EPA Method 1694: Agilent's 6410A LC/MS/MS Solution for Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS

Application Note

Environmental

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Abstract

An analytical methodology for screening and confirming the presence of 65 pharmaceuticals in water samples was developed using the Agilent G6410A Triple Quadrupole mass spectrometer (QQQ). The method was developed following the guidelines in EPA Method 1694. Four distinct chromatographic gradients and LC conditions were used according to the polarity and extraction of the different pharmaceuticals. Positive and negative ion electrospray were used with two multi-reaction monitoring (MRM) transitions (a quantifier and a qualifier ion for each compound), which adds extra confirmation in this methodology compared with the EPA method. Linearity of response of three orders of magnitude was demonstrated ($r^2 > 0.99$) for all the pharmaceuticals studied. The analytical performance of the method was evaluated for one wastewater sample collected from Boulder Creek, Colorado; positive identifications for carbamazepine and diphenhydramine were found for this sample using the methodology developed in this work.

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Introduction

The analytical challenge of measuring emerging contaminants in the environment has been a major research focus of scientists for the last 20 years. Pharmaceuticals and personal care products (PPCPs) are an important group of contaminants that have been targeted, especially in the last decade. In the area of PPCPs there are several methods addressing the analysis of these analytes, including EPA Method 1694 [1], which was recently published (December 2007). This EPA protocol uses solid-phase extraction (SPE) for water sample preparation [1]. The extracts are then analyzed directly by a tandem mass spectrometer using a single transition for each compound. This application note describes the Agilent solution to this method, which is demonstrated with the Agilent model 6410A LC/MS QQQ. 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 PPCPs that are analyzed each by a single MRM transition. (Note that the other compounds and internal standards could not be obtained at this time.) The method also uses Agilent C-18 and Hydrophilic Interaction Chromatography (HILIC) columns for all analytes. 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 identification than prescribed by the EPA. Table 1 shows the list of pharmaceuticals studied here.

Table 1. Analytes Studied in This Work

List of Group 1 Compounds EPA 1694: 46 Analytes

Acetaminophen	Codeine	Flumequine	Penicillin V	Sulfanilamide
Ampicillin	Cotinine	Fluoxetine	Roxithromycin	Thiabendazole
Azithromycin	Dehydronifedipine	Lincomycin	Sarafloxacin	Trimethoprim
Caffeine	Digoxigenin	Lomefloxacin	Sulfachloropyridazine	Tylosin
Carbadox	Diltiazem	Miconazole	Sulfadiazine	Virginiamycin
Carbamazepine	1,7-Dimethylxanthine	Norfloxacin	Sulfadimethoxine	Digoxin*
Cefotaxime	Diphenhydramine	Ofloxacin	Sulfamerazine	
Ciprofloxacin	Enrofloxacin	Oxacillin	Sulfamethazine	
Clarithromycin	Erythromycin	Oxolinic acid	Sulfamethizole	
Cloxacillin	Erythromycin anhydrate	Penicillin G	Sulfamethoxazole	

*Compound formed intractable Na adduct with current conditions.

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

Anhydrotetracycline (2)	Doxycycline (2)	Minocycline (2)	Triclocarban (3) Triclosan (3) Warfarin (3)
Chlorotetracycline (2)	4-Epianhydrotetracycline (2)	Tetracycline(2) Meclocycline (2)	Albuterol (4) Cimetidine (4) Metformin (4)
Demeclocycline(2)	4-Epitetracycline(2)	Gemfibrozil (3) Ibuprofen (3) Naproxen (3)	Ranitidine (4)

List of Labeled Internal Standards

$^{13}C_2$-^{15}N-Acetaminophen	$^{13}C_2$-Erythromycin	$^{13}C_6$-Sulfamethazine	$^{13}C_3$-Trimethoprim
$^{13}C_3$-Atrazine	Fluoxetine-d_6	$^{13}C_6$-Sulfamethoxazole	Warfarin-d_5
$^{13}C_3$-Caffeine	Gemfibrozil-d_6	$^{13}C_6$-2,4,5-Tricloro-phenoxyacetic acid	Carbamazepine-d_{10} (Extra compound, not EPA list)
$^{13}C_3$-^{15}N-Ciprofloxacin	$^{13}C_3$-Ibuprofen	$^{13}C_6$-Triclocarban	
Cotinine-d_3	^{13}C-Naproxen-d_3	$^{13}C_{12}$-Triclosan	

