Analysis of Acidic Pesticides using QuEChERS (EN15662) and acidified QuEChERS method

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Subject: Analytical observations report concerning the following…

- **Compound(s)**: Pesticides and metabolites entailing acidic functions (e.g. carboxy- or phenolic groups)
- **Commodities**: Cucumber, Grapes, Maize (exemplary)
- **Method(s)**: QuEChERS, acidified QuEChERS (using 1% formic acid), no cleanup

Brief description:

Two methods, the QuEChERS method (EN 15662) and the acidified-QuEChERS method (A-QuEChERS) were tested for the analysis of acidic pesticides. A-QuEChERS involved extraction with acetonitrile containing 1% formic acid and the use of partitioning salts composed of NaCl and $MgSO_4$ only. Recovery experiments were conducted on cucumber, grapes and maize. No alkaline hydrolysis step was conducted and thus the focus was on free acids only. Most compounds showed satisfactory recovery figures by both methods. There were some pesticides, however, where average recoveries using QuEChERS were unsatisfactory (< 70%) using QuEChERS but satisfactory using A-QuEChERS.

Apparatus and Consumables:

Refer to EN 15662. For mechanical shaking the GenoGrinder by Spex was used. Extracts were filtered through disposable polyester syringe filters of 0.45 µm pore size.

QuEChERS (EN 15662):

The procedure as described in EN 15662 was followed. Shaking was conducted for 15 minutes using the GenoGrinder. No cleanup step was conducted.

Acidified QuEChERS (A-QuEChERS):

The method corresponds to the EN 15662 procedure with the only difference being the use of 10 mL acetonitrile containing 1% formic acid instead of pure acetonitrile for extraction and of 4g $MgSO_4$ and 1g NaCl (no citrate buffer salts) for partitioning. Shaking was conducted for 15 minutes using the GenoGrinder. No cleanup was conducted.

Measurement:

Table 1: LC details for Acidic Pesticides (exemplary)

Instrument	Waters Acquity, ABSciex API 4000 QTrap			
Ionisation mode	ESI neg			
Column	Acquity UPLC BEH C18,1.7 µm; 2.1 x 100 mm			
Pre-column	Van Guard BEH C18, 1.7 um			
Eluent A	0.01 % acetic acid in water (with 5% acetonitrile)			
Eluent B	0.01 % acetic acid in acetonitrile			
Gradient	Time [min]	flow [µL/min]	A [%]	B [%]
	0	400	80	20
	4	400	70	30
	7	400	10	90
	8.5	400	10	90
	8.6	400	80	20
Internal Standard	BNPH			

Table 2: MS/MS details for Acidic Pesticides (ESI-negative mode, Tune-data ABSciex 4000Q) (exemplary)

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	CE	CXP	Note
1-Naphthylacetic acid	1	185	141	-11	-10	-7	ESI neg.
2,4,5-T	3	253	159	-50	-40	-7	ESI neg.
	1	253	195	-50	-18	-9	ESI neg.
	2	255	197	-55	-18	-11	ESI neg.
2,4,5-TP (Fenoprop)	3	267	159	-50	-40	-9	ESI neg.
	1	267	195	-50	-16	-9	ESI neg.
	2	269	197	-50	-18	-9	ESI neg.
2.4.6-Trichlorophenol	1	195	35	-70	-44	-3	ESI neg.
	2	197	35	-70	-42	-3	ESI neg.
	3	199	37	-70	-40	-3	ESI neg.
2.4-D	3	219	125	-50	-38	-7	ESI neg.
	1	219	161	-50	-18	-9	ESI neg.
	2	221	163	-50	-18	-9	ESI neg.
2.4-DB	3	247	125	-50	-38	-5	ESI neg.
	1	247	161	-50	-12	-9	ESI neg.
	2	249	163	-35	-14	-9	ESI neg.
2.4-DP (Dichlorprop)	3	233	125	-50	-38	-5	ESI neg.
	1	233	161	-50	-18	-9	ESI neg.
	2	235	163	-50	-18	-7	ESI neg.
2-Naphthoxyacetic acid	2	201	115	-55	-54	-7	ESI neg.
	1	201	143	-55	-22	-7	ESI neg.

