BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2024. Vol. **21**(3), p. 1001-1013

Analytical Method for the Development and Validation of Residual Solvents in Tigecycline by Gas Chromatography Using Headspace Sampling Technology

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http://dx.doi.org/10.13005/bbra/3280

(Received: 08 July 2024; accepted: 24 September 2024)

Residual solvents such as Dichloromethane, Acetone, Methanol, and Isopropanol in pharmaceutical samples of Tigecycline were monitored using gas chromatography with headspace sampling technology. The column used for this elution is DB-624, 30m X 0.32mm X 1.8μm, Nitrogen is used as carrier gas with FID detector. Split ratio is 30:1 and the injector temperature is 210 °C. Estimation of the residual solvents is mandatory for the release testing of all active pharmaceutical ingredients (API). So, in this study, the authors estimated the four residual solvents of Tigecycline using the Headspace sampling technology, and the method is validated and meets all required standards per the ICH revised guidelines. So, this method can be used for routine analysis in Quality control laboratories for routine estimation.

Keywords: GC-HS; Impurity profile; Residual solvents; Tigecycline.

Tigecycline is a glycylcycline antibiotic developed and marketed by Wyeth under the brand name Tygacil. It was developed in response to the growing prevalence of antibiotic resistance in bacteria such as Staphylococcus *aureus*.It is used to treat several susceptible bacterial infections[1]. Its-IUPAC-name-is-N-[(5aR,6aS,7S,9Z,10aS)-9-(amino-hydroxy-methylidene)-4,7-bis(dimethylamino)-1,10a,12-trihydroxy-8,10,11-trioxo-5a,6,6a,7-tetrahydro-5H-tetracen-2-yl]-2-(tert-butylamino) acetamide. The Molecular Formula of Tigecycline is $C_{29}H_{39}N_5O_{8,}$ and the molecular weight is 585.658 g·mol^{-1}. Tigecycline is practically soluble in water and its LogP value was found to be 0.66, with Protein binding from 71% to 89%—Tigecycline excretion 59% Bile, 33% kidney and the Elimination half-life 42.4 hours. The main mechanism of action of Tigecycline is similar to another tetracycline in that it acts as an inhibitor of bacterial protein translation (i.e., elongation of the peptide chain) via reversible binding to a helical region (H34) on the 30S subunit of bacterial ribosomes. Erava MIC values were nearly half of that of Tigecycline against the clinical isolates of S.Agalactiae from China and genetic mutations in the 30S ribosome units of Tet target sites (16SrRNA copies or 30S ribosome protein S10) participated in the resistance

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evolution of both Erava and Tig under the antibiotic pressure[2]. TIG could serve as a lead candidate for novel chemotherapy-cytotoxic drug development. In mechanism analysis, combining a small compound screen, yeast chemo genomic platform and further *in vitro* and *in vivo* experiments is conducive to identifying dysregulation signaling as the target for candidate compounds, such as TIG. Furthermore, given the issues with clinical application, future studies should focus on the combined effects between TIG and standard chemotherapy drugs to effectively treat cancer patients[3,4]. Several analytical techniques are available for a quality control tool for tigecycline, including HPLC without derivatization, whereas the fluorescence technique requires derivatization using acidic dye. A few methods require tedious pre-sample preparation techniques, become time-consuming, and involve using one or more organic solvents; there is a need to develop eco-friendlier methods for analyzing tigecycline[5]. The area under the curve spectrophotometric method was reported in the literature to estimate Tigecycline in the pharmaceutical dosage form. The principle for the AUC curve method was "the area under two points on the mixture spectra is directly proportional to the concentration of the component of interest". The area was selected between 249 to 256 nm for determination of Tigeccycline[6]. An ion-paired HPLC assay was reported in the literature to determine Tigecycline (GAR-936) concentrations in Hank's balanced salts solution, Tigecycline intra-cellular concentrations in human polymorph nuclear neutrophils (PMNs), and Tigecycline concentrations in human serum. Minocycline was used as the internal standard, 5% trichloroacetic acid was added to lyse PMNs and also precipitate proteins in PMNs and serum. The top aqueous layer was aspirated for HPLC assay. The chromatograms were performed with a reversed-phase C_{18} column with UV detector. The mobile phase consisted of acetonitrile, phosphate buffer (pH 3) and 1-octanesulfonic acid at a flow rate of 1 ml/min[7,8]. One more- HPLC was reported, elution was done by using C18 column (Kromasil ODS C-18 (150×4.6mm, 5µ) as the stationary phase and 83ml of Buffer (1-Hexane Sulphonic acid Sodium Monohydrate Salt and Potassium Dihydrogen Ortho Phosphate) and 17ml of Acetonitrile in the ratio of 83:17 v/v as the mobile phase[9]. An Ultraviolet (UV) and visible spectrophotometric method was reported in the literature to determine Tigecycline in lyophilized powder. In the UV method Tigecycline showed an absorption maximum at 245 nm, in an aqueous medium. In contrast, in the visible spectrophotometric method, it reacted with copper acetate reagent, under acid conditions, forming a greenish-colored solution with an absorption maximum at 378 nm. Thermogravimetric Analysis and Differential Scanning Calorimetry (TGA-DSC) techniques were studied to determine the thermal analysis of tigecycline[10]. An RP-HPLC method was reported in the literature for the estimation of the drug in Pharmaceutical dosage form in which elution was done by reversible phase C18 column (250 × 4.6 mm, 5µm) with a mobile phase consisting of a mixture of acetonitrile and acetic acid (0.1% aqueous solution, pH:3.5) in the ratio of 20:80[11]. The authors noticed that no method is reported in the literature for the estimation of synthetic residual solvents in bulk drugs and their dosage form, hence the authors proposed a validated method for the same purpose

