

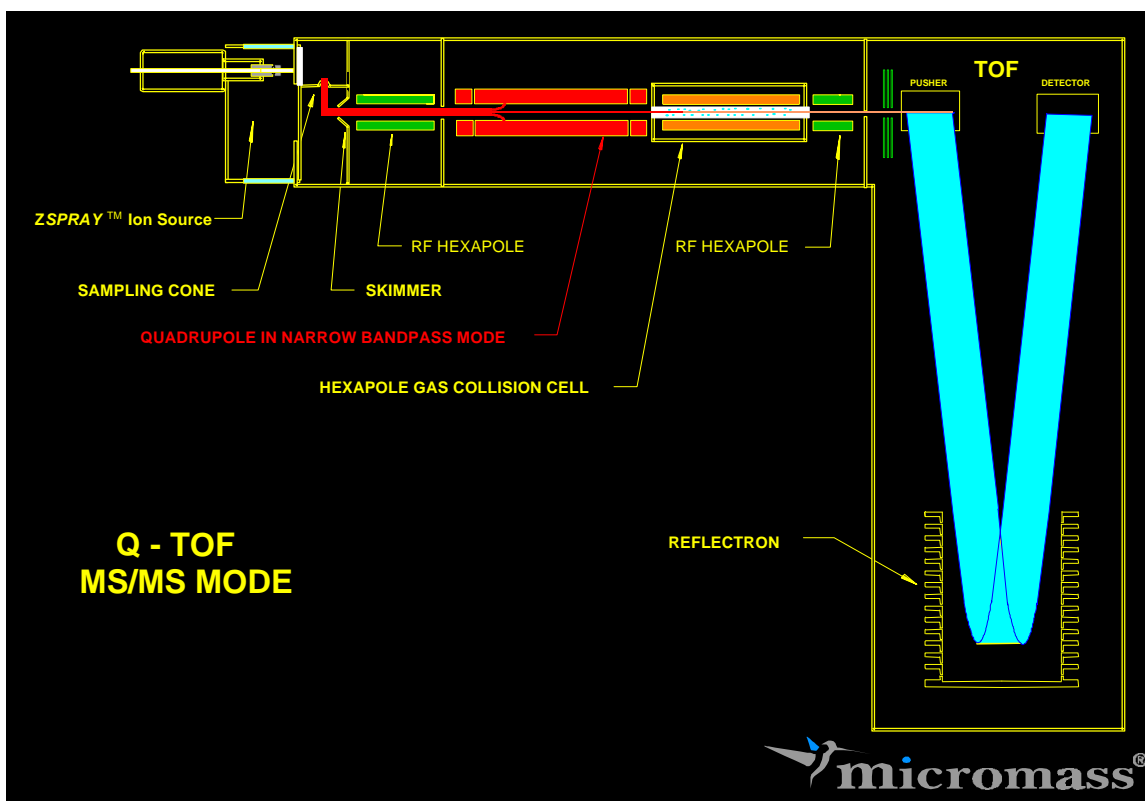
Impurity Identification using a Quadrupole - Time of Flight Mass Spectrometer

