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Mass Spectrometry and the Future of Routine Clinical Analysis

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Overview



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- Current analytical methods used and inherent problems
- Brief introduction to mass spectrometry
- Mass spectrometry and clinical analysis
- Developments for the future

Current principal methodology: immunoassay analysis



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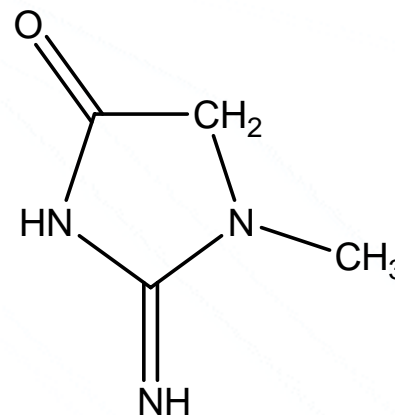
- Biochemical test to measure levels of substance in serum or urine
- Specific binding of antibody to antigen
- Antibody or antigen labelled
 - Enzyme, radioisotope, fluorescent chemical group
- Response quantified using calibration curve

Creatinine



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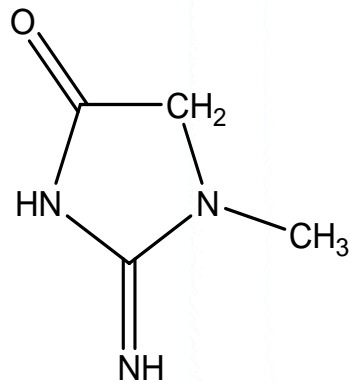
- Metabolite of creatine phosphate
- Monitored as a control for renal activity
- Filtered through kidneys
 - Typical values
 - 45-90 $\mu\text{mol/L}$ (women)
 - 60-110 $\mu\text{mol/L}$ (men)



creatinine

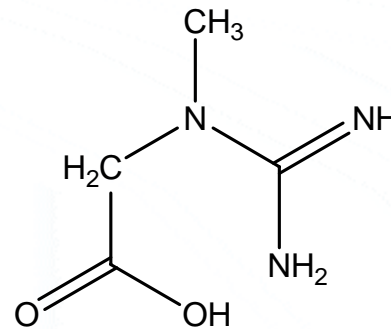
Immunoassay problems (1)

- Interference caused by structurally similar molecules
 - Jaffé reaction, creatine and other compounds also form picrate complexes

