PerkinElmer®
For the Better

As Speciation with HPLC-ICP-MS by Isocratic Method

JM Park, SK Ma
Inorganic Product Specialists
PerkinElmer Korea

25cm Anion Exchange Column

PerkinElmer®
For the Better

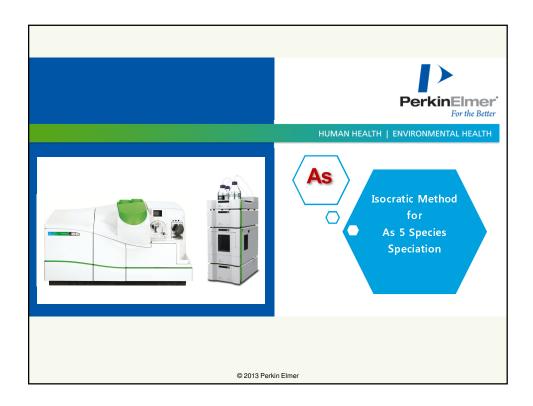| Mobile Phase Conditions	
Mobile Phase A	12mM NH4CO3
Mobile Phase B	20mM (NH4)2HPO4
HPLC Pump Conditions	
Separation Scheme	Gradient
Pump Program	- Equal time: 7 min / A 100% - Run 1: 1 min / A 100% / Curve 0 - Run 2: 7 min / B 100%
Pump Flow	2.0 mL/min
Injection Volume	50 microliter
ICP-MS Conditions	
Sample Introduction System	- Quartz Cyclonic Spray Chamber - Glass Concentric Nebulizer - 2.0 mm Quartz Injector
Analyze Mode	AsO 91 / DRC Mode
DRC Conditions	- O2 gas flow: 0.5ml/min - RPq: 0.45
Dwell Time	500ms

25cm Anion Exchange Column

PerkinElmer
For the Better

Calibration Test (1, 5, 10 ppb)

1ppb: AsO 91: 1   5ppb: AsO 91: 1   10ppb: AsO 91: 1

Intensity (cps)

100K
80K
60K
40K
20K
0

AsB
As3+
DMA
MMA
As5+

0   1   2   3   4   5   6   7   8
Time (min)

Analysis time = 15 min/injection

3

25cm Anion Exchange Column

PerkinElmer
For the Better

Calibration Test (1, 5, 10 ppb)

Component Name — AsB   AsO 91: AsB
Component Name — As3+   AsO 91: As3+
Component Name — DMA   AsO 91: DMA
Component Name — MMA   AsO 91: MMA
Component Name — As5+   AsO 91: As5+

Response(Counts) vs Amount(ppb)

Species	AsB	As3+	DMA	MMA	As5+
Slope/ppb	20819	20198	27356	26967	24754
r²	1.00000	0.99980	0.99965	0.99997	0.99999

4

25cm Anion Exchange Column

Results: NIST 1568a Rice Flour & Unknown Sample

Sample	AsB	As3+	DMA	MMA	As5+	Analysis Result (ppb)	Final Result (ppb)	Recovery (%)
NIST SRM	0.000	1.716	3.097	0.327	0.659	5.798	291.934	101
Unknown	0.000	3.049	0.466	0.157	0.000	3.672	183.580	-

* As in NIST SRM: 0.29 mg/Kg

Conclusion

1. **Very easy procedure of Mobile Phases**

2. 15 Cm, 25 Cm Anion Exchange Column can be used for Speciation

   - 15 Cm Column: PKI Order Made

   - 25 Cm Column: Commercialized Products

➢ Disadvantage #1: Long analysis time (13~15min/injection)

➢ Disadvantage #2: High base line = poorer Detection Limits

Preparing Your Lab.

