RIBMINS

3rd RIBMINS SCIENTIFIC CONFERENCE, 29-30 March 2023, Bucharest/Romania

A Multiclass method for the determination of veterinary drug residues in bovine meat using LC-MS/MS

Arzu YAVUZ*, **İsmail AZAR**, **Vesile ÇETİN**, **Ali ÖZCAN**, **Neslihan TURAN**

Central Research Institute of Food and Feed Control, Bursa, Turkey

Corresponding Author: arzu.yavuzyilmaz@tarimorman.gov.tr

INTRODUCTION

In modern animal husbandry applications, the use of veterinary drugs is extremely important for efficient and safe food production and also animal health. These veterinary drugs can cause residues in animal origin foods consumed by humans. Foods containing veterinary drug residues poses many risks to human health, especially antibiotic resistance. These risks highlight the importance of monitoring animal origin foods in terms of veterinary drug residues. Although sensitive and reliable analytical methods for the determination of veterinary drugs in meat are strongly required for meat safety assurance system, multiclass methods for veterinary drugs are still limited.

In this study, an analysis method optimization and validation was performed for the detection of veterinary drug residues in bovine meat using LC-MS/MS. Within the scope of the optimized method, veterinary drug residues from 9 different groups (sulfonamid, quinolone, tetracycline, penicillin, benzimidazoles, anticoccidials, anti-inflammatory nitroimidazole, amphenicol) were analyzed in bovine meat.

MATERIALS AND METHODS

Equipment

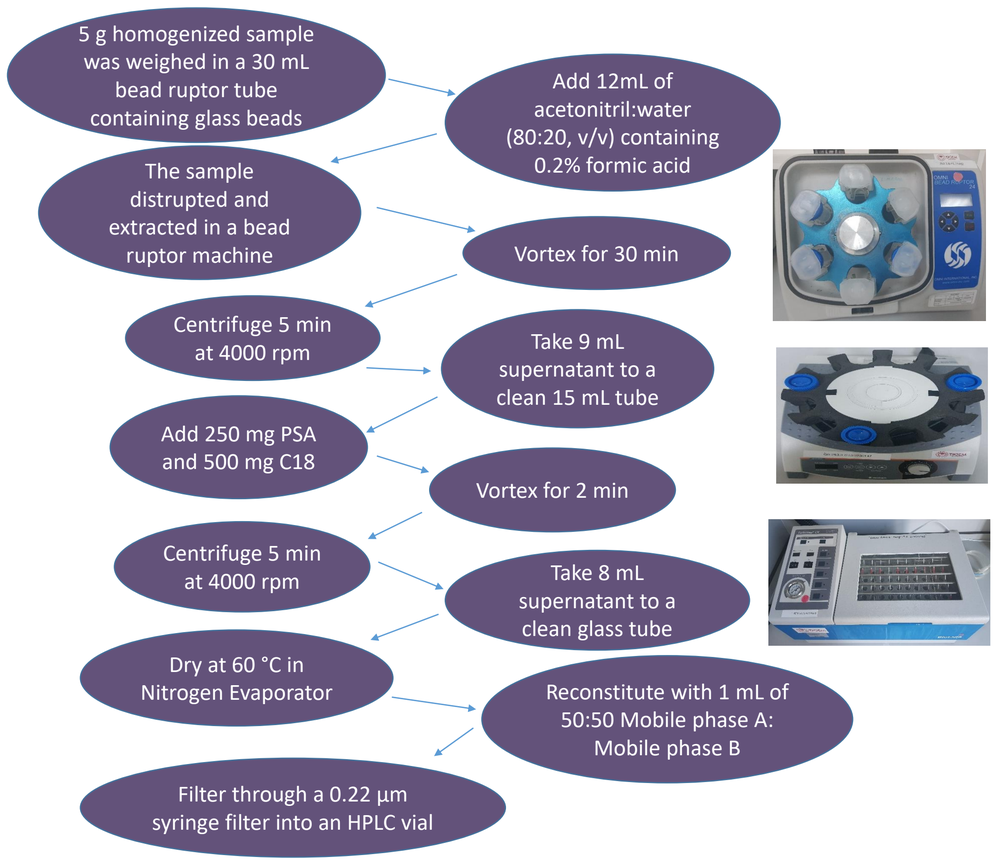
A Shimadzu LCMS-8040 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, a LC20AD pump, a Nexera X2 SIL-30AC autosampler, a CTO-10ASvp column oven, and PC software, LabSolutions ver. 5.97 (Shimadzu Corporation, Kyoto, Japan) was used for mass spectrometric detection.

