

Praziquantel determination in aquarium water by direct injection on a monolithic silica column.



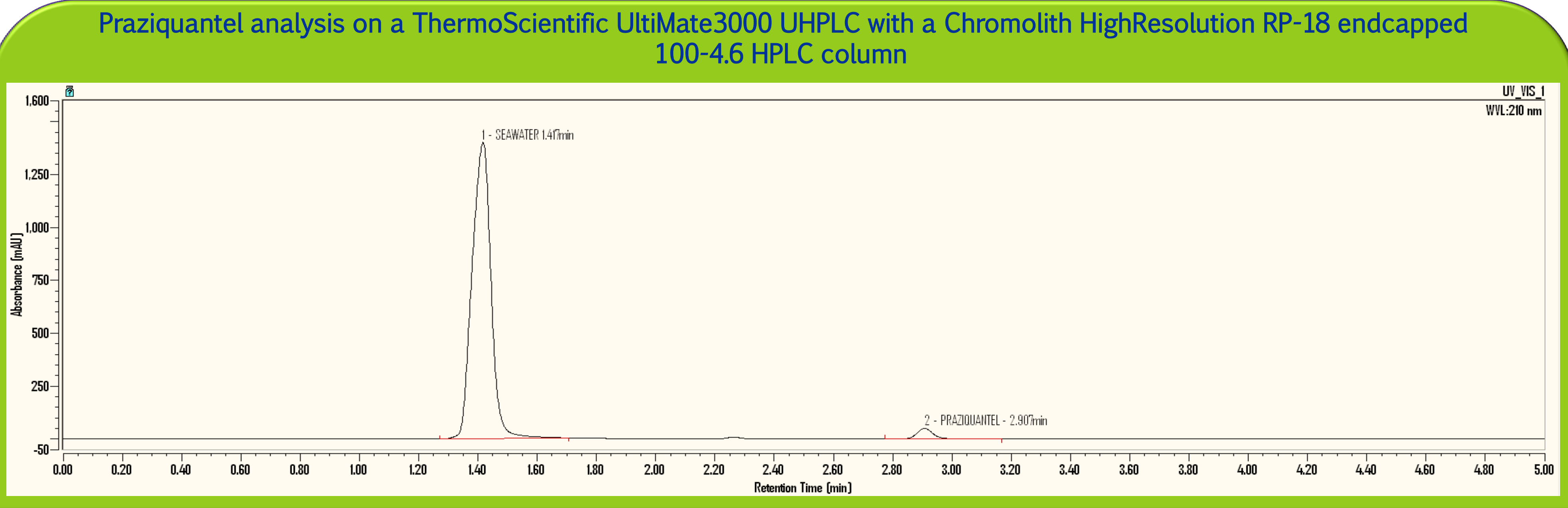
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Introduction

Praziquantel (PZQ) is used in the aquarium industry to treat monogeneas and other parasites, often through immersion treatments. The current method for measuring PZQ in aquarium water utilizes solid phase extraction (SPE) filtration to separate the PZQ from the sample. The extract is analyzed using high performance liquid chromatography (HPLC). Due to limits of time and cost, treatments are rarely monitored. The goal of this study was to decrease the time and cost of testing for PZQ by eliminating SPE extraction.

Objective

Demonstrate the HPLC determination and validation of Praziquantel in aquarium water with minimal sample preparation on a monolithic silica HPLC column, reducing both the time and materials required.



Methods

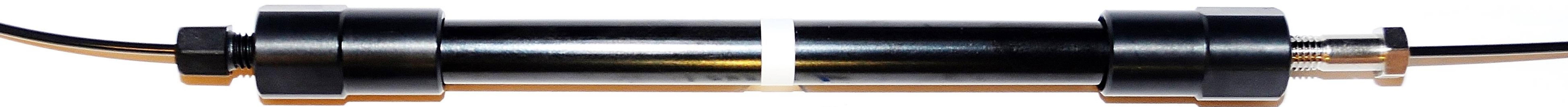
The SPE method of PZQ analysis in seawater was adapted by Crowder and Charanda (2004) from a method developed for blood serum (Xiao, Catto and Webster 1983) and run on a Whatman Partisil column. While HPLC determination of PZQ in serum requires extraction because of the sample matrix, a monolithic silica column should handle small injections of aquarium water without prior extraction.

Results

The monolithic silica column successfully separated PZQ from the saltwater matrix. A series of 5 standards were used to create a calibration curve, which had an excellent correlation coefficient of 1.000. Recoveries from spiked samples were >95%. The method detection limit (MDL) was 0.015mg/L PZQ. Repeated injection of saltwater had no effect on the results over time, although the column did need periodic flushing to prevent pressure buildup.