1. **Instruments: HPLC Speciation Kit, ICP-MS**
2. **Sample Preparation**
   - **Hot Block for 15 mL Tubes or Microwave, Centrifuge, Vortex Mixer**
3. **Bottles**
   - **15 mL PE or PP Tube for Extraction / 2 mL PE or PP Tube for HPLC**
   - **50 mL PP or Teflon Bottle for preparation for Organo-As Stock Standards**
4. **Column**
   - **Shiseido C18 MG II 5um, 250mm 4.6mm id**
5. **Stock Standards**
   - **Liquid Type As 3+ & 5+ (Purchased from ESI or SPEX or etc.)**
   - **Powder type "Arsenobetaine, Disodium methyl arsenate, Dimethylarsenic acid" (Purchased from Sigma Aldrich or etc.)**

Preparing Your Lab. (Chemicals)

PerkinElmer *For the Better*

All chemicals purchased from Sigma-Aldrich

1.1-Octanesulfonic acid sodium salt (~98%) / $C_8H_{17}NaO_3S$ / **CAS: 6324-84-5**

2.Malonic acid, ReagentPlus, 99% / $C_3H_4O_4$ / **CAS: 141-82-2**

3.Ammonium hydroxide, 5.0N in water / **EC: 215-647-6**

4.Analytical grade or Electronic grade Conc. HNO_3 (65%)

** Electronic grade Conc. HNO_3 from Dongwoo-Finechem in Korea
- Density: 1.42
- %: 63~65%
- Mole: 14.2 M

** **10% (v/v) HNO_3 & NH_4OH** for pH adjustment of Mobile Phase

---

Sample Extraction Procedure: HPLC

PerkinElmer *For the Better*

1. Uncooked rice ground into a fine powder (like wheat flour)
2. Prepare the **0.03M** (0.2% (v/v) HNO_3) **solution** ➔ 0.1~1 Litter
3. **0.5 gram Rice Flour** added to a **15mL Tube**
4. **4.5 gram 0.2% HNO_3 solution** added to a 15 mL Tube
5. **Vortex** (mixing) for **10 sec**
6. Heating & Extraction with Hot Block **@ 120 ℃, 4 Hour**
7. Cool down
8. Centrifuge **@ 4,000 rpm, 30 min**
9. Filter with **PTFE Membrane (0.45 um)**
10. **0.1 mL Extracted Solution & 0.9 mL DIW** added to a 2.0 mL **HPLC** Sample Tube
11. **Vortex** (mixing) for **10 sec**

** **Microwave: Extract with Same condition # 6 using Extraction Mode in Microwave**

Sample Digestion Procedure: Total As

1. **Uncooked rice ground into a fine powder (like wheat flour)**
2. **0.5 gram Rice Flour added to a 50 mL Tube**
3. **4 mL Conc. HNO$_3$ & 1 mL Conc. H$_2$O$_2$ added to a 50 mL Tube**
4. **Vortex (mixing) for 10 sec**
5. **Heating with Hot Block @ 120 ℃, 30 min**
6. **Cooling down**
7. **Bring to 50 mL with DIW**

ICP-MS Method & STDs: Total As

1. **Conditions for ICP-MS Method**
   - **- Mass: AsO 91**
   - **- DRC Gas: High purity Oxygen Gas**
   - **- CG / RPq: 0.5 / 0.65**
   - **- Internal Standard Element: Ir 193**

2. **STDs: 0.5, 1.0, 2.5 ppb (10 ppm ICP-MS Stock STD #3)**
3. **Internal STD: 5 ppb Ir (1,000 ppm Single Stock STD)**

Preparation for Analysis (Stock STDs)

1. **As 3+ & 5+ : Liquid Base**
2. **Organo-As: Powder Base / Need dilution with DIW**
3. **Preparation Method: Refer to below Equation**

$$\text{"As atom equivalent" conc. in mg/L} = \frac{\text{concentration of concentrated stock standard in mg/L} \times \text{\# As atoms per species molecule}}{\text{F.W. of As species}} \times 74.92 \text{ atomic wt. As}$$

