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PRINCIPLES OF GC AND GC-MS

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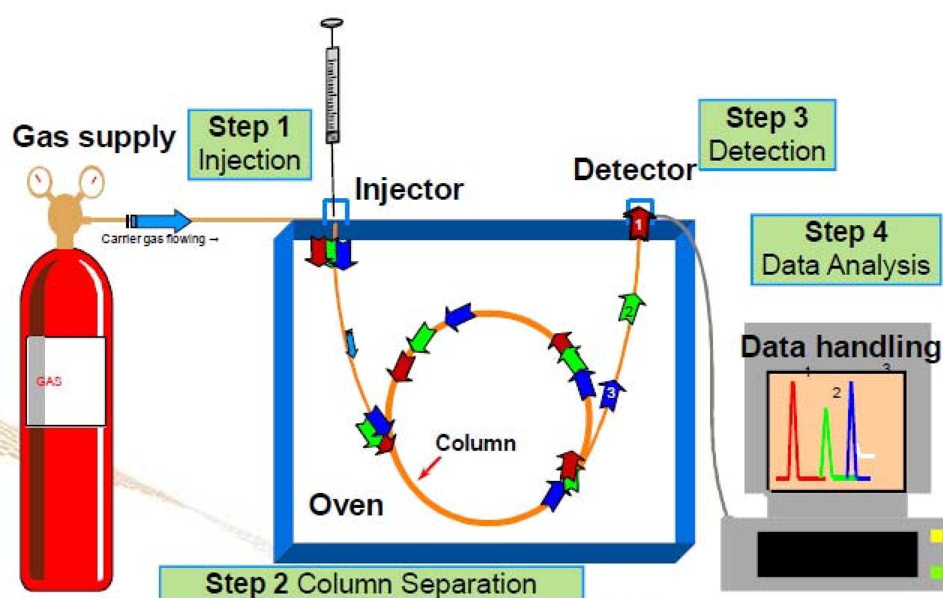
Why use GC?

- Gas chromatography - gas is more efficient than liquid as a mobile phase
- Increased column efficiency compared to HPLC
- *separates more compounds in unit time*
- *compounds must be volatile < approximately 350 °C*
- *usually low MW compounds <400 amu*
- *higher MW compounds only suitable for HPLC unless polarity/volatility is modified (e.g. derivatisation)*
- Compatible with a number of detector options

GC and LC are complimentary techniques

Typical GC system: schematic



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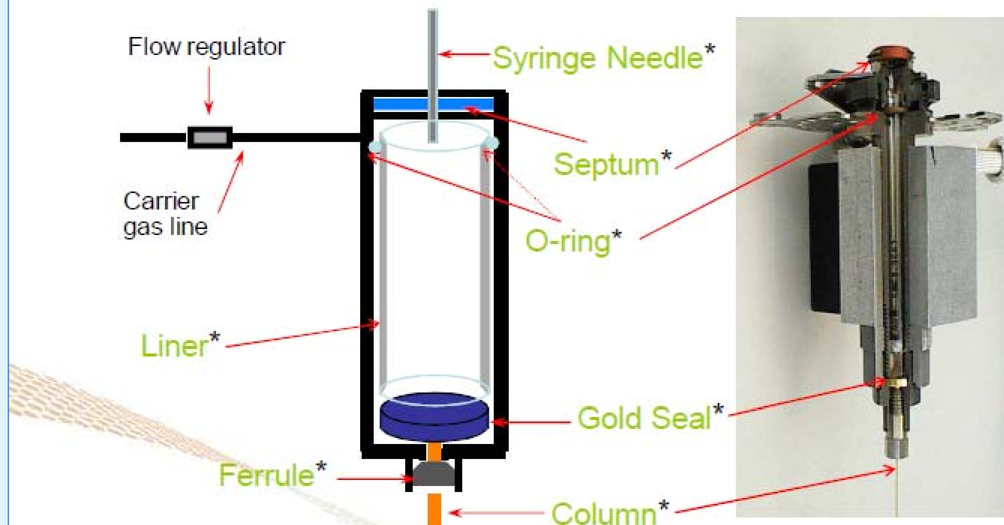
GC carrier gas

- The Carrier Gas (i.e., the mobile phase) provides continuous flow through the injector inlet that carries the compounds of interest through the column to the detector
- Use high purity gases
- Use gas purifiers or gas "traps" in line to remove contaminants and moisture.
- Typical capillary GC system carrier gases are Hydrogen (H_2), Helium (He) and, infrequently, Nitrogen (N_2).
- Beware of H&S with regard to cylinders, gas valves, gas under pressure etc.
- The Carrier Gas enters the GC at the injector

GC inlet (gas lines not shown)



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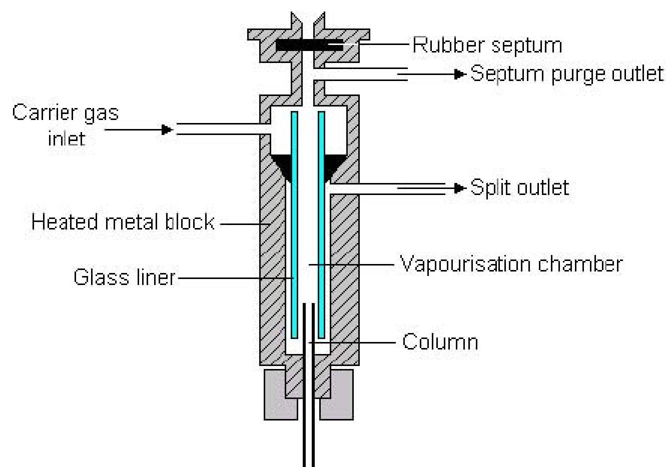
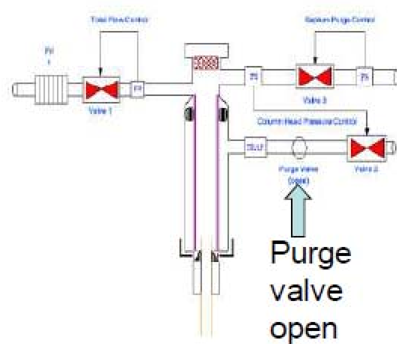
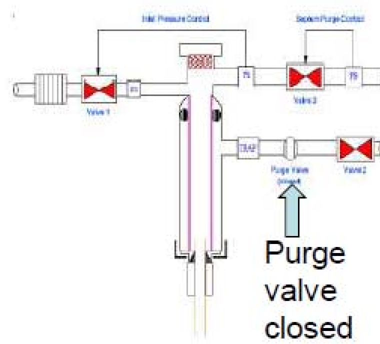
GC inlet with gas lines



- Minimise Discrimination
- Optimise sample transfer onto column

Split and splitless injection modes





Injection Modes – (1)

- Split

  - liquid sample vapourised in hot injector
  - only a small amount of sample vapour enters column
  - most of sample vapour exits via split/purge vent

Not used for trace analysis

- Splitless

  - liquid sample vapourised in hot injector
  - most of sample vapour enters column
  - purge valve opens after preset time to remove residual vapour (to prevent peak tailing)

Widely used for trace analysis

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Injection Modes –pulsed splitless

-As for splitless, except that a pressure pulse increases the inlet pressure just before injection of sample, and returns to 'normal' after preset time, before split valve is opened.

- allows injection of larger samples
- faster sample transfer from inlet to column, less time for analyte degradation to occur

Increasingly used for trace analysis

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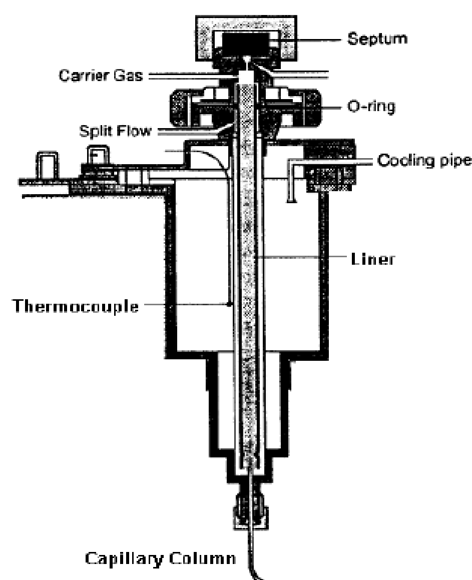
PTV Injector



- Minimise Discrimination
- Optimise sample transfer onto column

Injection Modes –PTV (2)

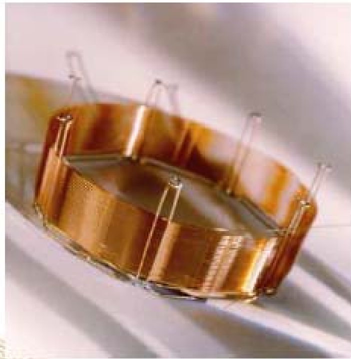
- PTV

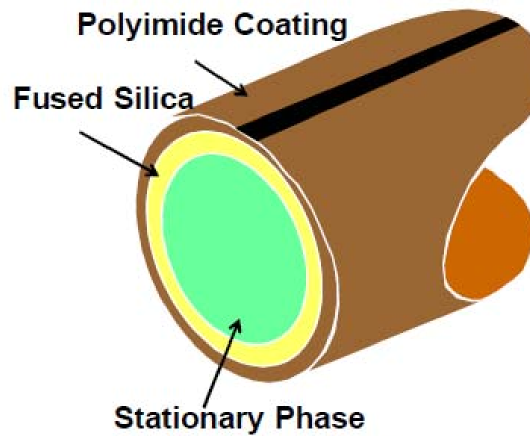
- Solvent vapourised in cold injector and exits via split/purge vent, analyte(s) not vapourised and remain in cold injector
- Purge valve closed after preset time, injector heated to vapourise analyte(s)
- Purge valve re-opens after preset time to remove residual vapour (to prevent peak tailing)

Requires optimisation of more parameters and specialised liners (packed liners must be used for large injection volumes >10-20 µl)

Increasingly used for trace analysis

GC column





Lots of column options:
30 m X 0.25 mm id x 0.25 µm film
5 % phenyl phase is good starting point for MRMs

Expanded view of a capillary GC column

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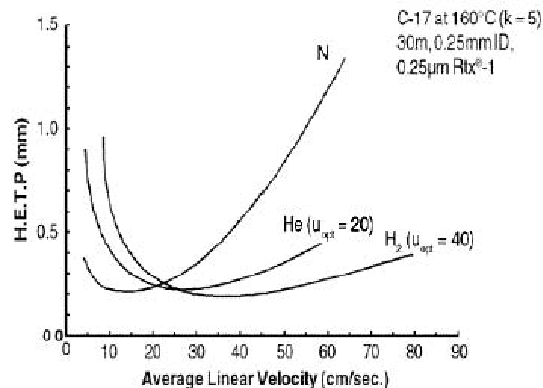
GC - Carrier gas selection

- Nitrogen
  cheap
  - inferior resolution
- Helium
  - expensive
  - easy to optimise
- Hydrogen
  - cheap
  - large operating range
  - good resolution
  - safety issues ??



