Application of High Resolution Mass Spectrum in Residue Analysis

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Sep. 2016

Contents

- Background
- Recent research
  - Pesticides Screening Method
  - Drugs Screening Method

The challenges in residue analysis

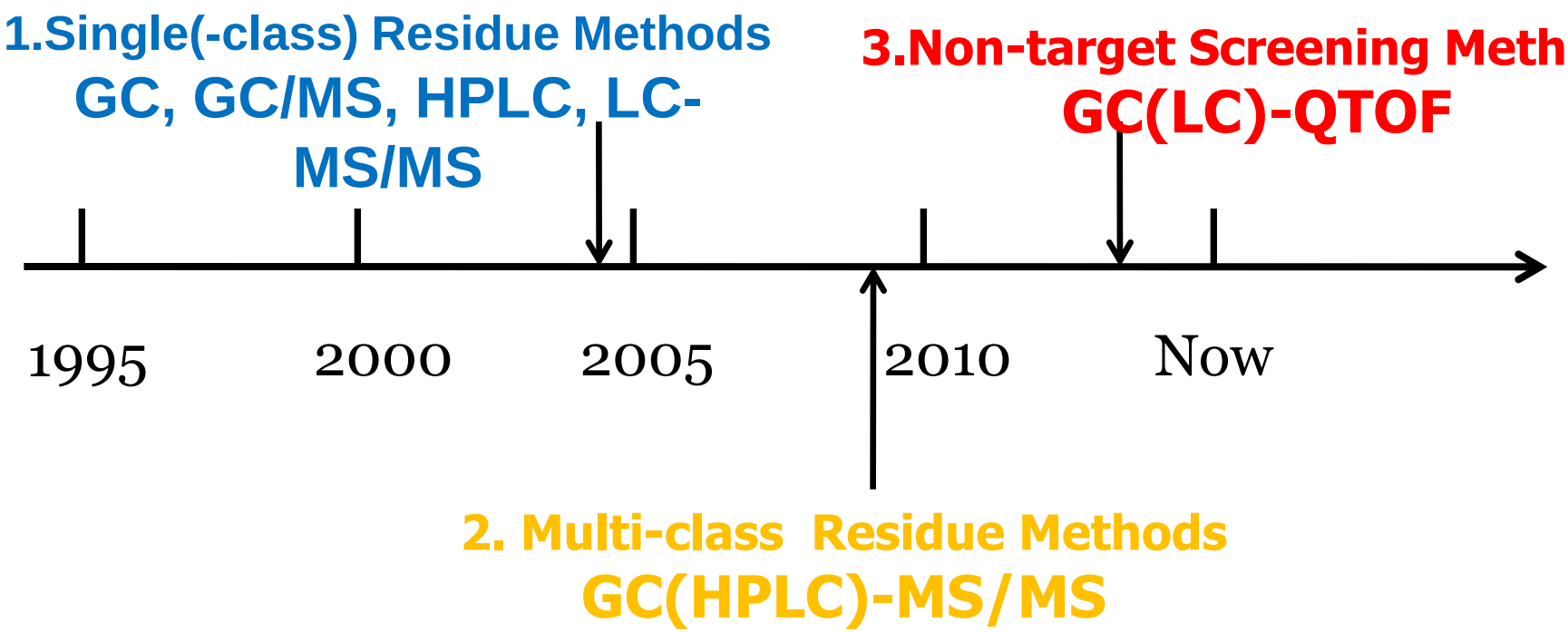
Diversity of the compounds

- More groups and classes
- Different physical/chemical properties（eg, polarity and pKa values）
- Parent drugs and metabolites

