International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Determination of Residual Solvents in Teneligliptin by Head Space Gas Chromatography

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Running Title: Residual Analysis of Teneligliptin

Abstract: ***A simple and selective HS-GC method is described for the determination & quantification of Residual Solvents in Teneligliptin API. Chromatographic separation was achieved on a DB-624 column, (30mx0.53mm) 3.0µm column using different temperature gradient of FID Detectors. Linearity was observed in the range 50-150 µg /ml for Methanol, Dimethyl formamide, Tetrahydrofuran, Dimethyl acetamide and 1,4Dioxane (r^2>0.999) for the amount of solvent estimated by the proposed methods was in good agreement. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered diluent and API. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 10 for Methanol, Dimethyl formamide, Tetrahydrofuran, Dimethyl acetamide and 1,4Dioxane. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical active ingredients for estimation of Residual Solvents of Methanol, DMF, THF, THF, DMA and 1,4 Dioxane in Teneligliptin.***

Keywords: HS-GC, Teneligliptin, residual solvents, dimethyl acetamide, flame ionization detector

1. Introduction

Teneligliptin is a third generation dipeptidyl peptidase-4 inhibitor used in the treatment of Type 2 Diabetes Mellitus. Gas Chromatography operates as follows:

Helium is used as the inert carrier gas which is used under controlled pressure .The regulated carrier gas is let into the detector. The sample is injected into the heated injection port where it is volatilized and carried into the column by the carrier gas. The sample is separated inside the column by differential partition of the analytes between the mobile and stationary phases, based on relative vapor pressure and solubility in the immobilized liquid stationary phase. On elution from the column, the carrier gas and analytes pass into a detector, which responds to some physicochemical property of the analyte and generates an electronic signal in response to the amount of analyte present. An integrated chromatogram is generated. The temperature of the GC oven typically ranges from 5°C to 400°C.

Flame-ionization detector (FID) has a nearly universal response to organic compounds, a low LOD and a wide linear response range.The FID response results from the combustion of organic compounds in a small hydrogen-air diffusion flame. The objective is to limit the acceptable amounts of residual solvents in pharmaceuticals for the safety of the patient. The term tolerable daily intake (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals, and the term acceptable daily intake (ADI).

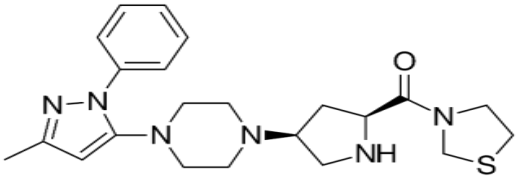


Figure 1: Chemical structure of Teneligliptin

No previous analytical method was developed to estimate the class 2 solvents in Teneligliptin by using head space gas chromatography as per the literature review. The aim is to determine a method for estimation and validation of class 2 residual solvents in Teneligliptin by HS-GC FID.

2. Experimental Set-up

2.1 Materials and reagents

Teneligliptin API was a sample gifted by Dr. Reddy's Laboratory, all other reagents were GC grade; Dimethyl sulfoxide and Methanol were purchased from Qualigens; Toulene, cycloheaxane and methyl isobutylketone were purchased from Sigma Aldrech.

2.2. Instruments

Agilent Infinity - 7697A model Gas chromatography was used in present study, Open labs EZchrome software used for data acquisition, Metler Toledo electronic balance and Dura Bond-624 column (30mX0.53mmX3.0 m) was used in HS-GC chromatography.

3. Method Development

3.1 Solubility studies for Teneligliptin at 25°C

The API of Teneligliptin is soluble in organic solvents such as Mehtanol, DMSO, dimethyl formamide(DMF), tetrahydrofuran, dimethyl acetamide and 1,4 dioxane.In these two solvents DMF and Methanol, DMSO has high solubility so DMSO has diluent.

