Sensitive Screening of Pharmaceuticals and Personal Care Products (PPCPs) in Water Using an Agilent 6545 Q-TOF LC/MS System

Application Note

Authors

Dan-Hui Dorothy Yang[1], Mark A. Murphy[2], Yue Song[3], Jimmy Chan[3]

[1]Agilent Technologies Inc., 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA

[2]EPA Region 8 Lab, 16194 West 45th Drive, Golden, CO 80403, USA

[3]Agilent Technologies Co., Ltd., Shanghai, PR China

Abstract

As a follow up to the Application Note on detection of pharmaceuticals and personal care products (PPCPs) in water using the Agilent 6495 Triple Quadrupole Mass Spectrometer[8], this Application Note describes two methods to screen and quantitate PPCPs in water at part per trillion (ppt) levels using the Agilent 6545 Q-TOF LC/MS System. Similarly, the methods were divided into positive ion mode and negative ion mode due to the unique mobile phases used for the two methods. The precise and accurate screening and quantitation of 118 compounds in positive ion mode and 22 compounds in negative mode was accomplished on the 6545 Q-TOF LC/MS using the Swarm tune parameters optimized for small fragile organic molecules. The high sensitivity slicer mode was selected to maximize instrument sensitivity. Most of the PPCPs could be detected without tedious analyte enrichment such as solid phase extraction (SPE). The extent of sample preparation included 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 Q-TOF LC/MS with reporting limits for the majority of analytes at 10 ppt. The limit of detection (LOD) and lower limit of quantitation (LLOQ) for most of the analytes are much lower than 10 ppt.

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Introduction

Pharmaceuticals and Personal Care Products (PPCPs) are comprised of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics. Several studies have shown that pharmaceuticals and their metabolites are present in our waterbodies[1,2]. PPCPs in surface waters can eventually enter drinking water systems when treatments are insufficient. Governmental agencies, such as the EPA and the European Water Framework, have proposed regulations to monitor water supply systems[3,4]. High performance liquid chromatography (HPLC) in combination with high resolution Q-TOF mass spectrometry is gaining traction to investigate the occurrence and fate of PPCPs in water systems. There are several advantages associated with the analysis of PPCPs by Q-TOF LC/MS:

- Screening of a large number of analytes within one run
- Retrospective data mining for new analytes
- No need for individual standards for fragmentation information
- Structure confirmation by MS/MS fragments

Compared with targeted analysis (for example, triple quadrupole), Q-TOF LC/MS has the added benefit of nontargeted or semitargeted screening for unknowns.

The detection limits for PPCPs in drinking water are typically in the low part per trillion (ppt) levels. This poses significant challenges in analytical methodology and instrumentation. Sample enrichment by solid phase extraction (SPE) is often performed to reach these levels in drinking water samples[5]. SPE requires large sample quantities, high consumption of solvents, and laborious procedures. PPCPs analysis also has the complexity of significant contamination, such as urban surface water sources, where some of the PPCPs can be found above part per billion (ppb) levels. In addition to higher analyte concentrations, total organic carbon levels in these samples also increase. This can add substantial interferences to the analytes. The instrumentation required for PPCPs analysis must have, not only a broad dynamic range, but also provide precise and accurate screening and quantitation through excellent mass accuracy and resolution.

The Agilent 6545 Q-TOF LC/MS, in combination with the Agilent Jet Stream Ionization source, meets the dynamic analytical demands for the occurrence and fate of PPCPs in water along with the convenience of direct sample injection. Several modifications associated with the 6545 Q-TOF LC/MS have resulted in higher analytical performance compared to previous model. Some of these improvements include:

- A new slicer design with the option to operate in high sensitivity or high resolution mode
- A new high performance high voltage power supply, along with a new pulser to improve mass accuracy and resolution
- A new enhanced gain-shifted detector that provides much better instrument robustness
- A new front end ion optics for increased precursor ion transmission

The most noteworthy change is the new Particle Swarm Optimization technology. For the first time, the Particle Swarm Optimization technology is used to optimize the 6545 Q-TOF LC/MS mass spectrometers (called Swarm autotune). Swarm autotune provides many choices to maximize sensitivity or mass resolution. First, it can optimize ion transmission at particular mass ranges (for example, 50–250 *m/z*, 50–750 *m/z*, or 50–1,700 *m/z*) based on application needs. Secondly, the improvements in ion transmission for small molecules has also resulted in enhanced mass accuracy below 100 *m/z*. Lastly, instrument parameters can be tuned according to the fragility of analytes, which requires milder ion transmission parameters to preserve their molecular masses. In combination with the modifications, and the ability to select the high sensitivity slicer mode, a substantial increase in signal response compared with the previous generation of the instrument has been achieved[6].

Experimental and Instrumentation

Reagents and chemicals

All reagents and solvents were HPLC-MS grade. Acetonitrile was purchased from Honeywell (015-4). Ultrapure water was obtained from a Milli-Q Integral system equipped with a 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 list of analytes and their internal standards are listed in Table 1 for the positive ion mode method and Table 2 for the negative ion mode method. Personal Compound Database Libraries (PCDLs) for analytes were created using the Agilent PCDL Manager (B.07.00) with retention time acquired with standards.

