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An environmentally sensitive method for rapid monitoring of 6PPD-quinone in aqueous samples using solid phase extraction and direct sample introduction with liquid chromatography and tandem mass spectrometry

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The tire rubber antioxidant derivative *N*-(1,3-dimethylbutyl)-*N*′-*p*-phenyl-phenylenediamine-quinone (6PPD-Q) has been linked to toxic injury and death of coho salmon (*Oncorhynchus kisutch*) in Northeastern Pacific urban watersheds. The chemical is known to be lethal to coho salmon at relatively low and environmentally relevant concentrations. We have developed a new and environmentally sensitive method for rapid monitoring of 6PPD-Q at concentrations ranging from less than 2 ng L^{-1} to over 1400 ng L^{-1} in water samples collected from creeks. Sample analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS) following solid phase extraction (SPE) or dilution and direct introduction (dilute-and-shoot or DnS) was investigated. Limits of quantification were 1.74 ng L^{-1} for DnS-LC-MS/MS and 0.03 ng L^{-1} for SPE-LC-MS/MS using 9.6 mL of water sample, which was 3.3 times lower than the lowest reported limit of quantification (0.1 ng L^{-1}) obtained with 500 mL of sample. The method used up to 99% less solvent during extraction than established procedures, leading to an equivalent reduction in the amount of waste generated. Sample storage space was also reduced due to the small volumes of sample required for analysis and the smaller bottles needed to collect these samples. The method was evaluated by comparing results with those obtained by a commercial laboratory using established procedures, which showed good agreement (r^2 = 0.982). This environmentally friendly and cost effective strategy for 6PPD-quinone analysis may be applied to other chemical monitoring studies in order to optimize sample storage and solvent usage while covering a wide range of analyte concentrations.

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Sustainability spotlight

6PPD-quinone was identified as the contaminant responsible for urban runoff mortality syndrome in coho salmon. Rapid profiling of 6PPD-quinone in water samples to support policy and regulatory decision making has been required, but current strategies are challenged by low sensitivity, large waste and storage space. Here, we report the use of dilute-and-shoot (DnS)-LC-MS/MS and (or) solid phase extraction (SPE)-LC-MS-MS to analyze water samples by using up to 99% less solvent and 9.6 mL sample, thus reducing up to 99% of generated waste, minimizing the overall time and cost of analysis, and saving sample storage space. Therefore, our work can help to realize the "Responsible Consumption and Production" of the Sustainable Development Goals (SDGs).

Introduction

The tire rubber antioxidant derivative *N*-(1,3-dimethylbutyl)-*N*′-*p*-phenyl-phenylenediamine-quinone (6PPD-Q) has been identified as the toxic agent responsible for urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*) in urban watersheds in the Northeastern Pacific.[1] The identification of 6PPD-Q, and its occurrence at lethal concentrations in urban aquatic systems[2–5] has prompted the need for environmental detection and monitoring that will assist with the prioritization of sites for mitigation.

Since the discovery of this toxic chemical in 2020 numerous analytical methods for the determination of 6PPD-Q have been developed.[1,6–11] These include methods based upon gas chromatography (GC) time-of-flight mass spectrometry (TOF-MS) and ultra high-performance liquid chromatography (UHPLC) combined with Orbitrap, TOF, and triple quadrupole mass

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spectrometry.[6,10] Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been applied to the analysis of 6PPD-Q in water samples obtained from toxicity studies[1,3,12–15] and in environmental samples including runoff water,[16–18] surface and storm water,[7–9,18] snowmelt and river water,[18] municipal waste water,[19] dust,[20] particulate matter[16,20–23] and soil.[16] LC-MS/MS has also been used to quantify 6PPD-Q in biological samples including human urine,[24] cell cultures,[25] and tissues like brain, gill, liver and muscle[15] collected from fish and mice during toxicity studies[26] as well as in food samples such as honey and fish.[27]

LC-MS/MS-based methods require sample preconcentration using solid-phase extraction (SPE) for determination of 6PPD-Q in aqueous matrices, in order to measure 6PPD-Q in water samples at concentrations below 2 ng L^{-1}. This represents the lower range of concentrations typically observed before and after rainfall events, whereas higher concentrations occurred during raining may be detected and quantified by LC-MS/MS following sample dilution and direct introduction, also known as dilute-and-shoot (DnS). To our knowledge the use of LC-MS/MS with DnS and SPE to monitor the wide range of 6PPD-Q concentrations observed in environmental water samples during rainfall events has not yet been reported.

