

BAB V

KESIMPULAN DAN SARAN

A. Kesimpulan

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

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

Kedua, penggunaan fosfatidilkolin pada konsentrasi tertinggi yaitu pada formula 4 sebesar 73,37 mg mampu menghasilkan ukuran partikel paling kecil yaitu 92,710 nm dan indeks polidisperditas terendah yaitu 0,284 dengan efisiensi penjerapan tertinggi 85,25% setara dengan 8,525 mg dari total 10 mg.

Ketiga, karakterisasi nanofitosom askorbil palmitat menghasilkan ukuran partikel rata-rata pada F1, F2, F3, F4, dan F5 berturut-turut sebesar 95,943; 115,833; 96,860; 92,710 dan 112,567 nm, dengan nilai indeks polidispersitas sekitar 0,284-0,388.

Keempat, nanofitosom askorbil palmitat tidak stabil selama penyimpanan lebih dari 3 minggu

B. Saran

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

Pertama, perlu dilakukan pembuatan nanofitosom dengan metode lainnya yang lebih bisa memberikan ukuran dibawah 100 nm serta metode preparasi untuk pengujian efisiensi penjerapan dan optimasi keadaan suhu, lama proses pada saat pembuatan fitosom sehingga dihasilkan nanofitosom dengan efisiensi penjerapan dan stabilias yang lebih baik.

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

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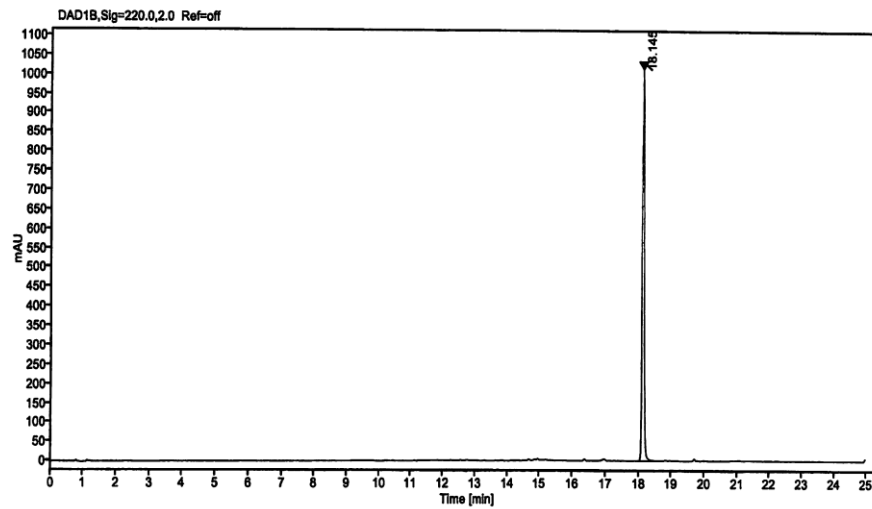
N

Lampiran 1. Serifikat Analisis Askorbil Palmitat

Certificate of Analysis		Dr. Ehrenstorfer 
Product Identification		Reference Materials for Residue Analysis
10303930 Ascorbyl palmitate		Expiry Date 05.10.2021
CA Ascorbic acid palmitate		Lot Number 155003
IUPAC Ascorbyl palmitate		Store at 20 °C ±4 °C
Formula C ₂₂ H ₃₈ O ₇		
Mol.Weight 414.54		
CAS No. 137-66-6		
Please note: The expiry date is valid under recommended storage conditions only.		
Toxicological Data	Physical Data	
	Phase crystalline solid	Vapour pressure N/A at °C
R Code	Color colourless	Solubility in water N/A g/l at °C
S Code	Melt.Range 114,3°C °C	Boiling Range (lit.)
LD50 (Rats female/male in mg/kg) 25000		
Analytical Data		Method Details:
Detection: HPLC/DAD	Eluent A: Acetonitrile:H ₂ O+0,5% H ₃ PO ₄ 1:9 for 1 min	
Column:	Eluent B: Acetonitrile 100% for 5 min	
Inj.-Vol.: 10.00 µl	Eluent A → Eluent B: 19 min	
Flow: 1.0 ml/min		
Ret.-Time: 18.14 min.		
Identity: IR, UV, RT, MS, NMR, EA		
Comment Column: YMC-Pack ODS-AQ, 3 µm, 150 x 3 mm		
Water Content Determined by Karl-Fischer Titration		
Det. Purity 99.0 % Tolerance/Uncertainty +/- 2.0 %		
The uncertainty/tolerance of this standard is calculated in accordance with the EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement - Second Edition. The uncertainty given is the expanded combined uncertainty and represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The expanded uncertainty is U which is Uc(y)*K, where K is the coverage factor at the 95% confidence level (K=2). The expanded uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of this product.		
Certified on 05.10.2017		
by M. Beck 		
<p>The Laboratory LGC Labor GmbH is accredited by DAkkS as indicated by the Accreditation Number D-RM-19883-01 & D-PL-19883-01 has shown competence based on ISO Guide 34:2009 with relevant parts of DIN EN ISO/IEC 17025:2005 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions organic pure substances.</p> <p>LGC Labor GmbH · Bgm.-Schlosser-Str. 6 A · 86199 Augsburg · Germany Phone +49 821 906080 · Fax +49 821 9060888 · augsburg.inquiry@lgcgroup.com The warranty for this product is limited to the purchasing price of this product.</p>		

05.10.2017
CF

Data file: 10303930-01.dx Instrument: DAD4
Sample name: 155003 Sequence Name: 05102017-YMC1
Inj. volume [µl]: 10.0 Injection date: 10/5/2017 11:43:54 AM
Acq. method: Gradient_10-100_PK_S2.amx Location: 45
Sample Description Ascorbyl palmitate
Column: YMC-Pack ODS-AQ, 3 µm, 150 x 3 mm




Signal: DAD1B,Sig=220.0,2.0 Ref=off

Nr.	RT [min]	Area	Height	Area%
1	18.145	4107.20960	1011.66	100.00
	Sum	4107.21		

M. Ben

Lampiran 2. Sertifikat Analisis Lipoid

				Lipoid	
				PHOSPHOLIPID GmbH - Member of the Lipoid Group	
		ANALYTICAL DATA		 AN30193645 - 1 -	
PHOSPHOLIPON 90 G					
Batch	228154-3180044	Recommended storage	n.m.l. 18 °C		
		Date of production	07/2018		
Sample for laboratory use only					
Parameter	Result	Specification		Unit	Method
		min	max		
Phosphatidylcholine	96.1	94.0	102.0	% (m/m)	05.P07.867
Identity (TLC)	conform to reference	conform to reference			05.P08.909
Lysophosphatidylcholine	1.4		4.0	% (m/m)	05.P07.867
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.P08.120
Water	0.2		1.5	% (m/m)	05.P10.013
Toluene insolubles	0.00		0.05	% (m/m)	05.P08.001
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 - Hattermarstraße 1 - D-50829 Köln - Tel. 0221-99745-0 - Fax 0221-99746-218					

Lipoid

PHOSPHOLIPID GmbH Member of the Lipoid Group

ANALYTICAL DATA

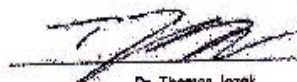
AN3019294.0
- 2 -

PHOSPHOLIPON 90 G

Batch 228154-3180044

Sample for laboratory use only

Köln, September 26, 2018



Dr. Thomas Jozek
Head of Quality Control



Anneli Tengerdek
Quality Assurance

Lampiran 3. Nanofitosom Askorbil Palmitat Sebelum Disonikasi



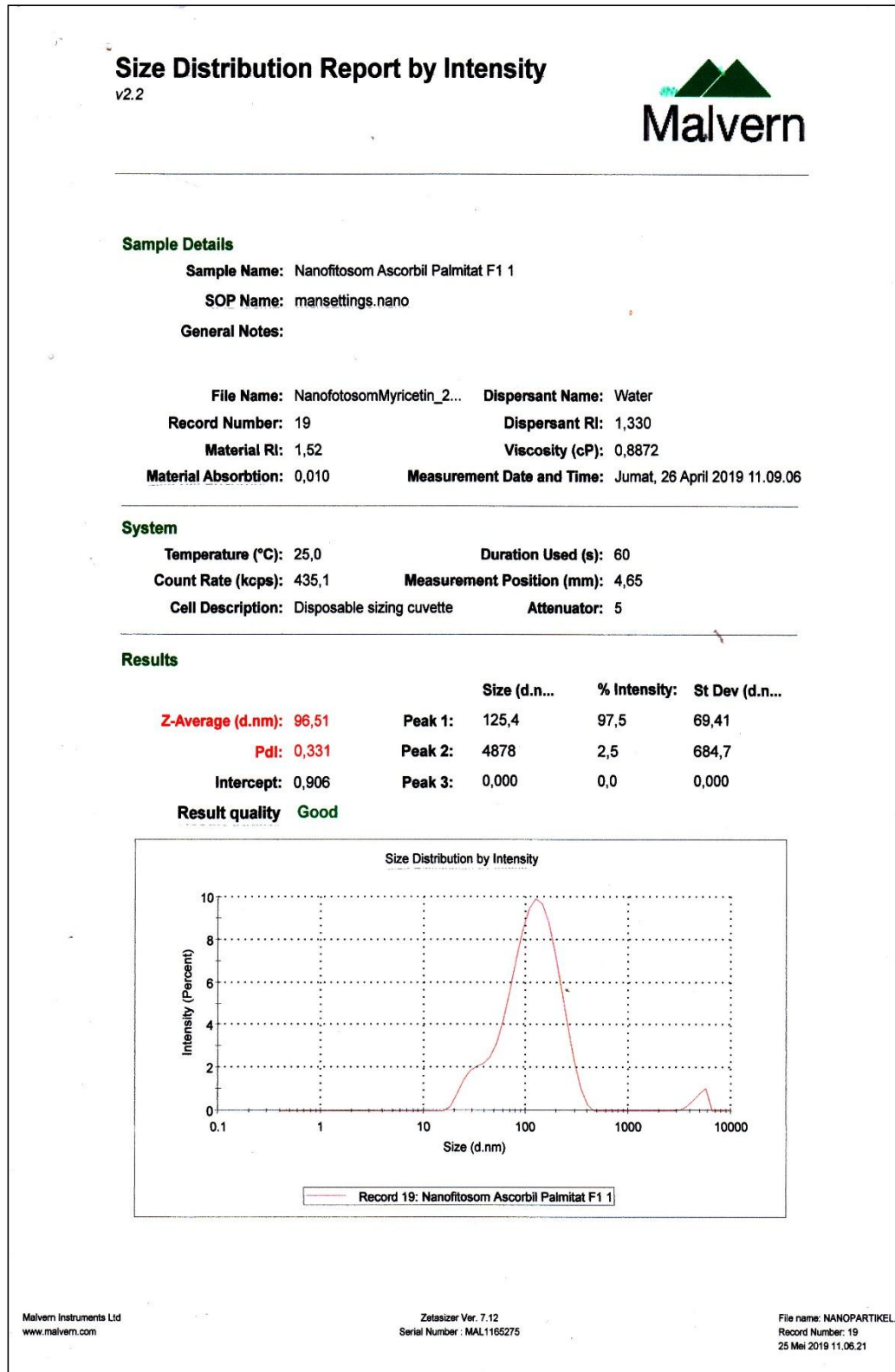
Lampiran 4. Nanofitosom Askorbil Palmitat Setelah Disonikasi