Experimental

Sample Preparation

Pharmaceutical analytical standards were purchased from Sigma, (St. Louis, MO). All stable isotope labeled compounds used as internal standards were obtained from Cambridge Isotope Laboratories (Andover, MA). Individual pharmaceutical stock solutions (approximately 1,000 µg/mL) were prepared in pure acetonitrile or methanol, depending on the solubility of each individual compound, and stored at –18 °C. From these solutions, working standard solutions were prepared by dilution with acetonitrile and water.

Water samples were collected from the wastewater treatment plant at the Boulder Creek outfall (Boulder, CO) and extracted as per the EPA method. Agilent has introduced a polymeric SPE sorbent with hydrophilic/lipophilic properties that may also be appropriate for this application. "Blank" wastewater extracts were used to prepare the matrix-matched standards for validation purposes. The wastewater extracts were spiked with the mix of pharmaceuticals at different concentrations (ranging from 0.1 to 500 ng/mL or ppb) and subsequently analyzed by LC/MS/MS.

LC/MS/MS Instrumentation

The analytes were subdivided in groups (according to EPA protocol for sample extraction) and LC conditions for the chromatographic separation of each group are as follows.

LC Conditions for Group 1-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2–0.3 mL/min
Gradient	t_0 = 10% ACN, 0.2 mL/min t_5 = 10% ACN, 0.2 mL/min t_6 = 10% ACN, 0.3 mL/min t_{24} = 60% ACN, 0.3 mL/min t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 2-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	10% ACN and 90% H_2O with 0.1% HCOOH
Flow rate	0.2 mL/min
Gradient	t_0 = 10% ACN t_{10} = 10% ACN t_{30} = 100% ACN
Injection volumes	15 µL

LC conditions for Group 3-acidic extraction, negative electrospray ionization (ESI–) instrument conditions

Column	Agilent ZORBAX Eclipse Plus C18 2.1 × 100 mm, 3.5 µ (p/n 959793-902)
Column temperature	25 °C
Mobile phase	40% MeOH and 60% H_2O with 5 mM ammonium acetate, pH 5.5
Flow rate	0.2 mL/min
Gradient	$t_{0.5}$ = 40% MeOH t_7 = 100% MeOH
Injection volumes	15 µL

LC conditions for Group 4-acidic extraction, positive electrospray ionization (ESI+) instrument conditions

Column	Agilent ZORBAX HILIC Plus 2.1 × 100 mm, 3.5 µm (p/n 959793-901 custom order until November 1, 2008)
Column temperature	25 °C
Mobile phase	98% ACN and 2% H_2O with 10 mM ammonium acetate, pH 6.7
Flow rate	0.25 mL/min
Gradient	t_0 = 98% ACN t_5 = 70% ACN t_{12} = 70% ACN
Injection volumes	15 µL

The mass spectrometer conditions were general to all groups and are as follows.

MS Conditions

Mode	Positive and negative (depending on group) ESI using the Agilent G6410A Triple Quadrupole mass spectrometer
Nebulizer	40 psig
Drying gas flow	9 L/min
V capillary	4000 V
Drying gas temperature	300 °C
Fragmentor voltage	70–130 V
Collision energy	5–35 V
MRM	2 transitions for every compound as shown in Table 1
Dwell time	10 msec

Results and Discussion

Optimization of LC/MS/MS Conditions

The initial study consisted of two parts. First was to optimize the fragmentor voltage for each of the pharmaceuticals studied in order to produce the largest signal for the precursor ion. Typically the protonated molecule was used for the precursor ion. Each compound was analyzed separately using an automated procedure (MassHunter Optimizer software, Agilent Technologies, Santa Clara, CA) to check the fragmentor at each voltage. The data was then selected for optimal fragmentor signal and each compound was optimized again to determine automatically the collision energies for both the quantifying and qualifying ions. Optimal collision energies varied between 5 and 35 V. The MRM transitions and optimized energies used for this study are shown in Tables 2A to 2D.

