ICMGP 2024

CAPE TOWN • SOUTH AFRICA • 21 - 26 JULY

CAPE TOWN INTERNATIONAL CONVENTION CENTRE

Deep Dive into Speciation Analysis

Mercury and Methylmercury in Fish

Presenter: Victoria Wagener

AGENDA

- Background
- Why perform speciated analysis?
- Instrumentation
- Application: *Determination of Methyl- and Inorganic Mercury in Fish with the NexSAR HPLC-ICP-MS Speciation Solution*
  - Importance of Mercury
  - Sample preparation & experimental conditions
  - Results
  - Conclusions

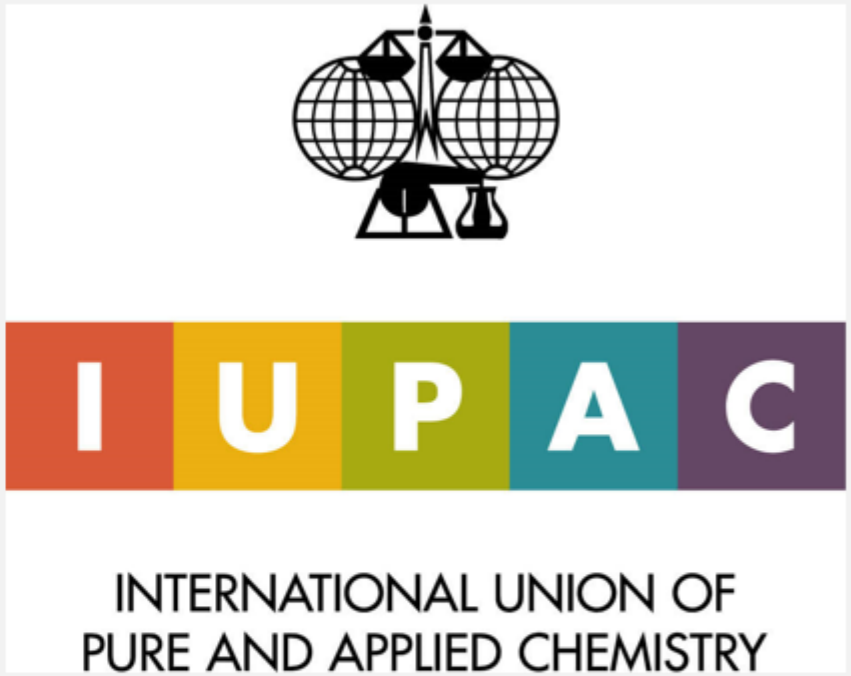
BACKGROUND

- **Speciation** - Allows separation and measurement of species (process)
- **High Performance Liquid Chromatography (HPLC)** - Separates the species, all the chemistry is done here
- **Inductively Coupled Plasma Mass Spectrometer (ICP-MS)** - Detects and measures metals, the detection is done here

WHAT IS SPECIATION?

Analytical activities of identifying and/or measuring the quantities of one or more individual **chemical species** in a sample.

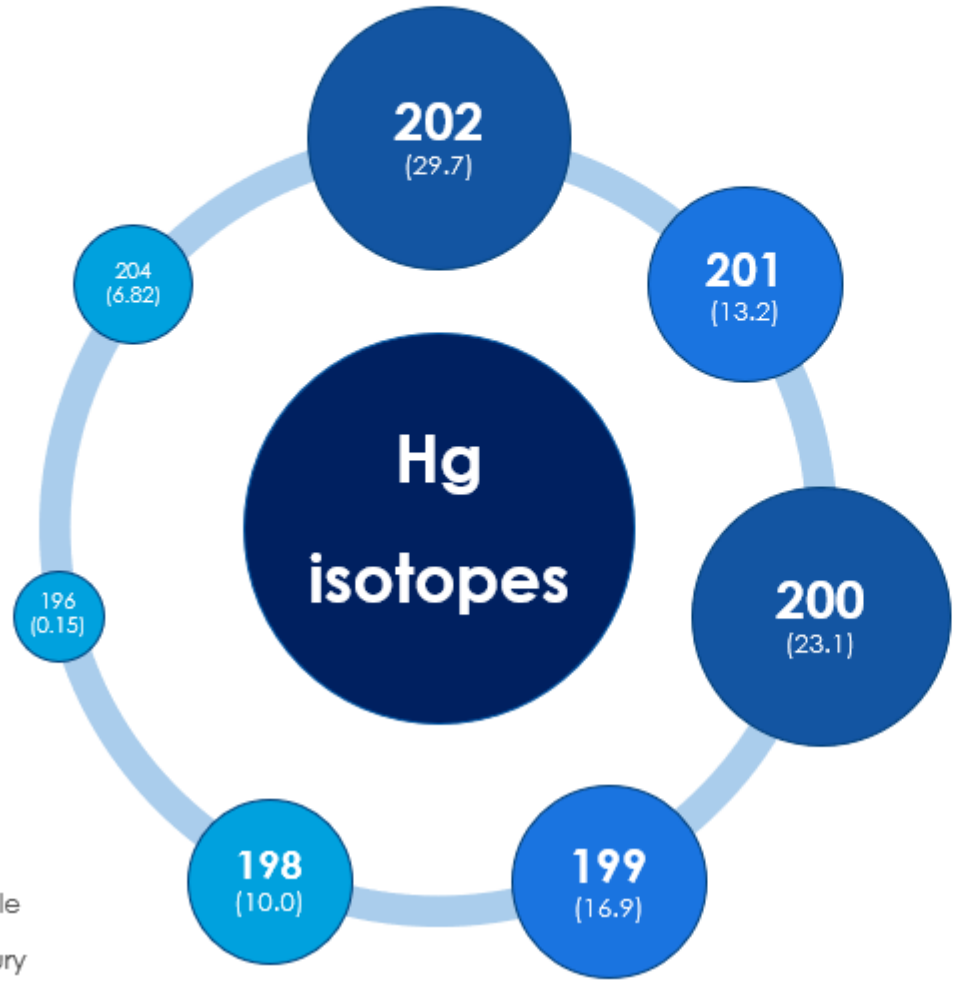
Specific form of an element defined as to **isotopic composition**, **electronic or oxidation state**, and/or **complex or molecular structure**.



Templeton et al., ***Pure App. Chem.*** 72, 1453 (2000)

WHAT IS A CHEMICAL SPECIES?

Specific form of an element defined as to **isotopic composition**



Most abundant and stable isotopes of natural mercury

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

Other Hg isotopes are synthetic, radioactive and unstable (Hg194; Hg195; Hg197; Hg203 etc)

Templeton et al., ***Pure App. Chem.*** 72, 1453 (2000)

WHAT IS A CHEMICAL SPECIES?

Specific form of an element defined as to **electronic or oxidation state**

- Hg^0 - Elemental Mercury
- Hg^+ - Mercurous Mercury
- Hg^{2+} - Mercuric Mercury

Oxidation state – determines chemical behavior (level of reactivity), toxicity (bioavailability) and environmental fate (potential for transformation within environmental systems).

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Templeton et al., ***Pure App. Chem.*** 72, 1453 (2000)

WHAT IS A CHEMICAL SPECIES?

