Hmotnostná spektrometria a jej úloha v metabolomike

Mariana Danková

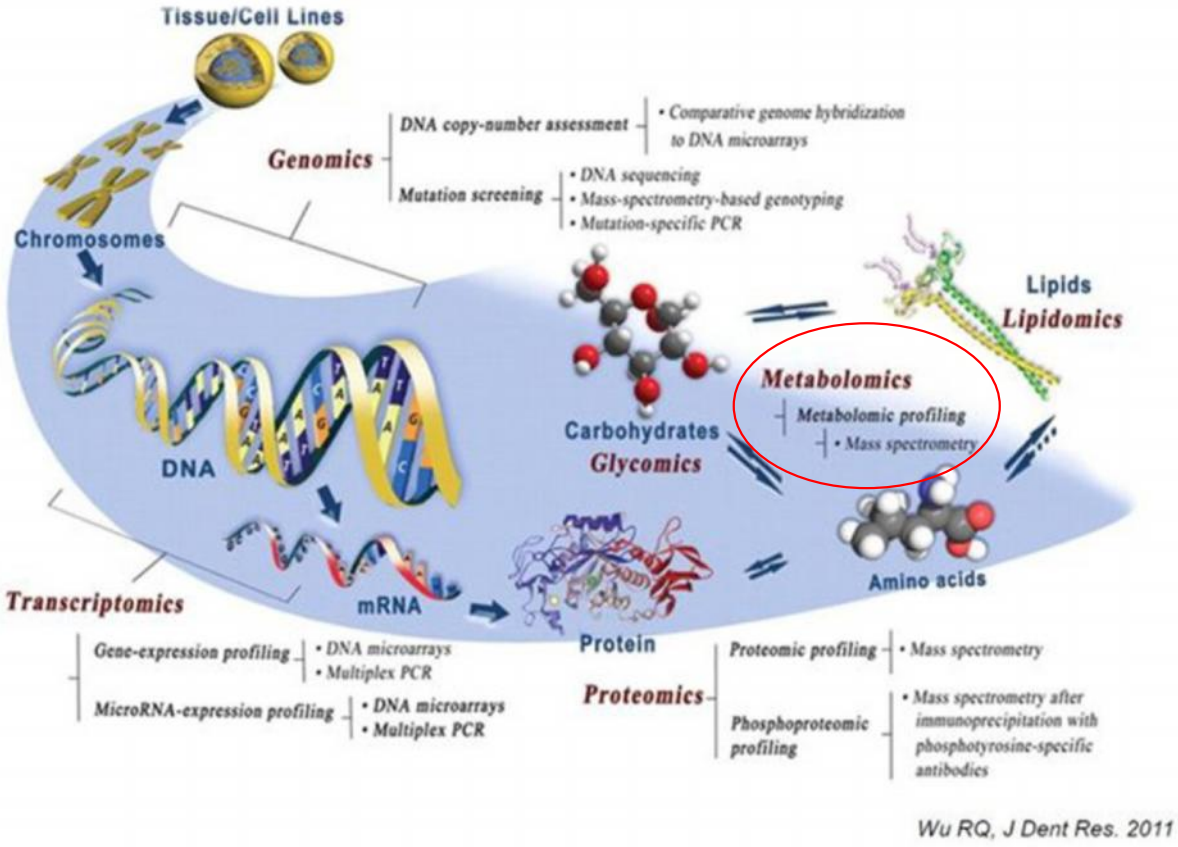
AGENDA

- METABOLOMICS
- MASS SPECTROMETRY
- RESOLUTION/ACCURATE MASS
- HRMS
- APPLICATIONS

METABOLOMICS

- komplexná analýza metabolomu v konkrétnom fyziologickom alebo vývojovom štádiu organizmu, tkaniva alebo bunky

- hladina metabolitov-metabolický "pool", nie je odpoveď iba génovej expresie, ale aj environmentálneho a vývojového stimulu alebo dôsledok genetickej mutácie



METABOLOMICS

Environment

Microbiome

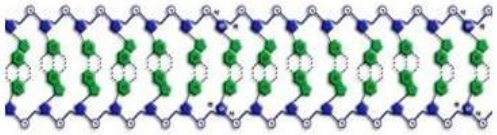
DNA

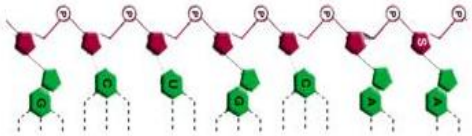
RNA

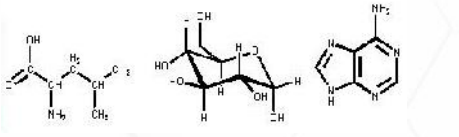
Proteins

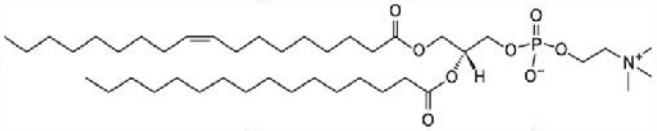
Metabolites

Lipids









Genomics – 22,000 genes
Biological potential

↓

Transcriptomics – 100,000 transcripts
Response to conditions

↓

Proteomics – 1,000,000 proteoforms
Biological Function

↓

Metabolomics >5,000 compounds
Lipidomics >30,000 species
Physiological state/phenotype

