Highly Sensitive Detection of Pharmaceuticals and Personal Care Products (PPCPs) in Water Using an Agilent 6495 Triple Quadrupole Mass Spectrometer

Application Note

Authors

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Abstract

This Application Note describes two methods to detect pharmaceuticals and personal care products (PPCPs) in water at part per trillion (ppt) levels using the Agilent 6495 Triple Quadrupole Mass Spectrometer. The methods are divided into positive ion mode method and negative ion mode method since different mobile phases are required. The precise and accurate quantitation of 118 compounds with 316 MRM transitions in positive ion mode, and 22 compounds with 62 MRM transitions in negative ion mode were accomplished by dynamic multiple reaction monitoring (DMRM). The highly sensitive 6495 Triple Quadrupole LC/MS system was used to streamline the analysis by direct injection of 40 µL water samples without tedious analyte enrichment by solid phase extraction (SPE).

Agilent Technologies

Introduction

Pharmaceuticals and Personal Care Products (PPCPs) comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics. Several studies have shown that pharmaceuticals are present in our water supply systems[1,2]. PPCPs in surface waters can eventually enter drinking water systems when treatments are insufficient. Governmental agencies, such as the EPA and European Water Framework, have proposed regulations to monitor water supply systems[3,4].

PPCPs exist at low concentrations in drinking water, typically at part per trillion (ppt) or ng/L levels. This poses significant analytical challenges. Sample enrichment by solid phase extraction (SPE) is often necessary where detection is performed using low to mid-range triple quadrupole mass spectrometers[5]. SPE requires large sample quantities, high consumption of solvents, and laborious procedures. With the advent of the highly sensitive Agilent 6495 Triple Quadrupole Mass Spectrometer in combination with the Agilent Jet Stream Ionization Source for more efficient ion generation and sampling, we were able to investigate the occurrence and fate of PPCPs in water supply systems from source water to tap water by direct injection of water samples at low ppt levels.

Improvements to the 6495 Triple Quadrupole include new front end ion optics for increased precursor ion transmission, a newly designed curved and tapered collision cell for improved MS/MS spectral fidelity, and a new ion detector operating at dynode accelerating voltages of up to 20 kV. With this increased sensitivity, analytical workflow can be simplified and throughput can be increased. The extent of sample preparation includes filtering approximately 3 mL of sample, adding internal standards to a 1.0-mL aliquot of the filtered sample and injecting 40 µL of sample for analysis by LC/MS/MS with reporting limits for all analytes at 10 ppt. Limit of detection (LOD) and lower limit of quantitation (LLOQ) for most of the analytes are much lower than 10 ppt.

Experimental

Reagents and chemicals

All reagents and solvents were of HPLC-MS grade. Acetonitrile was purchased from Honeywell (015-4). Ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Ammonium acetate, 5 M solution, was purchased from Fluka (09691-250ML). Acetic acid was purchased from Aldrich (338828-25ML). The PPCP standards and some of the internal standards were acquired from an outside collaborator. The analytes and their internal standards as well as their MRM transitions are listed in Table 1 for the positive ion mode method and Table 2 for the negative ion mode method, respectively.

Table 1. MRM transitions of analytes and internal standards in positive ion mode method.

Compound name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
10,11-dihydro-10-hydroxycarbamazepine	255.12	237	4				
10,11-dihydro-10-hydroxycarbamazepine	255.12	194.1	20				
6-Acetylmorphine	328.16	211.1	24	D6 6-Acetylmorphine	334.19	211.1	24
6-Acetylmorphine	328.16	165.1	44	D6 6-Acetylmorphine	334.19	165.1	44
Acebutolol	337.21	116	16				
Acebutolol	337.21	56	40				
Acetaminophen	152.07	110	12	D4 Acetaminophen	156.1	114.1	12
Acetaminophen	152.07	65.1	32				
Albuterol	240.16	222.2	0				
Albuterol	240.16	148	12				
Amitriptyline	278.19	202.2	68	D3 Amitriptyline	281.21	91.1	32
Amitriptyline	278.19	91	24				
Amitriptyline metabolite	294.19	276.2	8				
Amitriptyline metabolite	294.19	214.9	48				
Amphetamine	136.11	119.1	4	D5 Amphetamine	141.1	96.1	12
Amphetamine	136.11	91.1	12				
Aripiprazole	448.16	285.1	24	D8 Aripiprazole	456.21	293.1	24
Aripiprazole	448.16	98.2	40				
Atenolol	267.17	145.1	24	D7 Atenolol	274.22	145.1	24
Atenolol	267.17	74	20				
Atorvastatin	559.26	440.2	20				
Atorvastatin	559.26	250.2	40				
Atrazine	216.1	174.1	8	D5 Atrazine	221.14	179.2	8
Atrazine	216.1	68.2	36	D5 Atrazine	221.14	69.1	40
Benzoylecgonine	290.14	168.2	16	D3 Benzoylecgonine	293.16	171.2	12
Benzoylecgonine	290.14	77	64				
Buprenorphine	468.31	84.2	48	D4 Buprenorphine	472.34	59.2	52
Buprenorphine	468.31	55.1	52				
Bupropion	240.12	184	4				
Bupropion	240.12	131	20				
Caffeine	195.09	138.1	20	^{13}C3 Caffeine	198.1	140.1	20
Caffeine	195.09	110.3	20				
Carbamazepine	237.1	194.1	12	D10 Carbamazepine	247.17	204.2	20
Carbamazepine	237.1	193.1	28	D10 Carbamazepine	247.17	202.1	36
Carbamazepine 10,11 epoxide	253.1	210.2	8				
Carbamazepine 10,11 epoxide	253.1	180.1	24				
Carisoprodol	261.18	176.2	0	D7 Carisoprodol	268.23	183.1	0
Carisoprodol	261.18	55.1	24	D7 Carisoprodol	268.23	62.2	12
Chlorpheniramine	275.13	230	8				
Chlorpheniramine	275.13	167.1	44				
Clenbuterol	277.09	203	8	D9 Clenbuterol	286.15	204	8
Clenbuterol	277.09	132.1	28				
Clopidogrel carboxylic acid	308.05	198.1	8				
Clopidogrel carboxylic acid	308.05	76.9	64				
Cocaethylene	318.17	196.2	12	D3 Cocaethylene	321.19	199.2	12
Cocaethylene	318.17	82.2	28				
Cocaine	304.16	182.2	16	D3 Cocaine	307.18	185.1	12