Complex matrices

- Matrix effect
- Co-extracted matrix
- Extremely low part-per-billion levels

Methods Classification in Our Lab



Workflows

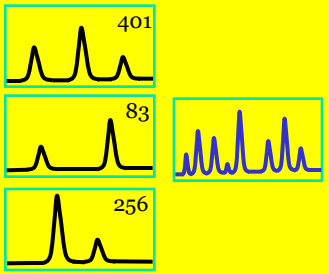
Representative Sample

Sample Extraction

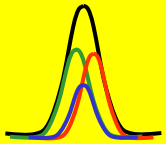
Extract Clean-up

GC/MS (PTV) – for known/unknown

SIM/Scan



Deconvolution

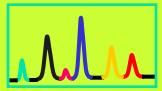


Final Report

Library Search

S C Q

LC/QQQ MRM – for known targets



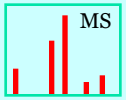
S C Q

Screen S

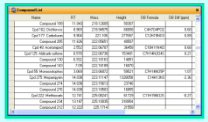
Confirm C

Quantify Q

LC/QTOF or TOF Full Spectrum – for unknowns or non-targeted



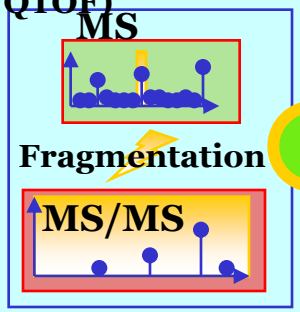
Accurate Mass Database Search



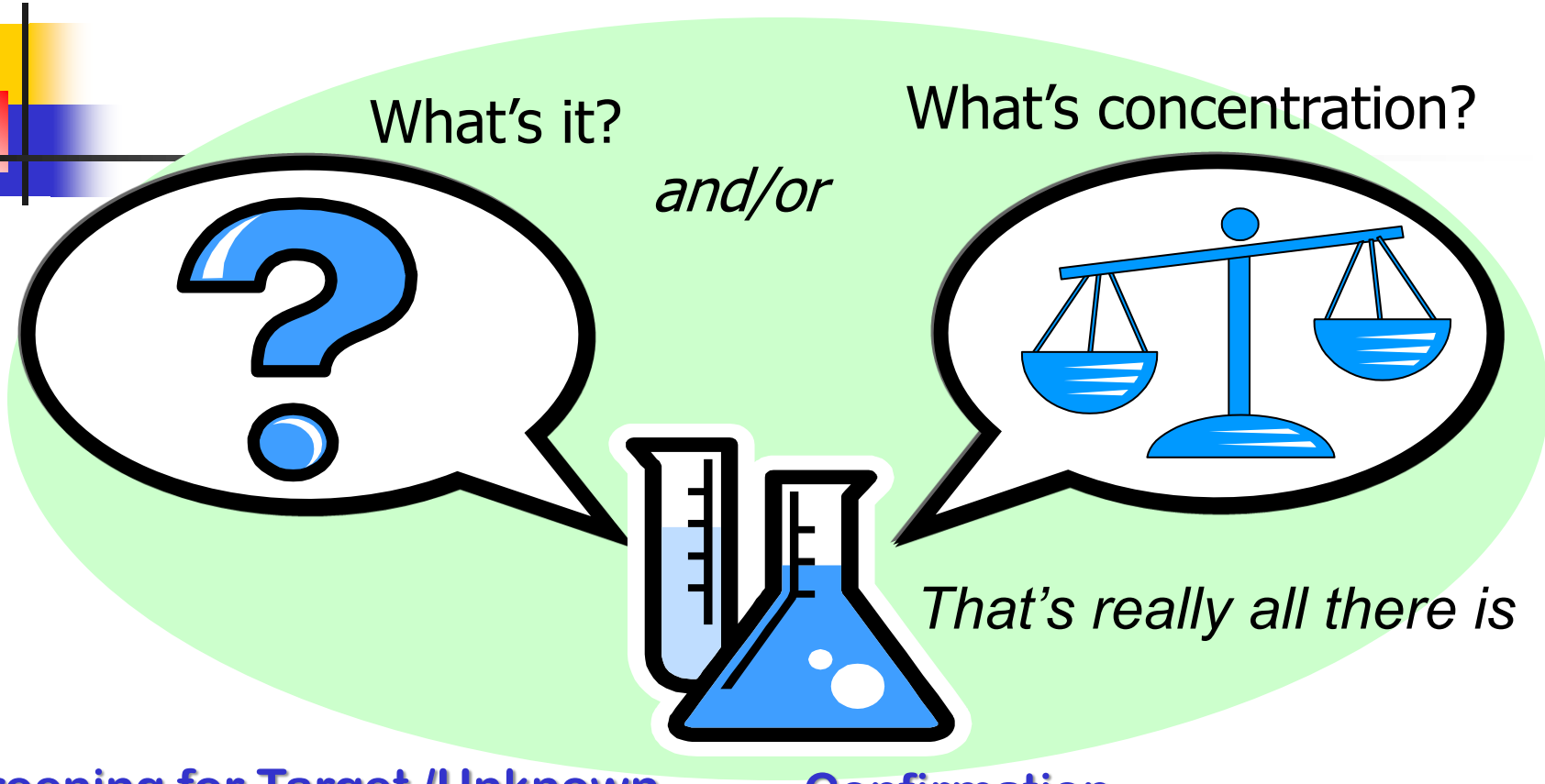
Molecular Formula Generation

S Q

Another injection for MS/MS (QQQ or QTOF)



What do we want from TOF/Q-TOF analysis



Screening for Target /Unknown

Identification with AM(RT) or PCDL

Confirmation with MS/MS

Quantification With TOF/Q-TOF/QQQ

Application 1: Pesticides Screening Method

Qualitative Screening and Quantitative Determination of 900+ Pesticides in Food Using High Performance Gas (Liquid) Chromatography Tandem Quadrupole Time-Of-Flight Mass Spectrometry

食品中多种农药残留的筛查测定方法-气相（液相）色谱-四级杆-飞行时间质谱法

Method overview

- **Screening of 900+ residue compounds**
- **Modified QuEChERS**
- **GC-Q-TOF & LC-Q-TOF**

Matrix selection

样品主类 Sample class	样品子类	样品基质 Sample matrix
高含水量 High water content	鳞茎类蔬菜	洋葱 onion
	果菜类/瓜类	黄瓜、西红柿 cucumber, tomato
	甘蓝类蔬菜	西兰花 broccoli
	叶菜类	菠菜spinach
	茎杆类蔬菜	韭菜 leek
	新鲜豆类蔬菜	黄豆 soybean
	根和块茎类蔬菜	胡萝卜 carrot
	鲜菇类	香菇 mushroom
低水分、低脂肪、高淀粉或高蛋白 Low water content, low fat, high starch or high protein	谷物	大米 rice
高酸、高含水量 High acid, high water content	柑橘类水果	柚子 grapefruit

Sample prepare

Sample(for cereal water must added)

↓

Formic acid Acetonitrile，vibrate

↓

MgSO4; NaCl；soudium citrate; sodium hydrogencitrate sesquihydrate

↓

vibrate，centrifugation

↓

C18， PSA，GCB， $MgSO_4$

↓

Supernatant，add formic acid

↓

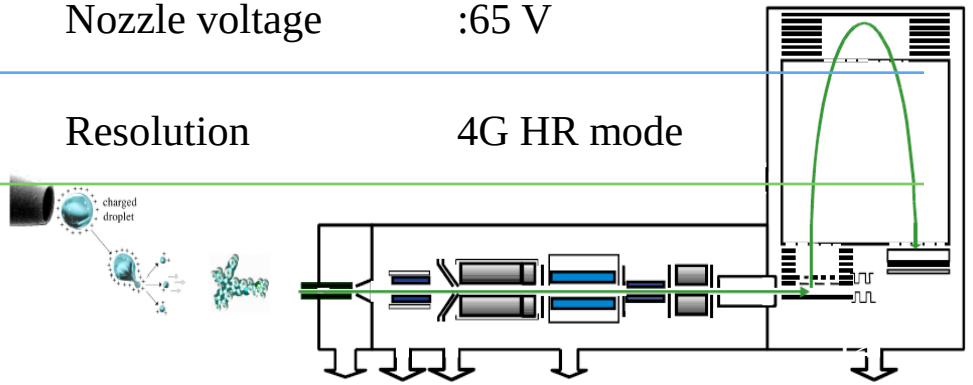
GC-Q-TOF and LC-Q-TOF detection

Method parameters—LC condition

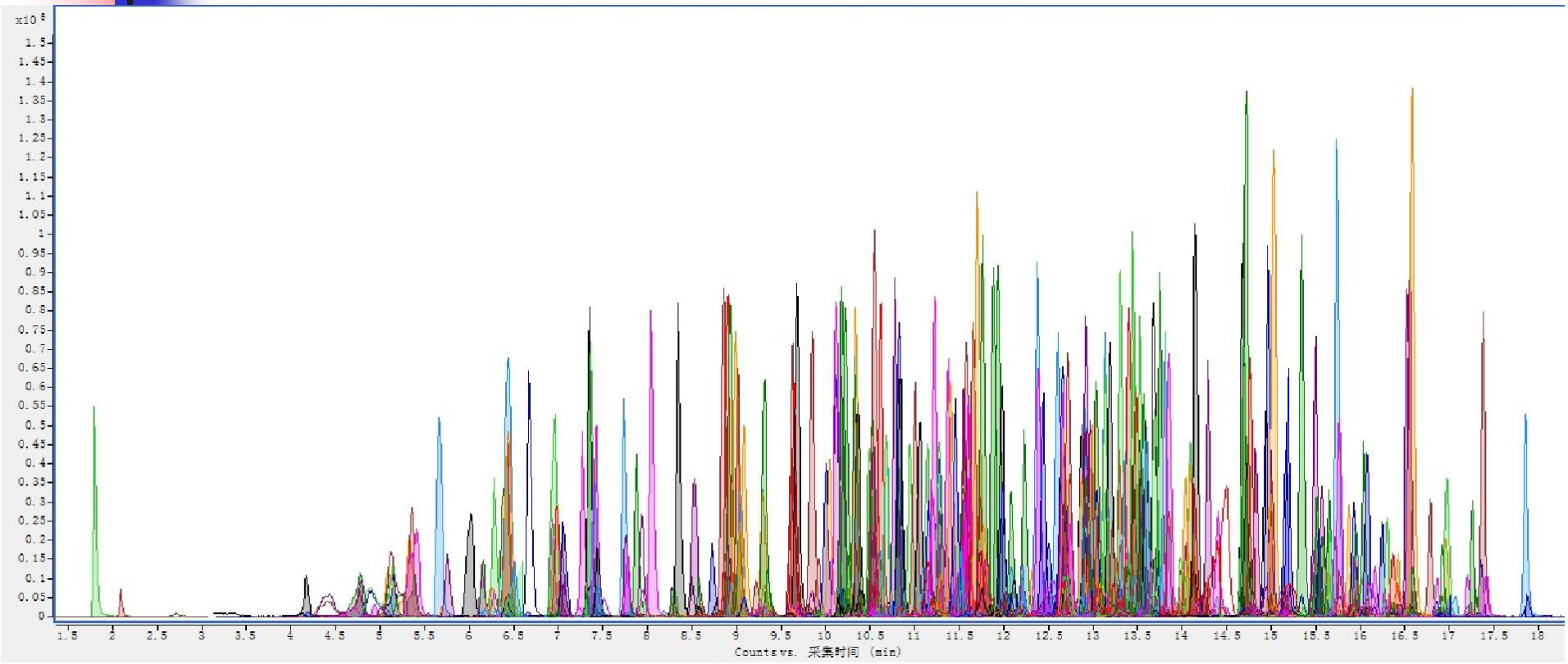
HPLC system	**: Agilent 6540 LC Q-TOF (Dual ESI)**	
Column	**: Poroshell 120 EC C18 150mm×3mmi.d. , 2.7 μm (核壳柱)**	
Injection volume	**: 3 μL**	
Flow rate	**: 0.30 mL/min**	
Mobile phase	**: A-0.1% Formic acid/5mM Ammonioun formate/water**	
	B-MeoH	
Gradient	**Time (min)**	**B %**
	0.0	**5**
	1.0	**5**
	6.0	**60**
	16.0	**100**
	20.0	**100**
	20.1	**5**
	25.0	**5**

