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Comprehensive Pesticide Screen for Fruit Juice using On-Line Sample Extraction with LC-MS/MS

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Objectives

- Develop a quick, automated on-line extraction LC-MS/MS method to assay pesticides in fruit juice (cranberry and orange juices).
- Compare detection sensitivity [LOD, LOQ] of pesticides extracted from fruit juice matrix using TurboFlow™ technology coupled to a Thermo Scientific TSQ Quantum Ultra MS/MS to the current US-EPA Specific Tolerances in raw fruit.
- Investigate matrix effect by comparing neat and juice samples.

Thermo Fisher Scientific On-Line Technology

- Developed in the late 1990's – patented technology
  - Originally for Bio-analysis
    - Zimmer (Bayer) J. Chrom A 1999; Herman (Cephalon) RCMS 2002.
  - Allows for the direct injection of biological fluids onto a chromatographic environment; no pre-extraction.
  - Adopted by major pharmaceutical companies worldwide.
    - Merck, Novartis, BMS, J&J, Sanofi-Aventis, Pfizer, Genentech, GSK, Cephalon, …
- Adopted by the Clinical Research field – 2002.
  - Quest Diagnostics, LabCorp, Mayo Clinic, Johns Hopkins, …
- More recently introduced in two more fields:
  - Environmental
    - Antibiotic screens, pesticide screens, personal care & pharma products screen.
  - Food Safety
    - Antibiotic screen (in honey, Nestle, Switzerland), β-agonists, melamine, pesticides
- Over 800 systems worldwide (NA, Europe, Asia, India, Australia)

Thermo Scientific solution vs “home grown” solutions

- Many labs devised their own online or 2D LC systems
  - Solution for a specific problem
  - Inexpensive, but with significant limitations
  - Primitive software control

- Thermo Scientific solution
  - Made for many laboratory environments
    - Online, 2D LC, various detectors (QQQ, Trap, Accurate Mass, UV, DAD)
  - Extreme versatility
    - Accommodate infinite amount of methods – all small molecules, peptides
    - Use the principles of both SPE and size exclusion, enabling the direct injection of complicated matrices and online sample clean-up.
    - Accommodate wide array of matrices (biofluids, buffers, foods, water, …)
    - Can ramp up throughput on short notice – **Multiplexing**
      - Can run same method in parallel or different methods – **unique feature**
  - Exceptional software control provide unparalleled versatility.

Sample Preparation Challenges

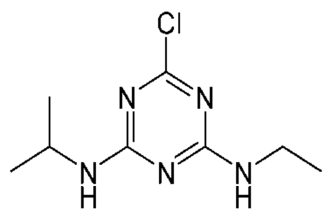
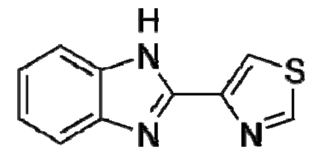
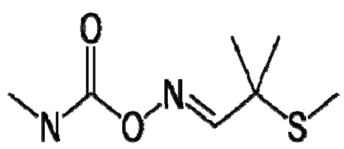
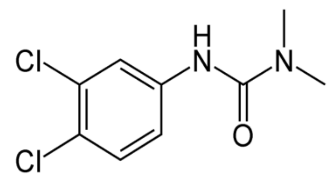
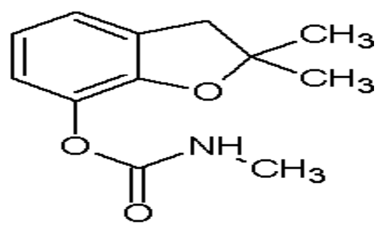
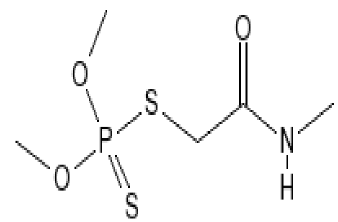
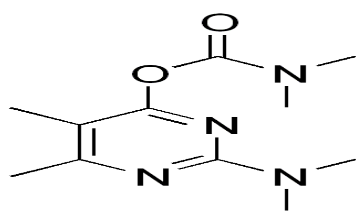
- Problems with Traditional Methodologies:
  - Long and complicated manual sample preparation procedures
  - Matrix interference with analyte detection

- Automated Solutions
  - Off line extraction – partial automation
  - ONLINE TURBOFLOW METHODS – full automation

What is TurboFlow?

