

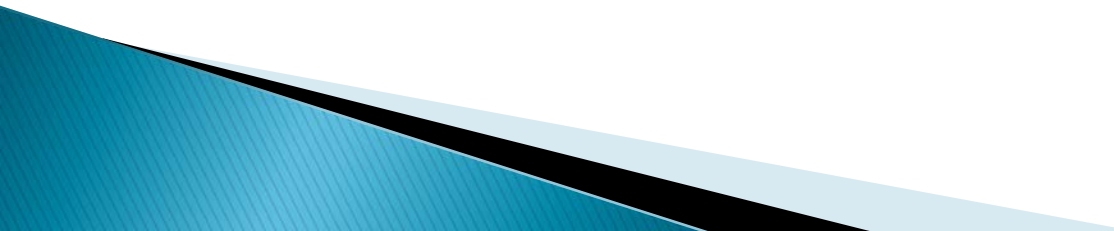
Identification and Quantification of 6 Illegal Antibiotics in Chinese Chicken Jerky Dog Treats

New York State Department of Agriculture & Markets
Food Laboratory
Robert Sheridan

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 - ▶ Certain drugs such as cidofovir, tenofovir, outdated tetracycline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
- ▶ Certain drugs: tetracycline
- ▶ Paraquat, Dic
- ▶ Lysol
- ▶ Certain organ
- ▶ Lysine
- ▶ Maleic acid
- ▶ Other chemical agents (organic)



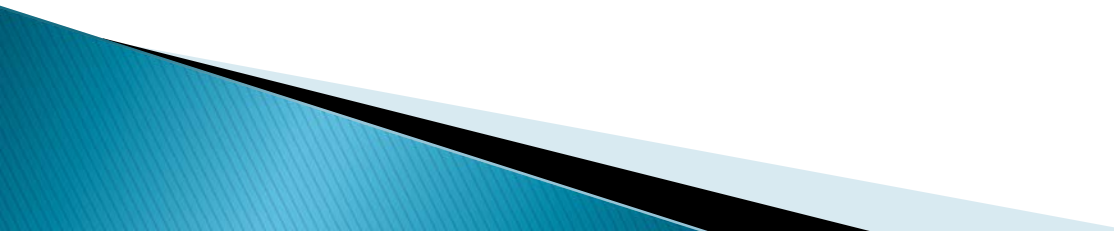
(Pb, Cd, U..)

tenofovir, outdated

toluene

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

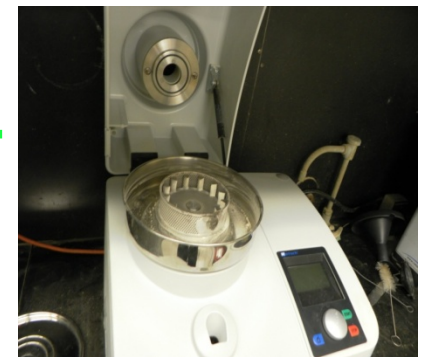
- ▶ Bags of suspect chicken jerky are received
- ▶ Given sample number
- ▶ Typically several pieces from a bag is ground together and considered to be one sample



analysis



Extraction
with
organic
solvent



Sample Preparation

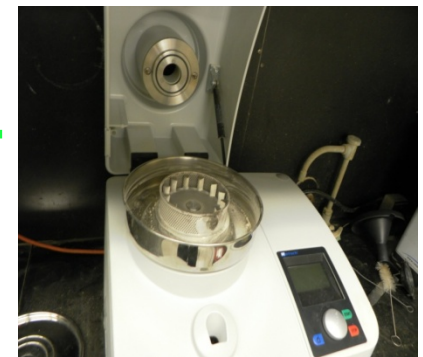
- ▶ We decided to grind each piece separately and give each piece a unique sample number



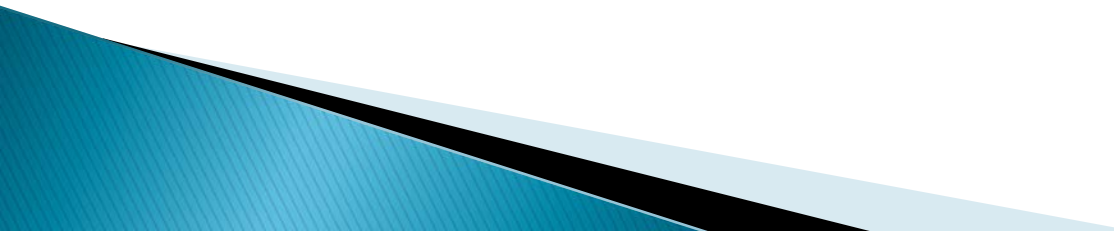
analysis



Extraction
with
organic
solvent



Why analyze treats individually?

