

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

On The Path To A Random Access LC/MS Workflow:

A Novel Approach To Calibration

Don Cooper, PhD
Clinical Business Operations
Waters Corporation
Manchester UK

©2013 Waters Corporation

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

Background (1)

- There has been increasing adoption of liquid chromatography coupled with mass spectrometry (LC/MS) by clinical laboratories over the last approx 10 years.
- The adoption has been driven by several factors including:
 - improved specificity and sensitivity over conventional assays for some analytes
 - reduced costs compared to conventional assays for some analytes
 - multiplex capability
 - open architecture allows laboratories to develop LDTs e.g., for research purposes
- There are limitations:
 - high complexity & requires some degree of operator skill
 - relatively high instrument costs
 - limited availability of reagents (kits)
- Successful in niche applications (e.g., for immunosuppressant drug monitoring, steroid analysis, 25OHVID etc)

©2013 Waters Corporation

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

Background (2)

- Some of the limitations are being addressed to facilitate wider adoption of LC/MS:
 - IVD marked LC & MS instruments and consumables (columns etc)
 - Development of CE/IVD certified and FDA cleared reagent kits
 - Education / training programs (AACC, MSACL, fellowship programs, degree courses etc)
 - Reduced complexity compact bench-top LC/MS instruments
- But the typical batch mode LC/MS operation remains alien to many modern clinical chemistry laboratories that typically rely on automated random access workflows.

©2013 Waters Corporation

Conventional LC/MS Calibration

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Instrument

Intet Method

Solvent Monitor

Edit Shutdown or Startup

Shutdown

Startup

Options

Queue Is Empty

| File Name | Sample ID | Sample Type | MS File | MS Tune File | Tune File | Bole | InjVol (µL) | Conc A |
|---------------------|---------------|-------------|-----------|--------------|--------------|------|-------------|--------|
| 1 Testosterone 001 | Blank | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.2 | 15 | 0.1 |
| 2 Testosterone 002 | CAL 0.1ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.2 | 15 | 0.1 |
| 3 Testosterone 003 | CAL 0.2ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.3 | 15 | 0.2 |
| 4 Testosterone 004 | CAL 1.0ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.4 | 15 | 1.0 |
| 5 Testosterone 005 | CAL 2.0ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.5 | 15 | 2.0 |
| 6 Testosterone 006 | CAL 5.0ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.6 | 15 | 5.0 |
| 7 Testosterone 007 | CAL 10.0ng/mL | Standard | Testm_095 | Testm_2012 | Testosterone | 1.7 | 15 | 10.0 |
| 8 Testosterone 008 | Sample 1 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.8 | 15 | |
| 9 Testosterone 009 | Sample 2 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.9 | 15 | |
| 10 Testosterone 010 | Sample 3 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.10 | 15 | |
| 11 Testosterone 011 | Sample 4 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.11 | 15 | |
| 12 Testosterone 012 | Sample 5 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.12 | 15 | |
| 13 Testosterone 013 | Sample 6 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.13 | 15 | |
| 14 Testosterone 014 | Sample 7 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.14 | 15 | |
| 15 Testosterone 015 | Sample 8 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.15 | 15 | |
| 16 Testosterone 016 | Sample 9 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.16 | 15 | |
| 17 Testosterone 017 | Sample 10 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.17 | 15 | |
| 18 Testosterone 018 | QC 1 | QC | Testm_095 | Testm_2012 | Testosterone | 1.18 | 15 | 30 |
| 19 Testosterone 019 | Sample 11 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.19 | 15 | |
| 20 Testosterone 019 | Sample 12 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.19 | 15 | |
| 21 Testosterone 020 | Sample 13 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.20 | 15 | |
| 22 Testosterone 021 | Sample 14 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.21 | 15 | |
| 23 Testosterone 022 | Sample 15 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.22 | 15 | |
| 24 Testosterone 023 | Sample 16 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.23 | 15 | |
| 25 Testosterone 024 | Sample 17 | Analyte | Testm_095 | Testm_2012 | Testosterone | 1.24 | 15 | |

Example sample list for a typical LC/MS assay. Samples are analysed in batches. Each batch includes a sequence of calibrators.

Conventional External Calibration Curve

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

TESTO

| Sample Name | Concentration (ppb) | Retention Time (min) |
|--------------------|---------------------|----------------------|
| External_Sample 01 | 0.1 | 1.18 |
| External_Sample 02 | 0.2 | 1.18 |
| External_Sample 03 | 1.0 | 1.18 |
| External_Sample 04 | 2.0 | 1.18 |
| External_Sample 05 | 5.0 | 1.18 |
| External_Sample 06 | 10.0 | 1.18 |
| External_Sample 07 | 30.0 | 1.18 |

Calibration 04 May 2013 16:30:06
 Chromatogram: TESTO
 Calibration equation: $y = 0.0002x + 0.0002$
 Correlation coefficient: $r^2 = 0.997259$
 Calibration curve: $1.17517 \times 10^{-5} [0.00044]$
 Response type: Internal [PP] Ref 1: 499 - 15 Conc: 10 Area 1
 Curve type: Linear Origin: External, Weighting: 1/A Area Ratio

Barriers to Random Access LC/MS

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Several barriers to random access quantitative mass spectrometry:
 - Manual review of results
 - Mobile phase switching & equilibration
 - Column switching & equilibration

- Can be addressed by:
 - Software / informatics
 - Instrument design / engineering
 - Assay design (e.g., common fit-for-purpose mobile phases)

- The need to run calibrators and batches of samples remains the major barrier

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

Internal Calibration

©2013 Waters Corporation

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

The Internal Calibration Concept*

- The internal calibrators are added directly to the individual sample which is then processed in the normal way (e.g., SPE, LLE etc ...)
- Each point on the calibration curve is derived from a unique internal calibrator that can be differentiated from the analyte of interest and from the other internal calibrators using mass spectrometry.
- The ideal situation is to use a different stable isotope labelled form of the analyte of interest for each calibration point so that each internal calibrator has a unique mass.
- The sample is then analysed by LC/MS(/MS), simultaneously monitoring the analyte and all the added internal calibrators.
- From that single analysis, the integrated peak areas for the internal calibrators can be used to construct a calibration line from which the analyte concentration can be calculated.

* Patent pending

©2013 Waters Corporation

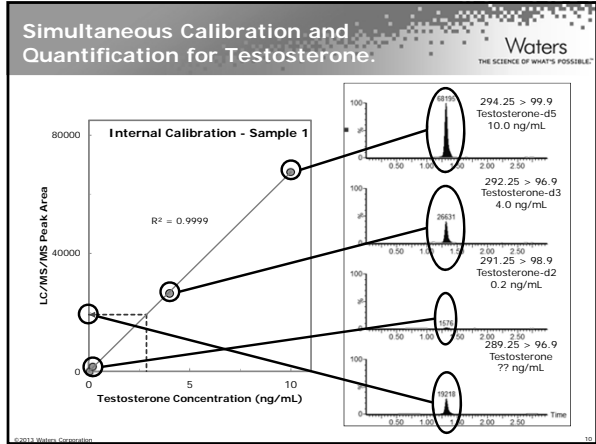
Waters
THE SCIENCE OF WHAT'S POSSIBLE™

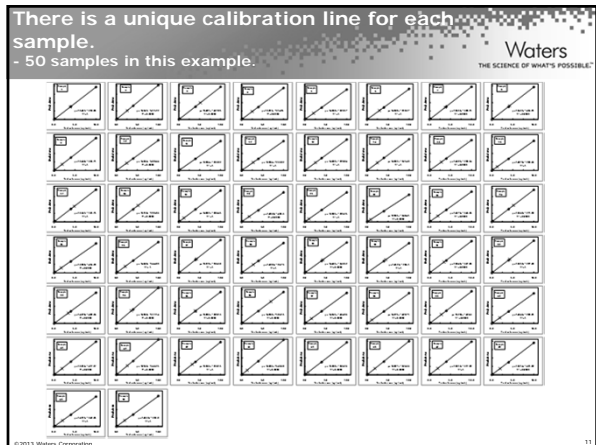
Example Internal Calibration for Testosterone

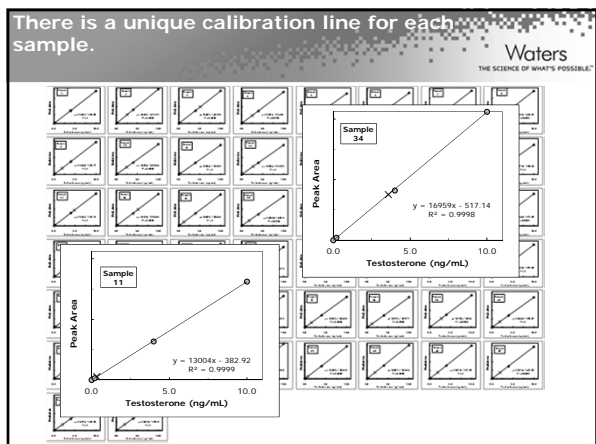
| Analyte */ Calibrator | MS/MS | Final Concentration (ng/mL) |
|-----------------------------|---------------|-----------------------------|
| Testosterone* | 289.25 > 96.9 | |
| Testosterone-D ₂ | 291.25 > 98.9 | 0.2 |
| Testosterone-D ₃ | 292.25 > 96.9 | 4.0 |
| Testosterone-D ₅ | 294.25 > 99.9 | 10.0 |

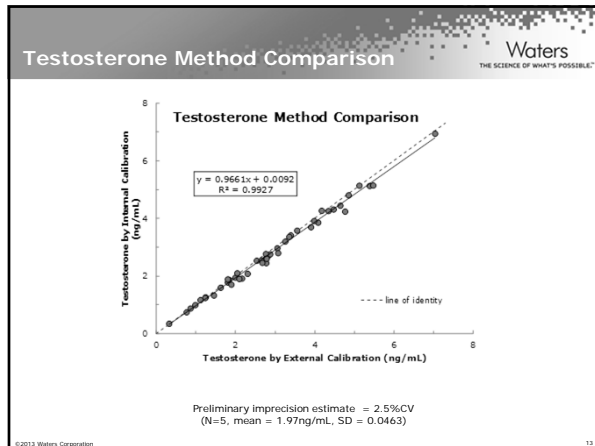
- A 20x concentrated mixture of internal calibrators was prepared.
- The internal calibrator mixture was added to each sample and the samples prepared as usual (liquid-liquid extraction).
- The samples were analysed by LC/MS/MS simultaneously monitoring the 4 MRMs above.

©2013 Waters Corporation









- Summary**
- Waters
THE SCIENCE OF WHAT'S POSSIBLE™
- We have developed an approach to quantitative LC/MS compatible with the demands of the routine clinical chemistry customer.
 - Acceptable preliminary validation performed for several different analytes.
 - No requirement to analyse separate, external calibrators
 - Simplified workflow
 - Calibration (internal) is perfectly matrix matched
 - Time to first result is reduced (4min vs. 32min for testosterone example)
 - Potential to develop random access LC/MS-based clinical analysers
 - Such an instrument linked to a LIS and with on-board reagents could be used to run personalised, multiplexed analyte panels.
- © 2013 Waters Corporation
