e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

IDENTIFICATION OF RP-HPLC METHOD FOR THE DETERMINATION OF GENOTOXIC IMPURITIES IN PALAVERATONE

Nagam Anusha*1, M. Ajitha*2

*1,2Department Of Pharmaceutical Analysis, Centre For Pharmaceutical Sciences, UCESTH, JNTUH, India.

DOI : https://www.doi.org/10.56726/IRJMETS63717

ABSTRACT

The study focused on assessing the applicability and precision of NDMA and NDEA using standard solutions. The %RSD for the peak locations were 5.14 and 2.40, respectively, remaining within the allowable limit of 15%. Specificity tests demonstrated no interference, and detection thresholds for NDMA and NDEA were established at 0.035 ppm, with a S/N ratio of 3:1. The quantitation limitations were set at 0.093 ppm, demonstrating precision %RSD values of 5.12 and 2.41. Investigations of linearity revealed correlation coefficients of 0.999 for both NDMA and NDEA. Accuracy evaluations at 50%, 100%, and 150% levels exhibited recovery rates between 70% and 130%. The investigation into method precision and intermediate precision confirmed the reliability of the analysis, with %RSD values being within acceptable limits.

Keywords: NDMA, NDEA, Quantitation limit, Standard Solutions, %RSD, Peak Location.

I. INTRODUCTION

Nitrosamine

Nitrosamines, or N-Nitrosamines, are organic compounds characterized by the chemical formula R2N−N=O, with R often representing an alkyl group. They comprise a Nitroso group (NO+) linked to a de-protonatedamine.

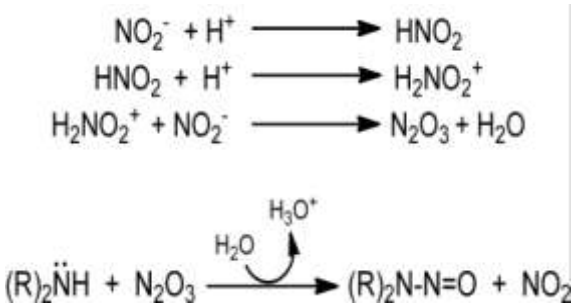

Figure 1: N-nitrosamine formation

AIM & PLAN OF WORK

Objective:

Development & validation of a HPLC technique for the quantification of NDMA and NDEA in PAE.

Plan of work:

1. Determination of chromatographic parameters (mobile phase, column, flow rate, etc.).
2. Optimization of the methodology.
3. Estimation of Selected Impurities in Conjunction with the Drug Molecule.
4. Selection of an Appropriate Method for Impurity Estimation.
5. Validation of the suggested RS methodology
6. Implementing the established approach on the commercial formulation, including impurities.

II. EXPERIMENTAL METHODOLOGY

Content Name:

1. N-Nitrosodimethylamine(NDMA)
2. N-Nitrosodiethylamine(NDEA)

Limit (ppm): 0.4 ppm

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Chemicals:

NDMA and NDEA: Chromatographic grade (or) equivalent

Chromatographic Conditions:

Instrument	: Waters-Make, 2695 seperation Module.
Column	: Platsil C_{18}, 250 x 4.6 mm, 5 µm or equivalent
Flow rate	: 1.0 mL/min
Injection volume	: 20µL
Column temperature	: N/A
Wavelength	: 296 nm
Auto sampler temperature	: 5°C
Elution mode	: Isocratic
Buffer	: KH2PO4 BUFFER FILTER THROUGH 0.22 µm membranefilter paper.
Solution A	: Buffer
Solution B	: ACN
Mobile Phase	: Solution A: Solution B (10:90) v/v
Run time	: 15 min
Rinse solution	: Methanol
Diluent	: ACN

Blank Solution Preparation: Transfer 1.0 mL of diluent into a vial and promptly seal the container.

Making of NDMA and NDEA Standard Solution I:

Transfer 20 mg of NDMA and NDEA standard solutions into a 20 mL VF containing 5 mL of diluent, then fill to the margin with diluent. Transfer 4 mL of the aforementioned solution into a 100 mL VF containing 25 mL of diluent & fill to the mark diluent.

Making of Standard Solution (0.4 ppm):

Take out 1.0 mL of standard solution-I into a 100 mL VF containing 25 mL of diluent and fill to the mark with diluent.

Transfer 1.0 mL of the standard solution into a vial and inject it into the HPLC system.

Making of the test sample: Accurately weigh approximately 10 mg of the test sample into a 10 mL volumetric flask. Add 7.0 mL of diluent, then dilute the solution to a final volume of 10 mL, achieving a concentration of 1 mg/mL.

Additionally Transfer 0.1 ml from the aforementioned solution and dilute with the same solution (0.01 mg/ml).

Procedure: Equilibrate the column for 16 minutes, inject the blank solution, and record the chromatogram. Adjust the data processor to ignore blank peaks. Inject standard solutions into the HPLC individually, six times, and document the chromatograms. Confirm system suitability criteria are met, then proceed.

III. PARAMETERS OF VALIDATION & PROCEDURE

3.1 SST / System Precision:

Established SST/precision by injecting standard solutions six times & calculating %RSD for NDMA & NDEA peak areas.

SST /System Precision Results:

Injection No.	Areas of NDMA	Areas of NDEA
1	36150	6688
2	35218	6529
3	35456	6432
4	38121	6726

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

5	39501	6560
6	34729	6874
Average	**36529.17**	**6634.833**
% RSD	**5.14**	**2.40**

Observation:

The %RSD for NDMA and NDEA peak areas from six injections were 5.14 and 2.40, respectively.

3.3 SPECIFICITY:

Conducted the specificity study for NDMA and NDEA.

Observation:

No interference was observed from the blank at the retention time of NDMA and NDEA.

3.3 Detection Limit (DL):

DL for NDMA and NDEA were established and solutions prepared, with DL peaks finalized using a visual method.

The Results were given below.

DL Outcomes:

Compound Name	Test Conc. w.r.t. (ppm)
NDMA & NDEA	0.035

Observation:

Quantitation limit for NDMA and NDEA impurity peak were visualized.

Acceptance limitations:

The S/N ratio should be about 3:1 (or) report the DL level values by visual method.

3.4 Quantitation Limit (QL):

QL for NDMA and NDEA were established and solutions prepared based on DL concentrations. QL peaks were finalized using a visual method; results are as follows.

QL Outcomes:

Compound Name	Test Conc. w.r.t. (ppm)
NDMA & NDEA	0.093

Observation:

Quantitation limit for NDMA and NDEA impurity peak were visualized.

Acceptance limitation:

The S/N ratio should be about 10:1 (or) report the QL level values by visual method.

Precision at QL:

QL solutions were injected six replicates (six) times & calculated % RSD for NDMA and NDEA.

The results were summarized table.

Outcomes for Precision at QL Level

QL Level	Peak Areas of NDMA	Peak Areas of NDEA
Inj-1	36146	6682
Inj-2	35258	6535
Inj-3	35472	6445
Inj-4	38124	6756
Inj-5	39510	6543

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Inj-6	34749	6872
Average	**36543.17**	**6638.833**
SD	**1869.60**	**159.68**
%RSD	**5.12**	**2.41**

The results were given table.

3.5 % Recoveries at QL level:

	Preparation	**%Recovery of NDMA**	**%Recovery of NDEA**
QL level	1	98.2	109.7
	2	102.5	115.2
	3	102.1	114.1

The percentage recovery of NDMA and NDEA at the quantitation limit was within acceptable parameters.

