APEC CHINA 2014

FSCF Food Safety Cooperation Forum
PTIN Partnership Training Institute Network

Analysis of Veterinary Drug Residues in China

Dr. Xionghai Yi（伊雄海）
Shanghai Entry-Exit Inspection and Quarantine Bureau of The People's Republic of China
SEP. 10, 2014

Contents

- Profile of the institution
- Analysis of veterinary drugs
- The newly research findings

About us

- The Technical Center for Animal, Plant and Food Inspection and Quarantine (AFTC) is one of the affiliated institutions of Shanghai Entry-Exit Inspection and Quarantine Bureau (SHCIQ).

- **Responsibilities:** Inspect the entry-exit foodstuffs, cosmetics and their products, and quarantine the animal and plant products.

Capacities

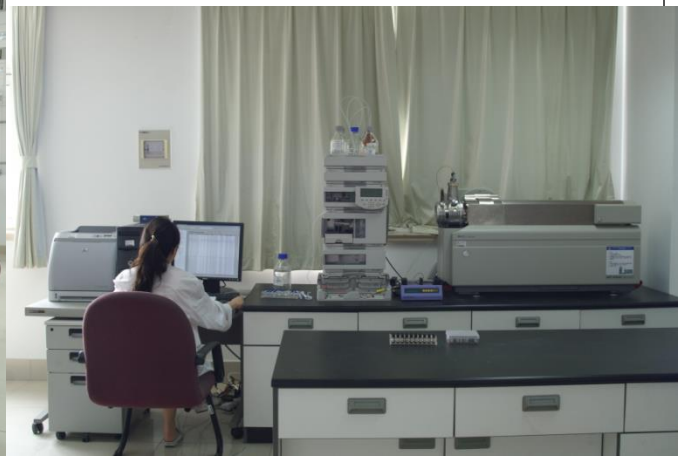
- State Key Laboratory (Shanghai) of Food Safety.
- Authorized reference laboratory of veterinary drugs (Triphenylmethanes, β-agonists, Resorcylic acid lactones and Steroids) by General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ).
- Coordinate PT's including Triphenylmethanes in animal feeds in 2013.

No.	2013-2014 PT's Project	Code	Result
1	Nitrofuran in shrimps	FAPAS PT02229	Satisfied
2	Chloramphenicol in milk	FAPAS PT1870	Satisfied
3	Trifluralin in fish meat	FAPAS PT0588	Satisfied
4	Antibiotics in egg	RILILT 1227295401	Satisfied
5	β-agonist pork	CNCA-13-A08	Satisfied

Personnel and Equipments

- 15 chemists and 35 technicians/assistants
- GC,GC-MS, GC*GC-MS, GC-QQQ, GC-QTOF; HPLC, HPLC-QQQ, HPLC-IT-TOF, HPLC-QTOF, HPLC-Oritrap; IRMS, ICP-MS, AAS, AFS, RT-IR, GPC, ASE, IC, etc.





Contents

- Profile of the institution
- Analysis of veterinary drugs
- The newly research findings

Regulations

- No. 193 Announcement from Ministry of Agriculture of China: The prohibited veterinary drugs and other chemicals in food animals.

- No. 235 Announcement from Ministry of Agriculture of China: The maximum residues limits of veterinary drugs in animals origin food.

Guidance on Method Validation

- GB/T 27404-2008 Criterion on quality control of laboratories-chemical testing of food.
- AQSIQ: The guidance of quality control in residue analysis （2002).

Reference:

- 657/2002/EC Implementing Coucil Directive 96/23/EC Concerning the performance of analyticl methods and the interpretation of results.
- No.SANCO/10684/2009 Method validation and quality control procedures for pesticides residues analysis in food and feed.
- CRL guidelines for the valid ation of screening methods for residues of veterinary medicines.
- AOAC Guidelines for single labortory validation of analytical methods for trace-level concentrations of organic chemicals.

The challenges in drug 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
- Coextracted matrix
- Extremely low part-per-billion levels

Workflows

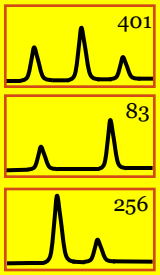
Representative Sample

Sample Extraction

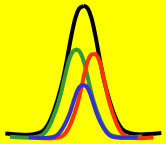
Extract Clean-up

GC/MS (PTV) – for known/unknown

SIM/Scan



Deconvolution

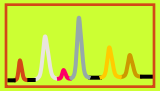


Final Report

Library Search

S C Q

LC/QQQ MRM – for known targets



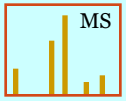
S C Q

Screen S

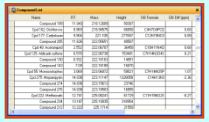
Confirm C

Quantify Q

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



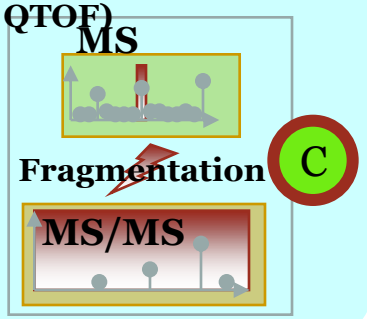
Accurate Mass Database Search



Molecular Formula Generation

S Q

Another injection for MS/MS (QQQ or QTOF)



