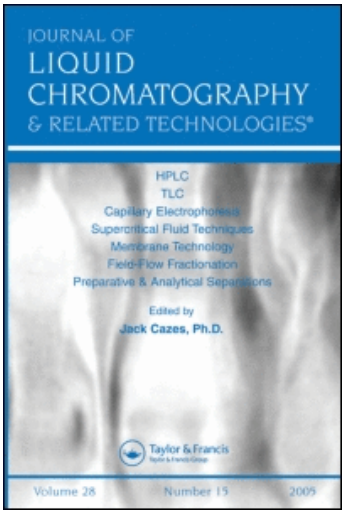

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ANALYSIS OF PALYTOXIN BY LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS

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ABSTRACT

Palytoxin (PTX) a non-protein molecule extracted from soft coral of the genus *Palythoa* is the most poisonous marine toxin known to date. We have established high-performance-capillary electrophoresis (HPCE) and liquid-chromatography (HPLC) methods for the analysis of PTX. The detection limit of the HPLC method was 125 ng/injection. The sensitivity of the HPCE method was 0.5 pg/injection, several times greater than the HPLC method. The detection sensitivity at 230 nm was two fold higher than that at 263 nm. The sample analysis time for both methods was about the same (10 min). The development of an HPCE method will allow the measurement of PTX at low concentrations in small sample volumes, which was not possible by HPLC.

INTRODUCTION

Palytoxin (PTX), a toxic macromolecule (Fig. 1) isolated from soft coral of the genus *Palythoa*, is the most poisonous marine compound known to date (1). PTX ($C_{129}129H_{223}N_3O_{54}$) consist of a long aliphatic, partially unsaturated chain with interspersed cyclic ether, 41 hydroxyl groups, 64 chiral centers (2) and its successful synthesis was recently achieved (3,4). Human fatalities due to ingestion of the sea food containing palytoxin-like toxin was reported (5). The molecular mechanism of palytoxin is still unknown, but it impairs the function of smooth muscle, skeletal muscle and neuronal cells (6). It is purified using powdered PE and sephadex columns and eluted with 50% ethanol (1). Analytical HPLC methods for PTX determination requires quantities in the range of 4-10 ug/injection (1,7). Recently, we have established a sensitive HPLC and HPCE methods for PTX at nanogram quantities as summarized herein.

MATERIALS AND METHODS

PTX (Hawaii Biotech, Honolulu, Hi) solutions were prepared in water and stored in glass at -20°C at 1 ug/ml. Solutions of PTX were scanned from 190 to 290 nm using a UV spectrophotometer (DU-70, Beckman Inst., Fullerton, CA).

Chromatographic analysis of PTX was performed on BIO-SIL 5 ODS, 250 X 4 mm i.d. (BIO-RAD, Richmond, CA) using an eluent of 52:48 mixture water:acetonitrile with 0.1% trifluoroacetic acid, 1 ml/min (Beckman Programmable Solvent Module 126), and monitored at 230 and 263 nm

