Development of robust High Resolution Mass Spectrometric (HRMS) method for detection of known mixed-contaminants (veterinary drugs, mycotoxins, and other residues) in raw milk

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Abstract

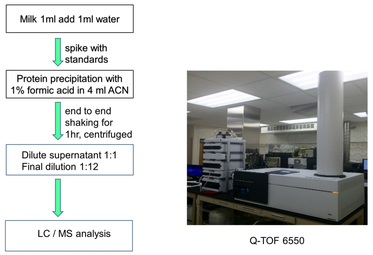
Milk and its byproducts constitute a major portion of the human diet. However, the misuse of veterinary drugs and pesticides during dairy farming along with the possibility of unidentified contaminants in feed can lead to chemical residue contamination of milk which can negatively impact human health. US FDA and other agencies worldwide have set tolerances or maximum residue limits (MRLs) and developed methods used to determine and quantitate residues in milk. There is an important unmet need for a single highly sensitive, robust, and fast analytical method to detect a wide range of mixed contaminants such as veterinary drugs, mycotoxins and pesticides in milk.
We have previously developed a method that detects 30 veterinary drugs in milk. We are extending the scope of this method by adding 46 additional compounds including mycotoxins, pesticides and other veterinary drugs. including aminoglycosides.

Introduction

- Veterinary drugs are used on dairy farms. Residue concentrations in raw milk must be determined to see whether their concentration exceeds the established tolerances.
- Some of the contaminants found in milk are chlorinated pesticides, organophosphates, herbicides, fungicides, anti-helminthic drugs, antibiotics, sulfonamides, mycotoxins, heavy metals, detergents, disinfectants, and persistent environmental pollutants such as poly-chlorinated biphenyls (PCBs), and dioxins. These enter the milk through both direct and indirect routes. Indirect contamination can result from agricultural and veterinary applications and from the feed stuff. Contaminants may also enter milk from equipment after milking. Many of these compounds are fat-soluble and are stored in adipose tissue or secreted in milk fat.
- In the 1980s, FDA undertook a major effort to develop methods for detecting drug residues in milk. Most of these methods are now functionally obsolete.
- Therefore, we are interested in the development of multi-residue HRMS method for this purpose. Our method includes 76 compounds. These are veterinary drugs of many classes, mycotoxins and pesticides (Table 1).

Materials and Methods

Milk samples were collected from the FDA Office of Research Animal Research Facilities. A generic extraction procedure of protein precipitation followed by dilution was carried out to minimize the loss of compounds during extraction. This method is a modification of an extraction procedure reported by Mol et al (2008)[1].



Q-TOF 6550

Figure 1. Sample preparation schematic and photograph of the LCMS apparatus used.

Data acquisition: The mass spectrometer was run in AIF (All ion fragmentation) (MS1) mode and scanned in the *m/z* range of 50—1500 at 3 spectra/second using three scanning segments.
The first segment was a full scan without inducing collision of ions in the quadrupole, while the second and third segment involved fragmentation of ions with collision energy values set at 20 and 40.

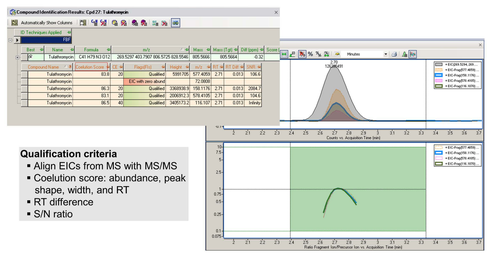
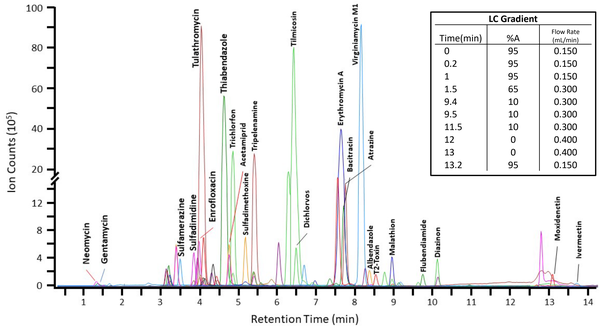


Figure 2. All-ions fragmentation target screening qualification process.

UPLC method: Chromatographic separation was performed on a 100 mm × 2.1 mm i.d., 1.8 µm ACQUITY UPLC HSS T3 Column (Waters) maintained at 40 °C. Mobile phase A was 100% water containing 5 mM ammonium formate and 0.1% formic acid and mobile phase B was 100% methanol containing 5 mM ammonium formate and 0.1% formic acid.

Results and Discussion

The major challenge was to develop a LC method that elutes all 76 compounds (Table 1) with different polarities within the short analysis time of 15 min. We were able to accomplish it by optimizing the step gradient. The extracted ion chromatograms are depicted in Fig 3.



LC Gradient

Time(min)	%A	Flow Rate (mL/min)
0	95	0.150
0.2	95	0.150
1	95	0.150
1.5	65	0.300
9.4	10	0.300
9.5	10	0.300
11.5	10	0.300
12	0	0.400
13	0	0.400
13.2	95	0.150

Figure 3. Extracted ion chromatogram for the 76-compound mixture spiked into 33% Acetonitrile/H_2O solvent, without 12x dilution.

Data analysis: The AIF data was analyzed by using Find by Formula (FBF) algorithm in Mass Hunter qualitative analysis software. The in-house generated compound library consisting of exact masses of 76 compounds and their retention times was used as a formula source.

