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Determination and Quantification of an N-Nitroso Desmethyl Olopatadine in the Olopatadine Hydrochloride Active Pharmaceutical Ingredient by LC-MS/MS

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ABSTRACT

A Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method was developed for the quantification of N-Nitroso Desmethyl Olopatadine in the Olopatadine Hydrochloride active pharmaceutical ingredient. Chromatographic separation was achieved using a phenyl hexyl column, with 2 mm ammonium formate in water and acetonitrile as mobile phase in gradient elution mode at a 0.5 ml/ min flow rate. Quantification was carried out using triple quadrupole mass detection with electrospray ionization in the multiple reaction monitoring modes. The method was validated with good linearity over the concentration range of 0.014 ppm-0.210 ppm. The correlation coefficient obtained in each case was >0.9990. The recoveries were found to be satisfactory over the range between 80.0 and 120.0% for N-Nitroso Desmethyl Olopatadine. The developed method was able to quantitate N-Nitroso Desmethyl Olopatadine at a concentration level of 0.14 ppm with respect to 0.5 mg mL^{-1} Olopatadine Hydrochloride.

Keywords: Nitrosamine; N-nitroso desmethyl olopatadine; LCMS/MS; Validation; Olopatadine hydrochloride

INTRODUCTION

N-nitroso compounds have been listed as one of the cohorts of concern as per the ICH M7 guidance and are internationally considered as a class of strong carcinogens as per the international agency for research on cancer. The issue of nitrosamine impurities first reported in the industry in 2018 by FDA and EMA in certain class of drugs specifically sartan 3, 4 series of active pharmaceutical ingredients. From the initial phase of nitrosamine impurities assessment formed due to reagent and solvents, in recent years regulatory agencies has shifted the focused from common nitrosamine impurities to nitrosamine impurities of drug products. Thus N-nitrosamine risk assessment of pharmaceuticals moved from small molecules N-Nitrosoamine to Nitrosamine Drug Substances Related Impurities (NDSRI).

Olopatadine Hydrochloride (trade name Patanol) 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 USFDA. It is usually used in finished formulation as ophthalmic solution as eye drops or as a nasal spray.

As per the latest guidance by EMA7 and USFDA8 the N-Nitroso Desmethyl Olopatadine (Figure 1) was reported as possible nitrosamine impurity due to desmethyl intermediate formed during the manufacturing process of Olopatadine Hydrochloride as an active pharmaceutical ingredient. As per the guidance carcinogenic potency categorization approach, N-Nitroso Desmethyl Olopatadine impurity falls in category 5 with Acceptable Intake (AI) 1500 ng/day. Based on the maximum daily dose of 5.32 mg/day, N-Nitroso Desmethyl Olopatadine need to control at 282 ppm in Olopatadine Hydrochloride [1-3].

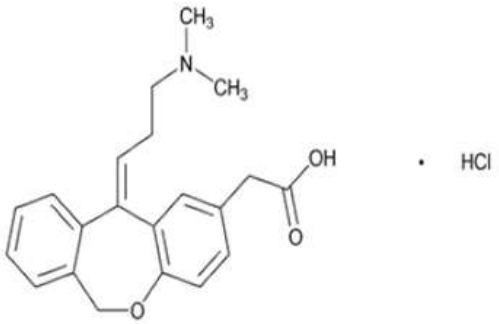

Figure 1: Chemical structure of Olopatadine hydrochloride.

We have developed a LC-MS/MS method for the quantification of the N-Nitroso Desmethyl Olopatadine as nitrosamine impurity in Olopatadine Hydrochloride active pharmaceutical ingredient. The method was further validated with respect to the specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, repeatability, accuracy and robustness, in accordance with ICH guidance.