- 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

No Artificial Flavors

No Fillers

Crude Fat (Minimum) 1
Crude Fat (Maximum) 5
Crude Fiber (Maximum) 1
Moisture (Maximum) 15

Feeding Instructions
Feed as a snack.
Recommended feeding instructions based on dog's weight:
Fresh drinking water should always be available.

Dog Size	# Pieces
Under 5 lbs	1/2 - 1
5 - 10 lbs	1 - 2
10 - 25 lbs	2 - 3
25 - 50 lbs	3 - 4
50 - 75 lbs	4 - 5
Over 75 lbs	5 - 8

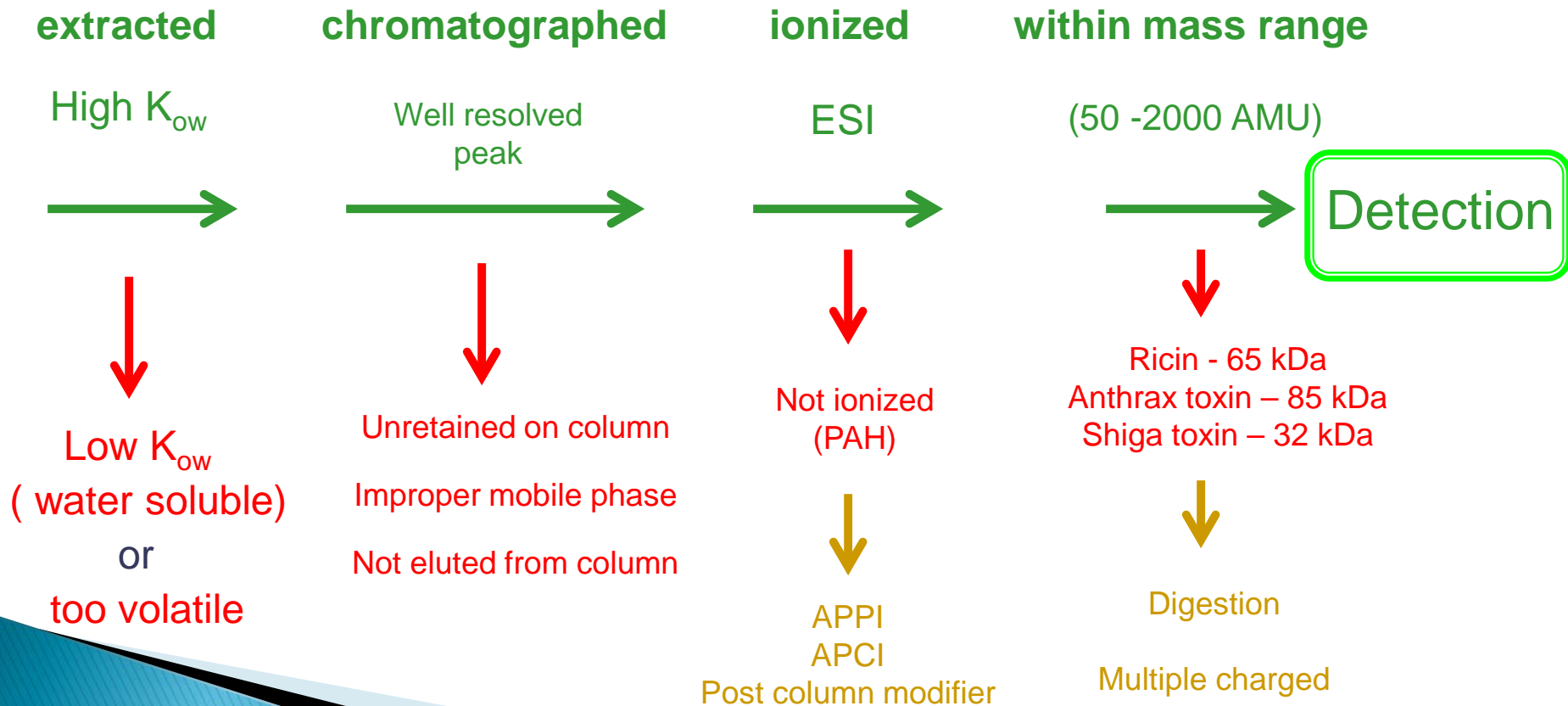
INGREDIENTS: Chicken Breast, Glycerin, Sugar, Salt, Natural Flavors, Mixed Tocopherols (a Preservative and Natural Source)

The makers of Milo's Kitchen™ dog treats do not use any artificial colors; color change in this product is

Comments or Questions?

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

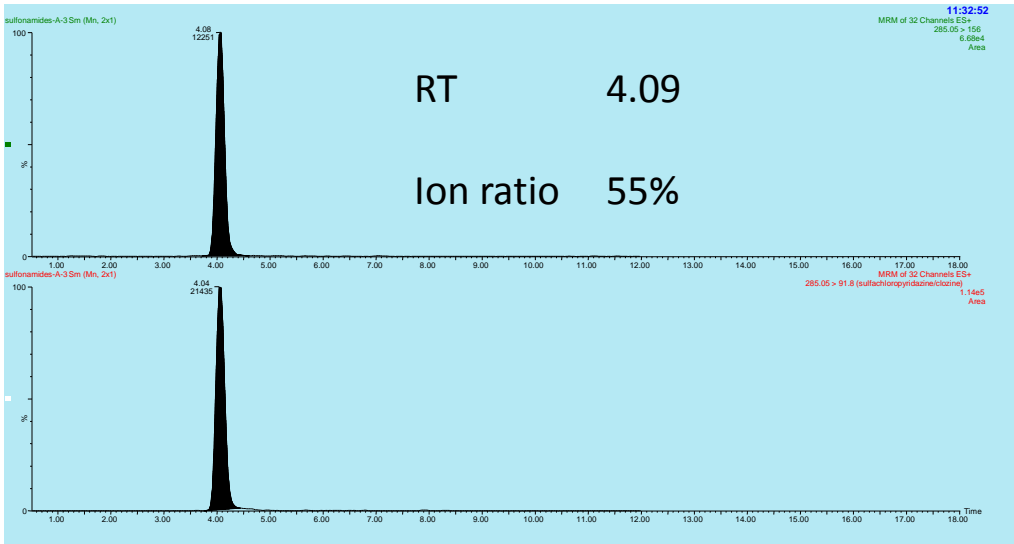
Targeted screens

▶ Results

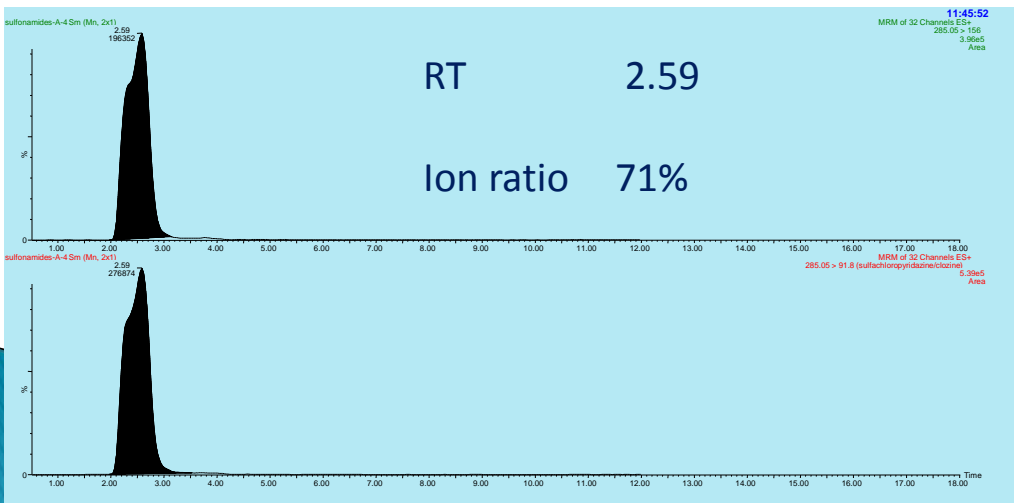
FDA 21CFR

- | | |
|--------------------|--------------|
| ◦ Sulfaquinoxaline | 100 ppb |
| ◦ Sulfamethoxazole | no tolerance |
| ◦ Enrofloxacin | no tolerance |
| ◦ Tilimicosin | 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)

