

## **Lampiran 1. Lembar Penjelasan Penelitian**

Nama Peneliti : Widya Dwi Astuti

NIM : P17334117087

Judul Penelitian : Hubungan kadar Nikotin Terhadap Glukosa Darah Pada Perokok Aktif

Peneliti adalah mahasiswa Program Diploma III Analis Kesehatan Poltekkes Kemenkes Bandung. Saudara telah diminta ikut berpartisipasi dalam penelitian ini. Responden dalam penelitian ini bersifat sukarela. Saudara berhak menolak berpartisipasi dalam penelitian ini. Penelitian ini dilakukan dengan cara mengambil sebanyak 3cc sampel darah yang selanjutnya dibuat plasma darah, kemudian dilakukan pengukuran kadar nikotin dan glukosa darah. Segala informasi yang saudara berikan akan digunakan sepenuhnya hanya dalam penelitian ini. Peneliti sepenuhnya akan menjaga kerahasiaan identitas saudara dan tidak dipublikasikan dalam bentuk apapun. Jika ada yang belum jelas, saudara boleh bertanya pada peneliti. Jika saudara sudah memahami penjelasan ini dan bersedia berpartisipasi dalam penelitian ini, silahkan saudara menandatangani lembar persetujuan yang akan dilampirkan.

Peneliti

Widya Dwi Astuti

**Lampiran 2. Salah satu Lembar Pernyataan Persetujuan (*Informed Consent*)  
Subjek Uji**

**Lampiran 2. Lembar Pernyataan Persetujuan (*Informed Consent*)**

Yang bertandatangan di bawah ini,

Nama : Rakha Prathama F  
Jenis Kelamin : Laki-laki  
No. Tlp/HP : 082121980478  
Umur : 22  
Alamat Rumah : JL. Getot Subroto

Semua penjelasan yang berkaitan dengan penelitian mengenai "Hubungan Kadar Nikotin Terhadap Glukosa Darah Pada Perokok Aktif", telah disampaikan kepada saya dan semua pertanyaan saya telah dijawab oleh peneliti. Saya mengerti bahwa bila memerlukan penjelasan, saya dapat menanyakan kepada Widya Dwi Astuti.

Dengan menandatangani lembar *informed consent* ini, saya setuju untuk ikut serta dalam penelitian ini.

Tandatangan subjek



Tanggal 27 Februari 2020

Tandatangan saksi



### Lampiran 3. Salah satu Kuesioner Subjek Uji yang masuk dalam Kriteria Inklusi

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#### Lampiran 3. Formulir Kuesioner Subjek Uji

Nama : Dzikra Pratiwara F  
Umur : 22  
Jenis Kelamin : Laki-laki  
Pekerjaan : -  
No. Tlp/HP : 082121480478  
Waktu pengambilan darah : 27 Februari 2020 / 21.15 WIB

#### Jawablah petanyaan berikut!

1. Berapa lama anda menjadi perokok aktif?  
Jawab: .....7.....th
2. Berapa banyak batang rokok perhari yang anda hisap?
  - a. <10 : ..... batang
  - ✓ b. 11 – 20 : .....12..... batang
  - c. 21 – 30 : ..... batang
3. Apakah anda memiliki riwayat penyakit berikut?
  - a. Diabetes Melitus (DM)
  - b. Hipertensi

✓ .....-
4. Pukul berapa terakhir kali anda merokok? 15.00
5. Berapa jumlah batang rokok yang anda konsumsi sehari sebelum pengambilan darah? 12
6. Pukul berapa terakhir kali anda makan? 13.00
7. Makanan/minuman apa yang anda konsumsi sehari sebelum pengambilan darah? Nasi

Bandung, 27 Februari 2020

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## Lampiran 4. Kit Insert Glucose GOD-PAP



**BIOLABO**  
[www.biolabo.fr](http://www.biolabo.fr)  
**MANUFACTURER:**  
**BIOLABO SA,**  
Les Hautes Rives  
02160, Maizy, France

REF 80009	R1	1 x 500 mL	R2	1 x 7.5 mL	R3	1 x 5 mL
REF 87109	R1	6 x 250 mL	R2	6 x 3.75 mL	R3	1 x 5 mL
REF 16GL8	R1	6 x 1000 mL	R2	6 x 15 mL	R3	1 x 10 mL

### TECHNICAL SUPPORT AND ORDERS

Tel : (33) 03 23 25 15 50

Fax : (33) 03 23 256 256



**IVD IN VITRO DIAGNOSTIC USE**

### CLINICAL SIGNIFICANCE (1) (6)

The glucose level in blood is maintained within a fairly narrow range under diverse conditions (feeding, fasting, or severe exercise) by regulatory hormones such as insulin, glucagon, or epinephrin. Measurement of glucose is one of the most frequently performed procedures in clinical chemistry laboratories in conjunction with other tolerance testing (Glucose tolerance test, Glucose 2h post-prandial...). The most frequently encountered disorder of carbohydrate metabolism in blood is hyperglycemia due to diabetes mellitus.

Hyperglycemia higher than 300 mg/dL (16.5 mmol/L) may induce ketoacidosis and hyperosmolar coma.

In prolonged hypoglycemia, lower than 30 mg/dL (1.7 mmol/L), severe irreversible encephalic damage may occurs.

### PRINCIPLE (4) (5)

Trinder Method. Glucose is oxidised by GOD to gluconic acid and hydrogen peroxide which in conjunction with POD, reacts with chloro-4-phenol and PAP to form a red quinonoidimine. The absorbance of the coloured complexe, proportional to the concentration of glucose in the specimen is measured at 500 nm.

### REAGENTS COMPOSITION

#### Vial R1 ENZYME-BUFFER

Phosphate Buffer	150 mmol/L
Glucose oxidase (GOD)	≥ 20 000 UI/L
Peroxidase (POD)	≥ 1000 UI/L
4-Amino-antipyrine (PAP)	0.8 mmol/L

#### Vial R2 CHROMOGEN

Chloro-4-phenol	2 mmol/L
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#### Vial R3 STANDARD

Glucose 100 mg/dL (5.55 mmol/L)

### SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

### REAGENTS PREPARATION

**Vial R1 and R2:** If appropriate, use a non-sharp instrument to remove aluminium cap.

Using a volumetric flask, measure the volume of demineralised water stated on the label of the vial R1 (Enzymes-Buffer).

Transfer the contents of vial R1 into the flask and mix gently until complete dissolution (approximately 2 minutes). Then, add the contents of vial R2 and mix gently. Store away from light in a plastic bottle free from contamination.

### STABILITY AND STORAGE

**Store at 2-8°C, away from light.**

- Standard (vial R3):** transfer requested quantity, well recap the vial and store at 2-8°C.
- Reagent R1 (unopened) and reagents R2 and R3 are stable until expiry date stated on the label of the kit when stored and used as described.
- Once reconstituted, working reagent is stable for at least 2 years when free from contamination.
- Discard reagent if cloudy or if reagent blank at 500 nm is > 0.400.
- Don't use working reagent after expiry date stated on the label of the kit.

### SPECIMEN COLLECTION AND HANDLING (2)

**Serum or plasma:**

Separate promptly from cells to prevent glycolysis. If fluoride is used as a preservative, a decrease of 9 mg/dL (0.5 mmol/L) is seen within the first 2 hours, then concentration stabilises.

Glucose is stable in serum or heparinised plasma :

- for 8 h at 25°C
- for 72 h at 2-8°C

Glucose is stable in plasma (Sodium fluoride or iodoacetate) :

- for 24 h at room temperature.

**CSF:**

Process immediately to avoid falsely low results. Store at -20°C.

**Urine:**

Collect in dark bottle and store at 2-8°C. Preserve 24 h urines with 5 mL glacial acetic acid or 5 g sodium benzoate or sodium fluoride.

### INTERFERENCES (3)

**Ascorbic acid:** No interference up to 10 mg/dL.

**Total bilirubin:** Negative interference above 20 mg/dL.

**Direct bilirubin:** No interference.

**Hemolysis:** No interference.

**Lipemia:** Positive interference above 626 mg/dL of triglycerides.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

### MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.

2. Normal and pathological control sera.

## CALIBRATION

- Standard enclosed in the Kit (vial R3) or BIOLABO-Multicalibrator REF 95015 traceable to SRM 965a.
- Or any calibrator traceable to a reference method or material. The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:

1. When changing batch of reagent.
2. After maintenance operations on the instrument.
3. If control values are out of range, even after using a new vial of fresh control.

## QUALITY CONTROL

- BIOLABO EXATROL-N (level I) REF 95010.
- BIOLABO EXATROL-P (level II) REF 95011.
- Assayed control referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
  - At least once within 24 hours.
  - When changing vial of reagent.
  - After maintenance operations on the instrument.
- If control is out of range, apply following actions:
1. Repeat the test with the same control.
  2. If control is still out of range, prepare a fresh control and repeat the test.
  3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
  4. If control is still out of range, calibrate with a new vial of reagent.
  5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

## EXPECTED VALUES (2)

In serum or plasma :	mg/dL	[mmol/L]
Newborn, 1 day	40-60	[ 2.2-3.3 ]
Newborn > 1 day	50-80	[ 2.8-4.4 ]
Children	60-100	[ 3.3-5.6 ]
Adult	74-106	[ 4.1-5.9 ]
60-90 years	82-115	[ 4.6-6.4 ]
> 90 years	75-121	[ 4.2-6.7 ]

In CSF :	mg/dL	[mmol/L]
Infant, Child	60-80	[ 3.3-4.4 ]
Adult	40-70	[ 2.2-3.9 ]

In 24 h urines : 1-15 mg/dL [0.1-0.8 mmol/L]

< 0.5 g/24 hours [<2.78 mmol/24 hours]

Each laboratory should establish its own normal ranges for the population that it serves.

## PERFORMANCES

Within run N = 30	Normal level	High level	Between run N = 60	Normal level	High level
Mean mg/dL	81	269	Mean mg/dL	81	284
S.D. mg/dL	1.05	1.80	S.D. mg/dL	0.97	3.01
C.V. %	1.3	0.67	C.V. %	1.2	1.06

Detection limit: approximately 10 mg/dL

Sensitivity for 100 mg/dL: approximately 0.420 Abs at 500 nm.

Comparison with a commercially available reagent :

y = 0.969 x + 1.33

r = 0.9984

## LINEARITY

The reaction is linear up to at least 500 mg/dL (28 mmol/L).

Above, dilute the specimen with saline solution and re-assay taking into account dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

## MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into well identified test tubes :	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL

Mix. Let stand for 10 minutes at 37°C or 20 minutes at room temperature. Read absorbance at 500 nm (460-560) against reagent blank. Coloration is stable for 15-20 minutes at 37°C, and then slowly decreases.

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

## CALCULATION

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

## REFERENCES

- (1) Tietz Textbook of clinical chemistry, 3<sup>rd</sup> Ed. C.A. Burtis, E.R. Ashwood. W.B. Saunders (1999) p. 750-785.
- (2) Clinical Guide to Laboratory Test, 4<sup>th</sup> Ed., N.W. Tietz (2006) p. 444-455
- (3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests. 4<sup>th</sup> Ed. (1995) p. 3-274 to 3-294.
- (4) FARRANCE I. Clin. Biochem. reviews (1987), 8, p.55 to 68.
- (5) TRINDER P.. Ann. Clin. Biochem.(1969), 6, p.24-27.
- (6) BERNARD S., Biochimie clinique, 2<sup>nd</sup> éd. Edition Maloine (1989), p.165-167
- (7) SRM : Standard Reference Material ®



**Lampiran 5. Hasil Pemeriksaan Kadar Nikotin dan Glukosa Darah**

NO	Kode Sampel	Usia (Tahun)	Kadar Nikotin (mg/L)	Kadar Glukosa Darah (mg/dL)
1	Sp01	22	Tidak Terdeteksi	75
2	Sp02	30	Tidak Terdeteksi	81
3	Sp03	24	0,4644	107
4	Sp04	30	0,4399	97
5	Sp05	22	0,3347	93
6	Sp06	21	Tidak Terdeteksi	80
7	Sp07	21	Tidak Terdeteksi	73
8	Sp08	22	Tidak Terdeteksi	100
9	Sp09	29	Tidak Terdeteksi	77
10	Sp10	22	Tidak Terdeteksi	91
11	Sp11	30	Tidak Terdeteksi	93
12	Sp12	24	Tidak Terdeteksi	73
13	Sp13	23	Tidak Terdeteksi	76
14	Sp14	21	Tidak Terdeteksi	76
15	Sp15	23	Tidak Terdeteksi	88
16	Sp16	30	0,2592	117
17	Sp17	30	0,0945	74
18	Sp18	29	0,2914	101
19	Sp19	21	0,0724	91
20	Sp20	30	0,1661	98
21	Sp21	21	Tidak Terdeteksi	85

## Lampiran 6. Hasil Uji Statistik Data Penelitian

Hasil Uji Statistik data penelitian menggunakan SPSS

### 1. Uji Statistik Gambaran Kadar Nikotin dan Glukosa Darah Perokok Aktif

#### Frequencies

Statistics		
	Kadar Nikotin	Kadar Glukosa Darah
N	21	21
Valid		
Missing	0	0
Mean	,101076	87,90
Std. Error of Mean	,0345212	2,721
Std. Deviation	,1581959	12,470
Variance	,025	155,490
Range	,4644	44
Minimum	,0000	73
Maximum	,4644	117

#### Frequency Table

Kadar Nikotin					
	Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	,0000	13	61,9	61,9	61,9
	,0724	1	4,8	4,8	66,7
	,0945	1	4,8	4,8	71,4
	,1661	1	4,8	4,8	76,2
	,2592	1	4,8	4,8	81,0
	,2914	1	4,8	4,8	85,7
	,3347	1	4,8	4,8	90,5
	,4399	1	4,8	4,8	95,2
	,4644	1	4,8	4,8	100,0
	Total	21	100,0	100,0	

**Kadar Glukosa Darah**

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	73	2	9,5	9,5
	74	1	4,8	14,3
	75	1	4,8	19,0
	76	2	9,5	28,6
	77	1	4,8	33,3
	80	1	4,8	38,1
	81	1	4,8	42,9
	85	1	4,8	47,6
	88	1	4,8	52,4
	91	2	9,5	61,9
	93	2	9,5	71,4
	97	1	4,8	76,2
	98	1	4,8	81,0
	100	1	4,8	85,7
	101	1	4,8	90,5
	107	1	4,8	95,2
	117	1	4,8	100,0
Total		21	100,0	100,0

**Case Processing Summary**

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Kadar Nikotin * Kadar Glukosa Darah	21	100,0%	0	0,0%	21	100,0%

**Kadar Nikotin \* Kadar Glukosa Crosstabulation**

Count

		Kadar Glukosa Darah			Total
		Rendah	Normal	Tinggi	
Kadar Nikotin	Tidak Terdeteksi	2	11	0	13
	Terdeteksi	0	6	2	8
Total		2	17	2	21

## 2. Korelasi Kadar Nikotin dan Glukosa Darah Perokok Aktif

		Correlations	
		Kadar Nikotin	Kadar Glukosa
Spearman's rho	Correlation Coefficient	1,000	,557**
	Kadar Nikotin	Sig. (2-tailed)	,009
		N	21
	Correlation Coefficient	,557**	1,000
	Kadar Glukosa	Sig. (2-tailed)	,
		N	21

\*\*. Correlation is significant at the 0.01 level (2-tailed).

