Automated Solid Phase Extraction (SPE) of EPA Method 1694 for Pharmaceuticals and Personal Care Products in Large Volume Water Samples

Application Note ENV0212

This collaboration study was performed jointly by Gilson, Inc. and Curtis Hedman, Assistant Researcher with the Wisconsin State Laboratory of Hygiene.

Keywords

Gilson Large-Volume Clean Water Solid Phase Extraction System, TRILUTION® LH Liquid Handling Software, Solid Phase Extraction (SPE), Pharmaceuticals and Personal Care Products (PPCPs) Gas Chromatography Mass Spectrometry (GC/MS)

Introduction

Contamination of clean water sources is a constant concern because of its impact on our agricultural industry and natural resources; ultimately affecting both humans and animals. In the USA, the Clean Water Act (CWA) regulates surface water quality and pollutant discharges. The Environmental Protection Agency (EPA) has implemented pollution control programs and water quality standards under the CWA. EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS-MS (December 2007) was developed for use in CWA programs to test for common over-the-counter topical products, dietary supplements, human pharmaceuticals, veterinary drugs, and other consumer products and compounds labeled as Pharmaceuticals and Personal Care Products (PPCPs).

The U.S. Geological Survey in 2002 reported PPCP levels found in a variety of stream samples taken from across the USA. Using EPA Method 1694, laboratories today are monitoring PPCPs levels in a variety of clean water samples and linking these levels to any potential impact on humans and animals, even at low contamination levels.

EPA Method 1694 measures target analytes in large volume water samples by groups according to acid or basic solid phase extraction conditions and ionization mode. In this application, clean water samples of 1000 mL and 500 mL were prepared and run, comparing results of a suite of 45 target analytes (see Table 1) from manual acid solid phase extraction with results from the automated acid solid phase extraction (using the Gilson Large-Volume Clean Water Solid Phase Extraction System). ESI positive ionization

mode HPLC/MS-MS analysis was used for final quantitation and recovery. This application 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 Large-Volume Clean Water Solid Phase Extraction System (GX-274 ASPEC System Base With Special 1931-Series Accessories & Operated Using TRILUTION® LH Liquid Handling Software).

Table 1. EPA Method 1694 Suite of 45 Target Acid Analytes.

Compound Name	Pharmacological Compound Description	Molecular Formula
Acetaminophen	Non-Narcotic Analgesic Antipyretic	C8H9NO2
Ampicillin	Anti-Bacterial Agent	$C_{16}H_{19}N_3O_4S$
Azithromycin	Anti-Bacterial Agent	$C_{38}H_{72}N_2O_{12}$
Caffeine	Central Nervous System Stimulant Phosphodiesterase Inhibitor Purinergic Pt Receptor Antagonist	$C_8H_{10}N_4O_2$
Carbadox	Anti-Infective Agent Carcinogen Mutagen	$C_{11}H_{10}N_4O_4$
Carbamazepine	Non-Narcotic Analgesic Anticonvulsant Antimanic Agent	$C_{15}H_{12}N_2O$
Cefotaxime	Anti-Bacterial Agent	$C_{16}H_{17}N_5O_7S_2$
Ciprofloxacin	Anti-Infective Agent Nucleic Acid Synthesis Inhibitor	$C_{17}H_{18}FN_3O_3$
Clarithromycin		
Cloxacillin		
Codeine	Opioid Analgesic Antitussive Agent Narcotic	$C_{18}H_{21}NO_3$
Cotinine	Indicator and Reagent	$C_{10}H_{12}N_2O$
Digoxigenin	Metabolite of Digoxin, a Cardiotonic Drug	$C_{23}H_{34}O_5$
Diltiazem	Antihypertensive Agent Calcium Channel Blocker Cardiovascular Agent Vasodilator Agent	$C_{22}H_{27}ClN_2O_4S$
Paraxanthine	Central Nervous System Stimulant	$C_7H_8N_4O_2$
Diphenhydramine	Local Anesthetic Anti-Allergic Agent Antiemetic Histamine H1 Antagonist Hypnotic and Sedative	$C_{17}H_{21}NO$
Enrofloxacin	Antineoplastic Agent	$C_{19}H_{22}FN_3O_3$
Erythromycin	Anti-Bacterial Agent Gastrointestinal Agent Protein Synthesis Inhibitor	$C_{37}H_{67}NO_{13}$
Flumequine	Urinary Anti-Infective Agent	$C_{14}H_{12}FNO_3$

Table 1, continued. EPA Method 1694 Suite of 45 Target Acid Analytes.

