Journal of Chromatography B 1072 (2018) 267–272

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Fast and quantitative determination of saxitoxin and neosaxitoxin in urine by ultra performance liquid chromatography-triple quadrupole mass spectrometry based on the cleanup of solid phase extraction with hydrophilic interaction mechanism

Xiao-min Xu*, Bai-fen Huang, Jiao-jiao Xu, Zeng-xuan Cai, Jing Zhang, Qing Chen, Jian-Long Han*

Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, 310051, China

ARTICLE INFO

Keywords:
Saxitoxin
Neosaxitoxin
Hydrophilic interaction
Solid phase extraction
Urine
Liquid chromatography-triple quadrupole mass spectrometry

ABSTRACT

Saxitoxin (STX) and neosaxitoxin (NEO) are water-soluble toxins and their cleanup in bio-matrix is a hot topic but difficult problem. A fast and quantitative determination method for STX and NEO in urine was developed using ultra performance liquid chromatography-triple quadrupole mass spectrometry (LC–MS/MS) based on the cleanup of solid phase extraction (SPE) with hydrophilic interaction (HILIC) mechanism. Acetonitrile/methanol/water mixture was used to extract the toxins in urine. Polyamide (PA) was used as HILIC SPE material to clean the toxins in sample matrix. The limits of detection were 0.2 ng mL^{-1} for STX and 1 ng mL^{-1} for NEO in urine. The linear ranges were 0.5 ng mL^{-1}–99.2 ng mL^{-1} with the correlation coefficient of $r = 0.9992$ for STX and 2.1 ng mL^{-1}–207 ng mL^{-1} with $r = 0.997$ for NEO in urine matrix. The recoveries at three spiking levels were 81.5%–117% with the relative standard deviations (RSDs) of 5.4%–8.5% for STX and 89.0%–118% with the RSDs of 6.7%–9.1% for NEO. STX was found in all the 6 patients' urines while NEO was only found in one sample from an intoxication case.

1. Introduction

Paralytic shellfish toxins (PSTs) are marine biotoxins naturally produced by dinoflagellates [1,2]. They are neurotoxins causing so called paralytic shellfish poisoning (PSP) and are even lethal to humans [2]. Poisoning cases have been reported for the consumption of PSTs contaminated bivalves [3–6]. Saxitoxin (STX) and neosaxitoxin (NEO) are typical PSTs. They are well applied as the bio-marker for the forensic diagnosis of related intoxication cases [3,7,8].

Toxin analysis in biomaterial is necessary for forensic diagnosis when poisoning case happens. Garcia et al. reported that STX and NEO were removed from the body by excretion in urine [9]. Comparing to the bio-material of blood, urine is easier to obtain without any harm to patient. In the reported intoxication cases, PSTs were found in all of the patients' urine [5,6,8–12]. However, the contents were very low and the toxins were hard to be detected in blood as reported by Rodrigues et al. [6]. Urine would be one of the most typical bio-specimens for the forensic diagnosis about PSTs intoxication cases.

Liquid chromatography-triple quadrupole mass spectrometry (LC–MS/MS) presents high sensitivity for the determination of STX and NEO in urine samples [5,8]. However, strong matrix interference makes it important for finding an effective sample preparation method before LC–MS/MS measurement. Solid phase extraction (SPE) with C18 was used for the cleanup of the analytes [6,13,14]. However, only hydrophobic components can be removed. Hydrophilic interferences, which are co-eluted with the toxins in sample matrix, cannot be cleaned by C18 [14]. SPE with weak cation exchange material can be used effectively for cleanup of sample matrix [7,8] while time consumed drying steps were required. It's important to develop an applicable SPE method for the fast, accurate and sensitive determination of STX and NEO in urine by LC–MS/MS.

Hydrophilic interaction chromatography (HILIC) columns were well applied for the chromatographic separation of water-soluble or polar compounds [1,15–20]. The HILIC mechanism was also used for the development of SPE material in recent years. Turrell et al. reported a computationally designed polymer (CDP) based on 2-(trifluoromethyl) acrylic acid as the HILIC SPE material to clean PSTs in shellfish sample [21]. Rodriguez et al. used a CDP based on the functional monomer

* Corresponding authors.
E-mail addresses: chemxuxm@163.com (X.-m. Xu), jlhan@cdc.zj.cn (J.-L. Han).

https://doi.org/10.1016/j.jchromb.2017.11.032
Received 1 June 2017; Received in revised form 22 November 2017; Accepted 24 November 2017
Available online 26 November 2017
1570-0232/

ethylene glycol methacrylate phosphate (EGMP) to purify STX and NEO [22].

In this study, an applicable and easy to be obtained HILIC SPE material with polyamide (PA) was introduced to clean STX and NEO in urine. The study included the sample preparation procedure, the HILIC SPE mechanism between the toxins and PA.

2. Experimental

2.1. Chemicals and reagents

All reagents and solvents were of analytical grade unless specified.

Certified calibration solution of STX and NEO were purchased from the National Research Council of Canada (Halifax, NS, Canada). HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Formic acid was supported by ROE Scientific Inc. (New Castle, DE, USA) and ammonium formate was provided by Sinopharm Chemical Reagent (Shanghai, China). Solid phase extraction (SPE) cartridge containing polyamide material (PA, 500 mg, 3 mL, JXA5003) was provided by Agela Technologies (Tianjin, China). Ultrapure water was prepared using a Millipore system (Bedford, MA, USA).

