ENVIRONMENTAL

Praziquantel Determination in Aquarium Water by Direct Injection of Samples Using a Monolithic Silica Column

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Introduction

Praziquantel (PZQ) is used in the aquarium industry to treat monogenea and other parasites. This is often done through a bath immersion treatment, where medications are dissolved in aquarium water rather than dosed directly to a target organism.

Depending on the details of the treatment, it can be done in a separate holding tank or directly in the system being treated. In order to determine whether the therapeutic level of the medication is reached, and for how long that level persists, it is important to test medication concentrations in the treatment water. The current methodology for the determination of PZQ in aquarium water utilizes solid phase extraction (SPE) filtration to separate the analyte from the aquarium water sample. The extract is then analyzed using high performance liquid chromatography (HPLC).

Treatments may not be monitored to the extent desirable due to the time and cost of materials involved in the analysis. The goal of this study was to simplify the analysis and to decrease overall analysis time primarily by eliminating the SPE extraction steps.

Experimental

The current method was developed by Walt Disney World Resort Epcot, The Living Seas,[1] which referenced a Journal of Chromatography article[2] for the determination of Praziquantel in serum. While the HPLC determination of this analyte in serum would require extraction because of the sample matrix, seawater in small injection volumes should not present a problem so long as the organic concentration of the mobile phase is kept low enough that salts will not precipitate from solution. At 40% acetonitrile in the mobile phase, the salts in the sample do not precipitate out of solution. By using a monolithic silica column, SPE sample prep can be eliminated because of this support's ability to tolerate dirty samples. Changes to acetonitrile concentration of the moblie phase, injection size, and a simplified sample prep increase the method's sensitivity and efficiency and add up to significant time savings. These improvements are further discussed below. Additionally, these changes reduce the acetonitrile consumption for sample processing and completely eliminate the use of methanol. This will reduce the amount of solvent waste generated by the method.

The following experiments were performed on a Waters ACQUITY Arc with PDA detection at 220 nm.

Results/Discussion

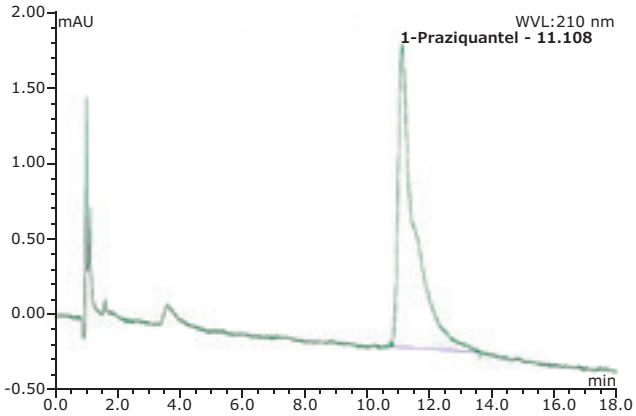
Separation Improvements

The chromatogram in **Figure 1** is from the Georgia Aquarium. It is a 50 µL injection, and mobile phase is 40% acetonitrile (ACN). Because of the fairly large injection volume, the Praziquantel peak is overloading the column as evidenced by poor peak shape.

A Chromolith® HighResolution RP-18 endcapped 100-4.6 HPLC column was used for the remainder of the chromatograms presented below.

The analysis in **Figure 1**, that utilized a Partisil ODS3 column, was duplicated on a Chromolith HR. **Figure 2** (purple line) is a 50 µL injection of a 2 mg/L PZQ standard in ACN. The analysis time was greatly reduced from 14 minutes in **Figure 1** to about 2 minutes on the Chromolith column. Peak symmetry was greatly improved with the Chromolith column. To simulate direct injection of a seawater sample, a standard was prepared in water containing 3.5% NaCl (**Figure 2**, green line). In this chromatogram you can see the baseline disturbance of the injection volume as the injection solvent elutes in the dead volume.