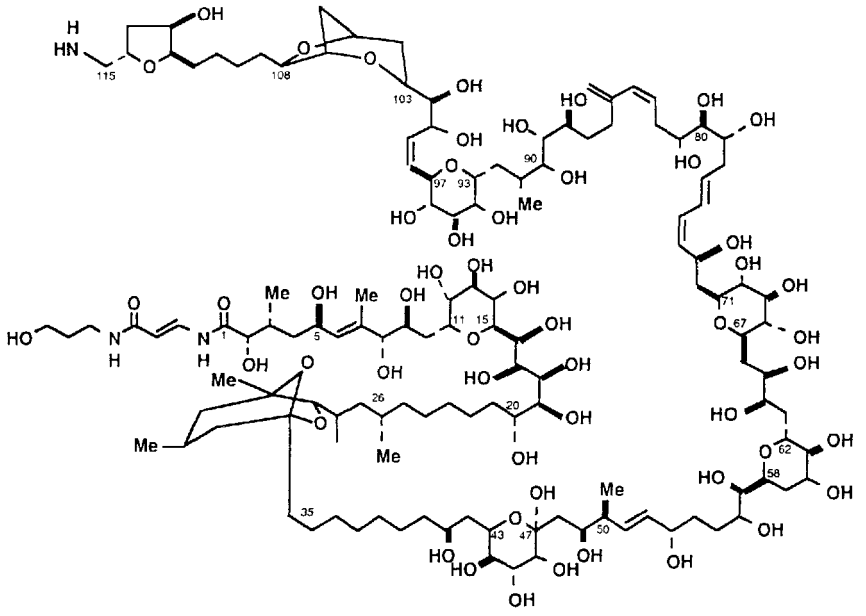

FIGURE 1. Structure of Palytoxin (from reference 2).

(Beckman Scanning Detector Module 167). Data were collected using Beckman System Gold™ Chromatography Software.

Electrophoresis was performed using an open-capillary (P/ACE System 2000, Beckman Inst., Fullerton, CA) and applying a voltage of 15kV across a 50 cm X 75 um column. The column temperature was maintained at 25°C, and a conducting buffer solution of 25 mM sodium borate at pH 8.5. A 5 nl samples of PTX was injected onto the capillary cartridge, eluted and detected at 230 and 263 nm. Data were collected using Beckman System Gold™ Chromatography Software.

RESULTS AND DISCUSSION

The maximum absorption wavelengths for PTX were found to be 230 and 263 nm (Fig. 2) as previously reported (1). The absorptions at either

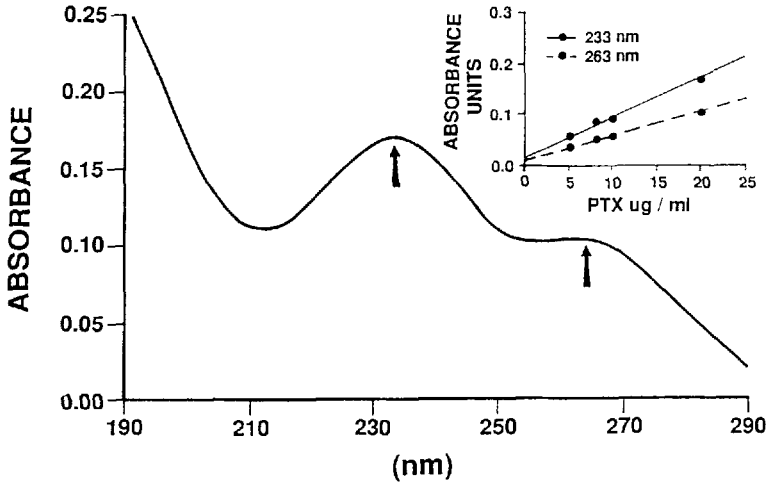

FIGURE 2. The ultraviolet spectrum of palytoxin at 20 ug/ml in water.

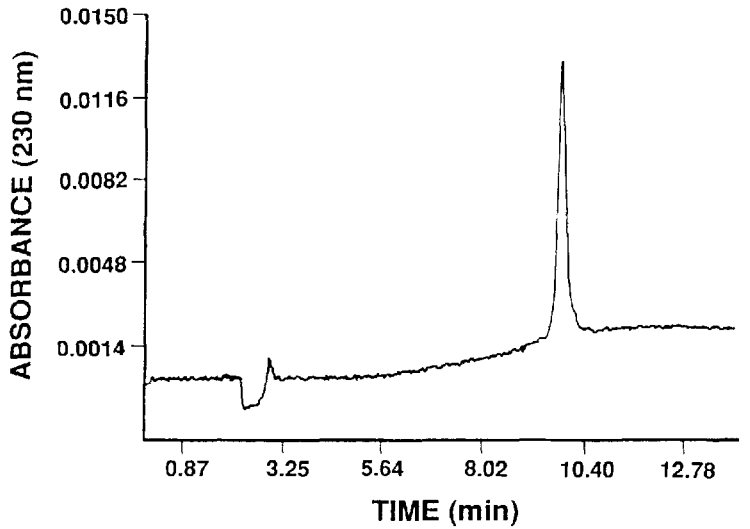

FIGURE 3. Chromatogram of palytoxin.

