Ultrasensitive EPA Method 1694 with the Agilent 6460 LC/MS/MS with Jet Stream Technology for Pharmaceuticals and Personal Care Products in Water

Application Note

Environmental

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Abstract

An analytical method for screening and confirming the presence of 84 pharmaceuticals and 23 labeled standards in water samples for a total of 107 components was developed using the Agilent 6460 Triple Quadrupole Mass Spectrometer (Triple Quad MS) with Jet Stream Technology. The method was developed for the compounds in EPA Method 1694 and an additional 14 pharmaceuticals commonly found in wastewater. The results were compared to the Agilent 6410A Triple Quadrupole and an enhancement in limits of detection of 10 to 100 times was shown for the 6460. Also rapid resolution and fast chromatography were used to obtain the same or better quality separations for EPA Method 1694 with 1.8-µm columns. Four distinct chromatographic gradients and LC conditions were used according to the polarity and extraction of the different pharmaceuticals. The chromatographic conditions were then altered to show that the four gradients may be collapsed to only two chromatographic runs with no loss of sensitivity or accuracy of determination with a savings in time from 90 min to only 30 min total time. The method was evaluated for a treated wastewater effluent and five different pharmaceuticals were identified at levels as low as 1 ng/L. The new Jet Stream technology is a valuable new tool for pharmaceutical analysis of water and wastewater with excellent sensitivity and limits of detection.

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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 are an important group of contaminants that have been targeted, especially in the last decade. In the area of pharmaceuticals and personal care products (PPCPs), there is one EPA method (although not yet promulgated) addressing the analysis of these analytes, which is EPA Method 1694 [1] published in December 2007. In this method, the standard EPA protocol uses solid-phase extraction (SPE) for water samples followed by analysis with LC/MS using a tandem mass spectrometer with a single transition for each compound. Recently, we published an application note that was an improvement on this EPA Method 1694 because it uses two transitions for each analyte, which is a standard analytical protocol, while still meeting the chromatographic conditions specified by EPA with all Agilent columns [2].

This application note describes the latest new Agilent solution to this EPA method, which is demonstrated with the Agilent 6460 LC/MS Triple Quad with Jet Stream technology. The number of compounds in the method has been increased by 14, which include not only the standard analytes in EPA Method 1694 (70 analytes of their 74 - four were not available to us) but also 14 commonly found pharmaceuticals and 23 labeled internal standards for a total of 107 compounds. The chromatography has been shortened by reducing the analysis from 4 groups to 2 groups of analytes, in spite of the increased number of compounds. Furthermore, the analysis times have been reduced from a total of 90 min to less than 30 min, while achieving a 10 to 100 times increase in sensitivity, depending upon the analyte detected. The result is a robust analytical method for PPCPs in water that may be analyzed rapidly and sensitively while maintaining the highest analytical standards for correct analysis.

Experimental

Sample Preparation

Pharmaceutical analytical standards were purchased from Sigma, (St. Louis, MO, USA). Individual pharmaceutical stock solutions (approximately 1000 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.

Wastewater samples were collected from an effluent site in Boulder Creek (Boulder, CO) and extracted with Oasis HLB cartridges using a modified EPA protocol. One-liter water samples were extracted directly onto a 500-mg cartridge without pH adjustment, dried for 10 min with air, and eluted with 8 mL of methanol. The methanol was evaporated to 1 mL and analyzed directly by LC/MS/MS as described below. "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 Conditions for Agilent 6460 with Jet Stream Technology

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 for the standard EPA Method Analysis.

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

Column:	Agilent ZORBAX Eclipse Plus C-18 narrow bore, 2.1 mm × 100 mm, 1.8 µm, p/n 959764-902
Column temp:	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 C-18 narrow bore, 2.1 mm × 100 mm, 1.8 µm, p/n 959764-902
Column temp:	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 C-18 narrow bore, 2.1 mm × 100 mm, 1.8 µm, p/n 959764-902
Column temp:	25 °C
Mobile phase:	40% MeOH and 60% H_2O with 0.1% ammonium formate pH 5.5
Flow-rate:	0.2 mL/min
Gradient:	t_0 = 40% MeOH $t_{0.5}$ = 40% MeOH t_7 = 100% MeOH
Injection volumes:	15 µL

LC Conditions for Group 4
Basic extraction, positive electrospray ionization (ESI+) instrument conditions.

Column:	Agilent ZORBAX Eclipse HILIC Plus narrow bore, 2.1 mm × 100 mm, 3.5 µm, p/n 959793-901
Column temp:	25 °C
Mobile phase:	5% ACN and 5% H_2O with 10 mM ammonium acetate, pH 6.5
Flow-rate:	0.25 mL/min
Gradient:	t_0 = 95% ACN t_9 = 70% ACN t_{15} = 70% ACN
Injection volumes:	15 µL

LC Conditions for Group 5
Extra compounds commonly found in wastewater but not part of EPA Method 1694, positive electrospray ionization (ESI+) instrument conditions. All conditions are the same as in Group 1.

Agilent Jet Stream conditions for the LC/MS/MS Model 6460.

Gas heater:	250 °C
Gas flow:	8 L/min
Nebulizer pressure:	35 psi
Sheath gas heater:	300 °C
Sheath gas flow:	10 L/min
V_{cap}:	4000 V
Nozzle voltage:	0
Delta EMV:	400 V

Results and Discussion

Optimization of LC/MS/MS conditions

Method development for LC/MS with a triple quadrupole always consists of two parts. The first step 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 (Agilent Optimizer software) to check the fragmentor at each voltage. The data was then selected for optimal fragmentor signal and each compound was optimized again to determine collision energies for both the quantifying and qualifying ions. The software does this automatically. Collision energies varied between 5 and 45 V. The energies were optimized for each of the ions and the voltages that gave the best sensitivity were selected. The list of PPCPs that were analyzed in EPA Method 1694 along with the additional 18 analytes and internal standards are shown in Table 1A to 1E along with the optimized MRM transitions used for this study.

Table 1A. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 1. In Bold are the Labeled Standards.

