Alternate Analytical Method Validation for the Determination of the Presence and Concentration of Pharmaceutical and Personal Care Products in Wastewater Discharges and Sources of Drinking Water

Summary

In the last two decades, environmental professionals have become increasingly aware of the presenceof Pharmaceutical and Personal Care Products in our bodies of water.This is due to the indiscriminate use of these products by our modern societies, where personal wellness is focused on consumerism and increased economic growth.These pollutants, also known as “Emerging Contaminants, reach our waters in three main ways: direct discharge of untreated wastewater, Wastewater Treatment Plants (WWTPs) effluents, and by inadequate disposal of expired or unused products.The resulting impact on our ecosystemsis still uncertain, as thereis no conclusive data on the magnitudeand extent of this impact in the Americas.At present, the U.S. Environmental Protection Agency (EPA) has developed an analytical method to determinethe presenceand concentration of these emerging contaminants, MethodEPA 1694. This method requiresexpensive and specialized instrumentation, as it uses the High Performance Liquid Chromatography (HPLC) technique, combined with two (2) Mass Spectrometry (MS) detectors,in tandem.There are only a few environmental laboratories equippedwith HPLC/MS/MS instrumentation.This paper will present the validation process of an alternate analytical method for the detection and quantification of Pharmaceutical and Personal Care Products in aqueous samples, using Gas Chromatography with Mass Spectrometry (GC/MS) technique.

Introduction

Recently, the presence of emerging contaminants in our source of drinking water, and other surface waters has been brought to the spotlight. Among these pollutants, we can find active and inactive ingredients of pharmaceutical and personal care products, and other by-products.The pharmaceutical products are widely used in our modern society, where emphasis is placed mostly on the treatment of symptoms, rather than on preventive health care.And for the more privileged, there is an extensive selection of beauty, hygiene and personal care productscontaining multiple chemical components, which can make matters even more complicated.

Pharmaceutical products, and other legal and illegal drugs, are metabolized in our bodies and expelled via the urine and fecal matter. The same happens with animals, where veterinary pharmaceuticals are used also extensively to ensure the health of our pets and food supplies.Both in humans and animals, these pharmaceutical products are expelled unaltered, going directly into our waters. Some are metabolized, becoming byproducts of the original chemicals. There is the potential for interactions and

recombination of these substances once mixed together. Therefore, their effects on aquatic life forms can be augmentedor magnified.

Human and animal waste products may enter our bodies of water via untreated wastewater discharges. However, even our WWTPs may be contributing to the accumulation of these pollutants in our ecosystems. The truth is that none of our existing WWTPs were designed to treat and removethese chemical and pharmaceutical products. In some cases, but in lower numbers, the pollutants of concern are entering our surface waters due to the inadequatedisposal of medicines and otherexpired products, which are typically flushed down our toilets, or poured directly into the sinks of many offices, building and homes.

Conventional WWTPs are generally designed to handle human residues of organic nature. In these treatment systems, the nutrients and organic matter is primarily reduced and degraded by microbiological organisms acclimated to these discharges. In addition, other contaminants are removed by their own precipitation and absorption into the residual sludge resultingfrom thesebiologicalprocesses.

For this processes to be effective, microorganisms must be acclimated to these new contaminantsin order to be able to biodegrade them. Due to the vast amount and diversity ofthe chemical and pharmaceutical products reaching our WWTPs, in relatively low and variable concentrations, the challenge on the micro-biota is constant. When we add the antibiotic products so widely used in our medical treatments, to this cocktail of chemical products, we are making the challenge a bigger one. We can say without a doubt, that our present WWTPs cannot effectively handle these emerging contaminants, due to the fact that they were not designed for this task.

To make matter more complicate, the fresh water available for human consumption is becoming scarce. Reuse of residual water may help reduce the effect of water scarcity in the future, specifically in areas where the problem might be more significant. The presence of these emerging contaminants shall be studied in depth, so we can gain a clear understandingof the potential risks our population might be exposed to.At present, the U.S. Environmental Protection Agency (EPA) has developed an analytical methodto address this concern. In other geographical areas,scientists are studying possible alternatives so they can adapt available instrumentation and existing technology to determine the presence y concentration ofpharmaceutical and personal care products, and their byproducts, in our surface water and other potential source for water reuse.

EPA Method 1694

In the United States of America, the EPAhas created ananalytical methodologyspecificallyfor PPCP’sas environmental contaminants. This method is applicable to the following matrices: water, soil, sediment and biosolids.This method,

EPA 1694, uses the High Performance Liquid Chromatography (HPLC) technique, combined with two (2) Mass Spectrometry (MS) detectors, in tandem.It was originally developedto be used for compliance purposes, specifically the Clean Water Act (CWA). However, it can be adapted for other purposes, as it was developed based on standard EPA protocols, and current Good Laboratory Practices (cGLP's).

EPA Method 1694 may be modified as new needs may arise in the future, as well as other applications. This task will be up to the laboratory, which shall validate and demonstrate compliance with all Quality Assurance / Quality Control procedures of the analytical method.Therefore, this analysis and potential modifications shall be performed by specially trained laboratory personnel, familiar with the HPLC/MS/MS technique, orunder the direct supervision of properly trained and qualified personnel.

Thesampling activities for analysis under EPA Method 1694 shall be performed using standard protocols for the collection of aqueous samples.The following is a summary of the specific requirements for simple collection, preservation and handling:

1. Samples shall be collected in Amber Glass,1-Liter containers. One (1) 1-Liter container shall be collected for each factions to be extracted: acidic, and alkaline.That is, two (2) Liters shall be collected for each sample.Containers shall not be pre-rinsed with the waster to be sampled prior to simple collection. This procedure, known as "acclimatization", is not recommended.
2. If high concentrations of the compounds of concern are expected, two (2) additional containers, each 100-mL, shall be collected per sample.
3. Samples may be collected either Grab or Composite. If a composite sampling if performed, the sample volume inside the collection device shall be keep refrigerated, at 4°C (± 2°C), during the entire duration of the sampling event.
4. If residual chlorine is detected in the sample, it shall be neutralized using Sodium Thiosulfate,at a rate of 80 mg per Liter.
5. Samples shall be kept at a temperatureof 4°C (± 2° C), until arrival at the laboratory. Samplestemperatureshall not exceed 6°C.
6. Samples may be frozen, as it is understood that it will not affect theanalytical test results. A small free space shall be provided inside the container, to allow for the expansion of water while freezing.

The Holding Time (HT) for the samples, under EPA Method 1694, have not formally been established by the EPA.It is suggested that the extraction process is completed within 7 days after sample collection. However, the EPA recommends to extract the samples within 48 hours of sample collection, or de-freezing (if applicable. After the extraction process is completed the holding time for analysis is 40 days. At present, exceeding any of these holding times does not invalidate the analytical test results obtained under this method.

The compounds of interest under EPA Method 1694 are divided in 4 specific groups, numberedfrom 1 through 4. Each Group requires an independent individual analysis procedure (or run). The extraction process follows:

1. Groups 1, 2 y 3 are extracted under acidic conditions, that is, at pH < 2.
2. Group 4 is extracted under alkaline conditions, that is, at pH > 10.

Once the extractions are completed, the analysis procedure is summarized:

1. Groups 1 and 2 are analyzed usingPositive Electrospray Ionization (ESI+).
2. Group 3 isanalyzed using Negative Electrospray Ionization (ESI-).
3. Group 4, is analyzed using Positive Electrospray Ionization (ESI+).

The following is a list of thepharmaceutical compounds included in the analytical method:

Table1: Pharmaceutical Compounds inEPA Method 1694 (HPLC/MS/MS)

Compound	CAS Register #
Acetaminophen	103-90-2
Albuterol	18559-94-9
Ampicilin	69-53-4
Anhydrochlortetracycline (ACTC)	4497-08-9
Anhydrotetracycline (ATC)	4496-85-9
Azithromycin	83905-01-5
Caffeine	58-08-2
Carbadox	6804-07-5
Carbamazepine	298-46-4
Cefotaxime	63527-52-6
Chlortetracycline (CTC)	57-62-5
Cimetidine	51481-61-9
Ciprofloxacin	85721-33-1
Clarithromycin	81103-11-9
Clinafloxacin	105956-97-6
Cloxacillin	61-72-3
Codeine	75-57-3
Cotinine	486-56-6
Dehydrofenidipine	67035-22-7
Democlocycline	127-33-3
Digoxigenin	1672-46-4
Digoxin	20830-75-5
Diltiazem	42399-41-7
1,7-Dimethylxanthine	611-59-6
Diphenhydramine	58-73-1

Doxycycline	564-25-0
Enrofloxacin	93106-60-6
4-Epianhydrochlortetracycline (EACTC)	158018-53-2
4-Epianhydrotetracycline (EATC)	4465-65-0
4-Epichlortetracycline (ECTC)	14297-93-9
4-Epioxytetracycline (EOTC)	14206-58-7
4-Epitetracycline (ETC)	23313-80-6
Erythromycin	114-07-8
Erythromycin anhydrate	59319-72-1
Flumequine	42835-25-6
Fluoxetine	54910-89-3
Gemfibrozil	25812-30-0
Ibuprofen	15687-27-1
Isochlortetracycline (ICTC)	514-53-4
Lincomycin	154-21-2
Lomefloxacin	98079-51-7
Metformin	657-24-9
Miconazole	22916-47-8
Minocycline	10118-91-8
Naproxen	22204-53-1
Norfloxacin	70458-96-7
Norgestimate	35189-28-7
Ofloxacin	82419-36-1
Ormetoprim	6981-18-6
Oxacillin	66-79-5
Oxolinic acid	14698-29-4
Oxytetracycline (OTC)	79-57-2
Penicillin V	87-08-1
Penicillin G	61-33-6
Ranitidine	66357-35-5
Roxithromycin	80214-83-1
Sarafloxacin	98105-99-8
Sulfachloropyridazine	80-32-0
Sulfadiazine	68-35-9
Sulfadimethoxine	122-11-2
Sulfamerazine	127-79-7
Sulfamethazine	57-68-1
Sulfamethizole	144-82-1
Sulfamethoxazole	723-46-6
Sulfanilamide	63-74-1
Sulfathiazole	72-14-0
Tetracycline (TC)	60-54-8
Thiabenzadole	148-79-8
Triclocarban	101-20-2
Triclosan	3380-34-5

Trimethropin	738-70-5
Tylosin	1401-69-0
Virginiamycin	11006-76-1
Warfarin	81-81-2

This analytical technique is complex. It requires of specially trained, qualified analytical personnel to obtain reliable tests results.Another factor to consider is the economic reality of the countries in the region. There is a limited number of environmental laboratories in the Caribbean. Thecost for the instrumentation required for the analysis under Method 1694 (HPLC/MS/MS) exceeds $100,000. There are no regulatory requirements at present, to analyze for these emerging contaminants in any of the Caribbean countries of the CWWA. Not even in the United States, where a limited list of these pollutants have been included in the so-called “Unregulated Contaminant Monitoring Rule” (UCMR’s). One note: “Unregulated” means that the EPA has not determinedaMaximum Contaminant Level (MCL) for the discharge of this contaminant into the environment. This may be so because the medical science has not determined yet how much of a concentration may be harmful to a human’s health. Or how much of a contaminant is acceptable in our drinking water supply. This may explain the lack of regulations at present time regarding these emerging contaminants.

With all these facts on the table, it is not justifiable to expend over $100,000in such a rare, complex and expensive piece of equipment, an HPLC/MS/MS. But the emerging contaminants have to be detected and quantified, for us to understand the risks we are, and will be facing. Therefore, we must study the possibility of adaptingexisting analytical methods and instrumentation, to effectively detect these emerging contaminants.

Alternate Analytical Method Validation

Conventional analytical methods utilizevery diverse instrumentation to provide reliable test results. Specifically, the Gas Chromatography with Mass Spectrometry (GC/MS) technique is widely used in the detection of many Volatile Organic Compounds (VOC’s), as well Semi-Volatile Organic Compounds (SVOC’s). This technique is highly reliable, providing results with adequate precision, accuracy and sensitivity. This translates to Minimum Detection Limits(MDL’s) in the range of0.5 a 1.0 µg/L (parts per billion), in general.

The non-volatile nature of these emerging contaminants of interest,indicates thatthe GC/MS technique for Semi-Volatile Organic Compounds (SVOC’s) analysis is the most adequate. The applicable method for the analysis of SVOC’s is EPA 8270C. Many laboratories in the Caribbean, including Environmental Quality Laboratories Inc. (EQ Lab) in Puerto Rico, are equipped to perform this analysis, and have successfully

performed the InitialDemonstration of Capabilities (IDC), and the Minimum Detection Limit validation, for several of the contaminants of concern.

Sample collection for analysis underEPA Method 8270C is similar to the procedure mentioned earlier for EPA 1694:

1. Samples shall be collected in Amber Glass, 1-Liter containers. Only one (1) 1-Liter container is needed, as the extraction process is performed on the same simple aliquot. The extraction is made at acid, neutral and alkaline pH values. “Acclimatization” of sample containers is also not recommended.
2. Samples may be collected either Grab or Composite. If a composite sampling if performed, the sample volume inside the collection device shall be keep refrigerated, at 4°C (± 2°C), during the entire duration of the sampling event.
3. If residual chlorine is detected in the sample, it shall be neutralized using Sodium Thiosulfate, at a rate of 80 mg per Liter.
4. Samples shall be kept at a temperature of 4°C (± 2° C), until arrival at the laboratory. Samples temperature shall not exceed 6°C. Samples shall not be frozen.