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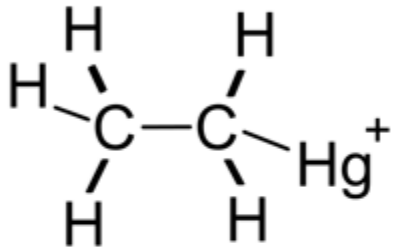
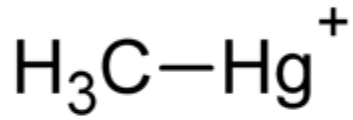
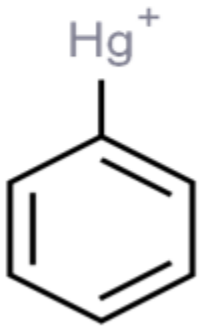


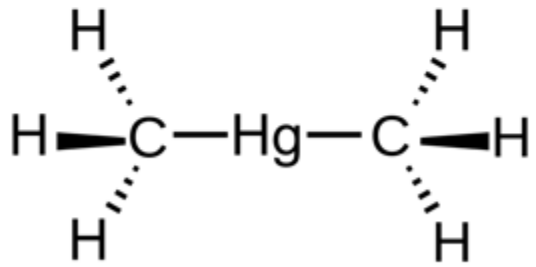
Ethyl mercury



Methylmercury



Phenylmercury



Dimethylmercury

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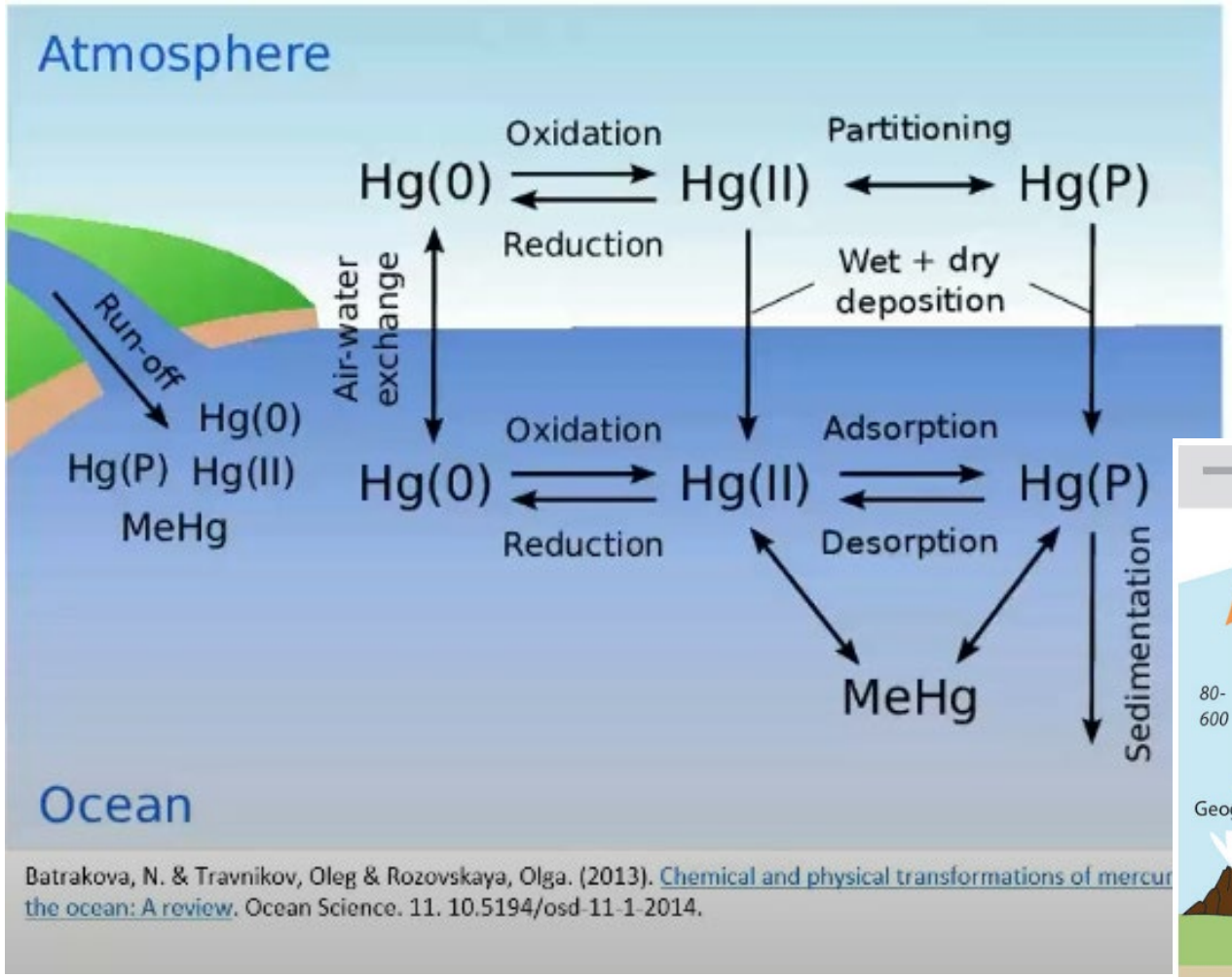
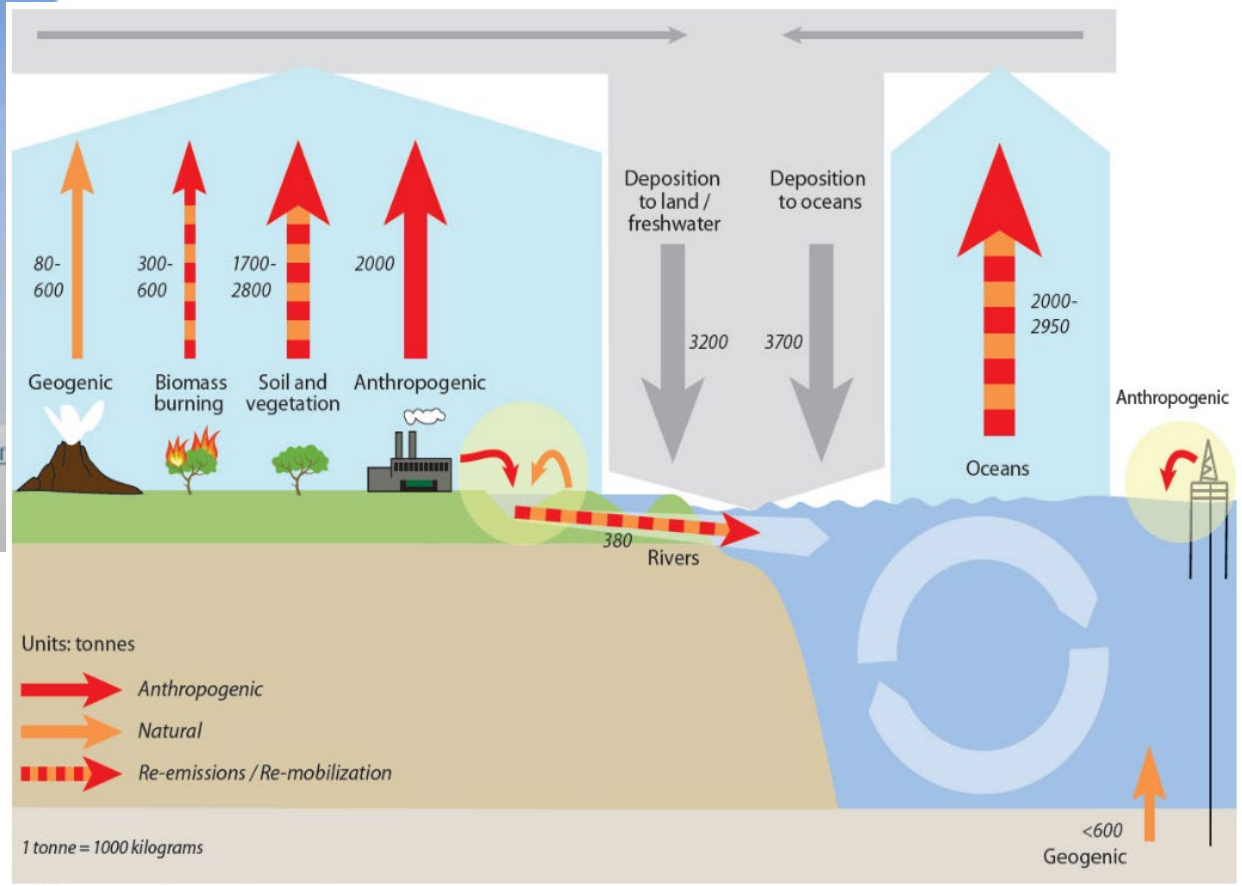
Templeton et al., ***Pure App. Chem.*** 72, 1453 (2000)



Batrakova, N. & Travnikov, Oleg & Rozovskaya, Olga. (2013). Chemical and physical transformations of mercur the ocean: A review. Ocean Science. 11. 10.5194/osd-11-1-2014.



Global mercury cycling (AMAP/UNEP 2013)

WHY MERCURY SPECIATION IN FISH?

1. **Health Risk Assessment**
   - Fish consumption is the major contributor to mercury risk in wildlife and humans
   - Methylmercury (MeHg) is more toxic and can be accumulated in biological tissues – Biomagnified
2. **Regulatory Compliance**
3. **Environmental Impact Assessment**
   - Inorganic mercury (Hg^{2+}) is main form
4. **Source Identification and Pathways**
   - **Typically comes from anthropogenic activities** and then becomes methylated via natural biomethylation processes
5. **Research and Scientific understanding**

THE NEXSAR SPECIATION SOLUTION FROM PERKINELMER

INTEGRATION	
SEPERATION	**DETECTION**
NexSAR HPLC – Truly inert technology for reproducible, repeatable, and reliable results	NexION ICP-MS – Most robust and versatile mass spectrometer in the industry
OPERATION	
Clarity™ Software – Simple, streamlined operator experience offering control of your entire system.	

