	on request		BOC Sciences	-
Trifloxystrobin met. CGA 321113	99.9%	252913-85-2	Sigma-Aldrich	34518-10MG
	99.9%		CHEMOS GmbH & Co. KG	WS252913852.5ml
	on request		BOC Sciences	-
Dimoxystrobin metabolite 505M09	96.8%	1418095-11-0	Sigma-Aldrich	93127-10MG
	on request		HPC Standards GmbH	681007
Propyzamid D3	99%	-	CDN Isotopes	D-6431
1,3-bis(4-nitrophenyl)urea (BNPU)	99.5%	330-95-0	Sigma-Aldrich	-

All other materials and chemicals used as listed in EN 15662

[1] used for experiments

Measurement conditions

Measurement was conducted by LC-MS/MS in the ESI-positive and ESI-negative mode depending on the compound. Details are given in *Table 2*, *Table 3* and *Table 4*.

Table 2: LC-instrumentation details

LC	WATERS Acquity UPLC		
MS/MS	SCIEX API 4000 Q-Trap, run in ESI positive mode or in ESI negative mode		
Column	Acquity BEH C18, 2.1x100 mm, 1.7 µm		
Pre-column	Acquity BEH C18, 2.1x5 mm, 1.7 µm		
Mobile Phase	ESI positive mode A: 5 mmol NH_4formate in purified water (5% methanol) B: 5 mmol NH_4formate in methanol ESI negative mode A: 0.01% acetic acid in purified water (5% acetonitrile) B: 0.01% acetic acid in acetonitrile		
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)
	0	95	5
	0.5	60	40
	4	10	90
	8	10	90
	8.1	95	5
Equilibration Time	5 min		
Run Time	13 min		
Flow	0.4 mL min^{-1}		
Injection volume	2 µL, partial loop with needle overfill		
Column temperature	40°C		

Table 3: MS/MS details (ESI-pos. mode using Sciex API 4000 QTrap[2]):

Compound	Intensity ranking	Q 1	Q 3	DP	CE	CXP
Fenpropidin met. CGA289267	**1**	304.2	107.1	36	69	4
	2	304.2	117.1	36	71	6
	3	304.2	105.0	36	69	2
Spiroxamine car-boxylic acid	**1**	328.2	144.1	71	31	8
	2	328.2	100.0	71	51	4
Fenpropimorph carboxylic acid (BF-421-2)	**1**	334.2	107.0	101	55	4
	2	334.2	105.1	101	59	4
	3	334.2	91.1	101	81	4
Trifloxystrobin met. CGA 321113	**1**	395.0	186.0	56	25	10
	2	395.0	145.0	56	61	8
	3	395.0	115.9	56	47	6
	4	395.0	89.2	56	95	4
Dimoxystrobin met. 505M09[3]	**1**	357.0	116.1	66	35	6
	2	357.0	205.1	66	19	10
	3	357.0	250.2	66	21	2
Propyzamid D3	**-**	259.2	193.0	41	21	10

[2] An older generation instrument API 4000 QTrap is used for validation studies in order to generate LOQs that can be monitored by the majority of laboratories. When using an **API 5500** instrument increase DP values by 20 in absolute terms.

[3] **Dimoxystrobin met. 505M09** can also be measured in ESI positive mode, at a slightly better sensitivity than in the ESI negative mode, but within this study it was measured at the ESI negative mode

Table 4: MS/MS details (ESI-neg. mode using Sciex API 4000 QTrap[4]):

Compound	Intensity ranking	Q 1	Q 3	DP	CE	CXP
Boscalid met. M510F01	**1**	357.0	244.0	-75	-28	-1
	2	359.0	246.0	-75	-28	-1
	3	359.0	244.0	-75	-26	-1
Isoxaflutole met. RPA202248	**1**	358.0	78.9	-60	-30	-1
	2	358.0	64.0	-60	-78	-1
	3	358.0	107.9	-60	-46	-5
	4	358.0	277.9	-60	-20	-7
Isoxaflutole met. RPA203328	**1**	267.0	222.8	-45	-14	-11
	2	267.0	158.9	-45	-26	-7
	3	267.0	64.1	-45	-70	-1
	4	267.0	79.1	-45	-46	-1
Dimoxystrobin met. 505M09	**1**	355.0	105.9	-75	-38	-3
	2	355.0	266.1	-75	-20	-1
	3	355.0	149.9	-75	-26	-1
	4	355.0	222.0	-75	-26	-9
1,3-bis(4-nitrophenyl)urea (BNPU)	**-**	301.1	136.9	-45	-16	-7
Propyzamid D3	**-**	257.0	231.0	-70	-20	-1

Experiments conducted and observations:

The validation experiments were based on the QuEChERS procedure (EN-15662[5]). The main focus was on muscle, liver, kidney and on full fat cow's milk. In all cases recovery experiments were conducted at two spiking levels (0.01 mg/kg and 0.05 mg/kg). The results of the validation experiments are shown in *Table 5* and

[4] An older generation instrument API 4000 QTrap is used for validation studies in order to generate LOQs that can be monitored by the majority of laboratories. When using an **API 5500** instrument increase DP values by 20 in absolute terms.

[5] Detailed instructions on the QuEChERS method are given in the CEN method EN 15662 (citrate buffered), see also brief description under www.quechers.de .

Table 6. Two mass transitions were evaluated for each compound.

Fenpropidin metab. CGA 289267, spiroxamine carboxylic acid, fenpropimorph carboxylic acid (BF-421-2) and trifloxystrobin metab. CGA 321113 were analyzed in the **ESI-positive** mode using propyzamid-D3 as internal standard. At the 0.05 mg/kg spiking level mean recovery rates were between 80-120% (the range within which no correction for recovery is required) and RSDs ≤ 12 % for all compounds and both mass transitions. At the 0.01 mg/kg recovery rates were outside the 80-120% range for one mass transition of CGA 321113 (rec. 79%) and one mass transition of CGA 289267 (rec. 124%). RSDs were ≤ 15 % in all cases except for one mass transition of CGA 321113 (RSD 22%).

Table 5: Recovery rates from various AO commodities using QuEChERS EN-15662 with C-18 cleanup (without PSA),, n=5 (ESI-pos. mode using Sciex API 4000 QTrap):

Matrix	Anal. Portion	Water added	Spiking level	Compound	Mass Transition [m/z]	Mean Recov. (n=5)	RSD %
Muscle (bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin metab. CGA 289267	304/107	93%	5
					304/117	97%	9
				Spiroxamine carboxylic acid	328/144	102%	6
					328/100	96%	2
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	98%	11
					334/105	88%	8
				Trifloxystrobin metab. CGA 321113	395/186	85%	10
					395/145	79%	9
Muscle (swine)	5 g	5 mL	0.05 mg/kg	Fenpropidin metab. CGA289267	304/107	100%	2
					304/117	100%	5
				Spiroxamine carboxylic acid	328/144	102%	2
					328/100	105%	2
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	94%	5
					334/105	94%	3
				Trifloxystrobin metab. CGA 321113	395/186	94%	2
					395/145	93%	3
Milk (cow's)	10 g	-	0.01 mg/kg	Fenpropidin metab. CGA 289267	304/107	104%	8
					304/117	124%	11
				Spiroxamine carboxylic acid	328/144	111%	8
					328/100	111%	9
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	93%	12
					334/105	94%	10
				Trifloxystrobin metab. CGA 321113	395/186	87%	9
					395/145	95%	13
Milk (cow's)	10 g	-	0.05 mg/kg	Fenpropidin metab. CGA 289267	304/107	111%	1
					304/117	105%	1
				Spiroxamine carboxylic acid	328/144	104%	3

