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Research

IDENTIFICATION AND QUANTIFICATION OF RELATED COMPOUNDS C&D IN VORICONAZOLE BY HPLC

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Abstract

An isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the identification & quantification of related substances C & D. The successful separation of voriconazole from its synthetic impurities and degradation products formed under stress conditions was achieved using 3.9mm x 15cm, 4µm Packing L1 or equivalent column maintained at 25°C with a mobile phase of a Acetonitrile: Methanol (55:15:30). The mobile phase flow rate was 1.0 mL/min, and the detection wavelength was 256 nm. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The developed HPLC method to determine the related substances and assay determination of voriconazole can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of voriconazole.

Key words: Voriconazole, HPLC, Identification, Quantification, Validation parameters

INTRODUCTION:

High-Performance Liquid Chromatography (HPLC): The fastest growing analytical technique for drug analysis is high performance liquid chromatography (HPLC). Its versatility, high quality and broad sensitivity make it the best way to evaluate many medicinal products in both dosage forms and biological fluids.
In analytical chemistry, HPLC is the preferred method since this is a specific, stable, linear, accurate and low detection limit with the various benefits.

The most comprehensive and important analytical method applied in all phases of drug exploration, growth and production in the modern pharmaceutical industry is high performance liquid chromatography (HPLC). Two main activities include the production of new chemical entities drug development and drug design. The purpose of the drug research programme is to analyze a large number of compounds using rapid screening techniques that generate active ingredients and then narrow down their range by means of precise synthetic methods and selective testing (lead optimization). This led to the final option of the most suitable therapeutic candidates for the production of medicines. Aspirants substances should be characterized by their metabolism, pre-clinical, clinical, and clinical trials in the principal tasks of drug production. The optimization of drug synthesis and formulation is carried out in accordance with the drug development process, which ultimately leads to an efficient and strong production process for the drug ingredient and drug. The rough analytical separation methods are developed and for each development category (i.e. early drug discovery; drug metabolism, pharmacokinetics, process research, pre-formulation and formulation) in this drug discovery and drug development technique.

A. Qualitative analysis: It is concerned with defined substances. It is concerned with determining the elements or compounds in the sample.

B. Quantitative analysis: includes numerical information concerning the sample quantity (analyte) in the sample's calculated quantity.

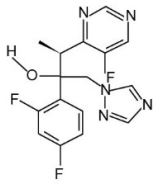
There are numerous pharmaceutical analytical techniques which are very sensitive, precise, accurate and comprehensive information on the sample.

Validation Parameters:

The method efficiency and reliability of the analytical results must be demonstrated by validation.The following fundamental parameters must be determined in compliance with the latest USFDA, ICH-GCP and current global bioanalytical industry guideline to validate the bio-analysis methodologies.i.e. Towards linearity (calibration curve), Specificity and precision Precision and accuracy interlocking Accuracy intra batch and exactness Towards the impact of matrix, The Factor Matrix Stability towards stability Stock &work solution stability at 22±5°C (2–8°C) and long term (2–8°C).Top stability for the bench at 2±5°CThe stability of the extract is -20±5°C or –70±10°C. Dryextract.Stability at -20±5 degrees and/or 70±10 degrees Celsius.Stability of the auto sampler 5±1°C Long term matrix stability at –20±5°C or -70±10°C.1/5 and 1/10 dilution, dilution integrityRe-injection reproducibility

DRUG PROFILE

Voriconazole



Molecular Formula	$C_{16}H_{14}F_3N_5O$
Molecular weight	349.31g/mol
Solubility	Insoluble in Water

MATERIALS AND METHOD

Details of Instruments, Column, Chemicals and Specification limits

Instruments: HPLC, with UV-Visible or PDA detector, Analytical Balance, Glassware Class-A. , Column: 3.9mmx15cm, 4µm Packing L1 or equivalent, Chemicals: Ammonium formate Formic acid, Methanol, Acetonitrile, HPLC grade Water

