Agilent LCMS products and the application strategies in veterinary drug analysis

Experience A New Level of Confidence

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LCMS Application Engineer

Two Great Companies from One 两个全新公司的诞生

Agilent Technologies and Keysight Technologies

Separation expected in early November 2014, subject to local legal procedures.

- Agilent has evolved into two distinct investment and business opportunities.
- As two separate companies, each will be able to direct resources to its own business and customer needs.
- Each company has the operational and financial scale to stand on its own.
- The companies can concentrate solely on distinct markets and growth drivers.
- Both companies are well positioned to maximize growth.

Agilent China at a Glance (since 1985)
安捷伦中国概略：在中国从1985年至今

- 21 Operations in14 cities
- 1800 employees
- 500 staff in R&D team

6 Legal Entities

- Agilent Technology Holding Co.
- Agilent Technology (China) Co. Ltd.
- Agilent Technologies (Shanghai) Co. Ltd.
- Agilent Technology Software Co.
- Agilent Technology Chengdu Co. Ltd.
- Agilent Technology Trading Co. Ltd.

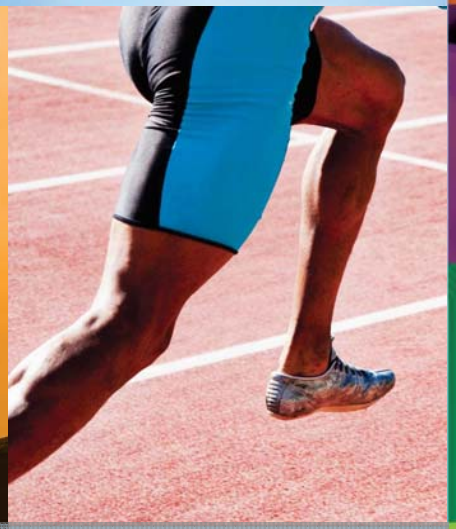
Agilent in Chemical Analysis 在化学分析主要应用领域

No. 1 in our core markets

Food
食品

Environment
环境

Chemical & energy
化学化工能源



Forensic & drug testing
法医毒物

CORE TECHNOLOGY PLATFORMS

Gas chromatography | Mass spectrometry | Spectroscopy | Chemistries

Continually Expanding Portfolio 持续拓展的产品技术平台

A few of our recent additions……this week 7900 & 4200

Q-TOF LC/MS

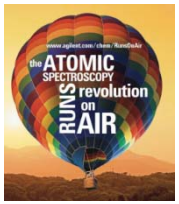
ICP-MS QQQ

WINNER: 2013 R&D 100 Product of the Year and Product of the Show at ASMS

GC/MS QQQ

GC/MSD

MP-AES



WINNER: 2012 R&D 100 Product of the Year

WINNER: Industrial Business Outlook 2012 Gold Award

FTIR Spectrometer

Customers of Third-party Testing Laborotory
第三方检测客户群

SYSTEM CERTIFICATION ISO 9001:2000 SGS

Sino Analytica 诺安检测服务有限公司

BUREAU VERITAS 1828 BUREAU VERITAS

Intertek Valued Quality. Delivered.

TÜV SÜD

BERLIN ORIENT 北京国立柏林医学科技发展有限公司 Beijing Orient Berlin Medical Technology Co.,Ltd.

金域检验 KingMed Diagnostics

PONY 谱尼测试 Pony Testing International Group

中国检验检疫科学研究院综合检测中心 Chinese Academy of Inspection and Quarantine Comprehensive Test Center

CTI 华测检测 CENTRE TESTING INTERNATIONAL

Analysis Strategy of Food Safety
食品安全分析策略

7200 GC-QTOF

Volatility
Thermally
stable

Thermally stable & Nonpolar
热稳定 & 非极性组分

非靶标化合物分析

Unknown Screening – what's in the sample?

靶标化合物分析

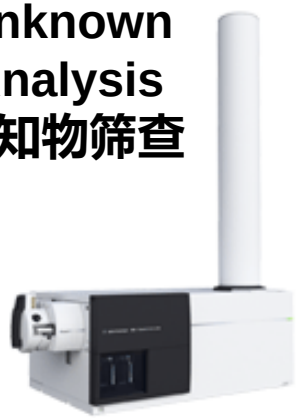
Target Screening – are specific compounds present & how much?

Non-Selective ⟷ Selective

Volatility
Thermally
stable

7000C GC-QQQ

Unknown Analysis
未知物筛查



6500 LC-QTOF

PCDL
MSC
All ion MSMS

Nonvolatile
Thermal
unstable

Nonvolatile
Thermal
unstable

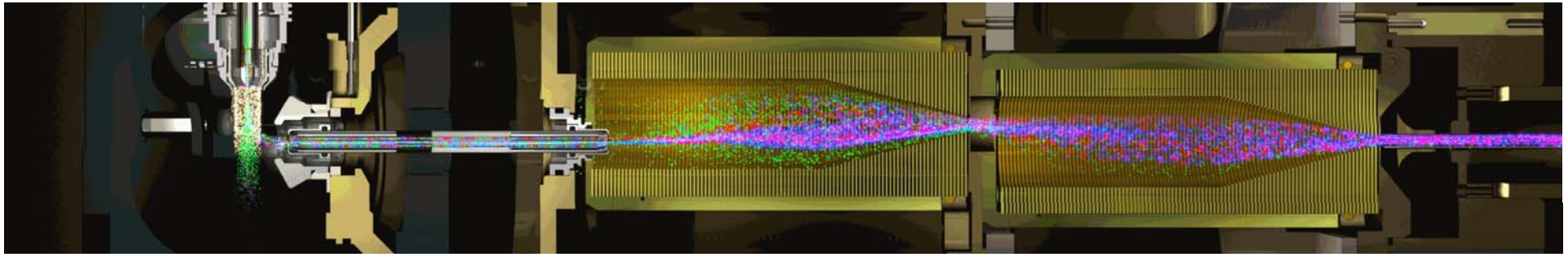
Target Analysis
已知物分析

6400 LC-QQQ

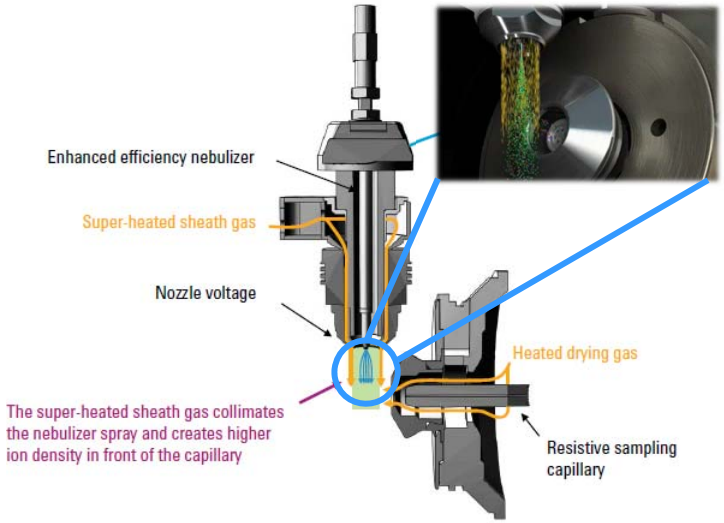
Dynamic MRM
tMRM

Thermal unstable & Polarity
热不稳定 & 极性组分

Proven iFunnel Technology

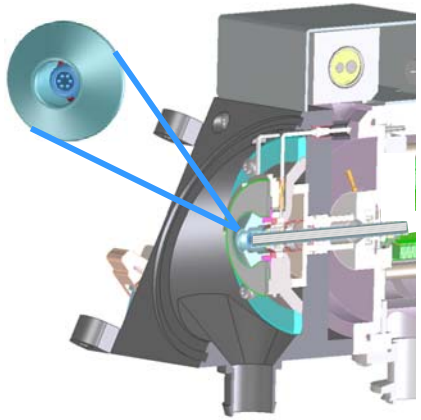


Agilent Jet Stream



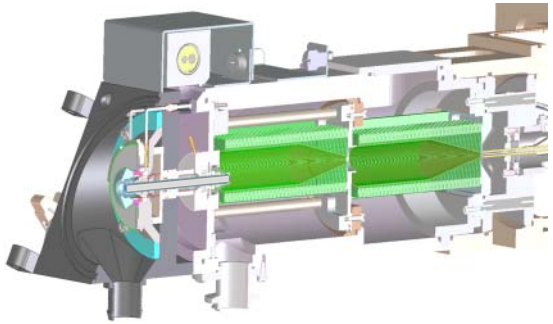
- Thermal gradient focusing
- Efficient desolvation
- Creates an ion rich zone

Hexabore Capillary



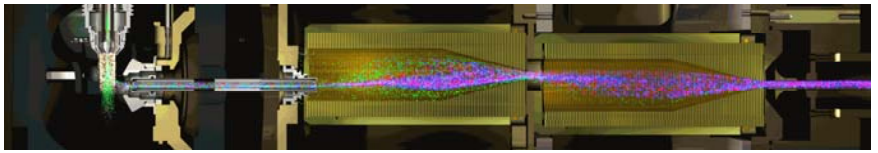
- Six capillary inlets
- Samples x10 times more ion rich gas

Dual Ion Funnel

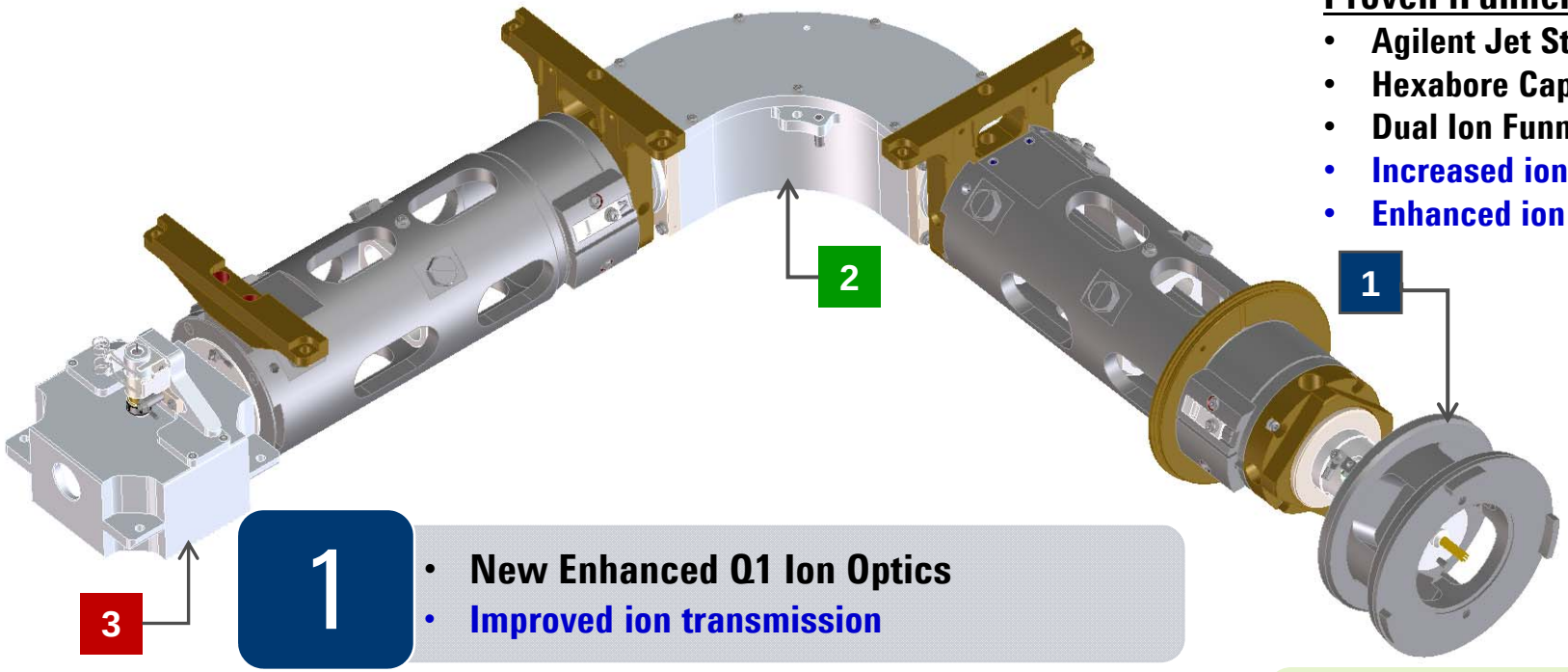


- Removes the gas but captures the ions
- Removes neutral noise

6495 QQQ Technologies
Continued Development



Proven iFunnel Technology
- Agilent Jet Stream
- Hexabore Capillary
- Dual Ion Funnel
- Increased ion generation
- Enhanced ion sampling