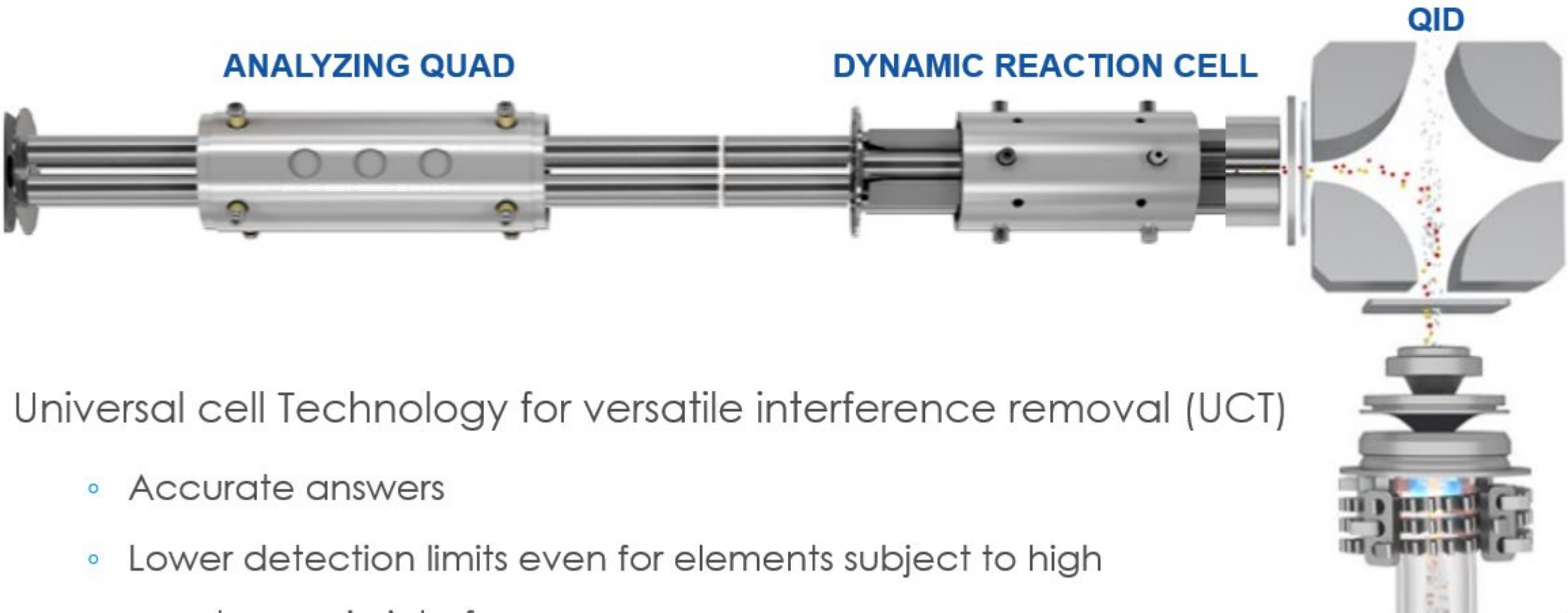
NexSAR™ HPLC SYSTEM SPECIATION ANALYSIS READY SYSTEMS

- Fluid path completely inert and metal free
- Modular design – configure the system to match your needs
  - Degasser & Solvent Tray/Solvent Tray
  - Cooled/Non-cooled Autosampler
  - Column Oven/Switching Valve/neither
- Integrated software and hardware
- Automation and reduced supervision
- Exceptionally low carryover and memory effects

NEXION® ICP-MS - ANY MATRIX, ANY INTERFERENCE

- Improved solvent tolerance
  - Best-in-class RF Generator
  - LumiCoil™ Technology
  - Better stability
- Robust interface (TCI + QID)
  - Triple Cone Interface (TCI)
  - Quadrupole Ion Deflector (QID)
  - Longer periods between routine maintenance

NEXION® ICP-MS - ANY MATRIX, ANY INTERFERENCE



Universal cell Technology for versatile interference removal (UCT)

- Accurate answers
- Lower detection limits even for elements subject to high spectroscopic interference

Determination of Methyl- and Inorganic Mercury in Fish with the NexSAR HPLC-ICP-MS Speciation Solution

OBJECTIVE

Rapid reliable measurement of methylmercury in fish

Simple, efficient **extraction of mercury** from fish **without changing the species**

Separate methylmercury from inorganic mercury with **HPLC**

Detect the species with **ICP-MS**

SAMPLE & CALIBRATION STANDARDS PREPARATION

- Sample
  - BCR-463 Freeze Dried Tuna Fish

    Certified for methylmercury and total mercury
  - 0.1 g sample extracted with L-cysteine·HCl
  - Final dilution of 4000x for analysis

- Calibration Standards
  - 0.5, 1, 2, 5 ppb prepared in mobile phase
  - Inorganic Hg standards from 10ppm Hg stock
  - Methylmercury standards from methylmercury chloride dissolved in deionized water

    (2-mercaptoethanol added to aid in methylmercury dissolution)

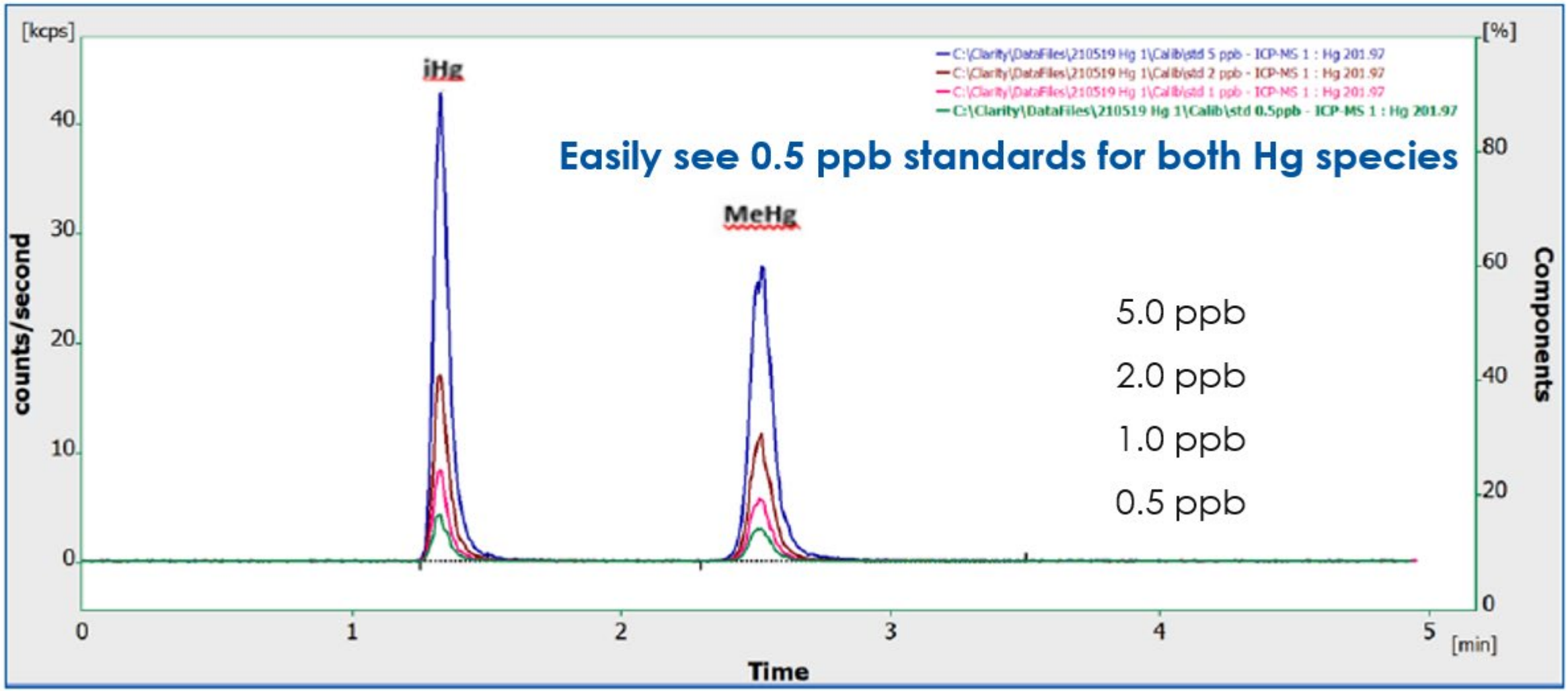
NEXSAR HPLC PARAMETERS

Component/Parameter	Value/Condition
Column	PerkinElmer C18
Mobile Phase	0.1% (w/v) L-Cysteine HCl (98%) + Methanol (2%)
pH	2.1
pH Adjustment	None
Flow Rate	1.5 mL/min
Separation Scheme	Isocratic
Column Temperature	35 °C
Injection Mode	Partial Loop
Injection Volume	50 µL
Loop Volume	100 µL
LC Vials	1.5 mL Polypropylene

