Analysis of Flonicamid-Metabolites TFNA and TFNG using acidified QuEChERS method

Reported by: EURL-SRM
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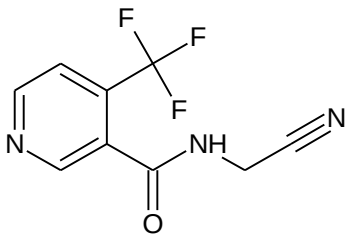
Brief description:

A QuEChERS-based method for the analysis of acidic flonicamid metabolites TFNA and TFNG in food commodities is presented. Recoveries using the standard, citrate-buffered, QuEChERS-procedure were too low, but practically quantitative recoveries could be achieved using an acidic version of the QuEChERS method. Acidification is achieved by using acetonitrtile containing 1% formic acid. No buffering salts are used. Using the normal procedure (EN-15662) the recoveries of TFNA and TFNG were too low. Parent flonicamid and the metabolite TFNA-AM can be analyzed by either the normal or the acidified QuEChERS procedure.

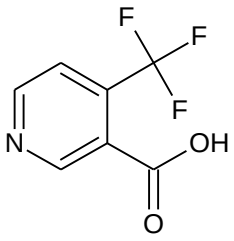
Compound profile:

The pesticide flonicamid was developed in 2000 as a selective agent against aphids and other sucking insects. The mode of action has been identified as suppressing feeding and movement by aphids. The use of this pesticide does not impair acetylcholine esterase or nicotinic acetylcholine receptors as compared with typical insecticides; thus, there is minimal chance of developing cross-resistance by insects.

Compound facts at a glance:

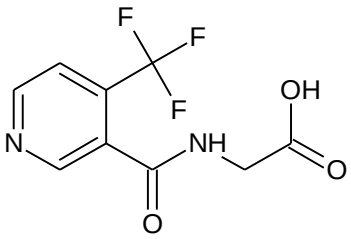
FLONCAMID (CAS: 158062-67-0), IUPAC: N-cyanomethyl-4-trifluoromethylnicotinamide			
Parameter	**Value**	**Notes**	
Molecular Formula:	C9H6F3N3O		
Molecular weight:	229.15		
Pka	11.6	at 20 °C (very weak base)	
LogPow	-0.24 (calculated)	at 20 °C (high polarity)	
Water solubility	5.2 g L^{-1}	at 20 °C	
Hydrolytic behavior (DT_{50})	pH 5: stable pH 7: stable pH 9: 204 days	at 25 °C	
Residue definition EU	Sum of flonicamid, TFNG and TFNA, expressed as flonicamid. For products of animal origin the residue definition includes TFNA-AM		
Approved in…	Approved within the EU		

TFNA (CAS: 158063-66-2); IUPAC: 4-trifluoromethyl nicotinic acid		
Parameter	**Value**	**Notes**
Molecular Formula:	C7H4F3NO2	
Molecular weight:	191.11	
Pka	3.99 (calculated*) 2.62 (calculated*)	Pyridine-group (Basic) Carboxy-group (acidic)
LogPow	Variable with pH	LogD at pH4 = ~ 0.8 (calculated*), (polar)
Water solubility	Variable with pH	
Hydrolytic behavior	No information	



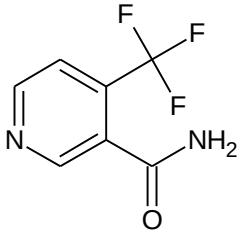
*using Chemicalize.org

TFNG (CAS: 207502-65-6); IUPAC: N-(4-trifluoromethylnicotinoyl)glycine)		
Parameter	**Value**	**Notes**
Molecular Formula:	C9H7F3N2O3	
Molecular weight:	248.16	
Pka	3.53 (calculated*) 2.84 (calculated*)	Pyridine group (basic) Carboxy-group (acidic)
LogPow	Variable with pH	LogD at pH4 = ~-0.8 (calculated*), (very polar)
Water solubility	Variable with pH	
Hydrolytic behavior	No information	



*using Chemicalize.org

TFNA-AM (CAS: 158062-71-6); IUPAC: 4-trifluoromethylnicotinamide		
Parameter	**Value**	**Notes**
Molecular Formula:	C7H5F3N2O	
Molecular weight:	190.12	
Pka	3.39	Pyridine-group (basic)
LogP	0.48	at pH7 (polar)
Water solubility	4.4 mg L^{-1}	at 20 °C (calculated**)
Hydrolytic behavior	No information	



*using Chemicalize.org; ** using ACD-labs software

Standard Materials Supply:

There is multitude of suppliers of flonicamid standard materials. Analytical standards of TFNA and TFNA-AM are provided by a few suppliers. The sources of TFNG are more limited. Some exemplary sources of TFNA, TFNG and TFNA-AM are listed below.

