METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF NITROSAMINE IMPURITIES IN OTESECONAZOLE CAPSULES BY USING RP HPLC

Ramya Sree.A, Vamsi Krishna.B, Sridevi.P

Associate Professor, Student, Professor

Department Of Pharmaceutical Analysis, Sri Venkateshwara college of Pharmacy, Madhapur, Hitech City Road, Hyderabad, 500081.

Abstract:

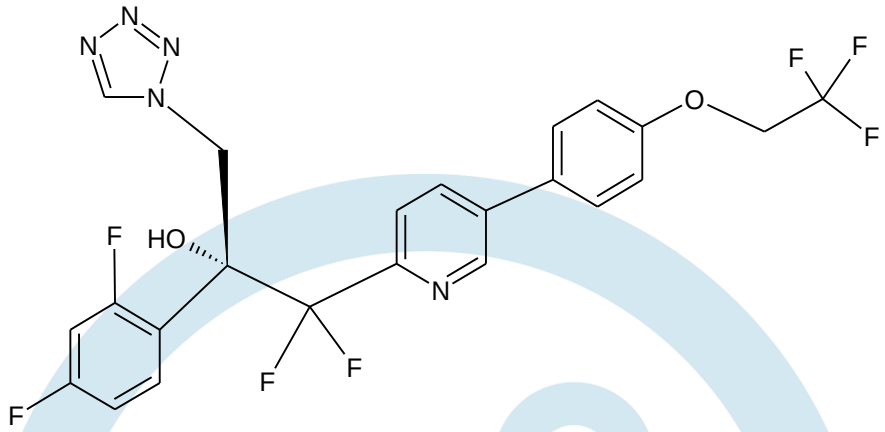
This research work outlines the advancement and verification of RP-HPLC techniques for impurity analysis in Otesecanazole using gradient elution with DIKMA Spursil C18 column, Potassium dihydrogen Orthophosphate buffer & Methanol as the mobile phase, techniques was optimized for specificity, precision, accuracy, linearity & robustness. Impurities including N-Nitroso-methylphenylamine, N-Nitrodiethylamine, N-Nitrodimethylamine were identified with calibration curves showing strong R2 =0.999. Precision, accuracy (101-122% recovery), SST parameters met all acceptance limitations. The technique also demonstrated LOD & LOQ limits, making it suitable for regular analysis of Oteseconazole and Its impurities to ensure quality and safety.

Keywords: Oteseconazole, Impurities, RP-HPLC (High Performance Liquid chromatography)

Introduction:

Oteseconazole is an anti-fungal drug, which is used to treat recurrent vulvovaginal candidiasis which is sold under the brand name of Vivjoa. Oteseconazole belongs to azole group it works by inhibiting the enzyme called fungal lanosterol 14α-dimethylase which is main component to produce the ergosterol. Where ergosterol is the vital component for fugal cell membrane. By causing the disruption for the production of cell membrane it causes the cell death.

The chemical formula of Oteseconazole:



Oteseconazole

Impurity profiling:

Impurity profiling is the process where series of analytical procedure involved for the detection, isolation, identification, elucidation and quantitative determination of organic, inorganic impurities and residual solvents present in the formulation (Oteseconazole Capsule). The impurity profiling plays an important role for the determination of the impurities in the drug substance and drug product for its control and efficacy

There are several methods for determination of the impurities in the pharmaceutical formulations those are Chromatographic methods like Thin Layer Chromatography, Gas Chromatography, High Performance Liquid Chromatography.

Materials and Methods:

High Performance Liquid Waters-Make, 2695 separation module equipped with PDA detector with column DIKMA Spursil C_{18}, 250 x 4.6 mm, 5 µm.

Preparation of Blank solution:

Take 1.0 mL of diluents into the vial and immediately place the vial

Preparation of NDMA and NMPA Standard Stock Solution I:

Take each 20 mg of NDMA and NMPA standard solutions into a 20 mL volumetric flask containing 5 mL diluent and make with diluent.

