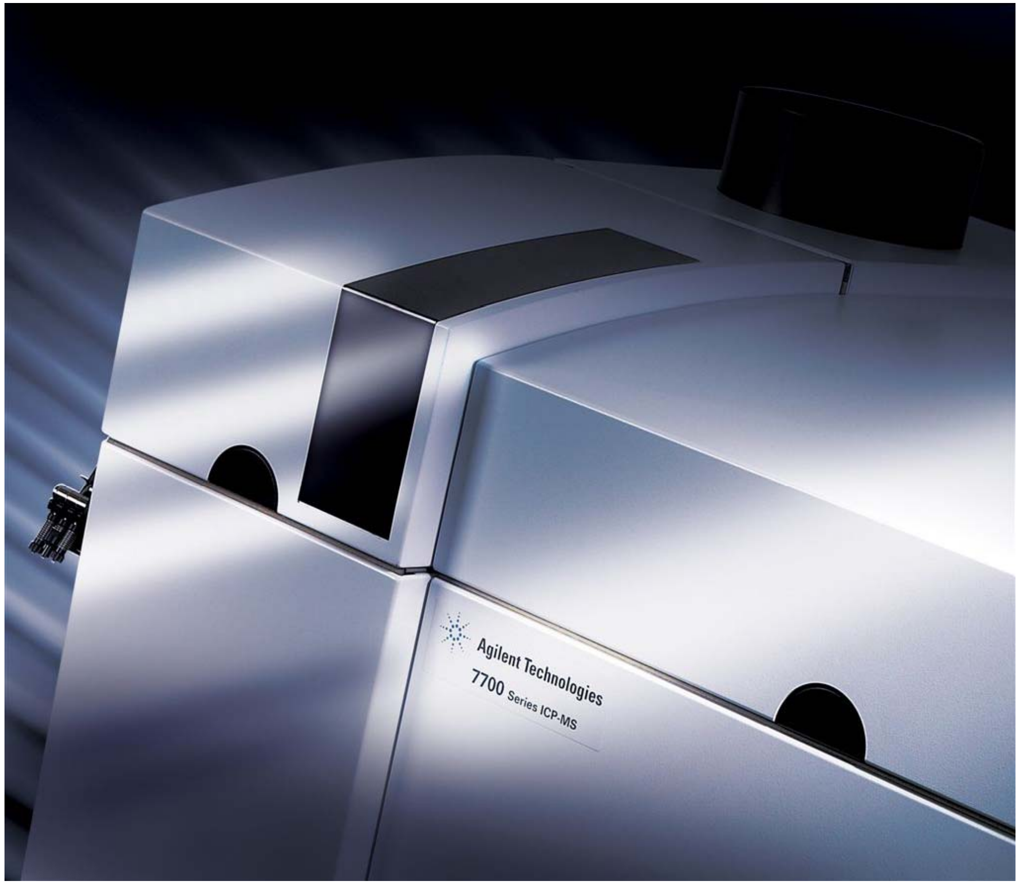


Hyphenated LC-ICP-MS Arsenic speciation in water

Agilent Technologies

Introduction – Speciation Why and How?

Why Speciation Rather than Total Concentration of Metals?

Almost all elements form species, with the different forms having different bioavailability, toxicity and mobility

- Cr^{III} vs Cr^{VI}; Inorganic As vs Organic As; Humic complexes …

Some species are specifically used for their toxic (or chemical) properties

- Organotin (OT) Species
  - marine anti-fouling paint; polymer stabilisers; fungicides …
- Brominated flame retardants (BFR's)
  - Eg PBDE's – poly-brominated diphenyl ethers (chemically similar to PCB's)
- Organo-phosphorus (OP) Species
  - pesticides; nerve agents

Whether natural or man-made, these species can find their way into the environment, food chain etc. If the compounds contain an inorganic component, it can be used to provide specific detection of the molecule

ICP-MS provides a simple, selective, rapid, sensitive and accurate detector for many of these species, via measurement of the inorganic element

Some Speciation Landmarks

- ”Fish arsenic” (Chapman 1926)
  ”…a different (and non-toxic) compound than As_2O_3”
- Minamata, Japan (1950s)
  $MeHg^+$ emissions from industry → fish → man
- Arcachon Bay, France (1980s)
  TBT from antifouling agents → oysters

- EU Thematic network ”speciation 21” (1997-2000)
  Participants from research, industry and authorities
- EVISA (European Virtual Institute for Speciation Analysis)(2003)
  Information and technology transfer in the framework of speciation

See more info at the EVISA homepage: www.speciation.net

Courtesy of Jens Sloth, DTU

ICP-MS Interfacing Options

LC-ICP-MS

GC-ICP-MS

CE-ICP-MS

Optional Conventional (Organic compound) Detector(s)

Key Analytical Requirements

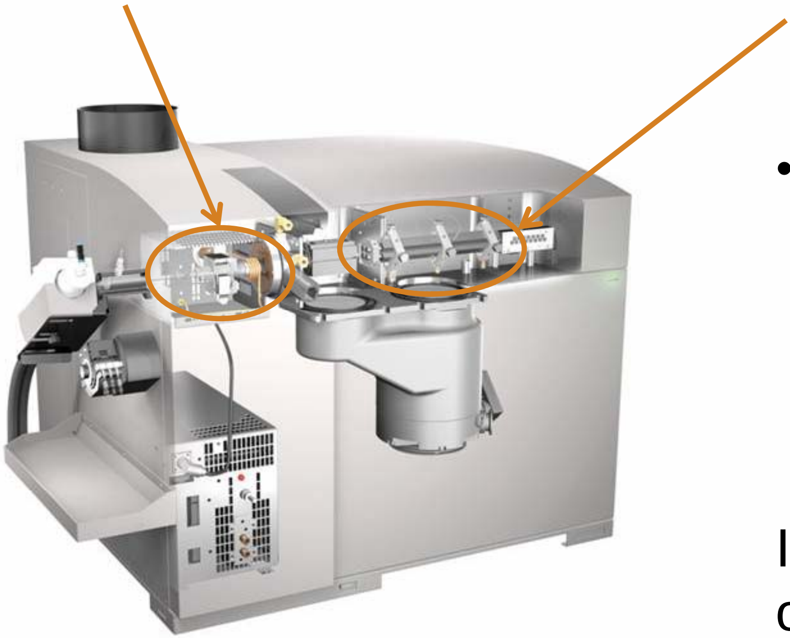
- Accuracy – toxic compounds in critical environmental, food pharma and clinical samples
  - Some analytes may suffer from spectral interferences – removed using collision/reaction cell technology
- Sensitivity – toxic effects may occur at low concentrations
- Stability – for long-term unattended sequencing
- Reproducibility
  - Elemental response is independent of compound – means unknown compounds can be quantified by reference to known compound
  - Consistent elemental response for different sample matrices
- Specificity – reliable quantification regardless of matrix
- Selectivity – certainty of MS on-mass detection
- Wide elemental coverage and dynamic range

Firstly, What is ICP-MS?

An inorganic (elemental) analysis technique

ICP - Inductively Coupled Plasma

- high temperature ion source
- decomposes, atomizes and ionizes the sample



MS - Mass Spectrometer

- Uses quadrupole mass analyzer
- Mass range from 7 to 250 amu (Li to U...)
  - separates all elements in rapid sequential scan
  - isotopic information available
- Measures ions, using dual mode detector
  - ppt to ppm levels

ICP-MS combines the detection limits of Graphite Furnace AA and the sample throughput of ICP-OES

Agilent's History of Innovation in ICP-MS – 1987 to 2009

1987 – PMS 100 introduced – First computer-controlled ICP-MS

1988 – PMS 200 introduced – Second generation ICP-MS with off-axis Qpole lens

1989 – 1st ETV accessory for semicon analysis by ICP-MS

1990 – PMS 2000 introduced – Omega off-axis lens. Lowest random background ICP-MS

1992 – ShieldTorch interface developed - Ar interferences virtually eliminated in cool plasma, enabling ppt analysis of K, Ca, Fe by ICP-MS

1994 – 4500 Series introduced - World's first benchtop system. Hyperbolic profile quad, motorized torch XYZ, cool plasma

1998 – First real time ICP-MS chromatographic software – PlasmaChrom. T-mode reaction interface introduced

1999 – 4500 Series 100, 200 & 300 introduced: 1st applications-specific ICP-MS.

2000 – Agilent 7500 Series introduced - 7500a, 7500i and 7500s - the next generation in ICP-MS instrumentation. 9 orders detector range

2001 – Agilent 7500c launched – 1st generation ORS for high matrix samples.

2002 – New digital generators and LAN control introduced. First commercial GC-ICP-MS interface.

2003 – Agilent 7500cs launched – 2nd generation ORS for high purity semicon samples.

2004 – Agilent 7500ce launched – 2nd generation ORS for high matrix samples.

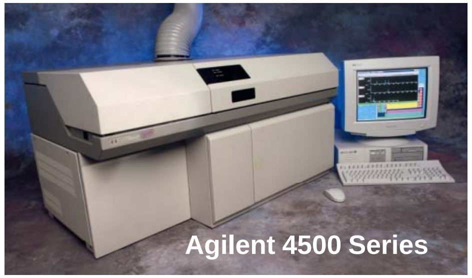
2005 – Low flow cell gas MFC's for Xe NH_3, O_2, etc added to 7500ce/cs.

2006 – Agilent acquires 100% of Agilent/Yokogawa joint venture

2007 – Agilent 7500cx introduced: He only mode ICP-MS

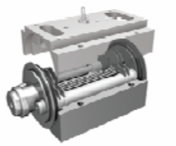
2008 – High Matrix Interface developed – enables 2% TDS samples to be run by ICP-MS

2009 – Agilent 7700 Series introduced – replaces 7500 Series. MassHunter Software introduced - common platform with other Agilent MS. ISIS-DS Discrete sampling system, for ultra high throughput analysis



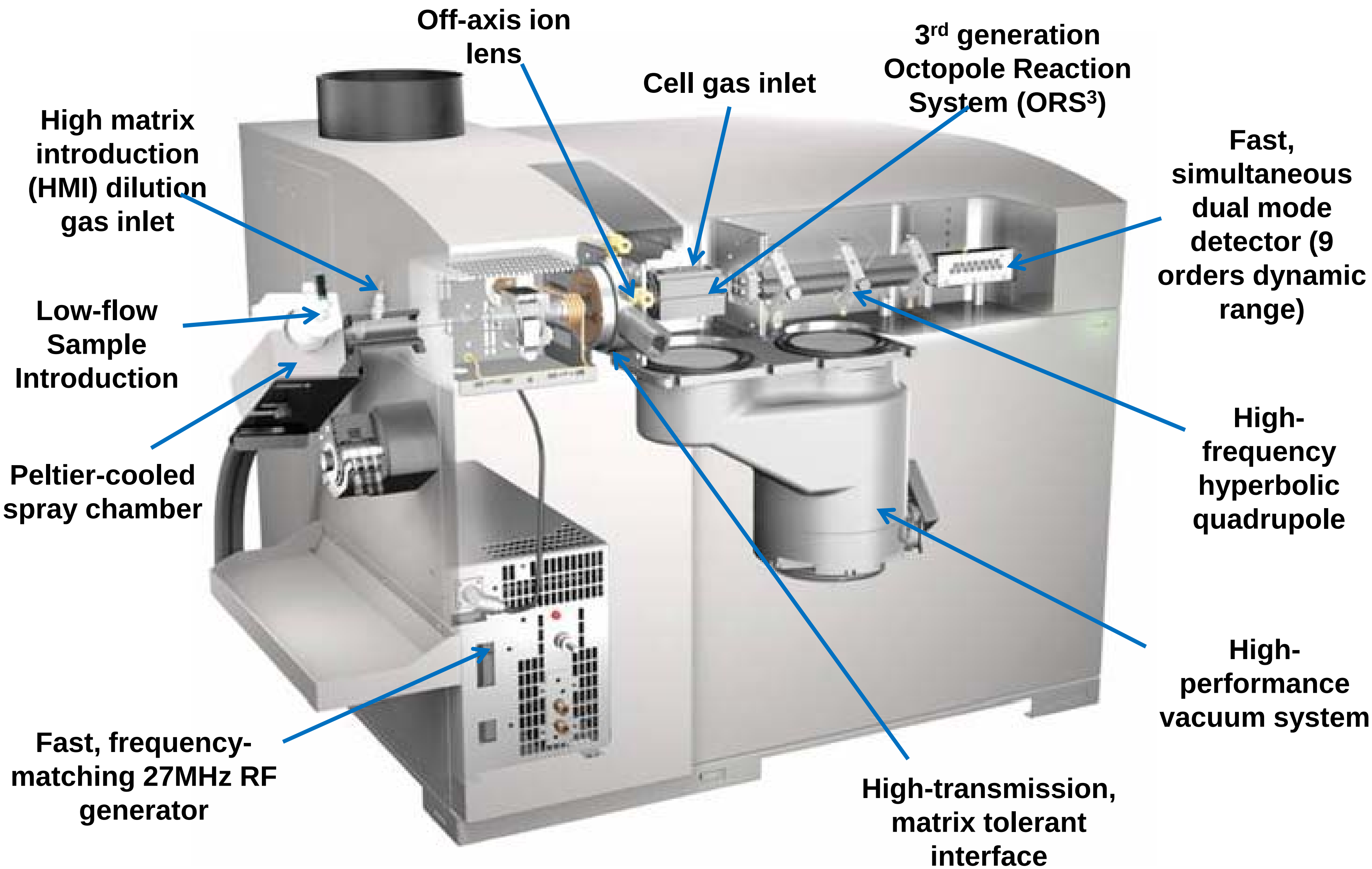
ShieldTorch System

Octopole Reaction System



Agilent 7700 Series

Agilent 7700x ICP-MS System



LC-ICP-MS Examples

- Arsenic speciation
- Organo-tin speciation
- Other, more unusual LC-ICP-MS applications

LC-ICP-MS Examples

- Arsenic speciation
- Organo-tin speciation
- Other, more unusual LC-ICP-MS applications

Effect of Chronic Exposure to As

Long-term exposure to As through drinking water causes:

