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Evaluation of MS Scanning Speeds with UHPLC Peak Widths

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Introduction

The trend toward high-speed LC analysis with reduced column particle sizes continues to increase in popularity. The narrow peak widths of high-speed LC have generally been handled adequately with UV detectors by increasing data acquisition rates. However, use of a MS will reduce the risk of misidentifying peaks when transferring methods from conventional to high-speed LC. Most MS instruments have had difficulty keeping up with these sharp peaks, which may have peak widths of 200 milliseconds or less. Accurate mass analysis of sharp chromatographic peaks obtained by high-speed LC requires ultra-fast MS detection capabilities. A number of high-speed separations will be evaluated using MS scanning speeds of up to 15,000 u/sec with polarity switching speeds of 15 msec.

Mass Spectrometer Design

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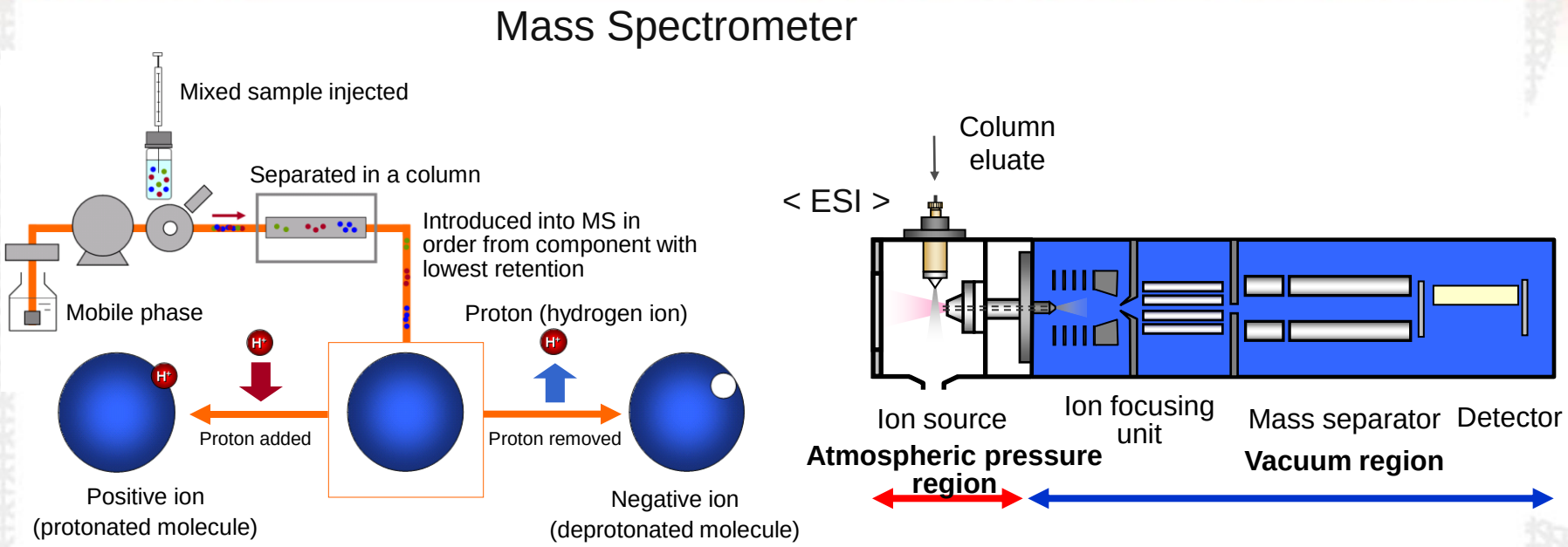

- High scanning speeds needed for sharp peaks
- Fast polarity switching time required for sharp peaks

Influence of Sampling Rates

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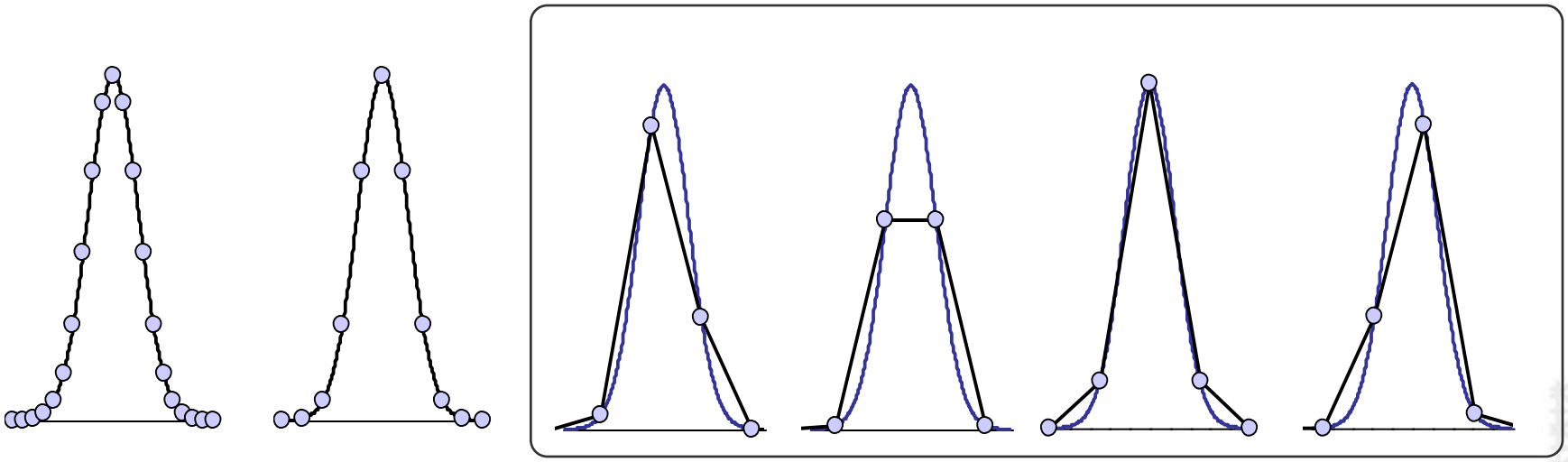
A higher sampling rate is required for detection of the narrow chromatographic peaks of UHPLC. The lowest sampling rate should be 10 points/peak.



