BAB V
KESIMPULAN DAN SARAN

A. Kesimpulan

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

Pertama, *retinyl palmitate* dapat dibuat sediaan serum pada semua formula sesuai dengan karakterisasi sediaan.

Kedua, serum *retinyl palmitate* dengan berbagai variasi konsentrasi sodium glukonat tidak stabil pada pengamatan visual selama proses penyimpanan.

B. Saran

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

Pertama, perlu dilakukan uji difusi sel menggunakan membran biologis untuk mengetahui serum *retinyl palmitate* yang dapat berpenetrasi ke dalam kulit

Kedua, perlu dilakukan uji DPPH sediaan serum untuk mengetahui serum *retinyl palmitate* mempunyai aktivitas antioksidan.

Ketiga, pengembangan sediaan serum *retinyl palmitate* dalam bentuk nanopartikel dan enkapuslasi.

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LAMPIRAN

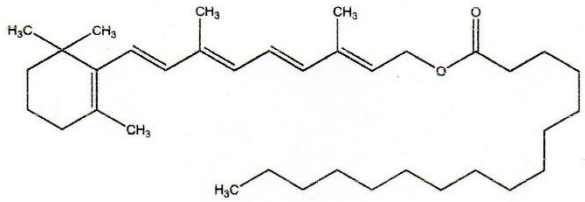
Lampiran 1. Sertifikat analisis retinyl palmitate

Certificate of Analysis

ISO GUIDE 34
ANAB Cert# AR-1470

ISO/IEC 17025
ANAB Cert# AT-1467

RETINYL PALMITATE
(Vitamin A Palmitate)
CERTIFIED REFERENCE MATERIAL



CERTIFIED PURITY: 99.7%, $U_{crm} = \pm 0.1\%$ k = 2
(Mass Balance/as is basis)

CERTIFIED ACTIVITY: 1756000IU/g, $U_{crm} = \pm 8297$IU/g k = 2.06
(UV Assay/as is basis)

NOMINAL PACKAGE SIZE: 1g

CATALOG #: PHR1235 **LOT #:** P500235

CERTIFICATE VERSION: 500235.4 **ISSUE DATE:** 19 May 2016
Note: Certificates may be updated due to Pharmacopeial Lot changes or the availability of new data.
Check our website at: www.sigma-aldrich.com *for the most current version.*

CRM EXPIRATION: 12 Months from Receipt (Proper Storage and Handling Required).

RECEIPT DATE: _____
Note: this space is provided for convenience only and its use is not required.

STORAGE: Store in a Refrigerator/Protect from Light, keep container tightly closed. Discard unused portions.

CHEMICAL FORMULA: $C_{36}H_{60}O_2$ **MW:** 524.9

SIGMA-ALDRICH

PHYSICAL DESCRIPTION: Pale yellow viscous oil in amber ampule **CAS #:** 79-81-2

HAZARDS: Read Safety Data Sheet before using. All chemical reference materials should be considered potentially hazardous and should be used only by qualified laboratory personnel.

INSTRUCTIONS FOR USE: Do not dry, use on the as is basis. The internal pressure of the container may be slightly different from the atmospheric pressure at the user's location. Open slowly and carefully to avoid dispersion of the material. This material is intended for R&D use only. Not for drug, household or other uses.

TRACEABILITY ASSAY

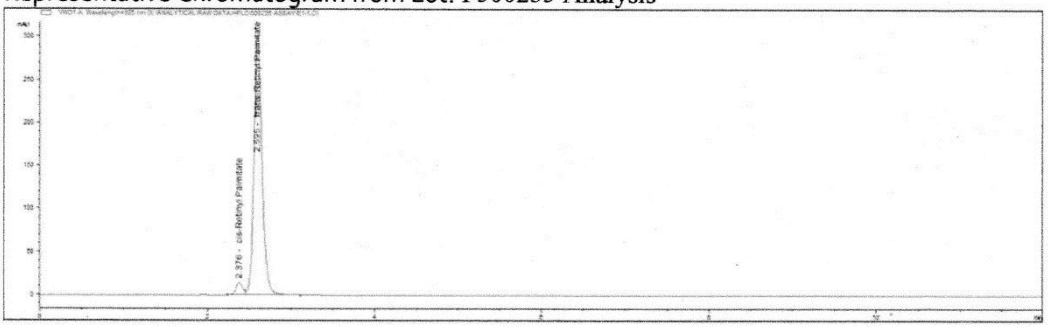
Comparative assay demonstrates direct traceability to Pharmacopeial Standards
Specification: None