2.2. Preparation of the standard solution

The concentrations (C_0) of the certified calibration solution are listed in Table 1. They were mixed and diluted with water to prepare the standard spiking solution (C_1 and C_2). The matrix-matched calibration curve of each analyte was prepared with seven concentration levels ranged from 0.5-99.2 ng mL^{-1} for STX and 2.1–207 ng mL^{-1} for NEO by diluting C_1 or C_2 with the matrix solution extracted from toxins free urine.

2.3. Sample preparation

2.3.1. Homogenized urine samples

Toxins free urine sample was obtained from 5 healthy volunteers. All urines were mixed together and centrifuged at 8000 rpm for 5 min. The supernatant was used as homogenized toxins free urine sample.

The urine samples containing toxins were obtained from six patients in a poisoning case caused by consumption of toxins contaminated mussels. Each sample was mixed and centrifuged at 8000 rpm for 5 min before use.

2.3.2. STX and NEO analysis in urine

An aliquot of 0.2 mL of urine, 0.2 mL of water, 0.1 mL of methanol and 1 mL of acetonitrile were mixed by vortexing for 0.2 min. The mixture was centrifuged at 14000 rpm for 2 min. The supernatant was then transferred to the PA SPE cartridge, which was pre-equilibrated with 2 mL of 50% (*v/v*) of acetonitrile/water and 2 mL of pure acetonitrile in sequence. The eluent was discarded. Then, 1 mL of acetonitrile, 2 mL of 80% (*v/v*) of acetonitrile/water and 0.5 mL of 80% (*v/v*) of acetonitrile/water containing 0.5% (*v/v*) formic acid were used to wash the SPE cartridge one by one. The eluent in each step was discarded again. Finally, the analytes were eluted with 2 mL of 80% (*v/v*) of acetonitrile/water containing 0.5% (*v/v*) formic acid. The total 2 mL of the eluate was collected and mixed for 0.1 min. The resulting mixture was centrifuged at 14000 rpm for 5 min and ready for LC–MS/MS measurement.

2.3.3. Optimization of the urine volume loaded onto the SPE cartridge

An aliquot of 0.2 mL, 0.3 mL and 0.4 mL of toxins free urine samples were added to different test tubes. Each sample was spiked with 5 μL of C_1 (Table 1) and vortex mixed for 5 min. For matrix effects study, toxins free samples were tested for corresponding conditions. The total water volume was adjusted to 0.4 mL by adding the pure water with 0.2, 0.1 and 0 mL, respectively, for each sample. The volumes of methanol and acetonitrile were the same of 0.1 and 1 mL, respectively. Each sample was vortex mixed and then loaded onto different PA cartridges. The next steps were operated the same as described in section *2.3.2*. Three repetitions were done for each sample.

2.3.4. Method recovery and precision

An aliquot of 5 μL of C_2 (2.5 ng mL^{-1}/2.6 ng mL^{-1}, STX/NEO), 20 μL of C_2 (9.9 ng mL^{-1}/10.3 ng mL^{-1}, STX/NEO) and 10 μL of C_1 (49.6 ng mL^{-1}/51.5 ng mL^{-1}, STX/NEO) of standard mixture solutions were spiked into 0.2 mL each of toxins free urine sample. The values of C_1 and C_2 are listed in Table 1. After vortex mixed for 5 min, each sample was added with 0.2 mL of water, 0.1 mL of methanol and 1 mL of acetonitrile and vortex mixed for 0.2 min. The next steps were performed the same as described in section 2.3.2. Six repetitions were done for each spiking level.

2.4. Instrumentation

STX and NEO were measured by an UPLC–MS/MS instrument equipped with an ESI source (8050, Shimadzu, Japan). UPLC separation was performed on an XBridge BEH Amide column (2.1 × 100 mm, 1.7 μm, Waters) with a flow rate of 0.4 mL min^{-1}. The oven was kept at 30 °C. The sample injection volume was of 5 μL. The mobile phase was consisted of water containing 3.6 mM formic acid/2 mM ammonium formate in channel A and acetonitrile in channel B. The gradient elution was programmed as follows: 85–40% B (0–4 min), 40% B (4-4.5 min), 40–85% B (4.5–5 min), 85% B (5–8 min). Argon was used as CID gas under 270 kPa. The flow rates of heating gas (compressed air), nebulizing gas (nitrogen) and drying gas (nitrogen) were of 10 L min^{-1}, 3 L min^{-1} and 10 L min^{-1}, respectively. The desolvation line (DL), MS interface and heat block temperatures were 200 °C, 300 °C and 400 °C, respectively. STX and NEO were detected under ESI^+ mode. Multiple reaction monitoring (MRM) mode was used for MS/MS measurement and the transitions are listed in Table 2 as consulted to the reference [23].

Table 1
Concentrations of STX and NEO in solvent.

Toxins	Formula	MW[a]	Molarity[b]	C_0[c]	C_1[d]	C_2[d]
			(μmol L^{-1})	(μg mL^{-1})	(ng mL^{-1})	(ng mL^{-1})
STX	$C_{10}H_{17}N_7O_4$	299.29	66.3	19.8	992	99.2
NEO	$C_{10}H_{17}N_7O_5$	315.29	65.6	20.7	1030	103

[a] MW: Molecular weight.
[b] Concentrations of the certified calibration solution with molarity.
[c] C_0: Concentrations calibrated from the molarity of the certified calibration solution.
[d] C_1 and C_2: Concentrations of the standard mixture spiking solution diluted from C_0 with water.