Falsely elevated plasma metanephrine on uHPLC-MS/MS by the metabolite of midodrine with a HILIC column

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**Introduction**

- Patients with phaeochromocytoma and paraganglioma (PPGLs) can present with non-specific symptoms including headache, sweating and postural hypotension.
- Midodrine is a sympathomimetic agent used for the treatment of postural hypotension; this symptom has been described in 10-50% of patients with newly diagnosed PPGL. ¹
- Midodrine is a prodrug with a half-life of 0.5 hour and is hydrolyzed to form the active metabolite, desglymidodrine which has a longer elimination half life of 2-3 hours.²

**Case**

- A 35 year old male presented to cardiology with severe postural hypotension and unexplained dilated cardiomyopathy and was treated with the medication midodrine.
- Plasma metanephrines were requested for the investigation of PPGL. Initial plasma metanephrine was 72,000 pmol/L (< 500 pmol/L).
- An abnormal qualifier to quantifier ion ratio was noted in the measurement of plasma metanephrine.
- Given the structural similarities between metanephrine, midodrine and desglymidodrine, we tested the hypothesis of analytical interference by midodrine or its metabolite as the cause of elevated plasma metanephrine.

**Results**

- Methanolic extract of a midodrine tablet (Douglas Pharmaceuticals, New Zealand) before and after hydrolysis demonstrated detection at the transitions for metanephrine. Baseline resolution for metanephrine could not be achieved (Figure 1).
- A longer HILIC column successfully separated the interfering midodrine metabolite from metanephrine in the patient specimen and in a mixture of partially hydrolyzed midodrine spiked with metanephrine (Figure 2).
- As a result of possible interference related to midodrine, plasma metanephrines were repeated following the discontinuation of this medication (Table 1).

**Discussion**

- Unexpected elevations of this magnitude in the plasma have previously been reported in a patient taking the medication midodrine. This group raised the possibility of analytical interference as the cause of elevation.³
- Our findings demonstrate the co-elution of midodrine metabolite with metanephrine as the cause of abnormal plasma metanephrine. It is likely other laboratories using similar methodologies will be affected.
- Three subsequent patients on midodrine have also had falsely elevated metanephrine. None of these four patients had a diagnosis of phaeochromocytoma and paraganglioma.
- We advise caution in the reporting of plasma metanephrine in patients on midodrine.

**Conclusion**

Patients taking midodrine may have falsely elevated plasma metanephrine as a result of analytical interference.

*References:

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**Free plasma metanephrines by uHPLC-MS/MS**

<table>
<thead>
<tr>
<th>Sample Preparation</th>
<th>Solid phase extraction Internal Standard</th>
<th>Mixed mode cation exchange (Phenomenex, Litosil) (Metanephrine D3/Normetanephrine D3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatography</td>
<td>Standard column</td>
<td>Phenomenex Kinetix® HILIC 50 x 2.1 mm, 2.6 µm (Phenomenex Luna® HILIC 150x4.6 mm, 5 µm)</td>
</tr>
<tr>
<td></td>
<td>Long column</td>
<td>Phosphoric acid with 0.1% formic acid</td>
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<tr>
<td></td>
<td>Mixed Phase A</td>
<td>50 mM ammonium formate with 1% formic acid</td>
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<tr>
<td></td>
<td>Mixed Phase B</td>
<td>Detection</td>
</tr>
<tr>
<td>Detection</td>
<td>Metanephrine (Qualifier, Quantifier)</td>
<td>Thermo Scientific Aria TLX</td>
</tr>
<tr>
<td>Instrument</td>
<td>Auto Sampler</td>
<td>Thermo Scientific TSQ Vantage</td>
</tr>
</tbody>
</table>

**Method**

**In vitro hydrolysis of midodrine**

- In vitro hydrolysis of midodrine (Douglas Pharmaceuticals, New Zealand) was performed using human hepatic cytosol (Gifted by Associate Prof. M. Tingle, Department of Pharmacology and Clinical Pharmacology, University of Auckland).

**Results**

- On midodrine
- Off midodrine

<table>
<thead>
<tr>
<th>Metanephrine (&lt; 500 pmol/L)</th>
<th>72,000 pmol/L</th>
<th>133 pmol/L</th>
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<tbody>
<tr>
<td>Ion Ratio: (Qualifier to Quantifier Ratio)</td>
<td>1.9 %</td>
<td>65 %</td>
</tr>
</tbody>
</table>

**Table 1: Plasma metanephrines while on and off midodrine**

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**Figure 1:** Chromatogram of metanephrine and midodrine (180.1 → 148.1) before and after incubation with human hepatic cytosol on the standard column.

**Figure 2:** Successful chromatographic separation of metanephrine (180.1 → 148.1) from midodrine and the metabolite of midodrine with the long HILIC column.

**Figure 3:** Putative mechanism of analytical interference with metanephrine by the formation of isobaric product ions from midodrine and desglymidodrine (active metabolite).