Note: *electronic pressure control permits constant flow*

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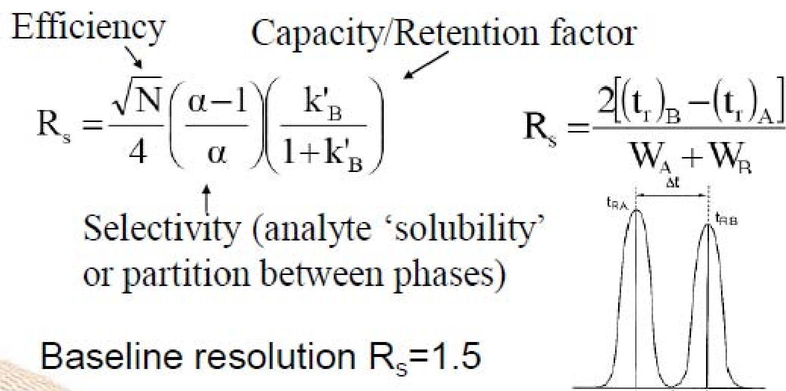
Chromatographic resolution



Baseline resolution R_s=1.5

Resolution is a compromise between separation of peaks and time

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Capillary GC column selection

- Stationary phase
  - *match to physicochemical properties of analyte*
- Film Thickness (0.05 µm – 5.0 µm)
  - *consider bleed in MS applications*
- Internal Diameter (0.1 mm – 0.53 mm)
- Length (10 m – 60 m)
- Separation also influenced by carrier gas velocity and column temperature
- In practice most residue analysis can be achieved using (95% dimethyl / 5%diphenyl phase or equivalent, typically *20-30m X 0.18 - 0.25mm i.d.*

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Capillary column film thickness

- Thin films
  - *high efficiency, low bleed, suitable for high MW compounds*
- Thick films
  - *lower efficiency, higher bleed, suitable for low MW compounds e.g. fumigants, CS_2 (dithiocarbamates)*
  - *but high bleed not suitable for MS*

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GC detectors

- FPD (Flame photometric)
- NPD (Nitrogen Phosphorous)
- ECD (Electron Capture)
- MS (Mass spectrometry) - *Quadrupole, Ion Trap, ToF*

Also available but less useful for residues are

- FID (Flame Ionization)
  - *used in the analysis of pesticide formulations*
  - *universally detect any organic sample*
- TCD (Thermal Conductivity)
- AED (Atomic Emission)

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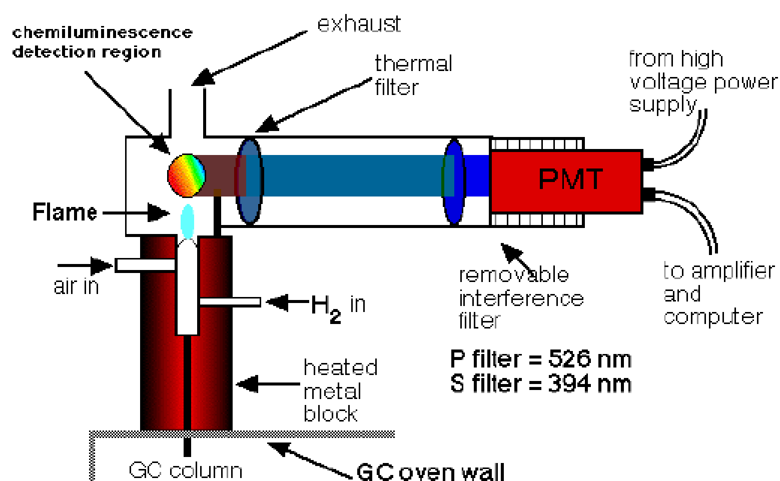
Flame photometric detector (FPD)

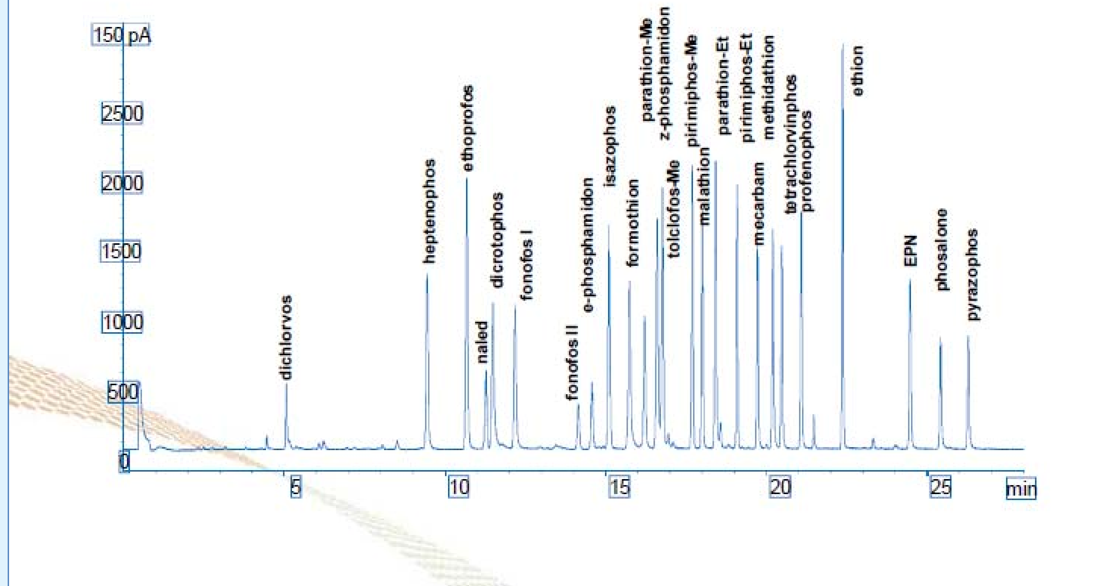


- Analytes burn in a flame producing ions which are collected and converted to an electric current

Flame photometric detector

- Sample burns in a hydrogen/air flame
- Sulphur and phosphorus chemiluminesce
- Emission filtered and detected
- Fundamentally non-linear for S, linear for P
- Still widely used (~100 pesticides respond to FPD)
- Also used for CS_2 detection (dithiocarbamates)

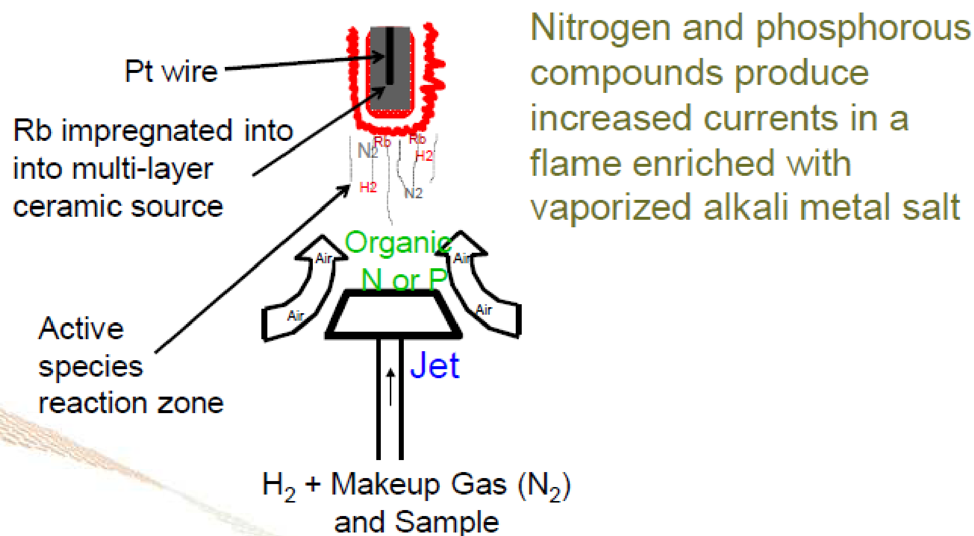
FPD – chromatogram of OPs



Pulsed flame photometric detector

- Better sensitivity and selectivity for sulfur and phosphorus containing compounds than the FPD
- Also detects N, As, Sn and Cu
- Because it was only recently developed has had to compete with MS

Nitrogen phosphorus detector (NPD)



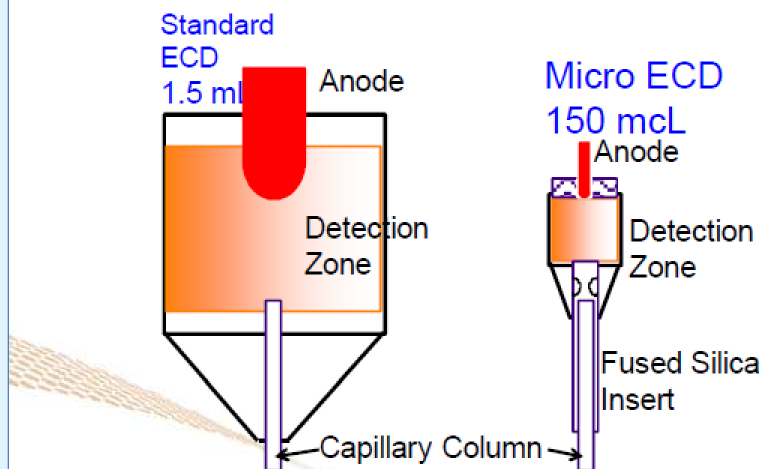
Nitrogen and phosphorous compounds produce increased currents in a flame enriched with vaporized alkali metal salt

Nitrogen Phosphorus detector (NPD)

Also called the alkaline FID or thermionic detector

- Based on an FID but contains a rubidium or caesium silicate (glass bead) in a heater coil close to the hydrogen flame
- The bead emits electrons by thermionic emission and the presence of N or P materials adsorbed onto the surface of the bead increases the electron flow
- The beads need regular replacement to avoid decrease in response

Electron capture detector (ECD)



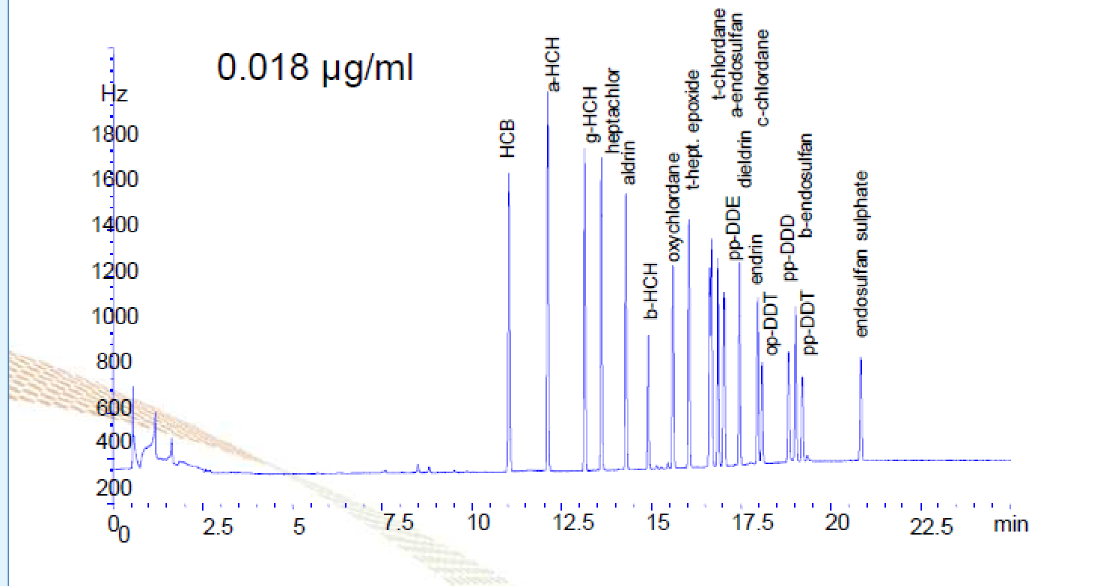
As electronegative species pass through the detector they capture low energy thermal electrons causing a decrease in cell current

Electron capture detector: ECD

'Selective' detector for OCs & synthetic pyrethroids

- Ni-63 electrons ionise make-up gas (N_2)
- The electrons produced generate current
- Electronegative compounds (particularly those containing halogen atoms) capture the electrons and reduce the standing signal
- Highly sensitive (still used by many laboratories)
- Limited dynamic linear range
- Non-specific (complex chromatograms) - effective clean-up required
- User maintenance not allowed - radioactive source

ECD: Chromatogram of OCs using DB1701



Basic components of a mass spectrometer

- Sample molecules are introduced into the instrument through a **sample inlet**
- Once inside the instrument, the sample molecules are converted to ions in the **ionisation source**, before being accelerated into the mass analyser
- Ions are then deflected by magnetic or electrostatic fields according to their mass and their charge within the **mass analyser**
- The **detector** converts the ion energy into electrical signals, which are then transmitted to a **computer** for data handling, quantification and interpretation

The mass to charge ratio

- Mass spectrometers do not directly give the mass of the ion
- Rather the instrument determines the mass to charge ratio or *m/z*
- For ions with either a +1 or –1 formal charge the mass of the ion is the same as the *m/z* ratio.