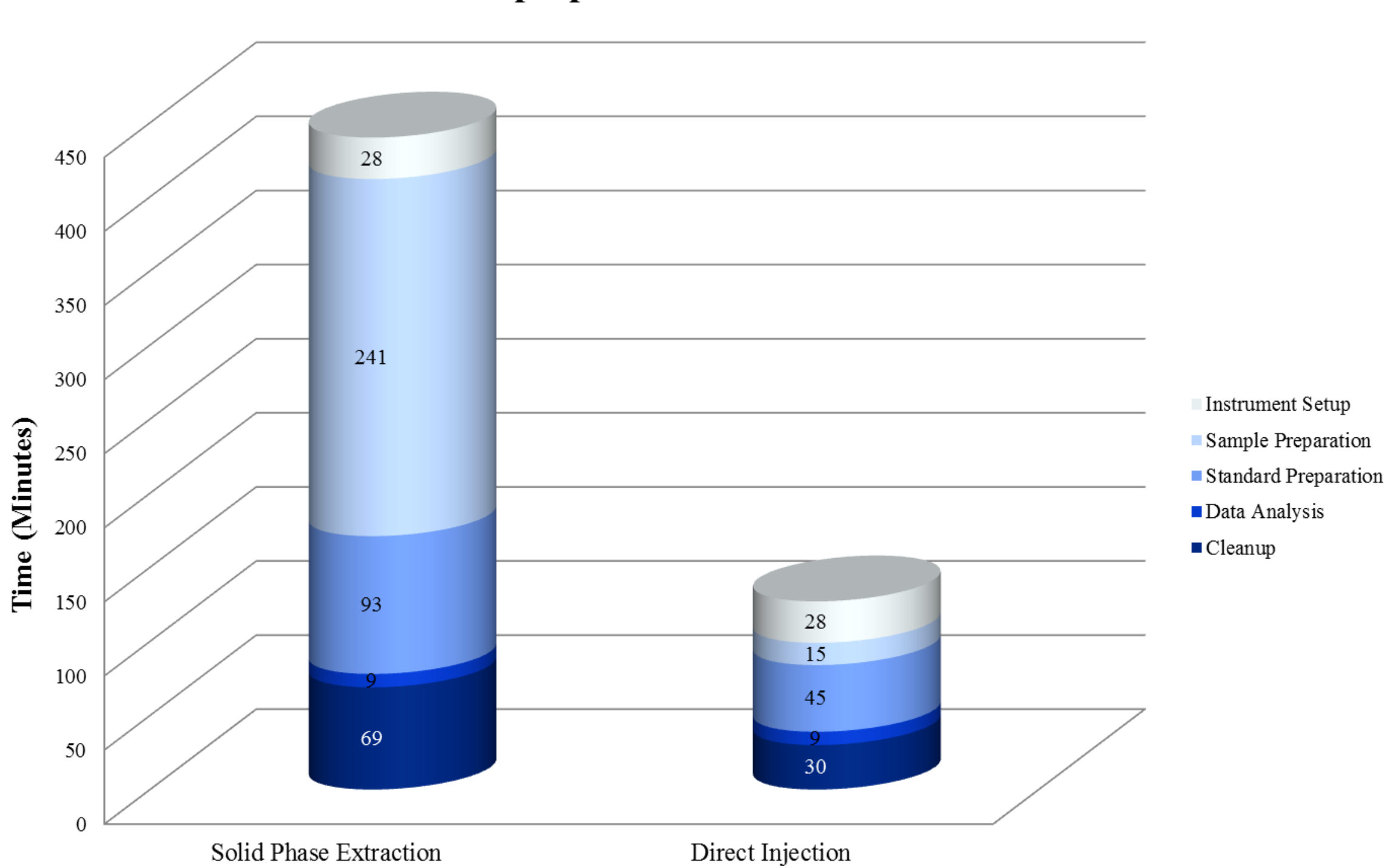
Comparison of Methods

Analysis of 15 samples using both methods showed that there was no significant difference in the results obtained (paired t-test, $p>0.05$). Peak shape was more symmetrical on the Chromolith column. Retention time was reduced from 11 minutes to 3 minutes without loss of resolution.

