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Direct Analysis of Pharmaceuticals and PPCPs in Environmental Waters using a Newly Developed QQQ MS

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Introduction

Pharmaceuticals and Personal Care Products (PPCPs), including drugs and metabolites, are an important water quality issue for both scientific community and governmental agencies. PPCPs in surface waters may have an adverse impact on wildlife and human, even at very low concentrations (ng/L). This poses significant analytical challenges. Sample enrichment by solid phase extraction (SPE) is often required prior to LC/MS/MS analysis. Unfortunately, SPE involves large sample quantities, high solvent consumption, and laborious procedures.

In this work, a newly designed triple quadrupole (QQQ) mass spectrometer with improved performance was used to streamline PPCP analyses in surface water by enabling direct injections of water samples without time consuming SPE enrichment. Twenty eight selected PPCPs, 24 in positive ion mode and 4 in negative ion mode, were accurately quantified at low-ng/L levels with excellent assay reproducibility and robustness.

The newly designed 6470 QQQ mass spectrometer includes the proven Agilent Jet Stream (AJS) ionization source, enhanced MS1 ion optics with optimized prefilter geometry, a curved and tapered hexapole collision cell, and a detector with high energy conversion dynode. All the design elements work in concert to improve ion transmission, ensures effective ion collection, and enhances the efficiency of ion detection and quantitation, resulting in enhanced area response and improved area precision. This ultimately leads to more sensitive and precise quantitation attaining lower limits of detection and quantitation compared to previous designs.

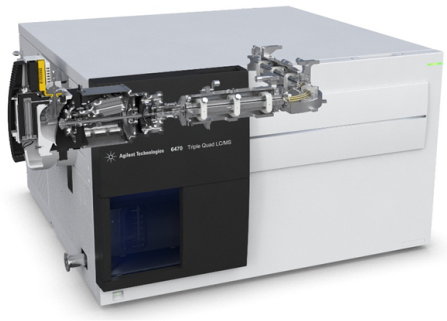


Figure 1 *The new 6470 Triple Quadrupole LC/MS System with new and enhanced MS1 ion optics, improved curved collision cell, and new ion detector for enhanced sensitivity, precision and system robustness .*

Experimental

UHPLC/MS/MS analysis was performed on a 1290 UHPLC system coupled to the 6470 mass spectrometer with electrospray ionization. Twenty eight selected PPCPs represent commonly occurring PPCPs in surface waters. Calibration standards containing these 28 PPCPs were prepared in water ranging from 0.1 - 5,000 ng/L. Surface water samples were also analyzed. Both standards and samples were directly injected to LC/MS/MS using an injection volume of 40µL.. The PPCPs were detected using MRM in polarity switching mode. For each compound, two MRMs transitions, one quantifier and one qualifier, were optimized and employed for quantitation.

1290 UHPLC Conditions	
Column	Zorbax Eclipse Plus RRHD C18 2.1 x 50 mm, (p/n 959741-902)
Column temp	35°C
Injection volume	40 µL
Autosampler temp	4°C
Needle wash	15 sec (80% MEOH/20% water)
Mobile phase	MPA: Water with 0.03% Formic acid) MPB: Acetonitrile
Flow rate	0.4 mL/min
Gradient program	Time B% 0.0 10 1.7 10 10.0 100 10.3 100 Post Time 4min

6470 QQQ MS Conditions	
Ion mode	Positive/Negative
Drying gas temperature	250°C
Drying gas flow	7L/min
Sheath gas temperature	370°C
Sheath gas flow	11 L/min
Nebulizer pressure	40 psi
Capillary voltage	2500 V(pos) / 3000 V (neg)
Nozzle voltage	0V(pos) / 300V (neg)
Delta EMV	400 (pos/neg)

Results and Discussion

Method Performance

The UHPLC-MS/MS method was validated for quantitation performance including sensitivity, precision, accuracy, linearity, and dynamic range.

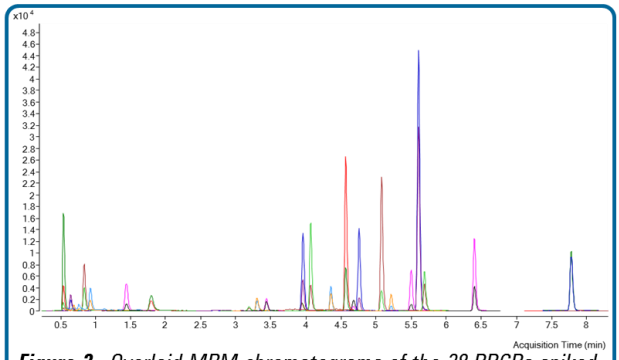


Figure 2. *Overlaid MRM chromatograms of the 28 PPCPs spiked in water at 10 ng/L. (ppt) analyzed on the 6470 QQQ LC/MS*

Improved Sensitivity, Precision, and Detection Limits

Increased peak area responses of PPCPs were observed on the 6470 QQQ LC/MS, with an average gain of **3.0x** compared to the previous design 6460 QQQ LC/MS

