

# An automatic workflow for analyzing untargeted stable isotope tracking experiments by GC-TOF MS

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## INTRODUCTION

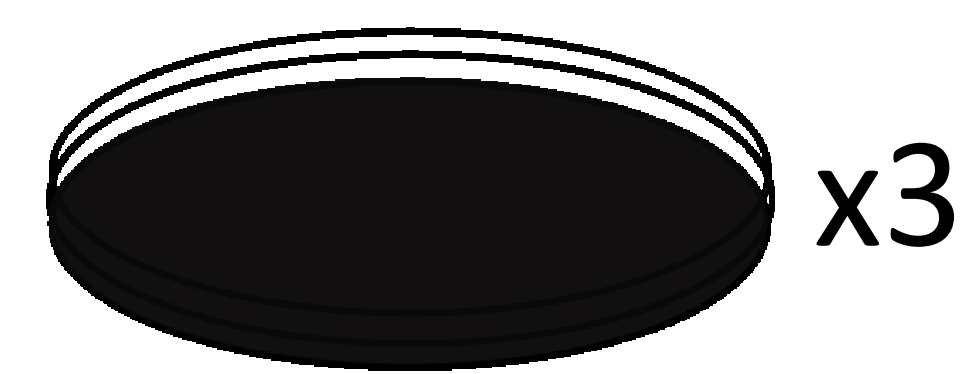
The use of GC-(EI)MS to study metabolic fluxes is hampered by the difficulty of analyzing the datasets derived.

*eRah* is a computational tool that allows the unbiased tracing of labelled atoms

We have analyzed an *in vitro* model of diabetic retinopathy that simulates hyperglycaemic conditions on the metabolism of a human retinal pigment epithelial cell line (ARPE-19)

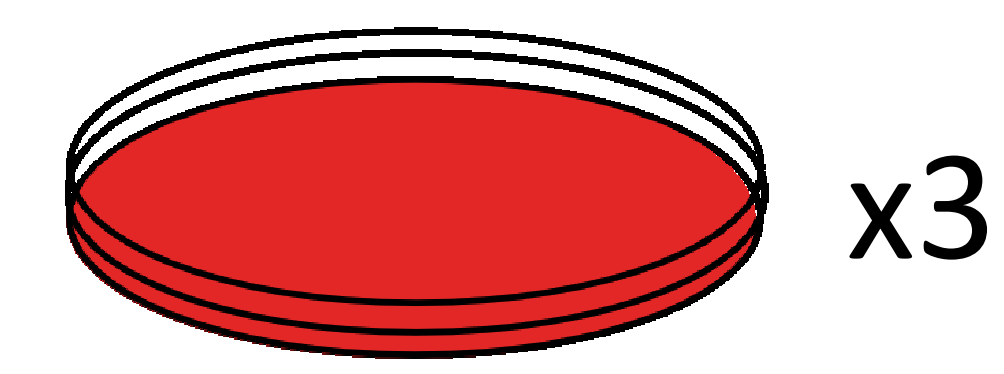
## MATERIALS AND METHODS

Unlabelled Samples



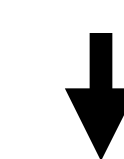
5.5 or 25 mM Glucose (N5 or N25)

Labelled Samples



5.5 or 25 mM [U-<sup>13</sup>C]Glucose (N5 or N25)

Extraction and derivatization of metabolites



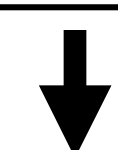
GC-qTOF MS

Electron Ionization and Chemical Ionization (using isobutane gas)

