Automated SPE for the Determination of Contaminants in Water According to Regulatory Requirements

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Introduction

Contamination of clean water sources causes impact on the agricultural industry and natural resources; ultimately affecting both humans and animals. With a constant concern because of this, the US Environmental Protection Agency (EPA) has implemented pollution control programs and water quality standards like the EPA Methods 1694 and 525.3 to test for pharmaceuticals and personal care products, and semi volatile organic chemicals in water, respectively. The determination of the pollutant concentrations in water by these methods is based on a solid phase extraction (SPE) of the target analytes and a subsequent separation and identification by chromatography-mass spectrometry.

SPE has become a technique of choice for sample clean-up, fractionation and trace-enrichment, but most manual SPE methods do not really optimize the extraction mechanism due to a rather simplified approach to method development. The automation and optimization of SPE provides more efficient and reproducible sample preparation, improves day-to-day precision and increases sample throughput compared to using manual fractionation. In addition, the automated SPE can be configured with on-line injection onto the chromatographic system.

This work presents automated SPE methods for pharmaceuticals & personal care products in large volume water samples and the extraction of hormones in surface water.

Automated SPE for Pharmaceuticals and Personal Care Products in Large Volume Water Samples

EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS-MS (December 2007) measures target analytes in large volume water samples by groups according to acid or basic SPE conditions and ionization mode. In this work, clean water samples of 1000 mL and 500 mL were prepared and run, comparing results of a suite of 45 target analytes from manual acid SPE with results from the automated acid SPE using the Gilson GX-274 ASPEC System for Large-Volume Clean Water SPE System (Figure 1). The manual SPE was done with a Vacuum manifold and vacuum pump. ESI positive ionization LC/MS-MS analysis was used for final quantitation and recovery. This poster ultimately shows comparable research results for 1000 mL and 500 mL large volume water samples to address two common issues faced by many laboratories: 1) efficiency of the SPE process and 2) data reproducibility.

Figure 1: Gilson GX-274 ASPEC Large-Volume Clean Water SPE System.

Automated Solid Phase Extraction Steps

The automated SPE steps are summarized for the 1000 mL and 500 mL clean water samples, with the schematic provided using TRILUTION LH Software (Figure 2).

500 mL samples were run in sequential mode according to the Automated Solid Phase Extraction Step method using one 6 mL SPE cartridge per sample. Samples of 1000 mL were run in batch mode according to the same method, using two 6 mL SPE cartridges per sample.

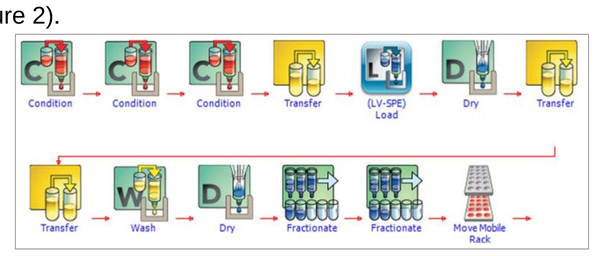

Figure 2: TRILUTION LH SPE Tasks for Fractionation of Large Volume Water Samples.

The summary of each step are as follows:

1. Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack.
2. Prime solvent lines from VALVEMATE II units.
3. Condition one 6 mL cartridge per sample with 20 mL methanol followed by an air push.
4. Condition the cartridge with 6 mL 18 Mohm/cm water followed by an air push.
5. Condition the cartridge with 6 mL of 18 Mohm/cm water @ pH 2 followed by an air push.
6. Prime Sample lines with sample (30mL)
7. Load 500 mL of sample to the cartridge at a dispense flow rate of 8 mL/min using a 0.7 minute air push.
8. Wash lines with Methanol (30mL)
9. Prime lines with Water (30mL)
10. Wash the cartridge with 10 mL 18 Mohm water.
11. Dry the cartridge for 5 minutes using an air purge.
12. Move the Gilson Mobile SPE Rack over the collection tubes.
13. Elute SPE cartridge into collect row 1 with 12 mL methanol at 3 mL/min followed by a 0.5 min air push
14. Elute SPE cartridge into collect row 2 with 6 mL (1:1) acetone:methanol at 3 mL/min followed by a 0.5 min air push.
15. Move Mobile Rack.

Clean water samples of 1000 mL and 500 mL were prepared and run, comparing results of a suite of 45 target analytes (Figure 3) from manual acid SPE with results from the automated acid SPE. As a summary of the full work, a smaller group selection of four target analytes, Erythromycin, Caffeine, Carbamazepine, and Fluoxetine, were randomly chosen to show that comparable research results.

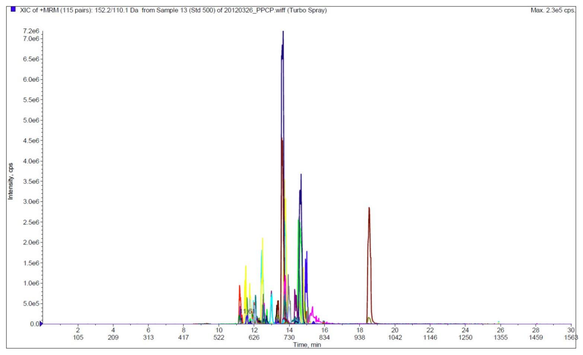

Figure 3: Example 500 ng/mL standard chromatogram showing 45 Target Analytes.

