Automatic Generation of Extract ion Chromatogram for Mass Spectral Peaks with Specific Isotope Labeled Patterns

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Overview

- Automated detection of drug metabolites based on ^{14}C labeled isotope patterns
- Novel algorithms to calibrate mass spectra for both mass and peak shape andautomatically calculate ^{12}C/^{14}C ratios
- Three case studies demonstrating better selectivity and sensitivity of current approach than on-line radio activity monitoring (RAM)

Introduction

In drug metabolism studies, using radioisotopes or stable isotopes to label drug molecules is an effective way to trace a parent drug and its major metabolites in complex biological mixtures. The isotope-labeled drug and metabolites usually coexist with their non-labeled counterparts and produce unique isotope profiles. While the unique isotope profiles can be reliably used for metabolite identification by LC/MS, to find such peaks of metabolites from the LC/MS analysis of biological samples is labor intensive and tedious. We report here a new method based on peak shape calibration to automatically filter out the metabolites that have unique isotope patterns.

Methods

Experimental

Carbon 14 labeled and non labeled test articles, at 1 and 10 µM with ^{12}C/^{14}C ratios of 50:50, 65:35, and 75:25, were incubated with human hepatocytes and liver microsome. At the completion of incubation, the incubation mixtures were extracted with 1 or 3 volume of ice-cold methanol and the methanol extracts were used for radio-profiling and LC/MS analysis. All LC/MS data were acquired on a ABI/Sciex API 4000 QTRAP and analyzed by MassWorks software[1].

Data Processing

The procedure to automatically detect mass spectral peaks with specific isotope patterns is based on novel mass spectral peak shape calibration technologies. It is straightforward to perform a calibration
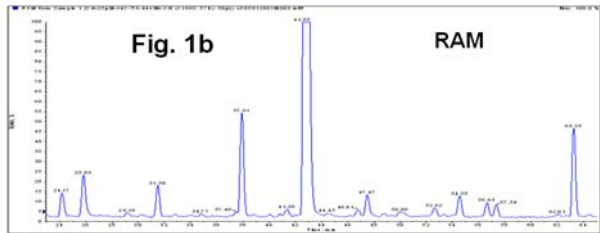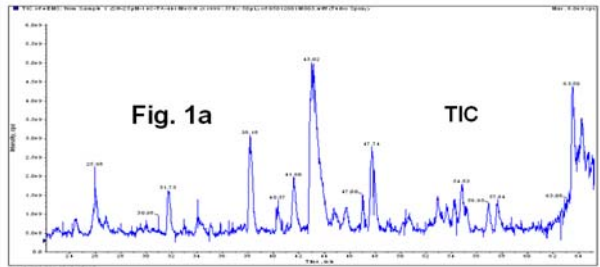
with pure compounds. In the case of $^{12}C/^{14}C$ mixtures, the peak shape calibration is based on two assumptions. One is that the isotope patterns of the $^{12}C/^{14}C$ mixtures are a linear combination of ^{14}C-labeled and non-labeled molecules. Another is that these two-amu apart peaks have the same isotope profiles. To perform the calibration, two formulas such as C12H30O2N and $^{14}CC11H30O2N$ are needed as input. MassWorks software can then automatically calculate the relative abundance for ^{12}C and ^{14}C molecules and calculate the peak shape of the mixtures to reliably detect all ^{14}C related peaks.
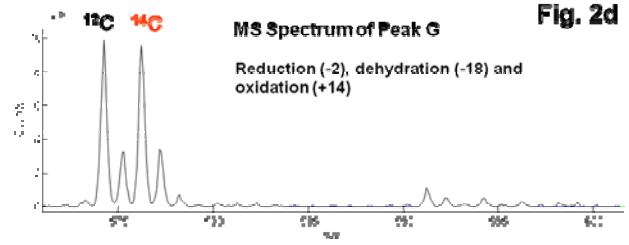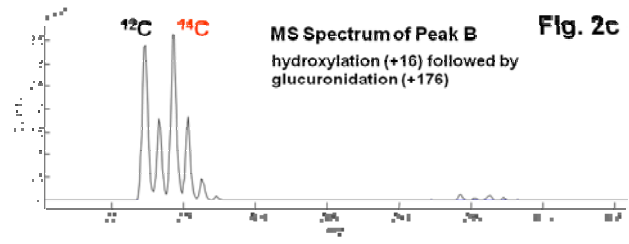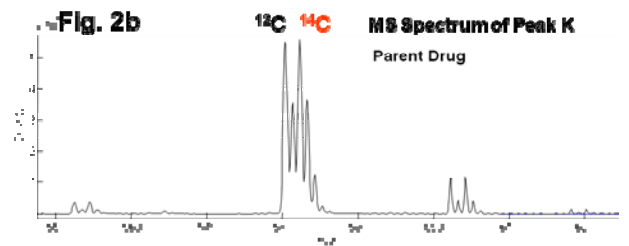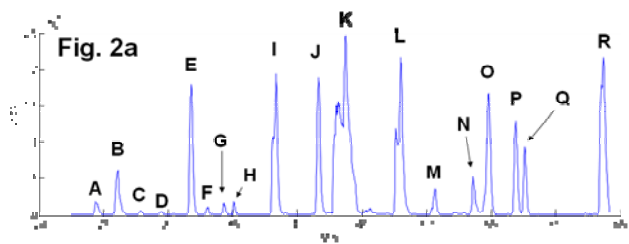
Results and Discussion