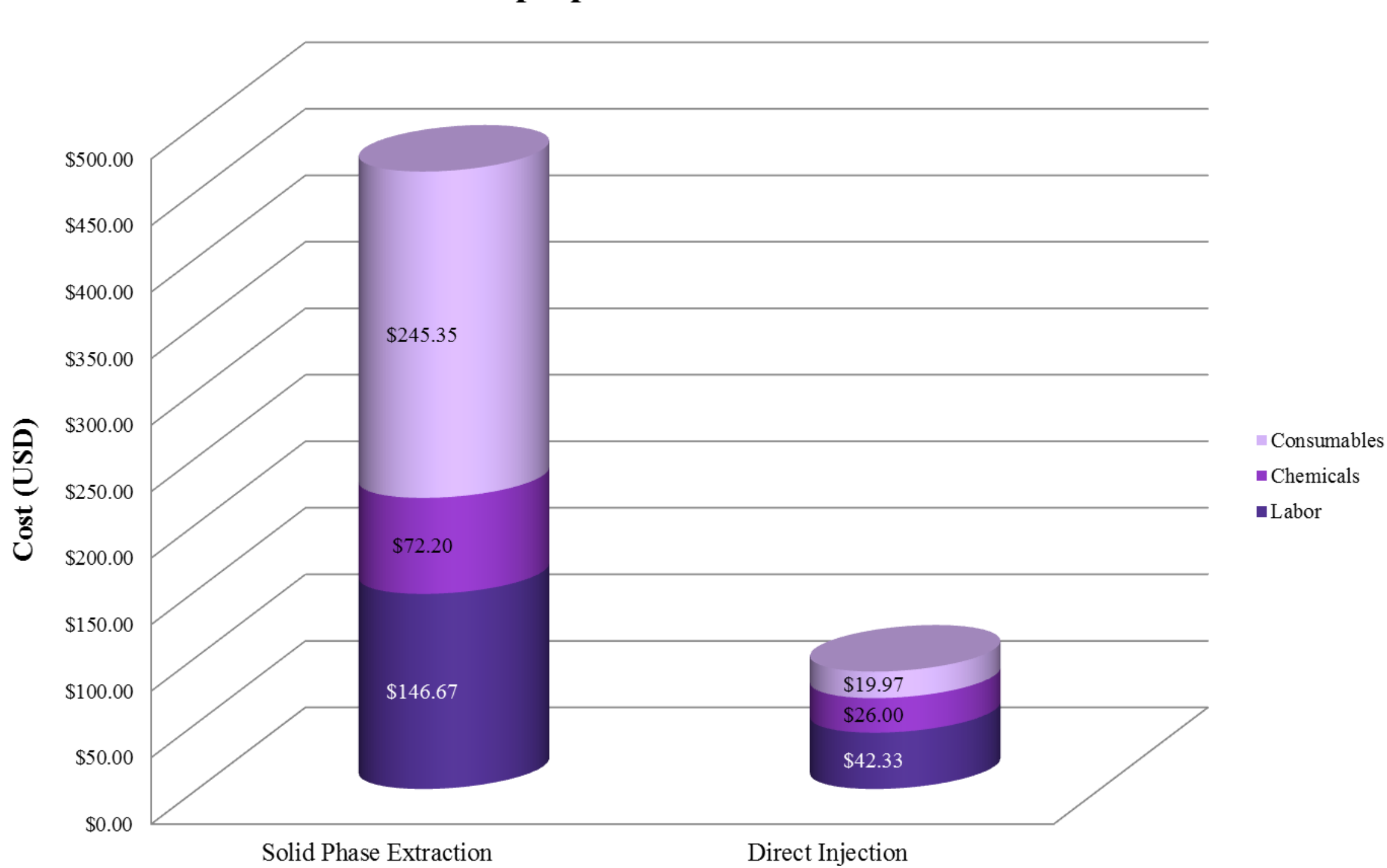


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Time spent on a 15-sample praziquantel analysis using two sample preparation methods