NEXION ICP-MS PARAMETERS

Component/Parameter	Value/Condition
Nebulizer	Glass Concentric
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Nebulizer Flow	Optimized for < 2% Oxides
Mode	Standard
Analyte	^{202}Hg
Dwell Time	250 ms
Sampling Rate	4 points/second

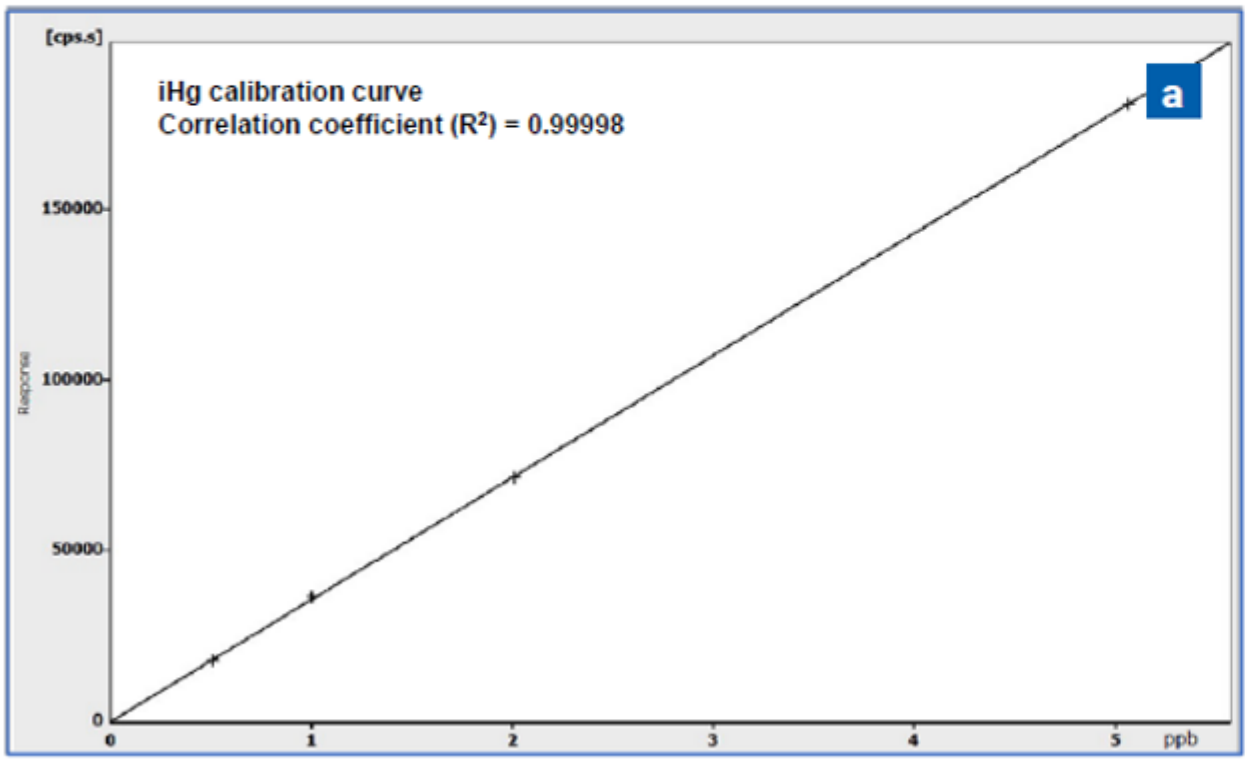
RESULTS: CALIBRATION OVERLAY



RESULTS: CALIBRATIONS