METHOD: HPLC (ref.: Vitamin A Tablets, USP34)
Column: Supelcosil LC-NH_2, 4.6 x 150mm, 3µm
Mobile Phase: Hexane, Ethyl Acetate (98:2)
Flow Rate: 1mL/min
Column Temperature: 30°C
Injection: 2.5µL
Detector: 325nm

ASSAY vs. USP REFERENCE STANDARD (as is basis)

ASSAY VALUE	vs. USP LOT
98.7%	**F0J106**
	Labeled Content = 0.957mg/mg

Representative Chromatogram from Lot: P500235 Analysis



ACTIVITY DETERMINATION

METHOD: UV (ref.: Vitamin A Concentrate, EP7)
Solvent: Dissolve in pentane, dilute with isopropanol to ~15IU/g
Cell Pathlength: 1cm
Wavelength: 326nm

CERTIFIED ACTIVITY

1756000IU/g, U_{crm} = ±8297IU/g k = 2.06
(as is basis)

PURITY DETERMINATION BY MASS BALANCE

CHROMATOGRAPHIC IMPURITY ANALYSIS
METHOD: HPLC

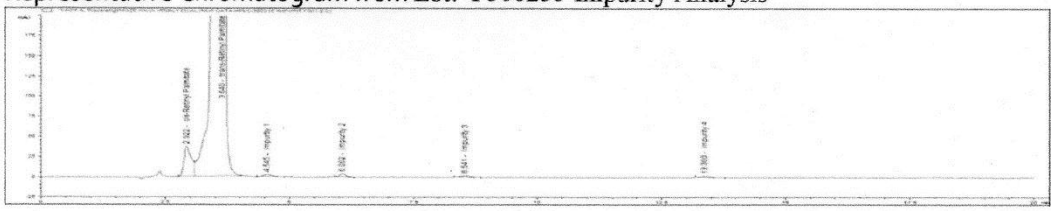
See Assay

Impurities Detected:

Impurity 1:	**0.1%**
Impurity 2:	**0.1%**
Impurity 3:	**0.07%**
Impurity 4:	**0.07%**

Total Impurities: **0.3%**

Representative Chromatogram from Lot: P500235 Impurity Analysis



RESIDUAL SOLVENTS
Method: GC-MS Headspace (ref.: Residual Solvents <467>, USP34)
Column: DB-1301
Carrier gas: He
Flow: 1.2mL/min
Split Ratio: 1:5
Injection/Temperature: 1µl/250°C
Temperature Program: 40°C for 20min, 10°C/min to 240°C, hold 20min

Solvents Detected: None

WATER DETERMINATION
Method: Karl Fisher titration
Mean of three measurements, Water Content = **0.002%**

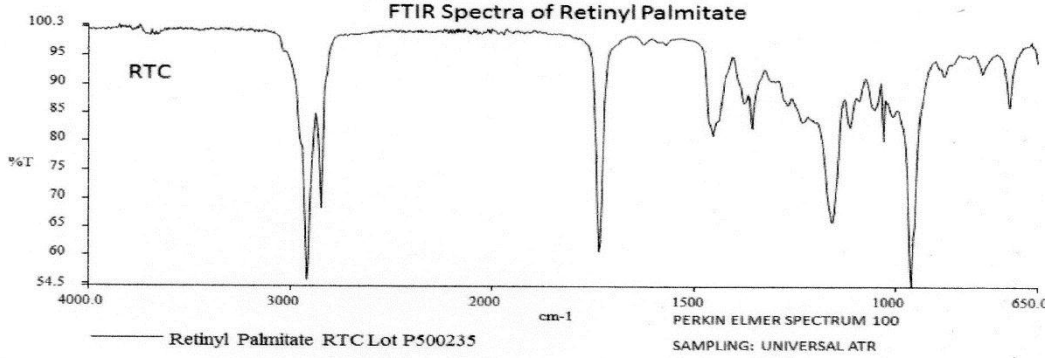
RESIDUE ANALYSIS
Method: Sulfated Ash
Sample Size: ~1g
Mean of three measurements, Residue = **0.02%**

CERTIFIED PURITY BY MASS BALANCE [100% - Impurities (normalized)]