TABLE 1: Optimized HPLC Conditions

Stationary phase	Shim-pack XR-ODSII 2.2µ 2.0x75mm
Mobile phase	A: 998 mL Water + 1 mL Formic Acid+Ammonium Formate
	B: 998 mL Methanol+1 mL Formic Acid+Ammonium Formate
Flow rate	0.2 ml /min
Column temperature	60˚c
Volume of injection	5 µl
Run time	12 min

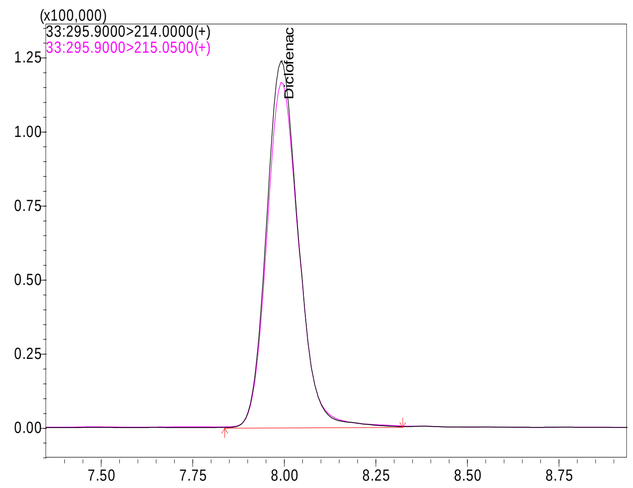
	Time	Module	Command	Value
1	1.00	Pumps	B.Conc	1
2	3.00	Pumps	B.Conc	30
3	6.00	Pumps	B.Conc	99
4	10.00	Pumps	B.Conc	99
5	10.01	Pumps	B.Conc	1
6	12.00	Controller	Stop	

Sample Preperation

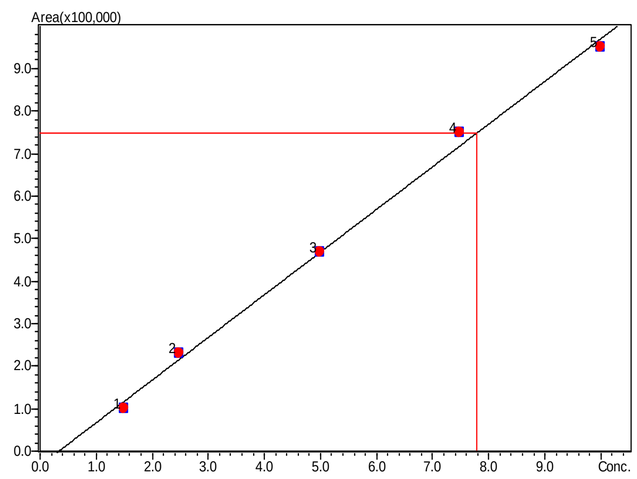


Validation

In the validation study, linearity, trueness/recovery, precision (repeatability, reproducibility), LOD, LOQ, CCα and CCβ parameters were performed. These parameters was carried out according to the requirements outlined in Commission Decision 2002/657/EC. If there is a maximum residue limit of analytes Commission Regulation (EU) No 37/2010 in bovine meat, it has been taken into account.



