

Derivatization for MS/MS

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University of Arizona

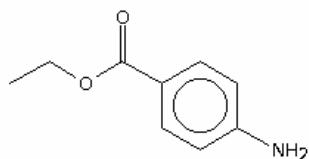
Summer Workshop

Why derivatize?

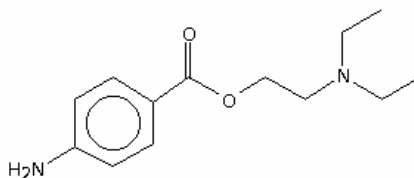
- To increase abundance of MH^+ for better MS/MS
 - Improve sensitivity for detection of oligosaccharides
- To achieve selectivity in a detection scheme
 - Newborn screening for disease: Detection of amino acid imbalance, a marker of inherited disease
- To distinguish an analyte from an interference
 - Analysis of methylmalonic acid in plasma & urine (differentiated from Succinic Acid)

Derivatization to improve sensitivity for detection of oligosaccharides

- Poor ionization efficiency of free carbohydrate chains in ESI limits the utility of ESI-MS in structural carbohydrate studies
- Ionization efficiency enhanced in positive-mode ESI-MS (improved 5000-fold)
- 2 derivatizing agents investigated



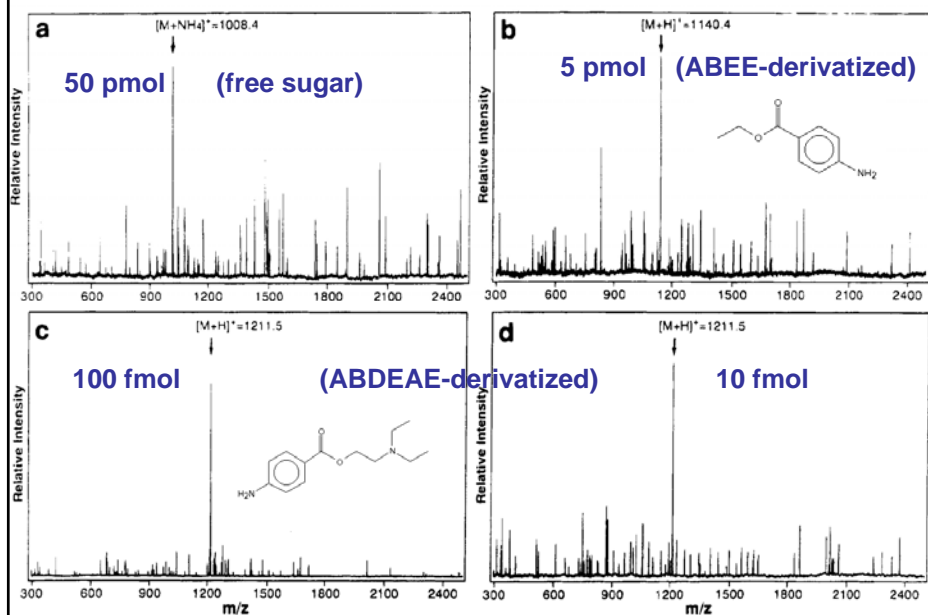
Benzoic acid, 4-amino-, ethyl ester (ABEE)



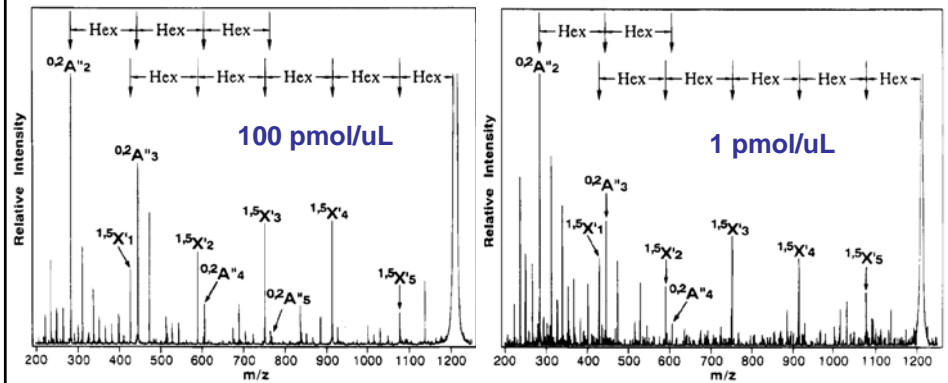
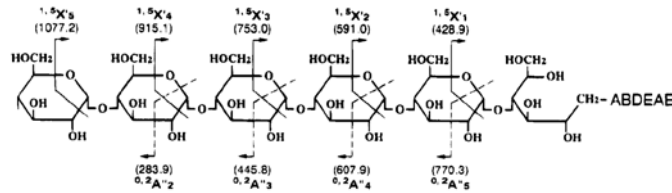
Benzoic acid, 4-amino-, 2-(diethylamino)ethyl ester (ABDEAE)

