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Determination of Multiclass Harmful Residues in Shellfish Using LC-MS

Xiaoming Gong^{a,*}, Ronghui Ma^a, Kai Li^a, Hongwei Zhang^b, Zhenxing Wu^b, Hongtao Wang^a, Jun Sun^a, Han Zhao^a

^aWeifang Entry-Exit Inspection and Quarantine Bureau of the Peoples Republic of China, Weifang, 261041 Shangdong, China ^bShandong Entry-Exit Inspection and Quarantine Bureau of the Peoples Republic of China, Qingdao, 266001 Shangdong, China

Abstract

A multi-residue procedure with liquid chromatography-tandem mass spectrometry analysis was developed to simultaneously detect the presence of 4 diarrheic shellfish poisoning toxins, 14 sulfonamides, chloramphenicol and thiamphenicol in shellfish tissues. Samples were first purified by a modified rapid procedure and separated on a ZOBRAX Eclipse Plus C18 column and quantified using electron spray ionizationmass spectrometry-mass spectrometry. The results showed that the limits of quantification (LOQ) of SPX1, OA-C, GYM, and PTX-2 were 1.0, 2, 0.5 and 2 μ g/kg, respectively. The LOQ for the antibiotics was below acceptable limits established by most countries. Compared to the external standard method, this matrix matched the calibration method effectively, overcame the matrix effects and gave better quantitative results. Recoveries of spiked compounds ranged between 67.6% and 109.8%, with relative standard deviations below 15% for most target analytes. Only sulfathiazole had a %RSD of 18.6% at the lowest spiked concentration. The proposed method was accurate, rapid and reliable.

Keywords: LC-MS/MS, diarrheic shellfish poisoning toxins, multiclass residues, matrix effects

1. Introduction

Shellfish such as oysters are filter feeders and continuously sample suspended particles in search for food. Due to this filtration mechanism, environmental pollutants such as heavy metals, pesticides and veterinary drugs can accumulate and persist in shellfish tissues. Human ingestion of contaminated shellfish poses a significant health risk especially since shellfish are now important contributors to many coastal economies.

In addition to environmental pollutants, a group of potent diarrheal shellfish poisoning (DSP) toxins produced by dinoflagellates and cyanobacteria have been identified in shellfish tissues. The most potent members of this group are the okadaic acid (OA) group and its analogues dinophysistoxins-1 and -2 (DTX1, DTX2) [3]. The DSP toxins cause gastrointestinal disease with symptoms including nausea, diarrhea, vomiting, and abdominal pain [14, 6].

A common method that is routinely used for DSP toxin detection is the mouse bioassay (MBA) [11], which is also the current reference method employed in the European Union (Regulation 2074/2005). There are many drawbacks to the MBA test, including ethical concerns, a lack of specificity and its expense [12]. LC-MS has been used successfully to identify DSP toxins and has become an effective and viable alternative to the MBA [10]. LC-MS has been widely used for many environmental pollutants that are important to the shellfish industry, including multi-residue and simultaneous analysis of pesticides, veterinary drugs, and antibiotics [2, 4, 5, 13].

One barrier to the use of LC-MS detection has been quantitative analyte extraction from shellfish tissues. Due to recent advances in fatty acid extraction techniques for pesticide analysis, we adopted octadecylsilane (ODS) as a sorbent to purify shellfish samples [8].

The focus of the present research was the development of a multiclass liquid chromatography-tandem mass spectrometry method for the qualitative detection of OA-C, SPX1, GYM, PTX-2, chloramphenicol, thiamphenicol and 14 sulfonamides (chemical structures are shown in Supplemental Figure A.1) in shellfish.

2. Materials and Methods

2.1. Chemicals and Materials

HPLC-grade acetonitrile, methanol, hexane and formic acid were purchased from Sigma-Aldrich (Germany). Water was obtained from a Milli-Q purification system (Millipore Corp., Milford, MA, USA). All antibiotics were purchased from Sigma-Aldrich (Germany). GYM, SPX1, OA, and PTX2 were purchased from the National Research Council of Canada (NRC).