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CHALLENGES FOR METABOLOMICS

Diversity in structures and physical chemical properties

- Require multiple technologies to capture a metabolome

Many isomeric and isobaric species

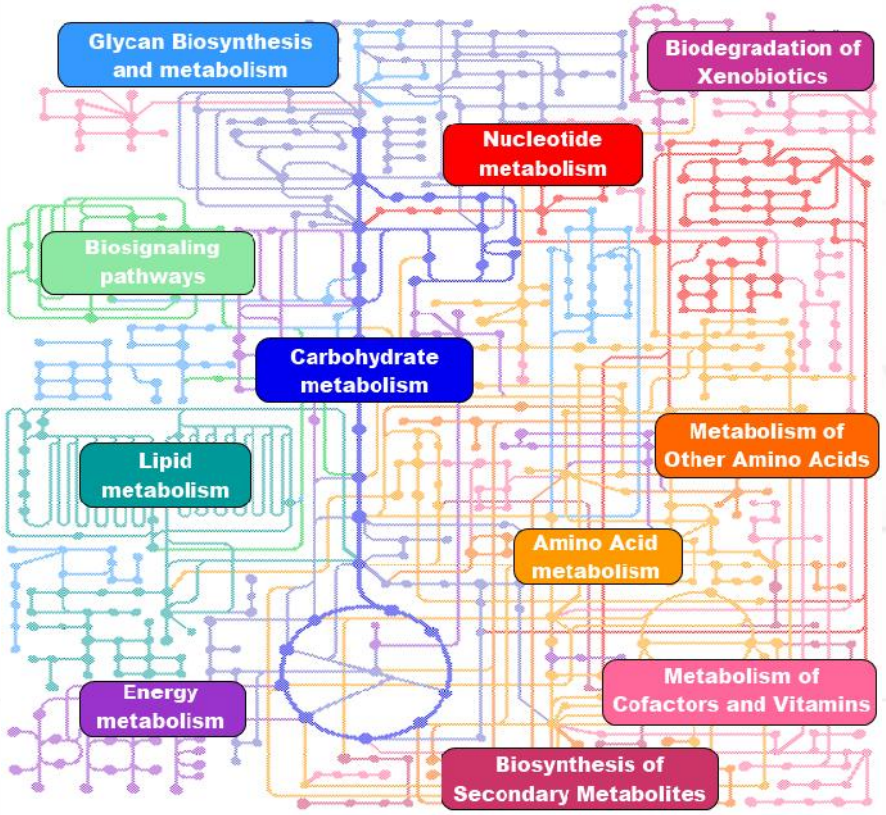
- Require high resolving power for correct ID

Very low to very high concentrations

- Require High sensitivity and wide dynamic range

No single database to identify all unknown metabolites

- Require extensive library or fragment ion prediction based on compound structure



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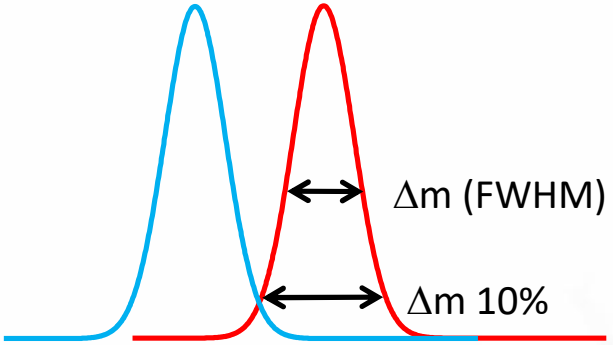
RESOLUTION (RESOLVING POWER)

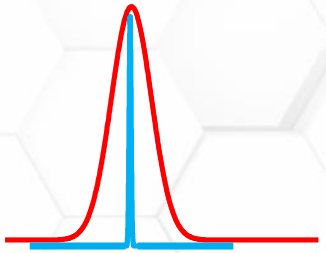
- **Resolution**

$$R = \frac{m}{\Delta m}$$



- Quadrupole MS

$$R = \frac{m}{\Delta m} = \frac{500}{0.6} = 833$$



- HRMS (Orbitrap MS)

$$R = \frac{m}{\Delta m} = \frac{500}{0.005} = 100000$$

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HOW ACCURATE IS YOUR MASS?

- **Mass accuracy**

$$\Delta m/z = \frac{m_{meas} - m_{true}}{m_{true}} \cdot 10^6$$

- Quadrupole MS

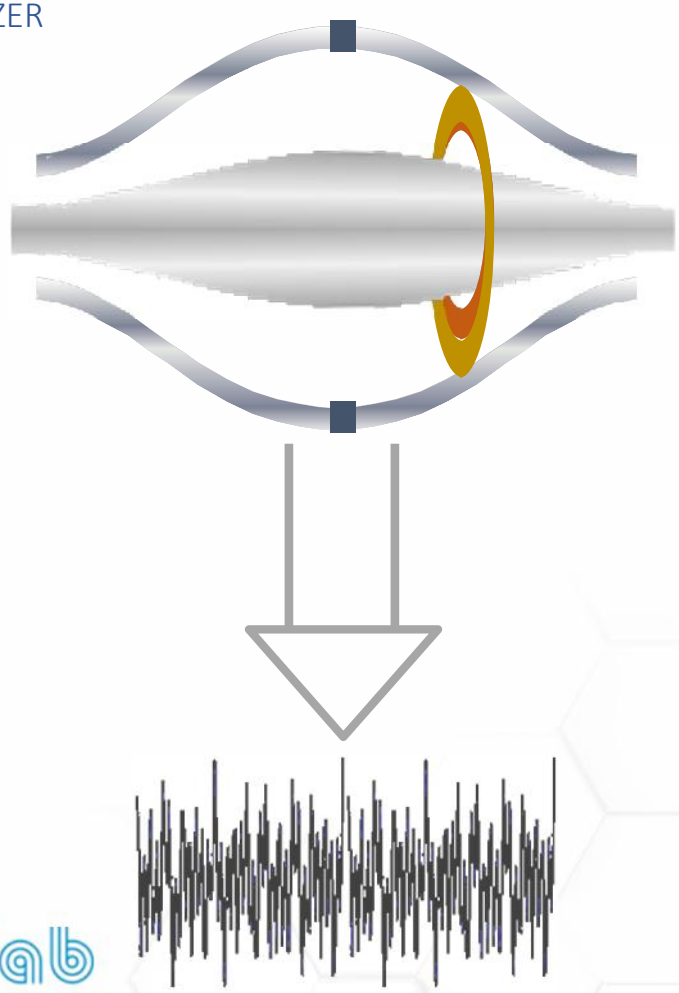
$$\frac{\Delta}{} = \frac{500.10 - 500.00}{500.00} \cdot 10 =$$

- HRMS (Orbitrap MS)

$$\frac{\Delta}{} = \frac{500.10314 - 500.10214}{500.10214} \cdot 10 =$$

ORBITRAP: THE PERFORMANCE LEADER SINCE 2006

ORBITRAP MASS ANALYZER



$$\mu = \sqrt{\frac{k}{m/z}}$$

- Ions injected into the Orbitrap are trapped in an electrostatic field
- Each ion oscillates axially with a frequency that is proportional to its mass
- An image current of these oscillations is measured using a split outer electrode
- This image is then converted to a mass spectrum using Fourier transform
- The longer a signal (transient) is measured, the higher the resolution

