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Working methods

Complete extraction of arsenic species: a worthwhile goal?

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Some issues regarding sample preparation for arsenic speciation analyses are briefly discussed. In particular, the use of a single set of extraction conditions for the many different arsenicals present in biological samples is questioned. Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

An international meeting,[1] held recently at Seggau Castle, Austria, focused on methods of preparing samples for chemical analysis. The presentations covered both organic and inorganic analyses, and highlighted the traditionally different approaches taken in these two areas: the former using mild conditions to extract intact compounds of interest, and the latter employing forcing conditions, to destroy all organic compounds or mineral phases and thereby release the analytes. There were also presentations in the fledgling field of speciation analyses, after which it was apparent that considerable improvements in sample preparation techniques are still needed in that research area. This letter expands on some of the discussion points at the meeting with regard to sample preparation for arsenic speciation analyses.

WATER- VERSUS LIPID-SOLUBLE ARSENIC

The recent literature contains several examples of optimization of extraction efficiencies using various combinations of solvents and heating/agitation devices, such as microwaves and sonication probes. Although different solvent mixtures are used in the initial extraction steps, the final extract for analysis is invariably prepared in water. Thus, only water-soluble compounds are covered by current sample preparation procedures. It is well recognized, however, that arsenic in biological samples can also exist as lipid-soluble compounds, and, indeed, the early work[2] on natural arsenic compounds focused on the lipids. The first arsenolipid to be identified was isolated from an alga by Morita and Shibata[3] in 1988, and in a subsequent comprehensive study[4] these researchers showed that lipid arsenic can contribute up to 50% of the total arsenic in algae. In the intervening 13 years, however, at least 100 papers have been published on naturally occurring water-soluble arsenicals, whereas only a handful of reports have dealt with the lipids, and the compound of Morita and Shibata still remains the only arsenolipid to be rigorously identified.

Analytical convenience appears to be the reason for this apparent bias in the literature. The most common techniques for arsenic speciation analyses use high-performance liquid chromatography to separate the species, followed by arsenic-specific detection. In all cases, the separation is performed in aqueous mobile phases (>95% water) using conditions suitable only for water-soluble arsenicals. Chromatographic separation of lipids is more difficult to achieve, and the mobile phases employed are currently not compatible with commonly used arsenic detection methods, such as inductively coupled plasma mass spectrometry. A substantial amount of method development is required for the analysis of arsenolipids, and the analytical community appears to consider this research investment unjustified. Perhaps it is time to question the research priorities in the field of arsenic speciation analyses.

ONE EXTRACTION PROCEDURE—ALL SPECIES?

Most people would accept that a single extraction procedure cannot efficiently remove both lipid- and water-soluble arsenic compounds. But perhaps we should also consider finer shades of polarity differences. Many sample preparation methods use methanol or methanol-based mixtures despite the fact that very polar arsenicals are poorly extracted by methanol. This effect was clearly shown in the work by

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Edmonds *et al*.,[5] which reported low recovery of arsenate from turtle tissues when the extractant was methanol rather than water—a result explained in terms of the greater polarity of arsenate compared with the other arsenicals, chiefly arsenobetaine and arsenocholine, in the sample. Several of the naturally occurring organoarsenic compounds are also very polar, some of the arsenosugars for example, and they may also be poorly extracted by solvents less polar than water.

MULTIPLE EXTRACTIONS. WHY?

