GC-HS Method Limited Verification for Residual Solvents in TGF-001 (non-GMP)

Project	**Pyronaridine_INV-054926**
Compound	**TGF-001**
Purpose	**Method Verification**
Category	**Methods**
Substance Type	**Drug Substance**
Report ID	**INV_054926_GC_V1 Version 1.0**

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1. Objective

The residual solvents in TGF-001 are analyzed by the newly developed GC-HS method 'INV_054926_GC_M1'. In this document, the limited verification of the new GC-HS method shall be performed at non-GMP level.

Related reports:

INV_054926_GC_M1: GC-HS Method Description for Residual Solvents in TGF-001

2. Summary and conclusion

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

- Specificity/ selectivity
- LOD
- LOQ
- Linearity
- Accuracy
- Repeatability
- Matrix effect

The obtained results are summarized in Table 1.

Table 1 non-GMP verification of the new developed GC method for residual solvents in TGF-001

Parameter	Acceptance criteria	Results	Conformity	Remark
Specificity/ selectivity	No interference between the blank peaks and the components of interest in blanks. All solvent peaks should be separated.	No interference peaks in blanks. All solvent peaks are separated.	Pass	See section 3.1
LOD	S/N ≥ 3	S/N (methanol, 3ppm) = 10 S/N (ethanol, 5ppm) = 18 S/N (IPA, 5ppm) = 7 S/N (EA, 5ppm) = 13 S/N (THF, 1ppm) = 10	Pass	See section 3.2
LOQ	S/N ≥ 10 %RSD (3 injections) ≤ 30 %	S/N (methanol, 9ppm) = 17, 22, 13 S/N (ethanol, 15ppm) = 28, 37, 23 S/N (IPA, 15ppm) = 15, 20, 12 S/N (EA, 15ppm) = 27, 38, 22 S/N (THF, 2ppm) = 11, 16, 10 %RSD (methanol) = 1.6% %RSD (ethanol) = 5.1% %RSD (IPA) = 1.2% %RSD (EA) = 0.2% %RSD (THF) = 4.4%	Pass	See section 3.2
Linearity	R^2 ≥ 0.99, Report R^2, y intercept, slope, figure	Methanol: R^2 = 0.9999 y intercept = -0.0861 Slope = 14799 Ethanol: R^2 = 1.0000 y intercept = -0.0829 Slope = 16504 IPA: R^2 = 1.0000 y intercept = 0.02309 Slope = 20310	Pass	See section 3.3

		EA: R^2 = 0.9999 y intercept = 1.1153 Slope = 49719 THF: R^2 = 0.9999 y intercept = 0.0319 Slope = 79380		
Accuracy	%RSD ≤ 10% for each concentration level	Methanol: %RSD (50%) = 0.1% %RSD (100%) = 0.6% %RSD (150%) = 0.2% Ethanol: %RSD (50%) = 0.2% %RSD (100%) = 0.5% %RSD (150%) = 0.2% IPA: %RSD (50%) = 0.2% %RSD (100%) = 0.5% %RSD (150%) = 0.2% EA: %RSD (50%) = 0.4% %RSD (100%) = 0.4% %RSD (150%) = 0.3% THF: %RSD (50%) = 0.4% %RSD (100%) = 0.4% %RSD (150%) = 0.3%	Pass	See section 3.4
Repeatability (with Matrix)	%RSD (n=6) ≤ 10%	%RSD (methanol) = 0.4% %RSD (ethanol) = 0.4%	Pass	See section 3.5

		%RSD (IPA) = 0.3% %RSD (EAI) = 0.3% %RSD (THF) = 0.4%		
Matrix effect	Report Matrix effect (M)	M (methanol) = 1.00 (1.11) M (ethanol) = 1.00 (1.05) M (IPA) = 1.00 (1.06) M (EA) = 1.00 (1.05) M (THF) = 1.00 (1.03) If 0.80 ≤ M ≤ 1.20, the matrix is considered as analytical irrelevant, and M is defined as 1.00	Pass	See section 3.6

3. Results

3.1. Specificity/ selectivity

Specificity/ selectivity was performed using a mixture of solvents i.e.: methanol, ethanol, IPA, EA and THF.

There is no interference between the blank peaks and the components of interest. All solvent peaks are separated.