20 points/peak

10 points/peak

5 points/peak

Loss of the apex brings unreliable results, such as a peak height becomes too low or a peak width becomes too broad.

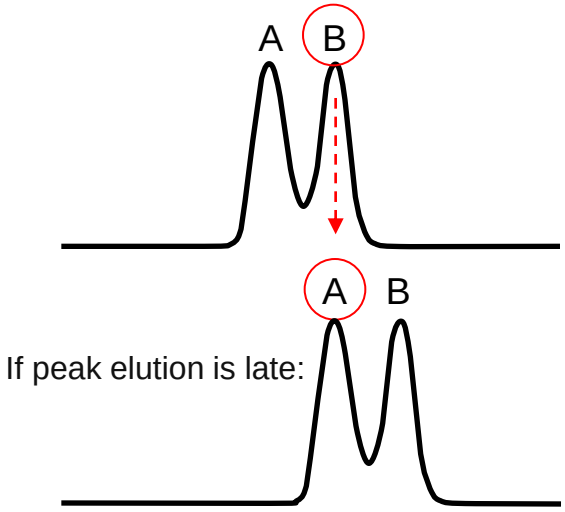
UHPLC/MS requires a higher sampling rate, i.e. both a faster scan speed and a faster polarity switching speed. If the number of data points decreases, the sensitivity also decreases. This adversely affects reproducibility.

Risks Associated with LC Detectors

Mobile phase preparation errors

Fluctuations in peak retention times

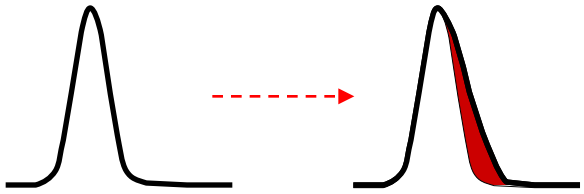
Peak misidentification



If an impurity coincides with the target component:

Changes in area value

Incorrect quantitation



Impurity

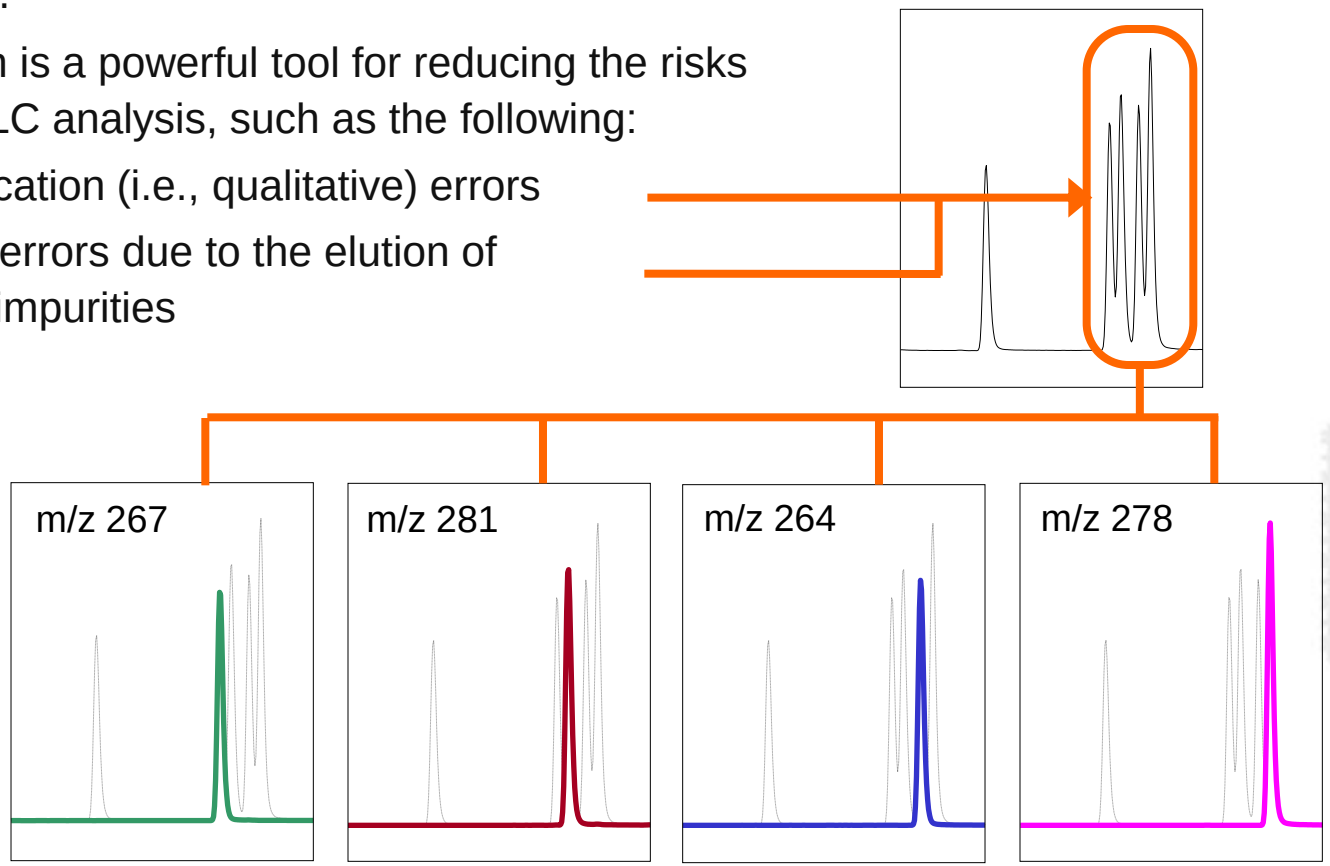
Merits of MS Detectors

The greatest merit in using an MS instrument as an LC detector:

In addition to retention times, mass information for each peak can be obtained simply at the same time.

Mass information is a powerful tool for reducing the risks associated with LC analysis, such as the following:

- Peak identification (i.e., qualitative) errors
- Quantitative errors due to the elution of unpredicted impurities



The peaks (including those that cannot be separated by time) can be separated using mass information.

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This reduces the risk of qualitative and quantitative errors.

UHPLC-MS

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Mass spectrometer requirements for UHPLC:

What makes an MS instrument suitable for UHPLC?
The ability to acquire data at high speed without sacrificing data quality.

The three things that enable ultrafast analysis:

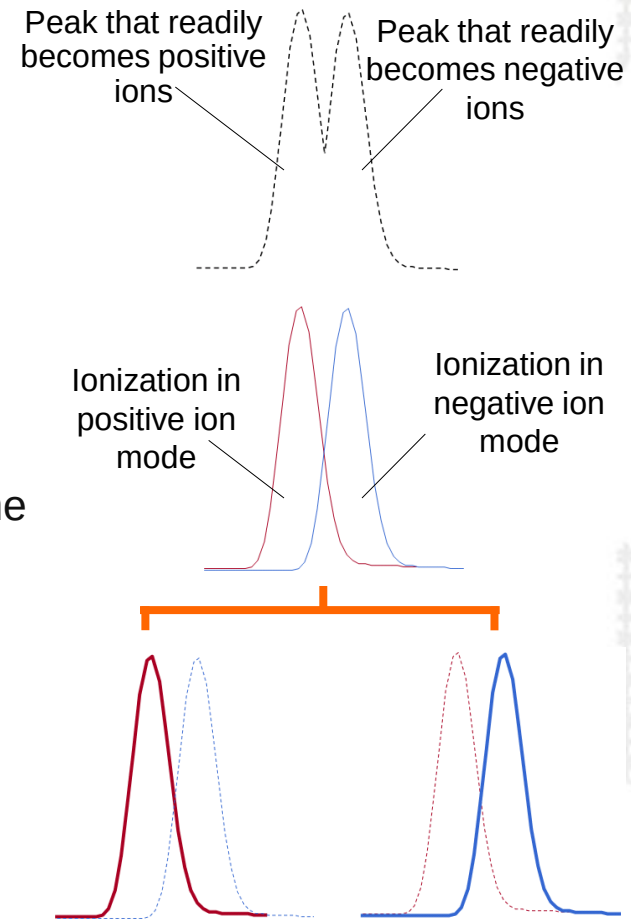
- **The ability to perform scan measurement at high speed** **15,000 u/sec**
- **The ability to switch between positive and negative ion measurement at high speed** **15 msec**
- **High sensitivity in high-speed measurement**

15,000 Da/second = 15 Da/millisecond = 1 Da/67microseconds

LC-MS: Positive/Negative Ion Measurement

- Simultaneous Measurement of Positive and Negative Ions

  - The ease with which positive/negative ions are created depends greatly on the compound characteristics.
  - With positive/negative polarity switching, both positive and negative ions are measured at the same time.
  - In simultaneous measurement, the number of sampling points is important.



Chromatograms for both positive and negative ions obtained in a single analysis.

