BAB V
KESIMPULAN DAN SARAN

A. KESIMPULAN

Berdasarkan dari hasil penelitian yang telah dilakukan dapat diperoleh kesimpulan bahwa:

Pertama, myricetin dapat dibuat nanofitosom dengan menggunakan metode hidrasi lapis tipis-sonikasi.

Kedua, karakterisasi nanofitosom dilihat dari ukuran partikel semua formula memiliki ukuran di antara 10-1000 nm, penggunaan fosfatidilkolin pada konsentrasi tertinggi sebesar 118 mg mampu menghasilkan ukuran partikel paling kecil yaitu 139 nm dan indeks polidipersitas terendah yaitu 0,380 dengan efisiensi penjerapan tertinggi 91,54%.

Ketiga, nanofitosom myricetin formula 1, 2, 3 dan 4 tidak stabil selama penyimpanan lebih dari 3 minggu, formula 5 memiliki nilai potensial zeta -6,350 mV, sehingga kurang stabil setelah penyimpanan karena nilai potensial zeta kurang dari ±30 mV.

B. SARAN

Penelitian ini masih banyak kekurangan, maka perlu dilakukan penelitian lebih lanjut mengenai:

Pertama, perlu dilakukan pembuatan nanofitosom dengan metode lainnya yang bisa memberikan ukuran partikel lebih kecil dan stabil.

Kedua, perlu dilakukan uji karakterisasi morfologi nanofitosom menggunakan alat *Transmission Electron Microscopy* (TEM).

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Lampiran 1. Sertifikat analisis myricetin

TOCRIS
a biotechne brand

Print Date: Jul 20th 2017

Certificate of Analysis

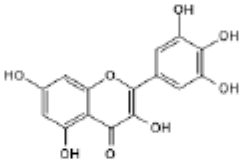
www.tocris.com

Product Name: Myricetin **Catalog No.:** 6189 **Batch No.:** 1

CAS Number: 529-44-2

IUPAC Name: 3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4H-1-benzopyran-4-one

1. PHYSICAL AND CHEMICAL PROPERTIES

Batch Molecular Formula:	$C_{15}H_{10}O_8.H_2O$
Batch Molecular Weight:	336.26
Physical Appearance:	Yellow solid
Solubility:	DMSO to 100 mM ethanol to 50 mM
Storage:	Store at -20°C
Batch Molecular Structure:	

2. ANALYTICAL DATA

HPLC:	Shows 97.7% purity
^{1}H NMR:	Consistent with structure
Mass Spectrum:	Consistent with structure
Microanalysis:	

	Carbon	Hydrogen	Nitrogen
Theoretical	53.58	3.6	
Found	53.6	3.57	

Lampiran 2. Sertifikat analisis fosfatidilkolin

Lipoid

PHOSPHOLIPID GmbH · Member of the Lipoid Group

ANALYTICAL DATA

AN33119384/8

- 1 -

PHOSPHOLIPON 90 G

Batch 228154-3180044

Recommended storage n.m.t. +8 °C
Date of production 07/2018

Sample for laboratory use only

Parameter	Result	Specification min	Specification max	Unit	Method
Phosphatidylcholine	96,1	94,0	102,0	% (m/m)	05.P07.857
Identity (TLC)	conform to reference	conform to reference			05.P08.309
Lysophosphatidylcholine	1,4		4,0	% (m/m)	05.P07.857
Nonpolar lipids	1,0		3,0	% (m/m)	05.P03.008
Tocopherol	0,21		0,30	% (m/m)	05.P07.142
Acid value	0,2		0,5		05.P03.002
Peroxide value	1,8		5,0		05.P06.120
Water	0,2		1,5	% (m/m)	05.P10.013
Toluene insolubles	0,00		0,05	% (m/m)	05.P06.001
Ethanol	0,1		0,2	% (m/m)	05.P05.049
Heavy metals	< 10		10	mg/kg	USP <231> method II
Arsenic	< 0,015		0,15	mg/kg	USP <232>/ USP <233>
Lead	< 0,015		0,10	mg/kg	USP <232>/ USP <233>
Appearance	yellowish, waxy	yellowish, waxy			05.P06.155

../2

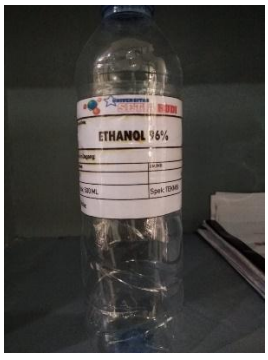
PHOSPHOLIPID GmbH · Nattermannallee 1 · D-50829 Köln · Tel. 0221 - 98 74 6 - 0 · Fax 0221 - 98 746 - 216

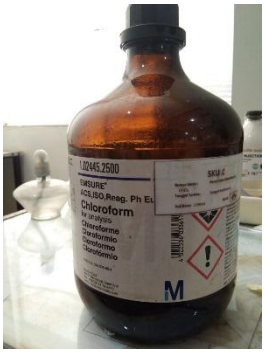
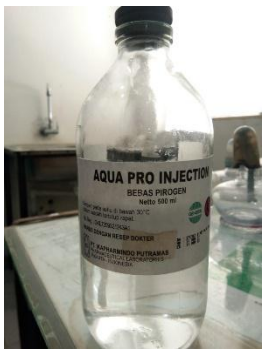
Lampiran 3. Alat yang digunakan dalam penelitian

Alat	Nama alat	Kegunaan
	Neraca analitik	Menimbang bahan baku dan eksipien
	Spektrofotometer UV-Vis	Membaca absorbansi, mencari lamda maksimum dan OT sampel
	*Magnetic stirrer*	Menghomogenkan formula dengan pengadukan
	*Rotary evaporator*	Menguapkan pelarut organik

	pH meter	Mengukur pH
	Sonikasi probe	Menghomogenkan dan memperkecil ukuran partikel
	*Particle size analyzer*	Mengukur ukuran partikel dan zeta potensial
	Sentrifugasi	Memisahkan obat bebas dengan obat yang terjerap dalam nanofitosom

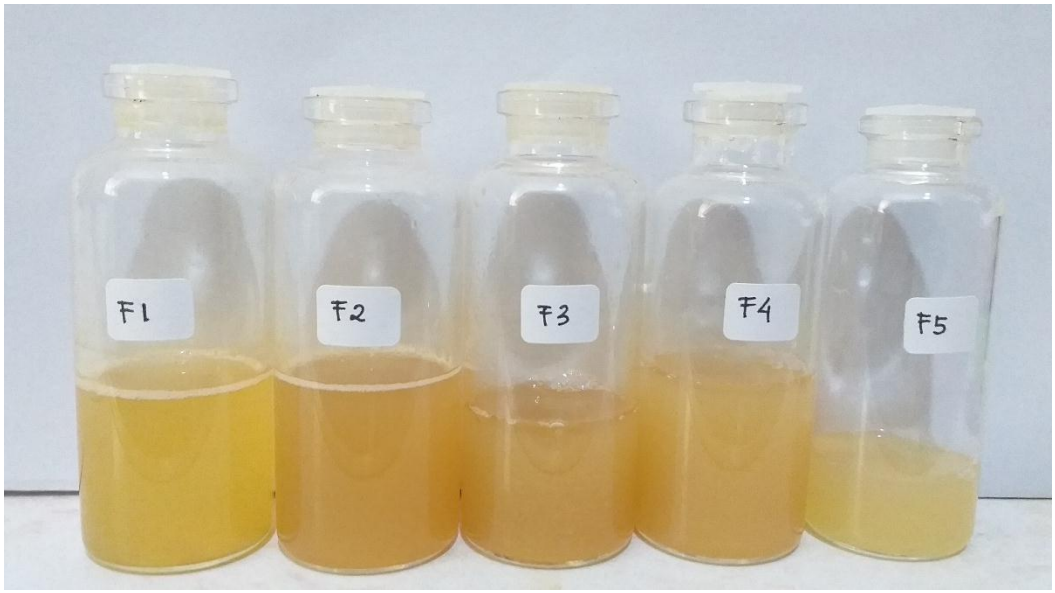
Lampiran 4. Bahan yang digunakan dalam penelitian