Figure 1 Example GC-HS chromatogram of blank, method INV_054926_GC_M1, FID

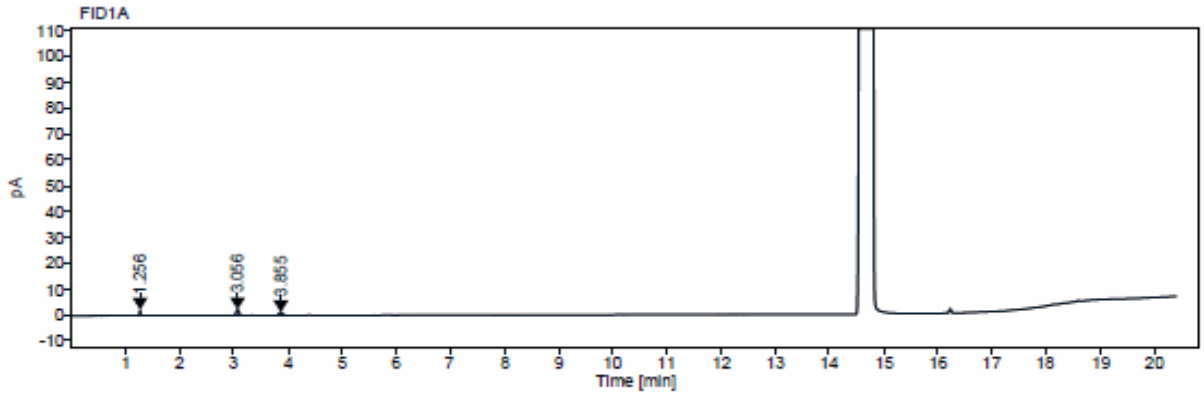
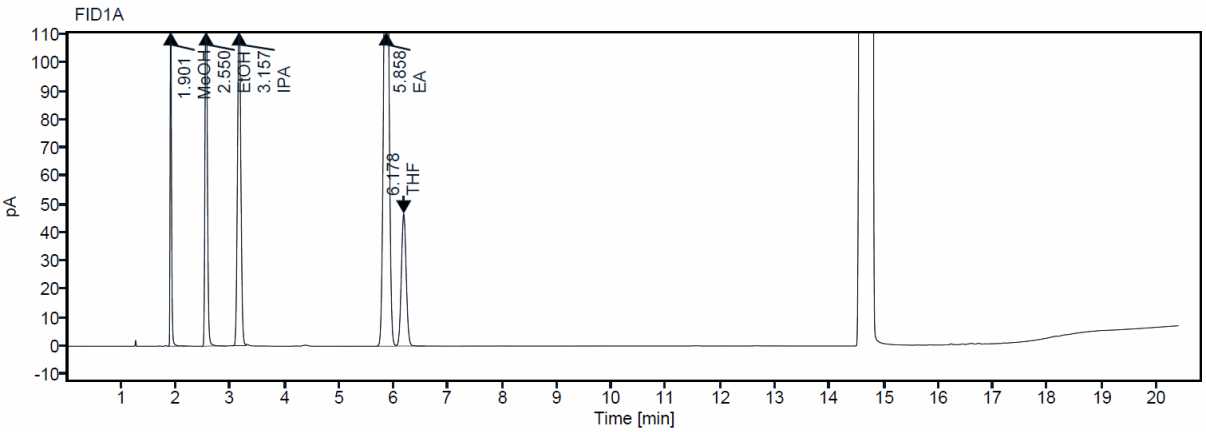


Figure 2 Example GC-HS chromatogram of mixed solvents, method INV_054926_GC_M1, FID



3.2. LOD/ LOQ

LOD and LOQ were performed using a mixture of solvents i.e.: methanol, ethanol, IPA, EA and THF. The signal to noise is calculated for peaks of methanol (10), ethanol (18), IPA (7), EA (13) and THF (10) in LOD solution. The signal to noise is calculated for peaks of methanol (17, 22, 13), ethanol (28, 37, 23), IPA (15, 20, 12), EA (27, 38, 22) and THF (11, 16, 10) in LOQ solutions.

