Application Note

Environmental

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# Simultaneous Targeted Quantitation and Suspect Screening of Environmental Contaminants in Sewage Sludge

## The Agilent 6546 LC/Q-TOF


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## Abstract

The list of environmental contaminants is growing and regulations are becoming increasingly stringent. Various environmental agencies require fast, accurate, and sensitive analytical tools. Environmental laboratories encounter samples that require a range of analyses, from trace analysis of highly toxic contaminants to protect ecosystems, to screening large libraries of emerging contaminants often manufactured and persisted beyond their intended period of use. Techniques and procedures to acquire data, and quantitate and report results have been established, typically using targeted LC-TQ technology. However, monitoring growing lists of new environmental toxicants continues to challenge environmental scientists.

This Application Note evaluates a combined quantitation and screening workflow using the Agilent 6546 LC/Q-TOF MS system. The 6546 has simultaneous extended dynamic range and high mass resolution capability, with uncompromised acquisition rate. In combination with highly curated MS/MS spectra and retention times in Agilent's Personal Compound Database and Libraries (PCDL), and updated MassHunter Quantitative Analysis software, a workflow describing seamless targeted quantitation with simultaneous suspect screening capability is presented. The software enhancements and hardware capabilities enable rapid and simplified quantitation of regulated compounds, while screening for thousands of emerging contaminants, from the same injection.

## Introduction

Sewage sludge is a concentrated, complex mixture of compounds that, in most instances, is treated for land application. Regulated monitoring of persistent toxic chemicals originating from consumer products is limited for land applied sewage sludge. However, toxicity values of many of these compounds remain unknown, which suggests a need to investigate and mitigate risks of ecosystem effects downstream of discharges. The challenge is that the list of toxicants and their transformation products are consistently increasing as more and more products are made available. Broad screening for these compounds can provide a more holistic picture of highly persistent chemicals originating in consumer products and their effects to downstream when they are unable to be removed during robust waste treatment techniques.

Endocrine disruptors are chemicals that interfere with biological systems controlled by hormones, and as such, are monitored in sewage treatment plant processes. An analytical method[1] describing the analysis of endocrine active organic environmental contaminants in sewage sludge was updated to make the best use of the 6546 quadrupole time-of-flight (Q-TOF) system. The 6546 LC/Q-TOF has simultaneous extended dynamic range and high mass resolution capability, without compromise to acquisition rate. Coupled with the Agilent 1290 Infinity II liquid chromatography (LC) system to apply fast LC gradients to increase chromatographic resolution also ensures that run times are amenable for high-throughput operations.

The combination of these capabilities enables rapid quantitation of known toxicants while monitoring the presence of many other suspected toxicants, adding value to work already done. The data independent acquisition (DIA) capability also allows retrospective analysis for new toxicants, as they are discovered.

We assessed the 6546 LC/Q-TOF system for quantitative capability by spiking carefully selected compounds into sewage sludge matrix: compounds previously detected in sewage sludge with suspected endocrine active characteristics were compiled into a list of approximately 50 compounds. Of these compounds, 12 surrogates were selected for method validation that were representative of the larger list's physiochemical properties. As the spiked surrogates are chemically diverse enough to elute throughout the chromatogram, we correlated the retention times (RTs) of the surrogate compounds with published analytical methods measuring the same compounds. A model was then used to project the RTs of a broader range of toxicants from the same data file. Additionally, compounds with no RT correlation were also monitored.

In total, 4,856 compounds with highly curated MS/MS spectra where monitored in addition to the spiked surrogates. A simplified data analysis workflow extracts a compound's known precursor and fragment masses sourced from highly curated compound libraries from the high mass resolution data and then:

- Measures mass accuracy and coelution of extracted masses
- Compares known theoretical isotope patterns to what was accurately measured
- Compares known or projected RTs to what was measured

The putative identifications follow basic identification criteria, as recommended by SANTE guidelines[3], while the software focuses the reviewing process and reduces the potential of false positives. After verifying the workflow by finding and quantifying the spiked surrogates through traditional quantitation processes (similar to LC/TQ workflows), we continue to find suspected contaminants, retrospectively, by further expanding our highly curated compound databases with new toxicants.

## Experimental

Where possible, the sample preparation procedure of sewage sludge samples was deliberately as nonchemically selective as possible, allowing the detection of a broad range environmental contaminants. High-quality solvents and consumables were used to reduce the introduction of compounds that could lead to false results.

### Reagents and chemicals

Solvents were prepared with Agilent LC/MS grade acetonitrile (p/n G2453-85050), formic acid (Merck, 5330020050) and ammonium fluoride (Sigma-Aldrich, 338869). Reference mass solution was prepared by adding 100 µL of Agilent HP-0921 and 200 µL of purine (p/n G1969-85001) to 100 mL of 5 % water in acetonitrile. ESI-L calibrant solution was prepared as specified in the instrument manual. Standards were prepared in 20 % methanol in water at UC Davis.

## Standard and sample preparation

Table 1 lists the target compounds. To evaluate the dynamic range and sensitivity of the 6546 Q-TOF LC/MS system, standards were prepared in 20 % methanol in water at calibration levels of 1,000, 750, 500, 250, 100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 ppb. No internal standards were used to normalize the data. Samples were sourced from a water treatment facility in California, USA, and prepared as previously described[1], and spiked with the target compounds at 200 ppb pre and post sample preparation to assess recovery and sample matrix suppression.

## Instrumentation

An Agilent 1290 Infinity II LC system consisting of the modules described in Table 2 was coupled to the 6546 Q-TOF LC/MS system (G6546A).