Matrix	Anal. Portion	Water added	Spiking level	Compound	Mass Transition [m/z]	Mean Recov. (n=5)	RSD %
					328/100	107%	4
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	98%	2
					334/105	101%	2
				Trifloxystrobin metab. CGA 321113	395/186	100%	2
					395/145	99%	3
Liver (bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin metab. CGA 289267	304/107	105%	5
					304/117	107%	13
				Spiroxamine carboxylic acid	328/144	108%	6
					328/100	113%	3
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	98%	10
					334/105	87%	8
				Trifloxystrobin metab. CGA 321113	395/186	90%	7
					395/145	89%	5
Liver (bovine)	5 g	5 mL	0.05 mg/kg	Fenpropidin metab. CGA 289267	304/107	99%	6
					304/117	98%	6
				Spiroxamine carboxylic acid	328/144	99%	7
					328/100	98%	6
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	89%	7
					334/105	91%	8
				Trifloxystrobin metab. CGA 321113	395/186	90%	7
					395/145	87%	12
Kidney mix (Swine+Bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin metab. CGA 289267	304/107	99%	4
					304/117	110%	13
				Spiroxamine carboxylic acid	328/144	99%	10
					328/100	102%	6
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	101%	15
					334/105	102%	9
				Trifloxystrobin metab. CGA 321113	395/186	106%	22
					395/145	94%	13
Kidney mix (Swine+Bovine)	5 g	5 mL	0.05 mg/kg	Fenpropidin metab. CGA 289267	304/107	110%	9
					304/117	98%	12
				Spiroxamine carboxylic acid	328/144	103%	6
					328/100	110%	5
				Fenpropimorph carboxylic acid (BF-421-2)	334/107	98%	12
					334/105	91%	8
				Trifloxystrobin metab. CGA 321113	395/186	99%	10
					395/145	93%	7

Boscalid metab. M510F01, isoxaflutole metab. RPA202248, isoxaflutole metab. RPA203328 and dimoxystrobin metab. 505M09 were analyzed in the **ESI-negative** mode using 1,3-bis(4-

nitrophenyl)urea (BNPU) as internal standard. At the 0.01 mg/kg spiking level mean recovery rates were between 80-120% and RSDs ≤ 19 % for all compounds and both mass transitions with exception of RPA203328, that showed recovery rates between 66 and 78% and RSDs ≤ 10 %. At the 0.05 mg/kg spiking level mean recovery rates were within 80-120% range and RSDs ≤ 8 % in all cases with exception of RPA203328 that showed recovery rates between 66 and 80% and RSDs ≤ 8 %.

Some exemplary chromatograms are shown in *Figure 1*-*Figure 8*.

Table 6: Recovery data for Substances from various AO commodities using QuEChERS EN-15662 with C-18 cleanup (without PSA), n=5 (ESI-neg. mode using ABSciex API 4000 QTrap):

Matrix	Anal. Portion	Water added	Spiking level	Compound	Mass Transition [m/z]	Mean Recov. (n=5)	RSD %
Muscle (bovine)	5 g	5 mL	0.01 mg/kg	Boscalid metab. M510F01	357/244	93%	5
					359/246	92%	10
				Isoxaflutole metab. RPA202248	358/79	95%	3
					358/64	96%	5
				Isoxaflutole metab. RPA203328	267/159	66%	10
					267/223	66%	6
				Dimoxystrobin metab. 505M09	355/106	96%	4
					355/266	97%	11
Muscle (swine)	5 g	5 mL	0.05 mg/kg	Boscalid metab. M510F01	357/244	92%	6
					359/246	91%	3
				Isoxaflutole metab. RPA202248	358/79	94%	2
					358/64	95%	4
				Isoxaflutole metab. RPA203328	267/159	67%	4
					267/223	68%	5
				Dimoxystrobin metab. 505M09	355/106	100%	4
					355/266	103%	5
Milk (cow's)	10 g	-	0.01 mg/kg	Boscalid metab. M510F01	357/244	95%	6
					359/246	91%	5
				Isoxaflutole metab. RPA202248	358/79	94%	2
					358/64	94%	5
				Isoxaflutole metab. RPA203328	267/159	78%	5
					267/223	77%	7
				Dimoxystrobin metab. 505M09	355/106	100%	3
					355/266	103%	6
Milk (cow's)	10 g	-	0.05 mg/kg	Boscalid metab. M510F01	357/244	98%	5
					359/246	105%	8
				Isoxaflutole metab. RPA202248	358/79	98%	3
					358/64	95%	1
				Isoxaflutole metab. RPA203328	267/159	79%	6
					267/223	80%	5
				Dimoxystrobin metab. 505M09	355/106	100%	5
					355/266	104%	5
Liver (bovine)	5 g	5 mL	0.01 mg/kg	Boscalid metab. M510F01	357/244	119%	2
					359/246	103%	11
				Isoxaflutole metab. RPA202248	358/79	96%	4
					358/64	94%	3
				Isoxaflutole metab. RPA203328	267/159	77%	8
					267/223	77%	3
				Dimoxystrobin metab. 505M09	355/106	109%	4
					355/266	110%	18

Matrix	Anal. Portion	Water added	Spiking level	Compound	Mass Transition [m/z]	Mean Recov. (n=5)	RSD %
Liver (bovine)	5 g	5 mL	0.05 mg/kg	Boscalid metab. M510F01	357/244	115%	3
					359/246	120%	8
				Isoxaflutole metab. RPA202248	358/79	98%	3
					358/64	98%	3
				Isoxaflutole metab. RPA203328	267/159	72%	8
					267/223	74%	6
				Dimoxystrobin metab. 505M09	355/106	112%	6
					355/266	108%	7
Kidney mix (Swine+Bovine)	5 g	5 mL	0.01 mg/kg	Boscalid metab. M510F01	357/244	102%	19
					359/246	101%	12
				Isoxaflutole metab. RPA202248	358/79	100%	8
					358/64	103%	4
				Isoxaflutole metab. RPA203328	267/159	72%	8
					267/223	72%	7
				Dimoxystrobin metab. 505M09	355/106	115%	4
					355/266	110%	5
Kidney mix (Swine+Bovine)	5 g	5 mL	0.05 mg/kg	Boscalid metab. M510F01	357/244	108%	6
					359/246	104%	6
				Isoxaflutole metab. RPA202248	358/79	97%	3
					358/64	96%	2
				Isoxaflutole metab. RPA203328	267/159	69%	6
					267/223	66%	2
				Dimoxystrobin metab. 505M09	355/106	100%	3
					355/266	106%	5

Experiments to improve recovery rates of Isoxaflutole metabolite RPA203328

Using standard QuEChERS (with C18 cleanup) recoveries of the isoxaflutole metab. RPA203328 ranged between 66-80%. The low recoveries could be explained by the high polarity of this compound at the pH of the QuEChERS partitioning step of ~4 (logD -0.38 at pH4). It was therefore decided to conduct further validation experiments using the FA-QuEChERS procedure, which entails the use of acetonitrile containing 1% formic acid and the addition of 4g MgSO4 and 1 g NaCl for phase-separation (without citrate buffering salts). Here the partitioning is accomplished at a pH of ca 2.2, which proved beneficial for increasing the partitioning of this analyte to the acetonitrile phase. The recoveries of RPA203328 increased to 85-96% (see *Table 7*).

Table 7: Recovery rates for Isoxaflutole met. RPA203328 from various AO commodities using QuEChERS EN15662 and FA-QuEChERS, n=5 (ESI-neg. mode using Sciex API 4000 QTrap):

Matrix	Anal. Portion	Water added	Method	Spiking level	Mass Transition [m/z]	Mean Recov. (n=5)	RSD %
Liver (bovine)	5 g	5 mL	QuEChERS EN15662 (citrate buffered)	0.01 mg/kg	267/159	77%	8
					267/223	77%	3
				0.05 mg/kg	267/159	72%	8
					267/223	74%	6
			FA-QuEChERS (with 1% FA)	**0.01 mg/kg**	**267/159**	**85%**	**6**
					267/223	**94%**	**4**
Kidney mix (Swine+Bovine)	5 g	5 mL	QuEChERS EN15662 (citrate buffered)	0.01 mg/kg	267/159	72%	8
					267/223	72%	7
				0.05 mg/kg	267/159	69%	6
					267/223	66%	2
			FA-QuEChERS (with 1% FA)	**0.01 mg/kg**	**267/159**	**91%**	**10**
					267/223	**96%**	**8**

Experiments to localize the source of overestimated recovery rates for spiroxamine carboxylic acid and the fenpropidin metabolite CGA 289267 (in pre-experiments)

Interestingly first experiments showed overestimated recovery rates at low spiking levels for CGA289267 (fenpropidin metabolite) and spiroxamine carboxylic acid (see

Table 8). As these compounds entail strong basic groups with pKa values of 10 and 9.3 respectively, it was hypothesized that this effect might be due to a tendency of these compounds to interact with the glass surfaces via those basic groups within standard mixtures (in the absence of water). It was hypothesized the stronger recovery overestimations observed at the lower spiking levels would be due to proportionally higher losses of these compounds within the lowest concentrated standard solutions. ***Table 9*** shows the results of an experiment confirming this hypothesis.

