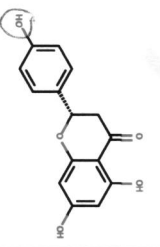
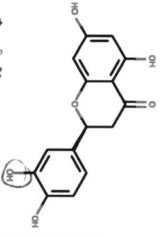
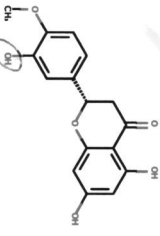
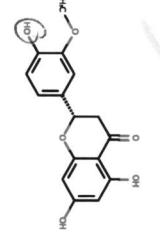
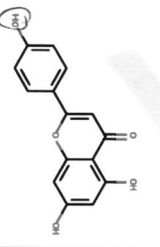
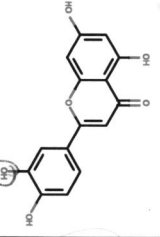
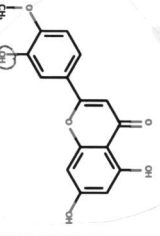
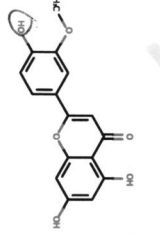
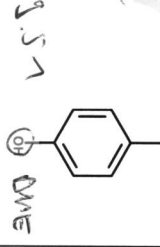
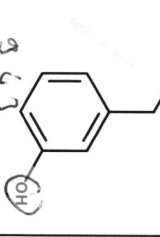
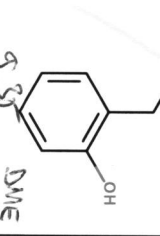
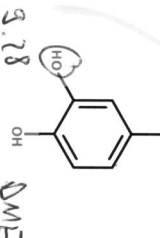
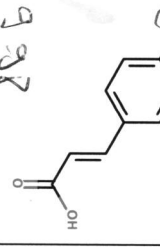
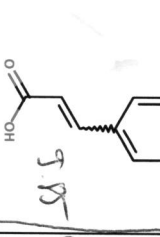
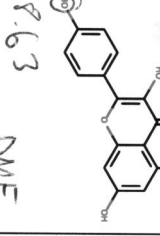
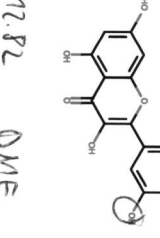
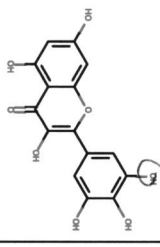
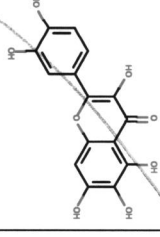


from

1	 9.75 DME	2	 9.69 DME	3	 9.98 DME	4	 10.06 DME
5	Naringenin	6	Eriodictyol	7	hesperetin	8	homoeriodictyol
9	 8.83 DME	10	 12.16 ✓	11	 9.57 ✓	12	 9.24 ✓
apigenin		luteolin		diosmetin		chrysoeriol	
13	 9.51 DME	14	 9.43 ✓	15	 9.37 DME	16	 9.28 DME
p-coumaric acid		m-coumaric acid		o-coumaric acid		caffeic acid	
17	 9.98	18	 5.15 ✓	19	 8.63 DME	20	 12.82 DME
ferulic acid		iso-ferulic acid		kaempferol		quercetin	

17	 11.19 DME	18	
myricetin		quercetagenin	

Pinocembrin (7-methyl)

lul. very fresh 0106.5.1.2
Folienon TLC

7-yl

1 Introduction

Determination of the pH optimum of PFOMT with different substrates (caffeic acid, eriodictyol and iso-ferulic acid) and in low and high magnesium conditions. Measure progresscurve using six (6) timepoints (0, 3, 6, 9, 30 min and 2 h). Two different Mg^{2+} -concentrations (0 and 10 mM), as well as five pH-points (5.5, 6.5, 7.5, 8.5, 9.5). For each substrate this amounts to $6 \times 5 \times 2 = 60$ measurements.

2 Methods

2.1 Assays

The standard reaction conditions for a total volume of 50 μ l are 50 mM MMT-buffer, 0.4 mM caffeic acid, 2.5 μ M GSH, 0.5 mM SAM, \pm 10 mM $MgCl_2$ and 20 μ g PFOMT, plus 0.1 mM flavon as ITSD.

