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Chromatographic Fractionation Protocol of Natural Extracts Using Diaion HP20-SS Resin and Isolera-Biotage System

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We use this protocol and it's working

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Abstract

This method processes natural extracts to separate secondary metabolites into distinct chemical classes, supporting their identification and biochemical assessment. A solvent gradient with increasing polarity enables selective elution, improving resolution and compound recovery. A non-ionic polymeric resin was selected for its strong adsorption capacity and versatility in separating compounds of varying polarities.
Using the automated Isolera-Biotage system increases reproducibility and efficiency, while reducing experimental time and minimizing material loss. The fractions obtained, being less complex than the crude extract, can be further analyzed by HPLC-DAD, LC-HRMS, and bioactivity assays to provide a broad chemical and functional profile.
The approach is applicable to metabolomics, bioprospecting, and bioactive compound isolation, contributing to projects that explore and add value to natural resources, and supporting the discovery of new products with economic, medicinal, or ecological importance.

Guidelines

Warnings and Precautions for Flash Chromatography Fractionation Using Diaion HP20-SS Resin
Resin Preparation and Handling:

Wash and equilibrate the Diaion HP20-SS resin properly before use to remove residual contaminants or impurities that could affect separation.

Do not reuse the resin for different samples to avoid cross-contamination from adsorbed compounds.
Sample Loading:

Completely dissolve the dry extract in the appropriate solvent before loading onto the column. Insoluble particles can clog the column and compromise fractionation.

Use controlled extract concentrations to prevent column overload, which reduces compound resolution.
Gradient Setup:

Carefully check gradient parameters (flow rate, pressure, solvent ratios) on the Isolera-Biotage system before starting the run. Incorrect settings can reduce separation efficiency.
Cross-Contamination Prevention:

Thoroughly clean tubes, racks, and equipment accessories between samples to avoid cross-contamination.
Fraction Collection and Identification:

Label all fraction tubes clearly during collection to ensure accurate sample tracking. Use solvent-resistant tags and code fractions according to the applied gradient.
Solvent Handling:

Work in a ventilated area and wear proper personal protective equipment (PPE) such as gloves, lab coat, and safety goggles due to the toxicity and flammability of solvents like methanol (MeOH) and ethyl acetate (AcOEt).
Fraction Drying:

Avoid overheating during drying with Speed-vac or nitrogen flow to prevent degradation of heat-sensitive compounds.
Documentation and Records:

Keep detailed records of all fractionation steps, including column preparation, sample loading, gradient parameters, and results.
Fraction Storage:

After drying, store fractions in tightly sealed vials under appropriate conditions (e.g., freezer or controlled environment) to prevent degradation or contamination.

Final System Check:

Before each run, inspect the Isolera-Biotage system for leaks, clogs, or calibration issues to ensure proper operation.

Before start

Steps and Checks Before Starting the Method
Work Area Preparation:

Ensure the workspace is clean, organized, and free of contaminants.

Check that all required equipment—Isolera-Biotage, Speed-vac, and nitrogen (N_2) flow source—are functioning properly.
Materials and Reagents Check:

Confirm availability, purity, and sufficient quantities of all reagents (ultrapure water, methanol, ethyl acetate).

Inspect tubes, vials, and columns/cartridges for cleanliness and any damage or residues.
Dry Extract Verification:

Make sure the dry extract is prepared in advance and properly stored. Verify its quantity and homogeneity.
Resin Quality Control:

Store Diaion HP20-SS resin under appropriate conditions. Check for contamination signs, such as color or odor changes.

Wash and activate the resin as needed before use.
Isolera-Biotage System Inspection:

Perform a full equipment check to rule out leaks, clogs, or failures in the gradient or collection systems.

Verify that collection racks are correctly positioned and aligned with fraction tubes.
System Calibration:

Set flow rate, pressure, and solvent gradient parameters according to the experimental protocol.

Run a quick blank test with solvents to confirm system stability and function.
Planning and Documentation:

Prepare a detailed fractionation plan, including sample order, solvent gradients, and collection parameters.

Keep a lab notebook or electronic spreadsheet ready to document all process steps.
Safety:

Wear appropriate personal protective equipment (PPE) such as gloves, lab coat, and safety goggles.

Ensure the fume hood is operating properly when handling volatile solvents.

Sample and Fraction Control:

Prepare identification labels with unique codes for samples and fractions to avoid mix-ups.

Organize fraction tubes in racks or holders for proper storage during collection.

Final Checklist:

Review the entire protocol and confirm that all materials, reagents, and equipment are available and ready for use.

