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

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# 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 mmi.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 mmi.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 |

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Table 1 List of veterinary drugs

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

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.

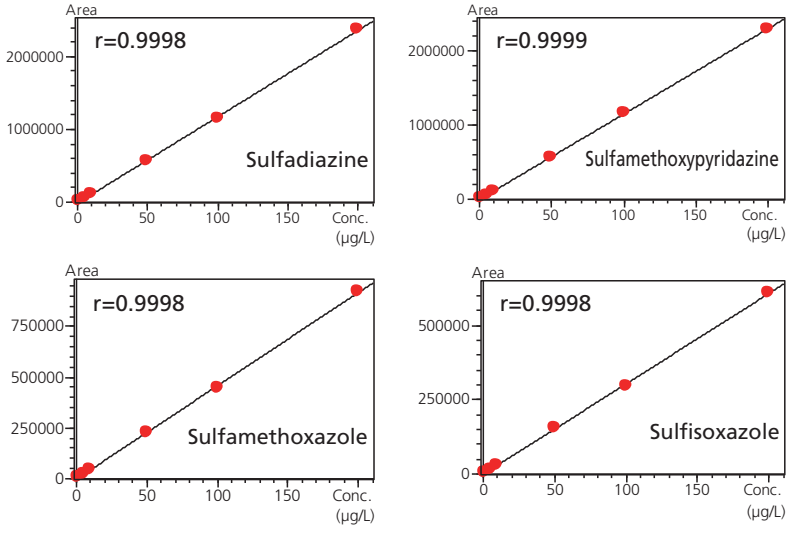


Fig. 1 Representative calibration curves of sulfonamides

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 | Ipronidazole | 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 | Chlorotetrachclie 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.

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Table 3 Recovery test of 52 compounds

| | Compound | Recovery (%) |
|---|---|---|
| Sulfonamides | Sulfacetamide | 88.6 |
| | Sulfadiazine | 96.4 |
| | Sulfathiazole | 91.1 |
| | Sulfapyridine | 106.3 |
| | Sulfamerazine | 94.0 |
| | Sulfamethazine | 97.2 |
| | Sulfamethoxypyridazine | 91.9 |
| | Sulfchloropyridazine | 95.3 |
| | Sulfamethoxazole | 103.1 |
| | Sulfisoxazole | 102.2 |
| | Sulfadimethoxine | 94.1 |
| | Sulfachinoxalin | 93.9 |
| Quinolones | Pipemidic acid | 81.0 |
| | Enoxacin sesquihydrate | 68.0 |
| | Ofloxacin | 88.0 |
| | Norfloxacin | 77.0 |
| | Ciprofloxacin hydrochloride | 95.5 |
| | Lomefloxacin | 92.0 |
| | Danofloxain | 83.0 |
| | Enrofloxacin | 74.0 |
| | Sarafloxacin hydrochloride | 76.0 |
| | Cinoxacin | 82.5 |
| Tetracyclines | Tetracycline hydrochloride | 105.0 |
| | Oxytetracycline | 120.0 |
| | Demeclocycline hydrochloride | 110.0 |
| | Chlorotetrachclie hydrochloride | 100.0 |
| | Doxycycline | 115.0 |

| | Compound | Recovery (%) |
|---|---|---|
| Hormones | 19-nor-4-androstene-3,17-dione | 106.0 |
| | 1-Dehydrotestosterone Sulfate | 99.0 |
| | Danazol | 93.0 |
| | Fluoxymesterone | 113.5 |
| | Testosterone | 106.0 |
| | 17-alpha-methyltestosterone | 94.7 |
| | Methadrostenolone | 90.5 |
| | Nandrolone | 95.0 |
| | 19-nor-4-androstene-3,17-dione | 80.0 |
| | Trenbolone | 96.1 |
| | Megestrol-17-acetate | 95.0 |
| | Medroxyprogesterone | 94.1 |
| | Medroxyprogesterone-17-acetate | 114.5 |
| | Norgestrel | 115.0 |
| | Chloromadinone 17-acetate | 110.5 |
| | Norethindrone | 111.6 |
| | Progesterone | 78.0 |
| Macrolides | Spiramycin | 89.0 |
| | leucomycin hydrate | 91.0 |
| | Erythromycin | 84.0 |
| | Tilmicosin | 104.0 |
| | Acetylisovaleryltylosin Tartrate | 93.0 |
| Chloramphenicols | Thiamphenicol | 105.0 |
| | Florfenicol | 81.5 |
| | Chloramphenicol | 93.3 |

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.

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