**Table 1.** Target compounds.

| Name | CAS | Formula | Neutral Mass |
|---|---|---|---|
| AHTN/Tonalide (Fixolide) | 1506-02-1 | $C_{18}H_{26}O$ | 258.19837 |
| Carbamazepine | 298-46-4 | $C_{15}H_{12}N_2O$ | 236.09496 |
| DEET/Diethyltoluamide | 134-62-3 | $C_{12}H_{17}NO$ | 191.13101 |
| Diclofenac | 15307-86-5 | $C_{14}H_{11}Cl_2NO_2$ | 295.01668 |
| Dihydrojasmonic acid, Methyl Ester | 24851-98-7 | $C_{13}H_{22}O_3$ | 226.15690 |
| Efavirenz | 154635-17-3 | $C_{14}H_9ClF_3NO_2$ | 315.02739 |
| Flunixin | 38677-85-9 | $C_{14}H_{11}F_3N_2O_2$ | 296.07726 |
| Fluoxetine | 54910-89-3 | $C_{17}H_{18}F_3NO$ | 309.13405 |
| Fluvoxamine | 54739-18-3 | $C_{15}H_{21}F_3N_2O_2$ | 318.15551 |
| Lamotrigine | 84057-84-1 | $C_9H_7Cl_2N_5$ | 255.00785 |
| Mefenamic Acid | 61-68-7 | $C_{15}H_{15}NO_2$ | 241.11028 |
| Metoprolol | 37350-58-6 | $C_{15}H_{25}NO_3$ | 267.18344 |
| Miconazole | 22916-47-8 | $C_{18}H_{14}Cl_4N_2O$ | 413.98602 |
| Norgestrel | 797-63-7 | $C_{21}H_{28}O_2$ | 312.20893 |
| Sulfamethoxazole | 723-46-6 | $C_{10}H_{11}N_3O_3S$ | 253.05211 |
| Triclocarban | 101-20-2 | $C_{13}H_9Cl_3N_2O$ | 313.97805 |
| Trimethoprim | 738-70-5 | $C_{14}H_{18}N_4O_3$ | 290.13789 |
| Estrone (E1) | 53-16-7 | $C_{18}H_{22}O_2$ | 270.16198 |
| Ethinylestradiol (EE2) | 57-63-6 | $C_{20}H_{24}O_2$ | 296.17763 |
| 2-Phenylphenol (Orthophenylphenol) | 90-43-7 | $C_{12}H_{10}O$ | 170.07316 |
| Gemfibrozil | 25812-30-0 | $C_{15}H_{22}O_3$ | 250.15689 |
| Estriol | 50-27-1 | $C_{18}H_{24}O_3$ | 288.17254 |
| 4-*tert*-Octylphenol (4-(1,1,3,3-tetramethylbutyl)phenol) | 140-66-9 | $C_{14}H_{22}O$ | 206.16707 |

**Table 2.** Agilent 1290 Infinity II UHPLC conditions.

| Module | Positive Ionization Parameters | Negative Ionization Parameters |
|---|---|---|
| High Speed Pump (G7120A) | Solvent A1) 0.1% formic acid in water<br>Solvent B1) 0.1% formic acid in acetonitrile | Solvent A2) 1 mM ammonium fluoride in water<br>Solvent B2) acetonitrile |
| | Flow Rate: 0.4 mL/min<br>Max Pressure Limit: 1,300 bar (the operating pressure was less than 450 bar) | |
| | Gradient:<br>Time (min) %B<br>0.00 2.00<br>0.50 2.00<br>15.50 100.00<br>19.50 100.00<br>20.00 2.00<br>Stop time: 20.00 minutes<br>Post time: 1.00 minute | |
| Multisampler (G7167B) | Injection Volume: 1 µL<br>Multiwash: Seat back flush and needle wash with 5 seconds each of 100 % isopropanol, then 100 % acetonitrile, then 100 % water | |
| Multicolumn Thermostat (G7116B) | Column Temperature: 30 °C<br>Column: Agilent InfinityLab Poroshell EC-C18 2.1 × 100 mm, 1.9 µm (p/n 685775-924) | |

The parameters stated in Table 2 produced an LC gradient represented in Figure 1A. The pump pressure curves of 160 injections of sewage sludge, blanks, and calibrators are overlaid (Figure 1B). A representative total ion current (TIC) chromatogram from positive ionization mode (in red) and negative ionization mode (in black) is also shown (Figure 1C).

Table 3 describes conditions used to measure compounds in a DIA mode on the 6546 LC/Q-TOF system.

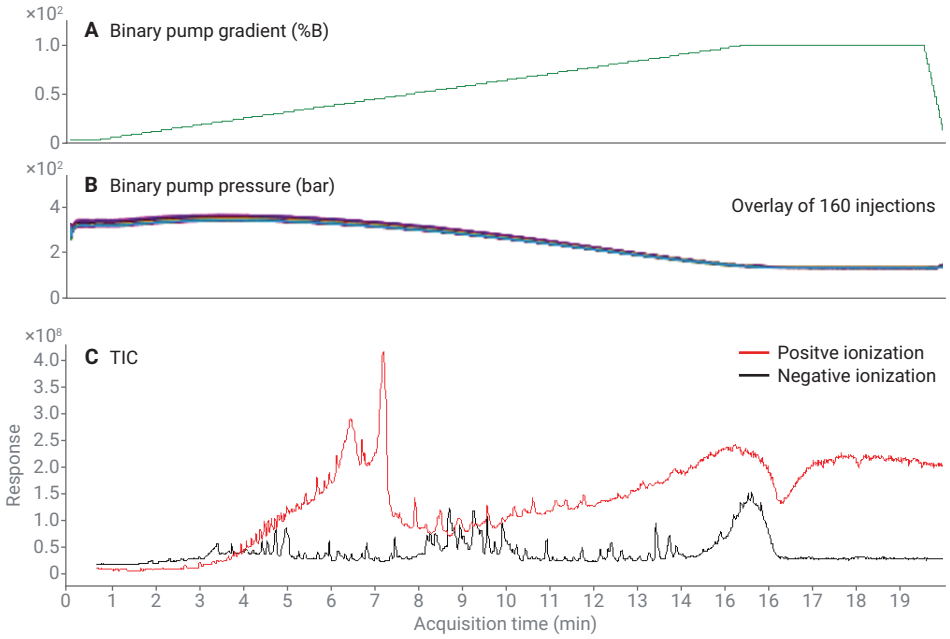


**Figure 1.** Chromatographic results from sewage sludge injected onto a 1290 Infinity II LC system.

**Table 3.** 6546 LC/Q-TOF LC/MS system (G6546A) conditions.

| Parameter | Positive Ionization Parameters | Negative Ionization Parameters |
|---|---|---|
| **Agilent Jet-Stream Ion Source** | | |
| Drying Gas Temperature | 225 | |
| Drying Gas Flow | 12 | |
| Nebulizer | 30 | |
| Sheath Gas Temperature | 350 | |
| Sheath Gas Flow | 350 | |
| Capillary Voltage | 3500 | |
| Nozzle Voltage | 500 | |
| Fragmentor Voltage | 110 | |
| **Tune Mode** | | |
| Ion Polarity | Positive | Negative |
| Mass Range | Low (1,700 *m/z*) | |
| Slicer Mode | High resolution | |
| **Acquisition Mode** | | |
| | 50 to 1,050 *m/z* | |
| Rate | 8 spectra/sec | |
| Collision Energy | 0, 10, 20, 40 V | |
| Reference Mass Correction | Enabled using bottle A | |
| Reference Masses | 121.050873 $(M+H)^+$ adduct of purine<br>922.009798 $(M+H)^+$ adduct of HP-0921 | 119.03632 $(M-H)^-$ adduct of purine<br>940.001473 $(M+F)^-$ adduct of HP-0921 |

