Fast Analysis of Flonicamid and Its Metabolites in Agricultural Foods by RPLC-MS/MS

ASMS 2009

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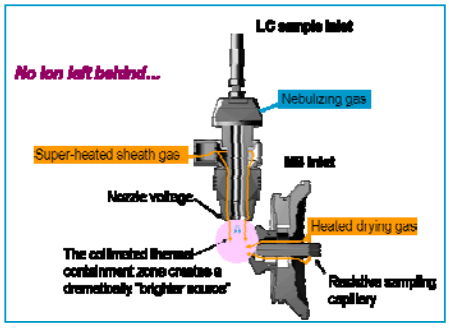
Introduction

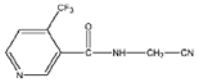
Novel Aspect: A fast and highly sensitive method that facilitates enforcement efforts to monitor the use of Flonicamid insecticide on foods.

The novel pesticide flonicamid was developed in 2000 as a selective agent against aphids and other sucking insects. The mode of action has been identified as blocking the A-type potassium channel with the biological effect of suppressing feeding and movement by aphids (1). The use of this pesticide is advantageous because it does not impair acetylcholine esterase or nicotinic acetylcholine receptors as compared with typical pesticides; thus, there is minimal chance of developing cross-resistance by insects. Flonicamid is also nontoxic to beneficial arthropods, so the use of this pesticide is ideal for pest management programs (2). To help enforce the use of this insecticide and monitor its presence in foods, a rapid and sensitive method using reversed-phase LC-MS/MS is needed.

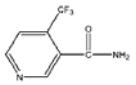


Samples Structures

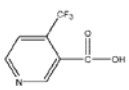
Flonicamid
MW 229.04 $C_9H_6F_3N_3O$



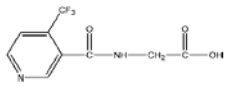
TFNA-AM
MW 190.04 $C_7H_5F_3N_3O$



TFNA
MW 191.02 $C_7H_4F_3N_3O_2$



TFNG
MW 248.04 $C_9H_7F_3N_3O_3$



Experimental

Sample Preparation

A standard mixture of Flonicamid and its metabolites, TFNA, TFNA-AM, and TFNG was prepared from 1 pptr to 1 ppm. To test for matrix effects, hops or canola seed oil was spiked with metabolites and the results were compared with the standards and matrix blank. Matrices were extracted using 50% acetonitrile, followed by SPE clean-up, and dilution with 0.1% formic acid in dH2O. Separations were performed using the Agilent Rapid Resolution LC (RRLC, 600 bar, 9000 psi) and either 2.1mm x 100mm XDB C-18 column (1.8 um) or 4.6mm x 100mm Zorbax XDB C-18 column (5 um). The LC was interfaced to either a standard ESI source or ESI with Agilent Jet Stream Technology (AJST) and MS/MS.

Given 4-10mg vials containing Flonicamid, TFNA, TFNA-AM, and TFNG. Combine all 4-10mg wts into a 10mL Volumetric flask (or 10mL graduated cyclinder) and bring up to 10 mL using Acetonitirle to give a stock solution of 1ug/uL. Create dilution series using appropriate buffer.

HPLC Conditions

Column: Zorbax XDB C18 2.1x100mm, 1.8 µm (P/N 928700-902)

Col. Temperature: 50°C

Mobile Phase: A = 0.2% formic acid in water

B = 0.2% formic acid in acetonitrile

Pump Flow: 0.25 mL/min

Isocratic: 85%A : 15%B

Gradient:

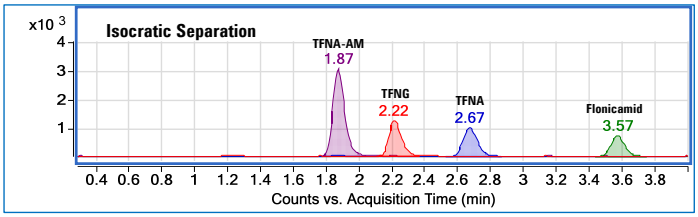
Time(min)	%B
0.00	10
8.00	20
8,20	10

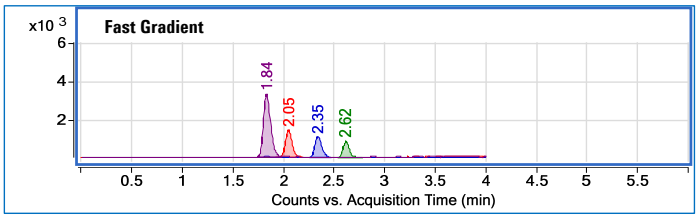
MS Parameters

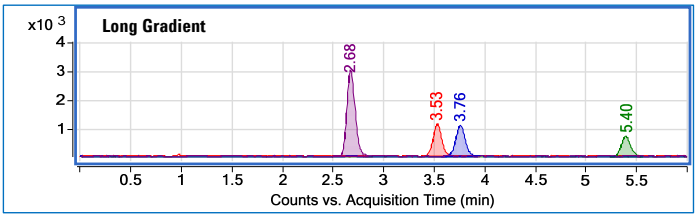
Compound	RT (min)	MRM	Frag (V)	Dwell (ms)	CE (V)
TFNA-AM	1.85	191 > 148	120	100	20
TFNG	2.20	249 > 203	125	100	16
TFNA	2.66	192 > 148	110	100	20
Flonicamid	3.55	230 > 203	125	100	12

