Summary

A speciation technique for anionic arsenic species has been applied using an ion pair reverse phase-high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (RP-HPLC-ICP-MS). Six arsenic species (arsenite, arsenate, dimethylarsinic acid, dimethylarsinous acid, momomethylarsonic acid, and monomethylarsonous acid) have been separated with isocratic elution within less than 6 minutes. Furthermore, a cation exchange column was used for separation of AsB, AsC, tetra, and TMAsO. The chemical form and oxidation state of arsenic is very important with regards to toxicity, therefore analysis of total arsenic is insufficient for complete toxicological and risk assessment evaluation. Thus, arsenic speciation has been studied on some urine samples of the children from an arsenic-affected area in Iron Quadrangle, Brazil. DMAs(V) and MMAs(V) were the major urinary metabolites in these samples which have been detected. The mean value for total arsenic concentration of all urine samples analysed (n=15) is 26.33 ng As/mL with a range from 16.1 to 55.2 ng As/mL. TMAsO and AsC (arsenocholin) were not detected in any urine samples in this study. In the most of these samples, monomethylarsonous acid [MMAs(III)] was detected up to 2.0 ng As/mL. DMAs(III) was not detected at any time, most probably due to volatilisation and some oxidation to DMAs(V). The chromatographic recoveries calculated from [sum(species)×100]/total arsenic in urine samples were from 77.4 to 94.9 percent. To validate the method, a certified reference material NIES CRM No.18 human urine (National Institute for Environmental Studies, Tsukuba, Japan), the only CRM available for arsenic species in urine was analysed. Good agreement was obtained between certified and analyzed values for DMAs(V) and AsB in NIES CRM No.18.

More investigation has been made on identification of monomethylarsonous acid (MMAs(III)): **(i)** The retention time interval of MMAs(III) from the HPLC run with urine samples from Brazilian children exposed to arsenic-rich drinking water was cut off, and then by hydride generation at pH 5 volatilized. The GC separation led to clear isolation of $MMAsH_2$ as proven by its mass frangmentogram compared with a library standard. This shows that the analyte is either MMAs(III) (MMAs(V) is separated by HPLC separation and not volatilized under the applied pH conditions) or a compound, which contains a MMAs(III) group that can be cleaved under the reaction conditions applied. **(ii)** Mass of 48 and 50 monitored as sulphur oxide (^{48}SO, ^{50}SO) during arsenic speciation. The sulphur amount within the retention time interval of MMAs(III) in urine sample was not significant on the background of the chromatogram.

Different cell types have different capabilities for the uptake of arsenic compounds. The oxidation state and the degree of methylation of the arsenicals determine the uptake and subsequently the toxicity of the compounds. Table 5.1 shows the comparison of uptake capabilities of different cell types which studied here.

Table 5.1 Comparison of uptake capabilities of different cell types

Cell type		Arsenic species						
		As(V)	As(III)	MMAs(V)	MMAs(III)	DMAs(V)	DMAs(III)	TMAsO
CHO-9	HP*	1.99	3.83	0.03	1.78	0.02	16.3	0.78
	C	1	1	100	25	1000	0.5	1
Hep G2	HP	0.97	0.63		1.16	0.63	4.17	
	C	0.5	0.5	ND**	5	0.5	5	ND
UROtsa	HP		0.13		0.18	0.02	13.27	
	C	ND	0.5	ND	5	0.5	0.1	ND
Hela S3	HP		0.02	0.05	1.22		0.46, 0.48	
	C	ND	0.5	0.5	0.5	ND	0.5, (5, 10)	ND
Ra Hep	HP	0.08	0.33		0.75		0.41, 0.46	
	C	0.5	0.5	ND	0.5	ND	1, 10	ND

*HP: Highest Percent (detected As in whole-cell extract) & C: concentration (μM)
**ND: not detected

As it has been shown in Table 5.1 trivalent methylated and inorganic arsenic species are more membrane-permeable than pentavalent arsenic metabolites and are taken up by these cells to a higher degree from the external medium. The present study revealed that trivalent arsenic metabolites [As(III), MMAs(III), and DMAs(III)] were best taken up by CHO cell lines and also in higher degree compared to other cell types which have been studied in this work.