Alat	Nama alat	Kegunaan
	Myricetin	Zat aktif
	Fosfatidilkolin	Zat pembawa
	Kolesterol	Penstabil
	Etanol 96%	Pelarut myricetin dan fosfatidilkolin

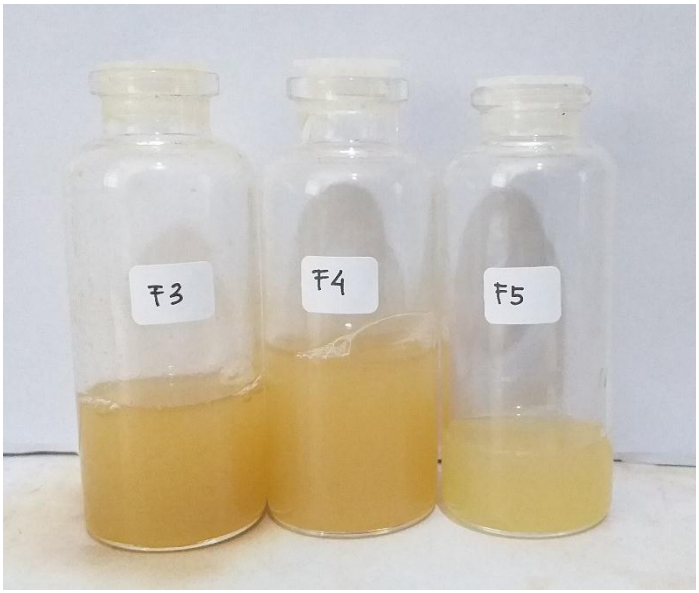
	Kloroform	Pelarut kolesterol
	*Phosphat Buffer Saline* (PBS) pH 7,4	Untuk menghidrasi lapisan tipis nanofitosom
	DPPH	Radikal bebas untuk mengukur aktivitas antioksidan sampel
	Etanol p.a	Pelarut DPPH
	Aqua p.a	Untuk membuat larutan *Phosphat Buffer Saline* (PBS)

	NaOH	Mengendalikan tingkat keasaman atau pH pada larutan PBS

Lampiran 5. Nanofitosom myricetin setelah disonikasi



Lampiran 6. Nanofitosom myricetin setelah uji stabilitas



Lampiran 7. Perhitungan formula

Kadar myricetin murni : 98%

Berat molekul myricetin : 318,2351 gram/mol

Berat molekul fosfatidilkolin : 768 gram/mol

Berat molekul kolesterol : 386,67 gram/mol

- Kandungan myricetin dalam 10 mg serbuk

  Myricetin (mg) = 10 mg x $\frac{98}{100}$

  = 9,8 mg

- Mol myricetin dalam 10 mg serbuk

  Mol myricetin (µmol) = $\frac{9{,}8\ mg}{318235\ mg \times\ 10^{-6}\mu mol}$

  = 30,7949 µmol

- Fosfatidilkolin

  a. Formula 1:1

     Fosfatidilkolin yang ditimbang = 30,7949 µmol × $\frac{768.000\ mg}{10^{6}\mu mol}$

     = 23,6505 mg

  b. Formula 1:2

     Fosfatidilkolin yang ditimbang = 61,5898 µmol × $\frac{768.000\ mg}{10^{6}\mu mol}$

     = 47,3010 mg

  c. Formula 1:3

     Fosfatidilkolin yang ditimbang = 92,3847 µmol × $\frac{768.000\ mg}{10^{6}\mu mol}$

     = 70,9514 mg

  d. Formula 1:4

     Fosfatidilkolin yang ditimbang = 123,1796 µmol × $\frac{768.000\ mg}{10^{6}\mu mol}$

     = 94,6019 mg

  e. Formula 1:5

     Fosfatidilkolin yang ditimbang = 153,9745 µmol × $\frac{768.000\ mg}{10^{6}\mu mol}$

     = 118,2524 mg

- Kolesterol

  Perbandingan myricetin:kolesterol (1:0,2), maka jumlah kolesterol yang dibutuhkan 30,7949 µmol × 0,2 = 6,1590 µmol

  Kolesterol yang ditimbang = 6,1590 µmol × $\frac{386.670\ mg}{10^6 \mu mol}$

  = 2,3815 mg

Lampiran 8. Ukuran partikel

Ukuran partikel	Formula				
	1	**2**	**3**	**4**	**5**
Replikasi 1	905,2	688,5	163,5	211,2	139,1
Replikasi 2	912,2	471,8	155,9	197,9	139,2
Replikasi 3	930,5	619,1	192,9	211,1	140,1
Rata-rata±SD	915,967 13,064	539,133 110,659	170,767 19,541	206,733 7,650	139,467 0,551

Lampiran 9. Indeks Polidispersitas

Indeks polidispersitas	Formula				
	1	**2**	**3**	**4**	**5**
Replikasi 1	0,572	1,000	0,474	0,385	0,375
Replikasi 2	0,484	1,000	0,417	0,405	0,387
Replikasi 3	0,575	0,924	0,431	0,454	0,377
Rata-rata±SD	0,544 0,052	0,975 0,044	0,441 0,030	0,415 0,036	0,380 0,006

Hasil Ukuran Partikel F1

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	Nanofitosom Myricetin F1 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	NanofotosomMyricetin_2...	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1,330
Material RI:	1,52	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,100	**Measurement Date and Time:**	Jumat, 26 April 2019 09.51....

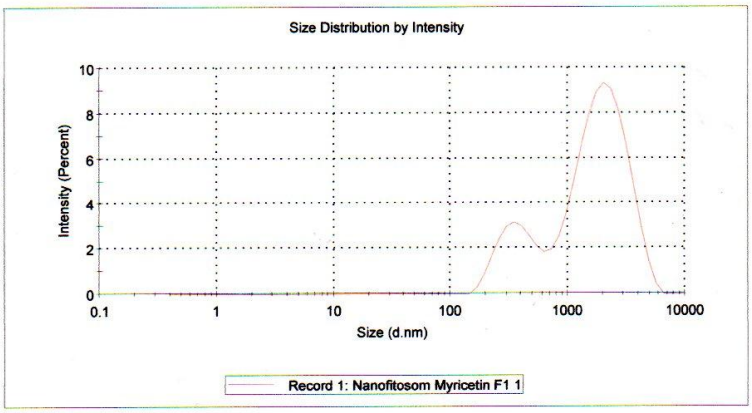
System

Temperature (°C):	25,0	**Duration Used (s):**	60
Count Rate (kcps):	343,8	**Measurement Position (mm):**	1,25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	4

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	905,2	**Peak 1:**	2091	79,5	987,1
PdI:	0,572	**Peak 2:**	372,4	20,5	123,1
Intercept:	0,860	**Peak 3:**	0,000	0,0	0,000

Result quality Good



Hasil Ukuran Partikel F2

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	Nanofitosom Myricetin F2 1
SOP Name:	mansettings.nano
General Notes:	

File Name:	NanofotosomMyricetin_2...	**Dispersant Name:**	Water
Record Number:	7	**Dispersant RI:**	1,330
Material RI:	1,52	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,100	**Measurement Date and Time:**	Jumat, 26 April 2019 10.21....

