Presented at the Eurachem 2019 workshop, 20.05.2019
"Validation of targeted and non-targeted methods of analysis"

TFTAK CENTER OF FOOD AND FERMENTATION TECHNOLOGIES

1989-2019 Eurachem 30
A focus for analytical chemistry in Europe

Detection of a multitude of (unknown) components in complex samples
Criteria for identification

Georg Arju

TFTAK, TalTech

Chromatography Vs MS

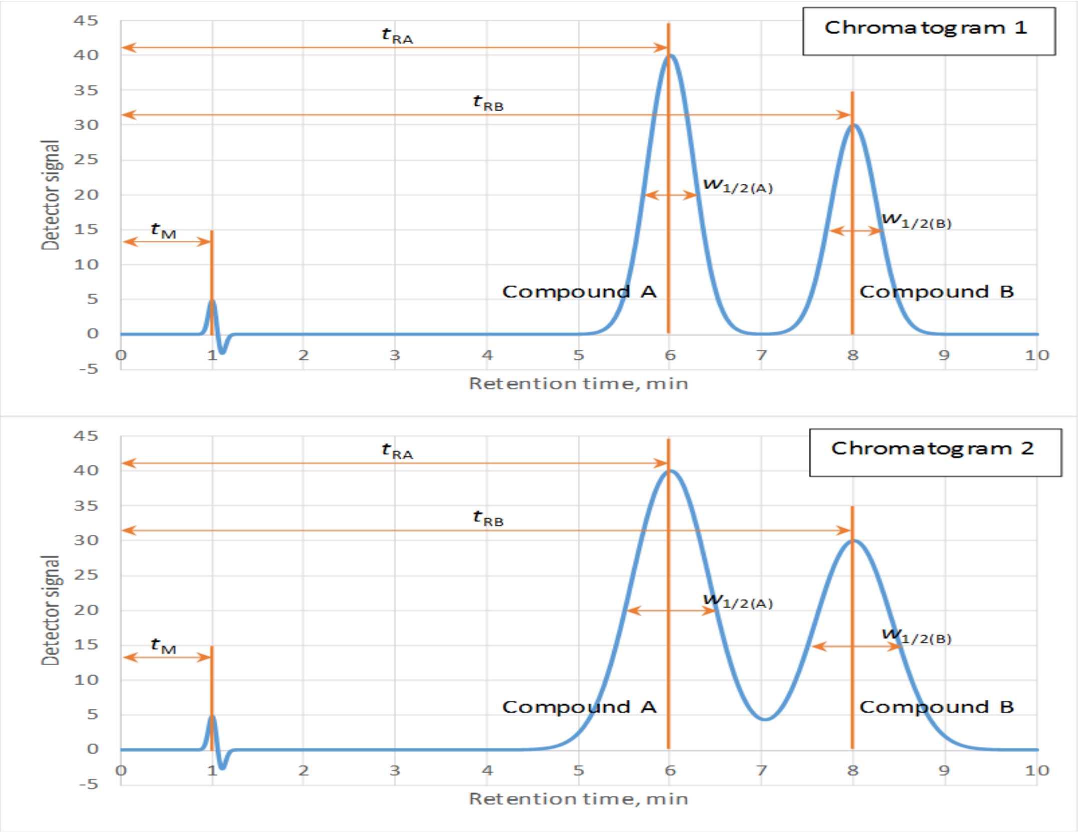
Schools of thought:

1. LC/GC is only an inlet for my mass spectrometer
2. MS is only a detector for my chromatography
3. Efficient use and understanding of both LC/GC and MS enhances overall performance of the system

Throughput Dilemma

What do we want to achieve:

- Throughput
- Selectivity
- Balance



https://sisu.ut.ee/sites/default/files/lcms_method_validation/files/image002_6.png

Throughput

Becomes an issue for large sample pool studies

Increase in throughput results in:

Use of shorter columns	Reduction of selectivity
Use of shorter gradients	Reduction of sensitivity
Use of higher flow rates	Reduction of reproducibility

Selectivity

Becomes an issue for complex samples

Increase in selectivity results in:

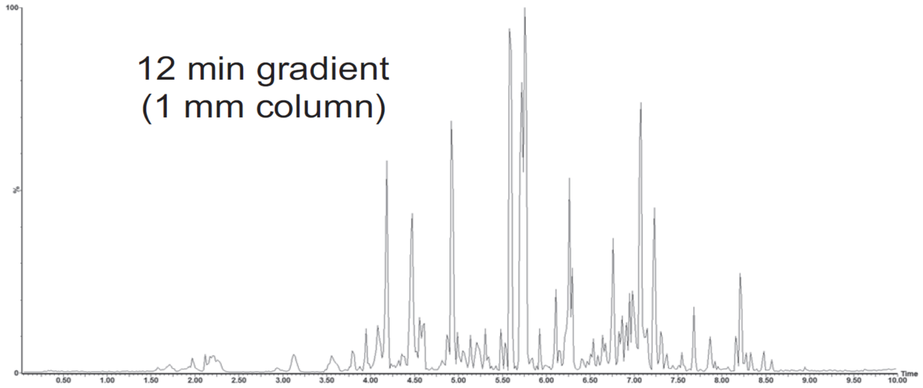
Use of longer columns	Increase of selectivity
Use of longer gradients	Increase of sensitivity
Use of lower flow rates	Increase of reproducibility

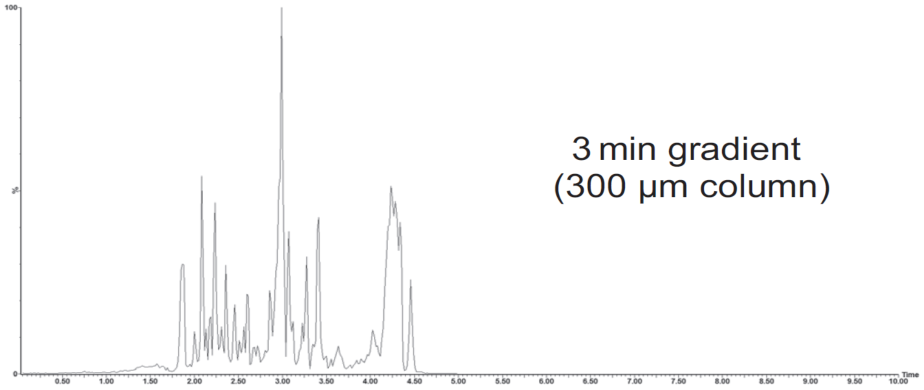
Balance

Large sample pools of high complexity

Selectivity and Throughput

- Use of narrower columns: 2.1 > 1 > 0.3 mm
- Use of shorter columns: 150 > 100 > 50 mm
- Use of columns with >2 µm particle size
- Use of columns at near pressure limit
- Use of LC at near pressure limit





Lee et al, 2017. Milford

Importance of Instrumentation

Challenges of complex sample analysis:

1. Sample complexity
2. Narrow peaks
3. Reproducibility
4. General coelution
5. Isobaric coelution

Data acquisition strategies

Acquired data quality

Importance of Instrumentation

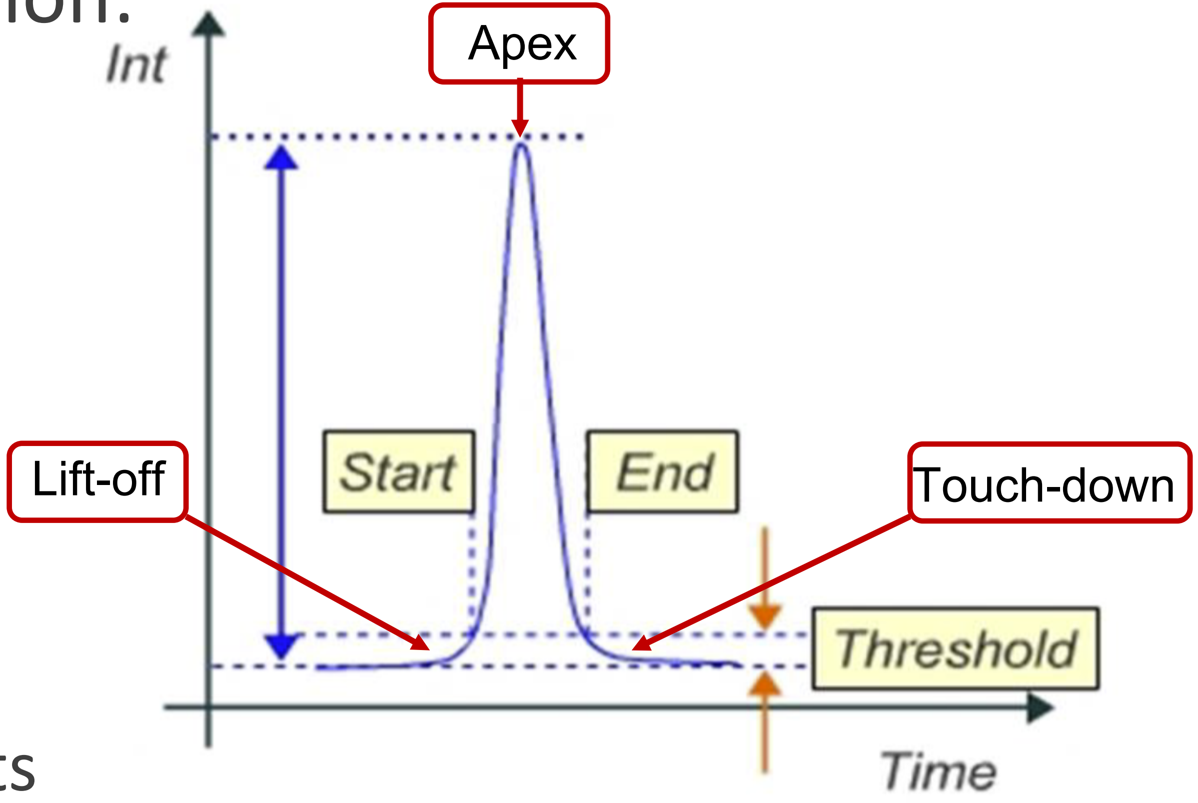
Peak detection and integration:

