Demonstration of the Utility of a 1.5 mm ID UHPLC column for Pesticide Analysis using the Multi-Analyte Method

Stephanie A. Schuster[1], Austin Whittington[2], Mary Ellen P. McNally[2]

[1]Advanced Materials Technology, Inc.; [2]FMC Corporation

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Outline

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Multi-analyte Method (MAM) for Pesticide Analysis

- Currently, 1000's of different methods used across the world for pesticide analysis of formulated materials.
- MAM designed for use by regulatory labs who test pesticide products available in the market to determine if they meet reported specifications.
    - Developed and validated at the Irish Department of Agriculture, Food and The Marine, and contributed to by regulatory labs in Belgium, the Czech Republic and FMC.
    - Goal is to have a method that works for a wide variety of pesticides using the same column.
    - To date, > 70 active ingredients have been analyzed using HPLC and > 35 active ingredients have been analyzed by UHPLC.
    - With this data we are adding six more actives with an alternative choice of UHPLC columns.

Multi-analyte Method (MAM) for Pesticide Analysis (continued)

	MAM Method	MAM Method	HALO 1.5 mm Method
Column	Kinetex® C18, 4.6 x 150 mm, 2.6 µm or equivalent	Kinetex® C18, 2.1 x 150 mm, 2.6 µm or equivalent	HALO 90 Å C18, 1.5 x 150 mm, 2.7 µm
Flow Rate (mL/min)	1.0	0.4	0.2
Injection Volume (µL)	0.5	0.5	1
Detector Wavelength (nm)	220-340	220-340	220-340
Column Temperature (°C)	25	25	30

Multi-analyte Method (MAM) for Pesticide Analysis (continued)

	4.6 mm MAM Method		2.1 mm MAM Method		HALO 1.5 mm Method	
Mobile Phase A	0.1% pH 2.0–2.2; o-phosphoric or formic acid-adjusted H_2O		0.1% pH 2.0–2.2; o-phosphoric or formic acid-adjusted H_2O		formic acid in H_2O, pH 2.7	
Mobile Phase B	ACN		ACN		ACN	
Gradient	Time (min)	%B	Time (min)	%B	Time (min)	%B
	0.00	35	0.00	35	0.00	35
	10.00	85	4.00	85	10.00	85
	16.00	85	6.00	85	14.00	85
	16.40	35	6.01	35	14.50	95
	18.00	35	7.00	35	17.50	95
					18.00	35
					23.00	35

Pesticides Tested

Name	Type	Formulation Type
Azimsulfuron	Herbicide	Technical concentrate (TC)
Bifenthrin	Insecticide	Water dispersible granules (WG)
Chlorsulfuron	Herbicide	Water dispersible granules (WG)
Flutriafol	Fungicide	Suspension concentrate (SC)
Indoxacarb	Insecticide	Suspension concentrate (SC)
Triflusulfuron-methyl	Herbicide	Water dispersible granules (WG)

Column Alternative for the MAM Method

- Method specifies a 2.1 mm ID column so how would a 1.5 mm column work with the intent of offering an alternative column for the MAM?

- 1.5 mm ID column is stainless steel and looks and feels like a 2.1 mm ID column