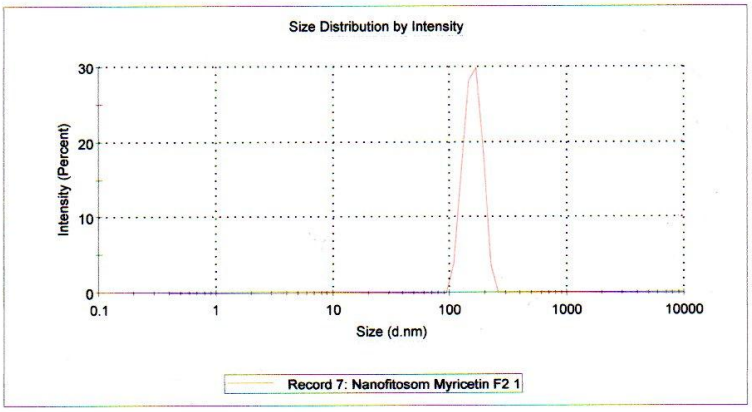
System

Temperature (°C):	25,0	**Duration Used (s):**	60
Count Rate (kcps):	323,3	**Measurement Position (mm):**	1,05
Cell Description:	Disposable sizing cuvette	**Attenuator:**	3

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	688,5	**Peak 1:**	155,8	100,0	26,93
PdI:	1,000	**Peak 2:**	0,000	0,0	0,000
Intercept:	0,795	**Peak 3:**	0,000	0,0	0,000

Result quality Refer to quality report



Hasil Ukuran Partikel F3

Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

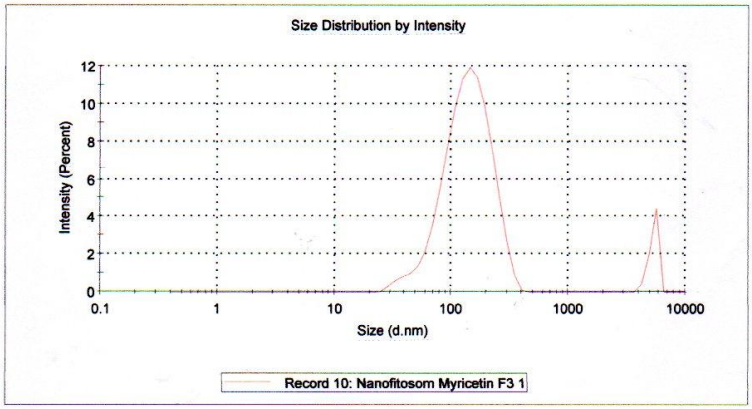
Sample Name:	Nanofitosom Myricetin F3 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	NanofotosomMyricetin_2...	**Dispersant Name:**	Water
Record Number:	10	**Dispersant RI:**	1,330
Material RI:	1,52	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,100	**Measurement Date and Time:**	Jumat, 26 April 2019 10.32....

System

Temperature (°C):	25,0	**Duration Used (s):**	60
Count Rate (kcps):	158,1	**Measurement Position (mm):**	1,05
Cell Description:	Disposable sizing cuvette	**Attenuator:**	3

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	163,5	**Peak 1:**	145,6	93,2	63,34
PdI:	0,474	**Peak 2:**	5249	6,8	443,5
Intercept:	0,931	**Peak 3:**	0,000	0,0	0,000
Result quality	Good				



Hasil Ukuran Partikel F4

Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	Nanofitosom Myricetin F4 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	NanofotosomMyricetin_2...	**Dispersant Name:**	Water
Record Number:	13	**Dispersant RI:**	1,330
Material RI:	1,52	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,100	**Measurement Date and Time:**	Jumat, 26 April 2019 10.44....

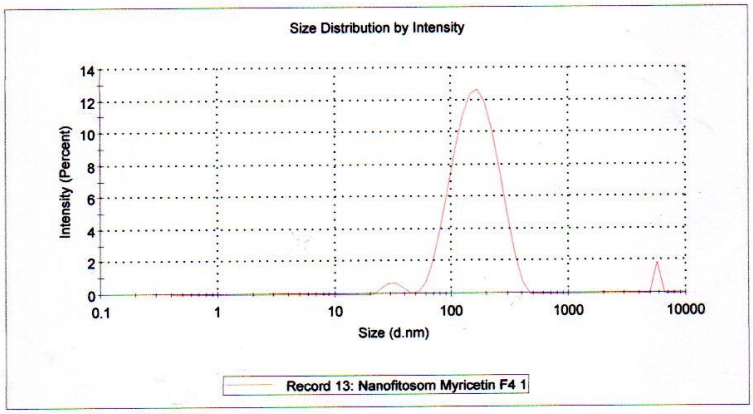
System

Temperature (°C):	25,0	**Duration Used (s):**	70
Count Rate (kcps):	170,5	**Measurement Position (mm):**	0,85
Cell Description:	Disposable sizing cuvette	**Attenuator:**	3

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	211,2	**Peak 1:**	168,5	95,8	68,40
PdI:	0,385	**Peak 2:**	32,23	2,3	6,163
Intercept:	0,889	**Peak 3:**	5560	1,9	0,000

Result quality Refer to quality report



Hasil Ukuran Partikel F5

Size Distribution Report by Intensity
v2.2

Malvern

Sample Details

Sample Name:	Nanofitosom Myricetin F5 1
SOP Name:	mansettings.nano
General Notes:	

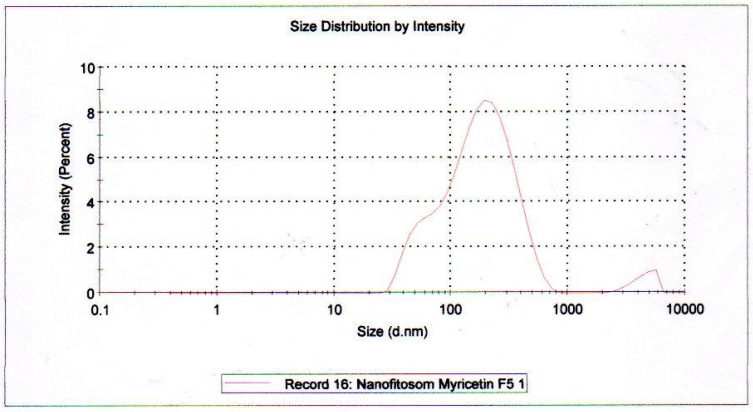
File Name:	NanofotosomMyricetin_2...	**Dispersant Name:**	Water
Record Number:	16	**Dispersant RI:**	1,330
Material RI:	1,52	**Viscosity (cP):**	0,8872
Material Absorbtion:	0,100	**Measurement Date and Time:**	Jumat, 26 April 2019 10.56....

