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Determination of Residual Solvents in Bictegravir by Headspace Gas Chromatography

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Abstract: ***A modest and discerning headspace gas chromatography (HS-GC) method is defined for the determination & quantification of Residual Solvents in Bictegravir API. Chromatographic parting was achieved on a D-B-624 column, (30mx0.53mm) 3.0micron column using various temperature gradients of Flame Ionisation Detectors (FID). Linearity in the series 50-150µg/ml using Methanol, Dimethyl formamide (DMF), Tetrahydrofuran, Dichloromethane and Acetonitrile (r^2>0.999) as solvents. The proposed methods were validated. Accuracy assessed by recovery studies at 3 diverse levels. Recovery experiments showed that diluent and API not interfered. The technique was found to be precise as indicated by the repeatability analysis, showing %RSD less than 10 for Methanol, DMF, Tetrahydrofuran, Dichloromethane and Acetonitrile. Therefore, it proves validity of the procedures and shall be utilised for routine study of pharmaceutical active ingredients for valuation of Residual Solvents of Methanol, DMF, Tetrahydrofuran, Dichloromethane and Acetonitrile in Bictegravir.***

Keywords: HS-GC, Residual solvents, Bictagravir, FID, Tetrahydrofuran

1. Introduction

Gas chromatography (GC) is analytical used in present research work. The main aim of gas chromatography is parting with the aid of gas, which acts as mobile phase. No two compounds have same affinity on the stationary phase. Therefore, compounds shall be separated by their nature of affinity. Its reputation chiefly owes to efficient separation of complex mixtures of various analytes. Although GC analysis is very common, but not all chromatographic equipment offers. Most researchers think of a GC method development as a simple modification of temperature program. The main theme of discrimination is with the help of selective detectors. Finally, the result can be recorded through a read out device. 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. In the absence of reference standards, FID can be used to predict relative response factors of known structures with reasonable accuracy using the effective carbon number concept, as shown with amphetamine-type compounds[2],[4].

Testing of drug substances, excipients, and drug products for residual solvents should be performed when production or purification processes are known to result of residual solvents. It is only necessary to test for residual solvents that are used or produced in the manufacture or purification of drug substances, excipients, or products. 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). Types of Residual solvents: Class 1, solvents to be avoided; Class 2, solvents to be limited; Class 3, solvents with low toxic potential. If Class 1 solvents, they should be identified and quantified. If solvents of Class 2 or 3 are present at greater than their Option 1 limits or 0.5%, respectively, they should be identified and quantified[9].

Bictegravir is a recently approved investigational drug that has been used in trials studying the treatment of HIV-1 and HIV-2 infection.

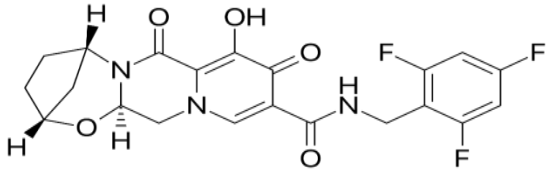


Figure 1: Organic structure of Bictegravir

The literature study shows no analytical method was reported before for the estimation of residual solvents of classs II in Bictegravir by Headspace Gas Chromatography. The purpose is to progress new approach of residual solvents in Bictegravir in pure drug by Head Space-Gas chromatography (HSGC-FID) as per the ICH guidelines[13].

2. Experimental

2.1. Materials and reagents

Bictegravir API was gifted sample from Dr Reddy's laboratory, USA Manufacturers -Gilead Sciences, Inc. remaining all reagents were GC grade; Dimethyl sulfoxide and Methanol were purchased from Qualigens; Tetrhydofuran, DMF, Dichloromethane and Acetonitrile 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 Bictegravir at 25^{0}C

The solubility of Bictegravir (active entity) is soluble in organic solvents such as Methanol, DMSO, dimethyl formamide (DMF), tetrahydrofuran, dichloromethane and acetonitrile. In these two solvents DMF and DMSO, DMSO has high solubility so DMSO as diluent.