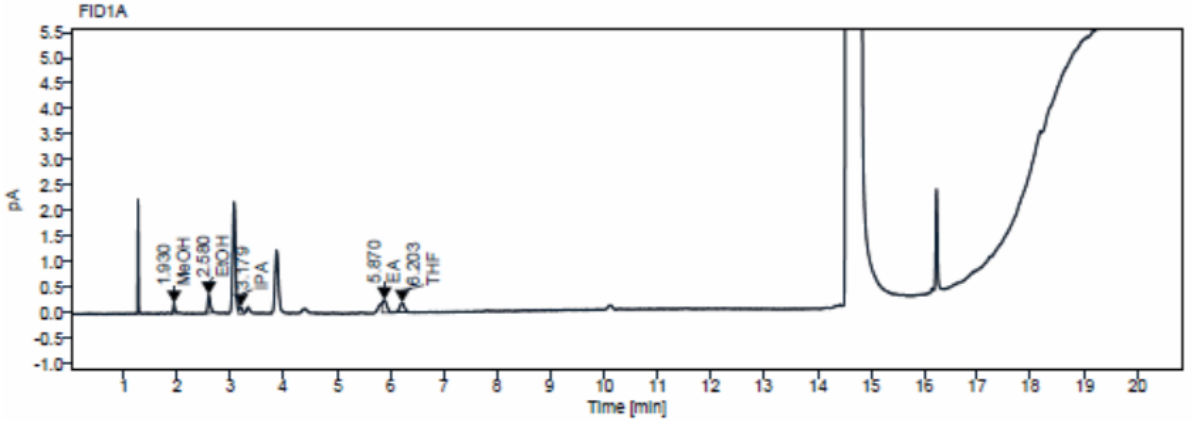
The %RSD of peak area in LOQ solutions (n=3) is found to be 1.6%, 5.1%, 1.2%, 0.2% and 4.4% for methanol, ethanol, IPA, EA and THF respectively.

All results meet the acceptance criteria.

Table 2 %RSD results for 3 injections of LOQ solutions

Reference solution 2	**Area counts [pA]**				
	Methanol (9 ppm)	Ethanol (15 ppm)	IPA (15 ppm)	EA (15 ppm)	THF (2 ppm)
Inj. 1	0.8506	2.3459	1.4880	4.1579	1.6897
Inj. 2	0.8238	2.1636	1.5174	4.1735	1.6546
Inj. 3	0.8363	2.3849	1.5212	4.1663	1.7983
Average	0.8369	2.2981	1.5089	4.1659	1.7142
STDEV	0.0134	0.1181	0.0182	0.0078	0.0749
%RSD	1.6%	5.1%	1.2%	0.2%	4.4%
Acceptance criteria	≤ 30%				

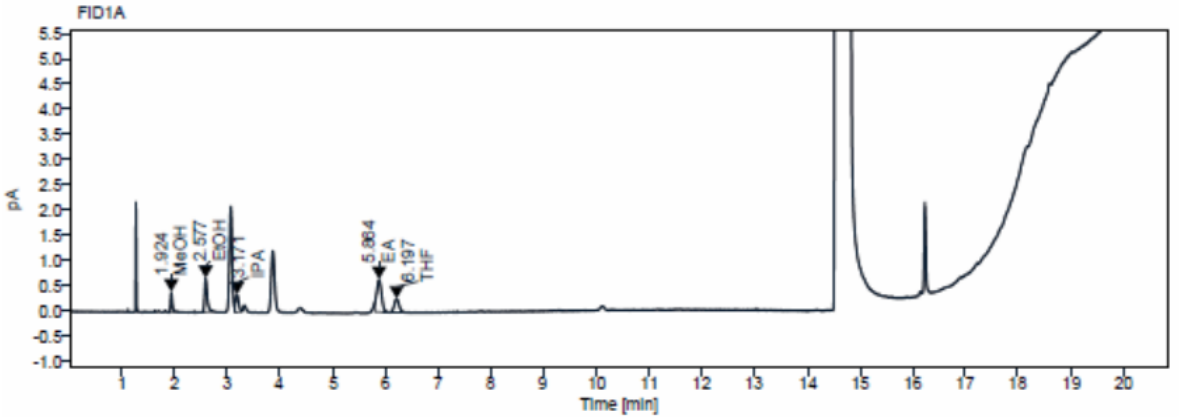
Figure 3 Example Chromatogram of signal to noise calculation for LOD solution.



