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Simultaneous Target Quantitation and Suspect Screening of Environmental Contaminants in Sewage Sludge

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Introduction

Sewage sludge is a concentrated, complex mixture of compounds that, in many 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 after land application. 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 environmental effects 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 commonly investigated (or researched, or something similar) in sewage treatment plant discharges. 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 new Agilent 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. When 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. MassHunter Workstation Software automates the workflow (Figure 1) which is acquiring All Ions MS/MS data, which is automatically processed utilizing Agilent SureMass technology[1] to allow rapid and more accurate quantitation of targets, and simultaneous detection of suspect compounds, before reporting results.

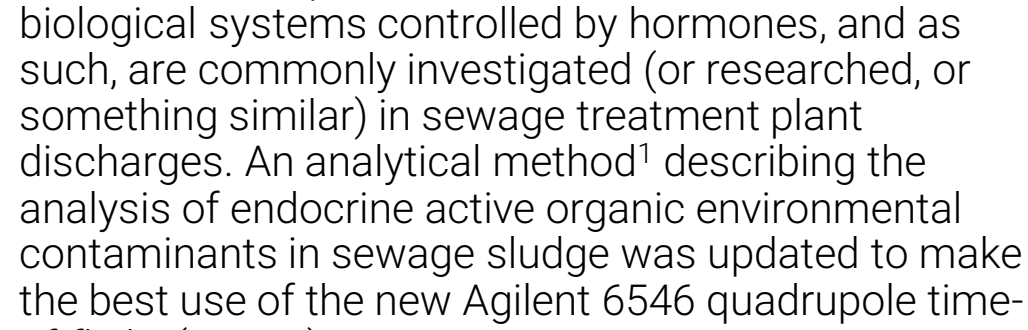


Figure 1. Target quantitation and suspect screening workflow.

We assessed the workflows quantitative capability with carefully selected compounds spiked into sewage sludge matrix while monitoring up to 4,856 suspect compounds with highly curated MS/MS spectra.

Experimental

Sample preparation

Samples were sourced from a water treatment facility in California, USA, and prepared as previously described[2]. 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.

Target preparation

23 targets (listed in Table 1) were selected for method validation that were representative of a larger list's physiochemical properties.

Table 1 : Target compounds and their quantitation limits

Compound Name	Chemical Formula	Neutral Mass	LLOD (µg/L)	LOS (µg/L)	RT (min)
AHTN / Tonalide	$C_{18}H_{26}O$	258.1984	5	1000	12.219
Carbamazepine	$C_{15}H_{12}N_2O$	236.0950	1	250	5.731
DEET / Diethyltoluamide	$C_{12}H_{17}NO$	191.1310	0.5	500	6.382
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295.0167	25	>1000	8.547
Dihydrojasmonic acid, methyl ester	$C_{13}H_{22}O_3$	226.1569	50	>1000	8.432
Efavirenz	$C_{14}H_9ClF_3NO_2$	315.0274	5	>1000	9.006
Flunixin	$C_{14}H_{11}F_3N_2O_2$	296.0773	0.5	2500	6.767
Fluoxetine	$C_{17}H_{18}F_3NO$	309.1341	0.5	>1000	6.476
Fluvoxamine	$C_{15}H_{21}F_3N_2O_2$	318.1555	1	>1000	6.081
Lamotrigine	$C_9H_7Cl_2N_5$	255.0079	0.1	100	3.532
Mefenamic acid	$C_{15}H_{15}NO_2$	241.1103	5	>1000	9.335
Metoprolol	$C_{15}H_{25}NO_3$	267.1834	0.5	>1000	3.708
Miconazole	$C_{18}H_{14}Cl_4N_2O$	413.9860	0.5	500	8.049
Norgestrel	$C_{21}H_{28}O_2$	312.2089	2.5	750	7.927
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.0521	50	>1000	4.109
Triclocarban	$C_{13}H_9Cl_3N_2O$	313.9781	50	>1000	10.15
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.1379	0.1	100	2.964
Estrone	$C_{18}H_{22}O_2$	270.1620	2.5	>1000	7.441
Ethinylestradiol	$C_{20}H_{24}O_2$	296.1776	5	>1000	7.343
2-Phenylphenol	$C_{12}H_{10}O$	170.0732	25	>1000	7.55
Gemfibrozil	$C_{15}H_{22}O_3$	250.1569	5	>1000	8.833
Estriol	$C_{18}H_{24}O_3$	288.1725	5	>1000	4.709
4-tert-octylphenol	$C_{14}H_{22}O$	206.1671	5	>1000	10.382

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 1000, 750, 500, 250, 100, 50, 25, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 µg/L. No internal standards were used to normalize the data.