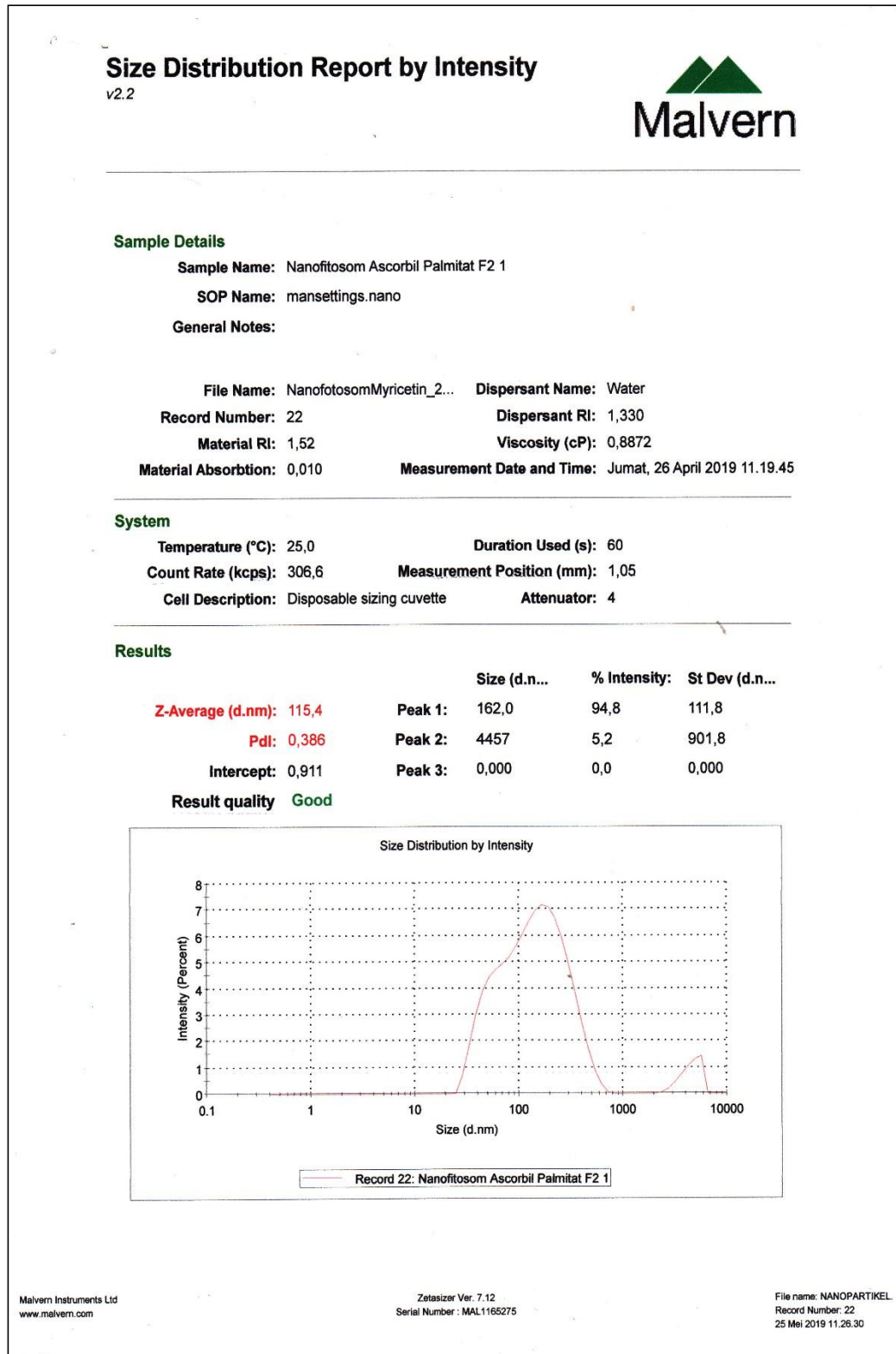
Lampiran 5. Nanofitosom Askorbil Palmitat Setelah Uji Stabilitas