Table 1. MRM transitions of analytes and internal standards in positive ion mode method (continued).

Compound name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
Cocaine	304.16	77	64				
Codeine	300.16	199.1	76	D6 Codeine	306.2	202	52
Codeine	300.16	152	72	D6 Codeine	306.2	153	52
Codeine	300.16	115.2	76				
Cotinine	177.1	98	24	D3 Cotinine	180.12	101	24
Cotinine	177.1	80.1	36	D3 Cotinine	180.12	79.8	28
DEET	192.14	119.3	16	D6 DEET	198.18	118.9	16
DEET	192.14	91	32	D6 DEET	198.18	90.9	32
Dehydroaripiprazole	446.14	285.1	24				
Dehydroaripiprazole	446.14	98.1	44				
Desmethylcitalopram	311.16	262.2	8	D3 Desmethylcitalopram	314.18	109.1	20
Desmethylcitalopram	311.16	109.1	20				
Desmethylvenlafaxine	264.2	246.2	4	D6 Desmethylvenlafaxine	270.24	64	12
Desmethylvenlafaxine	264.2	58.1	16				
Dextromethorphan	272.2	171.1	36	D3 Dextromethorphan	275.22	171.2	36
Dextromethorphan	272.2	128.1	64				
Diltiazem	415.17	178.1	20				
Diltiazem	415.17	109.1	76				
Diphenhydramine	256.17	165.1	48	D3 Diphenhydramine	259.19	167.2	8
Diphenhydramine	256.17	152.1	44				
Disopyramide	340.24	239.1	8				
Disopyramide	340.24	194.2	48				
Donepezil	380.22	91.1	40				
Donepezil	380.22	65.2	76				
Duloxetine	298.13	153.9	0	D3 Duloxetine	301.15	157.1	0
Duloxetine	298.13	44	12	D3 Duloxetine	301.15	46.9	16
Ecgonine methyl ester	200.13	182	12	D3 Ecgonine methyl ester	203.15	185.2	12
Ecgonine methyl ester	200.13	82.1	24				
EDDP	278.19	249	20	D3 EDDP	281.21	234	24
EDDP	278.19	234.1	24				
Erythromycin	734.47	158.1	24	^{13}C2 Erythromycin	736.48	160	24
Erythromycin	734.47	83.1	68				
Erythromycin-anhydro	716.46	158	24				
Erythromycin-anhydro	716.46	83.2	76				
Escitalopram	325.17	262.2	16				
Escitalopram	325.17	109.1	20				
Famotidine	338.05	189.1	12				
Famotidine	338.05	155	28				
Fentanyl	337.23	188.3	20	D5 Fentanyl	342.2	105.1	36
Fentanyl	337.23	105.1	36				
Fluoxetine	310.14	148.2	4	D6 Fluoxetine	316.18	44	16
Fluoxetine	310.14	44	16				
Fluticasone propionate	501.19	313	8				
Fluticasone propionate	501.19	293.2	12				
Gabapentin	172.14	154.1	8	D10 Gabapentin	182.2	164.1	12
Gabapentin	172.14	55	24				
Glyburide	494.15	369	12				

Table 1. MRM transitions of analytes and internal standards in positive ion mode method (continued).

Compound name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
Glyburide	494.15	169.1	36				
Hydrocodone	300.16	199.1	28	D6 Hydrocodone	306.2	202	24
Hydrocodone	300.16	171.1	40				
Hydromorphone	286.15	185.1	24	D3 Hydromorphone	289.17	185	32
Hydromorphone	286.15	157.1	48				
Hydroxybupropion	256.11	238.1	4	D6 Hydroxybupropion	262.15	244.1	4
Hydroxybupropion	256.11	130.1	48				
Ketoprofen	255.1	209.2	4				
Ketoprofen	255.1	77.1	52				
Lamotrigine	256.02	109	52	^{13}C-$^{15}N4$ Lamotrigine	261.01	74.2	76
Lamotrigine	256.02	74	76	$^{13}C3$ Lamotrigine	259.03	74.1	76
Levorphanol	258.19	199.1	20				
Levorphanol	258.19	157.2	32				
Lidocaine	235.18	86.2	8				
Lidocaine	235.18	58.1	32				
Loratadine	383.15	337.2	20				
Loratadine	383.15	266.9	32				
Lorazepam	321.02	275.1	12	D4 Lorazepam	325.05	279	20
Lorazepam	321.02	229.2	28				
MDA	180.1	163.2	4				
MDA	180.1	105.2	20				
MDEA	208.14	163.1	4				
MDEA	208.14	77.1	44				
MDMA	194.12	163.1	8	D5 MDMA	199.15	165.1	8
MDMA	194.12	77.1	40				
Mefenamic acid	242.12	224	16	D3 Mefenamic acid	245.14	227	16
Mefenamic acid	242.12	208	36	D3 Mefenamic acid	245.14	212	28
Meperidine	248.17	220.2	16	D4 Meperidine	252.19	224.2	16
Meperidine	248.17	174.1	16				
Meprobamate	219.14	158.1	0	D7 Meprobamate	226.18	165	0
Meprobamate	219.14	97	12				
Metformin	130.1	71.1	24				
Metformin	130.1	60	12				
Methadone	310.22	265.2	8	D9 Methadone	319.28	267.9	8
Methadone	310.22	104.9	28				
Methamphetamine	150.13	119.1	8	D11 Methamphetamine	161.2	127.2	8
Methotrexate	455.18	308.2	16	D3 Methotrexate	458.2	311.2	16
Methotrexate	455.18	175.1	36				
Methylphenidate	234.15	84.2	20	D9 Methylphenidate	243.21	93.2	20
Methylphenidate	234.15	56.2	52				
Metoprolol	268.19	76.9	56				
Metoprolol	268.19	56.2	24				
Mevastatin	391.25	185.1	8				
Mevastatin	391.25	159.1	24				
m-Hydroxybenzoylecgonine	306.14	168.1	12				
m-Hydroxybenzoylecgonine	306.14	65.2	72				
Modafinil	296.1	129.2	8	D10 Modafinil	306.14	129	4

