Minimizing matrix effects in multi-residue pesticide analysis using a new sensitivity-enhanced triple quadrupole instrument in combination with extensive sample dilution

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International Symposium on Food Safety and Quality:
Applications of Nuclear and Related Techniques

The Measure of Confidence

Pesticides screening – regulation and residue situation

- **EU regulation**

  - Maximum residue levels regulated in commision regulation (EC) 396/2005

    - appendix II – IV regulates >170 000 maximum residue limits (matrix-pesticide combinations)

  - European and national monitoring programs are in place also regulated by (EC) 396/2005

  - 121 notifications related to pesticide residues in tea have been entered in the Rapid Alert System for Food and Feed (RASFF)

Why do you want a 6495?

Utmost sensitivity is required for …

- the analysis of a large number of relevant compounds with a single injection

- dilution of samples for reduced matrix effects, better accuracy and improved robustness

- improved LOQs for pesticides in complex matrices

- highest precision

Experimental setup

- Small validation with tomato, orange, black tea

- QuEChERS extraction

- Dilution of black tea samples with acetonitrile prior injection

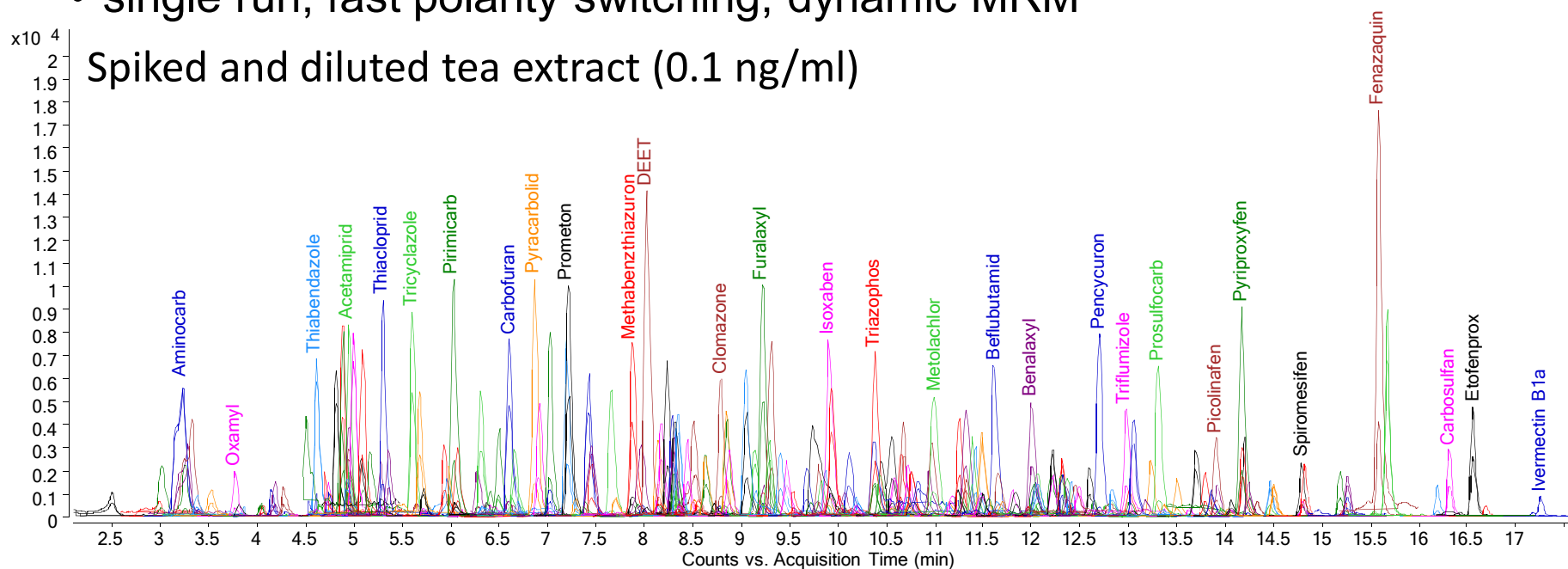- Quantitation with solvent calibration – one calibration for all matrices?

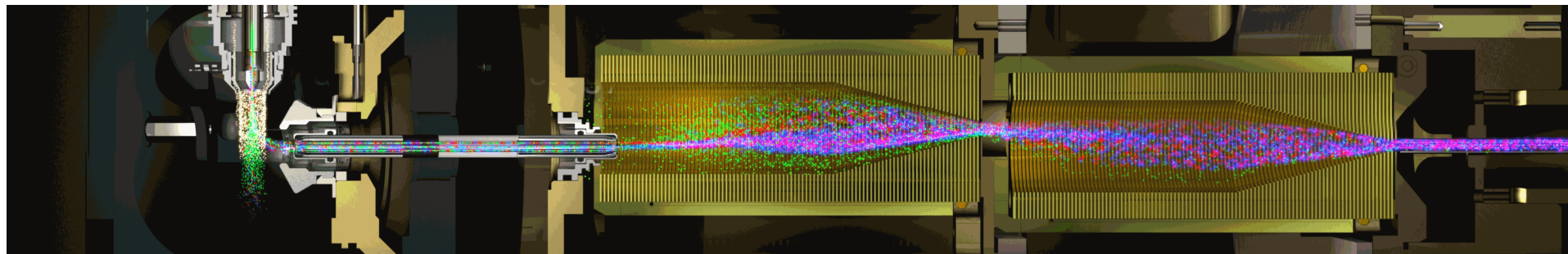# UHPLC/MS/MS Setup

1290 Infinity UHPLC
- column: Zorbax RRHD Eclipse Plus C18
- A: H_2O / 0.1% HCOOH / 5mM NH_4COOH
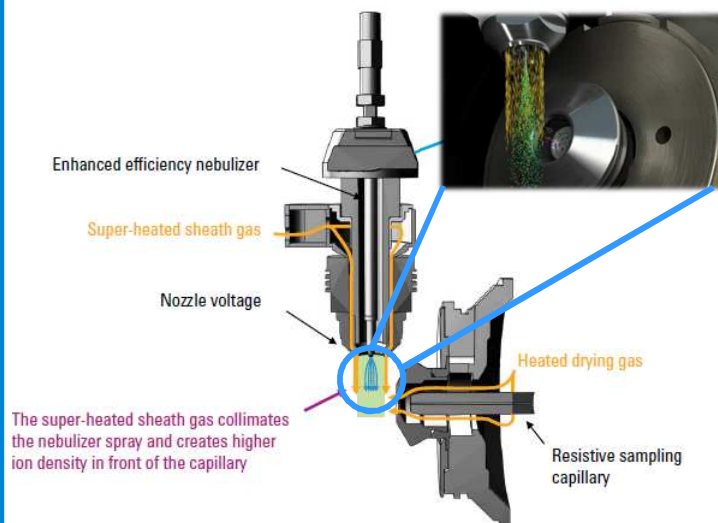- B: MeOH / 0.1% HCOOH / 5mM NH_4COOH

