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Innovation Applied

Automated Sample Preparation Tools in Modern Analytical Methodology

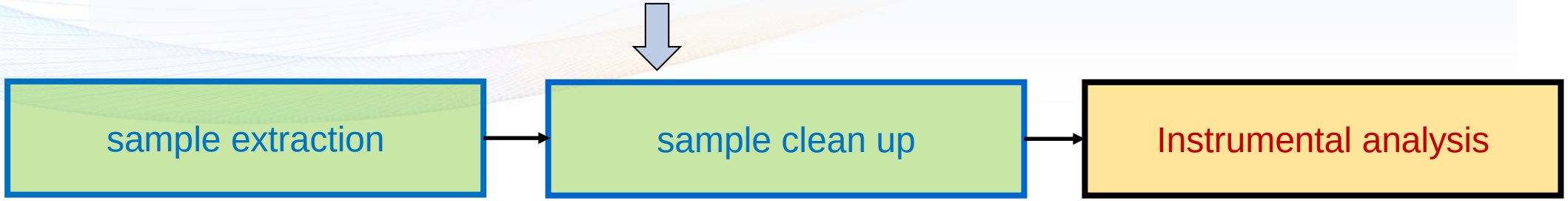
6-8th June , 2017

Discovery Days Tour 2017

Laszlo Hollosi, PhD

Automated sample preparation

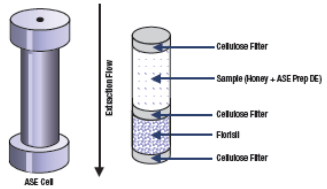
Most lab & time intense, **most error-prone** → Trend to reduce manual steps→ **automation**



Accelerated Solvent Extraction, ASE 350

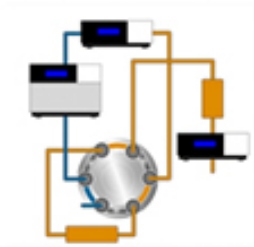
Solid samples

Inline extraction and clean-up



AutoTrace SPE

column coupling and switching



Liquid samples

Ultimate 3000 or Vanquish

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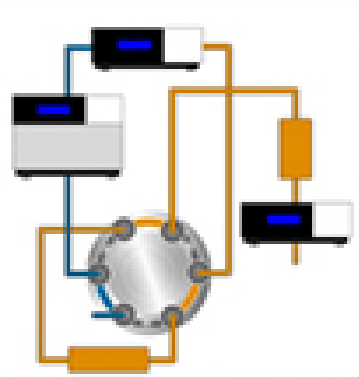
Advantages of column switching

- Minimal sample interaction
- Higher
  - accuracy
  - precision (no human error)
  - selectivity (higher peak capacity)
  - sensitivity (sample concentration)
- Lower analysis time – high throughput
- No ISD necessary
- Low organic consumption
- Quick handling of unstable compounds
- low risk with hazardous compounds

- Bit more investment
- Solvent system compatibility crucial
- Tendence for carry-over

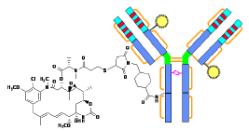


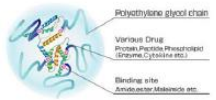
Column coupling strategies – plenty of opportunities!

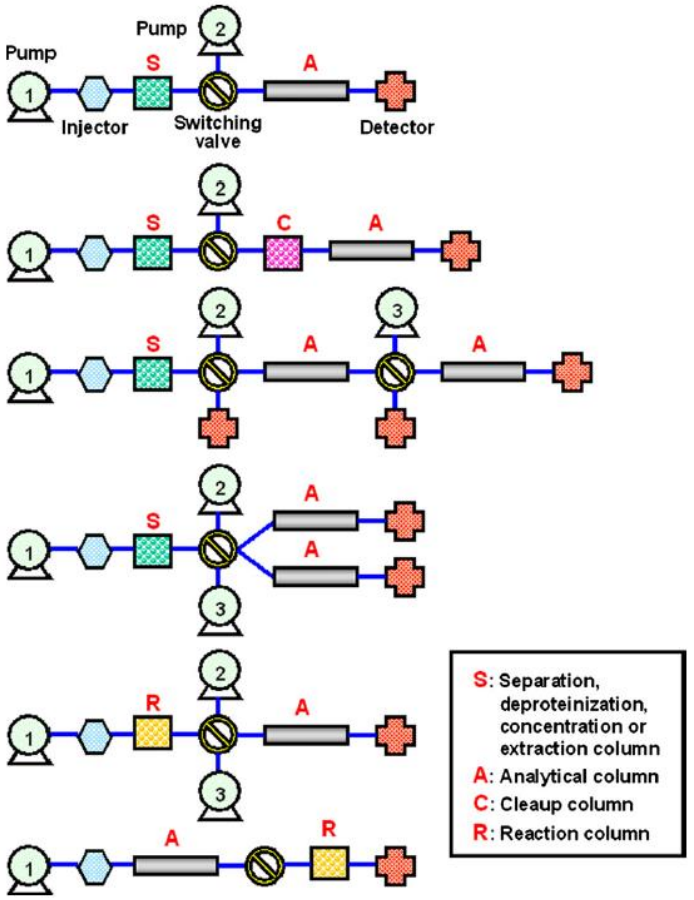
Orthogonality is important, but works without as well

Pre-column options:

- TLX
- RAM
- Ion Exchange
- Immuno Affinity
- MIP
- semipermeable membranes
- inline SPME

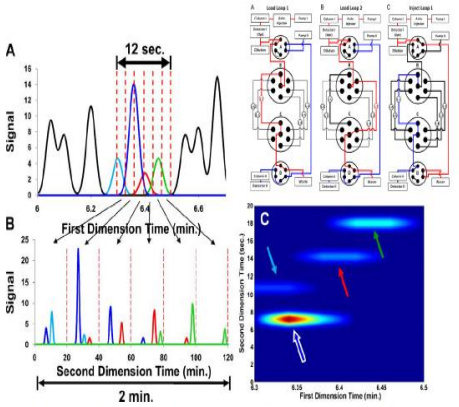






Typical couplings:

IE-RP
IE-IE
SEC-RP
SEC-IE
RP-RP



S.R. Groskreutz, M. M. Swenson, L. B. Secor, D. R. Stoll, J Chromatogr A, 2012, 1228, 31-40

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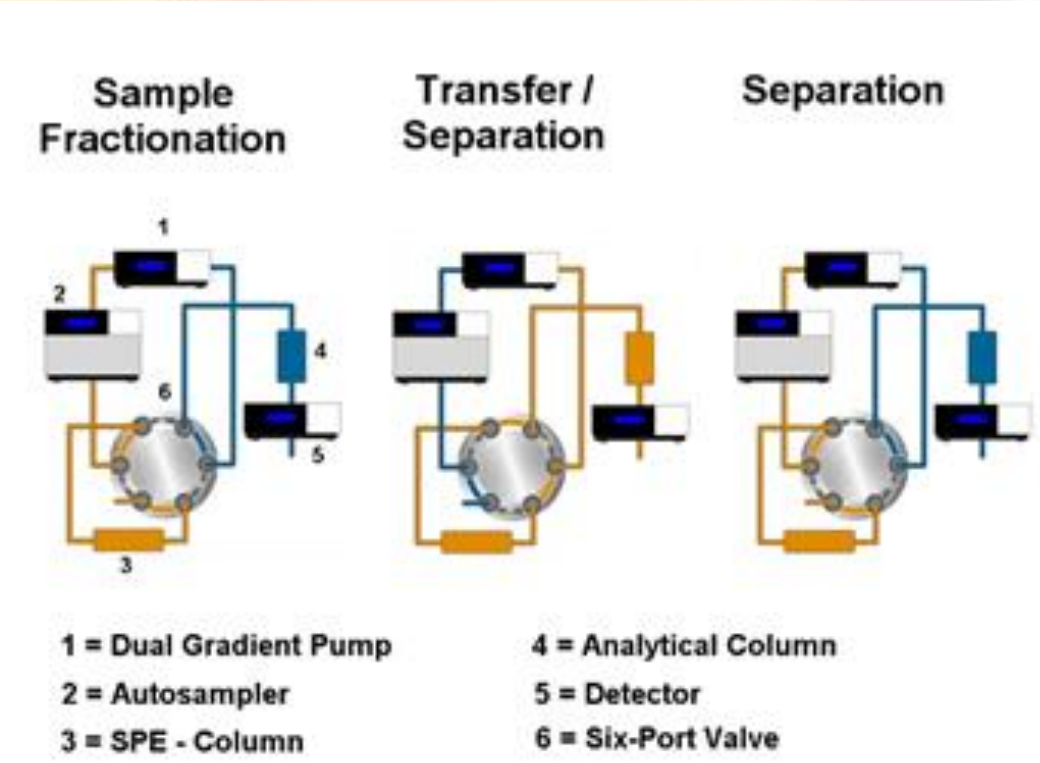
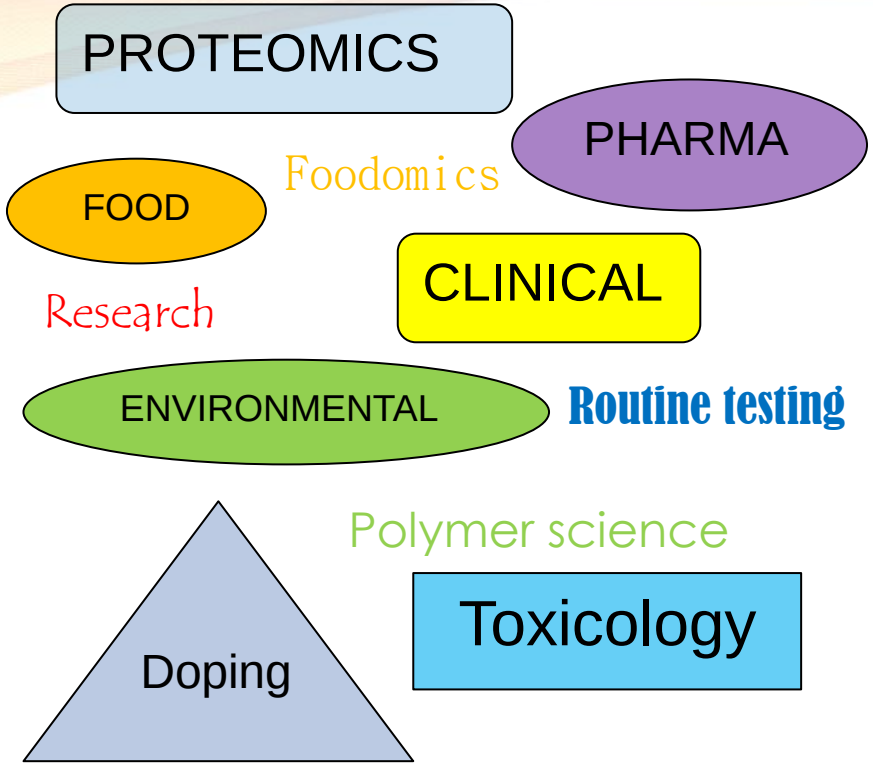
Typical workflow