Signal: FID1A

RT [min]	Peak Signal To Noise	Name	Type	Width [min]	Area	Height	Area%	Peak Signal To Noise
1.930	9.979 61	MeOH	MM m	0.0894	0.4127	0.1930	8.6684	9.98
2.580	18.489 66	EtOH	MM m	0.1478	1.2145	0.3575	25.5088	18.49
3.179	6.797 34	IPA	MM m	0.1174	0.5752	0.1314	12.0819	6.80
5.870	12.936 96	EA	MM m	0.1733	1.4616	0.2501	30.6984	12.94
6.203	9.716 10	THF	MM m	0.2104	1.0971	0.1879	23.0425	9.72

Figure 4 Example chromatogram of signal to noise calculation for LOQ solution.



Signal: FID1A

RT [min]	Peak Signal To Noise	Name	Type	Width [min]	Area	Height	Area%	Peak Signal To Noise
1.924	16.648 77	MeOH	MM m	0.1355	0.8506	0.3994	8.0760	16.65
2.577	27.987 76	EtOH	MM m	0.2208	2.3459	0.6714	22.2737	27.99
3.171	14.560 10	IPA	MM m	0.1363	1.4880	0.3493	14.1281	14.56
5.864	27.026 16	EA	MM m	0.2285	4.1579	0.6484	39.4789	27.03
6.197	11.265 49	THF	MM m	0.2309	1.6897	0.2703	16.0433	11.27

3.3. Linearity

Linearity was performed using a mixture of solvents i.e.: methanol, ethanol, IPA, EA and THF from 5% to 150% of each ICH limit concentration.

All results are reported in Table 3, and meet the acceptance criteria. Detailed results are listed in Table 4.

Table 3 Results for linearity of each solvent.

Items	Result	Acceptance criteria
Methanol		
R^2	0.9999	≥ 0.99
Slope	14799	report
y intercept	-0.0861	report
Ethanol		
R^2	1.0000	≥ 0.99
Slope	16504	report
y intercept	-0.0829	report
IPA		
R^2	1.0000	≥ 0.99
Slope	20310	report
y intercept	0.2309	report
EA		
R^2	0.9999	≥ 0.99
Slope	49719	report
y intercept	1.1153	report
THF		
R^2	0.9999	≥ 0.99
Slope	79380	report
y intercept	0.0319	report

Table 4 Concentration and area counts of linearity for each solvent

Methanol		Ethanol		IPA		EA		THF	
Conc. mg/mL	Area counts [pA]	Conc. mg/mL	Area counts [pA]	Conc. mg/mL	Area counts [pA]	Conc. mg/mL	Area counts [pA]	Conc. mg/mL	Area counts [pA]
0.0007	11.0994	0.0012	20.621	0.0012	25.6206	0.0013	62.0951	0.0002	14.0027
0.0015	21.9651	0.0025	40.6134	0.0025	50.3412	0.0025	123.0115	0.0004	28.0361
0.0037	55.035	0.0062	102.5873	0.0062	126.5444	0.0063	309.6876	0.0009	70.5281
0.0075	111.7875	0.0125	208.3544	0.0125	256.2485	0.0125	635.0496	0.0018	145.867
0.0150	219.9448	0.0250	410.0278	0.0250	505.0776	0.0251	1244.4415	0.0036	285.5132
0.0225	332.9602	0.0375	619.2713	0.0375	761.637	0.0376	1869.3375	0.0054	428.6657

Figure 5 Linearity of Methanol from 5% to 150% of ICH limit (3000 ppm)