Scan Speed Effect on Peak Height & Width

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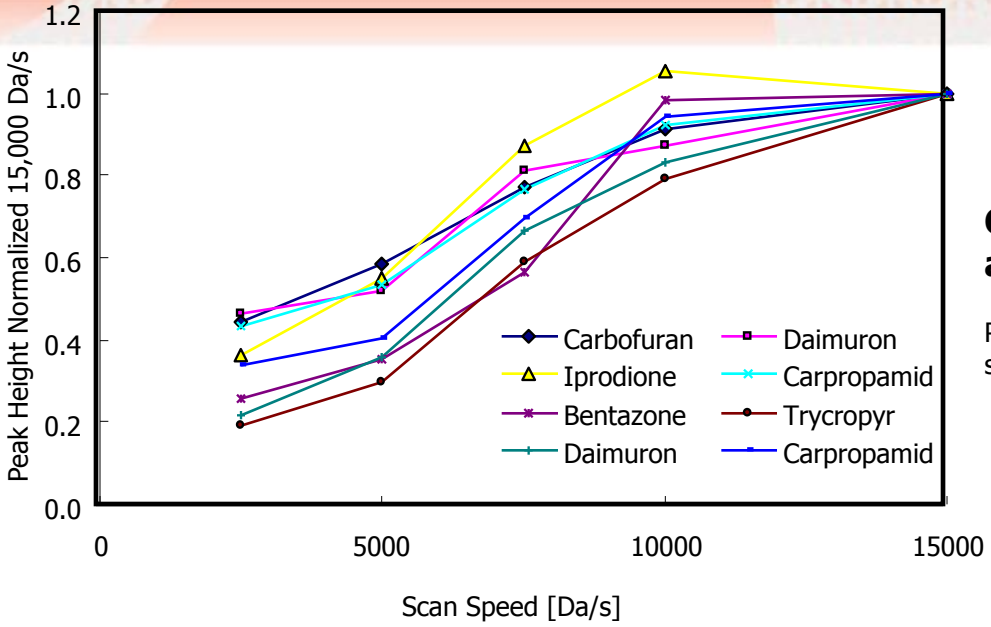
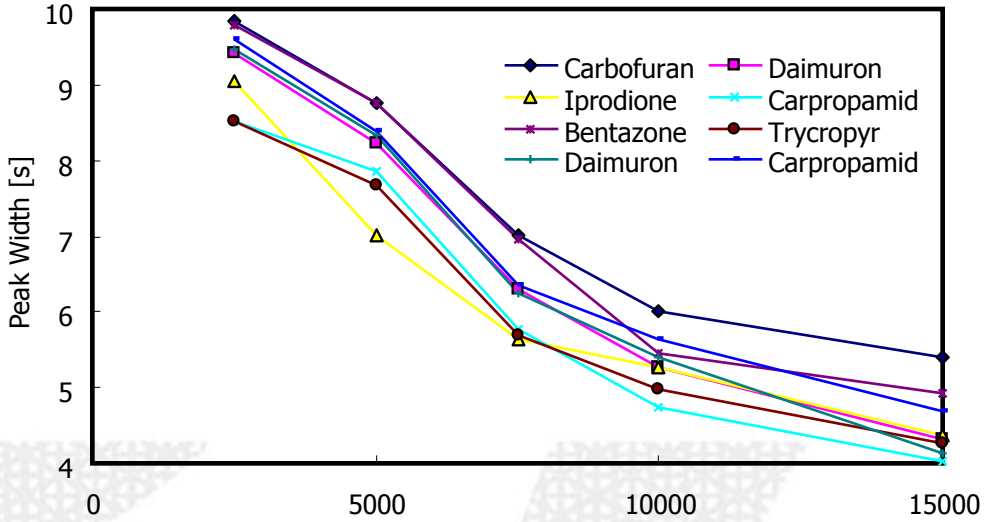


Correlation between Scan Speed and Peak Height

Peak height is lowered because of an insufficient sampling rate at a slow scan speed.



Correlation between Scan Speed and Peak Width

Peak width is broadened because of an insufficient sampling rate at a slow scan speed.

LC/MS: Scan Speed Problem

- Scan: Data is acquired in the desired m/z range.

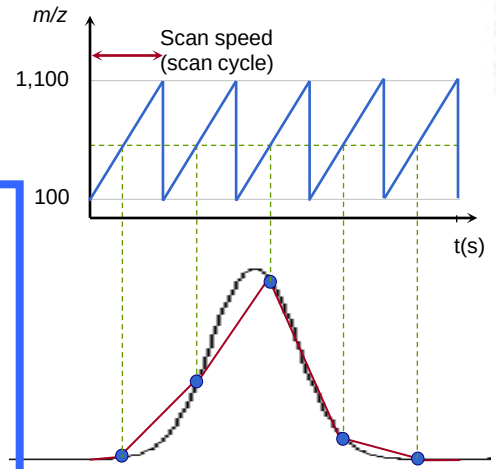
- Increase the scan speed.

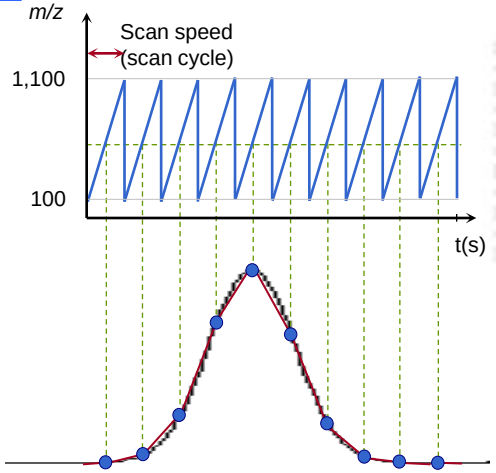
  With conventional instruments, the sensitivity decreases.

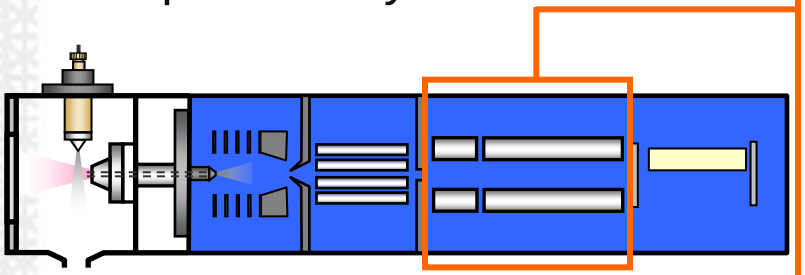
- Decrease the scan speed.

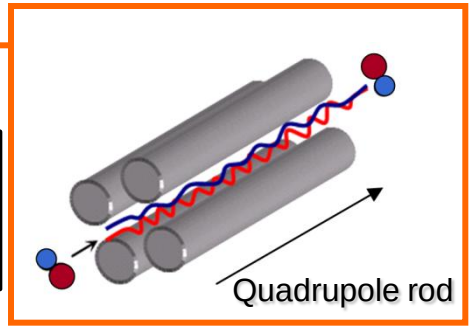
  It is difficult to handle high-speed analysis.

