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Novel Route of Synthesis and Characterization of Bilastine with Evaluation of Nitrosamine Impurities

C. Hazarathaiah Yadav[1*], Venkatesh Medarametla[2]

[1,2]Department of Chemistry, VelTech Rangarajan Dr Sagunthala R & D Institute of Science and Technology, Chennai, Taml Nadu, India

Corresponding Email: info@anaxlab.com

Abstract: The current paper proposes to prepare the novel route of the synthesis of Bilastine drug substance with structural characterization. In addition to this work, formation and evaluation of Nitrosamine impurities of Bilastine are described as per the current regulatory requirements.
It is an antihistamine medication used to treat hives, allergic rhinitis and itchy inflamed eyes (allergic conjunctivitis) caused by an allergy. The drug is anti-histamine and selectively inhibits the histamine H1 receptor, the drug product effectively prevents these allergic reactions.

Keywords: Anti-Histamine, Bilastine, Nitrosamine Impurities.

1. Introduction

This new route of synthesis describes the preparation and characterization of intermediates and active pharmaceutical ingredient preparation and characterization of intermediates and API drug substance. In addition, formation of nitrosamine impurities related to the drug substance "Bilastine" are evaluated and discussed. This proposal provides an idea about the nitrosamine impurities (NDSRIs) and their respective control limits as per the current regulatory acceptance criteria.

The synthesis and characterization of the Bilastine, 2- [4 (2-{4 [1 (2-ethoxy ethyl) -1H--benzimidazol-2-yl piperidin-1-yl} ethyl) phenyl]-2-methyl propionic acid) is marketed under the trade name Bilaxten by FAES.

The current work also elucidates its strong impact on product quality along with its feasibility for cost effective process development in the pharmaceuticals industry to produce high quality drugs with considerably lower cost raw material and utilities.

The first stage of the reaction predominantly has some advantages: Primarily, the price of the raw materials is comparatively less when compared with those which are used in the current market and observed yield is significantly high when compared to other processes. The proposed work is low cost, ease of operation and less pollution, and smoothly implementable at industriel production.

In this proposal, the advantages of the novel route of synthesis and the primitive route of synthesis are clearly explained. The technical problems in the prior part are the invention provides a preparation method of Bilastine, which solves the defects of harsh operation conditions, high toxicity, expensive raw materials and complex operation in the prior art, and has mild reaction conditions in each step, simple to operate, has high yield and purity, and this route is suitable for industrial production.

The novel method for synthesizing Bilastine provided by the invention adopts cheap and easily-obtained 2-nitroaniline as a raw material, and obtains Bilastine through reduction-ring closure reaction, alkylation reaction, hydrolysis, coupling and hydrolysis. The method solves the problems of harsh operating conditions, high toxicity, expensive raw materials and complex operation in the prior art, and has mild reaction conditions in each step, simple operation of the synthetic method, easy treatment, few byproducts, high yield and purity and low production cost, and is suitable for industrial production.

The existing work progressed to reach the industrial requirements as per the current regulatory bodies. The drug acceptance received from the Europe and USA and other Canada markets based on the controlled the impurities is one of the complicated task in the drug acceptance based on the risk evaluations of the nitrosamine impurities.

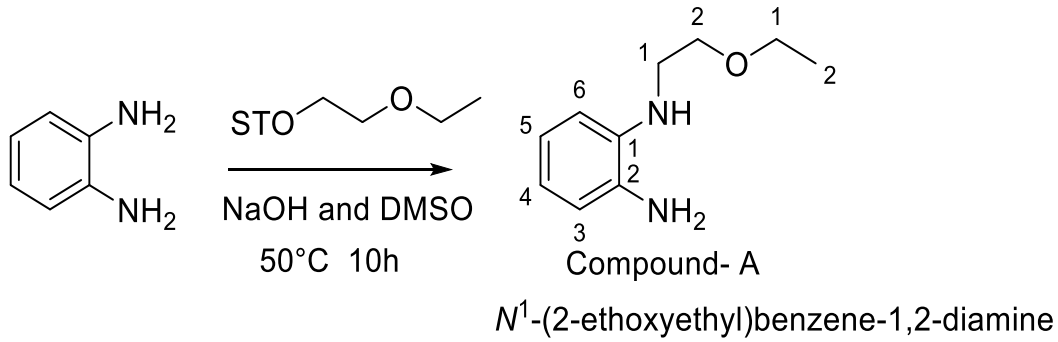
2. Experimental Procedures:

2.1 Compound-A: (N1-(2-ethoxyethyl) benzene-1,2-diamine) preparation:

O-Phenylene diamine ($C_6H_4(NH_2)_2$) is precursor to the preparation of the Compound-A and it is completely novel synthetic route with good yield due to low level of impurities are the advantage in the current suggested ROS.

High levels impurities could be the reason for decrease the actual yield, which in turn decreases the percentage yield.

This route of synthesis yielding high pure intermediate causing that increases the actual yield (output) and therefore increases the percentage yield. This commercially viable and acceptable by the scale up batches manufactured in the pharmaceutical industries.



Procedure:

Take O-Phenylenediamine into the round bottom flask and charge the DMSO (3.0 volumes).

Stir the mass for 5-10 min then charged Tosyl-diethyl ester (0.8 equivalents) in the RBF. Add the Sodiumhydroxide (NaOH) (2.0 equivalents) heat the mass to 50°C and stir for 10 hours.

Check the content of O-Phenylene diamine by TLC. Charge water into the reaction mass gets solid; stir the mass for 2 hours at room temperature, filter the solid sundry obtained the Compound-(A). Above described process yield obtained 75-85%with purity observed 99.5%.