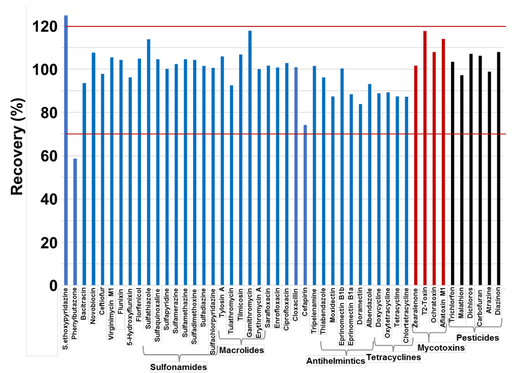


Figure 4. Recoveries of compounds after spiking the milk before extraction (prefortification) at twice the tolerance level

Disclaimer

The views expressed in this poster are those of the authors and may not reflect the official policy of the Department of Health and Human Services.

Table 1. Mixed contaminants included in the method.

	Compound	Class	Tolerance (ppb)		Compound	Class	Tolerance (ppb)
1	Albendazole	Antihelmintic	5	39	Chlorantraniliprole	Insecticide	5
2	Doramectin	Antihelmintic	5	40	Clothianidin	Insecticide	5
3	Eprinomectin	Antihelmintic	12	41	Deltamethrin	Insecticide	5
4	Ivermectin B1a	Antihelmintic	5	42	Diazinon	Insecticide	5
5	Moxidectin	Antihelmintic	40	43	Flubendiamide	Pesticide	5
6	Thiabendazole	Antihelmintic	50	44	Imidacloprid	Insecticide	5
7	Tripelenamine	Antiihstamine	20	45	Acetamidine	Metabolite	5
8	5-hydroxyflunixin	Anti-inflammatiry	2	46	Permethrin	Insecticide	5
9	Flunixin	Anti-inflammatory	2	47	Carbaryl	Pesticide	5
10	Phenylbutazone	Anti-inflammatory	5	48	Dichlorvos	Insecticide	5
11	Ceftiofur	Cephalosporin	100	49	Malathion	Pesticide	5
12	Erythromycin	Macrolide/Lincosamide	50	50	Phoxim	Pesticide	5
13	Gamithromycin	Macrolide/Lincosamide	5	51	Atrazine	Herbacide	5
14	Tilmicosin	Macrolide/Lincosamide	100	52	Chloramphenicol	Phenicol	0.3
15	Tulathromycin A	Macrolide/Lincosamide	100	53	Florfenicol	Phenicol	1
16	Tylosin	Macrolide/Lincosamide	50	54	Florfenicol amine	Metabolite	5
17	Bacitracin	Miscellaneous	500	55	Sulfachloropyridazine	Sulfonamide	10
18	Flunixin Impurity B	Miscellaneous	2	56	Sulfadiazine	Sulfonamide	10
19	Novobiocin	Miscellaneous	100	57	Sulfadimethoxine	Sulfonamide	10
20	Virginiamycin M1	Miscellaneous	100	58	Sulfaethoxypyridazine	Sulfonamide	0.3
21	Aflatoxin M1	Mycotoxin	0.5	59	Sulfamerazine	Sulfonamide	10
22	Deoxynivalenol	Mycotoxin	5	60	Sulfamethazine	Sulfonamide	10
23	Fumonisin B1	Mycotoxin	5	61	Sulfapyridine	Sulfonamide	10
24	Ochratoxin A	Mycotoxin	5	62	Sulfaquinoxaline	Sulfonamide	**10**
25	T2-Toxin	Mycotoxin	5	63	Sulfathiazole	Sulfonamide	10
26	Zearalenone	Mycotoxin	5	64	Chlortetracycline	Tetracycline	100
27	Amitraz	Insecticide	5	65	Doxycycline	Tetracycline	100
28	Carbofuran	Pesticide	5	66	Oxytetracycline	Tetracycline	100
29	Cypermethrin	Insecticide	5	67	Tetracycline	Tetracycline	100
30	Fipronil	Insecticide	5	68	Ampicillin	β-Lactam	10
31	Fipronil sulfone	Metabolite	5	69	Cephapirin	β-Lactam	20
32	Aldicarb	Insecticide	5	70	Cloxacillin	β-Lactam	10
33	Aldicarb sulfoxide	Metabolite	5	71	Penicillin G	β-Lactam	5
34	Aldicarb sulfone	Metabolite	5	72	Ciprofloxacin	Fluoroquinolone	5
35	Trichlorfon	Insecticide	5	73	Enrofloxacin	Fluoroquinolone	5
36	Bifenthrin	Insecticide	5	74	Sarafloxacin	Fluoroquinolone	5
37	Boscalid	Fungicide	5	75	Gentamicin	Aminoglycoside	30
38	Carbendazim	Fungicide	5	76	Neomycin	Aminoglycoside	150

Conclusions

- We are in the final stages of LC/MS method development for screening milk for the 76 mixed contaminants. Once finalized and validated, this method will provide a rugged sensitive method that has a potential to be used for future milk sampling surveys.
- Aminoglycosides are very polar compounds that are not retained in conventional LC columns. They are usually analyzed by HILIC chromatography or using ion pair reagents. However, we were able to retain these compounds on the HSS T3 column with our gradient.
- Tetracyclines have poor recoveries in analytical methods and need chelating agents to improve recoveries. But with our generic extraction procedure we were able to get excellent recoveries (>85%) for all tetracyclines.

How this work support FDA's mission

The method developed provides an efficient screening procedure using UPLC-HRMS instrumentation, providing support for FDA surveillance of mixed contaminants in raw milk.

REFERENCES:
1. Mol, H. G. J.; Plaza-Bolaños, P.; Zomer, P.; de Rijk, T. C.; Stolker, A. A. M.; Mulder, P. P. J., *Analytical Chemistry* **2008,** *80* (24), 9450-9459.