Analysis of TFA, DFA and MFA in Fruit and Vegetables

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Introduction

Trifluoroacetic acid (TFA) is a known breakdown product of numerous pesticides, hydrofluorocarbons, the anaesthetic halothane and many other chemicals. Difluoroacetic acid (DFA) is a metabolite of the pesticide flupyradifurone, while monofluoroacetic acid (MFA) is the main agent in the rodenticide "1080" (primarily used in New Zealand and Australia). Traces of all three compounds are reported to have been found in animal products, plants, tap water, ocean water and chemical waste.

EFSA recently indicated the need to collect data on the occurrence of the substances in order to potentially establish separate MRLs for TFA and DFA in food.

Analytical Method

We have developed a quick and easy-to-use method allowing the analysis of all three compounds simultaneously. The method involves addition of ILIS (isotopically labelled internal standards), extraction with acidified methanol, centrifugation, filtration and LC-MS/MS analysis using DMS (differential mobility spectrometry). LC-separation was achieved by the QuPPe method 7 ("Morpholine, Diethanolamine and Triethanol-amine", see QR code) employing a Dionex Acclaim Trinity P1 column. Mobile phase: A: 50 mmol NH_4-formate, adjusted to pH 3 with formic acid; B: acetonitrile (isocratic elution). The MS and DMS parameters are listed below.

Substance	MRM	R.T. [min]	DP	CE	CXP	COV [V]	SV [V]
TFA	113/69	3.8	-55	-18	-1	-5.5	2300
	113/113			-6	-5		
TFA-$^{13}C_2$ (ILIS)	115/70	3.8	-35	-14	-9	-5.5	2300
DFA	95/51	5.7	-35	-16	-7	-9.5	2500
	95/95			-6	-7		
DFA-$^{13}C_2$ (ILIS)	97/52	5.7	-65	-20	-7	-12	3000
MFA	77/57	6.8	-45	-16	-7	-15.5	2700
	77/77			-6	-7		
	77/33			-16	-15		
MFA-$^{13}C_2$, -D_2 (ILIS)	81/36	6.8	-40	-16	-17	-	-

The use of DMS using the SelexIon module improved selectivity, which is compromised by the fact that TFA and DFA exhibit only one useful mass-transition. By means of DMS the molecular ions could be separated from isobaric matrix components without fragmentation. Despite the considerable drop in ion intensity, DMS resulted in improved LOQs for TFA and DFA, compared to simple LC-MS/MS. In the case of MFA the very strong loss in sensitivity caused by DMS resulted in considerably higher LOQs.

The use of ILIS proved to be essential to overcome matrix effects.

Validation

MFA was deemed of minor interest, as it is currently neither used as a pesticide nor a relevant metabolite of pesticides. The method was thus only validated for TFA and DFA. Validation was done on four matrices at two levels each. The achieved recoveries and relative standard deviations (RSD) are listed below (using ILIS).

Compound	Spiking Level [mg kg⁻¹]	Mean Recoveries in % at n = 5, (RSDs in %)			
		Goosberry	Tomato	Rice	Avocado
TFA	0.02	63 (9)	73 (4.3)	-	62 (11)
	0.04	76 (4.3)	76 (8.3)	81 (2.3)	80 (3.5)
	0.08	-	-	78 (4.4)	-

Compound	Spiking Level [mg kg⁻¹]	Mean Recoveries in % at n = 5, (RSDs in %)			
		Grape	Cucumber	Rice	Avocado
DFA	0.01	98 (6.8)	105 (5.5)	-	-
	0.02	97 (3.4)	94 (3.4)	104 (6.2)	99 (3.5)
	0.04	-	-	88 (3.5)	103 (5.1)

The sensitivity for TFA is very good. However, considerable and fluctuating background levels of TFA did not allow validation at lower levels.

TFA and DFA findings

211 samples of fruit and vegetables were analyzed, 30 of them being organic. There were only traces of DFA in two conventional samples and no findings in organic samples. 23 % of the conventional and 13 % of the organic samples contained TFA levels above the validated LOQs.
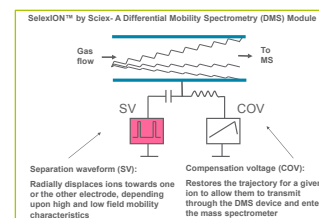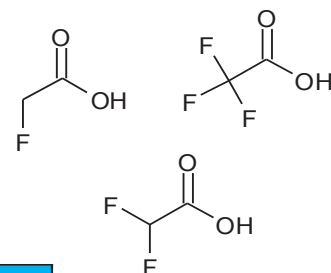
Compound	No. of Samples	No. of Positives	% of Positives	% of Samples ≥LOQ*	Max. [mg kg⁻¹]	Mean ≥LOQ* [mg kg⁻¹]
Conventional Samples						
TFA	181	129	71	23	0.47	0.050
DFA		2	1	0	-	-
Organic Samples						
TFA	30	26	87	13	0.051	0.034

*TFA: high water 0.02 mg kg⁻¹; high acid, high oil and dry 0.04 mg kg⁻¹;
  DFA: high water and high acid 0.01 mg kg⁻¹; high oil and dry 0.02 mg kg⁻¹;

SelexION™ by Sciex- A Differential Mobility Spectrometry (DMS) Module

Separation waveform (SV): Radially displaces ions towards one or the other electrode, depending upon high and low field mobility characteristics

Compensation voltage (COV): Restores the trajectory for a given ion to allow them to transmit through the DMS device and enter the mass spectrometer

Summary

An easy-to-use method for TFA, DFA and MFA was developed and validated for TFA and DFA in QuPPe extracts. TFA exceeded the LOQs in approximately one out of four samples. No DFA (and no MFA) residues were detected in any of our samples. Further measures to reduce TFA background levels are needed to enable monitoring with lower reporting limits.

Further optimization of the SelexIon settings, in particular the SV and the COV, may result in additional gains in selectivity and sensitivity.

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