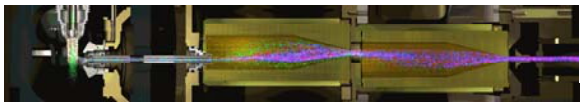
1
- New Enhanced Q1 Ion Optics
- Improved ion transmission

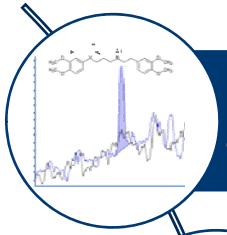
2
- New Tapered Hexapole Collision Cell
- Effective ion collection and transmission

3
- New Detector with High Energy Conversion Dynode
- Improved NEG ion detection with low noise

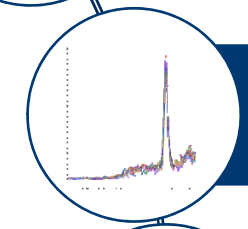
Quantitative Applications
- Enhanced peak area response
- Improved peak area %RSD
- More sensitive and precise
- Lower Limits of Detection (IDL)and Quantitation (LLOQ)
- More reliable and robust

6495 QQQ - Premium Performance

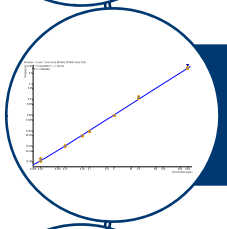




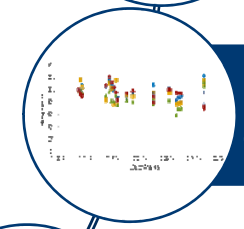
Improved sensitivity and lower LLOQs
– Average 3x in S/N specifications and applications



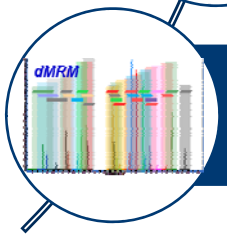
Improved precision and excellent accuracy at the lowest levels – 3x in IDL specifications



Proven 6 orders of linear dynamic range



Proven robustness in complex matrix
– Food matrix and biological matrix (plasma)



Improved mass range, fast scan speed and MRM acquisition rate

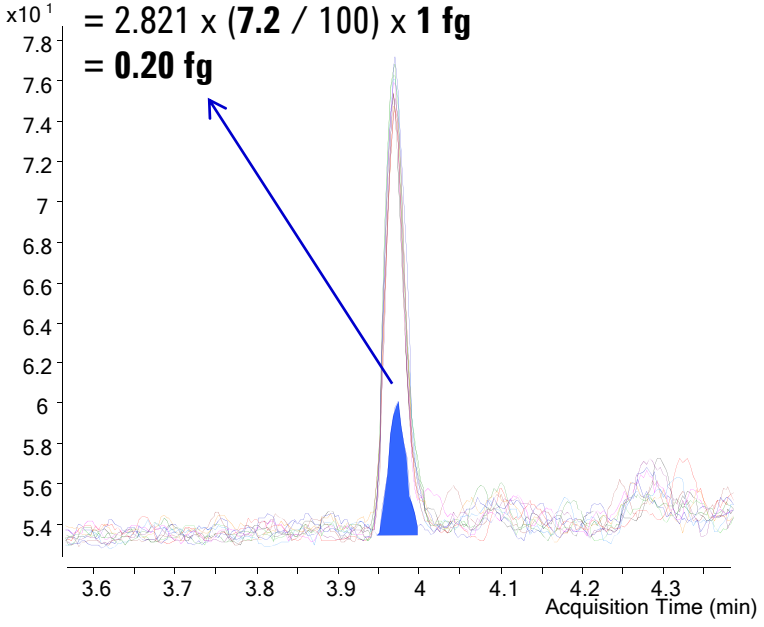
Improved Sensitivity and Precision – 6495 QQQ IDL

IDL for the Agilent 6495 QQQ LC/MS System

6495 QQQ IDL	Amount measured	Replicates	Area %RSD	t (99%)	IDL
Reserpine (+)	**1 fg** **5 fg**	n = 10	**7.2** **7.2**	2.821	**0.20 fg** **1.02 fg**
Chloramphenicol (-)	**1 fg** **5 fg**	n = 10	**9.7** **8.9**	2.821	**0.27 fg** **1.25 fg**

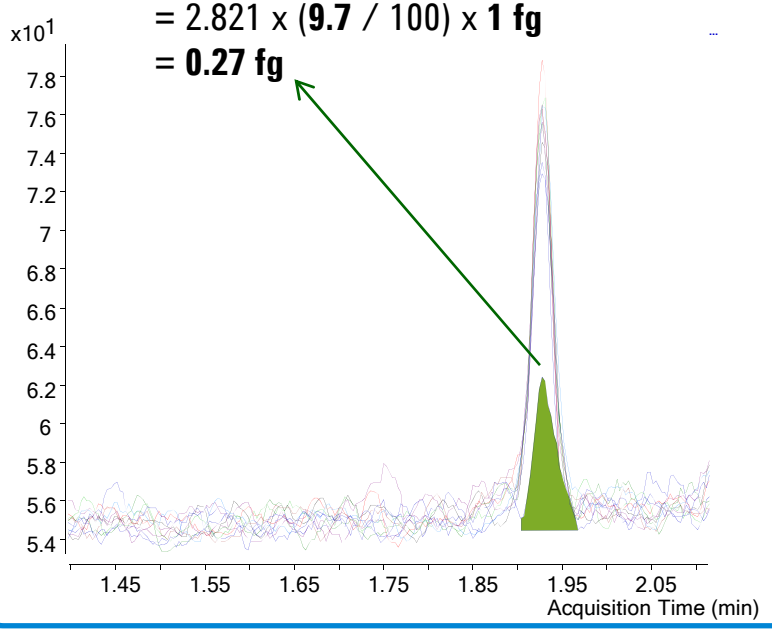
1 fg of reserpine used to measure IDL (+)

IDL = t x (%RSD / 100) x Amount measured
= 2.821 x (**7.2** / 100) x **1 fg**
= **0.20 fg**



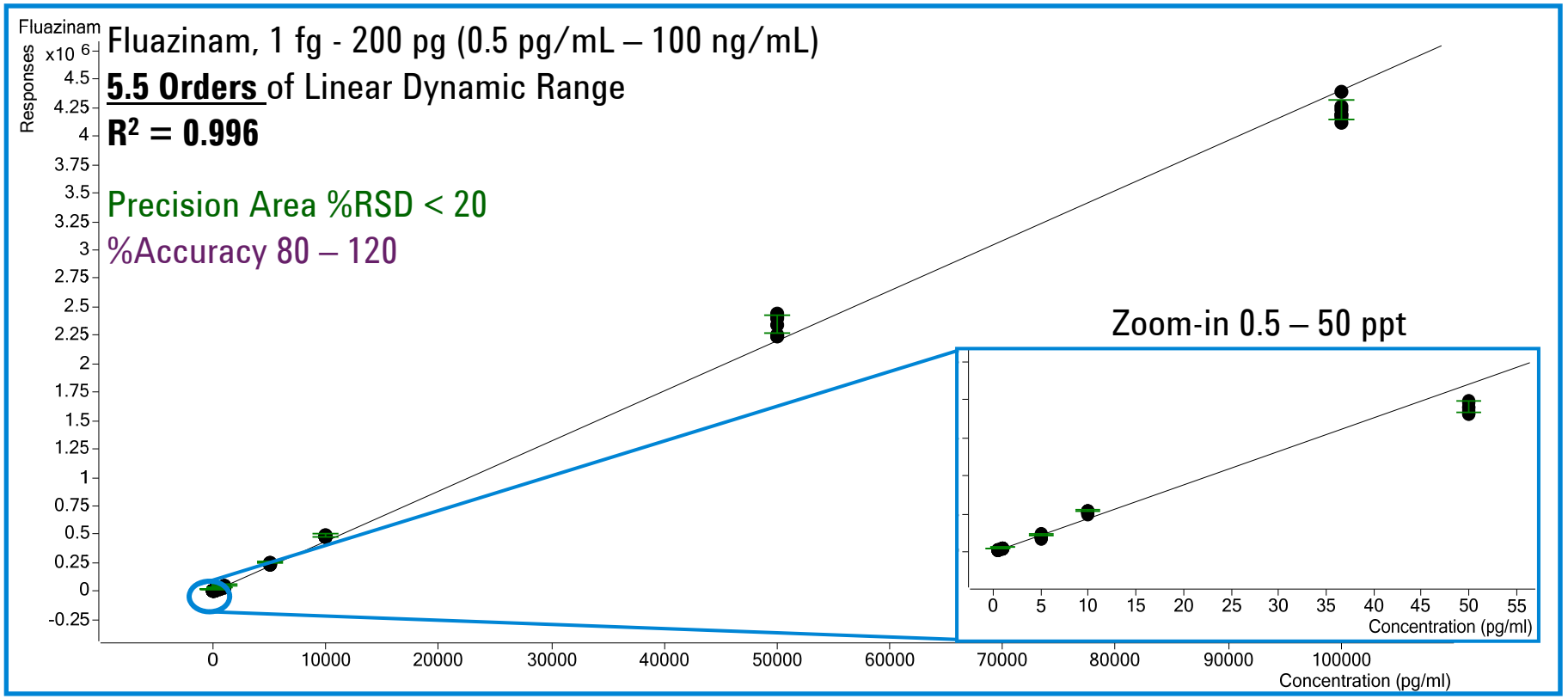
1 fg of chloramphenicol used to measure IDL (-)

IDL = t x (%RSD / 100) x Amount measured
= 2.821 x (**9.7** / 100) x **1 fg**
= **0.27 fg**



Improved **precision** (%RSD) and Instrument Detection Limit (**IDL**) achieved on the **6495** vs. the **6490**

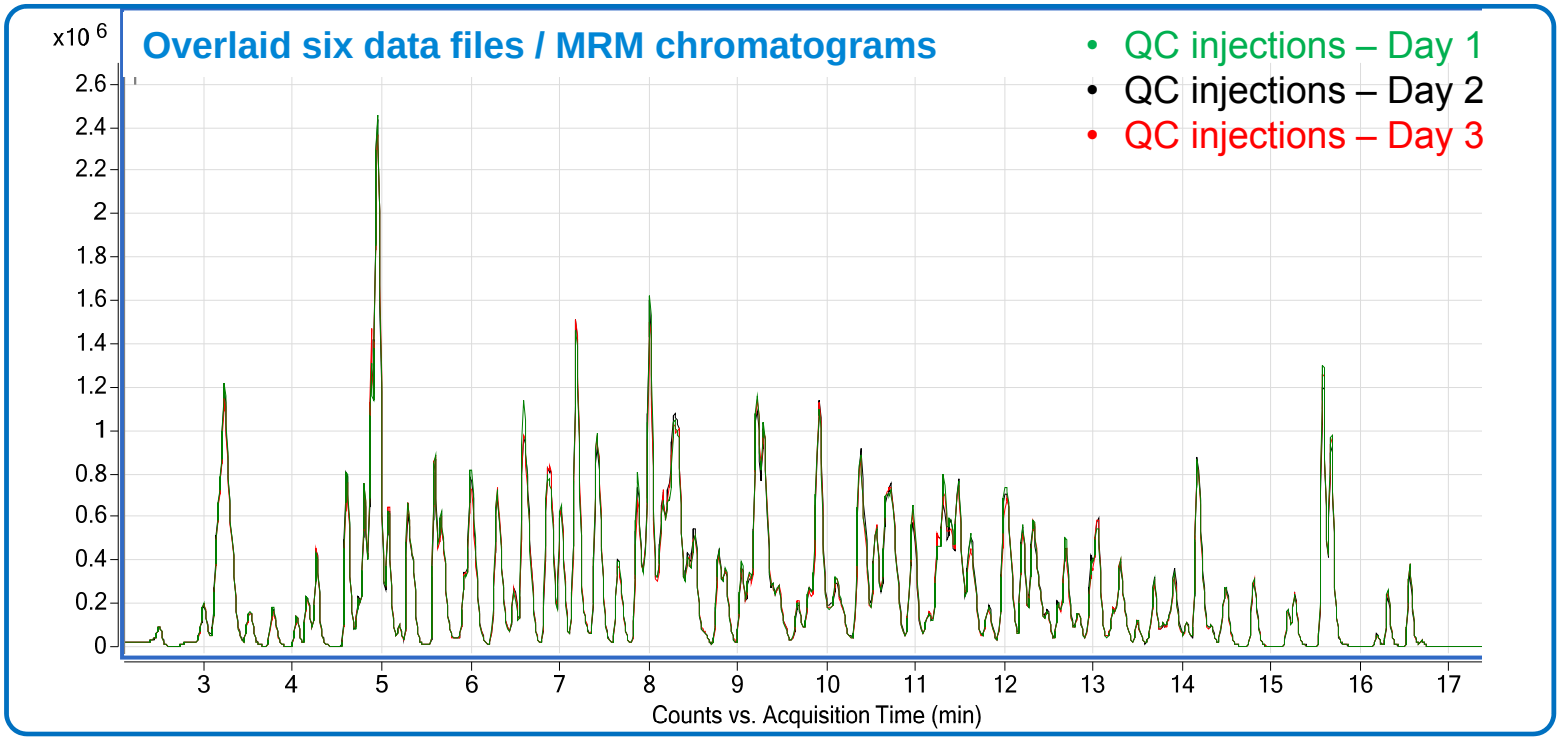
Wide Linear Dynamic Range, Pesticide Fluazinam



