

Agilent's 6410 LC/MS/MS Solution for EPA Method 1694:

Pharmaceuticals and Personal Care Products in Water, Soil,
Sediment, and Biosolids by HPLC/MS/MS

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About Emerging contaminants:

- Measuring emerging contaminants in the environment is an area of active environmental research.
- In the area of PPCPs there are several methods addressing these analytes including EPA Method 1694.
- This report describes the Agilent solution to Method 1694 (June 2008)

EPA Method 1694

1. The EPA Method 1694 is demonstrated with the Agilent QQQ model 6410.
2. The Agilent initial implementation for EPA method 1694 consists of 65 analytes (of 75 total analytes) and 17 labeled internal standards (of 20 total), which are a mixture of pharmaceuticals and personal care products that are analyzed each by a single MRM transition. (Note that the other compounds and internal standards could not be readily obtained at this time.)
3. The method also uses Agilent columns, C-18 and HILIC column for all analytes.

How does the EPA Method Work?

1. Standard EPA protocol is used for sample extraction, either solids extraction (acetonitrile, filtration, and centrifugation) or SPE for water samples (using Oasis HLB). See protocol PDF from EPA method for details.
2. Extracts are then analyzed directly by the Agilent 6410 LC/MS/MS using a single transition for each compound as prescribed by EPA.
3. Quantitation and QA/QC follow EPA protocol.

Agilent Method with Additional Confirmation

To provide additional confirmation, a second MRM transition was added for 60 of the 65 analytes analyzed. This gives an even greater assurance of correct analysis than prescribed by EPA.



List of Group 1 Compounds: 46 Analytes

Acetaminophen	Codeine	Lincomycin	Sarafloxacin	Sulfanilamide
Ampicillin	Cotinine	Lomefloxacin	Sulfachloro-pyridazine	Thiabendazole
Azithromycin	Dehydronifedipine	Miconazole	Sulfadiazine	Trimethoprim
Caffeine	Digoxigenin	Norfloxacin	Sulfadi-methoxine	Tylosin
Carbadox	Diltiazem	Ofloxacin	Sulfadiazine	Virginiamycin
Carbamazepine	1,7-Dimethyl-xanthine	Oxacillin	Sulfadi-methoxine	Digoxin*
Cefotaxime	Diphenhydramine	Oxolinic acid	Sulfamerazine	
Ciprofloxacin	Enrofloxacin	Penicillin G	Sulfamethazine	
	Erythromycin			
Clarithromycin	Flumequine	Penicillin V	Sulfamethizole	
Cloxacillin	Fluoxetine	Roxithromycin	Sulfa-methoxazole	

*Compound formed intractable Na adduct with current conditions.

List of EPA 1694 Group 2, 3 and 4 Compounds: 19 Analytes

Anhydrotetracycline	Doxycycline	Minocycline	Triclocarban
			Triclosan
			Warfarin
Chlorotetracycline	4-Epianhydrotetracycline	Tetracycline	Albuterol
		Meclocycline	Cimetidine
			Metformin
Demeclocycline	4-Epitetracycline	Gemfibrozil	Ranitidine
		Ibuprofen	
		Naproxen	

List of Labeled Internal Standards

13C-15N-Acetaminophen	13C2-Erythromycin	13C6-Sulfamethazine	13C3-Trimethoprim
13C3-Atrazine	Fluoxetine-d6	13C6-Sulfamethoxazole	Warfarin-d5
13C3-Caffeine	Gemfibrozil-d6	13C6-2,4,5-Trichlorophenoxyacetic acid	Carbamazepine-d10 (Extra compound, not EPA list)
13C3-15N-Ciprofloxacin	13C3-Ibuprofen	13C6-Triclocarban	
Cotinine-d3	13C-Naproxen-d3	13C12-Triclosan	



