# Novinky na poli hmotnostní spektrometrie

**Taťána Halešová**

*VIZE 2025*

*Hotel Hermitage, Praha*

# Novinky na poli MS

Waters™

Cílená analýza (targeted, QQQ, MRM)

**Xevo TQ Absolute XR**

- Kvantifikace známých látek při ultra nízkých koncentracích
- Rutinní analýzy – plnění legislativních povinností
- **Analýza** – mikropolutanty, rezidua, forenzní analýza, cílená omika

– **citlivost, robustnost** (rutinní provoz)

– **multireziduální analýzy** (100vky látek v nástřiku)

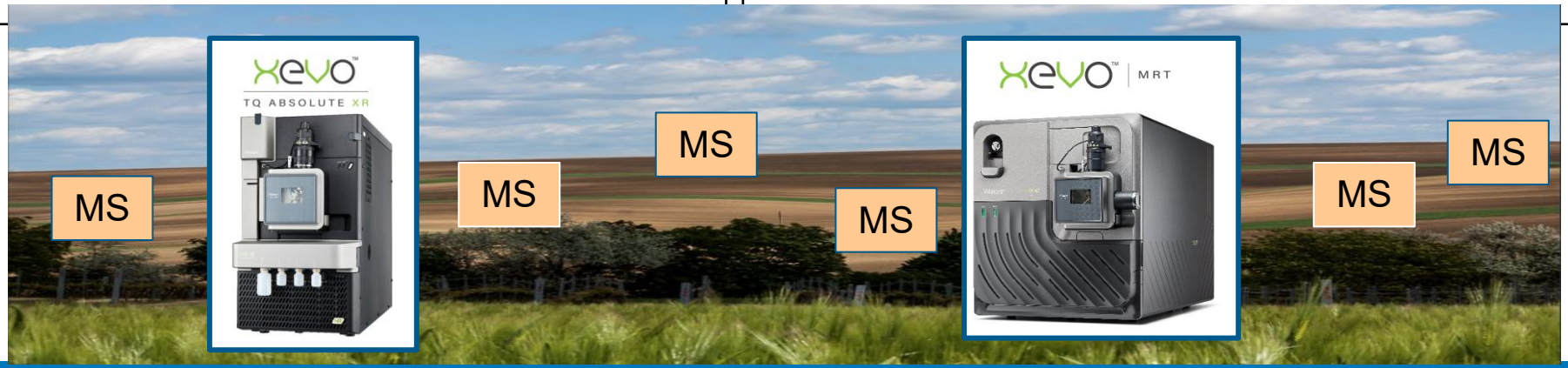
Necílená a suspect analýza (untargeted, HRMS)

**Xevo MRT**

- Identifikace látek, objev nových látek a metabolitů
- Vysoké rozlišení – možnost rozlišit izobarické interference
- **Analýza** – metabolomika, lipidomika, forenzní screening, screening emergentních polutantů, farmakokinetika a studium metabolismu léčiv

– **výzkumné a screeningové aplikace**

– **možnost vracet se k datům**



# Novinka _Waters Xevo TQ Family

Xevo TQ ABSOLUTE XR

Waters™

Xevo™ TQ-S cronos

Xevo™ TQ-S micro

Xevo™ TQ-XS

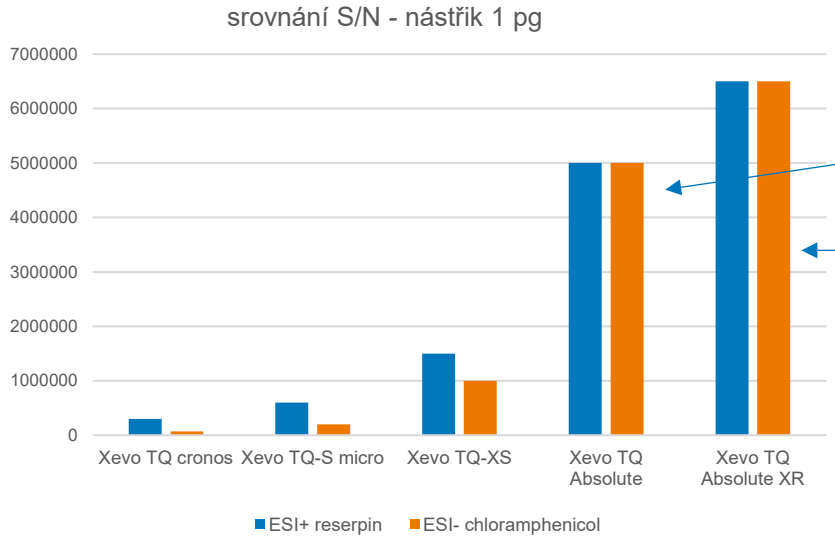
Xevo™ TQ ABSOLUTE

MassLynx™ +

waters_connect™

| 2014 | Výkon | 2025 |
|---|---|---|
| Rutinní úroveň - dostupný, spolehlivý výkon | Střední úroveň - citlivý, flexibilní a kompatibilní | Vysoká úroveň – maximální citlivost a flexibilita a robustnost |

# Srovnání citlivosti – Waters Xevo TQ Family

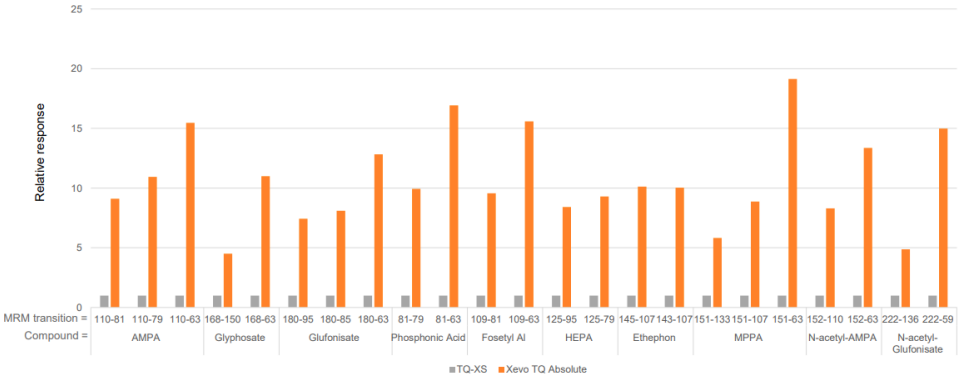



Nový vysokonapěťový fotonásobič
Nová iontová optika - StepWave XS

Nová iontová optika – StepWave XR

*Polární pesticidy (ESI -)*

- Xevo TQ Absolute vykazuje 4x až 19x lepší odezvu než Xevo TQ-XS pro řadu polárních pesticidů

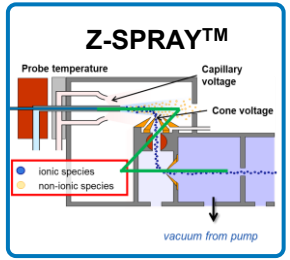


# Xevo TQ Absolute XR

- navazuje na Xevo TQ Absolute
- **XR = eXtreme Robustness**
- nová generace iontové optiky StepWave™ XR
- vhodný pro komplexní vzorky
- eliminace iontů s vyšší hmotností (matrice)
- bez ztráty výkonu a potřeby častého čištění
- 50% nižší produkce tepla, spotřeba $N_2$, el. energie
- rozměry: 43,0 cm (Š) × 79,0 cm (V) × 96,0 cm (H)
- Software – MassLynx, waters_connect


# Parametry **Xevo TQ Absolute XR**

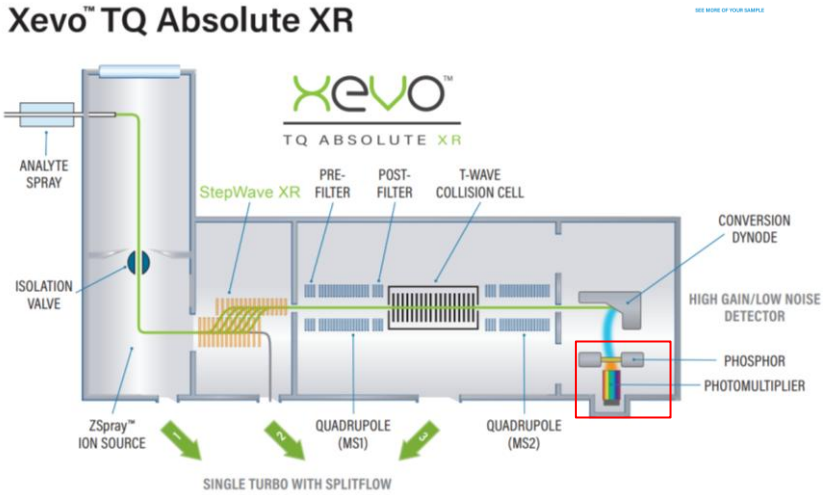
| Xevo TQ Absolute XR | |
|---|---|
| **Mass range** | 2 – 2050 m/z **(PFAS m/z 19)** |
| **Scan speed** | 20 000 Da/s<br>10 scan/s (m/z 2 -2050)<br>20 scan/s (m/z 50 -1000)<br>40 scan/s (m/z 50 - 500) |
| **Rozlišení** | 0.5 Da, 0.7 Da, 1.0 Da |
| **Lineární dynamický rozsah** | až 6 řádů |
| **Iontové zdroje** | ESCi, **UniSpray**, nanoFlow, ASAP, **APGC**, **DESI XS** |
| **ESCi mode switching time** | 20 ms ESI/APCI |
| **Polarity switching time** | 4 ms +/-mod |
| **Typy skenů** | Full scan, SIR, **MRM**, Produkt, Precursor ion scan, RADAR |
| **Dwell time** | min. 1 ms MRM; min. 1 ms **inter-channel delay** |
| **MRM acquisition rate** | max. 500 MRM/sec |
| **Software** | MassLynx, **waters_connect** |




Waters™

- ✓ Nižší kontaminace
- ✓ Lepší robustnost

UniSpray




MassLynx™

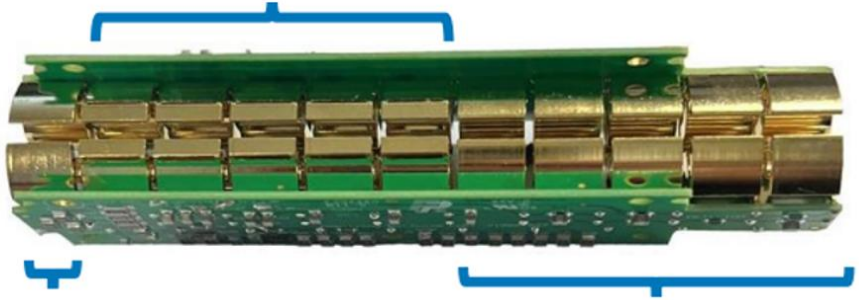
waters_connect™

# Xevo TQ Absolute XR - StepWave **XR**

Waters™

**Resolving section**

elementy mají štěrbiny, které umožňují odstranit ionty s vysokou hmotností úplně z dráhy iontů

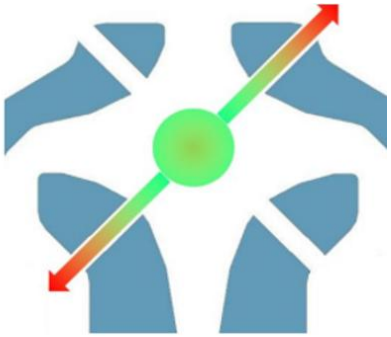


**Non-resolving entrance**

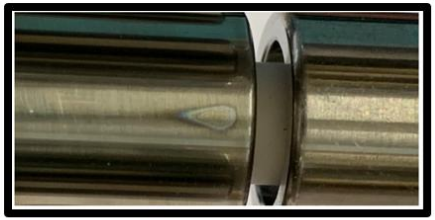
umožňuje efektivní zachycení iontů ze zdroje

**Non-resolving cooling**

umožňuje soustředit ionty zpět do středu iontové dráhy a účinně postupují dál do analyzátoru

