Identification and Quantification of 6 Illegal Antibiotics in Chinese Chicken Jerky Dog Treats

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Food Laboratory
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Dogs begin experiencing symptoms

- In 2007 several cases of Acquired Fanconi Syndrome in dogs were suspected to be associated with the consumption of chicken jerky treats imported from China

- Fanconi Syndrome – kidney malfunction and can lead to death
- Symptoms include
  - Weight loss
  - Reduced appetite
  - Excessive thirst
  - Lethargy
  - Vomiting
  - death

Causes of Acquired Fanconi Syndrome

- Exposure to heavy metals (Pb, Hg, Cd, U..)
- Certain drugs such as cidofovir, tenofovir, outdated tetracycline
- Paraquat, Diquat
- Lysol
- Certain organic solvents such as toluene
- Lysine
- Maleic acid
- Other chemical agents

Causes of Acquired Fanconi Syndrome

- Exposure to heavy metals, inorganics (Pb, Hg, Cd, U..)
  - Microwave digestion followed by analysis by ICP-MS
  - Relatively easy to determine if they are present because the periodic table is limited.

Causes of Acquired Fanconi Syndrome

- Exposure to l , Cd, U..)
- Certain drugs enofovir, outdated tetracycline
- Paraquat, Dic
- Lysol
- Certain orgar toluene
- Lysine
- Maleic acid
- Other chemical agents (organic)

About 9 million organic compounds are known to exist

Detection of organic compounds

- Gas or Liquid chromatography provides separation of analyte of interest from co-extracted matrix interference and from other analytes
- Tandem mass spectrometry provides unambiguous detection and quantitation

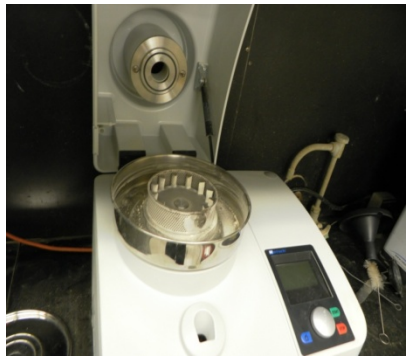
Detection of organic compounds

- **Targeted screen**
  - Analytes are determined
  - Analytical conditions are determined using standards (retention time, parent mass > product mass, ion ratio…)
  - Samples are run to determine presence and quantity of analytes

- **Unknown screen**
  - Analytes are detected using means other than comparison with a standard
    - Spectral examination of suspected analyte(GC-EI spectrum searching)
    - Exact mass determination – LC-HRMS

Sample Preparation

- Bags of suspect chicken jerky are received
- Given sample number
- Typically several pieces from a bag is ground together and considered to be one sample

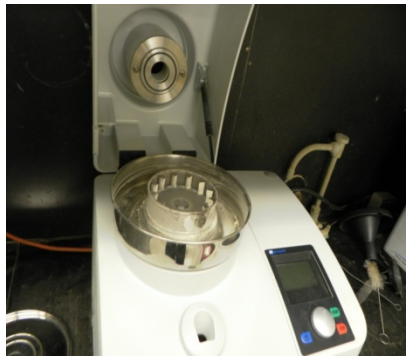


Extraction with organic solvent

analysis

Sample Preparation

- We decided to grind each piece separately and give each piece a unique sample number



Extraction with organic solvent

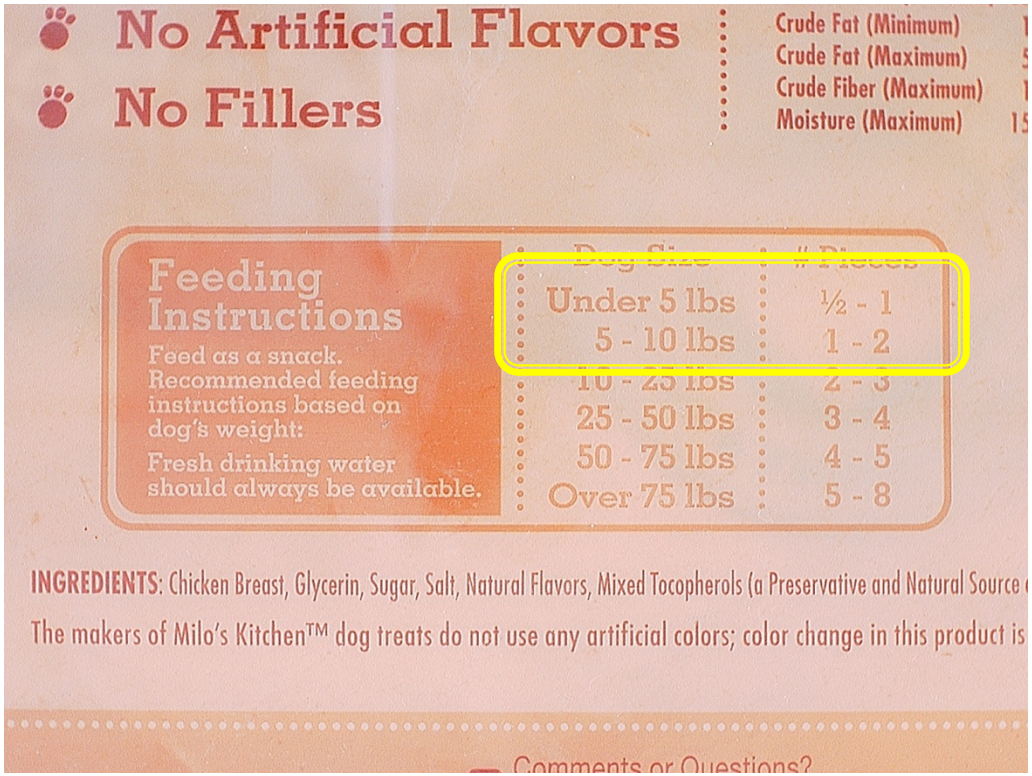
analysis

Why analyze treats individualy?

- Prevent possible dilution of an unknown contaminant if “hot spots” exist. This makes detection of contaminants easier.
- Possibly allow us to observe differences between treats from the same bag.
- Many times one treat is the recommended serving size