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-d10	**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**
Clarithromycin	110	748.5 > 158 748.5 > 590	25 15
Cloxacillin	90	436 > 160 436 > 277	15 15
Codeine	154	300 > 165 300 > 215	41 21
Codeine-d3	**162**	**303 > 165** **303 > 61**	**45** **25**
Cotinine	90	177 > 98 177 > 80	25 25
Cotinine-d3	**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

(Continued)

Compound	Fragmentor voltage	MRM transitions (m/z)	Collision energy (eV)
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-d6	**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
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

(Continued)

Compound	Fragmentor voltage	MRM transitions (m/z)	Collision energy (eV)
$^{13}C_6$-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
Sulfathiazole	108	256>156 256>92	9 21
Thiabendazole	130	202 > 175 202 > 131	25 35
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
Viginiamycin	110	526 > 508 526 > 355	5 15

Table 1B. 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)
Anhydrochlortetracycline	122	461 > 444 461 > 410	13 13
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-Epianhydrochlortetracycline	134	461>444 461 > 426	13 13
4-Epianhydrotetracycline (EATC)	90	427 > 410 427 >105	15 35
4-Epichlortetracycline	134	479 > 462 479 > 197	17 17
4-Epioxytetracycline	130	461 > 444 461 > 426	13 17
4-Epitetracycline (ETC)	110	445 > 410 445 > 427	15 5
Isochlotetracycline	138	479 > 462 479 > 252	17 45
Meclocycline	110	477 > 460	15
Minocycline	90	458 > 441	15
Tetracycline (TC)	110	445 > 410 445 > 427	15 5

Table 1C. 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-d6	**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-d3	**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-d5	**90**	**312 > 161** **312 > 255**	**15** **25**

Table 1D. 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 > 148 240 >166	15 5
Cimetidine	100	253 > 159 253 > 95	10 25
Metformin	80	130 > 60 130 >71	10 25
Ranitidine	110	315 > 176 315 > 130	15 25

Table 1E. MRM Transitions and MS Operating Parameters Selected for the Analysis of the Pharmaceutical Compounds in Group 5, which are the Additional Commonly Detected Pharmaceutical Analytes Added to the Method

Compound	Fragmentor Voltage	MRM transitions (m/z)	Collision energy (eV)
Amphetamine	70	136 > 91 136 > 119	13 5
Amphetamine-d5	**74**	**141 > 93** **141 > 124**	**13** **5**
Atenolol	134	267 > 145 267 > 190	21 13
Clonidine	150	230 > 44 230 > 213	25 21
Dextromethorphan	152	272 > 171 272 > 147	41 29
Diazepam	162	285 > 154 285 > 193	25 33
Diazepam-d5	**162**	**290 > 198** **290 > 154**	**33** **25**
Diclofenac	83 83	294 > 250 294 > 214	5 21
Furesemide	95 95	329 > 285 329 > 205	13 21
Hydrocodone	158	300 > 199 300 > 171	29 41
Hydrocodone-d6	**166**	**306 > 202** **306 > 174**	**29** **41**
Meprobamate	70	219 > 158 219 > 55	5 20
Metoprolol	136	268 > 116 268 > 56	13 29
Nordiazepam	158	271 > 140 271 > 165	25 25
Oxycodone	134	316 > 298 316 > 241	13 25
Oxycodone-d6	**134**	**322 > 304** **322 > 247**	**13** **29**
Propranolol	122	260 > 116 260 > 56	13 29
Sertaline	88	306 > 159 306 > 275	25 5
$^{13}C_6$-2,4,5-Trichlorophenoxyacetic acid	**110**	**259 > 201** **259 > 165**	**5** **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 were overlaid for each one of the target analytes according to their respective protonated molecule and product-ion MRM transitions.

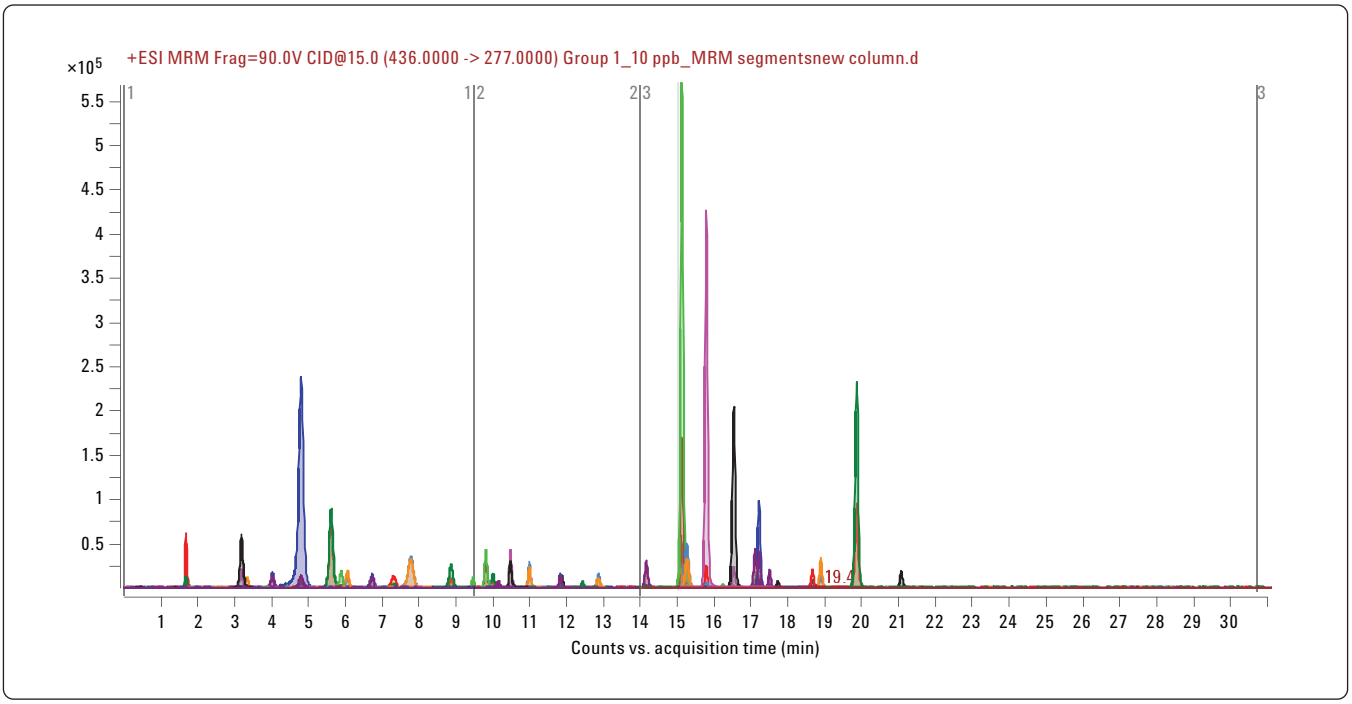

Figure 1A. MRM extracted chromatogram for pharmaceuticals in Group 1. Three time segments were used in this chromatographic separation using the 1.8 µm column and the Agilent Stream Technology. Concentrations were all at 10 ppb.