In view of the above, if the analysis is to restrict itself to water-soluble compounds, then the most efficient extracting solvent is probably water. The use of sonication probes removes concerns about not reaching the arsenic species within vesicles, and sonication techniques, together with microwave-assisted methods, are now commonly employed in sample extraction procedures. The next question is: how many times should the sample be extracted? Although usual practice employs multiple extraction/centrifugation steps, in most cases no justification for these time-consuming steps is provided. There are some papers, however, that seem to provide justification for multiple extractions by reporting that arsenic is present, in decreasing quantities, in successive supernatants. These methods (e.g. Tukai *et al*.[6]) describe how the supernatant is 'carefully pipetted off', and the (wet) pellet is re-extracted with a fresh portion of solvent. The quantities of arsenic in supernatants one, two, three etc. are then individually determined. No allowance is made, however, for what may appear to be a self-evident fact, namely that at each stage the wet pellet contains (arsenic-contaminated) extracting solvent from the previous supernatant, so fresh extractant merely combines with this residual interstitial solvent engulfing and carrying over its arsenic content. We have quantified this effect by sequential extraction of a homogeneous freeze-dried algal sample, and recording the volume (by weight) of the water retained interstitially in the pellet (Madsen and Francesconi, unpublished results). The tests clearly showed that the apparent 'additional' arsenic contained in second and subsequent extractions is completely accounted for by residual dissolved arsenic carried over from the previous extraction. In view of this result, a single extraction with a precise solvent/sample ratio is likely to be the most efficient procedure for preparing samples for speciation analysis of water-soluble arsenicals.

A LESSON FROM THE ORGANIC ANALYST—TARGETED SAMPLE PREPARATION

The organic analyst employs specific sample preparation procedures developed for particular compound classes. The extraction is designed to take advantage of the similar physical properties shown by compounds within a particular analyte group (e.g. polychlorinated biphenyls). The arsenic speciation analyst, rather naively, is treating arsenicals as if they also belong to the one class of compounds by trying to apply a single extraction procedure and aiming to achieve complete recovery of all species. Naturally occurring arsenic compounds, however, cover a wide range of polarities, so that extraction conditions ideal for one compound may be quite unsuitable for another. Of relevance to this discussion is the fact that some biological samples may contain 15 or more arsenic compounds.

The justification often cited for arsenic speciation analyses is based on toxicological considerations, particularly those dealing with human health, and most papers state the need to determine toxic arsenicals. One wonders, then, why so much effort is put into determining the precise concentration of arsenobetaine and other arsenicals of little toxicological significance, while more worthy targets are almost neglected. Recent work[7] has shown that the processes whereby humans methylate inorganic arsenic, leading to methylarsonate and dimethylarsinate (both arsenic(V) species), have, as intermediates, the corresponding methylated arsenic(III) compounds. These arsenic(III) species may play a key role in the observed toxic effects from chronic arsenic exposure.[8] Their quantitative analysis, however, presents a challenge to the analytical chemist because they have limited stability, occur in low concentrations, and their chromatographic behaviours are not readily explainable, which hinders attempts to optimize their separation from other arsenicals. Recent work[9] on the presence of arsenic(III) species in human urine appears to have succumbed to these analytical difficulties: the analyses were performed after inappropriate sample handling and storage, and the assignments and quantifications were based on unconvincing chromatographic signals alarmingly close to detection limits. More focused analytical methods are urgently needed to concentrate, identify and quantify these important arsenic metabolites reliably, and such methods would clearly benefit from targeted sample preparation steps designed to maximize the recovery of the arsenic(III) species. Development of such techniques would appear to have considerably more relevance to human toxicological research than trying to maximize extraction of the many harmless arsenicals found in nature.

REFERENCES

1. *Trends in Sample Preparation* 2002, Seggau Castle, Austria, 30 June–3 July 2002.
2. Lunde G. *Acta Chem. Scand.* 1973; **27**: 1586.
3. Morita M, Shibata Y. *Chemosphere* 1988; **17**: 1147.
4. Morita M, Shibata Y. *Appl. Organometal. Chem.* 1990; **4**: 181.
5. Edmonds JS, Shibata Y, Prince RIT, Francesconi KA, Morita M. *J. Mar. Biol. Assoc. U. K.* 1994; **74**: 463.
6. Tukai R, Maher WA, McNaught IJ, Ellwood MJ. *Anal. Chim. Acta.* 2002; **457**: 173.
7. Le XC, Ma MS, Lu XF, Cullen WR, Aposhian HV, Zheng BS. *Environ. Health Perspect.* 2000; **108**: 1015.
8. Kitchin KT. *Toxicol. Appl. Pharm.* 2001; **172**: 249.
9. Mandal BK, Ogra Y, Suzuki KT. *Chem. Res. Toxicol.* 2001; **14**: 371.