# GC-TOFMS Analysis of Urine Extract Samples Used for a Liver Drug-Induced Injury Study

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## 1. Introduction

The interest in comprehensive analysis of the metabolome has increased dramatically in the past decade. No single analytical technique can be used to accomplish this comprehensive study, but using various techniques in a complimentary way can make the task easier and enhance the chances of success. Gas chromatography coupled with mass spectrometry (GC-MS) is one of the techniques that are attractive to scientists involved in studies of the metabolome, due to the very good selectivity and sensitivity that it can provide. The only drawback was the lack of analysis speed. The addition of a Time-of-Flight Mass Spectrometer (TOFMS) with its high-speed capabilities overcame this difficulty. With acquisition speeds of up to 500 spectra/second, lack of spectral skewing, and unique Peak Find and Deconvolution algorithms, the LECO Pegasus® GC-TOFMS facilitates the analysis of thousands of samples every year.

The objective of the study presented here is to develop a suite of toxicity markers from GC-TOFMS analysis, for use in pre-clinical screening of drug candidates. Isoniazid is a drug used in the treatment of tuberculosis and may induce severe hepatotoxicity in treated patients. Hydrazine is produced on exposure to isoniazid, and hydrazine levels can be correlated with the severity of the hepatocellular damage.

*Pilot Study*

A group of rats was dosed by gavage with the studied drug at two levels of concentration (100 and 300 mg/kg) for either one or 14 days. Urine samples from the animals were collected pre-dose at 0 to 6 and 6 to 24 hours following dosage. Aliquots of the urine samples were then extracted with methylene chloride and dried under nitrogen. Eight different solvents and solvent mixtures were tested for efficiency and reproducibility, and methylene chloride was selected based on the study results. Prior to injection into the GC-TOFMS system, the samples were derivatized with 40 µl of MSTFA +1% TMSD at room temperature for one hour, and diluted with 40 µl of hexane.

## 2. Experimental Conditions

GC:
Agilent 6890 GC
Primary Column:
Rtx-5, 10 m, 0.18 mm id, 0.18 µm film thickness
Oven Program:
50°C (0.2 minute hold) to 320°C at 20°C/minute
Inlet Temperature: 270°C
Injection Size:
1 µl with a split ratio of 5:1
Carrier Gas:
He at a constant flow of 1.5 ml/minute

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| MS: | LECO Pegasus® GC-TOFMS |
| Ionization: | EI at 70eV |
| Mass Range (u): | 45 to 600 |
| Acquisition Rate: | 20 spectra/second |
| Source Temp: | 200°C |

## 3. Results

Figure 1 shows the total ion current (TIC) chromatogram for one of the samples analyzed. The total analysis time was 15.7 minutes, with the last peak of interest eluting in less than 14 minutes. 696 peaks were found automatically through data processing at a S/N ratio of 100. If the interest of the analyst is towards peaks of lower concentration, the S/N ratio can be decreased in order to increase the number of found peaks. Peak identification was performed using the NIST library. A more specialized library will most likely increase the number of identified peaks and improve the overall results.

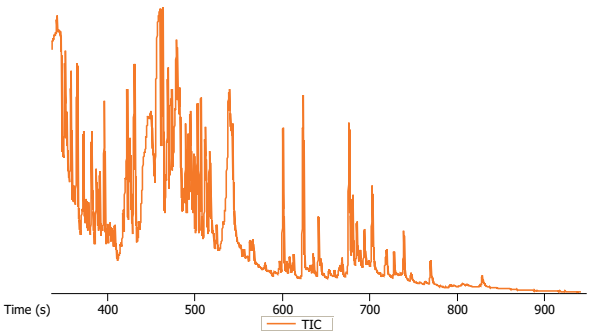


***Figure 1. TIC chromatogram of one of the urine extract samples.***

Figure 2 shows the ability of the Peak Find algorithms to locate multiple peaks in regions where severe coelution occur. A region spanning only 12 seconds in time is presented in this figure. While in part (a) of the figure only 4 to 6 peaks can be visually detected, part (b) of the same figure shows how the software located 23 peaks. To visually prove the presence of this many peaks, unique m/z values were plotted for each of them. It is now easy to see that each peak marker (vertical line) corresponds to the apex of a unique mass. The ability to peak find and deconvolute in "crowded" regions of the chromatogram allows compression of the chromatography (fast separation), with no loss in analytical resolution. Generation of large data sets for statistical analysis can now be rapidly accomplished.