Results and Discussion

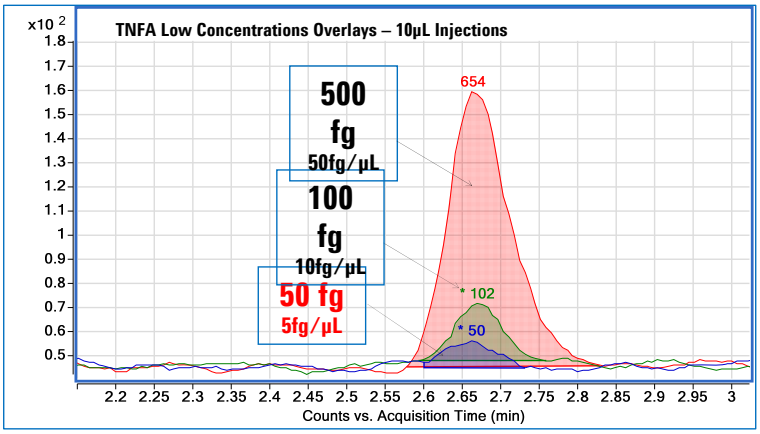
Chromatography



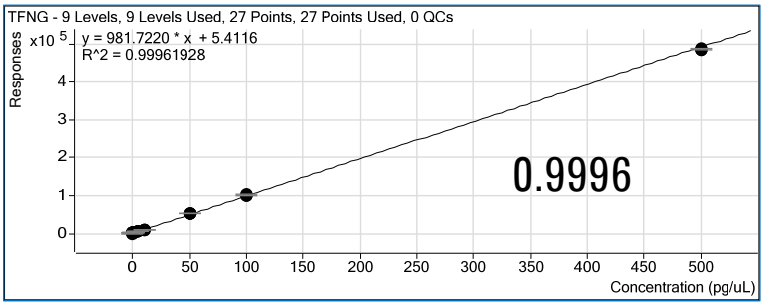


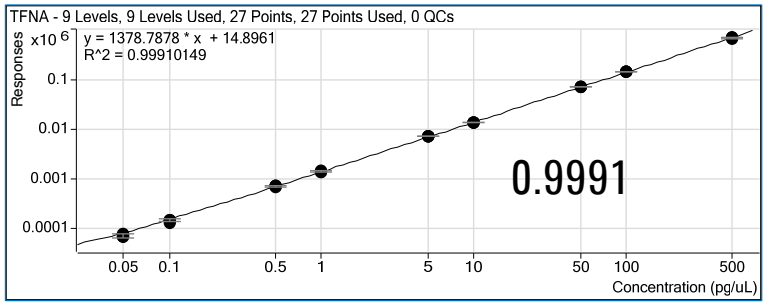


Verification of Low Levels Responses

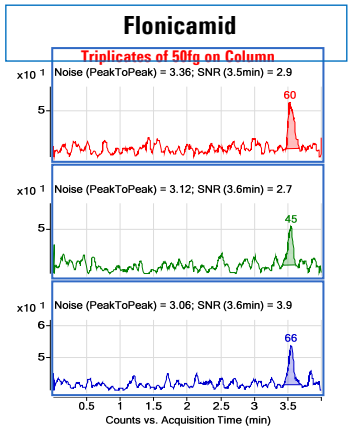


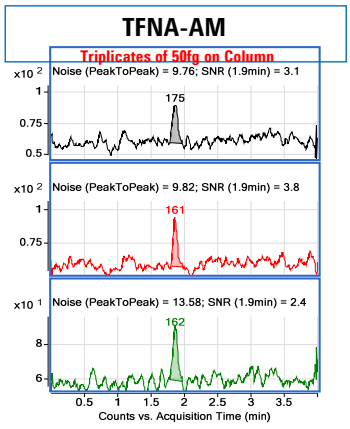
Quantitation

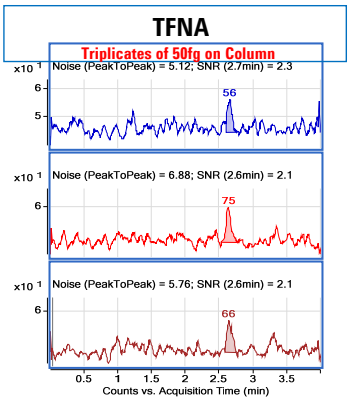


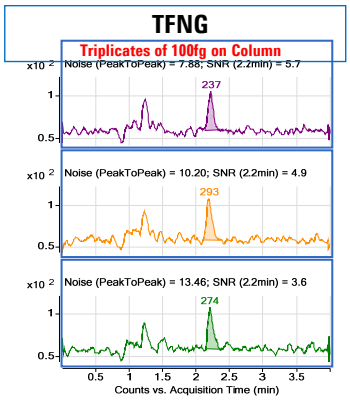


Limits of Detection (LOD)

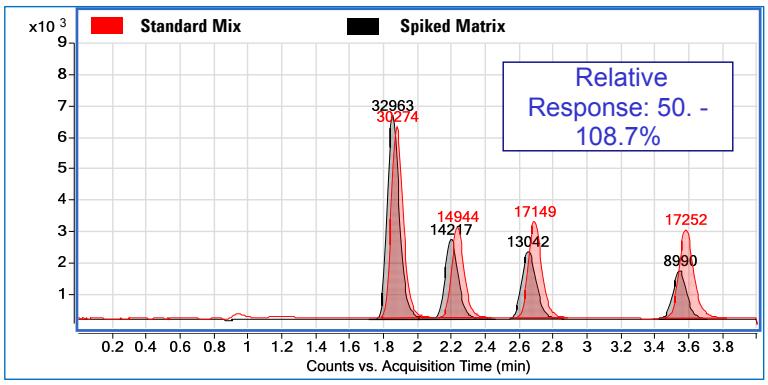








Matrix Effect



Results and Discussion

Flonicamid (CAS 158062-67-0), 4-trifluoromethylnicotinic acid (TFNA), 4-trifluoromethylnicotinamide (TFNA-AM), and N-(4-trifluoromethylnicotinoyl)glycine (TFNG) were evaluated for LOD, LOQ, and linearity. Mobile phases of 0.1% formic acid in H20 (A-phase) and 0.2% formic acid in acetonitrile (B-phase) and 0.2% formic acid were used to rapidly elute analytes off of a 2.1mm i.d. C18 column underc 4min with isocratic analysis (75% A-phase) at 0.25mL/min. MRM transitions were determined for each analyte: Flonicamid (230 > 203), TFNA (192 > 148), TFNA-AM (191 > 148), and TFNG (249 > 203).

Agilent Jet Stream with 6460 QQQ and RRLC and 250uL/min gave

Total run times of < 5 minutes with LOD: Flonicamid is 50 fg
TFNA-AM is 100 fg
TFNA is 50 fg
TFNG is 50 fg

In addition, an LOD range of 400-800 fg was obtained for the metabolites using flowrate of 1.0mL/min across a 4.6mm column (R^2 = 0.999) and < 5 %RSD,were obtained over 4 decades in concentration for each analyte.

Evaluations are currently underway to optimize the sample preparation method to optimize recovery and minimize ionization suppression effects using food matrices. Previous extraction recoveries ranged from 69-109% for these analytes in hops.