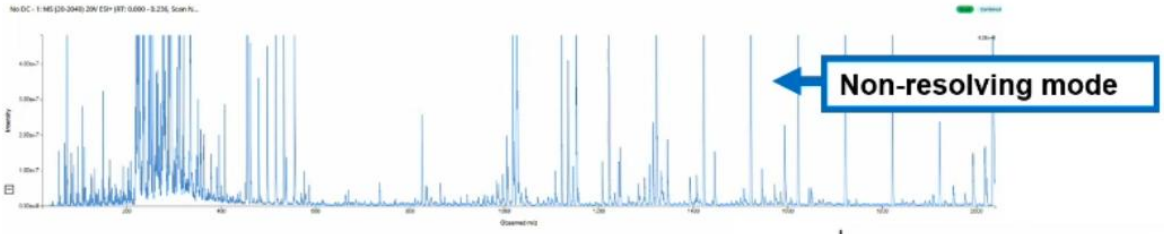


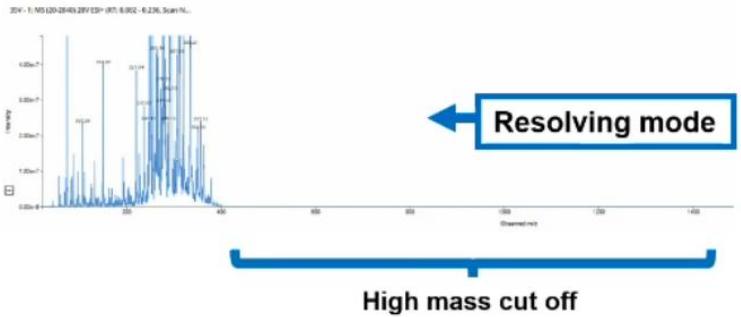
nežádoucí ionty jsou filtrovány štěrbinami ven z dráhy

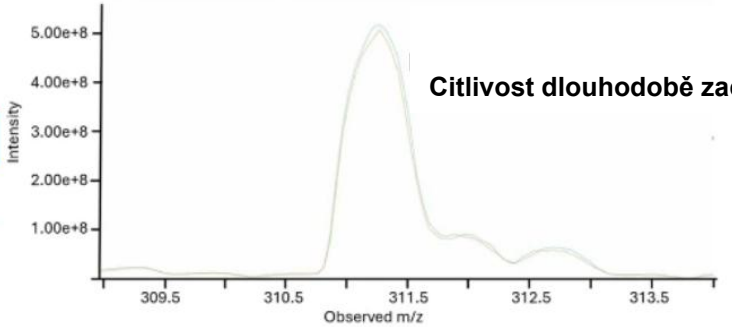


Cíl: eliminace kontaminace kvadrupólu
Praktický dopad: méně šumu, delší dobu bez ztráty citlivost a nutnosti čištění

# Xevo **TQ Absolute XR** - StepWave **XR**




**Citlivost dlouhodobě zachována**

Rušivé vlivy - eliminace
- Fosfolipidy
- Peptidy
- Sacharidy
- PEG

*6x robustnější, delší provozuschopnost a méně přerušení kvůli údržbě*
*S/N > 1,5x oproti Xevo TQ Absolute*

# Stanovení PFAS ve vodách

- **Přímý nástřik vzorku, ESI-**
- Objem nástřiku **10 µl**
- Menší zatížení stroje matricí – **Xevo TQ Absolute XR**
- **Matrice** (čistá voda, odpadní voda)
- **Linearita** 0,5 – 250 ng/L
- **Preciznost** (10 inj.,10 ng/L) RSD < 10%
- **Legislativa:** DW **suma 20 PFAS 0,1 µg/L, total PFAS 0,5 µg/L**

GW **suma 24 PFAS 4,4 ng/L (PFOA ekvivalent)**

| | Concentration (ng/L) | | | |
|---|---|---|---|---|
| | Wastewater | Drinking water | Ground water | Surface water |
| PFHxA | 17.4 | 4.6 | 3.8 | 3.8 |
| PFHpA | 4.1 | 2.0 | 2.0 | 2.2 |
| PFOA | 16.2 | 4.4 | 2.8 | 4.3 |
| PFNA | 2.5 | <LLOQ | – | – |
| PFDA | 1.6 | – | – | – |
| PFBS | 2.6 | 1.9 | 1.5 | <LLOQ |
| PFHxS | 1.2 | <LLOQ | – | <LLOQ |
| PFOS | 1.8 | – | – | 1.0 |
| FBSA | <LLOQ | <LLOQ | – | <LLOQ |
| FOSA | <LLOQ | – | – | – |
| NMeFOSAA | 2.7 | – | – | – |

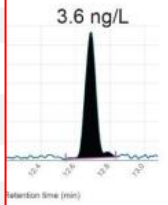
| | | | | |
|---|---|---|---|---|
| Suma | 50,1 ng/L | 12,9 ng/L | 10,1 → 4,2 ng/L(RPF) | 11,3 ng/L |

| Compound | Method detection limit (ng/L) | Compound | Method detection limit (ng/L) |
|---|---|---|---|
| PFBA | 21.9 | PFNS | 1.3 |
| PFPeA | 7.7 | PFDS | 1.1 |
| PFHxA | 1.6 | PFUnDS | 1.7 |
| PFHpA | 0.8 | PFDoDS | 1.0 |
| PFOA | 1.2 | PFTrDS | 1.5 |
| PFNA | 1.2 | GenX | 1.1 |
| PFDA | 1.5 | ADONA | 0.9 |
| PFUnDA | 2.0 | 9Cl-PF3ONS | 1.0 |
| PFDoDA | 1.5 | 11Cl-PF3OUdS | 1.5 |
| PFTriDA | 1.4 | 4:2 FTS | 1.4 |

**LOD**
32 látek v metodě

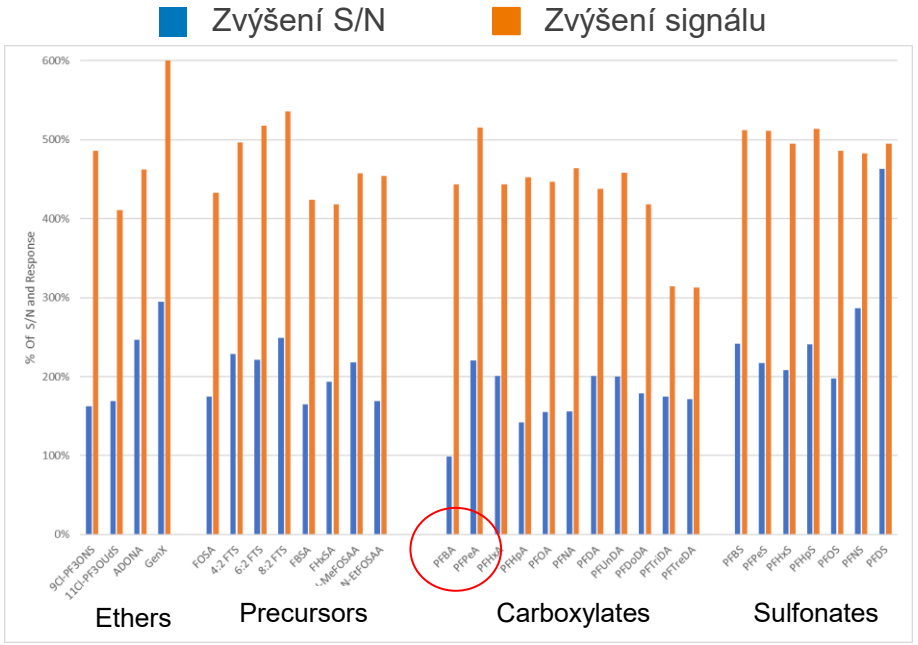
LOD pro většinu II.
≈
**1 ng/L**

| LEGISLATIVA - VODA | | | | | | |
|---|---|---|---|---|---|---|
| **PITNÁ VODA (0,1 µg/l)** | | **POVRCHOVÁ VODA** | **PODZEMNÍ VODA (4,4 ng/l)** RPF – PFOA ekvivalent | | | |
| PFBA | PFBS | PFOS | PFBA | 0.05 | PFBS | 0.001 |
| PFPeA | PFPeS | | PFPeA | 0.03 | PFPeS | 0.3 |
| PFHxA | PFHxS | | PFHxA | 0.01 | PFHxS | 0.6 |
| PFHpA | PFHpS | | PFHpA | 0.5 | PFHpS | 1.3 |
| PFOA | PFOS | | PFOA | 1 | PFOS | 2 |
| PFNA | PFNS | | PFNA | 10 | PFDS | 2 |
| PFDA | PFDS | | PFDA | 7 | | |
| PFUnDA | PFUnDS | | PFDoDA | 3 | 6:2 FTOH | 0.02 |
| PFDoDA | PFDoDS | | PFUnDA | 4 | 8:2 FTOH | 0.04 |
| PFTrDA | PFTrDS | | PFTrDA | 1.65 | Gen X | 0.06 |
| | | | PFTeDA | 0.3 | ADONA | 0.03 |
| | | | PFHxDA | 0.02 | C6O4 | 0.06 |
| | | | PFODA | 0.02 | | |

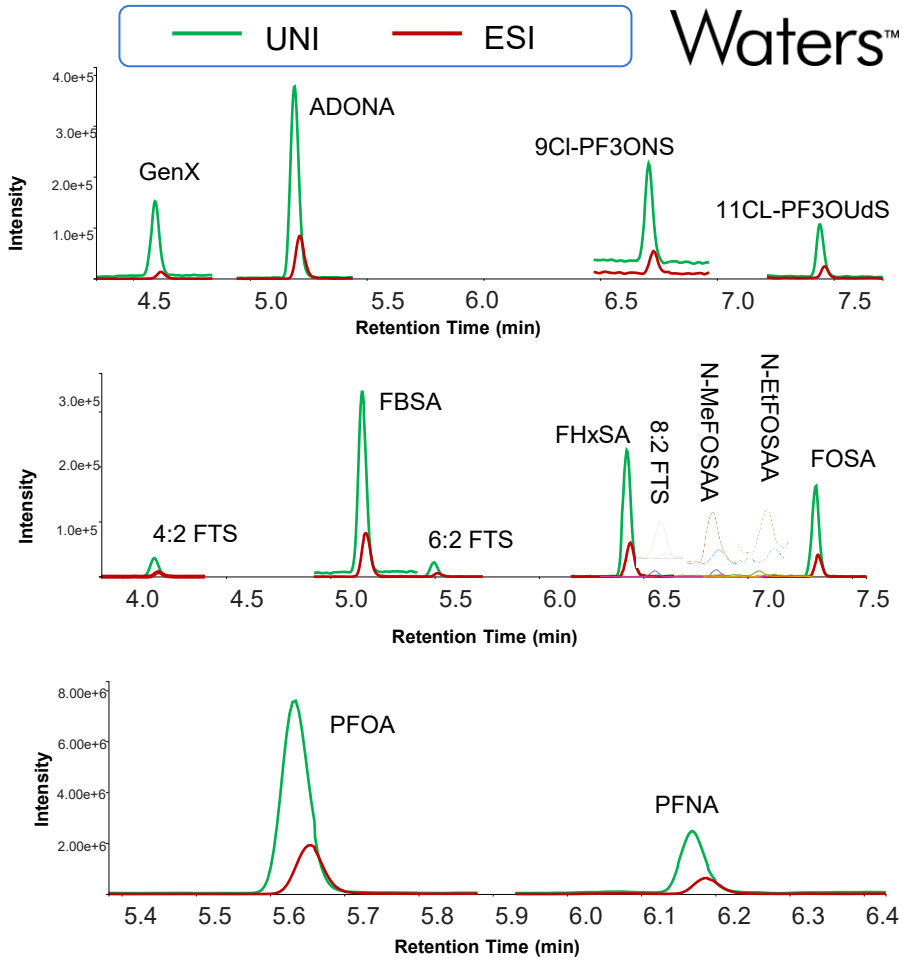


# PFAS - lepší citlivost s použitím UniSpray

*Zvýšená ionizace a účinnější desolvatace*

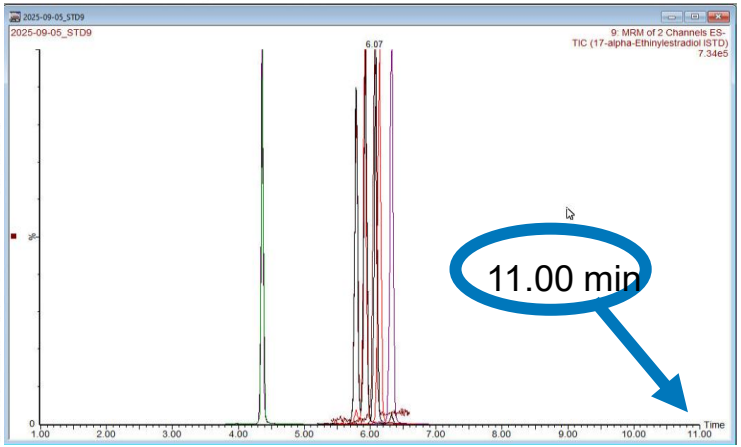



**UniSpray poskytuje minimálně 3x lepší signál a minimálně 2x vyšší S/N**



# Stanovení steroidních hormonů ve vodách

Pitná voda výhláška č. 252/2004 Sb. - 17β-estradiol **LOQ 1 ng/L**
Směrnice 2013/39/EU pro povrchové vody (Watch list) **LOQ 0,4 ng/L**




| Parameter | LOR (XTQA) | LOR (původní) |
|---|---|---|
| **17α-ESTRADIOL** | 1.0 ng/L | 1.0 ng/L |
| **17β-ESTRADIOL** | **0.4 ng/L** | 0.8 ng/L |
| **17α-ETHINYLESTRADIOL** | 0.8 ng/L | 0.8 ng/L |
| **ESTRON** | **0.4 ng/L** | 1.0 ng/L |
| **ESTRIOL** | **1.0 ng/L** | - |
| **EQUILIN** | 0.8 ng/L | 0.8 ng/L |