Compound-A with different solvents:

Solvent used for reaction	Yield observed
DMSO (Dimethyl sulfoxide)	80-85%
DMS (Dimethyl sulphide)	70-75 %
Acetone	55-60 %
DCM (Dichloro methane)	50-55 %
MIBK (Methyl isobutyl ketone)	50-55 %
Methanol	50-60 %

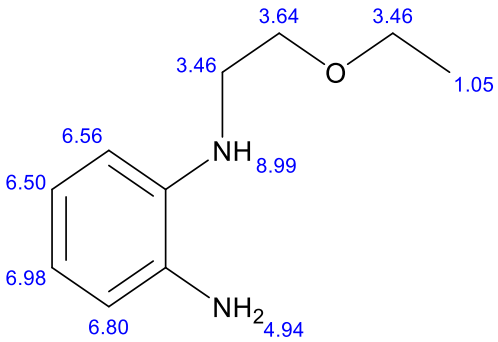
As per above table, it is concluded that DMSO is considered for best solvent for condensation of stage-1 with different solvents and conditions observed above summarized yields. Hence, it is concluded that DMSO is observed relatively high viable for this process when comparatively other solvents.

Evaluation of the Compound A with below characterization data:

Mass updated: m/z; 180.13

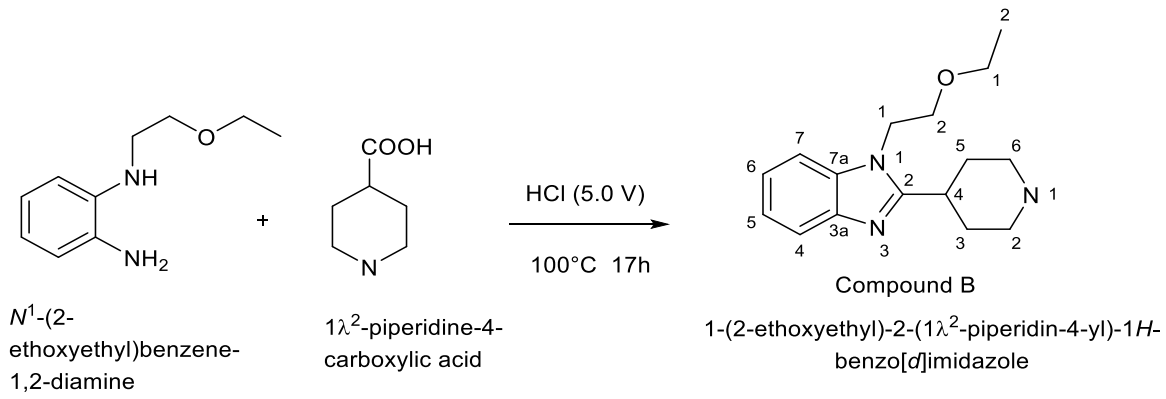
IR: N-H: 3400, C=C: 1600

NMR: N-H: 8.99, NH_2: 4.94, N-H: 8.99, Ar-C-H: 6.56-6.98 as shown below:



2.2 Preparation of Compound-B:

Compound-A 1.0 (eq.) is changed into the round bottom flask and charges the hydrochloride solution (5.0 volumes) into the RBF and stir for 10 min. Charge pipeline carboxylic acid (1.2 eq) in the RBF. Stir for 17hrs.at 100C. Slowly cool the reaction mass, solid observed was stir for two hours at 25°C. Filter thesolid and wash with water 2.0 volumes.



N[1]-(2-ethoxyethyl)benzene-1,2-diamine

1λ[2]-piperidine-4-carboxylic acid

1-(2-ethoxyethyl)-2-(1λ[2]-piperidin-4-yl)-1*H*-benzo[*d*]imidazole

Characterization of compound-B (Structure conformation data):

Mass updated: 273.18 m/z
IR:C=C:1433cm^{-1}, N-H: 3409 cm^{-1}
NMR: 2-[4-(2-Tolysulfonyl oxygen base ethylphenyl) embodiment 2 being prepared by nuclear-magnetism] methyl acetate detects, and result shows, H^{1}-NMR ($CDCl_3$, 500MHz, TMS) δ: 0.98 (T, 3H); 2.23 (d, 4H); 3.59 (s, 2H); 3.69 (S, 3H); 4.19 (t, 2H); 7.07 (d, 2H); 7.17 (d, 2H); 7.29 (d, 2H); 7.69 (d, 2H).

2.3 Preparation of Compound-C:

Charge compound-B 20.0 g (1.0 eq.) into the round bottom flask and charge 2-[4-(2-Chloro-ethyl)-phenyl]-2-methyl-propionic acid methyl ester reacting compound-B presence of base like sodium bicarbonate (3.0eq.). Heat the reaction mass to 100°C and stir the reaction mass for 15-16 hours then check TLC to conform the content of Compound-B, after completion of reaction charge NaOH (2.0 eq.) into the reaction mass and stir for 2 hours. Slowly cool the mass and add 2.0 N HCl solution into the reaction slowly solid formation observed on acidic condition. The obtained solid stir for 90 min at room temperature. Filter the reaction mass and suck dry well, wash the solid with water (2.0 volumes) after suck dry well dry the material in vacuum tray drier and dry weight observed 86% yield.
In compound-C preparation, crystallization is a simple procedure as per chemistry. Most acidic compounds crystallize under specific aqueous acidic conditions. Water is a universal solvent and is also a low-cost, readily available natural solvent. Therefore, this journal obviously encourages the development of maximum reactions and workups in bulk drug industries using water solvent conditions, which will be user-friendly and environmentally friendly. As the universe currently grapples with high levels of industrial pollution, this proposal aids in advancing green chemistry to mitigate the environmental impact caused by organic solvents.

