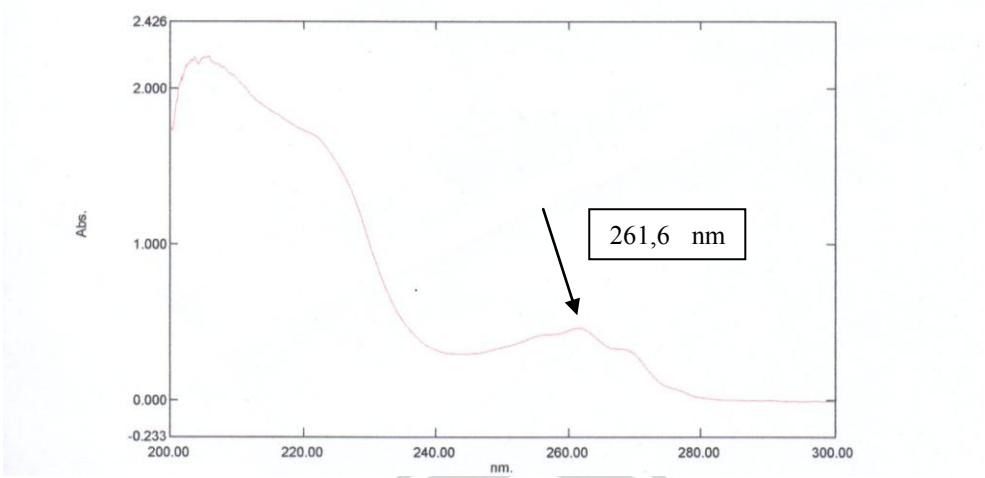
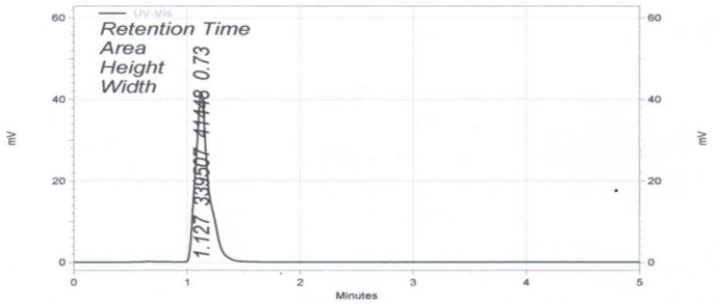
Lampiran 1. Data Absorbansi Fenilbutazon



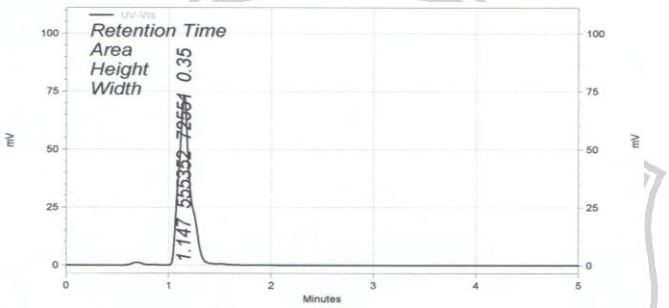
No.	P/V	Wavelength	Abs.	Description
1	↑	297.00	-0.009	
2	↑	294.00	-0.009	
3	↑	290.50	-0.004	
4	↑	288.20	-0.005	
5	↑	287.40	-0.005	
6	↑	284.60	-0.002	
7	↑	261.60	0.462	
8	↑	205.80	2.204	
9	↑	203.70	2.195	
10	↓	298.30	-0.012	
11	↓	289.00	-0.007	
12	↓	244.90	0.294	
13	↓	242.10	0.296	
14	↓	204.30	2.152	
15	↓	200.40	1.726	