Table 1. MRM transitions of analytes and internal standards in positive ion mode method (continued).

Compound name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
Monoethylglycinexylidide	207.15	122.2	8				
Monoethylglycinexylidide	207.15	58.2	4				
Montelukast	586.22	422.1	20				
Montelukast	586.22	278.1	28				
Morphine	286.15	152.2	64	D3 Morphine	289.17	152.1	64
Morphine	286.15	128	68				
Nifedipine	347.13	315.2	0				
Nifedipine	347.13	195.1	36				
Nifedipine oxidized	345.11	284	24				
Nifedipine oxidized	345.11	268.1	24				
Norfentanyl	233.17	84	12	D5 Norfentanyl	238.2	83.9	16
Norfentanyl	233.17	55	40	D5 Norfentanyl	238.2	55	44
Norfluoxetine	296.13	134.1	0	D6 Norfluoxetine	302.17	140.2	0
Norfluoxetine	296.13	30	8	D6 Norfluoxetine	302.17	30.1	16
Normeperidine	234.15	160.3	8	D4 Normeperidine	238.18	164.3	8
Normeperidine	234.15	91.2	48				
Normeperidine	234.15	56.1	20				
Norquetiapine	296.12	210.1	24				
Norquetiapine	296.12	139.1	60				
Norsertraline	275	159.1	16	^{13}C6 Norsertraline	281	158.9	16
Norsertraline	275	89	72				
Norverapamil	441.28	165	20				
Norverapamil	441.28	150.3	36				
Omeprazole	346.12	198.1	4				
Omeprazole	346.12	136	28				
Oxazepam	287.06	268.9	8				
Oxazepam	287.06	240.9	16				
Oxcarbazepine	253.1	208	16				
Oxcarbazepine	253.1	180.1	24				
Oxycodone	316.16	298.2	8				
Oxycodone	316.16	241.1	24				
Oxymorphone	302.14	284.1	12	D3 Oxymorphone	305.16	287.1	12
Oxymorphone	302.14	227	20				
Oxymorphone glucuronide	478.17	284.1	28	D3 Oxymorphone glucuronide	481.19	287.2	32
Oxymorphone glucuronide	478.17	227.1	48				
Paroxetine	330.15	192.1	16	D6 Paroxetine	336.19	76.1	32
Paroxetine	330.15	70.1	32				
Phenmetrazine	178.13	115	32				
Phenmetrazine	178.13	91	36				
Phentermine	150.13	133.1	4	D5 Phentermine	155.16	96	20
Phenylpropanolamine	152.11	134	4	D3 Phenylpropanolamine	155.13	136.9	8
Phenylpropanolamine	152.11	117	12				
Pioglitazone	357.13	133.9	24				
Pioglitazone	357.13	119	48				
Pregabalin	160.14	142.2	8	D6 Pregabalin	166.17	148	8
Pregabalin	160.14	55.2	20				
Primidone	219.12	162.1	4				

Table 1. MRM transitions of analytes and internal standards in positive ion mode method (continued).