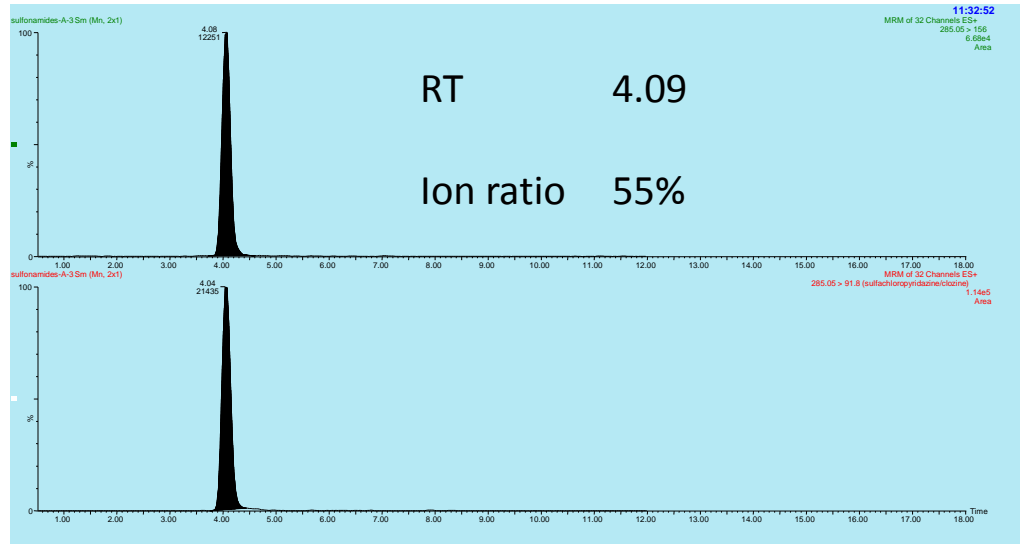
3-amino-N-(5-chloro-2-pyrimidinyl) benzenesulfonamide

4-amino-N-(6-chloro-3-pyrimidinyl) benzenesulfonamide

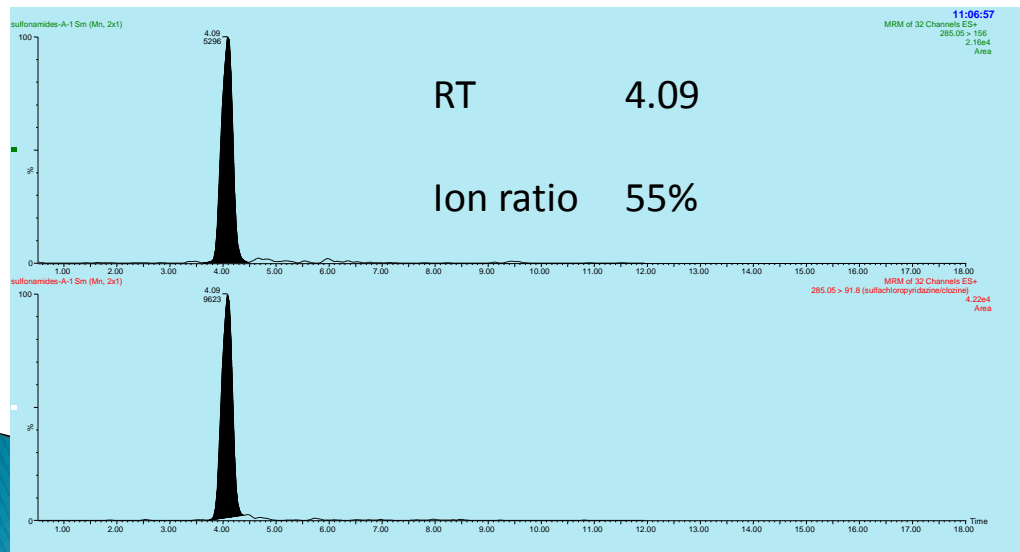
Sulfaclozine

antibiotic used in poultry production (not allowed in US)

Unknown peak found in chicken jerky same transitions as Sulfachloropyridazine



Unknown



Sulfachlorazine

Alibaba.com = Amazon.com for industrial chemicals

The screenshot shows the Alibaba.com website in a Mozilla Firefox browser. The browser's address bar displays the URL: `www.alibaba.com/?src=Google&albc=Google&albc=Search_Search-Better&albk=alibaba_USUKAU-Search-Trademark-P4P_none`. The page header includes navigation links: "Welcome Robert, Sign In (Not you?)", "Buy", "Sell", "Community", "My Alibaba", "Messages", "My Favorites", and "Help".

