QUANTIFICATION OF 17-HYDROXYPROGESTERONE IN PROTEIN-PRECIPITATED PLASMA USING THE QUATTRO PREMIER[™]

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INTRODUCTION

Atmospheric Pressure Chemical Ionization (APCI) is often cited as the technique of choice for improved linearity in quantitative LC/MS/MS. In this technical note we show the newly developed Waters® Micromass® Quattro Premier™ tandem quadrupole mass spectrometer in the analysis of 17hydroxyprogesterone in protein-precipitated plasma. The calibration curve was plotted over five orders linear dynamic range of concentration, indicating excellent dynamic range that will be beneficial in dose ranging studies.

EXPERIMENTAL

A standard solution of 17-hydroxyprogesterone was prepared in methanol (1 mg/ml). Subsequent dilutions were made in human plasma to generate a calibration curve from 1-100,000 pg/µL (5-500,000 pg on column). The calibration standards were then proteinprecipitated by adding acetonitrile (1:1), the resultant mixtures were centrifuged (ca 3000 rpm, 10 mins) and the supernatant taken for analysis by LC/MS/MS. Duplicate injections were made of each standard.



Waters Micromass Quattro Premier Mass Spectrometer configured with the Waters 1525µ Binary HPLC Pump, 2777 Sample Manager and MassLynx Software.

HPLC Conditions

| LC System: | | | | |
|--|--|--|--|--|
| Waters 1525µ binary HPLC pump | | | | |
| Sample Manager: Waters 2777 | | | | |
| Column: Waters Symmetry [®] C ₁₈ 4.6 x 50 mm | | | | |
| 3.5 μm | | | | |
| Solvents: | | | | |
| A - 70% water 30% MeOH 10 mM ammonium | | | | |
| acetate 0.005% acetic acid | | | | |
| B - 100% MeOH 10 mM ammonium acetate | | | | |
| 0.005% acetic acid | | | | |
| Gradient: | | | | |

%В

35

35

Flow rate

0.8

0.8 mL/min

MS Conditions

| Mass Spectrometer: | Waters Micromass | | | | |
|--------------------|-----------------------------------|--|--|--|--|
| | Quattro Premier | | | | |
| lon mode: | APCI+ve | | | | |
| Corona: | 5 mA | | | | |
| Cone Voltage: | 40 V | | | | |
| Collision energy: | 18 eV | | | | |
| Detection mode: | MRM (331.3 > 108.9) | | | | |
| Dwell: | 0.3 seconds | | | | |
| Collision gas: | Argon (2.7x10 ⁻³ mbar) | | | | |

0 0.5

Time

| 6.5 | 15 | 85 | 0.8 | |
|-----|----|-----|-----|--|
| 7.0 | 10 | 90 | 0.8 | |
| 7.1 | 0 | 100 | 0.8 | |
| 7.5 | 0 | 100 | 0.8 | |
| 7.6 | 65 | 35 | 0.8 | |

%A

65

65

Injection volume: 5 µL

RESULTS AND DISCUSSION

The plot of peak area against concentration showed good linearity over the range 5-500,000 pg on column. The calibration line was plotted using a linear fit with $1/x^2$ weighting and gave a correlation coefficient of >0.99 (Figure 1).

Figure 2 shows the QuanLynx[™] results table demonstrating that all the calibration points gave back-calculated values within ±6% of the theoretical concentrations at all concentrations except for the LLOQ which were within ± 13%.

CONCLUSION

The new Waters Quattro Premier tandem quadrupole mass spectrometer has been developed for quantitative LC/MS/MS. The results also show that the Quattro Premier can be used for quantitation over five orders of linear dynamic range, i.e. 5 to 500,000 pg on column for 17-hydroxyprogesterone.



Figure 1. A calibration line for hydroxyproges-terone over the range 5-500000 pg on column.

| Quantify Compound Summary Report | | | | | | | | | |
|----------------------------------|--------------|----------------------------|----------|-----------|------|----------|----------|-------|--|
| Printed Tue Dec 09 09:44:50 2003 | | | | | | | | | |
| Compound 1: hydroxyprogesterone | | | | | | | | | |
| | Name | Sample Text | Туре | Std. Conc | RT | Area | pg | %Dev | |
| 1 | hydroxy_2001 | hydroxyprogesterone 0 | Blank | | | | | | |
| 2 | hydroxy 2002 | hydroxyprogesterone 0 | Blank | | | | | | |
| 3 | hydroxy_2005 | hydroxyprogesterone 5 | Standard | 5 | 5.36 | 23.804 | 4.4 | -12.4 | |
| 4 | hydroxy 2006 | hydroxyprogesterone 5 | Standard | 5 | 5.37 | 28.273 | 5.6 | 11.7 | |
| 5 | hydroxy_2007 | hydroxyprogesterone 50 | Standard | 50 | 5.37 | 196.947 | 51.1 | 2.3 | |
| 6 | hydroxy 2008 | hydroxyprogesterone 50 | Standard | 50 | 5.38 | 200.588 | 52.1 | 4.2 | |
| 7 | hydroxy 2009 | hydroxyprogesterone 500 | Standard | 500 | 5.36 | 1818.96 | 489.2 | -2.2 | |
| 8 | hydroxy 2010 | hydroxyprogesterone 500 | Standard | 500 | 5.36 | 1944.986 | 523.2 | 4.6 | |
| 9 | hydroxy_2011 | hydroxyprogesterone 5000 | Standard | 5000 | 5.35 | 18161.74 | 4903 | -1.9 | |
| 10 | hydroxy 2012 | hydroxyprogesterone 5000 | Standard | 5000 | 5.35 | 19115.75 | 5160.7 | 3.2 | |
| 11 | hydroxy 2013 | hydroxyprogesterone 50000 | Standard | 50000 | 5.33 | 177533.7 | 47945.6 | -4.1 | |
| 12 | hydroxy 2014 | hydroxyprogesterone 50000 | Standard | 50000 | 5.34 | 188804.9 | 50989.7 | 2 | |
| 13 | hydroxy 2015 | hydroxyprogesterone 500000 | Standard | 500000 | 5.34 | 1740339 | 470022.3 | -6 | |
| 14 | hydroxy 2016 | hydroxyprogesterone 500000 | Standard | 500000 | 5.34 | 1823707 | 492538.2 | -1.5 | |

Figure 2. QuanLynx results table showing the % deviations for each standard.

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