Unified HRMS based workflows integrating the quantification and confirmation of veterinary drugs

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Overview

I Detecting and confirming

II Orthogonality of confirmation

III Data dependent modes (DDA)

IV Data independent modes (DIA)

V Requirements & Challenges

The need for a confirmatory signal

- **The more complex the matrix the more likely we obtain false positive findings**

- **Regardless if we use HRMS or MS/MS a signal appearing at the expected retention time is not yet a confirmed finding**

- **A HRMS signal >=50’000 FWHM is equally selective as a conventional MS/MS transition**

Conventional confirmation strategy (A)

- **Monitoring a single MS/MS transition or HRMS signal**

- **Analyzing the data**

- **Setting up a dedicated confirmatory method and doing a reinjection of the sample**

Conventional confirmation strategy (B)

- **Monitoring two or more MS/MS transitions for each analyte**

- **Using fast MS instruments and narrow retention time windows**

This becomes challenging for large multi-residue methods

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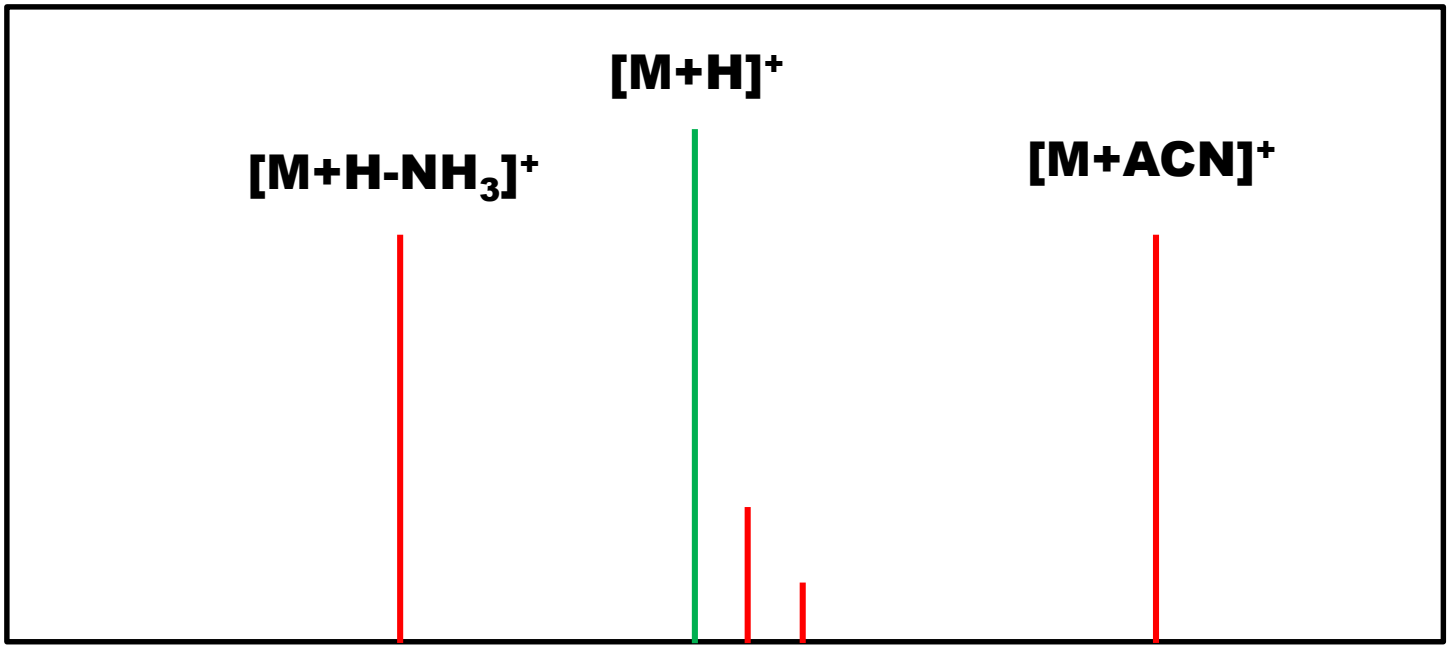
What is a second signal ? Orthogonality



Just using an other signal from the HRMS spectra is not sufficiently orthogonal

Quality of data

- **6-8 data points/across a chromatographic peak for quantitative trace**

- **Stable ion ratio or clean product ion spectra taken near the chromatographic peak apex**

- **Product ion traces showing identical chromatographical peak shape as the precursor ion**