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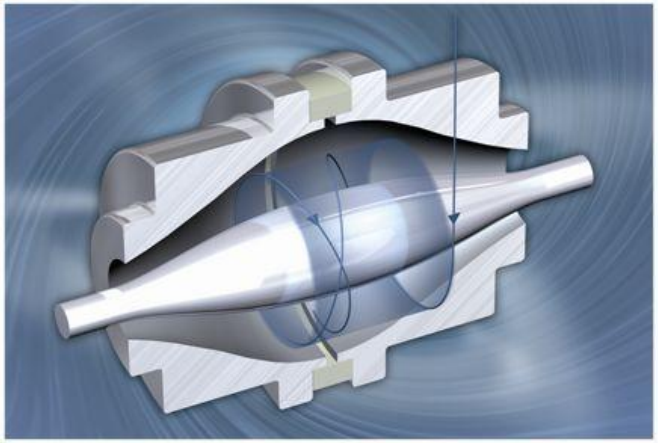
ORBITRAP: THE PERFORMANCE LEADER SINCE 2006

ORBITRAP MASS ANALYZER



What Orbitrap provides?

- Fundamental difference to other HRAM instruments
- Parameter measured is **frequency**, not time/voltage/etc
- Resolution for more accurate *m/z* determination
- Less prone to ambient conditions changes
- Stability within <1-2 ppm during several days
- No need for lock mass in “routine work”
- Small footprint
- Easy setup

Which applications?

- accurate identification, structural analysis, and quantification of organic molecules, lipids, carbohydrates, peptides & proteins in complex mixtures

Unmatched ultrahigh resolution, accurate mass performance

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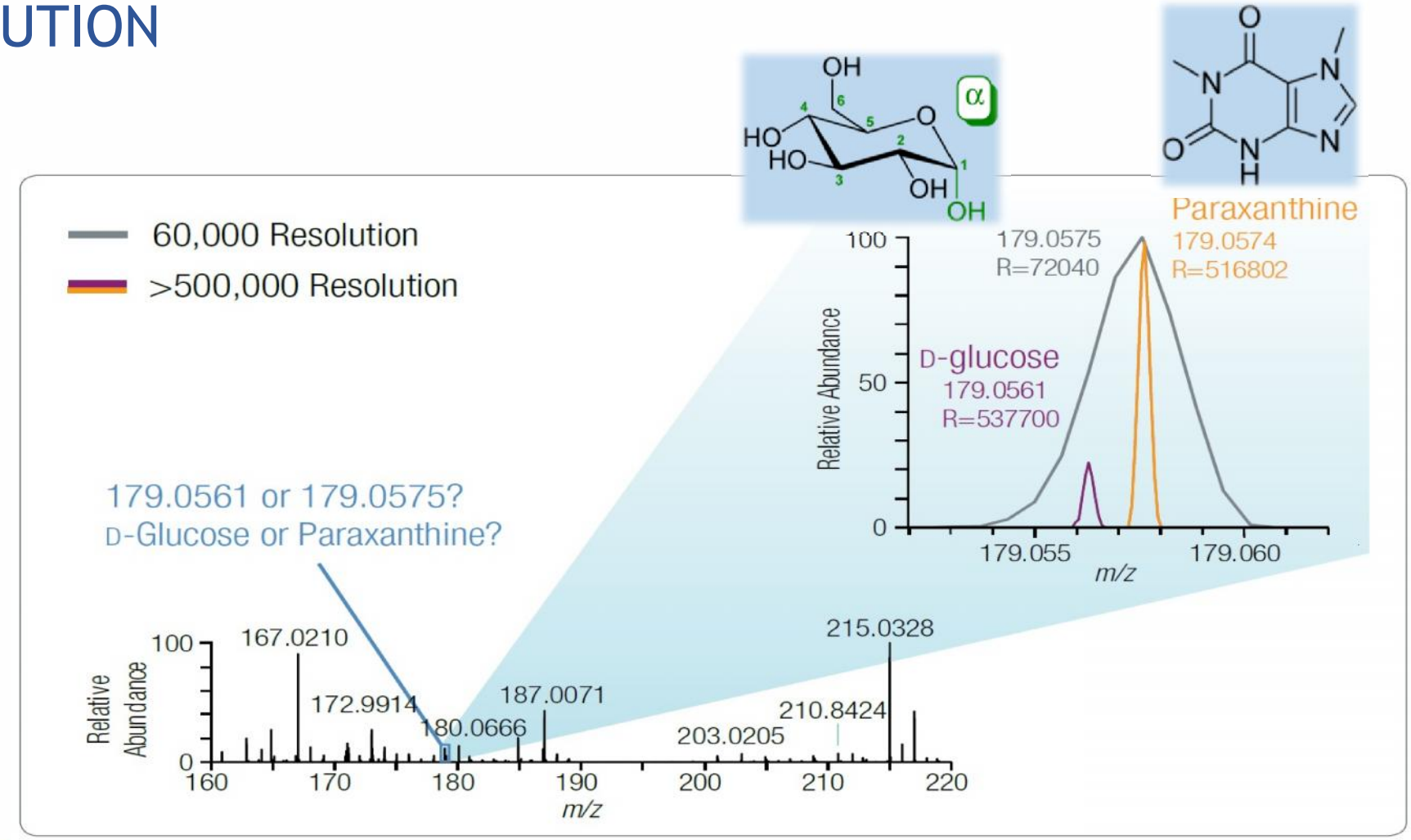
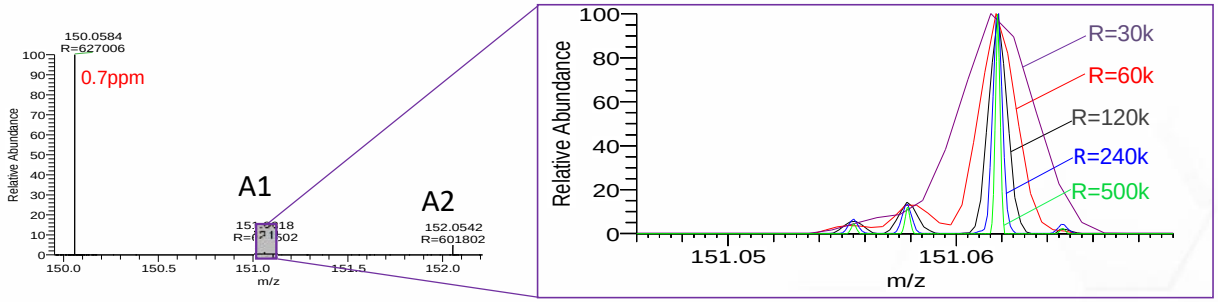
RESOLVING ISOBARIC METABOLITES WITH ORBITRAP HIGH RESOLUTION