Characterization of compound-C and conformation as per the below data described:

Mass $C_{28}H_{37}N_3O_3$, 463.62: m/z: M+H 466.78
HPLC: 99.23%
NMR:1H-NMR, 400MHz, CDCI3, ppm : δ1.08(t, 3H), 1.59(s, 6H)1 2.15-2.30(m. 4H), 2.64-2.79(m, 6H), 3.25(s, 1 H), 3.67(q, 2H), 3.47(m, 2H), 3.71 (t, 2H), 4.32(t, 2H), 6.79(d, 2H), 7.26(m, 2H), 7.29-7.32(m, 3H), 7.76(t, 1 H)

2.4. Purification of Compound-C:

Charge 25.0 g (1.0 eq.) of Compound-C into the round-bottom flask and add n-Butanol (5.0 volumes). Stir for 2-3 hours at 100°C. Allow the reaction mass to cool to room temperature, and then stir for one hour. Filter the reaction mass and wash with n-Butanol (2.0 volumes). Yield observed: 90-95%

Product Purity:

The final purity of the drug substance, as determined by chromatographic analysis, meets the high standards accepted by the API drug purity and the required levels in the industry. The analysis was performed using a Symmetry C18 column, with dimensions of 250*4.6mm and a particle size of 5 micrometers, for the analysis of the drug substance. The observed purity of the substance is 99.86%, with other impurities found within the acceptable levels outlined in the ICH Q3 regulatory guidance.
Purity of the Drug Substance: 99.86% with RT: 8.22 and RRT: 1.0

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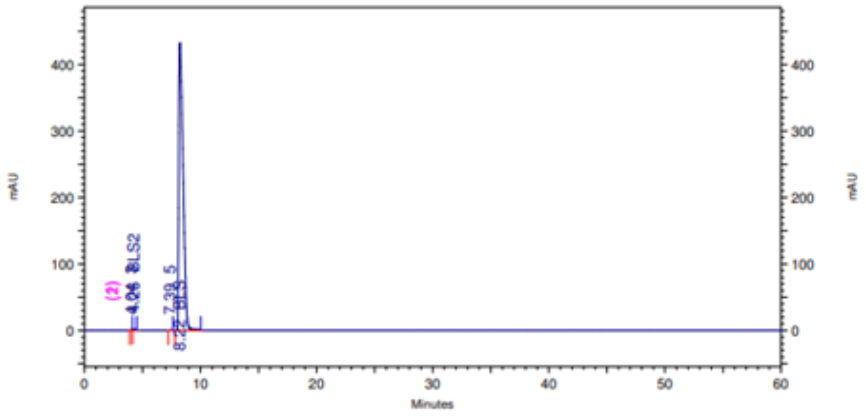
Description :Symmetry C18,250*4.6mm,5µm(ARD-LC014/2019)



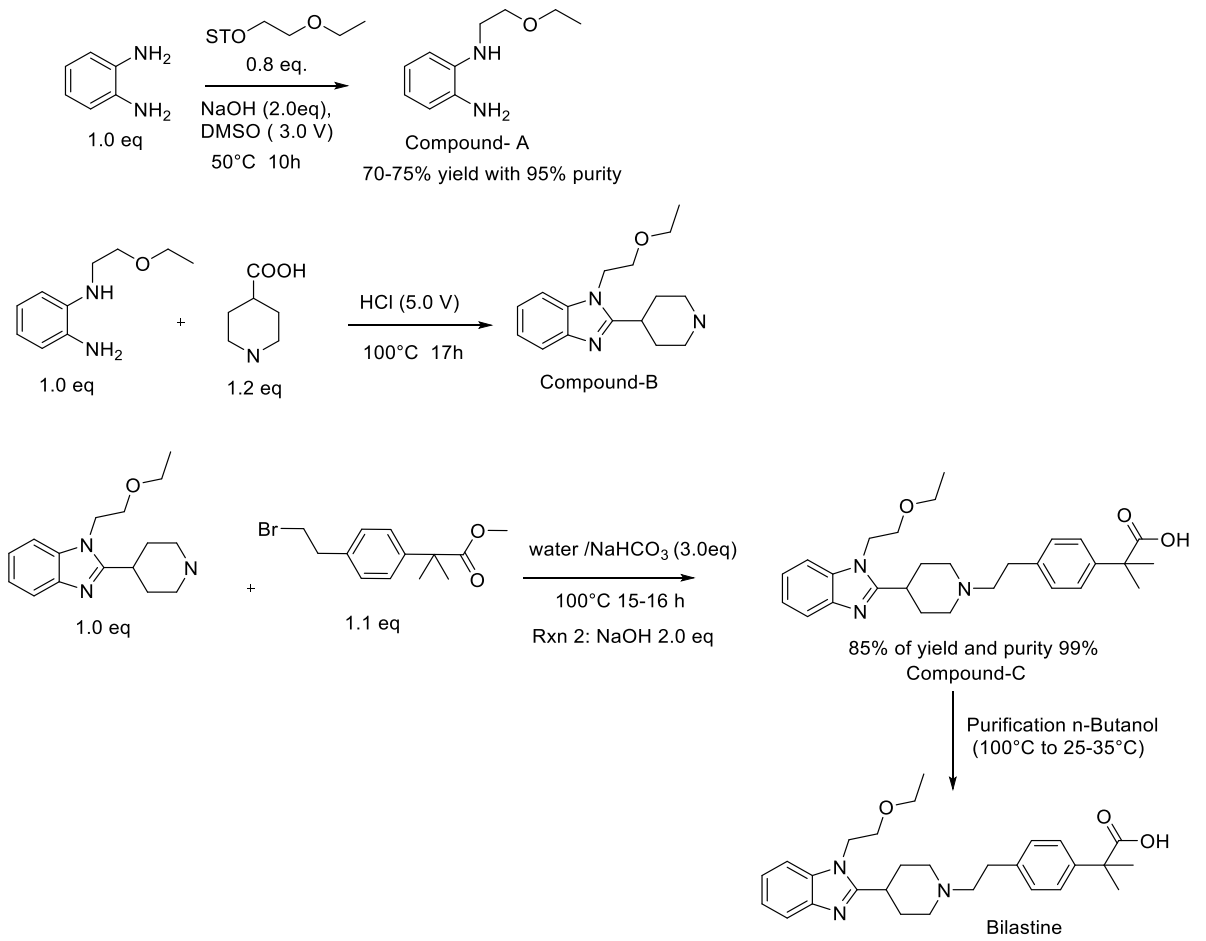
VWD: Signal A,
275 nm Results

Peak Number	Retention Time	Height	Area	Area Percent	Name	Relative RT
1	4.04	8866	60955	0.03	3	0.49
2	4.26	24446	179185	0.10	BLS2	0.52
3	7.39	1661	17604	0.01	5	0.90
4	8.22	7245763	187003825	99.86	BLS	1.00
Totals		7280736	187261569	100.00		

"High-Performance Liquid Chromatography (HPLC) is used for analyzing the purity of the Active Pharmaceutical Ingredient Bilastine. In the chromatograms, peaks correspond to the API and any impurities. The HPLC data is generally evaluated by the retention time of the API peak compared to that of a reference standard. The retention time should match within a specified tolerance limit. Also, parameters such as Peak Purity, Peak Area, and Height are evaluated consistently with the concentration of the sample. System Suitability, Calibration Curve, Limit of Detection (LOD), and Limit of Quantification (LOQ), along with impurity analysis with proper validation, verify that all calculations and interpretations are documented properly.