^{*}Corresponding author: Xiaoming Gong, Phone: +86 536 8582599. Email: 9647125@qq.com

Analyte	Ll	L2	L3	L4	L5	L6
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
GYM	1.0	2.0	3.0	4.0	5.0	10.0
SPX1	2.0	5.0	10.0	15.0	20.0	30.0
PTX2	10.0	15.0	20.0	30.0	40.0	50.0
OA-C	7.1	14.2	28.4	42.6	56.8	71.0
Sulfadiazine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfathiazole	5.0	10.0	20.0	30.0	40.0	50.0
Sulfapyridine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamerazine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamethazine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfameter	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamethizole	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamethoxypyridazine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfachloropyridazine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamonomethoxine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfamethoxazole	5.0	10.0	20.0	30.0	40.0	50.0
Sulfadimethoxine	5.0	10.0	20.0	30.0	40.0	50.0
Sulfaquinoxaline	5.0	10.0	20.0	30.0	40.0	50.0
sulfacetamide	5.0	10.0	20.0	30.0	40.0	50.0
Chloramphenicol	0.5	1.0	2.0	3.0	4.0	5.0
Thiamphenicol	0.5	1.0	2.0	3.0	4.0	5.0

Figure 1: Concentration of Standards.

Ameliate	Precursor ion Product ion		DP	CE	Ionization
Anaiyte	m/z	m/z	U/V	E/eV	mode
GYM	508.3	490.3*,174.1	100	35,60	ESI^+
SPX1	692.5	674.3*,444.3	100	40,50	ESI^+
PTX-2	876.0	823.2*,212.8	100	40,50	ESI^+
OA-C	803.3	255.0*,563.1	-120	-65, -55	ESI-
Sulfadiazine	251.1	155.8*,184.9	51	21,31	ESI^+
Sulfathiazloe	256.1	156.1*,107.9	26	19,31	ESI^+
Sulfapyridine	250.1	156.1*,107.9	70	21,33	ESI^+
Sulfamerazine	265.1	156.0*,110.1	65	23,31	ESI^+
Sulfameter	281.1	155.8*,107.9	31	23,35	ESI^+
Sulfamethizole	271.1	156.0*,108.1	106	19,35	ESI^+
Sulfamethoxypyridazine	281.1	155.8*,107.9	46	25,37	ESI^+
Sulfachloropyridazine	286.2	156.9*108.1	46	19,35	ESI^+
Sulfamonomethoxine	281.1	155.8*,107.9	26	23,35	ESI^+
Sulfamethoxazole	254.1	156.0*,107.9	81	21,33	ESI^+
Sulfadimethoxine	311.1	156.0*,107.9	126	27,43	ESI^+
Sulfaquinoxaline	301.1	156.0*,107.9	121	23,37	ESI^+
Sulfacetamide	215.1	156.0*,107.9	86	15,25	ESI^+
Sulfamethazine	279.2	186.0*,107.9	70	23,39	ESI^+
Chloramphenicol	321.0	152.0*,257.0	-120	-18, -30	ESI-
Thiamphenicol	356.0	185.0*,336.0	-120	-30, -40	ESI-

* Quantification ion

Figure 2: MS Parameters of Analytes.

The chemical structures of analytes in the present study are shown in Supplemental Figure A.1. NaCl, Al_2O_3 , primary secondary amine (PSA), graphitized carbon black (GCB), and ODS were obtained from Agela Technologies (Beijing, China).

2.2. Stock Standard Solution Preparation

All antibiotic stocks were made to 100 μ g/mL in MeOH. The tested antibiotics were chloramphenicol, thiamphenicol, sulfadiazine, sulfathiazole, sulfasalazine, sulfamerazine,



Figure 3: Extraction efficiency of four types of extraction solvents.

sulfamethoxydiazine, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, sulfaquinoxaline, sulfamethazine, and sulfacetamide. Working solutions were directly diluted from the stock solutions immediately before using. Shellfish toxin working-solutions were also freshly diluted to appropriate concentration from the original source (Figure 1).