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.)

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Acetaminophen	90	152 → 110 152 → 65	15 35
$^{13}C_2$-^{15}N-Acetaminophen	**90**	**155 → 111** **155 → 93**	**15** **25**
Ampicillin	70	350 → 160 350 → 106	10 15
$^{13}C_3$-Atrazine	**120**	**219 → 177** **219 → 98**	**15** **25**
Azithromycin	130	749.5 → 591.4 749.5 → 158	30 35
Caffeine	110	195 → 138 195 → 110	15 25
$^{13}C_3$-Caffeine	**110**	**198 → 140** **198 → 112**	**15** **25**
Carbadox	80	263 → 231 263 → 130	5 35
Carbamazepine	110	237 → 194 237 → 179	15 35
Carbamazepine-d_{10}	**110**	**247 → 204** **247 → 202**	**15** **35**
Cefotaxime	90	456 → 396 456 → 324	5 5
Ciprofloxacin	110	332 → 314 332 → 231	20 35
$^{13}C_3$-^{15}N-Ciprofloxacin	**110**	**336 → 318** **336 → 235**	**15** **35**

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Clarithromycin	110	748.5 → 158 748.5 → 590	25 15
Cloxacillin	90	436 → 160 436 → 277	15 15
Codeine	130	300 → 215 300 → 165	25 35
Cotinine	90	177 → 98 177 → 80	25 25
Cotinine-d_3	**90**	**180 → 80** **180 → 101**	**25** **25**
Dehydronifedipine	130	345 → 284 345 → 268	25 25
Digoxigenin	90	391 → 355 391 → 337	15 15
Digoxin	No response, Na adduct		
Diltiazem	130	415 → 178 415 → 150	25 25
1,7-Dimethylxanthine	90	181 → 124 181 → 99	15 15
Diphenhydramine	70	256 → 167 256 → 152	15 35
Enrofloxacin	130	360 → 316 360 → 342	15 15
Erythromycin	90	734.5 → 158 734.5 → 576	35 15
$^{13}C_2$-Erythromycin	**90**	**736.5 → 160** **736.5 → 578**	**25** **15**
Erythromycin anhydrate	90	716.5 → 158 716.5 → 116	25 25
Flumequine	90	262 → 174 262 → 244	35 15
Fluoxetine	90	310 → 148	5
Fluoxetine-d_6	**90**	**316 → 154**	**5**
Lincomycin	110	407 → 126 407 → 359	25 15
Lomefloxacin	130	352 → 308 352 → 265	15 25
Miconazole	90	415 → 159 415 → 69	35 25
Norfloxacin	70	320 → 302 320 → 276	15 15
Ofloxacin	110	362 → 318 362 → 261	15 25

Table 2A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1 (The labeled standards are bold.) continued

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Oxacillin	70	402 → 160 402 → 243	15 5
Oxolinic acid	90	262 → 244 262 → 216	15 25
Penicillin G	90	335 → 160 335 → 176	5 5
Penicillin V	70	351 → 160 351 → 114	5 25
Roxithromycin	130	837.5 → 679 837.5 → 158	15 35
Sarafloxacin	130	386 → 299 386 → 368	25 25
Sulfachloropyridazine	90	285 → 156 285 → 92	10 25
Sulfadiazine	110	251 → 156 251 → 92	15 25
Sulfadimethoxine	80	311 → 156 311 → 92	20 35
Sulfamerazine	110	265 → 156 265 → 92	15 25
Sulfamethazine	90	279 → 156 279 → 186	15 15
$^{13}C_6$-Sulfamethazine	**90**	**285 → 186** **285 → 162**	**25** **25**
Sulfamethizole	80	271 → 156 271 → 92	10 25
Sulfamethoxazole	110	254 → 156 254 → 92	15 25
$^{13}C_6$-Sulfamethoxazole	**110**	**260 → 162** **260 → 98**	**15** **25**
Sulfanilamide	70	173 → 156 173 → 92	5 15
Thiabendazole	130	202 → 175 202 → 131	25 35
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	**110**	**259 → 201** **259 → 165**	**5** **25**
Trimethoprim	110	291 → 230 291 → 261	25 25
$^{13}C_3$-Trimethoprim	**110**	**294 → 233** **294 → 264**	**25** **25**
Tylosin	110	916.5 → 174 916.5 → 772	35 35
Virginiamycin	110	526 → 508 526 → 355	5 15

