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

Project	**Pyronaridine_INV-054926**
Compound	**TGF-001**
Purpose	**Method Description**
Category	**Methods**
Substance Type	**Drug substance**
Report ID	**INV_054926_GC_M1 Version 1.0**

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1. Objective

This method 'INV_054926_GC_M1' for residual solvents in TGF-001 of Pyronaridine (INV-054926) project is developed by GC-HS. The parameters of the GC-HS method suitable for residual solvents in TGF-001 shall be described in this document.

Related reports:

INV_054926_GC_V1: GC-HS Method Limited Verification for Residual Solvents in TGF-001 (non-GMP)

2. Summary and conclusion

The method is applied for the GC-HS testing of residual solvents in TGF-001. This method is based on Headspace GC chromatography with FID detection and gradient elution using a DB-624, 30m length, 0.53 mm internal diameter, 3.0 µm film GC column.

Table 1 Retention time of each solvent

Solvent	Retention time (RT)	Matrix Factor (M)	LOQ	LOD
Methanol	ca. 1.90 min	1.00	15 ppm	5 ppm
Ethanol	ca. 2.55 min	1.00	15 ppm	5 ppm
Isopropanol	ca. 3.16 min	1.00	15 ppm	5 ppm
Ethyl acetate	ca. 5.86 min	1.00	9 ppm	3 ppm
Tetrahydrofuran	ca. 6.18 min	1.00	2 ppm	1 ppm

Matrix effect: the presence of TGF-001 in sample solution may affect the headspace response and a correction factor may be used. If $0.80 \leq M \leq 1.20$, the matrix is considered as analytical irrelevant, and M is defined as 1.00.

Specificity, LOD, LOQ, Linearity, Accuracy, Repeatability and Matrix effect were performed and reported in report 'INV_054926_GC_V1'.

3. Experimental

Equivalent equipment or grade of materials can be used.

3.1. GC-HS

GC-HS System: GC (e.g.: Agilent 7890B services)
Headspace (e.g.: 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

3.2. Equipment and reagents

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

3.3. Solutions

3.3.1. Diluent

DMSO.

3.3.2. Blank

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

3.3.3. Standard Solutions

Multi Solvent Stock Solution (5 solvents)

Accurately weight-in approx. 2.5g of ethanol, 2.5g of isopropanol, 2.5g of ethyl acetate, 1.5g of methanol and 0.36g THF into a 25-mL volumetric flask, and then dilute to volume with diluent. Mix well.

Standard Solution 1:

Transfer 1 mL of Multi Solvent Stock Solution into a 20-mL volumetric flask. Fill up to volume with diluent and mix well.

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

Pipette 1.0mL of Standard Solution 1 into headspace vials and seal each vial securely.

Standard Solution 2 at reporting threshold level:

Transfer 1.5 mL of Standard Solution 1 into a 25 mL volumetric flask. Fill up to volume with diluent and mix well.

Transfer 0.5 mL of above solution into a 10 mL volumetric flask. Fill up to volume with diluent and mix.

Pipette 1.0mL of Standard Solution 2 into headspace vials and seal each vial securely.

3.3.3.1. Sample solutions

Accurately weigh approx. 50 mg of TGF-001 sample into headspace vials and the add 1.0 mL of diluent, seal each vial securely. Prepare in duplicate.

All TGF-001 Materials must be weighted out in a hunidity-controlled environment (≤ 10%).

3.4. Proposed injection sequence and system suitability test

Table 2 Proposed injection sequence and SST criteria

Sample name	No. of injections [1]	SST acceptance criteria
Blank	1 + N [1]	No interference between the blank peaks and the components of interest
Standard Solution 2	1	S/N ≥ 10 for each solvent peak
Standard Solution 1	6	%RSD ≤ 10 % for peak area of each solvent
Sample solution prep.1	1	N/A
Sample solution prep.2	1	N/A
Standard Solution 1 [2]	1	Recovery: 80% - 120% (6 injection Std 1 to be used as reference)

[1] Additional blanks may be run until an acceptable baseline is obtained. - [2] For multiple sample analysis, 1 injection of standard solution 1 is recommended every 12 sample preparation injections.

3.5. Calculation and Reporting

Calculations should be performed individually for each sample weighing. Only then should the calculation of the average result be performed.

3.5.1. Identification by GC-HS

The Retention Time range for the Identification of every residual solvent in the Sample Solution is ± 1.0% of the Retention Time of each residual solvent in Reference Solution 2 at Reporting Threshold Level.