**Lampiran 7. Kromatogram Nikotin Sampel 01 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

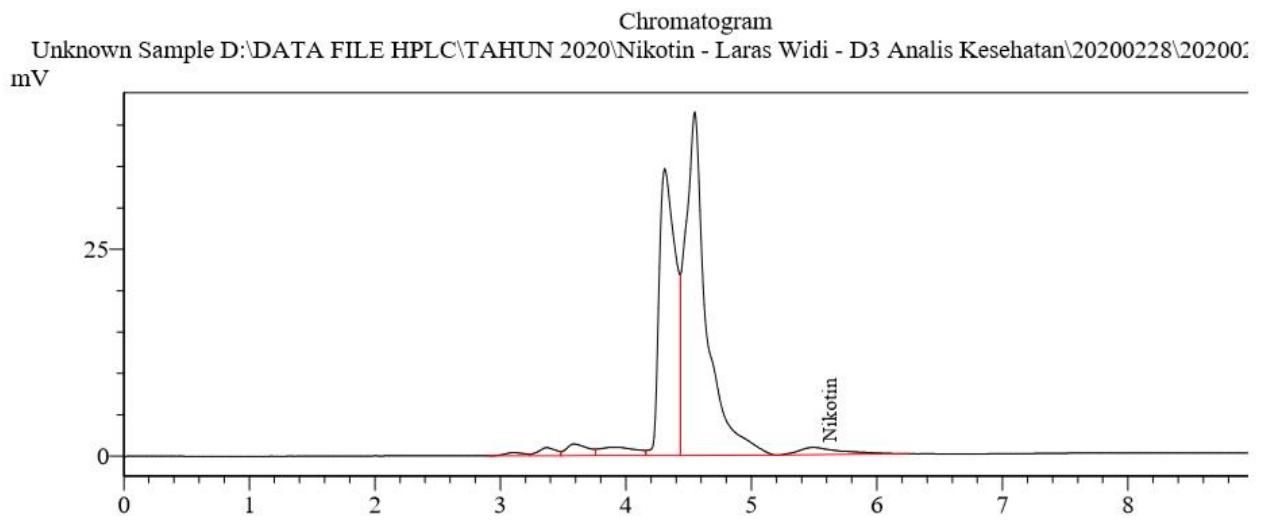
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 01  
Injection Volume : 20 mikron  
Data File Name : 20200228-19.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.486	21654	2.337	2.198	1.842	1558.059	0.769

**Lampiran 8. Kromatogram Nikotin Sampel 02 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

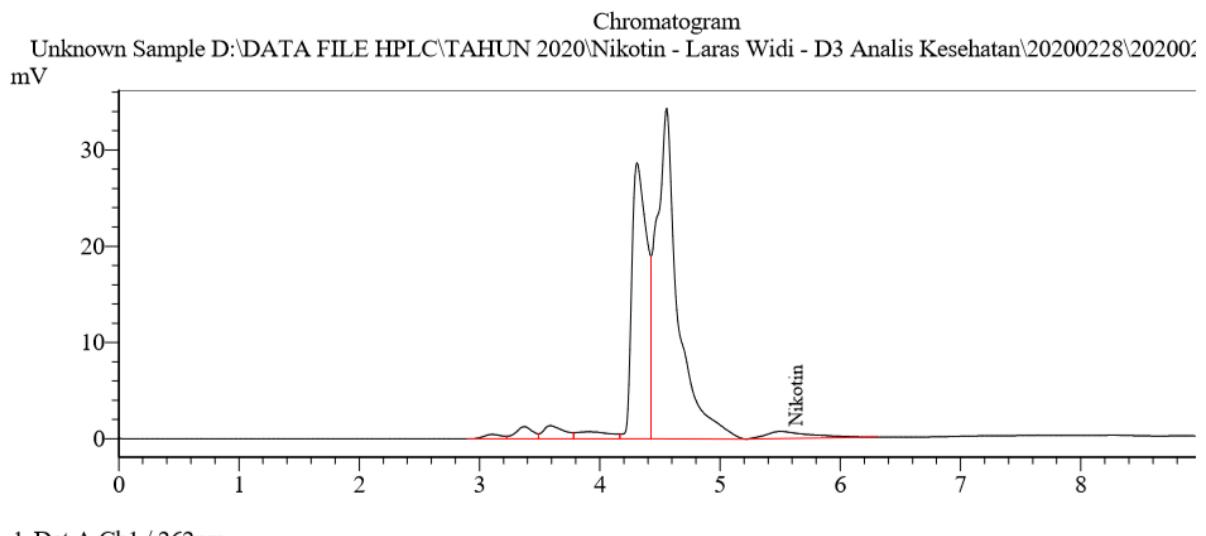
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 02  
Injection Volume : 20 mikron  
Data File Name : 20200228-20.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.497	19074	2.249	1.745	1.871	1264.572	0.772

**Lampiran 9. Kromatogram Nikotin Sampel 03 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

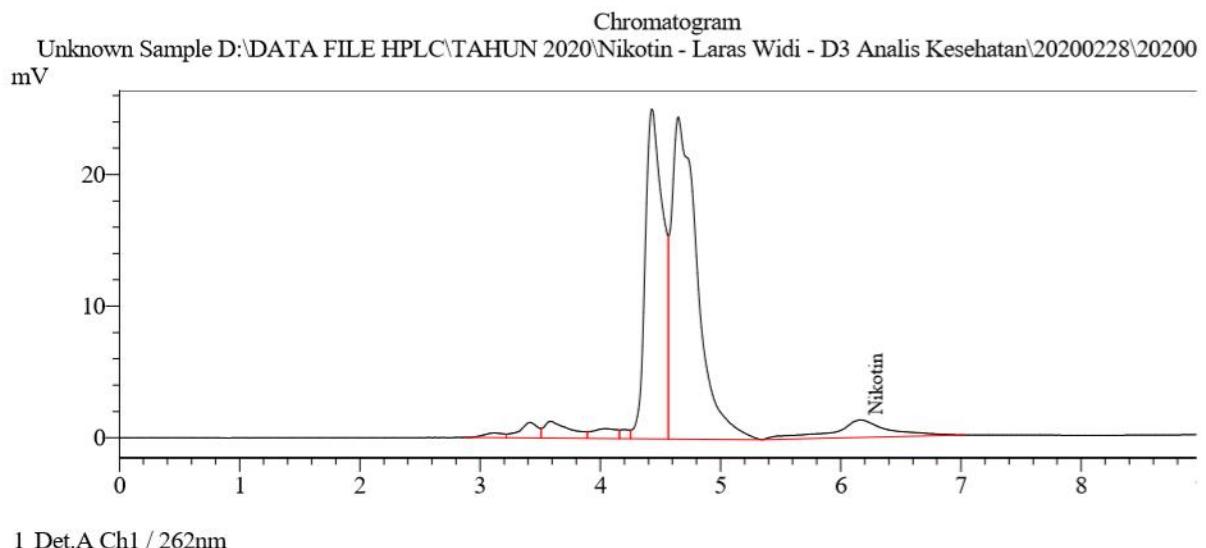
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 03  
Injection Volume : 20 mikron  
Data File Name : 20200228-5.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	6.159	42338	5.283	3.074	0.973	1903.585	0.979

**Lampiran 10. Kromatogram Nikotin Sampel 04 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

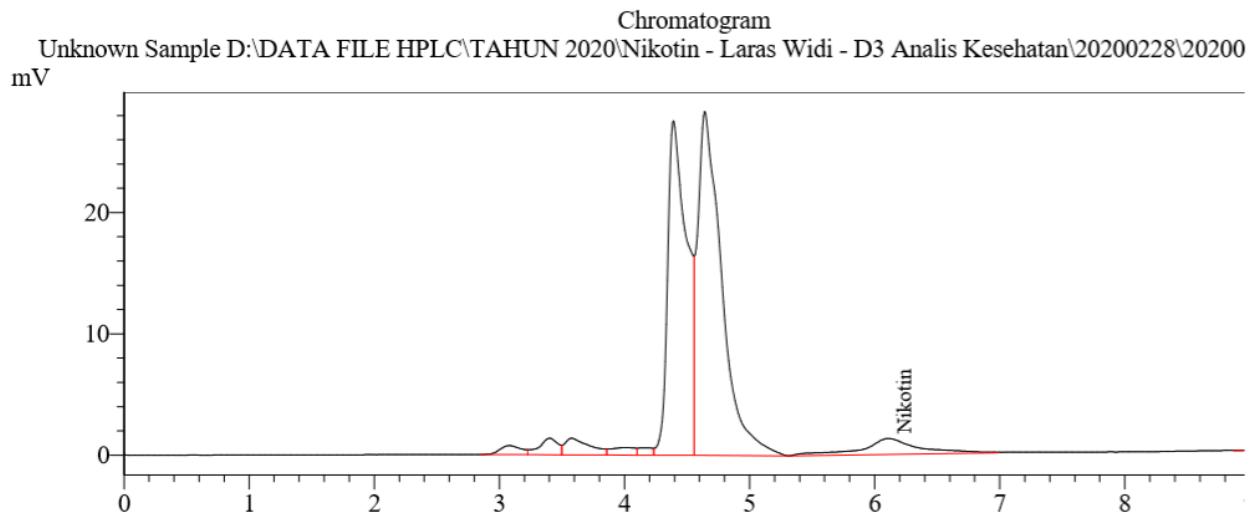
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 04  
Injection Volume : 20 mikron  
Data File Name : 20200228-6.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	6.103	41540	4.957	3.023	1.050	1814.035	0.985

**Lampiran 11. Kromatogram Nikotin Sampel 05 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

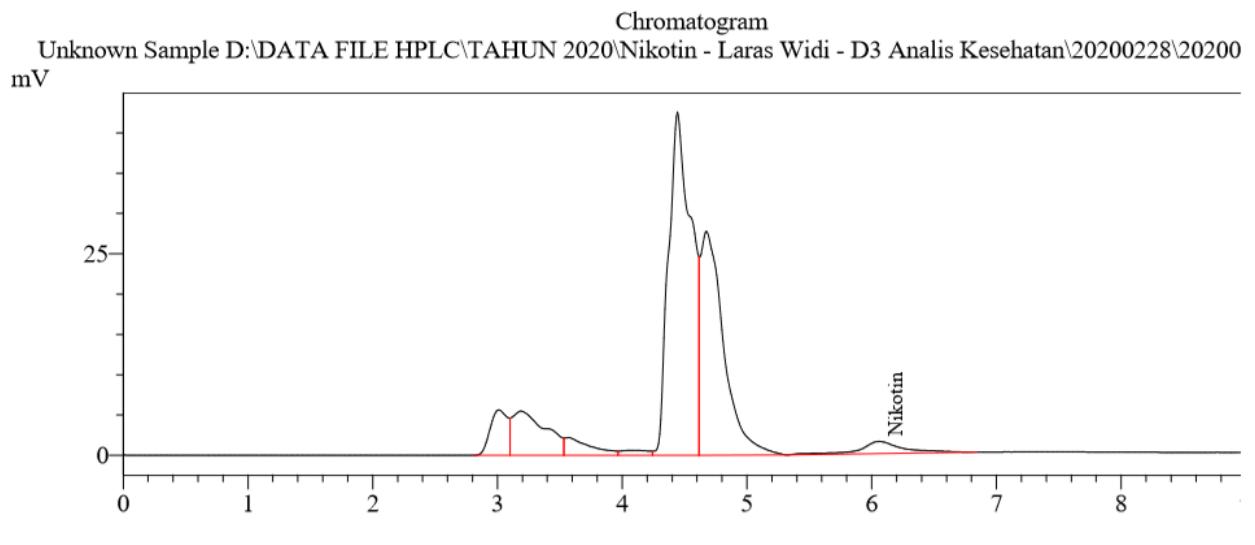
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 05  
Injection Volume : 20 mikron  
Data File Name : 20200228-7.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	6.056	38104	3.127	2.435	0.974	2414.700	1.014