Solvents to be quantified[14]

1) Methanol
2) Dimethyl formamide
3) Tetrahydrofuran
4) Dichloromethane
5) Acetonitrile

Determination of Boiling Points:

Table no.1 Determination of boiling points

S.No	*Solvents Name*	*Temperature(°C)*
01	Methanol	64.7
02	Dimethyl formamide	34.6
03	Tetrahydrofuran	39.6
04	Dichloromethane	66.0
05	Acetonitrile	82.1

3.2 Standard and sample preparation

Standard Sock-I Preparation: Weigh accurately about 500 mg of Methanol, 500 mg of DMF, 500 mg of Tetrahydrofuran, 500mg of mg Dichloromethane and 500 mg of Acetonitrile in 250ml flask containing about 180 ml of Dimethyl sulfoxide, make upto capacity with Dimethyl sulfoxide and shake well.

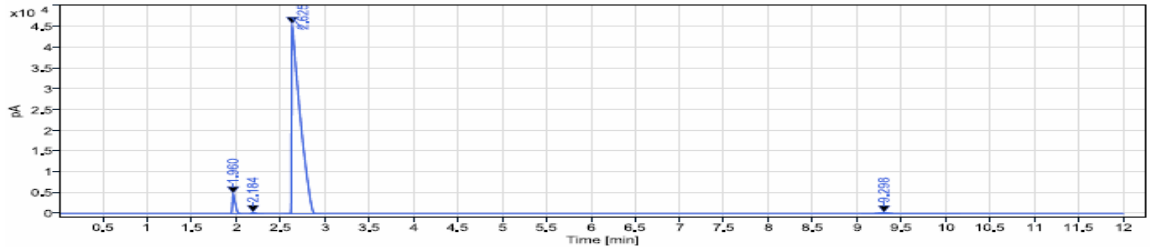
Standard Sock-II Preparation: Withdraw 10 ml of above solution in 200 ml flask containing about 20 ml Dimethyl sulfoxide, make up to volume with Dimethyl sulfoxide. Withdrawn 1ml prepared solution in headspace vial & seal the vial.

Test Sample Preparation: Weigh accurately about 500 mg of test sample (Bictegravir API) and transfer in to 50 mL flask add 35mL of Dimethyl sulfoxide, vortex it for 5min. Then volume mark with Dimethyl sulfoxide and mix well. Transfer 1 ml of above prepared solution in headspace vial and seal the vial.

3.3 Method development conditions for residual solvent determination

Table 2: HS-GC conditions for method development

GC conditions:	***Trial 1***	***Trial 2***	***Trial 3***
Column	DB624(20mX0.24mm)1.8µm	DB624(30mX0.24mm)1.8µm	DB624(30mX0.24mm)1.8µm
Inlet Temp.	145^{0}C	120^{0}C	160^{0}C
Detector Temp.	250^{0}C	230^{0}C	230^{0}C
Oven Temp.	250^{0}C	210^{0}C	Initial-60^{0}C,Final-150^{0}C
Carrier gas	Nitrogen	Nitrogen	Nitrogen
Flow	2.0ml/min	3.0ml/min	4.0ml/min
Split ratio	01:10	1:10	1:10
Headspace conditions:			
Oven Temp.	90^{0}C	80^{0}C	80^{0}C
Transfer line Temp.	90^{0}C	90^{0}C	90^{0}C
GC cycle time	40min	35min	20min
Loop fill Temp.	90^{0s}C	110^{0}C	100^{0}C



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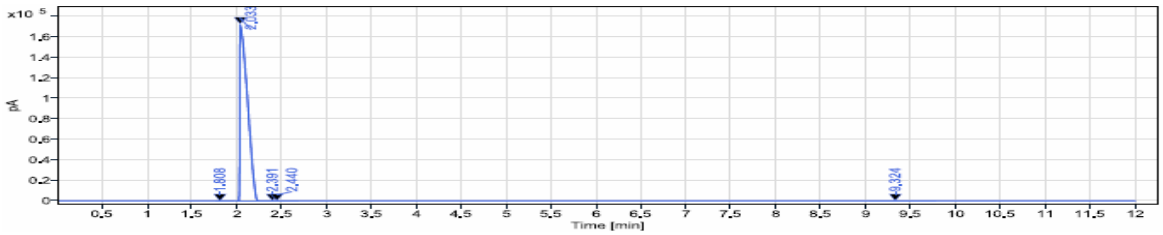