1. Lift-off

2. Apex

3. Touch-down

Qualitative	6+ data points
Quantitative	10+ data points



Chromacademy.com

<u>Data acquisition rate becomes highly important</u>

MS

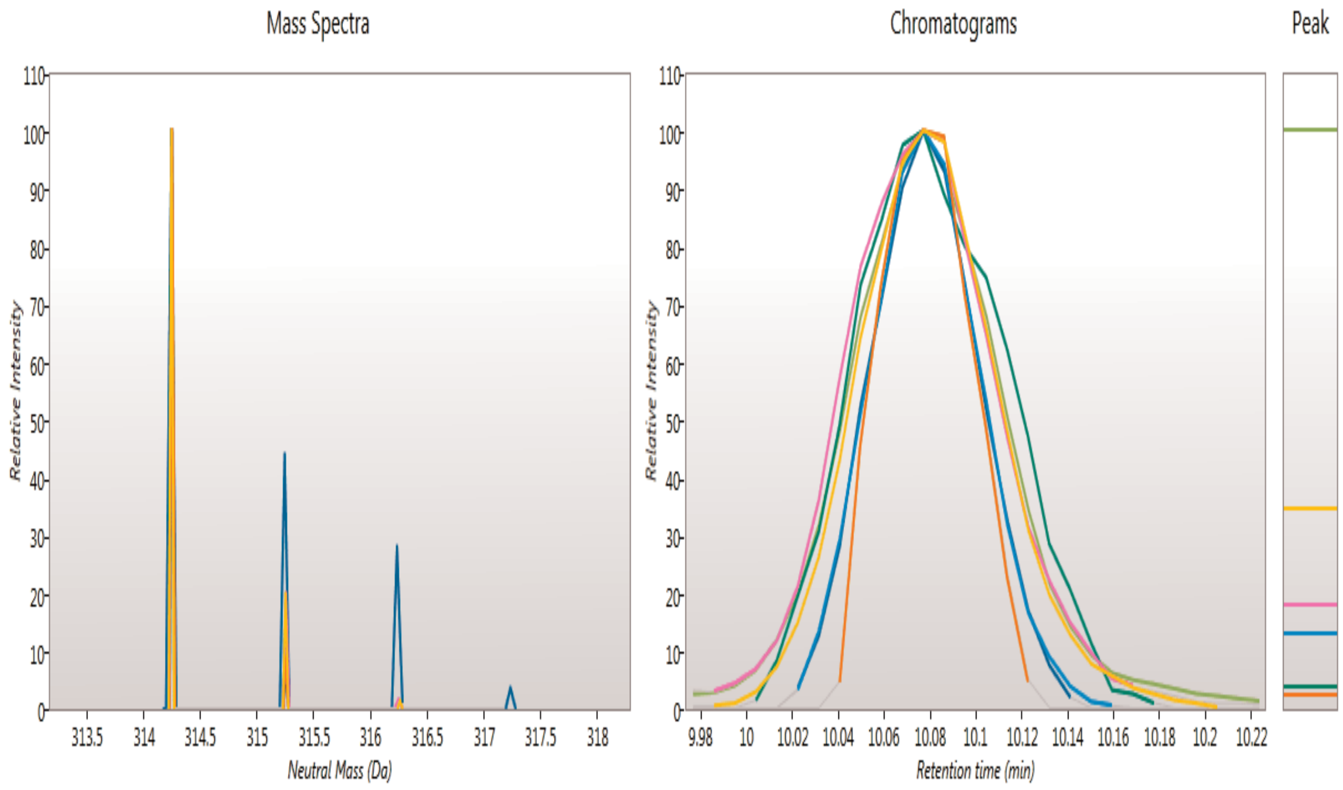
Mass accuracy

Mass resolution

Isotopic distribution

Peak deconvolution

Speed/Sensitivity



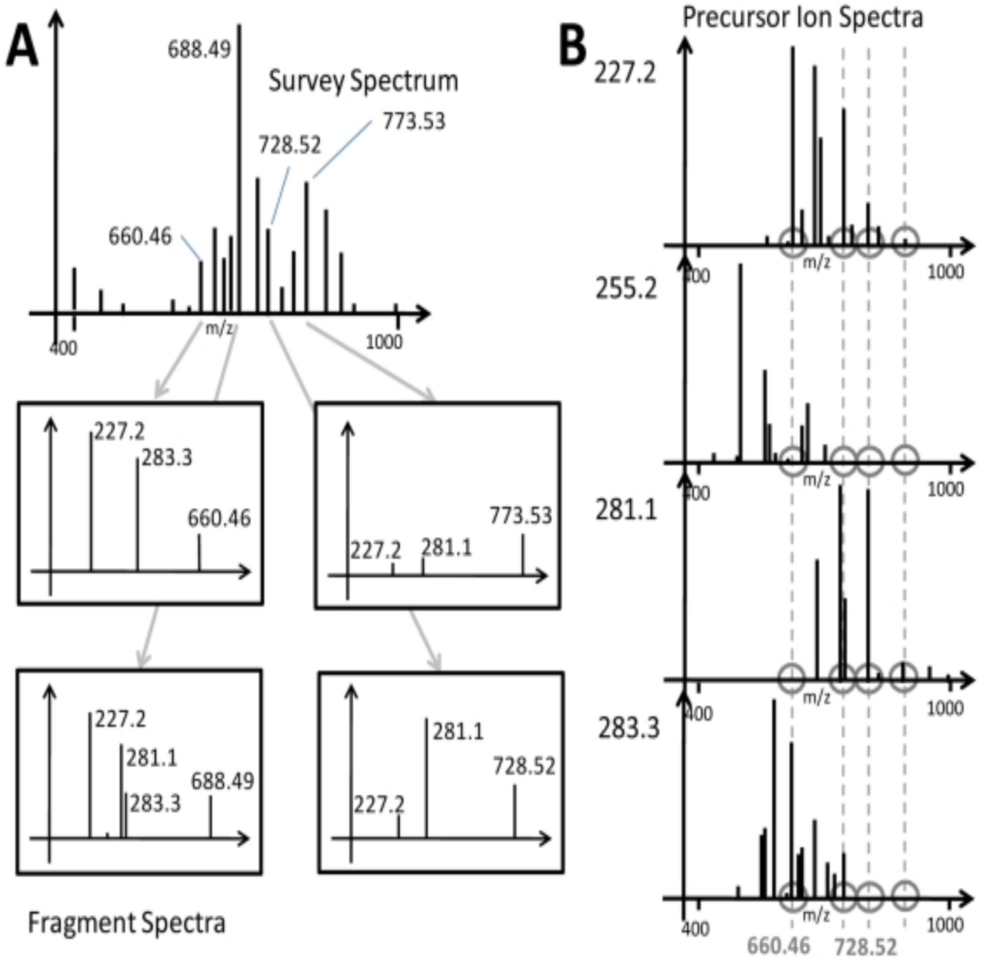
http://www.nonlinear.com/progenesis/qi/

MS/MS

DDA	Data Dependent Acquisition
DIA	Data Independent Acquisition
eDIA	Quadrupole-enhanced DIA
	Ion mobility-enhanced DIA

DDA

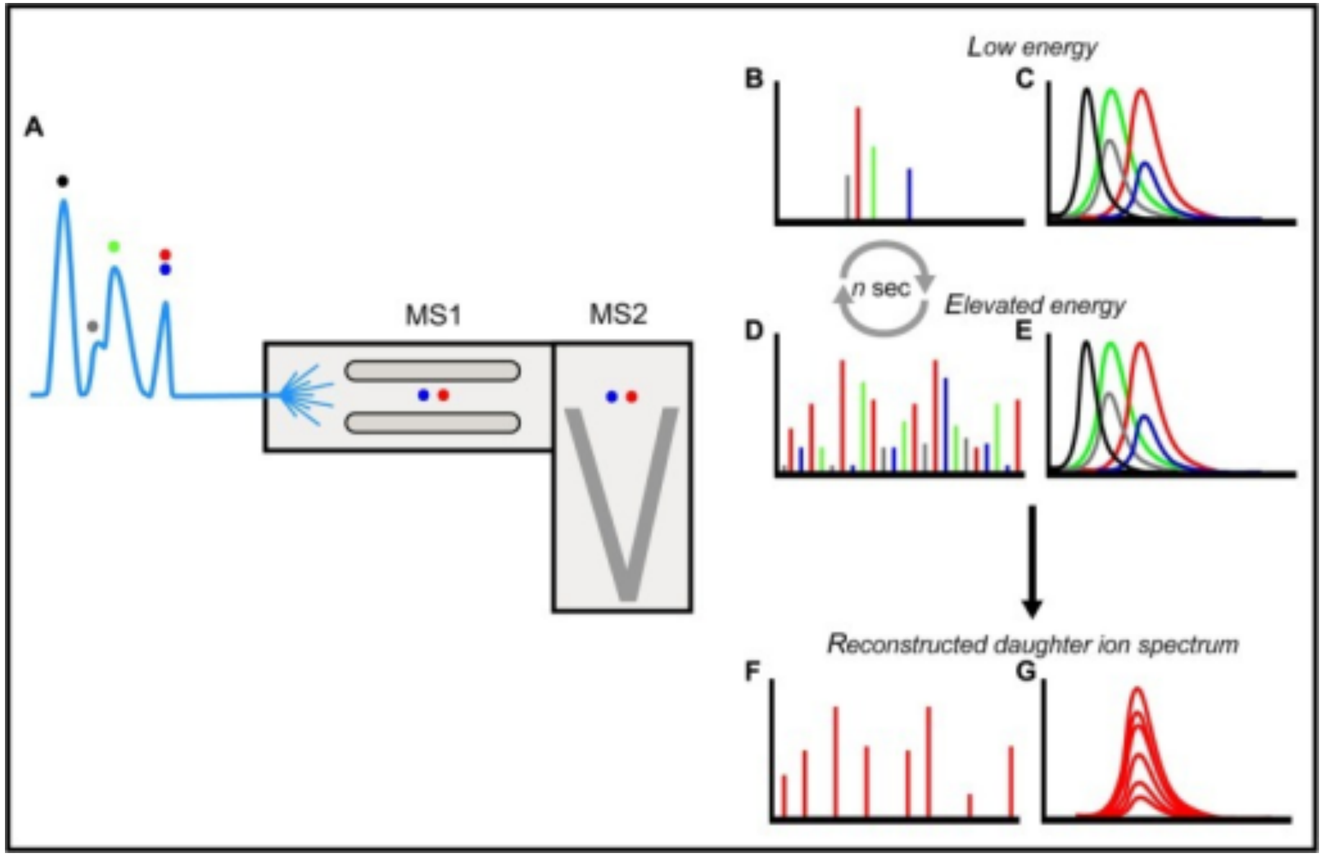
1. MS scan
2. Precursor selection
3. Precursor isolation on Q
4. Precursor fragmentation
5. Fragment scan



https://www.researchgate.net/profile/Julio_Sampaio/publication/221772729/figure/fig2/AS:202910513274881@1425389044734/The-scheme-explains-how-data-dependent-acquisition-of-full-MS-MS-spectra-DDA-driven.png