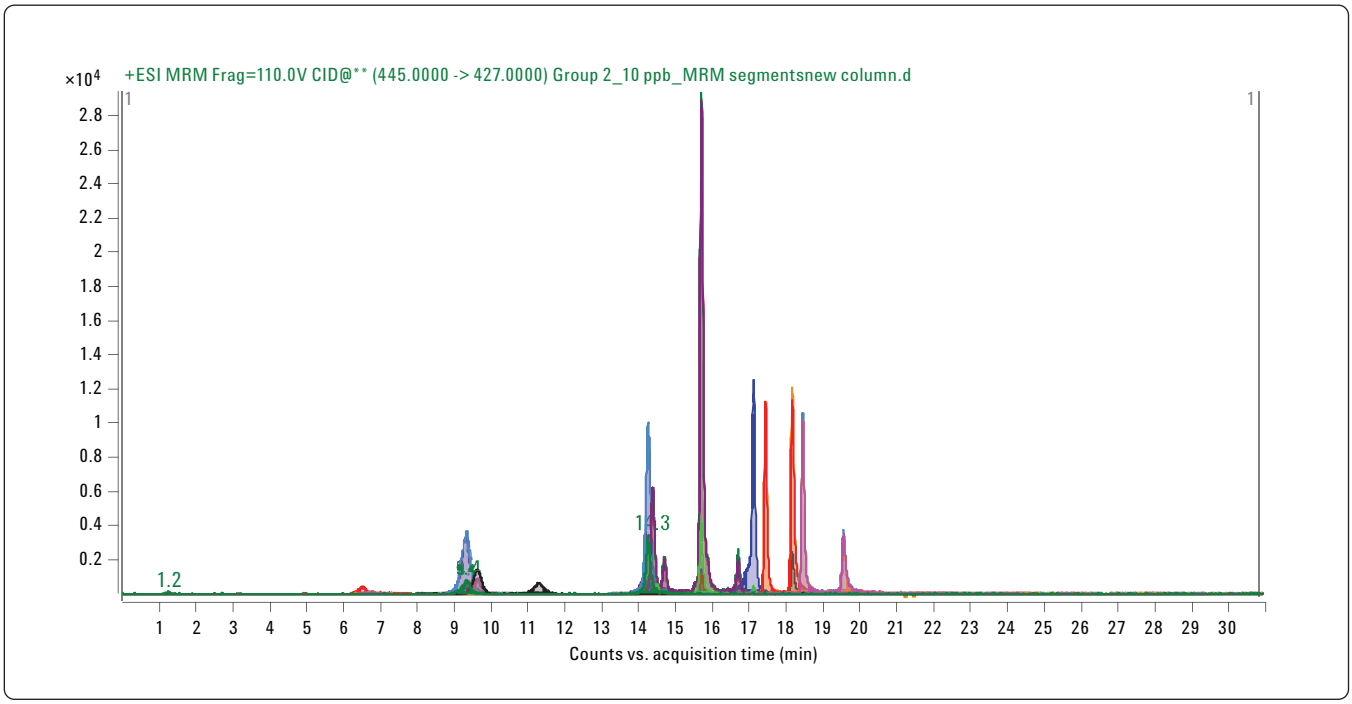

Figure 1B. MRM extracted chromatogram for pharmaceuticals in Group 2 at a concentration of 10 ppb.

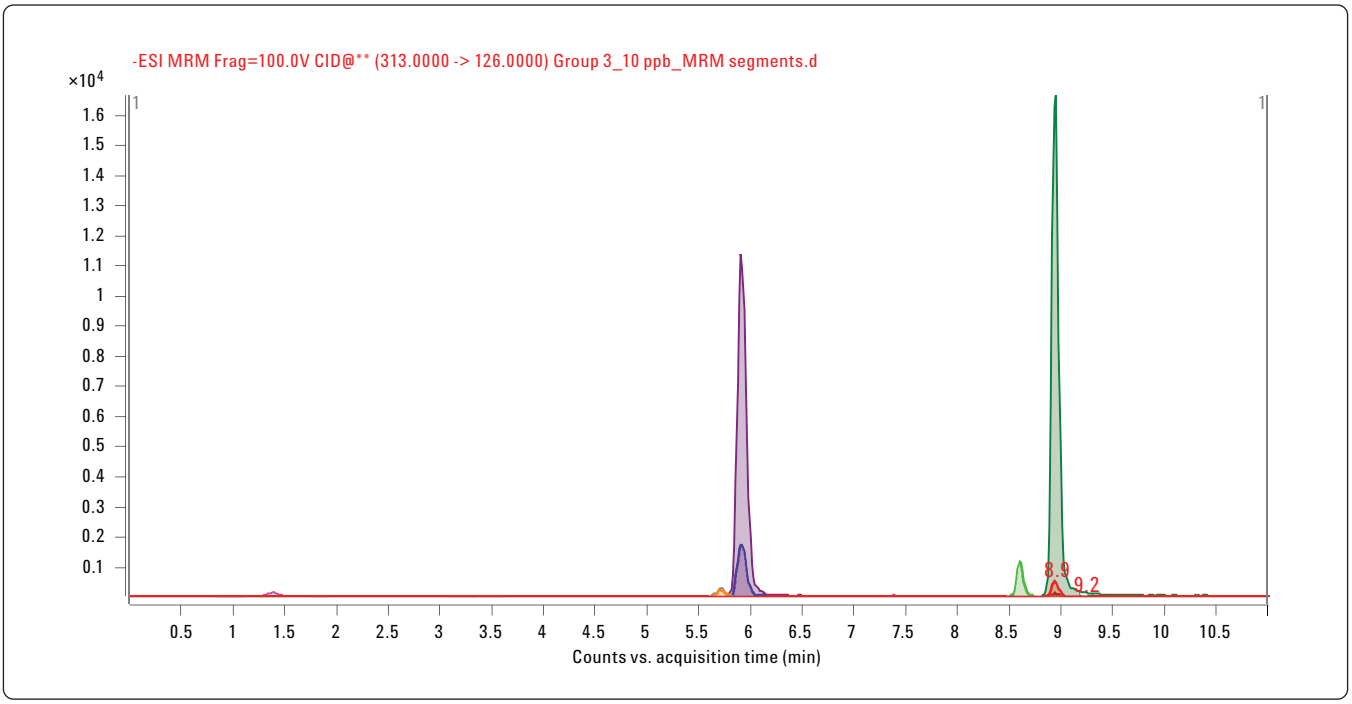

Figure 1C. MRM extracted chromatogram for pharmaceuticals in Group 3 at a concentration of 10 ppb.

