Analysis of ***6PPD-Q from Aqueous Samples*** Using Automated FREESTYLE XANA TableTop System using EluCLEAN® 6PPD-Q SPE Cartridges (US EPA 1634 Draft Method)

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Content

Key Features

- Excellent recovery rates and low standard deviations for 6 PPD-Q according to US EPA 1634 (draft) method
- No detectable background contamination or carryover in the system
- EluCLEAN 6PPD-Q cartridge is an equivalent to the SPE cartridge mentioned in US EPA 1634 draft method
- Reliable and robust automation with FREESTYLE XANA TableTop (Fully automated processing of up to 30 samples in one sequence)

LCTech Products

SPE cartridges

Part No.: 21641

EluCLEAN® 6PPD-Q, 30 Pcs/Pk, 200 mg/6 mL

Sorbent: Polymeric reversed-phase

FREESTYLE XANA TableTop

Part No.: 20600	FREESTYLE XANA TableTop (30 positions)
Part No.: 19372	SPE cartridge adapters for elution into 50 mL Falcon tubes (10 pcs.)
Part No.: 14923	Caps for 6 mL SPE cartridges, reusable (10 pcs.)
Part No.: 13156	Solvent bottle rack, 6 x 1 L
Part No.: 12709	Overflow Sensor for Waste Level Control, with GL 45 Cap