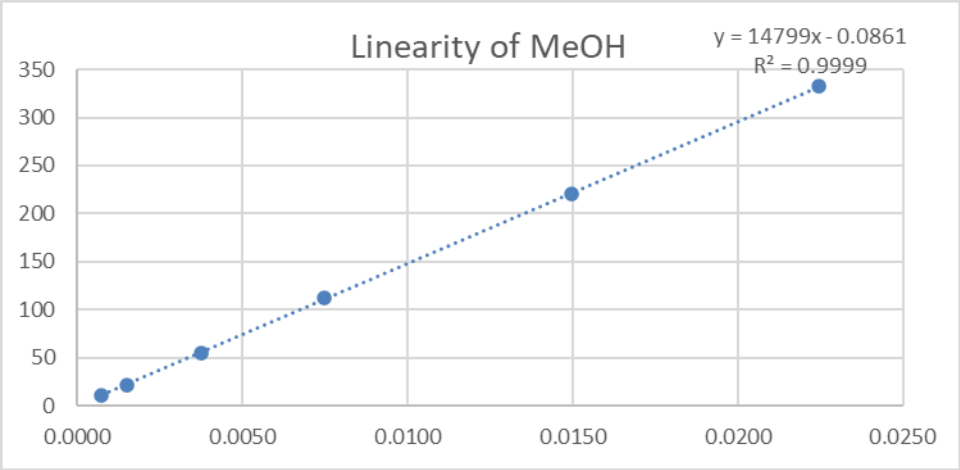


Figure 6 Linearity of Ethanol from 5% to 150% of ICH limit (5000 ppm)

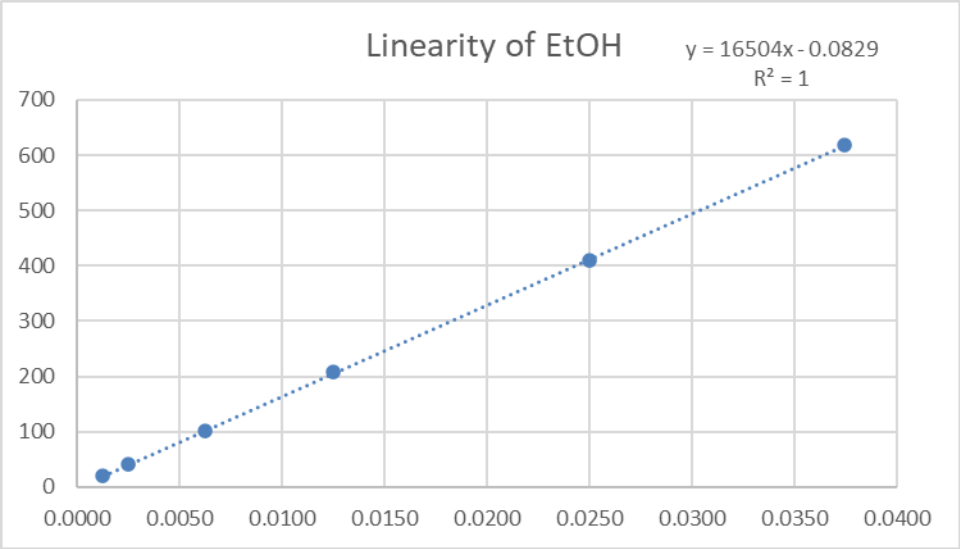


Figure 7 Linearity of IPA from 5% to 150% of ICH limit (5000 ppm)

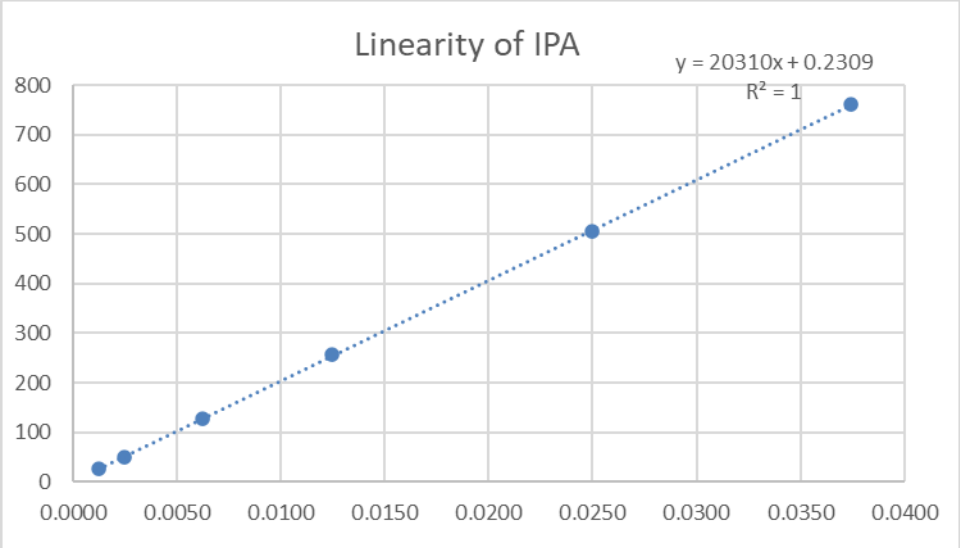


Figure 8 Linearity of EA from 5% to 150% of ICH limit (5000 ppm)