Transfer 0.75 mL of the above solution into a 100 mL volumetric flask containing 25 mL of diluent and make with diluent.

Preparation of NDEA Standard Stock solution-II:

Take 20 mg of NDEA standard solutions into a 20ml volumetric flask containing 5 mL diluent and makeup with diluent.

Transfer 0.75 mL of the above solution into a 100 volumetric flask containing 25 mL of diluent and make with diluent.

Preparation of Standard Solution:

Transfer 1.0 mL of standard stock solution-I and standard stock solution-II to a 100 mL volumetric flask containing 25 mL diluent and make up with diluent.

Add 1.0 mL of standard solution to a vial and inject it into the HPLC System.

Preparation of Test solution:

Weigh accurately about 10 mg of the test sample of oteseconazole into a vial, add 1.0 mL of diluent, and immediately place the vial.

Procedure:

Equilibrate the column for 16 minutes, inject blank solution into the system, and record the chromatogram. Then, program the data processor to inhibit the peaks due to the blank.

Inject standard solutions into the system separately six times and record the chromatograms. Check for the system suitability criteria; proceed further if the requirement is met.

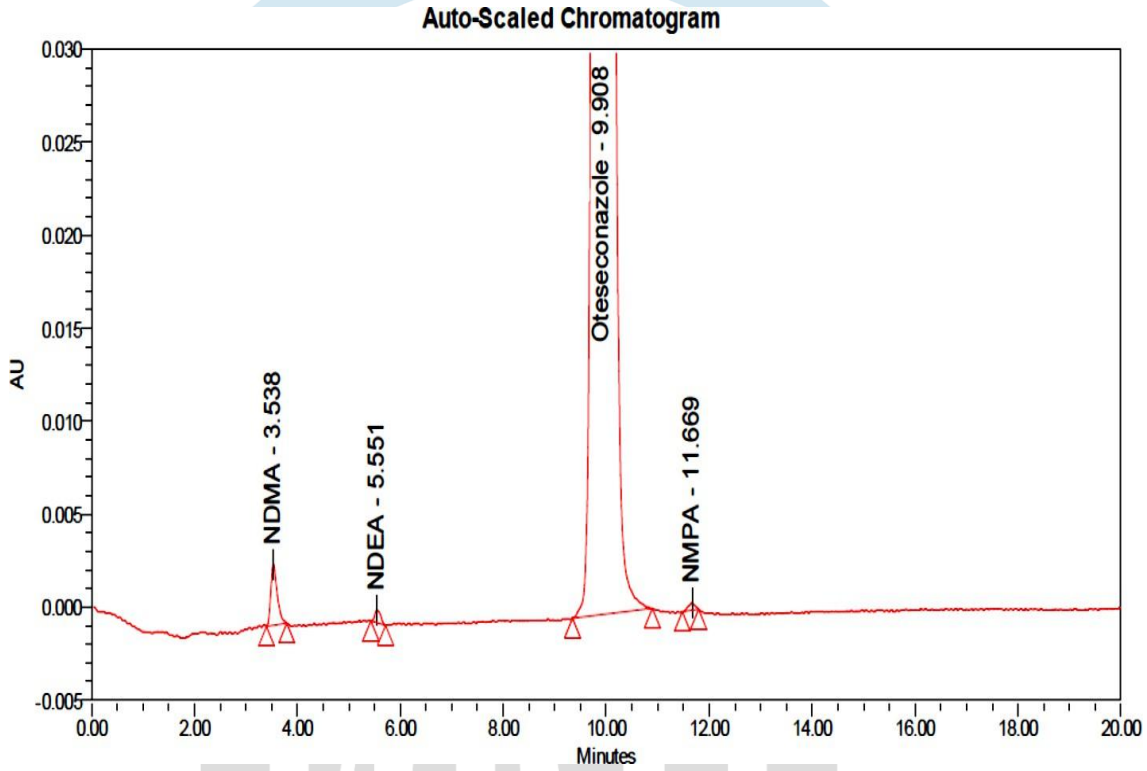
RESULTS AND DISCUSSION:

OPTIMISED METHOD

Chromatographic Conditions:

Chromatographic device	Waters-Make,2695 separation Module.996PDA
Packed column	DIKMA Spursil C_{18}, 250x4.6mm,5µm
Flowrate	1mL/min
Injection load	20µL
Thermal profile	N/A
Autosampler Temp	5^{o}C
Elution mode	Isocratic
Buffer	KH_2PO_4 (pH3.5)

Solution A	Buffer
Solution B	Methanol
Mobile Phase	SolutionA:SolutionB(80:20)
Elution duration	20 min



Linearity:

Perform linearity with different concentrations of NDMA, NDEA and NMPA by analyzing a minimum of six concentrations i.e. QL to 150% of limit level.

Preparation of:

50% Standard Solution:

Dilute 0.5 mL of standard stock solution into a 100 mL volumetric flask dilute to Volume with diluents and mix well

75% Standard Solution:

Dilute 0.75 mL of standard stock solution into a 100 mL volumetric flask dilute to Volume with diluents and mix well.

100% Standard Solution:

Dilute 1.0 mL of standard stock solution into a 100 mL volumetric flask dilute to Volume with diluents and mix well.

125% Standard Solution:

Dilute 1.25 mL of standard stock solution into a 100 mL volumetric flask dilute to Volume with diluents and mix well.

150% Standard Solution:

Dilute 1.5 mL of standard stock solution into a 100 mL volumetric flask dilute to Volume with diluents and mix well.

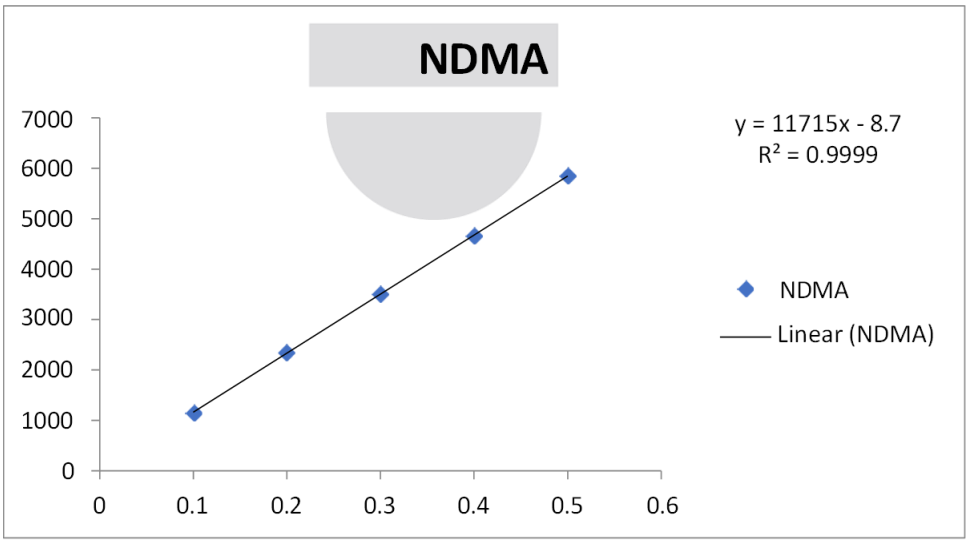
Linearity study was conducted for NDMA, NDEA and NMPA in the range from QL level to 150% level. Linearity graphs were drawn for NDMA, NDEA and NMPA in the range of QL to 150% of limit. Correlation coefficient value for NDMA, NDEA, and NMPA were derived from respective linearity graph

The results were given below.