Lampiran 2. Kromatogram Kurva Baku Fenilbutazon

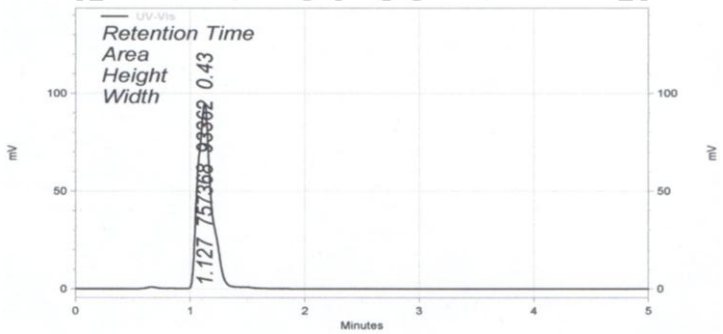
a. Larutan standar baku fenilbutazon 5 µg/mL



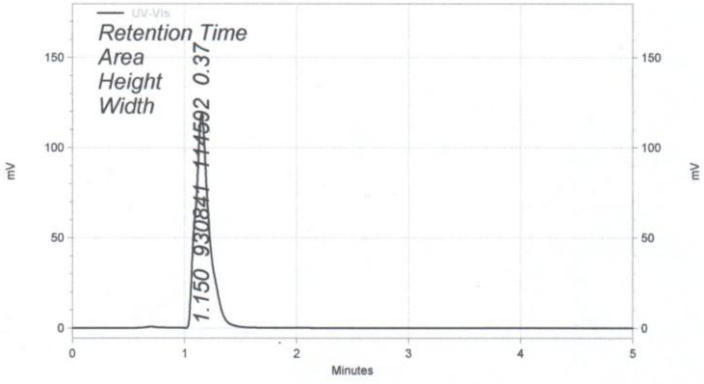
b. Larutan standar baku fenilbutazon 10 µg/mL



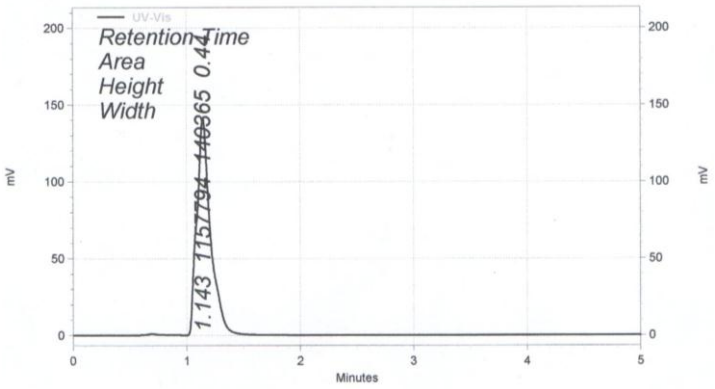
c. Larutan standar baku fenilbutazon 15 µg/mL



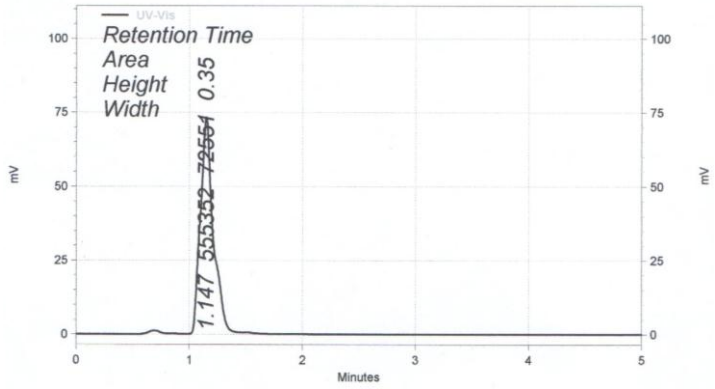
d. Larutan standar baku fenilbutazon 20 µg/mL



