SICRIT®-HRMS for Metabolic Profiling through Direct Breath Analysis

Summary

We demonstrate an easy extension of conventional capabilities for the SICRIT® ionization source, coupled to a high-resolution MS instrument, through a metabolomic-based breath analysis and how groups of target masses can be isolated and analyzed for potential biomarker identification. Additionally, we can see the sensitivity and broad ionization capabilities of the source through a comparison of different subjects, where the subtle differences in everyone's metabolic profile is given by the spectral distribution of metabolite density.

Introduction

The field of metabolomics along with experimental applications is vastly growing, where most questions and applications require the study of multiple biological systems. This is often observed from a holistic perspective, where the entire range of the metabolome is studied at once to determine patterns and changes between set conditions, with the result being a list of biomarkers that differentiate one state from another. One application that is gaining popularity in this regard, especially in the field of medical diagnostics is the use of breath metabolomic analysis. However, to effectively perform these experiments and determine biomarkers, a non-targeted approach is needed, with broad spectrum analysis of the entire breath profile. The issue that often arises from such studies is from the inherent nature of metabolomic processes; they are often rapid and to properly study the changes would require real-time analysis of these processes. Furthermore, these processes include reactants and products that range in both mass and polarity, which requires a measurement technique that can handle such a wide range of chemical diversity. To circumvent these pitfalls, it is necessary to have a technique that can not only provide real-time analysis, but also be able to measure a dynamic range of analytes. It is here that we present such a solution, with the SICRIT® ionization technology.

With the SICRIT® ionization technology there is a broad polarity range, where both non-polar and highly polar metabolites within the breath can be ionized, which enriches the overall metabolic distribution, expanding the potential compounds of interest and providing new insights into novel profile groupings for biomarker discovery. Additionally, with the source acting as an extension of the MS inlet capillary, the closed geometry of the system prevents the loss of charged species, increasing the sensitivity and visibility of compounds that could have been previously overlooked in conventional ionization techniques. Furthermore, with the source you can do direct analysis of a complex sample making breath analysis something that can be easily collected in real-time with the SICRIT ionization source and a simple apparatus set-up, without the need to consider storage or sample degradation.

Experimental Setup

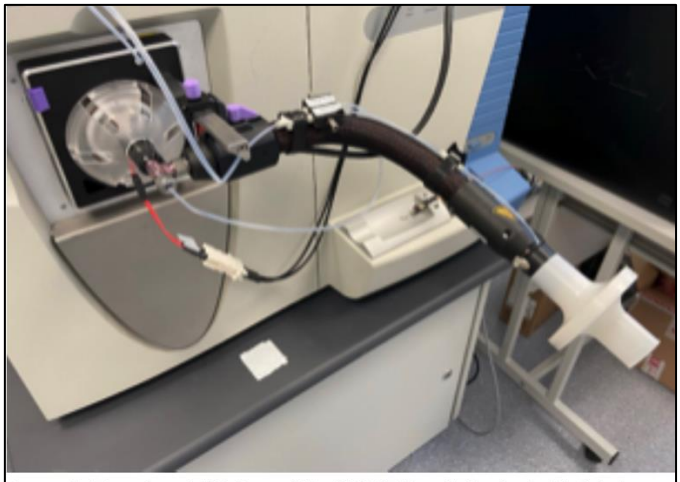

Image 1: Experimental Set-up of the SICRIT Breath Analysis Module in Diluted Breath Mode.

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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 monitored with a flowmeter where a disposable mouth piece was used for breath sampling. MS detection was performed in full-scan positive mode with a resolution of 25,000 FWHM (mass range 50-1000 m/z) and spectra were evaluated using a 10ppm window. For analysis, three consecutive exhalation replicates for each of the 5 test subjects were recorded and analyzed using an internal workflow within Python (Fig. 1).