Other Relevant LCTech Application Notes and Product Information

https://www.lctech.de/application-notes

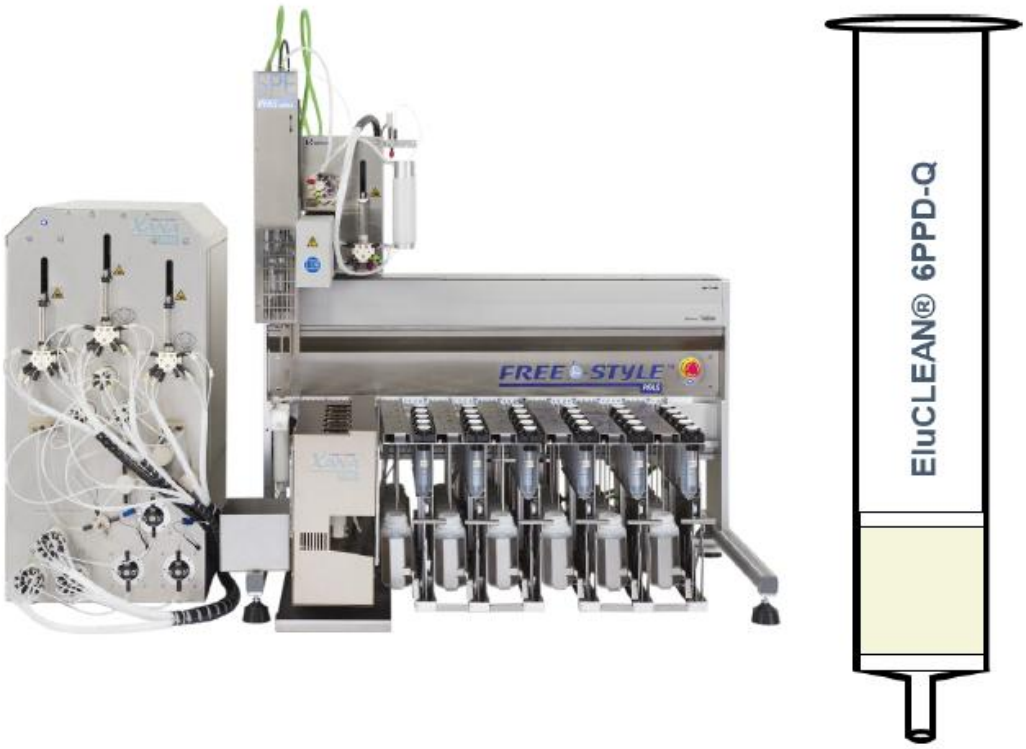


Figure 1. FREESTYLE XANA TableTop with EluCLEAN® 6PPD-Q – SPE cartridges

Figure 2. Equipping the bottle rack of the FREESTYLE XANA TableTop

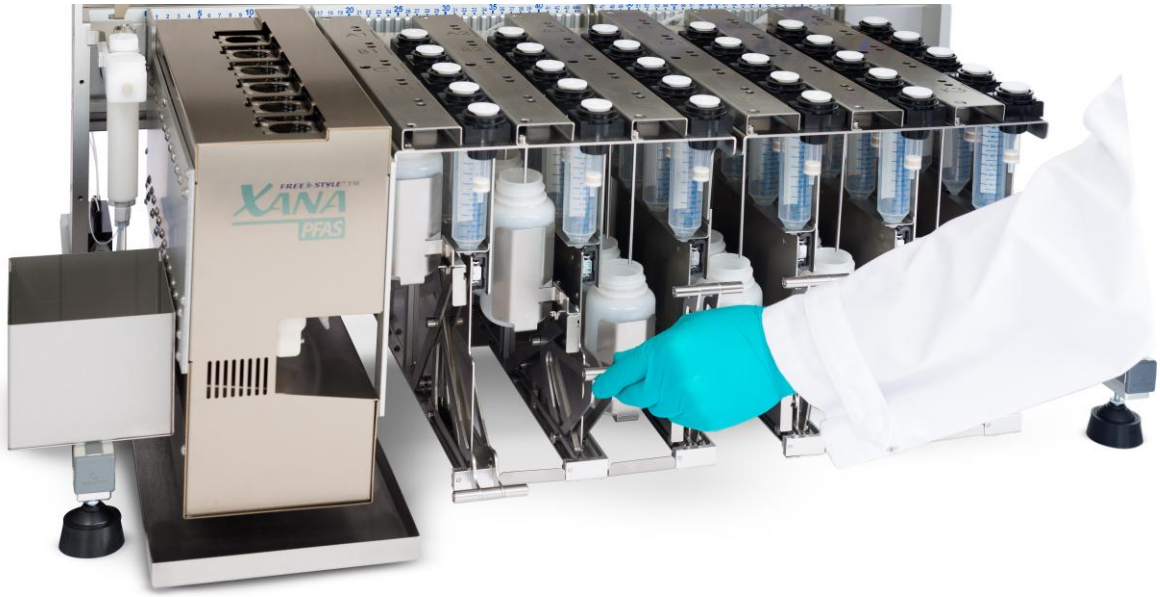


Figure 3. Loading the bottles of the FREESTYLE XANA TableTop

1. Introduction

6 PPD (*N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylendiamin*) is an organic chemical widely used a stabilizing additive in rubbers commonly used for vehicle tires (to prevent cracking). It reacts with ozone in the air to form protective layer. Oxidation of 6PPD leads to formation of 6PPD-Q. This compound can be washed away from tire wear and enter waterbodies via surface runoff during the event of rain. Hence, aquatic organisms can be exposed to 6PPD-Q. Research indicates the toxic effect of 6PPD-Q could be fatal to coho salmon[1]. The high toxicity of 6PPD-Q to coho salmon has raised interest in the analytical world for their rapid measurement in various surface aquatic bodies.

The US EPA has released the method (first draft of 1634 method) for the analysis of 6PPD-Q from water bodies[2]. This method requires solid phase extraction of aqueous samples and subsequent analysis in liquid chromatography tandem mass spectrometric analysis.

The following application note shows how aqueous samples can be prepared fully automated for LC-MS/MS analysis by applying SPE with FREESTYLE XANA TableTop robotic system according to US EPA 1634 (draft) method. By the application of fully automated parallel sample preparation, multiple samples can be processed at the same time. Thus, a high sample throughput at low demand of personnel resources is achieved.

In this application note, the FREESTYLE XANA Tabletop robotic system used a new single SPE cartridge solution containing 200mg of polymeric sorbent optimized for 6PPD-Q. The SPE cartridge shows excellent recovery rates in combination with low standard deviations and is therefore ideally suited for SPE of 6PPD-Q from various aqueous matrices.

FREESTYLE XANA Tabletop robotic system was able to achieve excellent recovery rates in combination with low standard deviation.

2. Experimental

2.1 Sample Preparation

250 mL of reagent water and river water is collected in 250 mL PE bottles. No further treatment was done. Native 6PPD-Q and 13C labelled surrogates standard were spiked to get the final concentrations of 40 ng/L.

250 mL of sample is extracted on the Freestyle Tabletop.