- LOADING (preconcentration)
- TRANSFER to ANALYTICAL COLUMN
- ANALYTICAL SEPARATION



Application areas

- **1) Sample cleanup**:
  deproteinisation, lipid or sugar removal, desalting…
- **2) Sample preconcentration**:
  drugs, trace level contaminants…
- **3) Profiling**:
  OMICs, fractionations, food authenticity
- **4) Parallel LC/multiplexing**:
  high throughput



Most Comprehensive and Powerful LC Portfolio

The first and only sample prep and HPLC system designed specifically for clinical research

Ease of Use

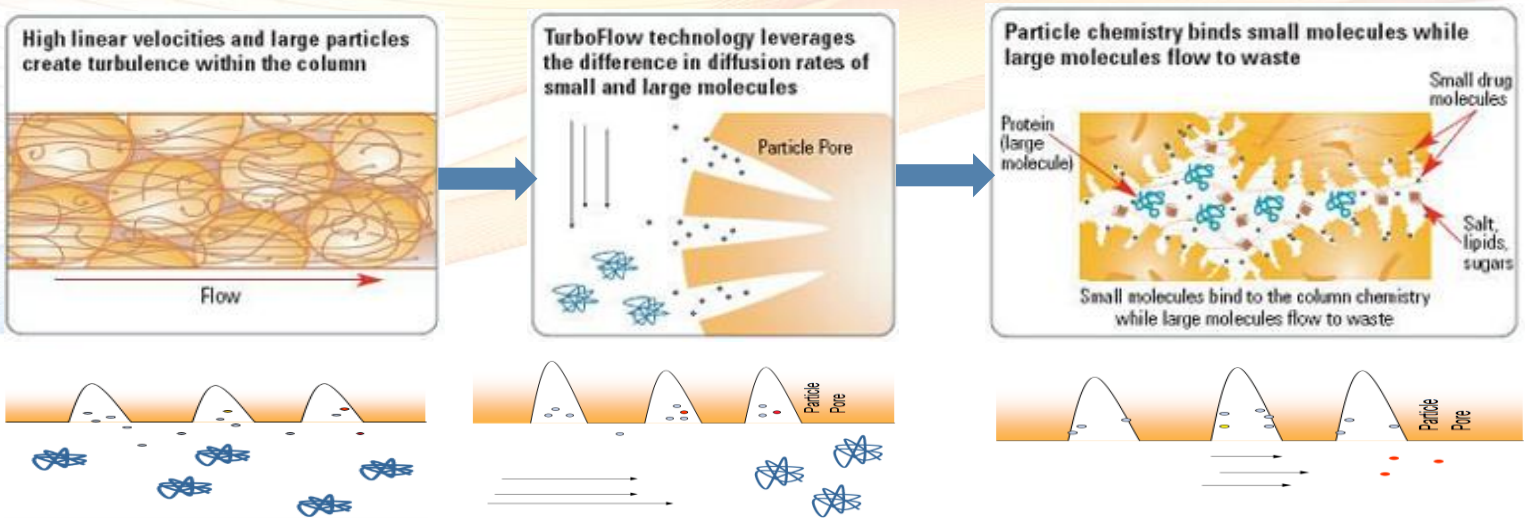
Thermo Scientific™ Dionex™ UltiMate™ Series HPLC

Thermo Scientific™ Prelude SPLC™ Sample Preparation and Liquid Chromatography System

Thermo Scientific™ Transcend™ II Systems

Channels/Throughput

Sample clean-up and/or concentration – TLX



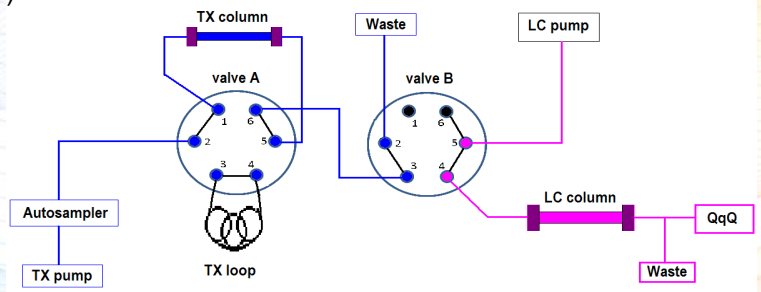
Separation based on:

- diffusion
- size exclusion

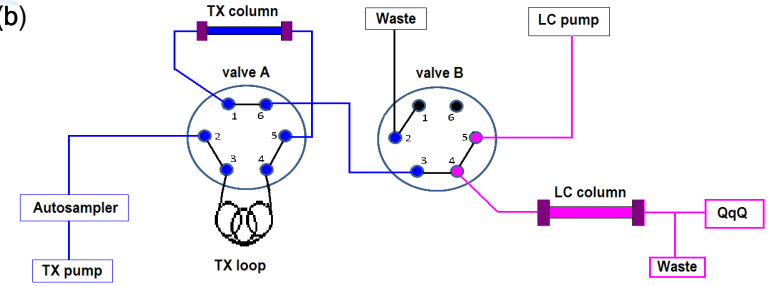
Separation of large and small molecules + SPE functionality (pre-concentration)

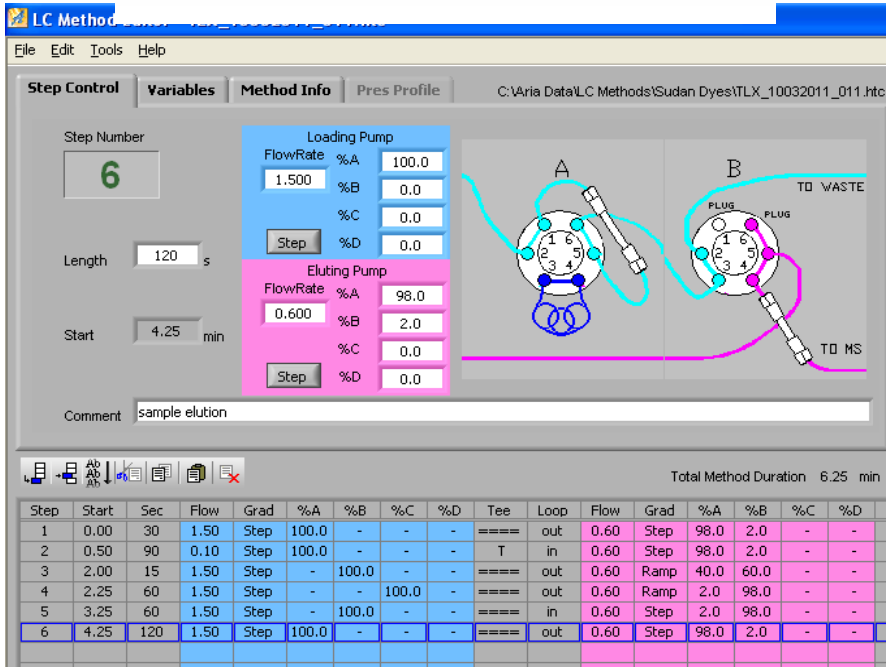
1) Sample clean-up by TLX

(a)



(b)





Special features, benefits:

- TX loop
- T-piece (unique feature!)

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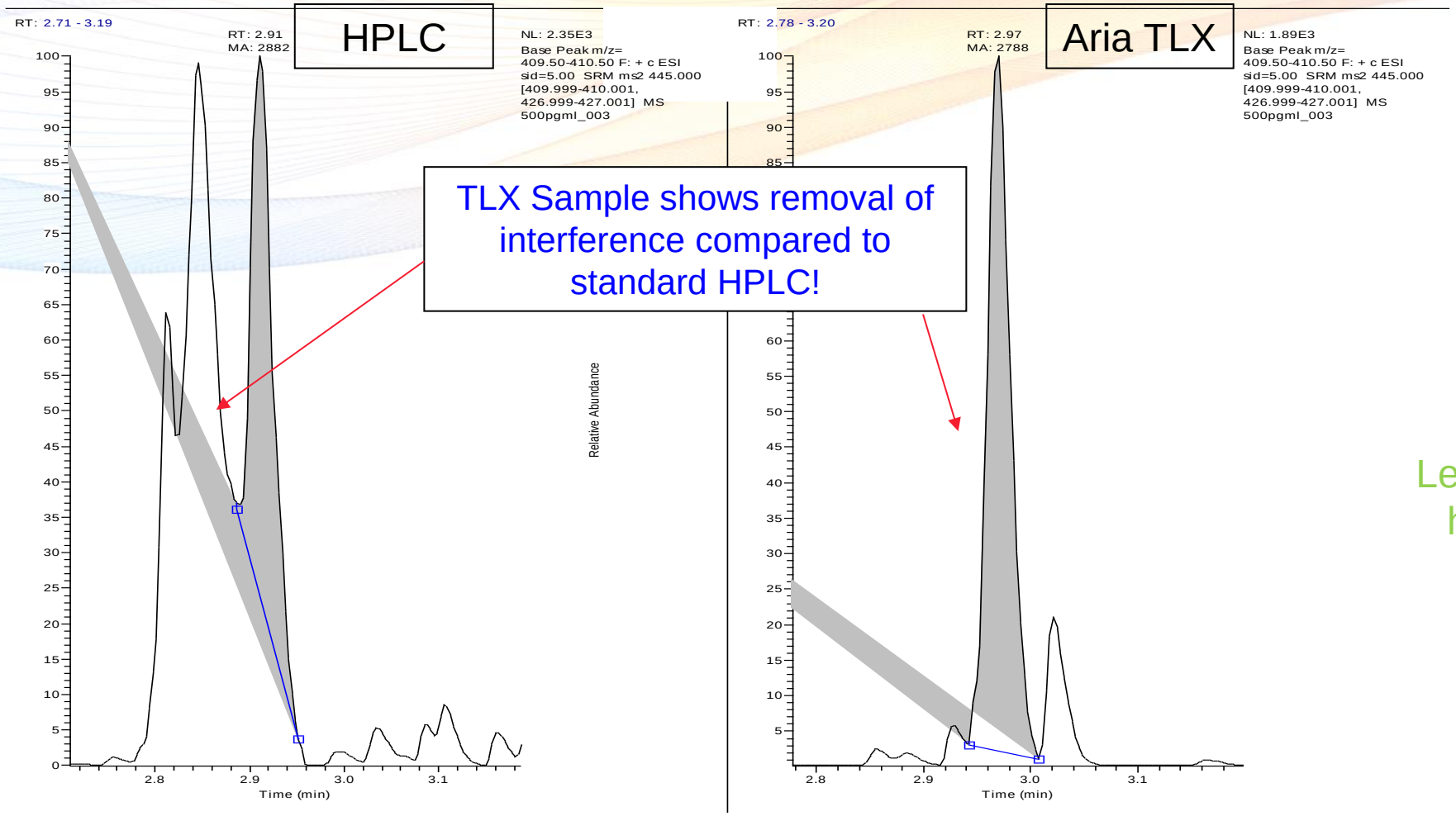
1) Sample clean-up by TLX