**Lampiran 12. Kromatogram Nikotin Sampel 06 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

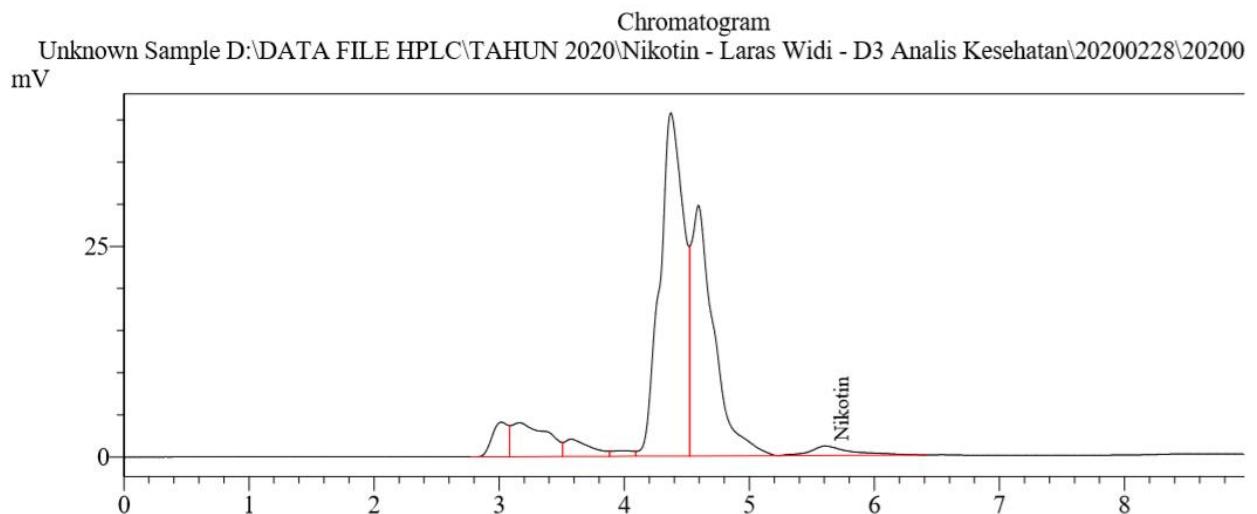
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 06  
Injection Volume : 20 mikron  
Data File Name : 20200228-8.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.605	24966	2.120	1.945	1.476	2187.276	0.860

**Lampiran 13. Kromatogram Nikotin Sampel 07 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

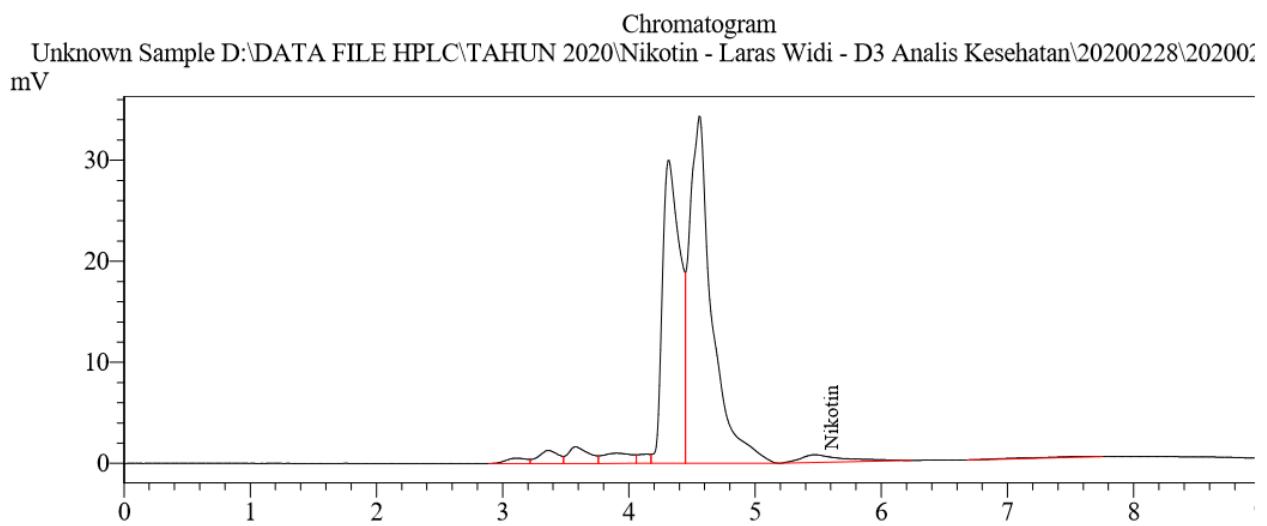
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 07  
Injection Volume : 20 mikron  
Data File Name : 20200228-10.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.468	18264	2.021	2.091	1.887	1707.785	0.760

**Lampiran 14. Kromatogram Nikotin Sampel 08 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

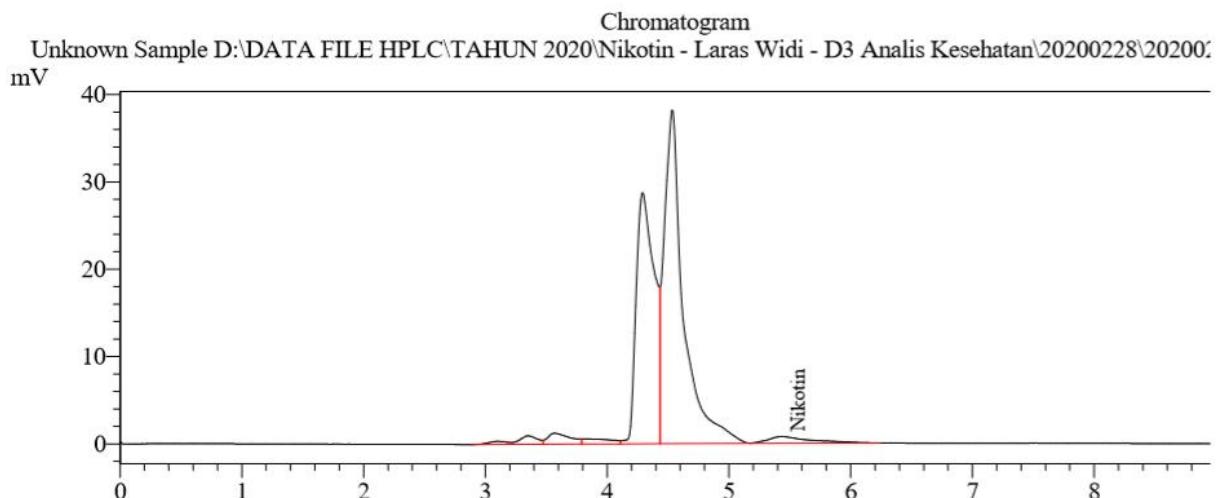
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 08  
Injection Volume : 20 mikron  
Data File Name : 20200228-11.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.431	19157	2.360	2.149	1.890	1570.272	0.753

**Lampiran 15. Kromatogram Nikotin Sampel 09 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

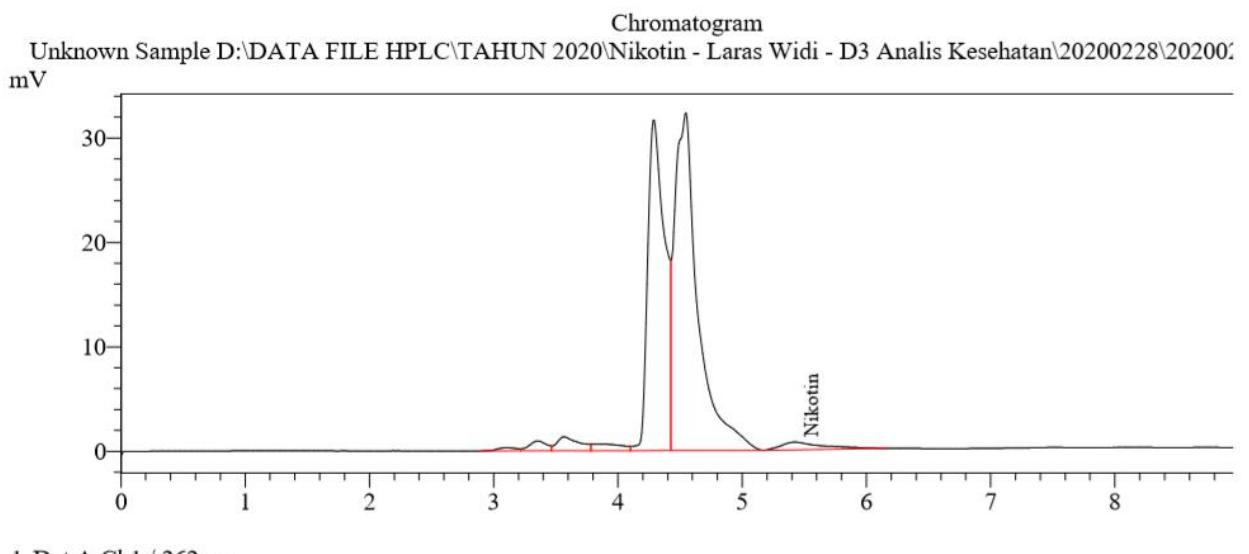
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 09  
Injection Volume : 20 mikron  
Data File Name : 20200228-12.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.420	17174	2.037	2.030	1.997	1753.717	0.750

**Lampiran 16. Kromatogram Nikotin Sampel 10 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

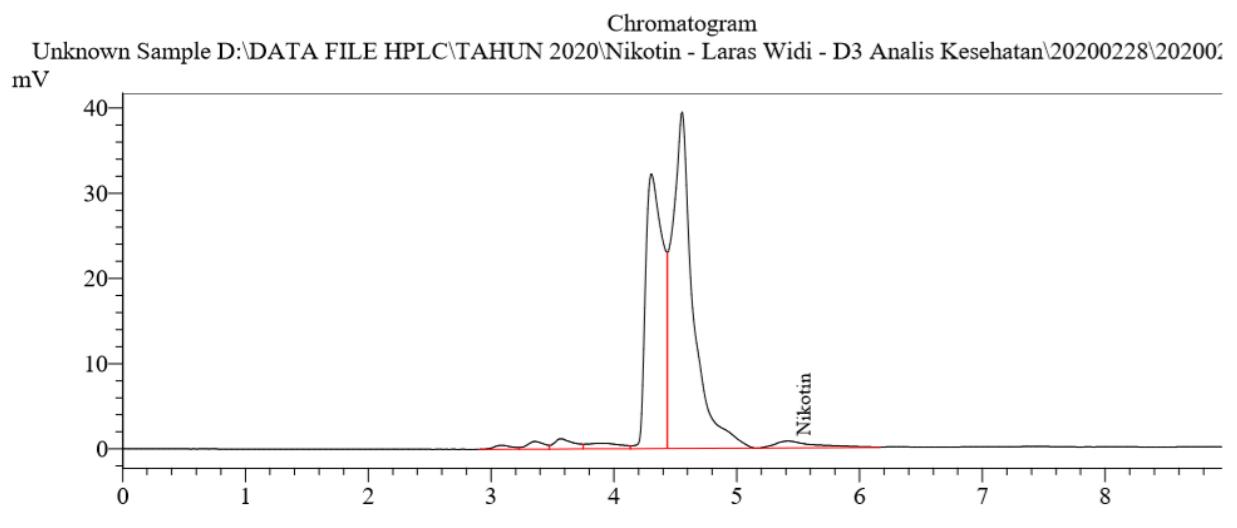
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 10  
Injection Volume : 20 mikron  
Data File Name : 20200228-13.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.410	19308	2.097	2.128	0.000	2011.973	0.757

**Lampiran 17. Kromatogram Nikotin Sampel 11 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

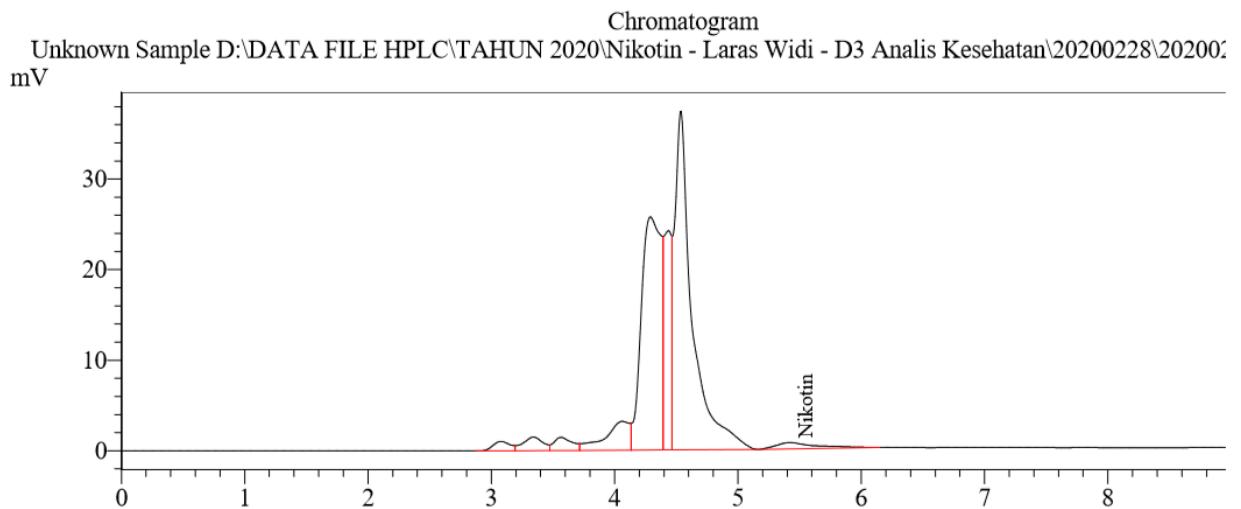
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 11  
Injection Volume : 20 mikron  
Data File Name : 20200228-14.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.416	15884	1.718	2.326	1.940	1903.444	0.763

**Lampiran 18. Kromatogram Nikotin Sampel 12 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

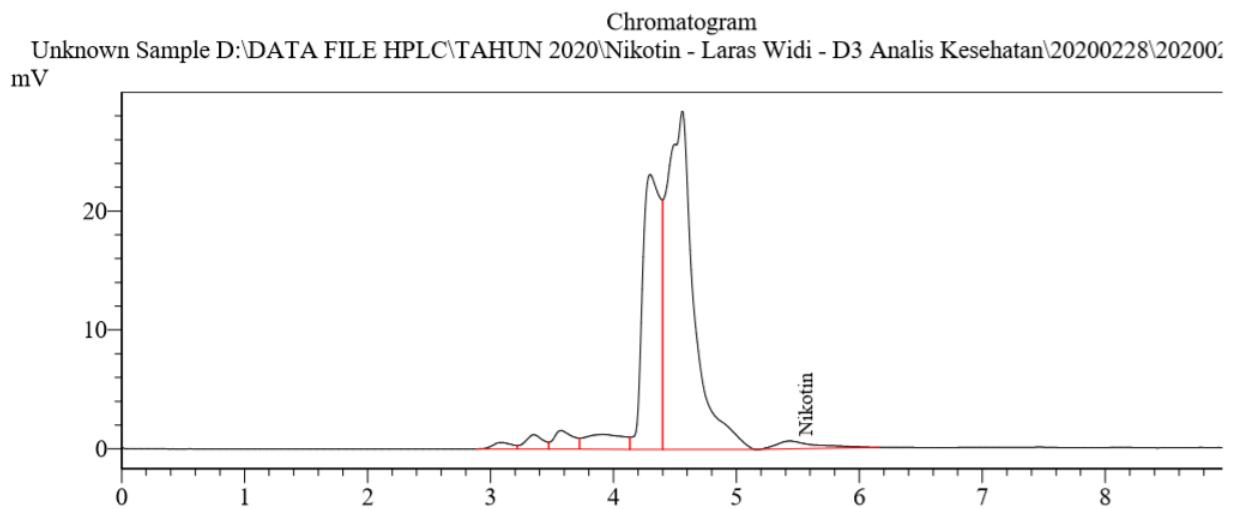
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 12  
Injection Volume : 20 mikron  
Data File Name : 20200228-15.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.428	15624	1.930	1.541	1.943	1778.003	0.761

