

## Analysis Report

<b>Project Name</b>	Gibberellins Quantification Analysis
<b>Sample Description</b>	Pine Pollen_Lot#BPP23AUG24 Pine Pollen Tincture_ Lot#PPT216AUG24
<b>Sample Quantity</b>	2
<b>Order Number</b>	CPZD11012402
<b>Client</b>	Zane Christopher
<b>Project Date</b>	2025-3
<b>Remark</b>	

## 1. Sample Information

2 pine pollen and pine pollen tincture samples for gibberellins analysis in the collected samples.

## 2. Sample Preparation and LC-MS Analysis

### 2.1 Chemicals and reagents

Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany).

MilliQ water (Millipore, Bradford, USA) was used in all experiments.

All of the standards were purchased from Olchemim Ltd. (Olomouc, Czech Republic).

Formic acid, triethylamine and 3-bromopropyltrimethylammonium bromide were bought from Sigma-Aldrich (St Louis, MO, USA).

The stock solutions of standards were prepared at the concentration of 1 mg/mL in methanol. All stock solutions were stored at -20°C.

### 2.2 Sample preparation

Transfer 50 mg or 50  $\mu$ L of sample into tube, extracted with 1 mL MeOH /H<sub>2</sub>O/formic acid (15:4:1, v/v/v). 10  $\mu$ L internal standard mixed solution (10 ng/mL) was added into the extract as internal standards (IS) for the quantitation. The mixture was vortexed for 15 min, then centrifugated for 10 min (12000 r/min, and 4°C). The supernatant was transferred to clean plastic microtubes and evaporated to dryness. 500  $\mu$ L H<sub>2</sub>O with 3.5% formic acid and 1 mL ethyl acetate were added to the residue. The sample was vortexed for 15 min and centrifugated for 5 min (12000 r/min, and 4°C). The supernatant was transferred to a brown injection vial. 500  $\mu$ L ethyl acetate was added to re-extracted and the supernatant was combined after centrifugated for 5 min (12000 r/min, and 4°C). The combined ethyl acetate part was evaporated to dryness and dissolved in ACN. To the resulting solution, 10  $\mu$ L triethylamine (TEA) and 10  $\mu$ L 3-bromopropyltrimethylammonium bromide (BPTAB) were added. The reaction solution was vortexed, incubated at 90°C for 1 h, and evaporated to dryness under nitrogen gas stream followed by redissolving in 100  $\mu$ L ACN/H<sub>2</sub>O (90:10, v/v), and filtered through a 0.22  $\mu$ m membrane filter for further LC-MS/MS analysis.

### 2.3 LC-MS conditions

LC parameters,

UPLC column, Waters ACQUITY UPLC CSH (1.7  $\mu$ m, 100 mm $\times$ 2.1mm);  
Mobile Phase A: 0.05% formic acid water (A), Mobile Phase B: 0.05% formic acid acetonitrile (B);  
The column temperature was held at 40°C, injection volume is 10  $\mu$ L. The elution gradient is as follows.

Time(min)	Flow Rate(mL/min)	(B)%
0.00	0.350	5
10.00	0.350	95
11.00	0.350	95
11.10	0.350	5
14.00	0.350	5

MS parameters,

Ion source, ESI+; source temperature 550°C; ion spray voltage (IS) 5500 V; curtain gas (CUR) was set at 35 psi, respectively. BRs were analyzed using scheduled multiple reaction monitoring (MRM). Data acquisitions were performed using Analyst 1.6.3 software (Sciex). Multiquant 3.0.3 software (Sciex) was used to quantify all metabolites. Mass spectrometer parameters including the declustering potentials (DP) and collision energies (CE) for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

### 3. Analytical Results

The results in ng/g or ng/mL of each compound are summarized.

Compounds	Q1 (Da)	Q3 (Da)	Pine Pollen	Pine Pollen Tincture
			Lot#BPP23AUG24	Lot#PPT216AUG24
			ng/g	ng/mL
GA1	448.3	389.2	3.449	2.708
GA3	446.3	387.2	N/A	N/A
GA4	432.4	373.3	4.007	4.491
GA8	464.3	405.2	N/A	N/A
GA9	416	357.3	N/A	N/A
GA12	416.2	253.2	408.404	368.301
GA19	281.3	118.2	19.579	13.941
GA20	432.4	373.2	2.270	1.844
GA24	273.2	243.7	1.956	11.138
GA29	448	389	N/A	N/A