Top = 2.1 mm

Bottom = 1.5 mm

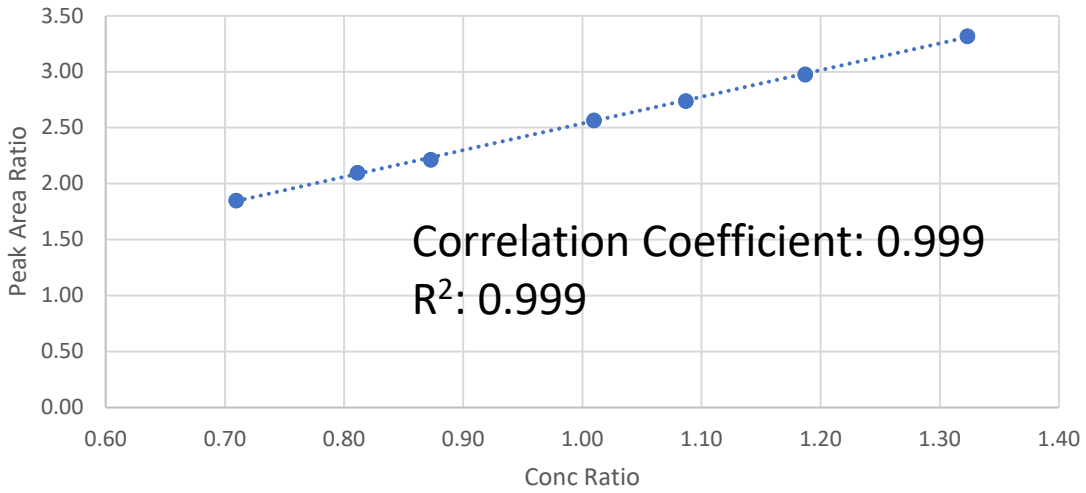
Experiments

- Standards or formulated product were weighed directly into glass scintillation vials using a 5 decimal place analytical balance
- Each sample was diluted with:
  - 22.5 mL Acetonitrile
  - 2.5 mL of 5 mg/mL dicyclohexyl phthalate in acetonitrile (internal standard)
  - Total volume of 25 mL
- Samples were vortexed and then sonicated for 15 minutes
- Samples were filtered directly into HPLC vials using 0.2 µm WW (water wettable) PTFE syringe filters
- Each sample was injected 2 times

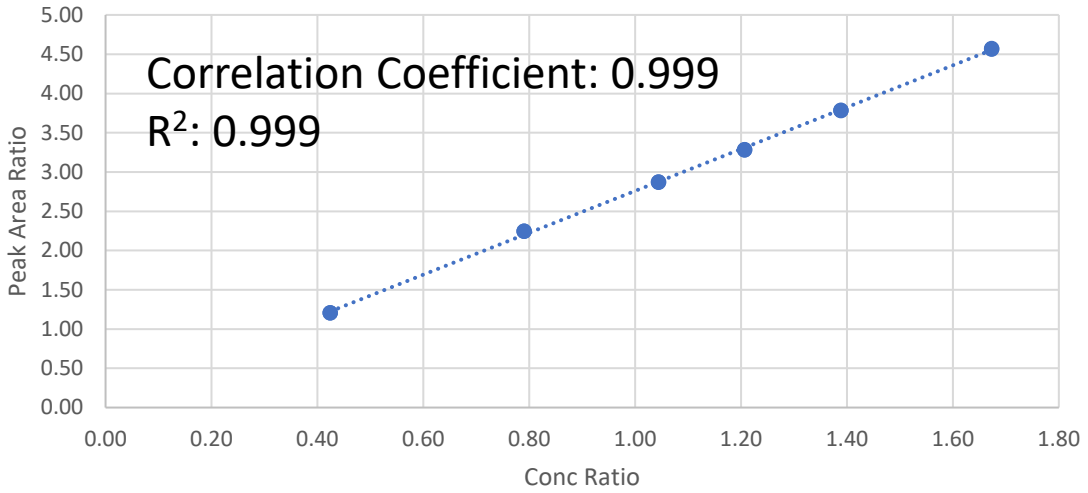
Linearity

Examples

Chlorsulfuron



Triflusulfuron



- For linearity a minimum of a 6 point calibration curve is constructed using primary analytical standards of the active ingredients.
- Passing results require a minimum correlation coefficient of 0.999 and a minimum R^2 of 0.999

Reproducibility (Precision)

- Modified Horwitz equation gives an estimation of intra-laboratory repeatability (RSD_r):

$$\%RSD_r = 0.67 \times 2^{(1-0.5\times\log(c))}$$ where c is the concentration

- Horrat (Horwitz ratio) is a parameter that gives the acceptability of a method with respect to precision:

$$H_r = \frac{\%RSD}{\%RSD_r}$$

- For acceptable reproducibility, Horrat must be between 0 and 1.

- If $1 < H_r \leq 2$, acceptable in case of a suggested explanation.

