Routine Analysis of Toxic Arsenic Species in Urine Using Agilent HPLC with 7500 Series ICP-MS

Agilent Technologies

By Agilent Technologies, Inc.

A new method using HPLC coupled to ICP-MS has been developed that is capable of separating all five important arsenic compounds in human urine within 12 minutes with detection limits ranging from 0.05 to 0.1 µg/L (50 µL injection). Furthermore, by using 5 µL injections, it is possible to achieve similar performance on undiluted urine samples, thereby significantly simplifying sample preparation and handling.

Arsenic exposure may lead to cancer or other adverse effects, but the toxicity is strongly dependent on the species. Of the five As species most commonly found in human urine, the order of toxicity is: As(III) (arsenite) > As(V) (arsenate) > DMA (dimethylarsenic acid) ≥ MMA (monomethylarsonic acid) >> AB (arsenobetaine). While HPLC-ICP-MS is well accepted as the analytical technique of choice for As speciation in urine, some remaining difficulties have prevented the technique from becoming routine. These are:

1) Finding chromatographic conditions that will separate the five most important species as well as inorganic chloride in a reasonable time, with good retention time reproducibility, dynamic range, and sensitivity.

2) Resolving or eliminating the ArCl interference on As formed as a result of the high NaCl concentration in urine samples.

3) Avoiding clogging of the ICP-MS interface from total dissolved solids (TDS) contained in the urine and HPLC buffers.

Experimental Conditions

An Agilent 1100 Series HPLC isocratic pump, with autosampler, thermostatted column compartment, and vacuum degasser, was coupled to an Agilent 7500ce ICP-MS system fitted with an Agilent MicroMist glass concentric nebulizer. Typical ICP-MS conditions were used for As analysis, including forward power: 1550 W, sample flow rate: 1 mL/min, carrier gas: 0.7 L/min, makeup gas: 0.42 L/min. As was monitored at its elemental mass: m/z = 75.

Column Selection

A new anion exchange column was developed and manufactured by Agilent.

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

The new Agilent column provides the advantages of excellent resolution of As(III) from both AB and DMA as well as good separation of MMA from Cl^- under isocratic conditions.

Mobile Phase

The mobile phase consisted of:

2 mM phosphate buffer solution (PBS), pH 11.00 adjusted with NaOH
0.2 mM EDTA
10 mM CH_3COONa
3.0 mM $NaNO_3$
1% EtOH

Purging the mobile phase with argon during analysis minimized the effects of pH changes due to absorption of atmospheric carbon dioxide.

Interference Removal

The new Agilent G3288-80000 column provides the necessary chromatographic resolution under isocratic conditions to completely separate inorganic chloride from the arsenic species, thereby eliminating the ArCl interference on As. As a result, this method is suitable for use with non-ORS Agilent 7500a systems as well.

Table I: Calculation of detection limits

Species	Height Counts	DL (S/N × 3)
Noise × 3 (average)	117.5	
AB*	2865	0.041 mg/L
DMA	3328	0.035 mg/L
As(III)	2255	0.052 mg/L
MMA	1574	0.075 mg/L
As(V)	1172	0.100 mg/L

* AB elutes with the void volume.

