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# Using tandem mass spectrometry and liquid chromatography in conjunction with hydrophilic interaction/ion-exchange mixed-mode solid phase extraction, it is simple and quick to determine the presence of tetrodotoxin in human plasma

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**AUTHORS' CONTRIBUTION: (A)** Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** No Fund Collection

**ABSTRACT**

In this study, we created and validated a straightforward, quick, and sensitive LC-MS/MS approach for tetrodotoxin detection in human plasma. We compared three solid phase extraction carriers of the HILIC type with various stationary phase functional groups. The Siphila HILIX SPE plate with multi-carboxyl groups was ultimately chosen because it had a clearly superior extraction recovery of TTX (about 80% of recovery from plasma samples) compared to the other two, and because no significant matrix effects were noticed. This was thought to be the result of mixed-mode synergistic effects of hydrophilic interaction and ion exchange. Acetonitrile with 1% trichloroacetic acid was used to quickly precipitate a 100 mL sample of plasma, and the filtrates were then put onto a Siphila i HILIX 96 well SPE plate. TTX was extracted with 200 mL of 50% acetonitrile after being washed with 95% acetonitrile. LC-MS MS and the whole run duration on a BEH amide column were both immediately injected with trichloroacetic acid. Of the elution solution. The procedure skips evaporation and ultrafiltration, which is straightforward and efficient. In positive mode, and were used to monitor the internal standard and TTX, respectively. The intra- and inter-assay accuracies of the method were in the ranges of 98.5%-99.8% and 98.8-99.5%, respectively, with the low limit of quantification. The method's good anti-interference property was demonstrated by the fact that biases of spiking analysis varied from -7.00% to 7.43% for healthy human plasma samples (RSDs 8.83%) and from -5.00% to 3.93% for hemolytic, high triglyceride, high cholesterol, and high bilirubin plasma samples. Human health is seriously threatened by tetrodotoxin. LC-MS MS and the whole run duration on a BEH amide column were both immediately injected with trichloroacetic acid of the elution solution.

Keywords: Tetrodotoxin; Hydrophilic-interaction/ion-exchange; Mixed-mode solid phase extraction; LC-MS/MS; Human plasma

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## INTRODUCTION

The procedure skips evaporation and ultrafiltration, which is straightforward and efficient. In positive mode, and were used to monitor the internal standard and TTX, respectively [1]. The intra- and inter-assay accuracies of the method were in the ranges of 98.5%-99.8% and 98.8-99.5%, respectively, with the low limit of quantification [2]. The method's good anti-interference property was demonstrated by the fact that spiking analysis biases varied from -7.00% to 7.43% for healthy human plasma samples and from -5.00% to 3.93% for hemolytic, high triglyceride, high cholesterol, and high bilirubin plasma samples [3]. Human health is seriously threatened by tetrodotoxin the coast, where the primary food source is marine food [4]. Results demonstrated that the approach is sensitive, accurate, specific, and reliable and can be used to monitor the concentration of TTX in plasma to suit the objectives of clinical research and poisoning screening [5]. Cases of food poisoning caused by TTX were recorded virtually every year [6]. Even though numerous techniques for TTX determination, including mouse bioassay, immunoassay, nuclear magnetic resonance, and liquid chromatography with fluorescence detection, have been described, the majority of them are for fish or shellfish tissue samples [7]. Dealing with human biological samples is substantially more complicated because the level of TTX in the urine and plasma is often extremely low [8]. The method of tandem mass spectrometry with liquid chromatography was thought to be the most widely used one for the identification and quantification of Hydrophilic interaction liquid chromatography demonstrated strong retention capabilities and enhanced ionisation efficiency in LC-MS, which could assist to overcome these problems [9]. A further difficulty in analysing TTX in biological samples was ion suppression [10]. In most cases, nondetectable interferences coelute and cause ion suppression. Ion suppression may generally be the sample preparation process is the most difficult analysis bottleneck. Because it is difficult to effectively extract and enrich it from complex human biological samples. Solid phase extraction, which often employs cation exchange extraction, is the most widely used method for the cleanup of TTX samples. TTX is strongly maintained on SPE cartridges thanks to interactions between its positively charged guanidinium group and the stationary phase's

