



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.

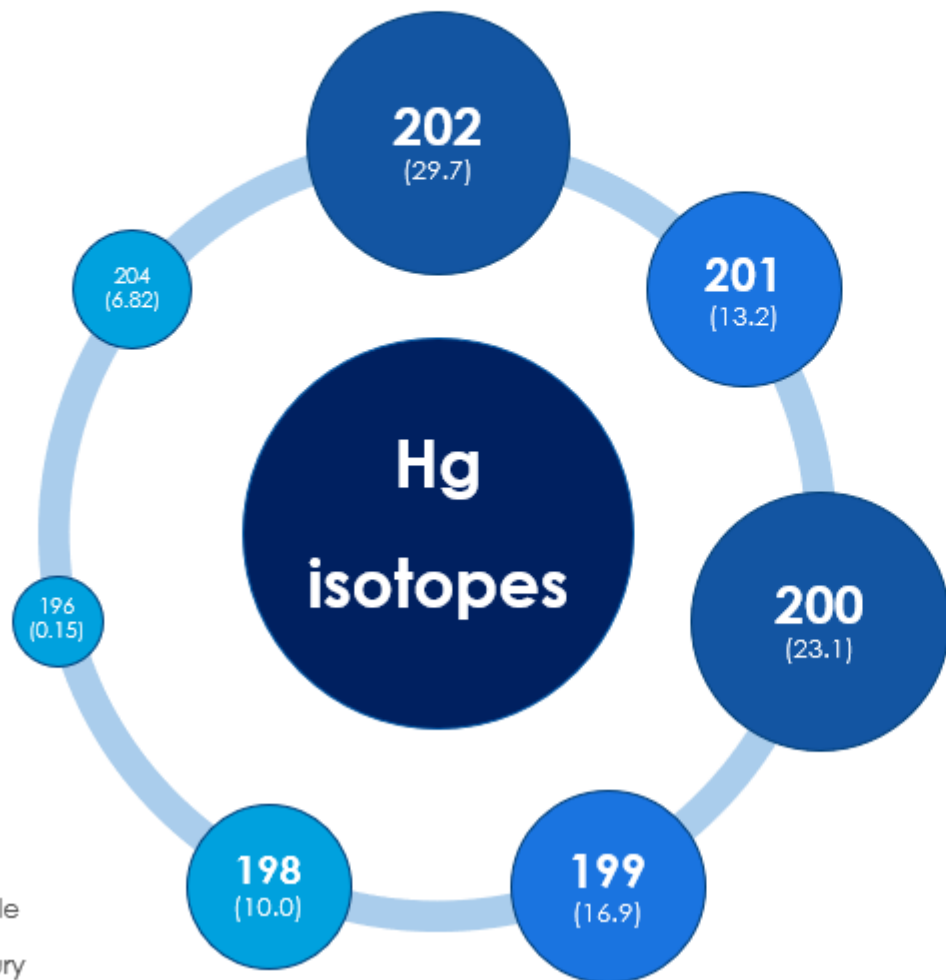
Specific form of an element defined as to **isotopic composition, electronic or oxidation state, and/or complex or molecular structure.**



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WHAT IS A CHEMICAL SPECIES?

Specific form of an element defined as to isotopic composition



Most abundant and stable
isotopes of natural mercury

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



I U P A C

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

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

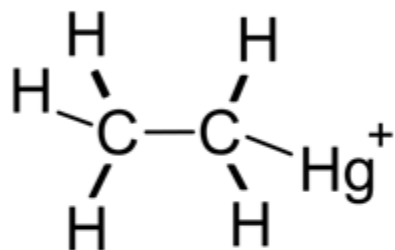
Oxidation state – determines chemical behavior (level of reactivity), toxicity (bioavailability) and environmental fate (potential for transformation within environmental systems).



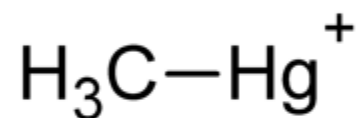
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WHAT IS A CHEMICAL SPECIES?

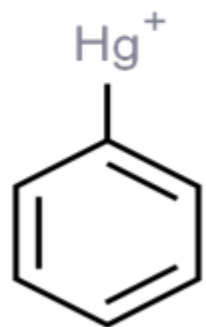
Specific form of an element defined as to complex or molecular structure.