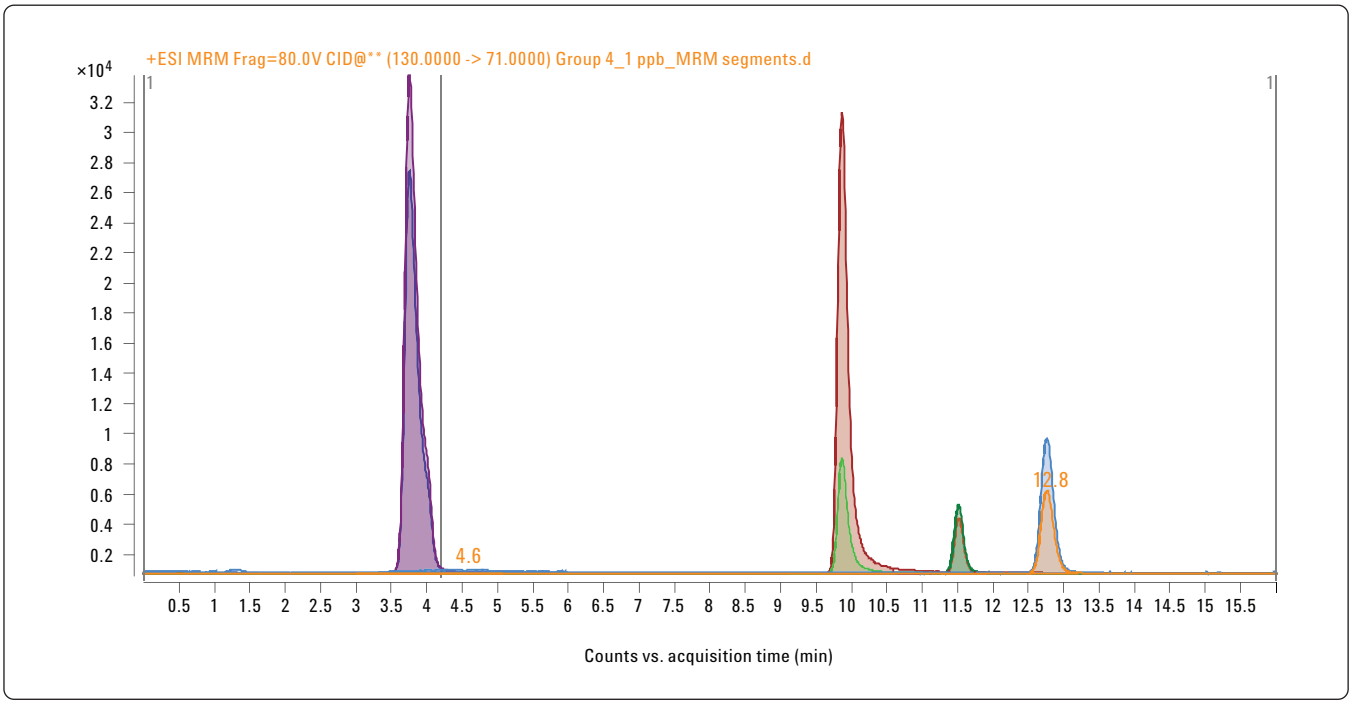

Figure 1D. MRM extracted chromatogram for pharmaceuticals in Group 4 at a concentration of 1 ppb.

Simplification of EPA Method 1694

Because EPA Method 1694 consists of four different groups, this means that four analyses are required with a total time of approximately 90 min. Therefore, the two goals in this application note are to simplify the chromatography to two analysis groups with the minimum time, and to increase the sensitivity to the maximum possible for the method. To accomplish this, the following changes to the method were made. First Groups 1, 2, 4, and 5 (additional commonly found analytes) were combined into one chromatographic run using the 1.8 µm Eclipse-C18 column, which gave good resolution for not only the EPA target analytes but also the 14 commonly found PPCPs in Group 5. This made our new Group I consist of 35 analytes and internal standards. These compounds were analyzed by positive electrospray in a 20-min run. The second group (Group II) consisted of six analytes with internal standards, which were the negative electrospray analytes in a 10-min run. While it is possible to combine both groups and do positive and negative ion switching at the same time, this is not a recommended procedure. It is necessary to use the same mobile phase (water with 0.1% formic acid/acetonitrile) for both analyses which results in a lower sensitivity for negative-ion analytes. Therefore, we recommend two groups but make use of fast chromatography for the negative ions in the original Group III. Thus, the total analysis time is reduced from 90 to 30 min, which is three times faster without loss of sensitivity or reliability of detection while increasing the number of analytes by about 25%.

Figures 2A and 2B show the chromatography for the new Group I and Group II analytes using the 1.8-µm columns and the combination of extended peak capacity in Group I and the fast chromatography of Group II.