The advantages of QQQ and QTOF

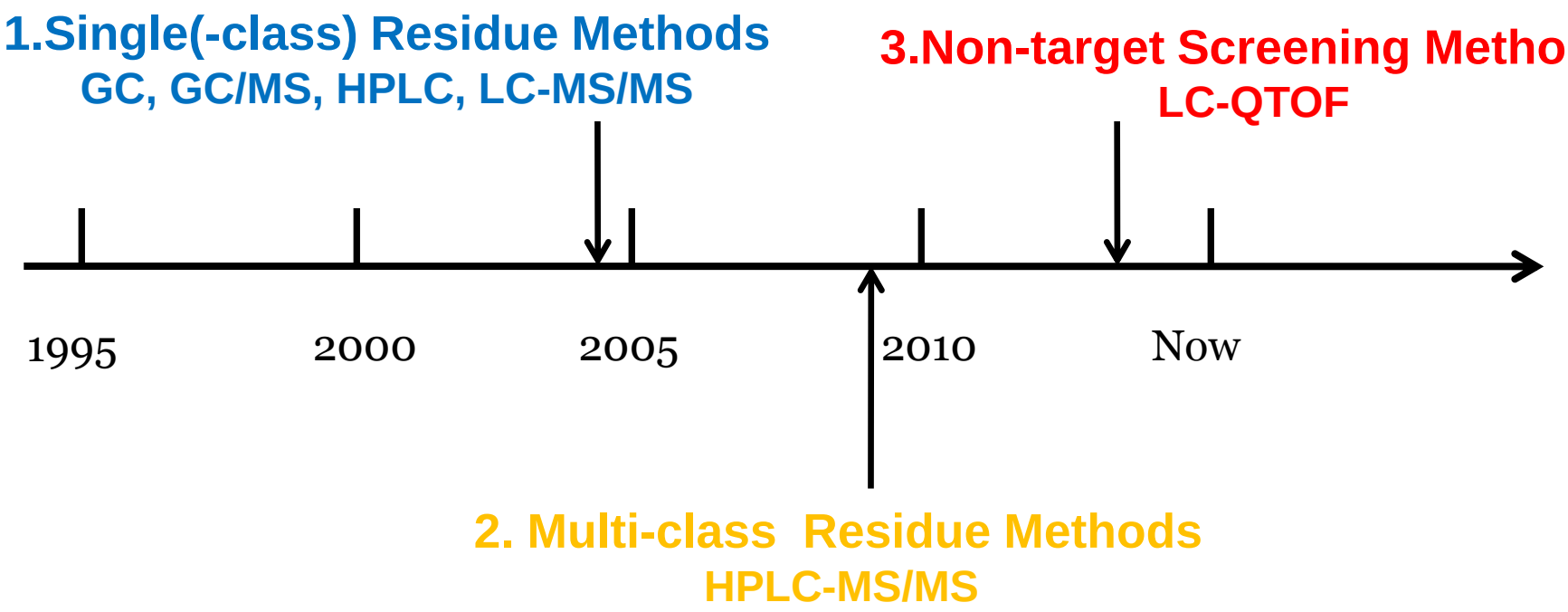
QTOF

- High resolution
- Accurate mass
- High scan speed
- Unknows in one injection
- High sensitive in Full scan

QQQ

- MS/MS mode
- Qualify and quantify both
- Low noise and high sensitive
- knows in one injection

Methods Classification in Our Lab



Single(-class) Residue Methods

- SN/T 1979-2007 Determination of praziquantel residue in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 1777.2-2007 Determination of macrolide antibiotic residues in foodstuffs animal oigin for export--LC-MS/MS method
- SN/T2113-2008 Determination of tranquillizer residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2190-2008 Determination of non-steroidal anti-inflammatory drugs residue in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2220-2008 Determination of benzodiazepine residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2222-2008 Determination of glucocorticosteroids residues in foodstuffs of animal origin for export--LC-MS/MS method
- More than 100+ other SRMs

Multi-class Residue Methods

- SN/T 2624-2010 Determination of basic veterinary drugs residues in foodstuffs of animal origin for export--LC-MS/MS method
- SN/T 2443-2010 Determination of multi-residues of acidic and neutral drugs in foodstuffs animal origin for import and export--LC-MS/MS method
- SN/T 3235-2012 Determination of multi-groups of banned drug residues in foodstuffs of animal origin for export-LC-MS/MS method
- ……

SN/T 2624-2010

- 76 basic veterinary drugs
- 6 classes (β-agonist, Benzodiazepine, Sulfonamide, Benzimidazole, Triphenylmethane, Nitroimidazole)

- Acetonitrile and Citrate buffer Extraction
- strong cation exchange SPE Cleanup
- LC-MS/MS in MRM mode

SN/T 2443-2010

- 64 acidic and neutral drugs
- 6 classes (corticosteroid, progestin, Androgens, hypoglycemic and non-steroidal anti-inflammatory drug)

- Acetonitrile extraction
- *n*-hexane Solvent exchange cleanup
- LC-MS/MS in MRM