Compound Name	Pharmacological Compound Description	Molecular Formula
Fluoxetine	Second Generation Antidepressive Agent Serotonin Uptake Inhibitor	$C_{17}H_{18}F_3NO$
Lincomycin	Anti-Infective Agent Protein Synthesis Inhibitor	$C_{18}H_{34}N_2O_6S$
Lomefloxacin	Anti-Infective Agent	$C_{17}H_{19}F_2N_3O_3$
Miconazole	14-alpha Demethylase Inhibitor Antifungal Agent	$C_{18}H_{14}Cl_4N_2O$
Norfloxacin	Anti-Bacterial Agent Enzyme Inhibitor Nucleic Acid Synthesis Inhibitor	$C_{16}H_{18}FN_3O_3$
Ofloxacin	Anti-Bacterial Agent Urinary Anti-Infective Agent Nucleic Acid Synthesis Inhibitor	$C_{18}H_{20}FN_3O_4$
Oxacillin	Anti-Bacterial Agent	$C_{19}H_{19}N_3O_5S$
Oxolinic acid	Urinary Anti-Infective Agent Nucleic Acid Synthesis Inhibitor	$C_{13}H_{11}NO_5$
Penicillin G	Anti-Bacterial Agent	$C_{16}H_{18}N_2O_4S$
Penicillin V	Anti-Bacterial Agent	$C_{16}H_{18}N_2O_5S$
Roxithromycin	Anti-Bacterial Agent	$C_{41}H_{76}N_2O_{15}$
Sarafloxacin	Anti-Bacterial Agent - Veterinary Fluroinated Qunolone Anti-Bacterial	$C_{20}H_{17}F_2N_3O_3$
Sulfachloropyridazine	Urinary Anti-Infective Agent	$C_{10}H_9ClN_4O_2S$
Sulfadiazine	Anti-Infective Agent Antiprotozoal Agent Coccidiostats	$C_{10}H_{10}N_4O_2S$
Sulfadimethoxine	Anti-Infective Agent	$C_{12}H_{14}N_4O_4S$
Sulfamerazine	Anti-Bacterial Agent	$C_{11}H_{12}N_4O_2S$
Sulfamethazine	Anti-Infective Agent	$C_{12}H_{14}N_4O_2S$
Sulfamethizole	Anti-Infective Agent	$C_9H_{10}N_4O_2S_2$
Sulfamethoxazole	Anti-Infective Agent	$C_{10}H_{11}N_3O_3S$
Sulfanilamide	Anti-Bacterial Agent	$C_6H_8N_2O_2S$
Sulfathiazole	Anti-Infective Agent	$C_9H_9N_3O_2S_2$
Thiabendazole	Anthelmintics	$C_{10}H_7N_3S$
Trimethoprim	Urinary Anti-Infective Agent Antimalarial Folic Acid Antagonist	$C_{14}H_{18}N_4O_3$
Tylosin	Anti-Bacterial Agent	$C_{46}H_{77}NO_{17}$
Virginiamycin	Anti-Micobial Agent	$C_{28}H_{35}N_3O_7$

Materials & Methods

Materials

Note: All solvents used were a grade suitable for GC, HPLC, pesticide residues analysis and spectrophotometry.