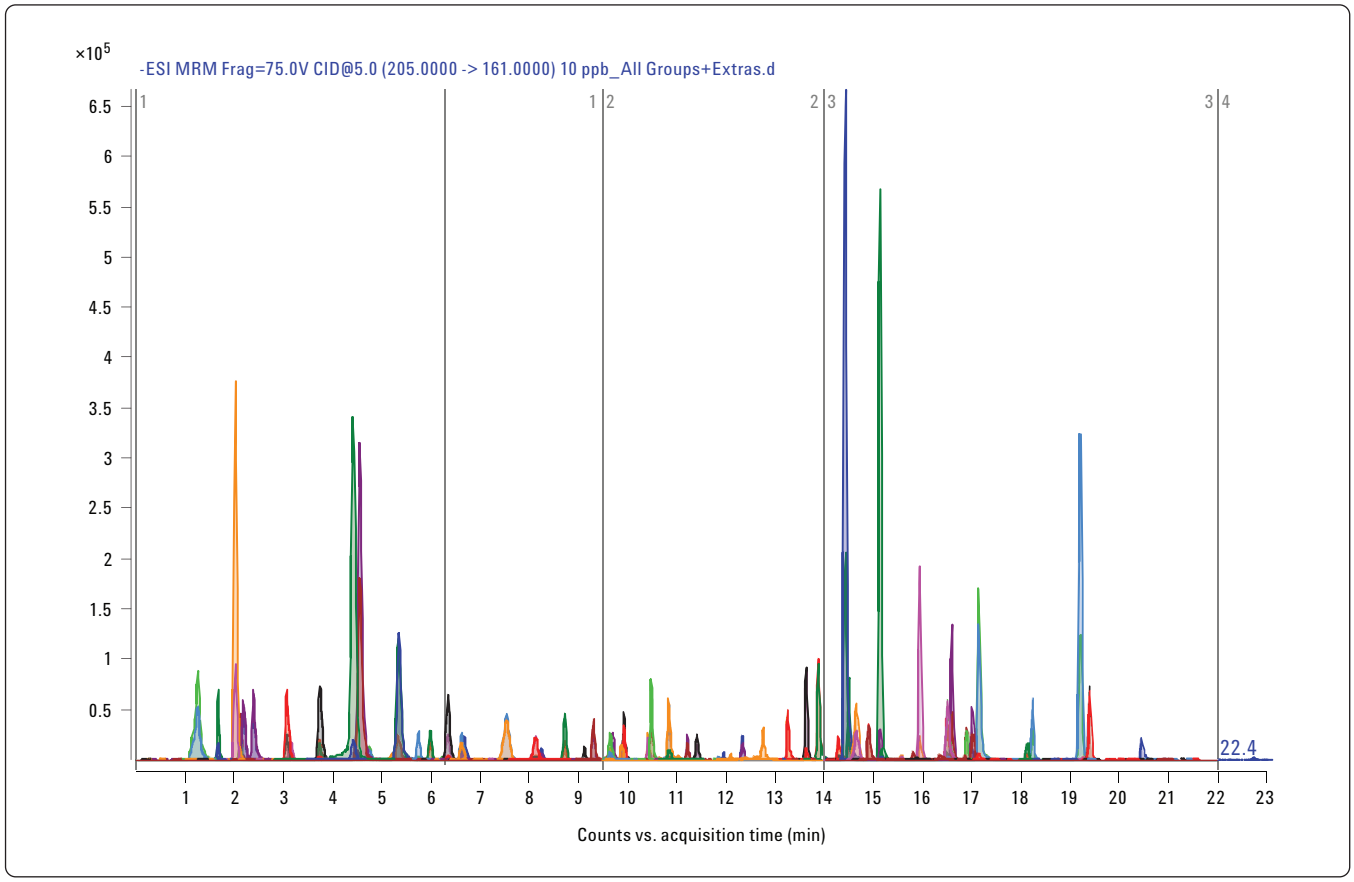

Figure 2A. New Group I analytes in positive ion electrospray. This group includes the original Group 1, 2, and 4 of EPA Method 1694 plus 14 commonly found pharmaceuticals for a total of 85 compounds, with internal standards.

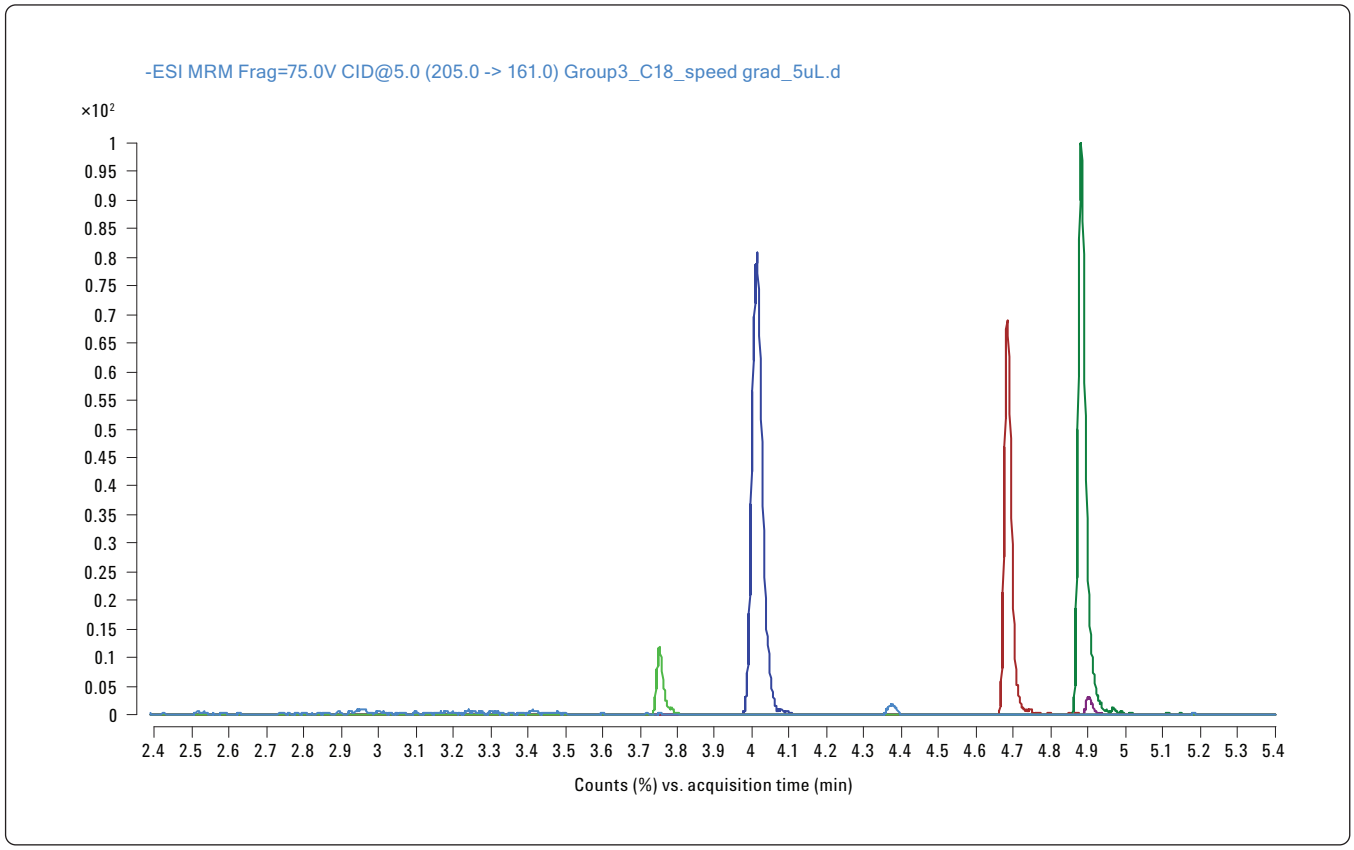

Figure 2B. Fast chromatography showing the analytes in the new Group II in negative ion using a 6-min run with 1.8-µm column.

