Application of Compound Independent Calibration (CIC) Software for the Quantitation of As-species in Undiluted Urine by LC-ICP-MS

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Summary

Liquid Chromatography (LC) coupled to Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) has gained increasing popularity for speciation studies during the last 15 years. ICP-MS offers sensitivity in the ng/L range for elements such as Sn, Se, As, Hg etc and their compounds. However it is not possible to tell the *form* of the element directly and therefore a separation technique has to be employed. LC (or ion chromatography (IC)) allows the use of separation chemistries to "identify" the species based upon their retention time. Provided the separation chemistry is reliable and reproducible, this is an elegant and simple solution to the problem of species identification.

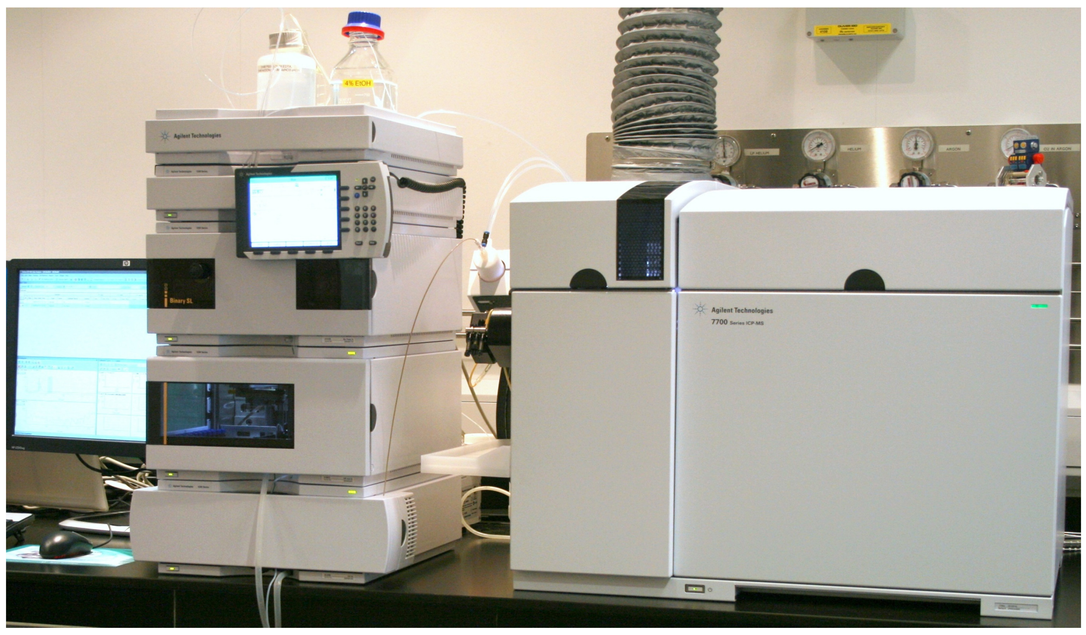

Figure 1. Agilent 1260 HPLC and Agilent 7700x ICP-MS used for speciation work

Provided the chemistry is stable the only real limitation to routine analysis becomes calibration of the interested species. Standards for some species might be not commercially available (or obtainable in a pure enough form for use as calibration standards) or are prohibitively expensive for routine use – for example arsenobetaine can be as expensive as €200 for 50mg. An alternative would be to use the ICP-MS's capability for compound independent calibration using the heteroatom – in this case arsenic.

Within the plasma all compounds are essentially converted to their component atoms before ionisation; therefore the compound's response is based solely upon the As signal and theoretically one should be able to calibrate on As regardless of the species.

This poster compares Compound Independent Calibration (CIC) to traditional Compound Specific Calibration (CSC).

Experimental

An Agilent 7700x ICP-MS was coupled with an Agilent 1260 HPLC fitted with an Agilent arsenic speciation column and guard column as described by Sakai et al[1]. This configuration allows the direct injection of undiluted urine. Instrumental conditions are displayed in table 1. Full control for both the HPLC and ICP-MS is provided by the MassHunter Workstation software (Figure 2).

Table 1. Instrumental conditions and mobile phase

Column	G3288-80000 (4.6 × 250 mm) G3154-65002 (Guard Column)
Mobile phase	2.0 mM PBS/0.2 mM EDTA/10 mM CH3COONa/3.0 mM NaNO3/1% EtOH pH 11.00 adjusted with NaOH Ar purged throughout run
Flow rate	1.0 mL/min
Injection volume	5 µL
RF power	1550 W
Sample depth	9.0 mm
Spray chamber temp	2 °C
Carrier gas	1.04 L/min
Makeup gas (to purge mobile phase)	0.3 L/min
Nebulizer	MicroMist

Results and Discussion

In order to test robustness over a typical analytical run, twelve patient urine samples were directly injected along with calibration standards. The samples and standards were repeated three times giving a total run time of over 13h.

Figure 2 MassHunter Workstation instrument control and Data Analysis

Repeatability for a 50µg l^{-1} standard is displayed in figure 3. A standard was used in order to display any signal drift or retention time shift for all 5 species after multiple injections of undiluted urine (x36) and standard solutions (x12).