Method parameters— MS condition

Mass system	: Q TOF MS	Ion source	: ESI
Nebulizer gas	: Nitrogen	Polarity	: Positive/
Nebulizer pressure	: 40 psi	Ion spray voltage	: 4000 V
Drying gas temperature	: 350 ℃	Drying gas flow rate	:9L/min
Sheath Gas temp	: 350 ℃	Sheath gas flow	:10mL/min
Fragmentor	: 125 V	Nozzle voltage	:65 V
Mass range	: m/z 80-1050	Resolution	4G HR mode



Overlaid EIC of LC-Q-TOF



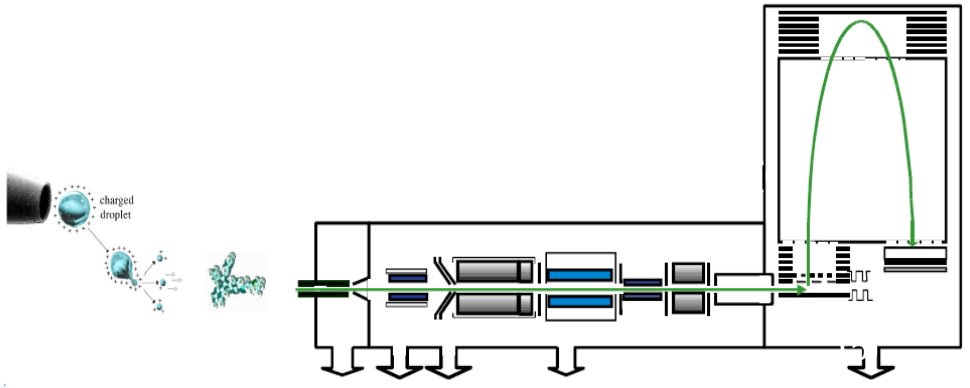
100 μg/kg 651 pesticides standard solution

Method parameters—GC condition

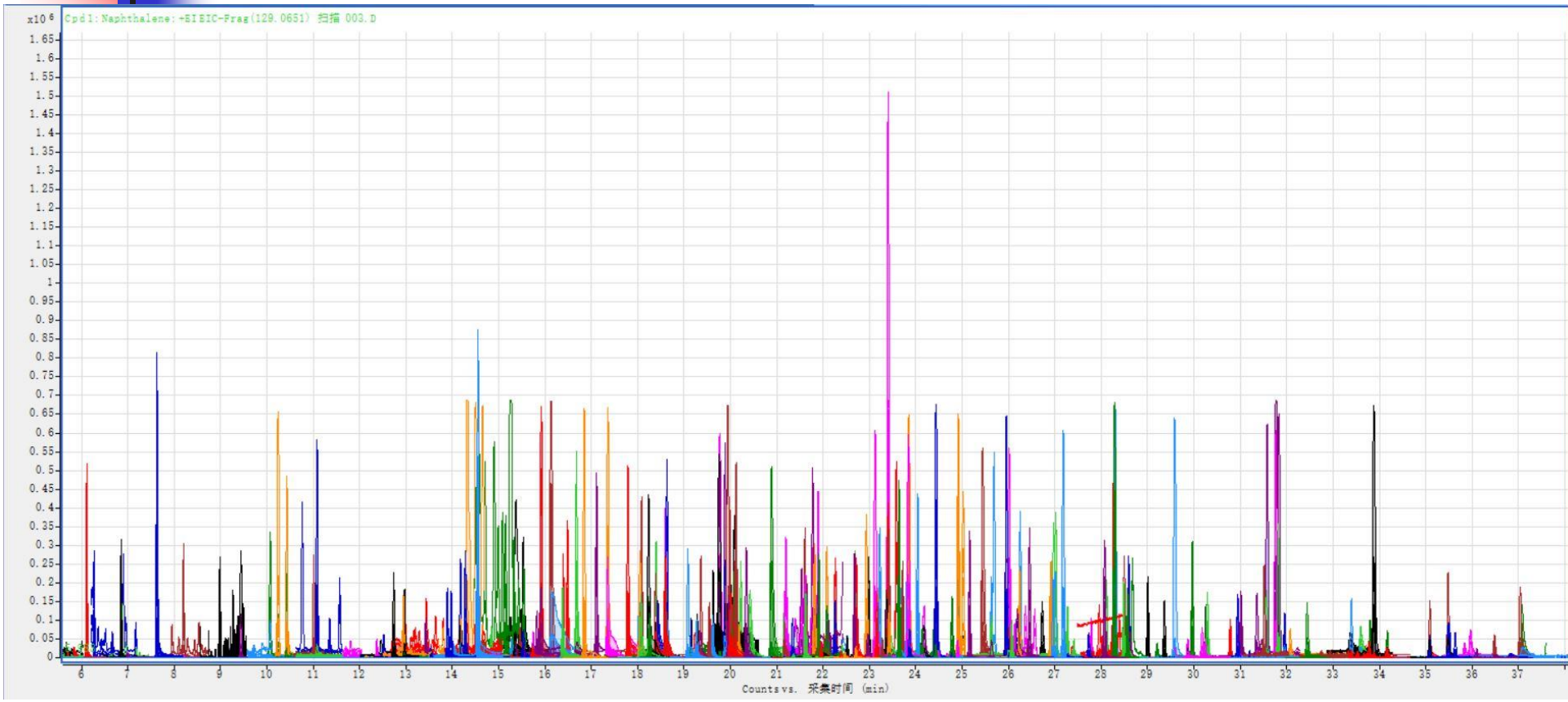
GC system	**: Agilent 7200 GC Q-TOF with EI source**	
Column	**: HP-5MS， 30m×0.25μm×0.25mm**	
Injection volume	**: 2 μL**	
Injection mode	**: MMI**	
Flow rate	**: 1.0 mL/min**	
Mobile phase	**: Nitrigen**	
Injection temp.	**280℃**	
Gradient	**Time (min)**	**Temp.**
	0.0	**60℃**
	1.0	**60℃**
	40℃/min	**120℃**
	5℃/min	**310℃**