Ref: Yoshino, 1995

MS (+ ESI) maltohexaose



MS/MS (+ ESI) maltohexaose-ABDEAE



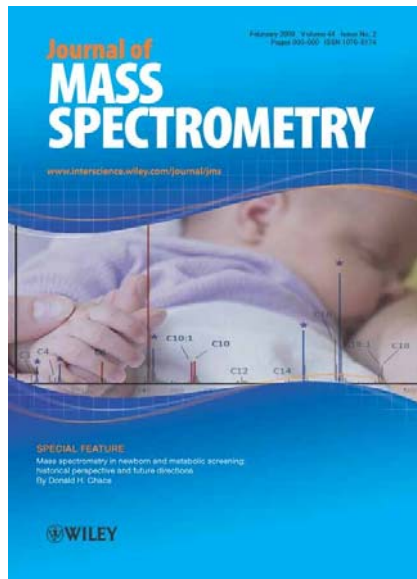
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side note: stable isotopes for quantitation by MS

- mass spectrometry is not quantitative
 - intensity is the result of *proton affinity* not concentration
- Method to quantify:
 - Add a standard of known concentration to your sample (isotope dilution)
 - This standard must have the same structure
 - and incorporate stable isotopes to vary the mass from your analyte – for example:
 - Add 1 mass unit: ^2H (D) ^{13}C ^{15}N
 - Add 2 mass units: ^{18}O
 - 1st step: vary concentration and measure peaks (area of total ion current in an HPLC-MS or MS/MS run)
 - determine concentration range for your method

February 2009 JMS Donald H. Chace newborn screening



Inherited metabolic diseases

Phenylketonuria (PKU)

Reduction in Phenylalanine to tyrosine metabolism

medium chain AcylCoA dehydrogenase (MCAD deficiency)

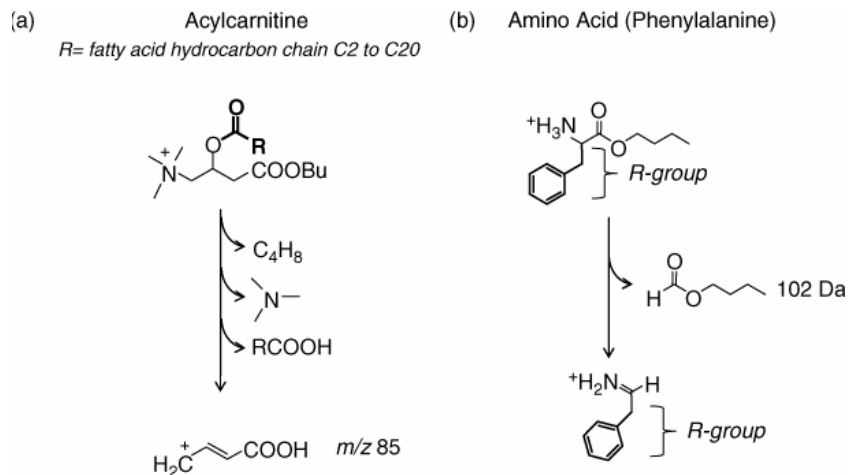
A fatty acid oxidation disorder involving the A985G gene mutation

Derivatization to achieve selectivity : Newborn Screening for Disease

- Blood sample from newborn collected onto dried filter paper
- Sample extracted and derivatized to butyl esters
 - acidified butanol (3N HCl) or butanol with acetyl chloride
- MS/MS is a fast, accurate, robust disease screening
 - method requires no chromatographic separation
 - extremely rapid: 2 min. per sample
 - methanol extraction of samples collected onto dried filter paper removes proteins and salts
- Extracted sample derivatized to butyl esters
 - acidified butanol (3N HCl) or butanol with acetyl chloride

