

Evaluation of Multi-Class, Multi-Residues screening and confirmation Method (MMM) for 77 Veterinary Drug Residues in Pork and Chicken Muscle using LC-MS/MS

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Introduction

Method of analysis for single analyte or one specific class of veterinary medicinal product (VMP) residues have been popularly replaced by Multiclass-Multiresidue Methods (MMM) in order to effectively enhance the detection of residues in sample for regulatory control purpose. MMMs have been increasingly developed as many VMP residues as the methods can cover by using various techniques for sample preparation and analytical instrumentation e.g. LC-TQD MS/MS. The effective MMMs should detect not only a big number of VMP residues from different classes but should also meet criteria of screening and/or confirmation of residue found in order to meet purposes of methods especially for monitoring and regulatory control to ensure food safety and protect consumer health. The presented MMM has followed the sample preparation of USDA FSIS, CLG MRM 1.04 and LC-MS/MS triple quadrupole was used for screening and confirmation 77 analytes from eleven classes of veterinary drug in pork and chicken. The performance criteria for screening and confirmation purposes were evaluated against CODEX CAC/GL 71-2009 rev. 2014.

Experiment

Twenty blank of pork (n=20) and twenty blank of chicken meat samples (n=20) were spiked with mixed standard solution at one level of which the concentration of analyte may equal to different level for each substance e.g. at 1/12, 1/8, 1/4, 1/2, MRL and 2xMRL and different concentration levels(X) for banned or no MRL substances. Perform testing and analysing results to determine and evaluate performance characteristic against CODEX & EU criteria i.e. Threshold (T), Cut Off Factor (Fm) and CC α for screening, CC α , Rt, Identification Point or IP = 4 and Ion Ratio for confirmation of this MMM. The results evaluation of MMM for pork and chicken meat were coded in colors as shown in Table 1

