Application Note

Food Testing

Multiclass residue screening of 217 veterinary drugs in milk and milk powder

Using liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS)

Authors

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Abstract

This Application Note describes a workflow solution that was developed for the screening of 217 veterinary drugs in milk and milk powder. These veterinary drugs, belonging to 31 different chemical classes, included macrolides, sulfonamides, tetracyclines, β-lactams, β-agonists, chloramphenicols, nitroimidazoles, cephems, avermectins, benzimidazoles, nonsteroid anti-inflammatory drugs (NSAIDs), hormones, aflatoxins, tranquilizers, and so forth.

Sample preparation involved a rapid and efficient protein precipitation extraction with Na_2EDTA-McIlvaine buffer solution and acetonitrile (ACN), followed by EMR—Lipid dSPE (p/n 5982-1010) and a polish kit (p/n 5982-0102) for further cleanup.

Introduction

Milk is a great natural food for consumers of all age groups due to its high nutritional value. It is high in protein, and is a valuable source of calcium, vitamins, and antioxidants[1]. Milk is also used as raw material for many other processed products, making milk one of the most consumed farm commodities around the world. Considering these facts, quality control of milk is an issue of concern for authorities.

Veterinary antibiotics are widely used to treat dairy cattle diseases such as mastitis, diarrhea, and pulmonary diseases, or to increase milk yield. However, the overuse or abuse of veterinary drugs results in drug residue problems in milk and milk products. These residues can cause adverse human health effects, such as allergic reactions, changes in the delicate balance of intestinal flora, and antibiotic resistance[2].

This Application Note describes a workflow solution that could simultaneously analyze 217 commonly monitored veterinary drugs (VDs) in milk and milk powder across multiple chemical classes. The list of VDs in this solution was based on general notice No. 235 (MOA, China)[3].

Experimental

Standards and reagents

Veterinary drug standards were purchased from Dr. Ehrenstorfer GmbH, WITEGA laboratorien Berlin-Adlershof GmbH, Toronto Research Chemicals (TRC), or AccuStandard, Inc. Ultrapure water (>18.2 MΩ, ELGA VEOLIA PureLab Chrous system), ammonium fluoride (eluent additive for LC/MS, ≥98.0 %, Fluka), acetonitrile (LC/MS grade, Fluka), and formic acid (~98 %, for mass spectrometry, Fluka) were used for mobile phase preparation.

Acetonitrile (ACN, HPLC grade, Sigma-Aldrich), citric acid (reagent grade, ~99 %, Vetec), phosphoric acid (85 %, Ashland), sodium hydroxide (>96.0 %, Sigma-Aldrich), disodium hydrogen phosphate (≥99.5 %, Merck), dimethyl sulfoxide (DMSO, >99.9 %, Sigma-Aldrich), ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2-EDTA, ACS reagent, 99.0–101.0 %, Sigma-Aldrich), ammonium acetate (≥98 %, Sigma-Aldrich), and formic acid (FA, ~98 %, HPLC grade, Fluka) were used during sample preparation.

Sample preparation

Milk powder was first reconstituted with warm water. A liquid milk sample was extracted with Na_2EDTA-McIlvaine buffer solution and acetonitrile, which served as a protein precipitation step. The extract was cleaned using EMR—Lipid dSPE (p/n 5982-1010), which could selectively remove lipid without trapping contaminants of interest. After cleaning, the extract was acidified with formic acid, and went through the final polishing step for further cleanup using a polish kit (p/n 5982-0102).

Instrumentation

Analysis was performed on an Agilent 1290 Infinity II LC consisting of:

- Agilent 1290 Infinity II binary pump with a 35 µL Jet Weaver (G7120A)
- Agilent 1290 multisampler with a 20 µL loop (G7167B)
- Agilent 1290 Infinity II MCT (G7116B)

The mass spectrometer used was an Agilent 6495 triple quadrupole LC/MS with Agilent iFunnel and Agilent Jet Stream technology (G6495A).

Results and Discussion

Table 1 lists the compounds covered in this solution.

Table 1. Veterinary drugs analyzed.