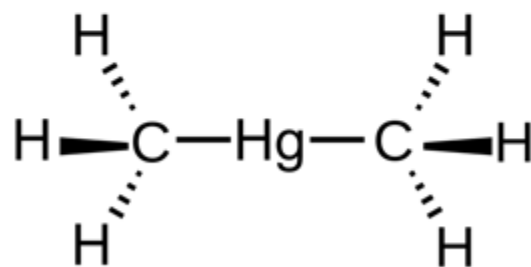
Ethyl mercury



Methylmercury



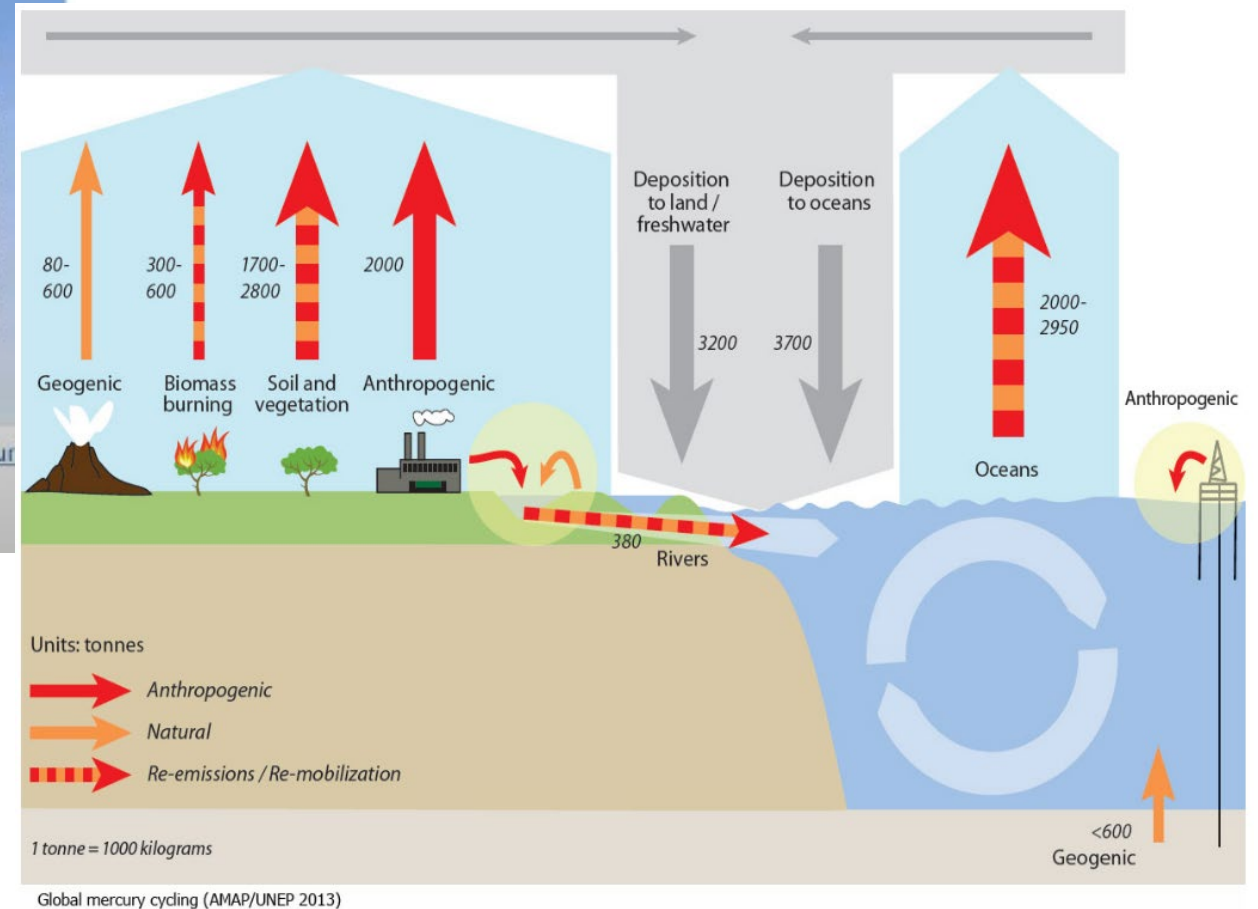
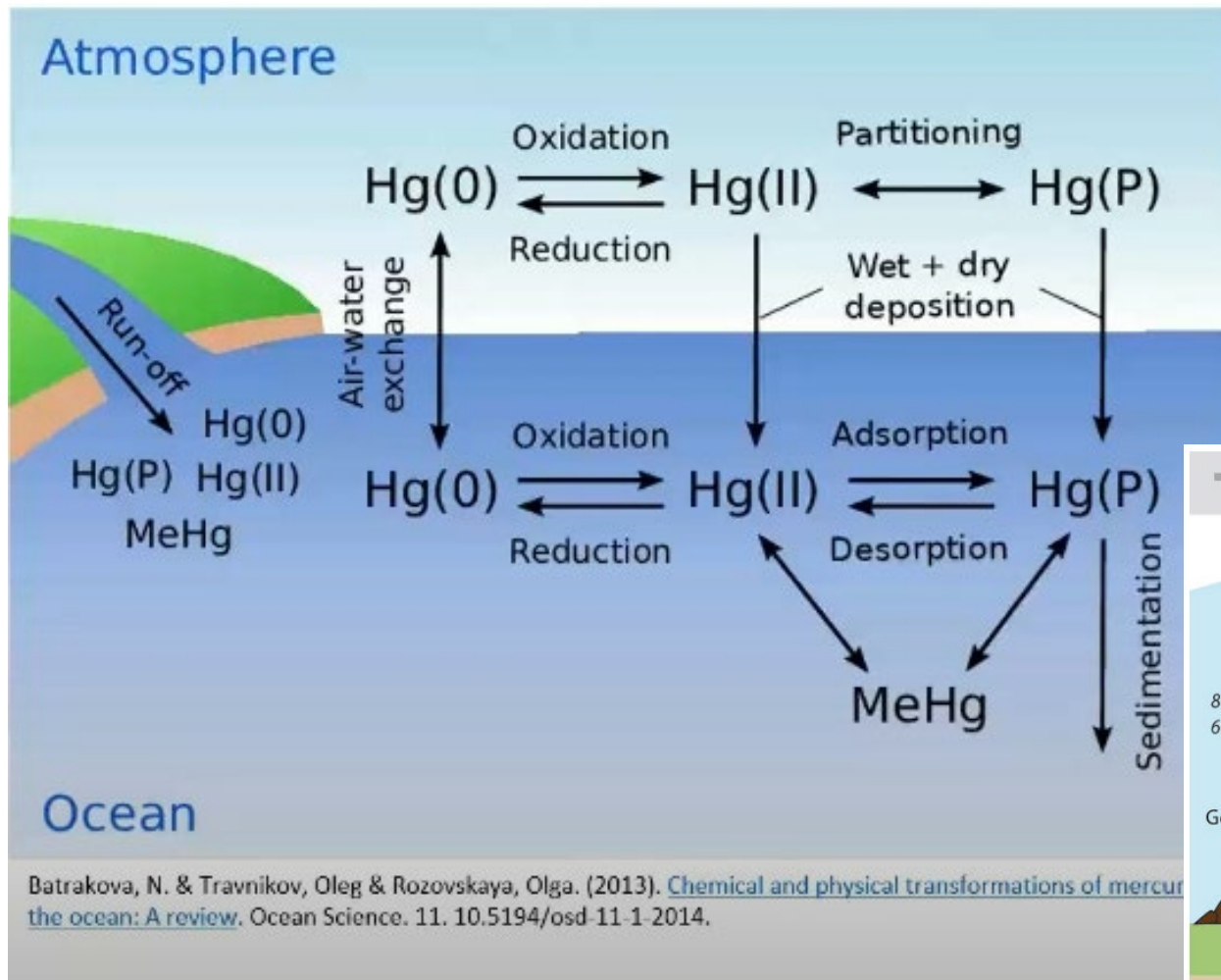
Phenylmercury



Dimethylmercury



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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

