ALTERNATE ANALYTICAL METHOD FOR THE DETERMINATION OF THE PRESENCE AND CONCENTRATION OF PHARMACEUTICAL AND PERSONAL CARE PRODUCTS IN WASTEWATER DISCHARGES AND SOURCES OF DRINKING WATER

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HYSTORICAL BACKGROUND

PHARMACEUTICAL AND PERSONAL CARE PRODUCTS (PPCP's)

- Prescription Drugs, Hormonal Products
- Antibiotics
- Beauty and Personal Hygiene Products
- Sun Blockers, Fragrances
- Human and Animal Treatment
- Active and Inactive Ingredients
- Byproducts, Metabolites
- Micro-Beads
- Also Known as "Emerging Contaminants"

HYSTORICAL BACKGROUND

"**Emerging contaminants**" can be broadly defined as any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and(or) human health effects. (June 17, 2016)

toxics.usgs.gov/investigations/cec/index.php

EMERGING CONTAMINANTS

HOW DO THEY ENTER OUR WATERS?

- Direct Industrial Discharges
- Untreated Domestic Wastewater Discharges
- Wastewater Treatment Plant (WWTP's) Effluents
- Inadequate Disposal of Expired Products

¿ARE THESE REALLY "CONTAMINANTS"?

- WWTP's Are Not Designed to Treat Them
- Biodegradation and/or Reactions Create Unintended, Undesired Byproducts
- "Micro-Beads" Plastics Are Not Biodegradable

EMERGING CONTAMINANTS

WHY ARE THEY AN ENVIRONMENTAL PROBLEM?

- Designed for Human and/or Animal Metabolic Routes
- Effects on Other Biological Organisms is Unknown
- Effects are Unintended, Undesired
- Their Introduction to the Environment is Constant
- Even at Low Concentration, Effects Could Be Severe, Dramatic and Long Lasting
- Continuous Cycles of Exposure on Aquatic Organisms Lead to Bioaccumulation
- **CAUTION:** Sources for Water Reuse, Drinking Water

HYSTORICAL BACKGROUND

Karen Kidd, New Brunswick University (2004-2007)

- Isolated Lake, “Fathead Minnow” *(Pimephales promelas)*
- Estrogen in Low Concentration / Effects in Males
  - 1st Summer: Male Minnows Were Producing Egg Proteins
  - 2nd Summer: Sperm Cells Were Undeveloped. Shortly After That Males Produced Eggs as Well = Population Collapse
  - 4th Summer: Population Failed to Recover, Persistent Effects

USGS Douglas Chambers, Potomac River (2007)

- Study Showed That Sharp Drop in the Minnow Population was Attributed Only to Synthetic Estrogen. Surprising.
- Hypothesis: Environmental Stresses Could Lead to Population Collapse. Conclusion: Feminization alone caused the decline.

