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Introduction

The concentration of an analyte in a sample can be determined from its MS response by reference to the relative response of a standard. For this to work effectively the response of the standard should be linear over the analytical concentration range. In practice MS response may be non-linear. This non-linearity will be dependent on the compound assayed and ionization technique employed and is associated with changes in ionization efficiency. To account for this phenomenon the assay is often referenced to a calibration line rather than a single standard. Few assays are performed with samples having concentrations differing by more than 4 orders.

Using the Waters® Micromass® Quattro Premier™ benchtop mass spectrometer the linearity of signal response, relative to sample concentration, of 5 orders of magnitude has been confirmed. This has been demonstrated for the LC/MS/MS analysis of hydroxyprogesterone in precipitated human plasma. Measurements were made from the limit of detection using positive ion atmospheric pressure chemical ionization (APCI).

Example Data

The data presented below was obtained using the Quattro Premier benchtop mass spectrometer. A sequence of samples was prepared to create a response vs. amount injected calibration line using hydroxyprogesterone spiked into precipitated human plasma. Duplicate 5 µL injections of each sample were made on a Waters Symmetry® C₁₈ column. Hydroxyprogesterone was detected in the multiple reaction-monitoring mode (MRM) using the parent ion to product ion transition of m/z 331 to m/z 109.

The LC/MS/MS data collected was processed using QuanLynx™. The resulting QuanLynx report file (Figure 1) displays a linear calibration line (correlation coefficient = >0.99, 1/x² weighting) over a sample loading range of 5 orders of magnitude (5-500000 pg). All points on the curve exhibit a back-calculated (amount loaded) deviation of less than +/-10%—well within the generally accepted maximum deviation of +/-15%.

The data presented demonstrates the potential of the Waters Quattro Premier mass spectrometer to accommodate LC/MS/MS assays where the sample concentration range is very large.

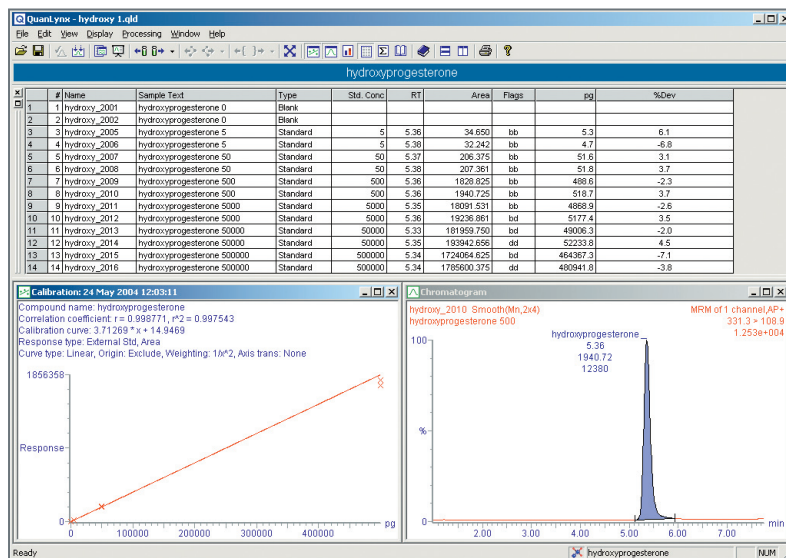


Figure 1. QuanLynx report, hydroxyprogesterone 5 pg to 500000 pg.

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