Table 1: Exemplary Providers of Flonicamid Metabolites (14.04.2015)[1]

Substance	CAS-No.	Source	Article-No.	Amount per unit	Prices
TFNA	158063-66-2	HPC	674703	10 mg	89 €
		Wako Pure Chemical Industries, Ltd.	205-16781	50 mg	US$ 165.00
		Interchim S.A.	26844	5 g	on application
		ABCR GmbH & CO. KG	AB106029	250 mg	56,20 €
		Apollo Scientific Ltd.	PC7692	1 g	£38.00
TFNG	207502-65-6	HPC	674702	10 mg	89 €
		Wako Pure Chemical Industries, Ltd.	208-16771	50 mg	US$ 165.00
		Apollo Scientific Ltd.	PC450018	on request	on application
TFNA-AM	158062-71-6	HPC	674706	10 mg	89 €
		Wako Pure Chemical Industries, Ltd.	202-16791	50 mg	US$ 165.00
		Interchim S.A.	66922	500 mg	on application
		ABCR GmbH & CO. KG	AB339696	500 mg	54,00 €
		Apollo Scientific Ltd.	PC10153	1 g	£70.00

Apparatus and Consumables:

Use materials described in the QuEChERS standard procedure (EN15662). As a mechanical shaker you can use a horizontally or vertically reciprocating shaker or a rotatory shaker (e.g. HS260 by IKA or GenoGrinder by Spex or SSL1 Labscale Orbital Shaker by Stuart). To filter the extract use e.g. polyester disposable syringe filters of 0.45 µm pore size.

Extraction Procedure for TFNA and TFNG:

Acidified QuEChERS: Weigh 10 g of frozen fruit or vegetable homogenate; add 10 mL acetonitrile containing 1 % (v/v) formic acid (conc.) and internal standard (e.g. 100µL of an appropriately concentrated solution of nicarbazin for ESI-neg. mode measurements or Chlorpyrifos-D10 for ESI-pos. mode measurements). Shake 15 min using a mechanical shaker. Add a mixture of 4g $MgSO_4$ and 1g NaCl (no citrate buffer salts), shake 1 min and centrifuge. Measure by LC-MS/MS. 1mL final extract will represent approximately 1 g matrix.

Extraction Procedure for Flonicamid and TFNA-AM (informative):

Flonicamid and TFNA-Am are both amenable to the normal QuEChERS procedure (EN-15662) and do not require acidification. Analysis by the acidified QuEChERS approach is also possible. In the case of milk and cereals the raw extract is cleaned-up using 25 mg C_{18} sorbent per mL extract.

[1] Disclaimer: this information is given solely for the convenience and information of the readers and it does not constitute any advertisement, preference or endorsement of the companies and products listed

Measurement Conditions:

Table 2: Exemplary LC details for TFNA and TFNG

Instrument	Waters Acquity connected to ABSciex API 4000QTrap			
Column	Acquity UPLC BEH Shield RP 18, 1.7 µm; 2.1 x 100 mm			
Pre-column	Van Guard BEH Shield RP 18, 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
	13.5	400	80	20
Internal Standard	Nicarbazin			

Table 3: Exemplary MS/MS details for TFNA and TFNG (ESI-negative mode)

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	EP	CEP	CE	CXP	Mode
Using ABSciex API 3200QTrap									
TFNA	1	190	146	-20	-7	-12	-18	-2	ESI neg.
	3	190	99	-20	-7	-12	-40	0	ESI neg.
	2	190	69	-20	-7	-12	-46	-8	ESI neg.
TFNG	2	247	146	-30	-5,5	-20	-24	-2	ESI neg.
	1	247	163	-30	-5,5	-20	-24	-2	ESI neg.
	3	247	183	-30	-5,5	-20	-18	0	ESI neg.
Using ABSciex 4000QTrap									
TFNA	3	190	126	-40	-12			-7	ESI neg.
	1	190	146	-40	-16			-5	ESI neg.
	2	190	69	-40	-20			-1	ESI neg.
TFNG	2	247	146	-55	-24			-7	ESI neg.
	3	247	163	-55	-24			-7	ESI neg.
	1	247	183	-55	-18			-9	ESI neg.