Level (ng/mL)	0.5	1	5	10	50	100	500	1,000	5,000	10,000	50,000	100,000
%RSD (n = 7)	9.2	11.4	8.1	2.13	3.59	1.19	3.01	3.44	3.53	1.86	3.66	2.13
%Accuracy	100.7	100.6	92.5	117.4	85.7	82.4	83.1	90.2	109.9	108.8	105.9	95.8

- Wide (5.5 orders) **linear dynamic range**, excellent assay **accuracy** and **precision** at all levels

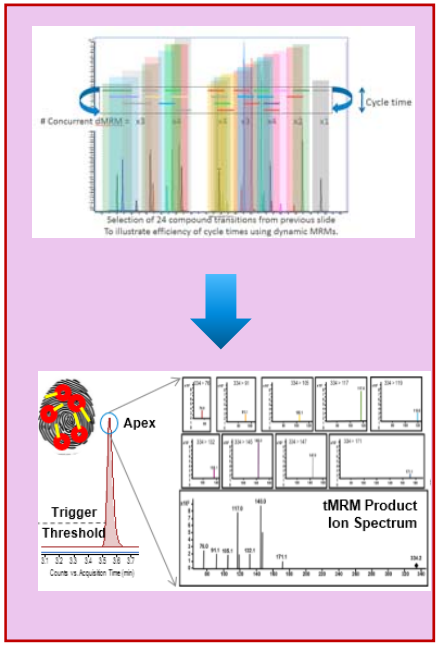
Proven System Robustness in Complex Food Matrix:
Multi-Residue Pesticide Analysis in Black Tea

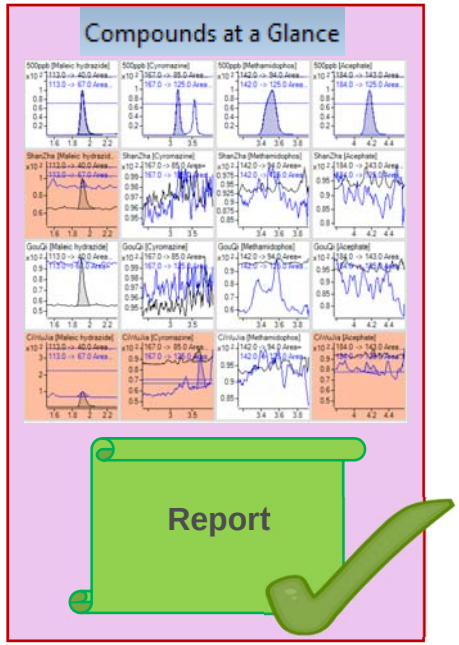


- Pesticide standards analyzed before, in the middle, and after matrix injections
- No signal decrease was observed after **3 days** of operation running with **heavy food matrix (black tea)**
- **%RSD < 6** (n = 6 data files) – Average results of > 250 pesticides analyzed

QQQ---Method development workflow of target compounds
目标化合物监测方法开发流程

Auto-optimizer MS parameters → Optimizer Chromatographic → Acquisition by dMRM/tMRM → Generate reports





1. Optimizer**自动优化软件自动优化化合物子离子，**Fragmentor**和碰撞能量**

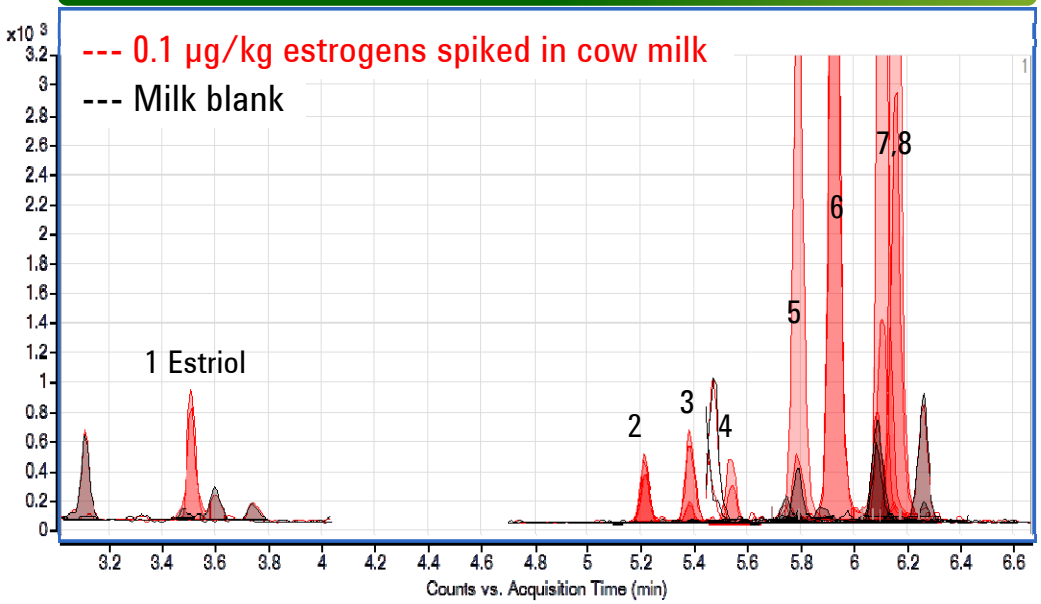
2. 优化色谱分离条件或利用iSET技术转化原有色谱条件

3.对于多农残或多兽残分析可以升级为动态或触发式采集模式，兼顾灵敏度及采集速率

4. 流程式定量报告和快速预览界面，灵活可编辑报告格式

Sensitive Quantitation of Estrogens in Cow Milk

Eight estrogens spiked in cow milk at 0.1 µg/kg

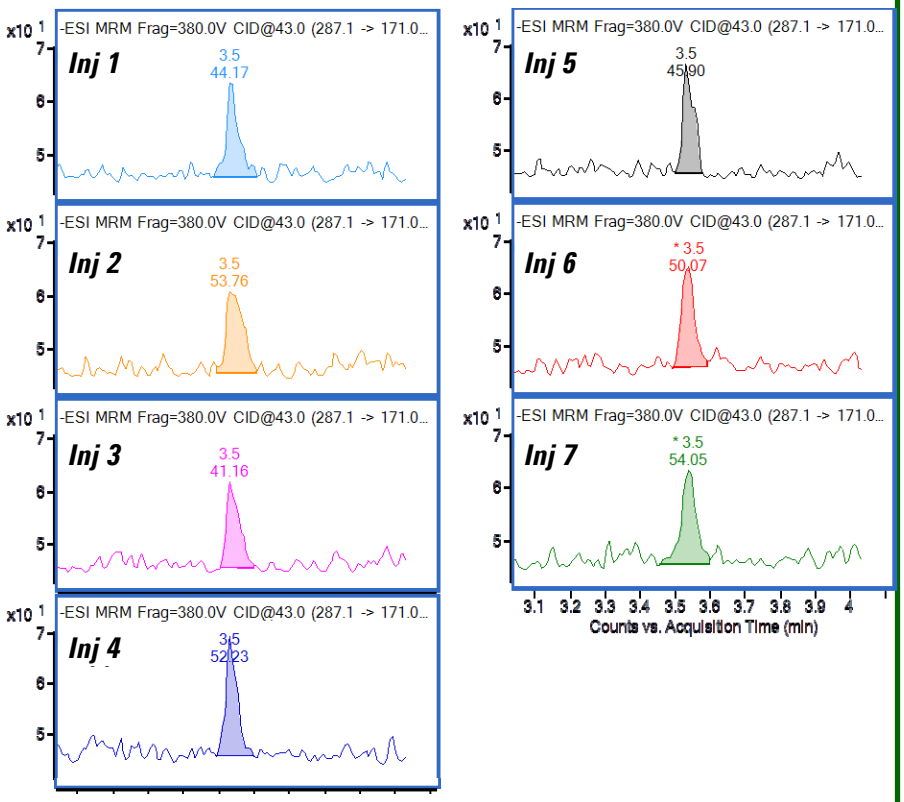


ID	Compounds	LLOQ (pg/mL)	%RSD n = 7	IDL (pg/mL)
1	Estriol	5	11.3	1.8
2	17-α-Estradiol	10	8.4	2.7
3	17-β-Estradiol	10	11.4	3.6
4	Ethynyl estradiol	10	11.7	3.7
5	Estrone	5	9.6	1.5
6	Diethylstilbestrol	10	12.8	2.0
7	Hexestrol	5	11.1	1.7
8	Dienestrol	5	7.6	1.2

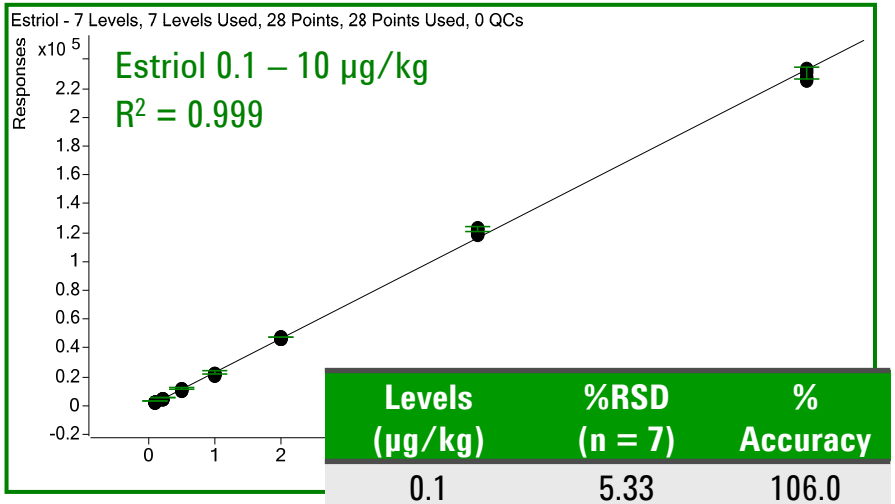
- Estrogen levels in milk products are regulated by government agencies down to 1 µg/kg
- Highly **sensitive** and **precise** quantitation of estrogens was achieved using the new 6495 with a simple QuEChERS procedure without derivatization or enrichment.
- **LLOQs** and **IDLs** are at low pg/mL level (low-fg on-column), far below the regulatory requirement

LLOQs and IDLs at pg/mL (fg) level

Estriol LLOQ level of 5 pg/mL, 25 fg on-column



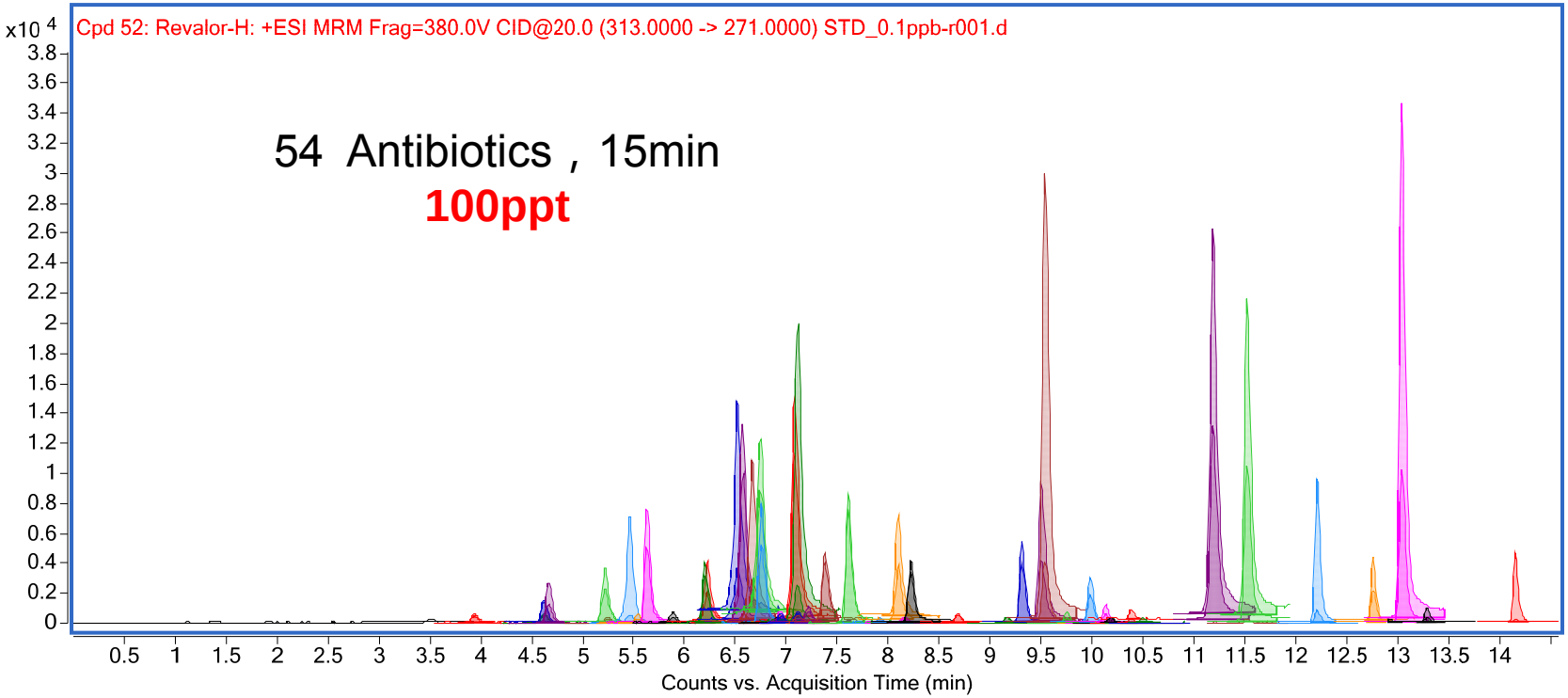
Replicates	%RSD	Estriol IDL
n = 7 injections	11.3	1.8 pg/mL, 8.9 fg