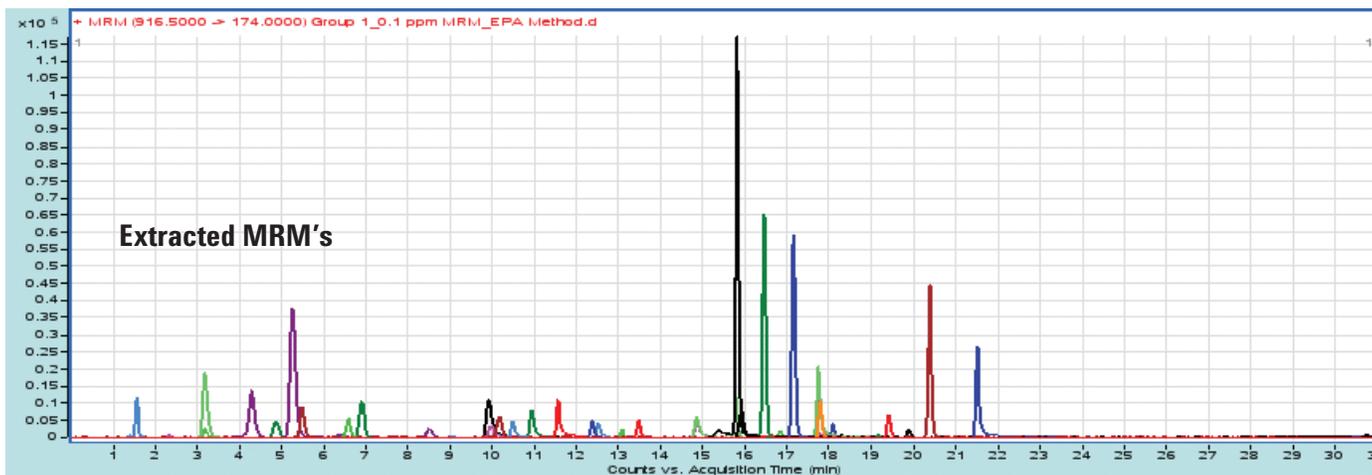
Columns and Gradients

- The column for group 1, 2, and 3 is a ZORBAX Eclipse Plus C18, narrow bore RR 2.1x100mm 3.5 micron column. Part number 959793-902.
- Group 1: The gradient is from 10% acetonitrile, 90% 0.1 % formic acid in water, held for 5 minutes at 0.2 mL/min, then to 0.3 mL/min in 1 minute. In 24 min go to 60% ACN and finally to 100% ACN in 30 minutes.
- Group 2: The gradient is from 10% acetonitrile 90% 0.1 % formic acid in water, held for 10 minutes, and then to 100% ACN in 30 minutes at a flow rate of 0.2 mL/min.
- Group 3: The gradient is from 40% acetonitrile/methanol and 60% water (0.1% ammonium acetate+0.1% Acetic Acid) to 100% ACN/MeOH in 12.5 minutes at a flow rate of 0.2 mL/min.
- Group 4: The gradient is from 98% acetonitrile for 5 min to 70% ACN in 12 minutes at a flow rate of 0.25 mins. The aqueous solvent is 10 mM ammonium acetate, pH 6.7. The column for HILIC is a ZORBAX Rx-SiL, narrow bore RR 2.1x100mm 3.5 micron column.
www.chem.Agilent.com/cag/lccustom_1page.asp

