Evaluation of Automated MS to MS/MS Function Switching for Comprehensive Drug Profiling Analysis Using a Quadrupole Time-of-Flight Mass Spectrometer

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Outline

- Introduction and goal
- Basis of Automatic Function Switching
- Parameters
  - Thresholds
  - Exclusion time
  - Number of components
  - Detection window
- Profiling analysis
- Results
- Conclusions

Introduction

- Toxicology: no foreknowledge

  ⇨ profiling analysis

  - ✓ High specificity
  - ✓ High selectivity
  - ✓ Detection of largest range of drugs

  ⇩ Mass Spectrometry (MS)

Introduction

- LC/MS
  - in-source fragmentation
    - ✗ interfering ions complicate interpretation
  - MS/MS
    - ✗ pre-experiment required

☞ **Solution:**

Automatic function switching

Goal

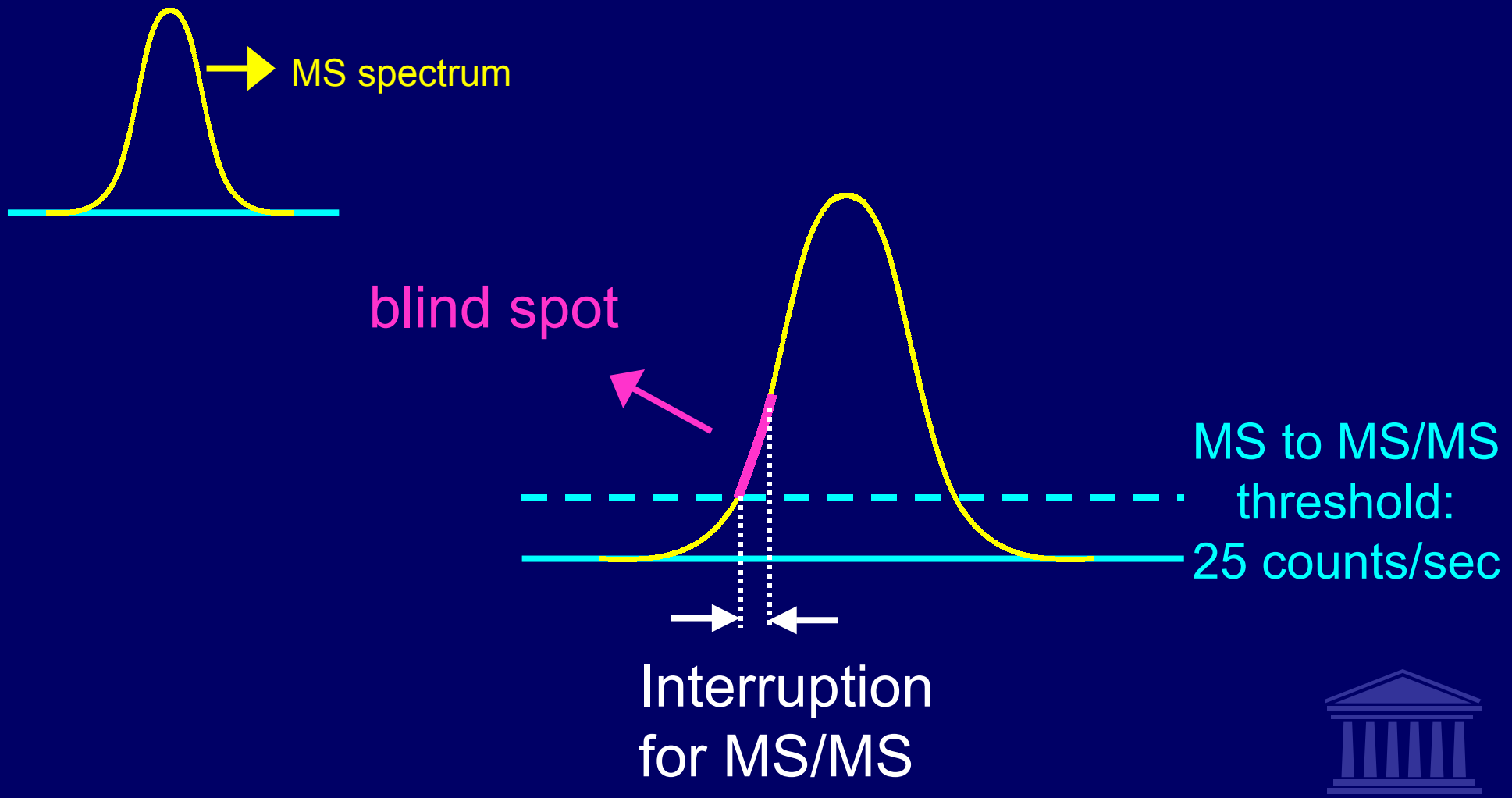
Development of a method for drug profiling analysis (i.e. screening and quantisation) in a single LC acquisition

