1.1 Determine the composition of the mobile phase and the whole cell biocatalyst.

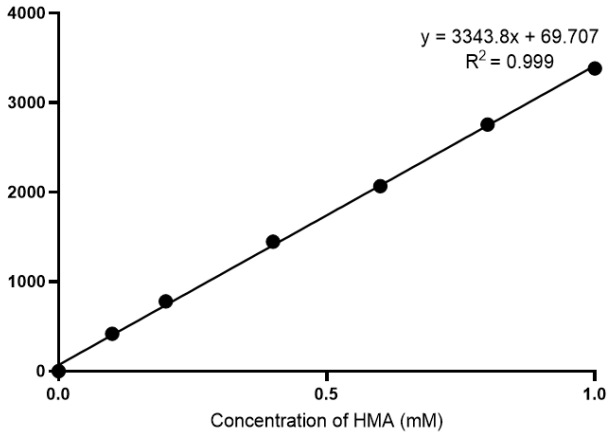
The mobile phase system used in the experiment included 10% methanol, 90% water and 20 mM PBS buffer, and the whole cell biocatalyst system consisted of 2.5 mM HPP and 50 mM Tris-Base buffer. The HPP and HMA standards were detected by high performance liquid chromatography (HPLC), the peak times were determined and the standard curves were plotted at the same time. Conditions for the sample assay: the whole cell catalytic product was diluted 10-fold using methanol before being measured on the machine.

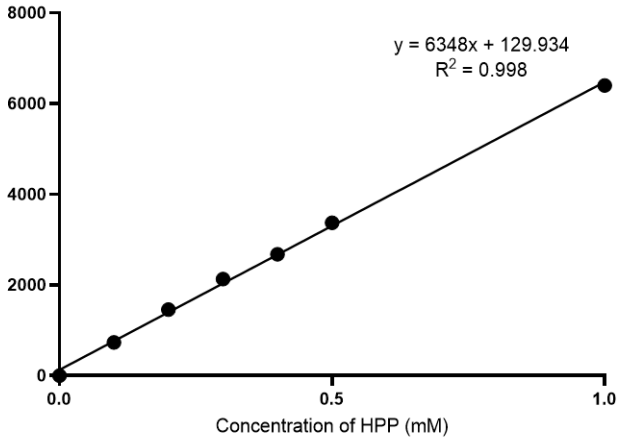
Mobile Phase and Whole Cell Biocatalyst System:

Mobile Phase System (1 L)	
$NaH_2PO_4 \cdot 2H_2O$	2.14 g
$Na2HPO_4 \cdot 2H_2O$	2.56 g
Methanol	100 mL
ddW	To 1 L

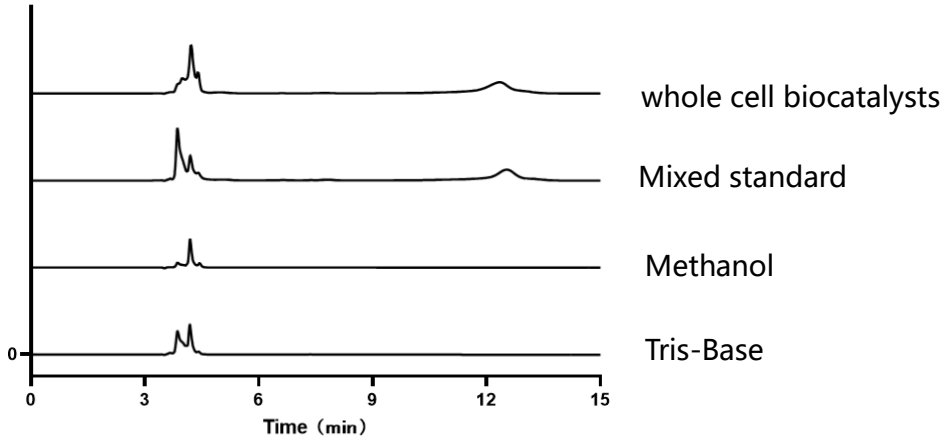
Whole Cell Biocatalyst (50 mL)	
HEPES	50 mM
HPP	9 mM
ddW	To 50 mL

Standard curves are as follows:





Chromatography results are as follows:



The initial peak time for HMA is approximately 3.9 minutes, while for HPP it is around 12.7 minutes.