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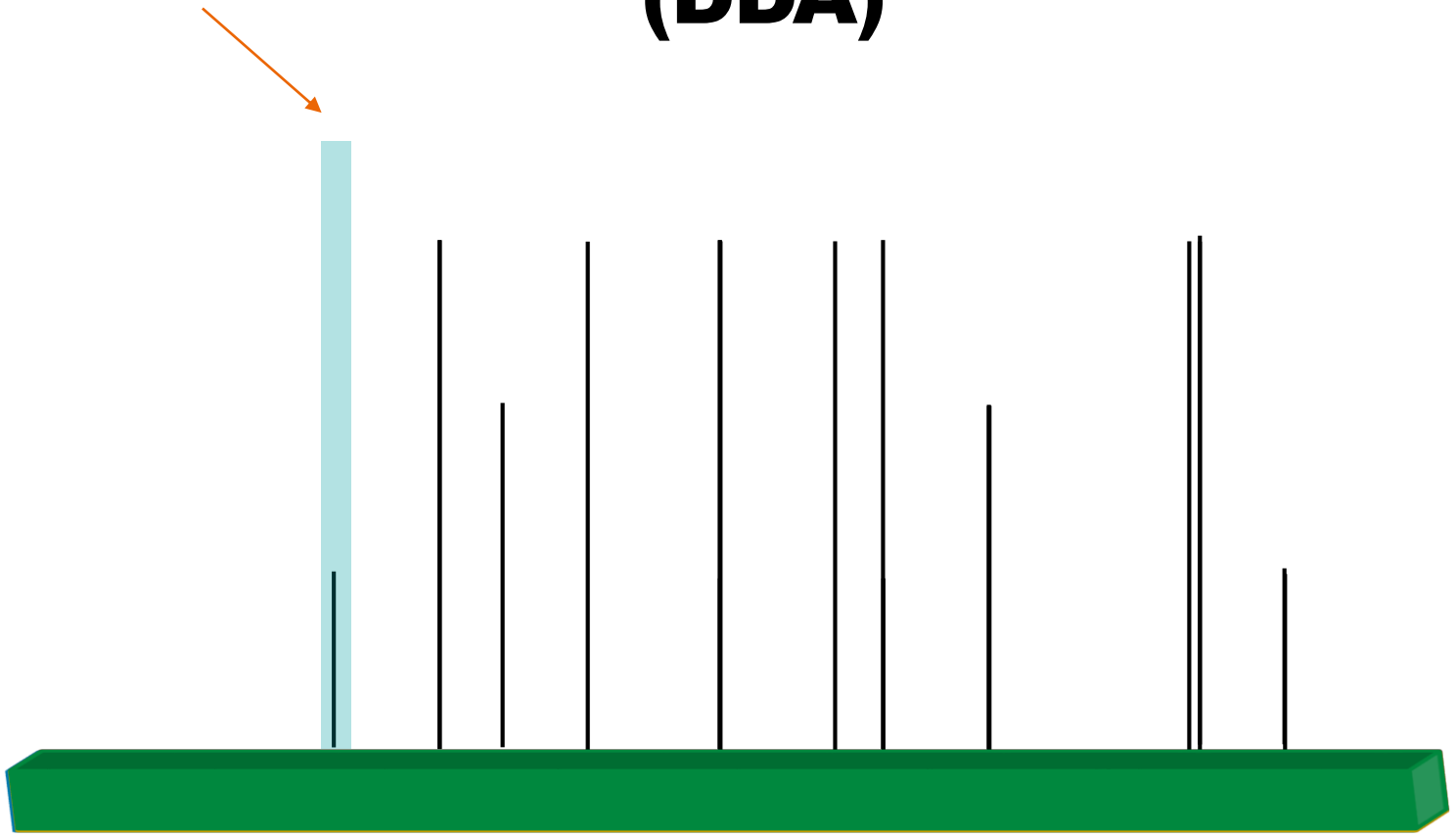
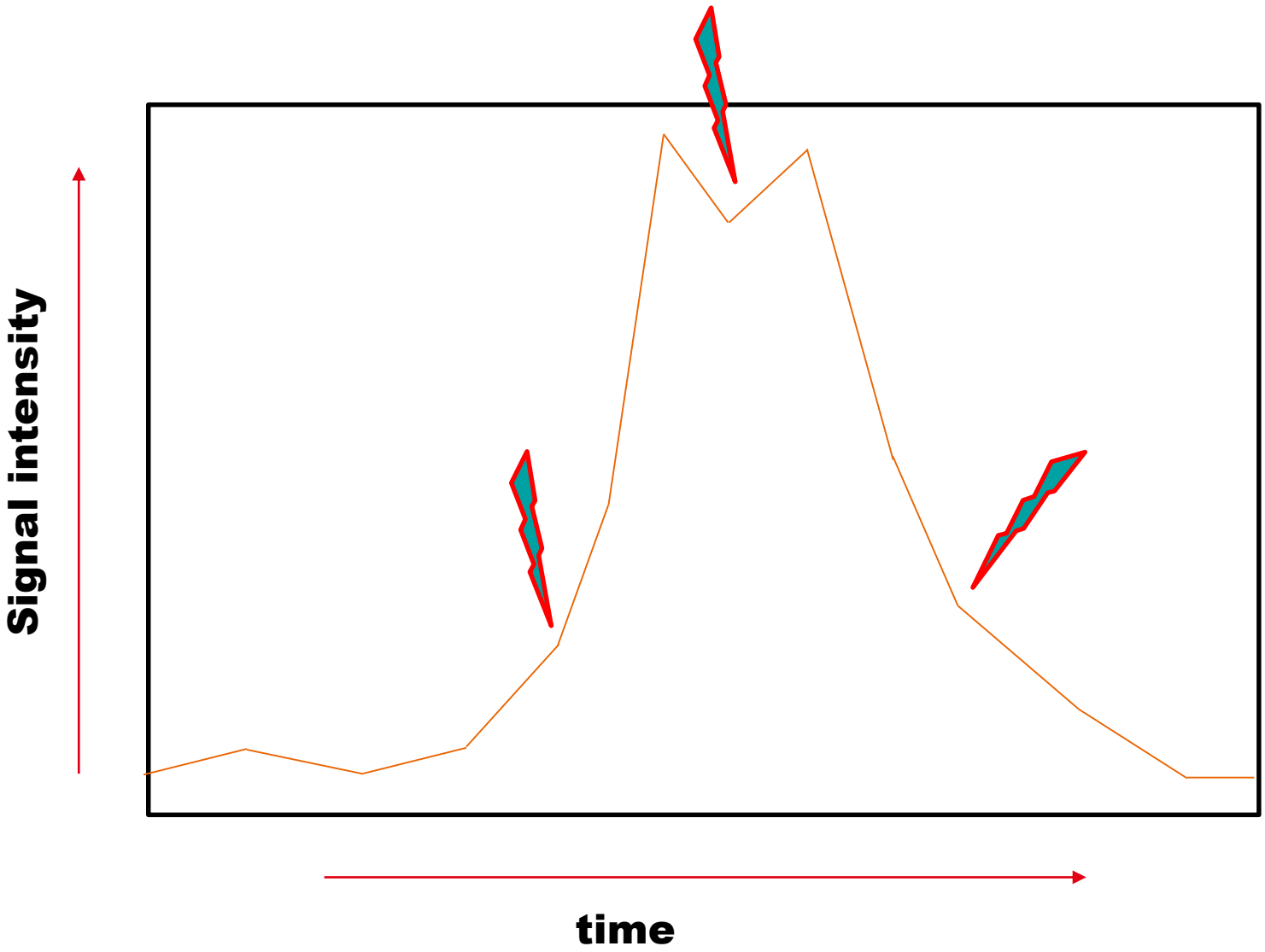
Data dependent acquisition (DDA)



Point of triggering



How to trigger

- **Monitoring a MS/MS by tandem quadrupole**

- **Monitoring sufficiently well revolved HRMS scans**

Everything else produces too many false triggering events

Problems related to DDA

- **Complex set-up**
- **Poor spectra quality (single scan)**
- **Thresholds (un-targeted)**
- **No guarantee that every compound of interest will be triggered**
  (how to validate limits of detection ?)

