Advances in Interference Removal for Accurate Arsenic Analysis in Food and Beverages

National Environmental Monitoring Conference 2013
Austin TX
Steve Wilbur and Amir Liba
Agilent Technologies




Let's Define the Problem -Medically, legally, analytically

Arsenic is toxic (but not all forms are toxic to the same degree)

Arsenic is present in drinking waters and many foods (but the form varies)

Toxicity depends on the form or species (but species conservation during sample prep is challenging)

Arsenic is challenging to determine by ICP-MS because it is monoisotopic, has a high ionization potential, and is subject to many common spectroscopic interferences.

Inorganic arsenic-related health effects

Inorganic arsenic is a well-characterized Group 1 human carcinogen

- Lung, bladder, skin and others
- Transplacental carcinogen

Noncancer health effects

- Cardiovascular disease
- Diabetes mellitus
- Dermal effects
- Neurological effects/deficits
- Immunologic effects
- Fertility effects
- Birth defects
- Respiratory effects

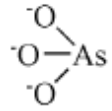
Acute toxicity

- Irritation of lungs, throat, stomach, intestines and skin
- Death within hours after ingestion of sufficient dose

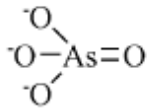
Arsenic in water – inorganic arsenic As(III) and As(V)

British Geological Survey

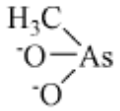
As species*



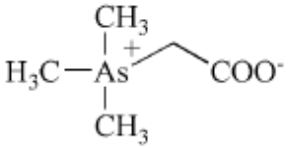
Arsenite
[As(III)]



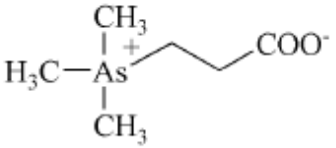
Arsenate
[As(V)]



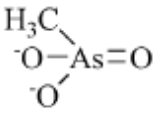
Methylarsonite
[MA(III)]



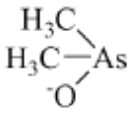
Trimethylarsonioacetate
(Arsenobetaine, AB)



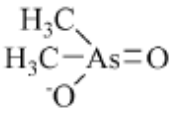
Trimethylarsoniopr
opionate
(TMAP)



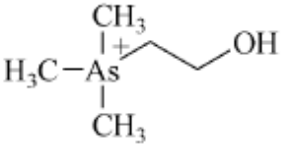
Methylarsonate
(MA)



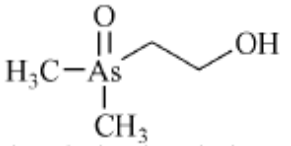
Dimethylarsinite
[DMA(III)]



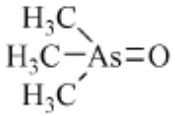
Dimethylarsinate
(DMA)



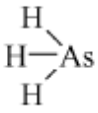
Arsenocholine
(AC)



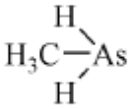
Dimethylarsinoylethanol
(DMAE)



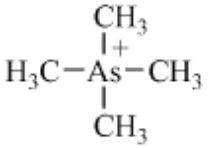
Trimethylarsine oxide
(TMAO)



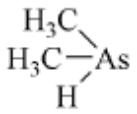
Arsine



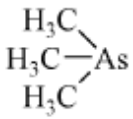
Methylarsine



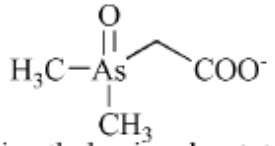
Tetramethylarsonium ion
(TETRA)



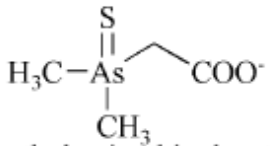
Dimethylarsine



Trimethylarsine



Dimethylarsinoylacetate
(DMAA)

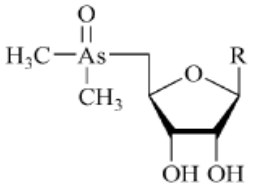


Dimethylarsinothioylacetate

*Analyst. 2004, 129, 373-395

More arsenic species*

Dimethylated Arsenosugars:



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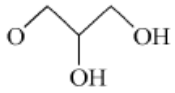
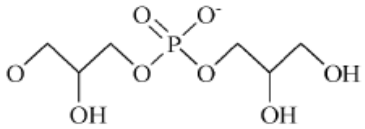
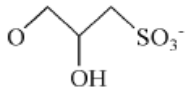


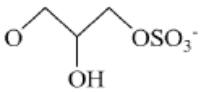
Arsenosugar **1**
(glycerol sugar)



Arsenosugar **2**
(phosphate sugar)



Arsenosugar **3**
(sulfonate sugar)



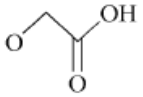
Arsenosugar **4**
(sulfate sugar)

H

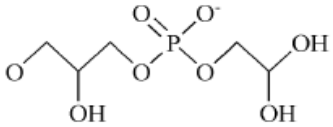
Arsenosugar **5**

OH

Arsenosugar **6**

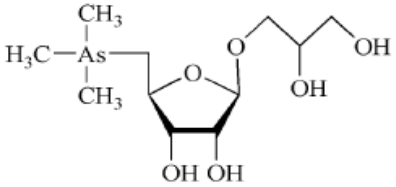


Arsenosugar **7**

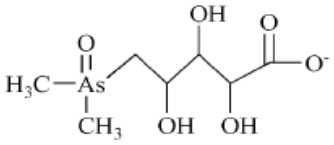


Arsenosugar **8**

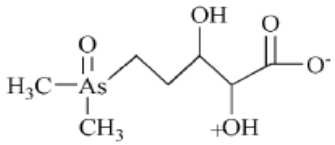
Trimethylated Arsenosugar:



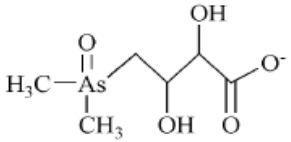
Arsenosugar **9**



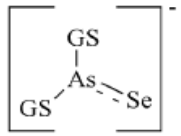
5-Dimethylarsinoyl-2,3,4-trihydroxypentanate



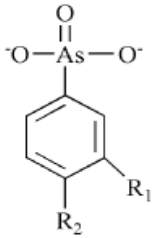
5-Dimethylarsinoyl-2,3-dihydroxypentanate



4-Dimethylarsinoyl-2,3-dihydroxybutanate



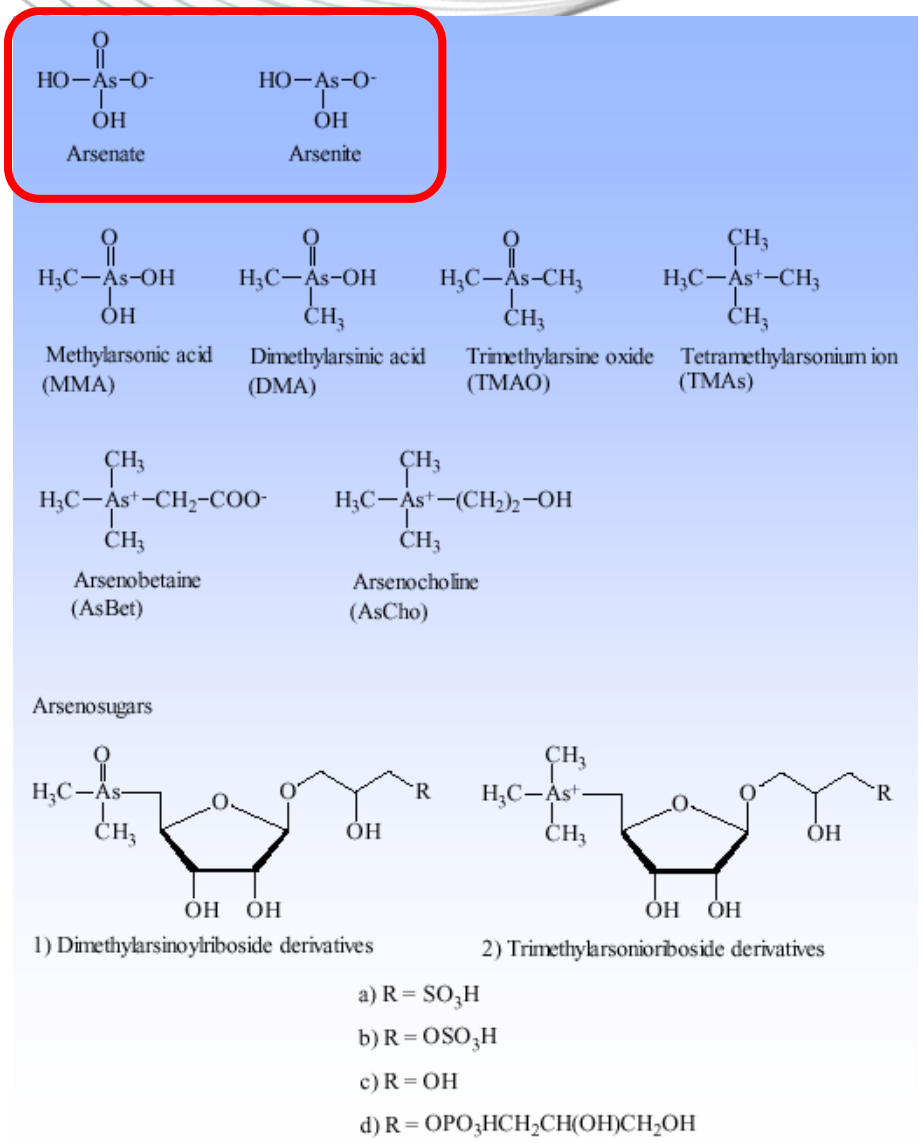
Seleno-bis(S-glutathionyl)arsinium ion
(GS = Glutathione)



$R_1, R_2 = H$	Phenylarsonate
$R_1 = H, R_2 = NH_2$	p-Arsanilate
$R_1 = NO_2, R_2 = OH$ itrophenylarsonate	4-Hydroxy-3- (Roxarsone)

*Analyst. 2004, 129, 373-395

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.

Arsenic in Food

Risk assessments are based on inorganic As

In food, high total As is not always equal to high inorganic As

Arsenic from water is 100% bioavailable; this might not be the case for food

Safe drinking water limits are set assuming long-term exposure

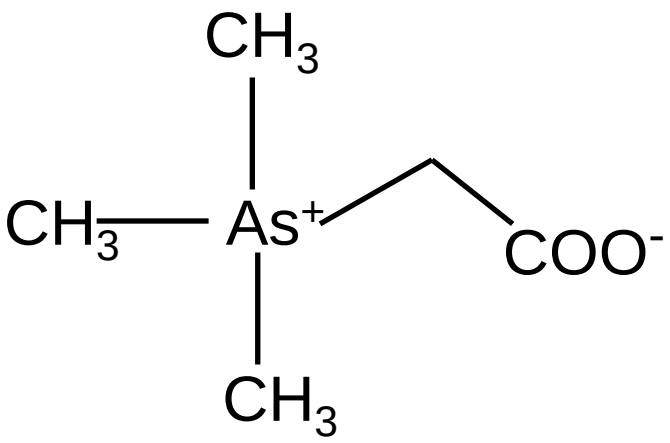
- Dietary choices are varied
- BUT rice-subsistence diets could be equal to or greater than ‘safe’ exposure limits
- Early life exposure is important, should limit exposure during this development period

