Identifying the impact of a pandemic on pharmaceutical river contamination by LC-MS/MS

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Overview

- River water samples were taken late 2019 and 2020 and measured by direct injection LC-MS/MS using TQ and HRMS Q-TOF platforms to assess the impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on pharmaceutical usage.

1. Introduction

In the UK, between October and December 2020 the prescription rates for antidepressants increased by 6% compared to the same three months in 2019. Evidence suggests there has been a 3-fold increase in the prevalence of depression in the US following the outbreak of the pandemic. In this work, targeted and untargeted high-resolution LC-MS/MS methods were applied to river water samples collected before and during a pandemic to assess population use of prescribed medications.

2. Methods

- **River Water Samples**. River water samples were taken in late 2019 and 2020 over spaced intervals on the River Thames and initially analyzed using a targeted rapid triple quadrupole LC-MS/MS method. Sample preparation involved filtration (0.2 µm PTFE) before 10 µL direct injection onto the LC-MS/MS system. A sub-set of samples previously analysed by a rapid injection triple quadrupole LC-MS/MS method were measured using a high-resolution LC-MS/MS QTOF system (8 samples taken in 2019 and 12 samples in 2020).
- **LC separation**.
  - Nexera LC system (Shimadzu Corporation); flow rate 0.4 mL/min
  - Biphenyl 100x2.1mm 2.7µm
  - Binary gradient: A - water +2 mM ammonium formate + 0.002% formic acid; B - methanol +2 mM ammonium formate + 0.002% formic acid
- **Mass Spectrometry Detection**. QTOF LCMS-9030 (Shimadzu Corporation) using external mass calibration for positive and negative ESI analysis;
  - TOF Survey MS mass scan m/z 100-920; 100 msecs
  - DIA-MS/MS mass scans m/z 40-920; 25 msecs; variable precursor isolation width (20 Da for m/z; 100-500; 35 Da for m/z 500-920). Collision energy spread 5-55V. Cycle time 0.9 second.
- **Data Processing**. LabSolutions Insight software was used in targeted and non-targeted screening.
  - **Targeted analysis**. The same compound list developed for the TQ screening method was used in QTOF data analysis (compound list with a ±5 ppm mass extraction window for each target using the TOF Survey mass scan data). Both the TQ and QTOF methods resulted in close agreement for reported concentrations (slope =0.8, R^2 ≥0.95; n=11).
  - **Non-targeted analysis**. Analyze component detection algorithm was used to locate ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner). A screening list of >1000 compounds was then used to find molecular ions. To identify each detected molecular ion the fragmentation information in high resolution libraries was used (in house toxicology database, Wiley and HighResNPS).

3. Results

- **Compound identification**. For each molecular feature detected in the suspect screening list, library searching was used to increase the reporting confidence in compound identification.

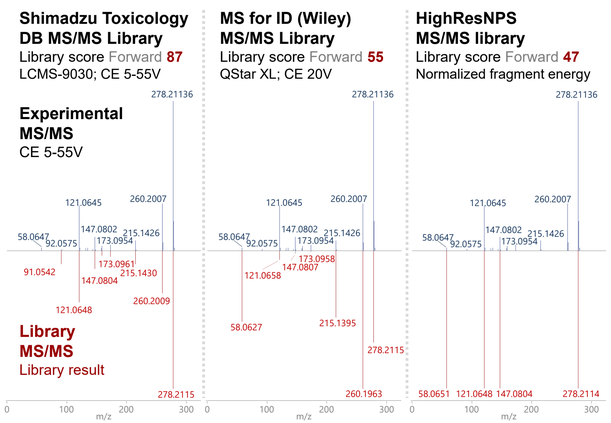

Figure 1. Library results for venlafaxine (antidepressant; $C_{17}H_{27}NO_2$, precursor m/z 278.2115) using multiple data repositories to help increase reporting confidence in compound identification. Although the data repositories use different QTOF platforms and different fragment ion intensities (CE spread 5-55V, fixed CE 20V and normalized fragment ion intensity) each MS/MS library can provide positive evidence for compound identification.

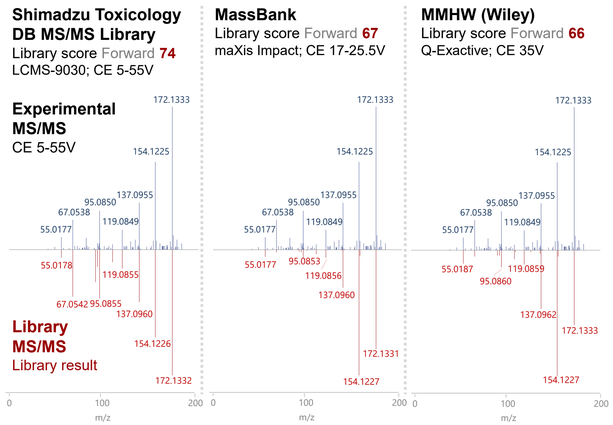

Figure 2. Library results for gabapentin (prescribed as an antidepressant, anticonvulsant and treatment for neuropathic pain; $C_9H_{17}NO_2$, precursor m/z 172.1332) using multiple data repositories with similar library matches.