- Changes in skin pigmentation and thickening (Arsenical keratosis; latency 10 Y)
- Cancer of the skin, lungs, urinary tract and kidney (cancer is a late phenomenon and usually takes 20 years to develop)
- Severe disease of blood vessels leading to gangrene (e.g. Black Foot Disease in Taiwan)
- Finally may lead to death from internal cancers and multi-organ failure

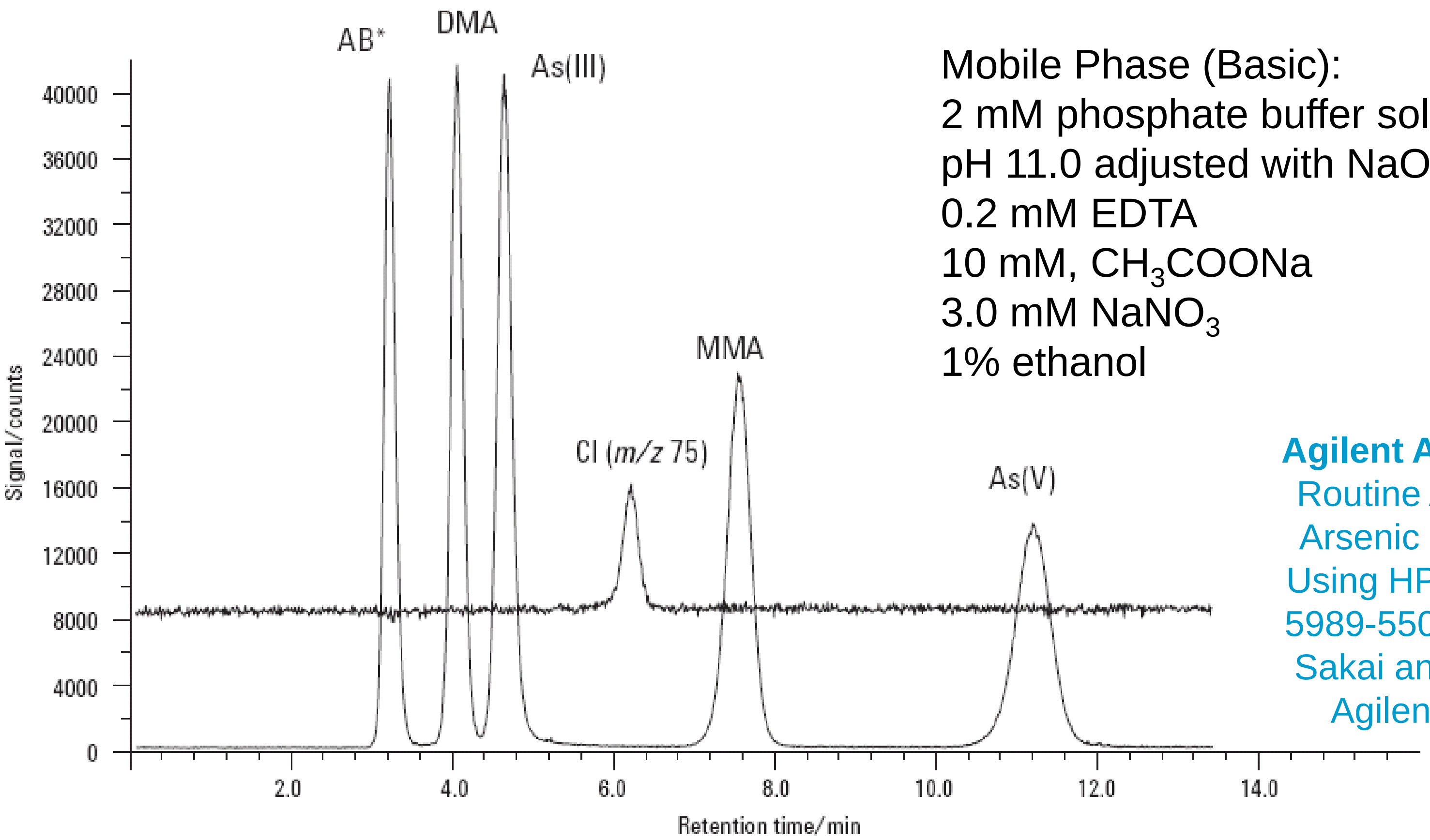
Arsenic Speciation with LC-ICP-MS

Complete isocratic separation of AsB & Cl from inorganic As, using a new column

***Surface Aminated Polymer Substrate**

Column G3288-80000 (4.6 x 250 mm)
Guard Column G3154-65002

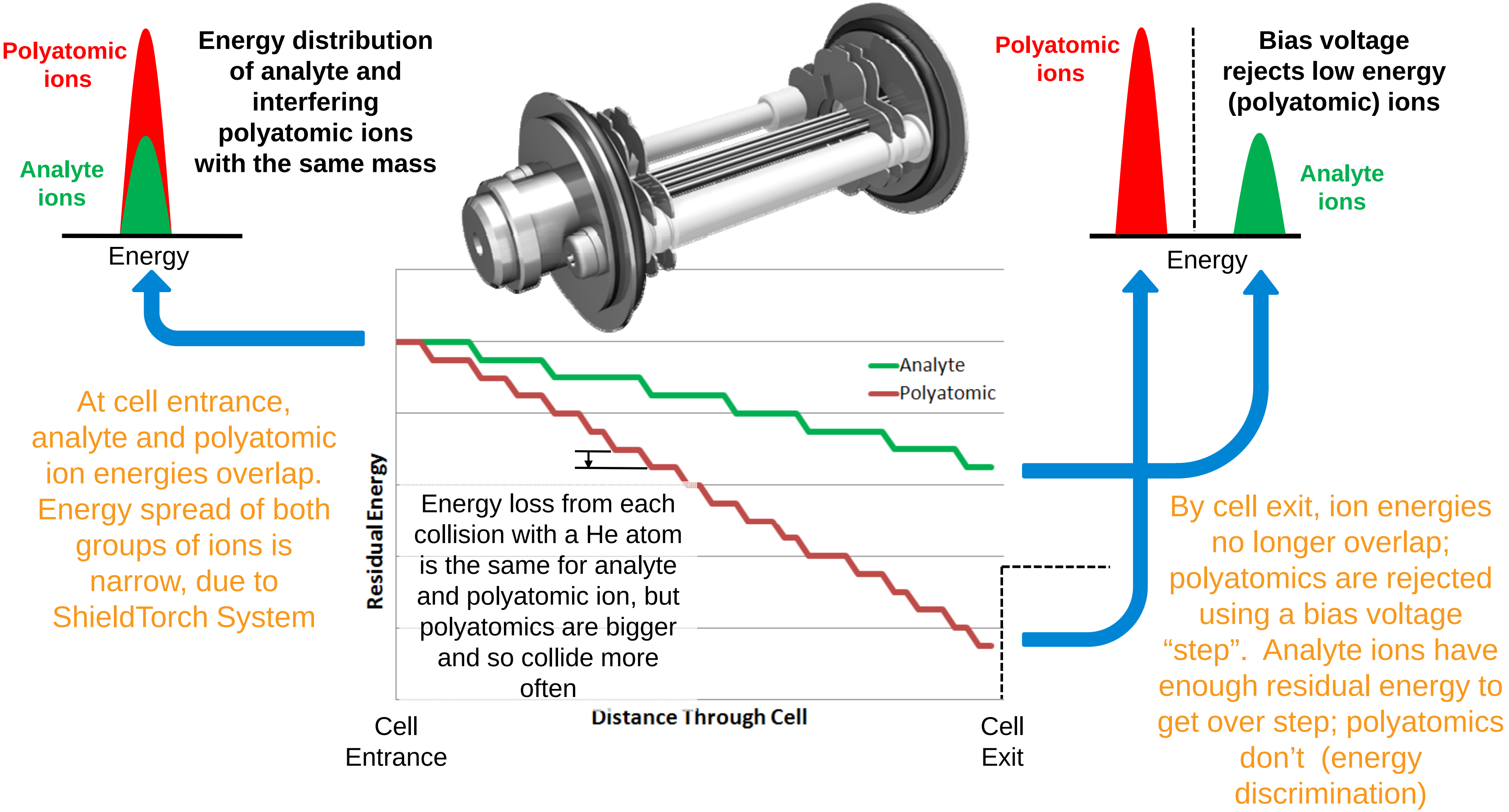
Mobile Phase (Basic):
2 mM phosphate buffer solution (PBS)
pH 11.0 adjusted with NaOH
0.2 mM EDTA
10 mM, CH_3COONa
3.0 mM $NaNO_3$
1% ethanol



Agilent Application Note:
Routine Analysis of Toxic Arsenic Species in Urine Using HPLC with ICP-MS, 5989-5505EN, by Tetsushi Sakai and Steven Wilbur, Agilent Technologies

*Adjusted to pH 11 to separate AsB. Ionic strength of mobile phase shortens elution of As V
10ug/L each As species

Principle of He Mode and KED*



*KED = Kinetic Energy Discrimination

Polyatomic Interferences in Complex Matrices

Some manufacturers claim that ICP-MS has only a few interferences, and that reaction gases can be used to remove them.

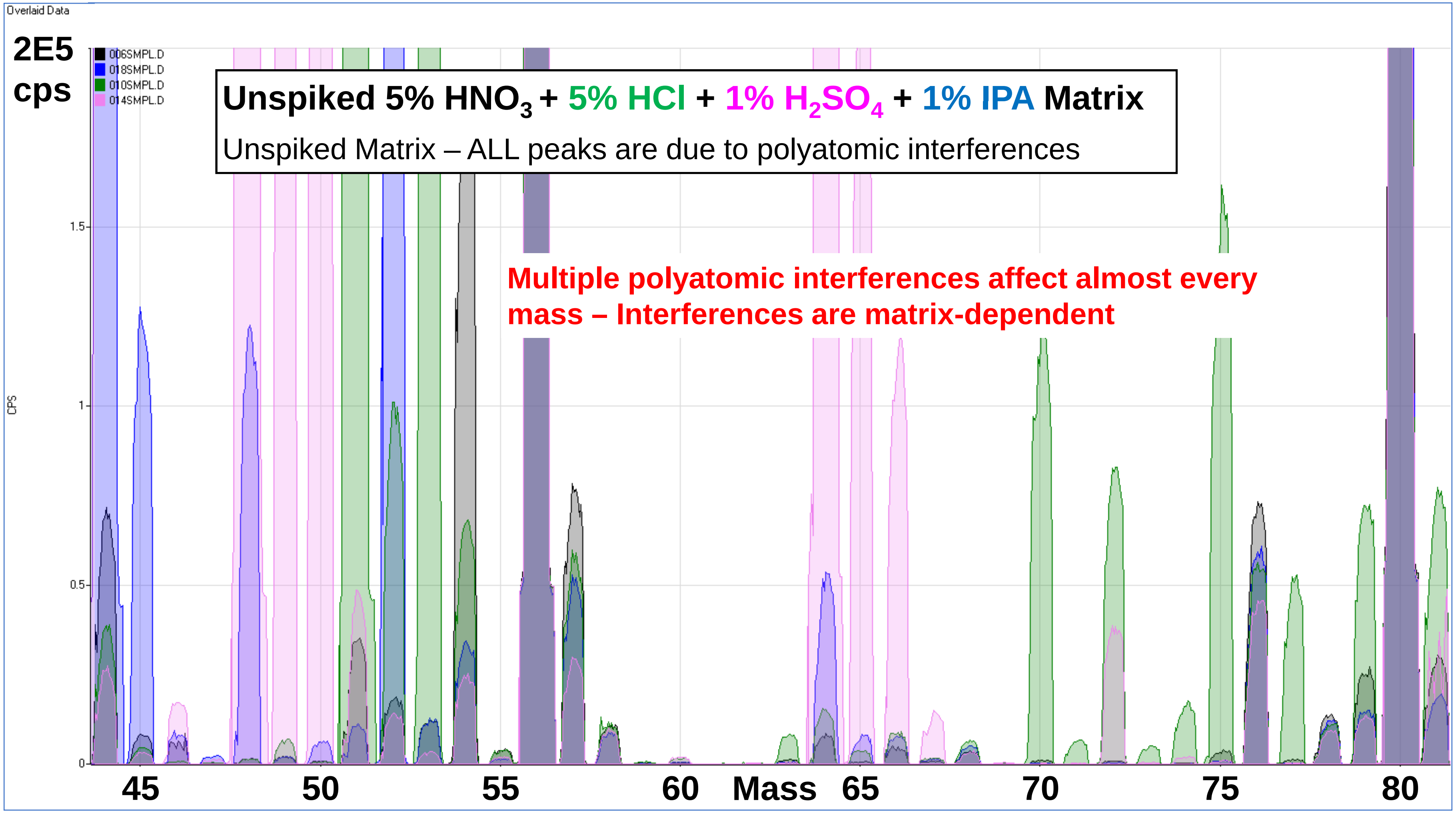
That's true in a simple sample, but in a complex sample there are many interferences, and no reaction gas can remove all of them

But He mode on the 7700 can!!

