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Note

High-performance liquid chromatographic assay for domoic acid in serum of different species

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In November and December of 1987 more than 200 people became ill after ingesting cultured blue mussels (*Mytilus edulis*) harvested from eastern Prince Edward Island. Within approximately three weeks researchers at the Atlantic Canada Division of the National Research Council of Canada isolated the molluscan toxin and identified it as domoic acid [1], the structure of which is shown in Fig. 1. Domoic acid is an amino acid that was originally isolated from the red algae *Chondria armata* in 1956 by Takemoto et al. [2] and has been

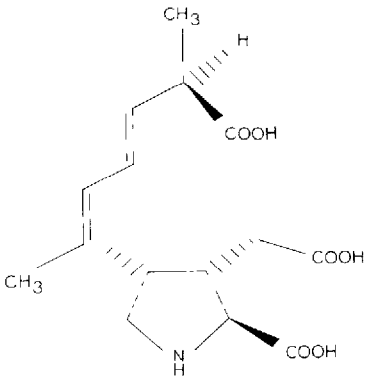

Fig 1. Structure of domoic acid.

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shown to have insecticidal and neurotoxic effects in vitro [3–6] and in vivo [7].

Following the outbreak of toxicity much research was directed towards producing qualitative and quantitative procedures for the detection of domoic acid in both mussel tissue and sea water. These procedures included a mouse bioassay previously used to detect other molluscan toxins [8], reversed-phase high-performance liquid chromatography (HPLC) with UV detection of domoic acid extracted from mussels [9] and more recently a fluorescence derivatization technique used for detecting concentrations of domoic acid in sea water [10].

To date, however, there have been no reports of methods to assay domoic acid in body fluids. A suitable method for quantifying domoic acid concentrations in body fluids, especially blood, is essential to studies on the pharmacodynamics and fate of domoic acid in vivo in different species. The present paper describes a simple preparation and chromatographic technique for the assay of domoic acid concentrations in serum.

EXPERIMENTAL

Apparatus and conditions

All samples were analysed using a Perkin-Elmer (Montreal, Canada) HPLC system consisting of an SEC-4 environmental control chamber, ISS-100 autoinjector, Series 410 LC pump, LC 90 UV spectrophotometric detector and an LCI-100 computing integrator. The UV detector was fixed at 242 nm (the absorption maximum for domoic acid) and the stainless-steel column (250 mm × 4.1 mm I.D.) was filled with 10-μm Partisil® ODS-3 packing (Whatman, Clifton, NJ, U.S.A.).

The mobile phase was an acetonitrile–distilled water (10.5:89.5, v/v) mixture adjusted to pH 3.0 with phosphoric acid. Solvent was delivered at a flow-rate of 1.5 ml/min for 19 min followed by 3.0 ml/min for 11 min. The injection volume was 20 μl.

Chemicals and solvents

HPLC-grade acetonitrile, phosphoric acid, chloroform and methanol were purchased from Fisher Scientific (Halifax, Canada). Serum was collected from male Long-Evans rats (250–350 g) (Charles River, Montreal, Canada), male English shorthair guinea pigs (400–500 g) (Charles River) and rainbow trout (*Salmo gairneri*) from a local hatchery. Domoic acid was obtained by acid extraction of contaminated mussels [9] with subsequent confirmation of purity and concentration by analytical HPLC [9].

Extraction procedure

The extraction procedure used was a modification of a lipid extraction method originally described by Bligh and Dyer [11] and later by Grimmelt [12]. Blank or spiked serum was placed in a culture tube. An aliquot (80 μl) of serum was removed and added to a second test tube containing chloroform (100 μl) and methanol (200 μl). This solution was then mixed using a vortex-mixer for 1 min. To the same tube were then added the remaining 100 μl of serum and an additional 100 μl of chloroform. Following an additional 1 min on a vortex-mixer the tubes were centrifuged (Model TJ-6, Beckman Instruments, Toronto, Canada) at 850 *g* for 12 min.

From the triphasic suspension the top phase (methanol–water) (approximately 200 μl) was removed for HPLC analysis (duplicate 20-μl injections via an autosampler).