All these parameters need to be confirmed before validating the process in the manufacturing of the drug substance. Hence, as per the current regulatory requirements, ensuring the presence of all types of impurities and human carcinogens in the drug substance needs to be evaluated and controlled.

2.5 Final Route of synthesis for drug substance:



The novel synthesis process route provides enhancements in both yield and quality compared to alternative synthetic routes. This means that the new method of synthesis not only increases the quantity of the desired product obtained but also enhances its overall quality. By employing this innovative approach, the process can achieve higher efficiency and purity, resulting in a more desirable final product. In contrast to other synthetic routes, which may be less effective or produce lower-quality outcomes, the novel route offers distinct advantages that contribute to improved results in terms of both yield and product quality.

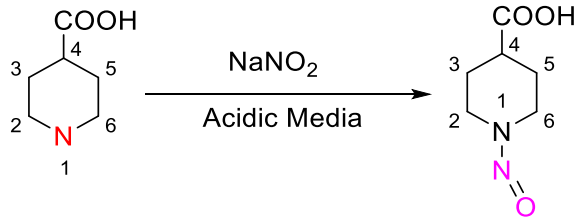
3. Discussion on the Nitrosamine Impurities (NDSRI) in the Bilastine:

As per the current guidelines and regulatory requirements nitrosamine impurities and nitrosamine drug substance related impurities are very impotent to control the drug substance and drug products.
Nitrosamine Drug Substance-Related Impurities (NDSRIs) are monitored and controlled as per the current requirements. As per the above Route of synthesis, it is investigated and identified the possibility of the NDSRIs, which is discussed below:
The present paper not only focused on seven most frequently reported N-nitrosamines in food, namely, NDMA, NMEA, NDEA, NDBA, NPIP, NPYR and NMOR but also other NDSRIs. As per the availability of nitrogen, Nitrate salts and other precursors in the drug substance there is possibility for identification of nitrosamine impurities, Based on the study of ROS of Bilastine drug substance and considering the guideline of US FDA,

Europe and Canadian regulatory bodies, risk assessment shall be evaluated. The outcome of the risk for the formation of nitrosamines and NDSRIs shall define the controls, like spiking study with purgeability.
NDSRIs are the impurities which are classified as most probable to human carcinogens on the basis of studies on the animals and other clinical studies.
Formation of the nitrosamine impurities from starting material used in the synthesis, Piperidine carboxylic acid, Benzene-1,2-diamine, intermediates and drug substance are discussed below:

3.1. Nitrosamine impurities as per the Raw-materials in the Drug substance:

a. The raw-material used in the Process of the subject API, is the Piperdine carboxylic acid, which is react with the nitrosating agent and give the related nitrosamine impurity in the drug substance or drug product shown the ROS below:

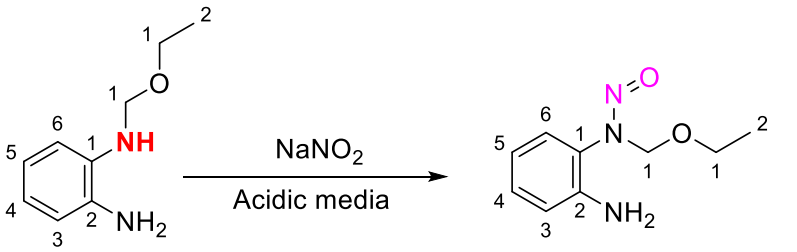


1λ²-piperidine-4-carboxylic acid

1-nitrosopiperidine-4-carboxylic acid

3.2. Nitrosamine impurities as per the Intermediates in the Drug substance:

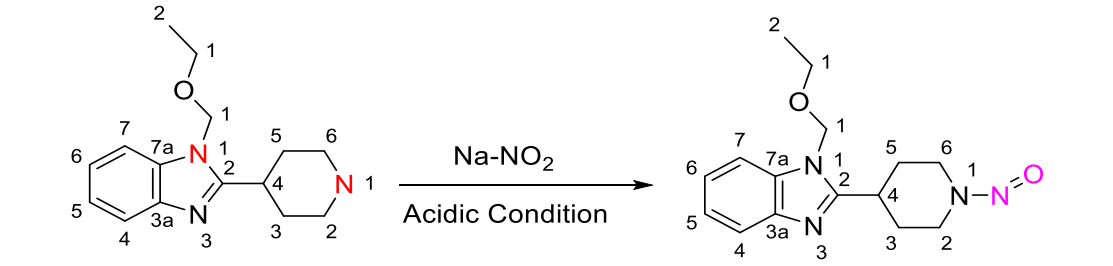
a. There is formation of the nitrosamine impurity with the Compound-A (benzene 1,2 diamine) react with sodium nitrate or other nitro sating agents are react in presence of the acidic conditions will provide the shown below structural possibility. Mainly in the below ROS highlighted secondary amine will give the nitrosamines.