3.5.2. Resiudal solvents by GC-HS in ppm

$$C_i \ (\text{ppm}) = \frac{\text{Area}_{\text{sam.}} \times W_{\text{std.}} \times V_{\text{sam.}}}{\text{Area}_{\text{std.}} \times W_{\text{sam.}} \times V_{\text{std.}}} \times M \times 1000000$$

Where:

Area $_{sam.}$ = Peak area of residual solvent obtained in the sample chromatogram
Area $_{std.}$ = Average peak area of residual solvent obtained for the 6 standard injections (std 1)
W $_{std}$ = Weight of residual solvent for Standard Solution 1 preparation (mg)
W $_{Sam}$ = Sample weight (mg)
V $_{sam}$ = Dilution volume of sample
V $_{std}$ = Dilution volume of standard solution 1
M = Matrix effect factor

Analyses with two sample weighings: (C1+C2)/2

- For residual solvent content below LOQ concentration, report "Less than LOQ';
- If residual solvent is not detected, report "Not detected'.

4. Figures

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

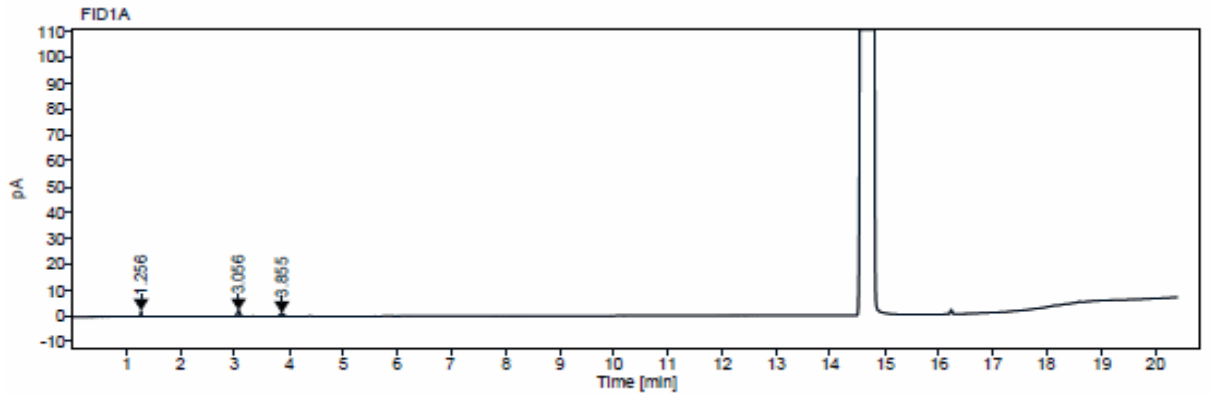

Figure 2 Example GC-HS chromatogram of Standard solution 1, method INV_054926_GC_M1, FID

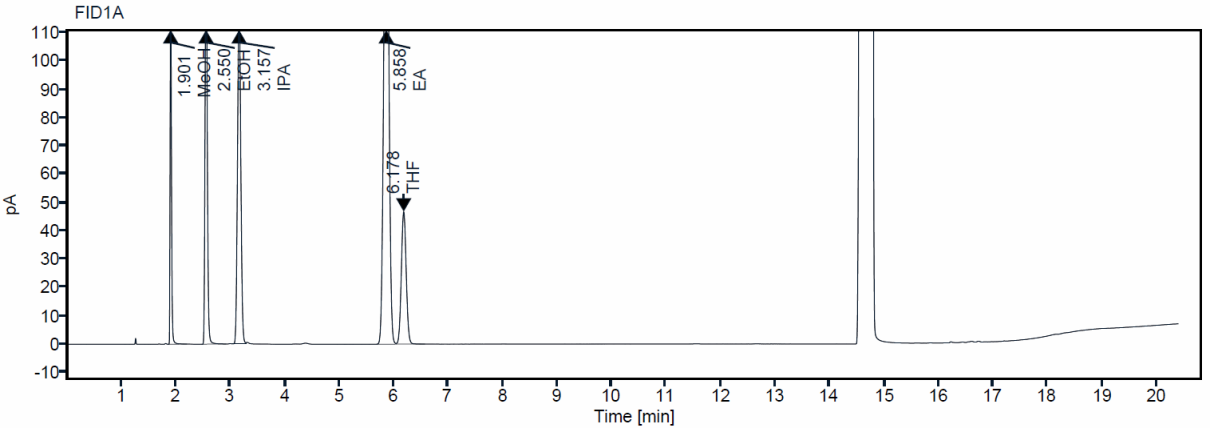

Figure 3 Example GC-HS chromatogram of TGF-001 sample solution, method INV_054926_GC_M1, FID