- Automated Solid Phase Extraction:
  - Gilson Large-Volume Clean Water Solid Phase Extraction System
    - GX-274 ASPEC System Base With Special 1931-Series Accessories & Operated Using TRILUTION® LH v3.0 Liquid Handling Software
- Manual Solid Phase Extraction:
  - Vacuum manifold and vacuum pump
- SPE Cartridges: Waters OASIS® HLB cartridges, 500 mg/6 mL using Gilson 6 mL Sealing Caps
- LC/MS-MS System:
  - Agilent 1100 HPLC System (Santa Clara, CA), consisting of an autosampler, binary pump, degasser, and column compartment.
  - Mass Spectrometer = AB/SCIEX API 4000 Triple Quadrupole Mass Spectrometer (Foster City, CA)
- HPLC Column: Phenomenex Synergi™ 4u MAX-RP, 4.6 x 250 mm
- Standards:
  - All standards, calibration solutions, matrix spiking solution and internal standards were prepared in accordance with the EPA Method 1694.
  - Atrazine was used as a labeled internal standard for monitoring injection stability
- Water Samples:
  - Water samples were taken from the Wisconsin State Laboratory of Hygiene (WSLH) water supply for all manual and automated tests.
  - System water blanks were run on the Gilson Large-Volume Clean Water Solid Phase Extraction System and analysis performed prior to running samples to determine if there was any compound bias present.
  - All water samples used for this application were prepared by the WSLH in accordance with the EPA Method 1694 for aqueous sample acid extraction.

Automated Solid Phase Extraction Steps

The fractionation protocol is entirely automated using the Gilson Large-Volume Clean Water Solid Phase Extraction System. The SPE steps are summarized for the 1000 mL and 500 mL clean water samples, with the schematic provided using TRILUTION LH Software (Figure 2).

Automated Solid Phase Extraction Step*

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.

Offline concentration of the two fractions was performed in accordance with EPA Method 1694.

**Note: 500 mL samples were run in sequential mode according to the Automated Solid Phase Extraction Step method (Figure 2), 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.*

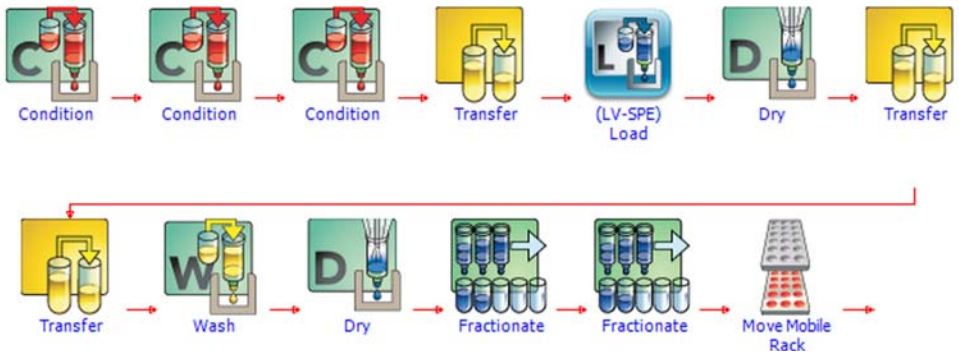

Figure 2. TRILUTION LH SPE Tasks for Fractionation of Large Volume Water Samples.

HPLC/MS-MS Analysis

System performance and calibration was verified each working day. A mid-level calibration standard was run after every 8 samples, along with duplicate methanol blanks before and after standard injections. An eight-point calibration curve was run at the beginning and end of each day.

Results

In this application, clean water samples of 1000 mL and 500 mL were prepared and run, comparing results of a suite of 45 target analytes (Figure 3 and Table 2) from manual acid solid phase extraction with results from the automated acid solid phase extraction (using the Gilson Large-Volume Clean Water Solid Phase Extraction System). As a summary of the full application, a smaller group selection of four target analytes, Erythromycin, Caffeine, Carbmazepine, and Fluoxetine, were randomly chosen to show that comparable research results.