Figure 2 presents the extracted ion chromatograms (EICs) of the target compounds.

### Data analysis workflow

The automated routine workflow acquires All Ions MS/MS data in positive and negative ionization modes using Agilent MassHunter Acquisition (version 10.0.111), which is automatically processed with Agilent SureMass technology[3] to allow rapid and more accurate quantitation of targets, and simultaneous detection of suspect compounds in MassHunter Quantitative Analysis (for TOF) (version 10.1 prototype).

MassHunter Quantitation Methods are set up by importing compounds from Agilent's highly curated Personal Compounds Database and Library (PCDL). The quality checking of data in the Agilent PCDL and the recommended process to add future emerging contaminates has been outlined[4]. Quantifier ions are set to the precursor ion, and at least two MS/MS fragment ions were set as qualifier ions for each compound.

Agilent's highly curated Environmental Water Screening PCDL has curated RTs from an analytical method described previously[5]. The Venn diagram in Figure 4 summarizes the contents.

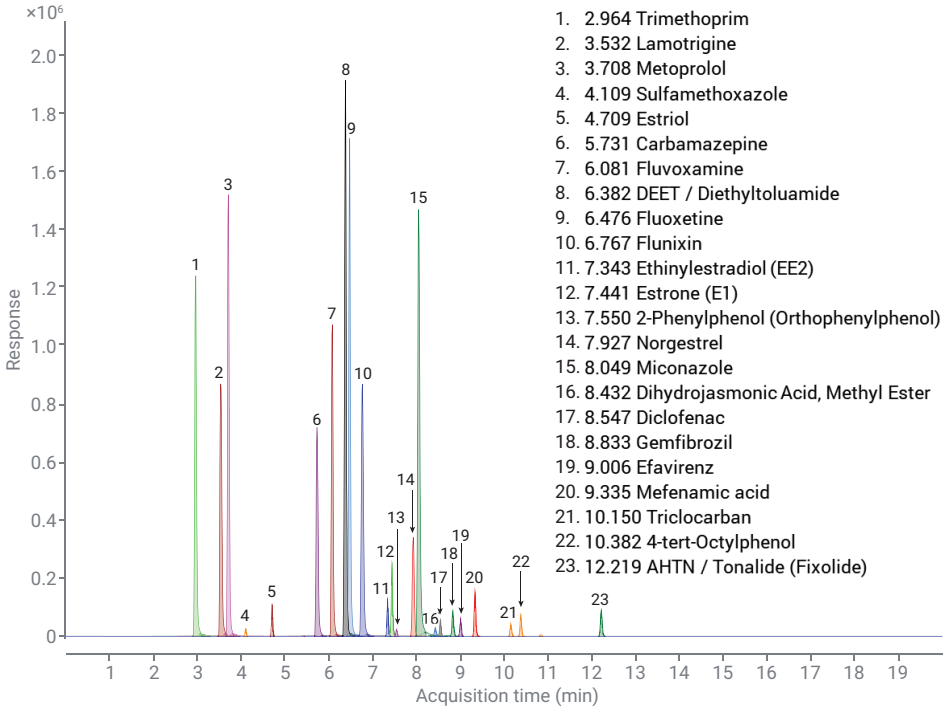


**Figure 2.** Extracted ion chromatograms for the target compounds defined in Table 1.

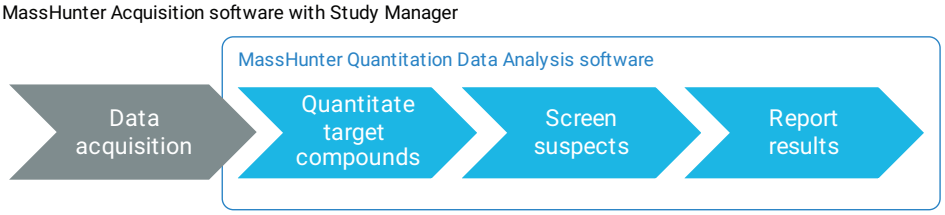


**Figure 3.** Target quantitation and suspect screening workflow.

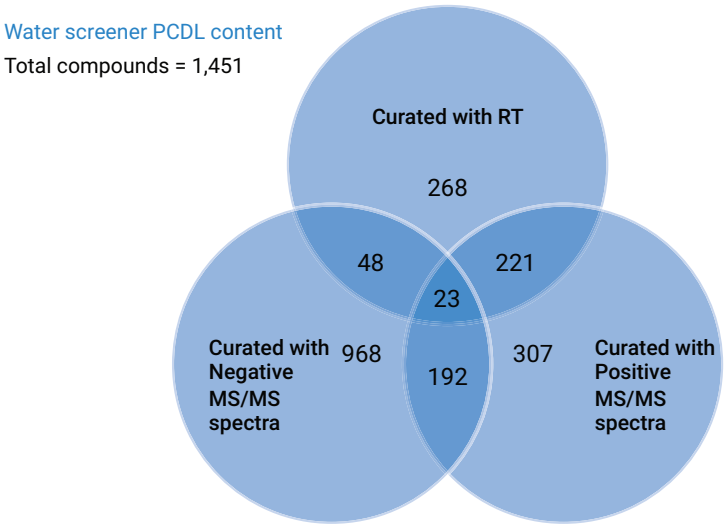


**Figure 4.** Content of the Water Screening Personal Compound Database and Library

As the LC method used in this application was different from that used to curate the RTs in the Environmental Water Screening PCDL, the common target compounds in both analyses were used to model RTs for suspect compounds in this analysis. Figure 5 shows nine compounds where the RT was known in both methods (black circles), a power curve force fitted to the origin, and suspected RT for compounds in the Environmental Water Screening PCDL when analyzed using the LC method described in this Application Note. The projected RTs (blue circles) are shown with error bars representing the RT window for which the suspect compounds were searched.

