BAB V
KESIMPULAN DAN SARAN

A. Kesimpulan

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

Pertama, *Solid Lipid Nanoparticles* (SLN) fisetin yang dibuat kombinasi belum bisa stabil, namun SLN fisetin memiliki efektifitas antioksidan yang sangat kuat karena memiliki rata-rata replikasi IC_{50} sebesar 12.14 ppm.

Kedua, konsentrasi lipid padat setil alkohol 0,15; 0,25%; dan 0,50% berpengaruh terhadap ukuran partikel karena semakin kecil konsentrasi yang digunakan semakin kecil juga ukuran partikel yang dihasilkan. Berbanding terbalik dengan efisiensi penjerapan, semakin besar konsentrasi yang digunakan semakin banyak obat yang terjerap dalam SLN.

Ketiga, formula terbaik dari SLN fisetin yang dihasilkan yaitu pada formula 6 untuk ukuran partikel dan tingkat keseragaman ukuran yang baik, sedangkan untuk efisiensi penjerapan terbaik dihasilkan oleh formula 5.

B. Saran

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

Pertama, perlu dilakukan analisis *screening lipid* dengan menggunakan jenis lipid yang lebih beragam.

Kedua, perlu dilakukan uji TEM untuk mengetahui morfologi atau bentuk dari SLN fisetin secara detail.

Ketiga, perlu dilakukan uji kelarutan kinetik dan uji disolusi untuk mengetahui kelarutan SLN zat aktif dan perlu dilakukan optimasi pada surfaktan untuk uji stabilitas penyimpanan dalam jangka waktu yang lebih lama minimal 4 minggu.

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LAMPIRAN

Lampiran 1. ***Certificate of analysis*** **(COA) fisetin**

TOCRIS
a bio-techne brand

Print Date: Jan 14th 2016

Certificate of Analysis

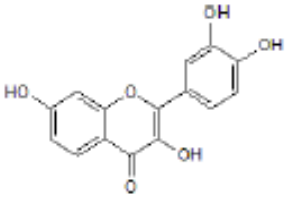
www.tocris.com

Product Name: Fisetin **Catalog No.:** 5016 **Batch No.:** 1

CAS Number: 528-48-3

IUPAC Name: 2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one

1. PHYSICAL AND CHEMICAL PROPERTIES

Batch Molecular Formula:	$C_{15}H_{10}O_6$
Batch Molecular Weight:	286.24
Physical Appearance:	Yellow solid
Solubility:	DMSO to 100 mM ethanol to 10 mM
Storage:	Store at -20°C
Batch Molecular Structure:	

2. ANALYTICAL DATA

HPLC:	Shows 98.1% purity
^{1}H NMR:	Consistent with structure
Mass Spectrum:	Consistent with structure

Microanalysis:

	Carbon	Hydrogen	Nitrogen
Theoretical	62.94	3.52	
Found	62.81	3.58	

Caution - Not Fully Tested • Research Use Only • Not For Human or Veterinary Use

bio-techne.com
info@bio-techne.com
techsupport@bio-techne.com

North America
Tel: (800) 343 7475

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Tel: +86 (21) 52380373

Europe Middle East Africa
Tel: +44 (0)1235 529449

Rest of World
www.tocris.com/distributors
Tel:+1 612 379 2956

TOCRIS
a biotechne brand

Print Date: Jan 14th 2016

Product Information

www.tocris.com

Product Name: Fisetin **Catalog No.:** 5016 **Batch No.:** 1

CAS Number: 528-48-3

IUPAC Name: 2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one

Description:

Naturally occuring flavonoid and antioxidant. Inhibits PI 3-K, Akt, mTOR and Cdk6. Displays antiproliferative activity in prostate cancer cells. Shown to activate ERK; exhibits neuroprotective activity in Huntington's disease models. Also a DNMT1 inhibitor.

Physical and Chemical Properties:

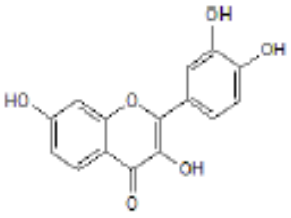
Batch Molecular Formula: $C_{15}H_{10}O_6$

Batch Molecular Weight: 286.24

Physical Appearance: Yellow solid

Minimum Purity: >98%

Batch Molecular Structure:



Storage: Store at -20°C

Solubility & Usage Info:

DMSO to 100 mM
ethanol to 10 mM

Stability and Solubility Advice:

Some solutions can be difficult to obtain and can be encouraged by rapid stirring, sonication or gentle warming (in a 45-60°C water bath).

Information concerning product stability, particularly in solution, has rarely been reported and in most cases we can only offer a general guide. Our standard recommendations are:

SOLIDS: Provided storage is as stated on the product label and the vial is kept tightly sealed, the product can be stored for up to 6 months from date of receipt.

SOLUTIONS: We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month. Wherever possible solutions should be made up and used on the same day.