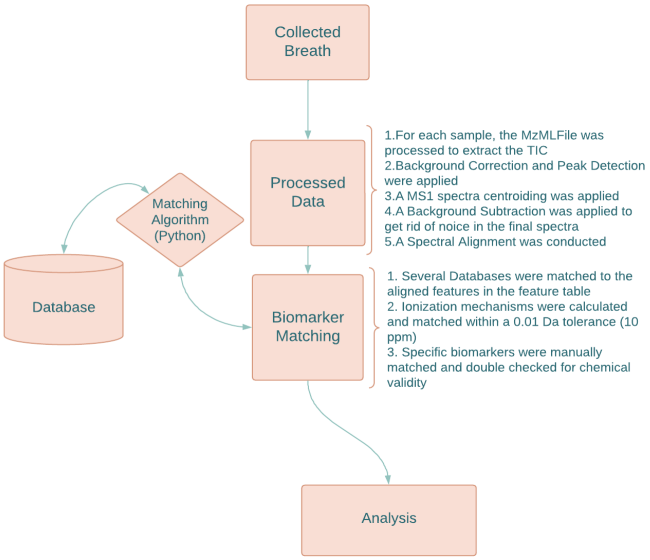

Figure 1: The python pipeline used to perform processing and analysis of the received data.

The workflow incorporates an automated TIC processing module, where the processed features are then matched against several open-source 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.

Results and Discussion

An initial look at the processed data through a standard density plot provides insight into the distribution of different metabolites for everyone's averaged replicates. Here, there are two immediate observations that can be determined; firstly, being that everyone has distinct metabolic differences that can be captured through direct analysis and secondly, that the range of possible metabolites detected is up to 1000 Da. This would effectively allow us to define the differences between individual breath profiles, where we can perform non-targeted analysis to identify potential biomarkers that span a larger range, providing useful applications for metabolomic and lipidomic studies.

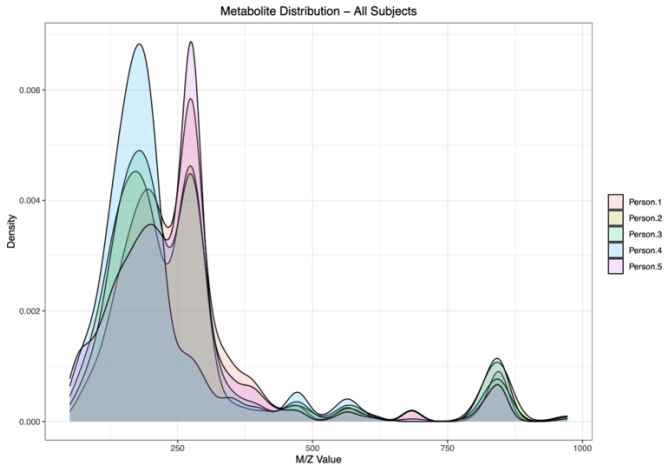

Figure 2: The distribution of M/Z Values for each individual's entire MS1 profile in the form of a density plot.

A deeper look into the MS1 data provides further insight into the categories of compounds that are often used for biomarker discovery and untargeted analysis. In a preliminary fashion, we were able to find several masses that matched to many Amino Acids and Hormone molecules that are often used in metabolomic or lipidomic analysis (Figure 3). This shows that with a broad, untargeted approach, we can identify potential

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compounds of interest that range from small polar to ionic molecules to larger, bulkier non-polar compounds, allowing us to effectively provide a larger range of compounds to study, even at the MS1 level.