Sample Preparation Flow Diagram



Table 1: Multiclass-Multiresidue Method data evaluation

CHICKEN										PORK										PORK										CHICKEN									
No.	STC (ug/kg)	MRL	FM (Threshold)	CC β (cut off)	% CV	Color code	No.	STC (ug/kg)	MRL	FM (Threshold)	CC β (cut off)	% CV	Color code	No.	Class of analyte/Analyte Standards	Rt (min)	MMR1	MMR2	Std. Ion Ratio (av. ± tolerance) %	No.	Color code	MRL	CC α	Recovery (%)	% CV	av. Ion Ratio (in sample) %	No.	Color code	MRL	CC α	Recovery (%)	% CV	av. Ion Ratio (in sample) %						
1	25	1/4	1.4	20.9	25	7.1	1	25	1/4	2.3	22.0	25	7.0	1	Sulfonamides (21)	4.69	279.20-124.30	279.20-186.20	44 ± 25	1	100	102.9	99	7.0	43	1	100	102.8	95	7.1	44								
2	25	1/4	4.0	21.8	25	7.8	2	25	1/4	2.9	21.1	25	7.0	2	Sulfacetamide	5.36	215.20-156.25	215.20-108.25	75 ± 20	2	100	102.7	96	7.0	78	2	100	103.2	100	7.8	76								
3	25	1/4	0.6	20.8	25	10.2	3	25	1/4	0.7	22.3	25	7.3	3	Sulfadiazine	5.52	251.20-108.20	251.20-156.15	75 ± 20	3	100	103.0	101	7.3	74	3	100	104.2	100	10.2	82								
4	25	1/4	1.0	21.6	25	11.2	4	25	1/4	1.0	21.6	25	7.4	4	Sulfathiazole	5.48	256.15-108.30	256.15-156.20	87 ± 20	4	100	102.8	98	7.4	90	4	100	104.4	97	11.2	85								
5	25	1/4	0.9	22.0	25	10.2	5	25	1/4	0.9	23.2	25	7.5	5	Sulfamonomethoxine	5.66	250.20-108.30	250.20-156.25	89 ± 20	5	100	103.3	106	7.5	86	5	100	104.4	106	10.2	91								
6	25	1/4	1.8	21.9	25	12.0	6	25	1/4	1.8	22.0	25	7.4	6	Sulfamazine	5.91	265.20-156.25	265.20-108.30	95 ± 20	6	100	104.6	107	7.4	97	6	100	105.4	109	12.0	98								
7	25	1/4	0.5	23.6	25	6.8	7	25	1/4	0.5	23.6	25	4.3	7	Sulfamoxole	6.75	268.20-156.25	268.20-108.30	78 ± 20	7	100	101.8	102	4.3	79	7	100	101.8	102	6.8	77								
8	25	1/4	0.2	24.4	25	3.9	8	25	1/4	0.2	24.2	25	3.4	8	Sulfamethazine	6.24	279.15-186.20	279.15-156.25	42 ± 25	8	100	101.4	103	3.4	63	8	100	101.7	104	3.9	60								
9	25	1/4	0.9	22.5	25	7.7	9	25	1/4	0.9	23.2	25	5.8	9	Sulfamethoxazole	6.08	271.15-156.25	271.15-108.30	58 ± 20	9	100	102.0	101	4.9	59	9	100	103.3	103	7.7	57								
10	25	1/4	0.4	23.4	25	7.3	10	25	1/4	0.4	23.3	25	6.0	10	Sulfameropyridazine	6.15	281.15-156.25	281.15-108.30	86 ± 20	10	100	102.5	103	6.0	87	10	100	103.2	106	7.3	86								
11	25	1/4	1.0	22.9	25	7.6	11	25	1/4	1.1	22.0	25	7.8	11	Sulfamerazine	6.29	281.10-92.30	281.10-156.25	55 ± 20	11	100	103.8	101	7.8	57	11	100	103.3	105	7.6	54								
12	25	1/4	0.3	22.8	25	8.8	12	25	1/4	0.4	25.3	25	5.2	12	Sulfamonomethoxine	6.43	281.15-156.25	281.15-108.30	90 ± 20	12	100	102.4	111	5.2	94	12	100	103.8	107	8.8	94								
13	25	1/4	0.1	21.9	25	11.0	13	25	1/4	0.2	21.8	25	10.9	13	Sulfadiazine	6.07	311.15-108.30	311.15-108.30	51 ± 20	13	100	104.7	106	10.9	51	13	100	104.8	107	11.0	50								
14	25	1/4	2.3	24.3	25	6.1	14	25	1/4	1.3	21.8	25	7.9	14	Sulfamethoxazole	6.67	254.15-108.30	254.15-156.20	70 ± 20	14	100	102.7	100	7.9	67	14	100	102.7	108	6.1	68								
15	25	1/4	0.3	22.0	25	6.9	15	25	1/4	0.4	22.1	25	6.2	15	Sulfisoxazole	6.75	268.20-156.25	268.20-108.30	59 ± 20	15	100	102.6	99	6.2	59	15	100	102.8	99	6.9	59								