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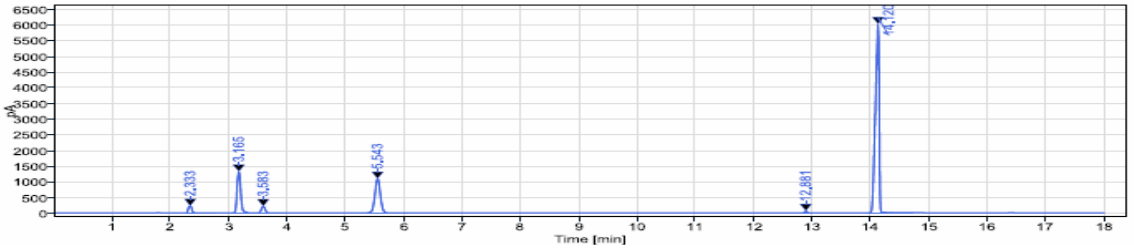


C

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

Observation: In Trial 1 & 2 Solvents Dichloromethane and Methanol were merged so resolution need to be optimised. In Trial 3 all solvent peaks were separated with good resolution and efficiency. Trial 3 taken as optimized Trial.

4. Validation

4.1 System suitability

4.1.1 Standard and sample preparation

Standard Sock-I Preparation: Weigh accurately about 500 mg of Methanol, 500 mg of DMF, 500 mg of Tetrahydrofuran, 500mg of mg Dichloromethane and 500 mg of Acetonitrile in 250ml flask containing 180 ml of DMSO, and volume mark with DMSO and shake well.

Standard Sock-II Preparation: Transfer 10ml of above in 200ml volumetric containing about 20ml DMSO, make to volume with DMSO. Transfer 1 ml of above prepared solution in headspace vial & seal the vial.

Table 3: System Suitability in different solvents

Solvents		*AVG*[a]	*SD*	*%RSD*
Methanol	Rt	2.3317	0.0008	0.04
	Area	915.795	14.276	1.56
DMF	Rt	3.165	0.001	0.02
	Area	5706.465	83.419	1.46
Tetrahydrofuran	Rt	3.5828	0.0008	0.02
	Area	934.680	13.979	1.50
Dichloromethan	Rt	5.5427	0.0010	0.02
	Area	7012.330	101.621	1.45
Acetonitrile	Rt	12.8812	0.0004	0.00
	Area	122.15	1.27	1.04

[a] average of six replicate analysis.

Observation: %RSD of each solvent were found to be less than 10%, hence followed acceptance criteria.

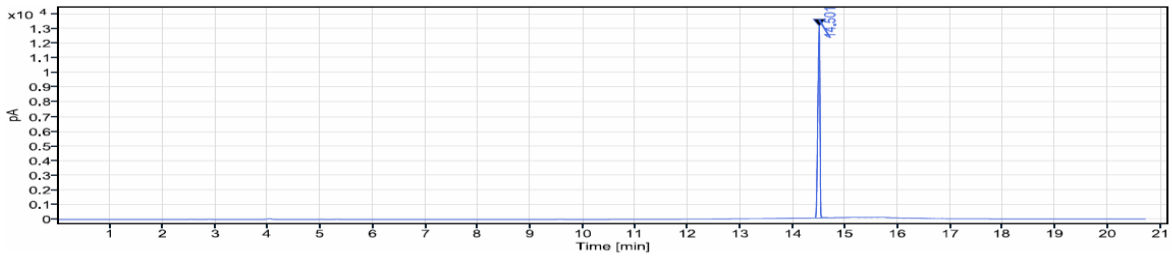
4.2 Specificity by direct comparison method

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

4.2.1 Standard and sample preparation

Standard Sock-I Preparation: Weigh precisely about 500 mg of Methanol, 500 mg of Dimethylformamide, 500 mg of Tetrahydrofuran, 500mg of mg Dichloromethane and 500 mg of Acetonitrile in 250ml flask containing about 180 ml of diluent, make capacity with diluent and shake well.

