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How Clean is "Clean"? Going Above and Beyond the Conventional Check-Clean with a Rapid, DART-MS Protocol

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INTRODUCTION

Fatty acids, oils, grease, and other industrial residues can negatively impact instrumentation that rely on clean components in order to perform optimally. That is why laboratories have in place specific cleaning and bake-out protocols in place. However, these protocols may not always be as effective as one may think. This is where high-resolution mass spectrometry comes to the forefront as a means for creating a "check-clean" process to insure cleanliness.

Through this work, a fast (< 30sec) solution is presented for direct comparison of unused, clean, and used components. Mass spectrometer components such as capillaries and nebulizers were sampled to demonstrate this workflow. The resulting mass spectra displayed hundreds of discrete peaks with several corresponding to hydrocarbons, fatty acids, and various polymers. The gathered data was then processed in MetaboScape® where unique features were identified. A principal component analysis was then performed to help detect if cleaned components were grouping with other clean components or showing signs of remaining contaminants.

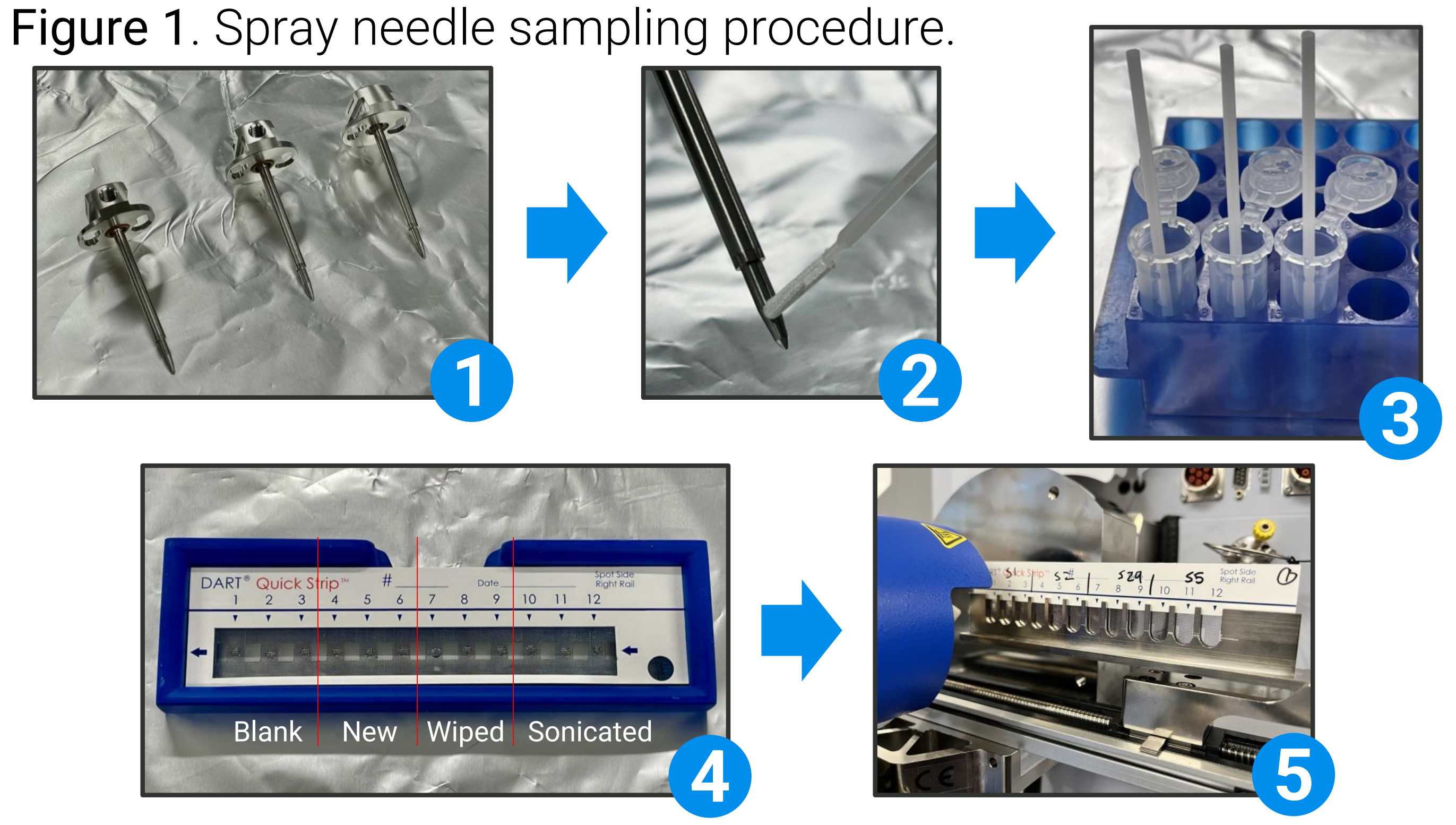
METHODS AND INSTRUMENTATION

2 spray needles of varying cleanliness were taken from ESI sources and directly sampled for this study. A brand-new needle was also sampled to provide a baseline comparison.

The used needles were cleaned. The first was cleaned with lint-free wipes and methanol/isopropanol and thoroughly wiped. The second needle was cleaned per Bruker's recommended procedure and placed in an ultrasonic bath with isopropanol/H_2O. Once cleaned, the three needles (new, sonicated, and wiped) were sampled by Contec Polyurethane Cleanroom Swabs that were dipped in IPA. The swabs were then soaked in 100µL of IPA to desorb any contaminants. (Fig. 1) The resulting solutions and a blank were analyzed in triplicate on the Bruker impact-II QTOF MS instrument, using a DART ion source with QuickStrip sampling module (Bruker Daltonics). All data analyses and statistic computations were made within MetaboScape (Bruker Daltonics).

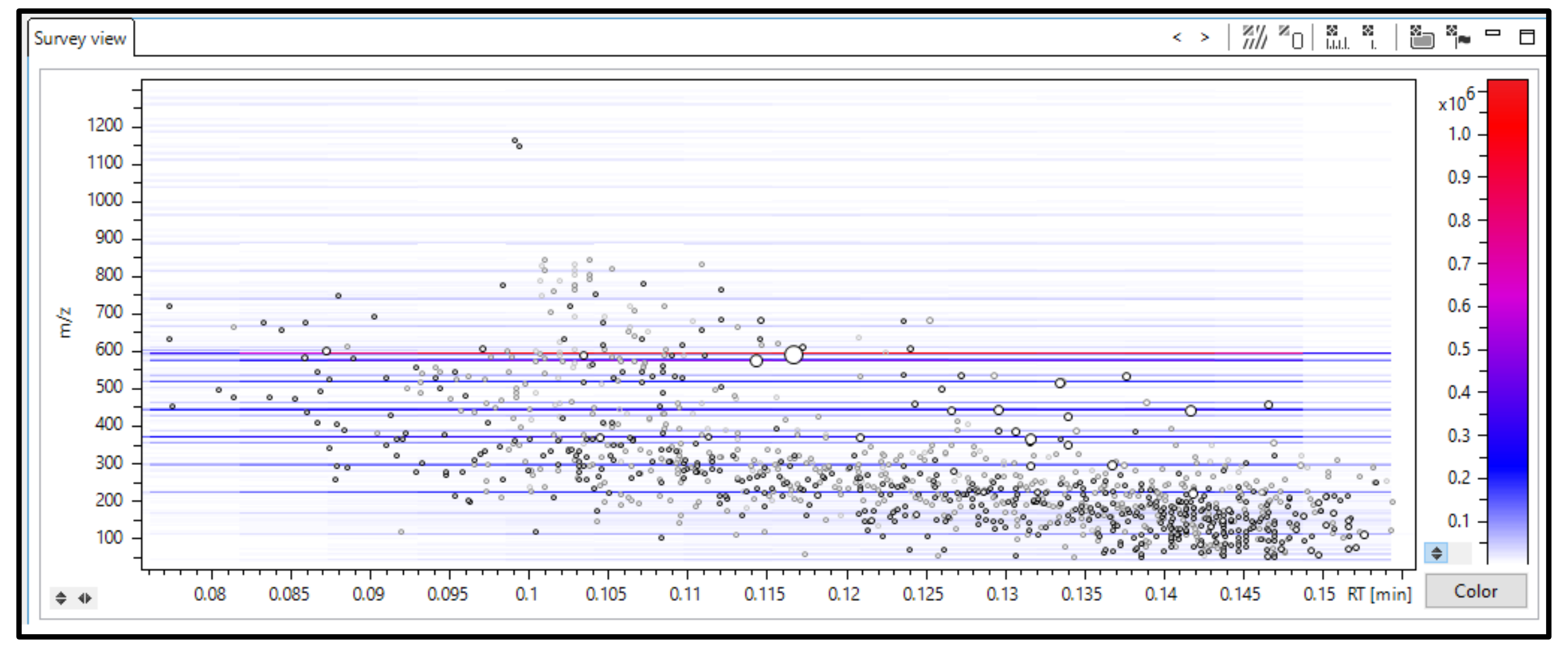
METHODS AND INSTRUMENTATION (cont.)