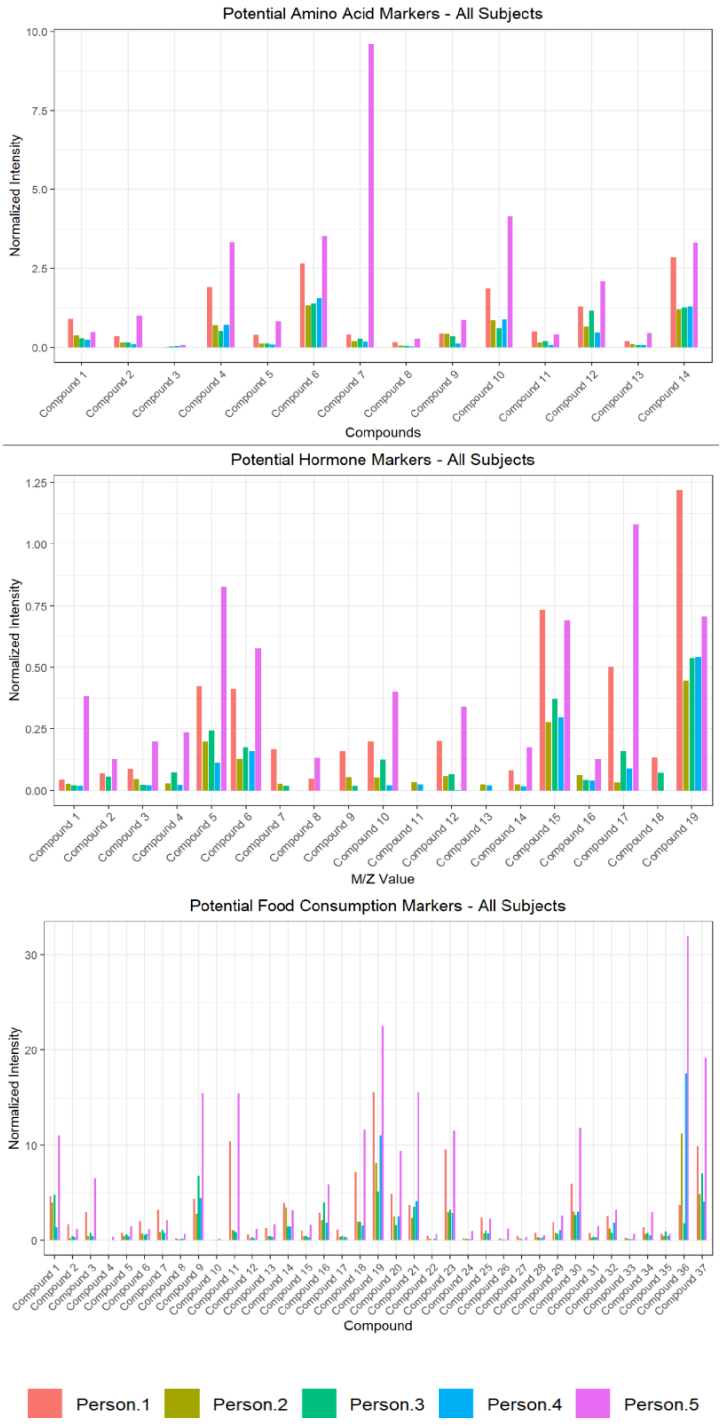

Figure 3: The normalized intensities of potential compounds of interest for three separate groups; small molecule amino acids, hormone compounds, and potential food biomarkers. See supplementary tables for tentative compound assignments.

Additionally, we analyzed the data through the lens of a holistic untargeted approach, where instead of looking at specific masses, we coupled our measurement results with a database matching pipeline (Figure 1) to determine a preliminary overview of the types of compounds that we are potentially ionizing. What we were able to find was a nice distribution of small molecule volatiles that splay across multiple compound classes of potential interest, such as Organic acid and derivatives, which are a super class of compounds that contain all the small molecule compounds found in all the major metabolic pathways, and are important compounds of interest in biomarker identification, particularly for disease and inflammatory mechanisms.

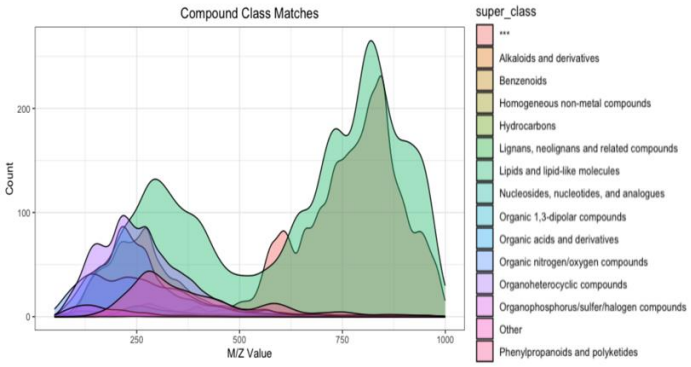

Figure 4: The compound class distributions that matched from the M/Z Values to the mono-isotopic masses found within HMDB can be viewed as a density plot.