Compound name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
Primidone	219.12	91.2	24				
Propranolol	260.17	116.1	12	D7 Propranolol	267.21	56	28
Propranolol	260.17	56.1	24				
Pseudoephedrine	166.13	115.1	24	D3 Pseudoephedrine	169.14	151.1	8
Pseudoephedrine	166.13	91	32				
Quetiapine	384.18	253.1	16	D8 Quetiapine	392.23	258	20
Quetiapine	384.18	221	36				
Ritalinic acid	220.14	84.2	16	D10 Ritalinic acid	230.2	93	24
Ritalinic acid	220.14	56.1	40				
Sertraline	306.08	275	4	D3 Sertraline	309.1	275	4
Sertraline	306.08	159.1	20				
Sildenafil	475.21	100	24				
Sildenafil	475.21	58.1	40				
Simvastatin	419.28	198.9	12				
Simvastatin	419.28	173	28				
Sotalol	273.13	255.1	4				
Sotalol	273.13	133.1	20				
Sulfamethazine	279.09	186	8	^{13}C6 Sulfamethazine	285.11	98	32
Sulfamethazine	279.09	92.1	28				
Sumatriptan	296.15	155.9	52				
Sumatriptan	296.15	58	12				
Tadalafil	390.15	268	0				
Tadalafil	390.15	204	80				
Temazepam	301.08	283	4	D5 Temazepam	306.11	288	4
Temazepam	301.08	255	16	D5 Temazepam	306.11	260	16
Thiabendazole	202.05	175	24	^{13}C6 Thiabendazole	208.07	181	28
Thiabendazole	202.05	131.1	32				
Tramadol	264.2	58.1	12	^{13}C-D3 Tramadol	268.22	58.1	12
Tramadol	264.2	56.1	68				
Trazadone	372.16	176.1	20	D6 Trazadone	378.2	182.1	20
Trazadone	372.16	148.1	36				
Triamterene	254.12	237.1	24				
Triamterene	254.12	104.1	32				
Trimethoprim	291.15	230.2	20	^{13}C3 Trimethoprim	294.16	233	20
Trimethoprim	291.15	123.2	24				
Tylosin	916.53	174.2	40				
Tylosin	916.53	83.1	60				
Valsartan	436.24	235.1	12				
Valsartan	436.24	207.2	20				
Venlafaxine	278.21	260.3	0	D6 Venlafaxine	284.25	64.1	16
Venlafaxine	278.21	58.2	20				
Verapamil	455.29	165.1	24				
Verapamil	455.29	150.1	40				
Zolpidem	308.18	235.2	32	D7 Zolpidem	315.22	242	36
Zolpidem	308.18	65.2	72				
Zolpidem phenyl-4-carboxylic acid	338.15	265	36				
Zolpidem phenyl-4-carboxylic acid	338.15	65.1	76				

Table 2. MRM transitions of analytes and internal standards in negative ion mode method.

Name	Precursor ion	Product ion	CE (eV)	ISTD	Precursor ion	Product ion	CE (eV)
(±)11-*nor*-9-carboxy-*delta*-THC	343.19	299.3	20	D9 (±)11-*nor*-9-carboxy-*delta*-THC	352.25	308.1	20
(±)11-*nor*-9-carboxy-*delta*-THC	343.19	245	24				
Bezafibrate	360.1	274	12				
Bezafibrate	360.1	154.1	28				
Celecoxib	380.1	316.1	20				
Celecoxib	380.1	276	28				
Chloramphenicol	321	152	12	D5 Chloramphenicol	326.03	157.1	12
Chloramphenicol	321	46	80	D5 Chloramphenicol	326.03	45.9	64
Diclofenac	294.01	250	8	D4 Diclofenac	298	254	8
Diclofenac	294.01	35	32	D4 Diclofenac	298	34.9	24
Diclofenac 4-hydroxy	310	265.9	8	^{13}C6 Diclofenac 4-hydroxy	316	272	8
Diclofenac 4-hydroxy	310	34.7	32	^{13}C6 Diclofenac 4-hydroxy	316	34.8	36
Fenbufen	253.08	153.1	20				
Fenbufen	253.08	55	24				
Furosemide	329	285	8				
Furosemide	329	204.9	16				
Gemfibrozil	249.15	127.1	4	D6 Gemfibrozil	255.18	121	4
Gemfibrozil	249.15	121	4				
Hydrochlorothiazide	295.95	268.9	12				
Hydrochlorothiazide	295.95	205.2	20				
Ibuprofen	205.12	161.2	2	^{13}C3 Ibuprofen	208.13	163.1	0
Methylparaben	151.04	135.9	8	^{13}C6 Methylparaben	157.1	141.8	8
Methylparaben	151.04	92	16	^{13}C6 Methylparaben	157.1	97.9	16
Modafinil acid	273.06	167.1	8				
Modafinil acid	273.06	165	36				
Naproxen	229.08	170.1	4				
Naproxen	229.08	168.9	28				
n-Butylparaben	193.08	136	12	^{13}C6 *n*-Butylparaben	199.1	141.9	12
n-Butylparaben	193.08	92	20	^{13}C6 *n*-Butylparaben	199.1	98	20
Phenobarbital	231.07	188.1	0	D5 Phenobarbital	236.11	42	12
Phenobarbital	231.07	42.1	12				
Phenytoin	251.1	102.1	20	D10 Phenytoin	261.1	106	20
Phenytoin	251.1	41.7	60	D10 Phenytoin	261.1	41.9	56
Pravastatin	423.2	320.9	12				
Pravastatin	423.2	303.2	16				
Sulfamethoxazole	252.04	156	8	^{13}C6 Sulfamethoxazole	258.06	162.1	8
Sulfamethoxazole	252.04	63.8	36	^{13}C6 Sulfamethoxazole	258.06	63.9	28
Triclocarban	312.97	160.1	8	^{13}C6 Triclocarban	319	159.9	8
Triclocarban	312.97	35	44				
Triclosan	286.94	35	4	^{13}C12 Triclosan	299	34.8	4
Warfarin	307.09	250	16				
Warfarin	307.09	161.2	16				

Instruments

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Standard Autosampler (G4226A) and sample cooler (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)

UHPLC conditions are listed in Table 3 and Table 4 for positive ion mode method and negative ion mode method, respectively.