**Lampiran 19. Kromatogram Nikotin Sampel 13 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

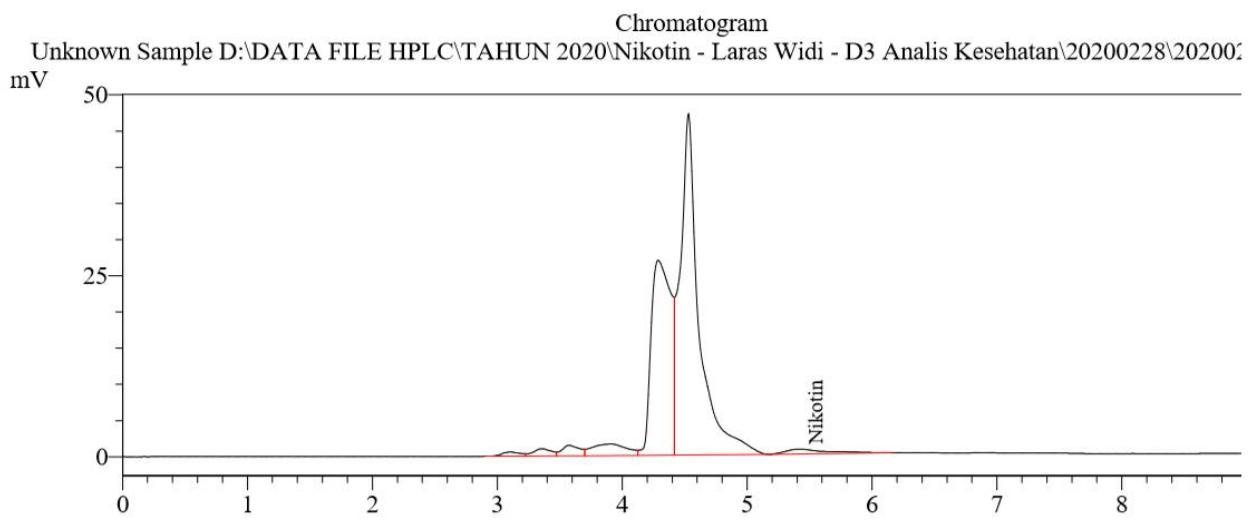
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 13  
Injection Volume : 20 mikron  
Data File Name : 20200228-16.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.414	15874	1.701	2.411	2.041	1889.879	0.748

**Lampiran 20. Kromatogram Nikotin Sampel 14 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

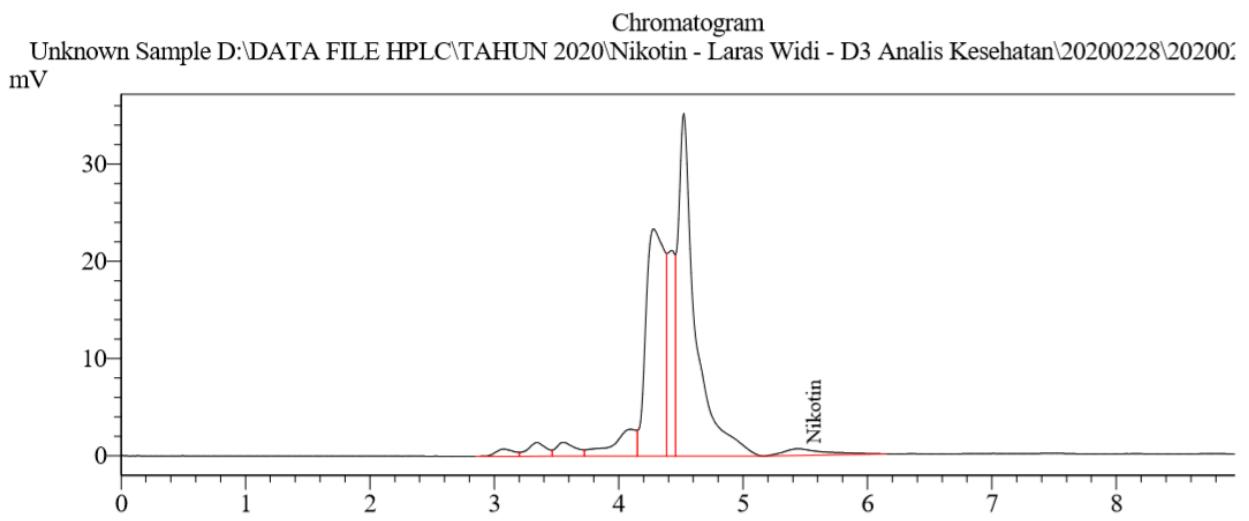
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 14  
Injection Volume : 20 mikron  
Data File Name : 20200228-17.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.436	16140	1.945	2.364	1.807	1689.861	0.769

**Lampiran 21. Kromatogram Nikotin Sampel 15 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

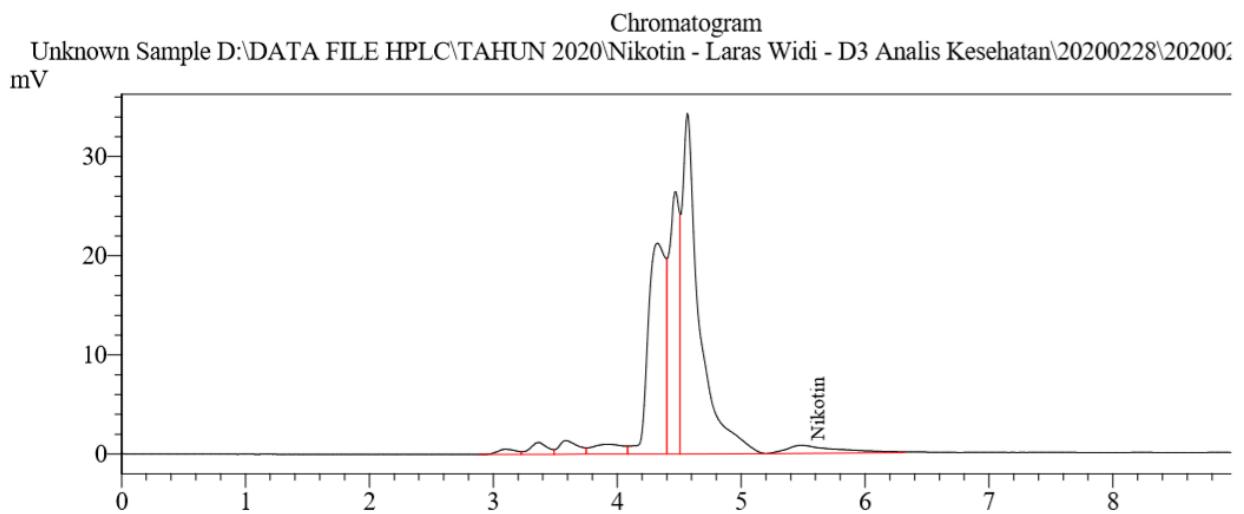
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 15  
Injection Volume : 20 mikron  
Data File Name : 20200228-18.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200228.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.482	23052	2.863	1.676	0.000	750.127	0.770

**Lampiran 22. Kromatogram Nikotin Sampel 16 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

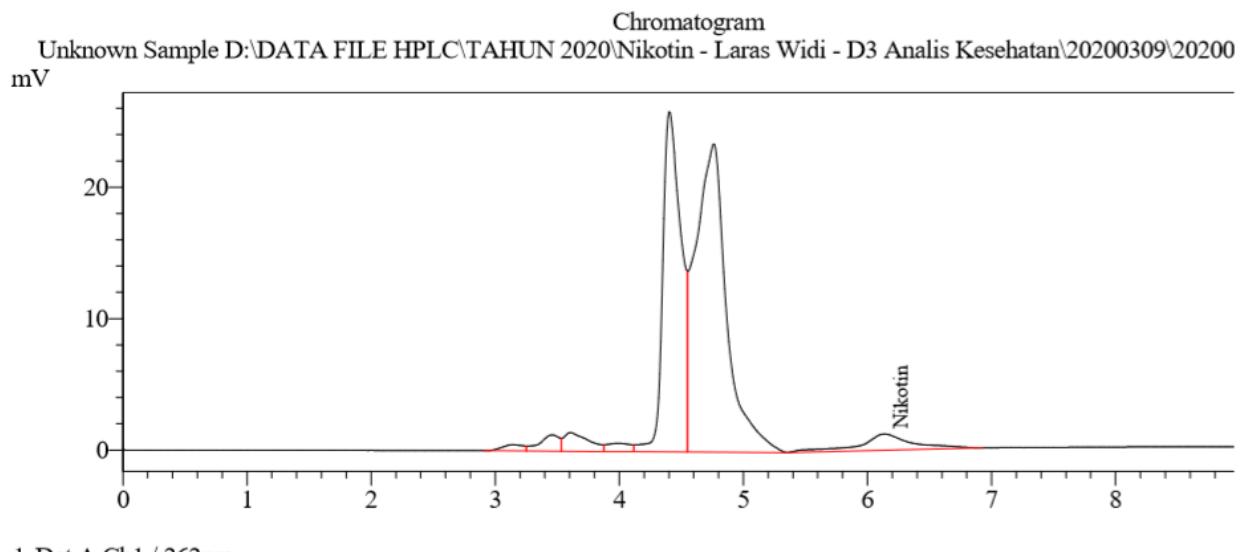
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 16  
Injection Volume : 20 mikron  
Data File Name : 20200309-2.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	6.134	35640	4.484	2.572	0.956	1984.092	0.956

**Lampiran 23. Kromatogram Nikotin Sampel 17 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

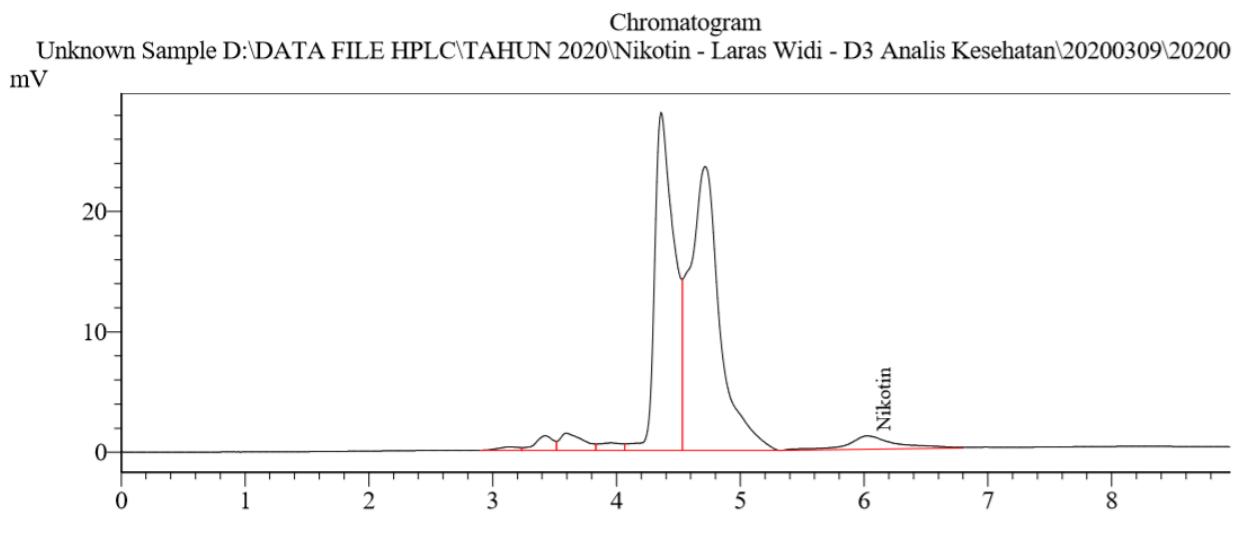
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 17  
Injection Volume : 20 mikron  
Data File Name : 20200309-3.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	6.023	30262	3.720	1.504	1.079	1962.958	0.927

**Lampiran 24. Kromatogram Nikotin Sampel 18 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

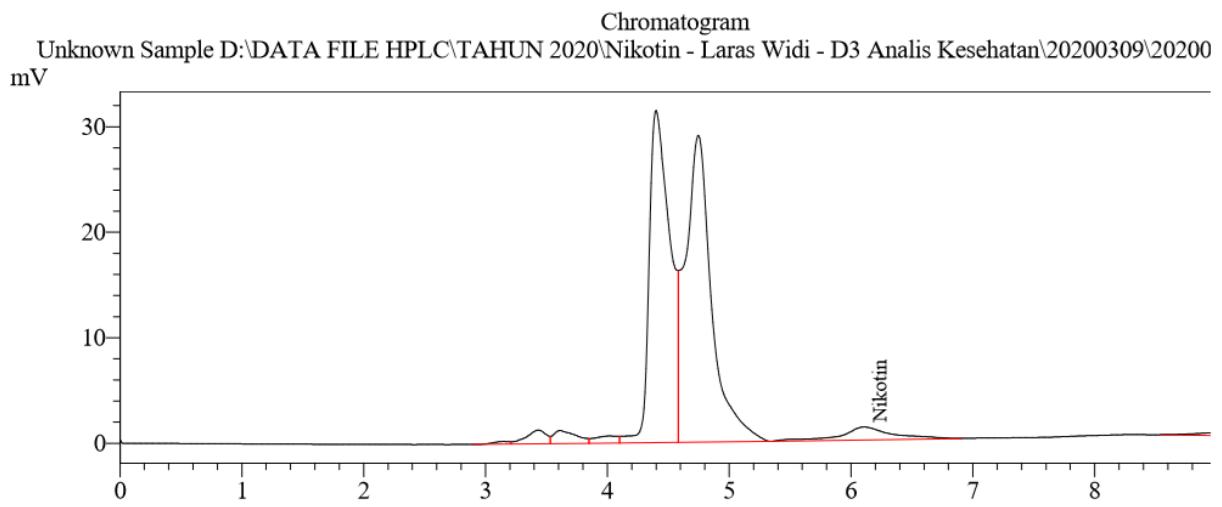
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 18  
Injection Volume : 20 mikron  
Data File Name : 20200309-4.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	6.104	36691	3.804	2.862	0.991	1824.697	0.943