Instrumentation

The 6546 LC/Q-TOF system was configured as previously described.[3]

Data analysis workflow

The simplified data analysis workflow (Figure 1) extracts a compound's known precursor and fragment masses sourced from Agilent's highly curated compound libraries of high resolution mass spectrometry data and reports identifications according to SANTE guidelines.[4]

Target quantitation capability

By monitoring spiked targets 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 is listed in Table 1.

Target compound results are shown in the same way as LC/TQ data (see Figure 2). The quantifier integration and expected RT (Figure 2A) and the coelution of qualifying ions (Figure 2B, scaled according to expected ratio determined from calibrators), are common between LC/TQ and LC/Q-TOF acquisition methods. The extra decimal places from an accurate mass measurement and ability to compare expected (Figure 2C, red boxes) versus measured isotope pattern (Figure 2C, black spectra), given a known chemical formula and natural isotope abundances, provides an extra level of confidence in a compound identification.

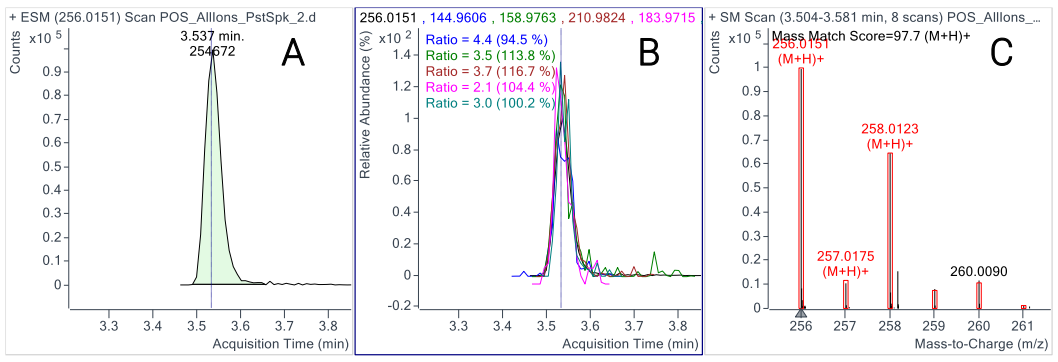


Figure 2. Lamotrigine results in 500 ppb spiked sewage sludge.

Compounds were fitted with a non-linear power curve regression, weighted 1/x (where x is the concentration) . The calibration curves used for the target compounds in this analysis are shown in Figure 3.

Suspect screening capability

Suspect compounds were appended to MassHunter Quantitation methods 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[5]. 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[6]. As the spiked targets in this analysis are chemically diverse enough to elute throughout the chromatogram, we correlated the retention times (RTs) of the target compounds with those published. A model was then used to project the RTs of a broader range of toxicants from the same data file (Figure 4). Additionally, compounds with no RT correlation were also monitored.

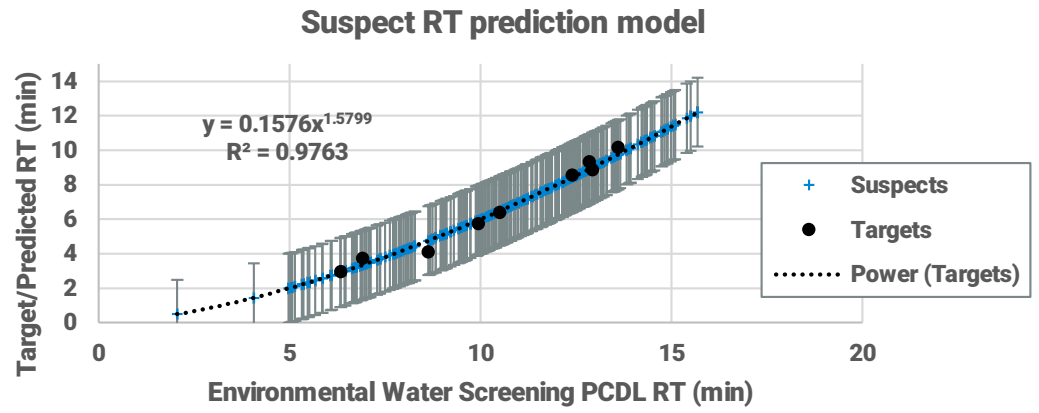


Figure 4. Correlation of surrogate retention times common between analytical methods.