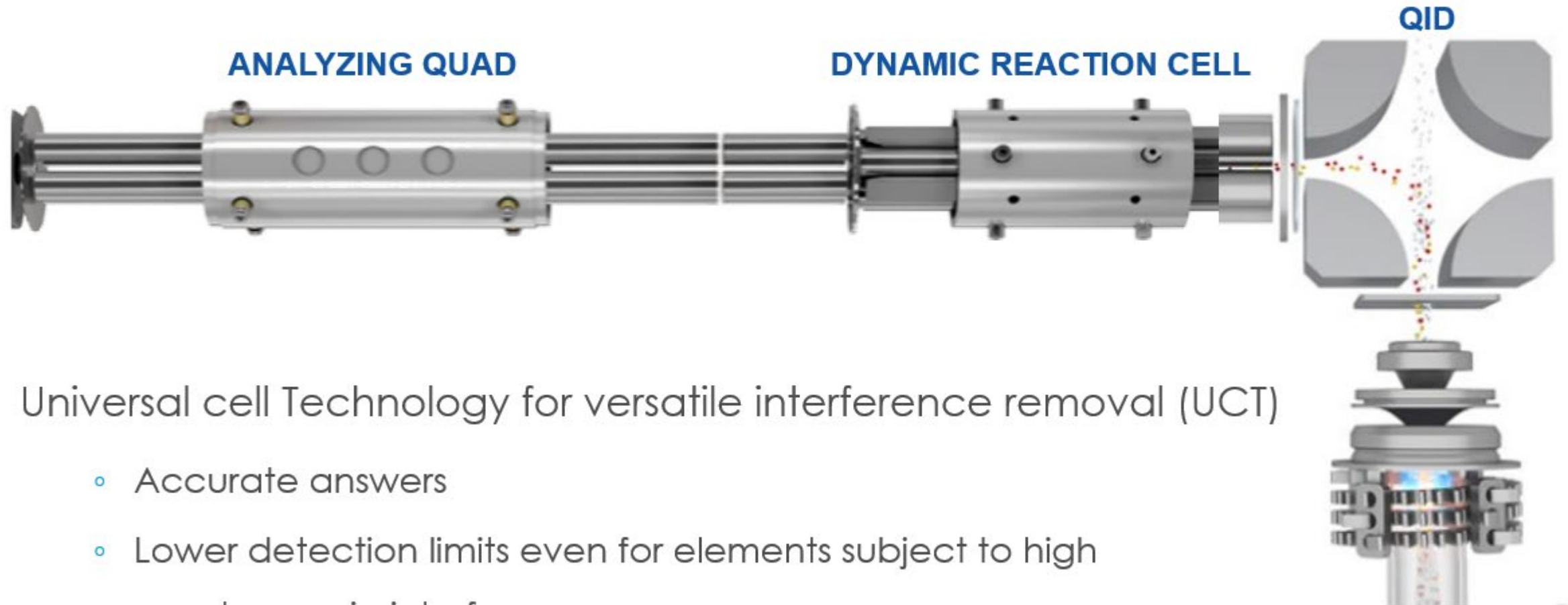


NEXION® ICP-MS - ANY MATRIX, ANY INTERFERENCE

- 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

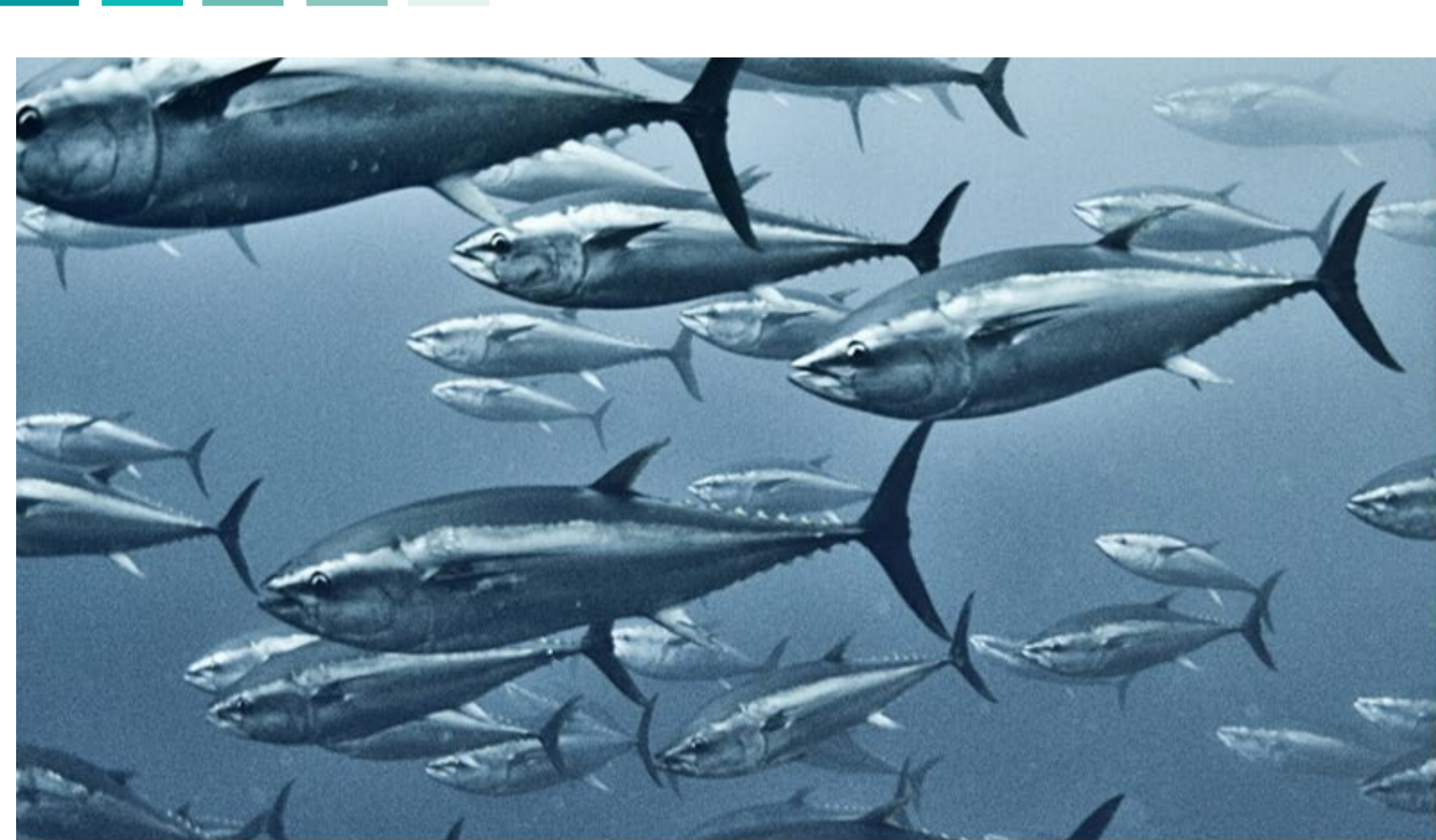


NEXION® ICP-MS - ANY MATRIX, ANY INTERFERENCE



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

APPLICATION NOTE

HPLC-ICP-MS

AUTHORS

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PerkinElmer, Inc., Singapore

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

Introduction
Several forms of mercury (Hg) occur naturally in the environment, the most common being metallic mercury, mercuric sulfide (cinnabar ore), mercuric chloride, and methylmercury. However, mercury can also enter the environment from anthropogenic sources.

Although it occurs naturally, methylmercury is also formed by biological activity in aquatic environments and is bio-magnified in the food chain, resulting in much higher concentrations in larger predatory fish and mammals than in water and smaller fish. Most of the total mercury concentration in fish is in the form of methylmercury, with the highest concentrations being present in mature fish. Consumption of seafood is the primary exposure route of methylmercury for humans, emphasizing the importance of accurately measuring methylmercury in fish.

Methylmercury neurotoxicity and carcinogenicity is well documented,^{1,2} with the most marked impact being on the developing brain. Methylmercury easily passes through both the placental barrier and the blood-brain barrier, making exposures during pregnancy a main concern.



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



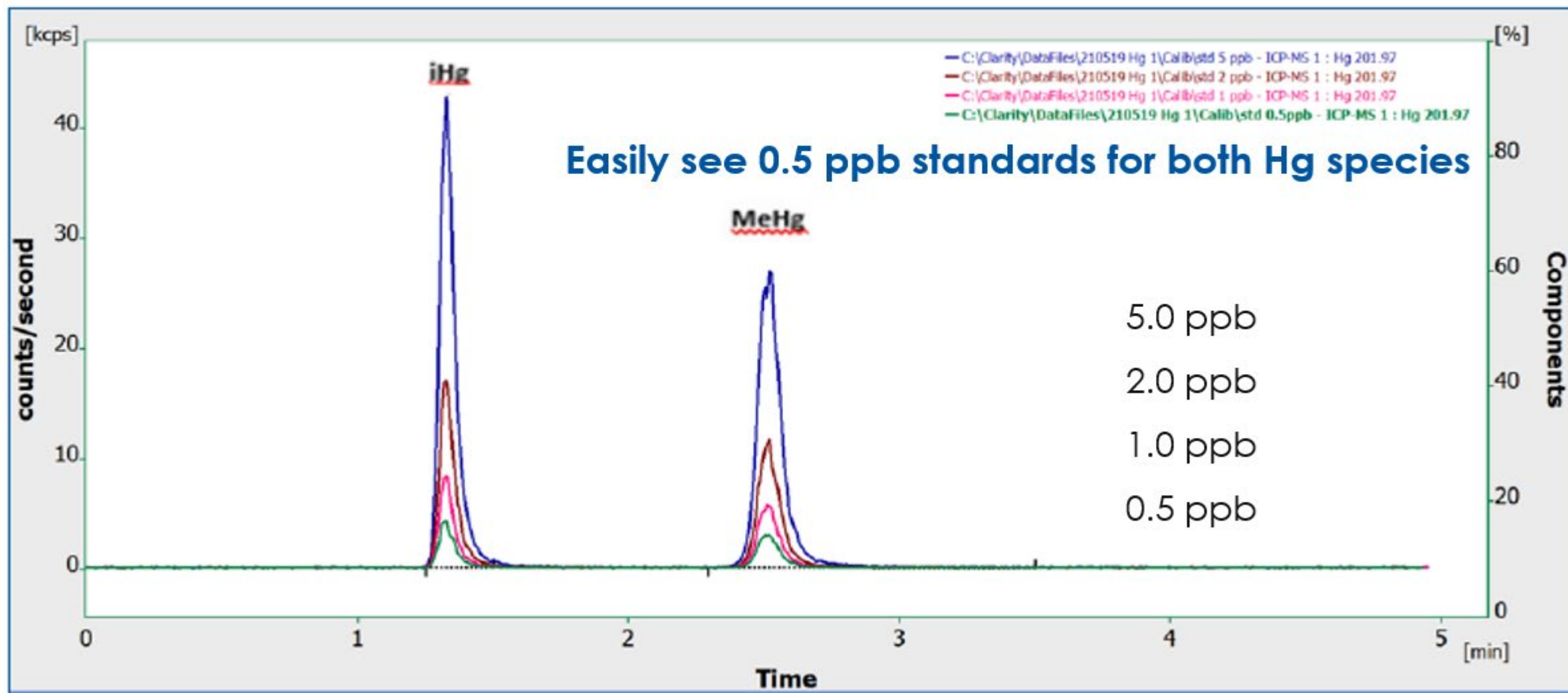
Component/Parameter	Value/Condition
Column	PerkinElmer C18
Mobile Phase	0.1% (w/v) L-Cysteine HCl (98%) + Methanol (2%)
pH	2.1
pH Adjustment	None
Flow Rate	1.5 mL/min
Separation Scheme	Isocratic
Column Temperature	35 °C
Injection Mode	Partial Loop
Injection Volume	50 µL
Loop Volume	100 µL
LC Vials	1.5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



