

Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

ASMS 2013 WP-368

Lingling Shen, Jinting Yao, Caiyong Lin,
Xiongxiong Qiu, Qisheng Zhong, Song Zhan,
Taohong Huang, Yuka Fujito, Shin-ichi Kawano,
Yuki Hashi
Shimadzu Global COE, Shimadzu (China) Co., Ltd.,
China

Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

1. Introduction

Veterinary drugs are widely used in breeding of marine products. However, residual veterinary drugs could enter human body and harm to human health. Therefore, those veterinary drugs in marine products have been strictly regulated in the world. In recent years, the China government continues to strengthen supervision and is developing quicker and highly sensitive analytical method.

Usually, the qualitative method of LC/MS/MS is based on the ratio of intensities between qualitative ion and quantitative ion. This paper describes ultra high performance liquid chromatography-triple quadrupole mass spectrometry for rapid screening of 68 veterinary drugs which belong to 12 categories.

2. Methods and Materials

Sample Preparation

Samples of marine products were extracted with acetonitrile. After centrifugation, concentration and filtration, the final extract was injected to the LC-MS/MS instrument.

LC/MS/MS Analysis

The analyses were performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A₃ degasser, and an SIL-30AC autosampler. A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was

connected to the Shimadzu UHPLC instrument via an ESI interface. Samples were separated on a Shim-pack XR-ODSIII (50 mmL. × 2.0 mm i.d., 1.6 μm). A flow rate of 0.4 mL/min was used together with a gradient elution.

Analytical Conditions

UHPLC (Nexera system)

Column	: Shim-pack XR-ODSIII (50 mmL. × 2.0 mm i.d., 1.6 μm)
Mobile phase A	: 0.1% formic acid
Mobile phase B	: acetonitrile
Flow rate	: 0.4 mL/min
Elution mode	: gradient elution
Column temperature	: 40°C
Injection volume	: 10 μL

MS/MS (LCMS-8040 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: positive & negative
Probe voltage	: +4.5 kV (positive), -3.5 kV (negative)
Nebulizing gas flow	: 1.5 L/min
Drying gas pressure	: 10 L/min
DL temperature	: 250°C
BH temperature	: 400°C



Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 1 List of veterinary drugs

Sulfonamides [12]		1-Dehydrotestosterone Sulfate	Nitro imidazoles [5]
Sulfacetamide		Danazol	Ronidazole
Sulfadiazine		Fluoxymesterone	2-methyl-5-nitroimidazole
Sulfathiazole		Testosterone	Metronidazole
Sulfapyridine		17-alpha-methyltestosterone	4-Nitroimidazole
Sulfamerazine		Methadrostenolone	Iprnidazole
Sulfamethazine		Nandrolone	
Sulfamethoxypyridazine		19-nor-4-androstene-3,17-dione	Nitrofurans metabolites [4]
Sulfchloropyridazine		Trenbolone	Furazolidone
Sulfamethoxazole		Megestrol-17-acetate	Furaltadone
Sulfisoxazole		Medroxyprogesterone	Nitrofurantoin
Sulfadimethoxine		Medroxyprogesterone-17-acetate	Furaclinum
Sulfachinoxalin		Norgestrel	
		Chloromadinone 17-acetate	Tetracyclines [5]
		Norethindrone	Tetracycline hydrochloride
		Progesterone	Oxytetracycline
Quinolones [10]			Demeclocycline hydrochloride
Pipemidic acid		Macrolides [5]	Chlorotetrachie hydrochloride
Enoxacin sesquihydrate		Spiramycin	Doxycycline
Ofloxacin		leucomylin hydrate	
Norfloxacin		Erythromycin	Lincosamides [2]
Ciprofloxacin hydrochloride		Tilmicosin	Lincolin Hydrochloride
Lomefloxacin		Acetylisovalerylyloisin Tartrate	Clindamycin
Danofloxain			
Enrofloxacin		Chloramphenicols [3]	Other drug [5]
Sarafloxacin hydrochloride		Thiamphenicol	Trimethoprin
Cinoxacin		Florfenicol	Malachite green oxalate
		Chloramphenicol	Leucomalachite green
Hormones [17]			Basic violet 3
19-nor-4-androstene-3,17-dione			Leucocrystal violet

Using a polarity switching speed of 15 msec and a scan speed of 15,000 u/sec, MRM spectra were generated in both positive and negative ionization. And fast polarity switching helps to provide information rich production spectra resulting in better detection and identification.