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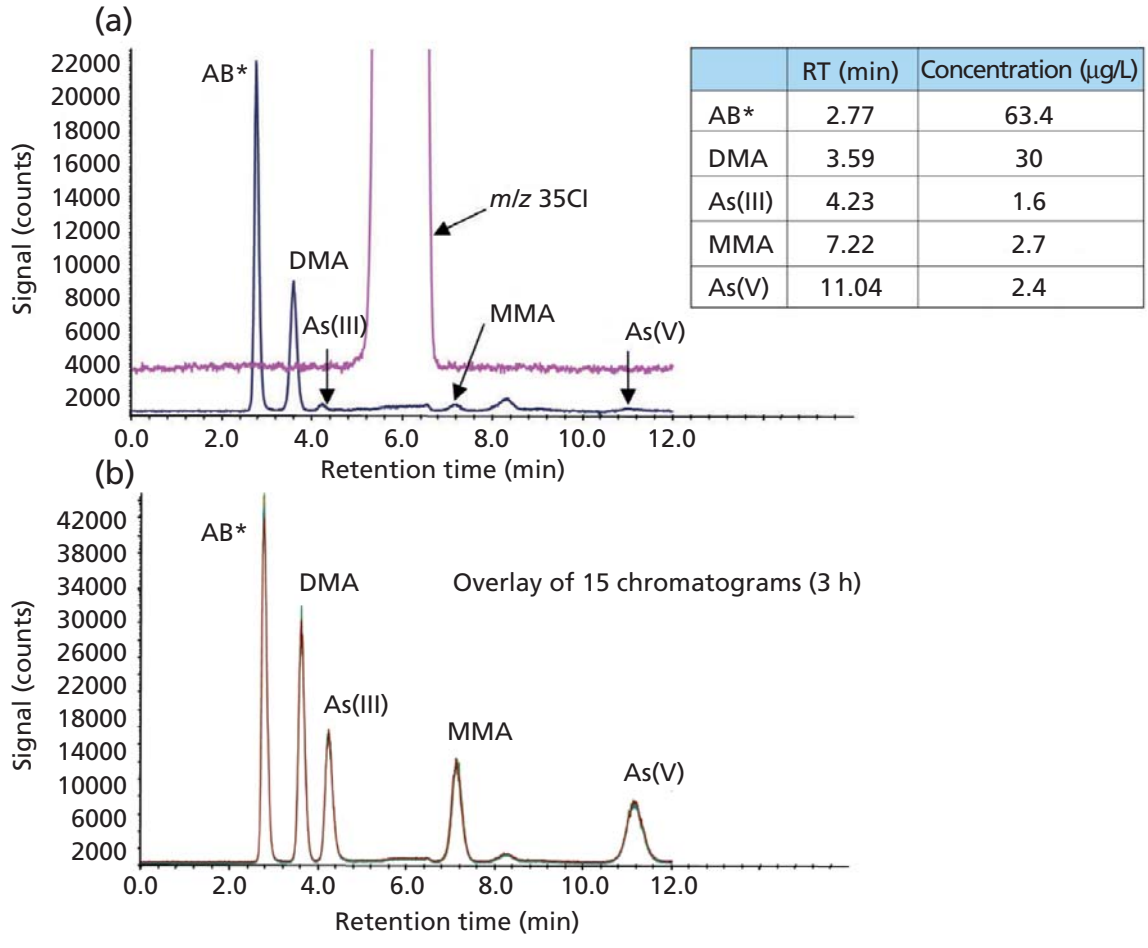

	RT (min)	Concentration (µg/L)
AB*	2.77	63.4
DMA	3.59	30
As(III)	4.23	1.6
MMA	7.22	2.7
As(V)	11.04	2.4

Figure 1: (a) Undiluted 5 µL injection of NIES CRM No.18 urine standard. (b) 5 µL injection of 5 µg/L standard.

* Arsenobetaine, while well separated from the four anionic species, elutes with the void volume and may co-elute with other neutral or cationic species, if present.

Results

The new methodology was applied to the analysis of undiluted NIES CRM No.18 urine, using a 5 µL injection (Figure 1A). The results agree well with the certified values (AB 66.0 µg/L, DMA 31.0 µg/L). Repeated injections (n = 15) of a 1/10 diluted human urine sample spiked at 5 µg/L demonstrated good long term stability and robustness of the method (Figure 1B). Detection limits for each arsenic species were calculated as three times the chromatographic peak-to-peak signal to noise. All species met the goal of ⩽ 0.1 µg/L (Table I).

Conclusions

A new HPLC-ICP-MS method capable of separating all five important arsenic compounds in human urine within 12 min has been developed through careful, systematic optimization of all parameters, including the development and manufacture of a new column. The method is robust enough for the analysis of undiluted urine with limits of detection of 0.1 µg/L or less for the individual As species.