**Lampiran 25. Kromatogram Nikotin Sampel 19 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

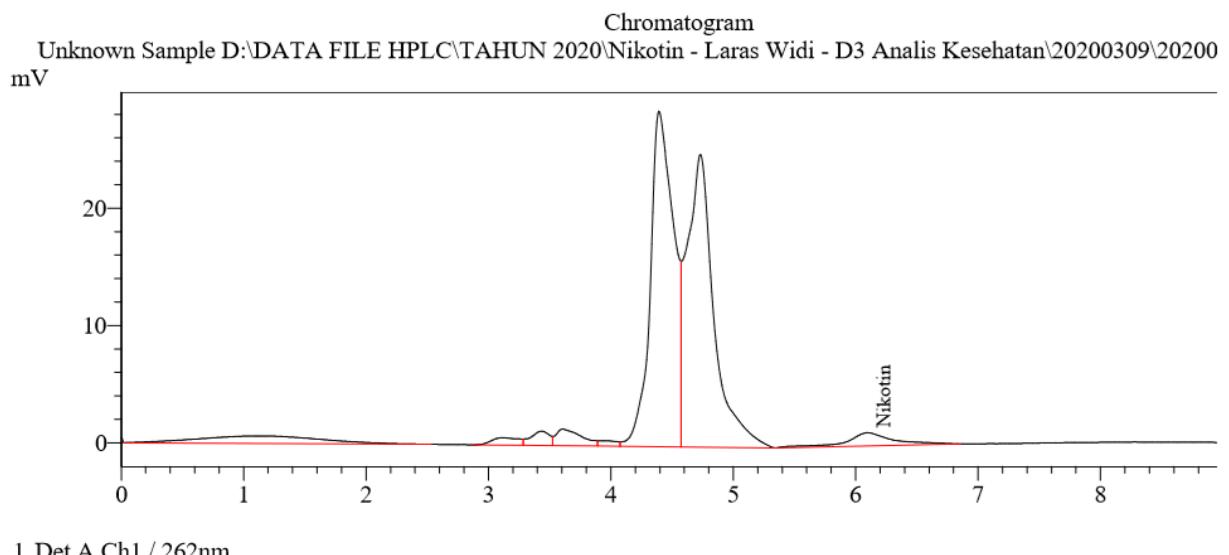
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 19  
Injection Volume : 20 mikron  
Data File Name : 20200309-5.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	6.094	29542	3.216	2.803	0.969	2100.873	4.377

**Lampiran 26. Kromatogram Nikotin Sampel 20 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

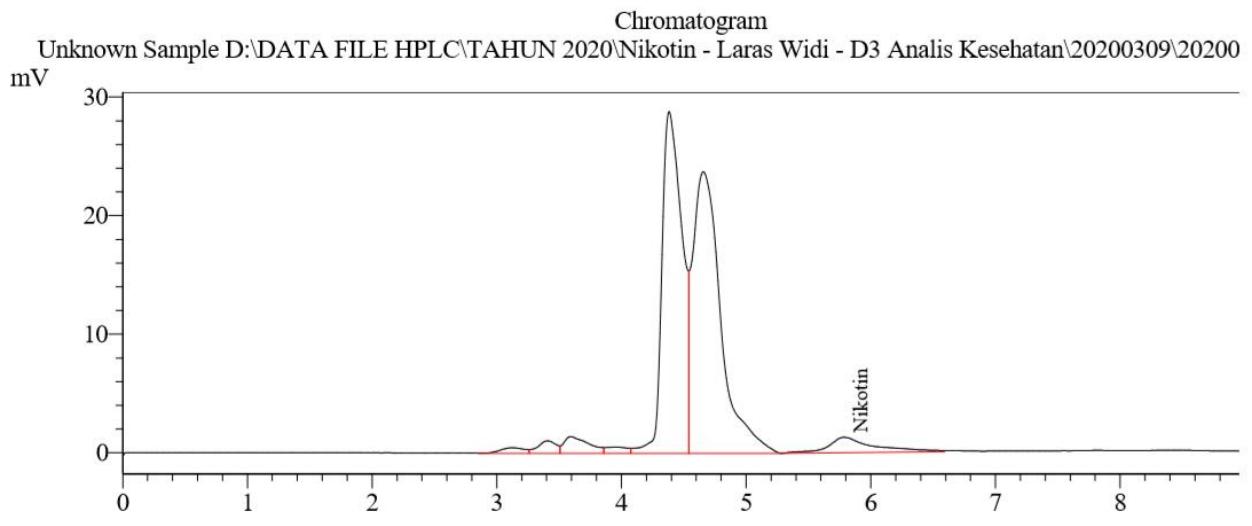
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 20  
Injection Volume : 20 mikron  
Data File Name : 20200309-6.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



1 Det.A Ch1 / 262nm

**Peak Table**

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.784	32600	3.936	2.383	0.000	2200.862	0.855

**Lampiran 27. Kromatogram Nikotin Sampel 21 Adisi 1 ppm dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

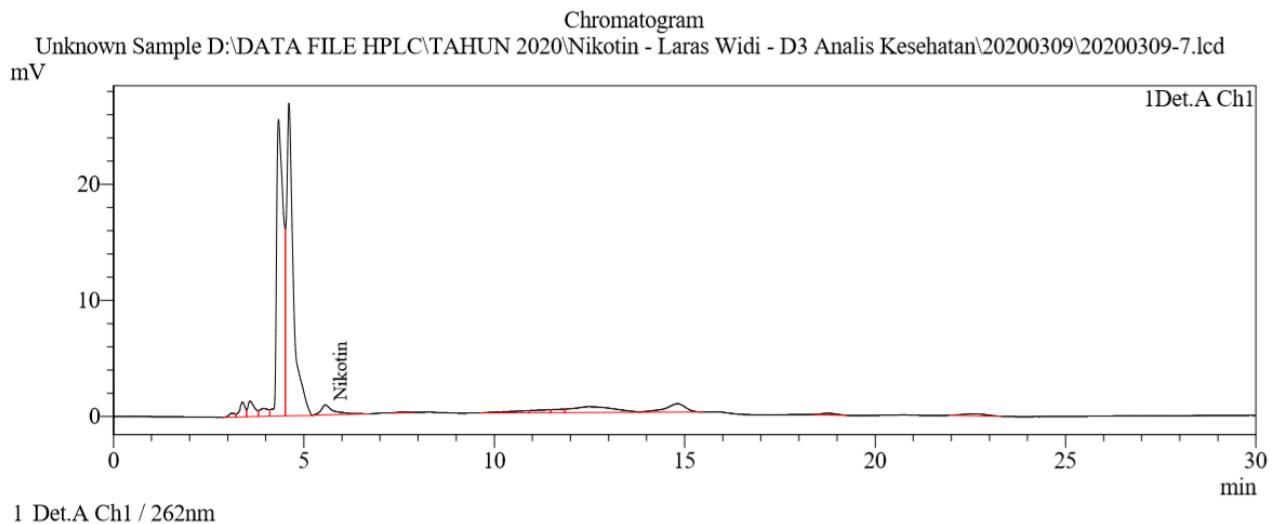
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Sampel  
Sample ID : 21  
Injection Volume : 20 mikron  
Data File Name : 20200309-7.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200309.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.560	20681	2.537	2.293	2.168	2202.628	0.782

**Lampiran 28. Kromatogram Optimasi Metode dengan Fasa Gerak Metanol  
: Air (60:40) Laju alir 1mL/menit Injeksi Sampel**

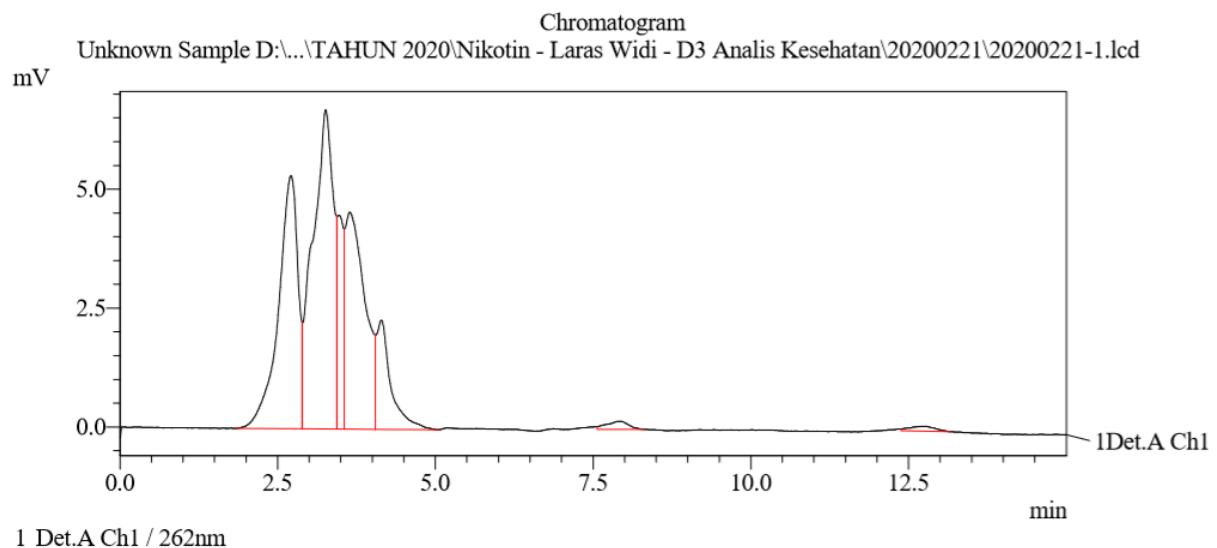
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-1.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi sampel dalam fasa gerak Methanol : Air (60:40)  
Injection Volume : 20 mikron  
Data File Name : 20200221-1.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-1.lcd  
Detector A

**Lampiran 29. Kromatogram Optimasi Metode dengan Fasa Gerak Metanol : Air (60:40) Laju alir 1mL/menit Injeksi Standar**

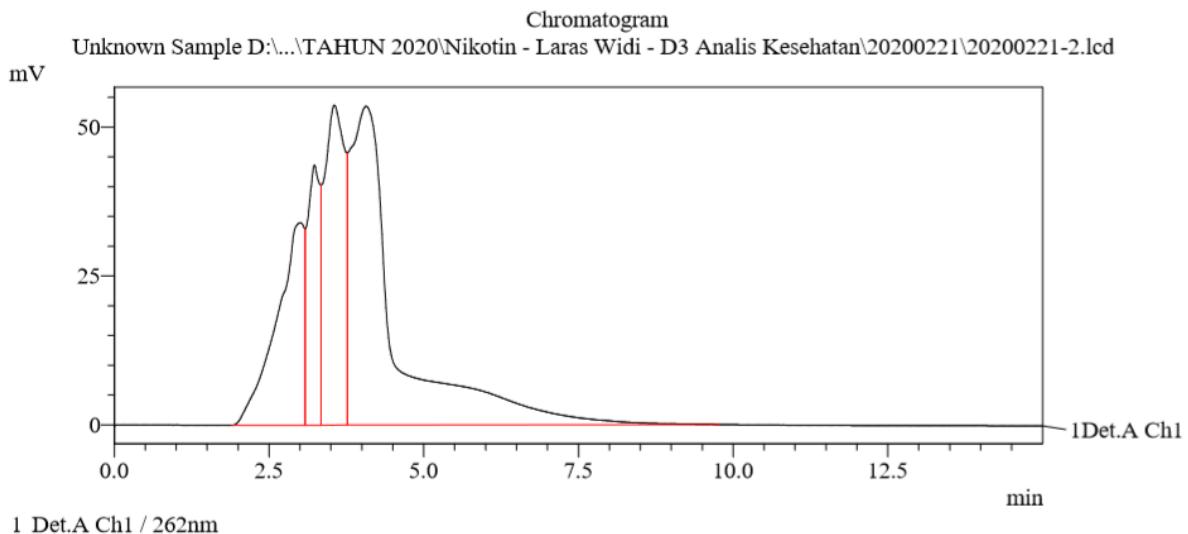
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-2.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Methanol : Air (60:40)  
Injection Volume : 20 mikron  
Data File Name : 20200221-2.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-2.lcd  
Detector A

**Lampiran 30 . Kromatogram Optimasi Metode dengan Fasa Gerak Metanol  
: Air (60:40) Laju alir 1mL/menit Injeksi Standar**