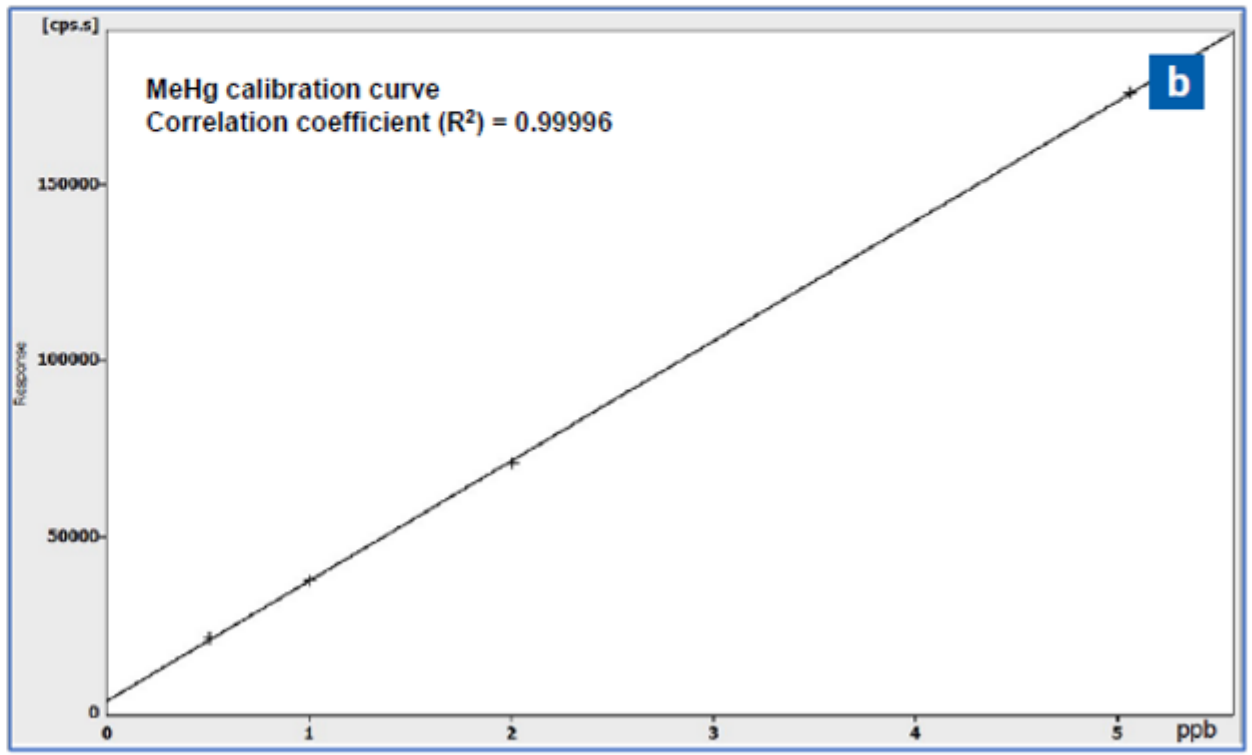
Inorganic Hg

$R^2 = 0.99998$

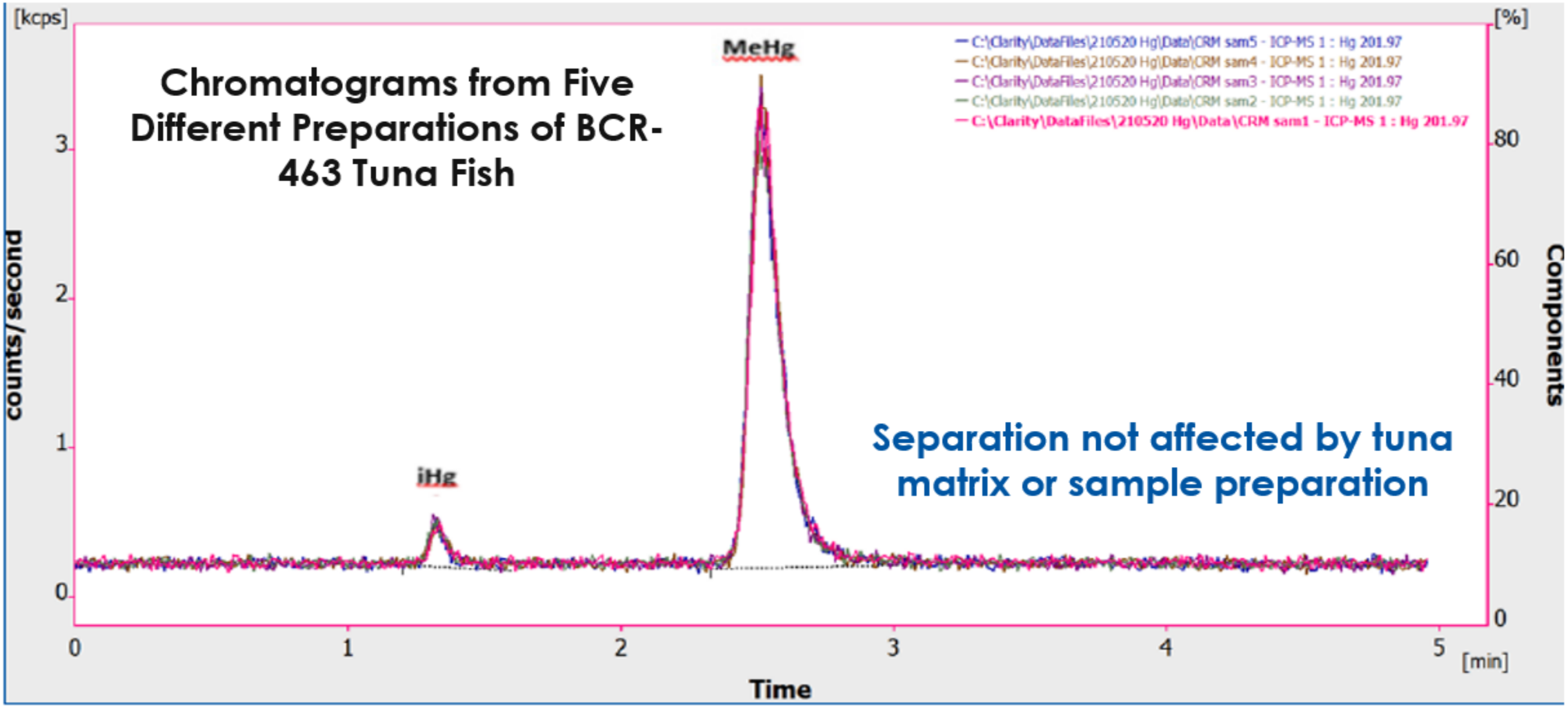


Methylmercury

$R^2 = 0.99996$



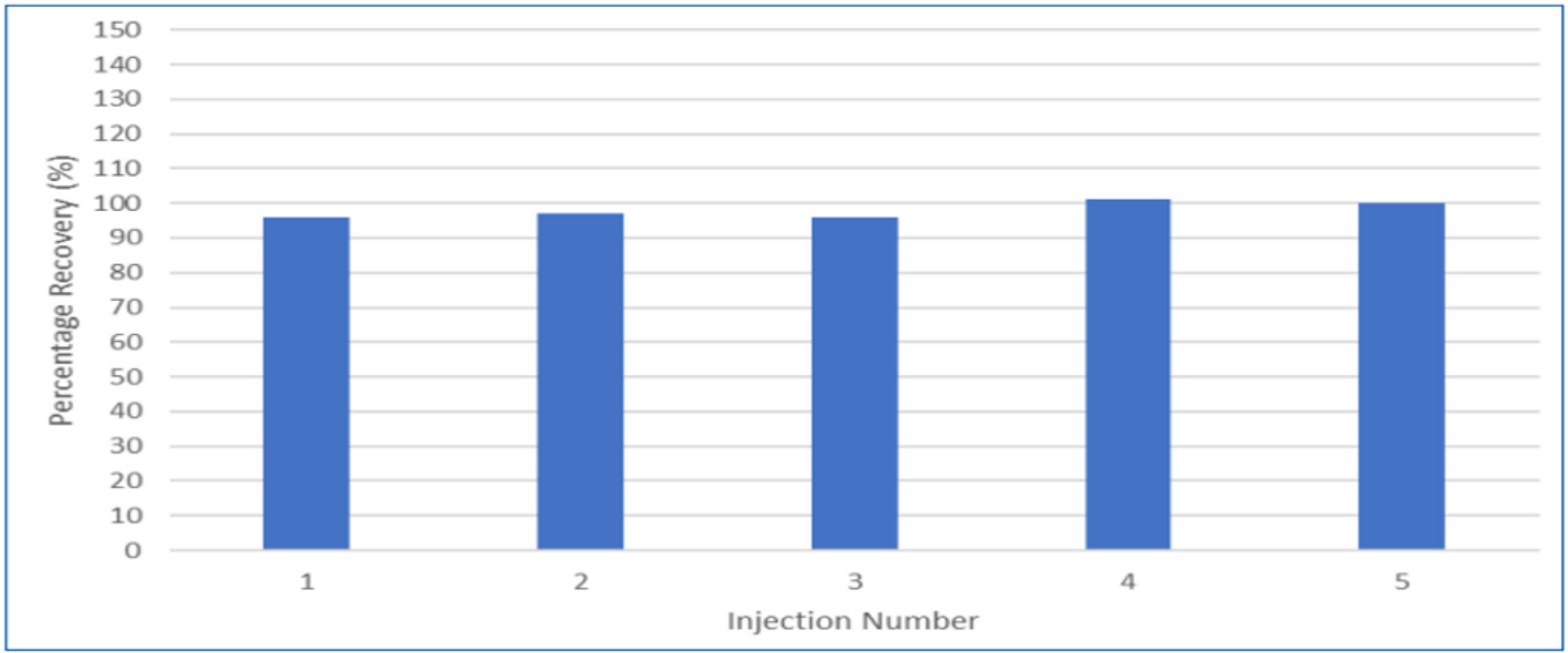
RESULTS: REPEATABILITY



RESULTS: ACCURACY

Methylmercury Recovery in BCR-463 Tuna Fish

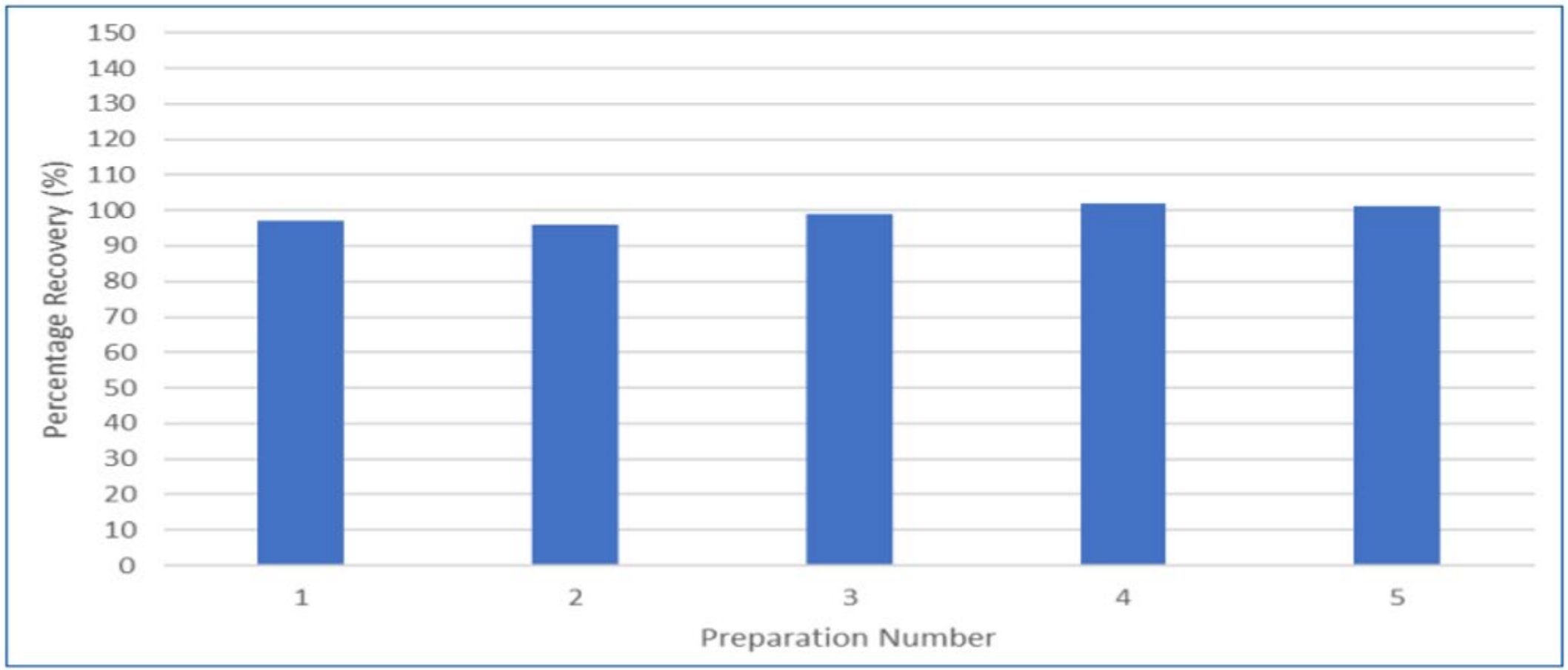
Certified Value = 3.04 µg/g - Recoveries within 5% of certified value