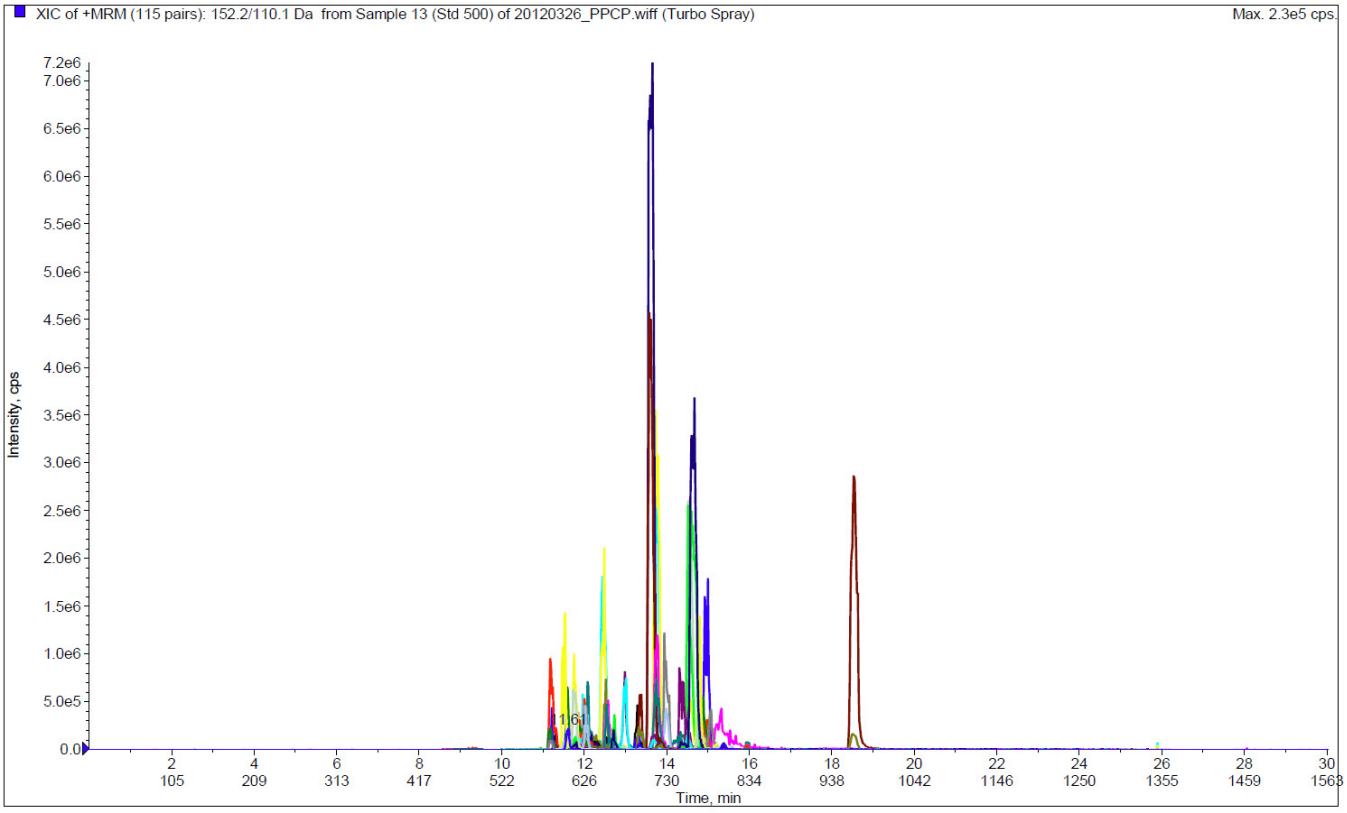

Figure 3. Example 500 ng/mL Standard Chromatogram Showing 45 Target Analytes.

Table 2. HPLC/MS-MS Analysis Retention Times of 45 Target Analytes.