Here we describe the development, optimization and validation of a robust method for rapid detection and quantification of 6PPD-Q in water samples at concentrations ranging from 0.03 to over 1000 ng L^{-1} based upon DnS-LC-MS/MS and SPE-LC-MS/MS. Specific objectives include (i) evaluation of the method with regard to method limit of quantification, linearity, precision, recovery and freedom from matrix interferences; (ii) adoption of an environmentally sensitive approach using small volumes of sample and solvent, (iii) evaluation of the method for analysis of 6PPD-Q in creek water samples and comparison with results obtained using a commercially available LC-MS/MS method and (iv) validation of the proposed environmental strategy on responsible consumption and production. This new method broadens the range over which 6PPD-Q concentrations can rapidly be monitored in natural waters, making it possible to determine the earliest onset and maximum levels of exposure to 6PPD-Q during rainfall, runoff, and other events.

Materials and methods

Samples

Water samples were collected before, during, and after rain events (≥5 mm precipitation) following dry periods (≥48 h) in creeks around Metro Vancouver and on Vancouver Island, British Columbia (B.C.). Samples were collected by hand directly into traceable precleaned 250 mL amber glass short jars with 70 mm Teflon lined cap (Systems Plus Ltd, ON, Canada) and then stored at −20 °C. Samples were shipped frozen prior to sample preparation for LC-MS/MS analysis at the Fisheries and Oceans Canada (DFO) Institute of Ocean Sciences (IOS) in Sidney, B.C. Water samples used for comparison with a commercial LC-MS method were shipped to SGS AXYS Analytical Ltd in Sidney, B.C. for analysis.

Chemicals and reagents

Acetonitrile and methanol were high-performance liquid chromatography (HPLC) grade and were purchased from VWR Canada (Mississauga, ON, Canada). Ammonium fluoride was purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Super-Q water was prepared using a Super-Q™ water isolation system (Millipore SAS, France). Oasis HLB (60 mg, 3 mL) solid-phase extraction cartridges were purchased from Waters Limited (Mississauga, ON, Canada). Native standard solution of 6PPD-Q (100 μg mL^{-1}) in acetonitrile and labelled 6PPD-quinone (phenyl-$^{13}C_6$, 99%) ($^{13}C_6$-6PPD-Q) (100 μg mL^{-1}) in acetonitrile were supplied by ACP Chemical Inc. (Montreal, QC, Canada). A standard mixture containing 6PPD-Q at a concentration of 100 ng mL^{-1} was prepared in 30 : 70 acetonitrile/water (v/v). Working calibration standard solutions (0.003 to 5 ng mL^{-1}) were obtained by diluting the appropriate amount of the standard mixture in 30 : 70 acetonitrile/water (v/v) containing $^{13}C_6$-6PPD-Q at 5 ng mL^{-1}. Spiking at various concentrations was achieved by adding different amounts of standard solution to creek water samples.

Dilute and shoot (DnS)

For water samples collected during and after raining which may have high 6PPD-Q concentrations (>2 ng L^{-1}), dilution was carried out prior to LC-MS/MS by mixing 70 μL of water sample with 30 μL of 16.66 ppb $^{13}C_6$-6PPD-Q prepared in acetonitrile.