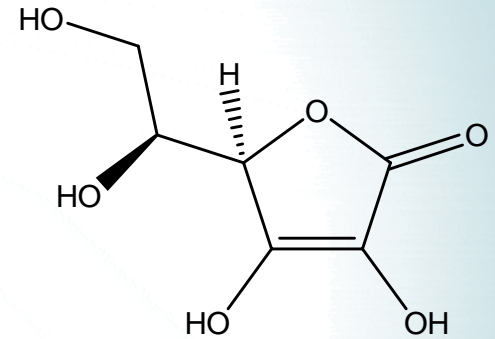


creatinine

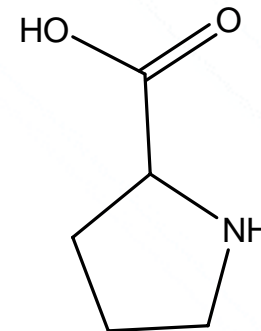
but also



creatine



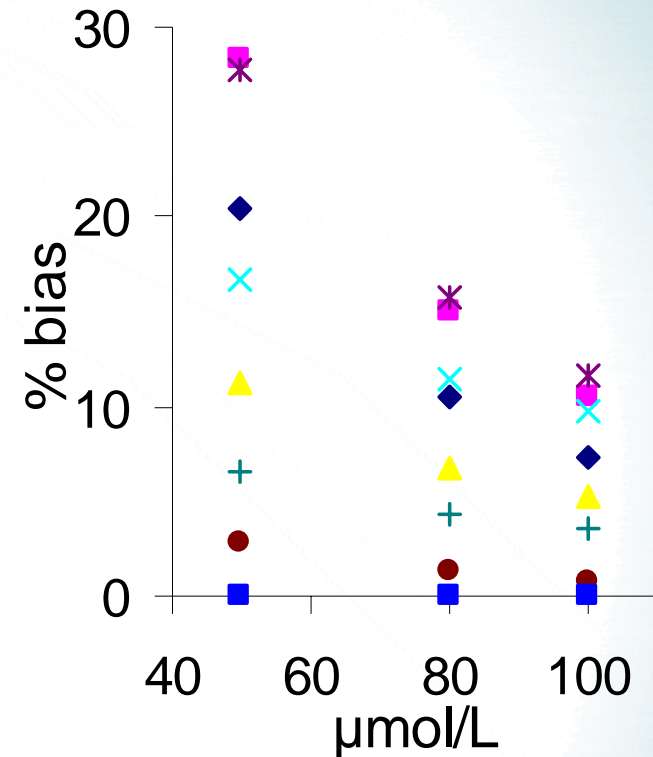
ascorbic acid



proline

Immunoassay problems (2)

- Biased
 - Creatinine example
- No standardisation between manufacturers
 - Different reagents

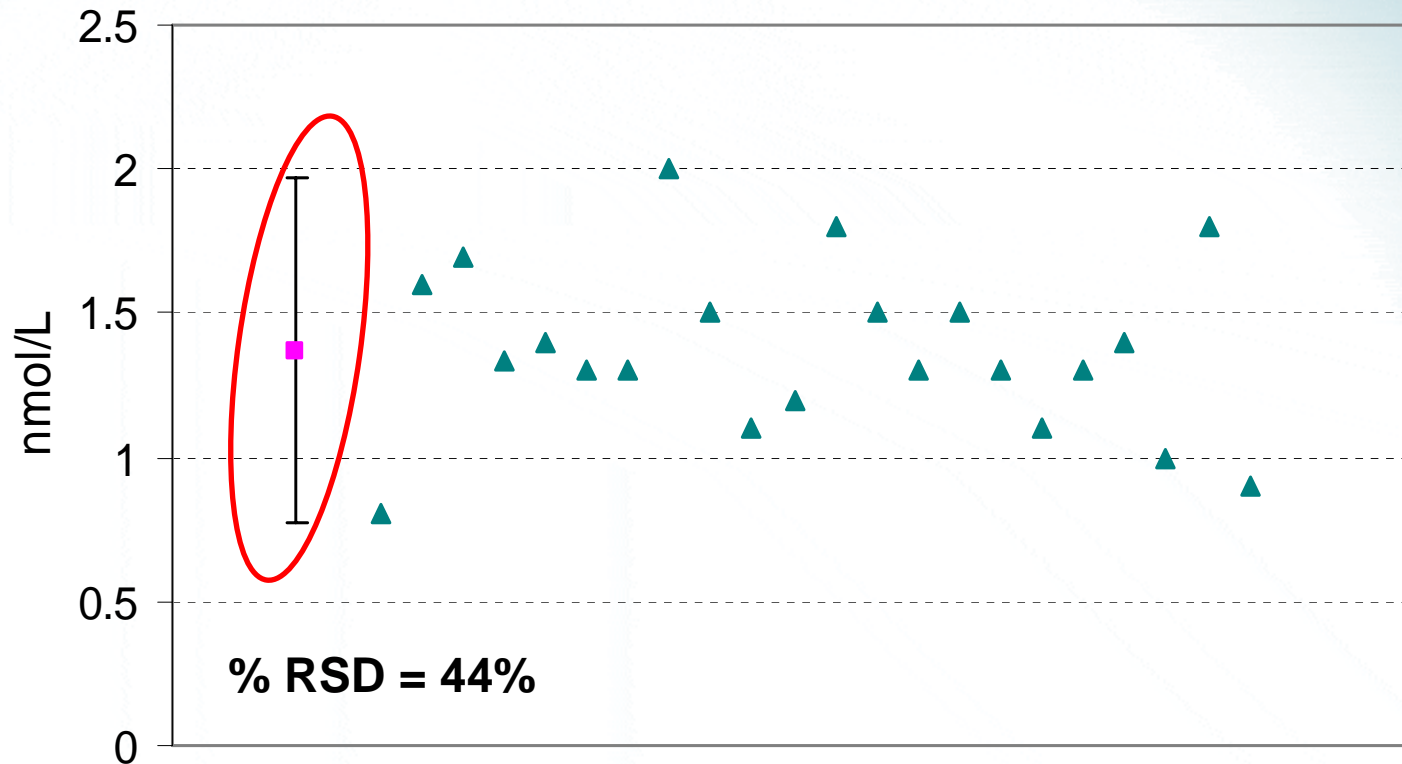


Bias on creatinine assay measurements

Immunoassay problems (3): testosterone example



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Low level testosterone in serum by immunoassay analysis

Importance of accurate results



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- Immunoassay results used for diagnosis
- Financial and human consequences
- Example: creatinine
 - Regular monitoring of creatinine levels over 55
 - Renal failure caused by Type 2 diabetes, old age
 - Early identification
 - Could prevent life-time on dialysis

What is the alternative?