2.3. Sample Preparation

Shellfish tissues were chopped and homogenized immediately after being removed from the freezer. Two gram samples were placed in a polypropylene centrifuge tubes (15 mL) containing 6 mL methanol. The tube was first vortexed for 1 min, then sonicated for 10 min, followed by a 5-min centrifugation (10,000 RPM). The supernatant was then carefully transferred to another tube. Extraction steps were repeated twice, and all the supernatants were combined into a 10-mL glass tube.

The supernatant was concentrated to approximately 1 mL under a gentle stream of nitrogen at 40°C. Two mL of hexane was then added followed by vortexing for 2 min. The remaining hexane layer was removed and the steps were repeated. The aqueous layer was then extracted twice with 2 mL of ethyl acetate as above. Three hundred mg of ODS sorbent was added to the aqueous mixture and vortexed at 2,000 r/min for 2 min. Two mL of the top layer were transferred to a new tube and concentrated to a nearly dry state. Finally, the residue was dissolved in 0.5 mL initial mobile phase (methanol/water 3/7 (v/v)/ v = 3:7) then filtered through a 0.22 μ m PTFE filter for LC-MS/MS analysis. See Supplemental Figure A.2 for an example of this process.

2.4. Liquid Chromatogrophy Analysis

An Agilent 1200 HPLC system with a Zorbax Eclipse Plus C18 (1.8 μ m, 2.1 mm 100 mm, Agilent, USA) column was used for sample separation. The mobile phase A was 0.1% formic acid, mobile phase B was acetonitrile, and mobile phase

C was methanol. The flow rate was 0.3 mL/min and the injection volume was 10 μ L. The column temperature was maintained at 35°C. Analytes were eluted using the following gradient: (duration in minutes; % A/B/C, respectively), Step 1: 2, 60/20/20; Step 2: 8, 35/45/20; Step 3: 9, 5/75/20; Step 4: 12, 5/75/20; Step 5: 12.5, 85/10/5; Step. 6: 16, 85/10/5.

2.5. MS/MS Analysis

An AB Qtrap 5500 mass spectrometer (AB SCIEX, USA) equipped with an electrospray ionization (ESI) interface system was operated in multiple reaction monitoring (MRM) mode. The mass spectrometer ion source parameters were: spray voltage 5500 Kv and capillary temperature 500°C. MS acquisition parameters are listed in Figure 2.

3. Discussion

3.1. Optimization of Extraction Method

In this study, four solvent systems – methanol, acetonitrile, 80% methanol, and ethyl acetate – were tested for the extraction of 20 different kinds of contamination compounds found in shellfish. The results indicated that the methanol solvent system had the highest extraction rate (Figure 3) of GYM, OA, DTX-1, and PTX-2 among the four solvent systems. The methanol and acetonitrile solvent systems had similar extraction rates for the other analytes. Here, we chose pure methanol as the extraction solvent.

3.2. Optomization of Purification Method

Shellfish tissues normally contain a large amount of proteins, fats, and pigments, which may have a considerable effect on the quality of the LC-MS results obtained. Hexane is an efficient fat-soluble solvent and is routinely used to remove fats and other impurities from animal-derived food. In this study, two hexane extractions were employed to eliminate most fats from shellfish tissues. In the liquid-liquid extraction step,



(c) Chromatograms of 1 sulfamethoxypyridazine, 2 sulfamonomethoxine, and 3 sulfameter in different mobile phase. A) acetonitrile system; B) methanol system; and C) acetonitrile-methanol system.

(d) Chromatograms of sulfapyridine in different mobile phase. A) methanol system; B) acetonitrile system; and C) acetonitrile-methanol system.