- Using automatic function switching and optimised parameter settings

Basis of Automatic Function Switching

- Initially: QUAD = wide band-pass filter
  - ◆ precursor ion(s) > MS threshold
    - ⇨ switch to MS/MS
      - ° fragment ions ⇨ TOF
  - ◆ fragment ion(s) < MS/MS threshold
    - ⇨ switch back to MS

MS to MS/MS threshold



MS/MS to MS (back)switching criterion

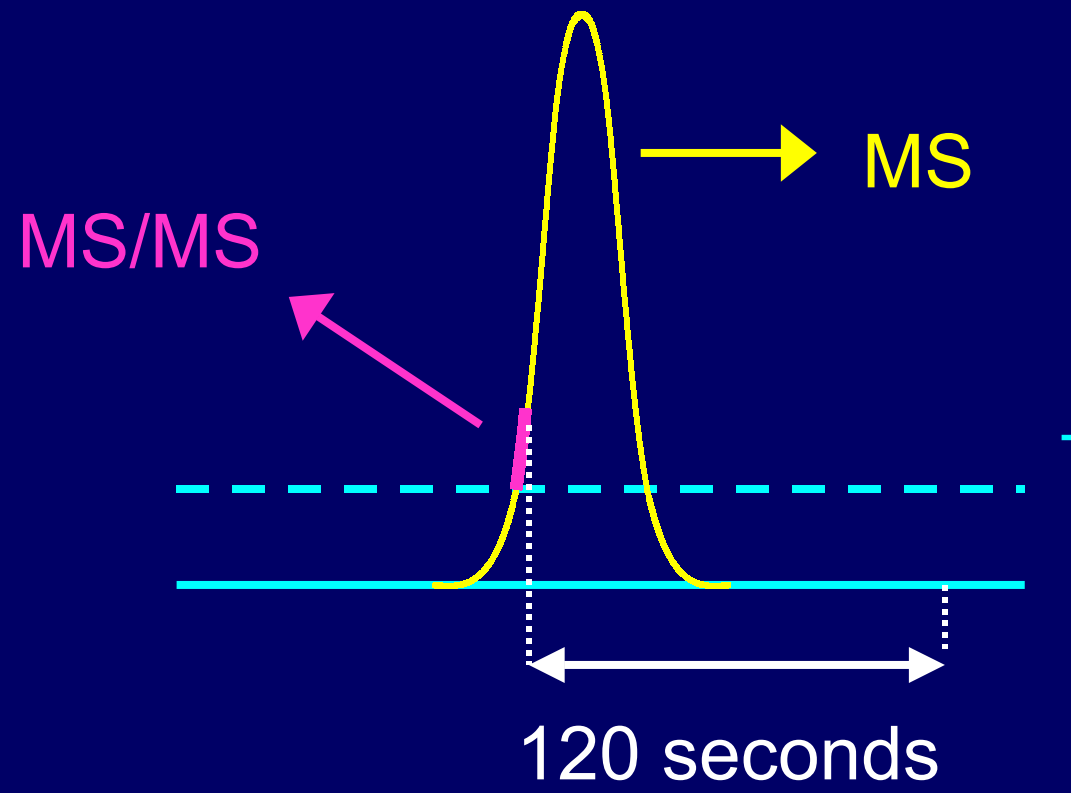
10 counts/sec or after 4 seconds

For the following reasons:

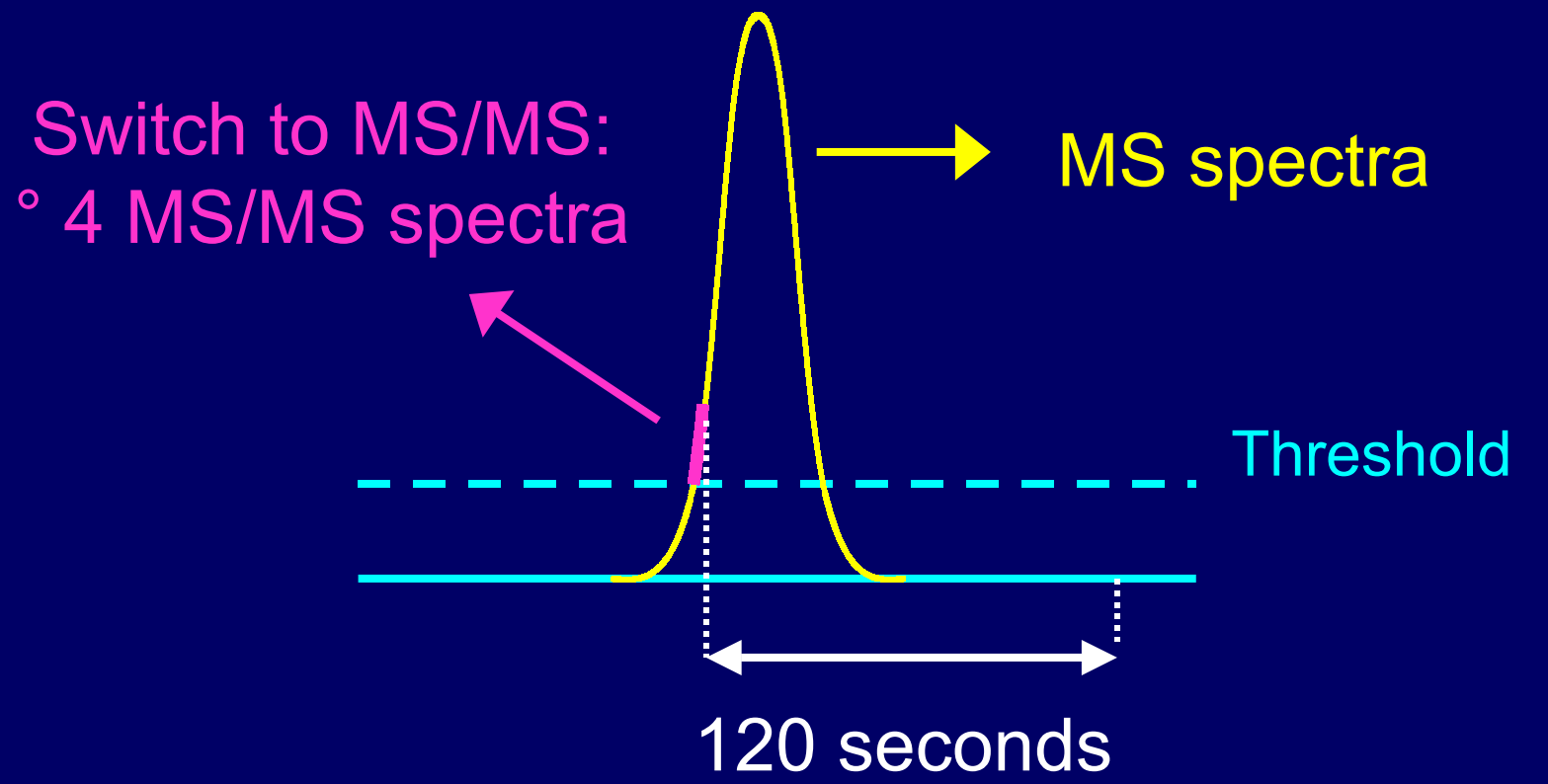
- ⇨ To generate MS/MS spectra (given 1 second TOF accumulations)
- ⇨ To obtain a well-defined chromatographic peak (MS mode)