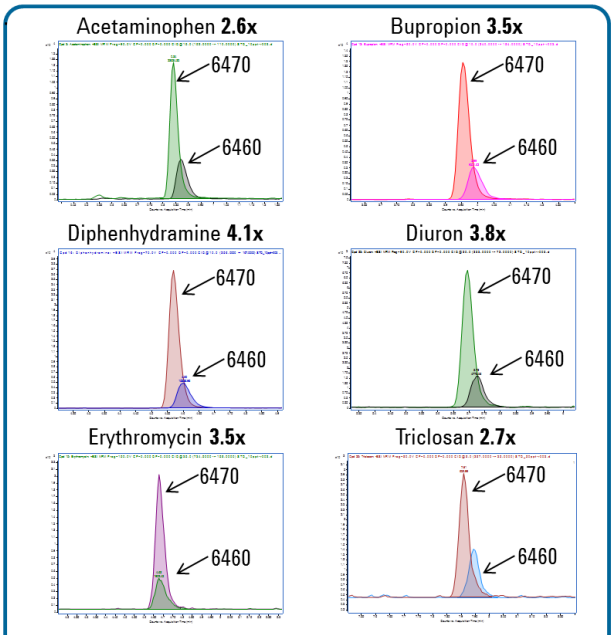


Figure 3. *Comparison of peak area responses for the 28 PPCPs spiked in water at 10 ng/L, analyzed using the 6470 QQQ LC/MS and the 6460 QQQ LC/MS*

Improved peak area precision (%RSD) was observed on the 6470 compared to the 6460, particularly at low levels. ***Figure 4*** demonstrates an average fold-improvement of **2.3x** across 28 PPCPs analyzed at 5 ng/L

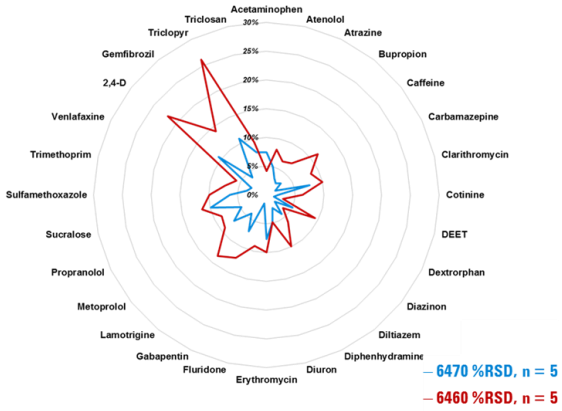


Figure 4. *Comparison of peak area %RSD (n = 5 replicate injections) for the 28 PPCPs spiked in water at 5 ng/L, analyzed using the 6470 QQQ LC/MS in **blue** and 6460 QQQ LC/MS in **red**.*

The peak area %RSD at a low concentration is a universal measure of ion efficiency and can be used to estimate the Instrument Detection Limit (IDL) as a superior sensitivity metric compared to the signal-to-noise (S/N). ***Table 1*** compares the IDLs for PPCPs observed on the 6470 versus the 6460 and illustrates a media fold-improvement of **3.6x.**

PPCPs	6460 IDL (ng/L)	6470 IDL (ng/L)	Fold-Improvement	PPCPs	6460 IDL (ng/L)	6470 IDL (ng/L)	Fold-Improvement
Acetaminophen	0.59	0.12	**4.9x**	Erythromycin	0.96	0.14	**6.8x**
Atenolol	0.64	0.45	**1.4x**	Fluridone	0.053	0.012	**4.5x**
Atrazine	0.44	0.28	**1.6x**	Gabapentin	1.89	1.05	**1.8x**
Bupropion	0.17	0.044	**3.9x**	Lamotrigine	2.15	0.94	**2.3x**
Caffeine	1.20	0.25	**4.8x**	Metoprolol	1.02	0.29	**3.5x**
Carbamazepine	0.32	0.082	**3.9x**	Propranolol	0.75	0.091	**8.2x**
Clarithromycin	3.58	1.14	**3.1x**	Sucralose	19.5	3.56	**5.5x**
Cotinine	0.57	0.068	**8.0x**	Sulfamethoxazole	0.87	0.41	**2.1x**
DEET	0.067	0.022	**3.1x**	Trimethoprim	0.64	0.39	**1.6x**
Dextrorphan	1.01	0.15	**6.7x**	Venlafaxine	0.22	0.076	**2.8x**
Diazinon	0.17	0.071	**2.4x**	2,4-D	34.4	8.69	**4.0x**
Diltiazem	0.13	0.030	**4.2x**	Gemfibrozil	22.1	11.5	**1.9x**
Diphenhydramine	0.13	0.052	**2.4x**	Triclopyr	38.0	16.3	**2.3x**
Diuron	0.56	0.28	**2.0x**	Triclosan	14.6	5.32	**2.7x**
Average Fold-Improvement							**3.6x**

Table 1. *IDLs for the 28 PPCPs analyzed using the 6470 compared to the 6460. Five replicate injections were used for IDL calculation.*

