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Development and Validation of a Multiclass, Multiresidue Method for Veterinary Drug Analysis in Infant Formula and Related Ingredients Using UHPLC-MS/MS

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ABSTRACT: A multiclass, multiresidue method based on ultrahigh-performance liquid chromatography−tandem mass spectrometry (UHPLC-MS/MS) has been developed and validated for the analysis of around 150 veterinary drugs in infant formula and related dairy ingredients. The included analytes belong to the following veterinary drug classes: anthelmintics, antibiotics (aminoglycoside, amphenicols, β-lactams—penicillins and cephalosporins, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, and others), antimicrobial growth promoters, antiprotozoals, β-agonists, coccidiostats, dyes, pesticides, and tranquilizers. The sample preparation procedure involves dispersing the sample in 0.05 M EDTA solution in water, followed by extraction with 0.1% formic acid in acetonitrile, drying down an aliquot of the extract, and reconstituting it in a water−acetonitrile mixture. The analyte detection, identification, and quantitation are performed by UHPLC-MS/MS using positive electrospray ionization mode. The method was validated in infant formula powder, whole milk powder, and whey protein isolate, typically achieving limits of quantitation (meeting acceptable recovery and precision validation criteria) at 1−10 ng/g.

KEYWORDS: *veterinary drugs, antibiotics, multiclass, multiresidue analysis, infant formula, milk powder, whey protein powder, UHPLC-MS/MS analysis, standard addition*

INTRODUCTION

Veterinary drugs are a complex group of different chemical classes and therapeutic agents. They are used within animal husbandry to treat and prevent diseases and ensure animal health and growth. The bulk of antimicrobials are not consumed by humans, but by animals.[1] In the United States, ∼80% of antimicrobial use is for agricultural and nonhuman uses.[1,2] Antimicrobials are often only partially metabolized in food-producing animals and can be excreted as the parent compounds.[2] There are also increasing concerns regarding environmental risk from residues. The main focus is on animal excrement of antimicrobials through manure as soil improver or direct excretion to pasture. Following the use of the drugs, they can enter and move through the environment and have a potential to adversely affect nontarget organisms, groundwater, freshwater, and terrestrial ecosystems.[3−5] Thus, veterinary drug residues can enter the human food supply chain from various sources.

Residues of veterinary drugs or their metabolites in animal edible tissues are undesirable as they pose a potential threat to consumer health if they are present above certain levels. Furthermore, the excessive use of antibiotics promotes antimicrobial-resistant bacteria strains, which are well-known to be a serious threat to public health worldwide.[4,6−11] Another undesirable effect is a potential inhibition of the fermentation-based food processing, which may compromise food quality.[12] Therefore, these substances are strictly regulated and monitored in food products to ensure food safety and prevent unnecessary exposure of consumers to veterinary drugs. The drug residues are monitored according to each government regulation based on maximum residues levels (MRL) or tolerances of animal drugs in food, for example, EU regulation 37/2010,[13] U.S. 21 CFR part 556,[14] or China 2002 235 announcement of the Ministry of Agriculture.[15]

Veterinary drug residues can be determined using traditional methods that include immunoassays,[16,17] microbial inhibition assays (for antibiotics),[18,19] or liquid chromatography.[20−23] These methods often suffer from poor sensitivity and selectivity or involve multiple assays/analytical runs. Multiclass, multiresidue methods based on liquid chromatography−mass spectrometry (LC-MS) are becoming increasingly popular and required in regulatory monitoring programs globally owing to their extended analytical scope and laboratory efficiency; however, robust multiclass methods are still limited.[24] Development of any large multiclass, multiresidue detection method poses significant challenges,[25] including a large number of analytes; coexistence of parent drugs and metabolites; different physical/chemical properties ranging from hydrophilic to hydrophobic and from acidic to neutral to basic; analyte stability and interaction with matrix components; compromise between analytical scope and performance characteristics; matrix effects and potential interference from coextractives.[26−32]

In this study, a modern large-scale method based on ultrahigh-performance liquid chromatography−tandem mass spectrometry (UHPLC-MS/MS) was developed and validated to provide screening, identification, and quantitation of

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Table 1. Analyte-Specific LC-MS/MS Conditions: Precursor to Product Ion Transitions, Collision Energies (CE), Cell Accelerator Voltage (CAV), and Retention Times (RT)

compound	precursor ion	product ion 1	CE (V)	CAV (V)	product ion 2	CE (V)	CAV (V)	product ion 3	CE (V)	CAV (V)	RT (min)	ΔRT (min)
albendazole	266.1	234.0	12	7	159.0	44	4	191.3	36	4	9.33	1.0
albendazole amino	208.1	79.0	52	6	105.1	52	5	110.9	44	5	8.32	1.0
albendazole sulfone	298.1	266.0	20	3	159.0	36	5	77.0	60	6	7.80	1.0
albendazole sulfoxide	282.1	159.1	48	5	240.0	8	3	208.0	24	4	7.30	1.0
albendazole-2-aminosulfone	240.1	198.0	20	4	133.1	28	5	105.0	72	4	4.18	1.5
cambendazole	303.1	190.0	44	5	261.1	20	4	217.0	28	4	8.43	1.0
febantel	447.1	383.0	16	7	415.2	8	6	280.1	36	4	10.70	1.0
fenbendazole	300.1	268.0	20	3	159.0	36	3	104.0	68	4	9.85	1.0
fenbendazole sulfone	332.1	300.1	28	4	159.0	48	5	104.0	72	5	8.74	1.0
fenbendazole sulfoxide	316.1	159.1	36	4	284.1	16	4	77.2	80	3	8.54	1.0
flubendazole	314.1	282.1	20	3	123.0	44	4	94.9	64	6	9.45	1.0
flubendazole-amine	256.1	95.1	40	6	123.0	24	7	75.0	76	4	7.63	1.0
levamisole	205.1	91.0	40	5	178.0	16	6	123.0	32	5	3.70	1.0
mebendazole	296.1	264.1	24	4	76.9	64	5	105.0	40	4	9.30	1.0
mebendazole-5-hydroxy	298.1	266.1	20	6	79.1	44	3	76.9	60	4	7.62	1.0
mebendazole-amine	238.1	105.0	28	4	77.0	52	6	51.1	80	3	7.30	1.0
oxibendazole	250.1	218.1	16	4	176.0	32	5	148.0	44	4	8.29	1.0
thiabendazole	202.1	175.0	24	6	130.9	36	5	65.1	52	4	5.14	1.0
thiabendazole-5-hydroxy	218.0	190.9	24	6	147.2	36	6	81.0	44	4	4.60	2.0
triclabendazole	359.0	274.1	44	4	344.0	28	4	171.1	56	6	11.00	1.0
triclabendazole-sulfone	391.0	242.0	44	3	312.0	32	4	276.9	32	3	10.80	1.0
triclabendazole sulfoxide	375.0	360.0	24	3	242.1	52	3	356.8	20	5	10.86	1.0
cefadroxil	364.1	113.9	24	6	208.1	8	5	86.2	56	4	3.50	1.5
cefazolin	455.0	323.1	8	5	156.1	16	5	112.0	56	6	5.96	1.0
cefoperazone	646.2	530.1	8	3	143.1	20	6	148.0	72	4	6.69	1.0
cefquinome	529.1	134.0	68	6	395.9	8	6	124.7	64	7	4.65	1.0
ceftiofur	524.0	241.1	16	4	125.0	60	4	125.9	40	4	8.33	1.0
desfuroylceftiofur	430.0	126.0	40	4	125.1	60	3	241.1	16	6	6.07	1.0
DCCD	549.0	183.2	36	5	241.1	20	5	181.9	48	5	4.70	2.0
cephacetrile	362.0	258.1	8	3	301.9	8	7	178.1	8	6	4.35	1.0
cephalexin	348.1	158.2	4	7	174.1	16	6	106.2	24	5	5.30	1.0
cephalonium	459.1	337.0	8	3	152.0	20	5	158.1	16	4	4.45	1.0
cephapirin	424.1	292.1	16	3	152.2	20	5	141.1	20	6	4.04	1.0
desacetyl cephapirin	382.1	152.0	24	4	111.9	28	5	291.9	12	3	2.59	1.5
amoxicillin	366.1	349.1	4	5	113.9	24	7	208.1	8	4	3.00	2.0
ampicillin	350.1	106.1	20	6	114.0	40	5	160.0	8	6	5.60	2.0
cloxacillin	436.1	277.0	12	4	160.1	16	4	114.0	48	6	9.62	1.0
dicloxacillin	470.0	311.0	12	3	160.1	12	3	114.0	40	4	9.80	1.0
nafcillin	415.1	198.9	16	5	115.1	76	5	171.1	40	5	9.85	1.0
oxacillin	402.1	243.1	8	6	160.0	8	4	77.2	72	5	9.49	1.0
penicillin G	335.1	176.2	12	4	160.1	8	5	114.1	40	5	9.00	1.0
penicillin V	351.1	159.9	8	6	113.9	40	5	53.2	72	4	9.43	1.0
florfenicol amine	248.1	230.0	8	3	91.0	56	4	130.0	28	6	0.95	2.0
clarithromycin	748.5	158.2	28	6	590.9	12	5	83.0	56	3	9.68	1.0
clindamycin	425.2	126.1	32	6	70.1	64	3	69.1	68	4	8.45	1.0
desmycosin	772.5	174.1	28	3	88.1	60	4	156.0	40	3	9.00	1.0
erythromycin A	734.5	158.2	24	4	576.1	16	4	82.9	60	5	9.28	1.0
josamycin	828.5	174.2	36	3	109.0	52	7	229.1	28	4	9.66	1.0
lincomycin	407.2	126.1	24	3	359.2	16	5	83.1	80	4	4.60	1.5
oleandomycin	688.4	158.1	32	3	544.1	12	3	116.0	56	4	8.86	1.0
roxithromycin	837.5	158.1	36	5	679.1	20	4	116.0	44	7	9.76	1.0
spiramycin I	422.3	174.1	20	5	101.2	16	4	88.0	40	5	7.63	1.0
tilmicosin	869.6	174.0	48	3	696.1	48	3	88.0	76	5	8.48	1.0
tulathromycin A	806.6	88.1	72	4	115.9	44	6	87.1	76	5	8.99	1.0
tylosin A	916.5	174.1	40	3	100.9	48	3	772.1	28	4	9.30	1.0
dapson	249.1	92.1	20	4	156.1	8	6	108.1	16	5	4.83	1.0
nifurstyrenate sodium	260.1	242.2	8	5	115.1	52	5	243.0	12	3	9.68	2.0
novobiocin	613.2	189.2	28	4	133.1	56	6	218.1	8	3	10.90	1.0
ormethoprim	275.2	259.2	28	4	123.1	40	5	81.0	52	3	5.41	1.0

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Table 1. continued

compound	precursor ion	product ion 1	CE (V)	CAV (V)	product ion 2	CE (V)	CAV (V)	product ion 3	CE (V)	CAV (V)	RT (min)	ΔRT (min)
rifaximin	786.4	754.1	20	6	151.1	36	3	95.1	60	6	10.20	1.0
tiamulin	494.3	192.1	20	3	119.0	40	4	73.0	60	5	9.06	1.0
trimethoprim	291.2	230.2	20	6	275.2	28	3	123.0	36	3	4.83	1.0
cinoxacin	263.1	189.0	28	3	217.1	20	6	245.1	8	3	7.51	2.0
ciprofloxacin	332.1	314.2	20	4	230.8	44	3	288.1	16	4	5.66	1.0
danofloxacin	358.2	340.1	24	3	82.1	44	3	255.1	44	3	5.91	1.0
difloxacin	400.2	382.1	24	4	356.2	16	3	299.2	28	5	6.14	1.0
enoxacin	321.1	303.1	20	4	232.0	32	3	204.1	48	4	5.40	1.0
enrofloxacin	360.2	316.2	20	5	342.2	16	4	245.1	24	3	5.86	1.0
flumequine	262.1	244.0	20	4	202.0	32	4	126.0	48	4	8.98	1.0
lomefloxacin	352.2	265.0	24	5	308.1	16	4	334.1	20	5	5.97	1.0
marbofloxacin	363.2	72.1	28	3	320.0	12	3	345.1	20	5	4.93	1.0
nalidixic acid	233.1	215.0	8	4	187.1	28	5	104.1	48	5	8.86	1.0
norfloxacin	320.1	302.1	20	3	231.1	40	4	276.1	16	4	5.47	1.0
ofloxacin/levofloxacin	362.2	318.1	16	4	261.1	28	4	344.2	16	6	5.33	1.0
oxolinic acid	262.1	244.1	16	3	160.0	44	3	216.0	28	3	7.90	1.0
sarafloxacin	386.1	368.0	20	3	299.0	28	5	342.1	20	3	6.30	1.0
sparfloxacin	393.2	229.8	44	4	195.0	60	4	84.1	32	4	7.03	1.0
sulfabenzamide	277.1	156.1	4	5	108.0	24	5	65.0	64	6	6.56	1.0
sulfacetamide	215.1	155.9	12	5	91.9	24	5	108.0	16	4	2.77	2.0
sulfachloropyridazine	285.0	155.9	16	5	108.0	24	6	91.8	28	4	5.62	1.0
sulfaclozine	285.0	156.1	12	3	65.2	60	3	92.0	32	4	7.04	1.0
sulfadiazine	251.1	155.9	12	3	65.0	56	3	108.0	24	3	3.40	2.0
sulfadimethoxine	311.1	156.0	20	3	92.0	28	4	108.0	28	3	7.53	1.0
sulfadoxin	311.1	156.0	16	5	92.1	36	4	65.0	60	4	6.20	1.0
sulfaguanidine	215.1	156.1	12	4	92.1	28	5	108.0	20	6	1.00	1.5
sulfamerazine	265.1	92.2	28	5	107.9	24	3	65.0	52	4	4.20	1.0
sulfameter	281.1	108.1	32	6	156.0	16	3	65.1	56	3	4.90	1.0
sulfamethazine	279.1	186.1	12	3	92.0	28	3	65.0	52	5	5.03	1.0
sulfamethizole	271.0	155.9	12	5	108.1	28	3	91.9	24	8	5.00	1.0
sulfamethoxazole	254.1	92.0	32	5	156.0	12	4	108.0	24	7	5.76	1.0
sulfamethoxypyridazine	281.1	155.9	16	6	108.1	28	6	65.0	52	6	5.36	1.0
sulfamonomethoxine	281.1	155.9	16	3	80.0	56	5	65.0	60	3	5.83	1.5
sulfamoxole	268.1	156.0	16	3	108.0	28	3	65.0	56	5	5.00	1.5
sulfanilamide	173.0	156.1	5	3	108.2	20	6	91.9	32	6	1.29	1.0
sulfaphenazole	315.1	158.1	28	8	160.1	20	5	131.1	56	7	7.10	1.0
sulfapyridine	250.1	156.0	12	5	65.0	56	3	108.0	20	3	4.24	2.0
sulfaquinoxaline	301.1	156.1	12	6	108.1	28	7	91.9	28	7	7.93	1.0
sulfathiazole	256.0	156.2	12	3	65.1	52	4	92.1	24	4	3.68	1.0
sulfatroxazole	268.1	92.1	32	4	108.0	24	3	65.0	60	3	5.90	1.0
sulfisomidine	279.1	123.9	32	8	65.0	52	5	91.9	32	3	3.45	1.0
sulfisoxazole	268.1	92.1	24	5	155.9	8	4	113.0	12	4	6.20	1.0
4-epichlortetracycline	479.1	444.1	24	3	462.0	16	4	97.9	36	4	6.20	2.0
4-epidemeclocycline	465.1	448.1	16	4	430.0	24	4	98.2	40	3	5.30	2.0
4-epioxytetracycline	461.2	426.1	20	3	444.1	12	3	98.0	48	3	5.15	2.0
4-epitetracycline	445.2	410.1	20	3	427.2	8	3	98.0	52	3	4.81	2.0
chlortetracycline	479.1	444.1	20	4	462.1	16	4	154.0	28	4	7.10	2.0
doxycycline	445.2	428.1	16	3	410.1	28	3	267.0	36	3	8.20	2.0
isochlorotetracycline	479.1	462.1	20	3	98.0	56	3	197.0	48	3	6.00	2.0
oxytetracycline	461.2	426.1	16	3	443.1	8	5	98.0	44	3	5.55	2.0
tetracycline	445.2	410.1	20	3	154.1	24	3	427.1	8	4	5.55	2.0
bacitracin	474.9	669.1	12	6	85.9	28	7	199.2	24	5	8.95	1.0
nitrovin	361.1	222.0	16	6	58.0	28	6	154.1	40	8	8.80	1.0
virginiamycin (M1)	526.3	133.1	44	4	105.2	60	3	108.9	48	7	9.77	1.0
isometamidium	460.2	313.1	16	4	298.1	36	4	269.0	60	5	8.03	1.5
cimaterol	220.2	202.1	4	4	143.0	20	4	160.0	12	4	3.00	2.0
clenbuterol	277.1	203.1	16	6	259.0	8	3	132.2	36	6	6.05	1.0
isoxsuprine	302.2	284.1	12	4	77.0	60	3	107.2	36	3	7.04	1.0
mabuterol	311.1	236.9	12	3	293.1	8	3	217.0	24	5	6.92	1.0
ractopamine	302.2	164.1	12	5	77.0	80	3	284.2	8	3	5.64	1.0