- ❖ Figure 1. Spray needle sampling procedure.

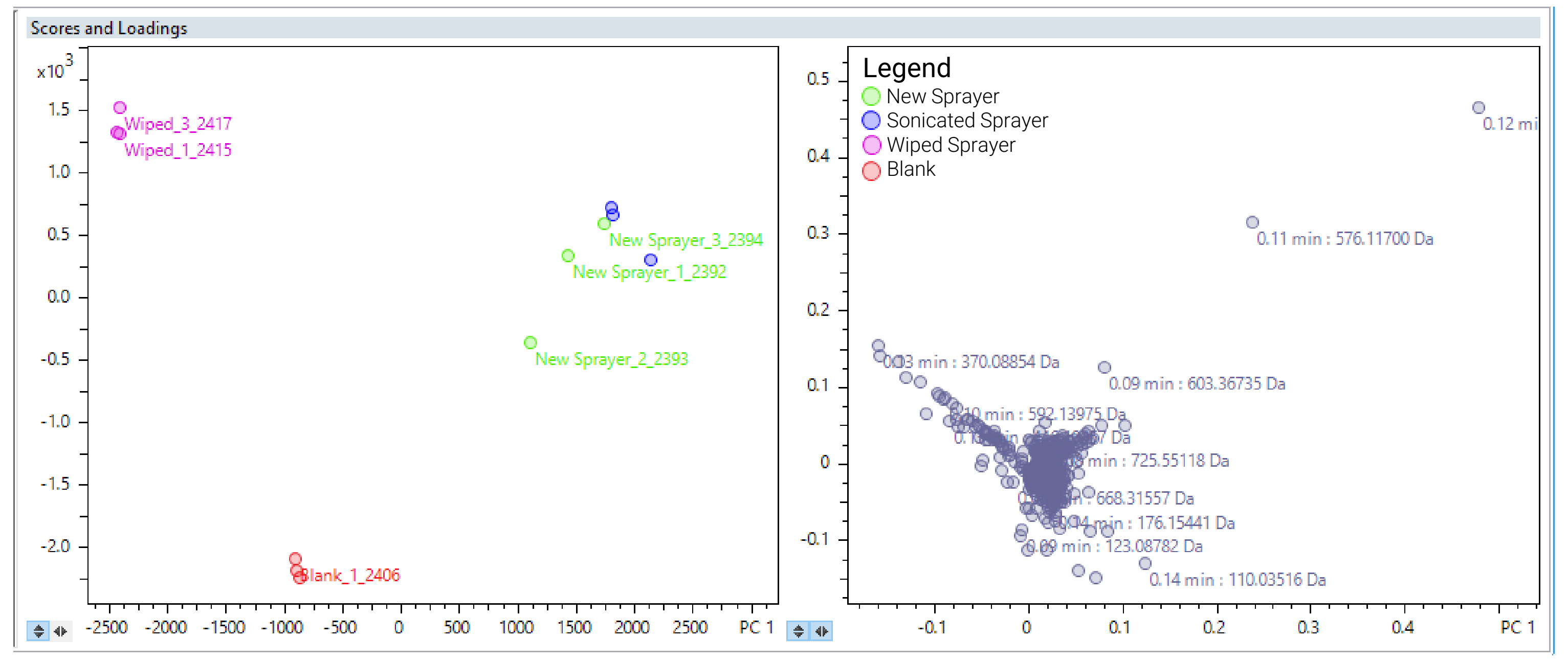


RESULTS

- ❖ Data processing in MetaboScape revealed > **1000** features



- ❖ A Principal Component Analysis (PCA) was generated to evaluate how the different cleaning methods compare to that of a new spray needle.
- ❖ Based on the clustering with the plot, results suggest that the sonicated needle is displaying similar results to that of the new needle.
- ❖ Whereas the wiped needle is grouping separately. Suggesting that the cleaning method is not sufficient.