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	CE	CXP	Note
	3	201	157	-55	-14	-7	ESI neg.
4-CPA	1	185	127	-55	-20	-7	ESI neg.
	2	187	129	-55	-20	-7	ESI neg.
Acifluorfen	2	360	286	-45	-22	-7	ESI neg.
	1	360	316	-45	-12	-9	ESI neg.
Bentazon	1	239	132	-75	-38	-7	ESI neg.
	2	239	175	-75	-28	-9	ESI neg.
	3	239	197	-75	-28	-11	ESI neg.
Bromacil	1	259	203	-46	-26	-11	ESI neg.
	3	261	205	-46	-28	-11	ESI neg.
	2	261	81	-41	-46	-5	ESI neg.
Bromoxynil	1	274	79	-60	-48	-1	ESI neg.
	2	276	81	-70	-42	-3	ESI neg.
	3	278	81	-60	-50	-3	ESI neg.
BTS 9608 (Metabolite of prochloraz)	2	253	195	-45	-18	-9	ESI neg.
	1	255	197	-45	-16	-11	ESI neg.
	3	257	199	-50	-18	-9	ESI neg.
Clopyralid	2	190	146	-35	-14	-7	ESI neg. similar MRM and RT as TFNA (metabolite of flonicamid)
	3	190	35	-35	-35	-3	ESI neg.
	1	192	148	-35	-14	-7	ESI neg.
	4	192	37	-35	-32	-5	ESI neg.
Dalapon	3	141	105	-30	-10	-10	ESI neg.
	1	141	97	-30	-12	-12	ESI neg.
	2	143	99	-30	-12	-5	ESI neg.
Dicamba	1	219	175	-25	-8	-8	ESI neg.
	2	221	177	-25	-8	-11	ESI neg.
Diflufenzopyr	2	333	134	-20	-22	-2	ESI neg.
	1	333	160	-20	-18	-2	ESI neg.
	3	333	204	-20	-12	-2	ESI neg.
Dikegulac	2	273	55	-45	-62	0	ESI neg.
	1	273	83	-45	-32	0	ESI neg.
Fenoxaprop-P	3	334	262	-70	-18	-1	ESI neg.
	2	332	152	-70	-32	-7	ESI neg.
	1	332	260	-70	-18	-13	ESI neg.
Fluazifop	3	326	108	-65	-56	-5	ESI neg.
	2	326	226	-65	-38	-11	ESI neg.
	1	326	254	-65	-22	-5	ESI neg.
Fluroxypyr	1	253	195	-50	-20	-9	ESI neg.
	3	253	233	-50	-10	-1	ESI neg.
	2	255	197	-55	-20	-11	ESI neg.

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	CE	CXP	Note
Gibberellic acid	1	345	143	-50	-42	0	ESI neg.
	2	345	221	-50	-34	0	ESI neg.
	3	345	239	-50	-22	-2	ESI neg.
Haloxyfop	3	360	196	-70	-52	-9	ESI neg.
	1	360	288	-70	-20	-15	ESI neg.
	2	362	290	-75	-20	-15	ESI neg.
Imazamox	3	304	186	-40	-44	-9	ESI neg.
	2	304	217	-40	-28	-9	ESI neg.
	1	304	260	-40	-18	-11	ESI neg.
Imazapyr	1	260	173	-50	-30	-9	ESI neg.
	2	260	216	-50	-18	-11	ESI neg.
Imazethapyr	3	288	186	-55	-50	-9	ESI neg.
	2	288	201	-55	-32	-11	ESI neg.
	1	288	244	-55	-20	-1	ESI neg.
Inabenfide	1	337	122	-75	-24	-7	ESI neg.
	3	337	231	-75	-30	-11	ESI neg.
	2	339	122	-75	-24	-7	ESI neg.
Ioxynil	1	370	127	-60	-44	-7	ESI neg.
	2	370	215	-60	-50	-13	ESI neg.
	3	370	243	-60	-32	-13	ESI neg.
MCPA	1	199	141	-55	-20	-7	ESI neg.
	2	201	143	-55	-20	-7	ESI neg.
MCPB	1	227	141	-50	-18	-7	ESI neg.
	2	229	143	-55	-16	-7	ESI neg.
MCPP	1	213	141	-55	-20	-7	ESI neg.
	3	213	71	-55	-14	-1	ESI neg.
	2	215	143	-55	-18	-7	ESI neg.
Pentachlorophenol	2	263	35	-80	-50	-3	ESI neg.
	1	265	35	-80	-52	-3	ESI neg.
	3	267	35	-80	-56	-3	ESI neg.
Quizalofop	1	343	271	-36	-22	-15	ESI neg.
	2	345	273	-41	-22	-13	ESI neg.
TFNA (Flonicamid-Metabolite)	4	190	119	-35	-36	-5	ESI neg.
	3	190	126	-35	-34	-5	ESI neg.
	2	190	69	-35	-46	-1	ESI neg.
	1	190	146	-35	-16	-7	ESI neg.
TFNG (Flonicamid-Metabolite)	2	247	146	-55	-24	-7	ESI neg.
	3	247	163	-55	-24	-7	ESI neg.
	1	247	183	-55	-18	-9	ESI neg.
Triclopyr	1	254	196	-50	-16	-11	ESI neg.
	3	254	218	-50	-8	-11	ESI neg.
	2	256	198	-50	-16	-9	ESI neg.