DIA

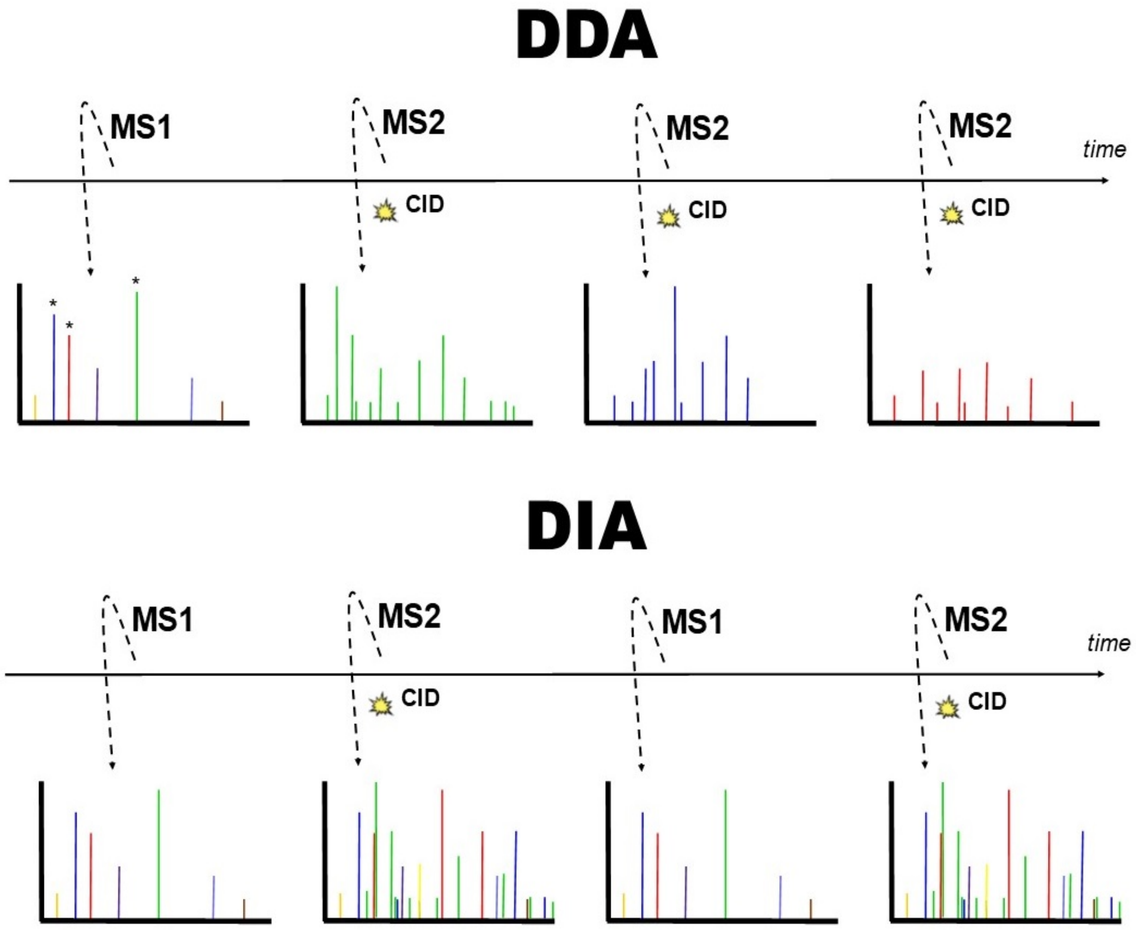
1. MS scan
2. Fragmentation
3. Fragment scan



https://openi.nlm.nih.gov/imgs/512/180/3389432/PMC3389432_fmicb-03-00240-g001.png

DDA vs DIA

	Pros	Cons
DDA	Higher specificity	Slow
DIA	No missed data	Lower specificity



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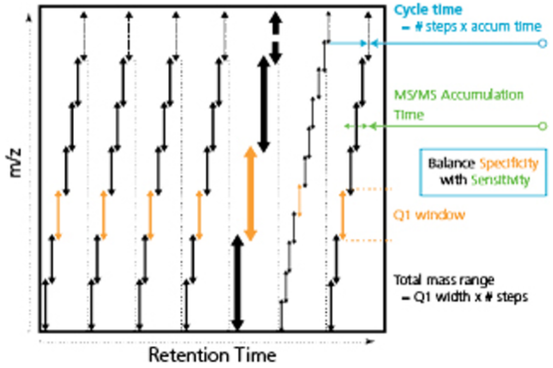
Enhanced-DIA