System

Temperature (°C):	25,0	**Duration Used (s):**	70
Count Rate (kcps):	237,5	**Measurement Position (mm):**	1,05
Cell Description:	Disposable sizing cuvette	**Attenuator:**	3

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	139,1	**Peak 1:**	194,5	96,4	121,5
PdI:	0,375	**Peak 2:**	4461	3,6	897,2
Intercept:	0,923	**Peak 3:**	0,000	0,0	0,000
Result quality	Good				



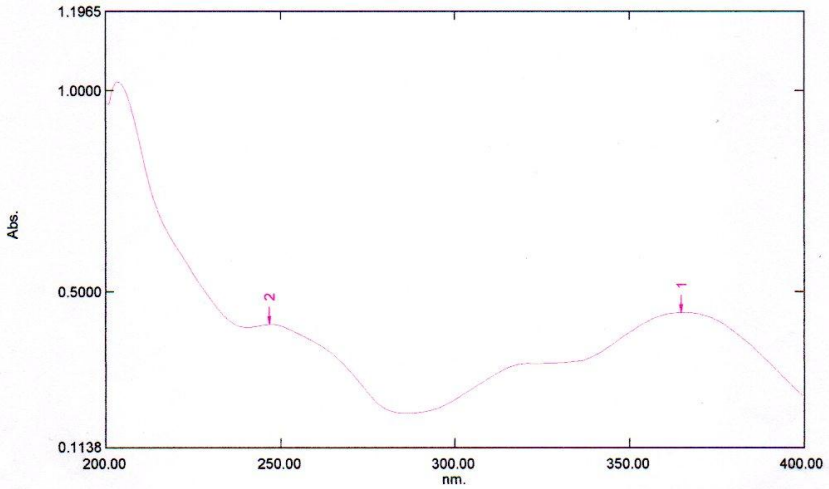
Lampiran 10. Kurva kalibrasi

a. Penetapan panjang gelombang myricetin dalam medium dapar pH 7,4

Spectrum Peak Pick Report

03/05/2019 08:46:50 AM

Data Set: MYRICETIN VILZA_084542 - RawData



No.	P/V	Wavelength	Abs.	Description
1	↑	365.00	0.4477	
2	↑	247.00	0.4174	
3	↓	286.00	0.1964	
4	↓	240.00	0.4097	

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Auto

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

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b. Penentuan *Operating Time* (OT)

Pembacaan *operating time* didapat nilai serapan yang stabil pada menit ke 14-17.

Kinetics Data Print Report

03/05/2019 09:21:35 AM

Time (Minute)	RawData ...
0.000	0.417
1.000	0.416
2.000	0.415
3.000	0.415
4.000	0.415
5.000	0.414
6.000	0.414
7.000	0.415
8.000	0.414
9.000	0.414
10.000	0.414
11.000	0.413
12.000	0.413
13.000	0.413
14.000	0.412
15.000	0.412
16.000	0.412
17.000	0.412
18.000	0.411
19.000	0.411
20.000	0.411
21.000	0.410
22.000	0.410
23.000	0.410
24.000	0.409
25.000	0.409
26.000	0.410
27.000	0.409
28.000	0.408
29.000	0.408
30.000	0.408

Page 1 / 1

c. Pembuatan larutan induk myricetin dalam medium dapar pH 7,4

Berat kertas kosong = 276,9 mg

Berat kertas + bahan = 287,2 mg

Berat bahan = 287,2 mg – 276,9 mg

= 10,3 mg

Berat kertas + sisa = 277,2 mg

Berat sisa = 277,2 mg – 276,9 mg

= 0,3 mg

Berat myricetin = 10,3 mg – 0,3 mg

= 10 mg

Volume dapar pH 7,4 = 100 mL

Larutan stok = 10 mg/100 mL

= 100 mg/1000 mL

= 100 ppm

d. Kurva baku myricetin dalam medium dapar pH 7,4

Larutan induk myricetin dibuat seri konsentrasi 6 ppm, 8 ppm, 10 ppm, 12 ppm dan 14 ppm dalam 25 mL.

1. 6 ppm

   $V_1 \times C_1 = V_2 \times C_2$

   $V_1 \times 100$ ppm = 10 mL x 6 ppm

   V_1 = 0,5 mL

2. 8 ppm

   $V_1 \times C_1 = V_2 \times C_2$

   $V_1 \times 100$ ppm = 10 mL x 8 ppm

   V_1 = 0,8 mL

3. 10 ppm

   $V_1 \times C_1 = V_2 \times C_2$

   $V_1 \times 100$ ppm = 10 mL x 10 ppm

   V_1 = 1 mL

4. 12 ppm

   $V_1 \times C_1 = V_2 \times C_2$

V_1 x 100 ppm = 10 mL x 12 ppm

V_1 = 1,2 mL

5. 14 ppm

V_1 x C_1 = V_2 x C_2

V_1 x 100 ppm = 10 mL x 14 ppm

V_1 = 1,4 mL

6. 16 ppm

V_1 x C_1 = V_2 x C_2

V_1 x 100 ppm = 10 mL x 16 ppm

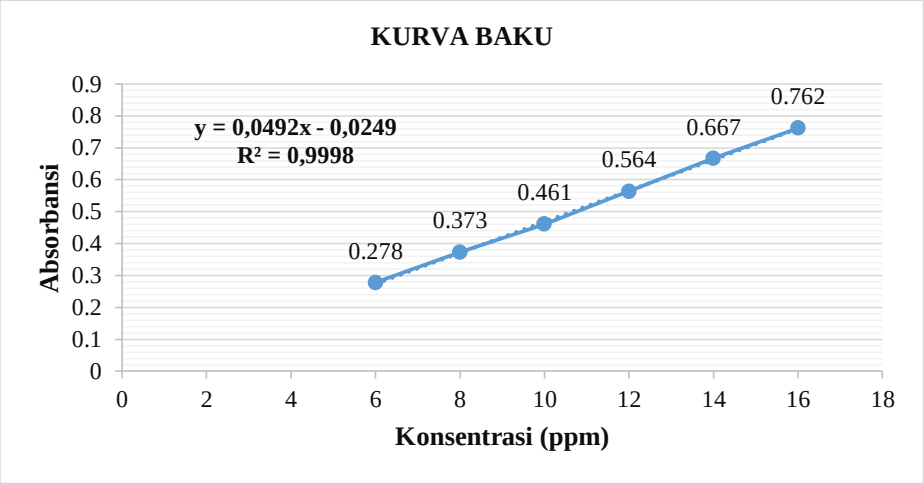
V_1 = 1,6 mL

e. Tabel kurva baku medium dapar pH

Larutan stok 100 ppm → 10 mg myricetin + 5 ml etanol p.a + ad 100 ml dapar fosfat pH 7,4

Konsentrasi (ppm)	Absorbansi
6	0,278
8	0,373
10	0,461
12	0,564
14	0,667
16	0,762

Persamaan regresi linier antara konsentrasi (ppm) dan serapan diperoleh:



a = -0,0249 b = 0,0492 r = 0,9998

y = a + b.x

y = -0,0249 + 0,0492.x

Keterangan:

x = konsentrasi

y = serapan

Lampiran 11. Verifikasi metode analisis

a. Linieritas

Konsentrasi (ppm)	Absorbansi
6	0,278
8	0,373
10	0,461
12	0,564
14	0,667
16	0,762

a = -0,0249

b = 0,0492

r = 0,9998

Hasil linearitas diperoleh R sebesar 0,9998, sehingga dapat disimpulkan bahwa data linier.

b. Penentuan LOD dan LOQ

KONSENTRASI (PPM)	ABSORBANSI (y)	$\hat{y}$	$\lvert y-\hat{y}\rvert$	$\lvert y-\hat{y}\rvert^2$
6	0,278	0,2703	0,0077	0,00005929
8	0,373	0,3687	0,0043	0,00001849
10	0,461	0,4671	-0,0061	0,00003721
12	0,564	0,5655	-0,0015	0,00000225
14	0,667	0,6639	0,0031	0,00000961
16	0,762	0,7623	-0,0003	0,00000009
Jumlah total $(\sum\lvert y-\hat{y}\rvert)^2$ = 0,00012694				

Nilai ŷ diperoleh dari substitusi konsentrasi dalam persamaan ŷ = -0,0249 +0,0492x dengan x adalah konsentrasi (ppm) dan ŷ adalah serapan.