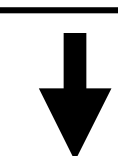


## *eRah* workflow\*\*

<sup>12</sup>C & <sup>13</sup>C Samples



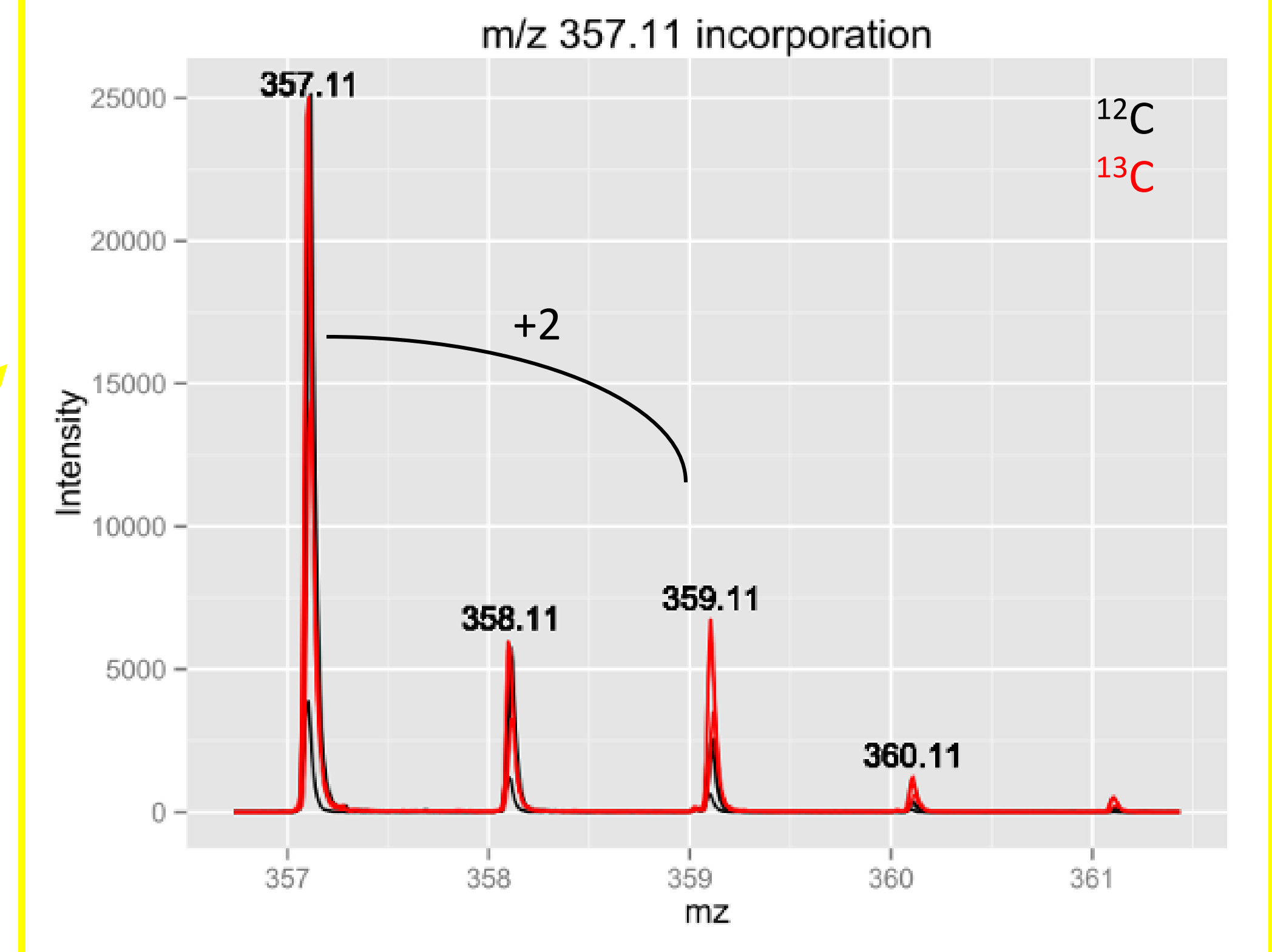