SN/T 2235-2012

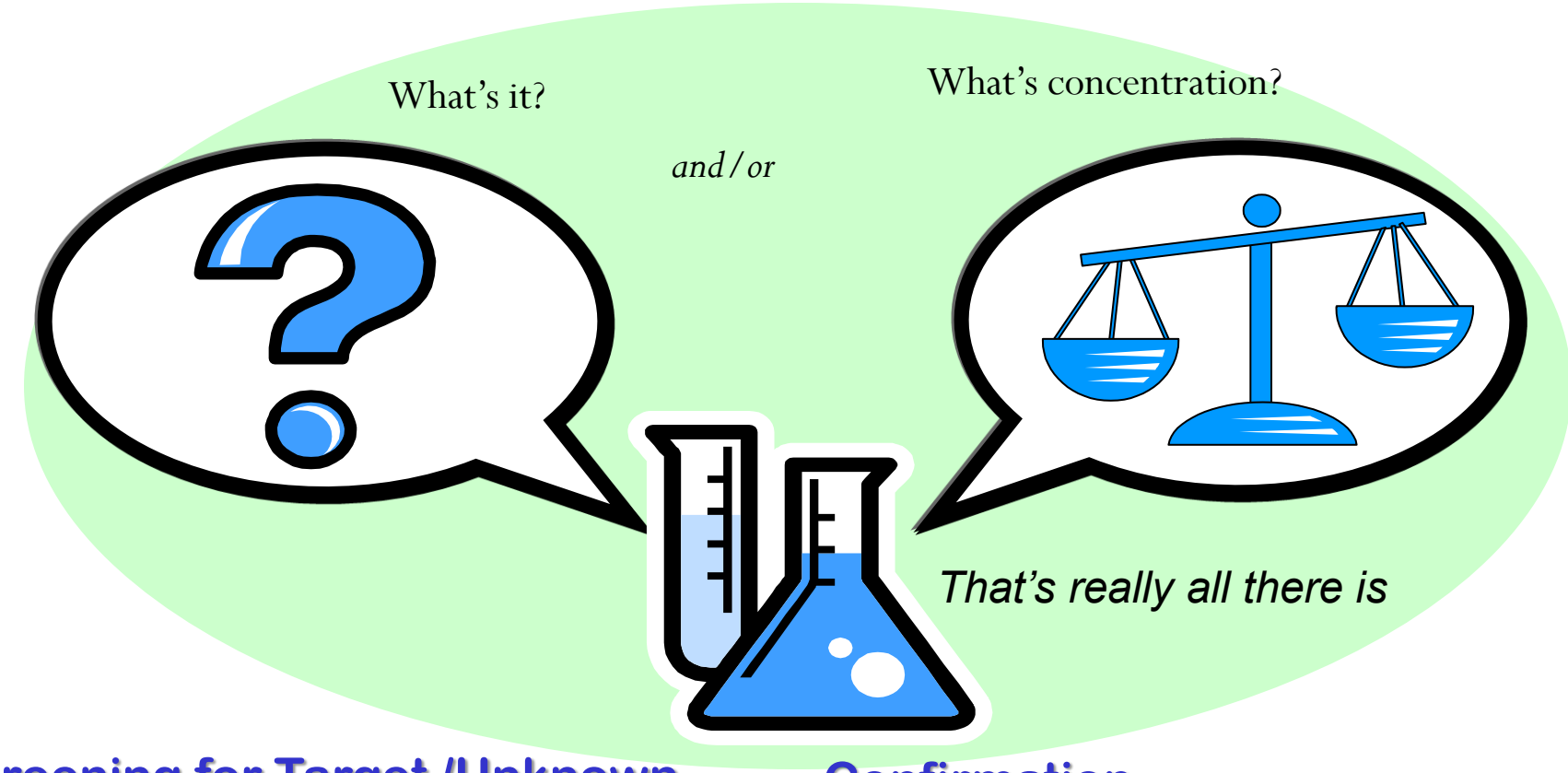
- 44 banned individual drugs
- 9 classes (β-agonist, Androgen, Glucocoritcoid, Estrogen, Nitroimidazols, Resorcylic acid lactone, Triphenylmethane, Sedative and Cloramphenicol)

