

Speciation of As and Cr in drinking waters - towards the development of a fully automated routine method

David Verstraeten, KVCV meeting 12 october 2006

- Chromium and arsenic are two naturally occurring elements whose toxicity depends on their chemical form/oxidation state.
- Trivalent chromium (Cr III) is an essential nutrient, while hexavalent chromium (Cr VI) is toxic, not naturally occurring, and results only from anthropogenic activities.
- Arsenic exists in a variety of forms with the trivalent form (As III) being the most toxic, followed by the pentavalent form (As V).

The goals of this work were:

- Simultaneous separation of As and Cr inorganic species in environmental water samples using HPLC-ICP-MS.
- Develop a very rapid separation method (less than five minutes).
- Eliminate potential interferences so that lower levels can be determined.
- Fully automate the process

Automation – Setup

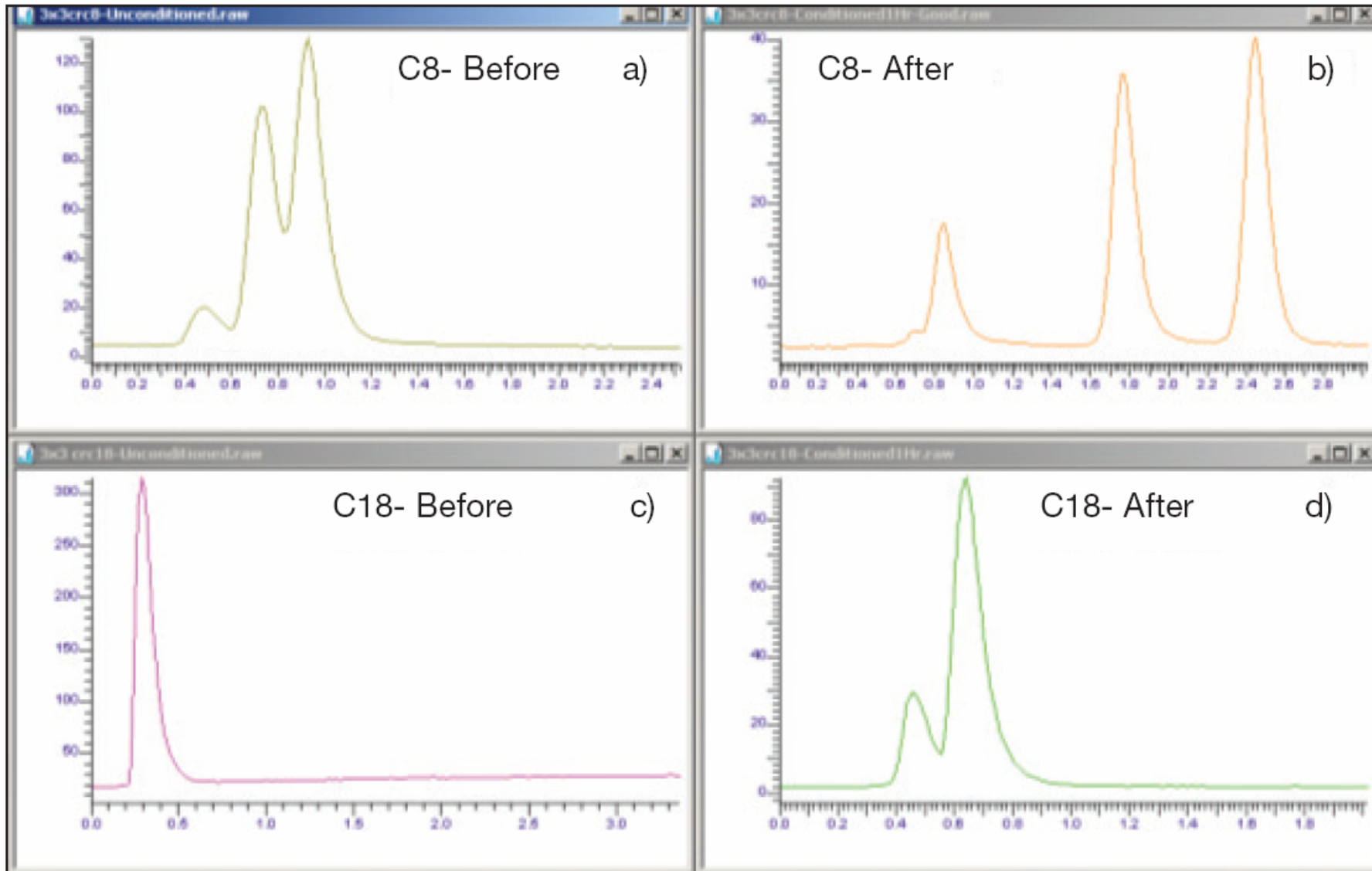


HPLC System	PerkinElmer Series 200 Binary Pump, Auto Sampler and Vacuum Degasser
Column	C8; 3 μm particles; 3 cm (PerkinElmer)
Mobile Phase	1 mM TBAH + 0.5 mM EDTA (potassium salt) + 5% methanol
pH	7.2
pH Adjustment	Dilute HNO_3 , NH_4OH
Injection Volume	50 μL
Flow Rate	1.5 mL/min
Samples	Various waters (non-acidified)
Sample Prep	Dilute with mobile phase let sit 30 min.

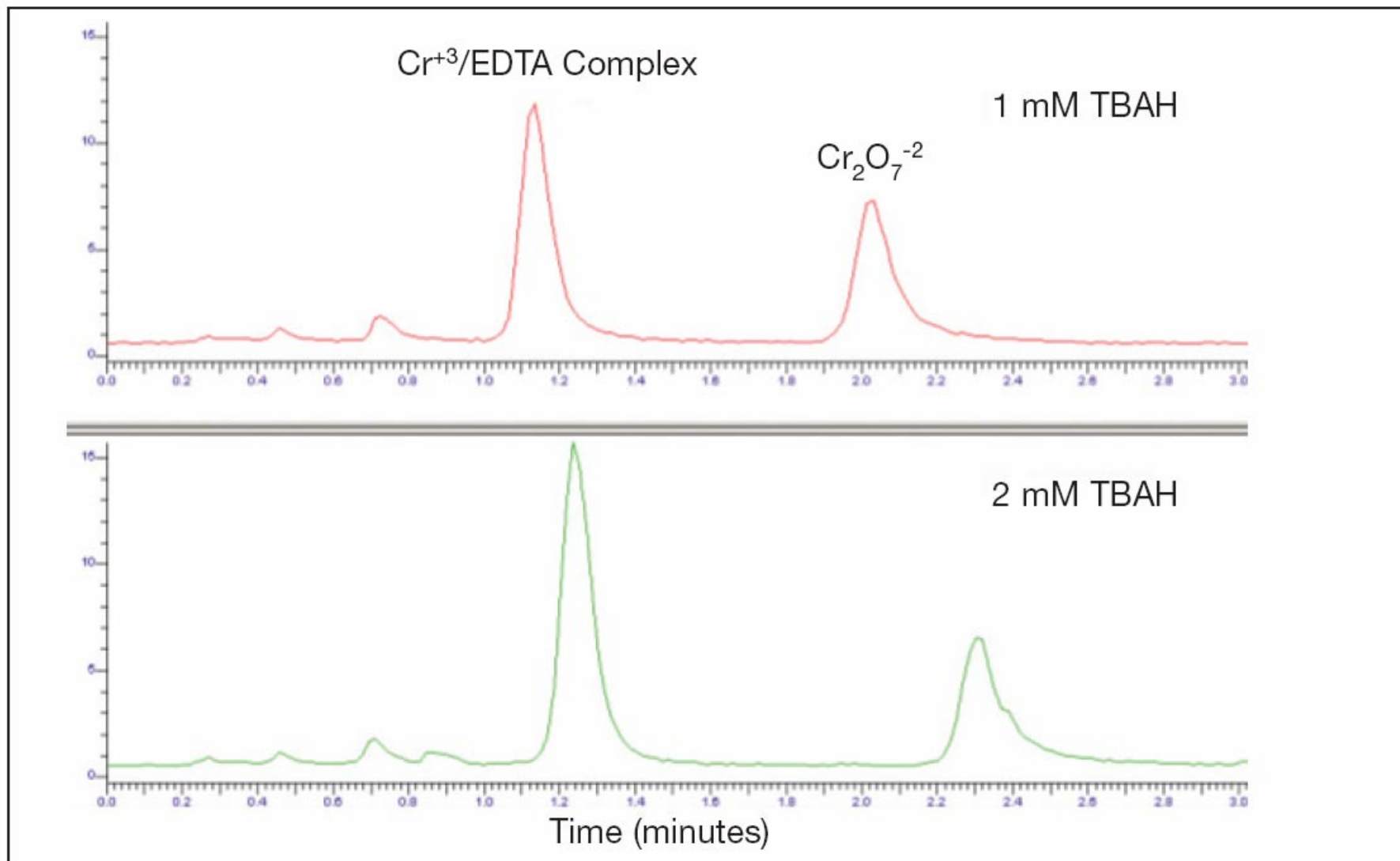
➤ **Choice of chromatographic separation.**