HPLC conditions:

Column:	BIO-SIL 5 ODS
Dimensions:	250 X 4 mm
Mobile Phase:	52:48 water:acetonitrile with 0.1% trifluoroacetic acid.
Flow Rate:	1.0 ml/min
Detection:	UV @ 230 nm
Sample:	50 ul from 2.5 ug/ml

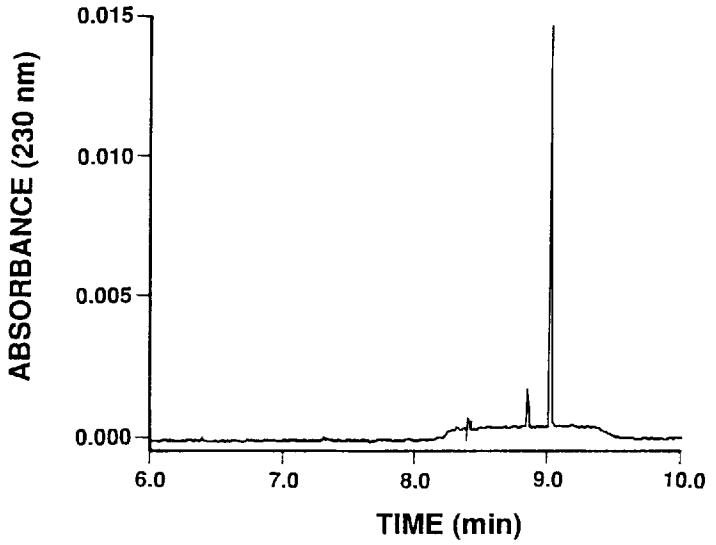

FIGURE 4. Electrogram of palytoxin.
HPCE conditions:
Column Dimension: 50 cm X 75 um
Electrolyte solution: 25 mM borate buffer at pH 8.5
Voltage applied: 15kV cross 50 cm
Temperature: 25 °C
Detection: UV @ 230 nm
Sample: 5 nl from 100 ng/ml.

230 or 263 nm were linearly related to PTX concentration (5-20 ug/ml) with a minimum detection limit of 5 ug/ml (Fig. 2). Spectrophotometric detection of PTX was at 5 ug/ml while toxicological and physiological effects were observed at concentrations of 0.05-0.1 ug/ml. For quality and quantity control of PTX in toxicological studies, detection at low concentration were needed. We have established a sensitive HPLC and HPCE methods for PTX as described herein (Fig. 3 & 4).

Elution times of PTX by HPLC (Fig. 3) or HPCE (Fig. 4) were 9.8 and 9.0 min, respectively. The detection limit of PTX analysis by HPLC was about 125 ng while the detection limit by HPCE was 0.5 pg. The detection sensitivity of PTX at 230 nm was two folds higher than at 263 nm (Fig. 2). The ratio of the absorbance at 230 and 263 nm can be used for peak identification and verification of sample homogeneity (6). The development of an HPCE method will allow measurement of PTX at low concentrations in small volumes which will reduce the risk of accidental exposure. In addition, HPCE method can be applied to determine PTX in biological fluids allowing future metabolic and kinetic studies of PTX *in vivo* and *in vitro*.

ACKNOWLEDGMENTS

The opinion and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. We thank Drs. A. Rose and A.B. Fajer for reviewing the manuscript. Portions of this study was presented at the First Annual Conference on Capillary Electrophoresis, Oct 15-16, 1990 at NCI-FCRDC, Frederick, MD.

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