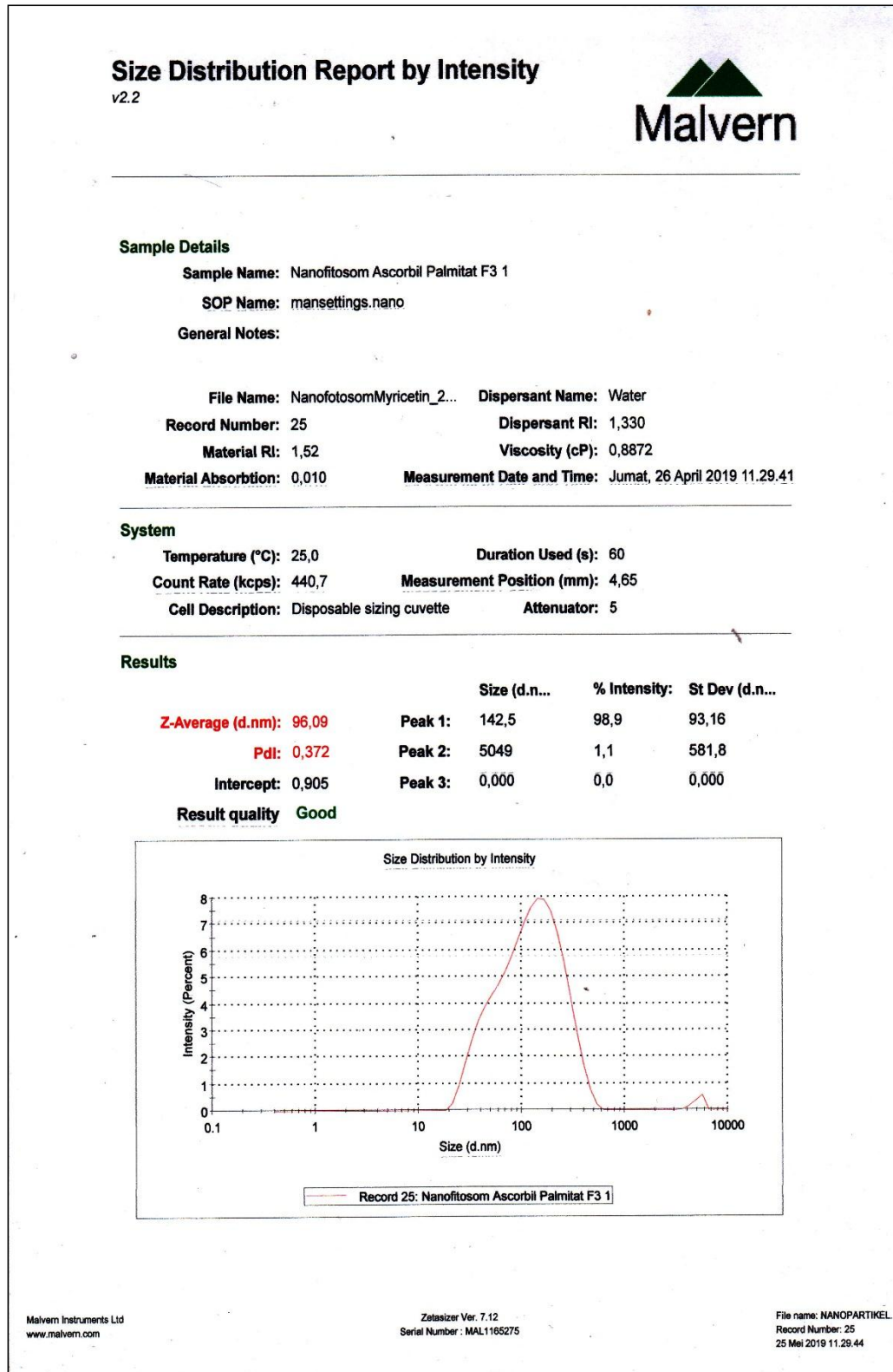
Lampiran 6. Hasil Ukuran Partikel F1



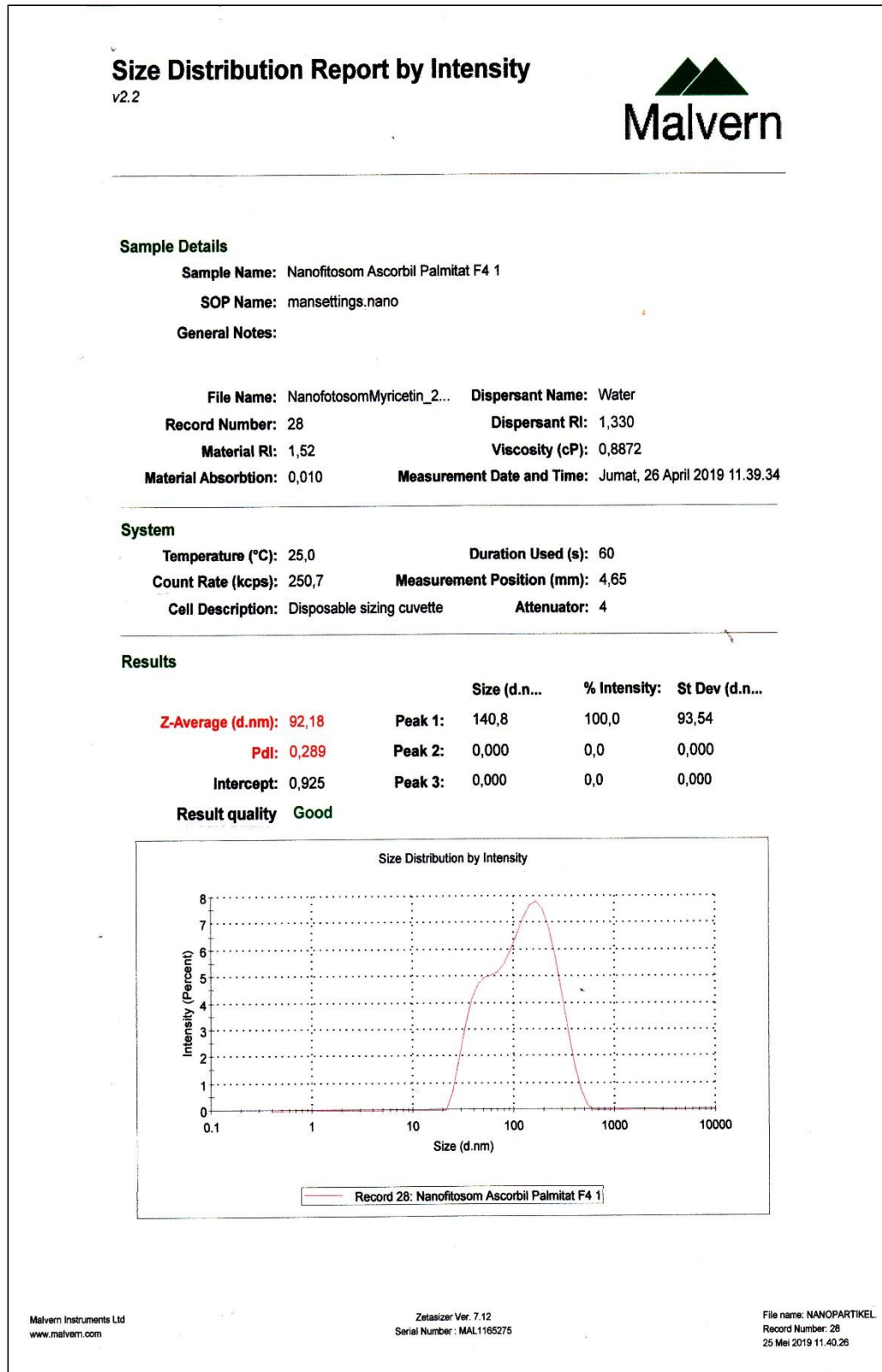
Lampiran 7. Hasil Ukuran Partikel F2



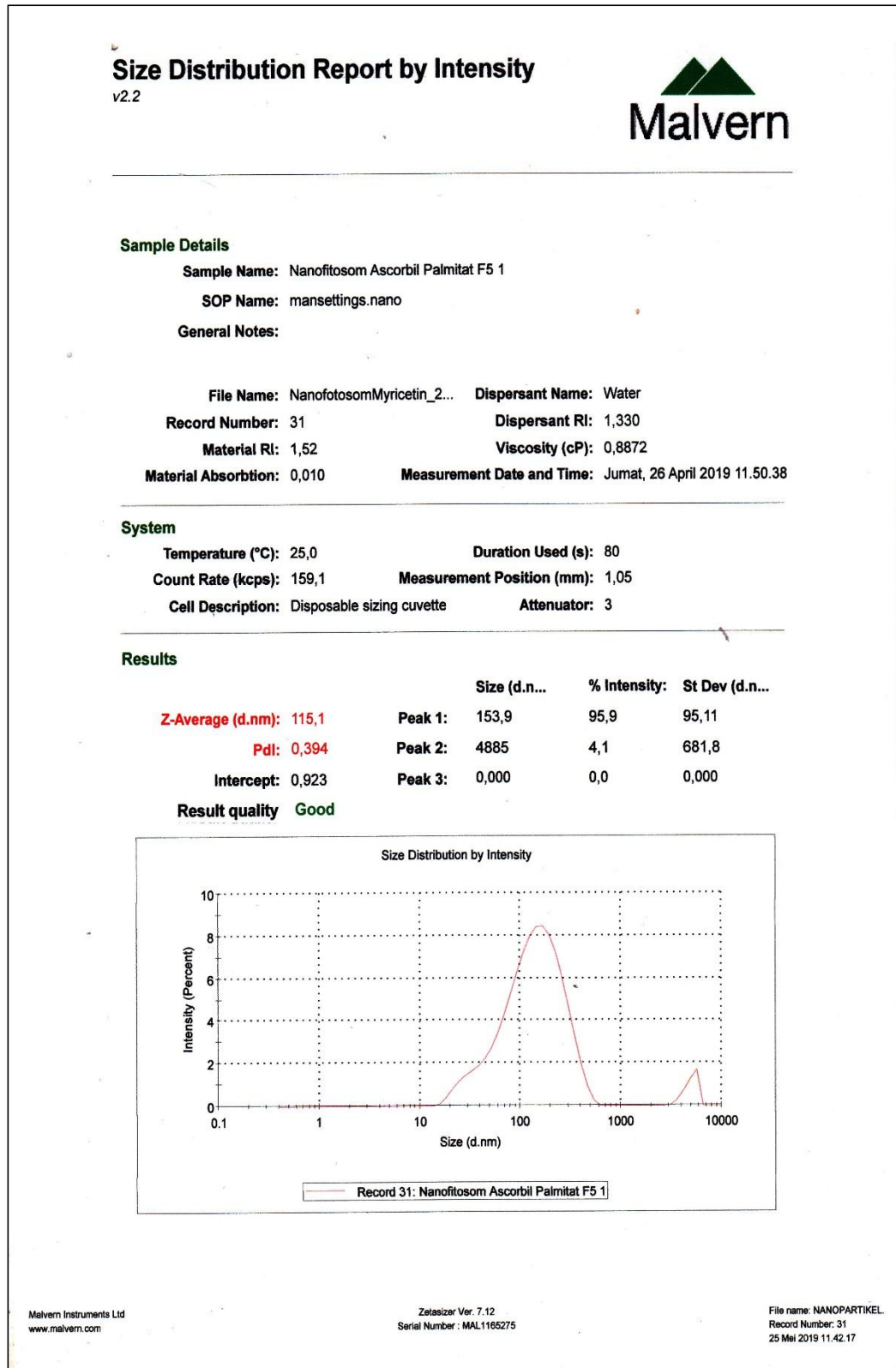
Lampiran 8. Hasil Ukuran Partikel F3



Lampiran 9. Hasil Ukuran Partikel F4



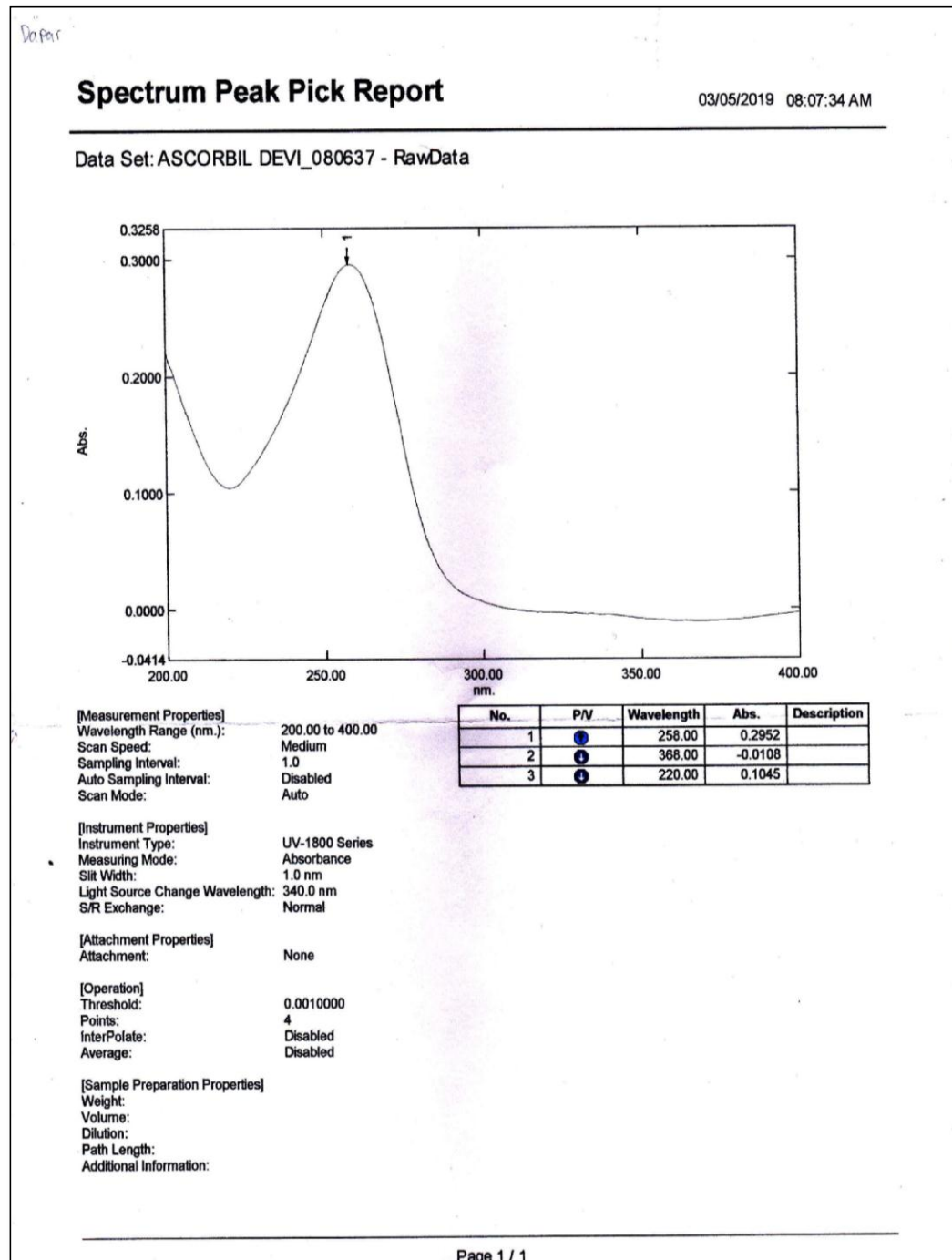
Lampiran 60. Hasil Ukuran Partikel F5



Lampiran 11. Pembuatan Kurva Kalibrasi dan Verifikasi Metode

1. Penentuan Panjang Gelombang Maksimum

Lamda maksimum \rightarrow 258 nm, dengan nilai serapan maksimum yaitu 0,2952



2. Penentuan *Operating Time*

Operating Time 21-30 menit dengan nilai serapan berturut-turut 0,287

Time (Minute)	RawData ...
0.000	0.288
1.000	0.289
2.000	0.289
3.000	0.290
4.000	0.289
5.000	0.289
6.000	0.288
7.000	0.288
8.000	0.289
9.000	0.288
10.000	0.288
11.000	0.288
12.000	0.287
13.000	0.288
14.000	0.288
15.000	0.288
16.000	0.288
17.000	0.288
18.000	0.288
19.000	0.288
20.000	0.288
21.000	0.287
22.000	0.287
23.000	0.287
24.000	0.287
25.000	0.287
26.000	0.287
27.000	0.287
28.000	0.287
29.000	0.287
30.000	0.287

3. Tabel Kurva Baku

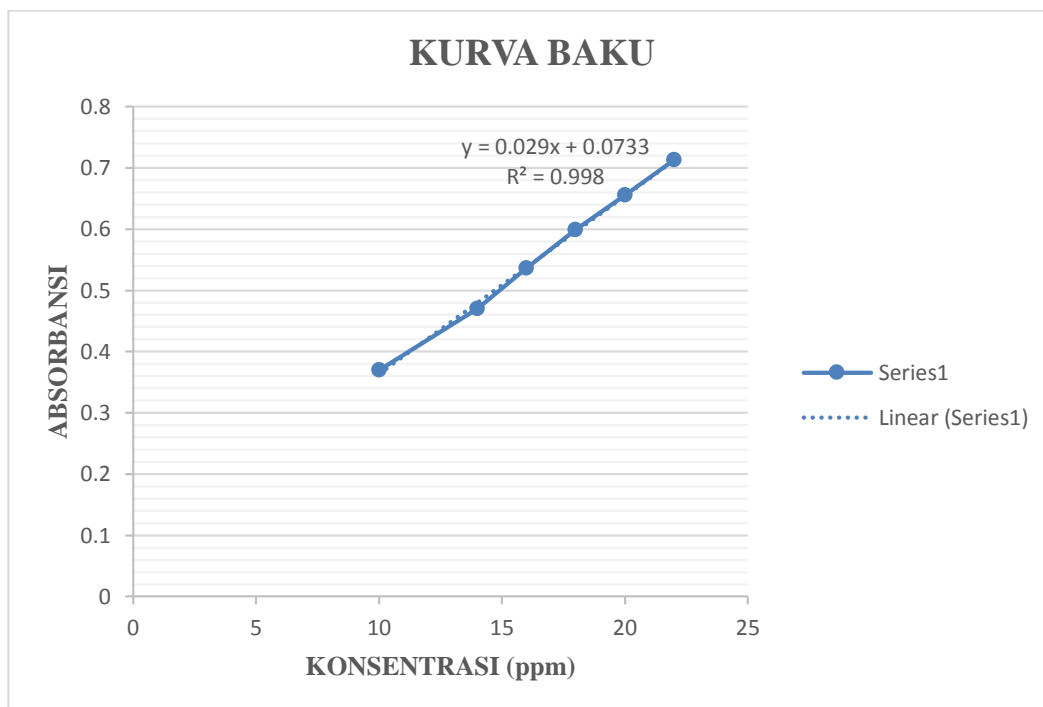
Larutan stok 100 ppm → 10 mg askorbil palmitat + 10 ml etanol 96% + dapar fosfat pH 7,4 ad 100 ml

Konsentrasi (ppm)	Absorbansi
10	0,370
14	0,470
16	0,536
18	0,599
20	0,656
22	0,713

$$a = 0,0733$$

$$b = 0,0290$$

$$r = 0,9990$$



- **Pembuatan larutan induk askorbil palmitat dalam medium dapar fosfat pH 7,4**

Berat Kertas kosong = 267,4 mg

Berat kertas + bahan = 278,7 mg

Berat kertas + sisa = 268,6 mg

$$\begin{aligned}
 \text{Berat zat aktif} &= 278,7 \text{ mg} - 268,6 \text{ mg} \\
 &= 10,1 \text{ mg} \\
 \text{Volume dapar } pH \ 7,4 &= 100 \text{ mL} \\
 \text{Larutan stok} &= 10,1 \text{ mg} / 100\text{mL} \\
 &= 100,1 \text{ mg} / 1000 \text{ mL} \\
 &= 100,1 \text{ ppm}
 \end{aligned}$$

- **Kurva baku askorbil palmitat dalam media dapar pH 7,4**