MATERIALS AND METHODS

Reagents and chemicals

LCMS grade ammonium formate, formic acid and acetonitrile procured from Fischer scientific and J.T. Baker, USA respectively. LCMS grade water used was from J.T. Baker. Sample of Olopatadine Hydrochloride and standard of N-Nitroso Desmethyl Olopatadine were synthesized and analyzed at FDC LTD Pharm., India.

Preparation of sample and standard solutions

The stock solution of the N-Nitroso Desmethyl Olopatadine and Olopatadine Hydrochloride were prepared individually by dissolving an appropriate amount of the substances in diluent. For quantitation of N-Nitroso Desmethyl Olopatadine in Olopatadine Hydrochloride a solution of 140.0 ng mL^{-1} (0.140 ppm) concentration was used. The target analytic concentration was fixed as 0.5 mg mL^{-1}.

Chromatographic conditions of LC-MS/MS

Analysis was performed on Nexa Shimadzu UPLC system equipped with a binary pump and an auto sampler and Sciex 5500+LC-MS/MS Triple Quad with an electrospray ionization interface. The analytical column used in the LCMS/MS study was Phenyl Hexyl, (100 × 4.6 mm, 2.1 µm) (Phenomenex, USA) employed in gradient mode using 2 mm ammonium formate, pH adjusted to 3.0 with formic acid in water as mobile phase A and acetonitrile as mobile phase B at a flow rate of 0.5 mL min^{-1}. The column oven temperature was maintained at 30°C. The sample injection volume was 10.0 µL. The auto sampler temperature was set at 15°C. The LC gradient program (time/% mobile phase A) was set as follows: 0.00/80, 3.0/80, 12.0/10, 15.0/10, 16/80 and 20.0/80. The negative Electrospray Ionization (ESI) probe was operated in MRM mode for the quantification of N-Nitroso Desmethyl Olopatadine in the form of protonated ions (M-H) at m/z 351.10>307.30.
The different voltage *i.e.,* Declustering Potential (DP), entrance potential and collision exit cell potential was maintained at 70 V, 10 V and 14 V respectively. The ion spray Voltage (V) was maintained at 4500 V. The curtain gas flow, ion source gas (GS1) and ion source gas (GS2) pressure was maintained at 40 psi, 50 psi and 70 psi. All parameters of LC and MS were controlled using Sciex Analyst version 1.7.3.

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 same solutions six times. Linearity of the method was evaluated from six concentration levels between the LOQ and 150% level. Calculate 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. The robustness of the method was tested by altering the mobile phase flow rate and column temperature. Further, the analysis of the sample solution at different intervals of time was compared against fresh samples to evaluate the stability of impurity in the sample solution [4].

RESULTS AND DISCUSSION

Method development

The aim of the study was to develop selective LC-MS/MS method that can able to quantitate N-Nitroso Desmethyl Olopatadine in the Olopatadine Hydrochloride. Columns were tested to obtain the most appropriate peak shape and separation. By using typical CSH, BEH C18 column the peak due to N-Nitroso Desmethyl Olopatadine and Olopatadine Hydrochloride are not retained properly and elutes very early and due to this the separation was not desire between the peaks. While on HSS T3 the retention improved slightly but the overall separation and peak shape was not up to the marked for the impurity. A Phenomenex, Phenyl Hexyl (100 × 4.6 mm, 2.6 µm) column was found to be the most suitable regarding both peak retention, shape and separation, as well as the response of analytes. The mobile phase was operated in gradient mode using 2 mm ammonium formate, pH adjusted to 3.0 with formic acid in water as mobile phase A and acetonitrile as mobile phase B. The flow rate of the mobile phase was maintained at 0.5 mL min^{-1}, with the column temperature set at 30°C. The auto sampler temperature was set at 15°C. The retention times of N-Nitroso Desmethyl Olopatadine were observed to be 14.14 min and the peak corresponding to Olopatadine Hydrochloride was eluted at 12.81 min. The chromatogram of standard solution of N-Nitroso Desmethyl Olopatadine is given in the Figure 2 [5-6].