  -this is typically the case for small molecules analysed by GC-MS and LC-MS

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Choice of ionisation for GC-MS?

- Electron ionisation (EI)
- Chemical ionisation (CI)

  Negative chemical ionisation or electron capture ionisation gives better selectivity than EI for “electron-trapping” compounds (*e.g.* halogen-containing and other heteroatomic compounds) and electron-deficient aromatic compounds

  CI cannot be used for comprehensive multi-residue methods containing

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Mass spectrum

- Plot of *m/z* (x axis) versus the intensity, frequently normalised to 100% for the most intense ion detected (y axis)
- Produced by scanning the analyser to transmit ions (or release them from a trap) for a predefined range of *m/z* values over a fixed period of time

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GC-MS options

- Ion-trap *(used for screening in full scan as no increase in sensitivity in SIM)*
- MS quadrupole *(good sensitivity and quantification in SIM*
- MS/MS (tandem quadrupole or ion trap)
- ToFMS (High Resolution or High speed)
- GC-GC (all detector options possible)
- GC x GC ToFMS

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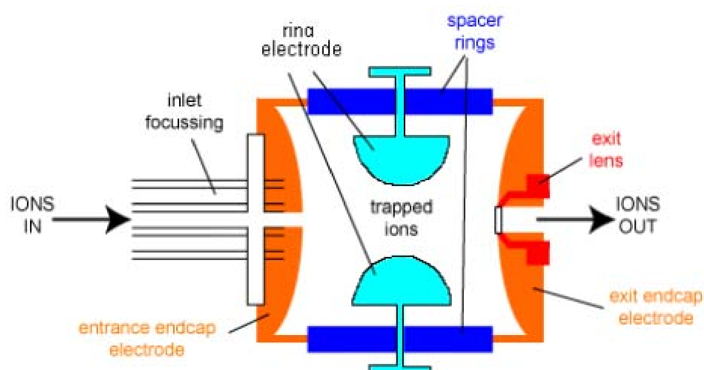
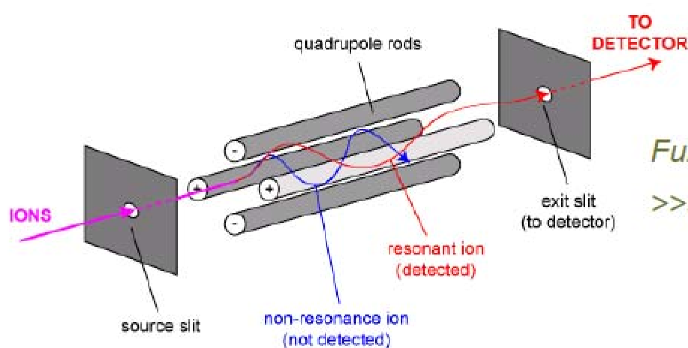
GC-MS: ion trap



Full scan data, low data acquisition rate, MS/MS option, relatively low senstivity, issues with quantification (space charging effects in trap) for complex samples

GC-MS: quadrupole

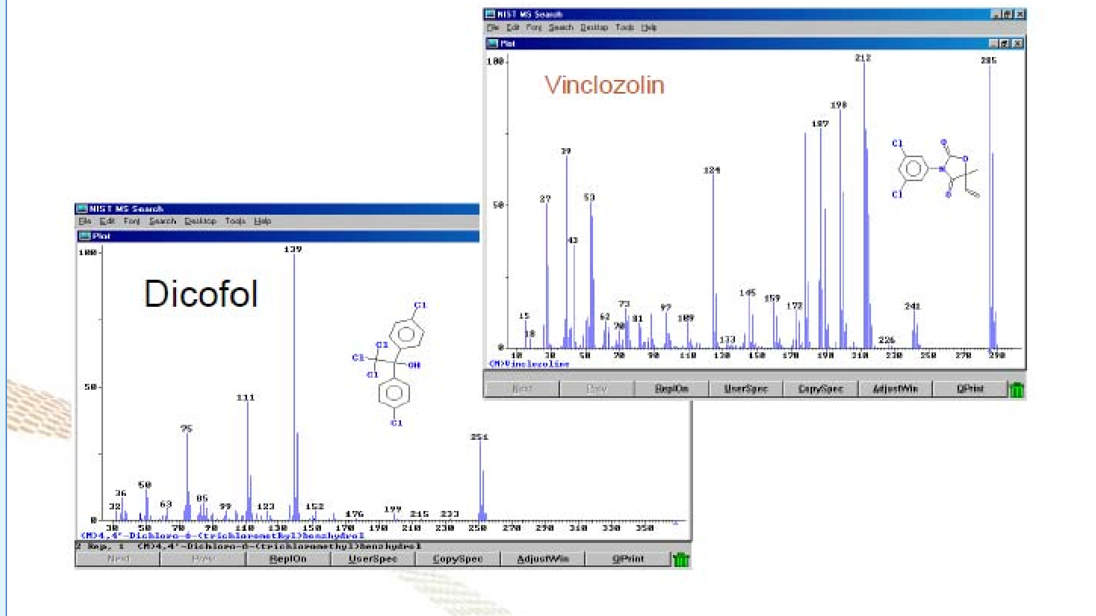


Full SCAN or SIM,

>>sensitivity in SIM

Mass Selective Detectors ionise analytes in a process that breaks the analytes apart into predictable fragments. The collection of fragments is very unique to a given chemical compound and thus provides precise identification of the analytes as they pass through the detector.

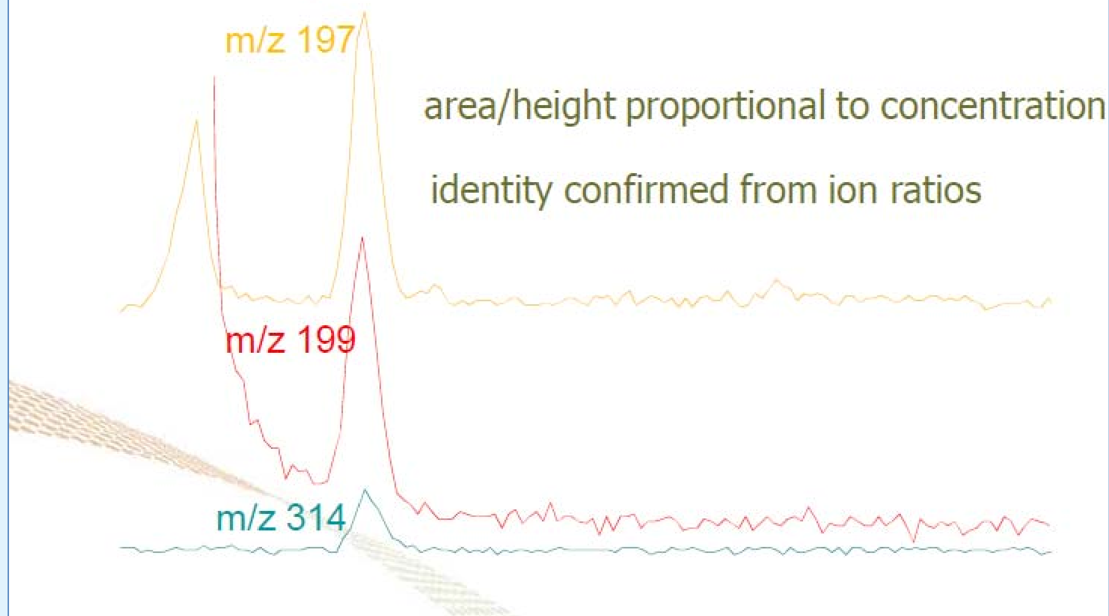
GC-MSD: full scan spectra



Selected ion monitoring (SIM)

- Valuable detection time is not wasted and efficiency of ion utilisation improves
- Provides the highest sensitivity for users interested in specific ions since more time can be spent on each mass (dwell time)

Chlorpyrifos - extracted ion chromatograms



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MS/MS using triple quadrupole analysers

- The analyser of a triple quadrupole instrument consists in two quadrupoles, separated by a collision cell
- The first quadrupole is used in SIM mode to select a first ion (precursor), which is fragmented in the collision cell
- This is typically achieved by accelerating the ions in the presence of a collision gas
  - The energy of the collision with the gas can be varied to allow different degrees of fragmentation
- The resulting fragments are analysed by the second quadrupole also typically used in SIM mode to monitor a specific fragment (product), the process known as multiple reaction monitoring (MRM)
  - also called selected reaction monitoring (SRM)

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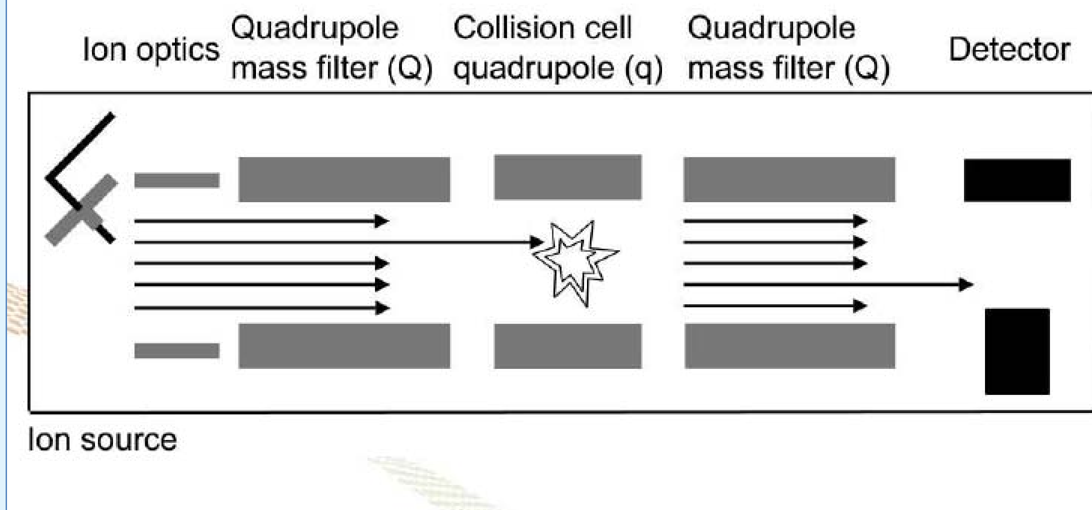
Triple quadrupole instrument in MRM mode



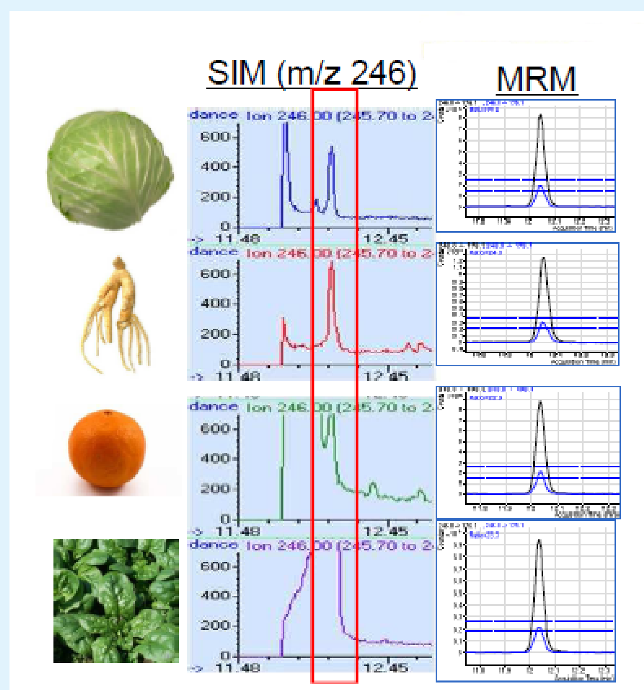
MS/MS using triple quadrupole analysers

- Having two analysers increases the selectivity that ensures interfering peaks from other analytes or matrix are rarely observed

