Application Brief

Veterinary Drugs

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A Fast Sample Preparation Workflow for Veterinary Drugs Analysis in Salmon

Using Agilent Captiva EMR—Lipid and LC/MS/MS

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Introduction

A simple, fast, robust sample preparation workflow and analytical method were developed for the analysis of seven classes of 53 drugs in salmon. These drugs are widely used and inspected in Europe, the United States, Canada, and Asian countries.[1] A one-step solvent extraction followed by pass-through cleanup with Agilent Captiva EMR—Lipid was used for the analysis of multiple veterinary drugs, resulting in satisfactory recovery and precision.

Equipment and materials

- Agilent Captiva EMR—Lipid cartridge, 3 mL, 300 mg (part number 5190-1003)
- SPEX sample preparation 2010 Geno/Grinder (Metuchen, NJ, USA)
- Agilent Vac Elut 20 Manifold with collection rack for 13 × 100 mm test tubes (part number 12234101)
- Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (part number 695975-302).
- Agilent 1290 Infinity LC
- Agilent 6495B triple quadrupole LC/MS system with an Agilent Jet Stream Electrospray ionization source

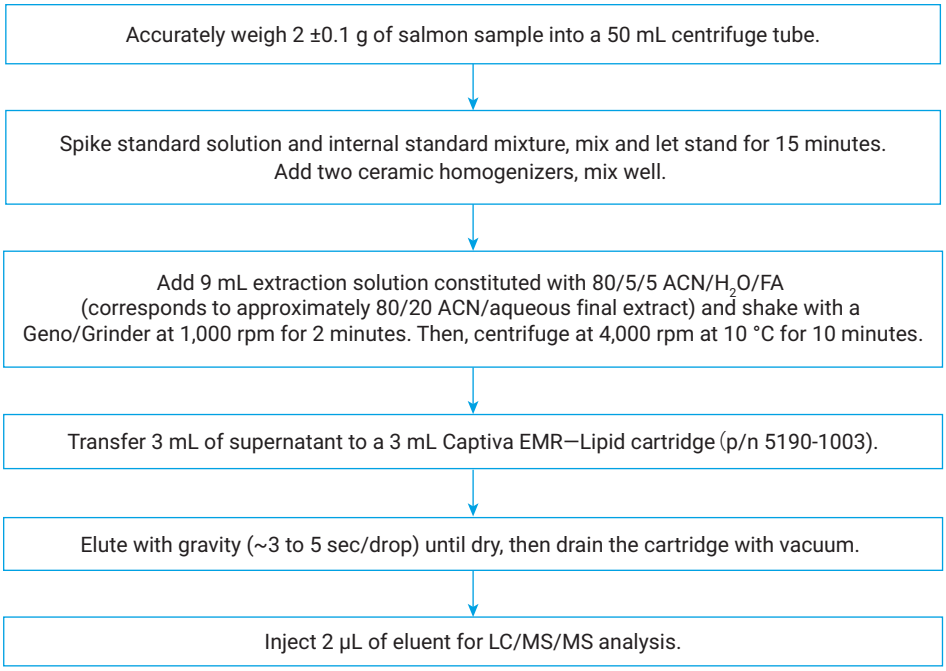

Figure 1. Salmon sample extraction and cleanup using an Agilent Captiva EMR—Lipid 3 mL cartridge (part number 5190-1003).

Sample preparation and optimization

Since salmon contains high levels of water, 9 mL of 80/5/5 acetonitrile (ACN)/H_2O/formic acid (FA), corresponding to approximately 80/20 ACN/water final extract, were added for veterinary drug extraction. Three extraction solvents (H_2O with 2% FA, 5% FA, and without FA) were investigated. The extraction solvent with 5% FA achieved the best extractability and recoveries (Figure 1).

Method validation

The solvent calibration standards for the standard curve were prepared at 0.1, 0.5, 1, 5, 10, 20, 50, and 100 ng/g. The internal standard mixture, which included malachite green-D_3, leucomalachite green-D_4, and chloramphenicol-D_6 was spiked at the level of 10 ng/g for selective calibration of the corresponding analytes. A matrix-matched calibration curve was prepared by spiking standard solution into the matrix blank, which was processed with the same sample preparation workflow.

The drugs were divided into three groups based on the limit of quantitation and linearity range. Prespiked QC samples were fortified by spiking the appropriate standard working solution into the homogenized salmon samples with six replicates at low, mid, and high levels for group 1 (5 ppb/10 ppb/50 ppb), group 2 (1 ppb/10 ppb/50 ppb), and group 3 (1 ppb/5 ppb/50 ppb).

Linearity

The data were processed with Agilent MassHunter quantification software. A calibration curve gave R^2 values between 0.993 and 0.999 for the veterinary drugs using linear regression fit and 1/x weighting.

