

Appendix A

Solubility Test of GLC02-521 andGLC02-568 in Phosphate Buffer (pH 11)

项目	GLP-1R	记录本	GLP1R
相对湿度	%	室温	℃
关键词		结论	
参与人	N/A		

Objective

To test the Solubility of GLC02-521 and GLC02-568 in Phosphate Buffer (pH 11)

Experimental Procedure

1. Preparation of Buffer (pH 11):
Weigh 14.20 g of anhydrous disodium hydrogen phosphate, dissolve in 800 mL water with sonication.
Add 1 mol/L NaOH solution dropwise while monitoring with a pH meter until the solution pH stabilizes at 11.
Add water to bring the total volume to 1000 mL, mix well, and set aside for use.
2. Sample Preparation:
Preparation of GLC02-521 sample: Weigh 10 mg of GLC02-521, add to 1 mL of buffer solution, vortex to mix, and sonicate vigorously for 2 hours (not completely dissolved, suspension formed). Centrifuge at 10,000 rpm for 10 minutes, collect the supernatant, and set aside for use.
Preparation of GLC02-568 sample: Weigh 10 mg of GLC02-568, add to 1 mL of buffer solution, vortex to mix, and sonicate vigorously for 2 hours (not completely dissolved, suspension formed, but majority clearly dissolved). Centrifugeat at 10,000 rpm for 10 minutes, collect the supernatant, and set aside for use.
3. Standard Solution Preparation:
Preparation of GLC02-521 standard solution: Accurately weigh approximately 1 mg of GLC02-521, dissolve in 1 mL methanol, sonicate until completely dissolved, and set aside for use.
Preparation of GLC02-568 standard solution: Accurately weigh approximately 1 mg of GLC02-568, dissolve in 1 mL methanol, sonicate until completely dissolved, and set aside for use.
4. Sample Testing (HPLC)
Test GLC02-521 andGLC02-568 standard solutions by HPLC (injection volume: 10 μL). Record the peak area of each standard.
Test GLC02-521 andGLC02-568 samples by HPLC (GLC02-521: injection volume 10 μL; GLC02-568: injection volume 1 μL). Record the peak area of each sample.

HPLC method

Instrument: HPLC Thermo U3000
Column: Welch Xtimate C18 (4.6×150mm, 3μm) or equivalent
Mobile Phase A: 0.05% Trifluoroacetic acid
Mobile Phase B: Acetonitrile
Parameters: Detection wavelength 254nm
Sample compartment temperature: RT
Column temperature: 45℃
Flow rate: 1ml/min
Needle wash solution: 50% acetonitrile in water
Gradient Program Time(min) Mobile Phase A (%) Mobile Phase B (%)
0 95 5
10 5 95
15 5 95
15.1 95 5
20 95 5

Results

HPLC Results of Standard Solutions:
GLC02-521: Standard Concentration: 0.90 mg/mL;Standard Peak Area: 217.090
GLC02-568: Standard Concentration: 1.32 mg/mL mg/mL;Standard Peak Area: 160.628
HPLC Results of Test Samples:
GLC02-521: Peak Area: 14.171
GLC02-568: Peak Area: 80.928

Calculation Formula: Csample=Cstd×Atest/Astd* F
Cstd is Standard Concentration;
Atest is Peak Area of Test Samples;
Astd is Standard Peak Area;
F is dilution factor (or injection volume ratio between standard and sample); (F = 1 for GLC02-521 (10 μL standard / 10 μL sample)); (F = 10 for GLC02-568 (10 μL standard / 1 μL sample)).
Solubility of GLC02-521: 14.171/217.090*0.90*1=0.0587 mg/mL.
Solubility of GLC02-568: 80.928/160.628*1.32*10=6.650 mg/mL.

Conclusion

The solubility of GLC02-521 in phosphate buffer (pH 11) is 0.0587 mg/mL;
The solubility of GLC02-568 in phosphate buffer (pH 11) is 6.650 mg/mL.
GLC02-568 exhibited a solubility of 6.650 mg/mL in pH 11 phosphate buffer, representing a 113-fold increase over GLC02-521 (0.0587 mg/mL). This substantially improved aqueous solubility at pH 11 offers significant advantages for formulation development.