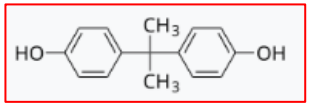
**UPLC I-Class**
**Xevo TQ Absolute**

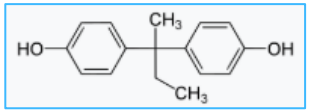
Cíl:

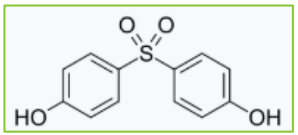
- ✓ převod metody na Xevo TQ Absolute
- ✓ dosáhnout nižších **LOQ**
- ✓ snížení objemu vzorku na analýzu (SPE Oasis® HLB) 250 ml → 100 ml)
- ✓ zkrátit čas analýzy (15 min. → 11 min.)
- ✓ snížení spotřeby organických rozpouštědel, dusíku a energie

# Analýza bisfenolů

- Směrnice EU 2020/2184 o pitné vodě; Bisfenol A (BPA): 2,5 µg/l
- **3 vzorky pitné kohoutkové vody + 1 balená voda, spike 5ng/L**
- **BPA –** nejlépe prozkoumaný, prokázané endokrinní účinky, vývojová toxicita (50 ng/L – 50x pod leg. limitem)
- **BPB –** pravděpodobně silnější estrogení aktivita než BPA, málo dat
- **BPS –** účinnější xenoestrogen než BPA, **má vyšší stabilitu v životním prostředí**, vyšší odolnost → vyšší riziko kumulace
- **LOQ pro bisfenoly 0,5 – 5 ng/L; linearita 0,5 -5000 ng/l, $R^2$ 0,9998; RSD < 10%**
- **LC-MS/MS, ESI-, 50 µl nástřik, MF: methanol:voda ($NH_4Ac$), 7 min. analýza**
- SZÚ: v roce 2016 byly bisfenoly v ČR zjišťovány v moči celkem 395 dětí ve věku 5 a 9 let.
- bisfenol A byl nalezen v moči téměř všech dětí (97 %) v největším množství, bisfenol S byl nalezen u poloviny dětí (53 %) (VZ SZÚ 2016,2019)




| Compound | Precursor | Product |
|---|---|---|
| BPS | 249.4 | 91.9 |
| | 249.4 | 107.9 |
| | 249.4 | 156.0 |
| BPA | 227.2 | 133.0 |
| | 227.2 | 211.1 |
| | 227.2 | 212.1 |
| BPF | 199.1 | 76.9 |
| | 199.1 | 92.9 |
| | 199.1 | 104.9 |
| BPB | 241.2 | 147.0 |
| | 241.2 | 211.1 |
| | 241.2 | 212.1 |
| BPAF | 335.1 | 68.9 |
| | 335.1 | 177.0 |
| | 335.1 | 197.0 |
| | 335.1 | 225.1 |
| | 335.1 | 265.1 |

| | Sample | BPF (ng/L) | BPA (ng/L) | BPB (ng/L) | BPS (ng/L) | BPAF (ng/L) |
|---|---|---|---|---|---|---|
| 1 | Unspiked | n.d. | 53.6 | n.d. | BLOQ | n.d. |
| | spiked | 5.89 | 57.3 | 4.83 | 5.15 | 4.04 |
| 2 | Unspiked | n.d. | 4.90 | n.d. | 3.39 | BLOQ |
| | spiked | 5.04 | 11.1 | 5.01 | 9.54 | 4.65 |
| 3 | Unspiked | n.d. | 2.55 | 0.34 | 0.263 | BLOQ |
| | spiked | 6.42 | 5.87 | 5.08 | 5.45 | 3.86 |
| 4 | Unspiked | n.d. | 4.23 | n.d. | 0.103 | BLOQ |
| | spiked | 4.67 | 7.97 | 4.78 | 5.19 | 4.49 |

# Analýza nitrosaminových nečistot ve farmacii

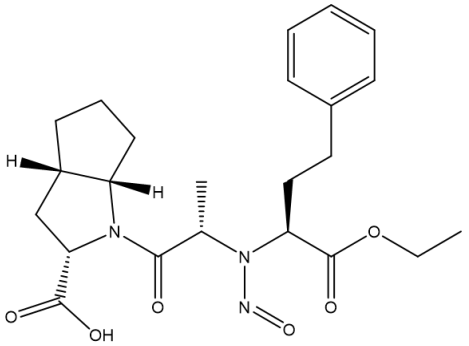
***N*-nitroso-ramipril**

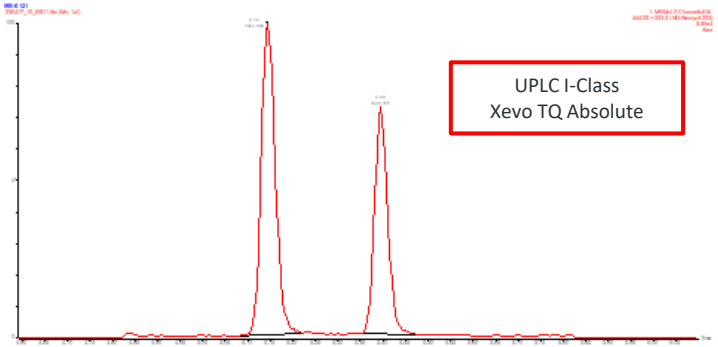
(*2S*,*3aS*,*6aS*)-1-(*N*-((*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl)-*N*-nitroso-*L*-alanyl)octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid

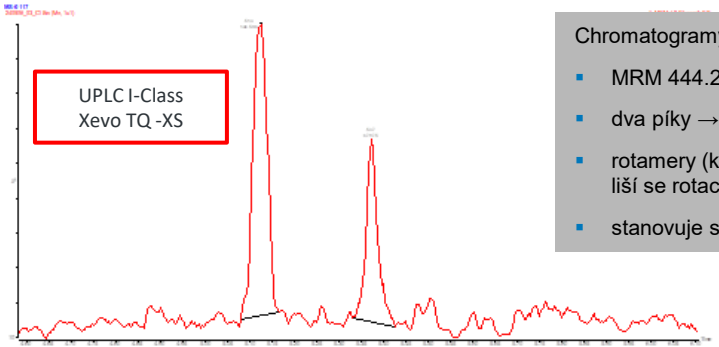
- Převod metody pro stanovení NO-Ramiprilu: UPLC I-Class Plus, **Xevo TQ-XS → Xevo TQ Absolute** ($ESI^-$)
- Stejná LC i MS metoda, chemikálie, nástřik **5 µl $c_{STD}$ 50 pg/ml** (50% methanol)

| Compound | Typical retention time (min) | Retention window (min) | Precursor (m/z) | Product (m/z) | Dwell time (s) | Cone voltage (V) | Collision energy (eV) |
|---|---|---|---|---|---|---|---|
| NO-Ramipril | 5.1 | 3.0 – 7.0 | 444.25 | 223.20* | 0.120 | 15 | 21 |
| | | | | 323.25 | 0.120 | 15 | 19 |

| *Xevo | S/N (Isomer-I) | Area (Isomer-I) | Area (Isomer-II) | Area (sum) |
|---|---|---|---|---|
| TQ Absolute | 75 | 1973 | 1338 | 3311 |
| TQ-XS | 7 | 135 | 68 | 203 |






Chromatogramy

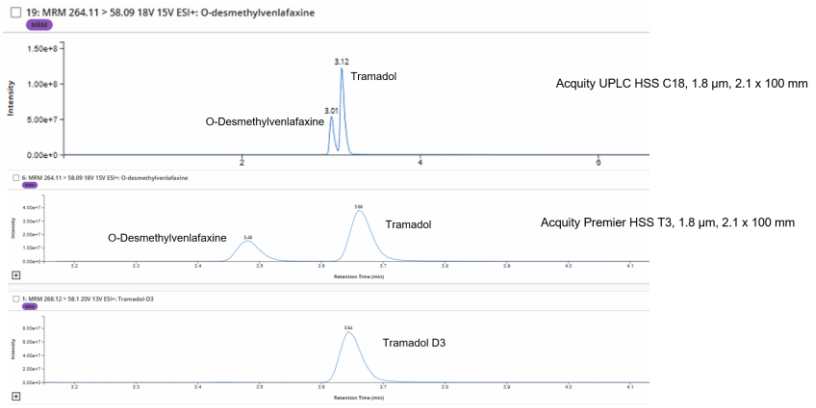
- MRM 444.25 → 223.2 CE (21 eV)
- dva píky → separace rotamerů
- rotamery (konformační izomery) – liší se rotací kolem N-N vazby
- stanovuje se jejich suma

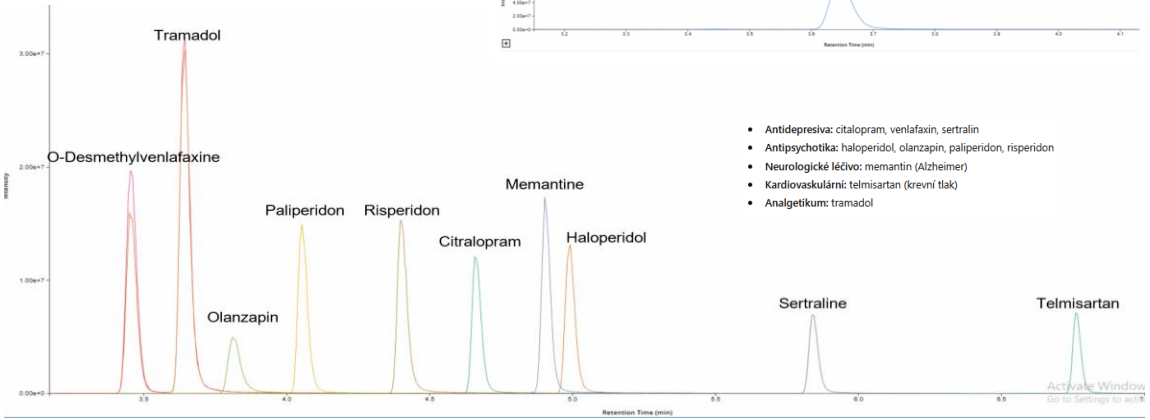
# Analýza terapeutických léků - TDM

*Analýza vybraných léčivých látek v séru*

- Xevo TQ Absolute with ACQUITY Premier UPLC
- **ESI+, 3 µl inj.**
- Požadavek na linearitu 0,01-1000 ng/ml
- Srovnání ESI+/UniSpray

| Compound | Precursor | Product |
|---|---|---|
| Citalopram | 325.13 | 109.03 |
| | | 261.99 |
| Haloperidol | 376.12 | 122.98 |
| | | 164.98 |
| Memantine | 180.20 | 107.0 |
| | | 163.1 |
| O-Desmethylvenlafaxine | 264.11 | 58.09 |
| | | 246.06 |
| Olazapin | 313.0 | 84.0 |
| | | 256.0 |
| Paliperidon | 427.14 | 110.03 |
| | | 206.97 |
| Risperidon | 411.13 | 110.01 |
| | | 191.01 |
| Sertraline | 306.05 | 158.87 |
| | | 274.93 |
| Telmisartan | 515.20 | 276.03 |
| | | 497.13 |
| Tramadol | 264.12 | 58.07 |
| | | 246.07 |
| | | 264.12 |