Method parameters— MS condition

Mass system	: Q TOF MS	Ion source	: EI
Transfer line	: 280℃	Polarity	: Positive
Current	: 2 mA	Scan speed	: 5 spectra/sec
Mass range	: m/z 80-1050	Resolution	4G HR mode



Overlaid EIC of GC-Q-TOF



100 μg/kg 628 pesticides standard solution

Database Establishment

- **Accurate MS：full scan mode，RT, accurate MW, precuror ion, ionization pattern, response.**

- **Targeted MS/MS：parent adduct ion, one or different CE, high response, more fragments.**

Detection mode	Compounds
LC-Q-TOF	628
GC-Q-TOF	651
total	904

Identification

An analyte was considered positively identified when criteria were confirmed:

- the accurate mass deviation of two selected ions of each analyte was less than 5 ppm.
- the ratio of the chromatographic retention time of the analyte to that of the same analyte in standard solution was within 2.5% tolerance.

ID Criteria（SANCO/12571/2013）

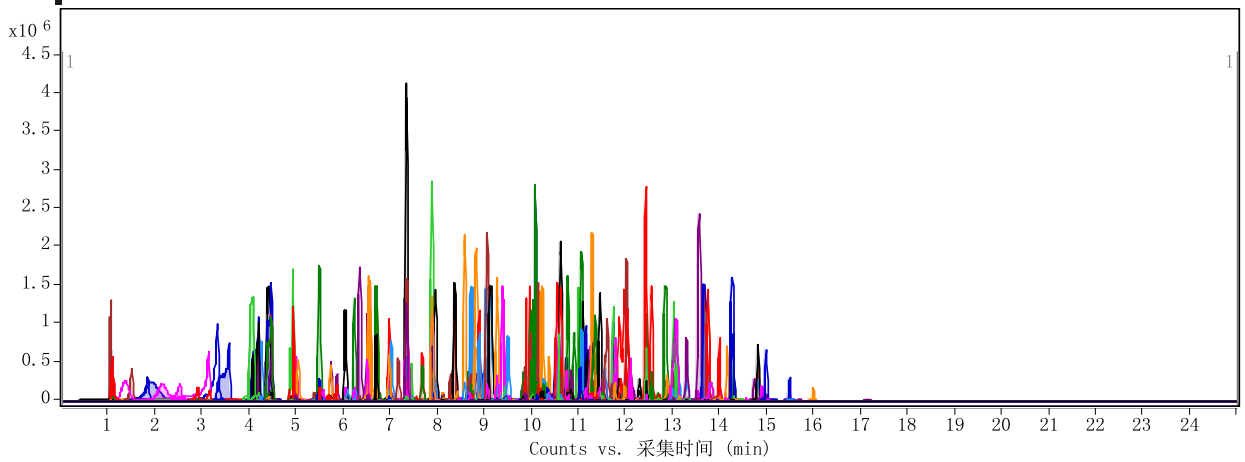
Table 4. Identification criteria for different MS techniques

MS mode:	Single-stage MS (unit mass resolution)	Single-stage MS (high resolution/high mass accuracy)	MS/MS
Typical systems (examples):	Quadrupole, ion trap, time-of-flight (TOF)	TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole, ion trap, hybrid MS (e.g. Q-TOF, Q-trap)
Acquisition mode:	Full scan, Limited *m/z* range, Selected ion monitoring (SIM)	Full scan, Limited *m/z* range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM),full scan product-ion spectra
Requirements for identification:	≥ 3 diagnostic ions, preferably including the (quasi) molecular ion	≥ 2 diagnostic ions, preferably including the (quasi) molecular ion; mass accuracy < 5 ppm; at least one fragment ion	≥ 2 product ions
	Ion ratio(s): according to Table 5		

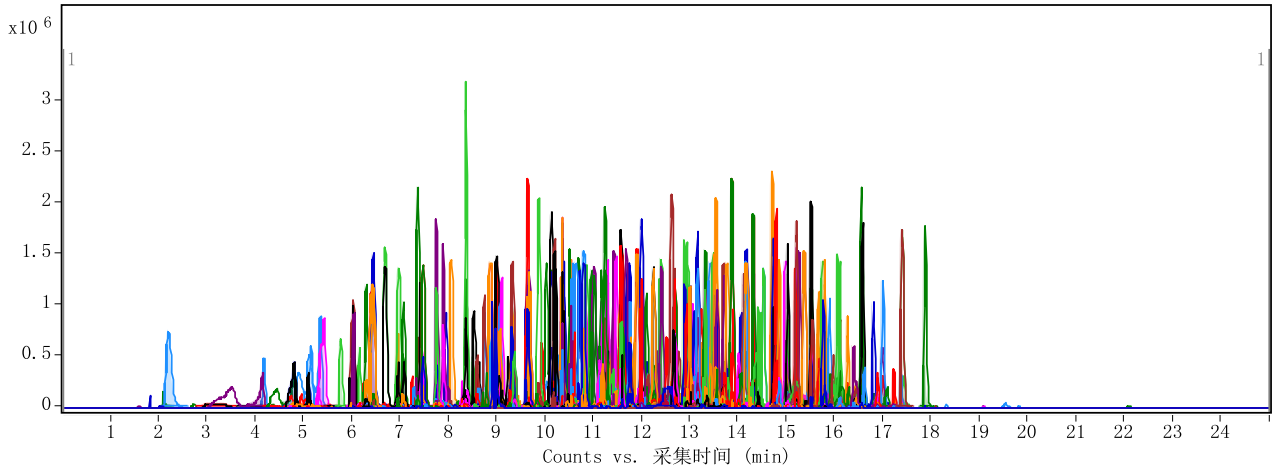
Table 5. Recommended maximum (default) tolerances for ion ratios using different MS techniques

Ion ratio (least/most intense ion)	Maximum tolerance (relative) for GC-EI-MS	Maximum tolerance (relative) for LC-MS^n, LC-MS, GC-MS^n, GC-CI-MS
0.50-1.00	± 10 %	± 30 %
0.20-0.50	± 15 %	± 30 %
0.10-0.20	± 20 %	± 30 %
<0.10	± 50 %	± 30 %

Mobile phase selection



Acetonitrile containing 0.1% formic acid (5mmol/L ammonium acetate)



Methnol containing 0.1% formic acid (5mmol/L ammonium acetate)

Precursor selection

- **$[M+H]^+$ (Most chemicals)**
- **$[M+NH4]^+$(Pyrethroids, >20 chemicals)**
- **$[M-CL+H]^+$(Chlormequat)**
- **$[M^{2+}H]^+$(Diquat)**
- **$[M-H_2O+H]^+$(Paraquat)**
- **$[M-CH_3+H]^+$(Cyhexatin)**