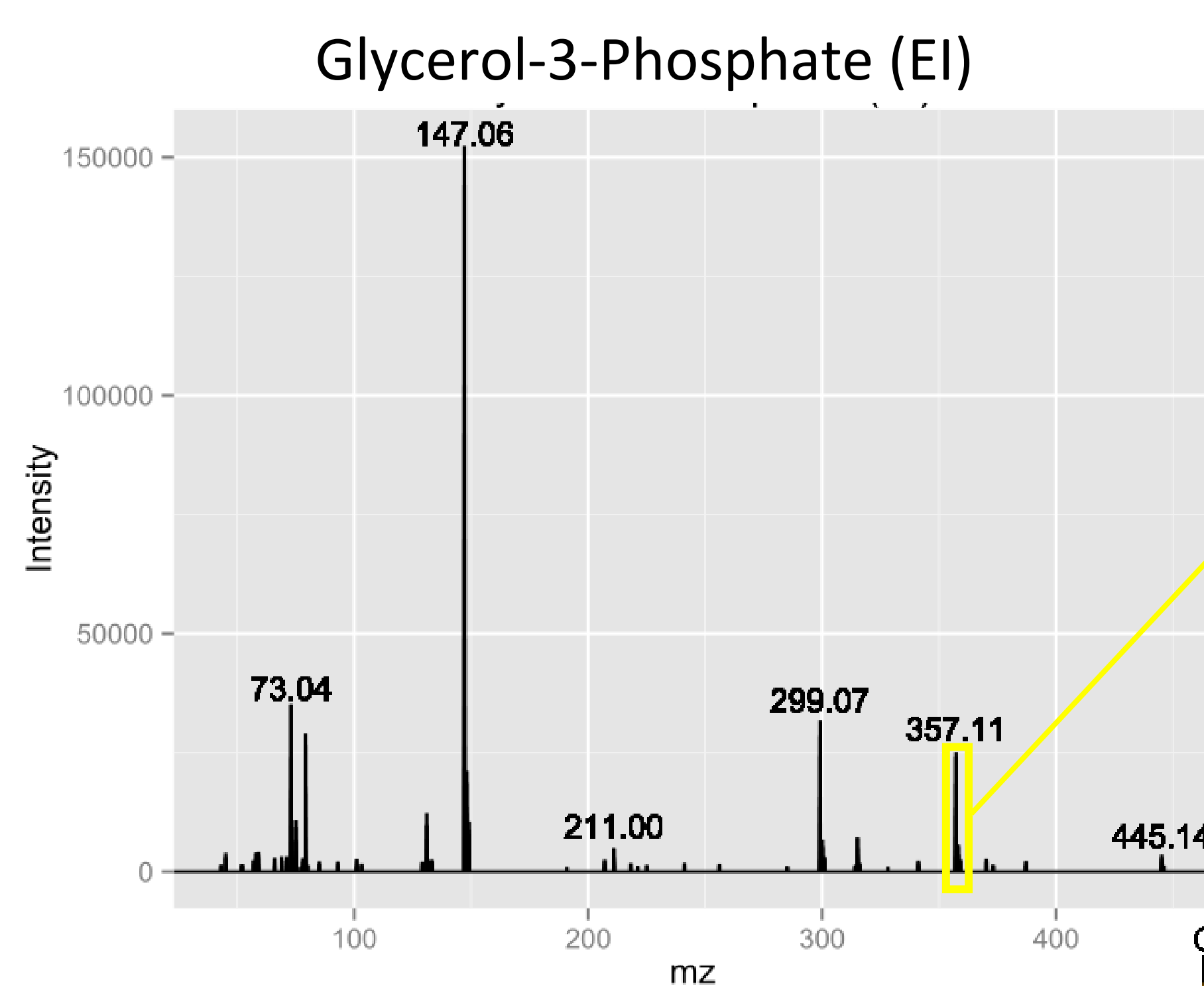
Deconvolution (ODS)  
Alignment  
Identification  
Compound recovery (PLS)



