Residue Analysis of Fish Exposed to Fishnets Coated with Antifouling Paint Containing Zinc or Copper Pyrithione

Abstract

Fish were exposed to fishnets treated with a coating containing copper pyrithione (CuPT) for a period of 45 days. Analysis was done by HPLC-MS/MS after extraction and derivatization of the pyrithione portion of the molecule from the fish muscle. The method was optimized to determine the concentration of pyrithione (as CuPT) in the range of 0.5 ng/g to 30 ng/g (ppb) in the fish. For the analysis, 1 gram of fish muscle was extracted with 3 mL of a solvent containing an internal standard, followed by derivatization of the pyrithione portion of the molecule. HPLC-MS/MS in the multiple reaction monitoring (MRM) mode was used for quantitation. The analyte response was linear over the concentration range of 0.1 to 10 ng/mL in the extraction solvent (R^2 = 0.99817). Six replicates each of fish muscle spiked with CuPT at concentrations of 2.19 ng/g and 2.57 ng/g, in between the limit of quantitation (LOQ = 0.5 ng/g) and 10X the LOQ for the method, gave recoveries of 87% (sd = 15) and 104% (sd = 11), respectively. Analysis of six exposed fish and three unexposed fish (controls) all showed levels below the LOQ of 0.5 ng/g.

Copper pyrithione

Results and Discussion

Analysis of the derivatized pyrithione was by HPLC-MS/MS with multiple reaction monitoring (MRM). The ion transition of the [M+Na]+ molecular adduct ion to a specific fragment ion was monitored for the pyrithione derivative and the internal standard. Figure 1 shows a typical MRM chromatogram of the CuPT derivative. The response ratio from the CuPT derivative and the internal standard was used to calculate the pyrithione concentration in the fish samples. The method, in combination with the use of an internal standard, is very sensitive and highly specific for pyrithione.

The calibration standards showed a linear response (Figure 2) over the entire concentration range. Figure 3 shows representative chromatograms from control fish muscle, control fish muscle spiked with CuPT at 2.19 ng/g, and exposed fish muscle. No quantifiable CuPT was seen in any of the control or treated fish. Table 1 shows the results from the three control and six exposed fish. The CuPT concentrations were all below the limit of quantification of 0.5 ng/g.

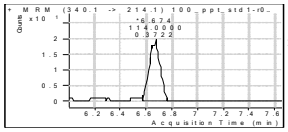


Figure 1. MRM chromatogram of the derivatized 0.1 ng/mL standard.

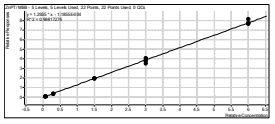


Figure 2. Standard calibration for derivatized CuPT using an internal standard

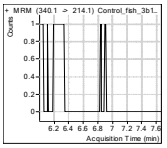
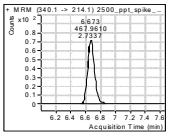
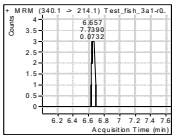


Figure 3. Representative MRM chromatograms from control fish #3 (top), control fish spiked with 2.19 ng/g of CuPT (middle), and exposed fish #3 (bottom).





Fish sample	Replicate	Injection	Peak area CuPT	Peak area IS	Conc. (ppb)
Control #1	A	1	-	769	< 0.5
	A	2	-	609	< 0.5
	B	1	-	476	< 0.5
	B	2	-	497	< 0.5
Control #2	A	1	15	806	< 0.5
	A	2	23	775	< 0.5
	B	1	-	773	< 0.5
	B	2	-	767	< 0.5
Control #3	A	1	-	605	< 0.5
	A	2	-	582	< 0.5
	B	1	-	600	< 0.5
	B	2	-	581	< 0.5
Exposed #1	A	1	42	976	< 0.5
	A	2	22	602	< 0.5
	B	1	24	468	< 0.5
	B	2	47	480	< 0.5
Exposed #2	A	1	88	739	< 0.5
	A	2	87	824	< 0.5
	B	1	34	703	< 0.5
	B	2	21	730	< 0.5
Exposed #3	A	1	-	436	< 0.5
	A	2	8	411	< 0.5
	B	1	-	384	< 0.5
	B	2	-	386	< 0.5
Exposed #4	A	1	-	370	< 0.5
	A	2	-	387	< 0.5
	B	1	9	439	< 0.5
	B	2	9	421	< 0.5
Exposed #5	A	1	-	295	< 0.5
	A	2	-	365	< 0.5
	B	1	-	339	< 0.5
	B	2	-	362	< 0.5
Exposed #6	A	1	37	566	< 0.5
	A	2	31	659	< 0.5
	B	1	33	615	< 0.5
	B	2	32	586	< 0.5
2.19-ppb spike control	A	1	468	662	2.37
	A	2	403	591	2.39
2.57-ppb spike control	B	1	318	495	2.53
	B	2	301	449	2.64