Table 8: Results of validation experiments of spiroxamine carboxylic acid and CGA 289267 (metabolite of fenpropidin); spiking level 0.01 mg/kg; n=5.
Notes: The working standard used to prepare the calibration solution (0.1 µg/mL acetonitrile) was prepared by diluting the working standard that was used for spiking the analytical portions prior to extraction (1 µg/mL acetonitrile). Both solutions were prepared in glass vessels. Spiking volume was 100 µL in each case.

Matrix	Anal. Portion	Water added	Spiking level	Compound	Mass Trans. [m/z]	Mean Recov. (n=5)	RSD %
Muscle (bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin met. CGA 289267	304/107	210%	8
					304/117	158%	10
				Spiroxamine carboxylic acid	328/144	156%	6
					328/100	169%	10
Liver (bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin met. CGA 289267	304/107	190%	10
					304/117	189%	10
				Spiroxamine carboxylic acid	328/144	153%	3
					328/100	175%	10
Kidney mix (Swine+Bovine)	5 g	5 mL	0.01 mg/kg	Fenpropidin met. CGA 289267	304/107	255%	4
					304/117	277%	13
				Spiroxamine carboxylic acid	328/144	179%	5
					328/100	178%	8

Table 9: Check of 0.05 µg/mL mix used for calibration, due to too high recoveries of Spiroxamine carboxylic acid and CGA 289267 (metabolite of fenpropidin)

Concentration of analytes in original mixture store in glass vessels (solvent: ACN)	Concentration of analytes in freshly prepared dilution in PP vial (solvent: ACN)	Spriroxamine carboxylic acid	CGA 289267 (metabolite of fenpropidin)	Dimoxystrobin 505M09 (example for a non-affecetd compound)
0,05 µg/mL	0.025 µg/mL	41	30	98
0,25 µg/mL	0.025 µg/mL	85	77	93
1 µg/mL	0.025 µg/mL	**100**	**100**	**100**

Figure 1: Exemplary chromatograms of Fenpropidin metabolite CGA 289267 in different matrices generated by API 4000

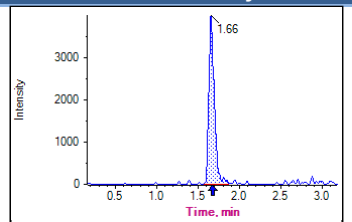
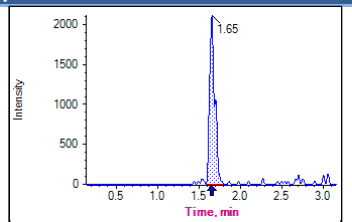
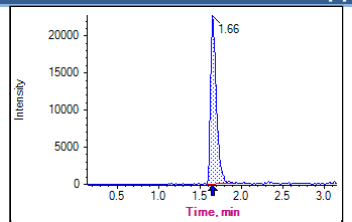
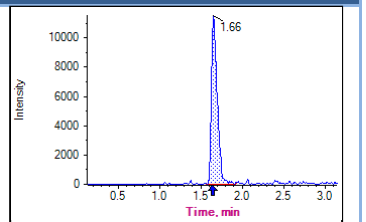
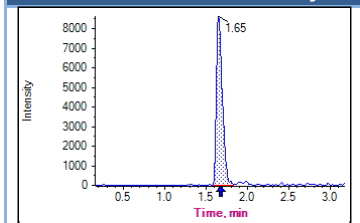
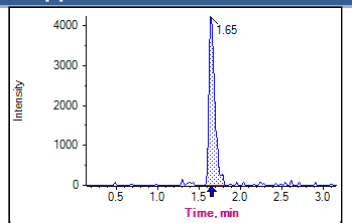
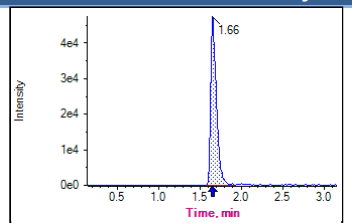
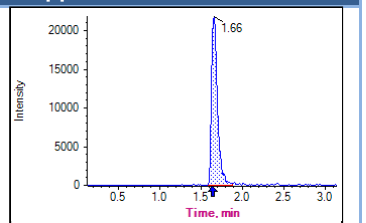
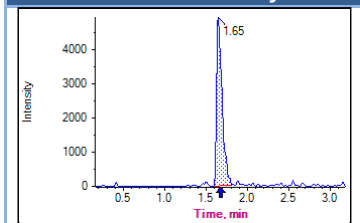
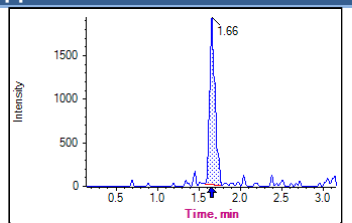
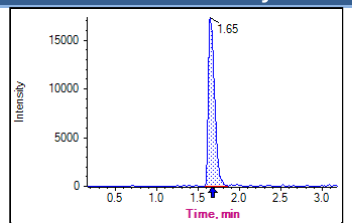
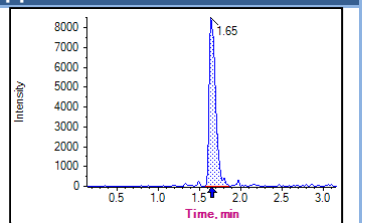
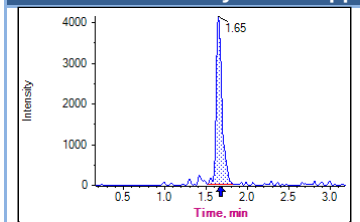
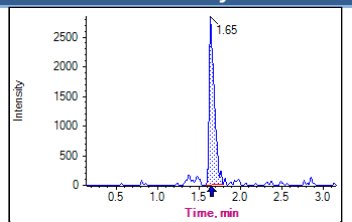
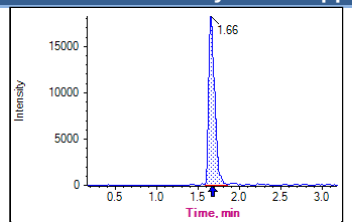
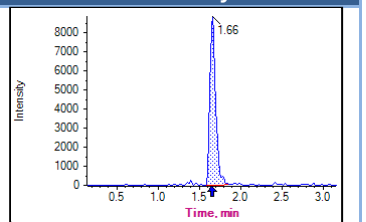
Substance	Recovery 1/5 0.01 ppm bovine muscle		Wdf 1/5 0.05 ppm swine muscle	
Fenpropidin metabolite CGA289267	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117	 CGA 289267 (metabolite of fenpropidin) 304/107	 CGA 289267 (metabolite of fenpropidin) 304/117

Figure 2: Exemplary chromatograms of Spiroxamine carboxylic acid in different matrices generated by API 4000