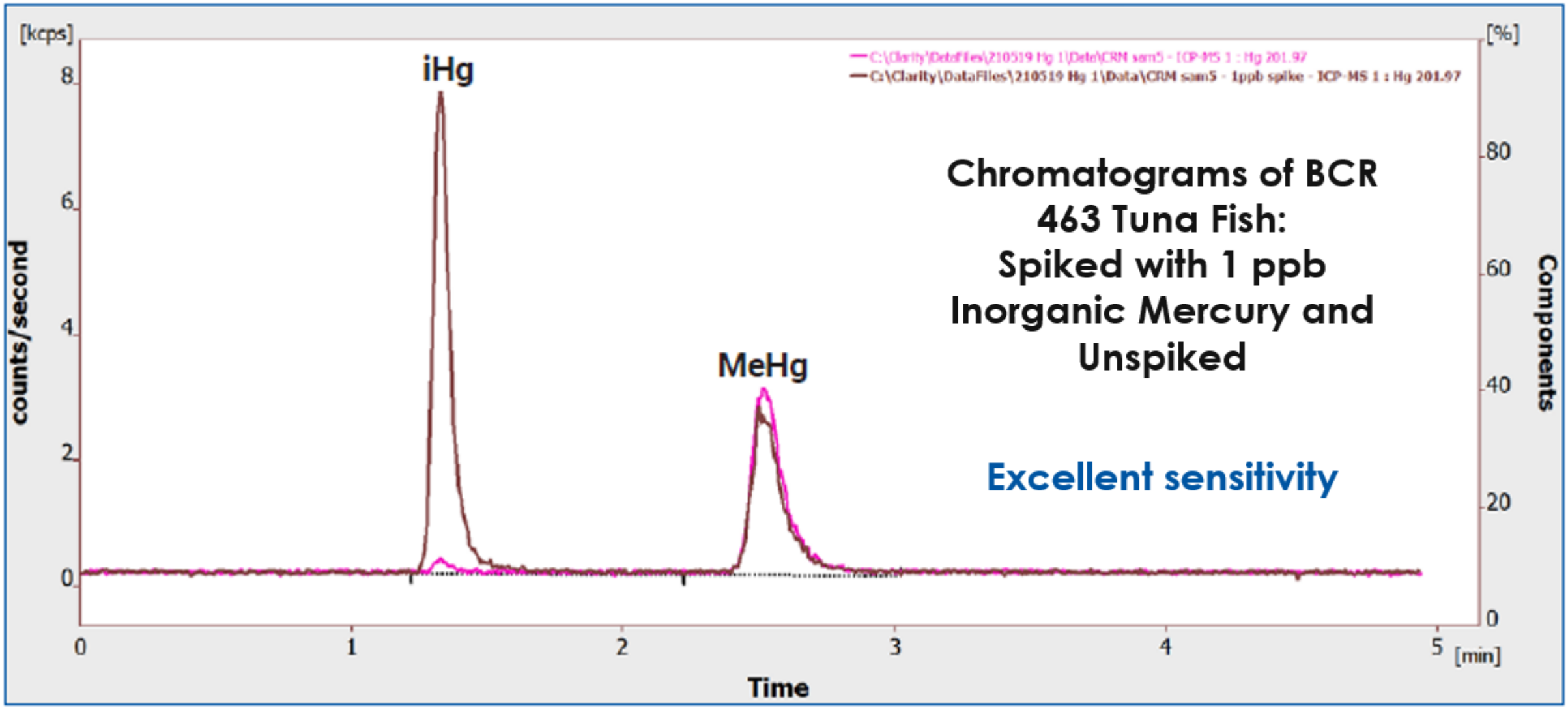
RESULTS: ACCURACY

Inorganic Mercury Spikes in BCR-463 Tuna Fish

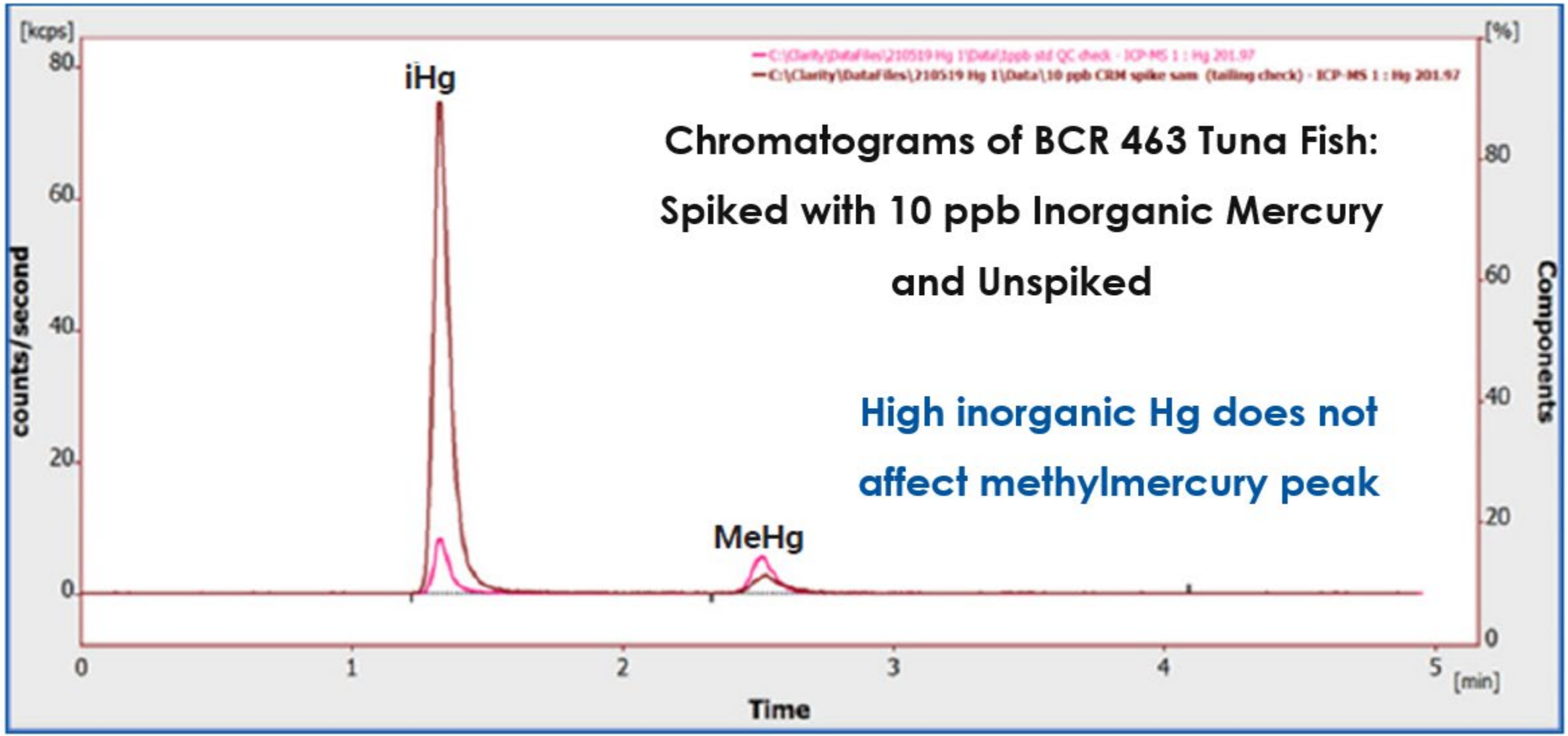
Spike = 1.0 ppb - Recoveries within 5% of spiked amount