Arsenic in seafood

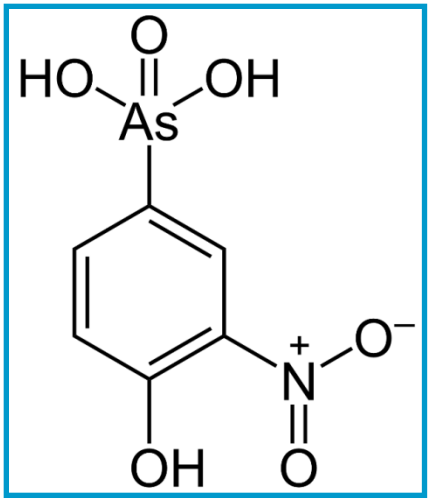
Main source of total As to diet

- Fish can be > 1 ppm – 40 ppm total As

Arsenic is present almost exclusively as arsenobetaine: NON-TOXIC



Arsenic in poultry

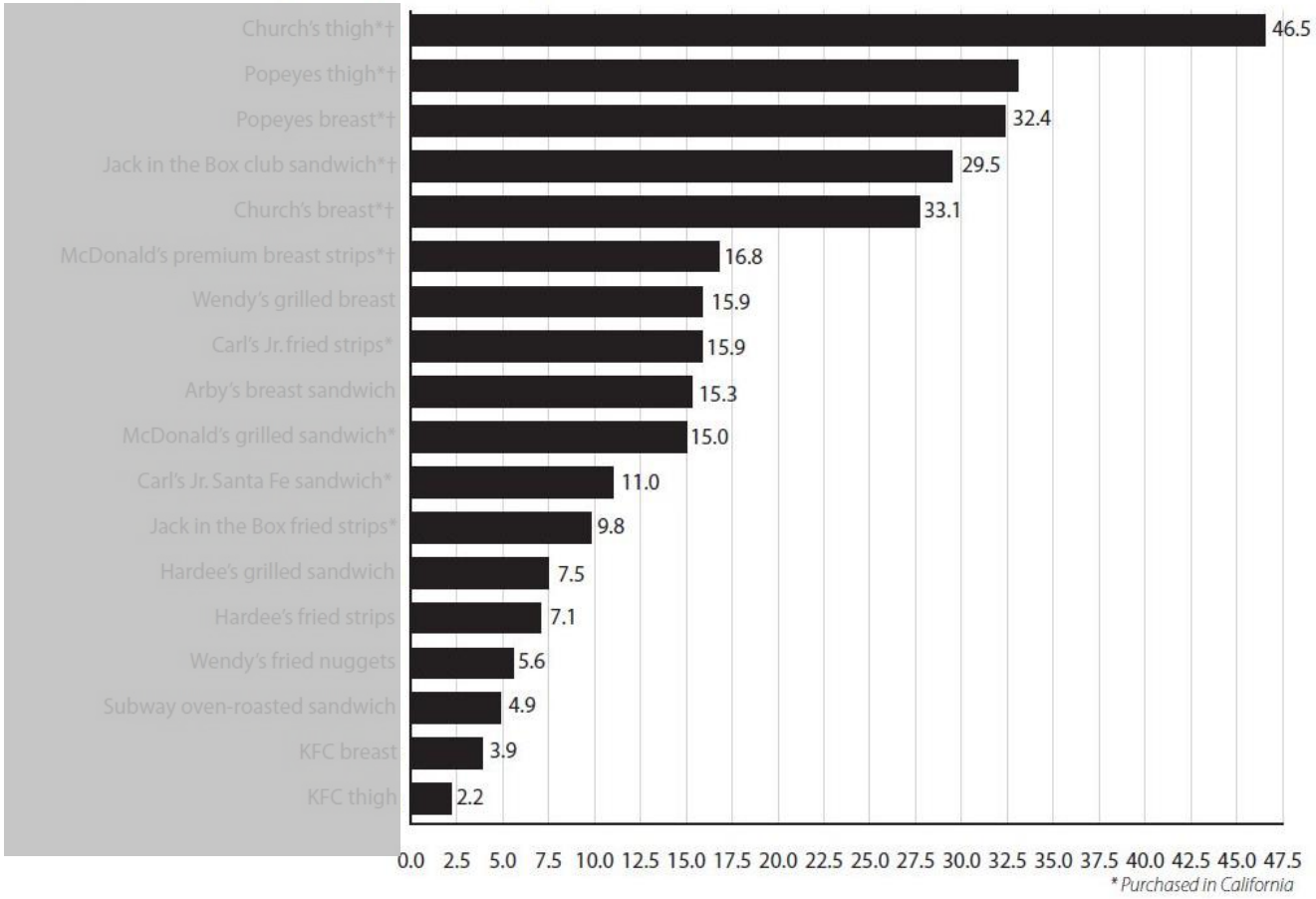


- **Roxarsone**
  - Also p-arsanilic acid, nitarsone, carbarsone, arsanilate sodium
- Additive in poultry and swine feed since mid-1940s
- Approved for growth promotion, improved pigmentation, coccidiostat, treatment of swine dysentery
- In poultry production: 88% raised using roxarsone
  - 2010 estimate
- In swine and turkey production: unknown %
- Single domestic producer
- July 8 2012 – Pfizer suspends marketing of roxarsone in the US

http://us.medage.net/upload/Roxarsone__USP24_20050704.jpg

What about arsenic in chicken meat

Figure B. Average total arsenic in select fast food chicken products (parts per billion)
Limit of detection = 2 ppb, 10 ppb if indicated (†)



2006 IATP: Playing Chicken: Avoiding Arsenic in Your Meat

Arsenic in rice

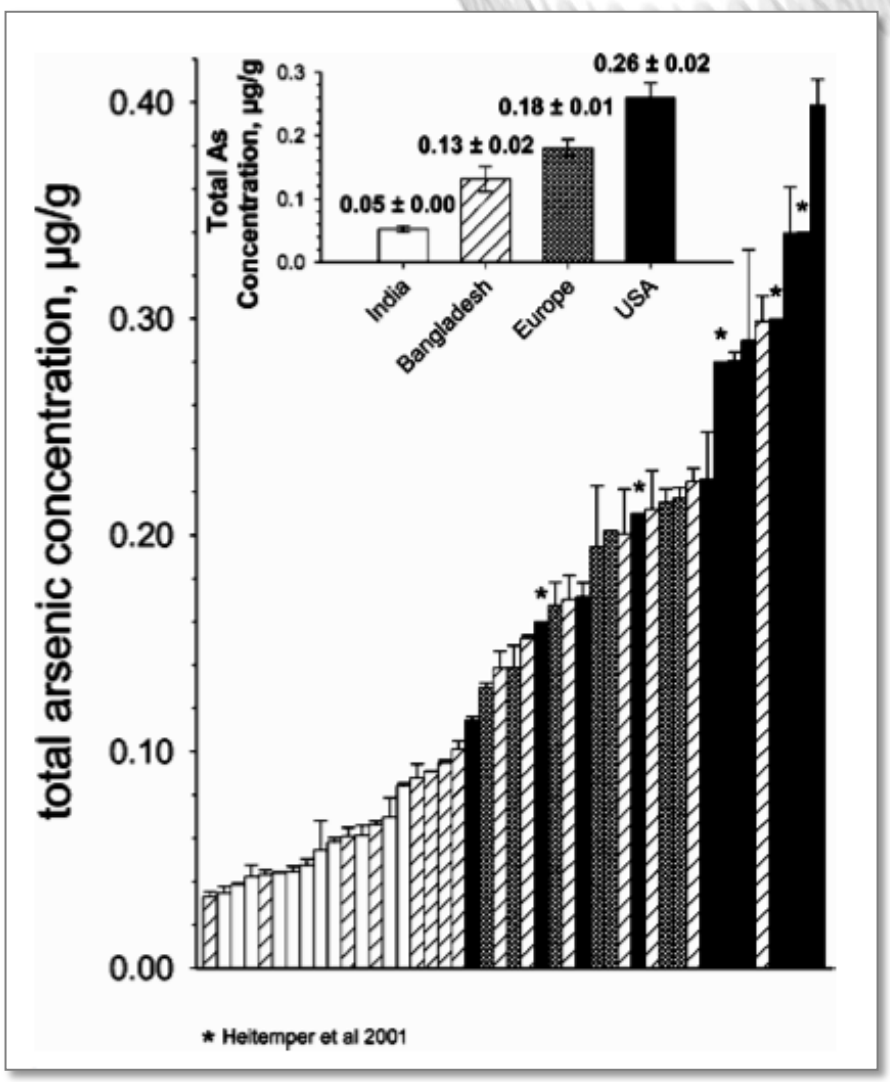
Variation in Arsenic Speciation and Concentration in Paddy Rice Related to Dietary Exposure

P. N. WILLIAMS,† A. H. PRICE,† A. RAAB,† S. A. HOSSAIN,†,§ J. FELDMANN,‡ AND A. A. MEHARG*,†

School of Biological Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK, and Department of Chemistry, University of Aberdeen, Aberdeen, AB24 3UE, UK

"USA long grain rice had the highest mean arsenic level in the grain at 0.26 µg As g^{-1}"

"arsenic in rice contributes considerably to arsenic ingestion in subsistence rice diets"



Williams et al ES&T 39: 2005

Surveys and ‘market basket’ studies

Survey of As and its speciation in rice products such as breakfast cereal, rice crackers and Japanese rice condiments.

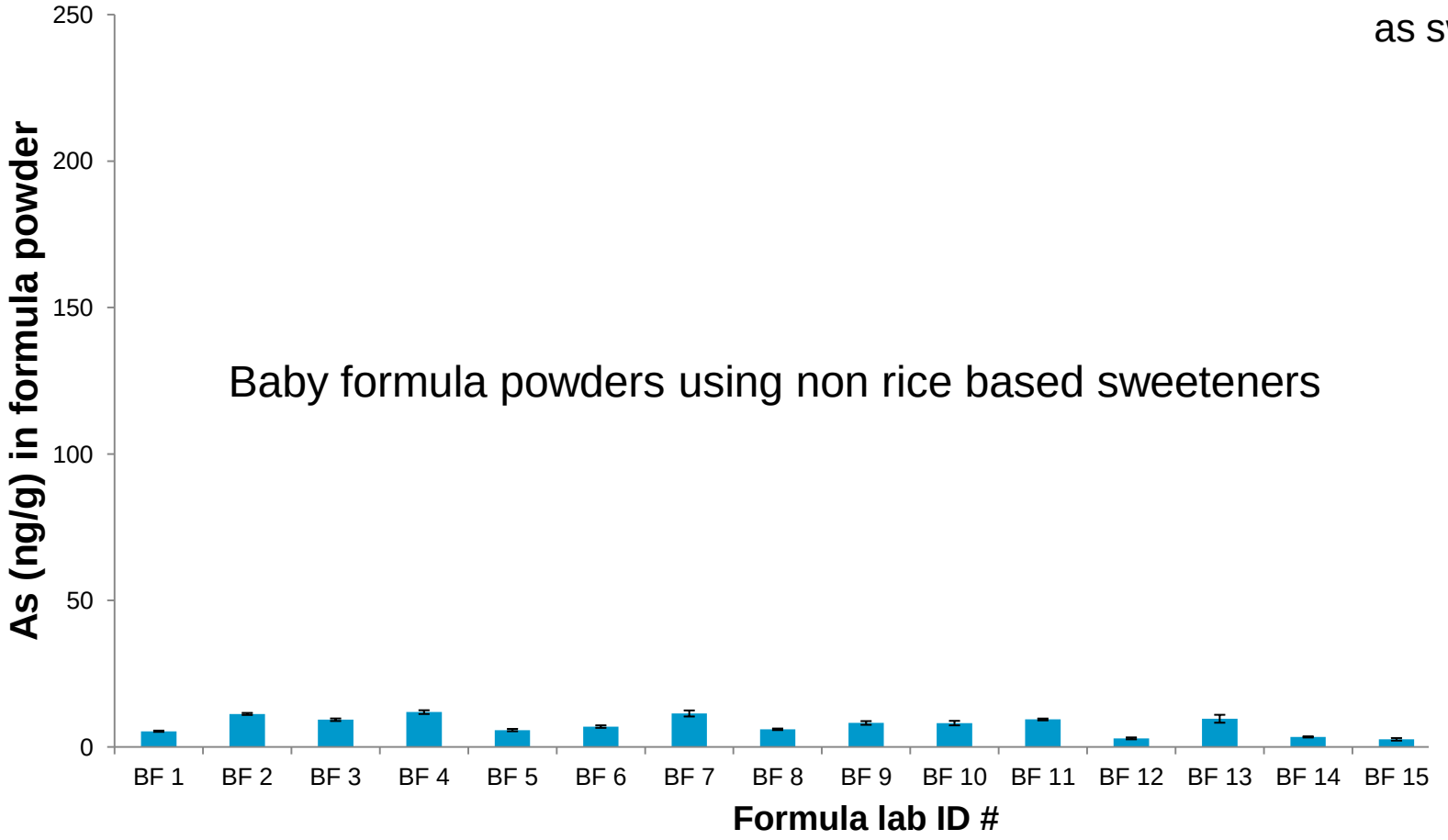
Table 1
Summary of arsenic level in rice products total arsenic

Product	*N*	Average concentration	Standard deviation
Crisped rice	3	0.21 mg/kg	0.01
Puffed rice	2	0.24 mg/kg	0.02
Rice malt	3	0.21 mg/kg	0.08
Noodles	6	0.12 mg/kg	0.09
Sweets	5	0.14 mg/kg	0.02
Rice cracker	11	0.28 mg/kg	0.03
Amazake	1	0.16 mg/kg	
Bran oil	3	0.03 mg/l	0.003
Vinegar	4	0.05 mg/l	0.023
Mirin	2	0.01 mg/l	0

Sun et al 2008, Environment International

Arsenic in baby formula powder

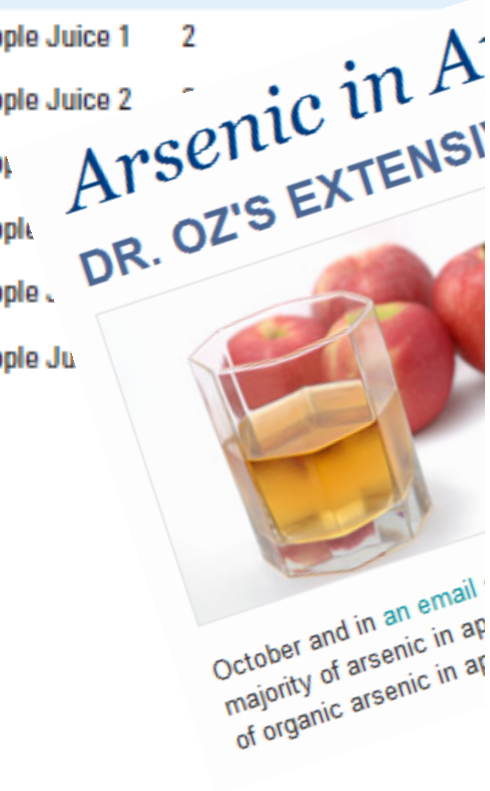
Toddler formulas using organic brown rice syrup as sweetener



Arsenic in Apple Juice

Table 3. Quantitative results (µg/L) for all five

Sample name	Dilution	AB
Apple Juice 1	2	
Apple Juice 2		
Apl		
Appl		
Apple		
Apple Ju		



Agilent application note

New Limits for Arsenic Proposed by F.D.A.

