

Tabisam Khan and Lisa J Calton
Waters Corporation, Atlas Park, Manchester, M23 9LZ

Introduction

Atmospheric 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 Waters® ACQUITY Ultra Performance LC™ System coupled with the Micromass® Quattro Premier™ tandem quadrupole mass spectrometer in the analysis of 17-hydroxyprogesterone in protein-precipitated plasma. The ACQUITY UPLC™ System runs at high pressure (up to 15,000 psi), resulting in very fast chromatography while maintaining excellent chromatographic resolution. This allows for excellent data quality with good linearity across large calibration ranges. A calibration curve was plotted over five orders linear dynamic range, indicating that a larger range of drug dosage can be analyzed in biological studies.

Experimental

A standard solution of 17-hydroxyprogesterone was made in methanol (1 mg/mL). Subsequent dilutions were made in human plasma to generate a calibration curve from 0.5-100000 pg/μL. The calibration standards were then protein-precipitated by adding acetonitrile (2:1 acetonitrile:plasma); the resultant mixture was centrifuged (ca 3000 rpm, 10 min) and the supernatant taken for analysis by LC/MS/MS. Duplicate injections were made of each standard.

LC Conditions

| | | | | |
|-------------------|---|----|----|-----------------------|
| LC System: | Waters ACQUITY UPLC | | | |
| Column: | Waters ACQUITY BEH C ₁₈ , 2.1 x 50 mm, 1.7 μm particles | | | |
| Solvents: | A - 70% water, 30% methanol (10mM ammonium acetate, 0.005% acetic acid) B - 100% methanol (10mM ammonium acetate 0.005% acetic acid) | | | |
| Gradient: | Time | %A | %B | Flow rate (mL/min) |
| | 0.5 | 65 | 35 | 0.6 |
| | 3.0 | 10 | 90 | 0.6 |
| | 4.0 | 10 | 90 | 0.6 |
| | 4.5 | 65 | 35 | 0.6 |
| Injection Volume: | 5 μL | | | |

MS Conditions

| | |
|-------------------|-------------------------------------|
| MS System: | Waters Micromass Quattro Premier |
| Ion Mode: | APCI+ve |
| Corona: | 8 μA |
| Cone Voltage: | 40 V |
| Collision Energy: | 18 eV |
| Detection Mode: | MRM (331.3 > 108.9) |
| Dwell: | 0.3 seconds |
| Collision gas: | Argon (3.2 x 10 ⁻³ mbar) |

Results and Discussion

The plot of peak area against concentration showed good linearity over the range of 5-50000 pg 17-hydroxyprogesterone 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).

The Quanlynx browser shown in Figure 1 also demonstrates that all the calibration points gave back-calculated values within $\pm 10\%$ of the theoretical concentrations.

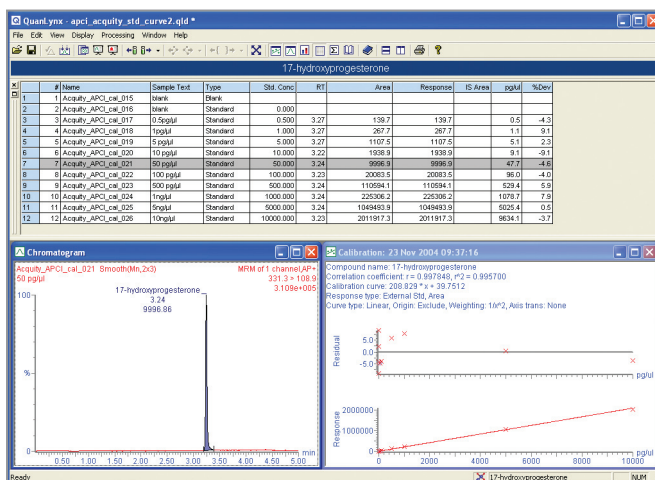


Figure 1. Quanlynx™ browser showing calibration curve linearity and percent deviations for each standard.

Conclusion

The Waters ACQUITY UPLC System with the Micromass Quattro Premier tandem quadrupole mass spectrometer has been developed for quantitative LC/MS/MS. The results show that excellent linear dynamic range can be obtained for pharmaceutical compounds in complex biological matrices. Advances in the chromatography have resulted from the improved resolving power and speed of the ACQUITY UPLC System.

The results also show that the ACQUITY UPLC with the Quattro Premier can be used to quantify 17-hydroxyprogesterone over five orders of linear dynamic range, i.e. 5 to 500000 pg on column.

Sales Offices:

AUSTRIA 43 1 877 18 07

AUSTRALIA 61 2 9933 1777

BELGIUM AND LUXEMBOURG 32 2 726 1000

BRAZIL 55 11 5543 7788

CANADA 800 252 4752 X2205

CZECH REPUBLIC 420 2 617 11384

DENMARK 45 46 59 8080

FINLAND 358 9 506 4140

FRANCE 33 1 3048 7200

GERMANY 49 6196 400600

HONG KONG 852 29 64 1800

HUNGARY 36 1 350 5086

INDIA 91 80 2837 1900

IRELAND 353 1 448 1500

ITALY 39 02 27 4211

JAPAN 81 3 3471 7191

KOREA 82 2 820 2700

MEXICO 52 55 5524 7636

THE NETHERLANDS 31 76 508 7200

NORWAY 47 6 384 6050

PEOPLE'S REPUBLIC OF CHINA 86 10 8451 8918

POLAND 48 22 833 4400

PUERTO RICO 787 747 8445

RUSSIA/CIS 7 095 931 9193

SINGAPORE 65 6278 7997

SPAIN 34 93 600 9300

SWEDEN 46 8 555 11 500

SWITZERLAND 41 62 889 2030

TAIWAN 886 2 2543 1898

UK 44 208 238 6100

US 800 252 4752

Waters

WATERS CORPORATION

34 Maple St.

Milford, MA 01757 U.S.A.

T: 508 478 2000

F: 508 872 1990

www.waters.com

For Complete Confidence

Waters, ACQUITY Ultra Performance LC, ACQUITY UPLC, Micromass, Quattro Premier and QuanLynx are trademarks of Waters Corporation.
All other trademarks are property of their respective owners.
©2005 Waters Corporation Produced in the USA March 2005 720001135EN LW-PDF