Table 3. Agilent 1290 UHPLC conditions for positive ion mode method.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Column temp	40 °C
Injection volume	40 µL
Speed	Draw 100 µL/min; Eject 100 µL/min
Autosampler temperature	6 °C
Needle wash	5 seconds (80 % MeOH/20 % water)
Mobile phase	A) Water with 5 mM ammonium acetate + 0.02 % acetic acid B) Acetonitrile
Flow rate	0.3 mL/min
Gradient program	Time B % 0 5 0.5 5 11 100 13 100 13.1 5
Stop time	15 minutes
Post time	1 minute

Table 4. Agilent 1290 UHPLC conditions for negative ion mode method.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Column temperature	40 °C
Injection volume	40 µL
Speed	Draw 100 µL/min; Eject 100 µL/min
Autosampler temperature	6 °C
Needle wash	5 seconds (80 % MeOH/20 % water)
Mobile phase	A) Water with 0.005 % acetic acid B) Acetonitrile
Flow rate	0.3 mL/min
Gradient program	Time B % 0 5 0.5 5 6 100 8 100 8.1 5
Stop time	10 minutes
Post time	1 minute

MS detection

Agilent 6495 Triple Quadrupole Mass Spectrometer with Agilent Jet Stream Electrospray Ionization Source

Agilent Jet Stream ionization source parameters and Funnel RF voltages are critical for the sensitive detection of analytes. Agilent MassHunter B.07 Acquisition Software includes the MassHunter Source and iFunnel Optimizer Software that allows the users to get the best conditions for analytes in an automated sequential fashion. Applying all the optimized parameters obtained by optimizer software, including both the low-pressure and high-pressure ion funnel RF voltages, provided a significant increase in analyte responses[6]. For multiple-analyte applications, parameters are typically weighted towards hard-to-detect analytes. Mass spectrometer source conditions generated by the Optimizer software are listed in Table 5 for the positive ion mode method and Table 6 for the negative ion mode method.

Software

- Agilent MassHunter Data Acquisition Software, for triple quadruple mass spectrometer, Version B.07.00
- Agilent MassHunter Qualitative Software, Version B.06.0.633.10 SP1
- Agilent MassHunter Quantitative Software, Version B.07.00/Build 7.0.457.0

Table 5. Agilent 6495 Triple Quadrupole Mass Spectrometer source parameters for positive ion mode method.

Parameter	Value
Ion mode	Positive
Drying gas temperature	250
Drying gas flow	16
Sheath gas temperature	400
Sheath gas flow	12
Nebulizer pressure	40
Capillary voltage	3,000
Nozzle voltage	0
Delta EMV	200
LPF RF	60
HPF RF	160
MS1 and MS2 resolution	Unit

Dilutions

Stock solutions for analyte standards and internal standards were prepared at 25 ppb in acetonitrile for each compound. All samples were fortified with internal standards at a constant concentration of 250 ppt, while calibration standards were spiked at 10 ppt, 25 ppt, 50 ppt, 100 ppt, 250 ppt, 500 ppt, and 1,000 ppt (7 levels) in MilliQ water.

Two of the three unknown samples were from an outside collaborator. One was from a remote site removed from significant anthropogenic sources, and one was from an urban surface water source. Another sample was local drinking tap water (Santa Clara, USA). All unknown samples were fortified with internal standards at 250 ppt after filtration.

Table 6. Agilent 6495 Triple Quadrupole Mass Spectrometer source parameters for negative ion mode method.

Parameter	Value
Ion mode	Negative
Drying gas temperature	200
Drying gas flow	12
Sheath gas temperature	400
Sheath gas flow	12
Nebulizer pressure	40
Capillary voltage	3,000
Nozzle voltage	2,000
Delta EMV	200
LPF RF	40
HPF RF	90
MS1 and MS2 resolution	Unit

Results and Discussion

Increased method performance

The 6495 Triple Quadrupole LC/MS design enhancements provide an efficient ion transmission[7]. Figure 1 and Figure 2 show the responses of 118 analytes in positive ion mode, and 22 analytes in negative ion mode at 10 ppt.

It is clearly demonstrated that most of the compounds can be detected at a concentration much lower than 10 ppt without sample enrichment.

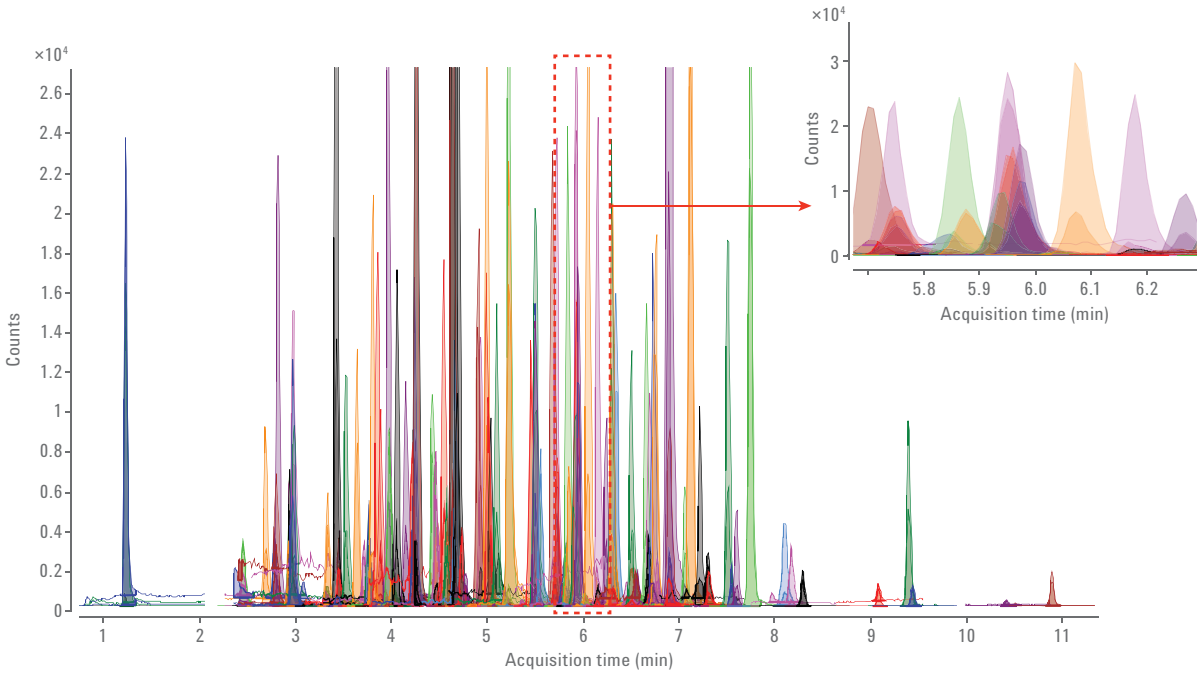

Figure 1. Signal response of the Agilent 6495 systems in positive ion mode (10 ppt at 40 µL direct injection).