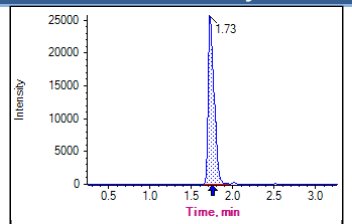
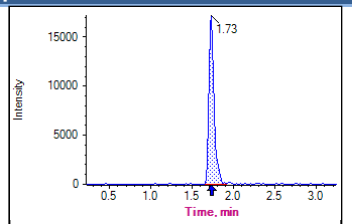
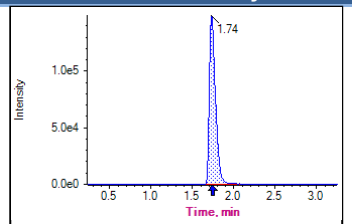
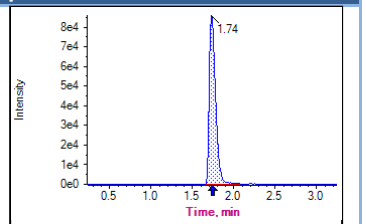
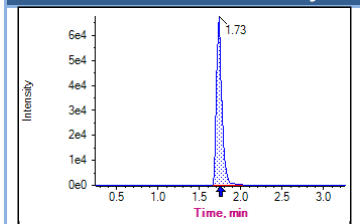
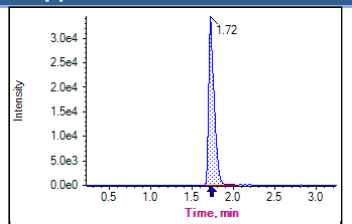
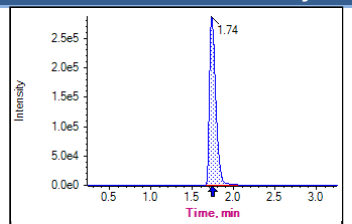
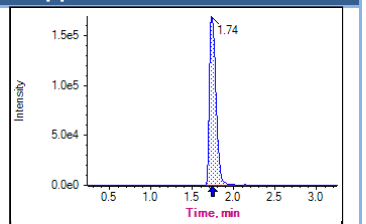
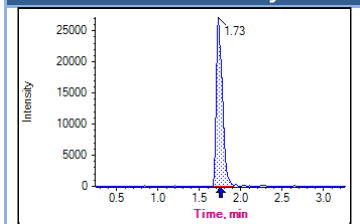
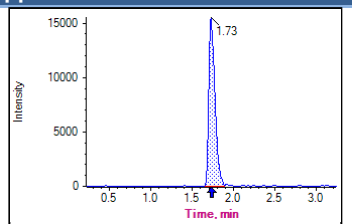
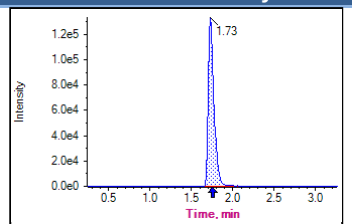
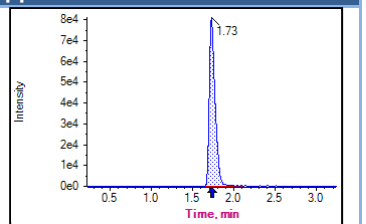
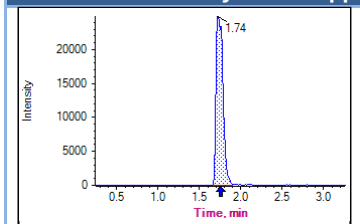
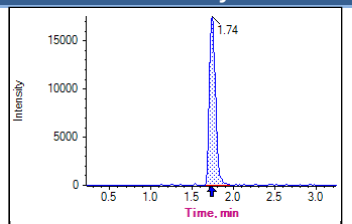
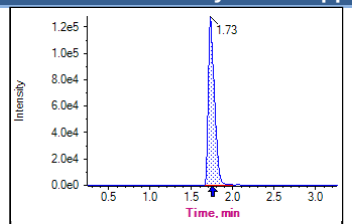
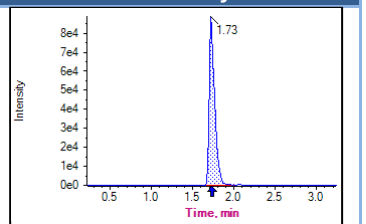
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Spiroxamine carboxylic acid	 Spiroxamine carboxylic acid 328/144 **Area:** 1.276e5	 Spiroxamine carboxylic acid 328/100 **Area:** 7.491e4	 Spiroxamine carboxylic acid 328/144 **Area:** 7.730e5	 Spiroxamine carboxylic acid 328/100 **Area:** 4.595e5
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 Spiroxamine carboxylic acid 328/144 **Area:** 3.072e5	 Spiroxamine carboxylic acid 328/100 **Area:** 1.577e5	 Spiroxamine carboxylic acid 328/144 **Area:** 1.532e6	 Spiroxamine carboxylic acid 328/100 **Area:** 9.455e5
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 Spiroxamine carboxylic acid 328/144 **Area:** 1.383e5	 Spiroxamine carboxylic acid 328/100 **Area:** 8.062e4	 Spiroxamine carboxylic acid 328/144 **Area:** 6.390e5	 Spiroxamine carboxylic acid 328/100 **Area:** 3.989e5
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 Spiroxamine carboxylic acid 328/144 **Area:** 1.462e5	 Spiroxamine carboxylic acid 328/100 **Area:** 9.149e4	 Spiroxamine carboxylic acid 328/144 **Area:** 6.593e5	 Spiroxamine carboxylic acid 328/100 **Area:** 4.073e5

Figure 3: Exemplary chromatograms of Fenpropimorph carboxylic acid (BF-421-2) in different matrices generated by API 4000

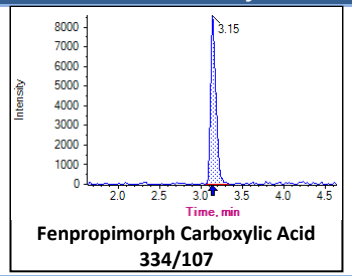
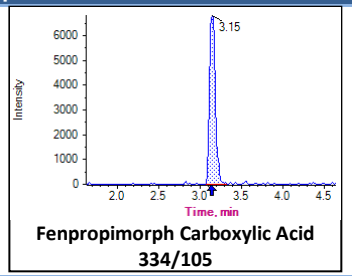
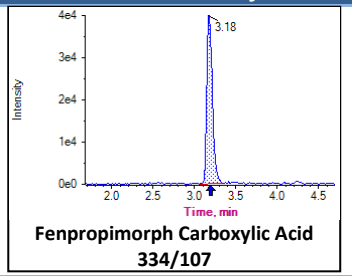
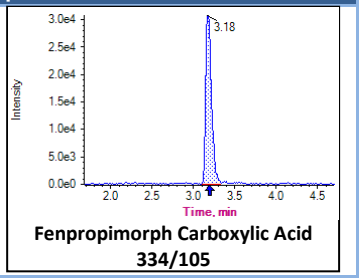
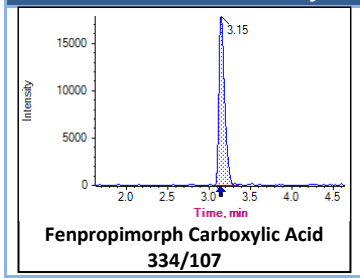
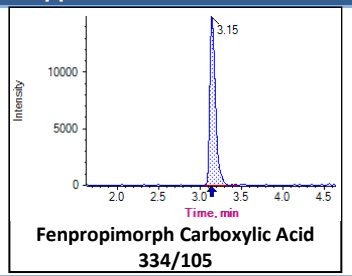
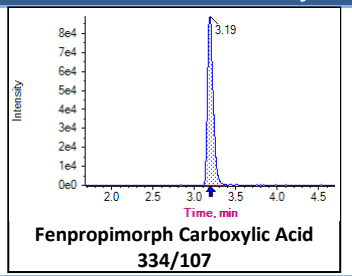
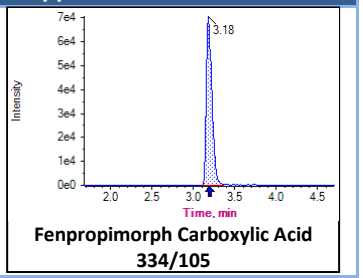
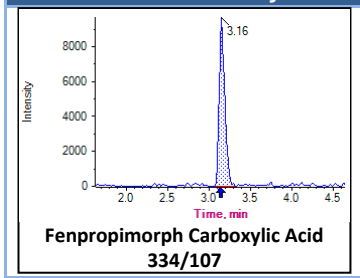
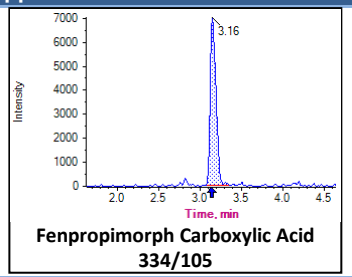
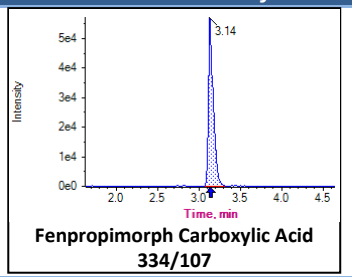
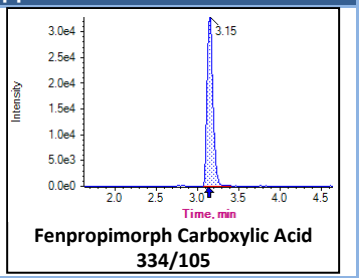
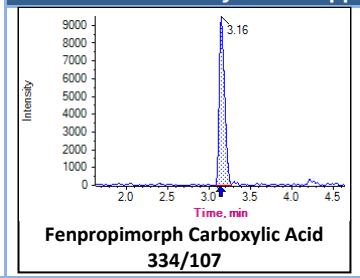
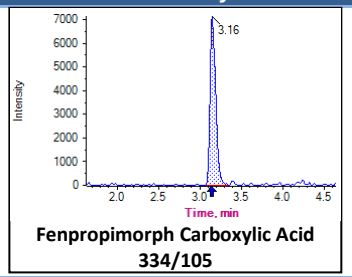
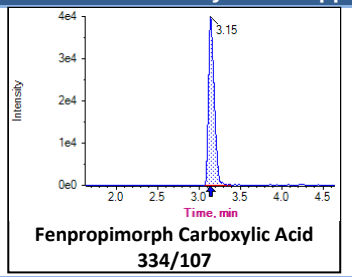
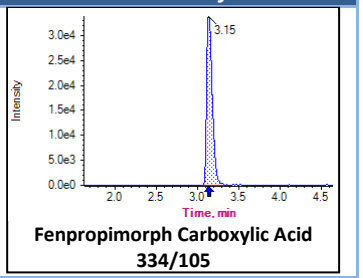
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Fenpropimorph carboxylic acid (BF-421-2)				
	Recovery 1/5 0.01 ppm milk		Recovery 1/5 0.05 ppm milk	
				