The main content area features the Alibaba.com logo with the tagline "Global trade starts here.™". Below the logo is a search bar with the placeholder text "Please input a keyword" and a "Search" button. To the right of the search bar is a "Post Buying Requests" button. Below the search bar is a "Supplier Assessment" section with the heading "Supplier Assessment" and a sub-heading "A powerful suite of tools for finding reliable and capable suppliers". The section includes three checked items: "Assessment Reports plus Verified Videos", "Details of supplier's production and trade capabilities", and "Verified onsite by leading verification companies". A "Find assessed suppliers now" link is provided below these items. To the right of the "Supplier Assessment" section is a sidebar titled "Our Sourcing Services" with links for "Customized Sourcing", "Supplier Assessment", "Escrow Service", "Inspection Service", and "Sourcing Hub".

At the bottom of the page is a "CYBER MONDAY" promotional banner with the text "AMAZING DEALS on Holiday Gifts! ALL 50% OFF". The banner includes a "Shop now" link and images of a jacket, a smartphone, and a tablet. The browser's taskbar at the bottom shows several open applications: "Start", "Inbox - Microsoft Outlook", "Microsoft PowerPoint - [...]", and "Manufacturers, Suppli...". The system clock in the bottom right corner shows "1:22 PM".

Products | tilmicosin poultry | Search | or | Post Buying Requests

Advanced Search

Related Searches: poultry equipment, Select 'Suppliers' to search by suppliers, fresh poultry More

Products > "tilmicosin poultry": 33 Product(s) from 9 Supplier(s)

Category

- Veterinary Medicine (31)
- Other Animal Drugs (13)
- Animal Pharmaceuticals (1)
- Antineoplastic Agents (1)
- Antibiotic and Antimicrobial Agents (1)

Need Help? Contact Us

Search Feedback
Did you find what you were looking for?
Yes | NO


Select Country/Region

China (Mainland) (33)

East Asia (33)

Gold Supplier Onsite Checked Assessed Supplier Online Safe Transactions

View: Group Products by Supplier Order Quantity 1 of 1 : Page




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.
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.
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.
China (Mainland)
No of Employees: 11 - 50 People

Contact Details

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

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

Contact Details

Contact Supplier Offline

Compare

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

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

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

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

Contact Details

Contact Supplier Offline

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

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

Contact Supplier Offline

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

Shenyang Tianpeng Animal
Feed Process Factory
China (Mainland)
No of Employees: 101 - 200 People
Management Certification: GMP certificate

Contact Details

Contact Supplier I'm Away

Compare

enrofloxacin 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

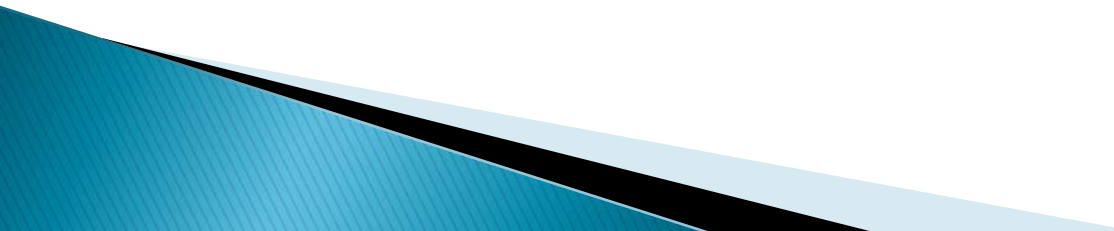
▶ 4

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
 - ▶ Consistently 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 Tom M. Chiller,¹ John H. Powers,¹ and Frederick J. Angulo

¹Enteric 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 Atlanta Research and Education Foundation, Atlanta, Georgia; and 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 powered by 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.

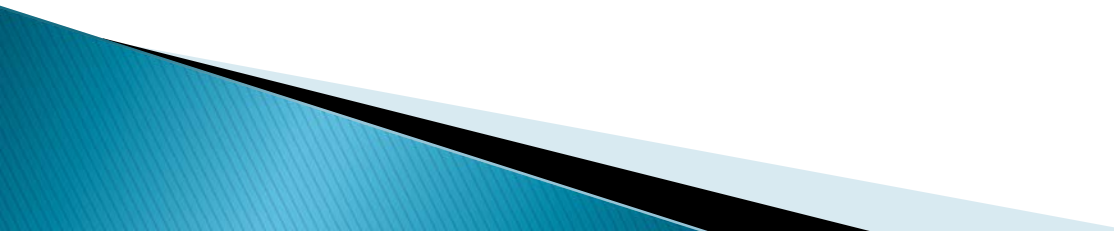
The main vehicle of transmission for antibiotic-resistant Salmonella Kentucky from African and Middle Eastern countries appears to be chickens and turkeys. The authors said the resistance is believed to be caused by “the massive overuse” of antibiotics in African poultry farming.