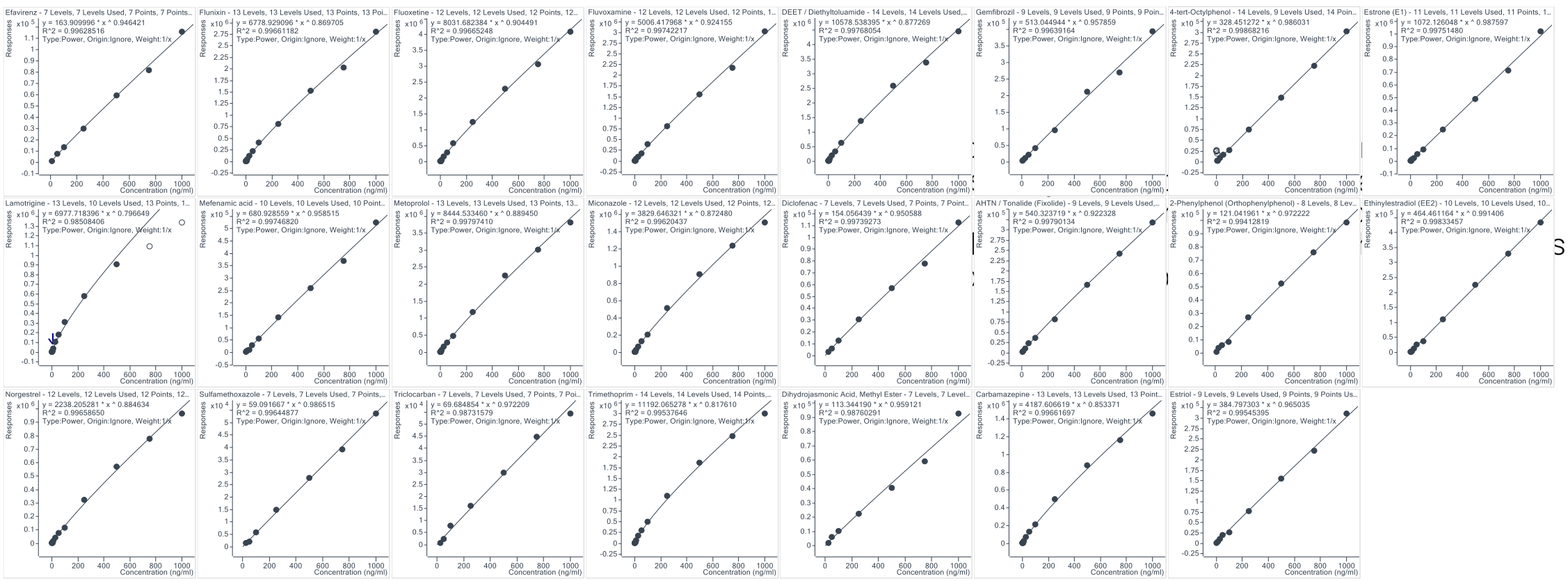
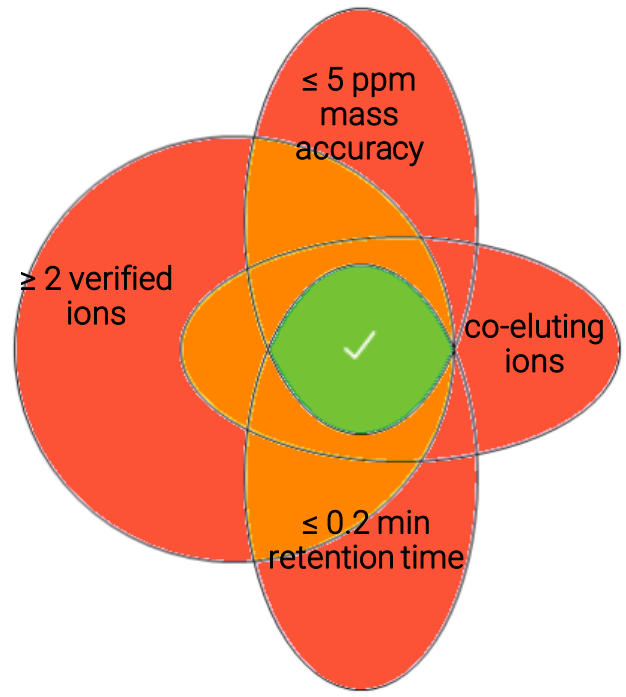


Figure 3. Calibration curves for target compounds.

Results and Discussion

Figure 5. Screening results following SANTE guidelines



	Positively ionized	Negatively ionized
Needs Review	159	47
Needs Review	159	47
Verified	18	8

The LC Screener tool (shown in Figure 5) built into MassHunter Quantitative software color codes putative identifications according to criteria that represent SANTE guidelines[4]. 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.

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 5. Two additional compounds were also verified in negative ionization mode.

Orange indicates a compound where the identification needs to be reviewed, and red indicates the compound was not detected in the selected sample. The total compounds measured with a level of identification according to SANTE guidelines and shown in Figure 5.

Conclusions

The combination of new hardware and software 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.

References

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