Furthermore, we compared the increase in sensitivity and the lower detection limits (LODs) possible with the new Agilent Jet Stream Technology. The Agilent Jet Stream uses a sheath gas to increase the number of ions that are directed into the source of the mass spectrometer. This is accomplished by increasing analyte ionization and capture using heated thermal gradient focusing, which employs a super-heated nitrogen sheath gas to increase desolvation efficiency in electrospray and reduce background ions. Figure 3 shows how the Jet Stream Technology works.

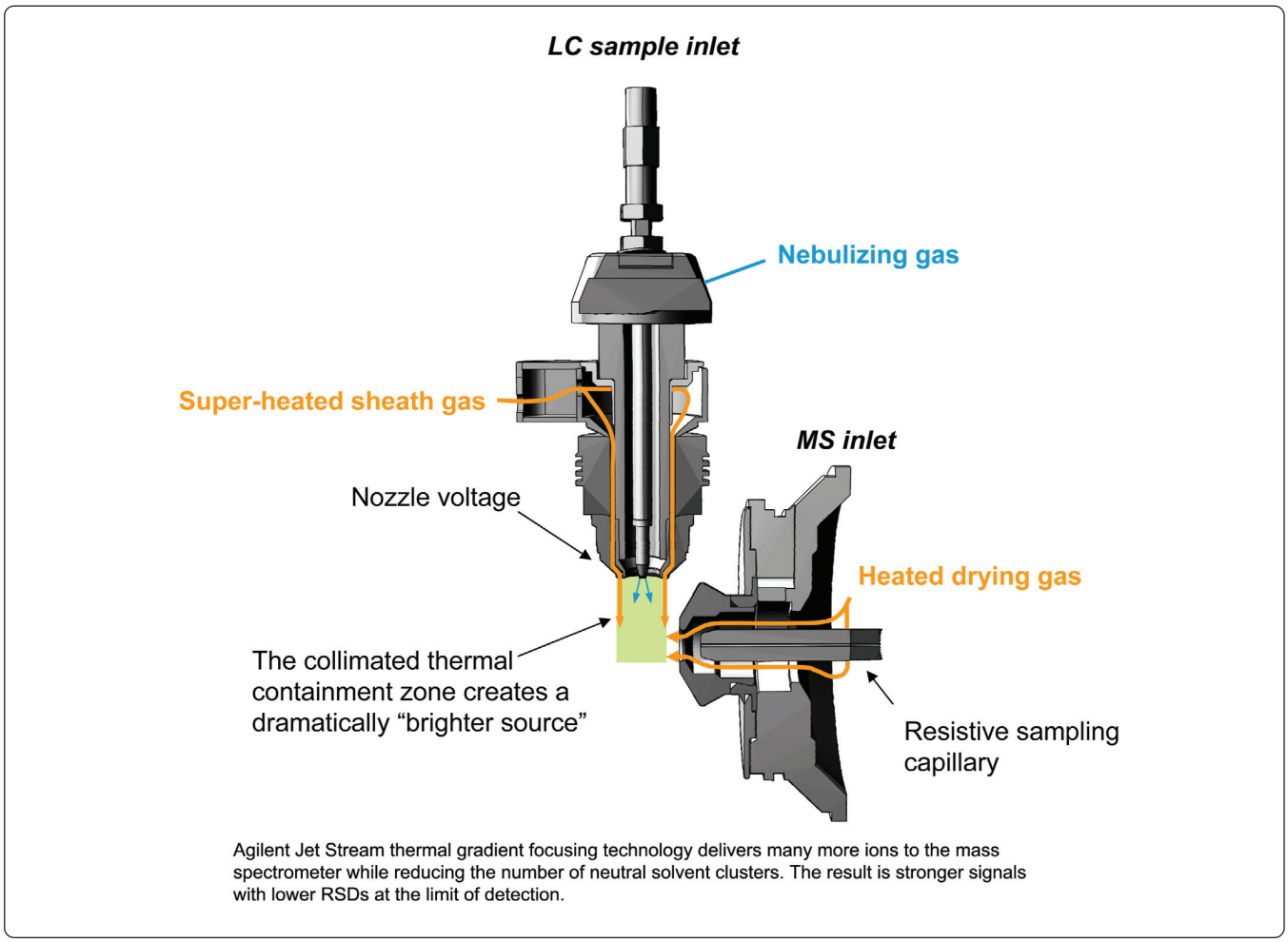

Figure 3. Example of how the Jet Stream Technology works.

The result of the Jet Stream Technology is that there is approximately a 5 to 10 times increase in the number of ions that are directed to the lens of the first octopole of the mass spectrometer. This results in an appreciable increase in sensitivity. Accompanying this is the individual effects of the analytes themselves. For example, each analyte has its own ionization efficiency, which is combined with the effects of its surface activity, its ability to accept or donate a proton, and its stability in the stream with electrospray potentials of 4000 volts. These combined effects increase the sensitivity and limit of detection of the PPCP analyte. We tested each of 70 analytes between the two instruments, the 6410A Triple Quad and the 6460 Triple Quad and the data from this comparison is shown in Table 2 with the original EPA groups (Groups 1 to 4). The solution of these compounds were injected directly with no concentration.

Table 2. PPCPs Analyzed by Group According to EPA Method 1694. Limits of Detection are Shown for Two Triple Quadrupoles, the Model 6410 and the Model 6460 with Agilent Jet Stream Technology (Note: 4 methyl esters were added to this table as compounds that form in the standard solution and are not part of the official EPA Method 1694.)

