Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2025, 17(2): 634-639
(http://www.derpharmachemica.com/archive.html)

A Comprehensive Approach for the Determination and Quantification of N-Nitroso Desmethyl Olopatadine in the Olopatadine Hydrochloride Ophthalmic Solution by RP-HPLC

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Received: 30-September-2024, Manuscript no: DPC-25-164834, **Editor assigned:** 03-October-2024, Pre QC No: DPC-25-164834 (PQ), **Reviewed:** 17-October-2024, QC No: DPC-25-164834, **Revised:** 01-April-2025, Manuscript No: DPC-25-164834 (R), **Published:** 28-April-2025, DOI: 10.4172/0975-413X.17.2.634-639

ABSTRACT

A High-performance liquid chromatography (HPLC) method was developed to quantify N-Nitroso Desmethyl Olopatadine impurity in an olopatadine hydrochloride ophthalmic solution. Chromatographic separation was achieved using a Poroshell 120l Phenyl Hexyl (150 × 4.6 mm, 2.7 µ) column with Mobile phase-A (1.36 g/L Potassium hydrogen phosphate pH-3.0): Acetonitrile (95:5 v/v) and mobile phase-B (Acetonitrile: water, 98:2 v/v) in gradient elution mode at a 0.8 mL/min flow rate. Quantification was performed using PDA/UV-visible detection; the method was validated with good linearity over the concentration range of 0.27 ppm-4.05 ppm. The correlation coefficient obtained in each case was >0.99. The recoveries were found to be satisfactory at the level of LOQ over the range between 70.0% to 130.0% and for the other levels between 80 %-120 % for N-nitroso desmethyl olopatadine. The developed method was able to quantitate N-nitroso desmethyl olopatadine at a concentration level of 0.27 ppm for 400 ppm olopatadine hydrochloride.

Keywords: Olopatadine Hydrochloride; HPLC; N-Nitroso Desmethyl; Nitrosamine; Validation

INTRODUCTION

N-nitrosamines are a class of molecules notable for the powerful carcinogenicity of several of its members and their pervasive prevalence throughout the human environment, from air and water to our meals and pharmaceuticals. Although a lot of work has gone into comprehending N-nitrosamines as pollutants, there are still issues with prevention, detection and remediation techniques. Addressing these issues will require an understanding of N-nitrosamine chemistry1. N-nitroso compounds have been listed as one of the cohorts of concern as per the ICH M7 guidance and are internationally considered a class of strong carcinogens by the International Agency for Cancer Research. The issue of nitrosamine impurities was first reported in the industry in 2018 by the FDA and EMA in certain classes of drugs specifically sartan 3, 4 series of active pharmaceutical ingredients. In recent years' regulatory agencies have shifted the focus from common nitrosamine impurities from the initial phase of nitrosamine impurities assessment formed due to reagents/solvents to nitrosamine impurities of drug products (NDSRI's). Thus N-nitrosamine risk assessment of pharmaceuticals moved from small molecules of N-Nitrosoamine to Nitrosamine Drug Substance Related Impurities (NDSRI)4.
Olopatadine hydrochloride (structure-1) is an antihistamine medication used to decrease the symptoms of allergic conjunctivitis and allergic rhinitis. It is an active pharmaceutical ingredient of an approved drug by the USFDA. It is usually used in finished formulations as an ophthalmic solution as eye drops or as a nasal spray. As per the latest guidance by EMA5 and USFDA6 the N-nitroso desmethyl olopatadine (Structure 2) was reported as a possible nitrosamine impurity due to the desmethyl intermediate formed during the manufacturing process of Olopatadine Hydrochloride as an active pharmaceutical ingredient. The risk assessment of olopatadine hydrochloride ophthalmic solution's manufacturing process, in accordance with the guidance carcinogenic potency categorization approach, considers all the variables or risk elements that may affect the development of N-nitrosamine impurities, including the drug substance used in manufacturing, material quality (risk of nitrosamine/nitrosating agent presence in excipients, solvents, etc.), cleaning methods, water treatment, solvent treatment (recovery/recycling), primary packaging materials and the kind of manufacture (dedicated/multipurpose). The appropriate level of specification for the impurity was set based on dosage and duration of treatment of the drug product under ICH M7 guidelines and declared permissible daily exposure. N-nitroso desmethyl olopatadine impurity falls in the category 4 as per the Carcinogenic Potency Categorization Approach (CPCA) with an Acceptable Intake (AI) of 1500 ng/day [1]. Based on the maximum

daily dose of 0.22 mg/day, N-nitroso desmethyl olopatadine needs to be controlled at 6818 ppm in olopatadine hydrochloride ophthalmic solution10.