Figure 1. 1 mg/L PZQ in salt water, 40% ACN, 50 µL injection



HPLC:	Dionex UltiMate 3000
Column:	Partisil 5µm ODS3 250x4.6mm
Mobile phase:	40% acetonitrile (ACN)
Gradient:	Isocratic
Flow Rate:	1.6 mL/min
Column Temp:	30°C
Detector:	UV/Vis at 210 nm
Injection:	50 µL
Sample:	1mg/L PZQ in artificial salt water

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Figure 3 is again a 2 mg/L PZQ sample injected on a Chromolith HR, but the injecton volume was reduced to 10µL. Peak resolution and shape are significantly improved while sensitivity is maintained even with the lower injection volume.

In **Figure 4**, a reduction in acetonitrile concentration in the mobile phase is studied. The mobile phase flow rate was also reduced to 1 mL/min. The acetonitrile concentrations are from 40% acetonitrile (purple trace) to 60% (yellow trace).

For the remainder of these experiments, a 50% acetonitrile mobile phase was used (represented by the green trace chromatogram in **Figure 4**) as it presents a good compromise of analysis time and solvent use.

At this point we have optimized the SPE separation from the original Disney technique to decrease organic solvent usage, and improve separation efficiency. In the next section, we demonstrate sample preparation without SPE.

Sample Preparation Improvements

Figure 5 is a 10 µL direct injection (no SPE) of a treatment water sample from Georgia Aquarium containing 5.2 mg/L PZQ. This demonstrated that the elimination of sample prep did not adversely affect the analysis.

Treatment samples that were received from Georgia Aquarium were spiked with 10mg/L PZQ. In **Figure 6** the original sample (green trace) containing 5.2mg/L PZQ and the spiked sample (purple trace) are overlaid.

Figure 2. PZQ in salt water (green) and in acetonitrile (purple), 2 mg/L PZQ, 40% ACN, 50 µL injection.

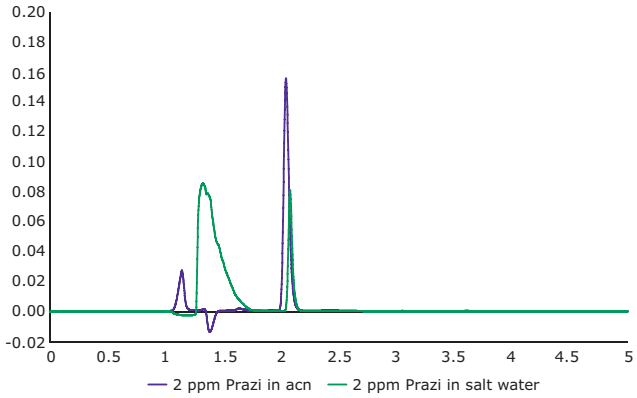


Figure 3. PZQ in salt water (green) and in acetonitrile (purple), 2 mg/L, 40% ACN, 10 µL injection.