Solid phase extraction (SPE)

For water samples collected before raining and those water samples collected during and after raining which have been confirmed to have low 6PPD-Q concentrations (<2 ng L^{-1}) by DnS-LC/MS/MS, SPE was carried out prior to LC-MS/MS using an automated SPE system (Caliper Life Science). SPE was performed using Oasis HLB cartridges (60 mg, 3 mL) in which 4.9 mL of methanol and 4.9 mL of water were used to condition sequentially prior to loading 9.6 mL of water sample containing 50 ng L^{-1} of $^{13}C_6$-6PPD-Q. After washing with 3.0 mL of water and drying column, compounds of interest were eluted with 2 × 2 mL of methanol and collected in a single fraction. The fractions were dried using a nitrogen flow and a temperature of 40 °C. The residues were reconstituted with 100 μL of 30 : 70 acetonitrile/water (v/v) and stored at −20 °C until LC-MS/MS analysis.

Liquid chromatography tandem mass spectrometry

Diluted water samples and SPE extracts were analysed by LC-MS/MS using an Agilent 1290 Infinity II liquid chromatograph fitted with a 2.1 × 50 mm, 1.9 μm Agilent InfinityLab Poroshell 120 EC-C18 column (Agilent, Mississauga, ON) and coupled to an API5000 triple-quadrupole tandem mass spectrometer fitted with a Turbo IonSpray source (AB Sciex, Concord, ON, Canada). The mobile phase consisted of ammonium fluoride in water and acetonitrile. The LC column temperature was 40 °C and the injection volume was 15 μL. Analyst 1.7.1 software (AB Sciex)

was used for operating the LC-MS/MS system, data acquisition and processing.

Method validation

Performance was validated with respect to linearity, sensitivity, precision, recovery, and freedom from matrix interferences. To evaluate sample preparation and LC-MS/MS analysis, water samples from various locations were processed and analysed. The concentrations of 6PPD-Q in these samples ranged from below the limit of detection to 1460 ng L^{-1}. Some samples were also spiked with native compounds. All spiked and non-spiked samples were prepared and analysed according to the procedures described above to check for recovery of the spike and the possibility of matrix effect.

Linearity and sensitivity. Linearity was determined using a seven-point calibration with analyte concentrations ranging from 0.003 to 5 ng mL^{-1} in 30 : 70 acetonitrile/water (v/v). A signal-to-noise (S/N) ratio of 10 : 1 was used to determine the method limit of quantification (MLOQ) for DnS-LC-MS/MS and SPE-LC-MS/MS.

Precision and recovery. Precision and recovery were determined using un-spiked water samples and identical samples spiked with an appropriate amount of 6PPD-Q based upon the concentration measured in the un-spiked sample.

Matrix effects and carryover. The peak areas of $^{13}C_6$-6PPD-Q in samples with both DnS-LC/MS/MS and SPE-LC-MS/MS were compared to those in calibration standards and the results were used to determine if there was matrix effect when analyzing samples. To determine carry-over, 6PPD-Q at 10 ng mL^{-1} in 30 : 70 acetonitrile/water (v/v) was analyzed by LC-MS/MS followed immediately by injection of a 30 : 70 acetonitrile/water (v/v) solvent blank, in which any residual 6PPD-Q was measured.

Blanks and contamination. 6PPD-quinone, which is derived from the ubiquitous tire rubber component 6PPD, has been found in air[16] and house dust.[20] Therefore, it is important to minimize the contamination during sample preparation, especially when dealing with ultra-trace levels (<2 ng L^{-1}) of 6PPD-Q in samples. Rinsing sample vials and pipette tips were investigated as a means of reducing contamination during analysis.

Sample stability. 6PPD-quinone is relatively new, emerging environmental contaminant for which few stability studies have so far been published.[9] Sample storage and preservation, including the addition of ascorbic acid, were therefore investigated as part of this study.