Arsenic Species	Formula Weight	Number of Arsenic Atoms per Molecule	Formula
Arsenobetaine	178.06	1	$(CH_3)_3AsCH_2COOH$
Arsenocholine bromide	244.99	1	$(CH_3)_3AsCH_2CH_2OH \cdot Br$
Disodium methyl arsenate	291.9	1	$CH_3AsO_3 \cdot 6H_2O$
Trimethylarsine oxide	136.03	1	$(CH_3)_3AsO$
Dimethylarsinic acid	138.01	1	$(CH_3)_2As(OH)_2$
Arsenic (V) oxide hydrate	229.84	2	$As_2O_5 \cdot xH_2O$
Arsenic (III) oxide	197.84	2	As_2O_3

13

Preparation for Analysis (Stock STDs)

3. **For example, prepare the 1,000 ppm As from AsB (50 mL)**

   1,000 ppm = (? mg x 1 x 74.92) / 178.06 x 0.05 L

   ? = 118.833 mg (0.1188 g)

   So, 0.1188 gram AsB added to a 50 mL Teflon Bottle, followed by DIW to 50 gram ➜ Finally, 1,000 ppm As from AsB Stock Solution

   ** Other Organo-As: Same procedure with AsB

4. **Mixed Stock Standard solution for Analysis: 10 ppm recommended**
   Dilute Solution: 0.2% HNO₃
   Expiration Date: 1 month

5. **Calibration Standard Solutions: 0.5, 2.5, 5.0 ppb recommended**
   Dilute Solution: DIW / Daily Preparation recommended

14
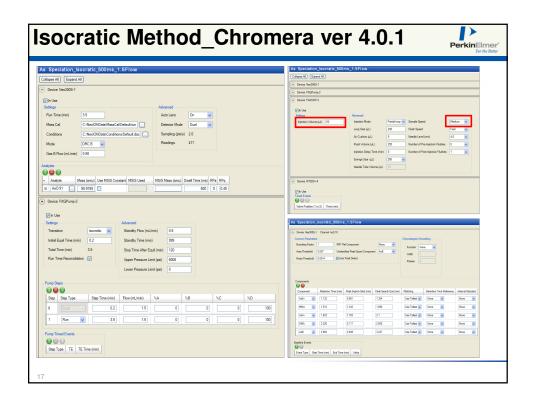
Preparation for Analysis
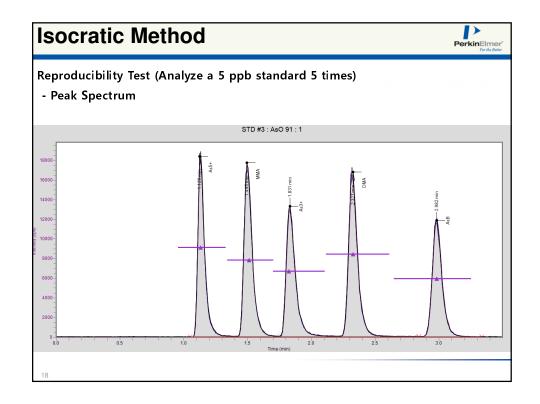
Prepare the Mobile Phase Solutions / **Daily Preparation recommended**
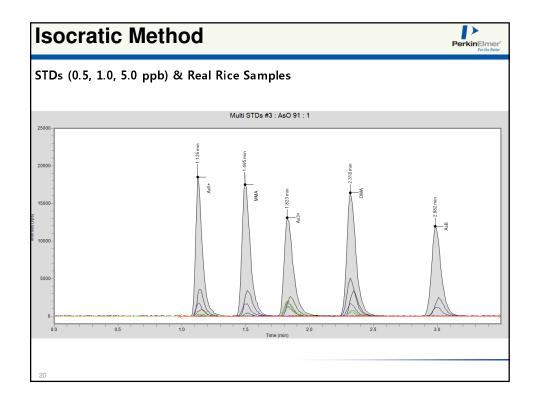
** **pH & Mole** Concentration are **Very Important Spec**