Ref: Chace, 2001

Collision induced dissociation of the butylester derivatives of generic acylcarnitines and phenylalanine (as an example of an alpha amino acid)



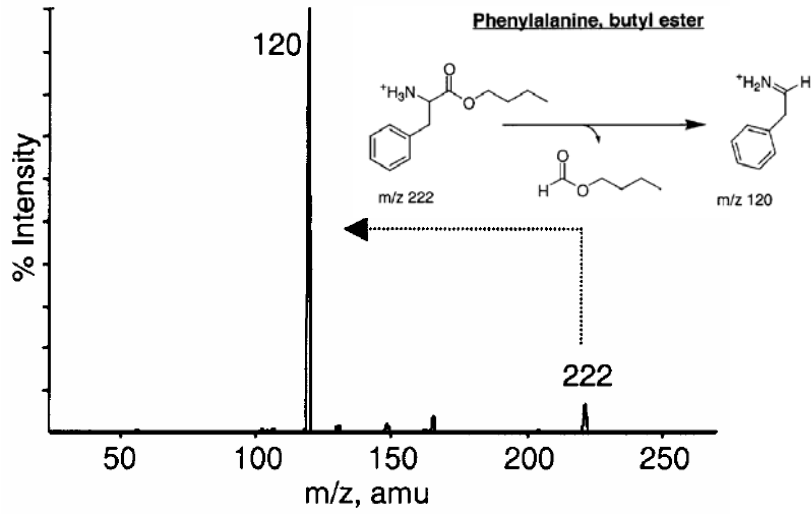
Hexanoyl carnitine $\text{R} = \text{CH}_3(\text{CH}_2)_3\text{CH}_2-$

Octanoyl carnitine $\text{R} = \text{CH}_3(\text{CH}_2)_5\text{CH}_2-$

Decanoyl carnitine $\text{R} = \text{CH}_3(\text{CH}_2)_7\text{CH}_2-$

Ref: Chace, 2009

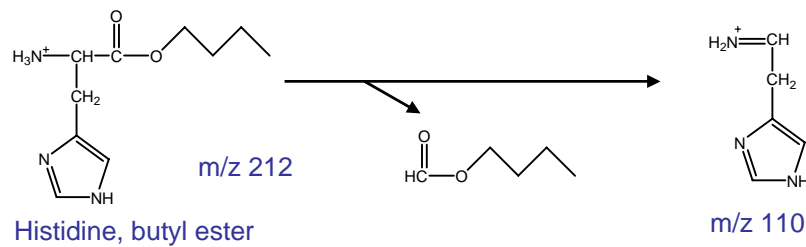
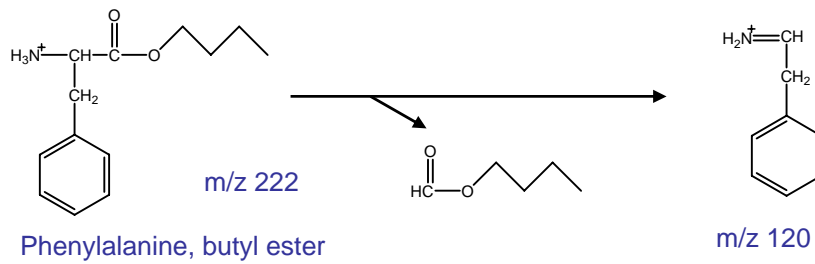
Product Ion Scan of Phenylalanine butyl ester shows loss of m/z 102



Ref: Chace, 2001

butyl ester amino acid derivatives lose 102 in MS/MS

Examples:



Learning Check: **FACT SHEET** Amino Acid Determination

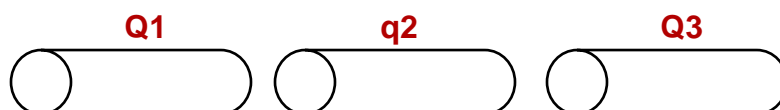
- Consider identification of a mixture of amino acids in a dried filter sample
- Select and describe a scan mode that may be used to identify and quantify amino acids in the sample
- What is the m/z that hits the detector? (1 or 2 examples)
- What happens in Q1, q2, Q3?
- Draw the spectrum

Histidine-butyl ester	212
Phenylalanine-butyl ester	222
Proline-butyl ester	172
Tryptophan-butyl ester	260
Tyrosine-butyl ester	238

stable isotope labeled amino acid standards are available

Learning Check: Amino Acid Determination

- Select a scan mode _____
- What m/z hits the detector? _____
- What happens in Q1, q2, Q3?