- Ammonia acetonitrile extraction
- QuEChERS cleanup
- LC-MS/MS in MRM

Non-target Screening Method

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

What do we want from TOF/Q-TOF analysis



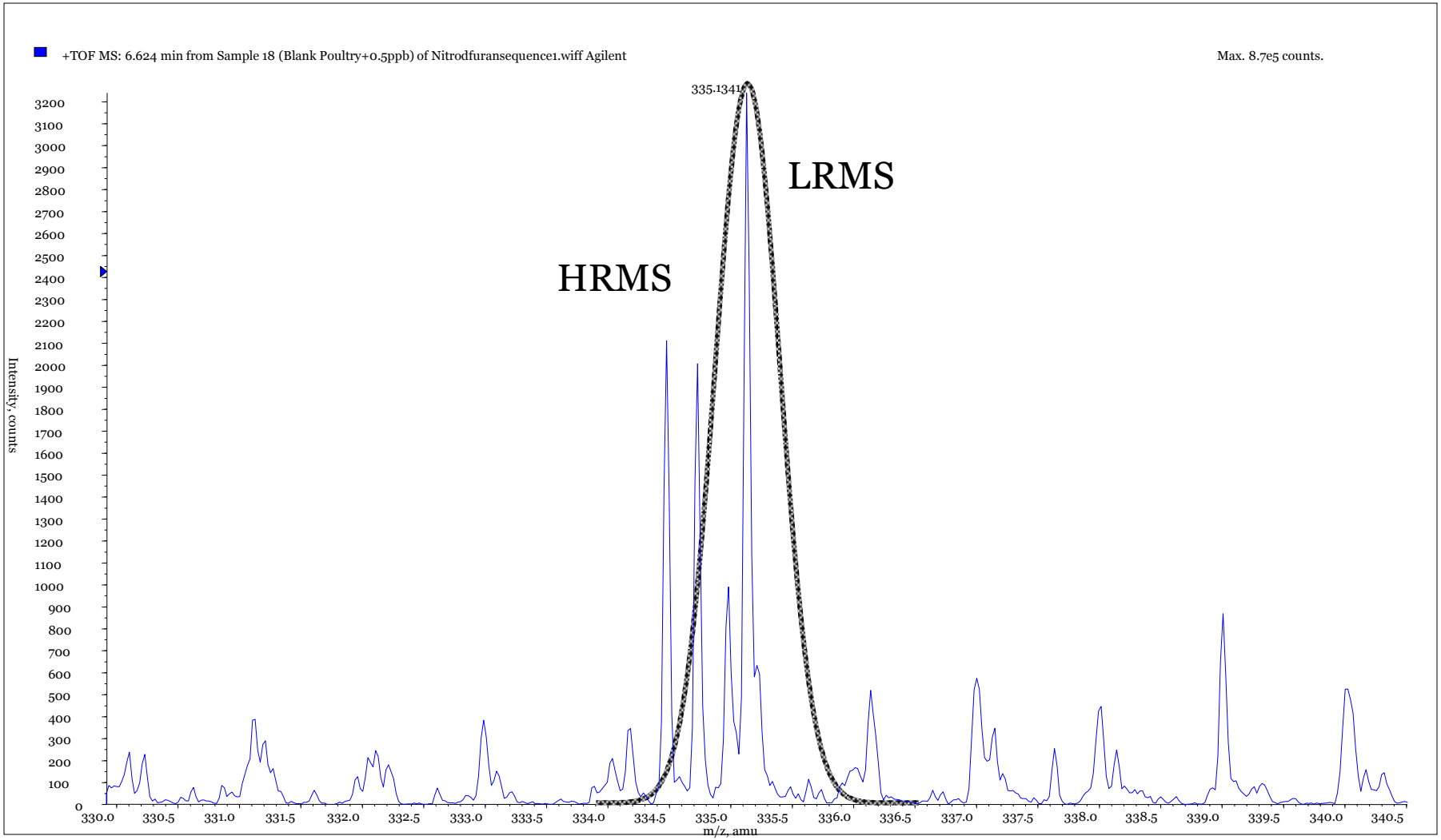
Screening for Target /Unknown

Identification with AM(RT) or PCDL

Confirmation with MS/MS

Quantification With TOF/Q-TOF/QQQ

LRMS v.s. HRMS


+TOF MS: 6.624 min from Sample 18 (Blank Poultry+0.5ppb) of Nitrodfuransequence1.wiff Agilent
Max. 8.7e5 counts.
335.1341
LRMS
HRMS
Intensity, counts
m/z, amu

Contents

- Profile of the institution
- Analysis of veterinary drugs in china
- The newly research findings

Veterinaries studied (total 105) 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

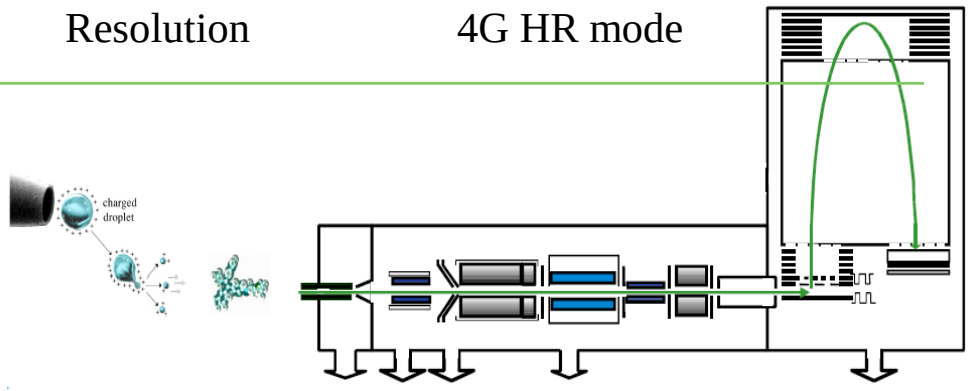
reconstituted with 5mL of 5% ammonia/methanol

Eluted with 8mL of 5% ammonia/methanol

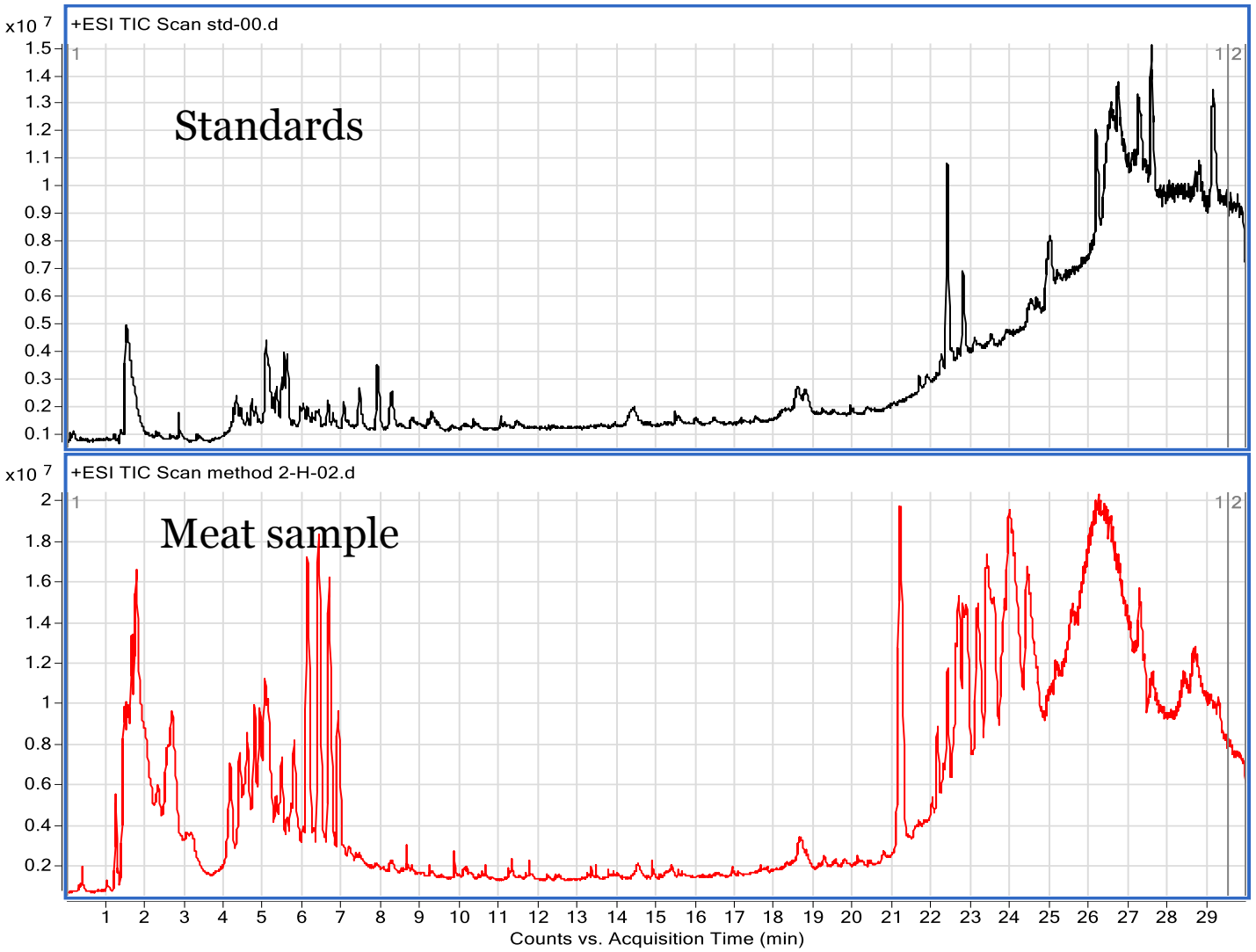
Collect all elution（HLB functions: Retain the interferences and filtrate）