Solvents to be quantified:
1.0 Methanol
2.0 Dimethylformamide
3.0 Tetrahydrofuran

4.0 Dimethyl acetamide
5.0 1,4 Dioxane

Determination of boiling points

Table 1: Determination of Boiling Points

S.No	Solvents Name	Temperature(°C)
01	Methanol	64.7
02	Dimethylformamide	34.6
03	Tetrahydrofuran	39.6
04	Dimethyl acetamide	165.0
05	1,4 Dioxane	101.0

3.2 Standard and Sample Preparation

Standard Sock-I Preparation
Weigh accurately about 500 mg of Methanol, 500 mg of Dimethylformamide, 500 mg of Tetrahydrofuran, 500mg of mg Dimethyl acetamide and 500 mg of 1,4 Dioxane in 250ml Volumetric flask containing about 180 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation
Pipette out 10 ml of the above solution in a 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

Pipette 1 ml of the above prepared solution in the headspace vial & then seal the vial.

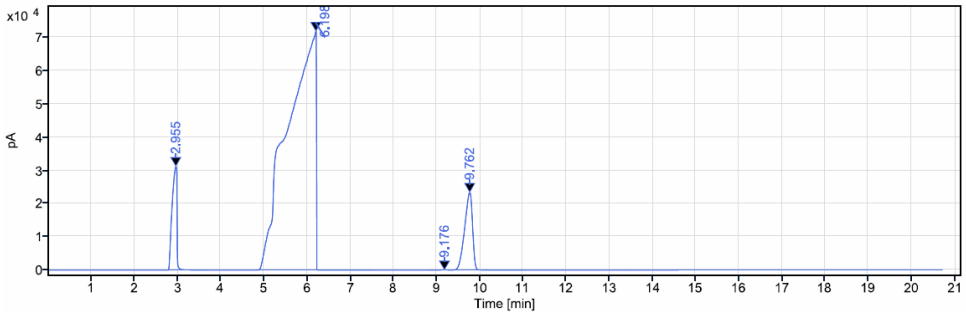
Test Sample Preparation
Weigh accurately about 500 mg of test sample (Teneligliptin API) and transfer in to 50mL volumetric flask add 35mL of diluent, vortex it for 5min. Then make up the volume with diluent and mix well.
Pipette 1 ml of the above prepared solution in the headspace vial and then seal the vial.

3.3 Method Development of Residual Solvents

Trial - 1
GC Parameter and Condition: Column: DB-620 column, (80mx0.22mm) 1.8µm, Inlet Temperature: 210°C, Detector Temperature: 200°C, Oven Temperature: 250°C, Carrier Gas: Nitrogen, Flow: 2.0 ml/min., Split Ratio: 1: 10
Head Space Conditions: Oven Temp.: 90°C, Transfer line Temp. : 80°C, GC cycle Time: 30 min, Loop Fill Temperature: 100 °C
Observation: From the above Trial Solvents Dimethyl formamide and tetrahydrofuran were merged so resolution needs to be optimized. Hence it was not taken for optimization.

Trial- 2
GC Parameter and Condition: Column: DB-620 column, (80mx0.22mm) 1.8µm, Inlet Temperature: 210°C, Detector Temperature: 220°C,Oven Temperature: 220°C, Carrier Gas: Nitrogen, Flow: 3.0 ml/min., Split Ratio: 1:1
Head Space Conditions:Oven Temp.: 70°C,Transfer line Temp. : 85°C,GC cycle Time: 35 min.,Loop Fill Temperature: 100 °C
Observation:From the above, Trial Solvents have a lower Resolution as in the case of the above trial, so resolution needs to be optimized.Hence it was not taken as an Optimization trial.

Trial- 3 (Optimized Trial):
GC Parameter and Condition: Column: DB-624 column, (50mx0.22mm) 1.8µm,Inlet Temperature: 220°C,Detector Temperature: 240°C,Initial Oven Temperature: 60°C,Final Oven Temperature: 220°C,Carrier Gas: Nitrogen,Flow: 4.0 ml/min.,Split Ratio: 2: 10
Head Space Conditions:Oven Temp.: 85°C,Transfer line Temp. : 95°C,GC cycle Time: 2 min.,Loop Fill Temperature: 105 °C
Observation: All Solvent Peaks were separated with good resolution and good efficiency, this Trial is taken as an Optimized Trial.