16	25	1/4	1.2	21.3	25	7.2	16	25	1/4	1.2	21.4	25	6.4	16	Sulfabenzamide	6.93	277.15-156.20	277.15-108.30	50 ± 20	16	100	102.9	96	6.4	50	16	100	102.9	97	7.2	51								
17	25	1/4	0.1	23.5	25	6.2	17	25	1/4	0.1	23.1	25	5.8	17	Sulfamethoxazole	6.95	311.15-108.30	311.15-108.30	40 ± 25	17	100	102.4	102	5.8	42	17	100	102.7	105	6.2	42								
18	25	1/4	0.2	24.5	25	4.5	18	25	1/4	0.3	24.9	25	3.1	18	Sulfaguanidine	6.93	301.15-156.20	301.15-108.30	67 ± 20	18	100	101.3	105	3.1	66	18	100	102.0	106	4.5	67								
19	50	1/2	2.2	30.3	50	14.3	19	50	1/2	2.1	34.3	50	15.6	19	Sulfaguanidine	3.62	215.20-108.25	215.20-156.20	75 ± 20	19	100	111.8	92	15.6	75	19	100	109.3	79	14.3	67								
20	50	1/2	17.0	46.1	50	4.0	20	50	1/2	15.8	44.3	50	5.1	20	Sulfachloropyridazine	6.57	285.10-156.25	285.10-108.30	78 ± 20	20	100	104.0	97	5.1	83	20	100	103.2	99	4.0	79								
21	200	2	0.1	110.2	200	98.8	21	200	2	0.1	114.0	200	22.7	21	Sulfantran	7.21	334.00-135.80	334.00-132.90	72 ± 20	21	100	100	91	22.7	72	21	100	100	103	28.3	72								
22	100	2	11.0	51.4	50	24.4	22	100	2	13.6	57.4	50	21.4	22	Amoxicillin	4.16	366.25-349.20	366.25-114.15	72 ± 20	22	50	63.5	88	21.4	76	22	50	63.5	86	24.3	75								
23	50	1	4.8	25.0	50	21.3	23	50	1	2.5	27.2	50	20.2	23	Ampicillin	4.61	350.20-106.25	350.20-160.20	26 ± 25	23	50	63.5	81	20.2	28	23	50	63.5	77	21.3	26								
24	100	2	15.5	62.7	100	15.2	24	100	2	16.6	55.5	100	21.8	24	Cefepim	5.17	424.25-292.20	424.25-152.20	59 ± 20	24	50	97	21.8	62	24	50	97	83	15.2	29									
25	100	2	1.5	49.6	100	22.6	25	100	2	3.0	63.8	100	17.9	25	Cefazolin	6.59	455.00-232.60	455.00-156.40	74 ± 20	25	50	90	17.9	77	25	50	90	79	22.6	24									
26	50	1/4	18.4	26.4	50	15.3	26	50	1/4	8.5	25.6	50	22.5	26	Cephalexin	4.63	348.15-158.05	348.15-174.05	59 ± 20	26	200	215.0	81	22.5	62	26	200	208.9	71	15.3	59								
27	75	1/2	6.7	51.5	75	24.7	27	75	1/2	5.1	58.3	75	23.4	27	Cloxacillin	8.2	436.15-277.25	436.15-160.25	83 ± 20	27	300	336.3	110	23.4	80	27	300	344.7	110	24.7	78								
28	150	1/2	9.3	103.4	150	19.6	28	150	1/2	12.1	79.1	150	29.5	28	Dicloxacillin	8.4	470.05-160.20	470.05-111.20	82 ± 20	28	300	374.1	102	29.5	83	28	300	349.0	102	19.6	83								
29	75**	1/4	18.0	46.2	75	13.0	29	75	1/4	16.1	30.0	75	48.4	29	Oxacillin	7.95	402.10-108.30	402.10-156.20	95 ± 20	29	200	126.2	100	13.0	97	29	200	132.8	78	13.0	96								
30	12.5	1/4	0.7	9.3	12.5	15.6	30	12.5	1/4	0.9	8.2	12.5	14.3	30	Penicillin G	7.35	335.10-160.10	335.10-176.15	91 ± 20	30	50	55.4	109	14.3	93	30	50	55.0	114	15.6	93								
31	50	2	8.4	17.4	50	43.2	31	50	2	6.9	37.2	50	18.2	31	Penicillin V	7.6	351.00-160.10	351.00-114.10	50 ± 20	31	25	106	18.2	54	31	25	106	119	43.2	56									
32	25	1/4	8.0	17.9	25	14.2	32	25	1/4	8.5	19.9	25	18.4	32	Lincosamide (1)	4.4	407.30-126.40	407.30-353.30	3 ± 50	32	100	106.6	114	18.4	3	32	100	106.4	93	14.2	3								
33	50	1/4	0.1	16.9	50	33.0	33	50	1/4	0.2	30.1	50	22.2	33	4. Macrolides (10)	4.82	843.45-174.30	843.50-540.35	14 ± 30	33	200	217.3	95	22.2	14	33													