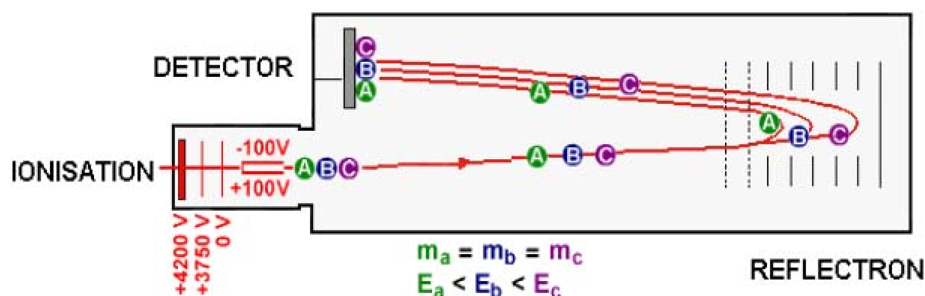
  The ion signal is reduced during the transmission, but the chemical noise, which is a major limitation for complex samples, is also largely decreased, leading to an improvement of the signal to noise ratio

- Use of other modes, such as neutral loss or precursor ion scanning, is limited by poor sensitivity and specificity
- MRM mode provides an order of magnitude better limit of quantification than product ion scanning on a 3D QIT instrument

GC/Q SIM Vs GC/QQQ



Schematic of TOF-MS



- Available options; *high speed, nominal mass acquisition, low speed, accurate mass*
- *Both give good results for most fruit and vegetables*
- *Complex samples e.g. Animal feeds require GC X GC*
- *Systems with mid-speed, mid-resolution being developed*
- *Large (Gbyte) data rich files require better data software*

Screening and identification

- Targeted analysis (detection and quantification) is possible by GC-MS (SIM) and GC-MS/MS (MRM)
- Non-targeted screening is possible with GC-MS using EI spectra and/or GC-TOFMS (full spectral acquisition at elevated resolution)
- Identification of unknowns by EI spectra and/or exact mass measurement (TOF) alone would be difficult

  Use of higher resolution/mass accuracy systems

  Combination with MS/MS or MS

  Combination with other techniques (*e.g.* NMR)

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Summary

- Most laboratories are transferring analyses from selective detectors to mass spectrometers
- ECD still useful for determining low concentrations of organochlorines
- FPD still useful for determining low concentrations of polar organophosphorus compounds

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QUESTIONS ?

THANK YOU

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PRINCIPLES OF LC AND LC-MS

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Overview

- Basics
- Ionisation
- Mass analysis
- Operational issues
- Conclusions

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Why LC-MS/MS?

- Why Liquid Chromatography?
  - Analysis of thermally labile analytes
  - Analysis of polar compounds
- Why MS/MS?
  - **Increased Specificity**
  - **Increased S/N**

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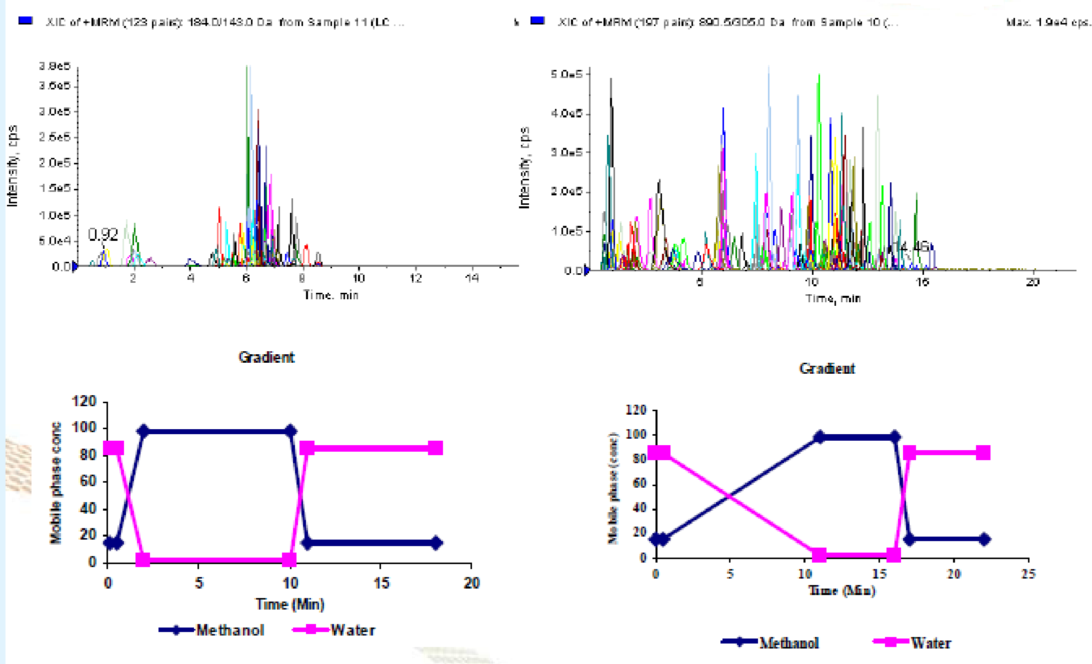
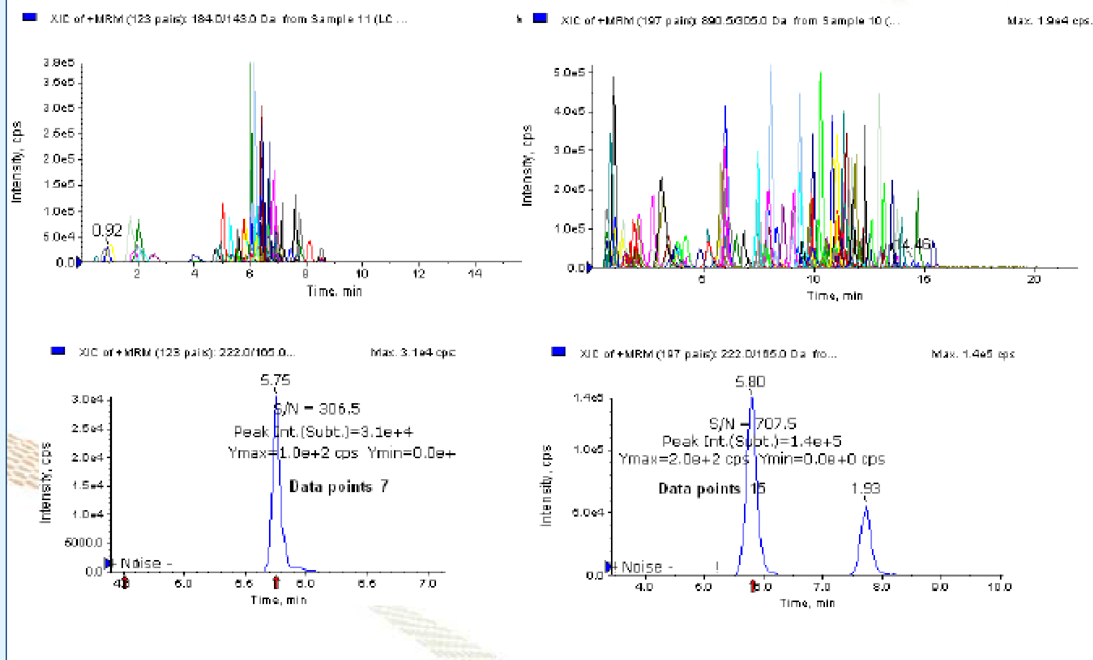
Optimisation of LC-MS/MS method

- Suitable column
- Suitable mobile phase composition and flow rate
- Proper gradient chromatography.
- Use of sMRM mode
- Use of screening methods
  - ✓MRM based method
  - ✓MRM to EPI