References:

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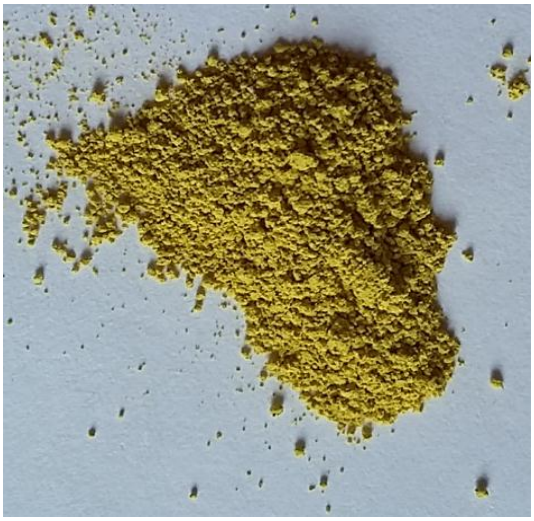
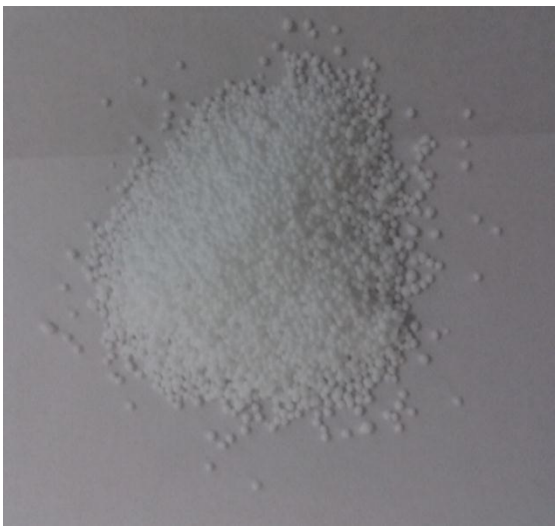
Lampiran 2. Foto serbuk fisetin



Lampiran 3. Foto Lipid Setil Alkohol

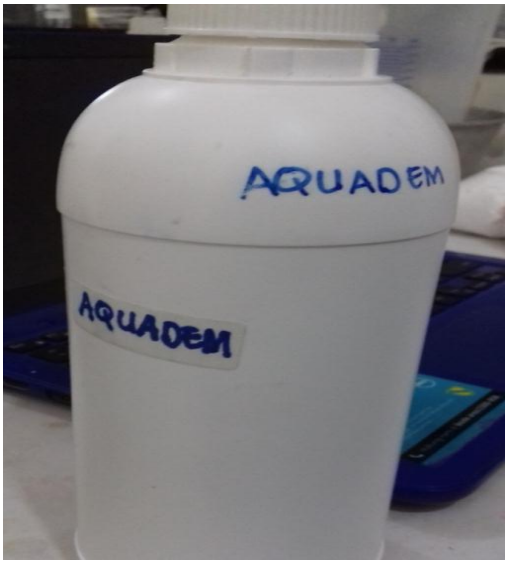


Lampiran 4. Foto Lipid Stearil Alkohol

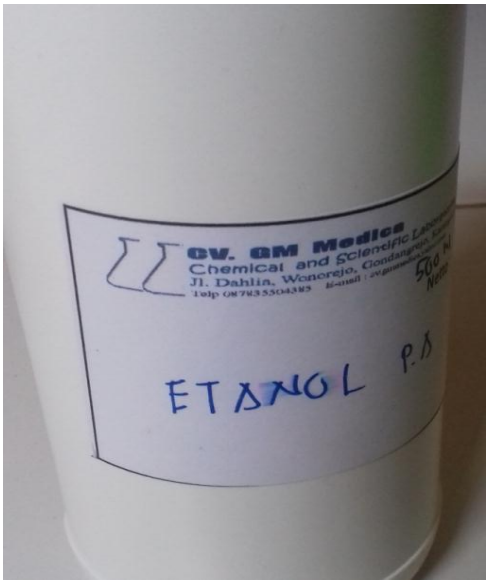
Lampiran 5. Foto lipid Setostearil Alkohol