Table 2B. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 2

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Anhydrotetracycline	90	427 → 410 427 → 154	15 25
Chlorotetracycline	110	479 → 462 479 → 197	15 35
Demeclocycline	130	465 → 430 465 → 448	25 15
Doxycycline	110	445 → 428 445 → 154	15 25
4-Epianhydrotetracycline (EATC)	90	427 → 410 427 → 105	15 35
4-Epitetracycline (ETC)	110	445 → 410 445 → 427	15 5
Minocycline	90	458 → 441	15
Tetracycline (TC)	110	445 → 410 445 → 427	15 5

Table 2C. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 3

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Gemfibrozil	100	249 → 121	5
Gemfibrozil-d_6	**100**	**255 → 121**	**5**
Ibuprofen	75	205 → 161	5
$^{13}C_3$-Ibuprofen	**75**	**208 → 163**	**5**
Naproxen	75	229 → 169 229 → 170	25 5
^{13}C-Naproxen-d_3	**75**	**233 → 169** **233 → 170**	**25** **5**
Triclocarban	100	313 → 160 313 → 126	10 25
$^{13}C_6$-Triclocarban	**90**	**319 → 160** **319 → 132**	**5** **25**
Triclosan	75	287 → 35	5
$^{13}C_{12}$-Triclosan	**75**	**299 → 35**	**5**
Warfarin	125	307 → 117 307 → 161	35 15
Warfarin-d_5	**90**	**312 → 161** **312 → 255**	**15** **25**

Table 2D. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 4

Compound	Fragmentor voltage	MRM transitions (*m/z*)	Collision energy (eV)
Albuterol (Salbutamol)	90	240 → 148	15
		240 → 166	5
Cimetidine	100	253 → 159	10
		253 → 95	25
Metformin	80	130 → 60	10
		130 → 71	25
Ranitidine	110	315 → 176	15
		315 → 130	25

Chromatographic separation was done independently for each group and a dwell time of 10 msec was used for every MRM transition. Figures 1A to 1D show the chromatograms corresponding to 100 ppb standard on column for all the pharmaceuticals studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective protonated molecule and product-ion MRM transitions.