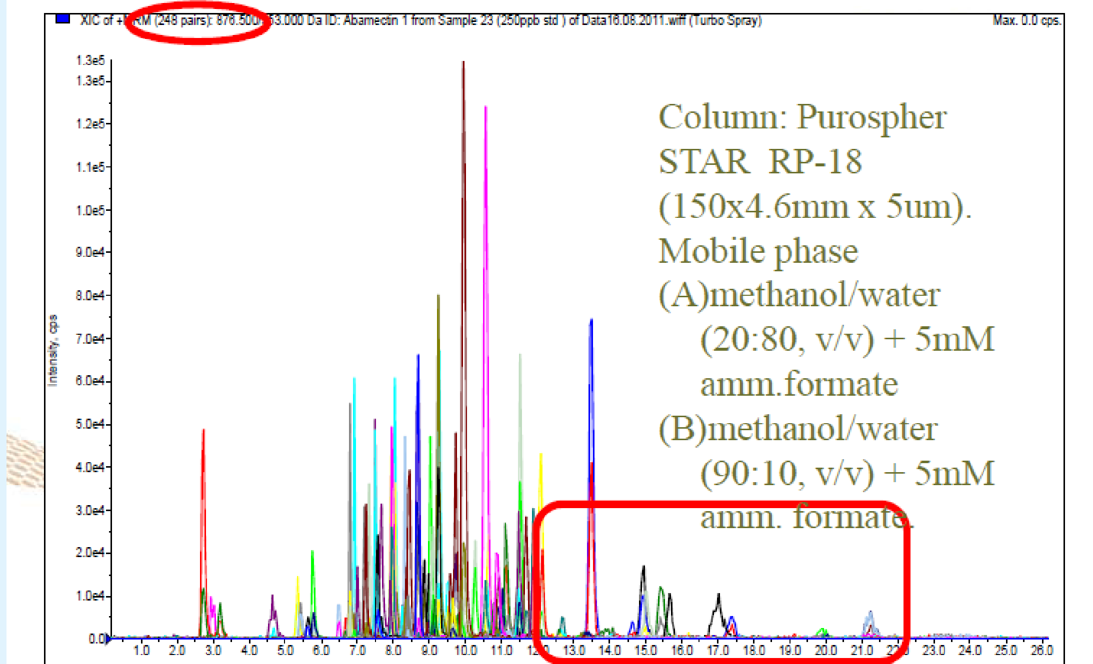
Effect of Gradient chromatography



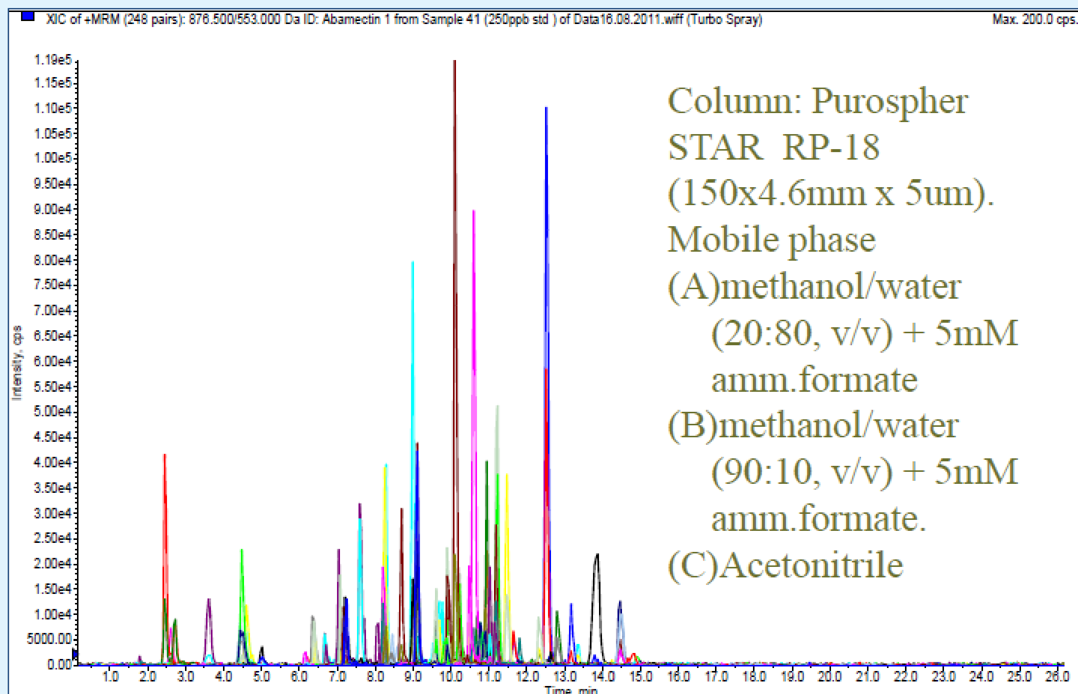
Effect of Gradient chromatography



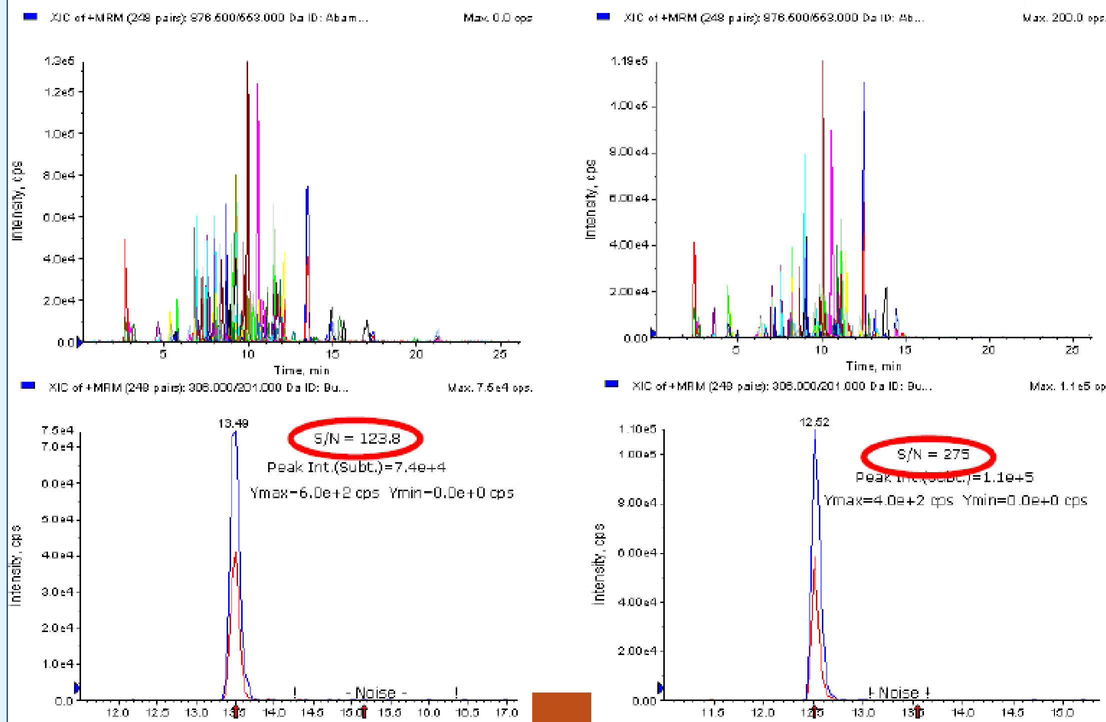
Effect of mobile phase



Effect- mobile phase combination

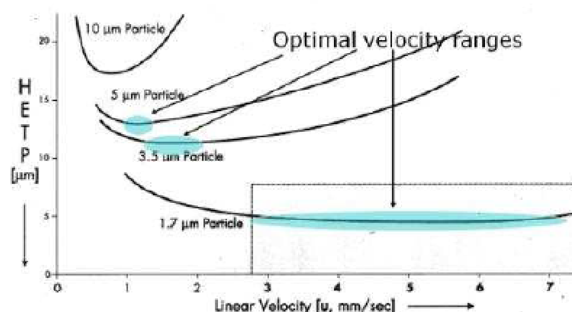


Combination of mobile phase



High efficiency at high flow rates – reducing particle size

- Reduces analyte dispersion by reducing pore depth and distance between pores
- Reduces analyte dispersion by minimising Eddy diffusion



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UHPLC instrumentation

- Increase in efficiency when using smaller particles but results in increased system back pressure
- New high pressure instrumentation platforms
  - Nexera, Infinity, Acquity, Ultimate, Accela *etc.*
  - Pump adaptations (14,500+ psi)
  - Reduction in extra column volume
  - Increase in MS acquisition speed required (*e.g.*<5 ms cycle times)

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Disadvantages of HPLC with sub 2 µm particle sizes

- Smaller frits (0.5µm) needed to retain the packing material
  - Increase in plugging of columns if extra steps are not implemented to filter all solvents and sample extracts
  - Increase in the contamination of the frit by co-extractives if an appropriate guard column not utilised

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Basic components of a mass spectrometer

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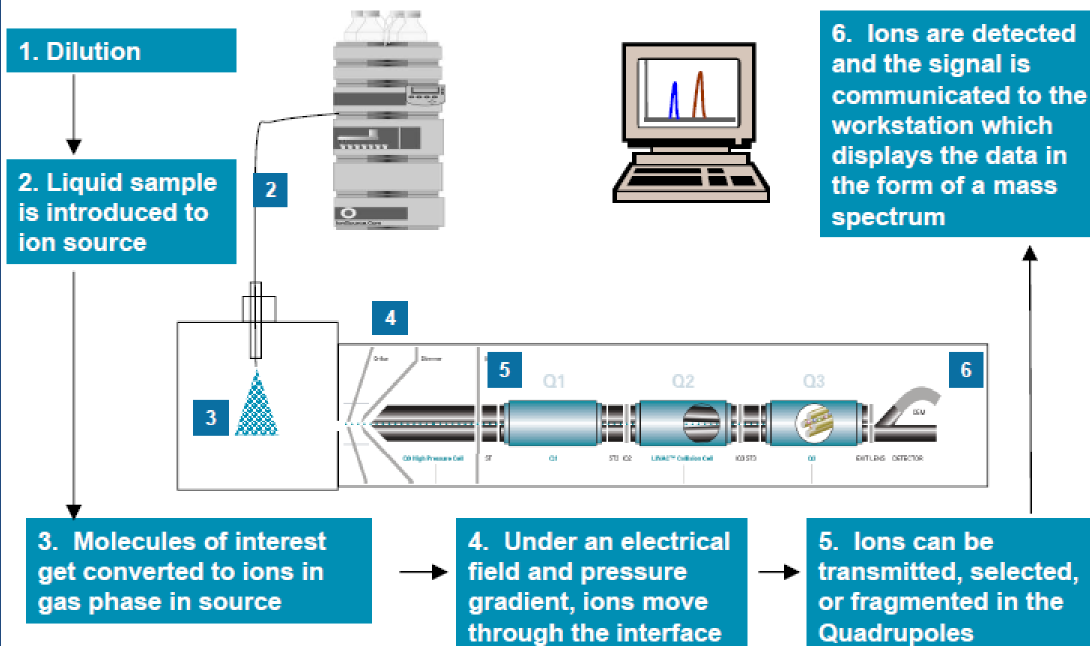
System Components



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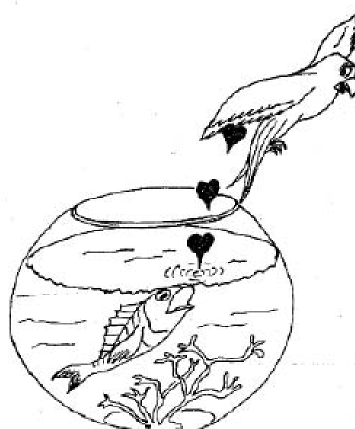
- Rather the instrument determines the mass to charge ratio or *m/z*
- For ions with either a +1 or –1 formal charge the mass of the ion is the same as the *m/z* ratio
  - The case most of the time with small molecules with LC-MS/MS

LC-MS: love between totally incompatible species

- LC involves a wet solvent as mobile-phase but
- Mass spectrometry requires ions in the gas phase and a vacuum
- Need a match-maker!



Arpino, Patrick J. *Trying to arrange a difficult marriage: A report on the workshop on liquid chromatography mass spectrometry*, held in Montreux, Switzerland, 22-23 October 1981, Biological Mass Spectrometry, Volume 9, Issue 4, Date: April 1982, Pages: 176-180.

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Transformation of solutes into gas phase ions

- Achieved by interfaces involving:
  - Pressure reduction
  - Desolvation (evaporative process) and ionisation processes
- Creation of ions at atmospheric pressure and then sample ions through so-called atmospheric pressure (AP) interfaces to vacuum

Bruins (1991). *Mass Spectrom. Rev.*, 10:53-77

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Common ionisation techniques for LC-MS

- **Electrospray** (ES)
- Atmospheric pressure chemical ionisation (APCI)[1,2]
- Atmospheric pressure photoionisation (APPI)
- Dual sources (ESI/APCI) or (APCI/APPI)

1. Willoughby *et al.* (2002). *A Global View of LC/MS, How to Solve Your Most Challenging Analytical Problems.*

2. Balogh, M. (2006). Ionization revisited. *LC-GC N. Am.*, 24 (12), 1284-1288.

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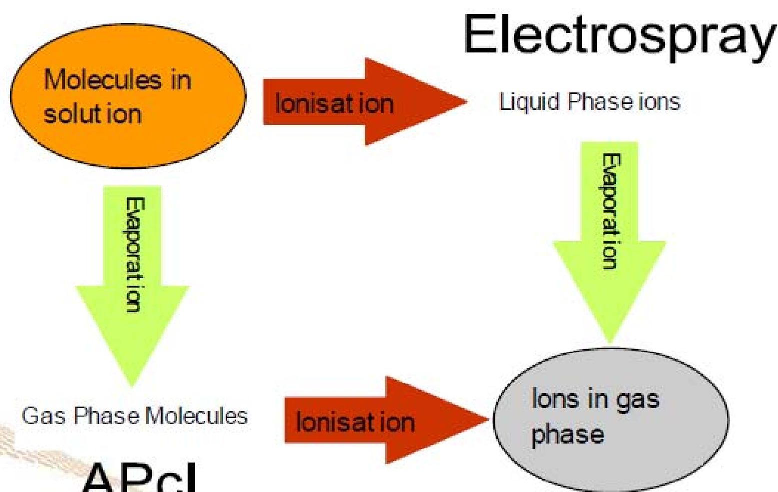
The two routes in ion production



In practice need more than one analysis but ES+ provides best response for more compounds

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Electrospray: the process

- Production of charged droplets
- Droplet size reduction, and fission
- Gas-phase ion formation

