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Chromatographic profile of aqueous extract by HPLC V.3

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ABSTRACT

This chromatographic detection method was developed in order to solve the most compounds from aqueous extracts leaves of dune and mangrove plant by HPLC.

MATERIALS

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⊗ Trifluoroacetic acid for HPLC > 99.0% **Sigma-aldrich Catalog #302031-100ML**

⊗ Acetonitrile HPLC **fisher Catalog #9012-03**

⊗ Methanol HPLC **Fisher Scientific Catalog #9093-03**

SAFETY WARNINGS

The trifluoroacetic acid is very high volatile, try to use a extraction cabinet to take it.

BEFORE START INSTRUCTIONS

Everything that goes into HPLC must be filtered first, through a 0.2 µm nylon filter and special glassware to remove particles that can get caught up on the column to avoid overpressure problems.

The potentiometer must be calibrated.

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Sample preparation

- 1 Each aqueous extract was added a 10% MeOH to improve solubility, and next it was filtered through 0.2 µm filter disc.

Movil phase

- 2 Movil phase consist:
 - Movil phase A contained ultrapure type 1 water (Simplicity® Water Purification System, Millipore) adjusted to pH 2.5 with trifluoroacetic acid (TFA).
 - Movil phase B contained acetonitrile (ACN).Each movil phase was filtered through 0.2 µm filter and finally was degassed during 30 min. in ultrasonic equipment.

Equipment

- 3
 - Profile was carried out on an Agilent Series 1290 Infinity HPLC System with vacuum degasser, quaternary pump, autosampler, thermostated column compartment and photodiode array detector (DAD).
 - Data analysis was performed with Agilent HPLC EZChrom software.

Column

- 4 Separation of analytes was performed on an Grace Alltima C18 column (4.6 mm × 250 mm, 5 µm), which was maintained at 35 °C during the analysis.

Elution gradient

- 5 The gradient was programmed as follows: 0-3 min, 5% B; 3-43 min, 5-30% B; 43-73 min, 30-85 %B, 73-75 min 85-5 %B.

Elution flow

- 6 Set the flow rate to 1 mL/min.

DAD monitoring

- 7 Set the DAD monitoring to 254 nm and 350 nm.

Injection

- 8 Inject 20 µL of the sample.