The highest intensities ions of each analyte were used for quantification, and the other ions were used for confirmation



The linearity for each analyte was determined by five-point standard calibration curves

TABLE 2: Optimised parameters of the LC-MS/MS system, and validation results

ID#	Name	Group	Molecular ion(m/z)	Product 1 (m/z)	Product 1 (m/z)	Ret. Time	ESI	Linear Range	LOD	LOQ	CCα	CCβ	Repeatability (%RSD)	Reproducibility (%RSD)	Recovery (%)
1	Metronidazole Hydroxy	Nitroimidazoles	188.0	123.1	125.8	4,03	(+)	2-10	0,38	1,27	2,45	2,77	4,60	4,08	101,24
2	Metronidazole	Nitroimidazoles	171.9	128.0	82.1	4,62	(+)	2-10	0,39	1,30	2,34	2,58	4,72	5,85	95,68
3	Levamisol	Benzimidaloles	204.9	178.0	91.0	4,41	(+)	2.5-20	1,14	3,80	12,22	13,78	7,27	10,55	101,26
4	Sulfadiazine	Sulfonamides	250.9	156.0	92.0	4,79	(+)	20-200	1,92	6,39	112,04	120,52	5,65	6,28	100,13
5	Thiabendazole 5 Hydroxy	Benzimidaloles	217.9	190.9	146.9	4,84	(+)	10-200	1,90	6,33	110,56	117,99	5,58	6,21	104,36
6	Marbofloxacin	Quinolones	362.9	72.1	320.0	4,98	(+)	25-300	4,12	13,74	161,65	169,85	5,45	5,17	101,53
7	Sulfathiazole	Sulfonamides	255.9	156.0	92.1	4,98	(+)	20-200	2,24	7,48	112,53	121,34	5,20	6,63	101,65
8	Sulfapyridine	Sulfonamides	249.9	156.0	108.0	5,23	(+)	20-200	2,84	9,46	110,69	118,22	6,37	7,24	100,96
9	Ciprofloxacin	Quinolones	331.9	314.0	230.9	5,28	(+)	20-200	3,42	11,41	110,15	117,29	5,18	3,81	101,79
10	Sulfamerazine	Sulfonamides	264.9	156.0	172.0	5,44	(+)	20-200	2,17	7,24	112,25	120,87	4,99	7,01	102,84
11	Enrofloxacin	Quinolones	360.0	342.1	316.1	5,43	(+)	20-200	3,08	10,28	108,86	115,10	4,64	5,23	103,60
12	Danofloxacin	Quinolones	358.0	340.1	82.1	5,35	(+)	30-400	7,30	24,32	217,22	229,33	5,97	4,17	102,30
13	Thiabendazole	Benzimidaloles	201.9	174.9	131.0	5,33	(+)	10-200	1,42	4,73	112,65	121,55	6,19	6,44	105,04
14	Difloxacin	Quinolones	399.9	382.0	299.0	5,48	(+)	100-700	19,22	64,07	425,62	443,66	5,01	6,36	103,01
15	Sarafloxacin	Quinolones	385.9	368.1	299.0	5,59	(+)	10-80	2,29	7,63	11,53	12,61	4,80	4,94	100,60
16	Sulfamethazine	Sulfonamides	278.7	186.0	124.1	5,86	(+)	10-200	1,40	4,68	44,73	48,06	6,73	7,08	102,66
17	Ampicillin	Penicillins	381.8	223.1	106.1	5,84	(+)	15-100	3,07	10,25	68,07	80,79	5,59	9,39	97,92
18	Sulfachloropyridazine	Sulfonamides	284.9	156.0	92.1	6,10	(+)	20-200	2,88	9,59	111,12	118,95	5,54	6,80	101,28
19	Sulfamethoxazole	Sulfonamides	253.9	156.0	92.0	6,15	(+)	20-200	2,32	7,74	110,12	117,24	4,71	8,86	100,93
20	Sulfadoxine	Sulfonamides	310.7	156.0	108.0	6,30	(+)	20-200	5,14	17,15	117,50	129,82	5,69	11,84	97,75
21	Ipronidazole Hydroxy	Nitroimidazoles	185.9	168.0	122.0	6,43	(+)	2-10	0,31	1,03	2,41	2,70	4,55	5,89	100,06