Tetracycline at 500 pg/ml with Aria TLX vs HPLC in Pig **Liver Matrix**

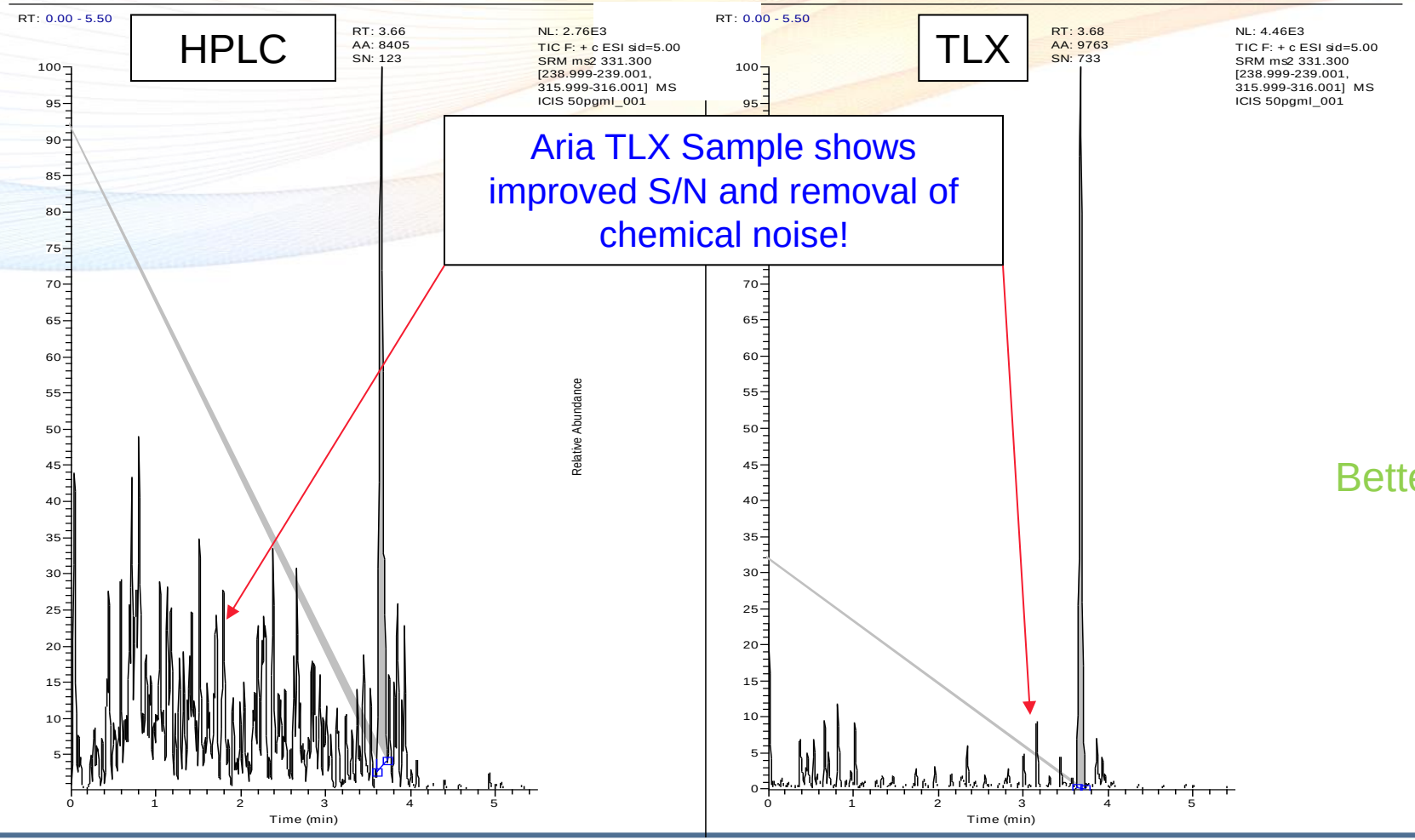


Less interference → higher selectivity

Courtesy of Dr. Charles Yang

1) Sample clean-up by TLX

Malachite Green at 50pg/ml with Aria TLX vs. HPLC in Pig Liver



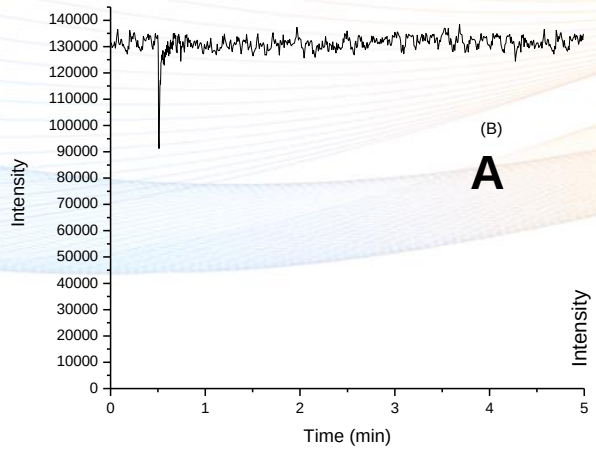
Better S/N → lower LOD

Courtesy of Dr. Charles Yang

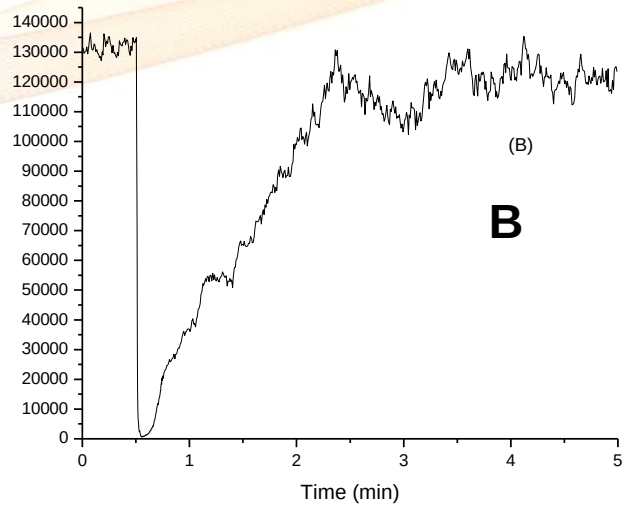
1) Sample clean-up by TLX

SPE, PPT and TurboFlow method comparison

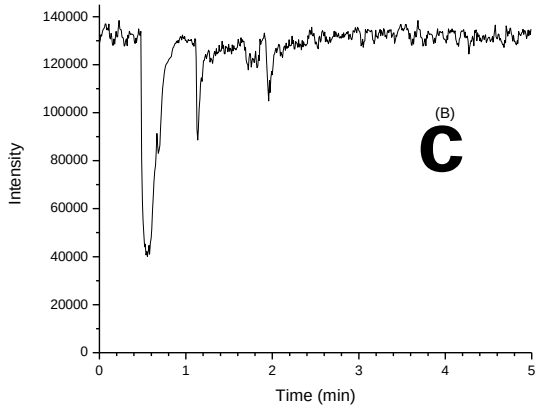
Rat Plasma



Buffered H20



PlasmaPrecipiTation
4:1 MeOH/H20



SPE

50:50 MeOH:H20

TLX System
With TurboFlow method has less ion suppression than other 2 methods

[J. L. Herman, Cephalon, Inc., Brandywine Parkway, PA]

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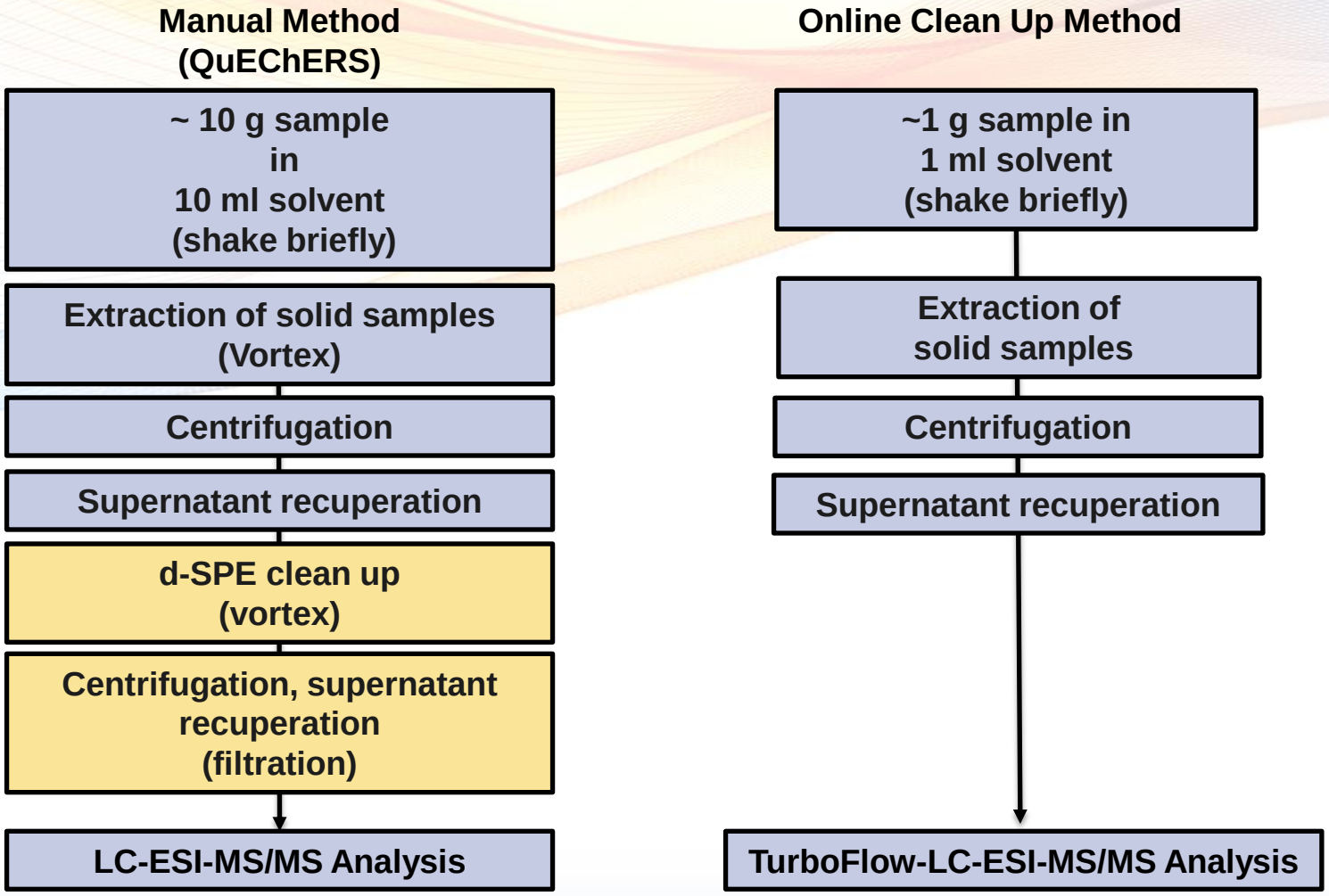
1) Advantages of TLX: speed of Analysis

pesticides in solid samples



~ 30 min/sample
(+ ~10 min LC analysis)