Experimental material and methods

Instruments Used

A gas chromatographic Instrument (Agilent model) was used for the proposed method and the analytical column of Mettler Toledo(XS205) was used throughout this research work.

Blank

Transfer 4ml of diluent into the headspace vials of about 20 mL capacities and add 6 mL of water to seal the vials immediately.

Preparation of standard stock solution: Weigh and transfer accurately about 300mg of Methanol, 500mg of Acetone, 500mg of isopropanol 60mg of Methylene chloride, into a 100 ml volumetric flask containing 10 ml diluent and make up to volume with the same diluent.

Preparation of standard solution

Transfer 1 mL of the stock solution into the headspace vials of about 20 capacities and add 3 mL of N, N-Dimethylformamide 6ml of water seal the vials immediately.

Preparation of Test solution

Weigh accurately about 2.5 g of substance to be examined in a 10 mL volumetric flask dissolved and diluent to volume with N,

N-Dimethylformamide, mix well accurately Transfer 4 mL of this solution to avail, add 6 mL of water, seal, and mix well.

Procedure

Condition the column for 2 hours at 200°C column oven temperature before starting the analysis. Inject standard solution and test solution respectively, Record chromatogram; calculate the content of residual solvent.

System suitability criteria

No interference in the blank solution was observed. The %RSD for the all peak area response of each solvent should be not more than 10.0 %. The Resolution of adjacent peaks is not less than 1.5. The number of theoretical plates calculated from the chromatogram from the first injection is not less than 5000.

Table 1. List of Chemicals

Name of TheMaterial	Make	Grade	Purity(%)
Milli-Q-Water	NA	NA	NA
Dimethylformamide	HONEYWELL	GC	99.98%
Methanol	MERCK	HPLC	100.00%
Acetone	MERCK	HPLC	99.99%
Isopropanol	MERCK	HPLC	99.9%
Dichloromethane	MERCK	HPLC	99.9%

Table 2. Optimized Chromatographic Conditions

Column	DB-624, 30m X 0.32mm X 1.8µm
Detector	FID
Carrier Gas	Nitrogen
Split Ratio	30:1
Injector Temperature	210 °C
Flow rate	2.0 mL/min
Linear velocity	38.2 cm/sec (constant flow mode)
Detector Temperature	280 °C

Table 3. Oven Programme

Rate (°C/min)	Temperature (°C)	Hold Time (min)
-	40	6
100	220	5

Run time:12.8Minutes

$$\text{Residual solvents} = \frac{\text{A sample x Cstandard}}{\text{A standard x Csample}} 100$$

A sample: Peak area of each residual solvent in the test solution

A standard: Peak area of each residual solvent in standard solution

C standard: Concentration of each residual

Table 4. Head Space conditions

Oven temperature	80 °C
Loop temperature	90 °C
Transfer line temperature	100 °C
GC cycle time	35 minutes
Equilibration time	30 minutes
Pressurization Time	5.0 minute
Loop fill time	0.20 minute
Loop equilibration	0.1 minute
Sample Inject	1 ml

Table 5. System Suitability Results

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	2.7	-	57494
Acetone	0.9	25	46070
Methanol	1.4	3	39245
Isopropanol	0.6	6	52364

solvent in standard solution, mg/mL C sample: Concentration of test solution, mg/mL

System Suitability

Inject six replicate injections of the standard solution into the chromatographic system as per the test method and evaluate the system suitability parameters.

Specificity

Blank Interference

The specificity study was conducted by preparing a blank solution and each solvent solution individually at the Specification level (Dichloromethane, Acetone, Methanol, Isopropanol), Sample solution, and by spiking the Sample solution with all solvents at specification level, and checked for the peak interference found due to blank and individual solvents at the retention time of Dichloromethane, Acetone, Methanol, and Isopropanol.