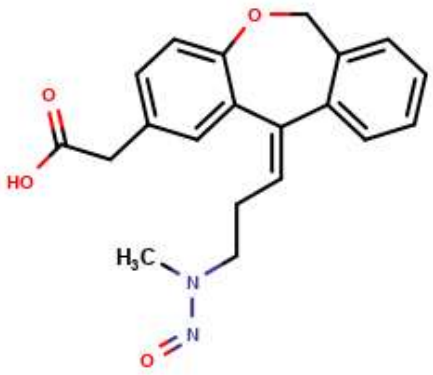

Figure 2: Chemical structure of N-Nitroso Desmethyl Olopatadine.

Operating conditions of LC-MS/MS

Initial optimization of the mass parameters for the detection of the N-Nitroso Desmethyl Olopatadine was performed at concentration level of 1 µg mL^{-1}. The intensity obtained with Electro Spray Ionization (ESI) in the negative mode was on higher side compare to that of positive mode for the impurity. As a part of optimization in the ESI conditions for N-Nitroso Desmethyl Olopatadine, fragmentation was carried out using different collision energy (10, 15, 20, 25 and 30 ev). The ion source parameters such as ion spray and collision gas were optimized to obtain a good response for the ions.

Method validation

The optimized LC-MS/MS method was successfully validated in accordance with the ICH guidelines. Method validation was carried out in terms of its adequate selectivity, linearity, LOD and LOQ, accuracy, repeatability, recovery and robustness.

Specificity

A single N-Nitroso Desmethyl Olopatadine solution was prepared at the specification level in the diluent. The spiked Olopatadine Hydrochloride solution was then subjected to LC-MS/MS analysis and the results revealed that there was no interference of the Olopatadine Hydrochloride peak with N-nitroso Desmethyl Olopatadine peak, and hence the specificity of the developed method was proven (Table 1) [7].

Table 1: System suitability criteria.

System suitability criteria	System suitability criteria	System suitability criteria
Olopatadine Hydrochloride	12.65	1
N-Nitroso Desmethyl Olopatadine	14.14	1.11

Determination of LOD and LOQ values

The Limit of Detection (LOD) and Limit of Quantification (LOQ) determined the sensitivity of the method. The LOD and LOQ value of N-Nitroso Desmethyl Olopatadine was determined based on S/N ratios of 3.0 and 10 by injecting standard solutions of known concentrations. The repeatability at the LOD and LOQ value was calculated by analyzing six replicate injections of N-Nitroso Desmethyl Olopatadine and calculating their RSD% values. The chromatograms of solutions of N-Nitroso Desmethyl Olopatadine with concentrations of LOD and LOQ shown in Figure 2 and Table 2 [8].

Linearity

Linearity of the method was studied by using the standard solution of N-Nitroso Desmethyl Olopatadine at different concentration level from the Limit of Quantification (LOQ) to 150% of the impurity. The slope, intercept, and correlation coefficient values were derived from the linear regression analysis of the average peak area versus the concentration of analytes. A good correlation between the peak area and concentration of analytes was obtained, as can be seen in Table 2.

Table 2: LOD, LOQ and Linearity results.

Validation parameter	Results
LOD-LOQ	
LOD (ng mL^{-1})	7
LOQ (ng mL^{-1})	14
Precision at LOQ (%RSD)	3.68

Linearity	
Regression (r)	0.9999
Calibration range (ng/mL^{-1})	14.0-210
Slope	13457952
Intercept	13531.64
% Intercept	0.77

Accuracy and recovery

The standard addition and recovery experiments were conducted for the N-Nitroso Desmethyl Olopatadine in bulk samples of Olopatadine Hydrochloride in triplicate at LOQ (0.014 ppm), 50% (0.07 ppm), 100% (0.14 ppm) and 150% (0.21 ppm) with respect to test concentration. The acceptance criterion for recovery was set at 80%-120%. The percentage recoveries for N-Nitroso Desmethyl Olopatadine are presented in Table 3.