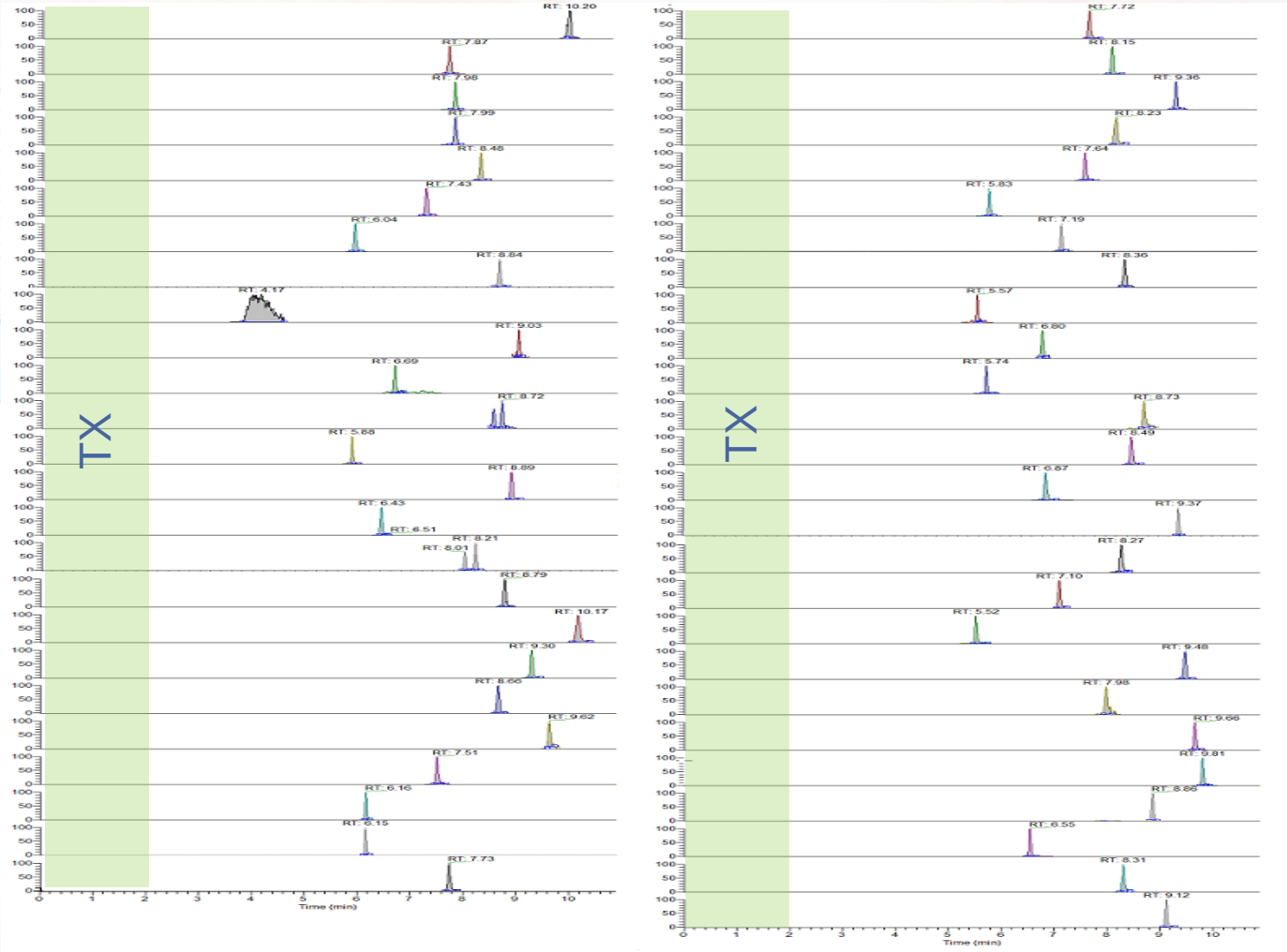
~ 15 min/sample
(+ 13 min online clean-up-LC analysis)

Pesticides in baby food, grape and wheat flour

50 pesticides

#	Analyte	Precursor ion
1	Abamectin	890.2
2	Ametryn	228.1
3	Azinphos methyl	339.8
4	Azoxystrobin	404.1
5	Bifenazate	301.1
6	Carbaryl	219.1
7	Carbendazim	191.8
8	Carfentrazone-ethyl	429.1
9	Chlormequat	122.1
10	Clofentezin	304.7
11	Cymoxanil	199.3
12	Cypermethrin	433.1
13	Dazomet	163.1
14	Diazinon	304.9
15	Dimethoate	230.2
16 / 17	Dimethomorph A&B	388.1
18	Ediphenfos	310.8
19	Fenazaquine	307.2
20	Fluazifop P	384.3
21	Fluzilazol	316.1
22	Hexithaizox	353.1
23	Imazalil	296.9
24	Imidacloprid	256.1
25	d4-Imidacloprid	259.9
26	Isoproturon	207.1
27	d6-Isoproturon	213.2
28	Isoxaben	333.1
29	Lactofen	479.1
30	Malathion	347.9
31	Metalaxyl	279.9
32	Methomyl	163.1
33	Metribuzin	215.2
34	Myclobutanyl	289.1
35	Omethoate	214.2
36	Oxadyxil	296.2
37	Oxamyl	236.9
38	d10-Parathion-ethyl	302.1
39	Pethoxamid	296.1
40	d6-Primicarb	245.2
41	Profenofos	374.8
42	Promecarb	225.2
43	Propoxur	210.1
44	Pymetrozin	218.0
45	Pyperonil-butoxide	356.0
46	Pyrimethanyl	200.1
47	Quinoxifen	307.9
48	Spirodiclofen	410.9
49	Tebuconazol	308.2
50	Thiacloprid	253.1
51	Triadimefon	294.1
52	Trifloxistrobin	409.5

