

## DAFTAR PUSTAKA

- Acton, Q.A. 2013. Xanthophylls—Advances in Research and Application: 2013 Edition: *ScholarlyPaper*. ScholarlyEditions.
- Agrios, G.N. 2005. Plant diseases caused by parasitic higher plants, invasive climbing plants, and parasitic green algae. *Plant Pathology*. (5<sup>a</sup> Ed.). San Diego. California. USA. *Elsevier Academic Press*. pp.705-722.
- Ahuja, S., & Dong, M. (Eds.). 2005. *Handbook of pharmaceutical analysis by HPLC*. Elsevier.
- Alves, C., Pinteus, S., Simoes, T., Horta, A., Silva, J., Tecelao, C. and Pedrosa, R. 2016. Bifurcaria bifurcata: a key macro-alga as a source of bioactive compounds and functional ingredients. *International Journal of Food Science & Technology*. 51(7). pp.1638-1646.
- Balasubramaniam, V., Chelyn, L.J., Vimala, S., Fairulnizal, M.M., Brownlee, I.A. and Amin, I. 2020. Carotenoid composition and antioxidant potential of Eucheuma denticulatum. *Sargassum polycystum* and *Caulerpa lentillifera*. *Helijon*. 6(8).
- Barbalace M.C. dkk. 2019. Anti-Inflammatory Activity of Marine Algae in Neurodegenerative Disease. *International Journal of Molecular Sciences*. 20(3061).
- Brunelle, R. 2003. *Advances In the Forensic Analysis And Dating of Writing Ink*. Charles C. Thomas.
- Camel, V. 2000. Microwave-assisted solvent extraction of environmental samples. *TrAC Trends in Analytical Chemistry*. 19(4). 229-248.
- Chuyen, H. V., & Eun, J. B. 2017. Marine carotenoids: Bioactivities and potential benefits to human health. *Critical reviews in food science and nutrition*. 57(12). 2600-2610.
- Endarini, L.H. 2016. *Farmakognosi dan Fitokimia*. Kementerian Kesehatan Republik Indonesia. Jakarta.
- Ermer, J., J. H. McB. Miller. 2005. *Method Validation in Pharmaceutical Analysis: A Guide to Best Practice* (Eds). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
- Firdaus M. 2019. *Pigmen Rumput Laut dan Manfaat Kesehatannya*. Malang: UB Press.

- Gandjar, G. I., dan Rohman, A. 2014. *Kimia Farmasi Analisis*. Pustaka Belajar, Yogyakarta.
- Garcia-Perez, P., Lourenço-Lopes, C., Silva, A., Pereira, A. G., Fraga-Corral, M., Zhao, C., ... & Prieto, M. A. 2022. Pigment composition of nine brown algae from the Iberian northwestern coastline: influence of the extraction solvent. *Marine Drugs*. 20(2). 113.
- Gholib, Ibnu. (2018). *Kimia Analisis Farmasi*. Universitas Gadjah Mada Yogyakarta. Pustaka Pelajar.
- Grinberg, N. and Rodriguez, S. 2019. *Ewing's Analytical Instrumentation Handbook*. Fourth Edition. CRC Press.
- Haminiuk, C. W., Maciel, G. M., Plata-Oviedo, M. S., & Peralta, R. M. 2012. Phenolic compounds in fruits—an overview. *International Journal of Food Science & Technology*. 47(10). 2024-2044.
- Hanyuda, T., Arai, S., Uchimura, M., Prathee, A., Draisma, S.G., Phang, S.M., Abbott, I.A., Millar, A.J. and Kawai, H. 2011. A taxonomic study of the genus *Padina* (Dictyotales, Phaeophyceae) including the descriptions of four new species from Japan, Hawaii and the Andamansea 1. *Journal of phycology*, 47(5), pp.1193-1209.
- Harmita. 2004. Petunjuk Pelaksanaan Validasi Metode dan Cara Perhitungannya. *Majalah Ilmu Kefarmasian*. I (3).
- Hastings, J., Owen, G., Dekker, A., Ennis, M., Kale, N., Muthukrishnan, V., Turner, S., Swainston, N., Mendes, P. and Steinbeck, C. 2016. ChEBI in 2016: Improved services and an expanding collection of metabolites. *Nucleic acids research*, 44(D1), pp. D1214-D1219.
- Heriyanto, Juliadiningtyas A.D, Shioi Y. Limantara L., Brotosudarmo T.H.P 2017. Analysis of Pigment Composition of Brown Seaweeds Collected from Panjang Island, Central Java, Indonesia. *Phillipine Journal of Science*. 143(3), pp. 323-330
- Hidayati J.R., Bahry M.S., Karlina I., Yudiat E. 2022. Antioxidant Activity and Bioactive Compounds of Tropical Brown Algae *Padina* sp. from Bintan Island, Indonesia. *Jurnal Kelautan Tropis*. 25(5), pp. 309-319
- Hidayati, Jelita Rahma., Ali Ridlo dan Rini Pramesti. 2017. Aktivitas Antioksidan Ekstrak Rumput Laut *Padina* sp. dari Perairan Bandengan Jepara Dengan Metode Transfer Elektron. *Buletin Osenografi Marina*, Vol 6 (1): 46 – 52.

- Higuera-Ciapara, I., Felix-Valenzuela, L. and Goycoolea, F.M. 2006. Astaxanthin: a review of its chemistry and applications. *Critical reviews in food science and nutrition.* 46(2), pp.185-196.
- Husni, A. and Budhiyanti, S.A. 2021. *Rumput Laut Sebagai Sumber Pangan, Kesehatan Dan Kosmetik.* UGM Press.
- ICH, 1994. *Text on Validation of Analytical Procedures: Q2A. Recommended for Adoption at Step 4 of the ICH Process.* International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use.
- Kanda, H., Kamo, Y., Machmudah, S., Wahyudiono and Goto, M. 2014. Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether. *Marine Drugs,* 12(5), pp.2383-2396.
- Kasanah, N., Seto, D.S. and Khotimah, H. 2022. *Rumput Laut Pangan: Kimia, Bioaktivitas, dan Toksisitas.* UGM PRESS.
- Kidd, P. 2011. Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. *Altern Med Rev.* 16(4), 355-364.
- Koduvayur Habeebullah, S.F., Surendraraj, A. and Jacobsen, C. 2018. Isolation of Fucoxanthin from Brown Algae and Its Antioxidant Activity: In Vitro and 5% Fish Oil-In-Water Emulsion. *Journal of the American Oil Chemists' Society.* 95(7), pp. 835–843.
- Komba, S., Kotake-Nara, E., & Machida, S. 2015. Fucoxanthin derivatives: Synthesis and their chemical properties. *Journal of Oleo Science.* 64(9), 1009-1018.
- Leba, M.A.U. 2017. *Ekstraksi dan Real Kromatografi.* Deepublish. Yogyakarta. 3 - 6.
- Li, J., Liu, Y., Liu, Y., Wang, Q., Gao, X., & Gong, Q. 2019. Effects of temperature and salinity on the growth and biochemical composition of the brown alga *Sargassum fusiforme* (Fucales, Phaeophyceae). *Journal of Applied Phycology.* 31(5), 3061–3068.
- Luthria, D. L. 2008. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. *Food chemistry.* 107(2), 745-752.
- Maharany F, Nurjanah, Suwandi R, Anwar E, H.T. 2017. Kandungan Senyawa Bioaktif Rumput Laut *Padina Australis* dan

- Eucheuma Cottonii Sebagai Bahan Baku Krim Tabir Surya. *Jphpi*, 20(1), pp. 10–17.
- Mukhriani. 2014. Ekstraksi, Pemisahan Senyawa dan Identifikasi Senyawa Aktif. *Jurnal Kesehatan*. 7. (2): 361 - 367.
- Muradian, K., Vaiserman, A., Min, K. J., & Fraifeld, V. E. 2015. Fucoxanthin and lipid metabolism: A minireview. *Nutrition, Metabolism and Cardiovascular Diseases*. 25(10), 891-897.
- Nasyanka, A.L., Na'imah, J., dan Aulia, R. 2020. *Pengantar Fitokimia D3 Farmasi 2020*. CV. Penerbit Qiara Media. Pasuruan, Jawa Timur. 31 - 41.
- Osbourn, A.E. and Lanzotti, V. 2009. *Plant-derived Natural Products: Synthesis, Function, and Application*. Springer New York.
- Pashkow, F. J., Watumull, D. G., & Campbell, C. L. 2008. Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *The American journal of cardiology*. 101(10), S58-S68.
- Pereira, A.G., Otero, P., Enhave, J., Carreira-Casais, A., Chamorro, F., Collazo, N., & Prieto, M.A. 2021. Xanthophylls from the Sea: Algae as Source of Bioactive Carotenoids. *Marine drugs*. 19(4), pp. 188
- Plaza, M., Santoyo, S., Jaime, L., Reina, G.G.B., Herrero, M., Señoráns, F.J. and Ibáñez, E. 2010. Screening for bioactive compounds from algae. *Journal of pharmaceutical and biomedical analysis*. 51(2), pp.450-455.
- Poole, C.F., 2011. Planar Chromatography. *Journal of chromatography*. 1218(19).
- Qin, Y. 2018. *Bioactive seaweeds for food applications: Natural ingredients for healthy diets, Bioactive Seaweeds for Food Applications: Natural Ingredients for Healthy Diets*. Elsevier Science.
- Rahman A., Ozkan S.A, Ahmed R. 2018. *Novel Developments in Pharmaceutical and Biomedical Analysis*. Bentham Science Publisher.
- Rao, A.R. and Ravishankar, G.A. 2022. *Sustainable Global Resources of Seaweeds Volume 2, Sustainable Global Resources of Seaweeds Volume 2*. Springer International Publishing.
- Sadvika I.G.A., Wulansari N.W.A., Suryaningsih N.P.E., & Mahendra A.N. 2022. Potensi Padina australis sebagai Marine Drug untuk

Aterosklerosis. *Smart Medical Journal.* 5(1), pp-1-10

Saloso, Y. A. Prajitno, A. L. Abadi & Alanni'am. 2011. Kajian Potensi Padina australis sebagai Antibakteri Alami dalam Pengendalian Bakteri Vibrio alginolisticus pada Budidaya Ikan Kerapu Tikus (Cromeleptus altivelis). *Jurnal Bahan Alam Indonesia.* (7). Pp. 365-369

Salosso, Y., Aisiah, S., Toruan, L. N. L., & Pasaribu, W. 2020. Nutrient content, active compound and antibacterial activity of Padina australis against Aeromonas hydrophila. *Pharmacognosy Journal.* 12(4), 771– 776.

Saputra, S.H. 2020. *Mikroemulsi Ekstrak Bawang Tiwai sebagai Pembawa Zat Warna, Antioksidan dan Antimikroba Pangan.* Deepublish. Yogyakarta.

Spigno, G., & De Faveri, D. M. 2009. Microwave-assisted extraction of tea phenols: A phenomenological study. *Journal of food engineering,* 93(2), 210-217.

Srivastava, M.M. 2010. *High-Performance Thin-Layer Chromatography (HPTLC).* Springer Berlin Heidelberg.

Waghmode, A.V., Narayankar, C.U., Nimbalkar, M.S. and Gaikwad, D.K. 2019. Exploration of fucoxanthin and astaxanthin in macro alga (*Sargassum sp.*) by high-performance liquid chromatography. *Indian Hydrobiol.* 18, pp.40-49.

