Purification and Analysis of Bioactive Extracts from Marine Yeast with the Gilson Semi-Micro 2D HPLC System

Application Note PHA0412

The data for this application note was provided by Y Hayashi, Applied Technical Department, M&S Instruments Inc., Japan

Keywords

2D HPLC (2 Dimensional High Pressure Liquid Chromatography), TRILUTION® LC Liquid Chromatography Software, Gilson 233XL, Bioactive, Marine Yeast.

Introduction

Marine yeast are found in a variety of natural water sources. Various marine organisms contain natural products that have bioactivity. Often these bioactive compounds are metabolites that are a natural part of the organism's life cycle, including self-defense. Many uses of purified naturally occuring bioactive compounds are being uncovered, with many projected for therapeutic applications. This application discusses a unique automated 2D technique for anion exchange purification of marine yeast extract prior to reverse phase analysis using a Gilson manual injection system coupled to a Gilson 233XL HPLC system (Figure 1). Note: A Gilson GX-271 Liquid Handler could be substituted for the 233XL in future applications.

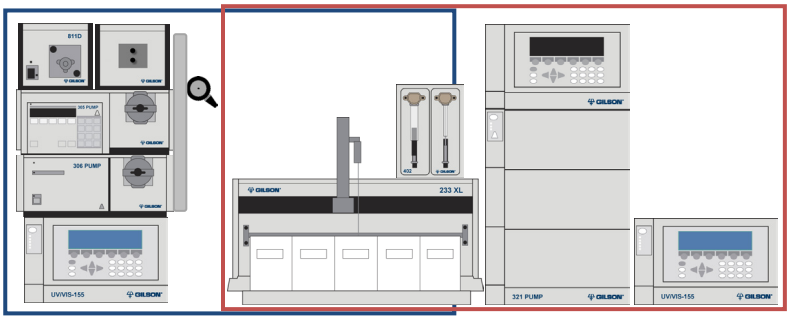

Figure 1. Phase 1 (Blue Box) Anion Exchange Purification. Phase 2 (Red Box) Reverse Phase Analysis

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Materials & Methods

Methods – Sample Preparation

A sample of marine yeast was treated with lysis buffer (20 mM Tris-acetate (pH 7.6), 1 mM EDTA, 5 mM DTT) to disrupt the cells. The marine yeast was treated for 30 min by Covaris S2 (Acoustic solubilizer, Covaris Inc. USA) for complete cellular disruption and lysis. The supernatant was injected using the phase 1 system components.

Methods – Phase 1 Purification

- Anion exchange chromatography was used in phase 1 as the primary purification step.
- Column: Sepax SAX NP1.7, 1.7 micron, non-porus, 3 x 50 mm (Sepax Technologies Inc.)
- Mobile Phase :
    - A: 20 mM Tris-acetate pH 7.5
    - B: A + 0.5 M Sodium acetate
    - Flowrate: 0.2 mL/min
    - Gradient: %B: 1 % - 99 % (1.6 min – 11.5 min)
    - Gilson Binary Pumping System (305 – 5 SC, 306 – 5 SC, 811D – 65 µL, 805)
- UV Detection:
    - 280 nm
    - Gilson 155 Dual Wavelength UV/VIS Detector
- Supernatant Manual Injection:
    - 20 µl
    - Rheodyne 9925i Manual Injection Valve
- Fraction Collection:
    - Time Based
    - Gilson 233XL with 3-way fraction collection valve

Note: system plumbing was optimized for minimal dead volume, with fraction delay volume strictly calculated.

Methods – Phase 2 Analysis

Each fraction collected in phase 1 was re-injected and analyzed with phase 2 system components.

- Column: Sepax GP C18, 2.2 micron, 120 A, 3 x 50 mm (Sepax Technologies Inc.)
- Mobile Phase :
  - A: 0.1% TFA in H2O
  - B: 0.1% TFA in Acetonitrile
  - Flowrate: 0.5 mL/min
  - Gradient: %B: 2% - 75% (1.8 min – 14.3 min)
  - Gilson 321 Binary Pumping System – H1
- UV Detection:
  - 280 nm
  - Gilson 155 Dual Wavelength UV/VIS Detector
- Fraction Re-Injection:
  - 60 µl
  - Gilson 233XL with injection valve and 293 µL custom injection loop

Results

2D chromatography allows for a bioactive sample to be purified and multi-dimensionally analyzed with a semi-micro Gilson HPLC system. The combination of the system components and the efficiency of the columns allows for compound separation under pressure. The high performance columns used are of nonporous and small particle sizes, creating very high backpressure (~300 bar) that enables faster compound resolution using a shorter column length. Simple time-based fraction collection allows for anion exchange purification into separate collection tubes for further analysis via phase 2 reverse phase chromatography (Figure 2).