	Recovery 1/5 0.01 ppm bovine liver		Recovery 1/5 0.05 ppm bovine liver	
				
	Recovery 1/5 0.01 ppm swine/bovine kidney		Recovery 1/5 0.05 ppm swine/bovine kidney	
				

Figure 4: Exemplary chromatograms of Trifloxystrobin met. CGA 321113 in different matrices generated by API 4000

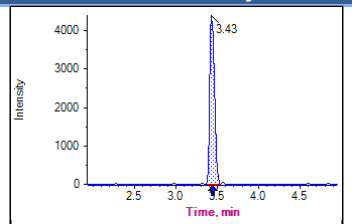
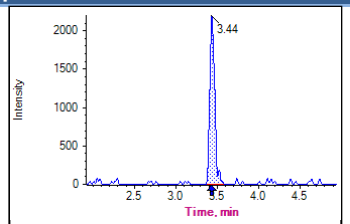
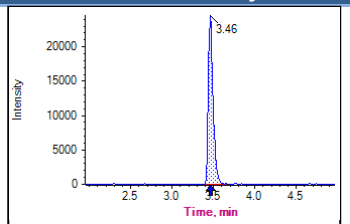
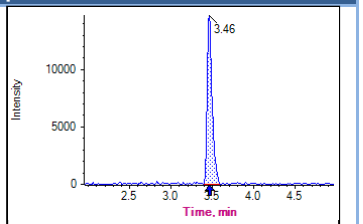
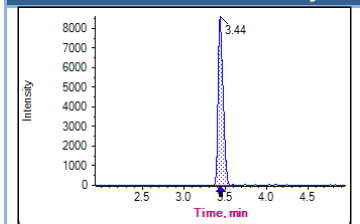
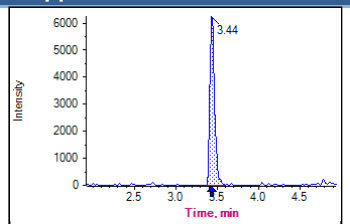
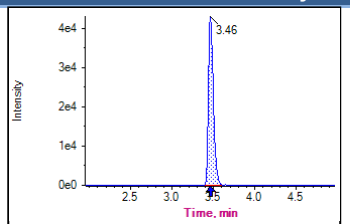
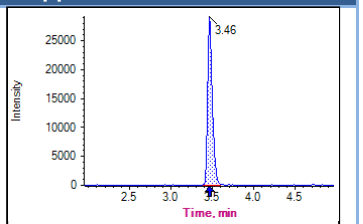
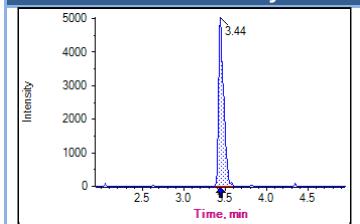
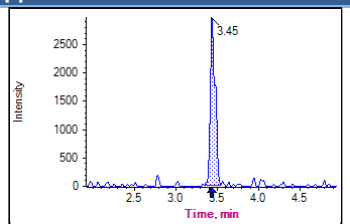
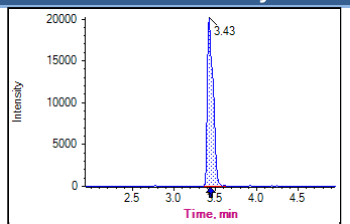
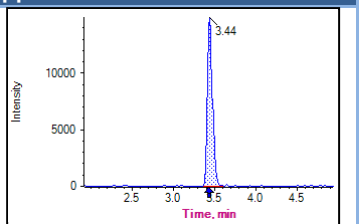
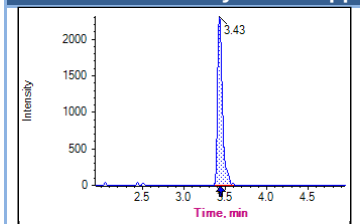
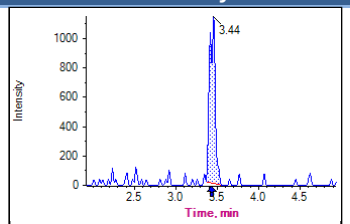
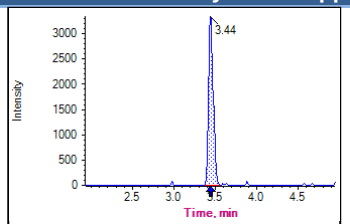
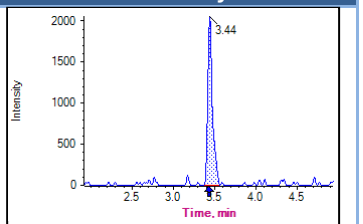
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Trifloxystrobin met. CGA 321113	 **Trifloxystrobin acid 395/186** **Area:** 1.786e4	 **Trifloxystrobin acid 395/145** **Area:** 9.136e3	 **Trifloxystrobin acid 395/186** **Area:** 9.875e4	 **Trifloxystrobin acid 395/145** **Area:** 6.131e4
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 **Trifloxystrobin acid 395/186** **Area:** 3.643e4	 **Trifloxystrobin acid 395/145** **Area:** 2.505e4	 **Trifloxystrobin acid 395/186** **Area:** 1.777e5	 **Trifloxystrobin acid 395/145** **Area:** 1.141e5
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 **Trifloxystrobin acid 395/186** **Area:** 2.126e4	 **Trifloxystrobin acid 395/145** **Area:** 1.201e4	 **Trifloxystrobin acid 395/186** **Area:** 9.722e4	 **Trifloxystrobin acid 395/145** **Area:** 6.407e4
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 **Trifloxystrobin acid 395/186** **Area:** 9.605e3	 **Trifloxystrobin acid 395/145** **Area:** 5.481e3	 **Trifloxystrobin acid 395/186** **Area:** 1.312e4	 **Trifloxystrobin acid 395/145** **Area:** 8.106e3

Figure 5: Exemplary chromatograms of Boscalid metabolite M510F01 in different matrices generated by API 4000

