Quantitative polarity switching LC-MS/MS method for pesticides and PPCPs in environmental water samples

Optimizing water analyses with SCIEX Triple Quad™ 5500+ System – QTRAP® Ready

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Pharmaceuticals and Personal Care Products (PPCPs) represent an ongoing relevant contaminant class in the environment and water samples which have been impacted by human activity. The ability to reliably and sensitively detect and quantify these diverse anthropogenic species in water and other systems is critical in the ongoing evolution of regulatory standards and frameworks around the world.

For years, triple quadrupole LC-MS/MS has been held as the highest standard for data quality and method performance in the quantitative analysis of PPCPs. Aspects of this technological approach make it particularly fit to purpose for this application. Specifically, the sensitivity to detect trace levels in dilute aquatic systems; the selectivity to confidently measure an analyte in the presence of complex environmental matrices; the linear dynamic response to encompass a range of potential environmental concentrations; robustness to handle throughput of important samples without degradation of data quality; and the accuracy and reproducibility in quantitation to underscore important scientific conclusions being drawn.

The SCIEX Triple Quad 5500+ System – QTRAP Ready is presented as a quantitative platform with exceptional performance in speed, polarity switching, linear dynamic range, and ease of use data handling with SCIEX OS-Q Software.

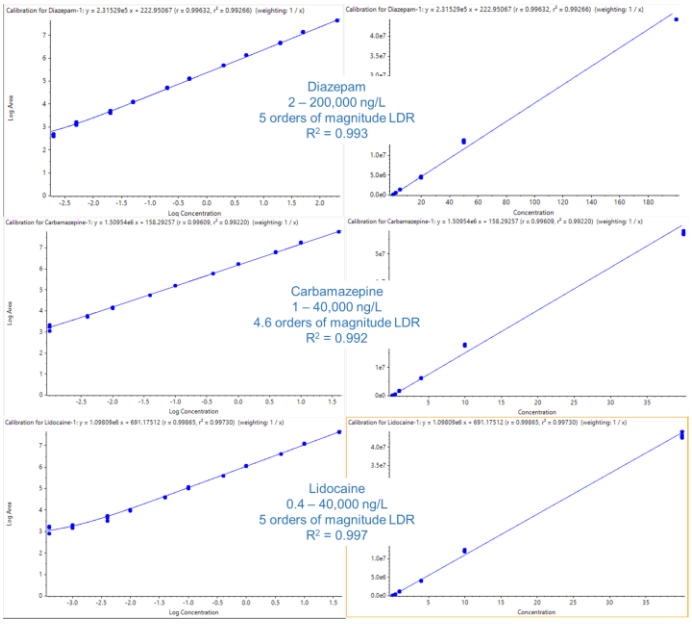


Figure 1. Linear dynamic range (LDR) demonstrated for a chemically diverse panel of analytes. Increased LDR in a quantitative workflow for residues in the environment allows the acquisition method to capture a greater potential of occurrence data in fewer injections (reduced need for re-runs following extra dilution, e.g.). Three example analytes are shown performing with excellent linear response from the very low concentration range (ng/L or sub- ng/L) up to the high end of the curve (ng/mL range), with potential for even higher concentrations to still behave linearly. Calibration curves in the left side panels are shown in log-log scale to visually see the full range of calibration point behavior. The linear space calibration curves typically utilized are shown in the right side panels.

Key advantages - SCIEX Triple Quad 5500+ System – QTRAP Ready for PPCPs Analysis

- Polarity switching performance of the SCIEX Triple Quad 5500+ – QTRAP Ready system is shown in the ability to combine large numbers of analytes (~300 MRM transitions) into a single method
- Wide Linear Dynamic Range (LDR) in the response for PPCPs in water is achieved, and allows for the advantage of screening over a wide potential of relevant environmental residue concentrations
- Under 10-minute chromatographic run time achievable with combined Scheduled MRM™ Algorithm and polarity switching during acquisition

Experimental Methods

Samples: Water samples were collected from a range of environmental and indoor sources to assess method performance in relevant matrices and report both occurrence and recovery data.

Data acquisition: The SCIEX Triple Quad 5500+ System – QTRAP Ready coupled with the ExionLC™ AD System was employed for sample separation, injection and MRM analysis. The Turbo V™ Ionization Source was operated in electrospray ionization (ESI) mode in both positive and negative polarities. A Phenomenex C18 Luna Omega Polar column demonstrated the level of separation and chromatographic resolution required; chromatographic and ion source conditions are shown in Table 1, and chromatographic gradient is shown in Table 2.