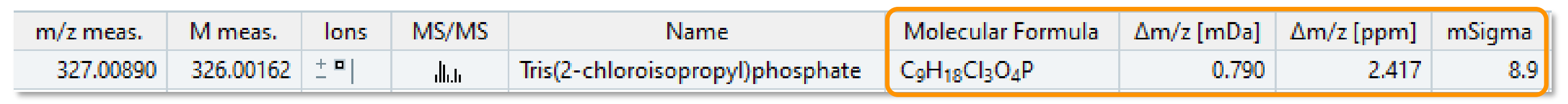


RESULTS

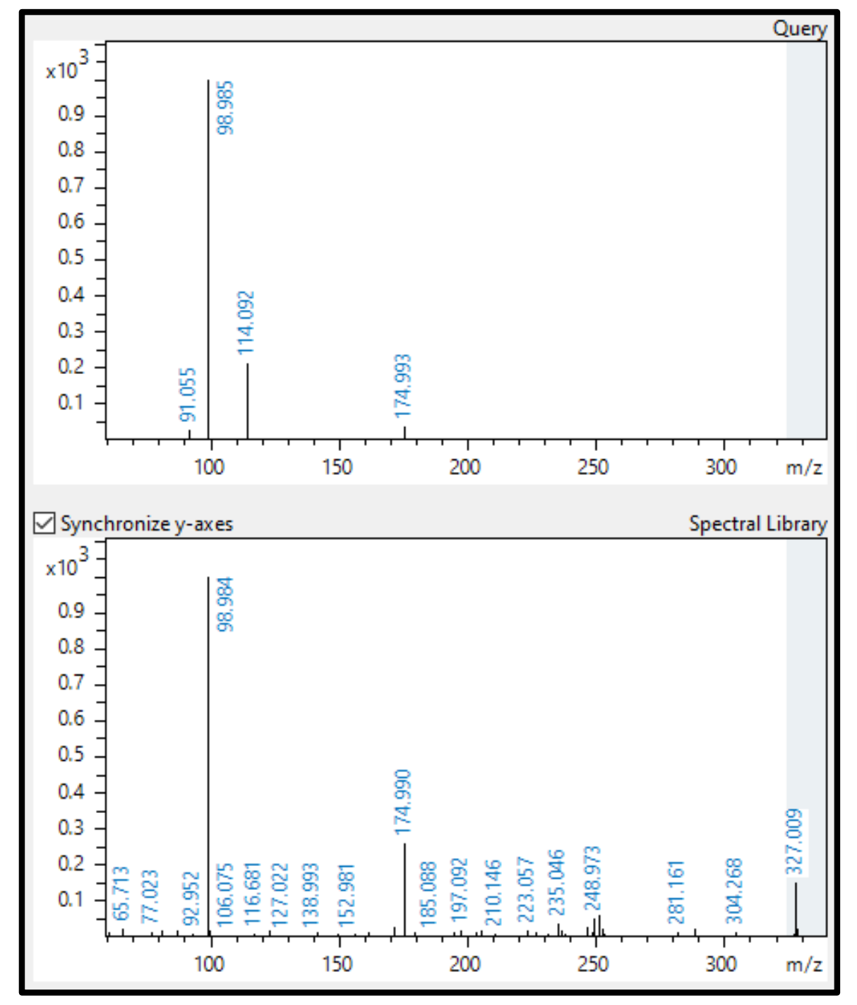
Automatic workflow for spectral library matching

- Exemplary identification of Tris(2-chloroisopropyl)Phosphate (TCEP)

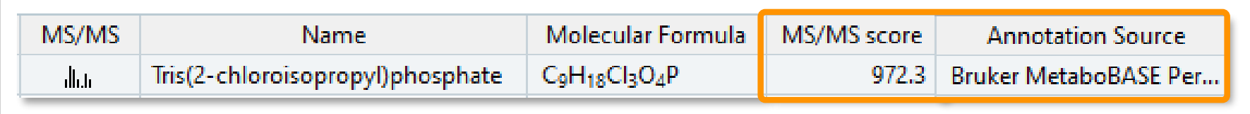
- ❖ Molecular formula matches with accurate mass (m/z) and isotope pattern (mSigma) of detected feature



m/z meas.	M meas.	Ions	MS/MS	Name	Molecular Formula	Δm/z [mDa]	Δm/z [ppm]	mSigma
327.00890	326.00162	± ▪		Tris(2-chloroisopropyl)phosphate	$C_9H_{18}Cl_3O_4P$	0.790	2.417	8.9