Table 6. Results of Blank interference

Sample Name	Peaks found at the RT of Dichloromethane, Acetone, Methanol, and Isopropanol peaks (Yes/No)
Blank Solution	No

Precision

System Precision

As per methodology, blank and six replicate injections of standard solution into the chromatographic system and calculated the % RSD for six replicate injections of Standard solution.

Method Precision

Determine the precision by preparing the six individual test preparations by spiking Dichloromethane, Acetone, Methanol, and Isopropanol at the specification level and analyzing as per the test method.

Limit of Detection/Limit of Quantification (LOD/LOQ) & LOQ Solutions

Accurately transfer 3 mL,4mL,5mL,6mL,7mL,8mL of standard stock solution into a series of 100 mL volumetric flasks containing 10 mL of diluent, dissolve, and dilute to volume with diluent. From the above solution 1.0mL transfer into an HS vial, add 3mL of N, N-Dimethyl formamide, and 6ml of water, seal, and mix well.

Establishment of Limit of Detection (LOD) and Limit of Quantification (LOQ) Inject

Table 7. Results of solvent Retention time in Standard & Spiked sample solution

Name of the solvents	Retention time of solvent peak from Standardsolution	Retention time from spiked sample solution(In minutes)
Dichloromethane	2.478	2.483
Acetone	3.907	3.911
Methanol	4.136	4.141
Isopropanol	4.636	4.640

Table 8. System precision results

Injection No.	Dichloromethane	Acetone	Methanol	Isopropanol
1	81.04	781.53	338.85	175.59
2	83.38	799.15	349.04	177.19
3	81.70	789.51	341.29	177.04
4	81.72	784.51	341.69	177.03
5	85.90	781.13	347.78	174.93
6	86.24	790.24	350.79	175.36
Mean	83.33	787.68	344.91	176.19
% RSD	2.7	0.9	1.4	0.6