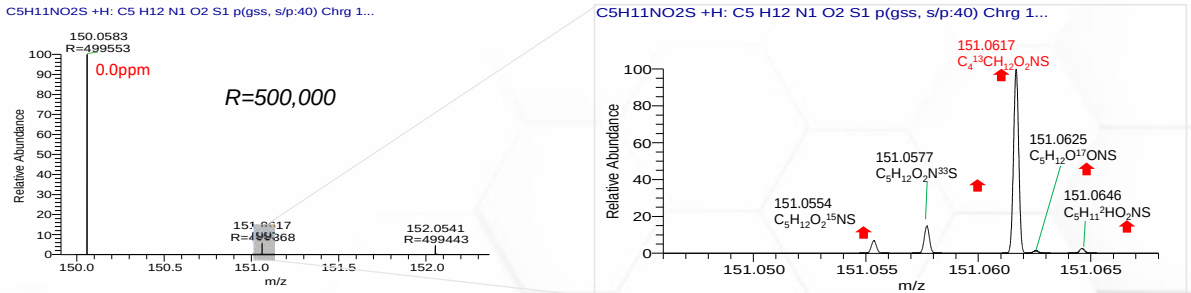
HIGH RESOLUTION IS FOR FINE ISOTOPIC PATTERN DETERMINATION & ISOTOPIC LABELING

- L-Methionine $C_5H_{11}NO_2S$ (+ mode)