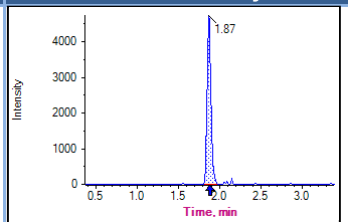
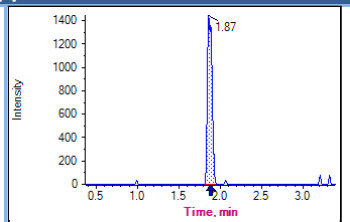
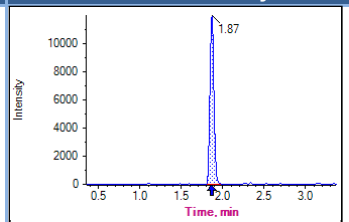
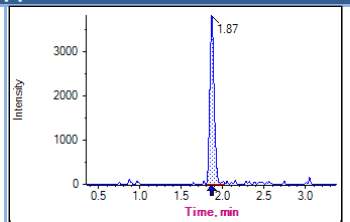
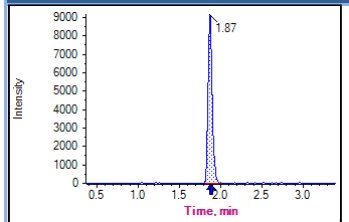
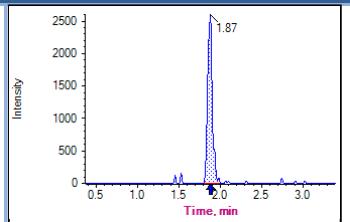
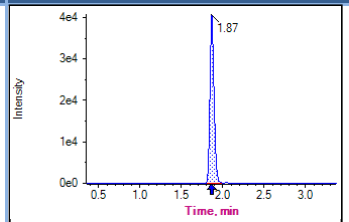
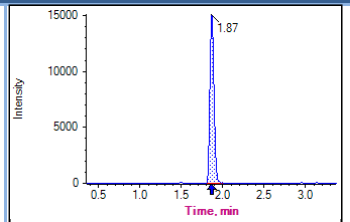
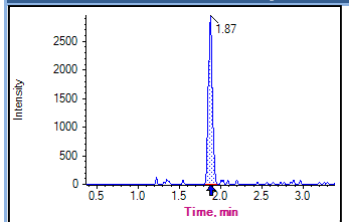
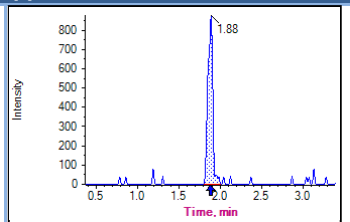
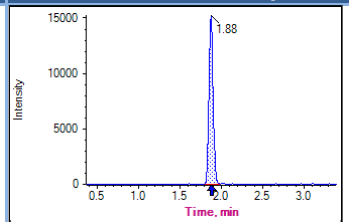
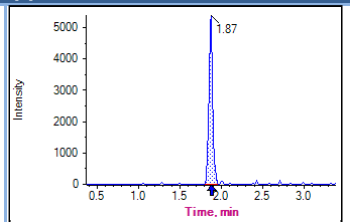
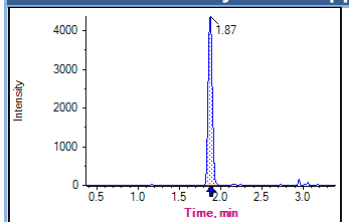
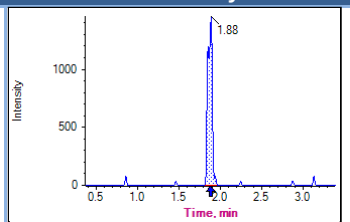
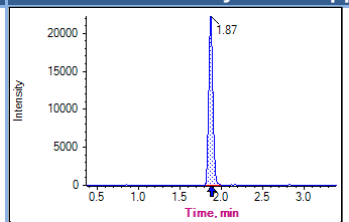
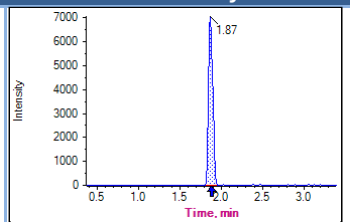
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Boscalid metabolite M510F01	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 1.537e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 5.700e3	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 4.050e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 1.409e4
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 3.247e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 1.026e4	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 1.389e5	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 5.246e4
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 1.011e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 3.619e3	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 5.310e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 1.691e4
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 1.561e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 5.502e3	 **M510F01 (Metab. Boscalid) 357/244** **Area:** 7.822e4	 **M510F01 (Metab. Boscalid) 359/246** **Area:** 2.553e4

Figure 6: Exemplary chromatograms of Isoxaflutole met. RPA202248 in different matrices generated by API 4000

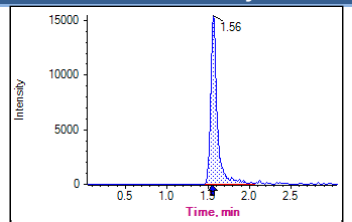
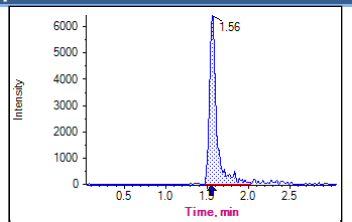
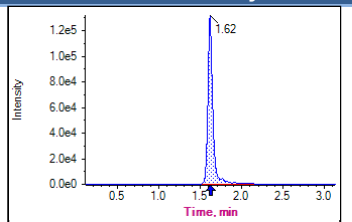
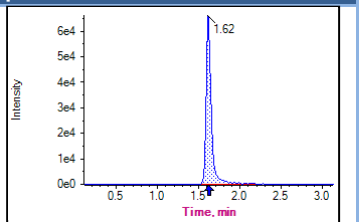
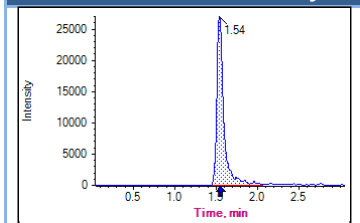
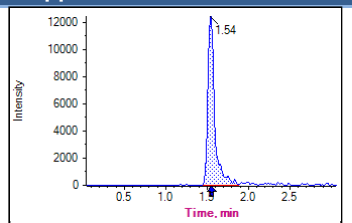
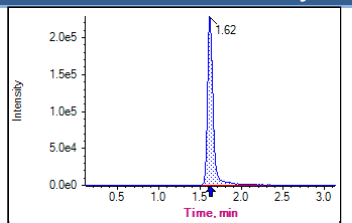
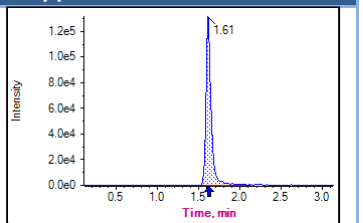
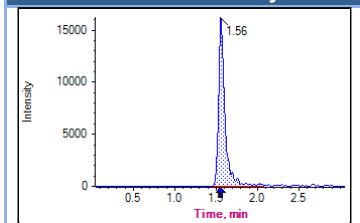
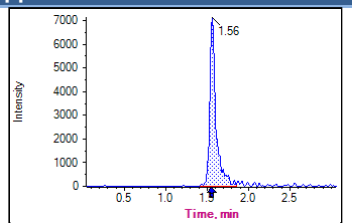
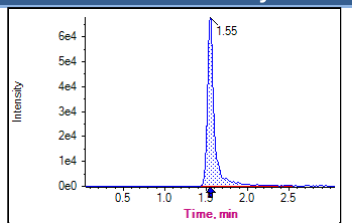
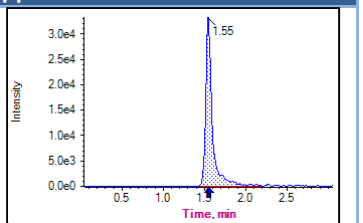
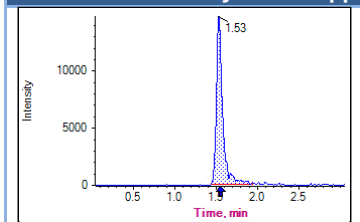
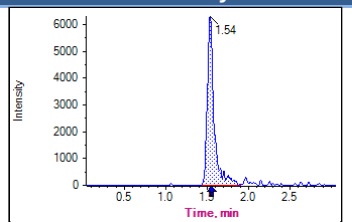
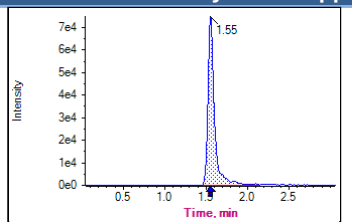
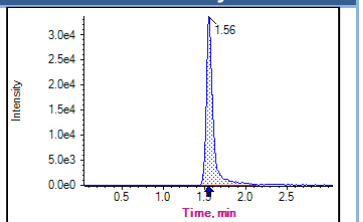
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Isoxaflutole met. RPA202248	 **Isoxaflutole,RPA 202248 358/79** **Area:** 8.542e4	 **Isoxaflutole,RPA 202248 358/64** **Area:** 3.894e4	 **Isoxaflutole,RPA 202248 358/79** **Area:** 5.524e5	 **Isoxaflutole,RPA 202248 358/64** **Area:** 2.883e5
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 **Isoxaflutole,RPA 202248 358/79** **Area:** 1.597e5	 **Isoxaflutole,RPA 202248 358/64** **Area:** 7.269e4	 **Isoxaflutole,RPA 202248 358/79** **Area:** 1.093e6	 **Isoxaflutole,RPA 202248 358/64** **Area:** 5.684e5
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 **Isoxaflutole,RPA 202248 358/79** **Area:** 8.668e4	 **Isoxaflutole,RPA 202248 358/64** **Area:** 3.756e4	 **Isoxaflutole,RPA 202248 358/79** **Area:** 4.001e5	 **Isoxaflutole,RPA 202248 358/64** **Area:** 1.848e5
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 **Isoxaflutole,RPA 202248 358/79** **Area:** 7.766e4	 **Isoxaflutole,RPA 202248 358/64** **Area:** 3.562e4	 **Isoxaflutole,RPA 202248 358/79** **Area:** 4.110e5	 **Isoxaflutole,RPA 202248 358/64** **Area:** 1.924e5