Wewengkang, D.S., dan Rotinsulu, H. 2021. *Fitofarmaka.* Penerbit Lakeisha. Klaten. 7 - 18.

Wulandari, L. 2011. *Kromatografi Lapis Tipis.* Taman Kampus Presindo.

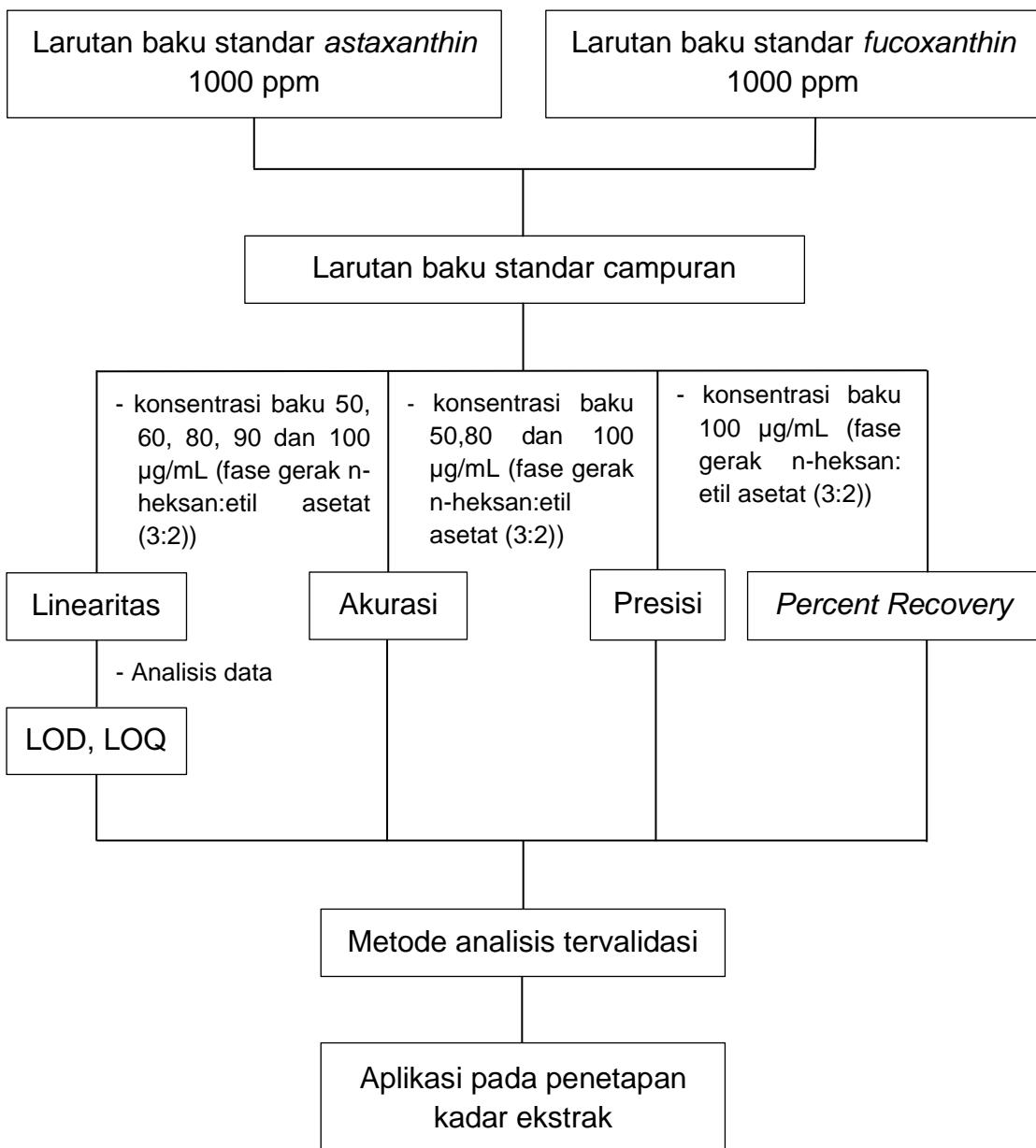
Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T. and Mori, N. 2011. Fucoxanthin and its deacetylated product, fucoxanthinol, induce apoptosis of primary effusion lymphomas. *Cancer letters.* 300(2), pp.225-234.

Zhang, Q.W., Lin, L.G., and Ye, W.C. 2018. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chinese Medicine.* 13. (1): 1 - 26.

## LAMPIRAN

### Lampiran 1. Skema Kerja Penelitian

Validasi Metode

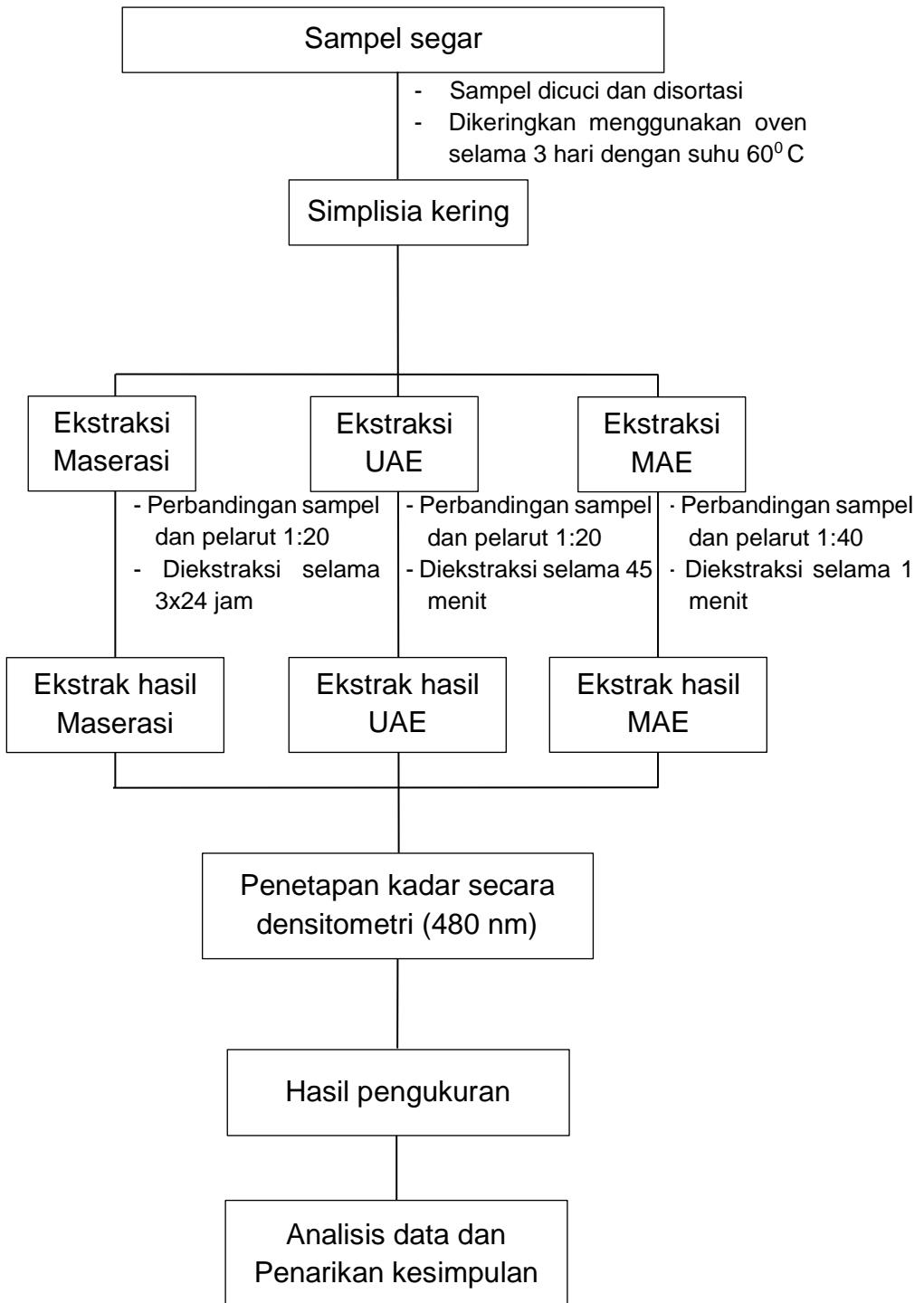


Daftar singkatan

LOD : *Limit of Detection*

LOQ : *Limit of Quantification*

### *Ekstraksi *Padina australis**



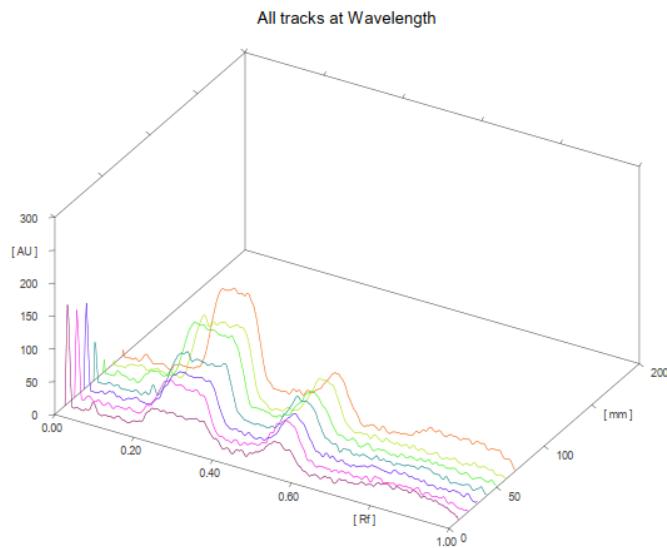
#### Daftar singkatan

UAE : *Ultrasonic Assisted Extraction*

MAE : *Microwave Assisted Extraction*

## Lampiran 2. Perhitungan

### 1. Linearitas



**Gambar 14. Densitogram linearitas**

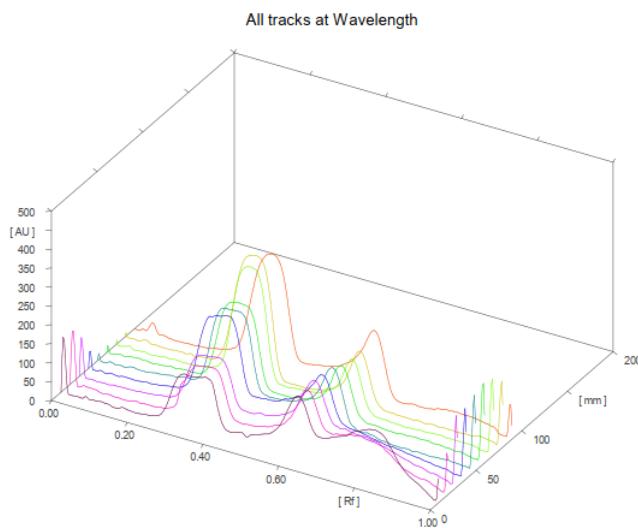
Baku *astaxanthin* dan *fucoxanthin* dibuat dalam konsentrasi 1000 µg/mL dengan menimbang masing-masing 1,0 mg baku dalam 1 mL *methanol*, lalu diencerkan menjadi konsentrasi 50,60,80,90 dan 100 µg/mL.