Levels (µg/kg)	%RSD (n = 7)	% Accuracy
0.1	5.33	106.0
0.2	2.96	101.2
0.5	2.99	94.6
1	2.75	95.3
2	0.94	100.3
5	1.68	104.1
10	1.64	98.5

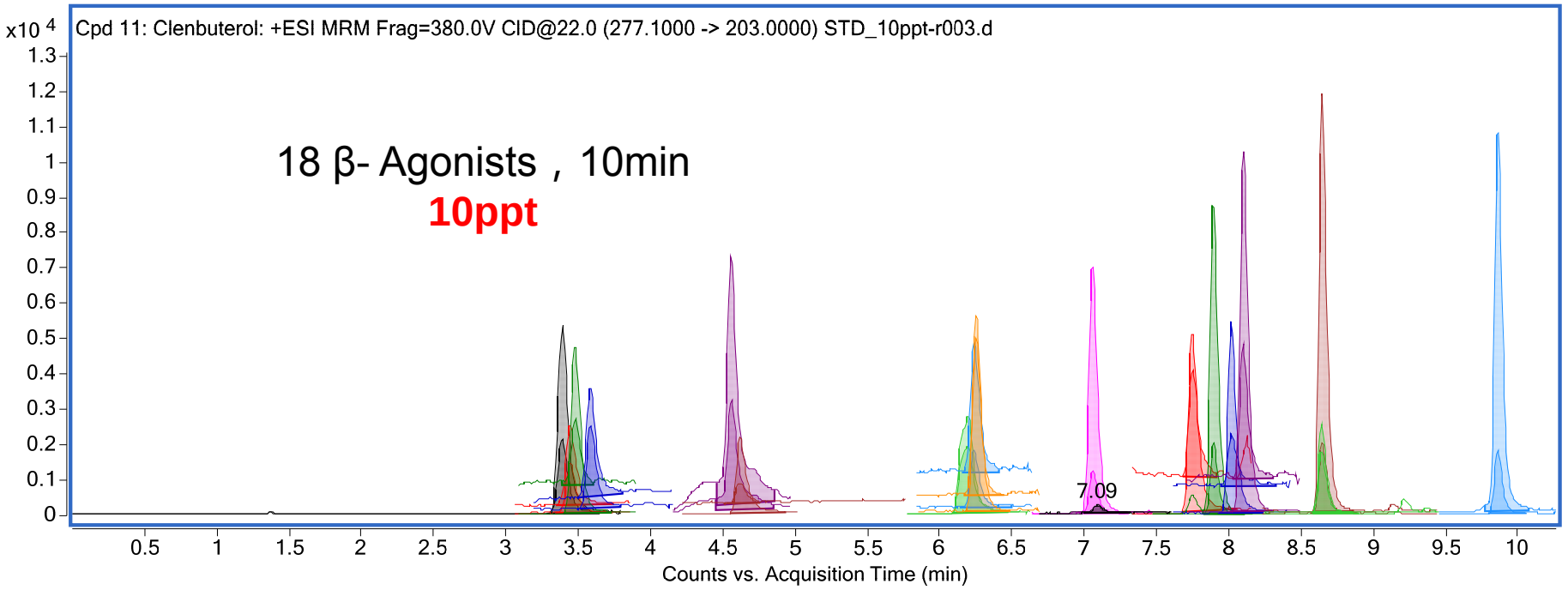
- Excellent **precision** and **accuracy** are observed at the lowest levels (LLOQs)
- Excellent **linearity** in quantitation range

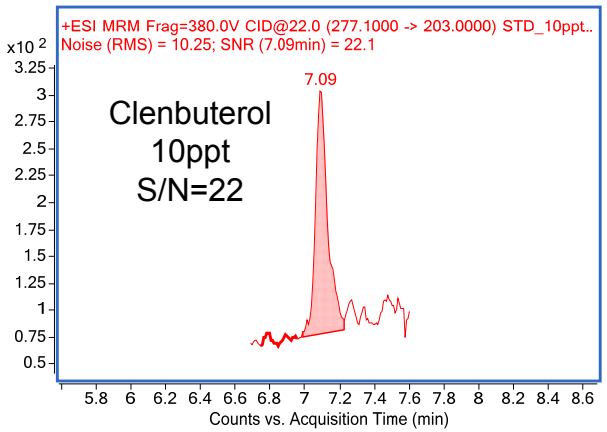
Multi- Veterinary Drugs Analysis by 6490QQQ

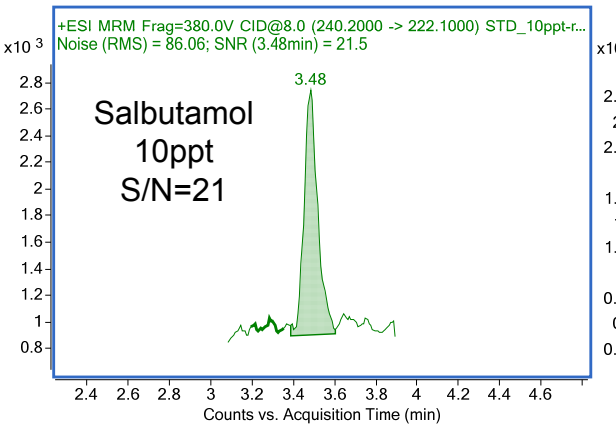


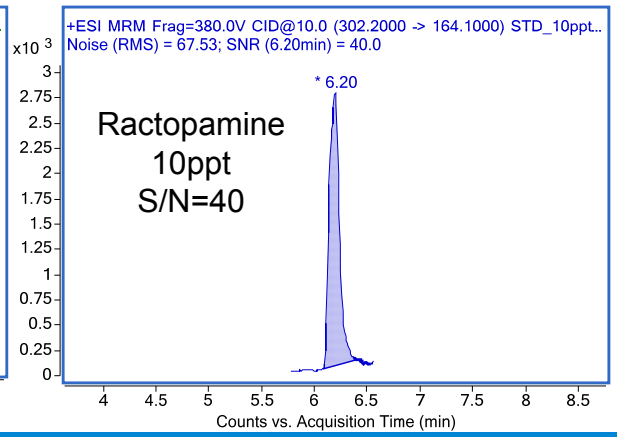
磺胺间甲氧嘧啶	磺胺氯哒嗪	恩诺沙星	奥比沙星	地塞米松	群勃龙醋酸酯
磺胺嘧啶	磺胺异恶唑	诺氟沙星	培氟沙星	氢化可的松	喹乙醇
磺胺甲氧嘧啶	磺胺二甲嘧啶	沙拉沙星	萘啶酸	泼尼松龙	曲吡那敏
磺胺甲噻二唑	苯甲酰磺胺	环丙沙星	氟甲喹	敌菌净	氟尼辛
磺胺噻唑	磺胺甲氧哒嗪	麻保沙星	恶喹酸	乙胺嘧啶	吡喹酮
磺胺二甲氧嘧啶	磺胺喹恶啉	二氟沙星	仲丁威	氯丙嗪	氨基比林
磺胺醋酰	磺胺吡啶	氧氟沙星	泰勒菌素	奥美普林	安替比林
磺胺甲恶唑	甲氧苄啶	洛美沙星	林可霉素	克球酚	左旋咪唑
磺胺甲基嘧啶	磺胺邻二甲氧嘧啶	丹诺沙星	替米考星	乙氧酰胺苯甲酯	氯舒龙

