



Quantification of plant hormones by standard addition method 20201203 2

DOI

<https://dx.doi.org/10.17504/protocols.io.bqctmswn>

Takuyu Hashiguchi¹, Masatsugu Hashiguchi¹, Hidenori Tanaka¹, Koki Fukushima¹, Takahiro Gondo¹, Ryo Akashi¹

¹University of Miyazaki



Takuyu Hashiguchi

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account



DOI: <https://dx.doi.org/10.17504/protocols.io.bqctmswn>

Protocol Citation: Takuyu Hashiguchi, Masatsugu Hashiguchi, Hidenori Tanaka, Koki Fukushima, Takahiro Gondo, Ryo Akashi .
Quantification of plant hormones by standard addition method 20201203 2. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bqctmswn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: December 03, 2020

Last Modified: December 03, 2020

Protocol Integer ID: 45171

Keywords: Plant hormone, Standard addition method, Lotus japonicus, quantification of plant hormone, simultaneous quantification of multiple plant hormone, plant hormone content by standard addition method, measuring plant hormone content, multiple plant hormone, plant hormones in plant tissue, plant hormone, plant physiology because hormone, quantifying hormone, quantification of various compound, understanding plant physiology, mass spectrometry, using liquid chromatography, molecules in multiple plant process, linked mass spectrometry, liquid chromatography, labeled compound, phytohormone, plant material such as lotus japonicus, multiple plant process, labeled chemical, hormone, standard addition method, plant material, various compound, pesticide, plant tissue, simultaneous quantification, lotus japonicus

Abstract

Phytohormones are essential signaling molecules in multiple plant processes, including growth, development, and stress response. Simultaneous quantification of multiple plant hormones is required for understanding plant physiology because hormones typically change in tandem in response to external stimuli. Sensitive and quantitative analysis using liquid chromatography linked mass spectrometry (LC-MS/MS) adopts stable isotope-labeled compounds in previously published works. However, quantifying hormones when isotopically labeled chemicals are not available remain to be established.

This protocol is used for measuring plant hormone content by standard addition method. Standard addition method has been used for quantification of various compounds such as drugs and pesticides especially when it is difficult to obtain stable isotope-labeled compound. Plant hormones is extracted from plant material such as Lotus japonicus with organic solvent and partially purified with reversed phase solid phase extraction and ready for LC-MS analysis. Calibration curve is constructed by spiking pure standard in the matrix. The method allows us to quantify plant hormones in plant tissue of interest by correcting matrix effects.

Materials

- Plant hormone (gibberellin A₄, abscisic acid, salicylic acid, jasmonic acid, indole-3-acetic acid, *trans*-zeatin, *cis*-Zeatin, brassinolide)
- 2 ml tube
- Methanol
- Acetonitrile
- Oasis HLB cartridge (Part number: WAT094225, Waters)
- Centrifuge
- Sonicator
- Tube rotator
- Vacuum concentrator
- Orbitrap Q-Exactive (Thermo Fisher Scientific)

Before start

Extraction for plant hormones has been conducted utilizing a previously established protocol with minor modifications (Simura, J., et al. Plant Physiol, 177, 476-489, 2018).

In standard addition method, some experimental condition such as the amount of starting material and dilution ratio have to be optimized when other tissues or plant species are used.

- 1 Plant tissues of interest such as leaves, roots, and stems are disrupted in liquid nitrogen using a homogenizer (e.g. TissueLyzer, Qiagen).
- 2 Collect 50 mg of the tissue and extract with 1 ml of cold 50 % acetonitrile. Prepare the tissue for both sample and standards. For example, when you make calibration curve with 5 different spiking solutions, prepare six homogenized-tissues in total (one for sample and five for standards).
- 3 Sonicate the solutions for 5 min at 4°C using a ultrasonicator (e.g. 1510J-MT, BRANSON) and then incubate on a tube rotator (SLRM-2M intelli mixer, My lab) for 60 min at 4°C.
- 4 After centrifuge at 15,000 g for 15 min, collect the supernatant to a new tube.
- 5 Activate Oasis HLB cartridge (Waters) with 1 ml of 100 % methanol and then wash 1 ml of ultrapure water.
- 6 Equilibrate the cartridge with 1ml of 50 % methanol. Load the sample collected onto the cartridge at step 4. Collect the flow-through into a 2 ml tube .
- 7 Elute with 1 ml of 30 % acetonitrile in the tube and evaporate to dryness in vacuum concentrator (e.g. SPD140DDA, Thermo Fisher Scientific) for 3 hours or overnight.
- 8 Dissolve dried pellets in 100 µl of 30 % acetonitrile.
- 9 Prepare stock solutions by adding each plant hormone into one tube. When you make five working solutions, prepare five stock solutions (e.g. 20, 200, 1000, 2000, 5000 ng/ml)
- 10 Make matrix calibration curve by diluting stock solutions with the matrix. For example, add 2.5 µl stock solutions to 47.5 µl matrix collected at step 8.
Note that dilution factor depends on plant material. If a sample contains high concentration of plant hormones, the sample should be diluted more.
- 11 Analyze 10 µl of samples by LC-MS/MS and obtain the peak area of each plant hormone. See the external link to our paper for analytical conditions used in Q-Exactive (Thermo Fisher Scientific).
- 12 Subtract the peak area of standard by the peak area of matrix and obtain the equation including the y-intercept and the slope of the calibration curve. Calculate the concentration of plant hormones by substituting the peak area of the sample for the equation.