3.1 Pharmaceutical river contamination

- **Changes during a pandemic**. As part of the study a subset of samples were analysed by high resolution MS/MS (8 samples from 2019, 12 samples from 2020). In this limited set of samples, the peak area data for several pharmaceutical compounds was marginally higher in 2020 compared to 2019 possibly reflecting a change in medicines used during the lockdown period or changes in environmental exposure through wastewater discharges, amongst other reasons.

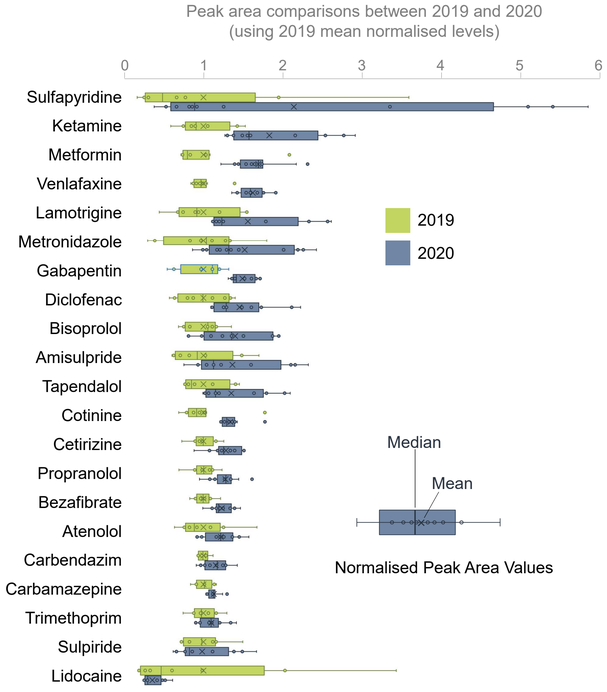

Figure 3. Applying a pharmaceutical screening panel targeting river water contamination resulted in detection of 21 pharmaceutical compounds. The reporting criteria considered the following parameters

- Present in over 20% of samples with peak height >1000 and a S/N>3
- Mass accuracy error within ±5 ppm, isotopic score >20
- Library verification with a matching score >40 (the matching score weighted both mass accuracy and fragment ion intensity)
- Box and whisker plot shows peak area data normalised to the group average for 2019 for each compound.
- With the exception of lidocaine, the concentration of pharmaceutical drugs detected in river water samples was marginally higher in 2020 compared to 2019.

3.3 Screening workflows

- **Using multiple data repositories in compound identification**
  - To increase reporting confidence in compound detection several criteria are considered in environmental screening including mass accuracy error, isotopic score and (if available) retention time using the TOF survey mass scan data. DIA-MS/MS mass scans are then used for product ion spectral matching to identify the compound with higher certainty and to reduce the possibility of false discovery and false negative reporting.
  - In this study, multiple data repositories were used in compound identification.
    - Shimadzu Toxicology Database; a highly curated database (authentic standards used with a fragmentation energy of 5-55V, all fragment ions verified using Assign fragmentation annotation application).
    - Wiley (Wiley Registry of Tandem Mass Spectral Data, MS for ID and LC-HR-MS/MS Library of Drugs, Poisons and Their Metabolites), HighResNPS, MassBank and other public domain repositories.
  - The advantage of using a highly-curated library created in-house enables retention time filtering and a matched fragmentation energy (CE spread of 5-55V) resulting in high confident reporting. However, there is considerable value in using alternative data repositories for product ion spectral matching, particularly with the capability to use alternative weighting for mass accuracy and fragment ion intensity in the dot product score (library similarity score).

3.4 Discussion

- As part of a wider sampling study, a targeted direct analysis LC-MS/MS MRM method was applied to a panel of pharmaceutical targets in 162 samples.
- Both the targeted LC-MS/MS MRM method (LCMS-8060) and high-resolution LC-MS/MS analysis (LCMS-9030 applied to a sub-set of samples) noted a marginal increase in diclofenac (non-steroidal anti-inflammatory), carbamazepine (antipsychotic), venlafaxine and gabapentin (more recently prescribed for anxiety in addition to epilepsy and nerve pain) river water concentrations in 2020 samples (mean concentrations for all were <100 ng/L).
- Peak areas for sulfapyridine (sulfasalazine is a combination of 5-aminosalicylic acid ('5-ASA') and sulfapyridine), ketamine and metformin showed the highest relative change from 2019 to 2020.
- It was noted that the peak area data for lidocaine (a known cocaine adulterant) was lower in 2020 compared to the previous year.

4. Conclusions

- DIA-MS/MS product ion spectral matching not only increases the probability of identifying the compound of interest with a higher certainty it also markedly reduces the impact of false discovery and false negative rates.
- Using multiple MS/MS data repositories is an advantage as product ion spectra across different instruments can be successfully used and provides further confidence in compound identification. Although product ion library match was the most critical factor for reducing the false discovery rate it is important to note the advantage of matched chromatographic retention time data.