Compound	LOD 6460 (µg/L)	LOD 6410 (µg/L)	Increase in LOD (times)
Acetaminophen	0.1	1.0	10
Ampicillin	0.6	5.0	8
Azithromycin	6.0	100	16
Caffeine	0.5	5	10
Carbadox	0.3	10	33
Carbamazepine	0.06	1.0	16
Cefotaxime	2	50	25
Ciprofloxacin	0.5	10	20
Clarithromycin	0.1	10	100
Cloxacillin	3.0	10	3
Cloxacillin Me-Ester	3.0	10	3
Codeine	0.3	10	33
Cotinine	0.05	1.0	20
Dehydronifedipine	0.03	1.0	33
Digoxigenin	0.4	2.0	5
Diltiazem	0.05	0.5	10
1,7-Dimethylxanthine	0.6	5.0	8
Diphenhydramine	0.05	0.2	4
Enrofloxacin	0.3	5.0	16
Erythromycin	0.3	10	30
Erythromycin Anhydrate	0.3	10	30
Flumequine	0.05	2.0	40
Fluoxetine	0.2	8.0	40
Lincomycin	0.05	1.0	20
Lomefloxacin	0.4	5.0	12
Miconazole	0.5	5.0	10
Norfloxacin	1.0	10	10
Ofloxacin	0.4	5.0	12
Ofloxacin Me-Ester	0.4	5.0	12
Oxacillin			
Oxolinic Acid	0.03	1.0	33
Penicillin G	1.0	5.0	5
Penicillin G Methyl Ester	1.0	5.0	5
Penicillin V	1.0	5.0	5
Penicillin V Methyl Ester	1.0	5.0	5
Roxithromycin	0.5	10	20
Sarafloxacin	0.5	5.0	10
Sulfachloropyridazine	0.2	5.0	25

(Continued)

Compound	LOD 6460 (µg/L)	LOD 6410 (µg/L)	Increase in LOD (times)
Sulfadiazine	0.5	5	10
Sulfadimethoxine	0.05	2.0	40
Sulfamerazine	0.1	3.0	30
Sulfamethazine	0.3	5.0	16
Sulfamethizole	0.3	3.0	10
Sulfamethoxazole	0.2	2.0	10
Sulfanilamide	4.0	20	5
Sulfathiazole	0.4	5.0	12
Thiabendazole	0.05	5.0	100
Trimethoprim	0.5	3.0	6
Tylosin	6.0	100	16
Virginiamycin	0.4	5	12

Group 2 Compounds.

Compound	LOD Jetstream 6460 (µg/L)	LOD 6410 (µg/L)	Increase in LOD (times)
Anhydrochlortetracycline	5.0	50	10
Anhydrotetracycline	1.0	50	50
Chlorotetracycline	0.5	10	20
Demeclocycline	4.0	100	25
Doxycycline	1.0	60	60
4-Epianhydrochlortetracycline	5.0	30	6
4-Epianhydrotetracycline	0.5	30	60
4-Epichlortetracycline	1.0	80	80
4-Epioxytetracycline	5.0	100	20
4-Epitetracycline	1.0	50	50
Isochlotetracycline	5.0	10	2
Minocycline	20	100	5
Tetracycline	0.8	60	75

Group 3 Compounds.

Compound	LOD Jetstream 6460 (µg/L)	LOD 6410 (µg/L)	Increase in LOD (times)
Gemfibrozil	0.1	0.1	1
Ibuprofen	5.0	5.0	1
Naproxen	1.0	1.0	1
Triclocarban	0.1	0.1	1
Triclosan	1.0	1.0	1
Warfarin	0.1	0.1	1

Group 4 Compounds.

Compound	LOD Jetstream 6460 (µg/L)	LOD 6410 (µg/L)	Increase in LOD (times)
Albuterol	0.05	0.05	1
Cimetidine	0.02	0.1	5
Metformin	0.05	0.1	2
Ranitidine	0.08	0.5	6

The result of this comparison shows that the Agilent Jet Stream technology increases the sensitivity for the PPCPs by at least 10 times and for many compounds this increase is on the order to 20 to 30 times. The detection limits for the majority of the PPCPs of EPA Method 1694 is in the ng/L or ppt range (approximately 50 of the 70 compounds or 71%). With these low LODs, it is possible to routinely monitor the majority of the PPCPs of EPA Method 1694 at the ng/L level or lower using a 1-L water sample and concentrating to 1-mL as directed by the EPA method.

Wastewater Analysis

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 4 shows an example standard curve for sulfamethoxazole 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.