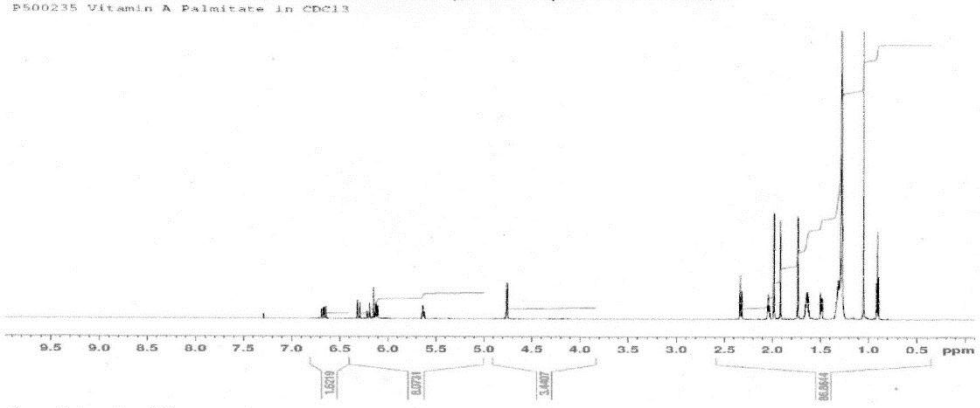
99.7% $U_{crm} = \pm 0.1\%$, k = 2
(as is basis)

IDENTIFICATION TESTS

INFRARED SPECTROPHOTOMETRY (This IR is for informational purposes only and shows good correlation to spectrum found in literature.)



^{1}H NMR (Data provided by an external laboratory; not in scope of accreditation)



Consistent with structure

ELEMENTAL ANALYSIS (Data provided by an external laboratory; not in scope of accreditation)
Exeter Analytical 440 Elemental Analyzer
Combustion method

%	Theoretical	Result 1	Result 2	Mean
C	82.38	82.36	80.47	81.42
H	11.52	11.49	11.15	11.32

HOMOGENEITY ASSESSMENT
Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

Analytical Method: HPLC Sample size: ~30mg

UNCERTAINTY STATEMENT
Uncertainty values in this document are expressed as Expanded Uncertainty (U_{crm}) corresponding to the 95% confidence interval. U_{crm} is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a *t*-distribution and degrees of freedom. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

STABILITY ASSESSMENT
Significance of the stability assessment will be demonstrated if the analytical result of the study and the range of values represented by the Expanded Uncertainty do not overlap the result of the original assay and the range of its values represented by the Expanded Uncertainty. The method employed will usually be the same method used to characterize the assay value in the initial evaluation.
Long Term Stability Evaluation - An assessment, or re-test, versus a Compendial Reference Standard may be scheduled, within the 3 year anniversary date of a release of a Secondary Standard. The re-test interval will be determined on a case-by-case basis.
Short Term Stability Study - It is useful to assess stability under reasonably anticipated, short term transport conditions by simulating exposure of the product to humidity and temperature stress. This type of study is conducted under controlled conditions of elevated temperature and humidity.

Operations Manager

QA Supervisor

APPENDIX

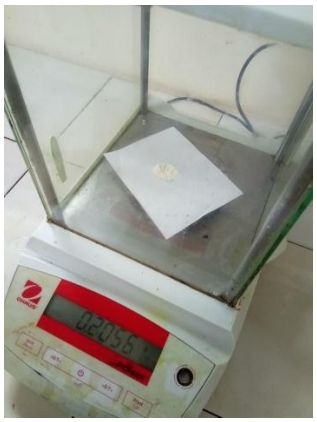
Original Release Date: 31 May 2012
Stability Test Date: 18 May 2015

Manufactured and certified by Sigma-Aldrich RTC, Inc.
2931 Soldier Springs Rd, Laramie WY, USA 82070
(Phone): 1-307-742-5452 (Fax): 1-855-831-9212
email: RTCTechGroup@sial.com