Isotope	Principal Interfering Species (mixed matrix)
^{45}Sc	$^{13}C^{16}O_2$, $^{12}C^{16}O_2H$, ^{44}CaH, $^{32}S^{12}CH$, $^{32}S^{13}C$, $^{33}S^{12}C$
^{47}Ti	$^{31}P^{16}O$, ^{46}CaH, $^{35}Cl^{12}C$, $^{32}S^{14}NH$, $^{33}S^{14}N$
^{49}Ti	$^{31}P^{18}O$, ^{48}CaH, $^{35}Cl^{14}N$, $^{37}Cl^{12}C$, $^{32}S^{16}OH$, $^{33}S^{16}O$
^{50}Ti	$^{34}S^{16}O$, $^{32}S^{18}O$, $^{35}Cl^{14}NH$, $^{37}Cl^{12}CH$
^{51}V	$^{35}Cl^{16}O$, $^{37}Cl^{14}N$, $^{34}S^{16}OH$
^{52}Cr	$^{36}Ar^{16}O$, $^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{37}Cl^{14}NH$, $^{34}S^{18}O$
^{53}Cr	$^{36}Ar^{16}OH$, $^{40}Ar^{13}C$, $^{37}Cl^{16}O$, $^{35}Cl^{18}O$, $^{40}Ar^{12}CH$
^{54}Fe	$^{40}Ar^{14}N$, $^{40}Ca^{14}N$, $^{23}Na^{31}P$
^{55}Mn	$^{37}Cl^{18}O$, $^{23}Na^{32}S$, $^{23}Na^{31}PH$
^{56}Fe	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$
^{57}Fe	$^{40}Ar^{16}OH$, $^{40}Ca^{16}OH$
^{58}Ni	$^{40}Ar^{18}O$, $^{40}Ca^{18}O$, $^{23}Na^{35}Cl$
^{59}Co	$^{40}Ar^{18}OH$, $^{43}Ca^{16}O$, $^{23}Na^{35}ClH$
^{60}Ni	$^{44}Ca^{16}O$, $^{23}Na^{37}Cl$
^{61}Ni	$^{44}Ca^{16}OH$, $^{38}Ar^{23}Na$, $^{23}Na^{37}ClH$
^{63}Cu	$^{40}Ar^{23}Na$, $^{12}C^{16}O^{35}Cl$, $^{12}C^{14}N^{37}Cl$, $^{31}P^{32}S$, $^{31}P^{16}O_2$
^{64}Zn	$^{32}S^{16}O_2$, $^{32}S_2$, $^{36}Ar^{12}C^{16}O$, $^{38}Ar^{12}C^{14}N$, $^{48}Ca^{16}O$
^{65}Cu	$^{32}S^{16}O_2H$, $^{32}S_2H$, $^{14}N^{16}O^{35}Cl$, $^{48}Ca^{16}OH$
^{66}Zn	$^{34}S^{16}O_2$, $^{32}S^{34}S$, $^{33}S_2$, $^{48}Ca^{18}O$
^{67}Zn	$^{32}S^{34}SH$, $^{33}S_2H$, $^{48}Ca^{18}OH$, $^{14}N^{16}O^{37}Cl$, $^{16}O_2{}^{35}Cl$
^{68}Zn	$^{32}S^{18}O_2$, $^{34}S_2$
^{69}Ga	$^{32}S^{18}O_2H$, $^{34}S_2H$, $^{16}O_2{}^{37}Cl$
^{70}Zn	$^{34}S^{18}O_2$, $^{35}Cl_2$
^{71}Ga	$^{34}S^{18}O_2H$, $^{35}Cl_2H$, $^{40}Ar^{31}P$
^{72}Ge	$^{40}Ar^{32}S$, $^{35}Cl^{37}Cl$, $^{40}Ar^{16}O_2$
^{73}Ge	$^{40}Ar^{32}SH$, $^{40}Ar^{33}S$, $^{35}Cl^{37}ClH$, $^{40}Ar^{16}O_2H$
^{74}Ge	$^{40}Ar^{34}S$, $^{37}Cl_2$
^{75}As	$^{40}Ar^{34}SH$, $^{40}Ar\,^{35}Cl$, $^{40}Ca\,^{35}Cl$, $^{37}Cl_2H$
^{77}Se	$^{40}Ar\,^{37}Cl$, $^{40}Ca\,^{37}Cl$
^{78}Se	$^{40}Ar\,^{38}Ar$
^{80}Se	$^{40}Ar_2$, $^{40}Ca_2$, $^{40}Ar^{40}Ca$, $^{32}S_2\,^{16}O$, $^{32}S^{16}O_3$

Blank Acid Matrices and IPA in No Gas Mode

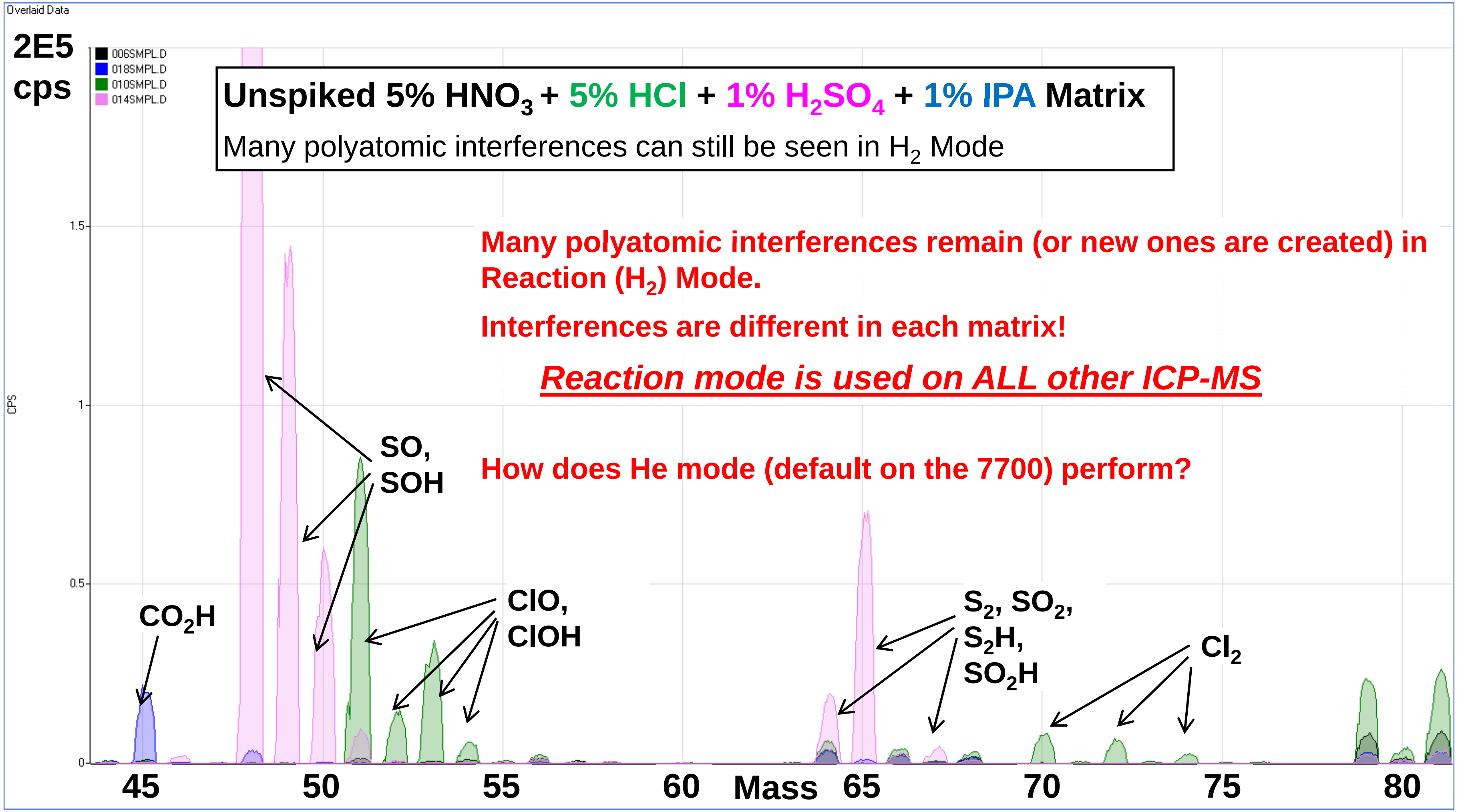
Color of spectrum indicates which matrix gave each interfering peak



No Gas Mode

Blank Acid Matrices and IPA in H_2 (Reaction) Mode

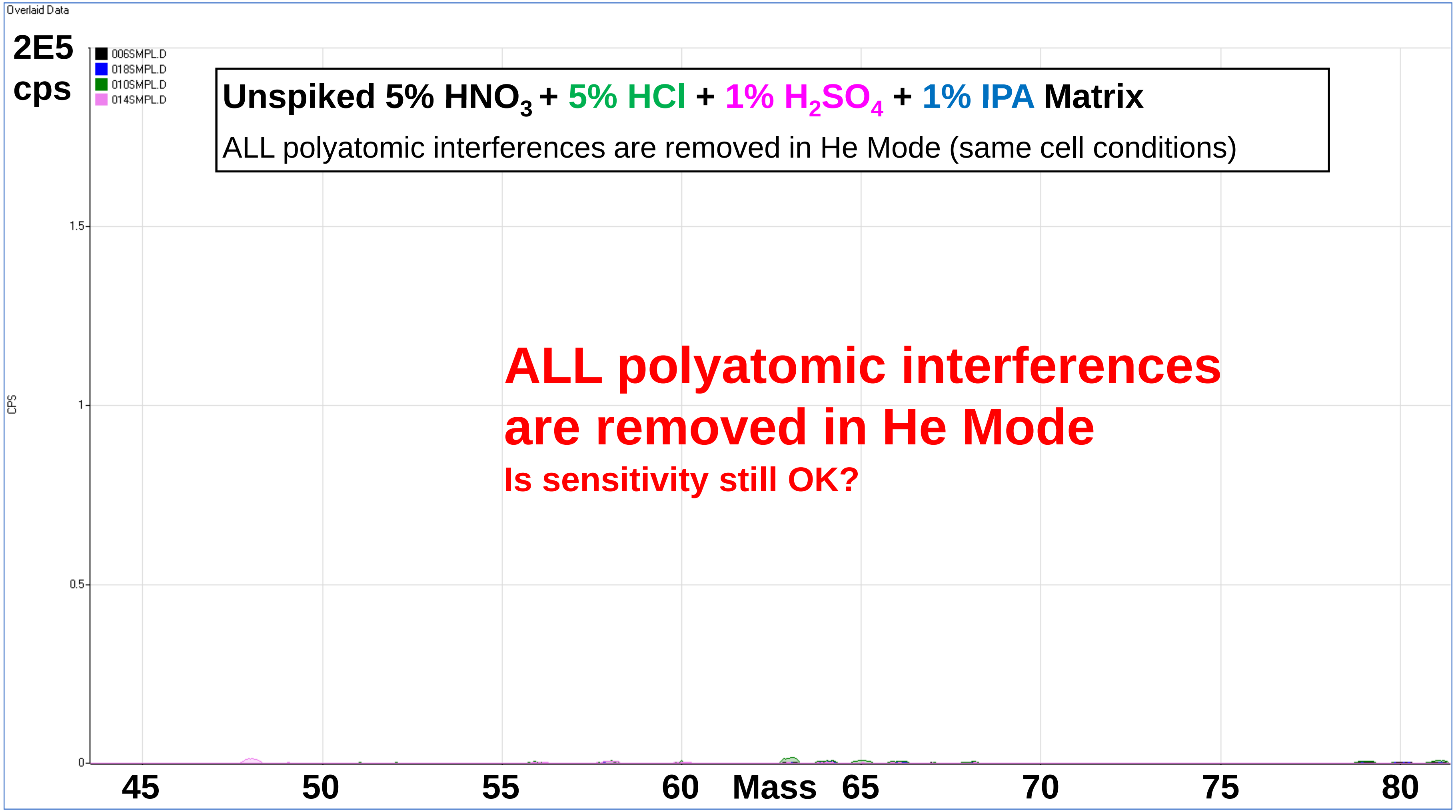
Color of spectrum indicates which matrix gave each interfering peak



H_2 Mode

Blank Acid Matrices and IPA in He Mode

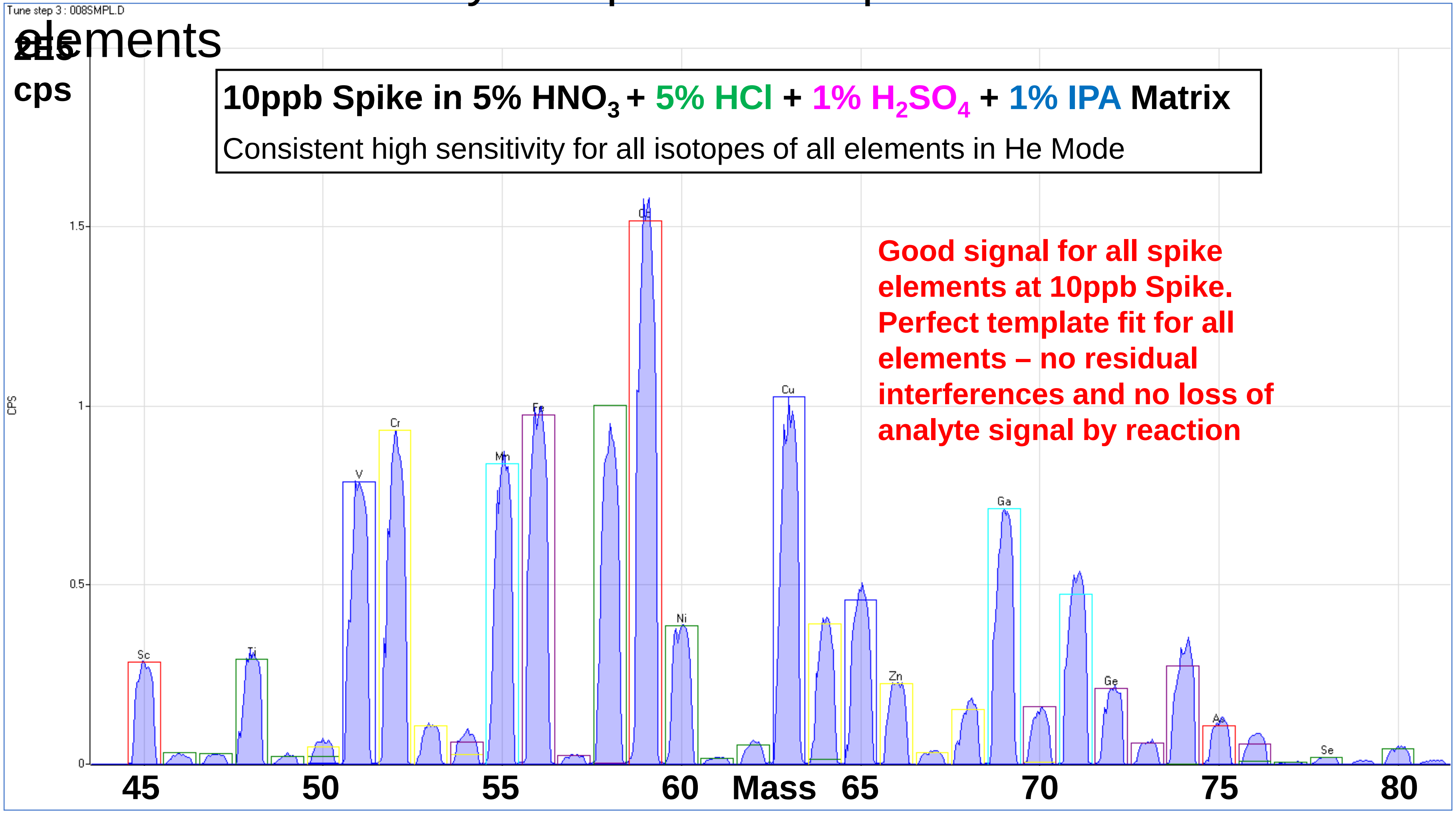
Color of spectrum indicates which matrix gave each interfering peak