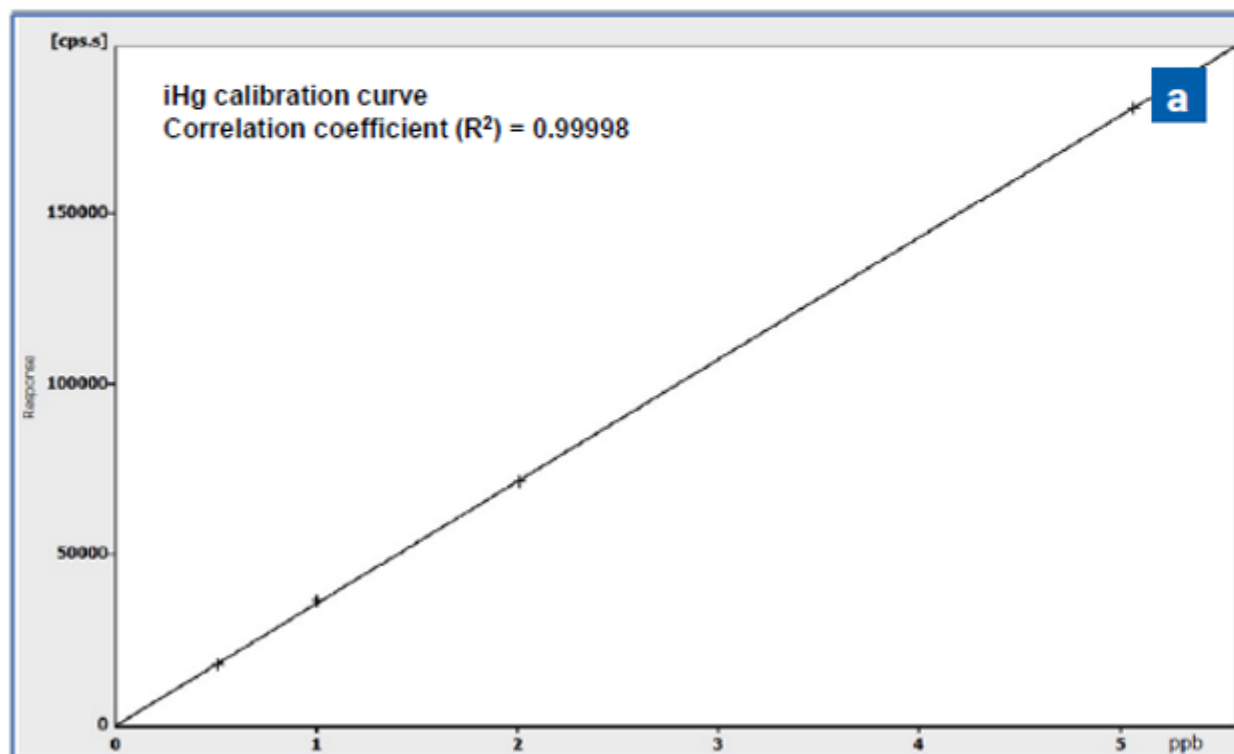
RESULTS: CALIBRATION OVERLAY



RESULTS: CALIBRATIONS

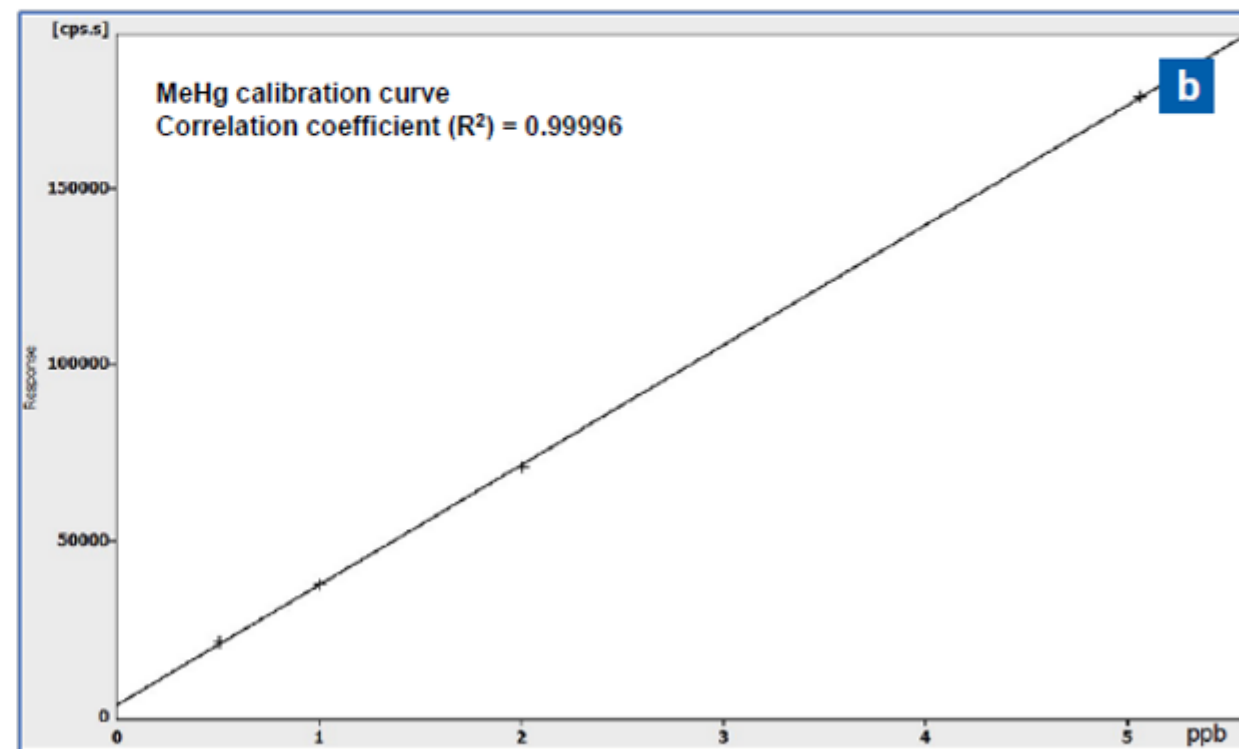
Inorganic Hg

$R^2 = 0.99998$

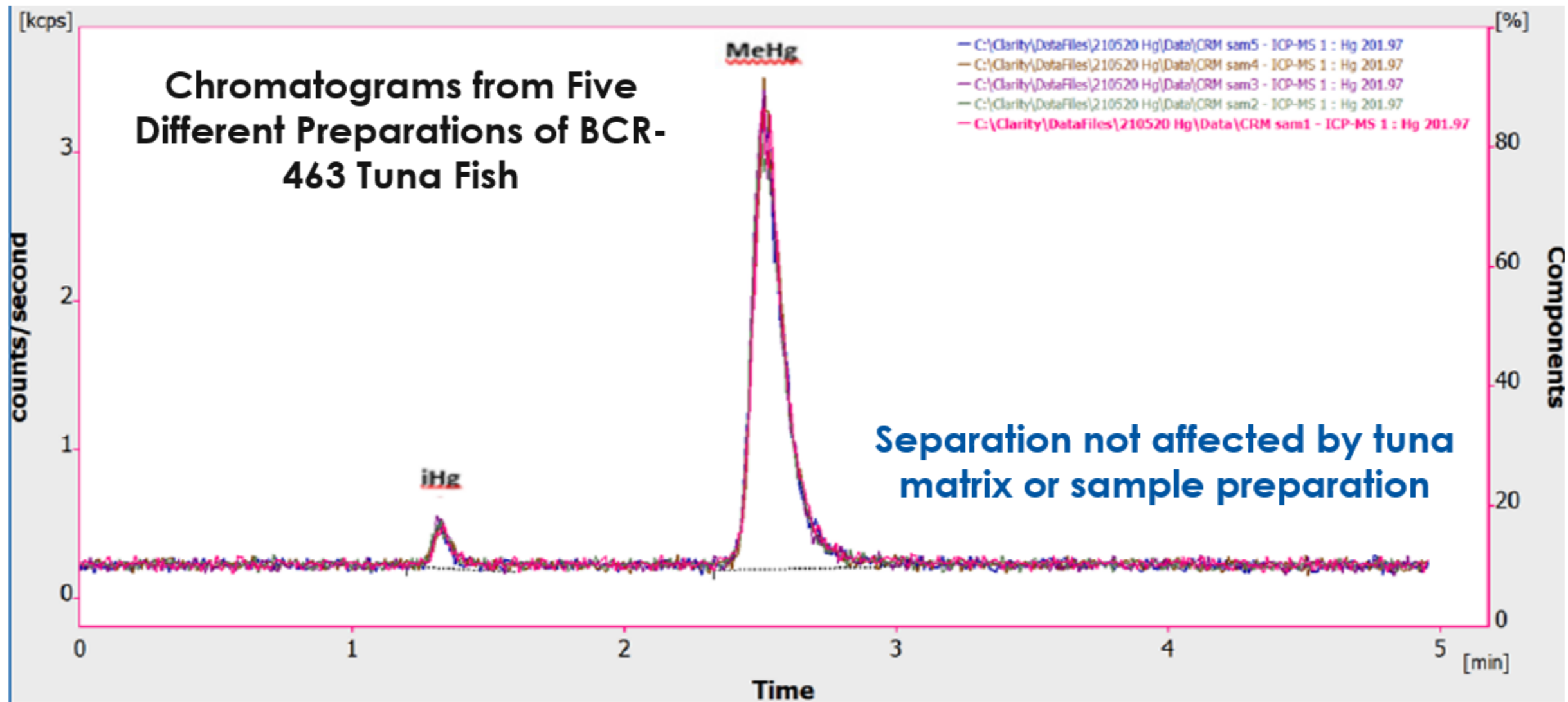


Methylmercury

$R^2 = 0.99996$