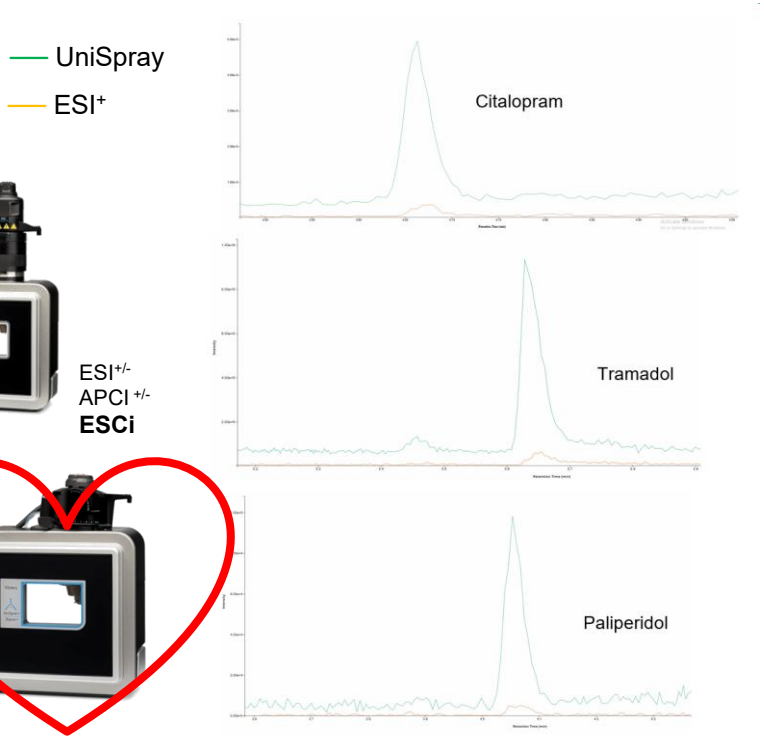



- **Antidepresiva:** citalopram, venlafaxin, sertralin
- **Antipsychotika:** haloperidol, olanzapin, paliperidon, risperidon
- **Neurologické léčivo:** memantin (Alzheimer)
- **Kardiovaskulární:** telmisartan (krevní tlak)
- **Analgetikum:** tramadol

# Analýza terapeutických léků - TDM

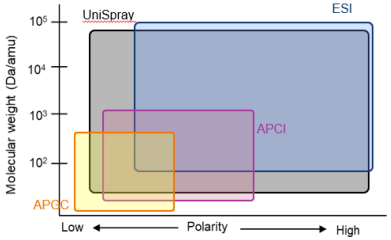
- Zlepšená citlivost ESCi **ESI+** x **UniSpray**




ESI+/-
APCI +/-
**ESCi**



**UniSpray**




| | ESI+ S/N | Unispray S/N |
|---|---|---|
| Citalopram | 40 | 81 |
| Tramadol | 25 | 82 |
| Paliperidon | 8 | 39 |
| Haloperidol | 6 | 310 |
| Memantine | 21 | 42 |
| O-desmethylvenlafaxine | 6 | 10 |
| Olanzapin | 9 | 27 |
| Risperidon | 15 | 49 |
| Sertraline | 5 | 9 |
| Telmisartan | 45 | 123 |

minimálně 2x vyšší S/N (3x, 5x, 50x)

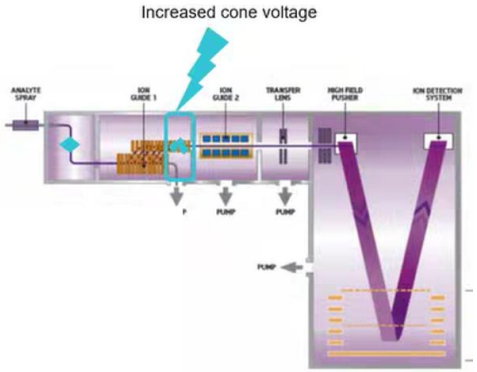
# Waters TOF; QTOF – Xevo MRT

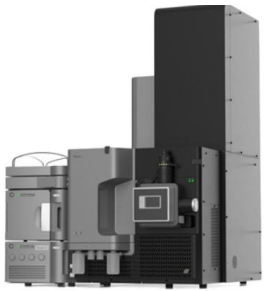
Waters™

> 10 000 FWHM
TOF 50-9000 m/z
Scan speed 1-20 Hz
Mass accurancy <2,5 ppm

*BioAccord System*

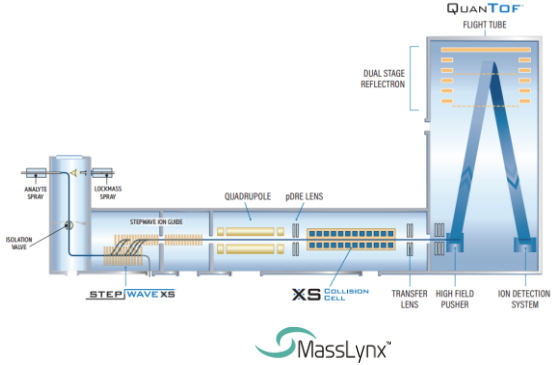
*ACQUITY RDa Detector*



> 40 000 FWHM
TOF 20 – 100 000 m/z
Q 20 – 4000 (16 000) m/z
Scan speed 1-30 Hz
Mass accurancy <1 ppm

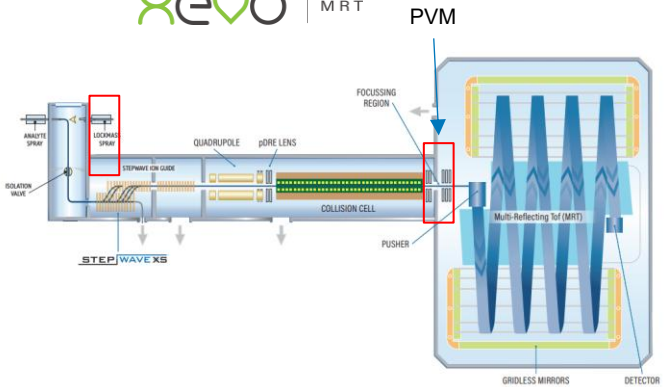
*Xevo G3 QTof*




MassLynx™

100 000 FWHM
QTOF 40 - 8000 m/z
Scan speed MS 50Hz
Scan speed MS/MS 100Hz
Mass accurancy < 0,5 ppm

XEVO™ MRT

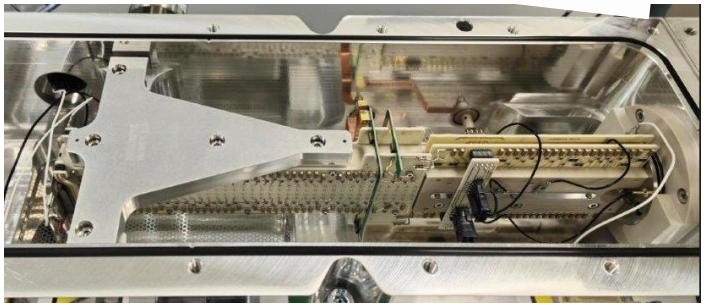


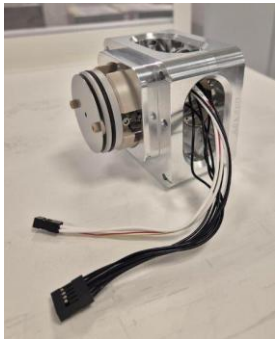

waters_connect™

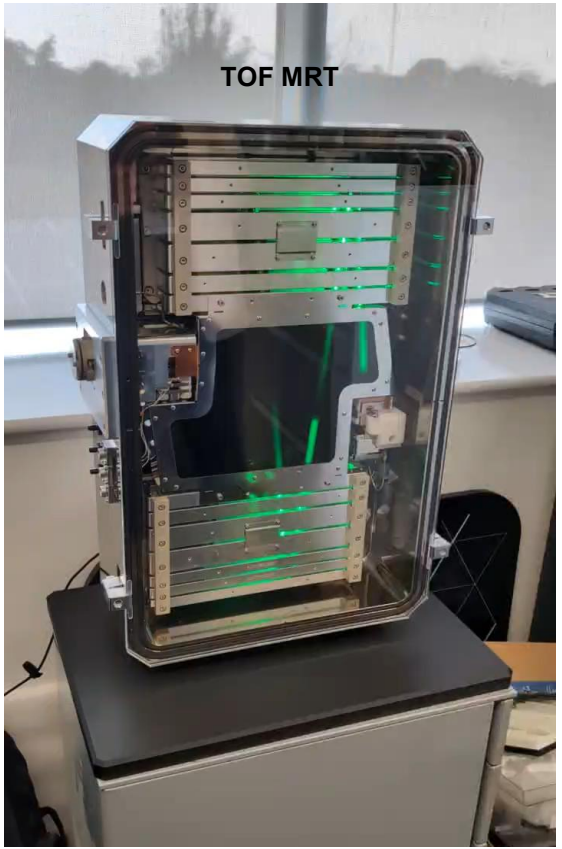
DATA convert mzML

# Hmotnostní spektrometr Xevo MRT – nová technologie

- systém optimalizován pro efektivní přenos iontů
- **menší ztráty signálu, lepší detekce stopových množství**






**Nová kolizní cela**

- rychlejší přepínání mezi různými kolizními energiemi
- **komprese iontového paprsku -** ionty se soustředí do užšího svazku
- více iontů se dostane do analyzátoru, což zvyšuje citlivost

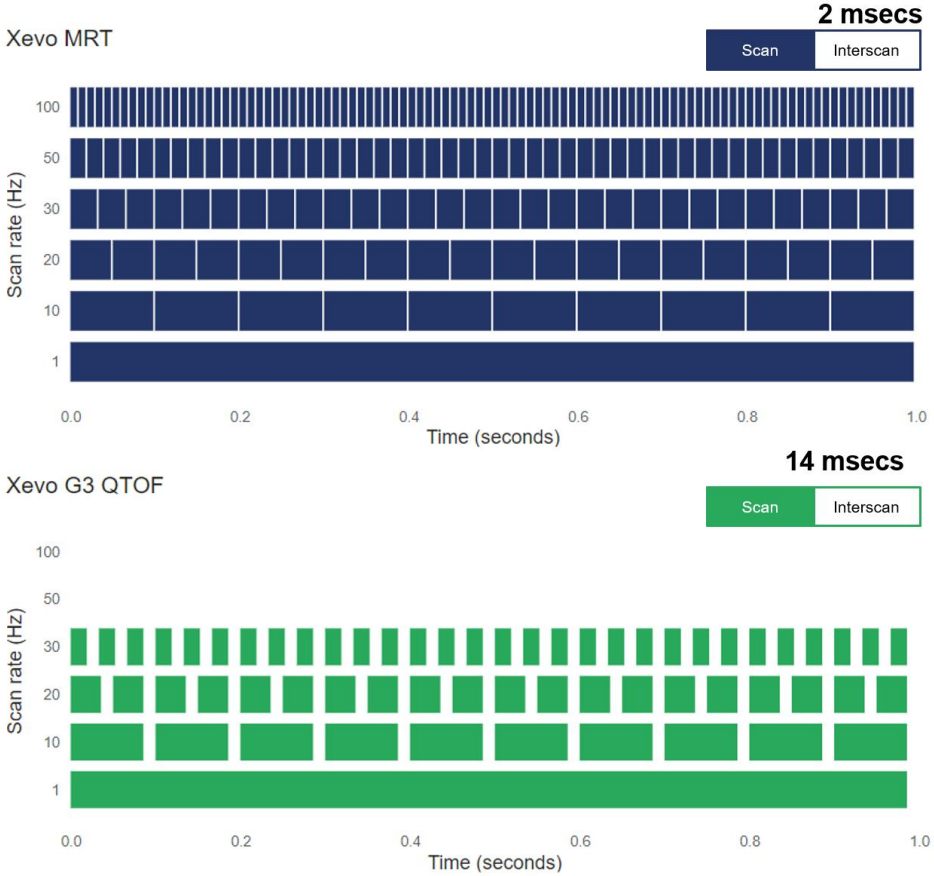
**PVM (Phase volume manipulator)**

- speciální iontová optika, která „řídí“čas průchodu iontů
- umožňuje dynamicky manipulovat s ionty – např. vybírat, zrychlovat nebo zpomalovat jejich průchod
- flexibilila - kombinace různých akvizičních režimů