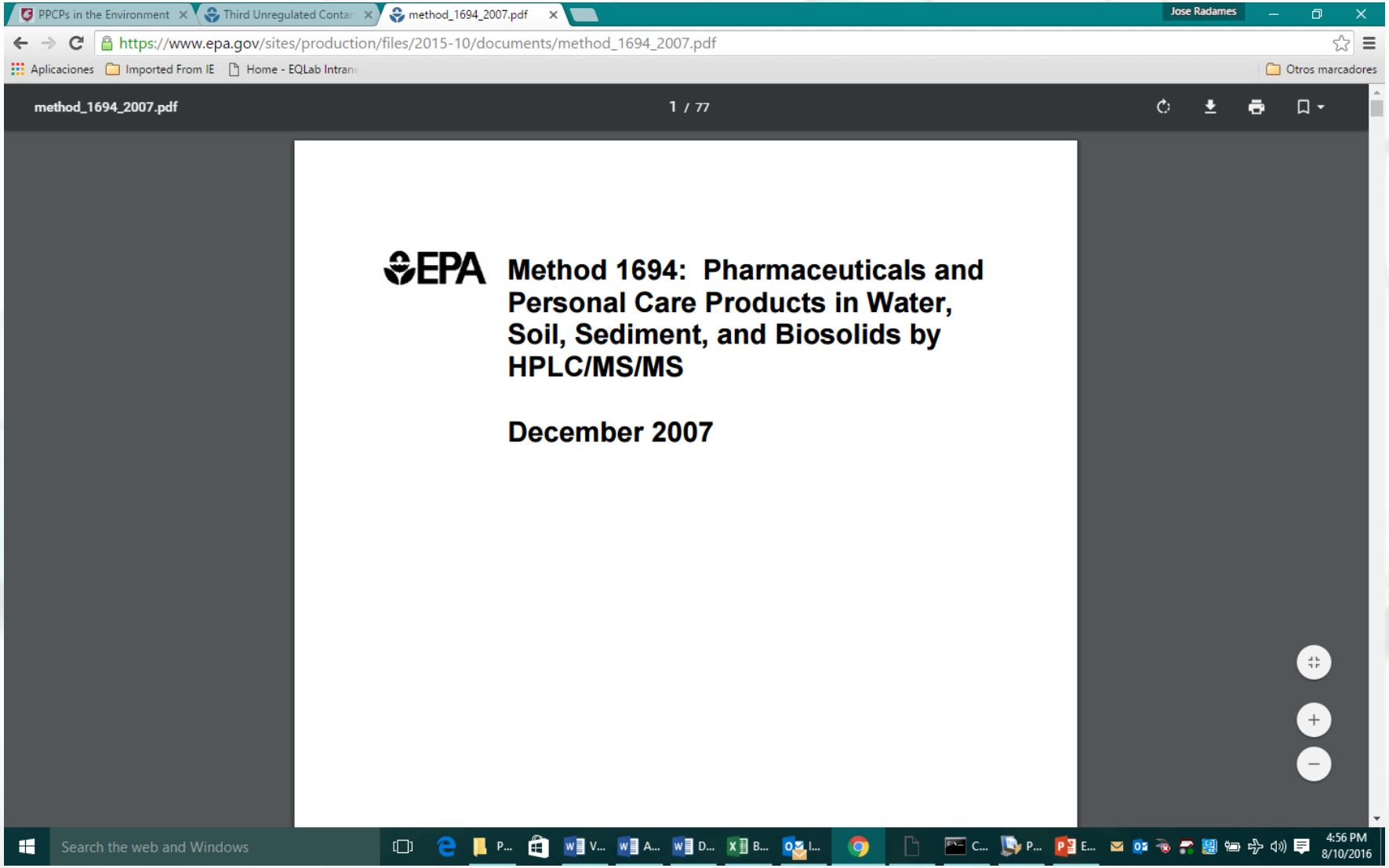
EMERGING CONTAMINANTS

Actual Situation USA / Environmental Protection Agency (EPA)

- No Existing Regulation for PPCP's Discharges to the Environment
- "Unregulated Contaminant Monitoring Rule" (UCMR 3, List 2), Only Applies to Hormones
  - Only Applies to Drinking Water
  - No Maximum Contaminant Limits are Specified
- EPA Published an Analytical Method for PPCP's (2007)
- Applicable to Water, Soil, Sediments y Biosolids

https://www.epa.gov/sites/production/files/2015-10/documents/method_1694_2007.pdf

EMERGING CONTAMINANTS



EMERGING CONTAMINANTS

23.0 Tables and Flowchart

Table 1. Names and CAS Registry numbers for pharmaceuticals and personal-care products (PPCPs) determined by isotope dilution and internal standard HPLC/MS/MS

Compound	CAS Registry	Labeled analog	CAS Registry
Acetaminophen	103-90-2	$^{13}C_2$-^{15}N-Acetaminophen	
Albuterol	18559-94-9	Albuterol-d_3	
Ampicillin	69-53-4		
Anhydrochlortetracycline (ACTC)	4497-08-9		
Anhydrotetracycline (ATC)	4496-85-9		
Azithromycin	83905-01-5		
Caffeine	58-08-2	$^{13}C_3$-Caffeine	
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	$^{13}C_3$-^{15}N-Ciprofloxacin	
Clarithromycin	81103-11-9		
Clinafloxacin	105956-97-6		
Cloxacillin	61-72-3		
Codeine	76-57-3		
Cotinine	486-56-6	Cotinine-d_3	
Dehydronifedipine	67035-22-7		
Demeclocycline	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	$^{13}C_2$-Erythromycin anhydrate	
Flumequine	42835-25-6		
Fluoxetine	54910-89-3	Fluoxetine-d_5	
Gemfibrozil	25812-30-0	Gemfibrozil-d_6	