HORWITZ & ALBERT: JOURNAL OF AOAC INTERNATIONAL VOL. 89, NO. 4, 2006

Reproducibility

- Same analyst weighed and prepped 8 samples of technical material on two consecutive days for a total of 16 samples.
- Data for indoxacarb shows RSD of 1.08 for 1 set of 8 preps

Prep	Injection	Sample wt%	Average sample wt % per prep	average wt%	Std. dev	RSD	Modified Horwitz	Horrat
1	1	27.09	27.09	26.80	0.29	1.08	1.63	0.66
	2	27.08						
2	1	26.83	26.85					
	2	26.87						
3	1	26.73	26.74					
	2	26.74						
4	1	26.52	26.51					
	2	26.51						
5	1	26.57	26.57					
	2	26.57						
6	1	26.45	26.44					
	2	26.43						
7	1	26.93	26.92					
	2	26.92						
8	1	27.31	27.31					
	2	27.31						

Reproducibility

- Same analyst weighed and prepped 8 samples of technical material on two consecutive days for a total of 16 samples.
- Data for Azimsulfuron shows RSD of 0.86 for 1 set of 8 preps

Prep	Injection	Sample wt%	Average sample wt % per prep	average wt%	Std. dev	RSD	Modified Horwitz	Horrat
1	1	48.34	48.38					
	2	48.43						
2	1	48.02	48.03	47.94	0.41	0.86	1.50	0.58
	2	48.05						
3	1	48.25	48.21					
	2	48.17						
4	1	48.07	48.05					
	2	48.03						
5	1	48.26	48.22					
	2	48.19						
6	1	47.31	47.26					
	2	47.21						
7	1	47.39	47.37					
	2	47.35						
8	1	47.33	47.29					
	2	47.26						

Reproducibility (Precision)

- All Horrat values were between 0 and 1, so reproducibility of the method is acceptable

	Average Horrat over 2 Days
Indoxacarb	0.518
Azimsulfuron	0.633
Flutriafol	0.570
Triflusulfuron	0.468
Bifenthrin	0.218

Accuracy

- Formulation blank was spiked with the active ingredient at 75%, 100%, and 125% of the target concentration.
- Passing criteria must be between 97 and 103%

Bifenthrin

Sample	Prep	Injection	Sample wt%
		1	101.05
	1	2	101.14
		1	100.36
100%	2	2	100.44
		1	99.81
	3	2	99.95
		1	99.48
75%	4	2	99.57
		1	100.64
	5	2	100.70
		1	100.30
125%	6	2	100.28
		average	100.31
		standard deviation	0.53
		% RSD	0.53

Flutriafol

Sample	Prep	Injection	Sample wt%
		1	97.77
	1	2	97.82
		1	100.73
100%	2	2	97.78
		1	96.78
	3	2	98.00
		1	98.36
75%	4	2	98.85
		1	98.31
	5	2	98.87
		1	99.44
125%	6	2	98.73
		average	98.45
		standard deviation	1.00
		% RSD	1.01

Tolerances

- Tolerances are limits that define an acceptable range of variability around a declared value for an active ingredient in a formulated product.
    - This recognizes that analytical error as well as other manufacturing errors are expected in the measurement of a product.

Declared content in g/kg or g/L at 20 °C	FAO Tolerances
Up to 25	± 15% homogeneous formulation
	± 25% non-homogeneous formulation
More than 25 up to 100	± 10% (Declared Content)
More than 100 up to 250	± 6% (Declared Content)*
More than 250 up to 500	± 5% (Declared Content)*
More than 500	± 25 g/kg or ± 25 g/L

* Pertinent for the formulations in this study

Examples of Acceptable FAO Specification Ranges

	Target Concentration wt%	Range for in Spec Material wt%	Measured Sample Concentration wt%	In Spec Material
Indoxacarb	27	25.65 – 28.35	27.37	☑
Azimsulfuron	50	47.50 – 52.50	48.58	☑
Triflusulfuron	50	47.50 – 52.50	47.97	☑