The aim of the study was to develop cost-effective RP-HPLC method that can be able to quantitate N-nitroso desmethyl olopatadine in the olopatadine hydrochloride ophthalmic solution (Figures 1 and 2).

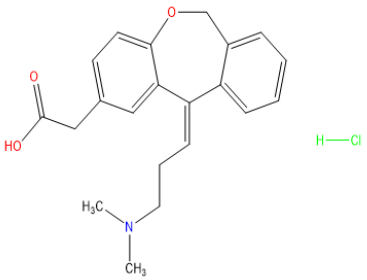

Figure 1: Olopatadine hydrochloride.

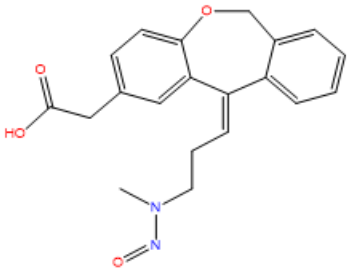

Figure 2: N-Nitroso desmethyl olopatadine.

MATERIALS AND METHODS

Reagents and chemicals

Potassium dihydrogen phosphate and ortho phosphoric acid (Emparta/ACS, Merck), acetonitrile and methanol HPLC (Gradient, Rankem), ultra-pure Milli-Q water, Merck Millipore. Standard of N-nitroso desmethyl olopatadine (Mixture of isomers) procured from Pure synth research chemicals and sample of olopatadine ophthalmic solution prepared at drug delivery research laboratory at USV Pvt Ltd, India.

Instrument

High-performance liquid chromatograph, Waters Alliance e2695-Separation modules equipped with 2498 PDA/2489 UV/Visible detector with empower3 software was used (Waters, USA). Analytical Balance (Model: XS205, Mettler Toledo), micro balance (Model: XP2U, Mettler Toledo), pH meter (Model: S210, Mettler Toledo).

Chromatographic conditions

The analytical column used in the HPLC study was Poroshell 120 phenyl hexyl, (150 × 4.6mm, 2.7 µ) (Agilent, USA), Mobile phase-A (buffer (1.36 g/L Potassium hydrogen phosphate, pH-3.0 with ortho phosphoric acid in water): Acetonitrile 95:5 mL v/v) and mobile phase-B (acetonitrile: water, 98:2 mL v/v) at a flow rate of 0.8 mL/ min. Column oven temperature was maintained at 35°C. Injection volume was 20.0 µL. Detection wavelength 220 nm. The LC gradient program (Time / % mobile phase-A) was set as 0.00/69, 28.0/69, 38.0/32, 50.0/69 and 60.0/69 [2].

Analytical solution preparation

Diluent: 20% acetonitrile in water.

Standard and sample solution

The standard stock solution of N-nitroso desmethyl olopatadine (mixture) was prepared in methanol (68 ppm), which was further diluted in diluent to prepare a final concentration of 2.720 ppm. The target analytic concentration was fixed at 400 ppm.

Analytical method validation

The developed method was successfully validated as per ICH guidance in terms of specificity, repeatability, linearity, accuracy, limit of detection, limit of quantification, robustness and solution stability. The repeatability at the determined limit of detection and limit of quantification values was

verified experimentally by injecting the exact solutions six times. The linearity of the method was evaluated from five concentration levels between the LOQ and 150% level. Calculated the slope, intercept and regression coefficient values. The specificity of the developed method was assessed with Olopatadine hydrochloride. Accuracy of the method was calculated in triplicate at LOQ to 150% concentration level by the standard addition method. The recoveries and RSD values were calculated for the N-nitroso desmethyl olopatadine impurity in olopatadine hydrochloride ophthalmic solution. The robustness of the method was tested by altering the pH of buffer, column temperature and different lots of HPLC columns. Further, the analysis of the standard and sample solution at different intervals of time was compared against fresh samples to evaluate the solution stability [3-5].