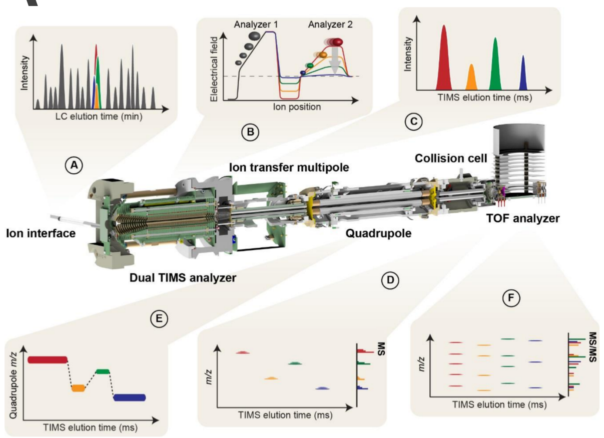
Quadrupole-eDIA

IMS-eDIA

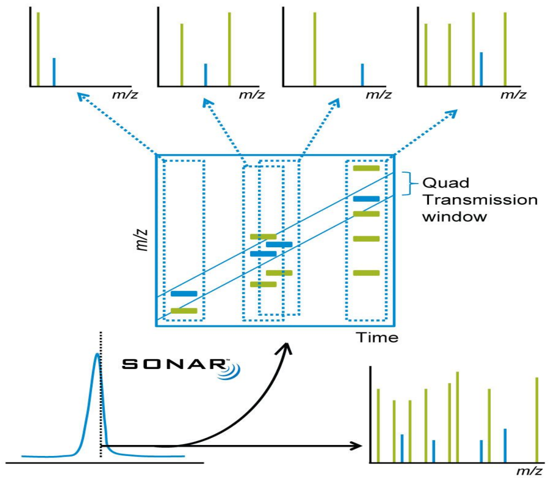
SWATH



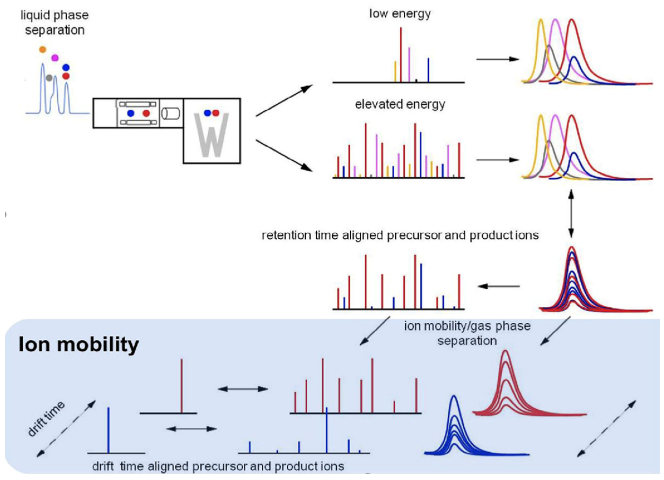
TIMS



SONAR



HDMSe



https://sciex.com/community/Asset/00003575/SWATH-Blog-Graphics_Image01jpg.jpg
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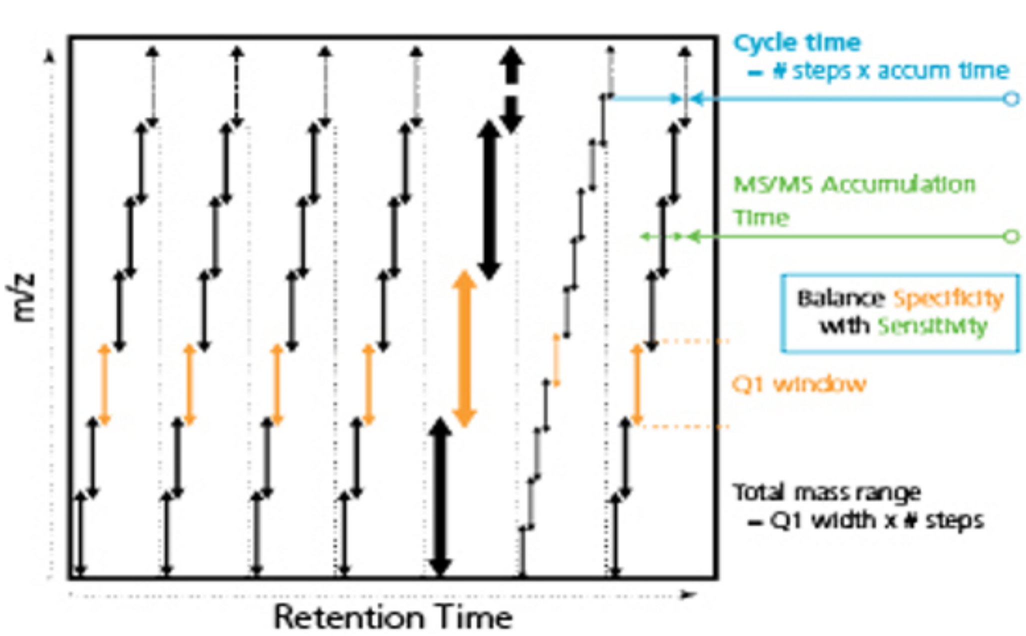
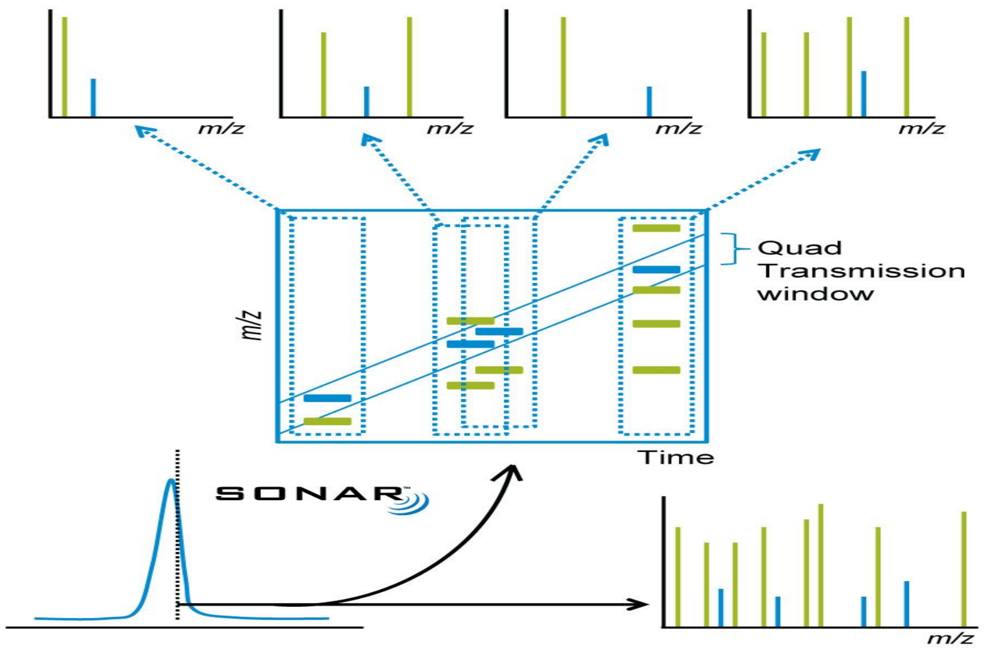
https://www.biorxiv.org/content/biorxiv/early/2018/06/01/336743.full.pdf
http://www.waters.com/waters/en_PR/TriWave/nav.htm?locale=en_PR&cid=134663660

Quadrupole-DIA

SWATH



SONAR



- Active switching/scanning quadrupole used for precursor isolation in a defined m/z range
- Similar to DDA in selectivity
- Unlike DDA, does not lose as much data