Specification limits

Name	Relative retention time (min)	Acceptance Criteria NMT (%)
Voriconazole related compound C	0.26	0.2
Voriconazole related compound D	0.61	0.1
Voriconazole	1.0	–
Any Unspecified impurity	–	0.1
Total impurities	–	0.5

RESULTS AND DISCUSSION:

Description of Analytical method (Methodology)

Chromatographic Conditions:

HPLC Column : 3.9mm ×15cm, 4µm Packing L1 or equivalent.
Wavelength : UV, 256 nm
Flow rate : 1.0 mL/minute
Injection Volume : 20 µL
Run time : 20 minutes
Diluent : Mobile phase

Preparation of Buffer: 1.9 µg/L of Ammonium formate in water. Adjust with formic acid to a pH of 4.0.

Preparation of Mobile Phase: Prepared a filtered and degassed mixture of Buffer: Acetonitrile: Methanol (55:15:30).

System Suitability solution: 0.25µg/mL of USP Voriconazole RS in mobile phase.

Preparation of Standard solution:2.5 µg/mL each of USP Voriconazole RS, USP Related compound C RS and USP Voriconazole Related compound D in mobile phase. (Note: sonicate to dissolve if necessary)

Preparation of Sample solution: 500µg/mL of Voriconazole in mobile phase. . (Note: sonicate to dissolve if necessary)

Procedure: 1. Equilibrate the column using mobile phase to get a stable base line. 2. Inject diluent as a blank (one injection), System suitability solution (Six times) and standard preparation (six injections) and check the system suitability parameters.

System suitability: The tailing factor for Voriconazole peak is NMT 2.0 from the standard solution: The Theoretical plates for Voriconazole peak is NLT 3500 from the standard solution, The % RSD of area response for six replicate injections of Voriconazole from System suitability preparation should be not more than 10.0.

Validation Plan: For Verification of Voriconazole USP, following parameters shall be verified.

S. No.	Verification Parameters
1	System Suitability
2	Specificity
3	Precision i) System Precision ii) Method Precision (repeatability) a) For Impurities b) For analyte at unknown level
4	Linearity a) Linearity of Impurities b) Linearity of Unknown peak (Voriconazole) at unknown level
5	Accuracy a) Accuracy of Impurities at Specification level b) Accuracy of unknown peak ay any unspecified level.

Description of Analytical Method

Chromatographic Conditions:

HPLC Column : 3.9mm × 15cm, 4µm Packing L1 or equivalent.
Wavelength : UV, 256 nm
Flow rate : 1.0 mL/minute
Injection Volume : 20 µL
Run time : 20 minutes
Diluent : Mobile phase

Preparation of Buffer:1.9 µg/L of Ammonium formate in water. Adjust with formic acid to a pH of 4.0.

Preparation of Mobile Phase": Prepared a filtered and degassed mixture of Buffer: Acetonitrile: Methanol (55:15:30).

System Suitability solution:0.25µg/mL of USP Voriconazole RS in mobile phase.

Preparation of Standard solution:2.5 µg/mL each of USP Voriconazole RS, USP Related compound C RS and USP Voriconazole Related compound D in mobile phase. (Note: sonicate to dissolve if necessary)

Preparation of Sample solution: 500µg/mL of Voriconazole in mobile phase. . (Note: sonicate to dissolve if necessary)

Procedure: Equilibrate the column using mobile phase to get a stable base line.Inject diluent as a blank (one injection), System suitability solution (Six times) and standard preparation (six injections) and check the system suitability parameters.

System suitability: The tailing factor for Voriconazole peak is NMT 2.0 from the standard solution. The Theoretical plates for Voriconazole peak is NLT 3500 from the standard solution, The % RSD of area response for six replicate injections of System suitability solution preparation should be not more than 1.0.

Validation Results

System Suitability:As per methodology, injected blank, six replicate injections of system suitability solution, and six replicate injections of standard solution into HPLC system. Calculated the % RSD of Voriconazole from six replicate injections of System suitability solution.

Results

Table 13: System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Conclusion: The above results reveal that the system meets the required system suitability criteria.

