

Amadori rearrangement products as potential biomarkers for inborn errors of amino-acid metabolism

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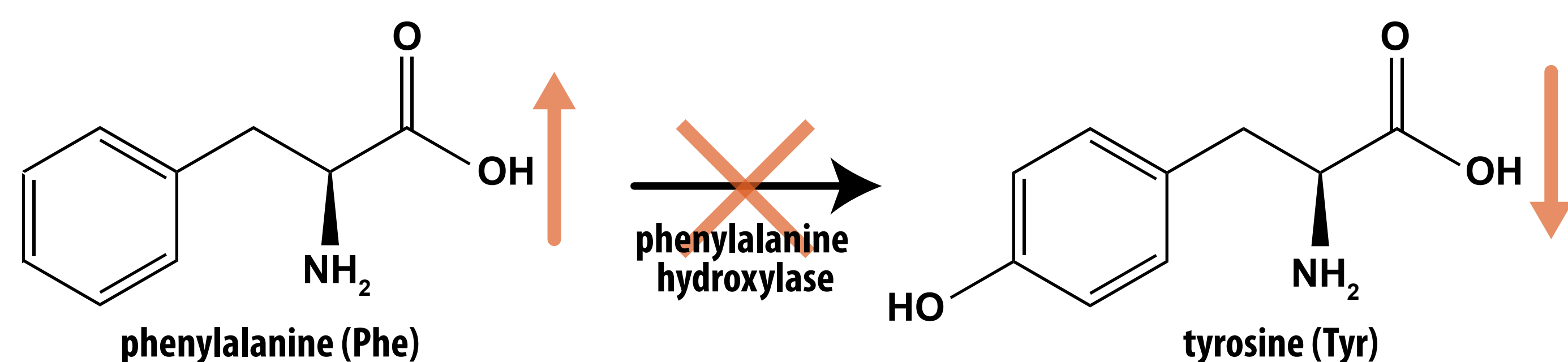


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Phenylketonuria (PKU)

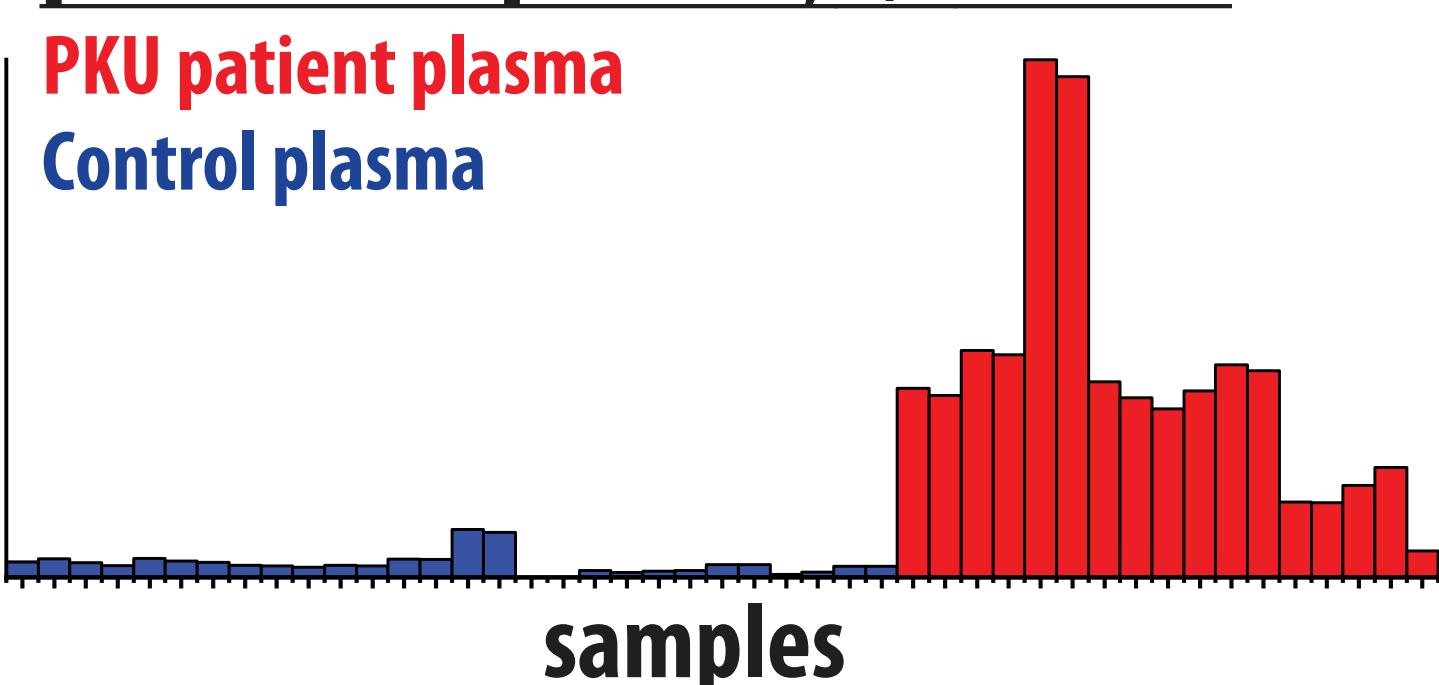
Inborn error of metabolism leading to **high levels of phenylalanine (Phe)**, causing neurological damage [1]. Phe levels observed among treated patients do not always relate to their clinical outcome, which presents a need for biomarkers that better represent clinical status.



