HPLC Method Limited Verification for TGF-001 (non-GMP)

Project	**Pyronaridine_INV-054926**
Compound	**TGF-001**
Purpose	**Method Verification**
Category	**Methods**
Substance Type	**Drug Substance**
Report ID	**INV_054926_HPLC_V4 Version 1.0**

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Distribution

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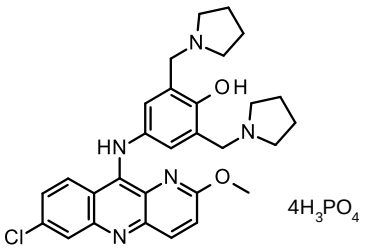
1. Objective

TGF-001 is analyzed by the newly developed HPLC method 'INV_054926_HPLC_M4' using a reversed phase C18 column, e.g.: Waters X-Bridge column. In this document, the limited verification of the new HPLC method shall be performed at non-GMP level.

Related reports:

INV_054926_HPLC_M4: HPLC Method Description for In-process control, Identity, Assay and Related Substances of TGF-001



TGF-001

2. Summary and conclusion

The method verification has been performed at non-GMP level. The following analytical parameters have been assessed:

- Autosampler stability
- Specificity/ selectivity
- LOD
- LOQ
- Linearity of TGF-001
- Linearity of DIA and DIN (RRF determination)
- Accuracy of TGF-001
- Repeatability

The peak purity showed that unknown peaks at RRT 0.85/ 0.87 should be coeluted peaks. The obtained results are summarized in Table 1.

Table 1 non-GMP verification of the new developed HPLC method for TGF-001

Parameter	Acceptance criteria	Results	Conformity	Remark
Autosampler stability	For TGF-001: %Δ ≤ 2% For impurities (report all ≥ LOQ): between LOQ and ≤ 0.1%: %Δ ≤ 40%, > 0.1% and ≤ 0.5%: %Δ ≤ 20%, > 0.5%: %Δ ≤ 15% Report limit time for autosampler stability	Stable at least 24 hours for assay Sample needs freshly prepared for impurity quantification	Pass	See section 3.1
Specificity/ selectivity	No interference between the blank peaks and the components of interest in blanks. All impurity peaks should be separated with TGF-001 peak	No interference peaks in blanks. All impurity peaks are separated with TGF-001 peak.	Pass	Unknown peaks at RRT 0.85/ 0.87 should be coeluted peaks. See section 3.2
LOD	S/N (0.03% TGF-001) ≥ 3 S/N (0.03% DIA) ≥ 3 S/N (0.03% DIN) ≥ 3	S/N (0.03% TGF-001) = 38.2 S/N (0.03% DIA) = 37.6 S/N (0.03% DIN) = 60.4	Pass	See section 3.3
LOQ	S/N (0.05% TGF-001) ≥ 10 S/N (0.05% DIA) ≥ 10 S/N (0.05% DIN) ≥ 10 %RSD (0.05% TGF-001 peak area) ≤ 30 % %RSD (0.05% DIA peak area) ≤ 30 % %RSD (0.05% DIN peak area) ≤ 30 %	S/N (0.05% TGF-001) = 60.5 S/N (0.05% DIA) = 73.0 S/N (0.05% DIN) = 83.2 %RSD (0.05% TGF-001) = 1.3% %RSD (0.05% DIA) = 0.9% %RSD (0.05% DIN) = 0.2%	Pass	See section 3.3
Linearity of TGF-001	a) LOQ to 140%: R^2 ≥ 0.995, y intercept ≤ 5% of the nominal concentration, Report slope, show figure. Report R^2, y intercept, slope, figure for: b) LOQ to 5% c) 50% to 140%	LOQ to 140%: R^2 = 0.9999 y intercept = 0.37% Slope = 52689 LOQ to 5%: R^2 = 0.9998 y intercept = 0.05% Slope = 51576 50% to 140%:	Pass	See section 3.4

		R^2 = 0.9998 y intercept = 2.19% Slope = 53579		
Linearity of DIA and DIN (RRF determination)	LOQ to 0.5%: $R^2 \geq 0.995$, Report slope, show figure. Calculate RRF value.	DIA: R^2 = 0.9995 Slope = 45379 RRF = 1.16 DIN: R^2 = 0.9993 Slope = 58071 RRF = 0.91	Pass	See section 3.5
Accuracy of TGF-001	%RSD ≤ 2% for each concentration level Single recovery: 95% - 105% Mean recovery for each concentration level: 98% - 102% (Recovery is calculated as determined concentration vs. nominal concentration)	%RSD (80%) = 0.6% %RSD (100%) = 1.7% %RSD (120%) = 2.0% Recovery (80% prep. 1) = 99% Recovery (80% prep. 2) = 99% Recovery (80% prep. 3) = 98% Recovery (100% prep. 1) = 100% Recovery (100% prep. 2) = 100% Recovery (100% prep. 3) = 97% Recovery (120% prep. 1) = 101% Recovery (120% prep. 2) = 98% Recovery (120% prep. 3) = 98% Mean recovery (80%) = 98% Mean recovery (100%) = 99% Mean recovery (120%) = 99%	Pass	See section 3.6
Repeatability	For TGF-001: %RSD (100% TGF-001, n=6) ≤ 2% For DIA: %RSD (0.14% DIA, n=6) ≤ 15% For DIN: %RSD (0.14% DIN, n=6) ≤ 15%	%RSD (100% TGF-001) = 0.1% %RSD (0.14% DIA in 100% TGF-001) = 0.6% %RSD (0.14% DIN in 100% TGF-001) = 0.9%	Pass	See section 3.7

3. Results

3.1. Autosampler stability

Autosampler stability was performed using TGF-001 batch 100144-200502-REF.

The sample solution was found to be stable at least 24 hours for assay purpose and instable for unknown impurity at RRT 0.82/ 0.85 which means fresh sample preparation is needed for impurity quantification.

Table 2 Results for autosampler stability of TGF-001

Time		0h	1h		2h		3h		4h		6h		9h		12h		18h		24h		Limit
RT, min	RRT	area%	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	area%	%Δ	%Δ
9.81	0.82	0.19%	0.24%	26%	0.26%	37%	0.28%	47%	0.29%	53%	0.31%	63%	0.32%	68%	0.33%	74%	0.34%	79%	0.35%	84%	≤ 20%
10.17	0.85	0.17%	0.15%	12%	0.14%	18%	0.13%	24%	0.13%	24%	0.12%	29%	0.12%	29%	0.11%	35%	0.12%	29%	0.11%	35%	≤ 20%
10.40	0.87	0.05%	0.05%	0%	0.04%	20%	0.04%	20%	0.04%	20%	0.04%	20%	0.04%	20%	0.03%	40%	0.03%	40%	0.03%	40%	≤ 40%
10.96	0.91	0.04%	0.04%	0%	0.04%	0%	0.04%	0%	0.04%	0%	0.04%	0%	0.05%	25%	0.05%	25%	0.05%	25%	0.05%	25%	-
11.24	0.94	0.05%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	≤ 40%
11.33	0.95	0.05%	0.06%	20%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	0.05%	0%	≤ 40%
11.99	1.00	98.25%	98.19%	0%	98.20%	0%	98.19%	0%	98.17%	0%	98.18%	0%	98.16%	0%	98.16%	0%	98.14%	0%	98.15%	0%	≤ 2%
12.80	1.07	0.34%	0.35%	3%	0.33%	3%	0.33%	3%	0.33%	3%	0.33%	3%	0.33%	3%	0.33%	3%	0.33%	3%	0.32%	6%	≤ 20%
16.77	1.40	0.08%	0.07%	13%	0.08%	0%	0.08%	0%	0.08%	0%	0.08%	0%	0.08%	0%	0.08%	0%	0.08%	0%	0.09%	13%	≤ 40%
23.46	1.96	0.09%	0.09%	0%	0.09%	0%	0.09%	0%	0.09%	0%	0.09%	0%	0.09%	0%	0.09%	0%	0.10%	11%	0.10%	11%	≤ 40%
24.83	2.07	0.52%	0.52%	0%	0.52%	0%	0.51%	2%	0.52%	0%	0.52%	0%	0.51%	2%	0.52%	0%	0.52%	0%	0.52%	0%	≤ 15%
29.92	2.50	0.20%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	0.20%	0%	≤ 20%

$\%\Delta = (\%area_{t=n} - \%area_{t=0}) / \%area_{t=0} * 100\%$

3.2. Specificity/ selectivity

Specificity/ selectivity was performed using TGF-001 batch 100144-200502-REF and TGF-001 spiked with impurity DIA or DIN.

There is no interference between the blank peaks and the components of interest. All impurity peaks are separated with TGF-001 peak. The peak purity showed that unknown peaks at RRT 0.85/ 0.87 should be coeluted peaks.

Table 3 Results for specificity/ selectivity of TGF-001

TGF-001, batch			**100144-200502-REF**		
Name	RT, min	RRT	Area	% Area	peak purity
	9.806	0.82	16.1742	0.19%	995.4
	10.166	0.85	14.0179	0.17%	844.8
	10.404	0.87	4.5063	0.05%	618.1
	10.962	0.91	2.9933	0.04%	922.1
	11.240	0.94	3.9864	0.05%	988.4
	11.333	0.95	4.3823	0.05%	973.4
TGF-001	11.988	1.00	8336.9955	98.25%	1000.0
	12.803	1.07	28.4520	0.34%	997.0
	16.768	1.40	6.5751	0.08%	961.9
	23.464	1.96	7.4326	0.09%	989.1
	24.830	2.07	43.7429	0.52%	999.5
	29.915	2.50	16.5870	0.20%	999.3

Figure 1 Example HPLC chromatogram of blank, 278 nm

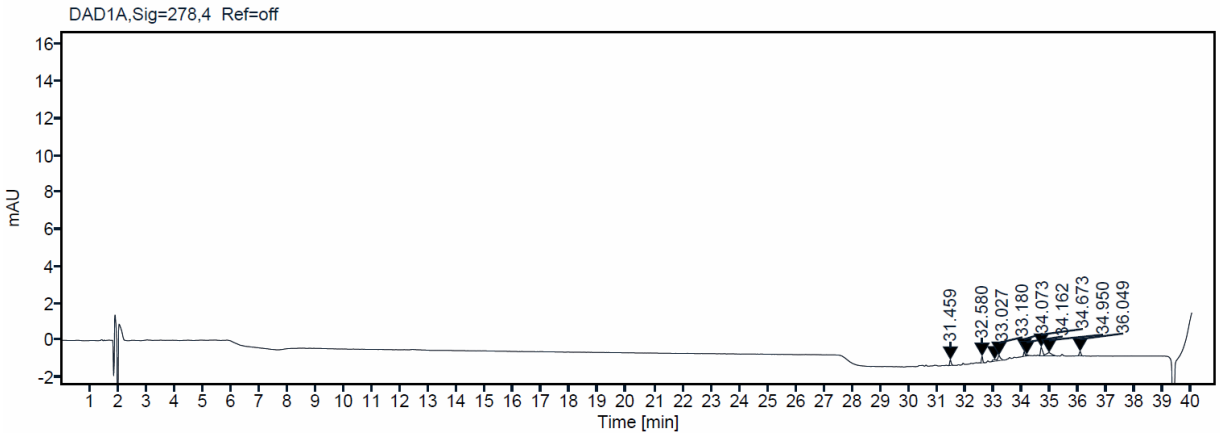


Figure 2 Example HPLC chromatogram of TGF-001 batch 100144-200502-REF, 278 nm

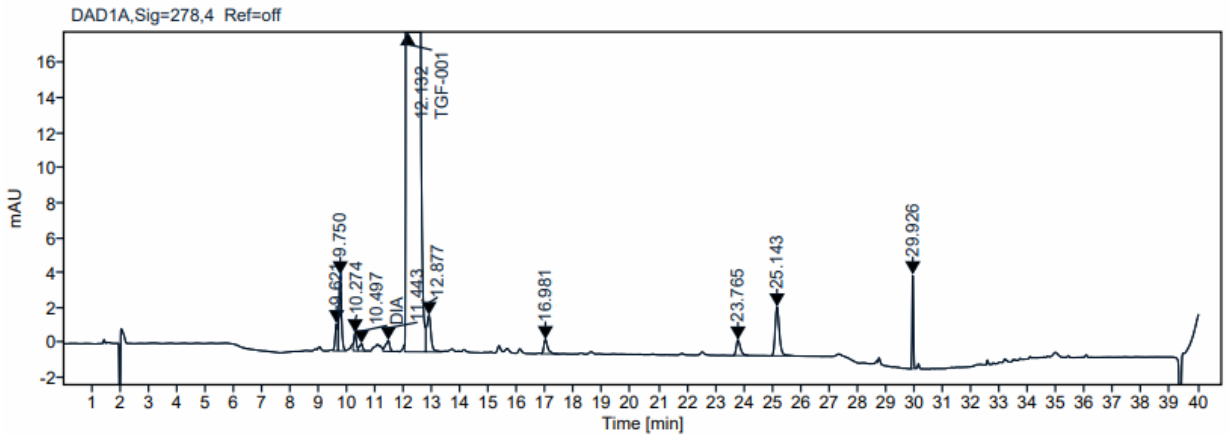


Figure 3 Example HPLC chromatogram of TGF-001 batch 100144-200502-REF spiked with impurity DIA batch PHTRACKD-394-REF, 278 nm

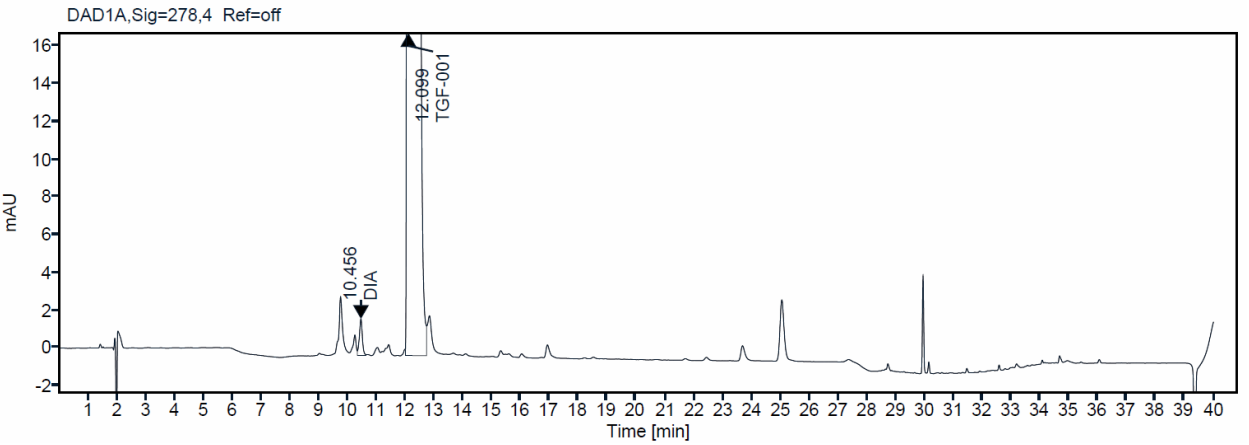
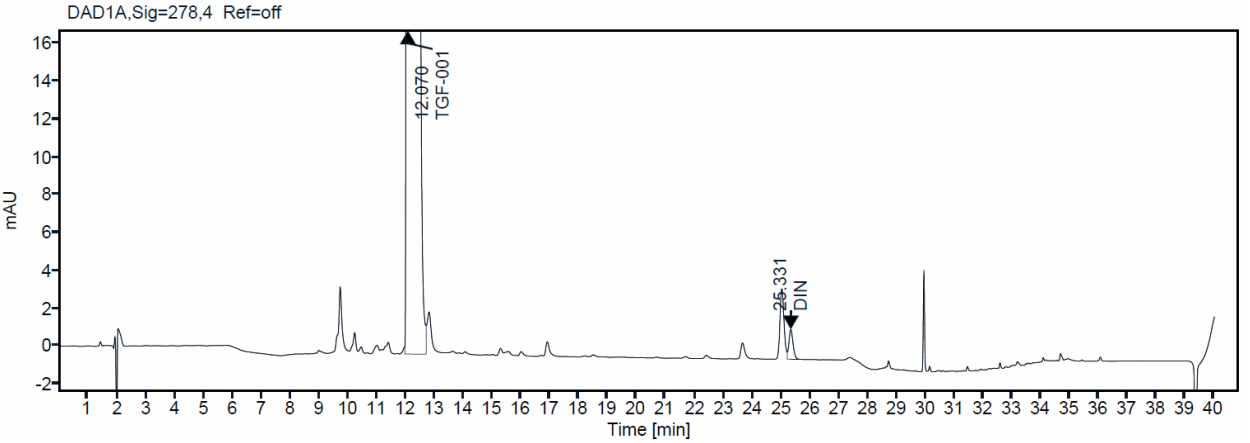


Figure 4 Example HPLC chromatogram of TGF-001 batch 100144-200502-REF spiked with impurity DIN batch PHTRACKD-389-REF, 278 nm



3.3. LOD/ LOQ

LOD and LOQ were performed using TGF-001 batch 100144-200502-REF, DIA batch PHTRACKD-394-REF and DIN batch PHTRACKD-389-REF.

The signal to noise of TGF-001 peak is calculated to be 38.2 in LOD solution (0.03%) and 60.5 in LOQ solution (0.05%) respectively. The %RSD of TGF-001 peak area in LOQ solutions (n=3) is found to be 1.3%.

The signal to noise of DIA peak is calculated to be 37.6 in LOD solution (0.03%) and 73.0 in LOQ solution (0.05%) respectively. The %RSD of DIA peak area in LOQ solutions (n=3) is found to be 0.9%.

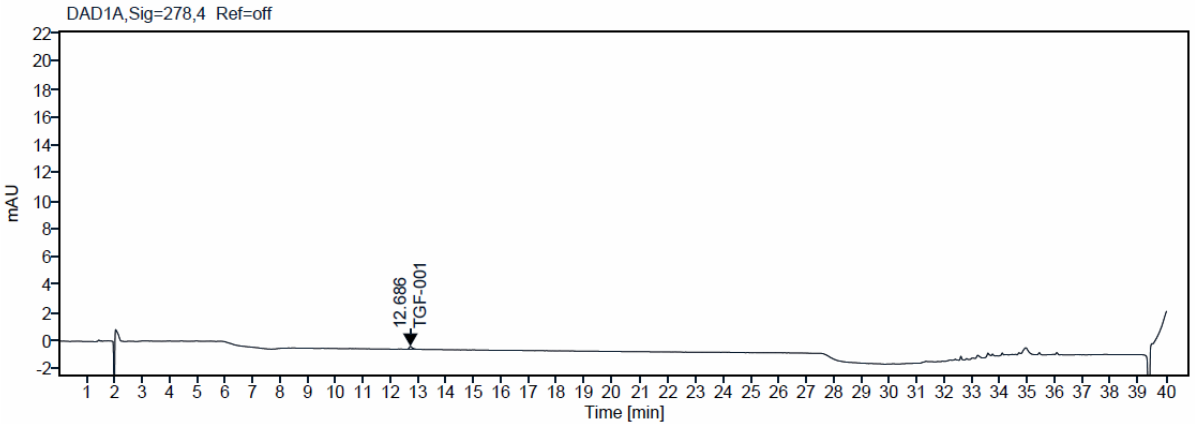
The signal to noise of DIN peak is calculated to be 60.4 in LOD solution (0.03%) and 83.2 in LOQ solution (0.05%) respectively. The %RSD of DIN peak area in LOQ solutions (n=3) is found to be 0.2%.

All results meet the acceptance criteria.

Table 4 %RSD results for 3 injections of 0.05% solution

Reference solution 2	**Area counts [pA]**		
	0.05% TGF-001	**0.05% DIA**	**0.05% DIN**
Inj. 1	2.8677	2.3974	4.6605
Inj. 2	2.8049	2.3868	4.6678
Inj. 3	2.8068	2.4287	4.6801
Average	2.8265	2.4043	4.6695
STDEV	0.0357	0.0218	0.0099
%RSD	1.3%	0.9%	0.2%
Acceptance criteria	≤ 30%		

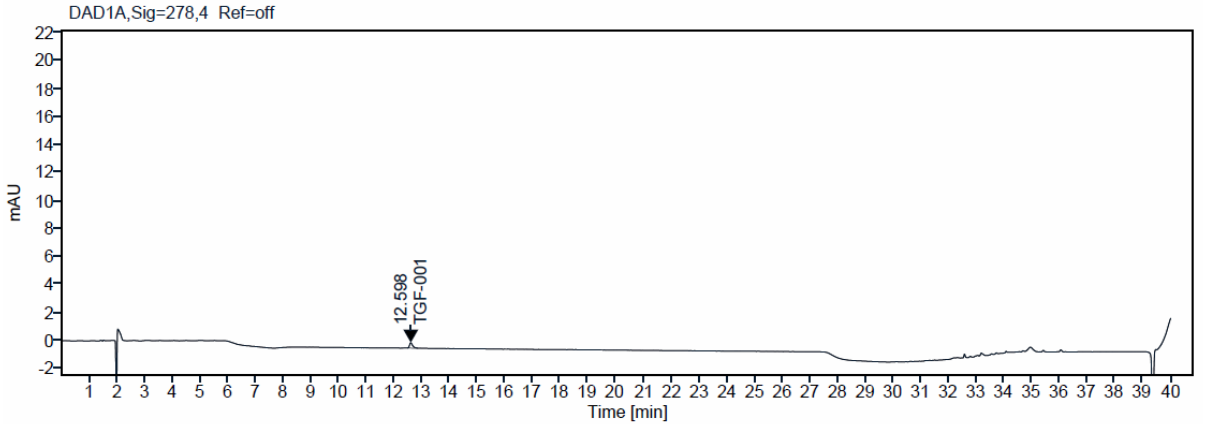
Figure 5 Example Chromatogram of signal to noise calculation for 0.03% TGF-001 solution (LOD).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
12.686	MM m	0.3773	1.5783	0.1995	100.00	TGF-001	38.150 09

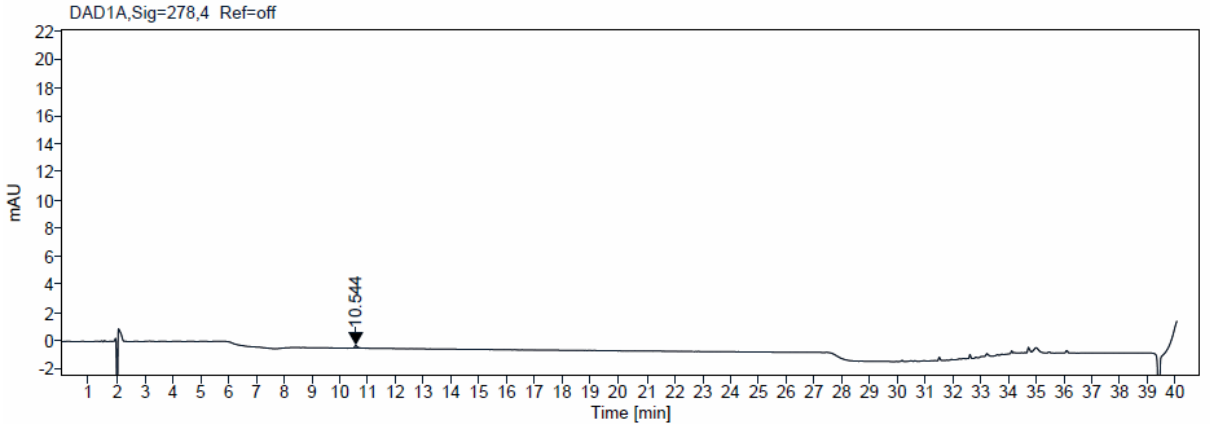
Figure 6 Example chromatogram of signal to noise calculation for 0.05% TGF-001 solution (LOQ).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
12.598	MM m	0.3746	2.8677	0.3789	100.00	TGF-001	60.54625

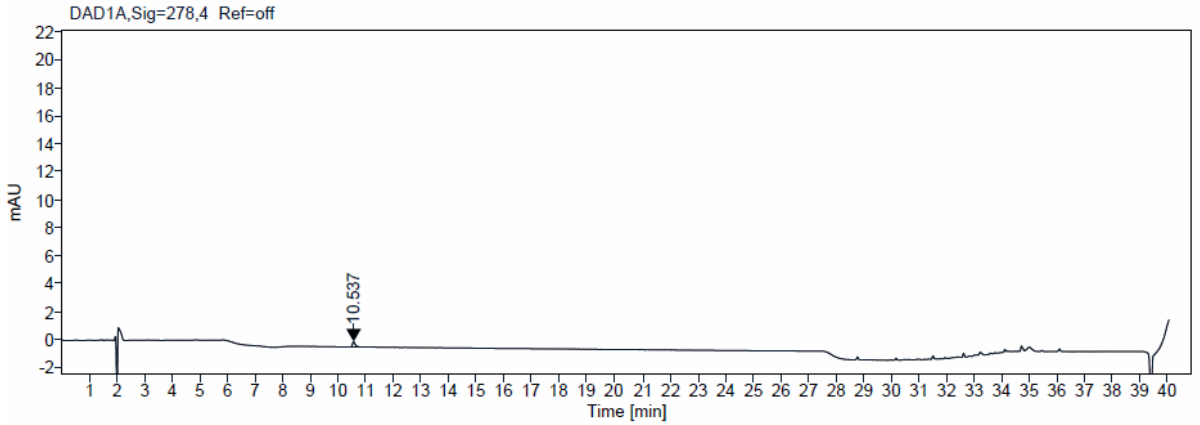
Figure 7 Example Chromatogram of signal to noise calculation for 0.03% DIA solution (LOD).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
10.544	MM m	0.4546	1.4910	0.1968	100.00		37.56624

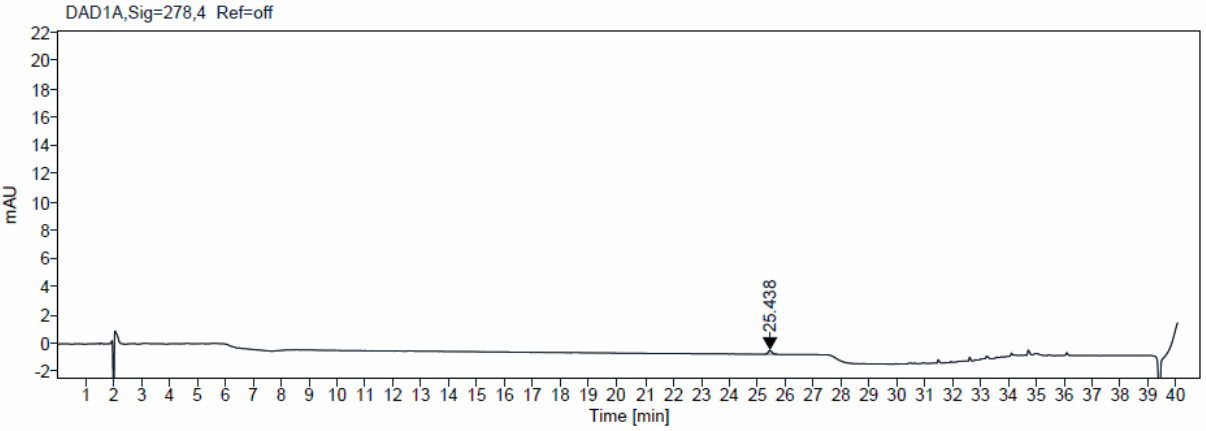
Figure 8 Example Chromatogram of signal to noise calculation for 0.05% DIA solution (LOQ).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
10.537	MM m	0.3644	2.3974	0.3585	100.00		72.965 22

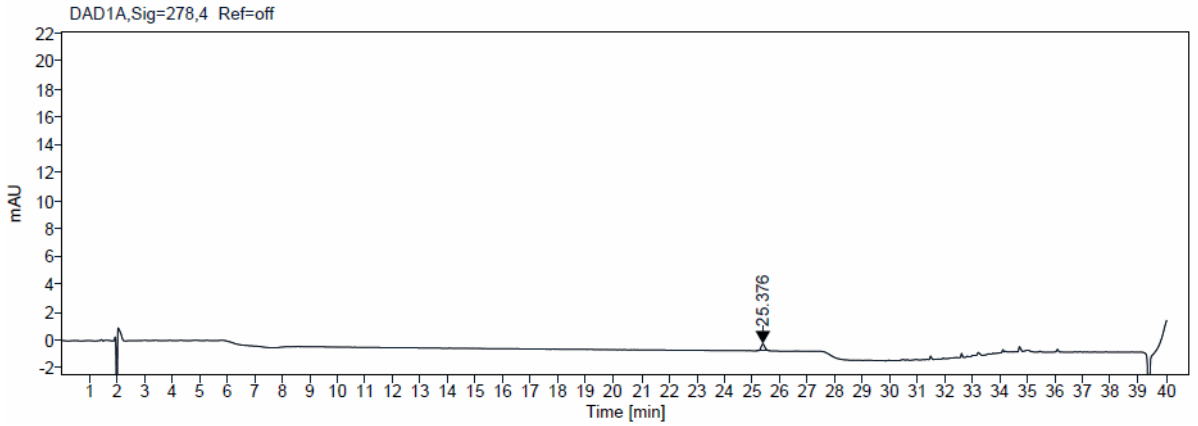
Figure 9 Example Chromatogram of signal to noise calculation for 0.03% DIN solution (LOD).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
25.438	MM m	0.4408	2.5680	0.2710	100.00		60.431 99

Figure 10 Example Chromatogram of signal to noise calculation for 0.05% DIN solution (LOQ).



Signal: DAD1A,Sig=278,4 Ref=off

RT [min]	Type	Width [min]	Area	Height	Area%	Name	Peak Signal To Noise
25.376	MM m	0.4515	4.6605	0.4860	100.00		83.166 14

3.4. Linearity of TGF-001

Linearity was performed using TGF-001 batch 100144-200502-REF.

The correlation coefficient (R^2) was found to be 0.9999 for the range from LOQ to 140%, 0.9998 for the range from LOQ to 5% and 0.9998 for the range from 50% to 140%. The y intercept and slope are reported in Table 5. All results meet the acceptance criteria.

Table 5 Results for linearity of TGF-001

Items	Result	Acceptance criteria
LOQ to 140%		
R^2	0.9999	≥ 0.995
y intercept	0.37%	≤ 5% of the nominal concentration
Slope	52689	report
LOQ to 5%		
R^2	0.9998	report
y intercept	0.05%	report
Slope	51576	report
50% to 140%		
R^2	0.9998	report
y intercept	2.19%	report
Slope	53579	report

Table 6 Concentration and area counts of linearity for TGF-001

% of the conc.	Conc. (mg/mL)	Area counts [pA]
0.05%	0.00008	2.879
0.1%	0.00017	5.636
0.5%	0.00083	34.856
5%	0.00829	423.865
20%	0.03315	1682.543
50%	0.08288	4294.905
80%	0.13261	6852.764
100%	0.16576	8660.748
120%	0.19891	10518.338
140%	0.23207	12242.546

Figure 11 Linearity of TGF-001 from LOQ to 140%

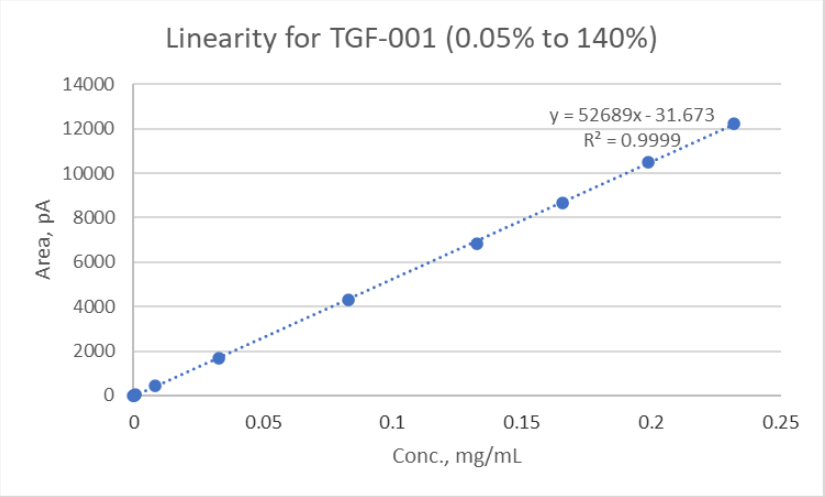


Figure 12 Linearity of TGF-001 from LOQ to 5%

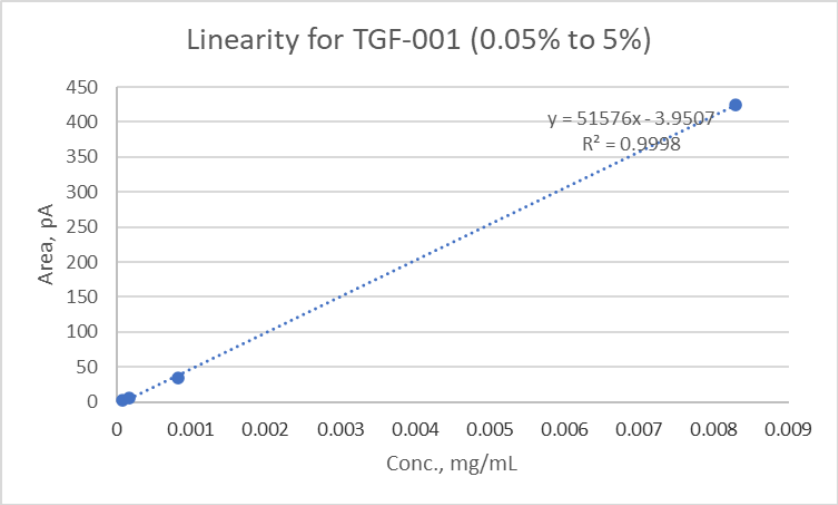
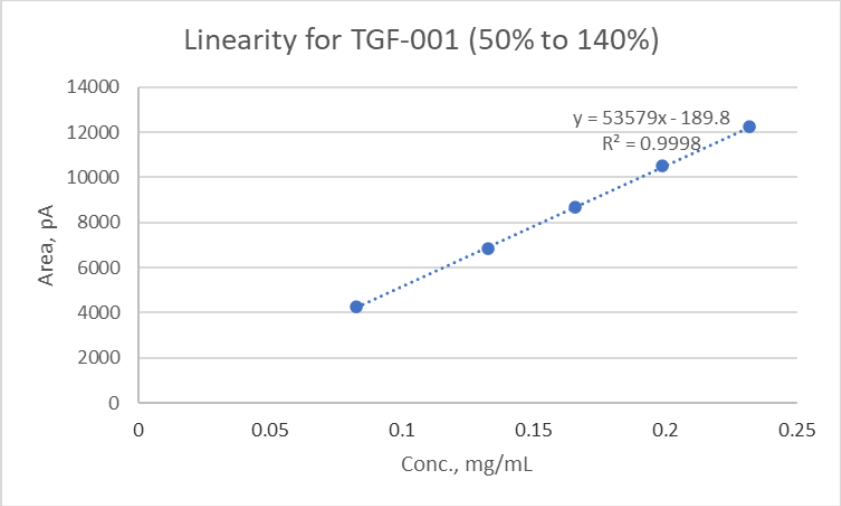


Figure 13 Linearity of TGF-001 from 50% to 140%



3.5. Linearity of DIA and DIN (RRF determination)

Linearity was performed using DIA batch PHTRACKD-394-REF and DIN batch PHTRACKD-389-REF. The correlation coefficient (R^2) was found to be 0.9995 for DIA from LOQ to 0.5% and 0.9993 for DIN from LOQ to 0.5%. All results meet the acceptance criteria.

The Relative Response Factor of DIA relative to TGF-001 was calculated to be '1.16' based on the slope of linearity and could be defined as '**1.00**'.

The Relative Response Factor of DIN relative to TGF-001 was calculated to be '0.91' based on the slope of linearity and could be defined as '**1.00**'.

Table 7 Results for linearity of DIA and DIN

Items	Result	Acceptance criteria
DIA		
R^2	0.9995	≥ 0.995
Slope	45379	report
RRF [1]	1.16	report
DIN		
R^2	0.9993	≥ 0.995
Slope	58071	report
RRF [1]	0.91	report

[1] RRF = slope main compound / slope impurity

Table 8 Concentration and area counts of linearity for DIA and DIN

% of the conc.	Conc. (mg/mL)	Area counts [AU*min]	
		DIA	DIN
0.05%	0.00008	2.3974	4.6605
0.08%	0.00014	4.1521	7.7411
0.1%	0.00017	5.6190	9.3238
0.14%	0.00024	8.5311	13.0105
0.17%	0.00029	10.7812	15.5249
0.3%	0.00051	21.1512	29.4748
0.5%	0.00085	36.6701	49.0316

Figure 14 Linearity of DIA from LOQ to 0.5%

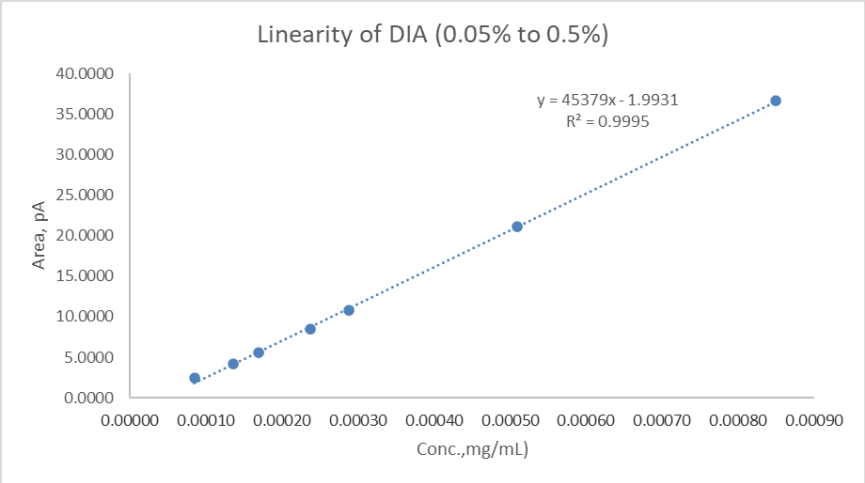
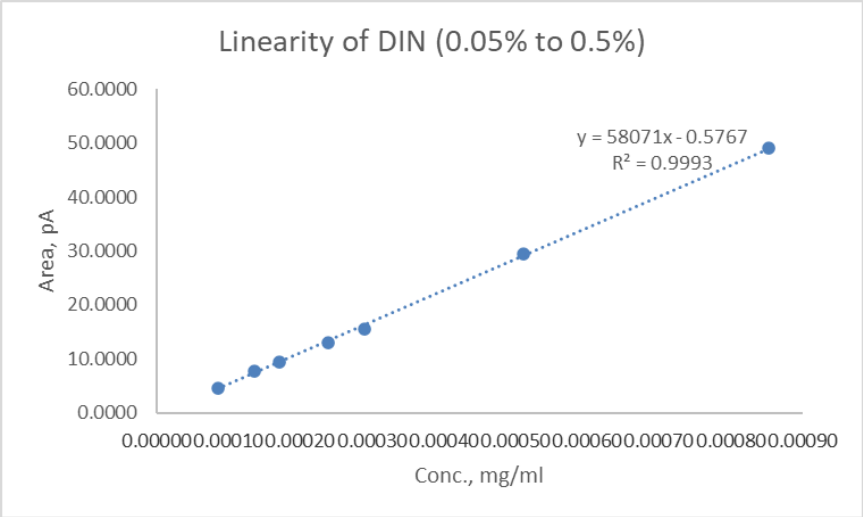


Figure 15 Linearity of DIN from LOQ to 0.5%



3.6. Accuracy of TGF-001

Accuracy was performed using TGF-001 batch 100144-200502-REF, DIA batch PHTRACKD-394-REF and DIN batch PHTRACKD-389-REF.

The %RSD of TGF-001 peak was determined to be 0.6% at 80% concentration level, 0.2% at 100% concentration level and 1.8% at 120% concentration level. The signal recovery and mean recovery for each concentration level are reported in Table 9, and all results meet the acceptance criteria.

Table 9 Results for accuracy of TGF-001

%RSD for each concentration level	**Result**	**Acceptance criteria**
%RSD (80%)	0.6%	≤ 2%
%RSD (100%)	1.7%	≤ 2%
%RSD (120%)	2.0%	≤ 2%
Single recovery	**Result**	**Acceptance criteria**
Recovery (80% prep.1)	99%	95% - 105%
Recovery (80% prep.2)	99%	95% - 105%
Recovery (80% prep.3)	98%	95% - 105%
Recovery (100% prep.1)	100%	95% - 105%
Recovery (100% prep.2)	100%	95% - 105%
Recovery (100% prep.3)	97%	95% - 105%
Recovery (120% prep.1)	101%	95% - 105%
Recovery (120% prep.2)	98%	95% - 105%
Recovery (120% prep.3)	98%	95% - 105%
Mean recovery for each concentration level	**Result**	**Acceptance criteria**
Recovery (80%)	98%	98% - 102%
Recovery (100%)	99%	98% - 102%
Recovery (120%)	99%	98% - 102%

Table 10 Calculations for accuracy of TGF-001

TGF-001	**Weigh in [mg]**	**Area counts [AU*min]**	**Response Factor (RF)**	**Recovery**
80% prep.1	11.99	6852.7640	28577.0	**99%**
80% prep.2	11.96	6821.5891	28518.3	**99%**
80% prep.3	11.86	6701.9588	28254.5	**98%**
Average	-	-	28449.9	**98%**
STDEV	-	-	171.8	**-**
%RSD	**-**	**-**	**0.60%**	**-**
100% prep.1	11.99	8660.7480	28893.2	**100%**
100% prep.2	11.96	8668.1695	28990.5	**100%**
100% prep.3	11.86	8335.2958	28112.3	**97%**
Average	-	-	28570.9	**99%**
STDEV	-	-	481.4	**-**
%RSD	**-**	**-**	**1.68%**	**-**
120% prep.1	11.99	10518.3380	29242.0	**101%**
120% prep.2	11.96	10176.5424	28362.7	**98%**
120% prep.3	11.86	10029.2460	28187.9	**98%**
Average	-	-	28597.5	**99%**
STDEV	-	-	564.9	**-**
%RSD	**-**	**-**	**1.98%**	**-**
100% 6 injections (average)	11.99	8663.1630	28901.2944	-

3.7. Repeatability

Repeatability was performed using TGF-001 batch 100144-200502-REF, DIA batch PHTRACKD-394-REF and DIN batch PHTRACKD-389-REF.

The results showed that the %RSD of peak area counts is 0.1% for TGF-001, 0.6% for DIA and 0.9% for DIN. All results meet the acceptance criteria.

Table 11 Results for repeatability for TGF-001, DIA and DIN

100% TGF-001	**Area of TGF-001, pA**	**0.14% DIA in 100% TGF-001**	**Area of DIA, pA**	**0.14% DIN in 100% TGF-001**	**Area of DIN, pA**
Inj. 1	8673.119	Inj. 1	13.987	Inj. 1	15.752
Inj. 2	8663.983	Inj. 2	14.085	Inj. 2	15.860
Inj. 3	8665.920	Inj. 3	13.953	Inj. 3	15.506
Inj. 4	8657.123	Inj. 4	14.122	Inj. 4	15.830
Inj. 5	8664.013	Inj. 5	13.972	Inj. 5	15.853
Inj. 6	8654.820	Inj. 6	14.131	Inj. 6	15.802
AVG	8663.1630	AVG	14.042	AVG	15.767
STDV	6.5453	STDV	0.0801	STDV	0.1337
RSD%	**0.08%**	RSD%	**0.57%**	RSD%	**0.85%**
Acceptance criteria	≤ 2%	-	≤ 15%	-	≤ 15%

4. Experimental

4.1. Equipment

- HPLC System: Agilent 1260 Infinity II system
  Open Lab CDS-control and integration software or equivalent
- Column: Waters X-Bridge C18, 150*4.6mm, 3.5µm PN: 186003034
- Flow rate: 1.0 mL/min
- Elution: Gradient mode
- Equilibrate time: 10.0 min
- Run time: 40.0 min
- Detection: 278nm
- Injection: 10 µL
- Column temp.: 35°C ± 2°C
- Auto sampler temp.: Room temperature
- Mobile phase:
  - **A:** 0.1% H_3PO_4 in Water
  - **B:** Acetonitrile
- Diluent: 0.1% H_3PO_4 in Water/Acetonitrile (80/20 v/v)
- Needle wash: Water/ Acetonitrile (50:50 v/v)
- Gradient:

Table 12 Gradient Table

Time (min)	% A	% B
0.0	98	2
3.0	98	2
5.0	92	8
25.0	85	15
32.0	15	85
37.0	15	85
37.1	98	2
40.0	98	2

4.2. Equipment and reagents

- Balance: Mettler Toledo XP56
- Acetonitrile: HPLC grade, Merck LiChrosolv, batch K51142730911
- Water: HPLC grade, from Milipore ultra-pure water system
- Phosphoric acid: HPLC grade, Sigmer-Aldrich, batch STBK2887.
- Glassware: 10, 20, 50 mL volumetric flasks, 1L graduated cylinders
- Pipette: 0.2, 1.0 mL, 5.0 mL Pipette

4.3. Solutions

4.3.1. Diluent

0.1% H_3PO_4 in Water/Acetonitrile (80/20 v/v):

Combine 800 mL of water and 200 mL of acetonitrile into a suitable container, and then add 1 mL of Phosphoric acid. Mix well.

4.3.2. Mobile phase

Preparation is described for a volume of 1 liter. Different volumes can be prepared as soon as the solvent ratio is the same.

Mobile phase A (0.1% H_3PO_4 in Water):

In a suitable container, add 1000 mL of water and 1 mL of Phosphoric acid. Mix well.

Mobile phase B (Acetonitrile):

Acetonitrile.

4.3.3. Standrad Solutions

- **Autosampler stability**

Accurately weigh approx. 6 mg of TGF-001 reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

- **Specificity/ selectivity and Repeatability**

Accurately weigh approx. 6 mg of TGF-001 reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

Accurately weigh approx. 6 mg of DIA reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well. Transfer 7µL of this solution into above 5mL of TGF-001 solution. Mix well.

Accurately weigh approx. 6 mg of DIN reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well. Transfer 7µL of this solution into above 5mL of TGF-001 solution. Mix well.

- **LOD, LOQ and Linearity**

TGF-001 solutions:

Stock Standard Solution (conc.: 0.6 mg/mL, 200%):

Accurately weigh approx. 12 mg of TGF-001 reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

140% standard Solution:

Transfer 7 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

120% standard Solution:

Transfer 6 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

100% standard Solution (conc.: 0.3 mg/mL):

Transfer 5 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

80% standard Solution:

Transfer 4 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

50% standard Solution:

Transfer 2.5 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

20% standard Solution:

Transfer 1 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

5% standard Solution:

Transfer 2.5 mL 20% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.5% standard Solution:

Transfer 1 mL 5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.1% standard Solution:

Transfer 1 mL 5% standard solution into a 50 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.05% standard Solution (LOQ solution):

Transfer 0.5 mL 5% standard solution into a 50 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.03% standard Solution (LOD solution):

Transfer 0.3 mL 5% standard solution into a 50 mL volumetric flask. Fill up to volume with sample diluent and mix well.

DIA solutions:

Stock Standard Solution (conc.: 0.16 mg/mL, 100%):

Accurately weigh approx. 8 mg of TGF-001 reference standard into a 50-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

0.5% standard Solution:

Transfer 0.5 mL stock standard solution into a 100 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.3% standard Solution:

Transfer 6 mL 0.5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.17% standard Solution:

Transfer 3.4 mL 0.5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.14% standard Solution:

Transfer 2.8 mL 0.5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.1% standard Solution:

Transfer 2 mL 5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.08% standard Solution:

Transfer 1.6 mL 5% standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.05% standard Solution (LOQ solution):

Transfer 0.5 mL 5% standard solution into a 50 mL volumetric flask. Fill up to volume with sample diluent and mix well.

0.03% standard Solution (LOD solution):

Transfer 0.3 mL 5% standard solution into a 50 mL volumetric flask. Fill up to volume with sample diluent and mix well.

DIN solutions:
Same as DIA solutions.

- **Accuracy**

Stock Standard Solution (conc.: 0.6 mg/mL, 200%):

Accurately weigh approx. 12 mg of TGF-001 reference standard into a 20-mL volumetric flask. Dissolve and dilute to volume with diluent. Mix well.

120% standard Solution:

Transfer 6 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

100% standard Solution (conc.: 0.3 mg/mL):

Transfer 5 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

80% standard Solution:

Transfer 4 mL stock standard solution into a 10 mL volumetric flask. Fill up to volume with sample diluent and mix well.

Prepare in triplicate.