Figure 7: Exemplary chromatograms of Isoxaflutole met. RPA203328 in different matrices generated by API 4000

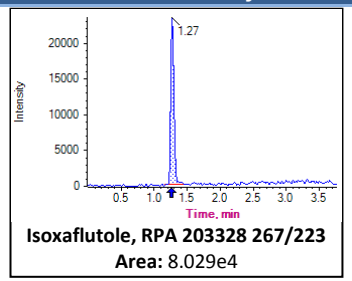
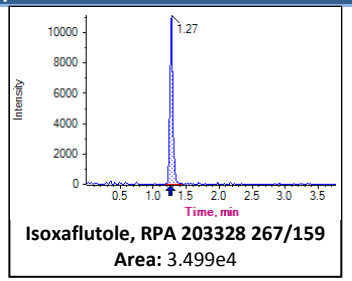
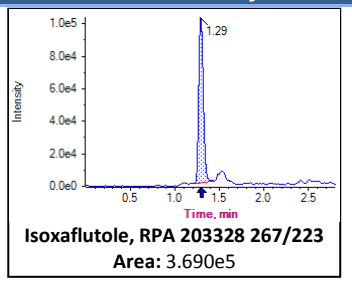
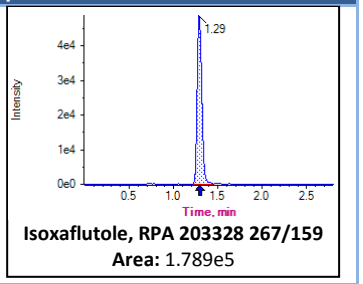
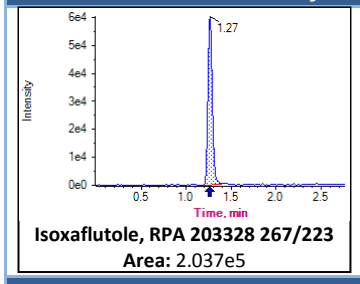
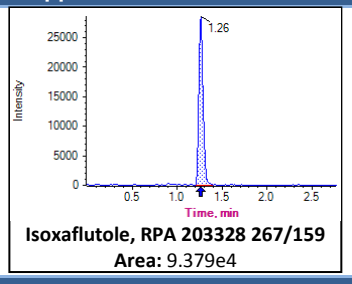
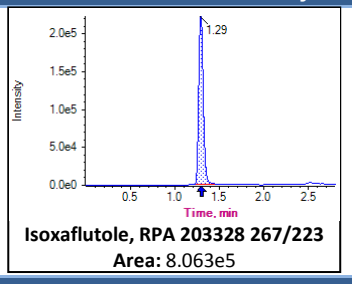
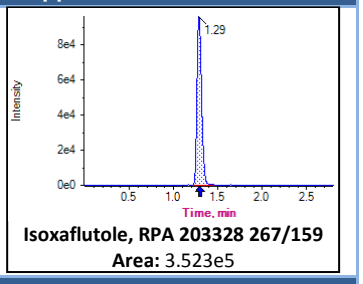
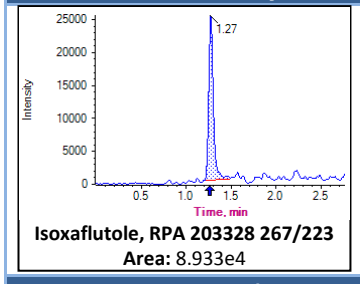
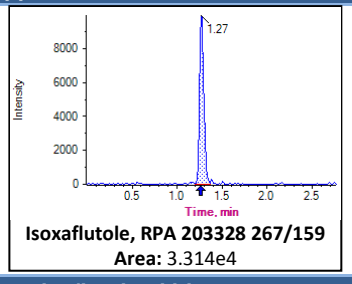
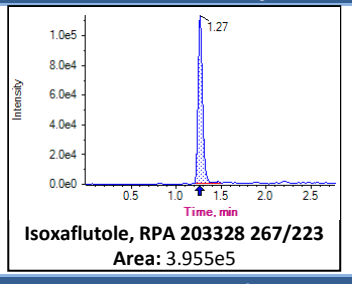
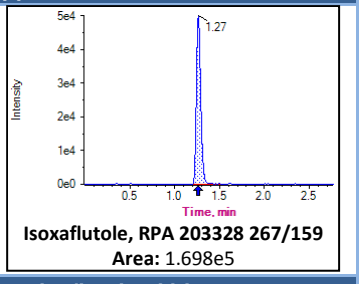
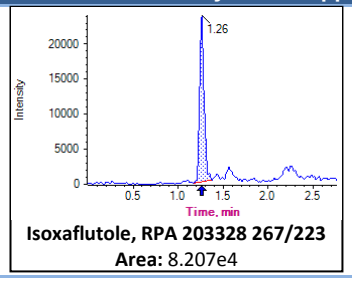
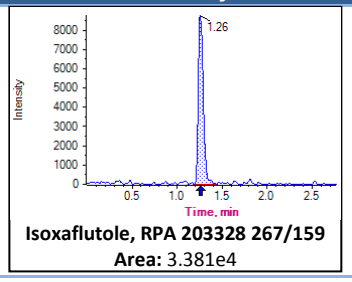
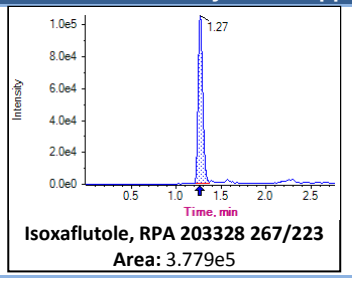
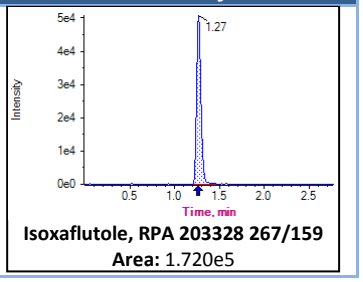
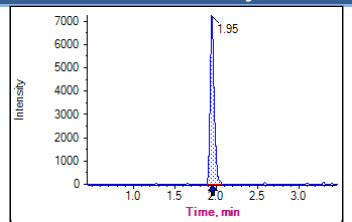
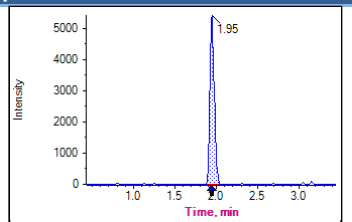
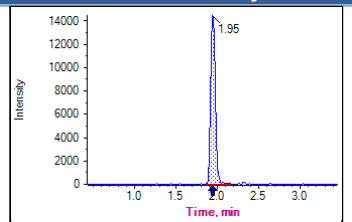
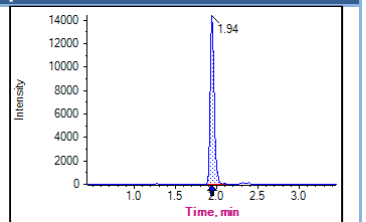
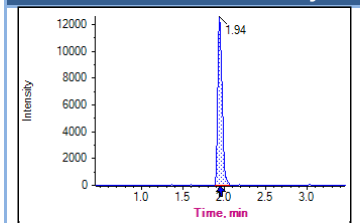
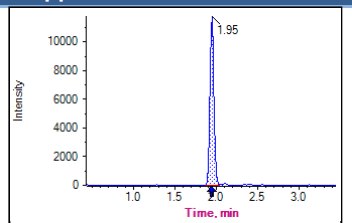
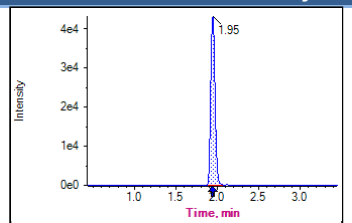
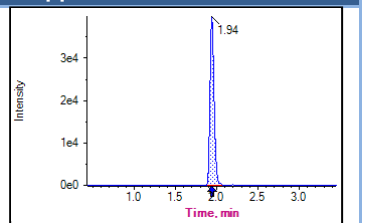
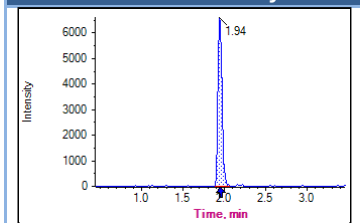
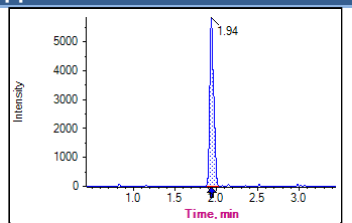
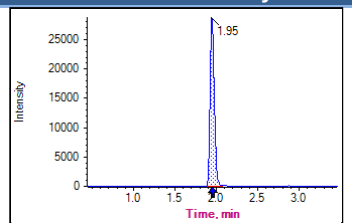
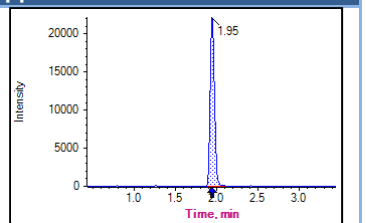
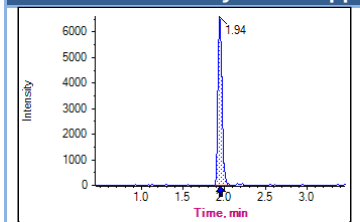
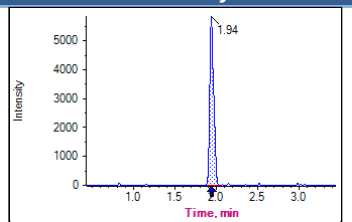
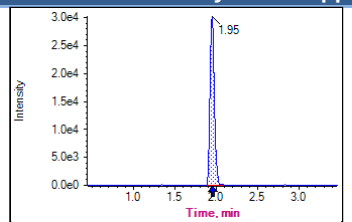
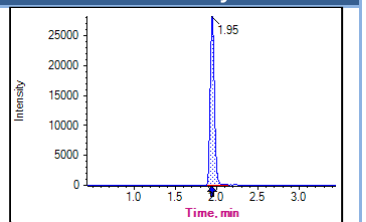
Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Isoxaflutole met. RPA203328	 **Isoxaflutole, RPA 203328 267/223** **Area:** 8.029e4	 **Isoxaflutole, RPA 203328 267/159** **Area:** 3.499e4	 **Isoxaflutole, RPA 203328 267/223** **Area:** 3.690e5	 **Isoxaflutole, RPA 203328 267/159** **Area:** 1.789e5
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 **Isoxaflutole, RPA 203328 267/223** **Area:** 2.037e5	 **Isoxaflutole, RPA 203328 267/159** **Area:** 9.379e4	 **Isoxaflutole, RPA 203328 267/223** **Area:** 8.063e5	 **Isoxaflutole, RPA 203328 267/159** **Area:** 3.523e5
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 **Isoxaflutole, RPA 203328 267/223** **Area:** 8.933e4	 **Isoxaflutole, RPA 203328 267/159** **Area:** 3.314e4	 **Isoxaflutole, RPA 203328 267/223** **Area:** 3.955e5	 **Isoxaflutole, RPA 203328 267/159** **Area:** 1.698e5
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 **Isoxaflutole, RPA 203328 267/223** **Area:** 8.207e4	 **Isoxaflutole, RPA 203328 267/159** **Area:** 3.381e4	 **Isoxaflutole, RPA 203328 267/223** **Area:** 3.779e5	 **Isoxaflutole, RPA 203328 267/159** **Area:** 1.720e5