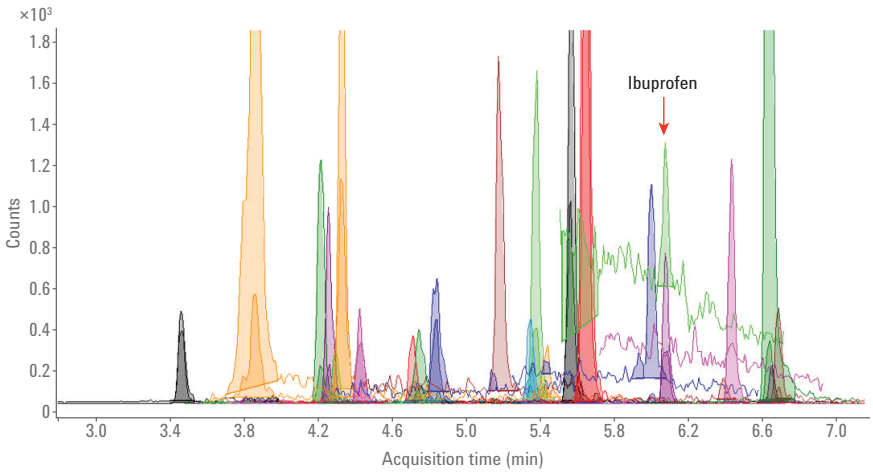

Figure 2. Signal response of the Agilent 6495 systems in negative ion mode (10 ppt at 40 µL direct injection).

Calibration curves

Calibration curves were assessed with PPCPs spiked in MilliQ water samples covering a concentration range from 10 ppt to 1,000 ppt. Examples of the calibration curves for metformin in positive ion mode and ibuprofen in negative ion mode are shown in Figure 3. The calibration equations were generated using a quadratic fit with a weighting factor of 1/x including the origin. The correlation coefficients (R^2) for all target analytes in both polarities were greater than 0.99, and most were greater than 0.995, except for quetiapine in positive ion mode (R^2 = 0.982) due to an interfering systematic peak nearby.

Precision and accuracy

Triplicate injections were made for calibration curves at each level. In most of the cases, the precision was very good. There were occasional cases in which accuracy was beyond the 80–120 % range. Five to six very hydrophobic compounds, such as statin drugs, buprenorphine, and montelukast, had accuracy outliers at the low levels. This may be due to the HPLC vial surface absorption of the compounds at lower spike levels. Overall, only 2.3 % of measurements had accuracy outliers beyond 80–120 % (< 1 accuracy outlier per two compounds with 21 measurements per compound) if five outlier compounds were removed from accuracy consideration in positive ion mode. In negative ion mode, accuracy was excellent for all compounds except for celecoxib. The accuracy issue for celecoxib may be caused by the uneven HPLC vial surface adsorption at low levels without a corresponding internal standard.

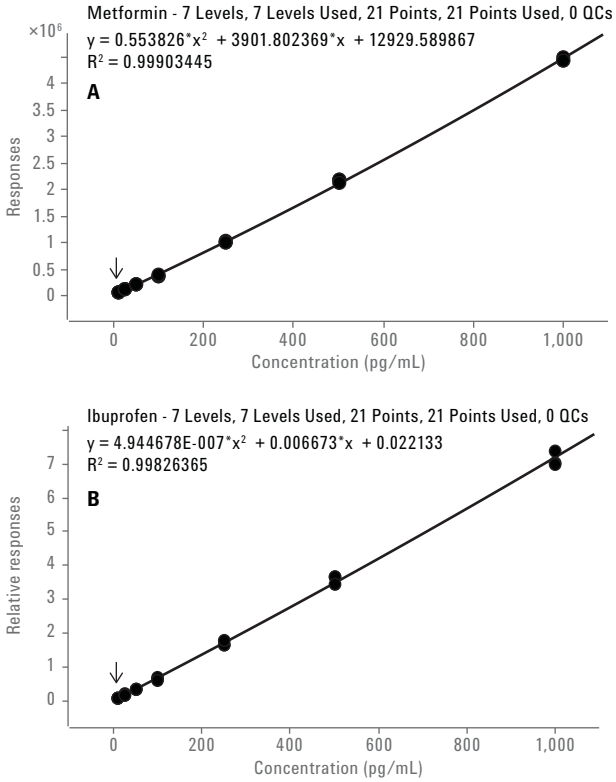

Figure 3. Calibration curves of metformin (positive) and ibuprofen (negative) in milliQ water.

Real-world samples

Three samples were tested. The first was from local tap water (Santa Clara, USA). The other two samples were from an outside collaborator: one from a remote site removed from significant anthropogenic sources and the other from an urban surface water source. Duplicate injections were run on each sample. The compound was considered positive if the average concentration of the two runs was greater than 10 ppt. The positive results are listed for these samples in Tables 7–10. Figure 4 and Figure 5 show the chromatographs for the Santa Clara tap water and remote source water, respectively. Three compounds were detected above 10 ppt in each sample.