- Ion pair separation on a “reverse-phase” type column (C8 or C18).
 - Lower cost
 - Flexible configuration (particle size, pore size, dimensions,...)

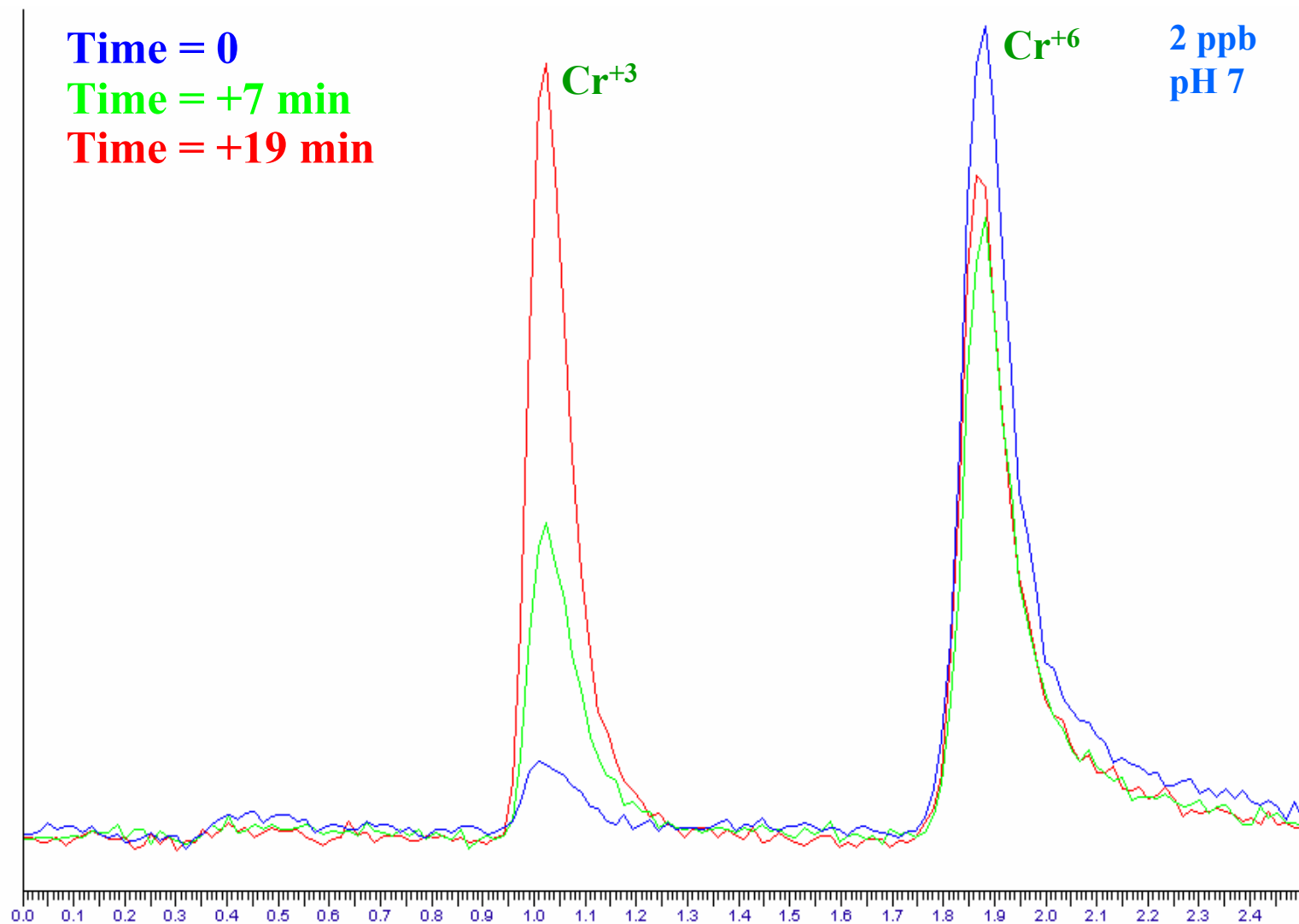
Method Development – Choice of column



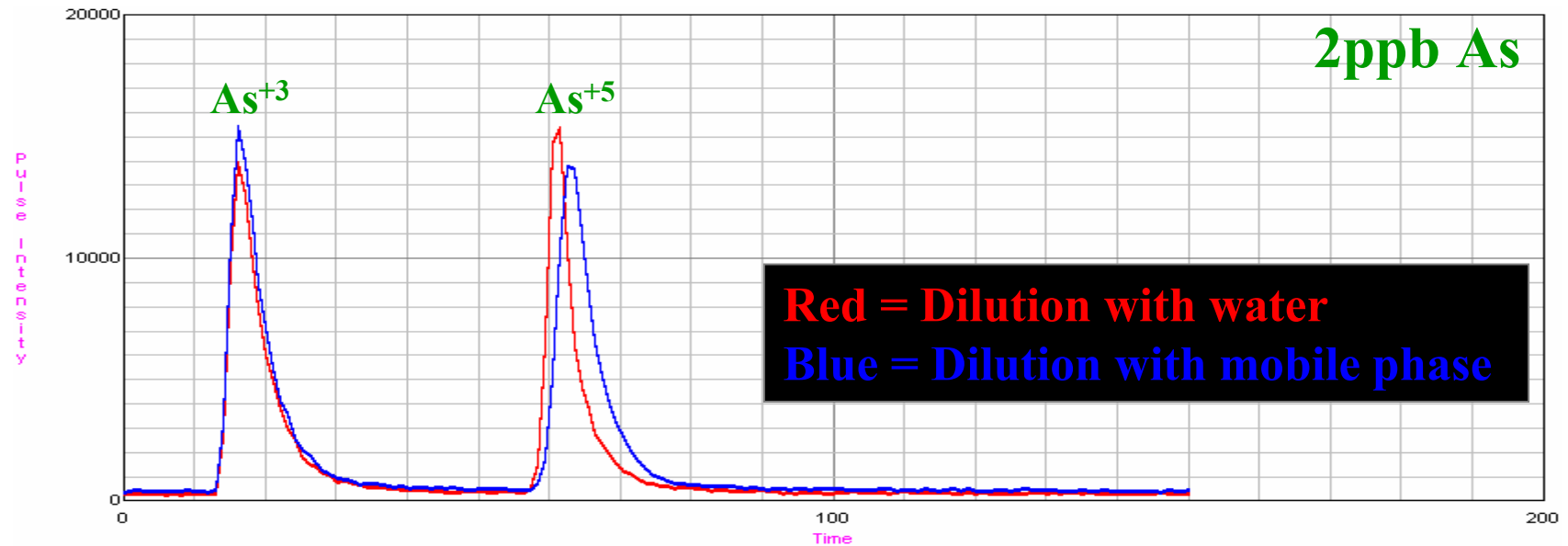
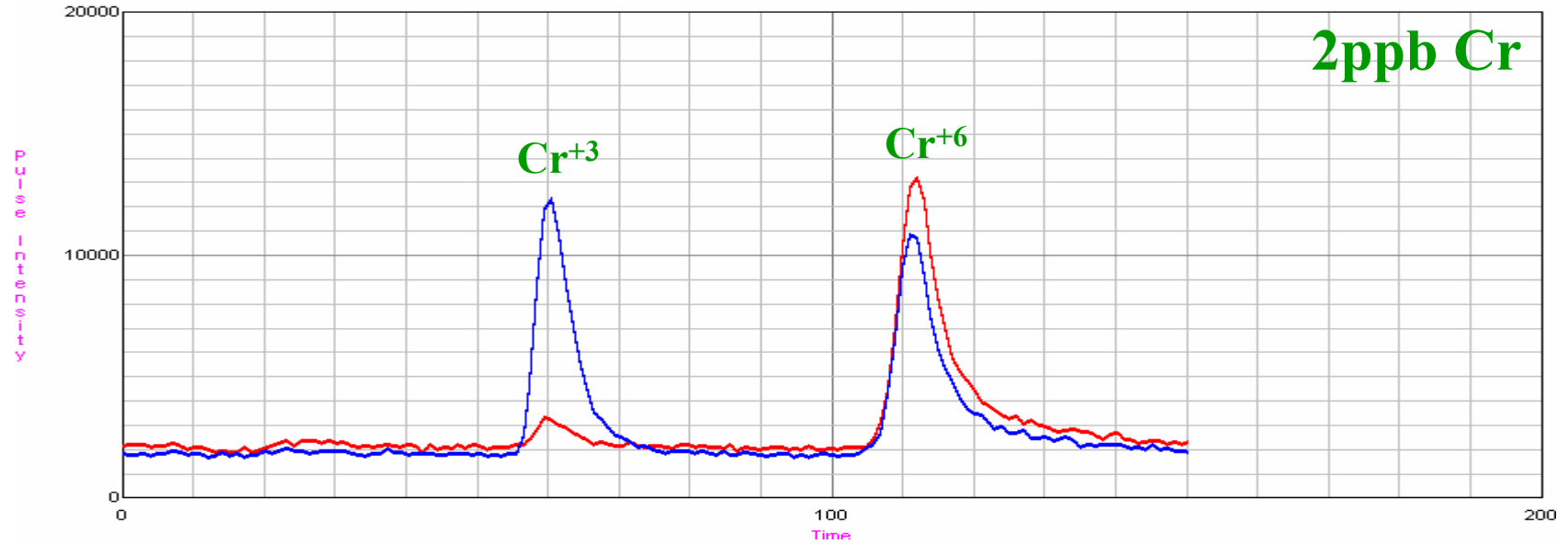
Method Development – Concentration of the ion pair reagent