Method Validation

- **LODs of all pesticides ranged from 10-100 μg/kg （S/N≥10）.**
- **Recovery and repeatability. Results with a range from32%-183%(cucumber), 20%-159%(onion)、34%-150%(tomato), 21%-119%(broccoli), 25%-165%(spinach)、26%-174%(leek)、28%-129%(soybean)、29%-161%(carrot)、20%-173%(mushroom)、21%-150%(rice)、28%-192%(grapefruit).**
- **The relative standard deviation was 5.9～47.5%.**
- **Quick, accurately, precisely.**

Application 2: Drugs Screening Method

Qualitative Screening and Quantitative Determination of 100+ Drugs in Food Using High Performance Liquid Chromatography Tandem Quadrupole Time-Of-Flight Mass Spectrometry

食品中多种兽药残留的筛查测定方法-液相色谱-四级杆-飞行时间质谱法

Veterinaries studied (100+) and their MRLs

Name	Number	Maximum residue levels	
		China	EU
Beta agonist	14	Banned(MRPL)	Banned(MRPL)
Benzimidazole	13	60 μg/kg(Mebendazole)	60 μg/kg(Mebendazole)
Benzodioxode	19	Banned(MRPL)	Banned(MRPL)
Nitroimidazole	10	100 μg/kg	100 μg/kg (Thiabendazole)
Sulfonamide	19	100 μg/kg	100 μg/kg
Triphenylmethane	4	Banned (MRPL)	Banned (MRPL)
Quinolone	14	10~200 μg/kg	10~200 μg/kg
Tetracycline	5	100 μg/kg (chlortetracycline)	100 μg/kg (chlortetracycline)
Sugar cortical	7	Banned (MRPL)	Banned (MRPL)

Sample prepare

2.0 g Sample

10mL 0.1% formic acid/acetonitrile，5g anhydrous NaSO4

homogeneous，shake 10min

4000 rpm for 5 min

Extracted again by 10 mL 0.1%acid/acetonitrile，followed by 10 mL ethyl acetate

Evaporating at 40℃ till dryness

reconstituted with 5mL of ammonia/methanol

Eluted with ammonia/methanol

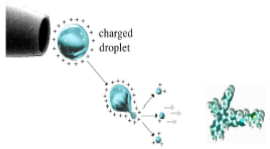
Collect all elution （HLB functions：Retain the interferences & filtrate）

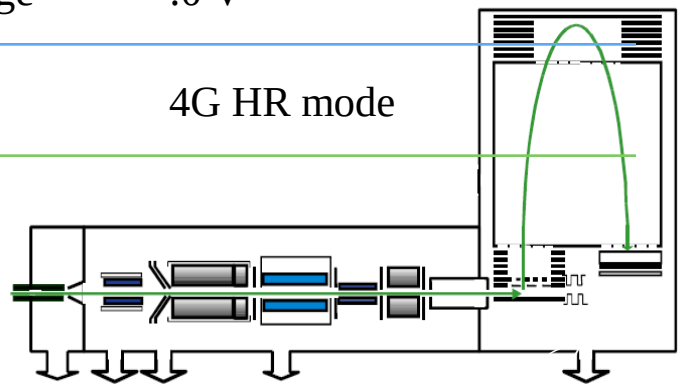
Method parameters—LC condition

HPLC system	**: Agilent 1200 RRLC series**	
Column	**: Zorbax XDB C18 3.0×100mm, 1.8 μm**	
Injection volume	**: 5 μL**	
Flow rate	**: 0.30 mL/min**	
Mobile phase	**: A-0.1% Formic acid/5mM Ammonioun formate/water**	
	B-0.1% Formic acid/ACN	
Gradient	**Time (min)**	**B %**
	0	**5**
	4.5	**30**
	12.5	**50**
	18	**50**
	20	**80**
	25	**100**
	30	**100**

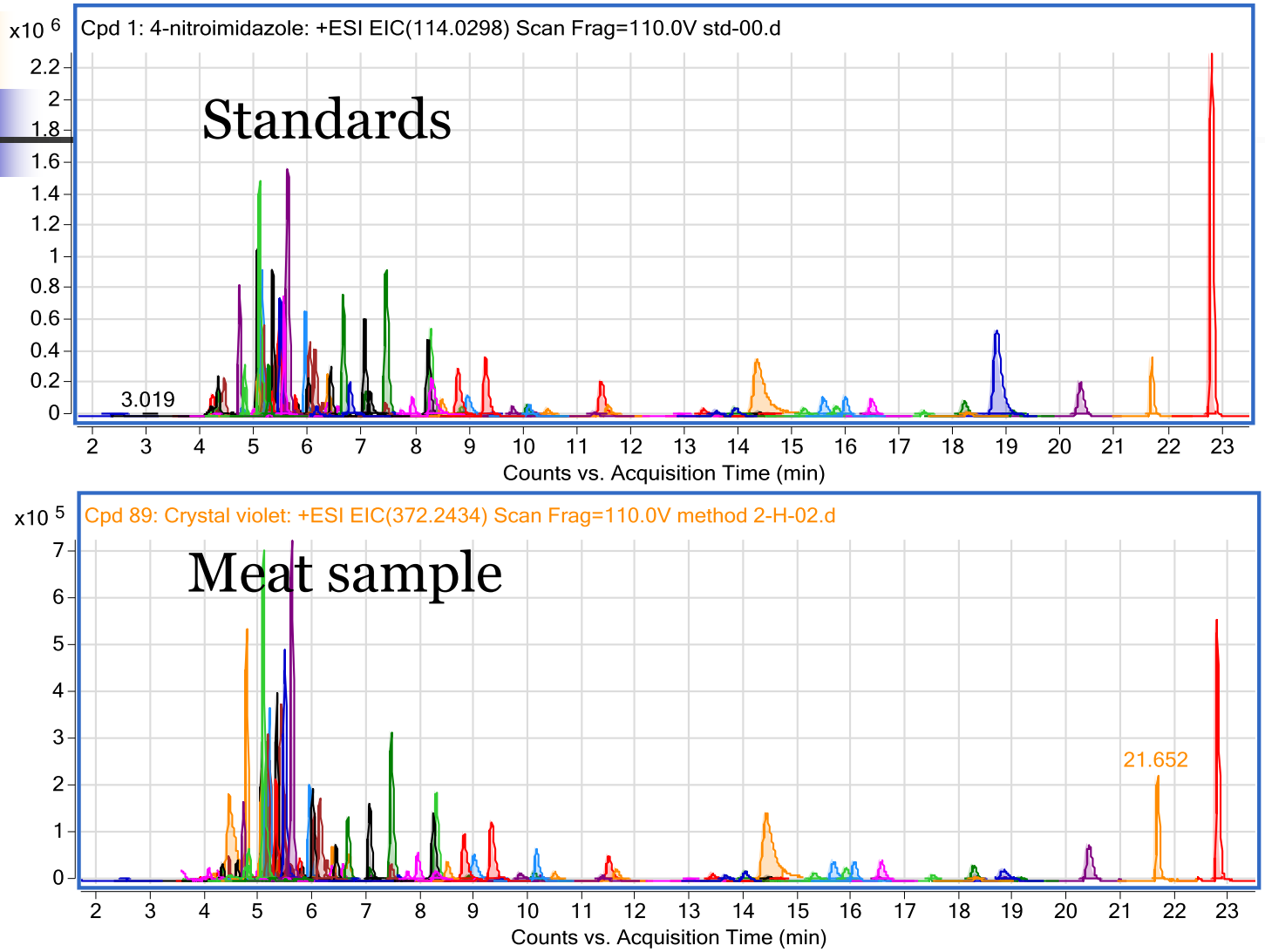
Method parameters— MS condition

Mass system	: Q TOF MS	Ion source	: ESI
Nebulizer gas	: Nitrogen	Polarity	: Positive/ Negative
Nebulizer pressure	: 45 psi	Ion spray voltage	: 4500 V/4000 V
Drying gas temperature	: 330 ℃	Drying gas flow rate	:5L/min
Sheath Gas temp	: 400 ℃	Sheath gas flow	:10mL/min
Fragmentor	: 110 V	Nozzle voltage	:0 V
Mass range	: m/z 80-1050	Resolution	4G HR mode