However, we noticed that the Tris-Base Peak is extremely close to the HMA peak, which interferes with the detection of HMA. Therefore, we made improvements to the mobile phase and whole cell biocatalysts system.

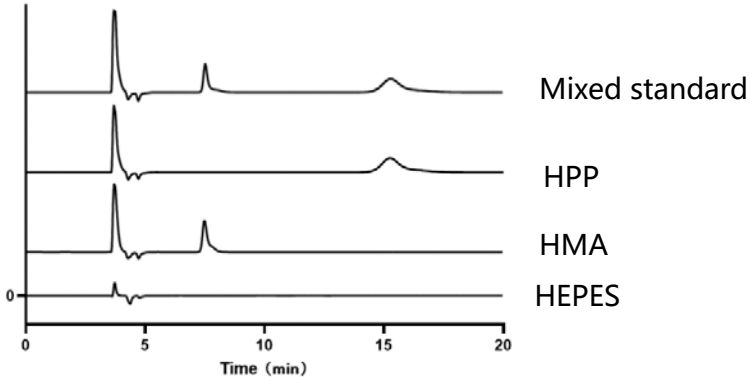
First, we replaced the Tris-Base buffer with HEPES buffer in the whole cell biocatalyst. Then we added 0.1% formic acid to the mobile phase to suppress the ionization of the sample and enhance the peak shape.

The improved system is as follows:

Mobile Phase System (1 L)	
$NaH_2PO_4 \cdot 2H_2O$	2.14 g
$Na2HPO_4 \cdot 2H_2O$	2.26 g
Methanol	100 mL
Formic acid	1 mL
ddW	To 1 L

Whole Cell Biocatalyst (50 mL)	
HEPES	50 mM
HPP	9 mM
ddW	To 50 mL

The improved mobile phase and the whole cell biocatalyst system are verified by HPLC. The chromatography is as follows. After the improvement of the system, the HMA peak can be distinguished from the HEPES and HPP peaks. The initial peak time for HMA is approximately 7.7 minutes, while for HPP it is around 16.0 minutes.



1.2 Drawing of the product generated curve.

The gY9s（Scpa1）-Bio177-Hmas/BWΔCD strain is induced at 30°C and performed whole cell catalysis. The time for the whole cell catalytic is set to 0.5h, 1h, 1.5h, 2h, 3h, 4h. Dilute catalytic products ten times with distilled water before machine detection. Then draw a curve diagram of the HMA concentration over time. The relevant systems and conditions are as follows:

Induction system (5 mL)	
ZY	4.8 mL
50 × 5052	100 μL
50 × M	100 μL
1M $MgSO_4$	10 μL
1000 × Microelement	10 μL
Bacterial solution	50 μL
IPTG	5 μL
Str	5 μL
Chl	5 μL

The gY9s（Scpa1）-Bio177-Hmas/BWΔCD strain showed the highest HMA production after 2 h of whole cell catalysis, with a conversion rate of 19.4%.

1.3 Drawing of the chassis strain HPP generated curve

The double-transformed strain (PSB1c-AroG-tktA-ppsA+gY9s(Scpa1)-Bio177-Hmas) were induced and fermented at 30°C. The fermentation time was set as 0.5h，1h，1.5h，2h, 3h, 4h. Samples were diluted tenfold using distilled water and assayed on the machine. The relevant systems and conditions were as follows:

Induction system (100 mL)	
ZY	96 mL
50 × 5052	2 ml
50 × M	2 ml
1M $MgSO_4$	200 μL
1000 × Microelement	200 μL
Bacterial solution	1 ml
Arabinose	1 ml
Str	100 μL
Chl	100 μL

Fermentation system (10 mL)	
5 x M9	2 mL
1M $MgSO_4$	20 μL
1M $CaCl_2$	1 μL
20% Glucose	500 μL
DDW	7.47ml
Str	10 μL
Chl	10 μL

The curve chart is as follows: