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

A Novel Approach To Calibration

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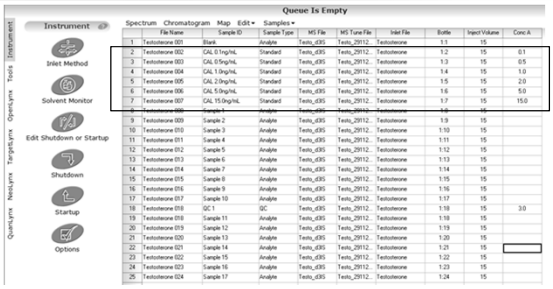
Background (1)

- 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)

Background (2)

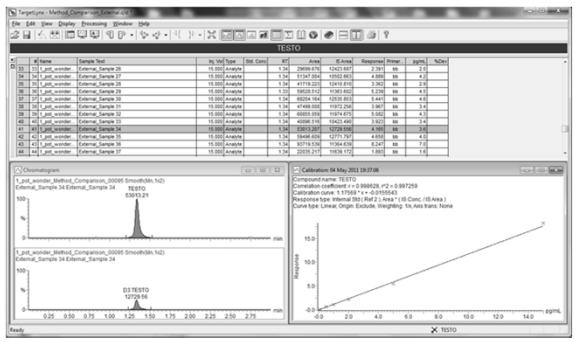
- Some of the limitations are being addressed to facilitate wider adoption of LC/MS:
  - IVD marked LC & MS instruments and consumables (columns etc)
  - Development of CE/IVD certified and FDA cleared reagent kits
  - Education / training programs (AACC, MSACL, fellowship programs, degree courses *etc*)
  - 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.

Conventional LC/MS Calibration



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



Barriers to Random Access LC/MS

- 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**

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Internal Calibration

The Internal Calibration Concept*

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- 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

Example Internal Calibration for Testosterone

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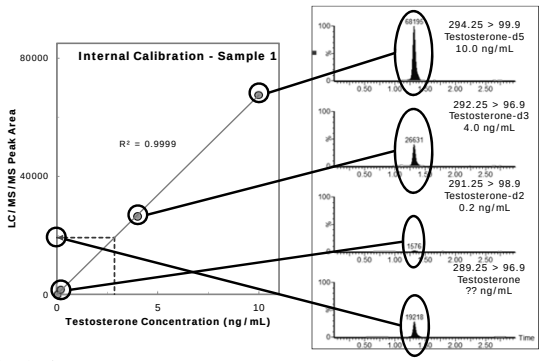
Analyte */ Calibrator	MS/MS	Final Concentration (ng/mL)
Testosterone*	289.25 > 96.9	
Testosterone-D_2	291.25 > 98.9	0.2
Testosterone-D_3	292.25 > 96.9	4.0
Testosterone-D_5	294.25 > 99.9	10.0

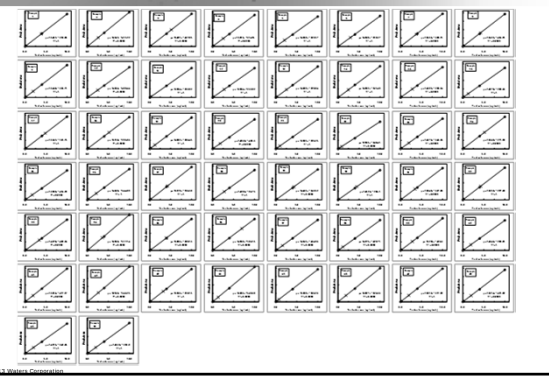
- A 20x concentrated mixture of internal calibrators was prepared.
- The internal calibrator mixture was added to each sample and the samples prepared as usual (liquid-liquid extraction).
- The samples were analysed by LC/MS/MS simultaneously monitoring the 4 MRMs above.

Simultaneous Calibration and Quantification for Testosterone.



There is a unique calibration line for each sample.

- 50 samples in this example.



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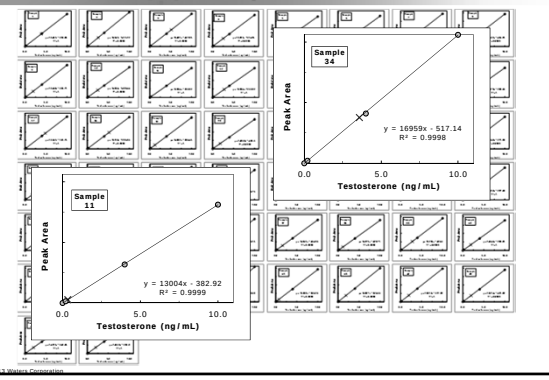
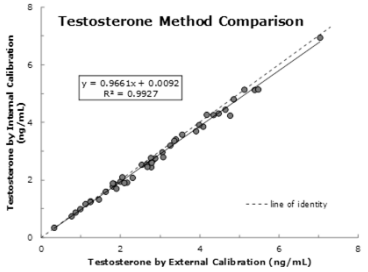


Testosterone Method Comparison



Preliminary imprecision estimate = 2.5%CV
(N=5, mean = 1.97ng/mL, SD = 0.0463)

Summary

- 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.