Instrument Parameters
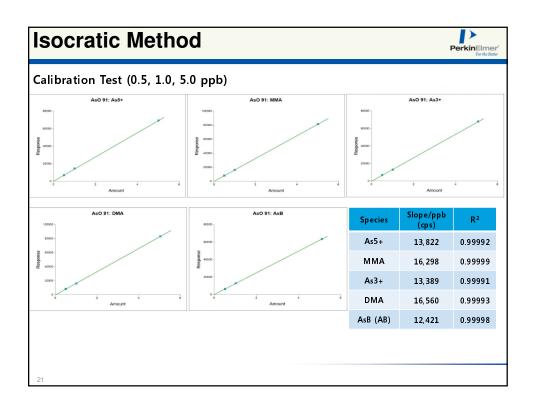
HPLC Pump Conditions	
Separation Scheme	Isocratic
Pump Program	- Equil time: 0.2 min / A 100% - Run: 3.5 min / A 100%
Pump Flow	1.5 mL/min
Injection Volume	50 microliter
ICP-MS Conditions	
Sample Introduction System	- Quartz Cyclonic Spray Chamber - Glass Concentric Nebulizer - 2.0 mm Quartz Injector
Analyze Mode	AsO 91 / DRC Mode
DRC Conditions	- O_2 gas flow: 0.5ml/min - RPq: 0.45
Dwell Time	500ms

Isocratic Method_Chromera ver 4.0.1

Isocratic Method

Reproducibility Test (Analyze a 5 ppb standard 5 times)
 - Peak Spectrum

STD #3 : AsO 91 : 1

Isocratic Method

Reproducibility Test (Analyze a 5 ppb standard 5 times)
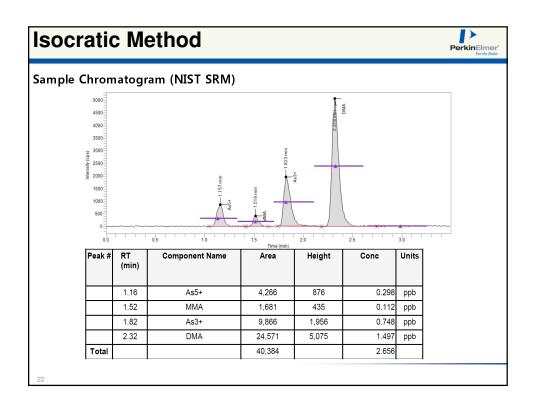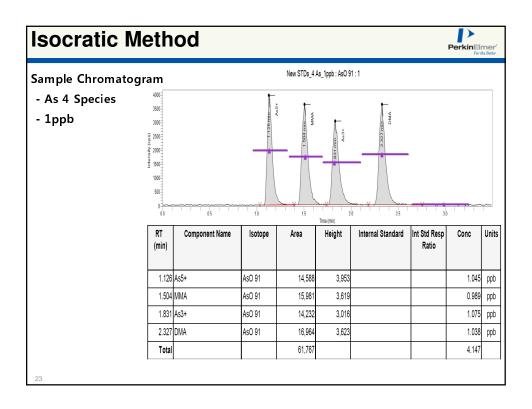
 - **Results**

Species	RT #1	RT #2	RT #3	RT #4	RT #5	AVG	SD	%RSD
As5+	1.126	1.126	1.134	1.134	1.134	1.131	0.004	0.387
MMA	1.495	1.495	1.504	1.495	1.504	1.499	0.005	0.329
As3+	1.831	1.831	1.823	1.831	1.831	1.829	0.004	0.196
DMA	2.318	2.327	2.318	2.327	2.318	2.322	0.005	0.212
AsB	2.982	2.982	2.974	2.982	2.982	2.980	0.004	0.120

Species	Results #1	Results #2	Results #3	Results #4	Results #5	AVG	SD	%RSD	% Recovery
As5+	4.928	4.931	4.899	5.008	4.913	4.936	0.042	0.858	98.716
MMA	4.910	4.924	4.913	4.912	4.916	4.915	0.005	0.111	98.300
As3+	4.974	4.960	4.927	5.010	5.021	4.978	0.038	0.766	99.568
DMA	5.007	4.998	4.960	4.931	4.973	4.974	0.030	0.612	99.476
AsB	5.032	5.075	5.081	5.108	5.100	5.079	0.030	0.583	101.584

Isocratic Method

STDs (0.5, 1.0, 5.0 ppb) & Real Rice Samples

Isocratic Method

Calibration Test (0.5, 1.0, 5.0 ppb)