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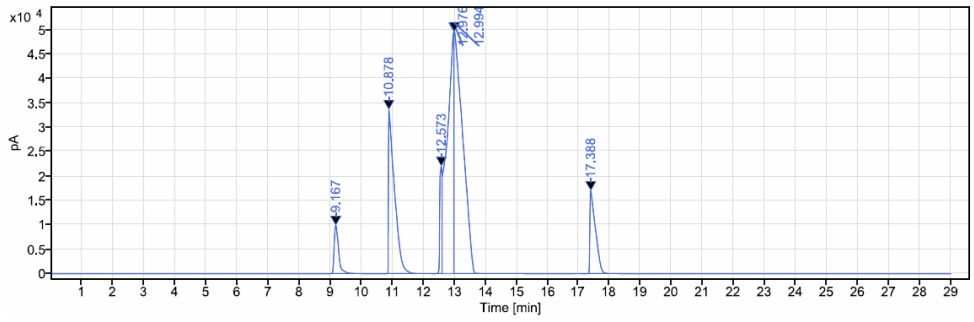

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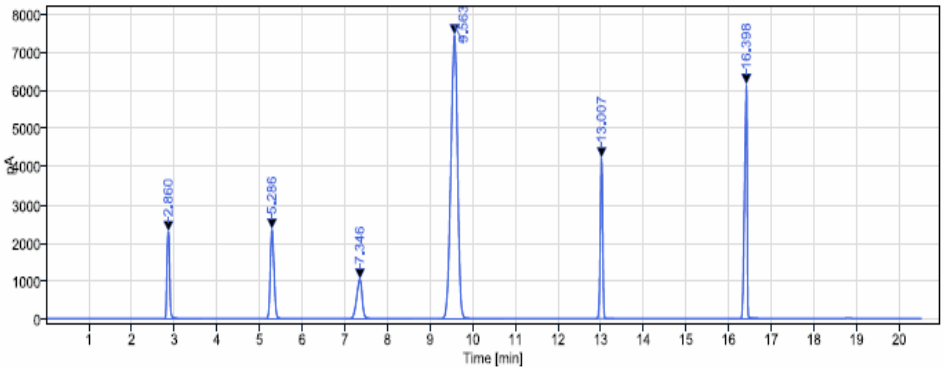

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Figure 2: Chromatogram of Trial 1(A), Trial 2(B), Trial 3(C)

4. Validation

4.1 System Suitability and System Precision

Standard Sock-I Preparation
Weigh accurately about 500 mg of Methanol, 500 mg of Dimethylformamide, 500 mg of Tetrahydrofuran, 500mg of mg Dimethyl acetamide and 500 mg of 1,4 Dioxane in 250ml Volumetric flask containing about 180 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation
Pipette out 10 ml of the above solution in a 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent. Pipette 1 ml of the above prepared solution in the headspace vial & seal the vial.

Table 2: System Suitability and System Precision

Solvent Name	Methanol		Dimethylformamide		Tetrahydrofuran		Dimethyl acetamide	
S. No.	**Rt**	**Area**	**Rt**	**Area**	**Rt**	**Area**	**Rt**	**Area**
Avg.[a]	2.8483	10258.658	5.275	14274.308	7.3393	9490.395	9.5538	81728.878
SD	0.0041	125.342	0.002	169.635	0.0039	155.428	0.005	1282.752
%RSD	**0.14**	**1.22**	**0.05**	**1.19**	**0.05**	**1.64**	**0.05**	**1.57**

1,4 Dioxane	
Rt	**Area**
13.0043	17413.592
0.0016	204.308
0.01	**1.17**

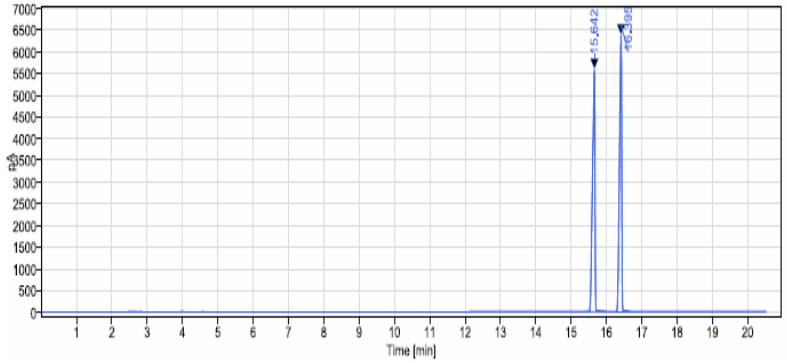
Observation: %RSD of responses of each of the solventswere found to be less than 10%.

