Waters™

Applikationsbericht

Quattro Premier: Linearity of Response

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the potential of the Waters Quattro Premier Mass Spectrometer to accommodate LC-MS/MS assays where the sample concentration range is very large.

Introduction

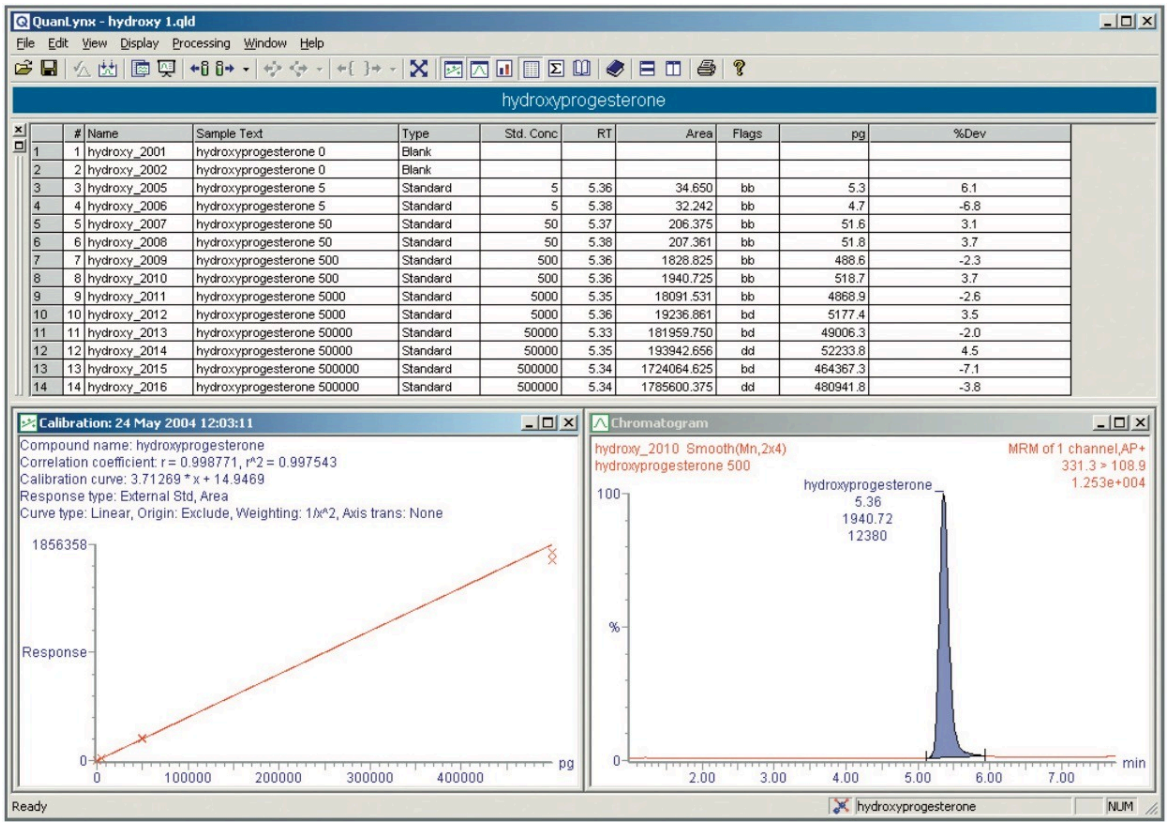
The concentration of an analyte in a sample can be determined from its MS response by reference to the relative response of a standard. For this to work effectively the response of the standard should be linear over the analytical concentration range. In practice MS response may be non-linear. This non-linearity 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).

Results and Discussion

The data presented below was obtained using the Quattro Premier benchtop Mass Spectrometer. A sequence of samples was prepared to create a response versus amount injected calibration line using hydroxyprogesterone spiked into precipitated human plasma. Duplicate 5 µL injections of each sample were made on a Waters Symmetry C_{18} 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, $1/x^2$ 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%.



#	Name	Sample Text	Type	Std. Conc	RT	Area	Flags	pg	%Dev
1	hydroxy_2001	hydroxyprogesterone 0	Blank						
2	hydroxy_2002	hydroxyprogesterone 0	Blank						
3	hydroxy_2005	hydroxyprogesterone 5	Standard	5	5.36	34.650	bb	5.3	6.1
4	hydroxy_2006	hydroxyprogesterone 5	Standard	5	5.38	32.242	bb	4.7	-6.8
5	hydroxy_2007	hydroxyprogesterone 50	Standard	50	5.37	206.375	bb	51.6	3.1
6	hydroxy_2008	hydroxyprogesterone 50	Standard	50	5.38	207.361	bb	51.8	3.7
7	hydroxy_2009	hydroxyprogesterone 500	Standard	500	5.36	1828.825	bb	488.6	-2.3
8	hydroxy_2010	hydroxyprogesterone 500	Standard	500	5.36	1940.725	bb	518.7	3.7
9	hydroxy_2011	hydroxyprogesterone 5000	Standard	5000	5.35	18091.531	bb	4868.9	-2.6
10	hydroxy_2012	hydroxyprogesterone 5000	Standard	5000	5.36	19236.861	bd	5177.4	3.5
11	hydroxy_2013	hydroxyprogesterone 50000	Standard	50000	5.33	181959.750	bd	49006.3	-2.0
12	hydroxy_2014	hydroxyprogesterone 50000	Standard	50000	5.35	193942.656	dd	52233.8	4.5
13	hydroxy_2015	hydroxyprogesterone 500000	Standard	500000	5.34	1724064.625	bd	464367.3	-7.1
14	hydroxy_2016	hydroxyprogesterone 500000	Standard	500000	5.34	1785600.375	dd	480941.8	-3.8

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

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.

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