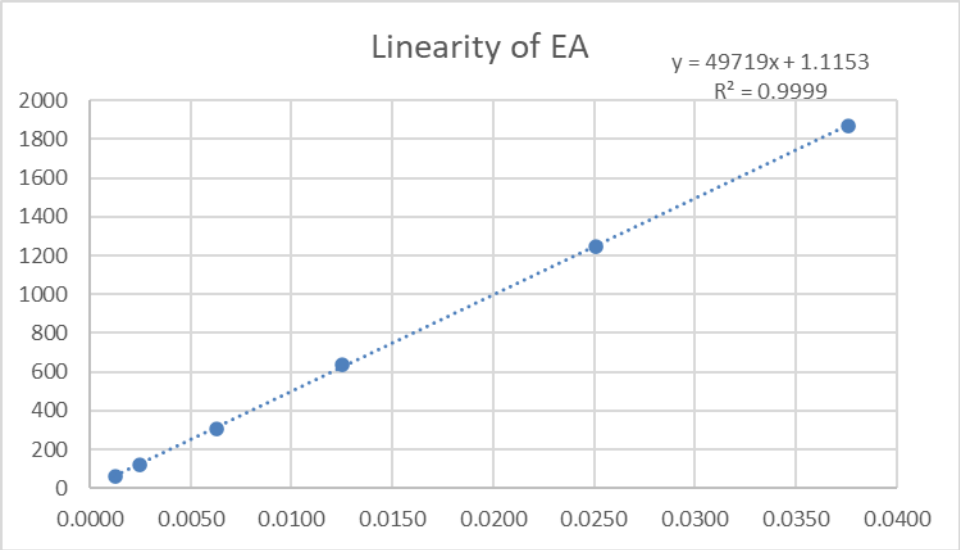
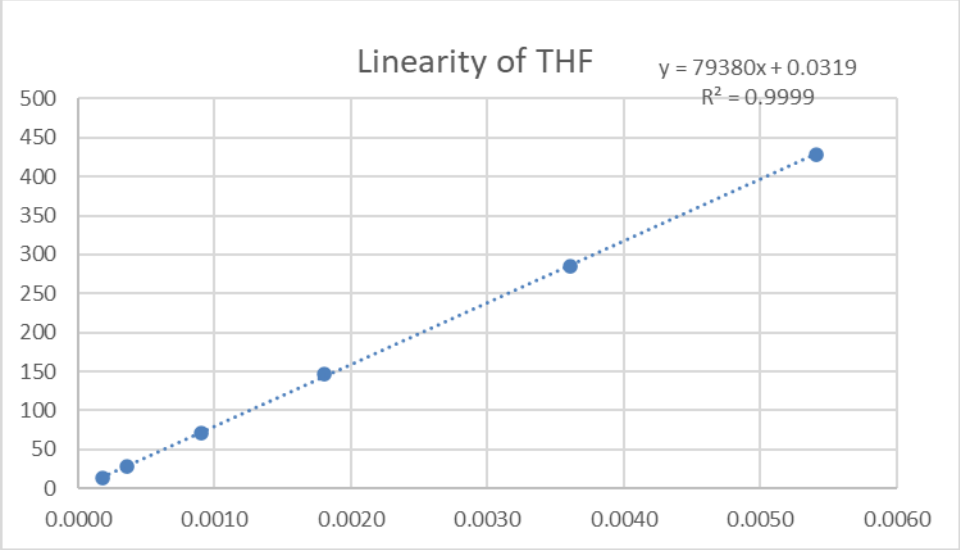


Figure 9 Linearity of THF from 5% to 150% of ICH limit (720 ppm)



3.4. Accuracy

Accuracy was performed using a mixture of solvents i.e.: methanol, ethanol, IPA, EA and THF at concentration level of 50%, 100%, 150% of each ICH limit.

All results are reported in Table 5, and meet the acceptance criteria.