Standard Sock-II Preparation: Take 10ml of above solution in 200ml flask containing about 20ml Dimethyl sulfoxide, diluted mark with DMSO. Pipette 1 ml of above prepared solution in headspace vial & seal the vial.



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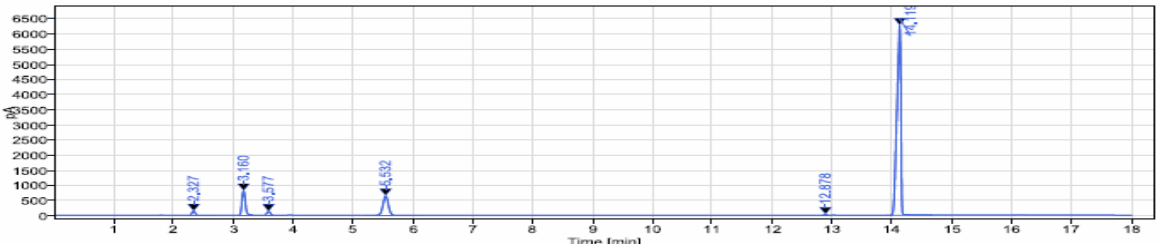

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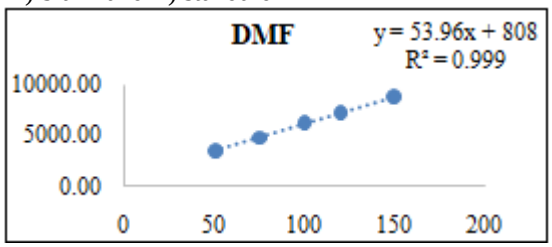
Figure 3: Chromatography for specificity D) blank and E) standard

Observation: It is observed from the above data (Fig.3), diluent or API peaks are not interfering with the Solvent peaks i.e., Methanol, DMF, Tetrahydrofuran, Dichloromethane, Acetonitrile.

4.3 Linearity

Preparation of Stock – I : Weigh precisely about 200mg of Methanol, 200mg of DMF, 200 mg of Tetrahydrofuran, 200mg of mg Dichloromethane and 200 mg of Acetonitrile in 100ml flask containing about 20ml of DMSO, volume made up with DMSO and shake well.

Preparations: 2.5 to 7.5ml stock solution is diluted to 100ml with DMSO to prepare 50 to 150% concentrated solutions respectively.



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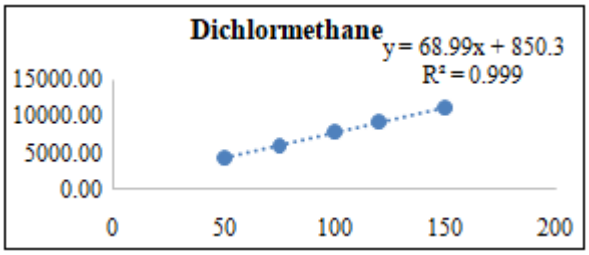

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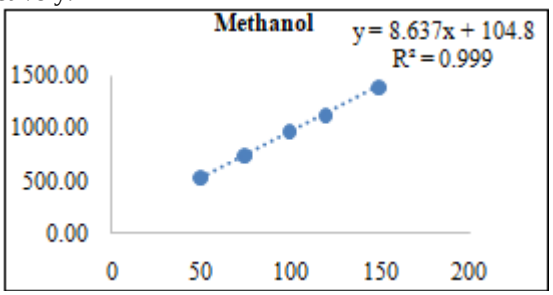

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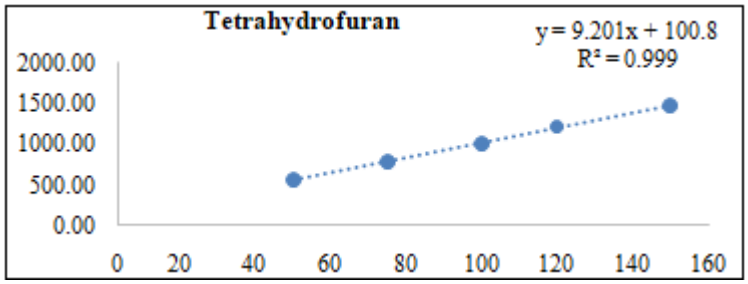