e. Larutan standar baku fenilbutazon 25 µg/mL

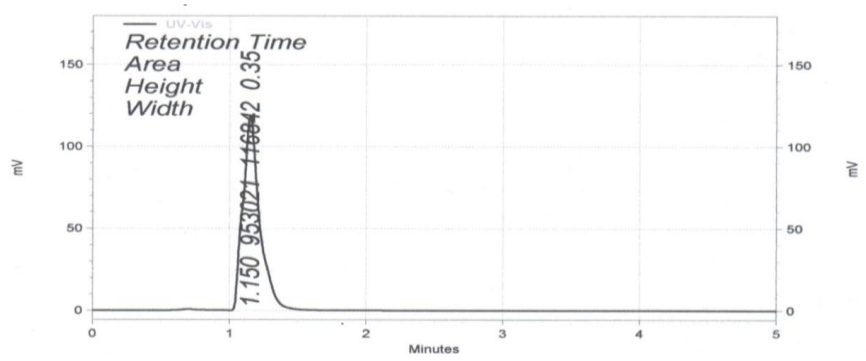


f. Larutan standar baku fenilbutazon 30 µg/mL

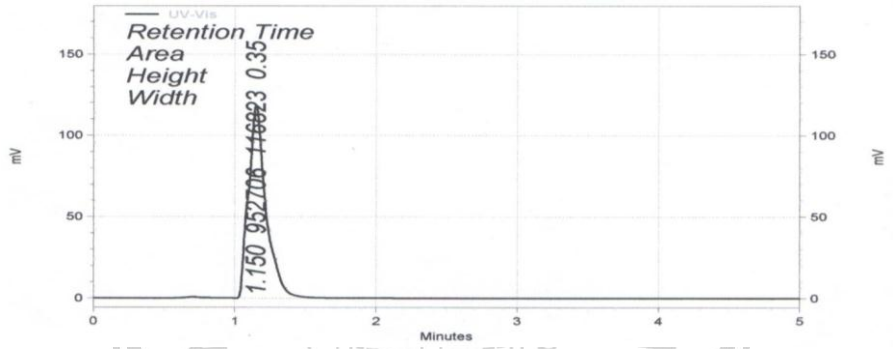


Lampiran 3. Kromatogram Sampel Fenilbutazon Replikasi 6 kali

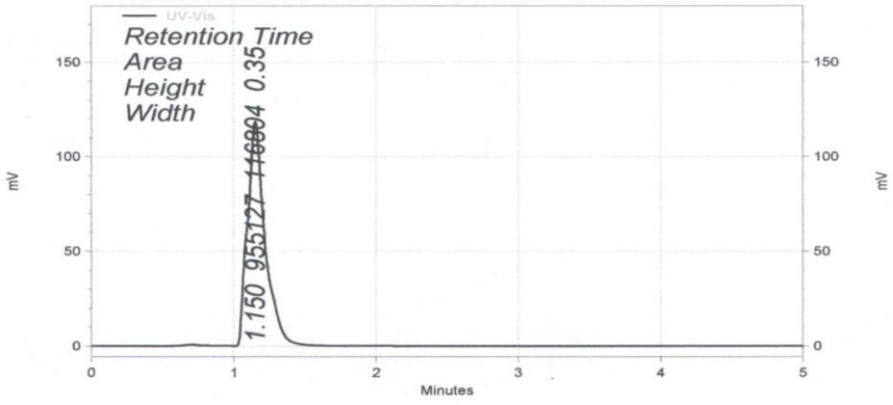
a. Kromatogram Sampel Fenilbutazon Replikasi 1



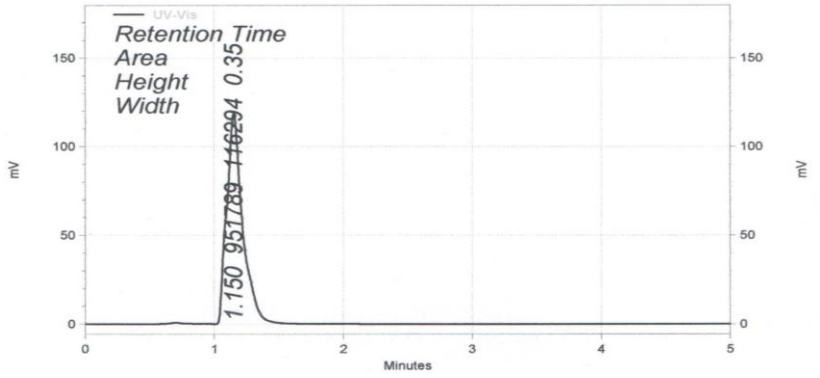
b. Kromatogram Sampel Fenilbutazon Replikasi 2