β- Agonists Analysis by 6490QQQ

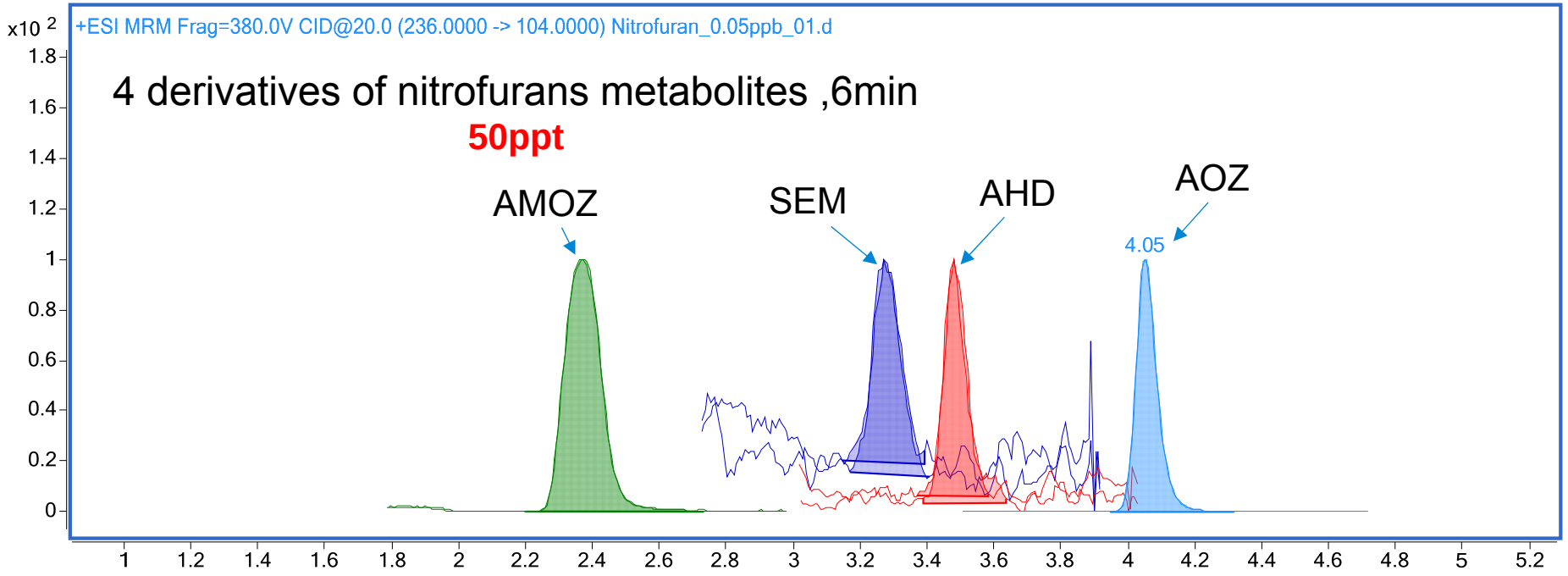


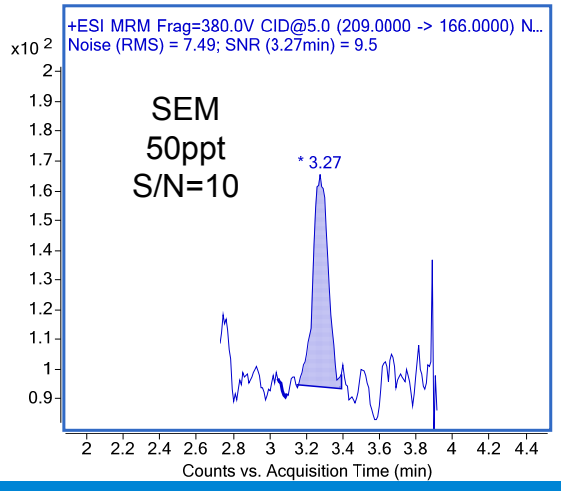


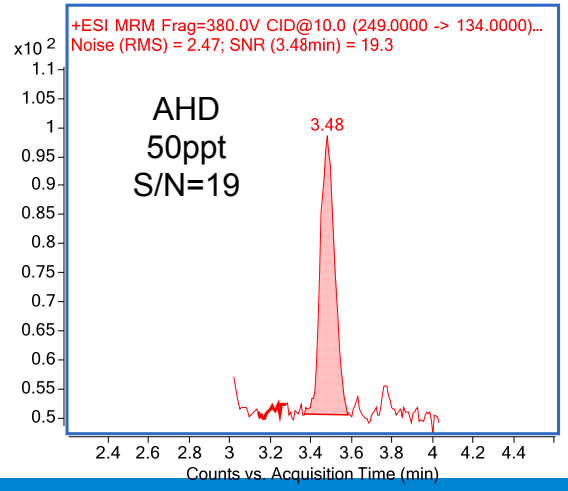




Nitrofurnans Analysis by 6490QQQ

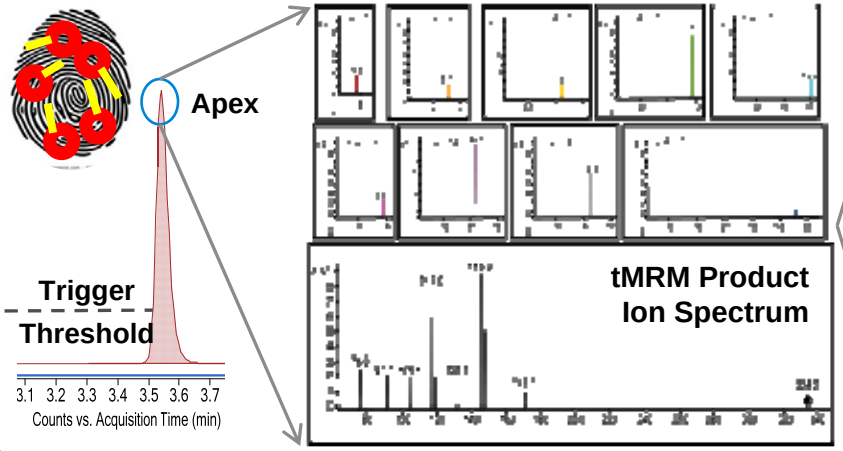


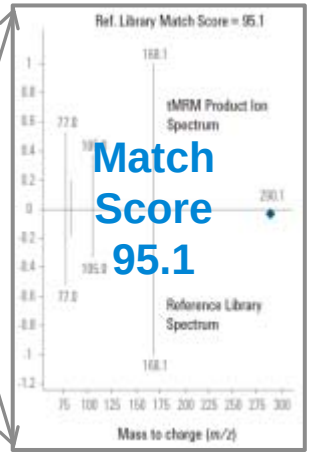




Triggered MRM (tMRM)-触发多反应通道监测

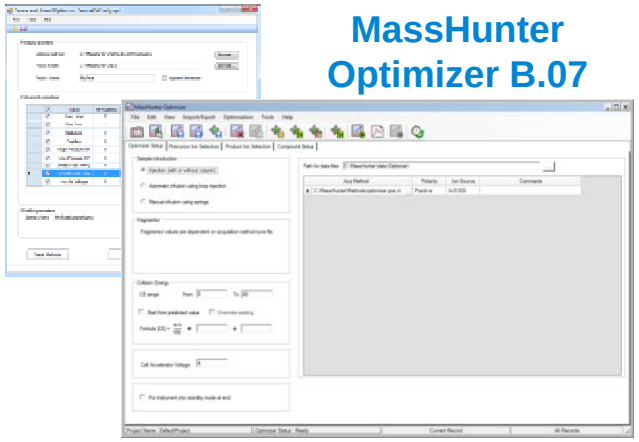
Triggered MRM, tMRM Database and Library





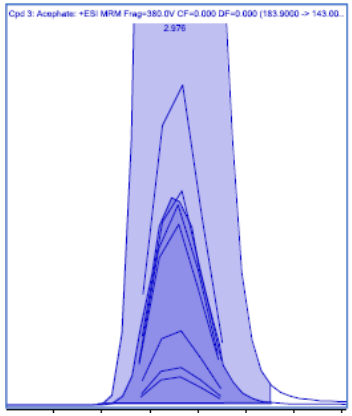
- tMRM database accelerates acquisition method setup
- tMRM library – Match Score for confirmation
- tMRM is Fast and Sensitive
- UHPLC compatible
- Simultaneous Quantitation, Screening and Confirmation

Enhanced Optimizer Workflow in MassHunter B.07 (to Better Support tMRM)



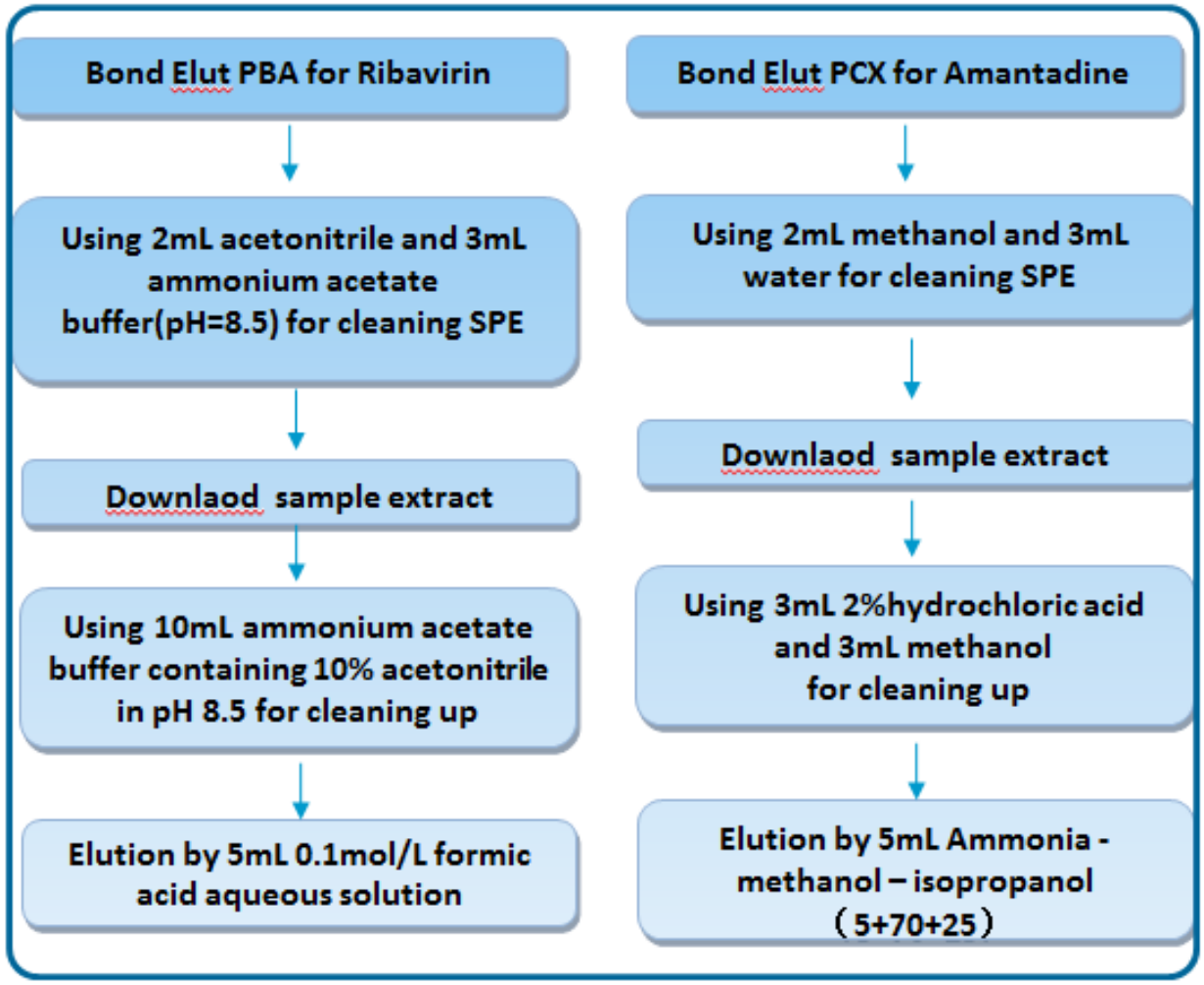
MassHunter Optimizer B.07

- Find 10 MRM transitions in Optimizer
- Injection volume flexibility
- Improved method editing

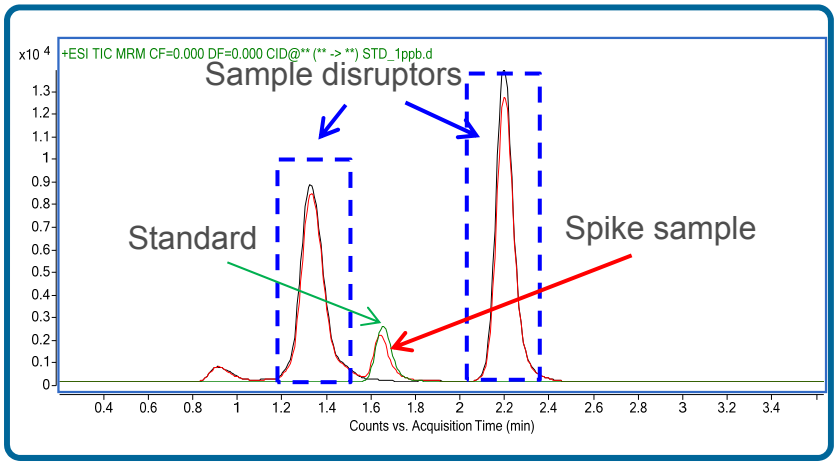


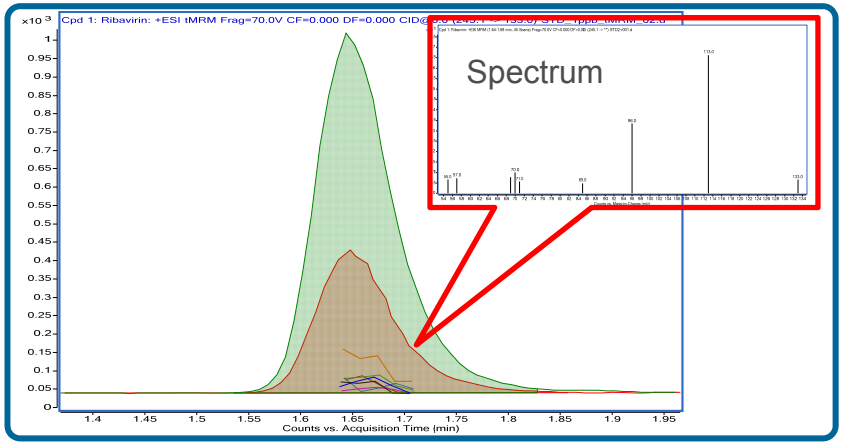
Quantitation of antiviral drugs in chickens by UHPLC-QQQ with triggered MRM for unprecedented confirmation