1. ŷ = -0,0249 + 0,0492x

   ŷ = -0,0249 + 0,0492 x 6

   ŷ = 0,2703

2. ŷ = -0,0249 + 0,0492x

   ŷ = -0,0249 + 0,0492 x 8

   ŷ = 0,3687

3. $\hat{y}$ = -0,0249 + 0,0492x

   $\hat{y}$ = -0,0249 + 0,0492 x 10

   $\hat{y}$ = 0,4671

4. $\hat{y}$ = -0,0249 + 0,0492x

   $\hat{y}$ = -0,0249 + 0,0492 x 12

   $\hat{y}$ = 0,5655

5. $\hat{y}$ = -0,0249 + 0,0492x

   $\hat{y}$ = -0,0249 + 0,0492 x 14

   $\hat{y}$ = 0,6639

6. $\hat{y}$ = -0,0249 + 0,0492x

   $\hat{y}$ = -0,0249 + 0,0492 x 16

   $\hat{y}$ = 0,7623

- $S_{x/y}$ = $\sqrt{\frac{(\sum|y-\hat{y}|)2}{n-2}}$

  $S_{x/y}$ = simpangan baku residual

  $(\sum|y-\hat{y}|)^2$ = jumlah kuadrat total residual

  n = jumlah data

  $S_{x/y}$ = $\sqrt{\frac{0,00012694}{5-2}}$ = 0,00650487

- LOD = 3,3 x $\frac{Sx/y}{b}$

  = 3,3 x $\frac{0,00650487}{0,0492}$

  = 0,4363 ppm

  y = -0,0249 + 0,0492x

  = -0,0249 + 0,0492 (0,4363)

  = -0,0249 + 0,0215

  Serapan LOD = -0,0034

- LOQ = 10 x $\frac{Sx/y}{b}$

  = 10 x $\frac{0{,}00650487}{0{,}0492}$

  = 1,3221 ppm

  y = -0,0249 + 0,0492x

  = -0,0249 + 0,0492 (1,3221)

  = -0,0249 + 0,0650

  Serapan LOQ = 0,0401

c. Akurasi

Konsentrasi	Absorbansi	Konsentrasi terukur (ppm)	Konsentrasi sebenarnya (ppm)	Konsentrasi (%)	% recovery	Rata-rata % recovery
	0,497	10,6112	10	106		
80%	0,499	10,6518	10	107	106%	
	0,492	10,5095	10	105		
	0,598	12,6646	12	106		
100%	0,596	12,6240	12	105	106%	105%
	0,601	12,7256	12	106		
	0,684	14,4132	14	103		
120%	0,687	14,4742	14	103	103%	
	0,689	14,5148	14	104		

a = -0,0249 b = 0,0492 r = 0,9998

Perhitungan konsentrasi (ppm)

- Konsentrasi 80%

  Replikasi 1 = $\frac{\text{Absorbansi} - \text{a}}{b}$ = $\frac{0{,}497 - (-0{,}0249)}{0{,}0492}$ = 10,6112

  Replikasi 2 = $\frac{\text{Absorbansi} - \text{a}}{b}$ = $\frac{0{,}499 - (-0{,}0249)}{0{,}0492}$ = 10,6518

  Replikasi 3 = $\frac{\text{Absorbansi} - \text{a}}{b}$ = $\frac{0{,}492 - (-0{,}0249)}{0{,}0492}$ = 10,5095

- Konsentrasi 100%

Replikasi 1 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}598 - (-0{,}0249)}{0{,}0492}$ = 12,6646

Replikasi 2 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}596 - (-0{,}0249)}{0{,}0492}$ = 12,6240

Replikasi 3 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}601 - (-0{,}0249)}{0{,}0492}$ = 12,7256

- Konsentrasi 120%

Replikasi 1 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}684 - (-0{,}0249)}{0{,}0492}$ = 14,4132

Replikasi 2 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}687 - (-0{,}0249)}{0{,}0492}$ = 14,4742

Replikasi 3 = $\frac{\text{Absorbansi} - \text{a}}{b} = \frac{0{,}689 - (-0{,}0249)}{0{,}0492}$ = 14,5148

d. Presisi

Replikasi	Absorbansi	Konsentrasi terukur (ppm)	Konsentrasi sebenarnya (ppm)
1	0,475	10,1639	10
2	0,480	10,2655	10
3	0,479	10,2452	10
4	0,489	10,4485	10
5	0,484	10,3468	10
6	0,488	10,4282	10
7	0,492	10,5095	10
8	0,480	10,2655	10
9	0,473	10,1232	10
10	0,484	10,3468	10

a = -0,0249 **b = 0,0492** **r = 0,9998**

- Rata-rata konsentrasi = 10,3143 ppm
- SD = 0,124671

- CV = $\frac{SD}{Rata-rata}$ x 100%

  = $\frac{0{,}124671}{10{,}3143}$ x 100%

  = 0,012087

- RSD = 1% < 2%

  Keterangan:

  SD = Simpangan baku

  RSD = Simpangan baku relatif

  CV = Koefisien variasi

Lampiran 12. Efisiensi penjerapan

y = -0,0249 + 0,0492.x

Efisiensi penjerapan	Formula		
	3	**4**	**5**
Replikasi 1	0,534	0,410	0,396
Replikasi 2	0,537	0,413	0,391
Replikasi 3	0,541	0,412	0,386
Rata-rata Absorbansi	0,537	0,412	0,391
%EE	88,57%	91,12%	91,54%

Formula 3

a. Perhitungan kadar myricetin terjerap menggunakan persamaan regresi linier yang menggunakan pelarut PBS:

   y = a + b.x

   0,5373 = -0,0249 + 0,0492.x

   0,0492.x = 0,5622

   x = 11,4303 ppm

b. Σ myricetin tidak terjerap $= \frac{11{,}4303\ ppm}{100\ ppm}$ x 10 mg

   = 1,14303 mg

c. % Efisiensi penjerapan $= \frac{TD-FD}{TD}$ x 100%

   $= \frac{10\ mg-1{,}14303\ mg}{10\ mg}$ x 100%

   = 88,57%

Formula 4

a. Perhitungan kadar myricetin terjerap menggunakan persamaan regresi linier yang menggunakan pelarut PBS:

   y = a + b.x

   0,4117 = -0,0249 + 0,0492.x

   0,0492.x = 0,4366

x = 8,8767 ppm

b. Σ myricetin tidak terjerap = $\frac{8{,}8767\ ppm}{100\ ppm}$ x 10 mg

= 0,88767 mg

c. % Efisiensi penjerapan = $\frac{TD-FD}{TD}$ x 100%

= $\frac{10\ mg-0{,}88767\ mg}{10\ mg}$ x 100%

= 91,12%

Formula 5

a. Perhitungan kadar myricetin terjerap menggunakan persamaan regresi linier yang menggunakan pelarut PBS:

y = a + b.x

0,391 = -0,0249 + 0,0492.x

0,0492.x = 0,4159

x = 8,4558 ppm

b. Σ myricetin tidak terjerap = $\frac{8{,}4558\ ppm}{100\ ppm}$ x 10 mg

= 0,84558 mg

c. % Efisiensi penjerapan = $\frac{TD-FD}{TD}$ x 100%

= $\frac{10\ mg-0{,}84558\ mg}{10\ mg}$ x 100%

= 91,54%

Keterangan:

TD = total jumlah myricetin yang terdapat dalam formula

FD = jumlah myricetin yang terdeteksi pada supernatan (tidak terjerap)

Lampiran 13. Uji stabilitas fisik selama 3 minggu

Formula	Minggu ke-1	Minggu ke-2	Minggu ke-3
1	Tidak ada endapan	Ada endapan	Ada endapan
2	Tidak ada endapan	Ada endapan	Ada endapan
3	Tidak ada endapan	Ada endapan	Ada endapan
4	Tidak ada endapan	Ada endapan	Ada endapan
5	Tidak ada endapan	Tidak ada endapan	Tidak ada endapan

Ket: Formula 1 menggunakan perbandingan mol Myricetin:Fosfatidilkolin:Kolesterol (1:1:0,2)
Formula 2 menggunakan perbandingan mol Myricetin:Fosfatidilkolin:Kolesterol (1:2:0,2)
Formula 3 menggunakan perbandingan mol Myricetin:Fosfatidilkolin:Kolesterol (1:3:0,2)
Formula 4 menggunakan perbandingan mol Myricetin:Fosfatidilkolin:Kolesterol (1:4:0,2)
Formula 5 menggunakan perbandingan mol Myricetin:Fosfatidilkolin:Kolesterol (1:5:0,2)

Lampiran 14. Ukuran dan distribusi partikel setelah penyimpanan

Formula	Ukuran Partikel (nm)		Indeks Polidispersitas	
	Sebelum	Sesudah	Sebelum	Sesudah
F5	139,467 nm	164,960 nm	0,380	0,393

Hasil Ukuran Partikel F5 Setelah Penyimpanan

Size Distribution Report by Number

v2.2

Malvern

Sample Details

Sample Name: F5 myricetin 1
SOP Name: mansettings.nano
General Notes:

File Name: Vilza Dwiki 2019.dts
Record Number: 21
Material RI: 1.52
Material Absorbtion: 0.100
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Tuesday, May 21, 2019 12...

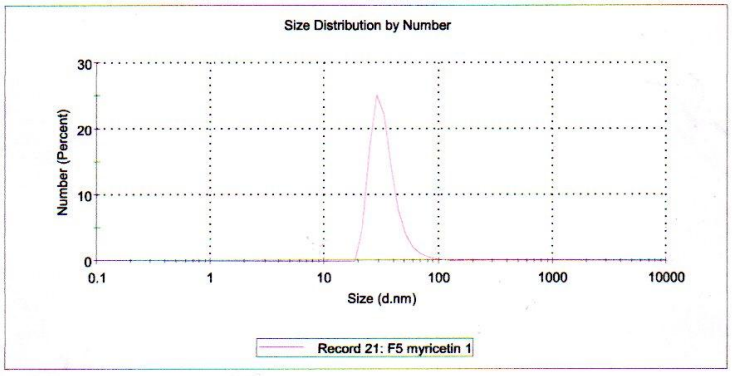
System

Temperature (°C): 25.0
Count Rate (kcps): 424.0
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 0.85
Attenuator: 5

Results

			Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm):	163.3	**Peak 1:**	34.15	100.0	14.53
PdI:	0.405	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.932	**Peak 3:**	0.000	0.0	0.000

Result quality Good



Lampiran 15. Zeta potensial nanofitosom myricetin setelah penyimpanan

	Potensial zeta (Mv)				
Formula 5	Repitasi 1	Repitasi 2	Repitasi 3	Rata-rata	SD
	-4,570	-5,930	-5,680	-5,393	0,724

Hasil Zeta Potensial F5 Setelah Penyimpanan

Zeta Potential Report
v2.3

Malvern

Sample Details

Sample Name: F5 myricetin 1
SOP Name: mansettings.nano
General Notes:

File Name: Vilza Dwiki 2019.dts
Record Number: 26
Date and Time: Tuesday, May 21, 2019 12:20:...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

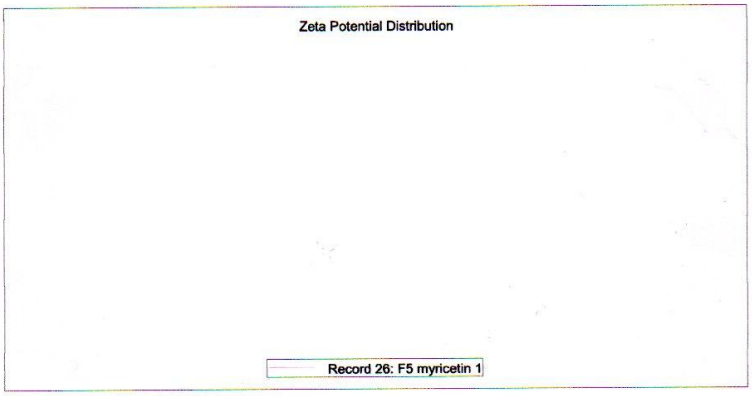
System

Temperature (°C): 25.0
Count Rate (kcps): 141.3
Cell Description: Zeta dip cell
Zeta Runs: 55
Measurement Position (mm): 4.50
Attenuator: 7

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -5.71	**Peak 1:**	0.00	0.0	0.00
Zeta Deviation (mV): 0.00	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 11.2	**Peak 3:**	0.00	0.0	0.00

Result quality Good



Lampiran 16. Hasil analisis statistik terhadap stabilitas ukuran partikel, indeks polidispersitas dan potensial zeta

1. Ukuran partikel

NPar Tests

One-Sample Kolmogorov-Smirnov Test

		Hasil ukuran partikel
N		8
Normal Parameters[a,,b]	Mean	155.40000
	Std. Deviation	13.546744
Most Extreme Differences	Absolute	.299
	Positive	.246
	Negative	-.299
Kolmogorov-Smirnov Z		.845
Asymp. Sig. (2-tailed)		.473

a. Test distribution is Normal.

b. Calculated from data.

T-Test

Group Statistics

	Waktu	N	Mean	Std. Deviation	Std. Error Mean
Hasil ukuran partikel	sebelum	3	139.46667	.550757	.317980
	sesudah	5	164.96000	4.043884	1.808480

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Hasil ukuran partikel	Equal variances assumed	2.574	.160	-10.524	6	.000	-25.493333	2.422463	-31.420886	-19.565780
	Equal variances not assumed			-13.884	4.243	.000	-25.493333	1.836222	-30.478378	-20.508289

2. Indeks polidispersitas

NPar Tests

One-Sample Kolmogorov-Smirnov Test

		Hasil indeks polidispersitas
N		8
Normal Parameters[a,,b]	Mean	.38813
	Std. Deviation	.016313
Most Extreme Differences	Absolute	.127
	Positive	.127
	Negative	-.100
Kolmogorov-Smirnov Z		.360
Asymp. Sig. (2-tailed)		.999

a. Test distribution is Normal.

b. Calculated from data.