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

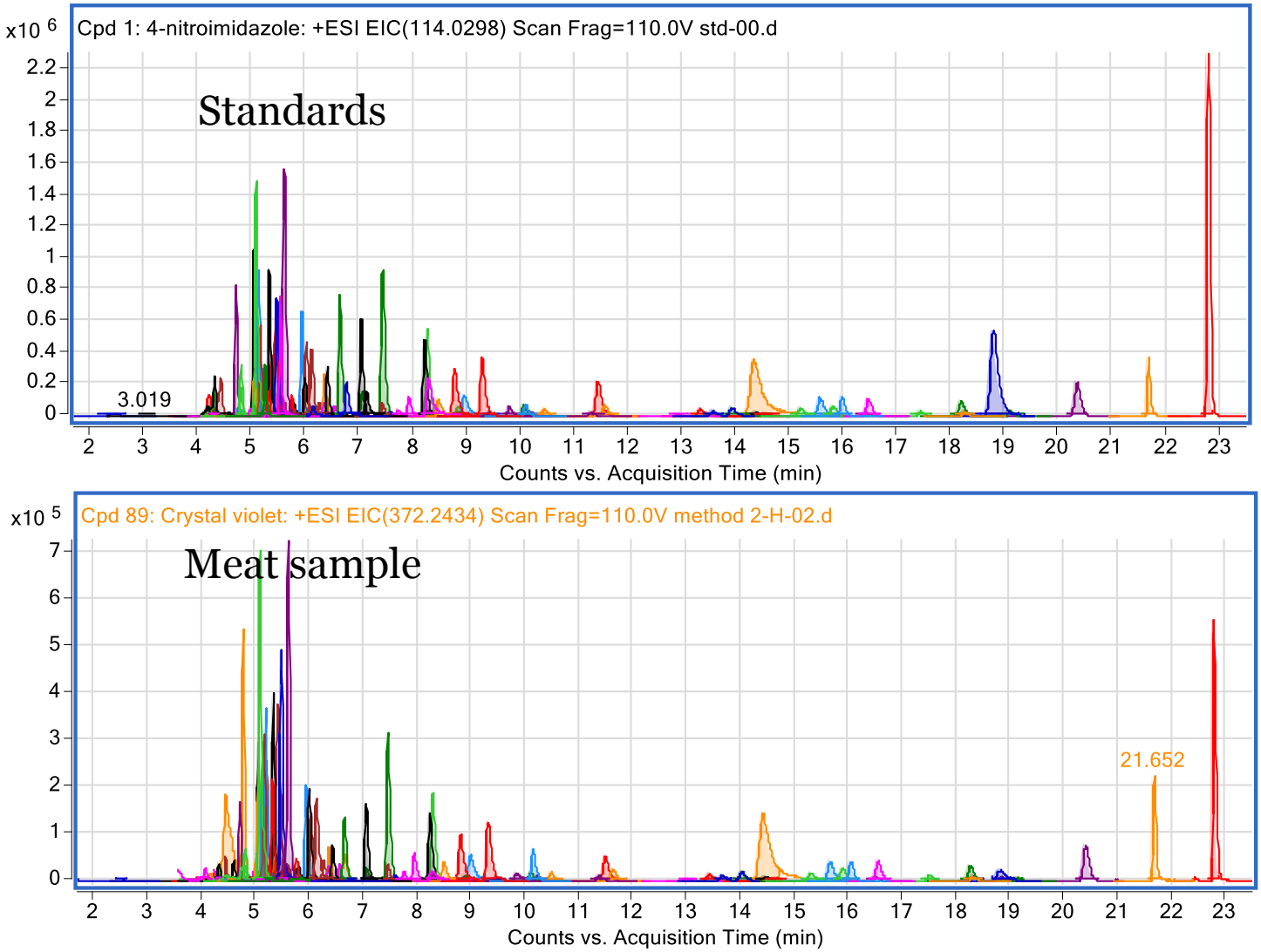


Results



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

Results



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

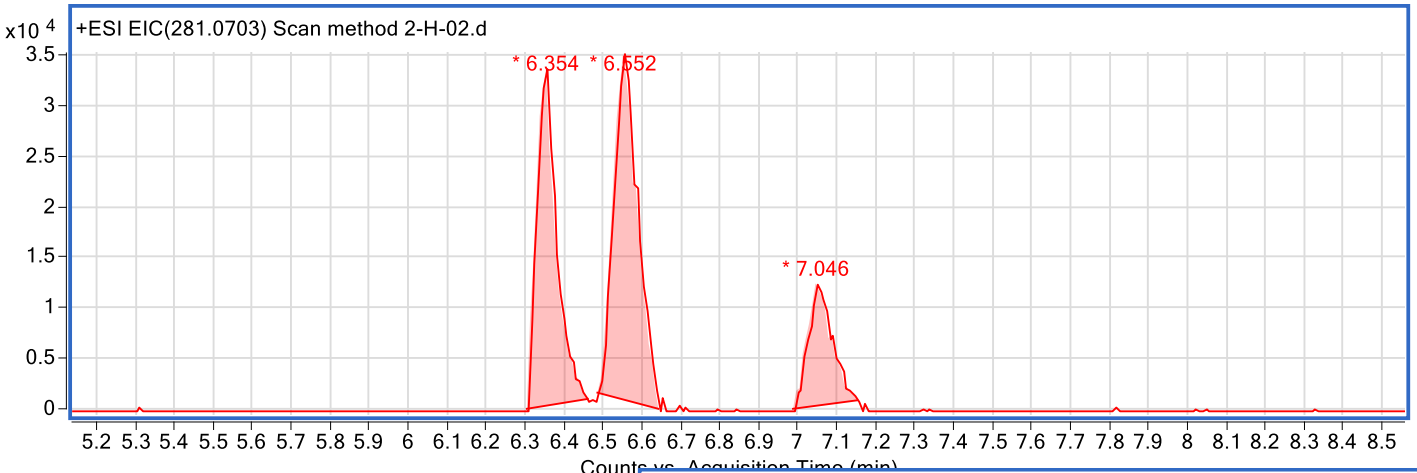
Identification

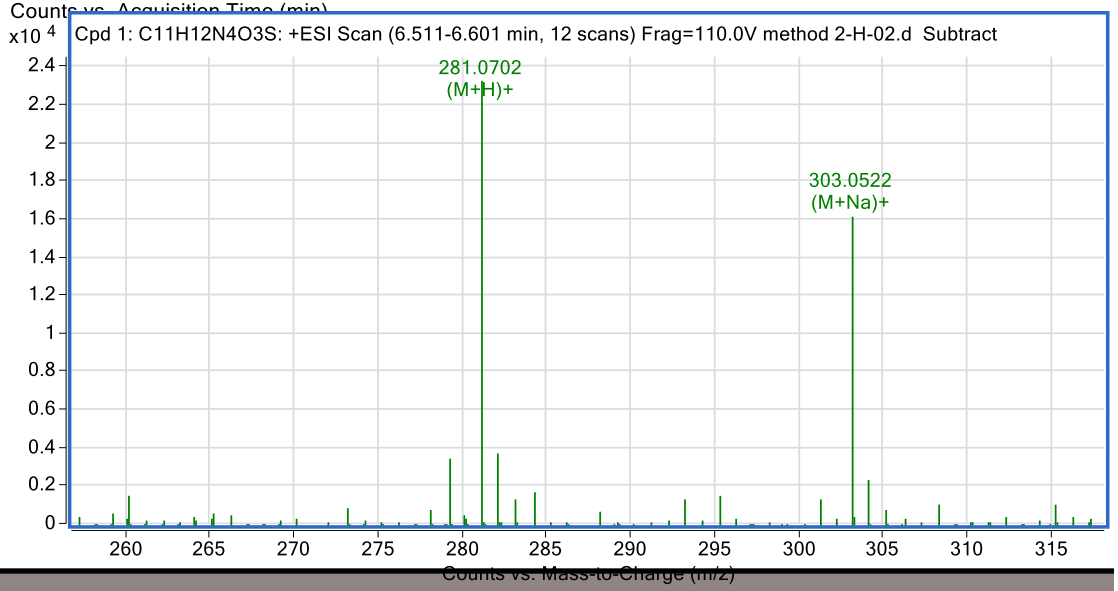
An analyte was considered positively identified when criteria were confirmed:

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