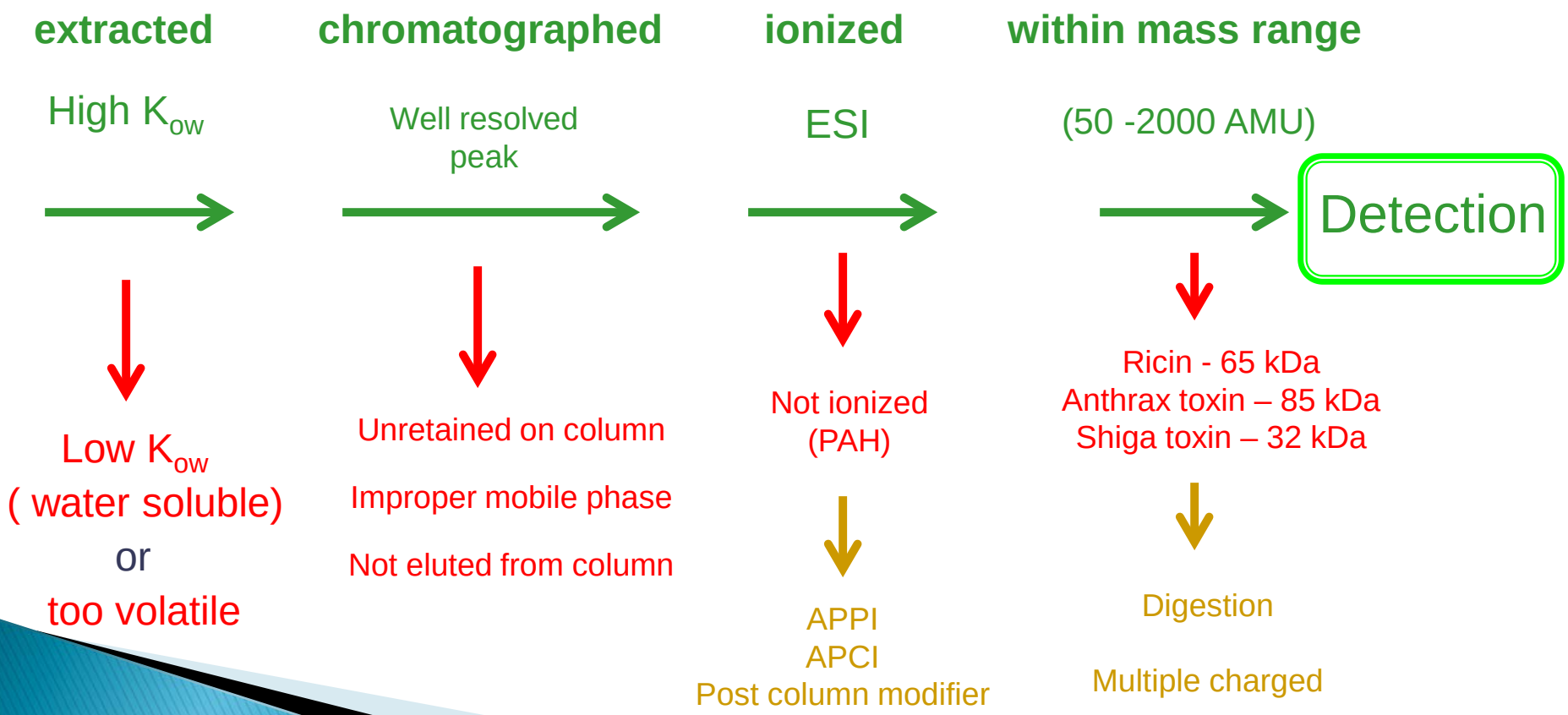
Chicken jerky label

Chicken jerky label



How likely are we to find the unknown compound?

- The compound must be



Targeted screens

- Toxin screen – 36 known toxins
  - Acetonitrile extraction
  - Analysis by LC/MS/MS
- Pesticide screen – 200 targeted pesticides
  - Acetonitrile extraction – solid phase clean up
  - Analysis by LC/MS/MS and GC/MS/MS
- Rodenticide screen – 10 targeted rodenticides
  - Acetonitrile extraction
  - Analysis by LC/MS/MS
- Mycotoxin screen – 9 mycotoxins
  - Elisa analysis
- Antibiotics screen – 38 legal and illegal veterinary drugs
  - 16 sulfonamides, 22 others
  - Acetonitrile extraction
  - UPLC/MS/MS analysis

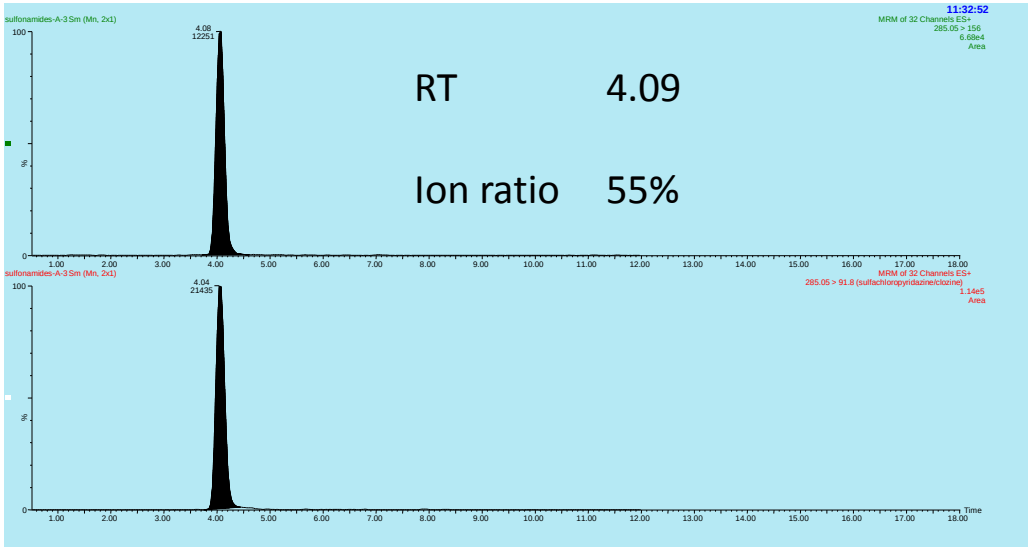
Targeted screens

- Results

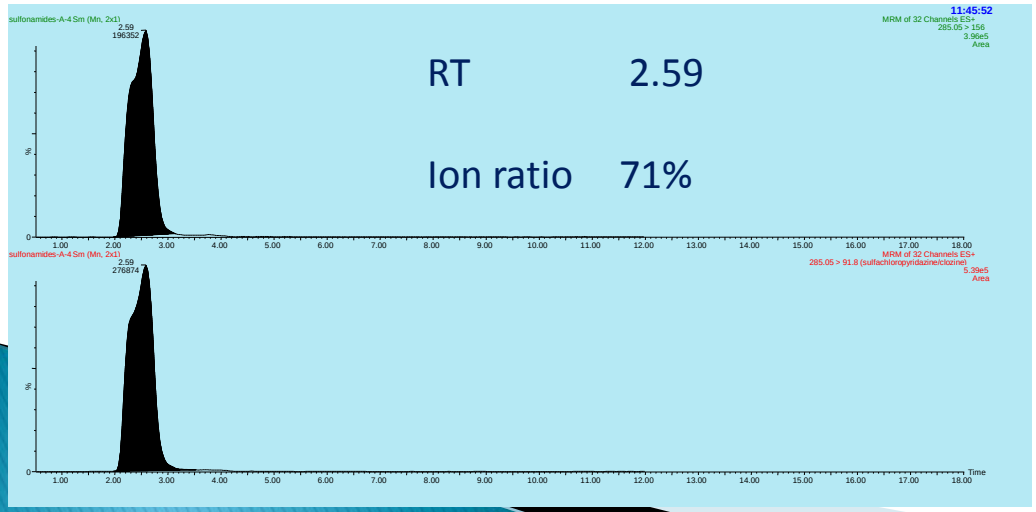
	FDA 21CFR
Sulfaquinoxaline	100 ppb
Sulfamethoxazole	no tolerance
Enrofloxacin	no tolerance
Tilmicosin	no tolerance
Trimethoprim	no tolerance

Unknown peak found in chicken jerky same transitions as Sulfachloropyridazine 285 > 156

285 > 91.8



Unknown



Sulfachloropyridazine

Unknown Identification

- Extract containing unknown peak was sent to Keith Goodman at AB Sciex (Framingham MA) for high resolution analysis using 5600 LC/QTOF