Table 4: Exemplary LC-details for Flonicamid and TFNA-AM (for information):

Instrument	**Waters Acquity connected to ABSciex API 3200QTrap**			
Column	Phenomenex Synergi 2.5u Fusion RP100A, 100x2 mm			
Pre-column	Fusion-RP, C18 Polar Embedded, 4x2mm			
Eluent A	5 mmol NH_4 formate in H_2O			
Eluent B	5 mmol NH_4 formate in MeOH			
Gradient	Time [min]	Flow [µL/min]	A [%]	B [%]
	0	300	100	0
	1	300	100	0
	2	300	60	40
	12	300	10	90
	17	300	10	90
	17.1	300	100	0
	22.1	300	100	0
Internal Standard	Chlorpyrifos D10			

Table 5: Exemplary MS/MS details for TFNA, TFNG, Flonicamid and TFNA-AM (ESI-positive mode)

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	EP	CEP	CE	CXP	Mode
Using ABSciex 4000QTrap									
TFNA	1	192	148	76	29			8	ESI pos.
	3	192	79	76	51			4	ESI pos.
	2	192	98	76	43			4	ESI pos.
TFNG	2	249	148	76	43			8	ESI pos.
	4	249	176	76	35			10	ESI pos.
	1	249	203	76	27			12	ESI pos.
	3	249	98	76	61			4	ESI pos.
TFNA-AM	1	191	148	81	31			8	ESI pos.
	3	191	79	81	53			2	ESI pos.
	2	191	98	81	45			6	ESI pos.
Flonicamid	2	230	148	61	39			8	ESI pos.
	3	230	174	61	27			10	ESI pos.
	1	230	203	61	25			10	ESI pos.
Using ABSciex 3200QTrap (for information)									
Flonicamid	1	230	203	41	12	16	25	4	ESI pos.
	2	230	148	41	12	16	37	4	ESI pos.
	3	230	174	41	12	16	27	4	ESI pos.
TFNA-AM	1	191	148	46	6,5	14	29	4	ESI pos.
	2	191	98	46	6,5	14	43	4	ESI pos.
	3	191	79	46	6,5	14	49	4	ESI pos.

Validation data

Table 6: Recovery figures for TFNA and TFNG in different commodities using acidified QuEChERS

Matrix	Level (mg/kg)	n	Extraction Method	Cleanup	TFNA		TFNG	
					Rec.(%)	RSD (%)	Rec.(%)	RSD (%)
Cucumber	0.1	5	QuEChERS (EN-15662)	None	37	4	53	6
		5	**QuEChERS + 1% FA**	None	**105**	**3**	**104**	**3**
Grape	0.1	5	QuEChERS (EN-15662)	None	39	6	58	5
		5	**QuEChERS + 1% FA**	None	**105**	**5**	**111**	**5**
Maize	0.1	5	QuEChERS (EN-15662)	None	34	6	43	6
		5	**QuEChERS + 1% FA**	None	**94**	**2**	**96**	**3**
Corn	0.01	5	QuEChERS (EN-15662)	None	33	14	53	6
		5	**QuEChERS + 1% FA**	None	**105**	**8**	**94**	**8**
	0.05	5	QuEChERS (EN-15662)	None	27	6	53	6
		5	**QuEChERS + 1% FA**	None	**86**	**2**	**96**	**2**
Brussels' sprouts	0.05	3	QuEChERS (EN-15662)	None	28	4	45	4
		3	**QuEChERS + 1% FA**	None	**80**	**8**	**92**	**5**

