Characterization of Electron Ionization and Chemical Ionization on a Novel High Resolution Gas Chromatography Time-of-Flight Mass Spectrometer—Tools for the Identification of Unknown Metabolites

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Introduction

Gas chromatography with mass spectrometry (GCMS) is a standard for metabolite detection and identification. Historically this analysis relies on established databases for analyte identification. Unknown analyte identification represents a principle challenge to metabolomics, and typically involves the combination of EI (database matching/classification), and CI (molecular formula assignment), to facilitate analyte identification. Here, a suite of analytes representing typical metabolites was used to characterize the performance attributes of importance to metabolomics using EI and CI on a novel high resolution time-of-flight platform. The significance of high performance MS has been a point of emphasis.[1] These attributes will help determine what else is in your samples.

The attributes to be evaluated in EI and/or CI include:

Relative Sensitivity	Dynamic Range
Spectral Matching	Mass Accuracy
Relative Isotope Abundance	Spectral Precision

Methods

A mixture of commercially available compounds (listed below) was created and included acids, diacids, amino acids, polyols, carbohydrates, and others of metabolic significance. The analytes were prepared at concentrations ranging between 0.1 and 10 µg/mL in aqueous solutions, and in a plant extract matrix (Arabidopsis). Portions of the extracts were dried under vacuum then lyophilized to remove residual water. The residue was derivatized using standard protocols including MSTFA and methoxylamine HCl. The derivatized samples were then analyzed by high resolution GC-TOFMS.

Analytes Spiked into Matrix

Pyruvic acid	Shikimic acid	Valine
Creatine	Citric acid	Alanine
Nicotinic acid	Succinic acid	Putrescine
4-Hydroxyproline	α-ketoglutarate	Salicylic acid
Stearic acid	Arachidic acid	α-tocopherol
Cholersterol	Glucose-6-Phosphate	Asparagine
Glucose	Glutamic acid	Sucrose
Lysine	Serine	Methionine

High Resolution GC-TOFMS

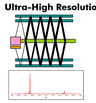
Chromatographic Parameters

- GC: Agilent Technologies 7890 and Gerstel MPS 2 Autosampler
- Column: Restek Rxi-5Sil MS (30 m x 0.25 mm x 0.25 µm) + 5 m Guard
- Carrier/Flow: He, 1.0 mL/min
- Injection: 1 mL, Splitless (CI: 2 mL, Splitless)
- Inlet Temp.: 250°C
- Temp. Prog.: 50°C (1 min hold) to 330°C (20°C/min, 5 min hold)

Mass Spectrometry Parameters

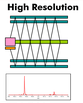
- Transfer Line Temp.: 300°C
- Ion Source Temp.: 250°C (CI: 180°C)
- Electron Energy: EI (70 eV); CI (140 eV)
- Range (m/z): 60 to 520 (CI: 100 to 1200)
- Flight Path: High Resolution (R = 25,000)
- Mass Calibration: PFTBA (Internal)
- CI Reagent Gas: 5% Ammonia in Methane

Pegasus® GC-HRT



Ultra-High Resolution

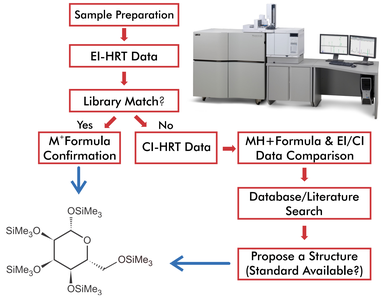
R=50,000
4:1 mass Range



High Resolution

R=25,000
m/z = 10 - 1500

Electron Ionization/ Chemical Ionization Workflow



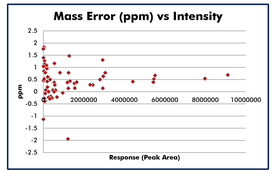
A representative workflow (above) shows the use of EI and CI data in the elucidation of unknown metabolites. This workflow forms the basis of unknown identification using high resolution GC-TOFMS. System performance attributes are key to its success. The combination of EI fragmentation for database searching and structural characterization and CI for molecular ion are leveraged for formula determination.

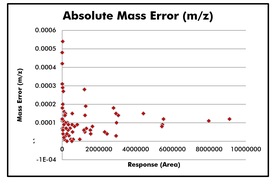
Mass Accuracy and Spectral Precision

Mass accuracy and spectral precision form the basis of high resolution GC-TOFMS analysis. Shown below are mass accuracies and between spectra reproducibilities from EI analyses for representative analytes. These attributes facilitate consistent selectivity and quantitation and provide more confidence that a specific analyte is being detected in the complexity of a typical metabolomic sample. Average mass errors of <1.8 ppm for ions even as low as m/z 74.0361 provide robust confidence in the selectivity of the analysis and the chemical nature of fragment ions.