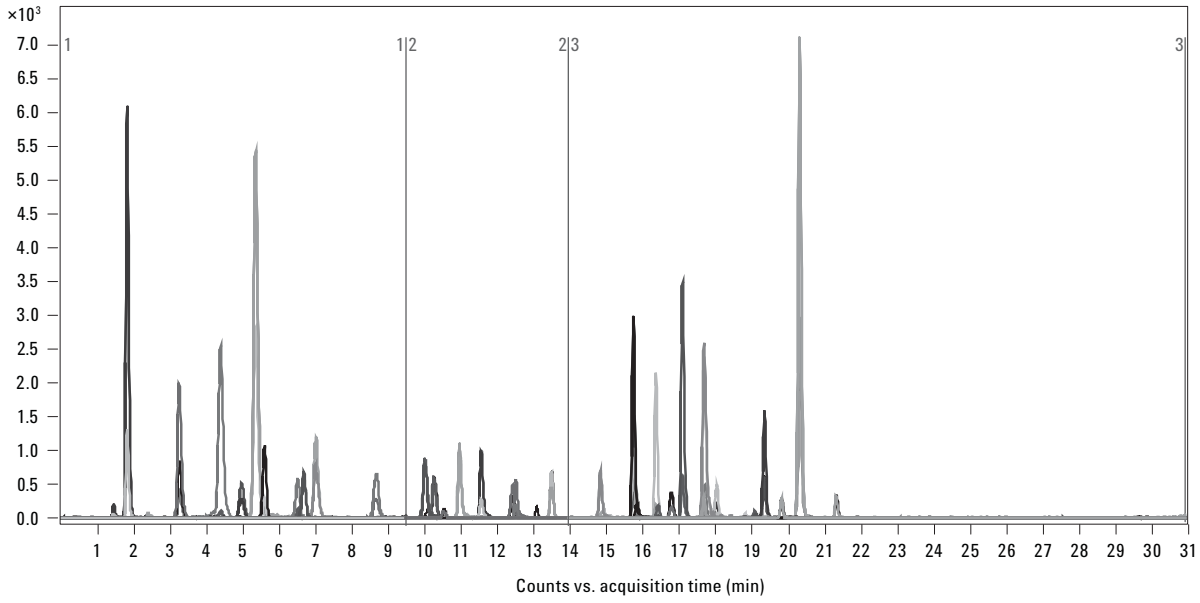

Figure 1A. MRM extracted chromatogram for pharmaceuticals in Group 1. Three time segments were used in this chromatographic separation.

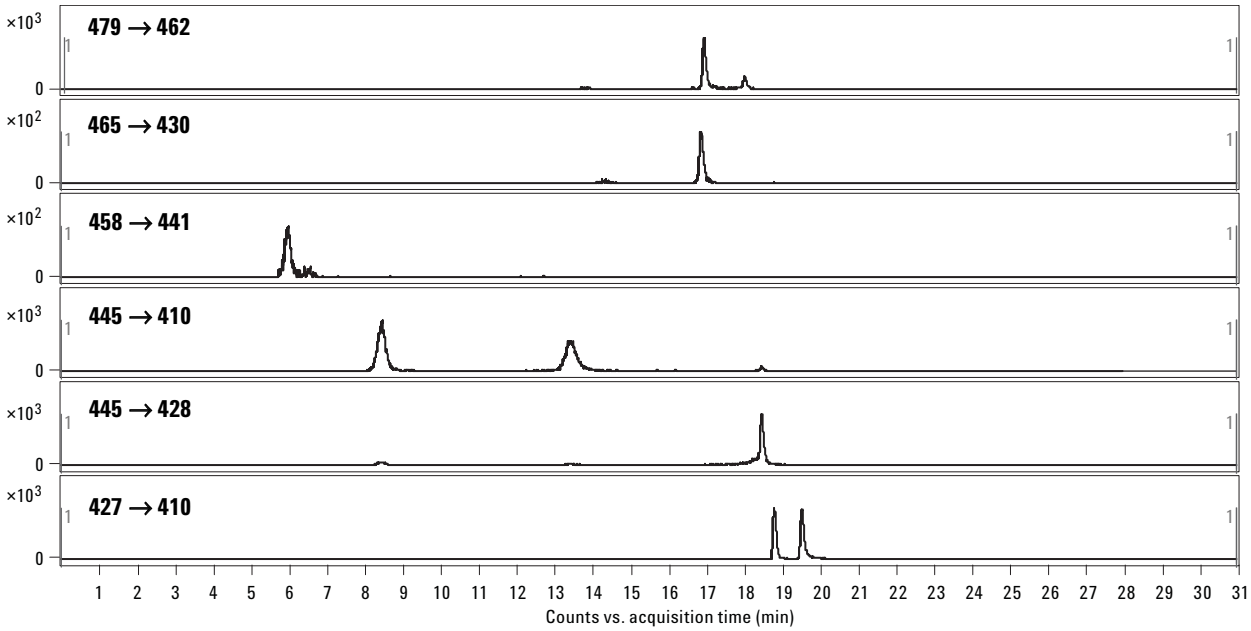

Figure 1B. MRM extracted chromatogram for pharmaceuticals in Group 2. Only one transition shown. See Table 2B for compound identification.