Initial Preparation

1 **Dry Crude Extract: Prepared Previously.**

Solvents:

- Ultrapure Water
- Methanol (MeOH)
- Ethyl Acetate (AcOEt)

Materials and Equipment:

- Diaion HP20-SS Resin
- Flash Chromatography Column/Cartridge
- Column Frits
- Syringe for sample injection (luer-lock)
- Glass Pipette
- 16 mL Carbonized Glass Tubes with Screw Caps: 40 units for each sample to be fractionated
- Pre-weighed 1.5 mL Vials (for mass balance) with Caps: 40 units per sample
- Evaporation Equipment (Speed-vac) or Nitrogen (N_2) Stream
- Rack for Tube and Vial Organization

Chromatography Column Preparation

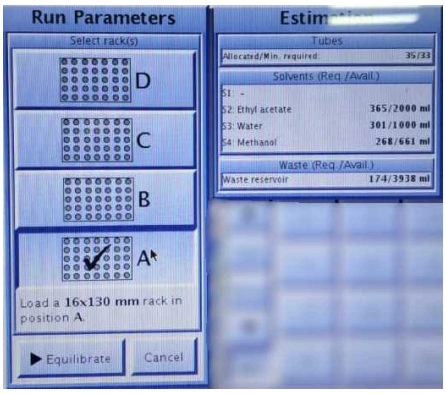
2 **Column/Cartridge Setup:**

- Place a frit at the bottom of the column.
- Carefully add 12 g of Diaion HP20-SS resin.

3 Method to be used: Wide-Range-Polarity (WRP), which covers a broad range of compound polarities.

4 **Fraction Tube Organization:**

- Label 16 mL tubes with solvent-resistant tags and arrange them in the appropriate racks of the Isolera-Biotage system.
- Prepare 40 tubes for each sample.

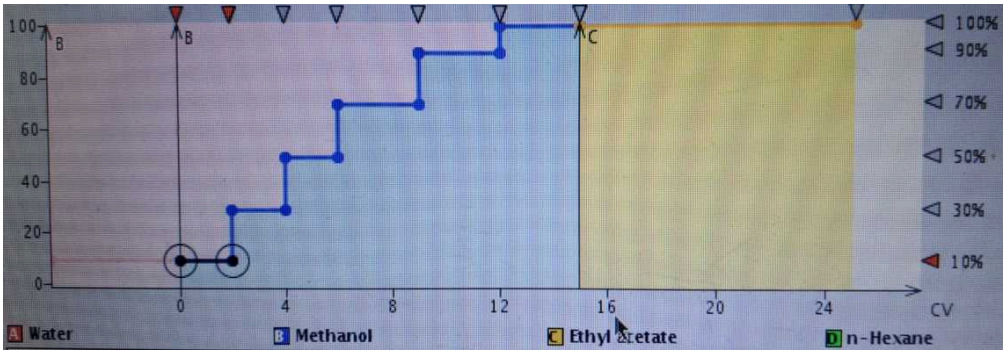


5 Gradient Preparation

Solvents: A = water, B = methanol, C = ethyl acetate
Flow rate: 8 mL/min
Length unit: CV (column volumes)

Equilibration: A/B 10% for 5.0 CV
Step 1: A/B 10% for 2.0 CV
Step 2: A/B 30% for 2.0 CV
Step 3: A/B 50% for 2.0 CV
Step 4: A/B 70% for 2.0 CV
Step 5: A/B 90% for 2.0 CV
Step 6: A/B 100% for 3.0 CV
Step 7 (apolar flush): B/C 100% for 10.0 CV
Quick notes:

- CV = total column/packing volume
- Monitor pressure and baseline; adjust if necessary



6 **Sample Cataloging:**
Record and catalog all generated fractions, including sample code, fraction number, and solvent used.

Isolera-Biotage Setup

7 Protocol Setup:
Refer to the "**Protocol for Using the Isolera-Biotage for "Wide-Polarity-Range" (WPR)**"

Select the fractionation method "Wide-Range Polarity" (WRP), which covers a broad range of compound polarities.

8 **Sample Preparation:**
Use an extract amount equivalent to 1 g of dry biomass per 12 g of Diaion HP20-SS resin.

Dissolve the dry extract in the appropriate loading solvent (e.g., MeOH-Dichloromethane).

Chromatographic Fractionation

9 **Gradient Execution:**
- Start the programmed gradient in the Isolera-Biotage method to begin fractionation and fraction collection.
- Ensure flow rate and pressure are properly adjusted to prevent material loss.

10 **Collection and Recording:**

- Remove the fraction tubes from the rack at the end of the run.
- **Save the generated chromatogram** to a storage device (e.g., flash drive) for analytical reference.

Fraction Concentration

11 **Drying:**

- Dry the fractions using a Speed-vac or nitrogen (N_2) flow.
- Ensure all solvent is completely removed.

12 **Identification and Weighing:**

- Transfer the dry fractions to 1.5 mL vials.
- Label the vials with specific codes and record the net weight of each fraction.
- Calculate the mass balance and yield of the procedure.

Fraction Analysis

13 **Analytical Characterization:**

- Submit the fractions (**including blank control and unfractionated crude extract**) for **HPLC-DAD** and **LC-HRMS** analysis.

14 **Biological Assays:**

- Send the fractions (**including blank control and unfractionated crude extract**) for biological activity testing to evaluate their biological potential.

15 **DAFDiscovery:**

- Use the DAFDiscovery software (**Streamlit**) to perform integrated analysis of the obtained data.

16

Protocol references

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