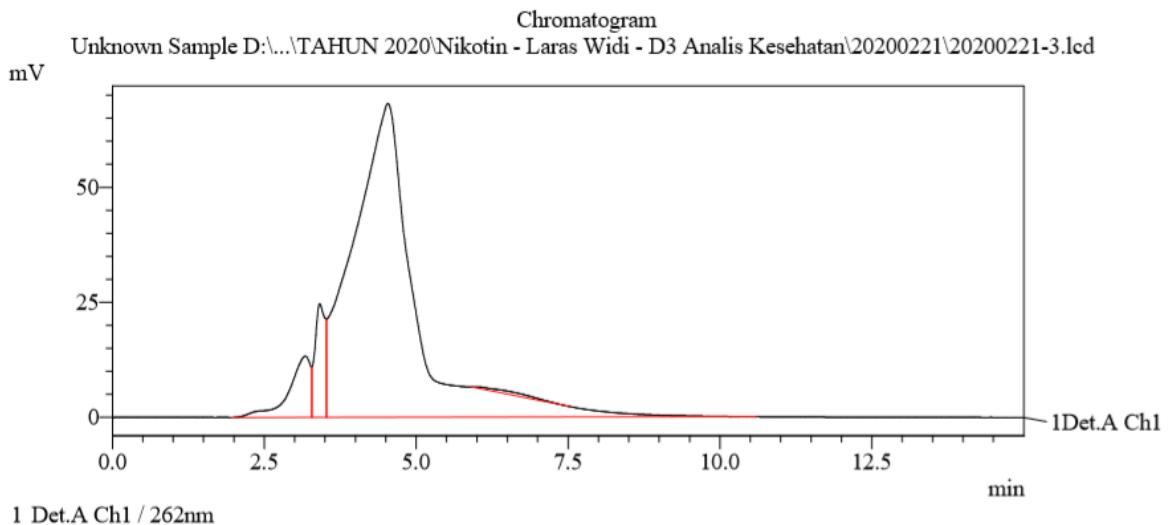
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-3.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Aqua pro injection dalam fasa gerak Methanol :  
Air (60:40)  
Injection Volume : 20 mikron  
Data File Name : 20200221-3.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-3.lcd  
Detector A

**Lampiran 31. Kromatogram Optimasi Metode dengan Fasa Gerak Air :  
Metanol : Buffer Asetat 0,1M pH 4 (10:60:30) Laju alir 1mL/menit**

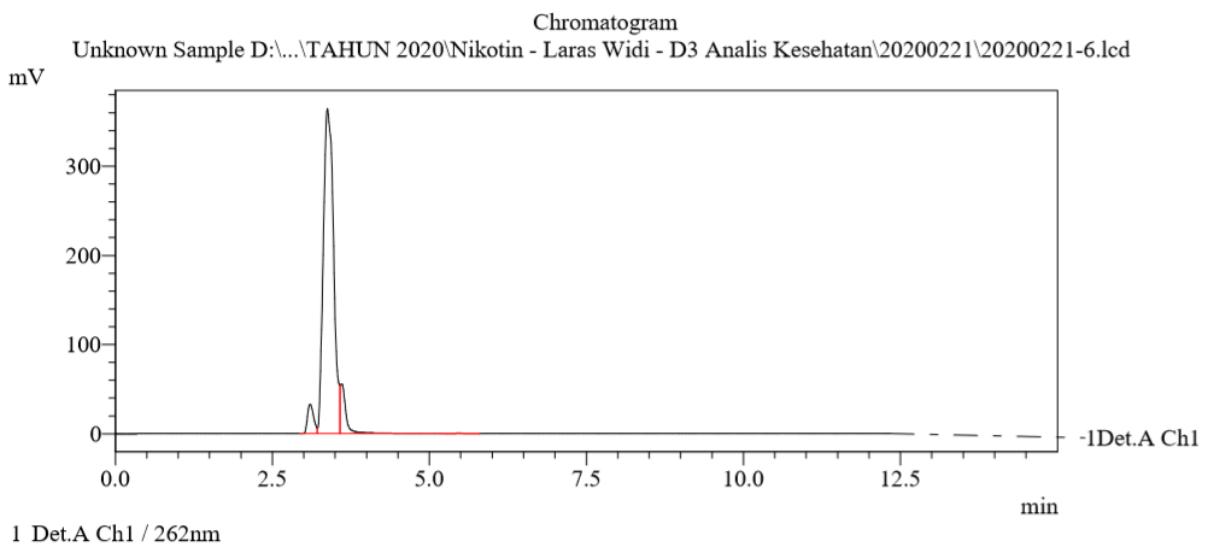
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-6.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer (10:60:30)  
Injection Volume : 20 mikron  
Data File Name : 20200221-6.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-6.lcd  
Detector A

**Lampiran 32. Kromatogram Optimasi Metode dengan Fasa Gerak Metanol :  
Buffer (70:30) Laju alir 1mL/menit**

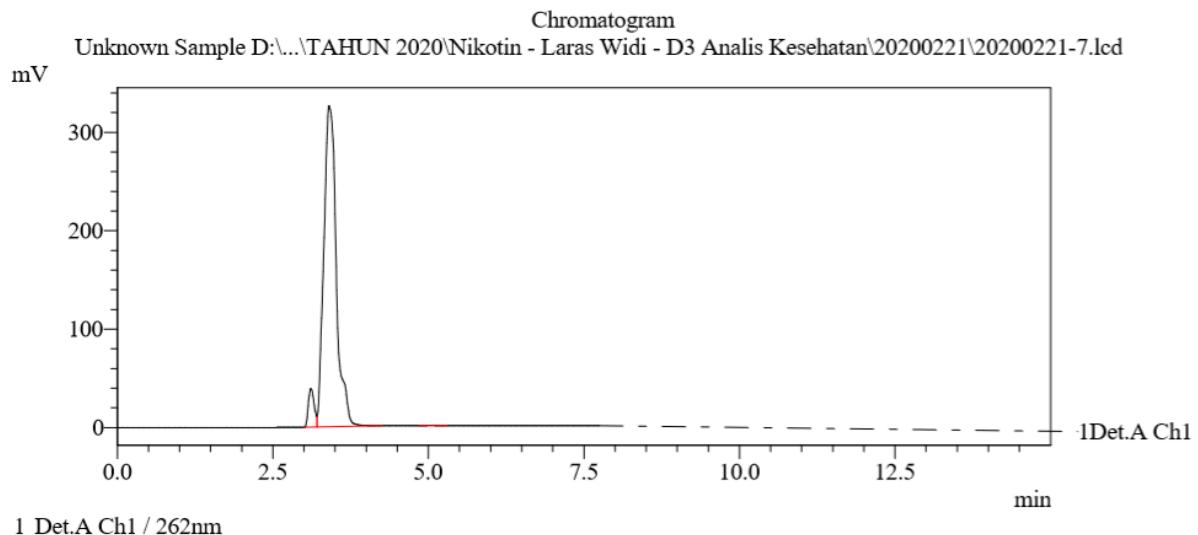
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-7.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Methanol : Buffer  
(70:30)  
Injection Volume : 20 mikron  
Data File Name : 20200221-7.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-7.lcd  
Detector A

**Lampiran 33. Kromatogram Optimasi Metode dengan Fasa Gerak Air :  
Metanol : Buffer Asetat 0,1M pH4 (40:40:20) Laju alir 1mL/menit**

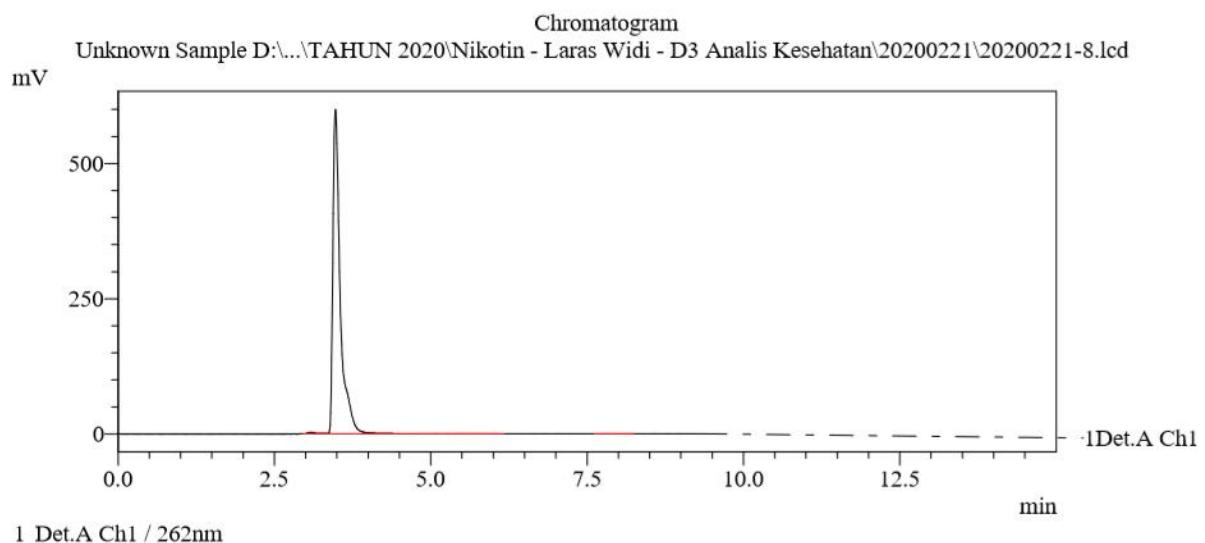
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-8.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer (40:40:20)  
Injection Volume : 20 mikron  
Data File Name : 20200221-8.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

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**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-8.lcd  
Detector A

**Lampiran 34. Kromatogram Optimasi Metode dengan Fasa Gerak Air :  
Metanol : Buffer Asetat 0,1M pH4 (60:20:20) Laju alir 1mL/menit**

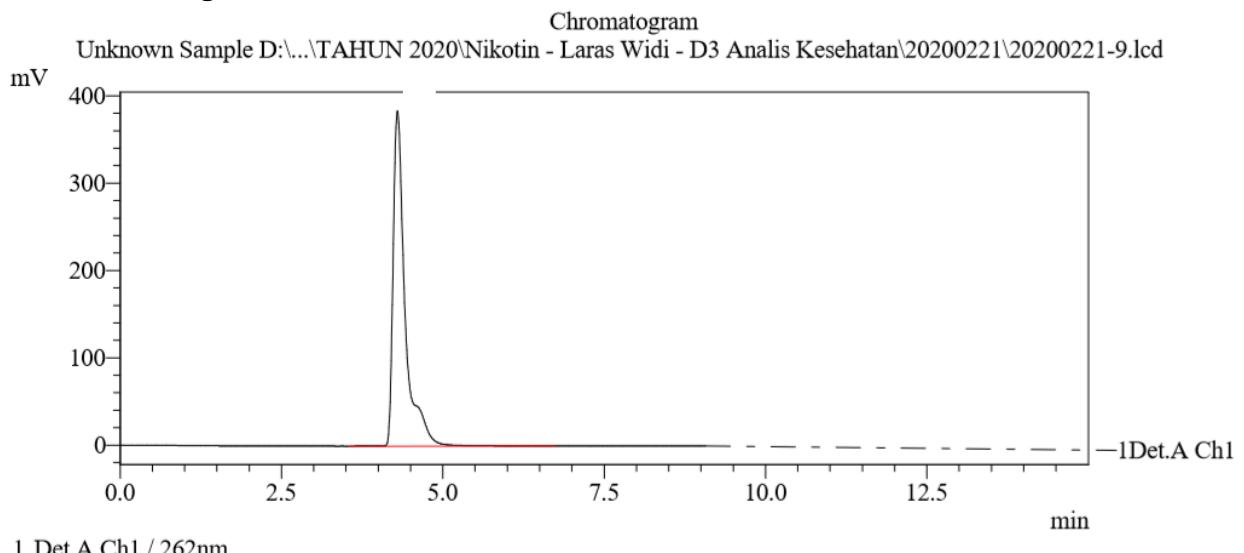
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-9.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer (60:20:20)  
Injection Volume : 20 mikron  
Data File Name : 20200221-9.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



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D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-9.lcd  
Detector A

**Lampiran 35. Kromatogram Optimasi Metode Kondisi Optimum dengan Fasa Gerak Air : Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:13:20:2) Laju alir 1mL/menit**

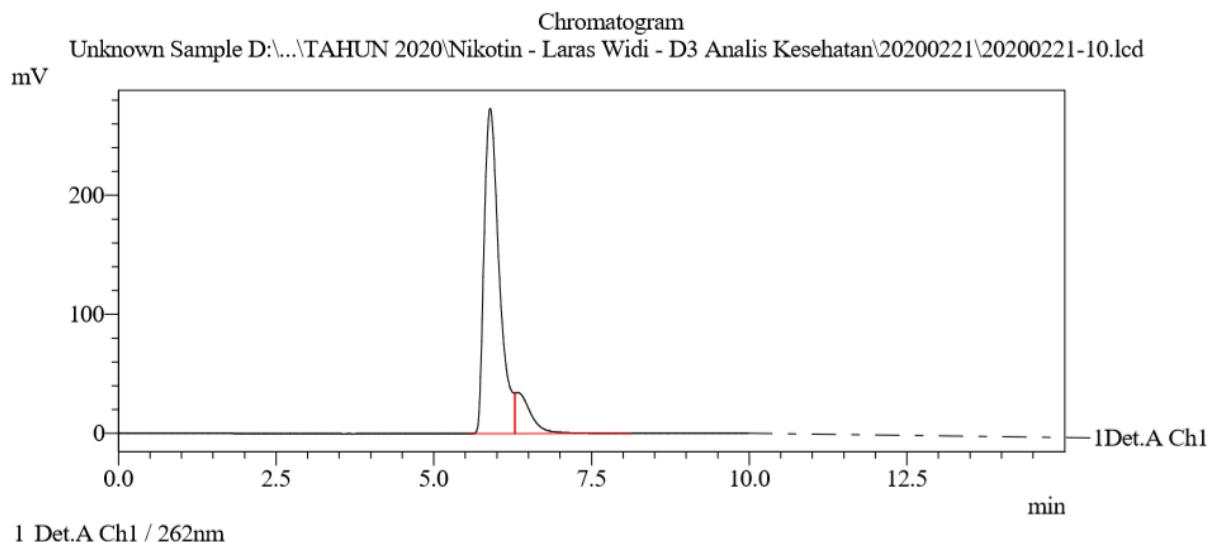
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221-10.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Injection Volume : Buffer : ACN (65:13:20:2)  
Data File Name : 20 mikron  
Method File Name : 20200221-10.lcd  
Batch File Name : OKA20200221.lcm  
Report File Name : 20200221.lcb  
: Default.lcr

---

**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221\20200221-10.lcd  
Detector A

**Lampiran 36. Kromatogram Optimasi Metode dengan Fasa Gerak Air :**  
**Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (62:13:20:5) Laju alir**  
**1mL/menit**