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Table 1. continued

compound	precursor ion	product ion 1	CE (V)	CAV (V)	product ion 2	CE (V)	CAV (V)	product ion 3	CE (V)	CAV (V)	RT (min)	ΔRT (min)
salbutamol	240.2	148.1	20	4	222.1	4	5	166.1	8	7	3.27	1.5
terbutaline	226.2	152.0	12	6	106.8	36	3	125.2	28	6	3.00	2.0
zilpaterol	262.2	244.0	8	7	185.1	28	5	202.0	16	7	3.18	2.0
clopidol	192.0	101.0	28	6	51.1	52	4	86.8	32	3	4.65	2.0
decoquinate	418.3	372.2	24	4	204.0	48	4	148.0	72	3	11.38	1.0
diminazine	282.2	119.1	12	4	101.9	40	5	134.9	12	5	3.80	1.0
dimetridazole	142.1	96.2	12	4	95.2	20	3	81.0	28	4	3.20	1.0
dimetridazole-hydroxy (HMMNI)	158.1	140.1	8	5	54.9	20	4	94.0	24	4	2.65	1.5
ipronidazole	170.1	124.1	16	4	109.1	24	4	122.9	32	4	6.60	1.0
ipronidazole-hydroxy	186.1	168.1	8	6	121.2	32	3	122.1	16	3	5.66	1.0
metronidazole	172.1	128.0	12	4	81.8	24	4	111.0	20	4	2.95	2.0
metronidazole-hydroxy	188.1	126.0	16	6	123.0	8	5	144.1	8	3	2.25	2.0
ronidazole	201.1	140.0	4	5	55.0	20	3	53.1	44	4	3.20	1.5
Brilliant green	385.3	341.1	44	4	297.2	60	4	241.2	80	3	9.98	1.0
Crystal violet	372.3	356.2	44	4	340.1	64	5	235.1	72	5	9.81	1.0
Leuco Crystal violet	374.3	238.0	28	4	358.2	28	7	239.1	32	3	8.67	2.0
Malachite green	329.2	313.2	36	6	208.2	40	5	165.1	80	4	9.38	1.0
Leuco Malachite green	331.2	223.0	56	4	313.2	36	6	208.2	40	5	10.28	2.0
colchicine	400.2	358.1	20	4	326.2	24	4	282.2	28	4	8.78	1.0
strychnine	335.2	184.0	48	3	156.1	52	4	129.0	72	5	5.08	1.0
chlorpromazine	319.1	85.9	16	6	58.1	48	4	246.1	24	3	9.54	1.0
diazepam	285.1	193.1	32	7	154.1	32	4	91.0	56	3	9.99	1.0
methaqualone	251.1	132.1	28	6	91.1	40	4	64.9	60	6	9.40	1.0
procaine	237.2	100.2	12	4	120.2	20	4	65.1	60	5	3.50	1.5
xylazine	221.1	89.9	20	6	77.1	60	4	164.1	24	5	5.91	1.0
streptomycin	582.3	263.1	36	3	246.2	44	3	220.9	40	3	0.50	1.0
chloramphenicol succinate	423.0	305.1	8	3	275.0	20	3	135.8	20	5	8.32	1.0
colistin A	585.4	101.1	40	7	86.0	44	5	240.8	20	4	6.86	1.0
colistin B	578.4	100.8	36	6	227.1	20	4	528.9	16	4	6.21	1.0
thiabendazole-d_4	206.1	179.1	28	5	135.2	40	5	–[a]	–	–	5.14	1.0
penicillin G-d_7	342.2	160.1	12	4	182.9	16	6	–	–	–	9.00	1.0
Leuco Malachite green-d_5	336.3	239.2	28	4	321.1	20	3	–	–	–	10.28	2.0
erythromycin-$^{13}C,d_3$	738.5	162.2	24	5	83.1	48	3	–	–	–	9.28	1.0
ronidazole-d_3	204.1	143.0	8	4	58.1	24	5	–	–	–	3.20	1.5
trimethoprim-d_9	300.2	234.1	28	3	264.1	28	3	–	–	–	4.83	1.0
sarafloxacin-d_8	394.2	350.2	20	3	376.2	28	3	–	–	–	6.30	1.0
sulfadoxin-d_3	314.1	156.1	16	3	92.0	40	3	–	–	–	6.20	1.0
demeclocycline (IS)	465.1	448.1	16	4	289.2	32	3	–	–	–	6.15	2.0
chlorpromazine-^{13}C, d_3	323.1	90.1	20	4	62.1	24	4	–	–	–	9.54	1.0

[a] –, only two product ions were selected for internal standard compounds.

approximately 150 compounds in infant formula and related dairy ingredients (milk powder and whey protein isolate). The analytes belong to the following veterinary drug classes: anthelmintics, antibiotics (aminoglycoside, amphenicols, β-lactams—penicillins and cephalosporins, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, and others), antimicrobial growth promoters, antiprotozoals, β-agonists, coccidiostats, dyes, pesticides, and tranquilizers. The method development and optimization were divided into five main phases: (i) MS/MS conditions for individual compounds; (ii) LC conditions; (iii) final LC-MS/MS method; (iv) sample preparation procedure; and (v) method validation, data acceptance criteria, and method implementation. Particular attention was devoted to mobile phase composition optimization and to comparison of different sample preparation approaches. Different concentrations of formic acid in the aqueous mobile phase and different ratios of acetonitrile and methanol in the organic mobile phase were evaluated to achieve a well-distributed elution profile and minimum analyte interferences. The sample preparation optimization was divided into three stages: (i) extraction procedure; (ii) different cleanup options (such as dispersive SPE cleanup or supported liquid extraction); and (iii) establishment of the sample extract dilution scheme.

MATERIALS AND METHODS

Safety and Cautionary Statements. Some veterinary drugs (e.g., triphenylmethane dyes and their leuco metabolites) are known or suspected carcinogens. Appropriate personal protective equipment must be used when handling them. Dyes, tetracyclines, and some other veterinary drugs are light sensitive. All of the standards, samples, and sample extracts were stored in the dark in amber glass or covered with foil. The sample preparation was done in an area with yellow lights.

Reagents and Standards. Acetonitrile, methanol, and water were of LC-MS grade, ethylenediaminetetraacetic acid (EDTA) disodium salt was of USP grade, and hexane and ammonium sulfate were of ACS grade and obtained from Thermo Fisher Scientific (Fair Lawn, NJ,

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USA). Formic acid (99%) was of LC-MS grade and dimethyl sulfoxide (99.9%) was of GC grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). PTFE syringe filters (0.2 μm) were from VWR (Arlington Heights, IL, USA).

Veterinary drug reference standards were of the highest available purity and were acquired from Sigma-Aldrich (St. Louis, MO, USA), Toronto Research Chemicals (Toronto, ON, Canada), LGC/Dr. Ehrenstorfer (Manchester, NH, USA/Augsburg, Germany), or 2A Pharma Chem (Chicago, IL, USA). The individual analyte and internal standard stock solutions were made at concentrations of 1000−2000 μg/mL and taking into account purity, water content, and counterions. These analytes were dissolved and diluted with appropriate solvent (acetonitrile, methanol, or water). Depending on the specific solubility properties, several analytes were dissolved completely with the addition of a small portion of dimethyl sulfoxide. The analytes were divided on the basis of their classes into nine different groups: (A) 22 anthelmintics; (B) 20 β-lactams; (C) 13 macrolides and lincosamides; (D) 23 quinolones and others; (E) 24 sulfonamides; (F) 9 tetracyclines; (G) 22 β-agonists; coccidiostats, and growth promoters; (H) 12 tranquilizers, dyes, and pesticides; and (I) 4 others (streptomycin, chloramphenicol succinate, and colistins A and B).

The analyte group composite stock solutions (mixes A−I) were prepared at 40−100 μg/mL. The internal standard composite stock solution, including 10 isotopically labeled compounds (see Table 1) representing different veterinary drug classes, was made at 1−20 μg/mL. All stock solutions were stored at −20 °C. The analyte spiking solutions were prepared at different concentration levels by mixing an appropriate volume of each of the 40−100 μg/mL composite stock solutions with 75:25 water/acetonitrile (v/v). The internal standard solution was made by diluting the internal standard composite stock solution with 75:25 water/acetonitrile (v/v). In the method validation study, three sets of standards (extracted matrix-matched standards, postextracted matrix-matched standards, and solvent-based working standards) were prepared fresh daily. All three types of standards were made at the same range of analyte concentrations of 0.5, 1, 5, 10, 50, and 100 ng/g as matrix equivalence.

Samples. Infant formula powder was obtained from a local grocery store, whole milk powder was purchased from Amazon, and whey protein powder was obtained from Sigma-Aldrich (St. Louis, MO, USA). The bulk samples were stored at approximately 5 °C, except when needed for laboratory analysis. They were analyzed prior to the fortification to verify that no targeted veterinary drug residues were present.

LC-MS/MS Analysis. The UHPLC-MS/MS analysis was performed with an Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity binary solvent delivery UHPLC system and autosampler. The UHPLC system was coupled with an Agilent 6495 triple-quadrupole mass spectrometer equipped with an Agilent Jet Stream electrospray ionization source and iFunnel technology. The instrument control, data acquisition, and analysis were performed with Agilent MassHunter software.

Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18 column (100 × 2.1 mm, 1.8 μm particle size) with an Agilent Eclipse Plus C18 guard column (5 × 2.1 mm, 1.8 μm particle size). Mobile phases A and B were 0.1% formic acid in water and 0.1% formic acid in methanol, respectively. The following gradient elution program (mobile phases A and B) was used: 0−0.75 min, 2% B; 0.75−7 min, 2−40% B; 7−11 min, 40−100% B; 11−14.5 min, 100% B; 14.5−17.5 min, 2% B. Flow rate was 0.5 mL/min. The column was maintained at 40 °C, and the autosampler was at 5 °C. The injection volume was 5 μL.

The dynamic MS/MS acquisition was carried out using ESI in positive mode. The MS parameters were optimized and set as follows: drying gas, N_2 (250 °C, 12 L/min); nebulizer gas, N_2 (60 psi); sheath gas, N_2 (350 °C, 10 L/min); capillary voltage, 4000 V; nozzle voltage, 500 V; positive high pressure RF, 75 V; positive low pressure RF, 60 V. Three MS/MS (multiple reaction monitoring, MRM) transitions of each analyte were chosen with optimized collision energy (CE) and cell accelerator voltage (CAV) parameters for quantification and identification (see Table 1).

Sample Preparation. Sample (1 g) was weighed into a 50 mL disposable centrifuge tube. To prepare fortified samples and extracted matrix calibration standards, blank samples were fortified with 25 μL of appropriate analyte spiking solutions and 25 μL of the internal standard solution and left interacting at room temperature for 15 min. Extraction solvent A (10 mL of 0.05 M EDTA in water) was added and vortexed briefly until the sample was homogeneous. Extraction solvent B (10 mL of 0.1% formic acid in acetonitrile) was added and vortexed and then shaken for 15 min. Sample was centrifuged at 2000 rcf for 10 min. A 2 mL aliquot of the supernatant was transferred to a 15 mL centrifuge tube, evaporated to dryness at 40 °C under a gentle flow of N_2, and then reconstituted in 1 mL of 75:25 water/acetonitrile (v/v). To prepare postextraction matrix-matched standards, evaporated blank matrix extracts were reconstituted using 25 μL of appropriate analyte spiking solutions and 25 μL of the internal standard solution plus 950 μL of the dilution solution. The sample was mixed thoroughly and then transferred to a microcentrifuge tube, centrifuged at 15000 rcf for 5 min, and filtered (0.2 μm PTFE) into an autosampler vial for LC-MS/MS analysis.

Method Validation. On the basis of the guidelines outlined in CAC/GL 71-2009,[33] the final method was validated in terms of identification, specificity, matrix effects, linearity, LOQs, accuracy, and precision. Infant formula powder was selected as a representative matrix for the initial method validation due to its high complexity. Method performance was evaluated by analyzing a representative infant formula powder sample in duplicate (as a matrix blank) together with five replicates of spikes at 0.5, 1, 5, and 10 ng/g on day 1 and with five replicates of spikes at 1, 5, 10, 50, and 100 ng/g on day 2. The quantitation was performed both using extracted matrix calibration curve[34] (prepared pre-extraction by spiking standards into the blank sample matrix) and matrix-matched calibration curve (prepared postextraction). The analysis was conducted by two different analysts on days 1 and 2. In addition to the infant formula powder, the method was also validated in two important infant formula ingredients: whole milk powder and whey protein isolate. Due to the similarity of these matrices to infant formula, the validation in whole milk powder and whey protein was conducted on a single day with the same evaluation of method performance.

RESULTS AND DISCUSSION

Analyte Selection. The majority of the analytes were selected on the basis of safety concerns and veterinary drug residue regulations in milk. In addition, compounds prohibited in all food-related items, such as dyes and β-agonists banned in China[15] and the European Union,[13] were also included to meet global regulatory requirements.