3.6 Linearity for NDMA:

Level	**Concentration of NDMA (ppm)**	**Average Areas of NDMA**
1-Level	0.2	39654
2-Level	0.3	79327
3-Level	0.4	119021
4-Level	0.5	158655
5-Level	0.6	198268
Correlation Coefficient		**0.999**

Linearity Graph for NDMA:

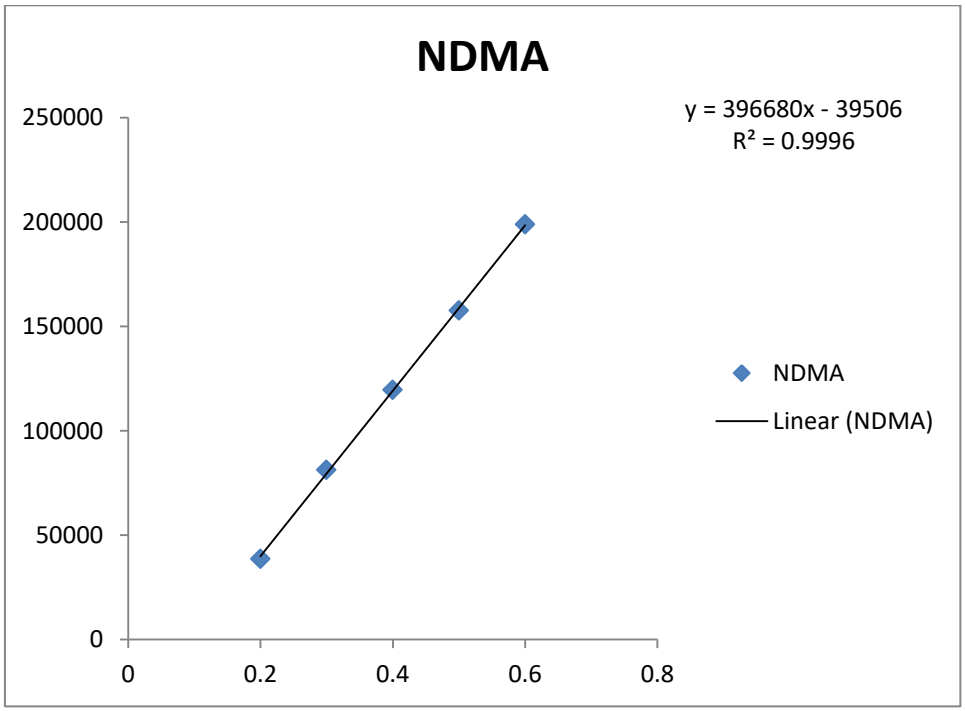

Figure 2: Linearity of NDMA

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

Linearity for NDEA:

Level	Concentration of NDEA (ppm)	Average Areas of NDEA
1-Level	0.2	26832
2-Level	0.3	53852
3-Level	0.4	80772
4-Level	0.5	107610
5-Level	0.6	136645
Correlation Coefficient		**0.999**

