SICRIT®-HRMS for Targeted Exposomic-Metabolomic Research through Direct Respiratory Analysis

Summary

We demonstrate an extension of conventional capabilities for the SICRIT® ionization source, coupled to a high-resolution MS instrument, through a metabolomic-based breath analysis and how efficient it is to conduct targeted studies of dynamic metabolomic profiles, of the lungs, for an individual person, in real-time. Here, the SICRIT® ionization technology can allow for real-time monitoring of an individuals' reaction to inhalants (Relvar) and the metabolism of such inhalants in the respiratory tract. This use-case provides a new application for the SICRIT® ionization technology.

Introduction

The field of exposomics and metabolomics are closely intertwined as we begin to uncover novel exposomes and their metabolic progression. Since the world of exposomics can become rather convoluted in its definition, we hold that the study of exposomics is the measure of all exposures of an individual in a lifetime and how these exposures effect overall health. This includes everything from toxic industrial chemicals to therapeutic pharmaceuticals and cosmetics. The average human is exposed to over 700,000 chemicals a day, where only a handful are purposeful. All these chemicals must be processed and metabolized by the enzymes within our cells, where each iteration of biotransformations decreases the hydrophobicity (and hopefully the toxicity), rendering an inert, water-soluble xenobiotic. For this process, the lungs can be the prime target of exposomes, both toxic and therapeutic. Rapid metabolism and absorption of most exposomes occurs within the lung endothelium due to an increase in surface area and increased volume of CYP450 enzymes within the tissue. With the rapid nature of these metabolic processes, we are often unable to effectively measure the minute changes that occur in real-time, this inability to do real-time monitoring in most methods presents an issue for those interested in such observations. However, with the SICRIT® ionization technology we are able to perform real-time online monitoring, which allows us to view dynamic systems, unlike other ionization methods. This provides a completely new way to study the world of metabolomics, particularly, exposomics. Additionally, with the SICRIT® ionization technology there is a broad range of ionizability from non-polar to highly polar compounds, which would allow for many different exposome classes to be targeted within a breath-lung analysis study. The source not only expands the range of novel compounds that could be detectable, but also due to the closed geometry of the system there is an increase in sensitivity. This could allow for the observation of trace exposomes in both a targeted and untargeted analysis.

For this particular experiment, we focused on monitoring the pharmaceutical absorption of Relvar and how the targeted compounds, along with the metabolic profile between different collection intervals differ.

Experimental Setup

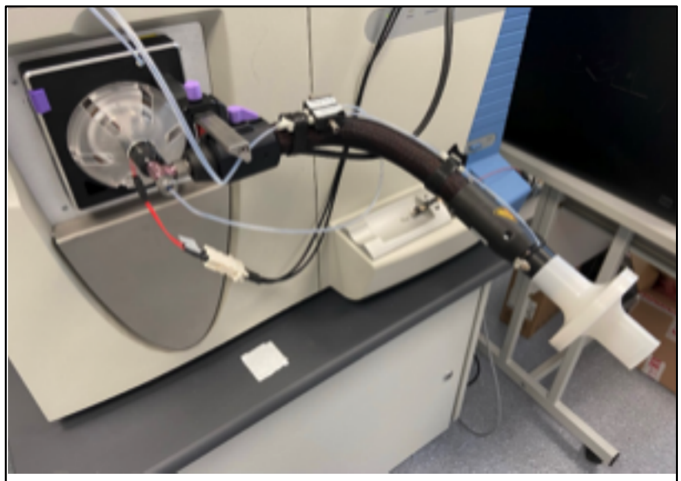

Image 1: Experimental Set-up of the SICRIT Breath Analysis Module in Diluted Breath Mode.

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The SICRIT® ionization source was interfaced with the atmospheric pressure inlet of a high-resolution mass spectrometer (Thermo Fisher LTQ Orbitrap XL), which was constantly drawing air through the source. In our tests, we used a SICRIT® Breath Analysis Module for transfer of the exhaled breath into the source (Image 1). The setup consisted of a deactivated solid steel tube encased in a heated hose connected to the ion source. To avoid condensation, the transfer tube was heated to 150°C. The exhaled air flow was kept at 7.5 L/min under a nitrogen overflow introduced via a Swagelok T-piece. For breath sampling, disposable mouth pieces with a reverse breathing lock were used. MS detection was performed in full-scan positive mode with a resolution of 30,000 FWHM (mass range 50-1000 m/z) and spectra were evaluated using a 10 ppm window. For analysis, two consecutive exhalation replicates for each stage (Control, Relvar, and after Relvar flushing) were recorded and analyzed using an internal workflow within Python (Fig 1).

