The price of exclusion: what gets lost when we don't hydrolyze urine and ignore sulfatase?

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Overview:

Purpose:

• What drugs and metabolites are missed when using a dilute-and-shoot method for comprehensive drug testing in the clinical setting?

Methods:

• We selected 60 patient samples that tested positive by routine toxicology testing at the San Francisco General Hospital.

• We hydrolyzed each sample using sulfatase and glucuronidase separately and run it without hydrolysis.

• We then acquired the samples using LC-HRMS and analyzed the data using our drug panel at the hospital with a library of over 270 drugs and metabolites.

Results:

• Samples differed quantitatively and qualitatively between the three sample types. We also observed patient to patient variability for the same compound.

Introduction:

Many toxicology LC-MS panels have been streamlined by the "dilute and shoot" method for ease of use and the capacity to see both drugs and their conjugates (glucuronides and to a lesser extent sulfates).

Labs that hydrolyze tend to only use β-glucuronidase, however drug metabolites can be present in both conjugated forms.

Objective: what drug metabolites are missed when using a dilute-and-shoot method for comprehensive drug testing in the clinical setting?

Methods:

Population

Sixty patient samples that tested positive for drugs by routine toxicology testing in a clinical laboratory were selected for evaluation. The patients ranged in age from 19 to 92 years where 70% identify as male and 30% as female (Fig. 1)

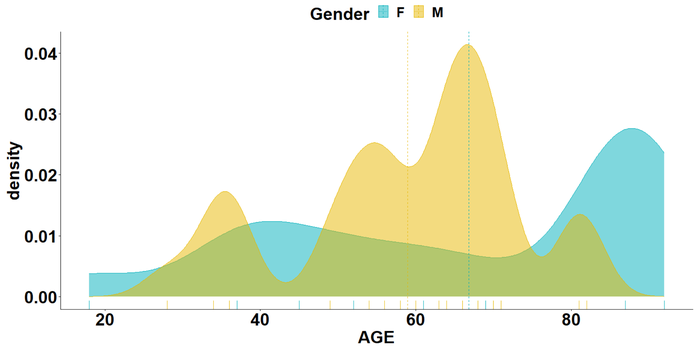

Figure 1: Age and gender distribution of the population analyzed

Sample Preparation

- Aliquot and spin 1 mL of urine samples at 15000 rpm to precipitate any materials in suspension.
- Prepare the Hydrolysis Solution (2:1:1) H_2O, Enzyme (IMCS Sulfatase or Glucuronidase), Buffer (IMCS provided buffer with compatible pH)
- Pipette 1:1 Hydrolysis Solution and Urine Sample into a 96 well plate divided to three sections:

1. Hydrolyzed with Sulfatase (Sulfazyme™, IMCSzyme®)
2. Hydrolyzed with recombinant β-glucuronidase (IMCSzyme®)
3. Non hydrolyzed urine

- Hydrolyze for one hour at room temperature and precipitate the enzyme with acetonitrile spiked with internal standard (1:3) Sample/ACN.
- Centrifuge the plates at 4000 rpm and transfer 60 µL to the injection plate containing 240 µL of the aqueous mobile phase (MPA) .

Quality control and blanks

A pool of all patients was prepared like all samples in three treatments and injected at regular intervals. A blank (drug free urine) and double blank (ddH_2O) were also included.

Instrumentation and Method Parameters

Samples were analyzed using the comprehensive drug test by LC-HRMS in the clinical laboratory at San Francisco General Hospital. A Phenomenex Kinetix method C18 (50x3 mm, 2.6µm) was used for the separation with a 15 min gradient of 5 mM ammonium formate, 0.05% formic acid as MPA and a mix of methanol and acetonitrile (1:1) with 0.05% formic acid as MPB. Data was acquired on ABSciex TripleTOF®5600 in positive-ion HRMS full scan mode with IDA triggered acquisition of HRMS product ion spectra was used for mass detection. PeakView® (AB Sciex) were used for targeted data analysis where an in-house library of 274 drugs and metabolites.