Konsentrasi baku <i>Astaxathin</i> (µg/mL)	AUC
50	1951,7
60	2455,3
80	3456,8
90	3744
100	4283,5

Konsentrasi baku <i>Fucoxathin</i> (µg/mL)	AUC
50	3619,3
60	4489,3
80	6344
90	7724,7
100	8652,6

## 2. Akurasi



**Gambar 15. Densitogram Akurasi**

Konsentrasi Baku	AUC	Konsentrasi Terdeteksi	% Recovery	Rata-Rata
50 µg/mL	2127,70	53,13	106,26	
	2085,40	52,21	104,42	102,19
	1889,70	47,95	95,90	
80 µg/mL	3495,00	82,89	103,61	
	3319,20	79,07	98,83	102,47
	3544,80	83,98	104,97	
100 µg/mL	5956,60	136,48	136,47	
	5211,20	120,25	120,25	127,19
	5423,00	124,86	124,86	
<hr/>				
Konsentrasi Baku	AUC	Konsentrasi Terdeteksi	% Recovery	Rata-Rata
50 µg/mL	3040,70	45,33	90,65	
	3262,10	47,50	95,00	94,94
	3474,80	49,59	99,17	
	6753,00	81,76	102,20	104,26

80 µg/mL	6600,10	80,26	100,32	
	7410,80	88,22	110,27	
	8322,20	97,16	97,16	
100 µg/mL	9436,70	108,10	108,10	105,55
	9773,00	111,40	111,40	

### A. Sampel astaxanthin

$$Y = 45,941x - 313,24$$

#### Perhitungan konsentrasi terdeteksi baku 50 µg/mL

$$X_1 = \frac{2127,7 + 313,24}{45,941} = 53,13$$

$$X_2 = \frac{2085,4 + 313,24}{45,941} = 52,21$$

$$X_3 = \frac{1889,7 + 313,24}{45,941} = 47,95$$

#### Perhitungan konsentrasi terdeteksi baku 80 µg/mL

$$X_1 = \frac{3495 + 313,24}{45,941} = 82,89$$

$$X_2 = \frac{3319,2 + 313,24}{45,941} = 79,06$$

$$X_3 = \frac{3544,8 + 313,24}{45,941} = 83,97$$

#### Perhitungan konsentrasi terdeteksi baku 100 µg/mL

$$X_1 = \frac{5956,6 + 313,24}{45,941} = 136,47$$

$$X_2 = \frac{5211,2 + 313,24}{45,941} = 120,25$$

$$X_3 = \frac{5423 + 313,24}{45,941} = 124,86$$

#### Perhitungan percent recovery

$$\text{percent recovery} = \frac{C_f}{C_a} \times 100\%$$

### **Perhitungan percent recovery baku 50 µg/mL**

$$\text{percent recovery } X1 = \frac{53,13206069}{50} \times 100\% = 106,26\%$$

$$\text{percent recovery } X2 = \frac{52,21131451}{50} \times 100\% = 104,42\%$$

$$\text{percent recovery } X3 = \frac{47,95150301}{50} \times 100\% = 95,90\%$$

### **Perhitungan percent recovery baku 80 µg/mL**

$$\text{percent recovery } X1 = \frac{82,89414684}{80} \times 100\% = 103,61\%$$

$$\text{percent recovery } X2 = \frac{79,06749962}{80} \times 100\% = 98,83\%$$

$$\text{percent recovery } X3 = \frac{83,97814588}{80} \times 100\% = 104,97\%$$

### **Perhitungan percent recovery baku 100 µg/mL**

$$\text{percent recovery } X1 = \frac{136,4759148}{100} \times 100\% = 136,47\%$$

$$\text{percent recovery } X2 = \frac{120,2507564}{100} \times 100\% = 120,25\%$$

$$\text{percent recovery } X3 = \frac{124,8610174}{100} \times 100\% = 124,86\%$$

### **B. Sampel fucoxanthin**

$$Y = 101,89x - 1577,8$$

### **Perhitungan konsentrasi terdeteksi baku 50 µg/mL**

$$X1 = \frac{3040,7 + 1577,8}{101,89} = 45,32$$

$$X2 = \frac{3262,1 + 1577,8}{101,89} = 47,50$$

$$X3 = \frac{3474,8 + 1577,8}{101,89} = 49,58$$

### **Perhitungan konsentrasi terdeteksi baku 80 µg/mL**

$$X1 = \frac{6753+1577,8}{101,89} = 81,76$$

$$X2 = \frac{6600,1+1577,8}{101,89} = 80,26$$

$$X3 = \frac{7410,8+1577,8}{101,89} = 88,21$$

### **Perhitungan konsentrasi terdeteksi baku 100 µg/mL**

$$X1 = \frac{8322,2+1577,8}{101,89} = 97,16$$

$$X2 = \frac{9436,7+1577,8}{101,89} = 108,10$$

$$X3 = \frac{9773+1577,8}{101,89} = 111,40$$

### **Perhitungan percent recovery**

$$\text{percent recovery} = \frac{C_f}{C_a} \times 100\%$$

### **Perhitungan percent recovery baku 50 µg/mL**

$$\text{percent recovery } X1 = \frac{45,32829522}{50} \times 100\% = 90,65\%$$

$$\text{percent recovery } X2 = \frac{47,50122681}{50} \times 100\% = 95,00\%$$

$$\text{percent recovery } X3 = \frac{49,58877221}{50} \times 100\% = 99,17\%$$

### **Perhitungan percent recovery baku 80 µg/mL**

$$\text{percent recovery } X1 = \frac{81,76268525}{80} \times 100\% = 102,20\%$$

$$\text{percent recovery } X2 = \frac{80,26204731}{80} \times 100\% = 100,32\%$$

$$\text{percent recovery } X3 = \frac{88,21866719}{80} \times 100\% = 110,27\%$$

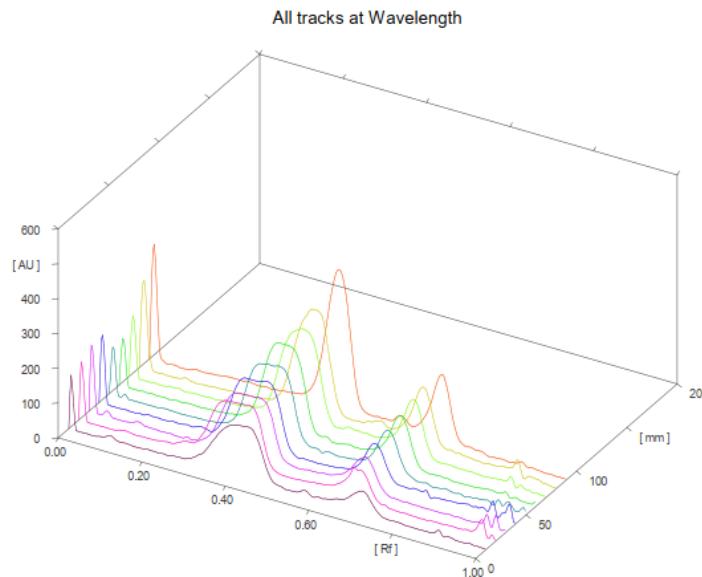
### **Perhitungan percent recovery baku 100 µg/mL**

$$\text{percent recovery } X1 = \frac{97,16360781}{100} \times 100\% = 97,16\%$$

$$\text{percent recovery } X2 = \frac{108,1018746}{100} \times 100\% = 108,10\%$$

$$\text{percent recovery } X3 = \frac{111,4024929}{100} \times 100\% = 111,40\%$$

### 3. Presisi



**Gambar 16. Densitogram Presisi**

Konsentrasi	Replikasi	AUC (X)	(X-Xi)	(X-Xi) <sup>2</sup>
100 µg/mL	1	4453,40	216,70	46958,89
	2	4360,40	123,70	15301,69
	3	4012,60	-224,10	50220,81
	4	3621,50	-615,20	378471,04
	5	3976,40	-260,30	67756,09
	6	4561,80	325,10	105690,01
<b>Rata-rata (Xi)</b>		4164,35	<b>Jumlah (X-Xi)<sup>2</sup></b>	664398,53
<b>SD</b>				364,53
<b>RSD</b>				8,75
Konsentrasi	Replikasi	AUC (X)	(X-Xi)	(X-Xi) <sup>2</sup>
100 µg/mL	1	8628,60	17,40	302,76
	2	8453,50	-157,70	24869,29

3	8672,20	61,00	3721,00
4	9455,80	844,60	713349,16
5	9624,60	1013,40	1026979,56
6	8402,30	-208,90	43639,21
<b>Rata-rata (Xi)</b>	<b>8872,83</b>	<b>Jumlah (X-Xi)<sup>2</sup></b>	<b>1812860,98</b>
	<b>SD</b>		<b>602,14</b>
	<b>RSD</b>		<b>6,79</b>

Analit pada matriks sampel 100 µg/mL

$$10 \text{ } \mu\text{g/mL} \text{ (untuk 0,001\%)} = 0,00001$$

$$\text{RSD} > 2^{(1-0.5 \log c)} \times 0,67$$

$$\text{RSD} > 2^{(1-0.5 \log 0,00001)} \times 0,67$$

$$\text{RSD} > 2^{(1-0.5(-3.5))} \times 0,67$$

$$\text{RSD} > 2^{3.5} \times 0,67$$

$$\text{RSD} > 7,58$$

Maka, untuk konsentrasi 100 µg/mL simpangan baku relatif yang diperbolehkan tidak boleh melebihi dari 7,58%.

#### **Perhitungan simpangan baku (SD) astaxanthin**

$$\text{SD} = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n-1}}$$

$$\text{SD} = \sqrt{\frac{664398.53}{6-1}}$$

$$\text{SD} = 364,52$$

#### **Perhitungan Simpangan Baku Relatif (RSD) astaxanthin**

$$RSD = \frac{SD}{x} \times 100\%$$

$$RSD = \frac{364,52}{4164,35} \times 100\%$$

$$RSD = 8,75\%$$

### Perhitungan simpangan baku (SD) *fucoxanthin*

$$SD = \sqrt{\frac{\sum (Xi - \bar{x})^2}{n-1}}$$

$$SD = \sqrt{\frac{1812860,98}{6-1}}$$

$$SD = 602,13$$

### Perhitungan Simpangan Baku Relatif (RSD) *astaxanthin*

$$RSD = \frac{SD}{x} \times 100\%$$

$$RSD = \frac{602,13}{8872,83} \times 100\%$$

$$RSD = 6,79\%$$

## 4. LOD dan LOQ

Konsentrasi	AUC (Y)	Y <sub>i</sub>	(Y <sub>i</sub> -Y)	(Y <sub>i</sub> -Y) <sup>2</sup>
50	1951,7	1983,81	32,11	1031,052
60	2455,3	2443,22	-12,08	145,9264
80	3456,8	3362,04	-94,76	8979,458
90	3744	3821,45	77,45	5998,502
100	4283,5	4280,86	-2,64	6,9696
<b>Jumlah (Y-Y<sub>i</sub>)<sup>2</sup></b>				<b>16161,91</b>

Konsentrasi	AUC (Y)	Y <sub>i</sub>	(Y <sub>i</sub> -Y)	(Y <sub>i</sub> -Y) <sup>2</sup>
50	3619,30	3516,70	-102,60	10526,76
60	4489,30	4535,60	46,30	2143,69
80	6344,00	6573,40	229,40	52624,36
90	7724,70	7592,30	-132,40	17529,76