Specificity:

Interference of Blank: As per methodology, injected Blank, system suitability solution, Standard solution, individual impurities solutions, Sample solution and spiked sample solution checked the peak interference of blank at the retention time of Voriconazole and its related impurities.

Results

System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Note: For Voriconazole retention time for individual solution considered from standard retention time.

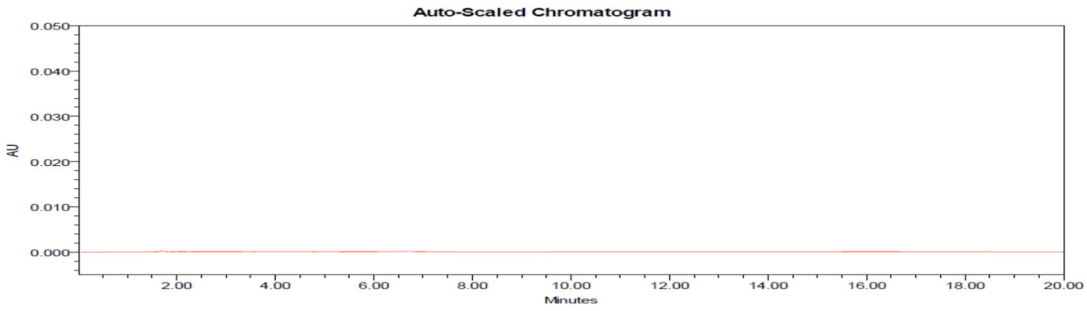


Figure 4: Typical chromatogram of Blank

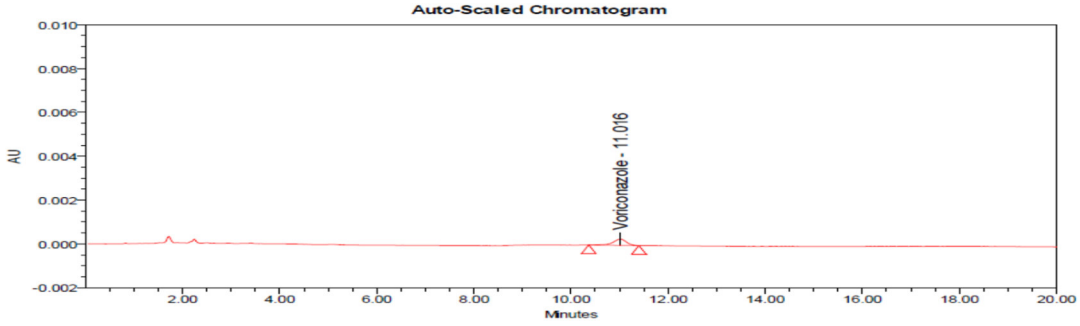


Figure 5: Typical chromatogram of System suitability solution

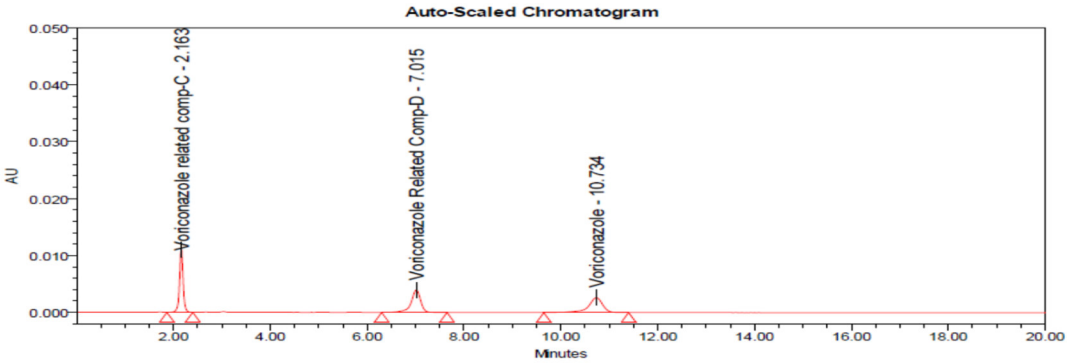


Figure 6: Typical chromatogram of Standard solution

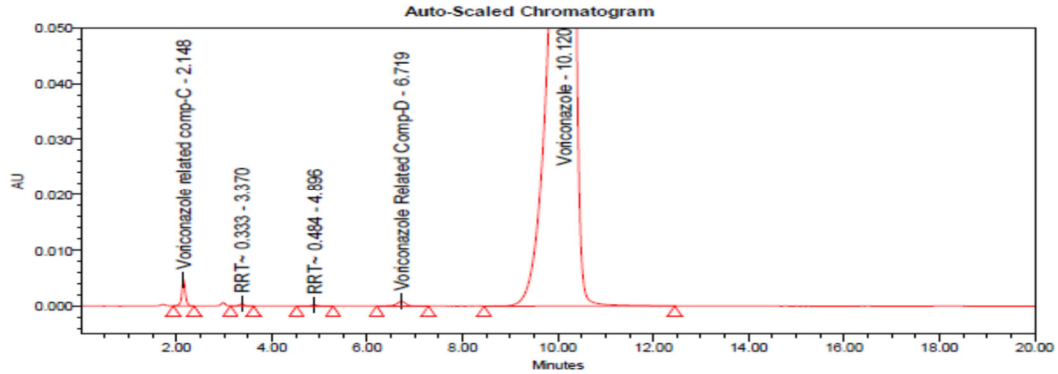


Figure 7: Typical chromatogram of Spiked sample solution

Conclusion: There is no interference observed from blank at the retention time of Voriconazole peak and no interference was observed with each individual impurity as well as with analyte peak.

Precision

System Precision: As per methodology, injected blank, six replicate injections of system suitability solution, and six replicate injections of standard solution into HPLC system. Calculated the % RSD of Voriconazole from six replicate injections of System suitability solution.

Results

Table 16: System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Method Precision

a) For Impurities: Analysed six spiked sample preparations of Voriconazole spiking with Voriconazole related compound-C and Voriconazole related compound-D as per specification level and established as per the methodology and determined the % RSD of Voriconazole related compound-C and Voriconazole related compound-D, Total impurities.