Overview of LC/MS/MS Fractions

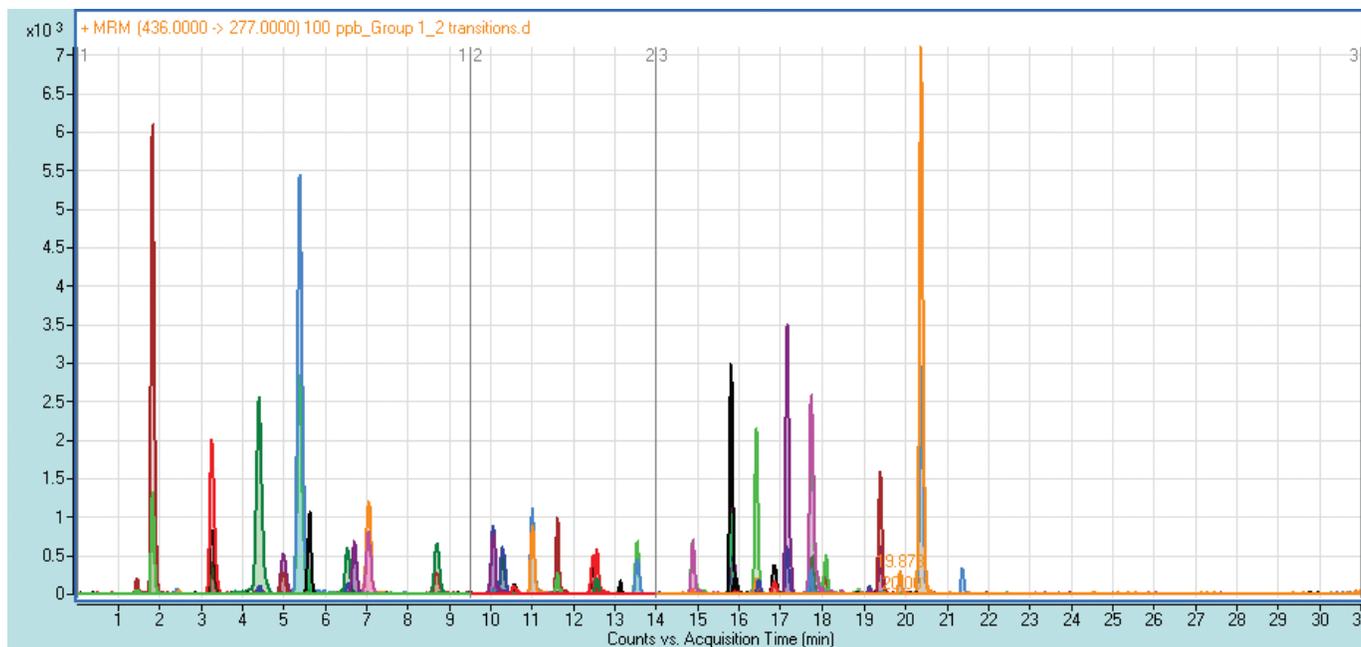
1. Group 1: Acidic Extraction by ESI + (C18 column)
2. Group 2: Acidic Extraction by ESI + (C18 column)
3. Group 3: Acidic Extraction by ESI – (C18 column)
4. Group 4: Basic Extraction by ESI + (HILIC column).

Group 1 Compounds



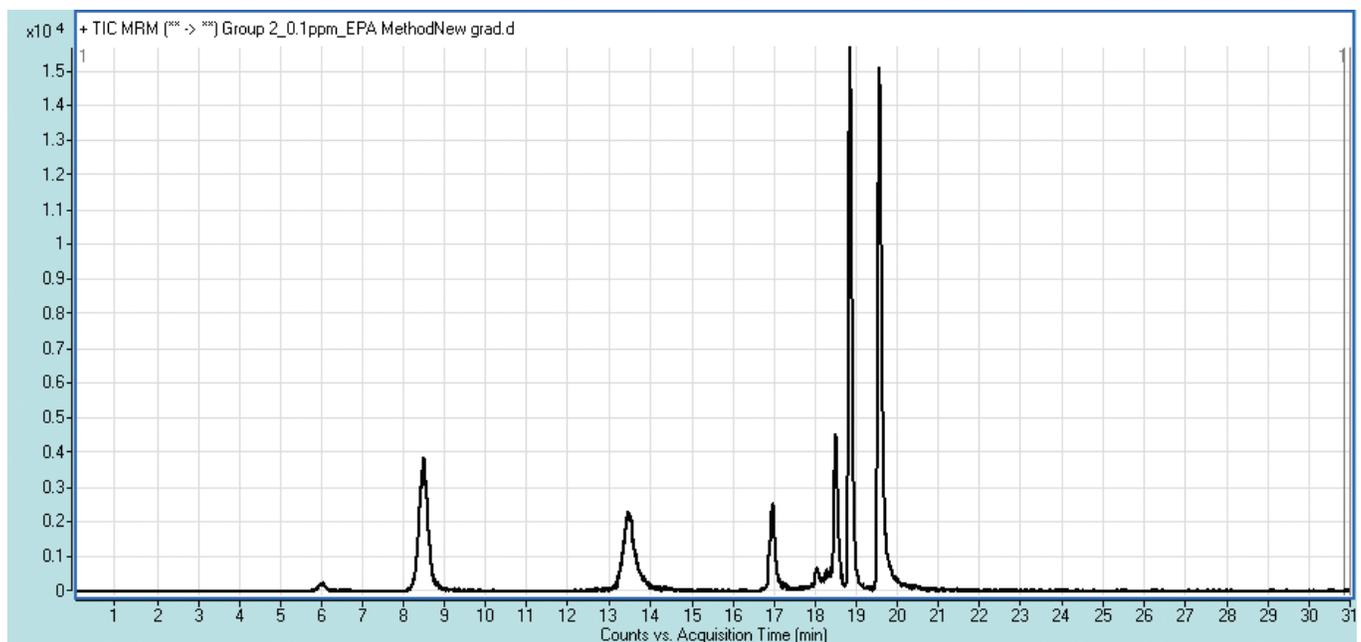
Note: Chromatogram for Extracted MRMs for Group 1 Compounds with one transition per compound using one time segment (per EPA Method 1694)