Table 5 Results for accuracy of each solvent.

each conc. level	Area counts [pA]	Average	STDEV	%RSD	Acceptance criteria
Methanol					
50% prep. 1	111.7875	111.6364	0.1363	0.1%	≤ 10%
50% prep. 2	111.5988				
50% prep. 3	111.5228				
100% prep. 1	219.9448	220.9322	1.3391	0.6%	≤ 10%
100% prep. 2	220.3954				
100% prep. 3	222.4564				
150% prep. 1	332.9602	333.1583	0.5884	0.2%	≤ 10%
150% prep. 2	333.8202				
150% prep. 3	332.6945				
Ethanol					
50% prep. 1	208.3544	207.8735	0.4246	0.2%	≤ 10%
50% prep. 2	207.5504				
50% prep. 3	207.7156				
100% prep. 1	410.0278	411.1161	2.0104	0.5%	≤ 10%
100% prep. 2	409.8844				
100% prep. 3	413.4360				
150% prep. 1	619.2713	620.0606	1.0580	0.2%	≤ 10%
150% prep. 2	621.2628				
150% prep. 3	619.6477				
IPA					
50% prep. 1	256.2485	255.9286	0.4544	0.2%	≤ 10%
50% prep. 2	256.1287				
50% prep. 3	255.4085				
100% prep. 1	505.0776	506.4222	2.3296	0.5%	≤ 10%
100% prep. 2	505.0767				
100% prep. 3	509.1122				
150% prep. 1	761.6370	762.8830	1.4541	0.2%	≤ 10%
150% prep. 2	764.4807				
150% prep. 3	762.5312				
EA					
50% prep. 1	635.0496	632.2529	2.4289	0.4%	≤ 10%
50% prep. 2	630.6710				
50% prep. 3	631.0382				
100% prep. 1	1244.4415	1246.2156	4.8672	0.4%	≤ 10%
100% prep. 2	1242.4844				
100% prep. 3	1251.7210				
150% prep. 1	1869.3375	1873.4441	5.3617	0.3%	≤ 10%
150% prep. 2	1879.5099				
150% prep. 3	1871.4849				
THF					

50% prep. 1	150.3150	149.6516	0.5842	0.4%	≤ 10%
50% prep. 2	149.2140				
50% prep. 3	149.4259				
100% prep. 1	289.9612	290.1544	1.0544	0.4%	≤ 10%
100% prep. 2	289.2100				
100% prep. 3	291.2920				
150% prep. 1	433.1137	433.5752	1.4674	0.3%	≤ 10%
150% prep. 2	435.2178				
150% prep. 3	432.3940				

3.5. Repeatability

Repeatability was performed using a mixture of solvent i.e.: methanol, ethanol, IPA, EA and THF at ICH limit concentration level spiked into 100% TGF-001.

All results are reported in Table 6, and meet the acceptance criteria (%RSD ≤ 10%).

Table 6 Results for repeatability for each solvent

Methanol		**Ethanol**		**IPA**		**EA**		**THF**	
No.	**Area, pA**	**No.**	**Area, pA**	**No.**	**Area, pA**	**No.**	**Area, pA**	**No.**	**Area, pA**
Inj. 1	245.6622	Inj. 1	431.0253	Inj. 1	538.9318	Inj. 1	1305.9106	Inj. 1	300.2533
Inj. 2	243.9681	Inj. 2	428.0942	Inj. 2	535.5121	Inj. 2	1299.6952	Inj. 2	298.2147
Inj. 3	246.4645	Inj. 3	432.7361	Inj. 3	541.0668	Inj. 3	1310.7249	Inj. 3	300.9556
Inj. 4	245.8587	Inj. 4	431.34	Inj. 4	538.7995	Inj. 4	1301.913	Inj. 4	299.2242
Inj. 5	245.5897	Inj. 5	430.926	Inj. 5	539.2093	Inj. 5	1309.6264	Inj. 5	300.8996
Inj. 6	246.5531	Inj. 6	431.7048	Inj. 6	539.608	Inj. 6	1305.2403	Inj. 6	299.7090
AVG	245.6827	AVG	430.9711	AVG	538.8546	AVG	1305.5184	AVG	299.8761
STDV	0.9326	STDV	1.5538	STDV	1.8313	STDV	1.0553	STDV	1.0553
RSD%	**0.4%**	RSD%	**0.4%**	RSD%	**0.3%**	RSD%	**0.3%**	RSD%	**0.4%**

3.6. Matrix effect

The matrix effect was evaluated based on the comparison between solvents with and without matrix at ICH limit concentration level.