Figure 4: Chromatograms.

dichloromethane and ethyl acetate were used as the extraction solvents. The results indicated that both solvents were able to collect toxins from water and simultaneously remove fats. Ethyl acetate was more efficient than dichloromethane and we chose ethyl acetate as the extraction solvent.

So far, many detection methods have proved to be efficient in removing the "matrix effect" from shellfish tissues. Methods using C18, Oasis HLB, MCX, and Strata TM-X are commonly employed. However, these methods are effective for removal of only one or a few toxins and are unreliable for multi-hazardous component analysis. To address this issue we chose a dispersive solid-phase extraction method. We tested three different sorbents (ODS, Alumina-N, and PSA) for the purification of analytes from shellfish tissues. Standards were dissolved in ethyl acetate and 150 mg of three the different sorbents were added and vortexed for 1 min. The supernatant was filtered through a $0.22 \,\mu m$ PTFE filter for LC-MS/MS analysis. This experiment was repeated six times and the recoveries are shown in Figure 5. Compared with the other two sorbents, we found that ODS had the highest impurity absorption capacity and possessed the lowest target analyte affinity (Supplemental Figure A.2).

3.3. Selection of LC Columns

The selection of LC columns with high separation efficiency is a prerequisite for a successful analysis. Therefore, two reverse phase LC columns with different carbon chain lengths C18 and C8 were tested for their separation efficiencies. The results of these experiments indicated that GYM, SPX-1, PTX-2, sulfamerazine, and sulfamethazine showed a better separation on the C-18 column although there were no great differences between the two columns for the remainder of the compounds (Figure 4(a)). Therefore, the Eclipse Plus C18 1.8μ m 3.0×100 mm was selected as the separation column.

3.4. Selection of Mobile Phase

The composition of the mobile phase will affect target compound response signals when using mass spectrum detection. In this study, methanol-water and acetonitrile-water systems were developed as the mobile phases for the separation of 20 compounds. As shown in Figure 4(b), GYM, SPX-1, PTX-2, and sulfamethazine, sulfamethoxazole, sulfacetamide, sulfamethoxazole, sulfamethazine and sulfachloropyridazine with acetonitrile-water elution system provided a better separation than did the methanol-water system. Sulfamethizole, sulfapyridine and sulfaquinoxaline worked better with the methanolwater elution system. The remainder of the compounds showed similar responses in both solvent systems.

Although compounds with an acetonitrile mobile phase eluted faster than with methanol, the acetonitrile-water system was not satisfactory for all the analytes. Sulfamethoxypyridazine, sulfamonomethoxine and sulfameter were, for example, difficult to identify because they shared the same molecular weight and ion fragments. Prolonging the retention time may solve this problem. Sulfamethoxypyridazine and sulfamonomethoxine could not be separated using the acetonitrileformic acid system but were resolved using methanol-formic acid (Figure 4(c)). For sulfapyridine, the methanol elution system offered better chromatographic intensity and shape than when using methanol and methanol-acetonitrile system (Figure 4(d)).

The C18 column along with the methanol-acetonitrile elution system was chosen to be the HPLC separation method. This method was able to provide higher response values and better LC peaks while effectively avoiding impurities (Supplemental Figure A.3).

3.5. Matrix Effects

In LC-MS/MS analysis, matrix refers to components of a sample other than the analyte of interest. The matrix can have a considerable effect on the way the analysis is conducted and the quality of the results obtained; such effects are called matrix effects (ME). These effects may significantly affect reproducibility and linearity of calibration curves, leading to inaccurate results.

To analyze ME we used a blank sample as the matrix and processed it using the purification steps from Section 2.3, above. The sample was then made to a constant volume using a standard solution. The ME value can be calculated with equation (1): ME=Ap/As x 100% where Ap represents the sample peak area and As represents the peak area of the standard containing the same concentration as the sample solution.

