



Method for Direct Analysis of Contaminants in Surface Waters With High Accuracy and Precision Using an Agilent 6470A Triple Quadrupole LC/MS System

Application Note

Environmental, Surface Water, Personal Care Products (PPCPs)

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Abstract

Using the Agilent 6470A Triple Quadrupole LC/MS System, 28 surface water contaminant standards including selected Personal Care Products (PPCPs), sucralose, 2,4-Dichlorophenoxyacetic acid (2,4-D), Atrazine, Diuron, Diazinon, and N,N-Diethyl-meta-toluamide (DEET), were accurately quantified at low-ng/L levels in surface water with excellent reproducibility and precision. Enhanced system performance enabled direct injection of water samples onto the LC/MS system to minimize sample preparation requirements.



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Introduction

The presence of pharmaceuticals and personal care products (PPCPs) and other contaminants in surface waters is an important quality concern for the scientific community and governmental regulatory agencies. According to the U.S. Environmental Protection Agency (EPA), PPCPs are products used by individuals for health and cosmetic purposes, and products used by agribusiness to enhance the growth and health of livestock. They comprise a very diverse collection of compounds, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics [1]. EPA Method 1694 was established for the determination of PPCPs in aqueous, solid, and bio-solid environmental samples using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS/MS) [2].

In the EU, the European Water Framework Directive governs chemical pollution of surface waters under Directive 2013/39/EU [3], Directive 2008/105/EC [4], and Decision 2455/2001/EC, which establishes the list of priority substances [5]. Commission Directive 2009/90/EC provides the technical specifications for the chemical analysis and monitoring of water status [6].

Although further research is necessary, there is growing concern that contaminants in surface waters may have an adverse impact on wildlife and humans, even at very low (ng/L) concentrations. For example, sucralose is a popular artificial sweetener used worldwide that ends up in waste and surface waters because it is not metabolized *in vivo*. Current wastewater treatment technologies do not address sucralose, so it has become ubiquitous in the environment. Studies have revealed biological effects that may have important toxicological consequences for aquatic organisms exposed to sucralose [7].

Steroid hormones that act as endocrine disruptors are also of interest. Research suggests that the compound ethinylestradiol, used in commonly prescribed contraceptives, can cause endocrine disruption in aquatic organisms and amphibians in concentrations as low as 1 ng/L [8].

The analysis of these diverse sets of contaminants at low ng/L levels poses significant analytical challenges. Sample enrichment using solid phase extraction (SPE) is often required prior to LC/MS/MS (LC/TQ) analysis. However, SPE involves large sample quantities, high solvent consumption, laborious procedures, and potential matrix effects. Because of these challenges, this application note explores a

method for the direct analysis of 28 selected contaminants including PPCPs in water at low-ng/L levels using an Agilent 1290 Infinity II UHPLC coupled to an Agilent 6470A triple quadrupole mass spectrometer equipped with Agilent Jet Stream (AJS) technology. LC/TQ performance, including sensitivity, accuracy, and precision, was evaluated.

Experimental

Standards, samples, and sample preparation

Calibration standards of 28 selected PPCPs and other contaminants commonly present in surface waters were spiked in ultrapure water obtained from a Milli-Q Integral system at 16 concentration levels ranging from 0.1–5,000 ng/L. Table 3 lists the analyzed standards. Matrix samples consisted of surface water samples collected from Colorado's Poudre and Big Thompson River's. The samples were obtained courtesy of the University of Colorado, Boulder. Prior to sample analysis, all samples were filtered through a 0.22 μm syringe filter and aliquoted to a 2.0-mL sample vial for analysis.

LC/TQ analysis

LC/TQ analyses were performed using a 1290 Infinity II UHPLC system coupled to a 6470A Triple Quadrupole LC/MS system equipped with an AJS ion source. Tables 1 and 2 give the UHPLC and mass spectrometer conditions used. After filtering, 40 μL of the spiked water samples were directly injected into the LC/TQ system for analysis.