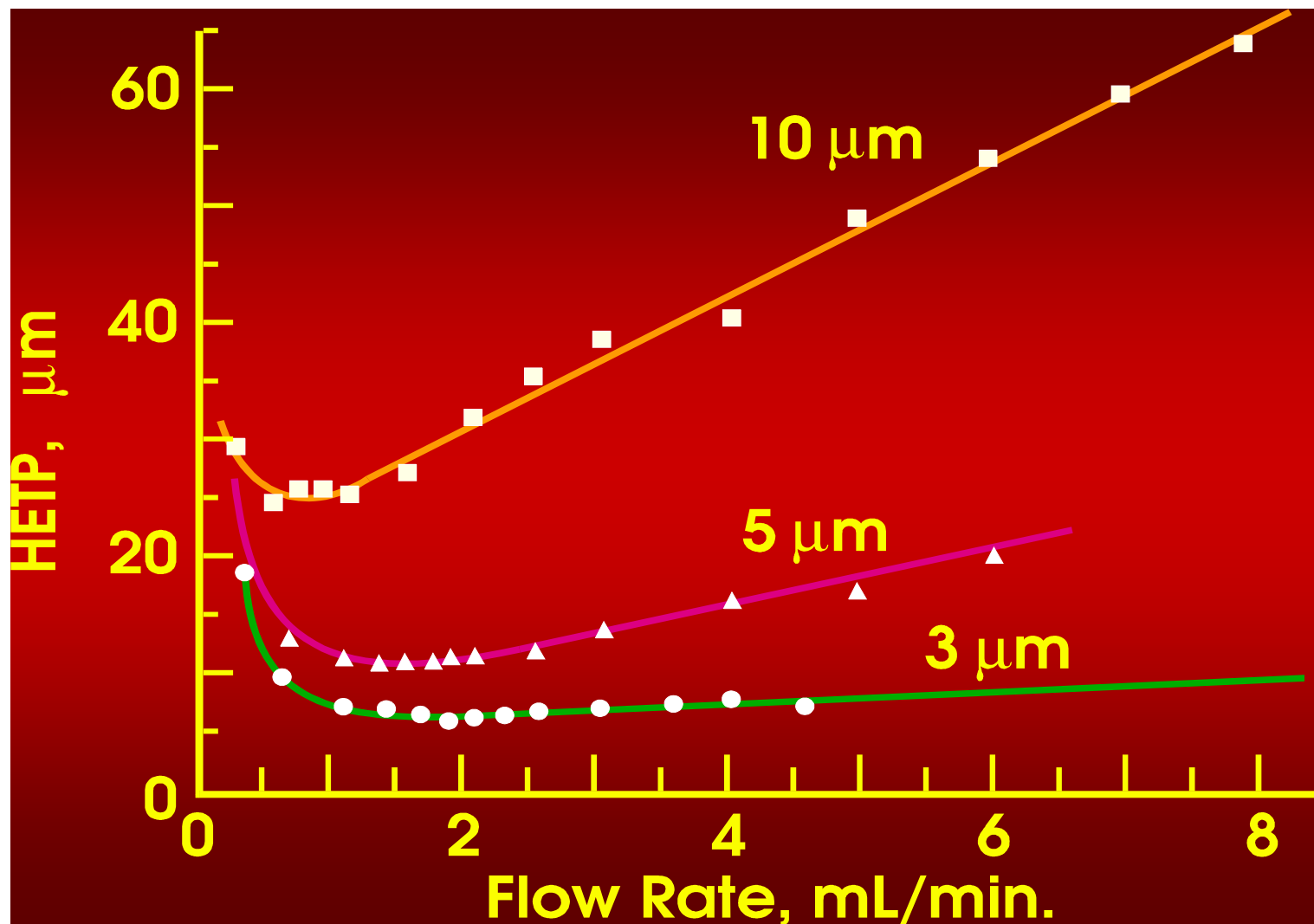


Method Development – Stabilisation time

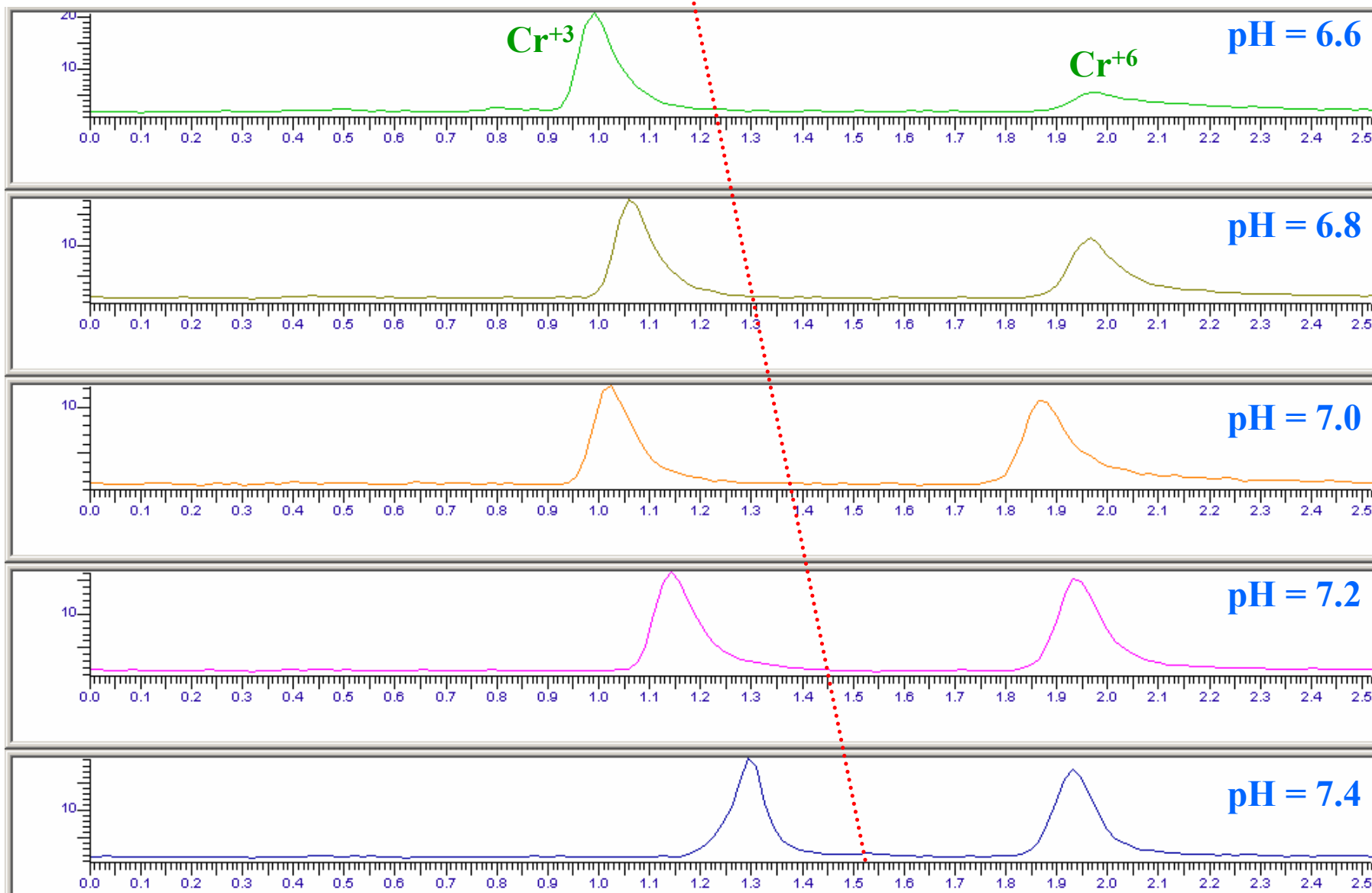


Method Development – Effect of Sample Diluent

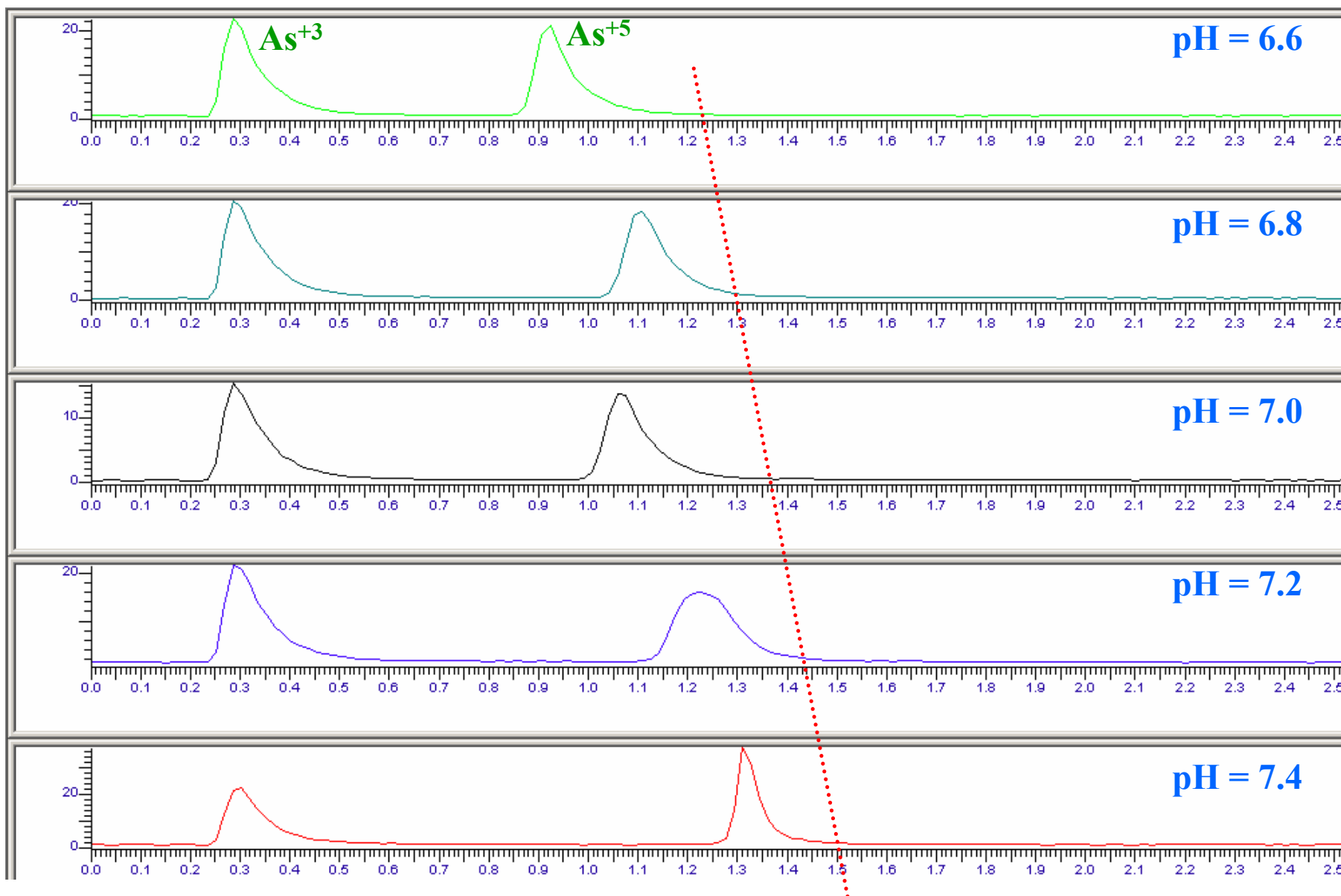




Method Development - Effect of pH on Cr



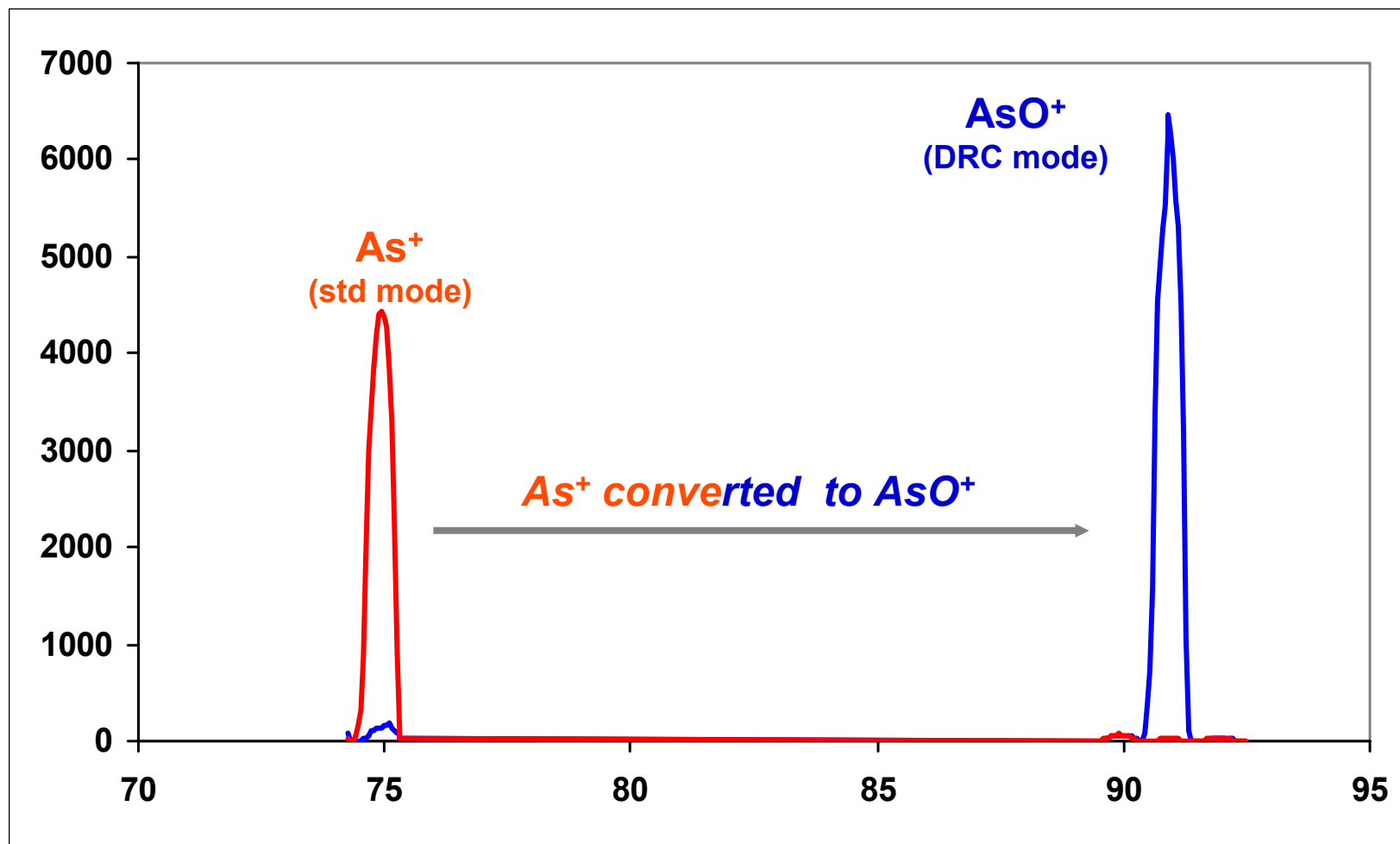
Method Development - Effect of pH on As