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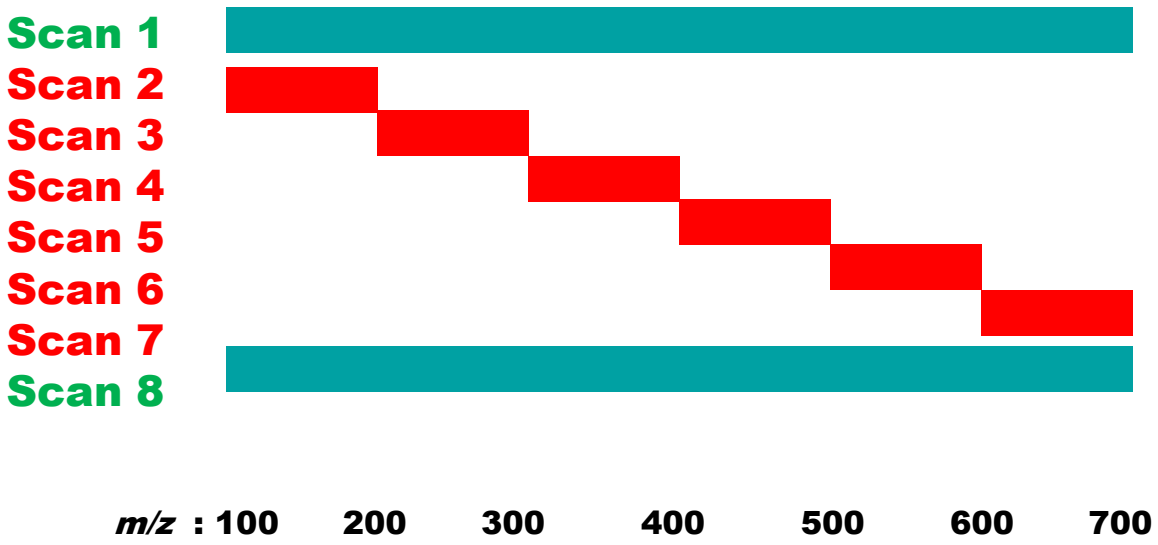
Data independent acquisition (DIA)

- **Monitoring different MS experiments**

Monitoring low and high collision energy traces (all ion fragmentation)

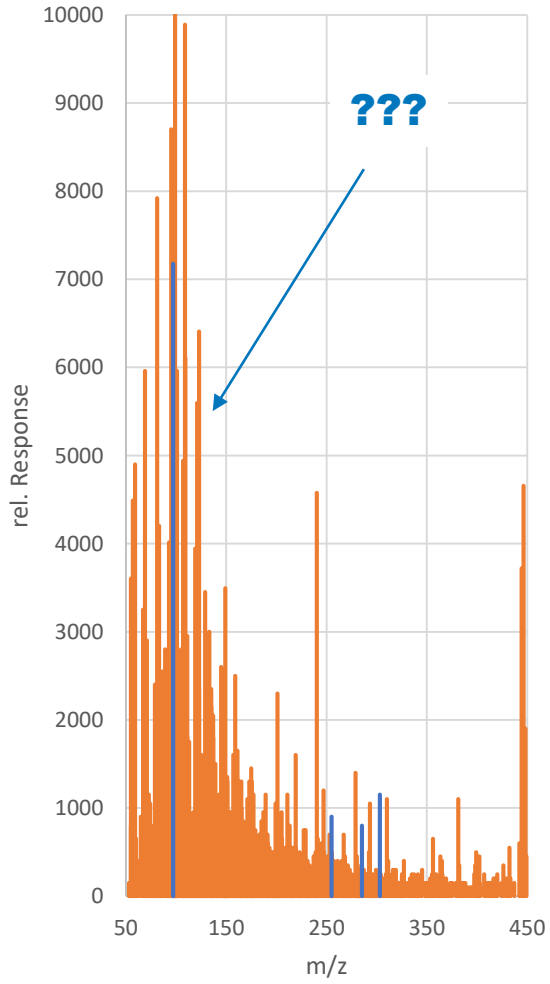
Sequentially isolating mass ranges which afterwards undergo fragmentation (SWATH)

SWATH

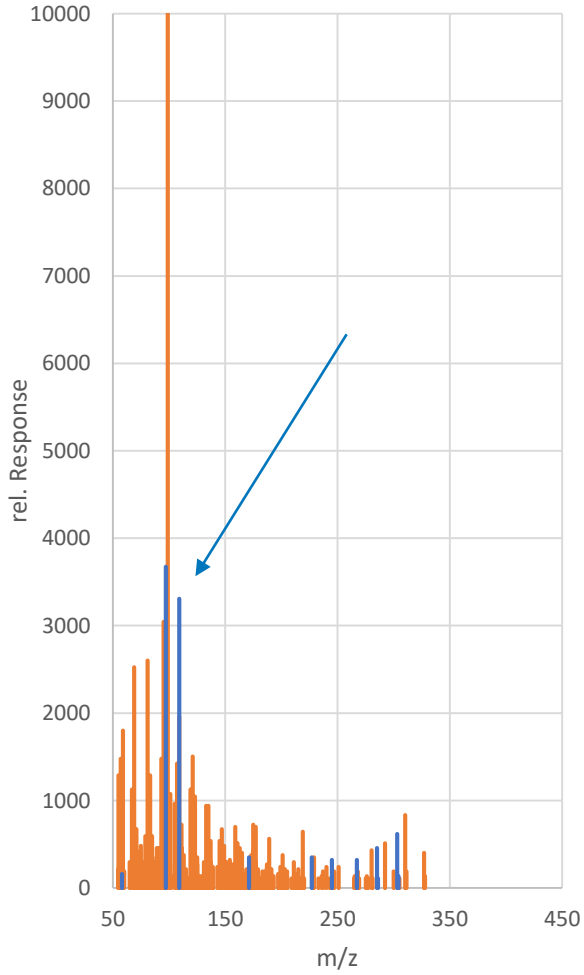

Scan 1
Scan 2
Scan 3
Scan 4
Scan 5
Scan 6
Scan 7
Scan 8
m/z : 100
200
300
400
500
600
700

5 µg/kg methyltestosterone in bovine liver

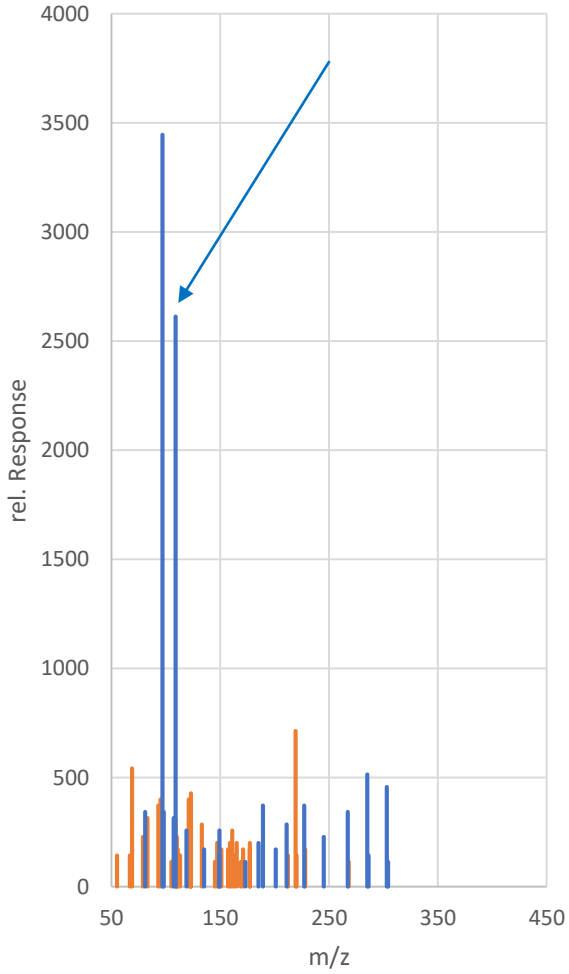
(AIF)