100	8652,60	8611,20	-41,40	1713,96
<b>Jumlah (Y-Yi)<sup>2</sup></b>				84538,53

Persamaan Garis	Koefisien Determinasi	Simpangan Baku Residual	Batas Deteksi ( $\mu\text{g/ml}$ )	Batas Kuantitasi ( $\mu\text{g/ml}$ )
-----------------	-----------------------	-------------------------	------------------------------------	---------------------------------------

$y = 45,941x - 313,24$	0,9956	73,40	4,79	15,98
------------------------	--------	-------	------	-------

Persamaan Garis	Koefisien Determiasi	Simpangan Baku Residual	Batas Deteksi ( $\mu\text{g/ml}$ )	Batas Kuantitasi ( $\mu\text{g/ml}$ )
-----------------	----------------------	-------------------------	------------------------------------	---------------------------------------

$y = 101,89x - 1577,8$	0,9953	167,87	4,94	16,48
------------------------	--------	--------	------	-------

### Simpangan Baku Residual *astaxanthin*

$$Sy/x = \sqrt{\frac{\sum(Y_i - Y)^2}{n-2}}$$

$$Sy/x = \sqrt{\frac{16161,9082}{5-2}}$$

$$Sy/x = 73,39$$

$$LOD = \frac{3 \times Sy/x}{\text{Slope}}$$

$$LOD = \frac{3 \times 73,39}{45,94}$$

$$LOD = 4,79 \mu\text{g/mL}$$

$$LOQ = \frac{10 \times Sy/x}{\text{Slope}}$$

$$LOQ = \frac{10 \times 58,97}{45,941}$$

$$LOQ = 15,97 \mu\text{g/mL}$$

### Simpangan Baku Residual *fucoxanthin*

$$Sy/x = \sqrt{\frac{\sum(Y_i - Y)^2}{n-2}}$$

$$Sy/x = \sqrt{\frac{84538,53}{5-2}}$$

$$Sy/x = 167,86$$

$$LOD = \frac{3 \times Sy/x}{\text{Slope}}$$

$$LOD = \frac{3 \times 167,86}{101,89}$$

$$LOD = 4,94 \mu\text{g/mL}$$

$$LOQ = \frac{10 \times Sy/x}{\text{Slope}}$$

$$LOQ = \frac{10 \times 167,86}{101,89}$$

$$LOQ = 16,47 \mu\text{g/mL}$$

## 5. Rendemen ekstrak

Nama Sampel	Bobot Simplisia (g)	Bobot Ekstrak (g)	Rendemen (%)
Maserasi	10,01	0,69	6,89
UAE	10,03	1,14	11,36
MAE	10,03	0,46	4,59

Ket:

UAE : Ultrasonic Assisted Extraction

MAE : Microwave Assisted Extraction

$$\text{Rendemen (\%)} \text{ hasil maserasi} = \frac{\text{Bobot akhir ekstrak (g)}}{\text{Bobot awal simplisia}} \times 100\%$$

$$= \frac{0,69 \text{ gram}}{10,01} \times 100\%$$

$$= 6,89\%$$

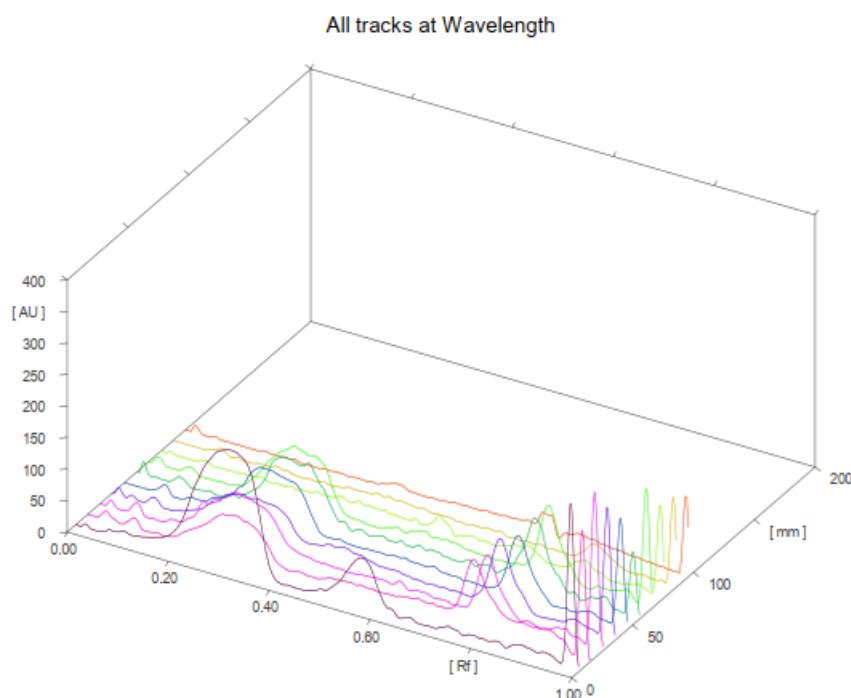
$$\text{Rendemen (\%)} \text{ hasil UAE} = \frac{\text{Bobot akhir ekstrak (g)}}{\text{Bobot awal simplisia}} \times 100\%$$

$$= \frac{1,14 \text{ gram}}{10,03} \times 100\%$$

$$= 11,36\%$$

$$\begin{aligned} \text{Rendemen (\%)} \text{ hasil MAE} &= \frac{\text{Bobot akhir ekstrak (g)}}{\text{Bobot awal simpisia}} \times 100\% \\ &= \frac{0,46 \text{ gram}}{10,03} \times 100\% \\ &= 4,59\% \end{aligned}$$

## 6. Hasil Perhitungan Penetapan Kadar



**Gambar 17. Densitogram kadar astaxanthin dan fucoxanthin**

Metode Ekstraksi	Persen rendemen (%)	AUC	Kadar astaxanthin (mg/g)	Rata-rata ± SD
Maserasi	6,89	-	-	-
UAE	11,36	-	-	-

MAE	4,59	-	-	-
		-	-	-
Metode Ekstraksi	Persen rendamen (%)	AUC	Kadar <i>fucoxanthin</i> (mg/g)	Rata-rata ± SD
Maserasi	6,89	2726,8 2538,2 2512,1	0,0654 0,0625 0,0621	0,63 ± 0,01
UAE	11,36	4069,8 4222,1 4183,6	0,0859 0,0882 0,087	0,87 ± 0,009
MAE	4,59	2790,7 2950,2 2665,5	0,0645 0,0664 0,0688	0,66 ± 0,017

### Perhitungan kadar *astaxanthin*

Persamaan linearitas  $y = 45,941x - 313,24$

Konsentrasi sampel = 10 mg/mL

### Perhitungan kadar *fucoxanthin*

Persamaan linearitas  $y = 101,89x - 1557,8$

Konsentrasi sampel = 10 mg/mL

$$\% \text{ Kadar} = \frac{\text{Konsentrasi fucoxanthin } (\mu\text{g/mL})}{\text{Konsentrasi sampel } (\mu\text{g/mL})} \times 100\%$$

### Ekstrak hasil maserasi replikasi 1

$$\text{Konsentrasi fucoxanthin} = \frac{2726,8 + 1557,8}{101,89} = 65,43 \text{ } \mu\text{g/mL}$$

$$\% \text{ Kadar} = \frac{65,43 \text{ } \mu\text{g/mL}}{10.000 \text{ } \mu\text{g/mL}} \times 100\%$$

$$= 0,65\%$$

$$= 0,0654 \text{ mg/g}$$

### **Ekstrak hasil maserasi replikasi 2**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{2538,2+1557,8}{101,89} = 62,55 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{62,55 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,625\% \\
 &= 0,0625 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi replikasi 3**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{2512,1+1557,8}{101,89} = 62,16 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{62,16 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,621\% \\
 &= 0,0621 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi UAE 1**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{4069,8+1557,8}{101,89} = 85,95 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{85,95 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,859\% \\
 &= 0,0859 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi UAE 2**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{4222,1+1557,8}{101,89} = 88,27 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{88,27 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,882\% \\
 &= 0,0882 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi UAE 3**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{4183,6+1557,8}{101,89} = 87,68 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{87,68 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,876\% \\
 &= 0,0876 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi MAE 1**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{2665,5+1557,8}{101,89} = 64,50 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{64,50 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,645\% \\
 &= 0,0645 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi MAE 2**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{2790,7+1557,8}{101,89} = 66,41 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{66,41 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,664\% \\
 &= 0,0664 \text{ mg/g}
 \end{aligned}$$

### **Ekstrak hasil maserasi MAE 3**

$$\begin{aligned}
 \text{Konsentrasi } fucoxanthin &= \frac{2950,2+1557,8}{101,89} = 68,85 \mu\text{g/mL} \\
 \% \text{ Kadar} &= \frac{82,27 \mu\text{g/mL}}{10.000 \mu\text{g/mL}} \times 100\% \\
 &= 0,688\% \\
 &= 0,0688 \text{ mg/g}
 \end{aligned}$$