Classification	Compound	CAS
β-Agonists	Cimaterol	54239-37-1
	Clenbuterol hydrochloride	21898-19-1
	Clorprenaline	3811-25-4
	Penbutolol	38363-40-5
	Propranolol hydrochloride	318-98-9
	Ractopamine	90274-24-1
	Salbutamol	18559-94-9
	Terbutaline hemisulfate salt	23031-32-5
	Tulobuterol hydrochloride	56776-01-3
Triphenylmethanes	Crystal Violet/Basic violet 3	548-62-9
	Leucomalachite green	129-73-7
Growth promoters	Quinoxaline-2-carboxylic acid	879-65-2
	3-Methyl quinoxaline-2-carboxylic acid	74003-63-7
	Olaquindox	23696-28-8
β-lactams	Amoxicillin	26787-78-0
	Ampicillin	69-53-4
	Cloxacillin	7081-44-9
	Dicloxacillin	13412-64-1
	Oxacillin	66-79-5
	Penicillin V	132-98-9
	Piperacillin	61477-96-1
	Sulbactam	68373-14-8
Diterpenes	Valnemulin hydrochloride	133868-46-9
Phenothiazines	Chlorpromazine	50-53-3
	Xylazine	7361-61-7
Furans	Nifurstyrenate	54992-23-3
	Nitrovin	2315-20-0
Quinolones	Ciprofloxacin	85721-33-1
	Danofloxacin	112398-08-0
	Difloxacin	98106-17-3
	Enoxacin	74011-58-8
	Enrofloxacin	93106-60-6
	Fleroxacin	79660-72-3
	Flumequin	42835-25-6
	Lomefloxacin	98079-51-7
	Marbofloxacin	115550-35-1
	Nalidixic acid	389-08-2
	Norfloxacin	70458-96-7
	Ofloxacin	82419-36-1
	Orbifloxacin	113617-63-3
	Oxolinic acid	14698-29-4
	Pefloxacin	70458-92-3
	Sarafloxacin	98105-99-8
	Sparfloxacin	110871-86-8
Tetracyclines	Chlortetracycline	57-62-5
	Doxycycline	564-25-0
	Oxytetracycline	6153-64-6
	Tetracycline	60-54-8
Peptides	Virginiamycin M1	211411-53-0

Classification	Compound	CAS
Macrolides	Erythromycin	59319-72-1
	Kitasamycin/Leucomycin	1392-21-8
	Oleandomycin	7060-74-4
	Pimaricin	7681-93-8
	Spiramycin	8025-81-8
	Tilmicosin	108050-54-0
	Tylosin	74610-55-2
	Acetylisovaleryltylosin/Tylvalosin	63409-12-1
Cephems	Cefaclor	53994-73-3
	Cefamandole	34444-01-4/58648-57-0
	Cefapirin	21593-23-7
	Cefazolin	25953-19-9
	Cefetamet pivoxyl	65243-33-6
	Cefoperazone	62893-19-0 / 62893-20-3
	Cefotaxime	63527-52-6
	Cefquinome	118443-89-3
	Ceftiofur	80370-57-6
	Cephalexin	15686-71-2
	Cephalonium	5575-21-3
	Cephradine	38821-53-3
Trematocides	Nitroxinil	1689-89-0
	Rafoxanide	22662-39-1
Anticoccidiosis	3-Amino-5-nitro-*o*-toluamide (ANOT)	3572-44-9
	Clopidol	2971-90-6
	Decoquinate	18507-89-6
	Diclazuril	101831-37-2
	Ethopabate	59-06-3
	Halofuginone	55837-20-2
	Nequinate	13997-19-8
	Nicarbazin	330-95-0
	Robenidine	25875-50-7
	Toltrazuril	69004-03-1
	Toltrazuril sulfone	69004-04-2
	Toltrazuril sulfoxide	69004-15-5
	Zoalene	148-01-6
Antivirus	Amantadine	768-94-5
Pesticides	Carbofuran	1563-66-2
	Chlordimeform	6164-98-3
	Closantel	57808-65-8
	Coumaphos	56-72-4
	Diazinon	333-41-5
	Dichlorvos	62-73-7
	Fenthion	55-38-9
	Fenthion sulfoxide	3761-41-9
	Fenthion sulfone	3761-42-0
	Phoxim	14816-18-3
	Trichlorfon	52-68-6
Lincomycins	Lincomycin	7179-49-9