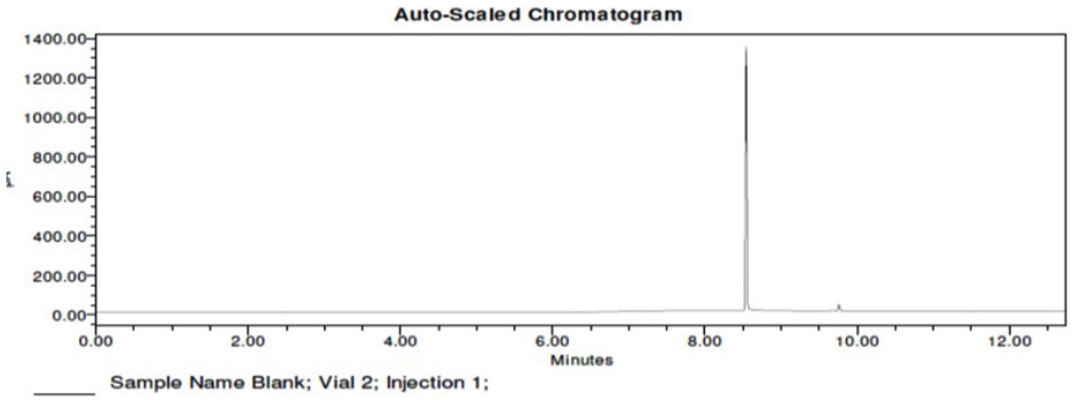

Fig. 1. Typical Chromatogram of Blank solution

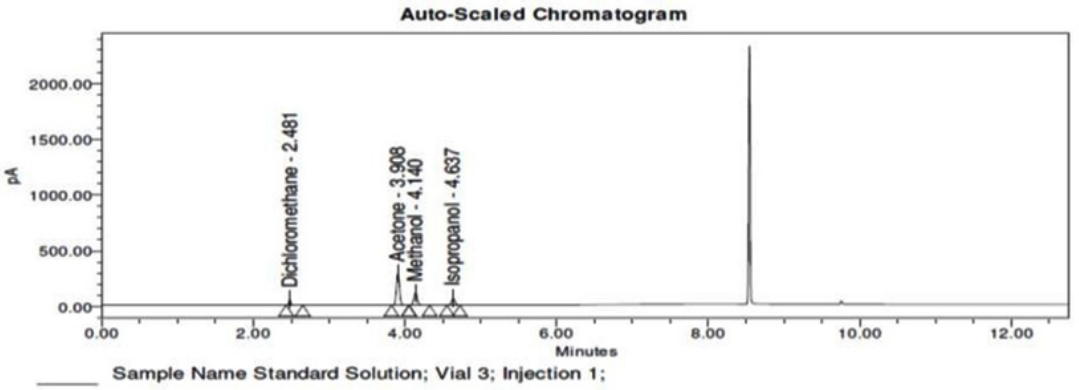

Fig. 2. Typical Chromatogram of Standard

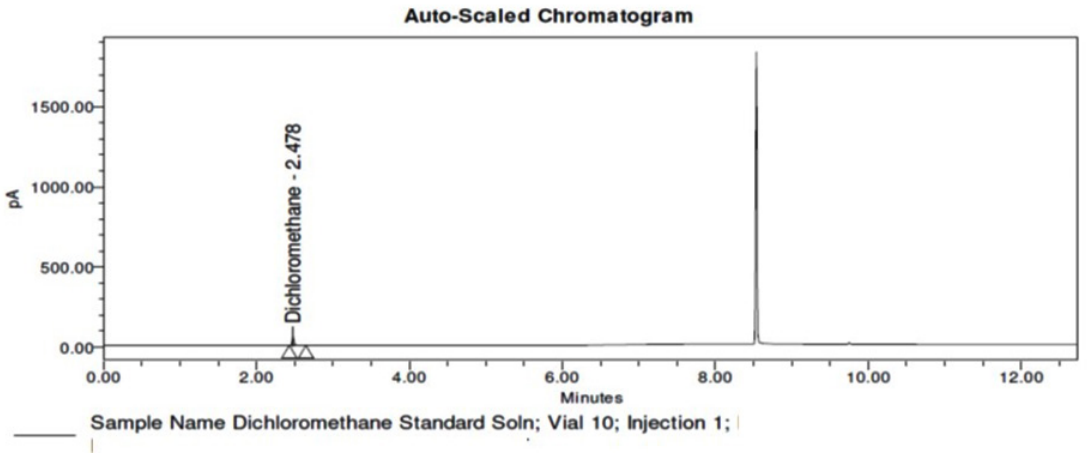

Fig. 3. Typical Chromatogram of Dichloromethane

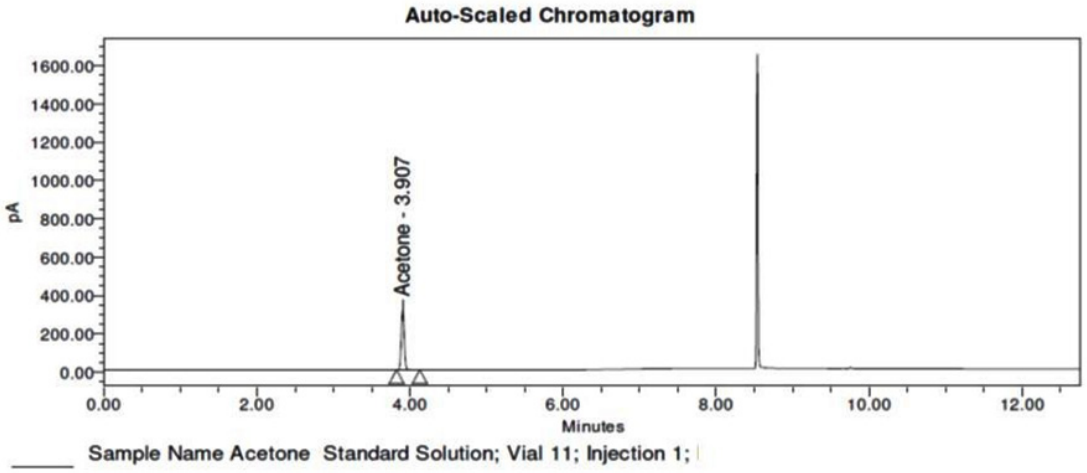

Fig. 4. Typical Chromatogram of Acetone

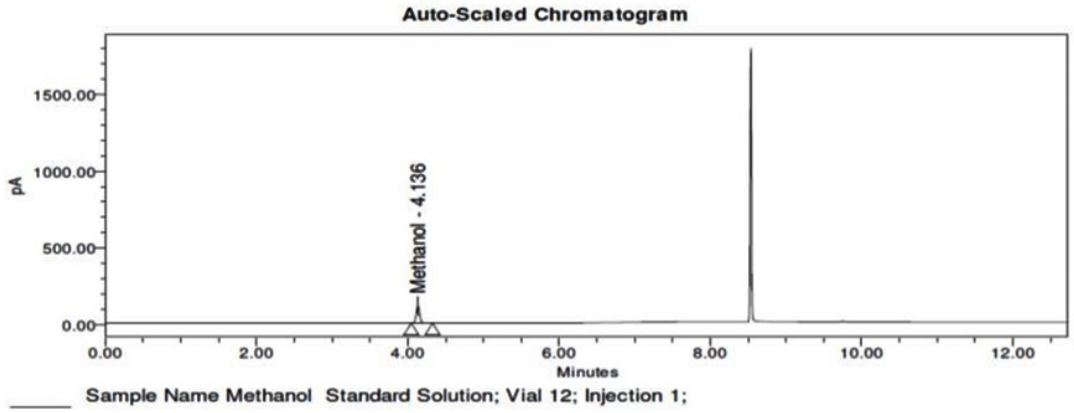

Fig. 5. Typical Chromatogram of Methanol

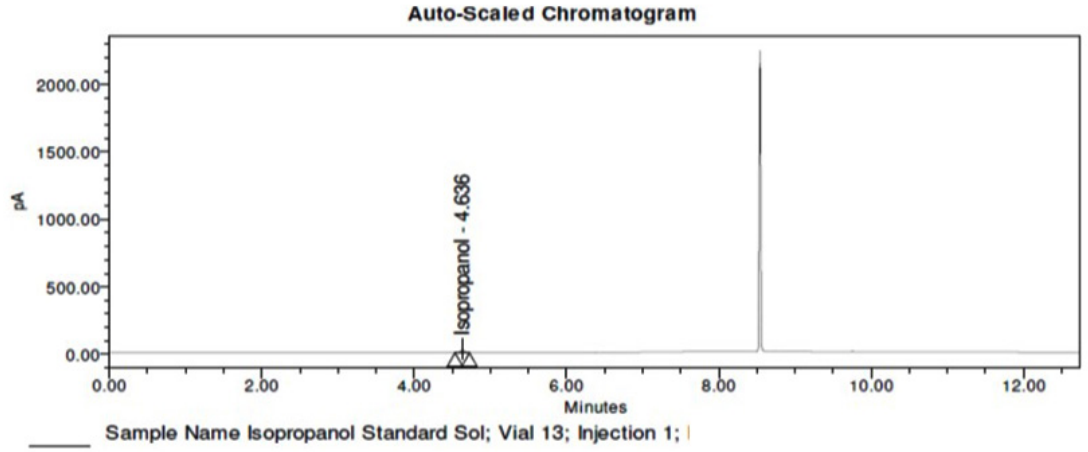

Fig. 6. Typical Chromatogram of Isopropanol

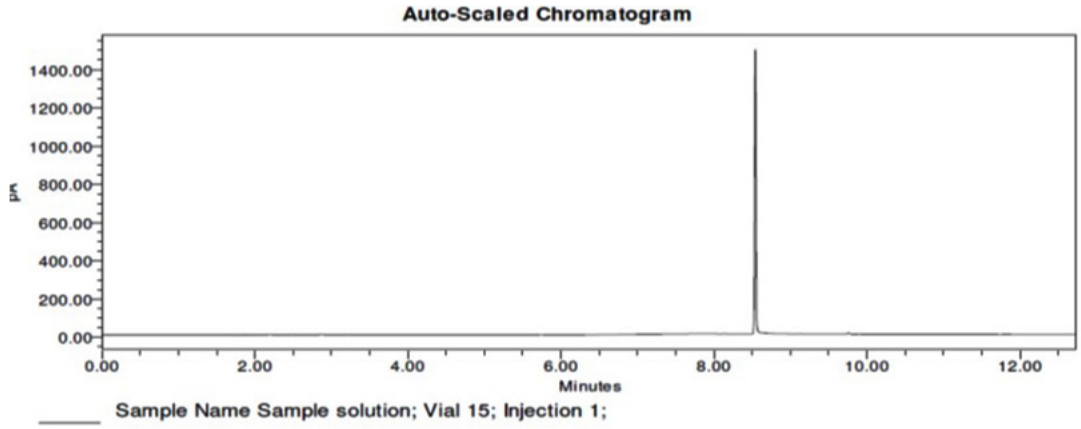

Fig. 7. Typical Chromatogram of sample Solution

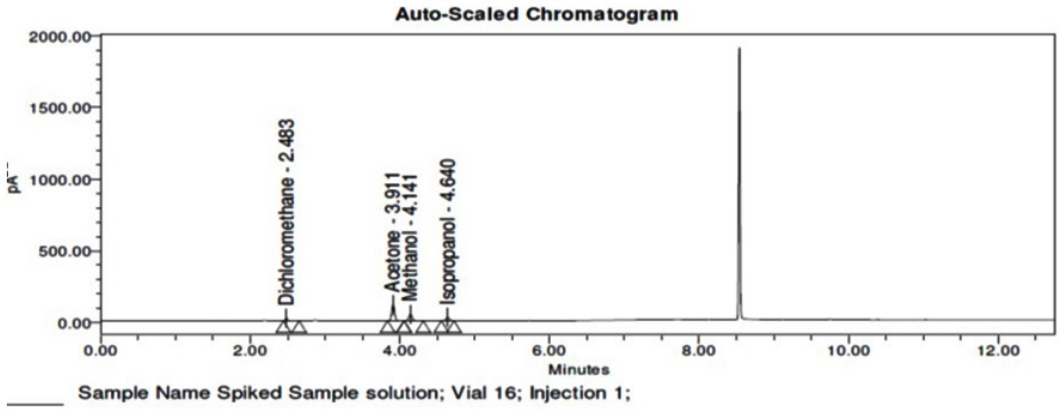

Fig. 8. Typical Chromatogram of Spiked sample

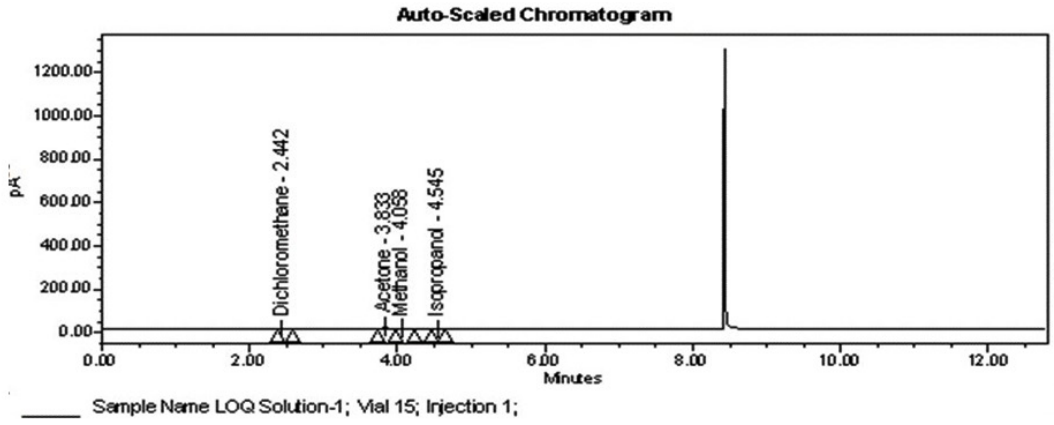

Fig. 9. Typical chromatogram of LOQ Solution

the known concentration of LOD & LOQ Solutions into the GC system for evaluation of LOD & LOQ values and calculated the LOD & LOQ Values based on S/N Ratio.

Precision at Limit of Quantitation

Inject the six injections of LOQ precision solution into the chromatographic system as per the test method and evaluate the precision of the LOQ solution.

Accuracy