Untargeted metabolomics using LC/MS

Liquid chromatography - mass spectrometry (LC-MS) is due to its high sensitivity the method of choice to find new biomarkers, however the identification of the biomarkers is challenging, as there are often multiple isobaric candidates. Here we use a novel strategy using infrared ion spectroscopy to identify the full structure of a Phe-hexose conjugate detected by LC-MS.

[Phe-hexose+H]⁺ - RT 3.80, m/z 328.1391

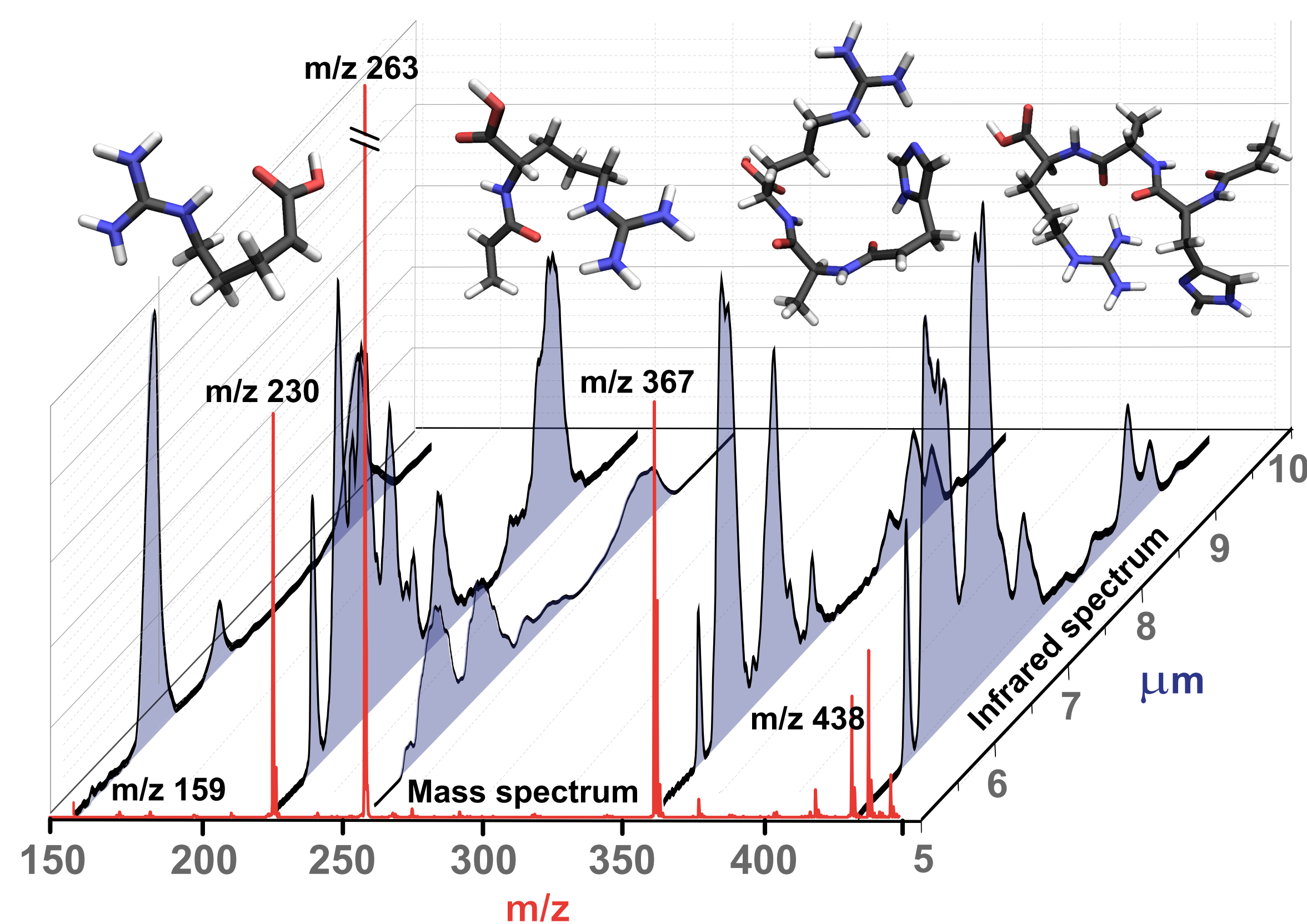


Unidentified by LC/MS-MS
(several isobaric candidates)

Undetected by
NMR spectroscopy
(too low abundance)

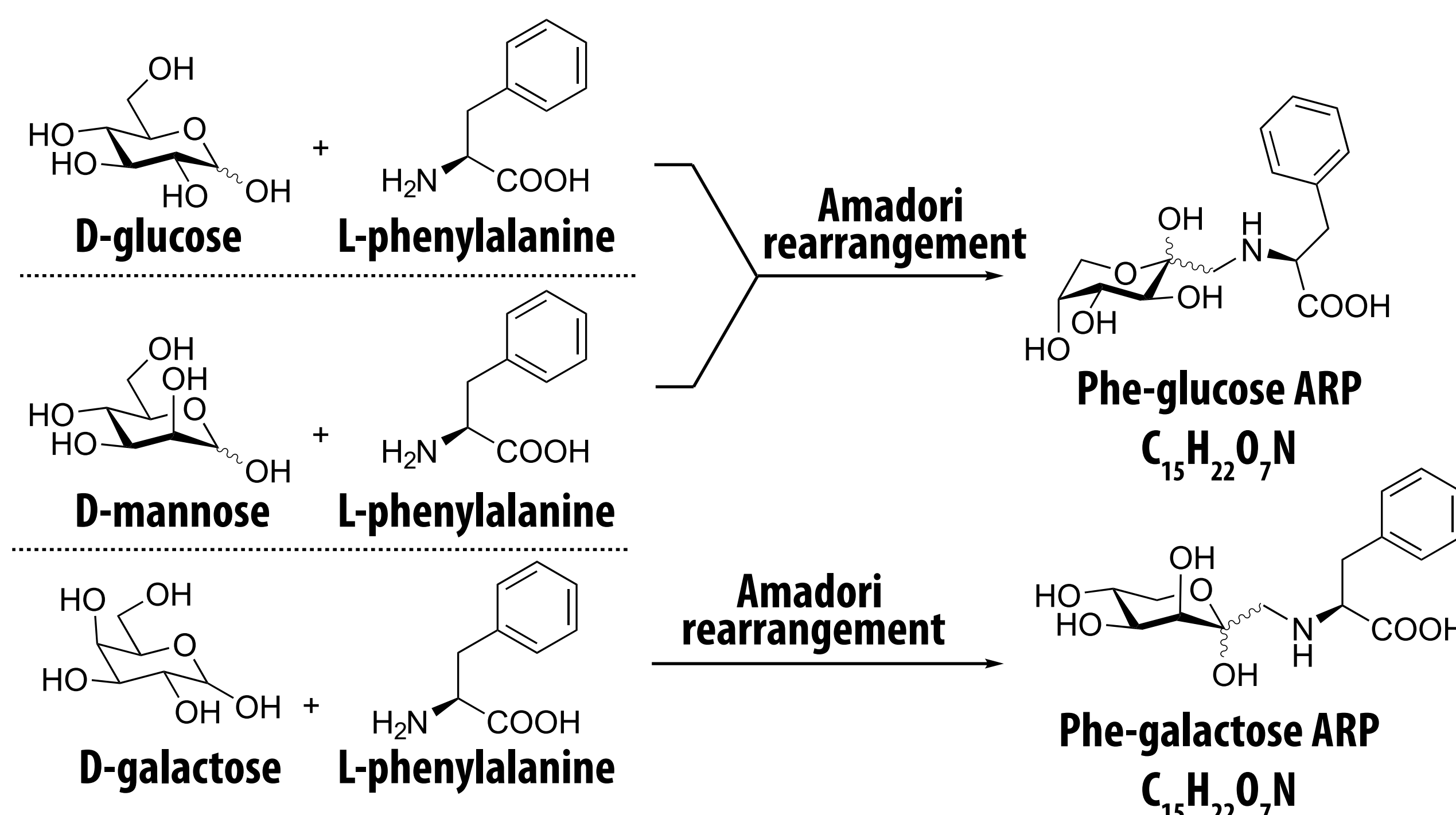
Analytical infrared (IR) ion spectroscopy