This method has 298 time scheduled MRM transitions with positive and negative polarity switching, as such, the 5 msec polarity switching time is critical to attaining comprehensive coverage and data quality in both positive and negative modes. Mixed stock standards containing 131 pharmaceutical and personal care product analytes were utilized for method optimization as well as building external calibration curves.

Data processing: The SCIEX OS-Q integrated software platform is used for data processing. The Analytics module is the primary interface for quantitative analysis and all calibration curves, LOQs, integration parameters, concentration calculations, accuracy calculations, and statistically analyses are performed within this module.

Table 1. Summary of LC-MS conditions.

Chromatography conditions			
LC Column	*Phenomenex C_{18} Luna Omega Polar 1.7 µm, 2.1 x 150 mm*		
Mobile Phase A	*Water + 0.1 % formic acid + 5 mM ammonium formate*		
Mobile Phase B	*Methanol*		
Flow Rate	*0.5 mL/min*		
Column Temperature	*40°C*		
Injection Volume	*100 µL*		
Mass spectrometry conditions			
CUR	*25 psi*	*CAD*	*10 psi*
IS Voltage	*2500V / -2500V*	*TEM*	*650°C*
GAS 1	*50 psi*	*GAS 2*	*70 psi*

Table 2. Chromatographic gradient.

Total time (min)	Flow rate (µL/min)	A%	B%
0.50	*500*	*100*	*0*
0.51	*500*	*75*	*25*
2.50	*500*	*40*	*60*
7.00	*500*	*0*	*100*
8.50	*500*	*0*	*100*
8.51	*500*	*100*	*0*
10.00	*500*	*100*	*0*

Method performance

1. Linearity, accuracy, precision

Maximizing linear dynamic range in environmental analyses is advantageous in the ability to screen over a wide potential range of relevant environmental residue concentrations, while minimizing sample preparation steps (such as dilution, concentration, or building multiple external calibration curves); minimizing data processing steps; and potentially mitigating the need for re-injecting samples which may otherwise fall outside of a lower range calibration curve. Many of the PPCPs in the target panel were quantifiable on a linear curve from the parts per trillion or sub- parts per trillion (ppt or ng/L) range up to the parts per billion (ppb or ug/L) range. The potential for drastic variation in detected occurrence levels of this diverse suite of chemicals in complex environmental water samples necessitates this capability for high range of linear response. Figure 1 shows calibration curves with concentration ranges, orders of magnitude linear range, and r^2 values from a few example compounds.

2. Sensitivity

Sensitivity of the SCIEX Triple Quad 5500+ system – QTRAP Ready was assessed by determining method lower limit of quantitation (LLOQ) values for the target panel of PPCPs. LOQ values were determined as the lowest concentration calibration standard which fit the standard performance requirements of: signal-to-noise ratio of at least 10; calculated concentration accuracy within 30% of 100%; and which fall on a linear calibration curve with an r^2 value of at least 0.995. Figure 2 displays observed peak intensity from very low level (0.1 ng/L) up through increasingly higher concentration standards for the example contaminant, caffeine, while Table 3 highlights the LLOQ values for a subset of compounds.