By SABRINA TAVERNISE
Published: July 12, 2013

WASHINGTON — Nearly two years after an outcry about arsenic in apple juice touched off by a segment on "The Dr. Oz Show," the federal Food and Drug Administration is proposing a new limit on acceptable levels.

The new standard for arsenic, a carcinogen when consumed in large enough quantities, is 10 parts per billion, equal to the level that the Environmental Protection Agency has set for arsenic in drinking water. Experts said the allowable amount was relatively conservative since people typically drink far less apple juice than water.

Apple juice with arsenic levels that exceed the new target might be subject to action by the agency, including seizure, said Dr. Margaret Hamburg, the F.D.A. commissioner. The proposed target will be finalized only after comments from industry and the public, she said.

Solving the Analytical Problems

- Separating the toxic from the non-toxic species with reliable species conservation and recovery
- Determining the concentration of toxic species (and others) in the presence of significant interferences and sometimes without access to species specific standards or reference materials
- Doing the above with sufficient accuracy, precision and method robustness

ICP-MS as an LC detector for As

Specific and Selective:

ICP-QMS Arsenic is determined at mass 75 and the chloride interference is separated, in time, by the chromatographic system or removed by the collision cell.

ICP-QQQ Arsenic is determined as AsO (m/z=91) after the first quad removes all other potential interferences at m/z=91

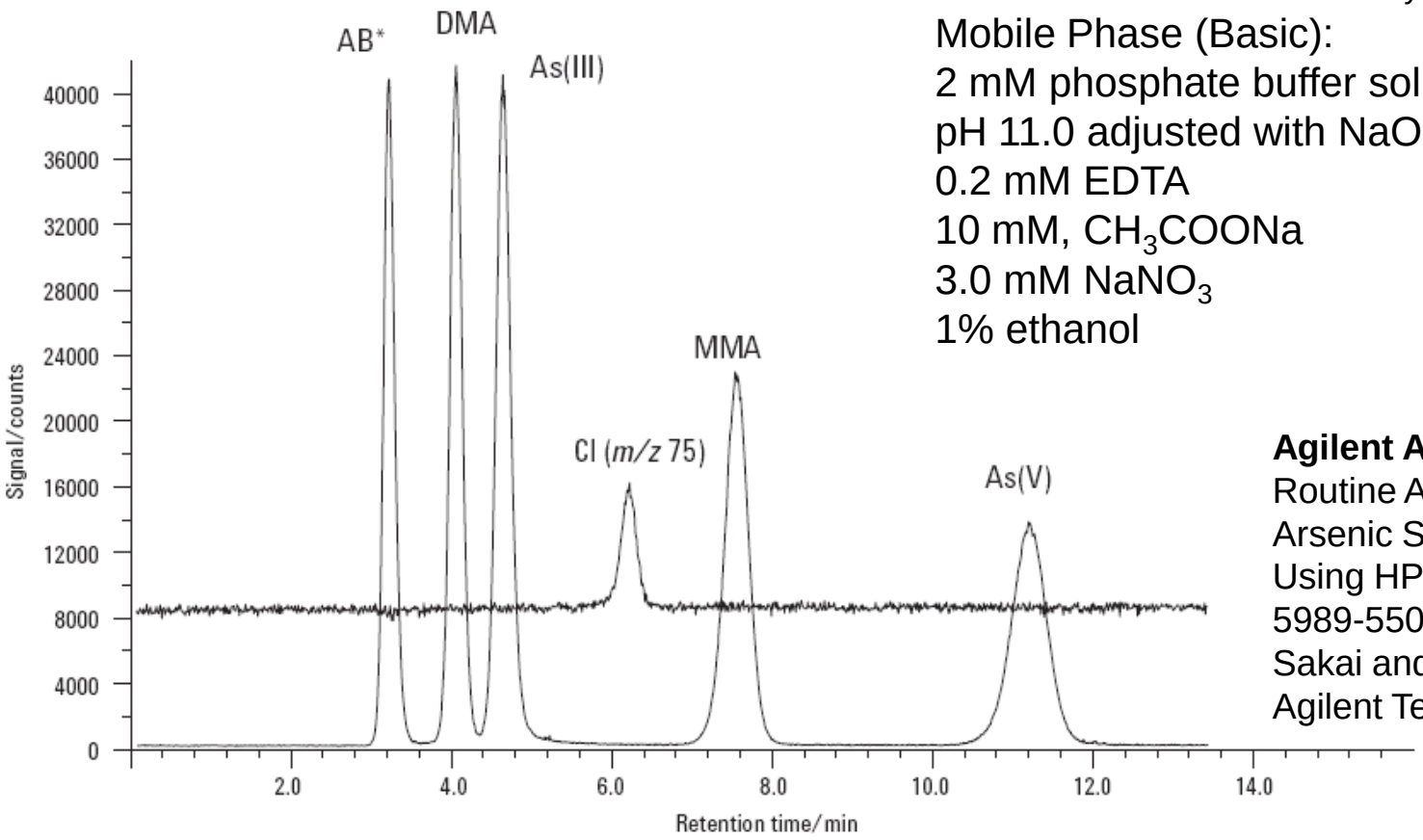
Wide dynamic Range: the ICP-MS detector is linear over large concentration range (10e9)
- range limited by chromatographic system

No structural information – ID by retention time

Accurate quantification for unknown As compounds possible using Compound Independent Calibration

Arsenic Speciation with LC-ICP-MS

Complete isocratic separation of AsB & Cl from inorganic As



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

*Surface Aminated Polymer Substrate

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

Arsenic species in apple juice
A simple, reliable method

A simple filtration (preserve the species, good recovery)

No sample digestion or stabilization was performed, as aggressive sample separation steps could cause species inter-conversion.

LC-ICP-MS Method	
Instrument	1200 LC
Column	G3288-80000 (4.6x250mm)
Mobile phase	2.0mM PBS, 0.2mM EDTA, 30mM CH3COONa, 2.0% EtOH pH 11.0 adjusted by 1M NaOH
Flow rate	1.0 mL/min
Injection Vol.	100µL
Oven Temp.	Ambient
Sample Preparation	
Filter1	Membrane filter: Millex-LH 0.45µm PTFE 25mm (Millipore corporation)
Filter2	DDS Cartridge: TOYOPAK ODS M (TOSOH corporation)
Dilution	1:2 with de-ionized water

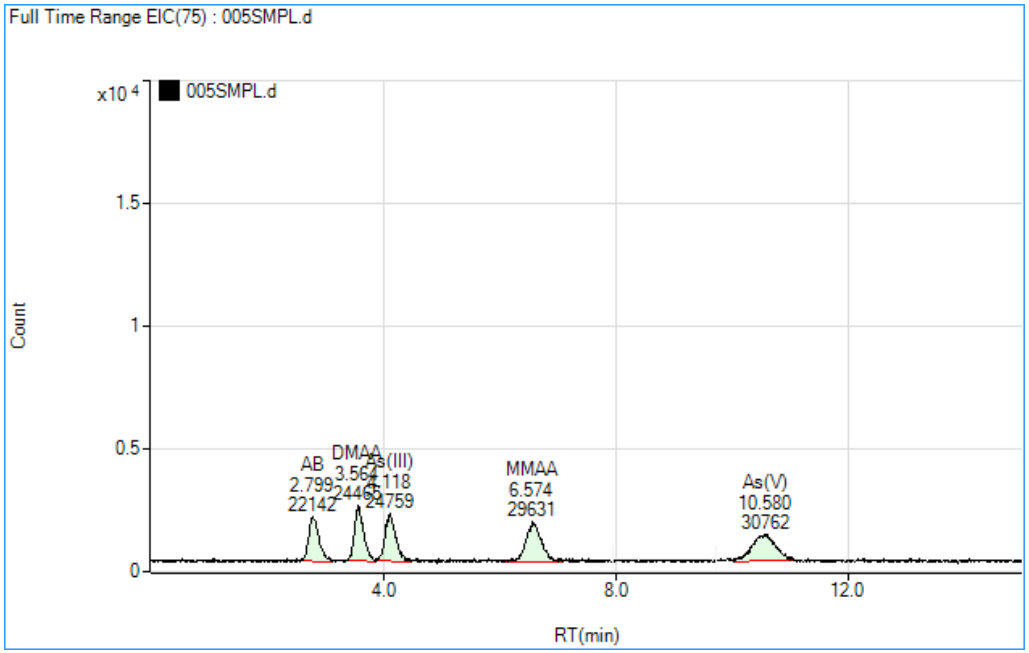
S/N for As Species in Apple Juice
Limit of Detection ~ 0.02ppb*

Peak#	Compound	RT[min]	Height	Area	Noise	S/N	Noise Type
1	AB	2.799	1797	22142	133	13.51	Peak-to-Peak
2	DMAA	3.564	2254	24465	133	16.95	Peak-to-Peak
3	As(III)	4.118	1910	24759	133	14.36	Peak-to-Peak
4	MMAA	6.574	1602	29631	133	12.04	Peak-to-Peak
5	As(V)	10.580	1082	30762	133	8.13	Peak-to-Peak

S/N report for 0.1ppb Standard – 7700x (intensity scale 20,000 counts)

S/N between 8 and 17 depending on compound

***proposed new FDA regulation is 10 ppb**

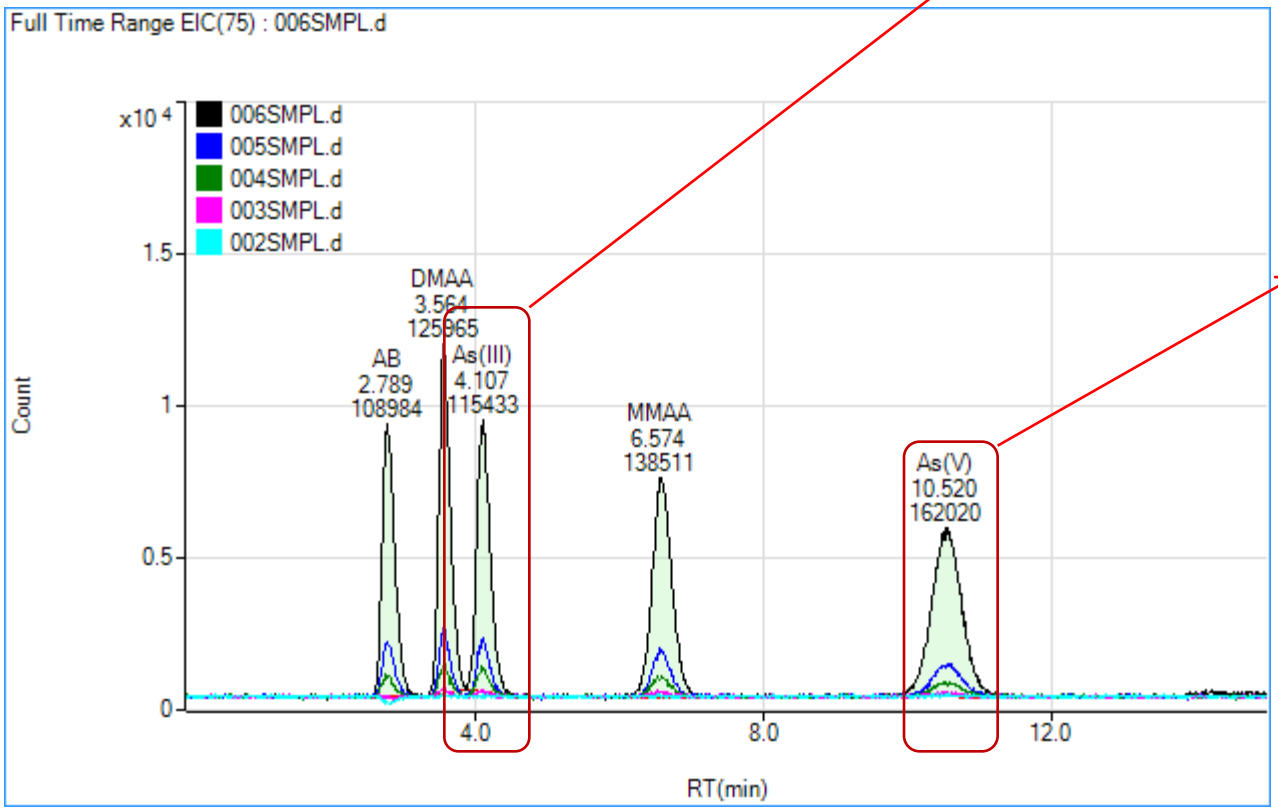


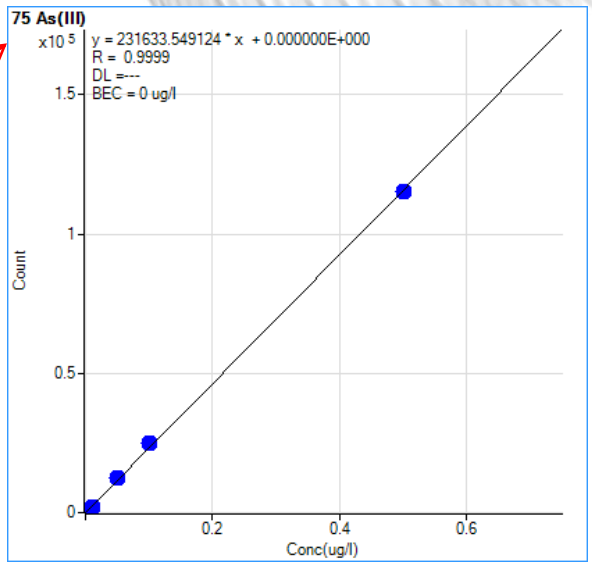
As Species Calibrations by LC 1200 with 7700x