(SWATH)

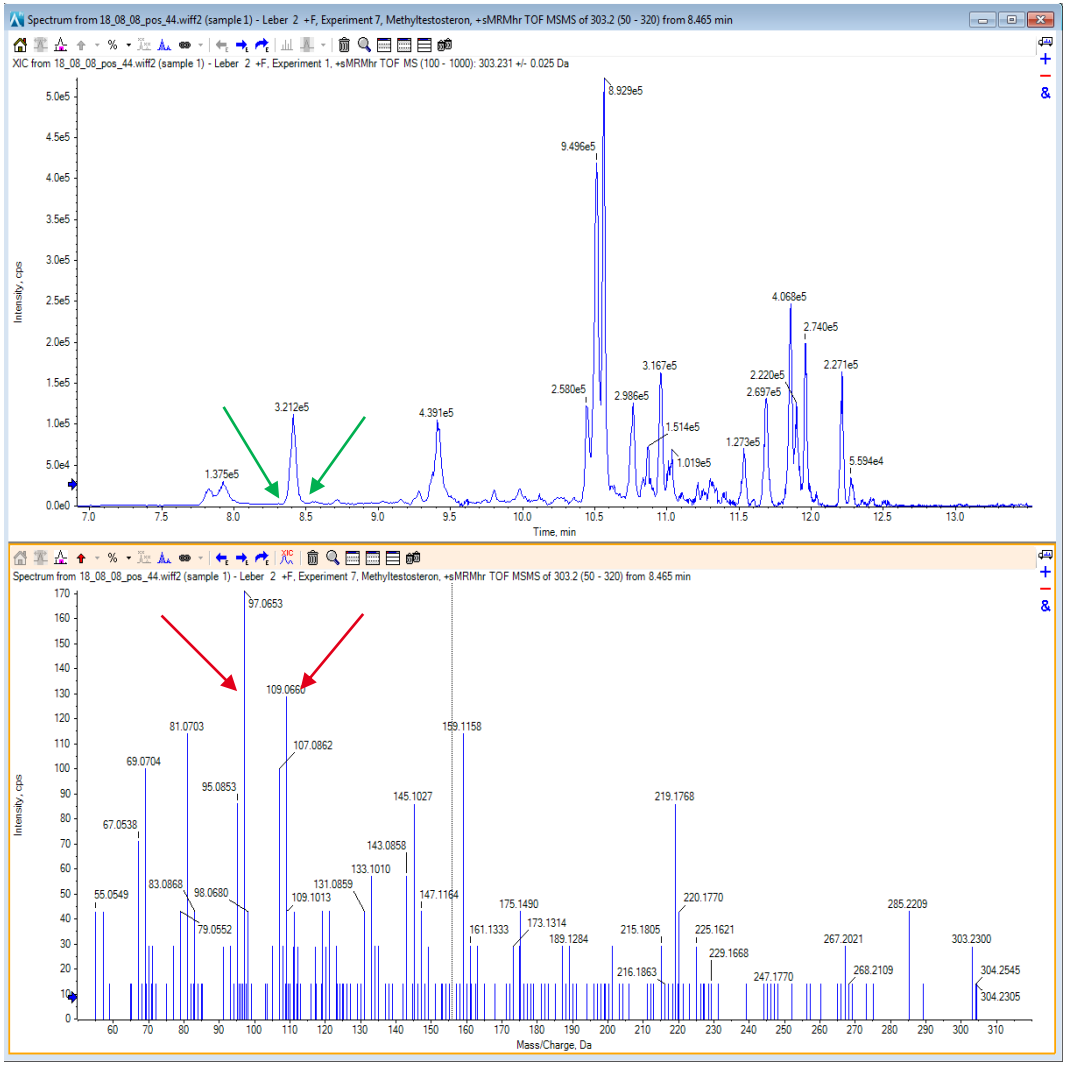


(PRM)



DDA versus DIA

Methlytestosterone in liver extract by DDA



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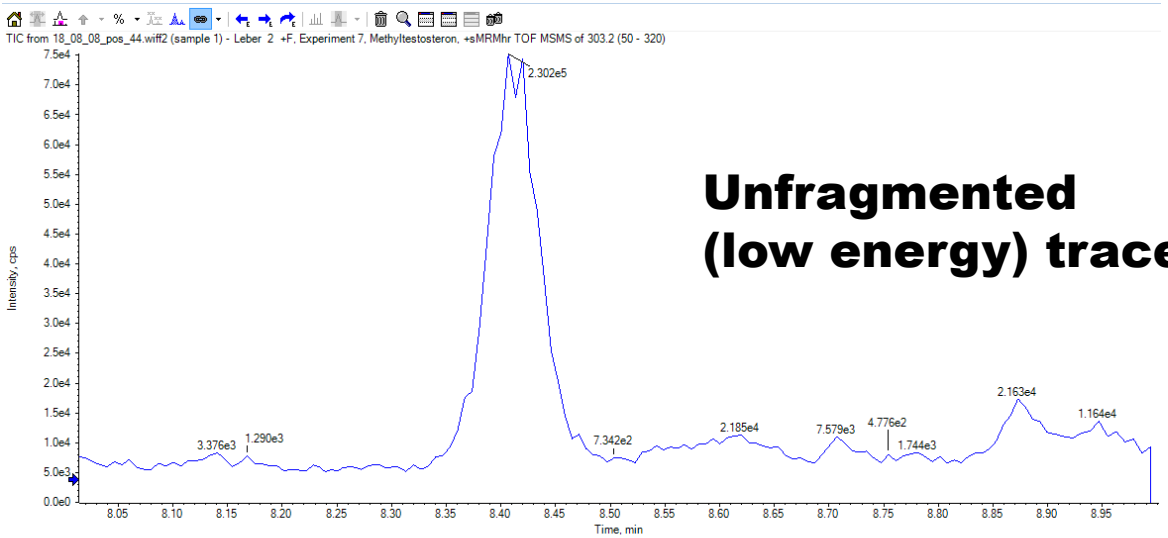
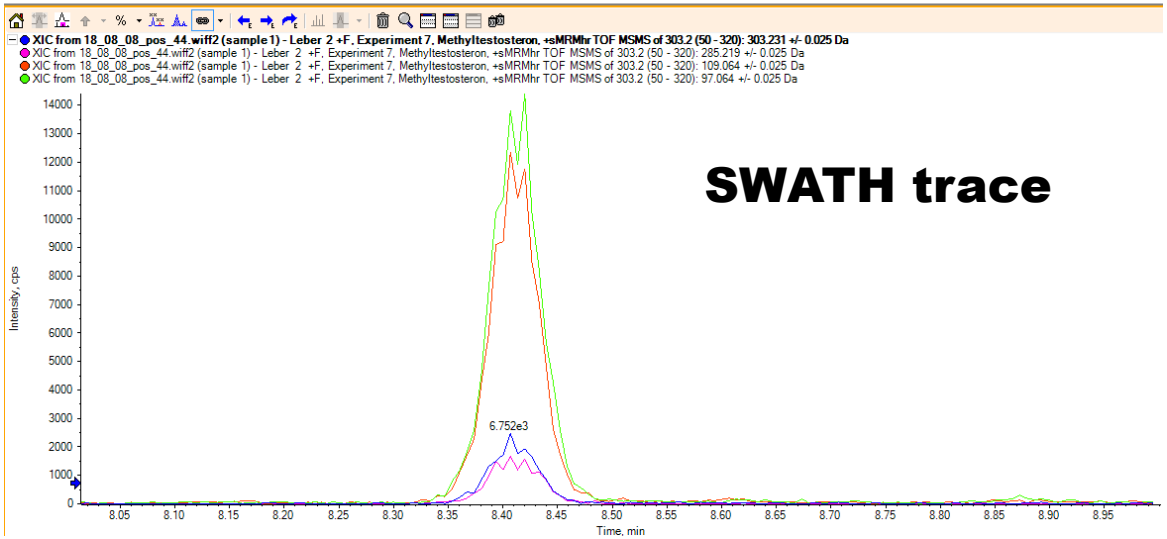


