Simultaneous Estimation of Total Homocysteine and Methylmalonic acid in Clinical Plasma/Serum Samples by Using Acquity UPLC-XEVO TQD

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INTRODUCTION

Clinical background:
Vitamin B12 (Cobalamin) deficiency is a common clinical problem in the elderly subjects, neonates and infants, its deficiency may lead to irreversible neurological damage.

In healthy population the coenzyme form of vitamin B12 participates in two key metabolic pathways. One is the conversion of methylmalonyl-coenzyme-A to succinyl-CoA & second one is formation of methionine from homocysteine (Hcy).

In case of vitamin B12 deficiency, methylmalonyl-CoA accumulates and subsequently increases Methylmalonic acid (MMA) and homocysteine concentration in blood. Monitoring of MMA and tHcy’s can be an indicative early biomarkers to characterize intracellular, functional vitamin B12 deficiency.

OBJECTIVE:
Development of simultaneous estimation of MMA & total Homocysteine using UPLC-XEVO TQD mass spectrometer.

CHALLENGES:
Both the analytes are highly polar and exist endogenously, having low molecular weight and low pKa values.

Simple and fast LC-MS/MS, MRM based assay was developed for the simultaneous determination of MMA & tHcy in plasma and serum samples.

Sample preparation demonstrated is simple, rapid, and analogous for biological matrices.

METHOD SUMMARY

Sample preparation: To the plasma/serum sample, two Internal standard was added with reduction reagent and vortexed for 5 minutes. Extraction solution was added and centrifuged, the supernatant was taken for LC-MS/MS analysis.

MRM CONDITIONS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent m/z</th>
<th>Daughter m/z</th>
<th>Cone (V)</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td>136.08</td>
<td>90.03</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Homocysteine-d4</td>
<td>140.08</td>
<td>94.03</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methylmalonic acid</td>
<td>117.00</td>
<td>73.00</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Methylmalonic acid-d3</td>
<td>120.00</td>
<td>76.00</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

MS Conditions:

MS System : Xevo TQD
Mode : ESI +Ve & -Ve
Cone gas : 50 L/Hr
Desolvation Temp : 300 °C
Source Temp : 150 °C
Desolvation gas flow : 600 L/hr
Data processed through TargetLynx application manager.

RESULTS AND DISCUSSION

1) Demonstrated simultaneous quantification method for MMA and tHcy, which is reliable, sensitive, precise, accurate and reproducible in serum and plasma matrix.
2) The method demonstrated showed less matrix effect and good sensitivity up to 50 ppb for MMA & 500 ppb for tHcy.
3) Opportunity for this method to be validated and used in routine analysis.

REFERENCES


CONCLUSION

ACKNOWLEDGMENT

The authors would like to thank Dr. Sujay Prasad (Chairman of Anand Diagnostics) and Mr. Prem Pal (Manager, Analytical Lab) for providing Reference standards and patient serum & plasma samples.