Table 1. Analytes and internal standards in positive ion mode method.

Compound	Mass	RT (min)	Compound	Mass	RT (min)
10,11-Dihydro-10-hydroxycarbamazepine	254.10553	5	MDMA	193.11028	3.91
6-Acetylmorphine	327.14706	3.72	MDMA-D5	198.14166	3.9
6-Acetylmorphine-D6	333.18472	3.71	Mefenamic acid	241.11028	8.15
Acebutolol	336.20491	4.39	Mefenamic acid-D3	244.12911	8.15
Acetaminophen	151.06333	2.92	Meperidine	247.15723	4.98
Acetaminophen-D4	155.08844	2.92	Meperidine-D4	251.18234	4.97
Albuterol	239.15214	2.77	Meprobamate	218.12666	5.15
Amitriptyline	277.18305	6.67	Meprobamate-D7	225.17059	5.14
Amitriptyline metabolite	293.17796	5.06	Metformin	129.10145	1
Amitriptyline-D3	280.20188	6.66	Methadone	309.20926	6.74
Amphetamine	135.1048	3.6	Methadone-D9	318.26576	6.71
Amphetamine-D5	140.13618	3.57	Methamphetamine	149.12045	3.82
Aripiprazole	447.14803	7.29	Methamphetamine-D11	160.18949	3.78
Aripiprazole-D8	455.19825	7.14	Methotrexate	454.17132	3.26
Atenolol	266.16304	2.88	Methotrexate-D3	457.19015	3.26
Atenolol-D7	273.20698	2.87	Methylphenidate	233.14158	4.65
Atorvastatin	558.253	7.51	Methylphenidate-D9	242.19807	4.64
Atrazine	215.09377	7.03	Metoprolol	267.18344	4.53
Atrazine-D5	220.12516	7	Mevastatin	390.24062	9.42
Benzoylecgonine	289.13141	4.01	*m*-Hydroxybenzoylecgonine	305.12632	3.73
Benzoylecgonine-D3	292.15024	4.01	Modafinil	273.08235	5.68
Buprenorphine	467.30356	8.07	Modafinil-D10	283.14512	5.65
Buprenorphine-D4	471.32867	7.72	Monoethylglycinexylidide	206.14191	3.8
Bupropion	239.10769	5.33	Montelukast	585.21044	10.88
Caffeine	194.08038	3.6	Morphine	285.13649	2.4
Caffeine-$^{13}C_3$	197.09044	3.6	Morphine-D3	288.15532	2.39
Carbamazepine	236.09496	6.28	Nifedipine	346.11649	7.57
Carbamazepine 10,11 epoxide	252.08988	5.47	Nifedipine oxidized	344.10084	7.48
Carbamazepine-D10	246.15773	6.22	Norfentanyl	232.15756	4.21
Carisoprodol	260.17361	6.75	Norfentanyl-D5	237.18895	4.19
Carisoprodol-D7	267.21754	6.72	Norfluoxetine	295.1184	6.55
Chlorpheniramine	274.12368	5.47	Norfluoxetine-D6	301.15606	6.53
Clenbuterol	276.07962	4.6	Normeperidine	233.14158	4.9
Clenbuterol-D9	285.13611	4.59	Normeperidine-D4	237.16669	4.89
Clopidogrel carboxylic acid	307.04338	4.69	Norquetiapine	295.11432	5.82
Cocaethylene	317.16271	5.42	Norsertraline	291.05815	6.87
Cocaethylene-D3	320.18154	5.41	Norsertraline-$^{13}C_6$	297.07828	6.71
Cocaine	303.14706	4.96	Norverapamil	440.26751	6.48
Cocaine-D3	306.16589	4.95	Omeprazole	345.11471	5.92
Codeine	299.15214	3.4	Oxazepam	286.05091	6.52
Codeine-D6	305.1898	3.39	Oxcarbazepine	252.08988	6.47
Cotinine	176.09496	3.69	Oxycodone	315.14706	3.68
Cotinine-D3	179.11379	3.38	Oxymorphone	301.13141	2.65
DEET	191.13101	7.1	Oxymorphone glucuronide	477.1635	1.13
DEET-D6	197.16867	7.06	Oxymorphone glucuronide-D3	480.18233	1.12
Dehydroaripiprazole	445.13238	6.87	Oxymorphone-D3	304.15024	2.63
Desmethylcitalopram	310.14814	5.81	Paroxetine	329.14272	6.22
Desmethylcitalopram-D3	313.16697	5.81	Paroxetine-D6	335.18038	6.21