Mass spectrometry (MS)

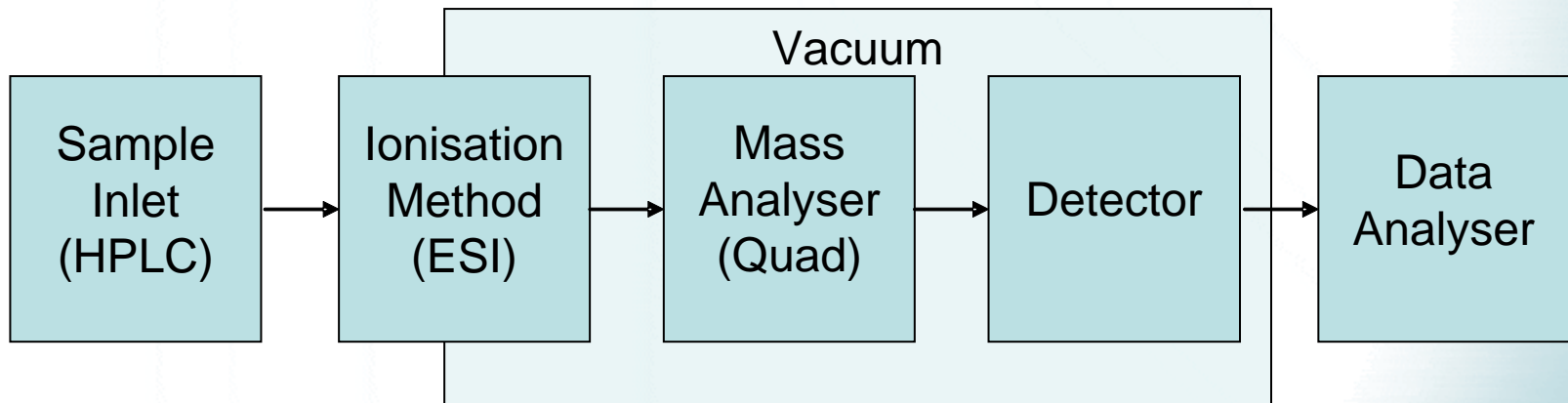


What is a mass spectrometer?



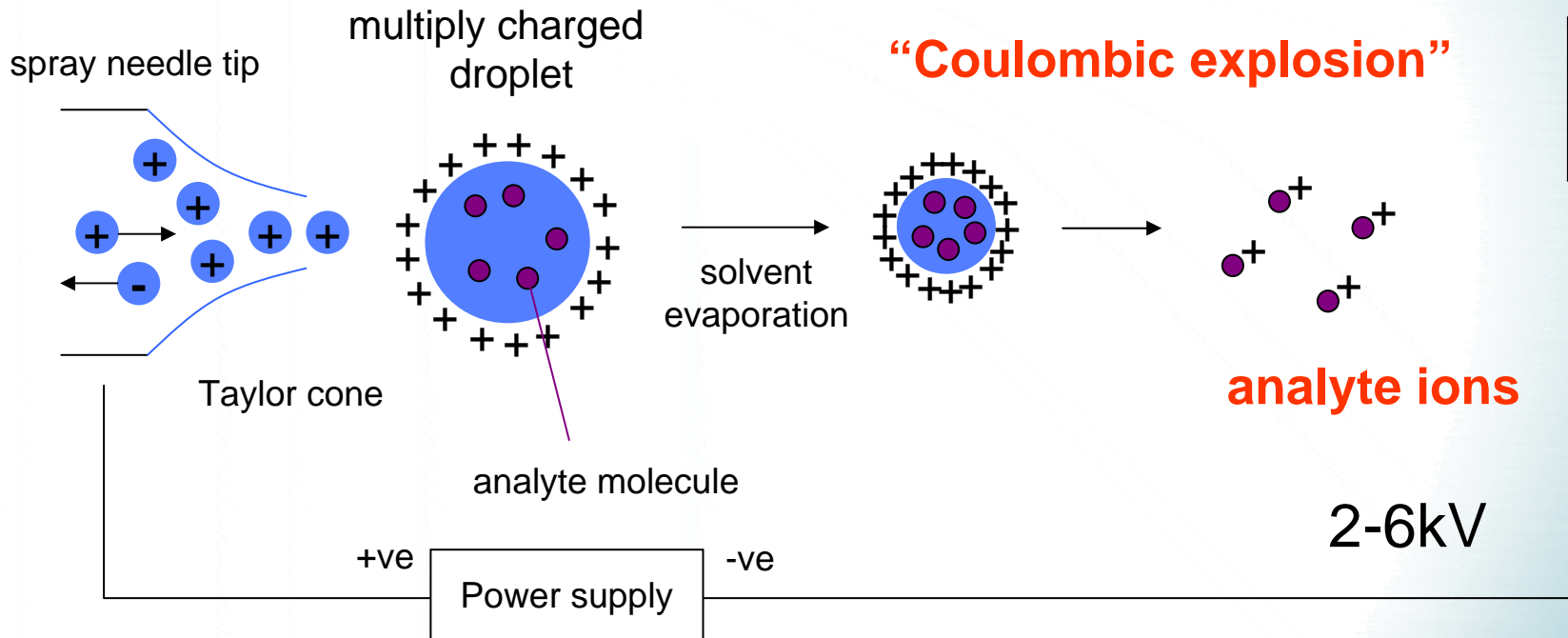
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- Separates charged gas phase species according to their **mass-to-charge** ratio (m/z)
- Once a molecule is charged (ion) it can be moved using electric and magnetic fields that either repel, attract or stabilise its path.
- Operates under vacuum