- Chromatographic technique
  - Exploits difference between large and small molecules
  - Incorporates column chemistry
- Large particle columns (30 µm or larger)
- High flow rate, low back pressure
- Efficient mass transfer created by turbulence
- Separates low MW analytes from high MW matrix
- Extract rinsed with 200 column volumes; complete removal of un-retained species (salts, sugars, acids, buffers)
- Allows for direct injection of a range of matrices

Molecular structure of the pesticides of interest

ATRAZINE		**THIABENDAZOLE**	
ALDICARB		**DIURON**	
CARBOFURAN		**DIMETHOATE**	
PIRIMICARB			

List of Pesticides, Internal Standards, SRM Transitions in Positive Heated Electrospray Ionization Mode, Retention Times, and their Chemical Class/Use

Analytes	Molecular Weight	Precusor Ion (m/z)	Product Ions (m/z)	Retention Time (mins.)	Chemical Class / Use
Dimethoate	229.26	230.0	199.0 (Q), 125.1 (C)	8.5	Aliphatic Amide Organothiophosphate Insecticide
Diuron	233.09	233.0	72.2 (Q) 46.2 (C)	9.7	Phenylurea Herbicide
Pirimicarb	238.29	239.1	182.3 (Q), 72.184 (C)	9.4	Dimethylcarbamate Insecticide
Carbofuran	221.25	222.1	165.2 (Q), 123.2 (C)	9.2	Benzofuranyl Methylcarbamate Insecticide
Atrazine	215.68	216.1	174.2 (Q), 104.1 (C)	9.6	Organochlorine Herbicide
Thiabendazole	201.25	202.0	175.2 (Q), 131.2 (C)	8.6	Benzimidazole Fungicide
Aldicarb	190.26	213.1 [Na+]	89.2 (Q), 116.2 (C)	8.9	Oxime Carbamate Insecticides
13C3-Atrazine	218.7	219.1	177.2	9.6	Internal Standard
13C6-Thiabendazole	207.20	208.0	137.2, 181.2	8.6	Internal Standard

Note: (Q) = Quantifier Ion, (C) = Qualifier Ion

Calibrator / QC Preparation Method

Spike Internal Standards into Blank Fruit Juice

↓

Vortex 2 mins

↓

Spike Pesticide Working Solution into Fruit Juice Aliquot

↓

Vortex 2 mins

↓

Centrifuge @ 10,000rpm, 4 deg C, 10 mins

↓

Aliquot Supernatant into Vial

↓

Inject 20ul

TurboFlow Method Parameters on Transcend TLX-1 System

TLX Plumbing Mode: Focus Mode

TurboFlow Columns: Two Cyclone-P, 0.5 x 50mm (in tandem)

TurboFlow Column Mobile Phases:

- **[B] 0.02% Acetic Acid in H_2O;**
- **[C] 0.5 % Formic Acid in 1:1 Acetonitrile: Methanol;**
- **[D] 1:1:1 Isopropanol : Acetonitrile : Acetone**

Analytical Column: Hypersil GOLD, 2.1 x 100 mm, 1.9 um

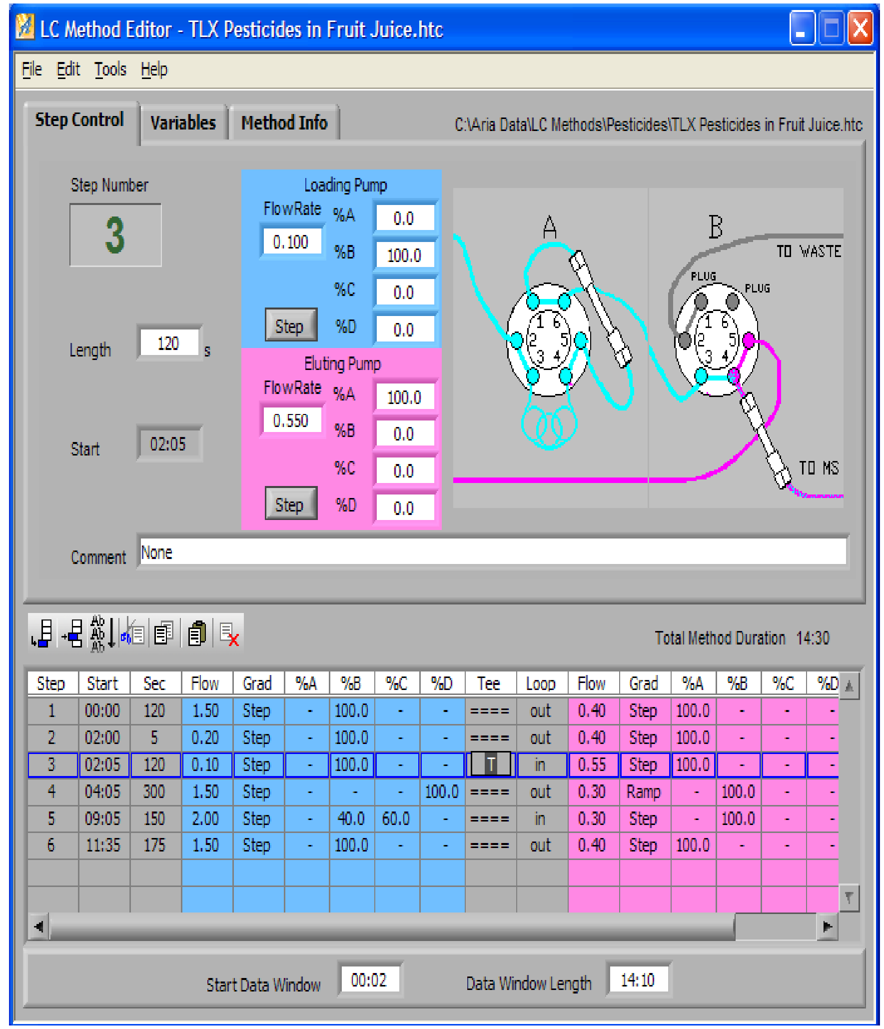
Analytical Column Mobile Phases:

- **[A] 0.02% Acetic Acid in H_2O;**
- **[B] 100 % Methanol**

Analytical Column Temp.: 28 C

Gradient Elution Flow Rate: 0.300 mL/min

Method Run Time: 14.5 minutes



Linear Regression (1/X weighting) and Extraction Efficiency Data for TurboFlow™ Assay of Pesticides in Fruit Juices

Pesticide Residue	Cranberry Juice				
	LOD (ppb) *	Dynamic Range (ng/mL or ppb)	R^2	Extraction Efficiency (%)***	US-EPA Title 40 e-CFR, Part 180, Subpart C: Specific Tolerances (Subpart C # in parentheses) #
Dimethoate	0.625	2.50 – 40.0	0.9979	63.7	1.0-5.0 ppm (180.204)
Diuron	0.156	0.625 – 40.0 **	0.9974	87.0	0.05-1.0 ppm (180.106)
Pirimicarb	0.0391	0.313 – 40.0 **	0.9978	111.1	revoked, foreign use
Carbofuran	0.0781	0.625 – 40.0 **	0.9991	83.4	0.1-2.0 ppm (180.254)
Atrazine	0.0195	0.625 – 40.0 **	0.9972	93.2	0.05 ppm in Guava (180.220)
Thiabendazole	0.0781	1.25 – 40.0 **	0.9997	77.7	3.0-15.0 ppm (180.242)
Aldicarb	0.0781	0.313 – 40.0	0.9981	81.3	0.3-0.6 ppm (180.269)