Compound	Mass	RT (min)	Compound	Mass	RT (min)
Desmethylvenlafaxine	263.18853	4.6	Phenmetrazine	177.11536	3.74
Desmethylvenlafaxine-D6	269.22619	4.23	Phentermine	149.12045	3.97
Dextromethorphan	271.19361	5.69	Phentermine-D5	154.15183	3.94
Dextromethorphan-D3	274.21244	5.68	Phenylpropanolamine	151.09971	2.93
Diltiazem	414.16133	6.14	Phenylpropanolamine-D3	154.11854	2.93
Diphenhydramine	255.16231	5.88	Pioglitazone	356.11946	7.72
Diphenhydramine-D3	258.18114	5.88	Pregabalin	159.12593	2.73
Disopyramide	339.23106	4.87	Pregabalin-D6	165.16359	2.76
Donepezil	379.21474	5.65	Primidone	218.10553	4.43
Duloxetine	297.11873	6.47	Propranolol	259.15723	5.52
Duloxetine-D3	300.13757	6.47	Propranolol-D7	266.20117	5.5
Ecgonine methyl ester	199.12084	1.15	Pseudoephedrine	165.11536	3.3
Ecgonine methyl ester-D3	202.13967	1.15	Pseudoephedrine-D3	168.13419	3.29
EDDP	277.18305	6.31	Quetiapine	383.16675	6.27
EDDP-D3	280.20188	6.31	Quetiapine-D8	391.21696	6.17
Erythromycin	733.46124	5.78	Ritalinic acid	219.12593	3.78
Erythromycin-$^{13}C_2$	735.46795	5.78	Ritalinic acid-D10	229.1887	3.75
Erythromycin-anhydro	715.45068	6.3	Sertraline	305.0738	6.88
Escitalopram	324.16379	5.92	Sertraline-D3	308.09264	6.87
Famotidine	337.04493	2.89	Sildenafil	474.20492	6.65
Fentanyl	336.22016	5.9	Simvastatin	418.27192	10.4
Fentanyl-D5	341.25155	5.88	Sotalol	272.11946	2.93
Fluoxetine	309.13405	6.7	Sulfamethazine	278.08375	4.45
Fluoxetine-D6	315.17171	6.69	Sulfamethazine-$^{13}C_6$	284.10388	4.45
Fluticasone propionate	500.18443	9.05	Sumatriptan	295.13545	3.5
Gabapentin	171.12593	2.75	Tadalafil	389.13756	6.86
Gabapentin-D10	181.1887	2.72	Temazepam	300.06656	7.2
Glyburide	493.14382	8.27	Temazepam-D5	305.09794	7.16
Hydrocodone	299.15214	3.84	Thiabendazole	201.03607	5.18
Hydrocodone-D6	305.1898	3.84	Thiabendazole-$^{13}C_6$	207.0562	5.19
Hydromorphone	285.13649	2.9	Tramadol	263.18853	4.6
Hydromorphone-D3	288.15532	2.89	Tramadol-^{13}C-D3	267.21071	4.58
Hydroxybupropion	255.10261	4.62	Trazadone	371.15129	5.9
Hydroxybupropion-D6	261.14027	4.61	Trazadone-D6	377.18895	5.81
Ketoprofen	254.09429	6.42	Triamterene	253.10759	4.12
Lamotrigine	255.00785	4.73	Trimethoprim	290.13789	3.95
Lamotrigine-$^{13}C^{15}N_4$	259.99935	4.74	Trimethoprim-$^{13}C_3$	293.14795	3.94
Lamotrigine-$^{13}C_3$	258.01792	4.73	Tylosin	915.51915	6.12
Levorphanol	257.17796	4.43	Valsartan	435.22704	5.97
Lidocaine	234.17321	4.51	Venlafaxine	277.20418	5.19
Loratadine	382.14481	9.38	Venlafaxine-D6	283.24184	5.19
Lorazepam	320.01193	6.67	Verapamil	454.28316	6.63
Lorazepam-D4	324.03704	6.67	Zolpidem	307.16846	6.02
MDA	179.09463	3.73	Zolpidem phenyl-4-carboxylic acid	337.14264	3.93
MDEA	207.12593	4.18	Zolpidem-D7	314.2124	5.98

Table 2. Analytes and internal standards in negative ion mode method.

Compound	Mass	RT (min)	Compound	Mass	RT (min)
(±)11-Nor-9-carboxy-*delta*-THC	344.19876	6.568	Diclofenac 4-hydroxy	311.0116	5.067
$^{13}C_{12}$ Triclosan	299.99142	6.535	Fenbufen	254.09429	5.317
$^{13}C_{3}$ Ibuprofen	209.14074	5.965	Furosemide	330.00772	4.712
$^{13}C_{6}$ Diclofenac 4-hydroxy	317.03173	5.066	Gemfibrozil	250.15689	6.32
$^{13}C_{6}$ Methylparaben	158.06747	4.216	Hydrochlorothiazide	296.96447	3.341
$^{13}C_{6}$ *n*-Butylparaben	200.11442	5.458	Ibuprofen	206.13068	5.958
$^{13}C_{6}$ Sulfamethoxazole	259.07224	4.096	Methylparaben	152.04734	4.21
$^{13}C_{6}$ Triclocarban	319.99818	6.512	Modafinil acid	274.06637	4.619
Bezafibrate	361.10809	5.257	Naproxen	230.09429	5.225
Celecoxib	381.07588	5.967	*n*-Butylparaben	194.09429	5.451
Chloramphenicol	322.01233	4.15	Phenobarbital	232.08479	4.184
D10 Phenytoin	262.15265	4.58	Phenytoin	252.08988	4.6
D4 Diclofenac	299.04179	5.87	Pravastatin	424.2461	4.326
D5 Chloramphenicol	327.04371	4.14	Sulfamethoxazole	253.05211	4.1
D5 Phenobarbital	237.11618	4.175	Triclocarban	313.97805	6.519
D6 Gemfibrozil	256.19456	6.304	Triclosan	287.95116	6.535
D9 (±)11-Nor-9-carboxy-*delta*-THC	353.25525	6.546	Warfarin	308.10486	5.532
Diclofenac	295.01668	5.88			

Instrumentation and conditions

- 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 for positive ion mode, and Table 4 for negative ion mode.