Figure 8: Exemplary chromatograms of Dimoxystrobin met. 505M09 in different matrices generated by API 4000

Substance	Recovery 1/5 0.01 ppm bovine muscle		Recovery 1/5 0.05 ppm swine muscle	
Dimoxystrobin met. 505M09	 **Dimoxystrobin 505M09 355/106** **Area:** 2.469e4	 **Dimoxystrobin 505M09 355/266** **Area:** 2.008e4	 **Dimoxystrobin 505M09 355/106** **Area:** 5.852e4	 **Dimoxystrobin 505M09 355/266** **Area:** 4.903e4
	Recovery 1/5 0.01 ppm milk		**Recovery 1/5 0.05 ppm milk**	
	 **Dimoxystrobin 505M09 355/106** **Area:** 4.604e4	 **Dimoxystrobin 505M09 355/266** **Area:** 3.922e4	 **Dimoxystrobin 505M09 355/106** **Area:** 1.580e5	 **Dimoxystrobin 505M09 355/266** **Area:** 1.376e5
	Recovery 1/5 0.01 ppm bovine liver		**Recovery 1/5 0.05 ppm bovine liver**	
	 **Dimoxystrobin 505M09 355/106** **Area:** 2.305e4	 **Dimoxystrobin 505M09 355/266** **Area:** 1.953e4	 **Dimoxystrobin 505M09 355/106** **Area:** 1.020e5	 **Dimoxystrobin 505M09 355/266** **Area:** 8.120e4
	Recovery 1/5 0.01 ppm swine/bovine kidney		**Recovery 1/5 0.05 ppm swine/bovine kidney**	
	 **Dimoxystrobin 505M09 355/106** **Area:** 2.305e4	 **Dimoxystrobin 505M09 355/266** **Area:** 1.953e4	 **Dimoxystrobin 505M09 355/106** **Area:** 1.110e5	 **Dimoxystrobin 505M09 355/266** **Area:** 1.025e5

Discussion and conclusions:

Metabolites of several pesticides relevant to food of animal origin were successfully validated using QuEChERS or variations of it. These analytes were the following: boscalid metabolite M510F01, dimoxystrobin metabolite 505M09, fenpropidin metabolite CGA289267, fenpropimorph metabolite BF421-2, isoxaflutole metabolite RPA 203328 and RPA 203348, spiroxamine carboxylic acid, and trifloxystrobin metabolite CGA 321113.

Lacking samples with incurred residues the validation of the boscalid metabolite M510F01 focused only on the parent compound and not the conjugates, which are also entailed in the residue definition.

The isoxaflutole metabolite RPA 203348 showed low recoveries using citrate buffered QuEChERS. Successfully validation was accomplished using the FA-QuEChERS (use of acetonitrile acidified with 1% formic acid).

Spiroxamine carboxylic acid and the fenpropidin metabolite CGA 289267 showed a strong tendency to interact with glass surface. It is thus indicated to use PP vials for these compounds.

History

Action	When	Document Version
Initial Experiments	Oct - Dec 2017	
Further Experiments	Jan – March 2018	
Observation document placed on-line	March 2018	V1