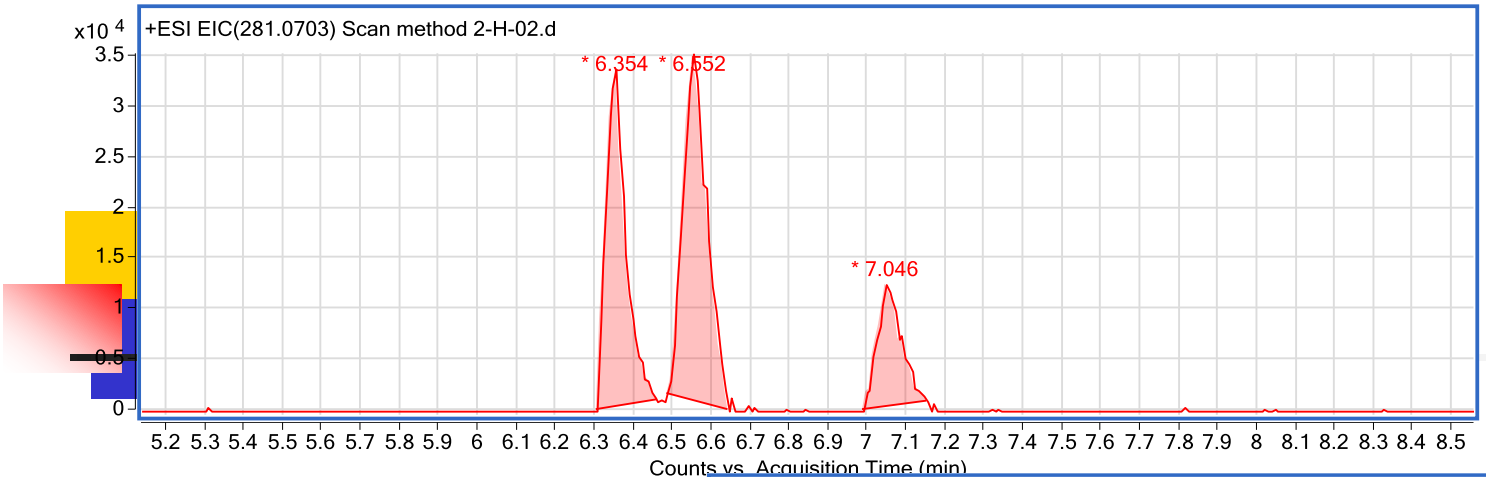
Results

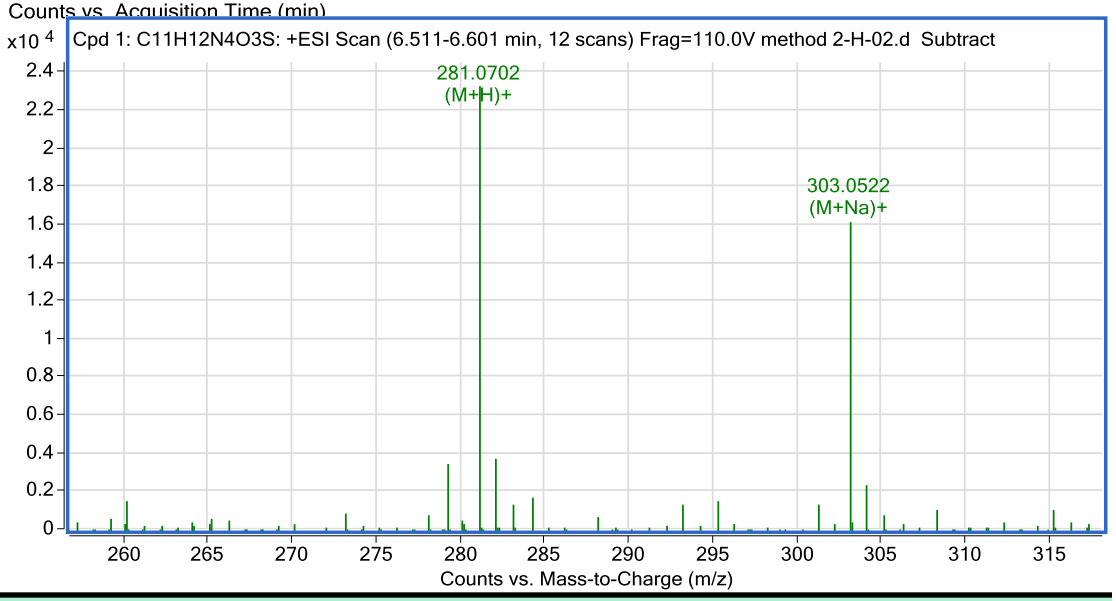


Overlaid EIC of 105 veterinary drugs standards (5 ng/mL) and sample (5 µg/kg)

Identification of compounds with the same nominal mass

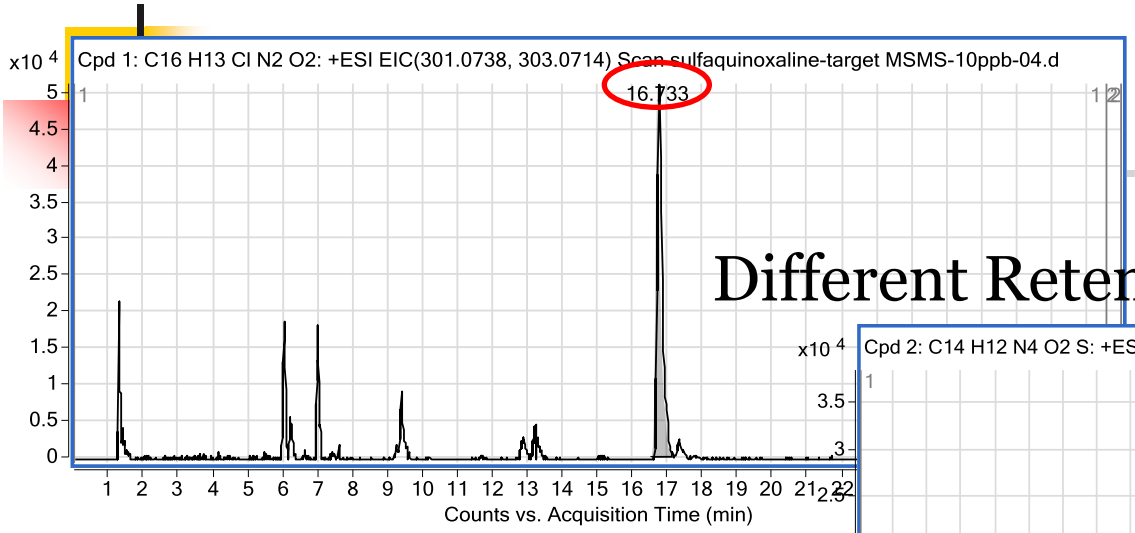
Group	Compound	Formula	Monoisotopic mass (Da)	Mass difference (ppm)	Identified by
1	Sulfameter	C11H12N4O3S	280.060301	0	Rt
	Sulfamethoxypridazine	C11H12N4O3S	280.060301		
	Sulfamonomethoxine	C11H12N4O3S	280.060301		
2	Temazepam	C16H13ClN2O2	300.06656	5.13	Rt and isotope match
	Sulfaquinoxaline	C14H12N4O2S	300.06810		