Table 3. Agilent 1290 Infinity UHPLC conditions for the positive 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 200 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

MS detection

An Agilent 6545 Q-TOF LC/MS with an Agilent Jet Stream electrospray ionization source was used.

Jet Stream ionization source parameters are critical for the sensitive detection of analytes[7]. For multiple analyte applications, parameters are typically weighted towards hard-to-detect analytes. In this case, source parameters were accessed based on the triple quadrupole data and other studies on the particular compounds[8]. Mass spectrometer source conditions 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 for Q-TOF mass spectrometer, Version B.06.01
- Agilent MassHunter Qualitative Software, Version B.07.00 Build 7.0.7024.0
- Agilent MassHunter Quantitative Software, Version B.07.00 Build 7.0.457.0

Table 4. Agilent Infinity 1290 UHPLC conditions for the 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 200 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

Table 5. Agilent 6545 Q-TOF LC/MS source parameters for positive ion mode method.

Parameter	Value
Mode	2 GHz Extended dynamic range; high sensitivity slicer mode
Tune	50–250 *m/z*; Fragile ions
Drying gas temperature	150 °C
Drying gas flow	10 L/min
Sheath gas temperature	375 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Capillary voltage	3,500 V
Nozzle voltage	200 V
Fragmentor	125 V
Skimmer	45 V
Oct1 RF Vpp	750 V
Acq mass range	100–1,000 *m/z* (MS only)
Acq rate	3 spectra/s
Ref mass ions	121.050873, 922.009798

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 100 ppt, while calibration standards were spiked at 10 ppt, 25 ppt, 50 ppt, 100 ppt, 250 ppt, 500 ppt, and 1,000 ppt (seven levels) in Milli-Q 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 freshly collected local tap water (Santa Clara, USA). All samples were fortified with internal standards at 100 ppt after filtration.

Results and Discussion

System stability

System stability was evaluated using 300 continuous injections of reserpine samples at 100 ppb in 70 % acetonitrile with a gradient of 1.5 minutes. The acquisition was set to 2 spectra per second in the presence of internal reference masses (*m/z* 121.0509 and 922.0098). The mass accuracy was obtained by Agilent MassHunter Qualitative Analysis. For all 300 injections, mass accuracy remained very stable, within 0.25 ppm, as illustrated by Figure 1. The area %RSD for 300 injections was 2.56 % with three separate sample preparations.

Table 6. Agilent 6545 Q-TOF LC/MS source parameters for negative ion mode method

Parameter	Value
Mode	2 GHz Extended dynamic range; high sensitivity slicer mode
Tune	50–250 *m/z*; Fragile ions
Drying gas temperature	200 °C
Drying gas flow	12 L/min
Sheath gas temperature	375 °C
Sheath gas flow	12 L/min
Nebulizer pressure	35 psi
Capillary voltage	4,000 V
Nozzle voltage	2,000 V
Fragmentor	110 V
Skimmer	40 V
Oct1 RF Vpp	750 V
Acq mass range	100–1,000 *m/z* (MS only)
Acq rate	2 spectra/s
Ref mass ions	119.03632, 966.000725

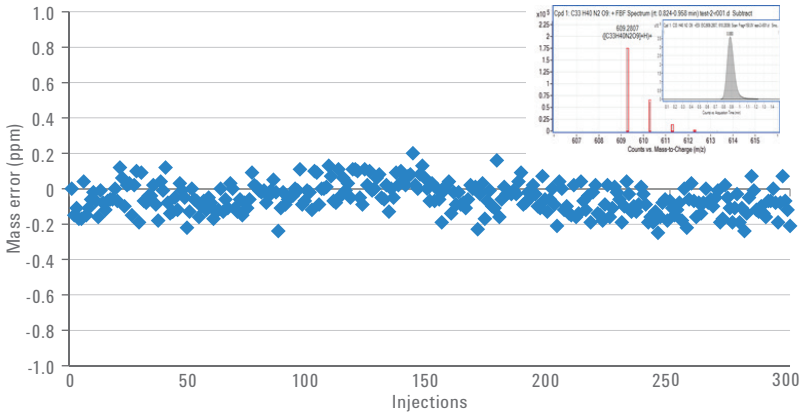

Figure 1. Excellent system stability. Mass accuracy was maintained within 0.25 ppm for 300 continuous injections of reserpine samples with area %RSD of 2.56 % .

Increased method performance

The sensitivity of the 6545 Q-TOF LC/MS was maximized by tuning the instrument to the 50–250 *m/z* range for this PPCPs application, and setting the slicer to high sensitivity mode. It is revolutionary that the user can optimize the ion transmission based on an analyte's *m/z*, especially for midrange mass spectrometers. The fragile ion option also prevents organic compounds from degrading during ion transmission. All these factors contribute to the sensitive detection of PPCPs at low ppt levels without tedious sample enrichment. Databases for the positive ion mode compounds and negative ion mode compounds were created using the PCDL manager (B.07.00) with retention time for the analytes and isotope-labeled internal standards. Data were initially evaluated in the Agilent MassHunter Qualitative Analysis Software (B.07.00) using Find by Formula with a mass error of 5 ppm and a retention time window of ± 0.5 minutes. Figure 2 shows the responses of the 118 analytes in positive ion mode, and Figure 3 shows the 22 analytes in negative ion mode at 25 ppt.

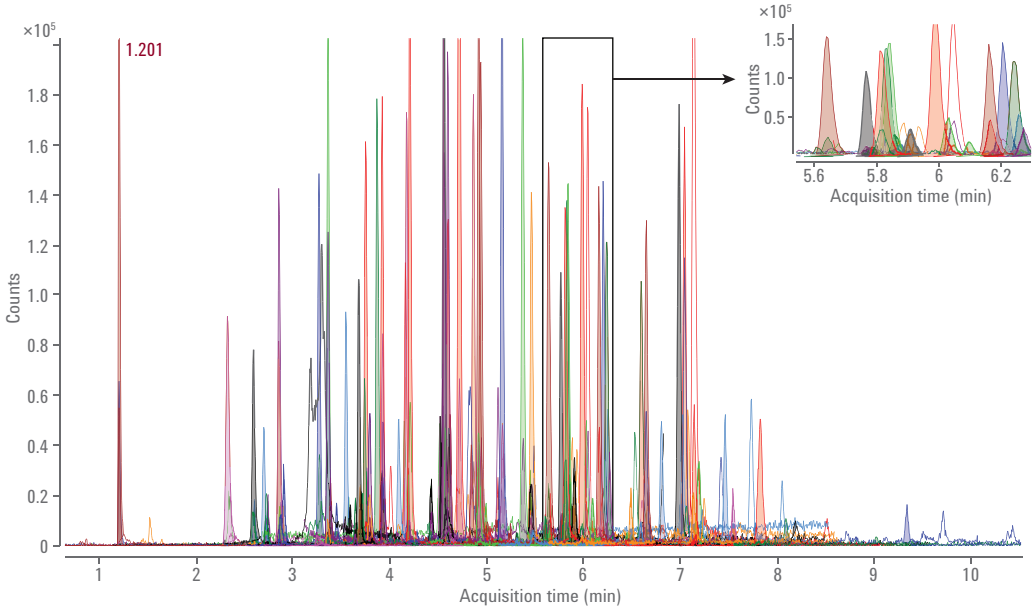

Figure 2. Signal response in positive ion mode (25 ppt at 40 µL direct injection).

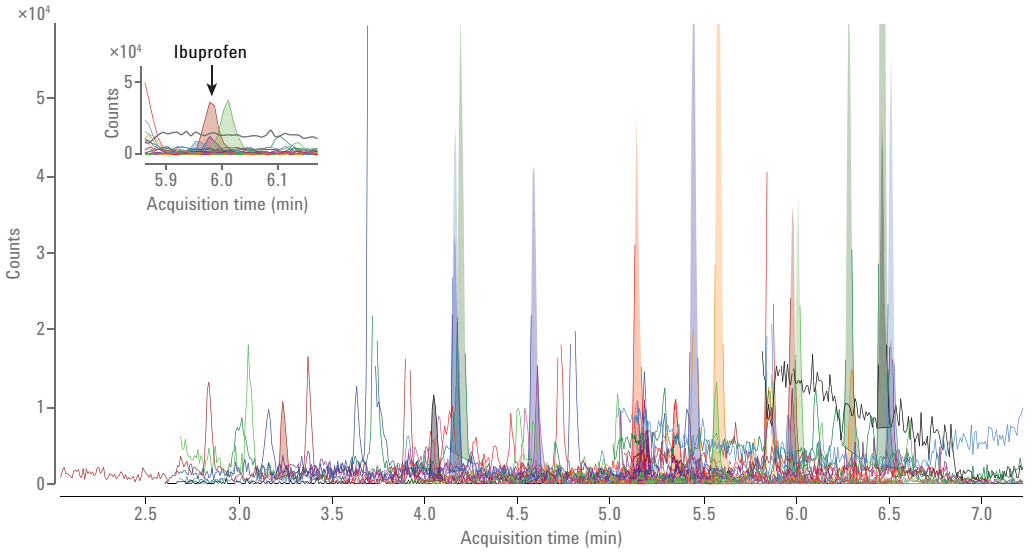

Figure 3. Signal response in negative ion mode (25 ppt at 40 µL direct injection).

Most of the compounds can be detected at a concentration much lower than 10 ppt without sample enrichment. Figure 4 shows the number of compounds that could be quantified, lower limit of quantitation (LLOQ), at each concentration level with accuracy between 80–120 % for at least three out of five replicates. There were 140 compounds, 118 in positive ion mode and 22 in negative ion mode. Norsertraline was not detected in all spiked concentrations, probably due to degradation since the stock standard was over three months old. Of the 44 compounds that failed to be quantified at 10 ppt, approximately 43 % failed due to quantitation accuracy beyond 80–120 %.