Prepared recovery samples by spiking Dichloromethane, Acetone, Methanol, and Isopropanol at LOQ level, 50 %, 100 %, and 150 % of Specification level concentration in the sample and inject into the chromatographic system and calculated the % individual recovery, % mean recovery and % RSD at each level.

Linearity Inject the linearity solutions from LOQ to 150% of the specification limit into the chromatographic system as per the test method and find the Correlation Coefficient.

Table 9. System suitability Results

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	1.2	-	50882
Acetone	1.1	24	45299
Methanol	1.2	3	38854
Isopropanol	1.2	6	51370

RESULTS AND DISCUSSION

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed 15.0 percent. The theoretical plates calculated from the chromatogram from the first injection are at least 5000. So, the results as mentioned earlier indicate that the system meets the required suitability criteria[12].

Table 10. Method Precision Results (in ppm)

Preparation.No.	Dichloromethane	Acetone	Methanol	Isopropanol
1	0.07	0.49	0.29	0.41
2	0.07	0.49	0.29	0.41
3	0.07	0.49	0.29	0.41
4	0.07	0.52	0.31	0.42
5	0.07	0.50	0.30	0.41
6	0.07	0.50	0.9	0.41
Mean	0.07	0.50	0.30	0.41
%RSD	0.0	2.3	2.8	1.0

Table 11. System Suitability Results

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	2.7	-	53.69
Acetone	2.1	24	44828
Methanol	1.9	3	37964
Isopropanol	1.6	6	50333

Table 12. LOD & LOQ Results

Name of the Solvent	LOD(%)	LOQ(%)
Dichloromethane	0.0003	0.0008
Acetone	0.0025	0.0077
Methanol	0.0012	0.0038
Isopropanol	0.0017	0.0057

Specificity results

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed 15.0 percent[13]. It is not less than 5000 than the number of theoretical plates that are computed from the chromatogram that was obtained from the initial injection. The Resolution of adjacent

peaks is not less than 1.5. The blank peak should not show any interference at the retention time of the Dichloromethane, Acetone, Methanol, and Isopropanol peaks in the standard and sample solutions. So, No Interference was observed due to the blank at the retention time of Dichloromethane, Acetone, Methanol, and Isopropanol in standard and sample solutions.Dichloromethane, Acetone, Methanol, and Isopropanol separated well from each other.