 micromass®

QTOF



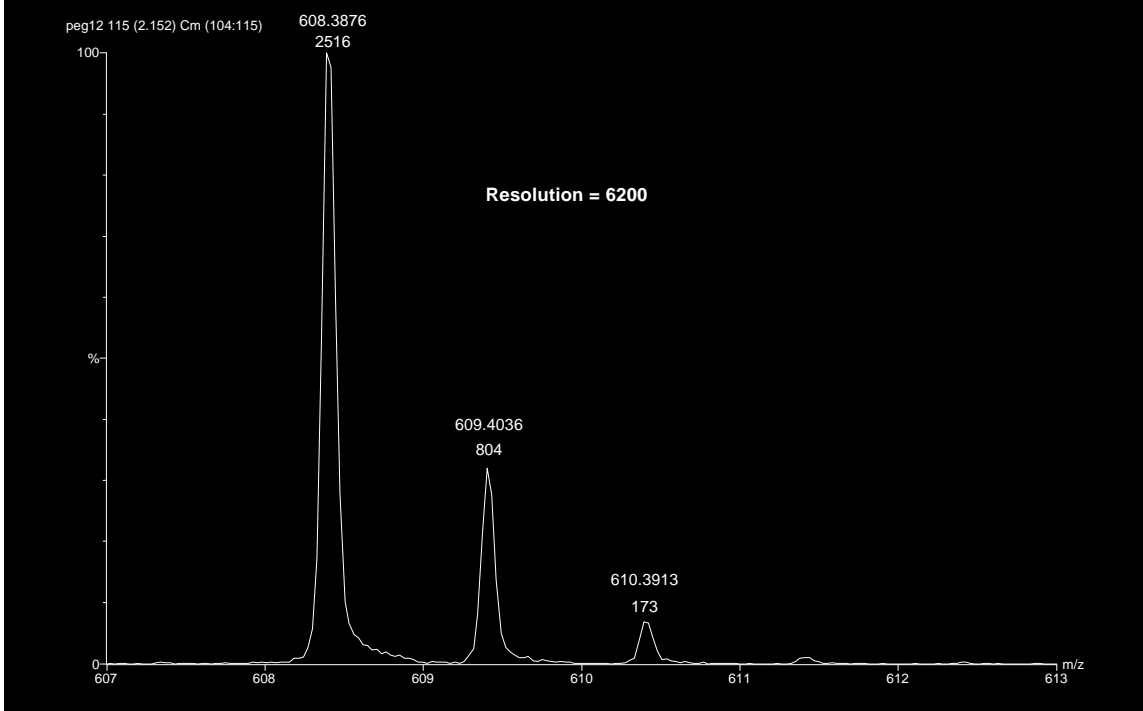
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Q-ToF Features

- ◆ Efficient duty cycle for enhanced sensitivity
- ◆ Mass range
- ◆ Acquisition of 10 scans/sec
- ◆ Resolution >5000 FWHM
- ◆ <5ppm mass measurement (MS)
- ◆ <5ppm mass measurement (MS/MS)
- ◆ Automated MS-MS/MS switching

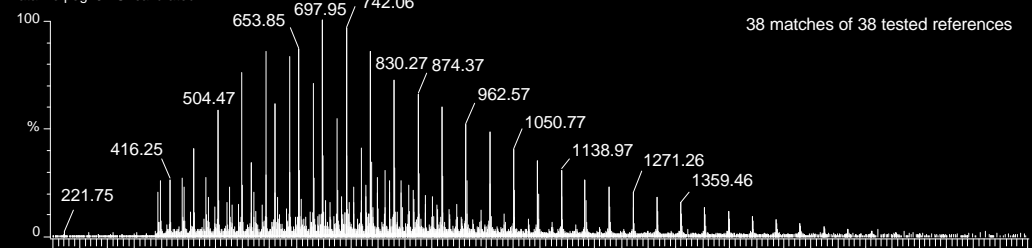
Typical Resolution achieved for accurate mass work



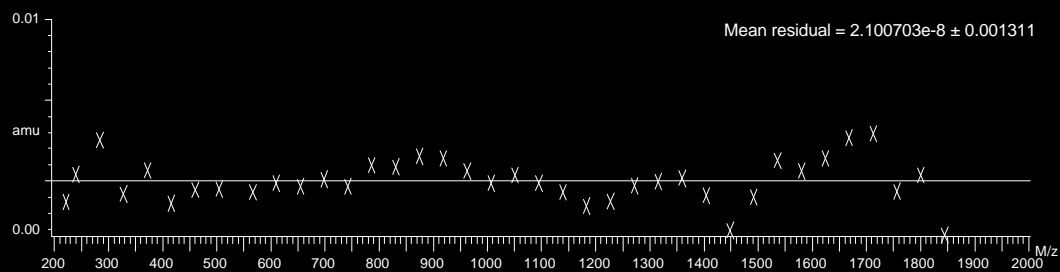
Fifth order best fit polynomial over 200-1800 Da range

Calibration Report

Data file peg13 - Uncalibrated



Residuals



Single Point Lock Mass Correction

$$\sqrt{\text{mass}} = A + Bt$$

“DRIFT”

$$\sqrt{\text{mass}} = A + B^1t$$

The diagram shows two equations for the square root of mass. The top equation is $\sqrt{\text{mass}} = A + Bt$. Below it, the word "DRIFT" is written in orange, with a vertical arrow pointing down to the second equation, $\sqrt{\text{mass}} = A + B^1t$. This illustrates how a single point lock mass correction (locking 'A') allows for the calculation of 'B¹' to correct for drift.