Lampiran 6. Foto Surfaktan Tween 80

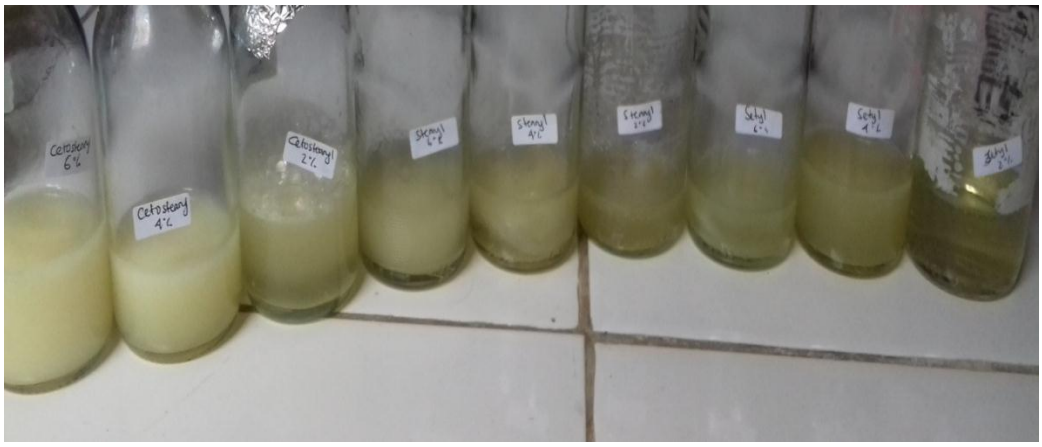
Lampiran 7. Foto Aquademineralisata



Lampiran 8. Foto etanol



Lampiran 9. Emulsi SLN Fisetin



Lampiran 10. Penentuan panjang gelombang dan pembuatan kurva baku

a. Penentuan panjang gelombang

Spectrum Peak Pick Report

03/23/2019 08:30:25 AM

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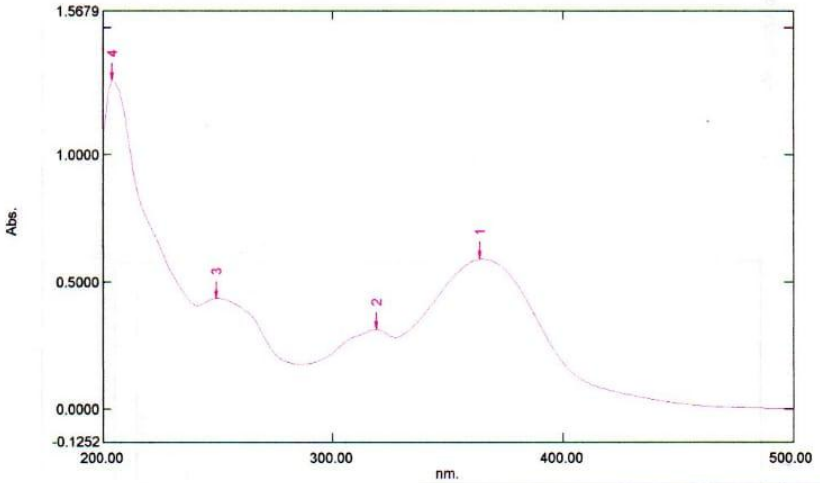


No.	P/V	Wavelength	Abs.	Description
1	↑	364.00	0.5917	
2	↑	319.00	0.3150	
3	↑	249.00	0.4369	
4	↑	204.00	1.2942	
5	↓	327.00	0.2818	
6	↓	286.00	0.1763	
7	↓	241.00	0.4067	

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 500.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

[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: 359
Volume:
Dilution:
Path Length:
Additional Information:

b. Penentuan operating time

Kinetics Data Print Report

05/15/2019 03:46:21 PM

Time (Minute)	RawData ...
0.000	0.457
1.000	0.458
2.000	0.459
3.000	0.460
4.000	0.459
5.000	0.461
6.000	0.460
7.000	0.461
8.000	0.460
9.000	0.460
10.000	0.462
11.000	0.462
12.000	0.462
13.000	0.462
14.000	0.463
15.000	0.463
16.000	0.464
17.000	0.464
18.000	0.463
19.000	0.464
20.000	0.464
21.000	0.465
22.000	0.465
23.000	0.464
24.000	0.464
25.000	0.466
26.000	0.465
27.000	0.466
28.000	0.466
29.000	0.466
30.000	0.466

c. Kurva kalibrasi (Linieritas (*linearity*)

- Membuat larutan induk sebesar 46 ppm dengan menimbang 4,6mg fisetin ditambahkan etanol *p.a* sampai 100 ml.

Kertas + sampel : 0,2786g

Kertas + sisa : 0,2740g

Serbuk fisetin : 0,0046g

- Perhitungan **seri konsentrasi**

1. 3,68 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 3,68 ppm x 10 ml

   V1 = 0,8 ml

2. 4,6 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 4,6 ppm x 10 ml

   V1 = 1 ml

3. 5,52 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 5,52 ppm x 10 ml

   V1 = 1,2 ml

4. 6,44 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 6,44 ppm x 10 ml

   V1 = 1,4 ml

5. 7,36 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 7,36 ppm x 10 ml

   V1 = 1,6 ml

6. 8,28 ppm

   C1 x V1 = C2 x V2

   46 ppm x V1 = 8,28 ppm x 10 ml

   V1 = 1,8 ml

Konsentrasi (ppm)	Absorbansi
3,68	0,246
4,6	0,305
5,52	0,371
6,44	0,424
7,36	0,478
8,28	0,541

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

a = 0,01401

b = 0,063571

r = 0,99951

y = a + bx

y = 0,01401 + 0,063571 x

keterangan :

x = konsentrasi (µg/ml)

y = serapan

Hasil linearitas diperoleh r = 0,99951; sehingga dapat disimpulkan bahwa data linear

d. Akurasi (*Accuracy*)

Konsentrasi Sebenarnya	Absorbansi	Konsentrasi Terukur	%recovery	Rata-rata
4,6	0,308	4,624	100,52%	
4,6	0,308	4,624	100,52%	100,19%
4,6	0,305	4,578	99,52%	
5,52	0,366	5,508	99,78%	
5,52	0,366	5,508	99,78%	
5,52	0,365	5,493	99,51%	99,69%
6,44	0,429	6,469	100,45%	
6,44	0,429	6,469	100,45%	
6,44	0,425	6,408	99,50%	100,13%

