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Automation for Improving the Workflows for LC-MS/MS

***Joe Di Bussolo*, Ph.D.**
Sr. Applications Scientist
Thermo Fisher Scientific
Applications Laboratory at
West Chester University of Pennsylvania

Discussion Overview

- Challenges of sample preparation in LC-MS analysis
- TurboFlow™ Technology
- Multiplexing Technology
- Automation / Workflow
  - Sample Prep with Various Matrices
  - Standard TurboFlow Method for Sample Prep
  - Customer examples

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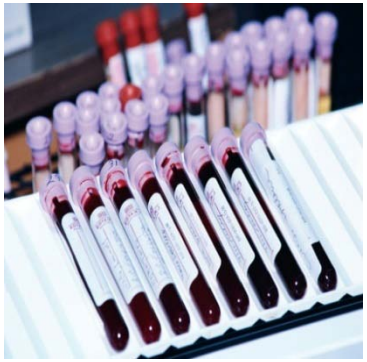
Challenges of Sample Preparation in LC-MS Analysis

- Many samples but not much time
- Wide varieties of matrices
- Matrix binding
- Labile drugs
- Intractable analytes
- Ion suppression
- Analytical Quality Assurance requirements
- LOD/LOQ, recovery, reproducibility, confirmation



Typical Sample Preparation Methods

- Protein precipitation (PPT)
- Liquid-liquid extraction (LLE)
- Solid Phase extraction (SPE)
- Dilute and shoot
- No sample prep
- Combined techniques

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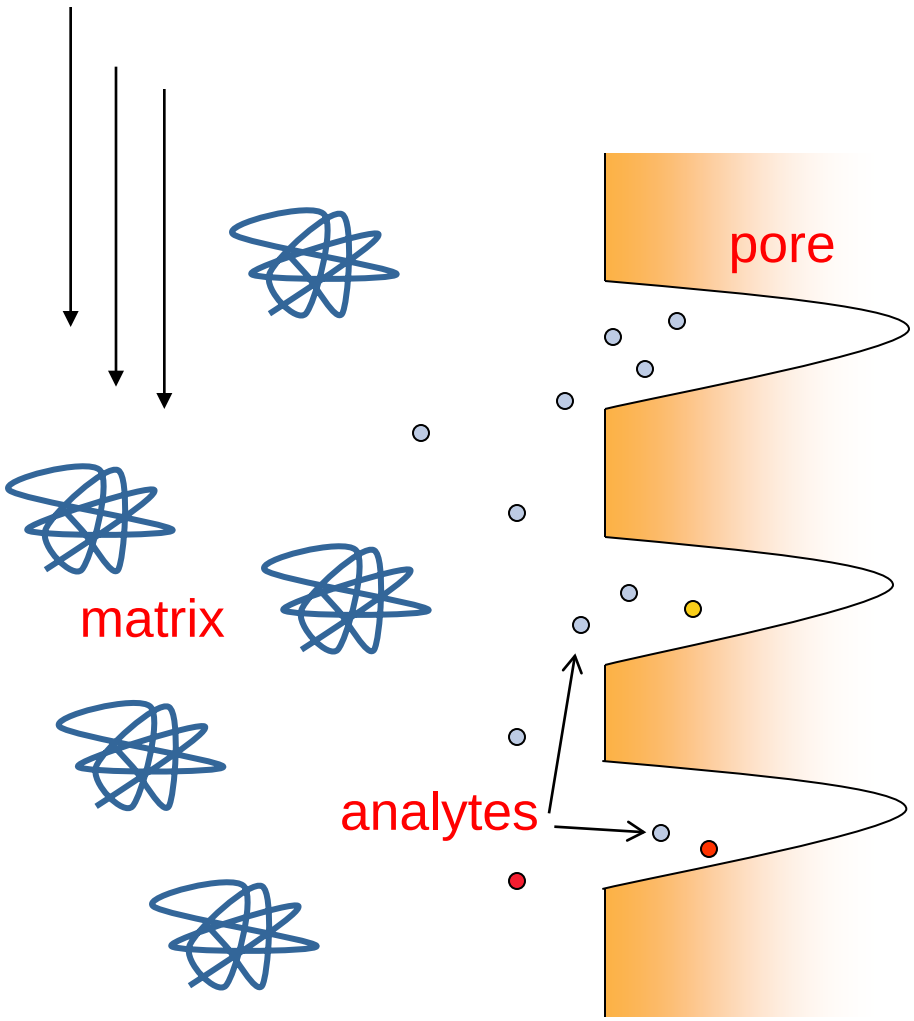
What is TurboFlow Technology?

- Chromatographic technique
  - Exploits difference between large and small molecules
  - Incorporates column chemistry
- Large particle packings
- High velocity, low back pressure
- Efficient mass transfer due to high velocity mixing
- Separates low MW analytes from high MW matrix
- Allows for the direct injection of complex matrices

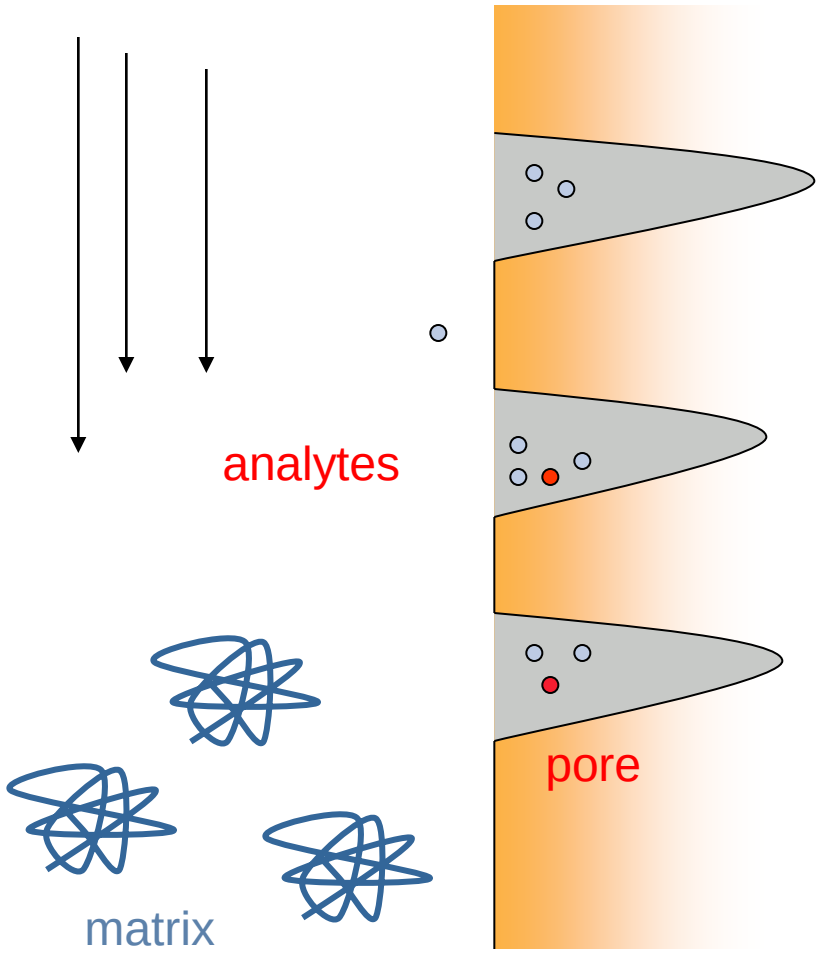
Small and Large Molecules Diffuse at Different Rates

- Small molecules diffuse into porous particles faster than large molecules
- Sample components of interest – analytes are well retained
- Less retained components (e.g. phospholipids, salts, and sugars) are rinsed away



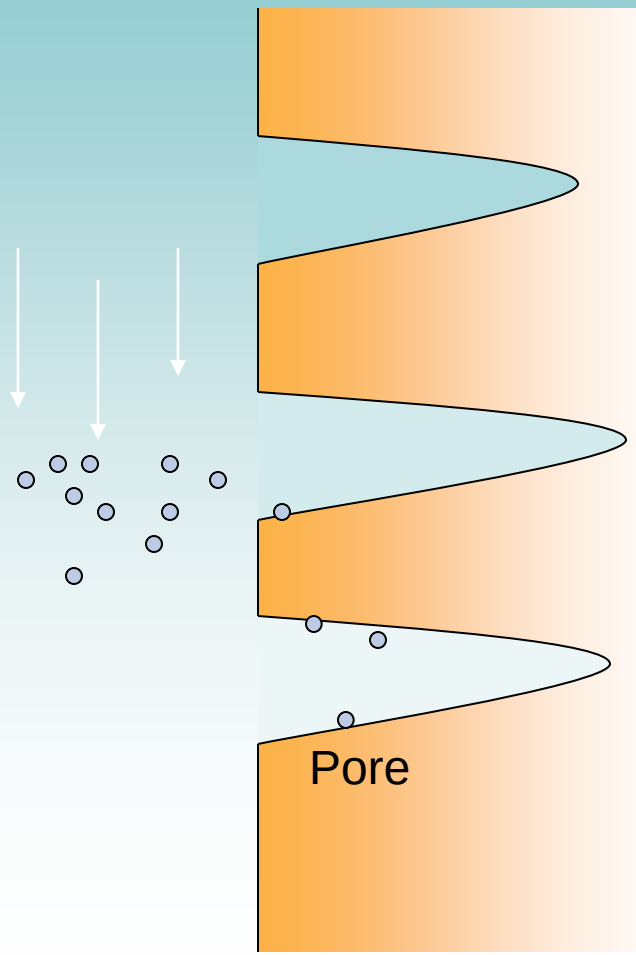
Large Molecules Flow to Waste

- Large molecules do not have time to diffuse into pores
- They are flushed through the column by the high-velocity mobile phase



Analyte Molecules are Eluted

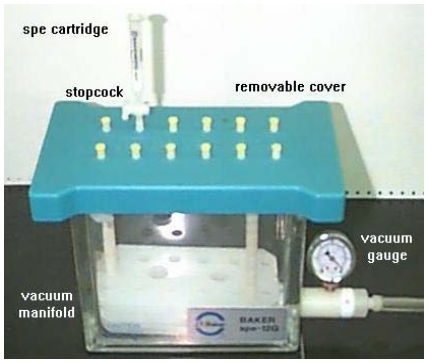
A change in mobile phase composition elutes analytes of interest to the detector or to an analytical column



Video of TurboFlow technology

- video

Less steps with TurboFlow technology



More Steps = More Time

Liquid-Liquid Extraction (LLE)	Solid Phase Extraction (SPE)	Protein Precipitation (PPT)	TurboFlow – 4 Steps
1. Aliquot of sample	1. Aliquot of sample	1. Aliquot of sample	1. Aliquot of sample
2. Spike with IS	2. Spike with IS	2. Spike with IS	2. Spike with IS
3. Add buffer	3. Add 0.1N HCL	3. Add acetonitrile	3. Centrifuge
4. Add MTBE	4. Condition sorbent	4. Centrifuge	4. Inject onto column
5. Shake 10 min	5. Add sample to sorbent	5. Remove supernatant	
6. Centrifuge	6. Wash	6. Reconstitute	
7. Remove organic	7. Evaporate	7. Transfer to plate	
8. Evaporate to dryness	8. Reconstitute	8. Inject onto column	
9. Reconstitute	9. Transfer		
10. Transfer to plate	10. Inject onto column		
11. Inject onto column			

FASTER RESULTS!

Using TurboFlow methods on the TLX System allows you to remove most of the time-consuming steps in the sample preparation process, which speeds sample throughput while minimizing errors and variability.

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Biological Tissues sample prep - TurboFlow methods

1. Place 5 g (+/- 5 mg) of flesh in a clean 50 mL polypropylene falcon test tube.
2. Cover the sample with 15 mL of 95:5 acetonitrile-water
3. Homogenize the sample using the Ultra Turrax until it becomes a smooth paste.
4. Add 250 uL of the internal standard (CAP-ISTD) and vortex the sample for 15 sec
5. Allow the mixture to stand at room temperature for 1 hour and then centrifuge the sample for 15 mins @ 7500 rpm.

Biological Tissues – Standard Sample Preparation

1. **Place 5 g (+/- 5 mg) of flesh in a clean 50 mL polypropylene falcon test tube.**
2. **Cover the sample with 15 mL of 95:5 acetonitrile-water**
3. **Homogenize the sample using the Ultra Turrax until it becomes a smooth paste.**
4. **Add 250 uL of the internal standard (CAP-ISTD) and vortex the sample for 15 sec**
5. **Allow the mixture to stand at room temperature for 1 hour and then centrifuge the sample for 15 mins @ 7500 rpm.**
6. Carefully decant the supernatant into a clean 50 ml polypropylene test tube.
7. Extract the supernatant with 10 ml of acetonitrile-saturated hexane (if an emulsion forms, centrifuge the sample for 10 mins @ 2500 rpm)
8. Remove and discard the top layer.
9. Repeat Step 7.
10. Transfer the bottom layer to a 50 mL round bottom flask and concentrate the solution to approximately 1-2 mL.
11. Add 10 mL of water and swirl the flask so that the water covers the entire surface of the flask.
12. Transfer the aqueous solution to a 30 mL screw cap centrifuge tube and add 5 mL of hexane.
13. Vigorously shake the test tube for 30 seconds and then centrifuge the mixture to resolve the layers.
14. Carefully remove the top layer and adjust the pH of the solution to 4 (5-4 is acceptable) with 1M hydrochloric acid (about 3-5 drops).
15. Add 2 grams of sodium chloride and vortex the mixture until the salt has dissolved.
16. Add 5 mL of pesticide-grade ethyl acetate and extract and shake the mixture for 1 minute.
17. Allow the phases to separate, then using a Pasteur pipette transfer the ethyl acetate to a screw cap test tube containing 1 gram of sodium sulfate
18. Repeat step 17.
19. Vortex the test tube containing the sodium sulfate for 30 sec, then centrifuge the sample for 15 mins @ 3500 rpm.
20. Decant the supernatant into a 15 mL test tube.
21. Place the test tube in a Reacti Therm-III™ hot block that has a stable temperature of 50 C (45-55 is acceptable)
22. Cautiously turn the nitrogen flow so that the meter reads 25 units (20-30 is acceptable)
23. Evaporate the solvent to dryness (takes about 20 minutes)

Benefits of TurboFlow Technology

- Simplify sample preparation by eliminating SPE/PPT/LLE
- Direct injection of fluid samples
- Reduces sample matrix interference and ion suppression
- Concentrates trace amounts from large injections
- Increase signal/noise ratio
- Automation—cost savings in time and labor

Matrix Interferences

- TurboFlow methods with 2 mechanisms of chromatography offers solutions for matrix interferences:

- TurboFlow methods significantly reduces matrix effects due to phospholipids, salts, sugars …

- Analytical column further separates analytes from interferences.