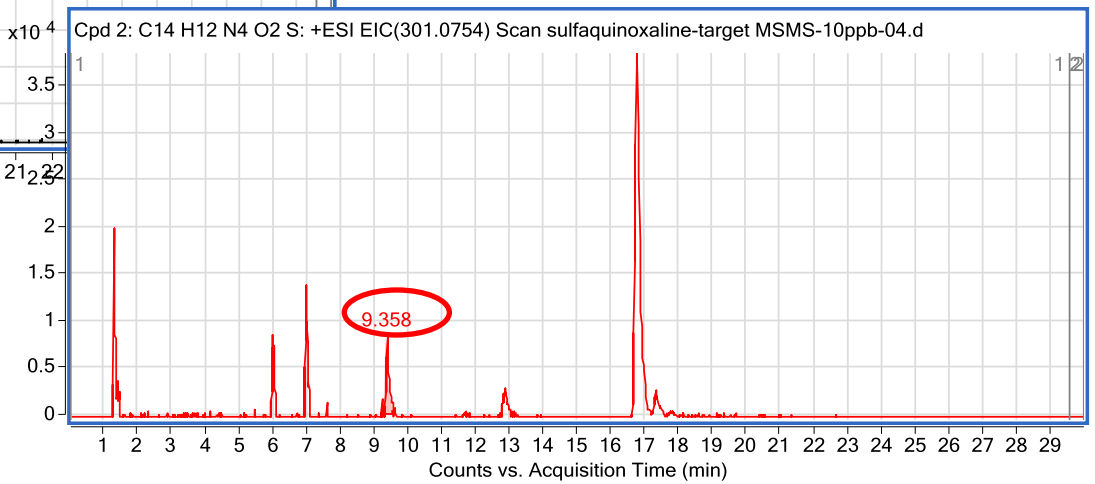
Compound	Formula	Rt
Sulfamethoxypyridazine	C11H12N4O3S	6.35 min
Sulfametere	C11H12N4O3S	6.55 min
Sulfamonomethoxine	C11H12N4O3S	7.05 min

Temazepam ($C_{16}H_{13}ClN_2O_2$) and Sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$)



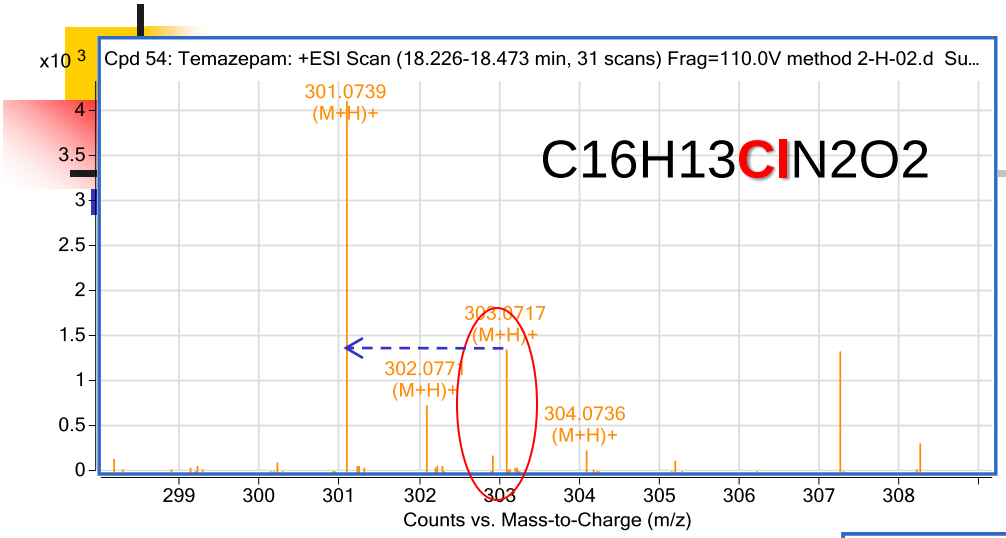
Mass accuracy

Different Retention time

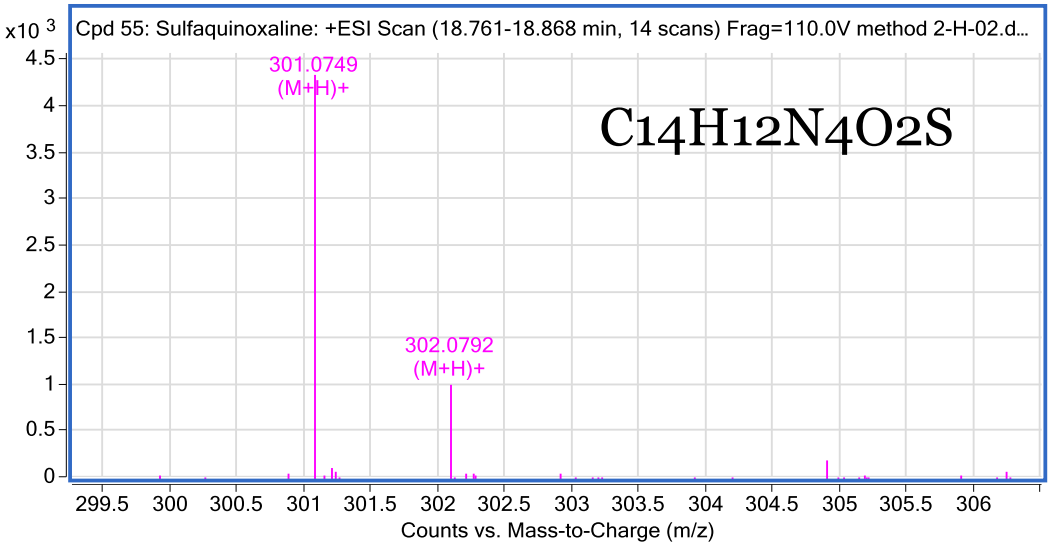


Compound	Formula	Solvent/Matrix	Measured Mass	Cal. Mass	Error
Temazepam	$C_{16}H_{13}ClN_2O_2$	Std	300.0661	300.0666	−1.62 ppm
		Meat	300.0666	300.0666	0.06 ppm
Sulfaquinoxaline	$C_{14}H_{12}N_4O_2S$	Std	300.0676	300.0681	−1.69 ppm
		Meat	300.0687	300.0681	−1.90 ppm