6495 LC/MS system
- single run, fast polarity switching, dynamic MRM

Spiked and diluted tea extract (0.1 ng/ml)

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Proven iFunnel Technology

Agilent Jet Stream

- Enhanced efficiency nebulizer
- Super-heated sheath gas
- Nozzle voltage
- The super-heated sheath gas collimates the nebulizer spray and creates higher ion density in front of the capillary
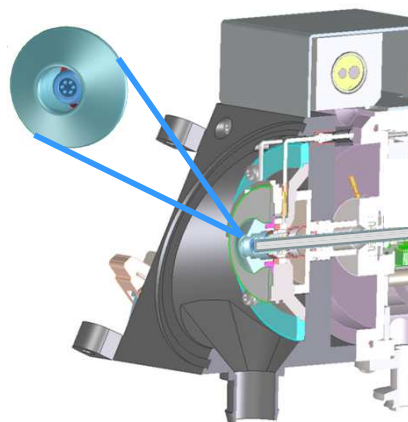- Heated drying gas
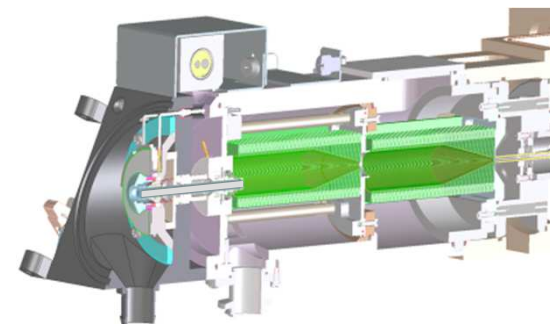- Resistive sampling capillary

- Thermal confinement of ESI plume
- Efficient desolvation
- Creates an ion rich zone

Hexabore Capillary

- Six capillary bores
- Samples x6 times more ion rich gas from the source
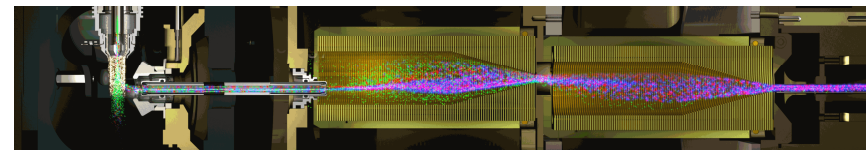
Dual Ion Funnel

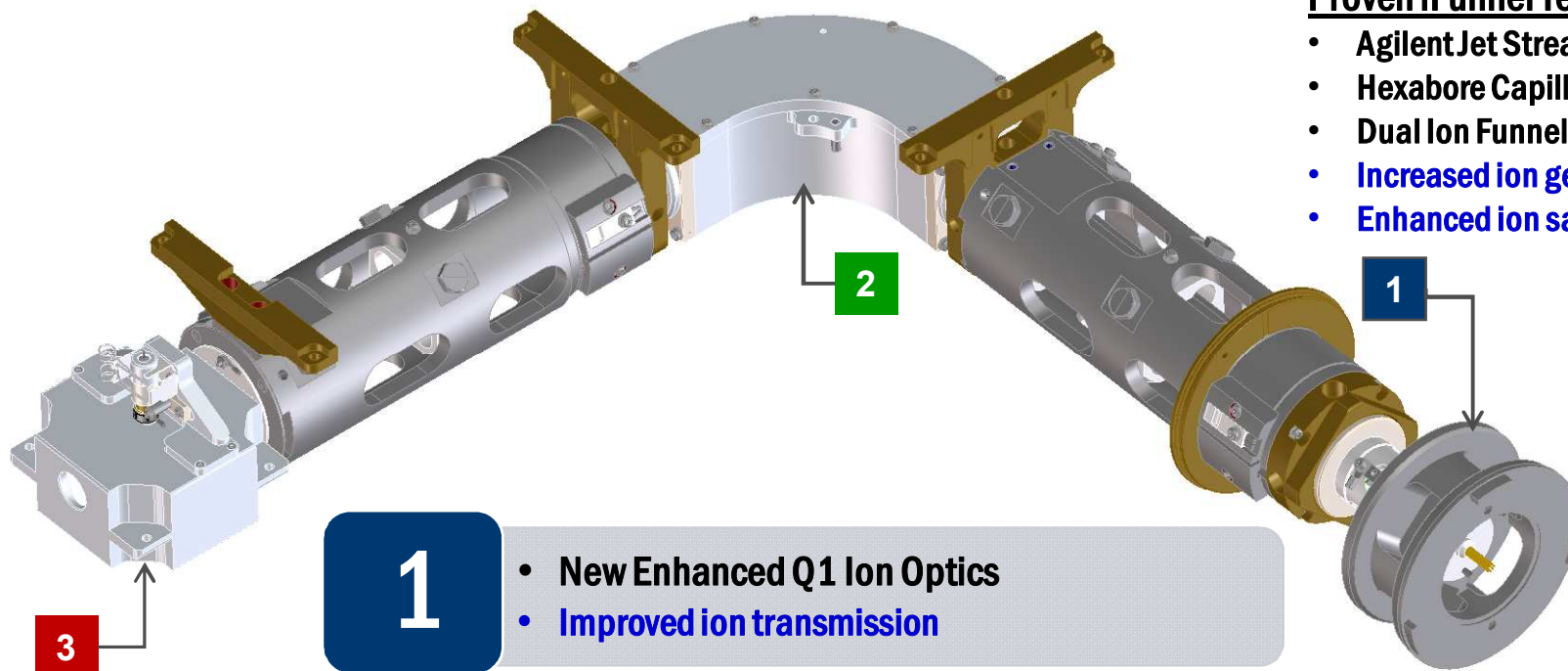- Removes the gas but captures the ions
- Removes neutral noise

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6495 QQQ Technologies
Continued Development

Proven iFunnel Technology

- **Agilent Jet Stream**
- **Hexabore Capillary**
- **Dual Ion Funnel**
- **Increased ion generation**
- **Enhanced ion sampling**

1
- **New Enhanced Q1 Ion Optics**
- **Improved ion transmission**

2
- **New Tapered Hexapole Collision Cell**
- **Effective ion collection and transmission**