Other important selection criteria included analytical considerations, mainly the analyte's ability to be included in a multiclass, multiresidue method from extraction, chromatographic separation, and MS detection/ionization perspectives. For this reason, we did not include aminoglycosides (except for streptomycin), avermectins, or compounds that do not ionize well in ESI positive mode. Aminoglycosides are highly polar antibiotic compounds that require different chromatographic conditions and thus are analyzed in a single-class method.[35] Avermectins are prone to forming sodium adducts and thus require a different mobile phase composition than what is the optimum for the majority of other veterinary drugs.[36] The majority of veterinary drugs can be analyzed using positive ESI, which is the ionization mode employed in the presented multiclass, multiresidue method. Therefore, compounds ionizing in ESI negative mode, such as most amphenicols (e.g., chloramphenicol) or nonsteroidal anti-inflammatory drugs (NSAIDs), are typically analyzed using separate, dedicated methods that provide optimum detection sensitivity for that type of compound. (Note: chloramphenicol succinate,

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Table 2. Sets of Analytes That Share the Same MS/MS Precursors and Even Product Ions but Were Chromatographically Separated As Demonstrated by Their Retention Times (RT)

set	compound	formula	precursor ion	product ion 1	product ion 2	product ion 3	RT (min)
		Set of Compounds Sharing Only Precursor Ion					
1	salbutamol	$C_{13}H_{21}NO_3$	240	148	222	166	3.27
	albendazole-2-aminosulfone	$C_{10}H_{13}N_3O_2S$	240	198	133	105	4.18
2	sulfapyridine	$C_{11}H_{11}N_3O_2S$	250	156	65	108	4.24
	oxibendazole	$C_{12}H_{15}N_3O_3$	250	218	176	148	8.29
3	sulfadiazine	$C_{10}H_{10}N_4O_2S$	251	156	65	108	3.40
	methaqualone	$C_{16}H_{14}N_2O$	251	132	91	65	9.40
4	sulfathiazole	$C_9H_9N_3O_2S_2$	256	156	65	92	3.68
	flubendazole-amine	$C_{14}H_{10}FN_3O$	256	95	123	75	7.63
5	clenbuterol	$C_{12}H_{18}C_{l2}N_2O$	277	203	259	132	6.05
	sulfabenzamide	$C_{13}H_{12}N_2O_3S$	277	156	108	65	6.56
6	diminazine	$C_{14}H_{15}N_7$	282	119	102	135	3.80
	albendazole sulfoxide	$C_{12}H_{15}N_3O_3S$	282	159	240	208	7.30
7	trimethoprim-d_9	$C_{14}H_9D_9N_4O_3$	300	234	264	–[a]	4.83
	fenbendazole	$C_{15}H_{13}N_3O_2S$	300	268	159	104	9.85
8	sulfadoxin-d_3	$C_{12}H_{11}D_3N_4O_4S$	314	156	92	–	6.20
	flubendazole	$C_{16}H_{12}FN_3O_3$	314	282	123	95	9.45
9	ciprofloxacin	$C_{17}H_{18}FN_3O_3$	332	314	231	288	5.66
	fenbendazole sulfone	$C_{15}H_{13}N_3O_4S$	332	300	159	104	8.74
10	strychnine	$C_{21}H_{22}N_2O2$	335	184	156	129	5.08
	penicillin G	$C_{16}H_{18}N_2O_4S$	335	176	160	114	9.00
11	difloxacin	$C_{21}H_{19}F_2N_3O_3$	400	382	356	299	6.14
	colchicine	$C_{22}H_{25}NO_6$	400	358	326	282	8.78
		Set of Compounds Sharing Precursor and at Least One Product Ion					
12	sulfaguanidine	$C_7H_{10}N_4O_2S$	215	156	92	108	1.00
	sulfacetamide	$C_8H_{10}N_2O_3S$	215	156	92	108	2.77
13	zilpaterol	$C_{14}H_{19}N_3O_2$	262	244	185	202	3.18
	oxolinic acid	$C_{13}H_{11}NO_5$	262	244	160	216	7.90
	flumequine	$C_{14}H_{12}FNO_3$	262	244	202	126	8.98
14	sulfamoxole	$C_{11}H_{13}N_3O_3S$	268	156	108	65	5.00
	sulfatroxazole	$C_{11}H_{13}N_3O_3S$	268	92	108	65	5.90
	sulfisoxazole	$C_{11}H_{13}N_3O_3S$	268	92	156	113	6.20
15	sulfisomidine	$C_{12}H_{14}N_4O_2S$	279	124	65	92	3.45
	sulfamethazine	$C_{12}H_{14}N_4O_2S$	279	186	92	65	5.03
16	sulfameter	$C_{11}H_{12}N_4O_3S$	281	108	156	65	4.90
	sulfamethoxypyridazine	$C_{11}H_{12}N_4O_3S$	281	156	108	65	5.36
	sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$	281	156	80	65	5.83
17	sulfachloropyridazine	$C_{10}H_9ClN_4O_2S$	285	156	108	92	5.62
	sulfaclozine	$C_{10}H_9ClN_4O_2S$	285	156	65	92	7.04
	diazepam	$C_{16}H_{13}ClN_2O$	285	193	154	91	9.99
18	mebendazole-5-hydroxy	$C_{16}H_{15}N_3O_3$	298	266	79	77	7.62
	albendazole sulfone	$C_{12}H_{15}N_3O_4S$	298	266	159	77	7.80
19	ractopamine	$C_{18}H_{23}NO_3$	302	164	77	284	5.64
	isoxsuprine	$C_{18}H_{23}NO_3$	302	284	77	107	7.04
20	sulfadoxin	$C_{12}H_{14}N_4O_4S$	311	156	92	65	6.20
	sulfadimethoxine	$C_{12}H_{14}N_4O_4S$	311	156	92	108	7.53
	mabuterol	$C_{13}H_{18}ClF_3N_2O$	311	237	293	217	6.92
21	4-epitetracycline	$C_{22}H_{24}N_2O_8$	445	410	427	98	4.81
	tetracycline	$C_{22}H_{24}N_2O_8$	445	410	154	427	5.55
	doxycycline	$C_{22}H_{24}N_2O_8$	445	428	410	267	8.20
22	4-epioxytetracycline	$C_{22}H_{24}N_2O_9$	461	426	444	98	5.15
	oxytetracycline	$C_{22}H_{24}N_2O_9$	461	426	443	98	5.55
23	4-epidemeclocycline	$C_{21}H_{21}ClN_2O_8$	465	448	430	98	5.30
	demeclocycline (IS)	$C_{21}H_{21}ClN_2O_8$	465	448	289	–	6.15
24	isochlorotetracycline	$C_{22}H_{23}ClN_2O_8$	479	462	98	197	6.00
	4-epichlortetracycline	$C_{22}H_{23}ClN_2O_8$	479	444	462	98	6.20
	chlortetracycline	$C_{22}H_{23}ClN_2O_8$	479	444	462	154	7.10

[a] –, only two product ions were selected for isotopically labeled internal standards.

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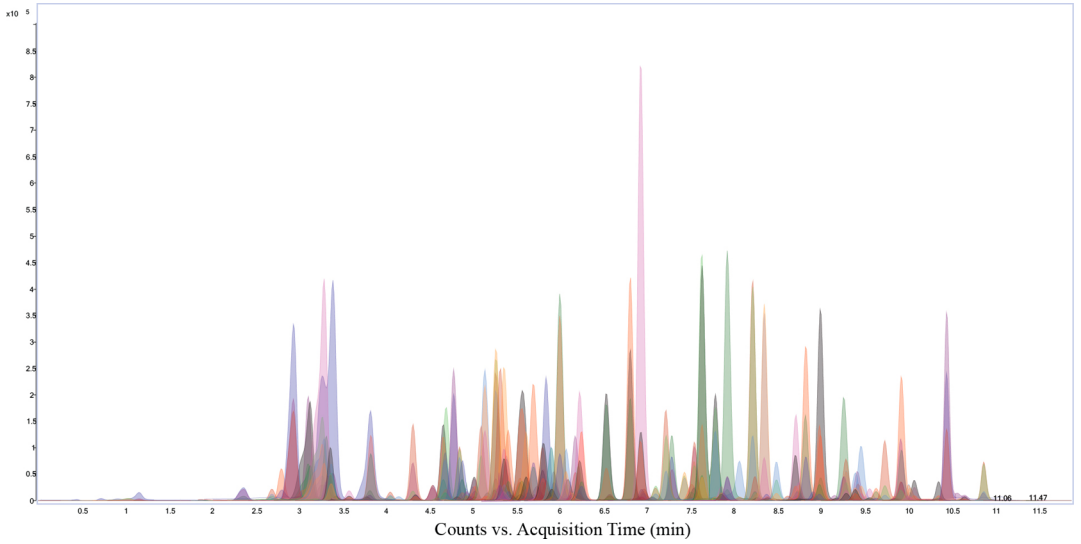

Figure 1. LC-MS/MS extracted ion chromatogram of an infant formula powder sample spiked at 100 ng/g with all analytes (equivalent to 10 ng/mL in the final extract).

regulated in China, and florfenicol amine are amphenicols analyzed in ESI positive and, thus, are included in our method.)

Overall, the presented multiclass, multiresidue veterinary drug method enables screening and quantitation of 143 analytes from 9 different veterinary drug classes, including 10 antibiotic subclasses.[37] In addition to this highly comprehensive scope of analytes, there has been, to our knowledge, no other method reported for multiresidue veterinary drug analysis in infant formula and related dairy ingredients. For the first time, a large scope of veterinary drugs was selected for method development and validation in infant formula powder, milk powder, and whey protein isolate.

LC-MS/MS Analysis. Agilent MassHunter Optimizer was used for MRM optimization of each individual veterinary drug, which included generation of product ion scans, selection of MRMs (up to 10 for each compound), and selection of the optimum collision energy for each MRM. The dynamic MRM (dMRM) acquisition method was further optimized including ESI source conditions, cell accelerator voltage, and evaluation of the MRMs for sensitivity and selectivity to choose the best three MRMs for the method. The selectivity of the MRMs was verified in infant formula powder using the final sample preparation procedure to make sure that the selected MRMs do not have any matrix interferences from closely eluting matrix components, which would affect accurate quantitation and/or analyte identification. The MRM transition with the highest intensity among the final three MRMs was used for quantification (quantifier), whereas the other transitions were used for identification (qualifier) by comparing ion ratios for samples to those of the reference standards, which should be within ±20% for relative ion intensity of >50%, within ±25% for relative ion intensity of 20−50%, or within ±30% for relative ion intensity of 10−20%.[33] Table 1 gives the specific MS/MS parameters and retention times of all the drugs in this study.

Suitable mobile phase composition and gradient are highly important to achieve good ionization efficiency and high sensitivity, reduce potential matrix interferences, and separate compounds that share the same precursor and main product ions (critical pairs/groups of analytes, e.g., selected tetracyclines). In this study, different organic mobile phase compositions were evaluated, including comparison of 0.1% formic acid in acetonitrile, 0.1% formic acid in methanol, and 0.1% formic acid in 1:1 acetonitrile/methanol (v/v) as organic mobile phases (mobile phase B). The best overall sensitivity and separation of critical pairs/groups of analytes were achieved using 0.1% formic acid in methanol. Studies have also shown that the use of methanol provided advantages over acetonitrile by removing more phospholipids from the system at a high content of the organic mobile phase. The mix of acetonitrile and isopropanol was used for rinsing the column at the end of each set to completely remove phospholipids.[38] In addition, different buffers, such as 0.1% formic acid, 0.3% formic acid, or 10 mM ammonium formate, in both mobile phase A and B were tested. The use of 0.1% formic acid provided the best sensitivity and separation selectivity results. Furthermore, the mobile phase gradient was optimized to achieve optimum chromatographic separation and peak shape. Despite the large number of targeted veterinary drugs included in the method, sufficient chromatographic separation of all critical analyte pairs/groups was achieved as demonstrated in Table 2. Figure 1 shows a typical extracted ion chromatogram of an infant formula powder sample spiked at 100 ng/g with all of the analytes.

Sample Preparation. Infant formula powder was chosen as the initial matrix in the method development process due to its high complexity (high protein, fat, and carbohydrate contents; and many additives, including metals) and strict requirements for low LOQs. Two main sample preparation steps were evaluated: sample extraction and sample cleanup.

Optimization of Sample Extraction. It is a great challenge to develop extraction conditions for a wide scope of veterinary drug analytes that show different physicochemical properties and at the same time achieve favorable operational characteristics, such as being simple, easy to use, high throughput, cost-effective, and safe and minimizing the use of hazardous reagents and generation of chemical waste.[31] Another difficulty stems from the complexity of the infant formula powder matrix.

Acetonitrile and water mixtures (75:25 and 50:50 acetonitrile/water, v/v) are frequently used in the extraction step in veterinary drug residue methods. Acetonitrile has the advantage of precipitating unwanted proteins. Various extraction solvent

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combinations were investigated in terms of the obtained absolute recoveries (calculated using postextraction matrix-matched calibration) of each individual analyte. The following three extraction solvent combinations were highly promising, providing acceptable absolute recoveries in the range of 70−120% for the majority of analytes: (A) 10 mL of 0.05 M EDTA in water + 10 mL of 0.1% formic acid in acetonitrile, (B) 10 mL of water + 10 mL of 0.1% formic acid in acetonitrile, and (C) 5 mL of water + 15 mL of 0.1% formic acid in acetonitrile. The problematic analytes with absolute recoveries outside the acceptable range included certain β-lactams, tetracyclines, and dyes; thus, extraction efficiency obtained for these compound classes served as the main extraction solvent selection factor. Figure 2 shows percentages of the evaluated β-lactams,

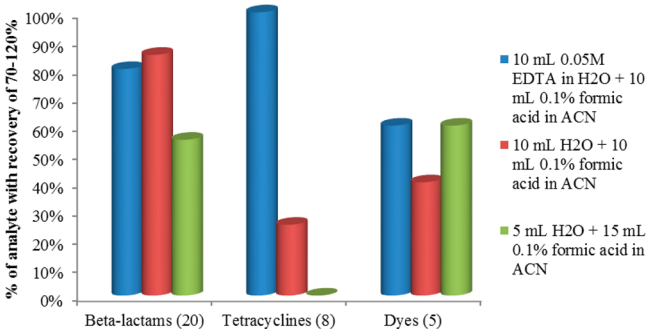

Figure 2. Comparison of extraction efficiency (shown as percent of analytes with absolute recovery within the range of 70−120%) obtained for β-lactams (20 analytes), tetracyclines (8 analytes) and dyes (5 analytes) fortified at 100 ng/g in infant formula powder using three different extraction solvents.

tetracyclines, and dyes for which acceptable absolute recoveries (within the range of 70−120%) were obtained in infant formula powder using the three promising extraction solvent combinations. Solvent combination A provided the best overall extraction efficiency and was selected as the extraction solvent combination in the final method. The use of EDTA (0.05 M) in this mixture is critical to prevent chelation of tetracyclines with metals,[39] leading to significantly increased recoveries of this antibiotic class.