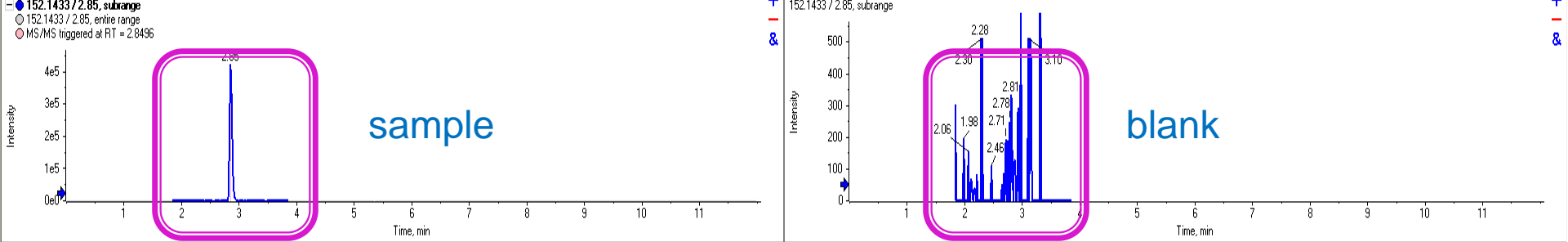
According to [a May 2013 report](#) by the Center for Science in the Public Interest, the U.S. saw [55 outbreaks of antibiotic-resistant pathogens](#) between 1973 and 2011. Contaminated [dairy products](#) and ground beef accounted for the majority of those outbreaks. Antibiotic-resistant Salmonella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

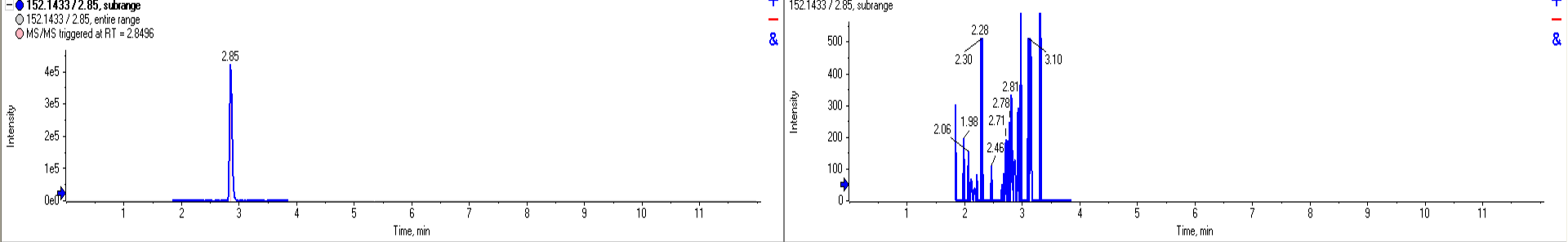
David Willetts, Britain’s science minister, is expected to use his platform at the G-8 meeting to propose new measures to curb the overuse of antibiotics by both healthcare professionals and farmers alike.

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
- 



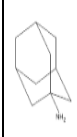
CTR	Wiff file Name	Sample Name	Number of positive results	#	Mass RT	Isomer	Library	Formula	Name	Extraction Mass (Da)	Width (Da)	RT Width (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak	Mol
	001 blank	Sample 1	0	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	✓	
	004 2069	Sample 1	69	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	C3H5N4OCI	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	C2N3OSCI	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	49.1	C9H14NS	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 / 2.70	153.10182	0.01	2	153.10155	2.73	224742	10	1000	44363	6-Mercaptourine	18.2	42.3	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	CH2N3P2CI	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	CH7N4O3CI	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	C8H12N2P	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	C10H13CI	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	C3H11N4O2CI	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	Metamfetramone	12.1	32.7	C9H9N7OF	74.7	✓	



MasterView navigation icons and search bar.