Species	Slope/ppb (cps)	R²
As5+	13,822	0.99992
MMA	16,298	0.99999
As3+	13,389	0.99991
DMA	16,560	0.99993
AsB (AB)	12,421	0.99998

Isocratic Method

Sample Chromatogram (NIST SRM)

Peak #	RT (min)	Component Name	Area	Height	Conc	Units
	1.16	As5+	4,266	876	0.298	ppb
	1.52	MMA	1,681	435	0.112	ppb
	1.82	As3+	9,866	1,956	0.748	ppb
	2.32	DMA	24,571	5,075	1.497	ppb
Total			40,384		2.656	

Isocratic Method

Sample Chromatogram
- As 4 Species
- 1ppb

New STDs_4 As_1ppb : AsO 91 : 1

RT (min)	Component Name	Isotope	Area	Height	Internal Standard	Int Std Resp Ratio	Conc	Units
1.126	As5+	AsO 91	14,588	3,953			1.045	ppb
1.504	MMA	AsO 91	15,981	3,619			0.989	ppb
1.831	As3+	AsO 91	14,232	3,016			1.075	ppb
2.327	DMA	AsO 91	16,964	3,623			1.038	ppb
	Total		61,767				4.147	

Final Results !!

Reference Material & Unknown Rice Sample

Results: ppb (ug/Kg)

#	Name	Acid Digestion AsO 91 (ppb)	Speciation AsO 91 (ppb)	Speciation/Digestion Matching Rate(%)	NIST SRM Recovery(%) Acid Digestion	NIST SRM Recovery(%) Speciation
1	NIST SRM	287.36	265.60	92.43	99.09	91.59
2	Cooked Rice #1	68.03	73.40	107.89		
3	Cooked Rice #2	84.66	81.80	96.62		
6	Market Rice #1	106.11	116.80	110.07	NA	NA
5	Market Rice #2	95.46	91.00	95.33		
4	Market Rice #3	95.91	90.10	93.94		

- **NIST SRM:** 1568a Rice Flour (As in SRM: 290 ug/Kg)
- Cooked Rice: Steam Cooked Rice for Instant Food
- **Market Rice #1: Cultivated from North Region of Korea**
- **Market Rice #2: Cultivated from Center Region of Korea**
- **Market Rice #3: Cultivated from South Region of Korea**

Special Attention & Advantage
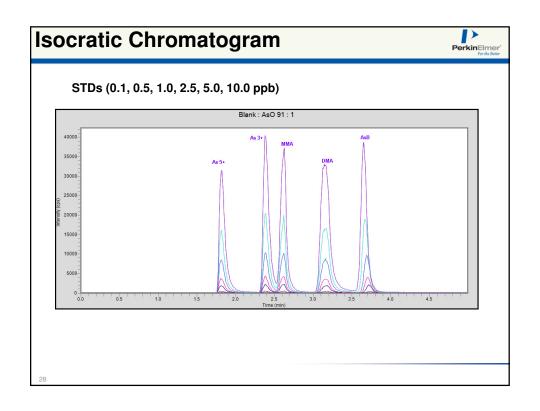
1. **Accurate MP's pH & Mole Conc.** are very important things