Different ratios of acetonitrile and water yielded different absolute recoveries for some compounds. The 75:25 acetonitrile/water (v/v) aqueous extraction solution C was capable of precipitating a larger fraction of proteins and improved the recoveries for some compounds but rendered lower recoveries (<50%) for some β-lactams, which is a highly important antibiotic class. Increasing the water content to 50:50 acetonitrile/water (v/v) was necessary to improve the recoveries of β-lactams.[40,41] Furthermore, increasing the water ratio in the extraction solvent prevents extensive coextraction of lipids and phospholipids. For infant formula, milk, and whey protein powders, the sample is first homogenized with water, followed by the addition of acetonitrile. Formic acid (0.1%) was added to the acetonitrile extraction solvent to further assist with the precipitation of proteins.

Investigation of Cleanup Options. A suitable cleanup step was desirable to improve method performance and maintain long-term instrument/column performance. With the large scope of the analytes, a cleanup option that could potentially change the chemistry of the sample environment should be avoided, such as the use of certain selective SPE phases (e.g., PSA, SCX, SAX, CN).[42] The ideal option is to remove coextracted proteins, peptides, phospholipids, lipids, and other matrix components that could reduce column lifetime, cause more frequent instrument maintenance, or potentially interfere with qualitative and quantitative analysis while having a minimum impact on the analyte recoveries.

Several cleanup procedures were assessed to meet this goal. To evaluate the cleanup efficiency, the sample extract was divided into aliquots and then subjected to various procedures, including (i) no cleanup; (ii) enhanced matrix removal (EMR) sorbent for lipid removal in dispersive SPE format (Agilent Bond Elut QuEChERS dSPE EMR-lipid coupled with QuEChERS final polish EMR-lipid Mg salt); (iii) salting-out supported liquid extraction[29] (SOSLE, Biotage ISOLUTE, 5 mL sorbent mass) for removal of proteins, phospholipids, and salts; (iv) C18 in a SPE cartridge format (UCT quick QuEChERS, 600 mg sorbent) for removal of lipids and other less polar compounds; (v) PLD+ (Biotage, 50 mg, 96-well plate format for phospholipid removal); and (vi) hexane defatting plus C18 cartridge SPE for removal of lipids and other less polar compounds. Three factors were utilized to evaluate the cleanup efficiency: recovery and precision; matrix coextractive removal efficiency by a gravimetric test; and ionization suppression/enhancement evaluation using postcolumn infusion of veterinary drug standards into the final extract with and without applying the different cleanup procedures.

On the basis of the gravimetric test, EMR and SOSLE provided the best coextractive removal efficiency. Figure 3 compares profiles of representative internal standards infused postcolumn into injected infant formula matrix blank samples subjected to the evaluated cleanup options, including the profile obtained without any cleanup. In addition, a postcolumn infusion profile of a solvent blank (75:25 water.acetonitrile, v/v) was included as a control. At <20% organic mobile phase (around 0.5−3 min in our LC run), the ionization suppression is caused mainly by salts and other polar ionic compounds, whereas at 40−70% organic mobile phase (around 7−9 min), it is mainly caused by proteins and peptides and later, at 70−100% organic mobile phase (around 9−12 min), mostly by phospholipids/lipids. Similarly to the gravimetric test, EMR and SOSLE provided the best coextractive removal efficiency based on the postcolumn infusion profiles. From the results of spiked (100 ng/g, n = 3) infant formula samples, acceptable absolute recoveries were observed for the no-cleanup option and the five different evaluated cleanup procedures for most analyte groups, except the already discussed classes of β-lactams, tetracyclines, and dyes. As for the precision (coefficient of variability, CV), all analytes had CV ≤ 20% for the procedure with no cleanup, whereas all of the cleanup options resulted in a certain percentage of analytes with CV > 20%. In particular, about 40% of analytes had CV > 20% when SOSLE was used and 30% in the case of EMR; 15% for hexane defatting plus C18 dSPE; and 10% of analytes had CV > 20% in the case of C18 dSPE and/or PLD+.

Similarly to the extraction efficiency evaluation, the percentage of absolute recoveries within the range of 70−120% and CV ≤ 20% obtained for the problematic classes of β-lactams, tetracyclines, and dyes was used for the evaluation of the various cleanup options. As shown in Figures 4 and 5, the sample preparation procedure without any cleanup provided the best recoveries and precision. All five evaluated cleanup options negatively affected the absolute recoveries of β-lactams, tetracyclines, and dyes. For β-lactams, the use of EMR gave low

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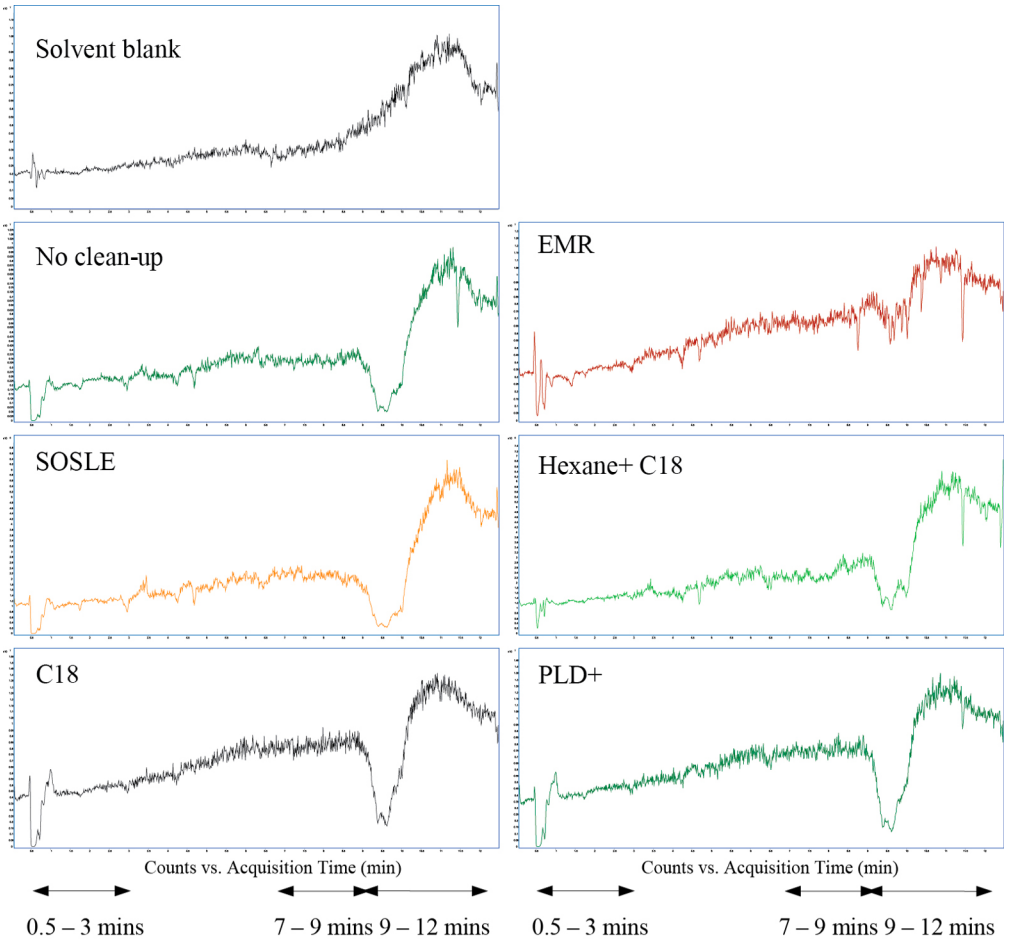

Figure 3. Postcolumn infusion profiles of infant formula matrix blank extracts obtained without any cleanup or using the five evaluated cleanup procedures. Infusion of a solvent blank is provided as a control for comparison purposes.

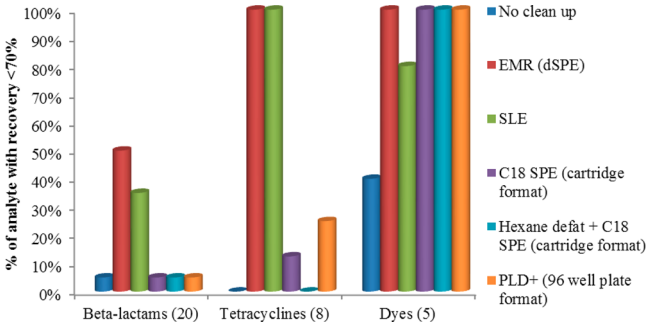

Figure 4. Comparison of recovery losses (shown as percent of analytes with absolute recovery <70%) obtained for β-lactams (20 analytes), tetracyclines (8 analytes), and dyes (5 analytes) fortified at 100 ng/g in infant formula powder using different cleanup procedures.

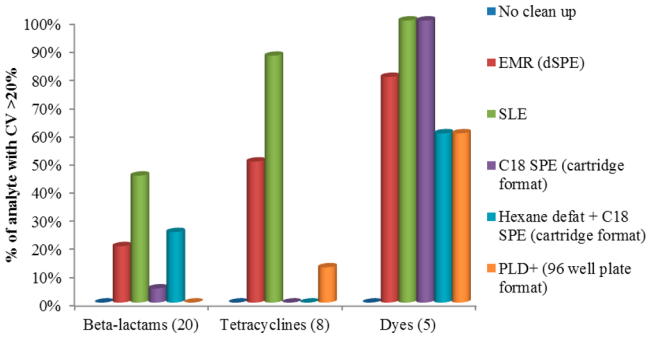

Figure 5. Comparison of precision problems (shown as percent of analytes with CV >20%) observed for β-lactams (20 analytes), tetracyclines (8 analytes), and dyes (5 analytes) fortified at 100 ng/g in infant formula powder using different cleanup procedures.

recoveries (<70%) for some of these compounds due to the final extract being partitioned from an aqueous into an acetonitrile layer, leading to the loss of some polar compounds such as certain β-lactams. For tetracyclines, employing SOSLE and EMR led to the lowest recoveries. The EMR cleanup procedure involved a "polishing" step with $MgSO_4$ to remove water and EMR sorbent residue from the final extract, which presumably also caused tetracycline losses due to their chelation and removal with the Mg^{2+} salt. SOSLE uses a porous solid support material, for example, diatomaceous earth, which is comparable to polar sorbents and may bind tetracyclines irreversibly.[43] For dyes, C18, hexane defatting plus C18, EMR, and PLD+ yielded low recoveries due to their retention of lipophilic compounds. Considering the lower recoveries observed for the critical compounds and an increased variability for additional analytes after application of the various cleanup procedures, a cleanup was omitted from the final method. The method, however, has other steps that contribute to matrix elimination/reduction, including acetonitrile-based extraction that coextracts minimum lipids and other lipophilic compounds and precipitates proteins, which are then removed by centrifugation. Also, the solvent exchange of the evaporated

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Table 3. Limits of Quantitation (LOQ), Accuracy (Percent Mean Spike Recovery Calculated Using Extracted Matrix Calibration Curves), and Precision (in Parentheses as Percent CV) at 0.5, 50, and 100 (n = 5 from 1 Day) and 1, 5, and 10 ng/g (n = 10 from 2 Days) in Infant Formula Powder (IF) and at 0.5, 1, 5, 10, 50, and 100 ng/g (n = 5) in Whole Milk Powder (MP) and Whey Protein Isolate (WP)[a]

				fortified level on sample								
	LOQ (ng/g)			0.5 ng/g			1.0 ng/g			5.0 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
anthelmintics												
albendazole	1	1	1	*132 (33)*	*108 (20)*	*99.4 (9.1)*	111 (20)	104 (26)	93.2 (3.0)	98.7 (14)	102 (12)	103 (3.2)
albendazole amino	5	5	1	*96.5 (13)*	–[b]	*116 (5.7)*	*102 (16)*	–	90.9 (5.5)	101 (7.4)	84.8 (14)	91.2 (9.4)
albendazole sulfone	1	1	1	*102 (19)*	*120 (2.6)*	*97.9 (6.7)*	105 (10)	102 (3.7)	104 (3.7)	95.1 (5.9)	93.3 (9.3)	93.3 (4.5)
albendazole sulfoxide	1	1	1	*108 (12)*	***131** (4.7)*	*109 (5.4)*	104 (14)	96.6 (7.1)	101 (4.5)	98.1 (7.1)	85.9 (4.6)	84.1 (10)
albendazole-2-aminosulfone	1	1	1	*100 (5.9)*	***126** (10)*	*101 (6.1)*	101 (6.4)	95.2 (7.5)	100 (6.7)	102 (4.2)	92.7 (6.1)	92.5 (8.1)
cambendazole	1	1	1	*106 (7.6)*	*105 (14)*	*110 (1.9)*	103 (9.4)	97.9 (5.2)	96.3 (7.7)	99.1 (6.2)	100 (4.7)	92.7 (10)
febantel	1	1	1	*95.7 (1.9)*	***125** (10)*	*117 (13)*	117 (12)	88.5 (14)	94.4 (7.2)	92.4 (10)	100 (7.5)	92.1 (6.6)
fenbendazole	1	1	1	*120 (27)*	*86.5 (15)*	***129** (7.2)*	89.7 (15)	90.5 (10)	111 (3.8)	99.4 (12)	108 (17)	82.0 (21)
fenbendazole sulfone	1	1	1	*100 (10)*	***124** (6.6)*	*93.1 (12)*	105 (10)	90.9 (8.9)	97.9 (8.3)	97.0 (9.0)	91.6 (6.0)	94.2 (11)
fenbendazole sulfoxide	1	1	1	*102 (8.1)*	***123** (9.2)*	*120 (7.8)*	103 (11)	94.7 (13)	95.0 (8.8)	100 (10)	94.4 (8.1)	85.1 (6.8)
flubendazole	1	1	1	*112 (14)*	***134** (12)*	***152** (4.7)*	107 (17)	101 (4.7)	97.0 (6.7)	88.7 (15)	76.8 (8.4)	69.9 (8.7)
flubendazole amine	1	1	1	*104 (6.6)*	***123** (10)*	*97.2 (7.5)*	102 (12)	95.5 (5.0)	102 (3.6)	100 (10)	93.6 (8.4)	99.3 (7.8)
levamisole	1	1	1	*96.0 (13)*	*112 (14)*	*111 (5.3)*	101 (10)	96.0 (11)	102 (4.4)	103 (11)	96.8 (8.1)	87.2 (4.9)
mebendazole	1	1	1	*105 (18)*	*115 (8.7)*	*111 (2.4)*	97.8 (17)	101 (11)	94.4 (23)	104 (13)	104 (14)	92.7 (14)
mebendazole-5 hydroxy	1	1	1	*100 (9.4)*	*121 (5.6)*	*105 (10)*	103 (10)	96.3 (7.9)	98.3 (5.5)	100 (8.0)	91.3 (5.1)	93.0 (8.2)
mebendazole amine	5	1	1	–	*125 (7.8)*	*94.2 (10)*	*107 (8.5)*	97.1 (10)	106 (11)	102 (10)	92.1 (5.6)	95.0 (9.0)
oxibendazole	1	1	1	*103 (2.2)*	*118 (5.4)*	*111 (3.1)*	99.3 (8.6)	98.3 (5.2)	94.4 (3.6)	101 (8.9)	94.0 (10)	90.5 (4.4)
thiabendazole	1	1	1	*105 (26)*	*120 (20)*	*106 (11)*	100 (12)	97.7 (6.3)	99.0 (10)	101 (11)	89.9 (12)	91.1 (4.8)
thiabendazole-5 hydroxy	1	1	1	*104 (12)*	***126** (15)*	*106 (4.3)*	95.1 (13)	95.1 (12)	97.6 (2.2)	99.1 (7.0)	93.1 (11)	93.2 (5.9)
triclabendazole	1	1	1	*108 (4.0)*	***125** (9.1)*	*105 (11)*	97.5 (10)	101 (11)	94.3 (11)	99.3 (10)	100 (6.6)	97.1 (9.0)
triclabendazolesulfone	5	5	1	*94.8 (10)*	***121** (12)*	*111 (29)*	*95.9 (12)*	*96.7 (22)*	97.0 (26)	104 (5.4)	96.1 (6.9)	96.4 (11)
triclabendazolesulfoxide	5	1	1	*110 (27)*	*115 (26)*	*92.3 (8.0)*	*98.1 (23)*	105 (6.9)	101 (8.2)	96.5 (7.4)	93.4 (11)	104 (3.9)
β-lactams												
cefadroxil	10	10	10	–	–	–	–	–	–	*102 (**38**)*	–	*93.3 (17)*
cefazolin	10	5	5	–	–	–	–	–	*70.1 (24)*	*113 (24)*	86.1 (22)	107 (6.2)
cefoperazone	10	5	5	–	–	–	–	–	–	*94.0 (15)*	119 (27)	94.7 (19)
cefquinome	10	10	10	–	–	–	–	–	–	–	–	*98.5 (**34**)*
ceftiofur	5	1	1	*82.7 (30)*	*120 (12)*	*110 (7.9)*	*114 (14)*	89.0 (23)	86.4 (8.9)	94.9 (7.6)	104 (5.5)	96.8 (8.5)
desfuroylceftiofur	100	50	50	–	–	–	–	–	–	–	–	–
DCCD	10	5	5	–	–	–	–	–	–	*109 (16)*	85.6 (17)	86.0 (4.9)
cefacetrile	10	10	10	–	–	–	–	–	–	–	*113 (32)*	*81.4 (18)*
cephalexin	5	1	1	–	***126** (26)*	*105 (31)*	***128** (27)*	100 (23)	96.1 (7.4)	97.7 (21)	93.2 (10)	87.1 (9.2)
cephalonium	10	1	1	–	–	*84.8 (4.1)*	–	112 (21)	105 (13)	*110 (19)*	101 (16)	94.8 (21)
cephapirin	10	5	1	–	*119 (7.3)*	*105 (21)*	–	–	104 (4.1)	*110 (11)*	93.8 (5.1)	86.6 (5.3)
desacetyl cephapirin	5	5	5	–	–	–	–	–	–	80.4 (23)	**129** (7.9)	95.7 (4.0)
amoxicillin	5	1	5	–	–	–	*112 (23)*	113 (6.5)	–	105 (7.2)	101 (6.0)	94.0 (4.7)
ampicillin	5	1	1	–	*117 (15)*	*115 (**39**)*	*107 (23)*	106 (8.6)	91.1 (7.2)	102 (12)	93.8 (11)	87.3 (10)