<sup>12</sup>C vs <sup>13</sup>C comparison  
Statistical analysis + FC

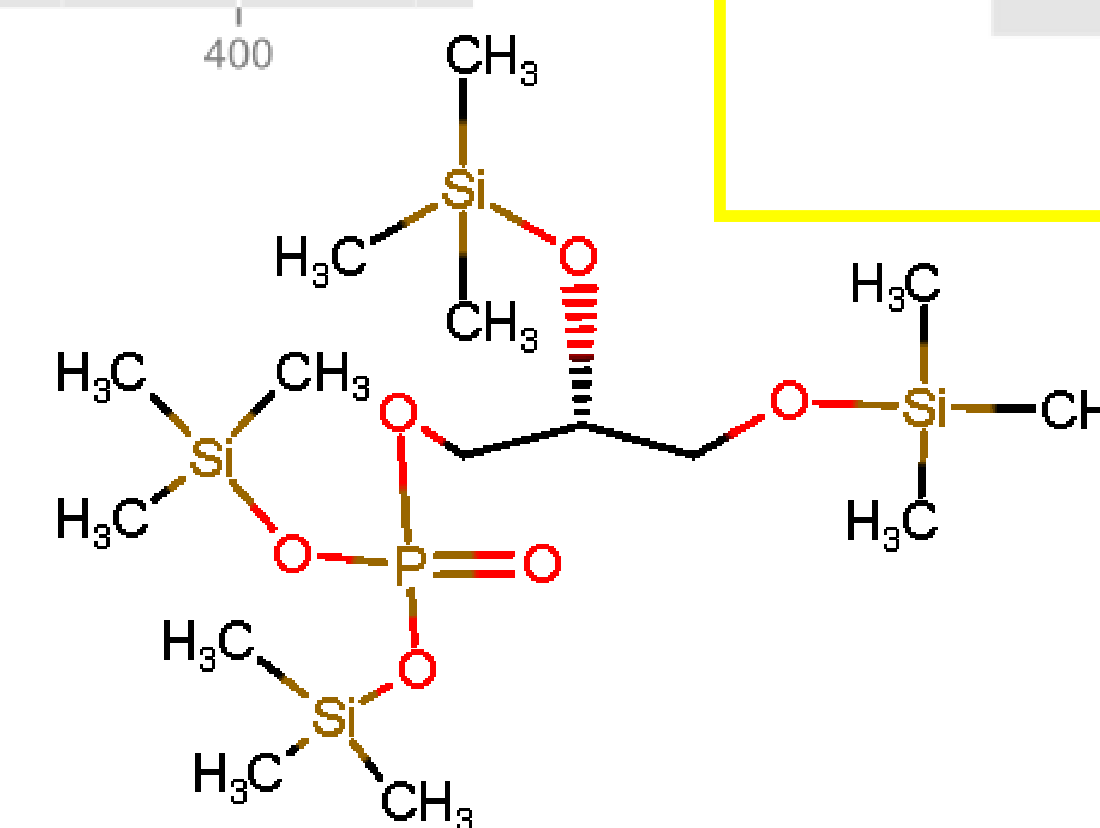
## RESULTS

### Incorporation of <sup>13</sup>C in downstream