3
- **New Detector with High Energy Conversion Dynode (up to 20 kV)**
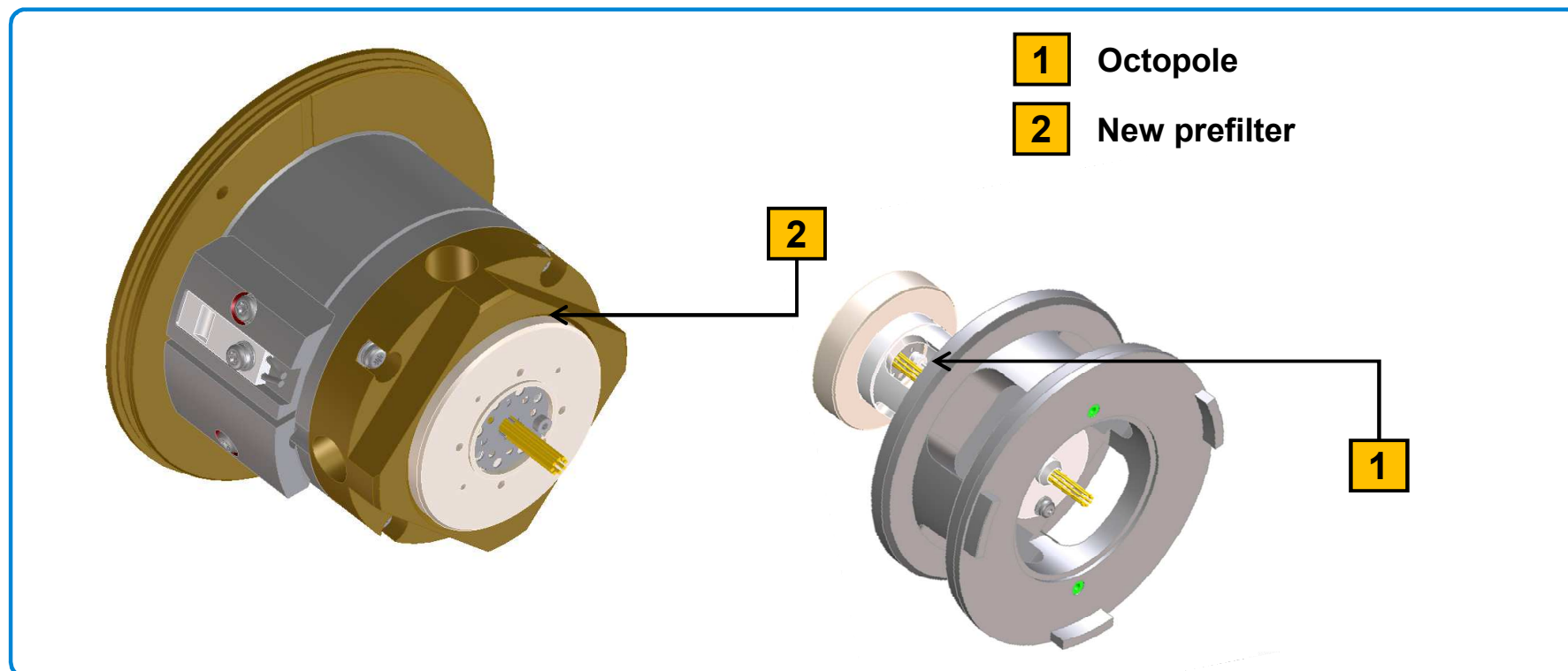- **Improved NEG ion detection with low noise**

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New Enhanced Q1 Ion Optics
Improved transmission of ions and system robustness

1	**Octopole**
2	**New prefilter**

- New optimized MS 1 prefilter geometry for improved precursor **ion transmission**
  - Improved peak area response and peak area %RSD, more **sensitive** and **precise**
- New optical lens elements for reduced contamination and easier autotune
  - More **reliable** and **robust** performance

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New Curved and Tapered Hexapole Collision Cell
## *Effective collection and transmission of ions*
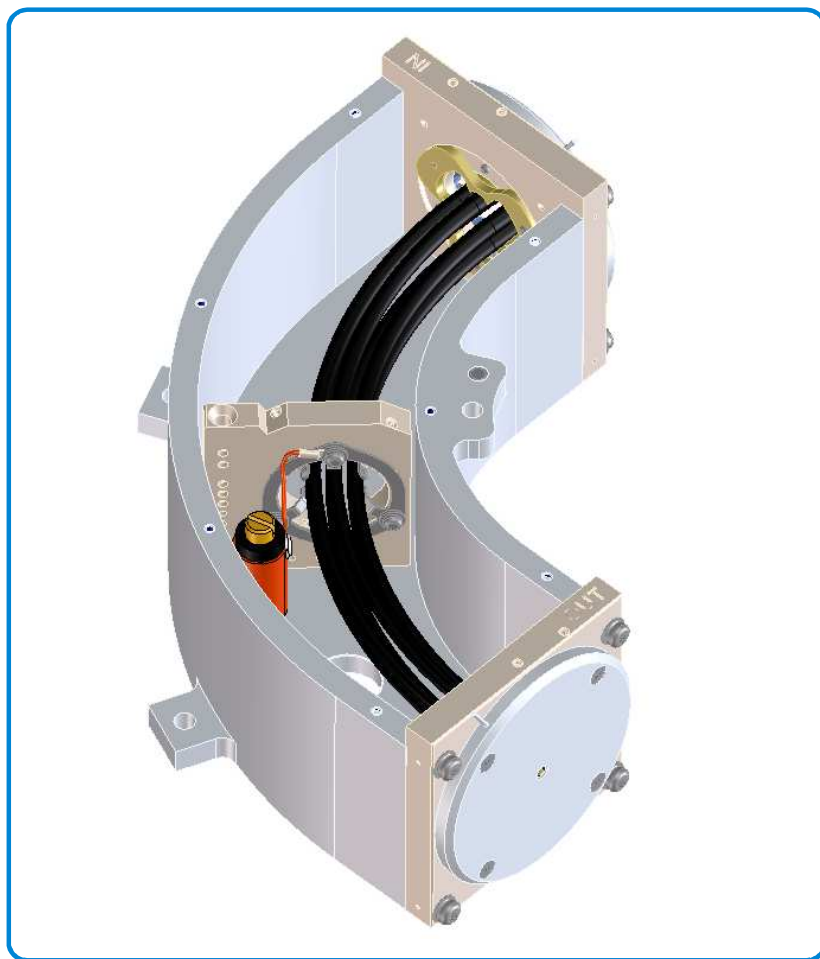
- Curved and Tapered Hexapole Assembly for efficient collection and transmission of product ions