He Mode

Matrix Mix with Spike (10ppb) in He Mode

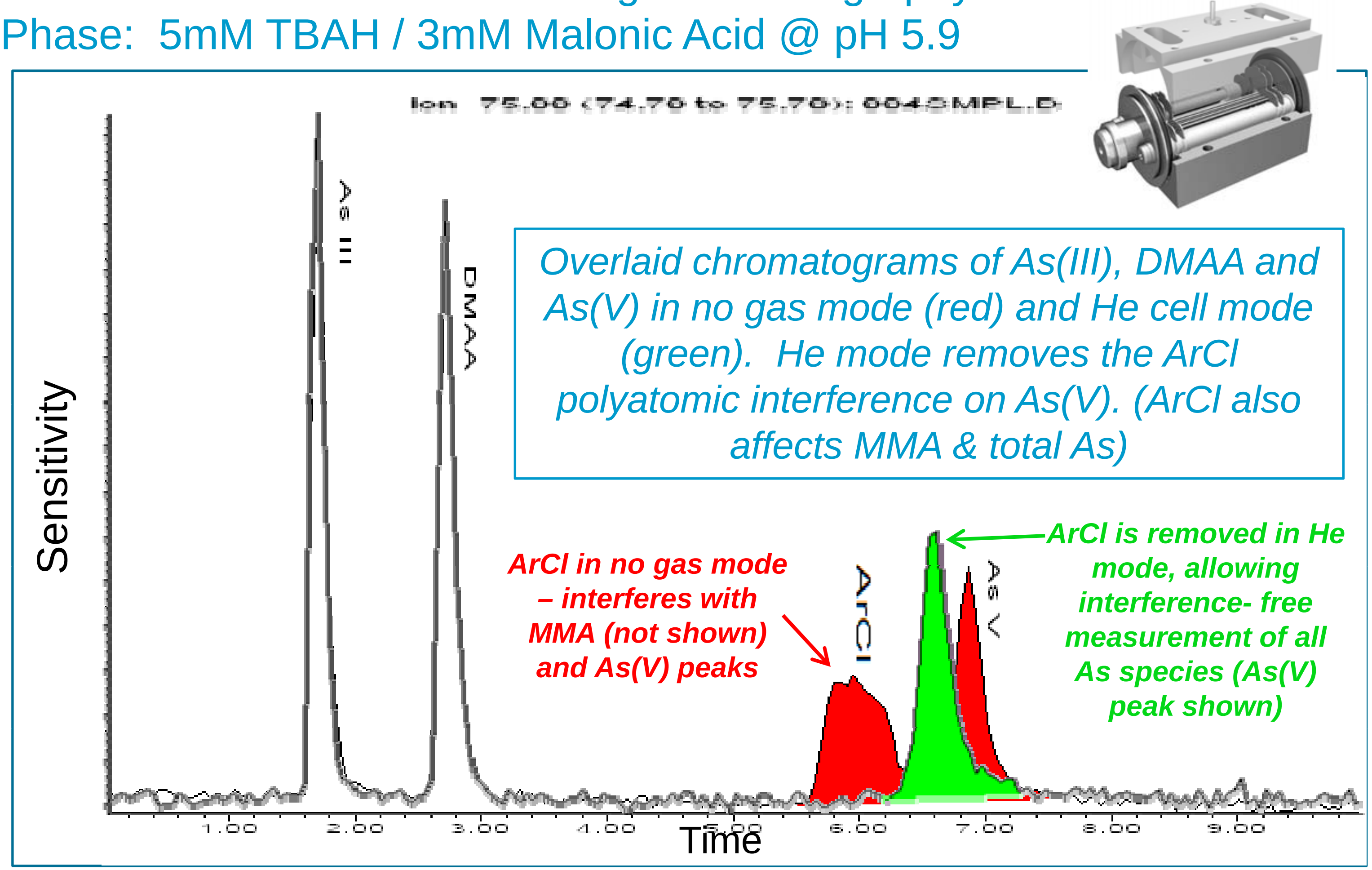
Consistent sensitivity and perfect template match for all elements



He Mode

Collision/Reaction Cell in He Mode –
Effective removal of ArCl for fast LC separation

1.0ppb As Mix in 0.3% NaCl. Ion-Pairing Chromatography with C18 column.
Mobile Phase: 5mM TBAH / 3mM Malonic Acid @ pH 5.9



*Chloride anion interferes with MMA, As(V) and total As measurement

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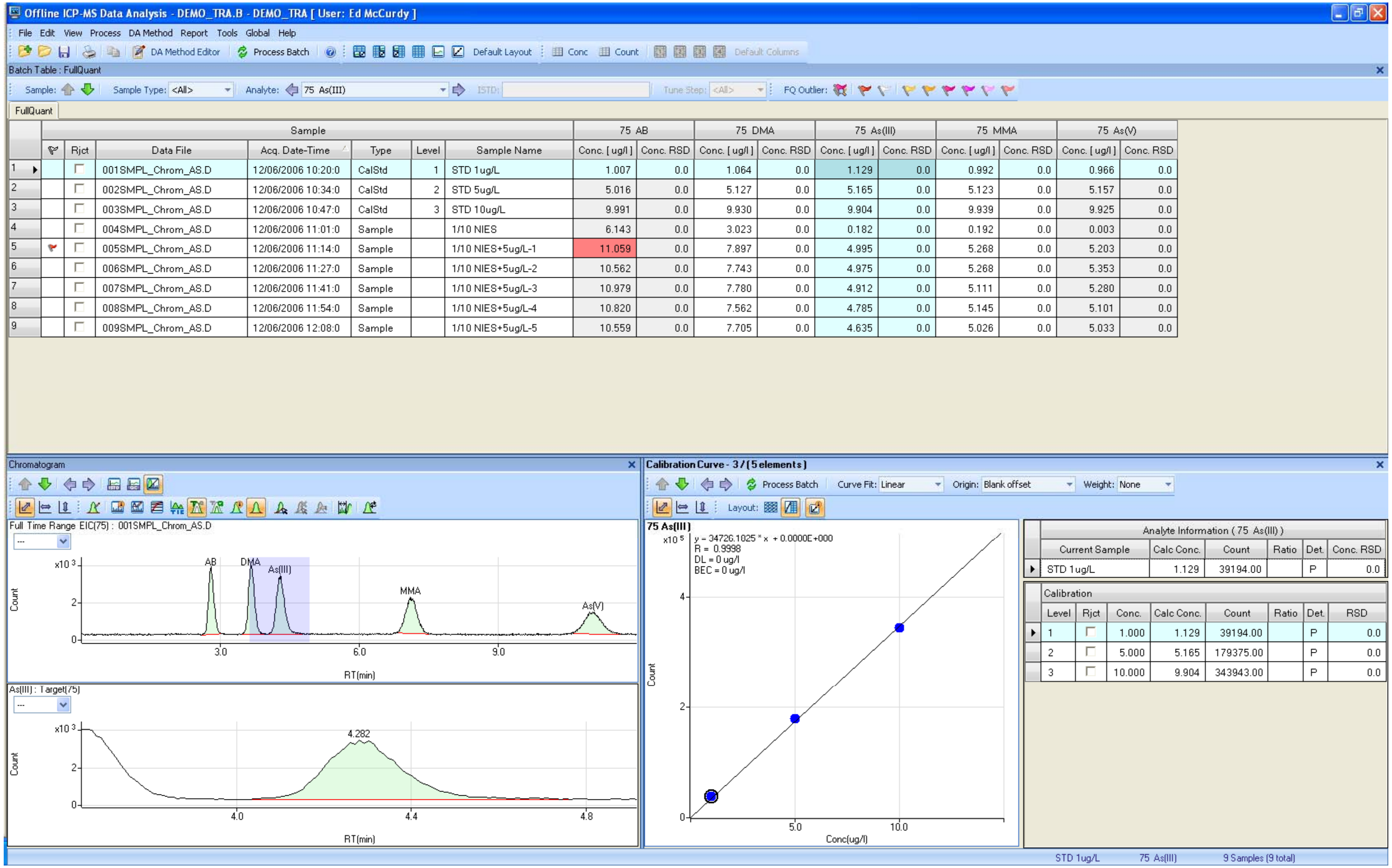
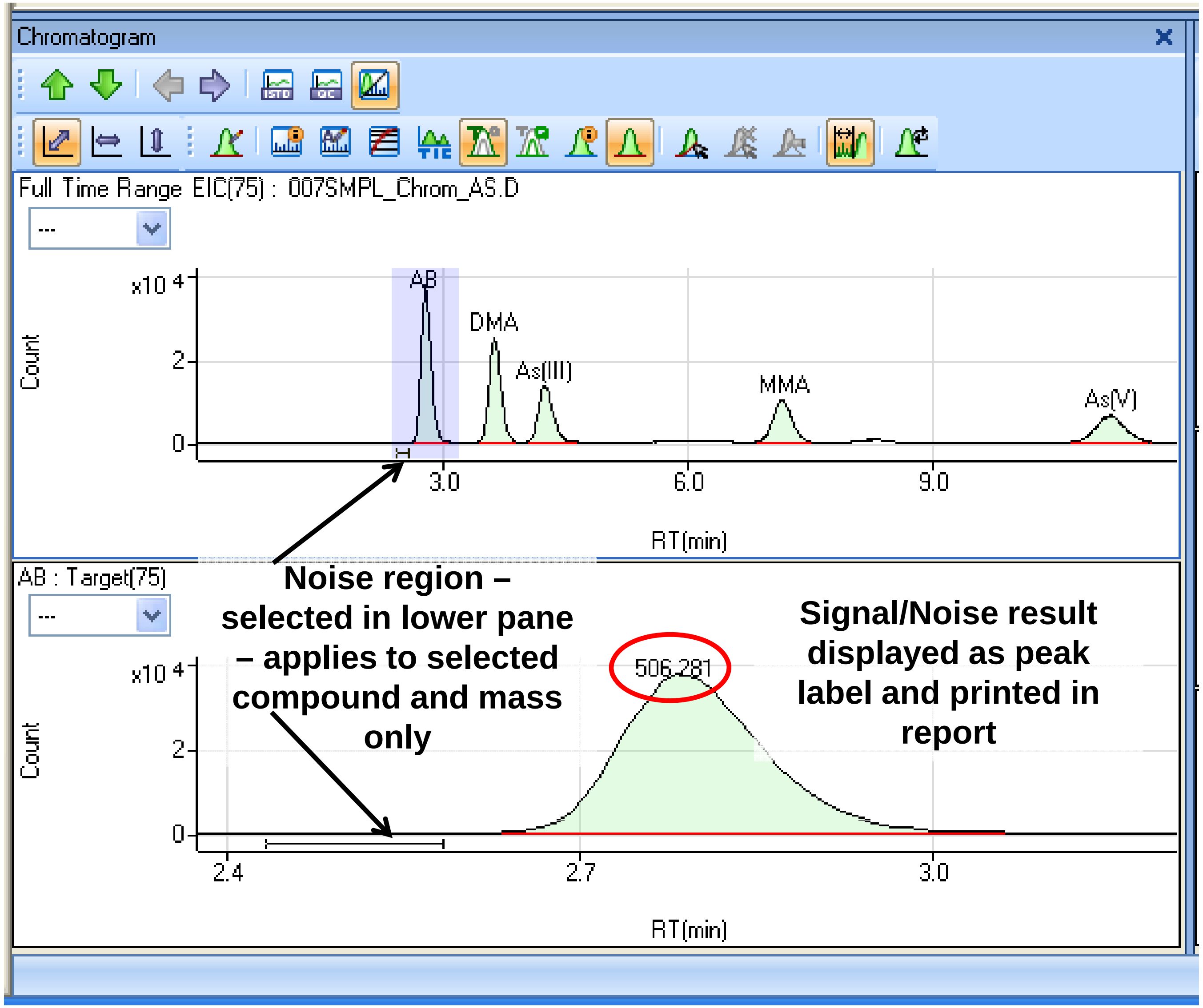
Chromatographic Data Analysis - Sequence

– Chromatographic Data Analysis in Batch table view, including real-time update, calib display and outlier flags.



	Sample						75 AB		75 DMA		75 As(III)		75 MMA		75 As(V)	
	Rjct	Data File	Acq. Date-Time	Type	Level	Sample Name	Conc. [ug/l]	Conc. RSD	Conc. [ug/l]	Conc. RSD	Conc. [ug/l]	Conc. RSD	Conc. [ug/l]	Conc. RSD	Conc. [ug/l]	Conc. RSD
1		001SMPL_Chrom_AS.D	12/06/2006 10:20:0	CalStd	1	STD 1ug/L	1.007	0.0	1.064	0.0	1.129	0.0	0.992	0.0	0.966	0.0
2		002SMPL_Chrom_AS.D	12/06/2006 10:34:0	CalStd	2	STD 5ug/L	5.016	0.0	5.127	0.0	5.165	0.0	5.123	0.0	5.157	0.0
3		003SMPL_Chrom_AS.D	12/06/2006 10:47:0	CalStd	3	STD 10ug/L	9.991	0.0	9.930	0.0	9.904	0.0	9.939	0.0	9.925	0.0
4		004SMPL_Chrom_AS.D	12/06/2006 11:01:0	Sample		1/10 NIES	6.143	0.0	3.023	0.0	0.182	0.0	0.192	0.0	0.003	0.0
5		005SMPL_Chrom_AS.D	12/06/2006 11:14:0	Sample		1/10 NIES+5ug/L-1	11.059	0.0	7.897	0.0	4.995	0.0	5.268	0.0	5.203	0.0
6		006SMPL_Chrom_AS.D	12/06/2006 11:27:0	Sample		1/10 NIES+5ug/L-2	10.562	0.0	7.743	0.0	4.975	0.0	5.268	0.0	5.353	0.0
7		007SMPL_Chrom_AS.D	12/06/2006 11:41:0	Sample		1/10 NIES+5ug/L-3	10.979	0.0	7.780	0.0	4.912	0.0	5.111	0.0	5.280	0.0
8		008SMPL_Chrom_AS.D	12/06/2006 11:54:0	Sample		1/10 NIES+5ug/L-4	10.820	0.0	7.562	0.0	4.785	0.0	5.145	0.0	5.101	0.0
9		009SMPL_Chrom_AS.D	12/06/2006 12:08:0	Sample		1/10 NIES+5ug/L-5	10.559	0.0	7.705	0.0	4.635	0.0	5.026	0.0	5.033	0.0

Chromatographic Data Analysis – Signal/Noise



Signal to Noise Calculation (per compound, per analyte or per sample)

Screen shows S/N for compound AB = S/N is shown above the selected peak.