Results and discussion

Method optimization

Liquid chromatography tandem mass spectrometry. Solutions containing 6PPD-Q or $^{13}C_6$-6PPD-Q were infused at 10 μL min^{-1} *via* the SI source using a syringe pump to allow optimization of MS/MS acquisition parameters. The main precursor (molecular) and product (fragment) ions produced in positive mode during MS and MS/MS were identified. The most intense precursor-to-product ion transitions for each compound were selected for MRM and optimized for sensitivity by adjusting parameters such as the declustering potential (DP), collision energy (CE), collision cell exit potential (CXP) and entrance potential (EP). Of the three MRM transitions selected for 6PPD-Q, one (299 > 215) was used for measuring analyte concentration and the others (299 > 187 and 299 > 241) to confirm the identity of the analyte.[14] The MRM transitions and MS/MS acquisition parameters for 6PPD-Q and $^{13}C_6$-6PPD-Q are listed in Table S1. A dwell time of 40 ms was used for each MRM transition.

LC-MS/MS conditions were optimized using various mobile-phase compositions including acetonitrile/water and methanol/water with and without the addition of formic acid and ammonium fluoride. Of the mobile phase compositions tested, acetonitrile and water containing 1 mM ammonium fluoride was found to achieve best results. Optimized instrumental conditions are shown in Table S2. The MRM chromatograms for 6PPD-Q at 50 ng L^{-1} in standard solution and 45 ng L^{-1} in water sample collected from creek, which is slightly above the 24 h median lethal concentration (LC50) of 41 ng L^{-1} for juvenile coho,[3] are shown in Fig. S1.

Solid phase extraction. Water samples collected from creeks with anticipated 6PPD-Q concentrations below 2 ng L^{-1} were subjected to automated SPE prior to LC-MS/MS. SPE procedures were similar to those reported elsewhere[1,17] except that only 9.6 mL (rather than 50 or 500 mL) of sample was required for automated SPE which reduces time, solvent and labor while ensuring precision and accuracy for 6PPD-Q analysis. Automated SPE took about 15 min per sample and satisfactory recoveries (80 to 95%) were achieved for spiked samples.

Dilute and shoot. 6PPD-Q has been found in soil[16] and dust[20] and has been detected at relatively high concentrations (above 1000 ng L^{-1}) during rain events in urban streams. Such concentrations are amenable to analysis using dilute and shoot (DnS), a technique that offers simplicity of analysis, minimal loss of analyte(s), and high sample throughput. Of the 81 samples selected for this study, 51 were successfully analyzed by DnS-LC-MS/MS with 6PPD-Q concentrations ranging from 3.5 to 1460 ng L^{-1}.

Method validation

Linearity and method limits of quantification. A linear calibration curve (r > 0.99) was generated by plotting the MRM response (peak area ratio) against concentration ratio (0.003 to 5 ng mL^{-1}) for 6PPD-quinone. The method limits of quantification were 1.74 ng L^{-1} for DnS-LC-MS/MS and 0.03 ng L^{-1} for SPE-LC-MS/MS which represent a significant improvement over previously published methods, as shown in Table 1 except one from Lane *et al.*[9]

Precision and recovery. Since certified reference materials for 6PPD-Q in water samples were not available, precision was estimated using duplicate samples, and recoveries were determined by comparing results obtained using spiked and un-spiked samples. Average precision (RSD) was 5% for DnS-LC-MS/MS and 7% for SPE-LC-MS/MS whereas average recoveries were 96% for DnS-LC-MS/MS and 87% for SPE-LC-MS/MS.

Matrix effect and carryover. Co-elution of analyte compounds with residual matrix components may lead to

Table 1 Method comparison for analysis of 6PPD-quinone in water samples

DnS-LC-MS/MS[a]		SPE-LC-MS/MS[b]		
MLOQ (ng L^{-1})	Ref.	Sample size (mL)	MLOQ (ng L^{-1})	Ref.
42.5	14	1000	Not applicable	1
24	7	500	6.6	18
		250	Not applicable	11
20	13	50	9.76	2 [c]
1.74	This study	11	5	17
1.33	9	500	0.1	Commercial lab
		9.6	0.03	This study

[a] DnS-LC-MS/MS: dilute and shoot liquid chromatography tandem mass spectrometry. [b] SPE-LC-MS/MS: solid phase extraction liquid chromatography tandem mass spectrometry. [c] Ultra-high pressure liquid chromatography with high resolution mass spectrometry was used.