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	CE	CXP	Note
2,4-D-D3 (IS)	x	222	164	-50	-18	-7	ESI neg.
BNPH (IS)	1	301	137	-45	-16	-7	ESI neg.

Experiments conducted and observations:

Recovery studies of acidic pesticides from cucumber, grapes and maize were conducted using the QuEChERS method and the acidified QuEChERS method. In both cases no cleanup was conducted. The recovery experiments were conducted in quintuplicate at a spiking level of 0.01 mg/kg in all cases. LC-MS/MS analysis was performed in ESI-negative mode.

Using A-QuEChERS the recovery rates were very satisfactory (>90% in all cases) in all cases.

Using standard QuEChERS (EN 15662), however, the average recovery rates were significantly lower and for certain compounds even dropped below 70%. In the case of dalapon, imazapyr, TFNA and TFNG recoveries from all 3 commodities were <70%; in the case of dicamba and dikegulac recoveries <70% were observed only in maize. Reduced but still acceptable recovery rates between 70 and 80% were observed for 4-CPA, BTS-9608 and gibberellic acid in maize; for dicamba and dikegulac in cucumber and for imazamox and imazethapyr in grapes.

Validation data

Table 3: Recovery figures for Acidic Pesticides in different commodities (n=5 in all cases)

Substance	Extraction Method	Cucumber 0.01 mg/kg		Grape 0.01 mg/kg		Maize 0.01 mg/kg	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
1-Naphthylacetic acid	QuEChERS	98	4	98	3	91	3
	QuEChERS + 1% FA	**102**	**4**	**104**	**5**	**101**	**5**
2.4.5-T	QuEChERS	92	6	99	6	85	3
	QuEChERS + 1% FA	**102**	**2**	**102**	**4**	**99**	**3**
2.4.5-TP (Fenoprop)	QuEChERS	96	3	99	3	91	2
	QuEChERS + 1% FA	**99**	**2**	**104**	**5**	**95**	**8**
2.4.6-Trichlorophenol	QuEChERS	95	10	104	4	105	5
	QuEChERS + 1% FA	**106**	**6**	**100**	**5**	**107**	**5**
2.4-D	QuEChERS	90	4	106	6	82	3
	QuEChERS + 1% FA	**101**	**3**	**108**	**3**	**98**	**5**
2.4-DB	QuEChERS	85	8	98	8	92	7
	QuEChERS + 1% FA	**101**	**6**	**90**	**6**	**98**	**12**
2.4-DP (Dichlorprop)	QuEChERS	95	4	100	3	87	4

