Enhanced Metabolite Profiling: Hard and Soft Ionization High Resolution Time-of-Flight Mass Spectrometry

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1. Introduction

Metabolomics and its toolset provide a foundation for quantitative biology and are indispensible for the detection of small molecules produced and/or transformed in the cells of living organisms.[1,2] Different estimates indicate that a majority of differentially expressed analytes remain unknowns. The high sensitivity, peak capacity and reproducibility of GC-MS have made it one of the most widely used techniques for plant and animal metabolite profiling. Time-of-flight mass spectrometry (TOFMS) provides additional benefits such as reduced analysis times, effective peak deconvolution and an ability to interrogate rich data sets repeatedly for novel materials. In addition, high resolution TOFMS instruments reduce matrix interferences and allow for production of high-quality, accurate mass data for robust formula determinations and library database comparisons. This application note demonstrates the value of both electron impact ionization (EI) and chemical ionization—high resolution time-of-flight mass spectrometry (HR-CI) in identifying analytes in these complex metabolomic samples. These ionization techniques result in a more confident identification of metabolites through the complementary information. The labile nature of TMS-derivatized metabolites and homologous nature of biological molecules make the need for molecular ion detection critical in metabolomics.

2. Results and Discussions

GC-MS compound identification in metabolomics relies primarily on retention indices and mass spectral library matching. In this study, the workflow included analysis by EI HR-CI to obtain a comprehensive profile for a mouse liver extract sample (Figure 1). Mass accuracies near 1 ppm for detected features resulted in robust elemental composition determinations for molecular, fragment, and adduct ions. Quality EI data facilitated searches against nominal mass libraries as evident by the similarity values for the representative set of compounds in mouse liver extract (Table 1). Similarity values for these trimethylsilyl derivatives ranged from 831 to 919 out of a possible score of 1000 in the absence of leveraging the mass accuracy.

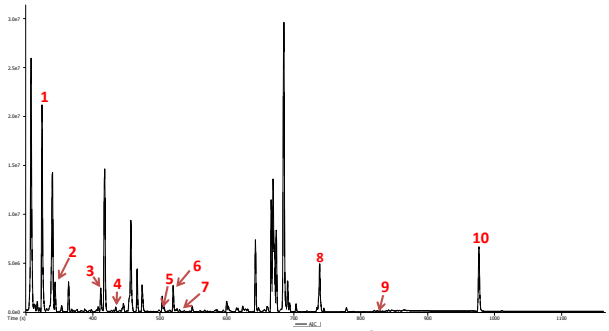

Figure 1. Analytical Ion Chromatogram (AIC) of Mouse Liver Extract (EI).

Table 1. Representative Compounds in Mouse Liver Extract.

Peak #	Name	Formula	R.T. (s)	Similarity
1	Lactic acid, bis(trimethylsilyl)oxy-, ester	$C_9H_{22}O_3Si_2$	324	**916**
2	l-Alanine, N-(trimethylsilyl)-, trimethylsilyl ester	$C_9H_{23}NO_2Si_2$	344	**904**
3	N,O-Bis-(trimethylsilyl)valine	$C_{11}H_{27}NO_2Si_2$	394	**831**
4	Glycine, N,N-bis(trimethylsilyl)-, trimethylsilyl ester	$C_{11}H_{29}NO_2Si_3$	434	**919**
5	DL-Malic acid, O-trimethylsilyl-, bis(trimethylsilyl) ester	$C_{13}H_{30}O_5Si_3$	504	**874**
6	L-Proline, 5-oxo-1-(trimethylsilyl)-, trimethylsilyl ester	$C_{11}H_{23}NO_3Si_2$	520	**915**
7	Erythronic acid, tetrakis(trimethylsilyl) deriv.	$C_{16}H_{40}O_5Si_4$	529	**888**
8	Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, D-	$C_{21}H_{52}O_6Si_5$	643	**911**
9	Arachidonic acid, trimethylsilyl ester	$C_{23}H_{40}O_2Si$	779	**859**
10	Cholesterol trimethylsilyl ether	$C_{30}H_{54}OSi$	977	**906**

Peak True (Deconvoluted) and NIST library spectra for two of the compounds, 5-oxo-proline and cholesterol, are shown below (Figures 2 and 3). The presence of molecular ions in these spectra make identification even more confident. Mass accuracy values for these TMS derivatives were 0.72 and -0.29 ppm respectively.

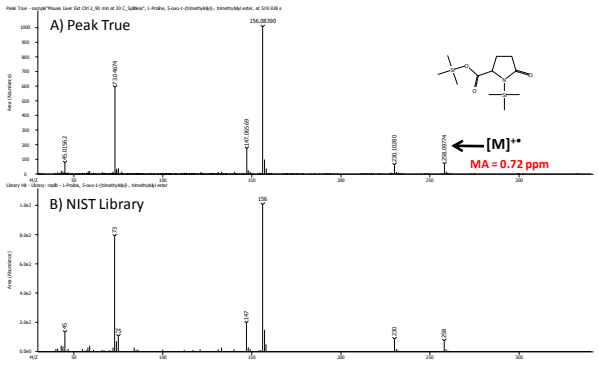

Figure 2. Peak True (A) and NIST library (B) Mass Spectra for the TMS Derivative of 5-oxo-Proline.