- Prior to analysis, the column was conditioned for 30 minutes with the mobile phase flowing at 1.5 mL/min; this was required to properly equilibrate the column.
- Upon completion of analyses at the end of each day, the column was washed with a 5/95 mixture of methanol/water to remove the buffer/salts from the column, followed by a 15 minute wash with a 70/30 methanol/water mixture to prevent the column from drying out.

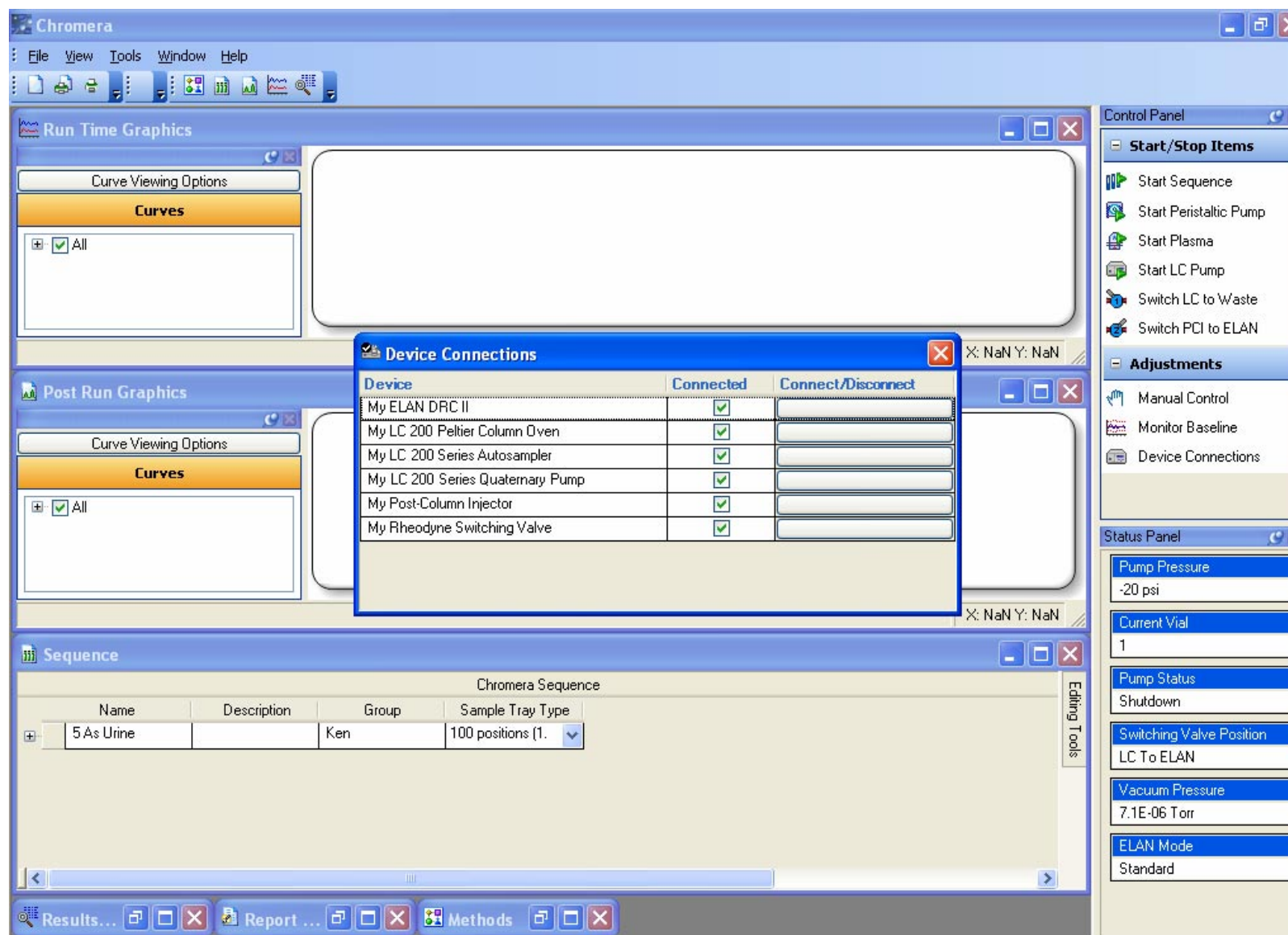
Instrument	ELAN DRC II (PerkinElmer SCIEX)
Nebulizer	Quartz Concentric
Spray Chamber	Quartz Cyclonic
RF Power	1500 W
Analytes	Cr ⁺ (m/z 52); AsO ⁺ (m/z 91)
Reaction Gas	O ₂ @ 0.6 mL/min
RPq	0.55
Dwell Time	500 ms (per analyte)
Analysis Time	210 seconds