Substance	Extraction Method	Cucumber 0.01 mg/kg		Grape 0.01 mg/kg		Maize 0.01 mg/kg	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
	QuEChERS + 1% FA	**99**	**2**	**102**	**3**	**99**	**3**
2-Naphthoxyacetic acid	QuEChERS	93	4	99	3	81	3
	QuEChERS + 1% FA	**102**	**2**	**106**	**5**	**101**	**5**
4-CPA	QuEChERS	90	3	96	4	76	2
	QuEChERS + 1% FA	**101**	**1**	**103**	**6**	**100**	**5**
Bentazon	QuEChERS	100	4	100	6	88	2
	QuEChERS + 1% FA	**105**	**3**	**105**	**4**	**98**	**3**
Bromacil	QuEChERS	100	4	99	5	95	4
	QuEChERS + 1% FA	**102**	**2**	**109**	**4**	**102**	**4**
Bromoxynil	QuEChERS	94	3	105	7	91	2
	QuEChERS + 1% FA	**104**	**2**	**106**	**4**	**103**	**2**
BTS 9608 (Metabolite of prochloraz)	QuEChERS	91	2	97	5	78	2
	QuEChERS + 1% FA	**103**	**2**	**101**	**5**	**98**	**3**
Dalapon	QuEChERS	38	13	31	8	26	10
	QuEChERS + 1% FA	**103**	**4**	**97**	**5**	**82**	**7**
Dicamba	QuEChERS	78	4	88	2	60	4
	QuEChERS + 1% FA	**102**	**4**	**103**	**4**	**96**	**2**
Diflufenzopyr	QuEChERS	97	6	97	3	91	5
	QuEChERS + 1% FA	**99**	**3**	**94**	**9**	**98**	**8**
Dikegulac	QuEChERS	72	6	84	6	65	1
	QuEChERS + 1% FA	**106**	**3**	**92**	**7**	**97**	**3**
Fenoxaprop-P	QuEChERS	95	5	96	4	92	5
	QuEChERS + 1% FA	**99**	**4**	**99**	**8**	**96**	**6**
Fluazifop	QuEChERS	94	4	102	4	98	2
	QuEChERS + 1% FA	**100**	**2**	**106**	**3**	**100**	**5**
Fluroxypyr	QuEChERS	95	4	102	5	82	2
	QuEChERS + 1% FA	**102**	**1**	**106**	**6**	**101**	**3**
Gibberellic acid	QuEChERS	90	3	89	4	79	3
	QuEChERS + 1% FA	**103**	**1**	**107**	**6**	**99**	**4**
Haloxyfop	QuEChERS	97	5	101	4	94	3
	QuEChERS + 1% FA	**103**	**2**	**105**	**3**	**106**	**7**
Imazamox	QuEChERS	86	3	72	3	83	3
	QuEChERS + 1% FA	**103**	**2**	**101**	**5**	**95**	**3**
Imazapyr	QuEChERS	67	5	45	6	67	2
	QuEChERS + 1% FA	**102**	**4**	**104**	**5**	**99**	**7**
Imazaquin	QuEChERS	97	4	101	4	93	2
	QuEChERS + 1% FA	**102**	**2**	**103**	**5**	**105**	**3**
Imazethapyr	QuEChERS	98	4	78	4	88	4
	QuEChERS + 1% FA	**102**	**1**	**98**	**4**	**99**	**4**
Inabenfide	QuEChERS	102	3	103	5	106	3
	QuEChERS + 1% FA	**102**	**2**	**113**	**7**	**104**	**3**
Ioxynil	QuEChERS	90	4	105	4	79	2
	QuEChERS + 1% FA	**102**	**4**	**104**	**4**	**99**	**4**