metabolites

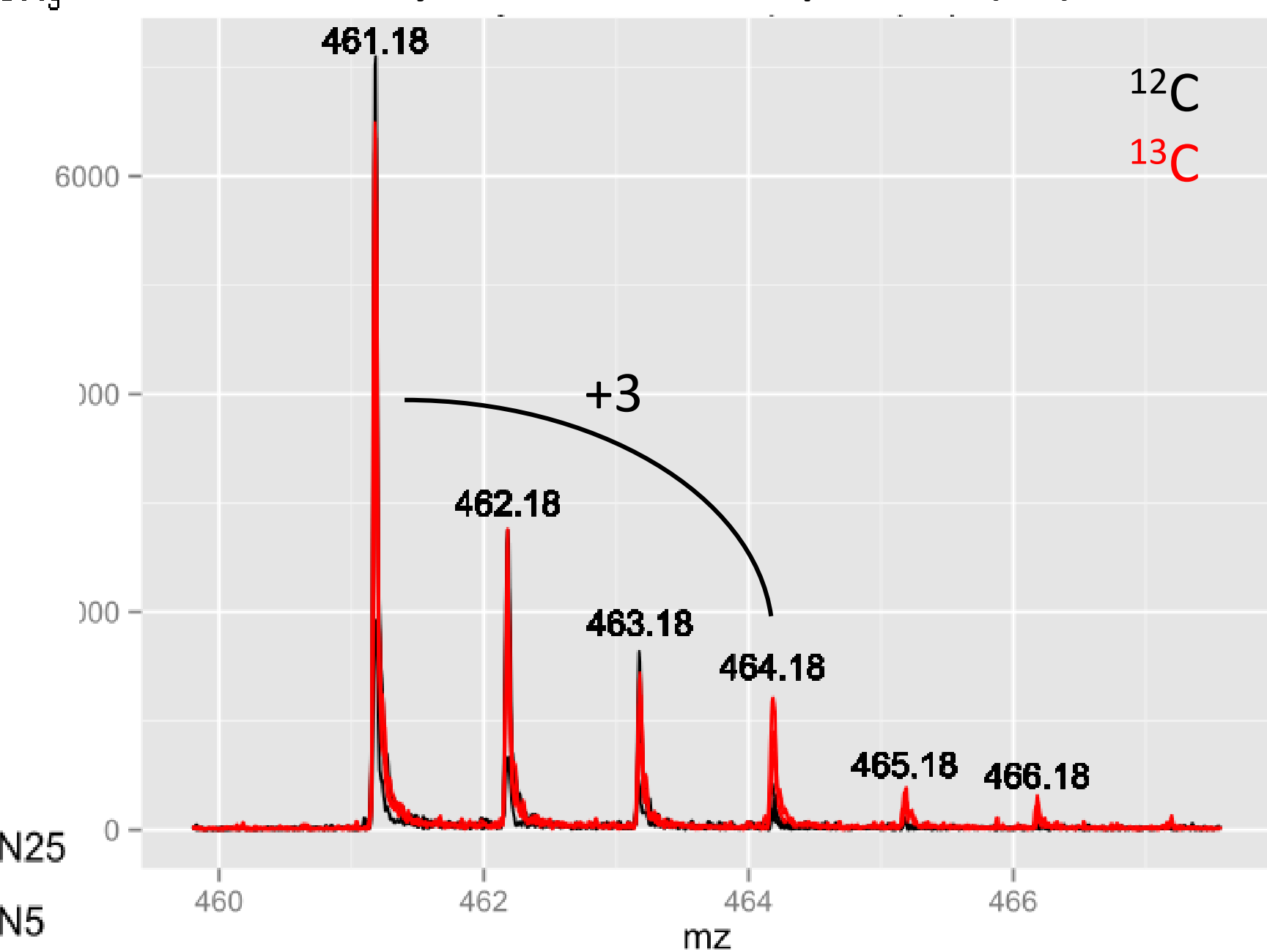


CI exact mass validation

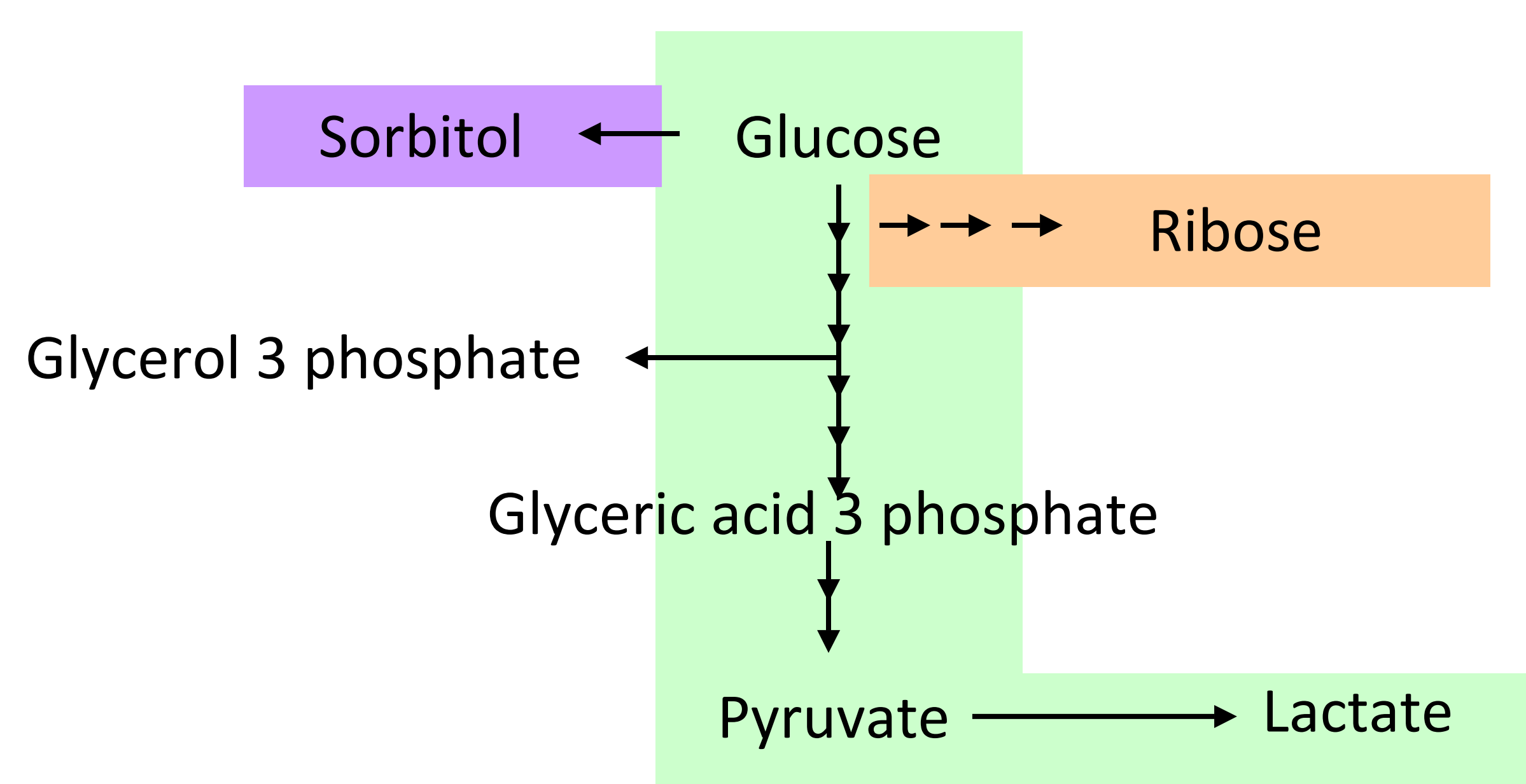
Glycerol-3-phosphate (4TMS)  
MW: 460.17