Due to the improvement in mass accuracy and the increased sensitivity as well as innate quantitation accuracy of the 6545 Q-TOF LC/MS, high confidence compound identification was achieved based not only on mass accuracy but also on isotopic abundance and spacing. An example is presented in Figure 5. 6-Acetylmorphine was identified with an overall target score of 93.43 out of 100 at 25 ppt in the presence of ~1,000x coeluting ions.

Calibration curves

Calibration curves were assessed with PPCPs spiked in Milli-Q water covering a concentration range from 10 ppt to 1,000 ppt. Some of the analytes had corresponding isotope-labeled internal standards. All samples were fortified with internal standards at a constant concentration of 100 ppt. Calibration curves were generated using a quadratic fit with a weighting factor of 1/x, including the origin. The correlation coefficients (R^2) for most of the target analytes in both polarities were equal to or greater than 0.99; most were greater than 0.995, except for methotrexate (R^2 = 0.978) and thiabendazole (R^2 = 0.984). The calibration curves for cotinine in positive ion mode and ibuprofen in negative ion mode are shown as examples in Figure 6.

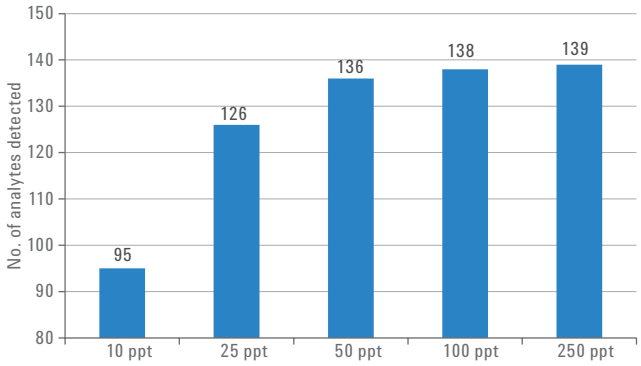

Figure 4. Number of compounds that could be quantified at each concentration level with 40 µL direct injection of water samples.

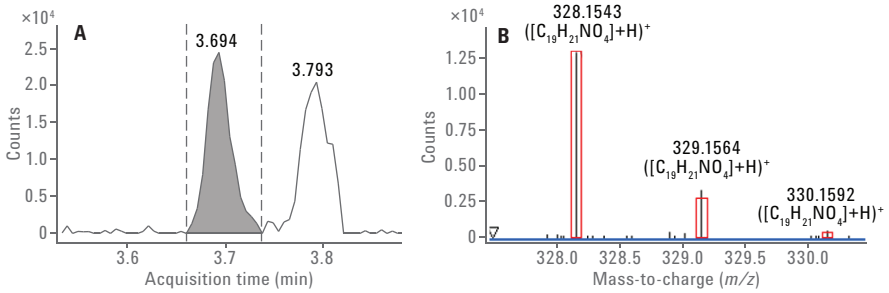

Figure 5. Identify with confidence. 6-acetylmorphine at 25 ppt can be detected with high confidence (target score 93.43 out of 100) in the presence of ~1,000x coeluting ions.

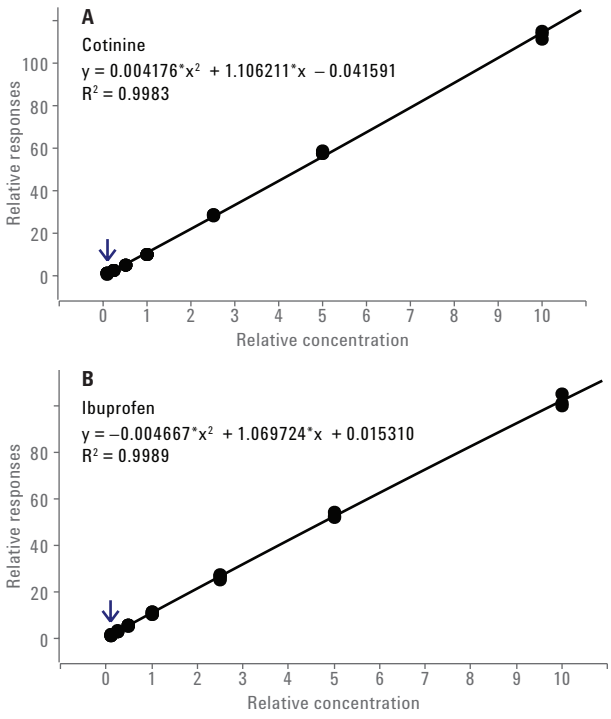

Figure 6. Calibration curves of cotinine (positive) and ibuprofen (negative) in Milli-Q water.