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Table 3. continued

compound	LOQ (ng/g)			fortified level on sample								
				0.5 ng/g			1.0 ng/g			5.0 ng/g		
	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
cloxacillin	50	10	5	–	–	–	–	–	*90.9 (**45**)*	*92.7 (**38**)*	–	101 (4.4)
dicloxacillin	50	5	10	–	–	–	–	–	–	***122** (30)*	82.4 (21)	***135** (39)*
nafcillin	5	1	5	–	*111 (28)*	–	–	91.0 (18)	***154** (11)*	106 (21)	91.9 (13)	105 (**32**)
oxacillin	10	10	10	–	–	–	–	–	–	–	–	*71.2 (**38**)*
penicillin G	5	5	5	–	–	*118 (**44**)*	–	–	*72.7 (33)*	**122** (24)	90.5 (23)	114 (3.7)
penicillin V	10	10	5	–	–	–	–	–	–	–	–	85.1 (24)
macrolides and lincosamides												
clarithromycin	10	5	1	*106 (26)*	*105 (16)*	*114 (19)*	*110 (26)*	*82.7 (19)*	93.8 (21)	*90.0 (14)*	103 (10)	94.1 (6.5)
clindamycin	1	1	1	–	*114 (11)*	*97.9 (11)*	113 (14)	104 (18)	102 (4.2)	96.6 (14)	93.9 (8.8)	95.3 (12)
desmycosin	5	1	1	–	*116 (16)*	*115 (26)*	*113 (25)*	95.5 (17)	93.5 (23)	96.4 (15)	96.7 (12)	88.6 (9.2)
erythromycin A	50	10	10	–	–	–	–	–	–	–	*103 (29)*	–
josamycin	10	5	5	–	–	–	–	*101 (19)*	*93.6 (35)*	*97.1 (20)*	89.7 (12)	101 (20)
lincomycin	1	1	1	*99.3 (5.0)*	***126** (3.9)*	*97.9 (4.4)*	104 (11)	93.6 (7.3)	100 (1.7)	103 (13)	92.9 (5.3)	95.5 (10)
oleandomycin	5	1	1	*116 (16)*	*120 (7.4)*	*107 (13)*	*101 (18)*	88.3 (14)	85.8 (11)	96.7 (5.6)	97.7 (19)	100 (11)
roxithromycin	5	1	1	*101 (34)*	*98.2 (33)*	*94.2 (26)*	*108 (21)*	96.2 (18)	112 (22)	92.3 (21)	104 (19)	90.5 (13)
spiramycin I	1	1	1	*101 (13)*	***122** (7.7)*	*118 (2.8)*	104 (15)	95.4 (12)	90.3 (8.2)	98.6 (12)	92.1 (4.9)	88.5 (8.4)
tilmicosin	10	5	5	–	–	–	*114 (24)*	*117 (14)*	*116 (15)*	*97.9 (9.4)*	97.0 (13)	92.8 (1.6)
tulathromycin A	100	100	100	–	–	–		–	–	–	–	–
tylosin A	50	10	10	–	–	–	–	–	–	*105 (**32**)*	*109 (12)*	*96.6 (5.4)*
quinolones												
cinoxacin	5	1	1	*100 (28)*	***129** (8.6)*	*91.6 (8.9)*	*102 (14)*	93.2 (6.4)	101 (9.0)	101 (8.0)	92.8 (5.1)	101 (13)
ciprofloxacin	1	1	1	*104 (18)*	***161** (30)*	*94.9 (1.2)*	101 (16)	77.9 (18)	96.3 (8.6)	96.7 (11)	81.9 (13)	102 (6.0)
danofloxacin	5	1	1	*95.8 (7.7)*	***134** (5.0)*	*100 (3.0)*	*114 (13)*	87.0 (2.6)	91.5 (11)	96.9 (14)	90.2 (7.1)	102 (14)
difloxacin	1	1	1	*113 (25)*	***127** (5.5)*	*101 (3.0)*	99.3 (19)	91.2 (10)	97.7 (6.9)	100 (11)	92.8 (12)	96.4 (14)
enoxacin	5	5	1	–	–	*100 (0.8)*	***122** (19)*	–	98.2 (3.4)	89.5 (17)	102 (5.4)	103 (12)
enrofloxacin	1	1	1	*111 (10)*	***127** (15)*	*92.5 (6.5)*	95.9 (17)	89.9 (6.3)	93.4 (4.6)	101 (8.9)	93.0 (10)	100 (16)
flumequine	1	1	1	*89.5 (13)*	*116 (6.6)*	*108 (5.2)*	102 (10)	93.8 (8.9)	109 (3.5)	113 (10)	95.8 (6.8)	89.2 (11)
lomefloxacin	1	1	1	*108 (32)*	***127** (10)*	*113 (5.0)*	104 (19)	87.1 (4.5)	91.6 (5.9)	94.5 (14)	95.3 (9.3)	90.7 (6.7)
marbofloxacin	1	1	1	*110 (11)*	***127** (7.2)*	*105 (5.8)*	98.2 (11)	90.7 (15)	94.9 (2.1)	96.3 (14)	95.3 (16)	101 (8.4)
nalidixic acid	1	1	1	*101 (15)*	*114 (14)*	*106 (6.7)*	98.9 (13)	97.9 (12)	100 (5.7)	102 (16)	95.3 (8.3)	92.1 (12)
norfloxacin	1	1	1	*106 (12)*	–	*101 (2.2)*	107 (20)	109 (19)	98.3 (11)	97.1 (13)	99.3 (6.0)	104 (13)
ofloxacin/levofloxacin	1	1	1	*104 (8.6)*	***128** (8.6)*	*104 (5.3)*	98.6 (12)	90.9 (1.0)	97.2 (2.3)	100 (10)	93.2 (5.2)	98.9 (3.2)
oxolinic acid	1	1	1	*98.5 (9.0)*	*115 (7.2)*	*107 (2.4)*	102 (7.9)	92.3 (4.3)	97.7 (5.2)	98.3 (7.1)	100 (6.0)	96.0 (7.0)
sarafloxacin	5	1	1	*96.8 (4.4)*	***128** (10)*	*104 (8.0)*	*114 (11)*	88.8 (12)	100 (5.7)	95.9 (6.1)	91.5 (16)	95.8 (16)
sparfloxacin	5	1	1	*103 (34)*	***129** (10)*	*106 (7.0)*	*106 (25)*	88.3 (7.8)	92.7 (4.3)	92.8 (14)	94.3 (8.3)	95.6 (7.6)
sulfonamides												
sulfabenzamide	5	1	1	*112 (28)*	–	*105 (6.4)*	*106 (22)*	99.0 (16)	98.5 (12)	96.5 (16)	108 (7.2)	95.4 (12)
sulfacetamide	1	1	1	*103 (25)*	*113 (13)*	*107 (7.7)*	93.9 (13)	98.0 (5.9)	105 (3.0)	108 (11)	95.2 (5.9)	89.2 (6.4)
sulfachloropyridazine	5	1	1	–	*105 (24)*	*90.6 (2.8)*	–	90.4 (20)	107 (12)	101 (14)	104 (10)	105 (2.5)
sulfaclozine	5	1	1	–	*119 (12)*	*100 (3.3)*	–	87.4 (22)	92.7 (3.5)	118 (6.5)	101 (11)	98.7 (6.6)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				0.5 ng/g			1.0 ng/g			5.0 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
sulfadiazine	5	1	5	–	*104 (14)*	*113 (21)*	*113 (16)*	96.6 (23)	*95.9 (11)*	103 (15)	95.7 (12)	92.4 (3.9)
sulfadimethoxine	1	1	1	*112 (17)*	*108 (16)*	*104 (11)*	100 (16)	96.1 (9.1)	95.1 (6.9)	97.8 (8.8)	101 (8.4)	93.5 (6.3)
sulfadoxine	1	1	1	*102 (10)*	*113 (21)*	*116 (6.1)*	104 (12)	93.8 (9.3)	97.5 (3.3)	102 (8.9)	91.9 (5.8)	91.3 (11)
sulfaguanidine	5	5	5	–	*102 (1.2)*	–	*109 (15)*	*69.3 (4.3)*	–	96.1 (5.2)	111 (2.0)	76.2 (7.4)
sulfamerazine	1	1	1	*103 (24)*	*109 (28)*	*101 (15)*	101 (12)	87.9 (7.9)	100 (7.2)	102 (16)	104 (3.3)	104 (7.4)
sulfameter	1	1	1	–	***123*** *(22)*	*61.4 (21)*	89.8 (24)	80.7 (18)	**122** (12)	104 (6.7)	104 (3.7)	105 (7.4)
sulfamethazine	5	5	1	–	–	*104 (8.5)*	*109* ***(40)***	–	99.4 (10)	98.7 (19)	106 (24)	95.3 (7.1)
sulfamethizole	5	1	1	*116 (10)*	*107 (19)*	*107 (15)*	*94.3 (32)*	96.9 (23)	99.0 (12)	98.9 (15)	97.9 (9.2)	95.5 (6.2)
sulfamethoxazole	5	1	1	*113 (25)*	*100 (31)*	*105 (5.8)*	*99.1 (31)*	111 (7.3)	105 (13)	103 (17)	100 (23)	95.7 (5.4)
sulfamethoxy-pyridazine	5	1	1	–	*115 (5.4)*	*112 (15)*	*120* ***(39)***	82.6 (7.8)	87.4 (9.0)	105 (12)	104 (6.8)	89.5 (11)
sulfamonomethoxine	5	5	5	***128*** *(7.1)*	***130*** *(22)*	*110 (12)*	*91.9 (42)*	*79.0 (12)*	*86.5 (12)*	104 (6.6)	97.9 (4.9)	96.6 (3.3)
sulfamoxole	1	1	1	*116 (18)*	*101 (10)*	*113 (20)*	87.8 (16)	86.3 (13)	97.9 (10)	110 (13)	109 (12)	94.0 (11)
sulfanilamide	100	100	100	–	–	–	–	–	–	–	–	–
sulfaphenazole	1	1	1	*103 (17)*	*118 (18)*	*105 (5.2)*	98.0 (10)	88.0 (7.0)	97.5 (5.3)	104 (7.7)	101 (7.2)	94.8 (6.5)
sulfapyridine	5	1	1	–	–	*103 (9.2)*	*112 (18)*	79.5 (11)	105 (7.8)	110 (9.0)	97.6 (11)	85.5 (14)
sulfaquinoxaline	1	1	1	*94.6 (22)*	*119 (11)*	*89.3 (5.0)*	105 (6.5)	93.2 (11)	108 (6.4)	103 (8.4)	97.5 (6.7)	101 (7.8)
sulfathiazole	5	1	5	*106* ***(37)***	*109 (18)*	*97.5 (10)*	*100 (21)*	91.3 (5.2)	*98.0 (23)*	107 (16)	104 (13)	94.2 (10)
sulfatroxazole	5	1	5	*109 (22)*	*112 (3.2)*	*104 (3.4)*	*86.3 (14)*	89.3 (8.7)	*96.2 (10)*	113 (10)	102 (8.3)	99.5 (7.3)
sulfisomidine	5	1	5	–	*110 (17)*	*111 (10)*	*94.2 (15)*	86.5 (11)	*111 (3.1)*	107 (12)	101 (7.9)	82.0 (5.5)
sulfisoxazole	5	1	5	–	*105 (30)*	*86.6 (43)*	*97.1 (26)*	103 (15)	*107 (32)*	103 (10)	96.1 (9.3)	98.7 (12)
tetracyclines												
4-epichlortetracycline	10	10	5	–	–	–	–	*104 (35)*	*103 (2.5)*	*95.2 (18)*	*65.8 (10)*	98.6 (6.0)
4-epioxytetracycline	5	5	5	*90.1* ***(39)***	–	–	*115 (22)*	–	–	101 (15)	78.0 (22)	93.3 (17)
4-epitetracycline	1	1	1	–	***144*** *(8.5)*	***141*** *(25)*	98.0 (16)	82.4 (6.1)	81.1 (3.0)	101 (9.0)	89.4 (6.0)	75.6 (11)
chlortetracycline	5	1	1	*108 (8.1)*	–	*116 (28)*	*102 (14)*	85.5 (16)	99.1 (3.3)	98.8 (10)	86.2 (2.4)	84.1 (1.6)
doxycycline	5	1	1	*97.7 (28)*	–	–	*110 (15)*	80.0 (12)	79.0 (2.3)	98.5 (16)	83.2 (11)	79.6 (13)
isochlorotetracycline	5	5	5	*100 (8.8)*	–	*76.6 (8.0)*	*106 (14)*	*86.8 (14)*	*110 (3.7)*	94.9 (11)	96.1 (7.9)	106 (7.9)
oxytetracycline	5	5	5	*100 (35)*	***152*** *(16)*	–	*102 (13)*	*83.7 (29)*	*93.7 (32)*	101 (10)	73.9 (4.3)	95.3 (8.2)
tetracycline	1	1	1	*106 (11)*	–	*119 (6.0)*	101 (7.8)	90.6 (12)	87.1 (8.3)	99.0 (12)	86.6 (7.6)	82.8 (3.1)
antibiotics—others												
dapson	1	1	1	*101 (19)*	*98.5 (16)*	*94.2 (33)*	105 (35)	92.9 (12)	110 (7.0)	98.1 (8.1)	113 (7.1)	96.1 (11)
nifurstyrenate sodium	50	NA	50	–	–	–	–	–	–	*120 (3.6)*	–	–
novobiocin	5	5	1	–	–	*104 (29)*	–	*100 (17)*	101 (23)	109 (8.4)	112 (10)	101 (3.5)
ormetoprim	1	1	1	*103 (14)*	*118 (8.9)*	*105 (12)*	107 (12)	94.2 (14)	89.2 (11)	93.7 (10)	96.7 (6.7)	98.6 (15)
rifaximin	1	1	1	*97.5 (34)*	***123*** *(11)*	*107 (11)*	106 (8.7)	87.8 (12)	110 (17)	100 (6.4)	95.3 (7.3)	93.4 (7.7)
tiamulin	1	1	1	*107 (11)*	*112 (7.0)*	*110 (4.2)*	104 (13)	97.6 (10)	102 (11)	96.6 (17)	96.7 (13)	91.0 (5.8)
trimethoprim	1	1	1	*101 (11)*	***121*** *(4.9)*	*110 (6.6)*	97.1 (8.8)	100 (8.2)	101 (6.9)	102 (10)	93.0 (8.5)	89.5 (11)
florfenicol amine	100	100	100	–	–	–	–	–	–	–	–	–
streptomycin	100	50	50	–	–	–	–	–	–	–	–	–
chloramphenicol succinate	50	50	5	–	–	–	–	–	–	*112 (25)*	*104 (33)*	92.9 (9.2)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				0.5 ng/g			1.0 ng/g			5.0 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
antimicrobial growth promoters												
bacitracin A	50	10	10	–	–	–	–	–	–	–	*100 (15)*	*96.1 (16)*