Identification of compounds with the same nominal mass

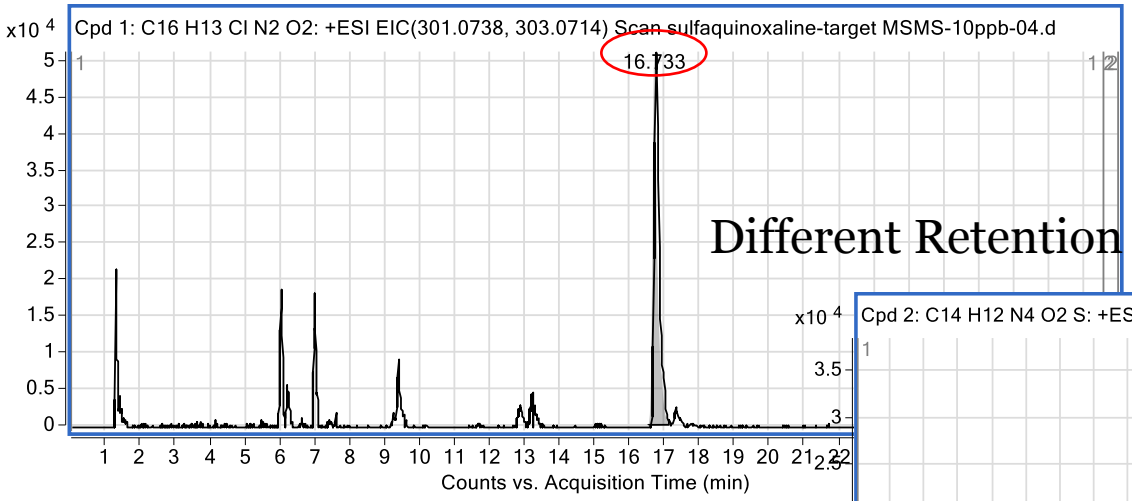
Group	Compound	Formula	Monoisotopic mass (Da)	Mass difference (ppm)	Identified by
1	Sulfameter	C11H12N4O3S	280.060301	0	Rt
	Sulfamethoxypridazine	C11H12N4O3S	280.060301		
	Sulfamonomethoxine	C11H12N4O3S	280.060301		
2	Temazepam	C16H13ClN2O2	300.06656	5.13	Rt and isotope match
	Sulfaquinoxaline	C14H12N4O2S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