sulfonic acid group. It can only be dissolved by strong acids like hydrochloric acid, which necessitates extra solvent evaporation and labour-intensive processes for residual dissolution. Strong cation exchange extraction techniques are thus currently infrequently employed for the detection of TTX. The usage of graphitized carbon carriers is another SPE method utilised for TTX detection. The TTX does, however, rebound.

## DISCUSSION

There are currently big needs in pharmaceutical drug discovery for the examination and purification of structurally varied samples before or after high-throughput screening. These procedures are necessary for the quick and precise biological profiling, structural analysis, and replenishment of new drug candidates. For several of these applications, electrospray ionisation mass spectrometry in combination with reversed-phase high-performance liquid chromatography has emerged as the go-to method for tiny molecule separation/detection. However, the hydrophobicity-based resolution of relatively nonpolar sample components has been the extent of the separation selectivity offered by RP-HPLC, and for high-throughput drug discovery applications, no adequate alternative approaches have been found. A mixed-mode anion-cation exchange/hydrophilic interaction chromatography technique has been created for this inquiry to enable both direct compatibility with Highly orthogonal to RP-HPLC in terms of detection and separation selectivity are ESI-MS and evaporative light scattering. The method used silica-based small-pore weak ion exchange resins that were eluted using an aqueous and pH gradient simultaneously. To clarify the relative contributions of three retention processes, a variety of dipeptide probes were used. For the analysis and purification of molecules from both biological and synthetic sources of molecular variety, ACE-HILIC-ESI-MS-ELSD should be helpful. Natural toxins comprise a variety of hazardous metabolites that are present in food and other items and pose a threat to consumer health. Several reliable and sensitive analytical techniques that can identify their presence in food have been developed in the recent several decades. Due to its benefits in terms of sensitivity and selectivity, liquid chromatography mass spectrometry is the most effective technique for the simultaneous detection of these poisons.

## CONCLUSION

This article provides a thorough evaluation of the most important studies on techniques based on liquid chromatography mass spectrometry for the detection of mycotoxins, alkaloids, marine toxins, glycoalkaloids, cyanogenic glycosides, and furocoumarins in food. In particular, a search of the literature from 2011 to 2021 was done, and 96 papers in all were chosen. Various methods for sample preparation, The detection mechanism and chromatographic separation are discussed. The analytical performance characteristics discovered during the validation procedure and the pertinent application to actual samples are given special consideration. Natural poisons are a diverse group of hazardous compounds produced by a variety of creatures, including mammals, specific plant species, and microbes. They can be created exogenously when they are produced during the metabolism of living things or endogenously when they are produced by organisms that are frequently found in food. In the final scenario, poisons appear in food as unintentional contamination. Others are unaffected by ordinary food processing techniques like baking, cooking, and frying. Some of them are only present in fresh crops and can be substantially removed by utilising adequate processing. These toxins can result in a variety of harmful effects due to their diverse chemical structures, biological roles, incidence, modes of action, and toxicity. health consequences, such as gastrointestinal or allergic reactions, and even death in the event of acute exposure. Immunosuppressive, reproductive, systemic, or cancer genic consequences could result from prolonged exposure. Consumers are constantly exposed to a vast variety of these natural poisons at varied levels due to the extremely variable foods and amounts consumed in daily meals, which is a major public issue. Depending on the dose of exposure, the chemical combinations may have a combination of consequences. Risk assessment for human exposure to complex mixes of natural poisons found in food, however, remains a significant difficulty. Consumption needs to be restricted when the poisons can't be eliminated or lowered. These natural poisons have been categorised using a number of different terminology.

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