suppression or apparent enhancement of the detected analyte signal. To investigate such matrix effects, the peak area for $^{13}C_6$-6PPD-Q in samples spiked with this compound at 2 ng mL^{-1} was determined for both DnS-LC/MS/MS and SPE-LC-MS/MS. A difference in peak area of about 10% was observed between calibration standards and spiked water samples analysed using DnS, suggesting that matrix effects for DnS-LC/MS/MS are relatively small. In contrast, Kryuchkov *et al.*[17] reported strong ion suppression during SPE-LC-MS/MS analysis of 6PPD-Q in water samples collected from tunnel-wash runoff, although this was attributed to the relatively high concentration of detergents present in the water. We observed differences of up to 50% between standard and sample peak areas when using SPE-LC-MS/MS, which is consistent with the proportionally greater impact of matrix effects on lower 6PPD-Q concentrations that require preconcentration by SPE, compared with the higher 6PPD-Q concentrations amenable to DnS. Calibration curves obtained for deionized (Super-Q) water and creek water were used to evaluate matrix effects. Using $^{13}C_6$-6PPD-Q as an internal standard, linear calibration curves with $1/x^2$ weighting were generated for both DnS-LC-MS/MS and SPE-LC/MS/MS. Calibrations obtained using standards prepared in 30 : 70 acetonitrile/Super-Q water (v/v) and 30 : 70 acetonitrile/creek water (v/v) has similar slopes (Table S3). However, differences in the intercept values are consistent with matrix effects and the presence of 6PPD-Q in the original creek water sample. These observations illustrate the importance of using isotopically labelled standards to account for matrix effects during sample preparation and analysis. No carryover was observed at 6PPD-Q concentrations up to 10 000 ng L^{-1} which is about 6 times higher than the highest concentration (1460 ng L^{-1}) measured in our samples.

Blanks and contamination control. During initial studies we detected background levels of 6PPD-Q consistent with previous reports that 6PPD-Q can be found in air and house dust.[16,20] Rinsing sample vials and pipette tips with water, methanol and acetonitrile were found to be effective in minimizing sample contamination during analysis.

Sample stability

Three concentrations of 6PPD-Q (~0.5, 5 and 50 μg L^{-1}) were spiked into well water samples collected at the Fisheries and Oceans Canada (DFO) Pacific Science Enterprise Centre (PSEC) in West Vancouver, British Columbia and held at room temperature to investigate the stability of natural water samples with and without addition of ascorbic acid. The results, presented in Table 2, show that 6PPD-Q remained relatively stable in the absence of ascorbic acid with losses occurring in proportion to initial 6PPD-Q concentration (*i.e.* 19, 21 and 34%, respectively, over 14 days) whereas the presence of ascorbic acid appeared to promote loss of 6PPD-Q from PSEC well water samples (pH 5–6).

Our findings are at odds with those of Hiki *et al.*[14] who observed that the concentration of spiked 6PPD-Q in dechlorinated tap water (pH 8) stored at 23 °C decreased from 68 to 4 μg L^{-1} after 5 days, although this may be due to differences in pH and/or other water properties between the two studies. We have also observed loss of spiked $^{13}C_6$-6PPD-Q and 6PPD-Q in some tap water samples we have analysed. Di *et al.*[28] reported that the half-life of 6PPD-quinone spiked at ~400 μg L^{-1} in river water (12.8 days) was significantly shorter than in pure water at pH 4 (15.5 days), pH 7 (15.2 days) and pH 9 (14.6 days), implying that 6PPD-Q is more stable in aqueous samples of lower pH and higher purity. It is worth noting that the 6PPD-Q concentrations investigated by Hiki *et al.* and Di *et al.* exceeded the experimental solubility of 6PPD-Q in water (67 ± 5 μg L^{-1}) at 23 °C[14,28] whereas the 6PPD-Q concentrations that we measured in samples collected from several creeks ranged from below the method limit of detection to 1460 ng L^{-1} (*i.e.* 1.46 μg L^{-1}). These creek samples, which contained 6PPD-Q at concentrations well below the limit of solubility, appeared to be stable for several days at room temperature and during long-term refrigerated storage. These observations are consistent with results reported by Lane *et al.* concerning the stability of 6PPD-Q in surface water under varying storage conditions.[9]

