Utilising Citrate QuEChERS extraction without dispersive SPE clean-up and LCMSMS (QTRAP) multi-residue method to include pesticides from the European Commission working document

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Introduction

SASA (Science and Advice for Scottish Agriculture) is one of the United Kingdom's (UK) official laboratories and we participate, on behalf of the Scottish Government, in the annual UK and EU statutory surveillance programmes that monitor various UK and imported food & drink for residues of pesticides, their metabolites and other degradation products.

It is mandatory for official laboratories to analyse all pesticides listed in the EU multiannual control programme and to also participate in UK and EU proficiency testing schemes. The European Commission released an updated revision in December 2015 of the working document (SANCO/12745/2013 rev. 6 (3)) which included pesticides to be considered for inclusion in future national control programmes. We are systematically replacing our ethyl acetate (crude extract) LC/MSMS (QqQ) method with the citrate QuEChERS extraction without dispersive SPE clean-up and LC/MSMS (QTRAP). The new method which incorporates polarity switching is being used routinely and successfully to extend our target lists and specifically to include amenable pesticides (Tables 1 & 2) highlighted in the EC working document.

In this poster we demonstrate the success of the method transfer with data generated from 39 of the pesticides highlighted in the working document. Presented data includes green bean validation data (Table 3), quantitative results from retail samples of speciality green beans (Table 4) which were analysed as part of the annual UK surveillance programme and EU Proficiency test data [EUPT-FV18 - Spinach] (Table 5).

Table 1. Positive MRM acquisition parameters.

Pesticide	Retention time	Q1	Q3	Declustering Potential (DP)	Collision Energy (CE)	Collision Exit Potential (CXP)
Ametoctradin 1	7.27	276.1	176.1	141	51	10
Ametoctradin 2	7.27	276.1	149	141	49	8
Amitraz 1	9.96	294	163	31	21	10
Amitraz 2	9.96	294	122	31	41	14
Clomazone 1	4.17	240	125	46	27	14
Clomazone 2	4.17	240	89	46	63	10
Cyazofamid 1	5.37	324.9	107.8	26	21	16
Cyazofamid 2	5.37	324.9	261	26	13	14
Cyhalofop-butyl 1	7.28	375	256	26	21	14
Cyhalofop-butyl 2	7.28	375	358	26	11	12
Emamectin benzoate 1	10.38	886.4	158.1	20	41	10
Emamectin benzoate 2	10.38	886.4	82	20	117	12
Etoxazole 1	9.22	360	141	106	39	10
Etoxazole 2	9.22	360	113	106	81	14
Fenpyrazamine 1	4.95	332	230	36	25	14
Fenpyrazamine 2	4.95	332	216	36	37	12
Flufenacet 1	5.3	363.9	194	31	15	10
Flufenacet 2	5.3	363.9	152	31	27	10
Fluopicollide 1	4.76	384.8	174.9	37	31	10
Fluopicollide 2	4.76	384.8	172.9	37	29	12
Fluxapyroxad 1	4.67	381.9	362	71	19	10
Fluxapyroxad 2	4.67	381.9	342	71	29	12
Isopyrazam 1	7.1	360	244	106	31	14
Isopyrazam 2	7.1	360	340.1	106	21	10
Isoxaflutole 1	3.64	360.1	251	111	23	14
Isoxaflutole 2	3.64	360.1	220	111	53	12
Metconazole 1	6.51	320	69.9	16	63	8
Metconazole 2	6.51	320	125	16	53	20
Molinate 1	5.06	188.07	126	38	19	6
Molinate 2	5.06	188.07	83	38	23	12
Oxadiargyl 1	6.58	340.9	222.9	131	21	12
Oxadiargyl 2	6.58	340.9	151	131	35	18
Oxasulfuron 1	2.2	407	150.1	61	23	8
Oxasulfuron 2	2.2	407	106.9	61	67	12
Penflufen 1	5.96	318	234.1	76	23	12
Penflufen 2	5.96	318	141	76	41	8
Penthiopyrad 1	6.04	360	276	76	19	14
Penthiopyrad 2	6.04	360	177	76	45	10
Picolinafen 1	8.28	377	238	71	35	14
Picolinafen 2	8.28	377	359	71	27	10
Prochloraz 1	6.68	375.9	307.9	23	17	8
Prochloraz 2	6.68	375.9	70	23	43	11
Propaquizafop 1	8.08	444	100	26	23	12
Propaquizafop 2	8.08	444	299	26	31	8
Proquinazid 1	9.59	373	330.9	11	19	18
Proquinazid 2	9.59	373	288.8	11	33	16
Prosulfocarb 1	7.62	252	91	81	33	10
Prosulfocarb 2	7.62	252	128	81	17	6
Prothioconazole Desthio 1	5.53	311.9	69.9	16	55	8
Prothioconazole Desthio 2	5.53	311.9	124.9	16	41	14
Pyrethrins Cinerin I 1	9.32	316.9	149	36	13	8
Pyrethrins Cinerin I 2	9.32	316.9	107	36	27	12
Pyrethrins Cinerin II 1	7.29	360.9	149	51	13	8
Pyrethrins Cinerin II 2	7.29	360.9	107	51	27	12
Pyrethrins Jasmolin I 1	10.07	330.8	163	41	10	5
Pyrethrins Jasmolin I 2	10.07	330.8	121	41	10	5
Pyrethrins Jasmolin II 1	8.19	375	162.9	51	13	12
Pyrethrins Jasmolin II 2	8.19	375	120.9	51	25	22
Pyrethrins Pyrethrin I 1	9.42	328.8	161	41	12	10
Pyrethrins Pyrethrin I 2	9.42	328.8	133	41	22	5
Pyrethrins Pyrethrin II 1	7.45	373	161	46	13	8
Pyrethrins Pyrethrin II 2	7.45	373	142.9	46	25	4
Quinoclamine 1	2.99	208	104.9	86	33	12
Quinoclamine 2	2.99	208	89.1	86	51	14
Quizalofop-P-ethyl 1	7.75	373	299	101	25	16
Quizalofop-P-ethyl 2	7.75	373	255	101	47	14
Rotenone 1	5.67	395	213	117	31	12
Rotenone 2	5.67	395	192	117	33	12
Spirotetramat 1	5.17	374	216	61	45	12
Spirotetramat 2	5.17	374	330.1	61	21	18
Tri-allate 1	8.82	303.9	142.9	41	35	8
Tri-allate 2	8.82	303.9	85.9	41	21	12
Triticonazole 1	5.22	317.9	70	16	37	8
Triticonazole 2	5.22	317.9	124.8	16	47	22