Target Analyte Name	Retention Time (Minutes)
Acetaminophen	11.6
Ampicillin	11.6
Azithromycin	12.4
Caffeine	12.3
Carbadox	12.5
Carbamazepine	14.9
Cefotaxime	12.1
Ciprofloxacin	12.1
Clarithromycin	14.5
Cloxacillin	15.1
Codeine	11.3
Cotinine	11.2
Digoxigenin	13.4
Diltiazem	13.7
Paraxanthine	11.6
Diphenhydramine	13.6
Enrofloxacin	12.3
Erythromycin	13.9
Flumequine	15.2
Fluoxetine	14.3
Lincomycin	11.5
Lomefloxacin	12.1
Miconazole	18.5
Norfloxacin	11.9
Ofloxacin	11.9
Oxacillin	15.1
Oxolinic acid	14.3
Penicillin G	14.5
Penicillin V	14.8
Roxithromycin	14.6
Sarafloxacin	12.5
Sulfachloropyridazine	13.3
Sulfadiazine	12.0
Sulfadimethoxine	14.0
Sulfamerazine	12.6
Sulfamethazine	13.0
Sulfamethizole	12.7
Sulfamethoxazole	13.4
Sulfanilamide	9.4
Sulfathiazole	12.0
Thiabendazole	13.8
Trimethoprim	11.8
Tylosin	13.8
Virginiamycin	15.4

Recoveries of the four target analytes for the 1000 mL manual SPE samples ranged from 90.7% - 125.6%, with all recovery values within the expected range (Table 3 and Figure 4). For the automated Gilson SPE samples, the recovery range of the same four target analytes for the 1000 mL samples was 91.0% - 105.1%, with all recovery values within the expected recovery ranged listed.

Table 3. Manual vs. Automated SPE Recovery Results for 1000 mL Samples for Four Target Analytes.

	Erythromycin	Caffeine	Carbamazepine	Fluoxetine
Manual WSLH SPE - 1000 mL Samples Mean (n=4) (theoretical value=125ng/mL)	157.0	116.5	113.3	125.1
Manual WSLH SPE - 1000 mL Samples % Recovery (n=4)	125.6	93.2	90.7	100.1
Manual WSLH SPE - 1000 mL Samples % RSD (n=4)	37.3	16.9	20.5	14.9
Automated Gilson SPE 1000 mL Mean (n=4) (theo.=125ng/mL)	131.4	128	113.7	131.2
Automated Gilson SPE 1000 mL %Recovery (n=4)	105.1	102.4	91.0	105.0
Automated Gilson SPE 1000 mL % RSD (n=4)	35.4	33.4	4.8	13.1