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Conclusions

A fast reversed-phase LC method was established using the 1200 series Rapid Resolution LC with a Zorbax XDB C-18 (1.8um) 2.1 mm x 100mm column (0.25mL/min) interfaced with the Agilent 6460A QQQ using the standard AJTS source.

Total run times of less than 5 minutes were obtained and a LOD of 50fg, 100fg, 50fg,and 50fg were obtained for TFNA-AM, TFNG, TFNA, and Flonicamid, respectively. To fully resolve each analyte a slow gradient could be used resulting in a run-time of 8 min. On the other hand, analysis times less than 3 minutes are also possible, but matrix effects should be considered.

Linear responses of R2 = 0.999 and low %RSDs were obtained over 4 decades in concentration for each analyte. Excellent reproducibility was obtained even at the LOD.

The MassHunter software also automatically and reproducibly performed peak area measurements and allowed for automated calibration curve fitting, quantification and report generation.

Overall, this new method allows for low femtogram detection of Flonicamid and its metabolites at flowrates as high as 1mL/min, while the ESI with Jet Stream Technology produced a 3-5X improvement in detection limits over the standard ESI.

References

(1) Morita, M.; Ueda, T.; Yoneda, Y.; Koyanagi, T.; Haga, T. Flonicamid, a novel insecticide with a rapid inhibitory effect on aphid feeding. Pesticide Science 2007, 63, 10, 969-973.

(2) Hengel, M.; Miller, M. Analysis of Floniamid and its metabolites in dried hops by LC-MS/MS. J. Agric. Food Chem. 2007, 55, 8033-8039.