2. Final solution base of STDs for calibration is **DIW**

3. Base of Extraction Solution is **0.03M (0.2% (v/v) HNO$_3$) solution** needed

4. Final Sample for Speciation is **10x dilution with DIW** needed

5. If used extraction solution's acidity is higher than recommended condition,

   all RT of peaks were delayed and increase the tailing ➔ **Poor Results**

1. Advantage

   - Very Short Speciation Time: **Totally 3.5 min**

   - Very Low Baseline: **AVG. 10~40 cps**

   - **Very Low Salt in Mobile Phase**
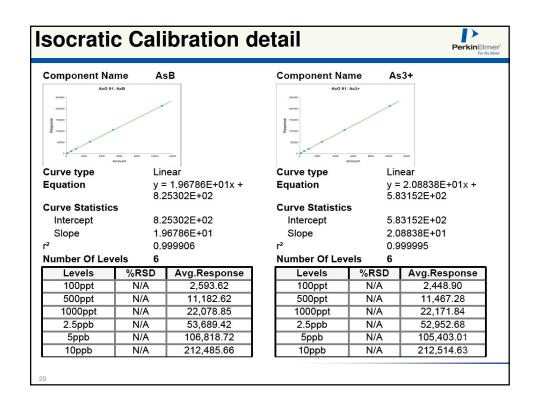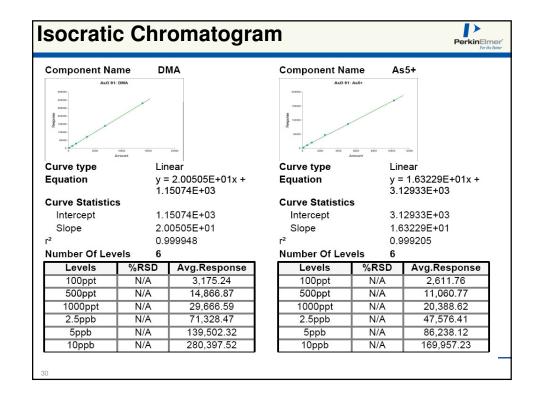
HUMAN HEALTH | ENVIRONMENTAL HEALTH
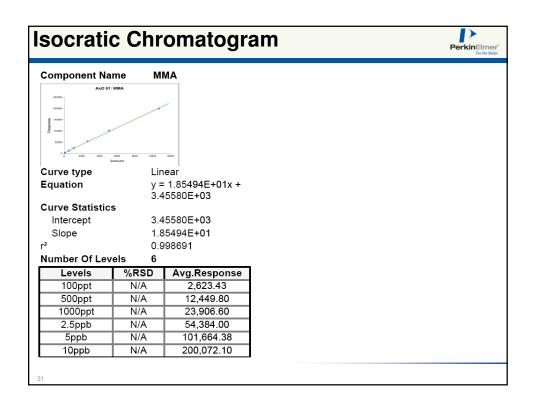
Work on Animal feed
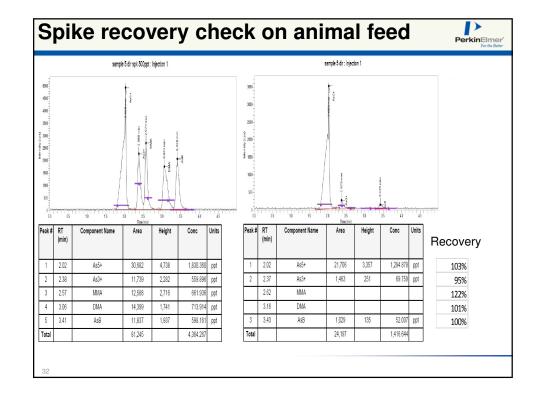
Instrument Parameters

HPLC Pump Conditions	
Separation Scheme	Isocratic
Pump Program	- Equil time: 0.5 min / A 100% - Run: 5 min / A 100%
Column	Brownlee Validated C18 250mm 4.6mm 5 um N9303561
Eluent	10mM Butanesulfonate 4mM Malonic + 4mM TMAH pH at 3 and 0.5% methanol
Pump Flow	1.0 mL/min
Injection Volume	50 microliter
ICP-MS Conditions	
Sample Introduction System	- Quartz Cyclonic Spray Chamber - Glass Concentric Nebulizer - 2.0 mm Quartz Injector
Analyze Mode	AsO 91 / DRC Mode
DRC Conditions	- O_2 gas flow: 0.8ml/min - RPq: 0.45
Dwell Time	500ms

Isocratic Chromatogram

STDs (0.1, 0.5, 1.0, 2.5, 5.0, 10.0 ppb)

Isocratic Calibration detail

Component Name   AsB

Curve type	Linear
Equation	y = 1.96786E+01x + 8.25302E+02
Curve Statistics	
Intercept	8.25302E+02
Slope	1.96786E+01
r²	0.999906
Number Of Levels	6