Larutan induk askorbil palmitat dibuat seri konsentrasi 10 ppm, 14 ppm, 16 ppm, 18 ppm, 20 ppm dan 22 ppm dalam 10 mL.

- **Konsentrasi 10 ppm**

$$\begin{aligned}
 V_1 \times C_1 &= V_2 \times C_2 \\
 V_1 \times 100 \text{ ppm} &= 10 \text{ mL} \times 10 \text{ ppm} \\
 V_1 &= 1 \text{ mL}
 \end{aligned}$$

Dipipet larutan induk askorbil palmitat 100 ppm sebanyak 1 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

- **Konsentrasi 14 ppm**

$$\begin{aligned}
 V_1 \times C_1 &= V_2 \times C_2 \\
 V_1 \times 100 \text{ ppm} &= 10 \text{ mL} \times 14 \text{ ppm} \\
 V_1 &= 1,4 \text{ mL}
 \end{aligned}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 1,4 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

- **Konsentrasi 16 ppm**

$$\begin{aligned}
 V_1 \times C_1 &= V_2 \times C_2 \\
 V_1 \times 100 \text{ ppm} &= 10 \text{ mL} \times 16 \text{ ppm} \\
 V_1 &= 1,6 \text{ mL}
 \end{aligned}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 1,6 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 18 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 18 \text{ ppm}$$

$$V_1 = 1,8 \text{ mL}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 1,8 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 20 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 20 \text{ ppm}$$

$$V_1 = 2 \text{ mL}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 2 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 22 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 22 \text{ ppm}$$

$$V_1 = 2,2 \text{ mL}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 2,2 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

A. Linearitas

Kosentrasi (ppm)	Absorbansi
10	0,370
14	0,470
16	0,536
18	0,599
20	0,656
22	0,713

$$a = 0,0733$$

$$b = 0,0290$$

$$r = 0,9990$$

$$y = a + bx$$

$$y = 0,0733 + 0,0290 x$$

Hasil linearitas diperoleh $r = 0,9990$, sehingga dapat disimpulkan bahwa data linear.