Group 1 Compounds: Agilent Addition: 2 Transitions and 3 Time Segments



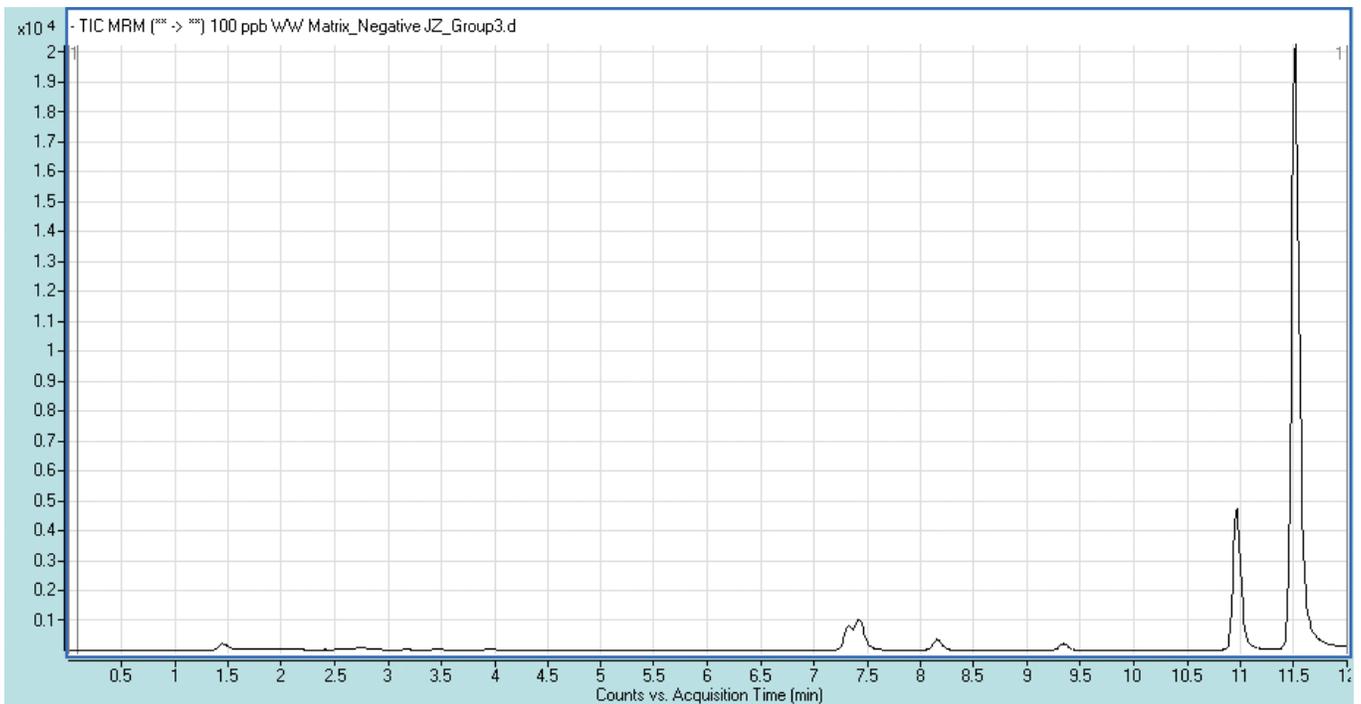
Note: Chromatogram for Extracted MRMs for Group 1 Compounds with two transition per compound using three time segments