实验文档

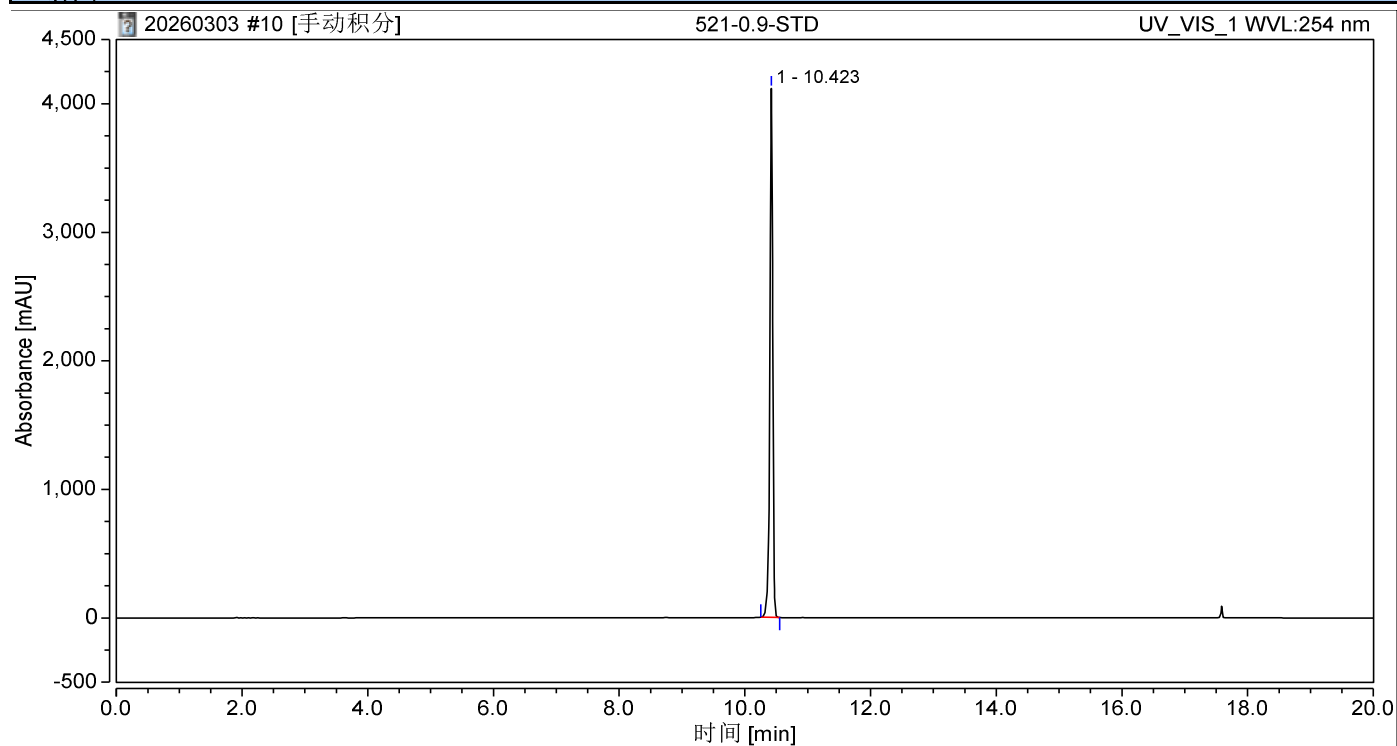
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521-PH 11.0.pdf	2026-03-04 16:29:29	梁斌	
568-1.32-STD.pdf	2026-03-04 16:29:36	梁斌	
568-pH 11.0.pdf	2026-03-04 16:29:42	梁斌	

色谱图和结果

进样信息

进样名称:	521-0.9-STD	运行时间 (min):	20.00
瓶号:	RD1	进样量:	10.00
进样类型:	未知	通道:	UV_VIS_1
校正级别:		波长:	254
仪器方法:	95-5 20 MIN	带宽:	4
处理方法:	TEST	稀释因子:	1.0000
进样日期/时间:	2026/03/03 13:39	样品重量:	1.0000

色谱图



积分结果

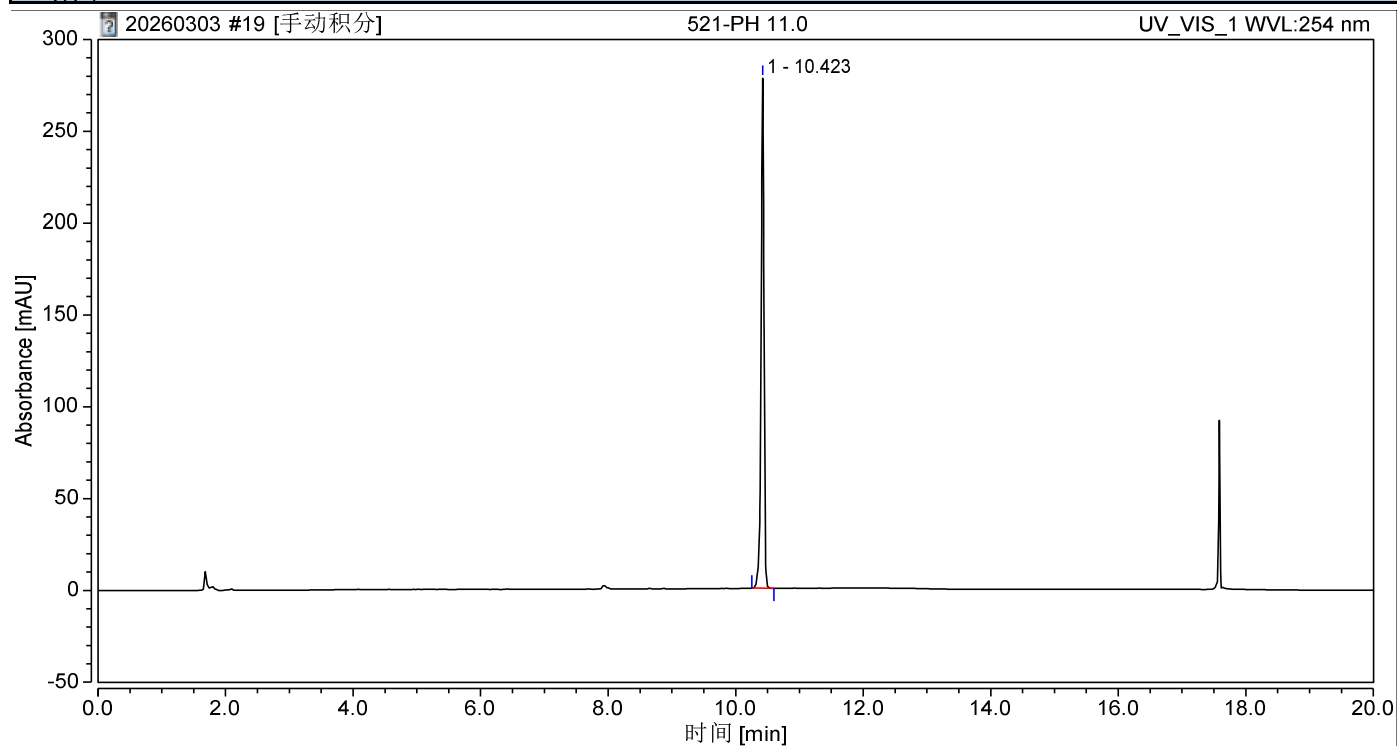
序号	峰名称	保留时间 min	峰面积 mAU*min	峰高 mAU	相对峰面积 %	相对峰高 %	样品量 n.a.
1		10.423	217.090	4113.530	100.00	100.00	n.a.
总和:			217.090	4113.530	100.00	100.00	

色谱图和结果

进样信息

进样名称:	521-PH 11.0	运行时间 (min):	20.00
瓶号:	RE1	进样量:	10.00
进样类型:	未知	通道:	UV_VIS_1
校正级别:		波长:	254
仪器方法:	95-5 20 MIN	带宽:	4
处理方法:	TEST	稀释因子:	1.0000
进样日期/时间:	2026/03/03 18:39	样品重量:	1.0000

色谱图



积分结果

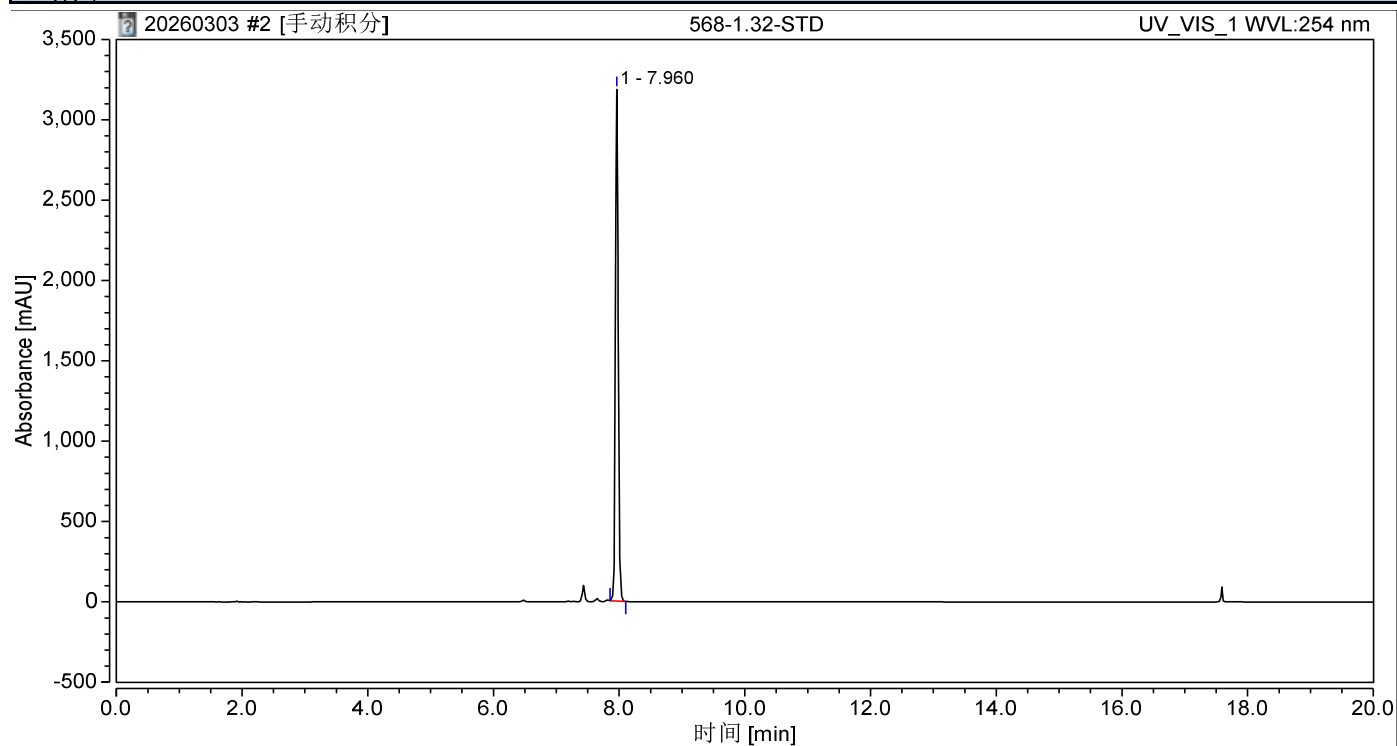
序号	峰名称	保留时间 min	峰面积 mAU*min	峰高 mAU	相对峰面积 %	相对峰高 %	样品量 n.a.
1		10.423	14.171	277.756	100.00	100.00	n.a.
总和:			14.171	277.756	100.00	100.00	

色谱图和结果

进样信息

进样名称:	568-1.32-STD	运行时间 (min):	20.00
瓶号:	RC1	进样量:	10.00
进样类型:	未知	通道:	UV_VIS_1
校正级别:		波长:	254
仪器方法:	95-5 20 MIN	带宽:	4
处理方法:	TEST	稀释因子:	1.0000
进样日期/时间:	2026/03/03 09:50	样品重量:	1.0000

色谱图



积分结果

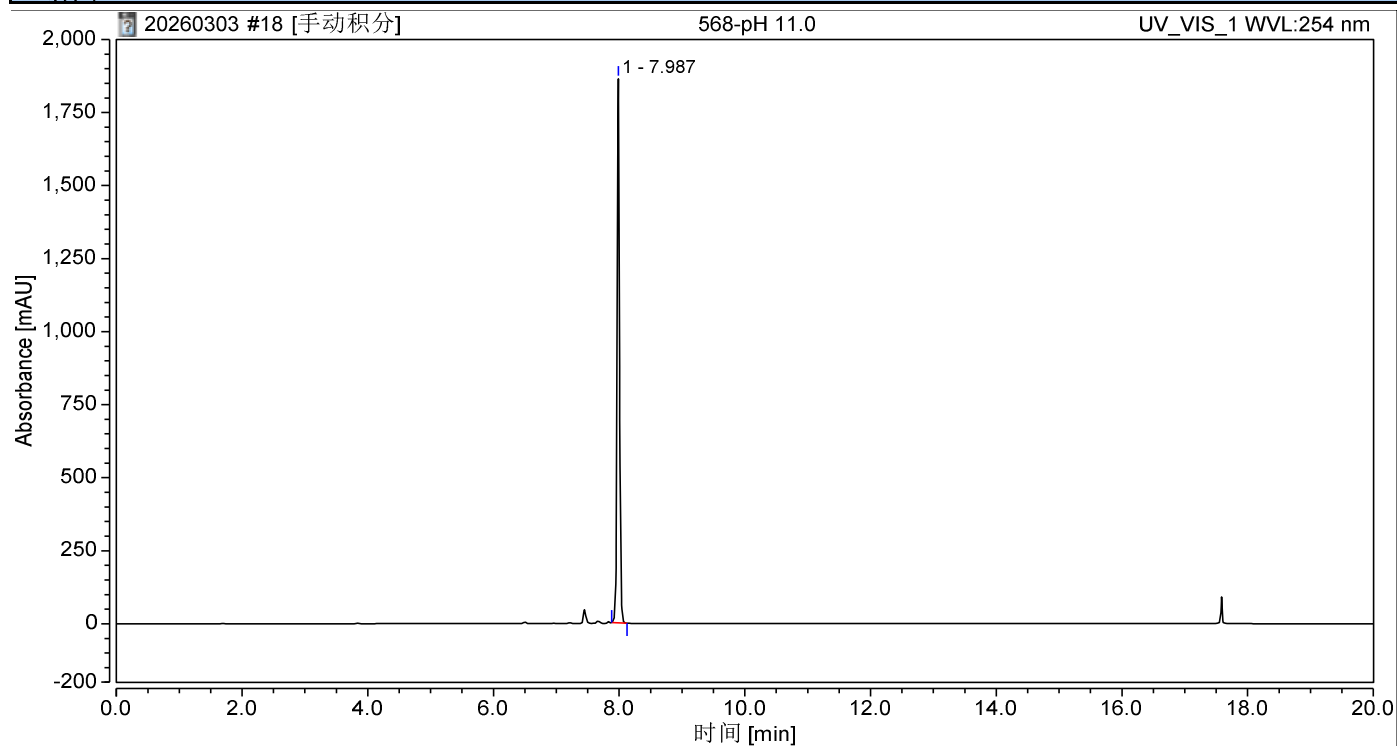
序号	峰名称	保留时间 min	峰面积 mAU*min	峰高 mAU	相对峰面积 %	相对峰高 %	样品量 n.a.
1		7.960	160.628	3183.916	100.00	100.00	n.a.
总和:			160.628	3183.916	100.00	100.00	

色谱图和结果

进样信息

进样名称:	568-pH 11.0	运行时间 (min):	20.00
瓶号:	RD4	进样量:	1.00
进样类型:	未知	通道:	UV_VIS_1
校正级别:		波长:	254
仪器方法:	95-5 20 MIN	带宽:	4
处理方法:	TEST	稀释因子:	1.0000
进样日期/时间:	2026/03/03 18:18	样品重量:	1.0000

色谱图



积分结果

序号	峰名称	保留时间 min	峰面积 mAU*min	峰高 mAU	相对峰面积 %	相对峰高 %	样品量 n.a.
1		7.987	80.928	1862.449	100.00	100.00	n.a.
总和:			80.928	1862.449	100.00	100.00	