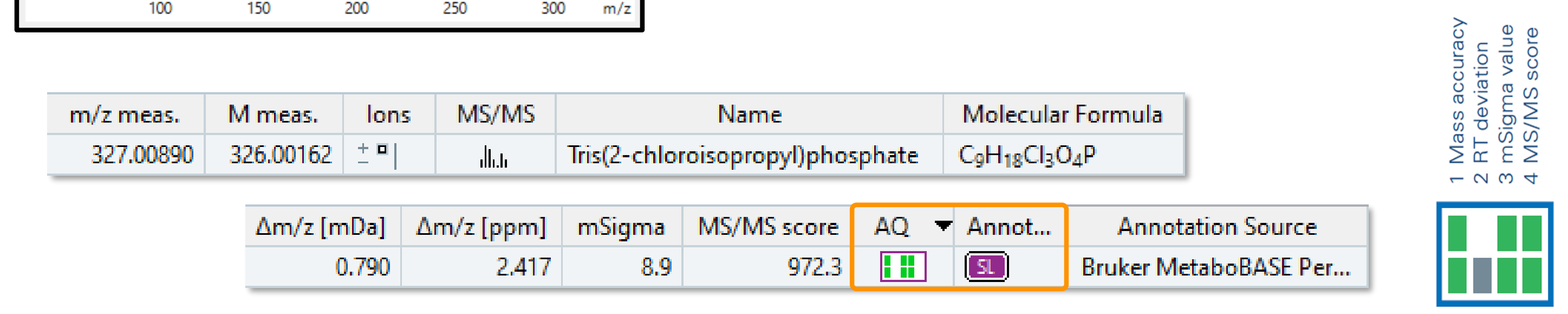


- ❖ MS/MS fragmentation patterns are matched against example spectra from the library



MS/MS	Name	Molecular Formula	MS/MS score	Annotation Source
	Tris(2-chloroisopropyl)phosphate	$C_9H_{18}Cl_3O_4P$	972.3	Bruker MetaboBASE Per...

- ❖ Once matched, a score of how well the mass accuracy, mSigma, and MS/MS pattern corresponds to the compound in the spectral library.



m/z meas.	M meas.	Ions	MS/MS	Name	Molecular Formula
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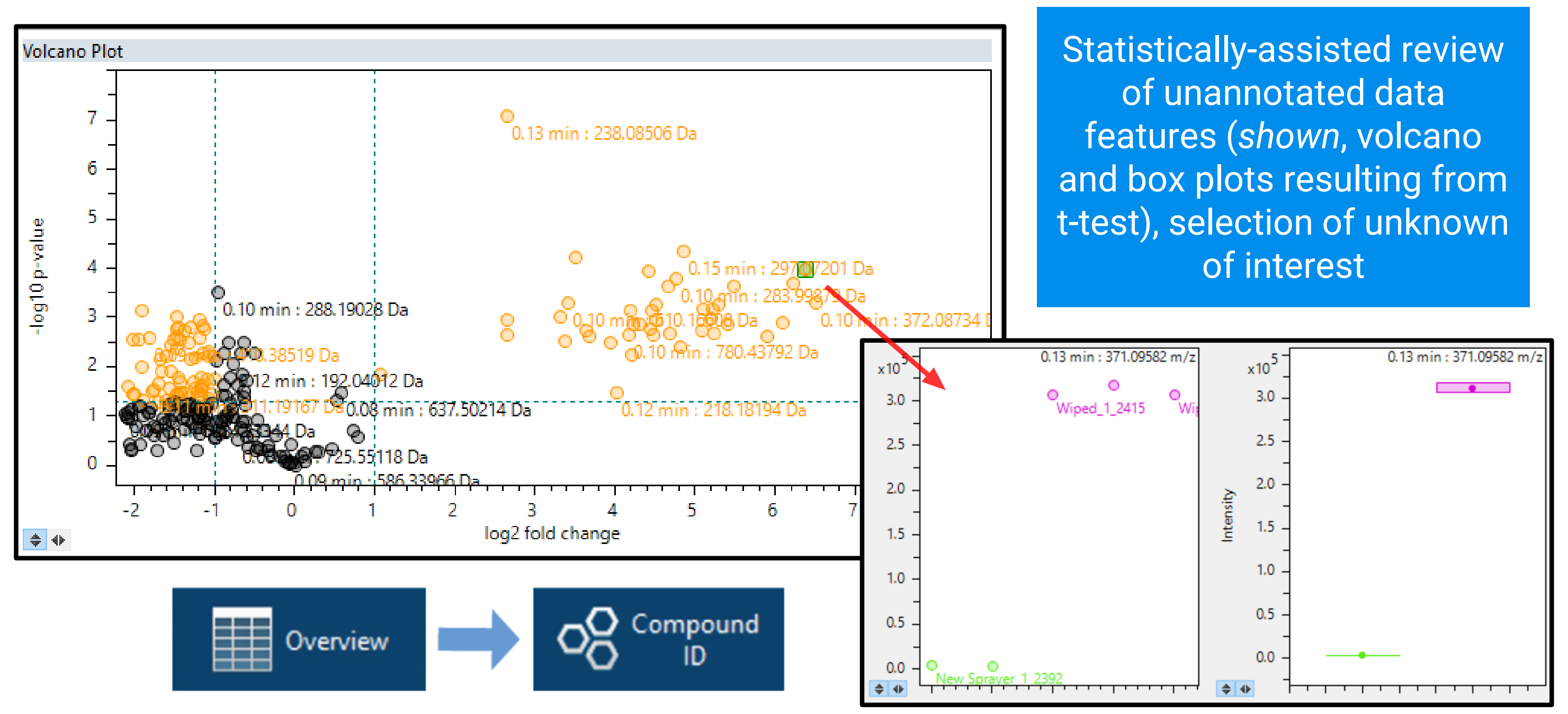
Δm/z [mDa]	Δm/z [ppm]	mSigma	MS/MS score	AQ	Annot...	Annotation Source
0.790	2.417	8.9	972.3	🟩	SL	Bruker MetaboBASE Per...