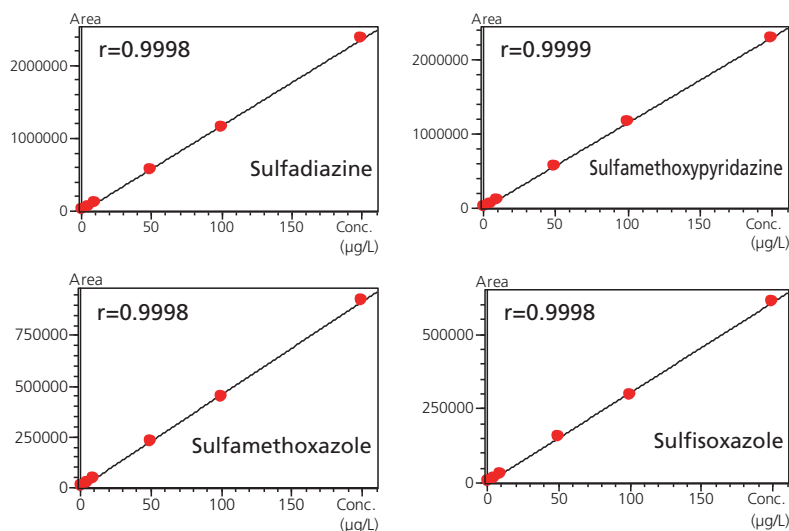


Fig. 1 Representative calibration curves of sulfonamides

Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 2 Repeatability of 68 drugs (n=6)

No.	Compound	%RSD		No.	Compound	%RSD	
		R.T	Area			R.T	Area
1	Sulfacetamide	0.40	4.43	35	Medroxyprogesterone-17-acetate	0.14	3.33
2	Sulfadiazine	0.27	3.91	36	Norgestrel	0.21	4.13
3	Sulfathiazole	0.21	3.13	37	Chloromadinone-17-acetate	0.14	4.09
4	Sulfapyridine	0.32	2.85	38	Norethindrone	0.27	3.46
5	Sulfamerazine	0.28	3.86	39	Progesterone	0.17	3.28
6	Sulfamethazine	0.18	2.40	40	Spiramycin	0.67	1.37
7	Sulfamethoxypyridazine	0.21	4.02	41	leucomycin hydrate	0.11	0.85
8	Sulfchloropyridazine	0.11	3.47	42	Erythromycin	0.08	1.88
9	Sulfamethoxazole	0.08	4.46	43	Tilmicosin	0.05	2.01
10	Sulfisoxazole	0.07	4.41	44	Acetylisovaleryltylosin tartrate	0.07	1.28
11	Sulfadimethoxine	0.10	2.06	45	Thiamphenicol	0.15	3.34
12	Sulfachinoxalin	0.09	4.00	46	Florfenicol	0.09	2.39
13	Pipemidic acid	0.23	2.82	47	Chloramphenicol	0.16	1.68
14	Enoxacin sesquihydrate	0.50	1.68	48	Ronidazole	0.82	1.25
15	Ofloxacin	0.14	2.88	49	2-methyl-5-nitroimidazole	0.69	1.34
16	Norfloxacin	0.09	2.61	50	Metronidazole	0.76	1.37
17	Ciprofloxacin hydrochloride	0.08	2.18	51	4-Nitroimidazole	0.51	1.10
18	Lomefloxacin	0.04	2.14	52	lpronidazole	0.07	0.91
19	Danofloxain	0.02	2.42	53	Furazolidone	0.15	2.36
20	Enrofloxacin	0.04	2.04	54	Furaltadone	0.09	2.13
21	Sarafloxacin hydrochloride	0.03	2.27	55	Nitrofurantion	0.27	3.49
22	Cinoxacin	0.04	1.94	56	Furacilinum	0.28	4.66
23	19-nor-4-androstene-3,17-dione	0.19	3.71	57	Tetracycline hydrochloride	0.65	1.60
24	1-Dehydrotestosterone sulfate	0.19	2.57	58	Oxytetracycline	0.65	1.60
25	Danazol	0.23	3.51	59	Demeclocycline hydrochloride	0.64	1.80
26	Fluoxymesterone	0.21	4.78	60	Chlorotetrachlie hydrochloride	0.65	1.60
27	Testosterone	0.22	3.05	61	Doxycycline	0.65	2.00
28	17-alpha-methyltestosterone	0.12	3.67	62	Lincocin hydrochloride	0.11	2.37
29	Methadrostenolone	0.20	1.44	63	Clindamycin	0.19	3.26
30	Nandrolone	0.19	2.89	64	Trimethoprin	0.29	1.72
31	19-nor-4-androstene-3,17-dione	0.18	3.83	65	Malachite green oxalate	0.08	0.75
32	Trenbolone	0.23	2.94	66	Leucomalachite green	0.10	1.29
33	Megestrol-17-acetate	0.10	3.02	67	Basic violet 3	0.04	0.68
34	Medroxyprogesterone	0.25	3.17	68	Leucocrystal violet	0.10	1.83