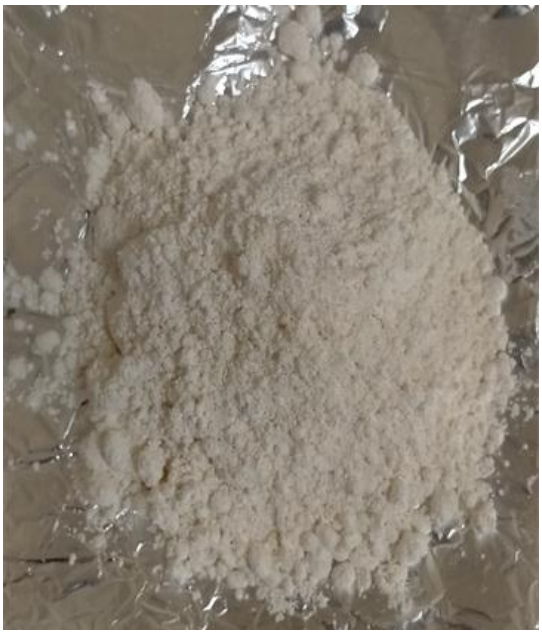
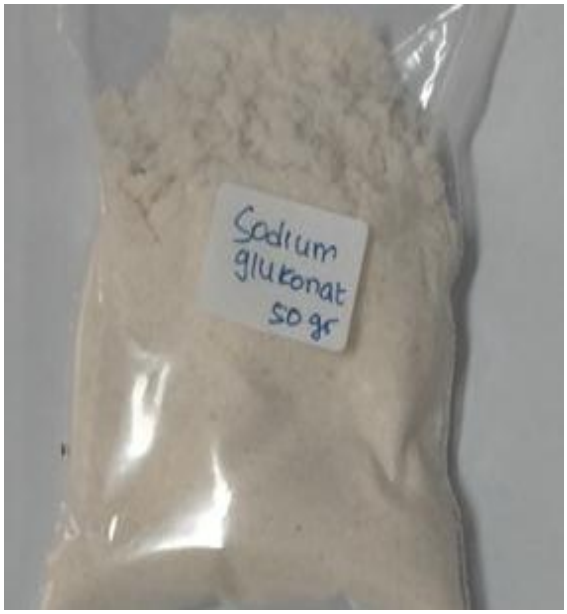
ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

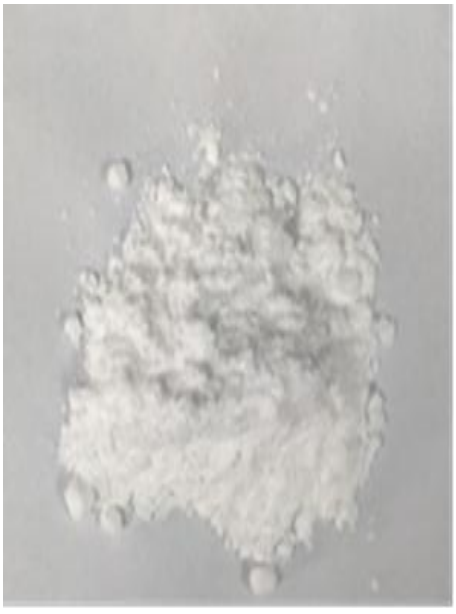
ANAB ACCREDITED ISO GUIDE 34 REFERENCE MATERIAL PRODUCER

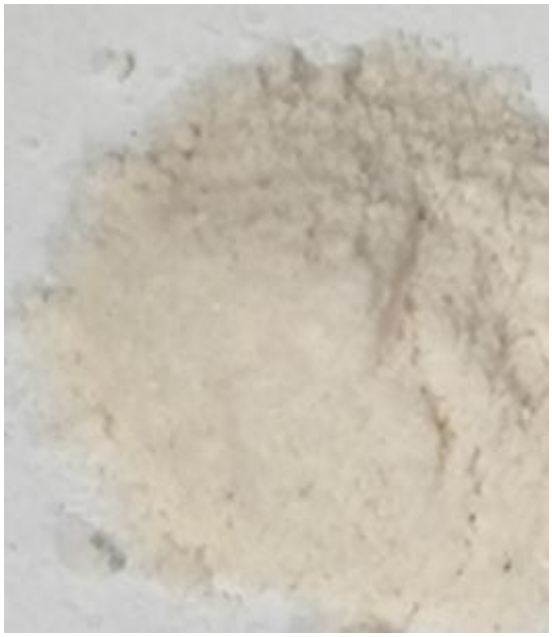
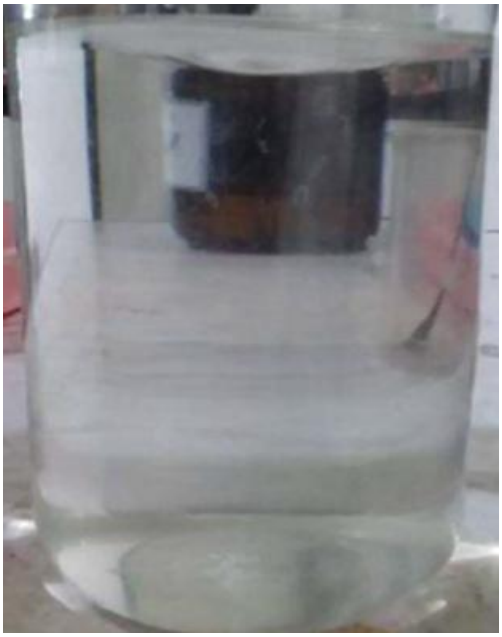
Lampiran 2. Alat dalam praktikum