% Recovery $= \frac{konsentrasi\ terukur}{konsentrasi\ sebenarnya} X100\%$

- **Konsentrasi 4,6 ppm**
  - % Recovery $=\frac{4,624}{4,6} X100\%$ = 100,52
  - % Recovery $=\frac{4,624}{4,6} X100\% = 100,52\%$
  - % Recovery $=\frac{4,578}{4,6} X100\% = 99,52\%$
- **Konsentrasi 5,52 ppm**
  - % Recovery $=\frac{5,508}{5,52} X100\% = 99,78\%$
  - % Recovery $=\frac{5,508}{5,52} X100\% = 99,78\%$
  - % Recovery $=\frac{5,493}{5,52} X100\% = 99,51\%$
- **Konsentrasi 6,44 ppm**
  - % Recovery $=\frac{6,469}{6,44} X100\% = 100,45\%$
  - % Recovery $=\frac{6,469}{6,44} X100\% = 100,45\%$
  - % Recovery $=\frac{6,408}{6,44} X100\% = 99,50\%$

Nilai rata- rata % Recovery diatas adalah 100%, hal ini menunjukkan nilai persen perolehan kembali yang baik.

e. Presisi

Konsentrasi	Absorbansi	Konsentrasi
4,6	0,367	6,131
4,6	0,365	6,098
4,6	0,369	6,164
4,6	0,370	6,181
4,6	0,366	6,114
4,6	0,368	6,148
4,6	0,369	6,164
4,6	0,371	6,197
4,6	0,370	6,181
4,6	0,366	6,114
	RATA-RATA	6,149
	SD	0,034
	CV	1%

Nilai CV dilihat dari data diatas adalah 1%, hasil ini sesuai dengan pernyataan bahwa syarat presisi adalah kurang dari 2%.

Lampiran 11. Hasil Uji Ukuran Partikel Formula Setil Alkohol 0,5%

Instrument

Serial Number:	3214-DMP
Model:	DelsaMax Pro
Pals Firmware Version:	1.1.0.6
DLS Firmware Version:	2.3.1.0
Assist Firmware Version:	1.0.0.9
Instrument Name:	BCI-3214-DMP
Laser Wavelength (nm):	532.0
Has DLS:	Yes
DLS Detector Angle (degrees):	163.5
Minimum Temperature (C):	3.5
Minimum Temperature without N2 (C):	20
Maximum Temperature (C):	70
Minimum Ramp Rate (C/min):	0
Maximum Ramp Rate (C/min):	1.5

Instrument Parameters: Measurements

Collect Data:	DLS and Pals (Simultaneous)
Acq Time (s):	20
Read Interval (s):	1
Number Acq:	3
Electric Field Frequency (Hz):	10.0
Voltage Amplitude (V):	2.5
Collection Period (s):	15.0
Auto-attenuation:	Yes
Attenuation Level (%):	0
Auto-attenuation Time Limit(s):	0
Laser Mode:	Normal
Set Temp On Connection:	No
Set Temp (C):	20
Temp Ramp Enabled:	Yes
Temp Ramp Rate (C/min):	1

Datalog Table: Measurements

	Item	Zeta Potential (mV)	Radius (nm)	Diameter (nm)
1	Meas 1	-19.22	109.2	218.3
2	Meas 2	-21.00	138.6	277.1
3	Meas 3	-20.51	138.7	277.5
Mean		-20.25	128.8	257.6
S		0.92	17.0	34.1
%S		4.55	13.2	13.2
S^2		0.85	289.9	1159.5
Min		-21.00	109.2	218.3
Max		-19.22	138.7	277.5

Lampiran 12. Hasil Uji Ukuran Partikel Formula Setil Alkohol 0,25%

Instrument

Serial Number:	3214-DMP
Model:	DelsaMax Pro
Pals Firmware Version:	1.1.0.6
DLS Firmware Version:	2.3.1.0
Assist Firmware Version:	1.0.0.9
Instrument Name:	BCI-3214-DMP
Laser Wavelength (nm):	532.0
Has DLS:	Yes
DLS Detector Angle (degrees):	163.5
Minimum Temperature (C):	3.5
Minimum Temperature without N2 (C):	20
Maximum Temperature (C):	70
Minimum Ramp Rate (C/min):	0
Maximum Ramp Rate (C/min):	1.5

Instrument Parameters: Measurements

Collect Data:	DLS and Pals (Simultaneous)
Acq Time (s):	20
Read Interval (s):	1
Number Acq:	3
Electric Field Frequency (Hz):	10.0
Voltage Amplitude (V):	2.5
Collection Period (s):	15.0
Auto-attenuation:	Yes
Attenuation Level (%):	0
Auto-attenuation Time Limit(s):	0
Laser Mode:	Normal
Set Temp On Connection:	No
Set Temp (C):	20
Temp Ramp Enabled:	Yes
Temp Ramp Rate (C/min):	1

Datalog Table: Measurements

	Item	Zeta Potential (mV)	Diameter (nm)
1	Meas 1	-19.68	26.1
2	Meas 2	-15.91	27.9
3	Meas 3	-19.41	31.9
Mean		-18.40	28.7
S		2.17	3.0
%S		11.80	10.3
S^2		4.71	8.8
Min		-19.68	26.1
Max		-15.91	31.9

