Assessment of C3-Epi-25-OH vitamin D concentrations in adult serum: LC-MS/MS determination using [2H₃] C3-epi-25OHD₃ as internal standard and NIST traceable commercial 3-epi-25OHD calibrators

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Introduction

- LC-MS/MS is currently considered the gold standard method for the measurement of 25OHD. It is able to distinguish 25OHD from 25OHD₃, providing a more accurate assessment of an individual’s vitamin D status.
- Interferences from co-eluting isobaric compounds of identical elemental composition but of different structure can result in overestimation of total 25OHD.
- C-3 Epimer of 25-hydroxy vitamin D₃ and D₂ (C3-Epi-25OHD₃/D₃) differs from 25OHD in configuration of the hydroxyl group at the third carbon (C-3) position. It has been shown to be more prevalent in infants and in adults with specific disease states.
- Due to the similarity in mass, charge and ionisation characteristics, conventional mass spectrometric systems are unable to separate the epimer according to the MRM transitions.

Aims and Objectives

- To resolve and quantify C3-Epi-25(OH)D₃ and 25(OH)D using LC-MS/MS technique.
- Analyse C3-Epi-25(OH)D₃/D₃ in patient samples received for 25(OH)D measurement at the Norfolk and Norwich University Hospital.

LC-MS/MS separations

Sample Preparations
1. 100µL of sample/Std/QC.
2. Add 100µL of 0.1M Zinc Sulphate.
3. Add 200µL of acetonitrile containing internal standards.

Gradient Timetable
Flow rate: 0.4 mL/min (A)Water : (B)methanol (both contains in 0.1% formic acid) 0 – 9.0 min 25%A: 70%B 9.0 – 10.0 min 100%B 10.0 – 11.0 min 25%A: 70%B

HPLC Column
Thermo Accucore 2.6µm 100 x 2.1mm I.D. pentfluorophenyl solid core particle column.

LC-MS/MS system
Micromass Ultima triple quadrupole tandem mass spectrometer.

Distribution of C3-epi-25OHD₃ concentration in cohort of 839 adult samples.

C3-epi-25OHD₃ – Prevalence and concentrations

- 4.8% of samples had C3-epi-25HD₃ level >10% of 25OHD₃ (average 5.1%).
- No correlation was found between C3-epi-25OHD₃ and 25OHD₃.
- 5% of samples reinterpreted as vitamin D insufficient with C3-epi-25OHD₃ is excluded from total 25OHD.

Assay imprecision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>CV%</th>
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<tbody>
<tr>
<td>Intra-assay imprecision</td>
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</tr>
<tr>
<td>10</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>42.4</td>
<td>3.0</td>
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<tr>
<td>Inter-assay imprecision</td>
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<td></td>
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<tr>
<td>10</td>
<td>64.8</td>
<td>6.4</td>
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<tr>
<td>12</td>
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<td>2.3</td>
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<td>Recovery efficiency</td>
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<tr>
<td>12</td>
<td>109.9</td>
<td>10.4</td>
</tr>
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</table>

Conclusions

- C3-epi-25OHD₃ was found in the majority of our sample cohort, but prevalence was low.
- C3-epi-25OHD₃ contributed to the overestimation of 25OHD₃ and resulted in misinterpretation of total vitamin D status.
- High prevalence in infant. Separation of epimer in neonatal samples is essential.
- DEQAS LC-MS/MS method group using NIST-aligned standards showed a positive bias against ALTM. NIST assay can resolve C3-epi-25OHD₃.

Biological activity and clinical utility of C3-epi-25OHD₃ remains to be elucidated.

References