Freestyle: EluCLEAN® 6PPD-Q Cartridges and 50 mL falcon tubes were placed on a SPE rack of the FREESTYLE system. Sample bottles were placed in the XANA bottle rack. Solvent bottles were connected with respective ports. The fully automated method (see below) was applied and the eluent was collected for subsequent evaporation

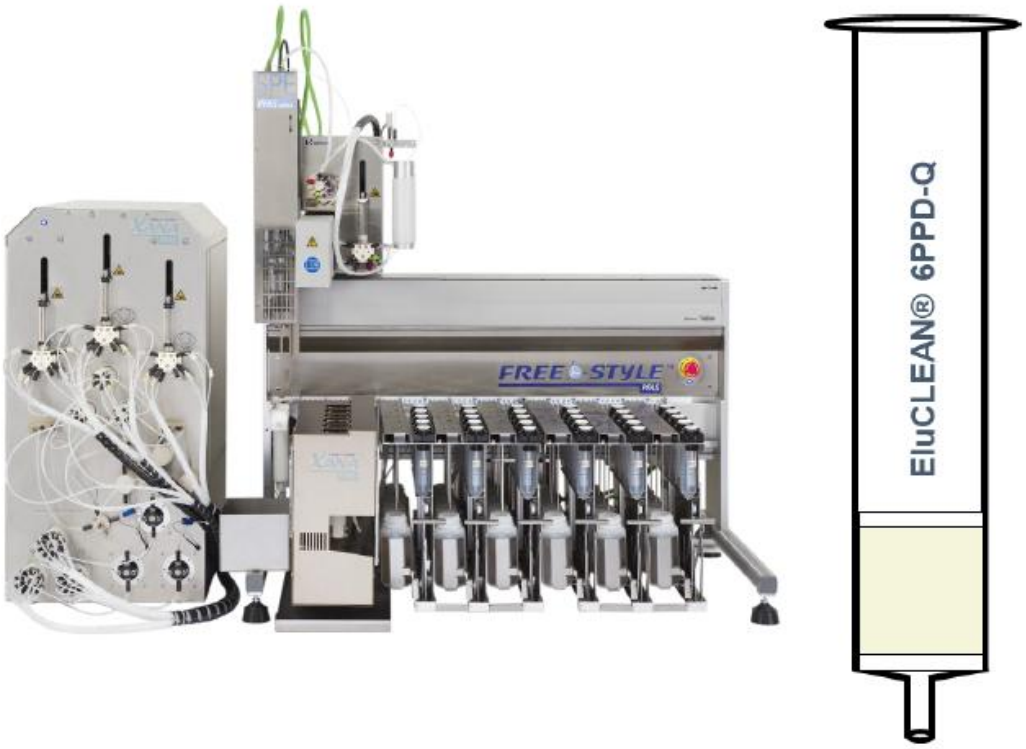


Figure 4. FREESTYLE XANA TableTop with EluCLEAN® 6PPD-Q – SPE cartridges

Table 1. FREESTYLE XANA TableTop conditions according to US EPA 1634 (4th draft) method

FREE STYLE™ AUTOMATED SAMPLE PREPARATION

LCTech FreeStyle - Report on Methods: WAT Type PFAS - EPA

Name: 6PPD-Q_Sequential.wat

Column:	LCTech_3ml.col		Extension cannula:	yes
Conditioning 1:	ON			
Volume:	6 ml	Dispensing Speed:	10 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0 min	Port : W11 ACN
Conditioning 2:	ON			
Volume:	10 ml	Dispensing Speed:	10 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0 min	Port : W10 H2O
Conditioning 3:	OFF			
Conditioning 4:	OFF			
Load 1:	ON	Typ:	empty	
Number of bottles:	1	Transfer Speed	10 ml / min	
1. rinsing cycle				
1x Rinsing volume:	6 ml	Suction Speed:	20 ml / min	
Dispensing Speed:	50 ml / min			Port : 10 MeOH:H2O; 50:50
Washing 1:	OFF			
Washing 2:	OFF			
Drying 1:	ON	stay on actual position		
Time:	20 min			
PFAS RinsElution	ON			
2x Volume:	6 ml	Dispensing Speed:	5 ml / min	
Suction Speed:	20 ml / min	Waiting time:	0 min	Port : 11 ACN

10 µL NIS (D5-6PPPD-Q) solution were added to each sample eluate and vortexed before LCMSMS analysis.