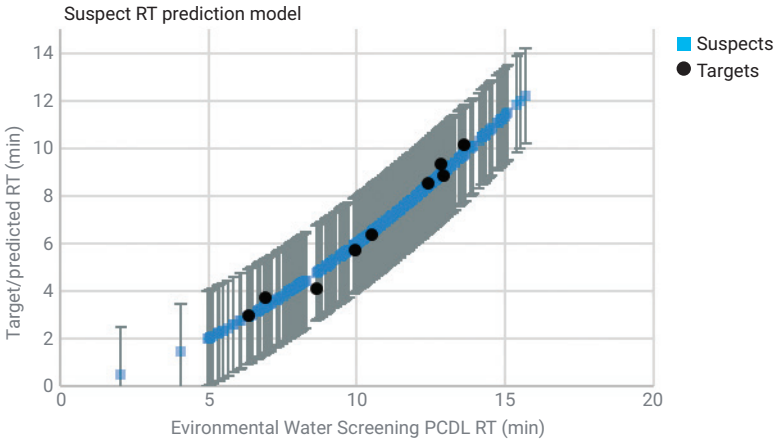


**Figure 5.** Correlation of surrogate retention times common between analytical methods.

## Results and discussion

### Target quantitation capability

By monitoring spiked surrogates in sewage sludge, we evaluated the quantitative capability of the analytical method applied to a 6546 LC/Q-TOF system. The linear dynamic range for compounds listed in Table 4 was assessed by linear regression of the calibration standards ranging from 0.1 to 1,000 ppb. Calibration levels where the signal-to-noise ratio (S/N) was below 3 were excluded, the lower limit of detection (LLOD) is reported in Table 4, where the lowest calibration level had a S/N ≥3. The limit of saturation (LOS) reported in Table 4 is the upper limit of the linear dynamic range; high concentration calibration points were removed until the coefficient of determination ($R^2$) was >0.99, and the quantitation accuracy of each calibrator was <±20 % when a linear curve was fitted with 1/x weighting (where x is the concentration).

**Table 4.** Quantitation capability of the 6546 LC/Q-TOF.

| Name | Quantifier Ion | $R^2$ | LLOD (ppb) | LOS (ppb) |
|---|---|---|---|---|
| AHTN/Tonalide (Fixolide) | $(M+H)^+$ | >0.99 | 5.0 | 1,000 |
| Carbamazepine | $(M+H)^+$ | >0.99 | 1.0 | 250 |
| DEET/Diethyltoluamide | $(M+H)^+$ | >0.99 | 0.50 | 500 |
| Diclofenac | $(M+H)^+$ | >0.99 | 25 | >1,000 |
| Dihydrojasmonic acid, methyl ester | $(M+H)^+$ | >0.99 | 50 | >1,000 |
| Efavirenz | $(M+H)^+$ | >0.99 | 5.0 | >1,000 |
| Flunixin | $(M+H)^+$ | >0.99 | 0.50 | 2,500 |
| Fluoxetine | $(M+H)^+$ | >0.99 | 0.50 | >1,000 |
| Fluvoxamine | $(M+H)^+$ | >0.99 | 1.0 | >1,000 |
| Lamotrigine | $(M+H)^+$ | >0.99 | 0.10 | 100 |
| Mefenamic acid | $(M+H)^+$ | >0.99 | 5.0 | >1,000 |
| Metoprolol | $(M+H)^+$ | >0.99 | 0.50 | >1,000 |
| Miconazole | $(M+H)^+$ | >0.99 | 0.50 | 500 |
| Norgestrel | $(M+H)^+$ | >0.99 | 2.5 | 750 |
| Sulfamethoxazole | $(M+H)^+$ | >0.99 | 50 | >1,000 |
| Triclocarban | $(M+H)^+$ | >0.99 | 50 | >1,000 |
| Trimethoprim | $(M+H)^+$ | >0.99 | 0.10 | 100 |
| Estrone (E1) | $(M-H)^-$ | >0.99 | 2.5 | >1,000 |
| Ethinylestradiol (EE2) | $(M-H)^-$ | >0.99 | 5.0 | >1,000 |
| 2-Phenylphenol (Orthophenylphenol) | $(M-H)^-$ | >0.99 | 25 | >1,000 |
| Gemfibrozil | $(M-H)^-$ | >0.99 | 5.0 | >1,000 |
| Estriol | $(M-H)^-$ | >0.99 | 5.0 | >1,000 |
| 4-*tert*-Octylphenol (4-(1,1,3,3-Tetramethylbutyl)phenol) | $(M-H)^-$ | >0.99 | 5.0 | >1,000 |

In most cases, the LOS was not observed when the linear curve fitted included the 1,000 ppb calibration sample. However, when compounds were fitted with a nonlinear power curve regression, weighted 1/x (where x is the concentration), in all cases except Lamotrigine (a compound that seems to ionize efficiently), the higher calibrations points could be included to accurately quantify compounds up to 1,000 ppb when 0.5 µL was injected. Figure 6 shows the calibration curves used for the target compounds in this analysis.