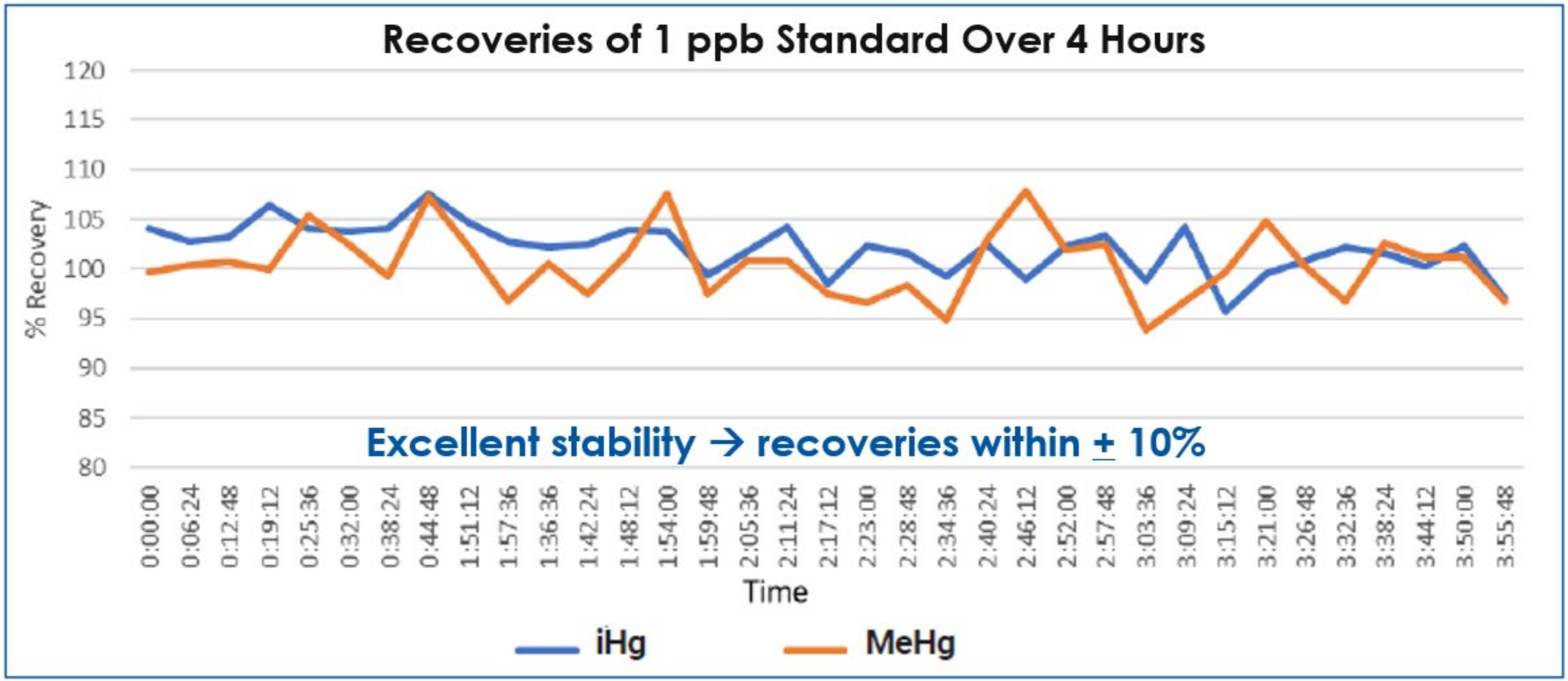
RESULTS: ACCURACY



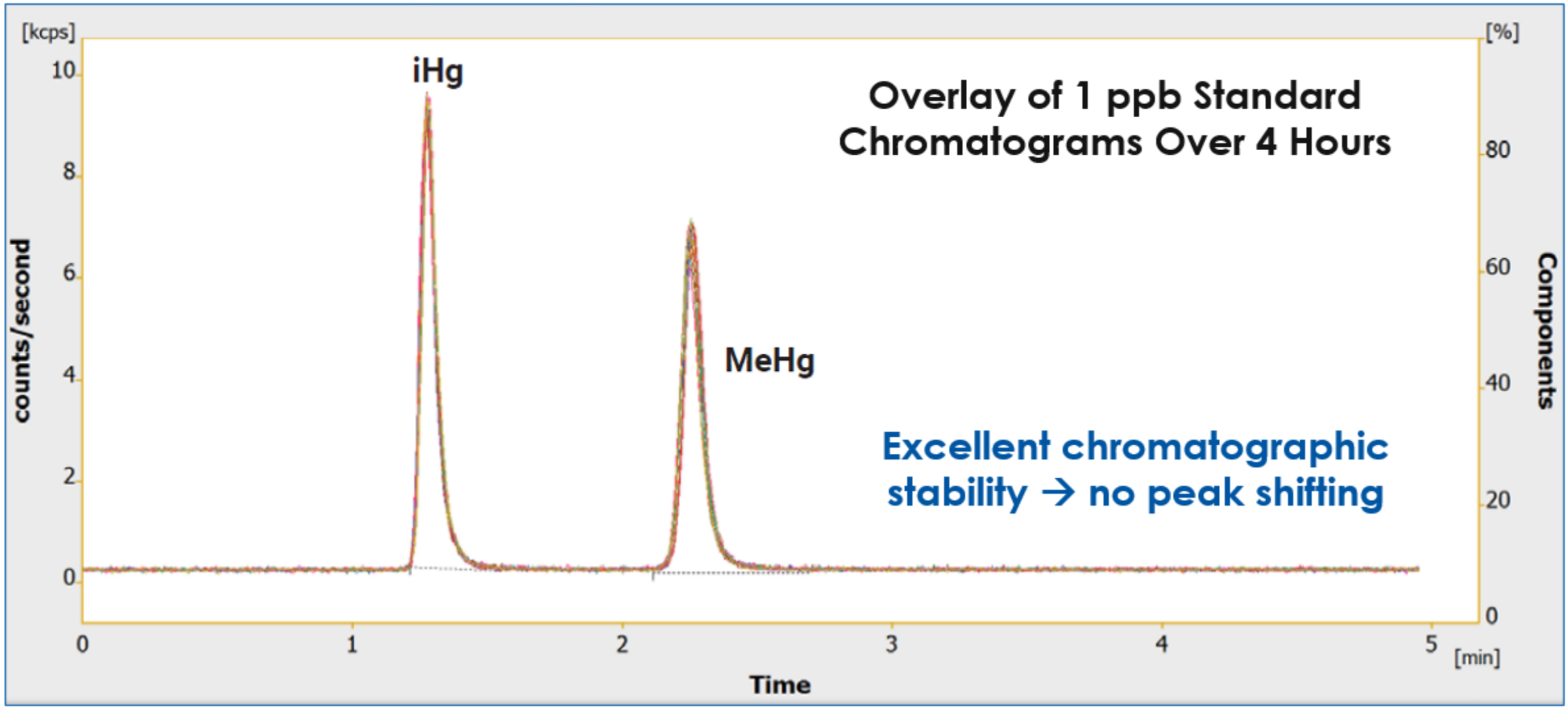
RESULTS: EFFECT OF HIGH INORGANIC MERCURY CONCENTRATIONS



RESULTS: STABILITY

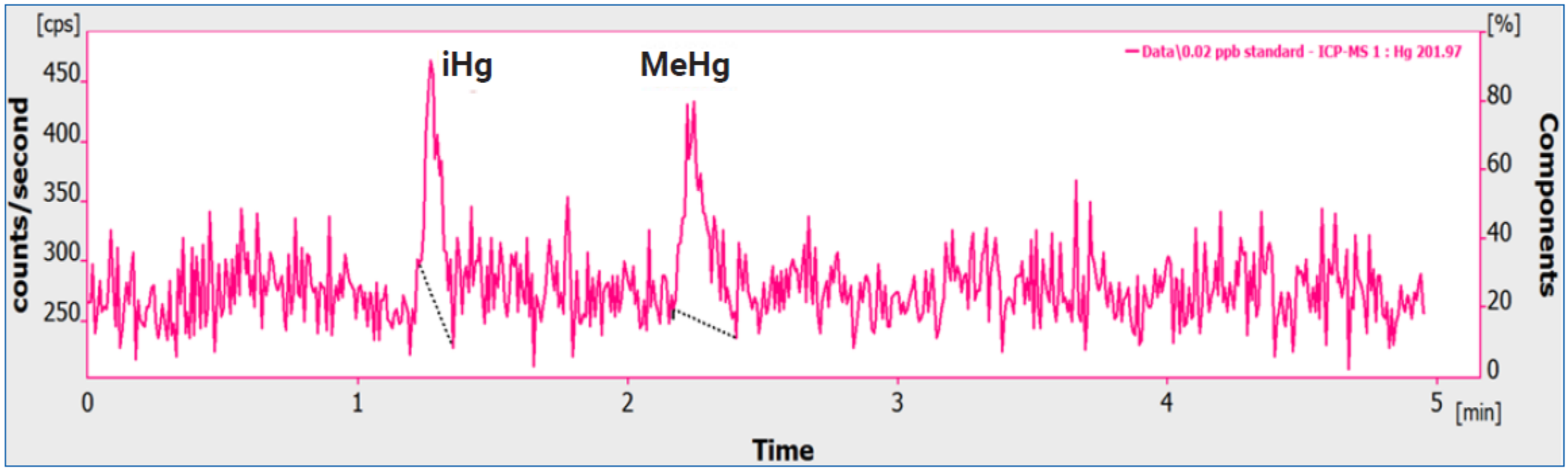


RESULTS: STABILITY



RESULTS: LOW-LEVEL MEASUREMENTS

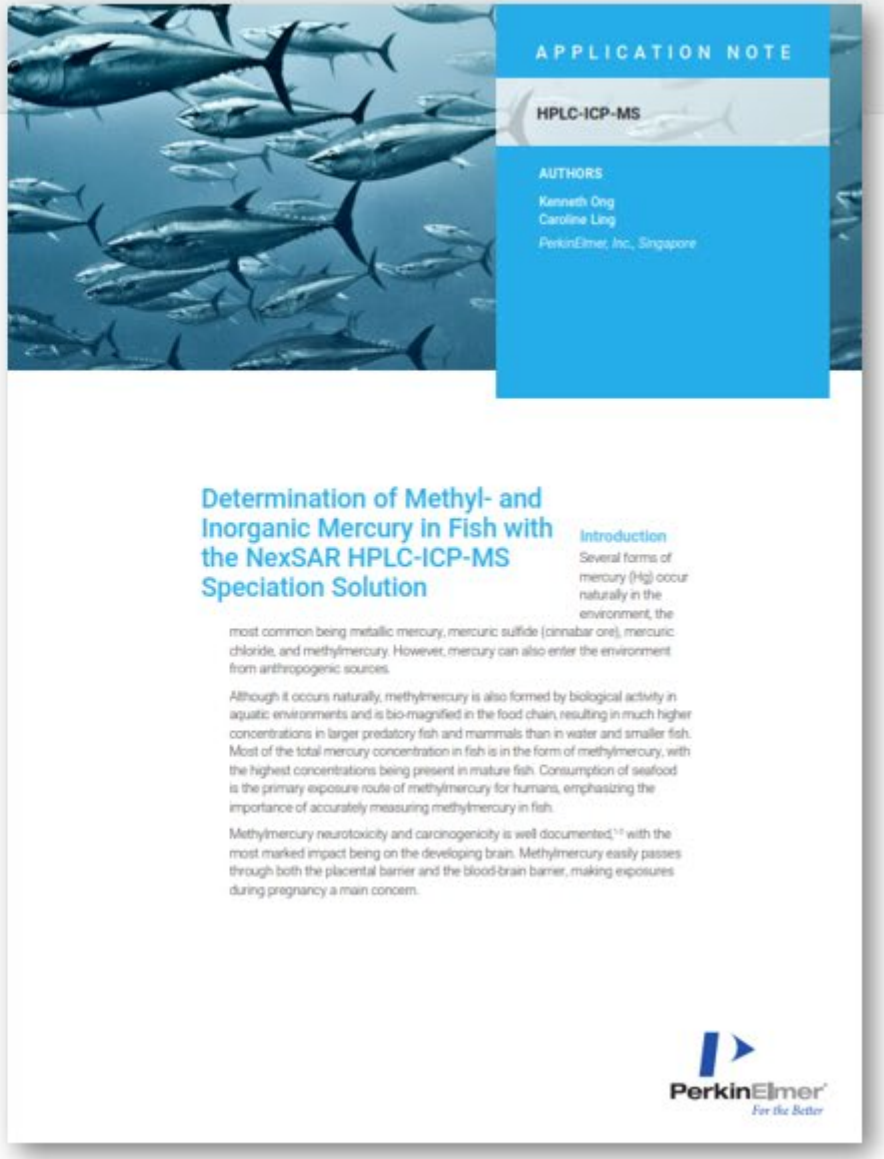
Chromatogram of 0.02 ppb iHg and MeHg Standard



Ability to measure down to 0.02 ppb

SUMMARY

- The presented data clearly demonstrates that NexSAR Speciation Solution and the proposed method can achieve:
  - Aqueous extraction
  - The rapid and accurate measurement of both MeHg and inorganic Hg in freeze dried fish within 3 minutes
  - Baseline separation of inorganic Hg and MeHg
  - Excellent robustness and repeatability



ACKNOWLEDGMENTS

- Kenneth Ong & Caroline Ling, PerkinElmer Singapore

ADDITIONAL NEXAR APPLICATIONS

Characterization of Arsenic Species in Apple Juice using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution

Quantification of Low Levels of Hexavalent Chromium in Water Using a NexSAR Inert HPLC-ICP-MS

Arsenic Speciation Analysis in Rice Following Method GB 5009.11-2014 to Ensure Safety

Determination of Selenium Species in Drinking Water by HPLC-ICP-MS

Speciation of Hexavalent Chromium in Children's Toys According to EN 71-3 Category II with the NexSAR HPLC-ICP-MS

Determination of Organotin in Tap Water by HPLC-ICP-MS in Accordance with Chinese Method HJ 1074-2019

Determination of Methyl- and Inorganic Mercury in Fish with the NexSAR HPLC-ICP-MS Speciation Solution

STREAMLINE YOUR SPECIATION WORKFLOW

NexSAR HPLC-ICP-MS Speciation Solution

Separation. Detection. Operation. Integration.