EMERGING CONTAMINANTS

Compound	CAS Registry	Labeled analog	CAS Registry
Ibuprofen	15687-27-1	$^{13}C_3$-Ibuprofen	
Isochlortetracycline (ICTC)	514-53-4		
Lincomycin	154-21-2		
Lomefloxacin	98079-51-7		
Metformin	657-24-9	Metformin-d_6	
Miconazole	22916-47-8		
Minocycline	10118-91-8		
Naproxen	22204-53-1	^{13}C-Naproxen-d_3	
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	$^{13}C_6$-Sulfamethazine	
Sulfamethizole	144-82-1		
Sulfamethoxazole	723-46-6	$^{13}C_6$-Sulfamethoxazole	
Sulfanilamide	63-74-1		
Sulfathiazole	72-14-0		
Tetracycline (TC)	60-54-8		
Thiabendazole	148-79-8	Thiabendazole-d_6	
Triclocarban	101-20-2	$^{13}C_6$-Triclocarban	
Triclosan	3380-34-5	$^{13}C_{12}$-Triclosan	
Trimethoprim	738-70-5	$^{13}C_3$-Trimethoprim	
Tylosin	1401-69-0		
Virginiamycin	11006-76-1		
Warfarin	81-81-2	Warfarin-d_5	
Other standards			
Unlabeled compound spiked into sample and used for recovery correction			
Meclocycline			
Labeled injection internal standard spiked into sample extract prior to injection into LC/MS/MS			
		$^{13}C_3$-Atrazine	
		$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid ($^{13}C_6$-TCPAA)	

EPA METHOD 1694

Analytical Method, Instrumentation Advantages

- High Performance Liquid Chromatography (HPLC)
- Double (2), Mass Spectrometer Detectors (MS/MS), Aligned in Tandem
- Ultra-High Sensitivity
- Very Low Detection Limits
- High Reliability of Results
- Precision
- Accuracy
- Reliable Technology

EPA METHOD 1694

Analytical Method, Instrumentation Disadvantages

- Highly Specialized Analytical Equipment
- EPA/USGS Use for Analysis of Pharmaceutical and Veterinary Medicine Residues, and Pesticides, as well as metabolites of these products.
- Pharmaceutical Products, Industrial Applications
- Equipment and Instrumentation are Very Expensive
- Not Common in the Environmental Analytical Field
- Few Analytical Laboratories Available (3-4 in USA)
- Recently Developed (2007)
- Complex Extraction and Analytical Process

EPA 1694 PROCEDURE

Sample Collection

- 1-Liter for Acid Fraction
- 1-Liter for Alkaline Fraction
- 2 Additional Containers (100 mls) if High Concentrations are Expected or Possible
- Neutralization of Residual Chlorine with Sodium Thiosulfate
- Ice to 4°C, ± 2°C
- Samples Can Be Frozen
- 7-Days Maximum Holding Time (HT) for Extractions, 48 Hours are Recommended

EPA 1694 PROCEDURE

Sample Analysis

- Groups 1, 2 y 3: Extract at pH < 2
- Group 4: Extract at pH > 10
- Groups 1 y 2: Are Analyzed in Positive Electro-Spray Ionization (ESI+) Mode
- Group 3 are Analyzed in Negative Electro-Spray Ionization (ESI-) Mode
- Group 4 is Analyzed in ESI+ Mode
- 2 Extraction Processes, Repeated 3 Times
- 4 Analytical Processes, 3-ESI+, 1-ESI-

EPA 1694 INSTRUMENTATION

ALTERNATE METHOD

EPA 8270C (SW-846)

- Gas Chromatography Technique, Mass Spectrometer Detector (GC/MS)
- Proven Technology, Reliable
- High Sensitivity, Low Detection Limits
- High Reliability of Results (i.e., Precision, Accuracy)
- Widely Used in the Environmental Analytical Field
  - VOC's
  - SVOC's
  - Confirmatory Analyses
- Available in Many Laboratories, Countries