Alat praktikum	
 **Neraca analitik**	 **Alat setirer**
 **Spektrofotometri Uv-Vis**	 **Pipet mikro**
 **Pipet volume**	 **Viskositas**

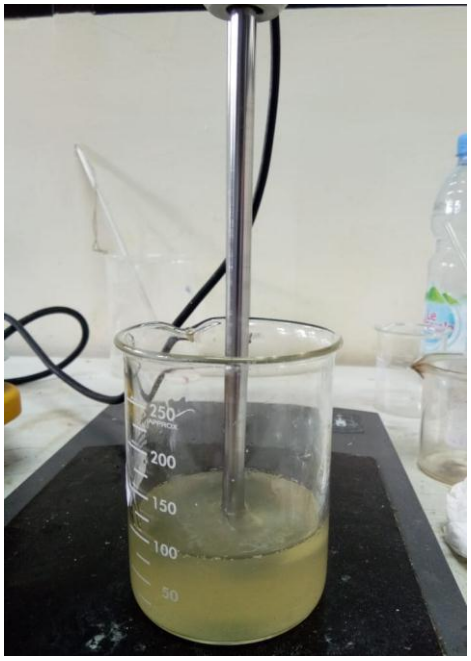
Lampiran 3. Bahan serum *retinyl palmitate*

<table>
<tr><th colspan="2">Bahan serum</th></tr>
<tr><td> 
Retinyl palmitate</td><td> 
Gliserin</td></tr>
<tr><td> 

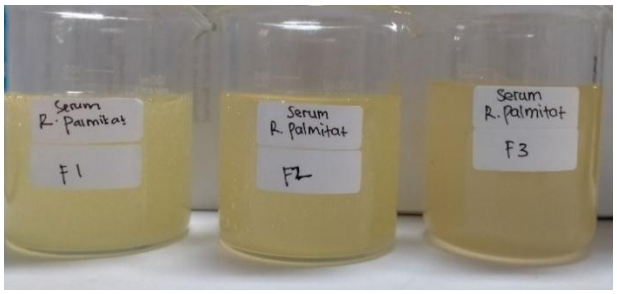
Sodium glukonat</td><td> 
Hyaluronic acid</td></tr>
</table>

 **Asam glikolat**	 **Optiphen**
 **Xanthan gum**	 **Aquadest**

Lampiran 4. Pembuatan serum *retinyl palmitate*

Pengembangan *hyaluronic acid*	Pengembangan xanthan gum
	
Pelarutan sodium glukonat dengan aquadest	Pencampuran menggunakan alat setirer
	

Lampiran 5. Hasil gambar karakterisasi serum *retinyl palmitate*

Uji Organoleptis

Uji Viskositas

Uji pH


Lampiran 6. Data uji viskositas dan *p*H

a.Viskositas

Formula	Replikasi	Hari ke-1	Hari ke-7	Hari ke-14	Hari ke-21
1	1	1000	750	650	600
	2	1100	850	700	550
	3	1000	800	750	650
	Rata-rata	1033	800	700	600
	SD	57,74	50	50	50
2	1	1000	750	650	600
	2	1000	800	600	500
	3	1100	750	550	550
	Rata-rata	1033	767	600	550
	SD	57,74	28,87	50	50
3	1	1000	700	550	450
	2	900	650	500	500
	3	800	600	500	400
	Rata-rata	900	650	517	450
	SD	100	50	28,87	50

b. *p*H

Formula	Replikasi	Hari ke-1	Hari ke-7	Hari ke-14	Hari ke-21
1	1	5,96	5,97	6,03	6,1
	2	5,96	5,98	6,05	6,12
	3	5,99	5,97	6,04	6,1
	Rata-rata	5,97	5,97	6,04	6,11
	SD	0,02	0,01	0,01	0,01
2	1	6,16	5,99	6,23	6,28
	2	6,14	6,07	6,2	6,22
	3	6,17	6,09	6,26	6,24
	Rata-rata	6,16	6,05	6,23	6,25
	SD	0,02	0,05	0,03	0,03
3	1	6,24	6,36	6,32	6,33
	2	6,27	6,31	6,28	6,36
	3	6,29	6,22	6,24	6,35
	Rata-rata	6,27	6,3	6,28	6,35
	SD	0,03	0,07	0,04	0,02