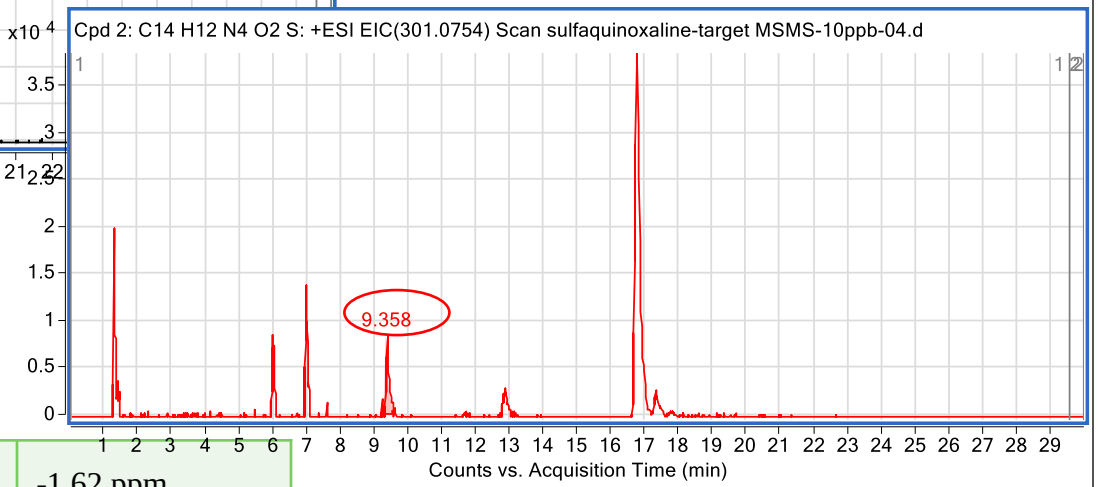
Compound	Formula	Rt
Sulfamethoxypyridazine	C11H12N4O3S	6.35 min
Sulfametere	C11H12N4O3S	6.55 min
Sulfamethazine	C11H12N4O3S	7.05 min

Temazepam (C16H13ClN2O2) and Sulfaquinoxaline (C14H12N4O2S)



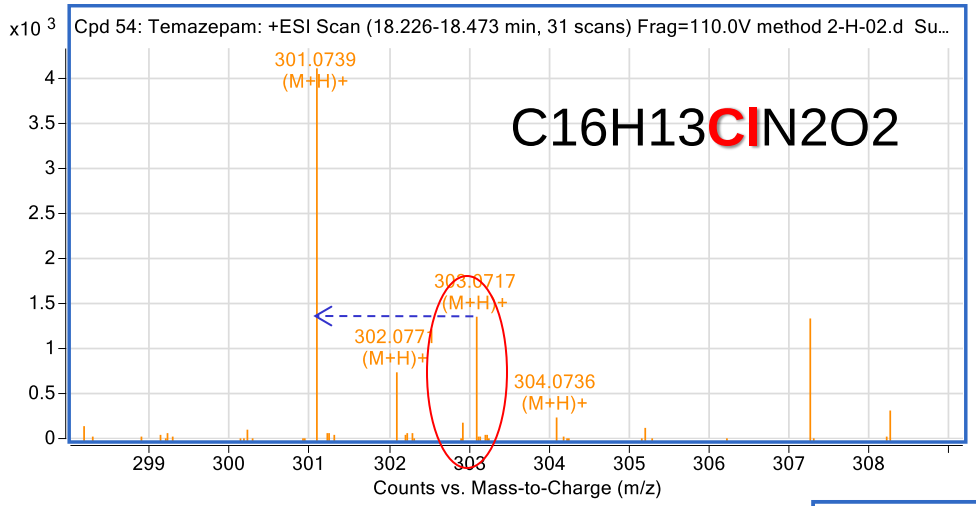
Mass accuracy

Different Retention time

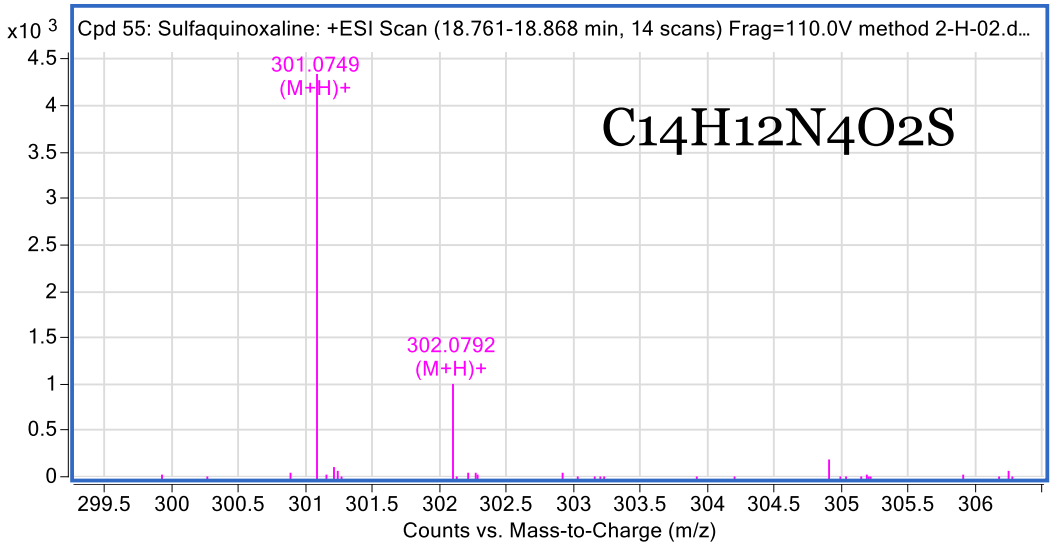


C16H13ClN2O2	Std	300.0661	300.0666	-1.62 ppm
	Meat	300.0666	300.0666	0.06 ppm
C14H12N4O2S	Std	300.0676	300.0681	-1.69 ppm
	Meat	300.0687	300.0681	-1.9 ppm