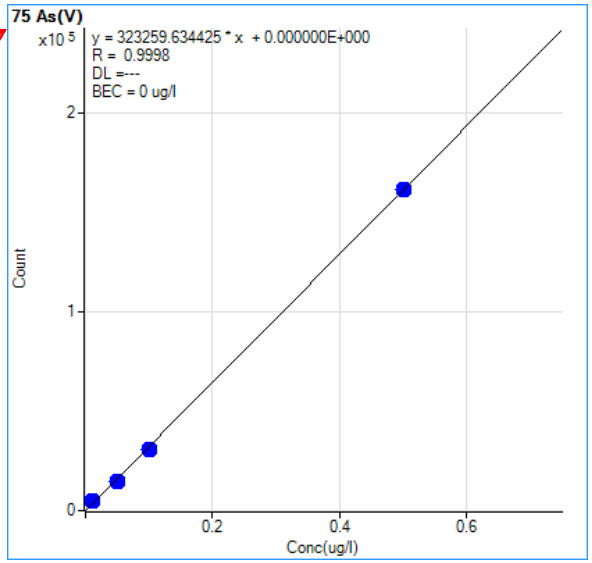
Overlaid chromatograms for As calibration standards:

- **0, 0.01, 0.05, 0.1, 0.5ppb As**

Intensity scale 20,000 counts







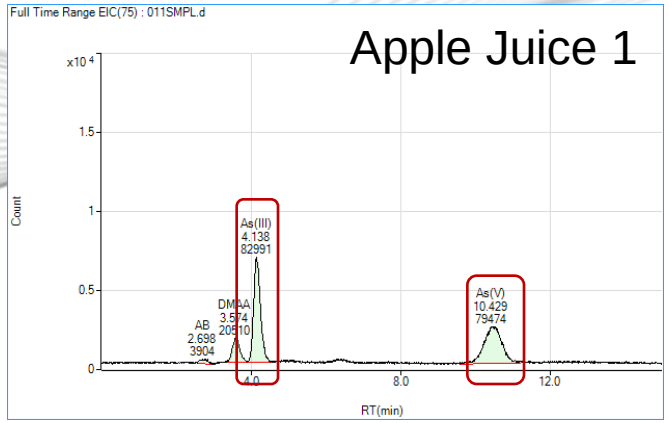
As Species in Apple Juice

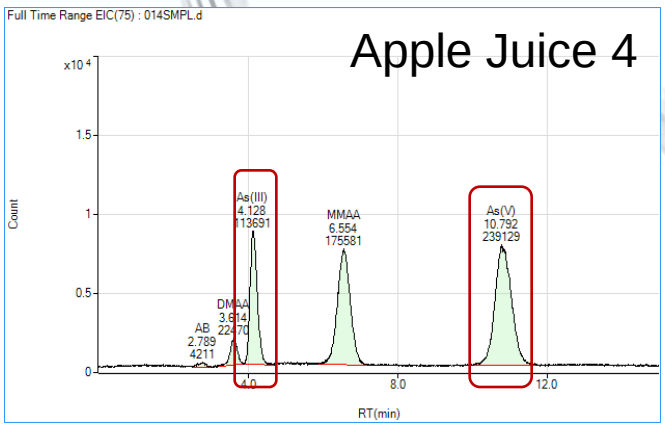
Chromatograms for 6 different commercially available apple juices (As(III) and As(V) highlighted).

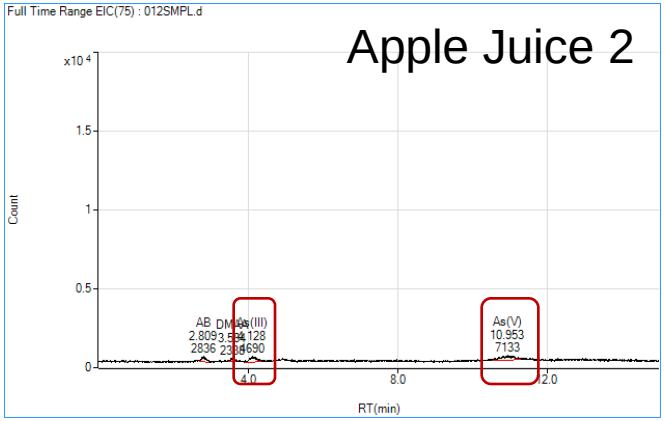
All chromatograms shown on an intensity scale of 20,000 counts

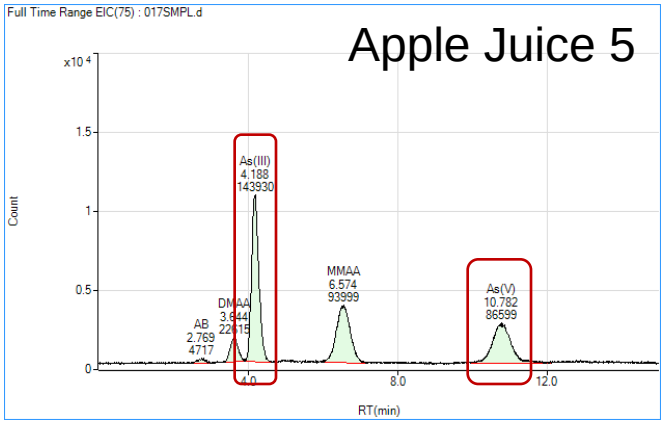
Samples diluted 2x with deionized water prior to analysis

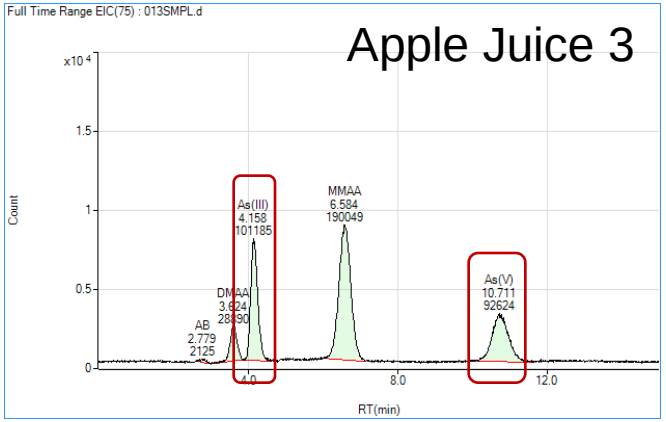
As species varied between samples, but all levels low (<1.5ppb each species in undiluted juice)

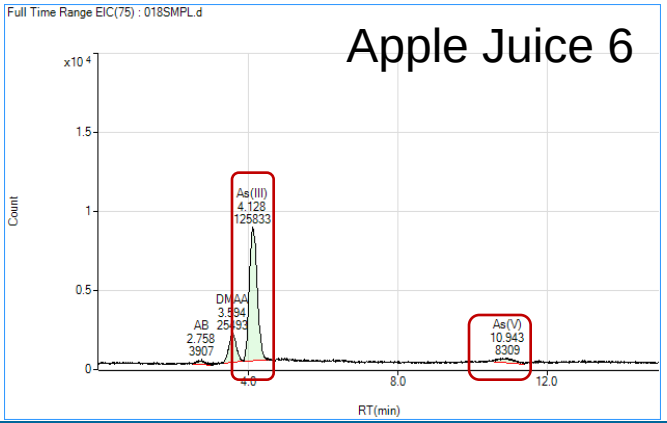






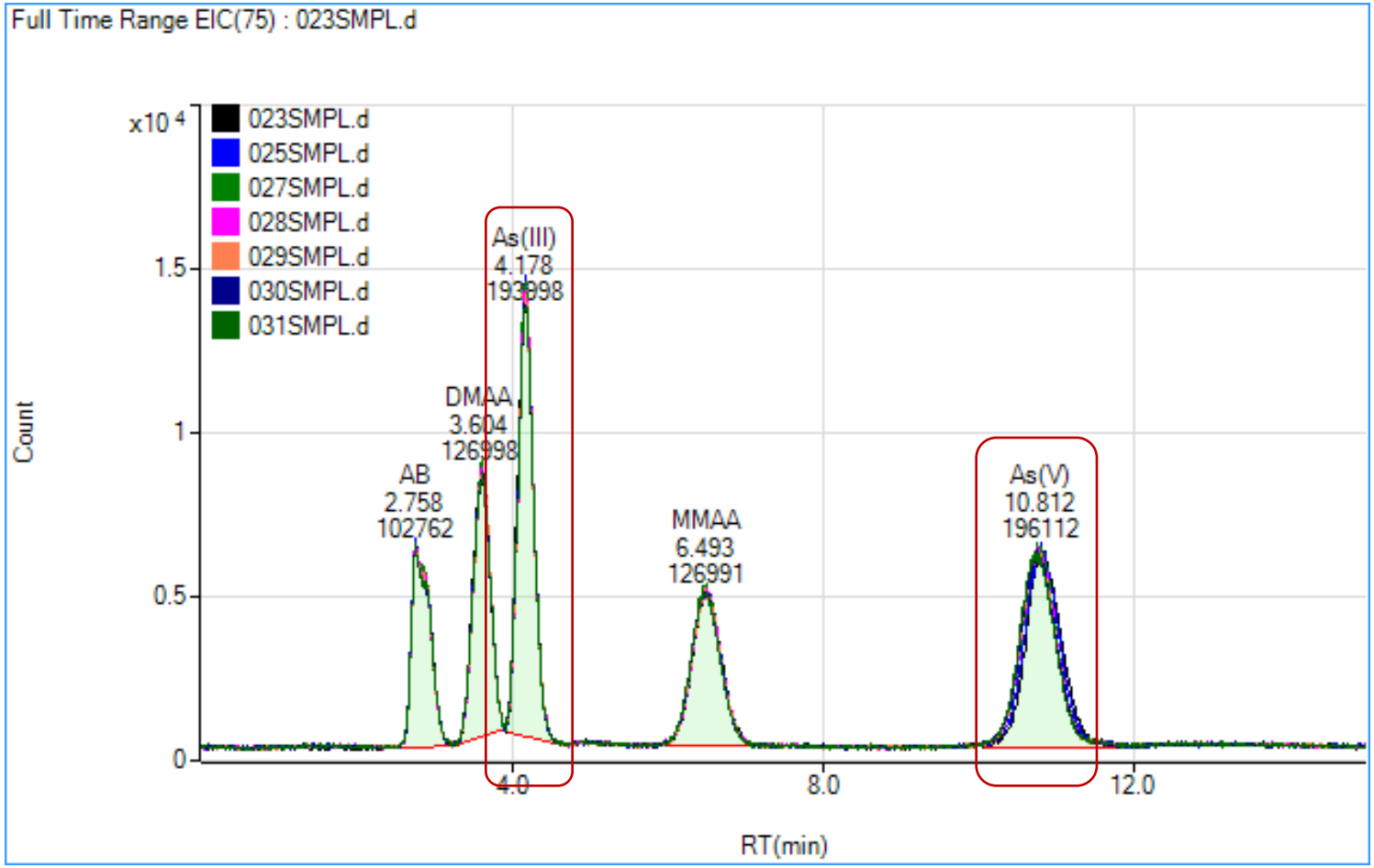






As Species Stability (precision and robustness)

Overlaid chromatograms for 7 separate injections of Apple Juice 1 spiked with 0.5ppb each As species



Quantitative Results for As Species in Apple Juice by single quad ICP-MS

		AB	DMAA	As(III)	MMAA	As(V)
Sample Name	Dilution	Conc. [ug/l]	Conc. [ug/l]	Conc. [ug/l]	Conc. [ug/l]	Conc. [ug/l]
Apple Juice 1	2	0.036	0.163	0.717	ND	0.492
Apple Juice 2	2	0.026	0.019	0.04	ND	0.044
Apple Juice 3	2	0.020	0.230	0.874	1.371	0.573
Apple Juice 4	2	0.039	0.179	0.982	1.266	1.479
Apple Juice 5	2	0.043	0.180	1.243	0.678	0.536
Apple Juice 6	2	0.036	0.203	1.086	ND	0.051

All individual species results less than 1.5ppb in the undiluted juice samples. Total inorganic As (sum of As(III) and As(VI) less than 2.5ppb in all samples

Precision for As Species in Apple Juice

Stability data (n=7) for spiked apple juice. RT and concentration results

RT stability around 0.2 to 0.3%RSD

Concentration stability ~1%RSD

	AB		DMAA		As(III)		MMAA		As(V)	
Sample Name	RT	Conc. [ug/l]	RT	Conc. [ug/l]	RT	Conc. [ug/l]	RT	Conc. [ug/l]	RT	Conc. [ug/l]
Spike 50-1	2.758	0.944	3.604	1.010	4.178	1.675	6.493	0.916	10.812	1.213
Spike 50-2	2.769	0.935	3.614	1.015	4.178	1.677	6.493	0.911	10.802	1.209
Spike 50-3	2.769	0.939	3.614	1.004	4.178	1.655	6.503	0.903	10.762	1.207
Spike 50-4	2.758	0.936	3.614	0.999	4.168	1.646	6.503	0.921	10.762	1.194
Spike 50-5	2.758	0.955	3.614	1.009	4.178	1.635	6.473	0.923	10.762	1.176
Spike 50-6	2.748	0.925	3.634	0.996	4.158	1.650	6.473	0.901	10.762	1.230
Spike 50-7	2.758	0.924	3.624	0.991	4.178	1.648	6.463	0.916	10.772	1.203
%RSD	**0.265**	**1.149**	**0.263**	**0.851**	**0.189**	**0.936**	**0.247**	**0.927**	**0.199**	**1.387**

Preliminary Conclusion – not too bad
Can this be improved using ICP-QQQ?