'A' is unchanged so a single point lock mass enables 'B¹' to be calculated

Why Exact Mass?

- ◆ Determination of elemental composition
 - ❖ molecular ion and fragments
- ◆ Differentiation of nominal isobars
 - ❖ combinatorial libraries
- ◆ Patent support and scientific journals
 - ❖ 5ppm accuracy required
- ◆ Efficient database searching
 - ❖ proteome elucidation

Measurement of ppm

'True' mass = 400.0000

Measured mass = 400.0020

Difference = 0.0020 2 mmu

ppm error = $\frac{0.002}{400} \times 10^6 = 5 \text{ ppm}$



The Experiment



Impurity Analysis

- ◆ Impurities in manufactured compound
- ◆ LC-MS analysis
- ◆ Exact mass measurement
- ◆ LC-MS/MS information
- ◆ Compound identification



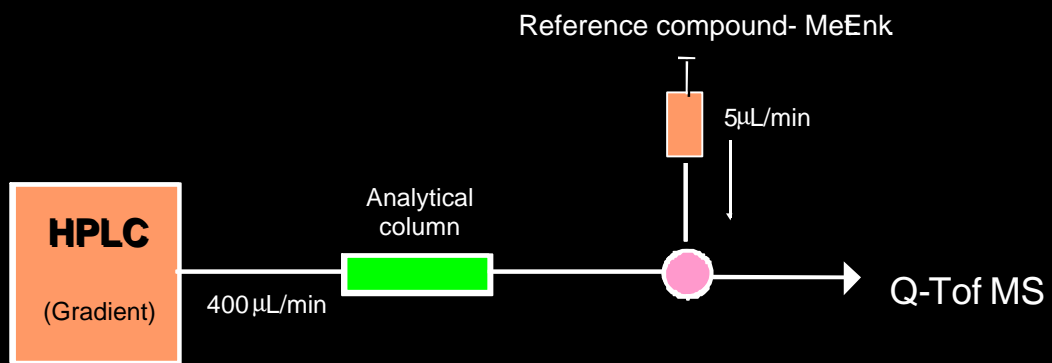
LC Conditions

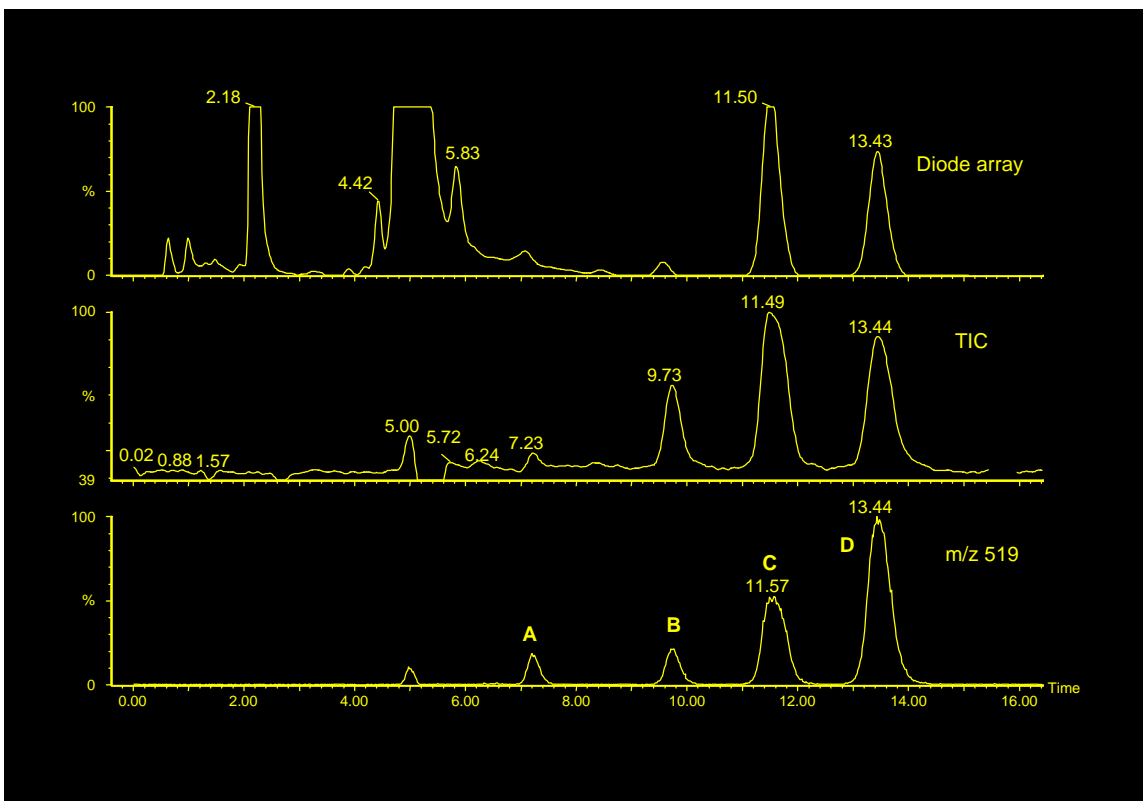
HPLC Waters Alliance
Column C8
Flow 400uL/min
Solvent 60:40 ACN/H₂O + 0.1% formic acid
Int ref Met Enkephalin (1ng/uL, 5uL/min)



MS Conditions

Ion mode Electrospray +
Cone 30V
Internal ref Met Enkephalin (m/z 574.2771)

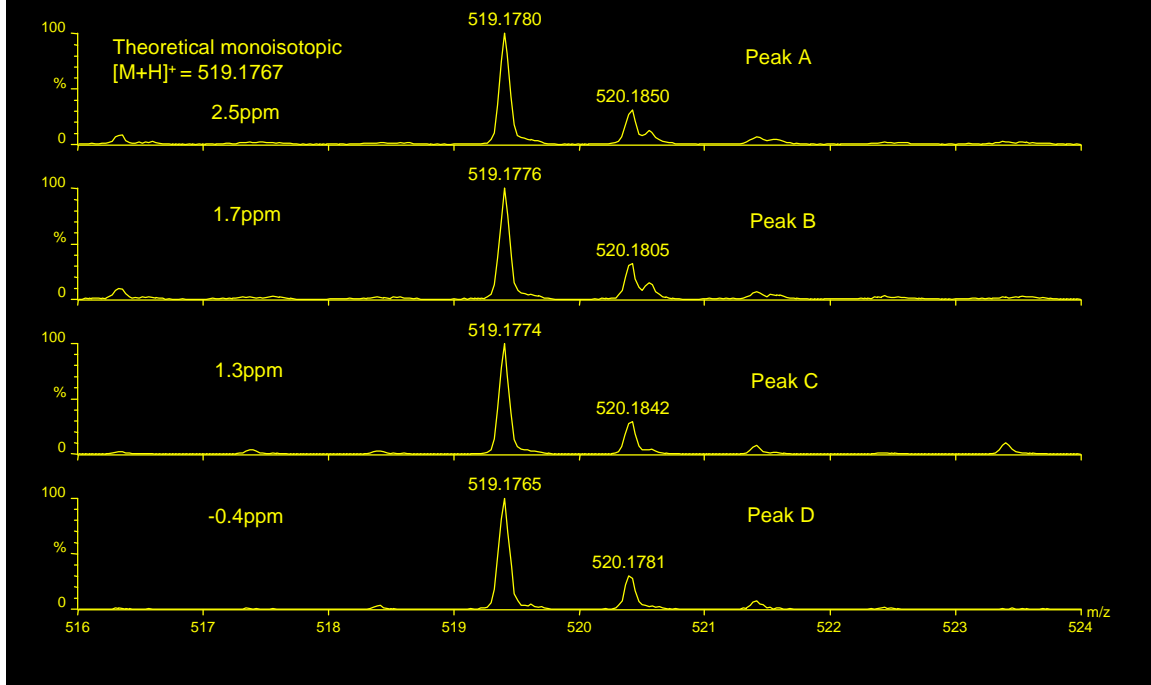




LC-MS Results

- ◆ Mass chromatogram at m/z 519
- ◆ Showed 4 components at nominal mass 518
- ◆ Need to identify these impurities