With new applied voltage technology, it is possible to maintain sensitivity when the scan speed is increased.









Ultrafast Scanning and Sensitivity

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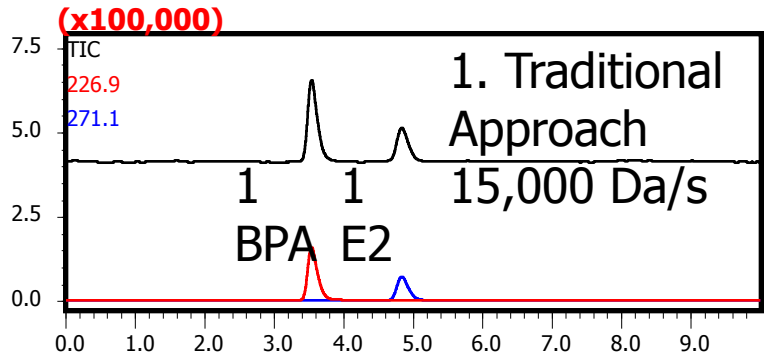

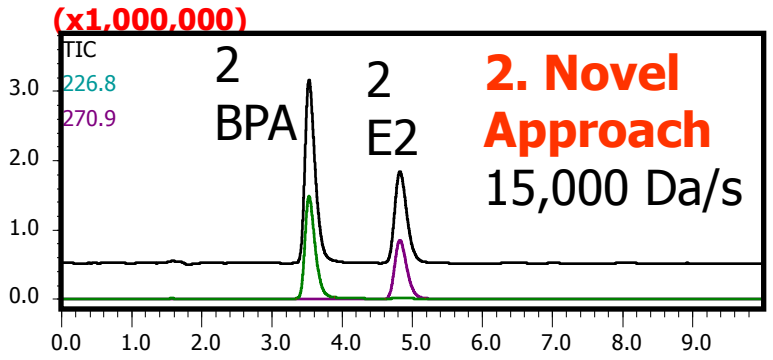

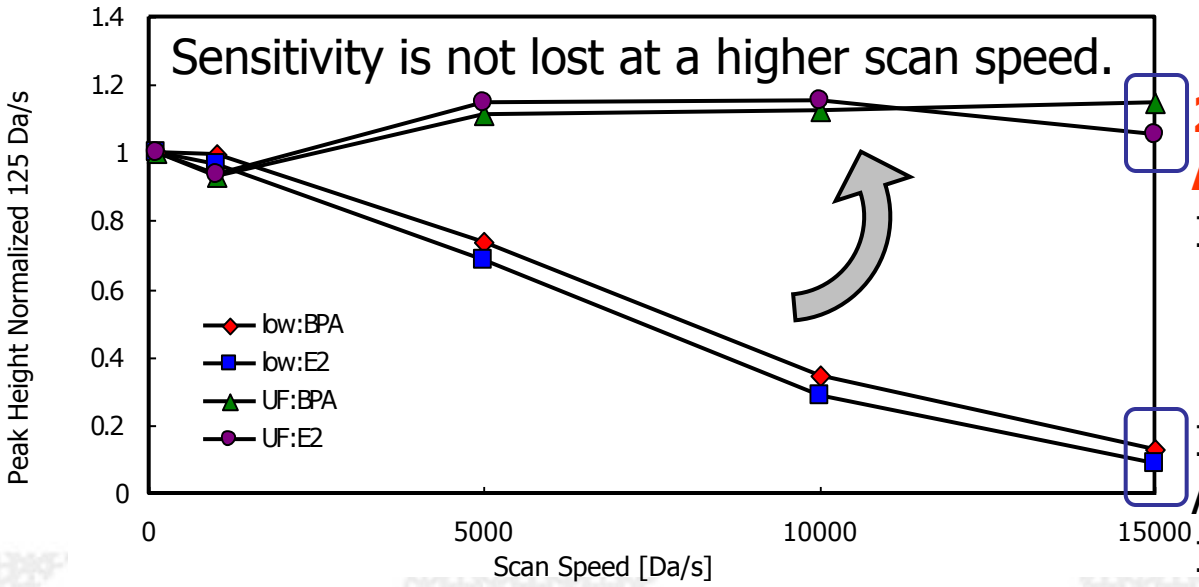

One Second Peak Data

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For a 500 mass range
At 15,000 mass units/second
Allowing for interscan delay (.005)

High Speed LCMS delivers…

Mass range	Scans/Second	Points/1 sec peak
500	26.08	26 data points/peak
200	54.55	54 data points/peak
100	85.71	85 data points/peak

Tested Instrument specifications:
LC/MS LC/MS/MS
Scan Speed: 15,000 u/sec
Polarity Switching time: 15 msec
LC/MS/MS
MRM: 500/sec
Dwell time: 1 msec
Pause time: 1 msec