Fast – the entire separation was complete in under 12 min, and sample to sample is about 14 min.

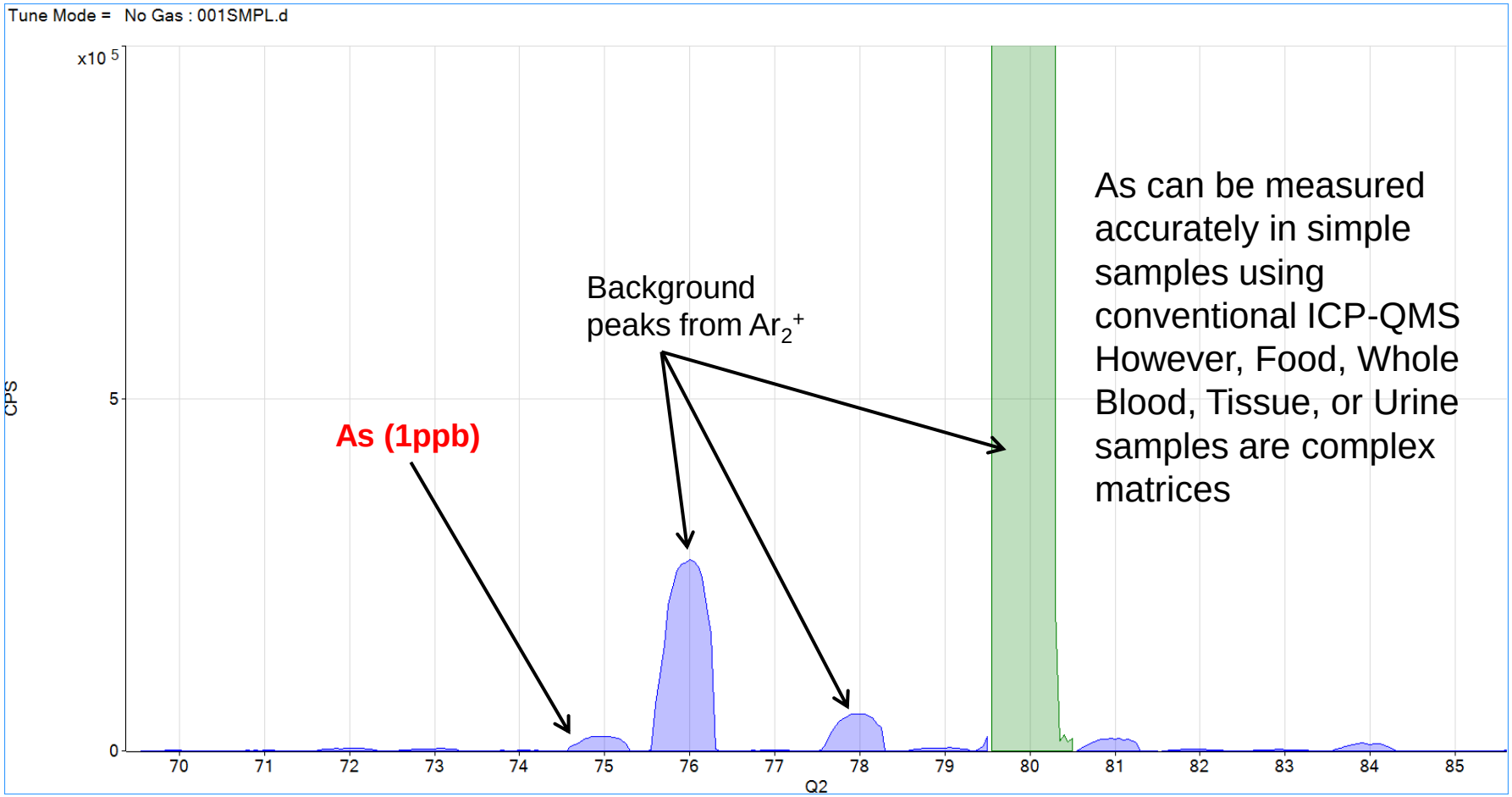
linearity – the MS detector shows excellent linearity across the analytical range

Specificity – the common non-toxic arsenic did not overlap with the species desired to be determined and the interfering peak from ArCl is also chromatographically separated.

Simplicity – the existing method used for As was modified to be tolerant of the changed matrix (apple juice) and was seen to be a very flexoble method.

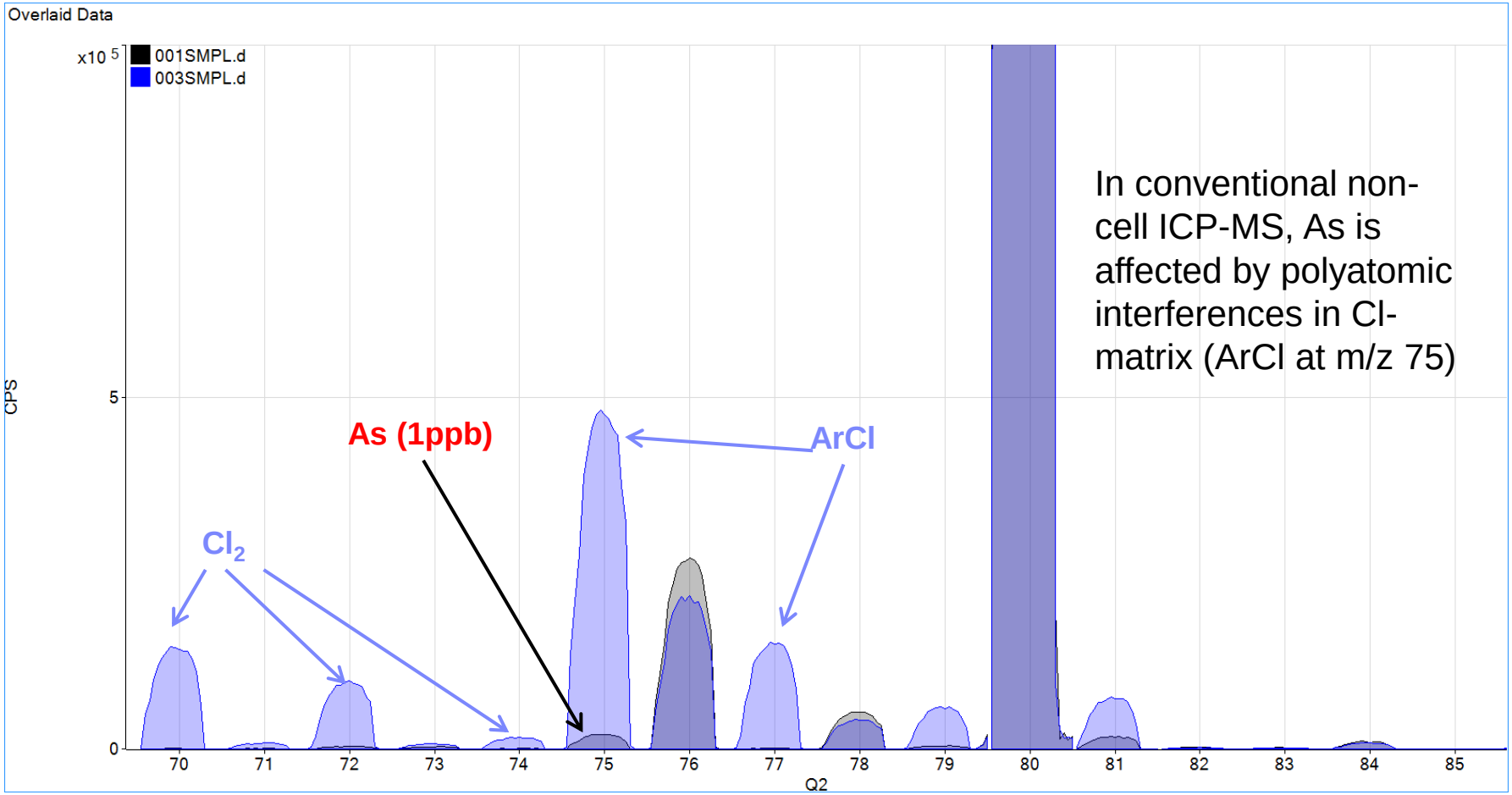
Reminder Why >90% of Quadrupole ICP-MS Have Collision/Reaction Cell – Interference Removal

Arsenic (mass 75) 1ppb Standard – <u>No Gas Mode</u>



As Measured at m/z 75 in Cl-Matrix is Overlapped by ArCl Polyatomic Interference

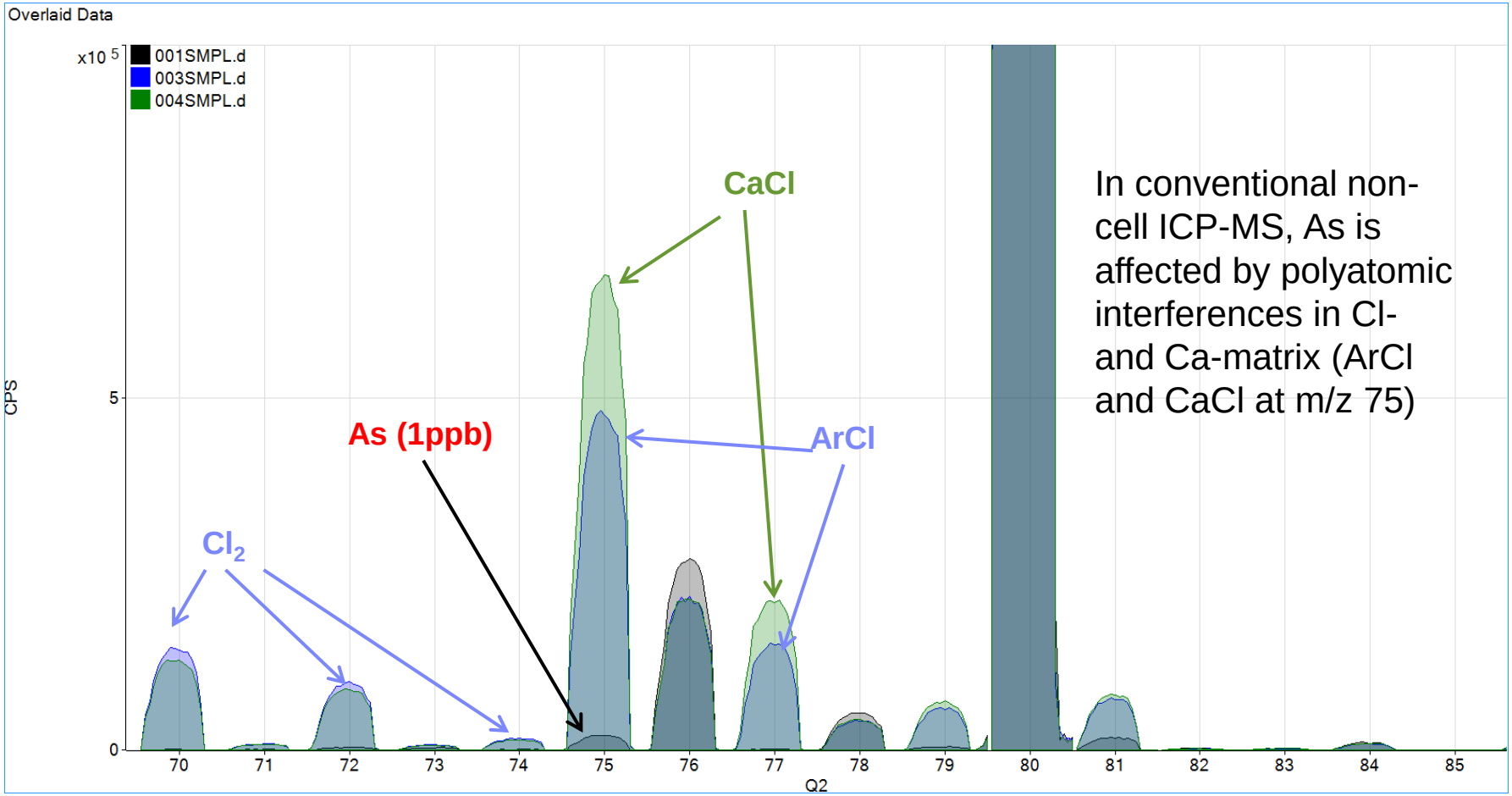
1ppb As in 5% HCl



In conventional non-cell ICP-MS, As is affected by polyatomic interferences in Cl-matrix (ArCl at m/z 75)

As Measured at m/z 75 in Cl & Ca-Matrix is Overlapped by ArCl/CaCl Polyatomic Interferences