Method comparison

Since no reference describing the use of both DnS-LC-MS/MS and SPE-LC-MS/MS for the analysis of 6PPD-Q in water samples was available, we compared our 6PPD-Q method with those used previously for toxicity studies[1,3,12–15] and environmental applications involving analysis of runoff and surface water, stormwater, snow and snowmelt, river and municipal waste water[7,9,16–19] in terms of method limit of quantification (MLOQ) and volume of sample required for MS/MS analysis. Our MLOQ for DnS-LC-MS/MS (1.74 ng L^{-1}) was 11 to 24 times

Table 2 6PPD-Q (μg L^{-1}) in well water stored at room temperature with and without the addition of ascorbic acid (AA)

Time	With AA	NO AA	With AA	NO AA	With AA	NO AA
Day 0	0.53	0.57	5	4.8	46	46
Day 12	0.15	0.53	0.99	4.46	9.79	31.2
Day 14	0.08	0.46	0.72	3.78	7.51	30.2

lower than for other published methods (Table 1) with the exception of Lane *et al.*[9] who were able to achieve a similar MLOQ (1.33 ng L^{-1}) using a more sensitive MS/MS instrument (Sciex API5500) than ours (Sciex API5000). Our MLOQ for SPE-LC-MS/MS was 3 to 325 times lower than those from other published methods (Table 1). These large differences in sensitivity may be due, at least in part, to our use of a relatively large injection volume injection (15 μL) compared to those typically used for DnS-LC-MS/MS (1 μL) and SPE-LC/MS/MS (5 μL) as well as the choice of analytical instrumentation, LC column, mobile phase composition (including the use of ammonium fluoride), and solvents used to dilute and reconstitute samples. As a result, our method was able to achieve good results with smaller volumes of sample (9.6 mL) than those typically used for SPE-LC-MS/MS analysis of 6PPD-Q (*e.g.* 50 mL for Johannessen *et al.*,[2] 250 mL for EPA Draft Method 1634,[11] and 500 mL for the commercial laboratory method). Such comparisons may be helpful in planning MS/MS analysis of 6PPD-Q in water samples based upon the available instrumentation, sample size, and the analytical sensitivity required.

Results comparison

Results obtained by DnS-LC-MS/MS analysis of 51 water samples containing greater than 2 ng L^{-1} 6PPD-Q and SPE-LC-MS/MS analysis of 30 water samples containing less than 2 ng L^{-1} 6PPD-Q were compared with those obtained for all 81 samples by a commercial laboratory (SGS AXYS Analytical Ltd, Sidney, BC, Canada.) using 500 mL of sample for SPE-LC-MS/MS. The results obtained by the commercial laboratory agreed well with those obtained using our method, a strong linear correlation ($r^2 = 0.982$) being observed between the two sets of results (Fig. S2). We also analysed some of the water samples containing greater than 2 ng L^{-1} 6PPD-Q using both DnS-LC-MS/MS and SPE-LC-MS/MS and found that the concentrations measured in each sample using the two methods agreed well with each other.

