Fractionation of Fungal Fermentation Broth using Solid-Phase Extraction

Application Note

Bioactive Pharmaceuticals, Agrochemicals

Authors

Liam Evans and Jonathan Steele
Hypha Discovery Ltd

Abstract

This application note describes a very efficient clean-up and fractionation method using a polymer-based, solid-phase extraction (SPE) product, Bond Elut Plexa.

This quick and effective method shows how unwanted sources of assay interferences, such as proteins and oligosaccharides, can be removed allowing the fractionation of the smaller bioactive pharmaceutical and agrochemical molecules of interest from tropical higher fungi into more precise groups of polarity.

Agilent Technologies

Introduction

Molecules from nature have an excellent proven track record of providing initial leads for development into new pharmaceutical and agrochemical products. To discover new bioactive pharmaceutical and agrochemical molecules from tropical higher fungi, a new method that stimulates fermentation of fungi has been developed. The huge increase in chemical production is shown in the chromatographic analysis (Figure 1).

The composed fermentation products vary greatly in molecule size and polarity and require an effective fractionation prior to identification and bioassay. For example, the macromolecules have to be removed before the compounds of interest can be selectively collected according to their polarities. Preparative chromatography is one technique, which can be used to clean-up and fractionate. However, the high price and the long analysis time make this technique inefficient for our work.

A very efficient method is the clean-up and fractionation on a new polymer-based, solid phase extraction (SPE) product, Bond Elut Plexa. The primary extracts generated from the proprietary method are processed through the SPE cartridges to remove proteins, enzymes, oligosaccharides and other biopolymers, which are known to cause interference in target-based screening.

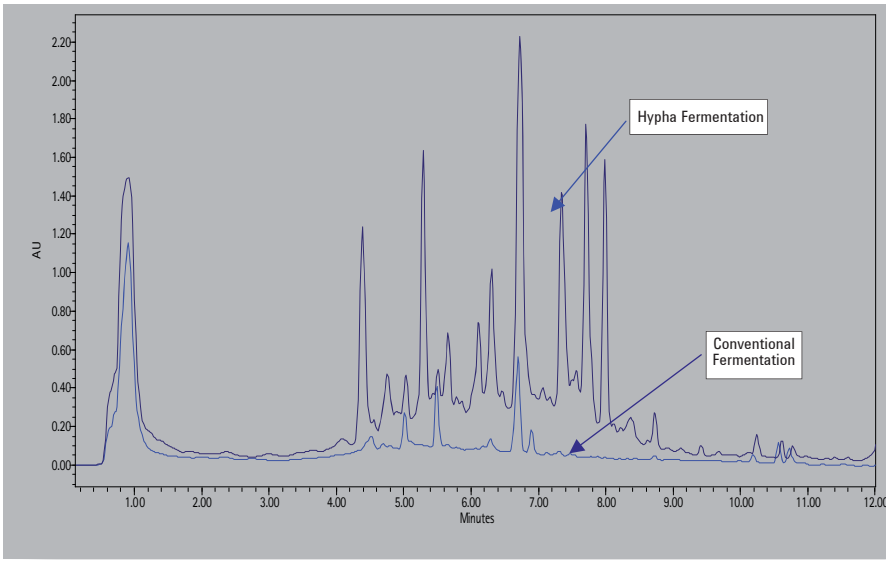


Figure 1. Chromatogram showing comparison of compound production using Hypha fermentation and conventional fermentation. Hypha's fermentation provides increased titres and expression of new molecules

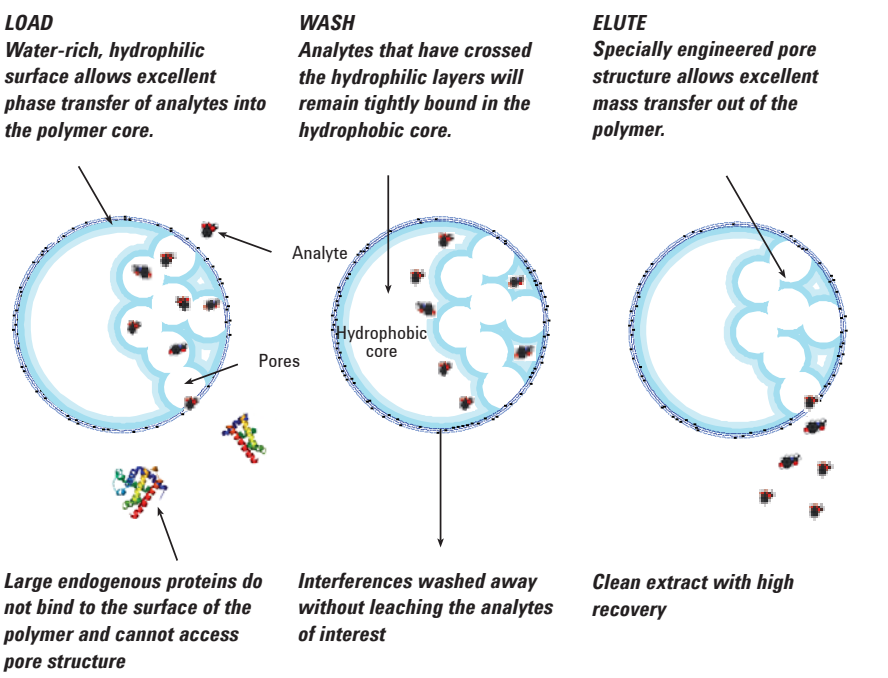


Figure 2. The water-wettable, hydroxylated exterior allows excellent flow of bio-fluid samples. A gradient of polarity on the polymer surface shunts small analytes to the more hydrophobic center of the polymer bead where they are retained. As the surface is highly polar and entirely amide-free, binding of proteins on the polymer surface is minimized, resulting in cleaner samples and reduced ion suppression