- Consistent collision cell pressure for higher quality MS/MS spectra

- Designed for consistent collision energies across all QQQ platforms

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New Detector with 20 kV High Energy Conversion Dynode
More efficient detection of ions with low noise characteristics

Anti-HER2/neu mAB Digest

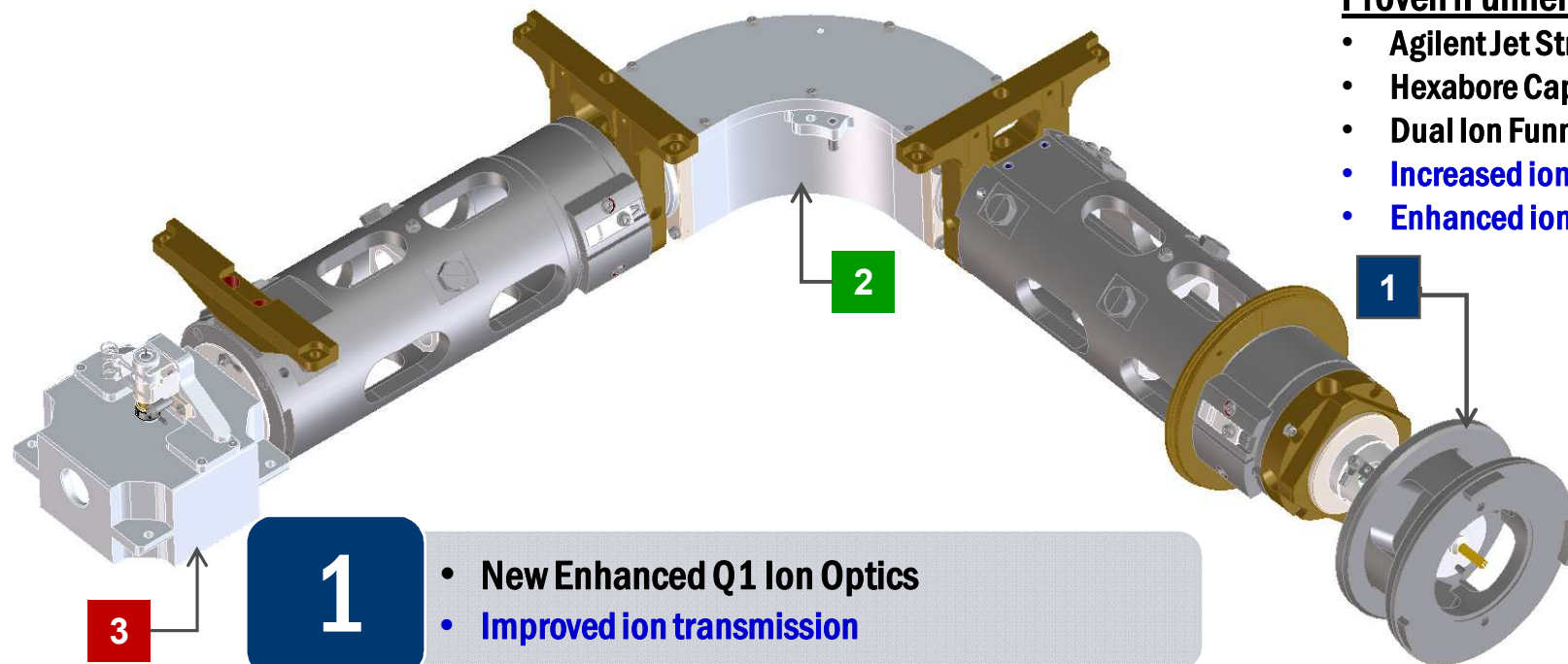**60 – 120% increase in response at 20kV**
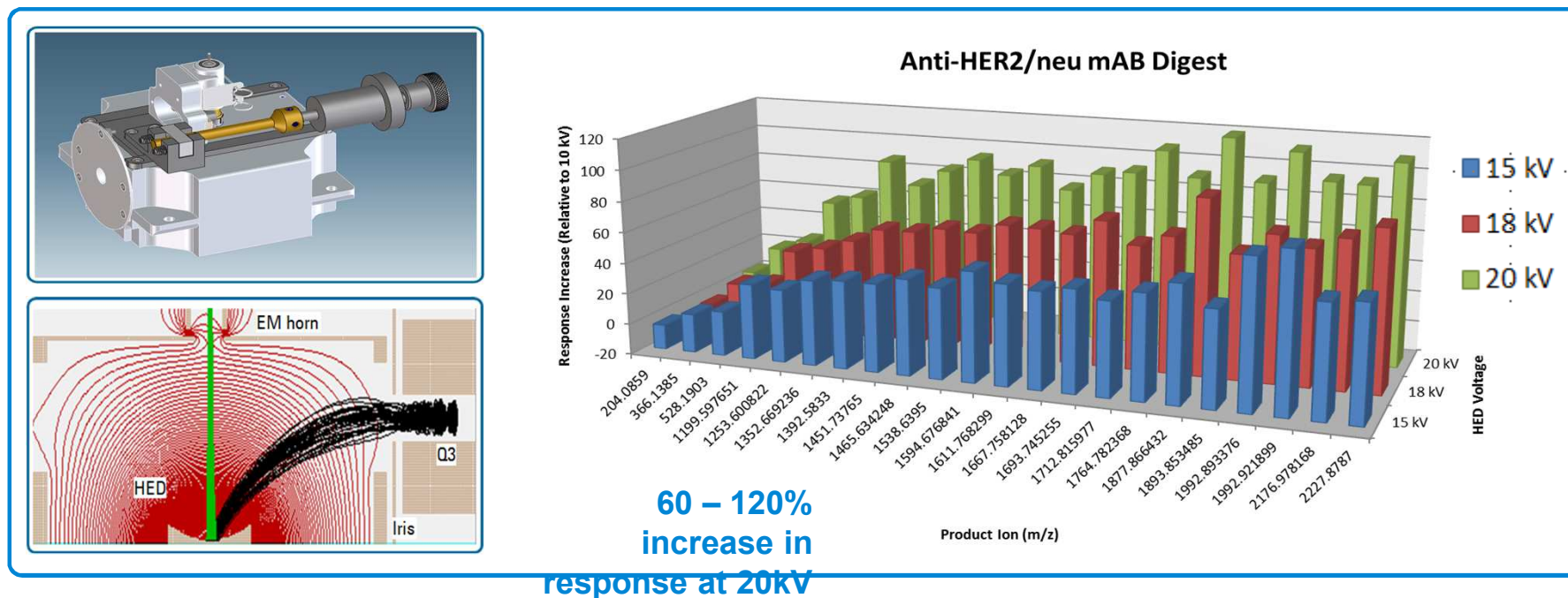
- Improved **ion detection** efficiency with High Energy Dynode (HED) voltage up to 20 kV
  - Improved peak area response and peak area %RSD in positive & negative ion mode
  - Improved **sensitivity** and **precision** for a wide m/z range of product ions
- Low noise level at 20 kV
  - Improved signal to noise
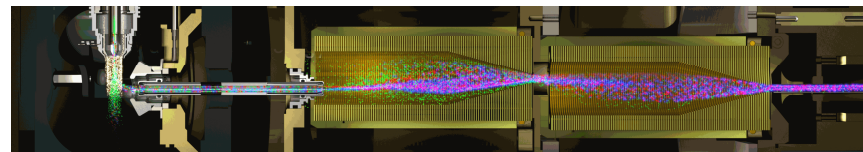
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6495 QQQ Technologies
Continued Development