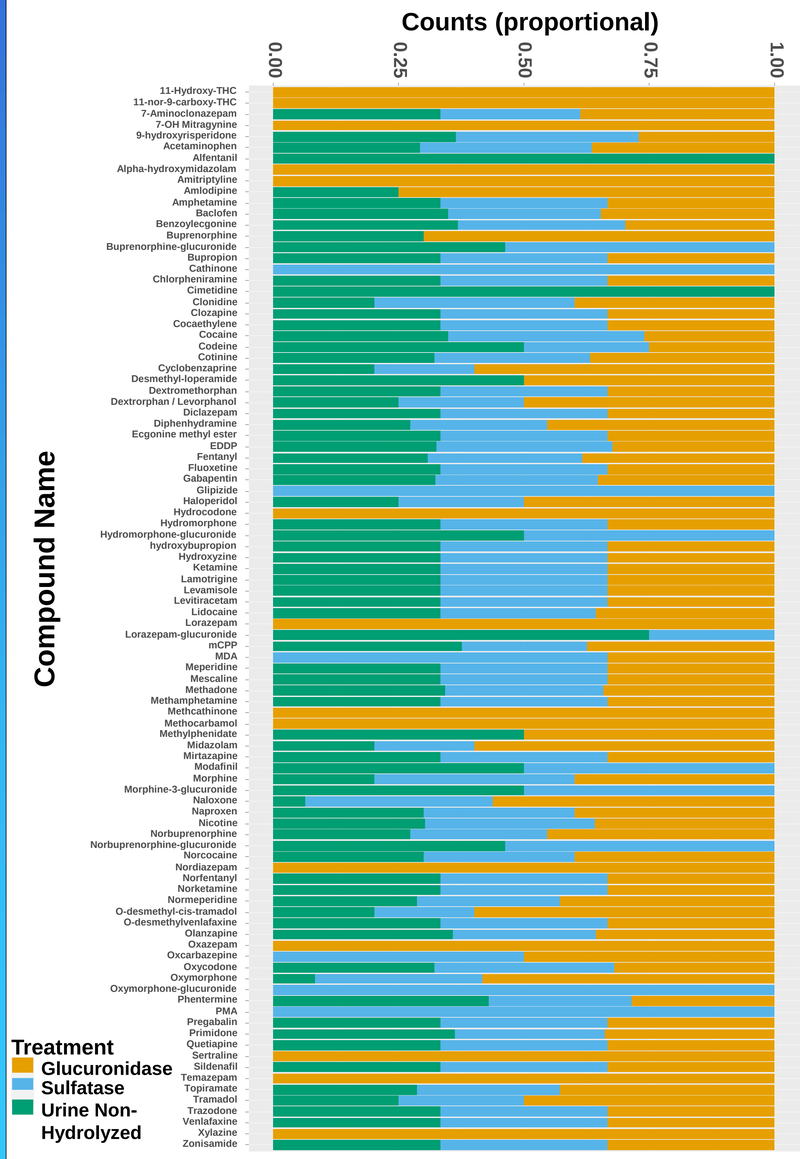

Figure 2: Proportion of patients showing the identified compound in each treatment category. When hydrolysis is not consequential the three bands will have the same length (e.g., Zonisamide)

Results

We observe two trends (1) patient to patient variability where compounds that show up in all treatments are more present in one form that the other in certain patients.

For instance, MDA is only seen upon hydrolysis. More patient samples show MDA when they are hydrolyzed with sulfatase (Fig. 2).