*N*¹-(ethoxymethyl)benzene-1,2-diamine

N-(2-aminophenyl)-*N*-(ethoxymethyl)nitrous amide

b. The Compound-B reacts with a nitrosating agent in the presence of acidic conditions, yielding the respective nitrosamine impurity. The structural route of synthesis is provided below, wherein only the secondary amine impurity is shown. Additionally, tertiary amines will also form nitrosamines



1-(ethoxymethyl)-2-(1λ²-piperidin-4-yl)-1*H*-benzo[*d*]imidazole

1-(ethoxymethyl)-2-(1-nitrosopiperidin-4-yl)-1*H*-benzo[*d*]imidazole

c. Formation of the NDSRI in Compound-C and compound-D

Like the compound-C, the drug substance (API) also has the 3° amine, present in these structures. This tertiary amine reacts with the Nitrosating groups and gives the corresponding nitrosamine impurities.
This present work discusses the NDSRIs, formed due to presence of Nitrosating agents, including sodium nitrite, nitrous acid, nitrous anhydride, and nitrosyl halides, etc. are causing the formation of the impurities. These levels are controlled strictly by the regulatory bodies like Canada, Europe and US FDA. Also the recycled solvents or reused catalysts are used in the process and inadequate cleaning of the equipment is observed in the manufacturing locations. And inappropriate monitoring of these impurities causes the failure of the drug substance in the final product. These culprits in Nitrosating agents are occurred by ROS/ any other source like pot-water, packing materials, Equipment, in-proper cleaning resulting contamination and may cause the formation of these nitrosamine impurities.
Hence these Impurities are needed to be controlled in the acceptable levels so that the API drug will be accepted for the usage by the customers. These impurities are needed to be discussed more elaborately and controls are needed to be shown in the final specifications as per the applicable requirements.

d. Control strategies of these impurities:

Based on the current drug substance, the regulatory requirements are clearly published these impurities in the drug substance and drug product. Control of these impurities is highly essential as these are they cause the human carcinogens. As per the Scope of Health Canada's, to evaluate the risk of the presence of nitrosamine impurities are discussed complete information' drug identification number (DIN) containing chemically synthesized and semi-synthetic APIs. The drugs substance and drug products which include:

- prescription and non-prescription (over-the-counter) drug pr1oducts
- chemically synthesized excipients and raw materials used in the manufacturing of drug products

Riskassessment to be performed for these impurities for specified above described. Regulatory required for drug substance risk assessment below stages are provided:
Step 1: Risk assessmentsfor nitrosamine impurities
Step 2: Confirmatory testing of manufacturing batches
Step 3: Changes to the market authorization to mitigate the risk of NDSRIs
In the first step, risk assessmentperformed for above discussed impurities as per the ROS and identify the risk of possibility of the impurities, and conform the testing with validated analytical methods with the well-established LOQ levels of the particular individual impurities.

Including routine testing for nitrosamine impurities in the API specification:

The API specification should include a test and acceptance criterion for each nitrosamine impurity when:
The presence of the risk for nitrosamine to be high and/or the concentration greater than 30% of the AI limit of the during confirmatory testing
Examples where the risk for nitrosamines is considered high:

- ✓ Potential for nitrosamine formation on storage
- ✓ Presence of nitrosamine precursor functional groups in the API
- ✓ The Formation of a nitrosamine impurity in the final stage of process

Control for nitrosamine impurities include routine testing in API as per ICH's M7.

During confirmatory testing, MAHs should test the drug product to determine the levels of nitrosamine impurities.
A scientific justification including experimental data which summarizes the efforts made to synthesize and/or isolate and purify the impurity should be included in the summary and discussion of the risk assessment in the regulatory submission and documented in the MAH's pharmaceutical quality system

Conclusion on the Nitrosamine Impurities in Bilastine:

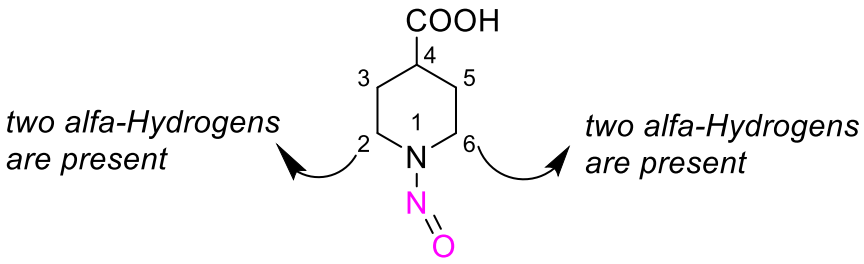
Based on the above all discussions and the potency score will be provided to the particular impurity and the impurity categorised based on the blow table:
Below data shall be extracted from published Guidelines:

The Five Predicted Potency Categories and Associated AI Limits for *N*-Nitrosamines		
Potency Category	**Recommended AI Limit(ng/day)**	**Comments**
1	**18**	The recommended AI limit of 18 ng/day is equal to the class-specific TTC for *N*-nitrosamine impurities. [Footnote] *N*-nitrosamines assigned to Category 1 are predicted to have high carcinogenic potency; however, the class-specific TTC for *N*-nitrosamine impurities is considered sufficiently protective to patients.
2	**100**	The recommended AI limit of 100 ng/day is representative of two potent, robustly tested *N*-nitrosamines, *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which have recommended AI limits of 96 ng/day and 100 ng/day, respectively. *N*-nitrosamines assigned to Category 2 are predicted to have carcinogenic potency no higher than NDMA and NNK.
3	**400**	Compared to Potency Category 2, *N*-nitrosamines in this category have lower carcinogenic potency due to, for example, the presence of a weakly deactivating structural feature. The recommended AI limit was set to reflect a 4-fold decrease in carcinogenic potency from Category 2.
4	**1500**	*N*-Nitrosamines assigned to Category 4 may be metabolically activated through an α-hydroxylation pathway but are predicted to be of low carcinogenic potency, for example, because the pathway is disfavored due to steric or electronic influences, or because clearance pathways are favored. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7
5	**1500**	*N*-Nitrosamines assigned to Category 5 are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens, or are predicted to form unstable species that will not react with DNA. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7.