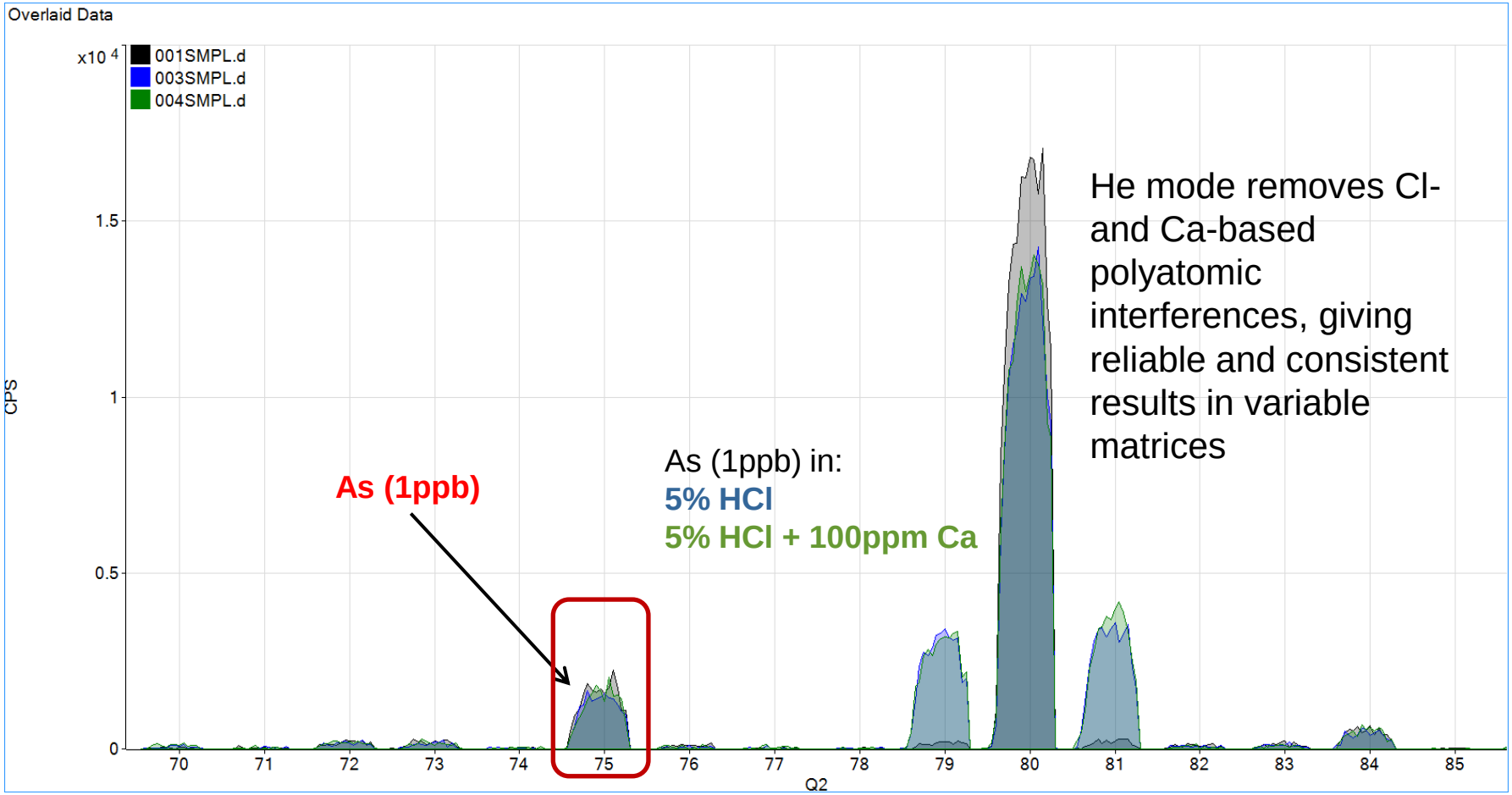
1ppb As in 5% HCl, 1ppb As in 5% HCl + 100ppm Ca



In conventional non-cell ICP-MS, As is affected by polyatomic interferences in Cl- and Ca-matrix (ArCl and CaCl at m/z 75)

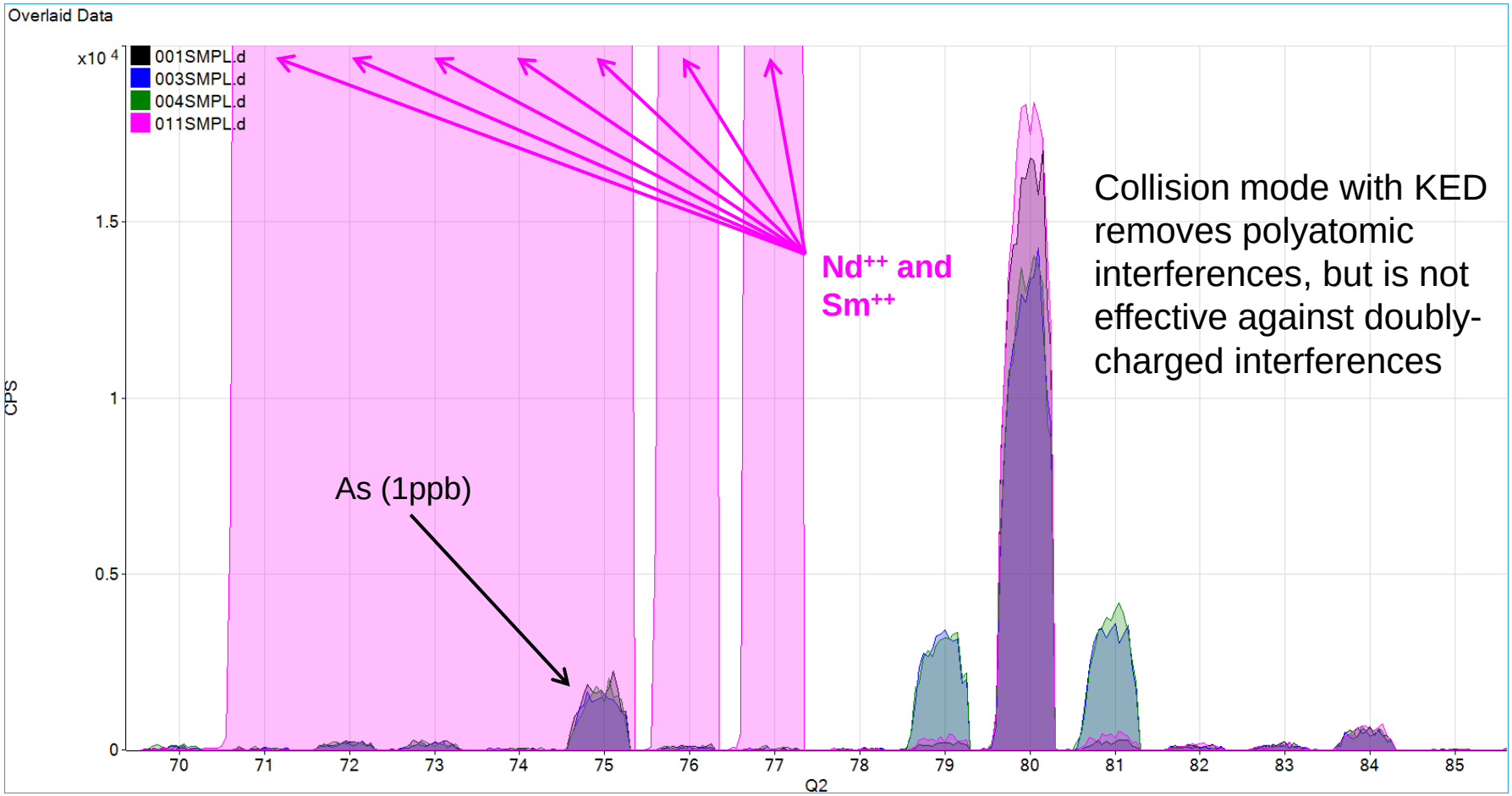
As Measured at m/z 75 in Ca/Cl-Matrix – He Mode Removes ArCl/CaCl Polyatomic Interferences

1ppb As in 5% HCl, 1ppb As in 5% HCl + 100ppm Ca – *all interferences removed – Problem solved…*



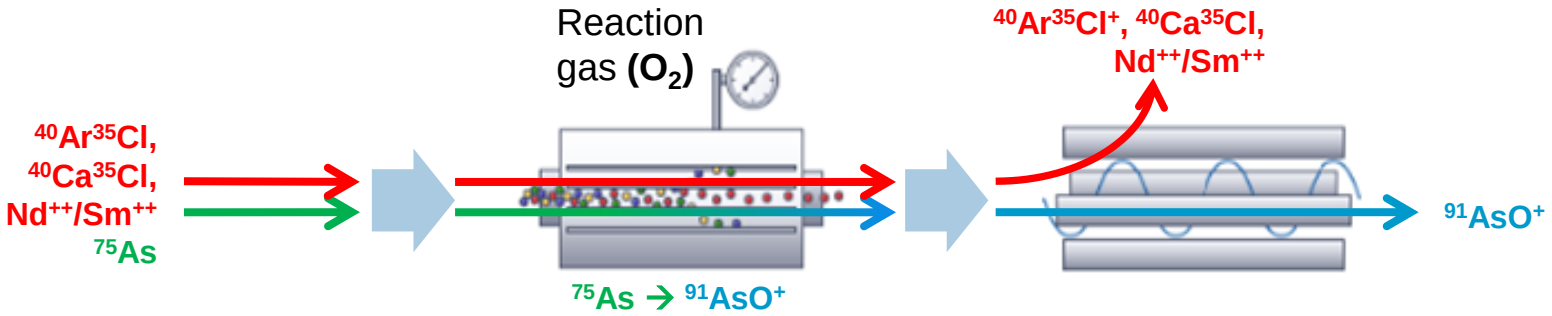
However,… He Mode Can't Remove ALL Possible Interferences

1ppb As + 1ppm Nd/Sm in He Mode – Nd & Sm form 2+ ions. Isotopes at mass 150 appear at 75 (half true mass) 2+ interferences not removed



Conventional ICP-QMS Can Use Reaction Mode to Avoid 2+ Interferences on ^{75}As

$^{75}As^{+} + O_2$ <cell gas> → $^{91}AsO^{+}$
$^{40}Ar^{35}Cl^{+}$, $^{40}Ca^{35}Cl^{+}$, Sm^{++}, $Nd^{++} + O_2$ → no (or very slow) reaction



Analyte and interfering ions enter reaction cell

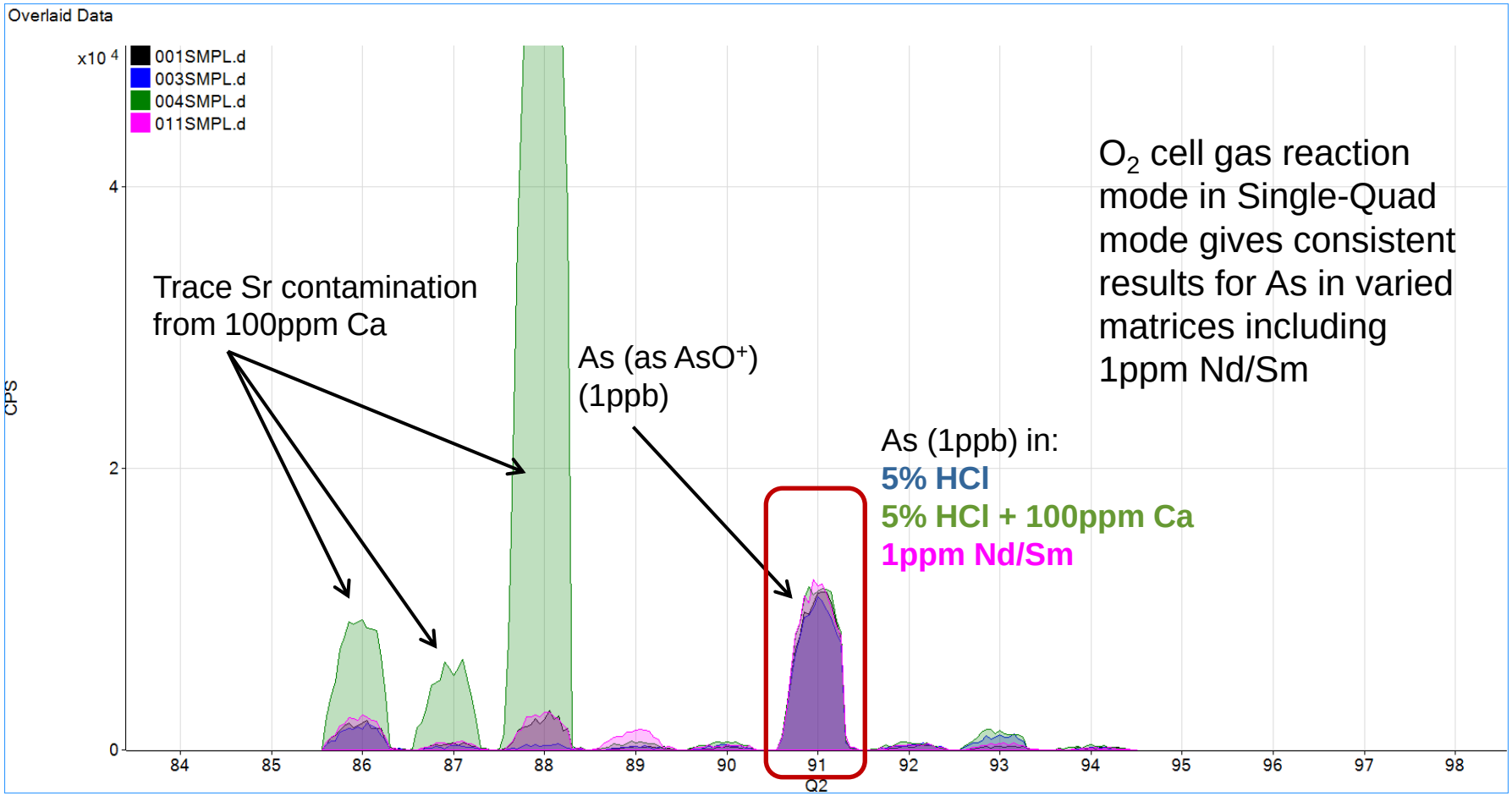
As^{+} reacts with O_2 cell gas to form AsO^{+} product ion