Results and Discussion

Method development and optimization

The study aimed to develop a cost-effective RP-HPLC method that can quantify N-nitroso desmethyl olopatadine in the olopatadine hydrochloride ophthalmic solution. Columns were tested to obtain the most appropriate peak shape and separation by using typical BEH, C8 and C18 columns. The Isomer peaks of the N-nitroso desmethyl olopatadine impurity mixture was not separated properly and the olopatadine peak eluted very early. While on the phenyl column the Separation improved slightly but the overall separation and peak shape were not up to the mark for the impurity. Poroshell 120 Phenyl Hexyl, (150 × 4.6mm, 2.7 µ) column was found to be the most suitable with respect to peak retention, shape and separation, as well as the response of analytes. The mobile phase was operated in gradient mode using buffer 1.36 g/L Potassium dihydrogen phosphate, pH adjusted to 3.0 with ortho phosphoric acid in water. Mobile phase A a mixture of (Buffer: Acetonitrile 95:5 mL v/v) and mobile phase-B (Acetonitrile: water, 98:2 mL v/v). The flow rate of the mobile phase was maintained at 0.8 mL/min, with the column temperature set at 35°C. The retention times of N-nitroso desmethyl olopatadine (Peak 1) and N-nitroso desmethyl olopatadine (Peak 2) were observed to be about 16.661 and 19.379 min respectively and the peak corresponding to olopatadine hydrochloride was eluted at about 4.379 min. This RP-HPLC method can be used for the determination of N-nitroso desmethyl olopatadine in olopatadine Hydrochloride API with the same chromatographic condition using initial isocratic mode for up to 28 minutes. The representative chromatogram of Blank, Placebo and standard solution of N-nitroso desmethyl olopatadine test and spiked solution is given below Figure 3 [6].

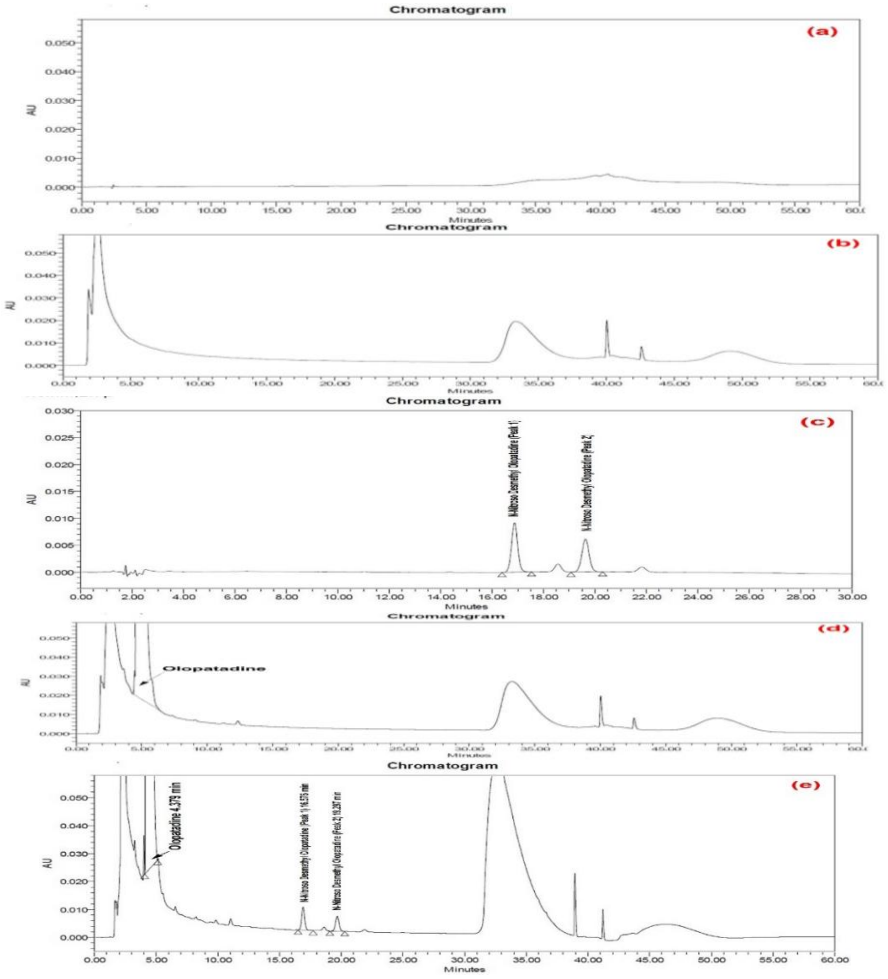

Figure 3: Chromatograph of a) Blank, b) Placebo, c) Standard, d) As such sample and e) Spike sample.

Specificity study

An individual solution of diluent, placebo, Standard, sample and spiked sample was assessed and the results revealed that there was no interference of the diluent, placebo and olopatadine Hydrochloride peak with N-nitroso desmethyl olopatadine peaks. As shown in Table 1. purity threshold is

greater than purity angle hence, the specificity of the developed analytical method was proven [7].