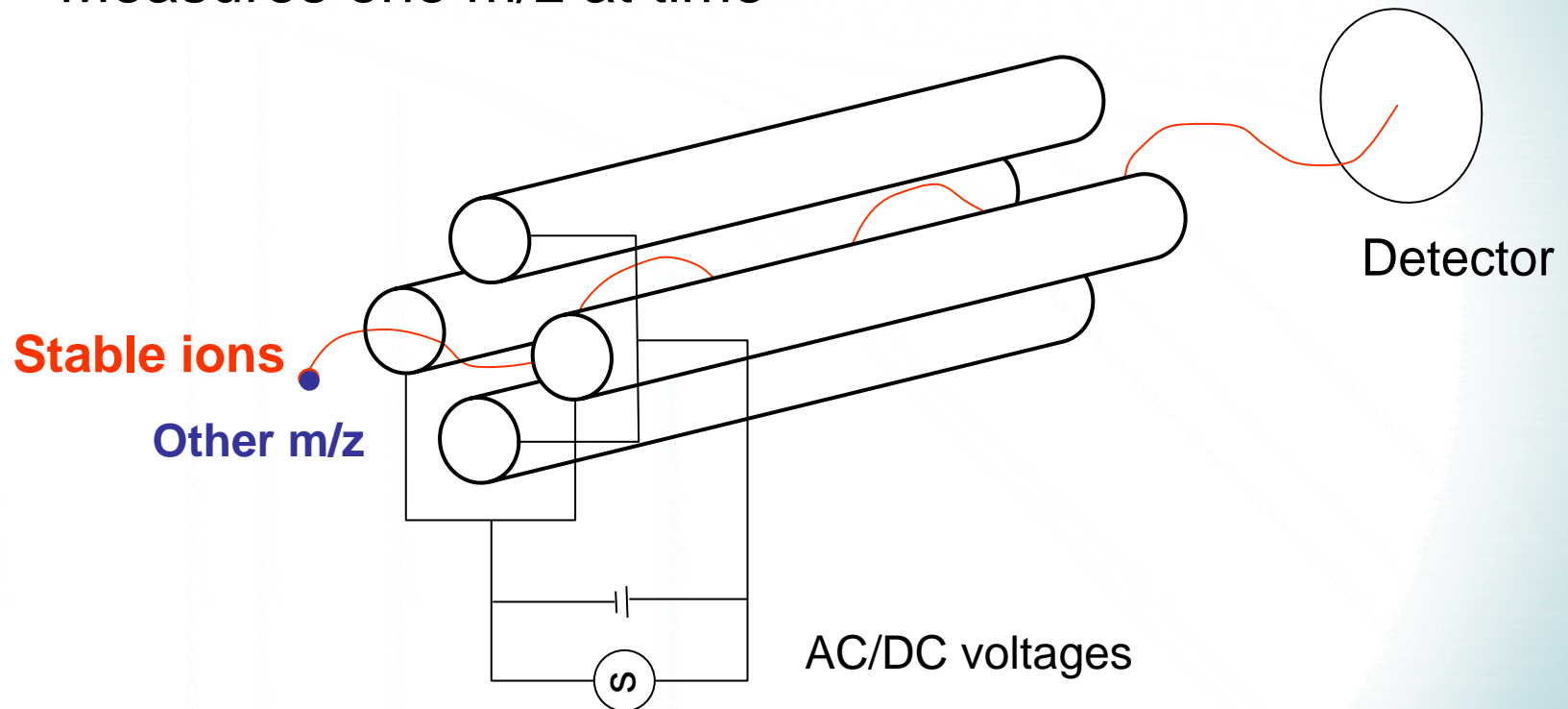
Electrospray ionisation (ESI)