4.2 Specificity by Direct Comparison Method

There is no interference of Diluent with the solvent peak and no interference of the API peak at the retention time of the solvent peaks.

Standard Sock-I Preparation
Weigh accurately about 500 mg of Methanol, 500 mg of Dimethylformamide, 500 mg of Tetrahydrofuran, 500mg of mg Dimethyl acetamide and 500 mg of 1,4 Dioxane in 250ml Volumetric flask containing about 180 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation
Pipette out 10 ml of above solution in 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent. Pipette 1 ml of the above prepared solution in the headspace vial &then seal the vial.



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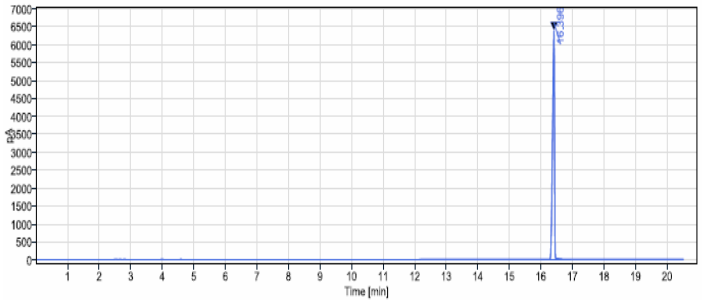

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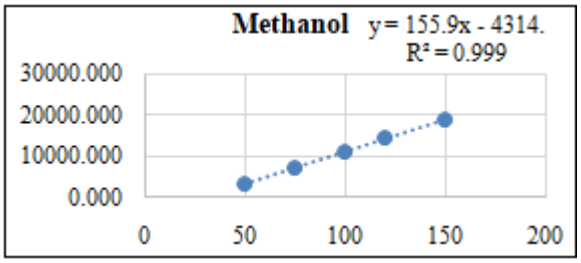
Figure 3: Chromatogram for specificity D) Blank E) Standard

Observation: It is noted from the above data, API or diluent peaks are not interfering with the Solvent peaks i.e., Methanol, Dimethylformamide, Tetrahydrofuran,Dimethyl acetamide, 1,4Dioxane.

4.3 Linearity

Standard Sock-I Preparation

Weigh accurately about 200 mg of Methanol, 200 mg of Dimethylformamide, 200 mg of Tetrahydrofuran, 200mg of mg Dimethyl acetamide and 200 mg of 1,4 Dioxane in 100ml Volumetric flask containing about 20 ml of diluent, make up to volume with diluent and shake well.



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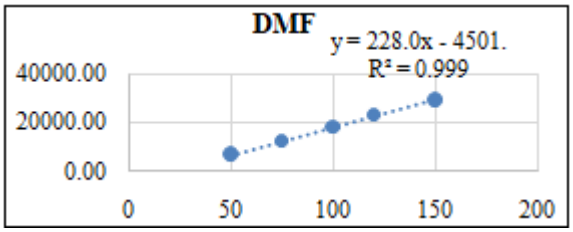

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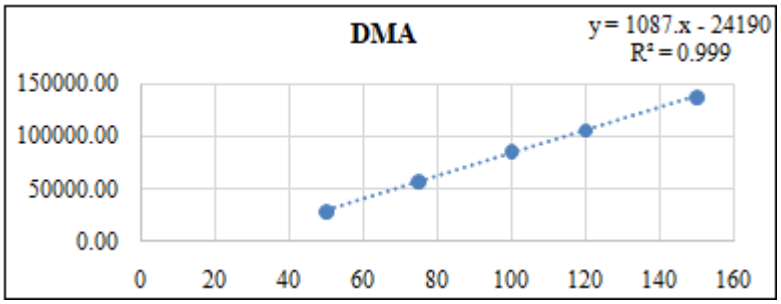