- Specific hardware configuration promotes reduction of residual matrix components on the system and results in a stable signal over time.

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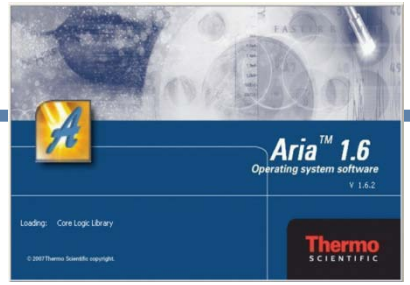
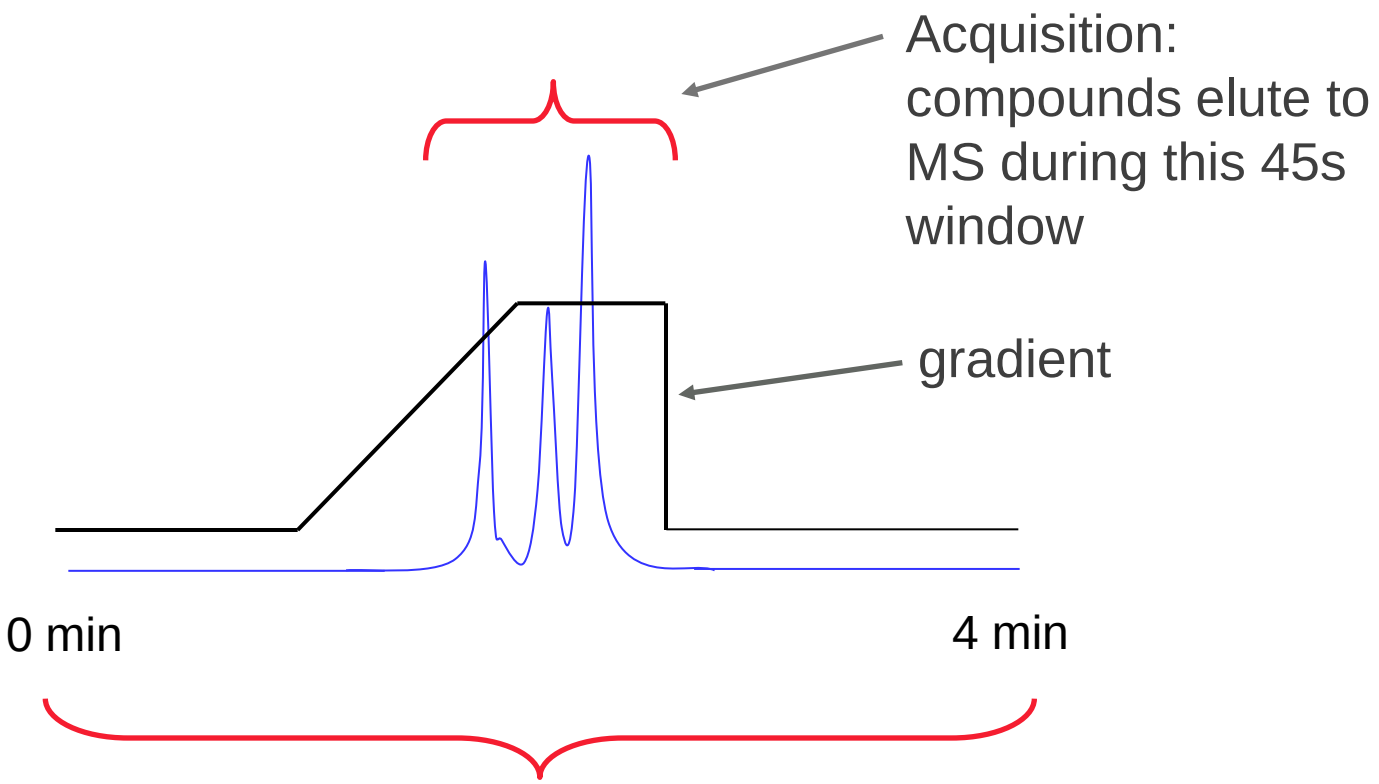
What is Multiplexing ?



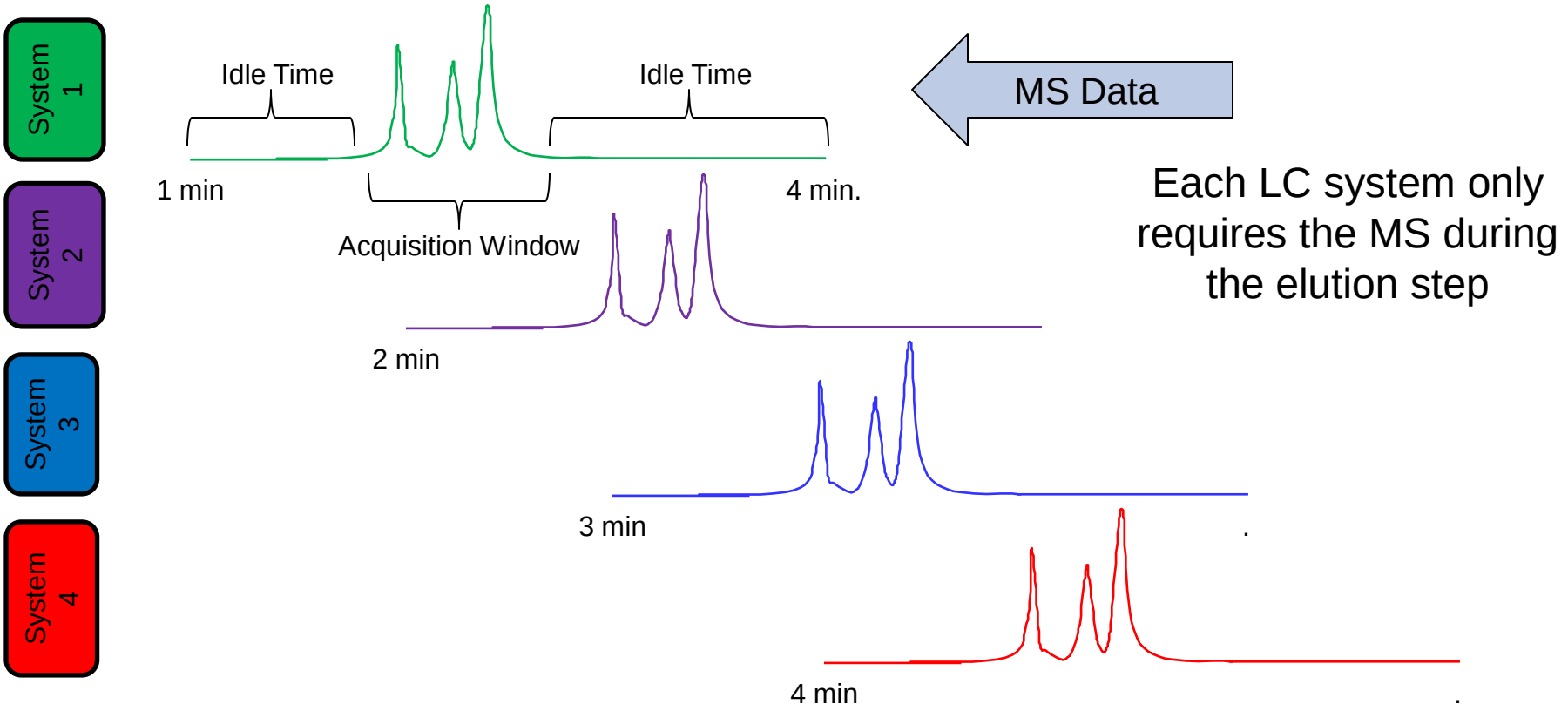
- Staggered parallel chromatography
- Multiplexing combines 2 or 4 LC units in front of a single MS.
- LC systems are independent of each other.
- LC systems functions and timing are controlled by Aria™ software.

Method and Gradient – LC-MS



Multiplexing increases throughput

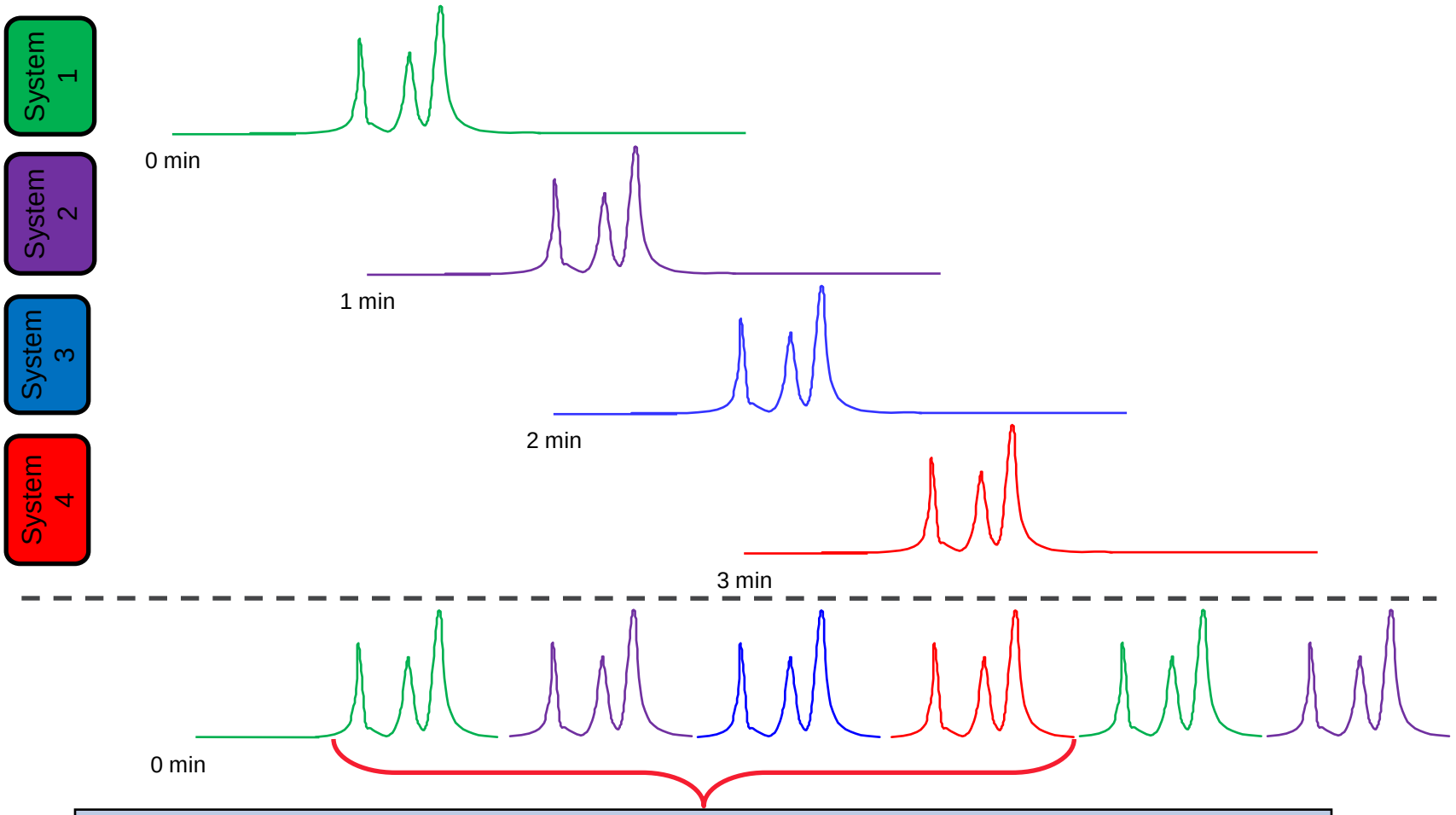
- Controlled by Aria software



Each LC system only requires the MS during the elution step

System diagram above is representative of a Thermo Scientific Transcend™ LX-4 system