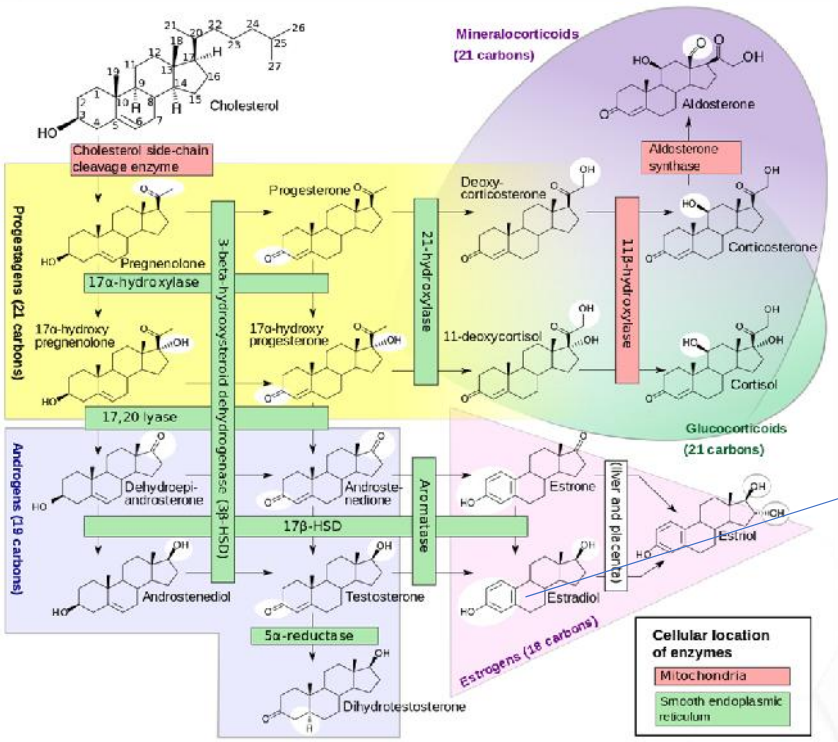
Observed



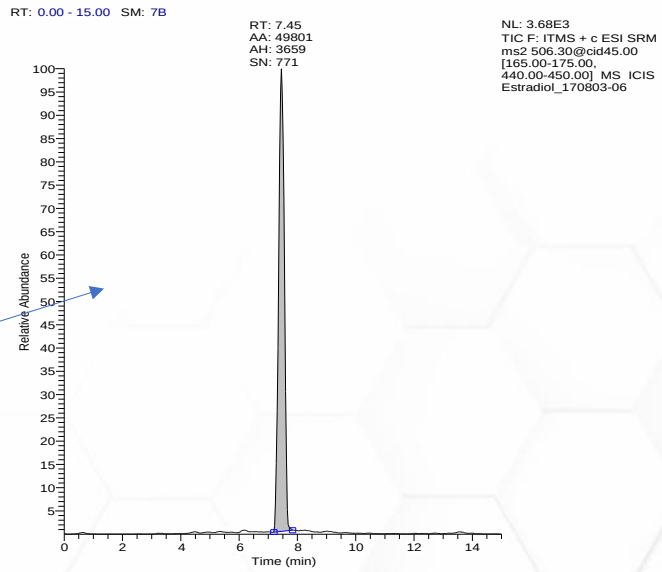
Simulated



SENSITIVITY



57,58 fg „on column“ in real sample



Estradiol is a steroid, an estrogen, and the primary female sex hormone

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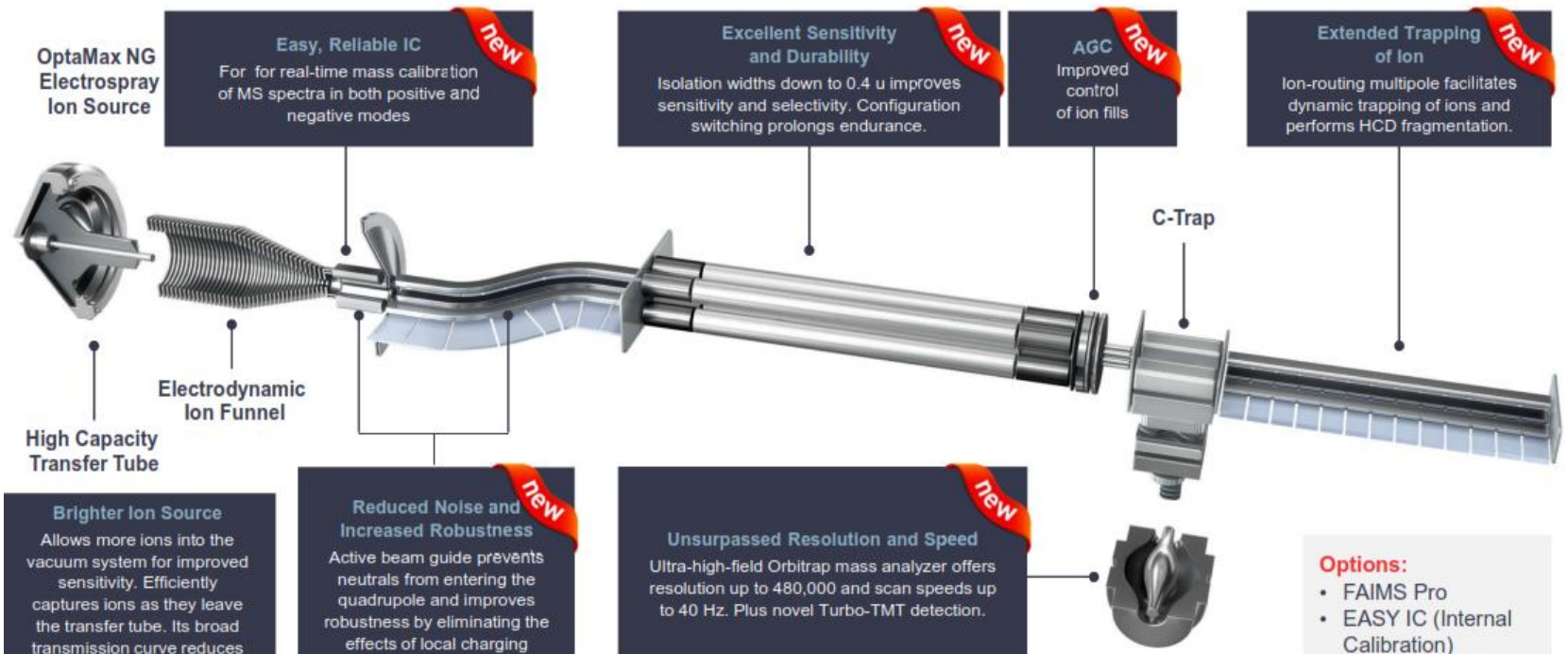
EXPLORIS 480



Options:

- FAIMS Pro
- EASY IC (Internal Calibration)
- BioPharma option (up to *m/z* 8,000)

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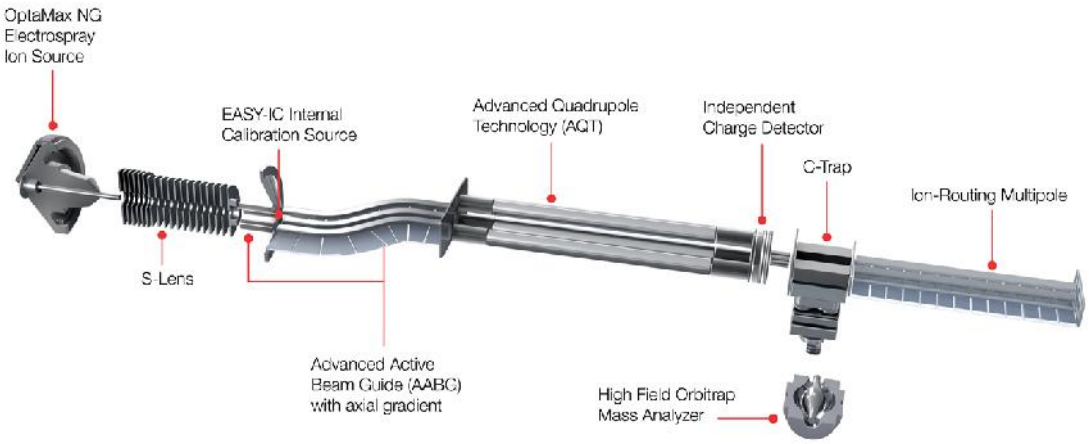
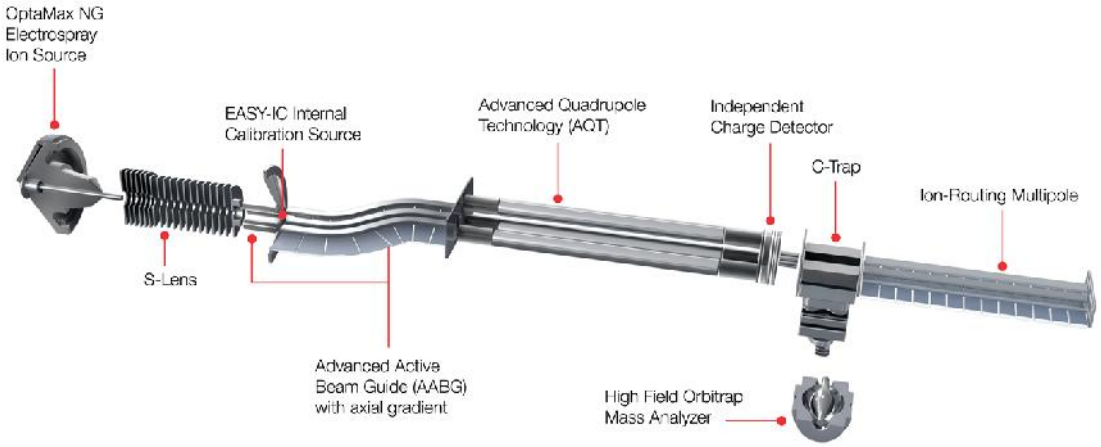
EXPLORIS 240

Mass Range
Standard mass range ***m/z* 40–6,000, up to *m/z* 8,000** with BioPharma option
Orbitrap Mass Analyzer Resolution
Up to **240,000** at *m/z* 200
Scan Rate*
Up to **22 Hz** at resolution setting 15,000 at *m/z* 200
Mass Accuracy*
External calibration achieves <3 ppm RMS drift over 24 hours;
Internal lock mass calibration achieves <1 ppm RMS drift over 24 hours;
EASY-IC achieves <1 ppm RMS drift for at least 5 days
Sensitivity
MS/MS: 200 fg reserpine on column S/N 100:1
tSIM: 200 fg reserpine on column S/N 250:1
Dynamic Range
>5,000 within a single Orbitrap mass analyzer spectrum
Polarity Switching
One Full Scan cycle** <700 ms equals >1.4 Hz
One tSIM Scan cycle** <600 ms equals >1.6 Hz
Multiplexing
Up to 20 precursors per scan