Lampiran 13. Hasil Uji Ukuran Partikel Formula Setil Alkohol 0,15%

Instrument

Serial Number:	3214-DMP
Model:	DelsaMax Pro
Pals Firmware Version:	1.1.0.6
DLS Firmware Version:	2.3.1.0
Assist Firmware Version:	1.0.0.9
Instrument Name:	BCI-3214-DMP
Laser Wavelength (nm):	532.0
Has DLS:	Yes
DLS Detector Angle (degrees):	163.5
Minimum Temperature (C):	3.5
Minimum Temperature without N2 (C):	20
Maximum Temperature (C):	70
Minimum Ramp Rate (C/min):	0
Maximum Ramp Rate (C/min):	1.5

Instrument Parameters: Measurements

Collect Data:	DLS and Pals (Simultaneous)
Acq Time (s):	20
Read Interval (s):	1
Number Acq:	3
Electric Field Frequency (Hz):	10.0
Voltage Amplitude (V):	2.5
Collection Period (s):	15.0
Auto-attenuation:	Yes
Attenuation Level (%):	0
Auto-attenuation Time Limit(s):	0
Laser Mode:	Normal
Set Temp On Connection:	No
Set Temp (C):	20
Temp Ramp Enabled:	Yes
Temp Ramp Rate (C/min):	1

Datalog Table: Measurements

	Item	Zeta Potential (mV)	Diameter (nm)
1	Meas 1	-17.25	15.6
2	Meas 2	-17.45	14.8
3	Meas 3	-19.52	14.9
Mean		-18.08	15.2
S		1.26	0.5
%S		6.95	3.4
S^2		1.58	0.3
Min		-19.52	14.8
Max		-17.25	15.6

Lampiran 14. Hasil Perhitungan Efisiensi Penjerapan SLN Fisetin

a. Formula 1 (Setil Alkohol 0,5%)

- Larutan induk → 200 mg SLN fisetin / 10 ml etanol p.a = 20.000 ppm
- Perhitungan teoritis

  Fisetin = 10 mg

  Eksipien (tween 80 + setil alcohol) = 10250 mg

  % kadar fisetin = $\frac{10}{10250+10} x100\% = 0{,}0975\%$
- Kadar dalam 200 mg SLN fisetin = 0,0975% x200 mg = 9,75 mg
- Perhitungan kadar fisetin terjerap menggunakan persamaan regresi linier :

  y = a + bx

  0,946 = 0,014 + 0,0636x

  0,0636x = 0,932

  X = 14,654 ppm
- % kadar =$\frac{14{,}654}{20000} x\ 100\% = 0{,}07327\%$
- Kadar dalam 200 mg SLN fisetin = 0,07327% x 200 mg = 7,327 mg
- % efisiensi penjerapan = $\frac{kadar\ terjerap}{kadar\ teoritis} \times 100\%$

  = $\frac{7{,}327}{9{,}75} x\ 100\%$

  = 75,15%

b. Formula 2 (Setil Alkohol 0,25%)

- Larutan induk → 200 mg SLN fisetin / 10 ml etanol p.a = 20.000 ppm
- Perhitungan teoritis

  Fisetin = 10 mg

  Eksipien (tween 80 + setil alcohol) = 11000 mg

  % kadar fisetin = $\frac{10}{11000+10} \times 100\% = 0.0908\%$

  Kadar dalam 200 mg SLN fisetin = 0,0908% x 200 mg = 9,08 mg
- Perhitungan kadar fisetin terjerap menggunakan persamaan regresi linier :

  y = a + bx

  0,548 = 0,014 + 0,0636x

  0,0636x = 0,534

X = 8,396 ppm

- % kadar $= \frac{8,396}{20000} \times 100\% = 0,04198\%$
- Kadar dalam 200 mg SLN fisetin = 0,04198% x 200 mg = 4,198 mg
- % efisiensi penjerapan $= \frac{kadar\ terjerap}{kadar\ teoritis} \times 100\%$

  $= \frac{4,198\ mg}{9,08\ mg}\ x\ 100\%$

  = 46,23%

c. Formula 3 (Setil Alkohol 0,15%)

- Larutan induk → 200 mg SLN fisetin / 10 ml etanol p.a = 20.000 ppm
- Perhitungan teoritis

  Fisetin = 10 mg

  Eksipien (tween 80 + setil alcohol) = 10750 mg

  % kadar fisetin $= \frac{10}{10750+10} \times 100\% = 0.09294\%$
- Kadar dalam 200 mg SLN fisetin = 0,09294% x 200 mg = 9,294 mg
- Perhitungan kadar fisetin terjerap menggunakan persamaan regresi linier :

  y = a + bx

  0,448 = 0,014 + 0,0636x

  0,0636x = 0,434

  X = 6,824 ppm
- % kadar $= \frac{6,824}{20000} \times 100\% = 0,03412\%$
- Kadar dalam 200 mg SLN fisetin = 0,03412% x 200 mg = 3,412 mg
- % efisiensi penjerapan $= \frac{kadar\ terjerap}{kadar\ teoritis} \times 100\%$