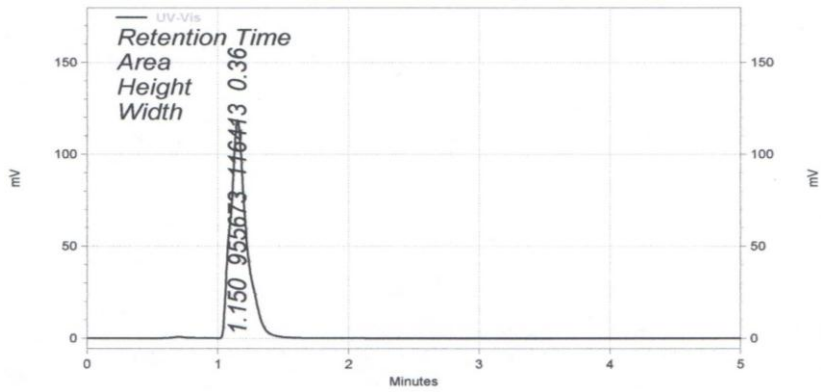
c. Kromatogram Sampel Fenilbutazon Replikasi 3



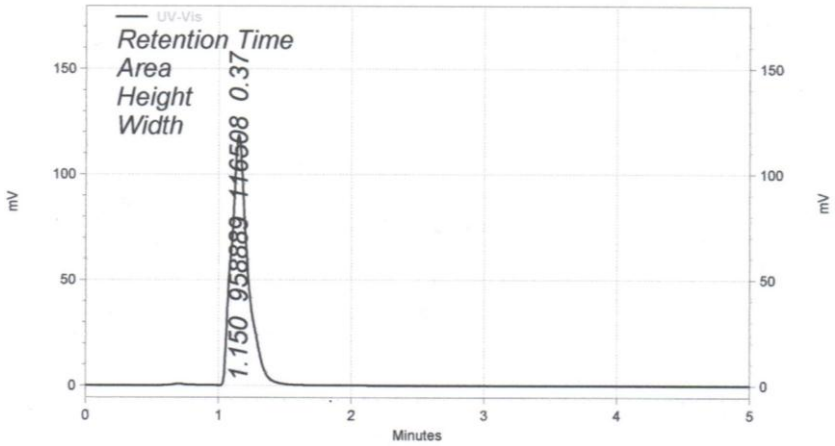
d. Kromatogram Sampel Fenilbutazon Replikasi 4



e. Kromatogram Sampel Fenilbutazon Replikasi 5



f. Kromatogram Sampel Fenilbutazon Replikasi 6



Lampiran 4. Contoh Perhitungan Perolehan Kembali Fenilbutazon dengan Metode *Standard Addition Method*

1. Perolehan kembali pada sampel yang ditambah baku sejumlah 80% dari target kadar analit dalam sampel

   a. Konsentrasi sampel sebelum penambahan bahan baku (B)

      1) Luas puncak fenilbutazon = 757368

      2) Kadar fenilbutazon berdasarkan persamaan garis

         Y = 40443,90x +142189,26 adalah 15,21 µg/mL

   b. Konsentrasi bahan baku yang ditambahkan (C)

      1) Luas puncak fenilbutazon = 555352

      3) Kadar fenilbutazon berdasarkan persamaan garis

         Y = 40443,90x +142189,26 adalah 10,22 µg/mL

   c. Konsentrasi sampel yang diperoleh setelah penambahan bahan baku (A)

      1) Luas puncak total analit 1 = 1171242

         Luas puncak total analit 2 = 1171414

         Luas puncak total analit 3 = 1171260

      2) Berdasarkan persamaan garis Y = 40443,90x +142189,26 maka :