Linearity Graph for NDEA

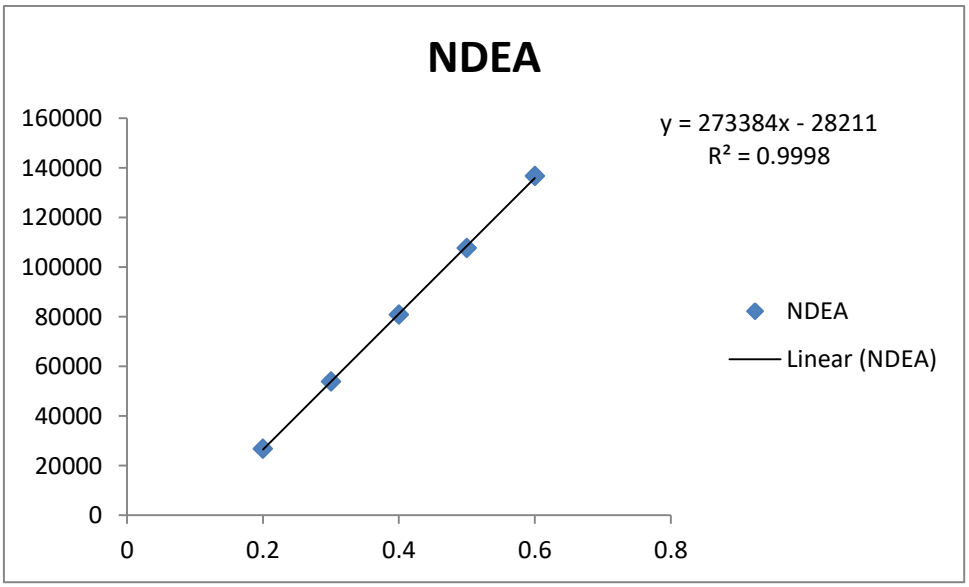

Figure 3: Linearity of NDEA

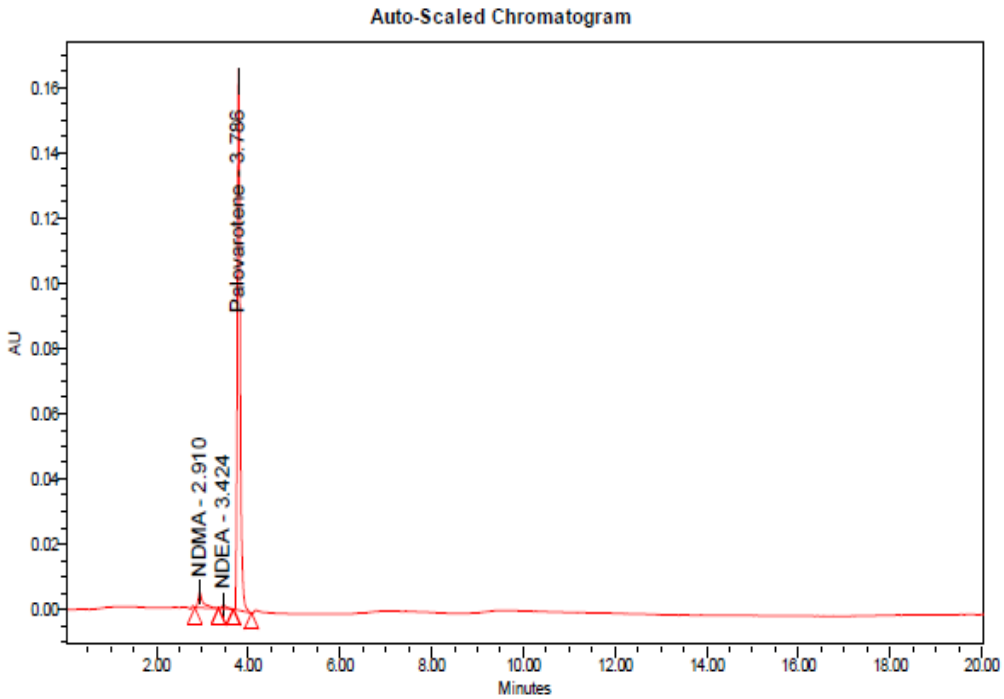

Figure 4: Linearity-1

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

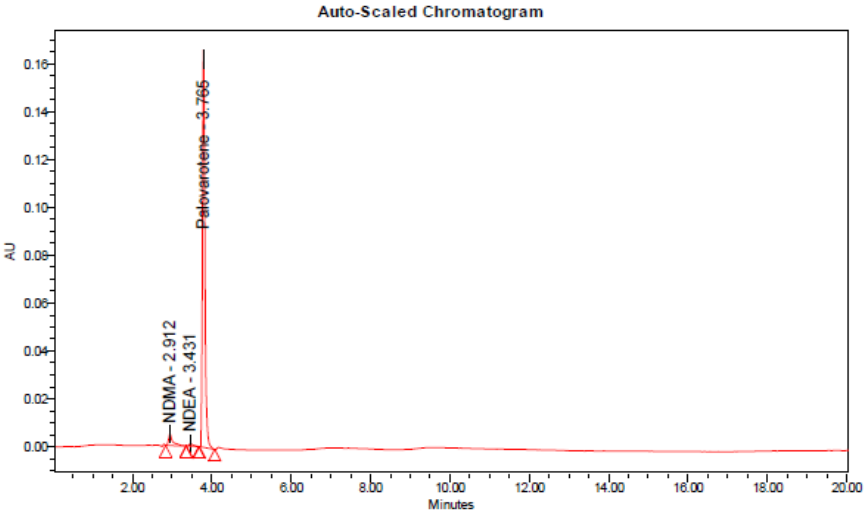

Figure 5: Linearity-2