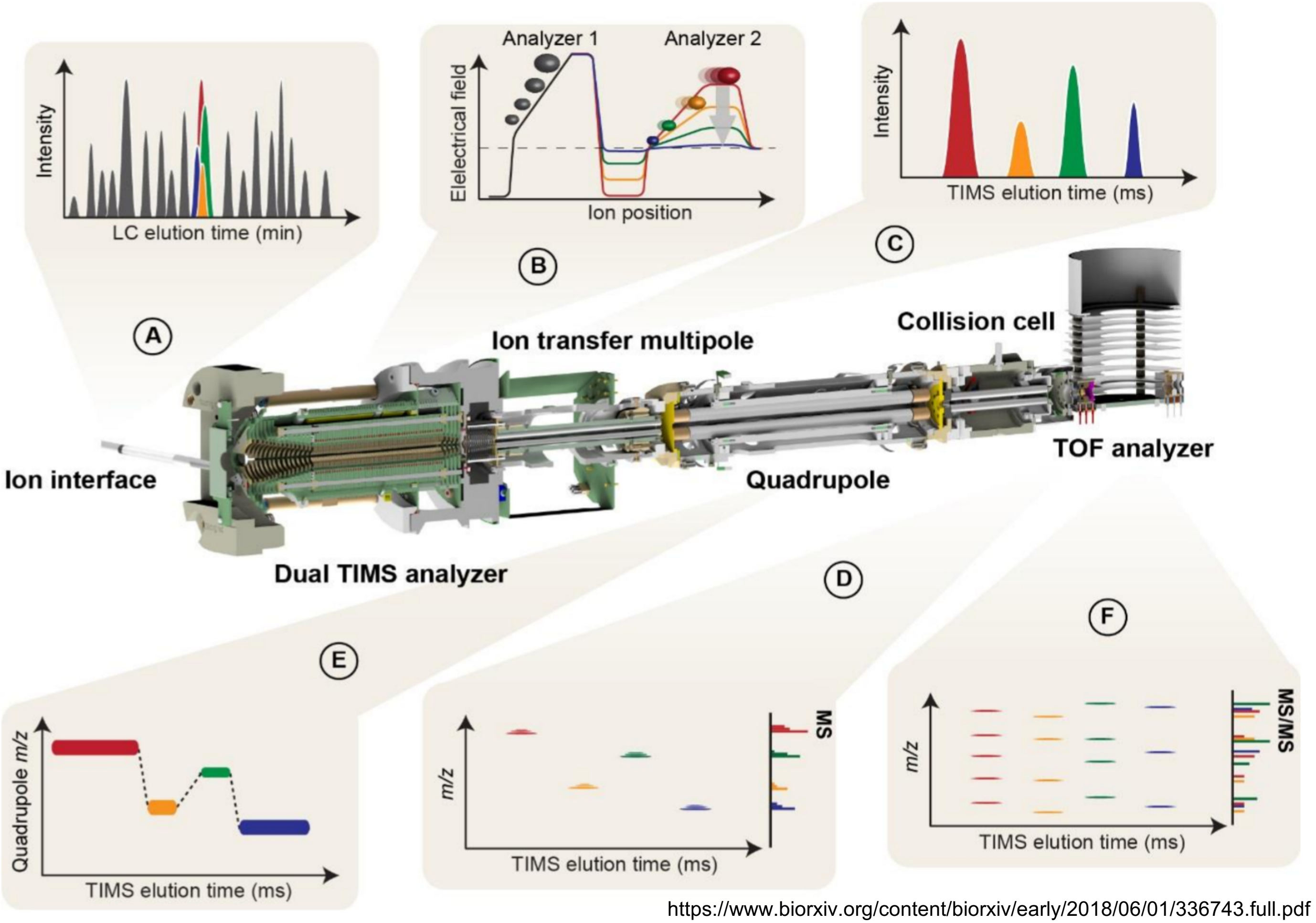
https://sciex.com/community/Asset/00003575/SWATH-Blog-Graphics_Image01jpg.jpg

http://www.waters.com/webassets/cms/category/media/content_blocks/wg_sonar_2.jpg

TIMS with PACEF

Trapped Ion Mobility Separation with Parallel Accumulation Serial Fragmentation

- Precursor trapping
- Ion mobility separation
- DIA with CE ramp

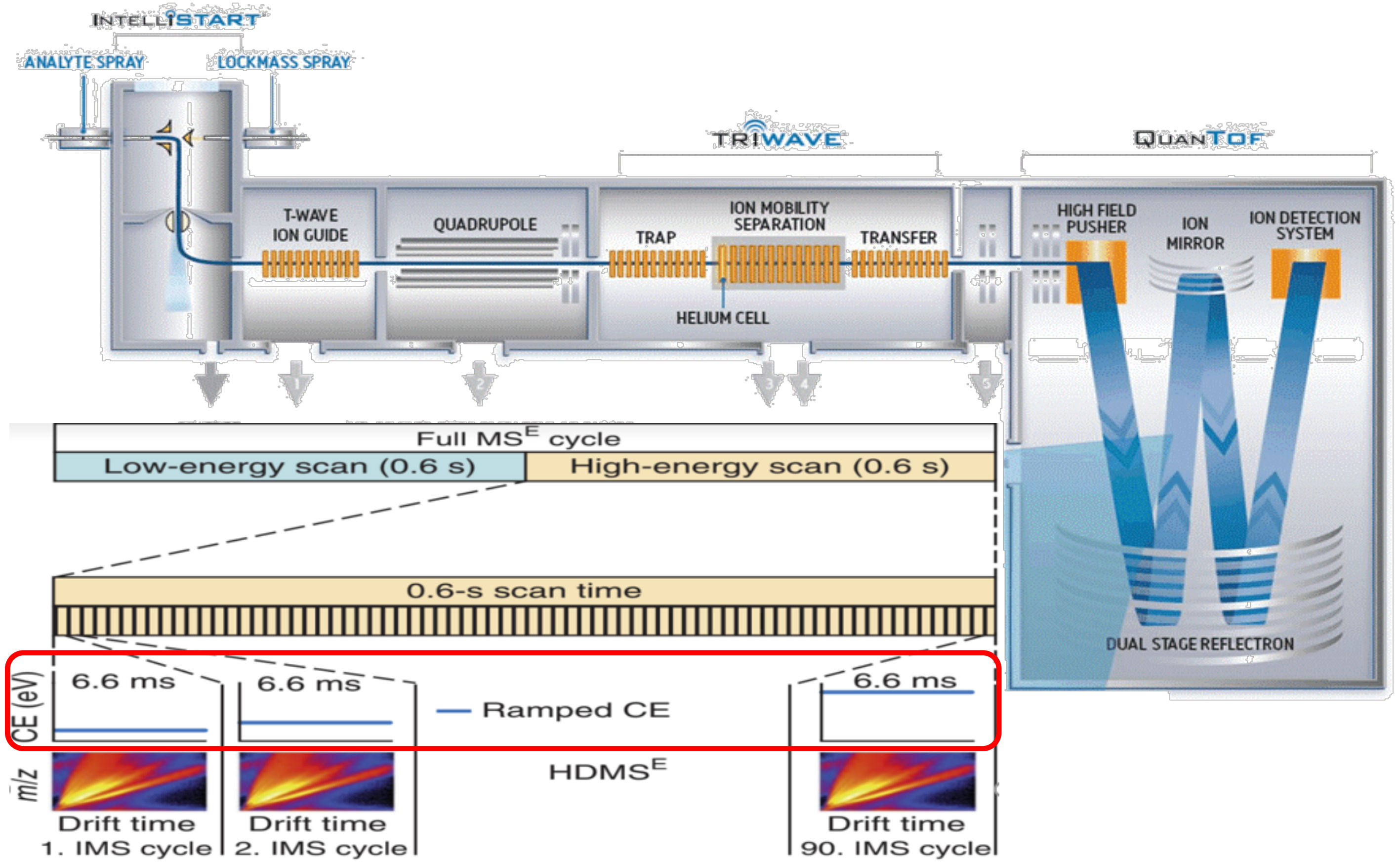


https://www.biorxiv.org/content/biorxiv/early/2018/06/01/336743.full.pdf

HDMSe

High Definition MSe

- Precursor trapping
- Ion mobility separation
- CE ramp per MS cycle
- Pre-/Post-IMS fragmentation