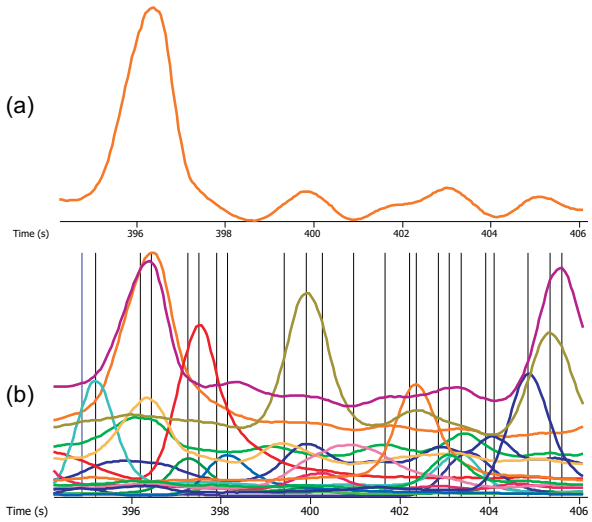


***Figure 2. A twelve-second region of the chromatogram shown in Figure 1 presented as (a) TIC and (b) extracted-ion chromatograms.***

While the Peak Find algorithm is able to locate hundreds of peaks in the chromatograms collected for this study, the Deconvolution algorithm is able to correctly extract the mass spectral information for the coeluting components. Not only are unique masses correctly assigned to each analyte, but the shared signal is correctly distributed between coeluting peaks as well. This allows easier identification based on comparison with data from the libraries. Figure 3 shows an example of how deconvolution extracted the spectral information for coeluting analytes in a region of the chromatogram presented in Figure 1. Spectral information before deconvolution (caliper spectrum), after deconvolution (peak true spectrum), as well as the spectrum of the first hit from the NIST library are all presented.

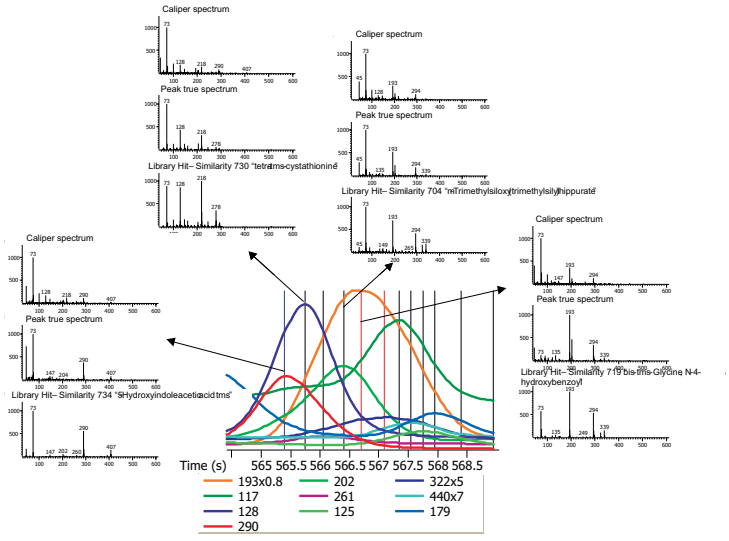


***Figure 3. Deconvolution results for four peaks coeluting in a 3-second region of the chromatogram presented in Figure 1.***

## 4. Conclusions

High-speed acquisition rates allowed the analysis of each urine sample in about 15 minutes. The fast analysis came with no loss in the ability to fully characterize the samples for the study. The spectral continuity across the chromatographic peak profile specific to TOFMS instruments, combined with unique ChromaTOF® Peak Find and Deconvolution algorithms, enabled detection and mass spectral characterization of more than 650 peaks in each of the samples studied. With more metabolites detected for any given sample, the potential of finding discriminatory peaks for each metabolome increases, and thus the ability to profile biological samples becomes more sensitive.

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