Quad set to AsO^{+} product ion mass (m/z 91) – rejects original $^{40}Ar^{35}Cl^{+}$, $^{40}Ar^{35}Cl^{+}$, Nd^{++}/Sm^{++} interfering ions

O_2 Reaction Mode on Conventional ICP-QMS - (8800 in single quad mode)

As^+ is converted to AsO^+ and moved to mass 91 – avoids interferences

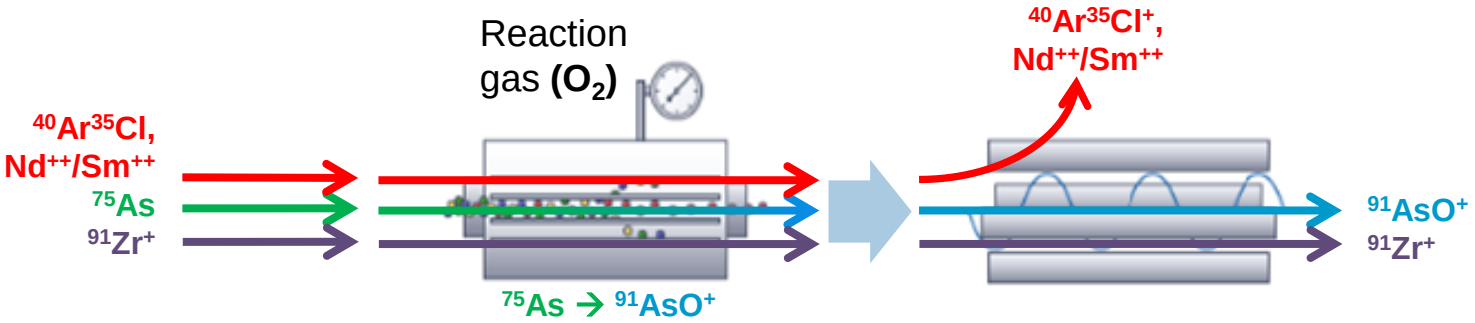
1ppb As, + 5% HCl + 100ppm Ca, + 1ppm Nd/Sm



So ICP-QMS Reaction Mode Solves the Problem?

Not Quite!

ICP-QMS with O_2 cell gas avoids $^{40}Ar^{35}Cl^+$ $^{40}Ca^{35}Cl^+$ and Nd^{++}/Sm^{++} overlap on $^{75}As^+$, but AsO^+ product ion at m/z 91 can be overlapped



Analyte and interfering ions enter reaction cell

As^+ reacts with O_2 cell gas to form AsO^+ product ion. Not all Zr^+ reacts to form ZrO^+ (some Zr^+ remains)

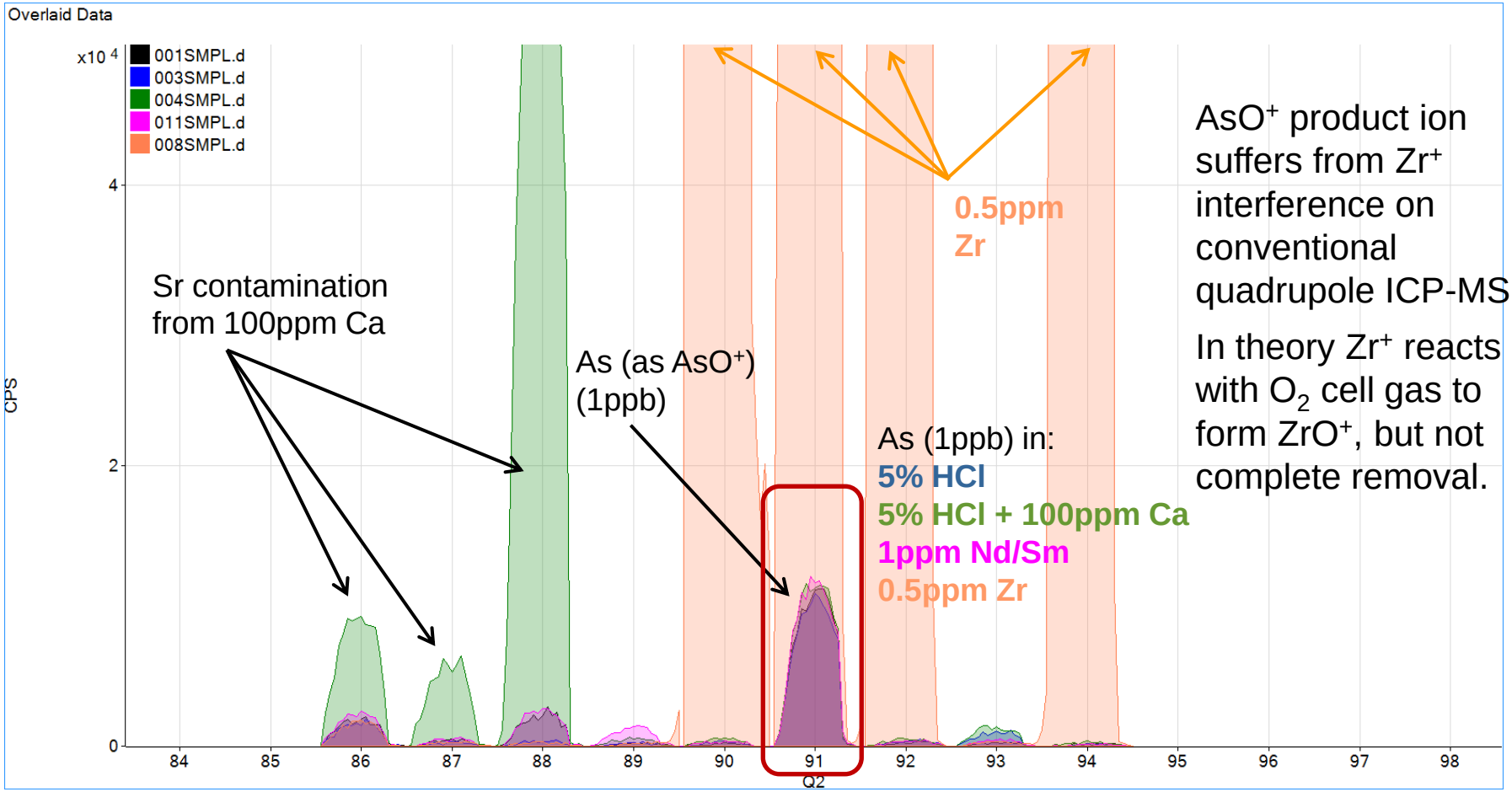
Quad set to AsO^+ product ion mass (m/z 91) – rejects original $^{40}Ar^{35}Cl^+$, $^{40}Ar^{35}Cl^+$, Nd^{++}/Sm^{++} interfering ions but cannot remove $^{91}Zr^+$

Conventional Quadrupole ICP-MS cannot reject on-mass interferences that overlap cell-formed analyte reaction product ions

Product Ion Overlaps with Conventional ICP-QMS O_2 Mass-Shift Mode

As^+ is converted to AsO^+ and measured at mass 91 – overlap from $^{91}Zr^+$

1ppb As, + 5% HCl, + 5% HCl + 100ppm Ca, + 1ppm Nd/Sm, +0.5ppm Zr



AsO^+ product ion suffers from Zr^+ interference on conventional quadrupole ICP-MS.

In theory Zr^+ reacts with O_2 cell gas to form ZrO^+, but not complete removal.

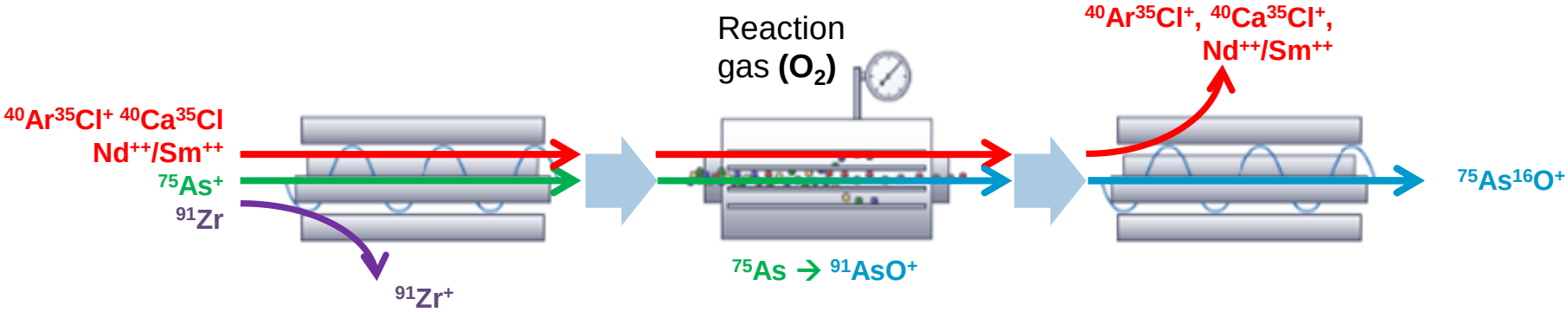
The solution: ICP-MS/MS Mass-Shift with O_2 Cell Gas

Same reaction with O_2 cell gas is also used on 8800 ICP-MS/MS:

$^{75}As^+ + O_2$ <cell gas> → $^{91}AsO^+$

$^{40}Ar^{35}Cl^+$, $^{40}Ca^{35}Cl^+$, Sm^{++}, $Nd^{++} + O_2$ → no reaction

<u>BUT</u> Q1 of 8800 rejects $^{91}Zr^+$ ions that would overlap AsO^+ at mass 91



Q1 set to m/z 75, so rejects all ions except m/z 75. ^{91}Zr at mass 91 is rejected

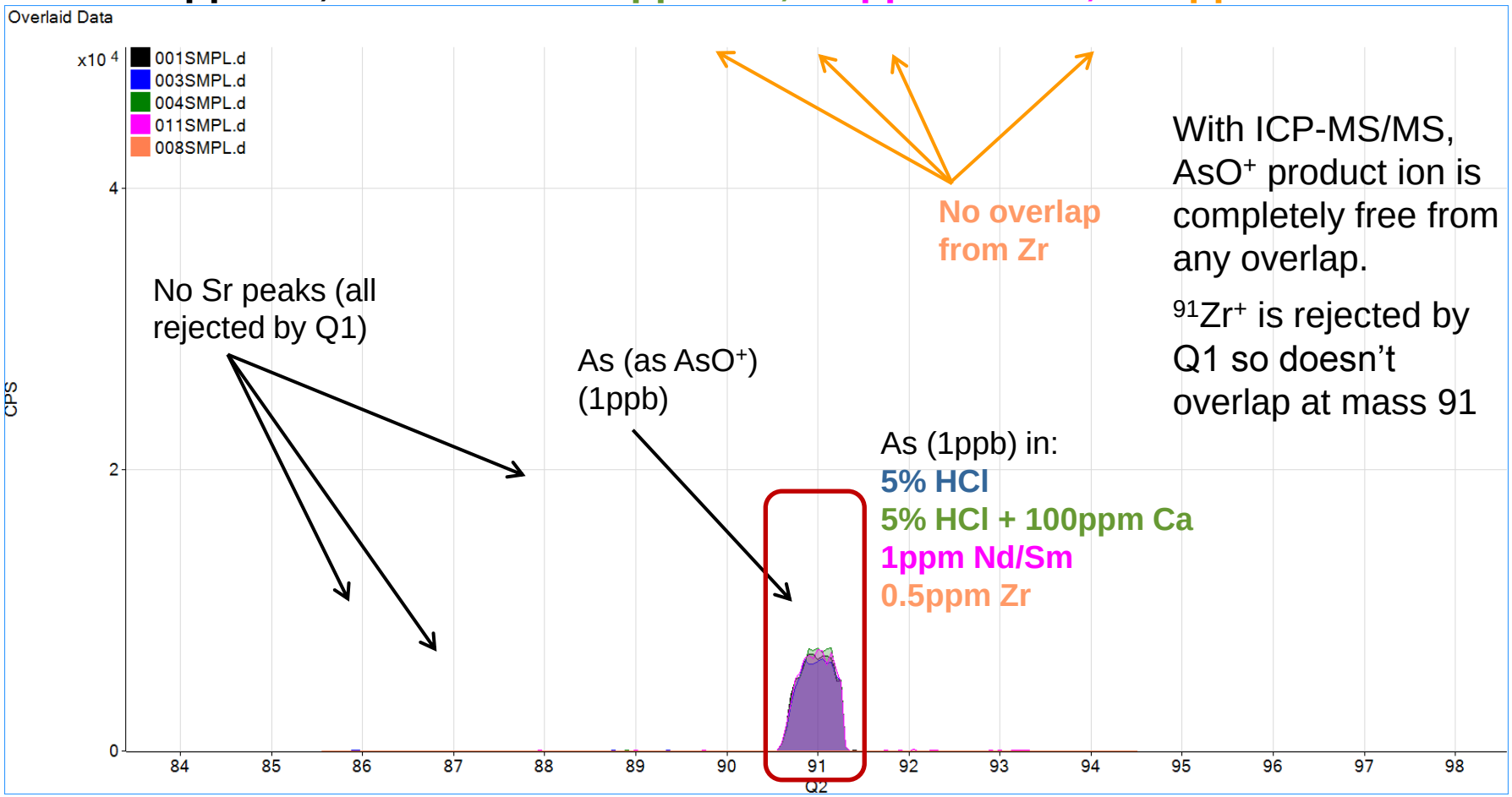
As^+ reacts with O_2 cell gas to form AsO^+ product ion. $^{40}Ar^{35}Cl^+$, $^{40}Ca^{35}Cl^+$, Nd^{++}/Sm^{++} don't react and stay at m/z 75