Spectra of the 4 Components



LC-MS Results

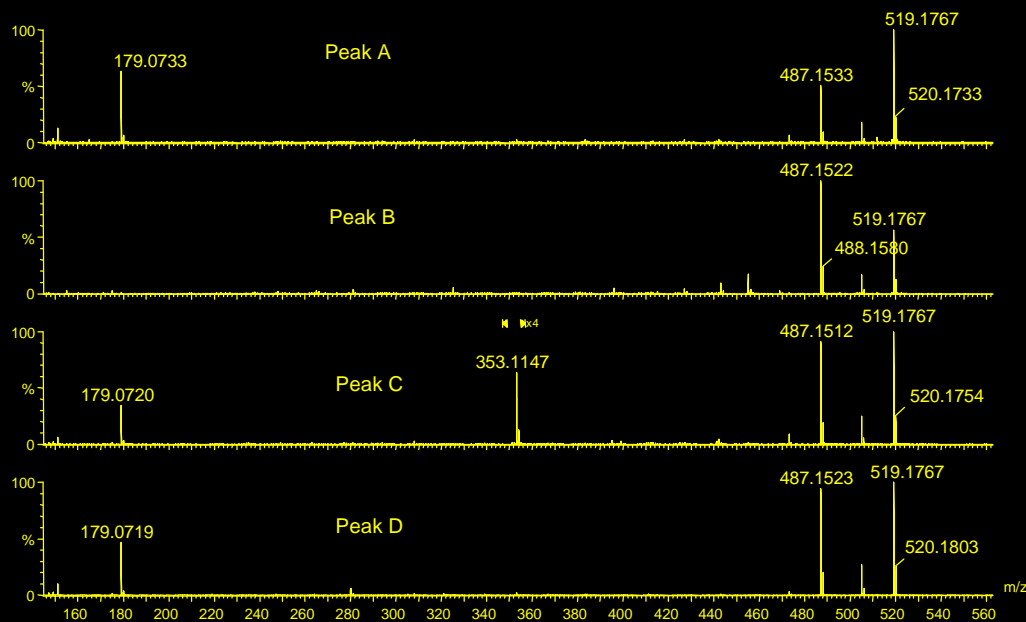
- ◆ Same exact mass for each component
- ◆ Agree to within 5ppm error
- ◆ Compounds are isomeric
- ◆ Need further information to identify
- ◆ Use LC-MS/MS

Data Dependent MS to MS/MS

- ◆ Instrument switched automatically to MS/MS
- ◆ Acquires product ion spectra
- ◆ Collision energy: 5, 10, 20, 30 and 40 eV
- ◆ Collision gas: argon
- ◆ Threshold: 50 counts/sec