TIC: Group 2 Compounds



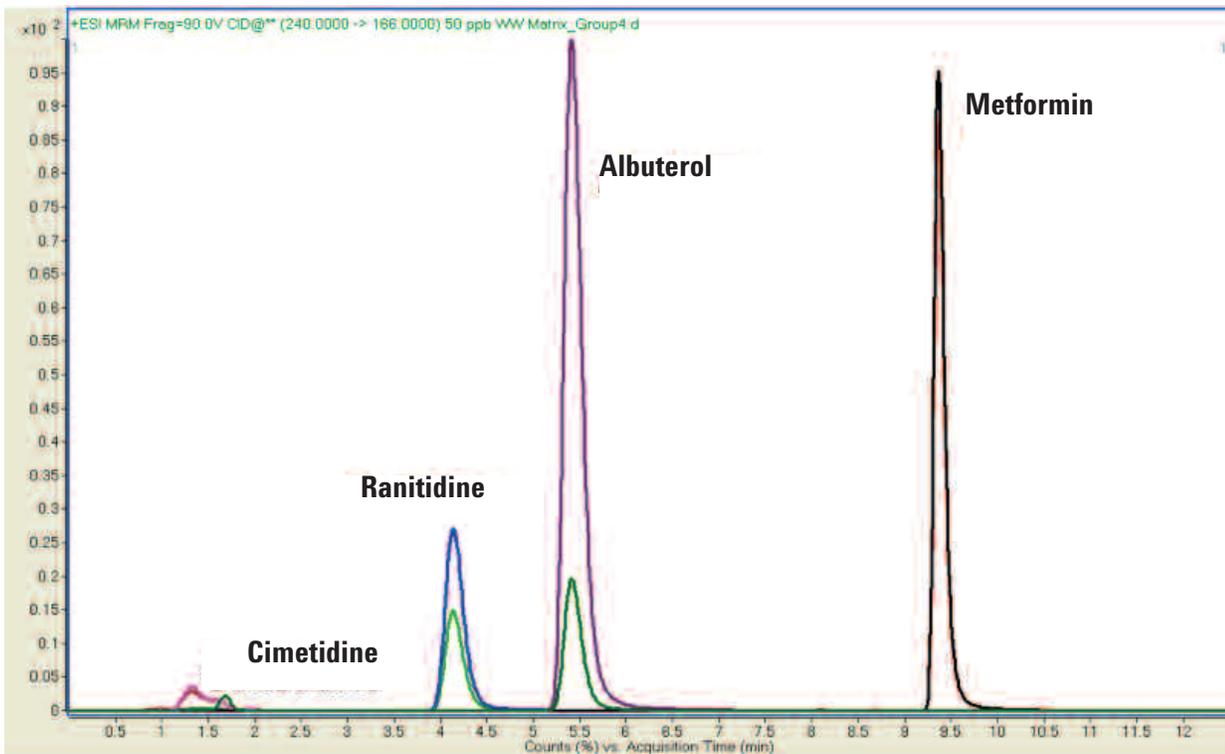
Note: TIC Chromatogram for for Group 2 Compounds with one transition per compound using one time segment (per EPA Method 1694)

TIC: Group 3 Compounds



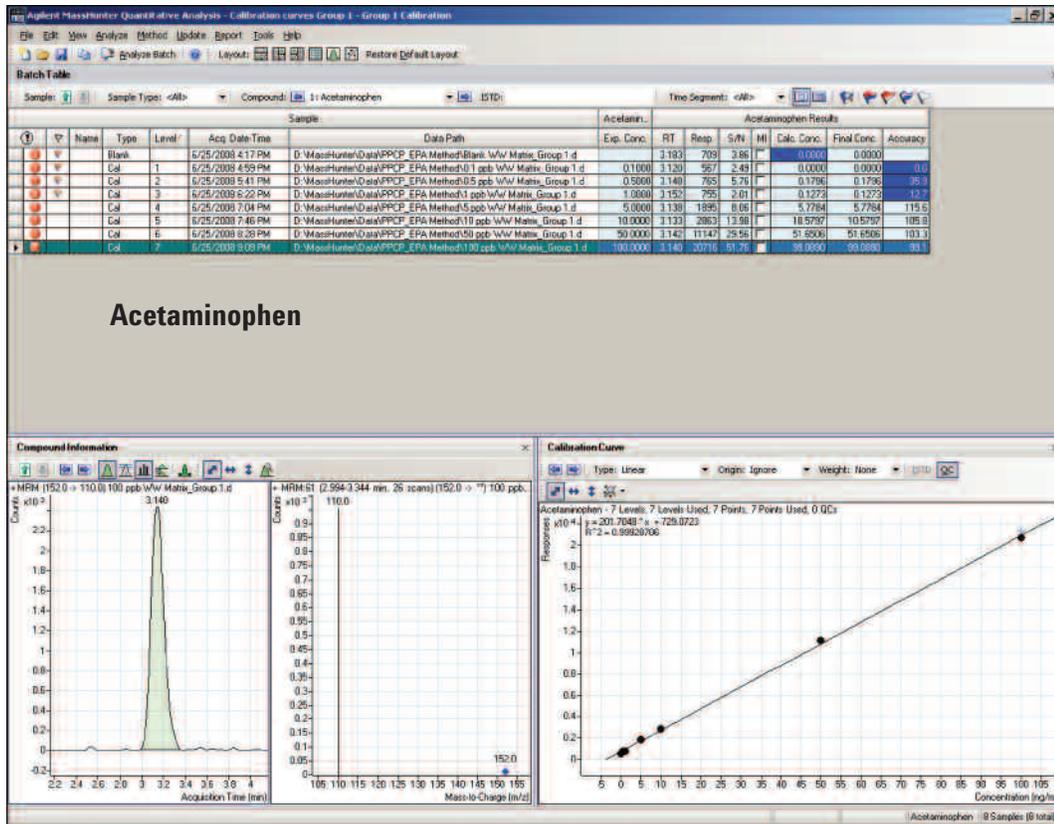
Note: TIC Chromatogram for for Group 3 Compounds with one transition per compound using one time segment (per EPA Method 1694)