					Mass Precision			
Metabolite	Tryptophan	Tyrosine	Lysine (3TMS)	Cholesterol	Octanoic Acid (Methyl ester)	Myoinositol	Glucose (5TMS)	Glutamine (4TMS)
	202.1045	218.1026	156.1202	129.0728	74.0361	147.0654	204.0996	147.0655
	202.1045	218.1027	156.1202	129.0728	74.0362	147.0654	204.0995	147.0655
	202.1045	218.1026	156.1201	129.0728	74.0362	147.0654	204.0996	147.0655
	202.1045	218.1027	156.1201	129.0728	74.0361	147.0653	204.0995	147.0655
	202.1045	218.1026	156.1200	129.0728	74.0361	147.0653	204.0996	147.0655
	202.1046	218.1026	156.1200	129.0728	74.0361	147.0653	204.0995	147.0655
	202.1045	218.1026	156.1202	129.0728	74.0360	147.0653	204.0994	147.0654
	202.1045	218.1026	156.1200	129.0728	74.0361	147.0653	204.0995	147.0656
	202.1043	218.1026	156.1201	129.0728	74.0362	147.0653	204.0994	147.0654
Average (mass)	**202.1045**	**218.1026**	**156.1201**	**129.0728**	**74.0361**	**147.0653**	**204.0995**	**147.0655**
std dev (ppm)	**0.3566**	**0.2050**	**0.4139**	**0.1455**	**0.6303**	**0.2040**	**0.3093**	**0.2611**
ppm (error)	**0.8150**	**0.4817**	**1.2757**	**1.8250**	**1.5608**	**-1.0993**	**0.5660**	**0.7339**
Theoretical	**202.1047**	**218.1027**	**156.1203**	**129.0730**	**74.0362**	**147.0652**	**204.0996**	**147.0656**

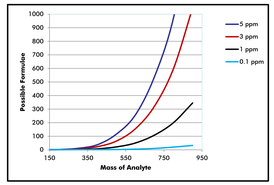
Mass Accuracy





Mass accuracy is critically important in the determination of formula or elemental composition. The lower the mass error, the smaller the number of possible formulae. This is particularly important in the application of CI for formula calculations. Here mass errors, both absolute (mm/z; 0.0001 Th) and relative (ppm) are shown from the GC-HRCI-TOFMS analysis of the suite of analytes spiked into *Arabidopsis* extract. Data from 0.1 up to 5 µg/mL are included and plotted versus response (96 data points). Even at VERY low signal intensity the observed relative mass errors are below 2 ppm. The absolute mass errors clearly demonstrate the need for robust measurement to 0.1 mm/z.

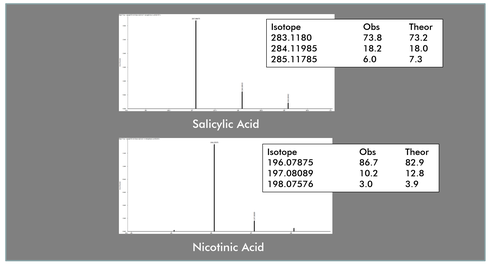
	Mass (mm/z)	Error (ppm)
Average	0.13	0.79
s.d.	0.17	1.25



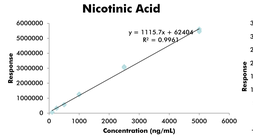
The significance of achieving low mass error is shown in the figure to the left which plots possible formulae vs m/z. The difference between 1 ppm and 5 ppm at 500 m/z is nearly 10 fold. Even a difference between 1 and 2 ppm will more than double the number of possible formulae.

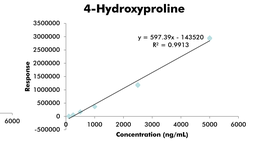
Relative Isotope Abundance

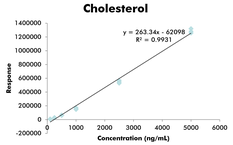
A determining factor in analyte identification is the ability to confirm formulae by isotopic contribution. This is particularly true using CI (HRCI), and is achieved using relative isotope abundance. Spectra and calculated abundances are shown for typical analytes. An error of <5% versus theory is demonstrated here and in others.



Linearity/Dynamic Range







Linear dynamic range is a key attribute for GCMS in metabolomic studies. This facilitates the detection of low-level unknowns which can then be further characterized. Here, the linearity of EI on the GC-HRT is demonstrated over a meaningful range of concentrations (0.05 to 5 µg/mL). The three analytes chosen (as TMS derivatives) provide different challenges to the system, but all show robust R^2 values in excess of 0.99 in a sample matrix.

Analytical Precision

Quantitative analysis is one of the strengths of EI and GCMS analysis. The ability to have precise analytical measurements allows for robust differential analysis in metabolomics. Here, the reproducibility of the measurement of several representative analytes are provided. The relative standard deviations for these range from 1.5 to 6.6% for 9 replicates. This allows for robust measurement of modulations of significance in metabolomic studies.