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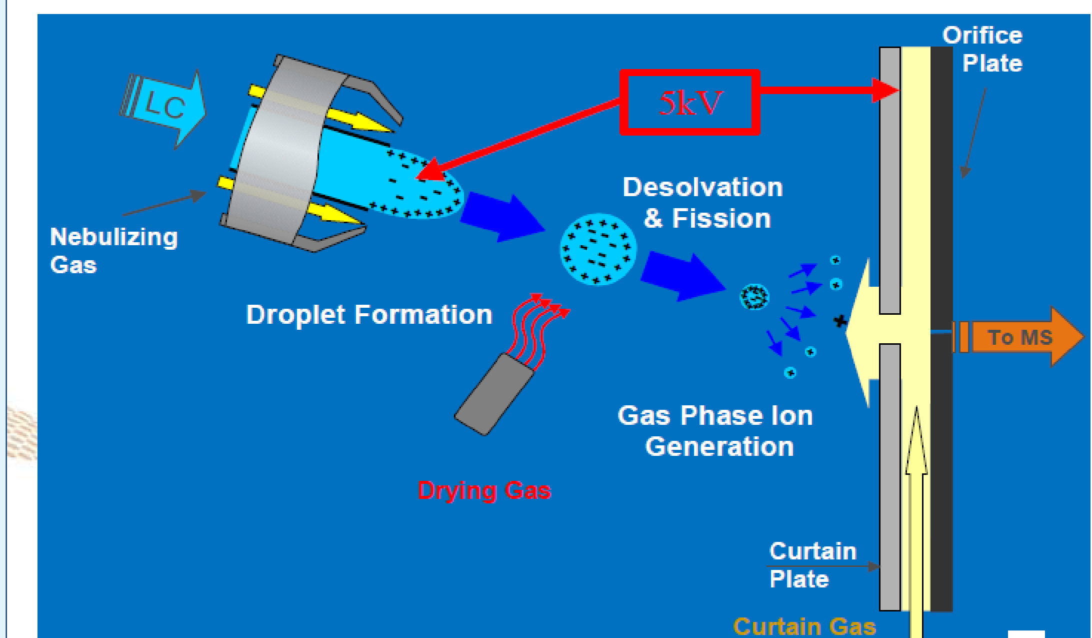
An overview of electrospray



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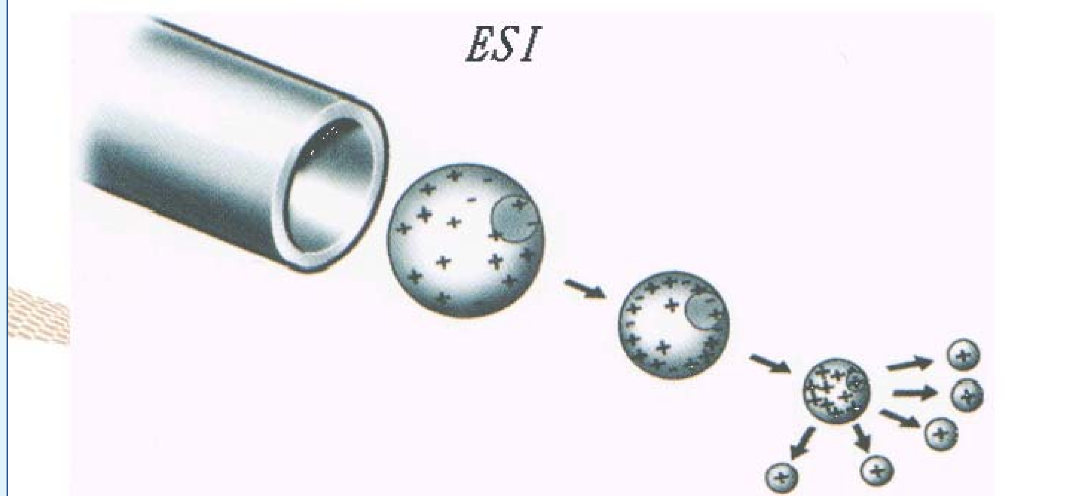
Droplet size reduction and fission



Electrospray ions

- Positive ion
  - *protonated molecule $[M + H]^+$*
  - *addition of NH_4^+*
  - *addition of Na^+*
  - *solvent adducts*
  - *virtually no fragmentation*
- Negative ion
  - *deprotonated molecule $[M - H]^-$*

"Spectrum" contains ions > molecular weight

Electrospray – advantages/ disadvantages

Advantages

- Higher MW possible due to multiple charges
- Good for volatile and non-volatile analytes
- Good for polar and ionic (very polar) analytes

Disadvantages

- Susceptible to ion suppression
- Sensitivity inhibited by high salt concentrations
- Sample matrix ions may compete with analyte ions
- **Best mobile phase for chromatography may not be best for ESI**

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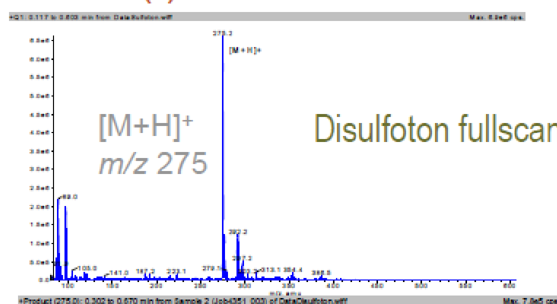
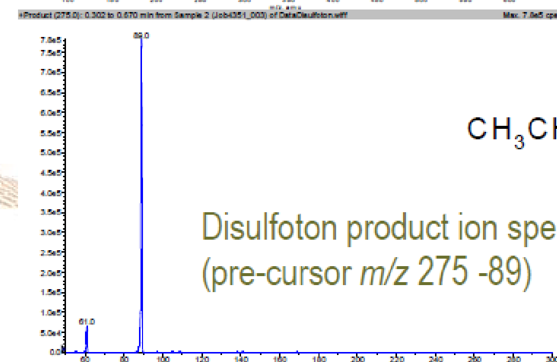
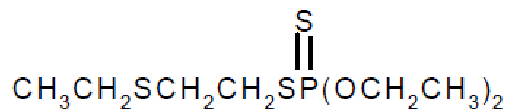
LC-MS/MS Analysis (ES+) of Disulfoton (I)



Disulfoton fullscan ES+ spectrum





Disulfoton product ion spectrum
(pre-cursor *m/z* 275 -89)

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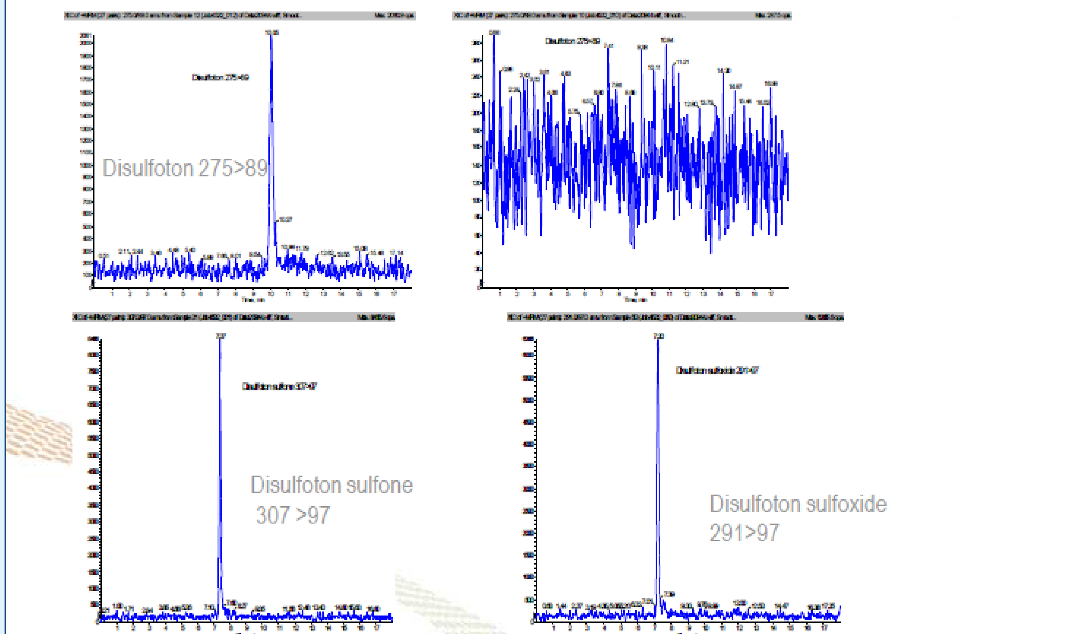
LC-MS/MS Analysis (ES+) of Disulfoton (II)



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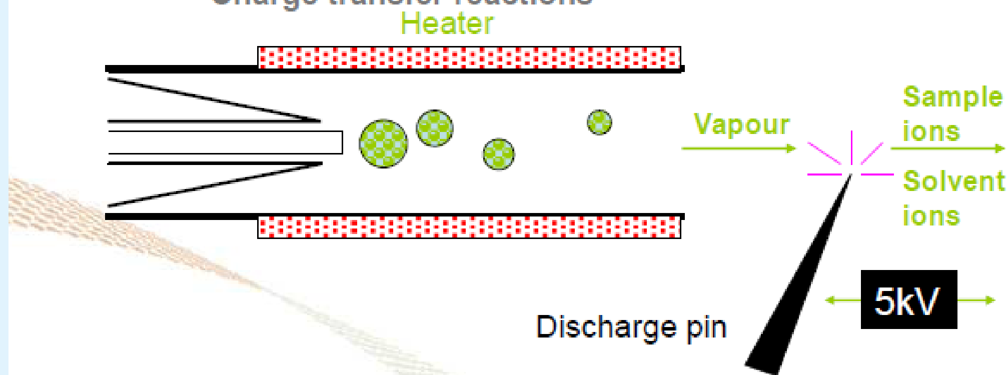
Atmospheric Pressure Chemical Ionisation

- **Flash vaporisation at 500-600°C**
  - Solvent and sample
- **Ionisation of solvent**
- **Charge transfer reactions**



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APCI - Ionisation Characteristics

- Gas phase ionisation
- Heat involved
- Accommodates 1 ml/min
- Mass flow sensitivity
- Mobile phase has little effect
- May be some fragmentation
- Predominantly protonation in positive
- May get [M + ammonium]
- Deprotonation or electron capture in negative mode

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Optimising APCI

- Solvent - MeOH better than MeCN
- Temperature - high temperature reduces solvation of reagent ions and makes stronger acids.
- Flow of 0.2 ml/min marginal for most interfaces. Higher flow is better.