Results

Table 18: System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Table 19: Method precision Results for Impurities:

Sample ID	Voriconazole related compound-C	Voriconazole related compound-D	Any individual unspecified	Total Impurities
	% w/w			
01	0.210	0.106	0.023	0.359
02	0.212	0.107	0.024	0.362
03	0.213	0.105	0.024	0.362
04	0.212	0.104	0.024	0.359
05	0.214	0.106	0.024	0.364
06	0.211	0.104	0.024	0.360
Average	0.212	0.105	0.024	0.361
STDEV	0.00	0.00	0.00	0.00
%RSD	0.7	**1.1**	1.7	0.6

Acceptance criteria: The tailing factor for Voriconazole peak is NMT 2.0 from standard solution.The Theoretical plates for Voriconazole peak is NLT 3500 from standard solution.The % RSD of area response for six replicate injections of Voriconazole from System suitability preparation should be not more than 10.0.The % RSD for the Voriconazole related compound-C, Voriconazole related compound-D, any individual unspecified impurity and Total impurities from the six preparations of the method precision solutions should be not more than 10.0.

b) For Analyte at Unknown specification Level:

Analysed six sample preparations of Voriconazole spiking at any individual unspecified impurity level on diluents as per the methodology and determined the % RSD of Voriconazole.

Table 20: Method precision Results for Analyte at Unknown Specification Level:

Sample ID	% w/w of Voriconazole
01	0.104
02	0.104
03	0.103
04	0.103
05	0.104
06	0.102
Average	0.103
STDEV	0.00
%RSD	0.8

Acceptance criteria: The % RSD for theVoriconazole from the six preparations of the method precision solutions should be not more than 10.0.

Conclusion: The above results reveal that the method is precise for impurities, total impurities and at unknown specification level.

