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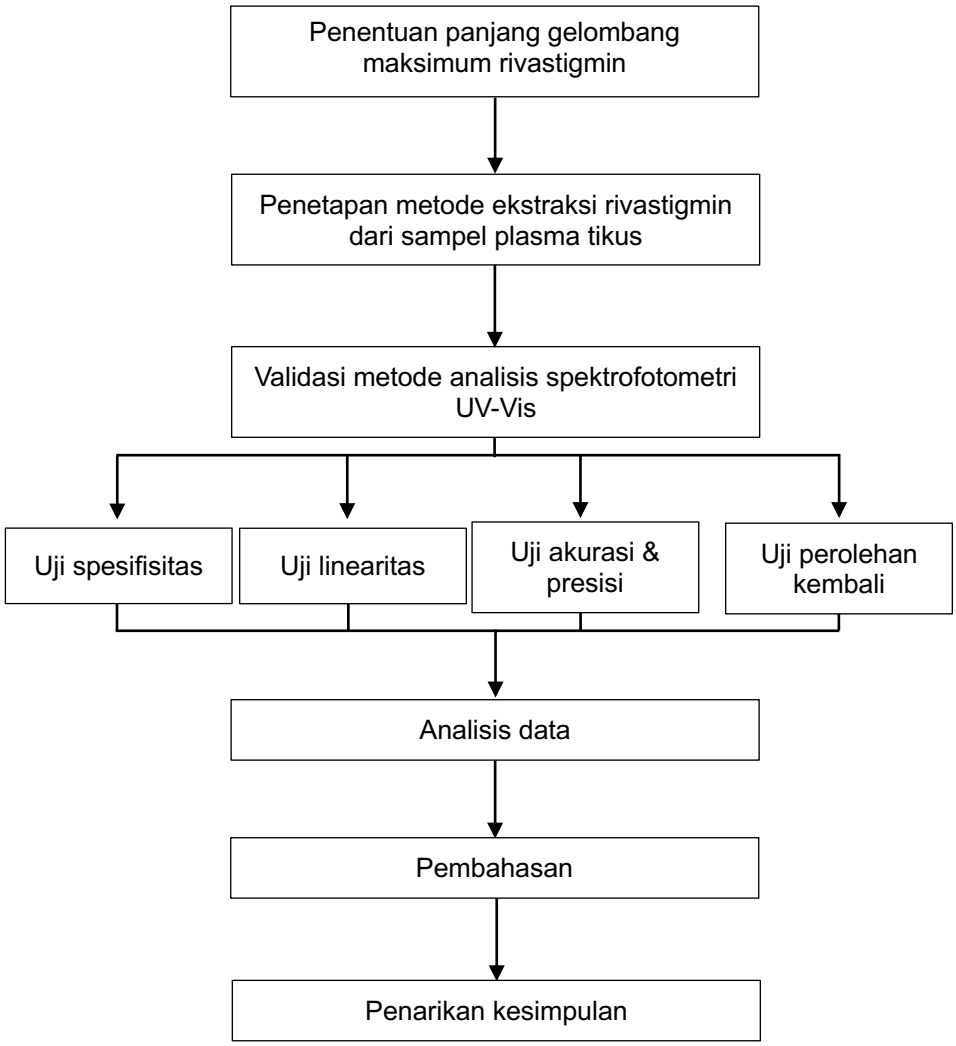
LAMPIRAN

Lampiran 1. Skema Kerja



Lampiran 2. Perhitungan pengenceran

Konsentrasi 2500 µg/mL → 2000 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
2500 µg/mL x V_1 = 2000 µg/mL x 1 mL
$V_1 = \frac{2000\ \mu g/mL\ x\ 1\ mL}{2500\ \mu g/mL}$
V_1 = 0,8 mL

Konsentrasi 2500 µg/mL → 1500 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
2500 µg/mL x V_1 = 1500 µg/mL x 1 mL
$V_1 = \frac{1500\ \mu g/mL\ x\ 1\ mL}{2500\ \mu g/mL}$
V_1 = 0,6 mL