Liquid mobile phase is pumped from the syringe through the tip of a spray needle. As the droplets increase in size, the repulsions of like charges on the surface of the droplet overcome the surface tension and cause the droplet to explode.



Quadrupole mass analyser

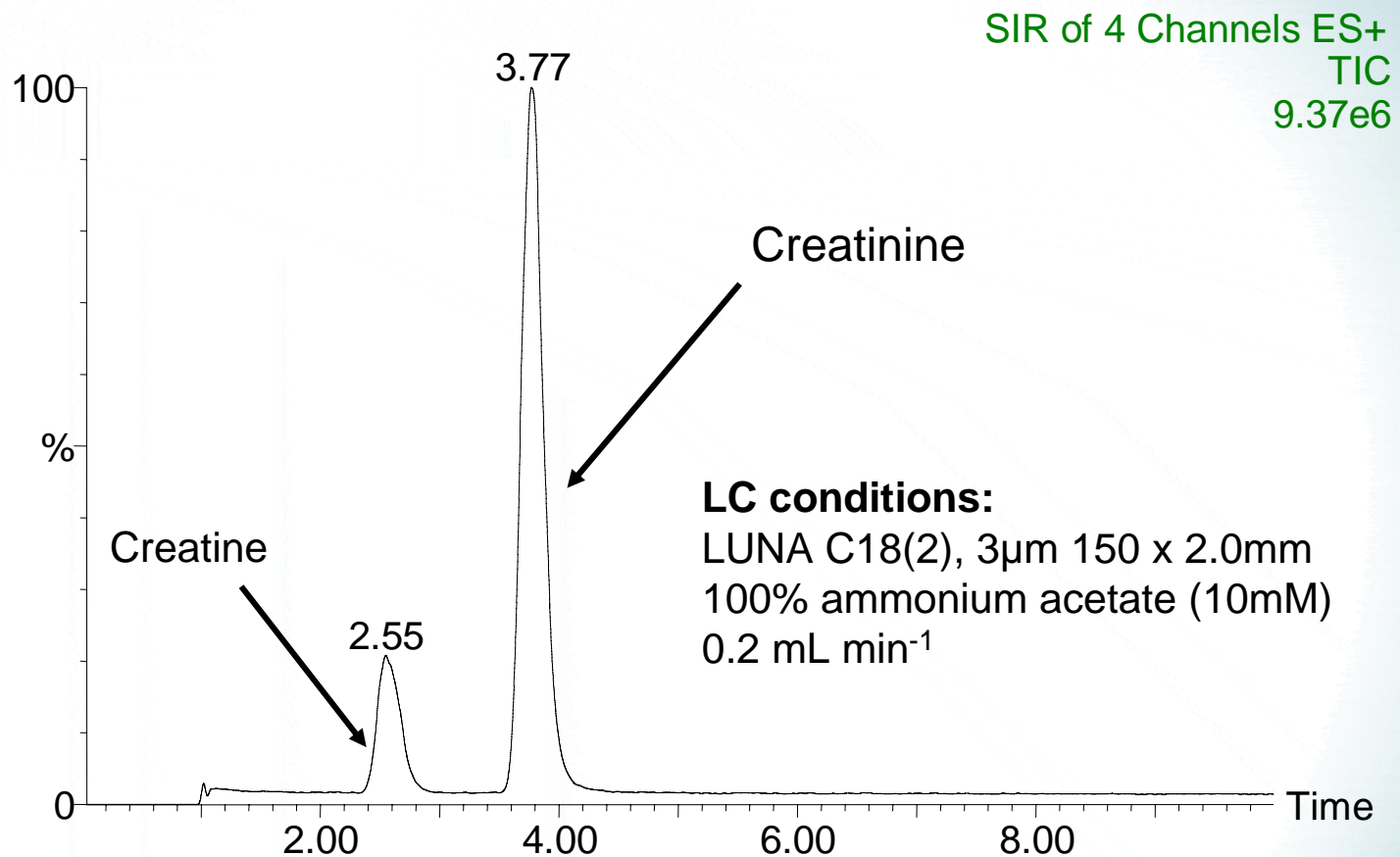
- High specificity detector
- Measures one m/z at time