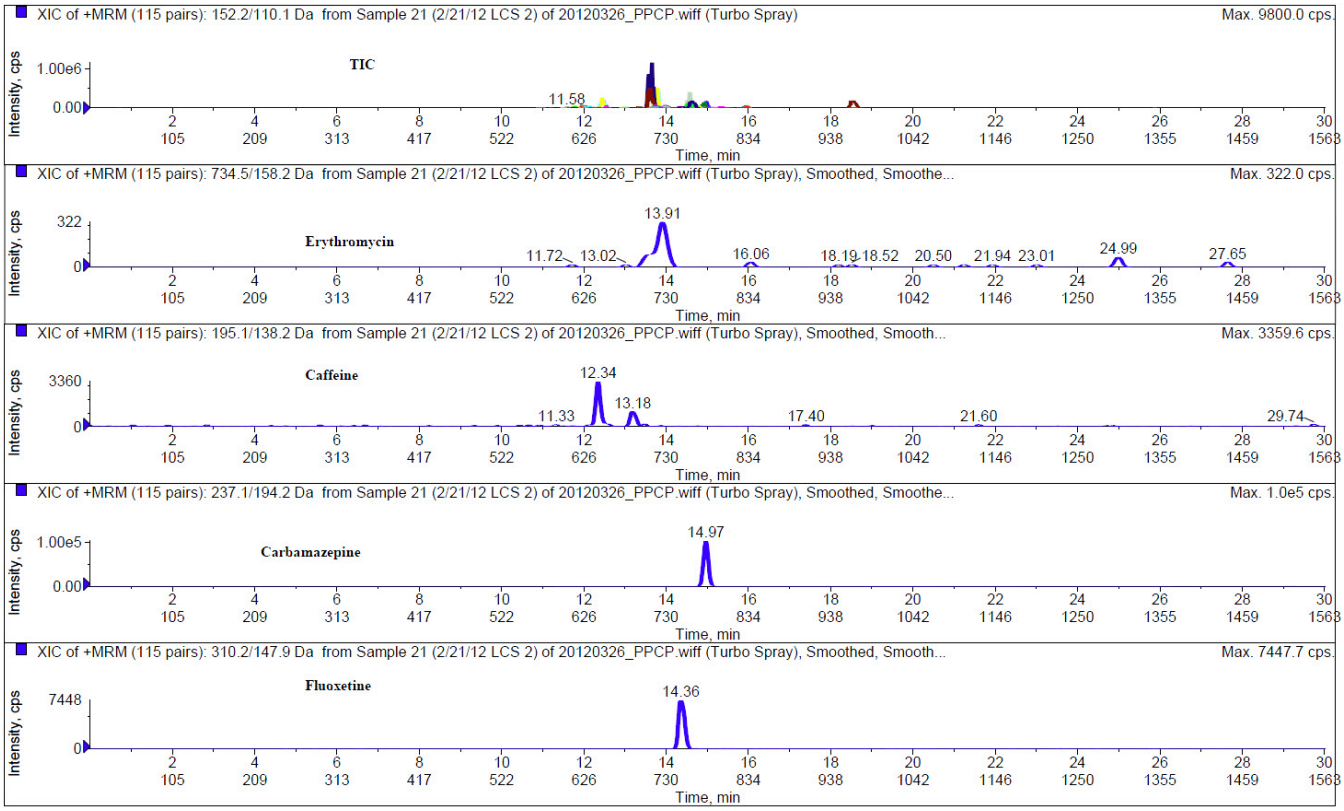

Figure 4. Manual SPE HPLC/MS-MS Analysis Results for 1000 mL Samples for Four Target Analytes.

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 (Table 4 and Figure 5). For the automated Gilson SPE samples, the recovery range of the same four target analytes for the 500 mL samples was 95.2% to 114.5%, with all recovery values within the expected recovery ranged listed.

Table 4. Manual vs. Automated SPE Recovery Results for 500 mL Samples for Four Target Analytes.

	Erythromycin	Caffeine	Carbamazepine	Fluoxetine
Manual WSLH SPE - 500 mL Samples Mean (n=4) (theoretical value=62.5ng/mL)	107	63.3	69.9	84.3
Manual WSLH SPE - 500 mL Samples % Recovery (n=4)	171.6	101.3	111.8	134.8
Manual WSLH SPE - 500 mL Samples % RSD (n=4)	52	26.7	17.8	52.8
Automated Gilson SPE 500 mL Mean (n=4) (theoretical value=62.5ng/mL)	59.5	71.5	67.2	71.6
Automated Gilson SPE 500 mL % Recovery (n=4)	95.2	114.5	107.6	114.5
Automated Gilson SPE 500 mL % RSD (n=4)	24.0	21.0	14.4	43.5

Automation of EPA Method 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 Solid Phase Extraction System. Mean sample values showed either no peaks detected or less than detectable reporting limits for all 45 target analytes.