Conclusion

With the use of both the SICRIT® ionization technology and an internal spectra processing pipeline we can generate a comprehensive breath metabolite profile and compare across multiple individuals. With the broader ionization capabilities and higher sensitivity, we can

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further exploit the subtle nuisances that differentiate each subject, which can be seen at the MS1 level and how certain M/Z values change between different individuals. This allows for a targeted analysis of groups of compounds to differentiate individuals and select for particular compounds of interest. Furthermore, we can apply this approach from a nontargeted perspective, where we can easily combine the use of experiment and analysis to determine the types of compounds being ionized and coming from breath, ranging beyond the volatile level. This could allow for novel biomarker identification or groupings of biomarkers, leading to the expansion or discovery of new fields of diagnostics. In the future, we hope that by combining both experimental and computational design we can efficiently process the additional ionized species produced by SICRIT®, allowing us to take full advantage of the technology has to offer from a computational perspective.

References

[1] Liang, S.; Nasir, R. F.; Bell-Anderson, K. S.; Toniutti, C. A.; O'Leary, F. M.; Skilton, M. R. Biomarkers of dietary patterns: A systematic review of randomized controlled trials

[2] Dhama, K.; Latheef, S. K.; Dadar, M.; Samad, H. A.; Munjal, A.; Khandia, R.; Karthik, K.; Tiwari, R.; Yatoo, M. I.; Bhatt, P.; Chakraborty, S.; Singh, K. P.; Iqbal, H. M.; Chaicumpa, W.; Joshi, S. K. Frontiers in Molecular Biosciences ***2019****, 6.*

[3] Home https://reactome.org.

Supplementary Information

Potential Amino Acid Markers	
Label	Compound
Compound 1	Alanine
Compound 2	Asparagine
Compound 3	Cysteine
Compound 4	Glutamine
Compound 5	Histidine
Compound 6	Isolucine, Leucine
Compound 7	Lysine
Compound 8	Methionine
Compound 9	Phenylalanine
Compound 10	Proline
Compound 11	Serine
Compound 12	Threonine
Compound 13	Tryptophan
Compound 14	Valine
Potential Hormone Markers	
Label	Compound
Compound 1	11beta-Hydroxytestosterone
Compound 2	16alpha-Hydroxyestrone
Compound 3	21-Deoxycortisol
Compound 4	3alpha,20alpha,21-Trihydroxy-5beta-pregnan-11-one
Compound 5	5alpha-Pregnane-3alpha,20alpha-diol, Pregnanediol
Compound 6	5beta-Dihydrotestosterone
Compound 7	Adrenosterone
Compound 8	Aldosterone, Cortisone
Compound 9	Androsterone
Compound 10	Cholesterol
Compound 11	Corticosterone
Compound 12	Cortisol
Compound 13	Cortol
Compound 14	Cortolone
Compound 15	Estradiol, Estradiol-17alpha
Compound 16	Estriol
Compound 17	Estrone
Compound 18	Progesterone
Compound 19	Testosterone
Potential Food Markers	
Label	Compound
Compound 1	4-Ethylcatechol
Compound 2	4-Methoxycinnamic acid
Compound 3	5_Acetylamino_6_amino_3_methyluracil
Compound 4	Anserine
Compound 5	Butyrylcarnitine
Compound 6	Caffeine
Compound 7	Carnitine
Compound 8	Carnosine
Compound 9	Cinnamic acid, Methione

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Compound 10	Cinnavalininate
Compound 11	cis-Piceid
Compound 12	Conjugated Linoleic Acid
Compound 13	Creatine
Compound 14	Cytosine
Compound 15	Dide-O-methylsimmondsin
Compound 16	Erythrose
Compound 17	Ethyl Acetate, Isobutyric acid
Compound 18	Ethyl butanoate, Phenylacetaldoxime
Compound 19	Glycerol
Compound 20	Hexanal, Tiglic Acid
Compound 21	Isobutyric Acid
Compound 22	Kynurenine
Compound 23	Linalool oxide
Compound 24	Melatonin
Compound 25	Oxalic Acid - Dimer
Compound 26	p-crestol
Compound 27	Paraxanthine
Compound 28	Picolinic Acid
Compound 29	Pyruvate?
Compound 30	Ribose
Compound 31	Skatole
Compound 32	Succinic Acid
Compound 33	Taurine
Compound 34	Theobromine
Compound 35	Theophylline
Compound 36	Tiglic acid
Compound 37	Tyrosine

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