Throughput – Typical LC/MS with Multiplexing

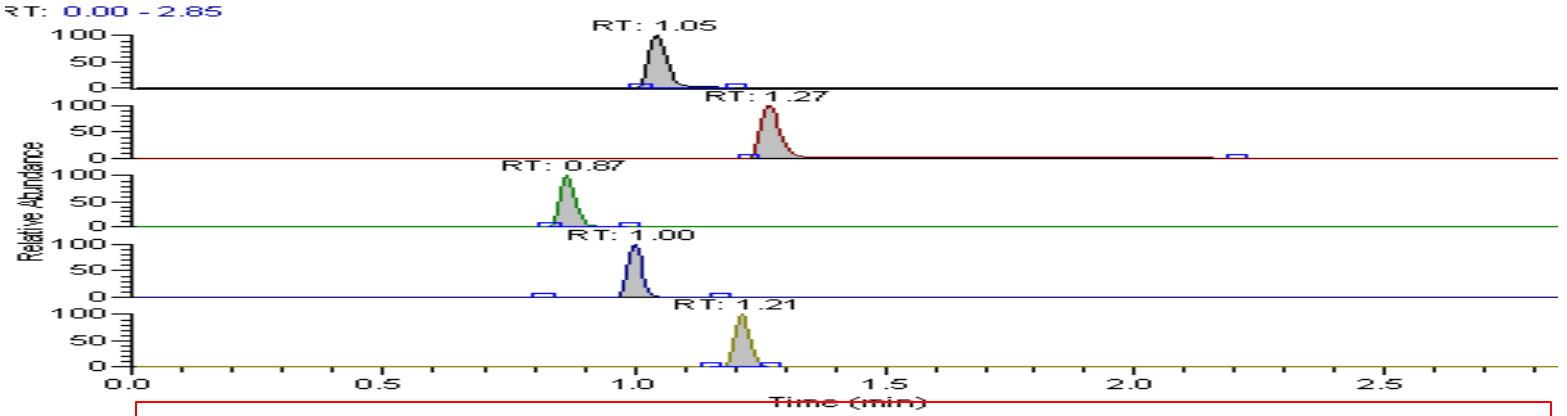


During this 4 min window – 4 sample data were completed

Equals 60 samples/hour. A 96 well plate complete in 1.6 hrs

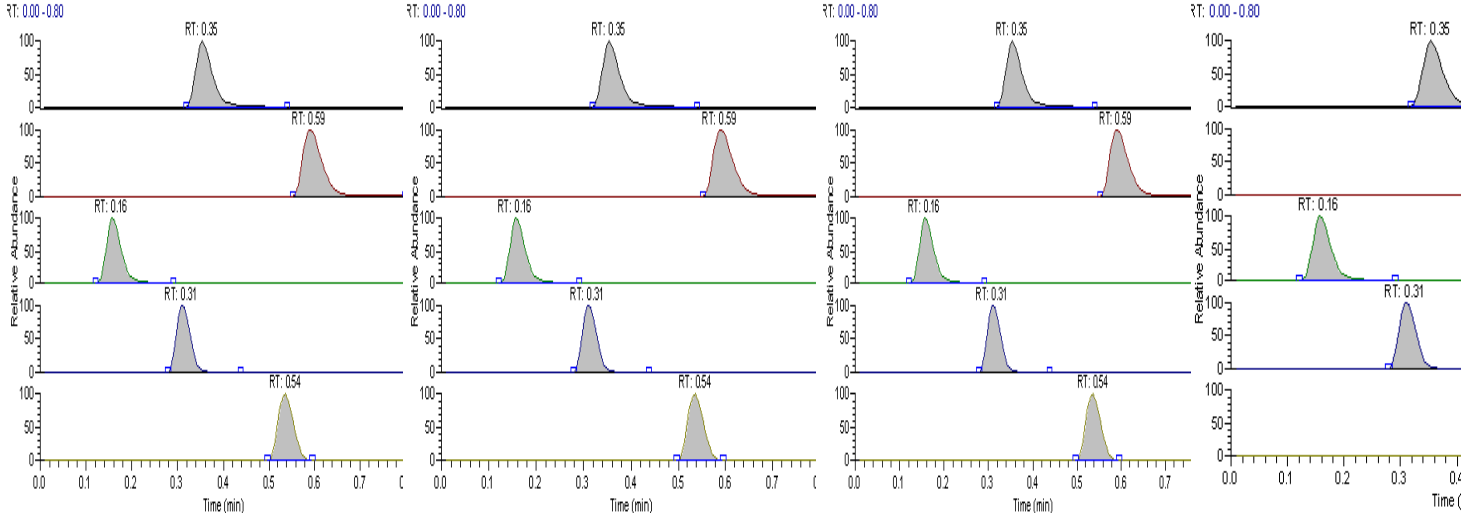
Data on LX-4 system – compare method data window

Single LC run vs. Transcend LX-4 each 48 sec.



1 LC

3.75 runs in the time it takes to collect 1 run



LX-4

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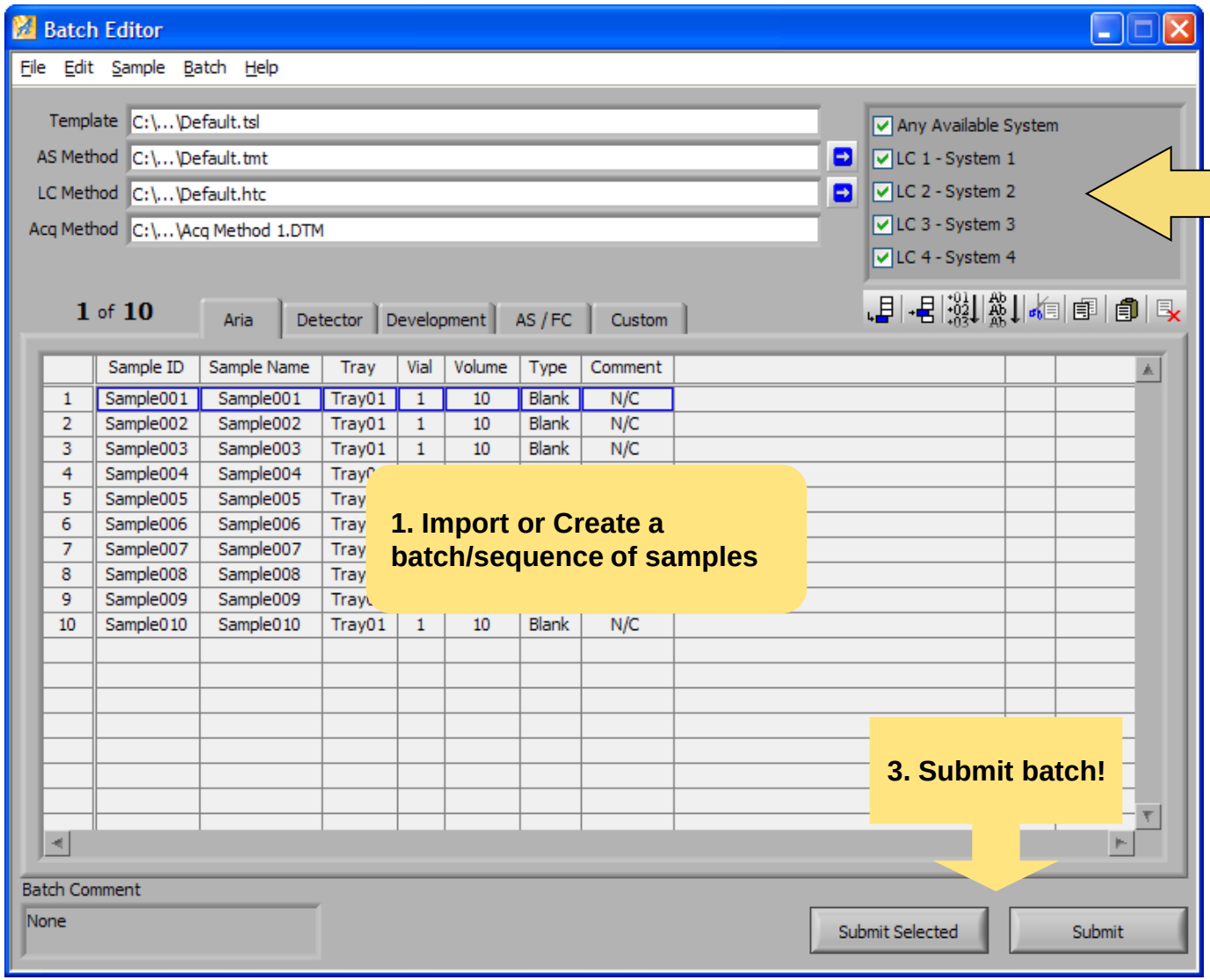
Multiplexing with Aria Software

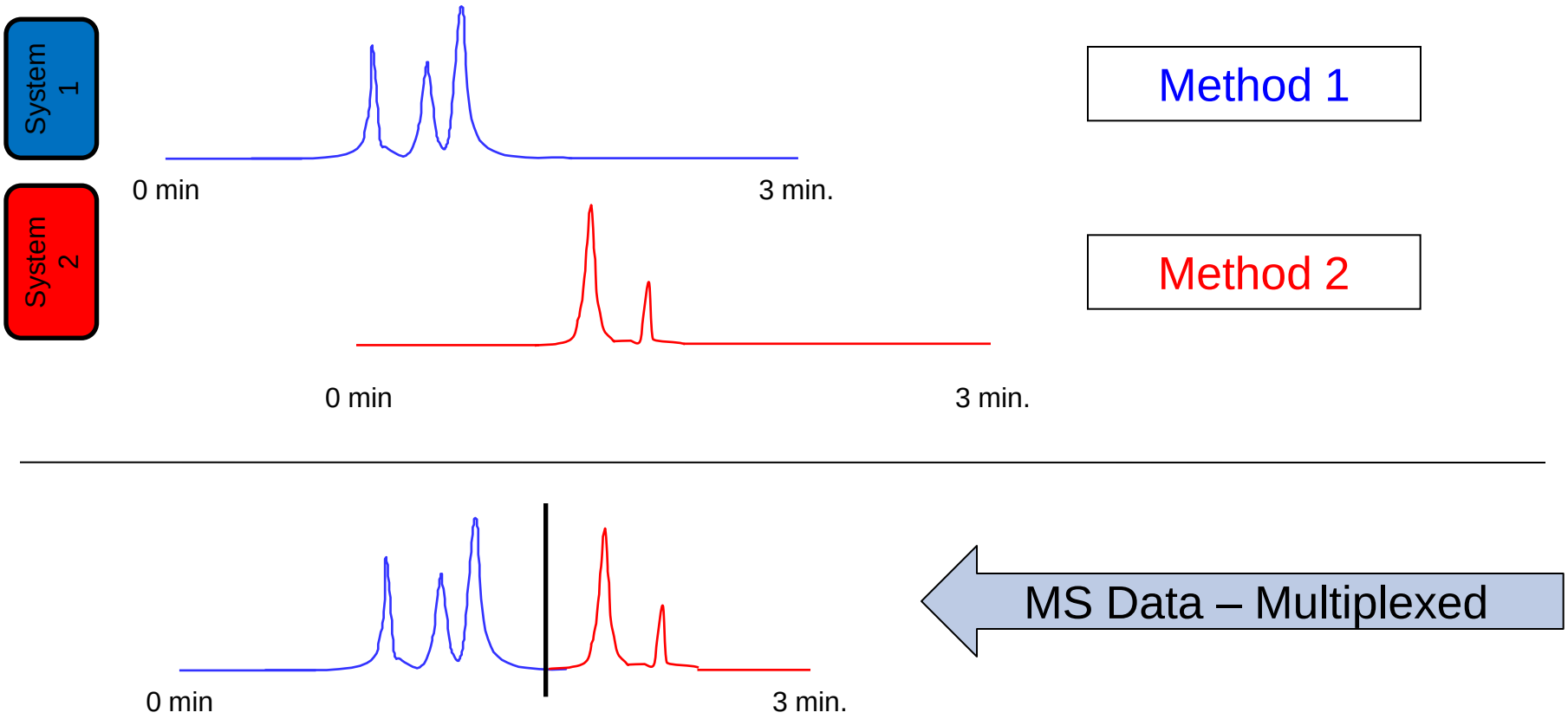


2. Select which LC channel(s) to run the samples or select "Any Available"

More batches can be submitted at any time in any order and the system will automatically Multiplex where possible

Cross sequential Multiplexing unique to Aria OS

Two methods to one MS - Same mobile phase, different samples



System diagram above is representative of a Thermo Scientific Transcend™ LX-2 system

Benefits of Multiplexing

- Accelerate results
- Getting answers/data faster

Throughput

- Reduce cost of capital equipment
- Reduce cost per sample

Less Cost

- Handles multiple methods
- Many options available

Flexibility

- Intuitive, quick learning curve

Ease of Use

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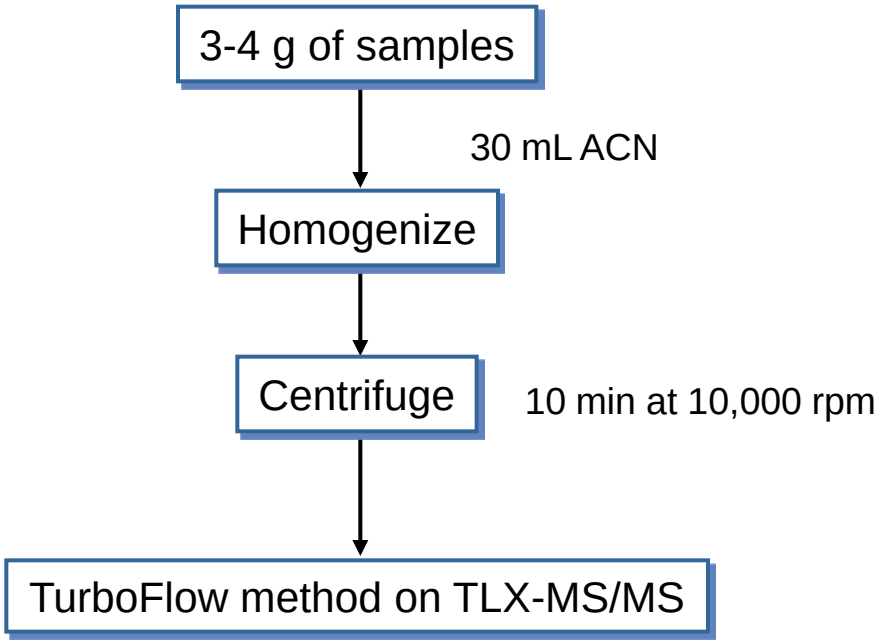
Examples