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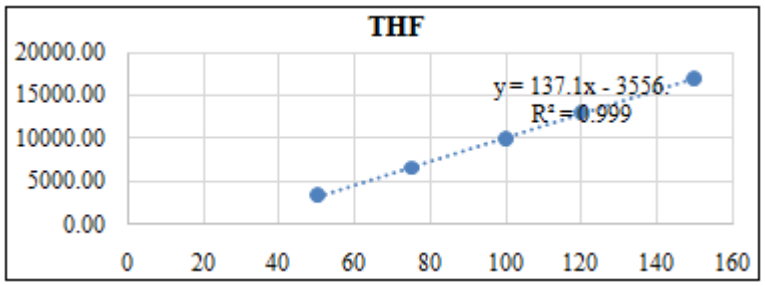

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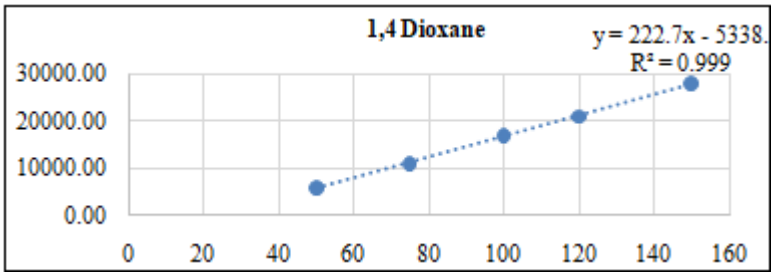

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Figure 4: F, G, H, I, J Linearity

Observation
The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of **Methanol, Dimethyl foramide, Tetrahydrofuran, Dimethyl acetamide and 1,4 Dioxane** is >0.999 is linear within the range examined in consideration of all the points lie in a straight line and the correlation coefficient is well within the limits.

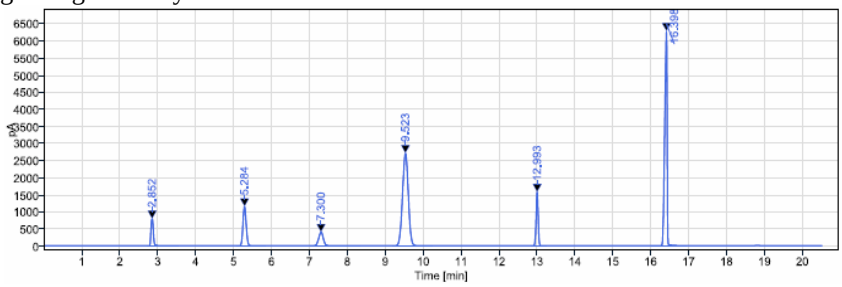
4.4 Accuracy

Accuracy of this method was found out by Recovery studies. To the API, the solvents were added at the level of 50%, 100%, and 150%.

Standard Sock-I Preparation Weigh accurately about 500 mg of Methanol, 500 mg of Dimethylformamide, 500 mg of Tetrahydrofuran, 500mg of mg Dimethyl acetamide and 500 mg of 1,4 Dioxane in 250ml Volumetric flask containing about 180 ml of diluent, make up to volume with diluent and shake well.

Preparations: 5-15ml stock 1 solution is diluted to 200ml with DMSO to prepare 50-150% concentrated solutions

Test Sample Preparation for 50% Accuracy Weigh accurately about 500 mg of test sample (Teneligliptin API) and transfer in to 25mL volumetric flask add 15mL of standard stock-II, vortex it for 5min. Then fill up the volume with standard stock-II for 50% Accuracy and mix well.Pipette 1 ml of the above prepared solution in the headspace vial and seal it.
***Above preparations were prepared three times and injected through head space