- Fish that were exposed to fishnets treated with zinc pyrithione (ZPT) containing coating were analyzed for the presence of ZPT in the fish muscle by *in-situ* chemical derivatization followed by LC-MS/MS analysis. The method has been optimized to determine the concentration of pyrithione at sub ppb levels (<1.0 µg/kg fish). ZPT in fish muscle was extracted with a solvent containing a derivatization reagent and an internal standard. The analyses of the derivatized ZPT and the internal standard were performed in multiple reaction monitoring (MRM) mode by LC-MS/MS. The linearity of the analyte response was obtained over the ZPT concentration range of 0.2 – 10 ng/mL, with the calculated regression coefficient of R^2=0.99947. Using blank and ZPT derivative-spiked fish extract, the limit of detection (LOD) was 0.15 ng/g fish and the limit of quantification (LOQ) was determined as 0.5 ng/g fish. Accuracy and precision were determined from six replicate samples spiked with known concentrations of ZPT in the presence of internal standard, and the measured concentrations of zinc pyrithione were 104 ± 0.6% and 99 ± 9.0% respectively, for LOQ and 10X LOQ samples. The quantification results show that ZPT concentrations were below the limit of quantification (0.5 ng/g fish) in both control and treated samples.

Zinc pyrithione

Results and Discussion

The analysis of chemically derivatized pyrithione was performed by LC-MS/MS using MRM, where the ion transition of molecular ion [M+1]+ to a fragmented ion with maximum peak intensity is monitored. Figure 1 shows the typical MRM chromatogram of ZPT derivative. The MRM response ratio of ZPT derivative to the internal standard was then used in the quantification of pyrithione in fish samples. This MS-based selective ion monitoring, in combination with the use of internal standard, provides absolute specificity for the analyte and absolute quantification of analyte concentration in the sample.

The results of standard calibration show the linear response of ZPT derivative as shown in Figure 2 in a range of ZPT concentration between 0.2 – 10.0 ng/mL. Figure 3 shows the representative MRM chromatograms of a control fish sample (A), a control sample spiked with 0.5 ng/g ZPT (B), and a treated sample (C). No detectable ZPT signatures were observed in the chromatograms of both control and treated samples, compared with the control sample spiked with ZPT. Table 1 presents the quantification results obtained for three control and twelve treated samples. The concentrations of ZPT were found to be below the limit of quantification (0.5 ng/g fish) in the samples from both control and treated groups.

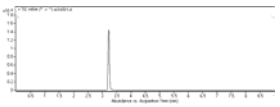


Figure 1. MRM chromatogram of ZPT derivative.

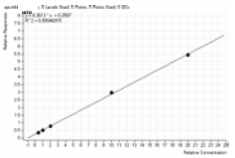


Figure 2. Typical calibration curve for ZPT derivative using an internal standard.

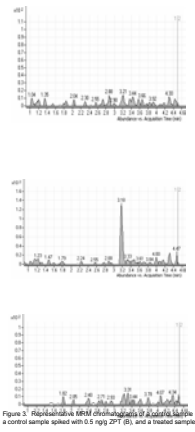


Figure 3. Representative MRM chromatograms of a control fish (A), a control sample spiked with 0.5 ng/g ZPT (B), and a treated sample (C).

Table 1. Concentrations of ZPT in the muscle of salmon fish in the control and the treated[a] groups.

Sample	No	Area of response ZPT	Area of response ISTD	Response ratio (ZPT/ISTD)	anal ZPT concentration (ng/g fish)
Control	1	97	665	0.1459	<LOQ[b]
	2	31	771	0.0402	<LOQ
	3	80	770	0.1039	<LOQ
Treated	1	39	721	0.0541	<LOQ
	2	49	673	0.0728	<LOQ
	3	30	601	0.0499	<LOQ
	4	67	721	0.0929	<LOQ
	5	84	640	0.1313	<LOQ
	6	96	606	0.1584	<LOQ
	7	106	632	0.1677	<LOQ
	8	36	580	0.0621	<LOQ
	9	20	657	0.0304	<LOQ
	10	16	615	0.026	<LOQ
	11	131	579	0.2263	<LOQ
	12	11	583	0.0189	<LOQ

[a] Salmon were exposed in the fishnet coated with ZPT antifouling paints for 7 months

[b] LOQ; limit of quantification (0.5 ng/g fish).