LC-MS analysis of creatine and creatinine

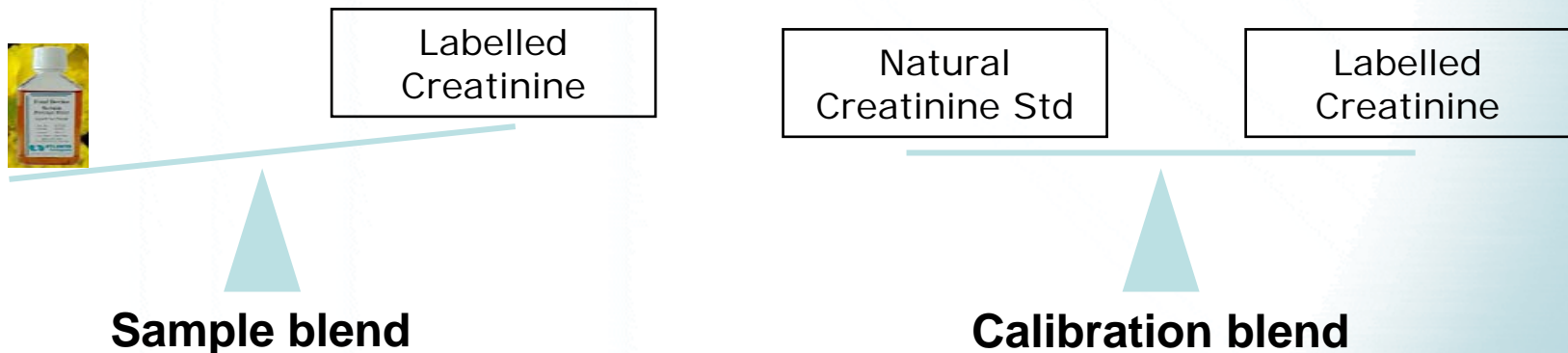


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Exact matching isotope dilution mass spectrometry (EM-IDMS)

- Iterative process used for reference values
- Sample blend
 - Sample + isotopically labelled analyte
- Calibration blend
 - Equal concentrations of standard + isotopically labelled analyte
 - Standard concentration equal to sample concentration



Advantages of isotope dilution mass spectrometry (IDMS)