Precision and accuracy

The %RSD calculation was based on five replicate injections of 138 compounds made from 10 ppt to 100 ppt (95 compounds at 10 ppt, 31 compounds at 25 ppt, 10 compounds at 50 ppt, and two compounds at 100 ppt). The %RSD results are shown in Figure 7. About 79 % of the compounds could be quantified with a %RSD of less than 10 %. Only four compounds had elevated %RSD of 20–25 %. These results clearly demonstrate the precise quantitative ability of the 6545 Q-TOF LC/MS due to the modifications, the high sensitivity slicer mode, and fast data acquisition.

Quantification accuracy on the 6545 Q-TOF LC/MS is exceptional due to excellent mass accuracy and mass resolution. This is reflected by the number of compounds that can be quantified at low ppt levels without sample enrichment. One requirement for the analytes to be considered detectable is that the concentration accuracy of at least three of the five replicates had to be within 80–120 %. At 10 ppt, 43 % of 44 compounds failed due to quantification accuracy beyond 80–120 % even though the signal-to-noise for these analytes was much greater than 5. Quantification accuracy was affected more drastically at lower levels mainly due to slight background influence on peak integration.

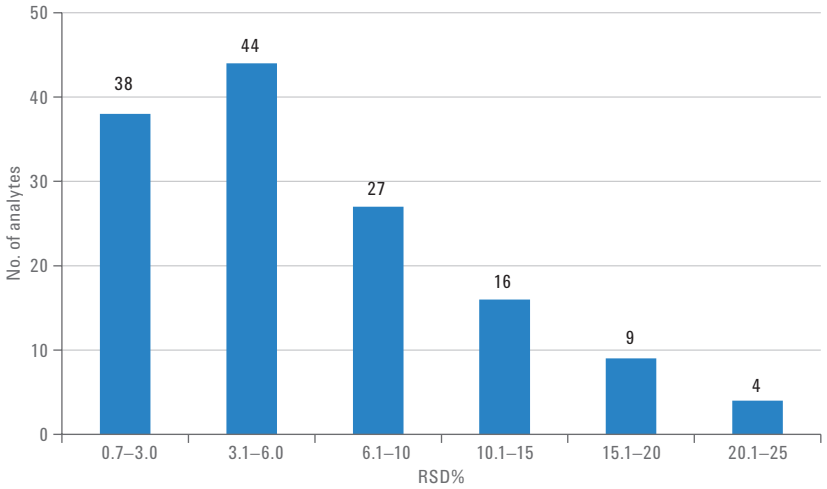

Figure 7. Measurement precision of five replicates at LLOQ levels from 10 ppt to 100 ppt; 79 % of analytes had %RSD less than 10 %.

Real-world samples

Three samples were tested. The first was freshly collected from local tap water. 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 reported if the average concentration of the two runs was greater than 10 ppt. The results are listed in Tables 7–10. Figure 8 and Figure 9 represent the chromatographs for the two unknown samples with only 2–3 PPCPs identified.

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

Name	Inj 1 (ppt)	Inj 2 (ppt)	Avg. (ppt)
Normeperidine	28.4	30.4	29.4
Temazepam	12.4	13.9	13.2

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

Name	Inj 1 (ppt)	Inj 2 (ppt)	Avg. (ppt)
6-Acetylmorphine	17.7	18.9	18.3
DEET	106.7	107.6	107.1
Temazepam	17.9	18.6	18.2

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

Name	Inj 1 (ppt)	Inj 2 (ppt)	Avg. (ppt)	Name	Inj 1 (ppt)	Inj 2 (ppt)	Avg. (ppt)
10,11-Dihydro-10-hydroxycarbamazepine	82.5	82.3	82.4	MDMA	18.5	14.1	16.3
Acebutolol	21.1	20.5	20.8	Meprobamate	105.6	116.5	111.0
Amitriptyline	33.9	35.3	34.6	Metformin	2796.1	2774.4	2785.3
Atenolol	1590.3	1476.8	1533.5	Methadone	42.6	42.4	42.5
Atrazine	40.1	40.2	40.1	Methamphetamine	250.8	249.5	250.1
Bupropion	167.9	160.2	164.0	Metoprolol	426.4	425.3	425.9
Caffeine	719.4	660.1	689.8	Modafinil	21.0	19.8	20.4
Carbamazepine	211.2	219.2	215.2	Monoethylglycinexylidide	44.5	52.3	48.4
Carbamazepine 10,11 epoxide	62.1	56.0	59.1	Norquetiapine	56.6	59.5	58.0
Carisoprodol	31.0	29.9	30.5	Oxazepam	25.6	24.1	24.8
Chlorpheniramine	30.7	29.9	30.3	Oxycodone	94.1	94.6	94.4
Clopidogrel carboxylic acid	144.2	142.8	143.5	Oxymorphone	32.5	31.8	32.2
Cotinine	10.0	10.8	10.4	Phentermine	124.5	121.9	123.2
DEET	564.9	567.2	566.1	Pregabalin	209.3	220.5	214.9
Dehydroaripiprazole	37.1	39.3	38.2	Propranolol	57.8	57.1	57.5
Desmethylcitalopram	100.3	96.8	98.6	Pseudoephedrine	110.8	104.8	107.8
Desmethylvenlafaxine	809.2	834.5	821.9	Ritalinic acid	112.8	123.0	117.9
Dextromethorphan	53.7	49.0	51.4	Sertraline	48.5	49.1	48.8
Diltiazem	76.1	79.4	77.7	Sildenafil	29.6	31.8	30.7
Diphenhydramine	163.5	164.3	163.9	Sotalol	79.3	76.6	78.0
Disopyramide	13.9	14.2	14.0	Temazepam	115.9	110.9	113.4
EDDP	322.8	312.8	317.8	Thiabendazole	76.4	51.9	64.1
Erythromycin	38.5	39.8	39.2	Tramadol	907.5	859.8	883.6
Erythromycin-anhydro	94.1	86.8	90.5	Trazadone	28.6	27.5	28.0
Escitalopram	226.8	225.7	226.3	Triamterene	108.5	113.1	110.8
Fluoxetine	34.3	33.6	33.9	Trimethoprim	269.9	278.1	274.0
Hydrocodone	30.6	31.9	31.3	Tylosin	50.1	48.9	49.5
Hydroxybupropion	165.9	142.1	154.0	Venlafaxine	397.9	405.9	401.9
Levorphanol	184.7	180.3	182.5	Verapamil	29.5	29.4	29.4
Lidocaine	377.8	375.5	376.7	Zolpidem phenyl-4-carboxylic acid	48.9	46.3	47.6
Loratadine	17.3	18.4	17.8				