EXPLORIS 120

Mass Range
Standard mass range ***m/z* 40–3,000**
Orbitrap mass analyzer Resolution
Up to **120,000** at *m/z* 200
Scan Rate*
Up to **22 Hz** at resolution setting 15,000 at *m/z* 200
Mass Accuracy*
External calibration achieves <3 ppm RMS drift over 24 hours;
Internal lock mass calibration achieves <1 ppm RMS drift over 24 hours;
EASY-IC achieves <1 ppm RMS drift to at least 5 days
Sensitivity
MS/MS: 200 fg reserpine on column S/N 100:1
tSIM: 200 fg reserpine on column S/N 250:1
Dynamic Range
>5,000 within a single Orbitrap mass analyzer spectrum
Polarity Switching
One Full Scan cycle** <700 ms equals >1.4 Hz
One tSIM Scan cycle** <600 ms equals >1.6 Hz
Multiplexing
Up to 2 precursors per scan for tMS2 and up to 20 compounds per scan for tSIM



MYCOTOXINS IN ANIMAL FEED

- Mycotoxins are toxic secondary metabolites produced by fungi that can grow on food products and crops
  - **These toxins account for the annual loss of millions of dollars worldwide in human health, animal health, and condemned agricultural products**
- Animal producers often test their feed in order to determine the level of mycotoxin contamination
  - **Producers use this information to determine how much to dilute the contaminated feed in order to safely feed their production animals.**
- The European Commission, US Food & Drug Administration and other regulations
  - **Have set up guidelines for the maximum tolerance levels for mycotoxins in products intended for use as animal feed**

Global guidance levels (ng/g) for mycotoxins in feed products intended for animal use

Mycotoxin	Canada	US	EU	China
Aflatoxin	20	20 - 300	5 - 20	10 – 50 (B1)
DON	1000 - 5000	5000 – 10000	900 - 12000	1000 - 5000
Fumonisin (total)		5000 – 100000	5000 - 60000	5000 – 60000 (B1+B2)
Ochratoxin A	200-2000		50 - 100	100000
Zearalenone	250-5000	1000-3000	100 - 3000	100 - 1500
T-2	1000			500
HT-2	25-100			

Note that the values above have a wide range because the tolerance of each mycotoxin varies widely with different animal species and stage of production.

TARGET COMPOUNDS

	Compound	Formula	Adduct	m/z	z	RT Time (min)
1	Aflatoxin B1	C17H12O6	+H	313.0707	1	7.07
2	Aflatoxin B2	C17H14O6	+H	315.0863	1	6.83
3	Aflatoxin G1	C17H12O7	+H	329.0656	1	6.53
4	Aflatoxin G2	C17H14O7	+H	331.0812	1	6.27
5	3-Acetyl DON	C17H22O7	+H	339.1438	1	5.5
6	Fumonisin B1	C34H59NO15	+H	722.3957	1	8.29
7	Fumonisin B2	C34H59NO14	+H	706.4008	1	9.17
8	Fumonisin B3	C34H59NO14	+H	706.4008	1	8.78
9	Ochratoxin A_M+H	C20H18ClNO6	+H	404.0895	1	8.86
10	T-2_M+NH4	C24H34O9	+NH4	484.2541	1	8.39
11	HT-2_M+NH4	C22H32O8	+NH4	442.2435	1	7.84
12	Zearalenone_M+H	C18H22O5	+H	319.154	1	8.95
13	Nivalenol_M+H	C15H20O7	+H	313.1282	1	2.63
14	Nivalenol_M+Na	C15H20O7	+Na	335.1101	1	2.63
15	DON_M+H	C15H20O6	+H	297.1333	1	3.59
16	Alpha-zearalenol_M-H	C18H24O5	-H	319.1551	1	8.86
17	HT-2_M+H	C22H32O8	+H	425.217	1	7.84
18	Zearalenone_M-H	C18H22O5	-H	317.1394	1	8.95

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SAMPLE PRETREATMENT IN CORN FEED SAMPLE

The QuEChERS method: Extraction with **no dSPE clean-up**

1. Weigh 5 g corn feed powder in 50 mL Teflon tube
2. Add 20 mL 70:30 v/v MeOH:H2O with 0.1% Formic acid
3. Shake on muti-tube vortexer for 30 minutes
4. Centrifuge 5 minutes @ 4700 rpm
5. Filter supernatant through 0.45 µm cellulose filters
6. Transfer 1 mL to A/S vial, inject 2.0µL
7. **HPLC separation**
8. **MS**

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ORBITRAP EXPLORIS™ 120 MS ACQUISITION AND DATA PROCESSING WORKFLOW

DIA

Data Independent Acquisition (DIA)

- No target list
- Precursor isolation windows w/ stepped NCE
- MS2 triggered across entire peak

ddMS²

Data Dependent Acquisition (DDA)

- Target inclusion list with retention times
- Specific precursor isolation and NCE
- MS2 trigger on single apex scan

tMS²

Targeted MS2 (tMS2 or PRM)

- Target inclusion list with retention times
- Specific precursor isolation and NCE
- All fragments are collected in a full scan high resolution mass analysis

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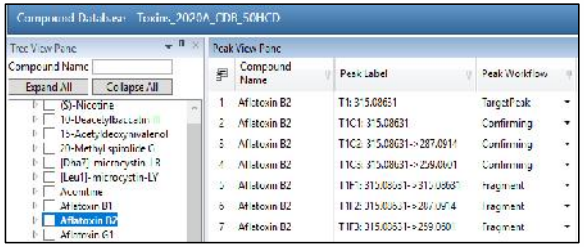
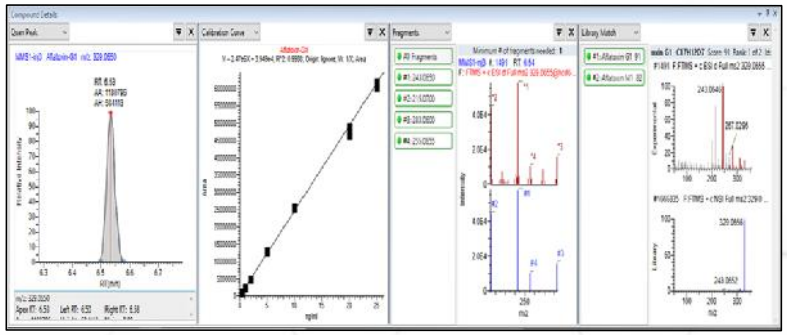
Screening and Quantitation