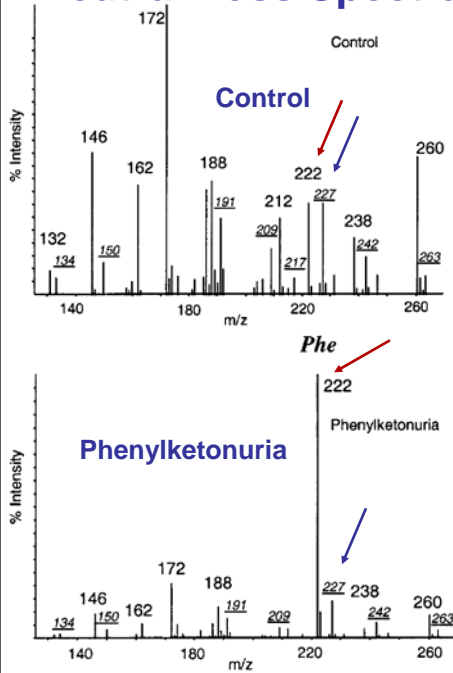


- Draw the spectrum

Relative
Intensity



Neutral Loss Spectra from Newborn Screen



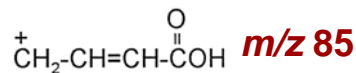
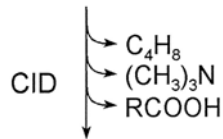
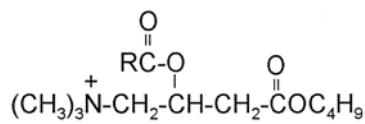
Blood sample from Normal Newborn

Masses of deuterated internal standards are underlined

Blood sample from newborn diagnosed with phenylketonuria

Ref: Chace, 2001

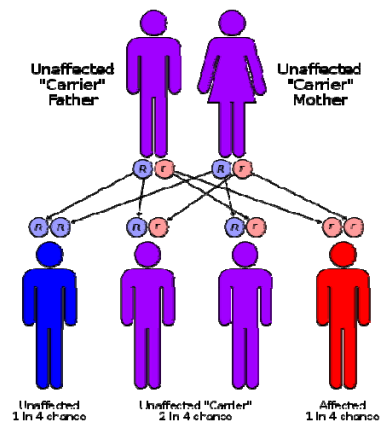
Same sample, another strategy MCAD -- butyl ester derivatives produce a fragment ion at $m/z = 85$



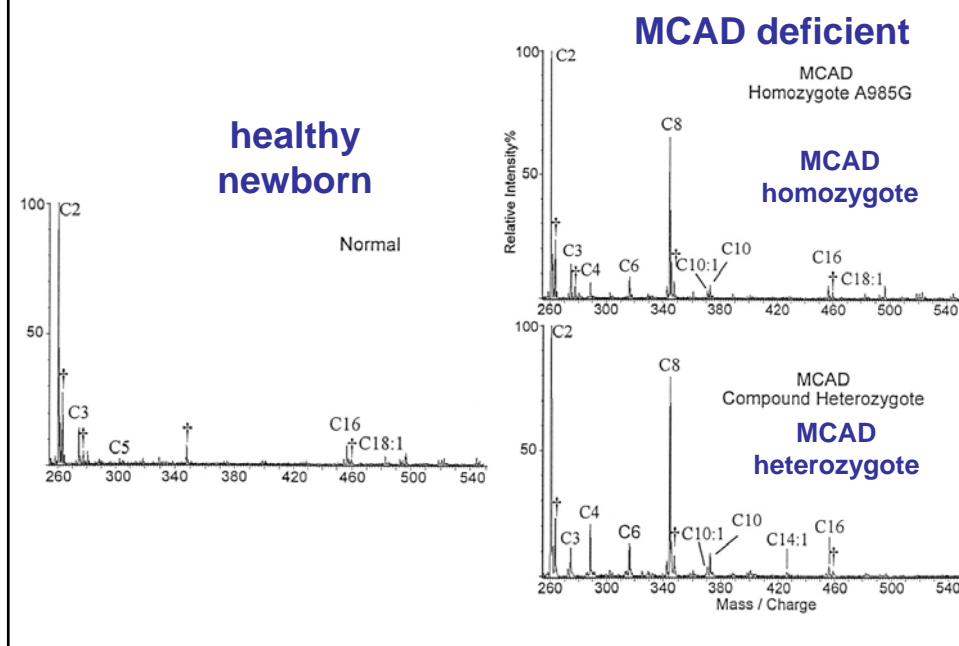
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Octanoyl carnitine R = $\text{CH}_3(\text{CH}_2)_5\text{CH}_2-$