Comparison of Scan Speeds

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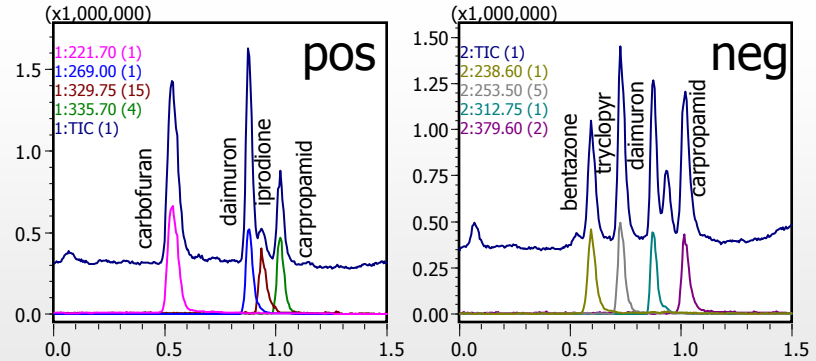
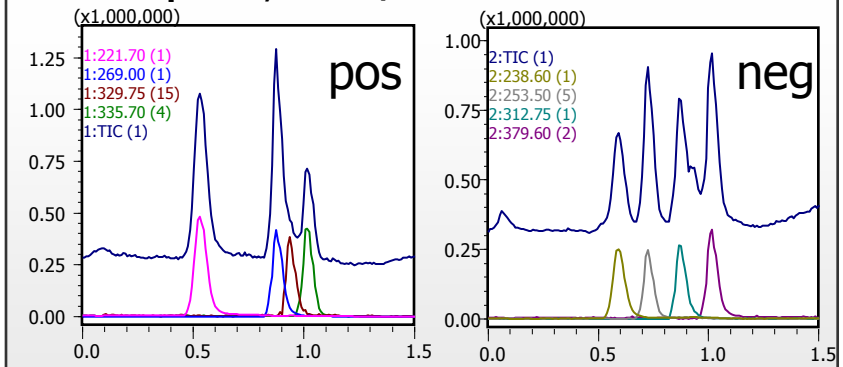
Polarity Switching Polarity Switching