- Empirical formula determined to be

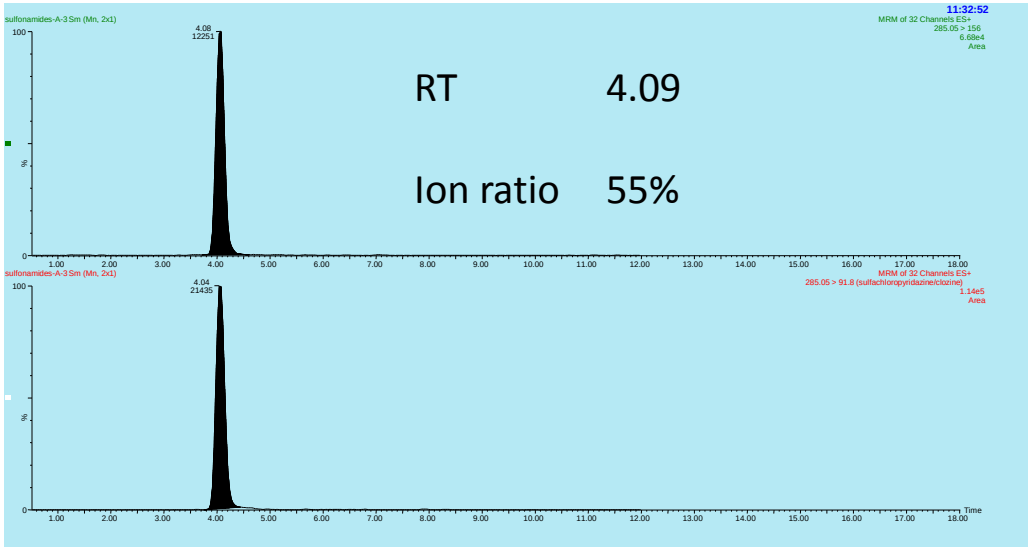
$C_{10}H_9N_4O_2SCl$ (same as sulfachloropyridazine)

Isomers of sulfachloropyridazine

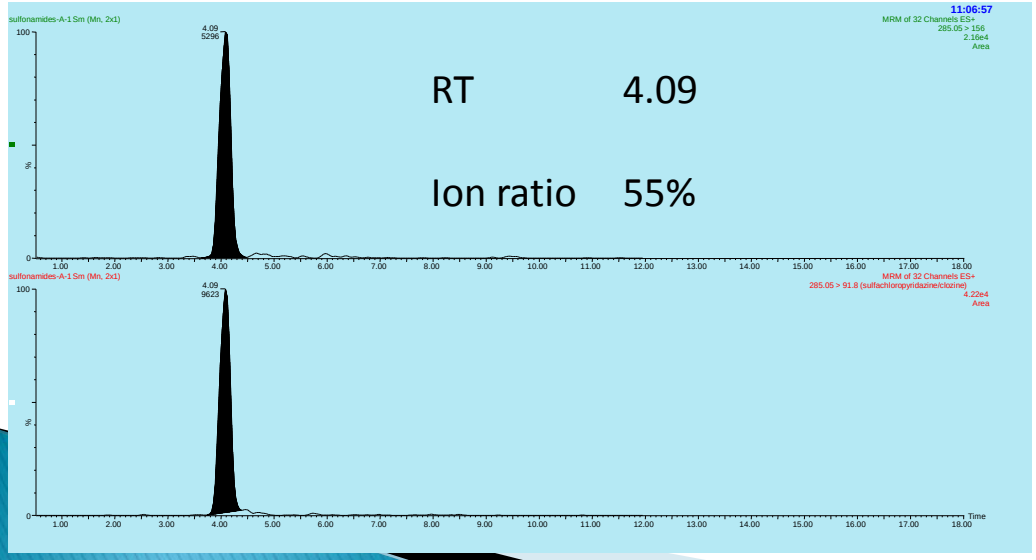
4-amino-N-(5-chloropyrimidin-2-yl) benzenesulfonamide
Metanilamide,N 1-5(chloro-2-pyrimidinyl)
3-amino-N-(5-chloro-2-pyrmidinyl) benzenesulfonamide
4-amino-N-(6-chloro-3-pyrmidinyl) benzenesulfonamide
Sulfaclozine

antibiotic used in poultry production (not allowed in US)

Unknown peak found in chicken jerky
same transitions as Sulfachloropyridazine

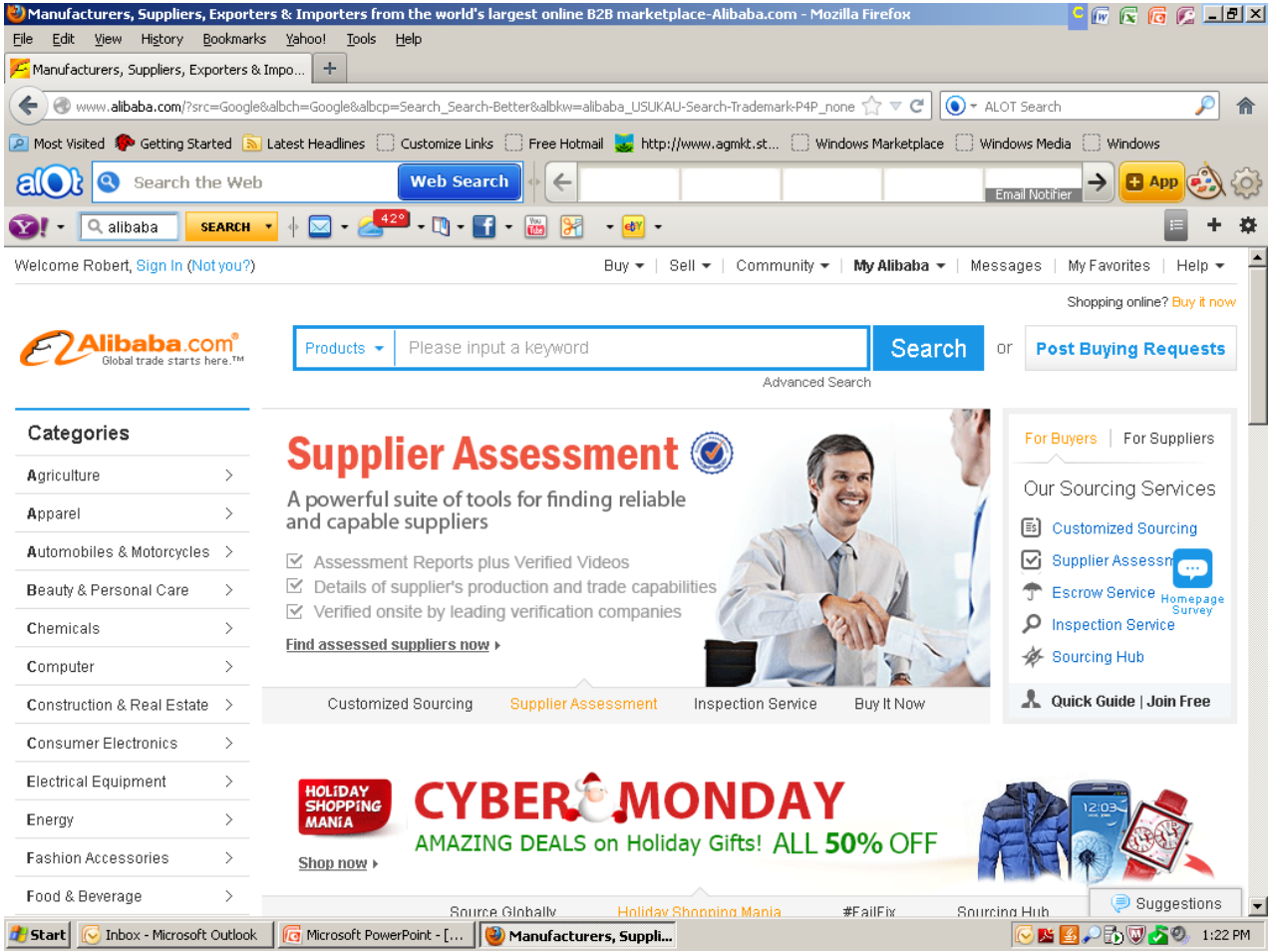


Unknown



Sulfaclozine

Alibaba.com = Amazon.com for industrial chemicals



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East Asia (33)

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25% tilmicosin oral solution for poultry
Min. Order: 3000 Barrels
FOB Price: US $2-30 / Barrel
Supply Ability: 10000 Barrel/Barrels per Day