**→ výsledkem je ještě vyšší kvalita dat a rychlejší, spolehlivější analýza**

# Xevo MRT Mass Spectrometer – Enhanced duty cycle (EDC)

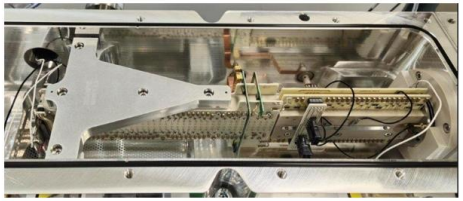
Waters™

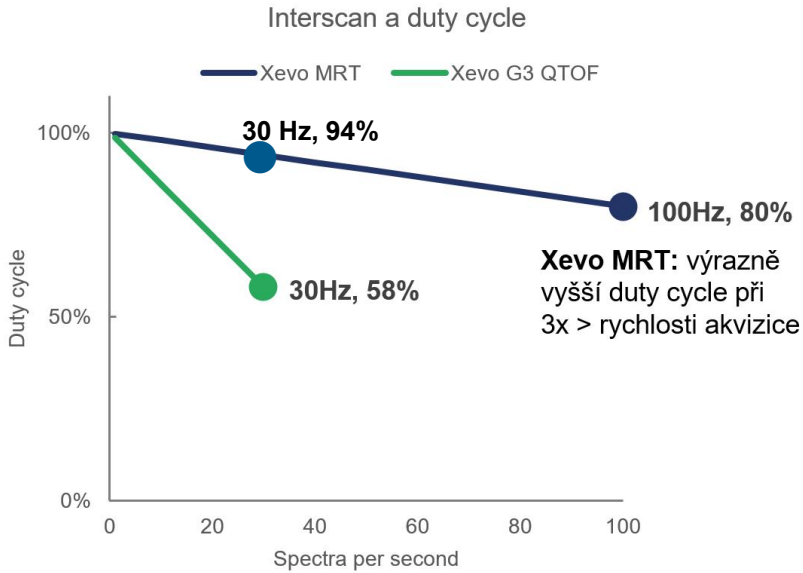



Kratší Interscan

Efektivnější sběr dat

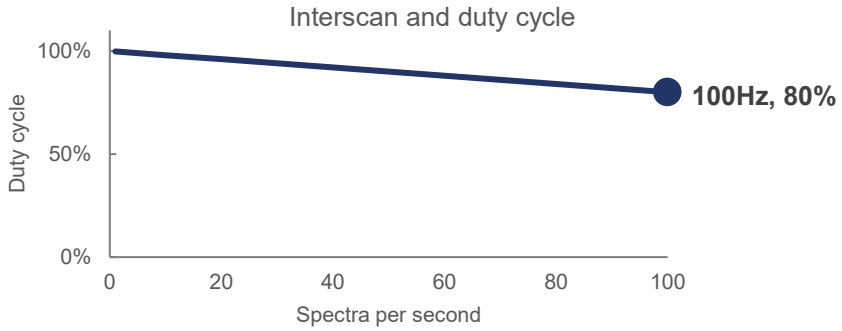
**EDC - vylepšený pracovní cyklus**
KZ + PVM – rychlé přepínání mezi scany, synchronizované vypouštění iontů do TOF → zamezení ztráty signálu, ↑ citlivost

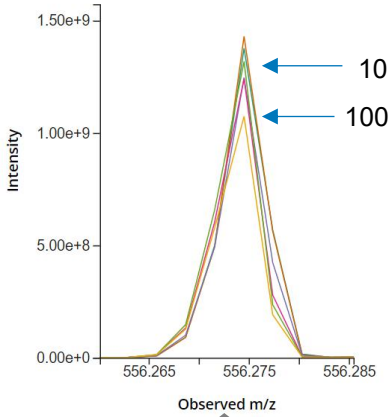


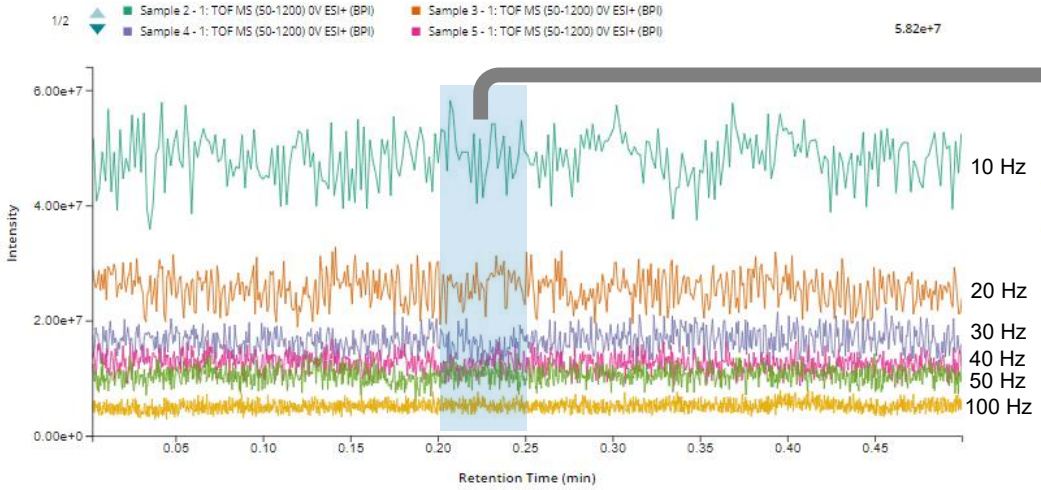


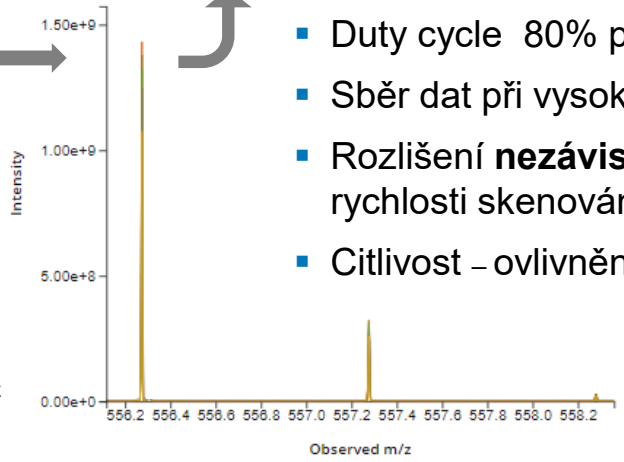
# Scan speed and sensitivity

*Série infuzního měření Leucine enkephalin 10 – 100 Hz*



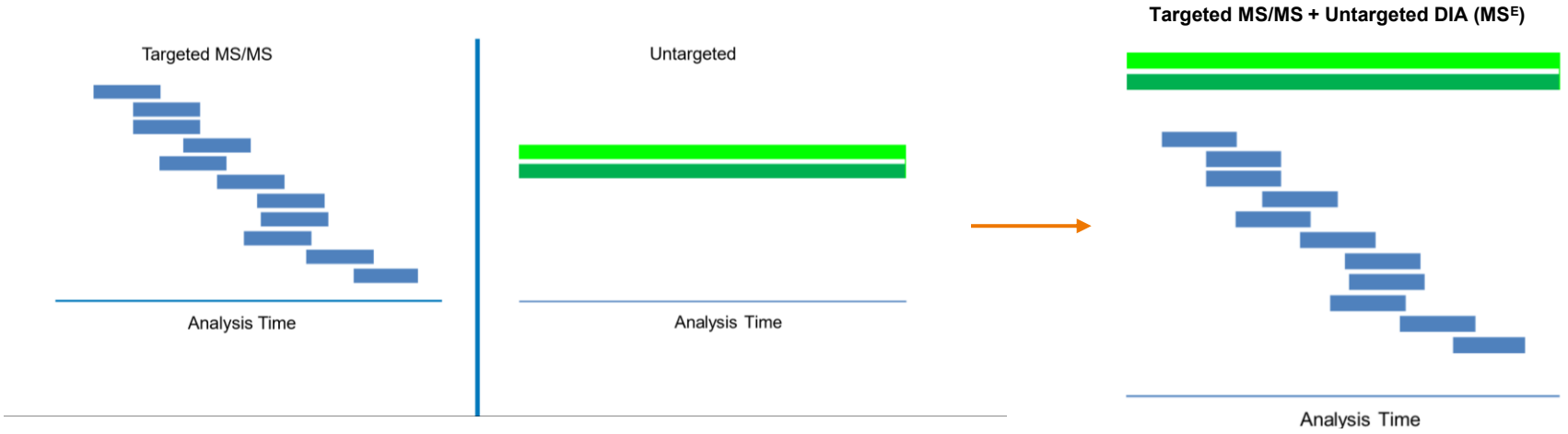




- Duty cycle 80% při 100 Hz
- Sběr dat při vysoké rychlosti
- Rozlišení **nezávislé** na rychlosti skenování
- Citlivost – ovlivněna cca 10%

# Xevo MRT – skenovací režimy

*Kombinace cíleného a necíleného přístupu - multifunkční metoda*

| Acquisition modes | Full scan MS, MS/MS, $MS^E$, DDA, TofMRM |
|---|---|

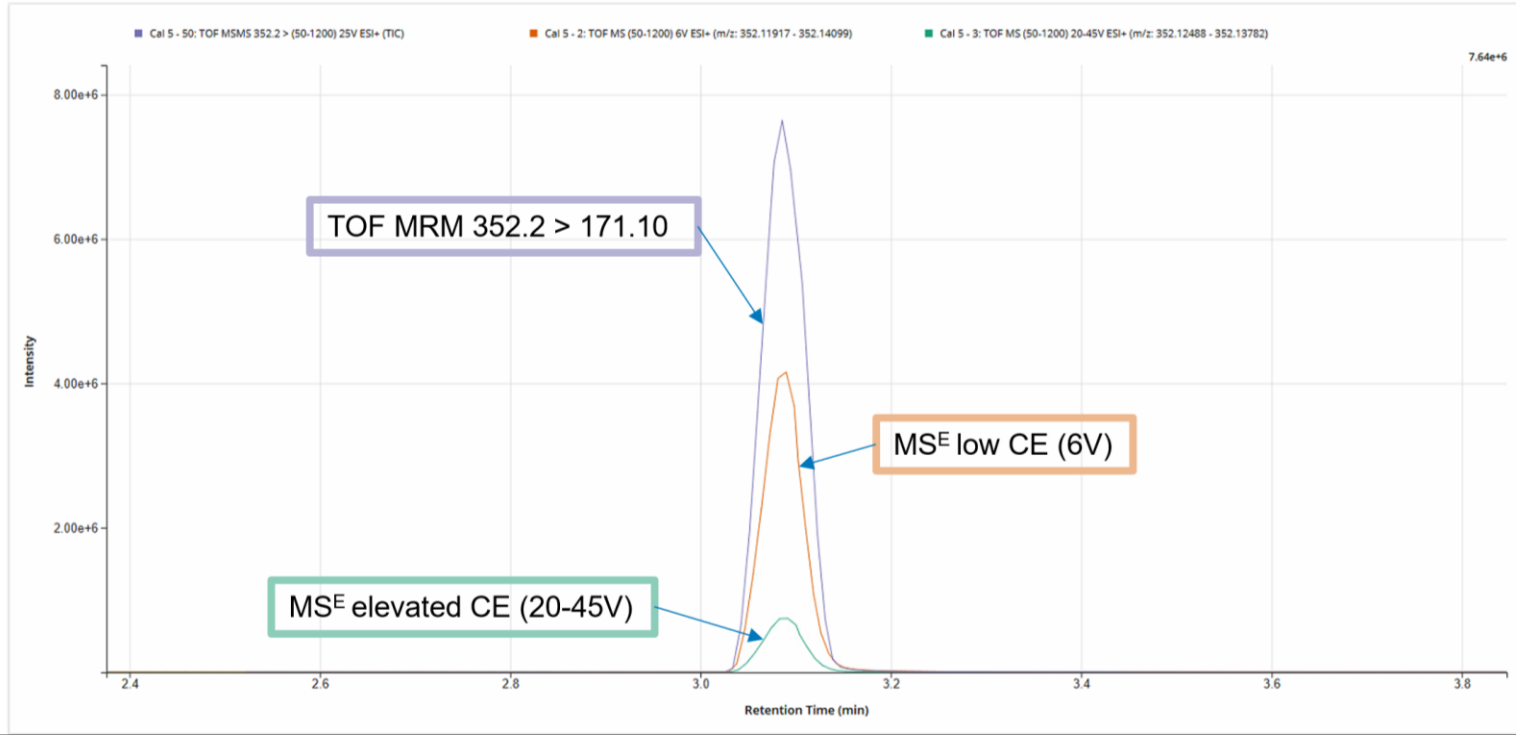


# Xevo MRT – skenovací režimy

Waters™

***Citlivost MS$^E$ x TOF MRM***

*Aminokyselina Tyrosin*



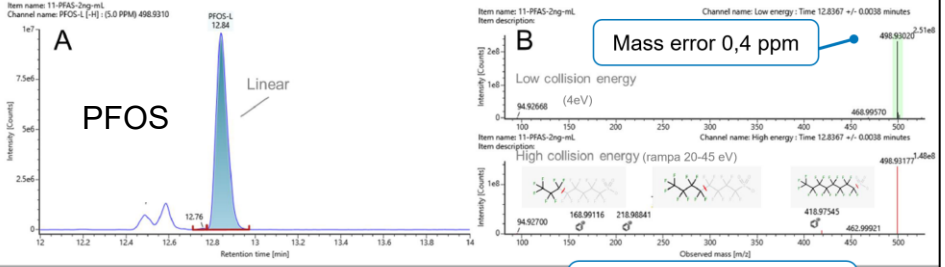
# Xevo MRT – environmentální analýza

Waters™

## Analýza PFAS – identifikace a kvantifikace

**MS$^E$** ~ DIA
- získání fragmentačních dat pro všechny nalezené ionty ve vzorku, bez výběru konkrétních prekurzorů
- umožňuje jednoznačnou identifikaci díky bohaté fragmentační informaci
- kvalitativní i kvantitativní analýza

