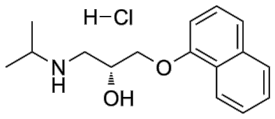
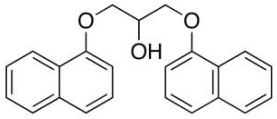
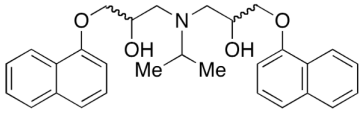
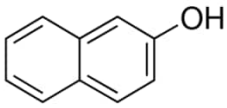
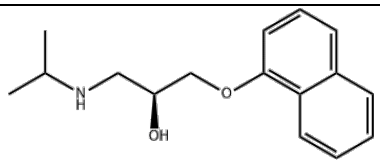
S.3.2 Impurities

1. Potential Process-Related Organic Impurities

Process related impurities include starting materials, intermediates, reagents, and solvents used during synthesis.

The structure and origin or potential related impurities of stelbatolol are provided in **Table 1**. Collated tables of process impurities for batches manufactured to date can be found in S.4.4 Batch Analyses. Degradation products observed in stress studies are detailed in S.7.3 Stability Data.

Table 1. Potential Process-Related Organic Impurities

Chemical Name / Descriptor	Structure	Molecular weight (g/mole)	Category	Impurity Characterization Method Summary	Levels Observed in the API (
R-enantiomer of stelbatalol CAS #		295.80	Chiral impurity	Enantiomeric purity by LC; Mass spectrometry	ND
Impurity 1 CAS#		344.4	Process impurity	Organic impurities by HPLC	Range: ND-0.2%
Impurity 2 CAS#		459.58	Process impurity	Organic impurities by LC	Range: ND – 0.3%
β-Naphthol CAS#		144.173	Starting material, forced degradation product	Organic impurities by LC; Chromatographic retention	Observed only during stress studies
1-(Ethylamino)-3-(1-naphthyloxy)-2-propanol CAS#		245.32	Process intermediate, forced degradation product	Impurity content by LC-MS; UV spectroscopy, NMR spectroscopy	Observed only during stress studies

Only Impurity 1 and Impurity 2 have been detected in stelbataolol drug substance lots manufactured to date. Impurity 1 has been observed at up to 0.7% and Impurity 2 has been observed at a level up to 0.2% in the API batches manufactured to date. 1-(Ethylamino)-3-(1-naphthyloxy)-2-propanol is the only impurity containing an alerting structure based on in-silico evaluation and in vitro testing for

genotoxicity. This impurity is controlled at under 0.15% per day as a Class 2 impurity under ICH M7 guidelines for Qualification Threshold.

The enantiomeric purity of the compound is assessed in the release specification. Any impurities arising from unwanted side reactions are as yet unknown but are likely to be detected by the HPLC method for batch release.

All other known or unknown impurities in stelbatalol batches to date have been within the at the ICH Q3A Qualification Limit of 0.15% based on a maximum daily dose of 20 mg/day.

2. Residual Solvents

The following solvents used in the manufacture of stelbatalol are potential residual solvents in the drug substance:

- Process A: methanol, acetic acid, ethyl acetate
- Process B: acetone, ethanol, acetic acid, hexane

All residual solvents are controlled or detected below the limits set forth in ICH Q3C guidance. No class 1 solvents are used in the drug substance manufacturing process. Methanol and hexane are class II solvents which are controlled to the <3000 ppm and <290 ppm concentration limits, respectively, as established by ICH Q3C.

3. Elemental Impurities

The assessment of elemental impurities in stelbatolol drug substance, as prescribed in ICH Q3D, includes As, Cd, Hg, Pb, Co, Ni, V, Pd, Li, Sb, and Cu. A Cu catalyst is the only metal catalyst used in the stelbatalol drug substance manufacturing process. Specifically, Cu is employed in the formation of 1-(isopropylamino)-3-(naphthalen-1-yloxy) propan-2-one with an acceptance criterion of not more than (NMT) 20 ppm as controlled in the release of stelbatalol drug substance. Additionally, an in-process control for Cu is employed in step 3a with an acceptance criterion of NMT 10 ppm. Elemental impurities are controlled in stelbatalol drug substance to ensure that the permitted daily exposure (PDE) limits set forth in ICH Q3D Guidance for Finished Drug Products with an oral route of administration are met.

In summary, release testing data show that the drug substance consistently meets the Cu and elemental impurities criteria, confirming that the risk of elemental impurities in stelbatalol is low and does not present safety and product quality concerns.

4. Potential Mutagenic Impurities

To evaluate potential mutagenic impurities in stelbatalol drug substance, ICH M7 guidance is applied to all potential mutagenic impurities to provide impurity classification and control strategies as well as in silico and in vitro Ames testing information.

A structural alert assessment was conducted on all starting materials, raw materials, intermediates, potential and observed impurities in both drug substance and process intermediates, degradants, and reagents in accordance with ICH M7 guidelines. In vitro mutagenicity assessment was completed for the stelbatalol drug substance, and was negative for mutagenicity, per GLP Ames study.

Potential and observed impurities were further assessed for their potential mutagenicity by review of literature data and in silico analyses using both rule-based and statistical models. The collective assessment and classification of the impurities according to ICH M7 is summarized in **Table 2**. Those impurities having positive mutagenic predictions from the in-silico analyses were further assessed by in

vitro tests. The in vitro genetic toxicity assessments included an in vitro bacterial reverse mutation assay (Ames).

The likelihood of Class 2 and Class 3 impurities to be present in the drug substance was assessed based on physical and chemical properties, batch analysis, and stage at which it is involved in the manufacturing process. Taking this into account and the stelbatolol dosage, mutagenic impurities will be managed using the ICH Q3A threshold of 0.15% per day. A summary of the ICH M7 Control Strategy options and how they will be used is presented in **Table 3**.

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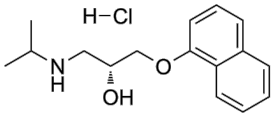
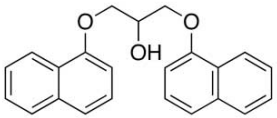
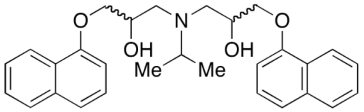
Table 2. Summary of (Q)SAR Results for All Known and Potential Mutagenic Impurites

Chemical Name	Structure	Origin in Process	Bacterial Mutagenicity Predictions or Knowledge			Comments
			Rule-Based (Q)SAR	Statistical (Q)SAR	ICH M7 Classification	
R-enantiomer of stelbatalol		Enantiomer of API SM	Negative	Negative	Class 5	Treated as non-mutagenic due to lack of alerting structure
Impurity 1		Step 1a Process Impurity	Negative	Negative	Class 5	Treated as non-mutagenic due to lack of alerting structure
Impurity 2		Step 1a Process Impurity	Negative	Negative	Class 5	Treated as non-mutagenic due to lack of alerting structure

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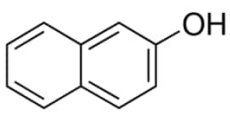
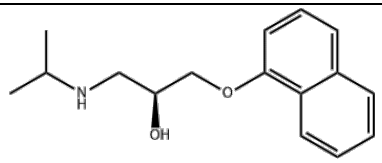
Chemical Name	Structure	Origin in Process	Bacterial Mutagenicity Predictions or Knowledge			Comments
			Rule-Based (Q)SAR	Statistical (Q)SAR	ICH M7 Classification	
β-Naphthol		API Starting material	Negative	Negative	Class 5	Treated as non-mutagenic due to lack of alerting structure
1-(Ethylamino)-3-(1-naphthyloxy)-2-propanol		API Process Intermediate	Positive	Positive	Class 2	Control at or below qualification threshold

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Table 3. Stelbatalol Mutagenic Impurity Control Strategy Summary

Compound Identifier	ICH M7 Control Strategy	Origin in Process	Predicted Purge factor	Calculated Purge Ratio[a]	Representative drug substance batch data	Control Explanation
1-(Ethylamino)-3-(1-naphthyloxy)-2-propanol	Option 3	API Process Intermediate	10^4	100	<10 ppm	Controlled on DS specification to NMT <50 ppm

5. Conclusions

This section provides a comprehensive overview of the organic impurities observed in stelbatalol as well as those impurities observed during long term stability studies. Batch data demonstrates that all elemental impurities assessed for stelbatalol drug substance are detected below the PDE limits set forth in ICH Q3D guidance. Additionally, all solvents used in the manufacture of stelbatalol have been controlled and detected below the limits set forth in ICH Q3C guidance. A mutagenic impurities assessment was performed and is presented above. The mutagenic impurities assessment demonstrates that all mutagenic impurities are controlled to below the limits set forth in the ICH Q3A/Q3B guidelines.