Table 2. Negative MRM acquisition parameters.

Pesticide	Retention time	Q1	Q3	Declustering Potential (DP)	Collision Energy (CE)	Collision Exit Potential (CXP)
2,4-DB neg 1	3.24	246.9	160.8	-5	-14	-9
2,4-DB neg 2	3.24	246.9	124.9	-5	-38	-9
loxynil 1	2.57	369.7	126.7	-75	-38	-15
loxynil 2	2.57	369.7	214.9	-75	-42	-9
MCPA neg 1	2.57	198.9	141	-15	-20	-9
MCPA neg 2	2.57	201	143	-15	-22	-8
MCPB neg 1	3.27	227	141	-20	-14	-9
MCPB neg 2	3.27	227	105	-20	-38	-13
Quizalofop neg 1	3.43	343	271	-20	-20	-9
Quizalofop neg 2	3.43	343	242.9	-20	-36	-13
Triclopyr 1	2.65	255.9	197.7	-10	-16	-9
Triclopyr 2	2.65	255.9	219.8	-10	-10	-13
Tritosulfuron neg 1	2.48	443.9	193	-15	-20	-11
Tritosulfuron neg 2	2.48	443.9	161	-15	-68	-11

1 denotes quantifier; 2 denotes qualifier

Experimental

Sample preparation

10g of cryomilled green beans samples were extracted using citrate QuEChERS method (matrix concentration ≡ 1g/ml). No clean-up was employed. Sample extracts were then filtered (0.45µm PTFE).
Calibration standards were prepared in (organic) green bean matrix.

Set-up for the ABSciex 6500 QTRAP mass spectrometer and Shimadzu Nexera UHPLC

- Run time: 17 min
- Flow rate: 0.4 mL/min
- Mobile phase A: Methanol/H_2O 5/95 v/v + 5mM ammonium acetate
- Mobile phase B: Methanol + 5mM ammonium acetate
- Column: Phenomenex Kinetex 2.6 µm, C18, 50 x 4.6 mm with Phenomenex Security Guard cartridge
- Injection volume: 3 µl

Results

Table 3. 2015-2016 green bean validation data.