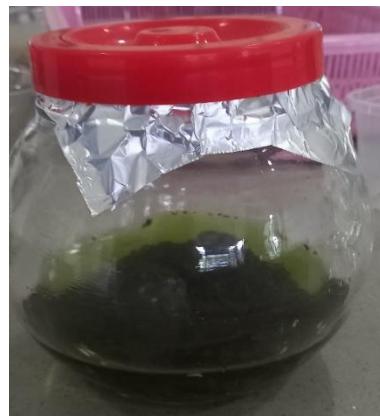
### Lampiran 3. Dokumentasi Penelitian



**Gambar 18. Sampel *Padina australis***



**Gambar 19. Proses pengeringan *Padina australis***



**Gambar 20. Proses maserasi**



**Gambar 21. Proses UAE**



**Gambar 22. Proses MAE**



**Gambar 23. Chamber CAMAG**



**Gambar 24.** Proses penyaringan ekstrak



**Gambar 25.** Proses penguapan pelarut



**Gambar 26.** Proses penotolan ekstrak



**Gambar 27.** Proses elusi sampel

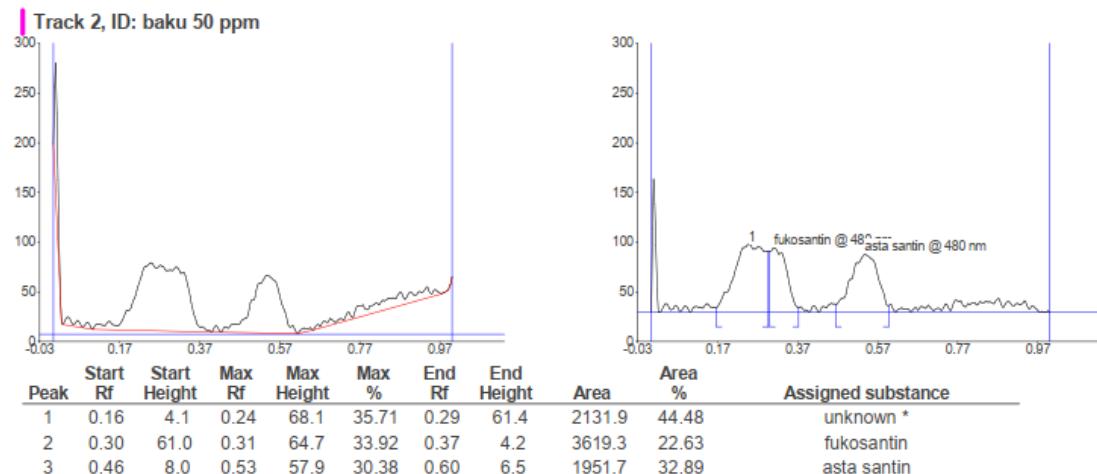


**Gambar 28.** Pengukuran luas area dengan KLT Densitometri

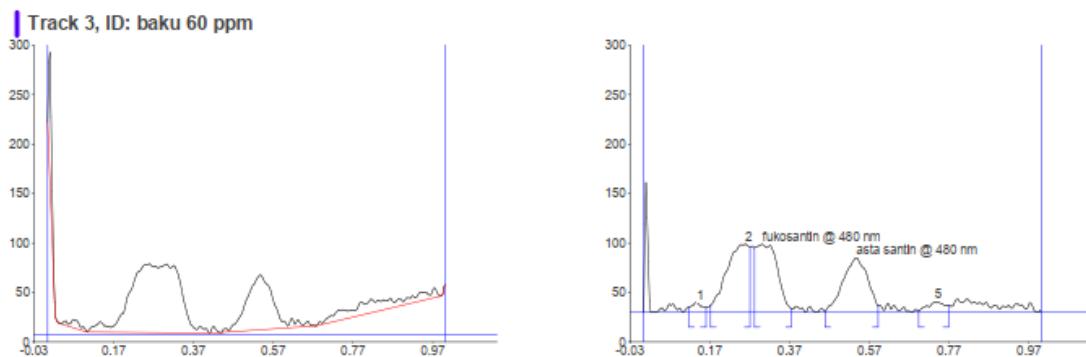
## Lampiran 4. Hasil Kromatogram Validasi Metode Analisis

### A. Linearitas

- Konsentrasi 50 µg/mL



- Konsentrasi 60 µg/mL



User : camag  
Saturday, January 01, 2005 12:16:43 AM

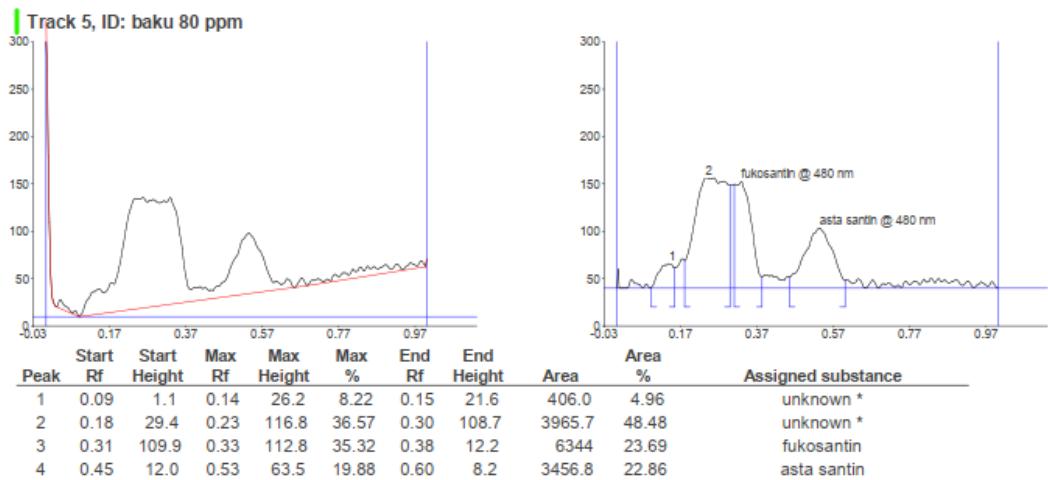
Approved : .....  
Report ID : 07D501010700101D

SN 1410W024, V1.4.3  
Page 3 of 7

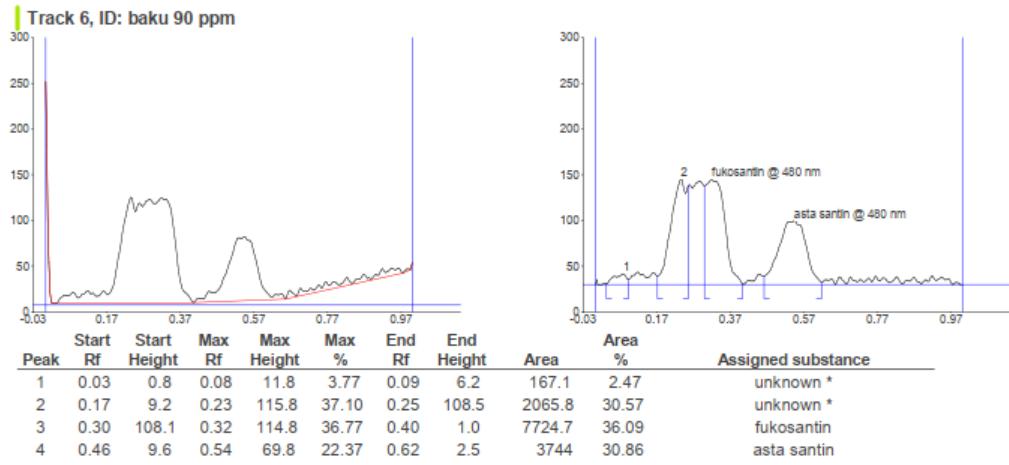
### winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	5.9	0.13	10.2	4.74	0.16	5.0	114.4	2.37	unknown *
2	0.17	7.4	0.26	69.6	32.31	0.27	66.4	1593.9	33.09	unknown *
3	0.28	65.6	0.30	69.3	32.17	0.37	4.1	4489.3	31.90	fukosantin
4	0.46	1.2	0.53	55.4	25.73	0.59	7.0	2455.3	28.57	asta santin
5	0.69	1.6	0.73	10.9	5.05	0.77	7.3	195.8	4.07	unknown *