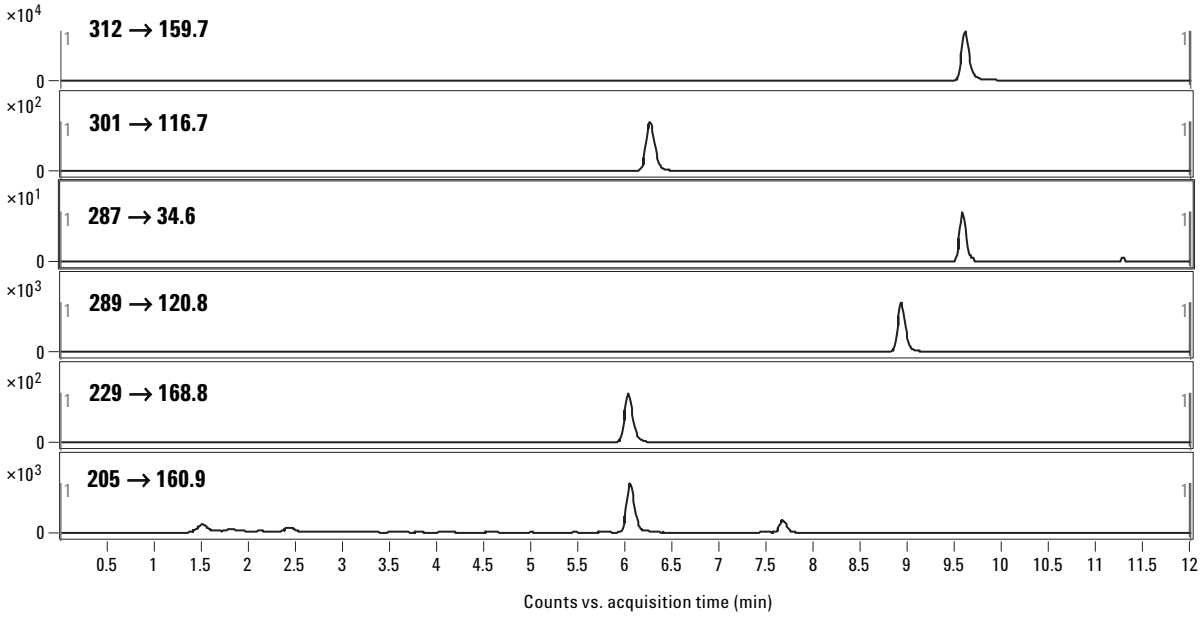

Figure 1C. MRM extracted chromatogram for pharmaceuticals in Group 3. Only one transition shown. See Table 2C for compound identification.

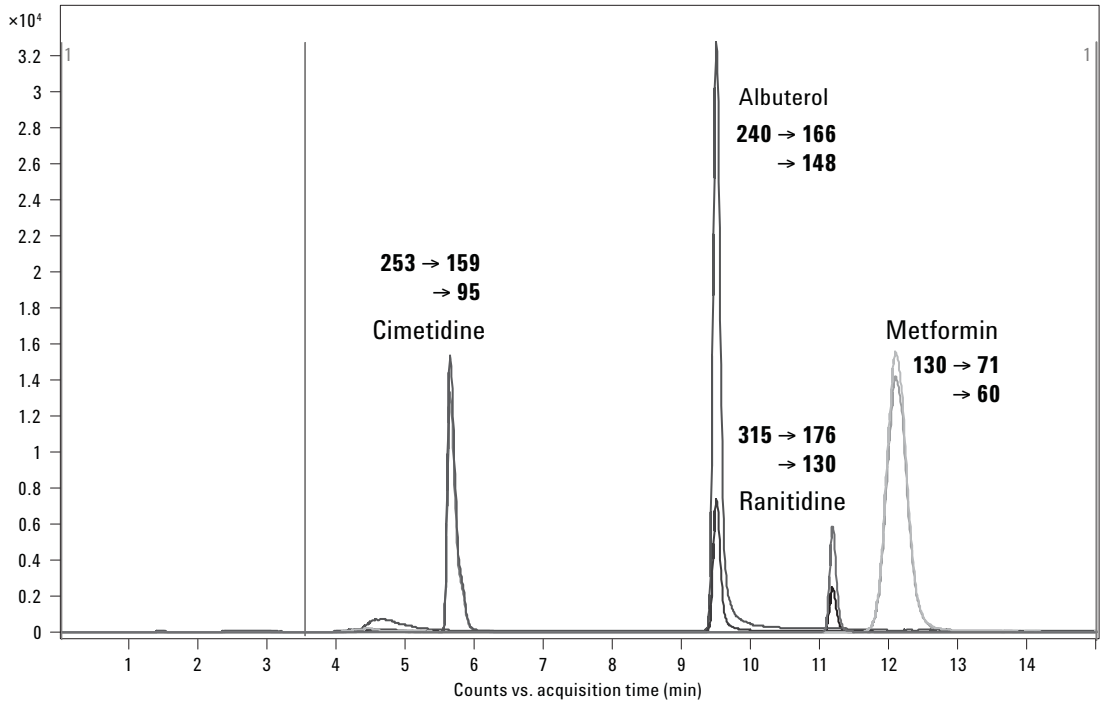

Figure 1D. MRM extracted chromatogram for pharmaceuticals in Group 4.