2.2 Background contamination vs Carryover of the system test

Background contamination: This test is to ensure that the system is free of 6PPD-Q contamination. 250 mL reagent water was extracted (without spiking) using EluCLEAN® 6PPD-Q Cartridges on FREESTYLE XANA Tabletop.

Carryover: This test is to ensure that the subsequent samples will not be contaminated from previous samples during automation. This test further ensures that the intermediate cleaning process implemented during the automation is effective.

250 mL reagent water blanks were extracted for subsequent extraction after intermediate cleaning process of the system.

2.2.1 Evaporation/Concentration

All samples were evaporated to 500 µL - 1 mL using D-EVA Rotational Vacuum Concentrator (temperature: 45°C, vacuum: 20 mbar) and transferred into a 1.5 mL polypropylene vial and kept at 0 - 4 °C for LC-MS/MS analysis.

2.3 Instrumentation

2.3.1 MS Conditions

Table 3. MS Conditions

Parameter	Value
MS	TSQ Quantis (Thermo)
Polarity	Positive
Spray voltage	3500 V
Sheath Gas	2 Arb
Aux Gas	2 Arb
CID Gas	2 mTorr
Ion transfer tube temp	325 °C
Vaporizer Temp	300 °C
Q1 resolution	0.7 FWHM
Q3 resolution	1.2 FWHM
Cycle time	0.5 sec
Chromatographic peak width	6 sec

2.3.2 LC Instrument Conditions

Table 4. LC Conditions

Parameter	Value	
LC	Thermo Scientific Vanquish Flex UHPLC system	
Analytical column	Accucore RP-MS, 2.1*100 mM, 2.6 µm	
Column temperature	50 °C	
Injection volume	5 µL	
Mobile Phase	A) 0.2 % Formic acid in Water B) Acetonitrile	
Gradient Flow rate	0.6 mL/min	
Gradient	Time (min) 0 1 3 5 6 9 10	% B 10 10 70 99 99 10 10

3. Results

3.1 Calibration curve

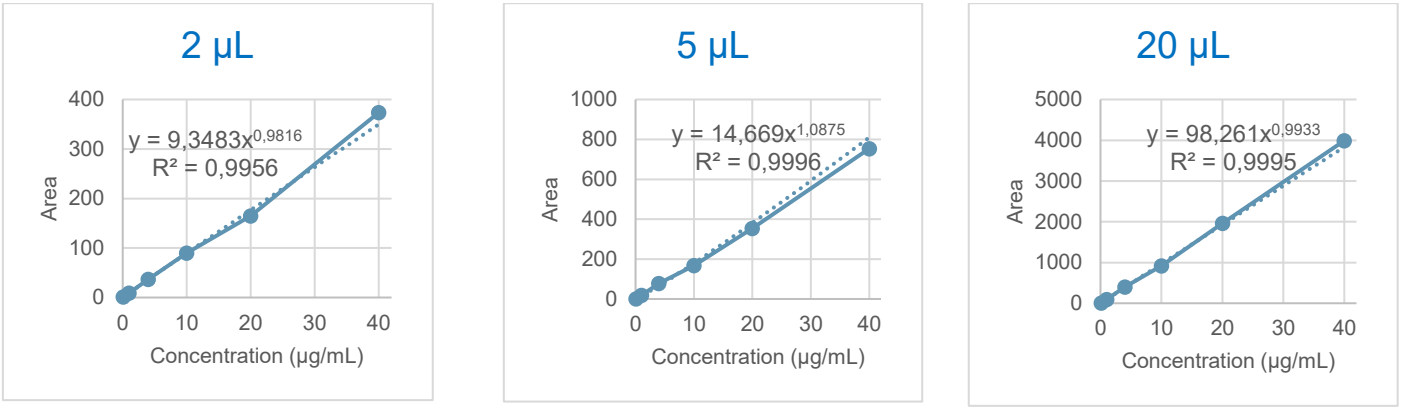


Figure 2. Calibration curve of 6PPD-Q in LCMSMS when using different injection volume

- ✓ Calibration curve is linear with various injection volume
- ✓ Various injection volume can be used to achieve required sensitivity