1 Mass accuracy
2 RT deviation
3 mSigma value
4 MS/MS score

Identification of unknowns using various tools

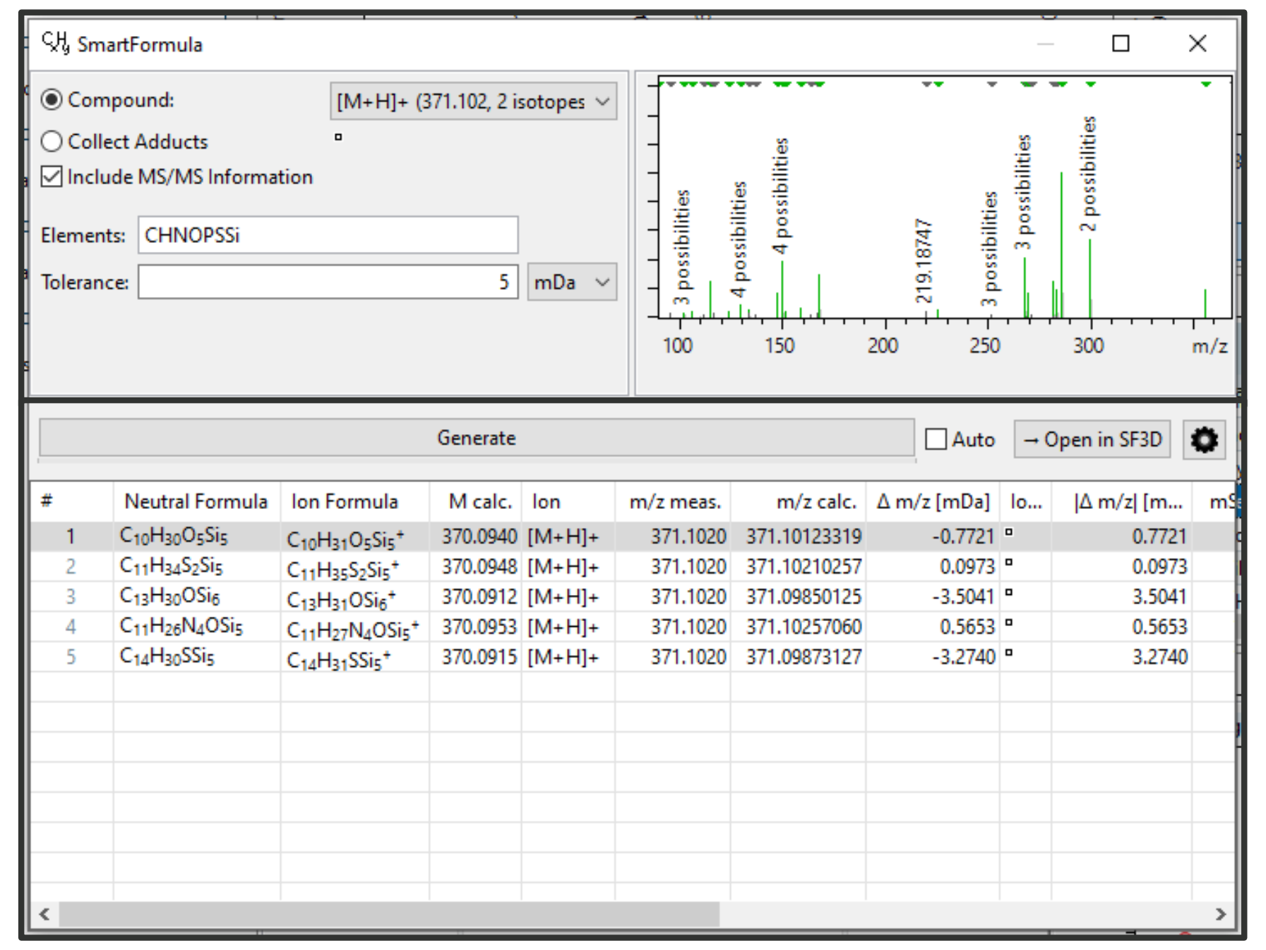
- ❖ The putative ID of compounds within the remaining (unannotated, filtered) signals focused on mass features of statistical significance between the New Sprayer and Wiped Sprayer.