Table 7. Compounds found in local tap water with positive ion mode method.

Name	Injection 1 (ppt)	Injection 2 (ppt)	Average (ppt)
Gabapentin	21	20	20
Metformin	31	30	31
Montelukast	13	12	13

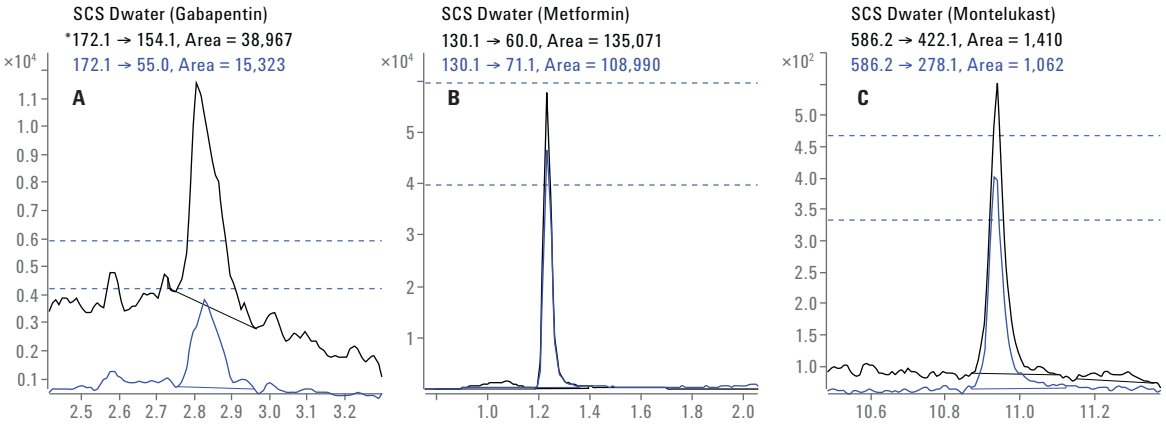

Figure 4. Chromatographs of PPCPs found in local tap water (Santa Clara, CA) with positive ion method.

Table 8. Compounds found in remote source sample with positive ion mode method.

Name	Injection 1 (ppt)	Injection 2 (ppt)	Average (ppt)
Montelukast	12	12	12
Caffeine	27	15	21
DEET	107	119	113

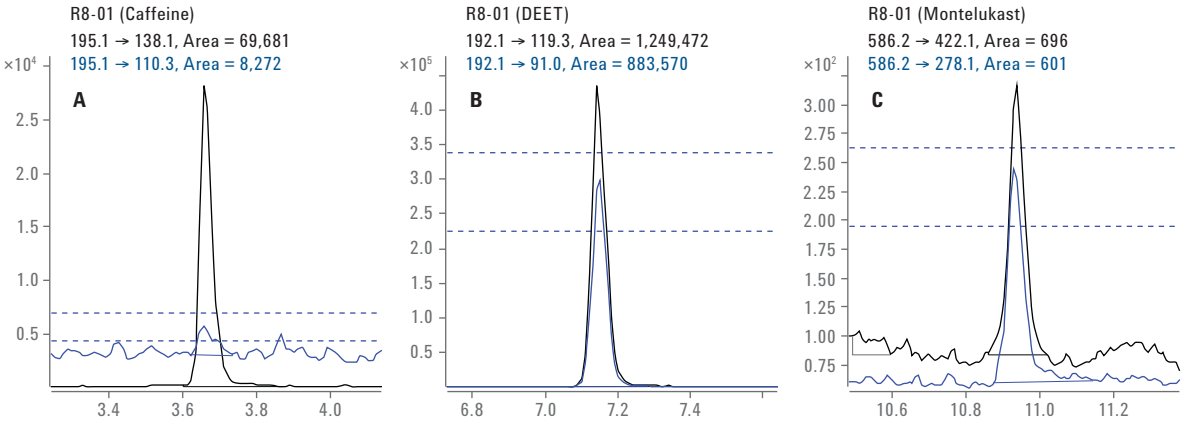

Figure 5. Chromatographs of PPCPs found in remote source sample with positive ion mode method.

Table 9. Compounds found in an urban surface water sample with positive ion mode method.

Name	Injection 1 (ppt)	Injection 2 (ppt)	Average (ppt)
10,11-dihydro-10-hydroxycarbamazepine	903	861	882
Amitriptyline metabolite	30	30	30
Amitriptyline	29	29	29
Atenolol	2,599	2,212	2,405
Atorvastatin	40	37	39
Atrazine	43	41	42
Benzoylecgonine	221	206	214
Bupropion	169	154	162
Caffeine	1,473	1,241	1,357
Carbamazepine 10,11 epoxide	38	36	37
Carbamazepine	214	229	221
Carisoprodol	27	28	28
Clopidogrel carboxylic acid	223	204	214
Cocaine	37	35	36
Codeine	67	67	67
Cotinine	98	90	94
DEET	503	570	536
Desmethylcitalopram	107	88	97
Desmethylvenlafaxine	744	827	786
Dextromethorphan	31	42	36
Diltiazem	55	61	58
Diphenhydramine	205	205	205
Ecgonine methyl ester	39	39	39
EDDP	102	100	101
Erythromycin	44	44	44
Erythromycin-anhydro	38	31	34
Escitalopram	192	179	186
Fluoxetine	30	28	29
Gabapentin	>>1,000	>>1,000	>>1,000
Hydrocodone	28	24	26
Hydroxybupropion	260	253	257
Ketoprofen	17	15	16
Lamotrigine	868	1,013	940
Levorphanol	213	205	209
Lidocaine	360	325	343
Loratadine	10	10	10
Lorazepam	137	143	140
Meprobamate	160	147	154
Metformin	3,956	3,956	3,956
Methadone	58	39	49
Methamphetamine	259	315	287
Metoprolol	295	334	315
Modafinil	16	14	15
Monoethylglycinexylidide	28	31	30
Montelukast	12	12	12
Norquetiapine	32	25	28
Norsertraline	32	24	28
Oxazepam	29	27	28
Oxcarbazepine	45	42	44
Oxycodone	95	83	89
Oxymorphone	17	14	15
Phentermine	117	117	117
Pregabalin	440	445	442
Primidone	77	58	68
Propranolol	70	71	70
Pseudoephedrine	211	236	223
Ritalinic acid	111	127	119
Sertraline	47	44	46
Sotalol	68	72	70
Sulfamethazine	10	13	11
Temazepam	89	83	86
Thiabendazole	37	43	40
Tramadol	708	727	717
Trazadone	35	30	33
Triamterene	100	111	106
Trimethoprim	277	321	299
Tylosin	13	10	11
Valsartan	475	517	496
Venlafaxine	446	384	415
Verapamil	11	10	11
Zolpidem phenyl-4-carboxylic acid	46	47	47