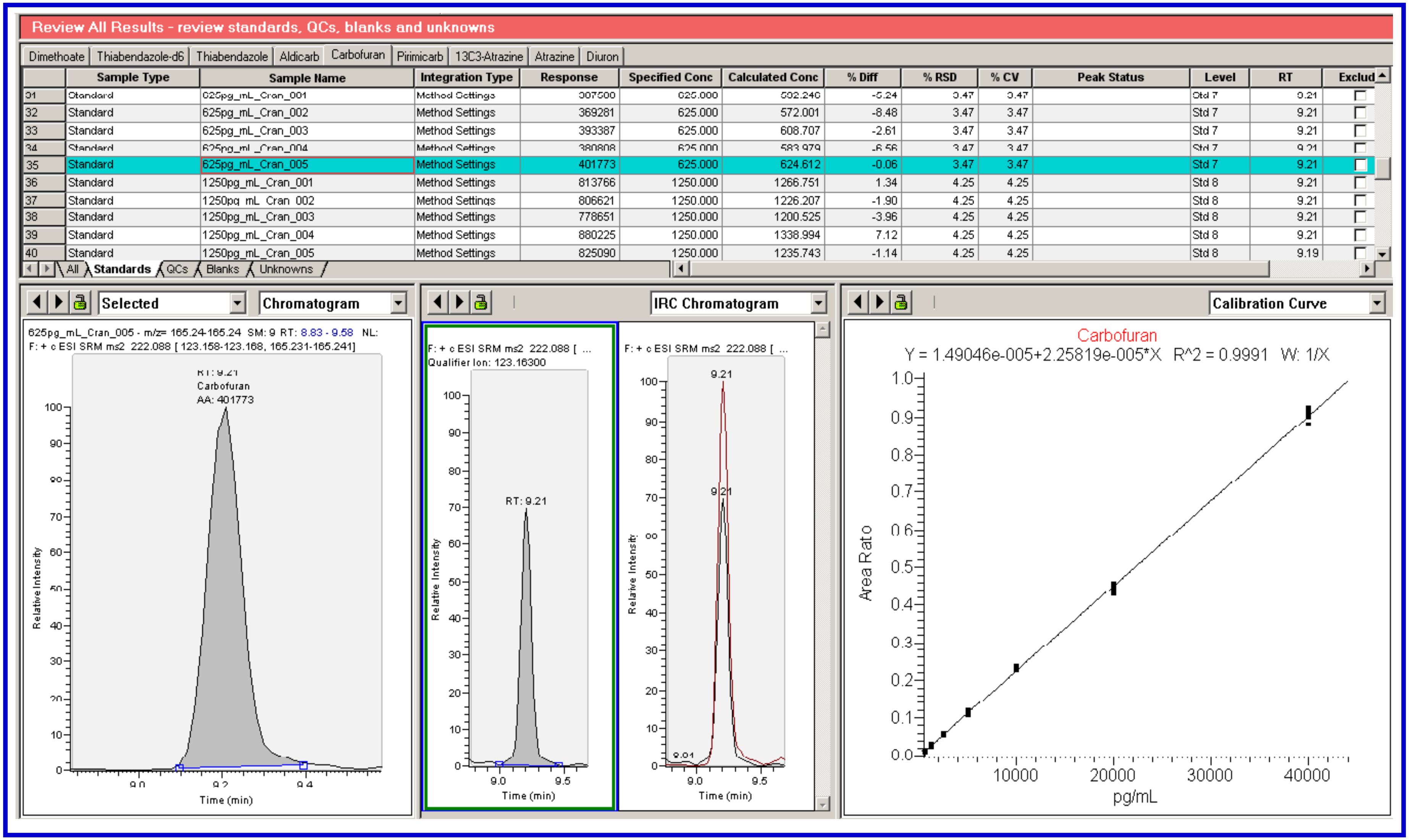
*A visual, non-instrumental method was used to determine LOD.

**Due to the presence of carryover with these compounds, LLOQ was determined by using criteria that carryover must be < 20% of LLOQ.

***Extraction Efficiency = Average (n=5) response of juice QC (5.00 ng/mL) divided by average (n=5) response of neat QC (H_2O) x 100.