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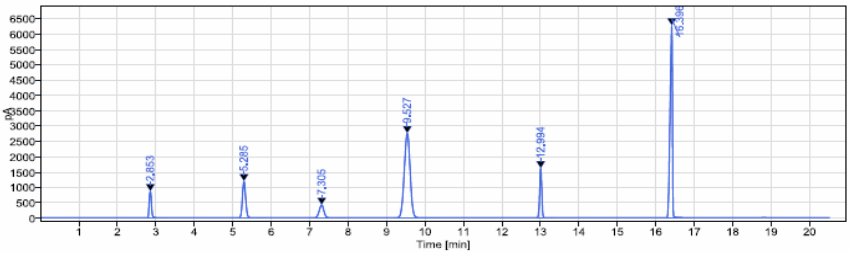

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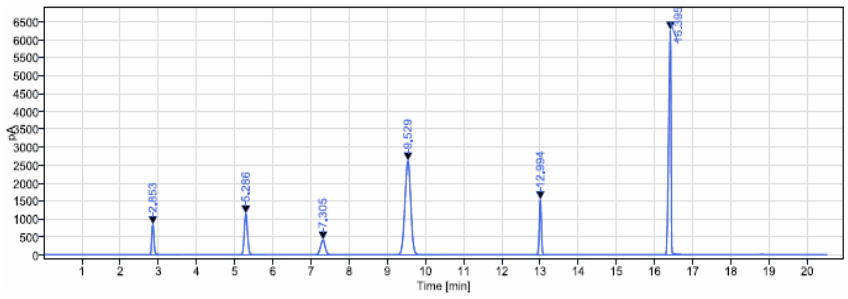

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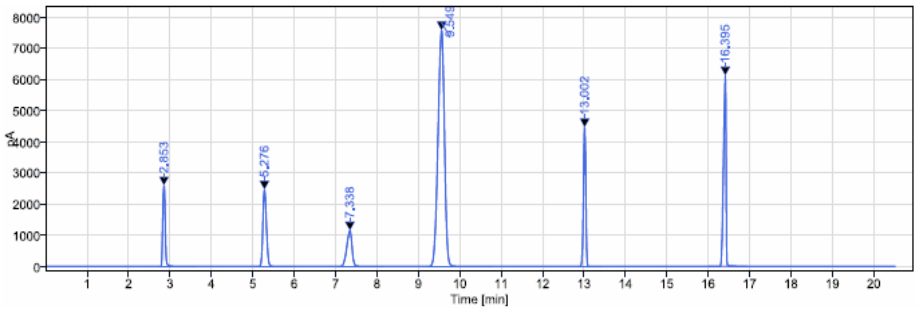

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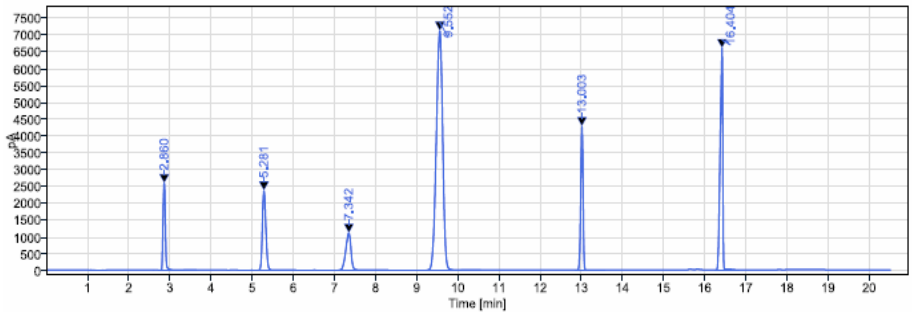

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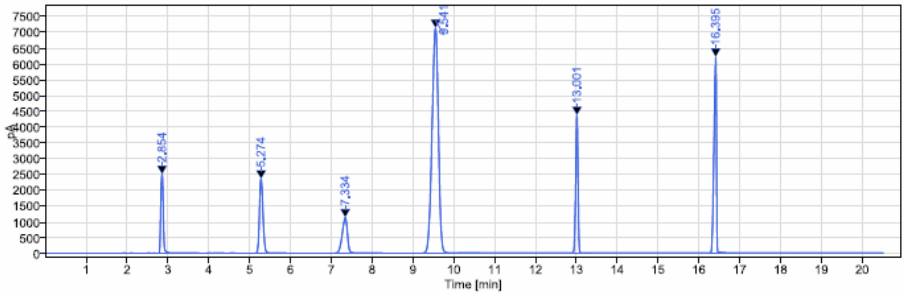