Potency Score = *α-**Hydrogen Score*** + ***Deactivating Feature Score*** *(sum all scores for features present in the* N-*nitrosamine)* + ***Activating Feature Score*** *(sum all scores for features present in the* N-*nitrosamine)*

As per above guidelines, the nitrosamine impurity for section-2.1 (1-nitrosopiperidine-4-carboxylic acid) will be categorized:

Nitrosamine Impurity (NDSRIs) evaluated structural represented nitrosamine impurity as per below shown



COOH group is electorn-withdrawing group present at 4th position

In the above structure both sides, 2 and 2 α-Hydrogen's (Score is +1) and 4th position acid group is withdrawing group present, hence (score is +1) and N-nitroso group in a 5- or 6-membered ring n- Nitro group in the 6 membered ring (Score is +2).
Hence total Potency score = 1 + 2+1 = 4; therefore: ***AI = 1500 ng/day****(acceptable intake of the drug)*
Hence, based on the above score the Nitrosamine impurities are categorized and controlled in the drug substance.

4. Biological activity and Antibacterial activity:

The antibacterial and antifungal activities of the title compounds were evaluated using various diffusion methods. Currently, the drug substance has sufficient biological and clinical data. Therefore, this study discusses the biological activities of existing publications, as described below:

Discussion on the Biological Activity of the Drug substance with previous Publications:

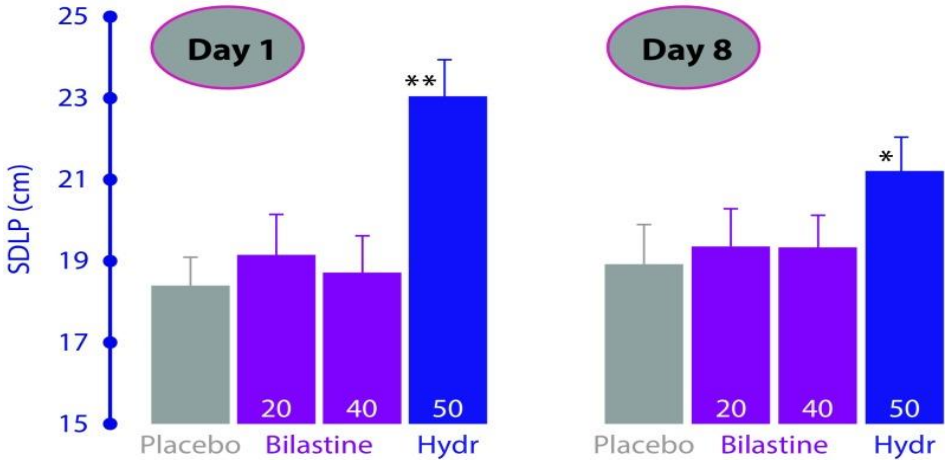
The drug substance subjected to review is Bilastine, a non-sedating second-generation oral antihistamine (OAH) used for treatment. This review aims to examine existing literature on Bilastine's efficacy as an antihistamine drug for treating patents and its biological activity, as identified in previous journals and patents. Furthermore, this review will discuss and extrapolate on how this drug substance acts on patient safety and efficacy, aiming for a simple understanding."
Bilastine is utilized for the treatment of allergic rhinitis (AR) patients. It has not been previously evaluated in a meta-analysis. This literature survey discusses findings from the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, Science Direct, and Google Scholar.
Bilastine demonstrated superiority over placebo in improving Total Symptom Score (TSS), Nasal Symptom Score (NSS), Non-Nasal Symptom Score (NNSS), rhinitis discomfort score, and Quality of Life (QOL). However, its efficacy is comparable to other Oral Antihistamines (OAHs) in TSS, NSS, NNSS, rhinitis discomfort score, and QOL. No significant difference was observed in hostility compared to placebo and other OAHs, except for a lower incidence of somnolence compared to cetirizine.
The quality of evidence ranged from moderate to high. Bilastine demonstrates high effectiveness and safety in treating AR, with efficacy and safety comparable to other OAHs. Additional data are described below
Randomized controlled trials (RCTs) comparing Bilastine with placebo or no treatment would be included. Additionally, trials involving Oral Antihistamines (OAHs), intranasal corticosteroid nasal sprays, and leukotriene receptor antagonists would be considered if available. Participants of all age groups, diagnosed with allergic rhinitis (AR), of any gender, or ethnicity, were eligible. The included studies must have had allergic rhinitis diagnosis confirmed by clinicians, and outcomes must have been measured over a minimum follow-up period of 2 week.

Study reference	Age (years)	Number of participants	Subject	Indication	Comparators	Outcome measures
Bachert et al. (2009)	12–70	721	SAR	At least 2-years history and positive skin prick test (common grass pollen and tree pollen, including perennial allergens	Desloratadine, placebo	TSS, NSS, NNSS, QOL, AE
Kuna et al. (2009)	12–70	683	SAR	At least 2-years history and positive skin prick test (season pollen allergens) or specific IgE to at least one seasonal allergen	Cetirizine, placebo	TSS, NSS, NNSS, AE
Novak et al. (2016)	2–12	509	Allergic rhinoconjunctivitis/ chronic urticaria	Positive skin prick test or specific IgE	Placebo	AE
Okubo et al. (2017)	18–74	765	PAR	At least 2-years history and positive nasal provocation test (house dust disc) or specific IgE (at least one house dust mite)	Placebo, fexofenadine	TSS, QOL,AE
Sastre et al. (2011)	12–70	651	PAR	At least 2-years history and positive skin prick test (house dust mites, cockroaches, molds or animal danders	Placebo, cetirizine	TSS, AE