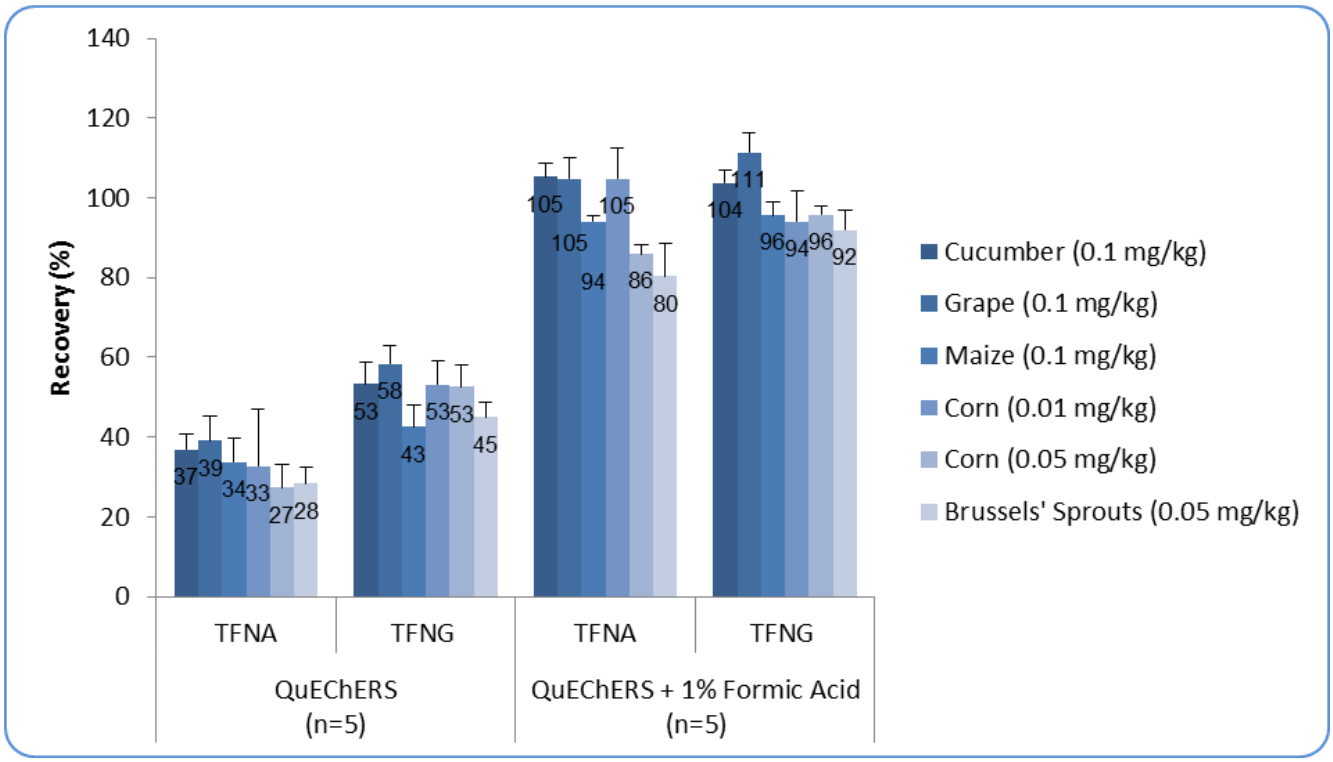


Figure 1: Comparison of recovery data for TFNA and TFNG using QuEChERS or acidified QuEChERS

Table 7: Recovery figures of TFNA-AM in different commodities (n=5) (for information)

Matrix	Level (mg/kg)	TFNA-AM recovery (%)	TFNA-AM recovery RSD (%)
Milk	0.01	110	3
Currant	0.01	99	4
Tomato	0.01	99	4
Rice	0.01	99	8
Barley	0.01	84	2

Supplementary Data

Matrix effects

The matrix effects on TFNA and TFNG were determined by comparing the peak areas of equally concentrated standards in pure solvent and blank extracts of two exemplary commodities (Brussels' sprouts and corn). Strong signal suppression was observed for both analytes with TFNG being stronger suppressed than TFNA (see **Figure 2**). The use of techniques that account for matrix-effects (e.g. matrix-matching, standard additions approach) is thus recommended.