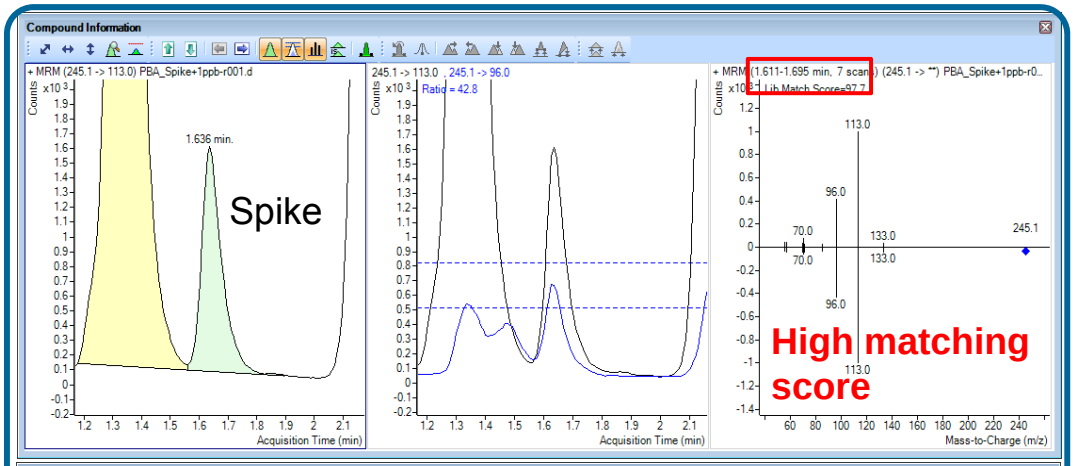
Sample Preparations

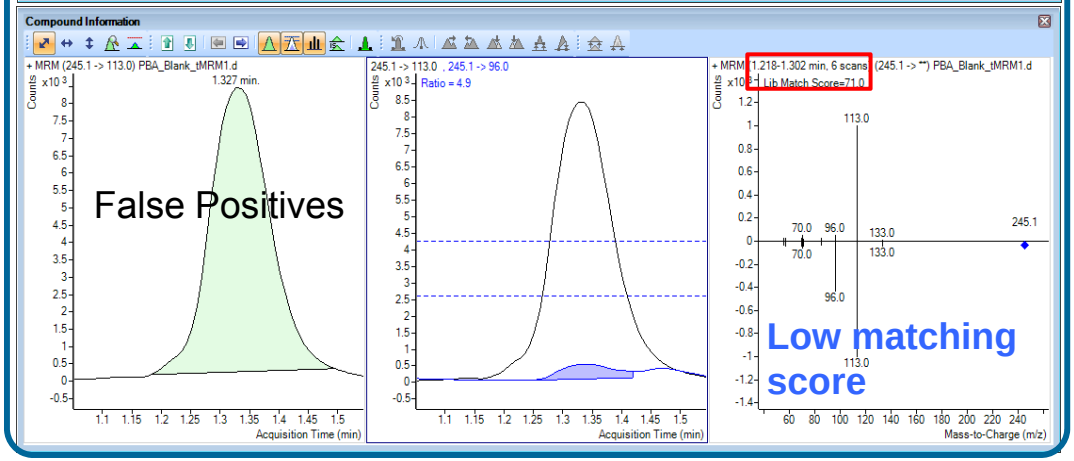


Improve the ability of the confirmation false positive results









Targeted Screening & Confirmation with QQQ

Pesticides

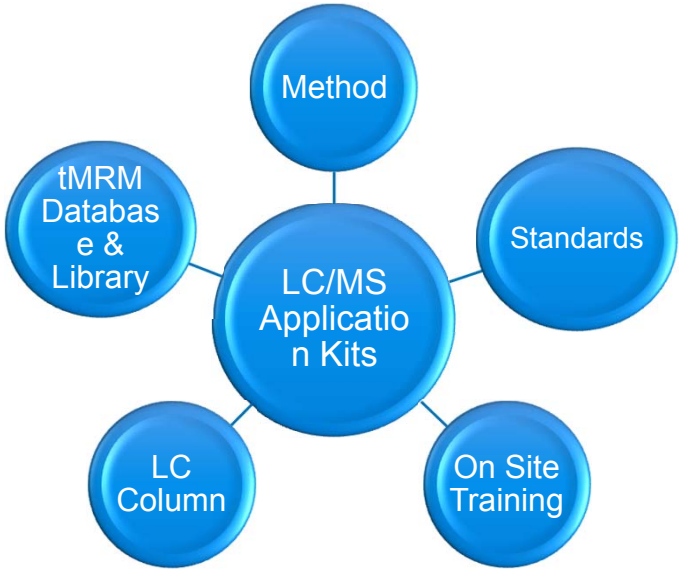
Test Mix: 254 compounds
DB: 700+ compounds
Library: 200+ compounds

Veterinary Drugs

Test Mix: 146 compounds
DB: 500+ compounds
Library: 100+ compounds

Forensic Toxicology

Test Mix: 139 compounds
DB: 2500+ compounds
Library: 100+ compounds



- ***Agilent unique data dependent acquisition for fast and sensitive compound screening, quantitation and confirmation.***

Analysis Strategy of Food Safety
食品安全分析策略

7200 GC-QTOF

Volatility
Thermally
stable

Thermally stable & Nonpolar
热稳定 & 非极性组分

非靶标化合物分析

Unknown Screening – what's in the sample?

靶标化合物分析

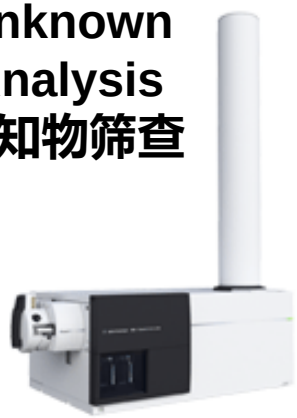
Target Screening – are specific compounds present & how much?

Non-Selective ⟷ Selective

Volatility
Thermally
stable

7000C GC-QQQ

Unknown Analysis
未知物筛查



6500 LC-QTOF

PCDL
MSC
All ion MSMS

Nonvolatile
Thermal
unstable

Nonvolatile
Thermal
unstable

Target Analysis
已知物分析

6400 LC-QQQ

Dynamic MRM
tMRM

Thermal unstable & Polarity
热不稳定 & 极性组分

6550 Instrument Technologies

Comprehensive Performance Enhancements

- Mass Resolution >40,000
- 50 spectra /sec MS and 33 spectra/sec MS/MS
- 5 orders of linear dynamic range
- <1 ppm MS mass accuracy; <2 ppm MS/MS
- Unrivalled sensitivity

Longer Invar flight tube (resolution + stable mass accuracy)

New- Ion Beam Compression and Shaping (resolution + sensitivity)

Dual Agilent JetStream orthogonal spray (robust and stable mass calibration)

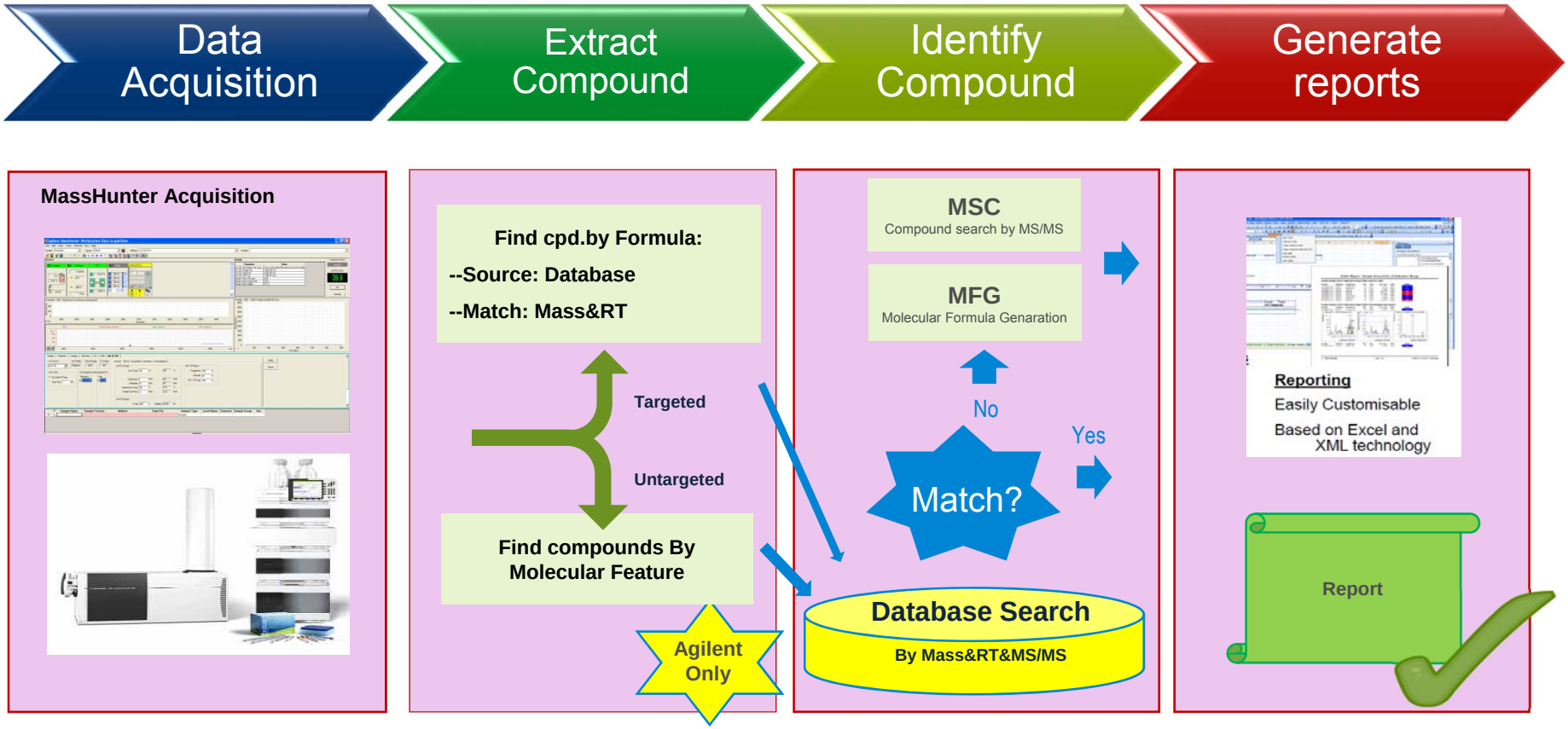
New embedded processor (50 spectra/sec)

New- iFunnel (10X sensitivity gain)

4 GHz digitizer + ADC detector (resolution, mass accuracy, dynamic range)

Hexapole axial focusing collision cell (faster MS/MS spectra)

QTOF---- Non target compounds screening identification workflow
非目标化合物筛查鉴定流程



1. Auto MS/MS自动二级质谱扫描模式同时完成一级二级质谱数据采集

2. 依据农残数据库，按精确分子量、同位素分布和保留时间提取化合物

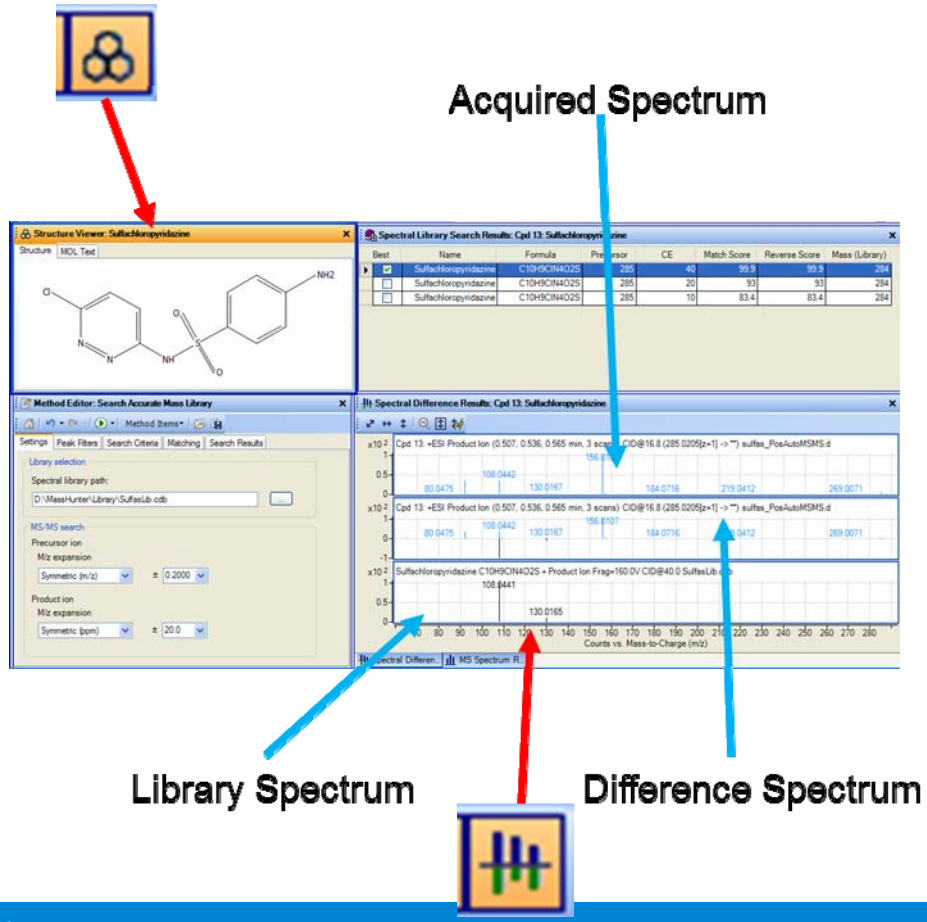
3. 二级谱图确认，如谱库中无标准谱图，可以使用MSC软件进行结构推测与确认

4. 生成报告

Identify with Confidence:
Personal Compound Database and Library (PCDL)