Unfragmented (low energy) trace



SWATH trace

Data independent acquisition is “harvesting” the whole raw data

- **No need to predefine masses of targeted analytes**

- **Any product ion of an analyte of interest can be extracted together with the unfragmented precursor ion**

- **We get a signal across a chromatographic peak and not just a single spectrum**

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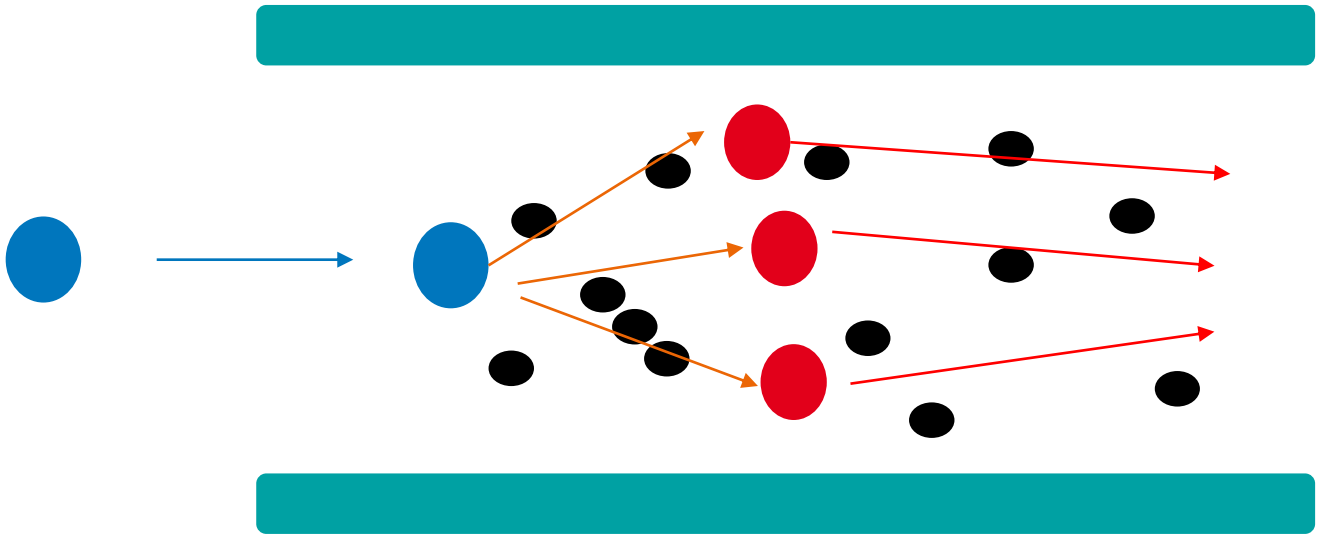
Data independent acquisition is superior because:

- **Narrow mass windows can improve fragmentation efficiency**

- **Narrow mass windows reduce isobaric interferences among product ions**

- **Narrow mass windows improve sensitivity of "automatic gain control" instruments**

Why is All Ion fragmentation problematic?