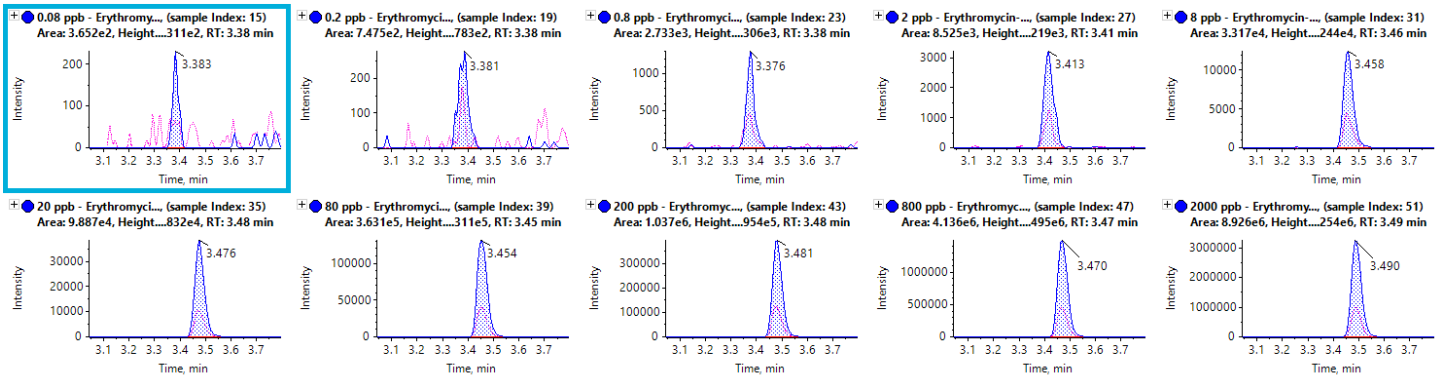


Figure 2. Sensitivity and dynamic range. Using Erythromycin as an example, the sensitivity and dynamic range of the LC-MS/MS method is illustrated. From top left to right, peaks for caffeine are shown from concentration of 80 ng/mL (0.08 ppb) up through 2,000,000 ng/mL (2,000 ppb). Even at the high concentration levels, it is observed that peak shape and quality are preserved, and the response (r^2=0.99447 for erythromycin) remains linear.

3. Polarity switching and Scheduled MRM Algorithm

Polarity switching is an enormously advantageous approach for the quantitative analysis of a large panel of chemically diverse contaminants such as PPCPs. The ability to ionize a greater panel of diverse chemical compounds proves time and again to be highly relevant to environmental analyses and the chemical contaminants encountered.

The speed at which the mass spectrometer is able to handle the polarity switching can have a direct impact on instrument cycle time. It is known that the integrity and quality of any quantitative results will rely on the consistent and accurate integration of the chromatographic peaks used to generate concentration data. To this end, it is critical that an adequate number of data points across the peak be collected to ensure reliable integration, quantitation, and confirmation of the peak. Figure 3 shows the data points collected across a few example chromatographic peaks for the PPCPs analysis, while Figure 4 shows a full chromatographic profile for both polarity modes.

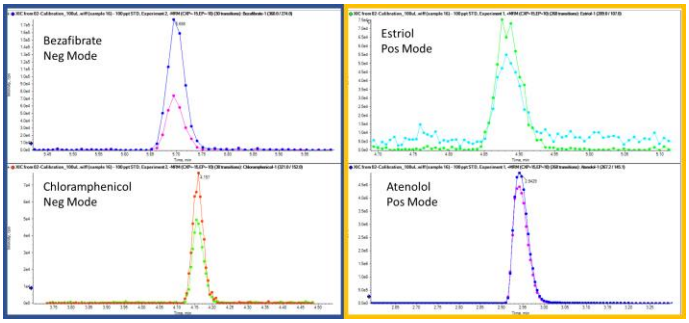


Figure 3. Fast polarity switching for good data sampling. Bezafibrate, Chloramphenicol, Estriol, and Atenolol show sufficient data point coverage for integration quality and confidence in quantitative results.

Crucial to ensuring reliable data point collection and peak shape is the method's cycle time. The speed of polarity switching on the SCIEX Triple Quad 5500+ system – QTRAP Ready plus the *Scheduled* MRM algorithm acquisition makes the reduction of the method cycle time while preserving data quality possible. This also allows reduction of the total method time per injection, by making shorter chromatographic run times with more peak co-elution possible without loss of quantitative capability. Achieving optimized peak shape via appropriate data point collection has the additional effect of providing maximum possible precision and reproducibility in the reported data.

Table 3. Limit of quantitation (LOQ) values and %CVs for a small subset of 15 example PPCPs.