Table 1: Results of specificity study.

Description	Retention Time (minute)	Spectral purity	
		Purity 1 angle	Purity 1 threshold
Standard solution			
N-nitroso desmethyl olopatadine (Peak 1)	16.661	0.315	0.598
N-nitroso desmethyl olopatadine (Peak 2)	19.379	0.423	0.773
Spike sample			
N-nitroso desmethyl olopatadine (Peak 1)	16.576	0.619	1.324
N-nitroso desmethyl olopatadine (Peak 2)	19.297	0.949	1.826

Determination of LOD and LOQ values

The LOD and LOQ value of N-nitroso desmethyl olopatadine was determined based on the S/N ratio by injecting standard solutions of known concentrations and the repeatability at the LOD and LOQ value was calculated by analyzing three and six replicate injections of N-nitroso desmethyl olopatadine and calculating their % RSD values as shown in Table 2. The actual concentration of N-nitroso desmethyl olopatadine for LOD is 0.08 ppm absolute and 204 ppm w.r.t. sample and for LOQ is 0.27 ppm absolute and 680 ppm w.r.t. sample.

Table 2: Results of LOD and LOQ.

Components	N-Nitroso Desmethyl olopatadine (Peak 1)	N-Nitroso Desmethyl olopatadine (Peak 2)
Limit of detection		
S/N ratio	55 to 82	36 to 53
% RSD of sum of area of N-nitroso desmethyl olopatadine	2.01%	
Limit of quantification		
S/N ratio	77 to 120	51 to 78
% RSD of sum of area of N-nitroso desmethyl olopatadine	0.48%	

Linearity

Linearity of the method was studied by using the standard solution of N-nitroso desmethyl olopatadine at five different concentration levels from the 10% limit of quantification (0.272 ppm), 50% (1.360 ppm), 100% (2.720 ppm), 120% (3.264 ppm) and 150% (4.080 ppm) of the impurity plotted a graph of actual concentration in ppm *vs.* corresponding peak area response and calculated correlation coefficient, the slope of the regression line, Y-intercept and residual sum of squares for N-nitoso desmethyl olopatadine as shown in Figure 4 and Table 3.

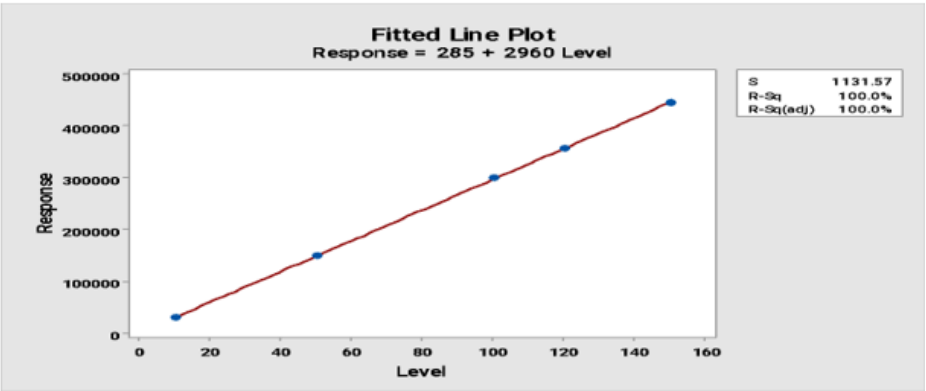

Figure 4. Linearity graph of N-nitroso desmethyl olopatadine solution.

Table 3: Results of linearity.

Correlation coefficient	1	(Limit: NLT 0.99)
Y-intercept	284.9306	To be reported only
The slope of regression line	43.8642	To be reported only
Residual sum of squares	3841365	To be reported only

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samples of a homogenous sample. It is a measure of either degree of reproducibility or repeatability of the method. The system and method precision for the N-nitroso desmethyl olopatadine were checked at its specification level (*i.e.*, 0.27 ppm with respect to analyte concentration, 400 ppm). The repeatability of the method has been checked by preparing six sample preparations as per method. The intermediate precision has been checked by comparing the results of six sample solutions prepared as per method of analysis on different days and by different analysts using different columns and on different HPLC instruments. The % RSD of method repeatability and system repeatability for the N-nitroso desmethyl olopatadine were reported (Table 4).

Table 4: Results of precision study.