Baby food sample spiked at 10 ppb



Hollosi et al., Chromatographia 75 (2012) 1377-1393

TX: Cyclone MCX-2

LX: Acccucore C18, 100x2.1, 2.6um

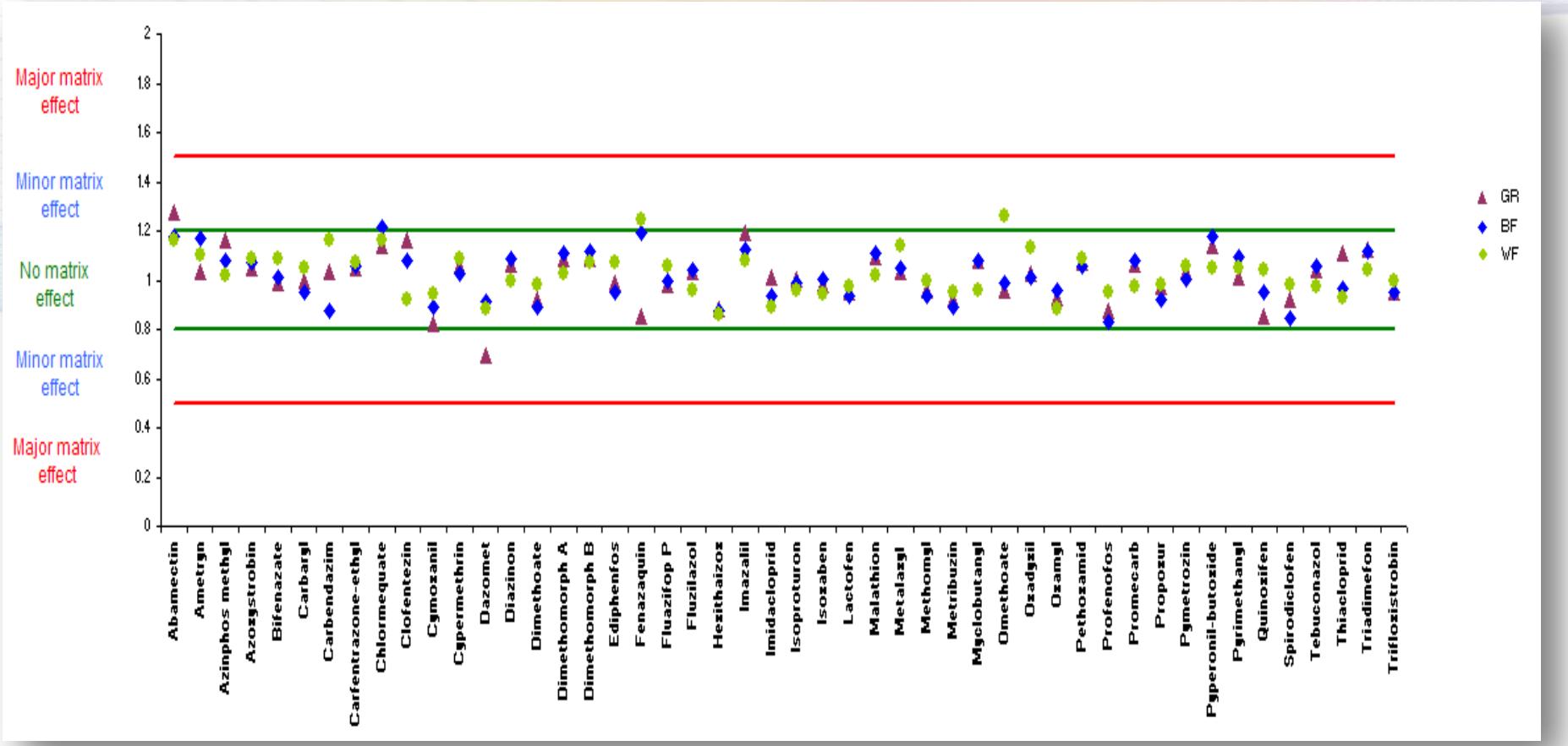
ELU: Aq-MeOH (0.1% FA) gradient

Vinj: 10 ul

DET: Themo TSQ Access Max

1) Advantages of TLX: reduction of matrix effect

pesticides in solid samples



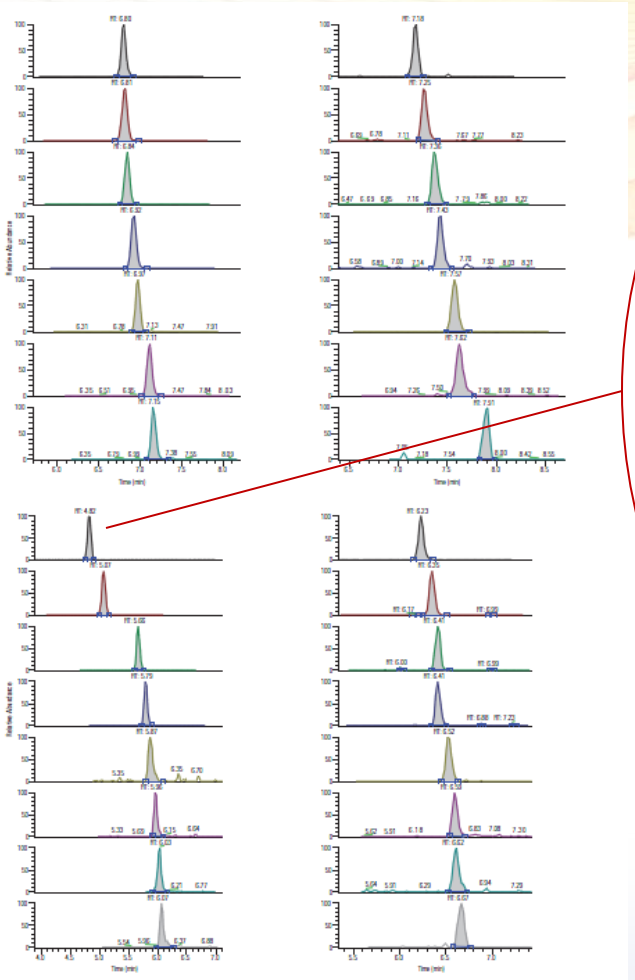
Hollosi et al., Chromatographia 75 (2012) 1377-1393

1) Advantages of TLX: reduction of matrix effect

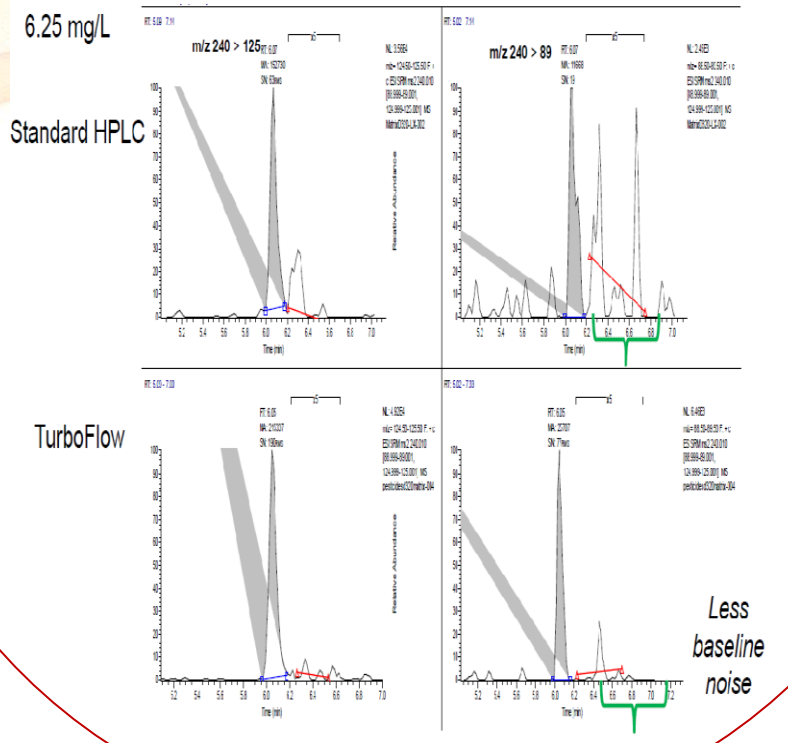
pesticides in difficult samples

30 pesticides in gren tea at 6.25 ug/L

Compound	RT (min)	Parent ion (m/z)
Prometon	4.82	226.0
Ametryn	5.07	228.0
Dimethametryn	5.68	256.1
Mefenoxam	5.79	280.0
Monolinuron	5.86	215.0
Isoproxarb	5.94	194.0
Dimethachlor	6.01	256.0
Clomazone	6.05	240.0
Furalaxyl	6.21	302.0
Azoxystrobin	6.33	404.0
Triadimefon	6.39	294.0
Ethoprophos	6.41	243.0
Isrobenfos	6.52	289.0
Isoprothiolane	6.57	291.0
Flutolanil	6.60	324.0
Propiconazole	6.65	342.0
Benalaxyl	6.78	326.0
Pirimiphos-methyl	6.81	306.0
Pirimiphos-methyl	6.87	168.0
Diazinon	6.90	305.0
Thiazopyr	6.95	397.0
Piperophos	7.09	354.0
Trifloxystrobin	7.13	409.0
Tebufenpyrad	7.16	334.0
Piperonyl butoxide	7.25	356.0
Pyriproxyfen	7.34	322.0
Trialkoxydim	7.39	330.0
Fenazaquin	7.55	307.0
Butralin	7.58	296.0
DEF	7.95	315.0



Standard HPLC vs. Turboflow™ Method



Clomazonein

Courtesy of Dr. Yang Shi

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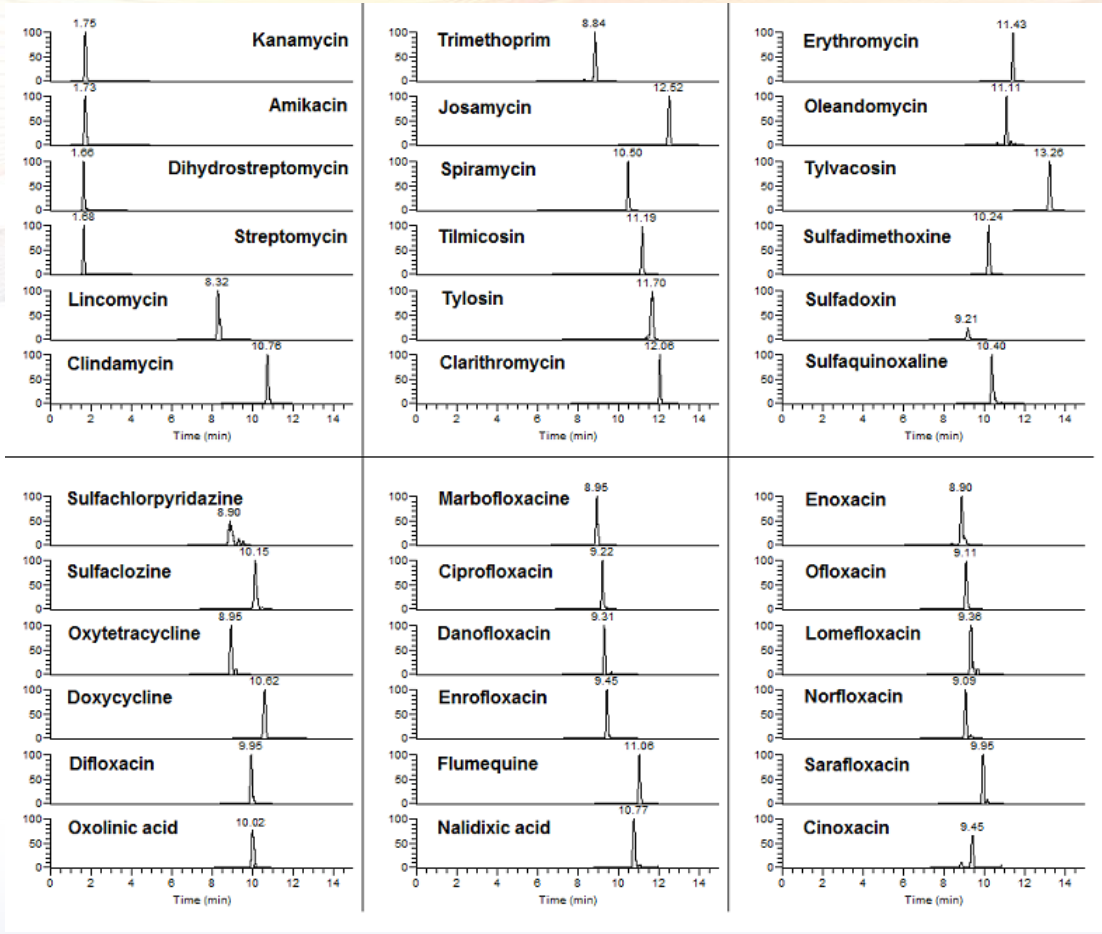
1) Clean-up by TLX
veterinary drugs in milk

Veterinary Drugs in Milk and Meat

36 antibiotics in milk
(100 µg/kg)

aminoglycosides
sulfonamides
quinolones
lincomycin
macrolides
trimethoprim
tetracyclines



Bousova et al., Food Add. Contamin. 29 (2012) 1901-1912

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1) TLX advantage: less ion source contamination