No compounds were found in the local tap water or the remote source water samples with negative ion mode method. The compounds found in the urban surface water sample in negative ion mode are listed in Table 10.

Flexible reporting enabled by MassHunter Quantitative Analysis Software B.07

Instead of exporting results and averaging the replicates in excel, users can use the Fast PDF reporting system in Quant Analysis Software B.07 to generate the result in the desired format: averaging replicates, inserting preferred logo, and defining sample layout etc. The average of replicates can be accomplished by grouping the replicates under **Sample Group**.

There are different choices of PDF report templates in the software product. Table 11 lists all of the relevant templates in Quant B07.

Table 10. Compounds found in an urban surface water sample with negative ion mode method.

Name	Injection 1 (ppt)	Injection 2 (ppt)	Average (ppt)
Celecoxib	45	41	43
Chloramphenicol	12	12	12
Diclofenac 4-hydroxy	41	45	43
Diclofenac	237	292	265
Furosemide	400	387	393
Gemfibrozil	309	337	323
Hydrochlorothiazide	503	487	495
Ibuprofen	140	139	139
Modafinil acid	118	114	116
Naproxen	354	347	350
Phenobarbital	55	53	54
Phenytoin	126	121	123
Pravastatin	57	52	54
Sulfamethoxazole	573	582	577
Triclocarban	40	39	39
Triclosan	242	268	255

Table 11. List of PDF report templates in Agilent MassHunter Quantitative Analysis B.07.

DIR	SUBDIR	Catogory	PDFTemplate
PDF-Reporting		Compliance	AuditTrail.report.xml
PDF-Reporting		Enviromental	Env_CC_Avg.report.xml
PDF-Reporting		Enviromental	Env_CC_MidPoint.report.xml
PDF-Reporting		Enviromental	Env_CC_Previous.report.xml
PDF-Reporting		Enviromental	Env_DualGCResults.report.xml
PDF-Reporting		Enviromental	Env_InitialCal.report.xml
PDF-Reporting		Enviromental	Env_LCSSpike.report.xml
PDF-Reporting		Enviromental	Env_MSD.report.xml
PDF-Reporting		Enviromental	Env_QA_Check.report.xml
PDF-Reporting		Enviromental	Env_Results.report.xml
PDF-Reporting		Enviromental	Env_Results_withGraphics.report.xml
PDF-Reporting		Enviromental	Env_TPH_Validation.report.xml
PDF-Reporting		General	Gen_ByCompound.report.xml
PDF-Reporting		General	Gen_BySample.report.xml
PDF-Reporting		General	Gen_BySample_withSN.report.xml
PDF-Reporting		General	Gen_Calibration.report.xml
PDF-Reporting		General	Gen_Complete.report.xml
PDF-Reporting		General	Gen_ResultsSummary.report.xml
PDF-Reporting		General	Gen_Samples.report.xml
PDF-Reporting		Special	Pesticide_Residues.report.xml
PDF-Reporting		Special	SIMScan.report.xml
PDF-Reporting		Special	TargetedDeconvolution.report.xml
PDF-Reporting	Unknowns	Unknowns	all-hits.report.xml
PDF-Reporting	Unknowns	Unknowns	best-hits.report.xml

Figure 6 shows an example report of one sample in this study by the new PDF reporting generating system. The results of each sample can be arranged in separate pages or in the same page.

Conclusion

Fast and simple LC/MS/MS methods for the accurate confirmation and quantitation of PPCPs in water have been developed. The methods leverage the full advantage of high sensitivity provided by the Agilent 6495 Triple Quadrupole Mass Spectrometer. It has been demonstrated that low ppt level LLOQs can be achieved for the quantitation of trace contaminants in water through direct injection. With these new design enhancements, tedious sample enrichment and cleanup processes can be avoided, which will increase sample throughput significantly.

Flexible PDF reporting system can facilitate users to generate high quality report with many choices of formats and layouts.

Quantitative Analysis Sample Report

Agilent Technologies

Batch name	D:\MassHunter\Data\091014_ESI+_Calibration_Dorthy\QuantResults\Sue.batch.bin
Sample name	R8-01

Compound	Injection 1	Injection 2	Avg
Caffeine	27.0	14.5	20.7
DEET	107.4	118.6	113.0
Montelukast	12.1	11.9	12.0

Figure 6. Example report of one sample by PDF reporting system.

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