Glycerol-3-Phosphate (CI)



### Glucose incorporation: N5 vs N25

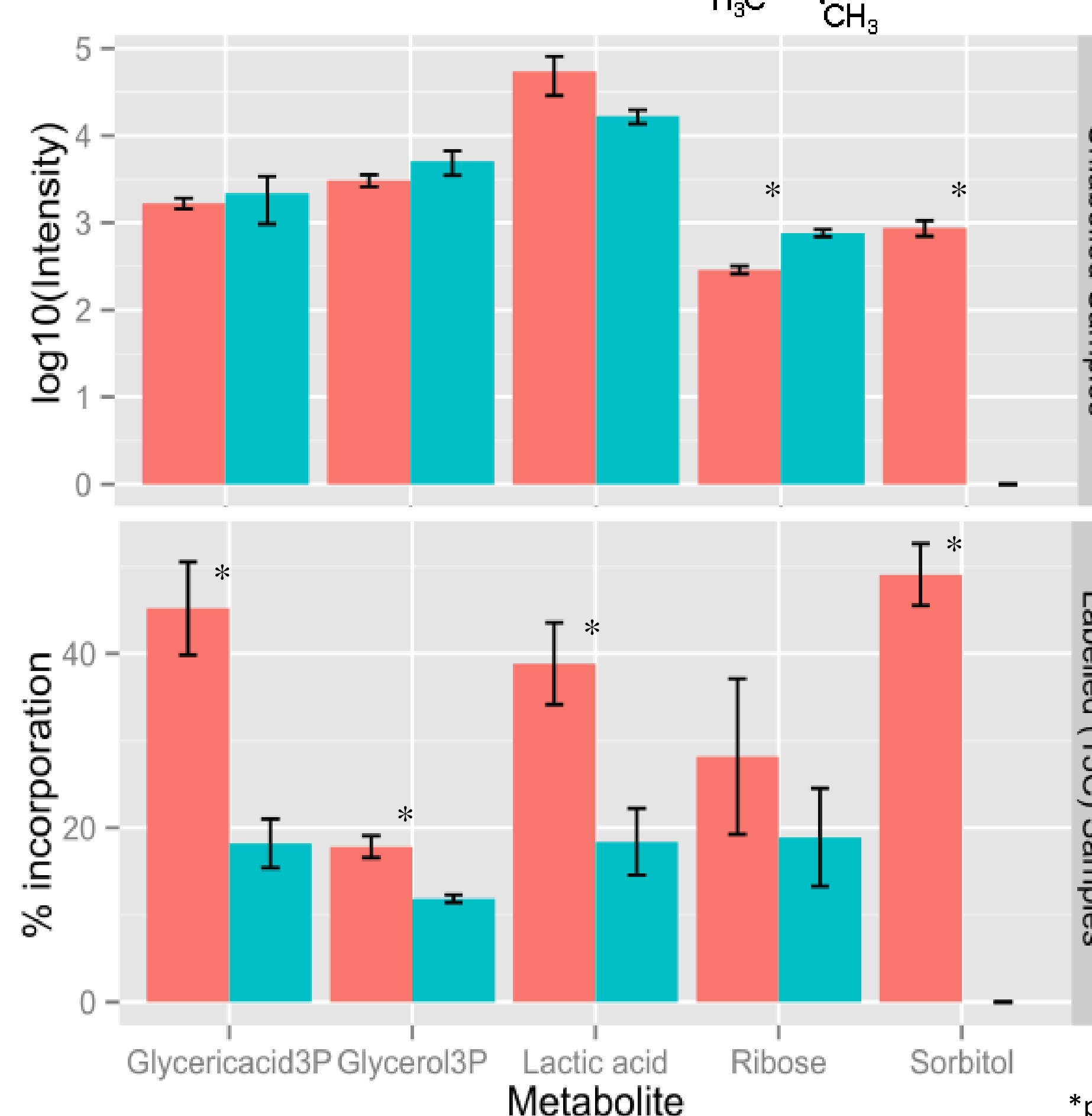


Pentose phosphate pathway

Polyol pathway

Glycolysis pathway

\*\*Please, see poster 308 for *eRah* details



\*p-value < 0.05