Range of EPA-listed fruit commodities

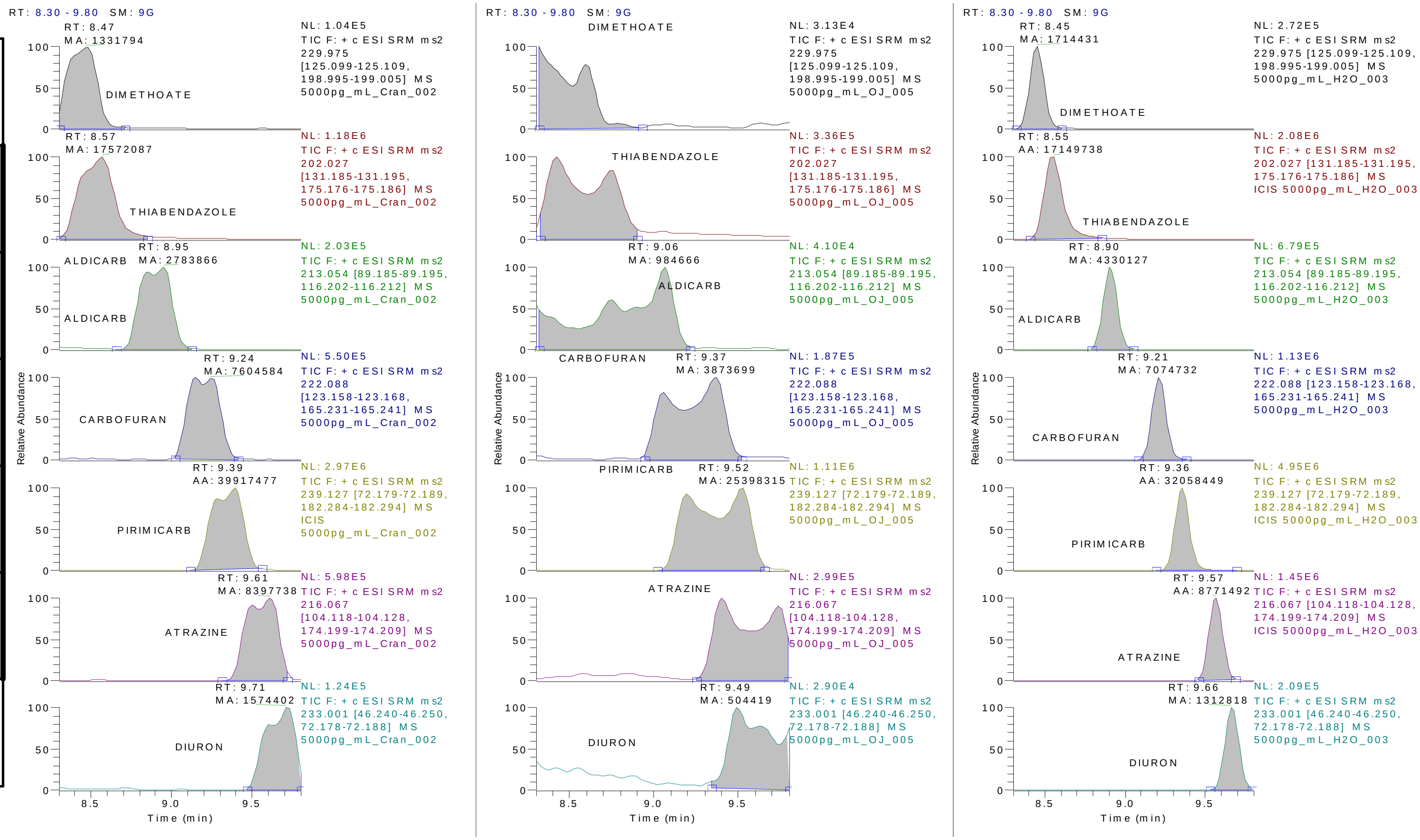
Carbofuran LOQ (0.625 ng/mL), IRC Confirmation, and Linear Regression in Cranberry Juice



	Sample Type	Sample Name	Integration Type	Response	Specified Conc	Calculated Conc	% Diff	% RSD	% CV	Peak Status	Level	RT	Exclude
31	Standard	625pg_mL_Cran_001	Method Settings	387500	625.000	592.240	-5.24	3.47	3.47		Std 7	9.21	☐
32	Standard	625pg_mL_Cran_002	Method Settings	369281	625.000	572.001	-8.48	3.47	3.47		Std 7	9.21	☐
33	Standard	625pg_mL_Cran_003	Method Settings	393387	625.000	608.707	-2.61	3.47	3.47		Std 7	9.21	☐
34	Standard	625pg_mL_Cran_004	Method Settings	380808	625.000	583.979	-6.56	3.47	3.47		Std 7	9.21	☐
35	Standard	625pg_mL_Cran_005	Method Settings	401773	625.000	624.612	-0.06	3.47	3.47		Std 7	9.21	☐
36	Standard	1250pg_mL_Cran_001	Method Settings	813766	1250.000	1266.751	1.34	4.25	4.25		Std 8	9.21	☐
37	Standard	1250pg_mL_Cran_002	Method Settings	806621	1250.000	1226.207	-1.90	4.25	4.25		Std 8	9.21	☐
38	Standard	1250pg_mL_Cran_003	Method Settings	778651	1250.000	1200.525	-3.96	4.25	4.25		Std 8	9.21	☐
39	Standard	1250pg_mL_Cran_004	Method Settings	880225	1250.000	1338.994	7.12	4.25	4.25		Std 8	9.21	☐
40	Standard	1250pg_mL_Cran_005	Method Settings	825090	1250.000	1235.743	-1.14	4.25	4.25		Std 8	9.19	☐

Laminar Flow (LX) Injection of QC Sample in Cranberry Juice, in Orange Juice, and in H_2O at 5.00 ng/mL.