Linearity for NDMA:

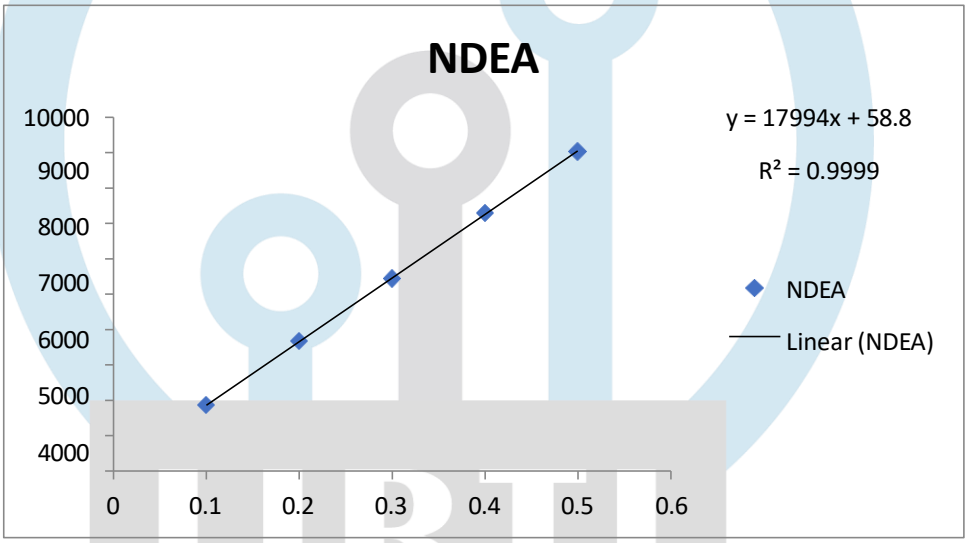
Level	Concentration of NDMA (ppm)	Average AreasofNDMA
Level-1	0.1	1151
Level-2	0.2	2351
Level-3	0.3	3512
Level-4	0.4	4662
Level-5	0.5	5853
Correlation Coefficient		**0.999**

Linearity Graph for NDMA:



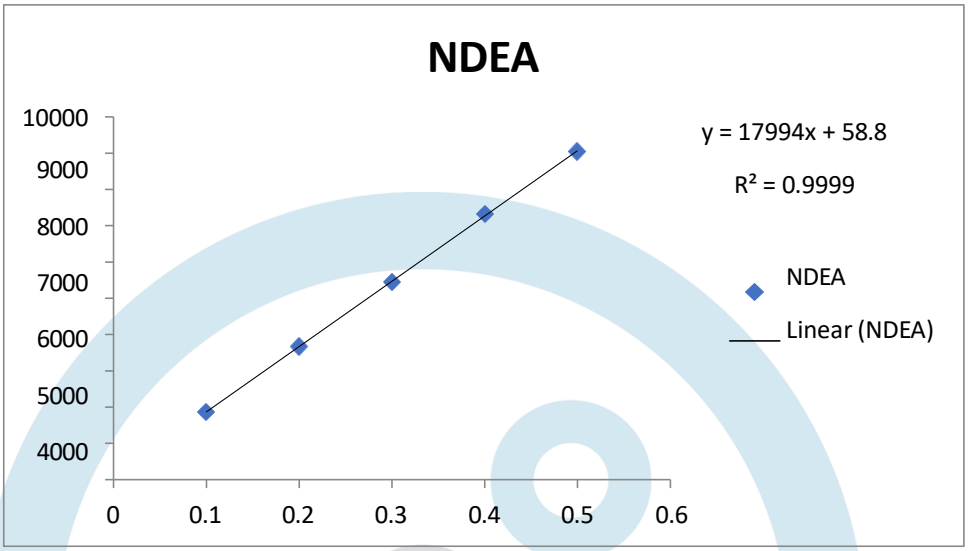
Linearity for NDEA:

Level	Concentration of NDEA (ppm)	Average Areas ofNDEA
Level-1	0.1	1852
Level-2	0.2	3665
Level-3	0.3	5437
Level-4	0.4	7299
Level-5	0.5	9032
Correlation Coefficient		**0.999**



Linearity for NMPA:

Level	Concentration of NDIPA (ppm)	Average Areas of NDIPA
Level-1	0.1	9558
Level-2	0.2	19136
Level-3	0.3	28554
Level-4	0.4	38292
Level-5	0.5	47450
Correlation Coefficient		**0.999**

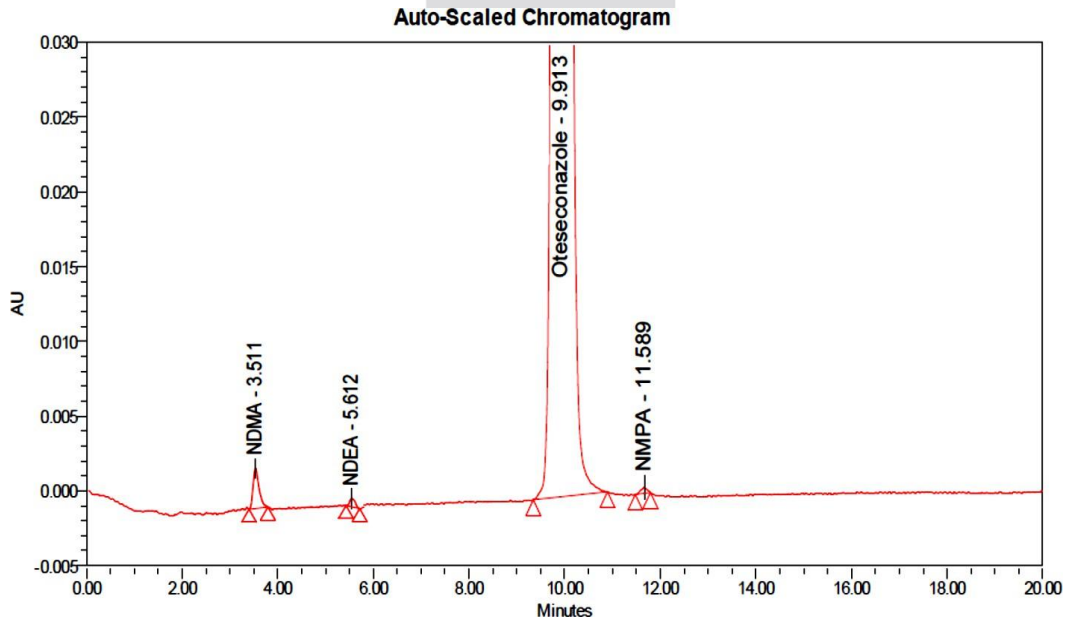


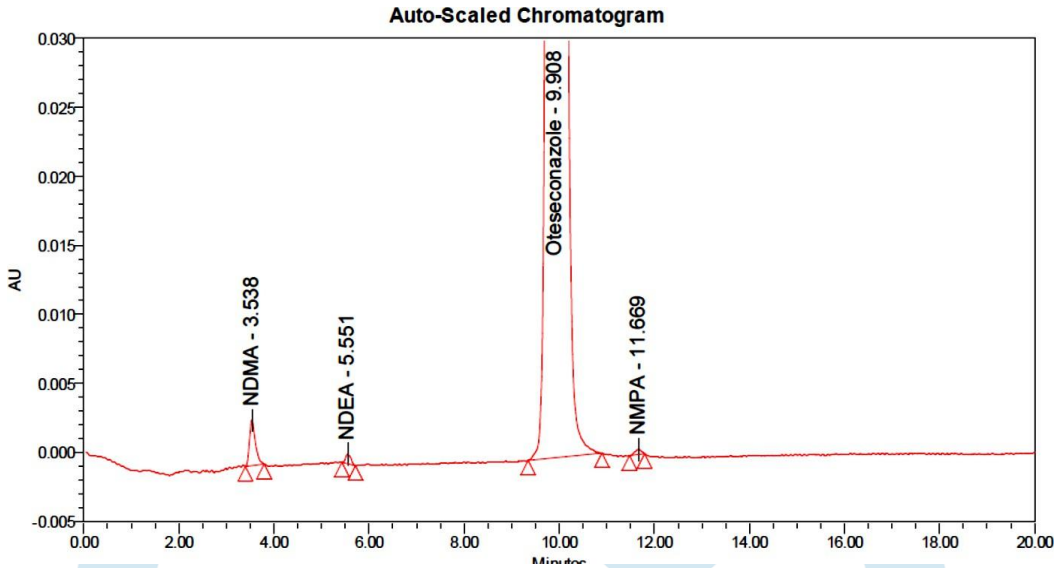
Accuracy:

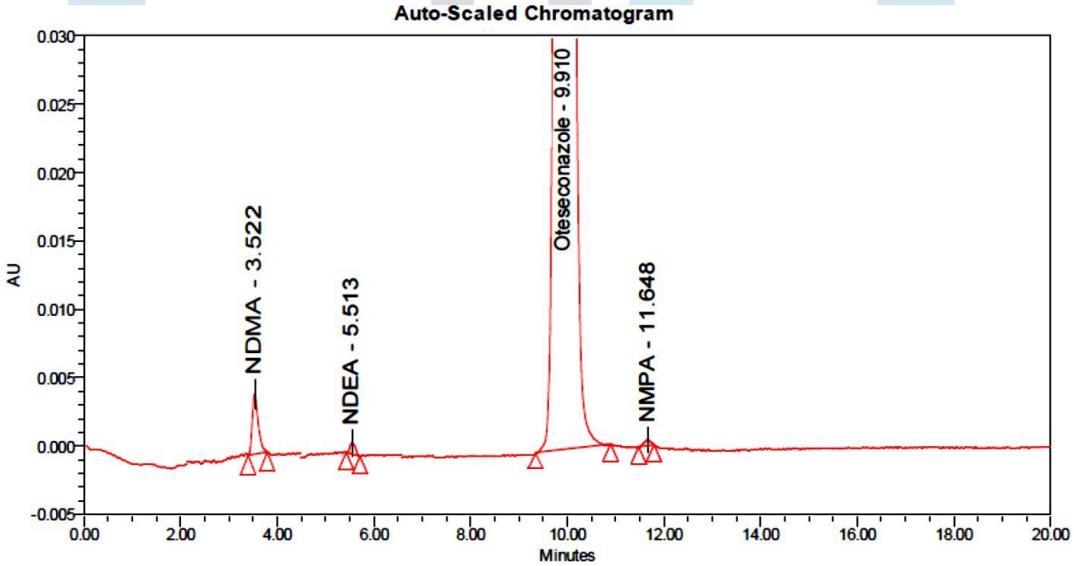
Accuracy of the method were proved by checking the % recovery of NDMA, NMPA, and NMPA.

Test solutions were spiked with NDMA, NDEA and NMPA at 50%, 100% and 150% level. Injected each Accuracy at 50% and 150% levels in triplicate preparations and Accuracy at 100% level in six preparations.

Accuracy level	Preparations	% Recovery of NDEA	% Recovery of NDEA	% Recovery of NDEA
50%	1	104.1	105.6	114.4
100 %	1	107.7	107.1	117.1
150%	1	107.0	106.9	113.7







Precision:

Method Precision:

The drug Substance test sample was spiked with NDMA, NDEA and NMPA at Specification level with respect to the test sample concentration injected six different preparations and analyzed for the method precision study as per the procedure mentioned in the protocol.

The results were as follows:

Summary results of Method Precision:

Method Precision	NDMA (ppm)	NDEA (ppm)	NDIPA (ppm)
Preparation-1	0.336	0.333	0.343
Preparation-2	0.331	0.329	0.339
Preparation-3	0.328	0.317	0.322
Preparation-4	0.335	0.320	0.324
Preparation-5	0.335	0.308	0.323

Preparation-6	0.340	0.337	0.333
Average	**0.334**	**0.324**	**0.331**
% RSD	**1.3**	**3.4**	**2.7**

Intermediate Precision:

Established the precision study on different day with different analyst and with freshly prepared solutions.