Positive Ion Scan → Negative Ion Scan → Positive Ion Scan ⋯→

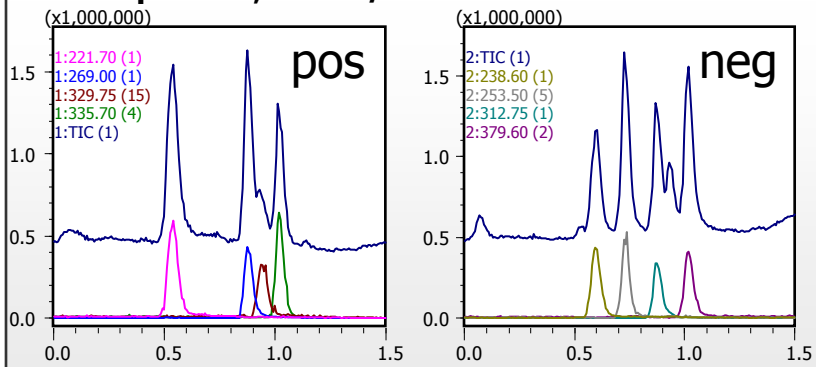
Scan Speed 15,000 Da/s



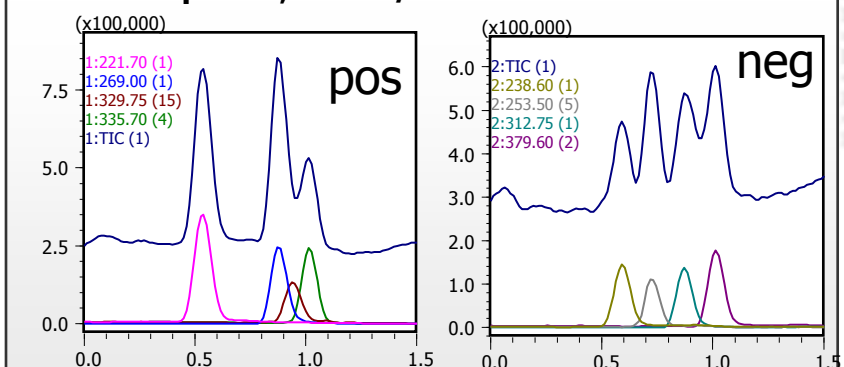
Scan Speed 7,500 Da/s



Scan Speed 10,000 Da/s



Scan Speed 5,000 Da/s



UHPLC/MS/MS Data

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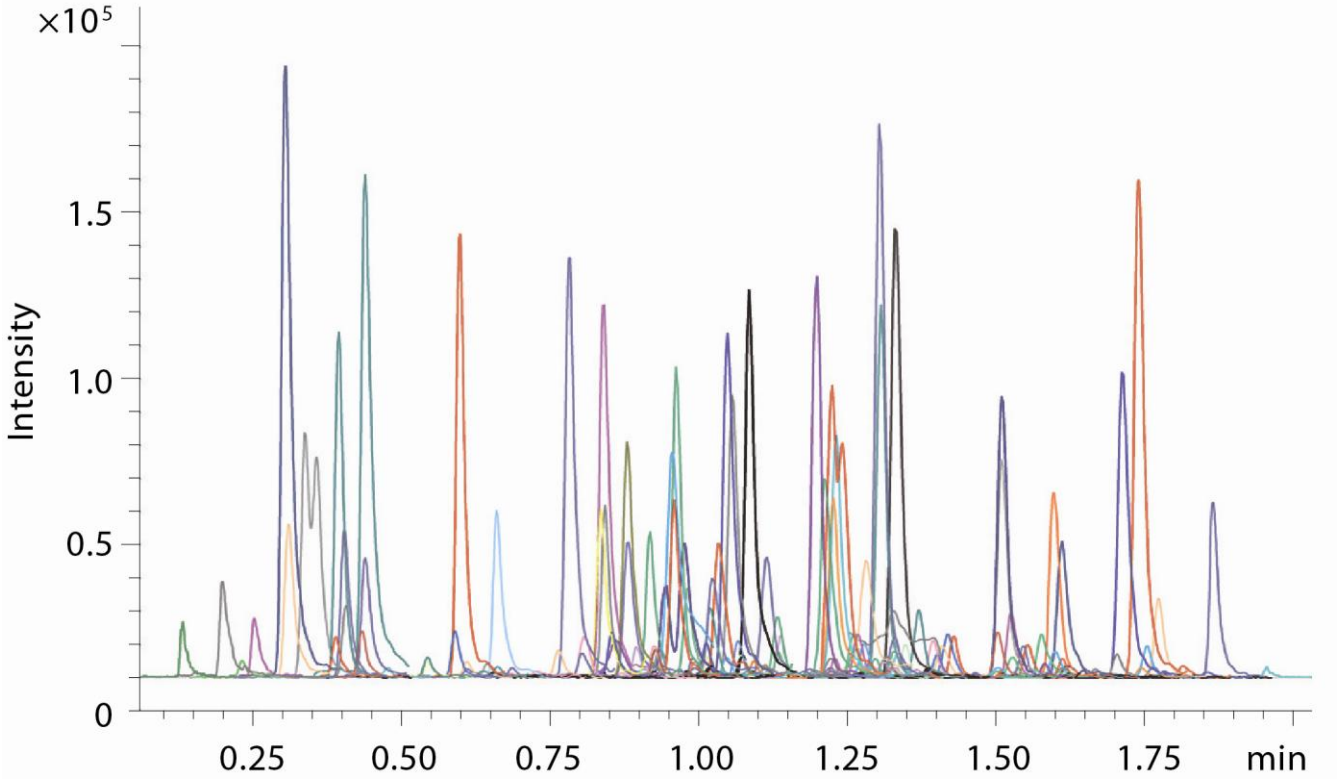
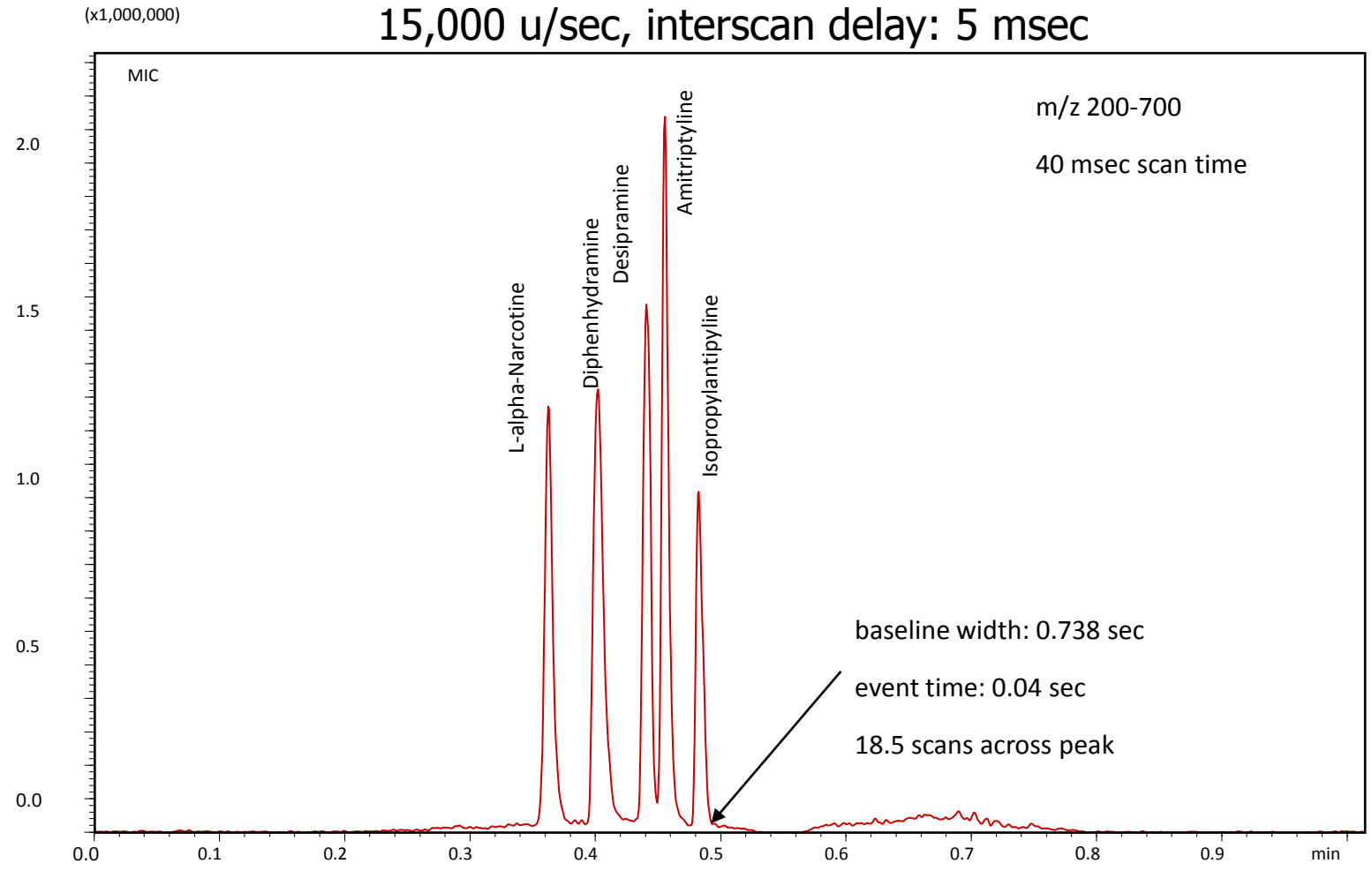
Standard Chromatogram of 226 Pesticides in Two Minutes



Ultrafast polarity switching (15 msec), a high-speed scanning rate of 15,000 u/sec, and Ultrafast MRM transitions allow full spectrum scans within a series of MRM measurements, providing confirmation of target compounds with information-rich product ion spectra.