Noise region can be selected for all compounds and all analyte masses, for all compounds of the selected mass, or just for the selected compound

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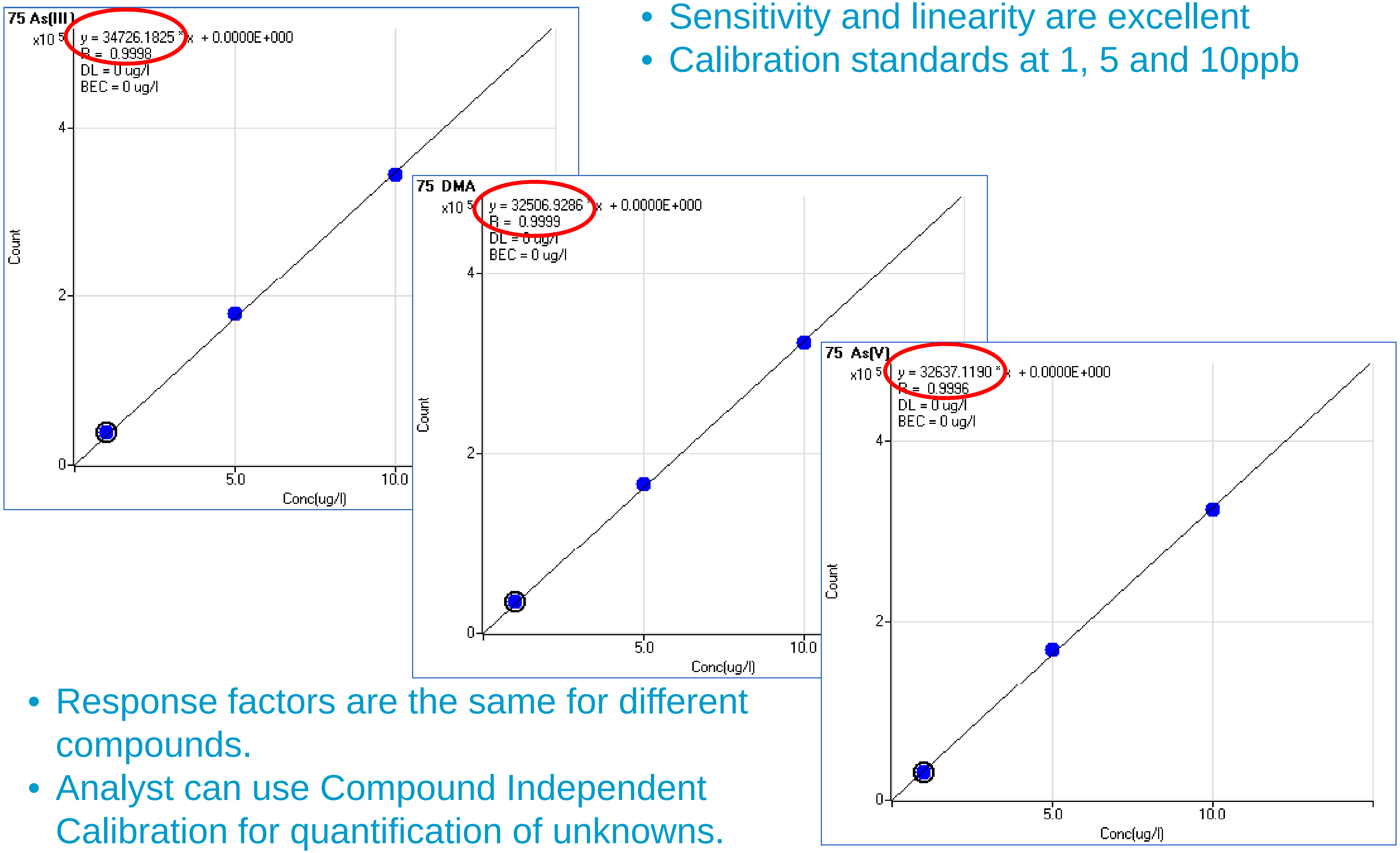
Calibrations for Different As Species

- Sensitivity and linearity are excellent
- Calibration standards at 1, 5 and 10ppb



- Response factors are the same for different compounds.
- Analyst can use Compound Independent Calibration for quantification of unknowns.

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Arsenic Occurence

High concentrations of arsenic has been found in samples from the marine environment.

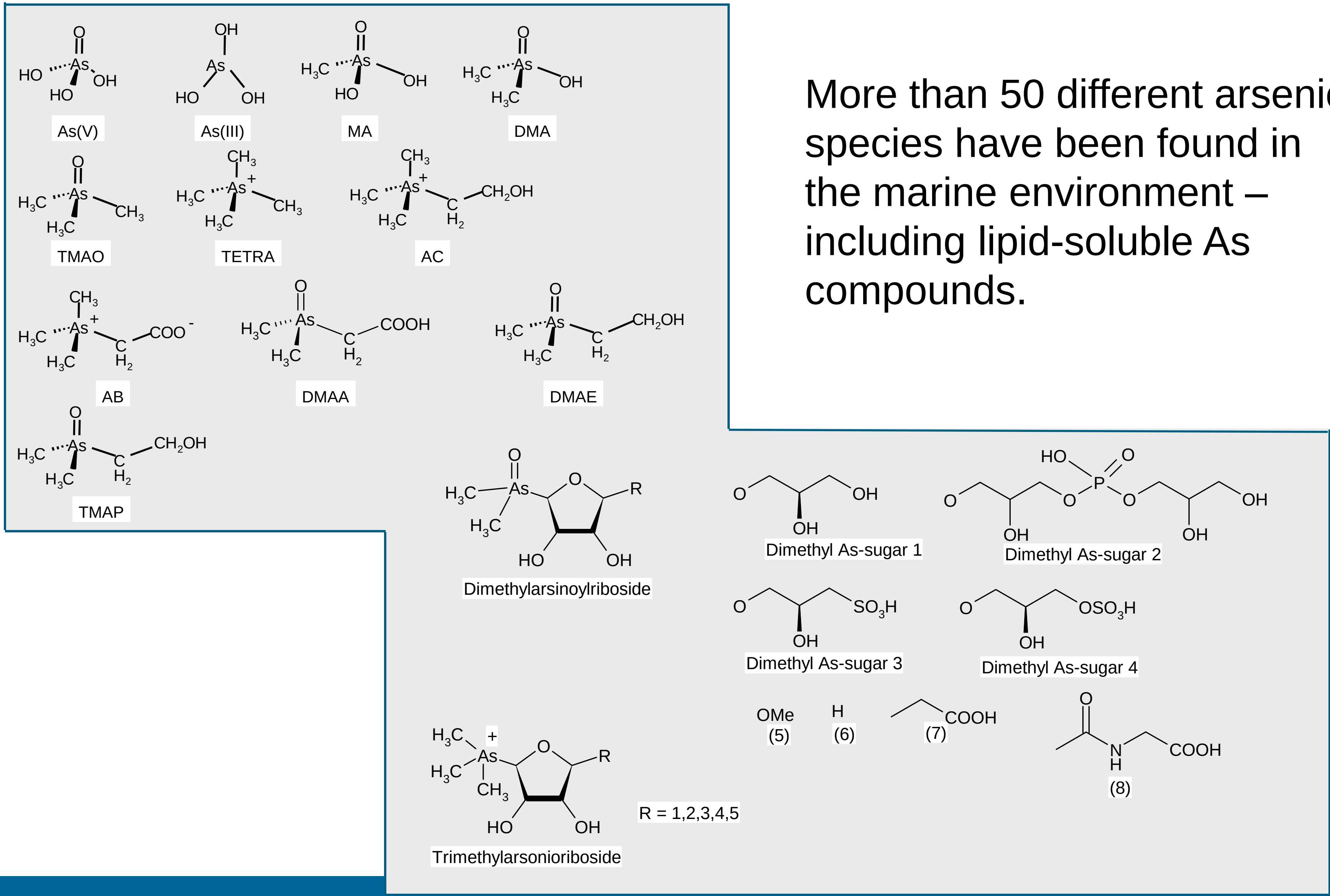
Seawater	1	-	2	µg/L
Marine fish	0.2	-	>100	mg/kg
Marine invertebrates	0.2	-	>100	mg/kg
Marine algae	0.02	-	40	mg/kg
Freshwater fish	<0.01	-	2	mg/kg
Terrestrial biota	<0.2			mg/kg

All results on wet weight basis

Marine organisms can bioaccumulate arsenic by a factor of up to 100,000 compared with seawater!!!

Courtesy of Jens Sloth, DTU

Arsenic compounds in the Marine Environment



More than 50 different arsenic species have been found in the marine environment – including lipid-soluble As compounds.

Courtesy of Jens Sloth, DTU

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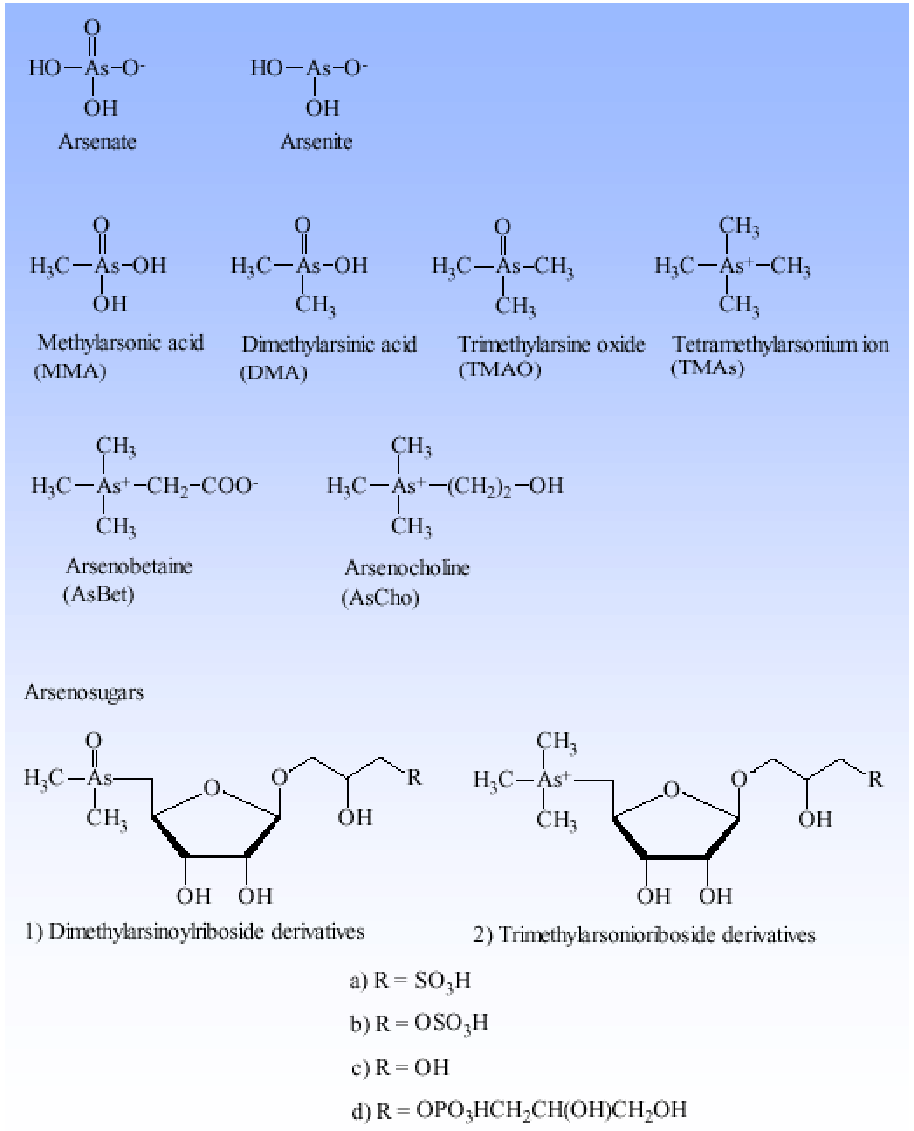
As Speciation - Toxicity



Toxic!