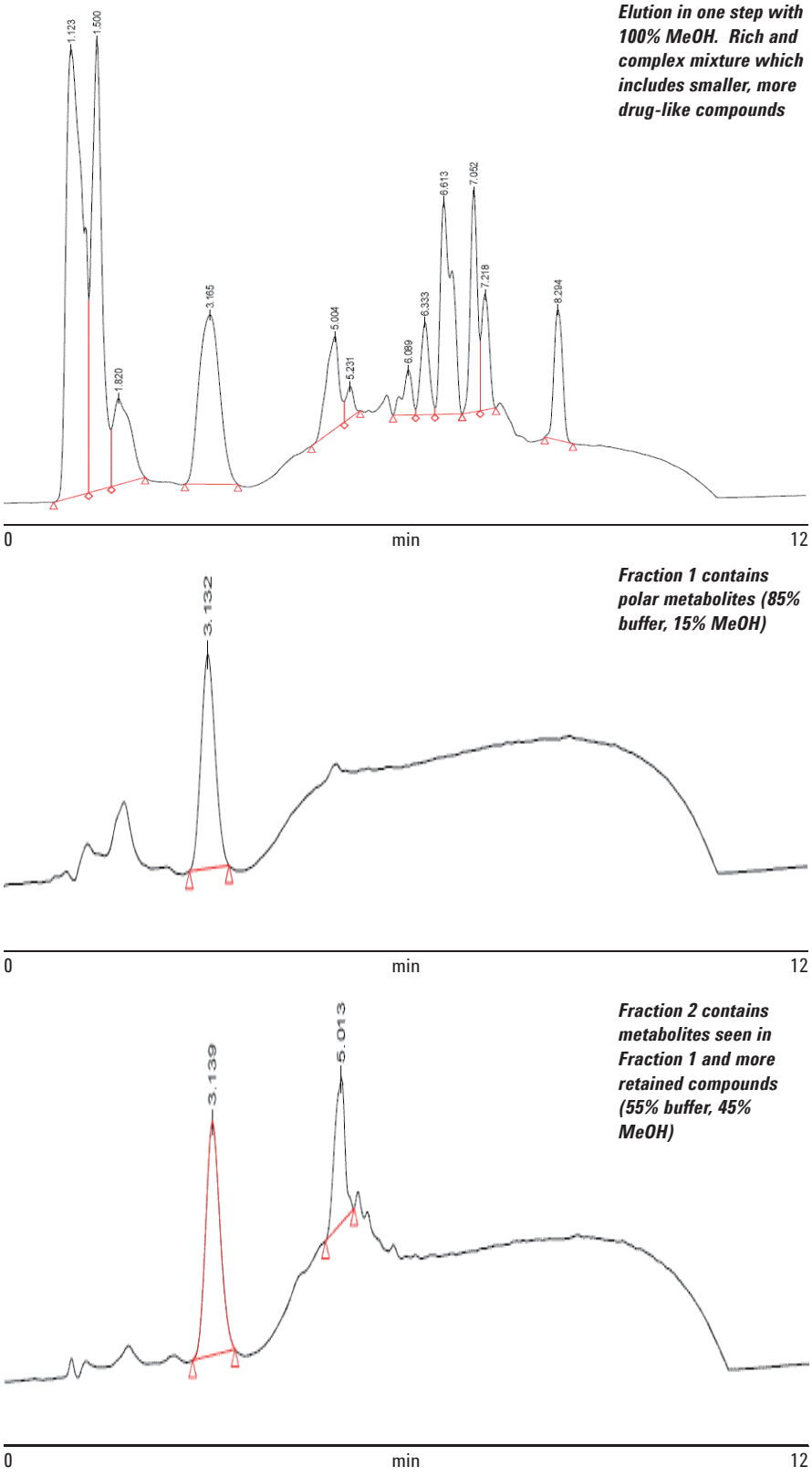
SPE Sorbent

Bond Elut Plexa is a new type of polymeric SPE product, designed for improved analytical performance and ease of use. The base material is a macroporous styrene divinylbenzene co-polymer. The monodisperse polymer particles are functionalized with hydrophilic substituents. These hydroxyl-containing moieties create a 'water-rich' environment on the surface of the polymeric bead and a polar gradient into the hydrophobic pores (Figure 2).

Large molecules cannot access the pore structure of the core. This facilitates a highly efficient sample throughput by allowing quick removal of unwanted assay interferences. The polarity gradient in the pore helps to fractionate some samples into different polarity groups.