Classification	Compound	CAS
Dapsones	Dapsone	80-08-0
	N-Acetyl dapsone	565-20-8
Contaminants	Bisphenol A (BPA)	80-05-7
	Perfluorooctanoic acid	335-67-1
	Heptadecafluorooctanesulfonic acid (PFOS)	1763-23-1
Hormones	Zearalanone	5975-78-0
	Estradiol	50-28-2
	Zearalanol	26538-44-3
	β-Zeranol/Zearalanol	42422-68-4
	Chlormadinone acetate	302-22-7
	Dienestrol	84-17-3
	Estradiol benzoate	50-50-0
	Mengestrol acetate	595-33-5
	Melengestrol acetate	2919-66-6
	Methyltestosterone	58-18-4
	Nandrolone phenylpropionate	62-90-8
	Progesterone	57-83-0
	Testosterone	58-22-0
	Testosterone propionate	57-85-2
	Trenbolone	10161-33-8
Aflatoxins	Aflatoxin B1	1162-65-8
	Aflatoxin B2	7220-81-7
	Aflatoxin G1	1165-39-5
	Aflatoxin G2	7241-98-7
	Aflatoxin M1	6795-23-9
Nitroimidazoles	Dimetridazole	551-92-8
	Dimetridazole-OH (HMMNI)	936-05-0
	Metronidazole-OH	4812-40-2
	Metronidazole	443-48-1
	Ronidazole	7681-76-7
Sulfonamides	Sulfabenzamide	127-71-9
	Sulfacetamide	144-80-9
	Sulfachloropyridazine	80-32-0
	Sulfaclozine	102-65-8
	Sulfadiazine	68-35-9
	Sulfadimethoxine	122-11-2
	Sulfamethazine/Sulfadimidine	57-68-1
	Sulfadoxin	2447-57-6
	Sulfaguanidine	57-67-0
	Sulfamerazine	127-79-7
	Sulfameter/Sulfamethoxydiazine	651-06-9
	Sulfamethizole	144-82-1
	Sulfamethoxazole	723-46-6
	Sulfamethoxypyridazine	80-35-3
	Sulfamonomethoxine	1220-83-3

Classification	Compound	CAS
Sulfonamides (continued)	Sulfamoxole	729-99-7
	Sulfanitran	122-16-7
	Sulfaphenazole	526-08-9
	Sulfapyridine	144-83-2
	Sulfaquinoxaline	59-40-5
	Sulfathiazole	72-14-0
	Sulfisomidine	515-64-0
	Sulfisoxazole/Sulfafurazole	127-69-5
	Trimethoprim	738-70-5
Glucocorticosteroids	Beclomethasone	4419-39-0
	Betamethasone	378-44-9
	Cortisone	53-06-5
	Cortisone acetate	50-04-4
	Dexamethasone	50-02-2
	Fludrocortsones acetate	514-36-3
	Flumethasone	2135-17-3
	Hydrocortisone	50-23-7
	Methylprednisolone	83-43-2
	Prednisolone	50-24-8
	Prednisone	53-03-2
	Triamcinolone	124-94-7
	Triamcinolone acetonide	76-25-5
Polyethers	Lasalocid	25999-20-6
	Maduramicin	61991-54-6
	Monensin	22373-78-0
	Nigericin	28643-80-3
	Salinomycin	55721-31-8
Benzimidazoles	2-Aminoflubendazole	82050-13-3
	5-Hydroxymebendazole	60254-95-7
	Albendazole	54965-21-8
	Albendazole sulfone	75184-71-3
	Albendazole sulfoxide	54029-12-8
	Albendazole-2-aminosulfone	80983-34-2
	Cambendazole	26097-80-3
	Fenbantel	58306-30-2
	Fenbendazole	43210-67-9
	Flubendazole	31430-15-6
	Hydroxy-thiabendazole	948-71-0
	Mebendazole	31431-39-7
	Mebendazole-amine	52329-60-9
	Oxfendazole	53716-50-0
	Oxfendazole sulfone/Fenbendazole sulfone	54029-20-8
	Oxibendazole	20559-55-1
	Thiabendazole	148-79-8
	Triclabendazole	68786-66-3