Contact Supplier | Offline

Hebei Kexing Pharmaceutical Co., Ltd. | Contact Details
China (Mainland)
No of Employees: 301 - 500 People
Management Certification: ISO 9001:2000; GMP

Compare

poultry medicine / tilmicosin premix
Min. Order: 2000 Kilograms
FOB Price: US $2-4 / Bag
Supply Ability: 500 Kilogram/Kilograms per Day

Contact Supplier | Offline

Shandong Soocom Animal Remedy Co., Ltd. | Contact Details
China (Mainland)
No of Employees: 301 - 500 People
Management Certification: ISO 9001:2000; ISO 9001:2008

Compare

Tilmicosin Solution veterinary medicine chemicals
Min. Order: 5000 Boxes
Supply Ability: 1000 Box/Boxes per Day

Hebei Depond Animal Health Care Science And Technology Co., Ltd. | Contact Details
China (Mainland)
No of Employees: 11 - 50 People

Suggestions

Compare

Chicken Medicine/poultry medicine/Multivitamin poultry powder(China (Mainland))

Chicken Medicine/poultry medicine/Multivitamin poultry powder

Min. Order: 4000 Packs
FOB Price: US $0.35-2.5 / Pack
Supply Ability: 10000 Pack/Packs per Day

Contact Supplier Offline

Hebei New Century Pharmaceutical Co., Ltd.
China (Mainland)
No of Employees: 301 - 500 People

Contact Details

Compare

Hi-effective and Veterinary and poultry medicines with Veterinary products with Vitamin AD3 E complex oral solution/Poutry drugs(China (Mainland))

Hi-effective and Veterinary and poultry medicines with Veterinary products with Vitamin AD3 E complex oral solution/Poutry drugs

Min. Order: 100 Liters
Supply Ability: 10000 Liter/Liters per Week

Contact Supplier Offline

Weifang Premier Animal Pharmaceutical Industries Co., Ltd.
China (Mainland)
No of Employees: 201 - 300 People

Contact Details

Compare

poultry medicines for prevention of avian flu(China (Mainland))

poultry medicines for prevention of avian flu

Min. Order: 200 Pieces
FOB Price: US $0.55-1.2 / Piece
Supply Ability: 200 Ton/Tons per Month

Contact Supplier Offline

Sichuan Jie Kang Plastic Technology Co., Ltd.
China (Mainland)
No of Employees: 11 - 50 People
Management Certification: ISO 9001:2008; ISO 14001:2004

Contact Details

Compare

Poultry medicine,(Enrofloxacin Soluble Powder)(China (Mainland))

Poultry medicine,(Enrofloxacin Soluble Powder)

Min. Order: 5000 Bags
FOB Price: US $0.5-1 / Bag
Supply Ability: 5000 Bag/Bags per Day

Contact Supplier I'm Away

Shenyang Tianpeng Animal Health Products Factory
China (Mainland)
No of Employees: 10 - 200 People
Management Certification: GMP certificate

Contact Details

Compare

norfloxacin poultry medicine

Shijiazhuang Reverence Animal Husbandry

Suggestions

Sample12C03337

Subsample	Analyte (ppb)
1	sulfaclozine 3.47 sulfaquinoxaline 7.54 enrofloxacin <3
2	sulfaclozine 751 sulfaquinoxaline 828 enrofloxacin <3
3	sulfaclozine 728 sulfaquinoxaline 828 enrofloxacin <3
4	sulfaclozine 12.0 sulfaquinoxaline 12.1 Tilmicosin <3 enrofloxacin <3

Highest concentrations found

Analyte	concentration	FDA tolerance
Sulfaclozine	2000 ppb	0
Sulfaquinoxaline	828 ppb	100ppb
Enrofloxacin	132 ppb	0
Sulfamethoxazole	5.2 ppb	0
Tilmicosin	528 ppb	0
Trimethoprim	41 ppb	0

All major brands voluntarily removed from sale throughout US

- No indication the illegal antibiotics were responsible for dog illnesses
- Consistantly above tolerance
- Antibiotic misuse could contribute to pathogenic bacteria resistance

Fluoroquinolone-Resistant *Campylobacter* Species and the Withdrawal of Fluoroquinolones from Use in Poultry: A Public Health Success Story

Jennifer M. Nelson,[1,2] Tom M. Chiller,[1] John H. Powers,[3] and Frederick J. Angulo[1]

[1]Enteric Diseases Epidemiology Branch, Division of Foodborne, Bacterial and Mycotic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Centers for Disease Control and Prevention, and [2]Atlanta Research and Education Foundation, Atlanta, Georgia; and [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

Campylobacter **species cause 1.4 million infections each year in the United States. Fluoroquinolones (e.g., ciprofloxacin) are commonly used in adults with** ***Campylobacter*** **infection and other infections. Fluoroquinolones (e.g., enrofloxacin) are also used in veterinary medicine. Human infections with fluoroquinolone-resistant** ***Campylobacter*** **species have become increasingly common and are associated with consumption of poultry. These findings, along with other data, prompted the US Food and Drug Administration to propose the withdrawal of fluoroquinolone use in poultry in 2000. A lengthy legal hearing concluded with an order to withdraw enrofloxacin from use in poultry (effective in September 2005). Clinicians are likely to continue to encounter patients with fluoroquinolone-resistant** ***Campylobacter*** **infection and other enteric infection because of the continued circulation of fluoroquinolone-resistant** ***Campylobacter*** **species in poultry flocks and in persons returning from foreign travel who have acquired a fluoroquinolone-resistant enteric infection while abroad. Judicious use of fluoroquinolones and other antimicrobial agents in human and veterinary medicine is essential to preserve the efficacy of these important chemotherapeutic agents.**

Food Safety News

Africa and EU See Rising Level of Antibiotic-Resistant Salmonella

By James Andrews | June 20, 2013

Strains of one increasingly antibiotic-resistant Salmonella serotype have seen a "rapid worldwide spread," according to a study published by researchers at the Institut Pasteur in Paris and Morocco.

Antibiotic-resistant Salmonella Kentucky, first isolated in 2002 in a French tourist who had visited Egypt, has now "spread at an astonishing rate throughout Africa and the Middle East in the space of only a few years," the study's authors claim.

The bacterium has also already been found in farmed-raised turkeys in Europe, though it is not clear based on available information if those turkeys were imported or grown domestically. In a summary of the study, the lead author said he worries that the resistant strain may soon spread to European poultry farms.

This study comes on the heels of a report out of Canada calling antibiotic-resistant Salmonella Kentucky a rare but "growing concern" in Canadian health. That study found that between 2003 and 2009, 30 percent of Salmonella Kentucky isolates from Canadian patients were resistant to the antibiotic ciprofloxacin.

Those Canadian infections, however, were not associated with any retail food sold in Canada. Instead, every patient with available travel information had visited an African country within a week of developing symptoms.

According to the authors of the Pasteur study, the resistant bacterium has continued to spread through Mediterranean countries, particularly Morocco, infecting hundreds of patients each year.

"In addition, the authors of this study made the troubling observation that a number of strains recently acquired in the Mediterranean Basin are showing a range of resistance towards all antibiotic classes used to treat severe cases of salmonellosis," the study's summary read.

The main vehicle of transmission for antibiotic-resistant Salmonella Kentucky from African and Middle Eastern countries appears to be chickens and turkeys. The authors said the resistance is believed to be caused by "the massive overuse" of antibiotics in African poultry farming.

According to a May 2013 report by the Center for Science in the Public Interest, the U.S. saw 55 outbreaks of antibiotic-resistant pathogens between 1973 and 2011. Contaminated dairy products and ground beef accounted for the majority of those outbreaks.