	Mean Recovery	%RSD	Mean Recovery	%RSD
Pesticide	0.02 mg/kg	0.02 mg/kg	0.01 mg/kg	0.01 mg/kg
2,4-DB	97	8	92	5
Ametoctradin	106	1	99	4
Amitraz	85	6	89	7
Clomazone	101	2	97	2
Cyazofamid	101	3	98	3
Cyhalofop-butyl	102	2	100	3
Emamectin benzoate	97	2	95	3
Etoxazole	96	2	94	3
Fenpyrazamine	93	2	95	2
Flufenacet	101	2	81	8
Fluopicolide	100	5	73	11
Fluxapyroxad	101	2	80	8
loxynil	98	1	79	7
Isopyrazam	100	2	98	4
Isoxaflutole	103	2	101	5
MCPA	94	2	94	3
MCPB	97	2	97	3
Metconazole	95	5	76	5
Molinate	102	2	98	2
Oxadiargyl	100	1	99	2
Oxasulfuron	103	3	100	6
Penflufen	92	1	96	2
Penthiopyrad	99	1	98	4
Picolinafen	97	1	96	3
Prochloraz	97	2	79	7
Propaquizafop	96	1	79	6
Proquinazid	98	2	96	2
Prosulfocarb	101	2	99	2
Prothioconazole Desthio	95	2	75	7
Pyrethrins Cinerin I	117	4	117	3
Pyrethrins Cinerin II	114	9	112	16
Pyrethrins Jasmolin I	113	8	107	8
Pyrethrins Jasmolin II	116	7	111	14
Pyrethrins Pyrethrin I	97	5	101	8
Pyrethrins Pyrethrin II	98	5	101	10
Quinoclamine	98	3	99	5
Quizalofop	94	2	92	3
Quizalofop-P-ethyl	99	1	97	2
Rotenone	91	4	75	12
Spirotetramat	99	2	98	3
Tri-allate	101	2	97	3
Triclopyr	93	1	91	2
Triticonazole 1	99	4	75	7
Tritosulfuron	102	1	99	2

n = 6

Table 4. Results from 2015 green bean samples.

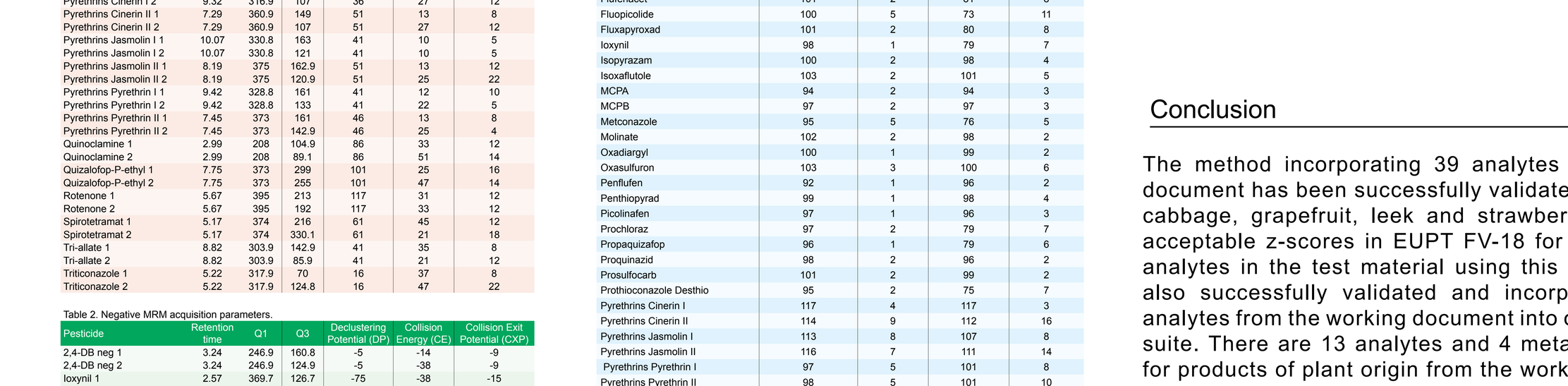
Sample Description	Country of Origin	Residue	Conc. (mg/kg)	Maximum Residue Level (mg/kg)
Papi Beans	Bangladesh	Emamectin Benzoate B1a	0.02	0.01
Uri beans	Malaysia	Emamectin Benzoate B1a	0.01	0.01
Hyacinth beans	Bangladesh	Emamectin Benzoate B1a	0.08	0.01

Table 5. Recent preliminary results form 2016 European Union Proficiency Test on spinach (EUPT-FV-18).

Pesticide	Reported Value	Assigned Value	Z-Score
Cyazofamid	0.25	0.279	-0.4
Fenpyrazamine	0.38	0.357	0.3

Conclusion

The method incorporating 39 analytes from the working document has been successfully validated for green bean, cabbage, grapefruit, leek and strawberry. We achieved acceptable z-scores in EUPT FV-18 for the two voluntary analytes in the test material using this method. We have also successfully validated and incorporated another 7 analytes from the working document into our existing GCMS suite. There are 13 analytes and 4 metabolites of interest for products of plant origin from the working document that we have not incorporated either because of cost/availability of reference standards or they are not amenable to multi-residue methods.



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