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Extraction Protocol for Collection

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Protocol status: In development

We are still developing and optimizing this protocol

Created: March 12, 2025

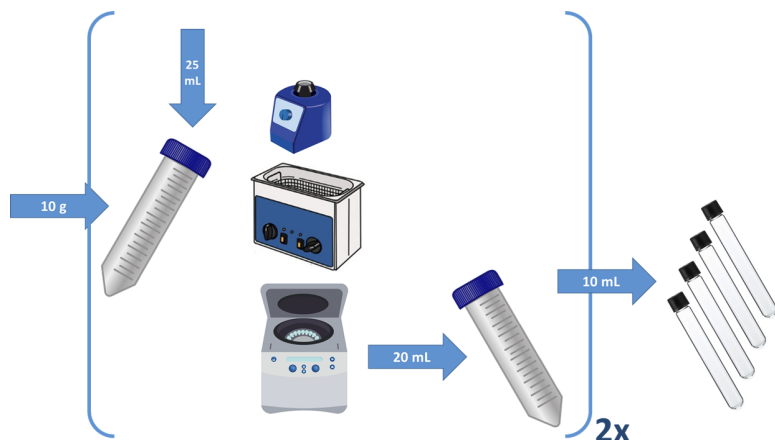
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Keywords: preparation of extract, extraction, extract, dried plant material, plant material, ultrapure water, preparation, plant, hplc grade, process, drying, extraction protocol, extraction protocol for collection, ethanol

Abstract

This protocol describes the preparation of extracts from plants. The process involves grinding the dried plant material, extraction with 70% ethanol (HPLC grade) and ultrapure water (Milli-Q), followed by agitation, ultrasound, centrifugation, drying, and storage. The aim is to obtain consistent samples for scientific studies.



Guidelines

Solvent Handling: Ethanol is flammable; handle it away from open flames or sparks and work in a well-ventilated area.

- 1. Personal Protective Equipment (PPE):** Wear gloves, safety goggles, and a lab coat to prevent contact with solvents and plant material.
- 2. Waste Disposal:** Follow proper disposal guidelines for chemical and biological waste.



Materials

Materials and Equipment

- Dried plant material
- Coffee Grinder
- 50 mL Falcon tubes
- 13 mL carbonized glass tubes
- 1.5 mL microtubes
- 1.5 mL glass vials
- Eppendorf tubes
- Vortex mixer
- Ultrasonic bath
- Centrifuge
- SpeedVac concentrator

Preparation of the Extraction Solution

- Prepare the extracting solution by mixing 70% ethanol (HPLC grade) with ultrapure water (Milli-Q).

Preparation of Plant Material:

- 1
 - Grind the entire dried plant material using a "coffee grinder" until a homogeneous powder is obtained.
 - Weigh and separate 10 grams for extraction into a 50 mL Falcon tubes.
 - Store the remaining ground material in Falcon tubes.

Extraction Steps

- 2 **First Extraction Step:**
 - Add 25 mL of the extraction solution to the 10 g of plant material.
 - Mix using a vortex mixer.
 - Sonicate for 5 minutes in an ultrasonic bath.
- 3 **Centrifugation:**
 - Centrifuge at 4000 RPM for 10 minutes at 4°C.
- 4 **First Supernatant Collection:**
 - Transfer 20 mL of the supernatant to a new 50 mL labeled Falcon tube.
- 5 **Second Extraction Step:**
 - Add another 25 mL of extraction solution to the plant residue.
 - Mix using a vortex mixer for 30 seconds.
 - Sonicate for another 5 minutes.
- 6 **Second Centrifugation:**
 - Centrifuge again at 4000 RPM for 10 minutes at 4°C.
- 7 **Second Supernatant Collection:**
 - Transfer 20 mL of the supernatant to the same 50 mL labeled Falcon tube for each sample respectively.
 - Mix using a vortex mixer for 10 seconds.
- 8 **Collect a small aliquot for Analysis:**
 - Collect 500 uL into a clean labeled vial for analytical data acquisition.

Note: This sample can be directed to LC-MS (or NMR or LC-DAD) analysis for catalogation.

- 9 **Aliquotes for use and storage:**

- Separate the final content (~40 mL) into four (4) new different 13 mL carbonized glass tubes.

Note: one of these 13 mL carbonized glass tubes can be weighted for reference. The other 3 tubes must have the same mass of the extract.

Drying:

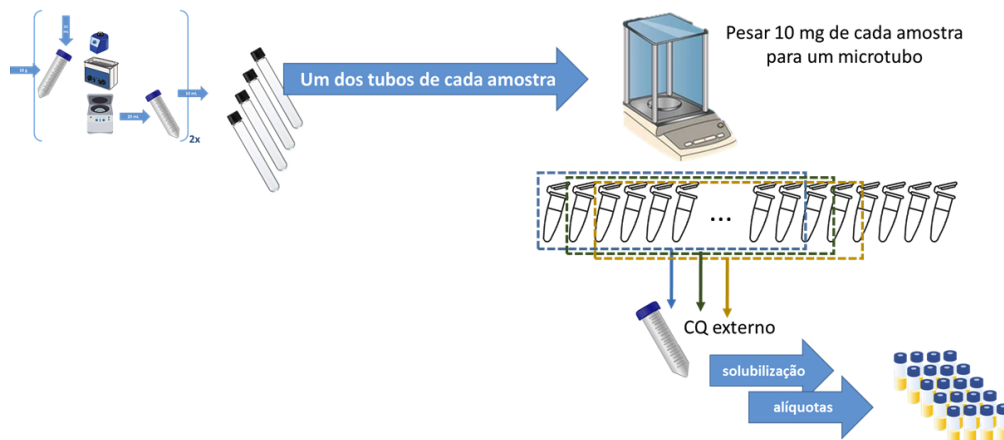
- 10
- Dry the extracts (13 mL carbonized glass tubes) using a SpeedVac concentrator.

Storage:

- 11
- Transfer the dried extract to Eppendorf tubes and record the weight for reference.

Controle de Qualidade

12



Esquema para amostra de Controle de Qualidade Externo

13 Pesagem para microtubos

- De cada tubo selecionado, é pesada uma alíquota de 10 mg.
- Essa alíquota é transferida para microtubos de 1.5 mL.

14 Controle de Qualidade Externo (CQ externo)



- Estas amostras serão combinadas a seco em quantidades iguais (10 mg) em um tubo tipo falcon de 50 mL
- Esta amostra combinada será solubilizada e distribuída em vials de vidro de 1.5 mL
- Estas alíquotas serão evaporadas a secura para serem usadas como amostra de QC externo através dos diferentes lotes.