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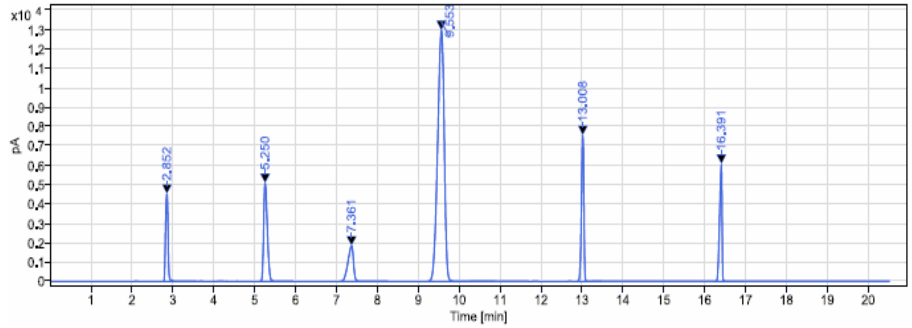

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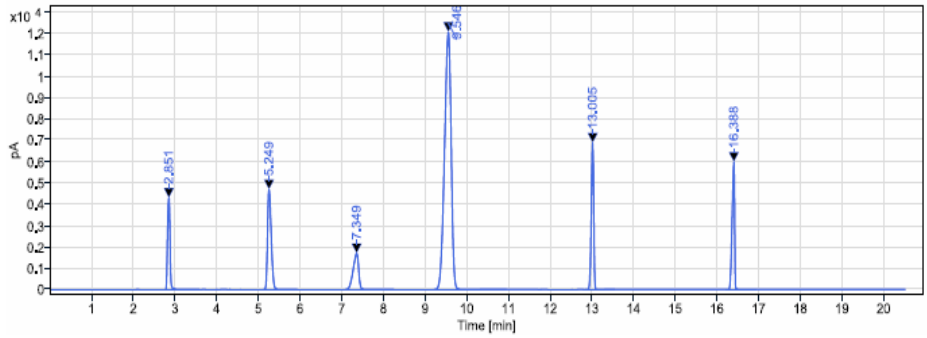

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Figure 5: (1,2,3)- 50% recovery; (4,5,6)- 100% recovery; (7,8)-150% recovery

Observation: The percentage mean recovery of all solvents was obtained between 80% to 120%.

4.5 Precision

Standard Sock-I Preparation Weigh accurately about 500 mg of Methanol, 500 mg of Diethylether, 500 mg of Dichloromethane 500mg of mg Tetrahydrofuran in 250ml Volumetric flask containing about 20 ml of diluent, make up to volume with diluent and shake well.

Standard Sock-II Preparation Pipette out 10 ml of the above solution in a 200 ml volumetric flask containing about 20 ml diluent, make up to volume with diluent.

Method Precision Sample-I Weigh accurately about 500 mg of test sample (Teneligliptin API) and transfer in to 25mL volumetric flask add 18mL of standard stock-II, vortex it for 5min. Then adjust the volume with standard stock-II and mix well.Pipette out 1 ml of the above prepared solution in the headspace vial and seal the vial.

***Above preparations were prepared six times and injected through head space.

Table 3: Results for Method Precision of Solvents

Sovent Name	Methanol		Dimethylformamide		Tetrahydrofuran		Dimethyl acetamide	
S.No	Rt	Area	Rt	Area	Rt	Area	Rt	Area
Avg	2.8565	10183.338	5.28	14212.82	7.3393	9394.803	9.5555	80718.448
SD	0.002	84.767	0.003	70.883	0.0039	57.831	0.0071	217.543
%RSD	**0.07**	**0.83**	**0.06**	**0.5**	**0.05**	**0.62**	**0.07**	**0.27**

1,4 Dioxane	
Rt	Area
13.0043	16340.68
0.0016	92.44
0.01	**0.57**

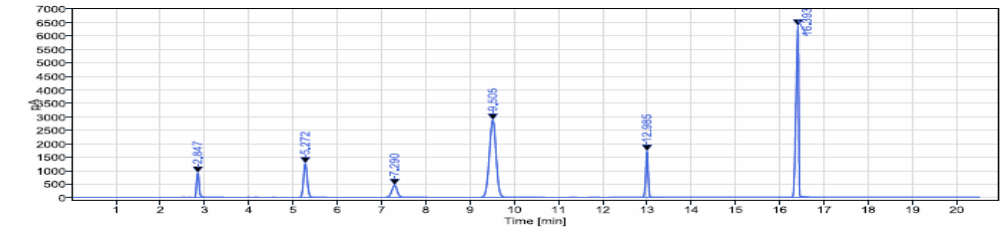
Observation: Test results for above solvents were showing that the % RSD of obtained results is within limits (2%).

4.6 Limit of Detection and Limit of Quantification

Table 4: LOD and LOQ values

Name of the Parameter	Methanol in ppm	DMF in ppm	THF in ppm	DMA in ppm	1,4 Dioxane in ppm
Limit of Detection	2.652	2.455	3.739	3.894	3.027
Limit of Quantification	8.036	7.439	11.33	11.799	9.174

4.7 Robustness



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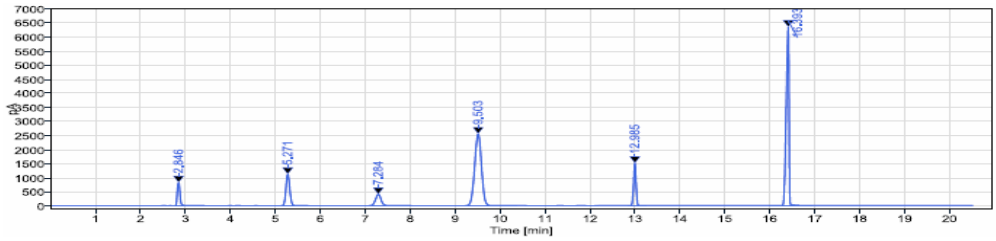

R2

Figure 6: Robustness at high flow rate (R1); Robustness at low flow rate (R2)

Table 5: Robustness of High and Low Flow Rate

Solvent Name		*Robustness of low flow rate*		
		Rt	*TP*	*Tailing factor*
Methanol	H	2.847	12372	1.14
	L	2.846	12149	1.15
DMF	H	5.272	18315	1.08
	L	5.271	18149	1.08
THF	H	7.290	16813	0.98
	L	7.284	16720	1.01
DMA	H	9.505	18258	0.97
	L	9.503	18186	0.99
1,4 dioxane	H	12.985	26510	0.68
	L	12.985	26134	0.69

Observation: From the above study upon changing the flow rates, theoretical plate count (NLT-2000) and tailing factor (NLT 2.0) were to be within limits.

4.8 Ruggedness

Table 6: Ruggedness

Sovent Name	Methanol		Dimethylformamide		Tetrahydrofuran		Dimethyl acetamide	
S.No	**Rt**	**Area**	**Rt**	**Area**	**Rt**	**Area**	**Rt**	**Area**
Avg	2.8565	10217.767	5.280	14211.968	7.3393	9413.858	9.5538	80724.307
SD	0.0020	97.064	0.003	66.428	0.0039	64.644	0.0050	216.537
%RSD	**0.07**	**0.95**	**0.06**	**0.47**	**0.05**	**0.69**	**0.05**	**0.27**

1,4 Dioxane	
Rt	Area
13.0043	16347.88
0.0016	93.71
0.01	**0.57**

Observation: %RSD of responses of each solventwere found to be less than 10%.

5. Conclusion

From the above experimental results and parameters, it was concluded that this newly developed method for the estimation of Residual Solvents including Methanol, Dimethyl formamide, Tetrahydrofuran, Dimethyl acetamide and 1,4 Dioxane in Teneligliptin API was found to be simple, precise, accurate, of high resolution and with a short retention time. This makes this method more acceptable and cost effective and it can be effectively adapted for routine analysis in research institutions, quality control departments in industries, approved testing laboratories, used in bio-pharmaceutical and bio-equivalence studies and in also in clinical pharmacokinetic studies in the near future

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Author Profile

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