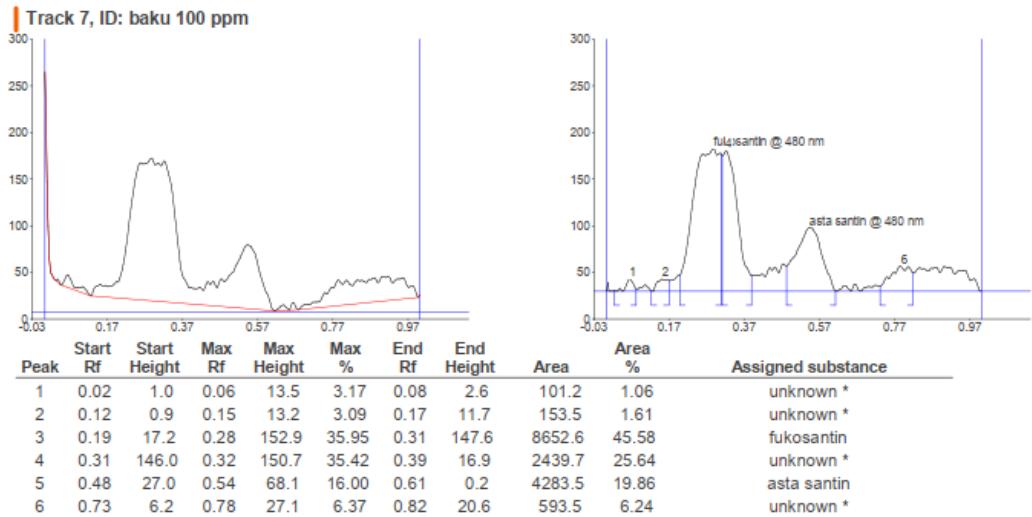
- Konsentrasi 80 µg/mL



- Konsentrasi 90 µg/mL

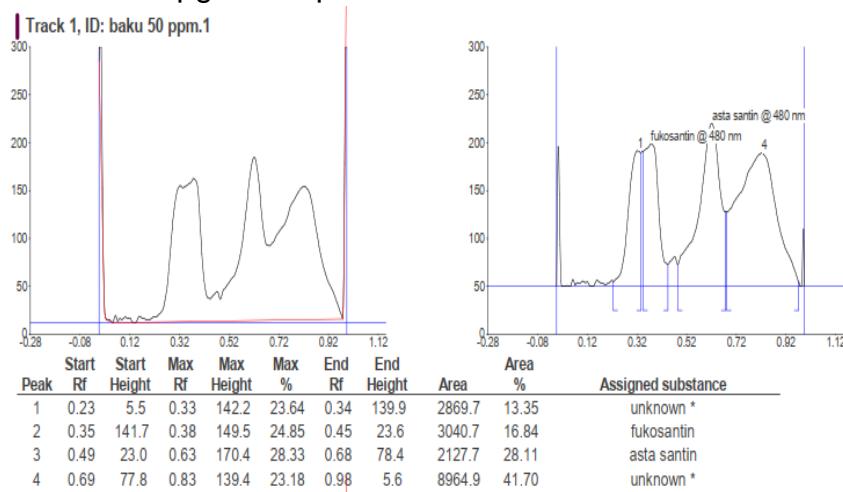


- Konsentrasi 100 µg/mL

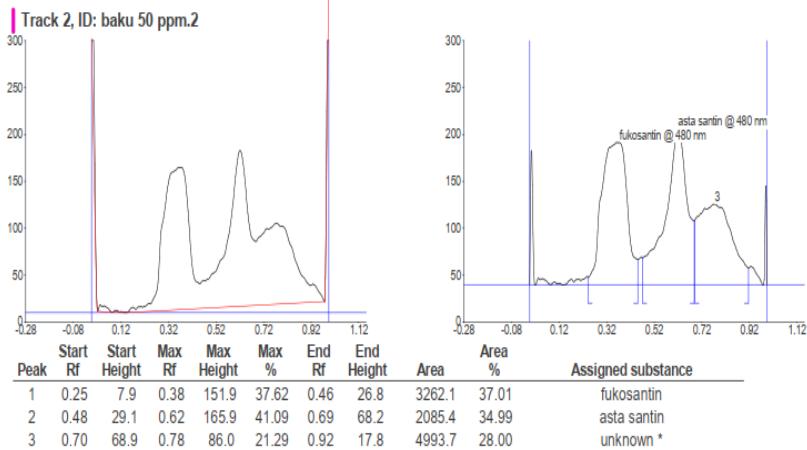


## B. Akurasi

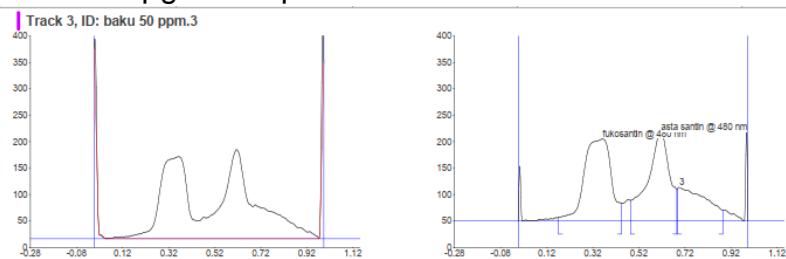
- Konsentrasi 50 µg/mL Replikasi 1



- Konsentrasi 50 µg/mL Replikasi 2



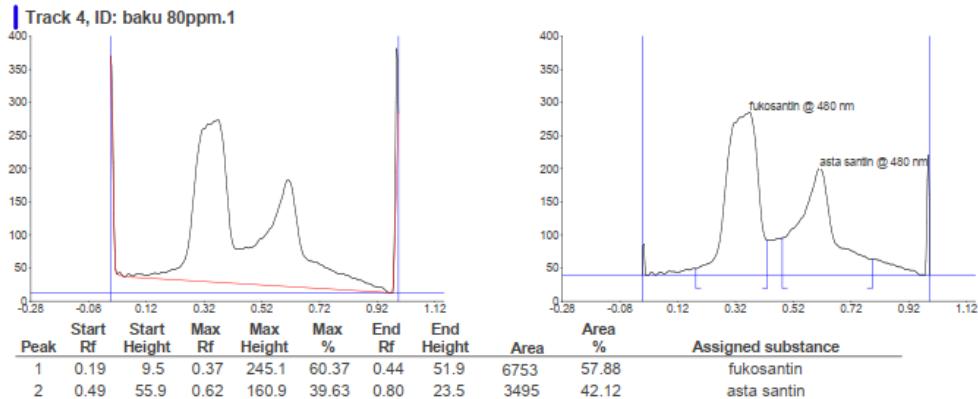
- Konsentrasi 50 µg/mL Replikasi 3



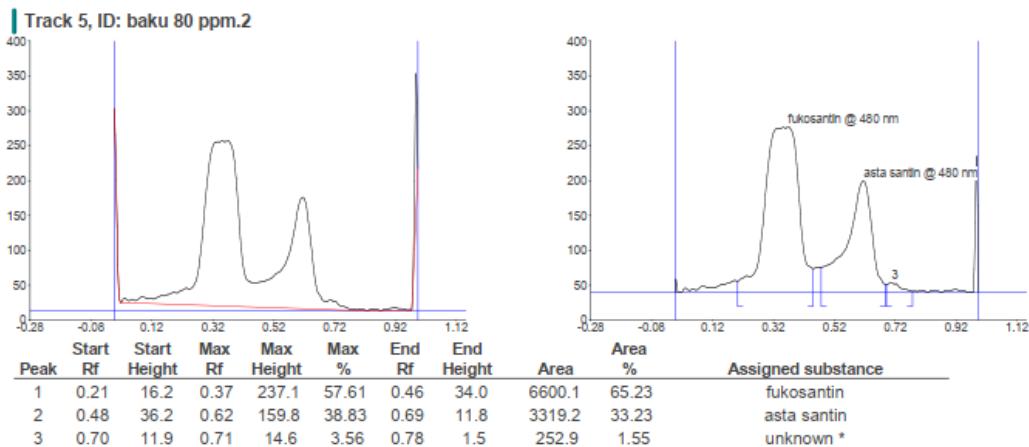
winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.17	6.7	0.37	155.5	40.04	0.45	33.8	3474.8	43.52	fukosantin
2	0.49	39.3	0.62	168.6	43.43	0.69	60.9	1889.7	37.75	asta santin
3	0.69	61.5	0.70	64.2	16.53	0.89	21.1	3189.3	18.73	unknown *

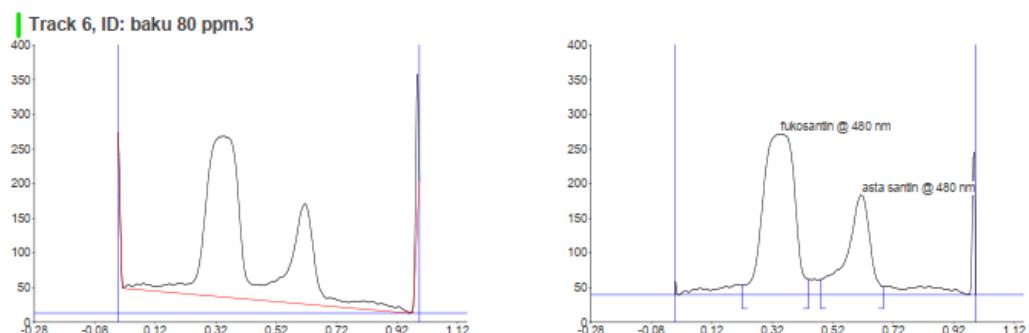
- Konsentrasi 80 µg/mL Replikasi 1



- Konsentrasi 80 µg/mL Replikasi 2



- Konsentrasi 80 µg/mL Replikasi 3



User : camag

Saturday, January 01, 2005 3:16:32 AM

Approved : .....

Report ID : 07D501010703101C

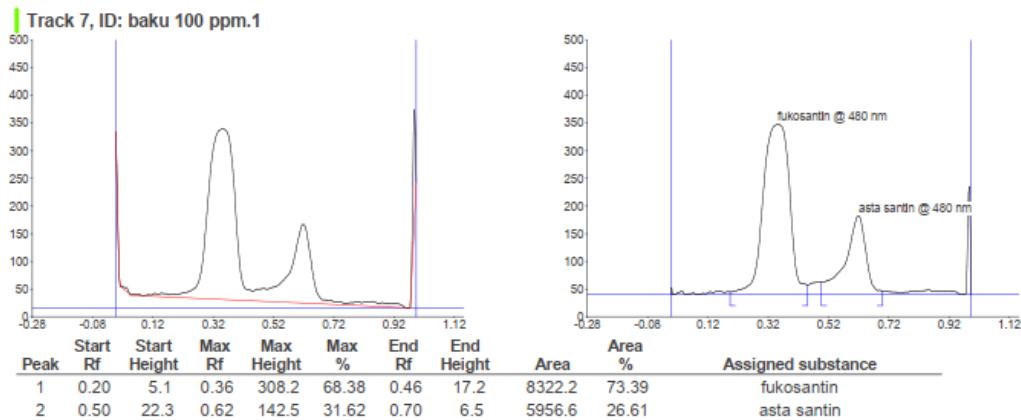
SN 1410W024, V1.4.3

Page 4 of 7

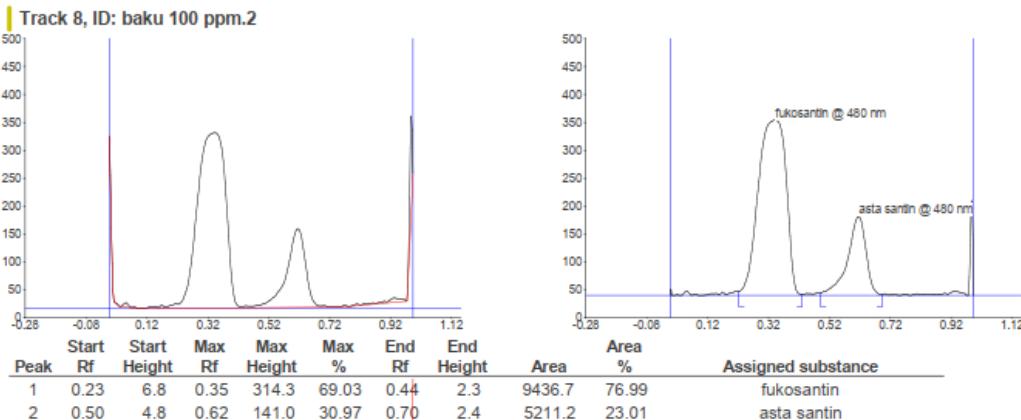
### winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.22	13.5	0.35	232.1	61.63	0.44	22.0	7410.8	68.70	fukosantin
2	0.48	22.0	0.62	144.5	38.37	0.69	11.4	3544.8	31.30	asta santin

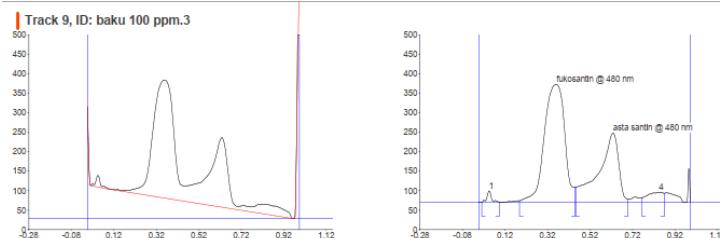
- Konsentrasi 100 µg/mL Replikasi 1



- Konsentrasi 100 µg/mL Replikasi 2



- Konsentrasi 100 µg/mL Replikasi 3



User : camag  
Saturday, January 01, 2005 3:16:32 AM

Approved : .....  
Report ID : 07D501010703101C

SN 1410W024, V1.4.3  
Page 5 of 7

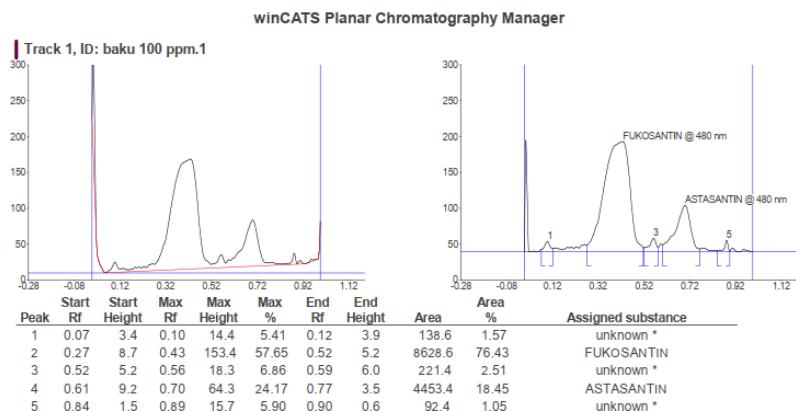


winCATS Planar Chromatography Manager

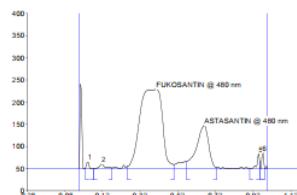
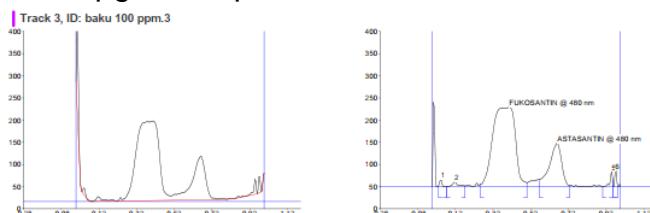
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.0	0.05	30.0	5.58	0.10	1.3	271.2	1.37	unknown *
2	0.19	4.8	0.37	303.1	56.37	0.46	39.2	977.3	59.97	fukosantin
3	0.46	39.3	0.63	178.4	33.17	0.70	8.7	5423	34.49	asta santin
4	0.77	12.9	0.85	26.2	4.88	0.88	23.9	827.2	4.17	unknown *

## C. Presisi

- Konsentrasi 100 µg/mL Replikasi 1



- Konsentrasi 100 µg/mL Replikasi 2



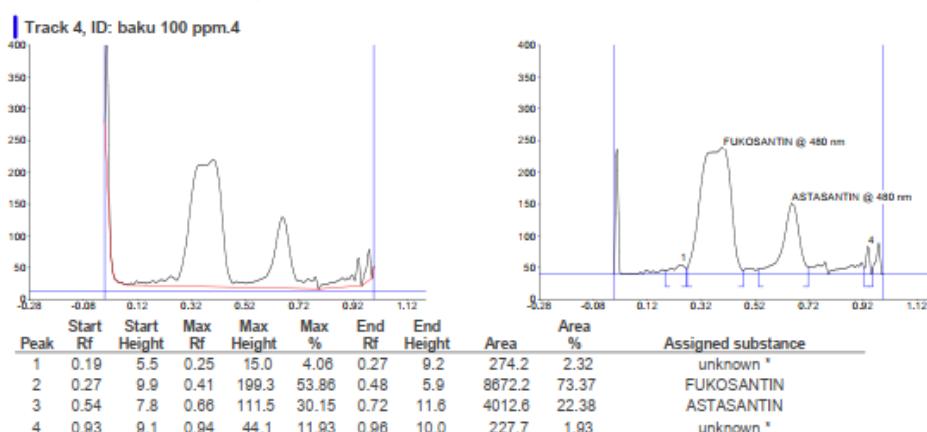
User : camag  
Saturday, January 01, 2005 12:35:49 AM  
Approved : .....  
Report ID : 0705010107002102