No compounds were found in the local tap water or the remote source water samples with the negative ion mode method, however, warfarin was detected at borderline in the remote source water sample. The compounds found in the urban surface water sample in negative ion mode are listed in Table 10.

The two surface water samples were also tested on the Agilent 6495 Triple Quadrupole LC/MS[8], however, the two studies were separated by several months during which the sample might have degraded. Even so, most compounds detected in both the targeted UHPLC-Triple Quadrupole method and the untargeted UHPLC-Q-TOF method overlapped well in terms of identified compounds and corresponding concentrations. This Application Note clearly demonstrates that the Agilent mass spectrometer portfolio can be used as a complete solution in environmental testing.

Conclusion

Fast and simple Q-TOF LC/MS methods for the screening of PPCPs in water have been developed. The methods leverage the full advantage of the sensitivity improvement provided by the hardware change of the Agilent 6545 Q-TOF LC/MS System and Swarm autotune on small fragile molecule ion transmission. The sensitivity can be further improved by the selection of the high sensitivity slicer mode. 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 design enhancements, tedious sample enrichment and cleanup processes can be avoided. This will increase sample throughput significantly.

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

Name	Inj 1 (ppt)	Inj 2 (ppt)	Avg. (ppt)
Celecoxib	40.2	36.6	38.4
Chloramphenicol	8.9	11.9	10.4
Diclofenac	277.2	235.2	256.2
Diclofenac 4-hydroxy	10.0	10.0	10.0
Furosemide	309.3	307.9	308.6
Gemfibrozil	223.7	225.6	224.7
Hydrochlorothiazide	532.7	539.8	536.3
Ibuprofen	47.5	46.8	47.2
Methylparaben	78.6	83.4	81.0
Naproxen	175.4	177.0	176.2
n-Butylparaben	10.2	12.5	11.3
Phenobarbital	43.3	26.2	34.7
Phenytoin	666.4	956.1	811.3
Sulfamethoxazole	649.8	599.2	624.5
Triclocarban	28.2	25.9	27.0
Triclosan	36.4	37.6	37.0

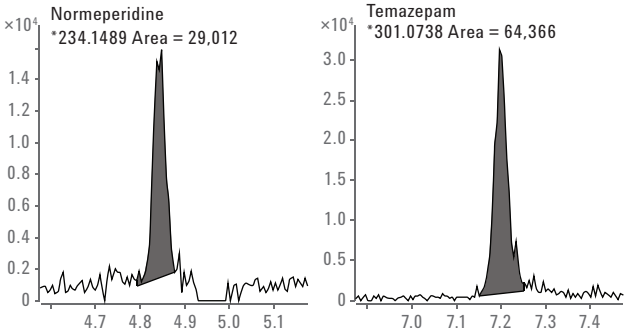

Figure 8. Chromatographs of PPCPs found in local tap water with positive ion method.

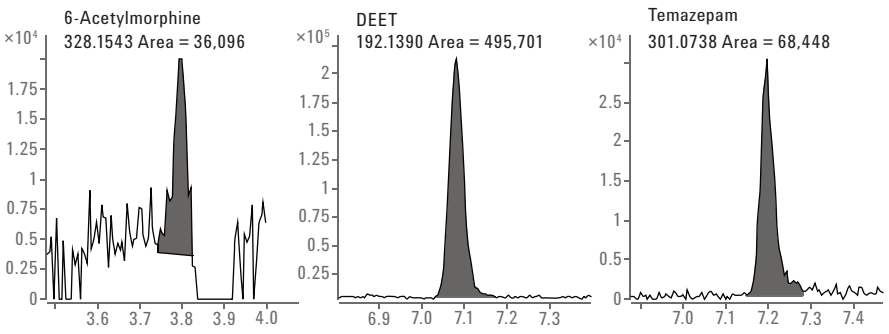

Figure 9. Chromatographs of PPCPs found in remote source sample with positive ion mode method.

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