3. Results and Discussion

The 68 veterinary drugs can be categorized into 12 groups. Fig.1 shows the representative calibration curves of sulfonamides. Excellent linearity was demonstrated in the range of 1 to 200 µg/L for sulfadiazine, sulfamethoxypyridazine, sulfamethoxazole and sulfisoxazole, with correlation

coefficients greater than 0.998. The repeatabilities of 68 drugs (1-10 µg/L) were investigated, and the %RSDs of peak area were less than 5%, and those for retention time were better than 0.9%, as shown in Table 2.

Determination of 68 Veterinary Drugs in Marine Products by Ultra High Performance Liquid Chromatography/Triple Quadrupole Mass Spectrometry

Table 3 Recovery test of 52 compounds

	Compound	Recovery (%)		Compound	Recovery (%)
Sulfonamides	Sulfacetamide	88.6	Hormones	19-nor-4-androstene-3,17-dione	106.0
	Sulfadiazine	96.4		1-Dehydrotestosterone Sulfate	99.0
	Sulfathiazole	91.1		Danazol	93.0
	Sulfapyridine	106.3		Fluoxymesterone	113.5
	Sulfamerazine	94.0		Testosterone	106.0
	Sulfamethazine	97.2		17-alpha-methyltestosterone	94.7
	Sulfamethoxyipyridazine	91.9		Methadrolone	90.5
	Sulfchloropyridazine	95.3		Nandrolone	95.0
	Sulfamethoxazole	103.1		19-nor-4-androstene-3,17-dione	80.0
	Sulfisoxazole	102.2		Trenbolone	96.1
	Sulfadimethoxine	94.1		Megestrol-17-acetate	95.0
	Sulfachinoxalin	93.9		Medroxyprogesterone	94.1
	Quinolones	Pipemidic acid		81.0	Medroxyprogesterone-17-acetate
Enoxacin sesquihydrate		68.0	Norgestrel	115.0	
Ofloxacin		88.0	Chloromadinone 17-acetate	110.5	
Norfloxacin		77.0	Norethindrone	111.6	
Ciprofloxacin hydrochloride		95.5	Progesterone	78.0	
Lomefloxacin		92.0	Macrolides	Spiramycin	89.0
Danofloxacin		83.0		leucomycin hydrate	91.0
Enrofloxacin		74.0		Erythromycin	84.0
Sarafloxacin hydrochloride		76.0		Tilmicosin	104.0
Cinoxacin		82.5		Acetylisovalerylytylosin Tartrate	93.0
Tetracyclines	Tetracycline hydrochloride	105.0	Chloramphenicols	Thiamphenicol	105.0
	Oxytetracycline	120.0		Florfenicol	81.5
	Demeclocycline hydrochloride	110.0		Chloramphenicol	93.3
	Chlorotetrachdie hydrochloride	100.0			
	Doxycycline	115.0			

In this study, we studied the different marine products. The recoveries of drugs in fish samples (the concentration of spiked drugs ranged from 0.1 to 2 µg/kg) were summarized

in Table 3. The average recovery range of 52 compounds was from 74 to 120%.

4. Conclusions

A simultaneous and cost-effective method of identification and quantification of 68 veterinary drugs in marine products was developed.

Improved selectivity and sensitivity of the instrumental analysis was achieved by LC/MS/MS technique.