Konsentrasi 2000 µg/mL → 1000 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
2000 µg/mL x V_1 = 1000 µg/mL x 1 mL
$V_1 = \frac{1000\ \mu g/mL\ x\ 1\ mL}{2000\ \mu g/mL}$
V_1 = 0,5 mL

Konsentrasi 1500 µg/mL → 1000 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
1500 µg/mL x V_1 = 1000 µg/mL x 1 mL
$V_1 = \frac{1000\ \mu g/mL\ x\ 1\ mL}{1500\ \mu g/mL}$
V_1 = 0,15 mL

Konsentrasi 1000 µg/mL → 500 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
1000 µg/mL x V_1 = 500 µg/mL x 1 mL
$V_1 = \frac{500\ \mu g/mL\ x\ 1\ mL}{1000\ \mu g/mL}$
V_1 = 0,5 mL

Konsentrasi 1500 µg/mL → 300 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
1500 µg/mL x V_1 = 300 µg/mL x 1 mL
$V_1 = \frac{300\ \mu g/mL\ x\ 1\ mL}{1500\ \mu g/mL}$
V_1 = 0,2 mL

Konsentrasi 500 µg/mL → 250 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
500 µg/mL x V_1 = 250 µg/mL x 1 mL
$V_1 = \frac{250\ \mu g/mL\ x\ 1\ mL}{500\ \mu g/mL}$
V_1 = 0,5 mL

Konsentrasi 1500 µg/mL → 67 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
1500 µg/mL x V_1 = 67 µg/mL x 1 mL
$V_1 = \frac{67\ \mu g/mL\ x\ 1\ mL}{1500\ \mu g/mL}$
V_1 = 0,0446 mL = 45 µL

Konsentrasi 250 µg/mL → 125 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
250 µg/mL x V_1 = 125 µg/mL x 1 mL
$V_1 = \frac{125\ \mu g/mL\ x\ 1\ mL}{250\ \mu g/mL}$
V_1 = 0,5 mL

Konsentrasi 125 µg/mL → 62,5 µg/mL
$M_1 \times V_1 = M_2 \times V_2$
125 µg/mL x V_1 = 62,5 µg/mL x 1 mL

Lampiran 3. Perhitungan persentase perolehan kembali ekstraksi (extraction recovery) penentuan metode ekstraksi

Tabel 8. Hasil pengukuran absorbansi larutan standar rivastigmin setelah penambahan larutan cobalt tiosionat

Konsentrasi larutan (µg/mL)	Absorbansi			
	Replikasi 1	Replikasi 2	Replikasi 3	Rata-rata
25	1,209	1,271	1,198	1,226

Tabel 9. Hasil pengukuran absorbansi penentuan metode ekstraksi

Pelarut organik	Metode	Volume (mL)	Absorbansi		
			Replikasi 1	Replikasi 2	Replikasi 3
Metanol	A	1	0,116	0,096	0,105
	B	3	0,361	0,508	0,427
	C	5	0,778	0,709	0,645
	D	7	0,911	0,920	1,002
Asetonitril	E	1	0,237	0,307	0,270
	F	3	0,709	0,780	0,543
	G	5	1,115	1,151	1,060
	H	7	1,222	1,105	1,127

Perhitungan persentase perolehan kembali (%R) metode A:

- Replikasi 1: $\%R = \frac{0,116}{1,226} \times 100\% = 9,48\ \%$
- Replikasi 2: $\%R = \frac{0,096}{1,226} \times 100\% = 7,82\ \%$
- Replikasi 3: $\%R = \frac{0,105}{1,226} \times 100\% = 8,57\ \%$

Rata-rata: $\overline{\%R_A} = \frac{9,48\ \% + 7,82\ \% + 8,57\ \%}{3} = 8,62\%$

Perhitungan persentase perolehan kembali (%R) metode B:

- Replikasi 1: $\%R = \frac{0,361}{1,226} \times 100\% = 29,45\ \%$
- Replikasi 2: $\%R = \frac{0,508}{1,226} \times 100\% = 41,43\ \%$
- Replikasi 3: $\%R = \frac{0,427}{1,226} \times 100\% = 34,86\ \%$