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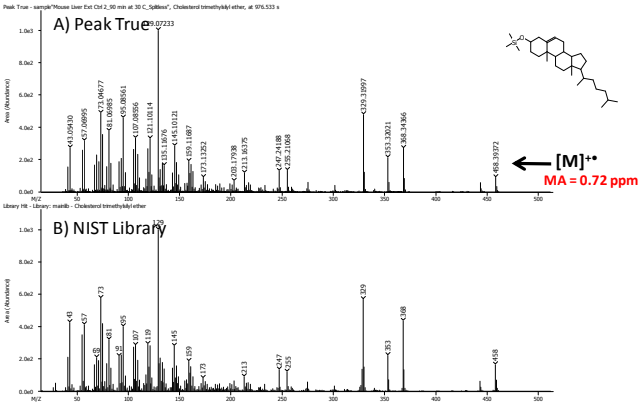

Figure 3. Peak True (A) and NIST Library (B) Mass Spectra for the TMS Derivative of Cholesterol.

Characterizing labile molecules present in low concentrations can often be difficult due to poor spectra and/or a lack of molecular ions in their mass spectra. This problem is addressed by soft ionization and accurate mass formula generation. In particular the utilization of soft and hard ionization methods with TMS derivatization offers a more reliable means for comprehensive metabolite profiling.[3] An example of the utility of HR-CI is illustrated by the TMS derivative of 1,5-Anhydroglucitol (1,5-AG), a marker used to identify glycemic variability in individuals with type 2 diabetes.[4] The EI library similarity for 1,5-AG (TMS) is marginal (679/1000) and its molecular ion (452.22603) is absent from the Peak True mass spectrum (Figure 4, Top). By comparison the subsequent analytes in the list of possible hits are Galactopyranoside TMS (628/1000), Arabinofuranoside TMS (628/1000) and a glucopyranose derivative (625/1000), each of which have very different molecular formula (10s of amu) than 1,5-AG. Below the HR-CI spectrum for the proposed 1,5-AG, TMS (Figure 4, Bottom) shows the intact protonated molecular ion at m/z = 453.23334 corresponding to a formula of $C_{18}H_{45}O_5Si_4$ whose mass error is -1.1 ppm. This additional soft ionization and accurate mass information allowed a confident identification of this analyte that would not have been possible with an EI spectrum and library match alone.

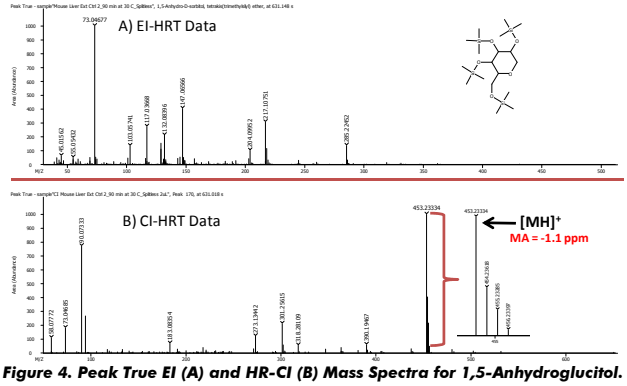

Figure 4. Peak True EI (A) and HR-CI (B) Mass Spectra for 1,5-Anhydroglucitol.

3. Conclusions

The GC-HRT is ideal for profiling metabolites in complex biological matrices. The combined utility of CI with EI ionization have demonstrated the clear identification of 1,5-AG. While the EI data supported the possible identification, the unequivocal match of the pseudo-molecular ion to the 1,5-AG formula removed any doubt on its identification and clearly differentiated it from the other matches to the EI spectrum. This approach provides a reliable mechanism for analyte identification and confident interpretation of biochemical pathways, particularly when analytes are differentially expressed. High quality spectral data and excellent mass accuracy values allowed for confident identification of most analytes; however, the addition of HR-CI data facilitated the characterization of low-level, labile compounds in samples.

4. References

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5. Sample Preparations

Samples were derivatized in two steps: 1) 20 µL of freshly prepared methoxylmaine hydrochloride (20 mg/mL in pyridine) was added to extracts (30°C for 90 min.); and 2) 45 µL of BSTFA with 1% chlorotrimethylsilane (37°C for 30 min.).

6. Experimental Conditions

Gas Chromatography

GC-MS analyses were performed using a LECO Pegasus® GC-HRT with an Agilent 7890A gas chromatograph and 7693 autosampler. Method details are provided in Table 2. HR-CI data was collected using a wider mass range, reagent gas consisting of 5% ammonia in methane, and PFTBA as an internal calibrant.

Table 2. GC-HRT Instrument Parameters

Injection	1 µL (2 µL CI), 250°C
Carrier Gas	He @ 1.0 mL/min.
Column	Rxi-5Sil MS, 30 m x 0.25 mm x 0.25 µm (Restek, Bellefonte, PA, USA)
Temp. Program	50°C (1 min.) to 320°C (5 min.) at 20°C/min.
Transfer Line	300°C
Ionization Energy	70 eV (EI), 140 eV (CI)
Mass Range (m/z)	30-510 (EI), 50-650 (CI)
Acq. Rate	6 sps
Source Temp.	250°C (EI), 180°C (CI)
Mode	High Resolution (R = 25,000)
CI Reagent Gas	5% Ammonia in Methane
Calibration	Internal (PFTBA)