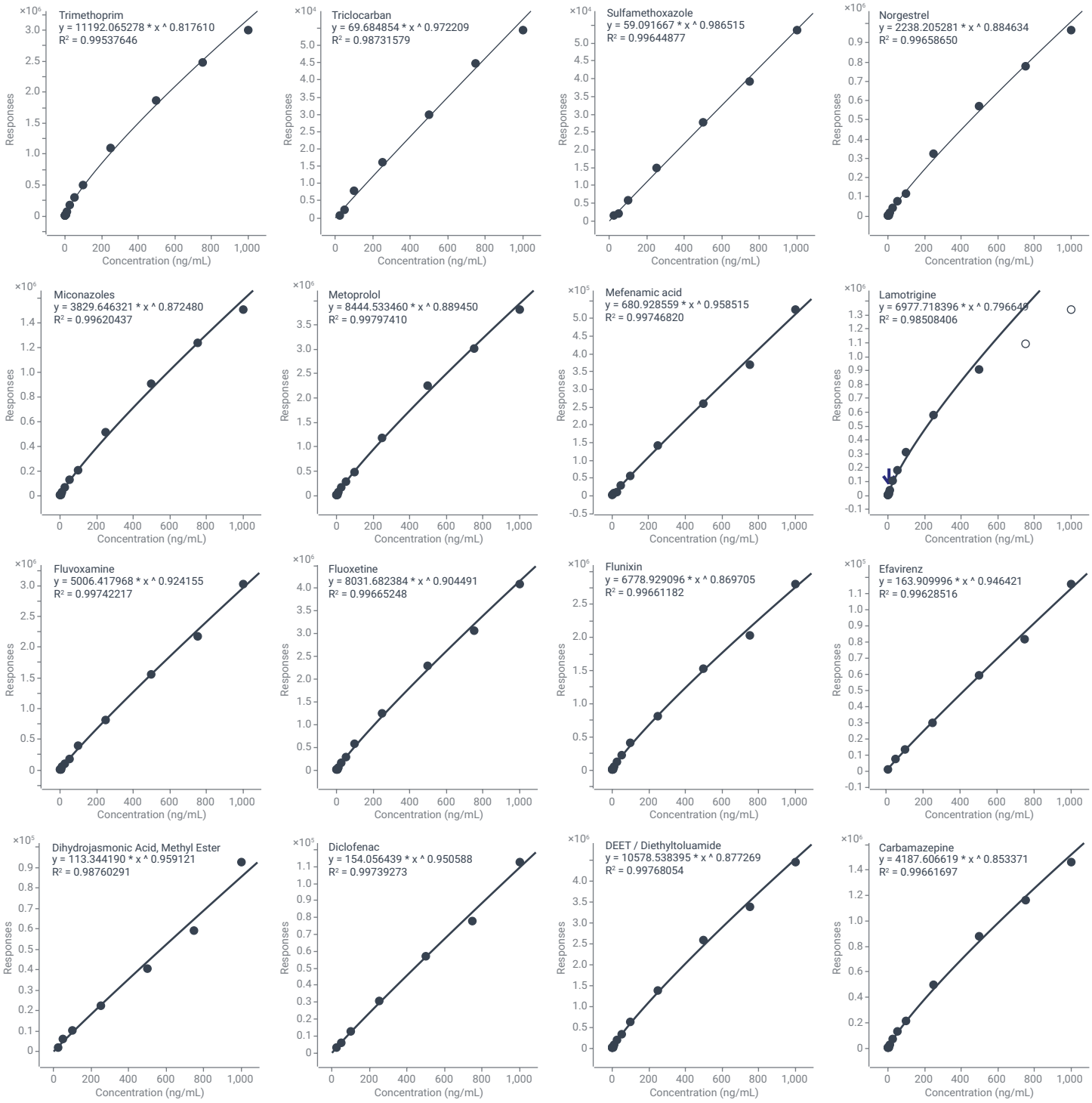


**Figure 6.** Calibration curves for target compounds.

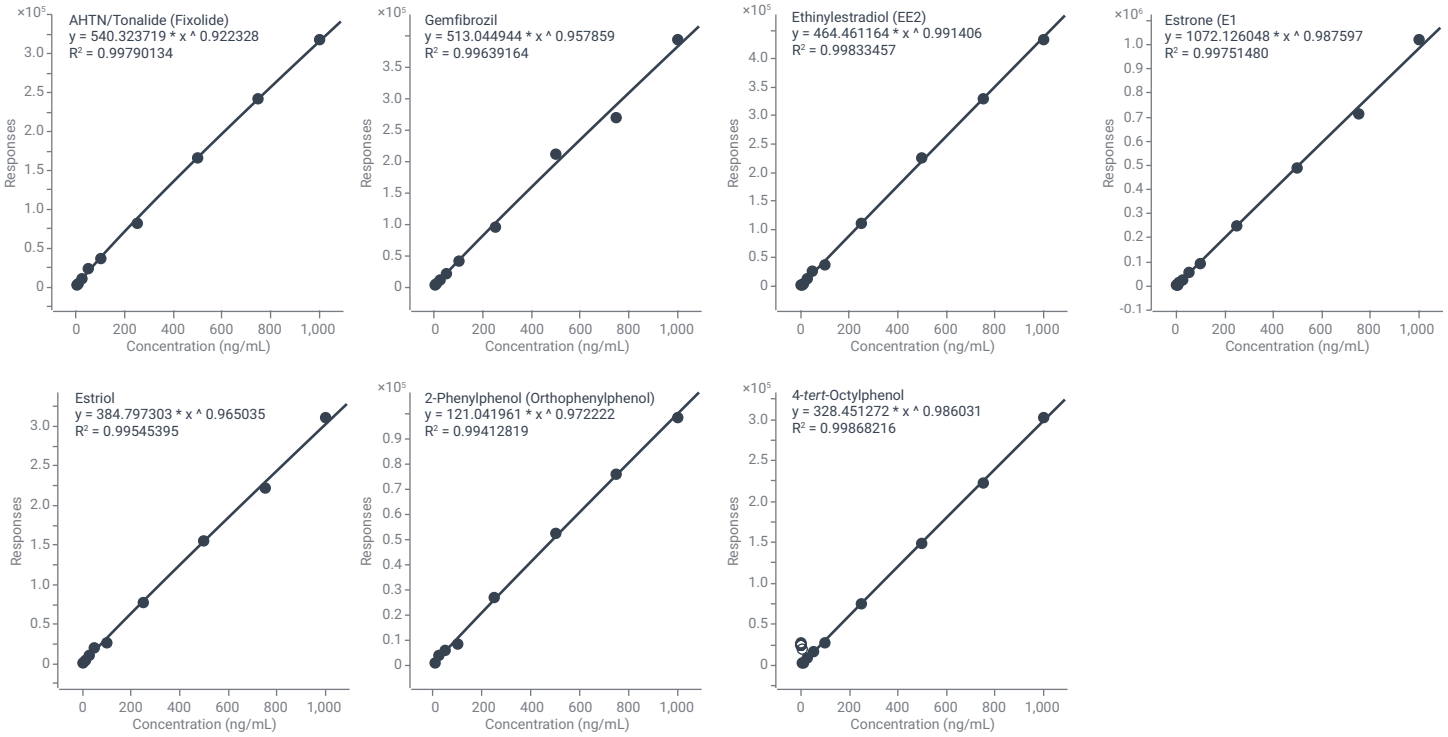


**Figure 6.** Calibration curves for target compounds (continued).

In the case of Lamotrigine, the limit of detection (LLOD) was not observed, as a peak with S/N >3 was observed when the lowest calibrator (0.1 ppb) was injected. A 1.0 µL injection volume was used in the analytical method, so a concentration of 100 ppb equates to 100 pg injected on column.