					Area Precision			
Metabolite	Tryptophan	Tyrosine	Lysine (3TMS)	Cholesterol	Octanoic Acid (Methyl ester)	Myoinositol	Glucose (5TMS)	Glutamine (4TMS)
	1209549	2483987	2026899	4625904	992727	241411	2180197	2934464
	1425203	2792543	1877508	4538291	1013148	251722	2326933	2626833
	1412940	2889500	2056316	4546540	1004118	249624	2327120	2603148
	1317243	2845047	2031254	4470378	982369	235453	2300872	2658024
	1317243	2785601	1985213	4435722	973077	230425	2264000	2469767
	1353960	2785601	1985213	4435722	973077	230425	2264000	2469767
	1357198	2813089	2007735	4456000	978288	240773	2289555	2583257
	1381092	2817299	2000210	4456000	987888	235517	2240697	2927248
	1382537	2807658	1986909	4320115	966635	226633	2275531	2525710
Average (area)	**1350774**	**2780036**	**1995251**	**4476075**	**985703**	**237998**	**2274323**	**2644246**
%RSD	**4.80**	**4.17**	**2.53**	**1.93**	**1.56**	**3.64**	**2.01**	**6.62**

Spectral Matching

The ability to achieve consistent scoring allows for the analyst to better determine whether an analyte is known or unknown (unsure ID). Spectral matching for representative standards (0.25 µg/mL) spiked into matrix are shown for 9 replicate analyses. Average scores (vs. Wiley/Fiehn) range from 726 up to 963. This consistency allows one to be confident in what is identified and what may require leveraging the mass accuracy of HRCI.

					Similarity Precision			
Metabolite	**Tryptophan**	**Tyrosine**	**Lysine (3TMS)**	**Cholesterol**	**Octanoic Acid (Methyl ester)**	**Myoinositol**	**Glucose (5TMS)**	**Glutamine (4TMS)**
	802	830	722	689	862	825	805	974
	988	586	729	889	906	975	852	974
	786	926	738	891	871	827	901	868
	804	585	738	900	890	869	929	975
	804	898	684	900	869	847	929	975
	691	898	733	900	869	847	929	975
	807	926	729	882	890	841	733	975
	950	832	727	897	874	863	734	974
	924	926	734	878	969	850	833	975
Average (Match)	**840**	**823**	**726**	**870**	**889**	**860**	**849**	**963**

Sensitivity Comparison (EI/CI)

The relative sensitivities of EI and CI on the Pegasus GC-HRT platform were compared using standards between 0.1 and 2.5 µg/mL. The criterion for detection for CI is the ability to automatically detect a pseudomolecular ion ($M+H^+$) and extract its spectrum. For the EI component of this study, all analytes produced matched peaks with scores >700. The results are using 5% ammonia in methane as the reagent gas. The results confirm a general reduced sensitivity for CI vs EI. CI does show good sensitivity in the range examined for many analytes (0.1 µg/mL) but many with significantly higher limits (>2.5 µg/mL). There are several factors which contribute to this observation, including pairing the right reagent gas. In general, the relative sensitivities are appropriate to allow for the detection of analytes using each technique with the need only for a larger injection volume, if that.

Analyte	EI	CI
Pyruvic Acid (MEOX TMS)	0.1	>2.5
Alanine (2 TMS)	0.1	1
Valine (2 TMS)	0.1	0.25
Nicotinic Acid (2TMS)	0.1	0.1
Succinic Acid (2TMS)	0.1	0.1
4-Hydroxyproline (2 TMS)	0.1	0.1
Salicyclic Acid (2 TMS)	0.1	0.25
Creatine (3TMS)	0.1	2.5
Alpha Keto Glutaric Acid (XTMS)	0.1	1
N-Acyl Asp (TMS)	0.1	>2.5
Putrescine (4 TMS)	0.1	0.1
Shikimic Acid (4 TMS)	0.1	1
Stearic Acid (TMS)	0.1	0.1
Arachidic Acid (TMS)	0.1	0.1
Alpha Tocopherol (3 TMS)	0.1	2.5

Conclusions

A mixture of metabolites spiked into matrix was analyzed using both EI and CI modes on a high resolution GC-TOFMS system to characterize its capabilities in facilitating analyte identification. The combination of technologies available in the Pegasus GC-HRT allow the facile and confident determination of metabolite identification. In applying high resolution GC-TOFMS, the metabolomic scientist can address concerns about obtaining critical analytical data and have a tool which has uniquely broad applicability. To determine **what else is in your samples**, the GC-HRT uniquely provides:

Relative Sensitivity (CI vs EI)
Dynamic Range
Spectral Matching
Mass Accuracy
Relative Isotope Abundance
Spectral Precision

References

[1]T. Kind and O. Fiehn, Tobias Kind and Oliver Fiehn , **Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry,** BMC Bioinformatics 2007, 8:105; Tobias Kind* and Oliver Fiehn, **Metabolomic database annotations *via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm,*** BMC Bioinformatics, 7, 234-243 (2006),