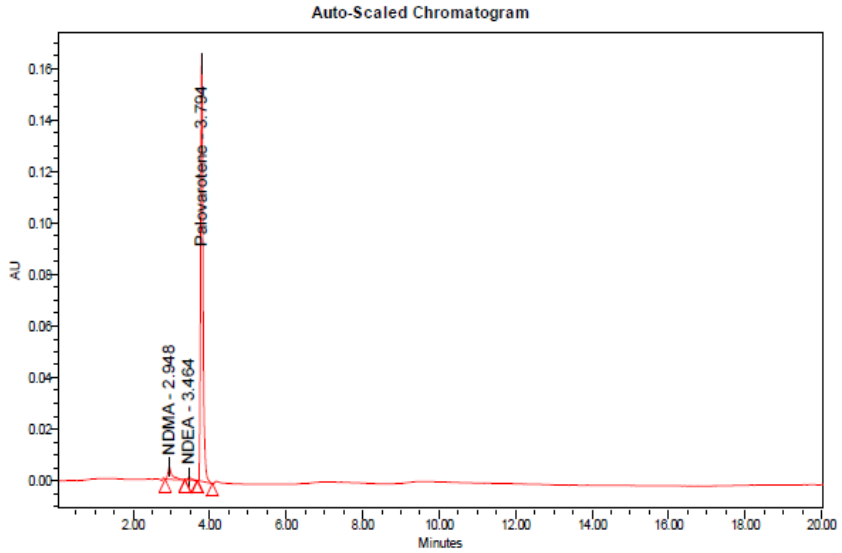

Figure 6: Linearity-3

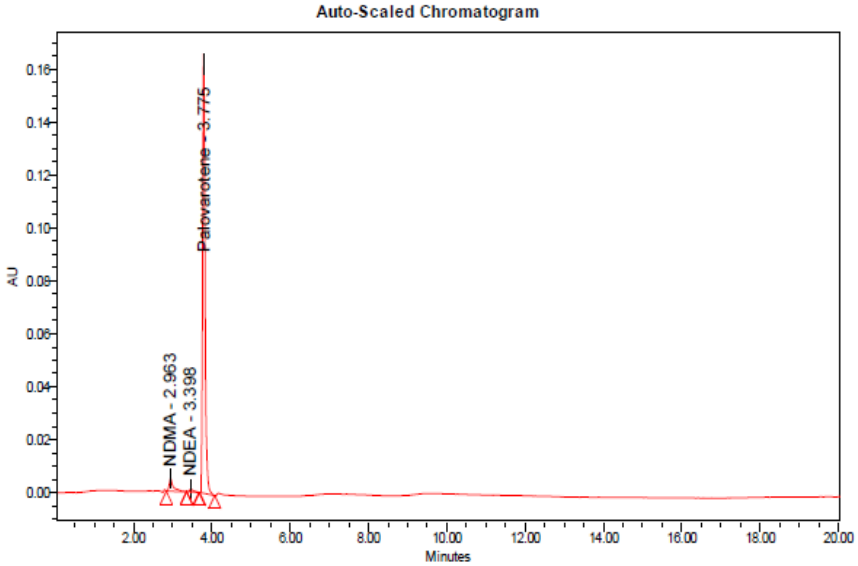

Figure 7: Linearity-4

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

% recoveries:

Accuracy level	Preparations	% Recovery of NDMA	% Recovery of NDEA
50%	1	102.1	100.4
100 %	1	100.7	100.2
150%	1	101.3	101.7