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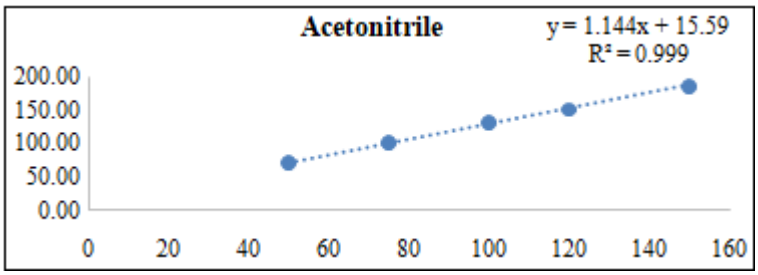

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Figure 4: Linearity curves

Observation: The correlation factor for linear curve found between concentrations vs. Area for preparations (50 - 150%) is greater than 0.99 and the correlation coefficient is well within limits.

4.4 Accuracy

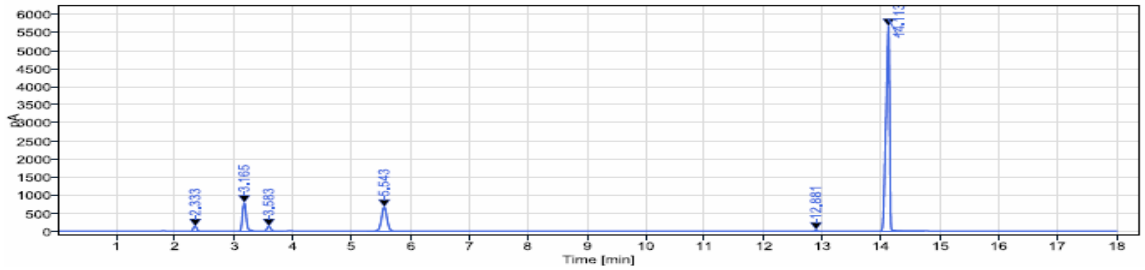
Accuracy of the method was determined by Recovery studies. To the API (pre analyzed sample), the solvents were added at the level of 50%, 100%, 150%.

4.4.1 Preparation of sample and standards

Stock-I Preparation: Weigh about 500mg of Methanol, 500mg of DMF, 500 mg of Tetrahydrofuran, 500mg of mg Dichloromethane and 500 mg of Acetonitrile in 250ml flask containing about 180 ml DMSO, and final mark done with DMSO and shake well.

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

Test Sample Preparation for 50% Accuracy: Weigh precisely about 500 mg of test sample (Bictegravir API) and transfer in to 25mL flask add 15mL of standard stock-II, vortex it for 5min. Then make up the size with standard stock-II for 50% Accuracy and mix well. Withdraw 1 ml of solution in headspace vial and seal the vial. Above measures were equipped 3 times and injected through head space.



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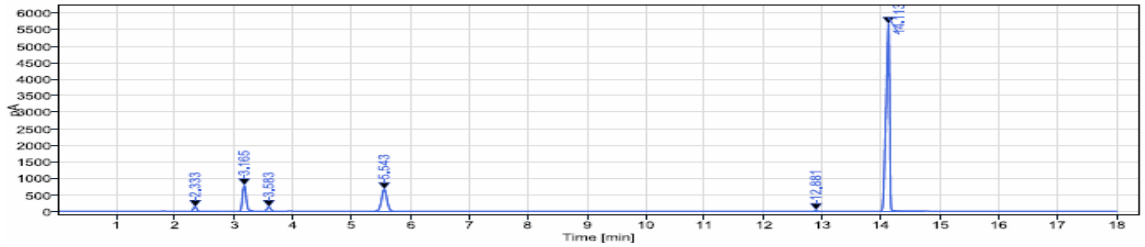