Less-Toxic

Non-Toxic

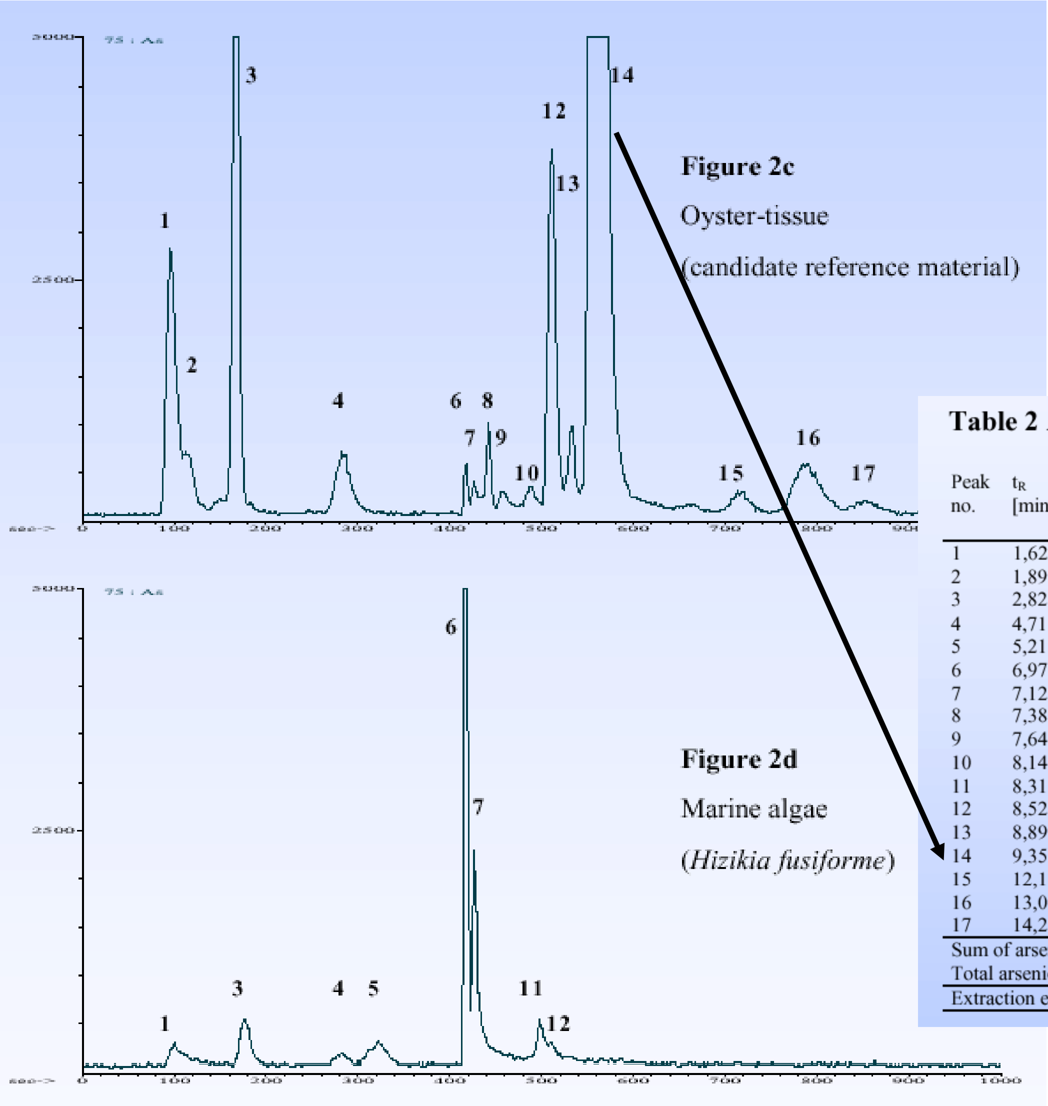
?

Many As species exist – the inorganic As species are known to be toxic and most organic species are relatively harmless to humans.

The potential toxicity of some species, such as the huge variety of arsenosugars, has not yet been established.

Courtesy Ute Kohlmeyer GALAB, Germany

LC-ICP-MS Determination of As Species



HPLC-ICP-MS chromatograms are shown for a marine animal (oyster tissue) and marine algae (*Hizikia fusiforme*).

The concentration (ug/g) of 17 different As species was measured

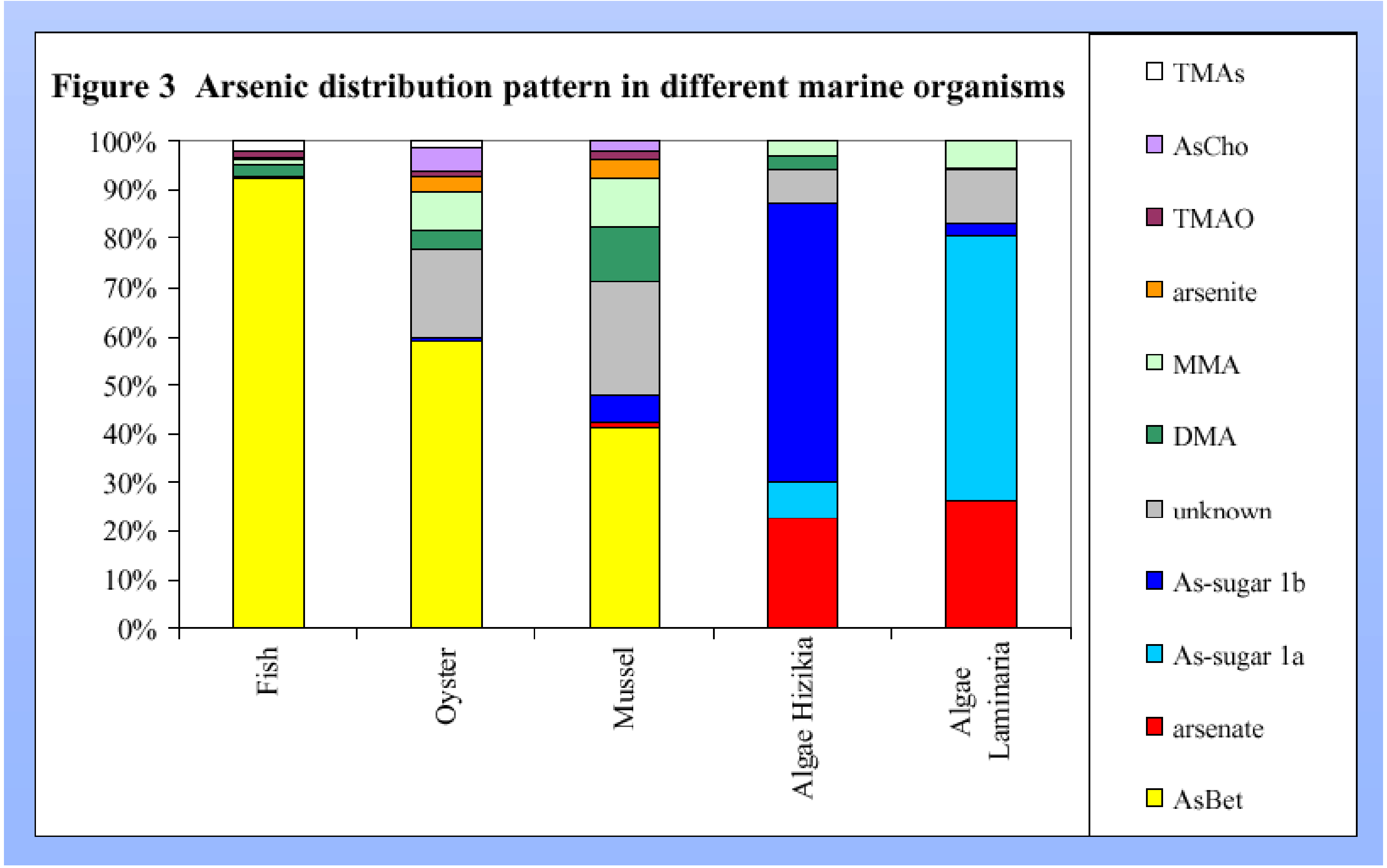
Table 2 Arsenic speciation – Results

Peak no.	t_R [min]	compound	**fish** µg.g^{-1} as As DORM-2	**mussel** µg.g^{-1} as As BCR477	**oyster** µg.g^{-1} as As candidate mat.	**algae** µg.g^{-1} as As *Hizikia fusif.*	**algae** µg.g^{-1} as As *Laminaria*
1	1,62	As(III)	0,05	0,35	0,71	-	-
2	1,89	*Unknown1*	-	-	0,13	-	-
3	2,82	MMA	0,14	0,84	2,10	1,75	2,74
4	4,71	DMA	0,49	0,94	0,97	1,32	0,27
5	5,21	As-sugar 1a	-	-	-	4,17	28,2
6	6,97	As-sugar 1b	-	0,49	0,17	31,4	1,37
7	7,12	As(V)	0,05	0,10	0,08	12,3	13,7
8	7,38	*Unknown2*	-	0,56	0,42	-	-
9	7,64	*Unknown3*	-	-	0,08	-	-
10	8,14	*Unknown4*	-	-	0,13	-	-
11	8,31	*Unknown5*	-	-	-	2,41	-
12	8,52	*Unknown6*	-	1,36	3,36	1,10	5,2
13	8,89	*Unknown7*	-	0,03	0,48	-	0,55
14	9,35	AsBet	16,1	3,49	15,1	-	-
15	12,1	TMAO	0,30	0,14	0,31	-	-
16	13,0	AsCho	-	0,17	1,24	-	-
17	14,2	TMAs	0,30	-	0,34	-	-
Sum of arsenic species			17,4	8,5	25,6	54,6	52,0
Total arsenic (digestion)			17,4	10,2	26,7	51,2	49,5
Extraction efficiency			100%	83%	96%	107%	105%

Courtesy Ute Kohlmeyer GALAB, Germany

LC-ICP-MS Determination of As Species

As species distribution varies dramatically in marine organisms. Non-toxic arsenobetaine is the main species found in marine animals (fish and shellfish), whereas toxic arsenate and arsenosugars are the main species in marine algae.



Courtesy Ute Kohlmeyer GALAB, Germany

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Analysis of Total Inorganic As by Anion Exchange HPLC-ICPMS – Sample Prep

Microwave assisted alkaline hydrolysis

Subsample + 0.9M NaOH in 50% EtOH

↓

Microwave treatment 20 min, 90°C

I: Solubilisation of sample matrix

II: Conversion of As(III) to As(V)

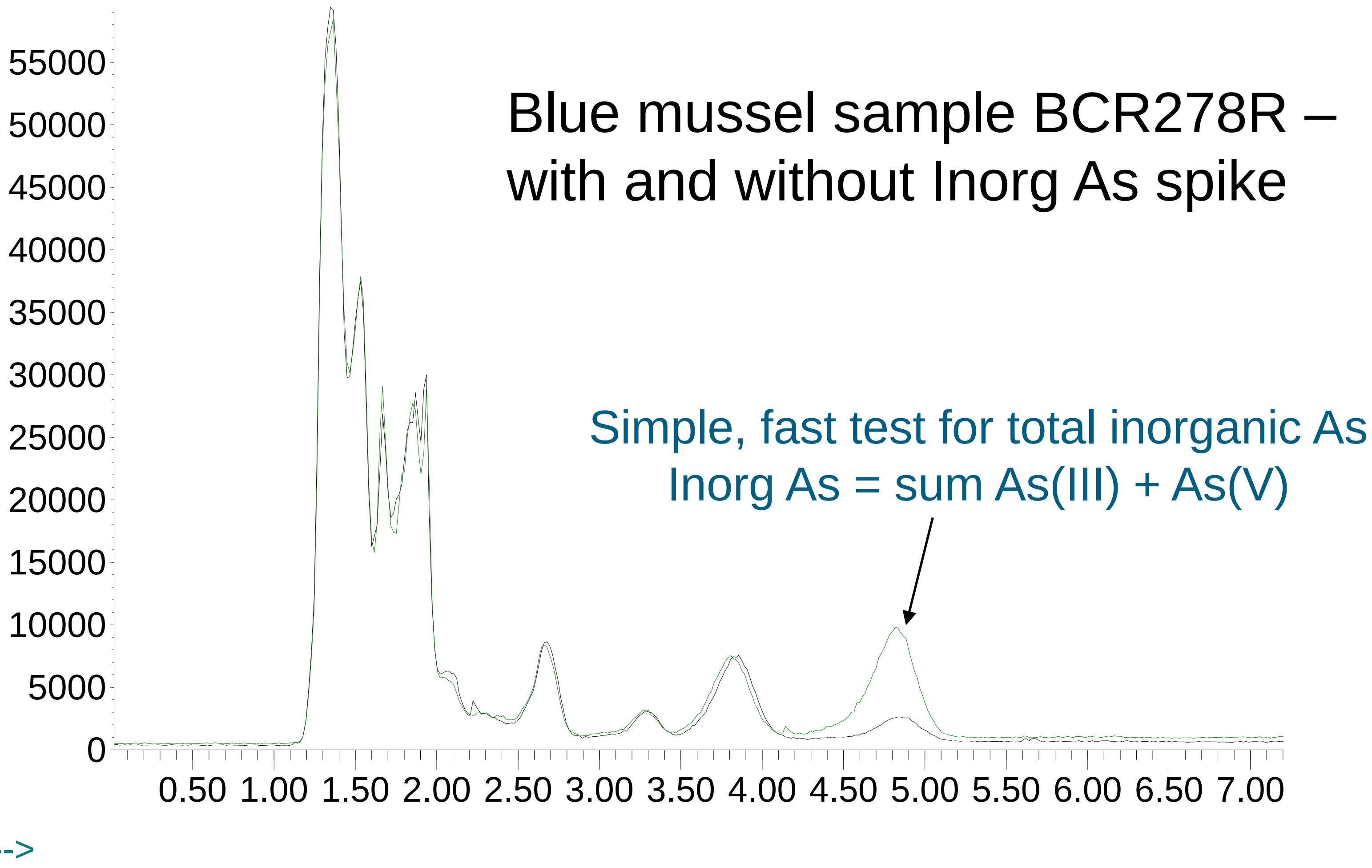
↓

Determination of total inorganic arsenic as As(V) by anion-exchange HPLC-ICP-MS

- No conversion of other arsenic compounds to inorganic arsenic

Sloth and Julshamn 2008, J. Agri.Food Chem.

Analysis of Inorganic As by Anion Exchange HPLC-ICPMS – Results



Sloth and Julshamn 2008, J. Agri.Food Chem.