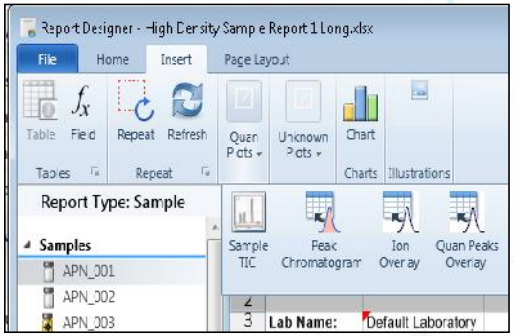


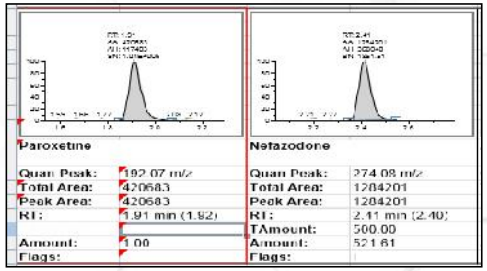
Compound Data Base

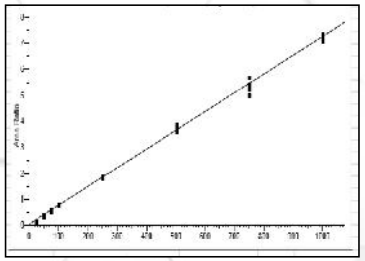


Fragment Match and Library Search

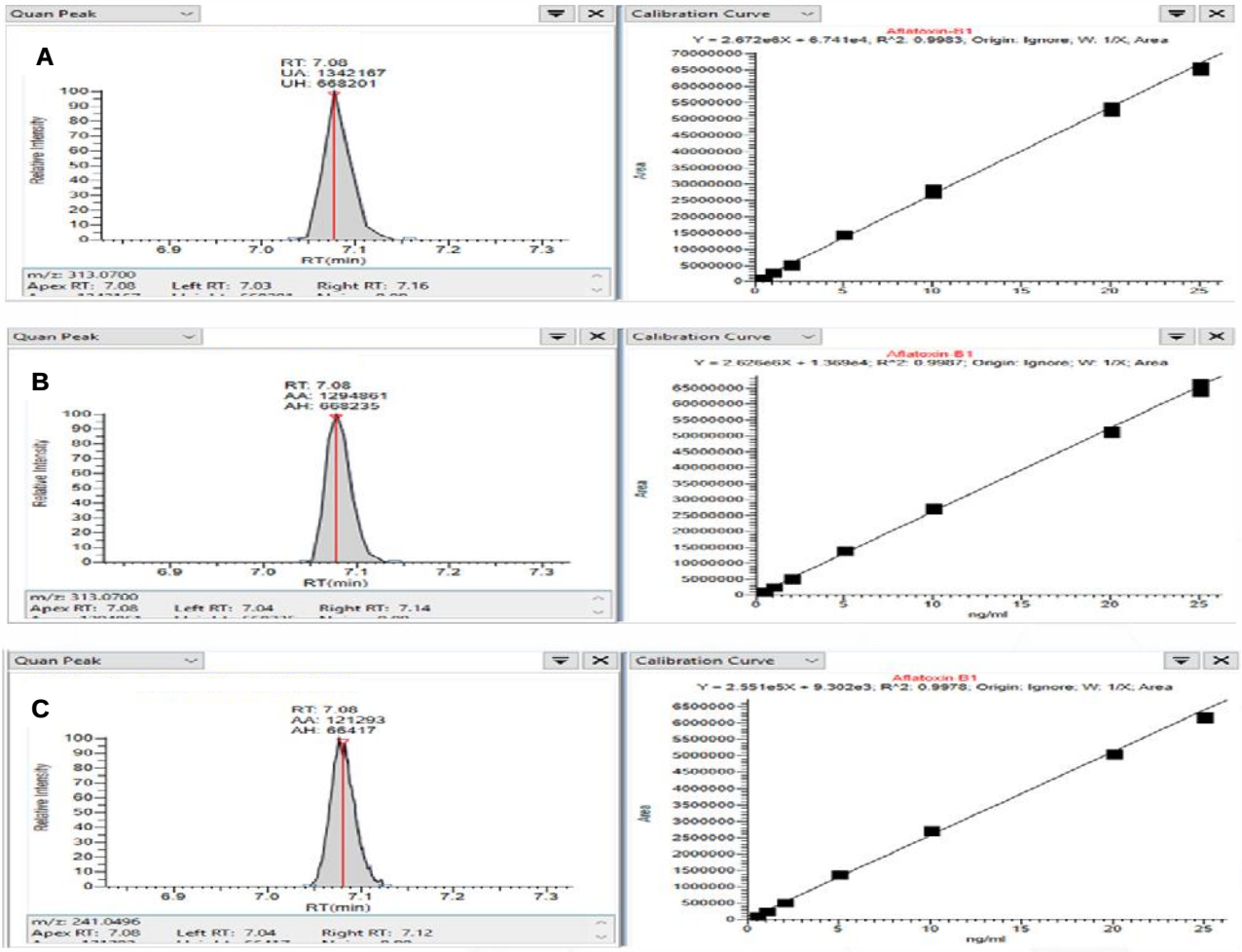
Reporting







MYCOTOXINS IN ANIMAL FEED- RESULTS



A: DIA with full scan used for quantitation

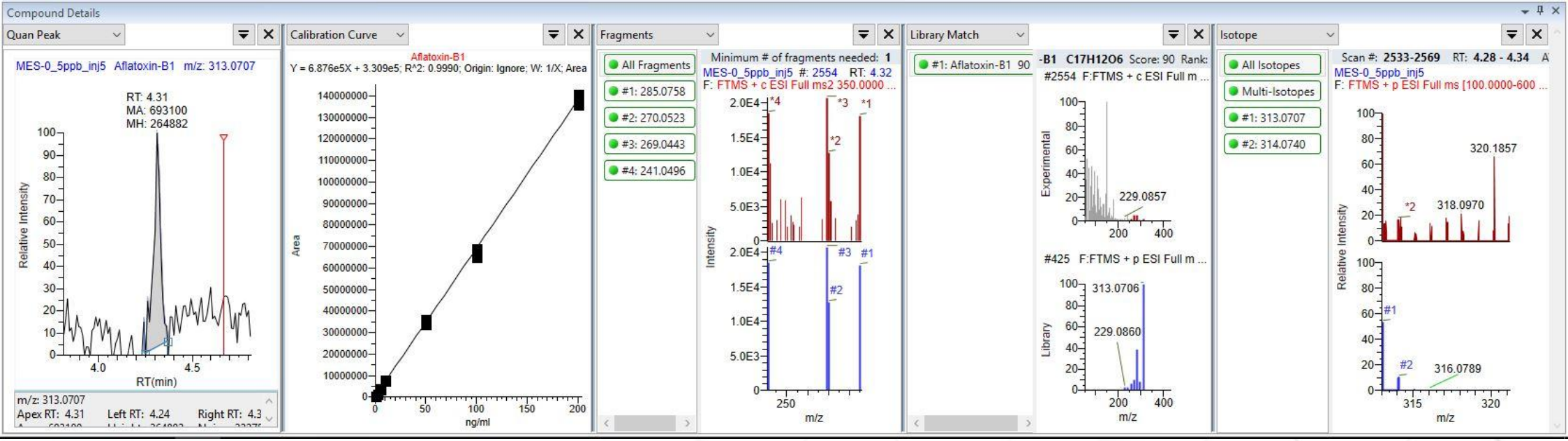
B: ddMS2 with full scan used for quantitation

C: tMS2 (PRM) with primary MS2 transition used for quantitation

Comparison of extracted ion chromatograms for Aflatoxin B1 at 0.5 ng/ml (2 ng/g) in corn feed matrix extracted spikes (MES). Calibration range: 2 ng/g-100 ng/g.

EXAMPLE FRAGMENT MATCH AND LIBRARY SEARCH RESULT FROM DIA EXPERIMENT

Aflatoxin B1 at 0.5 ppb in corn feed



MYCOTOXINS IN ANIMAL FEED- RESULTS

		DIA			ddMS2			tMS2		
Compound	Adduct	r2	Recovery	% RSD	r2	Recovery	% RSD	r2	Recovery	% RSD
3-acetyl-deoxynivalenol	M+H	0.997	104.6 ± 4.0	11.7	0.997	106.5 ± 2.8	7.4	0.998	103.9 ± 1.3	10.2
Aflatoxin B1	M+H	0.998	90.9 ± 1.5	3.7	0.999	92.2 ± 0.9	3.3	0.998	92.1 ± 0.5	3.1
Aflatoxin B2	M+H	0.998	96.1 ± 2.4	2.9	0.999	96.0 ± 0.6	4.8	0.999	95.4 ± 1.3	3.9
Aflatoxin G1	M+H	0.998	95.2 ± 1.4	4.7	0.999	96.3 ± 0.9	2.1	0.998	95.1 ± 0.5	2.9
Aflatoxin G2	M+H	0.998	99.0 ± 1.8	4.8	0.999	100.7 ± 1.1	3.4	0.998	99.2 ± 0.9	4
α-Zearalenol	M-H	0.999	98.9 ± 1.3	11.3	0.999	101.8 ± 1.0	9.4	0.998	96.8 ± 1.5	9.1
Deoxynivalenol	M+H	0.996	98.2 ± 2.2	7.6	0.997	104.0 ± 1.0	7.9	0.993	99.5 ± 1.0	10.7
Fumonisin B1	M+H	0.998	112.1 ± 2.6	9.9	0.998	113.1 ± 1.2	4.8	0.998	108.5 ± 3.7	2.6
Fumonisin B2	M+H	0.997	116.3 ± 4.8	9.2	0.998	119.2 ± 3.9	7.1	0.998	112.6 ± 1.2	3.2
Fumonisin B3	M+H	0.999	107.8 ± 2.5	7.1	0.999	110.0 ± 1.4	9.5	0.997	107.2 ± 2.0	8
HT2-Toxin	M+NH4	0.997	114.8 ± 3.2	6	0.998	111.5 ± 1.0	9.7	0.997	109.6 ± 0.9	6.5