As mixtures of ^{12}C and ^{14}C containing molecules, carbon 14 labeled drugs and their metabolites have unique isotope patterns. With the novel calibration, these patterns can be used for automated and rapid detection of the metabolites. This is demonstrated by three case studies where different sample concentrations and $^{12}C/^{14}C$ ratios were investigated.

Comparing RAM (Fig. 1b) with $^{12}C/^{14}C$ isotope pattern directed XIC (IPDXIC) (Fig. 2a), it is obvious that IPDXIC is much more selective and sensitive than RAM. The IPDXIC not only finds all the peaks which appear in RAM, but also detects those not shown in RAM such as peaks F, G, and H. Notice that peaks K and L (Figs. 2a&4a) look split in the middle of the peaks. This is due to a less than perfect isotope match caused by saturated signals. The highly selective and sensitive detection of IPDXIC is further demonstrated by a low concentration sample (case study 2). The on-line RAM (Fig.3b) has only one peak while corresponding IPDXIC (Fig. 4a) detects 9 drug metabolites. Another important feature of this approach is its fully automated operation. No matter what the $^{12}C/^{14}C$ ratios, one calibration can result in effective IPDXIC for the ratios of 50:50, 65:35, and 75:25 as shown in Figs. 5a-c.

Case Study 1
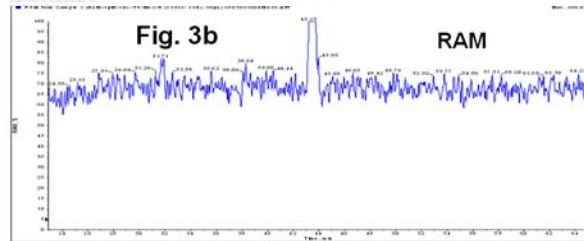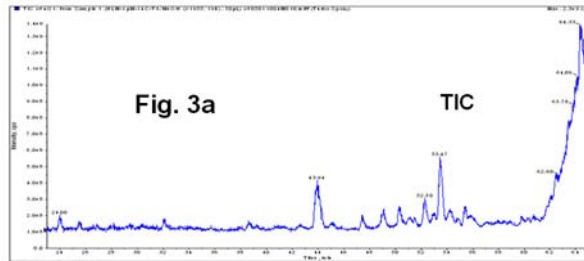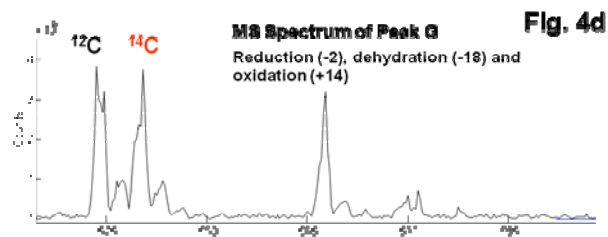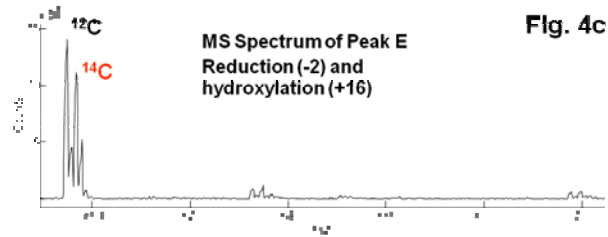## High Concentration Sample