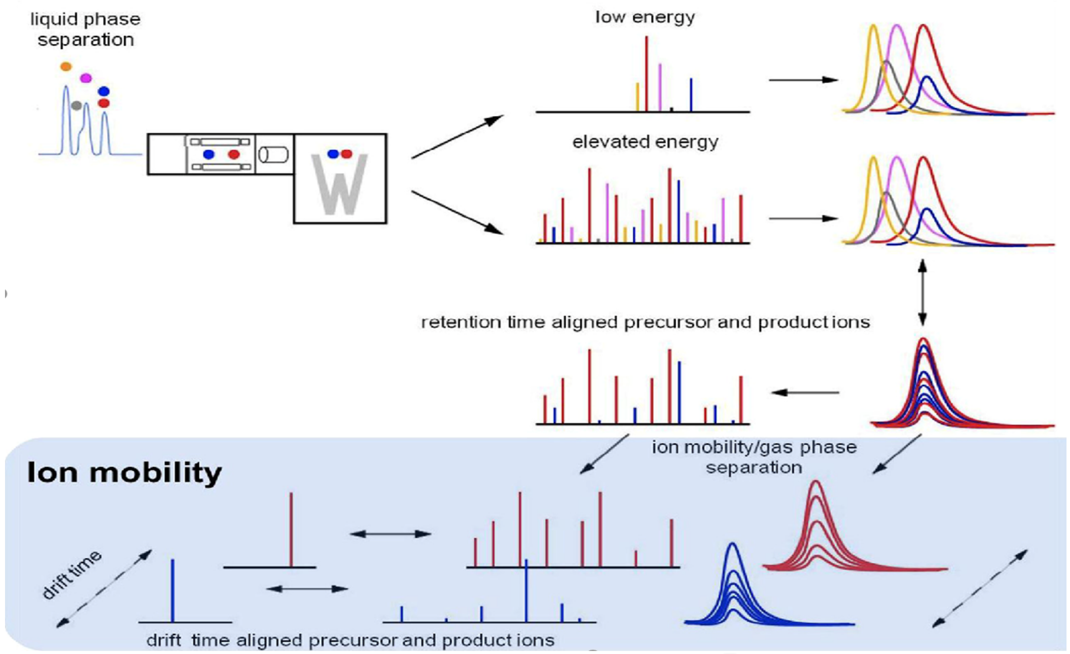


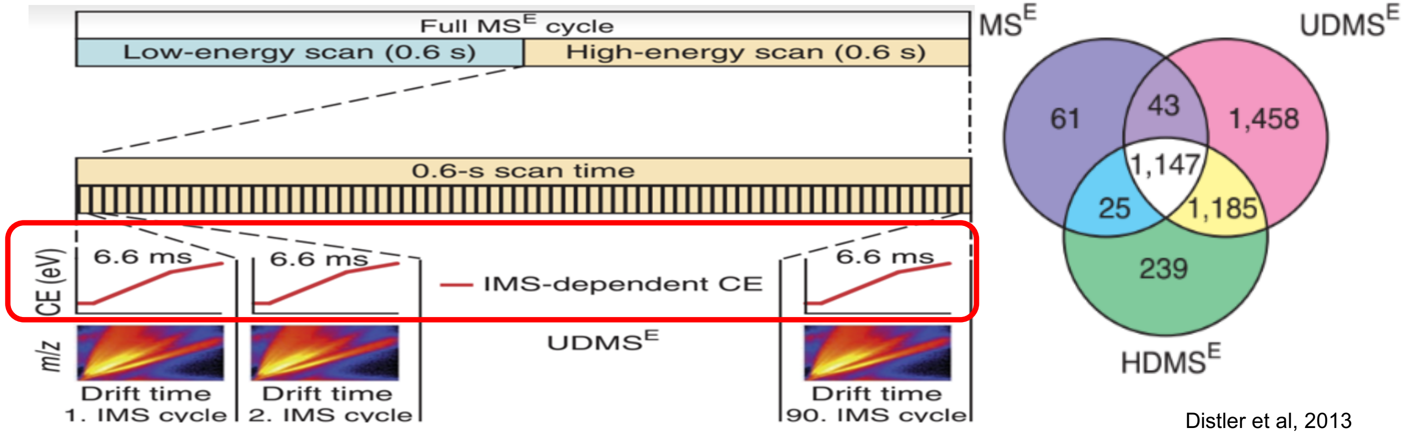
Distler et al, 2013

UDMSe

Ultra Definition MSe

- Precursor trapping
- Ion mobility separation
- CE ramp per IMS cycle
- Pre-/Post-IMS fragmentation