Name	LOQ (ng/L)	%CV at LOQ	%CV at 10x LOQ	Polarity
Atenolol	2	6%	1%	Pos
Carbadox	20	24%	17%	Pos
Carbamazepine	1	7%	2%	Pos
Chloramphenicol	2	13%	6%	Neg
Erythromycin	8	9%	9%	Pos
Glipizide	0.8	11%	2%	Neg
Hydrocortisone	4	16%	2%	Pos
Lidocaine	0.4	23%	3%	Pos
Lincomycin	0.4	23%	7%	Pos
Norethindrone	20	14%	7%	Pos
Progesterone	0.4	9%	5%	Pos
Sulfadiazine	1	17%	9%	Pos
Sulfamethoxazole	10	13%	8%	Pos
Trimethoprim	2	15%	12%	Pos
Warfarin	1	13%	13%	Neg

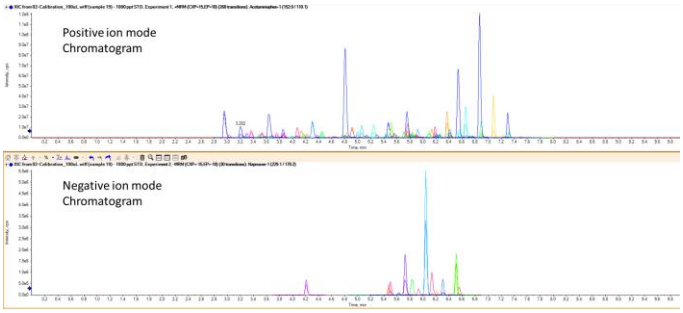


Figure 4. Overview of chromatography. Using *Scheduled* MRM Algorithm in Analyst 1.7 Software, 298 MRM transitions were used to detect all analytes within a 10-minute window in a single method.

4. Maximizing efficiency in data processing

The SCIEX OS-Q software for processing quantitative data was utilized, including the Automatic Outlier Removal feature, in order to efficiently reduce the amount of time managing the calibration curves and linear data for >200 MRM transitions in the analyte panel. Figure 5 (top) shows a screenshot from the software outlining the parameters which are able to be defined such that the calibration curves are defined by only those standard points which fit the appropriate criteria for accuracy, variability, and linearity.

As the Figure 5 shows, the user can define tolerance levels for %CV, accuracy, and r-value. The example calibration curve for the pesticide Thiacloprid shows the automatically excluded points at the low end of the curve (calibration curve is shown in log-log scale strictly to be able to visualize the low end easily). Because these outlier removal criteria can be applied to the entire panel at the time of data processing and saved as part of the processing method, this ensures consistent, objective treatment and handling of the standard points; a task which might otherwise take a trained analyst a significant amount of time and be inherently subjected to human bias.

Additionally, the user can define tolerance limits which define a “positive hit” in an Unknown sample, such as a tolerance around Ion Ratio or Retention Time delta, and these can also be handled rapidly in the software interface through advanced filtering and flagging options built into the results table. Again, these features address known bottlenecks to data processing while reducing human subjectivity in data handling.