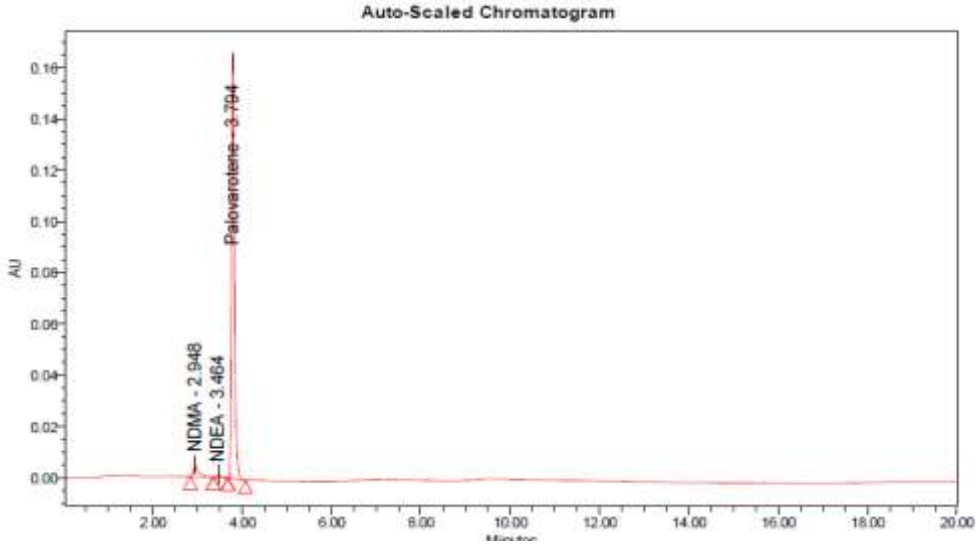

Figure 8: 150%

3.7 Method Precision:

Method Precision	NDMA (ppm)	NDEA (ppm)
Preparation-1	0.432	0.442
Preparation-2	0.441	0.436
Preparation-3	0.421	0.422
Preparation-4	0.410	0.425
Preparation-5	0.401	0.429
Preparation-6	0.408	0.433
Average	0.42	**0.43**
% RSD	**3.67**	**1.71**

System Suitability:

Injection No.	Peak Areas of NDMA	Peak Areas of NDEA
1	36820	6678
2	31885	6959
3	39124	6146
4	35895	6526
5	37052	6369

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

6	32386	6520
Average	**35527.00**	**6533.00**
% RSD	**7.98**	**4.22**

3.8 Intermediate Precision:

Intermediate Precision	NDMA(ppm)	NDEA (ppm)
Preparation-1	0.425	0.421
Preparation-2	0.412	0.425
Preparation-3	0.422	0.400
Preparation-4	0.411	0.429
Preparation-5	0.420	0.416
Preparation-6	0.411	0.405
Average	0.42	0.42
% RSD	1.50	2.75

Summary of cumulative %RSD results for method precision and intermediate precision.

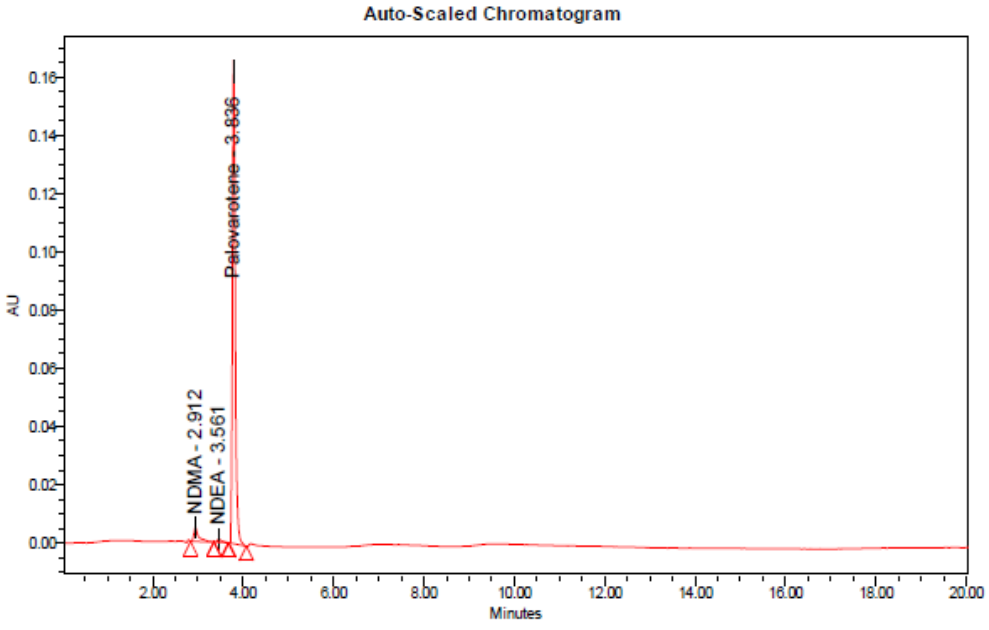

Figure 9: Method Precision and Intermediate Precision

IV. CONCLUSION

Overview:

The study assessed system applicability and precision for NDMA and NDEA, attaining acceptable %RSD values. Detection and quantification limits were determined, with linearity verified up to 150%. Accuracy assessments indicated percentage recoveries within permissible thresholds. The method and intermediate precision assessments confirmed the analysis's dependability.

Conclusion:

The aforementioned data demonstrate that the HPLC technique satisfies the acceptance requirements for the parameters chosen for the verification research. Therefore, the approach is appropriate for the quantification of NDMA and NDEA in Drug Substance using HPLC.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:06/Issue:11/November-2024 Impact Factor- 8.187 www.irjmets.com

V. REFERENCES

[1] Beard JC, Swager TM. N-Nitrosamines: their structure, reactivity, and role as contaminants: an organic chemist's guide. Journal of Organic Chemistry. 2021;86(3):2037-57.
[2] Yang CS, Yoo JS, Ishizaki H, Hong JY. Roles in nitrosamine metabolism and mechanisms of regulation: cytochrome P450IIE1. 1990;22(2-3):147-59. Drug Metab Rev. 3, Barnes JM, Magee PN. Some of the toxic properties of dimethylnitrosamine. British Journal of Indian Medicine. 1954;11(3):167-74.
[3] Barnes JM, Magee PN. "The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine." Br J Cancer. 1956;10(1):114-22. doi: 10.1038/bjc.1956.15, [PMID: 13342328].
[4] Scanlan RA. The relationship between malignancy and nitrosamines. "Nefab.com." Obtainable at: https://www.nefab. com/globalassets/sweden/products/4.-inneforpackningar/vci/nitrosamines-andcancer.pdf.
[5] Crossby NT, Sawyer R. N-Nitrosamines: a review of their chemical and biological properties and their determination in foods. Advances in Food Research. 1976;22:1-71.
[6] M. Iram. The history of nitrosamines: from valsartan to ranitidine. Indian Journal of Pharmacology, (Pr.), 2019, 13(1): 01-2.
[7] Scanlan R.A., Issenberg P. N-nitrosamines in foods. 5: 357–402. CRC Crit. Rev. Food Sci. Nutr. (1975).
[8] Cho, I., and Bratzler, L. The impact of sodium nitrite on the flavor of cured pork. (1970);35:668–670. Journal of Food Science.
[9] Walker, R. Nitrates, nitrites, and N-nitrosocompounds: a review of their toxicological implications and abundance in food and nutrition. (1990);7:717–768. Food Addit. Contam.
[10] K. Rostkowska, K. Zwierz, A. Różański, J. Moniuszko-Jako-niuk, and A. Roszczenko. The formation and metabolism of N-nitrosamines. Polish Journal of Environmental Studies, 7, 321–325, 1998.
[11] Gray J.I., Dugan L.R., Jr. The prevention of the formation of N-nitrosamine in food systems that serve as models. J. Food Sci. (1975);40:981–984.
[12] Douglas M.L., Kabacoff B.L., Anderson G.A., and Cheng M.C. The chemistry of the formation, inhibition, and annihilation of nitrosamine. J. Soc. Cosmet. Chem. 29:581–606.
[13] Mirvish S.S., Wallcave L., Eagen M., Shubik P. The ascorbate-nitrite reaction: a potential method for inhibiting the formation of carcinogenic N-nitroso compounds. Science. (1972);177:65–68.
[14] Sen N.P., Smith D.C., Schwinghamer L. The formation of N-nitrosamines from secondary amines and nitrite in the gastric fluid of humans and animals. Food and Cosmetic Toxicology, 1969, 7, 301-307.