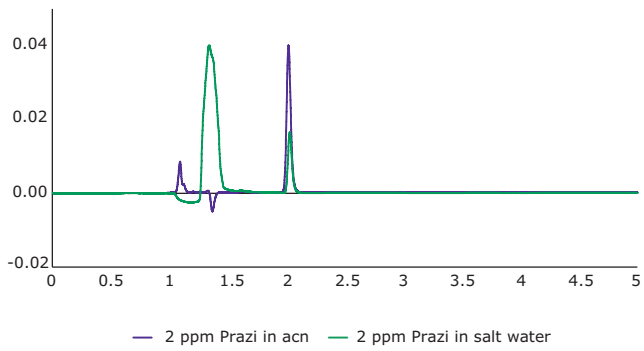


Figure 4. Acetonitrile concentration options, 2mg/L PZQ, 1 mL/min flow rate

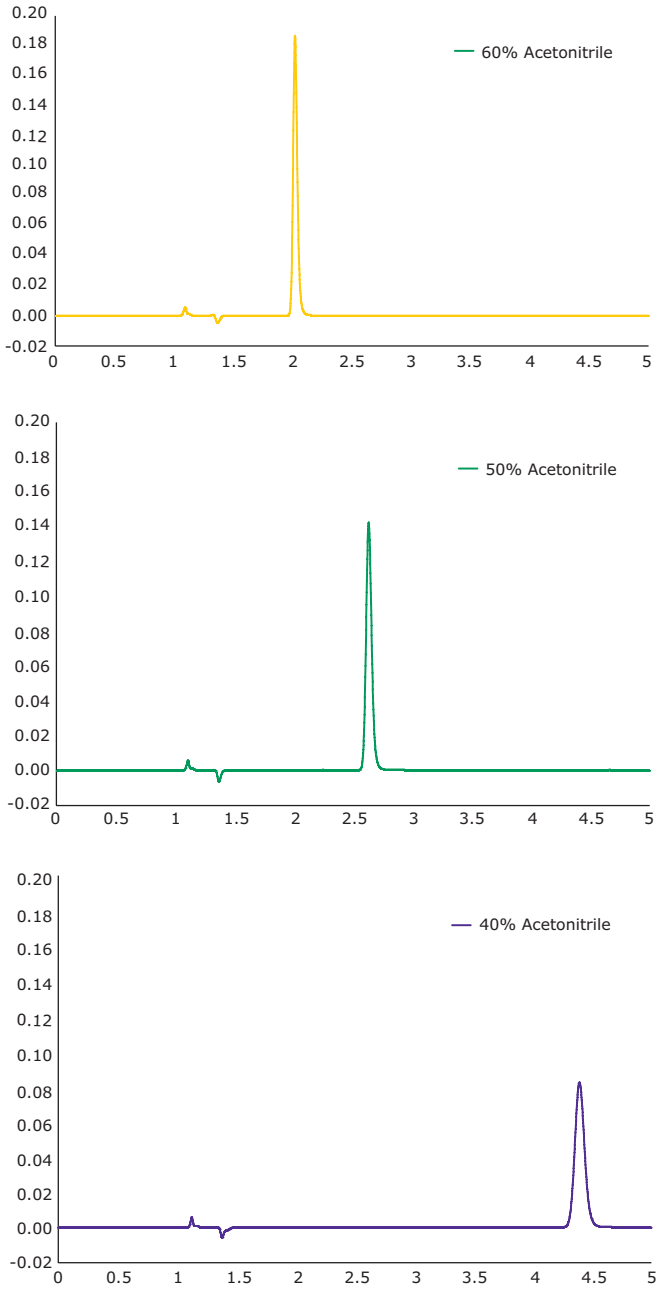


Figure 5. Sample NH03-3 from Georgia Aquarium analyzed without prior SPE extraction

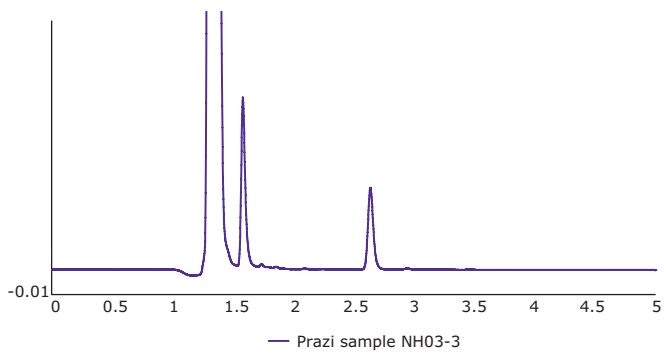


Figure 6. Sample NH03-3 from Georgia Aquarium, direct injection (green) and spiked with 10mg/L PZQ (purple)