Establishment of LOQ & LOD and Precision at LOQ Level:

LOQ and LOD of Voriconazole related compound C, Voriconazole related compound D and Voriconazole from the analytical method and ensure the s/n ratio of each individual peak as below,

Table 21: Precision at LOQ level

Sample ID	Voriconazole related compound-C	Voriconazole related compound-D	Voriconazole
	Area		
01	5987	2509	3208
02	5912	2546	3219
03	5946	2565	3235
04	5910	2498	3210
05	5946	2526	3209
06	5875	2556	3241
Average	5929	2533	3220
STDEV	38.75	26.73	14.36
%RSD	**0.7**	1.1	0.4

The % RSD for the %w/w of Voriconazole related compound-C and Voriconazole related compound-D, Voriconazole from the six preparations of LOQ solution should be not more than 10.0.

Linearity:For Impurities: Linearity for Voriconazole related compound-C and Voriconazole related compound-D and Voriconazole was determined in the concentration range from LOQ to 150 % levels of Specification level w.r.t test concentration levels.

Results

Table 22: System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Table 23: Linearity Results of Voriconazole related compound-C

Level (%w/w)	Voriconazole Related compound-C Concentration (ppm)	Voriconazole Related compound-C Peak Area
LOQ (L1)	0.250	5808
50 (L2)	0.500	12629
75 (L3)	0.750	18509
100 (L4)	1.000	24344
150 (L5)	1.500	36678
Correlation Coefficient	0.999738	
Slope	24470.11	
Y-Intercept	17.51351	
% Y-Intercept	0.071942	
Residual Sum of Squares	290574.7	

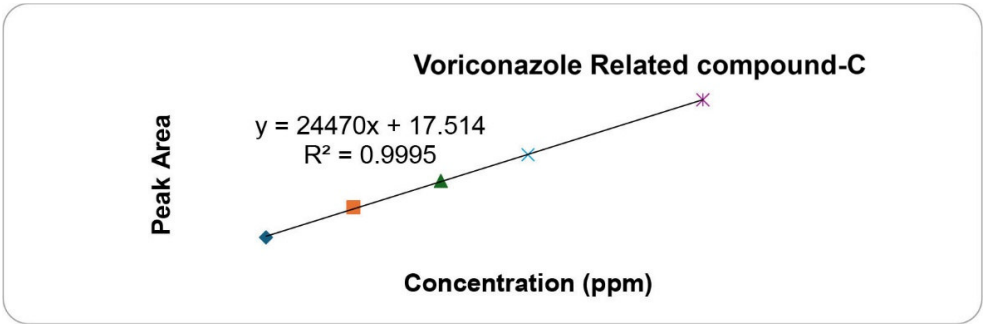


Figure 8: Linearity graph for Voriconazole related compound-C

Table 24: Linearity Results of Voriconazole related compound-D

Level (%w/w)	Voriconazole Related compound-D Concentration (ppm)	Voriconazole Related compound-D Peak Area
LOQ (L1)	0.150	2573
50 (L2)	0.250	5359
75 (L3)	0.375	7802
100 (L4)	0.500	10492
150 (L5)	0.750	15336
Correlation Coefficient	0.998251	
Slope	20948.81	
Y-Intercept	-171.867	
% Y-Intercept	-1.63808	
Residual Sum of Squares	335553.9	