Sample: 1 ppb As in 1% HNO₃



- **Having an automatically controlled switching allows the user to:**
 - **run the automatic tuning of the ICP-MS whilst the column is being conditioned.**
 - **Switch off the plasma whilst the column is being washed out**
 - **If need be avoid loading the plasma with unretained species.**

Automation – Software control of all devices



The screenshot displays the Chromera software interface with several panels and a central dialog box.

Device Connections Dialog Box:

Device	Connected	Connect/Disconnect
My ELAN DRC II	<input checked="" type="checkbox"/>	
My LC 200 Peltier Column Oven	<input checked="" type="checkbox"/>	
My LC 200 Series Autosampler	<input checked="" type="checkbox"/>	
My LC 200 Series Quaternary Pump	<input checked="" type="checkbox"/>	
My Post-Column Injector	<input checked="" type="checkbox"/>	
My Rheodyne Switching Valve	<input checked="" type="checkbox"/>	

Control Panel:

- Start/Stop Items:**
 - Start Sequence
 - Start Peristaltic Pump
 - Start Plasma
 - Start LC Pump
 - Switch LC to Waste
 - Switch PCI to ELAN
- Adjustments:**
 - Manual Control
 - Monitor Baseline
 - Device Connections

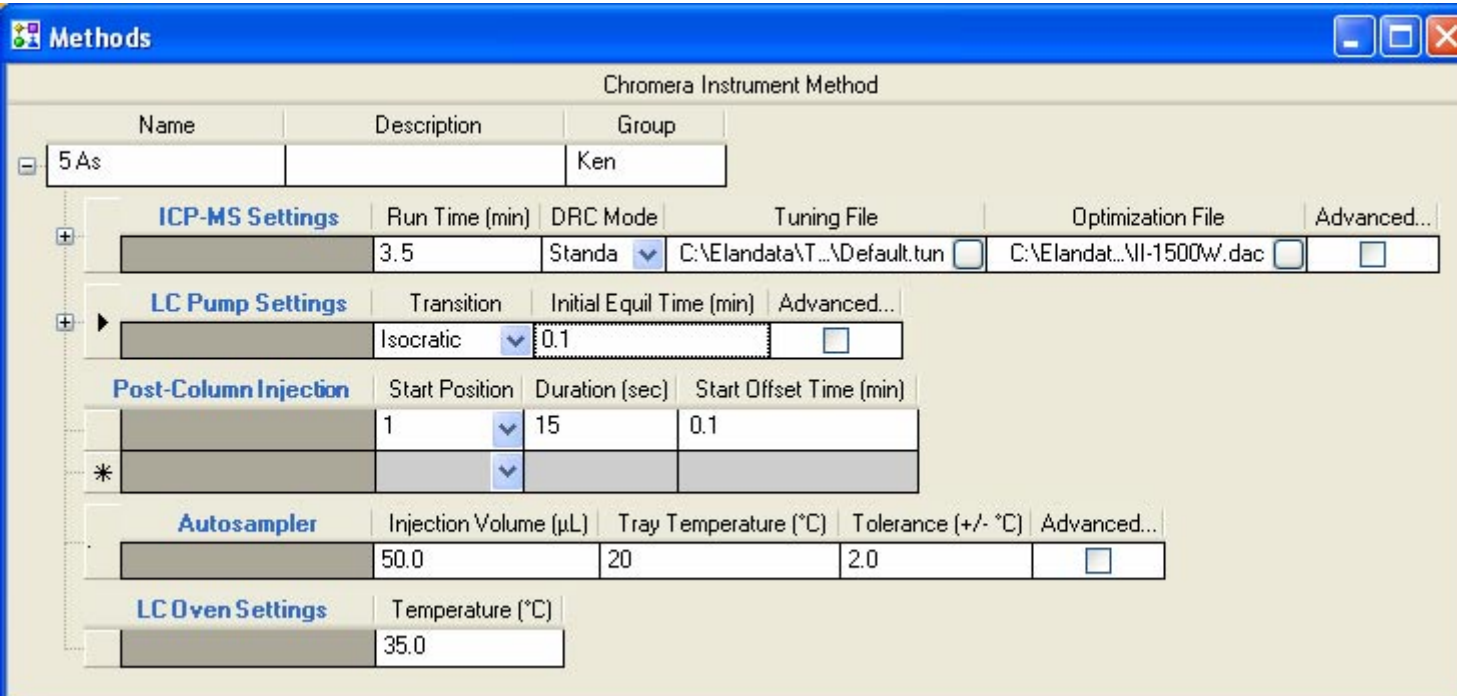
Status Panel:

- Pump Pressure: -20 psi
- Current Vial: 1
- Pump Status: Shutdown
- Switching Valve Position: LC To ELAN
- Vacuum Pressure: 7.1E-06 Torr
- ELAN Mode: Standard

Sequence Panel:

Name	Description	Group	Sample Tray Type
5 As Urine		Ken	100 positions (1)

Automation – Method setup



The screenshot shows the 'Methods' window with the following settings:

Name	Description	Group
5 As		Ken

ICP-MS Settings	Run Time (min)	DRC Mode	Tuning File	Optimization File	Advanced...
	3.5	Standa	C:\Elandata\T...\Default.tun	C:\Elandat...\II-1500w.dac	<input type="checkbox"/>

LC Pump Settings	Transition	Initial Equil Time (min)	Advanced...
	Isocratic	0.1	<input type="checkbox"/>

Post-Column Injection	Start Position	Duration (sec)	Start Offset Time (min)
	1	15	0.1
*			

Autosampler	Injection Volume (μL)	Tray Temperature (°C)	Tolerance (+/- °C)	Advanced...
	50.0	20	2.0	<input type="checkbox"/>