EPA 8270C PROCEDURE

Sample Collection

- 1-Liter Amber Glass / Teflon-Lined Cap for Liquid Extraction
- No Additional Containers, Only for Internal QA/QC Samples (DUP's, MS)
- Neutralization of Residual Chlorine with Sodium Thiosulfate
- Ice to a 4°C, ± 2°C
- Samples Can Not Be Frozen
- 7-Days Maximum Holding Time (HT) for Extractions

SAMPLE CONTAINERS

EPA 8270C PROCEDURE

Sample Extraction

- Extraction with Either Dichloromethane or Chloroform
  - At pH < 2. Repeated 3 Times.
  - At pH = 7. Repeated 3 Times.
  - At pH > 12. Repeated 3 Times.
- Then, All Extracts are Mixed Together
- Solvent Volume is Concentrated Down to 1.0 ml, Using Turbo-Evaporators, Gaseous Nitrogen
- Final Transfer to “Micro-Vial”
- 40 Days Maximum HT for Analysis

LIQUID EXTRACTION

EPA 8270C PROCEDURE

Sample Extraction

- Extraction with Either Dichloromethane or Chloroform
  - At pH < 2. Repeated 3 Times.
  - At pH = 7. Repeated 3 Times.
  - At pH > 12. Repeated 3 Times.
- Then, All Extracts are Mixed Together
- Solvent Volume is Concentrated Down to 1.0 ml, Using Turbo-Evaporators, Gaseous Nitrogen
- Final Transfer to “Micro-Vial”
- 40 Days Maximum HT for Analysis

TURBO-EVAPORACIÓN

EPA 8270C PROCEDURE

Sample Extraction

- Extraction with Either Dichloromethane or Chloroform
  - At pH < 2. Repeated 3 Times.
  - At pH = 7. Repeated 3 Times.
  - At pH > 12. Repeated 3 Times.
- Then, All Extracts are Mixed Together
- Solvent Volume is Concentrated Down to 1.0 ml, Using Turbo-Evaporators, Gaseous Nitrogen
- Final Transfer to “Micro-Vial”
- 40 Days Maximum HT for Analysis

“MICRO-VIAL”

EPA 8270C PROCEDURE

Sample Analysis

- Direct Liquid Injection
- Automated Sample-Injector 
- Capillary Column
- Long Distance Race
- ID by “Retention Time”
- ID by Mass/Ratio of Principal Ions
- No Confirmation Required
- **GC/MS Technology = A1+**