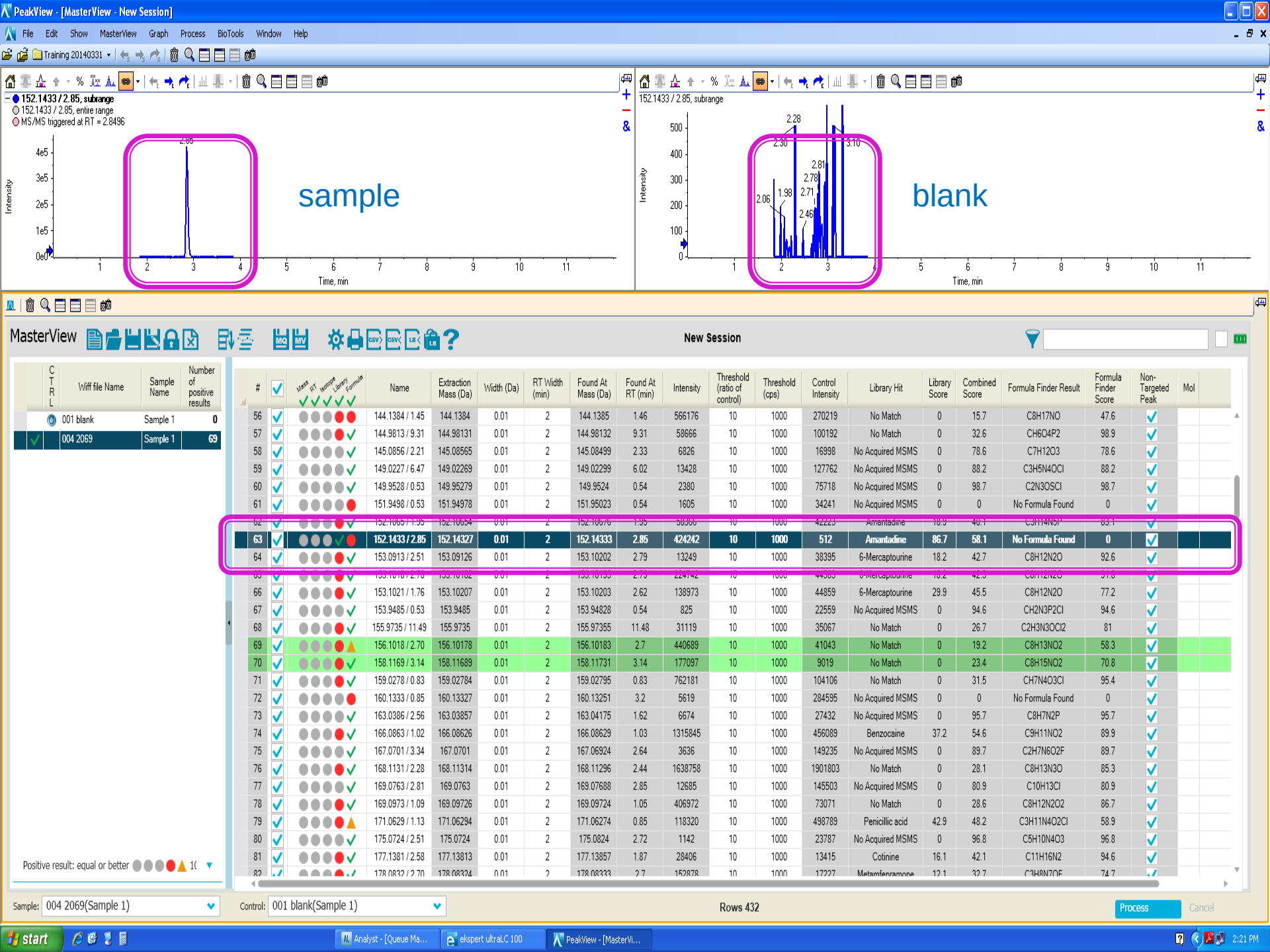
Antibiotic-resistant Salmonella strains accounted for 50 (91 percent) of those drug-resistant outbreaks, though none of them were Salmonella Kentucky. At least 35 (64 percent) of those were resistant to five or more antibiotics.

On Monday, Congresswoman Louise Slaughter (D-NY), the only microbiologist in Congress, wrote a letter to President Obama urging him to "pay special attention to issues of antibiotic resistance" at this week's G-8 Summit in Northern Ireland. Slaughter also suggested the President consider stronger limits on antibiotic use in animal agriculture.

David Willetts, Britain's science minister, is expected to use his platform at the G-8 meeting to propose new measures to curb the overuse of antibiotics by both healthcare professionals and farmers alike.

Recent findings

- Recent acquisition of Sciex 5600 triple TOF
- Allows for identification of compounds without comparison to a standard.
  - Exact mass determination
  - High resolution product ion library searching
  - Empirical formula finding



#	Name	Extraction Mass (Da)	Width (Da)	RT Width (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak	Mol
56	144.1384 / 1.45	144.1384	0.01	2	144.1385	1.46	566176	10	1000	270219	No Match	0	15.7	C8H17NO	47.6	✓	
57	144.9813 / 9.31	144.98131	0.01	2	144.98132	9.31	58666	10	1000	100192	No Match	0	32.6	CH6O4P2	98.9	✓	
58	145.0856 / 2.21	145.08565	0.01	2	145.08499	2.33	6826	10	1000	16998	No Acquired MSMS	0	78.6	C7H12O3	78.6	✓	
59	149.0227 / 6.47	149.02269	0.01	2	149.02299	6.02	13428	10	1000	127762	No Acquired MSMS	0	88.2	C3H5N4OCl	88.2	✓	
60	149.9528 / 0.53	149.95279	0.01	2	149.9524	0.54	2380	10	1000	75718	No Acquired MSMS	0	98.7	C2N3OSCl	98.7	✓	
61	151.9498 / 0.53	151.94978	0.01	2	151.95023	0.54	1605	10	1000	34241	No Acquired MSMS	0	0	No Formula Found	0	✓	
62	152.1065 / 1.95	152.10654	0.01	2	152.10676	1.95	58366	10	1000	42223	Amantadine	18.9	46.1	C3H14N5P	83.1	✓	
63	152.1433 / 2.85	152.14327	0.01	2	152.14333	2.85	424242	10	1000	512	Amantadine	86.7	58.1	No Formula Found	0	✓	
64	153.0913 / 2.51	153.09126	0.01	2	153.10202	2.79	13249	10	1000	38395	6-Mercaptourine	18.2	42.7	C8H12N2O	92.6	✓	
65	153.1018 / 2.78	153.10182	0.01	2	153.10193	2.79	224742	10	1000	44383	6-Mercaptourine	18.2	42.5	C8H12N2O	91.6	✓	
66	153.1021 / 1.76	153.10207	0.01	2	153.10203	2.62	138973	10	1000	44859	6-Mercaptourine	29.9	45.5	C8H12N2O	77.2	✓	
67	153.9485 / 0.53	153.9485	0.01	2	153.94828	0.54	825	10	1000	22559	No Acquired MSMS	0	94.6	CH2N3P2Cl	94.6	✓	
68	155.9735 / 11.49	155.9735	0.01	2	155.97355	11.48	31119	10	1000	35067	No Match	0	26.7	C2H3N3OCl2	81	✓	
69	156.1018 / 2.70	156.10178	0.01	2	156.10183	2.7	440689	10	1000	41043	No Match	0	19.2	C8H13NO2	58.3	✓	
70	158.1169 / 3.14	158.11689	0.01	2	158.11731	3.14	177097	10	1000	9019	No Match	0	23.4	C8H15NO2	70.8	✓	
71	159.0278 / 0.83	159.02784	0.01	2	159.02795	0.83	762181	10	1000	104106	No Match	0	31.5	CH7N4O3Cl	95.4	✓	
72	160.1333 / 0.85	160.13327	0.01	2	160.13251	3.2	5619	10	1000	284595	No Acquired MSMS	0	0	No Formula Found	0	✓	
73	163.0386 / 2.56	163.03857	0.01	2	163.04175	1.62	6674	10	1000	27432	No Acquired MSMS	0	95.7	C8H7N2P	95.7	✓	
74	166.0863 / 1.02	166.08626	0.01	2	166.08629	1.03	1315845	10	1000	456089	Benzocaine	37.2	54.6	C9H11NO2	89.9	✓	
75	167.0701 / 3.34	167.0701	0.01	2	167.06924	2.64	3636	10	1000	149235	No Acquired MSMS	0	89.7	C2H7N6O2F	89.7	✓	
76	168.1131 / 2.28	168.11314	0.01	2	168.11296	2.44	1638758	10	1000	1901803	No Match	0	28.1	C8H13N3O	85.3	✓	
77	169.0763 / 2.81	169.0763	0.01	2	169.07688	2.85	12685	10	1000	145503	No Acquired MSMS	0	80.9	C10H13Cl	80.9	✓	
78	169.0973 / 1.09	169.09726	0.01	2	169.09724	1.05	406972	10	1000	73071	No Match	0	28.6	C8H12N2O2	86.7	✓	
79	171.0629 / 1.13	171.06294	0.01	2	171.06274	0.85	118320	10	1000	498789	Penicillic acid	42.9	48.2	C3H11N4O2Cl	58.9	✓	
80	175.0724 / 2.51	175.0724	0.01	2	175.0824	2.72	1142	10	1000	23787	No Acquired MSMS	0	96.8	C5H10N4O3	96.8	✓	
81	177.1381 / 2.58	177.13813	0.01	2	177.13857	1.87	28406	10	1000	13415	Cotinine	16.1	42.1	C11H16N2	94.6	✓	
82	178.0832 / 2.70	178.08324	0.01	2	178.08333	2.7	152878	10	1000	17227	Metamfepramone	12.1	32.7	C3H8N7OF	74.7	✓	