3.2 Recovery Rates and RSD

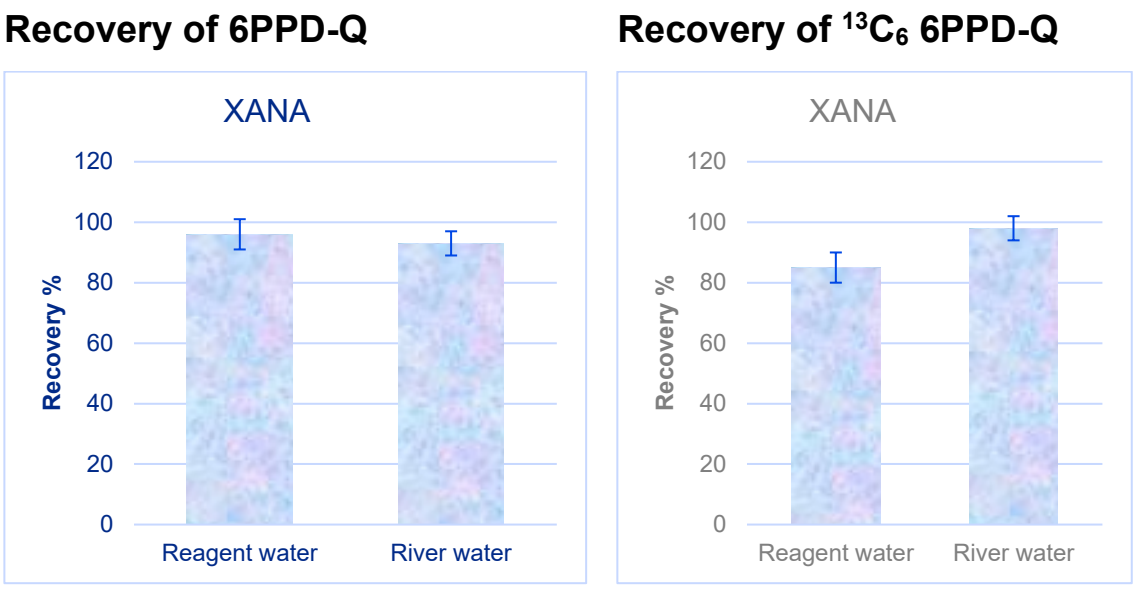


Figure 3. Recovery rates of 6PPD-Q (IS corrected) and ^{13}C6 6PPD-Q (net performance) from reagent water and river water (spiked concentration=10 ng in 250 mL) extracted with EluCLEAN® 6PPD-Q SPE cartridge using FREESTYLE XANA TableTop automated SPE XANA (n=3).

- ✓ Excellent recovery of 6PPD-Q
- ✓ EluCLEAN SPE (with glass fibre filter) was efficient for XANA (hence no clogging during the processing of samples, no pre-filtering of samples required)