Accuracy and precision results

As shown in Figure 3, the one-step solvent extraction with 5% FA and 80% ACN followed by Captiva EMR—Lipid pass-through cleanup, was verified by running spiked samples at three QC levels. The recoveries for this method were 60 to 120%. For 53 investigated veterinary drugs, 96% of the recovery data points, in three QC levels, were located between 70 to 120%, and only 4% of the data points were located between 60 to 70%.

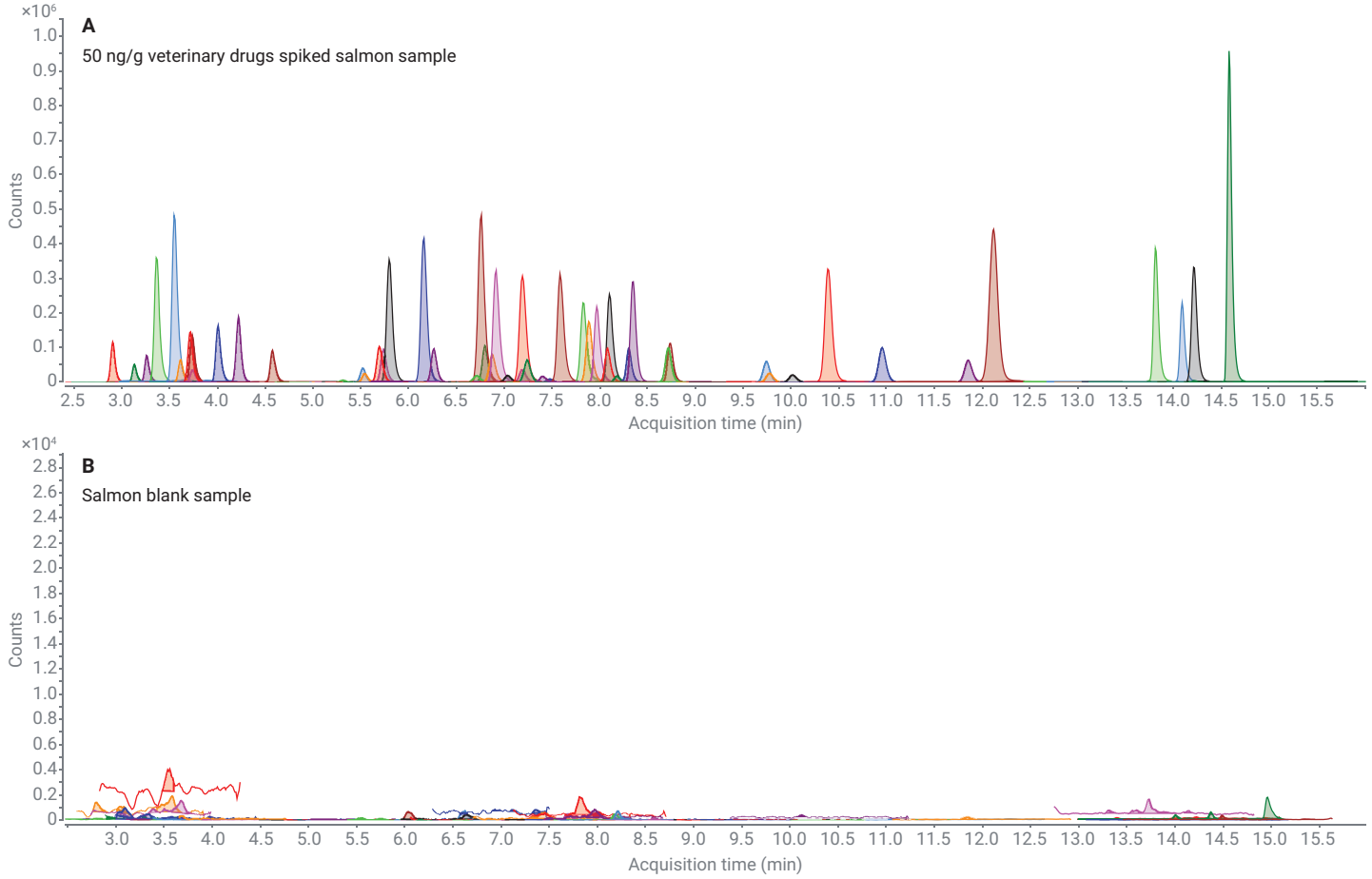

Figure 2. LC/MS/MS chromatograms for salmon extract fortified with 50 ng/g veterinary drug standards (A) and salmon extract matrix blank (B).