nitrovin	5	5	1	–	*88.9 (20)*	*112 (6.4)*	*87.5 (34)*	*85.8* (**39**)	102 (6.6)	110 (18)	112 (15)	94.6 (11)
virginiamycin (M1)	50	5	5	–	–	–	–	–	*74.9 (20)*	*108* (**38**)	118 (21)	95.5 (6.3)
antiprotozoal												
isometamidium	10	1	1	–	–	–	*121 (20)*	110 (6.5)	108 (4.3)	*86.8 (11)*	107 (5.5)	92.5 (2.5)
β-agonists												
cimaterol	1	1	1	*113 (8.9)*	*116 (13)*	*114 (14)*	97.5 (13)	97.9 (18)	90.6 (11)	101 (9.4)	95.4 (5.1)	90.1 (9.0)
clenbuterol	1	1	1	*106 (27)*	***124*** *(12)*	*109 (11)*	101 (16)	95.8 (5.8)	91.4 (3.6)	102 (13)	92.4 (5.0)	91.0 (14)
isoxsuprine	1	1	1	*100 (9.4)*	*119 (4.5)*	*107 (5.8)*	107 (17)	94.5 (13)	96.9 (6.7)	101 (9.1)	96.4 (7.2)	94.7 (6.7)
mabuterol	1	1	1	*109 (34)*	***128*** *(8.3)*	*126* (**41**)	96.5 (15)	93.4 (4.3)	76.9 (18)	101 (4.6)	93.0 (10)	91.2 (11)
ractopamine	1	1	1	*93.7 (12)*	***127*** *(11)*	*115 (19)*	97.9 (15)	93.6 (13)	86.3 (8.0)	104 (11)	94.8 (9.0)	91.7 (7.0)
salbutamol	1	1	1	*84.6 (18)*	*115 (9.1)*	*104 (6.0)*	110 (37)	101 (20)	94.1 (4.8)	98.9 (7.9)	93.7 (5.5)	96.7 (4.8)
terbutaline	1	1	1	–	***124*** *(13)*	*113 (16)*	113 (8.9)	94.2 (5.5)	91.8 (13)	103 (9.1)	94.7 (6.7)	90.2 (10)
zilpaterol	1	1	1	*96.8 (15)*	***128*** *(9.0)*	*103 (11)*	106 (27)	99.2 (5.2)	98.4 (1.7)	98.9 (15)	87.9 (3.8)	94.2 (7.4)
coccidiostats												
clopidol	5	5	1	–	–	***150*** *(8.7)*	*119 (25)*	*104 (29)*	90.7 (1.1)	96.0 (11)	97.1 (6.4)	80.8 (**34**)
dimetridazole	5	1	1	*94.3 (4.9)*	***131*** *(16)*	*111 (2.9)*	*101 (8.3)*	83.4 (11)	89.0 (4.2)	103 (6.6)	93.5 (2.8)	99.0 (6.5)
dimetridazole-hydroxy	10	10	10	–	–	–	–	*114 (26)*	–	*100 (9.0)*	*104 (11)*	*100 (2.1)*
ipronidazole	1	1	1	*102 (28)*	*116 (8.0)*	*107 (12)*	95.3 (14)	91.4 (5.3)	97.8 (7.1)	107 (10)	97.1 (2.9)	98.0 (16)
ipronidazole-hydroxy	1	1	1	*112 (11)*	*111 (14)*	*77.9 (25)*	97.8 (17)	103 (13)	**121** (5.8)	100 (12)	98.5 (13)	99.1 (9.3)
metronidazole	1	1	1	*103 (7.8)*	*117 (6.1)*	*93.4 (2.9)*	99.4 (10)	94.4 (1.9)	100 (4.5)	102 (5.7)	97.4 (4.3)	95.6 (5.0)
metronidazole-hydroxy	1	1	1	*96.6 (11)*	*118 (11)*	*115 (4.8)*	100 (6.5)	96.4 (0.9)	88.1 (8.4)	102 (5.9)	94.0 (2.6)	90.5 (4.4)
ronidazole	1	1	1	*107 (23)*	*105 (10)*	*94.0 (8.9)*	100 (14)	103 (8.9)	101 (9.3)	101 (6.7)	100 (5.2)	103 (14)
dyes												
Brilliant green	1	1	1	*103 (17)*	*108 (7.2)*	***130*** *(3.2)*	103 (9)	85.8 (15)	98.5 (5.6)	101 (14)	97.9 (4.3)	80.1 (7.2)
Crystal violet	5	5	5	–	–	–	–	–	–	83.7 (23)	94.8 (18)	**131 (38)**
Leuco Crystal violet	10	5	1	–	–	***125*** *(4.1)*	–	–	91.7 (1.4)	*110 (25)*	113 (23)	84.9 (4.9)
Malachite green	5	1	1	*108 (19)*	***126*** *(13)*	***138*** *(11)*	*103 (24)*	100 (20)	105 (11)	93.1 (11)	86.8 (14)	76.3 (11)
Leuco Malachite green	5	5	5	*116 (18)*	–	–	*112 (22)*	***135*** *(5.9)*	–	97.7 (18)	87.0 (4.3)	101 (1.7)
tranquilizers												
chlorpromazine	1	1	1	*108 (20)*	***124*** *(2.7)*	*112 (4.3)*	90.2 (26)	77.8 (17)	103 (9.2)	111 (21)	95.2 (19)	97.8 (15)
diazepam	1	1	5	*178 (16)*	*89.3 (19)*	–	102 (23)	98.3 (16)	–	97.5 (11)	98.3 (10)	103 (27)
methaqualone	5	1	1	*107 (19)*	***127*** *(6.7)*	*118 (15)*	*104 (17)*	102 (14)	96.6 (10)	101 (14)	85.0 (14)	95.0 (13)
procaine	1	1	1	*117 (22)*	*101 (16)*	*119 (5.0)*	91.0 (13)	90.6 (11)	93.0 (4.0)	102 (9.3)	103 (8.2)	85.5 (9.4)
xylazine	1	1	1	*102 (20)*	*118 (17)*	*90.8 (6.1)*	104 (21)	99.0 (4.9)	97.3 (2.5)	101 (12)	94.2 (8.3)	93.6 (11)
others												
colchicine	5	1	1	–	*113 (11)*	*104 (9.1)*	*109 (19)*	93.6 (17)	97.2 (10)	96.5 (11)	100 (9.5)	90.9 (8.0)
strychnine	1	1	1	*105 (28)*	*117 (16)*	*97.4 (27)*	101 (17)	94.0 (7.9)	96.4 (11)	103 (14)	97.4 (11)	91.9 (18)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				10.0 ng/g			50.0 ng/g			100 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
anthelmintics												
albendazole	1	1	1	101 (9.3)	84.5 (7.7)	103 (4.7)	97.0 (7.9)	103 (12)	104 (11)	102 (15)	100 (6.5)	97.4 (12)
albendazole amino	5	5	1	97.6 (7.1)	90.5 (3.0)	100 (4.9)	92.2 (11)	89.2 (2.6)	102 (2.1)	104 (12)	107 (8.3)	99.2 (12)
albendazole sulfone	1	1	1	99.0 (5.4)	87.6 (2.1)	104 (6.5)	94.9 (4.1)	89.8 (6.3)	102 (4.1)	103 (5.4)	107 (6.8)	98.9 (7.0)
albendazole sulfoxide	1	1	1	97.1 (8.2)	89.3 (5.5)	101 (7.9)	90.6 (8.8)	91.3 (10)	107 (2.2)	105 (15)	106 (7.9)	97.0 (10)
albendazole-2-aminosulfone	1	1	1	97.3 (8.2)	89.9 (8.4)	105 (5.9)	96.2 (7.5)	88.9 (7.4)	104 (4.7)	102 (11)	107 (5.3)	98.0 (12)
cambendazole	1	1	1	98.7 (8.5)	99.0 (6.5)	97.1 (5.2)	88.6 (3.2)	95.5 (7.9)	107 (3.0)	106 (10)	102 (4.2)	97.3 (10)
febantel	1	1	1	95.6 (6.7)	86.2 (9.0)	94.1 (7.8)	87.3 (4.2)	98.7 (11)	103 (5.8)	108 (7.8)	102 (10)	99.3 (9.4)
fenbendazole	1	1	1	103 (7.6)	117 (24)	74.7 (6.8)	94.0 (19)	**133** (8.5)	**160** (12)	103 (10)	98.1 (11)	103 (22)
fenbendazole sulfone	1	1	1	99.0 (8.2)	93.9 (12)	111 (8.0)	94.5 (3.6)	96.3 (15)	110 (10)	103 (7.6)	103 (3.7)	94.5 (15)
fenbendazole sulfoxide	1	1	1	97.2 (8.2)	88.4 (4.4)	97.5 (3.8)	97.7 (11)	97.2 (14)	103 (3.3)	102 (12)	103 (10)	100 (14)
flubendazole	1	1	1	99.3 (21)	85.2 (7.8)	77.5 (7.7)	92.9 (11)	72.4 (7.3)	76.4 (16)	105 (13)	102 (2.5)	104 (9.4)
flubendazole amine	1	1	1	97.2 (4.1)	88.7 (9.3)	99.3 (1.1)	91.6 (10)	94.9 (4.2)	105 (8.2)	105 (6.9)	104 (5.9)	97.8 (14)
levamisole	1	1	1	97.2 (10)	95.9 (2.3)	96.5 (2.9)	96.0 (13)	96.2 (7.3)	107 (0.7)	102 (13)	102 (6.8)	98.7 (5.0)
mebendazole	1	1	1	95.3 (11)	81.7 (8.8)	97.4 (7.0)	93.0 (7.0)	92.1 (15)	108 (14)	104 (14)	105 (15)	96.5 (6.7)
mebendazole-5-hydroxy	1	1	1	97.1 (8.2)	92.6 (13)	100 (8.6)	101 (3.7)	95.1 (6.1)	107 (3.6)	100 (8.9)	104 (6.6)	97.0 (11)
mebendazole amine	5	1	1	97.5 (7.4)	89.2 (4.8)	103 (9.1)	94.3 (2.4)	90.6 (6.2)	103 (4.1)	104 (14)	106 (4.6)	98.3 (16)
oxibendazole	1	1	1	98.2 (9.4)	93.1 (7.5)	104 (3.9)	97.9 (6.7)	90.4 (8.4)	101 (5.6)	101 (10)	106 (9.3)	100 (13)
thiabendazole	1	1	1	98.9 (8.7)	91.8 (6.0)	103 (3.5)	101 (14)	97.9 (11)	102 (8.0)	100 (11)	102 (3.6)	99.2 (7.8)
thiabendazole-5-hydroxy	1	1	1	103 (9.0)	90.0 (6.8)	99.5 (5.5)	102 (11)	89.8 (4.0)	107 (6.6)	98.6 (5.1)	106 (6.0)	96.8 (13)
triclabendazole	1	1	1	101 (3.3)	78.0 (11)	104 (10)	102 (5.0)	87.4 (6.8)	100 (4.7)	99.2 (16)	108 (4.8)	100 (6.2)
triclabendazole sulfone	5	5	1	103 (10)	91.0 (11)	93.3 (3.9)	94.8 (6.2)	88.9 (10)	103 (6.1)	102 (14)	107 (7.1)	99.1 (5.9)
triclabendazole sulfoxide	5	1	1	97.8 (16)	88.7 (11)	99.3 (8.0)	97.9 (3.9)	93.8 (4.8)	106 (4.7)	102 (13)	104 (4.9)	97.1 (4.5)
β-lactams												
cefadroxil	10	10	10	83.2 (18)	100 (10)	102 (0.8)	102 (7.6)	90.7 (8.8)	109 (4.6)	104 (13)	104 (4.2)	95.7 (10)
cefazolin	10	5	5	92.5 (17)	84.5 (9.2)	104 (18)	98.4 (7.0)	98.1 (6.3)	106 (11)	101 (16)	103 (8.0)	96.4 (11)
cefoperazone	10	5	5	109 (12)	96.8 (17)	115 (6.9)	97.0 (7.0)	90.5 (3.9)	107 (9.4)	101 (12)	104 (2.1)	95.5 (11)
cefquinome	10	10	10	96.0 (30)	97.2 (29)	97.3 (16)	88.6 (19)	86.5 (12)	93.2 (3.3)	106 (18)	107 (11)	104 (15)
ceftiofur	5	1	1	97.0 (8.2)	89.1 (4.5)	103 (3.7)	94.9 (4.9)	94.3 (4.6)	109 (6.8)	103 (7.5)	104 (6.9)	95.7 (5.5)
desfuroylceftiofur	100	50	50	–	–	–	*111* (**37**)	81.8 (8.6)	100 (**23**)	97.8 (13)	108 (8.1)	100 (20)
DCCD	10	5	5	96.5 (23)	91.6 (4.1)	113 (2.1)	98.8 (8.7)	85.6 (7.7)	103 (5.4)	103 (9.4)	109 (2.4)	98.0 (5.3)
cefacetrile	10	10	10	101 (14)	88.9 (14)	116 (7.8)	104 (13)	96.2 (12)	107 (4.4)	103 (5.1)	102 (10)	96.0 (9.2)
cephalexin	5	1	1	88.6 (16)	83.6 (9.3)	108 (5.1)	95.1 (8.1)	90.9 (12)	107 (9.3)	105 (5.9)	106 (5.9)	96.5 (8.5)
cephalonium	10	1	1	96.3 (8.4)	88.8 (11)	110 (6.6)	91.9 (4.8)	94.0 (13)	111 (2.5)	104 (10)	104 (10)	93.6 (13)
cephapirin	10	5	1	91.6 (7.7)	88.2 (3.2)	105 (7.8)	98.8 (7.3)	91.6 (7.0)	99.4 (5.1)	102 (10)	106 (4.8)	100 (15)
desacetyl cephapirin	5	5	5	105 (19)	105 (17)	97.9 (10)	103 (8.2)	90.1 (10)	112 (2.2)	98.7 (6.6)	103 (6.3)	94.5 (3.6)
amoxicillin	5	1	5	97.8 (6.4)	87.1 (5.3)	104 (0.3)	90.3 (3.6)	95.2 (11)	105 (3.0)	105 (7.4)	104 (7.6)	97.6 (8.4)
ampicillin	5	1	1	95.7 (8.0)	85.1 (12)	97.3 (6.0)	98.0 (10)	93.5 (14)	116 (6.9)	102 (9.0)	105 (14)	93.0 (3.9)
cloxacillin	50	10	5	*107 (21)*	93.7 (22)	106 (21)	101 (17)	102 (7.7)	104 (3.0)	100 (17)	99.4 (9.4)	97.2 (6.2)
dicloxacillin	50	5	10	–	80.5 (4.7)	80.5 (**32**)	100 (**21**)	98.6 (0.8)	**135** (**22**)	103 (16)	103 (10)	84.4 (**25**)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				10.0 ng/g			50.0 ng/g			100 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
nafcillin	5	1	5	95.8 (12)	102 (14)	61.0 (11)	96.8 (7.3)	108 (7.6)	**199** (18)	102 (2.0)	96.3 (15)	103 (**21**)
oxacillin	10	10	10	113 (**65**)	113 (18)	101 (10)	102 (11)	93.7 (6.5)	103 (3.6)	103 (26)	102 (16)	100 (17)