Dimethoate
Thiabendazole
Aldicarb
Carbofuran
Pirimicarb
Atrazine
Diuron



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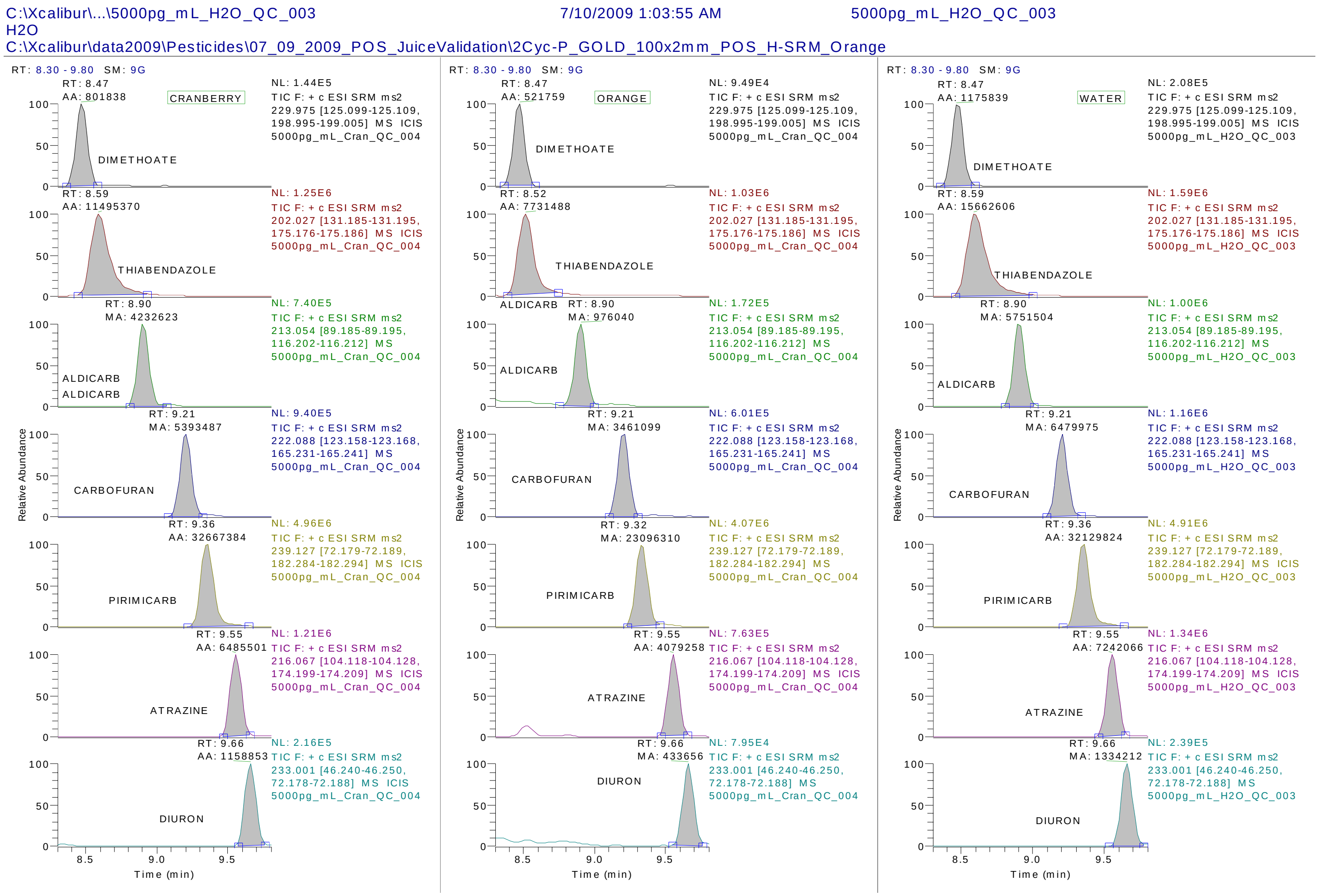
TurboFlow (TLX) Injection of QC Sample in Cranberry Juice, in Orange Juice, and in H_2O at 5.00 ng/mL

Dimethoate
Thiabendazole
Aldicarb
Carbofuran
Pirimicarb
Atrazine
Diuron



Conclusions

- **Using fruit juice as an initial model matrix, we produced a fast and highly selective alternative to currently used sample preparation methods for pesticide studies.**
- **TLX system can shorten sample process time as compared to standard QuEChERS, LLE or SPE extraction.**
- **Matrix Effect: As shown, signal suppression was the main matrix effect in this analysis. To compensate for this, matrix-matched calibration standards should always be employed. Interferences in orange juice reduced the analytical range of aldicarb, but still remained within EPA tolerances.**
- **We are currently actively testing other juice matrices and additional pesticides.**

Thank you for your attention

- www.thermo.com/turboflow