

Application Note

Quantification of 17-Hydroxyprogesterone in Protein-Precipitated Plasma Using the Quattro Premier

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Abstract

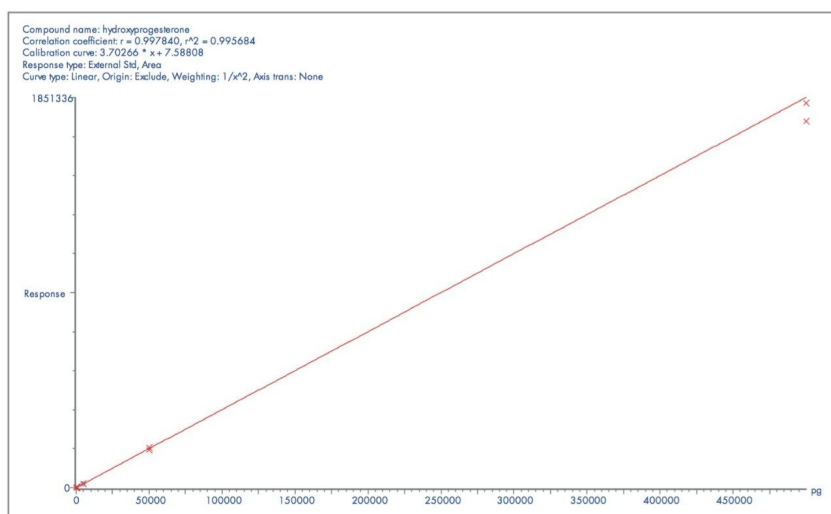
This application note shows the newly developed Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer for the analysis of 17- hydroxyprogesterone in protein-precipitated plasma.

Benefits

The Quattro Premier can be used for quantitation over five orders of linear dynamic range

Introduction

Atmospheric Pressure Chemical Ionization (APCI) is often cited as the technique of choice for improved linearity in quantitative LC-MS/MS. In this application note we show the newly developed Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer in the analysis of 17- hydroxyprogesterone 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.



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

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 protein-precipitated 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.

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
Injection volume:	5 μL

Gradient

Time (min)	%A	%B	Flow rate (mL/min)
0	65	35	0.8
0.5	65	35	0.8
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

MS Conditions

Mass spectrometer: Waters Micromass Quattro Premier

Ion 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 (2.7×10^{-3} mbar)

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).

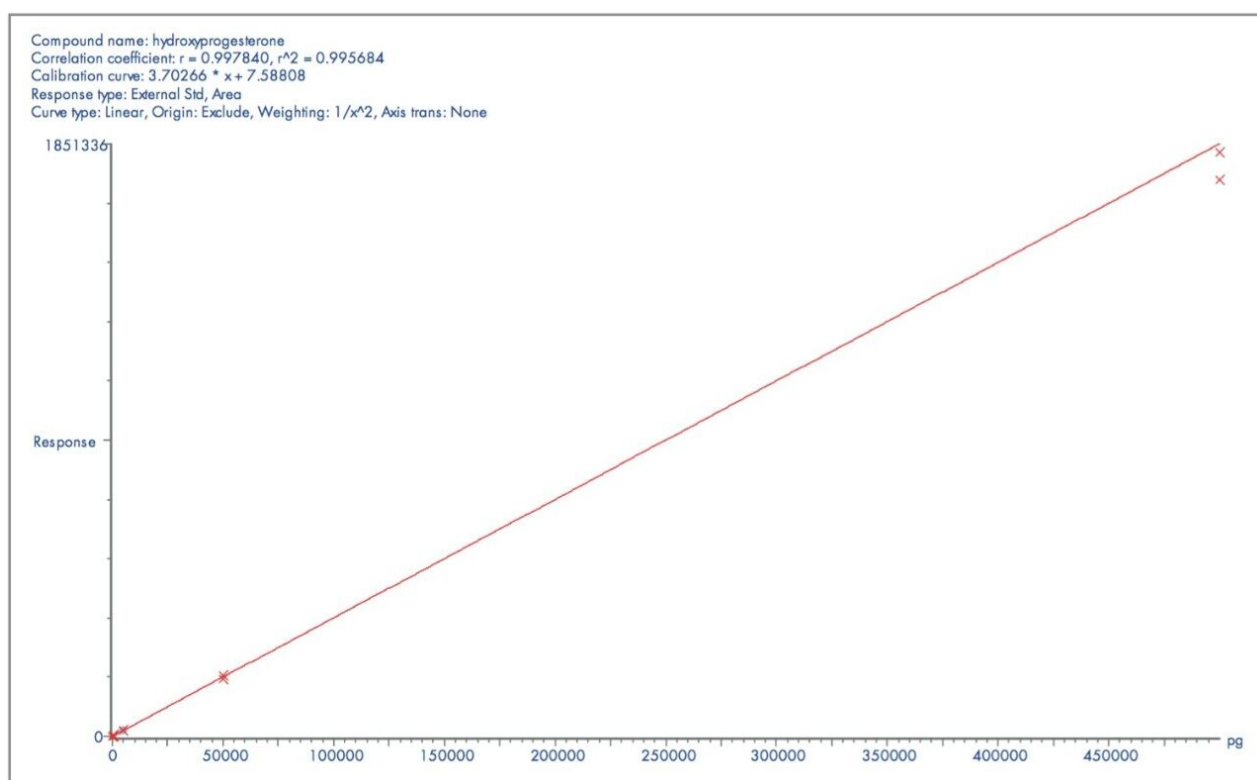


Figure 1. A calibration line for hydroxyprogesterone over the range 5–500000 pg on column.

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

Quantify Compound Summary Report								
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Compound 1: hydroxyprogesterone								
	Name	Sample Text	Type	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.

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.

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MassLynx MS Software <<https://www.waters.com/513662>>

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