Q2 set to m/z 91, AsO^+ product ion mass – rejects original on-mass interferences

ICP-MS/MS O_2 Mass-Shift Mode for As – Consistent Results in Any Matrix

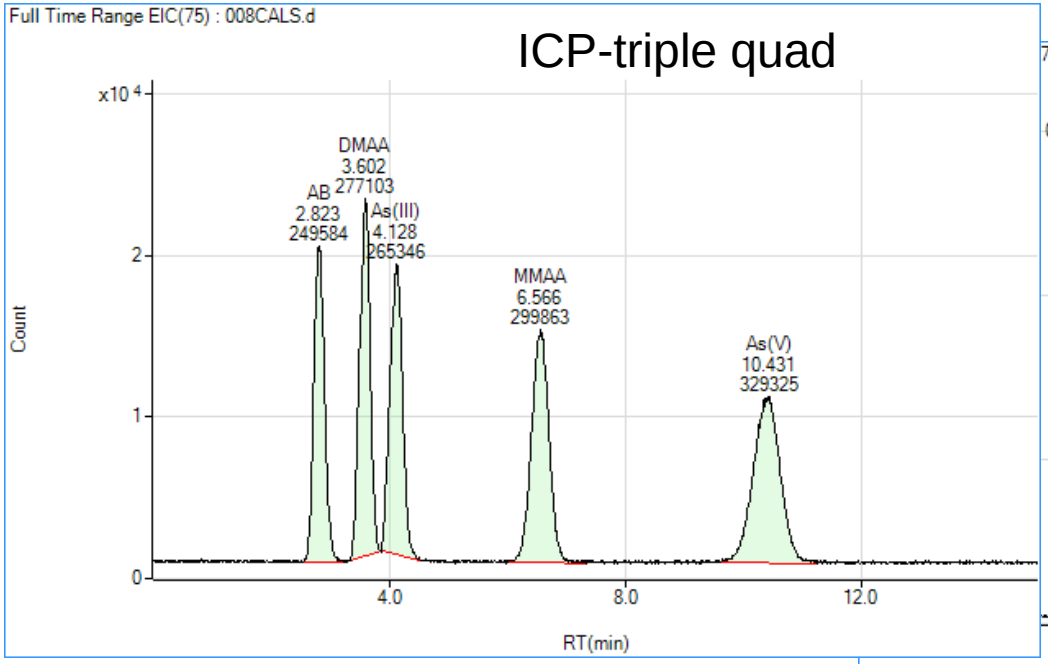
Same matrices as before – ALL overlaps are removed by MS/MS

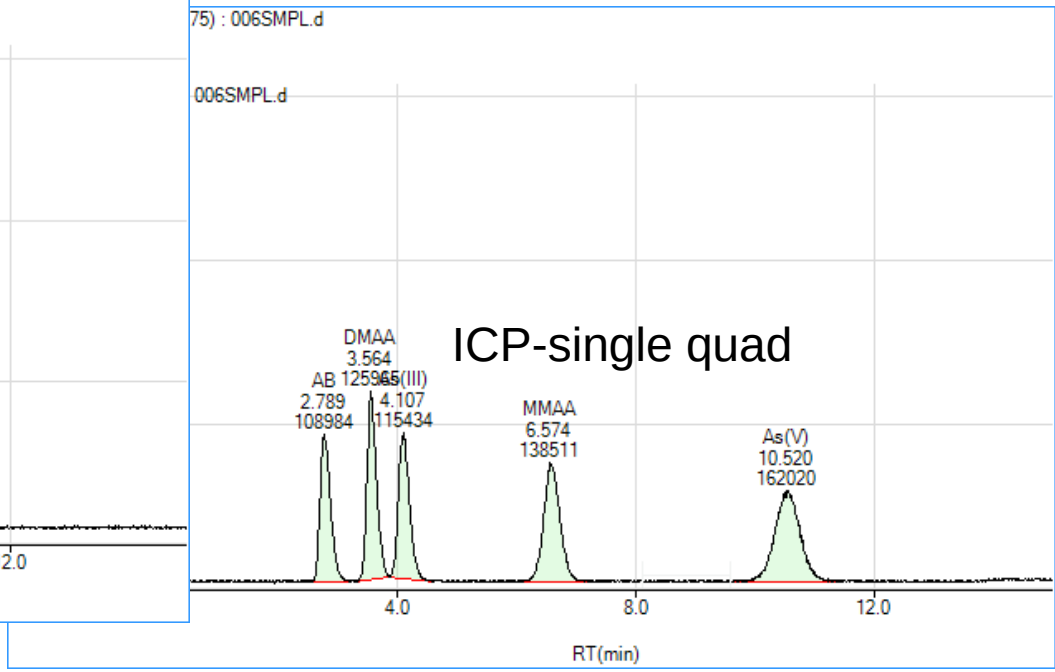
1ppb As, + 5% HCl + 100ppm Ca, + 1ppm Nd/Sm, +0.5ppm Zr



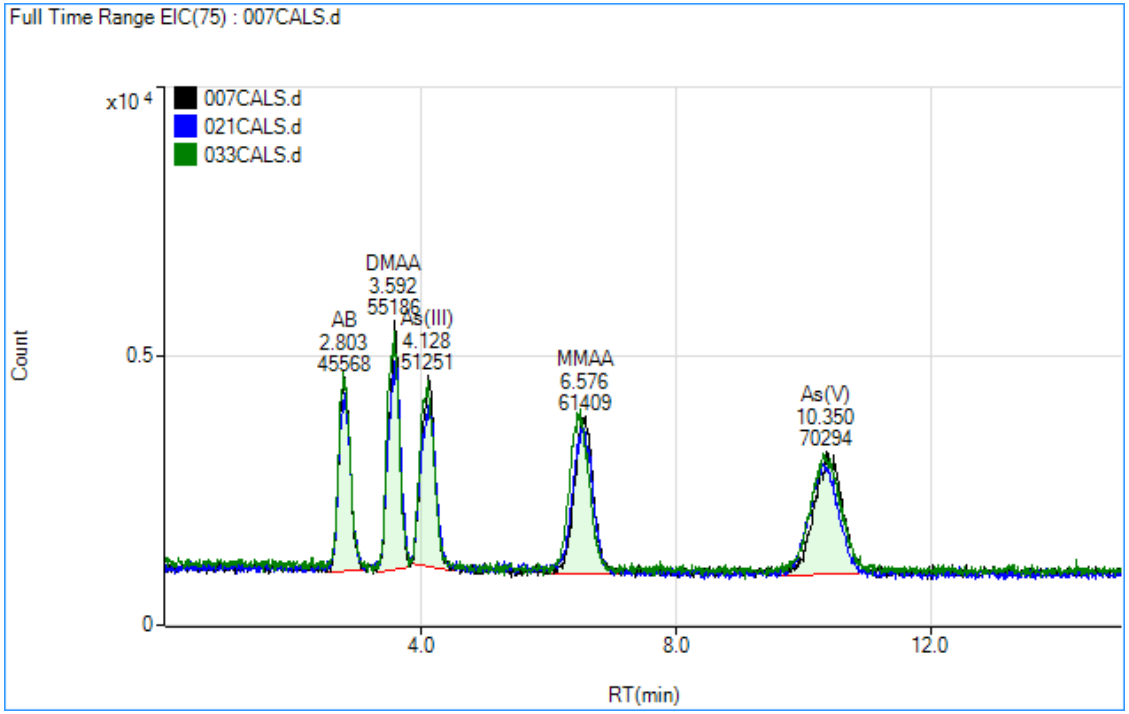
As Species Sensitivity with 8800 ICP-QQQ

Comparison of 0.5ppb As species standard with 8800 (left) and 7700x (right) –same intensity scale. Sensitivity ~ 2x higher with 8800 – significant improvement in S/N





8800 ICP-QQQ – As Species at Low-Levels



Overlaid chromatograms and stability data (n=3) for 0.1ppb standard repeated start, middle and end of sequence (7 hours total)

RT %RSD: 0.2% to 0.6%

Conc %RSD: 2.5% to 4%

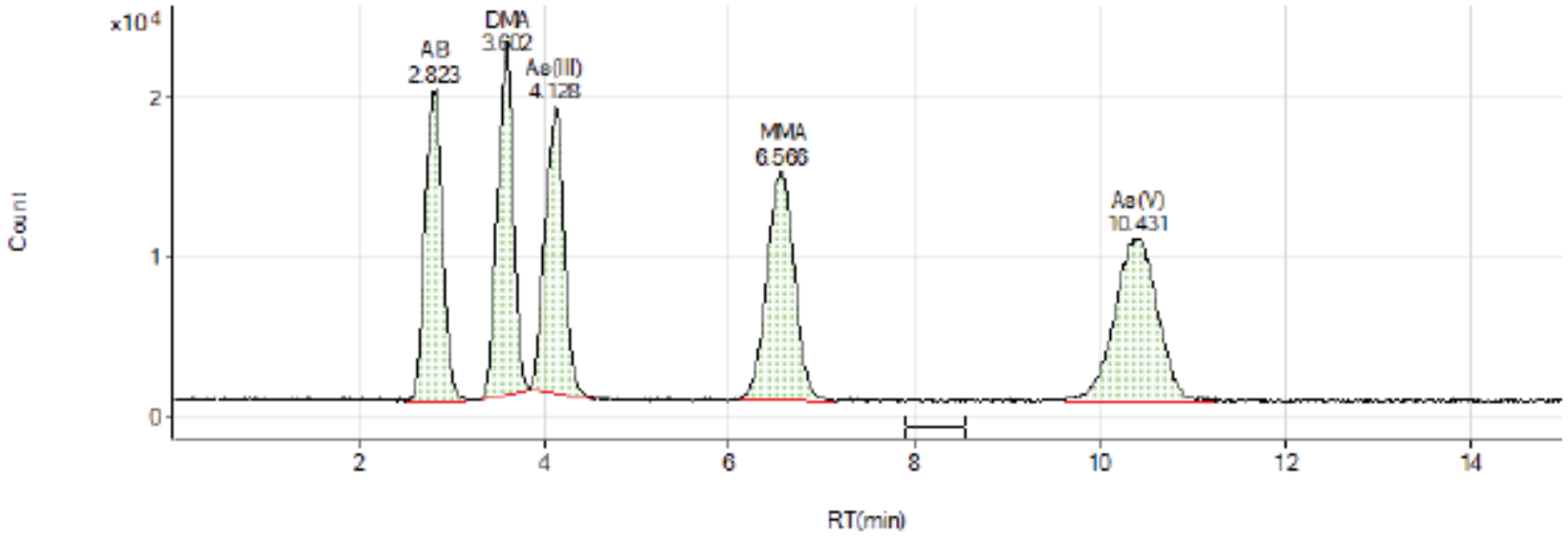
	75 AB			75 DMAA			75 As(III)			75 MMAA			75 As(V)		
	RT	Conc. [ug/l]	Area	RT	Conc. [ug/l]	Area	RT	Conc. [ug/l]	Area	RT	Conc. [ug/l]	Area	RT	Conc. [ug/l]	Area
0.1ppb	2.803	0.097	45568	3.592	0.098	55186	4.128	0.096	51251	6.576	0.100	61409	10.350	0.103	70294
0.1ppb	2.813	0.093	43686	3.602	0.093	52340	4.118	0.090	47972	6.546	0.098	59949	10.299	0.101	69028
0.1ppb	2.782	0.100	47263	3.592	0.100	56301	4.108	0.097	51792	6.495	0.105	64045	10.309	0.106	72305
%RSD	0.57	3.63	3.93	0.16	3.72	3.74	0.24	4.01	4.11	0.63	3.57	3.36	0.26	2.44	2.34

As Speciation in Apple Juice by ICP-QQQ
LOD ~ 2x better, and absolutely no interferences

Table 2. 3x S/N detection limits for arsenobetaine (AB), dimethylarsinic acid (DMA), As(III) (arsenite), monomethylarsonic acid (MMA), and As(V) (arsenate).
* Arsenobetaine (AB) elutes in the void volume and cannot be reliably quantified in the presence of some other co-eluting species.

Compound	RT (min)	Height	Area	Noise	S/N	LOD (ng/L)	Noise type
AB*	2.823	19584	249584	153	127.99	11.72	Peak-to-peak
DMA	3.602	22117	277103	153	144.54	10.38	Peak-to-peak
As(III)	4.128	18022	265346	153	117.78	12.74	Peak-to-peak
MMA	6.566	14421	299863	153	94.24	15.92	Peak-to-peak
As(V)	10.431	10265	329325	153	67.08	22.36	Peak-to-peak

Full Time Range EIC(75) : 008CALS.d



Conclusions

- LC-ICP-QMS is a simple, robust, sensitive technique for determining toxic arsenic species in foods and beverages
- Single quad ICP-MS using either He mode or O_2 reaction mode can be used effectively, but some risk of residual interferences remains
- LC-ICP-QQQ is just as simple and reliable as LC-ICP-QMS but has the added advantage of even lower LOD, and higher confidence that no unknown interferences are present.

Thank you

Any questions?