SN 1410W024, V1.4.3  
Page 3 of 8

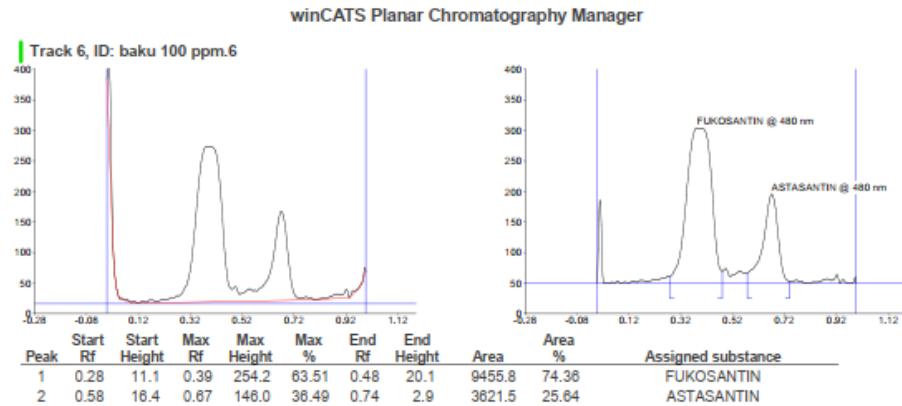
winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	1.8	0.04	15.4	4.10	0.07	0.0	78.3	0.68	unknown *
2	0.08	0.0	0.12	10.7	2.85	0.17	1.7	129.3	1.13	unknown *
3	0.26	5.8	0.41	179.4	47.95	0.50	9.2	8435.5	73.68	FUKOSANTIN
4	0.57	15.9	0.66	68.2	26.25	0.73	3.1	4360.4	21.79	ASTASANTIN
5	0.91	0.1	0.95	33.8	9.04	0.96	16.5	166.7	1.45	unknown *
6	0.96	3.7	0.97	36.7	9.81	0.99	0.0	144.6	1.26	unknown *

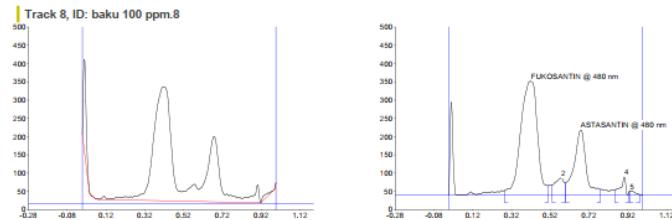
- Konsentrasi 100 µg/mL Replikasi 3



- Konsentrasi 100 µg/mL Replikasi 4



- Konsentrasi 100 µg/mL Replikasi 5

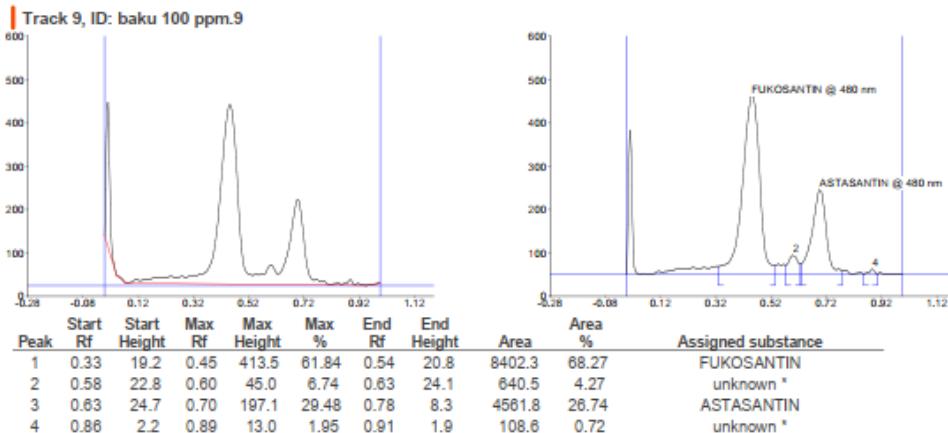


User : camag  
Saturday, January 01, 2005 12:35:49 AM      Approved : .....  
Report ID : 07D5010107002102      SN 1410W024, V1.4.3  
Page 5 of 8

winCATS Planar Chromatography Manager

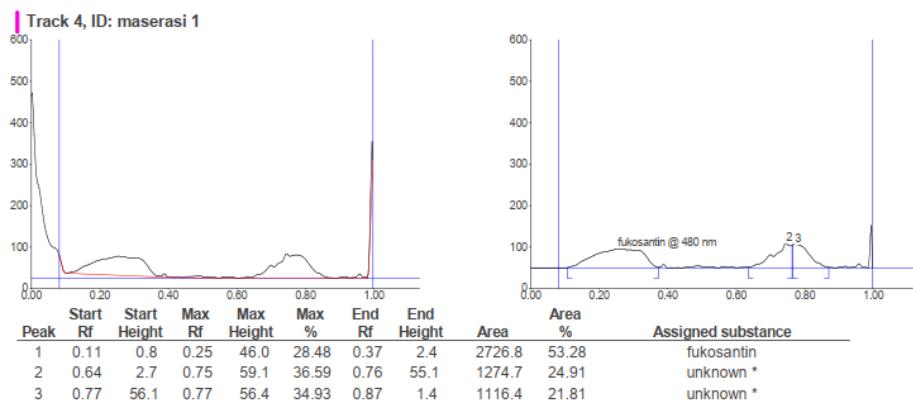
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.29	12.1	0.42	311.7	52.05	0.51	25.4	9824.6	64.22	FUKOSANTIN
2	0.53	27.7	0.58	46.9	7.84	0.60	32.9	906.8	5.47	unknown *
3	0.60	33.3	0.68	179.0	29.89	0.78	15.5	3976.4	26.33	ASTASANTIN
4	0.86	13.9	0.91	49.5	8.27	0.93	8.8	530.7	3.20	unknown *
5	0.93	11.3	0.94	11.6	1.95	0.99	0.4	128.9	0.78	unknown *

- Konsentrasi 100 µg/mL Replikasi 6

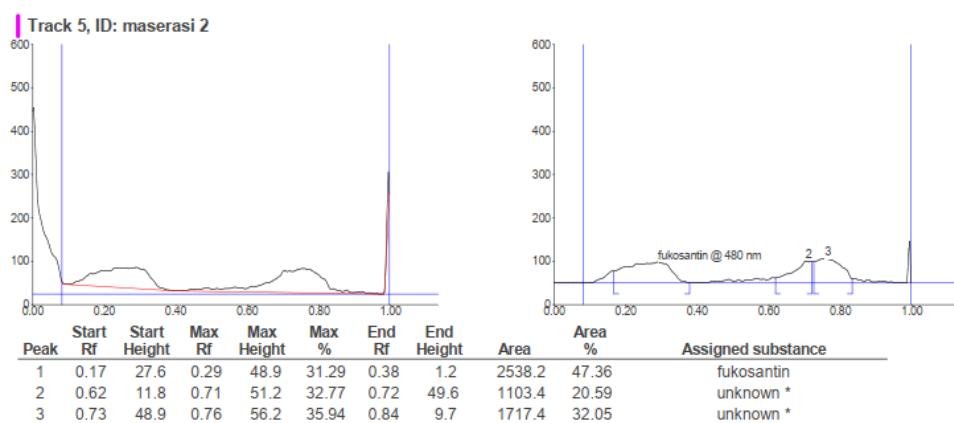


## Lampiran 5. Hasil Kromatogram Ekstrak

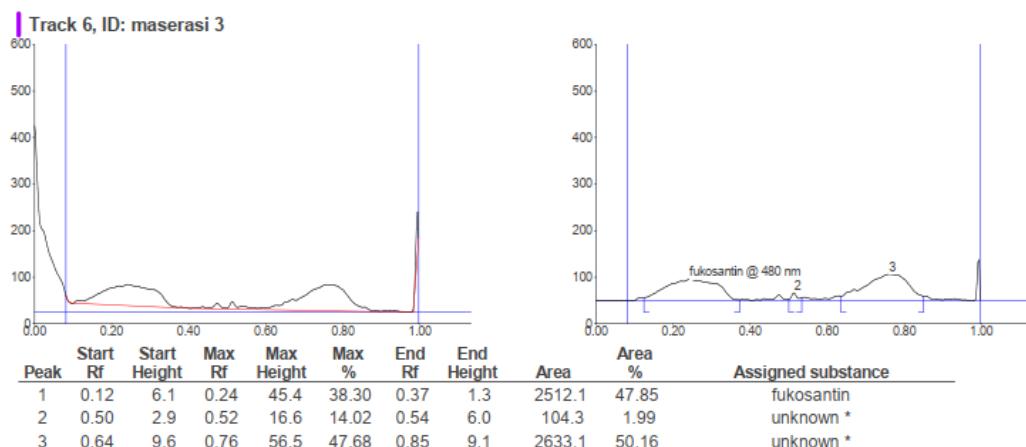
### Kromatogram *fucoxanthin* hasil maserasi 1



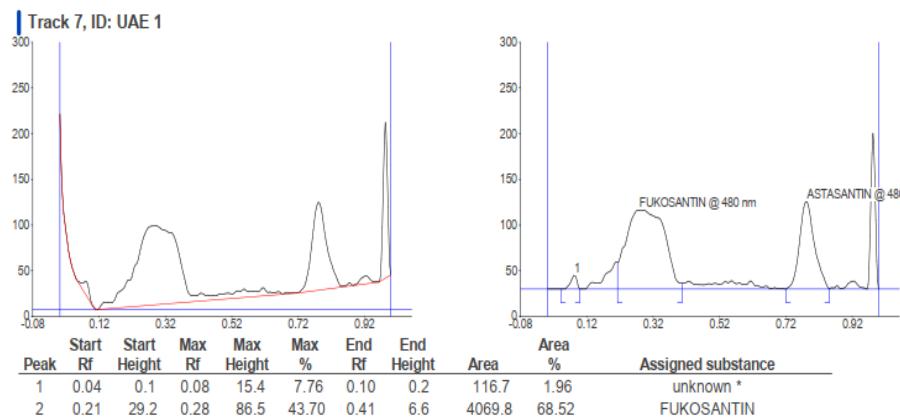
### Kromatogram *fucoxanthin* hasil maserasi 2