Lampiran 7. Data analisis *one way* ANOVA

a. Viskositas

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Hari	36	2,5000	1,13389	1,00	4,00
Formula	36	2,0000	,82808	1,00	3,00
Viskositas	36	713,8889	196,98119	400,00	1100,00

One-Sample Kolmogorov-Smirnov Test

		Hari	Formula	Viskositas
N		36	36	36
Normal Parameters[a,b]	Mean	2,5000	2,0000	713,8889
	Std. Deviation	1,13389	,82808	196,98119
Most Extreme Differences	Absolute	,170	,220	,127
	Positive	,170	,220	,127
	Negative	-,170	-,220	-,121
Kolmogorov-Smirnov Z		1,022	1,318	,763
Asymp. Sig. (2-tailed)		,247	,062	,605

a. Test distribution is Normal.

b. Calculated from data.

Test of Homogeneity of Variances

Viskositas

Levene Statistic	df1	df2	Sig.
,289	2	33	,751

ANOVA

Viskositas

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	146805,556	2	73402,778	2,000	,151
Within Groups	1211250,000	33	36704,545		
Total	1358055,556	35			

b. *p*H

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Hari	36	2.5000	1.13389	1.00	4.00
Formula	36	2.0000	.82808	1.00	3.00
pH	36	6.1628	.13199	5.96	6.36

One-Sample Kolmogorov-Smirnov Test

		Hari	Formula	pH
N		36	36	36
Normal Parameters[a,b]	Mean	2.5000	2.0000	6.1628
	Std. Deviation	1.13389	.82808	.13199
Most Extreme Differences	Absolute	.170	.220	.140
	Positive	.170	.220	.099
	Negative	-.170	-.220	-.140
Kolmogorov-Smirnov Z		1.022	1.318	.839
Asymp. Sig. (2-tailed)		.247	.062	.481

a. Test distribution is Normal.

b. Calculated from data.

Test of Homogeneity of Variances

pH

Levene Statistic	df1	df2	Sig.
1.738	2	33	.192

ANOVA

pH

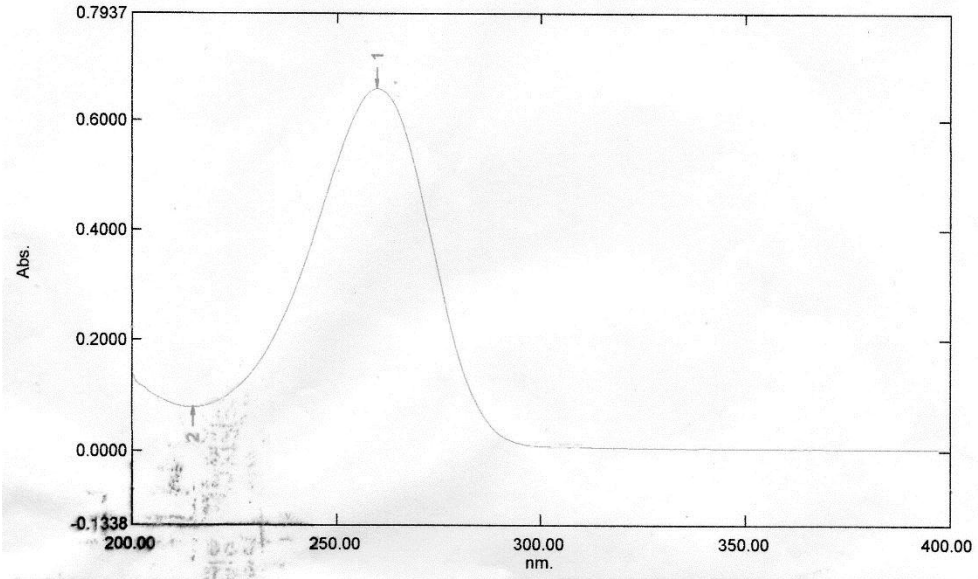
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.463	2	.232	52.164	.000
Within Groups	.147	33	.004		
Total	.610	35			