SAMPLE AUTO-INJECTOR

EPA 8270C PROCEDURE

Sample Analysis

- Direct Liquid Injection
- Automated Sample-Injector
- Capillary Column
- Long Distance Race
- ID by “Retention Time”
- ID by Mass/Ratio of Principal Ions
- No Confirmation Required
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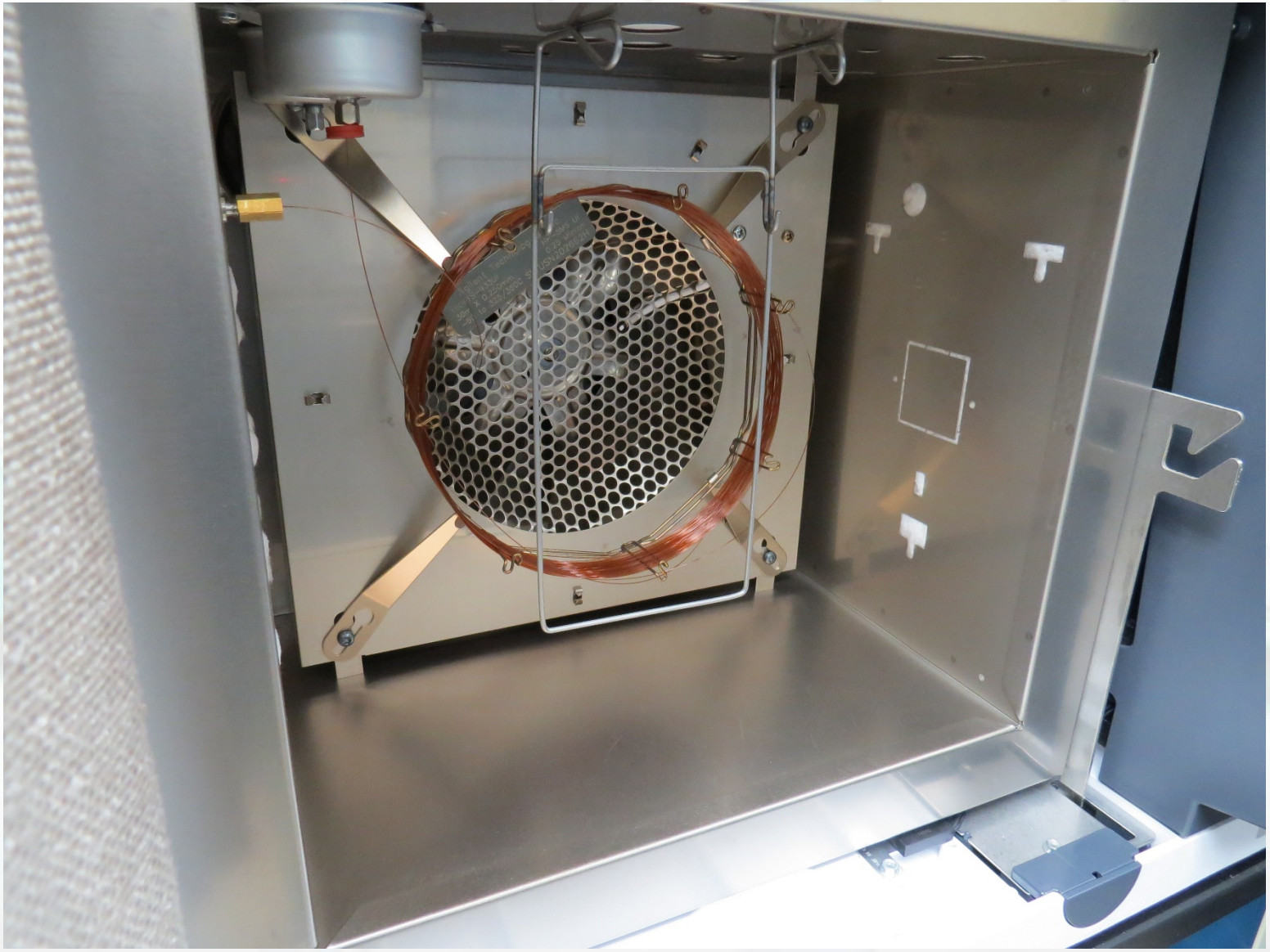
CAPILLARY COLUMN



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- **GC/MS Technology = A1+**

MASS SPECTROMETRY DETECTOR

GC/MS INSTRUMENTATION ARRAY

VALIDATED COMPOUNDS

Compounds	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

* Hydroxypropyl methyl cellulose

COMPUESTOS VALIDADOS

Compuesto	pH Extracción	Solvente
1,7 alpha - Ethynil estradiol	pH = 7, Neutral	Dichloromethane
Caffeine	pH = 7, Neutral	Dichloromethane
Cumarin	pH = 7, Neutral	Dichloromethane
Diphenhydramine	pH = 7, Neutral	Chloroform
HPMC*	pH = 7, Neutral	Chloroform
Hydroquinone	pH = 7, Neutral	Chloroform
Ibuprofen	pH = 7, Neutral	Chloroform
Maltodexin	pH = 7, Neutral	Dichloromethane
Propylene Glycol	pH = 7, Neutral	Chloroform
Pseudoepinephrine	pH = 7, Neutral	Chloroform
Quinine	pH = 7, Neutral	Dichloromethane

* Hydroxypropyl methyl cellulose

SUMMARY

PPCP's Analysis by GC/MS Technique

- Simplified Extraction Process
- Analytical Instrumentation, Reliable, Accessible
- Adequate Sensitivity
- Adequate Precision, Accuracy
- No Confirmation Required

Recommendations

- Specific Public Policies Regarding PPCP's
- Identify Potential Sources / Risk Assessment
- Identify Available Analytical Resources
- ¡Action!

¡THANKS!

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