Classification	Compound	CAS
Chloramphenicols	Chloramphenicol	56-75-7
	Florfenicol	73231-34-2
	Thiamphenicol	15318-45-3
Tranquilizer	Acetopromaizine	3598-37-6
	Azaperol	2804-05-9
	Azaperone	1649-18-9
	Carazolol	57775-29-8
	Haloperidol	52-86-8
	Propionylpromazine	7681-67-6
Avermectins	Avermectin B1a	65195-55-3
	Doramectin	117704-25-3
	Eprinomectin	123997-26-2
	Ivermectin	70288-86-7

Classification	Compound	CAS
NSAID	4-Acetylamino antipyrine	83-15-8
	4-Formylaminoantipyrine	1672-58-8
	Sodium nitrophenolate	63317-67-9
	Carprofen	53716-49-7
	Diclofenac/Diclofenac acid	15307-86-5
	Indomethacin	53-86-1
	Indoprofen	31842-01-0
	Ketoprofen	22071-15-4
	Mefenamic acid	61-68-7
	Meloxicam	71125-38-7
	Piroxicam	36322-90-4
	Sasapyrine	552-94-3
	Sulindac	38194-50-2
	Tenoxicam	59804-37-4
	Tolfenamic acid	13710-19-5
	Tolmetin	26171-23-3
Parasiticide	Levamisole	14769-73-4

A set of nine matrix-spiked calibration standards (0.1 ng/g, 0.2 ng/g, 0.5 ng/g, 1.0 ng/g, 2.0 ng/g, 5.0 ng/g, 10 ng/g, 20 ng/g, and 40 ng/g) were analyzed consecutively, and linear fittings were generated with coefficient of correlation values (R^2). Figures 1 and 2 show representative chromatographs in milk at 5.0 ng/g spiked level. Figures 3 and 4 show calibration curves obtained in milk on a G6495A tandem mass spectrometer for 10 compounds. These compounds belong to different chemical classes: amoxicillin, cephalexin, tetracycline, tilmicosin, chloramphenicol, clenbuterol, betamethasone, enrofloxacin, metronidazole, and sulfadimidine.