As shown in Figure 6, ME values of 20 compounds are listed. Matrix effects were inconspicuous when ME values ranged from 80% to 120%, while positive matrix effects were observed when ME values were above 120%. Negative matrix effects can be found when the ME values are below 80% which indicates that the signal of targeting compound was inhibited.

From the data in Figure 6, only sulfachloropyridazine had minimal matrix effects although chloramphenicol and thiamphenicol were similar. Four saxitoxins expressed negative matrix effects, with OA-C showing the lowest ME value, 27.5 - 31.7. Recoveries ranged from 21.0%-78.0% indicating ME as the most probable cause.

With regard to the detrimental effects that ion suppression/enhancement may have on important method performance parameters, they must be prevented wherever possible. Matrix effects that may arise from the endogenous compounds extracted from the sample matrix can usually be eliminated in several ways. Considering extraction and purification, liquidliquid extraction is used to remove big macromolecules such as protein and fat, while QuEChERS method is created to get rid of micromolecules. Considering quantitative analysis, internal standard method and standard spiking method are normally used for minimizing matrix effects. In this experiment, none suitable internal standard can be found, only standard spiking method matching the situation.

The matrix matching standard correction method effectively eliminate matrix effects. Recoveries were increased to 67.6% -109.8% when using this method. Therefore, matrix-matching should be employed for multi-residue analysis of shellfish contaminants.

C1		Recoveries (%)
Compound	ODS	Alumina-N	PSA
GYM	90.0	58.0	80.0
SPX1	91.0	66.2	96.0
PTX2	93.0	56.5	105.0
OA-C	93.0	89.1	23.0
sulfadiazine	114.0	22.3	31.2
sulfathiazole	105.0	6.71	3.65
sulfapyridine	113.0	81.3	80.3
sulfamerazine	111.0	28.6	24.1
sulfamethazine	113.0	63.7	78.8
sulfameter	105.0	46.3	48.3
sulfamethizole	138.0	1.32	1.45
sulfamethoxypyridazine	112.0	48.7	50.3
sulfachloropyridazine	121.0	3.76	4.68
sulfamonomethoxine	112.0	48.7	50.3
sulfamethoxazole	114.0	19.4	20.3
sulfadimethoxine	117.0	19.1	21.1
sulfaquinoxaline	124.0	9.44	10.8
sulfacetamide	97.1	7.69	10.8
Chloramphenicol	93.1	32.4	83.7
Thiamphenicol	86.6	84.6	87.3

Figure 5: Recovery of 20 Analytes Adsorbed by ODS, Alumina-N and PSA.

