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Working

Chromatographic profile of aqueous extract by HPLC

Version 3

PLOS One

Luis Wiliunfo Torres Tapia[1]

[1]Centro de Investigación Científica de Yucatán A. C.

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Luis Wiliunfo Torres Tapia
Centro de Investigación Científica de Yucatán A. C.

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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0213493

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Rodríguez-García CM, Ruiz-Ruiz JC, Peraza-Echeverría L, Peraza-Sánchez SR, Torres-Tapia LW, Pérez-Brito D, Tapia-Tussell R, Herrera-Chalé FG, Segura-Campos MR, Quijano-Ramayo A, Ramón-Sierra JM, Ortiz-Vázquez E (2019) Antioxidant, antihypertensive, anti-hyperglycemic, and antimicrobial activity of aqueous extracts from twelve native plants of the Yucatan coast. PLoS ONE 14(3): e0213493. doi: 10.1371/journal.pone.0213493

PROTOCOL STATUS

Working

MATERIALS

NAME	CATALOG #	VENDOR
Trifluoroacetic acid for HPLC > 99.0%	302031-100ML	Sigma-aldrich
Acetonitrile HPLC	9012-03	fisher
Methanol HPLC	9093-03	Fisher Scientific

SAFETY WARNINGS

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

BEFORE STARTING

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.

Sample preparation

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

Movil phase

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2 Movil phase consist:
- Movil phase A contaned 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 degassered 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.

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