Levels	%RSD	Avg.Response
100ppt	N/A	2,593.62
500ppt	N/A	11,182.62
1000ppt	N/A	22,078.85
2.5ppb	N/A	53,689.42
5ppb	N/A	106,818.72
10ppb	N/A	212,485.66

Component Name   As3+

Curve type	Linear
Equation	y = 2.08838E+01x + 5.83152E+02
Curve Statistics	
Intercept	5.83152E+02
Slope	2.08838E+01
r²	0.999995
Number Of Levels	6

Levels	%RSD	Avg.Response
100ppt	N/A	2,448.90
500ppt	N/A	11,467.28
1000ppt	N/A	22,171.84
2.5ppb	N/A	52,952.68
5ppb	N/A	105,403.01
10ppb	N/A	212,514.63

Isocratic Chromatogram

Component Name   DMA

Curve type	Linear
Equation	y = 2.00505E+01x + 1.15074E+03
Curve Statistics	
Intercept	1.15074E+03
Slope	2.00505E+01
r²	0.999948
Number Of Levels	6

Levels	%RSD	Avg.Response
100ppt	N/A	3,175.24
500ppt	N/A	14,866.87
1000ppt	N/A	29,666.59
2.5ppb	N/A	71,328.47
5ppb	N/A	139,502.32
10ppb	N/A	280,397.52

Component Name   As5+

Curve type	Linear
Equation	y = 1.63229E+01x + 3.12933E+03
Curve Statistics	
Intercept	3.12933E+03
Slope	1.63229E+01
r²	0.999205
Number Of Levels	6

Levels	%RSD	Avg.Response
100ppt	N/A	2,611.76
500ppt	N/A	11,060.77
1000ppt	N/A	20,388.62
2.5ppb	N/A	47,576.41
5ppb	N/A	86,238.12
10ppb	N/A	169,957.23

Isocratic Chromatogram

Component Name   MMA

AsO 91: MMA

Curve type   Linear
Equation   y = 1.85494E+01x + 3.45580E+03

Curve Statistics
  Intercept   3.45580E+03
  Slope   1.85494E+01
r^2 0.998691
Number Of Levels   6

Levels	%RSD	Avg.Response
100ppt	N/A	2,623.43
500ppt	N/A	12,449.80
1000ppt	N/A	23,906.60
2.5ppb	N/A	54,384.00
5ppb	N/A	101,664.38
10ppb	N/A	200,072.10

31

Spike recovery check on animal feed

sample 5 dir spk 500ppt : Injection 1

sample 5 dir : Injection 1

Peak #	RT (min)	Component Name	Area	Height	Conc	Units
1	2.02	As5+	30,682	4,738	1,830.360	ppt
2	2.38	As3+	11,739	2,282	559.896	ppt
3	2.57	MMA	12,588	2,716	661.936	ppt
4	3.06	DMA	14,399	1,741	713.914	ppt
5	3.41	AsB	11,837	1,937	598.161	ppt
Total			81,245		4,364.267	

Peak #	RT (min)	Component Name	Area	Height	Conc	Units
1	2.02	As5+	21,706	3,357	1,294.878	ppt
2	2.37	As3+	1,463	251	69.759	ppt
	2.62	MMA				
	3.16	DMA				
3	3.43	AsB	1,029	135	52.007	ppt
Total			24,197		1,416.644	

Recovery

103%
95%
122%
101%
100%

32

As speciation in urine

PerkinElmer Taiwan
Inorganic Specialist
Jenny Chen
陳 煜

© 2013 Perkin Elmer

Chromatogram and condition

STD3 : As 75 : 1

HPLC Pump Conditions	
Separation Scheme	Isocratic
Pump Program	- Equil time: 0.5 min / A 100% - Run: 5 min / A 100%
Column	CAPCELL PAK C18 MGII S5 250 x 4.6 mm [Shiseido]
Eluent	10mM Butanesulfonate 4mM Malonic + 4mM TMAH pH at 3 and 0.05% methanol
Pump Flow	1.2 mL/min
Injection Volume	20 microliter