T-Test

Group Statistics

	Waktu	N	Mean	Std. Deviation	Std. Error Mean
Hasil indeks polidispersitas	Sebelum	3	.37967	.006429	.003712
	Sesudah	5	.39320	.018953	.008476

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Hasil indeks polidispersitas	Equal variances assumed	1.767	.232	-1.164	6	.288	-.013533	.011622	-.041971	.014904
	Equal variances not assumed			-1.463	5.292	.200	-.013533	.009253	-.036929	.009863

3. Potensial zeta

NPar Tests

One-Sample Kolmogorov-Smirnov Test

		Zeta potensial
N		8
Normal Parameters[a,,b]	Mean	-5.9913
	Std. Deviation	.70548
Most Extreme Differences	Absolute	.205
	Positive	.205
	Negative	-.148
Kolmogorov-Smirnov Z		.579
Asymp. Sig. (2-tailed)		.891

a. Test distribution is Normal.

b. Calculated from data.

T-Test

Group Statistics

	Waktu	N	Mean	Std. Deviation	Std. Error Mean
Zetapotensial	Sebelum	3	-5.3933	.72390	.41794
	Sesudah	5	-6.3500	.42421	.18971

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zeta potensial	Equal variances assumed	1.716	.238	2.413	6	.052	.95667	.39641	-.01332	1.92666
	Equal variances not assumed			2.084	2.849	.133	.95667	.45899	-.54895	2.46229

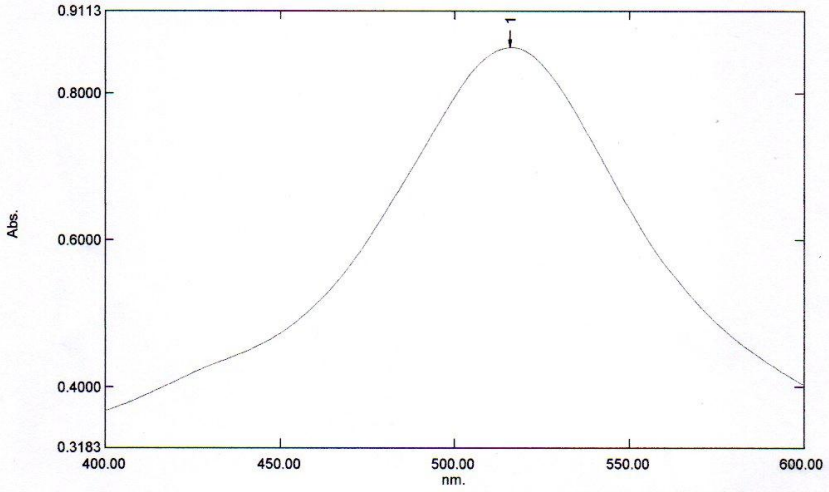
Lampiran 17. Uji DPPH

a. Penetapan panjang gelombang DPPH

Spectrum Peak Pick Report

03/13/2019 09:19:37 AM

Data Set: DPPH_091227 - RawData



No.	P/V	Wavelength	Abs.	Description
1		516.00	0.8619	

[Measurement Properties]
Wavelength Range (nm.): 400.00 to 600.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Auto

[Instrument Properties]
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

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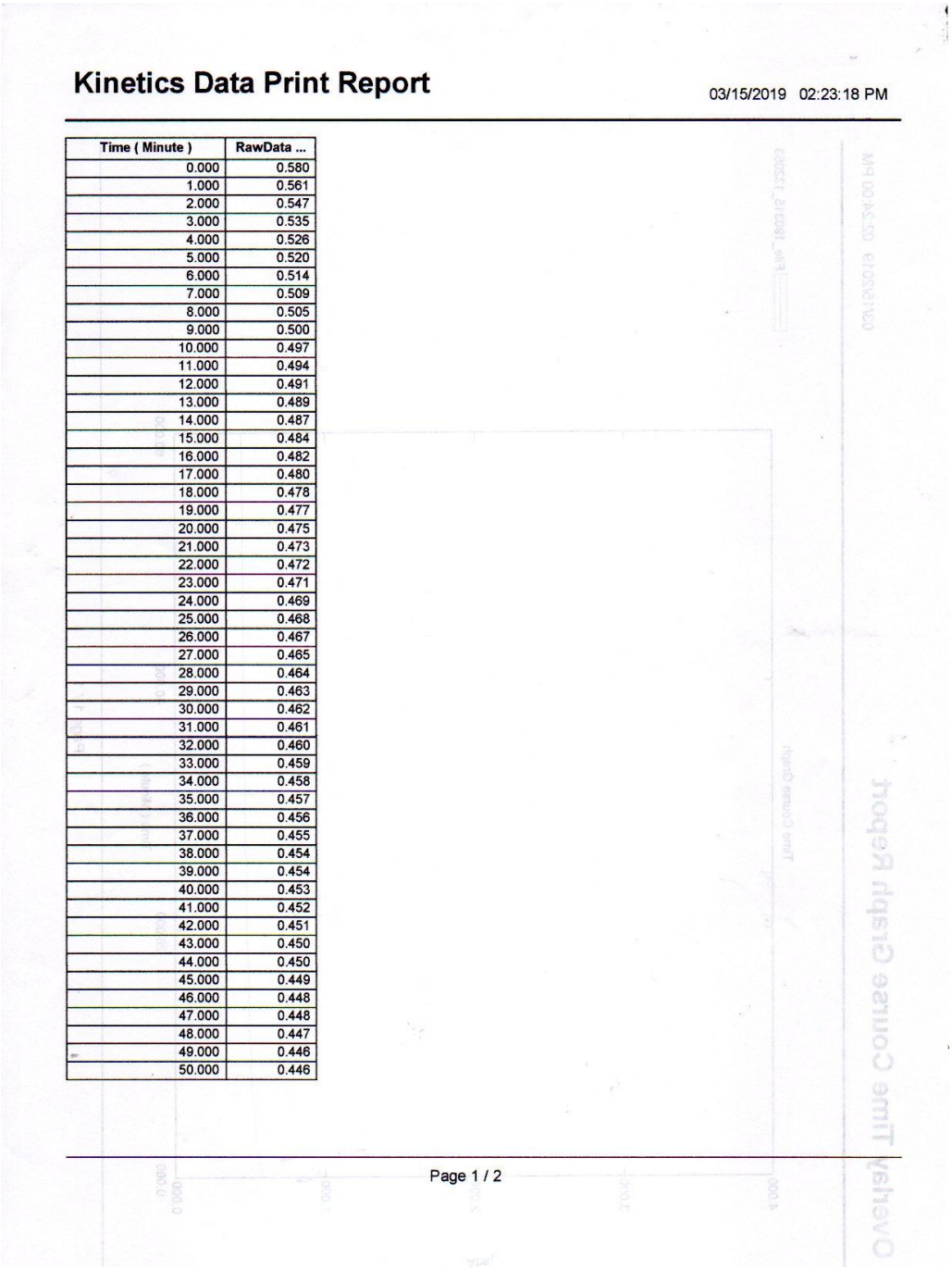
b. Penetapan *Operating Time* (OT)