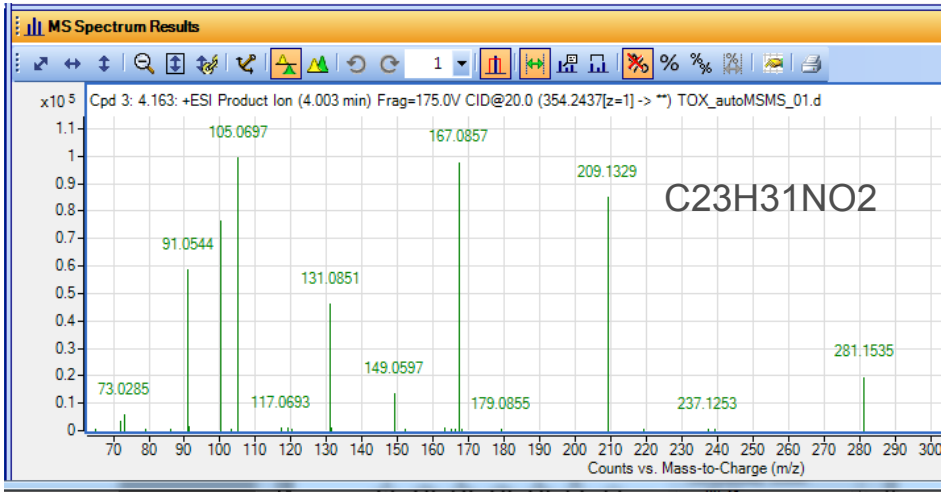
Agilent is the first vendor to sell accurate mass MS/MS libraries

- Higher confidence identifications
- Run library searches from Qual
  or PCDL Manager
- Display structures and difference
  spectra of hits in Qual
- Customers can create their own libraries
  or append to a purchased library

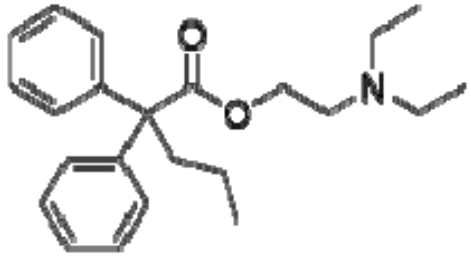


Identify with Confidence: Molecular Structure Correlator (MSC)

- Use MS/MS spectra to go from formula to structures
  - Match substructures to specific fragments for positive identifications
  - Access formula and structures from Agilent PCD/PCDLs or the ChemSpider database (26 million compounds) to provide the most likely structures (only Agilent offers this capability!)



MSC

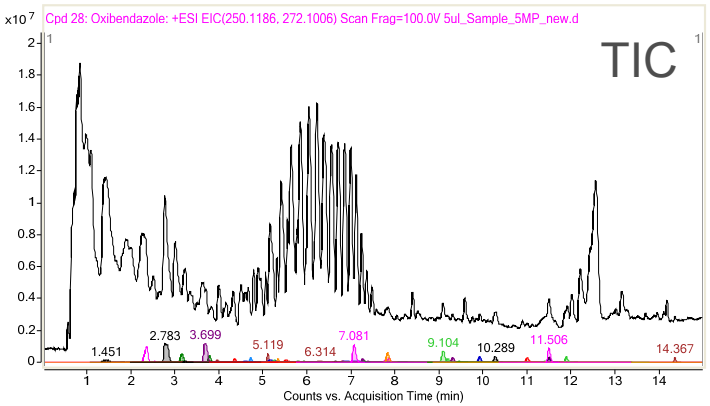


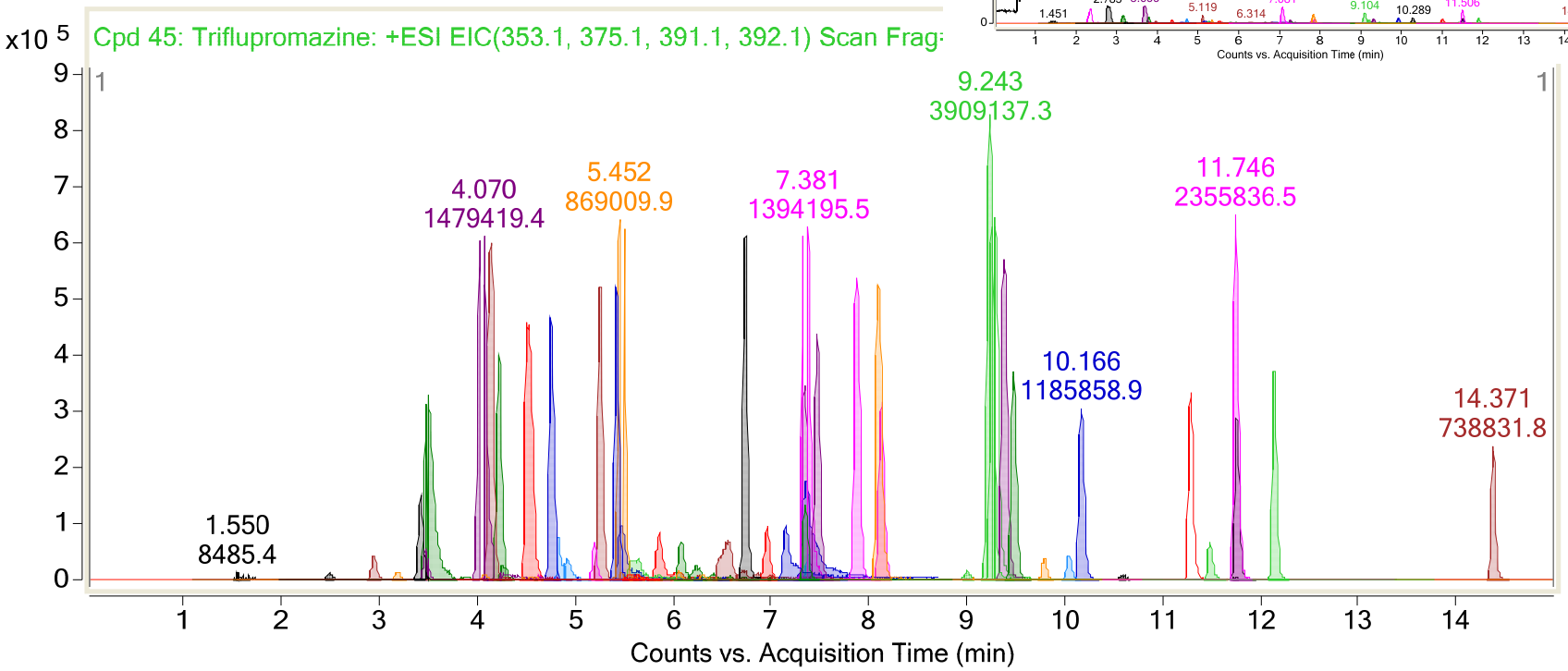
Proadifen

Veterinary drug screening project

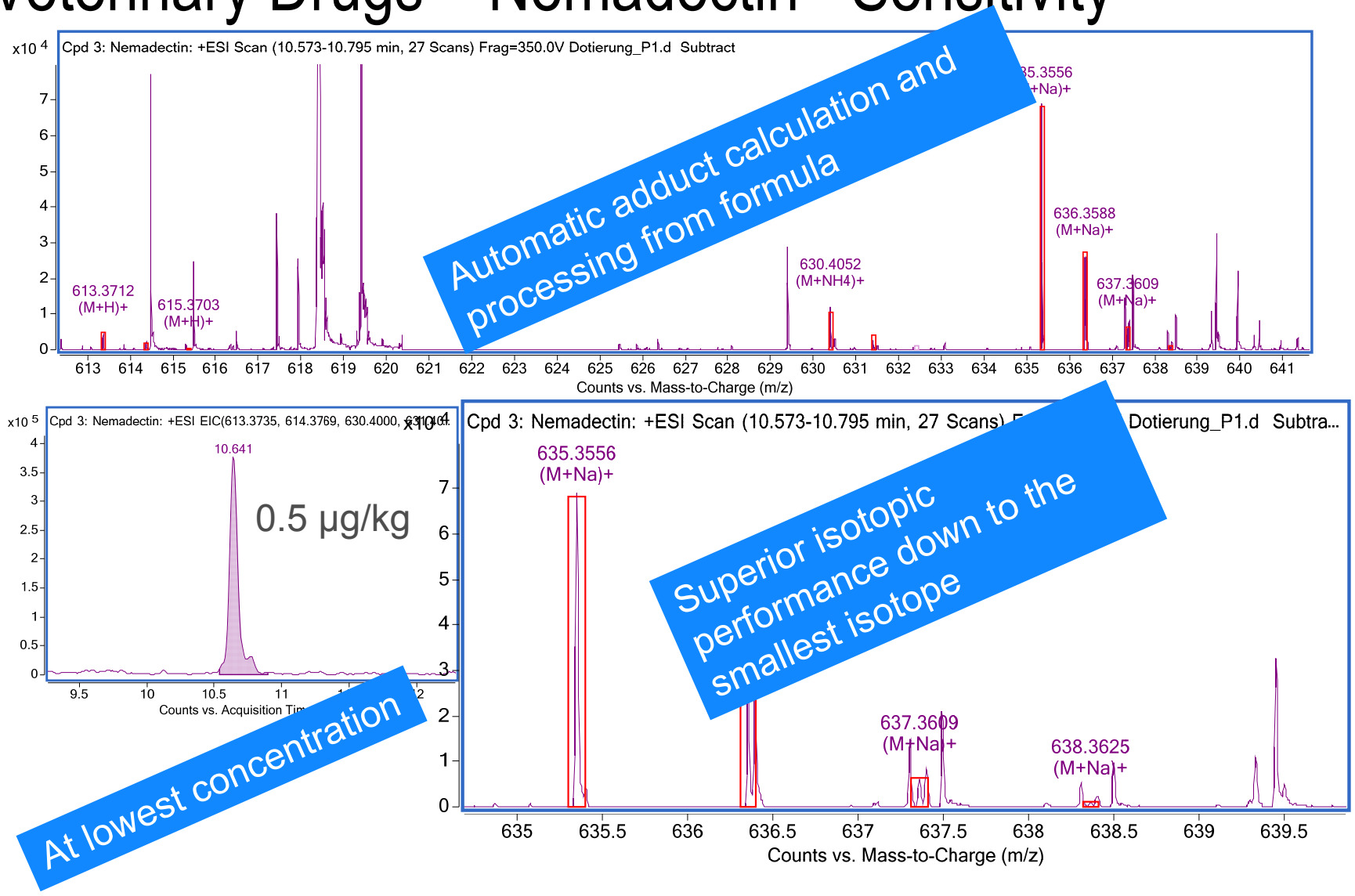
UHPLC-(Q)TOF screening

- Pig muscle extract spiked with 50 compounds
- Type A compounds: not authorized or forbidden; spiked @ 20 ng/ml
- Type B compounds: listed in 37/2010/EC table 1; spiked @ 200 ng/ml