Summary – Arsenic in the Marine Environment

High levels of arsenic in some marine samples

Complex chemistry

Inorganic arsenic most toxic, but usually low in seafood

HPLC-ICPMS versatile tool for speciation analysis

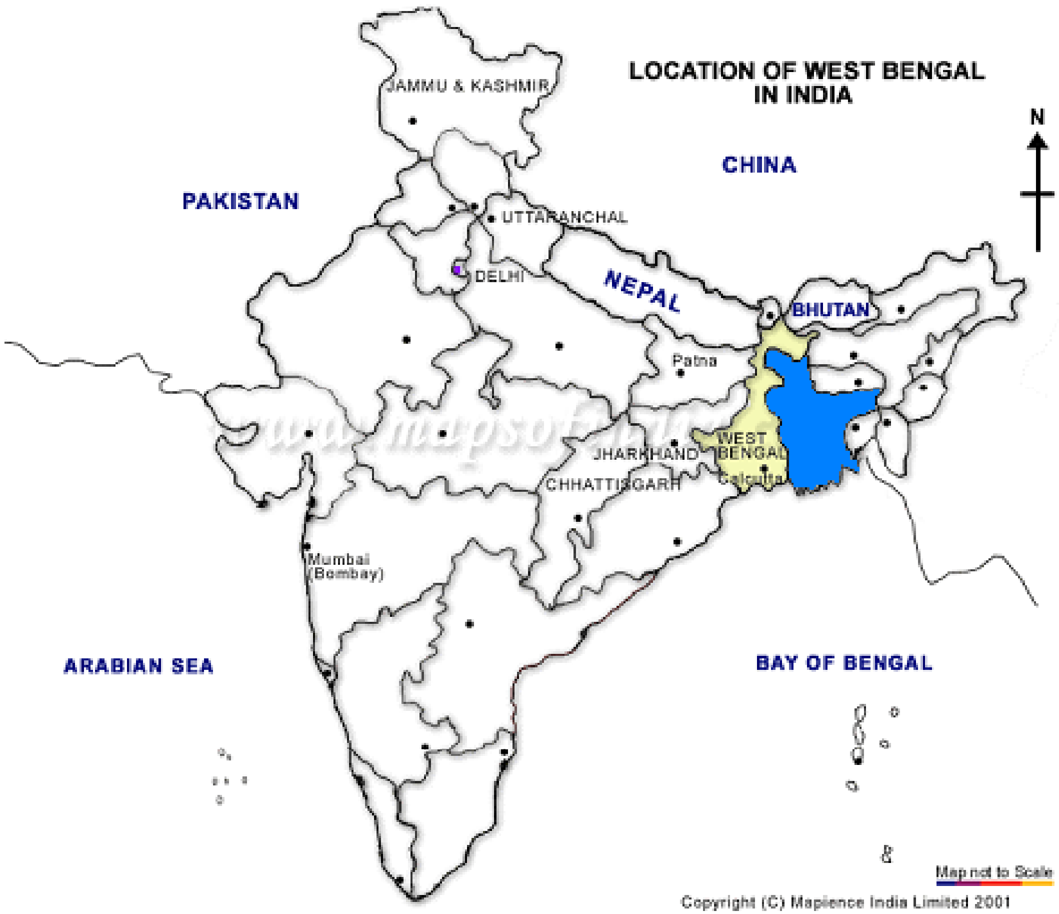
No maximum allowable As levels in food currently defined in the EU

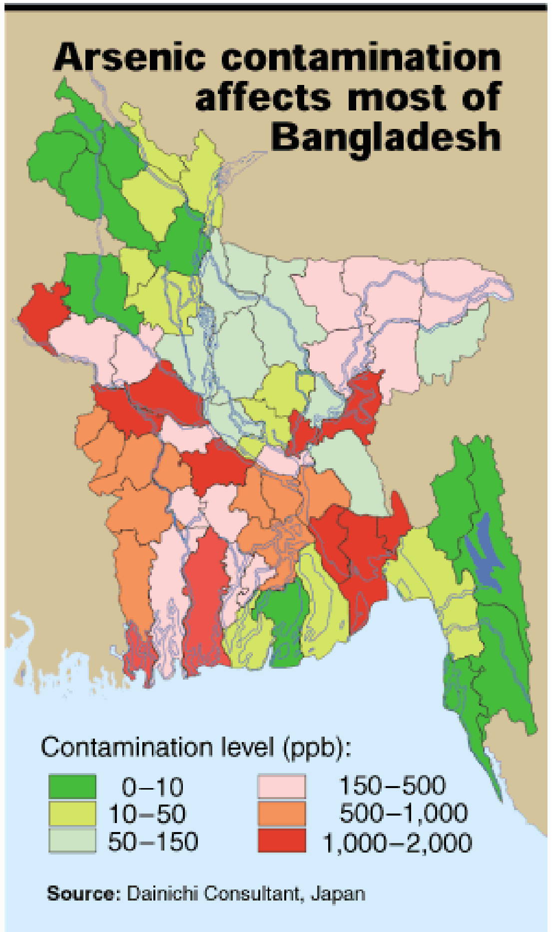
EFSA opionion on arsenic in food

European Food Safety Authority

www.efsa.europa.eu

Arsenic Contamination of Ground Water in Bangladesh & West Bengal - India





Courtesy of Amjad Shraim, Uni Queensland

- Worst human mass-poisoning in history
- More than 80 millions of people are drinking water highly contaminated with As
- Tens of thousands are diagnosed with symptoms of As poisoning
- Arsenic in drinking water may result in 0.2 – 0.3 million deaths from cancer in Bangladesh alone
- Highest reported As concentrations in GW 5 ppm
- More than 95% of arsenic is either arsenite or arsenate (inorganic forms = toxic)
- 1st cases: 1983 West Bengal
  1993 Bangladesh

Courtesy of Amjad Shraim, Uni Queensland

LC-ICP-MS Method for Arsenic in Groundwater

HPLC ICP-MS

Reversed-phase C18 (ODS-3, 150 x 4.6 mm, 3 μm)

Conditions

- 5 mM TBAH + 3 mM malonic acid + 5% methanol
- Flow Rate 1.5 $ml.min^{-1}$
- 50 ^{o}C
- 50 uL injection

Method's DL:

- 0.07 - 0.10 μg/L

Courtesy of Amjad Shraim, Uni Queensland

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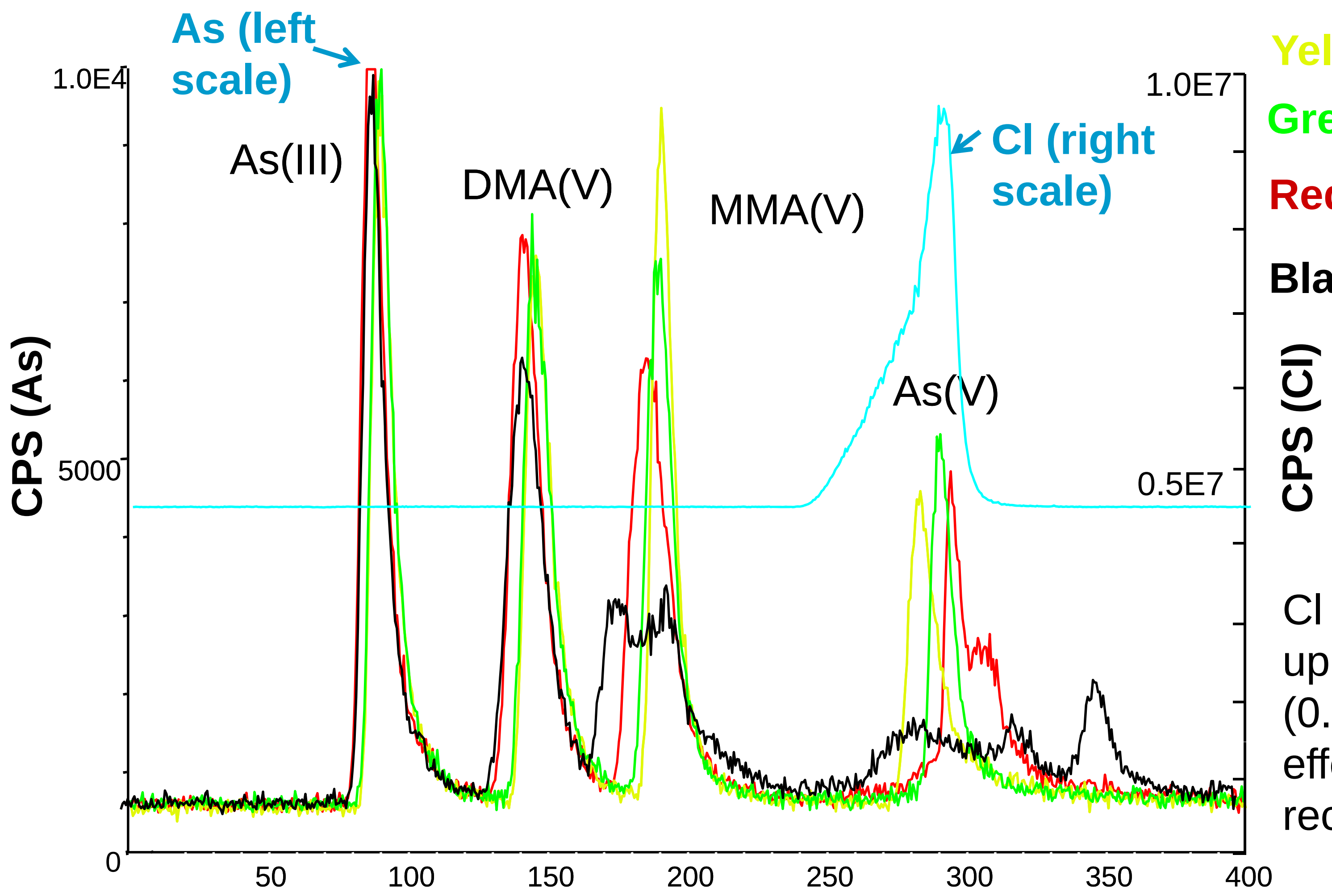
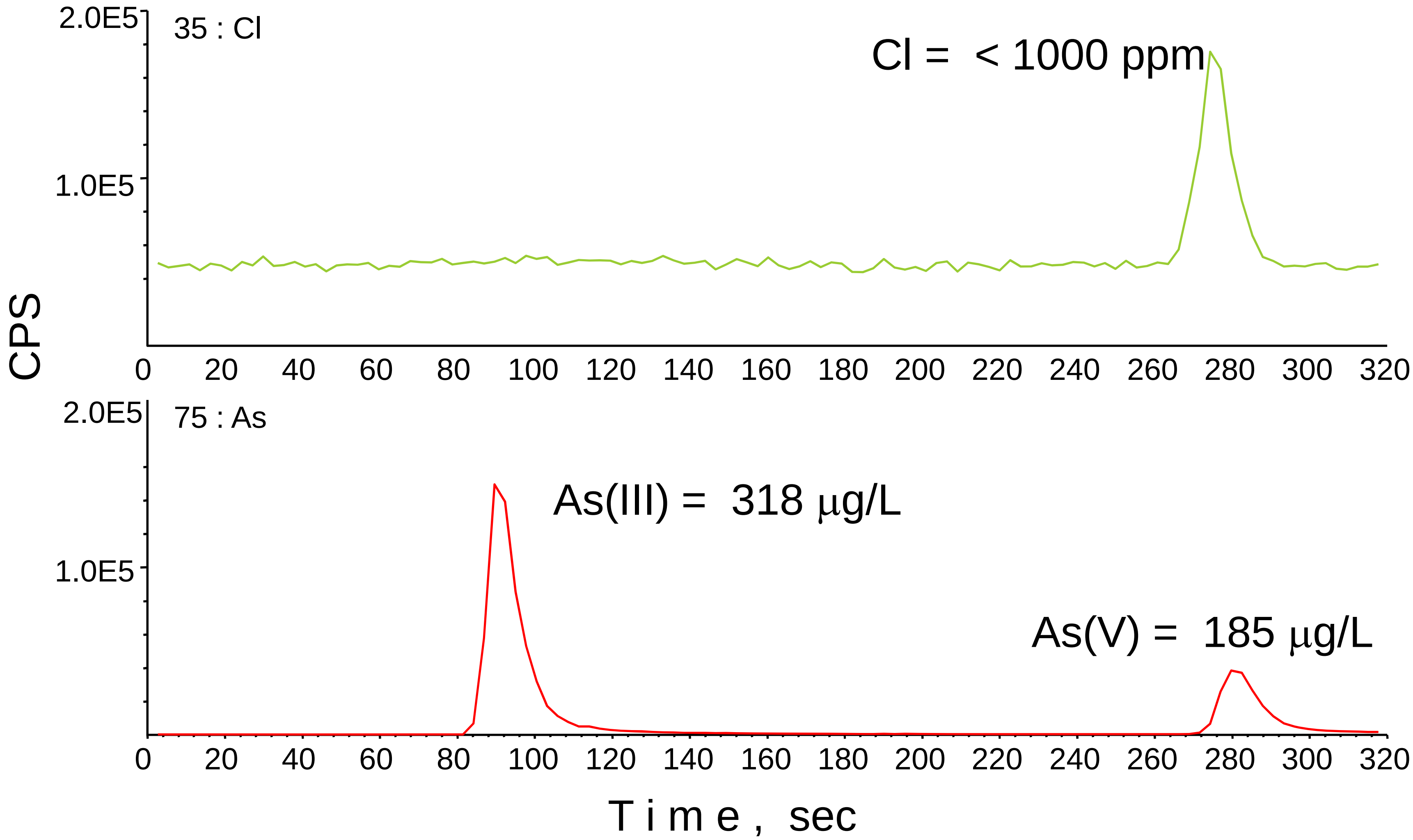


Cl, ppm

Yellow	**0.0**
Green	**1,000**
Red	**3,000**
Black	**10,000**

Cl concentration up to 1000ppm (0.1%) has no effect on As(V) recovery

Tube-Well Water Sample, Undiluted



Courtesy of Amjad Shraim, Uni Queensland

As Concentration in Groundwater

No	As(III)	DMA(V)	MMA(V)	As(V)	**Sum**
1	93.5	0.7	0.5	32.0	**125.9**
2	19.1	0.1	0.1	9.9	**29.1**
3	257.2	ND	ND	76.2	**333.4**
4	6.8	0.2	0.2	7.7	**15.4**
5	501.7	0.1	0.1	24.5	**526.3**
6	418.4	ND	0.1	184.5	**602.9**
7	23.6	ND	ND	9.9	**33.5**
8	461.9	ND	ND	183.9	**645.8**
9	317.5	ND	2.1	184.5	**504.1**

Tube-well water samples from Murshidabad district - West Bengal
All results in ug/L (ppb) in the well-water