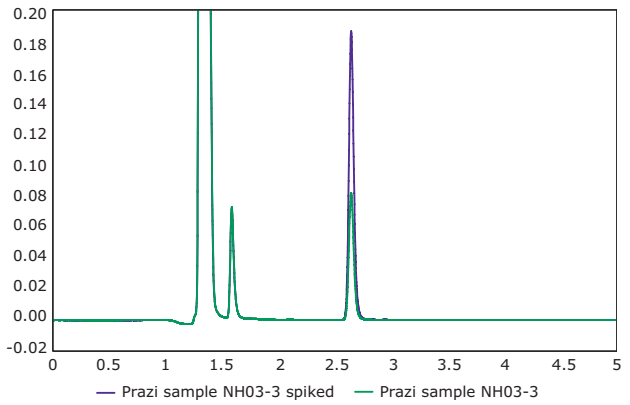


Figure 7. Calibration curve of 5 standards from 1.25mg/L to 20mg/L PZQ in ACN

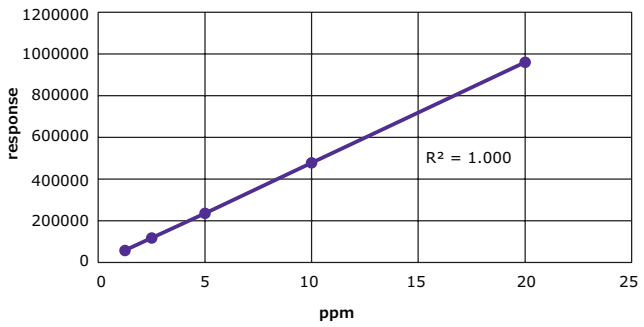


Figure 8. Chromatogram overlay of 6 PZQ standards, 0.625mg/L

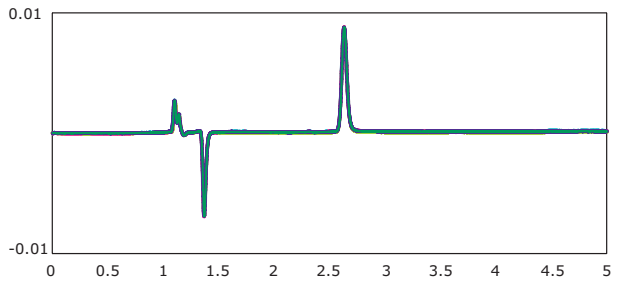
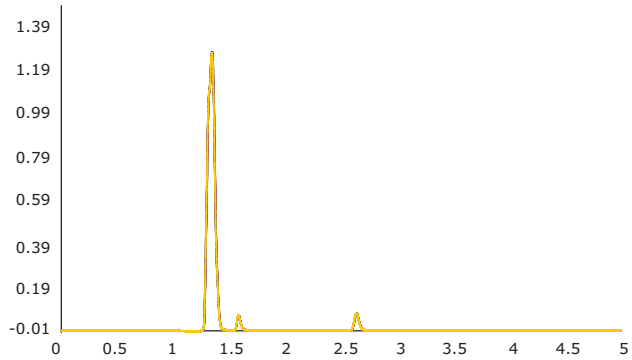


Figure 9. 10 injections from a series of 500 repeat injections of a composite sample from PZQ treatments at Georgia Aquarium



Spike recovery was calculated and is displayed in **Table 1** for several treatment samples.

Table 1. Spiked recoveries of treatment samples from Georgia Aquarium

Sample	Determined Amount	Spike Amount	Spike Recovery
NH03-6 spiked	10.02	10.00	96.92%
NH03-6	0.33		
NH03-4 spiked	14.96	10.00	96.50%
NH03-4	5.31		
Water blank spiked	9.71	10.00	97.11%
Water blank	0.00		
3.5% Salt Water blank spiked	9.75	10.00	97.49%
3.5% Salt Water blank	0.00		

The HPLC system was calibrated using 5 standards prepared in ACN between 1.25 and 20 mg/L Praziquantel. The calibration curve and data are displayed in **Figure 7** and the correlation is 1.000.

The method detection limit (MDL) was calculated from 6 replicate injections of a 0.625mg/L standard. MDL was calculated to be 0.015mg/L using the students' t-test method based on the data represented in **Table 2** and **Figure 8**.