IR structural information with the sensitivity and selectivity of LC/MS [2]



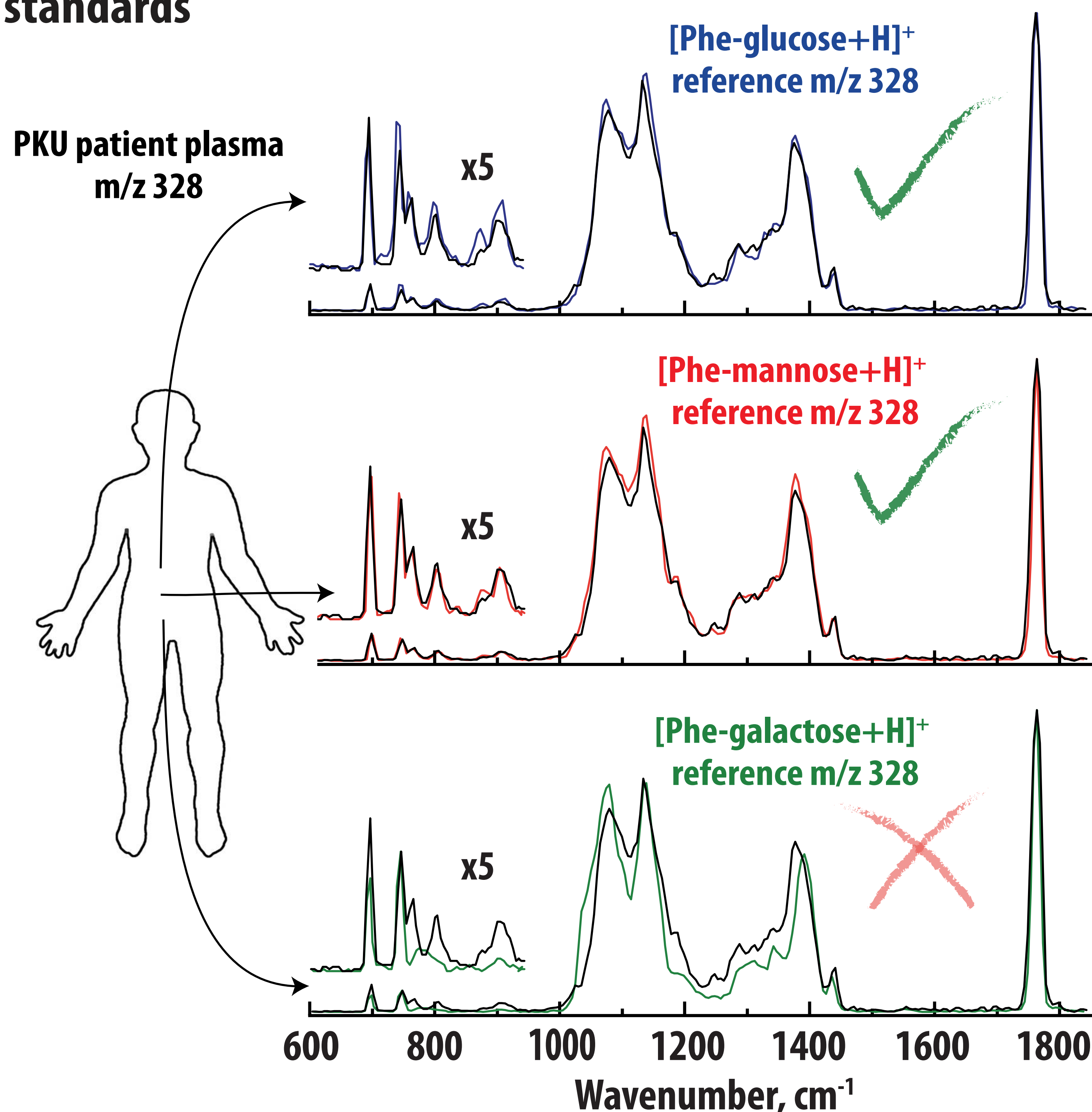
NMR spectroscopy of the reference standards