Compounds were detected using MRM's in both positive and negative ionization modes. Table 3 presents the optimized MRM transitions and operating parameters for each contaminant measured. Two transitions were monitored for each compound (one quantifier and one qualifier ion) with the exception of Gemfibrozil. This compound is a therapeutic drug administered for treatment of hyperlipoproteinemia and hypercholesterolemia [9]. Due to matrix interferences, only one selective transition was monitored; m/z 249 \rightarrow m/z 121 (2,5-dimethylphenol functionality), which agrees with 5990-6431EN methodology.

MS1 and MS2 resolutions were set to Wide and Unit respectively, except for Triclopyr. It was analyzed at the MS1 resolution setting of Widest, and the MS2 resolution setting of Wide. These resolution settings can be used to improve detectability if the MRM transition is not negatively impacted by chemical noise. Detection limits were measured based on the presence of both quantifier and qualifier ions.

Table 1. UHPLC Conditions

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm (p/n 959741-902)
Column temperature	35 °C
Injection volume	40 µL
Autosampler temperature	4 °C
Needle wash	15 seconds (80 % MEOH/20 % water)
Mobile phase	A) Water with 0.03 % formic acid B) Acetonitrile
Flow rate	0.4 mL/min
Gradient	Time B% 0.0 10 1.7 10 10.0 100 10.3 100 Post time 4 minutes

Table 2. Mass Spectrometer Conditions

Parameter	Value
Ion mode	Positive/Negative
Drying gas temperature	250 °C
Drying gas flow	7 L/min
Sheath gas temperature	370 °C
Sheath gas flow	11 L/min
Nebulizer pressure	40 psi
Capillary voltage	2,500 V (pos)/3,000 V (neg)
Nozzle voltage	0 V (pos)/300 V (neg)
Delta EMV	400 (pos/neg)

Table 3. MRM Transitions and Operating Parameters for Each Contaminant Measured

Compound	MRM transition (m/z)	Fragmentor voltage	Collision energy	Polarity	Compound	MRM transition (m/z)	Fragmentor voltage	Collision energy	Polarity
Acetaminophen	152 → 110	90	18	+	Erythromycin	734.5 → 576	150	20	+
	152 → 65		35			734.5 → 158		35	
Atenolol	267 → 190	110	20	+	Fluridone	330 → 310	110	35	+
	267 → 145		30			330 → 294		55	
Atrazine	216 → 174	120	20	+	Gabapentin	172 → 154	90	15	+
	216 → 146		25			172 → 137			
Bupropion	240 → 184	80	12	+	Lamotrigine	258 → 213	120	30	+
	240 → 166		20			256 → 211			
Caffeine	195 → 138	110	20	+	Metoprolol	268 → 116	110	20	+
	195 → 110		28			268 → 56		33	
Carbamazepine	237 → 194	120	20	+	Propranolol	260 → 116	110	20	+
	237 → 179		40			260 → 56		33	
Clarithromycin	748.5 → 590	110	20	+	Sucralose	419 → 239	110	20	+
	748.5 → 158		28			419 → 221			
Cotinine	177 → 98	90	22	+	Sulfamethoxazole	254 → 156	80	15	+
	177 → 80		27			254 → 92		30	
DEET	192 → 119	110	20	+	Trimethoprim	291 → 261	110	28	+
	192 → 91		35			291 → 230		25	
Dextrorphan	258.1 → 201	120	25	+	Venlafaxine	278 → 260	90	10	+
	258.1 → 133		35			278 → 58		20	
Diazinon	305 → 169	90	20	+	2,4-D	219 → 161	70	13	-
	305 → 153		23			221 → 163			
Diltiazem	415 → 178	130	25	+	Gemfibrozil	249 → 121	70	12	-
	415 → 150		35			256 → 198		70	
Diphenhydramine	256 → 167	70	12	+	Triclopyr	254 → 196	70	10	-
	256 → 152		40						
Diuron	235 → 72	90	20	+	Triclosan	289 → 37	80	10	-
	233 → 72					287 → 35			

Results and Discussion

The quantitative performance of the 6470A Triple Quadrupole LC/MS System using direct injection of water samples, including sensitivity, precision, accuracy, linearity, and dynamic range, was evaluated.

