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A Mini Review on Solid Phase Micro-Extraction Applications in Mass Spectrometry Detection of Toxins

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Abstract This review highlights developments of Solid Phase MicroExtraction in toxins analysis after 2005. It covers the major mycotoxins such as aflatoxins and ochratoxins where applications have been reported in the literature and some cyanobacteria toxins. Solid Phase MicroExtraction can act as an important sampling and sample preparation tool for the analysis of toxins in various matrices expanding its existing broad spectrum of analytes.

Keywords: *SPME, chromatography, toxins, mass spectrometry*

1. Introduction

Solid Phase Micro-Extraction (SPME) is a prominent sampling and sample preparation method which has gained interest the last two decades after its inauguration by Pawliszyn and coworkers [1]. Many applications have been reported in various scientific fields such as Environmental Sciences, Chemistry and Food Science.

SPME has been successively reviewed last years by eminent scientists highlighting its recent developments, strategies to complex matrices and various technologies facilitating this technique [2].

Mycotoxins are toxic secondary metabolites produced by organisms of the fungi kingdom, commonly known as molds such as *Aspergillus, Penicillium, Byssochlamys*. A versatile category of compounds belong to mycotoxins, with natural origin that can affect after exposure main mammals organs such as liver or kidneys exerting hepatotoxic, nephrotoxic, neurotoxic and carcinogenic effetcs. Major mycotoxins are afaltoxins, ochratoxins, fusarium toxins, ergot alkaloids, patulin, trichothecenes and zearalenone. Other important toxins are cyanobacteria toxins which are produced from cyanobacteria blooms and scums.

The chemical properties of these compounds favor analysis via Liquid Chromatography (LC) which is coupled with mass spectrometry (MS) -functioning in simple or tandem mode-, fluorescence (FD) or ultraviolet (UV) detectors. For these techniques many research papers have been published with LC-MS/MS routinely and HPLC-FD in less extent possessing predominant role in terms of analytical performance. However it is noted that some methods using HPLC with UV or FD face problems with sensitivity or require tedious sample preparation steps.

Gas Chromatography (GC) with its well-known advantages is used as an efficient tool for analyses of volatile and semi volatile compounds. Nevertheless the derivatization reaction has been proposed as an effective medium to transform polar and non-volatile compounds to non or semi polar compounds. This gained ground also for some toxins which could be derivatized and then be compatible to GC instrumentation.

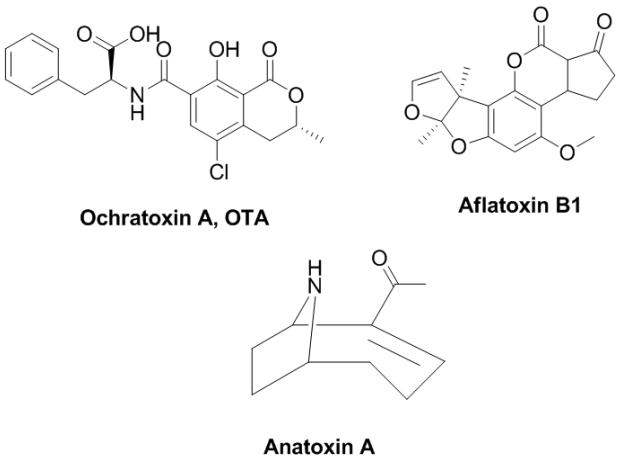

Figure 1. Chemical Structures of Ochratoxin A, Aflatoxin B1 and Anatoxin-α

Shephard et al. have reported in 2012 the developments in mycotoxin analysis updating data until 2011 [3]. This review was quite extensive including immunochemical methods, LC-MS methodology and more conventional ones.

Sample preparation and cleanup are key steps in toxins analysis. In the majority of works, these are performed by liquid-liquid extraction with methanol/water or chloroform [4], solid liquid extraction [5], column chromatography with Florisil or silica gel [6,7], solid phase extraction [8], matrix solid phase dispersion and QuEChERS [9].

It is noteworthy that SPME in toxins field is frequently implicated in the analyses of the volatile metabolites /intermediates produced by food commodities and

compounds therein (eg. cereals) and not the parent compounds [10,11,12].

In this context this mini-review aspires to comment on papers after 2005 with advances on toxins extraction-detection by SPME coupled with GC-MS or LC-MS in various commodities.

2. Main Text

2.1. Anatoxin-α, Microcystins and Saxitoxins

2.1.1. Anatoxin-α Case

Anatoxin-α is a bicyclic secondary amine with low molecular weight. It is a postsynaptic nicotinic agonist acting as a neuromuscular blocking agent. Anatoxin-α has an LD50 value of 200-250μg/kg in mouse, indicating its high toxicity [13].