- Multi-Residue methods for veterinary drugs
- Contamination – clenbuterol—lean meat powder
- Pesticides screening
- Clinical research vitamin D
- Pain management
- Pharmaceutical high throughput screening

Veterinary Drugs

Multi-Residue Methods for Veterinary Drugs

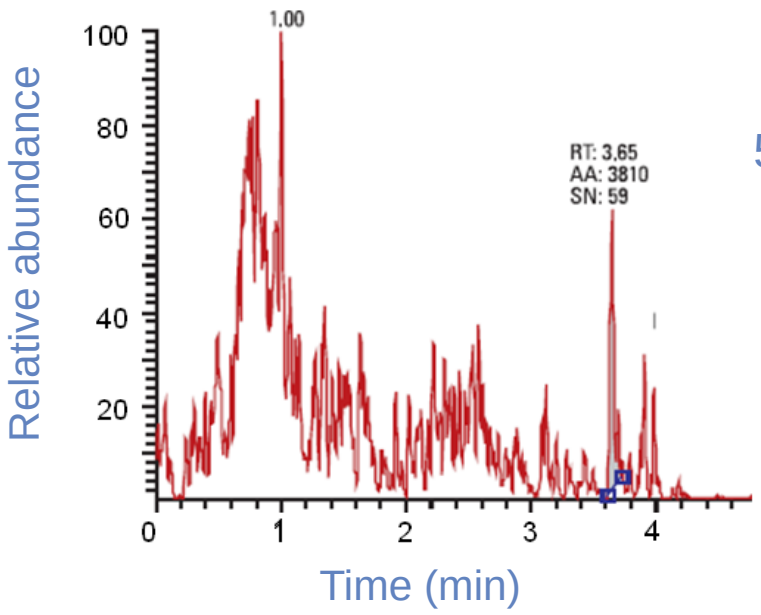
For: Malachite green, leucomalachite green, ciprofloxacin and tetracycline
In: Shrimp, tilapia and pig liver
LOD: 0.05-0.1 µg/kg



Thermo Fisher Scientific App Note 442

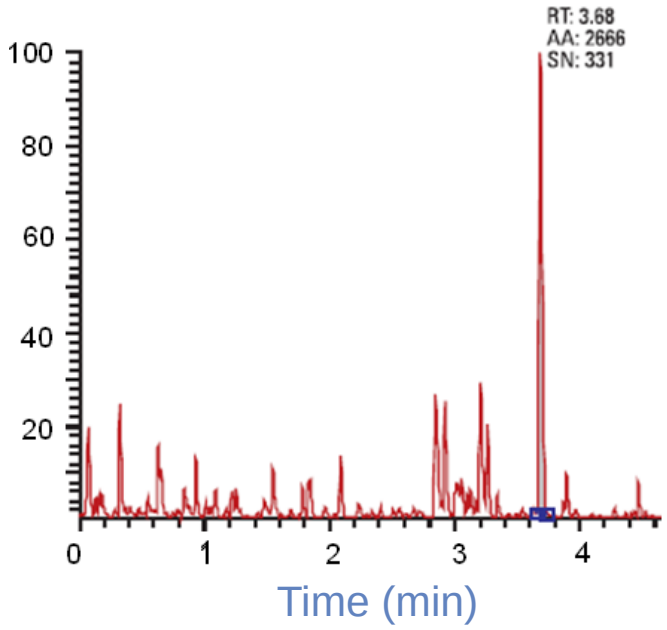
Comparison of methods for Leucomalachite Green in Shrimp

Standard HPLC without sample cleanup

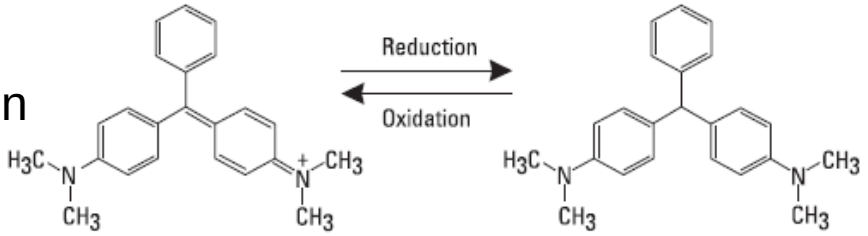


50 ng/kg

TurboFlow method with online sample cleanup



Malachite Green



Leucomalachite Green

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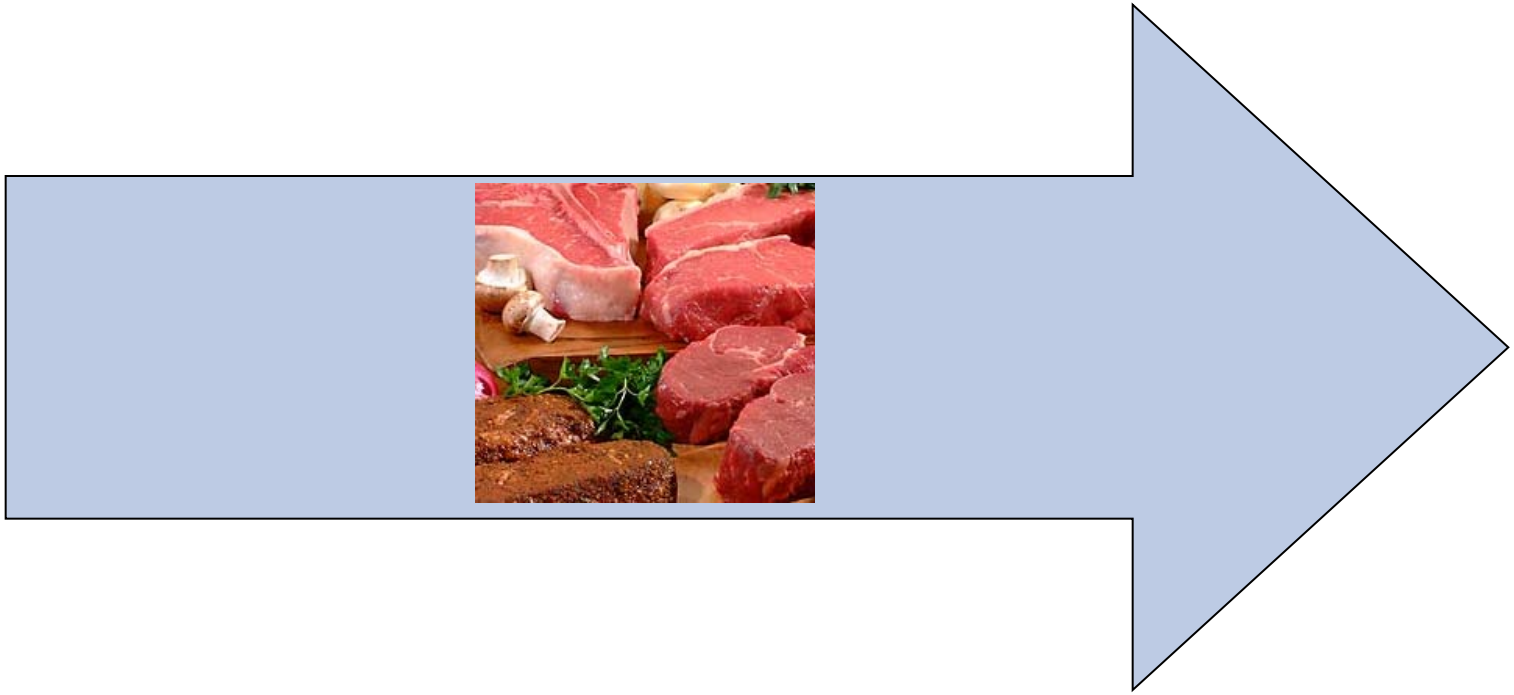
Clenbuterol – Lean Meat Powder



Clenbuterol –Lean Meat Powder

Pigs fed on bodybuilder steroids cause food poisoning in Shanghai

Sep 19 01:53 AM US/Eastern

Comments (0) Email to a friend Share on Facebook Tweet this SHARE

Over 330 Shanghai residents have been poisoned by pork tainted with a weight-loss steroid popular among bodybuilders.

Clenbuterol, a product normally marketed to fitness fanatics, was used by farmers in east China to produce leaner pork meat, the China Daily reported.

As a result, a total of 336 people have been poisoned in Shanghai since September 13, in the city's largest clenbuterol poisoning case, the paper said.

The chemical can reportedly cause damage to the human nervous and cardiovascular system. However, all those affected in Shanghai have been released from hospital, the paper said.

Most of the pigs have been traced to neighboring Zhejiang where rearing pigs on clenbuterol, known locally as "lean meat powder", is widespread, the paper said.

The government banned the use of the chemical in the 1990s, it added.

The incident has exposed many loopholes in China's food safety inspection system, the paper said.

"Many people are still using 'lean meat powder'," the local Oriental Morning Post quoted a Zhejiang farmer as saying.

"I've raised pigs for 10 years and almost all of them have been fed it."

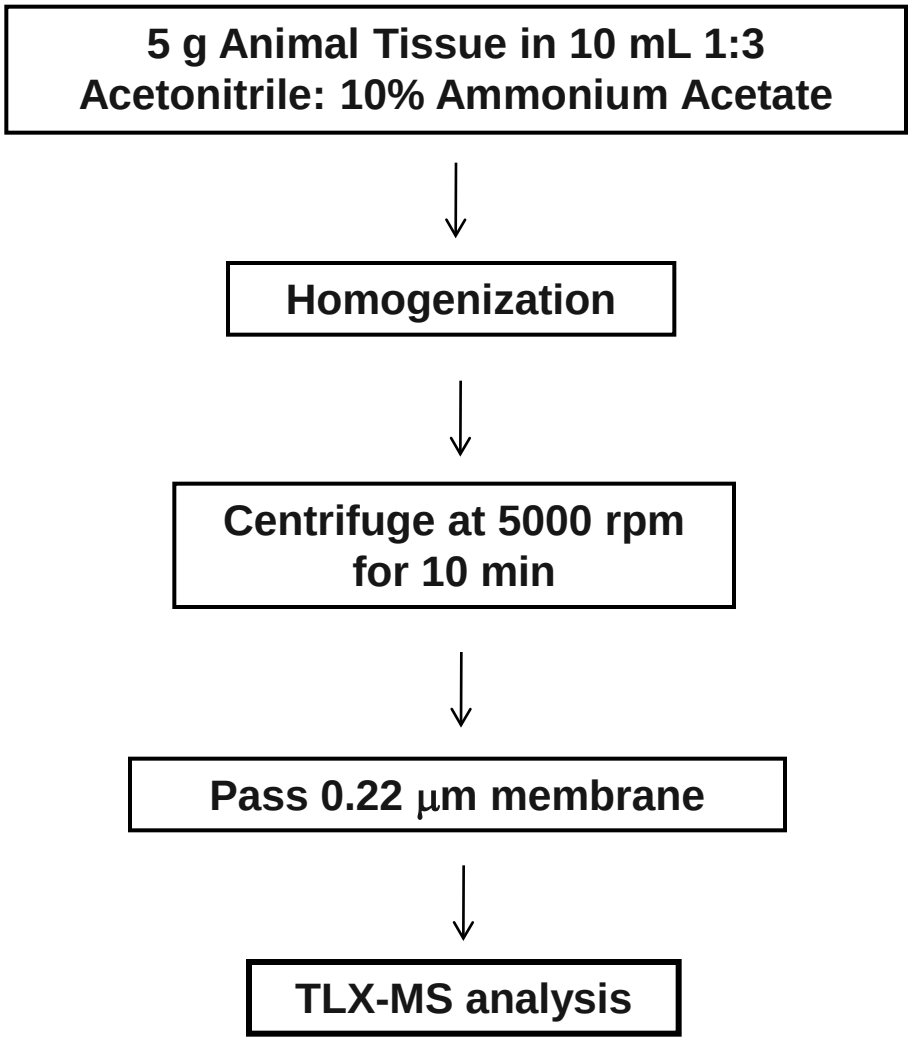
According to the report, many farmers stop feeding the pigs with the drug several weeks before they are slaughtered, making detection difficult for food inspectors.

Shanghai officials downplayed the scare, saying it is unlikely that more contaminated pigs are in city markets, the China Daily said.

Clenbuterol is a medication that has been used to treat bronchial diseases such as asthma, but is largely used by bodybuilders or as a weight-loss supplement for the overweight.