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Precursor of m/z 85 Da function

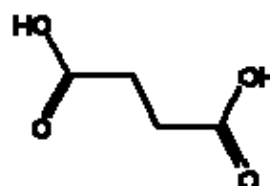
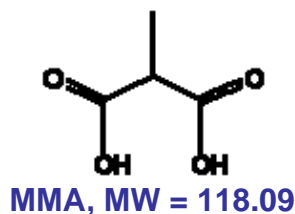


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Derivatization to distinguish an analyte from an impurity: Methylmalonic acid (MMA) in plasma & urine (differentiated from Succinic Acid)

- Increased MMA is a specific diagnostic marker for propionate metabolism and acquired vitamin B₁₂ deficiency
- Must overcome interference from the isomer succinic acid



	plasma (umol/L)	urine (mmol/mol creatinine)
MMA	0-0.4	0-3.6
Succinic acid	0-32	0.5-16

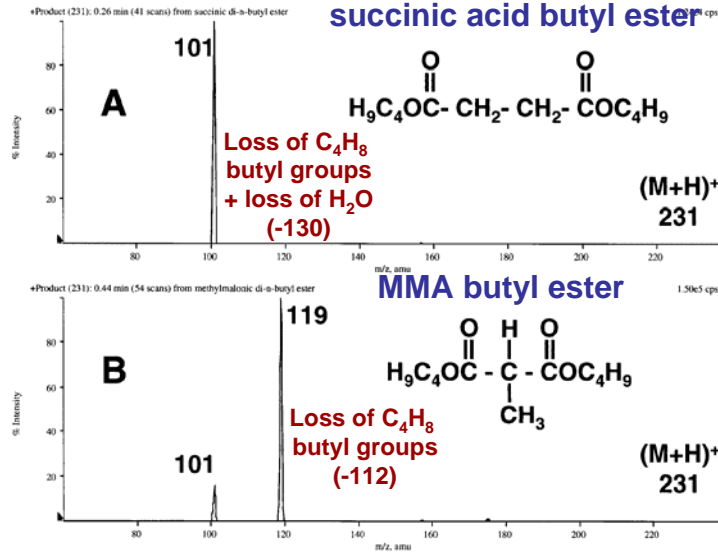
Ref: Magera, 2000;
Schmedes, 2006

Methylmalonic acid detected in plasma & urine (differentiated from Succinic Acid)

- HPLC-MS/MS methods have been developed (QQQ)
 - replace GC-MS methods in a high-throughput environment
- extracted sample (SPE) derivatized to butyl esters
 - extracted from plasma or urine, eluted and derivatized with HCl in n-butanol
- the method is demonstrated in this example using standards
- results of plasma & urine samples are in agreement with the same samples analyzed by the standard method (GC/MS)

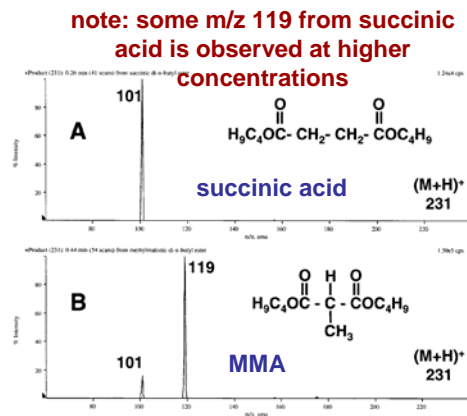
Ref: Magera, 2000;
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MS/MS of derivatized MMA and succinic acid



Learning Check: FACT SHEET MMA Determination

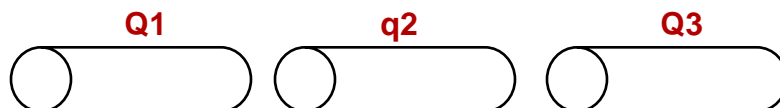
- Consider identification of a methylmalonic acid in plasma or urine
- Select and describe a scan mode that may be used to identify and quantify MMA
- What is the m/z that hits the detector?
- What happens in Q1, q2, Q3?
- Draw the spectrum



MMA is available as
MMA- d_3

Learning Check: MMA Determination

- Select a scan mode _____
- What m/z hits the detector? _____
- What happens in Q1, q2, Q3?