B. LOD dan LOQ

Kosentrasi (ppm)	Absorbansi (y)	y'	y-y'	y-y' ²
10	0,370	0,3637	0,0063	0,0000395
14	0,470	0,4799	-0,0099	0,0000978
16	0,536	0,5380	-0,0020	0,0000004
18	0,599	0,5961	0,0029	0,0000087
20	0,656	0,6541	0,0019	0,0000034
22	0,713	0,7122	0,0008	0,0000006
Jumlah total ($\sum y-y'$)² = 0,000150				

Nilai y' diperoleh dari substitusi kosentrasi dalam persamaan $y = 0,0733 + 0,0290 x$ dengan x adalah kosentrasi (ppm) dan y adalah serapan (y')

$$1. Y = 0,0733 + 0,0290 x$$

$$Y = 0,0733 + 0,0290 x 10$$

$$Y = 0,3637$$

2. $Y = 0,0733 + 0,0290 x$
 $Y = 0,0733 + 0,0290 \times 14$
 $Y = 0,4799$
3. $Y = 0,0733 + 0,0290 x$
 $Y = 0,0733 + 0,0290 \times 16$
 $Y = 0,5380$
4. $Y = 0,0733 + 0,0290 x$
 $Y = 0,0733 + 0,0290 \times 18$
 $Y = 0,5961$
5. $Y = 0,0733 + 0,0290 x$
 $Y = 0,0733 + 0,0290 \times 20$
 $Y = 0,6541$
6. $Y = 0,0733 + 0,0290 x$
 $Y = 0,0733 + 0,0290 \times 22$
 $Y = 0,7122$

- $S_{x/y} = \sqrt{\frac{(\sum |y-y'|)^2}{N-2}}$
 $S_{x/y}$ = simpangan baku residual
 N = jumlah data
 $(\sum |y-y'|)^2$ = jumlah kuadrat total residual

$$S_{x/y} = \sqrt{\frac{0,000150}{6-2}} = 0,0061$$

- $LOD = 3,3 \times \frac{S_x}{b}$
 $LOD = 3,3 \times \frac{0,0061}{0,0290}$
 $LOD = 0,6968 \text{ ppm}$

$$Y = 0,0733 + 0,0290 x$$

$$Y = 0,0733 + 0,0290 (0,6968)$$

$$Y = 0,0733 + 0,0202$$

Serapan LOD = 0,0935

$$\bullet \text{ LOQ} = 10 \times \frac{S_x}{b}$$

$$\text{LOQ} = 10 \times \frac{0,0061}{0,0290}$$

$$\text{LOQ} = 2,1114 \text{ ppm}$$

$$Y = 0,0733 + 0,0290 x$$

$$Y = 0,0733 + 0,0290 (2,1114)$$

$$Y = 0,0733 + 0,0612$$

$$\text{Serapan LOQ} = 0,1345$$

Lampiran 12. Penetapan Ukuran Partikel Sebelum Penyimpanan

Formula	Ukuran Partikel	Rata-rata \pm SD	Indeks Polidisperitas	Rata-rata \pm SD	Zeta potensial \pm SD
1	96,510	95,943 \pm	0,331	0,332 \pm	
	94,520	1,241	0,360	0,028	
	96,800		0,304		
2	115,400	115,833 \pm	0,386	0,386 \pm	
	117,500	1,498	0,387	0,001	
	114,600		0,386		
3	96,090	96,860 \pm	0,372	0,376 \pm	
	97,870	0,914	0,378	0,003	
	96,620		0,378		
4	92,180	92,710 \pm	0,289	0,284 \pm	-5,27
	92,770	0,503	0,283	0,004	-4,78 \pm 0,261
	93,180		0,281		-5,18
5	115,100	112,567 \pm	0,394	0,388 \pm	
	111,100	2,203	0,399	0,014	
	111,500		0,372		

Lampiran 13. Perhitungan Efisiensi Penjerapan

Formula	Replikasi	Absorbansi	Rata-rata absorbansi	% EE
1	1	0,778	0,779	75,67
	2	0,781		
	3	0,777		
2	1	0,669	0,667	79,53
	2	0,665		
	3	0,667		
3	1	0,605	0,605	81,67
	2	0,603		
	3	0,607		
4	1	0,497	0,501	85,25
	2	0,501		
	3	0,504		
5	1	0,549	0,551	83,53
	2	0,551		
	3	0,553		

Regresi linier :

$$a = 0,0733$$

$$b = 0,0290$$

$$r = 0,9990$$

Rumus Efisiensi Penjerapan :

$$\% EE = \frac{TD-FD}{TD} \times 100\%$$

Keterangan :

TD : total jumlah fenolat yang terdapat pada formula

FD : total senyawa fenolat yang terdeteksi pada supernatant (tidak terjerap)

- Larutan induk → 10 mg askorbil palmitat, supernatan atau askorbil yang tidak terjerap dicukupkan volumenya menjadi 10 mL sehingga konsentrasi menjadi 1000 ppm.

PERHITUNGAN :**FORMULA 1**

- $y = a + bx$
 $0,779 = 0,0733 + 0,0290x$
 $x = \frac{0,779-0,0733}{0,0290}$
 $x = 24,33 \text{ ppm}$
- Jumlah Fisetin yang tidak terjerap = $24,33 \text{ ppm} \times \text{faktor pengenceran}$
 $= 24,33 \text{ ppm} \times 10$
 $= 243,3 \text{ ppm}$
- $\% \text{ EE} = \frac{1000 \text{ ppm} - 243,3 \text{ ppm}}{1000 \text{ ppm}} \times 100 \%$
 $= 75,67 \%$

FORMULA 2

- $y = a + bx$
 $0,667 = 0,0733 + 0,0290x$
 $x = \frac{0,667-0,0733}{0,0290}$
 $x = 20,47 \text{ ppm}$
- Jumlah Fisetin yang tidak terjerap = $20,47 \text{ ppm} \times \text{faktor pengenceran}$
 $= 20,47 \text{ ppm} \times 10$
 $= 204,72 \text{ ppm}$
- $\% \text{ EE} = \frac{1000 \text{ ppm} - 204,72 \text{ ppm}}{1000 \text{ ppm}} \times 100 \%$
 $= 79,53 \%$

FORMULA 3

- $y = a + bx$
 $0,605 = 0,0733 + 0,0290x$
 $x = \frac{0,605-0,0733}{0,0290}$
 $x = 18,33 \text{ ppm}$

- Jumlah Fisetin yang tidak terjerap = 18,33 ppm x faktor pengenceran
= 18,33 ppm x 10
= 183,30 ppm
- % EE = $\frac{1000 \text{ ppm} - 183,30 \text{ ppm}}{1000 \text{ ppm}} \times 100 \%$
= 81,67 %

FORMULA 4

- $y = a + bx$
 $0,501 = 0,0733 + 0,0290x$
 $x = \frac{0,501 - 0,0733}{0,0290}$
 $x = 14,75 \text{ ppm}$
- Jumlah Fisetin yang tidak terjerap = 14,75 ppm x faktor pengenceran
= 14,75 ppm x 10
= 147,50 ppm
- % EE = $\frac{1000 \text{ ppm} - 147,50 \text{ ppm}}{1000 \text{ ppm}} \times 100 \%$
= 85,25 %

FORMULA 5

- $y = a + bx$
 $0,551 = 0,0733 + 0,0290x$
 $x = \frac{0,551 - 0,0733}{0,0290}$
 $x = 16,47 \text{ ppm}$
- Jumlah Fisetin yang tidak terjerap = 16,47 ppm x faktor pengenceran
= 16,47 ppm x 10
= 164,70 ppm
- % EE = $\frac{1000 \text{ ppm} - 164,70 \text{ ppm}}{1000 \text{ ppm}} \times 100 \%$
= 83,53 %

Lampiran 14. Uji Stabilitas Selama 3 minggu

A. Pembentukan Endapan

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	Tidak Ada endapan	Ada endapan
5	Tidak ada endapan	Ada endapan	Ada endapan

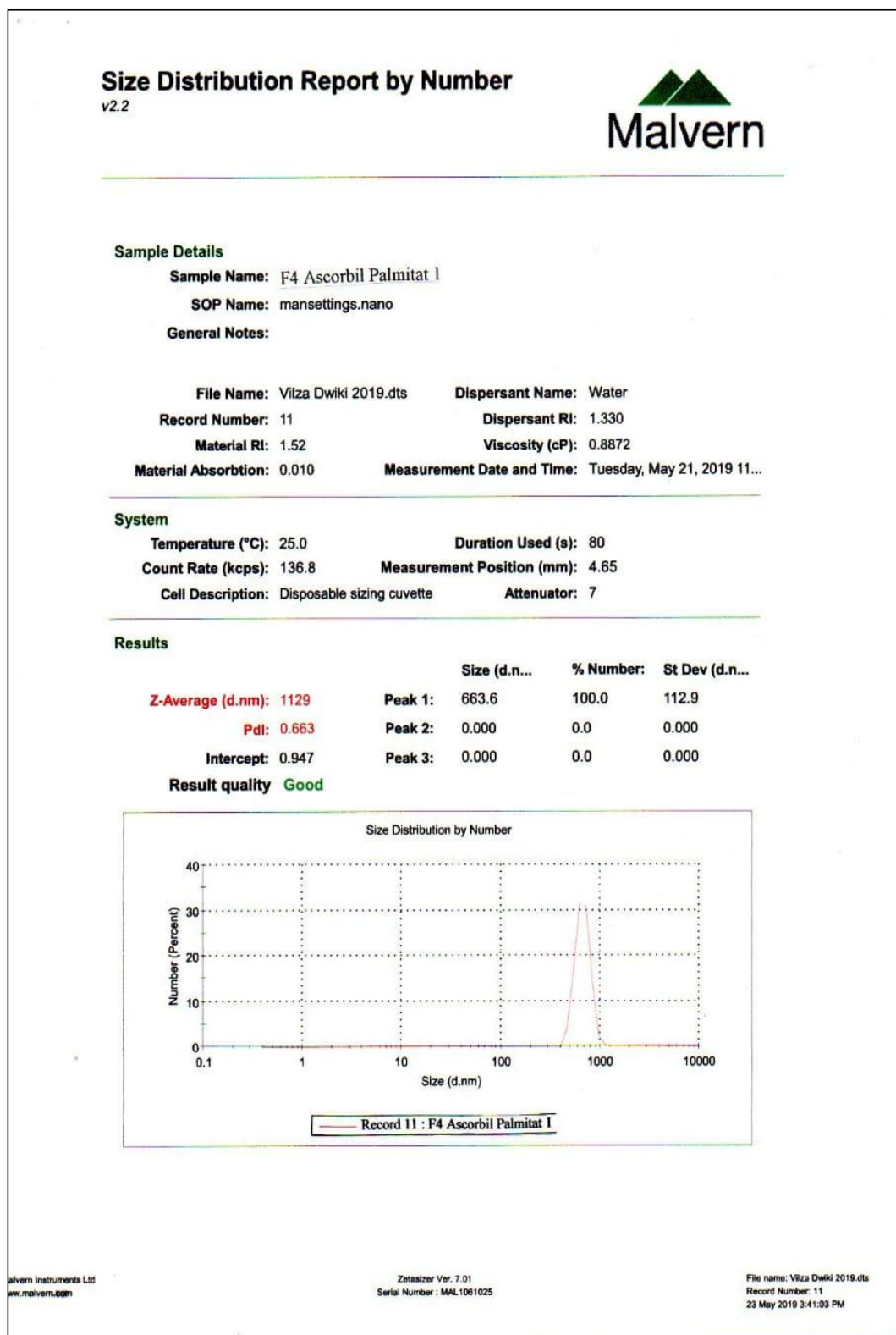
B. Ukuran Partikel Dan Distribusi Partikel Setelah Penyimpanan