	Concentration of Spikes ug/kg	Recoveries	Recoveries		
Analyte	and Working Solutions	(Matrix correction)	(Standards)	RSD %	ME%
	(ng/mL)				
Sulfadiazine	Ll, L3, L5	83.5, 87.6, 89.9	50.6, 52.4, 56.4	12.3, 9.6, 12.,2	60.6, 59.8, 62.7
Sulfathiazole	L1, L3, L5	67.6, 70.2, 70.8	51.5, 52.8, 55.6	18.6, 10.5, 11.2	76.2, 75.2, 78.5
Sulfapyridine	L1, L3, L5	75.5, 76.3, 79.8	30.9, 33.2, 35.8	10.3, 7.9, 6.8	40.9, 43.5, 44.9
Sulfamerazine	Ll, L3, L5	83.6, 86.9, 88.2,	52.4, 55.6, 57.9	11.5, 9.7, 10.4	62.7, 64.0, 65.6
Sulfamethazine	L1, L3, L5	77.2, 78.7, 82.0	48.2, 50.1, 52.4	12.3, 11.5, 11.1	62.4, 63.7, 63.9
Sulfameter	L1, L3, L5	96.4, 98.6, 100.2	48.2, 50.6, 52.4	12.9, 11.7, 11.3	50.0, 51.3, 52.3
Sulfamethizole	L1, L3, L5	93.4, 95.8, 97.7	71.2, 73.7, 75.8	11.7, 12.9, 14.5	76.2, 76.9, 77.6
Sulfamethoxypyridazine	L1, L3, L5	80.7, 82.4, 85.1	50.6, 52.4, 57.6	8.4, 9.5, 10.8	62.7, 63.6, 67.7
Sulfachloropyridazine	Ll, L3, L5	80.2, 83.6, 86.4	68.0, 69.5, 70.8	11.5, 10.9, 9.7	84.8, 83.1, 81.9
Sulfamonomethoxine	Ll, L3, L5	76.8, 77.4, 79.5	48.2, 49.5, 51.2	12.5, 10.8, 11.8	62.8, 64.0, 64.4
Sulfamethoxazole	Ll, L3, L5	68.3, 70.1, 74.6	50.7, 53.8, 55.4	8.5, 11.6, 9.4	74.2, 76.7, 74.3
Sulfadimethoxine	Ll, L3, L5	105.3, 108.0,109.8	63.2, 64.9, 65.2	13.5, 11.1, 10.7	60.0, 60.1, 59.4
Sulfaquinoxaline	Ll, L3, L5	67.4, 69.1, 70.5	47.8, 48.1, 51.7	10.4, 8.5, 7.9	70.9, 69.6, 73.3
sulfacetamide	L1, L3, L5	77.1, 75.5, 71.8	36.1, 34.5, 31.0	13.2, 12.5, 12.8	46.8, 45.7, 43.2
Chloramphenicol	L1, L3, L5	97.5, 100.2, 102.4	72.8, 75.2, 78.0	13.8, 12.9, 13.4	74.7, 75.0, 76.2
Thiamphenicol	L1, L3, L5	94.7, 97.5, 100.1	68.2, 71.2, 74.4	8.9, 10.1, 12.7	72.0, 73.0, 74.3
GYM	L1, L3, L5	73.9, 76.4, 79.9	38.8, 40.9, 44.1	14.1, 13.7, 12.0	52.5, 53.5, 55.2
SPX1	L1, L3, L5	77.4, 80.5, 83.1	37.4, 40.6, 42.1	12.6, 11.9, 11.5	48.3, 50.4, 50.7
PTX2	L1, L3, L5	80.2, 83.5, 86.0	44.7, 46.5, 48.4	12.7, 11.9, 12.8	55.7, 55.9, 56.3
OA-C	L1, L3, L5	76.5, 79.2, 83.2	21.0, 23.5, 26.4	12.6, 7.8, 11.6	27.5, 29.7, 31.7

Figure 6: Recovery and relative standard deviations of 20 analytes.

3.6. Calibration and Method Validation

Calibration curves of matrix-matched standards and solvent standards in the range 0.5 - 50 ng/mL for all 20 compounds at six calibration levels were produced by calculating the ratio of the quantitative ion chromatographic peak area to the chromatographic peak area of the internal standard as vertical coordinate (Y), and concentration (ng/mL) as horizontal coordinate (X). This analysis indicated that the curves were linear with correlation coefficients ≥ 0.998 .

The limits of quantification (LOQs) were estimated on the basis of 10/1 signal-to-noise ratios obtained using the lowest spiked sample level. LOQ values for the toxins SPX1, OA-C, GYM, and PTX-2 were 1.0, 2, 0.5 and 2 μ g/kg, respectively (data not shown). The LOQs of sulfadiazine, sulfathiazole, and sulfameter were 0.2 μ g/kg. LOQ of sulfamethoxypyridazine, sulfapyridine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, sulfaquinoxaline, and sulfamethazine was 0.1 μ g/kg. LOQ of sulfamerazine, sulfachloropyridazine, and sulfacetamide was 1 μ g/kg. LOQ of sulfamethizole was 0.01 μ g/kg. These LOQs were far lower than the limits of most countries, including Japan and the EU [7, 1, 9].