Table 13. System suitability results:

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	2.0	-	53021
Acetone	3.5	24	44746
Methanol	2.7	3	37968
Isopropanol	2.1	6	50298

The above results reveal that the method is specific.

System Precision

Discussion

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed

Table 14. LOQ Precision Results

Injection No.	Dichloromethane	Acetone	Methanol	Isopropanol
1	5.65	51.97	23.51	15.27
2	5.65	48.04	22.67	14.20
3	5.40	51.34	23.25	15.24
4	5.68	53.78	24.51	15.51
5	5.79	51.30	23.82	14.95
6	5.75	53.40	24.57	15.35
Mean	5.65	51.64	23.72	15.09
%RSD	2.4	4.0	3.1	3.1

Table 15. System Suitability Results:

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	2.2	-	53021
Acetone	3.7	24	44746
Methanol	3.1	3	37968
Isopropanol	2.7	6	50298

Table 16. Dichloromethane Accuracy Results

Sample No.	Spike Level	% found	% added	Individual % Recovery	Mean % Recovery	% RSD
1	LOQ%	0.0037	0.0032	115.6	110.4	4.3
2	LOQ%	0.0035	0.0032	109.4		
3	LOQ%	0.0034	0.0032	106.3		
1	50%	0.0335	0.0299	112.0	114.8	2.7
2	50%	0.0353	0.0299	118.1		
3	50%	0.0342	0.0299	114.4		
1	100%	0.07	0.06	1167	116.7	0.0
2	100%	0.07	0.06	116.7		
3	100%	0.07	0.06	116.7		
1	150%	0.1043	0.0970	107.5	109.7	2.1
2	150%	0.1063	0.0970	109.6		
3	150%	0.1086	0.0970	112.0		

Table 17. Acetone Accuracy Results

Sample No.	Spike Level	% found	% added	Individual % Recovery	Mean % Recovery	% RSD
1	LOQ%	0.0236	0.0251	94.0	94.0	0.9
2	LOQ%	0.0234	0.0251	93.2		
3	LOQ%	0.0238	0.0251	94.8		
1	50%	0.2642	0.2497	105.8	102.7	6.7
2	50%	0.2685	0.2497	107.5		
3	50%	0.2694	0.2497	107.9		
1	100%	0.49	0.51	96.1	96.1	0.0
2	100%	0.49	0.51	96.1		
3	100%	0.49	0.51	96.1		
1	150%	0.1043	0.740	99.0	101.2	2.3
2	150%	0.1063	0.740	100.9		
3	150%	0.1086	0.740	103.7		

Table 18. Methanol Accuracy Results

Sample No.	Spike Level	% found	% added	Individual % Recovery	Mean % Recovery	% RSD
1	LOQ%	0.0137	0.0121	113.2	110.2	2.4
2	LOQ%	0.0131	0.0121	108.3		
3	LOQ%	0.0132	0.0121	109.1		
1	50%	0.1592	0.1513	105.2	107.7	2.0
2	50%	0.1646	0.1513	108.8		
3	50%	0.1628	0.1513	107.6		
1	100%	0.29	0.32	90.6	90.6	0.0
2	100%	0.29	0.32	90.6		
3	100%	0.29	0.32	90.6		
1	150%	0.3554	0.429	82.8	85.1	2.7
2	150%	0.3653	0.429	85.2		
3	150%	0.3749	0.429	87.4		

Table 19. Isopropanol Accuracy Results

Sample No.	Spike Level	% found	% added	Individual % Recovery	Mean % Recovery	% RSD
1	LOQ%	0.0196	0.0201	97.5	98.8	1.3
2	LOQ%	0.0199	0.0201	99.0		
3	LOQ%	0.0201	0.0201	100.		
1	50%	0.2553	0.2512	101.6	100.6	0.8
2	50%	0.2512	0.2512	100.3		
3	50%	0.2548	0.2512	101.4		
1	100%	0.41	0.51	80.4	80.4	0.0
2	100%	0.41	0.51	80.4		
3	100%	0.41	0.51	80.4		
1	150%	0.6133	0.742	82.1	85.4	3.5
2	150%	0.6295	0.742	84.8		
3	150%	0.6576	0.742	88.6		

Table 20. System suitability results

Solvent name	% RSD	Resolution	Plate count
Dichloromethane	2.2	-	53021
Acetone	3.7	24	44746
Methanol	3.1	3	37968
Isopropanol	2.7	6	50298