Group 4 Compounds

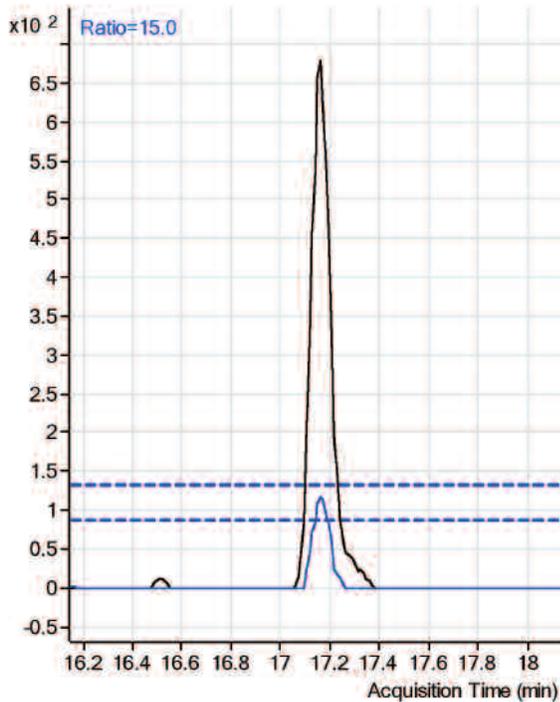


Note: Chromatogram for Extracted MRMs for Group 4 Compounds with two transition per compound using one time segment