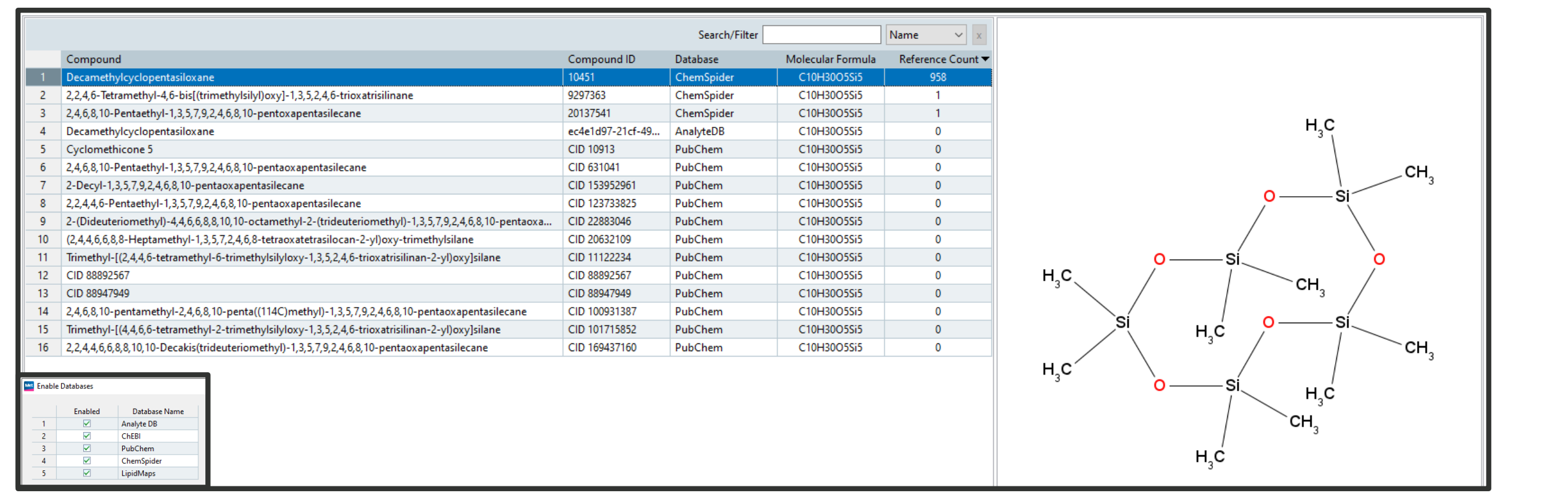
Statistically-assisted review of unannotated data features (*shown*, volcano and box plots resulting from t-test), selection of unknown of interest