PerkinElmer's NexSAR™ HPLC-ICP-MS Speciation Solution brings together our inert NexSAR Speciation Analysis Ready HPLC system and the revolutionary NexION® ICP-MS in a seamless platform. Integrated using Clarity™ software, all levels of expertise are catered to through an intuitive solution with multi-user and multi-permission functionality.

- Completely metal-free flow path NexSAR HPLC System
- Robust and versatile NexION ICP-MS system
- Flexible, tried and tested Clarity™ software
- Seamlessly integrated HPLC-ICP-MS solution

NexSAR HPLC – Lower Baselines, Remarkable Detection Limits

Speciation starts with separation. Discover the NexSAR HPLC, the next generation of analysis-ready speciation systems – engineered with a completely inert and metal-free fluid path, it enables you to meet the low chromatographic baselines required by your most challenging applications.

NexION ICP-MS – Ultimate Detection for Accurate Speciation

With an array of patented technologies designed to minimize maintenance even when working with heavy matrix samples and high total dissolved solids (TDS) mobile phase compositions, NexION is the world's most robust and flexible ICP-MS platform.

Clarity™ Software – Intuitive and Simple, Saving Time

At the center of the system is the proven Clarity™ chromatography software, which controls both the NexSAR HPLC and the NexION ICP-MS, allowing streamlined workflows, from method development through to reporting. Data processing, integration and calibration are straightforward, and for the most discerning users, advanced integration features are also available. Reporting is simple, allowing you to export data into a variety of different formats and personalize your reporting style to suit your individual needs.

To learn more, visit: www.perkinelmer.com/speciation

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