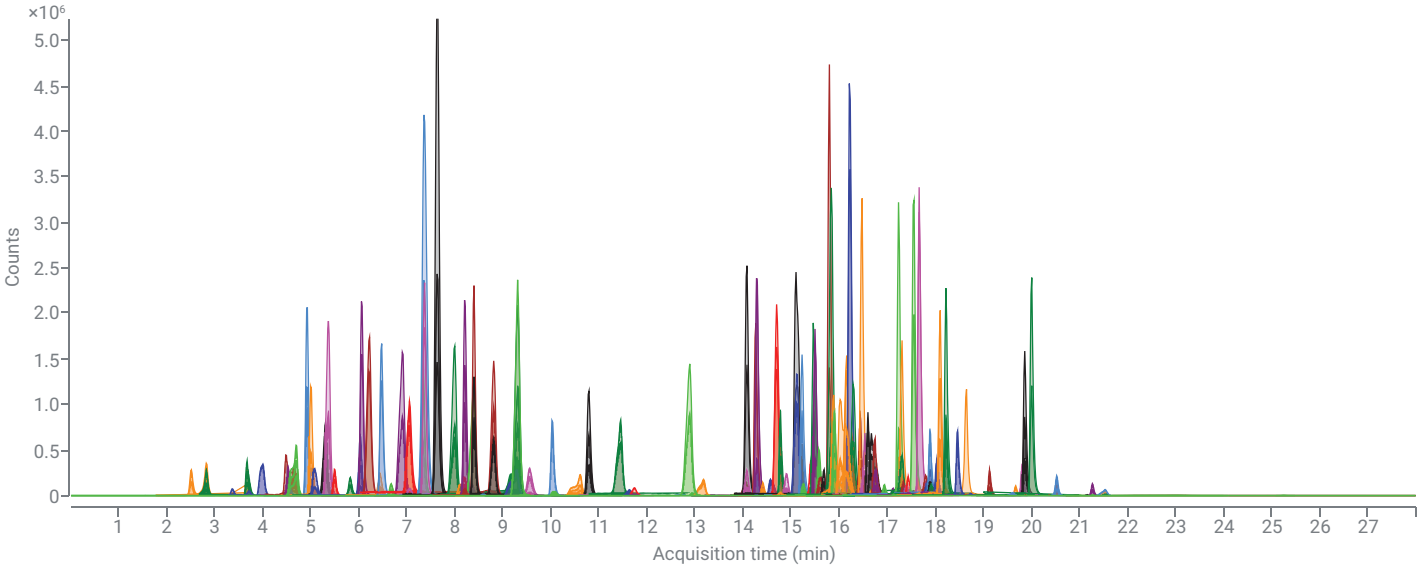

Figure 1. Representative chromatogram of Method 1 in milk at 5 ng/g.

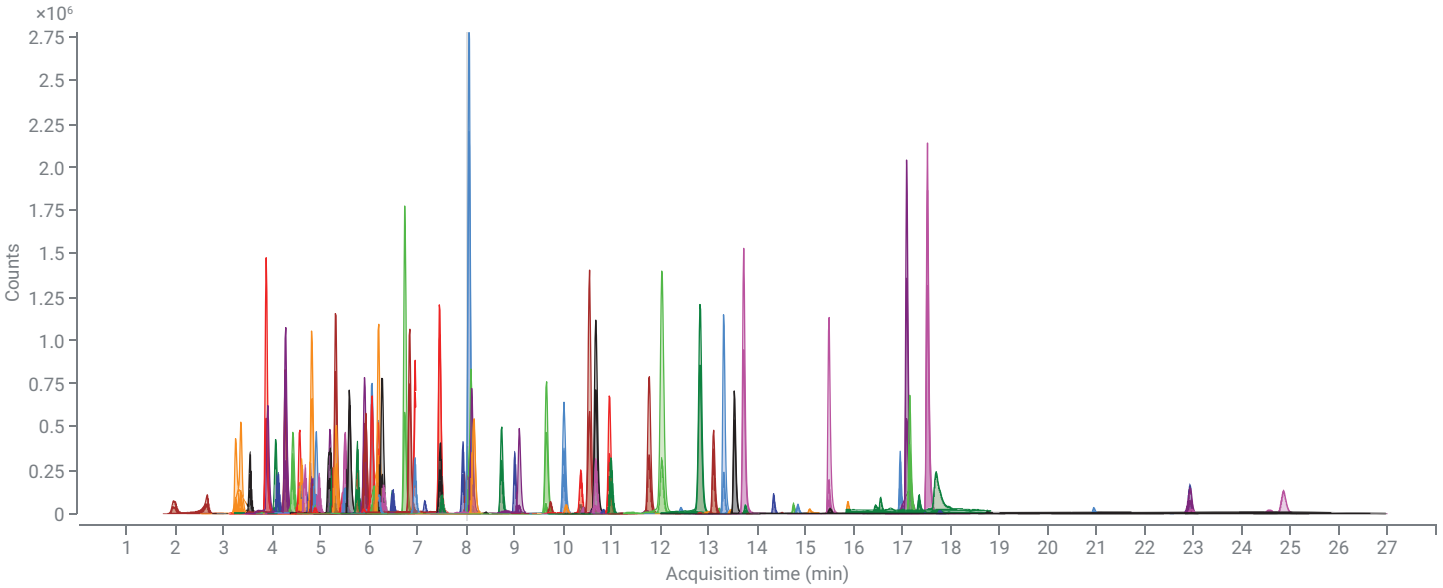

Figure 2. Representative chromatogram of Method 2 in milk at 5 ng/g.

