MAY 24, 2018

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.krncv5e](https://doi.org/10.17504/protocols.io.krncv5e)

External link:
<https://doi.org/10.1371/journal.pone.0196164>

Protocol Citation: Aparecido Almeida Conceição, Clemente Batista Soares Neto, José Antônio de Aquino Ribeiro, Felix Gonçalves de Siqueira, Robert Neil Gerard Miller, Simone Mendonça 2018. Extraction and detection of free-gossypol in cottonseed samples. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.krncv5e>

MANUSCRIPT CITATION:
 Conceição AA, Neto CBS, Ribeiro JAdA, Siqueira FGd, Miller RNG, Mendonça S (2018) Development of an RP-UHPLC-PDA method for quantification of free gossypol in cottonseed cake and fungal-treated cottonseed cake. PLoS ONE 13(5): e0196164. doi: [10.1371/journal.pone.0196164](https://doi.org/10.1371/journal.pone.0196164)

Extraction and detection of free-gossypol in cottonseed samples

PLOS One

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ABSTRACT

This simple and rapid chromatographic detection method was developed and validated in order to accurately quantify trace levels of free gossypol in different cotton materials, including cottonseed, cottonseed cake and cottonseed cake treated with macrofungi.

ATTACHMENTS

[Free gossypol extraction and detection protocol.docx](#)

GUIDELINES

Column and equipment used to develop this method was KINETEX® column and a Waters ACQUITY UPLC H-Class system, respectively.

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Protocol status: Working

Created: Nov 10, 2017

Last Modified: May 24, 2018

PROTOCOL integer ID: 8718

Keywords: Free gossypol, cottonseed cake, UHPLC

MATERIALS

MATERIALS

- ☒ Acetone **Contributed by users**
- ☒ Methanol **Contributed by users**
- ☒ Trifluoroacetic acid (TFA) **Contributed by users**

STEP MATERIALS

- ☒ Acetone **Contributed by users**
- ☒ 70% acetone **Contributed by users**
- ☒ Methanol **Contributed by users**
- ☒ Water 0.1% TFA **Contributed by users**
- ☒ Acetone **Contributed by users**
- ☒ 70% acetone **Contributed by users**
- ☒ Methanol **Contributed by users**
- ☒ Water 0.1% TFA **Contributed by users**

BEFORE START INSTRUCTIONS

Ice is needed for the sonicate step

Extraction protocol

- 1 Dry the cottonseed sample at 40 °C for 24 hours.

🕒 24:00:00 hours

Note

This protocol was tested with samples from cottonseed with linter, cottonseed without linter, cottonseed cake and cottonseed cake treated with basidiomycetes fungi.

🌡 40 °C

Expected result

The sample should be very well dried in order to proceed to the next step

- 2 Grind the dry sample to form a fine powder with an ultra centrifugal mill (0.5 mm)

Note

the samples can be grinded using bench mill if it is very well homogenized

- 3 Weight 1 gram of the powdered sample into a 15mL plastic centrifuge tube with screw.

1 μ L

- 4 Add 3 mL of ultrapure water into the tube.

3 mL

Note

Use Mili-Q water

- 5 Vortex vigorously for about 10 seconds.

Note

Make sure that the solution is very well homogenized.

00:00:10 seconds

- 6 Ultra-sonicate for 5 minutes using ice in the sonicator.

00:05:00 minutes

Note

The ice must fill the sonicator

- 7 Add 7 mL of acetone onto the mixture.

7 mL

Note

Use grade HPLC reagent

Acetone **Contributed by users**

- 8 Ultra-sonicate for 5 minutes using ice in the sonicator.

Note

The ice must fill the sonicator

00:05:00 minutes

- 9 Vortex vigorously for about 10 seconds.

00:00:10 seconds

Note

Make sure that the solution is very well homogenized

- 10 Centrifuge the tubes at 9000 rpm for 5 minutes at 8 °C.

00:05:00 minutes

8 °C

- 11 Transfer 2 mL of the supernatant to 15 mL centrifuge tube.

2 mL

- 12 Evaporate the solvent in a speed vacuum at room temperature.

Note

All solvent must be evaporated

The temperature should not be over 35°C

- 13 Resuspend the residue with 200 µL of 70% acetone.

200 µL

70% acetone **Contributed by users**

Note

Prepare 70% acetone using ultrapure water.

The pellet remaining in the tube must be entirely suspended with the solvent.

- 14 Transfer the solution to a 1.5 mL Eppendorf tube.

200 µL

- 15 Centrifuge at 14000 rpm for 10 minutes at 8 °C.

00:10:00 minutes

🌡 8 °C

- 16 Transfer the supernatant to vial.

Detection protocol

- 17 Set a reversed phase C18 column (100 x 2.1 mm, 2.6 µm) in a UHPLC equipped with a photodiode array (PDA).

- 18 Set column temperature to 35 °C.

🌡 35 °C

- 19 Use methanol as organic solvent (A) and 0.1% TFA in water as aqueous solvent (B) for mobile phase constituents.

⊗ Methanol **Contributed by users**

⊗ Water 0.1% TFA **Contributed by users**

- 20 Set elution as gradient. The elution starts with 60% of A, changing linearly to 100% of A, it is reached at 8 minutes and maintained until 10 minutes. After this, the mobile phase suddenly changes to 60% of A, maintaining this isocratic flow till the end of run at 14 minutes.

Note

Time (min)	Aqueous (%)	Organic (%)
Initial	40	60
8	0	100
10	0	100
10.01	40	60
14	40	60

21 Set the flow rate to 0.4 mL/min.

22 Set the sample manage to 8 °C.

23 Set the PDA monitoring to 254 nm.

24 Inject 2 µl of the sample.

Expected result

The gossypol peak should appear at 6.3 minutes using Empower software for analysis of chromatogram