In 2005 Ghassempour et al. have reported the analysis of anatoxin-α using polyaniline as a sorbent in SPME coupled with GC-MS [14]. Specifically three forms of polyaniline films and one polypyrrole film were prepared and applied for SPME. The leucoemeraldine form exhibited better selectivity for this molecule and then SPME conditions were optimized after selecting the respective extraction parameters. These included type of coating, salt concentration (salting-out effect), extraction time and stirring speed. The calibration was linear from 50 to 10000ng/ml and the detection limit (as signal to noise 3) was 11.2ng/mL, in the aqueous sample. The method was then applied for the analysis of anatoxin-α in cutured media of two cyanobacteria species. Quantification was carried out by peak area using the external standard calibration curve and the concentrations were expressed in mg/g of lyophilized cells used. In this work it was observed that exposure of standard and sample to light caused transformation of the parent compound.

The importance of SPME was communicated by Rodriguez et al. one year after by the report of an efficient method using SPME-GC-MS to determine anatoxin-α in environmental water samples [15]. This method was based on derivatization of the analyte by addition of hexylchloroformate in basic medium and subsequent extraction of the derivatized product with a PDMS fiber of 100μm. In this paper optimization of experimental conditions was presented assuring its good validation profile. The method was sensitive with an LOD of 2ng/mL and demonstrated that SPME-GC-MS can be used for monitoring anatoxin-α in water quality control.

2.1.2. Mycrocystins

Mycrocystins are cyanobacterial toxins which abandon in freshwater systems and have been associated to various acute and chronic health effects [16].

A comparison work on sample preparation for the evaluation of their extraction from surface water has been reported by Ammerman and Aldstadt in 2009 [17]. SPME was tested and connected to a LC-MS system and toxins were monitored however the monolithic SPE superseded in terms of loading sample volume the classical SPME.

Although their analysis in water is satisfactorily accomplished via LC-MS methods, in biological matrices is hindered by the strong binding of these molecules mainly to proteins

To circumvent these extraction problems research groups have pursued alternative ways to extract and detect these toxins. One proposed way was through the analysis of one surrogate compound which is a metabolite or a reaction product of mycrocystins that can be quantitavely extrapolated to the total content of the parent compound. The unique marker 2-methyl-3-methoxy-4-phenylbutanoic acid (MMPB), a product of Lemieux oxidation of mycrocystins was used recently by Suchy and Berry [18]. This group in order to enhance the recovery of the analyte it has applied a headspace SPME-GC-MS methodology to detect MMPB and its analogue 4-phenyl butanoic acid by esterifying the carboxylic acid group of these molecules. Indeed levels of MCs detected by this method were higher compared to levels by other conventional methods, indicating that the utilization of SPME can be beneficial in the determination of total mycrocystins content in complex biological matrices.

2.1.3. Saxitoxin

Saxitoxin is the most potent paralytic shellfish poisoning toxin. To our knowledge there is no reference on SPME-GC/LC-MS detection. However there is a reference on SPME of saxitoxin (using carbowax template resin) and subsequent detection by HPLC-post column fluorescent derivatization with a method detection limit of 0.11ng/mL [19]. It is a logical hypothesis that in forthcoming works saxitoxin will be included in SPME interface with mass spectrometry detector.

2.2. Ochratoxins, Aflatoxin and Patulin

2.2.1. Ochratoxins

Detection of ochratoxins A and B (OTA and OTB) in human biological fluids constitutes an intriguing target for researchers involved in exploration of toxic compounds and their implication to human health. A high-throughput method using SPME-LC-MS/MS for the analysis of OTA in human urine was developed by Pawliszyn's group and published in 2008 [20]. Specifically this was achieved by simultaneous preparation of up to 96 samples using multi-fiber SPME and multi-well plates. A carbon-tape coating was chosen for the first time as the best extracting phase for OTA after fiber selection comparison. Acidification of urine sample with phosphate-buffered saline was performed prior to SPME and then analyte was separated from matrix interferences on a 50mm Symmetry Shield RP column. The LC-MS/MS system functioned in the negative ion mode monitoring OTA with the 402.1 to 357.9 transition which corresponds to the loss of carbon dioxide from M-H parent ion. The instrumental LOD for OTA was 0.05ng/ml and LOQ 0.2ng/ml based on 3× and 10× signal to noise criteria. It is worth mentioning that authors during method validation did not encounter human urine samples free from OTA. For this reason blank subtraction method was used, and the LOD and LOQ were determined by spiking known ammounts of OTA in the naturally contaminated urine. By this way LOD and LOQ were 0.3ng/mL and 0.7ng/mL respectively. Other HPLC-FD methods as mentioned by authors are one order of magnitude more sensitive; however the LC-MS/MS

methodology shows better results in terms of specificity, with the inherent advantage of unambiguous identification. It is important to mention that this method can be applied for the sample preparation of more than 1500 samples, proving its capacity for large-scale screening.