Formula	Ukuran Partikel (nm)		Indeks Polidispersitas	
	Sebelum	Sesudah	Sebelum	Sesudah
F4	96,860	1105	0,376	0,643

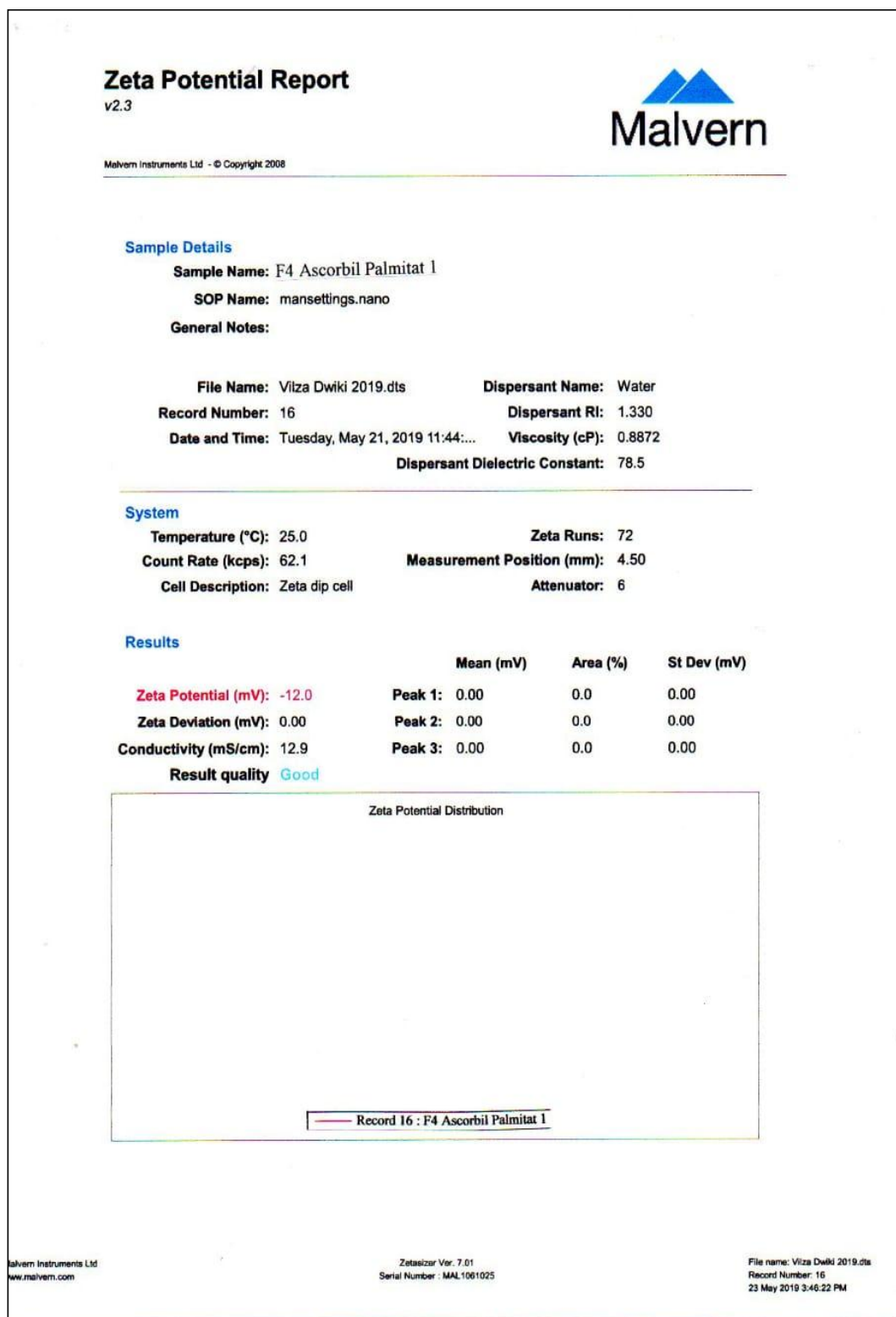
Lampiran 15. Potensial Zeta F4 Setelah Penyimpanan

Formula	Potensial Zeta (mV)	SD
F4	-13,040	± 0,658

Lampiran 16. Hasil ukuran Partikel F4 Setelah Penyimpanan



Lampiran 17. Hasil Potensial Zeta F4 Setelah Penyimpanan



Lampiran 18. Perhitungan Formula

Diketahui:

- Kadar askorbil palmitat = 99 %
- Mol askorbil palmitat = 414,54 g/mol
- Mol fosfatidilkolin = 768 g/mol
- Mol kolesterol = 386,67 g/mol

- Kandungan askorbil palmitat dalam 10 mg serbuk

$$\text{Askorbil palmitat (mg)} = 10 \text{ mg} \times \frac{99}{100} = 9,9 \text{ mg}$$

- Mol askorbil palmitat dalam 10 mg serbuk

$$\text{Mol askorbil palmitat } (\mu\text{mol}) = \frac{9,9 \text{ mg}}{414540 \times 10^{-6} \mu\text{mol}} = 23,8819 \mu\text{mol}$$

- Fosfatidilkolin

1. Formula 1:1

Maka, jumlah fosfatidilkolin yang dibutuhkan adalah sebesar 23,8819 μmol .

$$\text{Fosfatidilkolin yang ditimbang} = 23,8819 \mu\text{mol} \times \frac{768000 \text{ mg}}{10^{-6} \mu\text{mol}} = 18,34 \text{ mg}$$

2. Formula 1:2

Maka, jumlah fosfatidilkolin yang dibutuhkan adalah sebesar 47,7638 μmol .

$$\text{Fosfatidilkolin yang ditimbang} = 47,7638 \mu\text{mol} \times \frac{768000 \text{ mg}}{10^{-6} \mu\text{mol}} = 36,68 \text{ mg}$$

3. Formula 1:3

Maka, jumlah fosfatidilkolin yang dibutuhkan adalah sebesar 71,6457 μmol .

$$\text{Fosfatidilkolin yang ditimbang} = 71,6457 \mu\text{mol} \times \frac{768000 \text{ mg}}{10^{-6} \mu\text{mol}} = 55,02 \text{ mg}$$

4. Formula 1:4

Maka, jumlah fosfatidilkolin yang dibutuhkan adalah sebesar 95,5276 μmol .

$$\text{Fosfatidilkolin yang ditimbang} = 95,5276 \mu\text{mol} \times \frac{768000 \text{ mg}}{10^{-6} \mu\text{mol}} = 73,37 \text{ mg}$$

5. Formula 1:5

Maka, jumlah fosfatidilkolin yang dibutuhkan adalah sebesar 119,4095 μmol .

$$\text{Fosfatidilkolin yang ditimbang} = 119,4095 \mu\text{mol} \times \frac{768000 \text{ mg}}{10^{-6} \mu\text{mol}} = 91,71 \text{ mg}$$