Table 21. Linearity Solutions results

Linearity Levels	Dichloromethane	Acetone	Methanol	Isopropan ol
LOQ	5.25	45.35	21.13	14.35
50%	51.07	488.58	219.16	147.21
80%	79.53	753.99	340.25	236.31
100%	96.71	971.83	428.76	293.70
120%	121.52	1123.16	513.51	342.07
150%	144.14	1429.54	631.95	441.13
Correlation Coefficient	0.999	1.000	1.000	1.000
Slope	1622.71248	1898.91177	1386.42674	576.30796
Intercept	1.3872	3.7725	6.7201	2.5834
Y-Intercept at 100% bias	1.434	0.388	1.567	0.880

15%. The Resolution of adjacent peaks is not less than 1.5, Calculated with a chromatogram of the first injection. So, the above results reveal that the system is precise[14].

Method Precision

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed 15%. The Resolution of adjacent peaks is not less than 1.5, Calculated with a chromatogram of the first injection. The number of theoretical plates calculated from the chromatogram from the first injection is not less than 5000. The relative standard deviation (RSD) for each solvent content in the six preparations of the Method precision solutions should not exceed 15.0%. So, the above results reveal that the method is precise[16]

Establishment of Limit of Detection/Limit of Quantification (LOD/LOQ)

Discussion

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed 15 percent. The resolution of adjacent peaks is not less than 1.5, calculated with a chromatogram of the first injection. The number of theoretical plates calculated from the chromatogram from the first injection is not less than 5000. S/N ratios for LOD and LOQ, respectively, should not be less than 3 and 10. The LOQ concentrations for dichloromethane are 0.008%, Acetone 0.0077%, Methanol 0.0038%, and Isopropanol 0.0057% concerning sample concentration[17].

Precision at the Limit of Quantitation

Inject the six injections of a solution with a limit of quantification (LOQ) into the chromatographic system and assess the precision of the LOQ solution.

Acceptance criteria

The relative standard deviation for the area of respective solvent peaks from six replicate injections of the standard solution not exceed 15.0 percent. The Resolution of adjacent peaks is not less than 1.5, Calculated with a chromatogram of the first injection The theoretical plates calculated from the chromatogram from the first injection are at least 5000. The relative standard deviation (RSD) of the area of each solvent in the six preparations of the limit of quantification (LOQ) precision solutions should not exceed 15.0%. So, the above results reveal that the method is precise at the LOQ level.

Accuracy

Prepare recovery samples by spiking Dichloromethane, Acetone, Methanol, and Isopropanol at LOQ level, 50 %, 100 %, and 150 % of Specification level concentration in the sample and injected into the chromatographic system. Furthermore, the percentage of individual recovery, mean recovery, and relative standard deviation (RSD) for the individual recovery percentage were calculated at each level.

Acceptance criteria

The % RSD from six replicate injections of the standard solution does not exceed 15%. The Resolution of adjacent peaks is not less than 1.5. The number of theoretical plates calculated from the chromatogram of the first injection is not less than 5000. The individual percentage recovery and the mean percentage recovery result for each level should fall within the range of 80 to 120. The individual percentage recovery and the mean percentage recovery result at the limit of quantification (LOQ) level should fall within the range of 70 to 130. The relative standard deviation (RSD) for the individual recovery percentage at each level should not exceed 15.0%.

Conclusion: The above results indicate that the method's accuracy.

Linearity

Inject linearity solutions ranging from the Limit of Quantification (LOQ) to 150% of the Specification limit into the chromatographic system.

CONCLUSION

Estimation of the residual solvents is mandatory for the release testing of all active pharmaceutical ingredients (API). So, in this study, the authors estimated the four residual solvents of Tigecycline using the Headspace sampling technology, and the method is validated and meets all required standards per the ICH revised guidelines. Residual solvents such as Dichloromethane, Acetone, Methanol, and Isopropanol in pharmaceutical samples of Tigecycline were monitored using gas chromatography with headspace sampling technology. The column used for this elution is DB-624, 30m X 0.32mm X 1.8µm, Nitrogen is used as carrier gas with FID detector. Split ratio is 30:1 and the injector temperature is 210 °C. So, this method can be used for routine analysis in Quality control laboratories and bulk drug industries for estimation of impurities such as residual solvents.

ACKNOWLEDGEMENT

The authors thank the management of Sultan-ul-Uloom College of Pharmacy for providing research facilities for this work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Authors' Contribution

Syed Imam Pasha and Shaik Liyaqat : had done the method development and validation. Mushraff Ali khan, Anupama Koneru and Mohammed Abdul Farhan : helped draft the manuscript and provided the drug samples and reagents.

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