The 6470A Triple Quadrupole LC/MS System provided sub-ng/L detection and reliable quantitation over a wide linear dynamic range. Table 4 shows the instrument-enabled quantification of the tested contaminants at sub- to low-ng/L lower limits of quantitation (LLOQs).

Figure 1 shows the TIC and overlaid MRM chromatogram of the 28 standards spiked in water at 10 ng/L.

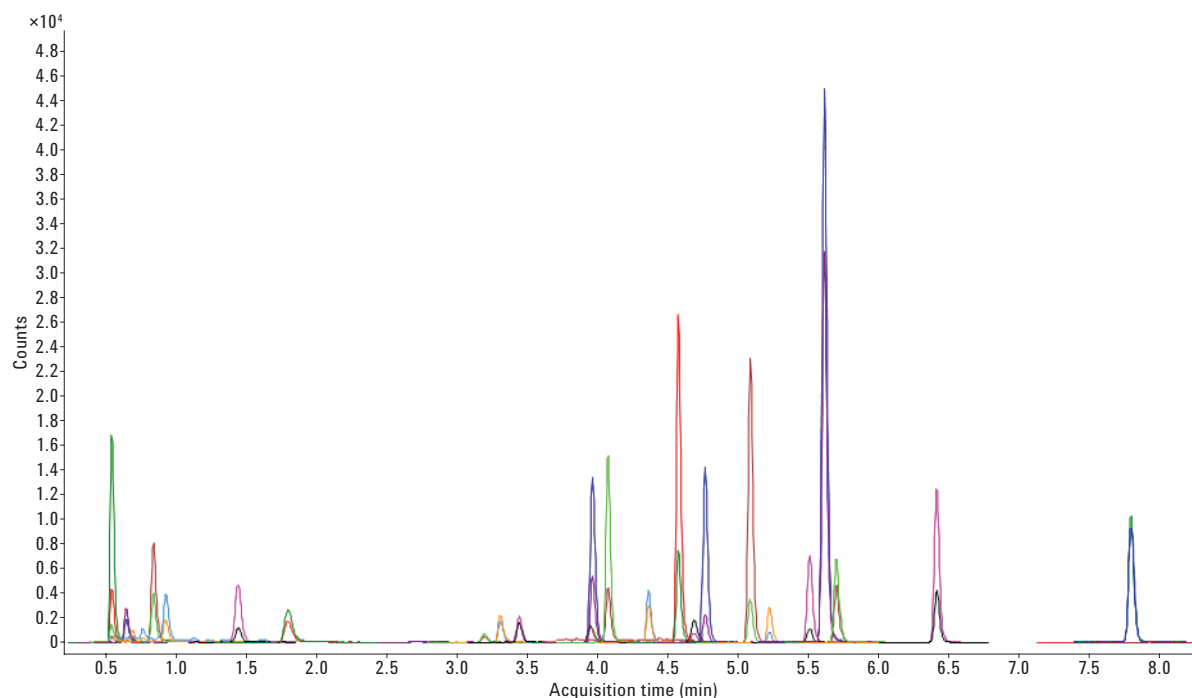


Figure 1. Overlaid MRM chromatograms of the 28 standards spiked in water at 10 ng/L.