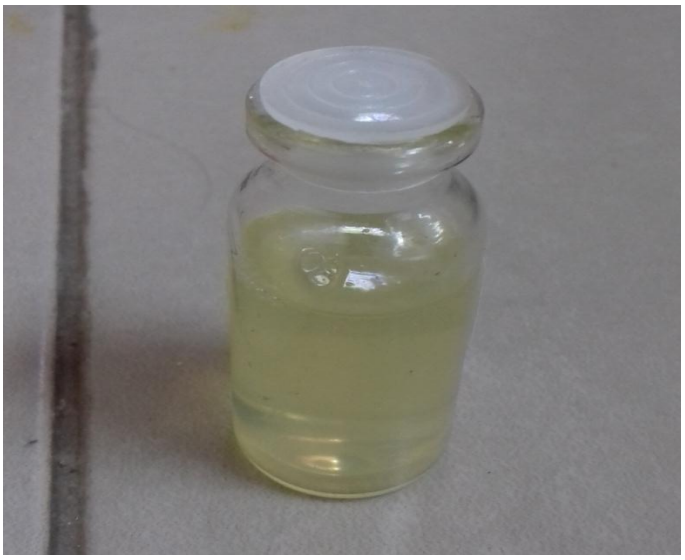
  $= \frac{3,412\ mg}{9,294\ mg}\ x\ 100\%$

  = 36,71%

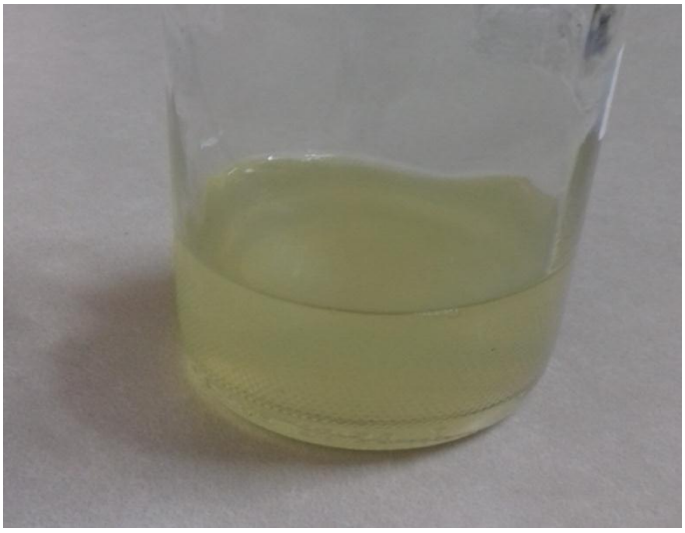
Lampiran 15. Uji stabilitas SLN fisetin dalam penyimpanan secara visual