penicillin G	5	5	5	105 (27)	106 (11)	94.7 (8.8)	105 (14)	99.2 (8.1)	**137** (7.5)	95.4 (14)	100 (7.5)	100 (5.7)
penicillin V	10	10	5	89.8 (**33**)	95.0 (26)	91.3 (6.8)	94.2 (16)	90.3 (7.2)	110 (20)	103 (10)	105 (13)	96.5 (14)
macrolides and lincosamides												
clarithromycin	10	5	1	96.1 (14)	107 (4.9)	95.6 (10)	102 (10)	107 (12)	103 (12)	100 (8.5)	95.8 (4.1)	99.3 (12)
clindamycin	1	1	1	96.8 (6.8)	90.6 (10)	106 (10)	94.2 (14)	92.3 (8.0)	100 (4.5)	104 (14)	105 (2.0)	100 (11)
desmycosin	5	1	1	96.7 (9.3)	95.9 (2.2)	100 (6.9)	87.9 (4.8)	90.4 (5.5)	104 (9.2)	107 (11)	105 (4.6)	98.5 (7.5)
erythromycin A	50	10	10	*109 (14)*	98.5 (**31**)	110 (18)	111 (15)	108 (12)	104 (8.6)	99.3 (15)	95.4 (6.8)	100 (12)
josamycin	10	5	5	106 (10)	113 (2.7)	102 (22)	96.3 (13)	95.4 (8.6)	107 (7.8)	101 (15)	102 (7.1)	96.3 (5.4)
lincomycin	1	1	1	98.1 (3.5)	90.7 (5.9)	104 (8.6)	90.5 (10)	91.1 (8.8)	105 (2.7)	105 (7.8)	106 (6.7)	97.3 (12)
oleandomycin	5	1	1	99.2 (8.5)	96.4 (12)	108 (10)	87.8 (11)	93.8 (12)	101 (6.8)	107 (9.2)	104 (5.5)	98.7 (11)
roxithromycin	5	1	1	102 (13)	106 (14)	101 (11)	99.3 (20)	92.9 (10)	104 (5.4)	101 (7.4)	103 (10)	98.2 (5.4)
spiramycin I	1	1	1	97.2 (5.6)	93.6 (8.6)	100 (5.3)	94.1 (4.8)	92.0 (2.8)	106 (0.9)	104 (6.4)	105 (4.1)	97.4 (9.2)
tilmicosin	10	5	5	97.4 (7.4)	91.0 (8.1)	87.3 (1.8)	94.6 (9.4)	88.2 (5.0)	106 (2.9)	103 (14)	107 (7.6)	98.6 (3.2)
tulathromycin A	100	100	100	–	–	–	*100 (13)*	*93.2 (**41**)*	*119 (8.6)*	102 (12)	103 (8.1)	91.6 (11)
tylosin A	50	10	10	*94.7 (17)*	94.2 (15)	98.2 (2.7)	90.3 (16)	87.9 (7.0)	110 (7.5)	106 (13)	106 (12)	95.5 (6.6)
quinolones												
cinoxacin	5	1	1	97.3 (8.7)	86.7 (7.5)	103 (9.0)	95.5 (11)	92.5 (11)	107 (4.4)	103 (8.5)	105 (5.3)	96.1 (18)
ciprofloxacin	1	1	1	97.3 (13)	81.7 (6.5)	106 (5.1)	100 (18)	88.9 (6.2)	103 (4.9)	101 (4.6)	108 (3.9)	97.8 (8.8)
danofloxacin	5	1	1	94.7 (7.9)	91.9 (6.2)	104 (3.1)	95.6 (15)	91.0 (10)	105 (11)	103 (12)	106 (8.8)	97.0 (7.8)
difloxacin	1	1	1	94.2 (11)	90.2 (9.0)	101 (11)	101 (10)	95.6 (7.3)	107 (5.6)	101 (14)	104 (11)	96.7 (12)
enoxacin	5	5	1	96.0 (10)	95.3 (6.5)	95.8 (6.3)	91.5 (17)	84.0 (11)	105 (5.4)	106 (8.4)	108 (6.1)	97.9 (11)
enrofloxacin	1	1	1	101 (12)	90.1 (5.9)	114 (4.4)	94.1 (12)	96.4 (5.1)	102 (5.2)	103 (6.7)	103 (8.7)	97.6 (4.6)
flumequine	1	1	1	91.1 (10)	99.2 (3.5)	92.8 (12)	92.9 (5.6)	90.1 (12)	101 (2.5)	104 (15)	105 (8.9)	101 (**22**)
lomefloxacin	1	1	1	96.9 (11)	91.7 (10)	104 (14)	100 (5.9)	95.3 (13)	102 (4.6)	101 (10)	103 (6.6)	99.2 (15)
marbofloxacin	1	1	1	103 (7.4)	89.5 (10)	98.7 (8.8)	92.8 (9.2)	92.6 (5.7)	100 (3.8)	103 (8.3)	105 (7.7)	100 (9.5)
nalidixic acid	1	1	1	97.1 (18)	96.7 (3.4)	98.8 (13)	93.6 (11)	91.1 (12)	104 (4.3)	103 (17)	105 (6.4)	98.5 (6.9)
norfloxacin	1	1	1	92.8 (10)	94.0 (1.1)	100 (6.5)	98.9 (4.1)	94.9 (4.0)	95.0 (7.8)	102 (15)	103 (14)	102 (11)
ofloxacin/levofloxacin	1	1	1	100 (6.8)	91.8 (3.0)	97.2 (2.9)	96.8 (10)	89.8 (13)	105 (7.6)	102 (8.4)	106 (6.6)	97.7 (11)
oxolinic acid	1	1	1	100 (7.3)	94.0 (8.7)	96.6 (3.4)	97.0 (4.1)	97.3 (8.3)	104 (4.3)	101 (14)	102 (11)	98.3 (7.4)
sarafloxacin	5	1	1	94.9 (12)	96.1 (7.3)	99.5 (6.7)	92.2 (6.4)	90.0 (10)	102 (2.4)	105 (21)	106 (8.2)	99.3 (10)
sparfloxacin	5	1	1	103 (7.0)	91.4 (4.6)	103 (8.7)	96.8 (7.6)	90.9 (6.0)	106 (3.7)	102 (8.0)	106 (8.4)	97.1 (12)
sulfonamides												
sulfabenzamide	5	1	1	96.3 (13)	96.7 (6.8)	98.1 (10)	98.6 (5.7)	99.3 (6.4)	104 (3.3)	102 (15)	100 (4.8)	98.2 (11)
sulfacetamide	1	1	1	94.6 (14)	90.4 (4.8)	91.6 (13)	98.0 (11)	105 (5.0)	113 (2.9)	101 (12)	98.7 (10)	94.9 (7.0)
sulfachloropyridazine	5	1	1	98.3 (18)	100 (8.5)	86.9 (4.2)	101 (20)	102 (11)	118 (5.6)	100 (14)	98.8 (11)	92.1 (14)
sulfaclozine	5	1	1	98.5 (9.1)	94.8 (5.2)	100 (10)	104 (11)	95.4 (6.9)	117 (5.2)	97.1 (16)	103 (8.4)	91.9 (6.4)
sulfadiazine	5	1	5	96.4 (12)	102 (19)	92.7 (12)	98.0 (18)	103 (10)	110 (6.7)	102 (13)	98.7 (6.4)	95.9 (6.0)
sulfadimethoxine	1	1	1	95.7 (11)	95.7 (6.3)	99.3 (7.0)	97.3 (7.2)	96.6 (8.3)	114 (7.0)	103 (7.9)	102 (5.4)	93.2 (13)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				10.0 ng/g			50.0 ng/g			100 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
sulfadoxine	1	1	1	92.4 (14)	97.0 (6.8)	88.1 (3.1)	100 (10)	108 (8.6)	111 (4.3)	101 (12)	96.7 (6.3)	96.0 (12)
sulfaguanidine	5	5	5	98.1 (13)	117 (4.8)	**132** (6.0)	87.7 (10)	107 (4.3)	106 (1.0)	107 (18)	94.6 (6.2)	95.0 (7.2)
sulfamerazine	1	1	1	97.1 (14)	98.1 (11)	86.1 (6.7)	97.4 (19)	102 (12)	114 (5.1)	102 (10)	99.1 (11)	94.1 (4.0)
sulfameter	1	1	1	94.8 (18)	93.2 (14)	113 (13)	104 (10)	97.4 (7.0)	**135** (12)	99.0 (13)	102 (6.2)	98.4 (8.9)
sulfamethazine	5	5	1	96.6 (16)	118 (30)	93.9 (2.5)	93.2 (20)	109 (17)	112 (16)	104 (19)	93.5 (14)	94.7 (12)
sulfamethizole	5	1	1	94.3 (16)	93.5 (10)	95.5 (6.2)	100 (**25**)	107 (9.0)	105 (3.7)	101 (15)	97.1 (11)	98.0 (4.1)
sulfamethoxazole	5	1	1	97.3 (16)	90.2 (17)	88.2 (8.8)	91.6 (14)	95.3 (7.4)	109 (4.8)	105 (16)	103 (8.7)	96.7 (11)
sulfamethoxy-pyridazine	1	1	1	95.0 (17)	100 (5.8)	103 (7.2)	97.9 (13)	98.6 (5.1)	116 (8.9)	102 (12)	101 (9.1)	92.4 (11)
sulfamonomethoxine	5	5	5	93.0 (14)	91.3 (15)	98.9 (6.7)	96.8 (8.7)	102 (5.8)	115 (6.4)	103 (8.8)	100 (11)	93.0 (10)
sulfamoxole	1	1	1	95.9 (17)	102 (15)	92.6 (6.0)	104 (16)	105 (10)	103 (7.1)	98.4 (20)	96.8 (10)	100 (12)
sulfanilamide	100	100	100	–	–	–	*92.1 (20)*	*95.4 (7.5)*	–	100 (11)	102 (6.1)	100 (4.3)
sulfaphenazole	1	1	1	96.8 (8.5)	92.2 (9.1)	97.4 (4.2)	100 (13)	101 (10)	110 (5.1)	100 (7.0)	100 (7.2)	95.4 (10)
sulfapyridine	5	1	1	96.7 (14)	102 (11)	103 (2.4)	88.8 (12)	108 (8.6)	106 (8.3)	106 (9.1)	96.1 (8.6)	97.3 (11)
sulfaquinoxaline	1	1	1	95.7 (10)	87.8 (5.3)	102 (2.1)	95.8 (10)	103 (8.9)	**129** (3.3)	103 (3.8)	100 (2.6)	100 (10)
sulfathiazole	5	1	5	93.3 (5.8)	93.7 (9.4)	107 (12)	90.6 (12)	103 (7.2)	108 (4.0)	105 (23)	98.8 (14)	95.8 (11)
sulfatroxazole	5	1	5	95.8 (8.8)	96.5 (8.1)	100 (4.7)	95.5 (11)	101 (7.0)	**136** (2.4)	102 (4.9)	100 (6.5)	100 (4.8)
sulfisomidine	5	1	5	93.5 (11)	100 (5.6)	91.7 (11)	100 (18)	106 (7.0)	106 (1.5)	101 (13)	97 (8.5)	98.5 (15)
sulfisoxazole	5	1	5	99.0 (16)	95.6 (8.1)	100 (8.6)	97.1 (17)	100 (9.1)	115 (8.3)	101 (14)	101 (7.8)	92.5 (22)
tetracyclines												
4-epichlortetracycline	10	10	5	99.1 (8.3)	79.9 (3.4)	98.1 (2.4)	96.7 (3.7)	86.8 (4.6)	101 (8.3)	103 (6.0)	110 (6.4)	100 (1.6)
4-epioxytetracycline	5	5	5	90.6 (10)	107 (3.1)	103 (4.5)	94.4 (10)	82.6 (3.7)	107 (2.5)	105 (13)	109 (12)	96.7 (2.8)
4-epitetracycline	1	1	1	97.2 (6.1)	88.8 (4.9)	100 (0.6)	97.0 (3.9)	87.8 (5.8)	102 (0.9)	102 (10)	108 (8.6)	100 (10)
chlortetracycline	5	1	1	99.2 (6.1)	92.0 (9.0)	96.6 (5.2)	94.5 (7.3)	86.8 (4.5)	106 (0.5)	103 (5.6)	108 (4.4)	97.9 (3.5)
doxycycline	5	1	1	94.6 (8.4)	89.4 (2.8)	93.0 (3.9)	93.8 (7.6)	88.8 (4.5)	95.8 (1.2)	104 (9.2)	107 (9.1)	104 (2.7)
isochlorotetracycline	5	5	5	99.2 (10)	91.9 (13)	102 (3.9)	93.5 (6.4)	85.9 (15)	111 (2.1)	104 (7.2)	108 (6.5)	93.9 (1.7)
oxytetracycline	5	5	5	96.9 (6.1)	92.6 (11)	108 (7.0)	98.4 (3.5)	91.5 (10)	106 (8.5)	101 (6.5)	106 (4.7)	96.3 (1.5)
tetracycline	1	1	1	100 (8.8)	83.6 (1.9)	107 (6.2)	96.9 (4.6)	86.0 (3.9)	106 (5.3)	102 (4.5)	109 (6.9)	97.0 (11)
antibiotics—others												
dapson	1	1	1	95.3 (13)	93.7 (8.1)	100 (6.4)	97.2 (14)	104 (14)	**128** (5.9)	102 (16)	97.9 (10)	100 (10)
nifurstyrenate sodium	50	NA	50	*105 (2.1)*	–	–	110 (20)	–	**147** (20)	111 (16)	–	79.1 (8.1)
novobiocin	5	5	1	101 (7.1)	88.9 (6.8)	91.9 (9.1)	105 (5.2)	96.6 (7.1)	103 (6.0)	97.9 (18)	102 (6.1)	99.0 (12)
ormetoprim	1	1	1	100 (7.6)	95.4 (14)	105 (5.5)	90.5 (8.3)	90.5 (8.0)	107 (6.8)	105 (15)	105 (5.2)	96.4 (18)
rifaximin	1	1	1	94.7 (6.2)	95.2 (4.0)	86.5 (6.1)	102 (1.2)	96.2 (6.7)	104 (3.6)	100 (5.1)	103 (6.7)	100 (10)
tiamulin	1	1	1	98.2 (13)	96.5 (12)	95.7 (6.3)	95.6 (16)	93.7 (8.1)	100 (4.6)	103 (5.4)	104 (9.1)	101 (15)
trimethoprim	1	1	1	101 (6.5)	88.7 (4.3)	97.0 (3.3)	94.8 (7.2)	91.9 (15)	104 (4.6)	102 (16)	105 (4.6)	98.8 (9.3)
florfenicol amine	100	100	100	–	–	–	*94.0 (19)*	*97.5 (12)*	*100 (**27**)*	109 (**22**)	100 (11)	100 (7.7)
streptomycin	100	50	50	–	–	–	*100 (**26**)*	98.9 (7.3)	85.3 (18)	100 (**25**)	100 (17)	103 (8.1)
chloramphenicol succinate	50	50	5	*100 (**32**)*	*96.4 (19)*	100 (1.7)	95.2 (14)	99.0 (17)	105 (2.6)	102 (10)	101 (4.4)	98.0 (17)
antimicrobial growth promoters												
bacitracin A	50	10	10	*98.3 (29)*	93.7 (11)	103 (20)	108 (9.1)	92.1 (14)	102 (7.7)	98.6 (5.0)	105 (10)	98.9 (1.3)