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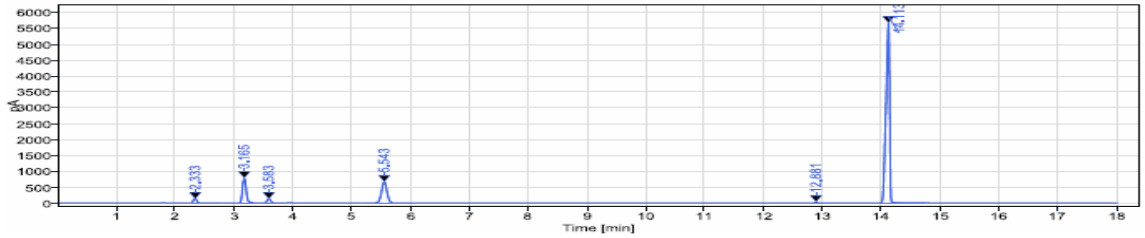

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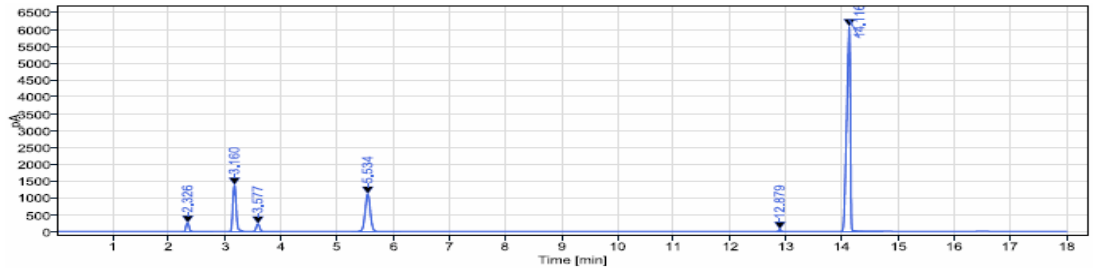
Figure 5: 50% recovery studies for injections 1, 2 and 3



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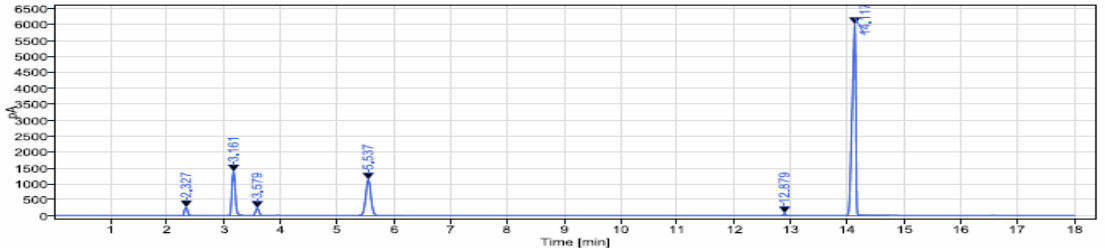

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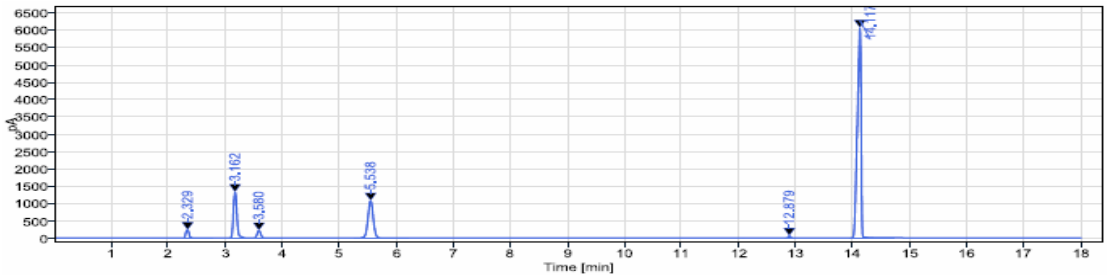