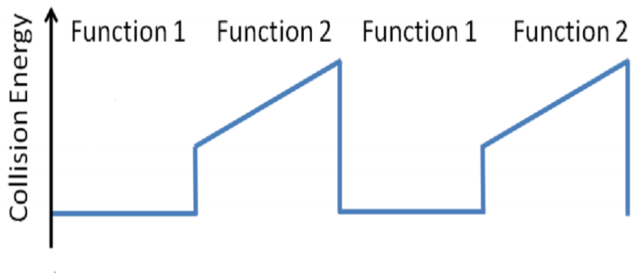


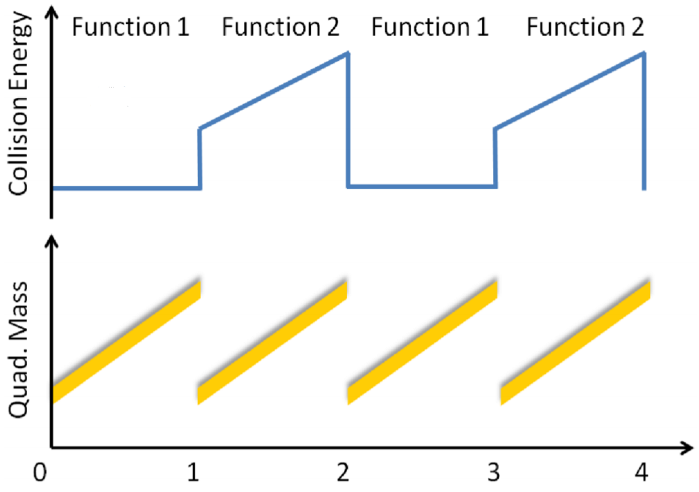


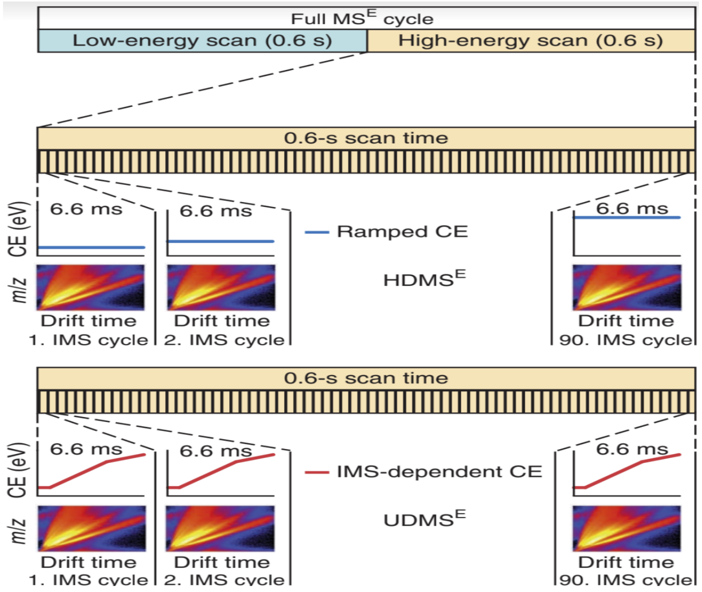
Distler et al, 2013

DIA vs eDIA

	DIA	Quad-eDIA	IMS-eDIA
Sensitivity	High	Low	High
Specificity	Low	High	High





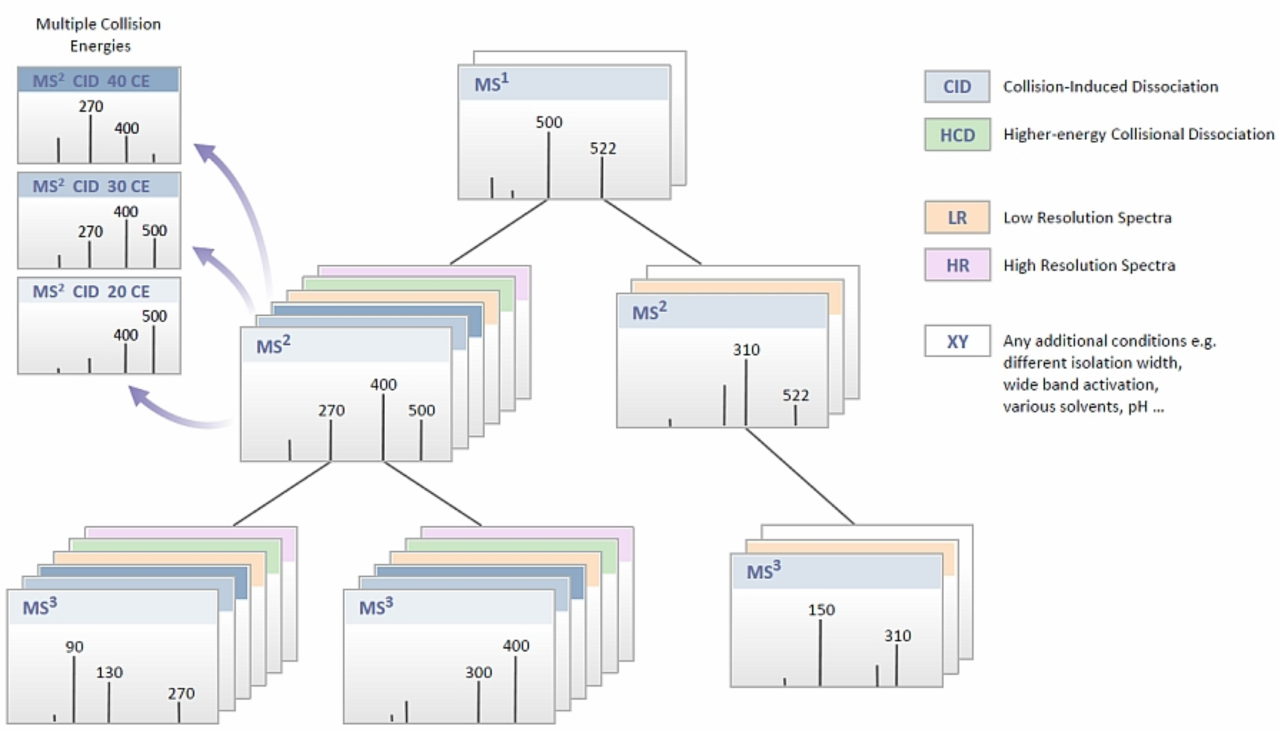


http://www.waters.com/webassets/cms/library/docs/2016imsc_vissers_system_level_omics.pdf

Distler et al, 2013

MS based criteria for ID

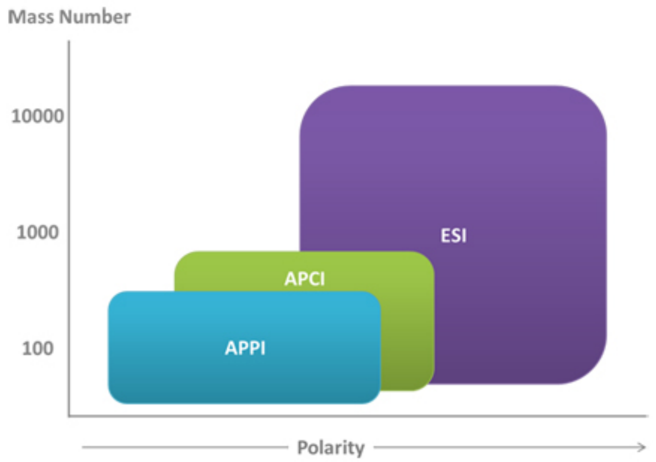
1. Mass
   - Accurate
   - Resolved
   - Peak shape
2. Isotopic distribution
   - Isotopes
   - Adducts
3. Fragmentation spectrum
   - Fragmentation mode
   - Accurate
   - Resolved

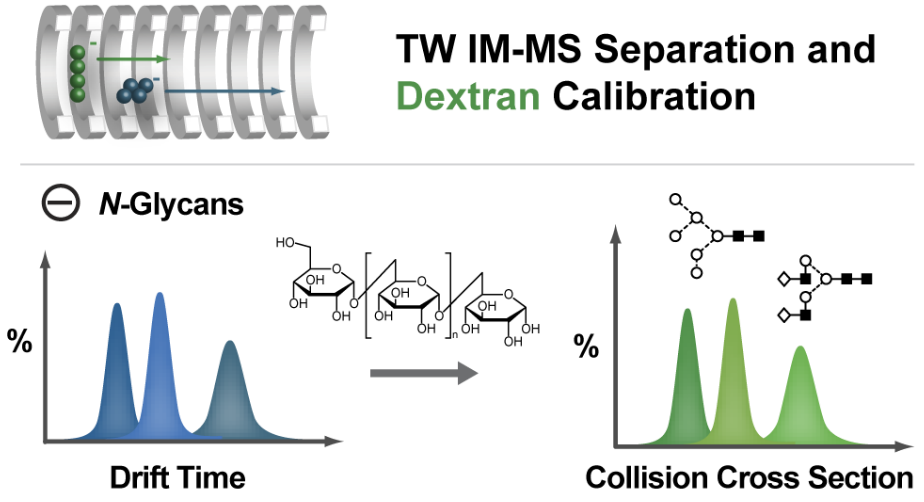


https://www.mzcloud.org/Images/features/spectralTreesImg.jpg

Orthogonal criteria for ID

1. Ionisation compatibility
   a) Ionisation mode
   b) Ionisation polarity
2. Ion mobility separation
   a) Drift time
   b) Collision Cross Section (CCS)
   ❖ DMS/FAIMS filter
3. Chromatographic separation
   a) Retention profile
   b) Elution profile





https://www.chromacademy.com/images/quick-guide/2016-Oct/LCMS-Ionisation-Modes.jpg
http://www.fhi-berlin.mpg.de/mp/helden/uploads/Main/neg_CCS_TOC.png

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CCS example

Collision Cross Section (CCS):

- 1.5% difference in CCS is sufficient for separation
- Useful for identification and quantification

	1	2	3	Av CCS	%RSD
Butyric Acid	147.11	147.42	147.42	147.32	0.12
Isobutyric Acid	149.35	149.02	149.11	149.16	0.11
Valeric Acid	160.89	160.95	160.68	160.84	0.09
Isovaleric Acid	160.38	160.30	160.17	160.28	0.07

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