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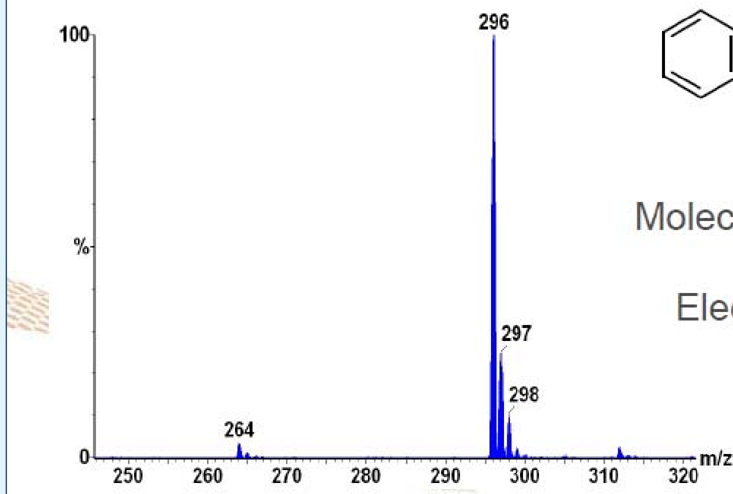
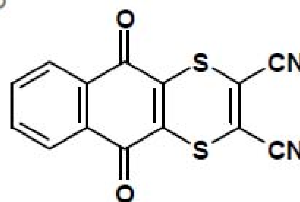
Dithianon – APCI negative mode

Weak response by +ve and -ve ES





Molecular weight 296

Electron capture

APCI – advantages/disadvantages

Advantages

- Higher sensitivity for some compunds
- Ion suppression much less of a problem
- Good for moderately volatile analytes
- Accommodates high flow rates (> 1 mL/min)

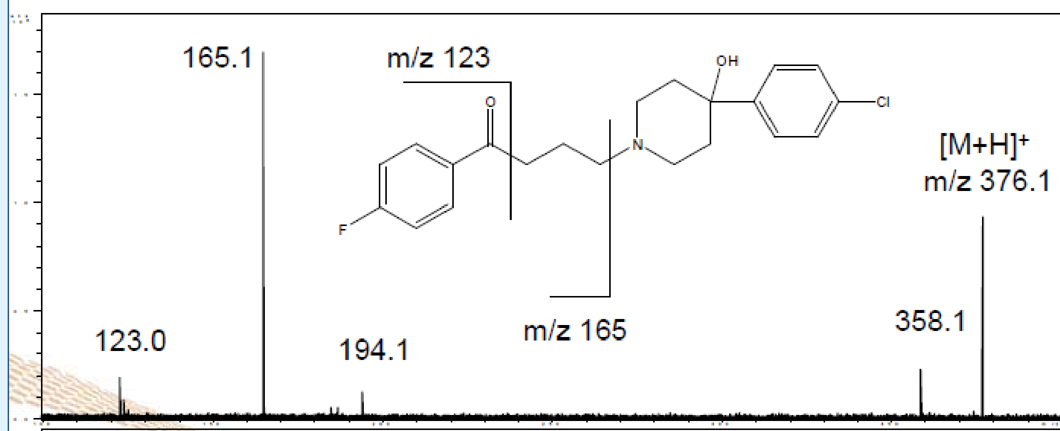
Disadvantages

- Single-charge ions limits mass range
- Low MW (<150 Da) analytes face problems with chemical noise (mobile phase ions)
- Thermal degradation of analytes can result, especially above 1000Da

Mass analysis

- Ions are separated according to their mass-to-charge ratios (*m/z*)
- A vacuum is needed as the ions must not collide with air molecules
- The relative abundances of ions of differing mass-to-charge ratios is measured and recorded as a spectrum that can provide molecular mass and even structural information

Mass spectrum and structure



Mass analysers for LC-MS

- Quadrupoles (MS, MS/MS)
  - Single and **triple quadrupole** instruments
- Ion traps (MS, MS/MS and MS^n)
  - 3D ion traps
  - Linear ion traps
  - Orbitraps
- Time of flight (Tof)
- Combinations of the above
  - Hyphenated instruments

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Sample preparation considerations

- Final extract solvent must be similar to initial HPLC conditions
- Filters can retain analytes
- Injection volume depends on sample solvent
- Correct vial and cap can be important
- Clean-up

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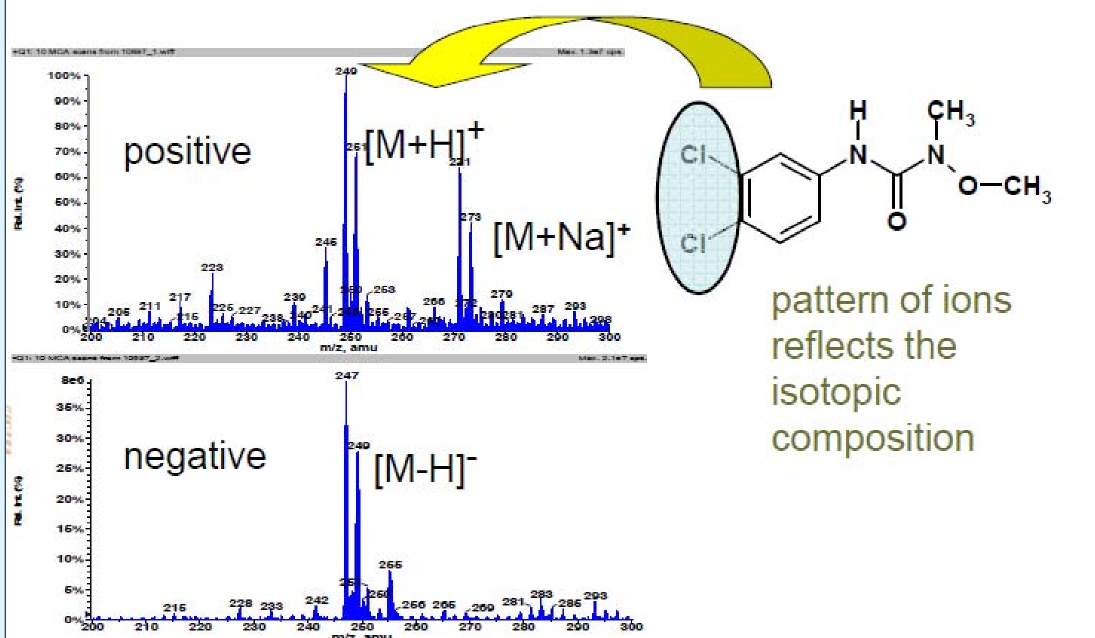
Types of Ions Formed

- Instruments can operate with either positive or negative polarity and so can detect ions with either a positive or negative charge
  - Charging is usually accomplished by adding or removing protons but cation or anion attachment generating adduct ions is also common
  - Basic Compounds tend to gain a proton
    $(M+H)^+$, $(M+NH_4)^+$, $(M+Na)^+$
  - Acidic Compounds tend to loose a proton
    $(M-H)^-$, $(M+acetate)^-$

Electrospray spectra - linuron



Quadrupole analysers

- The quadrupole consists of four parallel metal rods
- Each opposing rod pair is connected together electrically and a radio frequency (RF) voltage is applied between one pair of rods, and the other
- A direct current voltage (DC) is then supeimposed on the RF voltage
- Ions travel down the quadrupole in between the rods
- Masses are sorted by the motion of their ions induced by the DC and RF fields

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Quadrupole analyser

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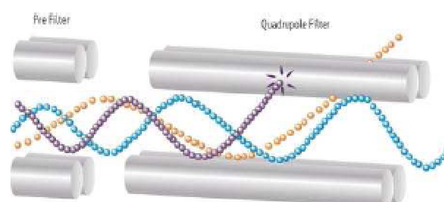
Quadrupole analysers

- Systematically changing the field strength via the operating software in effect alters which ion is filtered or transmitted through to the detector at any given time
- Varying the voltages allows:
  - "scanning" all the ions
  - selection of a particular ion
  - "jumping" between a selection of specific ions



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Tandem mass spectrometry (MS/MS)

- Can provide increased selectivity, which reduces the contribution to the analyte signal from isobaric interference leading to improvements in sensitivity
- Lower limits of detection become achievable
- Provides a greater degree of confidence for confirmation of identity than SIM, which can suffer from isobaric interferences

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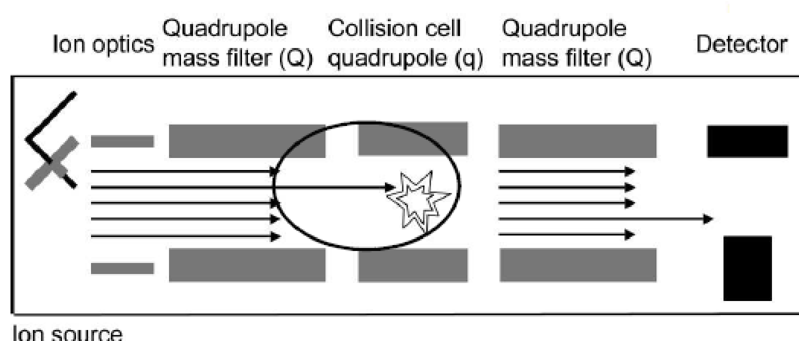
Mass analysers for tandem mass spectrometry

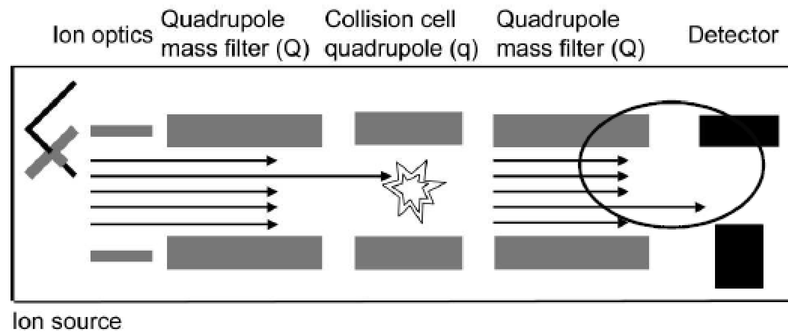
- Ion trap instruments
  - Three dimensional quadrupole ion traps (QIT or 3D IT)
  - Linear ion traps (LIT)
    - Ion accumulation devices in combination with other devices (Q-Trap, Orbitrap)
    - Stand-alone mass spectrometers with MS^n capabilities (LTQ)
- Hybrids
  - **Triple quadrupole instruments (QqQ)**
  - QqTof
  - LIT-Orbitrap

MS/MS using triple quadrupole



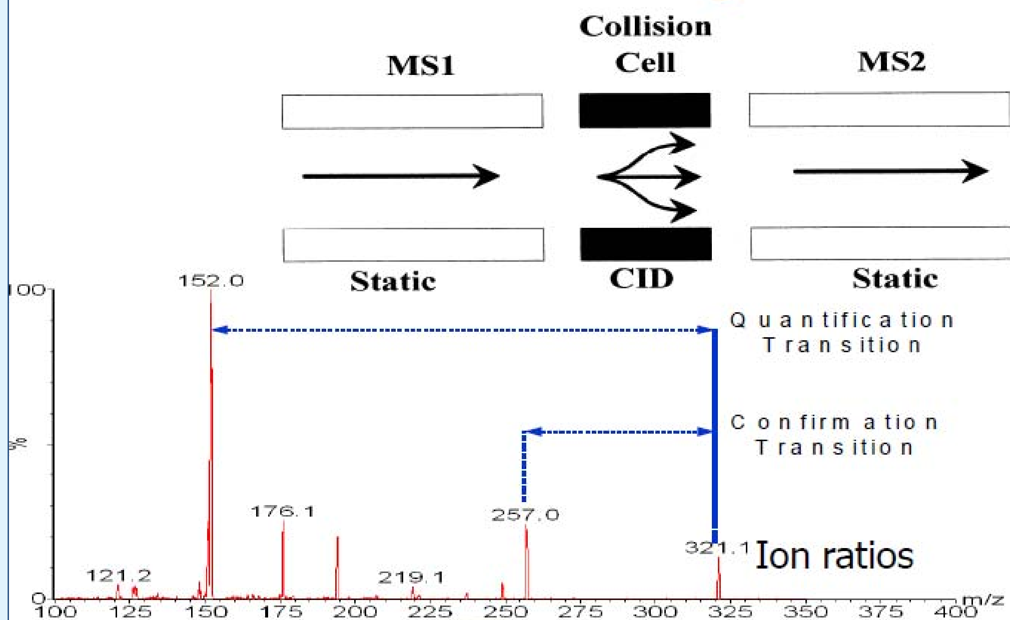
- Q1 is used in SIM mode to select a precursor ion, which is fragmented in the collision cell (q)
- This is typically achieved by accelerating the ions in the presence of a collision gas
- The energy of the collision with the gas can be varied to allow different degrees of fragmentation

MS/MS using triple quadrupole



- The resulting product ion are analysed by Q3 either by:
  - Scanning to monitor all product ions or
  - Monitor specific product ions, known as multiple reaction monitoring (MRM) or selected reaction monitoring (SRM)