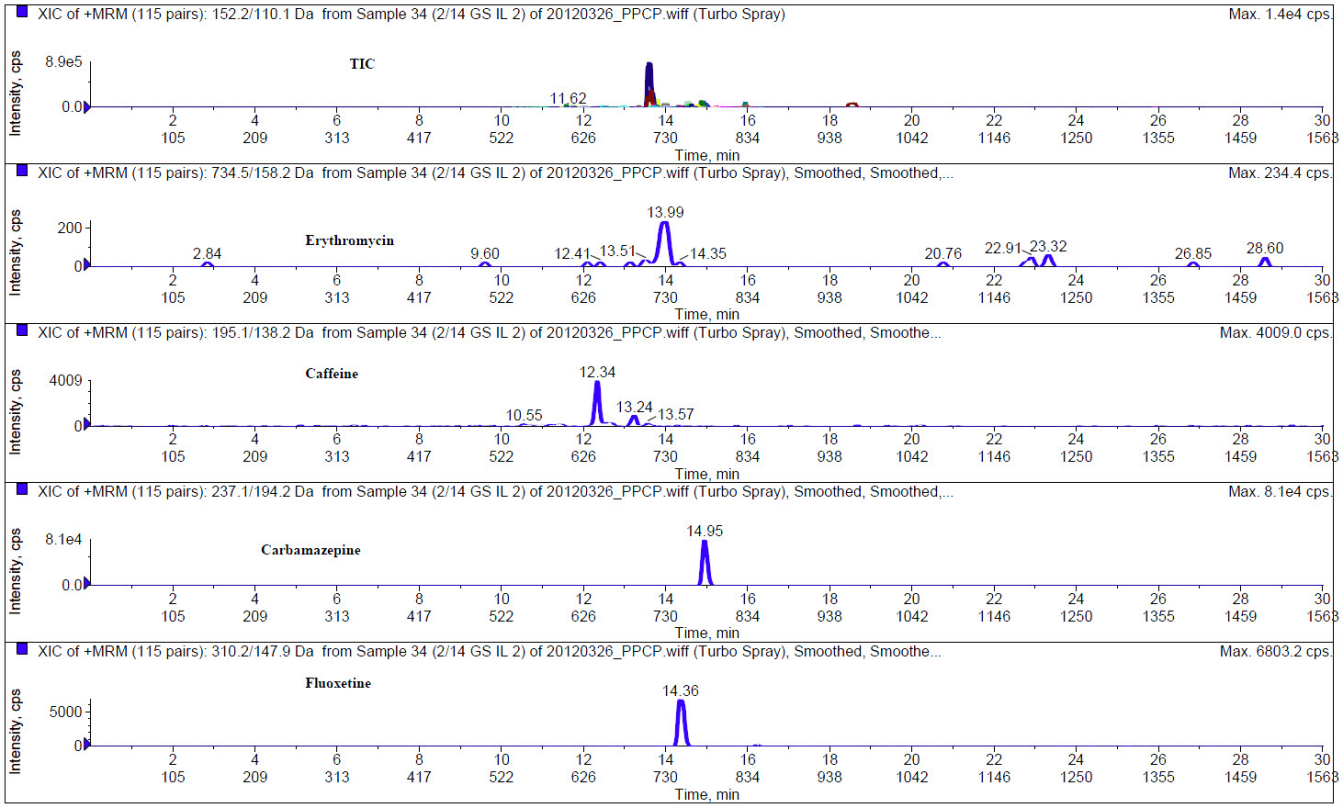

Figure 5. Automated SPE HPLC/MS-MS Analysis Results for 500 mL Samples for Four Target Analytes.

Summary

Efficiency with the SPE process for large volume water samples is addressed by comparing manual SPE to automated SPE, as well as comparing data that reduces the overall sample load volume and load time in half from 1000 mL to 500 mL. Recovery value for all 1000 mL automated SPE samples were within the expected EPA Method 1694 range, where the 500 mL manual SPE samples showed Erythromycin and Fluoxetine had reported values higher than the expected range. Comparability of mean recovery values between 1000 mL SPE samples and 500 mL SPE samples varies by less than 17% for the automated SPE samples, where the manual SPE samples vary by nearly three times from what the automated SPE samples reported, or up to 46% for the four target analytes when the sample volumes are compared.

Data reproducibility is a consideration when running samples. A robust method that eliminates environmental variables, technician variables, etc. can reduce the potential number of sample repeats performed. Comparing %RSD values from manual SPE samples and automated SPE samples provides a statistical representation of reproducibility. In all but one compound, % RSD values were lower for automated SPE samples vs. manual SPE samples. Caffeine reported from 1000 mL automated SPE samples showed nearly double the %RSD of the manual SPE samples reported for the same compound. Significant %RSD changes are visible with a five-fold reduction of Carbamazepine with the 1000 mL SPE samples and the two-fold reduction of Erythromycin with the 500 mL SPE samples.

This application provides good insight into 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.

References

1. Environmental Protection Agency (2007). EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS-MS.

2. The Groundwater Foundation: http://www.groundwater.org/gi/.