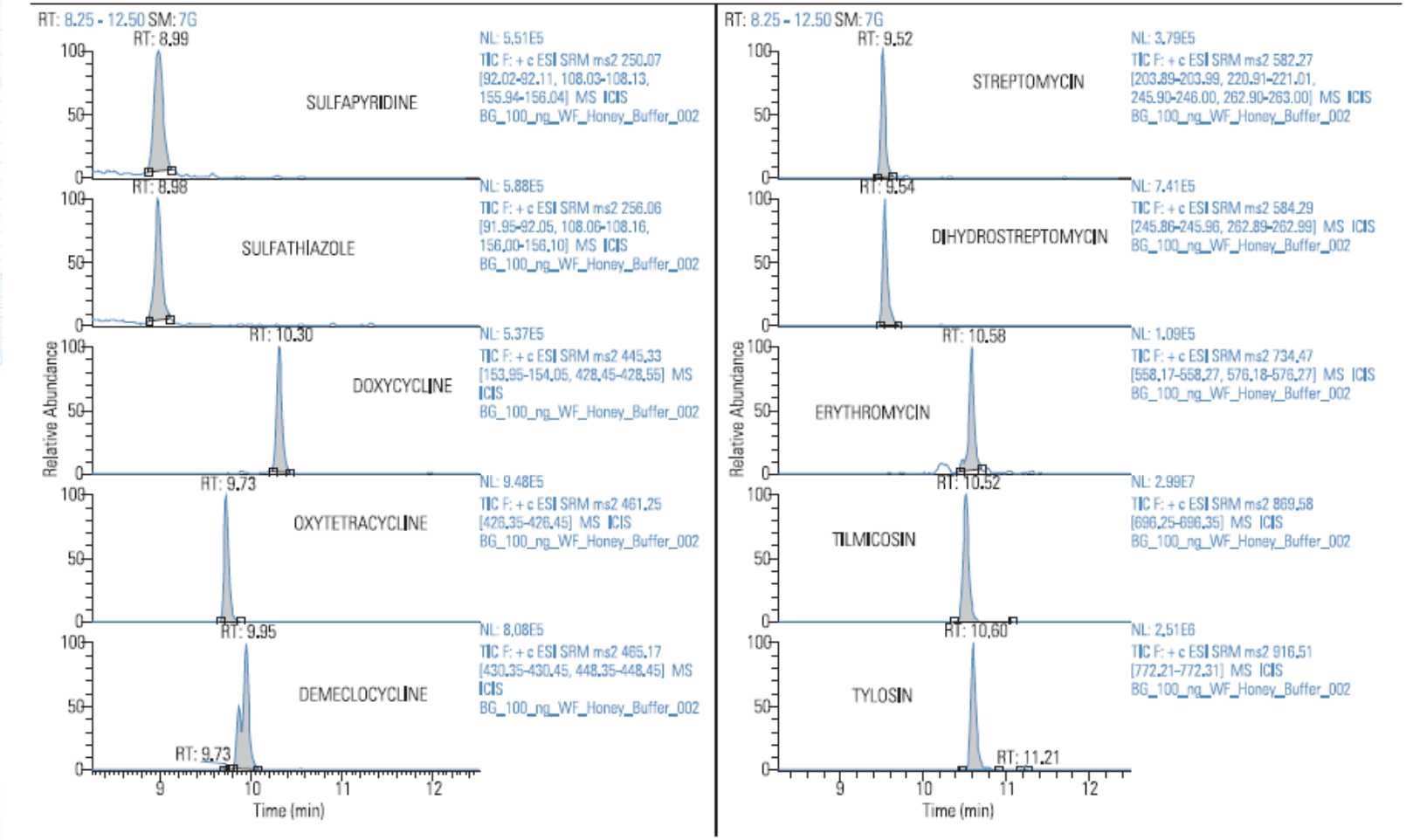
veterinary drugs in milk

after cleaning

without TLX after 32 injections

after 120 injections with TLX

Antibiotics in honey



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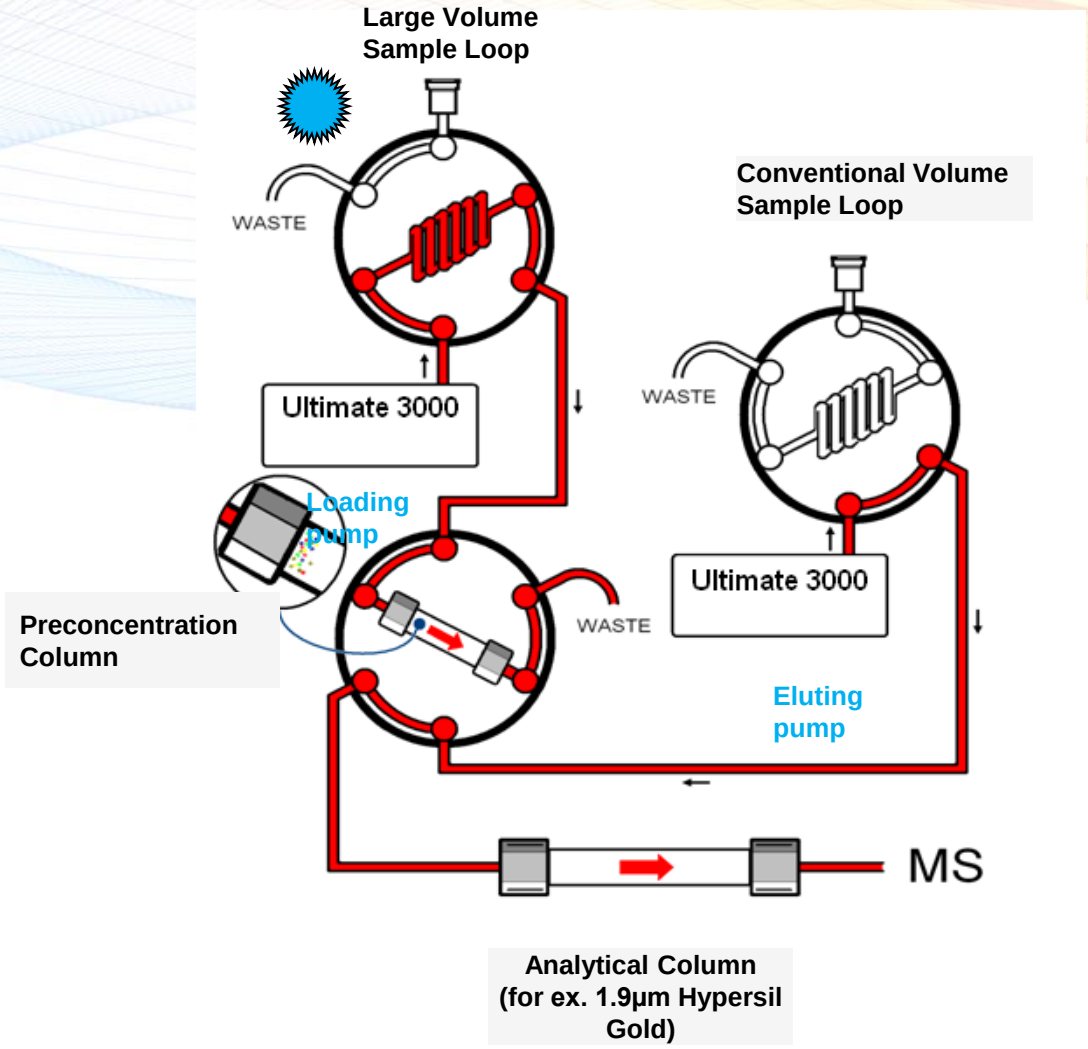
1) TLX EFS Applications Method Performance Parameters

Method Parameters	Pesticides	Veterinary drugs	Plasticisers	Mycotoxins
Multiresidue assay	yes (50)	Yes (36)	Yes (11)	Yes (6)
Matrix type	Baby food, whaet flour, strawberry	Milk, meat	Chilli souce, hot souce	flours
Sensitivity (LOD)	< 10 ug/kg	0.3-40 ug/kg	5-20 ug/kg	2-150 ug/kg
Linearity	0-500 ug/kg	0-400 ug/kg	0.05-100 mg/L	5-5000* ug/kg
Precision	< 25% (intermediate)	< 27%	<15%	<19%
Method accuracy (recovery)	75-110%	71-120%	72-115%	72-120%
Matrix effect	5 minor / 144	20 / 72	1 major / 11	n.d.
Fit for purpose EU	yes	yes	yes	yes
Reference	Chromatographia 75 (2012) 1377-1393	J. Chromatogr. A 1274 (2013) 19-27	J.AOAC (2013)	Food Add. Contamin. 30 (2013) 156-165

2) Sample concentration by column coupling – EQuan System



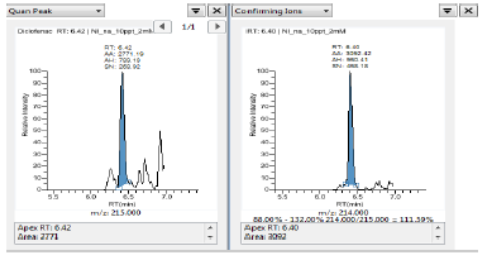
E-Quan System

For high injection volume and sample concentration needs (>100 ul)

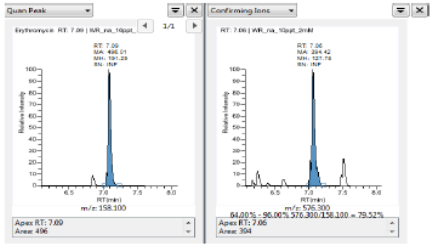
2) Sample concentration by column coupling

Water Framework Directive

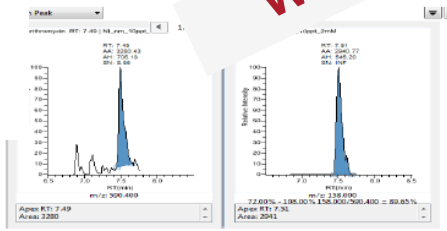
Compounds	CIP 2		River LOD (pg on column)	Effluent (pg on column)	Influent (pg on column)
	Required LOD (µg/L)	LOD (pg on column)			
Diclofenac (WFD)	0.01	0.1	0.03	0.1	0.03
Erythromycin	0.1	1	0.6	0.15	0.15
Azithromycin	0.005	0.05	2	tbd	0.4
Clarithromycin	0.01	0.1	0.1	0.1	0.03



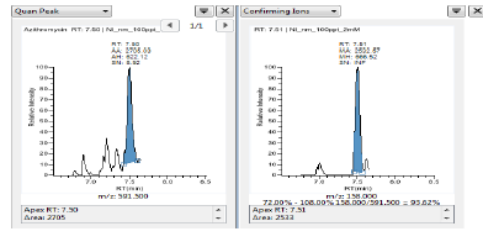
Diclofenac



Erythromycin



Clarithromycin



Azithromycin

Where 10-100x concentration needed

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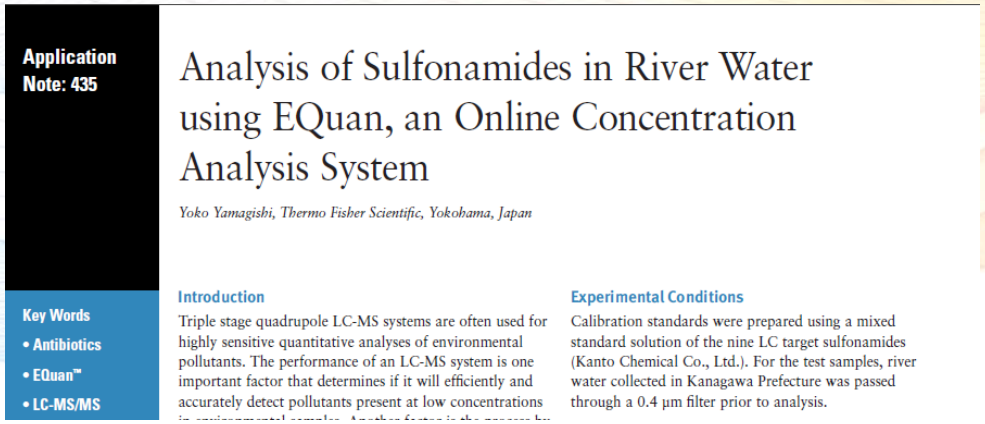
2) Water contaminant enrichment



Application Note: 435

Analysis of Sulfonamides in River Water using EQuan, an Online Concentration Analysis System

Yoko Yamagishi, Thermo Fisher Scientific, Yokohama, Japan

Key Words
- Antibiotics
- EQuan™
- LC-MS/MS

Introduction

Triple stage quadrupole LC-MS systems are often used for highly sensitive quantitative analyses of environmental pollutants. The performance of an LC-MS system is one important factor that determines if it will efficiently and accurately detect pollutants present at low concentrations

Experimental Conditions

Calibration standards were prepared using a mixed standard solution of the nine LC target sulfonamides (Kanto Chemical Co., Ltd.). For the test samples, river water collected in Kanagawa Prefecture was passed through a 0.4 µm filter prior to analysis.