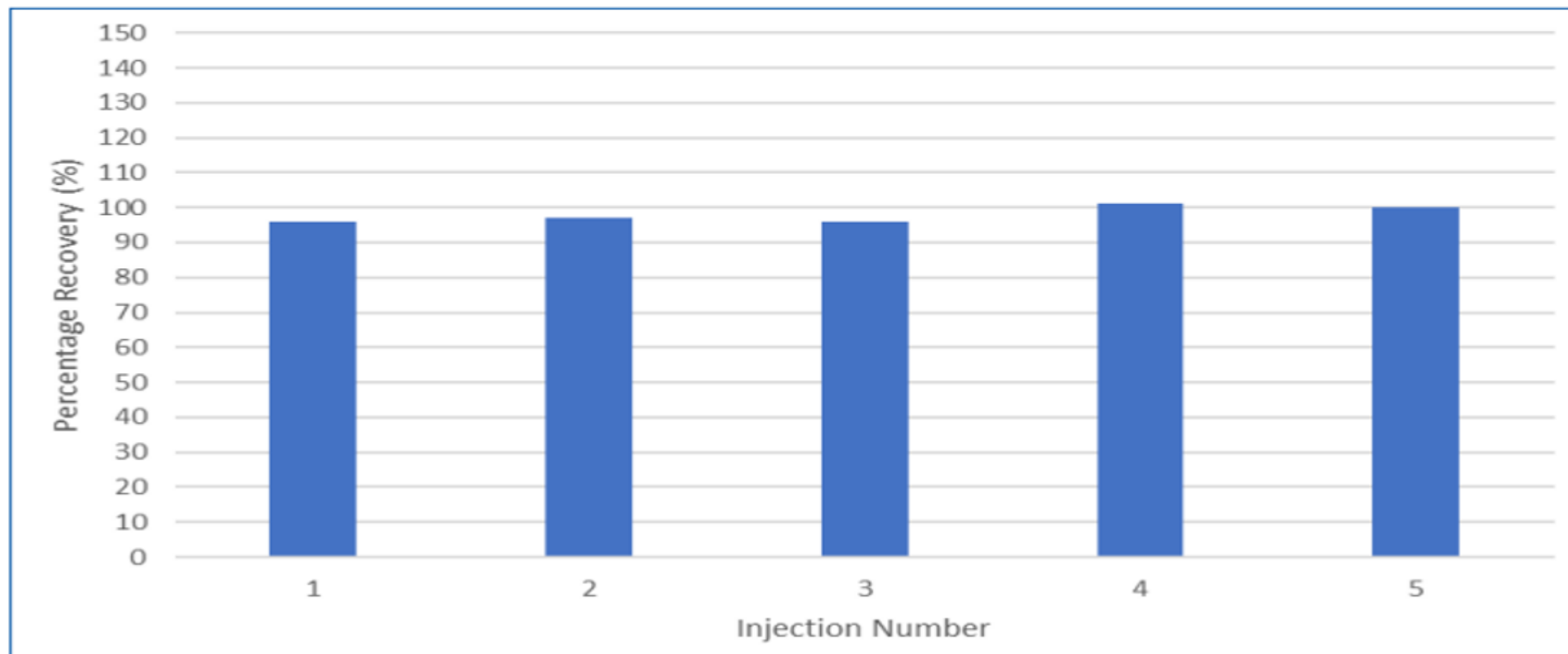
RESULTS: REPEATABILITY



RESULTS: ACCURACY

Methylmercury Recovery in BCR-463 Tuna Fish

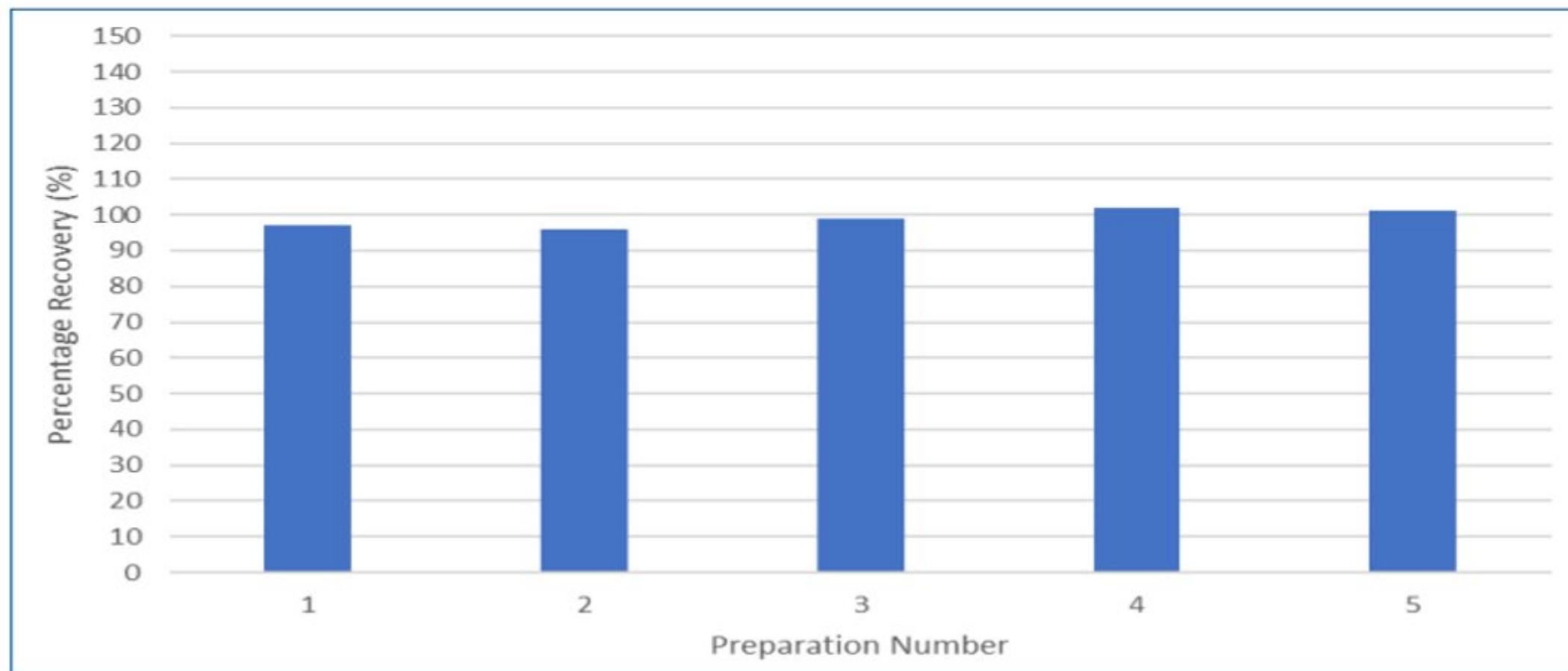
Certified Value = 3.04 µg/g - Recoveries within 5% of certified value