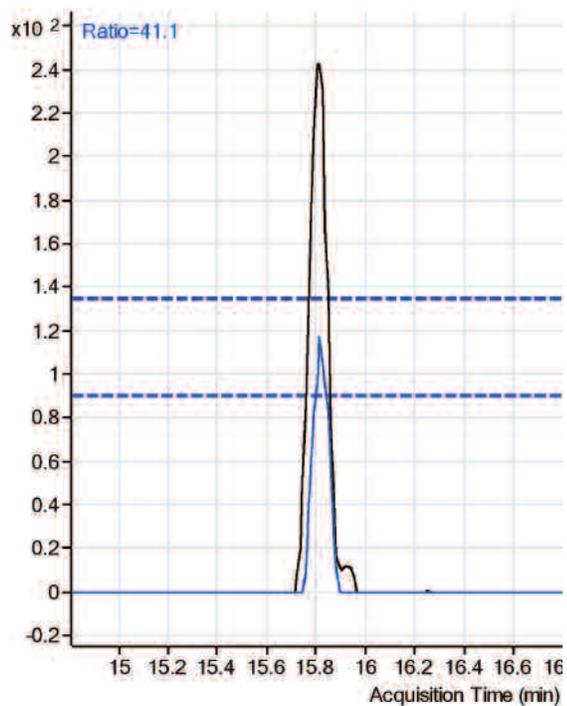
Calibration Curves in Wastewater Matrix



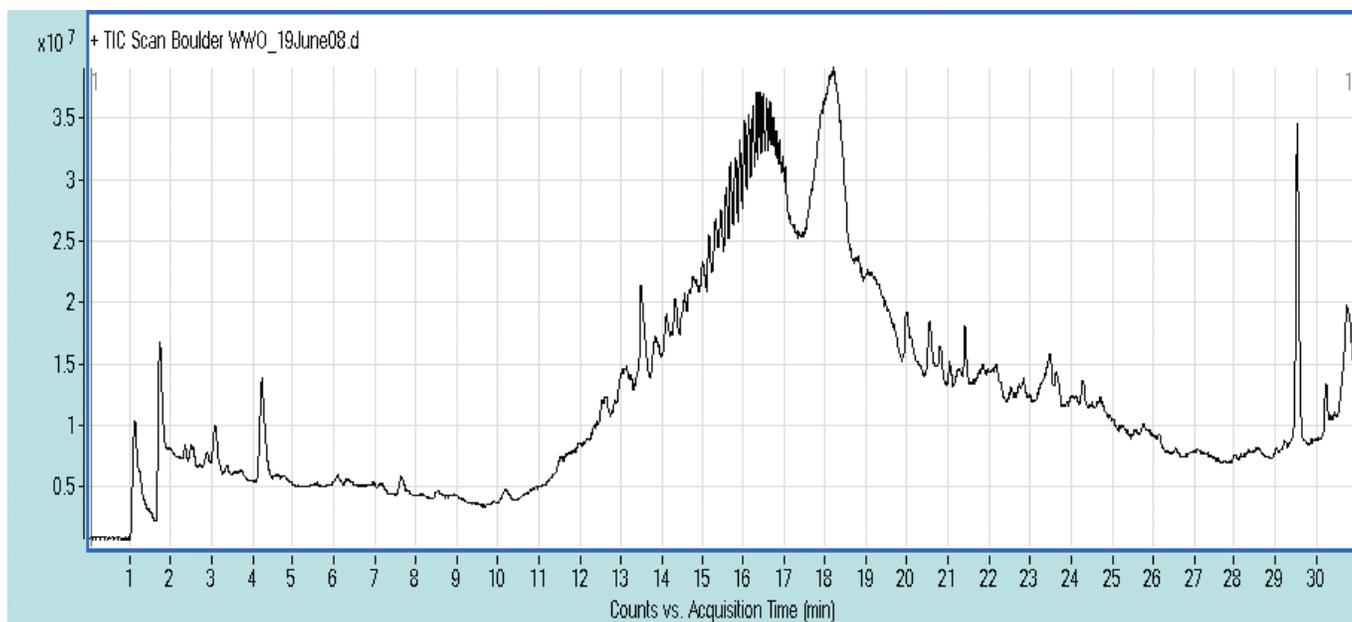
Agilent Addition using qualifier ions: Carbamazepine with a qualifier transition



Agilent Addition using qualifier ions: Sulfadimethoxine with a qualifier transition