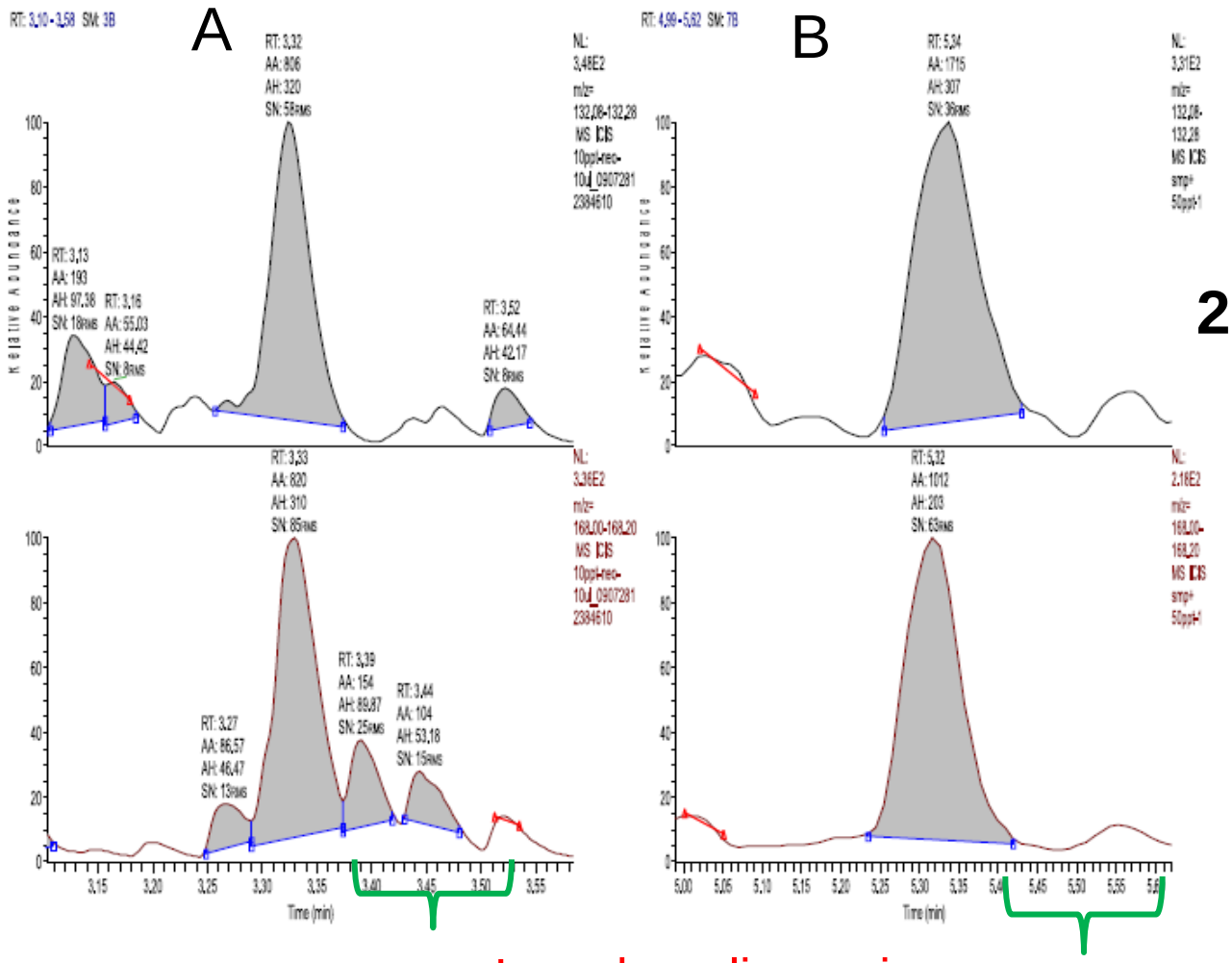
- 336 people have been poisoned
- Many farmers are still using it

Sample Preparation



Total sample prep time: 5 min

MCX SPE (A) vs. TurboFlow method (B)



20 pg/g

Less baseline noise

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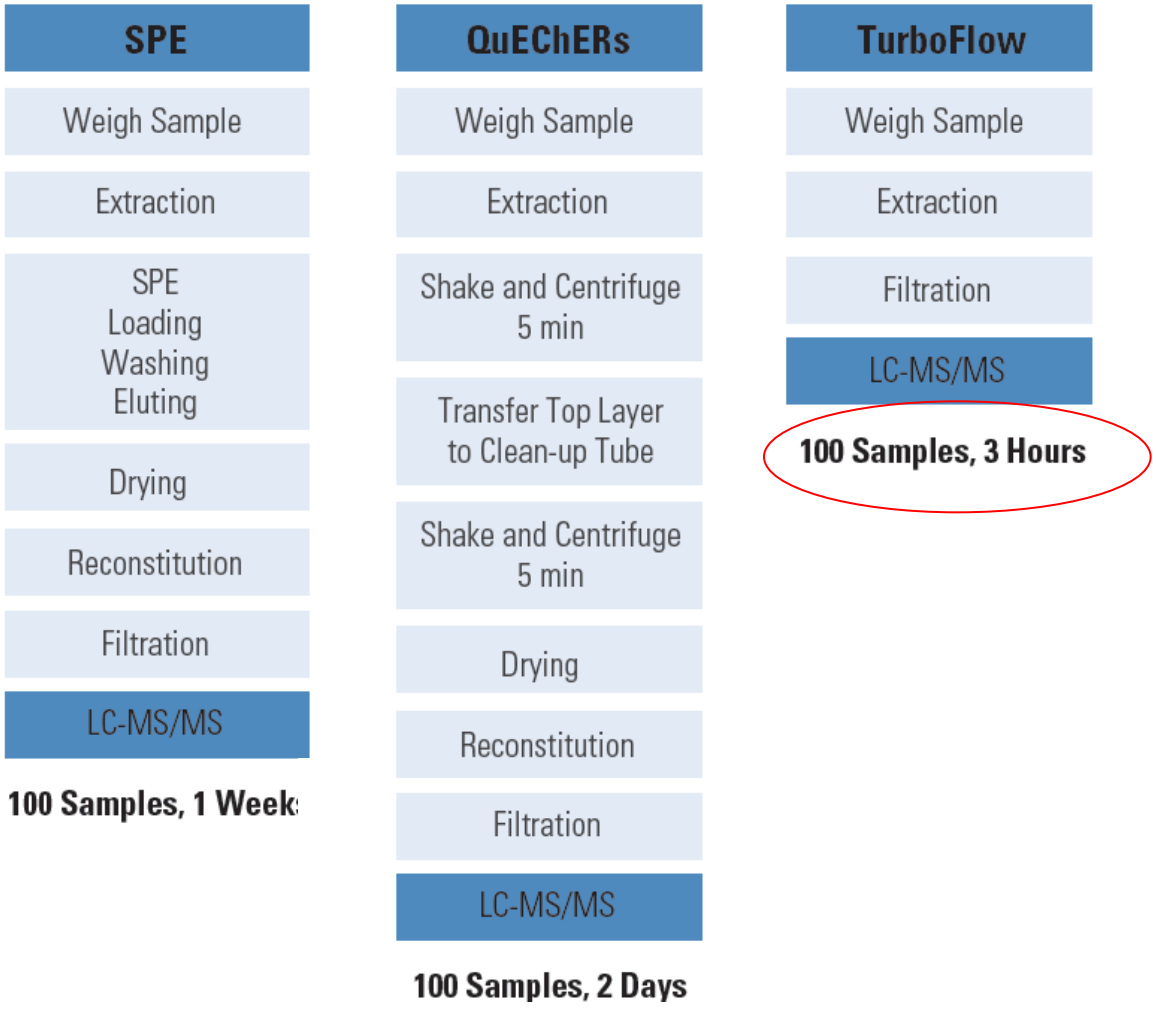
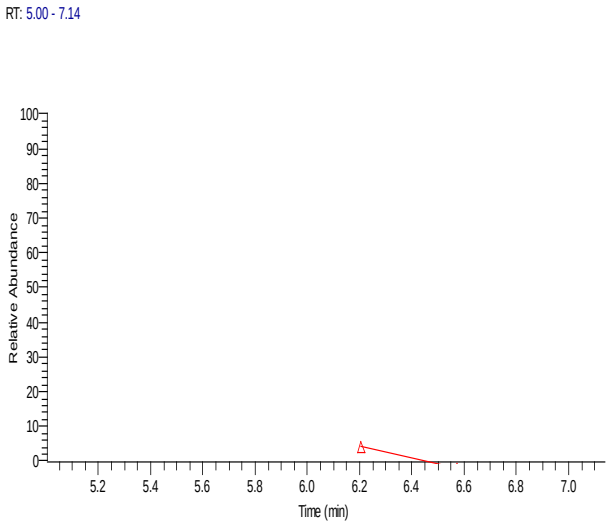
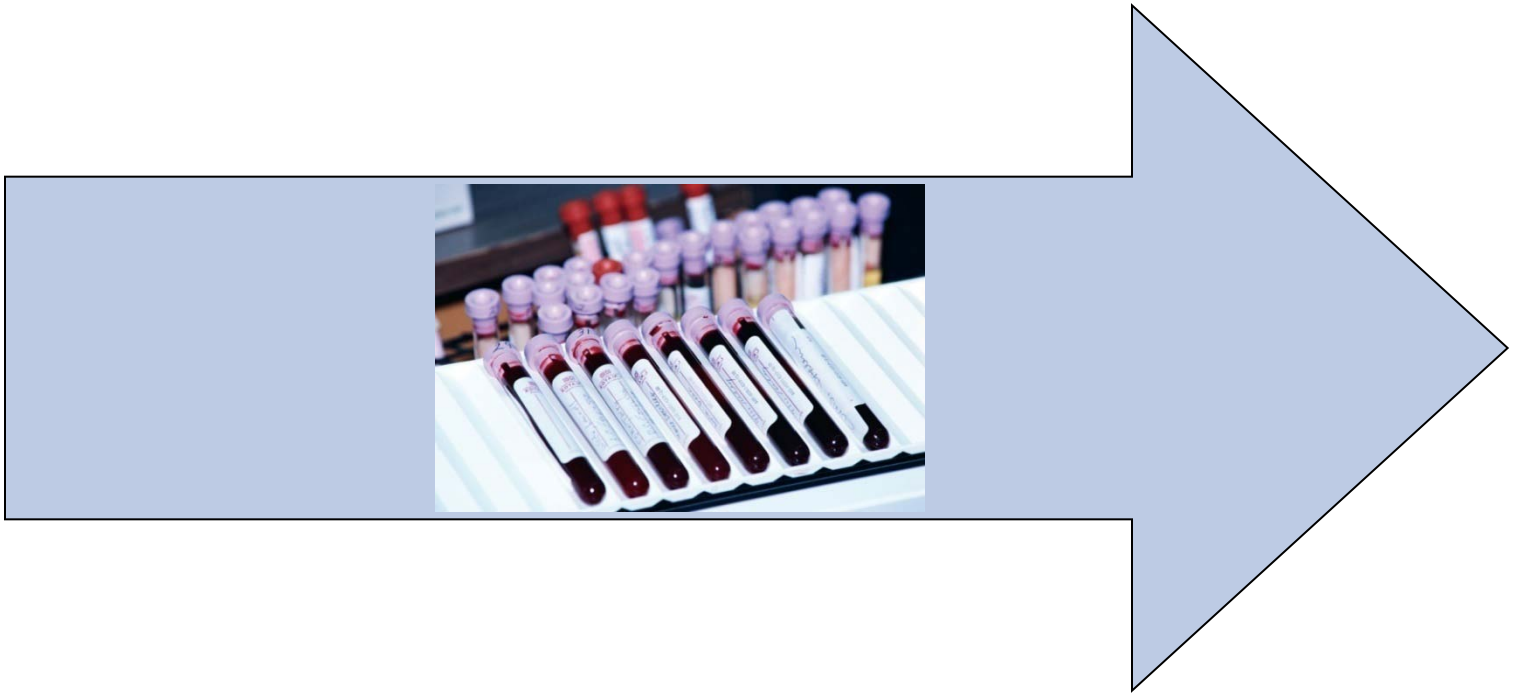
Pesticides Screening in Green Tea Extract