Product Ion Spectra for 4 Components

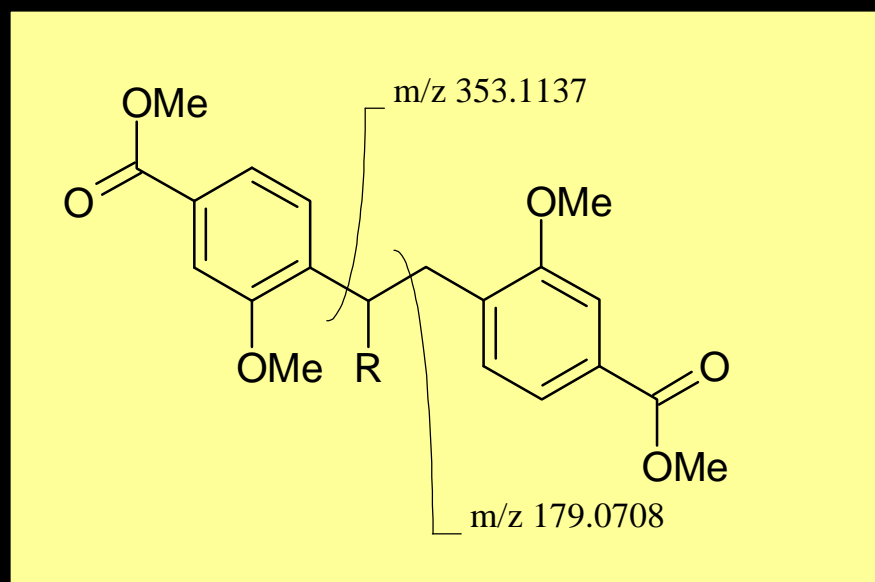


LC-MS/MS Results - Compound C

- ◆ Exact mass measurement
- ◆ Use precursor as lock mass
- ◆ Elemental composition of fragments
- ◆ Allowed structural elucidation at 0.03% level



Structure of Peak C



Conclusions

- ◆ Q-ToF sensitivity required for detection of impurities at 0.1% level
- ◆ Exact mass measurement determined that impurities had same elemental composition
- ◆ Exact mass MS/MS allows elemental composition of fragments to be determined
- ◆ NMR required for other impurities

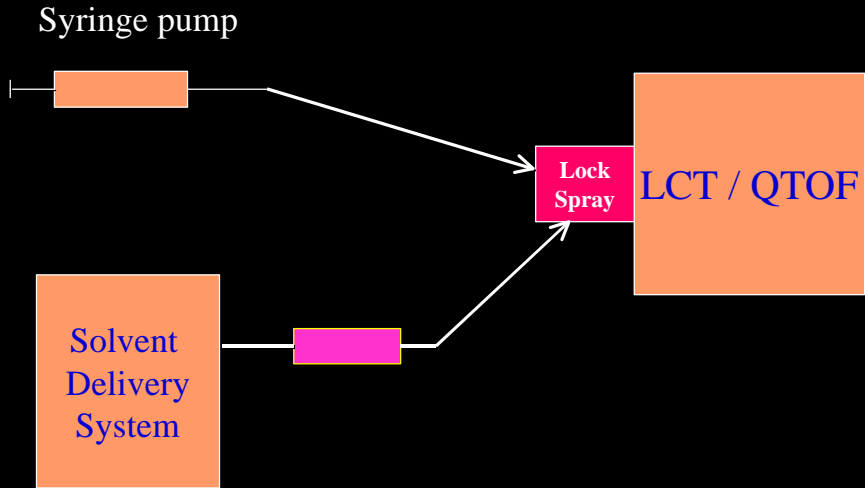


Considerations

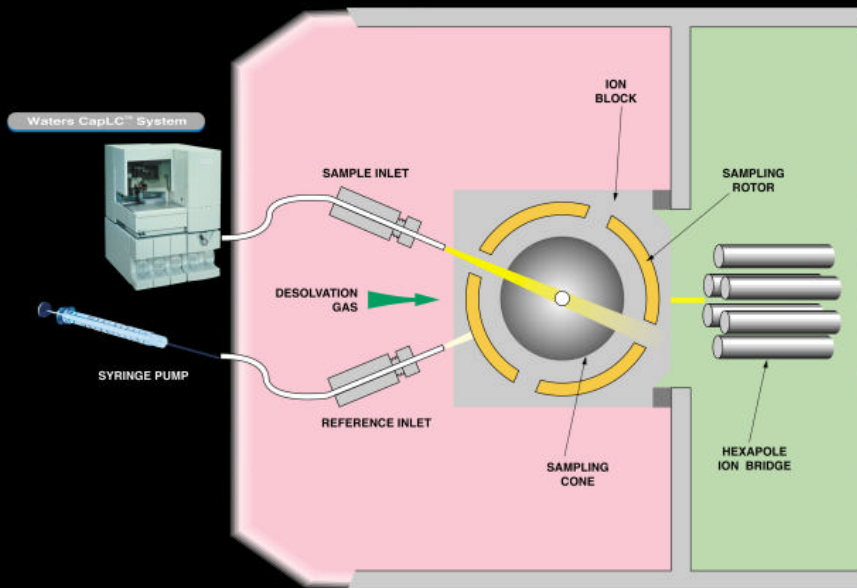
- ◆ Lock mass may suppress ionisation of analyte (and vice versa).
- ◆ Solvent gradient effects may interfere with post column addition of lock mass compound.
- ◆ Lock mass contributes to TIC signal and may mask smaller analyte components.