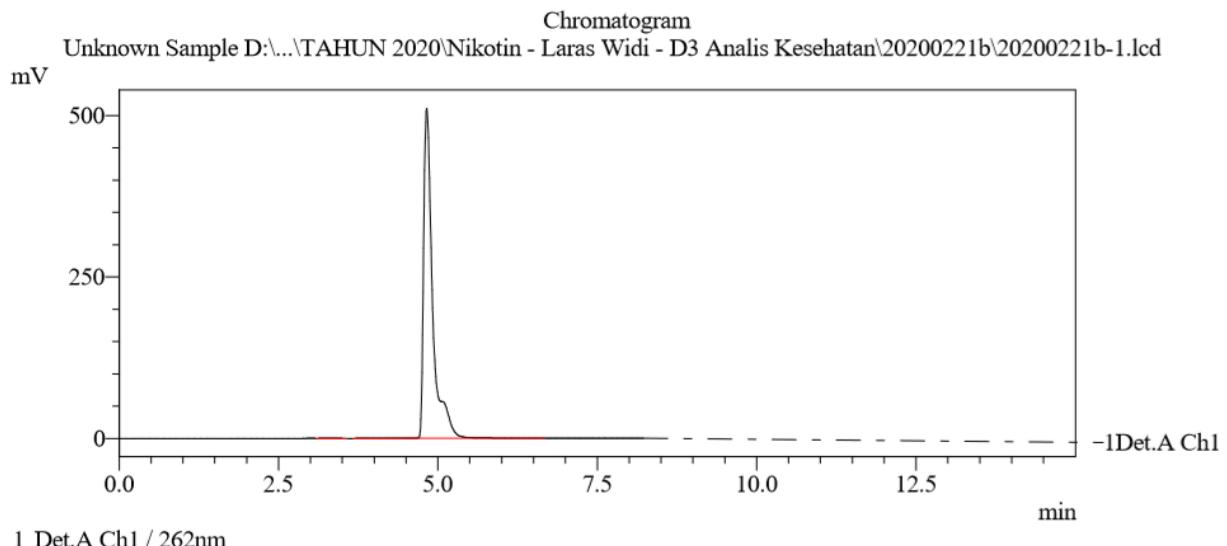
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221b-1.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer : ACN (62:13:20:5)  
Injection Volume : 20 mikron  
Data File Name : 20200221b-1  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221b\20200221b-1.lcd  
Detector A

**Lampiran 37. Kromatogram Optimasi Metode dengan Fasa Gerak Air :**  
**Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (65:10:20:5) Laju alir**  
**1mL/menit**

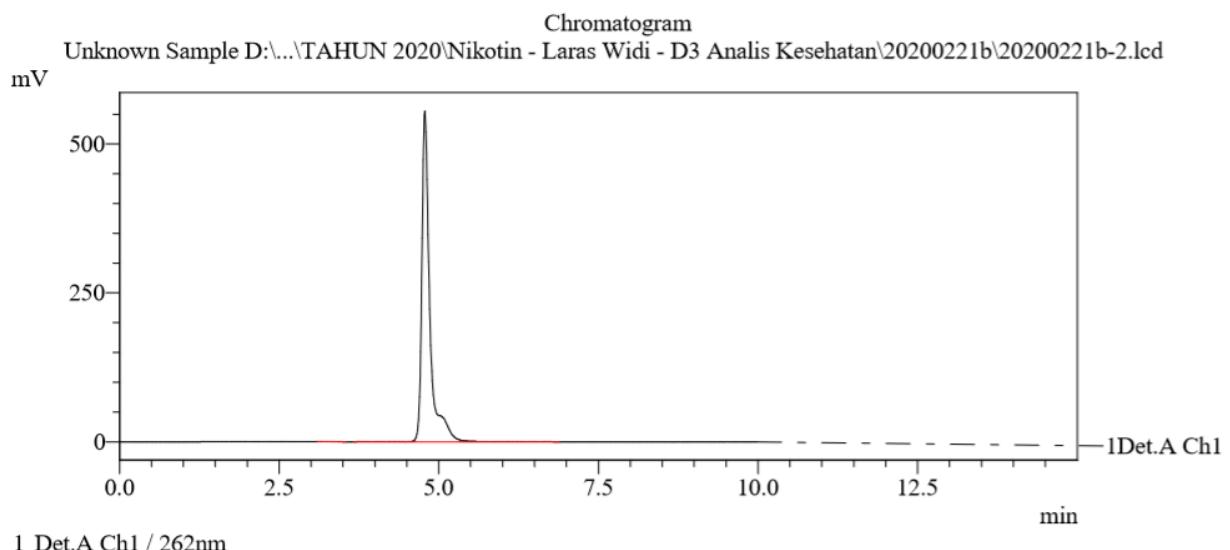
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221b-2.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer : ACN (65:10:20:5)  
Injection Volume : 20 mikron  
Data File Name : 20200221b-2  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221b\20200221b-2.lcd  
Detector A

**Lampiran 38. Kromatogram Optimasi Metode dengan Fasa Gerak Air :**  
**Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (60:10:20:10) Laju alir**  
**1mL/menit**

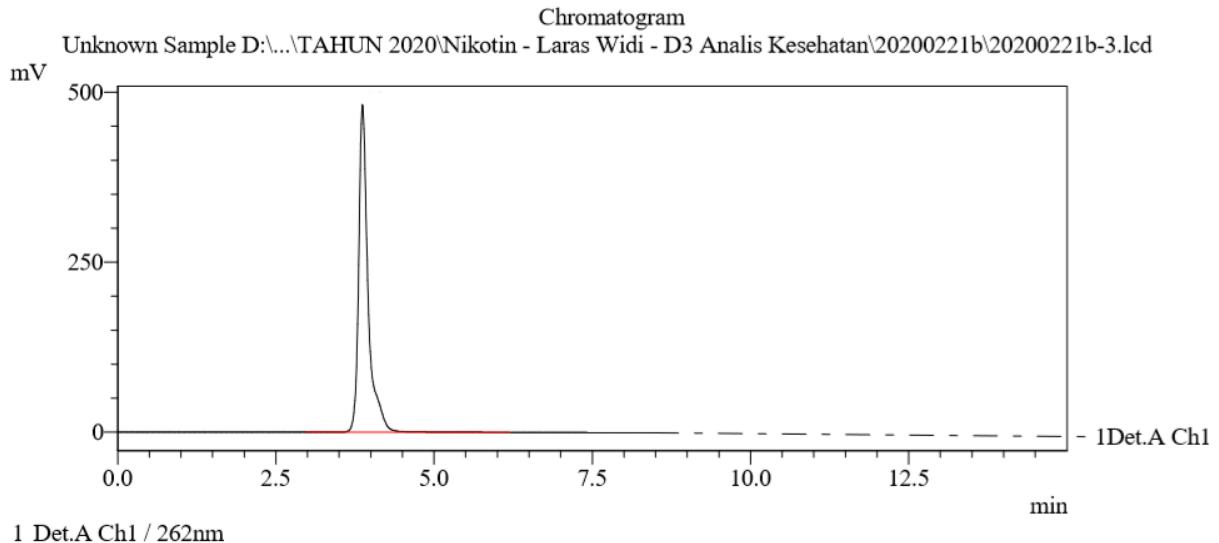
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221b-3.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Buffer : ACN (60:10:20:10)  
Injection Volume : 20 mikron  
Data File Name : 20200221b-3  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221b\20200221b-3.lcd  
Detector A

**Lampiran 39. Kromatogram Optimasi Metode dengan Fasa Gerak Air :**  
**Metanol : Buffer Asetat 0,1M pH4 : Asetonitril (60:20:15:5) Laju alir**  
**1mL/menit**

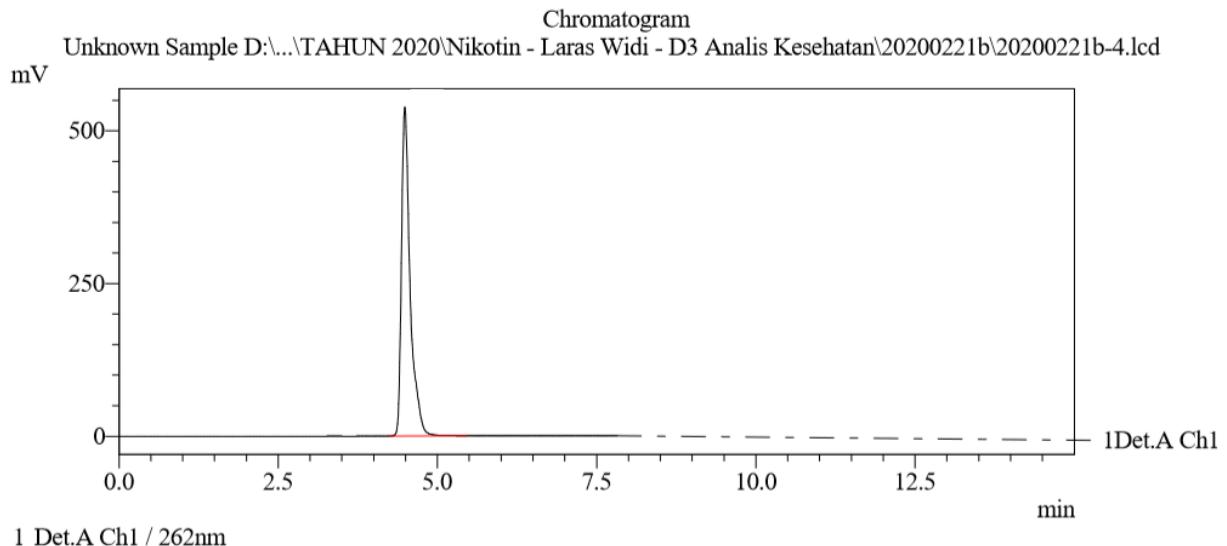
**POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU**

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200221\20200221b-4.lcd

Acquired by : Widya  
Sample Name : Optimasi Metode  
Sample ID : Injeksi standar pelarut Asetonitril dalam fasa gerak Air : Methanol :  
Injection Volume : 20 mikron  
Data File Name : 20200221b-4  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200221.lcb  
Report File Name : Default.lcr

---

**<Chromatogram>**



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200221b\20200221b-4.lcd  
Detector A

## Lampiran 40. Kromatogram Blanko Pelarut Asetonitril

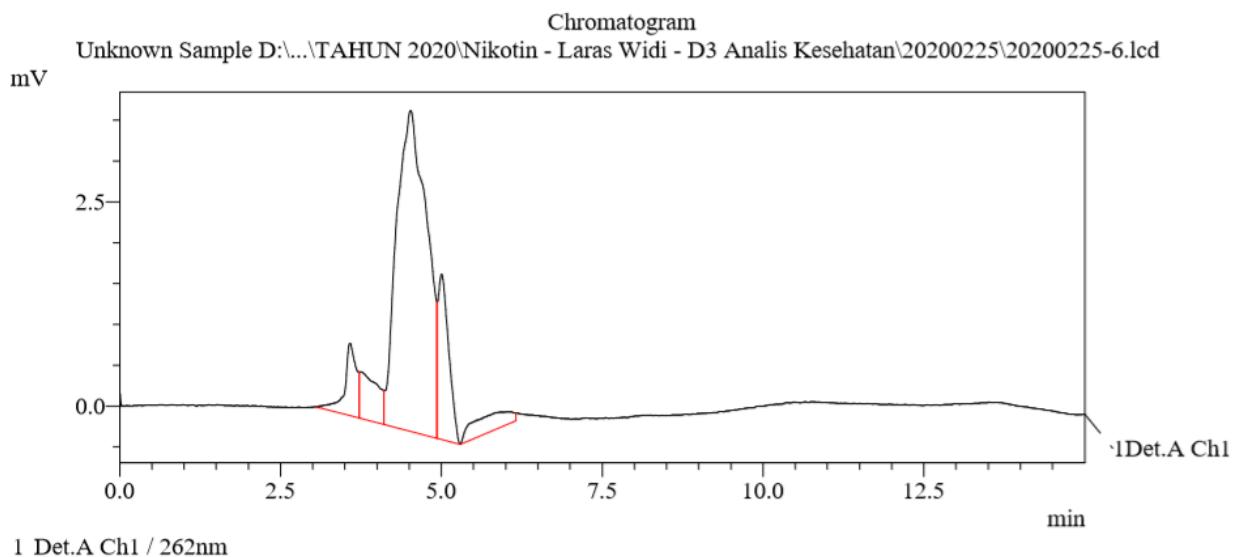
### POLITEKNIK KESEHATAN BANDUNG UNIT LABORATORIUM - LABORATORIUM TERPADU

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Blanko  
Sample ID : Blanko ACN  
Injection Volume : 20 mikron  
Data File Name : 20200225-6.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200225b.lcb  
Report File Name : Default.lcr

---

#### <Chromatogram>



D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan\20200225\20200225-6.lcd  
Detector A

## Lampiran 41. Kromatogram Standar Baku Nikotin 1,0 mg/L

### POLITEKNIK KESEHATAN BANDUNG UNIT LABORATORIUM - LABORATORIUM TERPADU

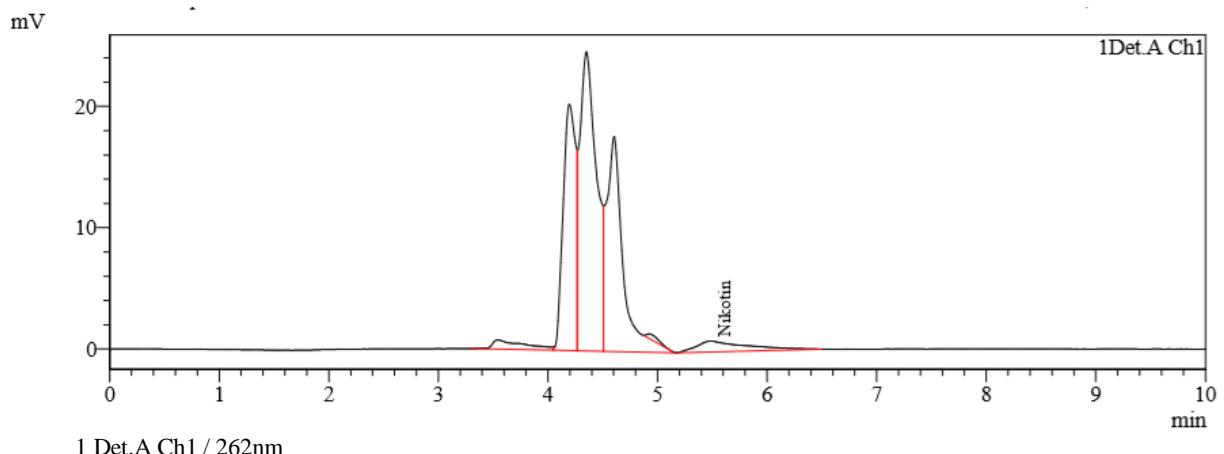
D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Standar  
Sample ID : Nikotin 1 ppm  
Injection Volume : 20 mikron  
Data File Name : Std 1ppm.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200225c.lcb  
Report File Name : Default.lcr

