# Extraction and detection of free-gossypol in cottonseed samples

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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.

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## Guidelines

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

## Before start

Ice is needed for the sonicate step

## Materials

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

## Protocol

Extraction protocol

**Step 1.**

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

TEMPERATURE
40 °C Additional info:

EXPECTED RESULTS

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

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

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

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

**Step 3.**

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

AMOUNT
1 µl Additional info:

ANNOTATIONS
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1g

**Step 4.**
Add 3 mL of ultrapure water into the tube.

AMOUNT
3 ml Additional info:

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Use Mili-Q water

**Step 5.**
Vortex vigorously for about 10 seconds.

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Make sure that the solution is very well homogenized.

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

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The ice must fill the sonicator

**Step 7.**
Add 7 mL of acetone onto the mixture.

AMOUNT
7 ml Additional info:

REAGENTS
Acetone by Contributed by users

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Use grade HPLC reagent

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

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The ice must fill the sonicator

**Step 9.**
Vortex vigorously for about 10 seconds.

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Make sure that the solution is very well homogenized

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

TEMPERATURE
8 °C Additional info:

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

AMOUNT

2 ml Additional info:

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

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All solvent must be evaporated

The temperature should not be over 35ºC

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

AMOUNT
200 µl Additional info:

REAGENTS
70% acetone by Contributed by users

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Prepare 70% acetone using ultrapure water.

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

**Step 14.**
Transfer the solution to a 1.5 mL Eppendorf tube.

AMOUNT
200 µl Additional info:

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

TEMPERATURE
8 °C Additional info:

**Step 16.**
Transfer the supernatant to vial.

## Detection protocol

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

**Step 18.**
Set column temperature to 35 °C.

TEMPERATURE
35 °C Additional info:

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

constituents.

REAGENTS

- Methanol by Contributed by users
- Water 0.1% TFA by Contributed by users

**Step 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.

NOTES

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

**Step 21.**

Set the flow rate to 0.4 mL/min.

**Step 22.**

Set the sample manage to 8 °C.

TEMPERATURE

8 °C Additional info:

**Step 23.**

Set the PDA monitoring to 254 nm.

**Step 24.**

Inject 2 µl of the sample.

AMOUNT

2 µl Additional info:

EXPECTED RESULTS

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