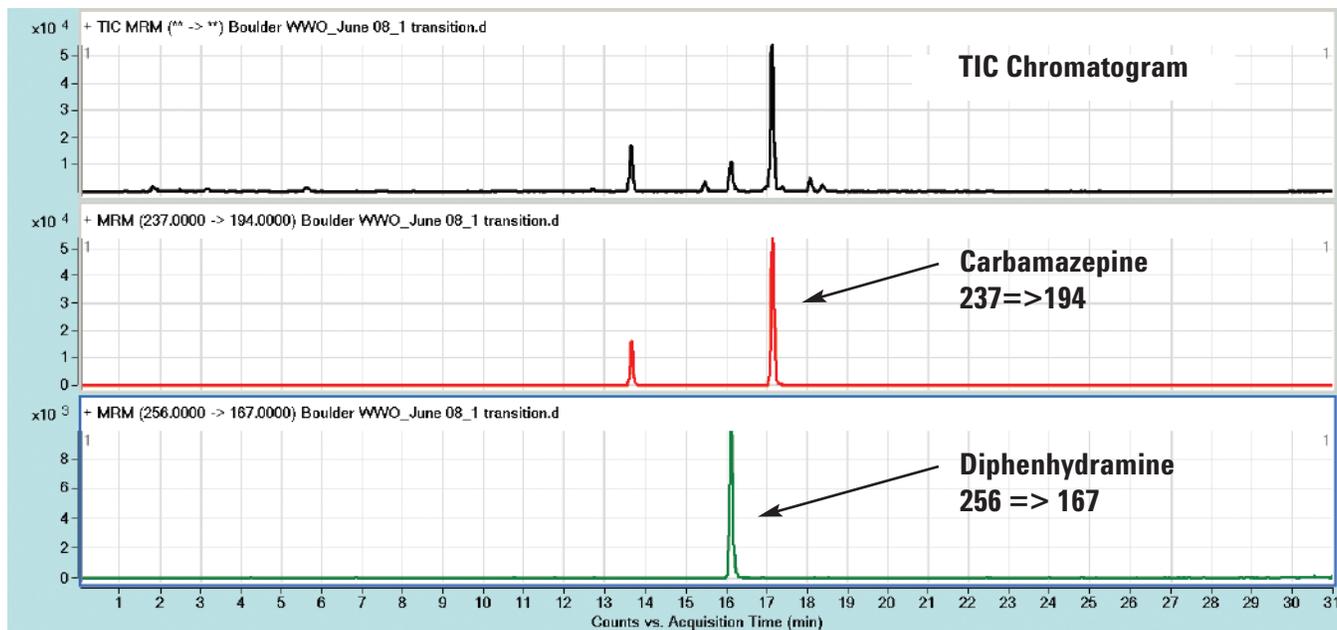


LC/TOF-MS Analysis of Boulder, Colorado, Wastewater Outfall



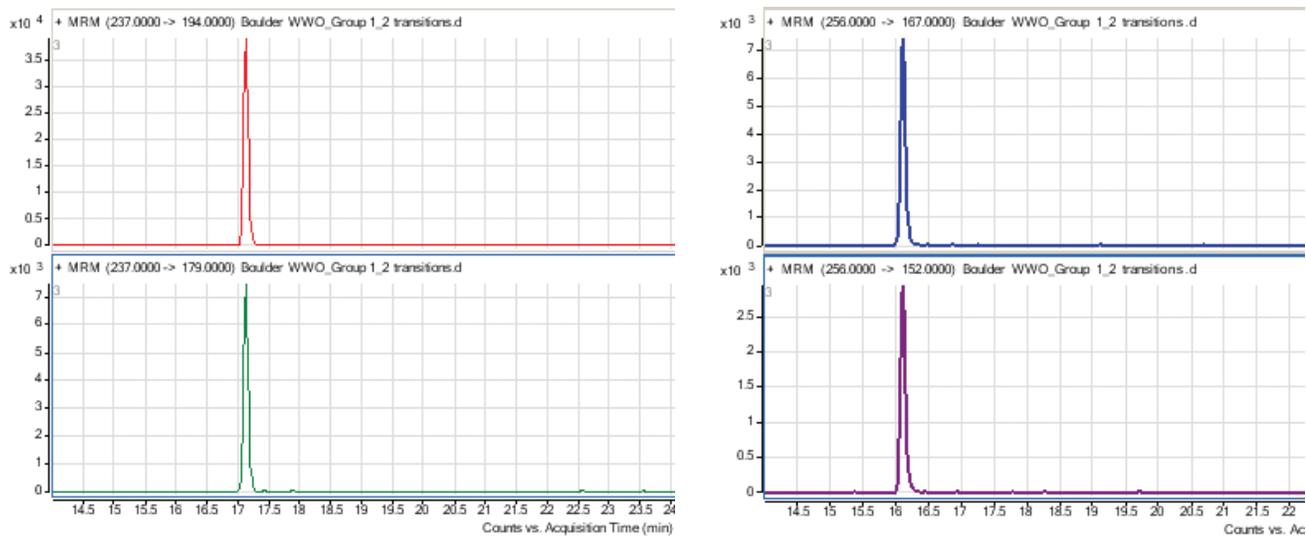
Note: To illustrate the complexity of wastewater samples, Boulder Colorado Wastewater was run using LC/TOF. Above is the TIC for this analysis.

LC/MS/MS Analysis of Boulder Wastewater Outfall for EPA 1694 Group 1



Note: Using LCQQQ MS/MS with one transition per compound, two compounds are clearly differentiated.

LC/MS/MS Analysis of Boulder Wastewater Outfall for EPA 1694 Group 1 with 2 transitions



Note: Chromatogram for Extracted MRMs for Carbamazepine and Diphenhydramine with two transition per compound.

Conclusions

- Agilent 6410 LC/MS/MS has been set up to run the EPA method 1694 for pharmaceuticals and personal care products using Agilent LC supplies (C18 and HILIC column).
- Two transitions are available for definitive identification of PPCPs in water.
- The method will soon have a SOP and Application Note describing the entire procedure.