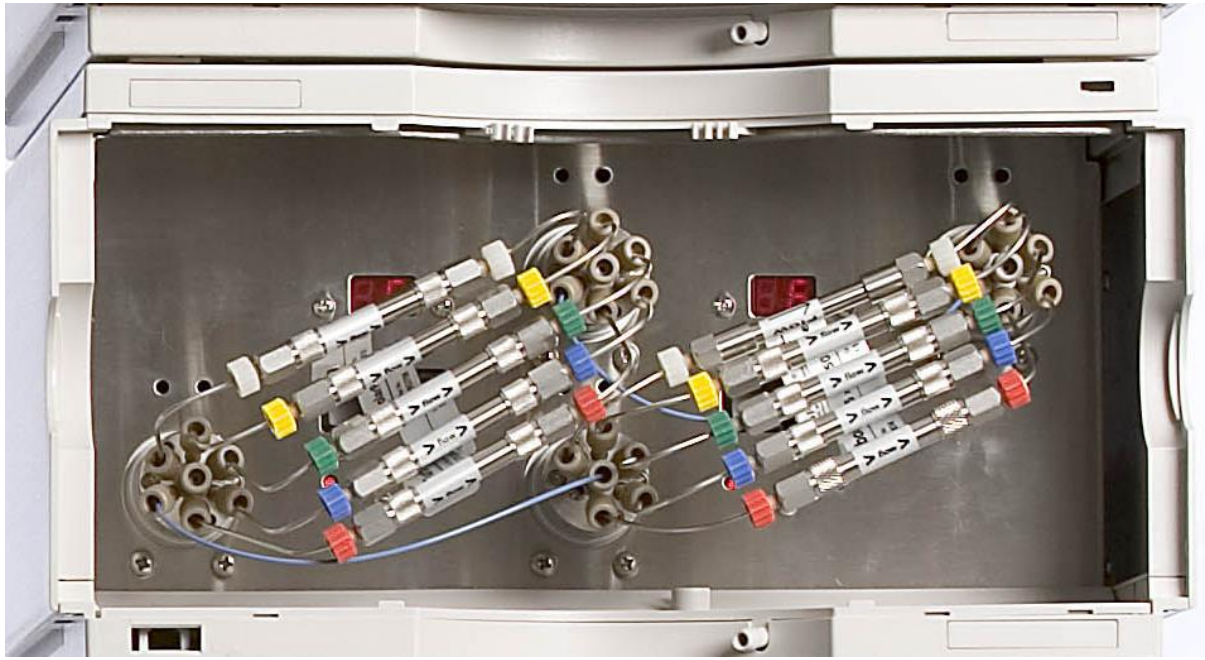
Pushing down LOD/LOQ values (~10-100x)

		1.0 ppt spiked samples (n = 4)		
Compound	Concentration in river water sample (ppt)	Concentration in spiked samples (ppt)	Recovery rate (%)	CV (%)
Sulfadiazine	0.35	1.19	84	10.4
Sulfamerazine	0.39	1.13	73	5.1
Sulfadimidine	NF	0.98	98	11.3
Sulfamethoxypyridazine	NF	0.87	87	7.1
Sulfamethoxazole	12.35	19.35*	70	2.8
Sulfamonomethoxine	1.11	1.85	74	3.3
Sulfisoxazole	NF	0.95	95	8.5
Sulfadimethoxine	1.42	2.19	77	1.4
Sulfaquinoxaline	NF	0.85	85	3.3

** The spiking concentration was set at 10 ppt because sulfamethoxazole was detected in the river water samples at concentrations higher than 10 ppt.*

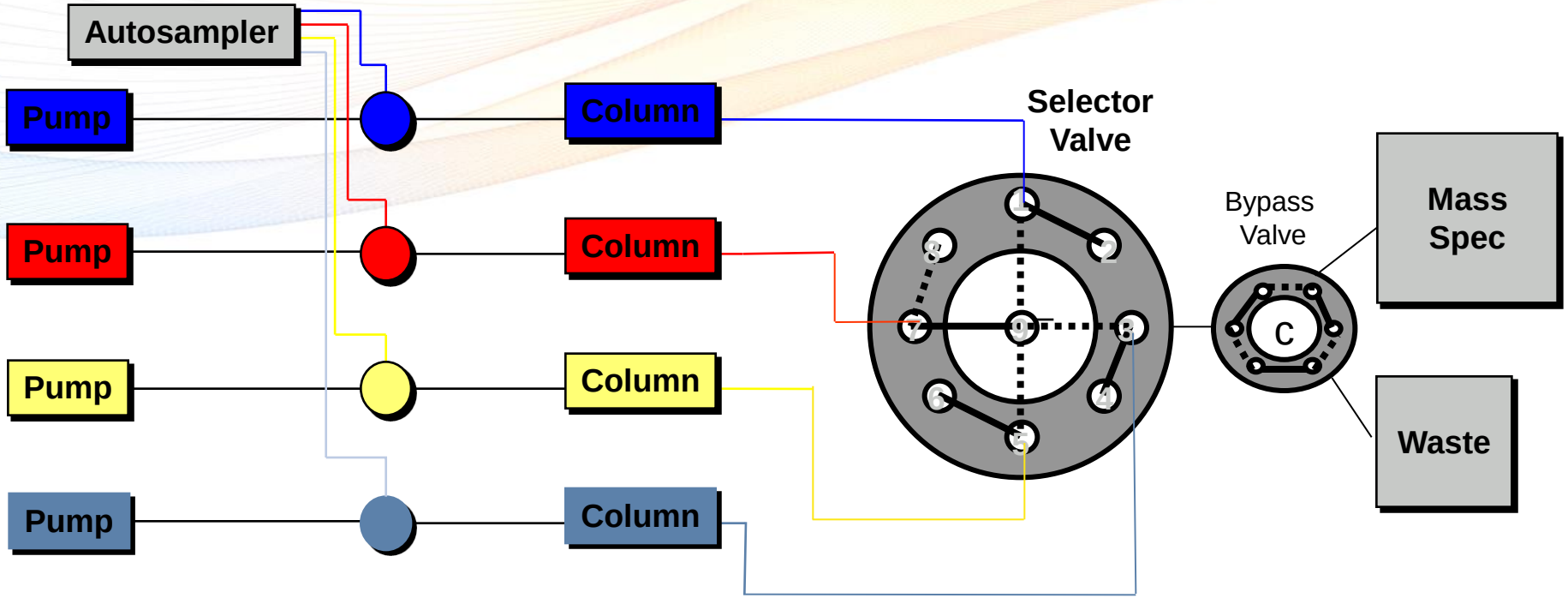
4) Multiplexing: MCM Multiple Column Module

- Simplifies method development
- For use of up to 12 columns
- Use 6 TurboFlow columns and 5 Analytical columns
- Controlled by Aria MX