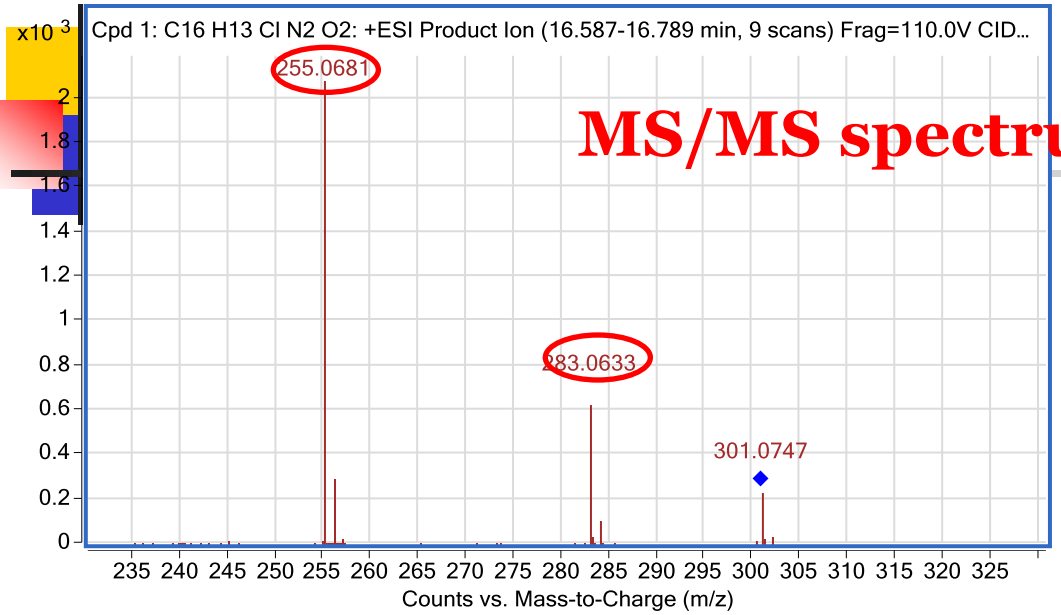
Temazepam (C16H13ClN2O2) and Sulfaquinoxaline (C14H12N4O2S)



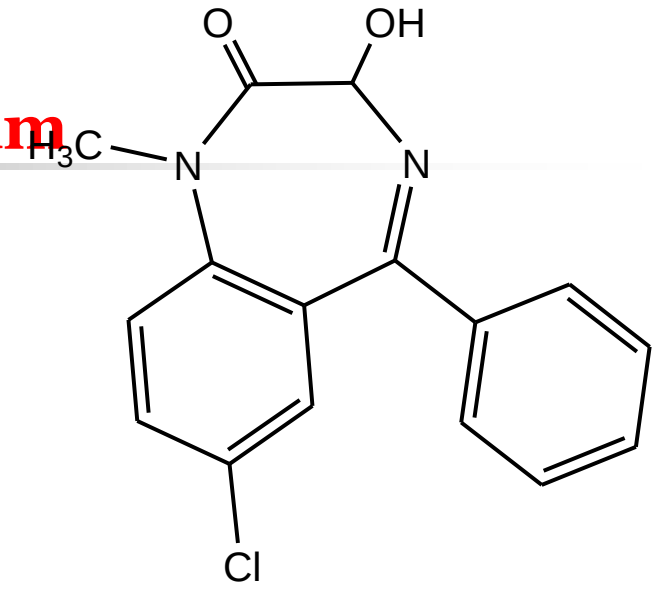
Isotope match



Confirmation of Temazepam



MS/MS spectrum

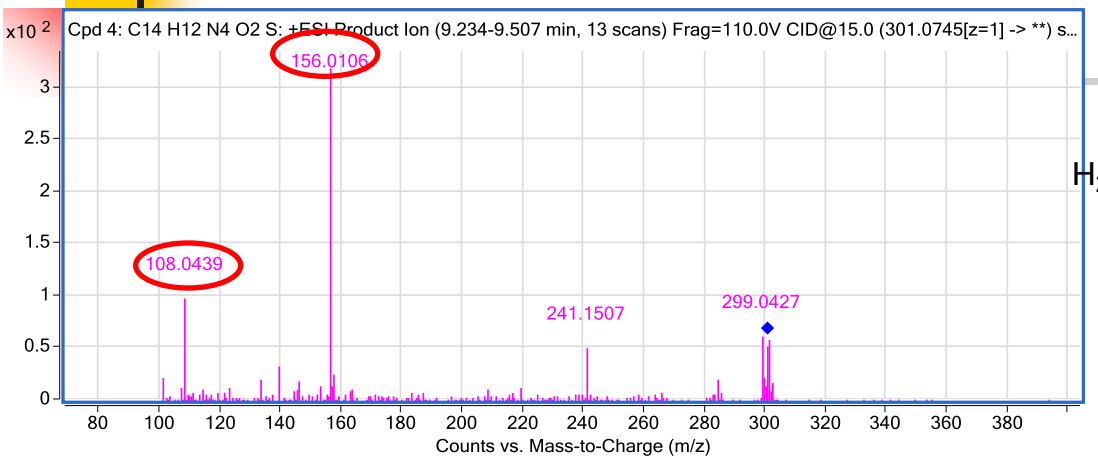


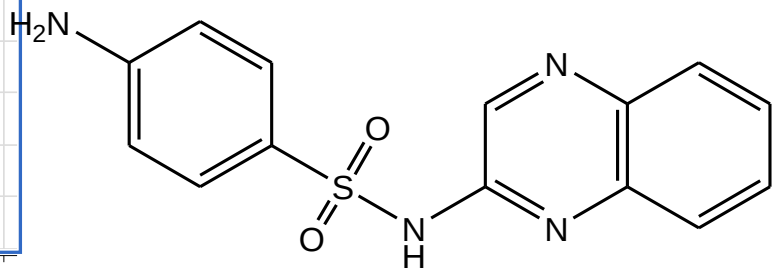
MS/MS Formula Details: Cpd1: C16 H13 Cl N2 O2 - target msms temazepam and sulfaquionxacline-02.d C

m/z	Formula	Abund%	Diff (ppm)	Loss Mass	Loss Formula
283.0633	C16 H12 Cl N2 O	20.86	-0.2	18.0106	H2 O
255.0681	C15 H12 Cl N2	79.14	1.16	46.0055	C H2 O2

Information of Fragment ion

Confirmation of Sulfaquinoxaline





MS/MS Formula Details: Cpd 4: C14H12N4O2S C14H12N4O2S

m/z	Formula	Abund%	Diff (ppm)	Loss Mass	Loss Formula
108.0439	C6 H6 N O	16.95	4.4	193.031	C8 H7 N3 O S
108.0439	C3 H10 N O S	16.95	35.59	193.0276	C11 H3 N3 O
156.0106	C6 H6 N O2 S	54.81	5.03	145.064	C8 H7 N3
156.0106	C9 H2 N O2	54.81	-16.57	145.0674	C5 H11 N3 S
241.1507		8.66			

Method Validation

- Recovery and repeatability. Results with a range from 41.1–120.9% (meat), 52.4–91.9% (milk), and 57.3–118.9% (egg), and the relative standard deviation was less than 20%.
- LODs and LOQs of all drugs ranged from 0.01 μg/kg to 5.96 μg/kg and from 0.04 μg/kg to 18.45 μg/kg, respectively.

Thank you for you attention!

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