         Kadar total analit 1 = 25,44 µg/mL

         Kadar total analit 2 = 25,45 µg/mL

         Kadar total analit 3 = 25,44 µg/mL

2. Perhitungan perolehan kembali

$$\%\ \text{perolehan kembali} = \frac{A - B}{C} \times 100\ \%$$

a. Analit 1

$$\%\ \text{perolehan kembali} = \frac{25{,}444 - 15{,}211}{10{,}216} \times 100\ \%$$

$$= 100{,}17\ \%$$

b. Analit 2

$$\%\ \text{perolehan kembali} = \frac{25{,}448 - 15{,}211}{10{,}216} \times 100\ \%$$

$$= 100{,}21\ \%$$

c. Analit 3

$$\%\ \text{perolehan kembali} = \frac{25{,}444 - 15{,}211}{10{,}216} \times 100\ \%$$

$$= 100{,}18\%$$

Lampiran 5. Perhitungan LOD dan LOQ Fenilbutazon

N o	X	Xi^2	$Xi-\bar{X}$	$(Xi-\bar{X})^2$	Yi	Yc	(Yi-Yc)	$(Yi-Yc)^2$
1	5	25	-12,5	156,25	339507	344408,8	-4901,81	24027736,86
2	10	100	-17,5	56,25	555352	546628,4	8723,648	76102027,45
3	15	225	-2,5	6,25	757368	748847,9	8520,105	72592184,95
4	20	400	2,5	6,25	930841	951067,4	-20226,4	409108798,2
5	25	625	7,5	56,25	1157794	1153287	4507,019	20313220,72
6	30	900	12,5	156,25	1358884	1355507	3377,476	11407345,48
	17,5	2275		437,5				613551313

Dari persamaan Y= 40443,90x + 142189,26 maka Yc dapat dihitung :

1. Y = 40443,90x+142189,26

   Y = 40443,90 (5) +142189,26

   Y = 334408,8

2. Y = 40443,90x +142189,26

   Y = 40443,90 (10) + 142189,26

   Y = 546628,4

3. Y = 40443,90x +142189,26

   Y = 40443,90 (15) + 142189,26

   Y = 748847,9

4. Y = 40443,90x +142189,26

   Y = 40443,90 (20) + 142189,26

   Y = 951067,4

5. Y = 40443,90x+142189,26

   Y = 40443,90 (25) + 142189,26

   Y = 1153287

6. Y = 40443,90x +142189,26

Y = 40443,90 (30) + 142189,26

Y = 1355507

7. Persamaan kurva baku : Y = 40443,90 x+142189,26 (r = 0,99)

$$S_{y/x} = \left\{\frac{\sum(Y_i - Y_c)^2}{n-2}\right\}^{1/2}$$

= $(6135513137/4)^{½}$

= 12384,98

$$S_a = S_{y/x}\sqrt{\frac{\sum X_i^2}{n\sum(X_i - X_{rata-rata})^2}}$$

= $12384{,}98 x \frac{\sqrt{2275}}{6 \times 437{,}5}$

= 12384,98 x 0,93

= 11529,79

Perhitungan nilai LOD :

Nilai Y pada batas deteksi ditentukan dengan persamaan $Y = Y_B + 3\ S_B$

Y = nilai intersept (a) pada persamaan kurva kalibrasi

S_B = simpangan baku intersept (a) (S_a)

Y = 142189,26 + 3 (11529,79)

= 142189,26 + 34589,37

= 176778,63

Maka nilai LOD

Y = 40443,90 x+142189,26

176778,63= 40443,90 x+142189,26

X = 0,85 µg/mL

Perhitungan nilai LOQ :

Nilai Y pada batas kuantifikasi ditentukan dengan persamaan $Y = Y_B + 10\ S_B$

Y = nilai intersept (a) pada persamaan kurva kalibrasi

S_B = simpangan baku intersept (a) (S_a)

Y = 142189,26+ 10 (11529,79)

= 142189,26 + 115297,9

= 257487,16

Maka nilai LOQ:

Y = 40443,90 x+142189,26

257487,16 = 40443,90 x+142189,26

LOQ = X = 2,85 µg/mL

Lampiran 6. Contoh Perhitungan Kadar Fenilbutazon dalam sediaan Obat Tradisional Pegal Linu

Persamaan regresi linier kurva baku adalah

Y = BX + A

Y = 40443,90x +142189,26

Replikasi 1

Y = 40443,90x +142189,26

953021 = 40443,90x +142189,26

X = 20,05 µg/mL

Sampel dilarutkan dalam 10 mL, sehingga kadar obat tradisional pegal linu yang ditambahkan fenilbutazon adalah

X = 20,05µg/mL x 10 ml x 5

= 10024µg / 1000

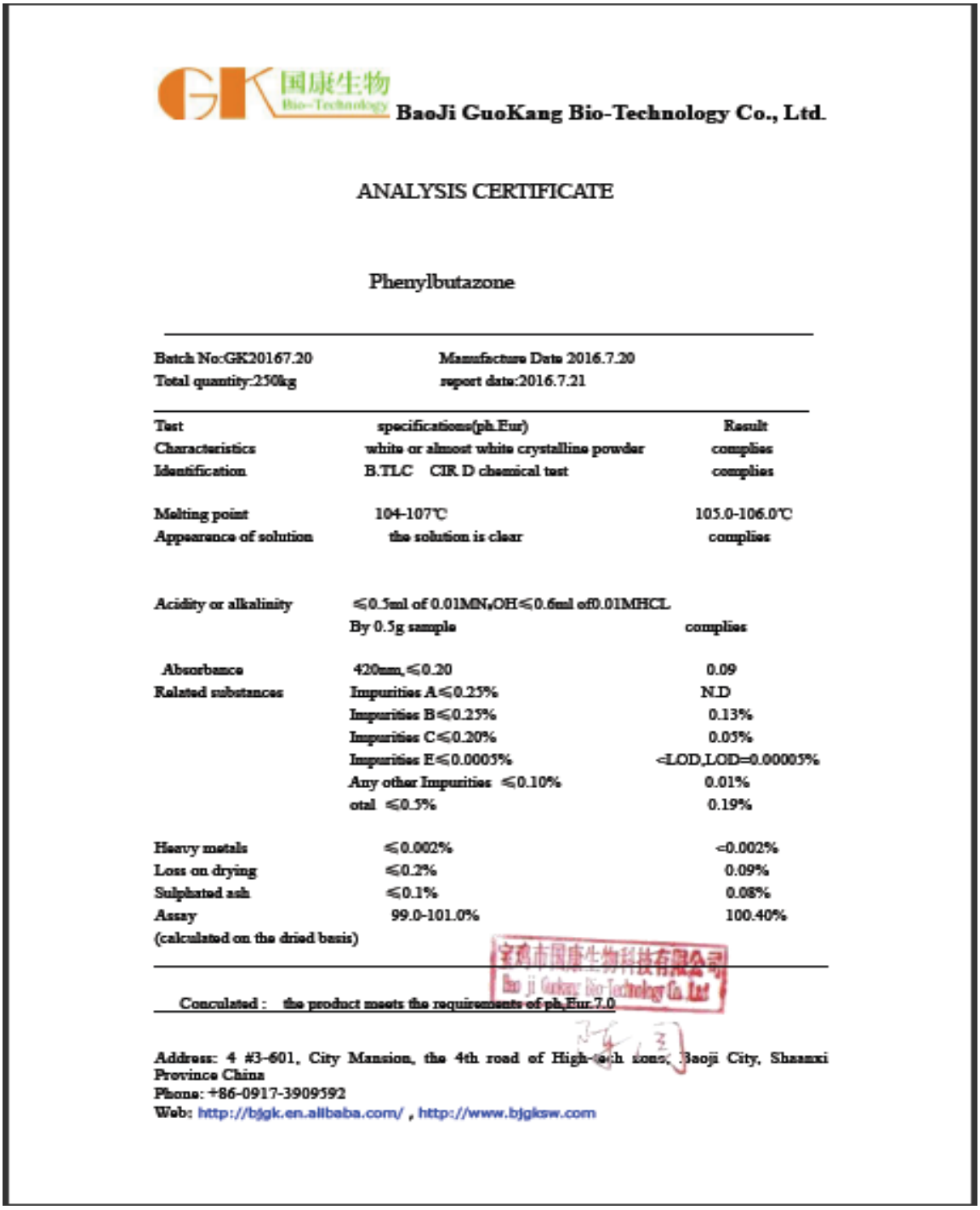
= 10,02 mg

% Kadar fenilbutazon dalam sediaan obat tradisional pegal linu

% Kadar fenilbutazon = $\frac{10{,}024\ mg}{10\ mg} x100\%$

= 100,34 %

Lampiran 7. *Certificate of Analysis* Fenilbutazon



国康生物 Bio-Technology

BaoJi GuoKang Bio-Technology Co., Ltd.

ANALYSIS CERTIFICATE

Phenylbutazone

Batch No:GK20167.20 Manufacture Date 2016.7.20
Total quantity:250kg report date:2016.7.21

Test	specifications(ph.Eur)	Result
Characteristics	white or almost white crystalline powder	complies
Identification	B.TLC CIR D chemical test	complies
Melting point	104-107℃	105.0-106.0℃
Appearance of solution	the solution is clear	complies
Acidity or alkalinity	≤0.5ml of 0.01MNaOH≤0.6ml of0.01MHCL By 0.5g sample	complies
Absorbance	420nm,≤0.20	0.09
Related substances	Impurities A≤0.25%	N.D
	Impurities B≤0.25%	0.13%
	Impurities C≤0.20%	0.05%
	Impurities E≤0.0005%	<LOD,LOD=0.00005%
	Any other Impurities ≤0.10%	0.01%
	otal ≤0.5%	0.19%
Heavy metals	≤0.002%	<0.002%