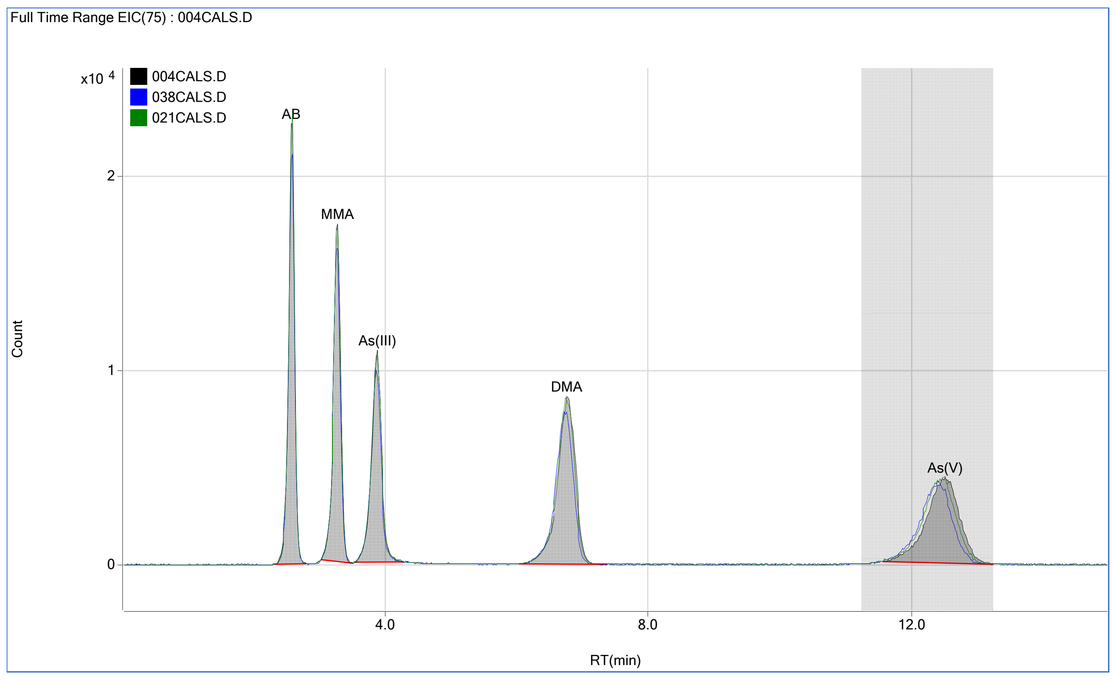

Figure 3 Overlaid chromatograms (50µg l^{-1} standard) over a 13h run of undiluted urine (highlight displays As(V) integration window)

The samples were quantitated using CSC and recalculated using CIC based upon the most readily available inorganic arsenic standard – As (V). Data are presented in table 2. The ratio of CIC/CSC displays the closeness of fit between the two calibration strategies.

Table 2. comparison of CSC and CIC data (all peaks calibrated using As(V) in µg l^{-1})

	AB		MMA		As(III)		DMA		As(V)	
Sample	**CSC**	**CIC**	**CSC**	**CIC**	**CSC**	**CIC**	**CSC**	**CIC**	**CSC**	**CIC**
Patient 1	49.41	48.72	3.69	3.32	0.36	0.32	0.53	0.61	0.42	0.42
Patient 2	514.55	507.33	6.82	6.13			0.67	0.77	0.92	0.92
Patient 3	10.33	10.19	7.60	6.83	0.66	0.59	0.90	1.04	0.75	0.75
Patient 4	21.85	21.55	1.54	1.38					0.66	0.66
Patient 5	21.63	21.32	1.49	1.34					1.01	1.01
Patient A	1.26	1.24	200.62	180.34	4.21	3.76	21.99	25.42	5.27	5.27
Patient B	63.36	62.47	33.22	29.86	0.60	0.54	1.95	2.25	1.11	1.11
Patient C	158.89	156.66	20.33	18.27	0.39	0.35	0.68	0.79	1.71	1.71
Patient D	63.07	62.18	5.95	5.35	0.35	0.31	0.62	0.71	0.74	0.74
Patient E	981.72	967.95	25.61	23.02	0.96	0.86	2.13	2.46	1.23	1.23
Patient F	3.54	3.49	8.43	7.58			0.83	0.96	2.76	2.76
Patient G	43.18	42.58	46.11	41.45			2.38	2.75	3.53	3.53
CIC/CSC	0.986		0.899		0.891		1.16		1.00	

Conclusions

A direct comparison to CIC and CSC demonstrates its feasibility as an alternative to compound specific calibration; a calibration regime using two or more of the readily available (or cheaper) As species combined with CIC would undoubtedly yield even closer agreement.

The benefit of CIC can also be extended to unexpected/unknown peaks (e.g. arsenosugars).

[1] Agilent application note: "Routine Analysis of Toxic Arsenic Species in Urine Using HPLC with ICP-MS"; T. Sakai, S. Wilbur. Pub Number: 5989-5505EN

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