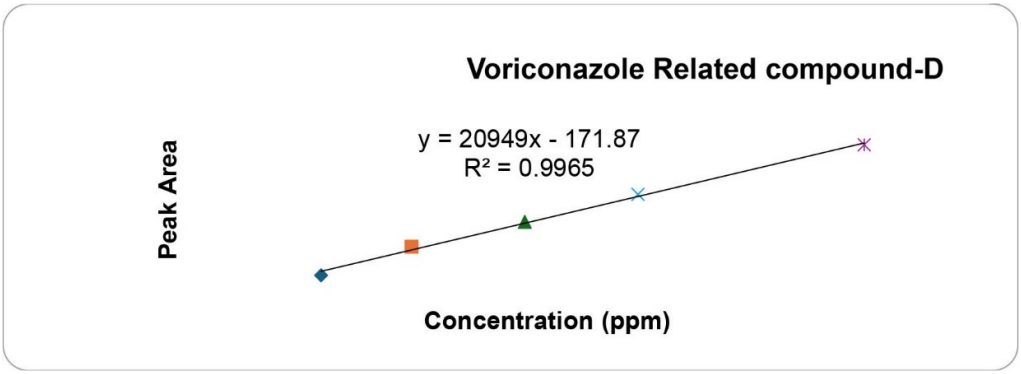


Figure 9: Linearity graph for Voriconazole related compound-D

Table 25: Linearity Results of Voriconazole

Level (%w/w)	Voriconazole Concentration (ppm)	Voriconazole Peak Area
LOQ (L1)	0.151	3292
50 (L2)	0.252	6502
75 (L3)	0.378	9830
100 (L4)	0.503	12860
150 (L5)	0.755	18994
Correlation Coefficient	0.998759	
Slope	25678.18	
Y-Intercept	-175.963	
% Y-Intercept	-1.3683	
Residual Sum of Squares	361761.7	

a) For Impurities &Analyte at Unspecified Level.

As per methodology, injected blank, System suitability solution in six replicate injections and Standard preparation in six replicates followed by LOQ to 150% sample solutions and injected into the HPLC system and demonstrated the accuracy of the method. Calculated the system suitability parameters and % Individual recovery and % mean recovery.

Note: Accuracy at LOQ level was considered from precision at LOQ Level.

Results:

Table 26: System suitability

Parameter	From Standard solution		System suitability solution
Result	0.9	7842	7.3
Acceptance Criteria	Tailing factor NMT 2.0 of Voriconazole peak	Theoretical plates NLT 3500 of Voriconazole	% RSD for Voriconazole is NMT 10.0

Table 27: Accuracy of Voriconazole related compound-C

S.No	Spike level	Added %w/w	Found %w/w	'%' Recovery	'%' Mean recovery	%RSD
1	LOQ-1	0.050	0.054	108.0	106.3	0.8
2	LOQ-2	0.050	0.053	106.0		
3	LOQ-3	0.050	0.053	106.0		
4	LOQ-4	0.050	0.053	106.0		
5	LOQ-5	0.050	0.053	106.0		
6	LOQ-6	0.050	0.053	106.0		
7	100%-1	0.200	0.210	105.0	105.8	0.7
8	100%-2	0.200	0.212	106.0		
9	100%-3	0.200	0.213	106.5		
10	150%-1	0.3	0.325	108.3	108.7	0.4
11	150%-2	0.3	0.326	108.7		

12	150%-3	0.3	0.327	109.0
13	150%-4	0.3	0.327	109.0
14	150%-5	0.3	0.327	109.0
15	150%-6	0.3	0.324	108.0

Table 28: Accuracy of Voriconazole related compound-D

S.No	Spike level	Added %w/w	Found %w/w	'%' Recovery	'%' Mean recovery	%RSD
1	LOQ-1	0.03	0.029	96.7	96.1	1.4
2	LOQ-2	0.03	0.029	96.7		
3	LOQ-3	0.03	0.029	96.7		
4	LOQ-4	0.03	0.028	93.3		
5	LOQ-5	0.03	0.029	96.7		
6	LOQ-6	0.03	0.029	96.7		
7	100%-1	0.1	0.106	106.0	106.0	0.9
8	100%-2	0.1	0.107	107.0		
9	100%-3	0.1	0.105	105.0		
10	150%-1	0.15	0.155	103.3	102.9	0.3
11	150%-2	0.15	0.154	102.7		
12	150%-3	0.15	0.154	102.7		
13	150%-4	0.15	0.155	103.3		
14	150%-5	0.15	0.154	102.7		
15	150%-6	0.15	0.154	102.7		

Conclusion: The above results reveal that the method is accurate.

CONCLUSION

The current analytical method was validated according to the protocol, and it passes the acceptance criteria. Thus, it was determined that the analytical approach is particular, precise, linear, accurate, rugged, and robust. As a result, the current analytical approach is suitable for regular analysis and serves its intended function.

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