Loss on drying	≤0.2%	0.09%
Sulphated ash	≤0.1%	0.08%
Assay (calculated on the dried basis)	99.0-101.0%	100.40%

宝鸡市国康生物科技有限公司 Bao ji Guokang Bio-Technology Co. Ltd

Conculated : the product meets the requirements of ph.Eur.7.0

Address: 4 #3-601, City Mansion, the 4th road of High-tech zone, Baoji City, Shaanxi Province China
Phone: +86-0917-3909592
Web: http://bjgk.en.alibaba.com/ , http://www.bjgksw.com

Lampiran 8. Surat Keterangan Laboratorium

UNIVERSITAS WAHID HASYIM
FAKULTAS FARMASI
BAGIAN KIMIA FARMASI
Jl. Menoreh Tengah X / 22 Sampangan – Semarang 50236 Telp. (024) 8505680 – 8505681 fax. (024) 8505680

SURAT KETERANGAN
No.02/Lab. Kimia Farmasi/ C.05/UWH/V/ 2018

Assalamu'alaikum Wr. Wb.

Yang bertanda tangan dibawah ini, Kepala Bagian Kimia Farmasi Fakultas Farmasi Universitas Wahid Hasyim Semarang menerangkan bahwa :

Nama : Syafa'atun Ni'mah
NIM : 135010978
Fak/ Univ/ Sekolah : Farmasi / Universitas Wahid Hasyim

Telah melakukan Penelitian Uji Validasi Kandungan Senyawa Aktif menggunakan HPLC di Laboratorium Kimia Analisa, Fakultas Farmasi Universitas Wahid Hasyim Semarang, dengan judul penelitian :
"Validsi Metode Penetapan Kadar Fenilbutazon Menggunakan Kromatografi Cair Kinerja Tinggi dan Aplikasinya Pada Obat Tradisional Pegal Linu"

Demikian surat keterangan ini dibuat untuk dipergunakan semestinya.

Wassalamu'alaikum Wr. Wb.

Semarang, Mei 2018
Ka. Bag Kimia Farmasi

Ulfah, M.Sc, Apt

Lampiran 9. Dokumentasi Penelitian

UNIVERSITAS WAHID HASYIM
SEMARANG