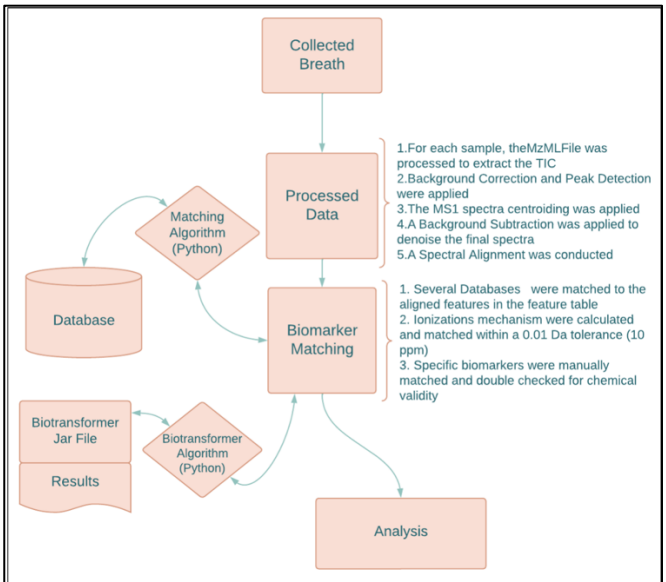

Fig. 1 : A python workflow was established with multiple steps to not only process the data received from the experiment, but to also derive some meaning for the features found.

The workflow incorporates an automated TIC processing module, where the processed features are then matched against several open-sourced databases. Once completed, those matched features were put through BioTransformer to see if the resulting transformations matched any of the remaining features that did not find a match in the first round. This allows us to view both experimental results and predicted results. For this experiment however, we focused on a targeted approach and as a result did not employ the BioTransformer module.

Results and Discussion

An initial look at the preprocessed data in the form of a PCA allows us to view how closely correlated the features are between the duplicates (Fig. 2). Here we are able to see that there are three distinct clusters that can be observed, which means that there exists reproducibility with our method.

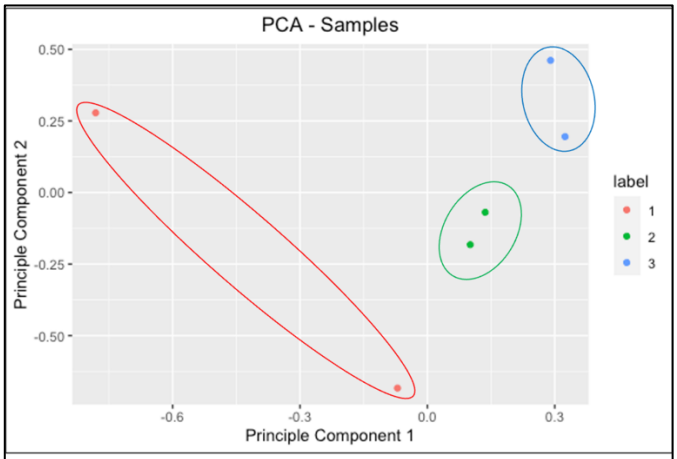

Fig. 2 : Duplicate samples for each time period were collected. Here it is possible to see two, even three separate clusters where the blue and green refer to the flush and medication stage, respectively. The red is the control.

Diving into an overall view of the features or masses present in the data we employed the same technique as with previous breath analyses where we observe the distribution of masses across a spectrum. Here, the entire metabolic profile was captured at each stage of the study (Control, Relvar, Relvar Flush), which allows for a direct comparison of the metabolite distributions, providing insight into the shifts that may occur in real-time (Fig. 3).