Pawliszyn's group also published in 2009 an *in situ* application of SPME as a sampling and sample preparation method coupled to LC-MS/MS for direct monitoring of OTA's distribution at different locations in a single cheese piece [21]. To be suited to the acidic analyte, the extraction phase (carbon-tape SPME fiber) was acidified with aqueous solution of HCl at pH 2, as a replacement for the traditional sample pre-treatment with acids before SPME sampling. OTA was separated in Waters Symmetry Shield RP18 column and two multiple reaction monitoring (MRM) transitions were monitored (402.1 to 357.9 as quantitation ion and 402.1 to 314.0 as confirmation ion). A miniaturized SPME fiber was adopted so that the concentration distribution of OTA in a small-sized cheese piece could be directly investigated. This direct analysis displayed comparable precision and accuracy to the conventional liquid extraction. The latter was reflected in its relatively high sensitivity (LOD = 1.5ng/mL) sufficient for OTA determination in real samples.

A sensitive method for the detection of OTA and OTB in nuts and grain was developed recently by Saito et al. [22]. It was based on an automated in-tube SPME - which uses an open tubular fused-silica capillary with an inner surface coating as the SPME device- coupled with LC-MS. Separation of toxins was achieved using an Inertsil ODS-3 column with a mobile phase consisting of ammonium acetate and acetonitrile within 5min. Electrospray ionization was performed in positive mode, selecting the pseudo molecular ion in selected ion monitoring mode. Sensitivity was in the pg/mL scale superseding the direct injection method, and recoveries approaching 90%. Application of this method to nuts and grain samples was effective in detecting residues of ochratoxins at ng/g level.

2.2.2. Aflatoxins

Nonaka et al. have published in 2009 a pertinent work based on in-tube SPME regarding determination of aflatoxins [23]. In this work in-tube SPME has been used in conjunction with LC-MS and aflatoxins were separated within 8 min in a Zorbax Eclipse XDB-C8 column. As mobile phase MeOH/acetonitrile and ammonium formate was used. Good linearity was obtained in a range of 0.05-2.0ng/ml and with LODs ranging from 2.1 to 2.8pg/mL. In commercial food samples analyzed toxins were detected below 10ng/g, verifying the detection capacity of the method.

2.2.3. Patulin

Patulin is analyzed mainly by GC-MS, HPLC and LC-MS. The GC-MS requires derivatization prior to analysis which set some limitations despite the low LODs observed. Thus in-tube SPME has been applied for patulin in conjunction with LC-MS for its determination in fruit juice and dried fruit samples [24]. The automation of this method was corroborated and it is in line with other in-tube SPME works regarding their efficiency and sensitivity. With regard to mass spectrometry detection this was performed in Selected Ion Monitoring (SIM) mode by monitoring the pseudo molecular ion in negative mode.

2.3. SPME and Other Extraction Tools

Hitherto, one main concern of analytical laboratories is to reduce the extraction time of compounds from matrices by the minimum use of organic solvents. A parallel target also is to reach satisfactory levels of detection apart from the acceptability in terms of recovery. In this frame SPME is a complementary tool that can comply with the above criteria.

The usual liquid extraction or modifications of it - such as accelerated solvent extaction - are still the most popular ways to extract toxins form various matrices as recently reported in various papers [25,26]. Other contemporary techniques have been elaborated for aflatoxins extraction such as microwave assisted extraction assisting in the accurate quantitation of these compounds [27].

In this regard, SPME supersedes other extraction methods in terms of simplicity and it can be implemented in specific applications especially in complex mediums as reported in this review. Hence SPME is a valuable extraction tool for toxins still other extraction procedures possesses the advantage of the inclusion of larger number of chemically diverse analytes.

Conclusions

Toxins constitute chemical compounds of priority interest. Improving their sampling and sample preparation methods is detrimental for research community which is in rigorous efforts to facilitate the sample preparation and clean-up steps and minimize as much as possible the detection limits of these compounds. SPME can be used as a satisfactory alternative to the conventional extraction techniques and include toxins in its broad spectrum of analytes.

Statement of Competing Interests

The authors have no competing interests.

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