Certified	Total As		Sum of Species	
	Conc	% Recovery	Conc	% Recovery
290	287	99	266	92

NIST SRM: 1568a Rice Flour

Calibration-AsIII and AsV

- **Calibration curve: 0.1, 0.5, 1, 5, 10 ppb**
- **Sample volume: 10 uL**

PerkinElmer
For the Better

Component Name   AsV

As 75: AsV

Curve type	Linear
Equation	y = 1.28773E+03x - 8.11066E+01

Curve Statistics

Intercept	-8.11066E+01
Slope	1.28773E+03
r^2	0.997630
Number Of Levels	5

Levels	%RSD	Avg.Response
STD1	N/A	201.24
STD2	N/A	464.69
STD3	4.237	1,360.68
STD4	2.695	6,040.98
STD5	1.872	12,940.17

Component Name   AsIII

As 75: AsIII

Curve type	Linear
Equation	y = 1.01811E+03x + 8.57067E-01
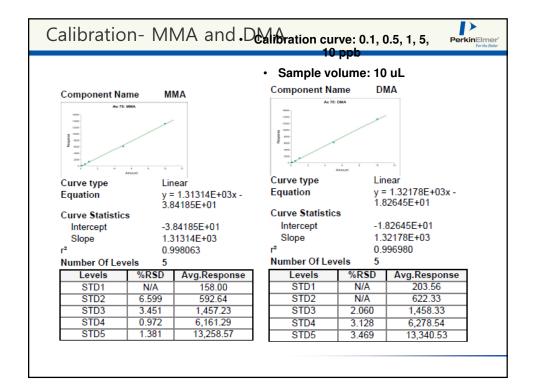
Curve Statistics

Intercept	8.57067E-01
Slope	1.01811E+03
r^2	0.999010
Number Of Levels	5

Levels	%RSD	Avg.Response
STD1	N/A	142.70
STD2	4.401	522.70
STD3	5.263	1,090.15
STD4	0.897	4,912.55
STD5	1.541	10,263.49

Calibration- MMA and DMA

- **Calibration curve: 0.1, 0.5, 1, 5, 10 ppb**
- **Sample volume: 10 uL**

PerkinElmer
For the Better

Component Name   MMA

As 75: MMA

Curve type	Linear
Equation	y = 1.31314E+03x - 3.84185E+01

Curve Statistics

Intercept	-3.84185E+01
Slope	1.31314E+03
r^2	0.998063
Number Of Levels	5

Levels	%RSD	Avg.Response
STD1	N/A	158.00
STD2	6.599	592.64
STD3	3.451	1,457.23
STD4	0.972	6,161.29
STD5	1.381	13,258.57

Component Name   DMA

As 75: DMA

Curve type	Linear
Equation	y = 1.32178E+03x - 1.82645E+01

Curve Statistics

Intercept	-1.82645E+01
Slope	1.32178E+03
r^2	0.996980
Number Of Levels	5

Levels	%RSD	Avg.Response
STD1	N/A	203.56
STD2	N/A	622.33
STD3	2.060	1,458.33
STD4	3.128	6,278.54
STD5	3.469	13,340.53

Calibration- AsB

Component Name   AsB

Curve type	Linear
Equation	y = 1.10913E+03x − 3.17514E+01

Curve Statistics

Intercept	-3.17514E+01
Slope	1.10913E+03
r²	0.996850
Number Of Levels	5

Levels	%RSD	Avg.Response
STD1	N/A	135.74
STD2	0.976	605.62
STD3	N/A	1,084.79
STD4	1.368	5,277.43
STD5	4.400	11,173.01

- Calibration curve: 0.1, 0.5, 1, 5, 10 ppb
- Sample volume: 10 uL

Arsenic speciation – Urine real sample analysis

As species	Urine sample (ppb)	Spike Recovery%
AsV	4.93	98.8 %
AsIII	0.78	107.3 %
MMA	1.56	105.0 %
DMA	5.44	105.3 %
AsB	9.15	100.0%

38

Thank You

3