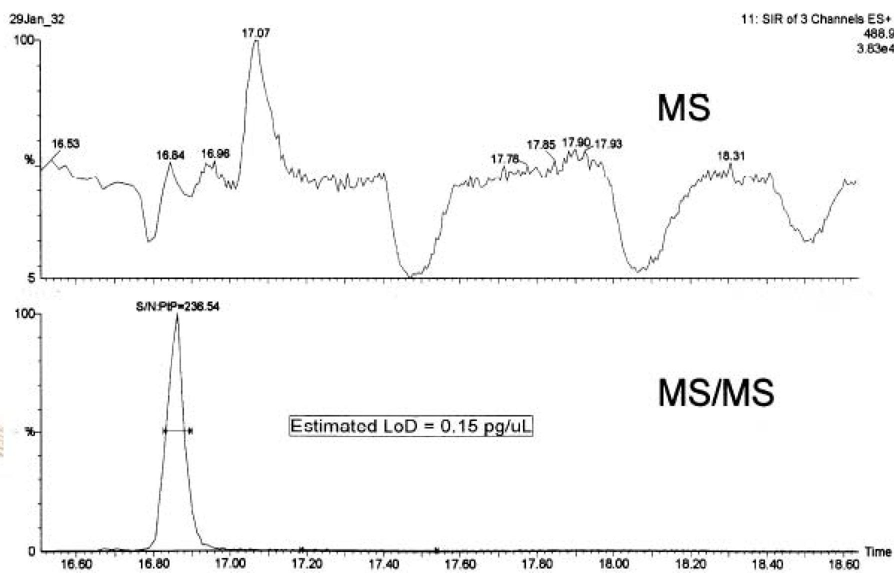
Selective reaction monitoring



Advantage of SRM analysis

- Having two analysers increases the selectivity that ensures interfering peaks from other analytes or matrix are rarely observed
- The ion signal is reduced during the transmission, but the chemical noise, which is a major limitation for complex samples, is also largely decreased, leading to an improvement of the signal to noise ratio

Advantage of SRM analysis



Solvents and additives

- Volatility an issue
- Useful Solvents

  Water, Acetonitrile, Methanol, Ethanol, Propanol, Isopropanol
- Acceptable additives

  Acetic acid, Formic acid, Ammonium hydroxide, Ammonium formate (≤10 mM), Ammonium acetate (≤10 mM)
- Avoid surface-active agents (surfactants)
- Non-volatile salts (phosphate, borate, citrate, *etc.*) can deposit in source and plug capillaries thus requiring more cleaning and maintenance operations

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Data acquisition rate and quantification

- Acquisition of data points in SRM depends on:
  - Dwell time
    - The time spent accumulating ion signal for each channel
    - Has little impact upon S/N on modern instruments
    - Can now be automated
  - Switching time
    - An intrinsic property of the mass analyser (electronics and clearing gas cell)
  - Total acquisition/cycle time termed duty cycle

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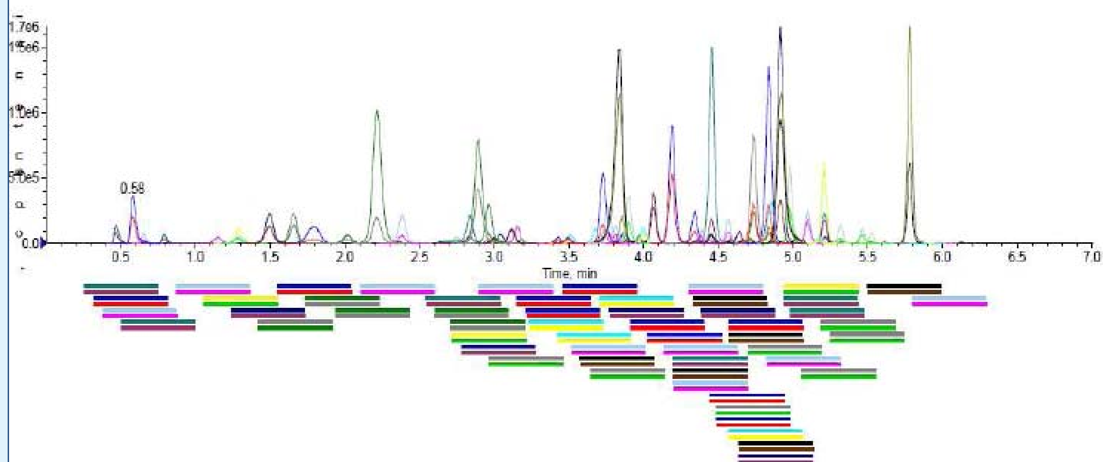
Data acquisition rate and quantification

- 10-15 data points needed for accurate and reliable quantification
  - Insufficient acquisition rate will result in the incorrect sampling of the peak and missing of the peak apex
- With peak widths from the sub 2 μm particles in the region of 1-2 seconds at the base, this means that data acquisition rates of < 50 ms
  - If more than one analyte is required during analysis then the acquisition times are much lower

Optimisation of data acquisition



Adjusts detection windows and dwell time automatically depending on retention time, peak width and how many data points you want across a peak

Optimised data acquisition

- Benefits
  - Optimises dwell time for each analyte & cycle time
  - Allows more transitions
  - Allows more data points across peak
  - Describes peak shape better
  - Improved repeatability
  - Longer dwell times
  - Improved signal to noise
- Important for coupling with fast LC
- Automated

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Limitations of selected reaction monitoring

- Confirmation of identity is based upon the ratio of SRM transitions
  - No spectra for comparison with universal or in-house reference library
  - Can acquire MS/MS product ion spectra
- The "target analysis" approach fails to detect other pesticides present in the sample
  - Unable to go back and "mine" the data later
  - LC-HRMS

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Matrix effects

- The electrospray source is susceptible to sample matrix-related ion-suppression effects from:

  Co-extractives, buffers, salts, mobile phase modifiers, other analytes

- Matrix effects should always be investigated as they impact on:

  "Detectability" and hence true reporting limit

  Possible reporting of false negative results

  Accuracy of quantification

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Mechanism of the matrix effect

- Such ions produce a **competition effect** in the ionisation which can result in suppression of ions from the targeted analytes
  - Present at high concentration
  - Having high surface potential or high proton affinity
    - Change in droplet surface tension leads to larger droplets and insufficient desolvation
    - Surfactants gathering at droplet surface lead to preferential ion evaporation

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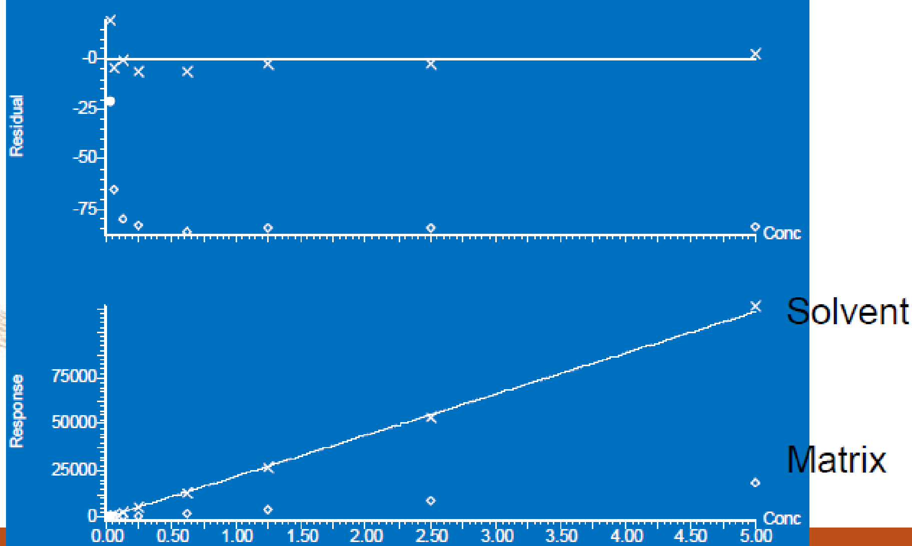
Minimising matrix effects

- Improve chromatography
  Separate analyte from matrix components
- Improve sample preparation
  Remove matrix components
  But these are limited in multi-residue analysis
- Dilute the sample extract or take less sample
- Change ionisation mechanism
  APCI is a gas-phase ionisation technique
  Need to re-evaluate matrix effects

Correcting for matrix effects

- Matrix-matched standards
  - Known amounts of targeted analytes added to extracts of an appropriate sample previously shown to be blank
  - Need to “bracket” samples: cals/samples/cals

Matrix matching



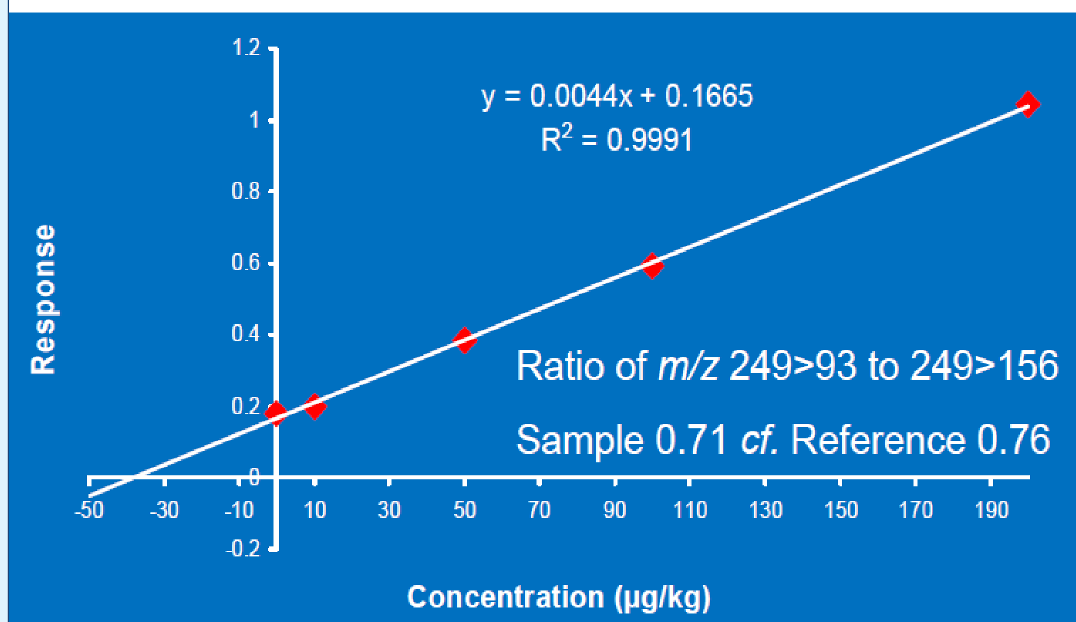
Correcting for matrix effects

- Internal standard
  - A compound, not found in the matrix, which, when added to each sample extract, attempts to inherently correct the variation in response of all targeted analytes caused by matrix effects

Correcting for matrix effects

- Standard addition
  - Addition of known amounts of analyte to sample or sample extract
  - Single concentration at reporting limit
  - Multiple levels for quantification

Use of standard addition



Conclusions

- Use of LC-MS for residue analysis has moved from a number of single residue methods to multi-residue UHPLC-MS/MS approaches offering
  - Good selectivity
  - High sensitivity
  - Robustness
  - Increased analyte coverage
  - Quantification
  - Concurrent confirmation of identity

QUESTIONS ?

THANK YOU

Conclusion

❖ There are a number of steps associated with analytical procedure

❖ Need to control sources of errors at all stages to ensure that data are 'fit-for-purpose'

❖ Further reading

- *Quality in the Food Analysis Laboratory, Wood, Nilsson & Wallin, RSC*
- *Quality Assurance Principles for Analytical Laboratories, Garfield, AOAC*
- *Statics for Analytical Chemists, Miller & Miller, J. Wiley & Sons*

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QUESTIONS ?

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THANK YOU

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