Rata-rata: $\overline{\%R_B} = \frac{29,45\ \% + 41,43\ \% + 34,86\ \%}{3} = 35,25\%$

Perhitungan persentase perolehan kembali (%R) metode C:

- Replikasi 1: $\%R = \frac{0,778}{1,226} \times 100\% = 63,48\ \%$
- Replikasi 2: $\%R = \frac{0,709}{1,226} \times 100\% = 57,82\ \%$
- Replikasi 3: $\%R = \frac{0,645}{1,226} \times 100\% = 52,58\ \%$

$\frac{63,48\% + 57,82\% + 52,58\%}{3} = 57,96\ \%$

se perolehan kembali (%R) metode D:

$\%R = \frac{0,911}{1,226} \times 100\% = 74,30\ \%$

$\%R = \frac{0,920}{1,226} \times 100\% = 75,04\ \%$

- Replikasi 3: % R = $\frac{1,002}{1,226}$ x 100% = 81,70 %

Rata-rata: $\overline{\%R_A} = \frac{74,3\ \% + 75,04\ \% + 81,7\ \%}{3} = 77,01\ \%$

Perhitungan persentase perolehan kembali (%R) metode E:

- Replikasi 1: % R = $\frac{0,237}{1,226}$ x 100% = 19,30 %
- Replikasi 2: % R = $\frac{0,307}{1,226}$ x 100% = 25,04 %
- Replikasi 3: % R = $\frac{0,270}{1,226}$ x 100% = 22,05 %

Rata-rata: $\overline{\%R_E} = \frac{19,30\ \% + 25,04\ \% + 22,05\ \%}{3} = 22,13\ \%$

Perhitungan persentase perolehan kembali (%R) metode F:

- Replikasi 1: % R = $\frac{0,709}{1,226}$ x 100% = 11,16 %
- Replikasi 2: % R = $\frac{0,780}{1,226}$ x 100% =63,65 %
- Replikasi 3: % R = $\frac{0,543}{1,226}$ x 100% = 44,26 %

Rata-rata: $\overline{\%R_F} = \frac{11,16\ \% + 63,65\ \% + 44,26\ \%}{3} = 39,69\ \%$

Perhitungan persentase perolehan kembali (%R) metode G:

- Replikasi 1: % R = $\frac{1,115}{1,226}$ x 100% = 90,93 %
- Replikasi 2: % R = $\frac{1,151}{1,226}$ x 100% = 93,85 %
- Replikasi 3: % R = $\frac{1,060}{1,226}$ x 100% = 86,44 %

Rata-rata: $\overline{\%R_G} = \frac{90,93\ \% + 93,85\ \% + 86,44\ \%}{3} = 90,41\%$

Perhitungan persentase perolehan kembali (%R) metode H:

- Replikasi 1: % R = $\frac{1,222}{1,226}$ x 100% = 99,67 %
- Replikasi 2: % R = $\frac{1,105}{1,226}$ x 100% = 90,10 %
- Replikasi 3: % R = $\frac{1,127}{1,226}$ x 100% =91,93 %

Rata-rata: $\overline{\%R_H} = \frac{99,67\ \% + 90,10\ \% + 91,93\ \%}{3} = 93,90\ \%$

Lampiran 4. Perhitungan Uji Linearitas

Tabel 10. Perhitungan linearitas

Konsentrasi (µg/mL)	Absorbansi			
	Replikasi 1	Replikasi 2	Replikasi 3	Rata-rata
20	1,079	1,113	1,025	1,072
10	0,514	0,557	0,513	0,527
5	0,257	0,278	0,256	0,263
2,5	0,128	0,139	0,128	0,131
1,25	0,064	0,070	0,064	0,065
0,625	0,032	0,035	0,032	0,032

Persamaan garis linear $y = a + bx$

y = absorbansi (A)

x = konsentrasi (µg/mL)

Berdasarkan hasil perhitungan regresi diperoleh:

a = -0,0026

b = 0,0536

r = 0,9999

Sehingga diperoleh persamaan:

$y = -0,0026 + 0,0536x$

Lampiran 5. Perhitungan Batas Deteksi (LOD) dan Batas Kuantitasi (LLOQ)

Tabel 11. Perhitungan LOD dan LLOQ

No	Konsentrasi (µg/mL)	Y	Yi	Yi-Y	$(Yi-Y)^2$
1	20	1,0694	1,072	0,00314	0,0000098596
2	10	0,5334	0,527	-0,00569183	0,000032397
3	5	0,2654	0,263	-0,00154592	0,00000238986
4	2,5	0,1314	0,131	0,000527	0,00000027777
5	1,25	0,0644	0,065	0,0015635	0,0000024446
6	0,625	0,0309	0,032	0,0020818	0,00000433373
					$\sum$ = 0,0000129256

$$sy = \sqrt{\frac{\sum (Yi-Y)^2}{N-2}}$$

$$= \sqrt{\frac{0,0000129256}{6-2}}$$

$$= 0,003595223$$

$$LOD = \frac{3,3 \times sy}{b}$$

$$= \frac{3,3 \times 0,003595223}{0,0536}$$

$$= 0,22130678$$

$$LLOQ = \frac{10 \times sy}{b}$$

$$= \frac{10 \times 0,003595223}{0,0536}$$

$$= 0,67062659$$

Keterangan:

Y = Absorbansi dari persamaan regresi
Yi = Absorbansi hasil pengukuran
N = Jumlah data
sy = simpangan baku
b = kemiringan (*slope*) dari persamaan regresi y = a + bx
LOD = *Limit of Detection* (LOD/Batas deteksi)
LLOQ = *Lower Limit of Quantification* (LLOQ/Batas kuantitasi)

Lampiran 6. Perhitungan Uji Akurasi dan Presisi

Tabel 12. Hasil uji akurasi dan presisi *intraday*

Replikasi	Konsentrasi sampel sebenarnya (µg/mL)	Absorbansi		
		Replikasi 1	Replikasi 2	Replikasi 3
1	0,67	0,027	0,031	0,029
	3	0,139	0,169	0,162
	10	0,498	0,548	0,559
	15	0,808	0,826	0,813
2	0,67	0,035	0,028	0,033
	3	0,148	0,162	0,172
	10	0,515	0,552	0,557
	15	0,828	0,797	0,817
3	0,67	0,029	0,033	0,035
	3	0,156	0,181	0,170
	10	0,548	0,537	0,570
	15	0,848	0,799	0,828

Tabel 13. Hasil uji akurasi dan presisi *interday*

Hari	Konsentrasi sampel sebenarnya (µg/mL)	Absorbansi		
		Replikasi 1	Replikasi 2	Replikasi 3
1	0,67	0,027	0,032	0,029
	3	0,137	0,173	0,160
	10	0,492	0,559	0,552
	15	0,798	0,843	0,803
2	0,67	0,035	0,029	0,033
	3	0,146	0,166	0,170
	10	0,509	0,563	0,550
	15	0,817	0,813	0,807
3	0,67	0,029	0,034	0,035
	3	0,154	0,185	0,168
	10	0,541	0,548	0,563
	15	0,837	0,815	0,817

Konsentrasi sampel untuk akurasi dan presisi dihitung menggunakan persamaan regresi:

y = -0,0026 + 0,0536x.

sorbansi

nsentrasi sampel (µg/mL)

n konsentrasi sampel, selanjutnya dihitung %RE untuk

an %RSD untuk penentuan presisi.