Střídání se nízké a vysoké kolizní energie
**Low-energy scan** → zachycuje **prekurzorové ionty** (intaktní molekuly)
**High-energy scan** → fragmentuje **všechny ionty současně**, generuje fragmentační spektra
Výsledkem jsou dvě paralelní datové sady: **prekurzory + fragmenty všech iontů**
**Fragmenty (high energy)** → potvrzení identity
**Prekurzorové ionty (low energy)** → kvantifikace



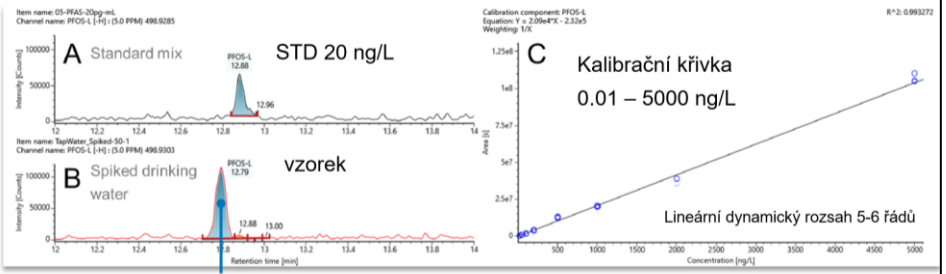

Mass error 0,4 ppm

Waters PFAS knihovna: 140 látek

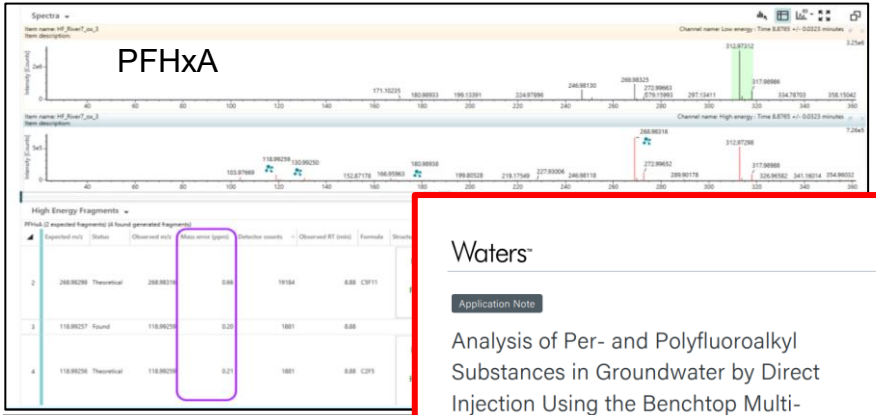
EPA knihovna: > 4,000 látek

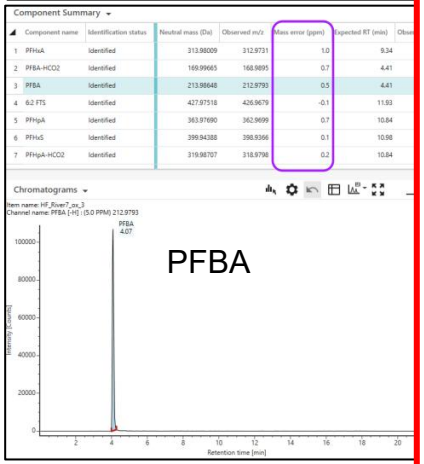
Fragmentační spektrum - otisk

## Identifikace a kvantifikace PFAS v jednom pracovním postupu




PFOS identifikován s přesností hmoty ± 0,4 ppm
S/N = 17 (PtP)
vzorek≈30 ng/L (10 µl objem nástřiku vzorku)




Waters™

Application Note

### Analysis of Per- and Polyfluoroalkyl Substances in Groundwater by Direct Injection Using the Benchtop Multi-Reflecting Time-of-Flight Xevo™ MRT Mass Spectrometer


Hania Khoury-Hollins, Sara Stead, Jayne Kirk, Richard Lock

Waters Corporation, United States



*Published on November 20, 2025*



Abstract

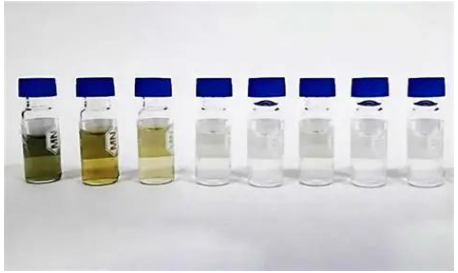
The increasing regulatory scrutiny and scientific interest in per- and polyfluoroalkyl substances (PFAS) are driving a shift in analytical workflows from traditional tandem quadrupole (TQ) methods toward high-resolution mass spectrometry (HRMS) platforms. While TQ systems are the preferred choice for absolute quantification, they are restricted by the availability of commercialized standards and the limited number of compounds that can be identified and quantified within a single method. In contrast, HRMS enables both absolute quantification and non-targeted screening (NTS) workflows simultaneously, offering a more holistic understanding of PFAS profiles in complex environmental matrices.

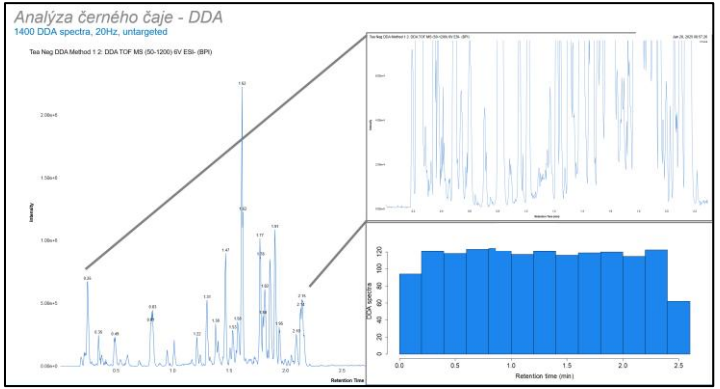
To demonstrate the capabilities of HRMS, the Xevo MRT Mass Spectrometer was evaluated for sensitivity and dynamic range by direct injection of groundwater samples and standards. 30 PFAS compounds were detected

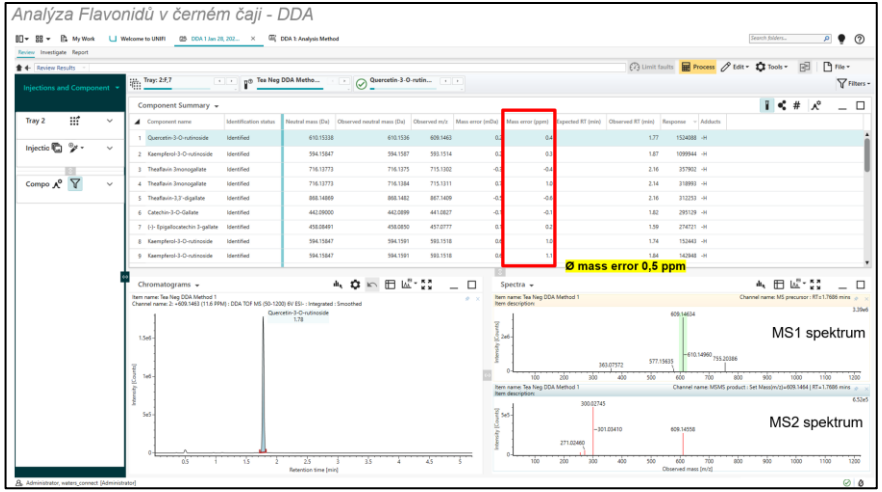
# Xevo MRT – analýza potravin

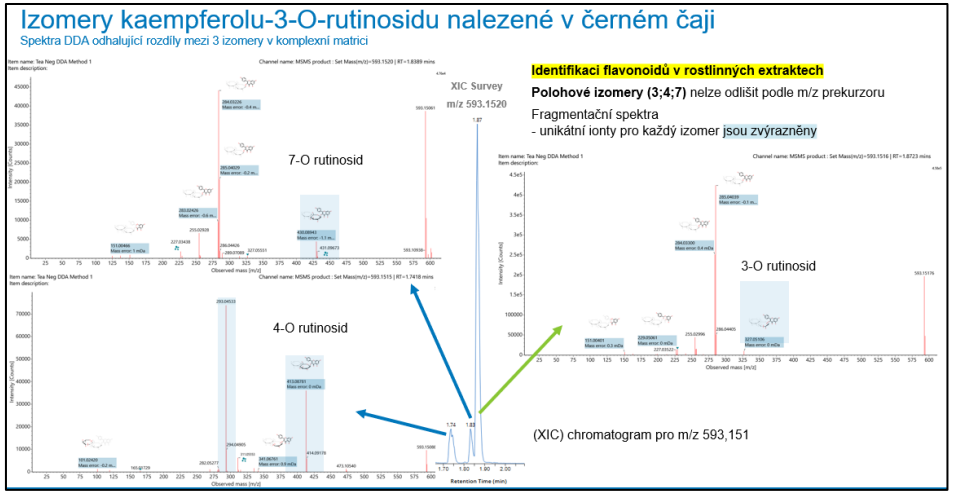
*DDA, negative*

*Identifikace – Natural products*
*Tea library*





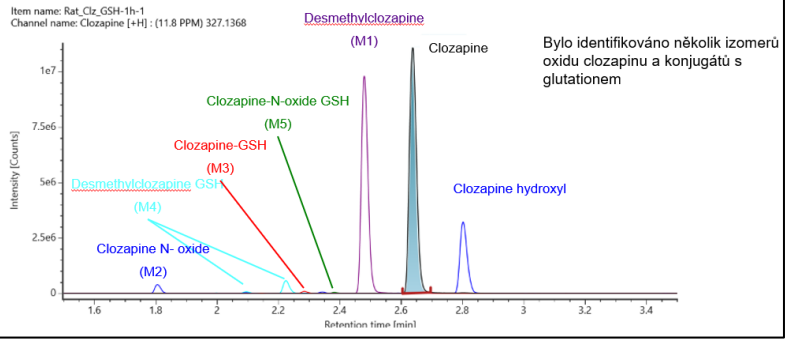



# Xevo MRT – klinická analýza

Waters™

## Clozapine a jeho hlavní metabolity v mikrosomech potkana

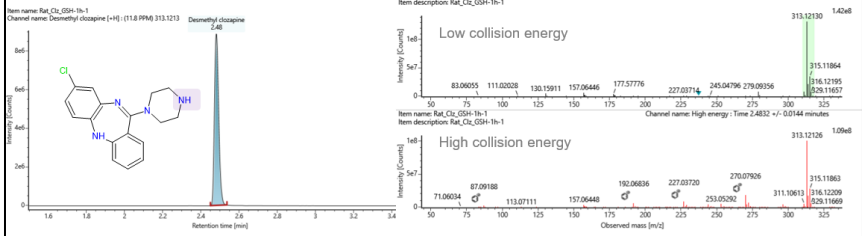
*Xevo MRT, MS$^E$ Clozapine a jeho hlavních pět metabolitů*




Bylo identifikováno několik izomerů oxidu clozapinu a konjugátů s glutationem

## N-Desmethylclozapine (M1)

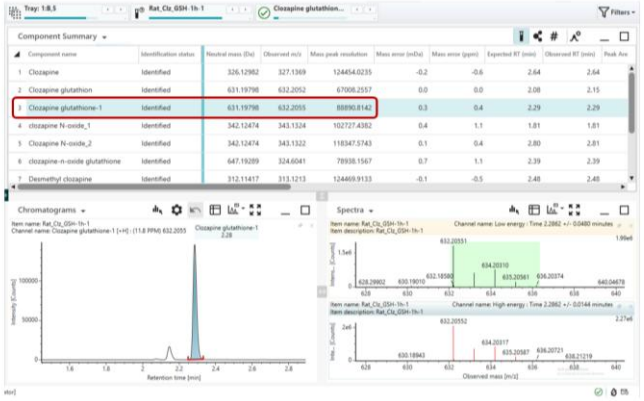
*XIC a spektra při vysokých a nízkých kolizních energiích.*