CTRL	Wiff file Name	Sample Name	Number of positive results	#	Match	RT	Income	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
	001 blank	Sample 1	0	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	✓
	004 2069	Sample 1	69	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	C3H5N4OCI	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	C2N3O5CI	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	✓

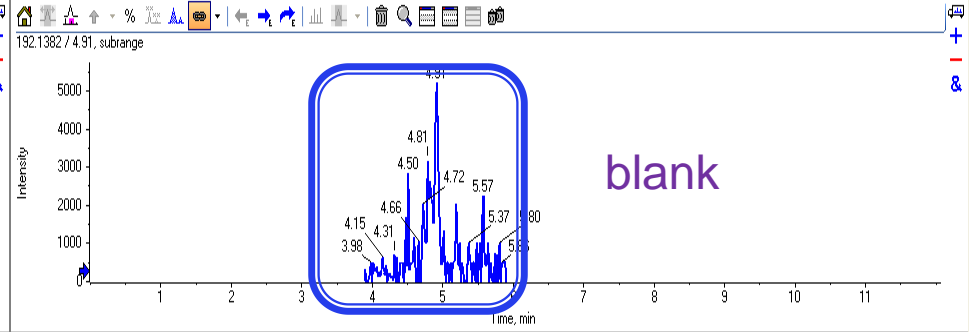
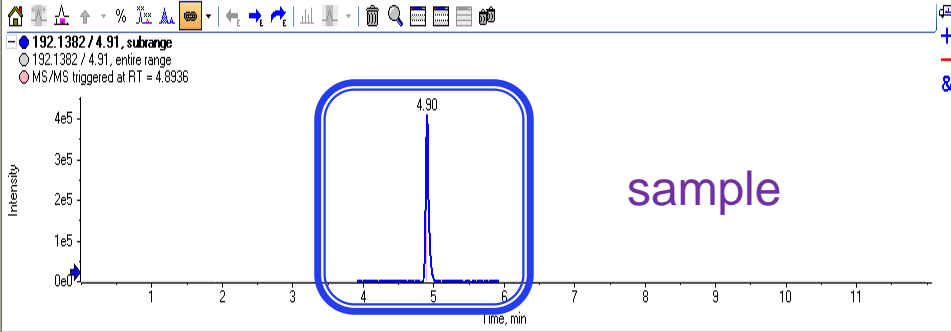
Library Search Results

Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	Cf
 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	22859	No Acquired MSMS	0	94.6	CH2N3P2CI	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	CH7N4O3CI	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	✓



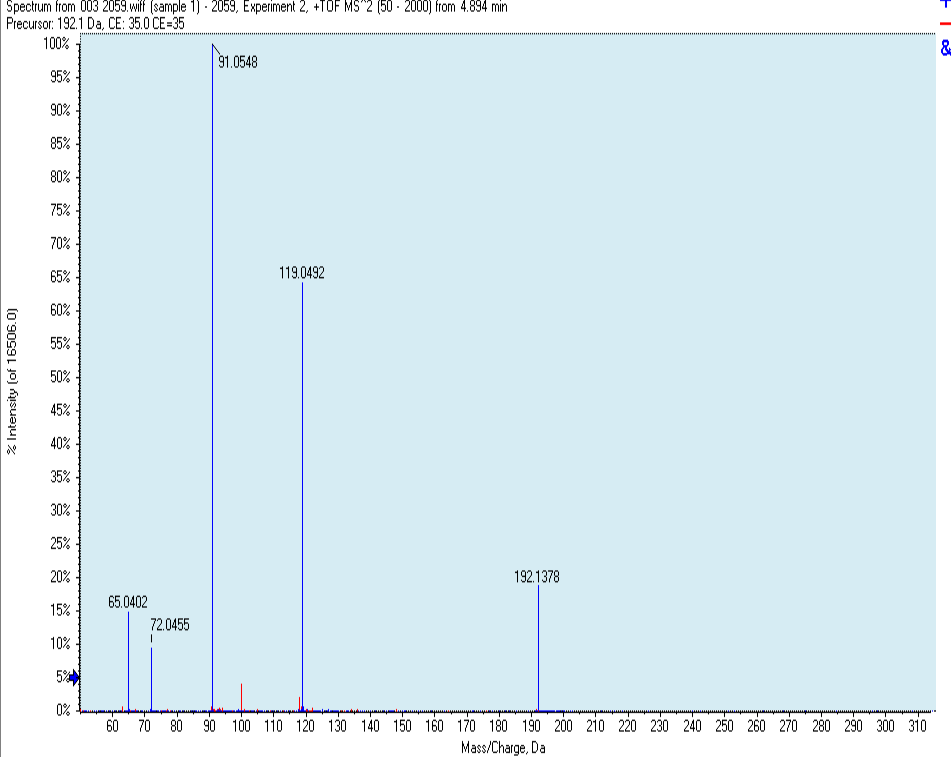
CTR	Wiff file Name	Sample Name	Number of positive results	#	Mass RT	Library Formula	Name	Extraction Mass (Da)	Width (Da)	RT (min)	Found At Mass (Da)	Found At RT (min)	Intensity	Threshold (ratio of control)	Threshold (cps)	Control Intensity	Library Hit	Library Score	Combined Score	Formula Finder Result	Formula Finder Score	Non-Targeted Peak	Mol
	001 blank	Sample 1	0	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	✓	
	003 2059	Sample 1	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	C2N3OSCI	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	✓	✓	188.0787 / 1.39	188.07872	0.01	2	188.07881	1.39	971224	10	1000	568319	No Match	0	31.3	C11H9NO2	94.1	✓	
				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								Formula Finder Results					
Compound Name	CAS #	Formula	MW (Da)	Fit	Rev. F	Purity	CI	Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS/MS (ppm)
DEET	134-62-3	C12H17NO	191.27327	100	99.7	99.7	35	C12H17NO	89.9	192.13829	0	6	
DEET	134-62-3	C12H17NO	191.27327	100	100	100	35	C6H18NSP	15.6	192.13726	5.3	7.9	
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						