The accuracy and precision of the methods were evaluated by analyzing analyte recoveries using three spike concentrations in shellfish homogenates, and repeating this procedure seven times. Analyte recoveries ranged between 67.6% and 109.8% and were significant, with standard deviations between 7.8% and 15.0% (only Sulfathiazole had a %RSD of 18.6% at the lowest concentration of spike) (Figure 6).

4. Conclusion

Commercially available liquid-liquid extractions using a modified QuEChERS method has been established to remove most fats, proteins and pigments from shellfish homogenates. The results indicate that the selection of columns, mobile phases and QuEChERS sorbents are necessary for optimal LC-MS results. The pre-treatment method combined with LC-MS/MS technology has proved to be precise, accurate, and applicable to the routine analysis of multiclass residues in shellfish.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest. Research was funded by Shandong Entry-Exit Inspection and Quarantine Bureau of the Peoples Republic of China.

6. Disclaimer

The views expressed are those of the authors and should not be construed to represent the views or policies of the Weifang Entry-Exit Inspection and Quarantine Bureau. Any reference to a specific commercial product, manufacturer, or otherwise, is for the information and convenience of the public and does not constitute an endorsement, recommendation or favoring by the Weifang Entry-Exit Inspection and Quarantine Bureau.

7. Article Information

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8. References

- Amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the permitted limits of yessotoxins in live bivalve molluses Text with EEA relevance, (EU) No 786/2013 (2013).
- [2] Bittencourt, M. S., Martins, M. T., de Albuquerque, F. G. S., Barreto, F., & Hoff, R. (2012). High-throughput multiclass screening method for antibiotic residue analysis in meat using liquid chromatography-tandem mass spectrometry: a novel minimum sample preparation procedure. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment, 29(4),* 508-516.
- [3] Fusetani, N., & Kem, W. (2009). Marine toxins: an overview. Progress in Molecular and Subcellular Biology, 46, 1-44.
- [4] Fux, E., McMillan, D., Bire, R., & Hess, P. (2007). Development of an ultra-performance liquid chromatography-mass spectrometry method for the detection of lipophilic marine toxins. *Journal of Chromatogrophy A*, 1157(1-2), 273-280.
- [5] Gerssen, A., Mulder, P. P., McElhinney, M. A., & de Boer, J. (2009). Liquid chromatography-tandem mass spectrometry method for the detection of marine lipophilic toxins under alkaline conditions. *Journal of Chromatogrophy A*, 1216(9), 1421-1430.
- [6] James, K. J., Lehane, M., Moroney, C., Fernandez-Puente, P., Satake, M., Yasumoto, T., & Furey, A. (2002). Azaspiracid shellfish poisoning: unusual toxin dynamics in shellfish and the increased risk of acute human intoxications. *Food Additives and Contaminants*, 19(6), 555-561.
- [7] Laying Down Specic Hygiene Rules for Food of Animal Origin, (EC) No 853/2004 (2004).
- [8] Lehotay, S. J. (2011). QuEChERS sample preparation approach for mass spectrometric analysis of pesticide residues in foods. *Methods in Molecular Biology*, 747, 65-91.
- [9] Pharmacologically Active Substances and Their Classification Regarding Maximum Residue Limits in Foodstuffs of Animal Origin, (EU) No 37/2010 (2009).
- [10] Quilliam, M. A. (2003). The role of chromatography in the hunt for red tide toxins. *Journal of Chromatography A*, 1000(1-2), 527-548.
- [11] Suzuki, H. (2013). Differences in Susceptibility to Okadaic Acid, a Diarrhetic Shellfish Poisoning Toxin, between Male and Female Mice. *Toxins*, 5(1), 9-15.
- [12] Suzuki, T., & Quilliam, M. A. (2011). LC-MS/MS Analysis of Diarrhetic Shellfish Poisoning (DSP) Toxins, Okadaic Acid and Dinophysistoxin Analogues, and Other Lipophilic Toxins. *Analytical Sciences*, 27(6), 571-584.
- [13] Tian, W. L., Gao, L. Y., Zhao, Y. Z., Peng, W. J., & Chen, Z. Z. (2013). Simultaneous determination of metronidazole, chloramphenicol and 10 sulfonamide residues in honey by LC-MS/MS. *Analytical Methods*, 5(5), 1283-1288.
- [14] Yasumoto, T., Oshima, Y., & Yamaguchi, M. (1978). Occurrence of a New Type of Shellfish Poisoning in the Tohoku District. *Nippon Suisan Gakkaishi*, 44(11), 1249-1255.