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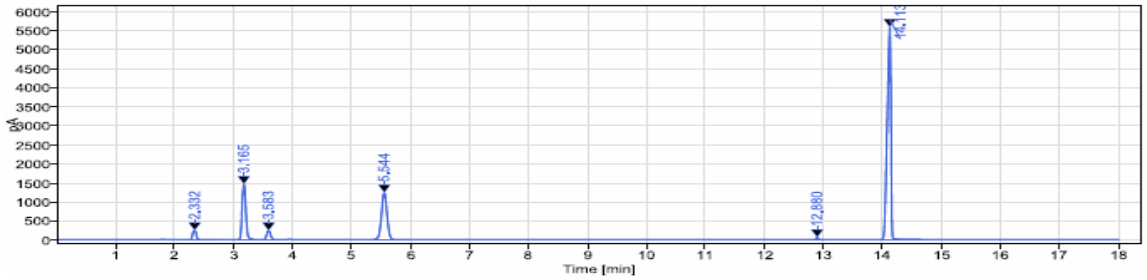
Figure 6: 100% recovery studies for injections 1, 2 and 3



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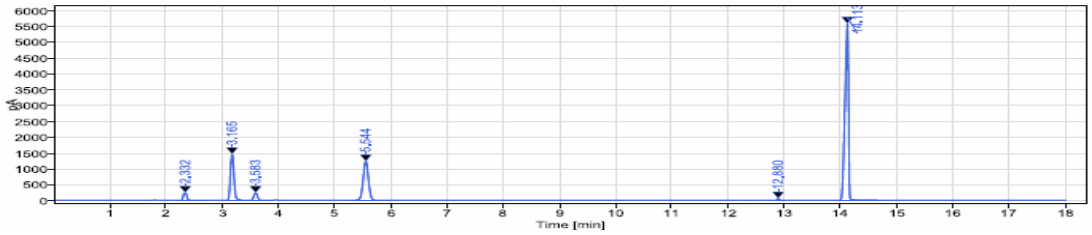

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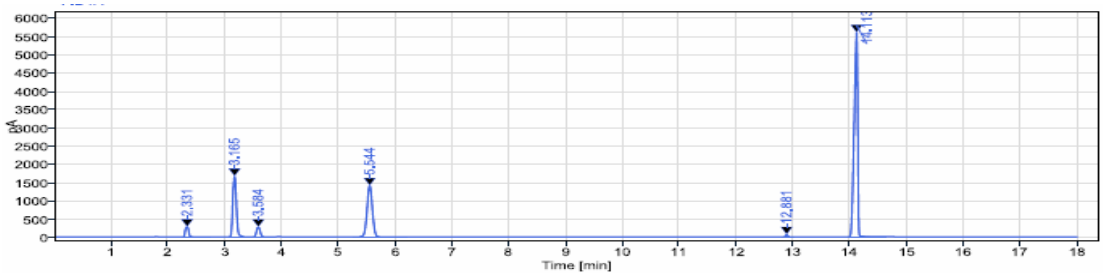

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Figure 7: 150% recovery studies for injection 1, 2 and 3

Table 4: Recovery results for Solvents

Level	*Methanol*[b]	*DMF*[b]	*Tetrahydrofuran*[b]	*Dichloromethane*[b]	*Acetonitrile*[b]
50 %	100.55	100.47	100.75	100.86	103.27
100 %	101.27	100.22	103.45	100.34	103.64
150 %	99.86	103.27	100.78	101.82	101.18

[b] average of 3 replicate analysis

Observation: The % mean recovery of all solvents was obtained at 80% to 120%, with in the acceptance limits.

4.5 Precision

4.5.1 Preparation of Stock and sample

Sock-I Preparation: Weigh accurately about 500 mg of Methanol, 500 mg of Diethylether, 500 mg of Dichloromethane 500 mg of Tetrahydrofuran in 250ml flask containing 20 ml of diluent, make up the mark with DMSO and shake well.

Sock-II Preparation: Pipette out 10 ml of above solution in 200 ml flask containing about 20 ml DMSO, make up to volume with DMSO.

Method Precision Sample-I: Weigh accurately about 500 mg of test sample (Bictegravir API) and transfer in to 25mL flask add 18mL of stock-II, vortex it for 5min. Then make up the volume with stock-II and mix well. Pipette 1 ml of above prepared solution in headspace vial and seal the vial. Above preparations were prepared six times and injected through head space.