PeakView - [MasterView - New Session]

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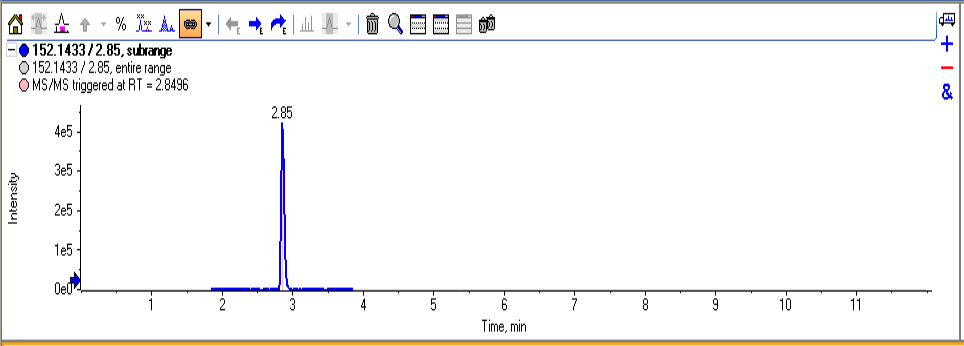
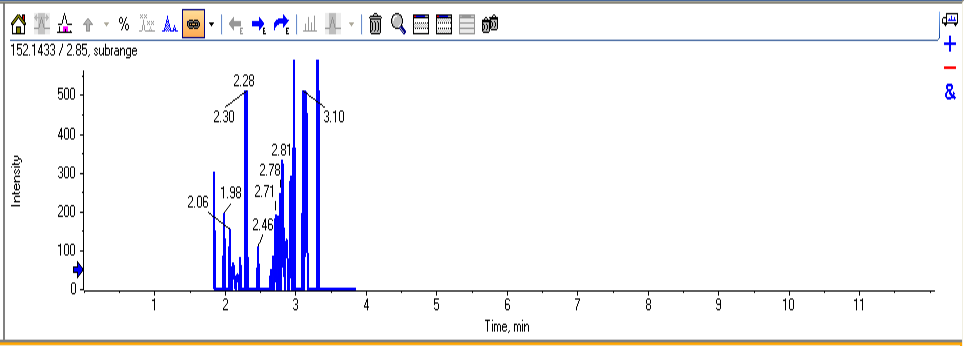
Training 20140331





MasterView

New Session

CTRL	Wiff file Name	Sample Name	Number of positive results
	001 blank	Sample 1	0
✓	004 2069	Sample 1	69

Positive result: equal or better

#	✓	Mass	RT	Isotope	Library	Formula	Name	Extraction Mass (Da)	Width (Da)	RT Width (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak
53	✓	✓	✓	✓	✓	✓	141.9587 / 0.04	141.95869	0.01	2	141.95816	10.77	198814	10	1000	189911	No Acquired MSMS	0	72.2	C4H-1NO3S	72.2	✓
54	✓	✓	✓	✓	✓	✓	144.0480 / 1.19	144.04798	0.01	2	144.04753	1.28	209494	10	1000	266901	No Match	0	29.7	C6H9NOS	90	✓
55	✓	✓	✓	✓	✓	✓	144.1014 / 2.21	144.10141	0.01	2	144.10149	2.22	115587	10	1000	2461	No Match	0	16.4	C7H13NO2	49.8	✓
56	✓	✓	✓	✓	✓	✓	144.1384 / 1.45	144.1384	0.01	2	144.1385	1.46	566176	10	1000	270219	No Match	0	15.7	C8H17NO	47.6	✓
57	✓	✓	✓	✓	✓	✓	144.9813 / 9.31	144.98131	0.01	2	144.98132	9.31	58666	10	1000	100192	No Match	0	32.6	CH6O4P2	98.9	✓
58	✓	✓	✓	✓	✓	✓	145.0856 / 2.21	145.08565	0.01	2	145.08499	2.33	6826	10	1000	16998	No Acquired MSMS	0	78.6	C7H12O3	78.6	✓
59	✓	✓	✓	✓	✓	✓	149.0227 / 6.47	149.02269	0.01	2	149.02299	6.02	13428	10	1000	127762	No Acquired MSMS	0	88.2	C3H5N4OCl	88.2	✓
60	✓	✓	✓	✓	✓	✓	149.9528 / 0.53	149.95279	0.01	2	149.9524	0.54	2380	10	1000	75718	No Acquired MSMS	0	98.7	C2N3OSCl	98.7	✓
61	✓	✓	✓	✓	✓	✓	151.9498 / 0.53	151.94978	0.01	2	151.95023	0.54	1605	10	1000	34241	No Acquired MSMS	0	0	No Formula Found	0	✓
62	✓	✓	✓	✓	✓	✓	152.1065 / 1.95	152.10654	0.01	2	152.10676	1.95	58366	10	1000	42223	Amantadine	18.9	40.1	C3H14N5P	83.1	✓
63	✓	✓	✓	✓	✓	✓	**152.1433 / 2.85**	**152.14327**	**0.01**	**2**	**152.14333**	**2.85**	**424242**	**10**	**1000**	**512**	**Amantadine**	**86.7**	**58.1**	**No Formula Found**	**0**	✓

Library Search Results

Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	CI
Amantadine	768-94-5	C10H17N	151.25187	89.5	86.8	86.7	35
Gepefrine	18840-47-6	C9H13NO	151.20845	69.2	34.6	34.6	35
p-(Aminomethyl)benzoic acid	56-91-7	C8H9NO2	151.16504	67.3	38.8	38.4	35

Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS/MS (ppm)

64	✓	✓	✓	✓	✓	✓	153.0913 / 2.51	153.09126	0.01	2	153.10202	2.79	13249	10	1000	38395	6-Mercaptourine	18.2	42.7	C8H12N2O	92.6	✓
65	✓	✓	✓	✓	✓	✓	153.1018 / 2.78	153.10182	0.01	2	153.10195	2.79	224742	10	1000	44583	6-Mercaptourine	18.2	42.5	C8H12N2O	91.8	✓
66	✓	✓	✓	✓	✓	✓	153.1021 / 1.76	153.10207	0.01	2	153.10203	2.62	138973	10	1000	44859	6-Mercaptourine	29.9	45.5	C8H12N2O	77.2	✓
67	✓	✓	✓	✓	✓	✓	153.9485 / 0.53	153.9485	0.01	2	153.94828	0.54	825	10	1000	22559	No Acquired MSMS	0	94.6	CH2N3P2Cl	94.6	✓
68	✓	✓	✓	✓	✓	✓	155.9735 / 11.49	155.9735	0.01	2	155.97355	11.48	31119	10	1000	35067	No Match	0	26.7	C2H3N3OCl2	81	✓
69	✓	✓	✓	✓	✓	✓	156.1018 / 2.70	156.10178	0.01	2	156.10183	2.7	440689	10	1000	41043	No Match	0	19.2	C8H13NO2	58.3	✓
70	✓	✓	✓	✓	✓	✓	158.1169 / 3.14	158.11689	0.01	2	158.11731	3.14	177097	10	1000	9019	No Match	0	23.4	C8H15NO2	70.8	✓
71	✓	✓	✓	✓	✓	✓	159.0278 / 0.83	159.02784	0.01	2	159.02795	0.83	762181	10	1000	104106	No Match	0	31.5	CH7N4O3Cl	95.4	✓
72	✓	✓	✓	✓	✓	✓	160.1333 / 0.85	160.13327	0.01	2	160.13251	3.2	5619	10	1000	284595	No Acquired MSMS	0	0	No Formula Found	0	✓

Sample: 004 2069(Sample 1) Control: 001 blank(Sample 1) Rows 432 Process Cancel

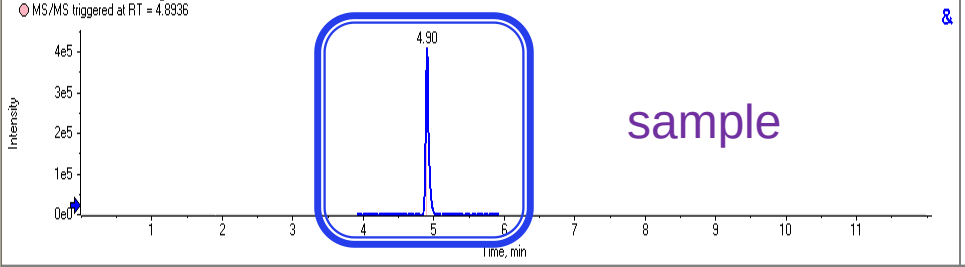
start Analyst - [Queue Ma... ekspert ultraLC 100 PeakView - [MasterVi... F:\ 2:28 PM

PeakView - [MasterView - New Session]

File Edit Show MasterView Graph Process BioTools Window Help

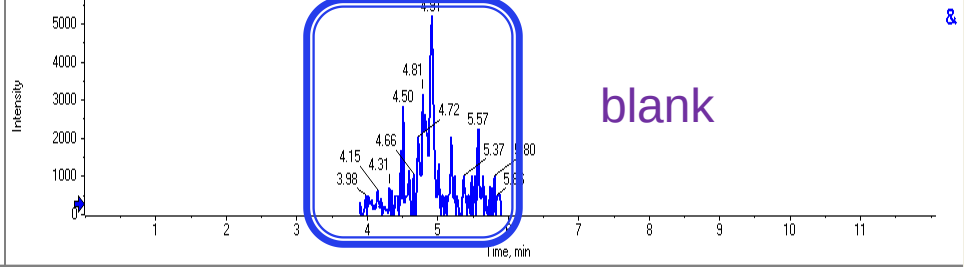
Training 20140331

192.1382 / 4.91, subrange
192.1382 / 4.91, entire range
MS/MS triggered at RT = 4.8936



sample

192.1382 / 4.91, subrange



blank

MasterView

New Session

CTRL	Wiff file Name	Sample Name	Number of positive results
	001 blank	Sample 1	0
✓	003 2059	Sample 1	7

Positive result: equal or better

#	Name	Extraction Mass (Da)	Width (Da)	RT Width (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak
19	149.0232 / 5.32	149.02323	0.01	2	149.02331	5.32	1201239	10	1000	85590	No Match	0	23	C8H4O3	69.7	✓
20	149.9526 / 0.53	149.95257	0.01	2	149.95247	0.54	3300	10	1000	75416	No Acquired MSMS	0	97.2	C2N3OSCl	97.2	✓
21	163.1326 / 3.68	163.13264	0.01	2	163.13286	3.68	1187163	10	1000	1010	No Match	0	16.4	C8H18O3	49.8	✓
22	166.0861 / 1.02	166.08608	0.01	2	166.08631	1.02	1300981	10	1000	454464	Benzocaine	33.7	52.3	C9H11NO2	90.2	✓
23	168.1131 / 2.28	168.11308	0.01	2	168.11326	2.46	378505	10	1000	1900377	No Match	0	30.3	C8H13N3O	91.9	✓
24	177.0544 / 5.32	177.05436	0.01	2	177.05452	5.32	874283	10	1000	19107	No Match	0	26.6	C10H8O3	80.7	✓
25	181.1219 / 4.93	181.1219	0.01	2	181.12143	4.93	14811	10	1000	1098877	Theobromine	26.3	45.6	C11H16O2	84.7	✓
26	182.1286 / 3.20	182.12862	0.01	2	182.12903	3.26	1886638	10	1000	4367721	No Match	0	28.7	C9H15N3O	86.9	✓
27	185.1147 / 3.66	185.11471	0.01	2	185.11471	3.68	673424	10	1000	3654	Fuberidazole	78.9	80.1	C6H12N6O	82.6	✓
28	[illegible]	[illegible]	0.01	2	[illegible]	[illegible]	[illegible]	10	1000	[illegible]	No Match	0	[illegible]	[illegible]	[illegible]	✓
29	192.1382 / 4.91	192.13824	0.01	2	192.13828	4.9	412026	10	1000	5211	DEET	99.7	96.5	C12H17NO	89.9	✓

Library Search Results

Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	CI
DEET	134-62-3	C12H17NO	191.27327	100	99.7	99.7	35
DEET	134-62-3	C12H17NO	191.27327	100	100	100	35
DEET	134-62-3	C12H17NO	191.27327	100	96.4	96.4	35
N,N-Diethyl-m-toluamide	134-62-3	C12H17NO	191.27327	98.7	96.4	95.1	35
Phendimetrazine		C12H17NO	191.27327	55.4	61.1	33.9	35

Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS/MS (ppm)
	C12H17NO	89.9	192.13829	0	6
	C6H18N5P	15.6	192.13726	5.3	7.9