Proven iFunnel Technology

- **Agilent Jet Stream**
- **Hexabore Capillary**
- **Dual Ion Funnel**
- **Increased ion generation**
- **Enhanced ion sampling**

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- **New Enhanced Q1 Ion Optics**
- **Improved ion transmission**

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- **Effective ion collection and transmission**

3
- **New Detector with High Energy Conversion Dynode**
- **Improved NEG ion detection with low noise**

Quantitative Applications

- **Enhanced peak area response**
- **Improved peak area %RSD**
- **More sensitive and precise**
- **Lower Limits of Detection (IDL)and Quantitation (LLOQ)**
- **More reliable and robust**

Bentazon: Superb Precision & Sub-fg IDL in Black Tea

Amount measured	Replicates	%RSD	t (99%)	IDL
1 pg/mL, 2.0 fg (LLOQ)	n = 7 injections	11.4	3.143	0.36 pg/mL, 0.72 fg

$$IDL = t \times (\%RSD/100) \times Amount = 3.143 \times (11.4/100) \times 2.0\,fg = 0.72\,fg$$

Injection volume = 2 µL

Inj #	Peak Area	S/N (Peak to peak)
1	82	20.9
2	96	13.2
3	70	12.7
4	86	13.4
5	92	10.3
6	72	12.7
7	83	29.4
%RSD	11.4	Ave 16.1
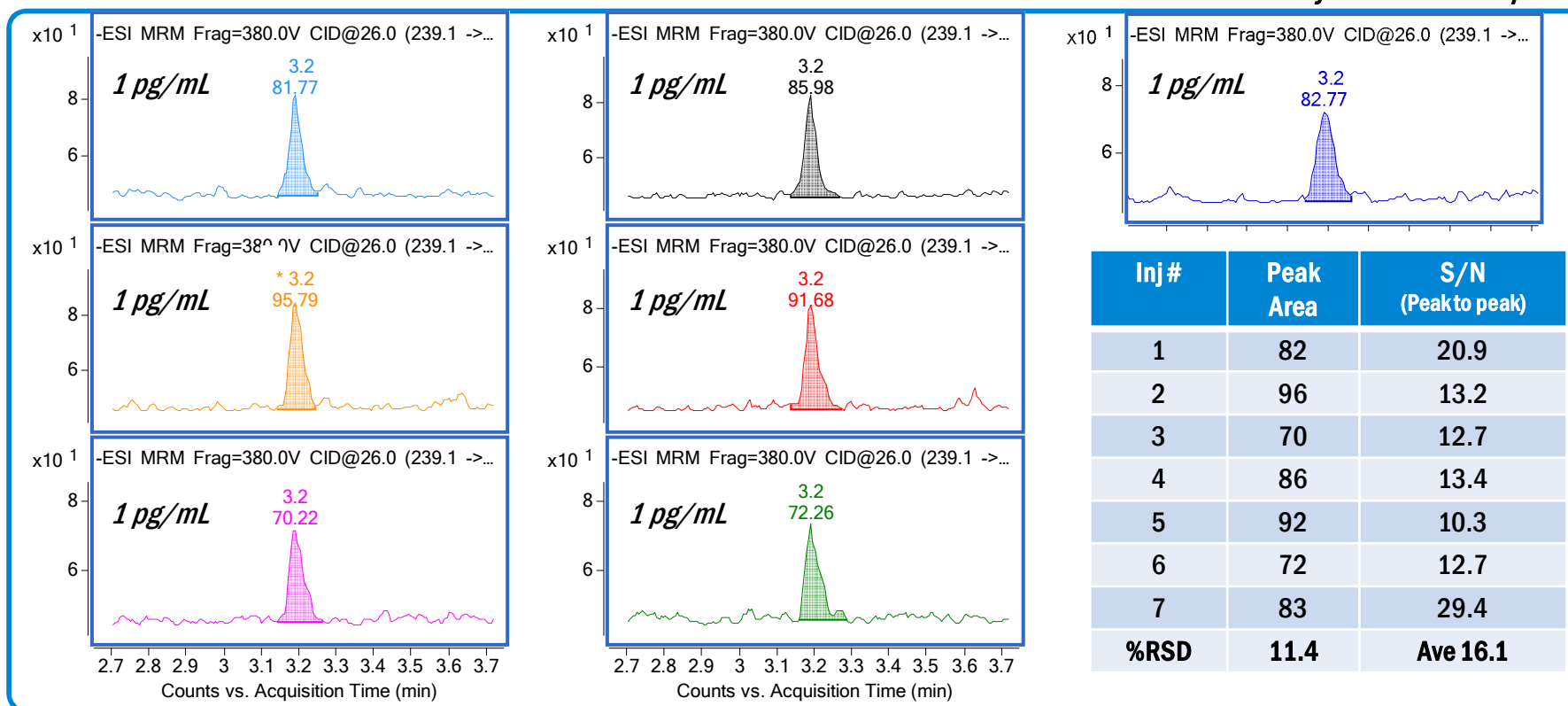
- Excellent peak area **precision** (%RSD) are observed at the lowest level in black tea matrix