Contoh perhitungan akurasi dan presisi

- Akurasi dan presisi *intraday* konsentrasi 0,67 (LLOQ) Replikasi 1
  - Replikasi 1.1

    Konsentrasi terukur = $\frac{0,027+0,0026}{0,0536}$

    = 0,567164179 µg/mL
  - Replikasi 1.2

    Konsentrasi terukur = $\frac{0,031+0,0026}{0,0536}$

    = 0,641791045 µg/mL
  - Replikasi 1.3

    Konsentrasi terukur = $\frac{0,029+0,0026}{0,0536}$

    = 0,604477612 µg/mL

Rata-rata konsentrasi terukur = $\frac{0,567164179 + 0,641791045 + 0,604477612}{3} = 0,6044776$

$$\%RE = \left[\frac{\text{Konsentrasi terukur - konsentrasi sampel sebenarnya}}{\text{Konsentrasi sampel sebenarnya}}\right] \text{x } 100\%$$

$$= \left[\frac{0,6044776 - 0,67}{0,67}\right] \text{x } 100\%$$

$$= -9,78\%$$

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

$$= \sqrt{\frac{(0,567-0,604)^2 + (0,642-0,604)^2 + (0,604-0,604)^2}{3-1}}$$

$$= 0,04$$

$$RSD = \frac{SD}{\bar{X}} \text{ x } 100\%$$

$$= \frac{0,04}{0,604} \text{ x } 100\%$$

$$= 6,17\%$$

Lampiran 7. Perhitungan Uji Perolehan Kembali

Tabel 14. Hasil pengukuran absorbansi larutan standar rivastigmin setelah penambahan larutan cobalt tiosionat

Konsentrasi larutan (µg/mL)	Absorbansi			
	Replikasi 1	Replikasi 2	Replikasi 3	Rata-rata
0,67	0,039	0,041	0,039	0,03966667
3	0,181	0,178	0,187	0,182
10	0,598	0,587	0,578	0,58766667
15	0,898	0,843	0,943	0,89466667

Tabel 15. Hasil pengukuran absorbansi rivastigmin dari sampel plasma tikus

Konsentrasi larutan (µg/mL)	Absorbansi		
	Replikasi 1	Replikasi 2	Replikasi 3
0,67	0,033	0,037	0,035
3	0,145	0,175	0,168
10	0,504	0,554	0,565
15	0,814	0,832	0,819

Contoh Perhitungan Uji Perolehan Kembali

Perhitungan persentase perolehan kembali (%R) konsentrasi 0,67 µg/mL:

- Replikasi 1: $\%R = \frac{0,033}{0,03966667} \times 100\% = 83,19\ \%$
- Replikasi 2: $\%R = \frac{0,037}{0,03966667} \times 100\% = 93,28\ \%$
- Replikasi 3: $\%R = \frac{0,035}{0,03966667} \times 100\% = 88,24\ \%$

Rata-rata: $\overline{\%R} = \frac{83,19 + 93,28 + 88,24\ \%}{3} = 88,24\ \%$

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

$$= \sqrt{\frac{(83,19 - 88,24)^2 + (93,28 - 88,24)^2 + (88,24 - 88,24)^2}{3-1}}$$

$$= 5,04$$

$$RSD = \frac{SD}{\bar{X}} \times 100\%$$

$$= \frac{5,04}{88,24} \times 100\%$$

$$= 5,17\%$$

Lampiran 8. Analisis Statistik

Tabel 16. Hasil uji normalitas

Tests of Normality

	Metode	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Hasil Ekstraksi	A	,192	3	.	,997	3	,894
	B	,192	3	.	,997	3	,893
	C	,178	3	.	1,000	3	,958
	D	,353	3	.	,824	3	,174
	E	,178	3	.	,999	3	,954
	F	,235	3	.	,978	3	,714
	G	,222	3	.	,985	3	,768
	H	,318	3	.	,887	3	,346

a. Lilliefors Significance Correction

Tabel 17. Hasil uji *One Way-Annova*

ANOVA

Hasil Ekstraksi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21263,326	7	3037,618	29,087	,000
Within Groups	1670,911	16	104,432		
Total	22934,237	23			