#	Name	Extraction Mass (Da)	Width (Da)	RT Width (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak
30	195.1130 / 1.29	195.11301	0.01	2	195.11314	1.28	2375650	10	1000	788839	No Match	0	26.6	C10H14N2O2	80.7	✓
31	[illegible]	[illegible]	0.01	2	[illegible]	[illegible]	[illegible]	10	1000	[illegible]	amisometradine	15.3	[illegible]	[illegible]	89.3	✓
32	197.1285 / 2.06	197.12852	0.01	2	197.12864	2.08	1974558	10	1000	680994	1-(3-chlorophenyl)piperazine	16.7	36	C10H16N2O2	75	✓
33	198.1488 / 2.74	198.14879	0.01	2	198.148	3.5	5936	10	1000	635904	No Acquired MSMS	0	0	No Formula Found	0	✓
34	200.1391 / 1.76	200.13913	0.01	2	200.13901	1.89	68633	10	1000	208136	No Match	0	31.2	C9H17N3O2	94.5	✓
35	202.0975 / 3.15	202.09749	0.01	2	202.09765	3.15	259380	10	1000	1204703	Simazine	39.7	57.5	C11H11N3O	93.4	✓
36	205.0972 / 1.30	205.09717	0.01	2	205.09736	1.39	508111	10	1000	313587	No Match	0	31.3	C11H12N2O2	95	✓
37	211.1444 / 3.18	211.14445	0.01	2	211.14444	3.18	5381001	10	1000	1197625	No Match	0	26.3	C11H18N2O2	79.8	✓

Sample: 003 2059(Sample 1) Control: 001 blank(Sample 1) Rows 86 Process Cancel

start Analyst - [Queue Ma... ekspert ultraLC 100 PeakView - [MasterVi... G:\ 10:39 AM

PeakView - [Fragmentation]

File Edit Show MasterView Graph Process BioTools Window Help

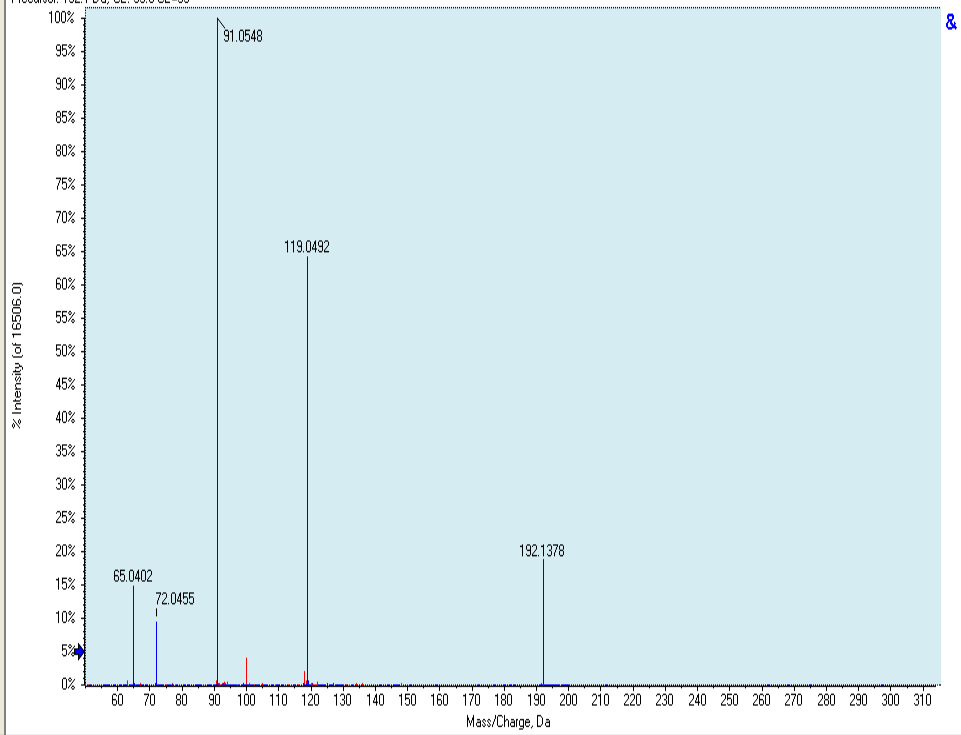
Training 20140331

ChemSpider results for: C12H17NO

1-40 of 1440

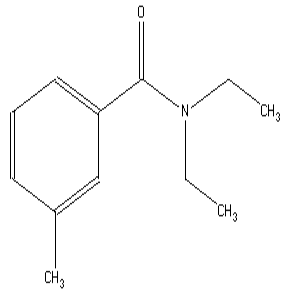
CSID	Common Name	Molecular Weight
4133	DEET	191.26948
16161	N,N-diethyl-2-phenylacetamide	191.26948
60061	o-Tolualdehyde, 4- (diethylamino)-	191.26948
70826	1-benzylpiperidin-4-ol	191.26948
71021	1-methyl-4-phenylpiperidin-4-ol	191.26948
77363	1-Benzyl-3-piperidinol	191.26948
84761	4-(4-Methylphenyl)-4-piperidinol	191.26948
88623	4-benzylpiperidin-4-ol	191.26948
122147	1-[4-(Diethylamino)phenyl]ethanone	191.26948
124073	p-tert-Butylacetanilide	191.26948
225069	propanamide, 2,2-dimethyl-N-(4-methylphenyl)-	191.2695
276553	N-benzyl-2,2-dimethylpropanamide	191.26948
300563	phenyl(piperidin-4-yl)methanol	191.2695
474138	4-(2-methoxyphenyl)piperidine	191.26948
475485	2,2-Dimethyl-N-(3-methylphenyl)propanamide	191.26948
489379	(1-Benzyl-2-pyrrolidinyl)methanol	191.26948
501565	benzamide, N-(1,1-dimethylethyl)-4-methyl-	191.2695
628233	[(2S)-1-Benzyl-2-pyrrolidinyl]methanol	191.26948
645434	2-(piperidin-1-ylmethyl)phenol	191.2695
722291	3-(1-piperidinomethyl)phenol	191.26948
2015763	2,6-Dimethylisobutyranilide	191.26948
2044612	3-(2-Methyl-2-propanyl)-3,4-dihydro-2H-1,4-benzoxazine	191.2695
2403051	2-furanmethanamine, tetrahydro-N-(phenylmethyl)-	191.2695

Spectrum from 003 2059.wiff (sample 1) - 2059, Experiment 2, +TOF MS^2 (50 - 2000) from 4.894 min
Precursor: 192.1 Da, CE: 35.0 CE=35



C N O P S F Cl Br I Na K Ca

selected composition: $C_7H_7^+$ (91.0542 Da)



Fragments | Peaks

Mass/Charge	Intensity (%)	Assigned	Error (Da)
51.0250	0.16		
67.0528	0.16		
91.2233	0.16		
91.4574	0.16		
91.5464	0.16		
91.6277	0.16		
91.9701	0.16		
92.0242	0.16		

Matches: 9 of 200 peaks, 88.1% of total intensity

Select Cancel

start | Analyst - [Queue Ma... | ekspert ultraLC 100 | PeakView - [Fragmen... | G:\ | DEET.rtf - WordPad | 10:42 AM

Quantification

- Standards are purchased
- LC/MS/MS analysis method is developed
- Extraction method is optimized
- Samples are extracted with method
- Samples are analyzed along with standards
- Analytes are confirmed with tandem MS
  - RT
  - Ion ratio comparison
- Analytes are quantified

Quantitation performed using UPLC/MS/MS

	Amantadine	DEET
Detection frequency	36%	38%
Highest concentration	882 ng/g	572 ng/g

- **Amantadine**
  - Antiviral drug only approved for human use
  - Chinese poultry farmers were suspected of misuse in 2005 for prevention of avian flu
  - H5N1 strains in China are now resistant

- **DEET**
  - Insect repellant/ pesticide
  - Acetylcholinesterase inhibitor in insects and mammals

- No connection has been made between any of the 8 compounds detected and the illnesses
- Many of the detections represent misuse
- Investigation continues

- Special thanks to :
  - Kristen Hafler
  - Jennifer Mirabile
  - Kendal Harr

- Questions ?