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# Paralytic shellfish toxins in clinical matrices: Extension of AOAC official method 2005.06 to human urine and serum and application to a 2007 case study in Maine

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## ABSTRACT

Paralytic shellfish poisoning (PSP), a potentially fatal foodborne illness, is often diagnosed anecdotally based on symptoms and dietary history. The neurotoxins responsible for PSP, collectively referred to as the saxitoxins or paralytic shellfish toxins (PSTs), are natural toxins, produced by certain dinoflagellates, that may accumulate in seafood, particularly filter-feeding bivalves. Illnesses are rare because of effective monitoring programs, yet occasional poisonings occur. Rarely are contaminated food and human clinical samples (e.g., urine and serum) available for testing. There are currently few methods, none of which are validated, for determining PSTs in clinical matrices. This study evaluated AOAC (Association of Analytical Communities) Official Method of Analysis (OMA) 2005.06. [AOAC Official Method 2005.06 Paralytic Shellfish Poisoning Toxins in Shellfish: Prechormatographic Oxidation and Liquid Chromatography with Fluorescence Detection. In Official Methods of Analysis of AOAC International 〈http://www.eoma.aoac.org〉], validated only for shellfish extracts, for its extension to human urine and serum samples. Initial assessment of control urine and serum matrices resulted in a sample cleanup modification when working with urine to remove hippuric acid, a natural urinary compound of environmental/dietary origin, which co-eluted with saxitoxin. Commercially available urine and serum matrices were then quantitatively spiked with PSTs that were available as certified reference materials (STX, dcSTX, B1, GTX2/3, C1/2, NEO, and GTX1/4) to assess method performance characteristics. The method was subsequently applied successfully to a PSP case study that occurred in July 2007 in Maine. Not only were PSTs identified in the patient urine and serum samples, the measured time series also led to the first report of human PST-specific urinary elimination rates. The LC-FD data generated from this case study compared remarkably well to results obtained using AOAC OMA 2011.27 [AOAC Official Method 2011.27 Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay. In Official Methods of Analysis of AOAC International 〈http://www.eoma.aoac.org〉], further demonstrating successful extension of the LC-FD method to these clinical matrices. Moreover, data generated from this poisoning event reiterated that urine is a preferable clinical matrix, compared to serum, for diagnostic purposes due to higher accumulation and longer residence times in urine.

## 1. Introduction

While paralytic shellfish poisoning (PSP) is a well-known human intoxication and there is an ever-expanding knowledge base on the suite of congeners comprising the paralytic shellfish toxins (PSTs), much less is known about specific toxin accumulation, metabolism, and elimination in humans. The required clinical data are sparse since outbreaks have been limited via effective monitoring programs. Shellfish harvesting is closed when toxin levels exceed the established action level of 80 µg saxitoxin (STX) equivalents (eq) 100 $g^{-1}$, which ensures safety of commercial seafood products. Despite successful management and monitoring programs, poisonings do occur, often the result of

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recreational harvesting (e.g., Etheridge et al., 2006; McLaughlin et al., 2011). Still, a very limited number of clinical accounts have been detailed, owing further to the facts that (1) cases are often not diagnosed or are underreported (e.g., Scallan et al., 2011), (2) meal remnants and clinical samples are rarely available for testing, (3) there is usually a lengthy time lapse between toxin ingestion and clinical sample collection, and (4) there are no well-characterized methods for testing PSTs in clinical matrices.

Sparse reports of human poisonings and clinical cases have been described in the literature (e.g., Gessner et al., 1997; Llewellyn et al., 2002; García et al., 2004, 2005), and many of these clinical samples of opportunity were only collected post-mortem and without concurrent analysis of the suspected contaminated food. Further, these studies employed a wide range of detection methods including native receptor and saxiphilin binding assays as well as multiple variations of liquid chromatography with fluorescence detection, without extensive characterization of the method applied to the matrices tested. To provide definitive, analytical confirmation of suspected PSP cases and to fill knowledge gaps regarding toxicokinetics in humans, having a well-characterized, simple analytical method that can determine both toxicity and toxin content in the meal remnants and clinical matrices is desirable.

In this study, AOAC (Association of Analytical Communities) Official Method of Analysis (OMA 2005.06) (referred to as pre-chromatographic oxidation and liquid chromatography with fluorescence detection [LC-FD]) was investigated for its extension to urine and serum matrices. This method was originally validated for shellfish (mussels, clams, oysters, and scallops) for the determination of saxitoxin (STX), neosaxitoxin (NEO), gonyautoxins 2 and 3 (GTX2/3), gonyautoxins 1 and 4 (GTX 1/4), decarbamoyl saxitoxin (dcSTX), B1 (GTX5), and C toxins (C1/2 and C3/4). Immediately following extension of OMA 2005.06 to urine and serum, the method was used to analyze samples from a case study that occurred in Maine in 2007, which represented the first PSP illness reported in the state in at least 30 years (HAN, 2007). The case study (1) demonstrated that the method was robust for PST determination in naturally contaminated human urine and serum samples, (2) provided a comparison of toxin profiles in the clinical samples compared to the meal remnants, which aided diagnosis and confirmed the suspected food as the cause, (3) elucidated novel PST-specific human urine elimination rates, and (4) contributed substantively to the limited knowledge base regarding PST toxicokinetics in humans.