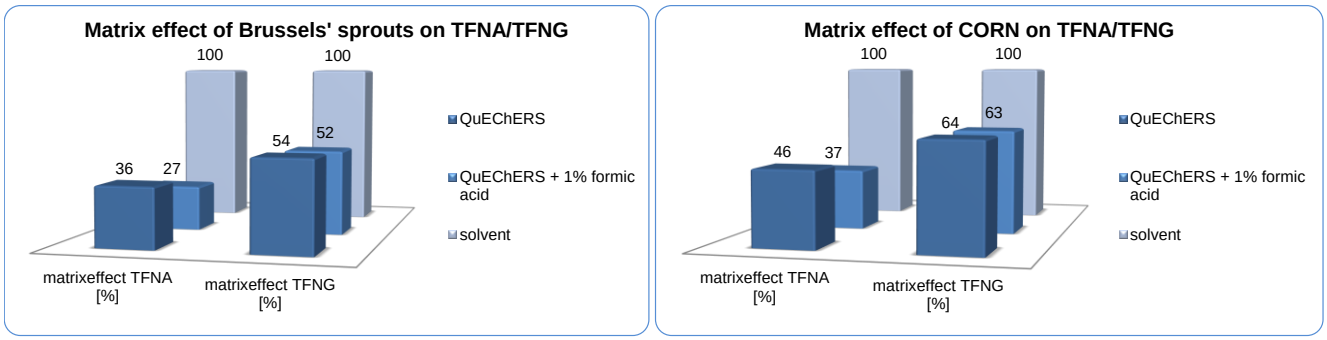


Figure 2: Matrix effects on TFNA and TFNG in Brussels' sprouts and corn extracts

Cleanup

Entailing carboxylic acid groups, TFNA and TFNG are prone to losses during cleanup with PSA sorbent. To assess the losses spiked raw extracts of cucumber, grapes and maize were subjected to dSPE-cleanup with PSA. Losses of both TFNA and TFNG were observed in all cases but were more pronounced in maize extracts. TFNG losses were more pronounced compared to TFNA showing an opposite trend that what was observed in the recovery experiments, where TFNA figures were lower. Steric constraints, due to the close proximity the carboxy and the trifluoromethyl-group may have influenced the PSA affinity to TFNA.

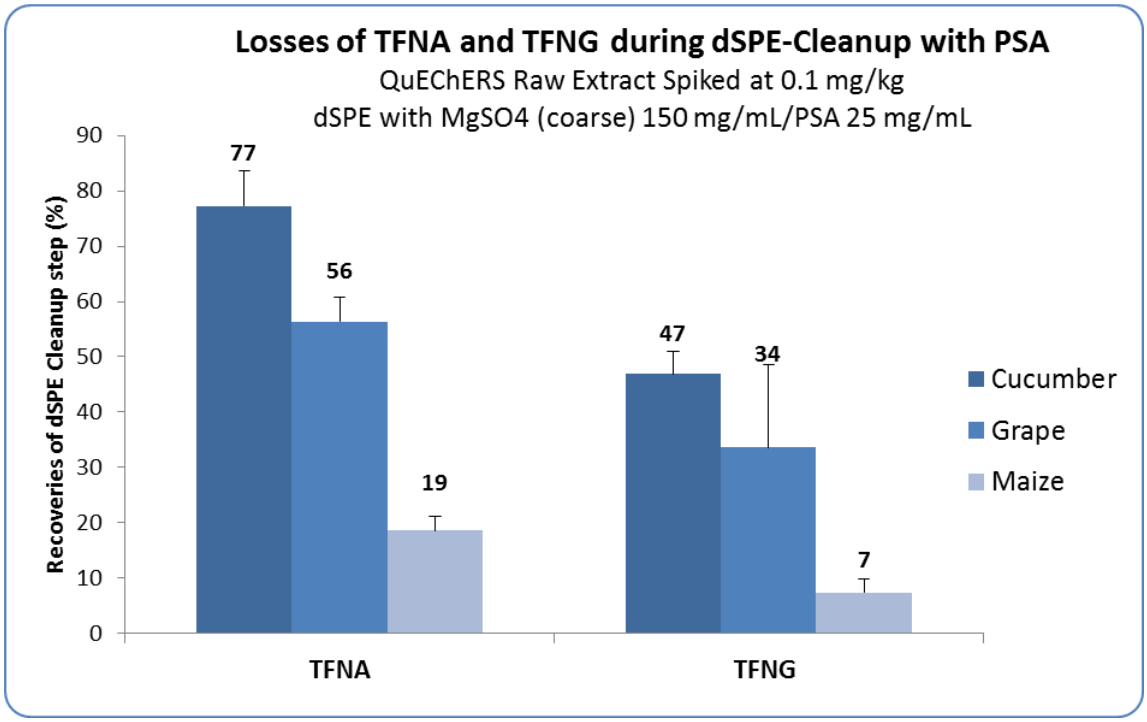


Figure 3 Losses of TFNA and TFNG during dSPE-Cleanup with PSA

References

- Modification of existing MRLs for Flonicamid in various crops, European Food Safety Authority, EFSA Journal 2010; 8(5): 1610
- Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding, Masayuki Morita et al., Pest Management Science, Volume 63, Issue 10, pages 969–973, October 2007
- Analysis of Flonicamid and its metabolites in dried hops by LC-MS/MS, Hengel, M. et al., J. Agric. Food Chem. 2007, 55, pages 8033-8039

Document History

Date	Action	Changes
Mar. 2014	Publication of V1	
May 2015	Publication of V2	General revision of the document
		New Table with Providers of TFNA and TFNG
		New results of recovery experiments for different commodities (the old recovery figures were removed as they were generated using another QuEChERS-variant using acetic acid)
		Results of experiments on matrix effects
		Results showing impact of cleanup with PSA on TFNA and TFNG recoveries