Fig. 1a   TIC

Fig. 1b   RAM

¹²C/¹⁴C Isotope Pattern Directed XIC

Fig. 2a

Fig. 2b   ¹²C ¹⁴C   MS Spectrum of Peak K
Parent Drug

¹²C ¹⁴C   MS Spectrum of Peak B   Fig. 2c
hydroxylation (+16) followed by
glucuronidation (+176)

¹²C ¹⁴C   MS Spectrum of Peak G   Fig. 2d
Reduction (-2), dehydration (-18) and
oxidation (+14)

Case Study 2
Low Concentration Sample

Fig. 3a   TIC

Fig. 3b   RAM

¹²C/¹⁴C Isotope Pattern Directed XIC

Fig. 4a

Fig. 4b

MS Spectrum of Peak L
Reduction (-2) and dehydration (-18)

Fig. 4c

MS Spectrum of Peak E
Reduction (-2) and
hydroxylation (+16)

Fig. 4d

MS Spectrum of Peak G
Reduction (-2), dehydration (-18) and
oxidation (+14)
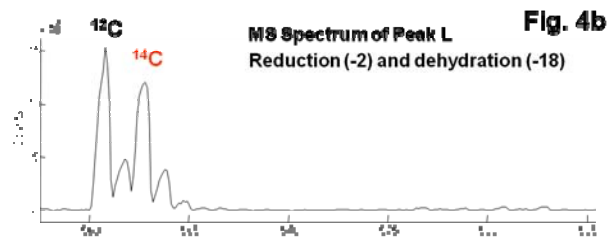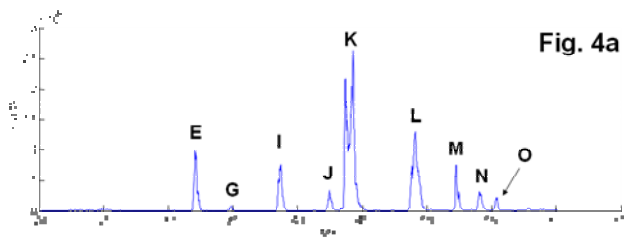
Case Study 3
Different ^{12}C/^{14}C Ratios