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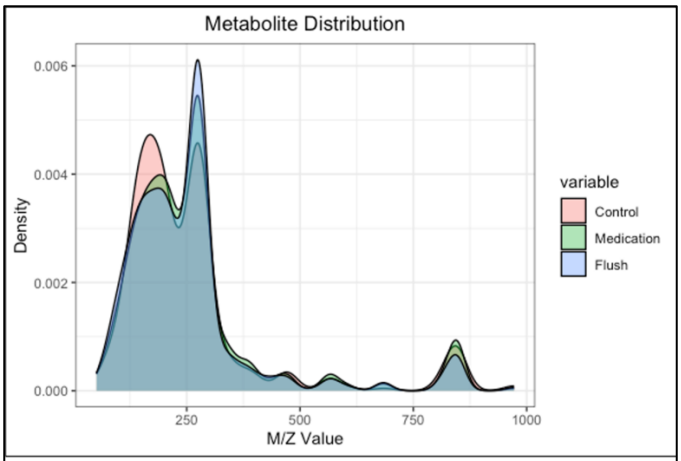

Fig. 3 : The differences in metabolite distribution depending on the sample time.

Here you can see that after the Relvar is taken there is a distinct shift in metabolite masses to the right, toward the masses of our target compounds.

The overarching goal of this experiment goes beyond the mapping of a metabolic profile. Essentially, we wanted to determine how well we could observe the metabolic changes of xenobiotics occurring within the lungs of specific compounds, particularly the active components within Relvar and their metabolites, respectively. Relvar, a pharmaceutical to treat asthma, contains two active ingredients: Vilanterol and Fluticasone Furoate. It should be noted that the understanding of how these two compounds metabolize is not fully understood and as a result that leads towards minimal targeted analysis and into an un-targeted approach for future studies. However, with the increased ionization range and sensitivity, we were able to find two known biotransformations at the proper targeted mass and observe the dynamic changes between each stage of study (Fig. 4).

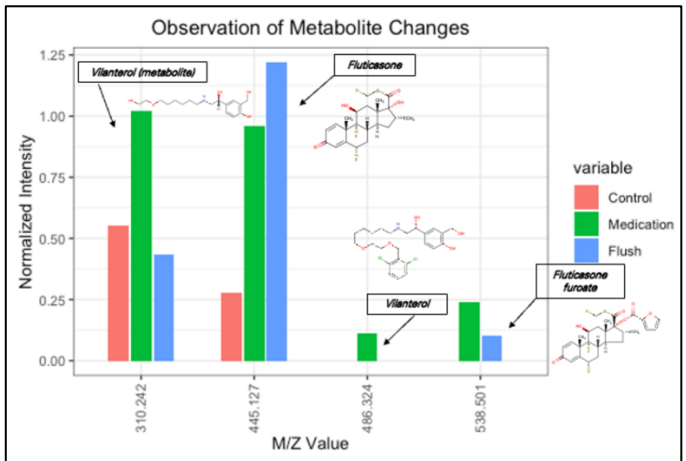

Fig. 4: The levels of each normalized peak corresponds to four different components present within either the medication itself or a metabolite from an active ingredient.

What makes this observation interesting is that with our technique we can validate those lesser-known metabolites that have been theorized or briefly observed, which is remarkable. Additionally, not only can we observe these metabolites, but we can find the relative abundances of each component depending on when the measurement was taken. For instance, we see a rise in all four compounds after medication introduction and flushing, which makes sense in terms of the time frame, where even the biotransformed metabolites (Fluticasone) increase after taking Relvar, which, again, makes sense since Fluticasone furoate has to be metabolized into Fluticasone (Fig. 4). This is an extremely important observation because with this knowledge we can begin to understand which metabolite are prevalent before medication, present immediately after inhalation, and those that metabolize after a set time, leading into kinetic studies of metabolism, observed in real-time.

Ultimately, from this experiment we have shown how you can view the differences and conduct preliminary targeted analysis by treating the data, observing general distribution trends in time-related changes, then going into detail to observe the changes of your target compounds.

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Conclusion

With the use of the SICRIT® ionization technology we can expand the typical breath analysis into a dynamic real-time analysis of metabolic changes of xenobiotics within the respiratory tract. This is possible with the broader ionization capabilities and increased sensitivity of the SICRIT® ionization source, as mentioned previously. From here, we can branch into exciting new territories of both exposomic and metabolomic research, where real-time observation can be conducted to provide powerful insights into metabolism for both targeted and untargeted respiratory analysis.

Acknowledgements

References

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[2] *DrugBank online: Database for Drug and Drug Target Info https://go.drugbank.com/ (accessed Apr 14, 2023).*

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