Lampiran 8. Hasil penentuan panjang gelombang maksimum *retinyl palmitate*

Spectrum Peak Pick Report

03/12/2020 02:33:36 PM

Data Set: File_200312_142730 - RawData



No.	P/V	Wavelength	Abs.	Description
1	↑	260.00	0.6576	
2	↓	215.00	0.0782	

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 400.00
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:
Volume:
Dilution:
Path Length:
Additional Information:

Lampiran 9. Hasil penetapan *operating time*

Kinetics Data Print Report

03/13/2020 09:07:44 AM

Time (Minute)	RawData ...
0.000	0.663
1.000	0.662
2.000	0.663
3.000	0.663
4.000	0.662
5.000	0.663
6.000	0.662
7.000	0.662
8.000	0.662
9.000	0.663
10.000	0.663
11.000	0.662
12.000	0.663
13.000	0.663
14.000	0.663
15.000	0.662
16.000	0.662
17.000	0.662
18.000	0.661
19.000	0.661
20.000	0.662
21.000	0.662
22.000	0.661
23.000	0.662
24.000	0.661
25.000	0.661
26.000	0.662
27.000	0.661
28.000	0.661
29.000	0.661
30.000	0.661

Lampiran 10. Pembuatan kurva kalibrasi dan validasi metode analisis

a. Kurva kalibrasi (Linieritas)

- Membuat larutan induk sebesar 103 ppm dengan menimbang 10,3 mg *retinyl palmitate* ditambahkan etanol *p.a* sampai 100 ml.

  Penimbangan *retinyl palmitate* :

  Kertas kosong + isi : 0,2864 g

  Kertas + sisa : 0,2761 g _

  Serbuk *retinyl palmitate* : 0,0103g

- Perhitungan seri konsentrasi

1. V1 x C1 = V2 x C2

   2,1 mL x 103 ppm = 10 mL x ppm

   C2 = 21,63 ppm

2. V1 x C1 = V2 x C2

   3,1 mLx 103 ppm = 10 mL x ppm

   C2 = 31,93 ppm

3. V1 x C1 = V2 x C2

   4,1 mL x 103 ppm = 10 mL x C2

   C2 = 42,23 ppm

4. V1 x C1 = V2 x C2

   5,1 mL x 103 ppm = 10 mL x C2

   C2 = 52,53 ppm

5. V1 x C1 = V2 x C2

   6,1 mL x 103 ppm = 10 mL x ppm

   C2 = 62,83 ppm

Kadar (ppm)	Absorbansi
21,63	0,234
31,93	0,374
42,23	0,492
52,53	0,621
62,83	0,761

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

a = -0,0314

b = 0,0125

r = 0,9993

y = a + bx

y = -0,0314 + 0,0125x

keterangan :

x = konsentrasi (µg/ml)

y = serapan

Hasil linearitas diperoleh r = 0,9993, sehingga dapat disimpulkan bahwa data linear baik mendekati 1.

b. Akurasi

Konsentrasi	Replikasi	Abs	Konsentrasi (ppm)	ppm sebenarnya	%	Rata-rata	Recovery
80%	1	0,489	41,5513	42,23	98%	98,08%	99,53%
	2	0,487	41,3916	42,23	98%		
	3	0,486	41,3118	42,23	98%		
100%	1	0,620	52,0110	52,53	99%	99,57%	
	2	0,626	52,4901	52,53	100%		
	3	0,625	52,4102	52,53	100%		
120%	1	0,766	63,6684	62,83	101%	100,95%	
	2	0,763	63,4288	62,83	101%		
	3	0,760	62,1893	62,83	101%		

Rumus konsentrasi ppm : $\frac{[Abs-(a)]}{b}$

- **Konsentrasi 80 %**
  - Konsentrasi ppm : $\frac{[0,489-(-0,0314)]}{0,0125}$ = 41,5513 ppm

- Konsentrasi ppm : $\frac{[0,487-(-0,0314)]}{0,0125}$ = 41,3916 ppm
- Konsentrasi ppm : $\frac{[0,486-(-0,0314)]}{0,0125}$ = 41,3118 ppm

❖ **Konsentrasi 100 %**

- Konsentrasi ppm : $\frac{[0,620-(-0,0314)]}{0,0125}$ = 52,0110 ppm
- Konsentrasi ppm : $\frac{[0,626-(-0,0314)]}{0,0125}$ = 52,4901 ppm
- Konsentrasi ppm : $\frac{[0,625-(-0,0314)]}{0,0125}$ = 52,4102 ppm