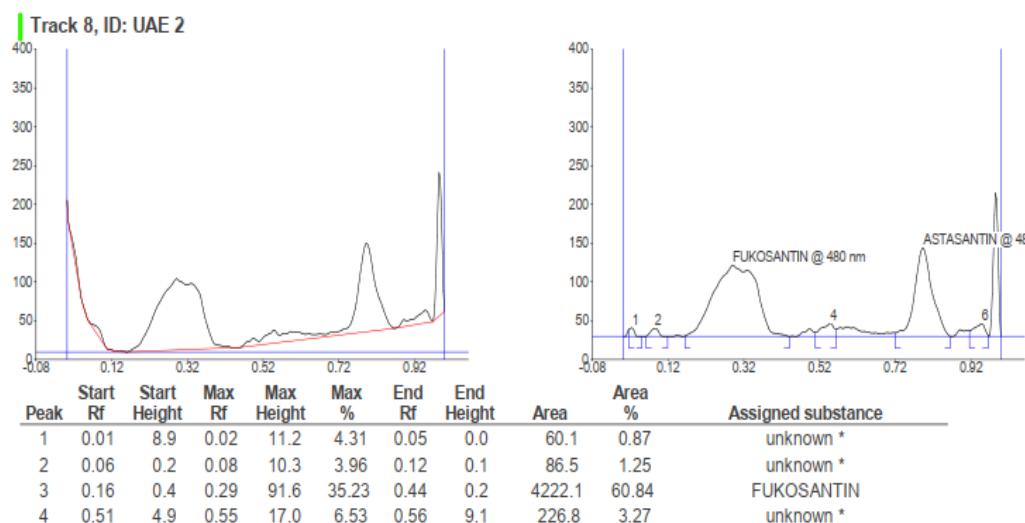
### Kromatogram *fucoxanthin* hasil maserasi 3



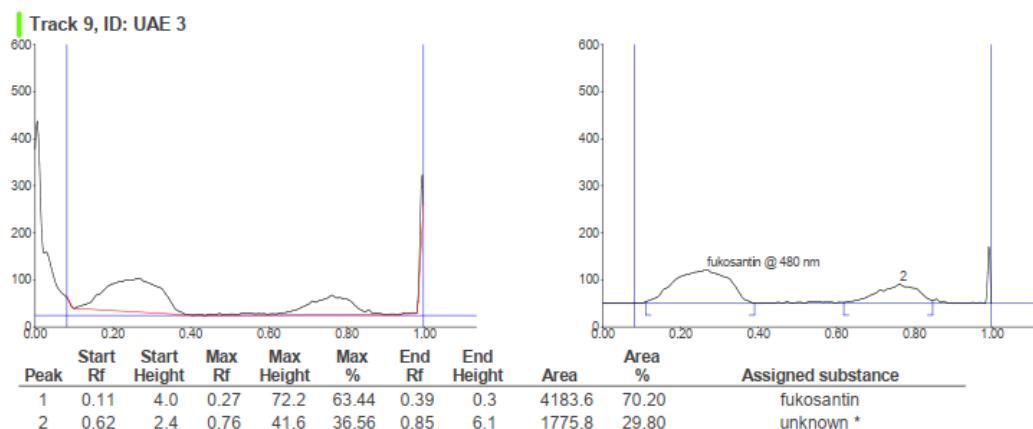
### Kromatogram fucoxanthin hasil UAE 1



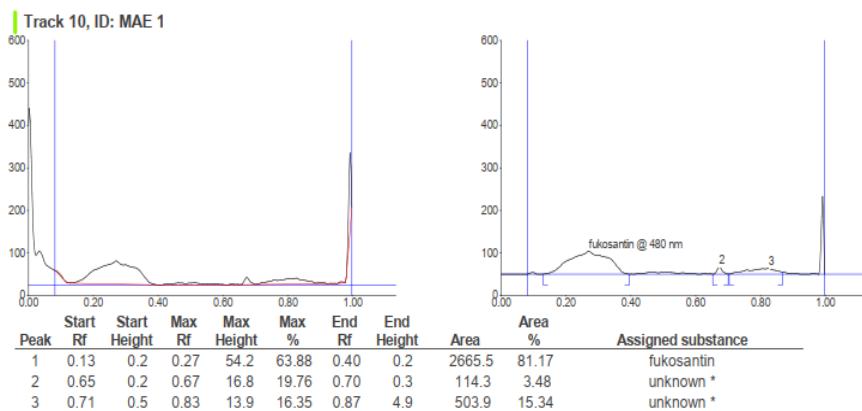
### Kromatogram fucoxanthin hasil UAE 2



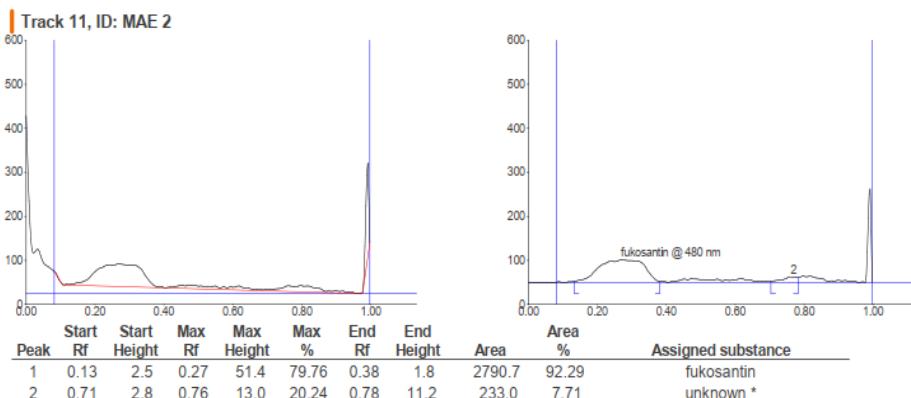
### Kromatogram fucoxanthin hasil UAE 3



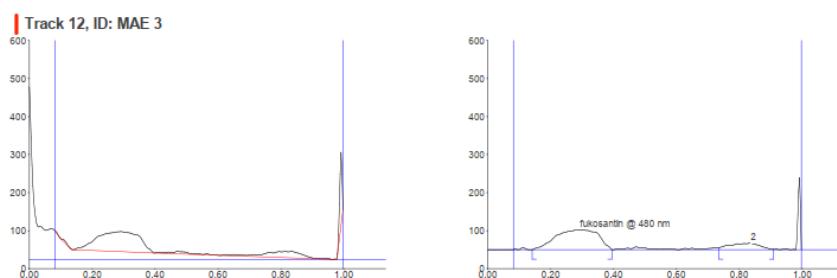
### Kromatogram *fucoxanthin* hasil MAE 1



### Kromatogram *fucoxanthin* hasil MAE 2



### Kromatogram *fucoxanthin* hasil MAE 3



User : camag

Saturday, January 01, 2005 12:26:49 AM

Approved : .....

Report ID : 07D501010700141A

SN 1410W024, V1.4.3

Page 9 of 11

#### winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.14	0.2	0.29	52.7	74.80	0.40	0.3	2950.2	80.95	fukosantin
2	0.74	5.5	0.84	17.7	25.20	0.91	1.0	694.2	19.05	unknown *

## Lampiran 6. Certificate of Analysis Baku

**Sigma-Aldrich.**

3050 Spruce Street, Saint Louis, MO 63103, USA

Website: [www.sigmaaldrich.com](http://www.sigmaaldrich.com)

Email USA: [techserv@sial.com](mailto:techserv@sial.com)

Outside USA: [eurtechserv@sial.com](mailto:eurtechserv@sial.com)

### Certificate of Analysis

Product Name:  
Fucoxanthin - carotenoid antioxidant

Product Number: F6932  
 Batch Number: MKCP1541  
 Brand: SIGMA  
 CAS Number: 3351-86-8  
 Formula: C<sub>42</sub>H<sub>58</sub>O<sub>6</sub>  
 Formula Weight: 658.91 g/mol  
 Storage Temperature: Store at -20 °C  
 Quality Release Date: 22 MAR 2021



Test	Specification	Result
Appearance (Color)	Red to Very Dark Red	Red
Appearance (Form)	Powder	Powder
Proton NMR Spectrum	Conforms to Structure	Conforms
<sup>13</sup> C NMR Spectrum	Conforms to Structure	Conforms
Purity (HPLC)	> 95 %	99 %
Solubility (Color)	Red to Very Dark Red	Dark Red
Solubility (Turbidity) 10 mg/mL, EtOH	Clear	Clear

Michael Grady, Manager  
 Quality Control  
 Milwaukee, WI US



**LABORATORIUM ILMU LINGKUNGAN DAN KELAUTAN  
DEPARTEMEN BIOLOGI  
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM  
UNIVERSITAS HASANUDDIN, KAMPUS TAMALANREA  
JL. PERINTIS KEMERDEKAAN KM.10, MAKASSAR**

No : 062/ILK.BIO/PP.13/10/2021  
 Hal : Identifikasi Algae  
 Lamp : 1 Lembar

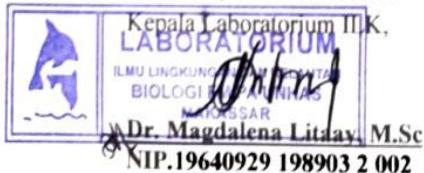
**SURAT KETERANGAN**

Yang bertanda tangan dibawah ini, menerangkan bahwa setelah mengkaji karakter sampel ganggang algae dan identifikasi maka terdapat tiga spesies yaitu :

**Alga Coklat (Phaeophyta)**

- |                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sampel         | : Terima tanggal 06/10/2022                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Kondisi sampel | : lembab                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| 1. Jenis       | : <i>Sargassum polycystum</i> C. Agardh                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Diskripsi      | : Tanaman cukup besar, panjangnya antara 10-40 cm. Alga berwarna coklat, melekat pada substrat keras. Stipula silindris, kaku, dapat tegak sepanjang thallus. Cabang utama kaku mengeluarkan cabang sekunder tumbuh selongseling dan pada cabang ini terdapat daun , thallus bercabang berbentuk lembaran seperti daun bergelombang, tepi daun bergerigi tidak beraturan, dengan permukaan licin dan agak kaku, dari nudus terdapat bulatan-bulatan banyak menyerupai buah. Tangkai vesikula oval, melekat banyak pada cabang tertier, tunggal atau bergerombol. |
| 2. Jenis       | : <i>Sargassum sp.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Diskripsi      | : Tanaman besar, panjang antara 20-40 cm, berwarna coklat. Bentuk daun besar. oval, dengan tepi bergerigi atau berombak dan ujung agak meruncing. Permukaan licin. Thallus silindris. Tidak memiliki organ pelekat ( <i>holdfast</i> ).                                                                                                                                                                                                                                                                                                                          |
| 3. Jenis       | : <i>Padina australis</i> Hanch, 1887                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Diskripsi      | : Thallus terdiri dari beberapa helaian bentuk kipas/filament berwarna coklat. Ukuran filament ini sedikit lebih besar dibandingkan jenis lain dari <i>Padina</i> . Tepi luar filament menebal dan permukaan atas filament mempunyai garis konsentris warna putih. Organ pelekat ( <i>holdfast</i> ) bentuk discoid.                                                                                                                                                                                                                                             |

Makassar, 10 Oktober 2022



**Tembusan :**  
 1. Arsip



LABORATORIUM ILMU LINGKUNGAN DAN KELAUTAN  
DEPARTEMEN BIOLOGI  
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM  
UNIVERSITAS HASANUDDIN, KAMPUS TAMALANREA  
JL. PERINTIS KEMERDEKAAN KM.10, MAKASSAR

Lampiran



Gambar 1. *Sargassum polycystum* C. Agardh



Gambar 2. *Sargassum sp.*



Gambar 3. *Padina australis* Hanch, 1887