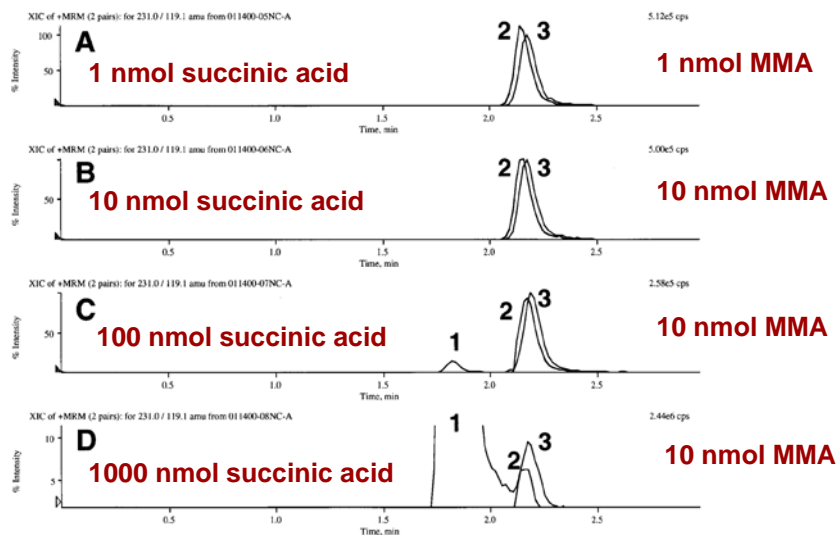
- Draw the spectrum

Relative
Intensity



MRM extracted ion chromatograms Selected transitions m/z = 231/119 and 234/122 (MMA-d₃)

1 = succinic acid 2 = MMA-d₃ 3 = MMA



References

Increased Sensitivity for detection of Oligosaccharides

Yoshino, K., Takao, T., Murata, H., Shimonishi, Y., *Use of the Derivatizing Agent 4-Aminobenzoic Acid 2-(Diethylamino)ethyl Ester for High-Sensitivity Detection of Oligosaccharides by Electrospray Ionization Mass Spectrometry*, *Anal.Chem.*, 1995, 67, 4028-4031.

Newborn Screening

Chace, D.H., *Mass Spectrometry in the Clinical Laboratory*, *Chem. Rev.*, 2001, 101, 445-477.

Chace, D.H., *Mass spectrometry in newborn and metabolic screening: historical perspective and future directions*, *J.Mass Spectrom.*, 2009, 44, 163-170

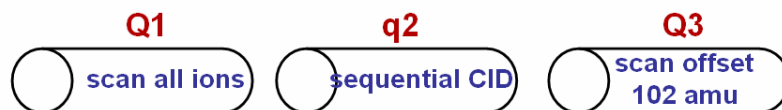
Detection of Methylmalonic Acid

Magera, M.J., Helgeson, J.K., Matern, D., Rinaldo, P., *Methylmalonic Acid Measured in Plasma and Urine by Stable-Isotope Dilution and Electrospray Tandem Mass Spectrometry*, *Clinical Chem.*, 2000, 46:11, 1804-1810.

Schmedes, A., Brandslund, I., *Analysis of Methylmalonic Acid in Plasma by Liquid Chromatography – Tandem Mass Spectrometry*, *Clinical Chem.*, 2006, 52:4, 754-757.

Learning Check: Amino Acid Determination

- Select a scan mode Neutral Loss
- What m/z hits the detector? $\frac{\text{m/z of Q1 less } 102}{\text{for example: phenylalanine} = 120}$
 $\text{histidine} = 110$
- What happens in Q1, q2, Q3?



- Draw the spectrum
-
- Relative Intensity
- 212 histidine butyl ester
- 222 phenylalanine butyl ester
- 100 150 200 250 300
- m/z

Learning Check: MMA Determination

- Select a scan mode MRM
- What m/z hits the detector? **119 = MMA & succinic acid frags**
122 = MMA-d₃ fragment
- What happens in Q1, q2, Q3?