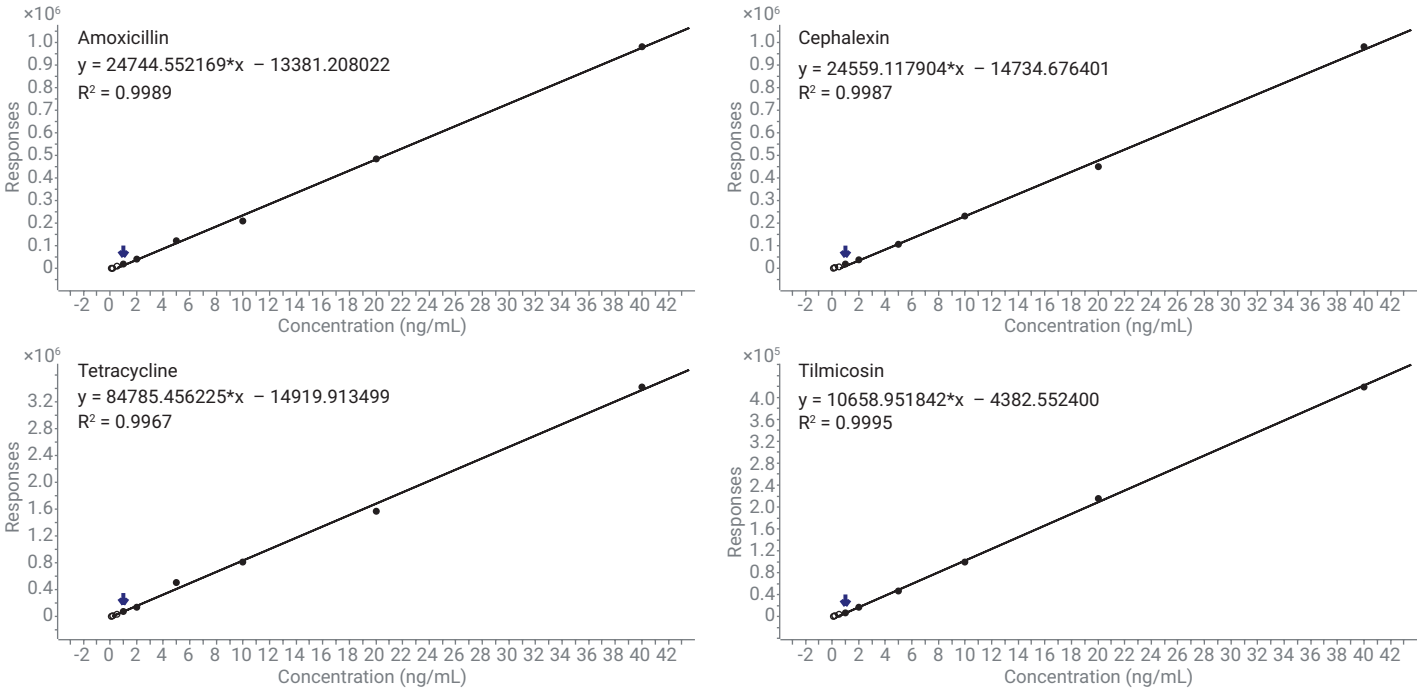

Figure 3. Calibration curves of amoxicillin, cephalexin, tetracycline, and tilmicosin from 1 to 40 ng/g in milk on an Agilent 6495 triple quadrupole LC/MS with the Agilent iFunnel and Agilent Jet Stream technology (G6495A).