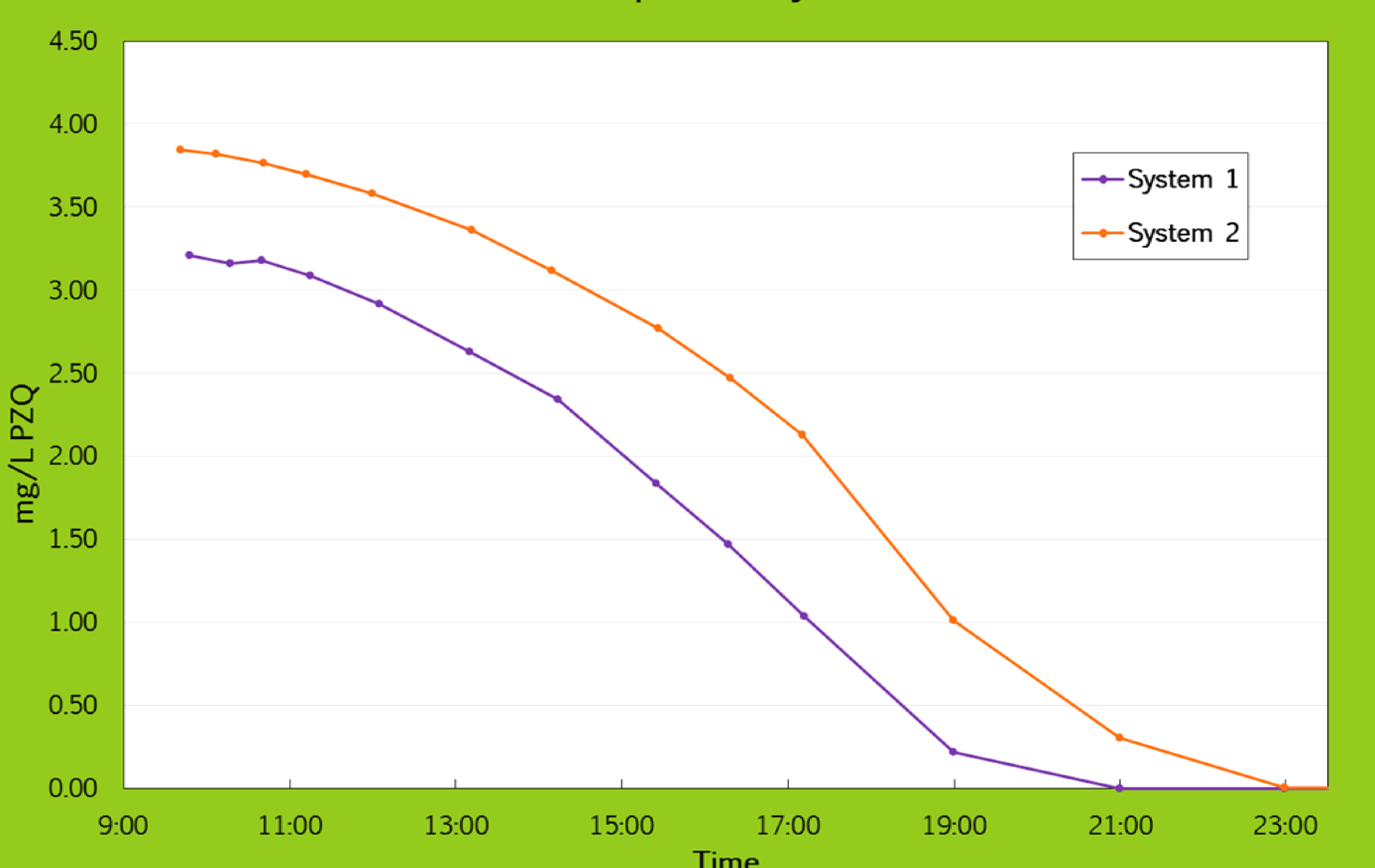
Cost of a 15-sample praziquantel analysis using two sample preparation methods



Application

The reduction in time and cost allowed Georgia Aquarium to effectively monitor the treatment of two 26,500 gallon systems with 5mg/L PZQ. PZQ levels reached non-detectable levels in both systems within 16 hours. Since PZQ concentrations can rapidly fall below therapeutic levels (Thomas et al 2016), increased PZQ analysis allows veterinary staff to make informed decisions about treatments, including treatment concentration, duration and repetition.

Praziquantel concentrations during a treatment for monogeneans in two aquarium systems



Previously, analyzing PZQ took 3.6 hours + 50 minutes per set of 3 samples. The total cost was \$154 + \$21 per sample.

The new direct injection method takes 1.7 hours + 2 minutes per sample and costs \$72 + \$1.10 per sample.

References

- Crowder J, Charanda T. 2004. Determination of Praziquantel in seawater . 1st AQUALITY symposium. Oceanario De Lisboa, Portugal.
- Thomas, A, MR Dawson, H Ellis and MA Stamper. 2016. Praziquantel degradation in aquarium water. PeerJ, 4:e1857.
- Xiao, S, BA Catto and LT Webster, Jr. 1983. Quantitative determination of praziquantel in serum by high-performance liquid chromatography. Journal of Chromatography, 275: 127 – 132.

Summary

Using a monolithic silica column allowed PZQ to be accurately tested on an HPLC without the costly and time consuming use of SPE. Without compromising the quality of the results, samples were analyzed with savings of approximately \$376 and 5 hours for a typical set of 15 samples. Direct injection yielded recoveries over 95%, had a method detection limit (MDL) of 0.015 mg/L and was constant over 500 repeated injections. Direct injection has allowed for increased sampling, which provided important information during real-world treatments at Georgia Aquarium. The demonstrated effectiveness of the direct injection method met the original goal of creating a simple, cost-effective method for monitoring PZQ in aquaria.