Table 3: Accuracy (Recovery) results of N-Nitroso Desmethyl Olopatadine in bulk sample.

Accuracy level	Mean recovery (%)	% RSD
LOQ%	113.58	4.61
50%	109.19	2.47
100%	105.95	3.02
150%	107.87	2.3

Precision (Repeatability)

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the N-Nitroso Desmethyl Olopatadine were checked at its specification level (*i.e.*, 0.14 ppm with respect to analyte concentration, 0.5 mg mL^{-1}). The percentage RSD of method repeatability and system repeatability for the N-Nitroso Desmethyl Olopatadine were reported (Table 4) confirms good precision of the method [9].

Table 4: Precision results of N-Nitroso Desmethyl Olopatadine.

Precision	% RSD
System precision	2.61
Method precision	2.58
Intermediate precision	3.52

Robustness

The robustness of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. The optimized flow rate of the mobile phase was 0.5 mL min^{-1} pH 3.0, and the column oven temperature was 30°C. The parameters are altered from 0.47 to 0.53 mL min^{-1}, 2.75 to 3.25 and 27°C and 33°C receptively. The data obtained confirms that these deliberately changed chromatographic conditions did not impact the chromatographic performance for N-Nitroso Desmethyl Olopatadine in spiked samples showing the robustness of the method [10].

Solution stability

The solution stability of Olopatadine Hydrochloride and N-Nitroso Desmethyl Olopatadine was carried out by leaving spiked and unspiked sample solutions in firmly capped LC vials at 15°C for about 24 h in an auto sampler. The concentration of N-Nitroso Desmethyl Olopatadine was determined against freshly prepared standard solutions and no significant changes were observed in the concentration for N-Nitroso Desmethyl Olopatadine. The data confirmed the stability of impurity in the sample solution for at least 24 h.

CONCLUSION

In this study, we have developed a LC-MS/MS method that is capable of quantifying N-Nitroso Desmethyl Olopatadine in Olopatadine Hydrochloride using the negative ionization mode with Multiple Reactions Monitoring (MRM). The method was validated as per ICH 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 of acceptable limit. The sample prepared in the analytical solution was found to be stable for at least 24 h. The method was fully validated and presents good linearity, accuracy, repeatability, and robustness. This method could be very useful for the determination of N-Nitroso Desmethyl Olopatadine in Olopatadine Hydrochloride during its manufacture and product release.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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REFERENCES

[1] Nickels AS, Dimov V, Wolf R. Expert Opin Drug Metab Toxicol. **2011**; 7(12): p. 1593-1599.
[2] Chu NN, Chen WL, Xu HR, et al. Clin Drug Investig. **2009**; 29: p. 451-457.
[3] Meier E, Narvekar A, Iyer GR, et al. Clin Ophthalmol. **2017**: 36: p. 669-681.
[4] Berger WE, Ratner PH, Casale TB, et al. Allergy Asthma Proc. **2009**; 30(6): p. 612-623.
[5] Roland PS, Marple BF, Wall GM. Exp Rev Clin Immunol. **2010**; 6(2): p. 197-204.
[6] Tashiro M, Mochizuki H, Sakurada, et al. Br J Clin Pharmacol. **2006**; 61(1): p.16-26.
[7] Roland PS, Ryan MW, Wall GM. Exp Opin Pharmacother. **2010**; 11(9): p. 1559-1567.
[8] Tashiro M, Duan X, Kato M, et al. Br J Clin Pharmacol. **2008**; 65(6): p. 811-821.
[9] Wang LA, Cohen-Wolkowiez M, Gonzalez D. Adv Pediat. **201**6; 63(1): p. 227-254.
[10] Toyohara J, Ishiwata K, Sakata M, et al. PloS One. **2010**; 5(2): p. 8961.