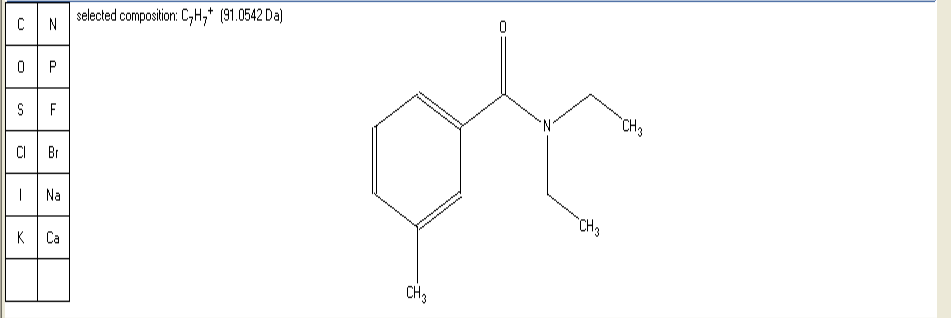
ChemSpider results for: C12H17NO 1-40 of 1440

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-yl)methylphenol	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 MS² (50 - 2000) from 4.894 min
Precursor: 192.1 Da, CE: 35.0 CE=35



selected composition: C₇H₇⁺ (91.0542 Da)



Fragments Peaks

Mass/Charge	Intensity (%)	Assigned	Error (Da)
51.0250	0.16	<input type="checkbox"/>	
67.0528	0.16	<input type="checkbox"/>	
91.2233	0.16	<input type="checkbox"/>	
91.4574	0.16	<input type="checkbox"/>	
91.5464	0.16	<input type="checkbox"/>	
91.6277	0.16	<input type="checkbox"/>	
91.9701	0.16	<input type="checkbox"/>	
92.0242	0.16	<input type="checkbox"/>	

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

Select Cancel

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

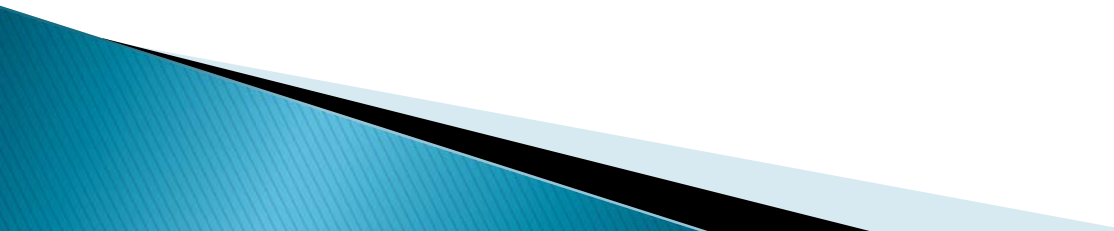
	<u>Amantadine</u>	<u>DEET</u>
Detection frequency	36%	38%
Highest concentration	882 ng/g	572 ng/g

▶ 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 repellent/ 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 ?