Table 2. Replicate analysis of a 0.625mg/L standard used to calculate MDL

Replicate	1	2	3	4	5	6	Avg	Sd	MDL*
ppm	0.674	0.675	0.676	0.686	0.678	0.675	0.677	0.004	0.015

*Students' t -test

To evaluate if column life will be affected by multiple injections of the seawater sample matrix, a series of 500 injections of a composite of the samples provided by Georgia Aquarium was performed. **Figure 9** is an overlay of 10 injections from the 500 injection sequence. These injections were equally spaced (every 50 injections) throughout the sequence. Both retention time and response remained stable over 500 injections. The column pressure did increase throughout the run from about 950 psi to 1800 psi. The lower column pressure at the beginning of the 500 injection series was easily restored by flushing the system with 90% water and 10% ACN. This showed that the column and HPLC system can be flushed clean. It is recommended to include a similar flush step at the end of each sequence of samples to properly maintain system pressure.

Table 3 is a comparison of the method described in this article with the original SPE method for detecting PZQ in seawater. The samples are from treatments carried out at Georgia Aquarium. The results of the two methods are comparable.

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Table 3. A comparison of the SPE method run at Georgia Aquarium and the Chromolith method described in this article.

Sample Name	Retention Time	Chromolith Method Result	Result from GA Aquarium SPE Method
Prazi sample NH03-1	2.635	nd	nd
Prazi sample NH03-2	2.645	5.17	5.1692
Prazi sample NH03-3	2.644	5.21	5.3906
Prazi sample NH03-4	2.644	5.32	5.6451
Prazi sample NH03-5	2.645	4.34	5.4443
Prazi sample NH03-6	2.646	0.20	1.3211
Prazi sample NH03-7	2.635	nd	nd
Prazi sample NQ01-1	2.635	nd	0.0627

Conclusions

With the new method, HPLC analysis time was reduced from 18 minutes per sample to 5 minutes, which provided time and cost savings for the lab. Because the monolithic column will tolerate "dirty" samples, seawater samples can be directly injected into the instrument, eliminating SPE sample prep. This reduces analysis time by an additional 20 minutes per sample. Total time saved is about 30 minutes per sample, as outlined in **Table 4**.

Table 4. A comparison of the time required for the SPE method and the method described in this article.

	Time Per Sample (min)	
Method Step	SPE Method	New Method
SPE Extraction	20	0
Sample Prep for HPLC	3	3
HPLC Analysis	18	5
Total Time Spent:	41	8

In addition, 84% less acetonitrile is consumed due to elimination of extraction solvents and shorter analysis time. This makes it a greener method by reducing solvent waste produced in the lab.

The new method provides reliable and sensitive results, with recoveries >95% and a method detection limit (MDL) of 0.015 mg/L. The development of the new method makes Praziquantel testing more efficient and cost effective, which will allow an increase in the measurement frequency for monitoring treatments.

Instrument and Conditions

Final Method:	
instrument:	Waters Acquity Arc with PDA
column:	Chromolith® High Resolution RP-18 Endcapped 100 x 4.6 mm (1.52022)
mobile Phase:	50% Acetonitrile (AX0142) / 50% Water (Milli-Q)
flow Rate:	1 mL/min.
column Temp:	30 °C
detector:	PDA @220 nm
injection Volume:	10 µL
sample:	Seawater (Filtered)

References

1. Crowder, J. and T. Charanda. 2004. Determination of Praziquantel in seawater. 1st AQUALITY symposium. Oceanario De Lisboa, Portugal.
2. Xiao, S., B.A. Catto, and L.T. Webster, Jr. 1983. Quantitative determination of Praziquantel in serum by high-performance liquid chromatography. Journal of Chromatography, 275: 127 – 132.

Featured Products

Description	Cat.No.
HPLC	
Chromolith® High Resolution RP-18 Endcapped 100-4.6	**1.52022**
Solvents	
Acetonitrile: Omnisolv®*	**AX0142**
Water was from a Milli-Q® Advantage lab water system	
Standard	
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*only available in North America

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Chromolith® HighResolution RP-18 Endcapped 5-4.6 guard cartridges (3 pieces)	**1.52025**
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Acetonitrile gradient grade for liquid chromatography LiChrosolv®	**1.00030**

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