Application to Wastewater Samples

To confirm the suitability of the method for analysis of real samples, matrix-matched standards were analyzed in a wastewater matrix from an effluent site, at eight concentrations (0.1, 0.5, 1, 5, 10, 50, 100, and 500 ng/mL or ppb concentrations). Figure 2 shows an example standard curve for acetaminophen in the wastewater matrix. In general, all compounds gave linear results with excellent sensitivity over three orders of magnitude, with r^2 values of 0.99 or greater.

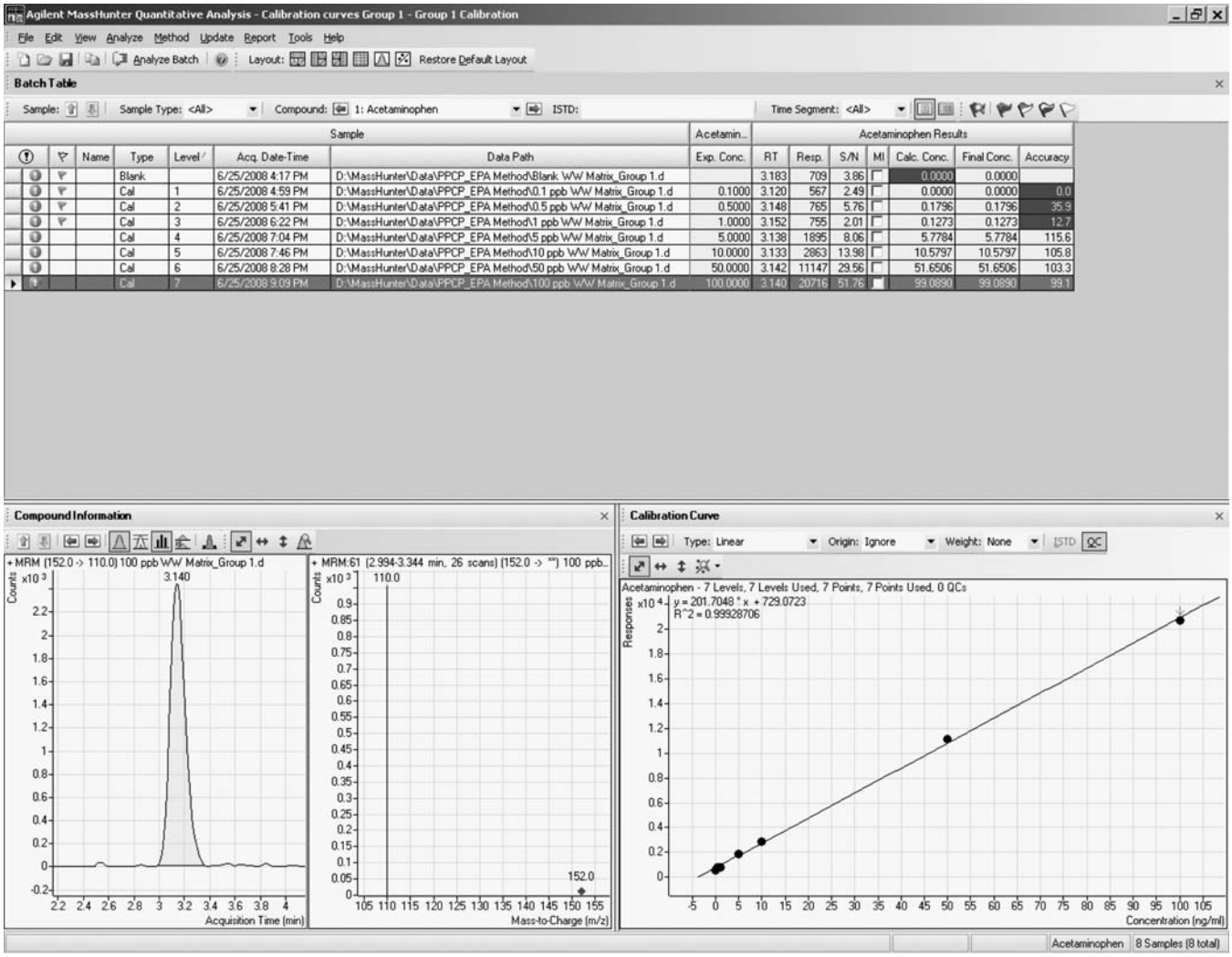

Figure 2. Calibration curve for acetaminophen in a wastewater matrix using a seven-point curve from 0.1 to 100 ng/mL (ppb) using a linear fit with no origin treatment.

Finally, a "blank" wastewater sample was analyzed and the presence of two pharmaceuticals, carbamazepine and diphenhydramine, could be confirmed with two MRM transitions. Figure 3 shows the