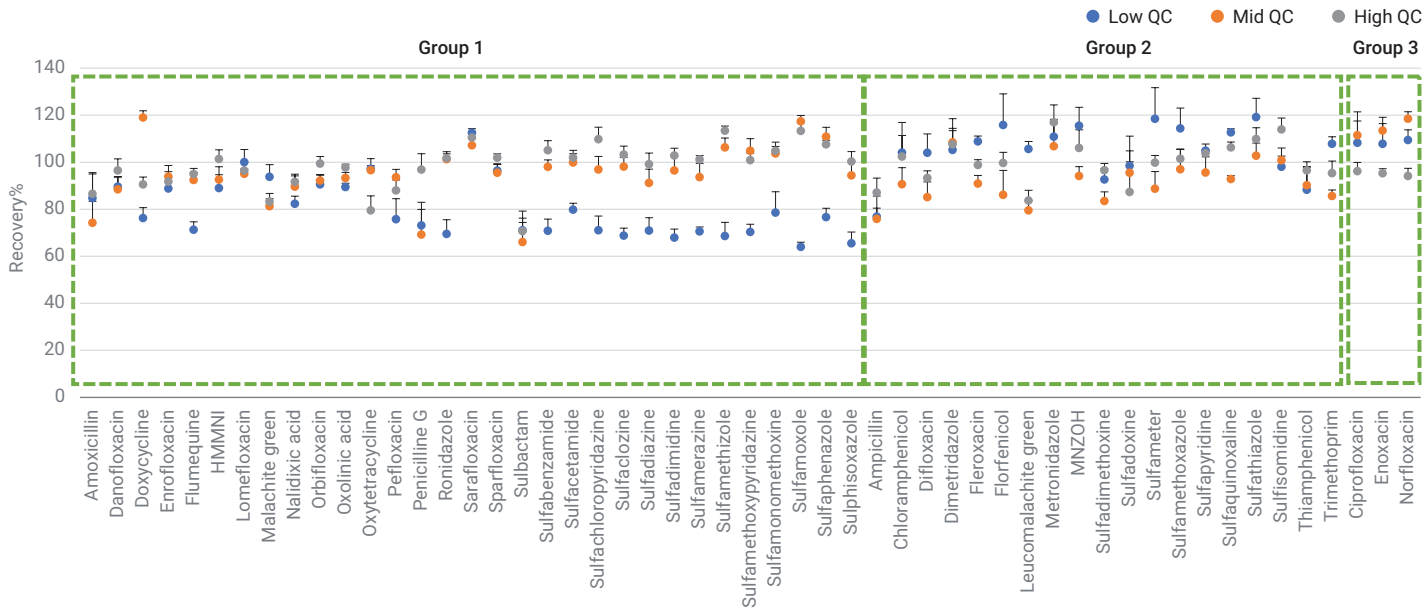

Figure 3. The recovery and precision data at three QC levels for the 53 investigated veterinary drugs in salmon.

Conclusion

This work demonstrates an easy, fast, and robust sample preparation workflow for the analysis of multiple veterinary drugs in salmon. The workflow only contains two steps: a one-step ACN solvent extraction with 5% FA, and a gravity pass-through cleanup with Agilent Captiva EMR—Lipid. This method provides acceptable recoveries and RSDs for commonly tested veterinary drug classes for seafood.

Reference

1. Love, D. C. *et al.* Veterinary drug residues in seafood inspected by the European Union, United States, Canada, and Japan from 2000 to 2009. *Environmental Science and Technology* **2011**, 45, 7232–7240.

