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Pretreatment Techniques for Arsenic Speciation Analysis in Biosamples

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Introduction

Coupled techniques such as HPLC–HG–AAS, HPLC–ICP–MS, HPLC–HG–AFS are widely used for arsenic speciation analysis in biosamples, during which pretreatment method usually plays a significant role. This paper introduces several typical pretreatment techniques such as traditional solvent extraction (SE), accelerated solvent extraction (ASE), enzymatic hydrolysis (EH), pressurized liquid extraction (PLE), liquid phase microextraction (LPME), solid phase microextraction (SPME) and also their applications.

Solvent extraction (SE) For arsenic speciation analysis in biological samples, solvent extraction commonly includes mixing, vigorously shaking on a wrist action shaker for some time to realize completely separation of the phases. H_3PO_4 is usually used as an extraction solvent to exact marine product samples (Geng et al, 2009), which presents good recovery for each arsenic species. In order to improve recoveries for arsenic species analysis and to reduce extraction time, optimizing the extraction procedures through choosing extraction solvent and assisted methods are often adopted. Microwave–assisted, ultrasonic water–bath and ultrasonic probe are the main assisted methods commonly used. Comparing the results from HNO_3/H_2O_2–digested cell lines, the ultrasonic probe yield the highest recovery (92%) among vortex, ultrasonic bath and ultrasonic probe (Yehiayan and Membreno, 2011)

Accelerated solvent extraction (ASE) Accelerated solvent extraction (ASE) is an automated method employing organic solvent extraction at high temperature and high pressure. The equipments include solvent bottles, pump, gas, heating stove, stainless steel extraction cell and collecting bottles. ASE has some advantages such as fewer organic solvent cost (commonly 15 mL is used to extract 10 g sample), faster (one procedure usually needs 15 min) and fewer matrix influence when compared with ultrasonic, microwave, supercritical and separatory funnel. Besides, ASE shows high extraction efficiency and selectivity for the extraction of arsenic in freeze-dried carrots. For example, Nohora et al (Nohora et al, 2001) evaluated several parameters including selection of the dispersing agent and extraction time so as to optimize the ASE method. The extraction efficiency ranged from 80 to 102% with arsenic concentration greater than the limit of quantitation of ICP–MS.

Enzymatic Hydrolysis (EH) Among these different sample pretreatments, enzymatic hydrolysis procedures are appealing methodologies for enzymes can break down specific bonds of the substrate (biomolecule hydrolysis, mainly protein hydrolysis), and they allow a selective analyte release from the sample matrix without chemical species changes (Piñeiro et al, 2010). Enzymes such as trypsin, pepsin, pronase E, pancreatin, protease type XIV, lipase type VII, protease XIV, lipase and protease are often used to extract various arsenic species, among which trypsin is most often used. For example, Simon et al (1994) used trypsin to extract fish, and arsenic species in the extracts were determined by using HPLC–ICP–MS. Recovery tests and representative residues analysis for total arsenic concentration were performed. The results were proved to be satisfied. Some optimized measures such as ultrasound assisted enzymatic hydrolysis (Piñeiroa et al, 2011), pressurization, microwave energy or ultrasound energy assisted-acid leaching process are often used to speed up enzymatic hydrolysis for relative long time cost during pretreatment step.

Pressurized liquid extraction (PLE) Pressurized liquid extraction (PLE) is a relatively new technique with fast, safe and automatic characteristics. It is on the basis of solvents (deionized water, organic solvents, and diluted organic acids, etc) at a high pressure and/or high temperature without reaching the sub–critical point. The

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extraction procedures commonly include: (1) mixing samples with extraction solvent and dispersing agent; (2) the mixture was transferred to the extracting cell between two cellulose filters at certain temperature and pressure for some static time; (3) the cell was purged for 60 s with N_2 gas after pressurization and the extracts were collected and stored at subzero until further analysis (Mato-Fernándeza et al, 2007). Pressurized assisted enzymatic hydrolysis extraction is often employed for arsenic speciation analysis in seafood samples before HPLC–ICP–MS analysis. Enzymatic hydrolysis (pepsin) can be completed after 2 cycles of 3.0 min each one at 50℃ and 1500 psi when assessed arsenic speciation in marine food products (Piñeiro et al, 2010).

Solid phase microextraction (SPME) Solid phase microextraction (SPME) was first proposed by Paw liszyn (Arthur and Paw-liszyn, 1990) and his co-workers. Compared with traditional methods such as solid-phase extraction (SPE), simultaneous distillation and extraction (SDE), solvent extraction, supercritical fluid extraction (SFE), SPME is more simple, faster, more inexpensive, safer, and solvent-free with high selectivity and sensitivity. GC-MS, HPLC, capillary electro–phoresis (CE) can be connected to SPME directly. Meanwhile, it is timesaving for it integrates of sampling, extraction, concentration and injection on-line. The mechanism is the "similar compatible" between organics and solvents. Components are extracted from the sample matrix because of the absorption of SPME fiber's chromatographic stationary phases (Liu and Zhou, 2003). Bogdan and Joseph. (1998) developed a method for the analysis of organoarsenical compounds by combining dithiol derivatization with solid–phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS). A precision of 10% R.S.D. was typical for the SPME–GC–MS procedure.

Liquid phase microextraction (LPME) In order to resolve the eluting problem of SPE before connecting to detectors, solvent extraction flow injection, SPME and LPME were developed. LPME integrates sample collection and extracting concentration in one procedure. Compared with liquid-liquid extraction and SPME, LPME can provide better sensitivity and concentrating effect with less solvent consumption (less to dozens μL). LPME is usually classified into drop–based LPME and hollow fiber based LPME. The latter is more common. For example, Jiang et al (Jiang et al, 2009) used hollow fiber based LPME to extract arsenic (III) and arsenic (V) in fresh waters and human hair. The results show good extraction efficiency.

It is noted that the new trend for trace and ultratrace arsenic speciation analysis in biosamples will focus on the simple, fast and environmental-friendly automatic online pretreatment methods and subsequently hyphened to relative detectors.

Key words: pretreatment techniques, biosamples, arsenic speciation

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