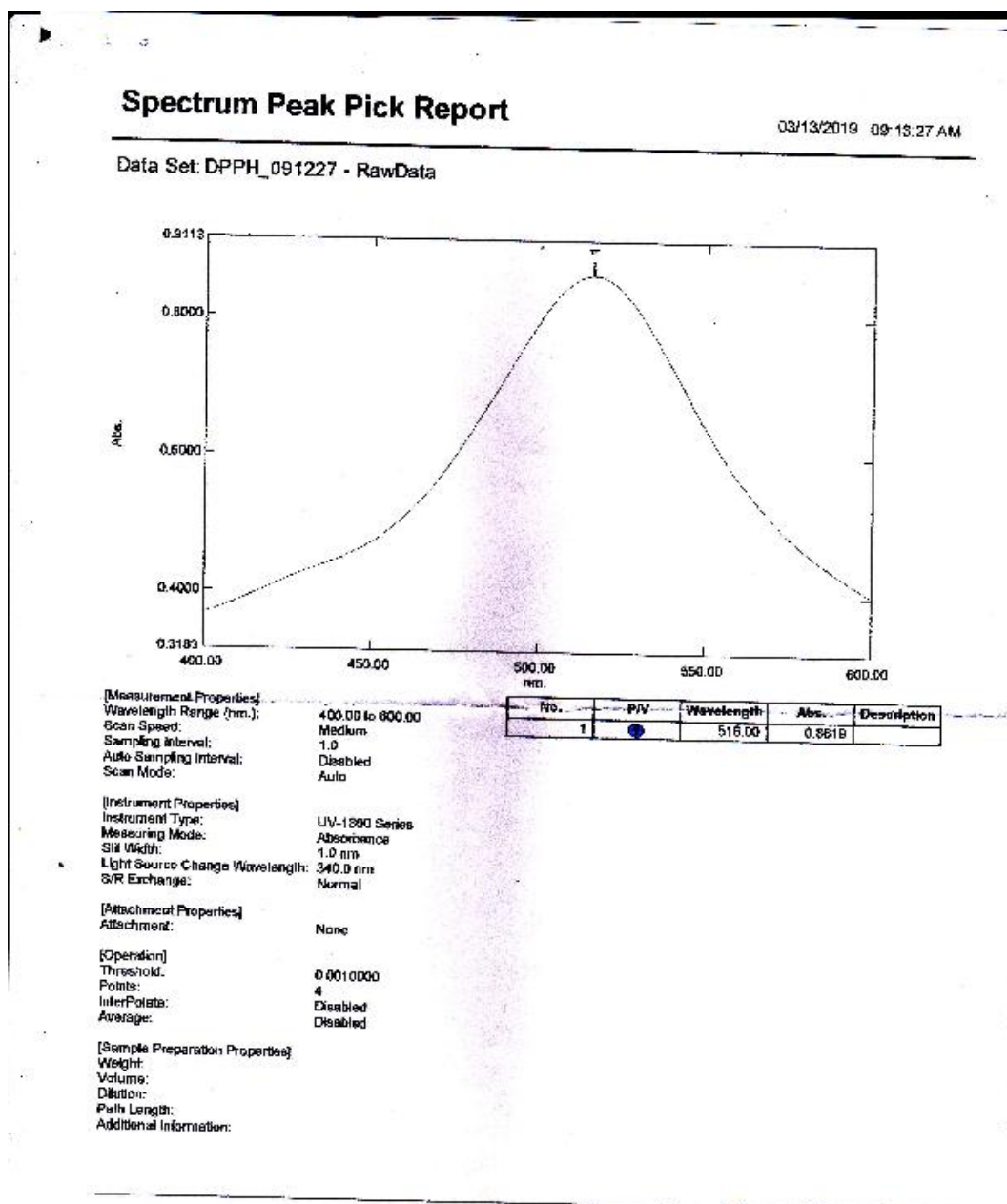
- Kolesterol

Perbandingan zat aktif : kolesterol \rightarrow 1:0,2. Maka jumlah kolesterol yang dibutuhkan adalah sebesar 4,7764 μmol .

$$\text{Kolesterol yang ditimbang} = 4,7764 \mu\text{mol} \times \frac{386670 \text{ mg}}{10^{-6} \mu\text{mol}} = 1,83 \text{ mg}$$

Lampiran 19. Penentuan Panjang Gelombang Maksimum DPPH dan *Operating Time*

1. Panjang Gelombang Maksimum DPPH



2. Penentuan *Operating Time*

Operating time 34-36 menit dengan nilai serapan berturut-turut 0,738

Kinetics Data Print Report

03/13/2019 02:50:15 PM

Time (Minute)	RawData ...
0.000	0.765
1.000	0.783
2.000	0.783
3.000	0.762
4.000	0.782
5.000	0.781
6.000	0.760
7.000	0.759
8.000	0.758
9.000	0.757
10.000	0.756
11.000	0.755
12.000	0.754
13.000	0.753
14.000	0.762
15.000	0.751
16.000	0.750
17.000	0.750
18.000	0.749
19.000	0.748
20.000	0.748
21.000	0.747
22.000	0.748
23.000	0.745
24.000	0.744
25.000	0.744
26.000	0.743
27.000	0.742
28.000	0.742
29.000	0.741
30.000	0.741
31.000	0.740
32.000	0.740
33.000	0.738
34.000	0.738
35.000	0.738
36.000	0.738
37.000	0.737
38.000	0.738
39.000	0.738
40.000	0.735
41.000	0.735
42.000	0.735
43.000	0.734
44.000	0.734
45.000	0.733
46.000	0.733
47.000	0.732
48.000	0.732
49.000	0.732
50.000	0.731

Lampiran 20. Penimbangan DPPH dan pembuatan larutan stok DPPH

Serbuk DPPH untuk uji aktivitas antioksidan ditimbang sesuai hasil perhitungan berikut :

$$\begin{aligned} \text{Penimbangan DPPH} &= \text{BM DPPH} \times \text{volume larutan} \times \text{molaritas DPPH} \\ &= 394,32 \text{ g/mol} \times 0,100 \text{ liter} \times 0,0004 \text{ M} \\ &= 0,01578 \text{ gram} \\ &= 15,78 \text{ mg} = 15,8 \text{ mg} \end{aligned}$$

Selanjutnya 15,8 mgserbuk DPPH dilarutkan dengan etanol p.a dalam labu takar 100 ml

Pembuatan larutan stok zat aktif askorbil palmitat

$$\begin{aligned} \text{Berat kertas + Askorbil palmitat} &= 288,3 \text{ mg} \\ \text{Berat kertas + sisa} &= 278,3 \text{ mg} \\ \text{Berat zat aktif} &= 288,3 \text{ mg} - 278,3 \text{ mg} \\ &= 10 \text{ mg} \end{aligned}$$

Pembuatan larutan stok dilakukan dengan cara ditimbang askorbil palmitat 10,4 mg dimasukkan ke dalam labu takar 100 mL kemudian ditambahkan etanol p.a sampai tanda batas, sehingga diperoleh konsentrasi 100 ppm.

$$\begin{aligned} \text{Konsentrasi askorbil palmitat} &= 10 \text{ mg}/100 \text{ mL} \\ &= 100 \text{ mg}/1000 \text{ mL} \\ &= 100 \text{ ppm} \end{aligned}$$

Larutan askorbil palmitat konsentrasi 100 ppm diencerkan menjadi 6 seri pengenceran konsentrasi, yaitu 26 ppm, 34 ppm, 42 ppm, 50 ppm, 58 ppm, 66 ppm.

➤ **Konsentrasi 26 ppm**

$$\begin{aligned} V_1 \times C_1 &= V_2 \times C_2 \\ V_1 \times 100 \text{ ppm} &= 10 \text{ mL} \times 26 \text{ ppm} \\ V_1 &= 2,6 \text{ mL} \end{aligned}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 2,6 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 34 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 34 \text{ ppm}$$

$$V_1 = 3,4 \text{ mL}$$

Dipipet larutan askorбил palmitat 100 ppm sebanyak 3,4 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 42 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 42 \text{ ppm}$$

$$V_1 = 4,2 \text{ mL}$$

Dipipet larutan askorбил palmitat 100 ppm sebanyak 4,2 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 50 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 50 \text{ ppm}$$

$$V_1 = 5 \text{ mL}$$

Dipipet larutan askorбил palmitat 100 ppm sebanyak 5 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 58 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 58 \text{ ppm}$$

$$V_1 = 5,8 \text{ mL}$$

Dipipet larutan askorбил palmitat 100 ppm sebanyak 5,8 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 66 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 100 \text{ ppm} = 10 \text{ mL} \times 66 \text{ ppm}$$

$$V_1 = 6,6 \text{ mL}$$

Dipipet larutan askorbil palmitat 100 ppm sebanyak 6,6 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

Pembuatan larutan stok nanofitosom askorbil palmitat (formula 4)

Pembuatan larutan stok nanofitosom dilakukan dengan cara dipipet larutan nanofitosom askorbil palmitat 5 mL dimasukkan ke dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas, sehingga diperoleh konsentrasi 250 ppm.

$$\begin{aligned} \text{Konsentrasi nanofitosom askorbil palmitat} &= 10 \text{ mg}/20 \text{ mL} \\ &= 500 \text{ mg}/1000 \text{ mL} \\ &= 500 \text{ ppm} \end{aligned}$$

Larutan askorbil palmitat konsentrasi 500 ppm diencerkan menjadi 6 seri pengenceran konsentrasi, yaitu 250 ppm, 125 ppm, 62,5 ppm, 31,25 ppm, 15,63 ppm, 7,81 ppm.

➤ **Konsentrasi 250 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 250 \text{ ppm}$$

$$V_1 = 5 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 500 ppm sebanyak 5 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 125 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 125 \text{ ppm}$$

$$V_1 = 2,5 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 250 ppm sebanyak 2,5 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 62,5 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 62,5 \text{ ppm}$$

$$V_1 = 1,25 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 125 ppm sebanyak 1,25 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 31,25 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 31,25 \text{ ppm}$$

$$V_1 = 0,625 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 62,5 ppm sebanyak 0,625 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 15,63 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 15,63 \text{ ppm}$$

$$V_1 = 0,31 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 31,25 ppm sebanyak 0,31 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

➤ **Konsentrasi 7,81 ppm**

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 500 \text{ ppm} = 10 \text{ mL} \times 7,81 \text{ ppm}$$

$$V_1 = 0,16 \text{ mL}$$

Dipipet larutan nanofitosom askorbil palmitat 15,63 ppm sebanyak 0,16 mL dimasukkan dalam labu takar 10 mL kemudian ditambahkan etanol p.a sampai tanda batas.