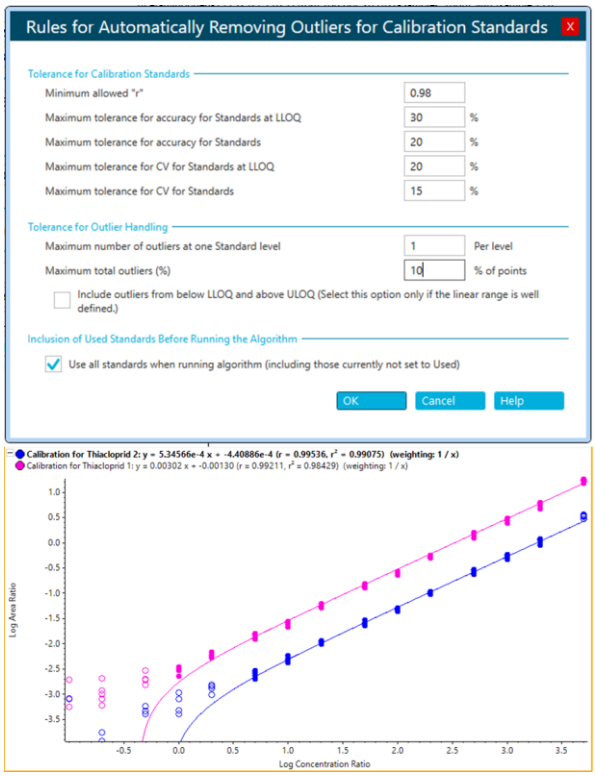
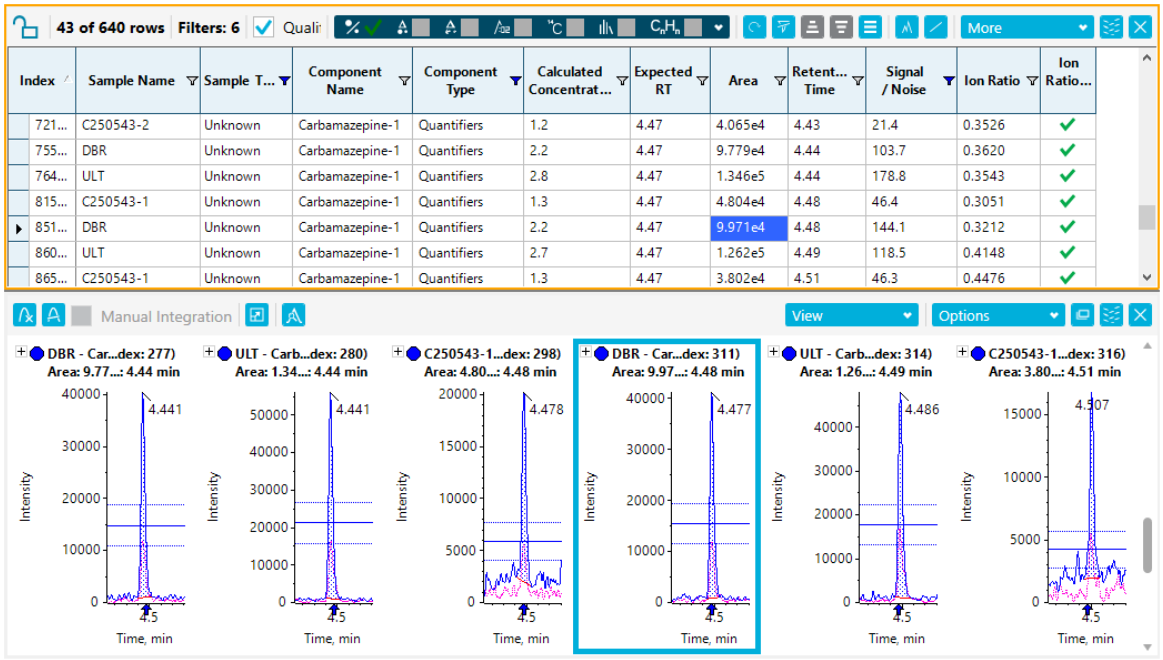


Figure 5. **Automatic outlier removal feature in SCIEX OS-Q Software**. Allows for the rapid, unbiased management of calibration data for hundreds of compounds and reduces time spent on manual manipulation. Open circles represent standard calibration curves automatically removed for not meeting defined performance criteria, allowing for greater reliability in the quantitative results. For the example compound shown thiacloprid, it can be seen that the linear response at the low end of the curve begins to fail; the response does not become significantly different from a blank response. The Automatic Outlier Removal tool was able to remove the low end points from the curve which are poor performing, to maintain a calibration curve which meets the desired criteria required for quantitative performance.

Experimental results

Water sample analysis for PPCPs

Unknown samples were collected from several surface water sampling sites, as well as a few indoor tap water sources, a distilled water, and one bottled water. These were analyzed using the data acquisition method described and the results demonstrate the occurrence of several common PPCPs at varying levels. Analytes detected in the waters were quantitated against the external calibration curves prepared in solvent, using deuterated internal standards for a subset of the panel, and confirmed using ion ratios for two MRM transitions for each compound. Up to 14 replicates were injected of each unknown sample in order to assess the method reproducibility and instrument ruggedness with a more complex matrix (surface water).



Index	Sample Name	Sample T...	Component Name	Component Type	Calculated Concentrat...	Expected RT	Area	Retent... Time	Signal / Noise	Ion Ratio	Ion Ratio...
721...	C250543-2	Unknown	Carbamazepine-1	Quantifiers	1.2	4.47	4.065e4	4.43	21.4	0.3526	✓
755...	DBR	Unknown	Carbamazepine-1	Quantifiers	2.2	4.47	9.779e4	4.44	103.7	0.3620	✓
764...	ULT	Unknown	Carbamazepine-1	Quantifiers	2.8	4.47	1.346e5	4.44	178.8	0.3543	✓
815...	C250543-1	Unknown	Carbamazepine-1	Quantifiers	1.3	4.47	4.804e4	4.48	46.4	0.3051	✓
851...	DBR	Unknown	Carbamazepine-1	Quantifiers	2.2	4.47	9.971e4	4.48	144.1	0.3212	✓
860...	ULT	Unknown	Carbamazepine-1	Quantifiers	2.7	4.47	1.262e5	4.49	118.5	0.4148	✓
865...	C250543-1	Unknown	Carbamazepine-1	Quantifiers	1.3	4.47	3.802e4	4.51	46.3	0.4476	✓