Method

The fermented sample is loaded onto the 500 mg Bond Elut Plexa cartridge in the presence of ion-pair reagent enabling retention of very polar compounds, even those of similar retention to aminoglycoside antibiotics. Drug-like molecules are retained within the porous particles at the hydrophobic core. Macromolecules are washed away in a buffer-wash step. With the macromolecules removed, the compounds of interest can then be selectively eluted according to their polarities. Component molecules are fractionated into four groups of polarity, allowing the operator to select subsets with different ranges of hydrophobicity (Figure 3).

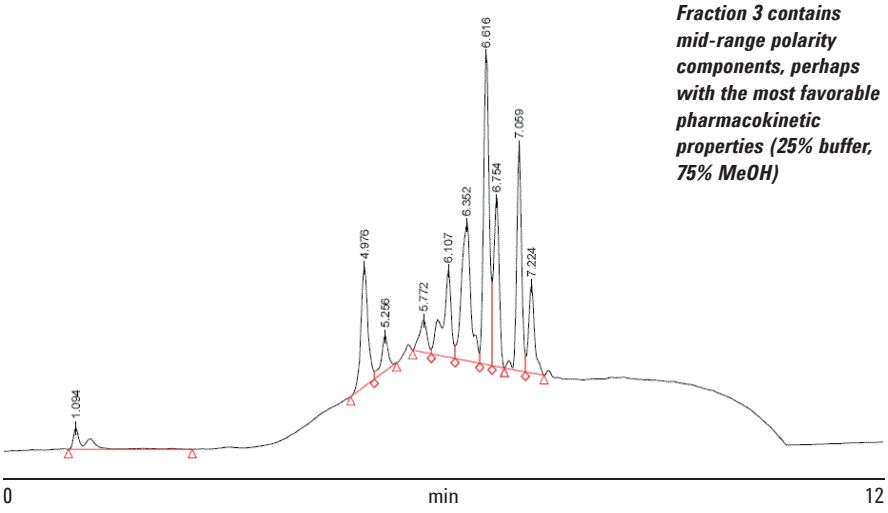


Elution in one step with 100% MeOH. Rich and complex mixture which includes smaller, more drug-like compounds

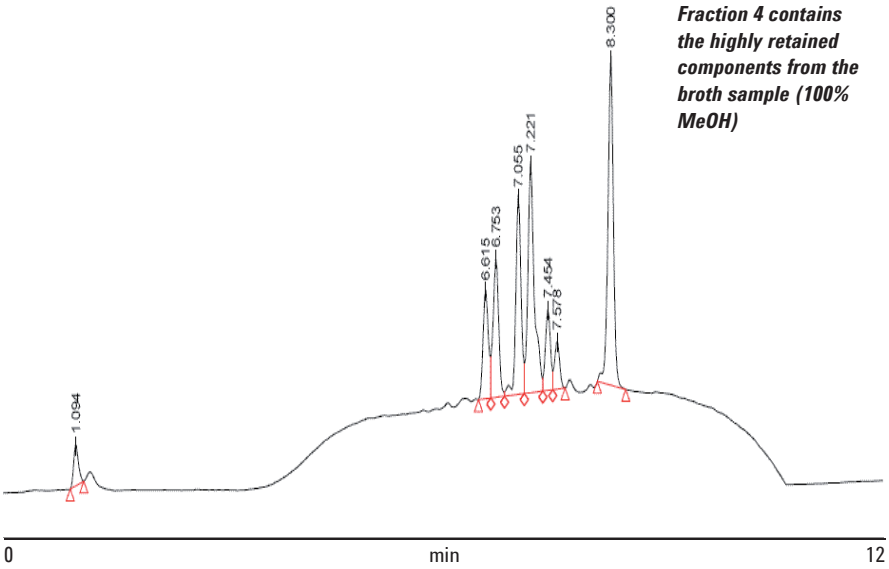
Fraction 1 contains polar metabolites (85% buffer, 15% MeOH)

Fraction 2 contains metabolites seen in Fraction 1 and more retained compounds (55% buffer, 45% MeOH)

Figure 3. Fractionated SPE of basidiomycetes fermentation with Bond Elut Plexa sample loading is performed in the presence of ion-pair reagent enabling retention of very polar N-compounds, even those of similar retention to aminoglycoside antibiotics ***(continued on page 4)***



Fraction 3 contains mid-range polarity components, perhaps with the most favorable pharmacokinetic properties (25% buffer, 75% MeOH)



Fraction 4 contains the highly retained components from the broth sample (100% MeOH)

Conclusion

SPE on Bond Elut Plexa works with a combination of mechanics. When compared to other polymeric materials that we have used at Hypha, this material greatly increases the precision and speed of our research. The advanced design of the pore facilitates a highly efficient sample throughput by allowing us to quickly remove unwanted sources of assay interferences such as proteins and oligosaccharides, while the polarity gradient in the pore enables us to fractionate samples into more precise groups of polarity. This results in more efficient targeting of small molecules of interest.

www.agilent.com/chem

Published in UK, August 17, 2010
SI-00969

Agilent Technologies