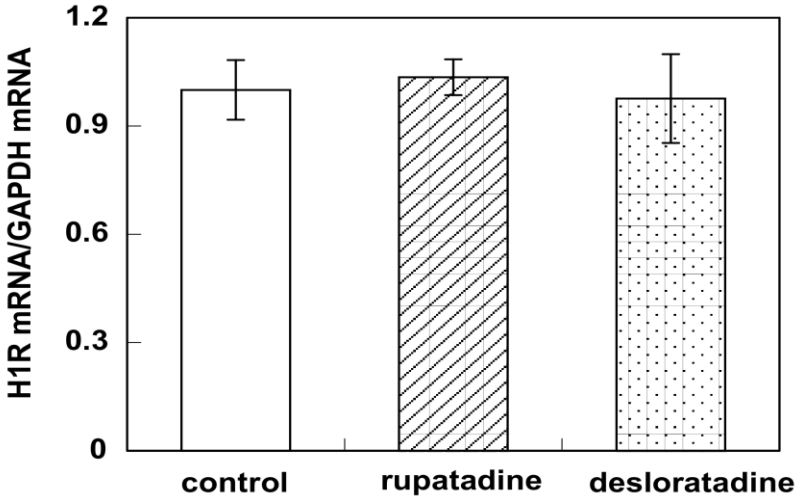
PAR, *perennial allergic rhinitis;* SAR, *seasonal allergic rhinitis;* TSS, *total symptom score;* NSS, *nasal symptom score;* NNSS, *non-nasal symptom score;* QOL, *quality of life;* AE, *adverse events.*

Comparisons and Effects of Interventions:

Four comparisons were assessed in this review, with primary and secondary outcomes evaluated for each. The comparisons included Bilastine versus placebo, Bilastine versus cetirizine, Bilastine versus desloratadine, and Bilastine versus fexofenadine.
This assessment aligns with the findings of 'Bilastine in allergic rhinoconjunctivitis and urticaria: a practical approach to treatment decisions based on queries received by the medical information department (doi: 10.7573/dic.212500)
The Medical Information Specialists at FaesFarma have received a number of queries regarding potential drug interactions involving Bilastine. The drug concentration and number of days are monitored in the journal and summarized in the diagram below:



Effect of antihistamines on H1R gene expression in HeLa cells. (A) Dose–response study: HeLa cells were incubated with or without 5 μM rottlerin or bilastine (0.3 μM to 3 μM) for 4 h. Subsequently, the cells were harvested, and H1R mRNA levels were determined by real-time RT-PCR. Data are expressed as means ± SEM (n = 8 for control and bilastine (0.3 and 3 μM); n = 4 for bilastine (1 μM) and rottlerin). **, $p < 0.01$ vs. control. (B) HeLa cells were treated with or without (represented as control) 3 μM H1-antihistamines for 4 h. Abbreviations: cont, control; bila, bilastine; fexo, fexofenadine; olop, olopatadine; oxat, oxatomide; levce, levocetirizine; bepo, bepotastine. Data are expressed as means ± SEM (n = 8 for control and olopatadine, n = 7 for bilastine, fexofenadine, levocetirizine, and bepotastine; n = 6 for oxatomide). **, $p < 0.01$ vs. control



The data discussed in the section: 3.0 (Biological activity Antibacterial activity) is based on various journals which have been reviewed to understand the bio-activity of Bilastine drug substance. Based on the above discussions, it is observed that all biological activities associated with the drug substance have been thoroughly discussed and concluded.

On evolution of the above data it is concluded that our data elucidate that Bilastine exhibits inverse agonist activity. The H1R gene is a disease-sensitive gene for pollinosis, and the suppression of up-regulated H1R gene expression improves nasal symptoms.

5. References:

1. Preparation method of Bilastine CN106146459 B
2. Okubo, K.; Kurono, Y.; Ichimura, K.; Enomoto, T.; Okamoto, Y.; Kawauchi, H.; Suzaki, H.; Fujieda, S.; Masuyama, K. Japanese Society of Allergology: Japanese guidelines for allergic rhinitis. Allrgol. Int. 2017, 66, 205–219. [Google Scholar] [CrossRef] [PubMed]
3. Reparation method of Bilastine CN10417733 1A
4. European Patent Application: EP 3 599 235 Al
5. Synthesis and characterization of Impurity F of Sulfomethoxazole And Their Derivatives Journal of Pharma Research, ISSN: 2319-5622, Vol. 8, Issue 6, 2019
6. Synthesis of cyclic carbonate - 4-(hydroxymethyl)-5-methyl-1,3-dioxolan-2-one and their derivatives GEDRAG & ORGANISATIE REVIEW - ISSN:0921-5077, VOLUME 34 : ISSUE 01 – 2021
7. 1,1,1,-Trifluoro methyl ketone and its reaction with Grignard reagent ISSN 0044-0477 in Volume 20 Issue 10(October 2021). YMER Journal A Scopus journal
8. Process and intermediates for the preparation of Bilastine: EP3599235A1
9. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the world health organization, GA (2) LEN and allerGe Allergy, 2008; 63 Suppl 86: 8-160.
10. Meltzer EO, Bukstein DA. The economic impact of allergic rhinitis and current guidelines for treatment. Ann Allergy Asthma Immunol, 2011; 106: S12-6.
11. Bilastine. Available from: https://www.pubchem.ncbi.nlm.nih.gov/compound/Bilastine. [Last accessed on 2018 Jun 06].
12. Product Monograph Including Patient Medication Information. Blexten®. Available from: https://www.aralez.com/wp-content/uploads/2016/12/BLEXTEN-ProductMonography-English.pdf. [Last accessed 2018 Jun 06].
13. Ridolo E, Montagni M, Bonzano L, Incorvaia C, Canonica GW. Bilastine: New insight into antihistamine treatment. Clin Mol Allergy, 2015;
14. https://patents.google.com/patent/CN106692090A/en
15. https://www.ncbi.nlm.nih.gov/pubmed/19438584 https://www.dovepress.com/treatment-of-allergic-rhinitis-and-urticaria-a-review-of-thenewest-an-peer-reviewed-fulltext-article-TCRM
16. https://pubchem.ncbi.nlm.nih.gov/compound/Bilastine