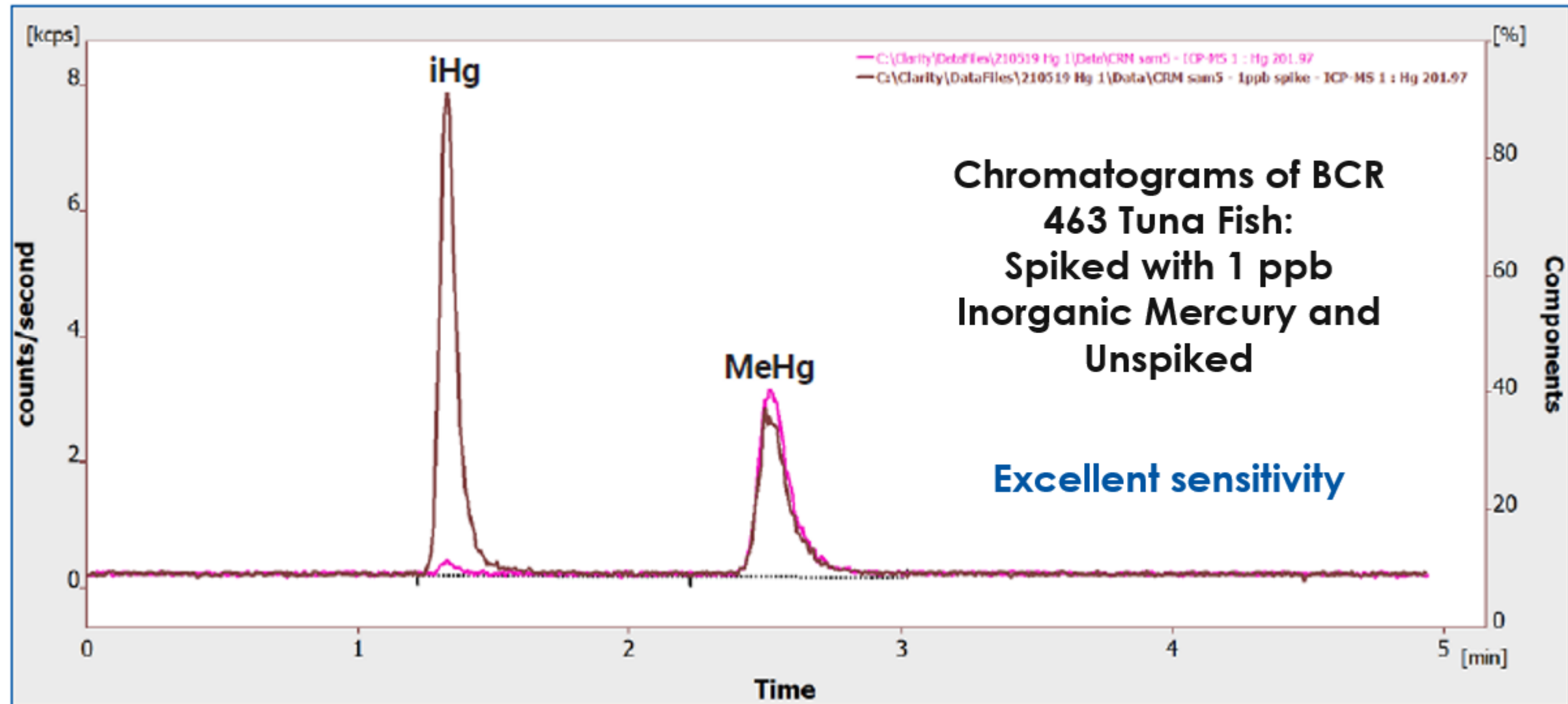
RESULTS: ACCURACY

Inorganic Mercury Spikes in BCR-463 Tuna Fish

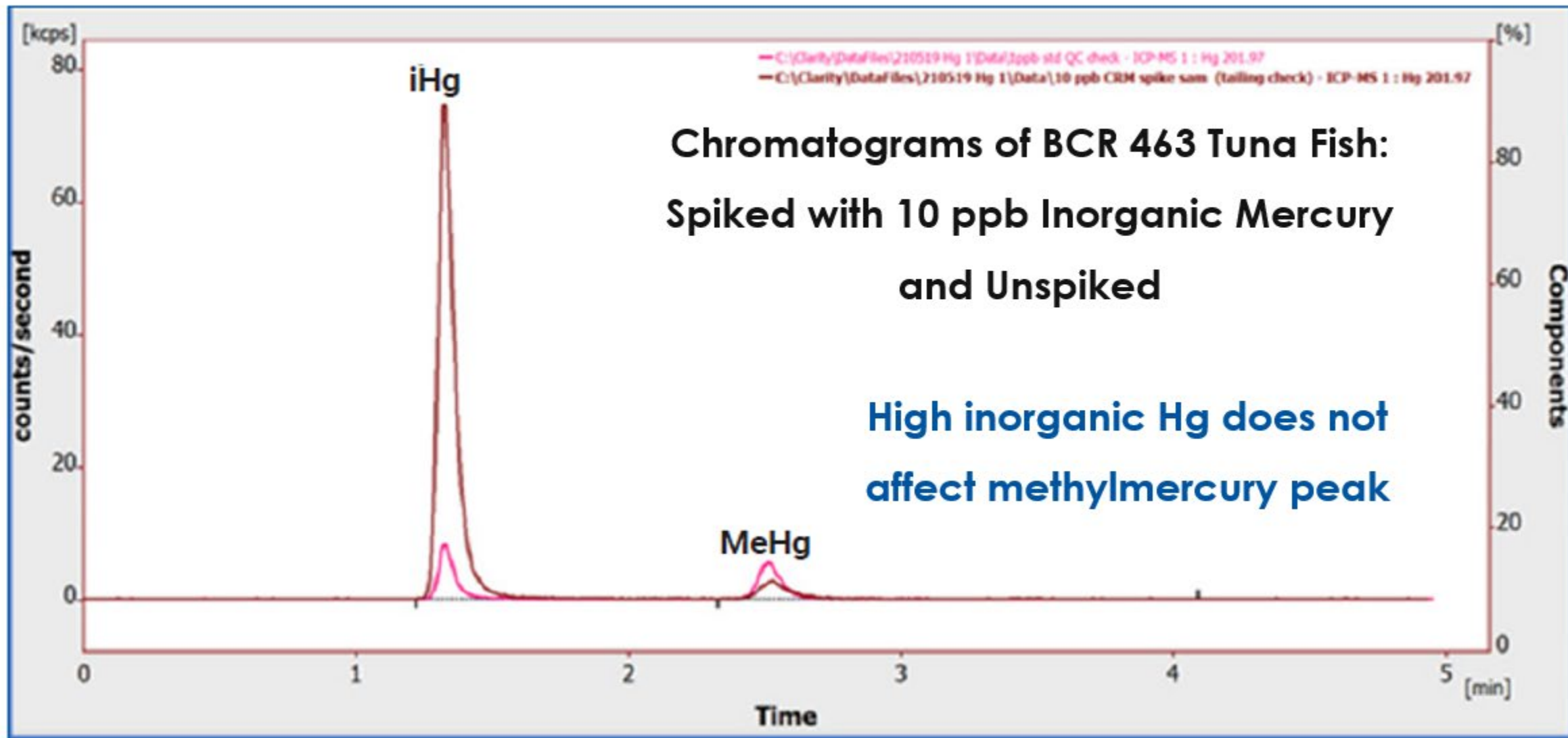
Spike = 1.0 ppb - Recoveries within 5% of spiked amount