↳ **TOF: “flash” mass analysis capability**

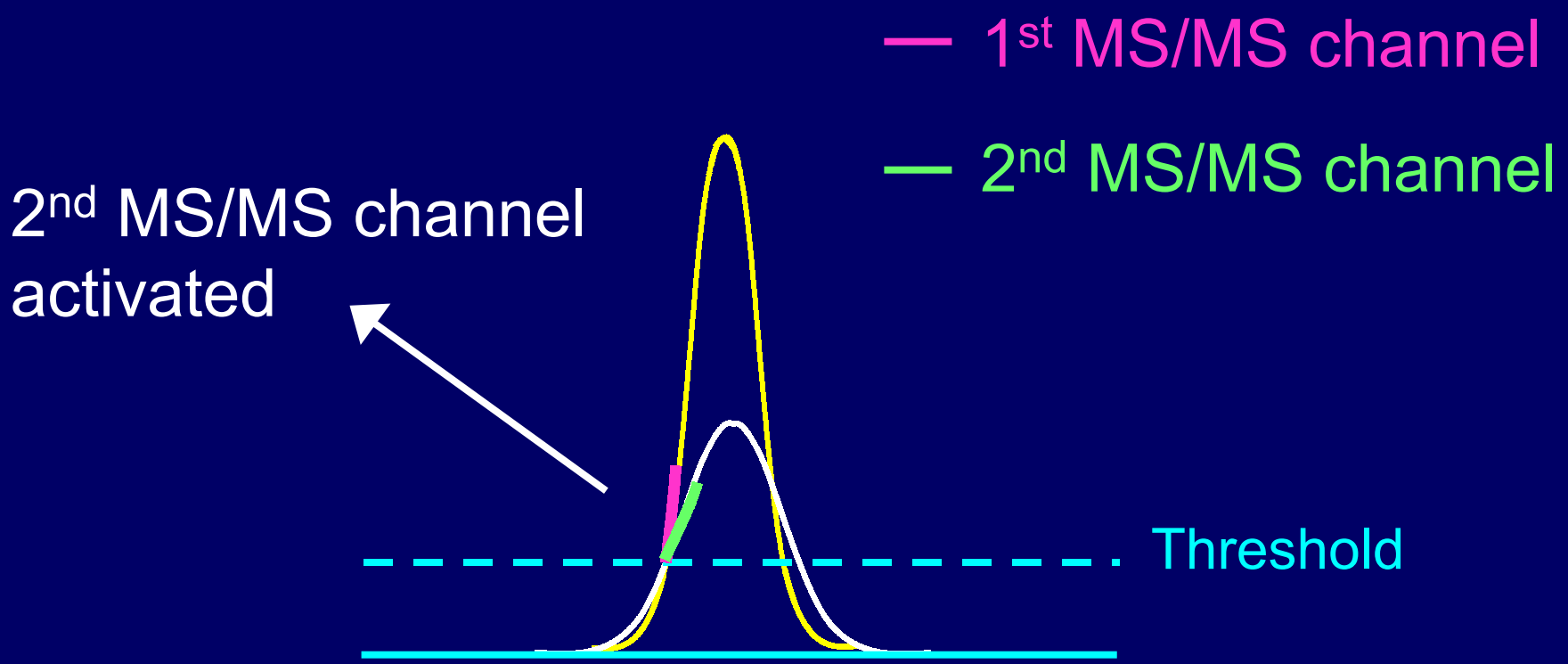
Exclusion of precursor ions



In complete…

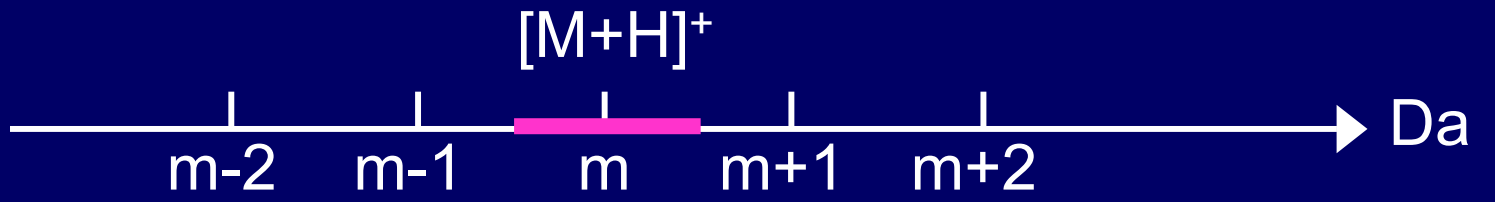

Switch to MS/MS:
° 4 MS/MS spectra
MS spectra
Threshold
120 seconds

Co-eluting peaks



Number of components: 4

Detection window



⇨ Exclusion of m+1 isotope peaks