Signal Improvements for High Relevance Pesticides
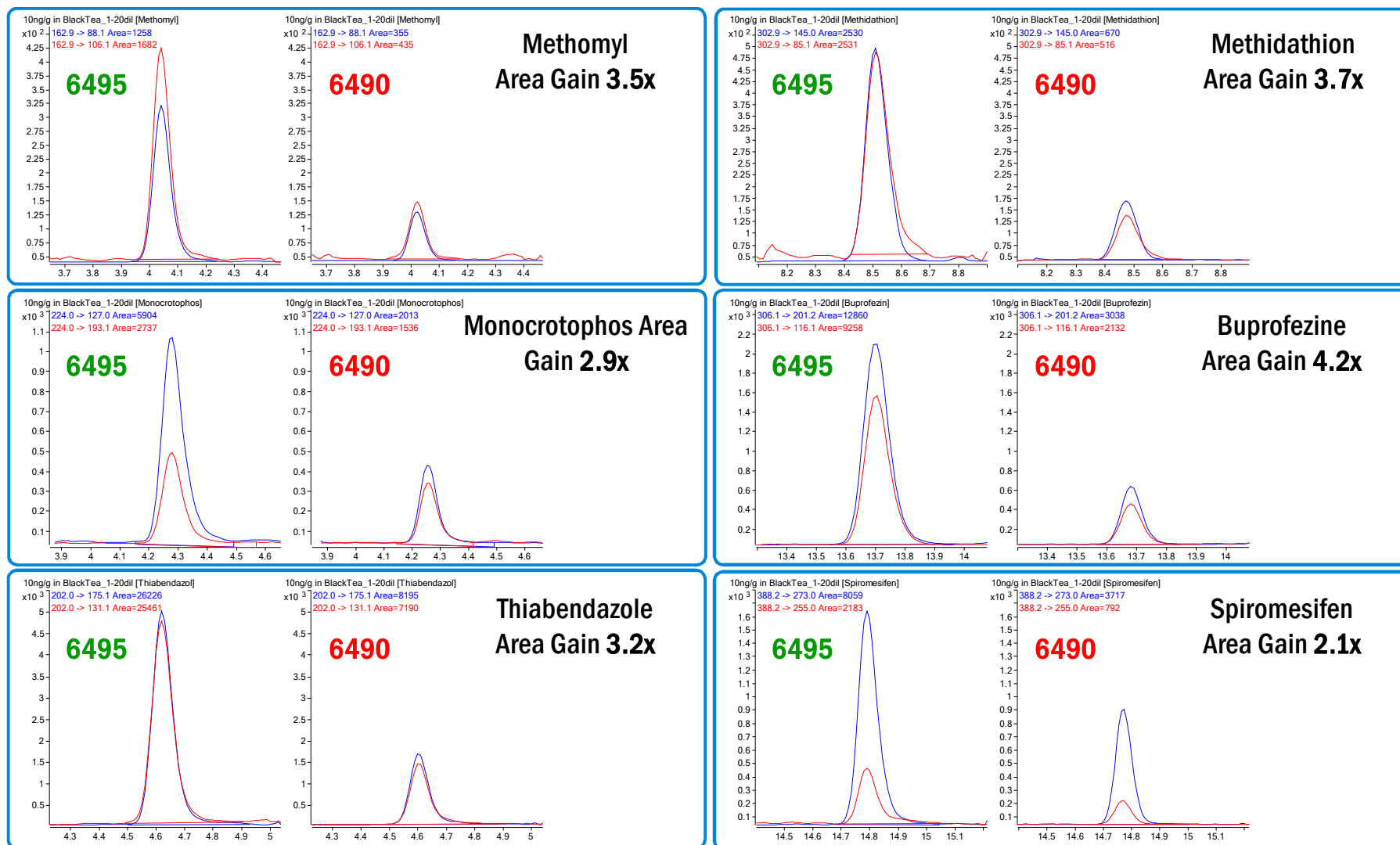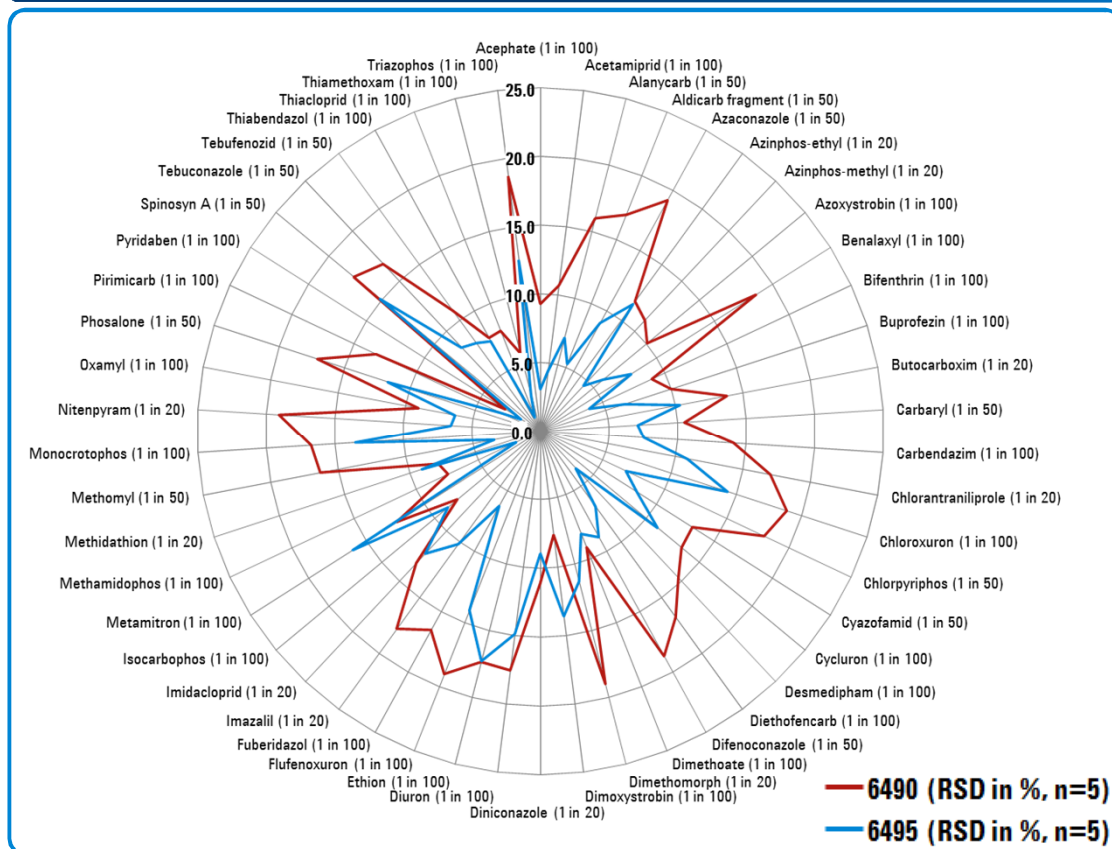

- Improved **sensitivity** (peak area gain of 3x) are observed on the new **6495** vs. the **6490**