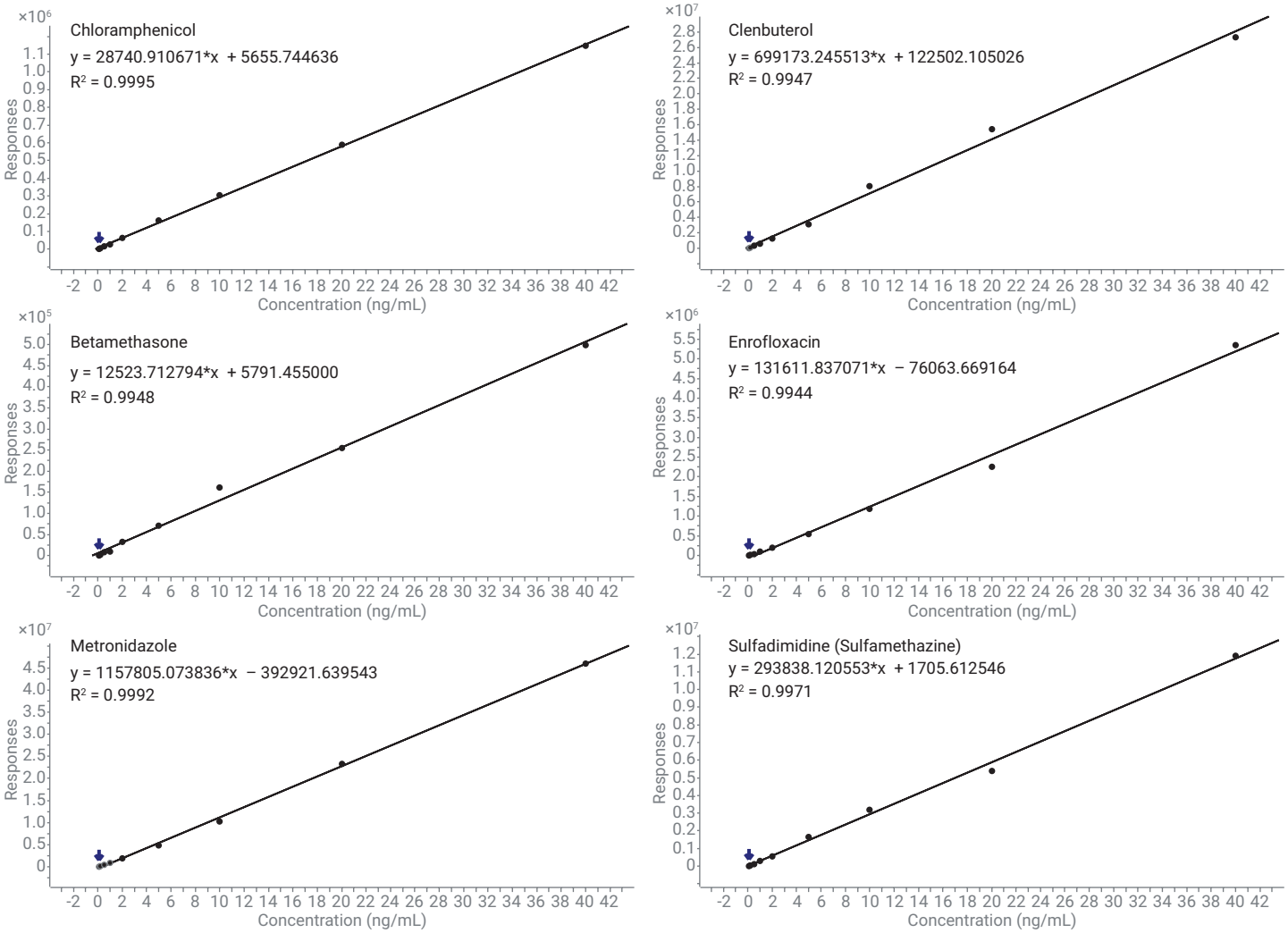

Figure 4. Calibration curves of chloramphenicol, clenbuterol, betamethasone, enrofloxacin, metronidazole, and sulfadimidine from 0.1 to 40 ng/g in milk on an Agilent 6495 triple quadrupole LC/MS with the Agilent iFunnel and Agilent Jet Stream technology (G6495A).