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⇨ Mixed MS/MS spectra…window too wide

⇨ Window choice : 2 Da

Profiling analysis

Qualitative + Quantitative

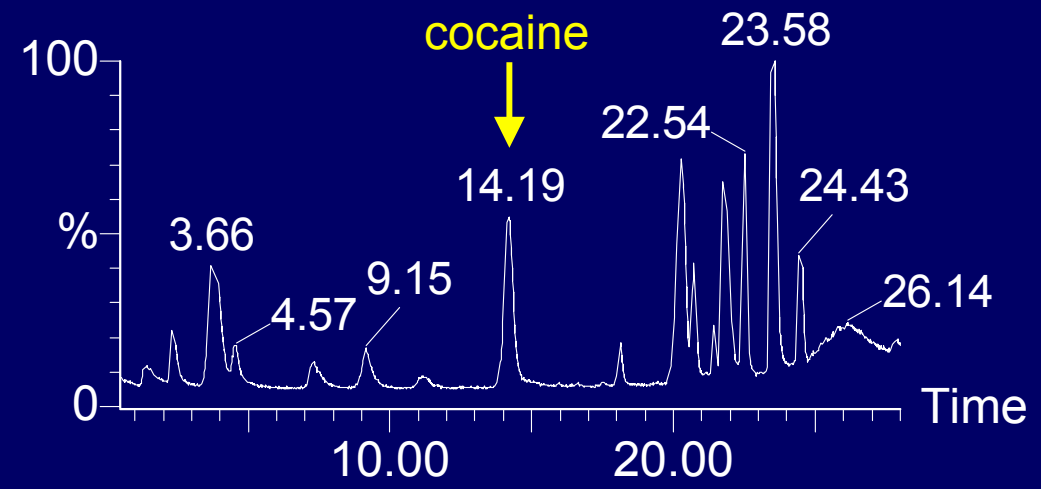
- ✓ MS spectrum ($[M+H]^+$)
- ✓ MS/MS spectrum

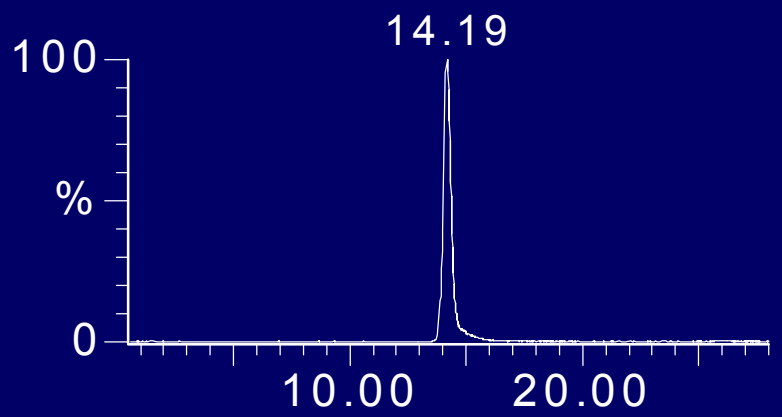
- ✓ Integration of MS extracted ion chromatogram