Recovery

Recoveries of the four target analytes for the 1000 mL manual SPE samples ranged from 90.7% - 125.6% (Figure 4). For the automated Gilson SPE samples, the recovery range of the same four target analytes was 91.0% - 105.1%.

Recoveries of the four target analytes for the 500 mL manual SPE samples ranged from 101.3% to 171.6%, with Erythromycin and Floxetine showing higher results than the expected recovery range listed in the EPA Method 1694 (Figure 5).

For the automated Gilson SPE samples, the recovery range of the same four target analytes was 95.2% to 114.5%, with all recovery values within the expected recovery ranged listed.

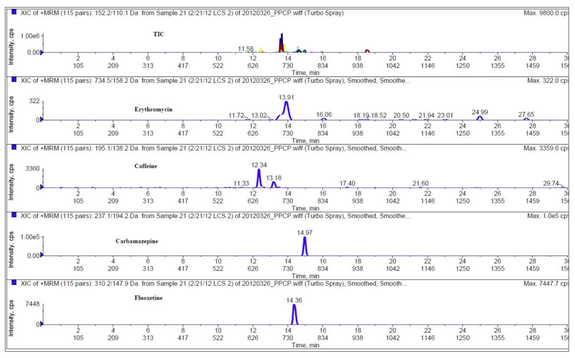

Figure 4: Manual SPE HPLC/MS-MS Analysis Results for 1000 mL Samples for Four Target Analytes.

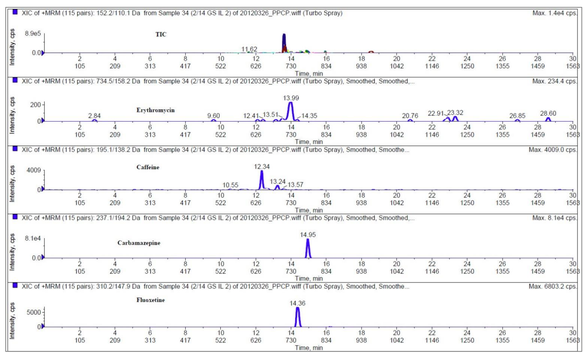

Figure 5: Automated SPE HPLC/MS-MS Analysis Results for 500 mL Samples for Four Target Analytes.

Automation of EPA 1694 included researching any carryover from the 45 target analytes. 1000 mL and 500 mL blank water samples were run through the Gilson Large-Volume Clean Water SPE System. Mean sample values showed either no peaks detected or less than detectable reporting limits for all 45 target analytes.

Summary

This work presents the simplicity of automating a manually intensive SPE process to provide efficiency in recovery and added efficiency with reduction of sample load volume with no negative impact on recoveries. Using the Gilson Large-Volume Clean Water Solid Phase Extraction System, carryover was tested, but not detected or seen for the 45 target analytes. With the exception of one analyte, the overall %RSD values show higher consistency with data generated from using automated SPE vs. manual SPE. Research through this application has shown that altering the sample load volume from 1000 mL to 500 mL has no impact on detection of the target 45 analytes. Reducing the sample load volume speeds up the load time, allowing for higher daily throughput of samples by a typical laboratory.

Quantification of Hormones in Surface Water

Measuring the concentrations of natural and synthetic hormones in water can be challenging. The extraction of hormones and hormone metabolites from surface water using the automated Gilson GX-271 ASPEC™ System (Figure 6) is presented.

The fractionation procedure used 200 mg/ 6mL ISOLUTE™ + polypropylene solid phase extraction cartridges (Biotage, USA). The SPE protocol is entirely automated using the Gilson GX-271 ASPEC system.

Figure 6: Gilson GX-271 ASPEC™ System with 406 Single Syringe Pump.

The SPE steps are summarized with the general schematic provided in the control software TRILUTION LH (Figure 7).

The summary of each step are as follows:

1. Initialization Step: Gilson Mobile SPE Racks are moved above the waste rack (Figure 8)
2. Condition the cartridge with 3 mL of methanol:ethyl acetate (1:1, v/v) at 3 mL/min
3. Condition the cartridge with 3 mL of methanol at 3 mL/min
4. Condition the cartridge with 3 mL of ultra-pure water at 3 mL/min
5. Load 50 mL of sample onto the SPE cartridge at a flow rate of 3 mL/min
6. Wash the cartridge with 10 mL of ultra-pure water at a flow rate of 3 mL/min
7. Dry the cartridge with a stream of air for 5 minutes
8. Move the Gilson Mobile SPE Rack over the collection tubes
9. Elute with 4 mL of methanol at a flow rate of 0.5 mL/min
10. Elute with 4 mL of methanol: ethyl acetate (1:1, v/v) at a flow rate of 0.5 mL/min
11. Concentrate the fractions with a gentle stream of N_2 gas to a volume of approximately 100 µL and reconstitute to a volume of 1.0 mL using methanol for HPLC-MS/MS analysis.

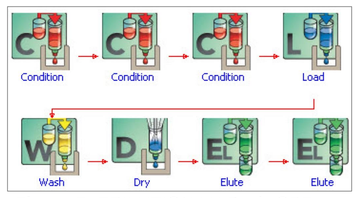

Figure 7: TRILUTION LH Basic SPE Tasks for SPE of Hormones from Water.

Figure 8: Gilson Mobile SPE Rack.

Summary

Using the Gilson GX-271 ASPEC for automation of the SPE process in the quantification of hormones in surface water increased sample throughput, reduced solvent usage and reduced the potential errors that may occur in during manual processing of samples.