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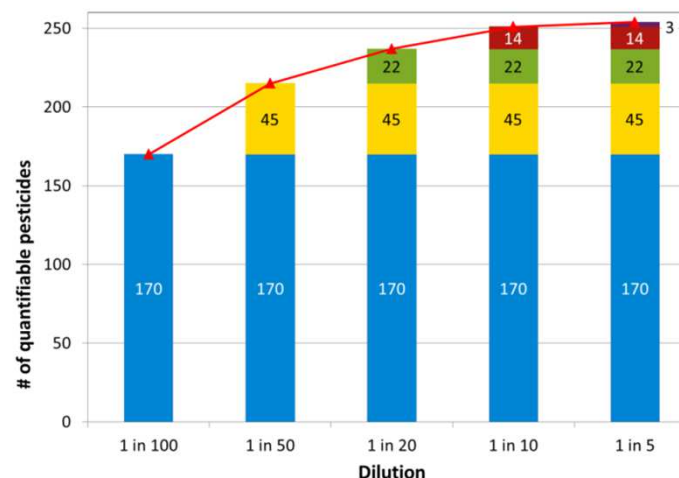
Improved Precision & Lower LLOQs in Black Tea

Comparison of Area %RSD for 50 Pesticides at MRL

- 6490 (RSD in %, n=5)
- 6495 (RSD in %, n=5)

of pesticides detected at MRL

- 67% of pesticides were easily detected with a %RSD < 20 with 1:100 dilution
- This means LLOQs < 20 pg/mL (ppt)
- %RSD < 20 meets SANCO guidelines

- Improved **precision** (%RSD) are observed, particularly at the lowest levels (LLOQs)
- The enhanced peak area response and improved precision (%RSD) means pesticides achieve **lower LLOQs** ($\leq$ MRL level) using the new 6495.
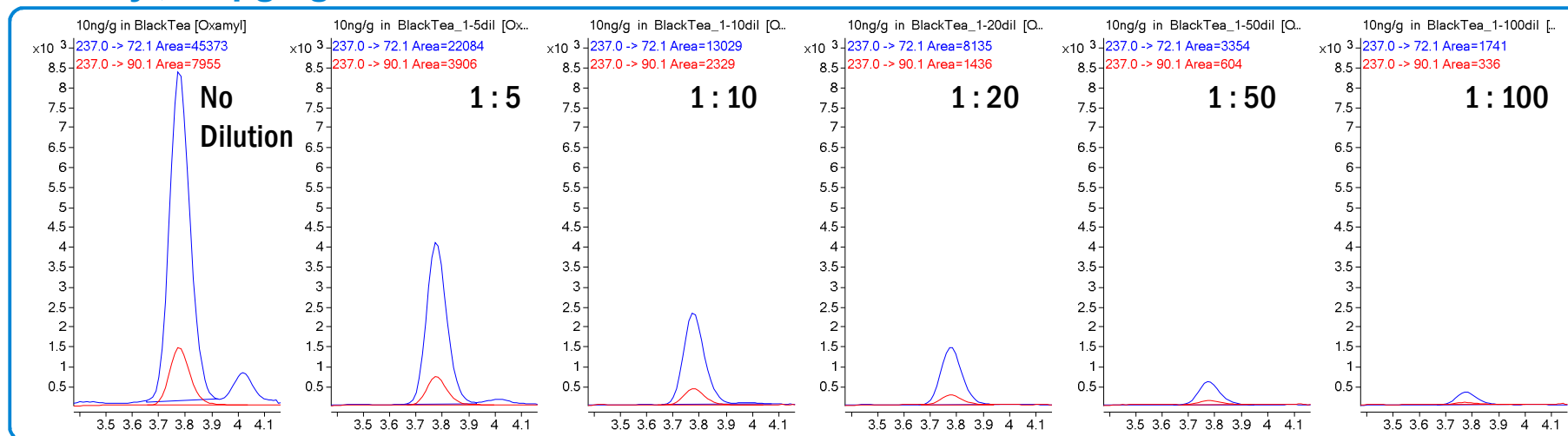
Dilution allows More Efficient Ionization of Pesticides

Alanycarb, 10 µg/kg in Black Tea
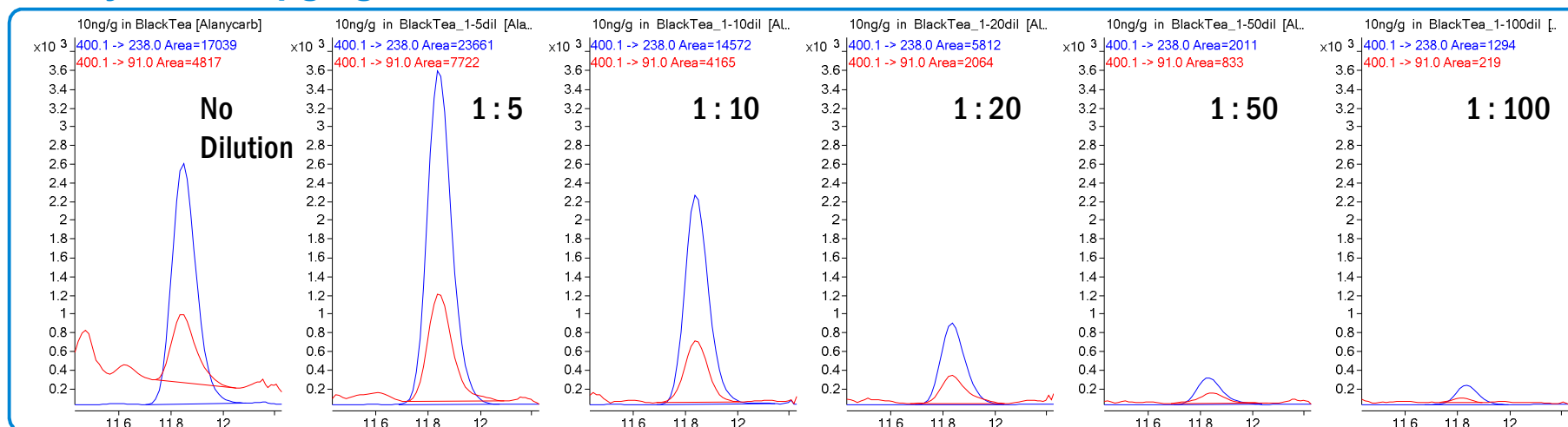
Oxamyl, 10 µg/kg in Black Tea