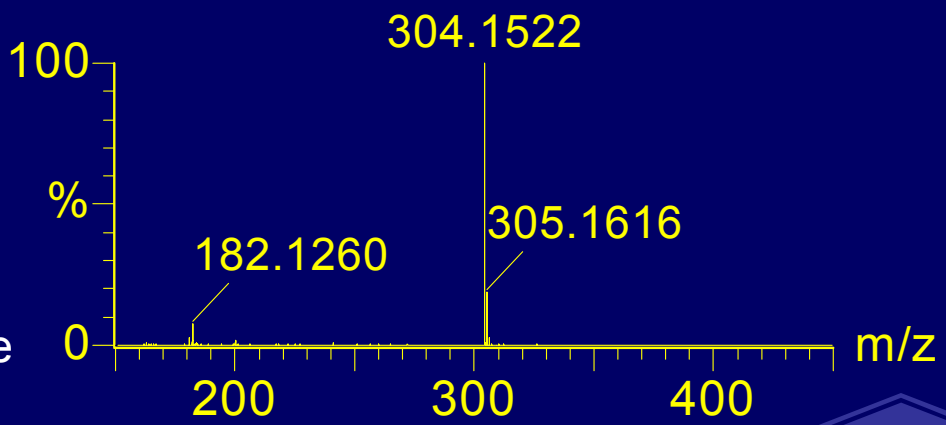
Results - Overview

- Standards
  - MS mode
  - MS/MS mode
  - Quantisation
- Benchmarking of our technique based on the analysis of a real sample