---

#### <Chromatogram>

Chromatogram  
Unknown Sample D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\Standar\Std 1ppm.lcd



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.476	28364	4.316	0.884	2.188	439.132	0.546

## Lampiran 42. Kromatogram Standar Baku Nikotin 2,0 mg/L

### POLITEKNIK KESEHATAN BANDUNG UNIT LABORATORIUM - LABORATORIUM TERPADU

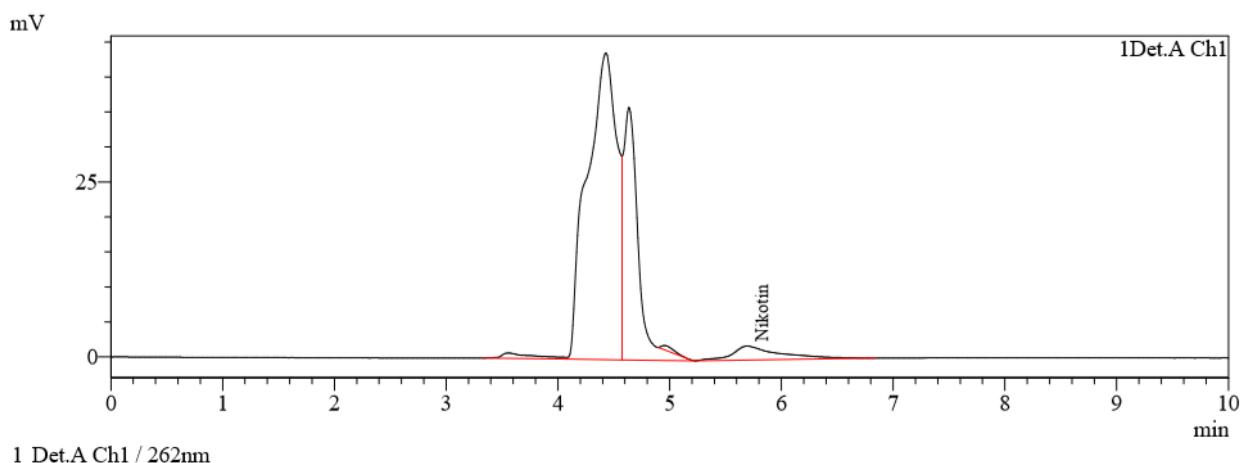
D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Standar  
Sample ID : Nikotin 2 ppm  
Injection Volume : 20 mikron  
Data File Name : Std 2ppm.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200225b.lcb  
Report File Name : Default.lcr

---

#### <Chromatogram>

Chromatogram  
Unknown Sample D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\Standar\Std 2ppm.lcd



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.691	58549	4.765	1.841	1.610	1490.507	0.603

### Lampiran 43. Kromatogram Standar Baku Nikotin 3,0 mg/L

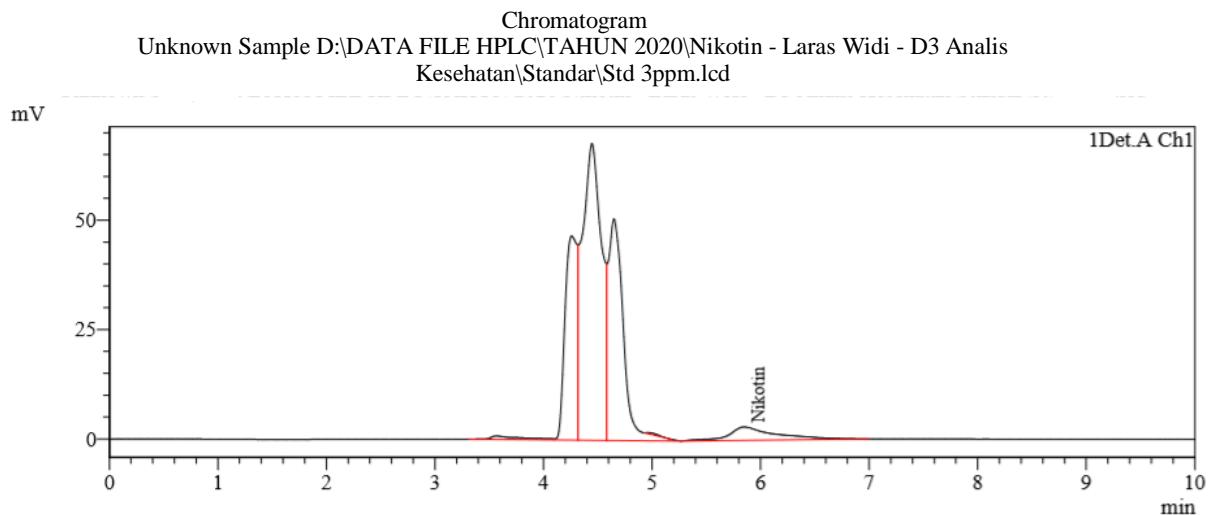
#### POLITEKNIK KESEHATAN BANDUNG UNIT LABORATORIUM - LABORATORIUM TERPADU

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Standar  
Sample ID : Nikotin 3 ppm  
Injection Volume : 20 mikron  
Data File Name : Std 3 ppm.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200225c.lcb  
Report File Name : Default.lcr

---

#### <Chromatogram>



1 Det.A Ch1 / 262nm

#### Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.848	91446	5.064	2.208	1.390	1518.993	0.641

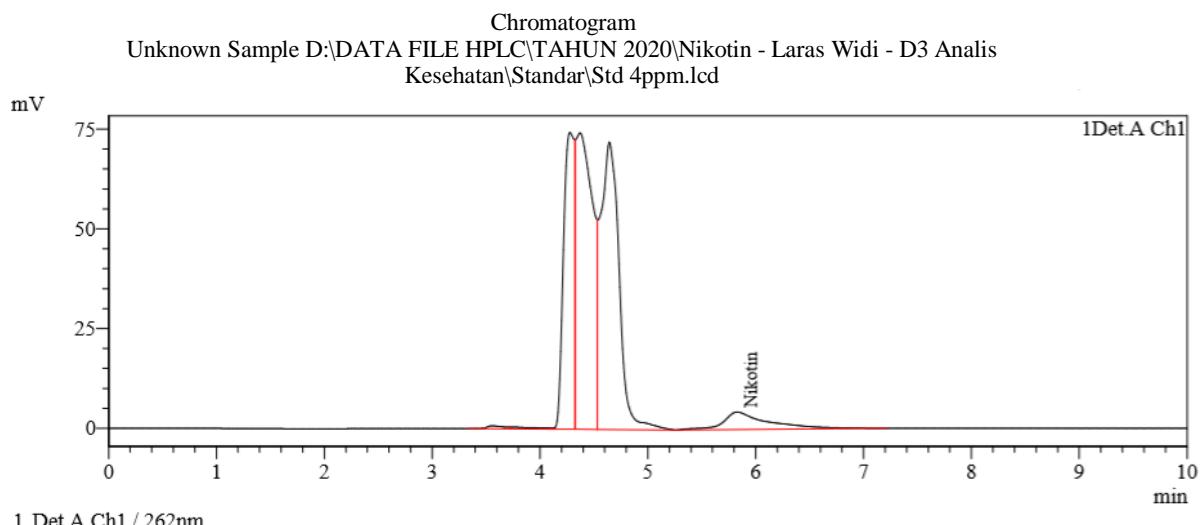
#### Lampiran 44. Kromatogram Standar Baku Nikotin 4,0 mg/L

POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU

D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Standar  
Sample ID : Nikotin 4 ppm  
Injection Volume : 20 mikron  
Data File Name : Std\_4\_ppm.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200225c.lcb  
Report File Name : Default.lcr

## <Chromatogram>



## Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
Nikotin	5.825	126202	5.388	2.419	1.479	1713.772	0.639

## Lampiran 45. Kromatogram Ekstrak Rokok Filter

POLITEKNIK KESEHATAN BANDUNG  
UNIT LABORATORIUM - LABORATORIUM TERPADU

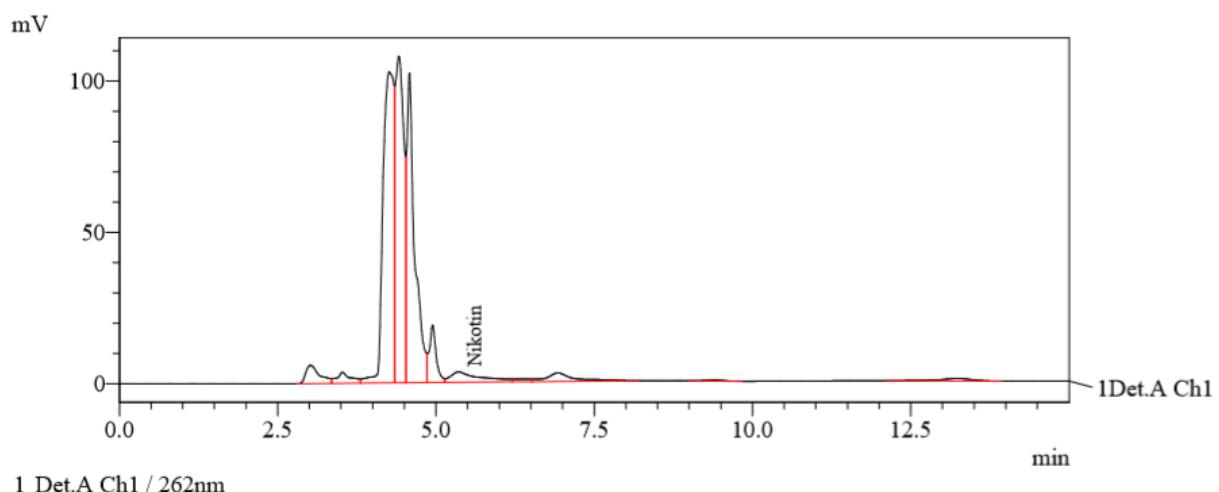
D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis Kesehatan

Acquired by : Widya  
Sample Name : Ekstrak rokok filter  
Sample ID : Ekstrak rokok filter  
Injection Volume : 20 mikron  
Data File Name : 20200226-1.lcd  
Method File Name : OKA20200221.lcm  
Batch File Name : 20200226.lcb  
Report File Name : Default.lcr

---

### <Chromatogram>

Chromatogram  
Unknown Sample D:\DATA FILE HPLC\TAHUN 2020\Nikotin - Laras Widi - D3 Analis  
Kesehatan\20200226\20200226-1.lcd



Peak Table

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	K'
Nikotin	5.354	112795	3.042	0.956	0.000	1086.614	0.775

#### Lampiran 46. Perhitungan Batas Deteksi dan Kuantitasi

No	Konsentrasi Baku Nikotin (mg/L)	Area	Yi	$(Y_i - \bar{Y})^2$
1	1,0	28.364	27177,5	1407782,25
2	2,0	58.549	59818,5	1611630,25
3	3,0	91.446	92459,5	1027182,25
4	4,0	126.202	125100,5	1213302,25

$$\Sigma = 5259897$$

Persamaan regresi:  $y = 32641x - 5462,5$

Yi didapat dari persamaan regresi, misalnya x = 1,0 maka

$$Y_i = 32641 (1,0) - 5462,5$$

$$= 27177,5$$

$S_{(y/x)^2}$  = Variasi variabel respon (y), didapat dari data-data yang dekat dengan garis regresi

$$= \frac{\sum (y_i - \bar{y})^2}{N}$$

$$= \frac{5259897}{4} = 1314974,25$$

$$S_{(y/x)} = \sqrt{1314974,25} = 1146,723267$$

$$\text{Batas deteksi (LOD)} = 3 \cdot SD / 625292$$

$$\text{Batas kuantitasi (LOQ)} = 10 \cdot SD / 625292$$

$$= \frac{3 \cdot 1146,723267}{625292}$$

$$= 0,0055 \text{ mg/L}$$

$$= \frac{10 \cdot 1146,723267}{625292}$$

$$= 0,0183 \text{ mg/L}$$

## Lampiran 47. Dokumentasi Penelitian

### Pemeriksaan Kadar Nikotin Perokok Aktif



Seperangkat Alat HPLC



Sampel Plasma Perokok Aktif



Fasa Gerak



Deret Standar Nikotin



Standar Nikotin 10000 ppm



pH meter



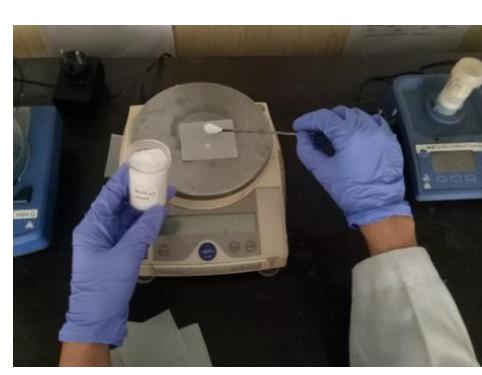
Pemipetan plasma+Asetonitril



Adisi Standar



Penimbangan  $\text{Na}_2\text{CO}_3$



Penimbangan  $\text{Na}_2\text{SO}_4$  anhidrat



Vortex



Mikro Centrifuge



Injeksi Sampel

## Pemeriksaan Glukosa Darah Perokok Aktif



Fotometer Kenzamax



Reagen dan Standar  
Glukosa GOD-PAP



Mikropipet



Tip kuning Tip biru



Plasma Perokok Aktif



Inkubasi sampel dan  
Pembacaan Hasil