Parameter	% RSD Sum of area of N-nitroso desmethyl olopatadine (Peak 1) and N-nitroso desmethyl olopatadine (Peak 2) peaks
System precision	0.68
Method precision	0.39
Intermediate precision	1.12

Accuracy

Accuracy of an analytical method expresses the closeness of agreement between the true value or an accepted reference value and the value found out on analysis as shown in table 5. The accuracy may be assessed, for quantitative purposes by spiking a known amount of impurity in a sample, at different levels and % recovery to be established. To study the accuracy, a known concentration of N-Nitroso Desmethyl Olopatadine solution spiked in the sample solution. Accuracy has been performed in triplicate for N-Nitroso Desmethyl Olopatadine w.r.t LOQ, 100% and 150% concentration levels (Table 5).

Table 5: Results of accuracy study.

Accuracy Level	% Recovery
LOQ	96 to 109
100%	100 to 102
150%	99 to 100

Robustness study

The robustness of an analytical procedure is defined as a measured of its capacity to remain unaffected by small but deliberate variation in procedural parameters. This was performed to establish the reliability of analysis to address deliberate variations in parameters such as Column Temperature, pH of Buffer and different lots of columns results as shown in Table 6.

Table 6: Results of robustness study.

Parameter	Set value	Varied value	% RSD N-nitroso desmethyl olopatadine	N-nitroso desmethyl olopatadine (ppm)
Column Temperature	35	30	0.2	6835
		40	0.3	6649
Buffer pH	3	2.8	0.6	6815
		3.2	0.2	6804
Different column Lots	NA	column-1	NA	6812
		column-2	NA	6867

Solution stability study

To establish the stability of the standard and sample solutions, the respective solutions were analyzed immediately after preparation and solutions at regular time intervals. The solutions were stored at room temperature. Calculated the percent change between the initial results and results at each time interval and no significant changes were observed in the concentration for N-nitroso desmethyl olopatadine. Which confirmed the stability of standard and sample solutions at least 58 hours and 48 hours respectively [8-10].

CONCLUSION

In this study, we have developed an HPLC method that is capable of quantifying N-nitroso desmethyl olopatadine in olopatadine Hydrochloride Ophthalmic solution. The method was validated as per ICH guideline recommendations and it was found to be specific and linear over the specified concentration range. The determined LOD and LOQ values for N-nitroso desmethyl olopatadine were set very low and well below that acceptable

limit. The standard and sample prepared in the analytical solution were found to be stable for at least 58 hours. and 48 hours respectively. The method was fully validated and presents good linearity, precision, accuracy, repeatability and robustness. Hence, this RP-HPLC method could be very useful for the determination of N-nitroso desmethyl olopatadine in olopatadine Hydrochloride pharmaceutical formulation and active pharmaceutical Ingredient during its manufacture product release and shelf-life studies.

DATA AVAILABILITY

All the data generated or analyzed during this study are included in this published article. All the data included in this manuscript have been generated at USV private limited and does not include any third-party data/analysis.

CONFLICTS OF INTEREST

There are no conflicts to declare. To the best of our knowledge, the contents of this manuscript do not conflict with any third-party rights/interests.

ACKNOWLEDGMENT

We acknowledge USV Private Limited, Management for support during the entire work of method development and method validation. The content and the information provided herein are proprietary and developed for USV private limited. Copying and circulation of the content are strictly prohibited.

REFERENCES

[1] Beard JC, Swager TM. J Org Chem. **2021**; 86(3): p. 2037-2057.
[2] Guideline IH. In International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva. **2014**. [Google Scholar]
[3] Nori LP, Rehana S, Penamacha A, et al. Int J Pharm Investig. **2023**; 13(4):680-687.
[4] Kay JE, Corrigan JJ, Armijo AL, et al. Cell Rep. **2021**; 34(11).
[5] Step. Committee for medicinal products for human use ICH M7(R2) guideline on assessment and control of DNA reactive (Mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. **2023**.
[6] Cber, C. M7(R2) Assessment and control of DNA reactive (Mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk guidance for industry ICH-multidisciplinary, **2023**.
[7] Pharma, D, Vele VT, More K, et al. Determination and quantification of an N-nitroso desmethyl olopatadine in the olopatadine hydrochloride active pharmaceutical ingredient by LC-MS/MS. **2024**; 16(4): p.362-366.
[8] Vikram HP, Kumar TP, Kumar G, et al. J Pharm Anal. **2024**; 14(5): p. 100919.
[9] Aboagye EA, Chea JD, Yenkie KM. Iscience. **2021**; 24(10).
[10] Rele RV. Pharm Lett. **2015**; 7(1): p. 258-262.