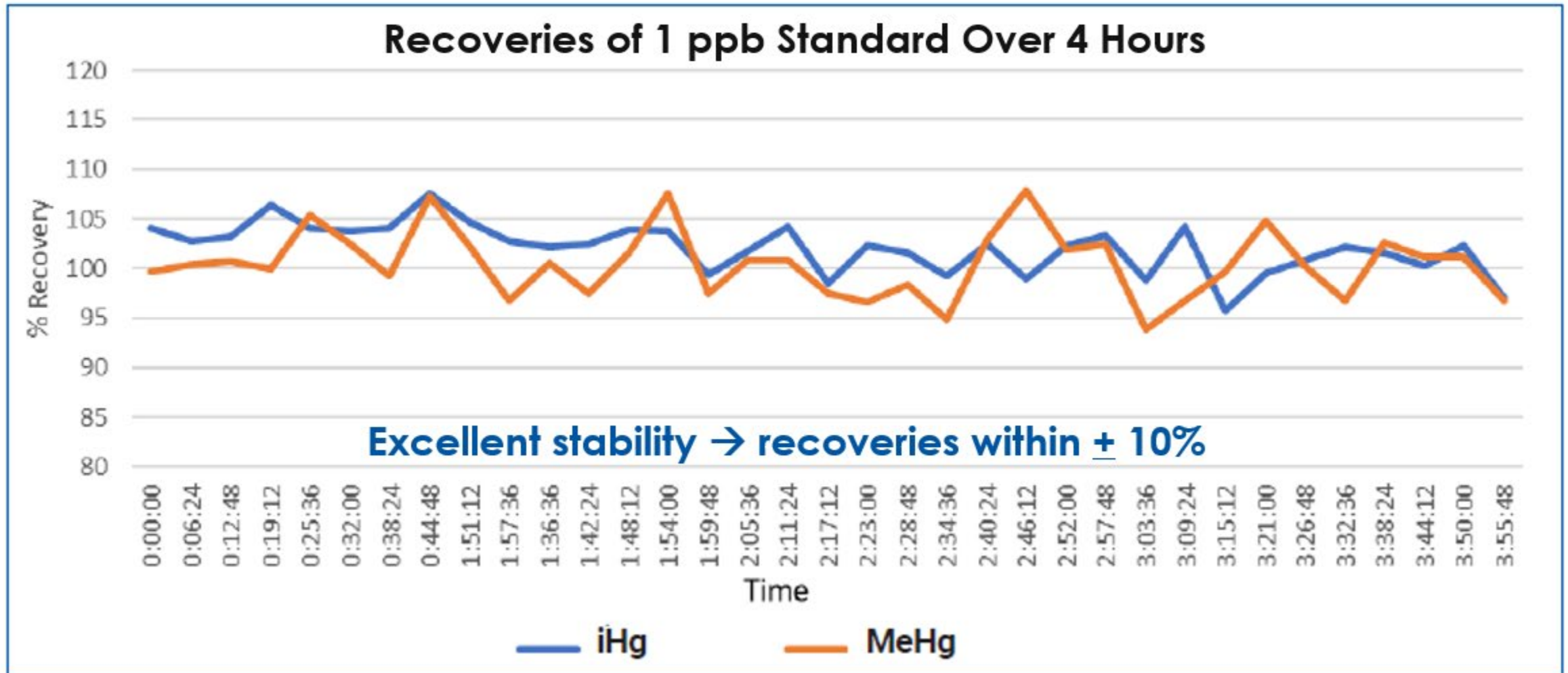
RESULTS: ACCURACY



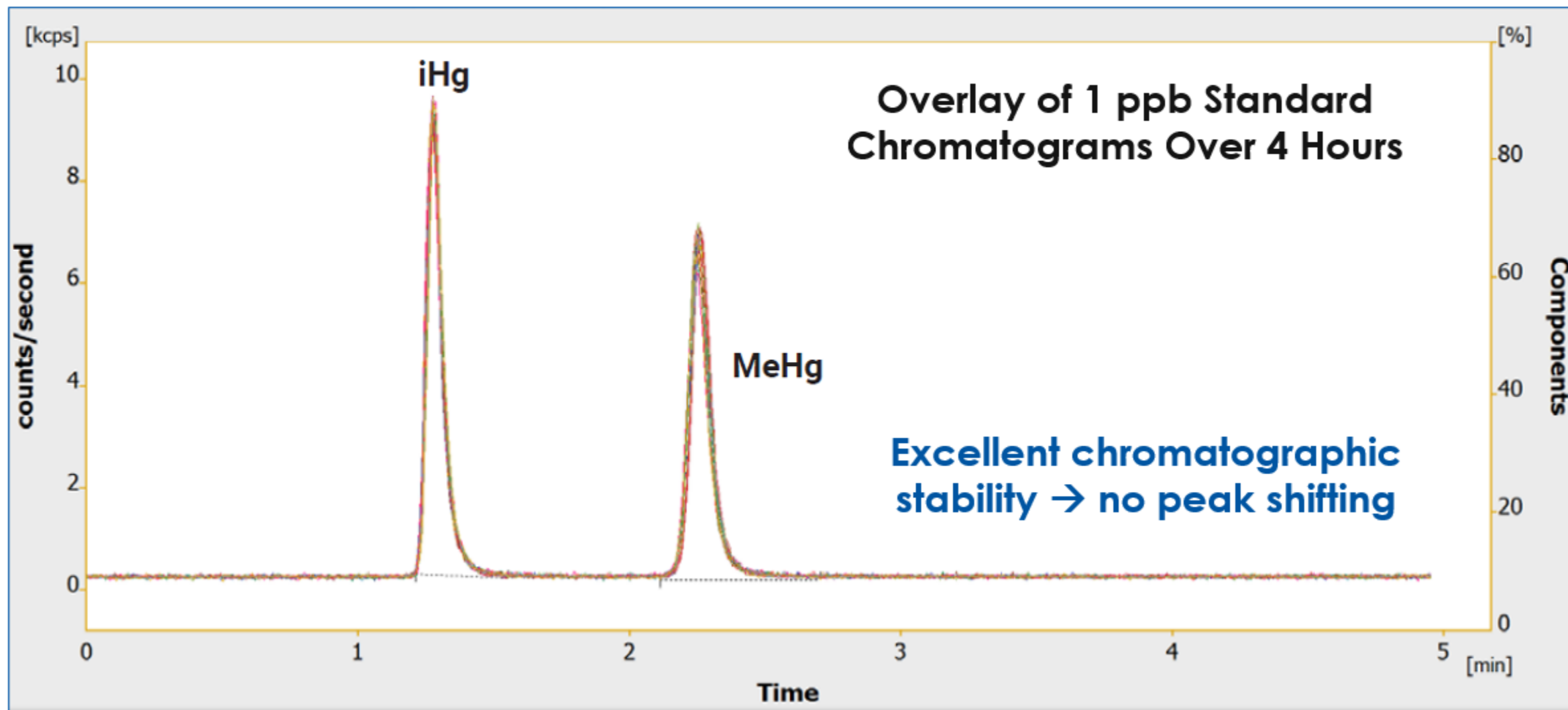
RESULTS: EFFECT OF HIGH INORGANIC MERCURY CONCENTRATIONS



RESULTS: STABILITY

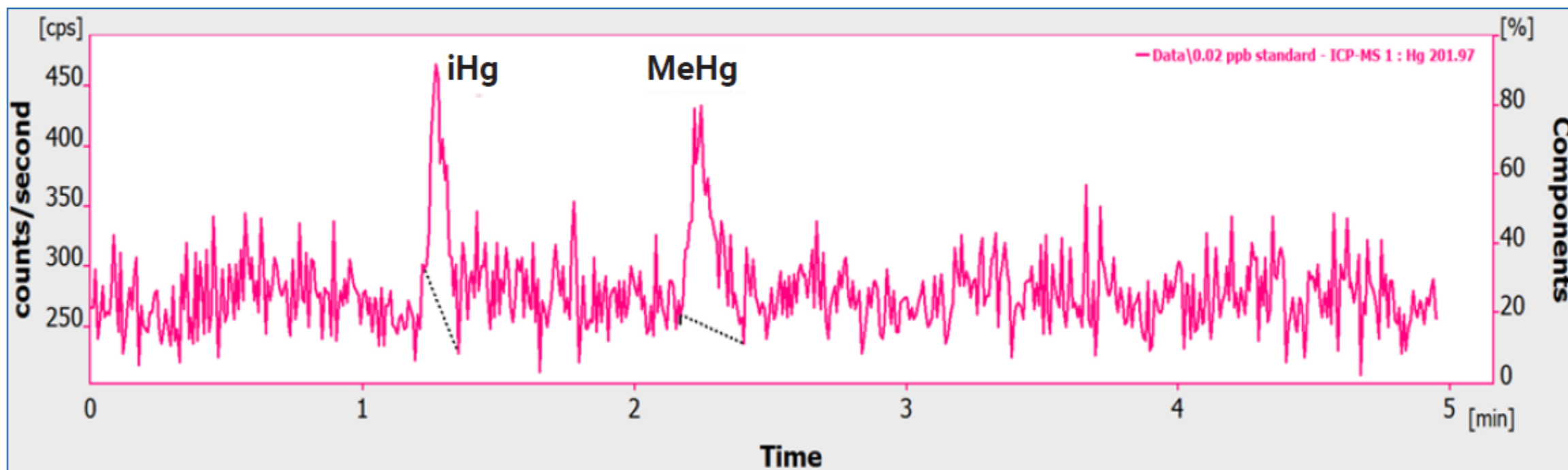


RESULTS: STABILITY



RESULTS: LOW-LEVEL MEASUREMENTS

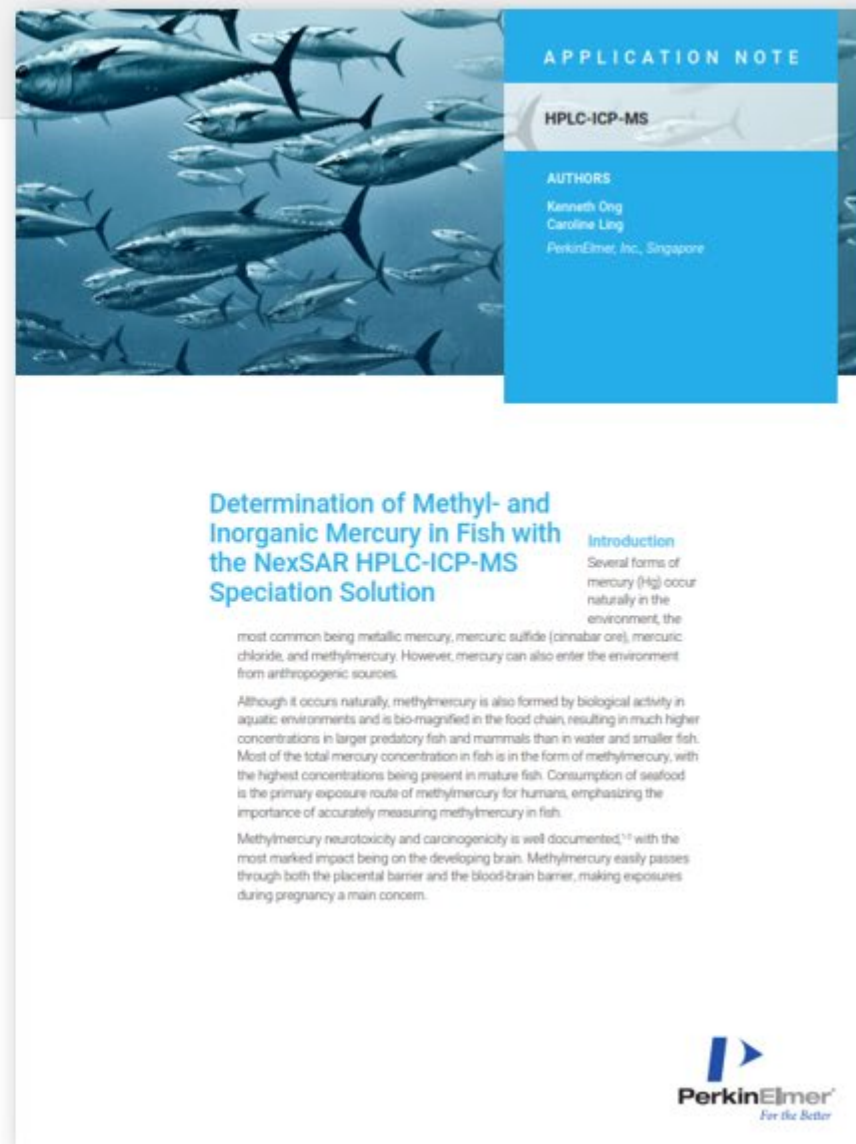
Chromatogram of 0.02 ppb iHg and MeHg Standard



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

being as a valuable source of chlorine, bromine, and fluorine." However, because this material was the source of water in such large quantities, it was both valued and feared.



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

Introduction There represents a striking divide for over half of the world's population. Due to its glutin-free, nonperishable (and high-calorie) content, the glutinous "rice" is often one of the first foods taught to an introduced nation. It is also the most popular grain for people in Asian parts. However, concern has been raised where the transgenic method can be inaccurate as well as a number of potential sources of As in rice, such



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

Introduction



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

Most current heavy metals/metalloids in aquatic systems are derived from natural (geogenic) and anthropogenic (non-point) sources. Heavy metals/metalloids in the environment have the potential to be toxic to organisms from anthropogenic sources.

Although it is well known that heavy metals/metalloids are also derived by biological activity in aquatic environments, and are re-mobilized in the food chain, resulting in much higher concentrations in larger predators than in primary producers and/or smaller herbivores, the role of the metal/metalloids in the food chain is not well understood. The role of the metal/metalloids in the food chain is not well understood. The role of the metal/metalloids in the food chain is not well understood.

The primary exposure route of heavy metals/metalloids to humans, and assessing the importance of secondary increasing heavy metals/metalloids in food.