As with EPA Method 1694, the holding time prior to the extractionunderEPA Method 3500B/3510C shall not exceed seven (7) days after sample collection. After the extraction process is completed the holding time for analysis is 40 days. The theoretical extraction process is as follows:

1. A first extraction under acidic conditions (pH < 2) is performed using either Methylene chloride or Chloroform (or both) depending on the solubility characteristics of the compounds of interest.The 2-minute procedure is repeated three (3) times.
2. A second extraction under neutral conditions (pH = 7) is performed using either Methylene chloride or Chloroform (or both) depending on the solubility characteristics of the compounds of interest. The 2-minute procedure is repeated three (3) times, but could be omitted if it is demonstrated that the compounds of concern can be recovered on the next extraction process.
3. Finally, an extraction under alkaline conditions (pH > 10) is performed using Methylene chloride or Chloroform (or both). As with the previous extractions, the 2-minute procedure is repeated three (3) times.
4. The extracts resulting from the three procedures are then mixed together, and ultimately concentrated to a volume of 1.0 mL, by Turbo-Evaporation with gaseous Nitrogen.

Once the extractions are completed, the analysis procedure by GC/MS is summarized

1. The concentrated solvent is introduced into the instrumentby Direct Liquid Injection (DAI). This is typically done by Auto-Injection systems integrated to the GC/MS.
2. A carrier gas, usually Ultra-High Purity Helium, moves the gasified solventthrough a capillary column. Generally, these columns are 30 meters long.
3. Compound separation occurs during this long “trip” inside the column. The more mobile compounds start moving ahead of the heavier compounds. These separation will allow the compounds to show up at the detector at a different time, called Retention Time.
4. When they reach the detector at the end of the column, this device identified the main ions within each compound.
5. Each compound is identified, under GC/MS technique, based on two (2) different criteria:
    a. The Retention Time (TR) which is compared to the RT determine for each compound during the initial calibration verification.And,
    b. La positive identification and confirmation of the compound, based on its main ions, which are compared with the data base in the computer integrated to the analytical instrumentation.

It is important to mention that this technique, GC/MS, does not require a confirmatory analysis under other techniques, due to its high reliability.

The following is a list of the compounds that have been validated by EQ Lab Inc, up to present, with their correspondingCAS Registry Number, and the respective Method Detection Limit:

Table2: Validated Compounds underEPA Method 8270C (GC/MS)

Compound	CAS Registry #	MDL (µg/L)
1,7 alpha - Ethynil estradiol	57-63-6	0.5
Caffeine	58-08-2	1.0
Cumarin	91-64-5	0.5
Diphenhydramine	58-73-1	0.5
HPMC	9004-65-3	1.0
Hydroquinone	123-31-9	0.5
Ibuprofen	15687-27-1	0.5
Maltodexin	9050-36-6	1.0
Propylene glycol	57-55-6	1.0
Pseudoepinephrine	98-82-4	0.5
Quinine	130-95-0	1.0

You may notice that this list is limited in number of compounds, when compared to the previous Table listed for EPA 1694. There is just one reason. EQ Lab is a commercial

laboratory. As there is no regulatory guidelines at present, we did engage in this validation process at the request of our clients. The industrial sector in Puerto Rico started to benchmark their discharges in order to understand the nature of their potential liabilities. We began validating these compounds, one at a time, upon individual requests of various pharmaceutical companies on the island. This has been an on-going process, and the Initial Demonstration of Capabilities (IDC), and the Minimum Detection Limit validation for each of the contaminants of concern have been performed successfully.

One highlight is worth mentioning. As these are pharmaceuticals compounds, EQ Lab did start the extraction process without pH adjustment, to avoid the possibility of altering the compounds with the pH change. Fortunately, all the compounds in this table have be recovered using only a neutral extraction, thus eliminating the need to repeat the process three times. This is a plus. And it is a good starting point.

Conclusions and Recommendations

Our past experience in the laboratory has proved that existing analytical instrumentation, when operated by qualified and skilled chemists and technicians, can be used to detect and quantify many of the compounds and emerging contaminants present is our wastewater discharges and surface water bodies.Many of these are not included in the list of applicable compounds found in the analytical methods, simply because the where not looked-for when these methods were created.The GC/MS technique is extremely powerful and versatile. Until clear regulations and guidelines are not established, mandating the use of highly specialized methods as EPA 1694, there is no justification for an environmental laboratory to expend over $100,000 in the acquisition of an HPLC/MS/MS. We have demonstrated that we can adapt existing, conventional and proven methods, to address the challenges presented by the presence of the emerging contaminants in our ecosystems.

It requires well trained professionals, with comprehensive knowledge of the instrumentation, its capabilities and its limitations. Well-developed analytical protocols and standard operating procedures. Robust Quality Assurance and Quality Control (QA/QC) programs. An adequate information management system, where complete and correct documentation of all activities related to the demonstration and validation processes is kept for auditing and future reference purposes.These are the key elements that will allow us to be ready for the reliable determination of the presence and concentration of pharmaceutical and personal care products in our wastewater discharges and sources of drinking water.

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