Lampiran 21. Perhitungan Aktivitas Antioksidan dan IC₅₀

A. Perhitungan aktivitas antioksidan Askorbil palmitat

Perhitungan presentase peredaman menggunakan rumus :

$$\text{Peredaman (\%)} = \frac{\text{absorbansi serapan larutan DPPH} - \text{absorbansi sampel}}{\text{absorbansi serapan larutan DPPH}} \times 100 \%$$

Peredaman replikasi 1

$$\text{➤ 26 ppm} = \frac{0,862 - 0,653}{0,862} \times 100 \% = 24,25 \%$$

$$\text{➤ 34 ppm} = \frac{0,862 - 0,591}{0,862} \times 100 \% = 31,44 \%$$

$$\text{➤ 42 ppm} = \frac{0,862 - 0,520}{0,862} \times 100 \% = 39,68 \%$$

$$\text{➤ 50 ppm} = \frac{0,862 - 0,457}{0,862} \times 100 \% = 46,98 \%$$

$$\text{➤ 58 ppm} = \frac{0,862 - 0,389}{0,862} \times 100 \% = 54,87 \%$$

$$\text{➤ 66 ppm} = \frac{0,862 - 0,322}{0,862} \times 100 \% = 62,65 \%$$

Peredaman replikasi 2

$$\text{➤ 26 ppm} = \frac{0,862 - 0,647}{0,862} \times 100 \% = 24,94 \%$$

$$\text{➤ 34 ppm} = \frac{0,862 - 0,595}{0,862} \times 100 \% = 30,97 \%$$

$$\text{➤ 42 ppm} = \frac{0,862 - 0,523}{0,862} \times 100 \% = 39,33 \%$$

$$\text{➤ 50 ppm} = \frac{0,862 - 0,454}{0,862} \times 100 \% = 47,33 \%$$

$$\text{➤ 58 ppm} = \frac{0,862 - 0,387}{0,862} \times 100 \% = 55,10 \%$$

$$\text{➤ 66 ppm} = \frac{0,862 - 0,326}{0,862} \times 100 \% = 62,18 \%$$

Peredaman replikasi 3

$$\text{➤ 26 ppm} = \frac{0,862 - 0,650}{0,862} \times 100 \% = 24,59 \%$$

$$\text{➤ 34 ppm} = \frac{0,862 - 0,592}{0,862} \times 100 \% = 31,32 \%$$

$$\text{➤ 42 ppm} = \frac{0,862 - 0,524}{0,862} \times 100 \% = 39,21 \%$$

$$\text{➤ 50 ppm} = \frac{0,862 - 0,456}{0,862} \times 100 \% = 47,10 \%$$

$$\text{➤ 58 ppm} = \frac{0,862 - 0,384}{0,862} \times 100 \% = 55,45 \%$$

$$\text{➤ 66 ppm} = \frac{0,862 - 0,323}{0,862} \times 100 \% = 62,53 \%$$

aktivitas antioksidan				
askorbil palmitat	konsentrasi	Replikasi	Absorbansi serapan larutan DPPH	absorbansi sampel
	26	Replikasi 1	0,862	0,653
	34			0,591
	42			0,520
	50			0,457
	58			0,389
	66			0,322
	26	Replikasi 2		0,647
	34			0,595
	42			0,523
	50			0,454
	58			0,387
	66	0,326		
	26	Replikasi 3		0,650
	34			0,592
	42			0,524
	50			0,456
	58			0,384
	66			0,323

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

askorbil palmitat	konsentrasi	% peredaman	hasil regresiliner	IC ₅₀	Rata-rata ± SD
	26	24,35	a = -0,9781 b = 0,9628 r = 0,9999	52,95	52,67 ± 0,55
	34	31,44			
	42	39,68			
	50	46,98			
	58	54,87			
	66	62,65			
	26	24,94	a = -0,4886 b = 0,9521 r = 0,9992	53,03	
	34	30,97			
	42	39,33			
	50	47,33			
	58	55,10			
	66	62,18			
	26	24,59	a = -2,9515 b = 1,0178 r = 0,9981	52,04	
	34	31,32			
	42	39,21			
	50	47,10			
	58	55,45			
	66	62,53			

Perhitungan IC₅₀ Askorbil Palmitat**IC₅₀ replikasi 1**

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = -0,9781$$

$$b = 0,9628$$

$$r = 0,9999$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = -0,9781 + 0,9628 x$$

$$x = 52,95$$

$$IC_{50} = 52,95 \text{ ppm}$$

IC₅₀ replikasi 2

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = -0,4886$$

$$b = 0,9521$$

$$r = 0,9992$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = -0,4886 + 0,9521 x$$

$$x = 53,03$$

$$IC_{50} = 53,03 \text{ ppm}$$

IC₅₀ replikasi 3

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = -2,9515$$

$$b = 1,0178$$

$$r = 0,9981$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = -2,9515 + 1,0178 x$$

$$x = 52,04$$

$$IC_{50} = 52,04 \text{ ppm}$$

B. Perhitungan aktivitas antioksidan dan IC₅₀ nanofitosom askorbil palmitat (formula 4)

Perhitungan presentase peredaman menggunakan rumus :

$$\text{Peredaman (\%)} = \frac{\text{absorbansi serapan larutan DPPH} - \text{absorbansi sampel}}{\text{absorbansi serapan larutan DPPH}} \times 100 \%$$

Peredaman replikasi 1

- 7,81 ppm = $\frac{0,862-0,659}{0,862} \times 100 \%$ = 23,55 %
- 15,62 ppm = $\frac{0,862-0,574}{0,862} \times 100 \%$ = 33,41 %
- 31,25 ppm = $\frac{0,862-0,498}{0,862} \times 100 \%$ = 42,23 %
- 62,5 ppm = $\frac{0,862-0,412}{0,862} \times 100 \%$ = 52,2%
- 125 ppm = $\frac{0,862-0,337}{0,862} \times 100 \%$ = 60,90 %
- 250 ppm = $\frac{0,862-0,261}{0,862} \times 100 \%$ = 69,72 %

Peredaman replikasi 2

- 7,81 ppm = $\frac{0,862-0,661}{0,862} \times 100 \%$ = 23,32 %
- 15,62 ppm = $\frac{0,862-0,576}{0,862} \times 100 \%$ = 33,18 %
- 31,25 ppm = $\frac{0,862-0,497}{0,862} \times 100 \%$ = 42,34 %
- 62,5 ppm = $\frac{0,862-0,410}{0,862} \times 100 \%$ = 52,44 %
- 125ppm = $\frac{0,862-0,339}{0,862} \times 100 \%$ = 60,67 %
- 250 ppm = $\frac{0,862-0,259}{0,862} \times 100 \%$ = 69,95 %

Peredaman replikasi 3

- 7,81 ppm = $\frac{0,862-0,658}{0,862} \times 100 \%$ = 23,67 %
- 15,62 ppm = $\frac{0,862-0,573}{0,862} \times 100 \%$ = 33,53 %
- 31,25 ppm = $\frac{0,862-0,496}{0,862} \times 100 \%$ = 42,46 %
- 62,5 ppm = $\frac{0,862-0,413}{0,862} \times 100 \%$ = 52,09 %
- 125ppm = $\frac{0,862-0,336}{0,862} \times 100 \%$ = 61,02 %
- 250 ppm = $\frac{0,862-0,262}{0,862} \times 100 \%$ = 69,61 %

aktivitas antioksidan				
	konsentrasi	Replikasi	absorbansi serapan larutan DPPH	absorbansi sampel
Nanofitosom F4	7,81	Replikasi 1	0,862	0,659
	15,62			0,574
	31,25			0,498
	62,5			0,412
	125			0,337
	250			0,261
	7,81	Replikasi 2		0,661
	15,62			0,576
	31,25			0,497
	62,5			0,410
	125			0,339
	250			0,259
	7,81	Replikasi 3		0,658
	15,62			0,573
	31,25			0,496
	62,5			0,413
	125			0,336
	250			0,262

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

	Konsentrasi	% peredaman	hasil regresilinier	IC ₅₀	Rata-rata ± SD
Nanofitosom F4	7,81	23,55	a = 33,2523 b = 0,1676 r = 0,8987	99,93	99,82 ± 0,16
	15,62	33,41			
	31,25	42,23			
	62,5	52,2			
	125	60,90			
	250	69,72			
	7,81	23,32	a = 33,1369 b = 0,1688 r = 0,8982	99,90	
	15,62	33,18			
	31,25	42,34			
	62,5	52,44			
	125	60,67			
	250	69,95			
	7,81	23,67	a = 33,3899 b = 0,1667 r = 0,8978	99,64	
	15,62	33,53			
	31,25	42,46			
	62,5	52,09			
	125	61,02			
	250	69,61			

Perhitungan IC₅₀ Askorbil Palmitat**IC₅₀ replikasi 1**

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = 33,2523$$

$$b = 0,1676$$

$$r = 0,8987$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = 33,2523 + 0,1676 x$$

$$x = 99,93$$

$$IC_{50} = 99,93 \text{ ppm}$$

IC₅₀ replikasi 2

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = 33,1369$$

$$b = 0,1688$$

$$r = 0,8982$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = 33,1369 + 0,1688 x$$

$$x = 99,90$$

$$IC_{50} = 99,90 \text{ ppm}$$

IC₅₀ replikasi 3

Hasil perhitungan regresi linier antara konsentrasi vs % peredaman

$$a = 33,3899$$

$$b = 0,1667$$

$$r = 0,8978$$

sehingga didapatkan persamaan : $y = a + bx$

$$50 = 33,3899 + 0,1667 x$$

$$x = 99,64$$

$$IC_{50} = 99,64 \text{ ppm}$$

Lampiran 22. Pengujian Aktivitas Antioksidan Zat Pembawa Nanofitosom

Pengujian aktivitas antioksidan zat pembawa nanofitosom dilakukan pembacaan pada 3 seri konsentrasi dan diamati kestabilan absorbansi yang diperoleh.

konsentrasi	Replikasi	Absorbansi	Rata-rata
250	1	1,051	1,051
	2	1,052	
	3	1,052	
125	1	1,050	1,051
	2	1,051	
	3	1,052	
62,5	1	1,051	1,051
	2	1,052	
	3	1,051	