Heavy metals/metalloids and carcinogenicity is well documented – with the most marked impact being on the drinking water. Heavy metals/metalloids enter systems through both the physical environment and the food chain, making important



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

[illegible]

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

Linking Water by ICP-MS

Introduction

Isotopes play a role in a number of different fields, from forensic science to the chemistry of adaptation to climate change (the paleoclimatology of isotopes).

For the determination of isotope species in water, the first practical approach is by mass spectrometry. The mass spectrometer is a device that separates ions according to their mass-to-charge ratio (m/z). Ions are created by ionizing the sample and are separated by a magnetic and/or electric field. Ion detectors are used to detect the ions.

The formation of polyatomic ions in the negative ion mode can cause interference with the detection of isotope species. One of the most common interferences is the formation of the H_2O^- ion, which has the same mass-to-charge ratio as the O^{16}H^- ion.

One way to reduce this interference is to use a technique called "ion optics" or "ion filtering". This involves using a series of electric and magnetic fields to filter out the H_2O^- ion and allow the O^{16}H^- ion to pass through. This technique is used in many mass spectrometers for the detection of isotope species in water.

Another way to reduce this interference is to use a technique called "ion counting". This involves using a series of detectors to count the ions. The detectors are arranged in a way that they can detect the ions at different mass-to-charge ratios. This technique is used in many mass spectrometers for the detection of isotope species in water.

In this study, the authors present a technique for detecting water composition in water by using a simple isotopic analysis method based on the detection of the H_2O^- ion.



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

[illegible]

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