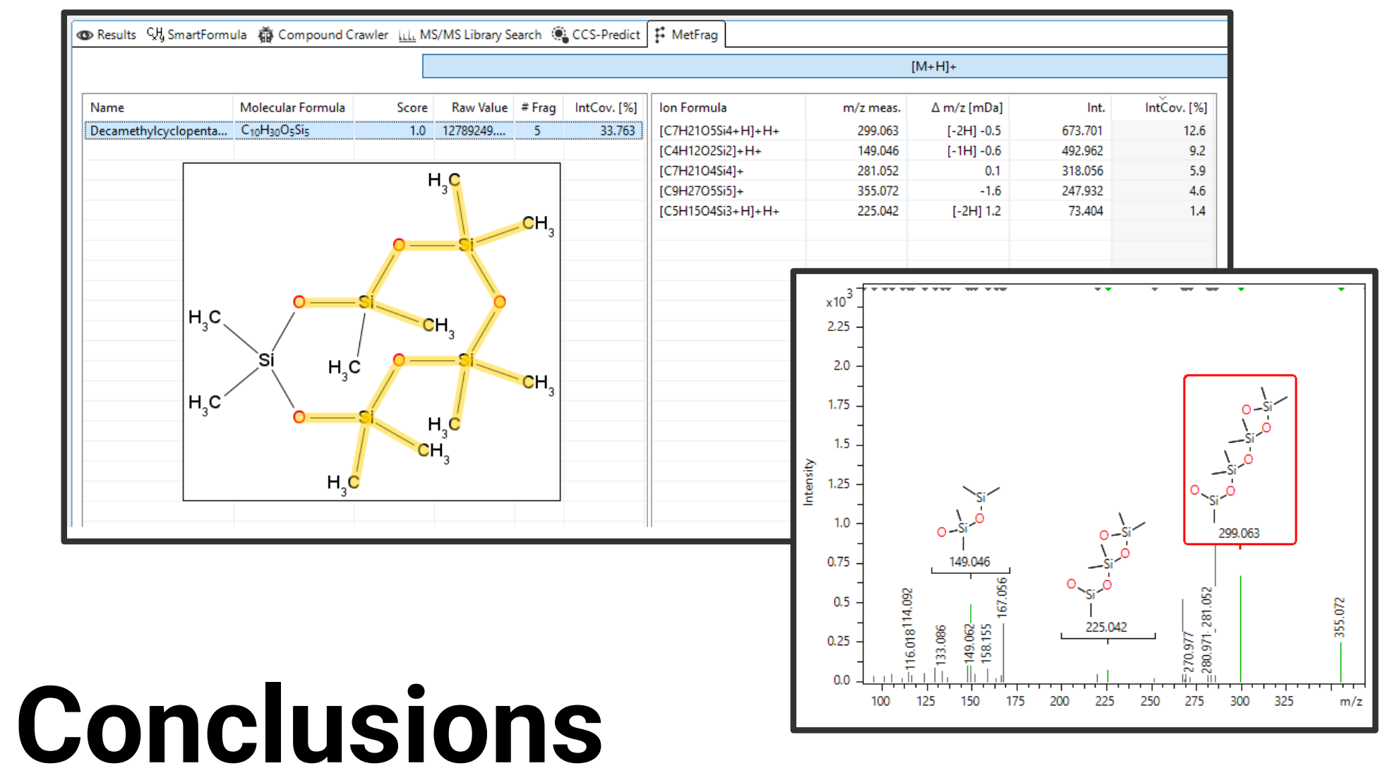
Identification of unknowns using various tools (cont.)

Automated calculation of potential elemental composition with SmartFormula. Selection of potential molecular formula.



Public database structure search based on derived formula using CompoundCrawler





In-silico fragmentation via MetFrag for MS/MS spectra matching. Resulting confirmation can lead to feature annotation and addition to in-house library for faster feature identification in future experiments.

Conclusions

- **DART-QTOF** provided a fast method of analysis with each sample taking less than ~ 1 minute to swab/desorb and then 6 seconds to ionize.
- Using the nontargeted software platform, **MetaboScape**, unique features were identified and used to generate a PCA plot. Showing that the extract from the Sonicated Sprayer groups closely to the extract from the New Sprayer. Some other uses can include…
  - ➢ Synthesis labs – verify that glassware/equipment are free of cleaning agents or previously used reagents.
  - ➢ Manufacturing/Production labs – Confirm that equipment is clean of cross-contaminants from previously manufactured compounds.
- Other feature identification tools (spectral library matching, SmartFormula, CompoundCrawler, and MetFrag) were also used to identify contaminants such as **TCEP** (common plasticizer) and **Decamethylcyclopentasiloxane** (extremely common in personal care products and industrial lubricants).