Table 5: Results for Method precision of solvents

Solvents		*AVG*[a]	*SD*	*%RSD*
Methanol	Rt	2.3278	0.0017	0.07
	Area	932.750	8.742	0.94
DMF	Rt	3.162	0.002	0.05
	Area	5758.657	68.150	1.18
Tetrahydrofuran	Rt	3.5793	0.0021	0.06
	Area	949.897	13.547	1.43
Dichloromethane	Rt	5.5372	0.0028	0.05
	Area	7121.932	88.982	1.25
Acetonitrile	Rt	12.8790	0.0000	0.00
	Area	143.74	1.76	1.22

[a] Average of six replicate analysis

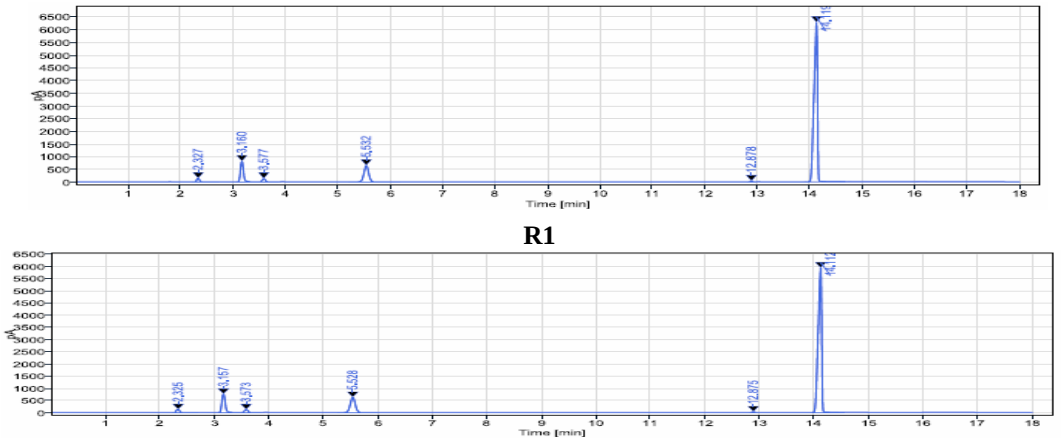
Observation: Test results for solvents were showing that the % RSD of obtained results were within limits (less than 2%).

4.6 Limit of detection and Limit of quantification

Table 6: LOD & LOQ Limits

Name of the Parameter	*Methanol in ppm*	*DMF in ppm*	*THF in ppm*	*DCM in ppm*	*Acetonitrile in ppm*
Detection limit	5.45	5.10	5.01	4.86	14.85
Quantification limit	16.52	15.45	15.19	14.72	45.00

4.7 Robustness



R1

R2

Figure 8: Robustness of Low flow rate (R1) and High flow rate (R2)

Table 7: Robustness of low (L) and high (H) flow rate

Solvents		*Rt*	*TP*	*Tailing factor*
Methanol	L	2.327	9354	1.10
	H	2.327	11313	1.17
DMF	L	3.157	12725	1.13
	H	3.160	14621	1.14
Tetrahydrofuran	L	3.573	15097	1.05
	H	3.577	17105	1.05
Dichloromethane	L	5.528	16995	1.00
	H	5.532	17758	1.00
Acetonitrile	L	12.875	60619	1.06
	H	12.878	660589	1.07

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

4.8 Ruggedness

Table 8: Ruggedness

Solvents		*AVG*[a]	*SD*	*%RSD*
Methanol	Rt	2.3242	0.0019	0.08
	Area	917.902	12.066	1.31
DMF	Rt	3.156	0.002	0.08
	Area	5712.107	79.008	1.38
Tetrahydrofuran	Rt	3.5727	0.0026	0.07
	Area	940.240	14.155	1.51
Dichloromethan	Rt	5.5275	0.0031	0.06
	Area	7009.668	104.479	1.49
Acetonitrile	Rt	12.8755	0.0021	0.02
	Area	123.08	1.66	1.35

[a] Average of six replicate analysis

Observation: %RSD of were found to be less than 10%

5. Conclusion

From the above tentative results and constraints it was determined that, this newly established method for the estimation of Residual solvents of Methanol, DMF, Tetrahydrofuran, Dichloromethane and Acetonitrile in Bictegravir API was selective, precise, accurate and high tenacity and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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