❖ **Konsentrasi 120%**

- Konsentrasi ppm : $\frac{[0,766-(-0,0314)]}{0,0125}$ = 63,6684 ppm
- Konsentrasi ppm : $\frac{[0,763-(-0,0314)]}{0,0125}$ = 63,4288 ppm
- Konsentrasi ppm : $\frac{[0,760-(-0,0314)]}{0,0125}$ = 63,1893 ppm

Rumus recovery : % Recovery = $\frac{konsentrasi\ terukur}{konsentrasi\ sebenarnya}$ × 100%

➢ **Konsentrasi 42,23 ppm**

- % Recovery = $\frac{41,5513}{42,23} X100\%$ = 98%
- % Recovery = $\frac{41,3916}{42,23} X100\% = 98\%$
- % Recovery = $\frac{41,3118}{42,23} X100\% = 98\%$

➢ **Konsentrasi 52,53 ppm**

- % Recovery = $\frac{52,0110}{52,53} X100\% = 99\%$
- % Recovery = $\frac{52,4901}{52,53} X100\% = 100\%$
- % Recovery = $\frac{51,4102}{52,53} X100\% = 100\%$

➢ **Konsentrasi 62,83 ppm**

- % Recovery = $\frac{63,6684}{62,83} X100\% = 101\%$
- % Recovery = $\frac{63,4288}{63,83} X100\% = 101\%$
- % Recovery = $\frac{63,1893}{63,83} X100\% = 101\%$

Nilai rata- rata % Recovery diatas adalah 99,53%, hal ini menunjukkan nilai persen perolehan kembali yang baik.

c. Presisi

Konsentrasi	Absorbansi	Konsentrasi (ppm)
21,63	0,254	22,7878
21,63	0,258	23,1071
21,63	0,267	23,8257
21,63	0,264	23,5862
21,63	0,256	22,9474
21,63	0,268	23,9056
21,63	0,258	23,1071
21,63	0,253	22,7079
21,63	0,269	23,9854
21,63	0,268	23,9056
	RATA-RATA	**23,3866**
	SD	**0,505334**
	CV	**0,021608%**

Rumus konsentrasi ppm : $\frac{[Abs-(a)]}{b}$

1. Konsentrasi ppm : $\frac{[0,254-(-0,0314)]}{0,0125}$ = 22,7878 ppm

2. Konsentrasi ppm : $\frac{[0,258-(-0,0314)]}{0,0125}$ = 23,1071 ppm

3. Konsentrasi ppm : $\frac{[0,267-(-0,0314)]}{0,0125}$ = 23,8257 ppm

4. Konsentrasi ppm : $\frac{[0,264-(-0,0314)]}{0,0125}$ = 23,5862 ppm

5. Konsentrasi ppm : $\frac{[0,256-(-0,0314)]}{0,0125}$ =22,9474 ppm

6. Konsentrasi ppm : $\frac{[0,268-(-0,0314)]}{0,0125}$ = 23,9056 ppm

7. Konsentrasi ppm : $\frac{[0,258-(-0,0314)]}{0,0125}$ = 23,1071 ppm

8. Konsentrasi ppm : $\frac{[0,253-(-0,0314)]}{0,0125}$ = 22,7079 ppm

9. Konsentrasi ppm : $\frac{[0,269-(-0,0314)]}{0,0125}$ = 23,9854 ppm

10. Konsentrasi ppm : $\frac{[0,268-(-0,0314)]}{0,0125}$ = 23,9056 ppm

Nilai CV dilihat dari data diatas adalah 0,021608%, hasil ini sesuai dengan pernyataan bahwa syarat presisi adalah kurang dari 2%.

Lampiran 11. Hasil data uji stabilitas penyimpanan

Formula	Siklus	Pada suhu dingin (4°C) dan suhu panas (40°C)		
		Bentuk	**Warna**	**Bau**
1	**1**	Gel encer	Kuning bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*
1	**2**	Gel encer	Kuning bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*
1	**3**	Gel encer	Kuning bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*
1	**4**	Gel encer	Putih bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*
1	**5**	Gel encer	Putih bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*
1	**6**	Gel encer	Putih bening	Khas *retinyl palmitate*
2		Gel encer	Kuning bening	Khas *retinyl palmitate*
3		Gel encer	Kuning bening	Khas *retinyl palmitate*