High Speed LC/MS Scanning



UHPLC with High Speed MS

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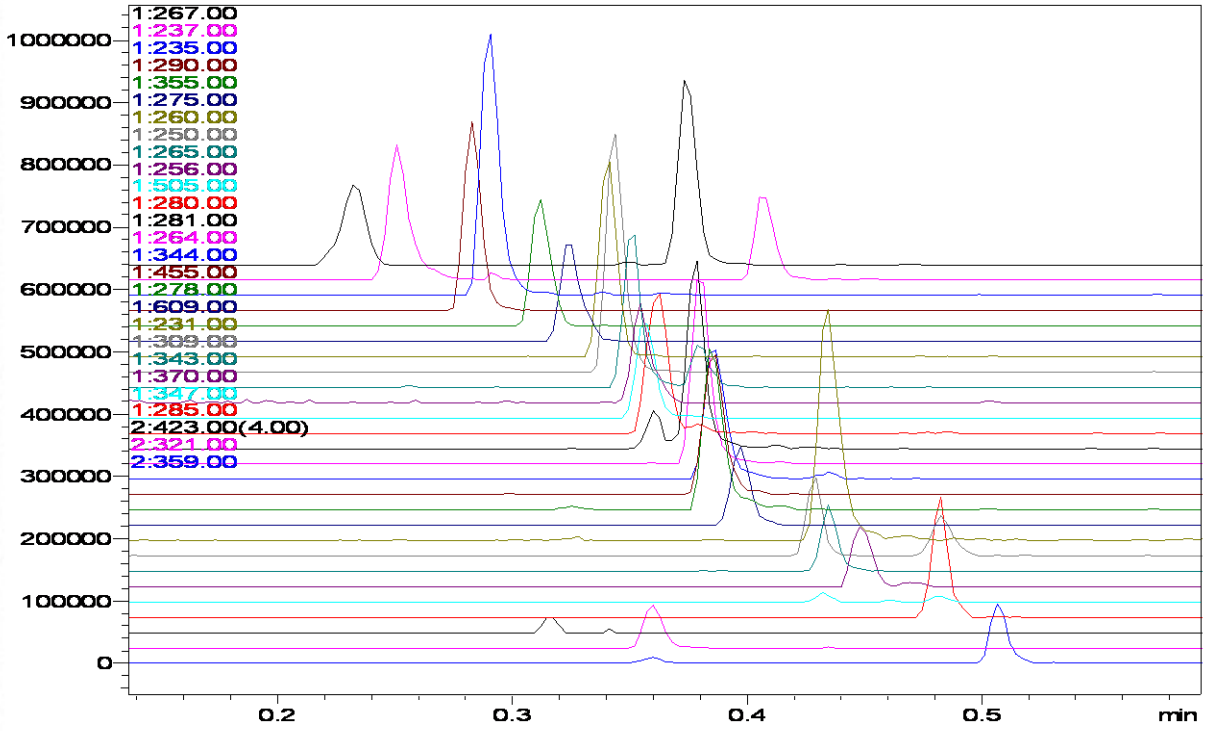
Analysis of 30 pharmaceuticals by high-speed polarity switching (15 msec) and high-speed scanning technology (15,000 u/sec)



Column	: C18 2.1mm, x 50mm, 1.8um
Mobile phase	: A : 0.1 % formic acid in water / B : **0.1 % formic acid in** acetonitrile
Gradient	: B 3% → 95 % (0.50 min)
Flow rate	: 1.8mL/min
Column temp.	: 50C
Detection	: ESI (+/-) Scan speed : 15000 u/sec

Positive

1. Atenolol (267)
2. Procaine (237)
3. Lidocaine (237)
4. Atropine (290)
5. Yohimbine (355)
6. Chlorpheniramine (275)
7. Propranolol (260)
8. Alprenolol (250)
9. Tetracaine (265)
10. Diphenhydramine (256)
11. Doxepin (280)
12. Dipyridamol (505)
13. Desipramine (267)
14. Imipramine (281)
15. Nortriptyline (264)
16. Amitriptyline (278)
17. Dibucaine (344)
18. Verapamil (455)
19. Reserpine (609)
20. Carbamazepine (237)
21. Isopropylantipyrine (231)
22. Alprazolam (309)
23. Trizolam (343)
24. Cilostazol (370)
25. Nifedipine (347)
26. Diazepam (285)
27. Warfarin (309)

Negative

1. Cefuroxime (423)
2. Chloramphenicol (321)
3. Nitrendipine (359)

Conclusions

Advantages of ultra high-speed MS detection:

- Ultrafast scanning and switching allows identification of unsuspected compounds in UHPLC analyses.
- Ultrafast scanning provides increased sensitivity and reduced peak widths during high-speed analysis.
- Resolution of compounds with identical molecular weights can be improved with UHPLC and MS with high-speed scanning.
- Reproducibility results and quantitative accuracy are improved with high-speed scanning.