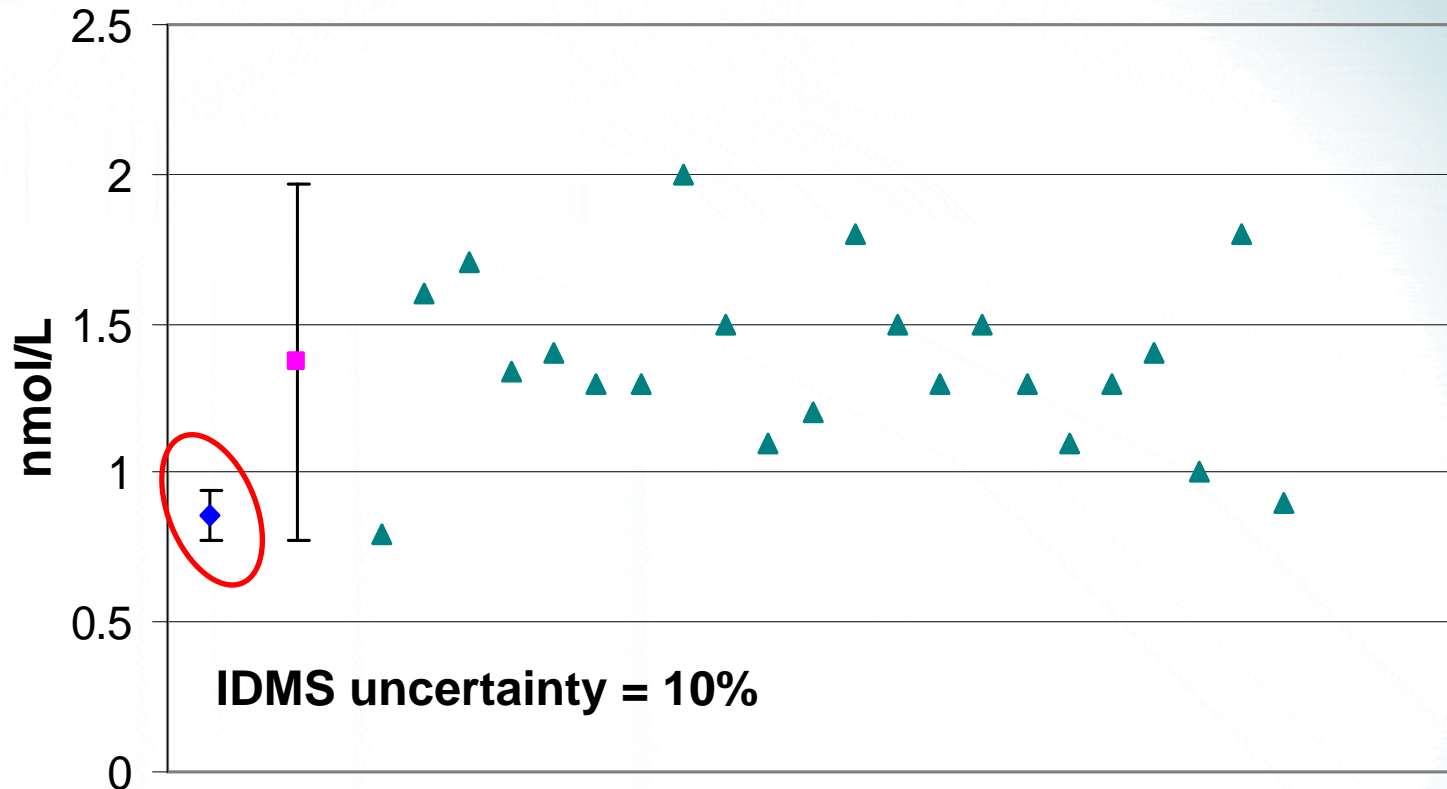
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- Traceable to SI units
 - Standards from a National Measurement Institute
 - Samples prepared gravimetrically
- Accurate and precise
- Low uncertainty
- Total sample recovery not necessary

Advantages of using LC-MS: testosterone example



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Low level testosterone in serum by IDMS analysis

Disadvantages of LC-MS for routine clinical analysis



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- Time intensive
 - Hospital labs currently running 500-1000 samples daily
 - Creatinine maximum of 130 daily
- Expensive
 - Initial outlay prohibitive (~ £150,000)
 - MS expertise necessary
 - Cost and availability of standards and isotopically labelled standards

