Use of Derivatization for LC-MS/MS Analysis in the Clinical Lab

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Outline

• Preliminaries
  – Background
  – Definitions
  – Etc.

• Application examples to illustrate pros, cons, and other issues
  – Sensitivity
  – MS/MS (tandem MS) quality
  – Separation (LC) quality
  – Compound class analysis
Purpose of derivatization

- Modify physical or chemical properties
- Improve analysis – detection, quantitation, separation, identification
FISH: Non-classical “Derivatization”

- Fluorescently labeled DNA sequences
- Target specific DNA sequences
- Targeted sequences visualized on chromosomes

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Derivatization in GC-MS and LC-MS

GC-MS

• Very common
• Improve volatility
• Improve thermal stability

LC-MS

• Less common than in GC and GC-MS
• Less often needed
• Avoid if possible
• Desirable in specific cases
Why derivatize in LC-MS?

- Improve ionization efficiency
- Improve fragmentation pattern (sometimes)
- Shift molecular weight of parent ion
- Improve separation properties (sometimes)
- Enable/improve quantitative analysis
- Class-specific detection
- Analyte confirmation
Why avoid derivatizing in LC-MS?

• Extra work, time, cost, and (possibly) error rate
• Supplier issues
• Yield (efficiency, isomer formation, side product formation)
• Poorer chromatography (sometimes)
• Poorer fragmentation patterns (sometimes)
Application examples/points to illustrate

- Keto-steroids
  - Ionization efficiency
  - LC separation issues
- Methylmalonic acid
  - Fragmentation pattern
  - Class-dependent ionization efficiency
- Hydroxy-steroids
  - Ionization efficiency
  - Fragmentation pattern issues
- Newborn screening
  - Class-selective analysis
- Peptides
  - Semi-quantitative analysis
Application: keto-steroids

- Oxime derivative
- Improved ionization efficiency (sensitivity)
- Poorer LC separation - sometimes
- Compound confirmation (possibly)
Oxime formation (testosterone)

Keto-steroid (testosterone)

Hydroxylamine

Oxime derivative
Protonation (ionization)
Sensitivity enhancement
Chromatography of testosterone-oxime derivative

Split peaks

Isomers
Difficulties and approaches

• More difficult peak integration
  – Approach: modify LC method to merge peaks
  – Approach: live with it

• Often results in poorer separation of related compounds
  – May require better LC separation
Application: methylmalonic acid

- Dibutyl ester
- More selective ionization
- Improved MS/MS spectrum
Biosynthetic Pathway

Propionyl-CoA

Propionyl-CoA carboxylase $\iff$ ATP + CO$_2$
ADP + Pi

$\text{(S)}$-Methylmalonyl-CoA

Methylmalonyl-CoA racemase $\downarrow$

$\text{(R)}$-Methylmalonyl-CoA $\rightarrow$ MMA

Methylmalonyl-CoA mutase (Coenzyme B$_{12}$) $\times$

Succinyl-CoA
Methylmalonic acid derivatization
Dibutyl esters of di-carboxylic acids more sensitive than monocarboxylic acids
Esters of di-carboxylic acids
Esters of mono-carboxylic acids
Non-selective MS/MS spectrum (underivatized)

Free Acid Ionization in the Negative Ion Mode
Selective MS/MS of derivatives

MMA

Parent ion

[MH - \text{C}_4\text{H}_8]^+ 

[MH - \text{C}_4\text{H}_8 - \text{H}_2\text{O}]^+ 

SA

Parent ion

[MH - \text{C}_4\text{H}_8]^+ 

[MH - \text{C}_4\text{H}_8 - \text{H}_2\text{O}]^+ 

Intensity, %

m/z
Application: hydroxy-steroids

- Dansyl chloride derivatization
- Improved ionization efficiency (sensitivity)
- Poorer MS/MS spectrum
Dansyl chloride derivatization

Dansyl chloride

Estradiol

Reaction site

Derivative
Ionization of dansyl derivatives

\[ \text{H}^+ \]
Dissociation of dansyl derivative of estradiol

m/z 171

m/z 156

m/z 506 “parent” ion

Non-specific “daughter” ions
Non-specific product ions of dansyl derivative of estradiol

m/z 171

Product ions from dansyl-end of ion.

m/z 156
Difficulties and approaches

• Lower specificity of MS/MS spectrum
  – Improve extraction
  – Improve LC
Application: newborn screening

- Butyl ester derivative

- MS/MS only

- Class sensitive compound detection
  - Acyl carnatines (fatty acids/organic acids)
  - Amino acids

- Scan functions
  - Precursor ion
  - Constant neutral loss
Acylcarnitines derivatization and fragmentation

Slide courtesy of Mike Morris, Waters Corp
Normal vs. MCAD deficiency

precursor ion scans

C2 carnitine

C3 carnitine

C16 carnitine

C8 carnitine

C6 carnitine

C10:1 carnitine

Normal
carnitine

MCAD
deficiency
carnitine

Slide courtesy of Mike Morris, Waters Corp
Application: relative peptide quantitation using derivatization

- ICAT
  - Cysteine

- ITraq & MTRAQ
  - Primary amines
ICAT reagents

Light ICAT reagent (hydrogen labels)

Heavy ICAT reagent (deuterium labels)

Adapted from http://www.bio.davidson.edu/people/macampbell/strategies/ICATdemo.html
ICAT™ quantitation

Control sample → label with ICAT™ mass tags → overnight trypsin digest → peptide extraction → combine samples → test sample

MS scan → quantitation

MS/MS scan → identification

Ratio d0/d8 ~ 3:1

Isotope-Coded Affinity Tags

Heavy reagent: D8-ICAT Reagent (X=deuterium)
Light reagent: DO-ICAT Reagent (X=hydrogen)
Conclusions

- Use derivatization only when advantages outweigh disadvantages
- Sensitivity
- MS/MS quality
- Separation quality
- Special applications
  - Compound class analysis
  - Quantitation of peptides
  - Etc.
Additional reading


• Handbook of Analytical Derivatization Reactions, by Daniel Knapp (Mostly oriented to GC-MS)
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• Additional source materials as listed on slides