## 2. Material and methods

### 2.1. Extension of AOAC official method of analysis 2005.06 to urine and serum

### 2.1.1. Chemicals and reagents

The standard chemicals and reagents described in OMA 2005.06 were used in this study. Certified reference materials for dc-STX, GTX2/3, B1, C1/2, NEO and GTX1/4 were purchased from the National Research Council, Institute for Marine Biosciences (Halifax, Canada), and FDA reference standard STX was used. The urine (Level 2, Product #U9631, Accumark$^{TM}$) and serum (Product #IPLA-SER) controls were procured from Sigma Diagnostics and Innovative Research, Inc. (Southfield, MI), respectively. Commercial clams (*Mercenaria mercenaria*) were purchased from Mobjack Bay Seafood Inc. (Ware Neck, VA). These clams were harvested from James River, VA, in a region where PSTs are not reported. They were tested using OMA 2005.06 and confirmed to be PST-free, and they were subsequently used as a control matrix.

### 2.1.2. Matrix and standard solution preparations

The commercial urine (reconstituted in distilled deionized water [Milli-Q water purification system] following product insert instructions) and serum matrices were prepared daily and adjusted from a pH of $\sim$6.5–7 to a pH 4 using dilute hydrochloric acid. The pH adjustment was primarily for ensuring toxin stability. Control clam matrix was prepared using 100 g of homogenized *M. mercenaria* according to the extraction procedure in OMA 2005.06. Toxins were then spiked into the pH-adjusted matrices (urine, serum, control clam, and 1% acetic acid) prior to sample cleanup. For initially assessing the ability to resolve toxins of interest in these matrices, the concentrations of toxins spiked into each matrix represented the middle of the toxin calibration curves and were as follows (in ng ml$^{-1}$): 63 STX; 152 dcSTX; 292/96 GTX2/3; 154 B1; 68/21C1/2; 79 NEO; and 136/45 GTX1/4.

The procedural steps from sample preparation through cleanup and LC-FD analysis for urine and serum matrices are outlined in Fig. 1. After adjusting urine and serum to pH 4, each standard solution was passed through a solid phase extraction (SPE) C18 cartridge. When working with the serum matrix, all

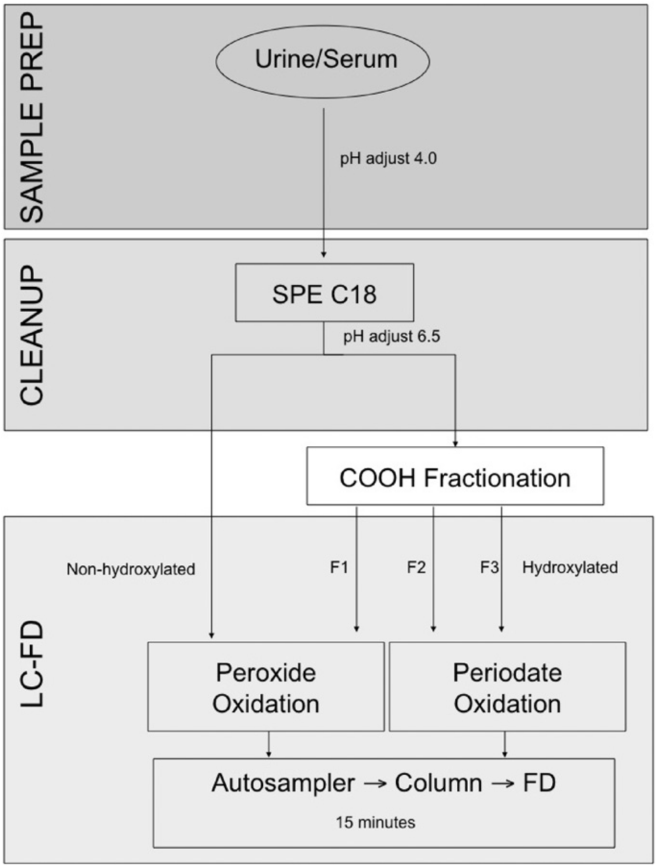

**Fig. 1.** Procedural steps from sample preparation through LC-FD analysis for urine and serum matrices. Urine and serum samples were adjusted to pH 4 prior to cleanup. Serum samples employed the same SPE C18 cartridges described in OMA 2005.06; however, the cartridge bed volume was doubled (6 ml) for urine. Toxins were washed from the SPE C18 cartridges and then adjusted to pH 6.5, and one aliquot was reserved for peroxide oxidation (this aliquot allowed for the identification of non-hydroxylated toxins present in the sample). Another aliquot of 6.5 pH-adjusted sample was passed through a carboxylic ion exchange cartridge (COOH) as described in OMA 2005.06 (without a matrix modifier) to separate toxins into three fractions: (F1) containing the C toxins, (F2) containing the GTXs, and (F3) containing STX, dcSTX, and NEO. From this fractionation, F2 and F3 were oxidized using periodate for the purpose of identifying hydroxylated toxins present in the sample. All oxidation products were analyzed immediately (completed within 24 h) using the LC-FD chromatography conditions as described in OMA 2005.06.

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