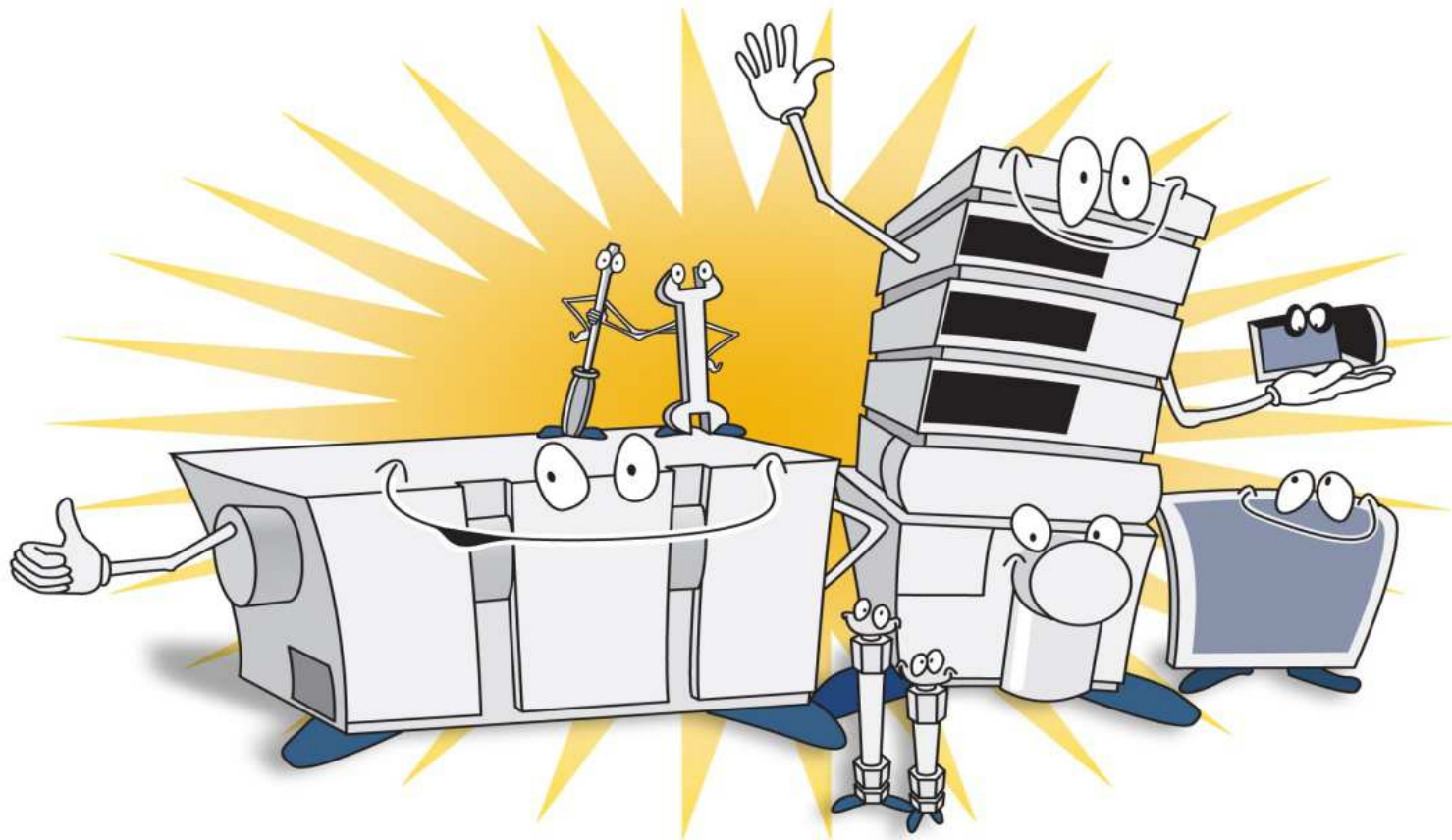# Recovery (%) in Black Tea with Different Dilutions

Pesticides	No dilution (n = 5)	Dilution 1:5 (n = 5)	Dilution 1:10 (n = 5)	Dilution 1:20 (n = 5)	Dilution 1:50 (n = 5)	Dilution 1:100 (n = 5)
Acetamiprid	29.4 ± 0.8	57.3 ± 1.4	67.5 ± 3.7	79.9 ± 2.9	91.8 ± 5.2	109.5 ± 3.4
Alanycarb	10.4 ± 1.3	73.9 ± 2.2	81.5 ± 14.3	85.7 ± 11.1	87.6 ± 4.7	121.7 ± 10.8
Aldicarb	36.9 ± 1.0	69.9 ± 1.4	78.0 ± 3.5	91.0 ± 4.2	95.2 ± 8.8	104.9 ± 14.1
Carbaryl	56.9 ± 1.8	80.1 ± 3.8	80.8 ± 4.1	96.1 ± 7.2	102.6 ± 6.6	116.4 ± 9.6
Dimethoate	33.9 ± 1.7	68.6 ± 2.4	84.1 ± 5.4	89.0 ± 7.9	88.2 ± 8.8	84.7 ± 7.5
Diuron	79.7 ± 4.0	90.4 ± 7.0	91.7 ± 4.9	94.9 ± 7.2	89.2 ± 7.3	100.9 ± 13.5
Flufenoxuron	95.4 ± 1.1	88.8 ± 1.6	89.4 ± 3.8	93.3 ± 5.8	100.0 ± 6.1	119.2 ± 13.9
Monocrotophos	4.6 ± 0.3	13.9 ± 0.3	21.8 ± 0.8	33.8 ± 1.1	58.5 ± 2.0	95.1 ± 5.7
Oxamyl	20.8 ± 0.7	52.6 ± 1.9	65.0 ± 2.0	79.7 ± 3.0	91.2 ± 4.6	110.6 ± 5.2
Thiamethoxam	40.0 ± 1.4	45.9 ± 0.9	46.6 ± 3.8	52.2 ± 1.7	70.9 ± 2.9	97.3 ± 2.0

- Cells shaded in green shows **%recovery** of 70 – 120, which are in compliance with SANCO requirements.
- Pesticides show full recovery and no signal suppression with 1:100 dilution
- Neat solvent calibration curve can be used for pesticides quantitation with 1:100 dilution

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Summary

- The method benefits from the highly sensitive Agilent 6495 Triple Quadrupole LC/MS System.

- It takes full advantage of the low delay volumes of the Agilent 1290 Infinity LC System.

- Enhanced sensitivity allowed for more flexibility in the degree of sample dilution.

- Extensive dilution of sample extracts was applied to minimize matrix effects

- With any dilution, a lower matrix amount is introduced into the LC/MS system

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Thank you for your attention!