System Suitability:

Injection No.	**Peak Areas of NDMA**	**Peak Areas of NDEA**	**Peak Areas of NDIPA**
1	3509	5468	28612
2	3525	5046	28052
3	3067	5942	27972
4	3651	5035	27243
5	3262	5531	35965
6	3692	5528	27025
Average	**3451**	**5425**	**29144.83**
% RSD	**6.4**	**5.76**	**10.62**

Summary Results of Intermediate Precision:

Intermediate Precision	**NDMA (ppm)**	**NDEA (ppm)**	**NDIPA (ppm)**
Preparation-1	0.319	0.323	0.324
Preparation-2	0.317	0.321	0.323
Preparation-3	0.316	0.336	0.299
Preparation-4	0.319	0.314	0.321
Preparation-5	0.321	0.333	0.317
Preparation-6	0.313	0.302	0.310
Average	**0.318**	**0.322**	**0.316**
% RSD	**0.9**	**3.9**	**3.0**

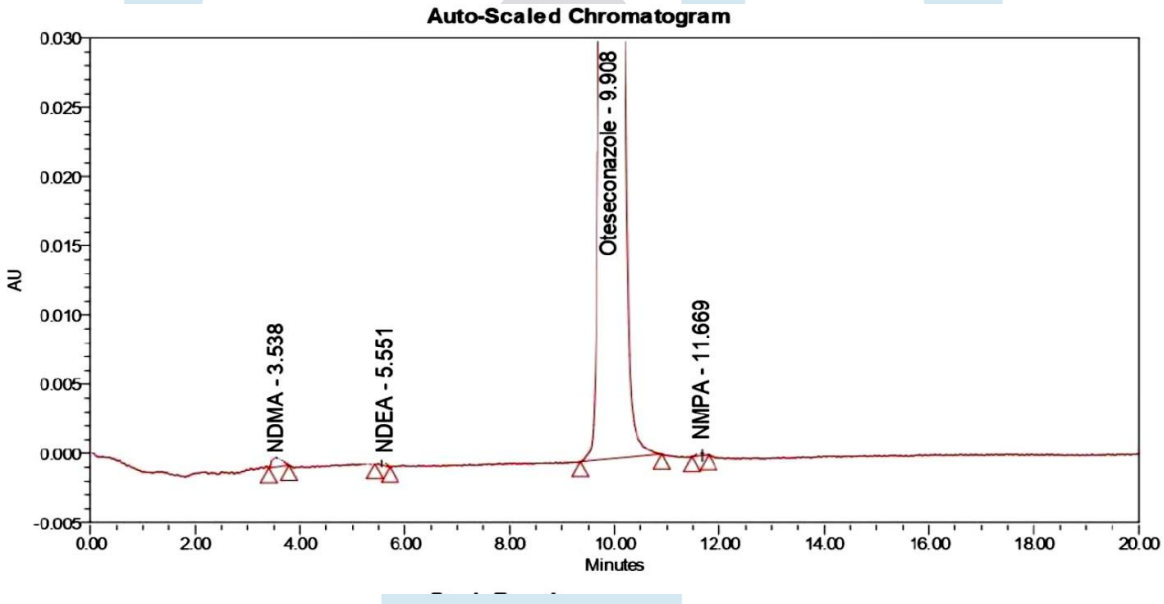
Limit of Detection or Detection Limit (DL):

Detection limit have been established for NDMA, NDEA and NMPA. DL solution were prepared. DL solution of NDMA, NDEA and NMPA peak were finalized by using visual method.

The results were given below.