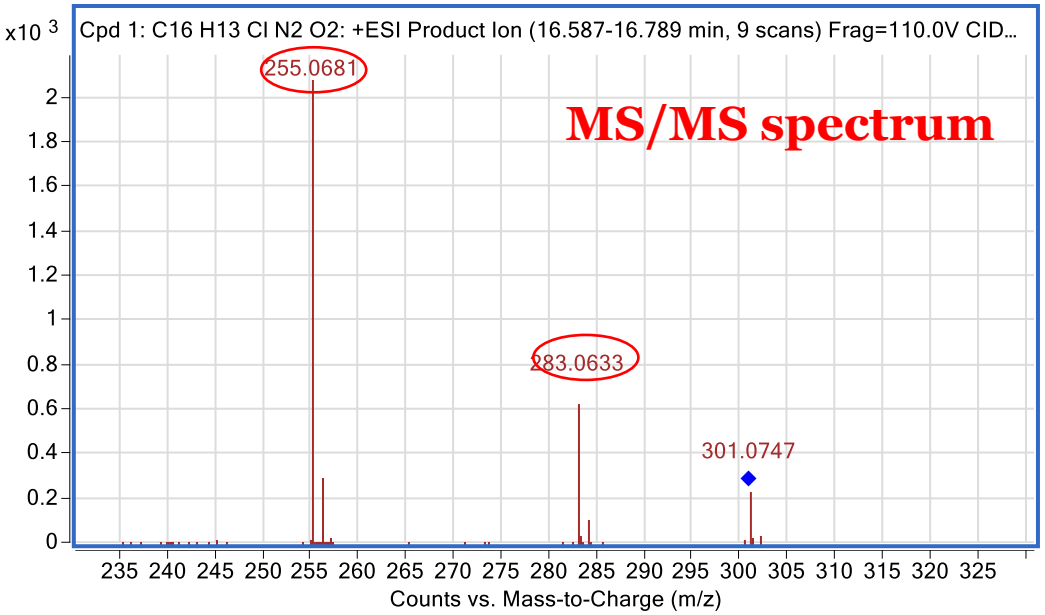
Temazepam ($C_{16}H_{13}ClN_2O_2$) and Sulfaquinoxaline ($C_{14}H_{12}N_4O_2S$)

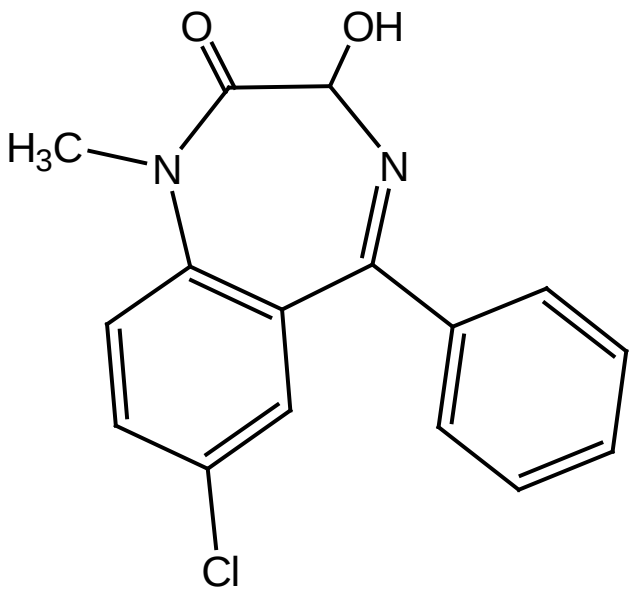


Isotope match



Confirmation of Temazepam



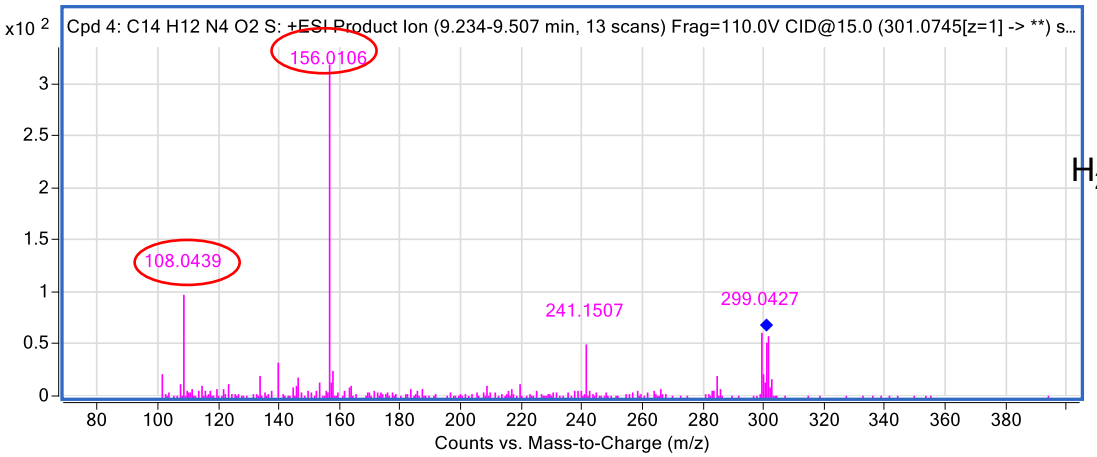


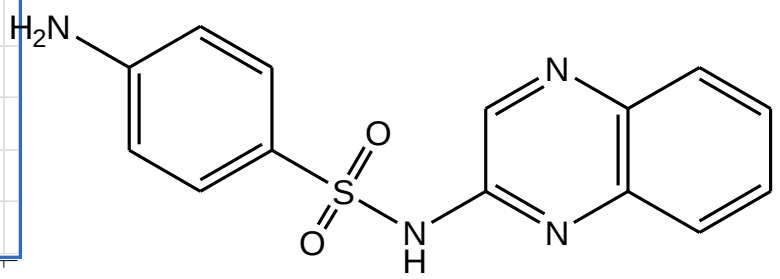
MS/MS Formula Details: Cpd1: C16 H13 Cl N2 O2 - target msms temazepam and sulfaquionxacline-02.d C

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			

Conclusion

- 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!

http://www.shciq.gov.cn/english/

Contact Us:
Shanghai Exit and Entry Inspection&Quarantine Bureau
Minsheng Road No.1208,
Shanghai, 200135
Phone:(86)021-38620517
Fax: (86)021-68549058