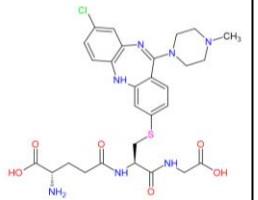

- N-desmethylclozapin byl detekován v mikrozomech potkana po 1hodinové inkubaci s klozapinem s přesností měření hmotnosti -0,42 ppm; -0,00013 Da

Monoisotopická [M+H]+ 313.12145

Observed [M+H]+ 313.12132




Clozapine-GSH
(M3)

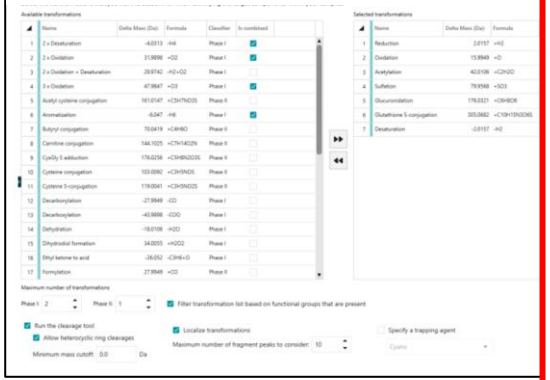


$C_{28}H_{34}ClN_7O_6S$

[M+H]+ 632.2055

Mass Peak Resolution >88k

## Met ID – metabolické transformace



Waters™

Application Note

Metabolomics Workflow using a Xevo™ MRT Mass Spectrometer

Lisa Reid, Matthew E. Daly, Lee A. Gethings, Jayne Kirk

Waters Corporation

Abstract

We demonstrate the acquisition and data processing to identify and pathway profile a typical metabolomic study acquired using a Xevo™ MRT Mass Spectrometer coupled with an ACQUITY™ Premier LC System. The resulting data shows a clear unsupervised PCA separation between the three study groups with exogenous or environmental markers being the most significant differences from the different volunteer lifestyles. The Xevo MRT MS delivers rapid, robust data acquisition for large cohort metabolomic studies, capable of providing a high mass resolution of up to 100,000 full width half maxim (FWHM) and consistent sub-ppm mass accuracy, ensuring the analyst has increased confidence in putative feature identification.

Benefits

- Simple and robust workflow delivering excellent data reproducibility and confident results
- A powerful benchtop mass spectrometer routinely providing high mass resolution for both precursor and product ions, enabling separation of compounds with near identical molecular weights e.g. isoforms
- Delivering a consistent mass accuracy <1 ppm RMS, increasing confidence in putative feature identification
- Ability to acquire data at extremely fast acquisition rates, maintaining mass resolution and mass accuracy benefits. Allowing compatibility with shorter chromatographic methods, increasing sample throughput

# Xevo MRT– toxikologický screening

Waters™

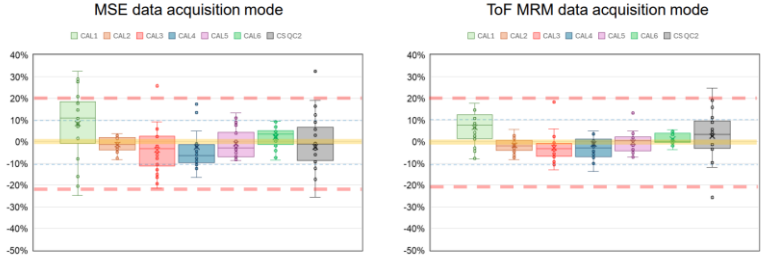
## LC-MS Conditions

LC/MS methods with ACQUITY UPLC I-Class Plus & Xevo MRT

| Ionization Mode | ESI Positive | ESI Negative |
|---|---|---|
| Run time | 15 min gradient elution | 7.5 min gradient elution |
| Mobile Phase A | 5mM ammonium formate pH 3.0 | Water with 0.001% formic acid |
| Mobile Phase B | Acetonitrile with 0.1% formic acid | Acetonitrile with 0.001% formic acid |
| Acquisition range | m/z 50-1000 | |
| $MS^E$ conditions | Collision energy ramp 10-40 eV | |
| Tof MRM conditions | 23 MRM transitions for compounds of interest | |
| Column | ACQUITY UPLC® HSS C18 Column | |
| Flow rate | 400 µL/min | |
| Injectionsvol. | 5 µL | |


## Quantitation Accuracy: Tof MRM vs. MSe

Measured quantitation deviation (%)* of a wide range of analytes at different concentrations

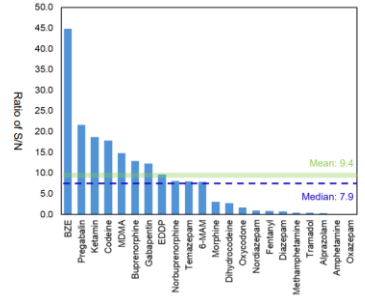



The Tof MRM method demonstrates greater quantification accuracy compared to MSe.

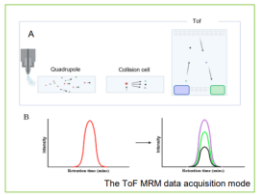
$* Deviation(\%) = \frac{(Measured - Expected)}{Expected}$, ng/mL *(míra odchylky mezi naměřenou a skutečnou koncentrací)*

## Quantitation Sensitivity: Tof MRM *vs.* MSe

Ratio of S/N measured between ToF MRM and MSe acquisition @ the same level




Tof MRM is approximately 9 times more sensitive than MSe for targeted quantitative analysis, although the actual improvement varies by analyte.

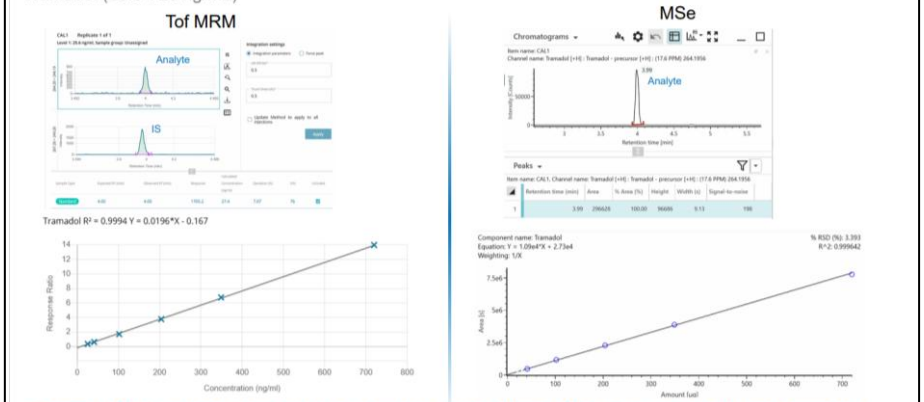



Ratio of S/N = Analyte S/N (Tof MRM) ÷ Analyte S/N (Mse)

Amphetamine and oxazepam are excluded, as their S/N in MSe mode is <3

THC-COOH is not detectable within the positive mode

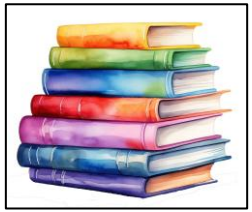
## Tramadol (25.6-720 ng/mL)




The Tof MRM method demonstrates greater linearity than MSe in the quantitation of tramadol.

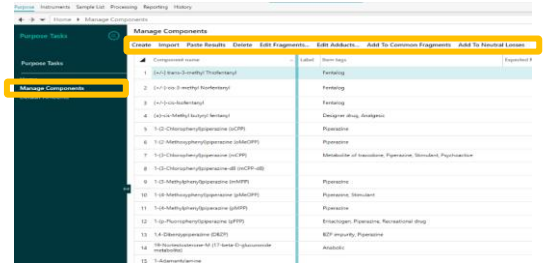
# HRMS - toxikologická knihovna

Waters™



Více než 2000 toxikologicky relevantních látek

- více než 200 analogů fentanylu
- obsahuje $t_R$, MS/MS fragmenty
- Custom library – lze upravovat




- Amphetamines
- Anaesthetic agents
- Analgesics
- Antiarrhythmics
- Anticonvulsants
- Antiepileptic
- Antimalarial
- Antipsychotics
- Anti-seizure
- Antidepressants
- Antihypertensive
- Benzodiazepines

- Cocaine + metabolites
- Opioids
- Fentanyl/Fentanyl analogues
- Novel Psychoactive Substances
- Cathinone's
- Hallucinogen's
- Nitazene's
- Synthetic Cannabinoid's
- Pesticides (136)
- Piperazines
- SSRIs /TCAs

**Toxikologický screening - Identifikace látek – tři přístupy:**

- **Targeted analysis**
  - analyzuje se přesně definovaný seznam (prekurzorů, fragmentů, tR)
  - vyhledávání Waters HRMS knihovna (Scientific Libraries → Master library, custom library)
  - obsahuje experimentální údaje měřená spektra, fragmenty, retenční časy, CCS hodnoty
- **Semi-targeted analysis**
  - analyzuje se širší seznam potenciálních látek, ale nejsou k dispozici experimentální údaje (tR, fragmenty)
  - k identifikaci využívá vyhledávání MOL. files (prekurzory, struktura)
  - Waters používá algoritmy (UNIFI fragment prediction), které umí „fragmentovat"molekulu podle chemicky realistických míst
  - vygeneruje **teoretické fragmenty (in-silico fragments)**
- **Non-targeted analysis**
  - vyhledává se vše, co je měřitelné
  - vyhledávání v knihovnách třetích stran (NPS, CHU Life, Wiley, NIST, CHemSpider)

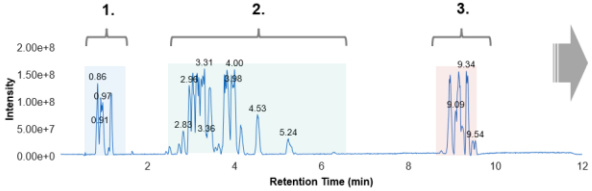
| Name of the library | NPS library[1] | CHU Lille Developed library[2] | Wiley Library Maurer, Meyer, Hefler, Weber: LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites |
|---|---|---|---|
| # of analytes | 2373 | 1,483 | Over 3,200 |

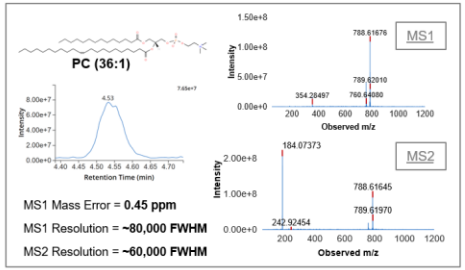
# Xevo MRT – lipidy

Waters™

## High Throughput
*Analýza lipidů*

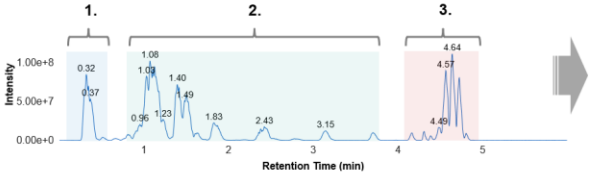
Conventional – 12 min

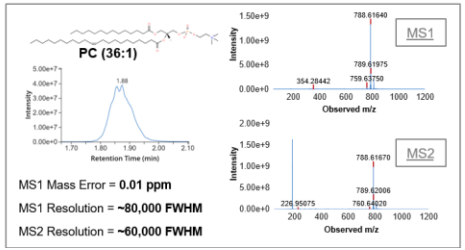



MS1 Mass Error = **0.45 ppm**
MS1 Resolution = **~80,000 FWHM**
MS2 Resolution = **~60,000 FWHM**

High-throughput – 5 min




MS1 Mass Error = **0.01 ppm**
MS1 Resolution = **~80,000 FWHM**
MS2 Resolution = **~60,000 FWHM**

1. FFA/Lysophospholipids; 2. Glycerophospholipids; 3. Glycerolipids

Waters™

Application Note

High Sensitivity, High Throughput LC-MS Analysis of Eicosanoids Using the Xevo™ MRT QTof

Nyasha Munjoma, Gemma Molyneux, Lee A. Gethings, Jayne Kirk, Richard Lock

Waters Corporation

Waters™

Application Note

The Achievability of Single Cell Lipidomics Utilizing the Novel Xevo™ MRT Mass Spectrometer

Scarlet Ferrinho, Nyasha Munjoma, Preeti Mourya, Shazneil Briones, Lee A. Gethings, Robert S. Plumb, Oliver Cexus, Paul A. Townsend

Waters Corporation, Faculty of Health and Medical Sciences, University of Surrey

[ PRODUCT SOLUTION ]

## A High-Throughput Lipodomic Workflow Using the Waters Xevo MRT Mass Spectrometer

### INTRODUCTION

The Xevo™ MRT Mass Spectrometer delivers an unrivaled combination of performance at speed, providing high resolution time-of-flight data independent of spectral acquisition rate. The MS specification of 100,000 (FWHM) resolution at scan rates of up to 100 Hz combined with <500 ppb mass accuracy enables confident identification of analytes at throughput levels that enable large cohort experiments.

