Arsenic Speciation in Urine Becomes Routine Using Agilent HPLC with 7500 Series ICP-MS

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 (dimethylarsinic acid) > MMA (monomethyl arsonic 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:

• Finding chromatographic conditions that will separate the 5 most important species as well as inorganic chloride in a reasonable time, with good retention time reproducibility, dynamic range and sensitivity.
• Resolving or eliminating the ArCl interference on As which is derived from the high NaCl concentration in urine samples
• Avoiding clogging of the ICP-MS interface from total dissolved solids (TDS) contained in the urine and HPLC buffers.

Optimized 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, total carrier gas flow: 1.12 L/min. As was monitored at its elemental mass: m/z = 75.

Column Selection
A new column was developed and manufactured by Agilent. Column G3288-80000 (4.6 x 250 mm) Guard Column G3154-65002

Mobile Phase
The basic mobile phase consisted of:
• 2 mM phosphate buffer solution (PBS), pH 11.0 adjusted with NaOH
• 0.2 mM EDTA
• 10 mM, CH₃COONa
• 3.0 mM NaNO₃
• 1% ethanol

Interference Removal
The new Agilent G3288-80000 column provides the necessary chromatographic resolution to completely separate inorganic chloride from the arsenic species under isocratic conditions, thereby eliminating the ArCl interference on As.

As a result, this method is suitable for use with the non-ORS 7500a ICP-MS as well.

Analysis of Urine Samples
The new methodology was applied to the analysis of NIES CRM No.18 urine, using a 5 µL injection of the undiluted sample (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 demonstrates good long term stability and robustness of the method (Figure 1B).

Table 1. RT (min) Conc. µg/L
<table>
<thead>
<tr>
<th></th>
<th>AB*</th>
<th>DMA</th>
<th>As (III)</th>
<th>MMA</th>
<th>As (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT (min)</td>
<td>2.77</td>
<td>3.59</td>
<td>4.23</td>
<td>7.22</td>
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<tr>
<td>Conc. µg/L</td>
<td>63.4</td>
<td>30</td>
<td>1.6</td>
<td>2.7</td>
<td>2.4</td>
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</tbody>
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Figure 1. (A) Undiluted 5 µL injection of NIES CRM No.18 urine standard. (B) Reproducibility of 15 x 1/10 human urine samples (spiked 5 µg/L) *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.

Conclusions
A new HPLC-ICP-MS method capable of separating all 5 important arsenic compounds in human urine within 12 minutes 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.