a. Minggu pertama penyimpanan

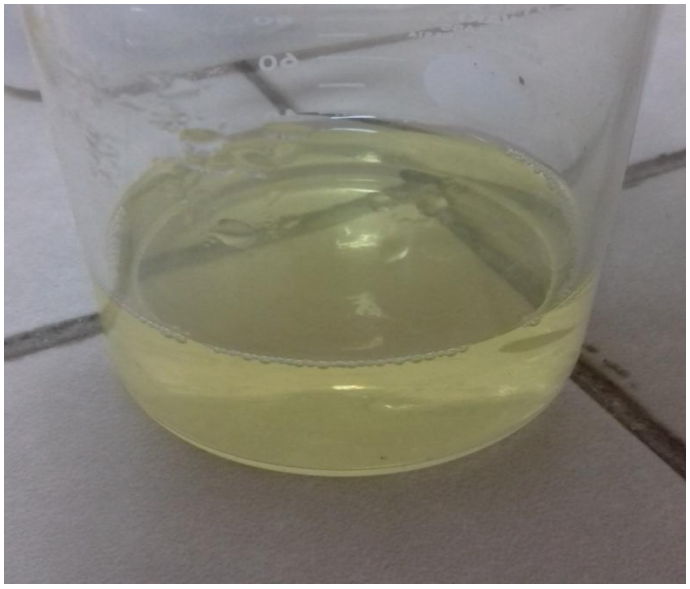
- ✓ **Setil Alkohol 0,5%**



- ✓ **Setil Alkohol 0,25%**



- ✓ **Setil Alkohol 0,15%**



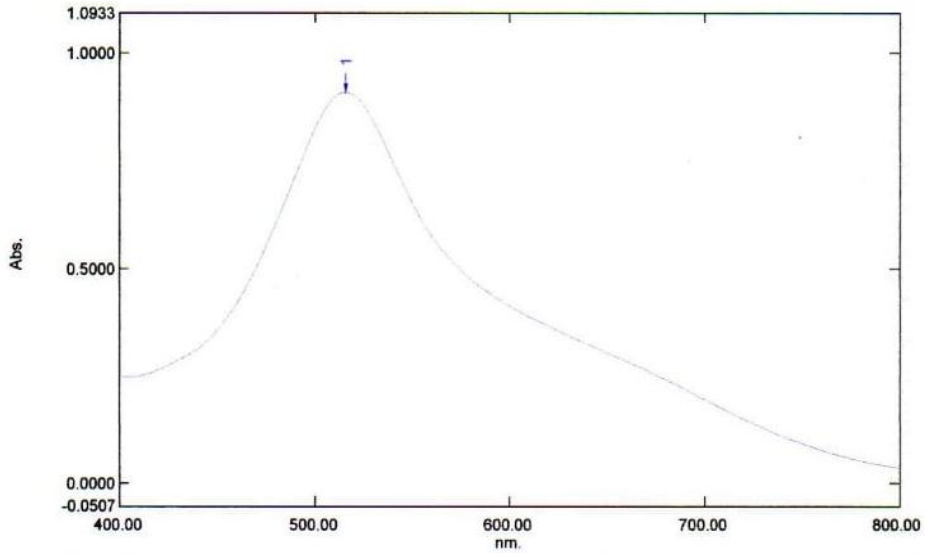
Lampiran 16. Uji aktifitas antioksidan

a. Penentuan panjang gelombang maks dpph

Spectrum Peak Pick Report

05/10/2019 10:03:42 AM

Data Set: lamda maks dpph fix 1 - RawData



No.	P/V	Wavelength	Abs.	Description
1	↑	516.00	0.9096	
2	↓	405.00	0.2479	

[Measurement Properties]
Wavelength Range (nm.): 400.00 to 800.00
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:

b. Penentuan operating time

OT Fisetin DPPH 4/5 19
60 MENIT

Kinetics Data Print Report

05/04/2019 12:24:22 PM

Time (Minute)	RawData ...
0.000	0.199
1.000	0.199
2.000	0.198
3.000	0.198
4.000	0.199
5.000	0.198
6.000	0.198
7.000	0.198
8.000	0.198
9.000	0.199
10.000	0.198
11.000	0.199
12.000	0.198
13.000	0.199
14.000	0.198
15.000	0.199
16.000	0.199
17.000	0.199
18.000	0.199
19.000	0.199
20.000	0.199
21.000	0.199
22.000	0.199
23.000	0.199
24.000	0.199
25.000	0.199
26.000	0.199
27.000	0.199
28.000	0.199
29.000	0.199
30.000	0.199
31.000	0.199
32.000	0.199
33.000	0.199
34.000	0.200
35.000	0.200
36.000	0.199
37.000	0.200
38.000	0.200
39.000	0.200
40.000	0.200
41.000	0.200
42.000	0.200
43.000	0.200
44.000	0.200
45.000	0.200
46.000	0.200
47.000	0.200
48.000	0.200
49.000	0.200
50.000	0.200

Kinetics Data Print Report 05/04/2019 12:24:22 PM

Time (Minute)	RawData ...
51.000	0.201
52.000	0.200
53.000	0.200
54.000	0.201
55.000	0.201
56.000	0.201
57.000	0.201
58.000	0.201
59.000	0.201
60.000	0.201

c. DPPH Fisetin murni

- **Perhitungan bahan fisetin :**

  50 mg/ 100 ml = 500 mg / 1000 ml = 500 ppm

  Yang ditimbang 49,8 mg sehingga didapat konsentrasi 498 ppm

- **Perhitungan konsentrasi (ppm)**

  - **$V_1 \times N_1 = V_2 \times N_2$**

    500 ppm x 0,3112 ml = V_2 x 10 ml

    V_2 = 15,56 ppm

  - **$V_1 \times N_1 = V_2 \times N_2$**

    500 ppm x 0,1556 ml = V_2 x 10 ml

    V_2 = 7,78 ppm

  - **$V_1 \times N_1 = V_2 \times N_2$**

    500 ppm x 0,0778 ml = V_2 x 10 ml

    V_2 = 3,89 ppm

  - **$V_1 \times N_1 = V_2 \times N_2$**

    500 ppm x 0,039 ml = V_2 x 10 ml

    V_2 = 1,95 ppm

  - **$V_1 \times N_1 = V_2 \times N_2$**

    500 ppm x 0,0194 ml = V_2 x 10 ml

    V_2 = 0,97 ppm

DPPH	Konsentrasi (ppm)	Volume (ml)	Etanol (ml)	Absorbansi replikasi 1	Absorbansi replikasi 2	Absorbansi replikasi 3
1 ml	160		4	0,909		
1 ml	15,56	1	3	0,212	0,221	0,224
1ml	7,78	1	3	0,405	0,416	0,418
1ml	3,89	1	3	0,507	0,509	0,511
1ml	1,95	1	3	0,555	0,557	0,559
1ml	0,97	1	3	0,578	0,578	0,588

$$\%\boldsymbol{inhibisi} = \frac{\boldsymbol{absorbansi\ DPPH - absorbansi\ sampel}}{\boldsymbol{absorbansi\ DPPH}}\ \boldsymbol{X}100\%$$

- **Konsentrasi 15,56 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,212}{0,909} x\ 100\% = 76,68\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,221}{0,909} x\ 100\% = 75,69\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,224}{0,909} x\ 100\% = 75,36\ \%$

- **Konsentrasi 7,78 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,405}{0,909} x\ 100\% = 55,45\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,416}{0,909} x\ 100\% = 54,24\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,418}{0,909} x\ 100\% = 54,02\ \%$

- **Konsentrasi 3,89 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,507}{0,909} x\ 100\% = 44,22\%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,509}{0,909} x\ 100\% = 44,00\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,511}{0,909} x\ 100\% = 43,78\ \%$

- **Konsentrasi 1,95 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,555}{0,909} x\ 100\% = 38,94\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,557}{0,909} x\ 100\% = 38,72\%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,559}{0,909} x\ 100\% = 38,50\ \%$