4) Multiplexing – what happens

For ultra high-throughput measurement - 1 minute per sample

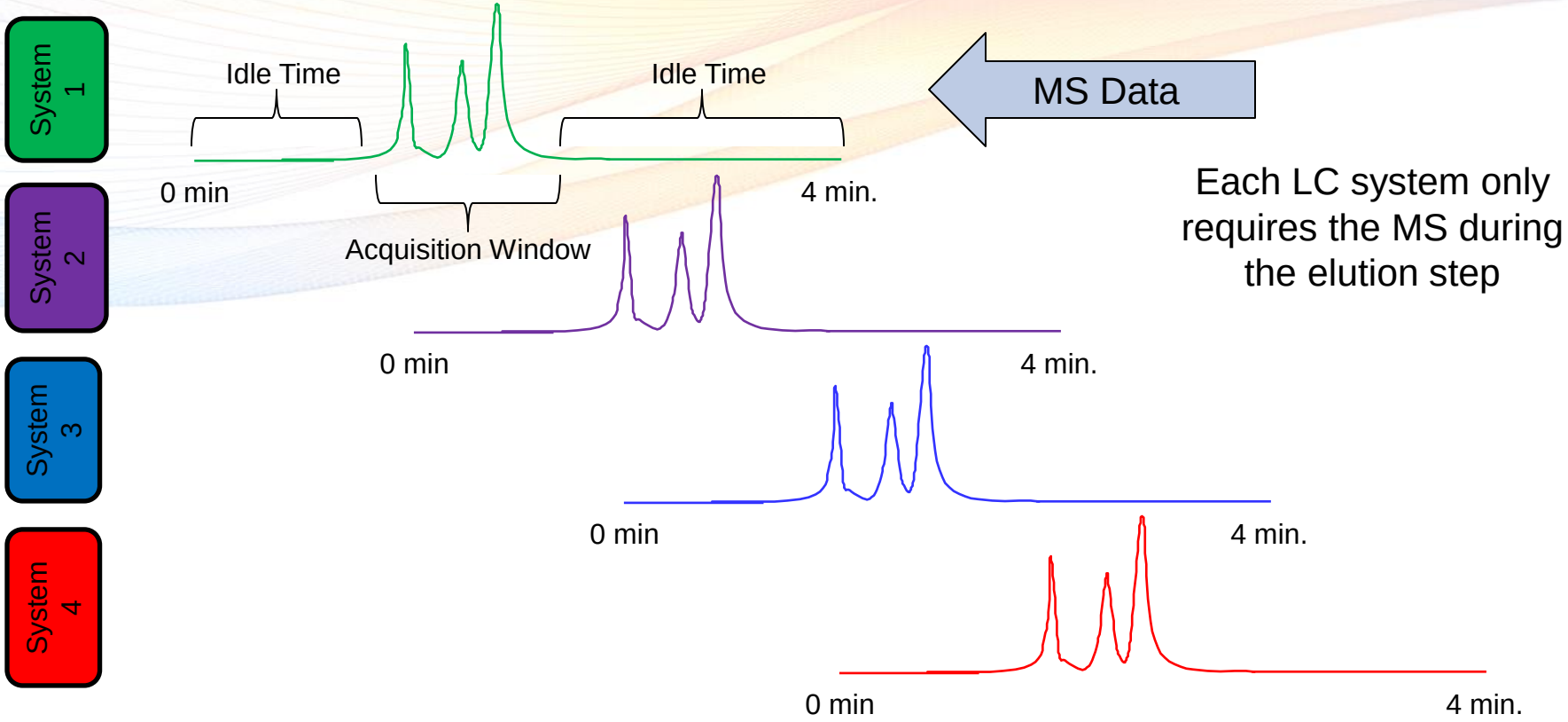


Parallel System, Serial Data

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

4) Multiplexing - increase throughput – 15 min screening

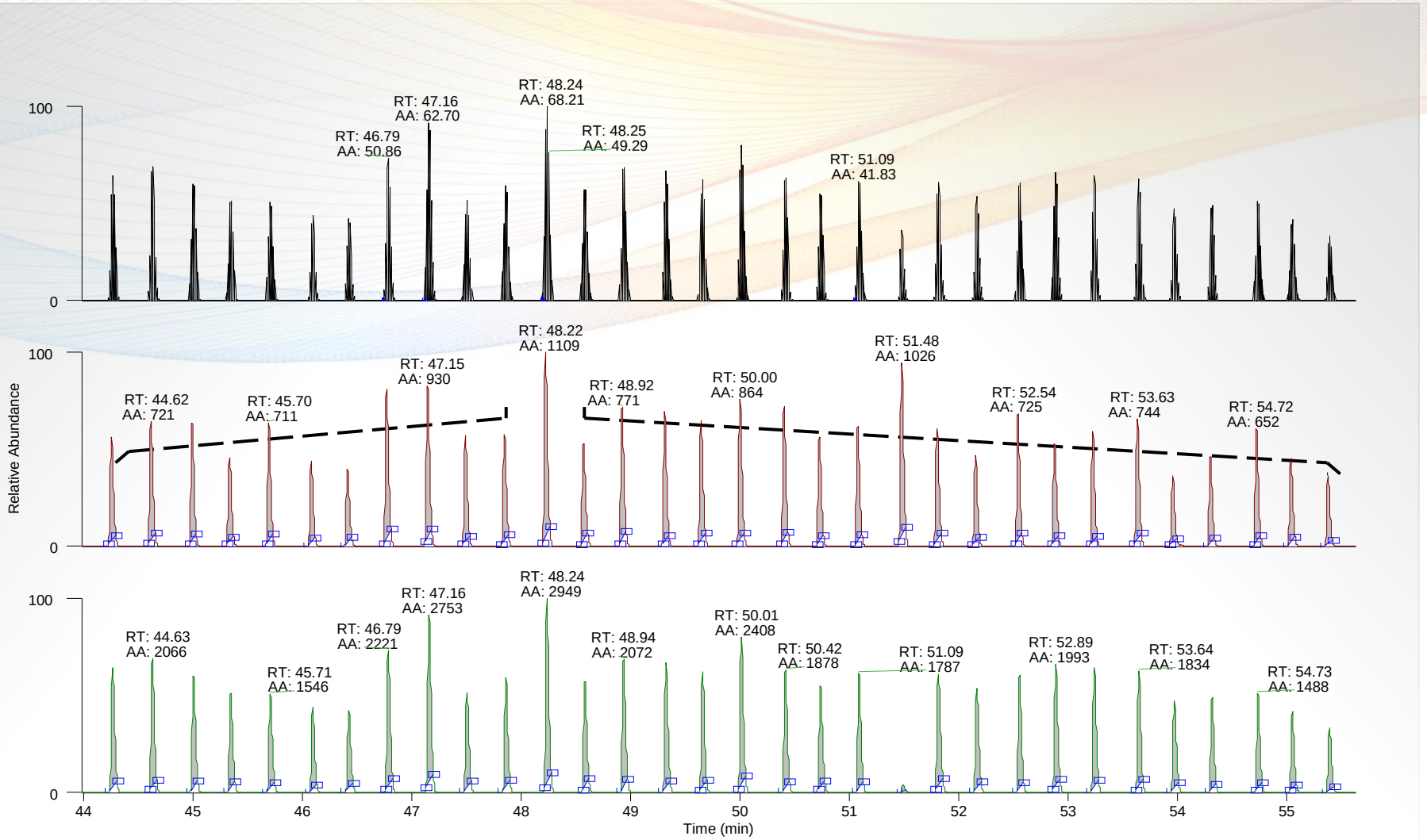
- Controlled by Aria MX



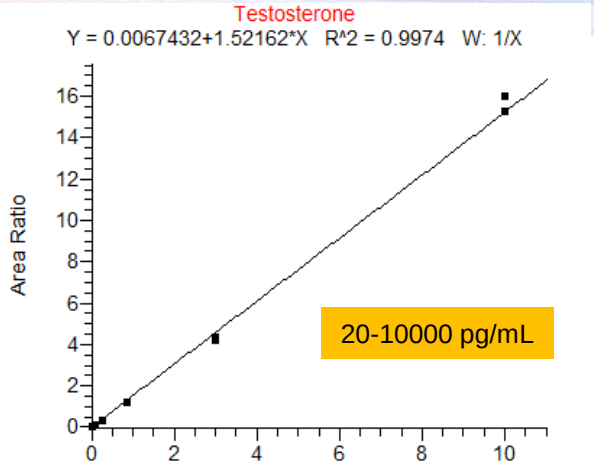
Each LC system only requires the MS during the elution step

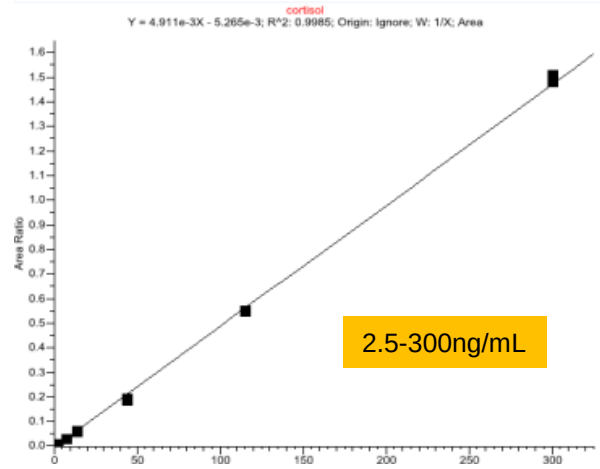
System diagram above is representative of a Thermo Scientific Transcend™ LX-4 system

4) Multiplexing - increase throughput – 15 min screening



CLINICAL

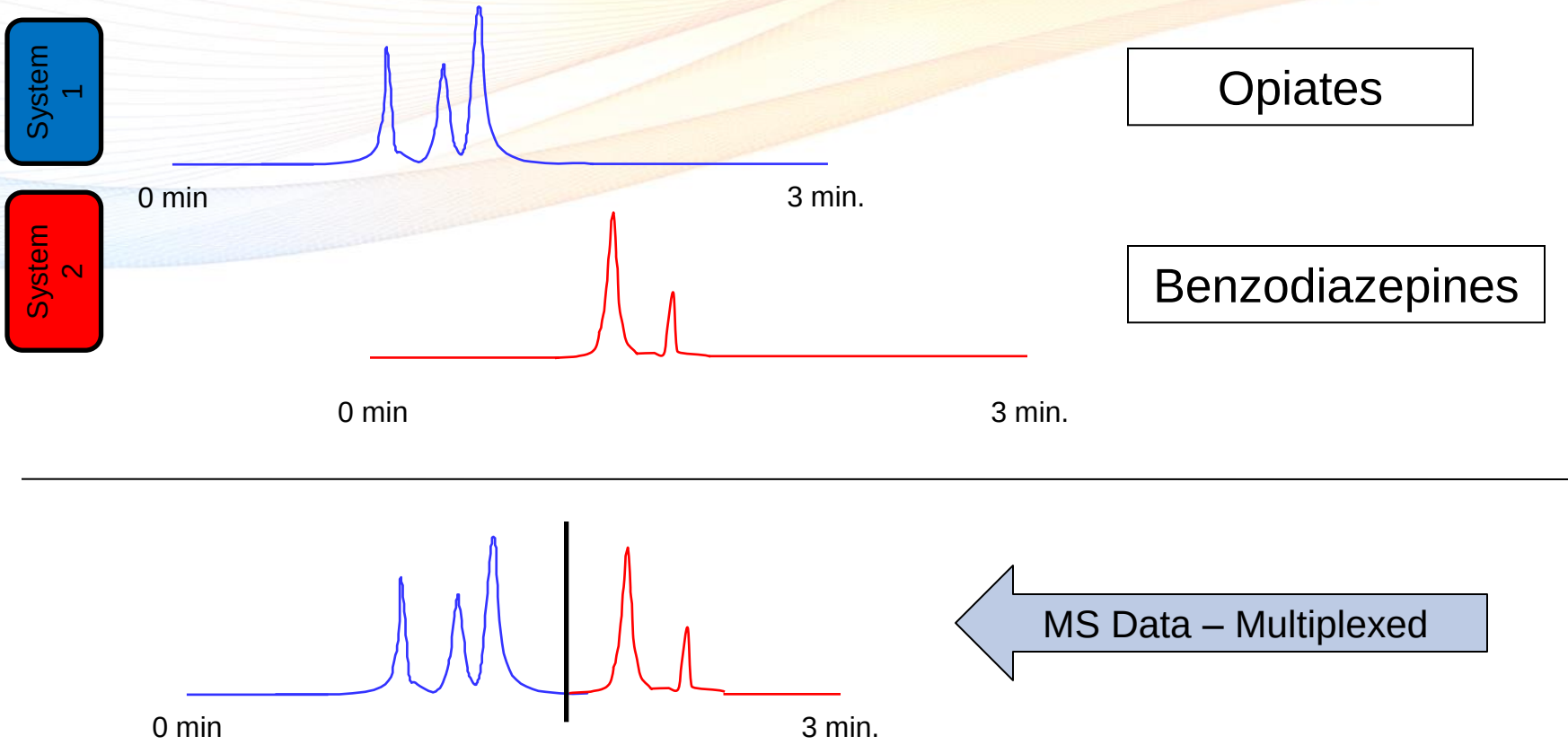




ThermoFisher SCIENTIFIC

4) Cross sequential Multiplexing is Unique to Aria OS

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



CLINICAL

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

SUMMARY

- **Online sample preparation systems**
  - **Versatile system setup** according to the specific needs
  - **Flexibility** online or offline LC
  - **Versatile application area** fit for purpose methods for EFS, ClinTox, Pharma
  - **Higher efficiency** human error eliminated
  - Easy method development
  - **Additional selectivity, sensitivity** with MSMS or HRMS detection
  - **Less MS maintenance**, stand by time
  - Expert support for method setup

- For details please contact us:

https://www.thermofisher.com/cz/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-systems/lc-ms-online-sample-prep-multiplexing-systems.html