LC Oven Settings	Temperature (°C)
	35.0

Automation – Method setup

Methods Chromera Instrument Method

Name	Description	Group
5 As		Ken

ICP-MS Settings		Run Time (min)	DRC Mode	Gas B Flow	RPq	Tuning File	Optimization File	Advanced...
		3.5	DRC-B	0.6	0.55	C:\Elandata\T...\Default.tun	C:\Elandat...\II-1500W.dac	<input type="checkbox"/>

Analyte	Mass	Use MSIS Constant	MSIS Used	MSIS Mass	View Equations	Equation In Use
▶ AsD	<input type="checkbox"/> 90.9	None	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
▶ Cr	<input type="checkbox"/> 51.9	None	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
*	<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>

LC Pump Settings		Transition	Initial Equil Time (min)	Advanced...
		Isocratic	0.1	<input type="checkbox"/>

Post-Column Injection		Start Position	Duration (sec)	Start Offset Time (min)
		1	15	0.1
*				

Autosampler		Injection Volume (µL)	Tray Temperature (°C)	Tolerance (+/- °C)	Advanced...
		50.0	20	2.0	<input type="checkbox"/>

LC Oven Settings		Temperature (°C)
		35.0

Automation – Method setup

Methods

Chromera Instrument Method

ICP-MS Settings		Run Time (min)	DRC Mode	Gas B Flow	RPq	Tuning File	Optimization Fi
		3.5	DRC-B	0.6	0.55	C:\Elandata\T...\Default.tun	C:\Elandat...NI-1500v

LC Pump Settings		Transition	Initial Equil Time (min)	Advanced...
		Isocratic	0.10	<input type="checkbox"/>

Step	Step Type	Step Time (min)	Flow (mL/min)	%A	%B	%C	%D
0	Equil	0.1	1.5	5.0	95.0	0.0	0.0
1	Run	3.0	1.5	5.0	95.0	0.0	0.0
2	Wash	0.3	1.5	5.0	95.0	0.0	0.0

Step Type	TE Time (min)	TE	Description
*			

Post-Column Injection		Start Position	Duration (sec)	Start Offset Time (min)
		1	15	0.1
*		1	0	0

Autosampler		Injection Volume (µL)	Tray Temperature (°C)	Tolerance (+/- °C)	Advanced...
		50.0	20	2.0	<input type="checkbox"/>