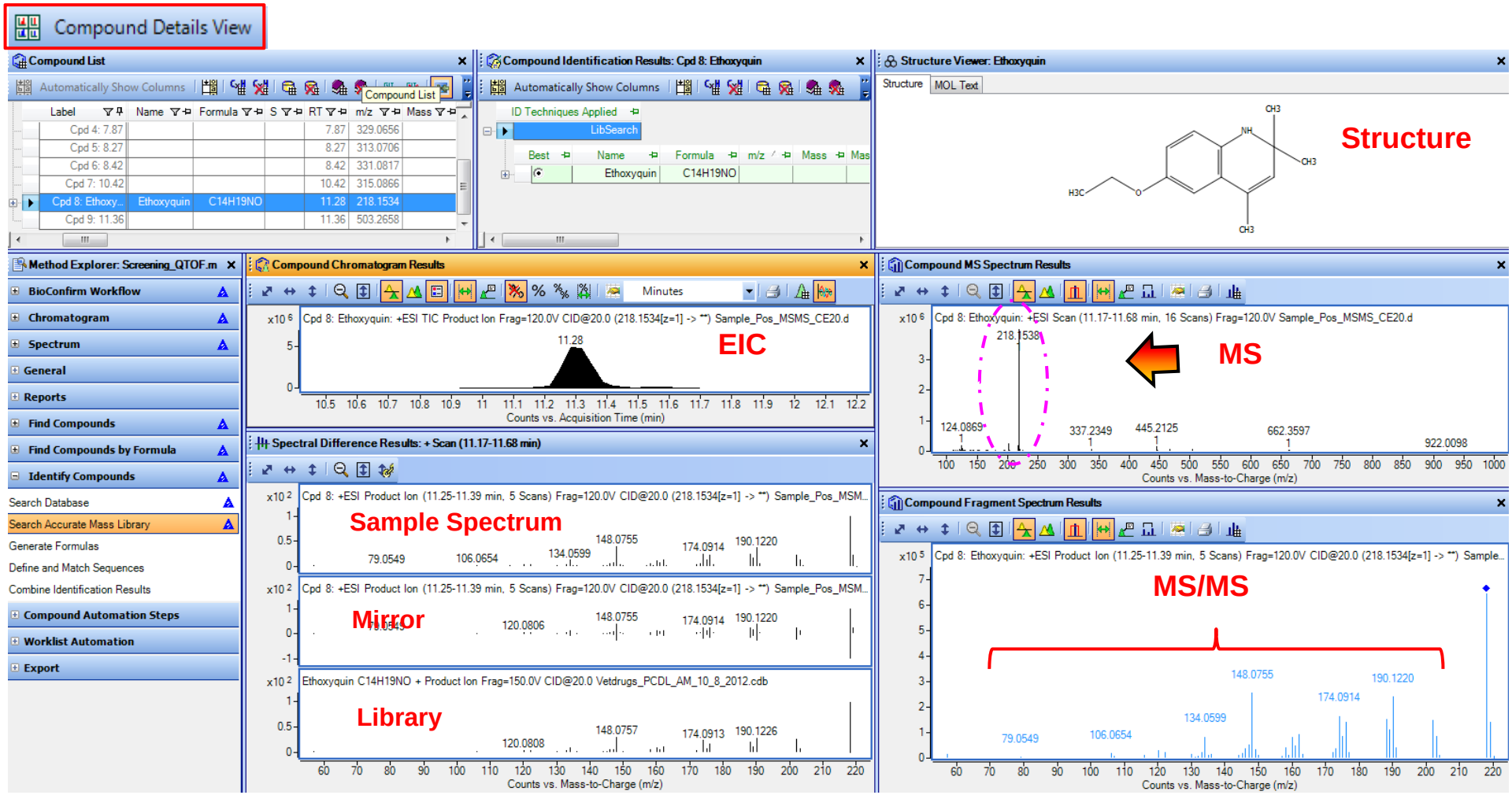




Veterinary Drugs – Nemadectin - Sensitivity



Screening results review



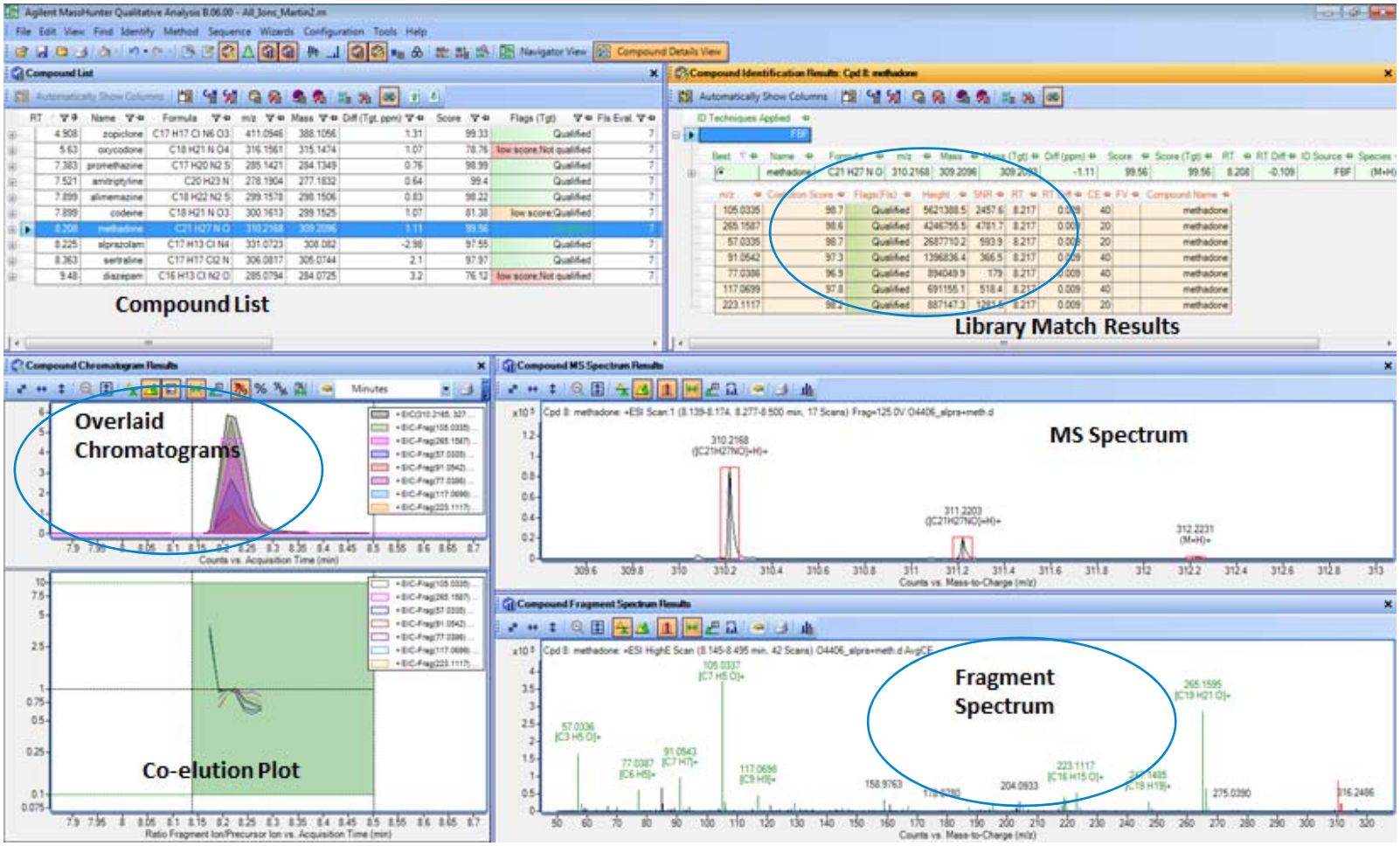
All Ions MS/MS

Create MS/MS Spectra in TOF with High Fragmentor Voltage

Great solution for screening in food, environmental and forensic/toxicology markets

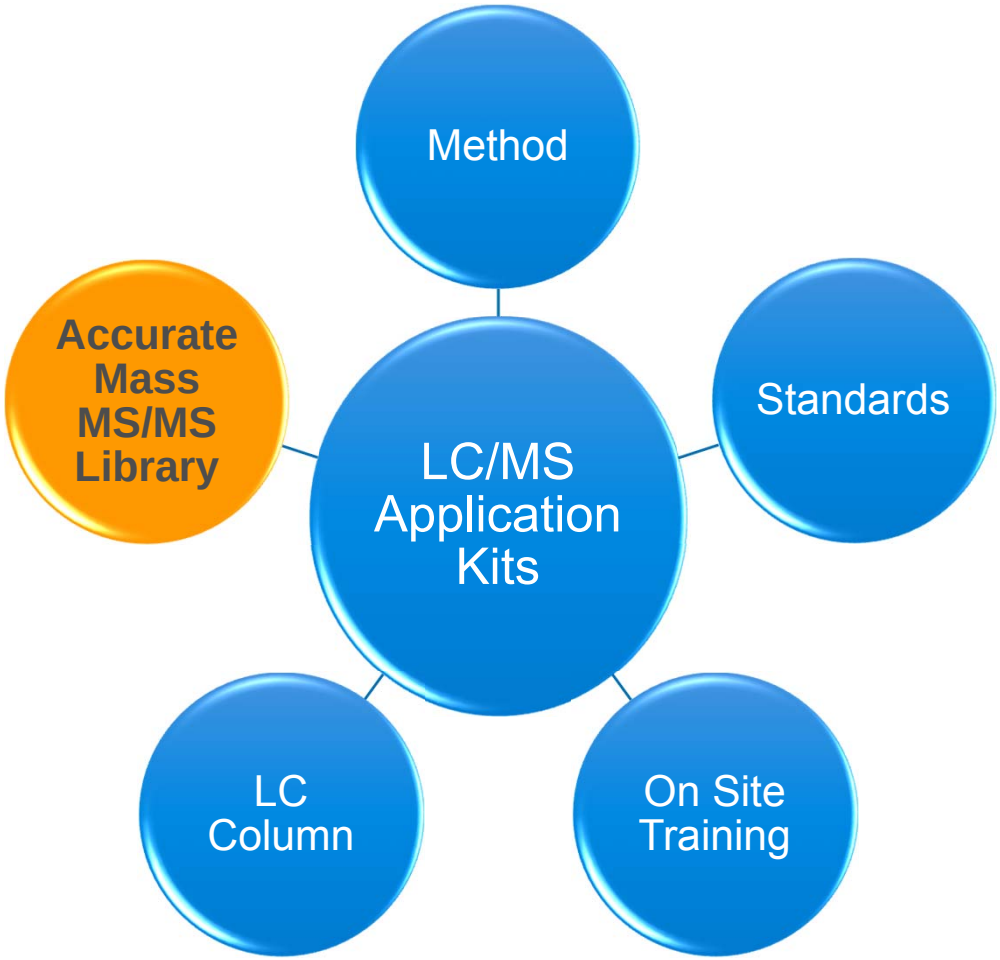
- Unique to Agilent:
  - MS/MS spectral libraries to identify fragments
  - coelution score to confirm fragments belong to the precursor ion
- Re-interrogate data for unknowns without reacquiring
- Quickly add unknowns to the MS/MS library and include screening
- Rapidly set up a quantitative method for multiple compound screening

Use of MSMS All Ions Browsing for High Confidence Data Review



Quick browsing compound details view to quickly inspect All Ions MSMS results

Untargeted Screening with TOF or QTOF



Pesticide Compounds

Test Mix: >250 (new)

Database: >1600

Library: >500 (new)

Veterinary Drugs - new

Test Mix: >200

Database: >1000

Library: >500

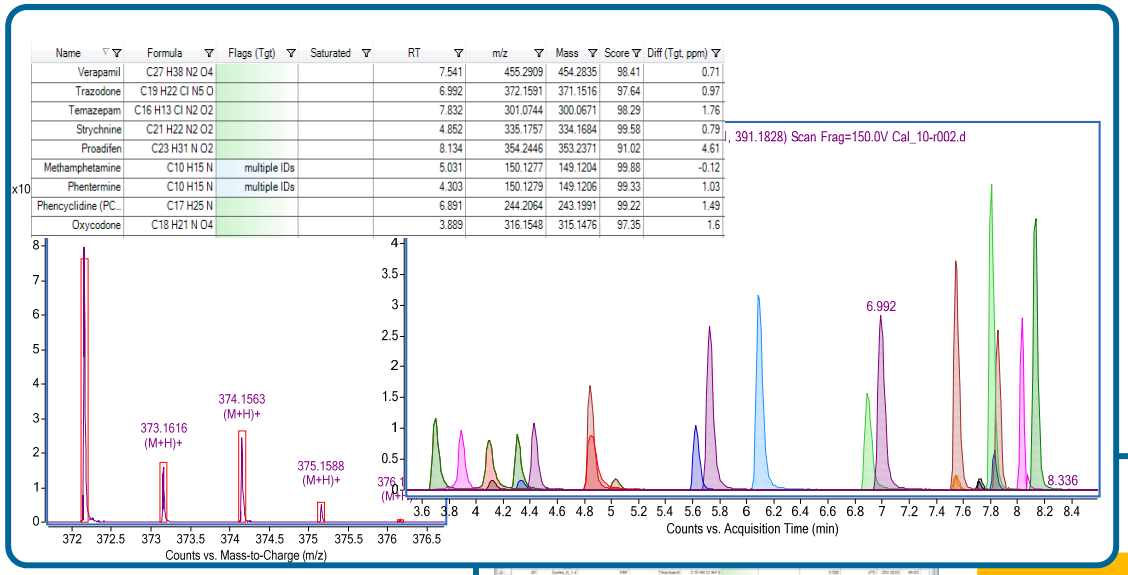
Forensic Toxicology

Test Mix: >100 (new)

Database: >9000

Library: >2500

Import and build Qual/Quant Method from Qual

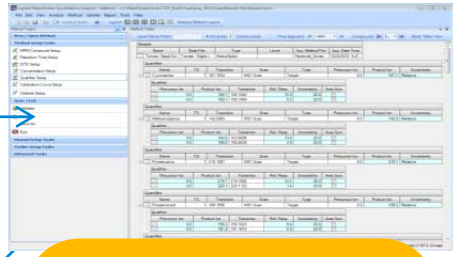


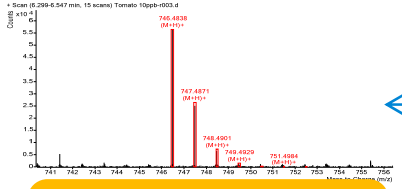
MH Qual

MH Quant

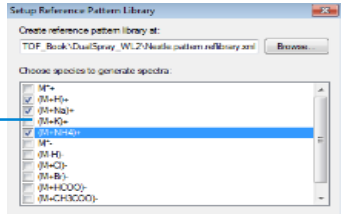
Compound results

.cef file





Reference pattern library



Adduct pattern

Autodynamic Quant method creation

- Accurate mass EICs for quantifier and qualifier
- Relative responses
- Retention times

Agilent Technologies

Thank You