Target compound results are shown in the same way as LC-TQ data (see Figure 7). The quantifier integration and expected RT (Figure 7A) and the coelution of qualifying ions (Figure 7B, scaled according to expected ratio determined from calibrators), are common between LC\TQ and LC\Q-TOF acquisition methods. The extra decimal places for an accurate mass measurement and ability to compare expected (Figure 7C, red boxes) versus measured isotope pattern (Figure 7C, black spectra), given a known chemical formula and natural isotope abundances, provides an extra level of confidence in a compound identification.

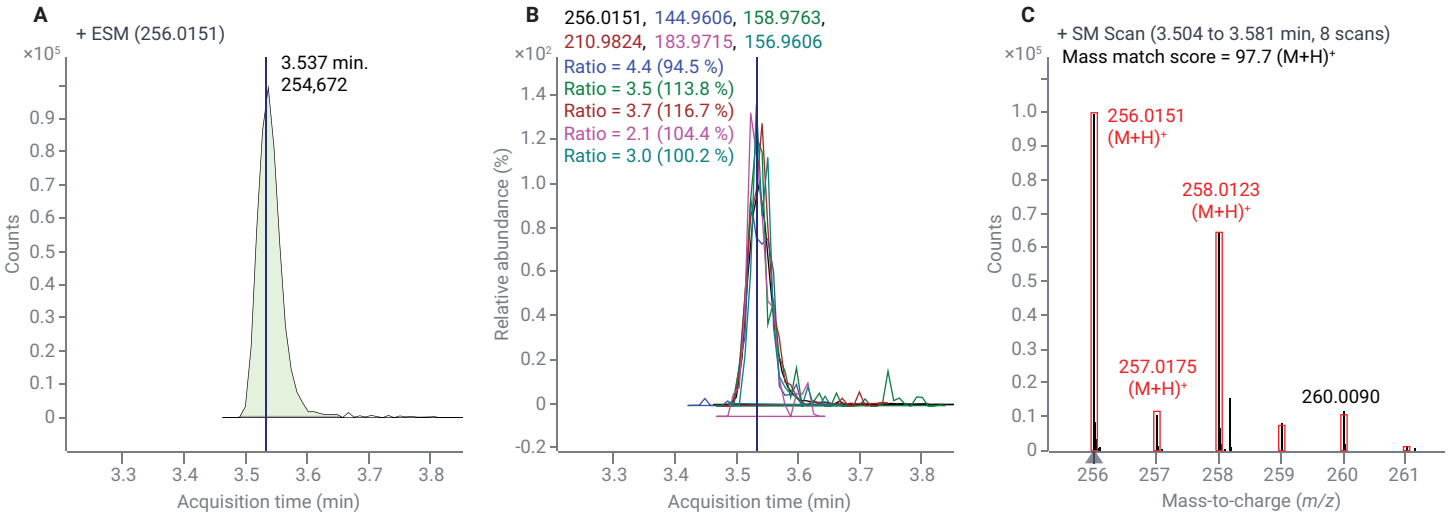


**Figure 7.** Lamotrigine results in 500 ppb spiked sewage sludge.

## Suspect screening

The LC Screener tool built into MassHunter Quantitative software color codes putative identifications according to criteria that represent SANTE guidelines[2]. In Figure 8, green indicates that more than two ions (precursor and/or fragment ions) were measured with the desired mass accuracy, were coeluting and within an expect RT range (when known). Additionally, the isotope pattern of the precursor ions were also verified. All six target compounds expected to be measured in negative ionization mode were verified, as shown in Figure 8. Two additional compounds were also verified in negative ionization mode. Orange indicates a compound needs to be reviewed, and red indicates the compound was not detected in the selected sample.