Substance	Extraction Method	Cucumber 0.01 mg/kg		Grape 0.01 mg/kg		Maize 0.01 mg/kg	
		Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
MCPA	QuEChERS	98	3	103	4	85	2
	QuEChERS + 1% FA	**105**	**2**	**100**	**2**	**97**	**4**
MCPB	QuEChERS	94	3	98	5	99	10
	QuEChERS + 1% FA	**95**	**6**	**94**	**7**	**100**	**3**
MCPP (Mecoprop)	QuEChERS	102	4	105	3	88	2
	QuEChERS + 1% FA	**104**	**2**	**103**	**3**	**102**	**3**
Pentachlorophenol	QuEChERS	90	5	108	7	76	8
	QuEChERS + 1% FA	**111**	**3**	**108**	**6**	**102**	**5**
Propoxycarbazone	QuEChERS	98	3	102	4	91	2
	QuEChERS + 1% FA	**105**	**1**	**104**	**5**	**104**	**5**
Quizalofop	QuEChERS	94	5	101	5	97	3
	QuEChERS + 1% FA	**102**	**2**	**105**	**4**	**96**	**4**
TFNA (Flonicamid-Metabolite)	QuEChERS	37	4	39	6	34	6
	QuEChERS + 1% FA	**105**	**3**	**105**	**5**	**94**	**2**
TFNG (Flonicamid-Metabolite)	QuEChERS	53	6	58	5	43	6
	QuEChERS + 1% FA	**104**	**3**	**111**	**5**	**96**	**3**
Triclopyr	QuEChERS	96	5	101	4	84	2
	QuEChERS + 1% FA	**99**	**2**	**102**	**4**	**98**	**2**

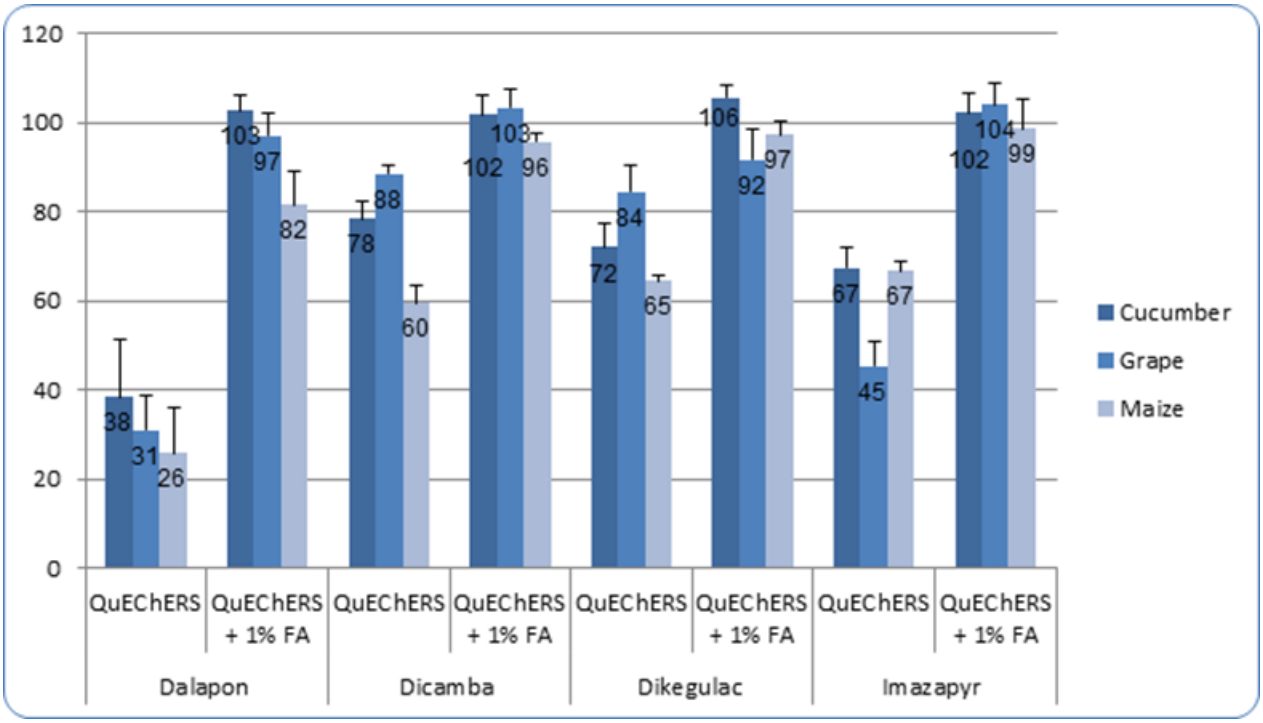

Figure 1: Comparison of recoveries of selected acidic pesticides using QuEChERS or A-QuEChERS

Document History

Date	Action	Changes
May. 2015	Publication of V1	