The cutting-edge multi-reflecting ToF technology that Waters™ Corporation pioneered in the SELECT SERIES™ MRT, has been scaled into a compact benchtop platform to solve the most challenging problems in biomedical research and epidemiological studies.

In large scale metabolic phenotyping experiments performance is critical, from instrument stability over large studies to precision of measurement, generating high quality experimental outcomes. The novel acquisition system, with dual gain analogue-to-digital converter, delivers long term system stability over a high dynamic range, ensuring exceptional quality data with consistent robustness and reproducibility.

Here we describe, using an exemplar sample set, the use of the Xevo MRT Mass Spectrometer for the analysis of colorectal cancer human serum samples[1]. This combined with market leading chemistry and separations, using premier technology, and informatics including LipoStar2 from Mass Analytica™ (MassAnalytica.com) describes a complete lipidomic workflow.

### EXPERIMENTAL CONDITIONS

**SAMPLES**

| Samples | |
|---|---|
| Colorectal cancer (CRC) human serum samples | 6 Healthy control plasma |
| | 4 Colon cancer plasma |
| | 2 Rectum cancer plasma |
| | NIST SRM 1950 plasma |
| | Study Reference/pooled (QC) |
| | A 100x dilution of EquiSPLASH |

| LC conditions | |
|---|---|
| LC System | Waters ACQUITY™ Premier |
| Column | ACQUITY Premier UPLC™ CSH™ $C_{18}$ 2.1 x 50 mm, 1.7 µm |
| Column Temperature | 55 °C |
| Sample temperature | 8 °C |
| Injection volume | 0.5 µL |
| Mobile phase A | 50:25:25 H20:IPA:MeCN w/5 mM ammonium acetate and 0.05% acetic acid |
| Mobile phase B | 50:50 IPA:MeCN w/5 mM ammonium acetate and 0.05% acetic acid |
| Flow rate | 0.4 mL/min |

Lipostar2

# Xevo MRT – DESI XS (2025)

*HD Imaging (rozlišení 5 μm)*

waters_connect™

[ SOLUTION SHEET ]

Waters™

## DESI XS with Xevo MRT Mass Spectrometer for MS Imaging

**Accelerating imaging. Amplifying sensitivity. Advancing visualization.**

### INTRODUCTION

This product solution highlights the capabilities of the Xevo™ MRT mass spectrometer equipped with the DESI™ (Desorption Electrospray Ionization) XS source (Figure 1), demonstrating its ability to provide rapid acquisitions while maintaining high sensitivity for MS imaging. With combined high mass resolution and mass accuracy, your lab can achieve greater spectral quality, improved analyte detection, and higher confidence in compound identification. The data presented includes data acquired in both positive and negative ionization modes and compares various acquisition speeds.

### THE POWER OF DESI XS

Analysis on the DESI XS source offers a powerful and versatile approach to mass spectrometry imaging, delivering a range of benefits that enhance a wide range of applications, including pharma, chemical materials, and pre-clinical research applications. One of the most significant advantages of DESI is its ability to perform ambient image acquisitions without the need for extensive sample preparation or matrix application. The direct sample analysis without the application of matrices makes DESI ionization particularly valuable for high spatial resolution acquisitions where application of a matrix could lead to analyte movement. Whilst simple, rapid sample preparation makes it ideal for real-time analysis applications.

The DESI XS source supports high spatial and high mass resolution imaging, currently offering analyses with pixel sizes as small as 5 µm, enabling single-cell image resolution. This level of detail allows researchers to visualize molecular distributions with exceptional fidelity, crucial for understanding complex biochemical processes and tissue heterogeneity. The integration of Microscope Mode within High-Definition Imaging (HDI™) Software further enhances efficiency by allowing rapid survey scans followed by targeted high-resolution re-acquisition of regions of interest [1].

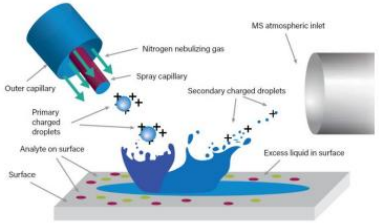
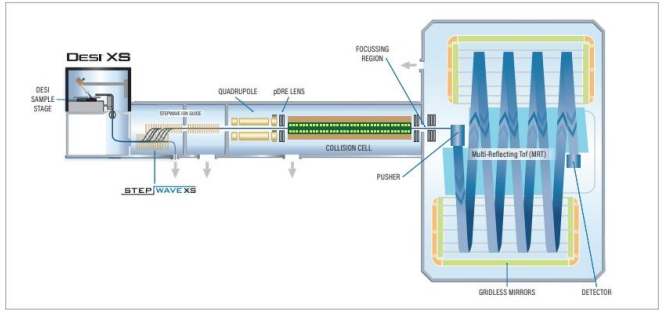
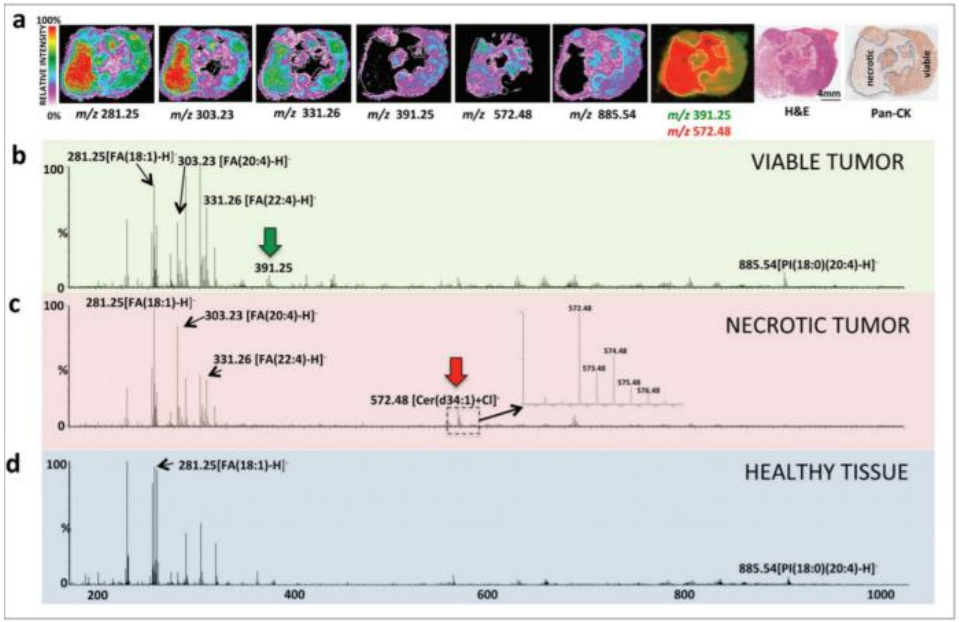



*Figure 1. Next-generation solution for MS imaging: DESI XS with Xevo MRT mass spectrometer.*




Waters™

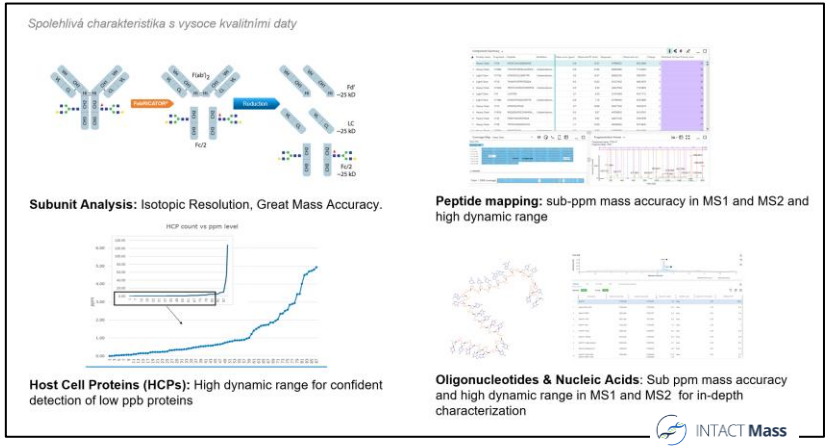


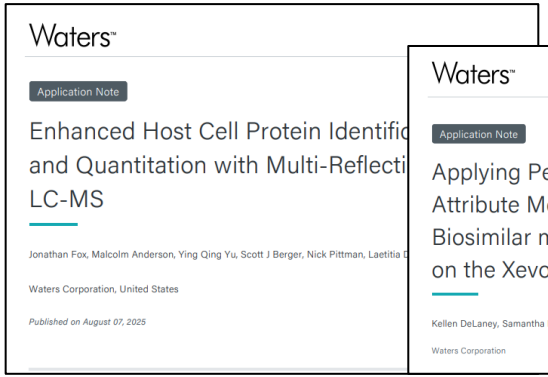


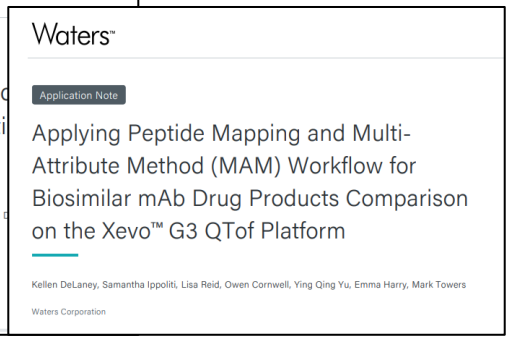

Scientific Reports | 6:35374 | DOI: 10.1038/srep35374

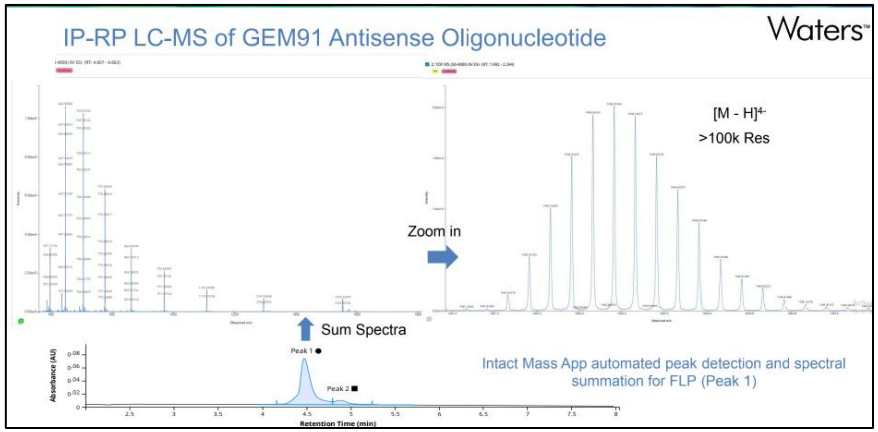
# Xevo MRT – mAb, proteiny, peptidy, oligonukleotidy

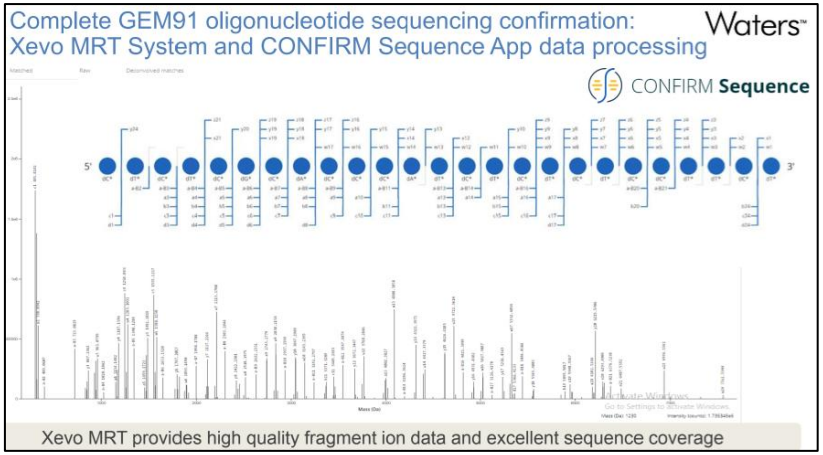
Waters™



UniSpray



DESI XS

Waters™

waters_connect™