Nivalenol	M+H	0.999	98.8 ± 1.1	9.6	0.999	98.6 ± 0.9	9.2	0.996	97.5 ± 2.5	9
Ochratoxin A	M+H	0.997	88.6 ± 1.7	8.1	0.997	92.6 ± 2.5	7.7	0.999	90.8 ± 1.9	3.3
T2-Toxin	M+NH4	0.998	111.9 ± 2.3	4.6	0.999	115.3 ± 1.1	2.5	0.997	111.4 ± 0.8	3.1
Zearalenone	M-H	0.998	96.2 ± 0.4	2.2	0.999	99.6 ± 0.4	9.4	0.997	95.3 ± 1.5	8.6

Polarity, coefficient of determination for linear regression curves, and recovery (mean ± SD, %) for mycotoxins spiked into control corn feed using three scan modes. Results show comparable calibration linearity (R2 > 0.990) and good spike recovery (80 – 120%) for all 15 mycotoxins in three acquisition modes with polarity switching. % RSD data represents matrix extracted spikes (MES) n = 5 replicates.

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EXAMPLE FRAGMENT MATCH AND LIBRARY SEARCH RESULT FROM DIA EXPERIMENT

	Nominal concen (ng/g)	Measured concentration (ng/g)		
		DIA	ddMS2	tMS2
Aflatoxin B1	12.1 ± 2.6	12.3 ± 2.9	14.1 ± 2.8	13.3 ± 3.8
DON	2200 ± 200	2258.2 ± 241.7	2333.7 ± 213.7	2156.3 ± 144.8
Total fumonisins	10200 ± 1000	-	-	-
HT-2	199.9 ± 21.9	230.7 ± 18.0	219.8 ± 24.1	236.5 ± 22.3
Ochratoxin A	9.9 ± 1.9	12.9 ± 1.6	13.0 ± 1.3	12.9 ± 0.6
T-2	254.0 ± 18.6	273.0 ± 32.2	291.3 ± 20.5	277.3 ± 20.1
Zearalenone	347.4 ± 23.6	351.4 ± 15.5	367.4 ± 16.9	336 ± 38.3

Nominal and measured concentrations (mean ± SD, ng/g) of mycotoxins in certified reference material using three different scan modes.
Results for fumonisins were excluded due to very high concentrations in the samples which were significantly outside of the calibration ranges.

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CONCLUSION

EXPLORE THE ORBITRAP FAMILY OF INSTRUMENTATI ON
HTTPS://PLANETORBITRAP.COM/PRODUCTS

ĎAKUJEM ZA POZORNOSŤ