- **Konsentrasi 0,97 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,578}{0,909} x\ 100\% = 36,41\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,578}{0,909} x\ 100\% = 36,41\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,588}{0,909} x\ 100\% = 35,31\ \%$

- **Konsentrasi dan % inhibisi**

Konsentrasi (ppm)	Replikasi 1 % inhibisi	Replikasi 2 % inhibisi	Replikasi 3 % inhibisi
15,56	76,68	75,69	75,36
7,78	55,45	54,24	54,02
3,89	44,22	44,00	43,78
1,95	38,94	38,72	38,50
0,97	36,41	36,41	35,31
A	33,625	33,533	32,969
B	2,772	2,700	2,724
R	0,9999	0,9999	0,9999

- $\boldsymbol{IC_{50}= \frac{50-a}{b}}$

  Replikasi 1 IC_{50} = $\frac{(50-33,625)}{2,772} = 5,91 ppm$

  Replikasi 2 IC_{50} = $\frac{(50-33,533)}{2,700} = 6,09 ppm$

  Replikasi 3 IC_{50} = $\frac{(50-32,969)}{2,724} = 6,25 ppm$

d. DPPH formula (Setil alkohol 0,5%)

DPPH	Konsentrasi (ppm)	Volume (ml)	Etanol (ml)	Absorbansi replikasi 1	Absorbansi replikasi 2	Absorbansi replikasi 3
1 ml	100		4	0,909		
1 ml	25	1	3	0,174	0,180	0,178
1ml	12,25	1	3	0,442	0,448	0,450
1ml	6,25	1	3	0,584	0,580	0,577
1ml	1,56	1	3	0,687	0,682	0,685
1ml	0,78	1	3	0,698	0,710	0,697

$$\%\boldsymbol{inhibisi} = \frac{\boldsymbol{absorbansi\ DPPH - absorbansi\ sampel}}{\boldsymbol{absorbansi\ DPPH}}\ \boldsymbol{X100\%}$$

- **Konsentrasi 25 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,174}{0,909} x\ 100\% = 80,86\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,180}{0,909} x\ 100\% = 80,20\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,178}{0,909} x\ 100\% = 80,42\ \%$

- **Konsentrasi 12,25 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,442}{0,909} x\ 100\% = 51,38\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,448}{0,909} x\ 100\% = 50,72\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,450}{0,909} x\ 100\% = 50,50\ \%$

- **Konsentrasi 6,25 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,584}{0,909} x\ 100\% = 35,75\%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,580}{0,909} x\ 100\% = 36,19\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,577}{0,909} x\ 100\% = 36,52\ \%$

- **Konsentrasi 1,56 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,687}{0,909} x\ 100\% = 24,42\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,682}{0,909} x\ 100\% = 24,97\%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,685}{0,909} x\ 100\% = 24,64\ \%$

- **Konsentrasi 0,78 ppm**

  Replikasi 1 %inhibisi = $\frac{0,909-0,698}{0,909} x\ 100\% = 23,21\ \%$

  Replikasi 2 %inhibisi = $\frac{0,909-0,710}{0,909} x\ 100\% = 21,89\ \%$

  Replikasi 3 %inhibisi = $\frac{0,909-0,697}{0,909} x\ 100\% = 23,32\ \%$

- **Konsentrasi dan % inhibisi**

Konsentrasi (ppm)	Replikasi 1 %inhibisi	Replikasi 2 %inhibisi	Replikasi 3 %inhibisi
25	80,86	80,20	80,42
12,25	51,38	50,72	50,50
6,25	35,75	36,19	36,52
1,56	24,42	24,97	24,64
0,78	23,21	21,89	23,32
A	20,228	20,923	21,385
B	2,449	2,386	2,366
R	0,9993	0,9997	0,9999

- $IC_{50} = \frac{50-a}{b}$

Replikasi 1 $IC_{50} = \frac{(50-20{,}228)}{2{,}449} = 12{,}15ppm$

Replikasi 2 $IC_{50} = \frac{(50-20{,}923)}{2{,}386} = 12{,}18ppm$

Replikasi 3 $IC_{50} = \frac{(50-21{,}385)}{2{,}366} = 12{,}09ppm$

e. Uji T-test Fisetin Murni

T-Test

[DataSet0]

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
IC50	3	6,0700	,17521	,10116

One-Sample Test

	Test Value = 9					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
IC50	-28,964	2	,001	-2,93000	-3,3653	-2,4947

Kesimpulan : Nilai sig. (2-tailed) yaitu 0,001 < 0,05 yang berarti terdapat perbedaan yang bermakna antara nilai IC50 hasil praktikum dengan nilai IC50 teoritis (IC50 fisetin 9ppm).

f. Uji T-test Formula Setil Alkohol 0,5%

T-Test

[DataSet0]

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
IC50	3	10,7633	,27465	,15857

One-Sample Test

	Test Value = 9					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
IC50	11,120	2	,008	1,76333	1,0811	2,4456

Kesimpulan : Nilai sig. (2-tailed) yaitu 0,008 < 0,05 yang berarti terdapat perbedaan yang bermakna antara nilai IC50 formula fisetin hasil praktikum dengan nilai IC50 teoritis (IC50 fisetin 9ppm).