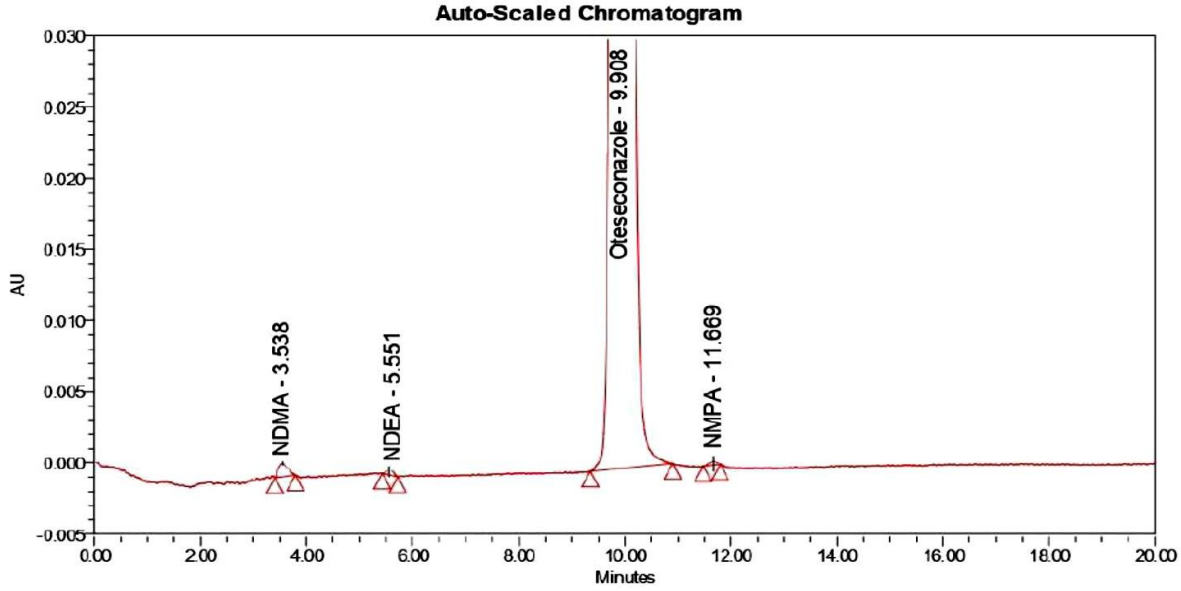
DL Results:

Name of the Compound	Conc. w.r.t. Test (ppm)
NDMA and NMPA	0.03
NDEA	0.02



Limit of Quantitation or Quantitation Limit (QL):

Quantitation limit have been established for NDMA, NDEA and NMPA Based on the concentration obtained from DL solutions, the QL solutions were prepared. QL solution of Drug Substance peak were finalized by using visual method. The results were given below.



QL Results:

Name of the Compound	Conc. w.r.t. Test (ppm)
NDMA and NMPA	0.09
NDEA	0.06

Conclusion:

Above observations indicate that the HPLC method meets the acceptance criteria for the parameters selected for validation study. Hence, the method is suitable for the determination of NDMA, NDEA, NMPA in Drug Substance by HPLC

References:

- Mark G Martens 2022Phase 3 study evaluating the safety and efficacy of oteseconazole in the treatment of recurrent vulvovaginal candidiasis and acute vulvovaginal candidiasis infections
- Egla Hasimllari August 8, 2022 VIVJOA (Oteseconazole) to Treat Recurrent vulvovaginal candidiasis, USA Drug Topics Journal
- S J Gerberding 11998 May 29 Preparative ion-exchange chromatography of proteins from dairy whey. NIH.
- Jack D. Sobel, M.D. , . Efficacy and Safety Efficacy and Safety of Oteseconazole in Recurrent Vulvovaginal Candidiasis. Published July 26, 2022jounarl NEJM
- Mark G.Martens,MD,july 18,2022.Phase 3 study evaluating the safety and efficacy of oteseconazole in the treatment recurrent vulvovaginal candidaisis and acute vulvovaginal candidiasis infenctions, AJOG
- List of Classifications, Volumes 1-115. IARC Monographs on the Evaluation of Risk to Humans. International Agency for Research on Cancer. February 22, 2016. Archived from the original on June 10, 2017. Retrieved July 26, 2016.