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Table 3. continued

	LOQ (ng/g)			fortified level on sample								
				10.0 ng/g			50.0 ng/g			100 ng/g		
compound	IF	MP	WP	IF	MP	WP	IF	MP	WP	IF	MP	WP
nitrovin	5	5	1	104 (21)	106 (5.5)	91.7 (5.0)	91.8 (5.7)	116 (13)	96.9 (4.4)	103 (13)	91.2 (9.3)	103 (13)
virginiamycin (M1)	50	5	5	*101 (17)*	89.6 (6.9)	72.3 (27)	100 (17)	93.3 (10)	115 (18)	100 (9.3)	100 (13)	95.4 (4.1)
antiprotozoal												
isometamidium	10	1	1	97.2 (18)	89.2 (12)	90.6 (3.3)	99.4 (1.7)	93.2 (11)	116 (5.5)	102 (11)	104 (5.6)	93.5 (10)
β-agonists												
cimaterol	1	1	1	98.6 (7.4)	91.9 (10)	96.5 (5.7)	91.1 (3.9)	95.1 (8.5)	115 (8.6)	105 (7.2)	103 (5.2)	93.4 (11)
clenbuterol	1	1	1	96.5 (8.9)	90.9 (6.4)	105 (13)	93.9 (12)	91.6 (13)	108 (8.1)	104 (7.9)	105 (8.2)	96.2 (16)
isoxsuprine	1	1	1	94.0 (8.4)	94.1 (5.1)	100 (4.2)	91.2 (11)	91.2 (6.8)	103 (2.6)	105 (12)	105 (8.3)	98.9 (15)
mabuterol	1	1	1	97.2 (7.0)	90.0 (3.2)	103 (7.3)	97.7 (9.4)	89.0 (10)	106 (1.0)	102 (6.1)	107 (8.0)	97.3 (13)
ractopamine	1	1	1	100 (23)	91.5 (4.0)	103 (13)	96.6 (8.4)	85.0 (9.4)	107 (5.2)	101 (15)	109 (3.3)	96.4 (14)
salbutamol	1	1	1	97.0 (7.5)	93.4 (2.8)	103 (8.0)	94.2 (3.1)	92.3 (10)	106 (3.8)	103 (8.8)	105 (5.5)	97.2 (8.6)
terbutaline	1	1	1	97.5 (6.0)	90.0 (8.5)	102 (7.3)	96.7 (6.9)	91.8 (10)	105 (3.9)	102 (6.9)	105 (6.4)	97.7 (12)
zilpaterol	1	1	1	97.1 (6.7)	88.4 (7.3)	103 (5.9)	97.1 (4.0)	90.0 (3.1)	103 (3.6)	102 (8.6)	107 (4.6)	98.4 (11)
coccidiostats												
clopidol	5	5	1	94.6 (8.9)	90.8 (5.6)	75.7 (4.7)	91.0 (3.2)	91.8 (12)	**65.4** (7.8)	106 (10)	105 (6.5)	103 (8.6)
dimetridazole	5	1	1	98.7 (5.4)	91.4 (7.1)	99.1 (8.3)	96.5 (8.7)	100 (5.3)	102 (3.1)	102 (11)	101 (2.5)	99.0 (5.2)
dimetridazole-hydroxy	10	10	10	100 (11)	100 (11)	98.8 (5.0)	96.9 (7.9)	104 (6.1)	103 (2.2)	102 (9.1)	97.5 (11)	98.7 (11)
ipronidazole	1	1	1	100 (7.1)	94.5 (8.9)	98.3 (11)	94.8 (10)	99.4 (10)	97.7 (2.0)	102 (9.1)	101 (10)	101 (12)
ipronidazole-hydroxy	1	1	1	98.5 (4.2)	87.6 (5.9)	99.0 (0.8)	93.7 (17)	97.1 (14)	106 (8.7)	103 (11)	103 (4.0)	97.0 (5.0)
metronidazole	1	1	1	97.6 (5.3)	90.2 (5.5)	113 (7.3)	96.3 (6.9)	100 (9.4)	99.0 (4.9)	102 (11)	101 (2.9)	99.4 (10)
metronidazole-hydroxy	1	1	1	100 (5.1)	91.1 (2.0)	106 (3.5)	99.4 (5.3)	98.7 (2.5)	101 (5.2)	100 (10)	102 (3.4)	99.4 (10)
ronidazole	1	1	1	97.5 (8.8)	91.9 (4.3)	104 (8.1)	94.9 (7.0)	97.4 (4.4)	98.4 (1.9)	103 (8.5)	102 (4.1)	100 (6.9)
dyes												
Brilliant green	1	1	1	98.3 (12)	101 (5.8)	81.8 (6.2)	97.2 (20)	115 (18)	113 (9.3)	102 (13)	92.4 (13)	96.2 (13)
Crystal violet	5	5	5	115 (13)	94.7 (14)	116 (**36**)	91.5 (21)	106 (6.6)	106 (8.8)	104 (10)	97.0 (14)	93.6 (12)
Leuco Crystal violet	10	5	1	104 (14)	85.8 (14)	95.6 (0.6)	95.5 (13)	77.3 (8.3)	103 (2.2)	103 (13)	101 (8.2)	100 (5.1)
Malachite green	5	1	1	97.1 (13)	85.3 (8.1)	81.9 (26)	106 (7.4)	71.6 (9.2)	91.0 (15)	98.2 (7.9)	102 (6.2)	107 (16)
Leuco Malachite green	5	5	5	96.3 (11)	82.5 (8.5)	100 (5.8)	78.1 (8.8)	87.4 (8.4)	98.7 (4.9)	112 (7.0)	108 (5.4)	101 (4.2)
tranquilizers												
chlorpromazine	1	1	1	95.3 (17)	100 (18)	88.8 (19)	103 (18)	105 (6.2)	95.2 (7.6)	98.6 (7.0)	98.0 (10)	104 (11)
diazepam	1	1	5	105 (11)	115 (14)	96.9 (7.0)	87.7 (8.1)	**129** (13)	**136** (12)	106 (4.5)	98.6 (8.3)	100 (22)
methaqualone	5	1	1	95.7 (10)	86.3 (6.2)	89.5 (14)	90.3 (13)	95.5 (4.6)	100 (13)	106 (17)	104 (5.2)	101 (15)
procaine	1	1	1	98.5 (18)	103 (3.5)	95.8 (5.4)	98.1 (16)	104 (10)	111 (3.1)	101 (10)	97.5 (5.6)	95.5 (8.5)
xylazine	1	1	1	92.1 (9.0)	91.0 (8.2)	116 (15)	93.6 (4.8)	93.4 (8.0)	107 (4.2)	105 (10)	104 (10)	95.1 (16)
others												
colchicine	5	1	1	103 (11)	93.2 (2.3)	105 (14)	98.1 (14)	100 (4.6)	105 (12)	100 (8.9)	101 (10)	97.3 (8.8)
strychnine	1	1	1	96.1 (18)	93.5 (6.8)	107 (8.7)	94.5 (17)	95.4 (7.8)	115 (2.7)	103 (12)	103 (7.4)	92.4 (8.6)

[a]Results not meeting the acceptable mean recovery and/or precision criteria are highlighted in bold. Results at spiking levels below the established LOQ levels are presented in italics. [b]–, accuracy and precision results not listed at levels well below the LOQs.

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residue into 3:1 (v/v) water/acetonitrile further eliminates less polar matrix components that are not soluble in this solvent mixture. Moreover, the use of the highly sensitive mass spectrometer allows significant sample dilution and, thus, low matrix introduction into the LC-MS/MS system. All of that combined with appropriate routine maintenance of the LC-MS/MS system provides very good routine method performance. As a result, a generic and simple procedure was established for the multiclass, multiresidue veterinary drug analysis providing reproducible and robust results and minimizing the potential analyte losses.

Validation. *Specificity.* Specificity was demonstrated by monitoring multiple MS/MS transitions together with the evaluation of their signal ratios, which allows distinguishing of the target analyte from potential interferences.

Linearity. To test the linearity of the method and compare quantification results, three sets of standards (extracted matrix calibration standards, postextraction matrix-matched standards, and standards in solvent) were prepared. All three types of standards were made with the same range of analyte concentrations equivalent to 0.5, 1, 5, 10, 50, and 100 ng/g in the infant formula powder, whole milk powder, and whey protein isolate. Six levels were chosen to bracket the optimal concentration range for every analyte given the analyte sensitivity differences. Solvent-based calibration standards were used to monitor matrix effects. Postextraction matrix-matched calibration was employed to determine absolute analyte recoveries. The extracted matrix curve was used to mimic the standard addition procedure, which was then implemented for routine quantitation of potential veterinary drug residues in the samples. The coefficient of determination (r^2) values and linear range were determined for the extracted matrix curves by using a linear calibration with $1/x$ weighting factor. The r^2 values were >0.990 for the majority of analytes at concentrations ranging from the method validated LOQ level (typically 1 ng/g) to 100 ng/g.

Accuracy, Precision, and Intermediate Precision. On the basis of the CAC/GL 71-2009 guideline,[33] the acceptable accuracy and precision (CV) were set as (i) mean recovery within 50−120% and CV ≤ 35% for the spike concentrations at 0.5 and 1 ng/g; (ii) mean recovery within 60−120% and CV ≤ 30% at 5 and 10 ng/g; and (iii) mean recovery within 70−120% and CV ≤ 20% at 50 and 100 ng/g. Table 3 provides the accuracy and precision results, which were obtained on two different days by two different analysts in the case of infant formula powder and on one day for whole milk powder and whey protein isolate using matrix-extracted calibration curves. This calibration approach is frequently employed in the veterinary drug analysis field.[34] It mimics the standard addition procedure and provides highly accurate results by compensating for both matrix effects and potential recovery losses. Ten labeled internal standards, representing different veterinary drug groups/classes, are used in the method to monitor routine performance but are not employed for response normalization. The precision was evaluated at six fortification levels of each matrix in five replicates. The intermediate precision of the method was investigated at three fortification levels of 1, 5, and 10 ng/g in five replicates on two different days for infant formula powder (listed in Table 3). The following analytes were excluded from the final method used for the routine analysis due to overall poor precision and/or recovery: 4-epidemeclocycline, decoquinate, diminazine, and colistins A and B. Acceptable analyte recoveries and precision meeting the CAC/GL 71-2009 criteria were obtained for the rest of the analytes (143 compounds) at and above their LOQs in all three matrices in the majority of cases as demonstrated in Table 3. A slight foam formation was observed during the extraction of some of the whey protein isolate samples,[24,44] presumably causing some of the slightly out of range results obtained in a small number of instances for this matrix.

Method-Validated LOQs. The method-validated LOQs (reporting limits) presented in Table 3 were determined for each analyte as the lowest spiking level that met the validation criteria for recoveries and CVs in the given matrix as well as identification criteria for at least two most abundant MRMs. The typical reporting limits obtained for the majority of analytes in infant formula powder, whole milk powder, and whey protein isolate were between 1 and 10 ng/g (see Figure 6).

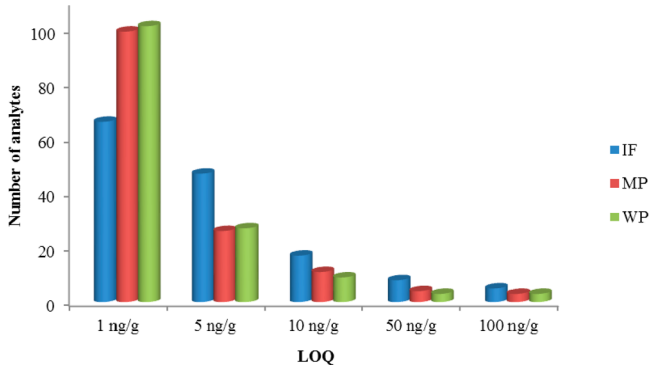

Figure 6. Comparison of LOQs (shown as the number of analytes at each validated LOQ level) obtained for infant formula powder (IF), milk powder (MP), and whey protein powder (WP).

The developed method was successfully implemented in our laboratory for a routine, cost- and time-effective analysis of a large number of important veterinary drug residues about a year ago. It has demonstrated a stable method performance in terms of analyte responses, linearity, and routinely checked recoveries in the application of extracted matrix-matched standards or standard addition in real matrices for quantification. The method utilizes a small solvent volume and generates minimum waste. It provides selective and sensitive LC-MS/MS-based detection, identification, and quantitation of individual analytes in infant formula powder and related dairy ingredients.

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Notes

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