Common Pesticides Sample Prep Methods



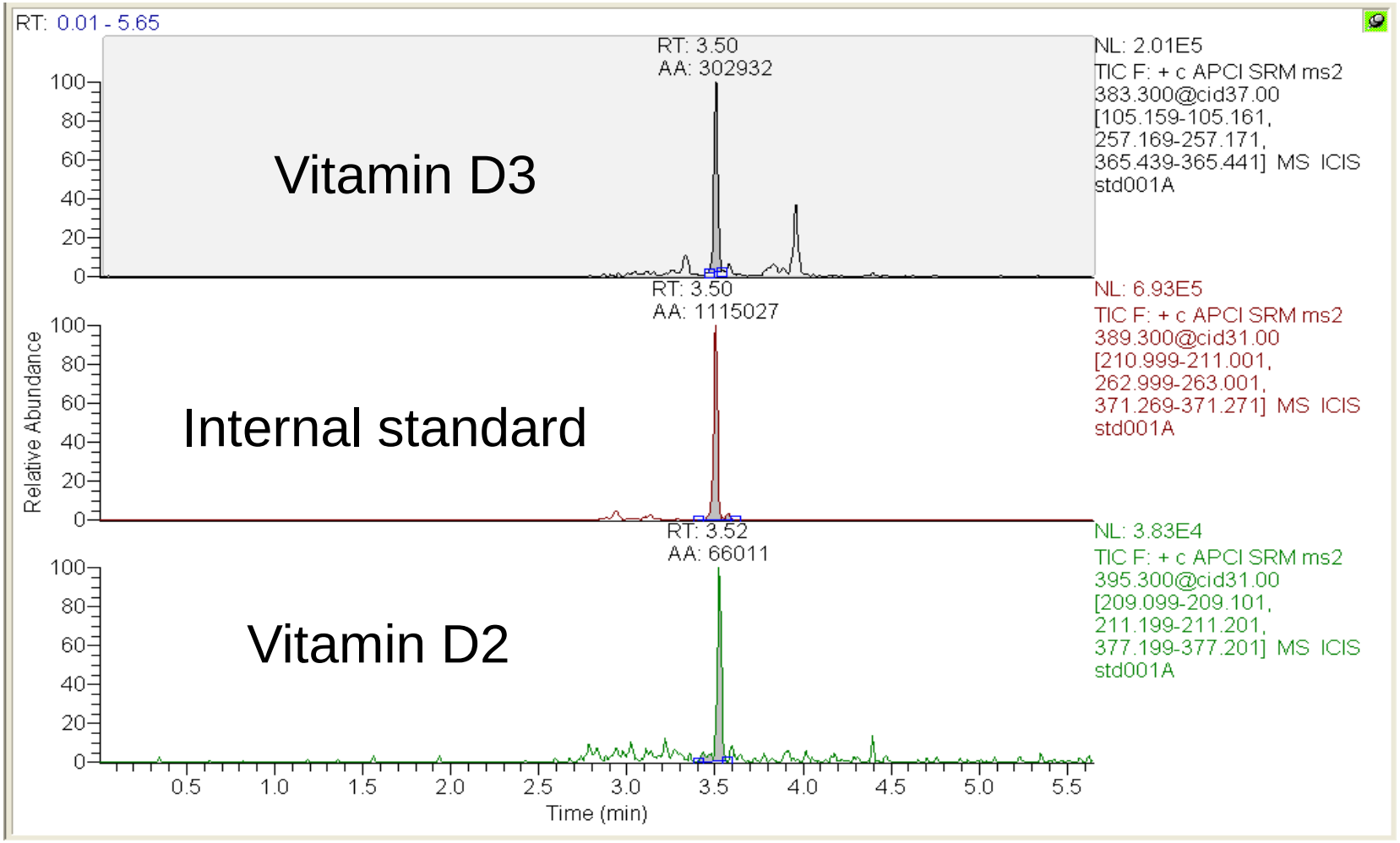


Clinical Research Vitamin D2 and D3 metabolites

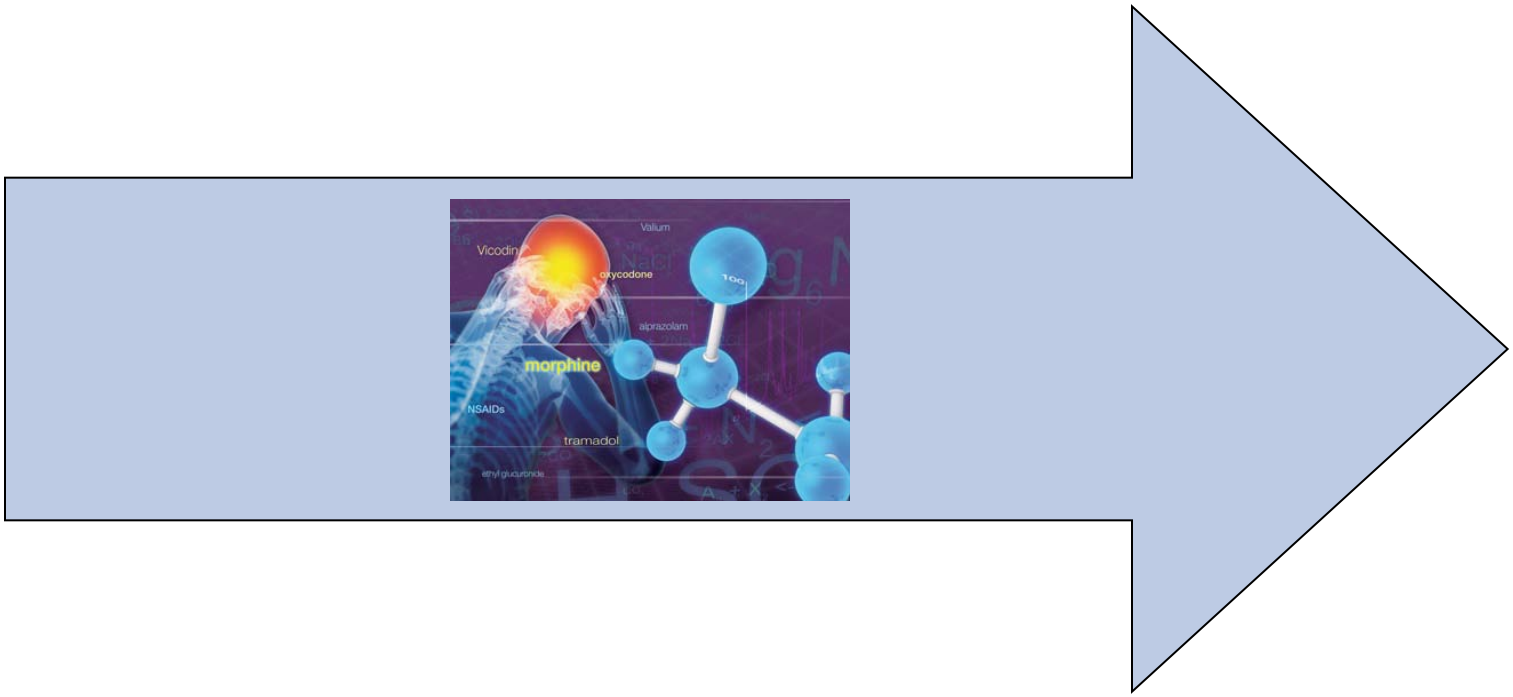


Vitamin D2 and D3 metabolites

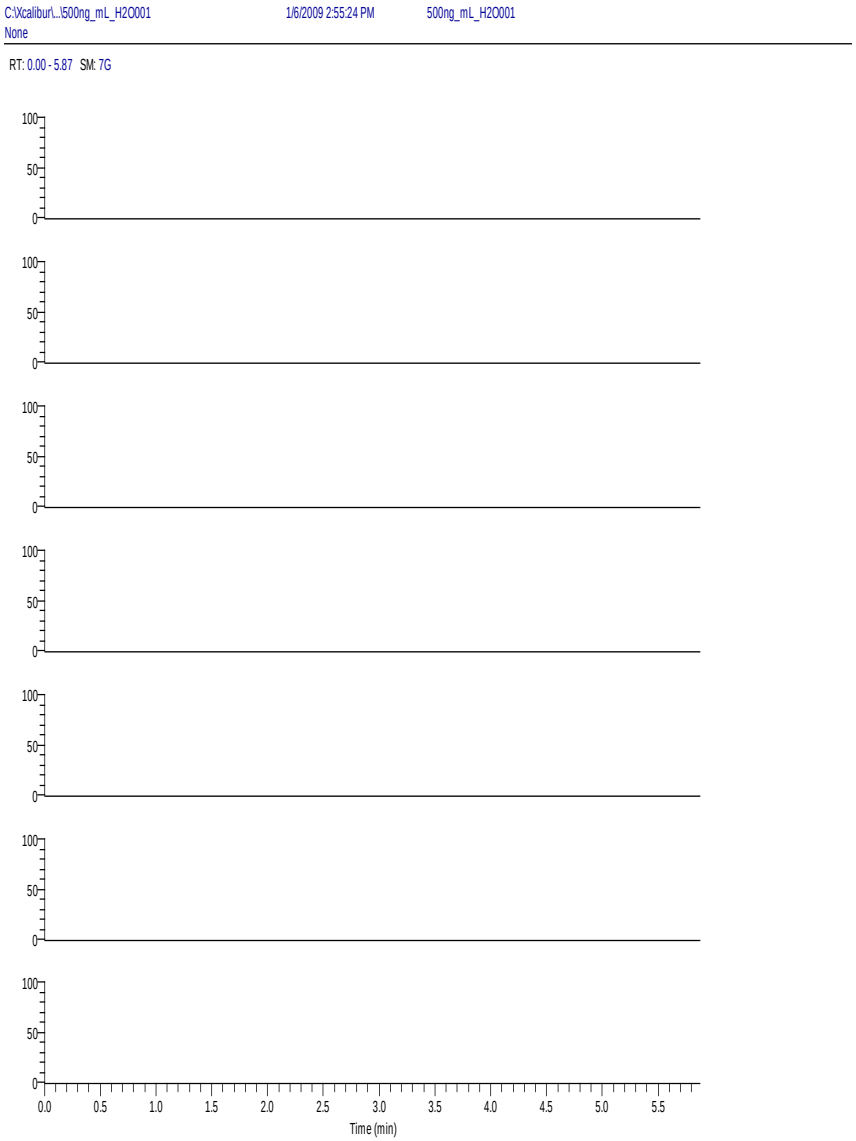
4ng standard in Plasma



Drugs of Abuse / Pain Management



Benzodiazepines LC run vs. Data Window
6.0 min. vs. 2.0 min.



Simplify Sample Preparation Protocol for 100 samples

Manual Solvent Extraction

- Punch 3/16” Disc
- Add IS Solution
- Mix
- Extract with Ether
- Remove Supernatant
- Repeat Extraction
- Combine Extracts
- Evaporate Ether
- Reconstitute in Mobile Phase
- Transfer
- Inject
- Inject onto HPLC. Sample Injection Every 12 Minutes
- 100 Samples @ 12 minutes = 1200
  390 min + 1200 min = 26.5 hours

Sample Prep Time = 390 minutes

Analysis time = 26.5 Hours

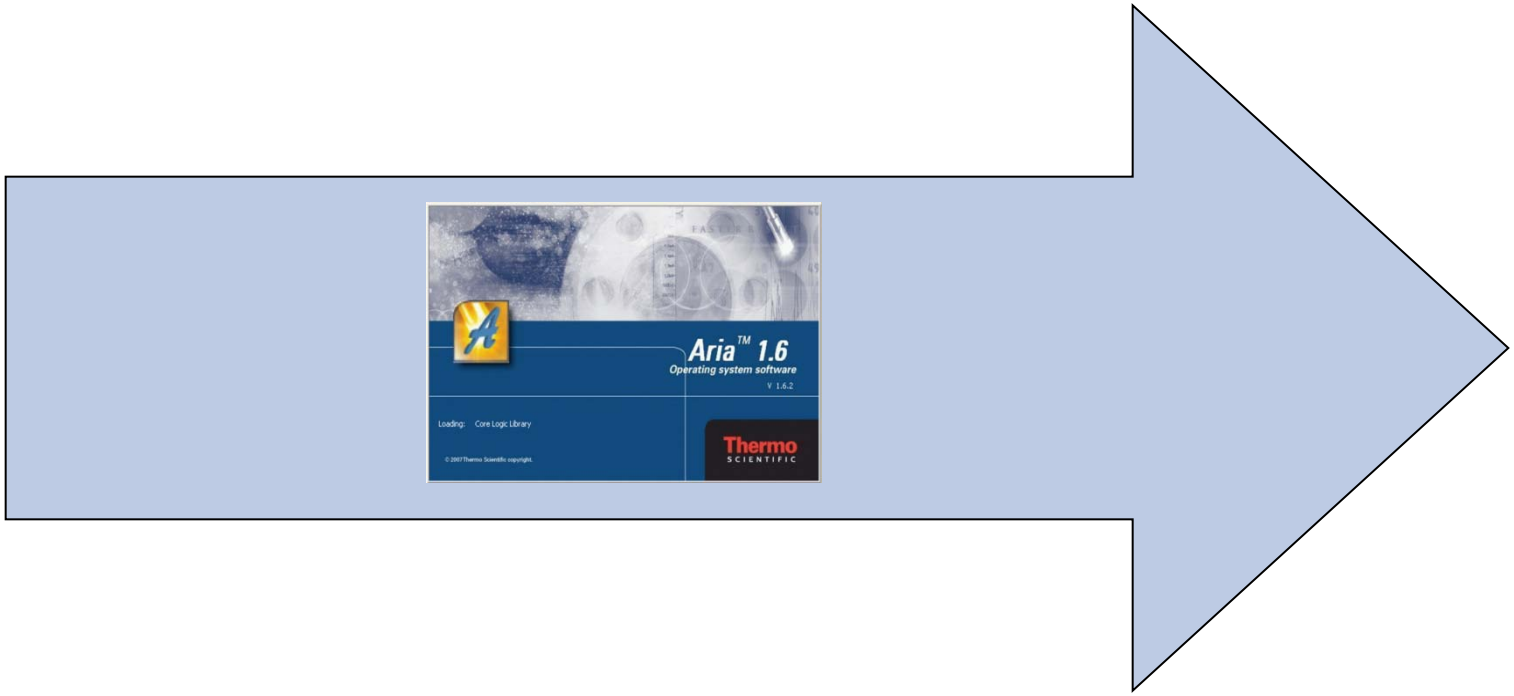
TLX with TurboFlow technology

- Punch 3/16” Disc
- Add IS / Extraction Solvent
- Mix
- Transfer
- Inject
- Inject onto Aria TLX-4 System. Sample Injection Every 2 Minutes
- 100 Samples @ 2 minutes = 200 Min
  60 min + 200 min = 4.3 hours

Sample Prep Time = 60 minutes

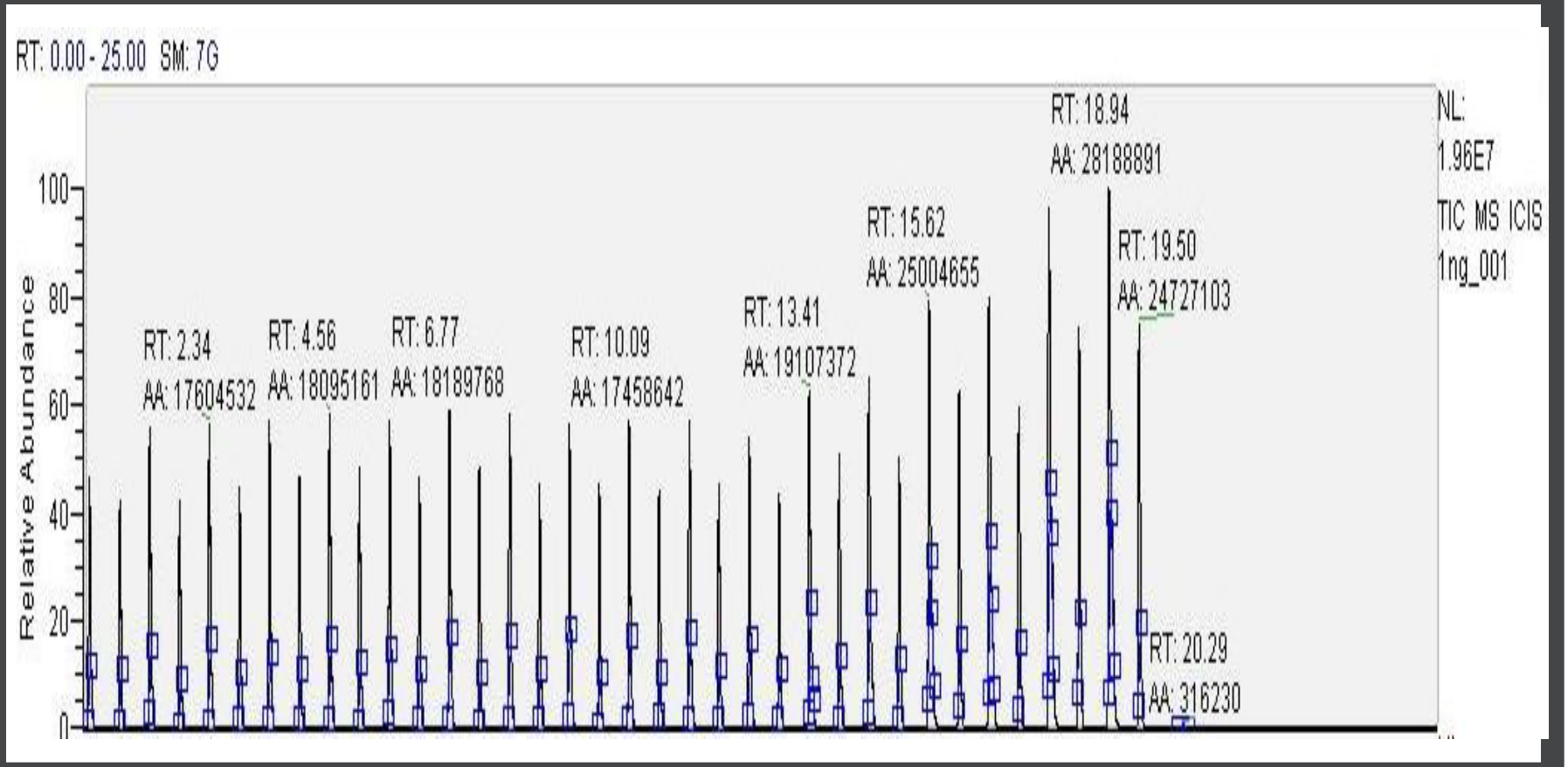
Analysis time = 4.3 Hours

Pharmaceutical Screening



Ultra High Throughput Assay - 4 injections per minute

Entire plate collected into a single data file, allowing higher sample throughput.



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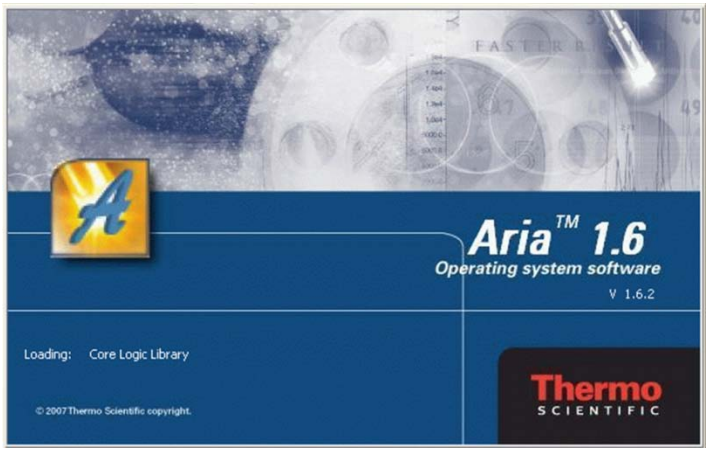
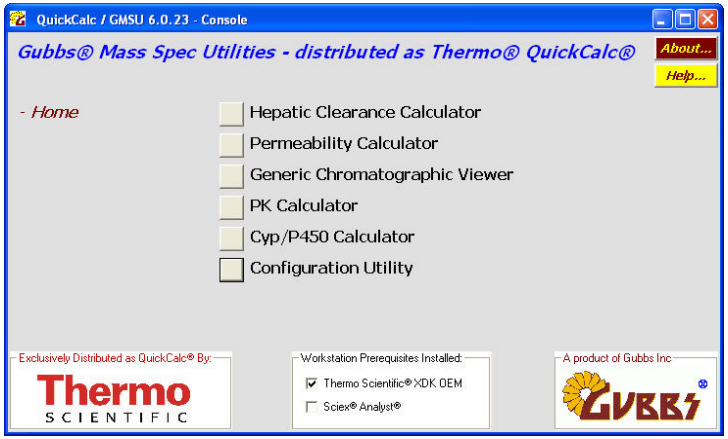
Aria + QuickCalc software = HTS capability





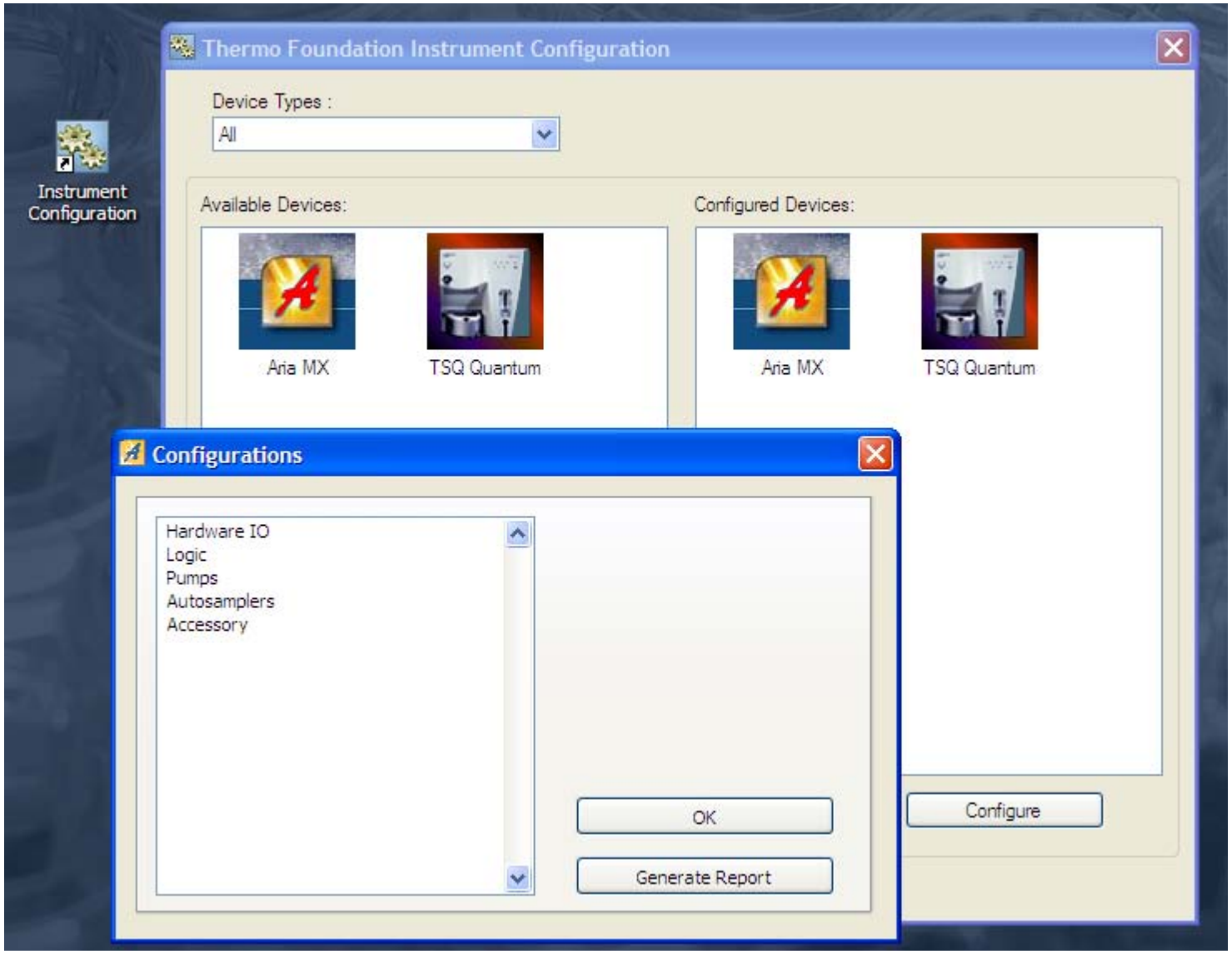
- 2 or 4 LC systems into single file
- Online sample extraction
- Multiplexing: intelligent injection timing without user intervention
- Automatic intelligence

- Quantitation of individual samples
- Creation of processing method
- Uses Aria sample information
- Prints or save chromatograms
- Easy report creation

Solutions for sample preparation in LC-MS/MS

- TurboFlow™ Technology
  - On line automation
  - Minimizes sample prep
  - Simplifies sample prep protocols
  - Saves time
- Multiplexing Technology
  - Accelerates results
  - Improves efficiency
  - Increases flexibility
- Automation / Workflow
  - Food
  - Biological Fluids
  - High throughput screening
- Integrated Software
  - Aria MX

Aria MX in Xcalibur, LCquan, TraceFinder…



Instrument Configuration

Add Aria MX and TSQ MS

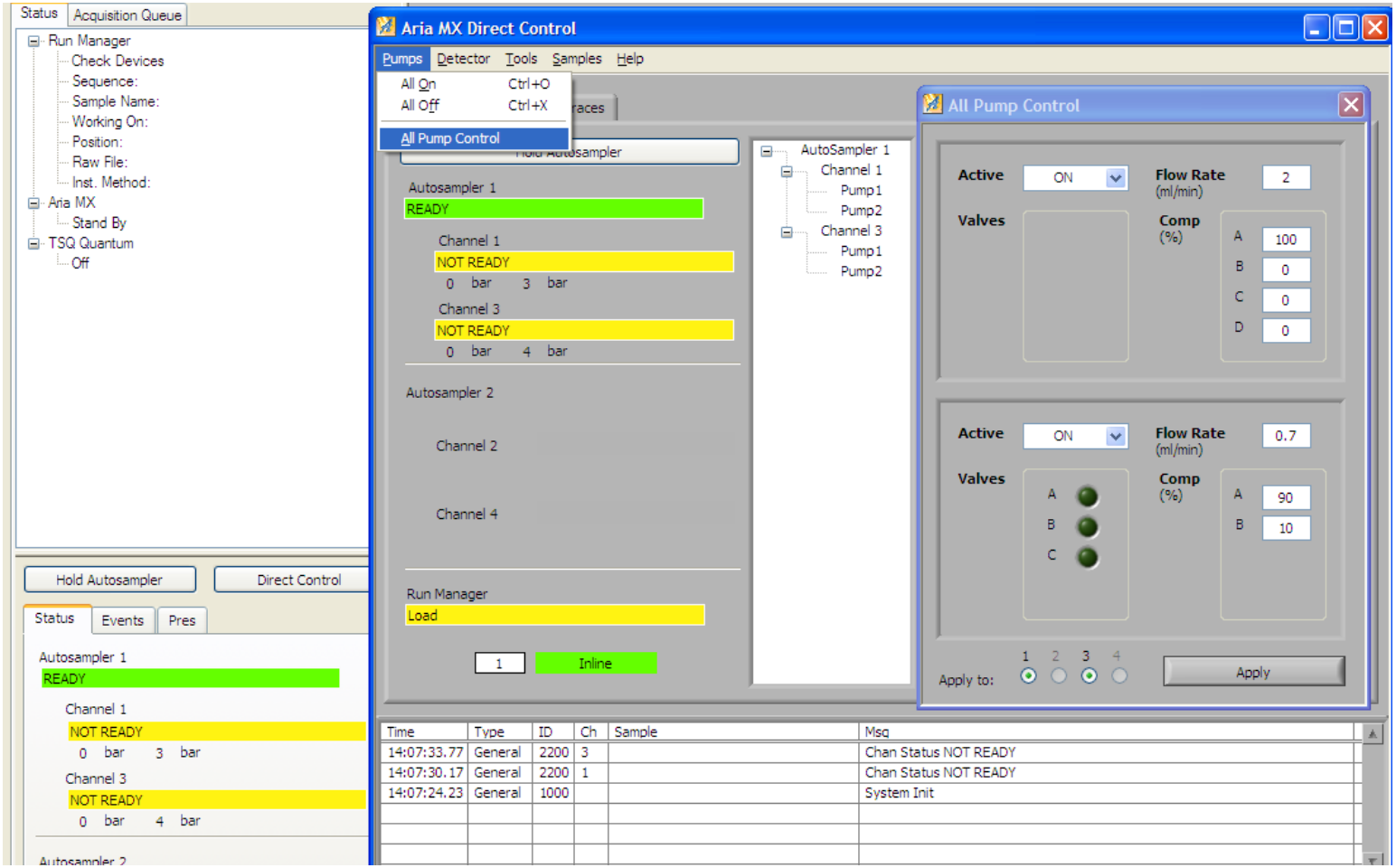
Configure Aria MX modules

Accessories

MCM

Temperature control

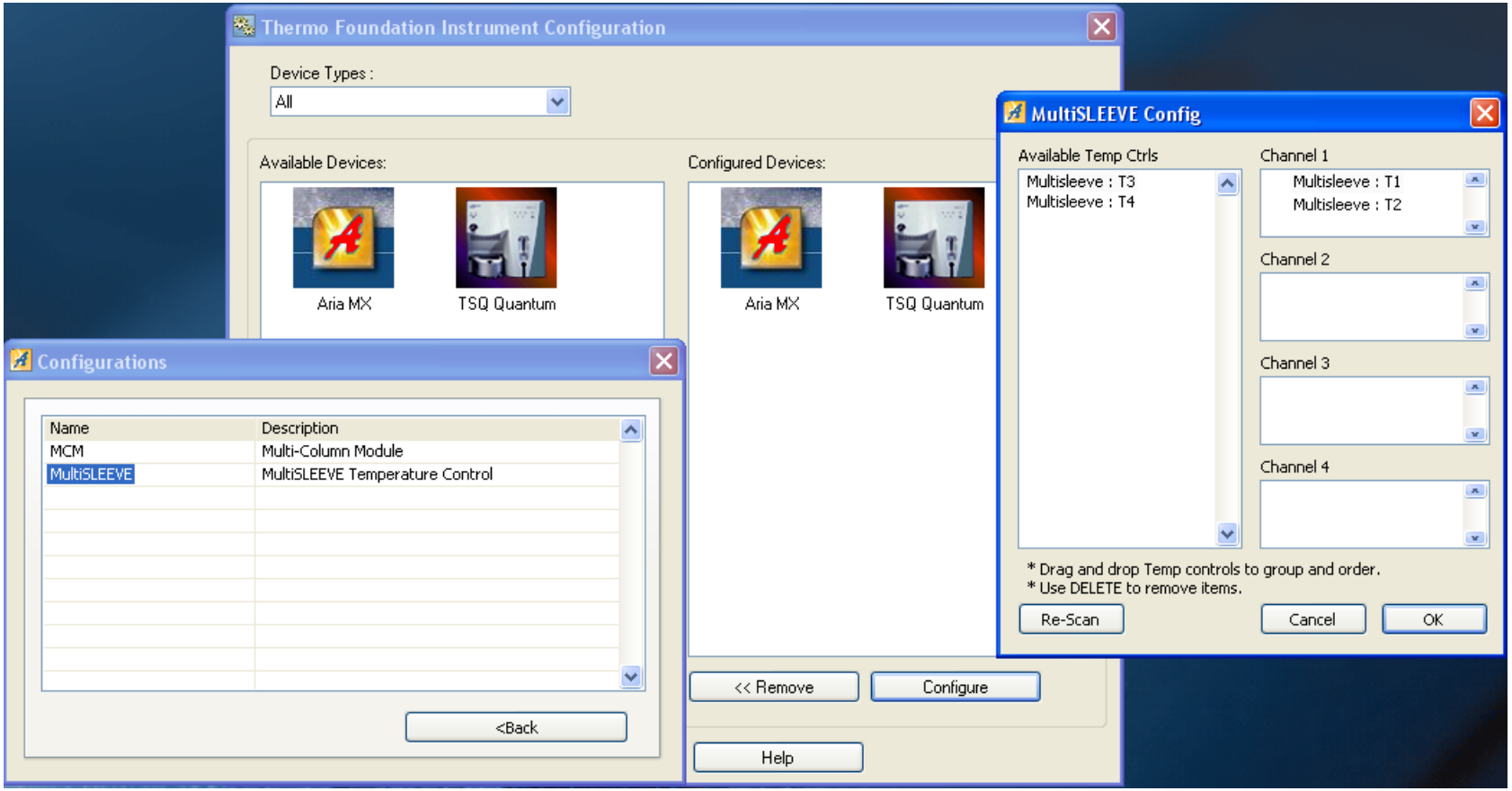
Aria MX with Xcalibur & LCquan



Status Tab:

Right-click on Aria MX will show Aria status at bottom. Click on Direct Control button and select All Pump Control to start up available pumps.

Aria MX – Control Column Temperature

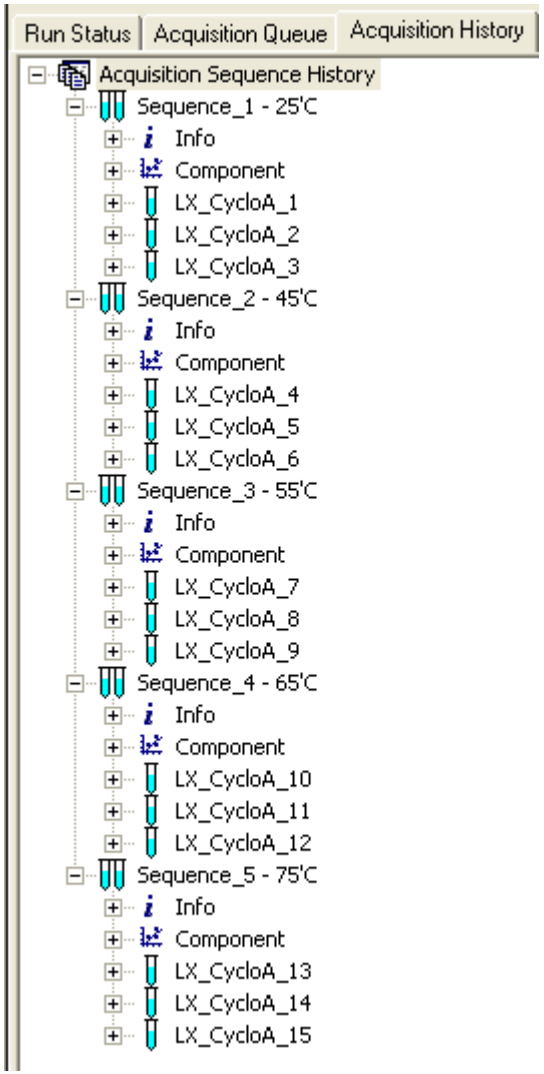


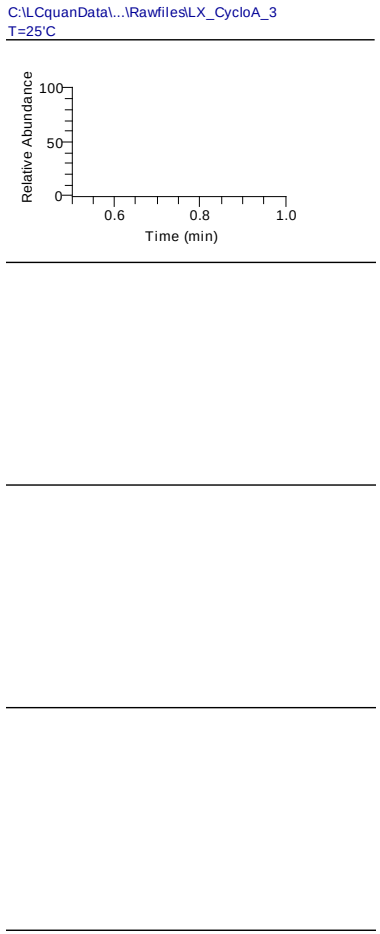
MultiSLEEVE controller utilizing 2 ports:

T1: AgileSLEEVE 25cm x 1/16” i.d. inlet tube heater

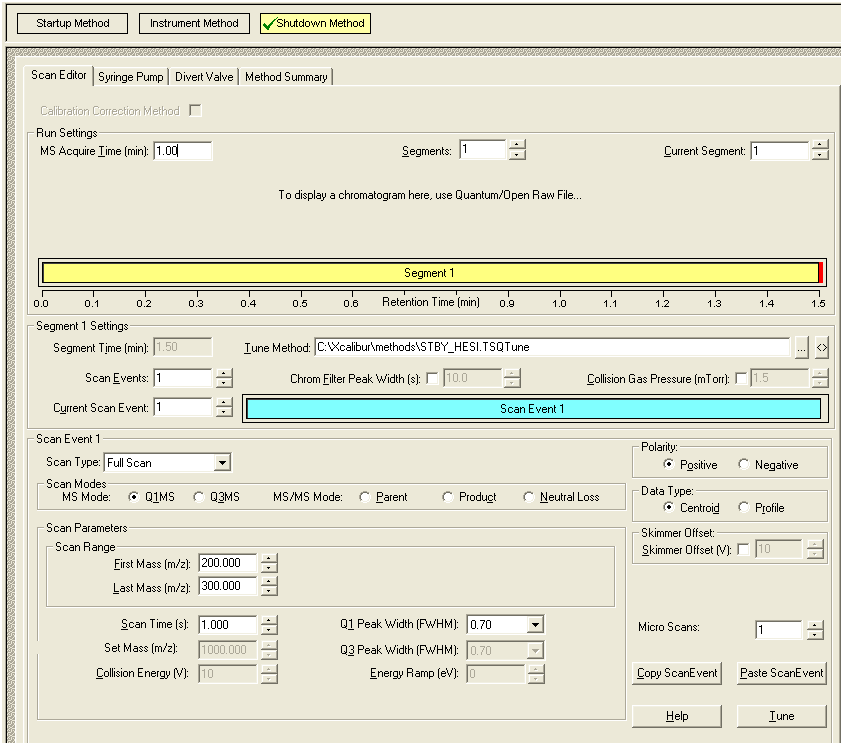
T2: IntelliSLEEVE 5 cm column heater

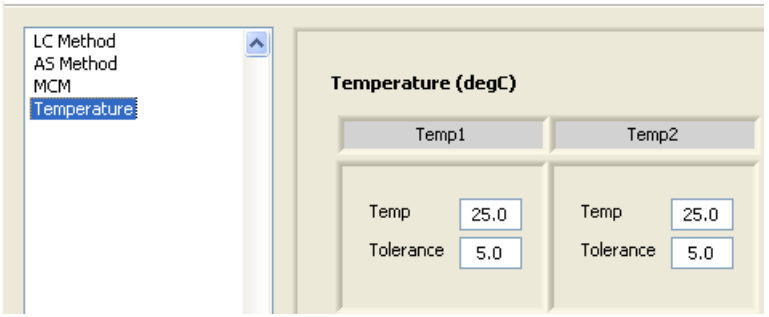
Optimizing Temperature for Cyclosporines

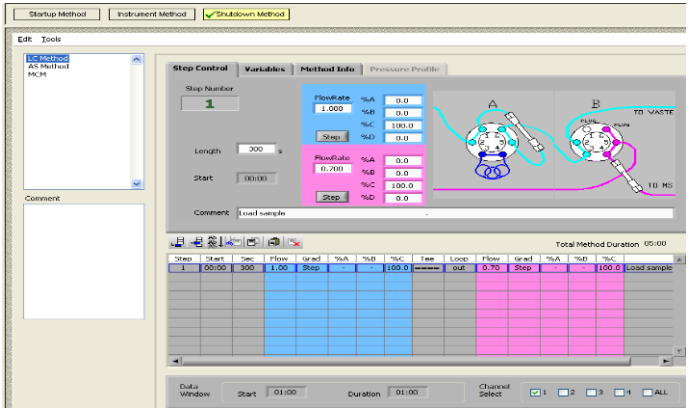




Shutdown Methods





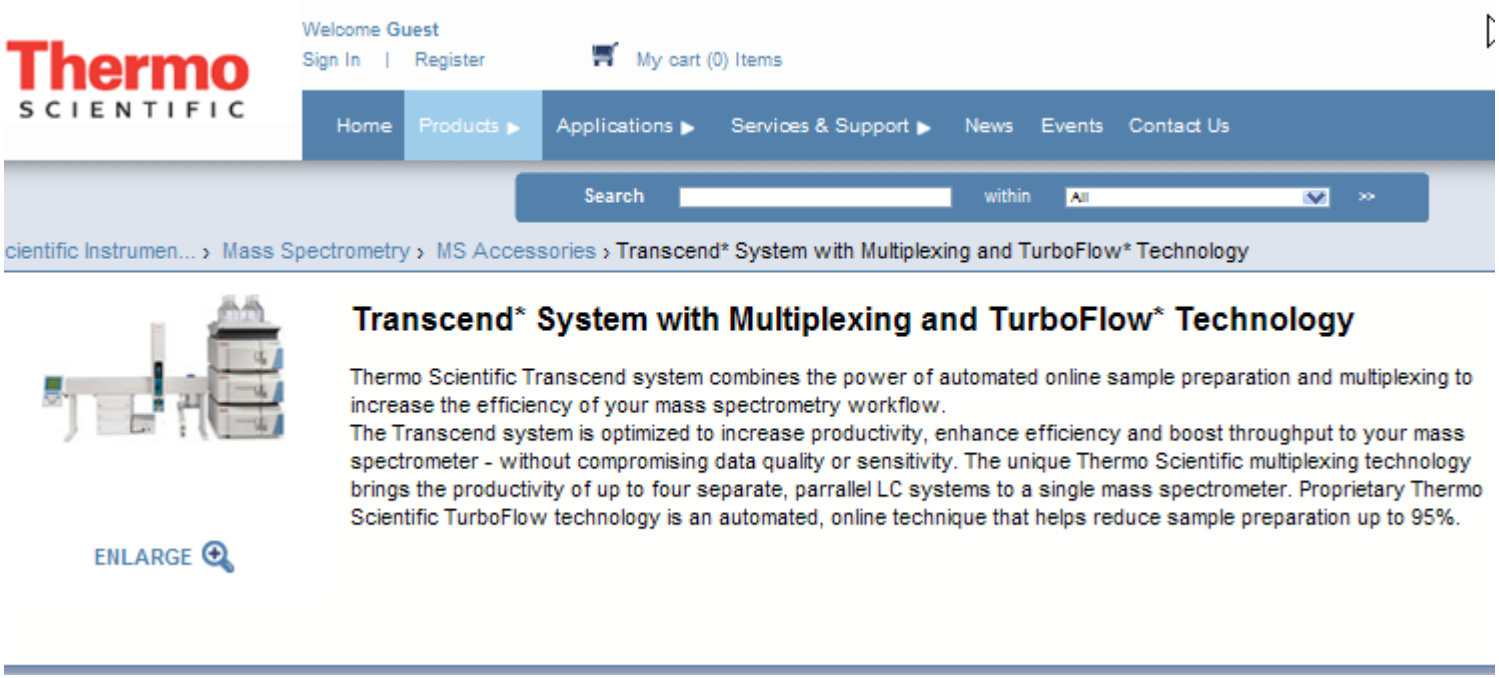


Columns washed with Magic Mix, Temperatures set to ambient and the TSQ's source set to standby conditions specified by tune method:

Voltage = 0, Temperatures = 100; Gas Pressures = 5

More Information

www.thermoscientific/turboflow



Thank You!

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