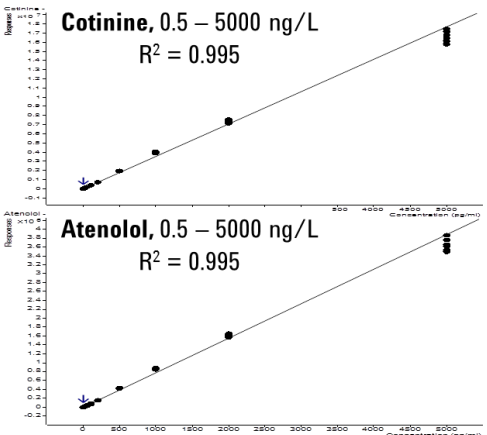
Results and Discussion

Sub-ng/L Sensitivity and Reliable Quantitation over a Wide Linear Dynamic Range

The newly developed ion optics and detector of the 6470 allowed the quantitation of PPCPs at Lower Limits of Quantitation (LLOQs) of sub- to low-ng/L. The calibration curves showed excellent linearity over four orders of dynamic range with $R^2 > 0.995$. The precision and accuracy of measurements were evaluated at up to thirteen standard concentrations ranging from the LLOQ as low as 0.1 ng/L (ppt) to the upper limit of quantitation (ULOQ) of 5 µg/L (ppb), and were calculated from five replicate injections at each level. Excellent assay precision (%RSD < 20% at LLOQ and < 15% at the rest of the levels) as well as average accuracy (80-120% at LLOQ and 85–115% at the rest of the levels) were obtained, which are well within the method validation criteria set by EPA method 1694.

PPCPs	6470 LLOQ (ng/L)	PPCPs	6470 LLOQ (ng/L)
Acetaminophen	0.5	Erythromycin	1.0
Atenolol	1.0	Fluridone	0.1
Atrazine	0.5	Gabapentin	5.0
Bupropion	0.2	Lamotrigine	2.0
Caffeine	1.0	Metoprolol	1.0
Carbamazepine	0.5	Propranolol	1.0
Clarithromycin	5.0	Sucralose	20.0
Cotinine	0.5	Sulfamethoxazole	1.0
DEET	0.2	Trimethoprim	1.0
Dextrorphan	1.0	Venlafaxine	0.5
Diazinon	0.5	2,4-D	20.0
Diltiazem	0.2	Gemfibrozil	20.0
Diphenhydramine	0.2	Triclopyr	50.0
Diuron	1.0	Triclosan	20.0

Table 2. *LLOQs achieved for the 28 PPCPs using the 6470 QQQ LC/MS.*

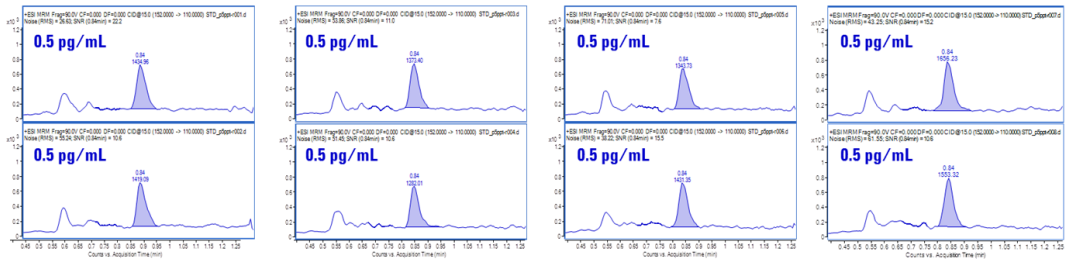


Cotinine Levels (ng/L)	%RSD (n = 5)	%Accuracy
0.5	4.53	111.2
1	2.37	95.7
2	2.58	89.8
5	2.13	91.1
10	1.09	95.3
20	0.84	94.9
50	1.00	98.8
100	0.59	102.1
200	0.48	101.9
500	0.73	109.1
1000	1.65	111.7
2000	2.14	104.0
5000	3.82	94.0

Figure 5. *Examples of the linear dynamic range achieved for PPCPs in water. Peak area precision %RSD and %accuracy were evaluated at 13 standard concentrations.*

Amount measured	Replicates	%RSD	t (99%)	IDL
0.5 pg/mL (LLOQ)	n = 8 injections	8.3	2.998	**0.12 pg/mL**

IDL = t x (%RSD/100) x Amount = 2.998 x (8.3/100) x 0.5 pg/mL = 0.12 pg/mL



Inj #	Peak Area
1	1435
2	1419
3	1373
4	1282
5	1343
6	1431
7	1656
8	1553
%RSD	**8.3**

Figure 6. Chromatograms from eight replicate injections of acetaminophen at the LLOQ level, analyzed using the new 6470 QQQ. The peak area %RSD from the LLOQ level of 0.5 pg/mL was used to calculate the IDL for acetaminophen.

Conclusions

- A UHPLC/MS/MS method has been developed for quantitation of 28 PPCPs in surface water
- Four orders linear dynamic range was achieved with excellent precision and accuracy at the lowest levels, e.g. the LLOQ
- The enhanced sensitivity achieved using the new 6470 QQQ LC/MS allowed for reliable quantitation of PPCPs in water at sub-ng level using direct injection and without SPE enrichment .