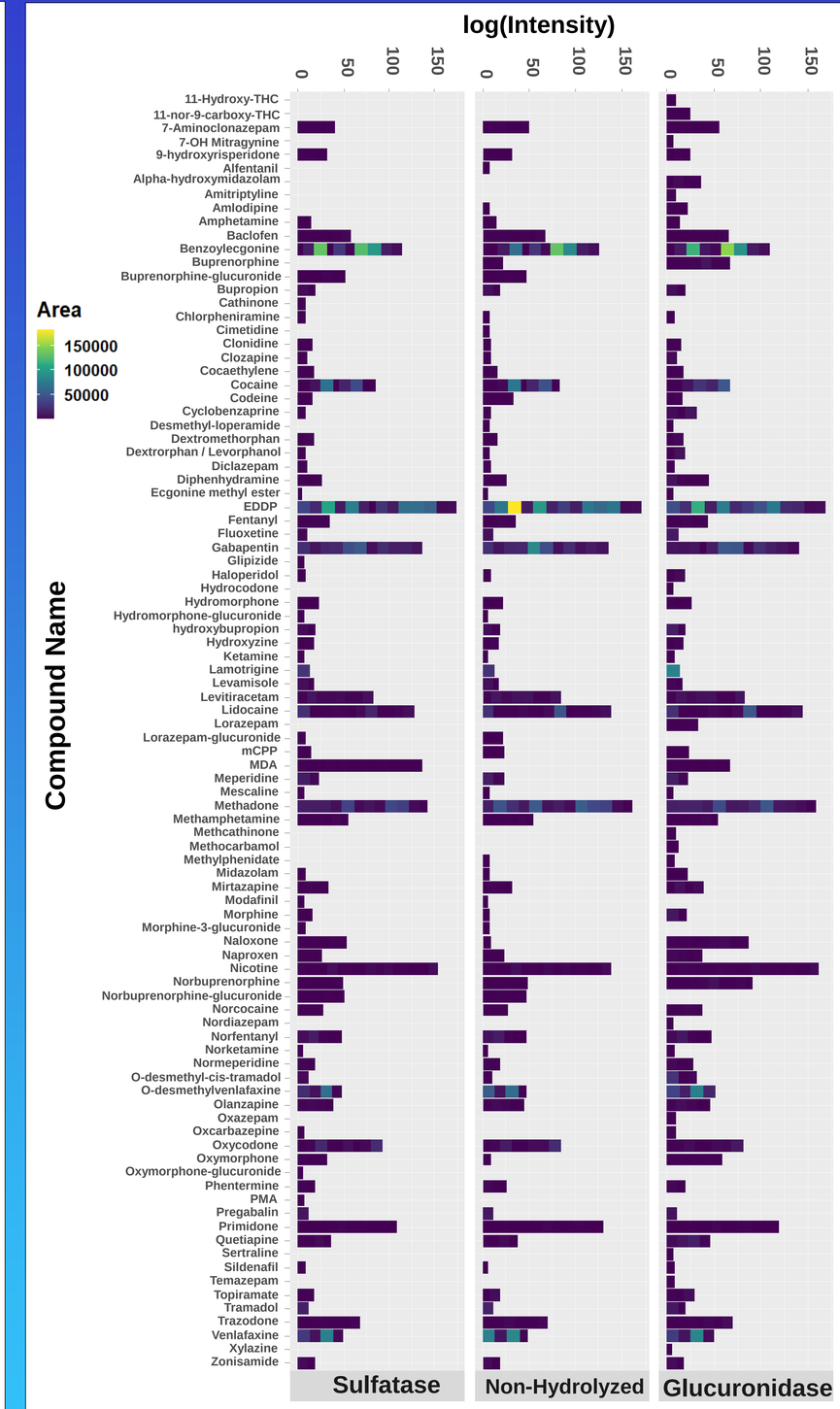

Figure 3: Normalized intensity of identified compounds across all treatments

(2) Enzyme dependent compounds, seen only upon hydrolysis: PMA, Xylazine, THC and Mytraginine metabolites, xylazine, Temazepam and Lorazepam, etc. The intensity of certain compounds changes substantially upon hydrolysis in certain patient samples suggesting a differential rate of glucuronidation and sulfation (Fig. 3)

Conclusion:

It seems that excluding hydrolysis for the operational ease of dilute and shoot method comes at the expense of losing some information that could provide further insight to practitioners.