3.3 Background contamination vs Carryover of the system

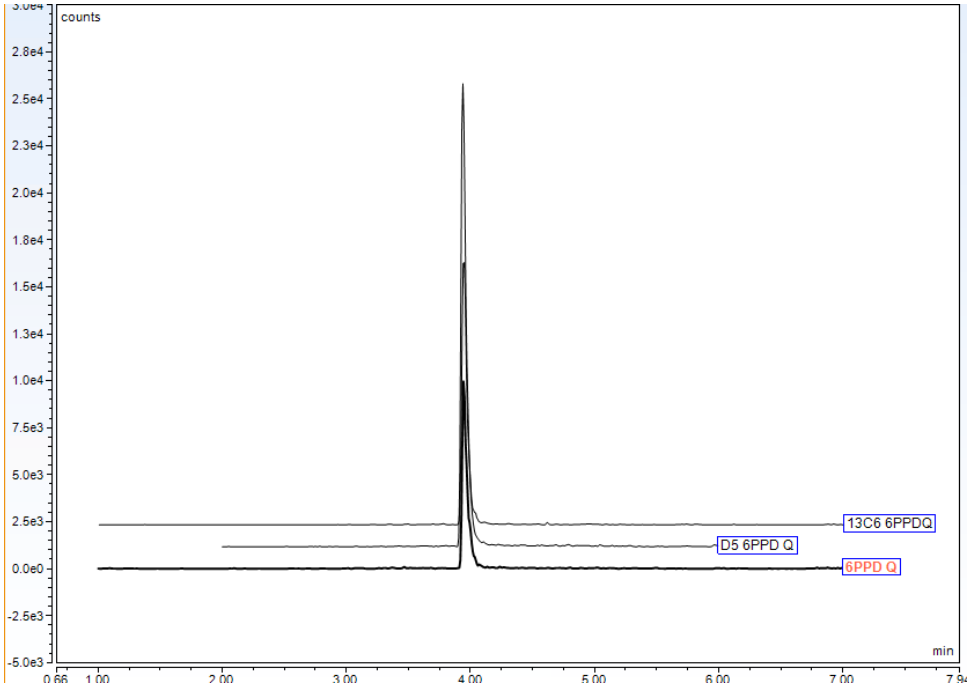


Figure 7. Chromatogram of 6PPD-Q spiked with 10 ng

Table 5. No peaks were detected in system for both carryover and background test

	6PPD-Q	$^{13}C_6$ 6PPD-Q
FREESTYLE XANA Tabletop	n.d.	n.d.

n.d. – not detected

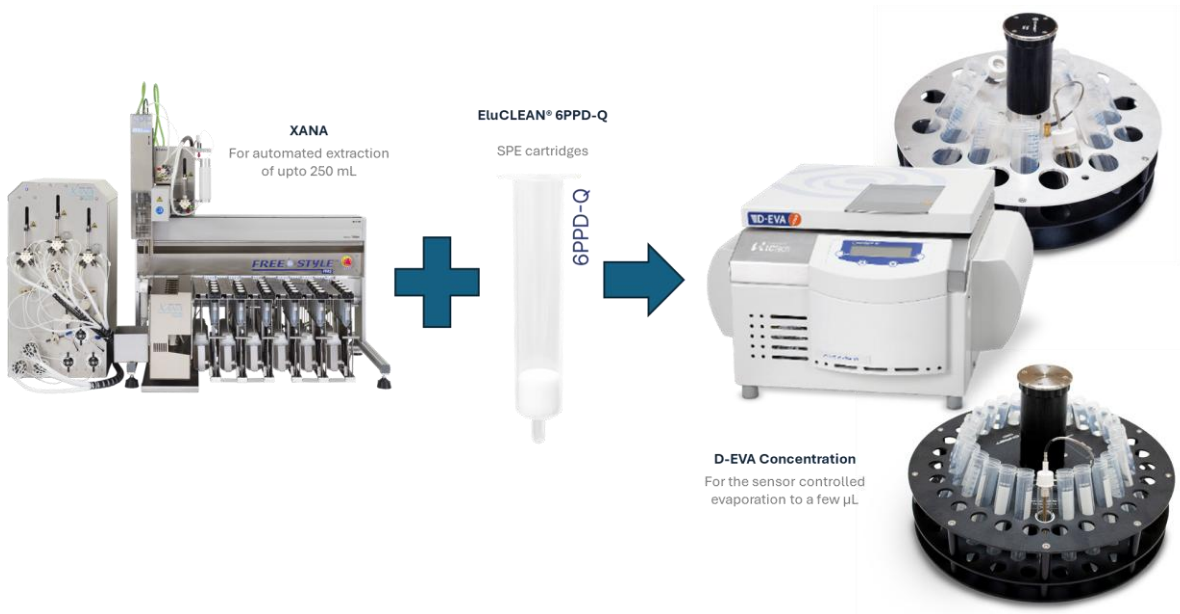
- ✓ No background contamination was detected from FREESTYLE XANA Tabletop system for 6PPD-Q
- ✓ No carryover of 6PPD-Q after spiked samples due to efficient intermediate cleaning
- ✓ Intermediate cleaning program is customizable to fit the need, depending upon types of matrices

4. Conclusion

EluCLEAN® 6PPD-Q cartridges are suitable for US EPA 1634 (draft) method according to their performance. Recoveries of samples are very well in between the acceptable criteria of 70 - 130 %. The results show that a reliable and robust 6PPD-Q analysis.

EluCLEAN® 6PPD-Q cartridges and FREESTYLE XANA TableTop have no detectable 6PPD-Q background contamination.

EluCLEAN® 6PPDQ cartridges combined with FREESTYLE XANA TableTop is therefore ideally suited to be used for the enrichment and clean-up of 6PPD-Q from aqueous matrices. This offers a time and cost saving alternative for laboratories.



5. References

[1] https://www.epa.gov/chemical-research/6ppd-quinone

[2] Draft Method 1634: Determination of 6PPD-Quinone in Aqueous Matrices Using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS); EPA Document No. EPA 821-D-24-001, December 2023

Any Questions?
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