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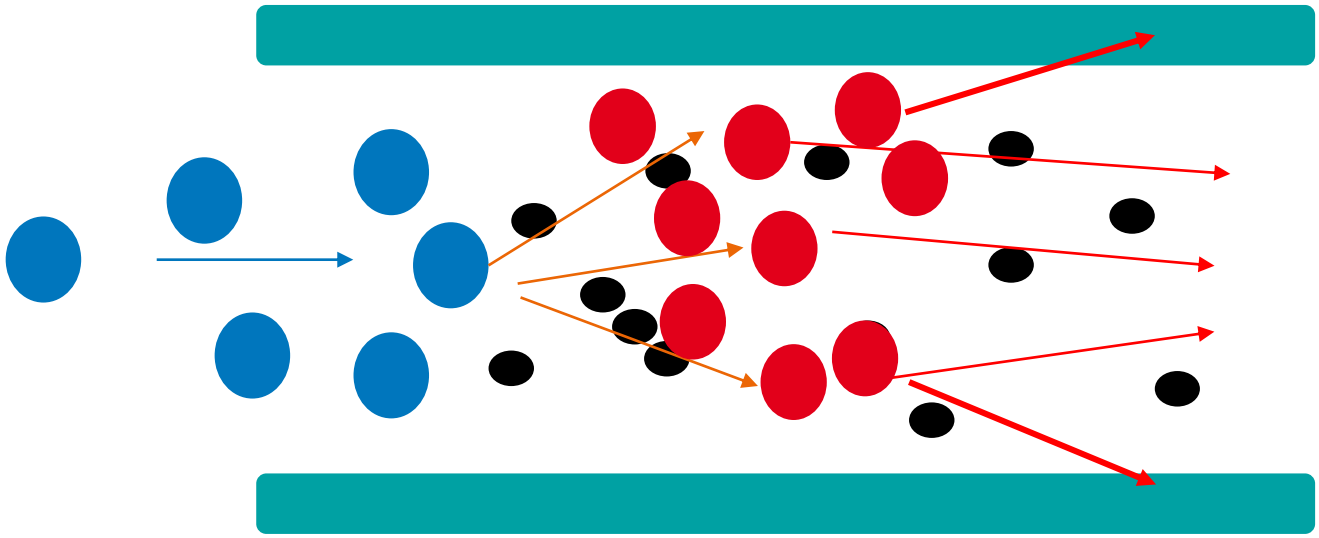
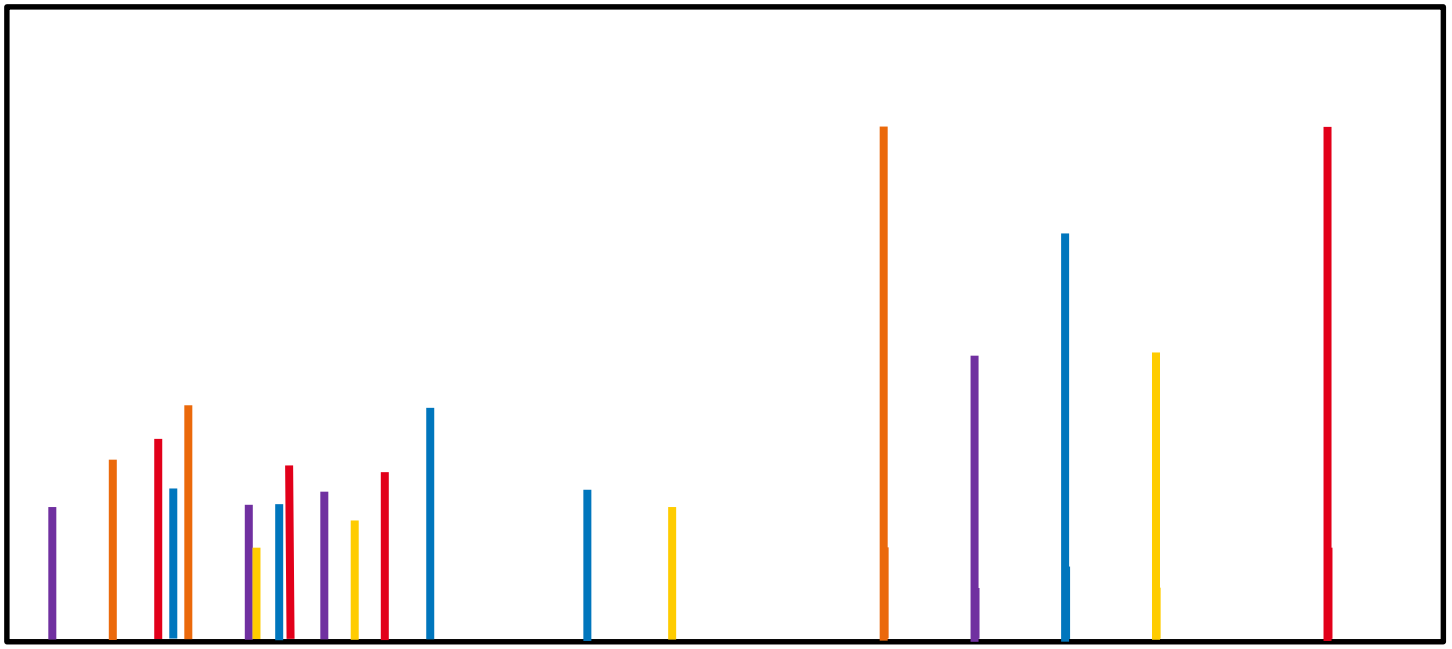
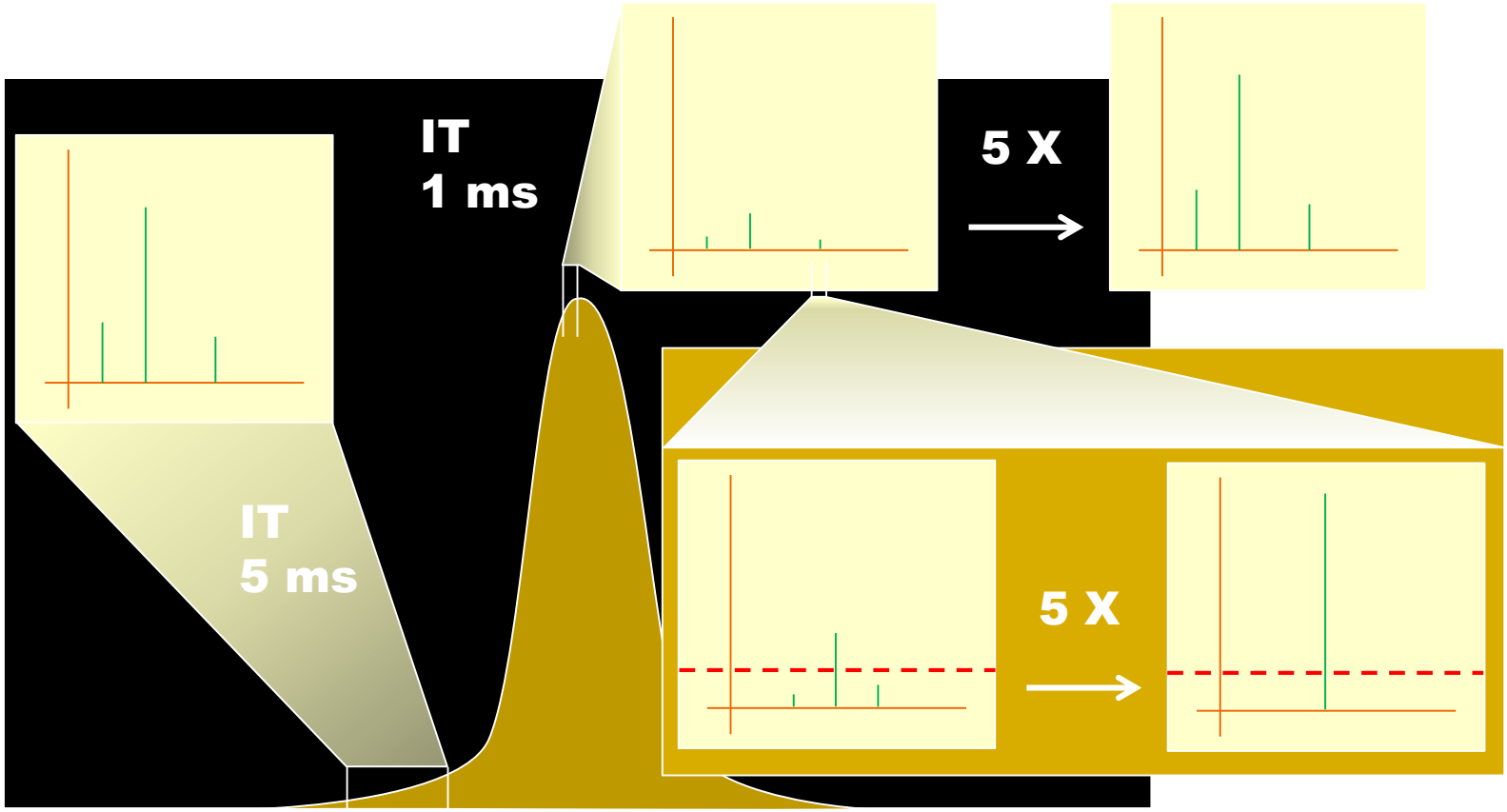