LC Oven Settings		Temperature (°C)
		35.0

Automation – Sequence setup

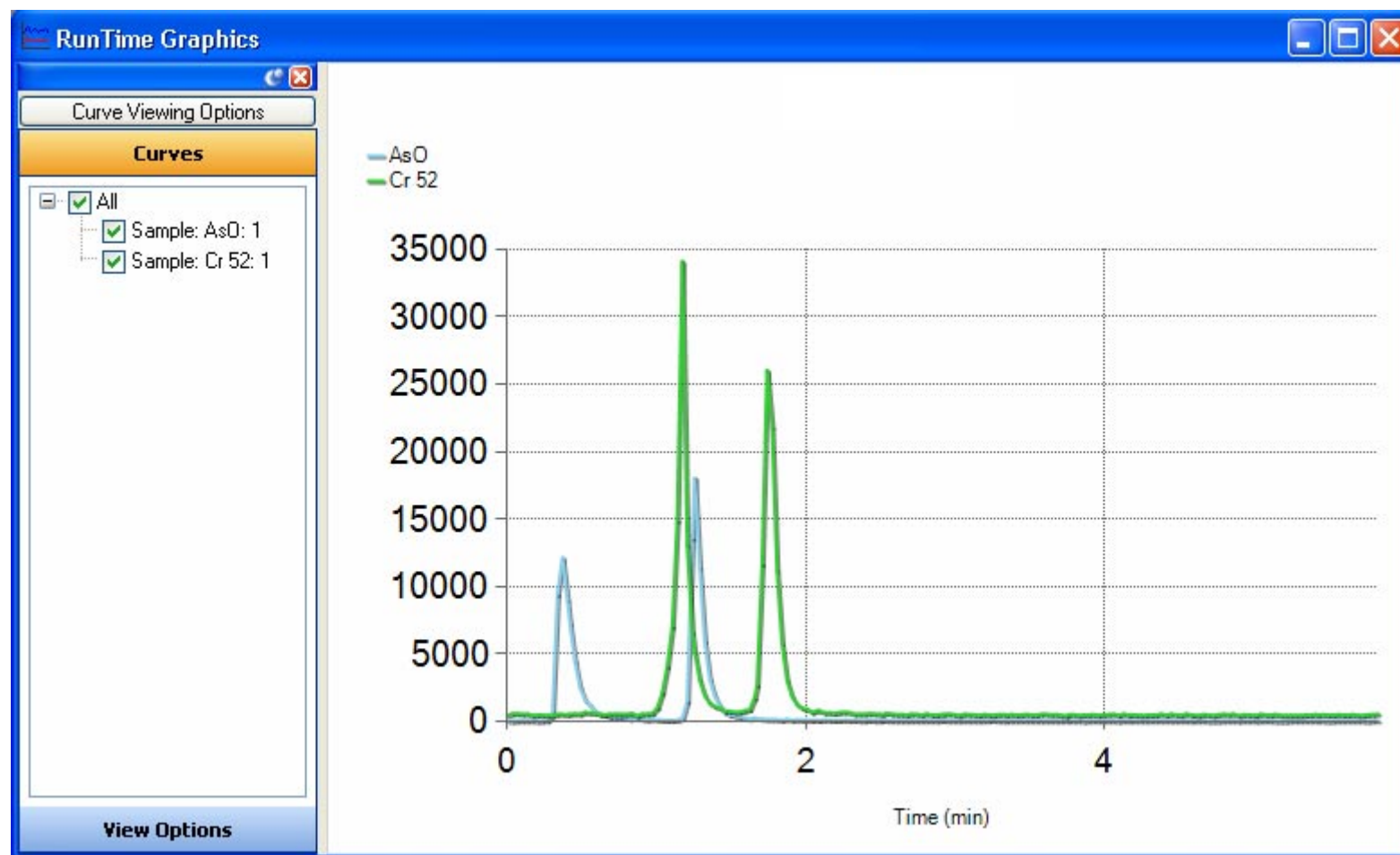
Sequence

Chromera Sequence

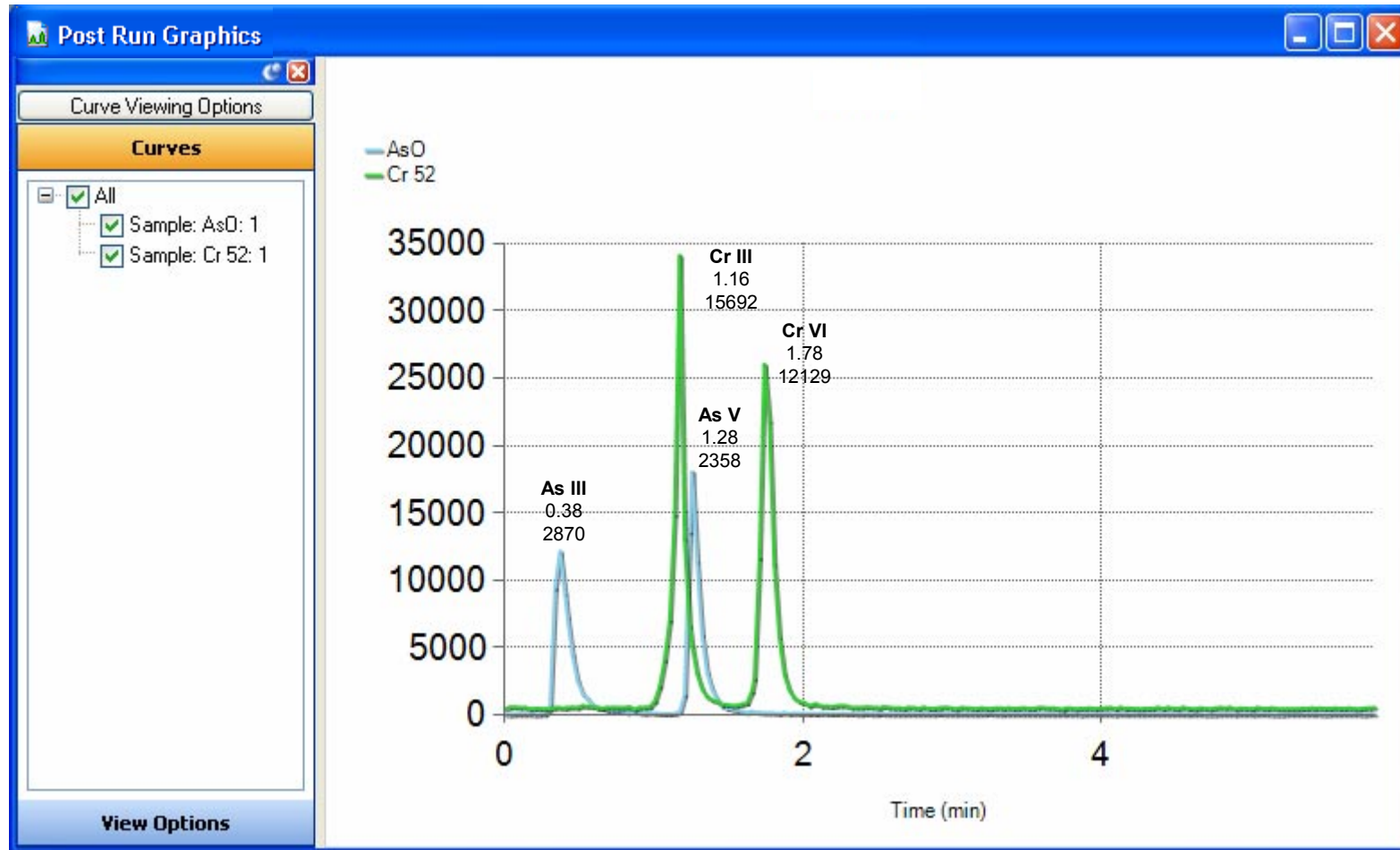
Name	Description	Group	Sample Tray Type
4As-Std		7-18	100 positions (1. ▾)

	Sample Type	Sample Name	Vial	Method	Standard	Injections	Dilution Factor
+	1 Blank (pt by pt) ▾	Cal Blank	1 ▾	4As-Std <input type="checkbox"/>		1	
+	2 Standard ▾	Cal 1 ppb	2 ▾	4As-Std <input type="checkbox"/>	Standard 1 ▾	1	
+	3 Standard ▾	Cal 5 ppb	3 ▾	4As-Std <input type="checkbox"/>	Standard 2 ▾	1	
+	4 Standard ▾	Cal 10 ppb	4 ▾	4As-Std <input type="checkbox"/>	Standard 3 ▾	1	
+	5 Sample ▾	5 ppb	3 ▾	4As-Std <input type="checkbox"/>		1	1
+	6 Sample ▾	1 ppb	2 ▾	4As-Std <input type="checkbox"/>		1	1
+	7 Wash ▾			Wash-As <input type="checkbox"/>			
	▾		▾	<input type="checkbox"/>	▾		

Automation – Real time data viewing



Automation – Post run data processing



Automation – Calibration

Species Calibration

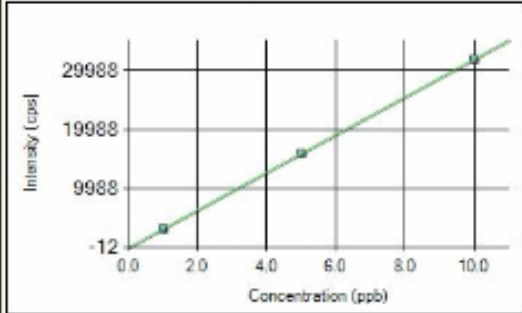
File Edit Display

Species Graphs

Species Calibrations

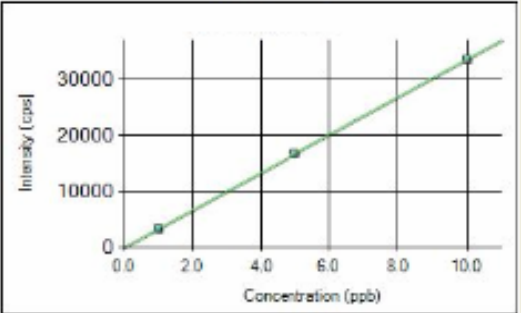
Analyte	Mass
Cr 52	51.9405

Cr III



$y = 3193.55x + -11.87$
0.992

Cr VI



$y = 3361.96x + 15.27$
0.9915

Analyte	Mass
As 75	74.9216

OK Cancel

Automation – Customisable data reporting

Results Quick Review

Print Preview Clear

Results accumulated during this sequence

Sample					
Cal 1 ppb					
Analyte	Species	Ret. Time	Peak Are	Peak Height	Concentration
As0 91	As 3	0.235	37813.13	11257.97	1.00
As0 91	As 5	1.912	40205.24	5675.24	1.00

Sample					
Cal 5 ppb					
Analyte	Species	Ret. Time	Peak Are	Peak Height	Concentration
As0 91	As 3	0.235	196053.7	58977.67	5.00
As0 91	As 5	1.923	205738.3	28256.13	5.00

Sample					
Cal 10 ppb					
Analyte	Species	Ret. Time	Peak Are	Peak Height	Concentration
As0 91	As 3	0.233	389537.3	117970.10	10.00
As0 91	As 5	1.914	390859.1	55577.46	10.00

<i>Chromium Results</i>			
Sample	Cr (III) ($\mu\text{g/L}$)	Cr (VI) $\mu\text{g/L}$	Total Cr ($\mu\text{g/L}$)
Connecticut River	—	—	0.07
Lake Mohegan	0.08	—	0.09
Shelton Water	0.14	—	0.38
Glendale Water	0.56	3.7	3.2
Well Water	—	—	0.003
Bottled Water - A	0.12	—	0.38
Bottled Water – B	0.31	—	0.58
Bottled Water – C	0.25	—	0.34

— = None detected

Arsenic Results			
Sample	As (III) (µg/L)	As (V) (µg/L)	Total As (µg/L)
Connecticut River	—	0.15	0.13
Lake Mohegan	—	0.17	0.26
Shelton Water	—	0.19	0.21
Glendale Water	—	0.65	0.57
Well Water	—	40	42
Bottled Water - A	—	0.45	0.45
Bottled Water – B	0.23	1.5	1.8
Bottled Water – C	—	1.9	1.6

— = None detected

It was possible to rapidly separate and detect Cr³⁺, Cr⁶⁺, As³⁺ and As⁵⁺ ions in drinking waters.

Measured speciated data agreed with total elemental content for elements in the samples under investigations

Using a state of the art software it is possible to automate the whole process in order to provide a rapid routine method for conducting this analysis

The use of an automated switching valve improved productivity by ensuring that both the HPLC column and the ICP-MS instrument can be optimised independently.