Alternative method

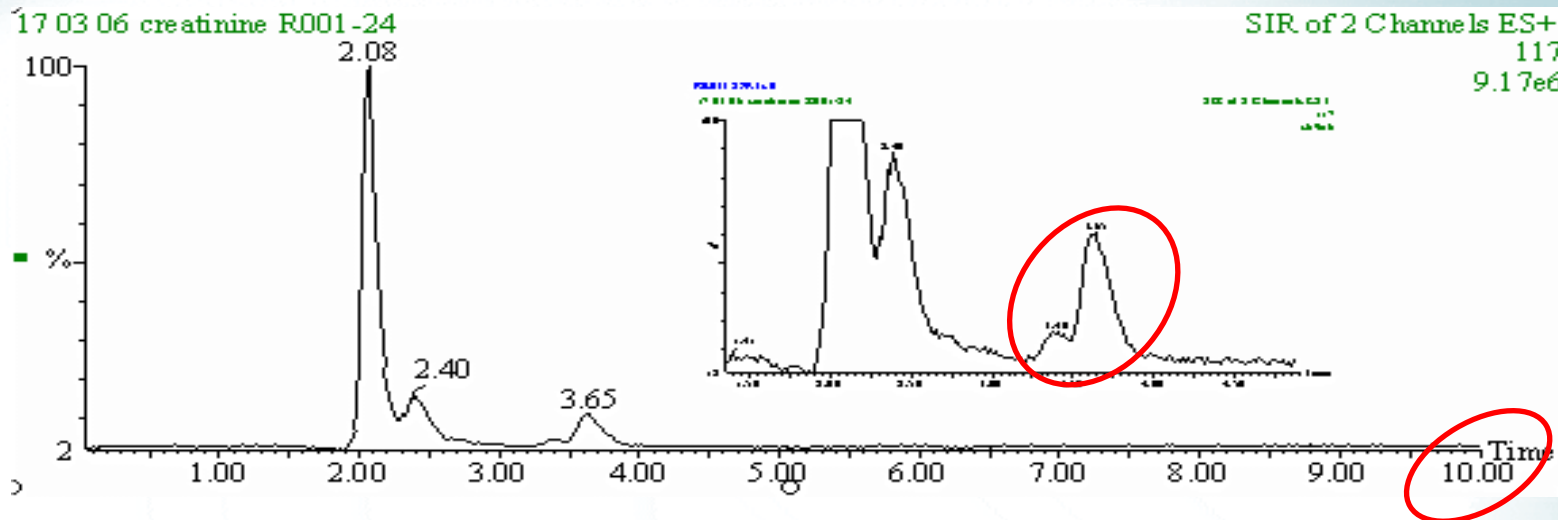
- Ultra Performance Liquid Chromatography (UPLC)
 - High pressure system
 - Narrower peaks, better definition
 - Requires fast-scan times
- Throughput increased as run-times shorter
- Greater signal to noise ratio for increased accuracy and precision
- Resolve more peaks per unit time
- Same confidence in results as for HPLC inlet

Creatinine HPLC v UPLC



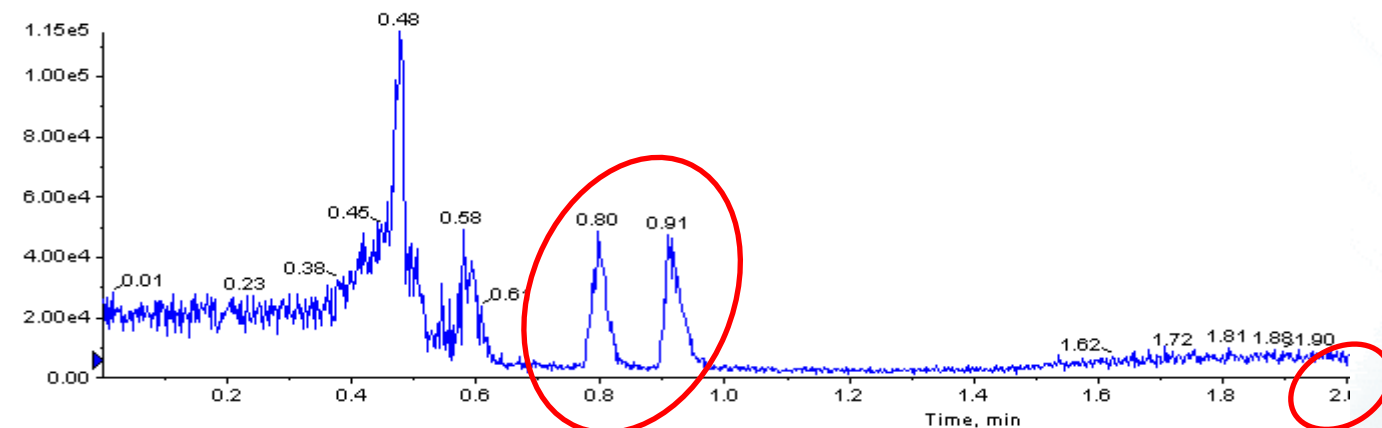
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HPLC



UPLC

XIC of +Q1 M1 (2 ions): 117.0 amu from Sample 10 (Creatinine SB1L2 100mm column 50ms dwell) of Data240306Creatinine.wiff (Turbo...



The future: UPLC-MS for routine clinical analysis

- High sample throughput levels
- Necessary accuracy and precision
- Regular monitoring to give early and accurate diagnoses
 - Reduced patient worrying
 - Eliminate unnecessary treatments
 - Financially beneficial
- Confidence in results



Acknowledgements



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- Chris Mussell, LGC
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