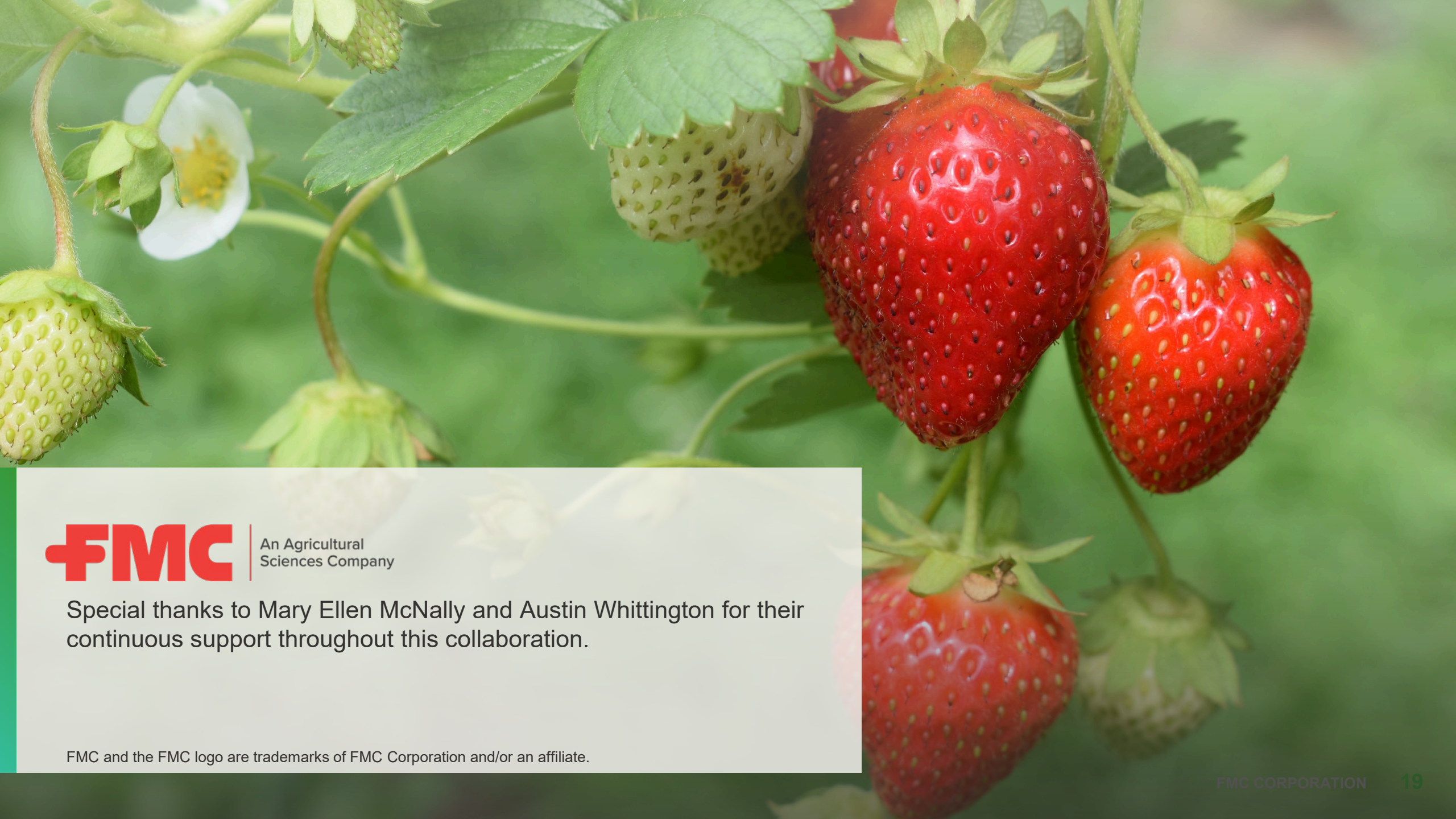
All Data

	Linearity		Reproducibility Horrat	Accuracy	
	Correlation Coefficient	R^2	Average over 2 Days	Accuracy	RSD
Indoxacarb	1.000	0.999	0.518	97.69%	1.28%
Azimsulfuron	1.000	0.999	0.633	99.87%	2.26%
Flutriafol	0.999	0.999	0.570	98.45%	1.01%
Triflusulfuron	1.000	1.000	0.468	97.85%	0.29%
Bifenthrin	1.000	1.000	0.218	100.31%	0.53%

Summary

- MAM was successfully run using a 1.5 mm HALO® C18 column
  - *Excellent linearity, reproducibility, and accuracy were achieved for 6 different pesticides of varying hydrophobicities.*

- Additional pesticide actives continue to be run using the current published MAM, now the 1.5 mm HALO® column is a viable alternative.
- To use the 1.5 mm HALO® C18 column for the MAM, small changes in the operating temperature and re-equilibration improved the robustness and reduced carryover.



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Questions?

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