ion ratios qualifying for these two compounds in a wastewater extract. As shown in Figure 3 in the two ion profiles, both pharmaceuticals were easily identified in this complex matrix due to the selectivity of the MRM transitions and instrument sensitivity.

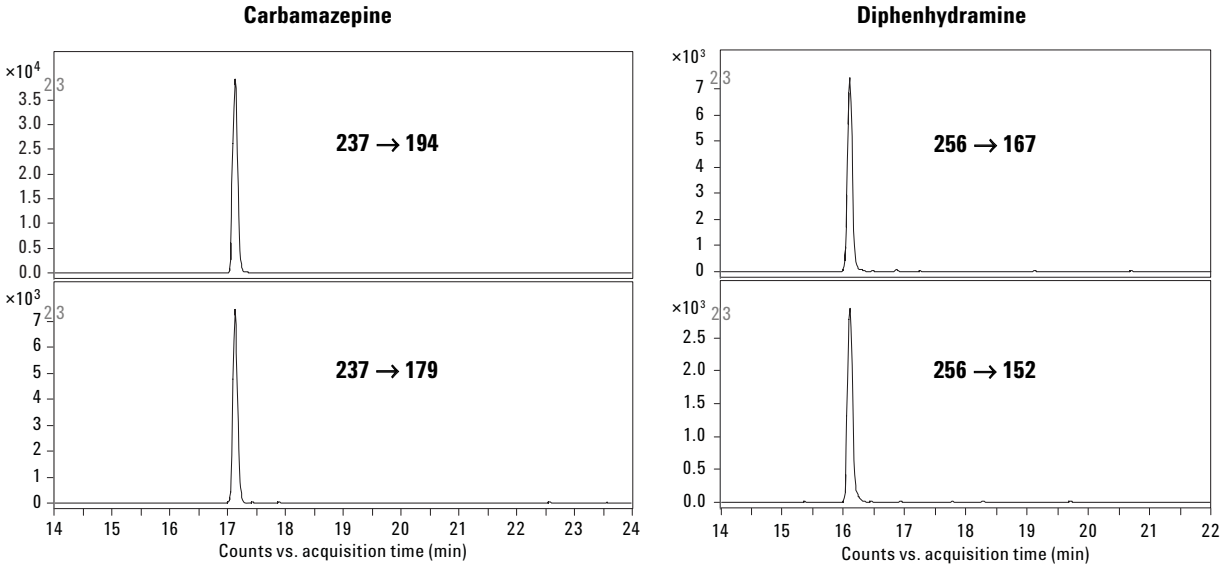

Figure 3. MRM chromatograms of a wastewater sample for carbamazepine and diphenhydramine using two transitions.

Conclusions

The results of this study show that the Agilent 6410A Triple Quadrupole is a robust, sensitive, and reliable instrument for the study of pharmaceuticals in water samples, using high throughput methods. The Agilent 6410A Triple Quadrupole has been shown to be a successful instrument for the implementation of EPA Method 1694.

References

1. EPA Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS, December 2007, EPA-821-R-08-002.

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