Figure 6. Detection of the pharmaceutical compound carbamazepine in several surface water samples. Agreement in ion ratios between unknowns and standards (green check marks in the results table designate ion ratio tolerance within 20%) provide qualitative confirmation of Carbamazepine. The chromatographic peaks for the two MRM transitions for the compound are also shown overlaid with the 20% tolerance lines for visualization of IR agreement. The blue peak is the quantifier ion trace, and the pink peak is the qualifier ion trace. Where the qualifier peak falls between the ion ratio tolerance lines, the ion ratio confirmation can be qualitatively visualized.

Sample preparation of these unknowns is as described in above sections and involved a direct injection without the inclusion of clean up steps. With such directly analyzed samples as these, it is especially important to characterize how the data quality is conserved over many injections. Findings from this experiment show that PPCPs are detectable and prevalent in our environmentally sampled waters.

Some PPCPs have very high frequency of occurrence; for example, Carbamazepine and Caffeine were detected in all or almost all surface water samples; Acetaminophen, Bisphenol A and DEET were also among the most frequently detected species. One surface water sample contained 14 detected species from the panel, including pesticides (imidacloprid, carbofuran, azoxystrobin); pharmaceuticals (acetaminophen, sulfamethoxazole, carbamazepine); and other compound classes (DEET, caffeine) with concentrations up to the hundreds of ng/L. One of the tap water sources had two hits for DEET and Bisphenol A, and the other had three for Acetaminophen, DEET, and Caffeine. In Figure 7, Carbamazepine, Sulfamethoxazole, Lidocaine and Caffeine detection and quantitation results in the collected water samples are shown are example compounds detected across multiple samples and sample types.

Figure 6 shows example compound Carbamazepine confirmed in several samples; the SCIEX OS-Q software features a green check mark visual display highlighting samples which meet or pass defined criteria; in this instance, which pass the 20% tolerance limit for ion ratio which was defined as qualitative identity confirmation. The software also allows for filtering on this column such that the results table displays only samples which pass (or fail) these criteria.

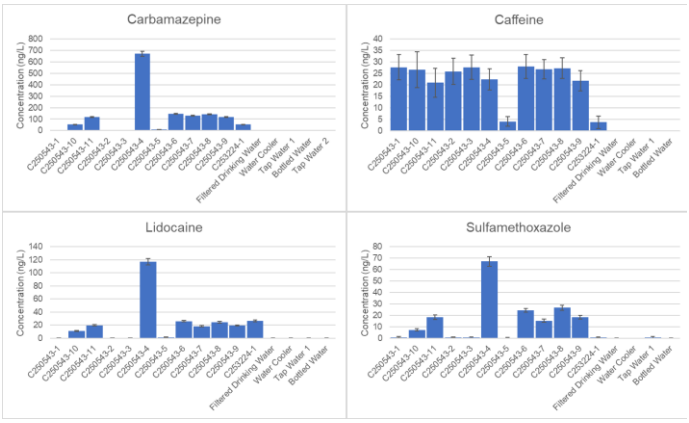


Figure 7. Quantitation across the various water samples. Four example analytes are shown which were detected in the water samples, at some very high measured levels (>100 ng/L). Standard deviation error bars also shown demonstrating excellent experimental reproducibility between replicates.

Summary

This report describes the use of the SCIEX Triple Quad 5500+ LC-MS/MS System – QTRAP Ready to establish an optimized method for the quantitative analysis of anthropogenic contaminants in environmental water samples. The method combines both the positive and negative polarity electrospray ionization modes into one comprehensive screening method, that scans across a vast range of analytes without any sacrifice or compromise to data quality and integrity. Exceptional sensitivity and linear dynamic range provide the basis for a robust quantitative methodology for this large panel of chemically diverse species.

Efficiency in data processing with SCIEX OS-Q Software is increased with the ability to employ streamlining features such as: Automatic Outlier Removal, confirmatory traffic light system for rapid scoring and review of data, and AutoPeak intelligent peak integration.

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