9. Supplemental Materials



Figure A.1: Chemical structures of analysts in the present study: 1 sulfadiazine, 2 sulfathiazole, 3 sulfapyridine,
4 sulfamerazine, 5 sulfamethazine, 6 sulfameter, 7 sulfamethizole, 8 sulfamethoxypyridazine, 9 sulfachloropyridazine,
10 sulfamonomethoxine, 11 sulfamethoxazole, 12 sulfadimethoxine, 13 sulfaquinoxaline, 14 sulfacetamide,
15 chloramphenicol, 16 thiamphenicol, 17 OA-C, 18 SPX1, 19 PTX2, and 20 GYM.



Figure A.2: Pictures of shellfish sample which was processed with nitrogen evaporation (A); hexane cleanup (B); ethyl acetate abstraction (C) and ODS purification (D).

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1.11 1.00	.2.24 .2.93	,4.28,4.77,5.27 ,5	.87						
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+ MRM (34 pairs): 271	1.088/156.000 D a I D	: sulfam ethizole i	from Sample 1	r (std) of	Time, min 20130717SET1.wi	lf(Turbo Sp	(29)	Max. 1.	4e0 ops.
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+MRM (34 pairs): 250	5.063/156.100 Dalb	: sulfathiazole fre	m Sample 7 (std) of 2	0130717SET1.witt	(Turbo Spra	0	Max. 6.	8 e5 ops.
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1					1				
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I+MRM (34 pairs): 301	1.121/156.000 D a I D	: su Haq uin oxalini	e from Sampk	17 (std)	of 20 1307 17 SET 1.4	vill (Turbo S	ipray)	Max. 3.	4e5 ops.
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rf + M R M (34 pairs): 280	5.189/156.900 D a I D	: sulfach loropyrid	azine from S.	ample 7 1 8.11	T im e, min s16) of 20 1307 17 Si	ET1.wiff (Tu	rbo Spray)	Max. 6.	
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+ MRM (34 pairs): 280	2.109/155.000 D = 10 3.30 4/0 1.111/155.800 D = 10 3.0 4/0 0.000/823.200 B = 10	sultach lorogyrid 5.0 6.0 6.0 sultam ater from 6.0 6.0 PTX-2 from Sar	azine from S. 1 7.0 Sample 7 (std) o nple 7 (std) o	8.08 8.08 1201307	Time, min 149 of 20 1007 17 5 100 Time, min 200 100 Time, min 200 17 5E Tiwett (Tubo	ET1.wiff (Tu 11.0 urbo Spray) 11.0 Spray)	(bo Spray)	Max, 6,	15.0 1e8 cps 15.0 3e4 ops 15.40
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Figure A.3: LC-MS chromatograms of twenty analysis standards and recovery acquired with positive (SPX1, GYM, PTX-2, sulfadiazine, sulfathiazole, sulfasalazine, sulfamerazine, sulfamethoxydiazine, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, sulfaquinoxaline, sulfamethazine and sulfacetamide) and negative (OA-C, chloramphenicol, florfenicol). A. Chromatograms of standards dissolved in mobile phase at the concentraion of L3 level; B. Chromatograms of standards dissolved in matrix extractions at the concentraion of L3 level; and C. Chromatograms of a spiked sample at the concentration of L3 level.