OMT-Reaction (50 μ l):

volume	compound	final concentration
25 μ l	100 mM buffer	50 mM
2 μ l	10 mM caffeic acid	0.4 mM
1.25 μ l	0.1 M GSH	2.5 μ M
6.13 μ l	81% 5 mM SAM (4.05 mM)	0.5 mM
10 μ l	1 mg/ml PFOMT	0.2 μ g/ μ l
ad to 50 μ l H_2O (5.62 μ l)		

+0.5 μ l 10 mM flavon

A standard substrate mastermix was prepared first. This mastermix was then added to an appropriate amount of buffer. 40 μ l of this resulting *reaction buffer* was pipetted into an 1.5 mL centrifuge tube and the reaction was started by addition of PFOMT.

substrate mastermix A (40 \times 15 μ l):

volume	compound	final concentration		
80 μ l	10 mM caffeic acid	1.33 mM	✓	✓
50 μ l	0.1 M GSH	8.33 μ M	✓	✓
245.25 μ l	81% 5 mM SAM	1.655 mM		✓
20 μ l	10 mM flavone	0.33 mM	✓	✓
ad to 600 μ l H_2O (204.75 μ l)			✓	✓

substrate mastermix B (40 \times 15 μ l):

volume	compound	final concentration		
80 μ l	10 mM caffeic acid	1.33 mM	✓	✓
50 μ l	0.1 M GSH	8.33 μ M	✓	✓
245.25 μ l	81% 5 mM SAM	1.655 mM		✓
20 μ l	1 M $MgCl_2$	33.3 mM	✓	✓
20 μ l	10 mM flavone	0.33 mM	✓	✓
ad to 600 μ l H_2O (184.75 μ l)			✓	✓

pH 5x
 ① ② ③ ④ ⑤
 5.4 6.5 7.5 8.5 9.5

AgI 2x

Substrate 1x (3x)

Carbonic acid

ED

(iso-leukic acid)

2.63 ml +

1.2086

+860

4 ml

time points 6

~~0 1 5 10 15 20~~

0 3 6 9 12 15
 30 2h

120 (180)

50 µl 80x

870

25 µl buffer

+

70x

20 µl

substrate run

8 x 75

20 µl

②

substrate mastermix ^A (40 × 15 μl):

volume	compound	final concentration
80 μl	10 mM eriodictyol	1.33 mM ✓
50 μl	0.1 M GSH	8.33 μM ✓
245.25 μl	81% 5 mM SAM	1.655 mM
20 μl	10 mM flavone	0.33 mM ✓
ad to 600 μl H ₂ O (204.75 μl)		

substrate mastermix ^B (40 × 15 μl):

volume	compound	final concentration
80 μl	10 mM eriodictyol	1.33 mM ✓
50 μl	0.1 M GSH	8.33 μM ✓
245.25 μl	81% 5 mM SAM	1.655 mM
20 μl	1 M MgCl ₂	33.3 mM ✓
20 μl	10 mM flavone	0.33 mM ✓
ad to 600 μl H ₂ O (184.75 μl)		

reaction buffer preparation:

1. add 105 μl (7 × 15) *substrate-MM* to 175 μl (7 × 25) of 100mM of each 100 mM buffer (pH 5.4, 6.5, 7.5, 8.5, 9.5)

Reaction:

1. 40 μl of reaction buffer (2.1) is pipetted into a 1.5 mL centrifuge tube, set at room temperature
2. reaction is started by addition of 10 μl 1 mg/mL PFOMT
3. incubate at 30 °C, 100 rpm
4. stop reaction by addition of 15 μl stop solution (10% TCA in 50% ACN)

+ 70 μl PFOMT → take 50 μl after each time p

Table 1: Sample times for activity test.

sample time <i>t_i</i> (min)	addition of PFOMT (<i>t₀</i> + ... min)	stop time (min)
<i>t₀</i> = 0		
3		
6		
9		
30		
120		

start enzyme



substrate mix → buffer → false samples

	Start	3	6	9	Re30	120
A1	0	3	6	9	30	120
A2	30	330	630	93	30.3	120.3
A3	1	4 30	7	10	31	121
A4	130	430	730	103	31.3	121.3
A5	2	5	8	11	32	122
A6	2.30					
B1	12	15	18	21	42	132
B2	12.3	15.3	18.3	21.3	42.3	132.3
B3	13	16	19	22	43	133
B4	13.3	16.3	19.3	22.3	43.3	133.3
B5	14	17	20	23	44	134

bei 30°C stalt Eis! → nicht dann Eis!

→ nach 18h 50 min

BC → nach 19h 50 min

20 μ l Substrate mix

25 μ l buffer (100 mM)
 + 15 μ l Substrate mix
 (0.3 mM Flavon
 1.3 mM substrate
 8.3 mM GSH
 1.6 mM SAM

pH	f (pH 9)	ml pH 9
3.97	0	
4.22	0.25	0.5
4.56	0.44	0.82
5.13	0.58	1.16
6	0.75	1.5
7.29	0.92	1.84
10.09	1	(2 ml)

Expt @ 30°C / substrate @ RT

