Simultaneous Analysis of Different Algal Toxins by LC-MS


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Summary
A method is proposed for the simultaneous determination of amnesic shellfish poisoning (ASP) toxin, diarrhetic shellfish poisoning (DSP) toxins, spirolydes, azaspiracids (AZP), pectenotoxins (PTX), brevetoxins (PbTx), and gymnodimine. After extraction of all these toxins with one solvent only the crude extract is subjected directly to reversed-phase LC-MS with atmospheric-pressure ionization.

The method was applied to bulk plankton samples obtained during a research cruise off the east coast of Scotland in May 2000. Contamination of plankton samples from one spot with different toxins from a variety of groups was determined.

Introduction
During the past two decades a variety of structurally different marine algal-borne toxins has been discovered [1]. Examples of the chemical structures of some of the most important algal toxins and an indication of the diversity of molecules involved are given in Figure 1. Figure 1a shows the structure of spirolyde toxins [2, 3]. Modifications of the molecule occur at position 31, where R can be either hydrogen or a methyl group. Between positions 2 and 3 a single bond or a double bond is possible. So-called desmethyl variations lack a methyl group other than at position 31 [4]. Figure 1b shows saxitoxin [5], the most prominent PSP toxin. Structural variations occur at position 1 (hydrogen or hydroxy group) and at position 11 (sulfate groups). The carbamoyl group at position 6 can also be replaced by a hydroxy group or a N-sulfo carbamoyl group [6]. Three modifications of azaspiracids (Figure 1c) have been described; these vary at R1 and R2, which can be a hydrogen or a methyl group [7] bound to the polyether basic structure [8]. Although numerous structural variations of domoic acid (Figure 1d) [9, 10], a glutamic acid agonist, have been described, their importance as naturally occurring algal toxins is negligible. The most important DSP toxins [11–16], which have a polyether structure [17], belong to the ‘okadaic acid type’ (Figure 1e), where R1 is a hydroxy group, R2 can be hydrogen or an acyl group from different fatty acids. Okadaic acid (OA) [18, 19] has a methyl group at position 31 and hydrogen at position 35. Its isomer dinophysistoxin 2 (DTX-2) [16] has the substitution at these positions reversed. Dinophysistoxin 1 (DTX1) has methyl groups at both positions. Figure 1f shows a typical brevetoxin (PbTx2) molecule [20, 21], with a polyether, ladder-like structure [22], and Figure 1g shows gymnodimine [3], an exotic toxin, found exclusively in the South Pacific [23–25].

The threat of algal toxins to human consumers and related economic losses by aquatic industries such as fish farming, mussel farming and fisheries have been described many times [1, 26–28]. Preventive measures for consumer protection, and basic scientific research on harmful algal blooms, require powerful analytical methods. Such methods were developed for almost all the individual groups of toxins when they were discovered. Because of structural differences between the molecules (Figure 1), methods are based on ion-pair chromatography, ion-exchange chromatography, and reversed-phase chromatography coupled with UV and fluorescence detection and pre- or post-column derivatization procedures had to be developed to label toxin molecules with chromophoric groups [29]. Complicated and labor intensive clean-up procedures are necessary before chromatographic se-
Figure 1. The structures of algal toxins found or sought in a survey off the east coast of Scotland during the cruise in May, 2000. (a) Spirolides: spirolide C, R = CH₃, double bond between positions 2 and 3; spirolide D, R = CH₃, single bond between positions 2 and 3; the so-called desmethyl variations lack a methyl group at a position other than 31, e.g. 13-desmethyl C-spirolide. (b) Saxitoxin: structural variations are possible at positions 1 (hydrogen, hydroxy group) and 11 (sulfate groups). The carbamoyl group at position 6 can be replaced by a hydroxy group or an N-sulfocarbamoyl group. (c) Azaspiracids (AZP); azaspiracid-1 (AZP-1), R₁ = H, R₂ = CH₃; azaspiracid-2 (AZP-2), R₁ = CH₃, R₂ = CH₃; azaspiracid-3 (AZP-3). (d) Domoic acid (DA); numerous structural variations whose role as natural occurring algal toxins is negligible. (e) Okadaic acid-type toxins: okadaic acid (OA), R₁ = OH, R₂ = H, R₃ = CH₃, R₄ = CH₃; dinophysistoxin 1 (DTX-1), R₁ = OH, R₂ = H, R₃ = CH₃, R₄ = CH₃; dinophysistoxin 2 (DTX-2), R₁ = OH, R₂ = H, R₃ = H, R₄ = CH₃. (f) Brevetoxins (PbTx): brevetoxin 2 (PbTx-2), R = CH₂C(=CH₂)CHO; brevetoxin 3 (PbTx-3), R = CH₂(=CH₂)CH₂OH. (g) Gymnodimine, an exotic toxin, found exclusively in the South Pacific.

From an analytical perspective determination of the PSP toxins is a very special problem. Because of their ionic character this group of toxins must be separated by either ion-pair or ion exchange chromatography [29]. The high concentrations of ions in both chromatographic procedures cause difficulties when the techniques are coupled on-line with mass spectrometry. The mobile phases used are also quite different from those used for chromatographic separation of other algal toxins. Simultaneous determination of different algal toxins by LC-MS did not, therefore, include PSP toxins.

When surveying the east coast of Scotland bulk plankton samples from approximately 200 different sampling sites from an extended marine area covering 240 by 60 nautical miles were collected within 3 weeks. All samples were analyzed independently for DSP, domoic acid (DA), spirolides, and for PSP, PTX, PbTx (the data are not given, and will be published separately). The possible occurrence of AZP to the east of Scotland was also checked (again the data are not shown).

The development of a fast and economical screening method enabling the on-board determination of numerous toxins with one chromatographic arrangement became an urgent need for further cruises.

The method described in this paper enables the simultaneous determination of ASP toxin, DSP toxins, spirolides, AZP, PTX, PbTx, and gymnodimine. It was validated for the toxins commercially available as standards (DA, OA, 35-methyl-okadaic acid (dinophysistoxin 1, DTX-1), brevetoxin 2 (PbTx-2), and brevetoxin 3 (PbTx-3)). Recovery and accuracy were tested with reference material containing DSP toxins. The method was applied to sample material obtained during a re-

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