Fig. 5a — ^{12}C/^{14}C = 50/50

Fig. 5b — ^{12}C/^{14}C = 65/35

Fig. 5c — ^{12}C/^{14}C = 75/25
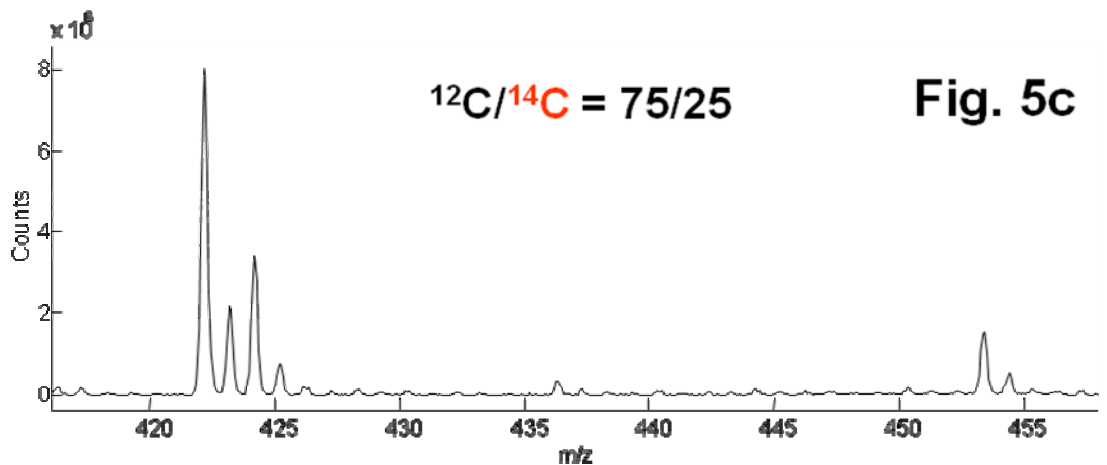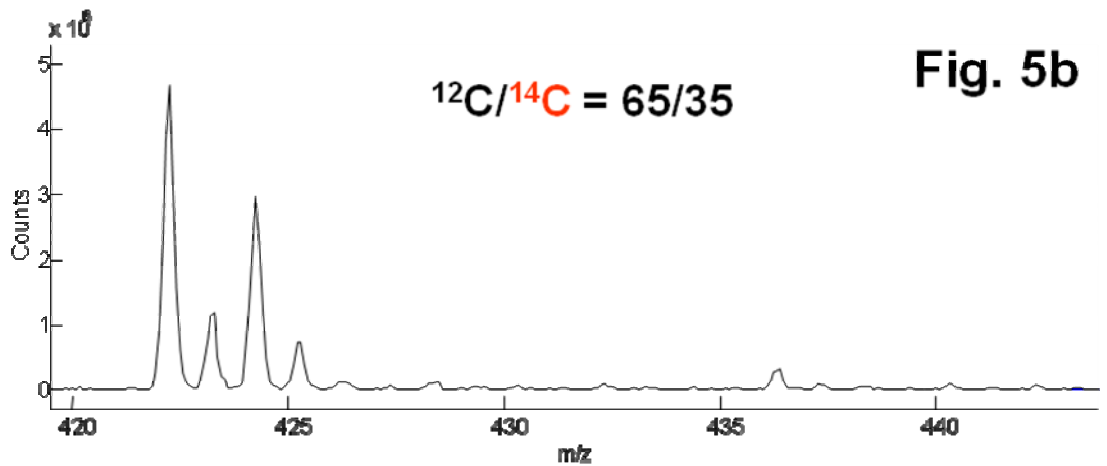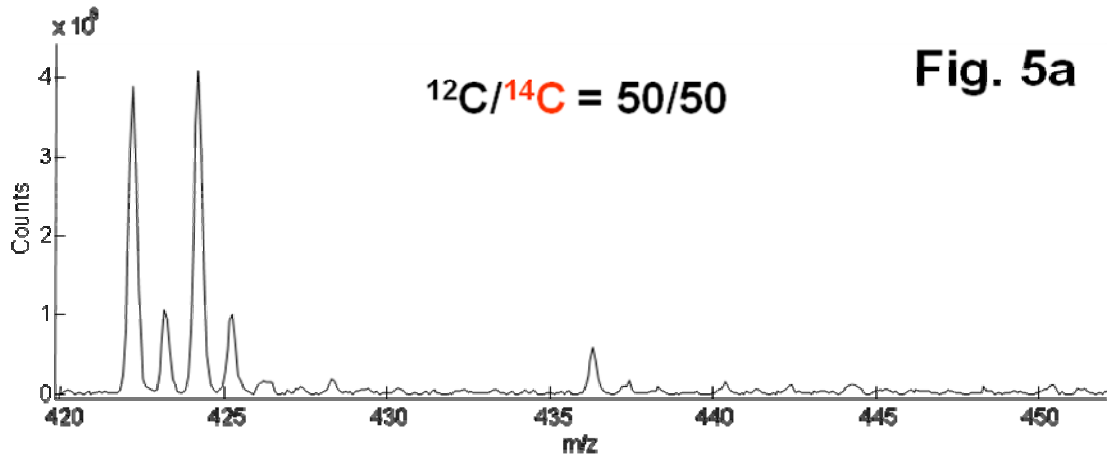
Conclusions

- ➢ The data processing for IPDXIC is fully automated and easy to perform.
- ➢ IPDXIC is much more sensitive than on-line RAM for detecting ^{14}C labeled metabolites.
- ➢ There is no need to specify ^{12}C/^{14}C ratios to find the isotope patterns with the unique calibration algorithms .
- ➢ The approach is also effective for identification of metabolites labeled with other radio isotopes, stable isotopes or the metabolites containing Br and Cl.

References

1) MassWorks software, Cerno Bioscience, Danbury, CT 06810