Courtesy of Amjad Shraim, Uni Queensland

Thank You

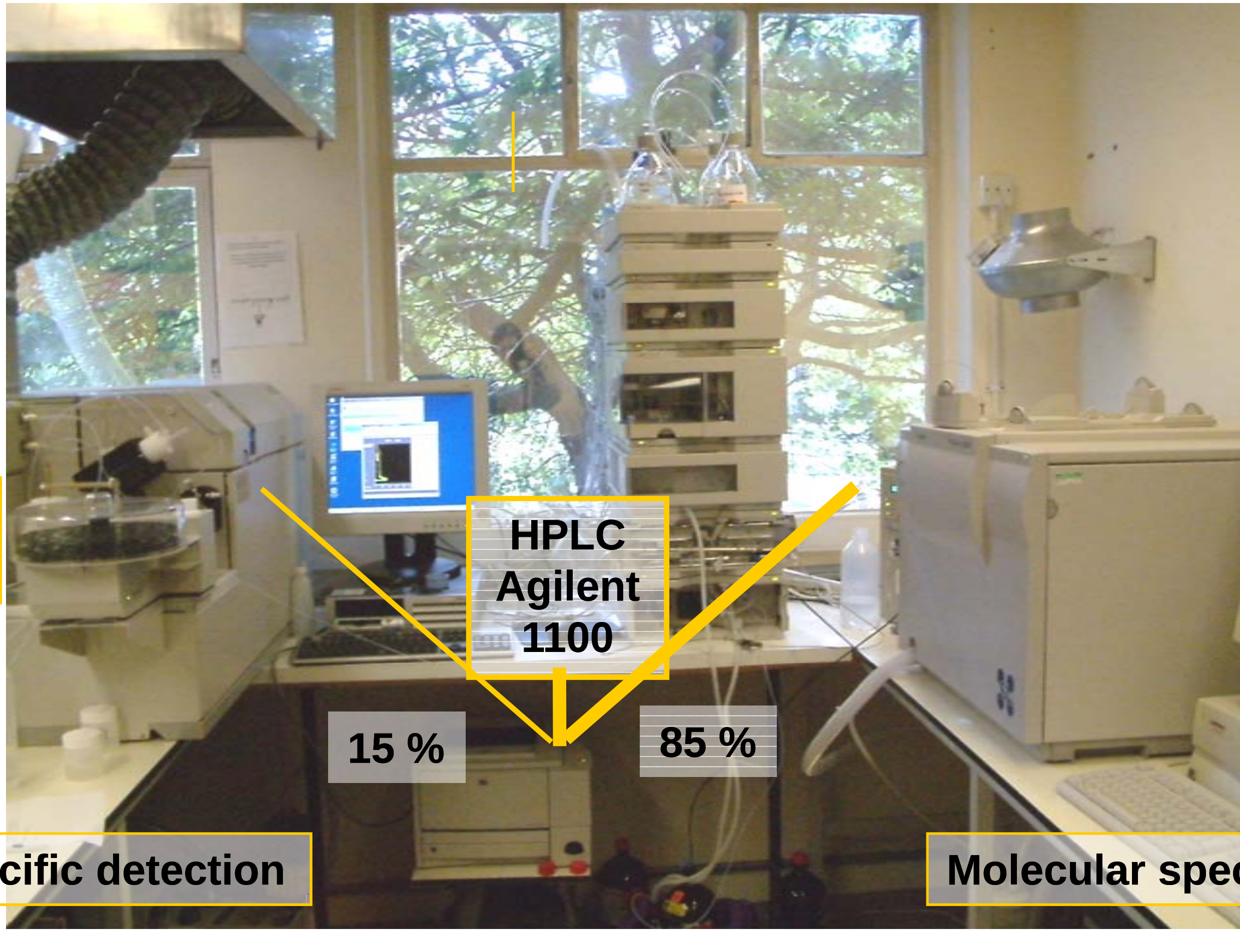
LC-ICP-MS Examples

- Arsenic speciation
- Organo-tin speciation
- Other, more unusual LC-ICP-MS applications

Determination of Complex Organo-As Species in Biological Systems

Small population of sheep on North Ronaldsay – outside enclosures → no grass to eat, so have lived on seaweed for many generations. How is high As content of seaweed metabolised?

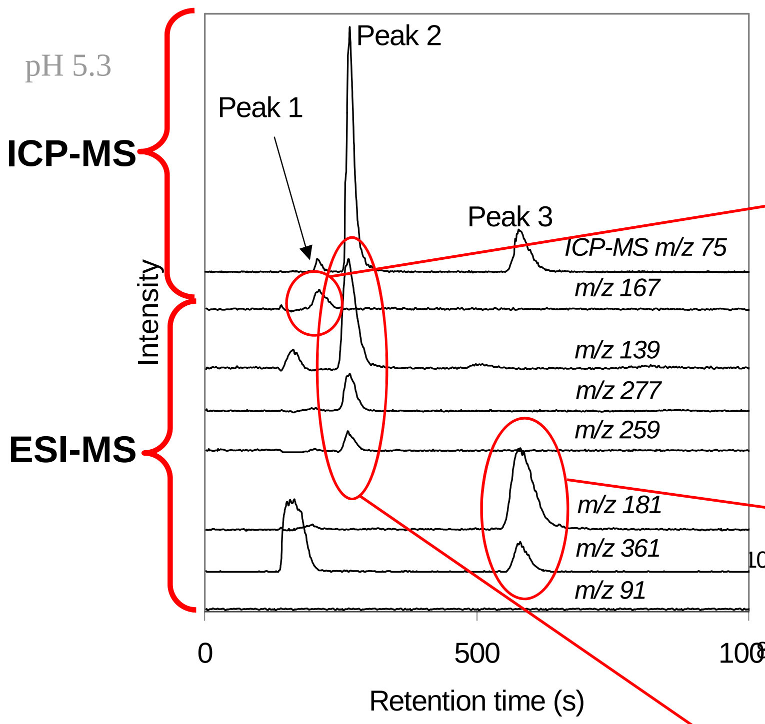
Determination of Organo-As Species
LC with ESI-MS & ICP-MS Detection (Split flow)

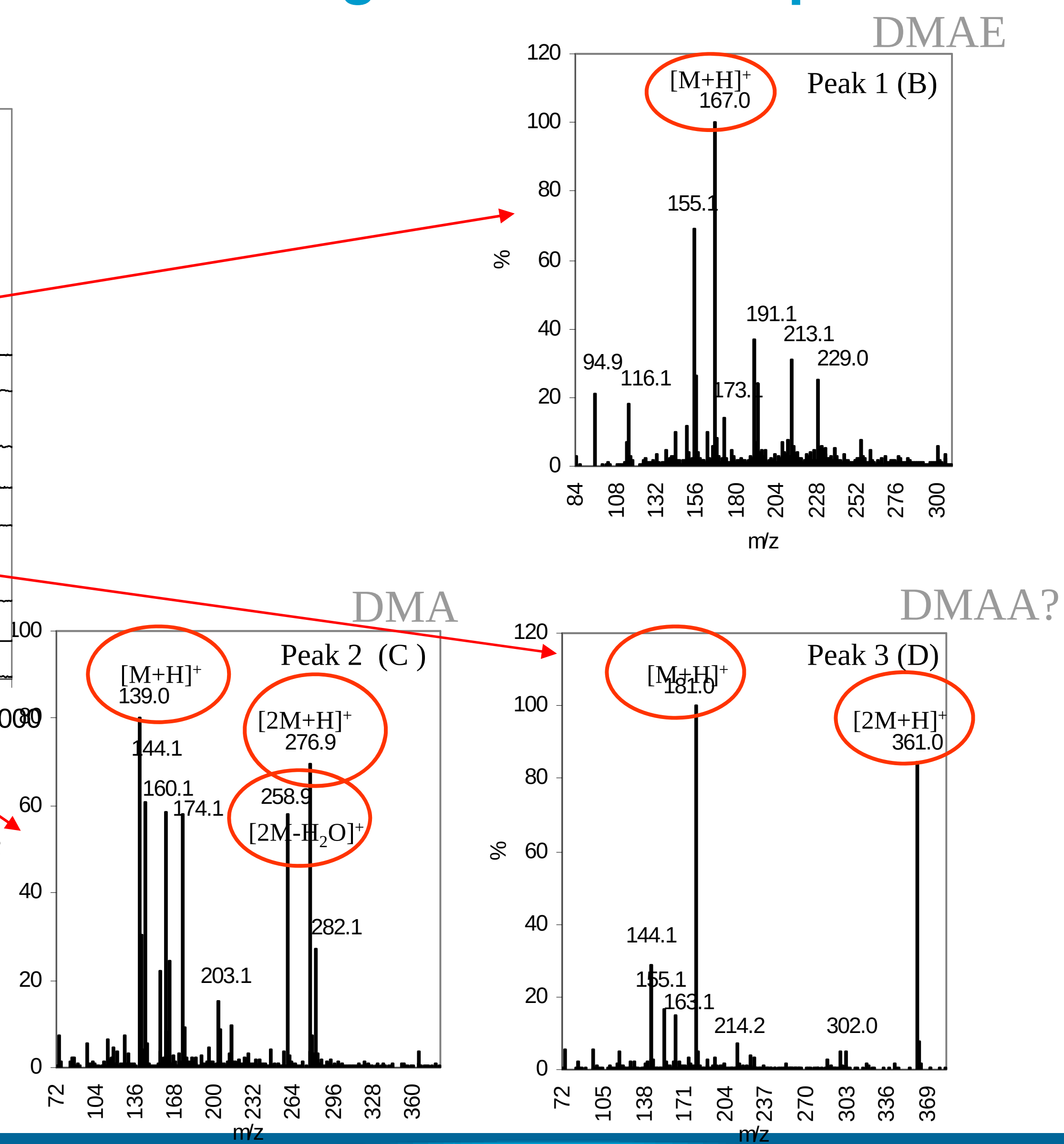


Single HPLC System with controlled split to provide sample flow to ESI-MS and ICP-MS.
Simultaneous identification/quantification of As-containing compounds (ICP-MS) and structural information on all the organic compounds (ESI-MS)

Courtesy Jörg Feldmann et al, Aberdeen Univ.

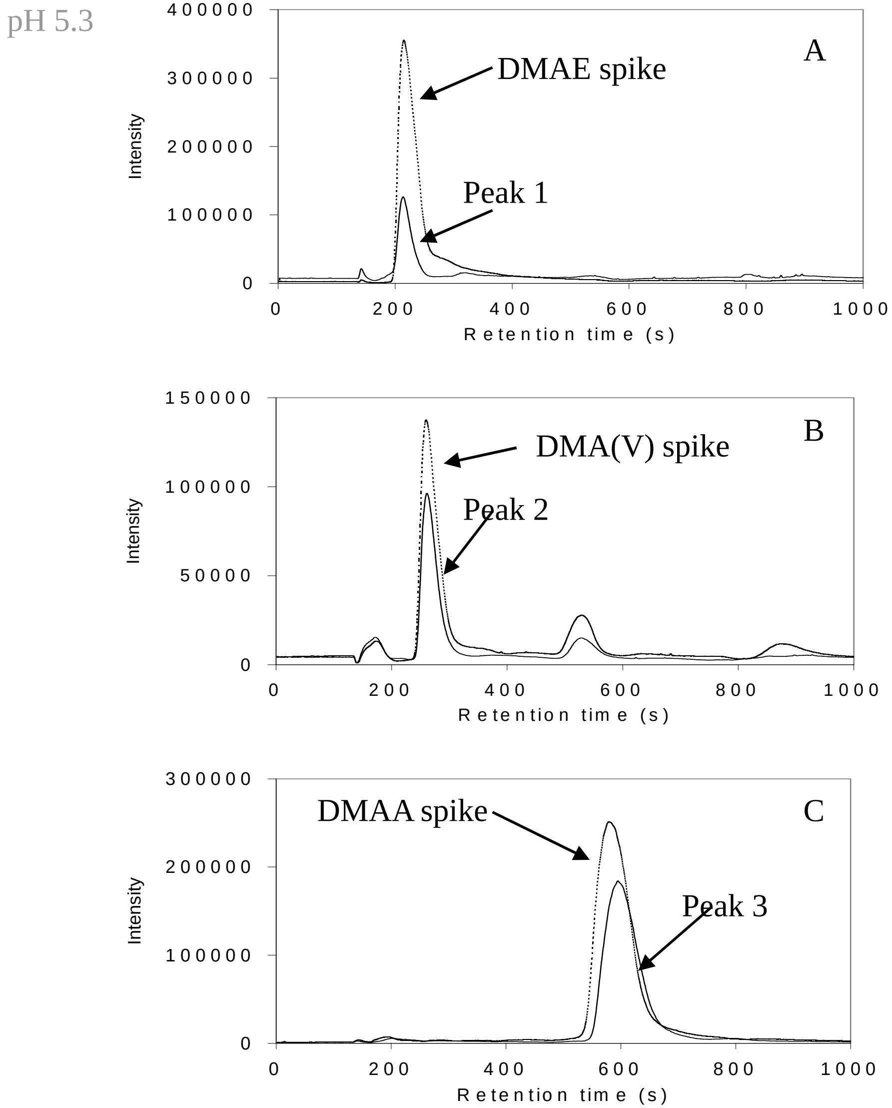
Separation and Identification of Organo-Arsenic Species

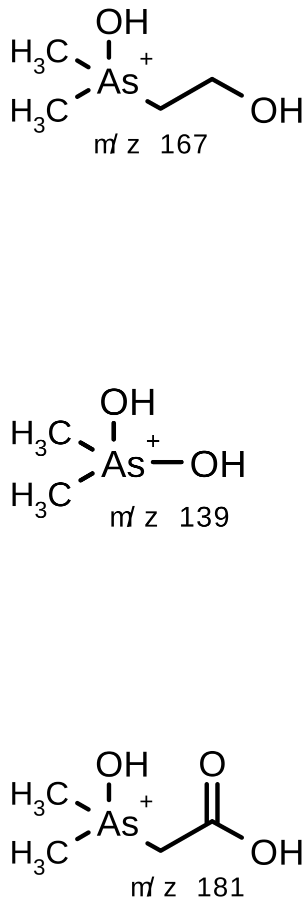




Chromatograms (above) for ICP-MS measurement of As (mass 75) and ESI-MS of various indicator masses. Mass spectra (ESI-MS) for the 3 peaks, showing fragmentation patterns for the identified species – DMAE, DMA and (maybe) DMAA

Confirmation of Organo-Arsenic Species in Sheep's Urine





Species identity confirmed through the analysis of species specific standard spikes by ESI-MS – same retention time and same fragmentation pattern.

Species identified in sheep's urine include DMAA (Dimethylarsinoyl acetic acid), which is known as a natural product in shellfish, but has not previously been reported as a urinary arsenic metabolite.

Hansen et al. J Anal At. Spectrom, 18, 474

Thank You