RESULTS AND DISCUSSION

Typical chromatograms for blank serum and serum containing domoic acid are shown in Fig. 2A and B. Peaks produced by unidentified serum components elute during either the initial 7.8 min or after about 14 min. No interfering peaks were seen in any sample at or near the elution time for domoic acid (11 min, see Fig. 2A). Fig. 2B shows a chromatogram obtained following extraction of rat serum spiked with 1.0 μg/ml domoic acid. The limit of quantitation for

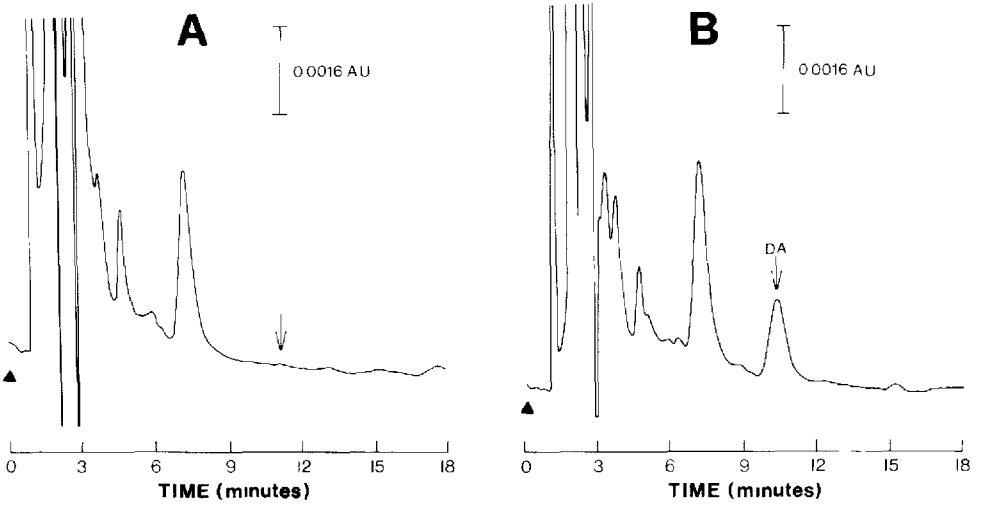

Fig. 2. (A) Chromatogram of control rat serum. Arrow indicates normal retention time of domoic acid. (B) Chromatogram of rat serum containing 1.00 μg/ml domoic acid (DA). Arrow indicates domoic acid ▲ indicates time of injection.

domoic acid in serum is 0.20 μg/ml with an extraction efficiency (mean $\pm$ S.D.) of 89.0 $\pm$ 1.73% for 1.0 μg/ml and 87.3 $\pm$ 5.86% for 0.5 μg/ml ($n=4$).

A composite ($n=10$) serum standard curve was described by the equation $y=0.0344x+0.0032$. Serum standard curves were linear throughout the range of concentrations tested (0.2–2.0 μg/ml) ($r^2=0.999$). Concentrations of domoic acid substantially greater than 2.0 μg/ml are approaching the threshold for toxicity in rodents and would, therefore, seldom be encountered in normal pharmacokinetic studies. To determine whether standard curves were reproducible, the regression equations for consecutive curves were compared using an analysis of variance (ANOVA). Standard curves for domoic acid in serum ($n=3$) were not significantly different either within day ($p \geq 0.27$) or between days ($p \geq 0.17$) making an internal standard unnecessary (overall within-day coefficient of variation = 6.5%).

It is important to note that the assay is also applicable to guinea pig and trout serum. Extracted blank guinea pig serum produced no interfering peaks after 8.4 min while trout serum produced peaks during the first 8.1 min and after 17.6 min. The elution times for domoic acid were 11.3 and 11.5 min, respectively.

The simple two-step extraction procedure described herein makes this assay well suited to situations in which a number of samples need to be analyzed in a reasonably short period of time (e.g. pharmacokinetic studies). Despite its apparent simplicity, this assay demonstrates a high degree of reproducibility and reasonable sensitivity and is applicable to blood samples from a variety of animal models. We believe that this procedure will facilitate many important studies of the in vivo pharmacology of domoic acid.

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