Table 4. LLOQs and IDLs for the 28 Surface Water Contaminants

Compound	LLOQ (ng/L)	IDL* (ng/L)	Compound	LLOQ (ng/L)	IDL* (ng/L)	Compound	LLOQ (ng/L)	IDL* (ng/L)
Acetaminophen	0.5	0.12	Diazinon	0.5	0.071	Sucralose	20.0	3.56
Atenolol	1.0	0.45	Diltiazem	0.2	0.030	Sulfamethoxazole	1.0	0.41
Atrazine	0.5	0.28	Diphenhydramine	0.2	0.052	Trimethoprim	1.0	0.39
Bupropion	0.2	0.044	Diuron	1.0	0.28	Venlafaxine	0.5	0.076
Caffeine	1.0	0.25	Erythromycin	1.0	0.14	2,4-D	20.0	8.69
Carbamazepine	0.5	0.082	Fluridone	0.1	0.012	Gemfibrozil	20.0	11.5
Clarithromycin	5.0	1.14	Gabapentin	5.0	1.05	Triclopyr	50.0	16.3
Cotinine	0.5	0.068	Lamotrigine	2.0	0.94	Triclosan	20.0	5.32
DEET	0.2	0.022	Metoprolol	1.0	0.29			
Dextrorphan	1.0	0.15	Propranolol	1.0	0.091			

* Five replicate injections were used to calculate IDL values.

With values of $R^2 > 0.995$, the resultant calibration curves showed excellent linearity over four orders of dynamic range. Figure 2A shows the calibration curve of cotinine, a representative example. Precision and accuracy were evaluated at 12 standard concentrations ranging from the LLOQ at 0.1 ng/L (ppt) to the upper limit of quantitation (ULOQ) at 2 µg/L (ppb), and were calculated using five replicate injections at each level (Figure 2B). Excellent assay precision (%RSD < 20 % at LLOQ and < 15 % for other levels) and average accuracy (80–120 % at LLOQ and 85–115 % for other levels) were well within the method validation criteria set by EPA Method 1694.

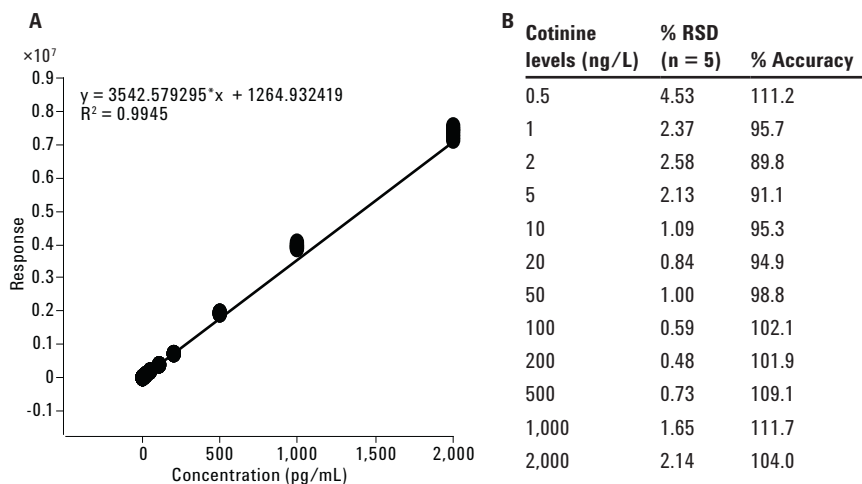


Figure 2. Cotinine example of linear dynamic range achieved. The table shows that area precision (%RSD) and accuracy were calculated at 13 standard concentrations.

Figure 3 shows that the assay precision at the 2 ng/L concentration level obtained using the 6470A Triple Quadruple LC/MS System generally exceeded that obtained using a similarly configured 6460 Triple Quadrupole LC/MS System. The improved area precision (%RSD) produced by the 6470A Triple Quadruple LC/MS System was particularly evident at low concentration levels, demonstrating the exceptional sensitivity of the new system.