“Squeezing” of product ions



Isobaric interferance is more likely to happen at the low mass end

Automatic gain control issues



Multiplying “0” by an attenuation factor produces still “0”

Data independent acquisition is superior but:

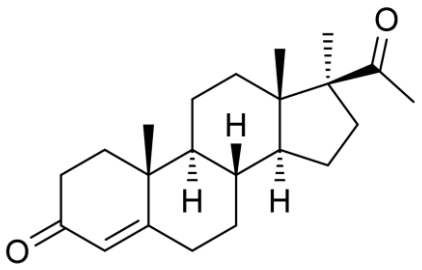
- **Sensitivity may be an issue**

- **Narrow mass windows require fast MS instrumentation**

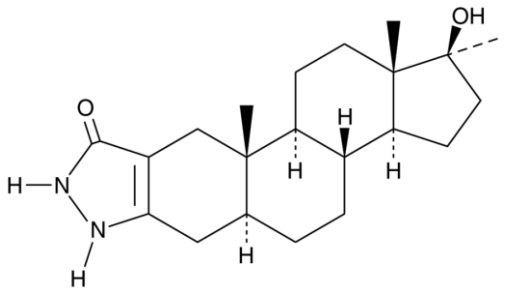
- **There is no universal fragmentation energy**

Some compounds produce “dust” instead of fragments

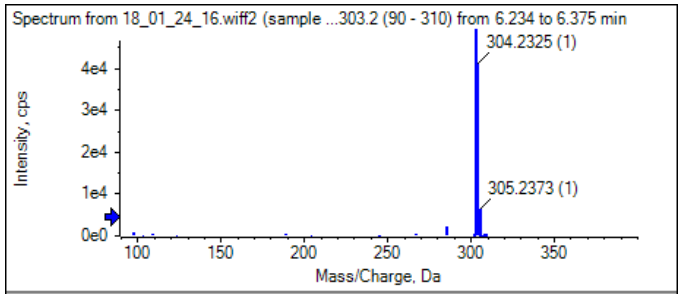
Methylprogesterone

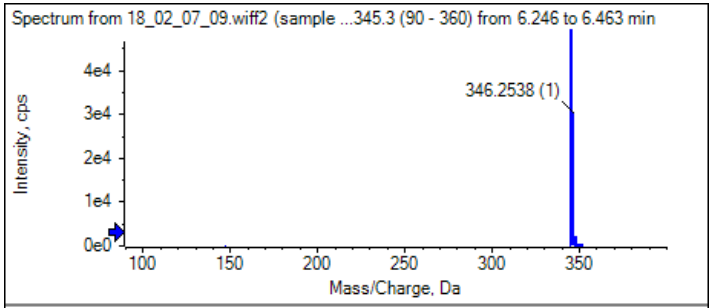


Hydroxy Stanozolol

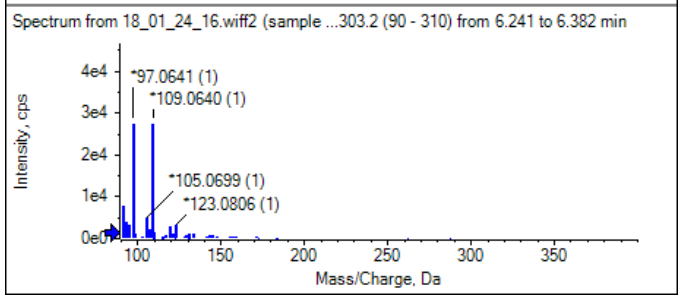


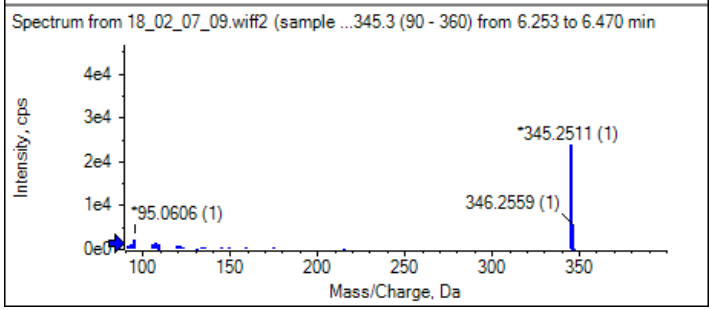
10 eV





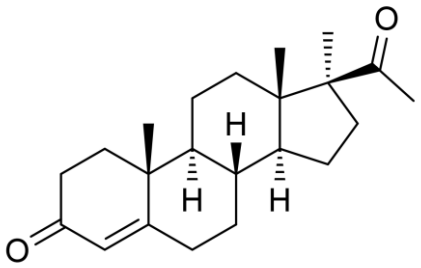
50 eV



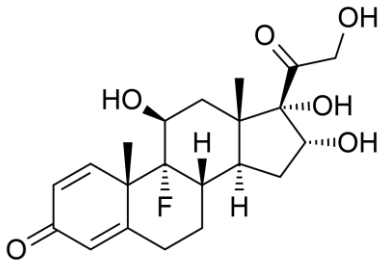


Efficient fragmentation but different fragmentation energy

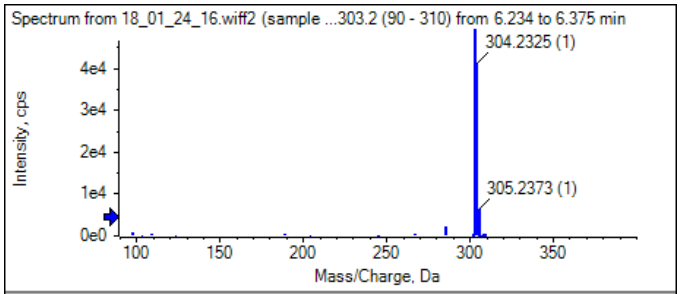
