Overall, there are two contamination issues – very high TIC intensity and two persistent contamination peaks on the BPC (385.08 and 403 m/z).

After the NE2 was upgraded, we made new mobile phases and added a new transfer line and column. The pump B check valve was not working, so we also replaced it.

This is the first blank we ran after NE2 was upgraded (TIC and BPC respectively).

A screenshot of a computer

Description automatically generated

A screenshot of a computer

Description automatically generated

Some steps we took to diagnose the contaminations:

*Autosampler testing*:

The injections were from a 384 well plate sealed with adhesive aluminum foil, we suspected that might be the source of contamination. To rule it out, we attempted no volume injection by leaving the injection volume blank. Unfortunately, the contaminations were still there after multiple days of flushing.

*Mobile phase testing*:

We then re-made the mobile phases and re-ran the blanks with no injection. Both TIC and the contamination were lower, but still higher than expected for a blank injection.

TIC and BPC are shown below. The two peaks from BPC are 385 and 403 m/z.

A screenshot of a computer

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We swapped the second batch mobile phases with the ones we are currently using for timsTOF pro. Then the TIC went back to the original high intensity (3x10^6).

We tried to diagnose if the contamination comes from the tubing by swapping the mobile phase A and B bottles and ran a direct flow of 70% pump B at 0.3ul/min. But no changes occurred.

*Flushing with ACN*:

We tried flushing the system at 95% B attempting to remove the contamination, but it did not work.

Then, we flushed it with 30% B and the contamination peaks seem to have decreased. But not fully.

We also noticed the retention time was shifting for the two contamination peaks.

A screenshot of a computer

Description automatically generated

Then, we changed the filter, but nothing improved.

Also note, the TIC intensity was also high when we observed the real time detection intensity when no method was run.

We plan to change the source as our next step.

This is how the blank looks with NE1 before all the contaminations appeared. A screenshot of a computer

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