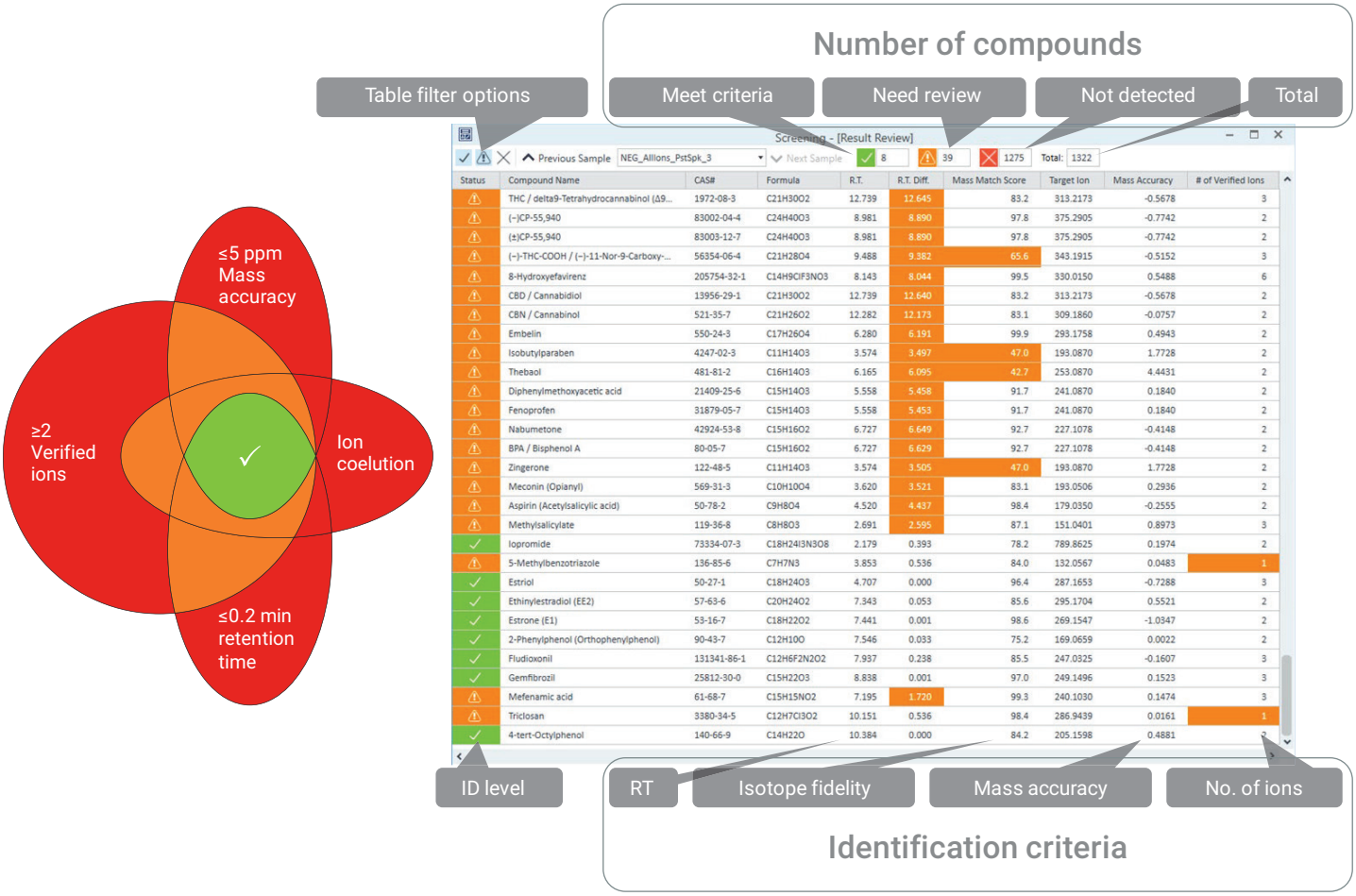


**Figure 8.** LC Screener tool representing criteria used to make a putative identification of a suspect compound.

After verifying the data analysis workflow by finding spiked surrogates in sewage sludge, the spiked compounds and other putatively identified negatively ionized compounds are reported, with an excerpt shown in Figure 9. The full reports for both negatively and positively ionized compounds are summarized in Table 4.

**Table 4.** Summary of compounds that were verified, need review, and not detected in a spiked sewage sludge sample.

| Ionization mode | Verified | Need review | Not detected |
|---|---|---|---|
| Positive | 18 | 159 | 3,998 |
| Negative | 8 | 47 | 1,267 |

## Screening Summary Report

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**Sample name:** NEG_AllIons_PstSpk_3 **Good** 8 **Warning** 47 **Error** 1267