Kinetics Data Print Report

03/15/2019 02:23:18 PM

Time (Minute)	RawData ...
0.000	0.580
1.000	0.561
2.000	0.547
3.000	0.535
4.000	0.526
5.000	0.520
6.000	0.514
7.000	0.509
8.000	0.505
9.000	0.500
10.000	0.497
11.000	0.494
12.000	0.491
13.000	0.489
14.000	0.487
15.000	0.484
16.000	0.482
17.000	0.480
18.000	0.478
19.000	0.477
20.000	0.475
21.000	0.473
22.000	0.472
23.000	0.471
24.000	0.469
25.000	0.468
26.000	0.467
27.000	0.465
28.000	0.464
29.000	0.463
30.000	0.462
31.000	0.461
32.000	0.460
33.000	0.459
34.000	0.458
35.000	0.457
36.000	0.456
37.000	0.455
38.000	0.454
39.000	0.454
40.000	0.453
41.000	0.452
42.000	0.451
43.000	0.450
44.000	0.450
45.000	0.449
46.000	0.448
47.000	0.448
48.000	0.447
49.000	0.446
50.000	0.446

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Kinetics Data Print Report

03/15/2019 02:23:18 PM

Time (Minute)	RawData ...
51.000	0.445
52.000	0.445
53.000	0.444
54.000	0.443
55.000	0.443
56.000	0.442
57.000	0.442
58.000	0.441
59.000	0.441
60.000	0.440

c. Uji DPPH myricetin

Absorbansi DPPH = 0,802

Lamda maksimal = 516 nm

Operating Time = 38-39 menit

Larutan stok 100 ppm → 10 mg myricetin + ad 100 ml etanol p.a

KONSENTRASI (PPM)	SERAPAN 1	SERAPAN 2	SERAPAN 3	RATA-RATA
2	0,700	0,701	0,705	0,702
4	0,625	0,624	0,624	0,624
6	0,560	0,563	0,570	0,564
8	0,525	0,535	0,566	0,542
10	0,478	0,481	0,484	0,481

Nilai IC_{50}

KONSENTRASI (PPM) x	ABSORBANSI	% PEREDAMAN y
2	0,702	18,56 %
4	0,624	27,61 %
6	0,564	34,57 %
8	0,542	37,12 %
10	0,481	44,20 %

a = 14,1750 b = 3,0395 r = 0,9852

- % Peredaman = $\frac{Absorbansi\ blangko - Absorbansi\ sampel}{Absorbansi\ blangko} x100\%$

1. 2 ppm

   % peredaman = $\frac{0,862-0,702}{0,862} x100\%$

   = 18,56 %

2. 4 ppm

   % peredaman = $\frac{0,862-0,624}{0,862} x100\%$

   = 27,61 %

3. 6 ppm

   % peredaman = $\frac{0,862-0,564}{0,862} x100\%$

   = 34,57 %

4. 8 ppm

   % peredaman = $\frac{0,862-0,542}{0,862} x100\%$

   = 37,12%

5. 10 ppm

   % peredaman = $\frac{0,862-0,481}{0,862} x100\%$

   = 44,20%

- IC_{50}

  Y = a + b.x

  50 = 14,1750 + 3,0395.x

  X = $\frac{50-14,1750}{3,0395}$

  IC_{50} = 11,7865 ppm

  Nilai IC_{50} Myricetin sebesar 11,7865 ppm sehingga memiliki aktivitas antioksidan yang sangat kuat karena suatu senyawa dikatakan sebagai antioksidan sangat kuat jika nilai IC_{50} kurang dari 50 ppm (IC_{50} < 50 ppm).

d. Uji DPPH sampel myricetin nanofitosom (Formula 5)

Absorbansi DPPH = 0,802

Lamda maksimal = 512 nm

Operating Time = 38-39 menit

Konsentrasi = 10 mg/20 ml = 500 mg/1000 ml = 500 ppm

Larutan stok 250 ppm → 5 ml sampel myricetin nanofitosom F5 + ad 10 ml etanol p.a

KONSENTRASI (PPM)	REPLIKASI 1	REPLIKASI 2	REPLIKASI 3	RATA-RATA
250	0,205	0,201	0,201	0,202
125	0,394	0,395	0,396	0,395
62,5	0,417	0,413	0,412	0,414
31,25	0,599	0,599	0,598	0,599
15,625	0,708	0,701	0,701	0,703
7,812	0,739	0,749	0,749	0,746

Perhitungan:

Sampel myricetin nanofitosom Formula 5 (konsentrasi 500 ppm) dibuat seri konsentrasi 250 ppm, 125 ppm, 62,5 ppm, 31,25 ppm, 15,625 ppm dan 7,812 ppm dalam 10 mL etanol p.a.

1. 250 ppm

   $V_1 \times C_1 = V_2 \times C_2$

   $V_1 \times 500$ ppm = 10 mL x 250 ppm

   V_1 = 5 mL

2. 125 ppm

   $V_1 \times C_1 = V_2 \times C_2$

   $V_1 \times 500$ ppm = 10 mL x 125 ppm

   V_1 = 2,5 mL

3. 62,5 ppm

V_1 x C_1	=	V_2 x C_2
V_1 x 500 ppm	=	10 mL x 62,5 ppm
V_1	=	1,25 mL

4. 31,25 ppm

V_1 x C_1	=	V_2 x C_2
V_1 x 500 ppm	=	10 mL x 31,25 ppm
V_1	=	0,625 mL

5. 15,625 ppm

V_1 x C_1	=	V_2 x C_2
V_1 x 500 ppm	=	10 mL x 15,625 ppm
V_1	=	0,312 mL

6. 7,812 ppm

V_1 x C_1	=	V_2 x C_2
V_1 x 500 ppm	=	10 mL x 7,812 ppm
V_1	=	0,156 mL

Nilai IC_{50}

KONSENTRASI (PPM) x	ABSORBANSI	% PEREDAMAN y
250	0,202	76,57 %
125	0,395	54,18 %
62,5	0,414	51,97 %
31,25	0,599	30,51 %
15,625	0,703	18,45 %
7,812	0,746	13,46 %

a = 20,9864 b = 0,2422 r = 0,9263

- % Peredaman = $\frac{Absorbansi\ blangko - Absorbansi\ sampel}{Absorbansi\ blangko} x100\%$

  1. 250 ppm

     % peredaman = $\frac{0,862-0,202}{0,862} x100\%$

     = 76,57 %

  2. 125 ppm

     % peredaman = $\frac{0,862-0,395}{0,862} x100\%$

     = 54,18 %

  3. 62,5 ppm

     % peredaman = $\frac{0,862-0,414}{0,862} x100\%$

     = 51,97 %

  4. 31,25 ppm

     % peredaman = $\frac{0,862-0,599}{0,862} x100\%$

     = 30,51 %

  5. 15,625 ppm

     % peredaman = $\frac{0,862-0,703}{0,862} x100\%$

     = 18,45 %

  6. 7,812 ppm

     % peredaman = $\frac{0,862-0,746}{0,862} x100\%$

     = 13,46 %

- IC_{50}

  Y = a + b.x

  50 = 20,9864 + 0,2422.x

  X = $\frac{50-20,9864}{0,2422}$

  IC_{50} = 119,7920 ppm

  Nilai IC_{50} myricetin nanofitosom Formula 5 sebesar 119,7920 ppm sehingga memiliki aktivitas antioksidan yang sedang karena nilai IC_{50} di atas 100 ppm (100 ppm < IC_{50} < 150 ppm).