Table 7 Calculation of matrix effect for each solvent

Solvent	**Methanol**	**Ethanol**	**IPA**	**EA**	**THF**
Average peak area of 3 injections **without** matrix (from accuracy test)	220.9322	430.9711	506.4222	1246.2156	290.1544
Average peak area of 6 injections **with** matrix (from repeatability test)	245.6827	411.1161	538.8546	1305.5184	299.8761
M	**1.11**	**1.05**	**1.06**	**1.05**	**1.03**

4. Experimental

4.1. GC-HS

GC-HS System: GC (Agilent 7890B services)
Headspace (Agilent 7697A services)
Open Lab CDS-control and integration software or equivalent

GC conditions:

- GC column: DB-624, 30m length x 0.53 mm internal diameter, 3.0 µm film
- Inlet temperature: 200°C
- Split ratio: 10:1
- Carrier gas: Nitrogen
- Column flow: 5.0 mL/min (constant flow)
- Detector temp.: 260°C (FID)
- H2 flow: 40 mL/min
- Air flow: 350 mL/min
- Makeup flow: 25 mL/min (Nitrogen)
- Injection Volume: 1000µL
- Run time: 20.4 min

Headspace conditions:

- Oven temperature: 100°C
- Loop temperature: 110°C
- Transfer line temp.: 120°C
- Inlet Temp: 230°C
- Vial equilibration: 30 min
- Injection time: 0.5 min
- GC cycle time: 35 min
- Fill pressure: 3 psi

Temperature Gradient:

Temp (°C)	Rate (°C/min)	Hold (min)
40	0	5
120	8	15
240	50	20.4

4.2. Equipment and reagents

- Balance: Mettler Toledo XP56
- DMSO: GC grade, Sigma-Aldrich
- Methanol: GC grade, Merck
- Ethanol: GC grade, Merck
- Isopropanol: GC grade, Merck
- Ethyl acetate: GC grade, Merck
- THF: GC grade, Merck
- Glassware: 20, 25-mL volumetric flasks, 1L graduated cylinders
- Pipette: 1.0 mL Pipette

4.3. Solutions

4.3.1. Diluent

DMSO.

4.3.2. Blank

Pipette 1.0mL of diluent into headspace vials and seal each vial securely.

4.3.3. Standard Solutions

- **Specificity/ selectivity and Repeatability**

Accurately weigh approx.50 mg of TGF-001 reference standard into a 20-mL into headspace vials. Pipette 1.0mL of 100% linearity Solution into the headspace vials. Seal each vial securely.

- **Linearity and accuracy**

Stock Standard Solution (2000%):

Accurately weigh approx. 2.5 of EtOH,2.5g of IPA ,2.5g of EA ,1.5g of MeOH ,0.36gTHF into a 25-mL volumetric flask. Dissolve and dilute to volume with diluent.

Transfer 1 mL above solution into a 20 mL volumetric flask. Fill up to volume with diluent and mix well.

150% linearity Solution:

Transfer 1.5 mL stock standard solution into a 20 mL volumetric flask. Fill up to volume with diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

100% linearity Solution:

Transfer 1.0 mL stock standard solution into a 20 mL volumetric flask. Fill up to volume with diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

50% linearity Solution:

Transfer 0.5 mL stock standard solution into a 20 mL volumetric flask. Fill up to volume with diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

25% linearity Solution:

Transfer 2.5 mL 100% linearity Solution into a 10 mL volumetric flask. Fill up to volume with diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

10% linearity Solution:

Transfer 1.0 mL 100% linearity Solution into a 10 mL volumetric flask. Fill up to volume with diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

5% linearity Solution:

Transfer 1 mL 25% linearity solution into a 5 mL volumetric flask. Fill up to volume with sample diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

- **LOD and LOQ**

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

LOQ Solution (0.3% of ICH limit concentration for each solvent):

Transfer 1.5 mL 1% linearity solution into a 5 mL volumetric flask. Fill up to volume with sample diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.

LOQ Solution (0.1% of ICH limit concentration for each solvent):

Transfer 1.0 mL 1% linearity solution into a 10 mL volumetric flask. Fill up to volume with sample diluent. Pipette 1.0mL into of this solution into headspace vials and seal each vial securely.