22	Doxycycline	Tetracyclines	444.9	428.0	154.0	6,52	(+)	25-200	5,05	16,82	111,74	120,00	4,38	4,77	99,87
23	Sulfadimethoxine	Sulfonamides	310.6	156.0	92.1	6,74	(+)	20-200	3,22	10,72	116,60	128,29	5,07	5,51	98,85
24	Ipronidazole	Nitroimidazoles	170.0	109.1	124.0	6,79	(+)	2-10	0,24	0,81	2,39	2,67	4,82	5,49	103,08
25	Sulfaquinoxaline	Sulfonamides	300.9	155.9	92.1	6,85	(+)	20-200	3,48	11,59	112,08	120,59	5,15	6,55	99,23
26	Oxolinic Acid	Quinolones	261.9	243.8	159.9	6,90	(+)	20-180	6,66	22,21	105,75	109,79	5,29	5,63	102,35
27	Nalidixic Acid	Quinolones	232.9	214.9	186.9	7,38	(+)	10-80	1,48	4,92	32,25	33,83	3,86	6,43	102,89
28	Flumequine	Quinolones	261.9	244.0	202.0	7,47	(+)	25-400	6,64	22,13	224,89	242,41	3,84	4,60	106,61
29	Oxacillin Sodium	Penicillins	434.0	159.9	144.0	7,62	(+)	50-600	6,98	23,27	339,59	367,46	4,17	4,00	99,69
30	Nafcillin Sodium	Penicillins	446.6	199.0	170.9	7,69	(+)	50-600	5,16	17,19	336,66	362,46	4,68	4,90	101,51
31	Meloxicam	NSAIDs	352.0	115.0	141.0	7,85	(+)	5-50	0,71	2,38	21,46	22,48	3,18	4,31	102,91
32	Diclofenac	NSAIDs	295.9	214.0	215.0	8,15	(+)	1,5-10	0,17	0,57	5,52	5,89	4,56	5,29	103,96
33	Salinomycin	Coccidiostats	773.4	431.1	531.2	8,82	(+)	0,5-4	0,21	0,70	2,54	2,92	9,73	11,32	99,87
34	Doramectin	Benzimidaloles	916.3	331.2	593.2	8,86	(+)	10-100	2,15	7,18	50,41	57,73	6,97	9,01	98,98
35	Moxidectin	Benzimidaloles	640.2	528.2	498.2	8,86	(+)	15-100	5,43	18,09	61,90	70,27	6,28	8,15	99,89
36	Monensin	Coccidiostats	687.9	635.3	461.3	8,79	(+)	0,5-4	0,17	0,58	2,65	3,11	9,77	12,61	100,15
37	Maduramicin	Coccidiostats	934.5	629.3	393.2	8,97	(+)	0,5-4	0,13	0,45	0,60	0,67	10,37	9,15	102,77
38	Narasin	Coccidiostats	787.3	431.2	531.2	9,31	(+)	1,25-10	0,50	1,67	6,61	7,74	10,97	11,53	98,68
39	Amoxicillin	Penicillins	395.9	318.1	224.0	5,25	(-)	15-100	5,57	18,56	63,32	72,69	9,12	9,24	96,63
40	Chloramfenikol	Amphenicoles	320.8	151.9	256.8	6,91	(-)	0,2-1	0,05	0,15	0,23	0,25	5,53	3,98	105,72
41	Penicillin G	Penicillins	364.9	287.0	169.0	7,36	(-)	10-100	3,50	11,68	61,95	70,36	4,24	9,89	98,74
42	Dicloxacillin	Penicillins	499.8	386.0	422.0	7,78	(-)	50-600	8,68	28,93	348,46	382,56	4,76	4,98	98,80
43	Nicarbazin	Coccidiostats	300.8	137.0	107.1	8,06	(-)	12,5-100	4,33	14,44	56,44	60,98	6,30	8,59	102,37
44	Lasalocid A	Coccidiostats	589.5	234.9	173.0	8,86	(-)	2,5-20	5,04	16,81	11,91	13,25	4,89	6,65	99,10

RESULTS

Acceptable validation results were obtained and summarized at Table 3.

A simple, low cost and fast method was optimized and validated.

Linear regression values ($r^2 > 0.99$) were obtained for all target analytes .

Matrix effect and uncertainties related with sample preparation and instrumental analysis were minimized by using matrix-matched calibration.