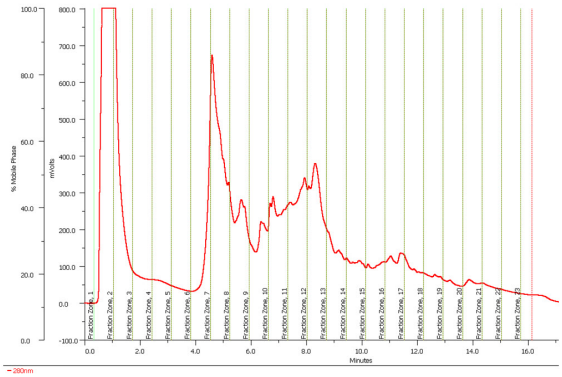

Figure 2. Marine Yeast Supernatant - Anion Exchange Purification Chromatography.

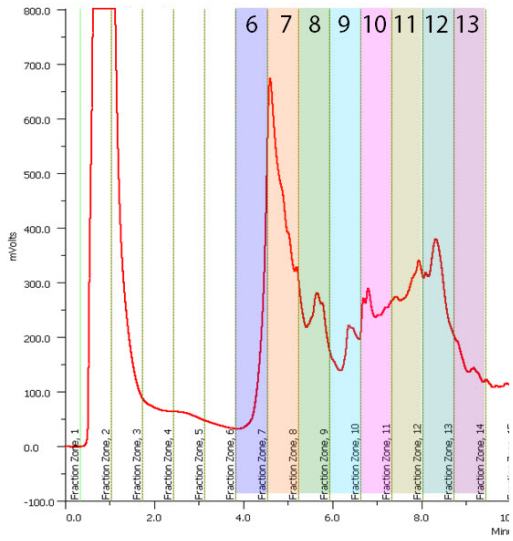

Figure 3. Select Fractions from Marine Yeast Supernatant - Anion Exchange Purification Chromatography, Used for Re-Injection.

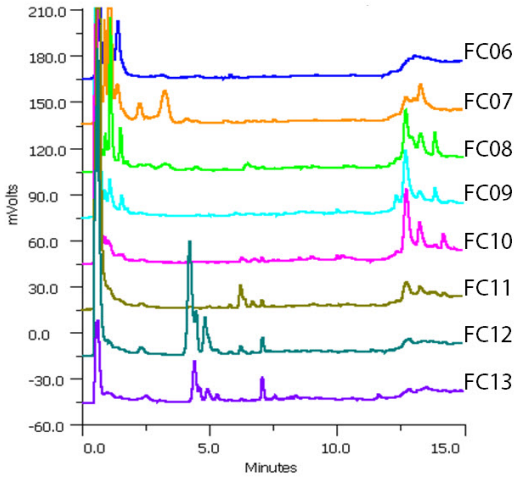

Figure 4. Re-injected Fractions from Marine Yeast Supernatant Analyzed by Reverse Phase Chromatography.

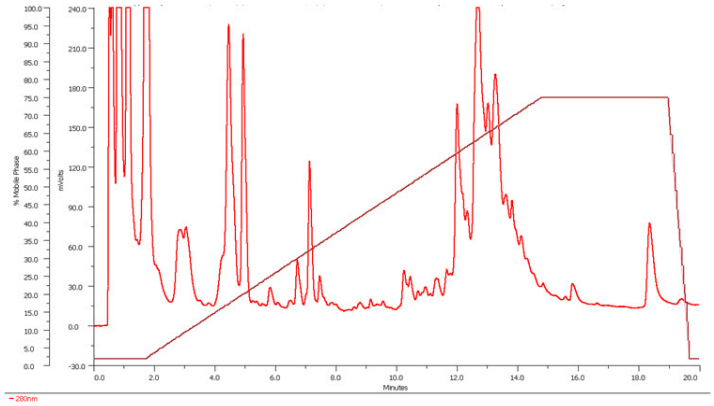

Figure 5. Unpurified Marine Yeast Lysate Sample Injected onto Reverse Phase Column.

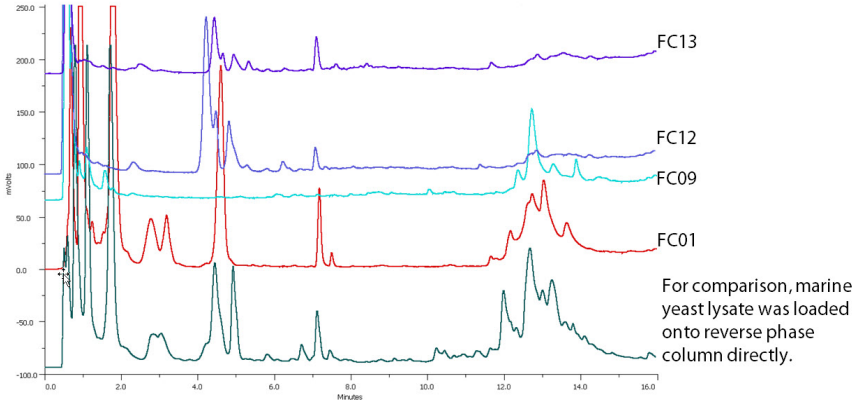

Figure 6. Selected Overlayed Re-injected Fractions Normalized Against Chromatography of Unpurified Marine Yeast Lysate Sample.