The validation results for milk using a G6495A tandem mass spectrometer were summarized to demonstrate the performance of our workflow solution:

- Good linearity results were achieved with 93 % of compounds having R^2 ≥0.990.
- The recovery data at three different levels were grouped into four categories:
  - % recovery between 50–79
  - % recovery between 80–120
  - % recovery between 121–150
  - % recovery greater than 150
- Figure 5 shows the results, which revealed that good recoveries were achieved through the matrix-spiked calibration method. For example, at the 5 ng/g spiking level, 86 % of the compounds had recoveries between 80–120 %.
- The repeatability at three different levels were also grouped into four categories:
  - %RSD between 0–10
  - %RSD between 11–15
  - %RSD between 16–20
  - %RSD greater than 20
- Figure 6 presents the results for all veterinary drugs tested in this method. Only a few compounds had %RSD greater than 20 %. For example, at the 5 ng/g spiking level, only two compounds (bisphenol A and toltrazuril-sulfoxide) had RSDs greater than 20 %, which could have been caused by weak signal responses.

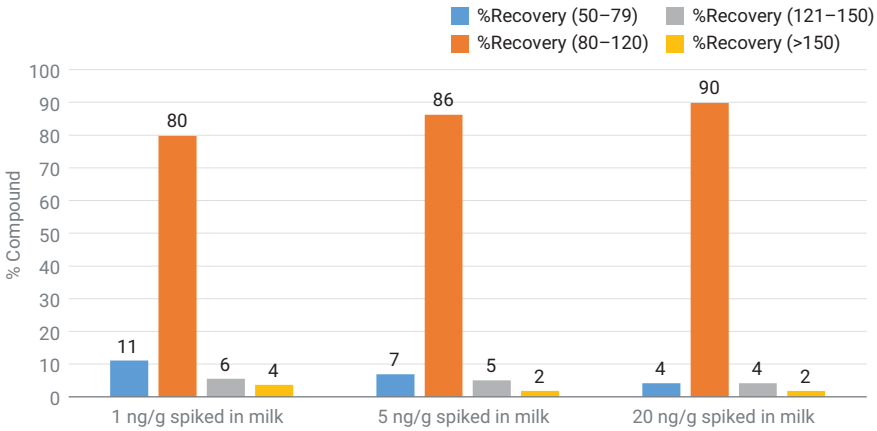

Figure 5. Analyte recoveries at different spiking levels in milk on an Agilent 6495 triple quadrupole LC/MS with the Agilent iFunnel and Agilent Jet Stream technology (G6495A).

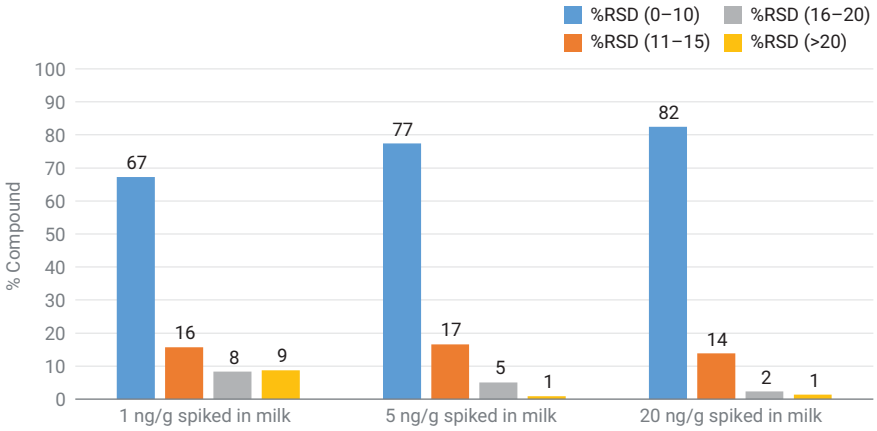

Figure 6. Analysis repeatability at different spiking levels in milk on an Agilent 6495 triple quadrupole LC/MS with the Agilent iFunnel and Agilent Jet Stream technology (G6495A).

Conclusions

This Application Note developed a workflow solution to screen multiclass veterinary drugs in milk and milk powder. It demonstrates that the total workflow solution provided highly sensitive, accurate, and precise results. The method would, therefore, significantly increase sample throughput without sacrificing data quality.

Acknowledgements

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