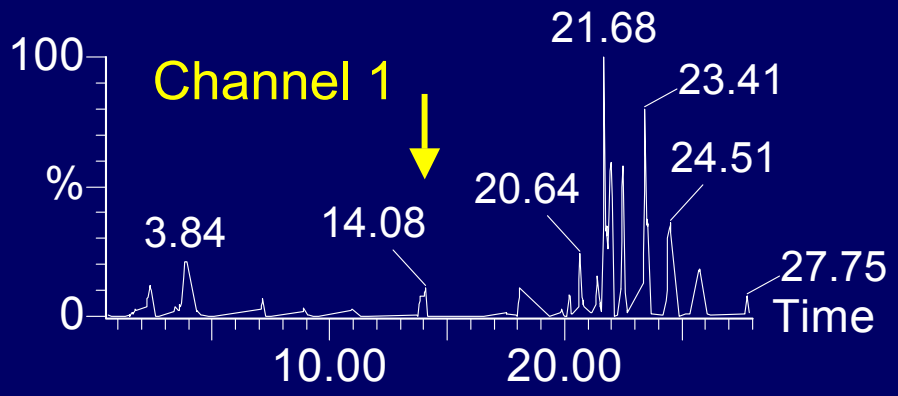
Standards - MS mode

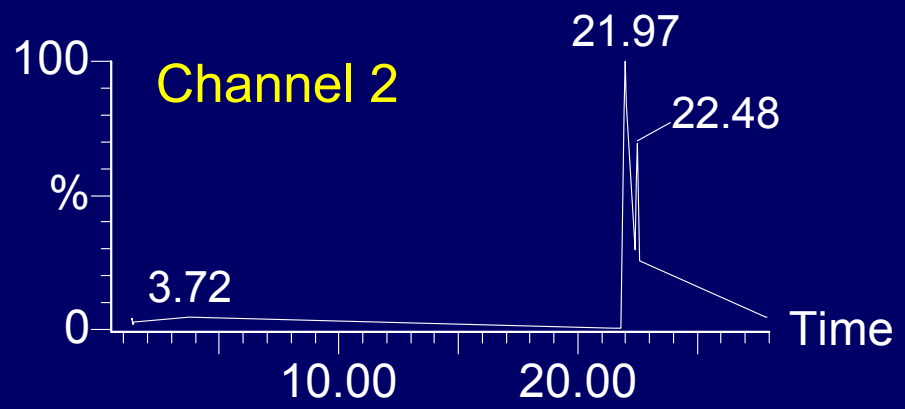


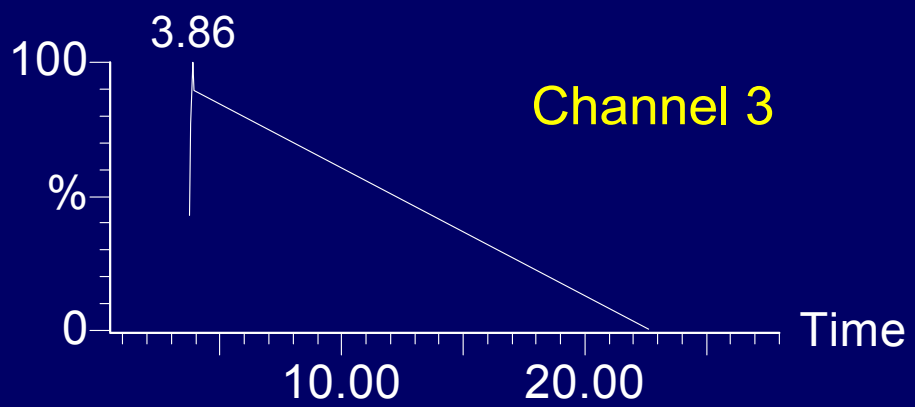


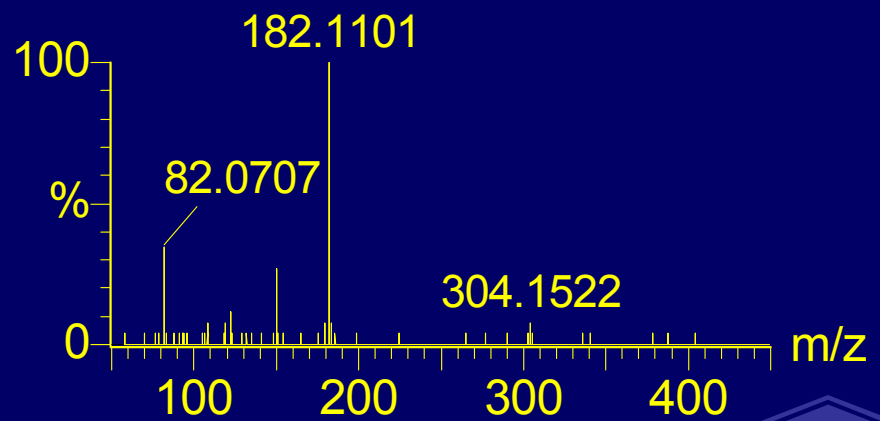


Standards – MS/MS mode

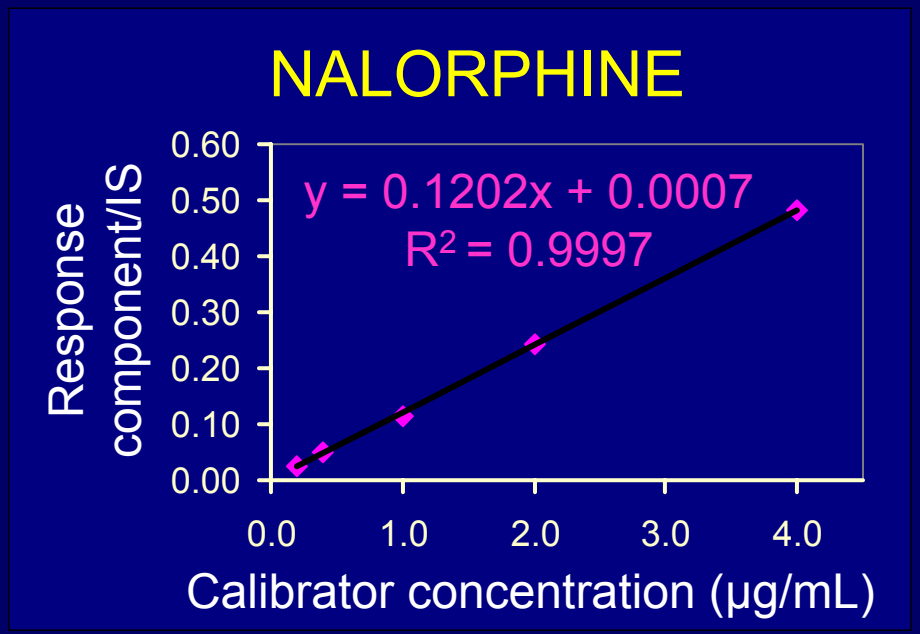


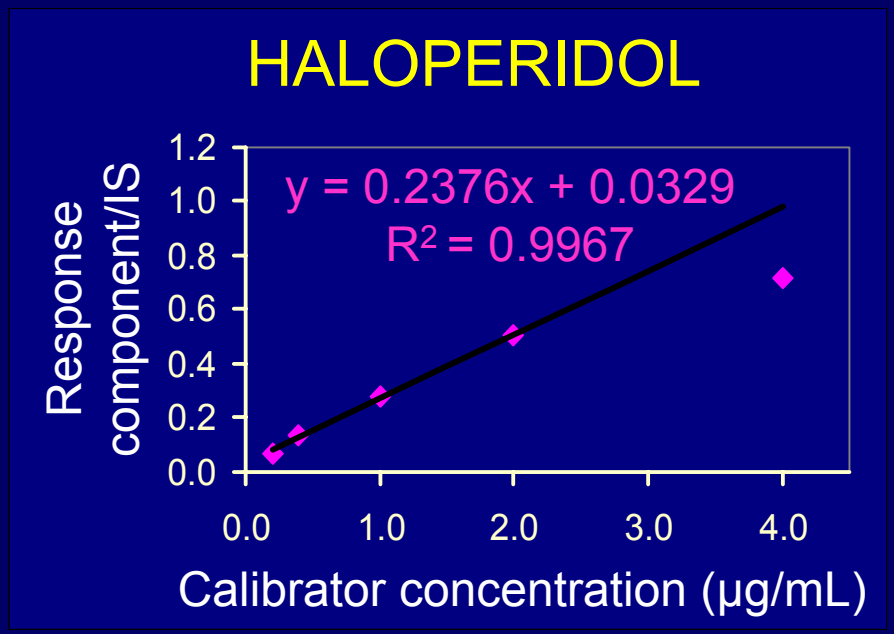






Standards - Quantisation





⇨ Sometimes deviation from linearity due to linear dynamic range constraints

Ref.: K. Clauwaert et al., *Rapid Commun. Mass Spectrom* **13**, 1540 (1999)

Benchmarking of our technique

⇨ Based on the analysis of a real sample

ROUTINE METHOD		LC-MS
EMIT	HPLC-DAD[1]	
Morphine		Morphine (0.28 µg/mL)
Caffeine		Caffeine (0.38 µg/mL)
	Codeine (4.40 µg/mL)	Codeine (5.20 µg/mL)
	Bromazepam (hy)[2]	Bromazepam (0.24 µg/mL)

[1] Ref. method on which quantisation is based

[2] Bromazepam benzophenone

Conclusions

- A wealth of information in 1 single analysis :
  - Qualitative: MS and MS/MS spectra
  - Quantitative: MS extracted ion chromatogram
    (thanks to the high acquisition speed of the Q-TOF)
- No interfering ions in the MS/MS spectra
- Negligible risk of missing co-eluting compounds