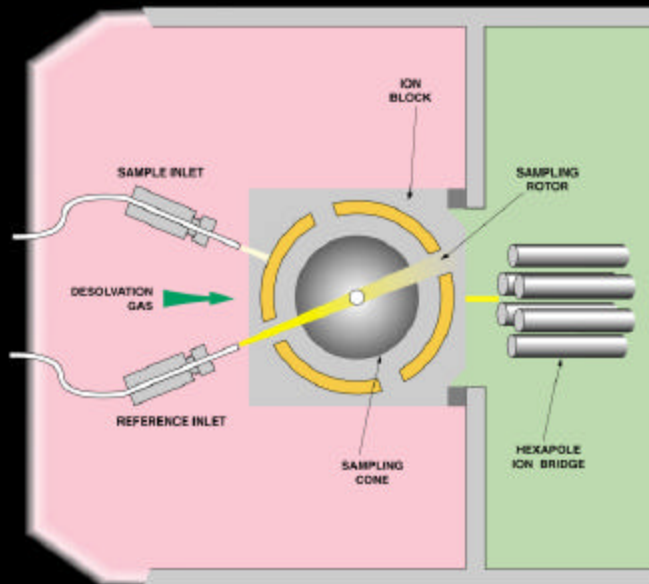
LC-MS with Lock Spray



LockSpray™ ESI LC-MS inlet

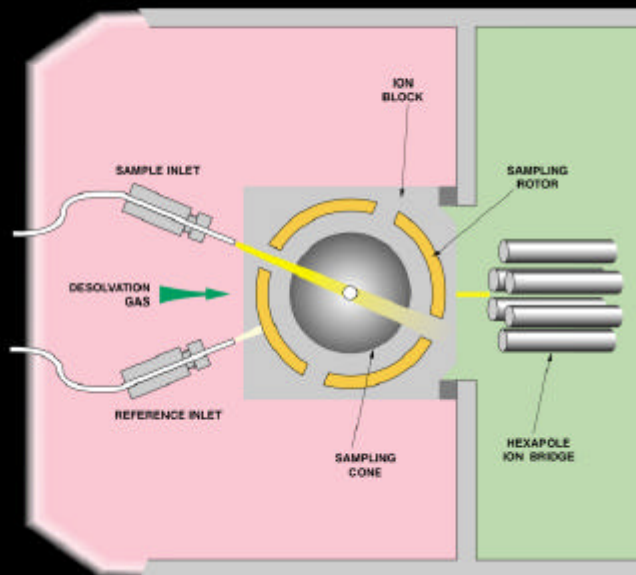


LockSpray™ ESI LC-MS Inlet



 micromass®

LockSpray™ ESI LC-MS Inlet



 micromass®

Lock Spray

- ◆ LockSpray allows one conventional (or microbore) HPLC column to be interfaced in parallel with a second liquid inlet for the introduction of a mass reference standard.
- ◆ Compatible with both isocratic and gradient LC
- ◆ The oaTOF-MS automatically monitors the 2 separate electrospray inlets.
- ◆ A sampling rotor within the ion source (propelled by a programmable stepping motor) allows the 2 electrosprays to be sampled exclusively in rapid succession.
- ◆ The position of the sampling rotor is monitored in real-time enabling the two liquid inlets to be indexed



Conclusions

- ◆ LC/MS and MS/MS can be performed in a single run
- ◆ Accurate mass measurements can be made on parent and daughter ions to suggest elemental composition
- ◆ Full scan sensitivity comparable to SIM sensitivity on quad systems