Variation in signal-to-noise ratio (S/N) results from variation in baseline noise, leading to uncertainty in determining the actual performance of the instrument being tested. For this reason, performance based on area response precision gives a much clearer indication of sensitivity and performance when assessing quantitative applications. In particular, the %RSD for peak area at low concentrations can be used

as an indirect measure of the relative number of ions in a chromatographic peak, and provides a more universal measure of sensitivity. Thus, the %RSD for peak area at low concentrations was used to calculate the instrument detection limit (IDL).

The IDL was defined as:

$$IDL_{LC-MS} = t \times \%RSD \times \text{amount measured}/100$$

t = student t-value, for the 99 % confidence level with n – 1 degrees of freedom (n = replicate injections)

%RSD = relative standard deviation (precision of signal response at the amount measured, from n replicate injections)

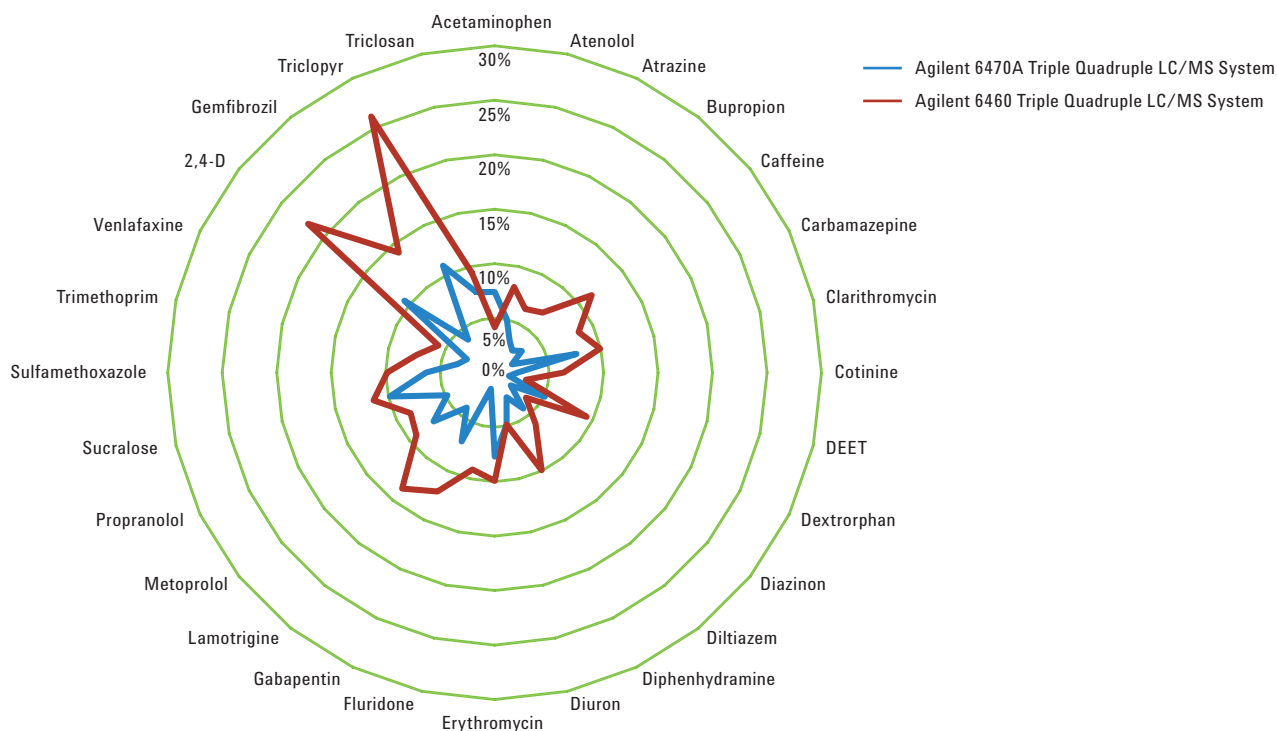


Figure 3. Comparison of assay precision (area %RSD, n = 5) obtained at 5 ng/L using the Agilent 6470A (blue) and Agilent 6460 (red) Triple Quadruple LC/MS Systems.