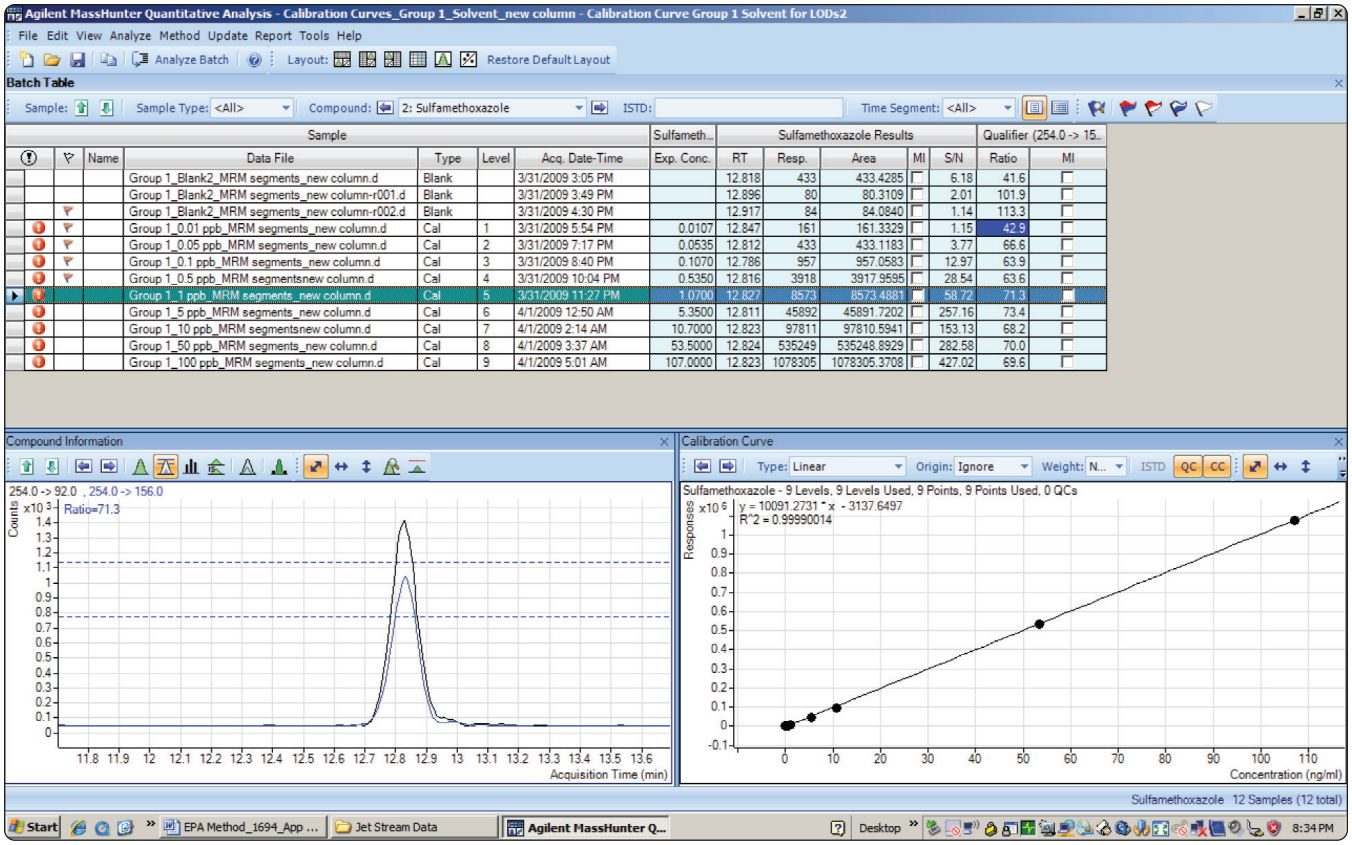

Figure 4. Calibration curve for sulfamethoxazole in a wastewater matrix using a six point curve from 0.1 to 100 ng/mL (ppb) using a linear fit with no origin treatment. Note how the software displays the ion ratios in the proper boundaries.

Finally, a "unspiked" wastewater sample was analyzed and the presence of 5 pharmaceuticals: carbamazepine, cotinine, diphenhydramine, thiabendazole, and trimethoprim could be confirmed with two MRM transitions. Figure 5 shows the ion ratios of the qualifying and the quantifying ion for two of these compounds in the wastewater extract. As shown in Figure 5 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.

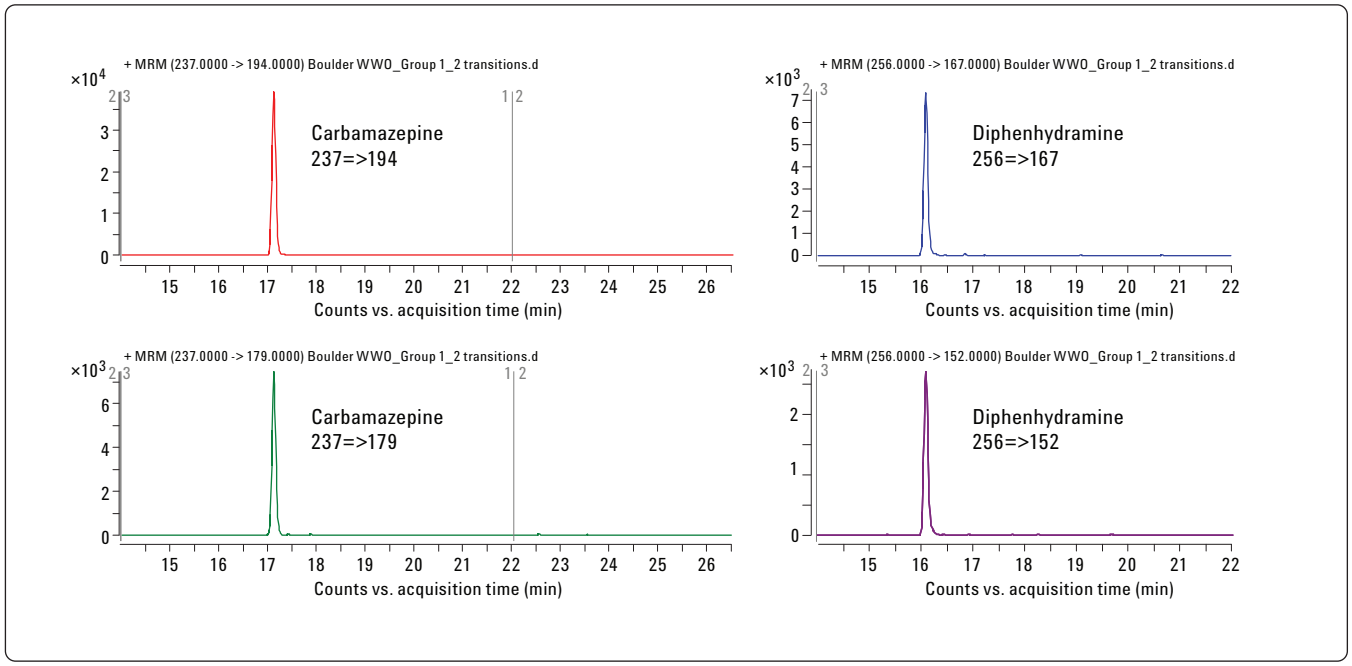

Figure 5. MRM chromatograms of a wastewater sample for carbamazepine and diphenhydramine using 2 transitions.

Conclusions

The results of this study show that the Agilent 6410 and 6460 Triple Quadrupoles are robust, sensitive, and repeatable instruments for the study of pharmaceuticals in water samples, using high throughput methods. The Jet Stream Technology will add another 10 to 20 times sensitivity for the PPCP compounds. It will also allow routine analysis at the ng/L or ppt level or lower in wastewater matrices using a 1-L water sample and concentrating to 1-mL as outlined in EPA Method 1694. Furthermore, we have shown that the combination of MRMs and rapid resolution will speed up the analysis times for pharmaceuticals in EPA Method 1694 from over 90 min to approximately a 30-min analysis time. Finally, the analysis of 18 commonly found pharmaceuticals and internal standards were added to the method for a total of 107 compounds.

References

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