| Status | Screening Summary Report | Formula | R.T. | R.T. Diff. | Match Score | Target Ion | Mass Accuracy | # of Qualified Ions | Final Conc. |
|---|---|---|---|---|---|---|---|---|---|
| ! | Xanthohumol | C21H22O5 | 9.411 | 9.310 | | 353.1394 | -0.61 PPM | 3 | |
| ! | Lauryl hydrogen sulfate | C12H26O4S | 8.249 | 8.144 | | 265.1479 | 0.11 PPM | 2 | |
| ! | Harmine | C13H12N2O | 4.923 | 4.825 | | 211.0877 | -1.28 PPM | 3 | |
| ! | THC / delta9-Tetrahydrocannabinol (Δ9-THC) | C21H30O2 | 12.739 | 12.645 | | 313.2173 | -0.57 PPM | 3 | |
| ! | (−)CP-55,940 | C24H40O3 | 8.981 | 8.890 | | 375.2905 | -0.77 PPM | 2 | |
| ! | (±)CP-55,940 | C24H40O3 | 8.981 | 8.890 | | 375.2905 | -0.77 PPM | 2 | |
| ! | (−)-THC-COOH / (−)-11-Nor-9-Carboxy-tetrahydrocannabinol | C21H28O4 | 9.488 | 9.382 | | 343.1915 | -0.52 PPM | 3 | |
| ! | 4-Methylphenol (p-Cresol) | C7H8O | 7.370 | 7.290 | | 107.0502 | 7.94 PPM | 2 | |
| ! | Fenofibric acid | C17H15ClO4 | 5.606 | 5.498 | | 317.0586 | -1.73 PPM | 2 | |
| ! | Bisphenol E | C14H14O2 | 4.634 | 4.532 | | 213.0921 | 0.19 PPM | 2 | |
| ! | BPS / Bisphenol S | C12H10O4S | 4.522 | 4.415 | | 249.0227 | 0.02 PPM | 4 | |
| ! | Silibinin | C25H22O10 | 5.707 | 5.610 | | 481.1140 | -1.58 PPM | 3 | |
| ! | Losartan | C22H23ClN6O | 5.482 | 5.379 | | 421.1549 | -0.12 PPM | 2 | |
| ! | Veratramine | C27H39NO2 | 14.185 | 14.087 | | 408.2908 | -0.96 PPM | 2 | |
| ! | BKF (Cyanox 2246) (2,2′-methylene-bis(6-tert-butyl-4-methylphenol)) | C23H32O2 | 12.898 | 12.796 | | 339.2330 | 0.43 PPM | 2 | |
| ! | Curcumin | C21H20O6 | 8.113 | 8.011 | | 367.1187 | 0.45 PPM | 2 | |
| ! | Iloprost | C22H32O4 | 6.119 | 6.037 | | 359.2228 | -0.23 PPM | 2 | |
| ! | Phenylpyruvic acid | C9H8O3 | 1.530 | 1.424 | | 163.0401 | 1.24 PPM | 3 | |
| ! | THC-COOH / 11-Nor-9-Carboxy-tetrahydrocannabinol | C21H28O4 | 9.488 | 9.382 | | 343.1915 | -0.52 PPM | 3 | |
| ! | 8-Hydroxyefavirenz | C14H9ClF3NO3 | 8.143 | 8.044 | | 330.0150 | 0.55 PPM | 6 | |
| ! | CBD / Cannabidiol | C21H30O2 | 12.739 | 12.640 | | 313.2173 | -0.57 PPM | 2 | |
| ! | CBN / Cannabinol | C21H26O2 | 12.282 | 12.173 | | 309.1860 | -0.08 PPM | 2 | |
| ! | Embelin | C17H26O4 | 6.280 | 6.191 | | 293.1758 | 0.49 PPM | 2 | |
| ! | Isobutylparaben | C11H14O3 | 3.574 | 3.497 | | 193.0870 | 1.77 PPM | 2 | |
| ! | Thebaol | C16H14O3 | 6.165 | 6.095 | | 253.0870 | 4.44 PPM | 2 | |
| ! | Diphenylmethoxyacetic acid | C15H14O3 | 5.558 | 5.458 | | 241.0870 | 0.18 PPM | 2 | |
| ! | Fenoprofen | C15H14O3 | 5.558 | 5.453 | | 241.0870 | 0.18 PPM | 2 | |
| ! | Nabumetone | C15H16O2 | 6.727 | 6.649 | | 227.1078 | -0.41 PPM | 2 | |
| ! | BPA / Bisphenol A | C15H16O2 | 6.727 | 6.629 | | 227.1078 | -0.41 PPM | 2 | |
| ! | Zingerone | C11H14O3 | 3.574 | 3.505 | | 193.0870 | 1.77 PPM | 2 | |
| ! | Meconin (Opianyl) | C10H10O4 | 3.620 | 3.521 | | 193.0506 | 0.29 PPM | 2 | |
| ! | Aspirin (Acetylsalicylic acid) | C9H8O4 | 4.520 | 4.437 | | 179.0350 | -0.26 PPM | 2 | |
| ! | Caffeic acid | C9H8O4 | 4.520 | 4.425 | | 179.0350 | -0.26 PPM | 2 | |
| ! | Phenacemide | C9H10N2O2 | 3.982 | 3.906 | | 177.0670 | 0.79 PPM | 2 | |
| ! | 2-Phenylphenol | C12H10O | 7.546 | 7.478 | | 169.0659 | 0.00 PPM | 2 | |
| ! | Homogentisic acid | C8H8O4 | 3.471 | 3.348 | | 167.0350 | -0.37 PPM | 2 | |
| ! | Methylsalicylate | C8H8O3 | 2.691 | 2.595 | | 151.0401 | 0.90 PPM | 3 | |
| + | Iopromide | C18H24I3N3O8 | 2.179 | 0.393 | | 789.8625 | 0.20 PPM | 2 | |
| ! | Primidone | C12H14N2O2 | 4.406 | 0.717 | | 217.0983 | 4.33 PPM | 1 | |
| ! | 5-Methylbenzotriazole | C7H7N3 | 3.853 | 0.536 | | 132.0567 | 0.05 PPM | 1 | |
| + | Estriol | C18H24O3 | 4.707 | 0.000 | | 287.1653 | -0.73 PPM | 3 | 225.9218 |
| ! | Oxazepam | C15H11ClN2O2 | 6.267 | 0.261 | | 285.0436 | -1.38 PPM | 1 | |
| ! | Isoproturon | C12H18N2O | 6.942 | 0.186 | | 205.1346 | -0.42 PPM | 1 | |
| ! | Diuron | C9H10Cl2N2O | 6.517 | 0.250 | | 231.0097 | 1.20 PPM | 1 | |
| ! | Naproxen | C14H14O3 | 6.835 | 0.390 | | 229.0870 | 0.92 PPM | 1 | |
| ! | Butyl 4-hydroxybenzoate (Butylparaben) | C11H14O3 | 6.913 | 0.463 | | 193.0870 | 1.19 PPM | 1 | |
| + | Ethinylestradiol (EE2) | C20H24O2 | 7.343 | 0.053 | | 295.1704 | 0.55 PPM | 2 | 166.2993 |
| + | Estrone (E1) | C18H22O2 | 7.441 | 0.001 | | 269.1547 | -1.03 PPM | 2 | 163.2462 |
| + | 2-Phenylphenol (Orthophenylphenol) | C12H10O | 7.546 | 0.033 | | 169.0659 | 0.00 PPM | 2 | 215.1786 |
| + | Fludioxonil | C12H6F2N2O2 | 7.937 | 0.238 | | 247.0325 | -0.16 PPM | 3 | |
| + | Gemfibrozil | C15H22O3 | 8.838 | 0.001 | | 249.1496 | 0.15 PPM | 3 | 382.1097 |
| ! | Mefenamic acid | C15H15NO2 | 7.195 | 1.720 | | 240.1030 | 0.15 PPM | 3 | |
| ! | Triclosan | C12H7Cl3O2 | 10.151 | 0.536 | | 286.9439 | 0.02 PPM | 1 | |
| ! | Fipronil | C12H4Cl2F6N4OS | 9.522 | 0.102 | | 434.9314 | -2.36 PPM | 1 | |
| + | 4-tert-Octylphenol | C14H22O | 10.384 | 0.000 | | 205.1598 | 0.49 PPM | 2 | 413.4864 |

**Figure 9.** Putatively identified compounds detected in negative ionization mode from sewage sludge spiked with target compounds.

## Conclusion

The workflow was able to quantitate the targets compounds spiked into sewage samples. With the increased resolution and dynamic range of the 6546 LC/Q-TOF system, out of 4,856 screened compounds from the PCDL, eight compounds were verified with high confidence in negative ionization mode, and 18 in positive mode. One hundred fifty-nine positively ionized compounds and 47 negatively ionized compounds needed an RT to be verified or the spectra to be reviewed. Many of the compounds that needed to be verified were related to the use of cannabis, which is legal to use in the sampling location. Some compounds, such as xanthohumol, with curated spectra from positive and negative ionization modes, were found in both negative and positive ionization modes to add another layer of added confidence in identification. Compounds with a projected retention time, such as thiabendazole, were also found. Users can continue to find suspected contaminants, retrospectively, by further curating our PCDL with retention time prediction and new toxicants. Predicting or projecting more RTs would likely further reduce the number of compounds to be reviewed, but the workflow does exclude a larger number of compounds that are unlikely to meet SANTE suspect identification criteria, reducing review burden.

As lists of environmental toxicants grow, nontargeted analysis adds value over traditional LC-TQ techniques, by offering the capability to monitor new compounds (even retrospectively) with minimal compromise to quantitation capability.

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