Table 4 presents IDLs obtained for the 28 surface water contaminants. Five replicate injections were used to calculate IDL values for these analytes. Design enhancements to the 6470A Triple Quadrupole LC/MS System led to improvements in response, generating lower area %RSDs as compared to previous designs. The superior analytical performance of the 6470A Triple Quadrupole LC/MS System is attributable to a number of design improvements which include: redesigned ion optics, improved curved and tapered collision cell, advanced detector operating at dynode accelerating voltages of up to ± 20 kV, and an autotune optimized for greater speed and sensitivity.

Figure 4 shows a representative example of how the sensitivity of the 6470A Triple quadrupole LC/MS system enabled streamlining the analytical workflow by using direct injection of a surface water sample, rather than SPE enrichment, while still achieving the LLOQ at 0.2 ng/L.

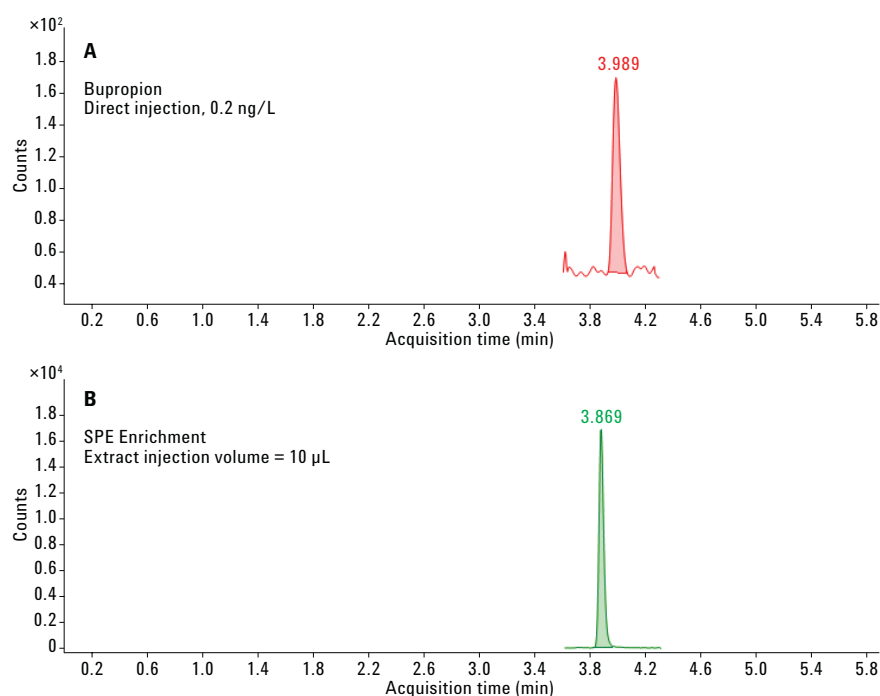


Figure 4. Agilent 6470A Triple Quadrupole LC/MS System response for bupropion spiked into surface water. A) 40 μ L direct injection of 0.2 ng/L bupropion. B) 10 μ L injection of a sample extract SPE enriched 200 times.

Conclusion

Twenty-eight water contaminant standards including selected PPCPs, sucralose, 2,4-D, Atrazine, Diuron, Diazinon, and DEET, were accurately quantified at low-ng/L levels with excellent assay reproducibility and precision using the Agilent 6470A Triple Quadrupole LC/MS System. The method achieved four orders of linear dynamic range, excellent assay precision (%RSD <20 % at LLOQ and <15 % for other levels), as well as average accuracy (80–120 % at LLOQ and 85–115 % for other levels), meeting the method validation criteria set by EPA Method 1694. The improved detection limits enabled reliable quantitation of contaminants in surface water samples at sub-ng levels using direct injection into the LC/MS system, minimizing the need for sample enrichment.

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