Appendix: MRM parameters

Classification	Analyte	Retention Time (min)	Polarity	Quant MRM	CE (V)	Qual MRM	CE (V)
Sulfonamides	Sulfabenzamide	8.5	Positive	277.1 → 155.9	12	277.1 → 92.0	36
	Sulfacetamide	3.2	Positive	215.0 → 156.0	8	215.0 → 108.0	16
	Sulfachloropyridazine	6.7	Positive	285.0 → 156.0	12	285.0 → 108.1	24
	Sulfaclozine	9.5	Positive	285.0 → 155.9	20	285.0 → 108.1	24
	Sulfadiazine	3.7	Positive	251.1 → 92.1	28	251.1 → 108.1	20
	Sulfadimidine	5.6	Positive	279.1 → 92.1	38	279.1 → 186.1	12
	Sulfamerazine	4.6	Positive	265.1 → 92.1	28	265.1 → 156.0	12
	Sulfamethizole	5.7	Positive	271.0 → 92.0	29	271.0 → 156.0	9
	Sulfamethoxypyridazine	6.2	Positive	281.1 → 92.1	32	281.1 → 108.1	28
	Sulfamonomethoxine	7.3	Positive	281.0 → 126.0	20	281.0 → 156.0	10
	Sulfamoxole	5.4	Positive	268.1 → 155.9	20	268.1 → 112.5	24
	Sulfaphenazole	9.4	Positive	315.1 → 158.1	40	315.1 → 92.0	40
	Sulfisoxazole	7.9	Positive	268.1 → 156.0	10	268.1 → 92.0	40
	Sulfadimethoxine	10.4	Positive	311.1 → 156.0	16	311.1 → 108.1	28
	Sulfadoxine	7.7	Positive	311.1 → 156.0	16	311.1 → 92.1	32
	Sulfameter	5.5	Positive	281.1 → 92.0	40	281.1 → 215.1	20
	Sulfamethoxazole	7.1	Positive	254.1 → 92.1	24	254.1 → 156.0	12
	Sulfapyridine	4.2	Positive	250.1 → 92.0	29	250.1 → 156.0	17
	Sulfaquinoxaline	11.2	Positive	301.1 → 156.0	16	301.1 → 92.0	32
	Sulfathiazole	4.0	Positive	256.0 → 156.0	12	256.0 → 92.1	28
	Sulfisomidine	3.8	Positive	279.1 → 124.1	20	279.1 → 186.0	20
	Trimethoprim	5.6	Positive	291.2 → 261.1	24	291.2 → 123.1	24
Tetracyclines	Doxycycline	6.5	Positive	445.2 → 410.0	24	445.2 → 428.1	16
	Oxytetracycline	6.8	Positive	461.2 → 426.1	14	461.2 → 201.1	48

Classification	Analyte	Retention Time (min)	Polarity	Quant MRM	CE (V)	Qual MRM	CE (V)
Quinolones	Danofloxacin	7.7	Positive	358.2 → 340.1	20	358.2 → 82.1	48
	Enrofloxacin	7.5	Positive	360.2 → 316.2	16	360.2 → 342.2	20
	Flumequine	13.9	Positive	262.1 → 244.1	12	262.1 → 202.0	32
	Lomefloxacin	7.8	Positive	352.2 → 308.2	16	352.2 → 265.0	20
	Nalidixic acid	13.6	Positive	233.1 → 215.1	20	233.1 → 159.1	40
	Orbifloxacin	8.1	Positive	396.2 → 352.1	16	396.2 → 295.1	28
	Oxolinic acid	11.5	Positive	262.1 → 243.9	16	262.1 → 159.9	40
	Pefloxacin	6.5	Positive	334.2 → 290.1	16	334.2 → 233.1	28
	Sarafloxacin	8.4	Positive	386.1 → 368.1	20	386.1 → 342.1	20
	Sparfloxacin	9.8	Positive	393.2 → 349.0	20	393.2 → 375.2	20
	Difloxacin	8.0	Positive	400.1 → 299.1	32	400.1 → 382.1	20
	Fleroxacin	6.0	Positive	370.1 → 326.0	20	370.1 → 268.9	24
	Ciprofloxacin	7.3	Positive	332.1 → 314.0	20	332.1 → 288.0	20
	Enoxacin	6.6	Positive	321.1 → 303.3	16	321.1 → 231.8	40
	Norfloxacin	6.9	Positive	320.1 → 302.1	20	320.1 → 282.1	40
Nitroimidazoles	Metronidazole	3.4	Positive	172.0 → 128.0	13	172.0 → 82.0	25
	MNZOH	3.0	Positive	188.0 → 123.0	13	188.0 → 126.0	21
	HMMNI	3.3	Positive	158.0 → 140.0	18	158.0 → 55.0	13
	Ronidazole	3.8	Positive	201.0 → 140.0	9	201.0 → 55.0	25
	Dimetridazole	3.6	Positive	142.0 → 96.0	21	142.0 → 54.0	40
Chloramphenicols	Chloramphenicol	9.8	Negative	321.0 → 151.9	21	321.0 → 257.0	9
	Florfenicol	7.3	Negative	356.0 → 185.1	12	356.0 → 336.0	0
	Thiamphenicol	5.3	Negative	354.0 → 289.9	8	354.0 → 185.0	16
β-lactams	Amoxicillin	3.8	Positive	366.1 → 349.1	4	366.1 → 114.1	32
	Penicilline G	14.0	Positive	335.1 → 160.1	8	335.1 → 176.0	8
	Sulbactam	3.8	Negative	232.0 → 140.1	12	232.0 → 64.1	44
	Ampicillin	8.0	Positive	350.0 → 106.0	16	350.0 → 114.0	36
Malachite green	Malachite green	14.3	Positive	329.3 → 313.3	40	329.3 → 284.0	50
	Leucomalachite green	14.2	Positive	331.2 →239.2	28	331.2 → 316.2	20

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