Methylprogesterone

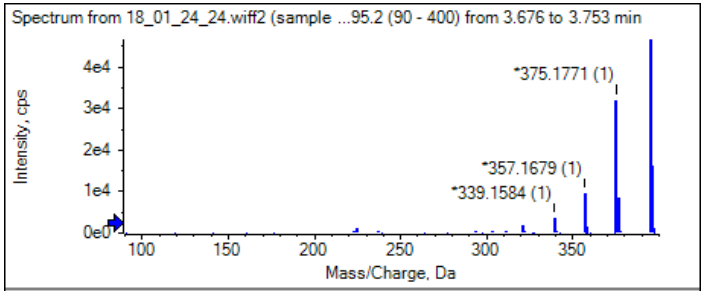


Triamcinolone

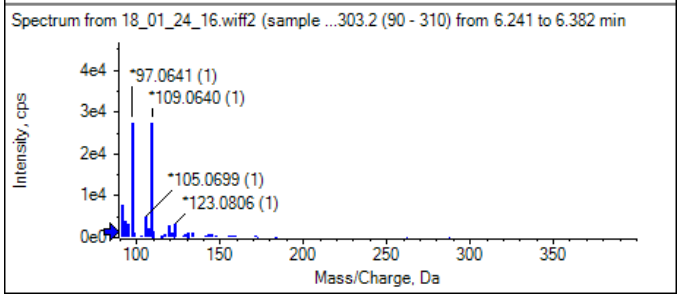


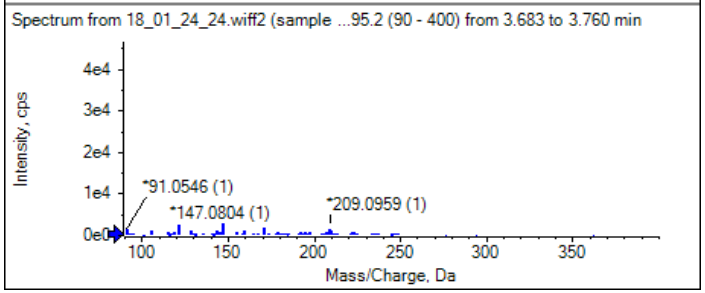
10 eV





50 eV



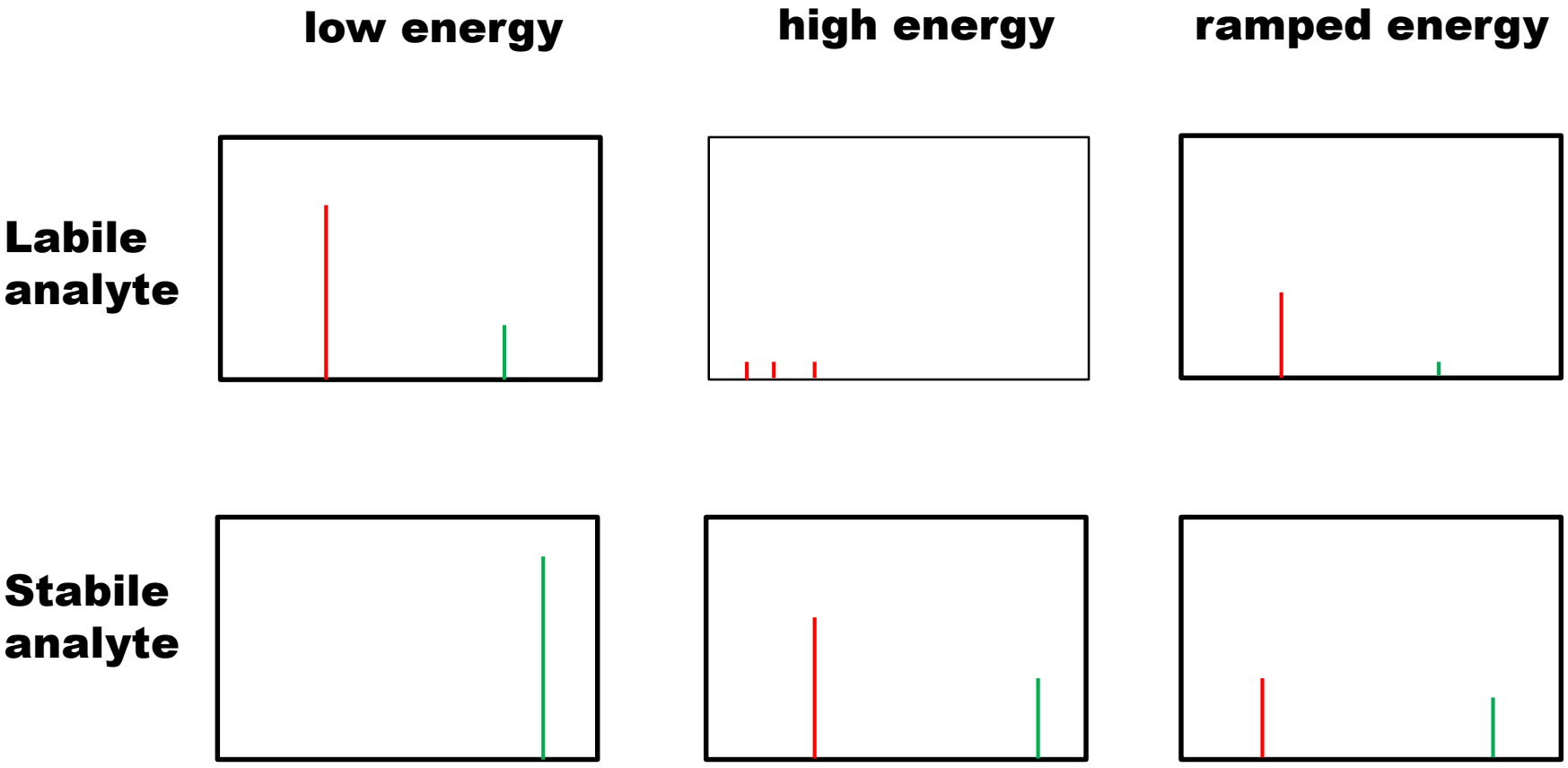


Ramping of collision energy

- **Produces useful fragments from labile and stable precursor ions**

- **The price to be paid: Some reduction in sensitivity (a single scan consist of low and high energy sub scans)**

Ramping of collision energy



Conclusion 1

- **Orthogonal detection selectivity still requires the use of MS/MS**

- **HRMS based MS/MS is clearly more selective, but slightly less sensitive than tandem quadrupole based instrumentation**

- **Data independent (DIA) is replacing DDA**

Conclusion 2

- **Fast acquisition speed is essential in order to:**

permit as many experiments as possible

(narrow SWATH windows
and
maybe even multiple collision energies)