Tabel 18. Hasil uji *post-hoc*

Multiple Comparisons

Dependent Variable: Hasil Ekstraksi

	(I) Metode	(J) Metode	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	A	B	-26,62333	8,34394	,082	-55,5113	2,2646
		C	-49,33667*	8,34394	,000	-78,2246	-20,4487
		D	-68,39000*	8,34394	,000	-97,2780	-39,5020
		E	-13,50667	8,34394	,734	-42,3946	15,3813
		F	-31,06667*	8,34394	,030	-59,9546	-2,1787
		G	-81,78333*	8,34394	,000	-110,6713	-52,8954
		H	-85,27667*	8,34394	,000	-114,1646	-56,3887
	B	A	26,62333	8,34394	,082	-2,2646	55,5113
			-22,71333	8,34394	,185	-51,6013	6,1746
			-41,76667*	8,34394	,003	-70,6546	-12,8787
			13,11667	8,34394	,759	-15,7713	42,0046
			-4,44333	8,34394	,999	-33,3313	24,4446
			-55,16000*	8,34394	,000	-84,0480	-26,2720
			-58,65333*	8,34394	,000	-87,5413	-29,7654
			49,33667*	8,34394	,000	20,4487	78,2246
			22,71333	8,34394	,185	-6,1746	51,6013

Lanjutan Tabel 18

	D	-19,05333	8,34394	,358	-47,9413	9,8346
	E	35,83000*	8,34394	,010	6,9420	64,7180
	F	18,27000	8,34394	,406	-10,6180	47,1580
	G	-32,44667*	8,34394	,022	-61,3346	-3,5587
	H	-35,94000*	8,34394	,010	-64,8280	-7,0520
D	A	68,39000*	8,34394	,000	39,5020	97,2780
	B	41,76667*	8,34394	,003	12,8787	70,6546
	C	19,05333	8,34394	,358	-9,8346	47,9413
	E	54,88333*	8,34394	,000	25,9954	83,7713
	F	37,32333*	8,34394	,007	8,4354	66,2113
	G	-13,39333	8,34394	,741	-42,2813	15,4946
	H	-16,88667	8,34394	,497	-45,7746	12,0013
E	A	13,50667	8,34394	,734	-15,3813	42,3946
	B	-13,11667	8,34394	,759	-42,0046	15,7713
	C	-35,83000*	8,34394	,010	-64,7180	-6,9420
	D	-54,88333*	8,34394	,000	-83,7713	-25,9954
	F	-17,56000	8,34394	,452	-46,4480	11,3280
	G	-68,27667*	8,34394	,000	-97,1646	-39,3887
	H	-71,77000*	8,34394	,000	-100,6580	-42,8820
F	A	31,06667*	8,34394	,030	2,1787	59,9546
	B	4,44333	8,34394	,999	-24,4446	33,3313
	C	-18,27000	8,34394	,406	-47,1580	10,6180
	D	-37,32333*	8,34394	,007	-66,2113	-8,4354
	E	17,56000	8,34394	,452	-11,3280	46,4480
	G	-50,71667*	8,34394	,000	-79,6046	-21,8287
	H	-54,21000*	8,34394	,000	-83,0980	-25,3220
G	A	81,78333*	8,34394	,000	52,8954	110,6713
	B	55,16000*	8,34394	,000	26,2720	84,0480
	C	32,44667*	8,34394	,022	3,5587	61,3346
	D	13,39333	8,34394	,741	-15,4946	42,2813
	E	68,27667*	8,34394	,000	39,3887	97,1646
	F	50,71667*	8,34394	,000	21,8287	79,6046
	H	-3,49333	8,34394	1,000	-32,3813	25,3946
H	A	85,27667*	8,34394	,000	56,3887	114,1646
	B	58,65333*	8,34394	,000	29,7654	87,5413
	C	35,94000*	8,34394	,010	7,0520	64,8280
	D	16,88667	8,34394	,497	-12,0013	45,7746
	E	71,77000*	8,34394	,000	42,8820	100,6580
	F	54,21000*	8,34394	,000	25,3220	83,0980
	G	3,49333	8,34394	1,000	-25,3946	32,3813

*. The mean difference is significant at the 0.05 level.

Lampiran 9. Dokumentasi Penelitian

Gambar 5. Pengambilan sampel darah

Gambar 6. Ekstraksi rivastigmin dari sampel

engukuran menggunakan spektrofotometer UV-Vis