17. World Journal of Pharmaceutical Research SJIF Impact Factor 8.074 Volume 8, Issue 7, 2197-2224, ISSN 2277– 7105
18. Bachert, C., Kuna, P., Sanquer, F., Ivan, P., Dimitrov, V., Gorina, M. M., et al. (2009). Comparison of the Efficacy and Safety of Bilastine 20 Mg vs Desloratadine 5 Mg in Seasonal Allergic Rhinitis Patients. *Allergy* 64, 158–165. doi:10.1111/j.1398-9995.2008.01813.
19. Bousquet, J., Ansótegui, I., Canonica, G. W., Zuberbier, T., Baena-Cagnani, C. E., Bachert, C., et al. (2012). Establishing the Place in Therapy of Bilastine in the Treatment of Allergic Rhinitis According to ARIA: Evidence Review. *Curr. Med. Res. Opin.* 28, 131–139. doi:10.1185/03007995.2011.648263
20. Bousquet, J., Khaltaev, N., Cruz, A. A., Denburg, J., Fokkens, W. J., Togias, A., et al. (2008). Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 Update (In Collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 63 (Suppl. 86), 8–160. doi:10.1111/j.1398-9995.2007. 01620.x
21. Braido, F., Sclifò, F., Ferrando, M., and Canonica, G. W. (2014). New Therapies for Allergic Rhinitis. *Curr. Allergy Asthma Rep.* 14, 422. doi:10.1007/s11882-014-0422-z
22. Burbach, G. J., Heinzerling, L. M., Edenharter, G., Bachert, C., Bindslev-Jensen, C., Bonini, S., et al. (2009). GA(2)LEN Skin Test Study II: Clinical Relevance of Inhalant Allergen Sensitizations in Europe. *Allergy* 64, 1507–1515. doi:10.1111/j.1398-9995.2009.02089.x
23. Church, M. K., and Church, D. S. (2013). Pharmacology of Antihistamines. *Indian J. Dermatol.* 58, 219–224. doi:10.4103/0019-5154.110832
24. Cumpston, M., Li, T., Page, M. J., Chandler, J., Welch, V. A., Higgins, J. P., et al. (2019). Updated Guidance for Trusted Systematic Reviews: a New Edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database Syst. Rev.* 10, ED000142. doi:10.1002/14651858.ED000142
25. Demonte, A., Guanti, M. B., Liberati, S., Biffi, A., Fernando, F., Fainello, M., et al. (2018). Bilastine Safety in Drivers Who Need Antihistamines: New Evidence from High-Speed Simulator Driving Test on Allergic Patients. *Eur. Rev. Med. Pharmacol. Sci.* 22, 820–828. doi:10.26355/eurrev_201802_14318
26. Guyatt, G. H., Oxman, A. D., Kunz, R., Vist, G. E., Falck-Ytter, Y., Schünemann, H. J., et al. (2008). What is "Quality of Evidence" and Why is it Important to Clinicians? *BMJ.* 336, 995–998. doi:10.1136/bmj.39490.551019.BE
27. Horak, F., Zieglmayer, P., Zieglmayer, R., and Lemell, P. (2010). The Effects of Bilastine Compared with Cetirizine, Fexofenadine, and Placebo on Allergen-Induced Nasal and Ocular Symptoms in Patients Exposed to Aeroallergen in the Vienna Challenge Chamber. *Inflamm. Res.* 59, 391–398. doi:10.1007/s00011-009-0117-4
28. Kowal, K., and DuBuske, L. (2014). Bilastine as a Potential Treatment in Allergic Rhinitis. *Am. J. Rhinol Allergy* 28, 312–316. doi:10.2500/ajra.2014.28.4049
29. Kruszewski, J. (2009). LekiPrzeciwhistaminowe W LeczeniuAlergicznegoNieżytuNosa - Uaktualnienie 2008/2009. *Otolaryngologiapolska* 63, 5–10. doi:10.1016/S0030-6657(09)70180-5
30. Kuna, P., Bachert, C., Nowacki, Z., van Cauwenberge, P., Agache, I., Fouquert, L., et al. (2009). Efficacy and Safety of Bilastine 20 Mg Compared with Cetirizine 10 Mg and Placebo for the Symptomatic Treatment of Seasonal Allergic Rhinitis: a Randomized, Double-Blind, Parallel-Group Study. *Clin. Exp. Allergy* 39, 1338–1347. doi:10.1111/j.1365-2222.2009.03257.x
31. Lynde, C. W., Sussman, G., Dion, P. L., Guenther, L., Hébert, J., Rao, J., et al. (2020). Multidisciplinary Real-World Experience with Bilastine, a Second Generation Antihistamine. *J. Drugs Dermatol.* 19, 145–154. doi:10.36849/JDD.2020.4835
32. Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G.PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA Statement. *BMJ* 339, b2535–269. doi:10.1371/journal.pmed.1000097
33. Novák, Z., Yáñez, A., Kiss, I., Kuna, P., Tortajada-Girbés, M., Valiente, R., et al. (2016). Safety and Tolerability of Bilastine 10 Mg Administered for 12 Weeks in Children with Allergic Diseases. *Pediatr. Allergy Immunol.* 27, 493–498. doi:10.1111/pai.12555
34. Okubo, K., Gotoh, M., Asako, M., Nomura, Y., Togawa, M., Saito, A., et al. (2017). Efficacy and Safety of Bilastine in Japanese Patients with Perennial Allergic Rhinitis: A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Phase III Study. *Allergol. Int.* 66, 97–105. doi:10.1016/j.alit.2016.05.014
35. Bousquet J, Van Cauwenberge P, Khaltaev N, ARIA Workshop Group World health organization. Allergic rhinitis and its impact on asthma. *J Allergy ClinImmunol.* 2001;**108**(5 Suppl):S147–334. doi: 10.1067/mai.2001.118891.

36. Paśko, P., Rodacki, T., Domagała-Rodacka, R., Palimonka, K., Marcinkowska, M., and Owczarek, D. (2017). Second Generation H1 - Antihistamines Interaction with Food and Alcohol-A Systematic Review. *Biomed*. *Pharmacother*. 93, 27–39. doi:10.1016/j.biopha.2017.06.008
37. Valk, P. J., Simons, R., Jetten, A. M., Valiente, R., and Labeaga, L. (2016). Cognitive *Med*. *Hum.*Performance Effects of Bilastine 20 Mg during 6 hours at 8000 Ft Cabin Altitude. *Aerosp Perform.* 87, 622–627. doi:10.3357/AMHP.4522.2016