Impurity Identification using a Quadrupole - Time of Flight Mass Spectrometer
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

Resolution = 6200

Fifth order best fit polynomial over 200-1800 Da range

Calibration Report

Data file peg13 - Uncalibrated

38 matches of 38 tested references

Mean residual = 2.100703e-8 ± 0.001311
Single Point Lock Mass Correction

\[ \sqrt{\text{mass}} = A + B t \]

“DRIFT”

\[ \sqrt{\text{mass}} = A + B^t t \]

‘A’ is unchanged so a single point lock mass enables ‘B’ 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

Theoretical monoisotopic $[M+H]^+$ = 519.1767

Peak A: 519.1780, 520.1850
Peak B: 519.1776, 520.1805
Peak C: 519.1774, 520.1842
Peak D: 519.1765, 520.1781

-2.5 ppm, 1.3 ppm, 1.7 ppm, 2.5 ppm

LC-MS Results

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

![Chemical Structure](attachment:image.png)
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

Syringe pump

Solvent Delivery System

Lock Spray

LCT / QTOF
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