The abundance of the Phe-hexose in plasma is too low for NMR spectroscopy, but we recorded NMR spectra of the synthesized reference standards



The Phe-hexose conjugates undergo an Amadori rearrangement to Amadori products. This product is identical for Phe-glucose and Phe-mannose. We can identify the metabolite as the Phe-mannose/glucose Amadori product.

Infrared ion spectroscopy of the metabolite and reference standards



The IR spectrum of the metabolite is identical to that of both the Phe-glucose and Phe-mannose conjugate, whose spectra are identical to each other. How is that possible?

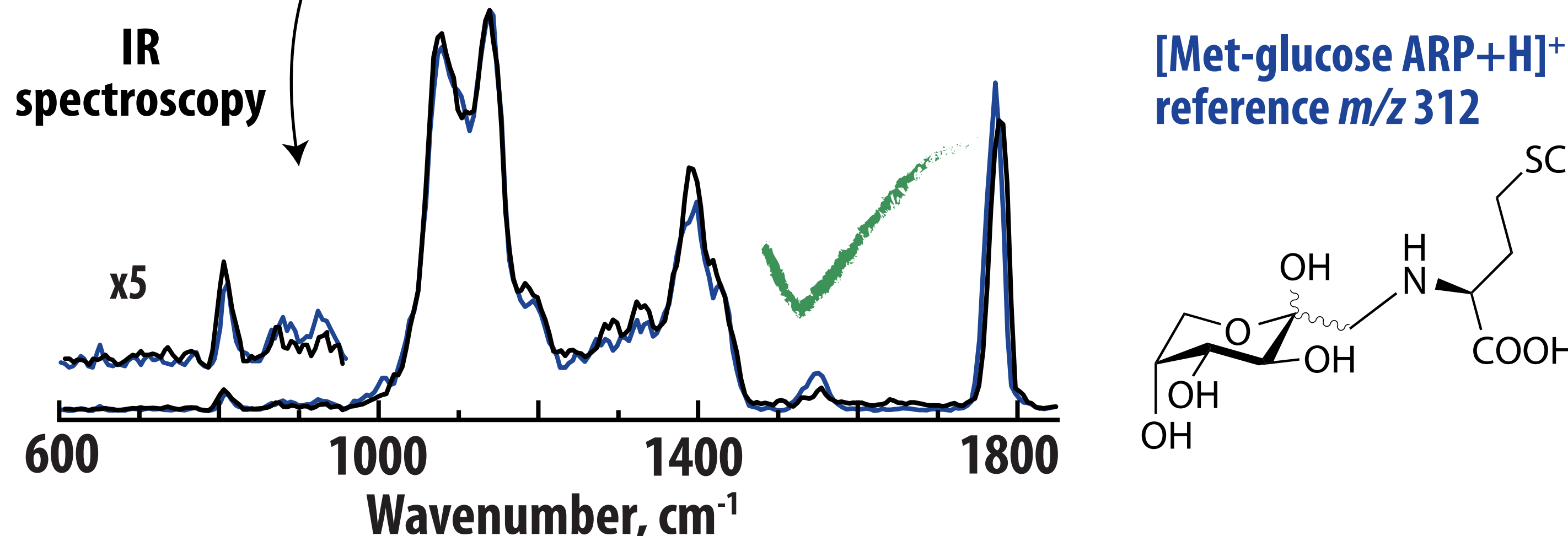
Other inborn errors of amino acid metabolism

Amadori products can be formed from any amino acid, thus we looked for Amadori products in plasma from patients with other diseases associated with elevated amino acid levels, such as CBS and MAT (high methionine).

[Met-hexose+H]⁺ - RT 1.56, m/z 312.1111

MAT patient plasma
CBS patient plasma
Control plasma

Observed as well for
proline, lysine and citrulline!



Amadori products can be expected to be biomarkers for any disease associated with high amino acid levels.

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