Environmental impact on greenness

As well as providing greater sensitivity for high-throughput analysis of 6PPD-Q in creek waters, our method supports the environment by reducing solvent consumption and the associated generation of waste through (i) the adoption of DnS-LC-MS/MS analysis first for samples collected during raining and after raining which may contain 6PPD-Q at concentrations greater than 2 ng L^{-1}, and (ii) the use of smaller volumes (9.6 mL) for SPE-LC-MS/MS analysis of samples collected from before raining and those water samples collected during and after raining which have been confirmed to have low 6PPD-Q concentrations (<2 ng L^{-1}) by DnS-LC/MS/MS. For example, this strategy reduced the amount of solvent required to analyse 81 creek samples by up to 99%, and the amount of required sample storage space by up to 90%, as a result of being able to use DnS for the 51 samples containing >2 ng L^{-1} 6PPD-Q and 9.6 mL (rather than 50 or 500 mL) of sample for SPE of the remaining 30 samples, with a corresponding reduction of up to 99% in the amount of solvent waste generated (Table 3). The requirement for less sample enables the use of smaller (*e.g.* 50 mL) rather than larger (*e.g.* 250 mL or 500 mL) amber bottles to collect creek or other natural water samples for analysis, reducing storage space as well as material and transportation costs. In addition, running all samples collected during and after raining events by DnS-LC-MS/MS can quickly identify those that require SPE-LC-MS/MS, thereby minimizing the overall time and cost of analysis.

Sample analysis

In accordance with EPA Draft Method 1634, creek water samples were not filtered prior to analysis. However, it is generally accepted that only the freely dissolved fraction of 6PPD-Q is bioavailable and able to elicit toxicological effects. While most creek water samples only contained small amounts of particulate matter that were found to contain less than 10% of the total 6PPD-Q in these samples (Table S4), this increased to about 20% for samples containing larger amounts of particulate matter (*i.e.*, runoff water). Depending on sample type and objective of the study (*i.e.*, comparison with toxicity thresholds *versus* overall mass loading estimations), it may or may not be necessary to filter the samples prior to analysis.

Following optimization and validation the method was applied to the detection and quantification of 6PPD-Q in 160 creek water samples, as well as over 250 water samples prepared as part of a holding study in Pacific Sciences Enterprise Centre,

Table 3 Solvents consumed and waste generated when using our method and a commercial lab for 6PPD-Q analysis

	Our method		Commercial laboratory
	DnS-LC-MS/MS[a]	SPE-LC-MS/MS[b]	SPE-LC-MS/MS[b]
Number of samples	51	30	81
Solvent used (mL)	15.3	261	36 936
Water used (mL)	51	582	40 500
Total solvent used (mL)	276.3		36 936
Total waste generated (mL)	909.3		77 436
Solvent saved (%)	99		
Waste reduced (%)	99		

[a] DnS-LC-MS/MS: dilute and shoot liquid chromatography tandem mass spectrometry. [b] SPE-LC-MS/MS: solid phase extraction liquid chromatography tandem mass spectrometry.

West Vancouver, British Columbia. The concentration of 6PPD-Q in creek water samples ranged from below the MLOQ of 0.003 ng L^{-1} to 1460 ng L^{-1}, a dynamic range spanning 5 orders of magnitude. A representative MRM chromatogram for a creek water sample containing 45 ng L^{-1} 6PPD-Q is shown in Fig. S1.

Conclusions

A new and improved method for rapid monitoring of 6PPD-Q in creek waters has been developed, and an environmental strategy to analyse the samples was employed in order to reduce solvent consumption and waste generated. Quantification of 6PPD-Q in creek water samples at concentrations ranging from less than 2 ng L^{-1} to over 1400 ng L^{-1} has been demonstrated. Limits of quantification are significantly lower than those achieved using other methods, allowing smaller volumes of sample and solvent to be used and reducing the environmental impact of the method. The use of both direct sample introduction and solid-phase extraction in combination with liquid chromatography and tandem mass spectrometry provides a dynamic range spanning 5 orders of magnitude, making it possible to determine both the earliest onset and the maximum levels of exposure to 6PPD-Q for salmon and other aquatic organisms during rainfall and other events, while reducing solvent consumption and waste production during analysis.

Author contributions

X. Liao.: conceptualization, data curation, methodology, formal analysis, visualization, writing. A. R. S. Ross.: conceptualization, manuscript review. T. M. Brown.: funding acquisition, resources, conceptualization, manuscript review.

Data availability

The data supporting this article have been included as part of the SI. See DOI: https://doi.org/10.1039/d5su00170f.

Conflicts of interest

The authors declare no competing interests.

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